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Implications of Climate Change for Bioassessment Programs and Approaches to Account for Effects

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ABSTRACT

Climate change will affect stream ecosystems directly, indirectly, and through interactions with other stressors. Biological responses to these changes include altered community composition, interactions and functions. Effects will vary regionally and present heretofore unaccounted influences on biomonitoring, which water-quality agencies use to assess the status and health of ecosystems as required by the Clean Water Act. Biomonitoring, which uses biological indicators and metrics to assess ecosystem condition, are anchored in comparisons to regionally established reference benchmarks of ecological condition. Climate change will affect responses and interpretation of these indicators and metrics at both reference and non-reference sites, and therefore has the potential to confound the diagnosis of ecological condition. This report analyzes four regionally-distributed state biomonitoring data sets to inform on how biological indicators respond to the effects of climate change, what climate-specific indicators may be available to detect effects, how well current sampling detects climate-driven changes, and how program designs can continue to detect impairment. Results can be used to identify methods that assist with detecting climate-related effects and highlight steps that can be taken to ensure that programs continue to meet resource protection goals.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
BCG	Biological Condition Gradient
BI	Biotic Index
CCA	Canonical Correlation Analysis
cfs	cubic feet per second
CWA	Clean Water Act
DEP	Department of Environmental Protection
DEQ	Department of Environmental Quality
EMAP	Environmental Monitoring and Assessment Program
ENSO	El Niño/Southern Oscillation
EPT	Ephemeroptera, Plecoptera, Trichoptera
GCRP	Global Change Research Program
HBI	Hilsenhoff Biotic Index
IBI	Index of Biotic Integrity
ICI	Invertebrate Community Index
IHA	Indicators of Hydrologic Alteration
MMI	Multi-metric index
NAO	North Atlantic Oscillation
NCAR	National Center for Atmospheric Research
NCBI	North Carolina Biotic Index
NCDENR	North Carolina Department of the Environment and Natural Resources
NMDS	Non-metric Multidimensional Scaling
O/E	Observed to Expected ratio
OCH	Odonata, Coleoptera, Hemiptera
OTU	Operational Taxonomic Unit
PDO	Pacific Decadal Oscillation
PRISM	Parameter-elevation Regressions on Independent Slopes Model
RBI	Richards-Baker flashiness Index
RIVPACS	River InVertebrate Prediction And Classification System
UAA	Use attainability analyses
USGS	United States Geological Service
WSV	Weighted Stressor Values

PREFACE

This report was prepared by Tetra Tech, Inc. and the Global Change Research Program (GRCP) in the National Center for Environmental Assessment of the Office of Research and Development at the U.S. Environmental Protection Agency (U.S. EPA). It is intended for managers and scientists working on biological indicators, bioassessment, and biocriteria, particularly in the EPA's Office of Water and Regions, and also at state agencies. The results presented in this report are based on data primarily from four states, Maine, North Carolina, Ohio, and Utah. The main findings of interest to manager and policymakers, the supporting evidence, and management responses are presented in a separate summary at the beginning of this report. The remainder of the report provides more detail to substantiate each of the findings. Descriptions of specific analysis methods, underlying data, and supporting analyses are in the appendices to this report.

AUTHORS, CONTRIBUTORS, AND REVIEWERS

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EXECUTIVE SUMMARY

Bioassessment is used for resource management to determine the ecological consequences of environmental stressors. All states utilize some form of bioassessment as part of their implementation of the Clean Water Act. This assessment identifies the components of state and tribal bioassessment programs that may be affected by climate change. The study investigates the potential to identify biological response signals to climate change within existing bioassessment data sets; analyzes how biological responses can be categorized and interpreted; and assesses how they may influence decision-making processes. This study focused on benthic macroinvertebrates, important indicators used in bioassessments of wadeable rivers and streams. The ultimate goals of the report are to provide a foundation for understanding the potential climatic vulnerability of bioassessment indicators and advancing the development of specific strategies to ensure the effectiveness of monitoring and management plans under changing conditions.

We selected four regionally distributed state bioassessment data sets from Maine, North Carolina, Ohio, and Utah for this analysis. Bioassessment data were analyzed to determine the relative sensitivity of benthic community characteristics and traits to historical trends in temperature, precipitation, and other environmental drivers. The analysis allowed community characteristics and traits to be classified as either sensitive or insensitive to climate change effects.

Bioassessment programs rely on reference sites, often the most natural or pristine sites available, to help provide a basis for comparison with impaired sites. However, climate change will impact all sites in a region. Consequently, it will be necessary to understand the potential impacts of climate change for the use of reference sites in bioassessments. We examined the vulnerability of reference conditions to changes in climate and interactions between climate change and other landscape-level stressors, especially land use.

This study describes biological responses to changes in temperature, precipitation, and flow that will, in the long term, affect the metrics and indices used to define ecological status. Not all regions are equally threatened or responsive because of large-scale variability in climate and other environmental factors. We found that climatically vulnerable components of bioassessment programs include:

- Assessment design (e.g., multi-metric indices (MMIs), selection of reference sites, determination of reference condition)
- Implementation (e.g., data collection and analysis)
- Environmental management (e.g., determination of impairment and water quality standards)

Ecological traits are useful tools for these analyses since traits are not location specific, unlike some species. This facilitates comparisons among the state data sets used. This study mainly focuses on traits related to temperature and hydrologic sensitivities. Effective bioassessment designs rely on MMIs and predictive models to detect impairment. The effectiveness of widely-used MMIs and predictive models may be undermined by changing climatic conditions through the ecological trait of temperature sensitivity. Taxa with cold water- and warm water-preferences are used in many MMIs and predictive models. The climate responsiveness of these traits groups varies between states and ecoregions; however, they are generally found to be highly sensitive to changing temperature conditions. Consequently, MMIs and predictive models, which rely heavily on these sensitive taxa are likely to be vulnerable to climate change. In many cases, it may be feasible to develop new MMIs and modify variables in predictive models to partition sensitive taxa and reduce the potential for changing conditions to confound efforts to detect impairment.

Another widespread and related finding is the moderate but significant relationship between temperature sensitivity and sensitivity to organic pollution. These findings show that metrics selected because the composite taxa are considered to be generally sensitive or because they respond to conventional pollutants also have demonstrable sensitivities to climate-related changes in temperature and flow conditions. These results reinforce the need to partition taxa with climate-sensitive traits from MMIs and to account for these responses in predictive models.

The implementation of bioassessment programs often involves flexible sampling systems, such as rotating basin designs. These systems ensure statistically adequate sampling over five-year periods, at the expense of continuous monitoring of specific locations. This type of probabilistic sampling creates challenges for reference-based comparisons to assess condition, detect impairment, and identify causes of impairment under changing climatic conditions. While rotating, probabilistic systems sample numerous reference locations across a state, detection of climate change requires evaluation of trends at least at a few specific locations over time. Consequently, states may have many reference locations, but lack enough stable, long-term stations needed to detect climate-driven changes in biotic condition.

Climate change can cause other problems for reference-based bioassessment systems. We note that climate change can drive changes in community composition that vary by location, potentially further compounded by non-climatic landscape stressors. The result is variation in responses between locations that can confound efforts to establish statistically significant relationships or detect impairment. For example, our results show that high-flow metrics (e.g., flashiness, high pulse-count duration, one-day maximum flow) tend to reflect urbanization, swamping climate change effects; whereas low flow metrics (e.g., short-duration minimum flows, low pulse count) respond to climate change effects more so than to land use.

Responses to low-flow parameters were also documented using long-term water temperature trends at USGS gaging stations. Most of the long-term stations in our study showed slightly to distinctly increasing trends in benthic inferred temperatures over time, though not all trends were significant. Inferred-temperature responses are evidence of climate change-related increases in temperature, in that they reflect a progressive shift over time in composition of temperature preferences integrated across the entire benthic community. The response over time of any one taxon with a particular temperature preference (e.g., a cold-preference taxon) may or may not be significant despite the expectation, but it is significant if the community as a whole is reflecting an overall progressive shift in temperature preferences. This response was slightly greater at higher elevation locations. Results from these analyses corroborate the results from the landscape analyses that low flow parameters have better performance than high flow/pulse event parameters in detecting climate change trends.

A synthesis of all results leads to several recommendations for bioassessment programs in terms of modifying assessment design, implementation, and environmental management. With respect to metrics and indices, it will be useful to partition climatically vulnerable indicators into new metrics that account for temperature preferences of the component taxa. Analyzing bioassessment data according to temperature preferences will facilitate tracking climate change-related taxa losses and replacements. This traits-based approach for detecting and tracking climate change effects is promising, given that there were few specific species that showed consistent climate-related trends across multiple sites and states analyzed.

Although data limitations prevent explicit differentiation between inter-annual, cyclical, and long-term directional climate effects, the net response of benthic community metrics to climate-sensitive variables (i.e., water temperature, hydrologic patterns) provides useful information. The responses can be used to (1) define the direction and nature of effects expected due to climate change; (2) identify the most sensitive indicators to climate change; and (3) understand implications to MMIs or predictive models and their application by managers to characterize condition of stream resources for decision making.

The limited long-term data also illustrate that annual monitoring at least at some fixed reference locations is needed to account for climate change effects. The ability to detect a real trend is affected by signal-to-noise ratio and by the amount of data available to account for this variation. Evidence from this study of the high among-site variability within ecoregions suggests a trade-off in sampling effort between sampling many stations using a probability-based design to understand regional variations and sampling selected locations more frequently to document long-term trends. A mixture of targeted reference sites that can be maintained over the long-term along with probabilistic sampling may be more appropriate for monitoring the effects of climate change. This more comprehensive monitoring design will increase the robustness of water program assessments to the confounding effects of climate change.

Long-term monitoring also requires that these reference locations are as protected as possible from other stressors and landscape influences. Our analyses show that reference conditions may be more vulnerable than impaired sites to climate-change effects, a result that undermines the current methods of condition assessment. Two approaches that can assist with condition assessments in the context of climate change are to: 1) implement the Biological Condition Gradient (BCG) framework, within which changes in condition of both high quality and impaired locations can be more rigorously defined and tracked; and 2) promote protection of high quality stream reaches that define reference conditions. Protection should focus on minimization, mitigation, and/or buffering from non-point source runoff, erosion, and hydrologic changes.

Documenting existing land use conditions surrounding established reference locations is also important to establish a baseline for tracking future changes. Urbanization surrounding reference stations will interfere with the ability to detect climate change and separate climate responses from conventional stressors; this can interfere with managing aquatic resources, setting permit limits, and meeting Clean Water Act requirements. Our results show that hydrologic monitoring, especially using low-flow parameters, can assist with distinguishing changes due to urbanization versus climate.

Reference sites that remain unprotected from stressors or land use changes are vulnerable to deterioration due to conventional stressors as well as climate change. The deterioration of reference conditions and climate impacts on biological indicators, metrics, and indices together will affect the determination of stream reach impairment. Unless metrics are modified so that climate effects can be tracked, thresholds for defining impairment are re-evaluated, and actions to document and protect reference station conditions are taken, it is likely that in vulnerable watersheds there may be fewer listings of impaired stream reaches and progressive under-protection of water resources.

Actions that are associated with the listing of a stream reach as impaired, including stressor identification and development of TMDLs, are also affected by climate changes. Stressor identification should include biological responses to climate change effects. Climate-related changes to flow may also need to be integrated into loading calculations and limits for new or revised TMDLs.

Water quality standards that are resilient to changes in climate-related variables will remain protective and should be identified. Climate change can be expected to alter some designated uses and their attainability, especially in vulnerable streams or regions. Refinement of aquatic life uses can be applied to guard against lowering of water quality protective standards.

The results from the analyses conducted as part of this assessment show that climate change will affect many of the activities in bioassessment programs. Our results also identify methods that can assist with detecting these effects and controlling for them analytically. Implementing these recommendations will allow programs to continue to meet their resource protection and restoration goals in the context of climate change.

SUMMARY FOR MANAGERS AND POLICYMAKERS

Climate change is an important consideration for bioassessment programs because it can affect almost all activities associated with these programs (Fig. SMP-1). This report uses data from four pilot study states, Maine, North Carolina, Ohio, and Utah, to examine the implications of climate change on these activities. This summary is intended for managers and policymakers working with bioassessment data and results, who are making decisions about resource impairment, designated uses, water quality standards, use attainability, and total maximum daily loads (TMDLs). This summary highlights the ten most important findings of the underlying report:

- Multi-metric indices are vulnerable to climate change
- Predictive models used in bioassessment may be less vulnerable to climate change
- Detection of climate change effects requires a specifically designed climate change monitoring network
- Reference stations are vulnerable from changes in community composition
- Vulnerability varies by location
- Reference sites need protection from other stressors
- Collecting abiotic data is also necessary
- Reference station degradation diminishes the ability to detect impairment
- Climate change may make TMDL development more difficult
- Climate change may alter designated uses and their attainability

Following each finding is a brief description of the evidence to support each finding and discussion of potential responses that can assist managers and policymakers in adapting bioassessment programs to climate-change effects. The findings in this summary and the body of the report are organized according to the steps shown in Fig. SMP-1.

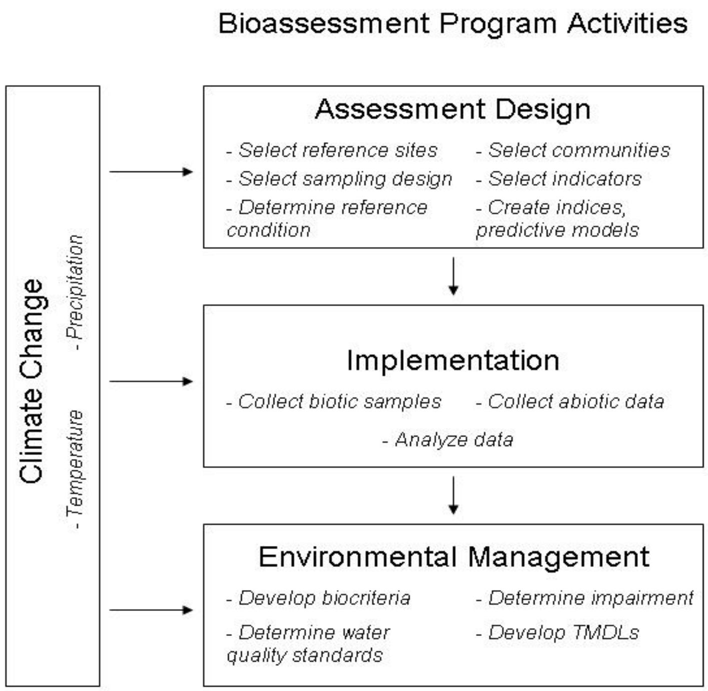

Figure SMP-1. Climate change can affect many bioassessment program activities from the initial assessment design, to collecting and analyzing data, and to developing responses to assessment outcomes.

Findings influencing assessment design

1. Multi-metric indices are vulnerable to climate change

Finding: Climate change affects specific biological metrics used in multi-metric indices (MMIs). This is important because MMIs are used by many states as a basis for comparing between high quality and potentially impaired sites and to assign site ratings.

Evidence: In the four states analyzed, though not at all stations or in all regions within each state, cold water-preference taxa decreased in richness and/or abundance with increasing temperatures, and in some areas, warm water taxa increased. Some responses are fairly widespread, including total taxa richness; Ephemeroptera, Plecoptera, and Ephemeroptera/Plecoptera/ Trichoptera (EPT) richness; and richness of cold or warm sensitive taxa. Changes in these metrics alter MMIs through shifts in the proportion of cold to warm water-preference taxa. Further evidence for this finding is presented for each state analyzed.

Maine: Maine's longest-term reference location is at a relatively low elevation and has a higher proportion of warm-preference taxa, including warm-preference EPT taxa. Therefore, an increase in EPT taxa with increasing temperatures could improve overall station rating, unlike in Utah and North Carolina (described below). This is because one metric, Ephemeroptera abundance, does not have a linear relationship with station class (see Section 3.1). In Maine there is an additional consideration associated with the use of a group of "Class A indicator taxa" as one of the ways of separating Class A from B condition ratings. Maine's Class A indicator taxa are fairly evenly divided between cold and warm-water-preference taxa. Therefore, application of this metric with increasing temperature could confound results, because some of the Class A indicators could decline with increasing temperatures, while others could increase.

North Carolina: In North Carolina, EPT taxa richness is one of two indices used for bioassessment, along with the Hilsenhoff Biotic Index (HBI). Both indices contain cold water-preference taxa. Though the loss of all cold water-preference EPT taxa due to increasing temperatures is highly unlikely, this scenario would lead to a reduction of reference station condition equivalent to one full category (e.g., from excellent to good). The HBI in North Carolina is vulnerable to the loss of cold-preference taxa and gain in warm water-preference taxa. This is due to the relationship between temperature preference and sensitivity to organic pollution (Section 3.2). Since a high proportion of cold water-preference taxa have low pollution tolerance ratings, the loss of cold water-preference taxa at reference stations due to climate change also increases the HBI index value for that station, making its assessed condition appear degraded.

Ohio: The Ohio MMI and the determination of the final station rating are also vulnerable to climate change because of the positive association between temperature sensitivity and pollution tolerance. Percent of tolerant taxa is one of the metrics used in the Ohio MMI. There are also several EPT metrics in the Ohio MMI, including EPT taxa richness, Ephemeroptera and Trichoptera richness, and relative abundance of Ephemeroptera and Trichoptera taxa. These metrics contribute to the vulnerability of the Ohio MMI through the relative contribution of cold-preference taxa within these groups (Section 3.3).

Utah: Fairly predictable losses in EPT taxa richness (especially cold water-preference taxa) with increasing temperatures are found in both higher and lower elevation ecoregions at reference stations in Utah. Up to a 25% loss of EPT taxa could occur with current scenarios of temperature increases by 2050 (Section 3.4). A lower EPT richness metric value will cause an overall decrease in the MMI, reducing the condition status of that station. Because the relative composition of cold water-preference taxa among EPT taxa, as well as in the total community, is associated with elevation, higher elevation regions with a greater proportion of cold-preference taxa may have a greater vulnerability to this effect.

Adaptation response: The liability of using existing metrics is due to the inability to separate temperature responses from other conventional pollution responses. Vulnerable and influential metrics, such as EPT richness, the HBI, other metrics related to EPT taxa, should be separated into new metrics that account for temperature preferences (e.g., a 'cold-EPT richness' metric, etc). This would allow climate-related taxa losses or taxa replacements to be tracked. For example, a ratio of cold-EPT-richness to warm-EPT-richness would provide a benchmark for changes related to climate variables compared to conventional stressors. The ability to compare cold metrics to warm or total metrics between reference and non-reference locations will help support detection of climate change responses.

Adding a climate-tolerant metric can also assist in separating climate change and conventional stressor responses. In this study, responses of Odonata, Coleoptera, and Hemiptera (OCH) taxa were examined based on their reported tolerance to summer conditions, higher temperatures, and lower flows (Bonada et al. 2007). OCH taxa showed positive trends over time, with increasing temperatures, and/or with lower precipitation in some locations (Section 3 and Appendix I); this supports their use as a climate-tolerant metric.

Current limitations to developing climate-sensitive or climate-tolerant metrics are due to a lack of information on temperature preferences for some taxa and hydrologic data necessary to determine flow-related preferences. This study used biomonitoring data to develop temperature preference and tolerance information for many taxa common to Maine, Utah and North Carolina (see Stamp et al., 2010, USEPA, 2011). However, this type of analysis is needed nationwide. In addition, much of the extant temperature preference information is at the generic operational taxonomic unit (OTU) level, rather than the species-level. Hydrologic data was also examined, but these variables were even more limited and could not be developed into flow-related preference information for taxa.

2. Predictive models used in bioassessment may be less vulnerable to climate change

Finding: Some predictive models (e.g., River InVertebrate Prediction and Classification System (RIVPACS)) used by states may be more resilient to climate change than MMIs because they incorporate long-term (e.g., 30-year) averages of environmental predictor variables, including climate parameters, and also because this baseline is recalibrated to a more recent timeframe. Most RIVPACS models are currently not designed to consider changes in climate, but they could be. Predictive modeling could be used to associate ranges of biological responses with the natural range of variation for various climate parameters, and perhaps then differentiate this from long-term changes. A limitation of modeling is that it assumes freedom of movement, when in reality, dispersal barriers exist.

Evidence: The greatest vulnerability in applying the RIVPACS model in Utah for decision making lies in the measured "observed" communities, since they change as a result of shifts in cold- (and warm-) preference taxa; this drives differences in the observed/expected (O/E) quantity used in the model. The model predictor variables themselves appear relatively robust to near-term climate changes in temperature, especially if long-term averaging periods for predictor variables are used (see Section 3.4). Thus the predictive modeling approach can track changes without detecting the trend in expected ("reference") communities. However, changes in climate-related parameters used as predictor variables will alter model precision in assigning the probability of occurrence of a taxon in a class. Without model recalibration, this could alter the expectation for inclusion of taxa in a community, and may therefore create larger differences between observed and expected communities.

Adaptation response: Periodic model recalibration may address this vulnerability. Recalibration allows shifts in the expected community to be incorporated in O/E calculations. Wider use of the predictive model approach may be a good adaptation for state biomonitoring programs to climate change influences on data interpretation. However, as 'expected' assemblages become more 'tolerant,' assemblages may be less likely to show responses to other stressors (i.e. nutrients). This may reduce differences between expected communities (i.e., the reference baseline) and observed communities exposed to anthropogenic stressors.

3. Detection of climate change effects requires a specifically designed climate change monitoring network

Finding: Detection of climate change requires evaluation of changes at some specific locations or strata over time; despite the relatively large number of reference stations, there are very few with long-term data, thus limiting the power of current monitoring schemes to detect effects due to climate change.

Evidence: Inherent biomonitoring program characteristics tend to limit regular, long-term sampling at reference locations. These include random sampling within a stream reach or watershed that tends to maximize spatial sources of variation; infrequent sampling (e.g., once every 5 years) in a rotating basin design; lack of replication (one sample per location per year); and lack of measurements of covariates (Section 4.1). An additional consideration is the high vulnerability of existing reference locations to impairment from encroaching land uses (Finding 6). These considerations illustrate the value of designing a monitoring scheme to account for climate change within the biomonitoring framework.

Adaptation response: Climatic changes, as well as aquatic ecosystem vulnerability to climate change, vary regionally. Some of the variability is related to elevation, topography, and geology. Such conditions often cross state and tribal boundaries. Establishment of climate-specific networks, their monitoring, and subsequent data analysis may require collaboration among states with regard to technical considerations (e.g., site selection, sampling methods) and funding. Regional or national support may be important to facilitate this process.

An initial climate-specific monitoring network could focus on climatically vulnerable locations. Sites should be sampled at least annually. Less frequent data collection would extend the time needed to detect climate change responses, because of interannual and cyclic climate variability. Monitoring should occur at some fixed locations, rather than only using a probability-based sampling approach. It is valid to identify fixed but representative reference locations within a target stratum (e.g., ecoregion/watershed /vulnerability zone) for detection of trends and evaluation of biological responses to climate change.

To separate climate change effects from other stressors both should be measured over time; thus, climate-specific monitoring should be established along part of the stressor gradient and be anchored in reference conditions (Finding 4). This allows temporal trends at reference sites to be compared to temporal trends at impaired sites as a mechanism for differentiating between climate effects and conventional stressors. Sampling the stressor gradient would potentially allow different levels of stressor effects to be compared and synergistic effects to be considered. A less resource-intensive alternative would be to establish long-term sentinel sites only at high-quality reference locations.

Many different groups are considering, or have already started, monitoring for climate change effects. Collaboration across groups avoids duplication, saves resources, and encourages consistency in data collection and use of a common database. Consistency among monitoring networks will enhance the ability to detect climate-related changes.

4. Reference stations are vulnerable from changes in community composition

Finding: Climate change increases the vulnerability of reference (high quality) stations through shifts in biological communities that lead to degraded states.

Evidence: This study documents climate change effects that can degrade reference station status to be more similar to non-reference stations, at least in some regions more vulnerable to climate change effects (e.g., high elevation sites, head-water or low order streams) (Section 4.2). In addition, at non-reference stations, effects of climate change may be additive with other stressors, or interactions between climate change and other stressors may augment or ameliorate responses (Figure SMP-2).

Comparison of long-term trends between reference and impaired sites can assist in separating climate change effects from other stressor effects. This implies the need for long-term monitoring at more than just high quality sites (see Finding 3). In the absence of climate change-specific monitoring data, long-term trend analysis to characterize climate change effects should be conducted on data from reference locations to minimize confounding effects (Sections 4 and 5). This is important, because impacts from land use, nutrient runoff, and other sources are often not measured and cannot be easily controlled in analyses.

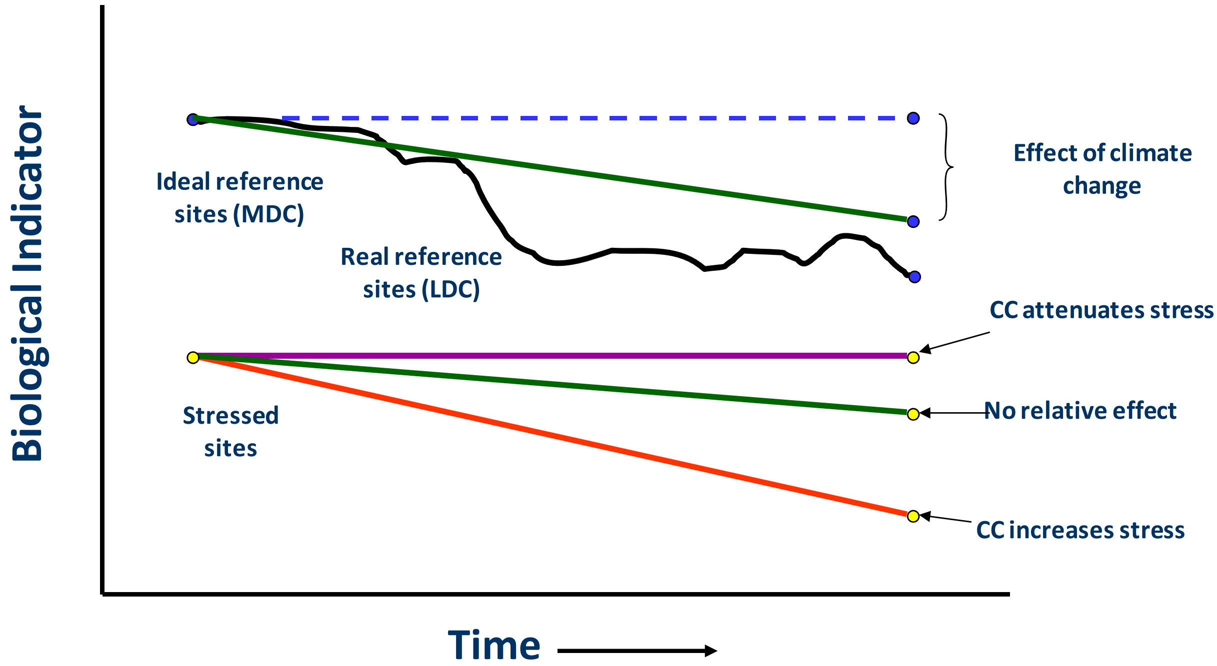

Figure SMP-2. Conceptual model showing relationship between climate change trends and reference and stressed sites with an overlay of temporal variation on the trend (black line). "MDC" = minimally disturbed condition; "LDC" = least disturbed condition.

Adaptation response: Reference station condition should be documented using a consistent framework such as the Biological Condition Gradient (BCG), which captures a more subtle range of biological conditions with regulatory significance, compared to an "impaired/not impaired" decision approach. Changing conditions can then be judged against a common framework. The BCG delineates a meaningful and scaled framework within which the degree of degradation attributable to climate change can be characterized (Figure SMP-3). A predictive modeling approach is another framework that can be used to judge a gradient of changes in condition against a reference baseline in a manner that could support differentiating climate change effects from other stressor effects. The modeling approach of classifying regions based on major predictive variables, and using those predictor variables to define expectations for taxa occurrences within a class (region) uses a wide spatial distribution of reference samples to define the range of 'natural' variability in each predictor variable. This is essentially doing a space for time substitution, to the extent that the spatial range of variation can be used to characterize the expected range of temporal variation. Predictive modeling could be used to associate ranges of biological responses with the natural range of variation for various climate parameters, and perhaps then be used to differentiate this from long-term changes.

5. Vulnerability varies by location

Finding: Elevation seems to determine the relative vulnerability of community metrics and MMIs to climate change effects; the magnitude of this response varies regionally. Other contributing factors appear to be stream order (size) and watershed size.

Evidence: Trends in biota are more distinct in Utah at more locations than Maine or North Carolina. This is likely related to regional differences in climate change scenarios. For example, projections for temperature increases are lower for the southeast, including North Carolina, than they are for the other three states[1]. Temperatures in the southwest (Utah) are expected to increase slightly more than for the northeast (Maine) and central states (Ohio), although the differences in projected temperature among those three areas are very small. Other factors may contribute to observed regional differences in biological trends and apparent vulnerability, such as differences in groundwater contribution to flow, stream order, or watershed size. There also are some artifacts of the available biomonitoring data sets, such as the lack of long-term reference locations in the northeast highlands ecoregion in Maine, where higher elevations and greater proportion of cold-preference taxa in the community might have shown stronger trends and provided a better comparison to the Utah results.

[1] See NCAR website: http://rcpm.ucar.edu

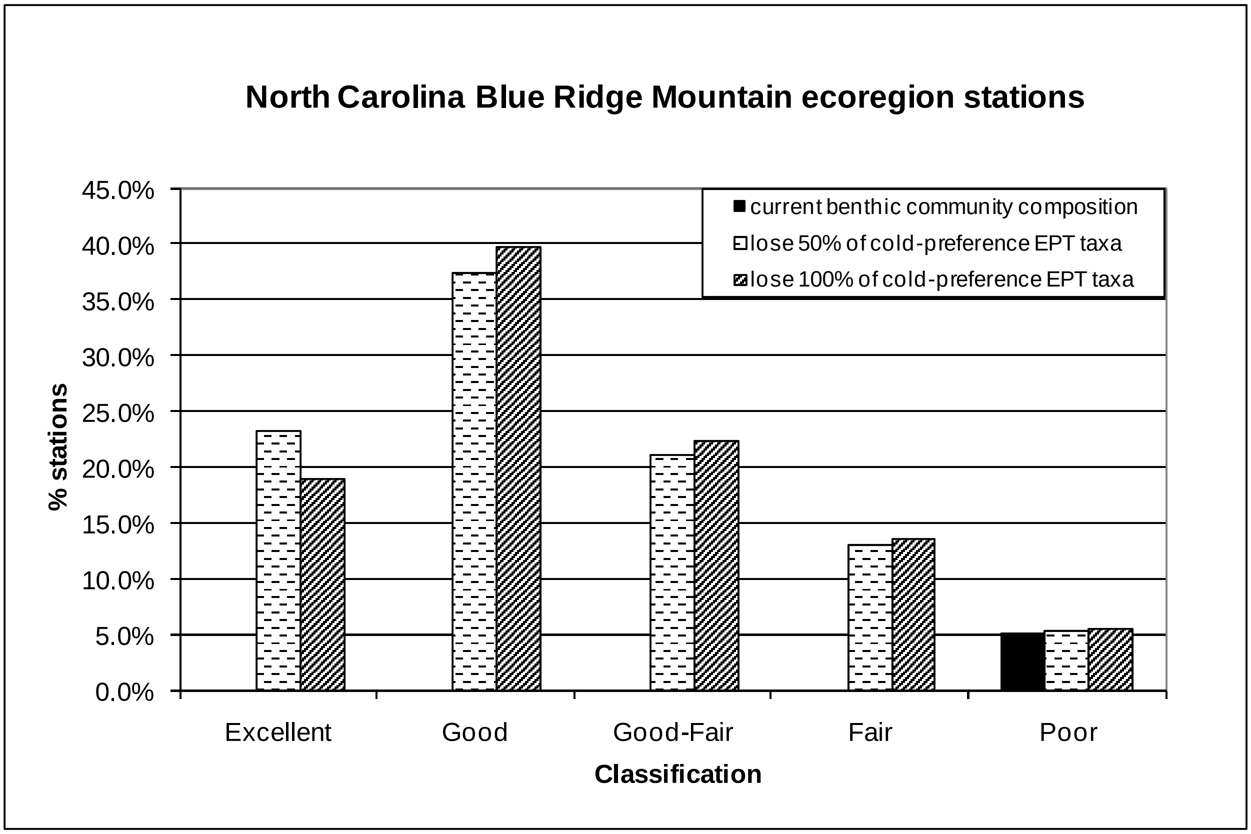

Figure SMP-3. Reference station drift (degradation of assessed site condition) over time at Blue Ridge Mountain ecoregion stations as cold-preference taxa are lost over time due to climate change.

Adaptation response: If detection of climate change effects becomes a goal, then sampling at sites more sensitive to these effects becomes important. Elevation may be the first criteria to identify these sites. The importance of other criteria, such as groundwater contributions and riparian conditions, still needs to be assessed.

Sites that are more sensitive to climate change can also be used to identify sensitive indicators and test hypotheses on the relationships among habitat, abiotic variables and species traits. These indicators, along with cold water-preference indices (Finding 1), can be used in a monitoring network (Finding 3) to document changes in reference condition (Finding 4).

Findings influencing assessment design and implementation

6. Reference sites need protection from other stressors

Finding: Encroachments of landscape-scale anthropogenic influences, in particular increasing urban/suburban development, on reference sites over time threaten reference conditions. This threatens both the documentation of climate change-associated biological trends and spatial comparisons to reference conditions for impairment detection.

Evidence: Our analyses of reference sites (Section 5) show that existing vulnerabilities to land-use effects are much greater and more widely distributed than previously conceived or quantified. Urbanization may affect 20-25% of reference stations currently. By 2100 urbanization could cause measurable degradation at almost 50% of reference stations. This level of land use encroachment would imperil the foundation of the reference condition approach.

Adaptation response: The high vulnerability of reference locations to land-use effects, along with the importance of identifying and separating climate-change effects, emphasizes the need to characterize reference conditions and document current status. Two aspects of reference stations must be considered. One is the selection and siting of reference stations and the other is their protection. Candidate reference stations should be screened using land-use data. Land-use distribution by major category should be documented for all stations. Criteria related to the maximum extent of developed and agricultural land uses should be created and applied to define reference conditions; however, the criteria may need to be state or region specific and accommodate existing realities of extent of development. Criteria should recognize that "unaffected" reference locations may not exist. If current land-use data show low urban and agricultural uses, it is a reasonable assumption that associated impacts, including urban-associated hydrologic impacts and agriculture-associated nutrient loadings, are minimal. In addition, temporal changes in land-use characteristics surrounding a reference site become important information for judging degradation of condition that is separate from climate change.

Concepts for protection of reference stations are primarily related to land-use changes and must involve social, political, and economic components in addition to technical considerations. In general, an appropriate scale for protecting reference sites is within a watershed management scheme. Implementing protection actions such as zoning regulations, incentives for limiting development in riparian areas or near headwater streams, or other strategies to directly protect high-quality stream reaches from land-use encroachment is inherently difficult. Identification of the most vulnerable and important sets of reference sites within a state could be an initial strategy to better target protective actions.

7. Collecting abiotic data is necessary

Finding: Abiotic data, such as air and water temperatures, precipitation, flow, and water chemistry, along with habitat characteristics, need to be collected frequently and over time at biotic sampling locations.

Evidence: Establishing a relationship between climate change and biotic responses is contingent upon understanding the relationship between climate variables and hydrologic variables like water temperature and flow. Long-term, continuous data were not available for all reference stations (Section 5 and Appendix A). This makes it more difficult, sometimes impossible, to establish relationships between observed changes in climate and the benthic community.

Adaptation response: Data loggers should be used at all reference stations, especially at sites within a climate-change monitoring network. Water temperature and flow are the minimum variables that should be collected; additional data about water chemistry and habitat characteristics would be useful, although these data may be collected with biological samples.

Findings influencing environmental management

8. Reference station degradation diminishes the ability to detect impairment

Finding: Changes in biological metrics are sufficient to downgrade reference station condition. Degradation of reference station condition is essentially causing references stations to become more similar to non-reference stations and diminishes the ability to detect impairment due to conventional stressors.

Evidence: Climate change does not discriminate between reference and non-reference stations. This diminishes the effectiveness of reference comparisons to determine impairment. This study documents changes in biological indicators, which are reasonably attributable to climate change effects. Sections 2 and 3 document changes in cold- and warm-preference taxa at reference stations due to climate-change-related trends in temperature and precipitation; these trends result in changes in MMIs (Finding 1). Unless metrics are modified so that climate effects can be tracked, and thresholds for defining impairment re-evaluated, degraded reference conditions will cause fewer stream reaches to be defined as impaired. This will lead to less corrective action and greater long-term degradation of stream conditions.

Adaptation response: Maintaining the ability to detect impairment will require modifications of biological metrics (Finding 1), re-evaluation of impairment thresholds, and reference station classification and protection (Findings 4 and 6). These actions, along with a monitoring network

(Finding 3), will improve tracking effects of climate change and comparing effects between reference and non-reference locations to differentiate climate change from other stressors and detect conventional stressor impairment.

9. Climate change may make TMDL development more difficult

Finding: Climate change scenarios show greater variability in runoff and flow, which may result in greater uncertainty in loadings expected from non-point sources. Critical low flows also drive TMDLs, and these may become more uncertain and more difficult to predict.

Evidence: Changes in biological metrics are sufficient to downgrade reference station condition (Section 4). This degradation causes reference sites to become more similar to impaired sites, thereby diminishing the ability to detect impairment. Therefore, unless climate change effects are tracked using modified metrics, degradation of reference sites will cause fewer stream reaches to be defined as impaired, at least in the most vulnerable watersheds (Section 6.1).

Adaptation response: In addition to modifying metrics, watershed-specific modeling to predict how flow dynamics change with climate is needed to provide support for estimating future changes in low flows, and to modify loading calculations and limitations accordingly.

10. Climate change may alter designated uses and their attainability

Finding: Climate change can be expected to alter some uses and their attainability, especially in vulnerable streams or regions. Biological responses to climate change will likely impact water quality standards and biocriteria through shifts in baseline conditions.

Evidence: Climate change will affect biological communities at reference locations, thereby altering the characterization of expected levels of ecological integrity. Some cold water streams could take on cool water characteristics, with declining abundances or richness of sensitive cold water taxa and possible increases in warm-water taxa. Regulated parameters such as temperature, dissolved oxygen, and ammonia, may also be sensitive to climate change effects, and their values may need to be adjusted relative to revised designated uses.

This study illustrates several avenues through which climate change is affecting stream communities in ways that have implications for biocriteria programs. Section 2 discusses how trait groups, taxonomic groups, and to some extent, individual taxa appear to be responding over time to climate drivers in ways that are predicable and consistent with expectations relative to climate change. Section 3 discusses implications of these changes to various MMIs and predictive models (Findings 1 and 2). The cascading effects of climate change-related trends in temperature and precipitation on watershed conditions, water quality, and aquatic biological communities, will lead to shifting, most often degrading, baseline conditions (Finding 4). Decreases in mean abundances or species richness of cold-preference or other sensitive taxa and trait groups, increases in warm-preference or other tolerant taxa and groups, and also increases in variability of these indicators drive reference sites to greater similarity with non-reference areas, as well as greater difficulty in establishing statistical differentiation (USEPA, 2008). As a result, reference-based standards will be liable to progressive under-protection (Section 6.2).

Adaptation response: There are numerous criteria, both biological and chemical, that are addressed in water quality standards and which may be affected by climate change (Table SMP-1). Biocriteria are of particular interest, as they tie closely to the indices and thresholds used to determine condition and impairment. The climate-related causes of drifting (degrading) baseline conditions cannot be directly controlled, but can be assessed. The concepts that support this include clear documentation of reference conditions, tracking of changes in reference conditions over time (Finding 4), and protection of reference conditions from other stressors, particularly land-use changes (Finding 6). This may include monitoring a network of sites designed to detect climate-change effects (Finding 3).

For watersheds that are particularly vulnerable to climate-change effects, including those characterized by particularly vulnerable trait groups, more refined aquatic life uses should be considered. Refinement of aquatic life uses can be applied to guard against lowering of water quality-protective standards. More refined aquatic uses could create more narrowly defined categories, which could accommodate potentially “irreversible” changes, but with sufficient scope to maintain protection and support anti-degradation from regulated causes.

Further efforts to address climate change impacts to standards require examination of which water quality standards are resilient to climate change effects and therefore remain protective, and identification of susceptible standards that may need adjustment. Climate change effects that contribute to degradation of water quality and biological resource condition bring into question how anti-degradation policies can be managed considering the additional influences of climate change. High quality water bodies may be most vulnerable to climate change degradation, making application of anti-degradation policies in vulnerable water bodies important. Management approaches and special considerations for implementation of anti-degradation policies may need attention. In addition, the application of use attainability analyses (UAA) on vulnerable water bodies may be pertinent for characterizing climate impacts.

Table SMP-1. Variables addressed in criteria and pathways through which they may be affected by climate change (from Hamilton et al. 2010)

Criteria	Climate change impacts
Pathogens	Increased heavy precipitation and warming water temperatures may require the evaluation of potential pathogen viability, growth, and migration.
Sediments	Changing runoff patterns and more intense precipitation events will alter sediment transport by potentially increasing erosion and runoff.
Temperature	Warming water temperatures from warming air temperatures may directly threaten the thermal tolerances of temperature-sensitive aquatic life and result in the emergence of harmful algal blooms (HABs), invasion of exotic species, and habitat alteration.
Nutrients	Warming temperatures may enhance the deleterious effects of nutrients by decreasing oxygen levels (hypoxia) through eutrophication, intensified stratification, and extended growing seasons.
Chemical	Some pollutants (e.g., ammonia) are made more toxic by higher temperatures.
Biological	Climate changes such as temperature increases may impact species distribution and population abundance, especially of sensitive and cold-water species in favor of warm-tolerant species including invasive species. This could have cascading effects throughout the ecosystem.
Flow	Changing flow patterns from altered precipitation regimes is projected to increase erosion, sediment and nutrient loads, pathogen transport, and stress infrastructure. Depending on region it is also projected to change flood patterns and/or drought and associated habitat disturbance.
Salinity	Sea level rise will inundate natural and manmade systems resulting in alteration and/or loss of coastal and estuarine wetland, decreased storm buffering capacity, greater shoreline erosion, and loss of habitat of high value aquatic resources such as coral reefs and barrier islands. Salt water intrusion may also affect groundwater.
pH	Ocean pH levels have risen from increased atmospheric CO_2, resulting in deleterious effects on calcium formation of marine organisms and dependent communities and may also reverse calcification of coral skeletons.

1. CLIMATE CHANGE AND BIOASSESSMENTS

In the US, the Clean Water Act (CWA) of 1972 identified the restoration and maintenance of physical, chemical and biological integrity as a long-term goal (Barbour et al., 2000). Biological assessment has been recognized around the world as a valuable and necessary tool for resource managers to determine biological integrity (Norris and Barbour, 2009). Research on biological assessment approaches is ongoing, including studies on the development and evaluation of the most effective bioindicators, metrics, indices, and models (Bressler et al., 2009; Johnson and Hering, 2009; Stevenson et al., 2008; Stepenuck et al., 2008; Stribling et al., 2008; Hawkins, 2006; Clarke and Murphy, 2006; Fellows et al., 2006; Hering et al., 2004; Dale and Beyeler, 2001; Johnson et al., 2006; Sandin and Johnson, 2000); consideration of the best assemblage groups or system attributes to monitor (Resh, 2008; Bonada et al., 2006; Hering et al., 2006); regional classification within which to structure monitoring (Archaimbault et al., 2005; Moog et al., 2004; Hawkins and Norris, 2000); and establishment of reference conditions (Verdonschot, 2006; Stoddard et al., 2006; Walin et al., 2003; Bailey et al., 1998; Reynoldson et al., 1997). Overall, incorporation of biological assessment methods to preserve ecological integrity of waterways has proved to be far more effective than sampling only chemical parameters (Karr, 2006). Because the structure and function of aquatic assemblages reflect all sources of environmental disturbance to which they are exposed over time, assessment of biological communities can provide information that may not be revealed by measurement of concentrations of chemical pollutants or toxicity tests (Barbour et al., 1999; Rosenberg and Resh, 1993; Resh and Rosenberg, 1984). Biological assessment, coupled with multi-metric or other predictive modeling analyses, is the strongest approach for diagnosing diminished ecological integrity, and minimizing or preventing degradation of river systems (Karr and Chu, 2000).

The effectiveness of biological assessment to more reliably detect impairment of ecological integrity than chemical sampling has been demonstrated in state programs over the years (Yoder and Rankin, 1998). When biological assessment was incorporated into Ohio's CWA assessment scheme, almost half of stream segments statewide that met the standards of their designated uses based on chemical sampling were found to be impaired based on assessment of biological indicators (Yoder and Rankin, 1998). In contrast, only a few (<3% of) stream segments that had acceptable biological condition had chemical exceedances (Yoder and Rankin, 1998).

Stream benthic invertebrates are the most common assemblage used for biomonitoring (USEPA, 2002a), although fish and algal assemblages also are frequently applied in the United States (Resh, 2008). In this study, benthic communities are the primary focus of analysis using state biomonitoring data sets. Their integrative characteristics make benthic assemblages effective monitoring tools if all major sources of stress are accounted for in order to reliably attribute observed responses to particular sources.

The main goal of this study is to determine what components of bioassessment programs are threatened by climate change, a stressor that is currently not considered. Related objectives are to investigate whether biological response signals to climate change are discernable within existing bioassessment data sets, how responses can be categorized and interpreted, and how they influence the decision-making process. There is substantial evidence that climate change is affecting the environment (IPCC 2007), including aquatic ecosystems, and therefore reasons to account for climate change within the context of bioassessment programs.

A growing number of studies document climate change responses in freshwater ecosystems. Increased prevalence and/or distribution of warm water (thermophilic) taxa, and changes in species richness have been found in fish communities (Daufresne and Boet, 2007; Buisson et al., 2008; Hiddink and Hofstede, 2008). Long-term responses of benthic invertebrate communities have included changes in stability and persistence, changes in community composition, increases or decreases in prevalence of taxa groups based on thermophily and rheophily, species replacements and range shifts, and changes in resilience of community states (Chessman, 2009; Collier, 2008; Burgmer et al., 2007; Beche and Resh, 2007; Woodward et al., 2002; Daufresne et al., 2007; Durance and Ormerod, 2007; Mouthon and Daufresne, 2006; Daufresne et al., 2003). Climate change effects on stream benthos can be seen as long-term, progressive changes that overlay other natural sources of variability, including other climate drivers. As an example, patterns of stream benthic community persistence in England were found to be related to fluctuations in the North Atlantic Oscillation (NAO), as well as to directional climate change (Bradley and Ormerod, 2001). The magnitude of changes associated with directional climate change are often subtle compared to other large-scale spatial (e.g., land use) and temporal (e.g., the NAO) influences (Chessman, 2009; Sandin, 2009; Collier, 2008; Bradley and Ormerod, 2001).

Climate change effects may be small and long term from certain perspectives, but they are pervasive. This study documents biological responses to changes in temperature, precipitation, and flow that will, in the long term, affect the metrics and indices used to define ecological status. Not all regions are equally threatened or responsive, because of regional variability in climate combined with spatial variability in vulnerability[2] and resilience of the affected aquatic ecosystems. Many factors can influence susceptibility to changing water temperature or hydrologic regime from climate change, such as elevation (Chessman, 2009; Diaz et al., 2008; Cereghino et al., 2003), stream order (Cereghino et al., 2003; Minshall et al., 1985), degree of ground water influence, or factors that affect water depth and flow rate, such as water withdrawals (Chessman, 2009; Poff et al., 2006a; Poff 1997).

The components of bioassessment programs that may be affected by climate change include assessment design, implementation, and environmental management (Figure 1-1). Awareness that climate change can have widespread effects on biological communities introduces additional uncertainty into a system that requires interpretable patterns of biological indicator responses to "conventional" stressors. This has the potential to cast doubt on all claims of stressor-response relationships that are being evaluated within a regulatory context. It also highlights that the biomonitoring tools applied must be appropriately tailored to the types of stressors expected. With increasing knowledge of the types of climate change effects that are appearing to different degrees in regions around the country, and of the categories of organisms that are showing the most predictable responses, it becomes important to adjust assessment tools to changing biota to enable a clearer interpretation of stressor identification and causal analysis.

This study investigates the potential effects of climate change on indicator organisms and consequences for benthic communities. The results will provide insights on how climate change may hinder the ability of state and tribal bioassessment and biocriteria programs to meet their goals of: 1) detecting impairment within reasonable temporal and spatial frames, 2) identifying probable causes of impairment, and 3) meeting a variety of resource management objectives. The ultimate goal of this study is to further the development of strategies for adapting monitoring and management plans to accommodate these expected environmental changes.

[2] Vulnerability is generally defined as a combination of exposure (e.g., the expected climate changes in temperature and precipitation); sensitivity or the degree of responses to the exposures; and resilience or ability of the communities (or habitats) to adapt and cope with the exposures and responses (see also Poff et al. 2010). We refer to the vulnerability of the habitat (features of the natural landscape), as well as the vulnerability of the biotic communities. Vulnerability can be thought about on at different scales, e.g. the biological assemblage as a whole, individual species, particular sites, stream types, etc.

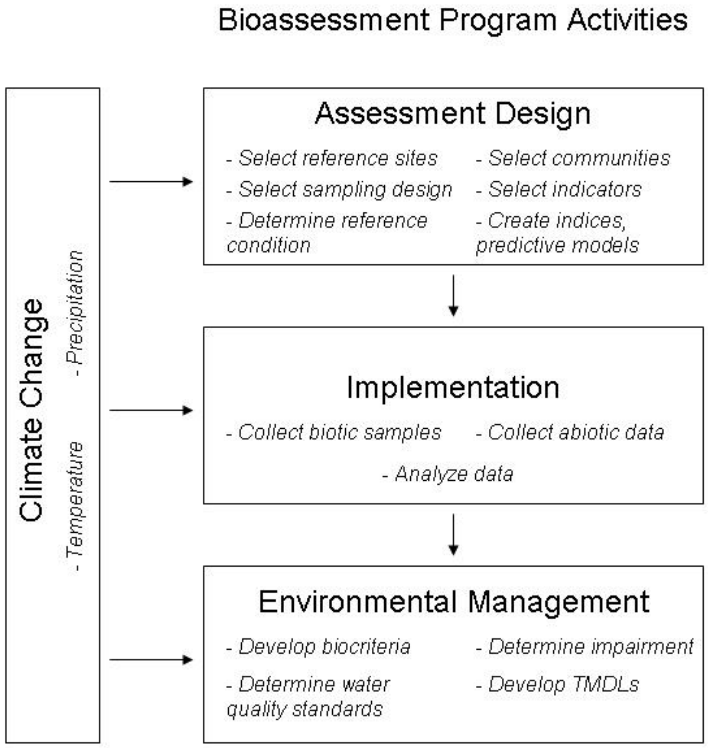

Figure 1-1. Climate change can affect many bioassessment program activities from the initial assessment design, to collecting and analyzing data, and to developing responses to assessment outcomes.

Using four regionally distributed state bioassessment data sets from Maine, North Carolina, Ohio, and Utah, historical trends are examined in relation to temperature, precipitation, and other environmental drivers. Community and traits analyses are used to identify potential indicators, both sensitive and insensitive (robust) to climate change effects. Examination of climate-sensitive traits is used to facilitate transfer of analysis results to other places. These results are supplemented with additional analyses focusing on the vulnerability of reference conditions, and the interactions between climate change and other landscape-level stressors, especially land use. This study builds on the results of a preliminary analysis (EPA 2008) and feedback from a workshop convened in 2009 with state and tribal scientists and resource managers, academic and agency experts, and decision makers to explore the following issues: the effects of climate change on endpoints of concern; methods for integrating climate change into existing state and tribal water quality programs; and ways to create opportunities for adaptation.

Study findings are summarized in the beginning of this report in the Summary for Managers and Policymakers (SMP). The body of the report expands on the analyses that support these findings. Section 2 describes analyses using ecological traits and Section 3 applies these results to indices and predictive models used in state and tribal water programs. Section 4 examines reference station vulnerabilities and discusses design considerations for a monitoring network to detect climate-change effects. Section 5 describes additional characteristics of biomonitoring programs that are relevant to discerning climate-change effects. Finally, Section 6 analyzes implications to environmental management, including development of total maximum daily loads (TMDLs) and water quality standards. Detailed results of all analyses are compiled in appendices.

2. ANALYSIS OF ECOLOGICAL TRAITS TO DETECT CLIMATE CHANGE EFFECTS

The underlying objective of conducting detailed analyses of several state bioassessment data sets is to understand how assessments of biological condition may be affected due to climate change. Several specific research questions contribute to this objective:

- Are there biological responses, illustrated in temporal patterns or relationships with climate variables, already discernable in long-term biomonitoring data?
- What biological indicators, e.g., trait or taxonomic groups, are sensitive or robust to climate change effects?
- Are there spatial patterns or associations that help elucidate climate vulnerabilities that may be important to bioassessment programs?
- What modifications to metric analyses might help separate and track climate change effects?

As more research is conducted and more trait information becomes available, it is likely that more traits-based metrics will become good candidates for detecting responses to climate change. In the United States, the value of traits-based versus taxa-based approaches is becoming more widely recognized (Olden et al., 2008; Beche and Resh, 2007; Poff et al., 2006b). In Europe, traits–based approaches are currently being used in researching climate-related trends on aquatic ecosystems (Bonada et al., 2007b). There are many values to traits-based approaches. Categorization by traits rather than species (or other taxonomic level) reduces variation across geographic areas, making traits better suited for regional analyses. Traits can be less susceptible to taxonomic ambiguities or inconsistencies (Moulton et al. 2000) in long-term datasets. Traits also can be used to detect changes in functional community characteristics (e.g., Bonada et al. 2006, 2007, Beche and Resh 2007) and provide a consistent framework for assessing community responses to gradients across local and regional scales (Vieira et al. 2006). Finally, use of trait categories allows for aggregation of data into fewer categories, which can simplify analyses.

2.1. BACKGROUND ANALYSES

In order to begin answering these questions, several foundational analyses were needed to establish the magnitude and direction of climate change trends in specific locations. The analyses of long-term air temperature, precipitation, water temperature, and flow records assist in partitioning bioassessment data into relevant groupings. These analyses also set up an expectation for the strength of the biological responses that may be discerned in the available data. For example, long-term air temperature increases are evident from PRISM[3] annual air temperatures; these show gradual, but significant increases from 1974 to 2006 in three of the states analyzed, Utah, Maine, and North Carolina (Appendix A). Air temperatures differed between ecoregions in each state, but the rates of increase in air temperature over time were similar across ecoregions. No significant long-term trends in annual precipitation could be defined using PRISM data.

Long-term water temperature trends are also evident from USGS gaging station records. The rate of water temperature increases averaged 0.76 °C per 10-year period, but varied around the country, partly in relation to stream size. It should be noted that the North Carolina stream analyzed had a higher water temperature increase than the Utah stream, even though climate change-related temperature projections are slightly greater for Utah[4] (Appendix A), suggesting that differences in stream size have a greater effect in this case.

Extensive and iterative analyses were conducted using the large bioassessment data sets from multiple states (see Appendix B for details on data preparation). These data also informed the selection of long-term stations (Appendix C). Most of the long-term stations or station groups within ecoregions of all states that were tested showed slightly to distinctly increasing trends in benthic inferred temperatures over time, though not all the trends were statistically significant (Appendix A). Inferred temperature responses are evidence of climate change increases in temperature, with slightly greater responses at higher elevation locations. The benthic inferred temperature trends in Utah were statistically significant, equivalent to a rate of increase of approximately 3 °C in 25 years. Using benthic invertebrate occurrence and abundance coupled with temperature preferences is a reliable means of estimating water temperature at the time of collection, and conversely, provides evidence of benthic community changes over time related to long-term changes in temperature.

[3] PRISM Climate Group, Oregon State University, http://www.prismclimate.org. Documentation: http://prism.oregonstate.edu/docs/index.phtml

[4] See NCAR website: http://rcpm.ucar.edu

An initial set of analyses was done to assure temporal consistency of the bioassessment data (Appendix B), as well as to evaluate reference station[5] conditions for possible contributions of other stressors (especially from land use; Appendix J). Table 2-1 summarizes the overall analysis approach, organized by question being addressed and analysis type. These analyses support the discussion of results presented in this chapter on the usefulness of ecological traits to track biological responses to climate change. They are also the foundation for assessing implications of climate change to multi-metric indices (MMIs) and predictive models used by state and tribal bioassessment programs, summarized in Section 3. Details about the analysis methods are presented in Appendix D.

2.2. TRENDS IN ECOLOGICAL TRAIT GROUPS

The reason for evaluating traits is that it comes closer to a mechanistic understanding of interactions and provides a pathway toward describing the functional implications of climate-change effects on aquatic communities. The ecological traits of temperature and hydrologic preferences or sensitivities (e.g., Poff et al. 2006) provide the most direct link to climate impacts. Other traits such as feeding types, habit, or morphology are also important, but defining expectations for responses to the effects of climate change is more challenging. For example, responses of some feeding types to climate change may be indirect through effects on food resources (phytoplankton, periphyton, allochthonous organic matter) (e.g., Hargrave et al., 2009; Monters-Hugo et al., 2009; Moline et al., 2004; Tuchman et al., 2002). This study evaluated many traits and trait suites for relationships to climate change effects, though not all potentially relevant and fruitful analyses were possible due to limitations of the available biomonitoring data. In addition to trait groups, analyses also focused on various taxonomic metrics and indices commonly incorporated in biomonitoring programs.

[5] We selected reference sites based on guidance from the respective state agencies. Selection criteria vary across states. In Utah, reference criteria are based on a combination of a reference scoring sheet (multiple lines of scoring (i.e., habitat, land use, chemistry) and independent ranking of sites from field crew/scientists. In North Carolina, land use land cover in the upstream catchment area is an important selection criterion. In Maine, for purposes of our analyses, we categorized Class A sites (these classifications are based on biology) as reference. Class A sites are not necessarily designated as reference sites by Maine DEP. Maine DEP is in the process of developing strict reference criteria; considerations will include factors such as land use land cover and proximity to NPDES discharges.

Table 2-1. Summary of analysis approach, by analysis type, main methods, and overlying questions.

Analysis Type	Relationship of (or patterns in):	Relationship to:	Method Highlights	Questions Addressed
Correlations	Biological indicators, Taxa; Taxa groups; Trait groups; Indices; Index components; Predictive model parameters.	Time (year) Climate variables (temperature, precipitation)	Pearson product moment; calculated using Statistica software (Version 8.0, Copyright StatSoft, Inc., 1984-2007); considered significant if $p < 0.05$.	Are there biological responses, illustrated in temporal patterns or relationships with climate variables, already discernable in long-term biomonitoring data?
ANOVA	Biological indicators, Taxa; Taxa groups; Trait groups (including cold and warm water temperature indicator taxa); Indices; Index components; Predictive model parameters.	Hot/cold/normal years; and wet/dry/normal years: defined these using extremes in climate variables among existing data as proxies for future climate conditions. Partitioned data at long-term reference stations in each state into years characterized by hotter (>75th percentile of the temperature distribution during years of biological collections), colder (<25th percentile of temperature), and normal (25th to 75th percentile) average annual air temperatures. Using similar thresholds, years were partitioned based on average annual precipitation into wetter, drier, and normal years.	One-way ANOVA; calculated using Statistica software (Version 8.0, Copyright StatSoft, Inc., 1984-2007); differences considered significant if: F statistics $p < 0.05$, and Tukey honest significant difference (HSD) test for unequal sample size (N) (Spjotvoll/Stoline) $p < 0.05$.	Same as above. Are certain metrics more likely to be affected by climate change than others?
ANOVA	Trait groups (esp. cold and warm water temperature indicator taxa)	Elevation categories; Ecoregions. Size (Strahler order or watershed area).	Same as above	Are there spatial patterns or associations that determine climate vulnerabilities important to bioassessment programs?
ANOVA	Maine station classification	Station classes	Same as above	How do model input metric values differ among

	descriminant model metrics			the different station classifications? How much do metric values have to change for a sample to change classification?

Table 2-1. continued.

Analysis Type	Relationship of (or patterns in):	Relationship to:	Methods Highlights	Questions Addressed
NMDS	Taxa composition of benthic communities collected each year at long-term reference stations, by state.	Hot/cold/normal years; Several temperature and precipitation variables (annual mean, departure from mean).	Non-parametric multidimensional scaling; performed using PCOrd: McCune, B. and M. J. Mefford. 1999. PC-ORD. Multivariate Analysis of Ecological Data. Version 4.41 MjM Software, Gleneden Beach, Oregon, U.S.A.); using a Sorensen distance measure.	Do changes in community composition over time reflect patterns consistent with climate change effects? Are taxa associated with observed changes sensitive/robust, as expected, to those climate change effects?
Weighted average (WA) modeling	All major taxa, by state.	temperature (and/or precipitation, flow when available)	Weighted average modeling or related approaches (e.g., maximum likelihood estimates, general linear modeling) to estimate the optima and range of temperatures of occurrence for each taxon from each state that had a sufficient distribution and number of observations to support the analysis (Yuan 2006); performed in R code.	What are the temperature (and/or precipitation, flow when available) preferences and tolerances of taxa collected in each state?
Benthic inferred temperature modeling	Taxon temperature preferences, occurrence and abundances	Time (long-term trends), temperature	Use WA model results of temperature optima for each taxon, and taxon occurrence and abundance by station, to do a weighted-average estimate of temperature [optimum temperature for each taxon at a station, times the abundance of that taxon, summed over all taxa, and divided by the sum of taxa abundances].	Do benthic communities reflect water temperatures at the time of collection? Do long-term changes in inferred temperatures provide evidence of benthic community changes over time related to temperature?
Re-running of Utah RIVPACS model	Model input parameters; Model outputs.	Climate changes in temperature, precipitation, other climate variables.	Done in R, using Utah DEQ model code, and revising modify climate-related input parameters.	Are O/E predictive model predictor variables and O, E, and O/E predictions sensitive to climate change alterations in temperature, precipitation, and other pertinent variables?

Evaluation of climate change effects is a fundamentally temporal question. Trend analysis is used to investigate long-term patterns in temperature, precipitation, flow, other habitat variables, and in biologic response variables. Results are used as evidence of whether global changes are contributing to the trends, and for considering other possible contributions. This type of post-facto analysis of historic data sets is widely used to determine whether climate change effects are already discernable in ecosystem responses (e.g., Daufresne et al., 2003; Durance and Ormerod, 2007; Burgmer et al., 2007; Murphy et al., 2007). Long-term stream benthic data from state biomonitoring programs are used in this study to look for temporal trends in various benthic community characteristics as evidence of existing biological responses to climate change. Limitations in the extent of statistically significant trends within the historic biological data are, in part, related to characteristics of the existing biomonitoring data sets, and should be understood in the context of the requirements and limitations of typical biomonitoring programs. This will inform on how biomonitoring and biocriteria programs are likely to be affected in the future.

Grouping macroinvertebrates based on temperature preferences and tolerances is expected to (1) have a greater chance of detecting temperature-related climate-change effects if they exist, (2) be interpretable with regard to causal relationships, (3) offer predictive ability and transferability to other regions, and (4) serve as a basis for developing adaptive responses (Verbeck et al., 2008a, 2008b; Poff et al., 2006b; Lamouroux et al., 2004). We developed temperature indicator metrics by designating cold and warm-water-preference taxa derived from weighted average or maximum likelihood modeling (methods in Appendix D), case studies, literature reviews and best professional judgment of regional workgroups[6]. Hydrologic indicator metrics were based on literature (e.g., the North Carolina perennial taxa list, Bonada et al., 2007a) and trait information (i.e., rheophily, drought resistance) related to flow permanence and current preference. Among the hydrologic indicator metrics were various ‘scenario’ metrics (drier-vulnerable, drier-robust, wetter-vulnerable, wetter-robust). These scenario metrics capture combinations of traits expected to impart an adaptive advantage (or not) under projected climate change conditions. After developing a list of traits believed to be favorable for each future climate change scenario, taxa that possessed the most number of those traits states were

[6] See Appendix Attachments E2, F2, and G1, Temperature Indicator descriptions for each state, for more detailed descriptions of the process followed to develop the temperature indicator taxa lists.

considered potentially ‘robust’ and those that had the fewest favorable trait states and the most number of unfavorable trait states were considered potentially ‘vulnerable.’ In addition, several scenario metrics were created that take both temperature and hydrologic regime into consideration (warmer drier vulnerable, warmer drier robust, warmer wetter vulnerable, warmer wetter robust).

2.2.1. Ecological Trait Groups and Climate Patterns

In Utah, results of ANOVA on ecological trait and scenario metrics varied by site. Two long-term reference stations, 4927250 (Weber) in the Wasatch Uinta Mountains and 4951200 (Virgin) in the Colorado Plateau, showed relatively strong temperature patterns, while two other long-term reference sites, 5940440 (Beaver) in the Wasatch Uinta Mountains, and 4936750 (Duchesne) in the Colorado Plateau, showed no patterns (Figure 2-1). At Stations 4927250 (Weber) and 4951200 (Virgin), hottest-year (see Table 2-1 and Appendix D for definition of hot and cold year definitions and proxy analysis approach) samples had significantly fewer cold-water-preference taxa than coldest-year samples[7] (Table 2-2). The greatest differences generally occurred between hottest- and coldest-year samples, while normal-year samples were variable.

Warm-water-preference taxa showed even fewer responses, increasing during hottest years only at Colorado Plateau station 4951200 (Virgin) of the four reference stations tested (Figure 2-2). Neither cold- nor warm-water-preference taxa responded differently among wettest, driest and normal years (see Table 2-1 and Appendix D for definition of wet and dry year definitions and proxy analysis approach, and Appendix F for additional details of Utah analysis results).

[7] In data preparation and analyses, the attempt was to identify and limit potential confounding factors as much as possible. However, factors other than (or in addition to) climate-related factors (such as changes in water chemistry, which were documented to show significant yearly trends at some of the sites) also potentially influenced assemblage composition. It should be emphasized that results for such confounding factors were generally site-specific, and it is uncertain whether similar patterns are occurring at other sites (see also Section 3.4).

Table 2-2. Mean (±1 SD) richness and % individuals with cold- or warm-thermal preferences in coldest (group 1), normal (group 2), and hottest (group 3) years at long-term biological monitoring sites in Utah (UT), Maine (ME), and North Carolina (NC). Year groups were based on Parameter-elevation Regressions on Independent Slopes Model (PRISM) mean annual air temperature values at each site. One-way analysis of variance (ANOVA) was done to evaluate differences in mean thermal-preference metric values. Groups with the same superscripts within a site are not significantly different ($p < 0.05$). NA = not applicable (warm-water-preference taxa absent).

		Cold		Warm	
Site	Group	Richness	% individuals	Richness	% individuals
UT 4927250 (Weber)	1	4.9 ± 1.1^{A}	6.5 ± 5.3^{A}	2.3 ± 0.8^{A}	0.6 ± 0.5^{A}
	2	3.4 ± 1.1^{A}	6.7 ± 7.3^{A}	1.1 ± 0.7^{A}	0.4 ± 0.3^{A}
	3	1.0 ± 0.7^{B}	1.0 ± 1.1^{A}	1.0 ± 1.2^{A}	0.3 ± 0.4^{A}
UT 4951200 (Virgin)	1	4.5 ± 2.4^{A}	15.7 ± 10.9^{AB}	1.5 ± 0.6^{A}	7.7 ± 6.7^{A}
	2	5.3 ± 1.2^{A}	23.4 ± 15.6^{A}	1.5 ± 0.8^{A}	18.1 ± 15.3^{A}
	3	0.8 ± 0.1^{B}	0.2 ± 0.2^{B}	3.8 ± 1.3^{B}	27.8 ± 19.4^{A}
UT 4936750 (Duchesne)	1	6.3 ± 1.5^{A}	24.3 ± 4.1^{A}	0.3 ± 0.6^{A}	0.03 ± 0.1^{A}
	2	6.3 ± 1.0^{A}	14.9 ± 6.8^{A}	0.7 ± 0.8^{A}	0.1 ± 0.2^{A}
	3	5.7 ± 2.9^{A}	17.7 ± 8.5^{A}	0.7 ± 1.2^{A}	0.1 ± 0.2^{A}
UT 5940440 (Beaver)	1	4.0 ± 2.6^{A}	12.1 ± 6.2^{A}	NA	NA
	2	3.3 ± 0.6^{A}	10.0 ± 9.2^{A}	NA	NA
	3	3.3 ± 1.2^{A}	8.4 ± 5.9^{A}	NA	NA
ME 56817 (Sheepscot)	1	0.5 ± 0.5^{A}	0.6 ± 0.6^{A}	6.4 ± 2.4^{A}	15.6 ± 7.4^{A}
	2	0.5 ± 0.8^{A}	0.7 ± 1.7^{A}	8.0 ± 1.4^{A}	21.2 ± 11.5^{A}
	3	1.1 ± 0.5^{A}	1.0 ± 0.8^{A}	8.5 ± 2.7^{A}	19.6 ± 10.7^{A}
ME 57011 (W.Br. Sheepscot)	1	0.7 ± 0.3^{A}	0.4 ± 0.2^{A}	7.2 ± 1.5^{A}	16.1 ± 7.3^{A}
	2	1.5 ± 0.5^{A}	6.3 ± 5.4^{A}	7.3 ± 2.8^{A}	48.4 ± 9.6^{B}
	3	1.0 ± 0.7^{A}	1.7 ± 0.3^{A}	7.8 ± 0.8^{A}	39.5 ± 15.4^{AB}
ME 57065 (Duck)	1	2.4 ± 1.2^{A}	7.8 ± 6.4^{A}	6.3 ± 0.6^{A}	44.0 ± 22.5^{A}
	2	1.7 ± 0.3^{A}	5.3 ± 5.9^{A}	6.8 ± 1.5^{A}	32.8 ± 10.8^{A}
	3	1.6 ± 0.7^{A}	5.0 ± 3.3^{A}	4.8 ± 1.3^{A}	46.6 ± 17.6^{A}
NC 0109 (New)	1	4.3 ± 1.5^{A}	2.3 ± 0.7^{A}	8.3 ± 0.6^{A}	7.7 ± 2.5^{A}
	2	5.4 ± 1.7^{A}	3.6 ± 2.9^{A}	7.4 ± 1.7^{A}	7.6 ± 2.5^{A}
	3	4.0 ± 1.7^{A}	2.2 ± 1.0^{A}	7.3 ± 2.3^{A}	7.0 ± 1.3^{A}

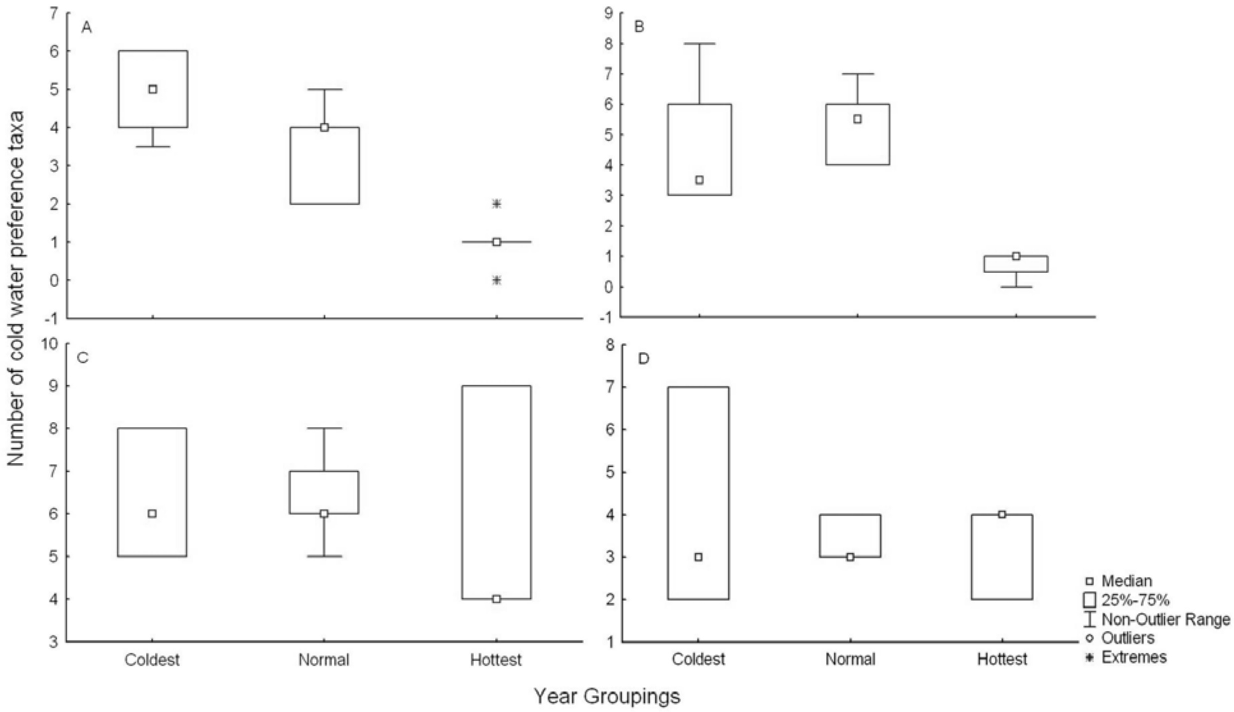

Figure 2-1. Distributions of cold-water-preference taxa richness values in coldest-, normal-, and hottest-year samples at Utah sites 4927250 (Weber) (A), 4951200 (Virgin) (B), 4936750 (Duchesne) (C), and 5940440 (Beaver) (D). Year groupings are based on PRISM mean annual air temperatures from each site during time periods for which biological data were available. Average temperatures in hottest-year samples were 1.1 to 2.7˚C higher than coldest year samples. Mean metric values for cold-water-preference taxa were significantly higher in coldest-year samples than in hottest-year samples at sites 4927250 and 4951200. Data used in these analyses were limited to autumn (September–November) kick-method samples.

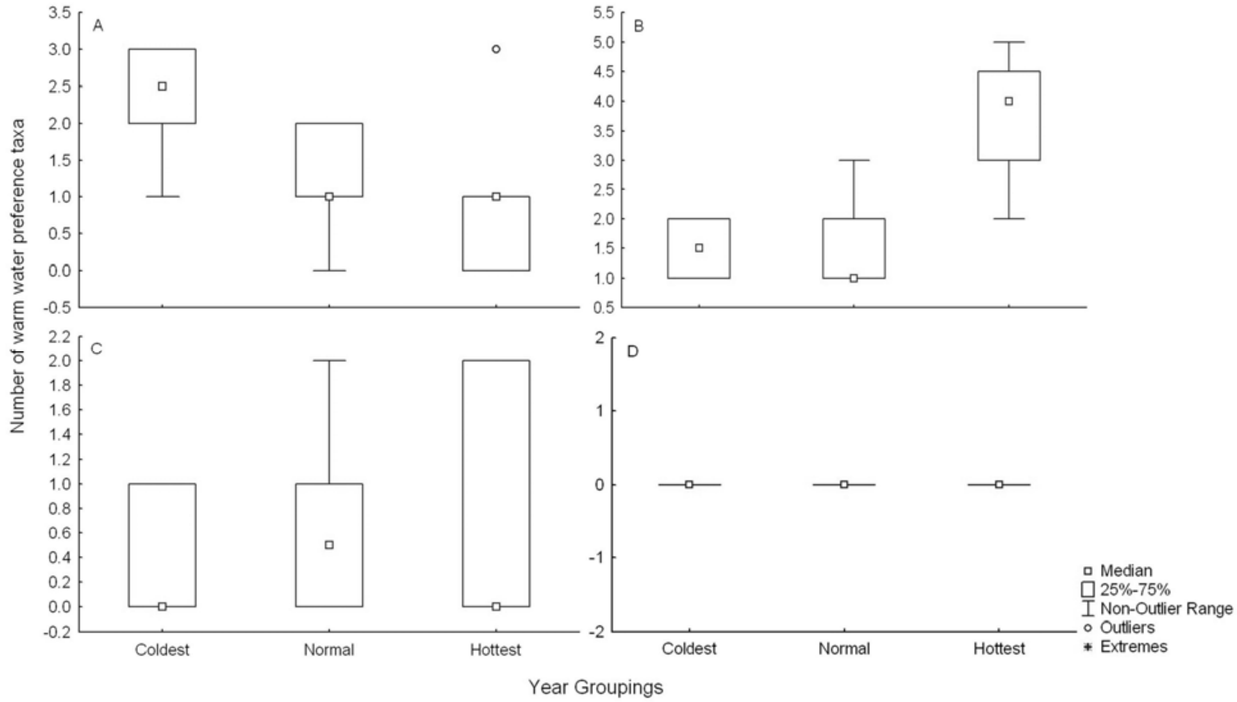

Figure 2-2. Distributions of warm-water-preference richness values in coldest-, normal-, and hottest-year samples at Utah sites 4927250 (Weber) (A), 4951200 (Virgin) (B), 4936750 (Duchesne) (C), and 5940440 (Beaver) (D). Year groupings are based on Parameter-elevation Regressions on Independent Slopes Model (PRISM) mean annual air temperatures from each site during time periods for which biological data were available. Average temperatures in hottest-year samples were 1.1 to 2.7°C higher than coldest year samples. Mean metric values for warm-water-preference taxa were significantly higher in hottest year samples than in coldest year samples at site 4951200. No warm-water-preference taxa were present at site 5940440. Data used in these analyses were limited to autumn (September–November) kick-method samples

In contrast to Utah, in Maine there was greater response to wet/dry years than to temperature differences. Cold-water-preference taxa, which were present in low numbers at the sites evaluated, were slightly more abundant and diverse during wet years at the longest-term reference station (Sheepscot) in the Laurentian Hills and Plains, though warm-water-preference taxa showed no response to a range of annual precipitation (Figure 2-3); the response of cold-water-preference taxa was not found at the few other reference stations that could be tested (Appendix E). Warm-water-preference taxa were generally more abundant and diverse during hottest and normal years at this Maine location (Sheepscot) (Figure 2-4). There also was a significant increase over time in richness and abundance of warm-water taxa at Station 56817 (Sheepscot) (Figure 2-5). This appears consistent with climate change expectations, given the predominance of warm-water-preference taxa at this station, plus increasing temperatures over time. However, neither abundance nor richness of warm-water-preference taxa was directly correlated with temperature at this station. In addition, the temporal trend was not spatially consistent. For example, the warm-water-preference taxa metrics did not increase at another Laurentian Hills and Plains reference location (site 57011 – W.Br. Sheepscot) (Appendix E).

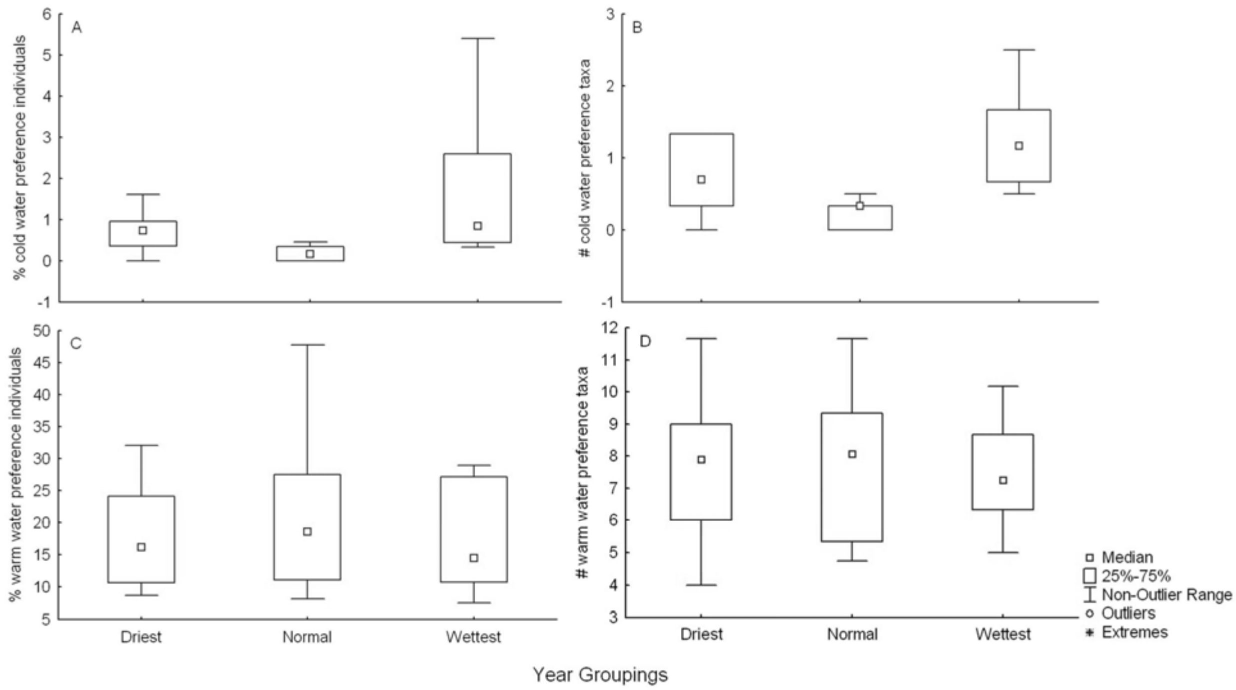

Figure 2-3. Distributions of thermal preference metric values in driest-, normal-, and wettest-year samples at Maine site 56817 (Sheepscot). Plot (A) shows % cold-water-preference individuals, (B) number of cold-water-preference taxa, (C) % warm-water-preference individuals, and (D) number of warm-water-preference taxa. Year groupings are based on PRISM mean annual precipitation from each site during time periods for which biological data were available. Data used in these analyses were limited to summer (July–September) rock-basket samples.

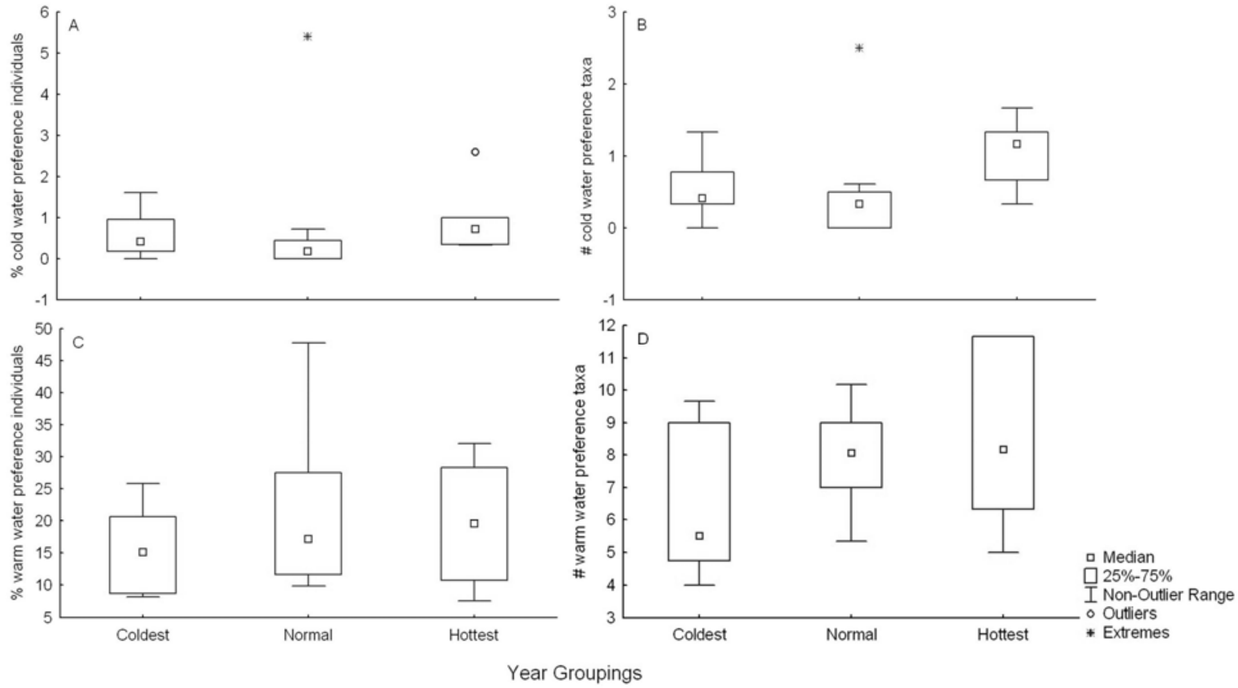

Figure 2-4. Distributions of thermal preference metric values in coldest-, normal-, and hottest-year samples at Maine site 56817 (Sheepscot). Plot (A) shows % cold-water-preference individuals, (B) number of cold-water-preference taxa, (C) % warm-water-preference individuals, and (D) number of warm-water-preference taxa. Year groupings are based on PRISM mean annual air temperatures during time periods for which biological data were available. Data used in these analyses were limited to summer (July–September) rock-basket samples.

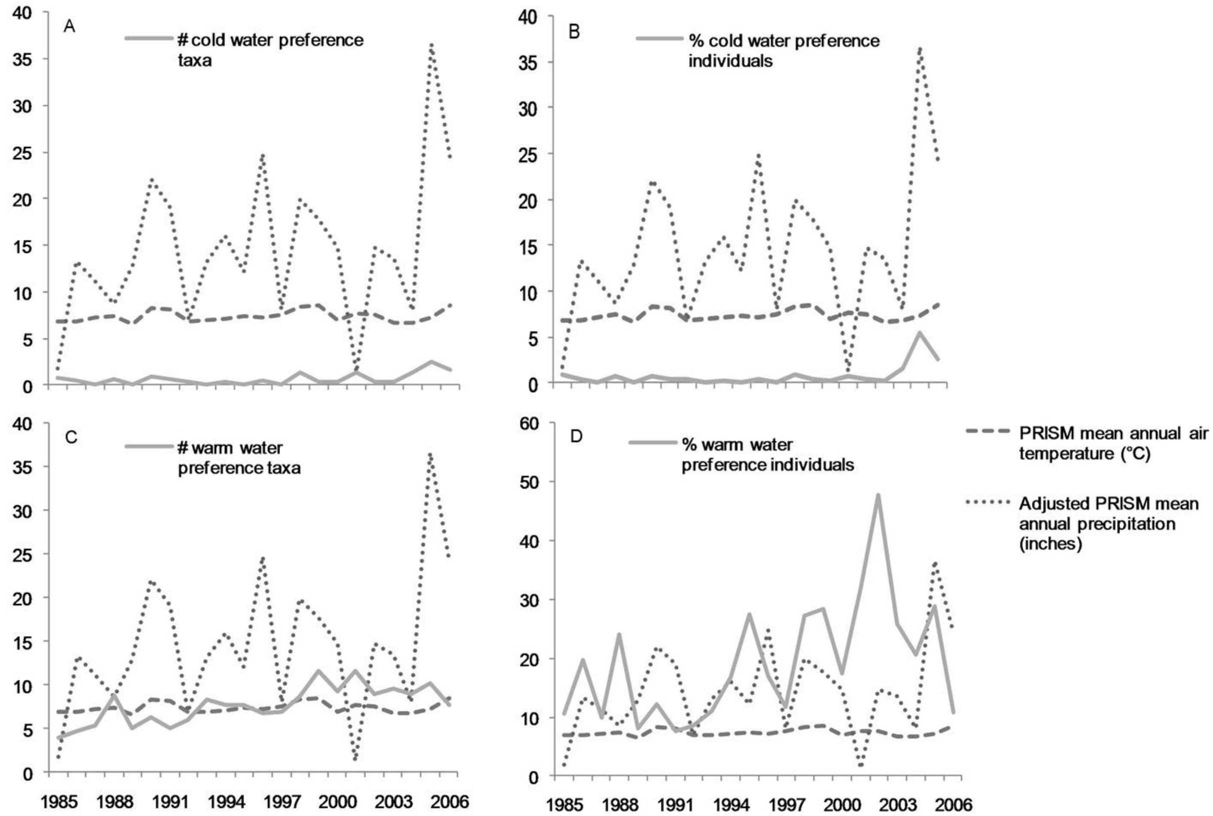

Figure 2-5. Trends in the thermal preference metrics and PRISM climatic variables over time at Maine site 56817 (Sheepscot). Plot (A) shows number of cold-water-preference taxa, (B) % cold-water-preference individuals, (C) number of warm-water-preference taxa, and (D) % warm-water-preference individuals. Data used in these analyses were limited to summer (July–September) rock-basket samples. In the plots, PRISM mean annual precipitation values were adjusted to fit the scale by subtracting 30 from the original values.

2.2.2. Ecological Trait Groups – Spatial Patterns, Elevation, and Size

We found differences in the distributions of thermal preference taxa between ecoregions, largely related to elevation differences, in all states tested. In Utah, distributions of the cold-water-preference taxa were significantly higher in the Wasatch Uinta ecoregion and at higher elevation sites (Figures 2-6 and 2-7). Sites in the Colorado Plateau ecoregion and at lower elevations had significantly more warm-water-preference taxa, but numbers of warm-water-preference taxa were low at the Utah reference sites[8]. The prevalence and distribution of cold-

[8] The relatively low number of taxa on the Utah warm-water-preference list was partially a consequence of the need to use a family-level OTU for Chironomidae because of inconsistencies in the long-term data set that arose from a change in taxonomic laboratories.

and warm-water-preference taxa also varied predictably with stream order (Figure 2-8). First and second order streams in Utah had slightly greater relative abundance and richness of cold-water-preference taxa, and fewer warm-preference taxa, compared to 3rd or higher order streams. These results suggest that effects are likely to vary spatially within states, potentially reflecting spatial differences in vulnerabilities. Biotic assemblages in the Wasatch and Uinta Mountains and at higher elevations may be more vulnerable to increasing temperatures that are predicted to occur. On the other hand, many of the higher elevation stations evaluated in Utah were also mid-order streams, and may not contain the greatest proportion of cold-preference taxa, but may represent transitional areas to higher elevation headwater reaches that may be vulnerable if they harbor taxa near thermal thresholds.

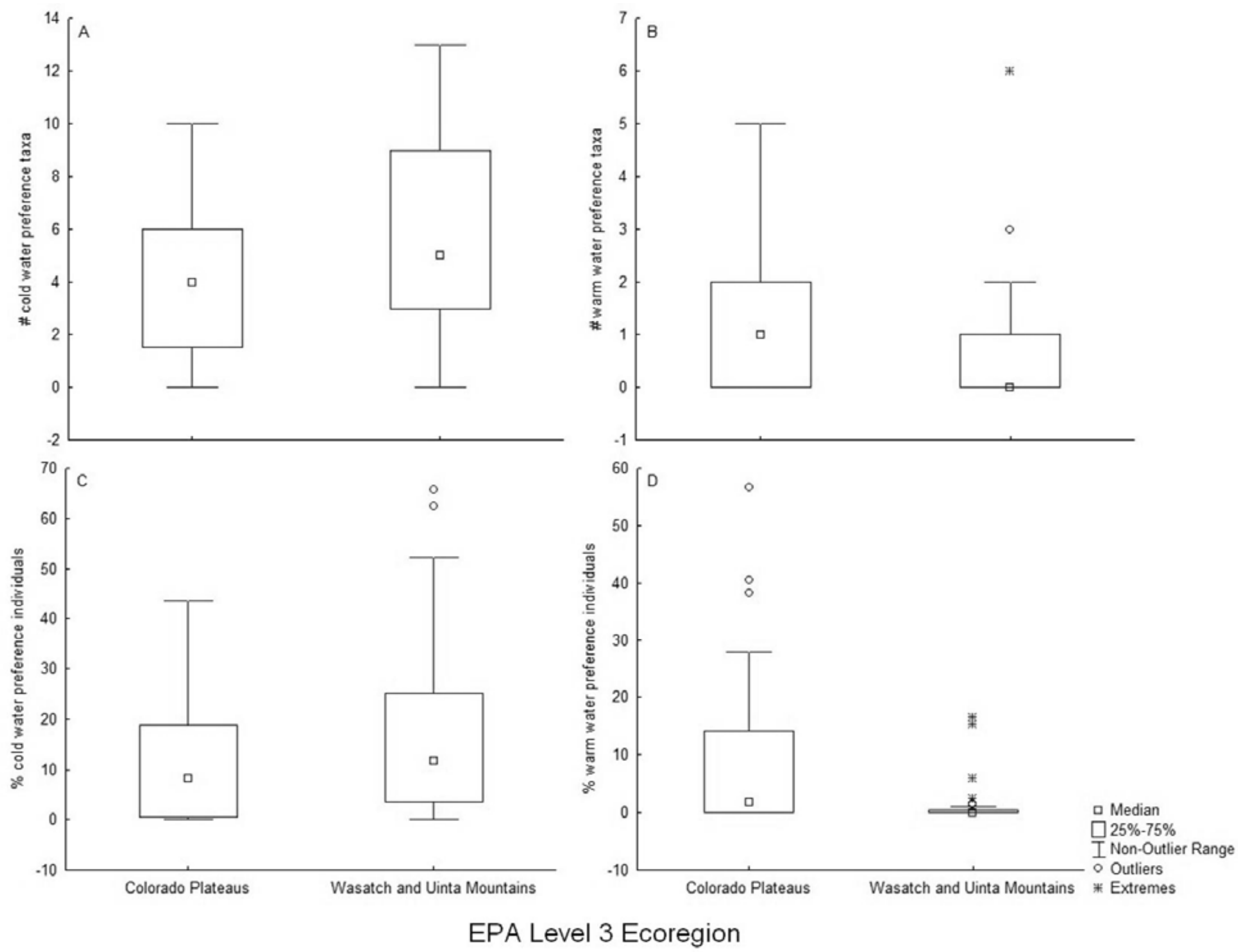

Figure 2-6. Distributions of thermal preference metric values in Utah reference samples in the Wasatch and Uinta Mountains and Colorado Plateaus ecoregions. Plot (A) shows number of cold-water-preference taxa, (B) number of warm-water-preference taxa, (C) % cold-water-preference individuals, and (D) % warm-water-preference individuals. Data

used in these analyses were limited to autumn (September–November) kick-method samples. The sample size (n) of the Wasatch and Uinta Mountains data set was 74 and n=44 for the Colorado Plateaus.

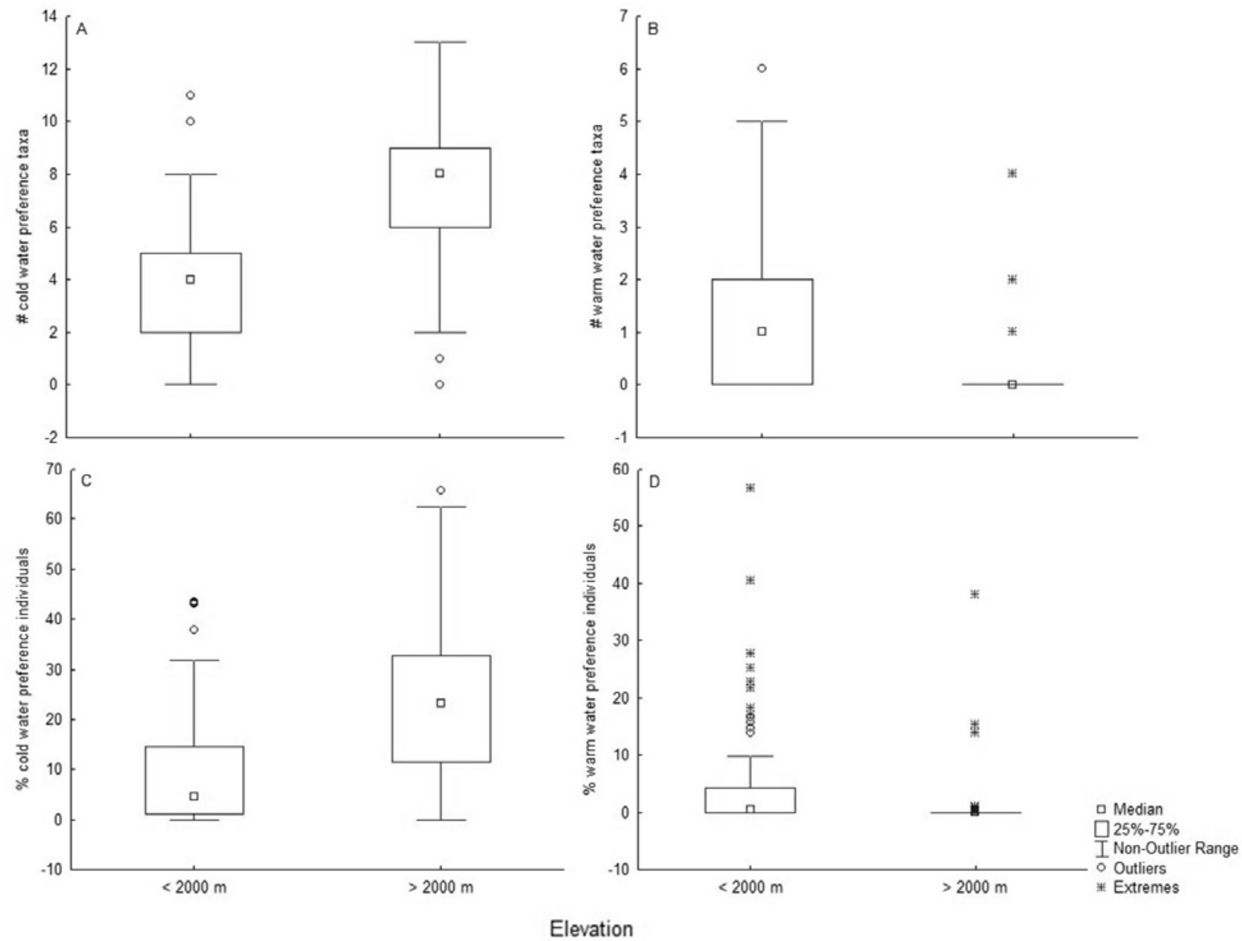

Figure 2-7. Distributions of thermal preference metric values in Utah reference samples in two elevation groups (< 2000 m and > 2000 m). Plot (A) shows number of cold-water-preference taxa, (B) number of warm-water-preference taxa, (C) % cold-water-preference individuals, and (D) % warm-water-preference individuals. Data used in these analyses were limited to autumn (September–November) kick-method samples. The sample size (n) of the <2000 m data set is 74 and n=55 for the >2000 m data set.

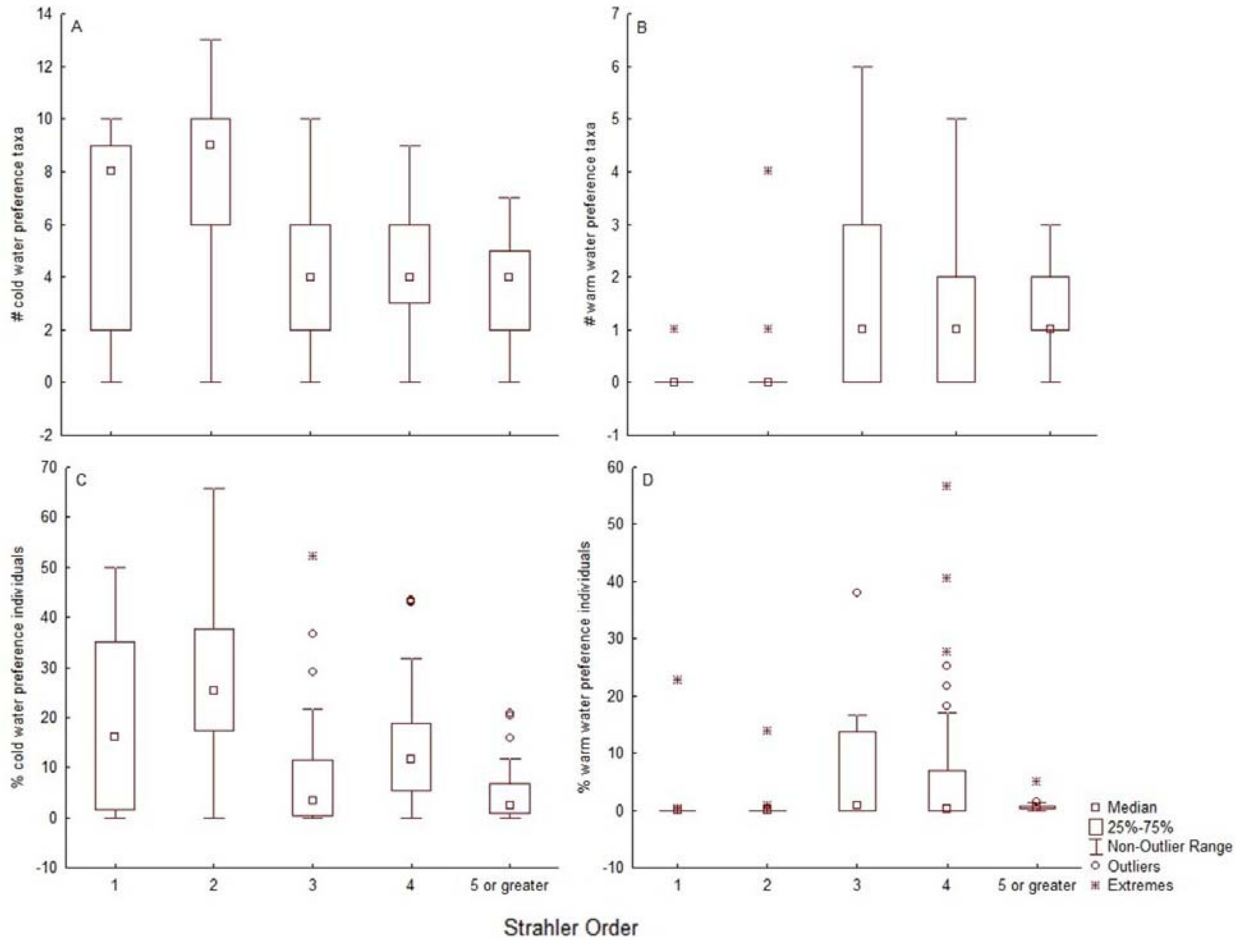

Figure 2-8. Distributions of thermal preference metric values in Utah reference samples grouped by Strahler order. Plot (A) shows number of cold-water-preference taxa, (B) number of warm-water-preference taxa, (C) % cold-water-preference individuals, and (D) % warm-water-preference individuals. Data used in these analyses were limited to autumn (September–November) kick-method samples. Sample size for 1^{st} order samples is 11, 2^{nd} order =29, 3^{rd} order = 22, 4^{th} order = 41, ≥ 5^{th} order = 21.

In Maine, the Northeastern Highlands sites had the highest mean number of cold-water-preference taxa, followed closely by the Northeastern Coastal Zone sites (Figure 2-9). Overall, the number of cold-water taxa in all the Maine ecoregions evaluated was low (1 to 2 taxa). The mean number of warm-water-preference taxa at sites in the Laurentian Plains and Hills was significantly higher than at sites in other ecoregions, while the Northeastern Highlands sites had the lowest mean number of warm-water-preference taxa. These observed ecoregional differences appear to be driven by elevation: there are more cold-water-preference taxa at higher elevation (> 150 m) sites and more warm-water-preference taxa at lower elevation (< 150 m) sites (Figure 2-

10). Although it was originally assumed that the high (northern) latitude of Maine also would influence composition by cold water taxa, apparently elevation is a more influential factor.

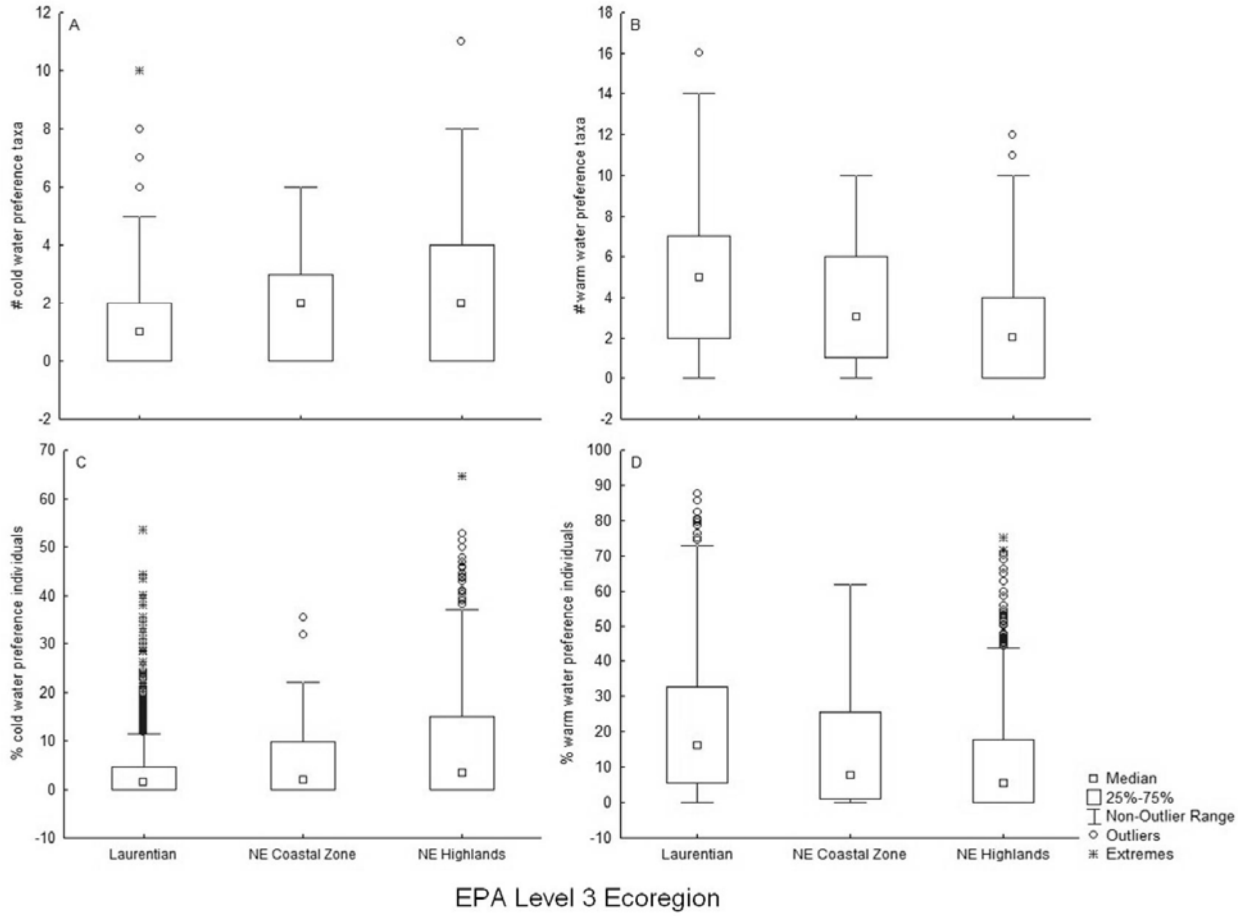

Figure 2-9. Distributions of thermal-preference-metric values in Maine Class A and AA samples in the Laurentian Plains and Hills, Northeastern Coastal Zone and Northeastern Highlands ecoregions. Plot (A) shows number of cold-water-preference taxa, (B) number of warm-water-preference taxa, (C) % cold-water-preference individuals, and (D) % warm-water-preference individuals. Data used in these analyses were limited to June-October rock-basket samples, and each replicate was treated as an individual sample. The sample size (n) of the Laurentian Plains and Hills data set was 747, n=41 for the Northeastern Coastal Zone and n=433 for the Northeastern Highlands.

As observed in Utah, first and second order streams in Maine had slightly greater relative abundances and richness of cold-water-preference taxa, while 4th and higher order streams had more warm-water-preference taxa (Figure 2-11). Third order streams appeared transitional in temperature preference composition. Based on the distribution of cold-water-preference taxa, it might be expected that biotic assemblages at Northeastern Highland and other higher elevation locations, especially in lower order streams, will be more vulnerable to increasing temperatures. Unfortunately, none of the reference sites located in the Northeastern Highlands have enough long-term data to support trend analyses. The three reference sites that had enough data to analyze were located in the Laurentian Plains and Hills and Northeast Coastal Zone ecoregions and were dominated by warmer-water-preference taxa.

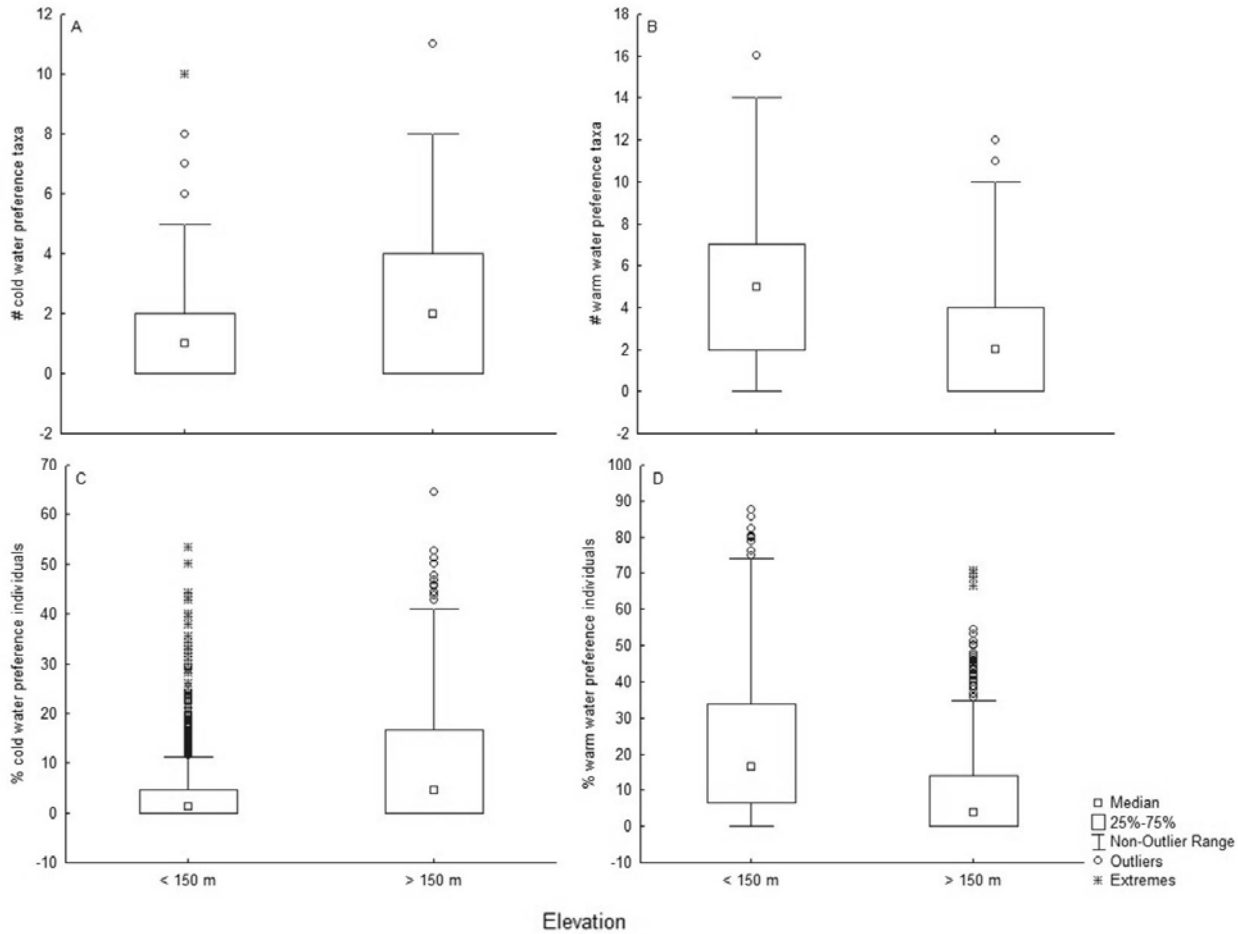

Figure 2-10. Distributions of thermal preference metric values in Maine Class A and AA samples in the two elevation groups (< 150 m and > 150 m). Plot (A) shows number of cold-water-preference taxa, (B) number of warm-water-preference taxa, (C) % cold-water-preference individuals, and (D) % warm-water-preference individuals. Data used in these analyses were limited to June-October rock-basket samples, and each replicate was treated as an individual sample. The sample size (n) of the <150 m data set is 817 and n=404 for the >150 m data set.

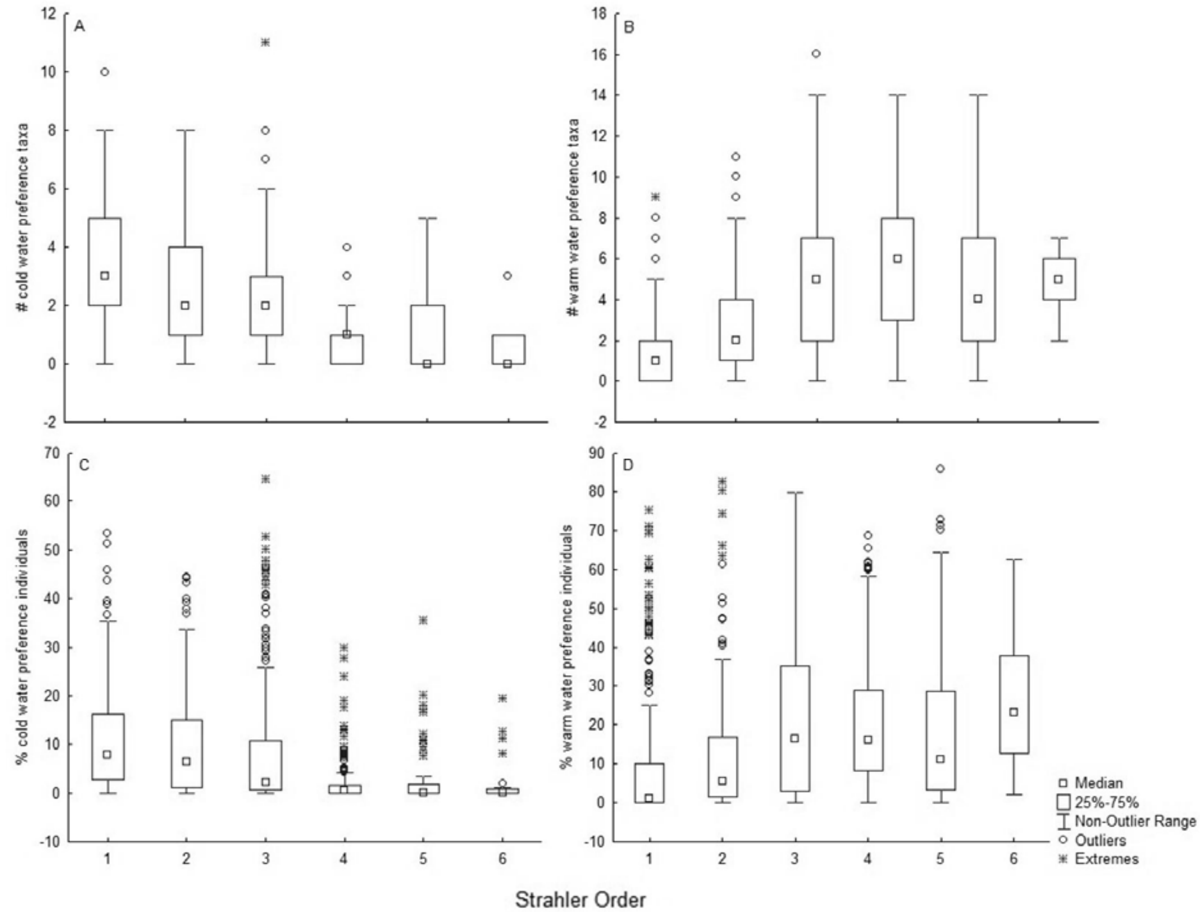

Figure 2-11. Distributions of thermal preference metric values in Maine Class A and AA samples grouped by Strahler order. Plot (A) shows number of cold-water-preference taxa, (B) number of warm-water-preference taxa, (C) % cold-water-preference individuals, and (D) % warm-water-preference individuals. Data used in these analyses were limited to June-October rock-basket samples, and each replicate was treated as an individual sample. Sample size of 1st order samples is 230, 2nd order =149, 3rd order = 273, 4th order = 284, 5th order = 95, 6th order = 32.

In North Carolina, ecoregions also vary in the predominance of cold- and warm-water-preference taxa. The richness of cold-water-preference taxa is, on average, higher in the Mountain ecoregion than in the other two ecoregions (Figure 2-12). The distribution of warm-water-preference taxa is significantly different between all three ecoregions, with the highest abundance occurring in the Coastal ecoregion and the lowest number occurring in the Mountain ecoregion. This distributional pattern is reinforced by the finding that significantly more cold-water taxa occur at higher elevation sites than at lower elevations (Figure 2-13). Conversely, median richness and abundance of warm-water taxa is greater at lower elevation sites. Distribution of cold-and warm-water-preference taxa was also related to watershed size. The smaller watersheds in North Carolina (<35 mi^2) had a greater proportion of cold-water-preference taxa (based on both abundance and richness), while larger watersheds (>100 mi^2) had a greater proportion of warm-water-preference taxa (Figure 2-14). Based on the results from the cold- and warm-water taxa distribution analysis, it is likely that biotic assemblages at Mountain and higher elevation sites, and in smaller watersheds, will be more vulnerable to increasing temperatures than others because greater numbers of cold-water taxa inhabit these sites. However, in North Carolina, few trends over time were found for cold- or warm-water-preference taxa. This may be attributable to the more limited time series of data available from North Carolina, as well as to the use of categorical rather than abundance data (though this would not affect evaluation of richness trends).

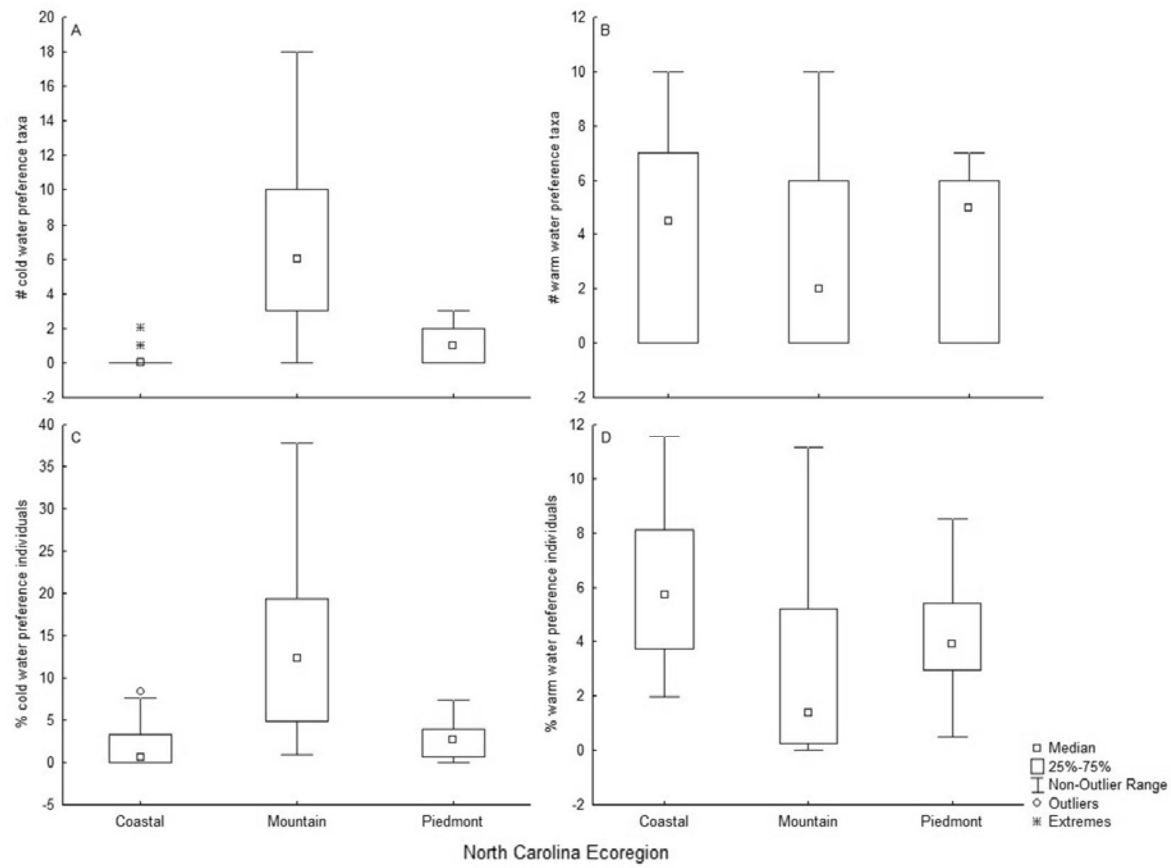

Figure 2-12. Distributions of thermal preference metric values in North Carolina reference samples in the Coastal, Mountain and Piedmont ecoregions. Plot (A) shows number of cold-water-preference taxa, (B) number of warm-water-preference taxa, (C) % cold-water-preference individuals, and (D) % warm-water-preference individuals. Data used in these analyses were limited to June–September standard qualitative samples. The sample size (n) of the Coastal data set is 20, n=61 for the Mountain ecoregion and n=21 for the Piedmont data set.

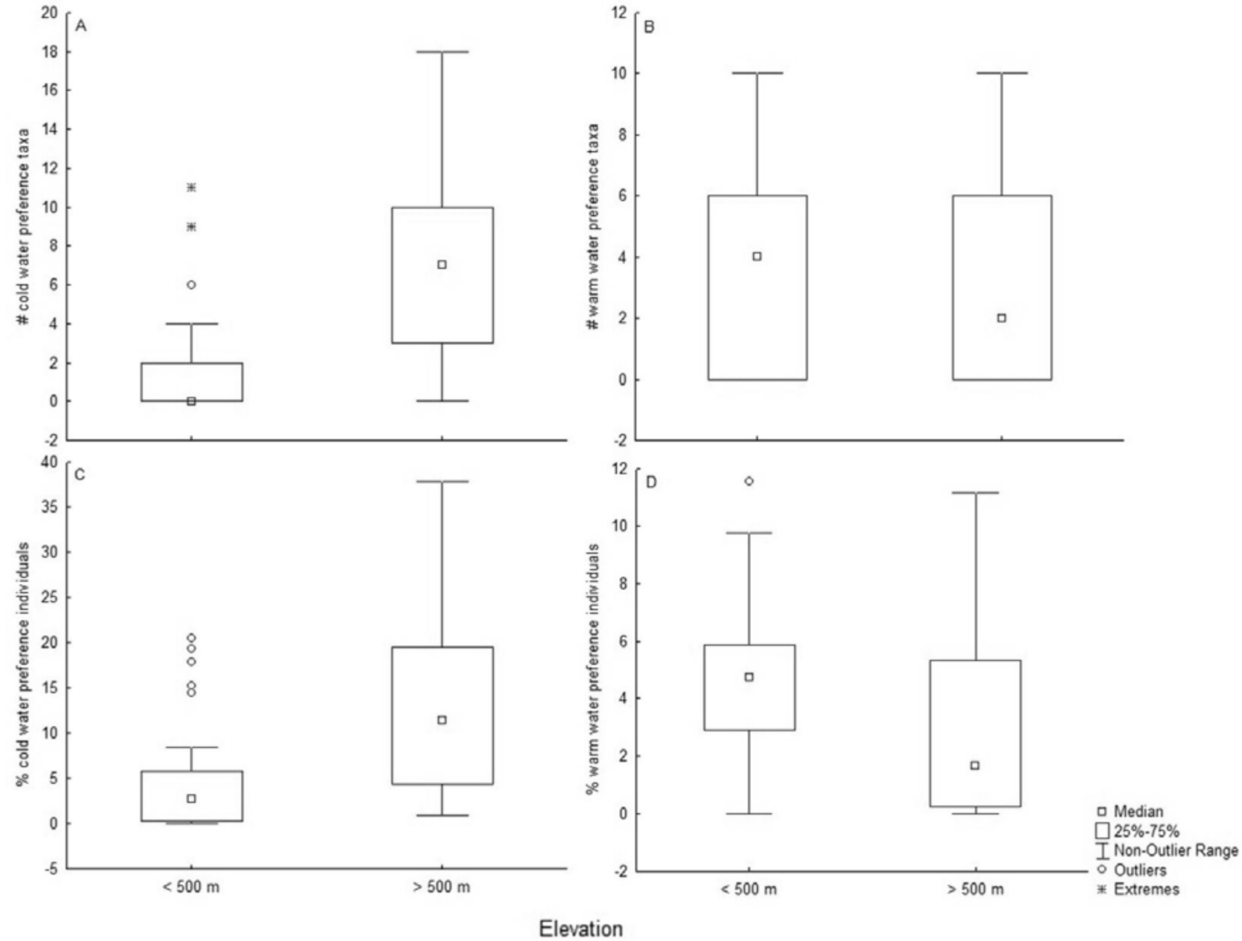

Figure 2-13. Distributions of thermal preference metric values in North Carolina reference samples in two elevation groups (< 500 m and > 500 m). Plot (A) shows number of cold-water-preference taxa, (B) number of warm-water-preference taxa, (C) % cold-water-preference individuals, and (D) % warm-water-preference individuals. Data used in these analyses were limited to June–September standard qualitative samples. The sample size (n) of the <500 m data set is 49 and n=50 for the >500 m data set.

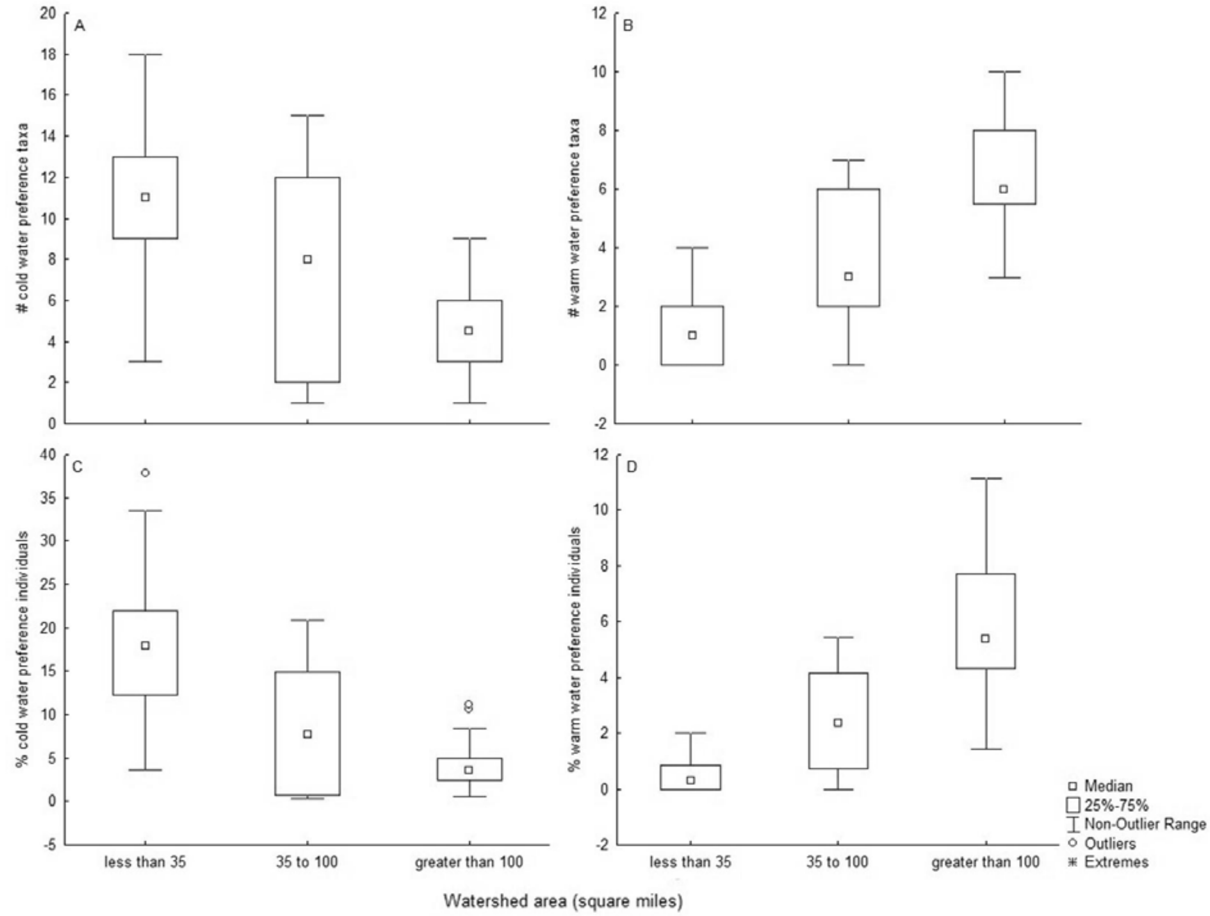

Figure 2-14. Distributions of thermal preference metric values in North Carolina reference samples grouped by watershed area. Plot (A) shows number of cold-water-preference taxa, (B) number of warm-water-preference taxa, (C) % cold-water-preference individuals, and (D) % warm-water-preference individuals. Data used in these analyses were limited to June–September standard qualitative samples. The sample size for the < 35 sq mi group is 17, 35 to 100 sq mi =15 and > 35 sq mi = 24.

2.2.3. Potential biological indicators of climate-related hydrologic changes

This section focuses on analyses of paired hydrologic-biological datasets in Utah, Maine and North Carolina. In the Utah analyses, weighted averaging was used to calculate taxa optima and tolerance values for selected Indicators of Hydrologic Alteration (IHA) (Richter et al., 1996) parameters derived from USGS flow data (Appendix K) and year. Data from 43 biological sampling sites (=159 fall samples) and their associated USGS gages were used in the analyses. Results showed that several low flow parameters performed well compared to high flow/pulse event parameters. Indicator values for the IHA 3-day minima flow values show potential for detecting climate change effects. Results for taxa that had more than 20 occurrences in the dataset (which is statistically an adequate sample size) show that Leuctridae (rolled wing stoneflies), Asellidae (isopod crustaceans referred to as sowbugs) and *Zapada* (a stonefly in the family Nemouridae) had the lowest 3-day minima optimum values (0.056 cfs or less), while *Hyalella* (an amphipod crustacean) and *Helicopsyche* had the highest (0.11 cfs). Leuctridae and *Zapada* had relatively low tolerance ranges, while *Hyalella* and *Helicopysche* (a caddisfly) had large tolerance ranges. These results suggest that the stoneflies Leuctridae and *Zapada* (in the family Nemouridae) are better adapted, perhaps partly due to their smaller sizes, to lower flow conditions than other organisms that appeared in the Utah data set.

When taxonomic trends were examined using NMDS and CCA, both analyses indicated that year had the strongest influence on taxonomic composition. The IHA parameters that were used in the analysis had a weaker effect, although the high pulse and 3-day minima parameters also explained a fair amount of variation. Results of the CCA are shown in Figure 2-15. The plot shows which taxa were most closely associated with year, high pulse and 3-day minima values. Correlation analyses were also performed on the seven sites in Utah that had the most number of years of biological and hydrological data, which were analyzed individually. Results showed that there were no taxa or metrics that were consistently associated with the IHA parameters across all sites (Leuctridae was not present at any of the sites, and at the one site where *Zapada* was present, it was not significantly correlated with the IHA parameters). The same was true for the flashiness index. Therefore, no taxa or metrics emerged as good candidates for 'disturbance indicator' metrics related to hydrology in Utah. Disturbance is of particular interest because hydrologic regimes are expected to change (i.e. duration and/or frequency of high and/or low flow events) as a result of climate change, so taxa that are better able to adapt to the changing conditions are generally believed to have a greater chance for success.

NMDS ordinations were also performed on the North Carolina data (440 samples, selected based on matches between biological sampling locations and USGS gaging stations, for all available sampling dates). Results from one of the analyses are shown in Figure 2-16. In the plot, samples are grouped by level 3 ecoregion. NMDS results show that baseflow index, which is a parameter that represents low flow influence, had the strongest correlation with macroinvertebrate species composition, though this strong relationship may be mostly due to ecoregional distribution of taxa. A number of covariates, such as elevation, temperature, and other factors may co-affect the observed pattern. The second factor that related to taxonomic composition was number of reversals, which is a measurement of flashiness. The flashiness index (RBI) had weaker correlation with the species axes. Other factors that showed correlations were low pulse and high pulse parameters (Table 2-3).

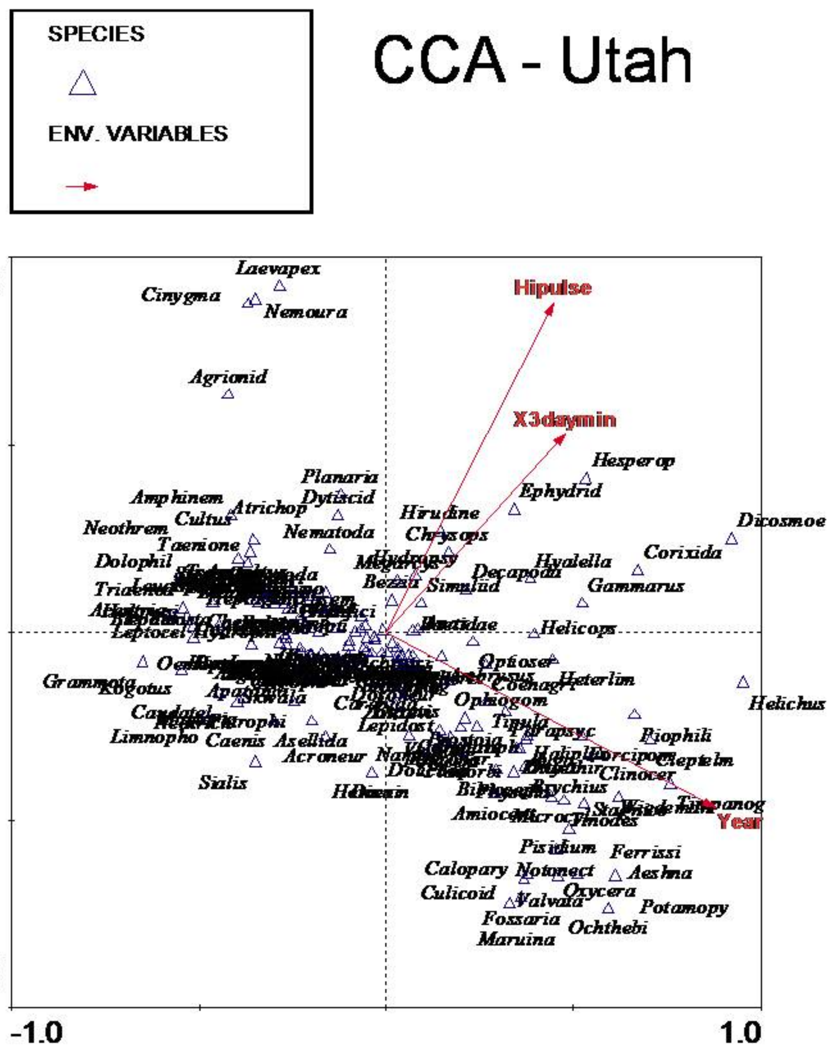

Figure 2-15. CCA plot of a selected subset of the Utah biological-hydrological data.

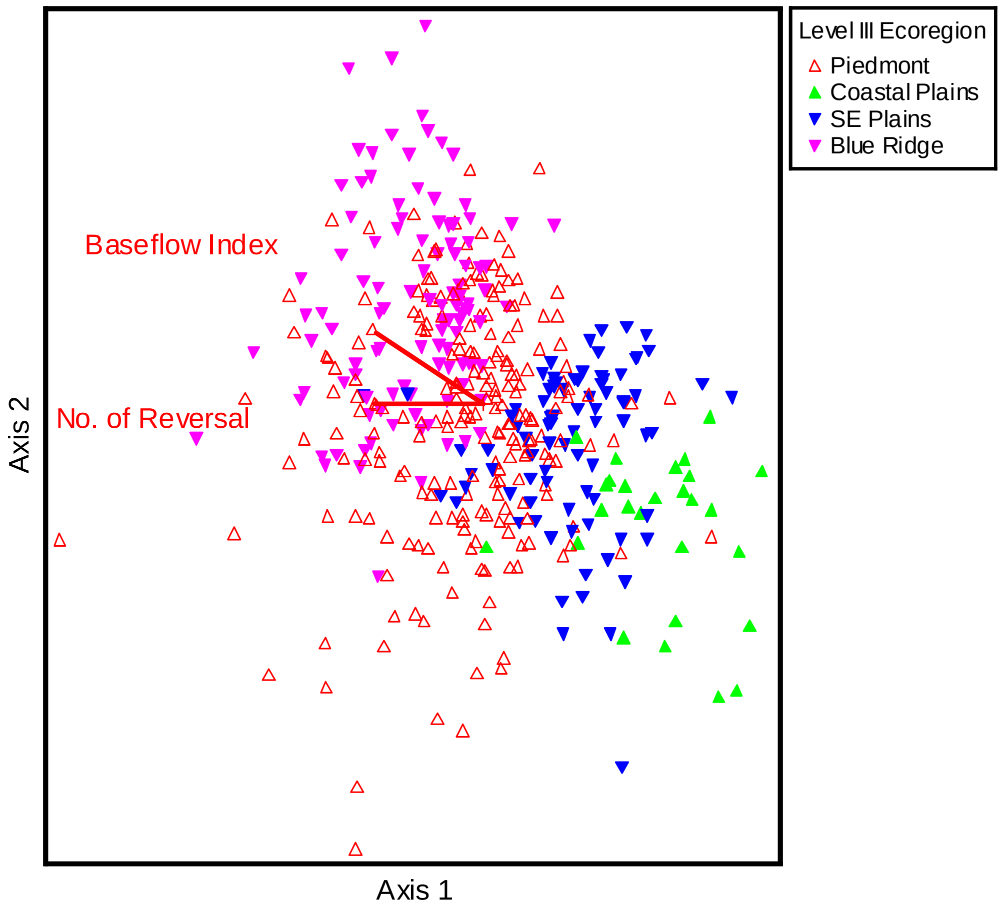

Figure 2-16. NMDS plot of macroinvertebrate taxonomic composition and its relationship with hydrologic parameters for a subset of North Carolina data. Baseflow index and number of reversals were associated with Axis 2.

Table 2-3. Selected results from the Pearson and Kendall Correlations with Ordination Axes (N= 440). Only those variables that had strong correlations are included in this table. Correlations with r or tau values greater than 0.3 are in bold print. Note: significance values are not available for these analyses because the correlations associated with the ordinations are not meant for testing hypotheses.

Axis:		1			2			3	
	r	r-sq	tau	r	r-sq	tau	r	r-sq	tau
January	-0.029	0.001	-0.074	-0.037	0.001	0.1	**0.364**	0.132	**0.312**
February	-0.039	0.002	-0.07	-0.059	0.003	0.073	**0.361**	0.13	**0.33**
March	-0.061	0.004	-0.086	-0.052	0.003	0.094	**0.368**	0.135	**0.327**
April	-0.097	0.009	-0.118	-0.045	0.002	0.129	**0.322**	0.104	0.285
X1daymin	**-0.373**	0.139	-0.282	0.105	0.011	0.254	0.177	0.031	0.088
X3daymin	**-0.375**	0.14	-0.283	0.095	0.009	0.241	0.197	0.039	0.101
X7daymin	**-0.327**	0.107	-0.281	0.061	0.004	0.235	0.228	0.052	0.111
X1daymax	-0.086	0.007	-0.09	-0.042	0.002	0.041	**0.363**	0.132	**0.338**
X3daymax	-0.064	0.004	-0.049	-0.055	0.003	0.026	**0.375**	0.141	**0.368**
X7daymax	-0.044	0.002	-0.037	-0.06	0.004	0.024	**0.4**	0.16	**0.375**
X30daymax	-0.047	0.002	-0.057	-0.061	0.004	0.037	**0.395**	0.156	**0.37**
X90daymax	-0.062	0.004	-0.069	-0.054	0.003	0.062	**0.387**	0.15	**0.359**
Baseflow	**-0.51**	0.26	**-0.382**	**0.416**	0.173	**-0.317**	**0.365**	0.134	-0.245
LopulseL	**0.364**	0.133	0.229	-0.037	-0.001	0.017	0.153	0.024	0.078
Hipulse	**-0.328**	0.108	-0.212	-0.042	0.002	-0.028	0.125	0.016	-0.101
HipulseL	**0.442**	0.196	0.242	-0.098	-0.01	0.073	0.3	0.09	0.23
Riserate	-0.076	0.006	-0.144	-0.047	0.002	0.11	0.284	0.081	**0.303**
Fallrate	0.048	0.002	0.151	0.059	-0.004	-0.081	0.24	0.058	**-0.332**
Reversals	**-0.512**	0.262	**-0.358**	0.039	0.002	0.011	-0.044	0.002	-0.083
High1peak	-0.062	0.004	-0.074	-0.032	0.001	0.072	**0.443**	0.196	**0.358**
High1dur	**0.362**	0.131	0.227	-0.066	-0.004	0.068	0.205	0.042	0.232
High1freq	**-0.302**	0.091	-0.18	-0.046	0.002	-0.014	-0.086	0.007	-0.079
High1rise	-0.162	0.026	-0.138	-0.035	0.001	0.071	**0.383**	0.147	**0.323**
High1fall	0.069	0.005	0.116	0.062	0.004	-0.038	**-0.338**	0.114	**-0.359**
RBI	-0.046	0.002	0.017	**-0.391**	-0.153	-0.211	0.001	0	-0.025

Similar analyses were attempted in Maine, but there were not enough USGS gages associated with biological sampling sites to estimate flow variable optima using weighted averaging. Instead, correlation analyses were performed on data from Station 56817 (Sheepscot) (22 samples). Some taxa and some metrics were significantly correlated with some IHA parameters. For example, *Hydropsyche* (spotted sedge caddisfly), *Promoresia* (an elmid beetle) and *Rhyacophila* (green sedge caddisfly) were significantly and positively correlated with 1- and 3-day minima flow values. However, it is difficult to draw conclusions about consistency of patterns statewide based on data from one site, and also because unambiguous causal relationships between the biological and hydrologic data cannot be established based on correlation analyses.

2.3. TEMPORAL TRENDS IN TAXA AND COMMONLY USED METRICS

Although different states use different methods for assessing sites, there are certain metrics, such as those related to EPT taxa, which are commonly used in state multi-metric indices (MMIs). To provide regional relevance and a common basis for regional comparisons, analyses were performed that examined associations between these commonly-used metrics and climate-related variables. ANOVAs were used to determine differences in biological metrics among hottest, coldest, wettest, driest, and normal years (Table 2-1) for reference sites that had adequate long-term data. Correlation analyses were used to evaluate associations between biological variables, annual air temperature and precipitation, variables related to inter-annual climate variability, and these variables with lagged year effects.

NMDS were used to show in ordination space how samples collected over years at long-term stations varied in species composition over time. Reference locations with sufficient long-term data to perform ordinations included two Utah reference sites (Station 4927250 - Weber and Station 4951200 - Virgin) and one Maine reference site (Station 56817 - Sheepscot). The ordinations were used to evaluate differences in taxonomic composition among samples collected during hottest, coldest, wettest, driest and normal years. Other environmental variables were used to group the data while looking for trends, including temperature and precipitation categories, PRISM[9] mean annual air temperature and precipitation from the year the sample was collected, PRISM mean annual air temperature and precipitation from the year prior to sample collection (to look for lagged effects), and absolute difference between the PRISM mean annual air temperature value and PRISM mean annual precipitation value from the year of the sample collection and the year prior (to look for effects of climate variability). In addition, several IHA parameters were included in the ordination of the Maine data: average of median monthly flows from sample collection months (July-September), Richards-Baker Flashiness Index (which uses flow data to quantify the frequency and rapidity of short-term changes in stream flow) (Baker et al., 2004), and 1- and 3-day minimum and maximum flows. Presence/absence data from fall

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[9] PRISM data downloaded from http://www.prism.oregonstate.edu/.

samples in Utah and summer/fall samples in Maine were used to analyze changes in the biological assemblage.

2.3.1. Trends and Patterns– Utah and Western States

In Utah, the NMDS ordinations tend to corroborate ANOVA findings regarding benthic responses among years partitioned by climate parameters. At the two long-term Utah reference stations tested, ‘hottest year’ samples formed distinct clusters from the ‘coldest’ and ‘normal’ year samples (Figures 2-17 and 2-18).

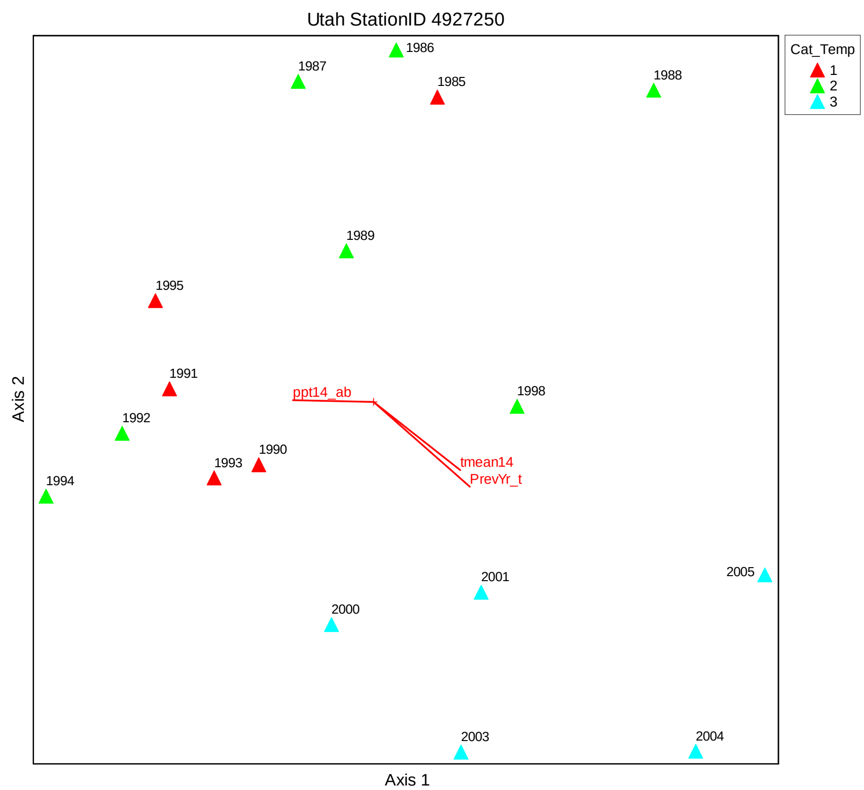


Figure 2-17. NMDS plot (Axis 1-2) for Utah Station 4927250 (Weber). Cat_Temp refers to the temperature categories, which are: 1=coldest years; 2=normal years; 3=hottest years. Samples are labeled by collection year. tmean14=PRISM mean annual air temperature from the year the sample was collected, PrevYr_t= PRISM mean annual air temperature from the year prior to sample collection, and ppt14_ab= absolute difference between the PRISM mean annual precipitation value from the year of the sample collection and the year prior.

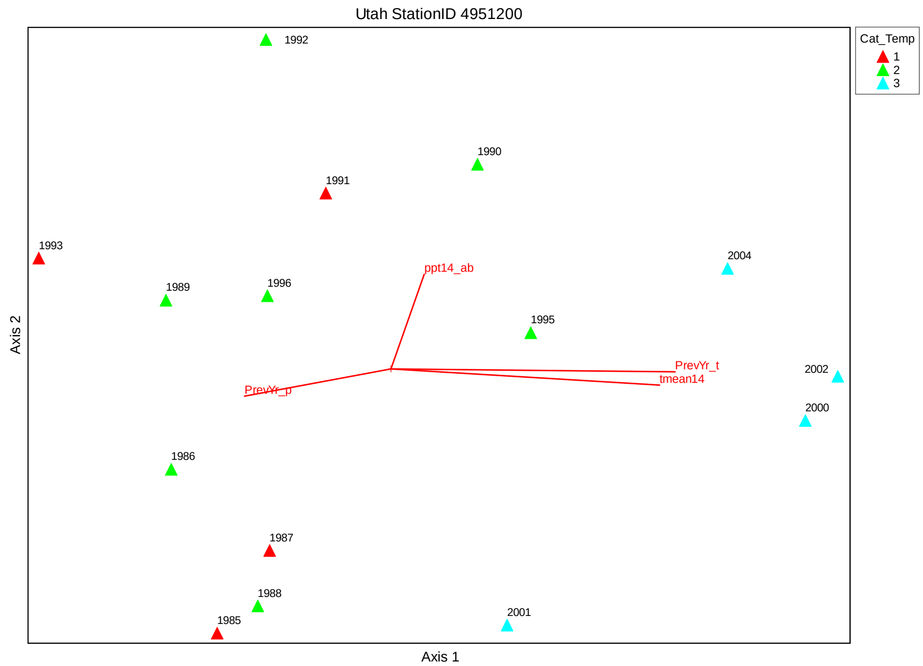


Figure 2-18. NMDS plot (Axis 1-2) for Utah Station 4951200. Cat_Temp refers to the temperature categories, which are: 1=cold years; 2=normal years; 3=hot years. Samples are labeled by collection year. tmean14=PRISM mean annual air temperature from the year the sample was collected, PrevYr_t= PRISM mean annual air temperature from the year prior to sample collection, ppt14_ab= absolute difference between the PRISM mean annual precipitation value from the year of the sample collection and the year prior, and PrevYr_p= PRISM mean annual precipitation from the year prior to sample collection.

Figure 2-19 shows which taxa are the strongest drivers along Axes 1-2 at Station 4927250 (Weber). *Pteronarcys*, Chloroperlidae and *Ephemerella* have the strongest positive correlations with Axis 2, and *Optioservus*, *Lepidostoma* and *Hyallela* have the strongest negative correlations with Axis 2. The three taxa positively associated with Axis 2 tend toward cold-water-preference - Chloroperlidae and *Pteronarcys* are absent from the 'hottest year' samples and *Ephemerella* is present in all the 'coldest year' and 'normal year' samples and is only present in one 'hottest year' sample. Some additional taxa that occurred during multiple years and were not found in 'hottest year' samples include *Rhithrogena*, Nematoda, and Tubificidae. Warm-water-preference taxa that are present in at least 4 of the 5 'hottest year' samples include *Optioservus*, *Lepidostoma* and *Hyallela*, though they also are present in 'coldest year' and/or 'normal year' samples.

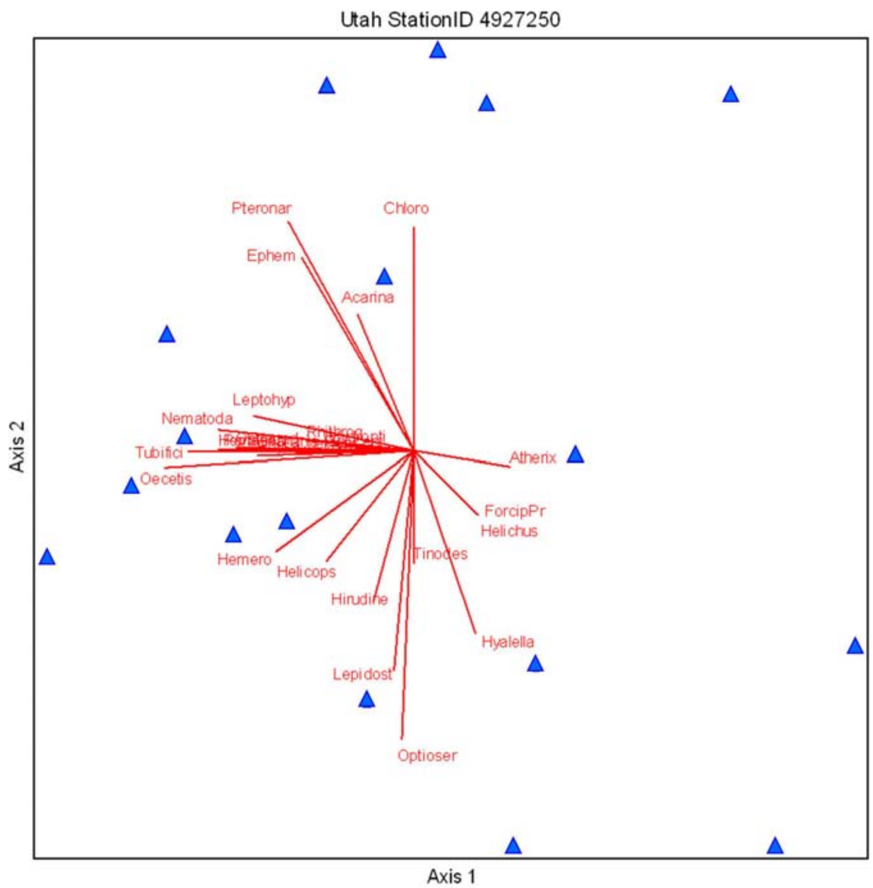


Figure 2-19. NMDS plot (Axis 1-2) for Utah Station 4927250 (Weber) that shows which taxa are most highly correlated with each axis.

Figure 2-20 shows which taxa are the strongest drivers along Axes 1-2 at Station 4951200 (Virgin). *Ephemerella*, Nematoda and *Heptagenia* have the strongest negative correlations with Axis 1, and appear to tend toward a cold-water-preference. Nematoda is absent from the 'hottest year' samples, and *Ephemerella* and *Heptagenia* are present in all 'coldest year' samples, 6 of the 7 'normal year' samples and only 1 of the 'hottest year' samples. *Forcipomyia*/*Probezzia*, *Microcylloepus*, *Caloparyphus* and *Chimarra* have the strongest positive correlations with Axis 1, and appear to be warm tolerant. These taxa are present in at least 2 of the 4 'hottest year' samples and are absent from the 'coldest year' and/or 'normal year' samples.

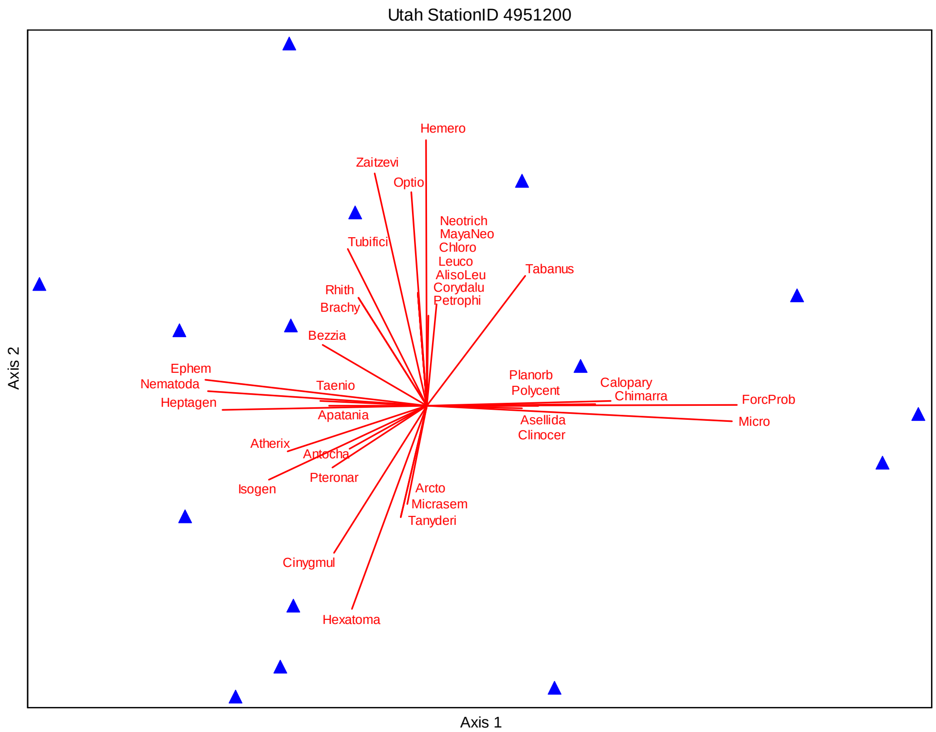


Figure 2-20. NMDS plot (Axis 1-2) for Utah Station 4951200 (Virgin) that shows which taxa are most highly correlated with each axis.

Only five of the metrics tested were significantly different between hottest, coldest, wettest, driest, and normal year samples at more than one site. At Stations 4927250 (Weber) and 4951200 (Virgin) in Utah, the hottest year samples had significantly fewer total taxa, EPT taxa and cold-water-preference taxa than the coldest year samples (Figures 2-21 and 2-22, and Figure 2-1). Figure 2-23 illustrates the relationship between EPT richness and PRISM mean annual air temperature at Utah sites 4927250 (Weber) and 4951200 (Virgin). If a linear regression is used to infer the relationship at site 4927250 (which is located in the Wasatch and Uinta Mountains), approximately 3 EPT taxa are lost for every 1°C increase in (air) temperature. If the same is done for site 4951200, which is a lower elevation site located in the Colorado Plateaus ecoregion, the inferred loss rate is ~1.5 EPT taxa for every 1°C increase in (air) temperature. If one were to take this a step further, the median number of EPT taxa at site 4927250 (Weber) is ~13 to 14 taxa. Based on a projected temperature increase of 2°C over the next 40 y (i.e., by 2050[10]), an average of 6 taxa could be lost (>40% of total EPT richness). There is no particular reason to assume that the actual rate of taxa losses will be linear over time, especially considering year-to-year and

[10] Based on data from the National Center for Atmospheric Research website: http://rcpm.ucar.edu.

decadal-scale climate variations (see Section 2). However, if these types of trends were to occur, they would likely affect MMIs.

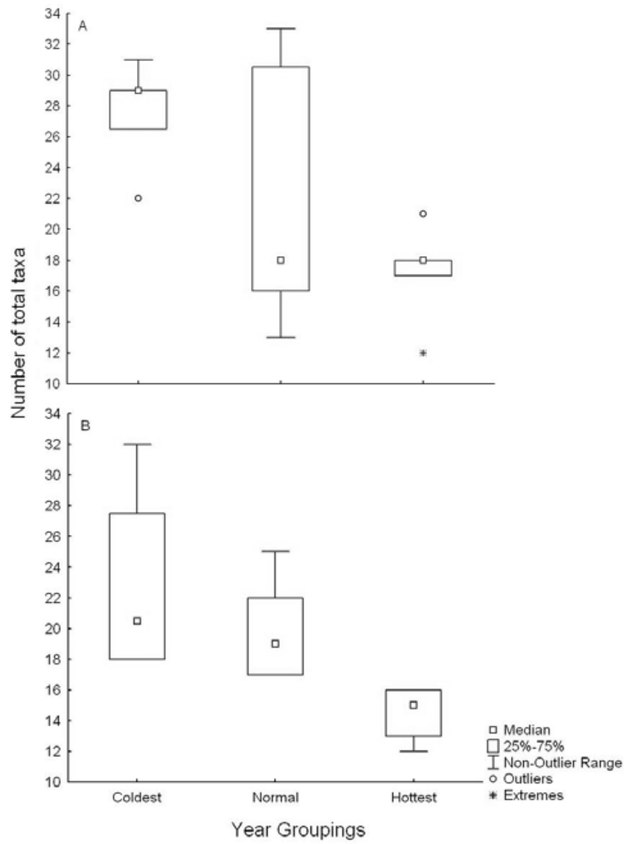


Figure 2-21. Distributions of total taxa richness values in coldest-, normal-, and hottest-year samples at Utah sites 4927250 (Weber) (A) and 4951200 (Virgin) (B). Year groupings are based on PRISM mean annual air temperatures from each site during time periods for which biological data were available. Average temperatures in hottest-year samples were 1.1 to 2.7˚C higher than coldest year samples. At both sites, mean total taxa metric values were significantly higher in coldest year samples than in hottest year samples. Data used in these analyses were limited to autumn (September–November) kick-method samples.

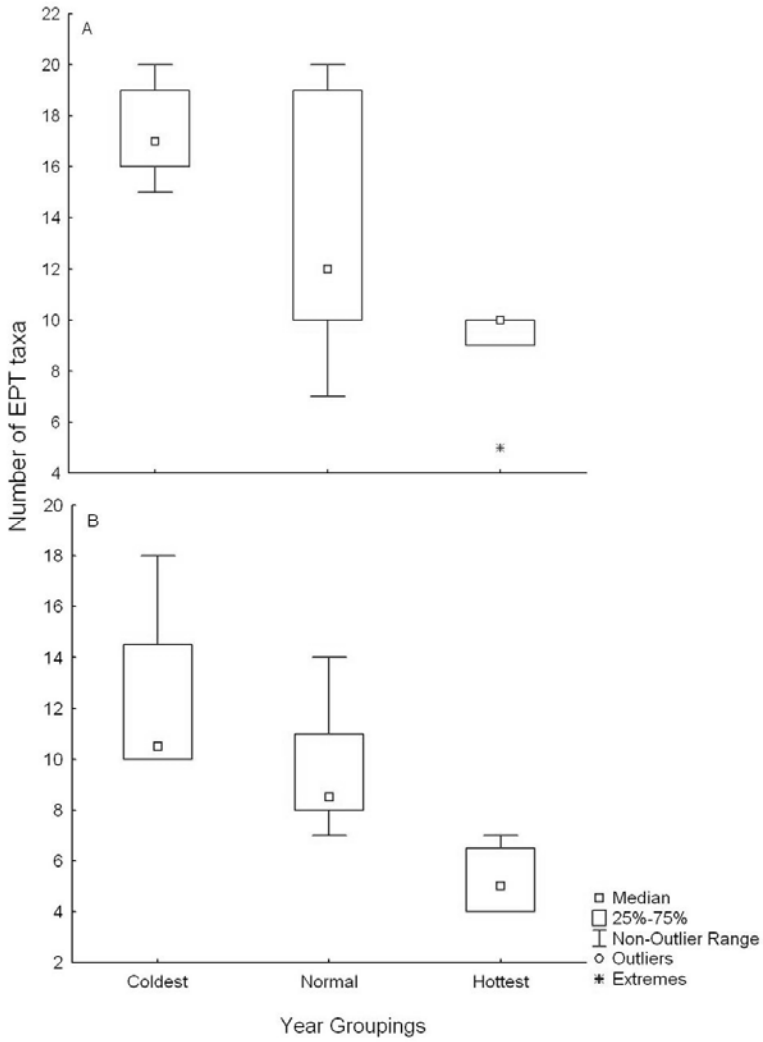


Figure 2-22. Distributions of EPT richness values in coldest-, normal-, and hottest-year samples at Utah sites 4927250 (Weber) (A) and 4951200 (Virgin) (B). Year groupings are based on PRISM mean annual air temperatures from each site during time periods for which biological data were available. Average temperatures in hottest-year samples were 1.1 to 2.7˚C higher than coldest year samples. At both sites, mean EPT richness metric values were significantly higher in coldest and normal year samples than in hottest year samples. Data used in these analyses were limited to autumn (September–November) kick-method samples.

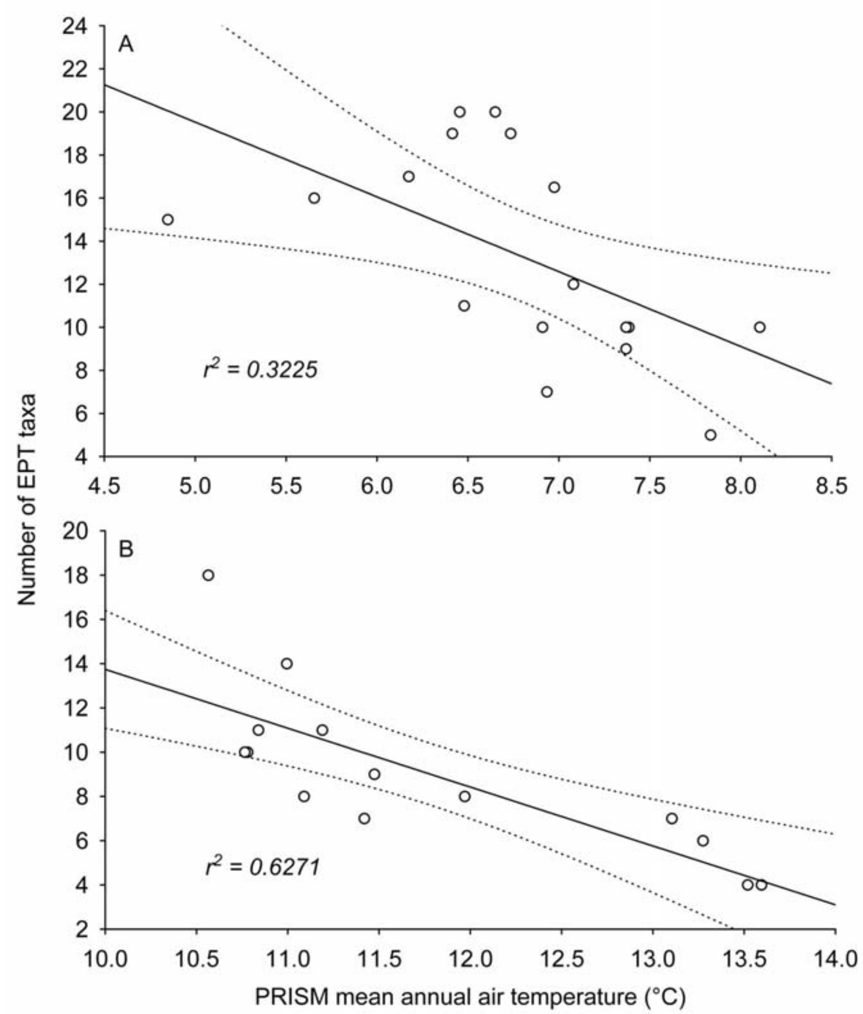


Figure 2-23. Relationship between EPT taxa richness and PRISM mean annual air temperature (°C) at (A) Utah site 4927250 (Weber) (r=0.57, p=0.01) and (B) site 4951200 (Virgin) (r=0.79, p<0.01). The dotted lines represent 95th confidence intervals.

2.3.2. Trends and Patterns– Maine and New England States

There were few consistent patterns in Maine that clearly relate trait or taxonomic metrics to climate condition variables. Unlike Utah, the NMDS ordination at Maine's longest term Station 56817 (Sheepscot) (in the Laurentian Hills and Plains) showed no distinct clusters reflecting hottest, coldest, wettest, driest, and/or normal year groups (Figure 2-24).

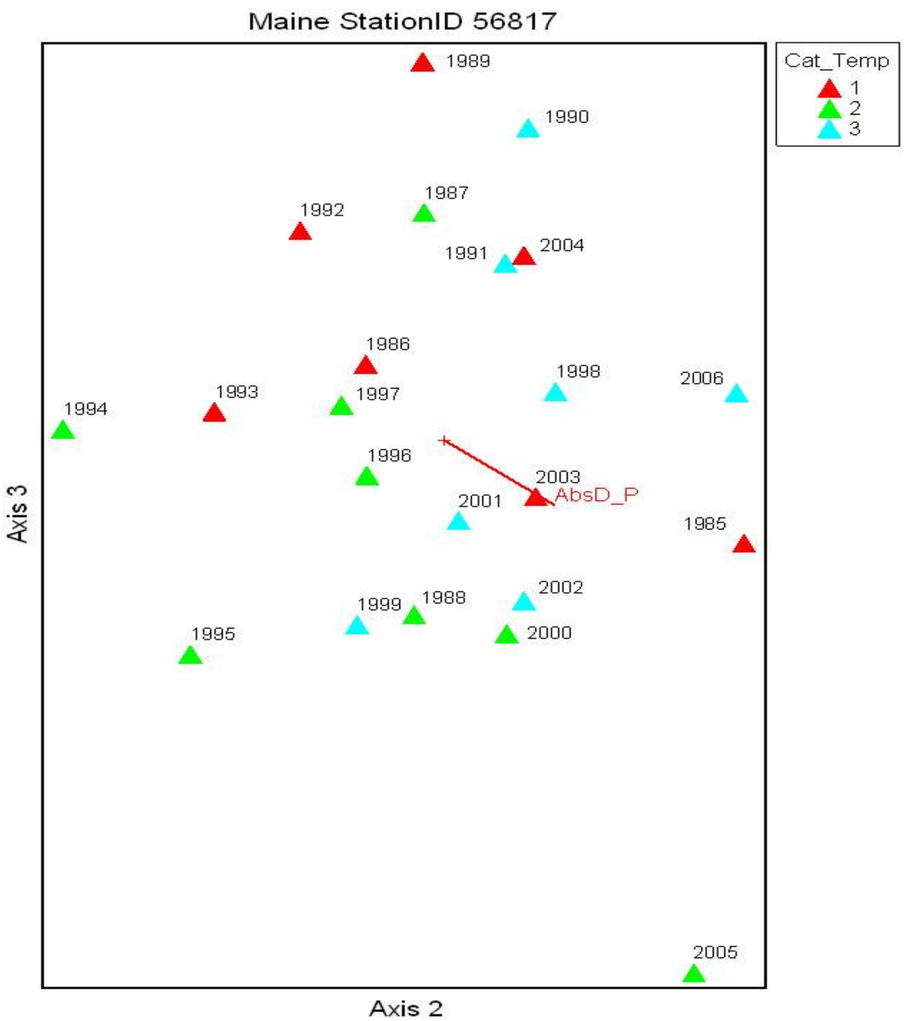


Figure 2-24. NMDS plot (Axis 3-2). Cat_Temp refers to the temperature categories, which are: 1=coldest years; 2=normal years; 3=hottest years. Samples are labeled by collection year. Absolute difference between the PRISM mean annual precipitation from the sampling year and the previous year (AbsD_P) is the most strongly correlated environmental variable with Axes 2 and 3.

Species composition did not change in a consistent way among hottest, coldest, wettest, driest, and/or normal year groups. Several of the taxa that were drivers of interannual patterns in community composition (*Ablabesmyia* (a midge), *Tricorythodes* (a mayfly), and *Pseudocloeon* (blue-winged olive mayflies) occurred most often during "normal" precipitation years, suggesting potential importance of hydrologic conditions in affecting community patterns in Maine. Overall, taxa could not be consistently categorized as having singular temperature or precipitation preferences. The lack of strong association between ecological trait groups and community patterns of responses, in combination with the lack of regional consistency in ecological trait group trends, makes it difficult to recommend a particular group of ‘climate change indicators’ as being regionally important.

At Station 56817 (Sheepscot), precipitation and flow appear to have a greater influence on the biotic assemblage than temperature. Five of the Maine bioclassification model input metrics were significantly correlated with flow category (Appendix E). The mean richness and abundance of cold-water-preference taxa were slightly higher during the wettest years (Figure 2-3). On average, more Class A indicator taxa were present during wetter years (Figure 2-25), as were EPT taxa relative to Diptera taxa (Figure 2-26). These patterns are consistent with expectation if the wettest years provide a more thermally stable and hospitable environment for the more sensitive cold-water-preference, Class A, and EPT taxa. The relative abundance of collector-gatherers was higher during higher flow years (Appendix E), though this may reflect a relationship between higher flows and the distribution of more fine and course particulate organic matter for food. In contrast, Diptera richness and Tanypodinae abundance decreased during higher flow years (Figure 2-26). These taxonomic groups include many environmentally tolerant taxa, which may do well during more stressful low flow years (higher relative abundance and richness), and decrease during wet years relative to the increase in sensitive taxa.

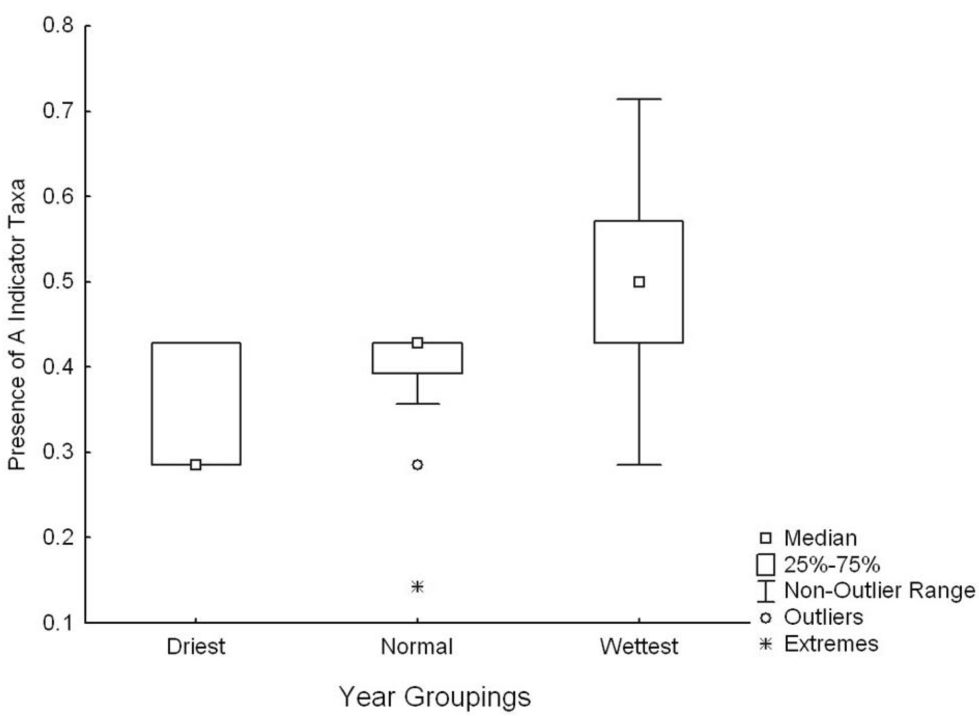


Figure 2-25. Distributions of Class A indicator taxa metric values in driest-, normal-, and wettest-year samples at Maine site 56817 (Sheepscot). Year groupings are based on PRISM mean annual precipitation from each site during time periods for which biological data were available. Data used in these analyses were limited to summer (July–September) rock-basket samples.

While Station 56817 in the Laurentian Hills and Plains showed faunal responses to precipitation, responses related to temperature were more evident in ANOVA analyses conducted on data from Station 57011, (W.Br. Sheepscot), also in the Laurentian Plains and Hills (Appendix E Table E3-2). This reflects the importance of site-specific variability in Maine. At Station 57011, a range of metrics, including percent abundance of collector-filterers, Hilsenhoff Biotic Index (HBI) scores, Shannon-Wiener diversity index scores, and percent abundance of Odonata (dragon/damsel flies), Coleoptera (beetles), and Hemiptera (true bugs) (OCH) taxa, showed significant differences between samples grouped by hottest, coldest and normal years. Some responses were logically consistent with climate change expectations (i.e. the mean percent of warm-water-preference individuals was lower in the coldest year samples). Others were not, perhaps because they were driven more by non-climatic factors, such as nutrient enrichment, or may reflect indirect mechanisms (e.g., flow or temperature effects on food resource availability, which then affects certain feeding groups), which cannot be directly assessed using the biomonitoring data sets. Since we do not yet fully understand the mechanisms behind these responses, interpreting these relationships and establishing expectations in the context of climate change is complex.

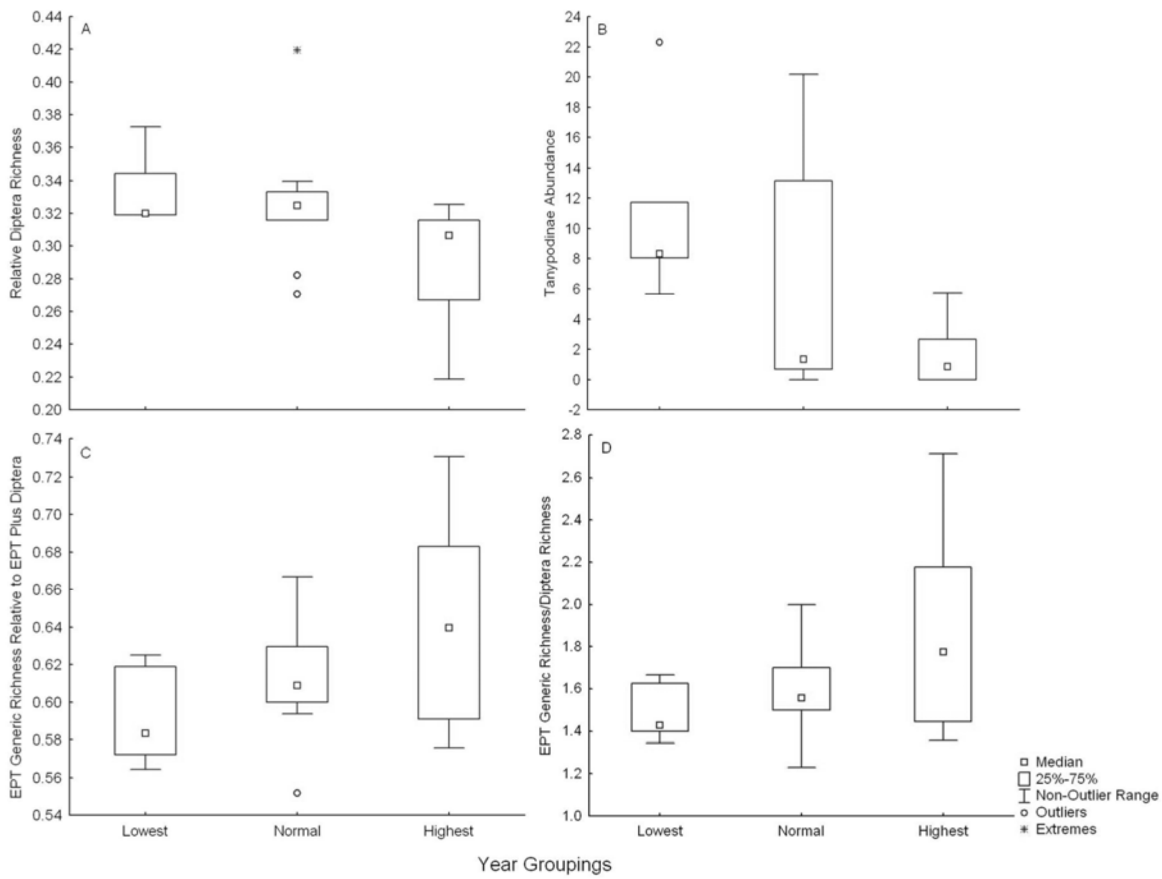


Figure 2-26. Distributions of EPT and Dipteran-related metric values in lowest-, normal-, and highest-flow year samples at Maine site 56817 (Sheepscot). Plot (A) shows relative Diptera richness, (B) Tanypodinae abundance, (C) EPT generic richness relative to EPT plus Diptera, and (D) EPT generic richness/Diptera richness. Year groupings are based on IHA median monthly flows averaged across July-September. Data used in these analyses were limited to summer (July–September) rock-basket samples.

2.3.3. Trends and Patterns – North Carolina and Southeastern States

Not many relationships were discerned between various biological metrics and annual air temperature variables based on North Carolina biomonitoring data; results are mostly site-specific. For example, cold-water-preference taxa were negatively correlated with temperature, as would be expected in response to climate change, but only at one Piedmont station (Appendix G). Only four trait group metrics showed significant correlations with air temperature at more than one of the tested reference locations. The percent of climbers (life habit trait category; see Stamp et al., 2010; Poff et al., 2006 for a description) was negatively correlated with mean annual air temperature from the year prior to sampling at two Blue Ridge sites. The percent of predators (feeding type trait category) was positively correlated with mean annual air temperature at one Blue Ridge site and negatively correlated with it at a Piedmont site. Appendices G and I shows the range of significant trends found, most only occurring at single reference sites.

More metrics were significantly correlated with annual precipitation variables than with temperature variables; however, inter-site variability is still strong, even within an ecoregion. Biological metrics that were related to precipitation at more than one site include the Hilsenhoff Biotic Index (HBI; negatively correlated with mean annual precipitation at two Blue Ridge sites); the percentage of climbers (negatively correlated with precipitation at one Blue Ridge and one Piedmont site); the percentage of shredders (negatively correlated with the previous year's precipitation at one Blue Ridge and one Piedmont site); and the percentage of burrowers (negatively correlated with the precipitation difference (sampling year – previous year) at one Blue Ridge site and positively correlated at one Piedmont site). Most of these relationships to precipitation are consistent with functional expectations. For instance, lower HBI scores during wetter years are consistent with the observed tendency for cold-water-preference taxa to have lower HBI tolerance values and to be more abundant during wetter years. The climbing habit may be disadvantaged during wetter years (e.g., more easily dislodged). On the other hand, the relationship of invertebrates that burrow to precipitation cannot be interpreted based on the limited information available and in any case exhibits contrasting responses among sites. Among trait groups selected for their expected hydrologic relationships, the abundance of perennial taxa was greater when precipitation was higher, and the richness of intermittent taxa was lower when precipitation was greater, both as would be expected (Appendix G). Furthermore, both the abundance and richness of cold-water-preference taxa increased as precipitation increased, which is consistent with the generally inverse relationship between precipitation and temperature. However, all these correlations were only significant at one Blue Ridge station. In addition, abundance of drought-tolerant taxa increased with increasing precipitation at that same station. This may not be entirely counter to expectation, in that taxa that can tolerate drought may still do better in more favorable conditions. However, it calls into question the value and sensitivity of the trait group designation for distinguishing precipitation trends related to climate change.

This notable spatial inconsistency in trends may result from high variability in site-specific habitat or other environmental conditions (e.g., in factors not specifically accounted for using biomonitoring data, such as groundwater contribution, riparian cover, substrate conditions, etc.), and from the relatively limited (short duration) data that are currently available for North Carolina. Variability between sites even within an ecoregion may also indicate variations in factors that affect vulnerability to climate change. Elevation and its effect on the relative contribution of cold-water-preference taxa to the community have been shown in other states to help define vulnerability to climate-change effects. In the North Carolina Blue Ridge, the higher elevations were associated with more cold-water taxa on average, but some Blue Ridge locations, such as the long-term reference Station NC0109 (New River) tended to have more warm-water-preference taxa. The reasons for this are not clear and warrant further consideration. The strength of the relatively short (mostly one decade or less at any one station) duration of the available data is too limited in the face of the magnitude of both spatial and temporal variation to discern the more subtle long-term trends and relationships needed to define any existing climate-change responses, and also to define the most effective climate-change indicators.

2.4. CONFOUNDING SOURCES OF TEMPORAL VARIATION

The ability to detect trends in a rigorous manner is affected by the amount and sources of variation contained in the data, and the ability to control or account for the variation. Interannual variation is expected to be larger in magnitude than incremental climate change responses (at least with respect to near-term linear projections based on historic data), and seasonal variation is often larger than that.

2.4.1. Seasonal variation

In a biomonitoring framework, seasonal variation is typically accounted for by limiting sampling to a single season or index period. This is the case for the four states (Maine, Utah, North Carolina, and Ohio) evaluated here, although not all focus on the same index period. In addition, over the two or more decades of data examined, the range of months during which sampling was actually conducted within any one state was found to vary over a wider range of seasons than expected based on current definitions of index period for each state. For example, Utah's defined index period is late summer to fall; however, sampling dates actually ranged from March through November (see Appendix F). Incorporation of the full range of available data over years would have introduced substantial variation in taxon occurrences and abundances due primarily to seasonal variation. This predictable increase in variation would further obscure other trends or patterns. The approach to minimizing seasonal variation was to subset data by season and for most analyses focus only on the predominant index period sampled in each state. For example for Utah data, sampling months were limited to August through November. Specifics on sampling months included for each set of trend, correlation, community, and weighted average modeling analyses conducted can be found in corresponding appendices (mainly see Appendices E through I, and K). It should be noted that in many cases, this approach reduced the amount of data available for long-term trend analyses. That is, elimination of "outlying" seasons frequently eliminated one or more years of data at some locations. While deemed an important procedure to account for predictable sources of variation, there is a practical impact of reducing the number of data points for trend analysis, and thus reducing the power to detect trends.

The selection of an index period will also be affected by climate change. Projected climate changes are likely to impact seasonal patterns through changes in flow conditions as well as in temperature regimes. These will influence a variety of biological processes, including rates of development, timing of emergence, and other components of reproduction (Seebens et al., 2009; Harper and Pecarsky, 2006; Poff et al., 2002; Vannote and Sweeney, 1980). This may have several ramifications to biomonitoring designs. If samples are collected at a fixed time during the year, then in the future sampling may yield lower abundances of some species, different species composition, or different relative abundances. This impacts temporal comparisons. Also, spatial comparisons may now be based on communities of more limited seasonal diversity. More extreme or extended summer low flows may, over the long term, become an impediment to sampling for states that use summer or fall index periods. This may be a particular concern in perennial streams vulnerable to a shift to intermittent conditions in the future. Biological responses to reductions in flow can represent legitimate responses to climate change. However, the eventual inability to sample during a late-season index period in some stream locations must be considered and planned for. Though highly unlikely due to resource limitations, sampling more than once per year, including once during the spring/high flow index period, could provide valuable information on components of the benthic community that emerge early in summer.

2.4.2. Interannual and multi-decadal climatic variation

Biological data also reflect responses to interannual variations (e.g., year-to-year variations in temperature, precipitation regime, etc); and to multi-year to multi-decadal “cyclic” climate variations, such as the North Atlantic Oscillation (NAO), the Pacific Decadal Oscillation (PDO), or the El Niño Southern Oscillation (ENSO) that drive differences in water temperature and hydrologic regimes in a manner similar to the mechanisms linking to long-term climate change responses. The NAO, for example, affects mainly winter weather conditions on decadal time scales (Hurrell, 1995). A rigorous approach, were it supported by available data, could examine what components of observable temporal variation in biological responses are attributable to long-term directional climate change, and then apply general linear modeling or another comparable approach to partition the variation within the observed biological responses between interannual or cyclic and long-term directional climatic sources. However, most state biomonitoring data sets, even the most critically developed (sensu Yoder and Barbour, 2009) and long term, such as those analyzed in these pilot studies, are limited in duration and frequency of sampling. These data are not able to support linear modeling, especially of several separate variables, because the average scope of available data is typically 20 years or less, with 10 to fewer than 20 annual data points over that time span. In addition, it often is the case that needed covariates, including flow variables, continuous temperatures, and water chemistry parameters such as nutrients, etc. are not available concurrently with the biological collections.

An alternative approach used here was to test for significant correlations between indices of known cyclic climatic variation (e.g., the NAO, PDO, and ENSO) with biological metrics, focusing on those that also showed long-term temporal responses, or correlations with temperature or precipitation. Details of results can be found in Appendices E through G, and I. In general, responses varied by state and region, as well as by taxon and trait group. Analyses were limited to representative reference stations with long-term data sets. In North Carolina, there were no strong correlations of major trait groups, especially cold- or warm-water-preference taxa, with annual or winter NAO indices. This is very likely a reflection of the shorter data sets available in North Carolina among reference stations in the Blue Ridge and Piedmont ecoregions.

In Maine, only one reference station, 56817 (Sheepscot) in the Laurentian Hills and Plains, was of sufficient length (23 years) to consider NAO effects. A few interesting relationships appear, though none are significant. None of the major climate variables, precipitation, air temperature, or water temperature are substantially related to either the mean annual or the winter (DJFM) NAO index (Figure 2-27).

With regard to benthic community characteristics, clustering of years at Station 56817 (Sheepscot) based on the Bray-Curtis (Sorensen) similarity index show some evidence of a temporal pattern (Figure 2-28), with the first four sampling years in cluster 1, and more of the early sampling years (e.g., 1984-1992 inclusive) within clusters 1 and 2. Later years of sampling occur more frequently in clusters 3 and 4. This suggests changes in community composition over time that reflect progressive changes in similarity. However, there are several “misplaced” years, e.g., 2004 and 2006 are in cluster 2, more similar to the mid- to late 1990’s sampling years. One of these, 2006, is a year with a low NAO index. But 2004 is an “average” NAO index year, and overall, there is no pattern associating the distribution of years among clusters with the NAO index (Figure 2-28).

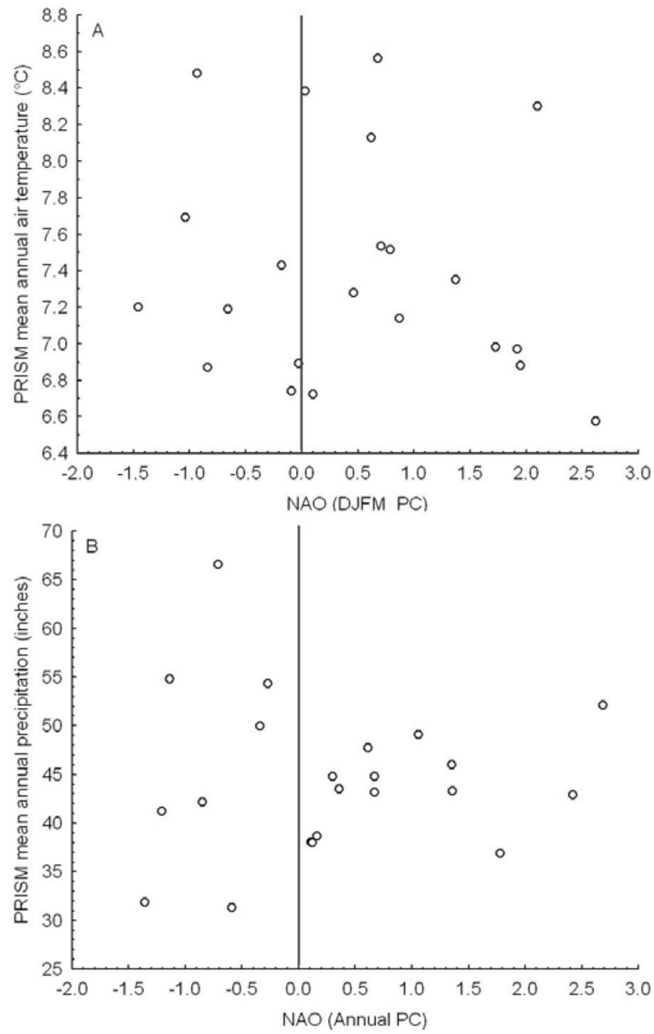


Figure 2-27. Relationships between North Atlantic Oscillation (NAO) indices and PRISM climatic variables at Maine site 56817 (Sheepscot). Plot (A) shows NAO winter index (December-January-February-March (DJFM)) vs. PRISM mean annual temperature (°C) (r=-0.15, p=0.51) and (B) shows PRISM mean annual precipitation (inches) vs. NAO annual index (r=0.03, p=0.90).

There is a modest relationship between benthic assemblages at Maine Station 56817 (Sheepscot) and NAO patterns when stability or persistence of the community is tested, based on degree of change in community similarity among years. The community was more stable, that is more similar between years, based on Euclidean distances during negative NAO phases, and more variable during positive phases (Figure 2-29). This is consistent with findings in Wales, where benthic community persistence was related to both long-term climate effects and cyclic effects of the NAO (Bradley and Ormerod, 2001). Persistence reflected environmental variability, with high persistence during negative NAO phases (cold, dry winters in northern

Europe) and low persistence (high variability) during positive NAO phases (milder, wetter winters). In New England, positive winter indices are associated with more winter storms, while negative winter indices are associated with fewer storms and drier winter conditions (New England Regional Assessment Group, 2001). Apparently the greater environmental variability introduced through more frequent winter storms to stream temperatures, flow conditions, and water quality translate to more variable benthic assemblages. This suggests that the NAO may be an important, climate-related influence on interannual patterns in benthic community responses, even though in most of the correlations of NAO index with community, trait group, or taxonomic group parameters were relatively weak.

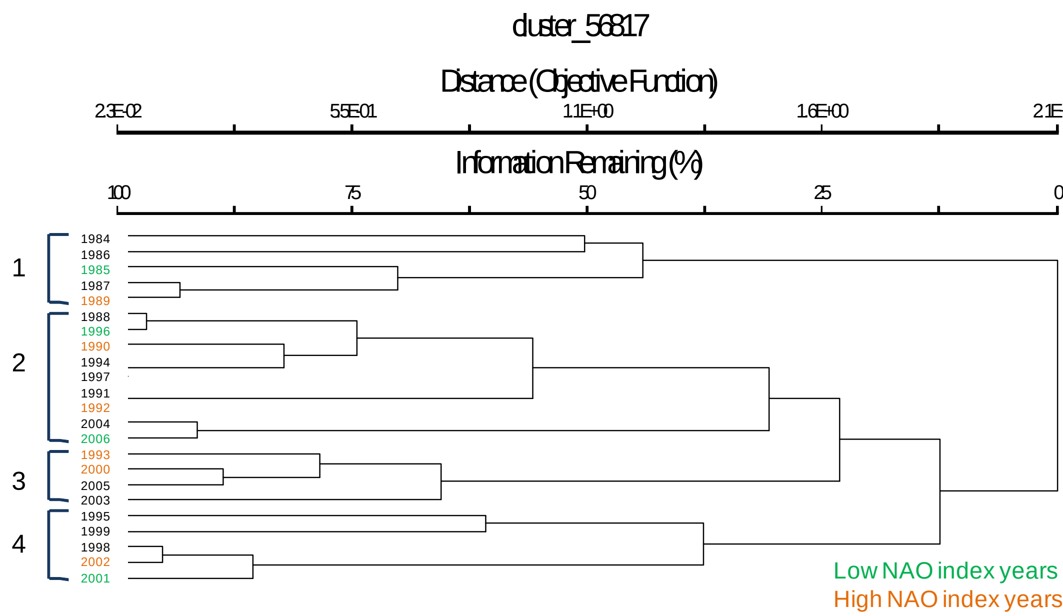


Figure 2-28. Cluster analysis using Bray-Curtis (Sorensen) similarity index, based on benthic invertebrate composition using genus-level OTUs, at Maine station 56817 (Sheepscot).

The PDO, which influences western and southwestern regions, is generally considered to be a much longer term, multi-decadal phenomenon (Brown and Comrie, 2004; Mantua et al., 1997), while ENSO is found to vary in the range of multiple years to a decade or more. In Utah, there were some intriguing relationships found at individual long-term reference stations between trait groups (e.g., warm-water-preference taxa, perennial taxa, etc. - see earlier sections of this chapter and Appendix I) and either the ENSO or PDO annual or monthly indices (see Appendix I). However, none of these were consistent spatially; therefore, no particular trait or taxonomic group is a strong indicator of PDO or ENSO responses. The complexity of the patterns compared to the relatively short (20 years or fewer) data sets suggests the importance of further investigation and long-term monitoring, including further study on the relative contributions of each index.

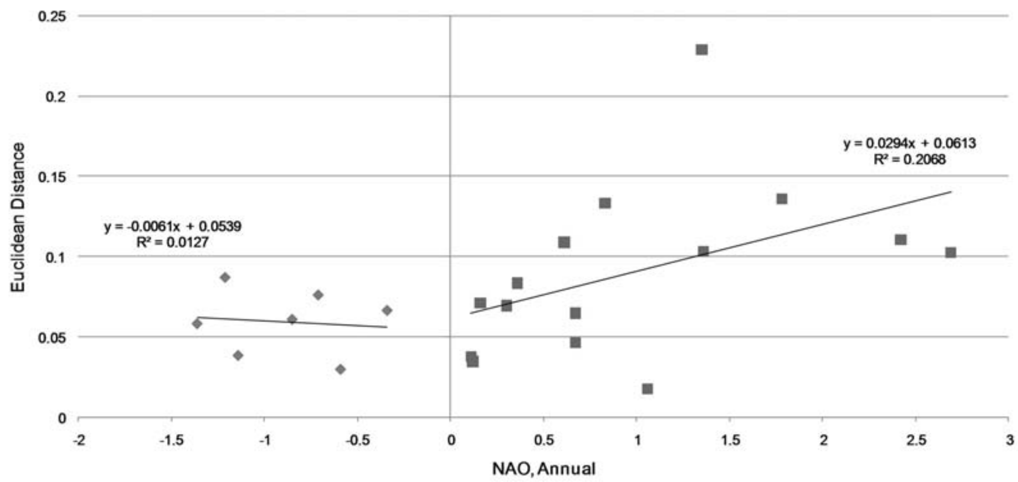


Figure 2-29. Correlation between Euclidean distance calculated as a difference between successive sampling years, as a measure of similarity between benthic assemblages, and the NAO annual index. In this case, Euclidean distance is plotted against the NAO index for the first year (e.g., 1984-85 comparison against 1984 NAO index), creating a 1-year lag.

While it is important to consider NAO, PDO, and/or ENSO when evaluating biomonitoring (or any other) data sets for climate change effects, there are still some practical limitations, particularly in the biomonitoring application. Fundamentally, the analyses require data spanning multiple (2-3) multi-decadal cycles to be able to model the cycle-associated responses and extract the residual long-term trend on a rigorous basis. The Maine Station 56817 (Sheepscot) data series spanned 23 years, and this is long compared to most existing available biomonitoring data. It also is likely that variations in the effects of the NAO interact with long-term climate change effects, potentially damping increasing temperatures in negative years and augmenting them in positive years (Durance and Ormerod, 2007). This is important, because the composite of climate effects may underestimate long-term climate impacts during some periods, or overestimate them during others. It would take proportionately more (longer-term) data to separate these and confidently define the long-term climate change component.

2.4.3. Interpretation of directional climate change effects

Since the nature of most bioassessment data limit the ability to separate the magnitude of observed trends among interannual, cyclical, and long-term directional climate effects, the results obtained in this study cannot be interpreted as entirely attributable to directional climate change. However, the net response of benthic or other aquatic community metrics to climate sensitive variables, including water temperature and hydrologic patterns, can reasonably and effectively be used to address the primary questions of this study. The direction and nature of the observed climate responses can be applied to characterize the types of responses that can be expected due to climate change, to identify the most sensitive indicators to climate change, and to understand implications to multimetric indices or predictive models and their application by managers to characterize condition of stream resources for decision making. These effects may be viewed in some respects as maximum estimates of probable effects, since multiple components of climate change could be included, though at times, the resulting estimates may also be undervalued.

2.5. OTHER SOURCES OF POTENTIAL SPATIAL CONFOUNDING

There are other potential sources of spatial confounding of temporal trends, which were tested in this study. Land use and land cover within a 1 km buffer of the individual reference sites indicated that anthropogenic influences were higher than desired (>5% urban or >10% agricultural) at most sites. The urban land uses surrounding these sites generally consisted of low-intensity and open-space development, and the agricultural land uses were mostly pasture/hay, with occasional cultivated crops. We further explored these relationships by using correlation analyses to determine whether any available chemistry and habitat variables were significantly correlated with biological metrics. Data availability limited this pursuit. For example, Utah only had chemistry data. At the two Utah long-term reference stations that showed strong temperature-related trends (Stations 4927250 - Weber and 4951200 - Virgin), some of the temperature preference metrics were significantly correlated with water chemistry variables. Many of the correlations were driven by outliers, but a few of the water chemistry variables, notably chloride, may have influenced trends in the biological assemblage (Figure 2-30; Appendix I). Chloride could be an indirect indicator of human development, as increases are sometimes associated with increasing road development and/or increasing application of road salt over time (NRC, 1991). However, chloride concentrations may also vary naturally with drought conditions.

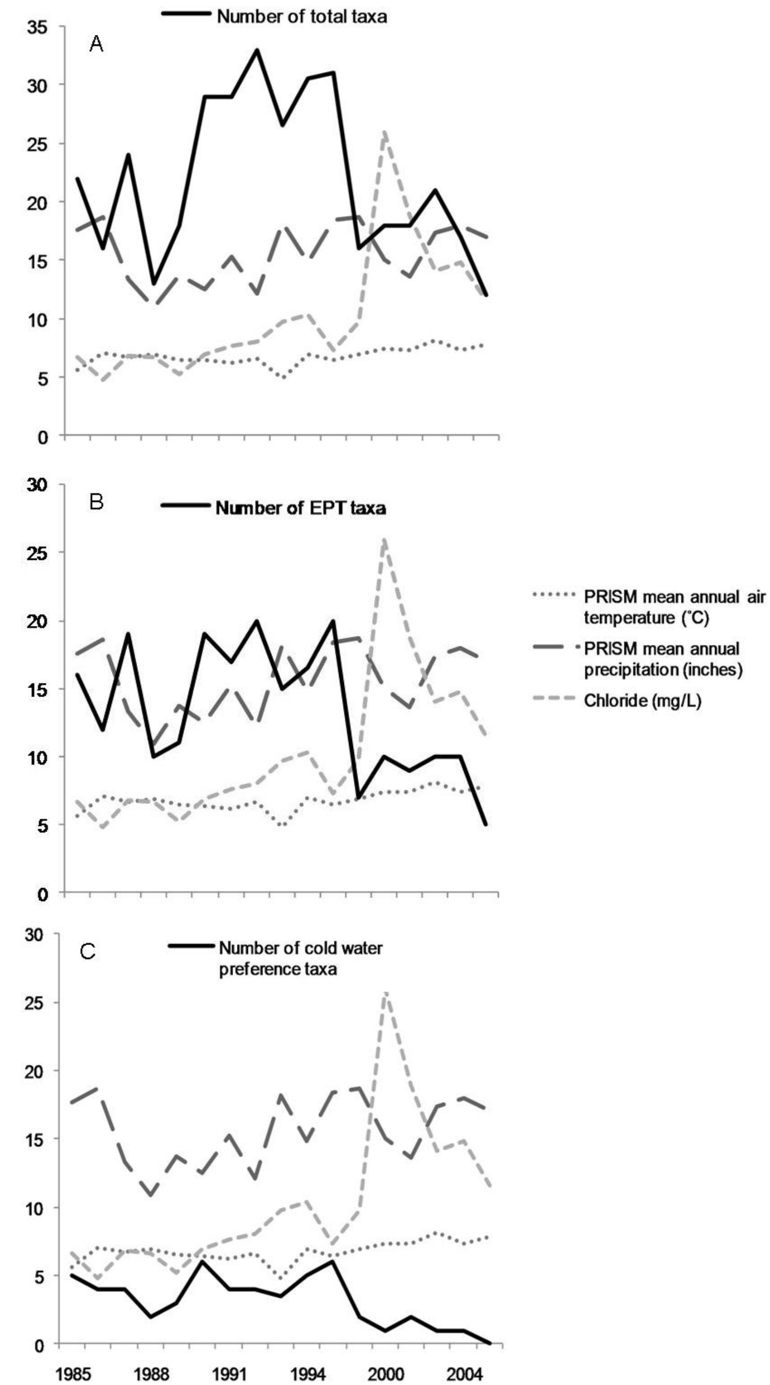


Figure 2-30. Trends in selected metrics, PRISM climatic variables and chloride concentrations over time at Utah site 4927250 (Weber). Plot (A) shows number of total taxa, (B) number of EPT taxa, and (C) number of cold-water-preference taxa. Data used in these analyses were limited to autumn (September–November) kick-method samples.

In Maine, limited chemistry and habitat information were available (mainly *in situ* water quality measurements and visual substrate estimates). At site 56187 (Sheepscot), yearly trends in the biological data were likely influenced by nonpoint-source pollution (pers.comm. Maine DEP), but we lack the long-term chemistry data necessary to confirm this possibility. Some of the habitat variables at site 56817 also showed trends over time. Percent boulders and % gravel were significantly correlated with some of the biological variables. However, based on conversations with Maine DEP, it appears that this 'trend' actually reflects observer bias, and it is not considered a real change over time in substrate characteristics. A similar example occurred in North Carolina, where visual substrate estimates for one site showed a fairly dramatic yearly trend. Scientists at NCDENR believe this also to be observer bias. More problematically, there were some fairly dramatic trends in canopy cover and water chemistry found at some North Carolina sites, which turned out to be due to data entry errors. This seems a minor but important cautionary note, as the "false" trend in canopy cover seemed feasible (increasing cover over time would be possible if there were an earlier instance of logging), and a (non-significant) trend of decreasing water temperature over time appeared to be logically consistent with increasing canopy cover. In the end, this very "appealing" discovery was false.

2.6. COMPARISON OF REGIONAL TRENDS, VULNERABILITIES, AND INDICATORS

Trends over time and in association with climate variables have been found within the state bioassessment data sets and in particular with ecological or life history trait groups (Section 2.2) (Gallardo et al., 2009; Beche and Resh, 2007; Bonada et al., 2007b). Unfortunately, there are numerous examples within this study in which observed trends were significant in some places but not in others. Spatial consistency can be used as evidence that a particular trend or relationship is important. But even with the extensive biomonitoring data sets analyzed in this study, it was rare to have more than one or two reference sites within a region with sufficient data to conduct satisfactory long-term trends analyses. The focus on reference stations is needed to minimize contributions of effects from sources other than climate change (potential confounding factors such as urban or agricultural land use, see Appendix C for land use and other criteria used for reference station screening). We used an alternative approach of grouping reference stations within an ecoregion (Appendix C) to increase spatial coverage for trend analyses. However, this was marginally successful, and there was still high inter-site variability in factors that affect community comparability (e.g., elevation, stream size, general geography/topography). As a result, variation among sites was almost always greater than the magnitude of any long-term temporal trends. Some component of spatial variability between sites may reflect real spatial variation in degree of vulnerability to climate change effects.

Regional variation in trends for ecological trait groups defined by temperature preferences also reflects the lack of spatial consistency in results. The number of warm-water-preference taxa increased significantly over time at lower elevation locations in both Maine (site 56187 – Sheepscot, site 57011 – W. Br. Sheepscot) and Utah (site 4951200 – Virgin), but not in North Carolina, and not at all stations (Table 2-4). The increasing temporal trend in warm-water-preference taxa was corroborated by correlation with temperature in Utah, but not in Maine (Table 2-5). At the longest-term station in Maine (56817) cold-water taxa also increased, but this is generally counter to climate change expectations. Though significant (see Table 2-2), the number of taxa was so low as to make the apparent trend largely meaningless. Cold-water-preference taxa decreased over time at one of the higher elevation sites in Utah (site 4927250 – Weber), and was also negatively correlated with temperature, as would be expected for a trait group responding to climate change increases in temperature. Comparable associations with temperature were not found in Maine or North Carolina. For these two states, cold-water-preference taxa were instead more often related to precipitation (Table 2-6).

Table 2-4. Results of Pearson product moment correlation analyses done to examine associations between *year* and a selected group of *metrics* at long-term biological monitoring sites in Utah (UT), Maine (ME), and North Carolina (NC). Significant relationships at p < 0.05 are shown in bold with shading; at p<0.1 in bold. NA=not available (we did not calculate the Shannon-Wiener diversity index for North Carolina samples because the abundance data were categorical).

	Utah								**Maine**						**NC**	
Biological Metric vs. YEAR	**4927250**		**4951200**		**4936750**		**5940440**		**56817**		**57011**		**57065**		**NC0109**	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
Cold water taxa richness	**-0.71**	0.00	**-0.62**	0.02	-0.38	0.23	**-0.64**	0.07	**0.49**	0.02	0.04	0.90	0.54	0.13	**0.55**	0.08
Cold water taxa relative abundance	**-0.72**	0.00	**-0.63**	0.02	-0.15	0.64	-0.12	0.76	**0.47**	0.03	**-0.67**	0.02	0.45	0.23	**0.57**	0.07
Warm water taxa richness	-0.21	0.42	**0.85**	0.00	0.38	0.22	NA	NA	**0.78**	0.00	**0.65**	0.02	0.58	0.10	**-0.58**	0.06
Warm water taxa relative abundance	-0.21	0.42	0.41	0.15	0.42	0.17	NA	NA	**0.55**	0.01	**-0.59**	0.04	-0.36	0.34	-0.04	0.90
Total taxa richness	-0.29	0.26	-0.28	0.34	0.08	0.81	-0.54	0.14	**0.75**	0.00	**0.81**	0.00	0.56	0.11	**-0.67**	**0.02**
EPT taxa richness	**-0.59**	0.01	**-0.49**	0.08	-0.21	0.52	**-0.65**	0.06	**0.75**	0.00	**0.76**	0.00	0.51	0.16	0.30	0.36
EPT relative abundance	0.06	0.81	0.06	0.85	-0.26	0.42	0.44	0.23	0.06	0.80	**-0.52**	0.08	-0.36	0.34	**0.74**	**0.01**
Ephemeroptera taxa richness	**-0.57**	0.02	**-0.60**	0.02	-0.04	0.89	-0.57	0.11	**0.58**	0.01	**0.63**	0.03	0.37	0.33	0.22	0.52
Plecoptera taxa richness	**-0.76**	0.00	**-0.53**	0.05	-0.29	0.36	**-0.71**	0.03	-0.16	0.47	0.05	0.88	0.44	0.23	0.45	0.16
Shannon Wiener diversity index	0.13	0.62	-0.43	0.12	-0.08	0.81	-0.25	0.52	**0.64**	0.00	0.12	0.72	0.43	0.25	NA	NA
OCH taxa richness	**0.61**	0.01	0.46	0.10	**0.83**	0.00	0.28	0.47	**0.43**	0.04	0.43	0.16	0.28	0.47	0.10	0.78
OCH taxa relative abundance	**0.66**	0.00	0.40	0.15	0.32	0.31	-0.59	0.10	0.28	0.20	**-0.52**	0.09	0.37	0.33	0.14	0.68
HBI	-0.19	0.47	0.28	0.34	0.32	0.31	-0.46	0.21	-0.13	0.54	**0.75**	0.01	0.18	0.65	**-0.63**	**0.04**

Table 2-5. Results of Pearson product moment correlation analyses done to examine associations between Parameter-elevation Regressions on Independent Slopes Model (PRISM) mean annual air temperatures and a selected group of metrics at long-term biological monitoring sites in Utah (UT), Maine (ME), and North Carolina (NC). Significant relationships at *p* < 0.05 are shown in bold with shading; at p<0.1 in bold. NA=not available (we did not calculate the Shannon-Wiener diversity index for North Carolina samples because the abundance data were categorical).

Biological Metric vs. TEMP	4927250		4951200		4936750		5940440		56817		57011		57065		NC0109	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Cold water taxa richness	**-0.63**	0.01	**-0.73**	0.00	-0.08	0.82	-0.14	0.73	0.31	0.15	0.02	0.95	-0.58	0.10	-0.38	0.25
Cold water taxa relative abundance	-0.30	0.24	**-0.56**	0.04	-0.20	0.53	-0.29	0.46	0.15	0.50	-0.16	0.62	-0.27	0.48	-0.32	0.34
Warm water taxa richness	**-0.44**	0.08	**0.76**	0.00	-0.03	0.93	NA	NA	0.21	0.34	0.27	0.39	**-0.73**	0.02	-0.18	0.59
Warm water taxa relative abundance	-0.35	0.17	**0.62**	0.02	0.01	0.98	NA	NA	0.13	0.55	0.37	0.23	0.05	0.90	0.00	1.00
Total taxa richness	**-0.48**	0.05	**-0.68**	0.01	-0.08	0.81	-0.20	0.60	0.29	0.18	0.10	0.76	-0.52	0.15	0.04	0.91
EPT taxa richness	**-0.57**	0.02	**-0.79**	0.00	-0.09	0.77	-0.43	0.25	0.17	0.44	0.25	0.43	**-0.64**	0.06	0.00	0.99
EPT relative abundance	0.03	0.91	0.29	0.32	0.04	0.90	0.07	0.86	0.08	0.71	**0.64**	0.03	-0.07	0.87	-0.09	0.80
Ephemeroptera taxa richness	**-0.59**	0.01	**-0.81**	0.00	-0.26	0.41	-0.22	0.56	0.19	0.39	**0.51**	0.09	**-0.62**	0.08	-0.18	0.60
Plecoptera taxa richness	**-0.45**	0.07	**-0.65**	0.01	0.17	0.61	**-0.72**	0.03	0.09	0.70	-0.06	0.85	-0.56	0.12	0.12	0.73
Shannon Wiener diversity index	-0.13	0.62	**-0.67**	0.01	-0.12	0.71	-0.29	0.46	0.45	0.38	0.45	0.14	**-0.59**	0.09	NA	NA
OCH taxa richness	0.11	0.68	0.12	0.68	0.27	0.40	**0.59**	0.09	0.13	0.54	0.35	0.26	-0.10	0.80	0.14	0.68
OCH taxa relative abundance	**0.44**	0.07	0.27	0.36	-0.11	0.74	-0.01	0.98	0.01	0.98	-0.09	0.79	-0.33	0.38	0.30	0.37
HBI	-0.32	0.21	0.04	0.89	0.09	0.77	0.09	0.82	-0.07	0.76	-0.21	0.51	0.13	0.75	0.13	0.71

Table 2-6. Results of Pearson product moment correlation analyses done to examine associations between Parameter-elevation Regressions on Independent Slopes Model (PRISM) mean annual precipitation and a selected group of metrics at long-term biological monitoring sites in Utah (UT), Maine (ME), and North Carolina (NC). Significant relationships at *p* < 0.05 are shown in bold with shading; at p<0.1 in bold. NA=not available (we did not calculate the Shannon-Wiener diversity index for North Carolina samples because the abundance data were categorical).

	Utah								**Maine**						**NC**	
	4927250		**4951200**		**4936750**		**5940440**		**56817**		**57011**		**57065**		**NC0109**	
Biological Metric vs. PRECIP	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
Cold water taxa richness	-0.11	0.68	0.44	0.12	0.42	0.17	0.01	0.98	**0.44**	0.04	0.18	0.59	-0.51	0.16	**0.85**	0.00
Cold water taxa relative abundance	0.08	0.75	0.23	0.42	0.30	0.35	0.54	0.14	**0.58**	0.00	0.03	0.93	-0.02	0.97	**0.63**	0.04
Warm water taxa richness	-0.05	0.84	-0.18	0.53	0.21	0.50	NA	NA	0.07	0.75	-0.04	0.91	-0.13	0.73	**-0.65**	0.03
Warm water taxa relative abundance	-0.14	0.60	-0.34	0.23	0.33	0.29	NA	NA	0.04	0.85	-0.10	0.76	-0.44	0.23	**-0.57**	0.07
Total taxa richness	-0.15	0.56	**0.57**	0.04	0.43	0.16	-0.07	0.85	0.28	0.21	0.15	0.63	-0.28	0.47	**-0.64**	0.04
EPT taxa richness	-0.25	0.34	**0.68**	0.01	0.45	0.14	0.17	0.66	0.20	0.37	0.24	0.45	-0.12	0.76	0.36	0.28
EPT relative abundance	-0.29	0.27	0.07	0.82	0.32	0.30	0.32	0.40	0.01	0.97	-0.05	0.88	0.17	0.66	**0.82**	0.00
Ephemeroptera taxa richness	-0.20	0.45	**0.58**	0.03	0.34	0.28	0.02	0.97	0.35	0.11	0.45	0.14	-0.09	0.83	0.24	0.47
Plecoptera taxa richness	-0.34	0.18	0.40	0.16	0.22	0.49	0.29	0.45	0.18	0.43	-0.19	0.56	-0.32	0.41	**0.62**	0.04
Shannon Wiener diversity index	**-0.49**	0.05	0.10	0.74	0.49	0.10	-0.14	0.72	0.25	0.27	0.09	0.79	0.01	0.97	NA	NA
OCH taxa richness	**0.46**	0.06	0.27	0.35	0.15	0.64	-0.30	0.44	0.28	0.20	0.07	0.82	-0.44	0.24	-0.06	0.86
OCH taxa relative abundance	-0.04	0.89	-0.29	0.32	0.25	0.43	-0.28	0.47	0.25	0.24	0.29	0.36	-0.33	0.39	-0.22	0.52
HBI	0.16	0.53	0.11	0.71	**-0.55**	0.06	-0.37	0.32	-0.22	0.31	0.25	0.43	-0.27	0.49	**-0.86**	0.00

As presented above, the distributions of cold-water-preference taxa (richness and relative abundance) were significantly associated with elevation, stream size (order) and watershed size, such that more cold-water-preference taxa were present at higher elevations and in smaller streams and watersheds, and warm-water-preference taxa were more common at lower elevations, and in larger streams and watersheds (Table 2-7, figures in Section 2.2.2). Though not consistently demonstrated in all states, some higher elevation ecoregions with a greater predominance of cold-water-preference taxa exhibited greater responsiveness (e.g., more significant trends) to changes in climate variables (Appendix A). The sizes of streams sampled in those ecoregions probably interacted with elevation differences. For example, in most of the states, low-order streams tended to be under-sampled. Mid-order streams even at relatively higher elevations might have fewer cold-water-preference taxa than lower order streams would. Nevertheless, higher elevation regions, as well as areas subsetted by stream and watershed size, should be evaluated for vulnerability to climate changes in temperature and hydrologic conditions. This factor should be accounted for in assessing climate change monitoring priorities.

The hydrologic indicator metrics generally failed to show significant trends for a number of reasons. The metrics as developed might not be effective at detecting shifts in hydrologic regimes and may need to be further refined. Limited knowledge about life history, mobility, morphology, and temperature preference and tolerance information is currently one of the major limitations of traits-based metrics; more information could improve metric performance. It also is possible that the metrics are effective and are simply documenting that there are no consistent patterns yet. Precipitation tends to be highly variable and can be difficult to predict or model (e.g., Brown and Comrie, 2004; 2002). Analyses conducted on Piedmont sites in North Carolina as an adjunct to this study (Appendix J) indicate that natural stream communities appear to be resilient within the range of natural hydrologic variability. Because of this resilience, effects from hydrologic changes associated with climate change may not be seen unless these changes are large. This may happen as the magnitude of effects increases. Analyses were conducted on reference site data with natural flow regimes; it is possible that the metrics may be effective, but that shifts in hydrology over the short periods of record have not followed consistent patterns.

Results of this study, as well as other research (Webb et al., 2009; Dewson et al., 2007; Suren and Jowett, 2006; Lind et al., 2006; Poff, 2002; Extence et al., 1999; Stanley et al., 1994) have demonstrated the importance of hydrologic changes on biological responses, and it will be worthwhile to consider both “scenario-based” and hydrologic metrics further in the future. These classes of indicators may be most valuable in regions, such as North Carolina, where associations with precipitation are already strong, and where other evidence suggests the dominance of hydrologic drivers (NCDENR, 2005). Hydrologic metrics are also likely to be valuable in regions with strong future vulnerability to hydrologic impacts due to the combination of climate change predictions for temperature and precipitation, such as in the arid west and southwest.

Analyses testing for relevant biological responses to climate patterns often lacked spatial consistency both within and across states. Several biological metrics, evaluated for differences between years partitioned based on temperature (hottest/coldest/normal years) or precipitation (wettest/driest/normal years) regime showed patterns in one or another state (see the above subsections of this chapter), but only a few showed statistically significant patterns at sites in more than one state, and none showed common patterns among all states. Overall, more metrics were significantly associated with temperature-related variables than with precipitation variables (Appendix I). While long-term increasing trends in temperature already can be demonstrated for many regions (see Section 2.1 and Appendix A), this is seldom the case for precipitation or flow-related variables (Appendix I). Long-term data for flow (e.g., IHA) variables tend to be scarcer; and climate change projections for precipitation are small in magnitude and variable for many regions. Nevertheless, the importance of ongoing changes in precipitation effects on flow regime should not be discounted.

Other biological metrics that were sometimes responsive to climate variables include functional feeding groups (e.g., predators, collector-filterers) or life history habits (e.g., swimmers, climbers) (Appendix I). Feeding, life habit, and other functional trait groups are often included as metrics in state MMIs. It is thus recommended that, on a case by case basis, the vulnerability of this class of metrics be evaluated through trend and correlation analysis, as well as through assessment of composition by temperature sensitive taxa.

Table 2-7. Summary of differences in elevation, PRISM mean annual air temperature and precipitation and mean number and percent of cold and warm-water-preference taxa across and within major ecoregions in each state. Only full-scale samples were used to derive the numbers for North Carolina. Samples were not limited to particular seasons in Utah and North Carolina. Mean % individuals of cold and warm water in the North Carolina Coastal ecoregion were not calculated (our analyses were concentrated in the Mountain and Piedmont ecoregions).

State	Ecoregion	# Samples	Elevation (m)	Air temperature (°C)	Richness		Relative Abundance	
					Cold water	Warm water	Cold water	Warm water
Maine	Northeastern Coastal Zone	576	29.3	8.3	1.7 ± 1.9	3.3 ± 2.8	5.4 ± 9.9	17.0 ± 20.6
	Laurentian Plains &Hills	2830	65.2	6.5	1.1 ± 1.4	4.7 ± 3.3	2.8 ± 6.6	22.4 ± 22.0
	Northeastern Highlands	857	210.4	5.8	1.7 ± 2.0	3.2 ± 2.7	7.1 ± 11.8	15.1 ± 17.5
Utah	Mojave Basin & Range	13	736.6	16.8	2.8 ± 2.4	1.3 ± 0.9	6.6 ± 8.9	5.5 ± 8.7
	Central Basin & Range	177	1411.7	10.0	1.4 ± 2.0	2.4 ± 1.4	2.1 ± 7.0	10.8 ± 16.5
	Colorado Plateaus	205	1729.4	9.1	3.8 ± 2.8	1.2 ± 1.2	9.8 ± 11.5	6.1 ± 11.6
	Northern Basin & Range	6	1769.7	8.6	4.7 ± 1.0	1.2 ± 0.8	3.2 ± 2.9	12 ± 20.1
	Wyoming Basin	27	2002.0	5.7	6.1 ± 4.0	1.3 ± 0.9	13.2 ± 13.2	1.1 ± 2.4
	Wasatch & Uinta Mountains	644	2131.1	5.4	5.5 ± 4.0	1.0 ± 1.3	13.1 ± 15.4	3.8 ± 11.0
	Southern Rockies	7	2535.2	6.3	9.1 ± 0.7	0 ± 0	30.6 ± 14.6	0 ± 0
North Carolina	Middle Atlantic Coastal Plain	173	4.7	16.7	0.1 ± 0.2	4.7 ± 5.1	0.1 ± 0.4	12.3 ± 6.4
	Southeastern Plains	317	34.1	16.3	0.1 ± 0.4	8.8 ± 3.4	0.1 ± 0.4	12.1 ± 5.1
	Piedmont	1106	183.5	15.0	1.5 ± 2.0	5.2 ± 3.1	1.8 ± 2.7	6.7 ± 4.7
	Blue Ridge	631	714.5	12.1	8.0 ± 4.5	2.8 ± 2.4	11.4 ± 7.9	3.1 ± 3.7

The Shannon-Wiener diversity index is another metric that is often included in MMIs, which showed an inverse relationship with temperature at one Utah station (site 4951200 – Virgin) (Table 2-5). Both the value and the drawback of using overall community diversity as a metric of condition is that it is a composite response of all community components. This study shows that, in at least some regions, overall community diversity is reduced in hotter years due to suppression of cold-water-preference taxa (Table 2-5). This response is mediated by the relative composition of cold and warm taxa, which is also associated with elevation and stream size. Potential modification of this metric to help track climate change effects will have to be site or region specific, and should initially focus on the relative contribution of cold and warm-water-preference taxa within the community.

The abundance or richness of OCH taxa are more rarely incorporated as an MMI metric. It functions as a contrasting metric to EPT taxa, due to the generally high environmental tolerances of these taxa and expectation that they do better in the summer and in drier, more intermittent conditions (Bonada et al., 2007a). The potential robustness of OCH taxa to climate change effects was considered important. In fact, the abundance of OCH taxa was higher during hot years in some locations, though the trends were not statistically significant (Table 2-4, Appendix I). Still, this may be a valuable indicator to consider in the future.

Climate change “scenarios” (e.g., warmer and drier conditions) were used to combine temperature preference traits with other ecological (e.g., hydrologic preferences) and life history traits in an attempt to improve both the detection of responses to climate variables, and impart greater ability to explain the responses and use this information to develop more effective indicators. Overall, temperature-preference metrics by themselves were more responsive to climate change variables in more regions tested than were these composite scenario metrics. Only one of the scenario metrics, percent drier-vulnerable taxa, showed significant patterns at more than one site (Appendix I). Further consideration of this “climate scenario” trait suite approach may still be fruitful in the future. The limitation is that selection for multiple traits tends to reduce the number of member taxa, and therefore limits the amount of data for trend analyses.

3. IMPLICATIONS TO MULTIMETRIC INDICES, PREDICTIVE MODELS, AND IMPAIRMENT/LISTING DECISIONS

For states and tribes to assess stream condition, the extensive biological monitoring data collected on macroinvertebrate, fish, and/or other stream and river communities must be distilled to a format that accurately and reasonably reflects condition. That is, the result must be a good "indicator" or "index" and must be readily compared between reference and affected conditions. The main categories of such computational approaches are multi-metric indices (MMIs) and predictive models.

MMIs are generally structured as a composite of biological metrics selected to capture ecologically important community structural or functional characteristics and have been applied to fish and benthic macroinvertebrate communities (Norris and Barbour, 2009; Bohmer et al., 2004; Sandin and Johnson, 2000; Barbour et al, 1995; Yoder and Rankin, 1995; DeShonn, 1995; Karr, 1991). Component metrics are selected based on their responsiveness to the environmental impacts most often evaluated. Sites are assessed by comparing the test location MMI to that calculated for applicable reference locations, grounded in the assumption that degradation in the MMI reflects aquatic community responses to pertinent environmental stressors.

There is much variation among states and tribes in the particular components included in MMIs or predictive models, because, as a rule, they are calibrated to the state, or more often, to regions within a state to account for predictable (natural) variability (Barbour and Gerritsen, 2006). Added to this index variability is the regional variability in both climate change projections and associated biological responses. These sources of variability make generalizations about the implications of climate change for bioassessment indices challenging. However, there are some commonalities among states, such as the categories of metrics used, which we use to investigate vulnerabilities of these approaches to climate change.

Predictive models use regional reference conditions to develop relationships between environmental predictor variables and macroinvertabrate taxon occurrence from which predictions for an "expected" (E) community are based. A commonly applied model for macroinvertebrate communities is the River InVertebrate Prediction And Classification System (RIVPACS) (Wright, 2000). An important assumption is that the predictor variables are minimally affected by human disturbance and are relatively invariant over ecologically-relevant time (Utah State University, 2009; Tetra Tech, 2008; Hawkins et al., 2000; Wright, 2000; Wright et al., 1984). The E community is then compared to various "observed" (O) communities at non-reference locations. A basis for comparison is that any differences between O and E communities reflect biological responses to the range of environmental pollutants or alterations that are intended to be evaluated. This is similar to the MMI approach.

Among the four states evaluated in this study, three of them, Maine, North Carolina, and Ohio, use some form of MMI. Utah uses a predictive model, RIVPACS, for assessing wadeable streams. These states are representative of major regions of the US, encompassing large-scale variations in climate, climate change projections, geography, topography, geology, and hydrology. State-specific analysis results also inform a regional view of climate change implications to commonly used MMIs and predictive models.

3.1. MAINE AND THE NORTHEAST

Maine uses 4 linear discriminant models that incorporate 30 input metrics to assign sites to one of four classes (A, B, C, and NA, where A represents the best conditions, and NA is Non-Attainment), applying the same criteria to all sites. Vulnerabilities of the component metrics to climate change can be evaluated, but it is difficult to extend the results to impacts on station classifications, because the discriminant model inherently looks at multiple variables simultaneously. There are no firm thresholds or individual metric values at which a sample changes classification levels. Analyses of the differences in each component metric among rating classes (summarized in Appendix E) provide the basis for comparing climate-related sensitivities of these metrics. Overall, stations with the following characteristics received better ratings:

- High generic richness
- High richness and abundance of EPT taxa
- High Shannon-Wiener diversity index values
- Low HBI scores
- Low Chironomidae abundances
- Low relative Diptera richness
- Low relative Oligochaeta abundance
- Greater presence of Class A indicator taxa
- Greater scraper relative abundance

A variety of analyses were used to characterize possible vulnerabilities of Maine's discriminant model approach (see Appendix E for details). For instance, ANOVA was used to evaluate whether certain model components were more important than others in distinguishing between different classes. Temperature preferences and tolerances of Class A indicator taxa were examined, as were Biological Condition Gradient (BCG) assignments and tolerance values of cold- and warm-water-preference taxa.

Climate change effects are likely to influence a number of Maine's discriminant model input metrics. Eight of these are metrics related to EPT taxa, which are also used by other northeastern states. In Maine, the vulnerabilities of EPT taxa are largely related to the ecological trait of temperature preference. Twenty nine (29) of the Maine cold-water-preference taxa are EPT taxa (Table 3-1). There are also 18 EPT taxa on the warm-water-preference list (Table 3-2).

Table 3-1. Number of Maine cold-water taxa in each order with EPT taxa in *italics*.

Order	Total
Plecoptera	*16*
Trichoptera	*10*
Diptera	7
Ephemeroptera	*3*
Coleoptera	2
Odonata	2
Megaloptera	1

Table 3-2. Number of Maine warm-water taxa in each order with EPT taxa in *italics.*

Order	Total
Diptera	10
Ephemeroptera	*9*
Trichoptera	*6*
Basommatophora	4
Plecoptera	*3*
Arhynchobdellida	1
Coleoptera	1
Decapoda	1
Haplotaxida	1
Hoplonemertea	1
Hydroida	1
Mesogastropoda	1
Odonata	1

More of the ephmeropteran (mayfly) taxa are warm-water- than cold-water-preference taxa. Two of the model input metrics used by Maine are specifically related to ephemeropterans (metrics for both absolute and relative abundance). On average, higher values for the Ephemeroptera abundance metric occur at Class B sites (Figure 3-1A), while the highest relative abundances occur at Class A sites (Figure 3-2A). Thus, increases in warm-water ephemeropterans as temperature increases with climate change can affect station classification. For example,former Class C sites could become Class B sites due to the addition of these taxa, while the same trend of increasing Ephemeroptera abundance might degrade former Class A sites to Class B. If the relative abundance of ephemeropterans increases as their absolute abundance increases (which would, of course, depend on the relative responses of other taxa as well), then station classification of any condition class might increase. Increasing abundance of warm water ephemeropterans at Maine's longest term reference station (56817 - Sheepscot in the Laurentian Hills and Plains) over the 22-year sampling period has already resulted in a difference in these ephemeropteran metrics comparable to the average difference between these metrics at Class A and B sites (Figures 3-1 and 3-2). For example, ephemeropteran abundance (Figure 3-1B) increased from just under 100 per sample in the first 5-years to close to 200 per sample (but with high variability) in the last 5 years. This range approximates the mean difference between Class A and B stations, or between Class B and C stations (Figure 3-1A).

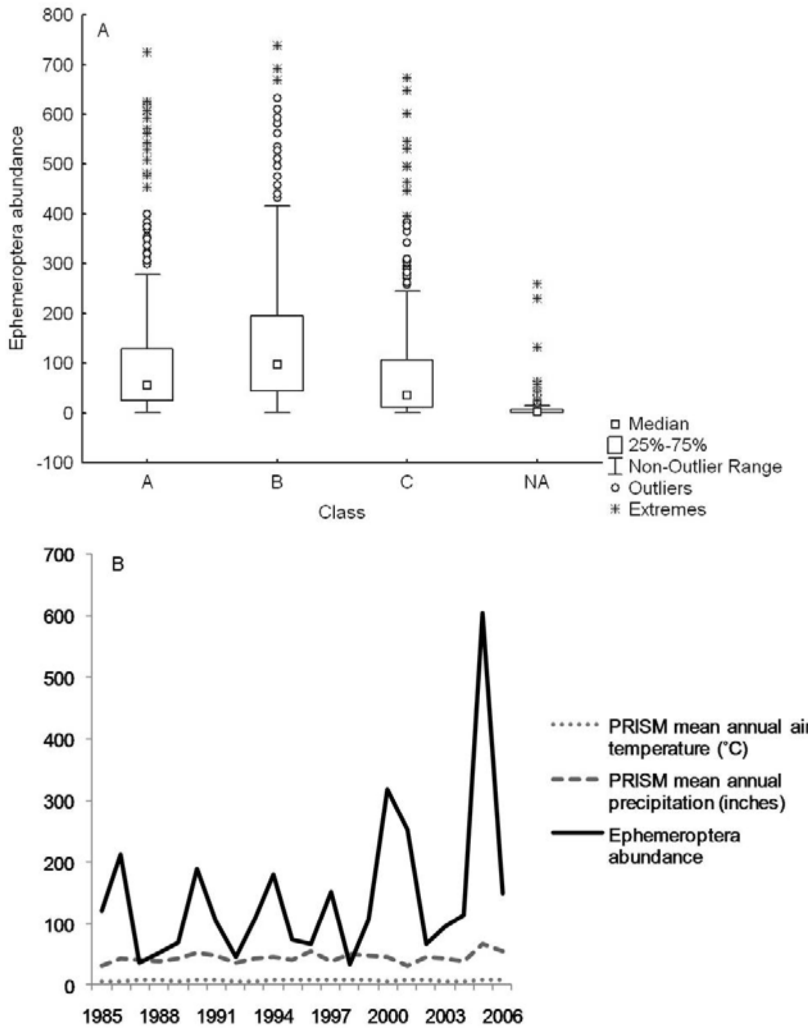


Figure 3-1. Ephemeroptera abundance in Maine. . Plot (A) shows distributions of Ephemeroptera abundance metric values across classifications (A, B, C, NA) and (B) shows trends in Ephemeroptera abundance and PRISM climatic variables over time at Maine site 56817 (Sheepscot). Data used in these analyses were limited to summer (July–September) rock-basket samples.

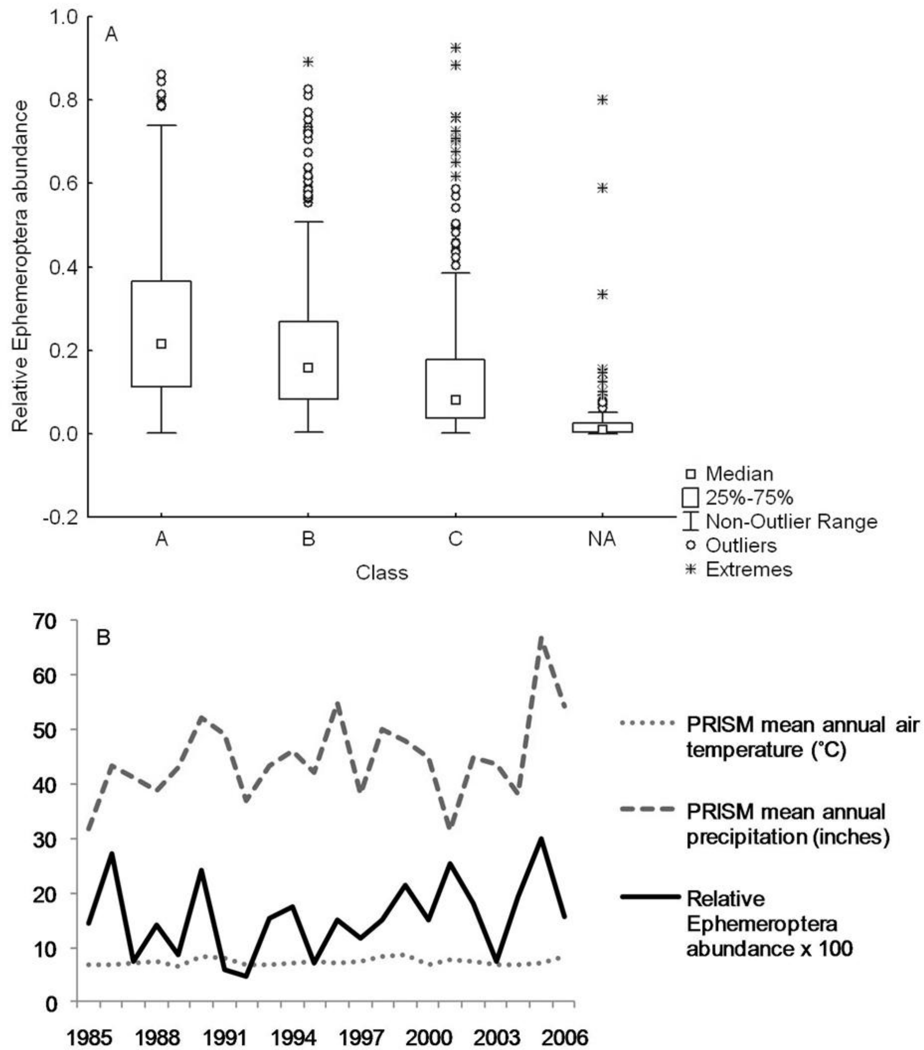


Figure 3-2. Relative Ephemeroptera abundance metric values in Maine.. Plot (A) shows distributions of relative Ephemeroptera abundance metric values across classifications (A, B, C, NA) and (B) shows trends in relative Ephemeroptera abundance and PRISM climatic variables over time at Maine site 56817 (Sheepscot). Data used in these analyses were limited to summer (July–September) rock-basket samples.

The Maine classification procedure also uses EPT taxa richness as a metric (as well as EPT richness divided by Diptera richness), which is also used in the MMIs of other states. At site 56817 (Sheepscot), EPT richness has increased over time, as has the number of warm-water EPT taxa (Figure 3-3a). When the first five years of data are compared to the last, results show that the number of EPT taxa has increased by approximately 6 taxa over time. This difference is much greater than the average difference in EPT taxa richness between Class A and B stations, which is approximately 2-3 taxa (Figure 3-3b). Based on this, station quality and ranking could improve due to warming temperatures that are projected to occur with climate change.

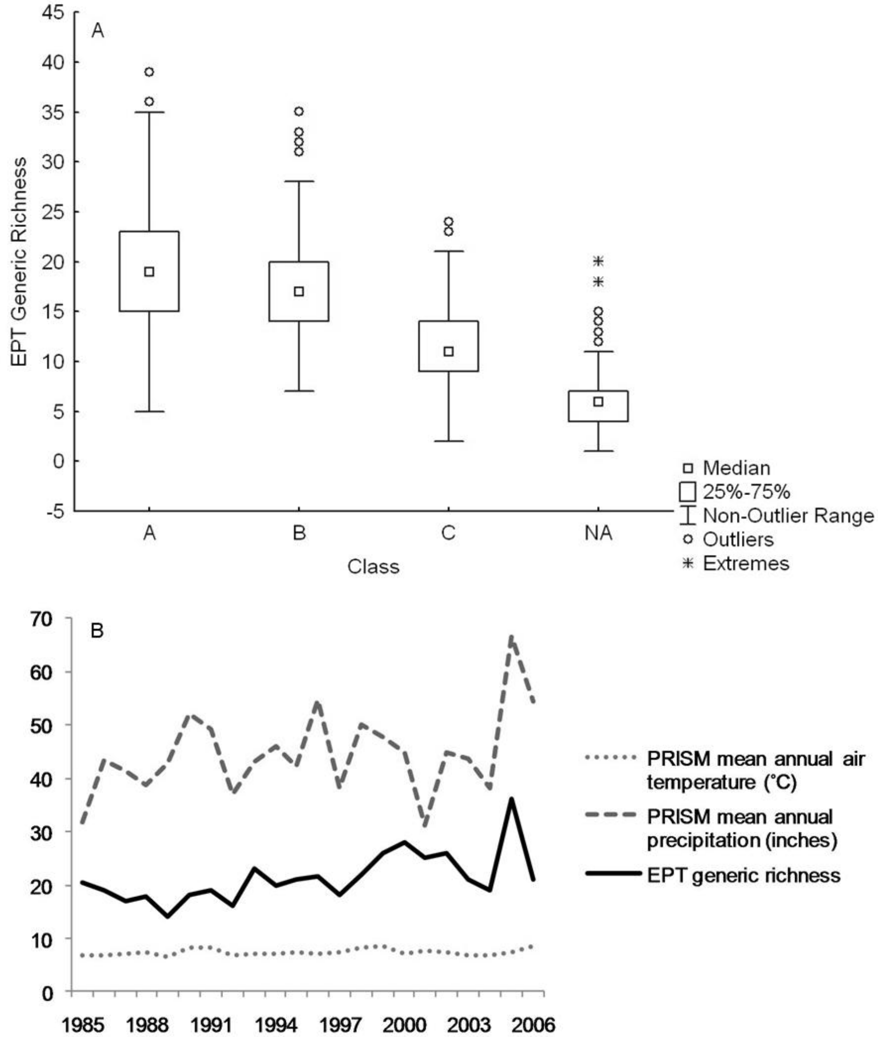


Figure 3-3. EPT generic richness metric values in Maine. Plot (A) shows distributions of EPT generic richness metric values across classifications (A, B, C, NA) and (B) shows trends in EPT generic richness and PRISM climatic variables over time at Maine site 56817 (Sheepscot). Data used in these analyses were limited to summer (July–September) rock-basket samples.

There are many more plecopteran (stonefly) taxa on the cold- than warm-water-preference list. Three of Maine's discriminant model input metrics involve plecopterans: Plecoptera abundance, Perlidae abundance and relative Plecoptera richness. For each metric, highest abundances or richness values occur at Class A sites, and ratings decrease as plecopteran abundance or richness values decrease. Although cold-water taxa like plecopterans should be sensitive to climate change effects, the Maine data did not reveal any trends. One explanation is that the longest-term reference station is a low elevation site where the benthic community is predominantly warm water tolerant. Also, PRISM mean annual air temperature trends at this site were small and variable over time, which suggests that water temperatures may not have exceeded the thermal tolerance limits of the cold-water-preference taxa at this site. For reference locations grouped in the Northeast Highlands, where cold-water taxa composed a much greater proportion of the community, the duration of data records was too limited to define significant trends.

Two other model input metrics are related to trichopterans: *Hydropsyche* abundance and *Cheumatopsyche* abundance. These trichopteran metrics are not currently viewed as particularly responsive to changes in temperature, because neither taxon is on the cold- or warm-water-preference lists. These taxa are likely to be more resilient to climate change effects. However, model metrics related to dipterans (true flies) may be vulnerable, as there are a large number of dipterans on both the cold- and warm-water-preference lists. Seven of the cold-water taxa are dipterans from the family Chironomidae (non-biting midges), and ten of the warm-water taxa are dipterans (Tables 3-1 and 3-2). Although several dipteran genera are cold-preference taxa, in the current Maine classification model a greater abundance or richness of dipterans tend to cause a station to receive a lower rating. As the cold- and warm-water-preference components of the dipterans are expected to respond differently to climate changes, the effects on outcomes of the Maine model are likely to be variable and somewhat unpredictable. Depending on whether there is any replacement of cold-water with warm-water dipteran taxa, increasing temperatures may not change the associated metric values much.

The HBI is also a component of the Maine discriminant model, and is also used in other northeastern states. Most of the Maine cold-water-preference taxa have low (≤ 3) HBI tolerance values (to organic pollution) (Figure 3-4). Exceptions include two chironomids, *Larsia* and *Natarsia*. There is a mix of tolerance values among the Maine warm-water taxa (Figure 3-4). This results in a significant but weak correlation between temperature optima values and pollution tolerance ($r=0.29$, $p=001$). The HBI metric is therefore also vulnerable to increases in water temperature; any responses that involve decreases in cold-water taxa with low HBI tolerance values or replacement by warm-water taxa with higher tolerance values could cause an increase in the HBI metric. Since higher HBI values impart a more impaired station rating, there would be a concomitant decrease in the station rating. Given the mixed relationship in Maine between warm-water-preference taxa and HBI tolerances, there should be regional (spatial) variability in HBI vulnerability, related to spatial differences in community composition of warm-water-preference taxa.

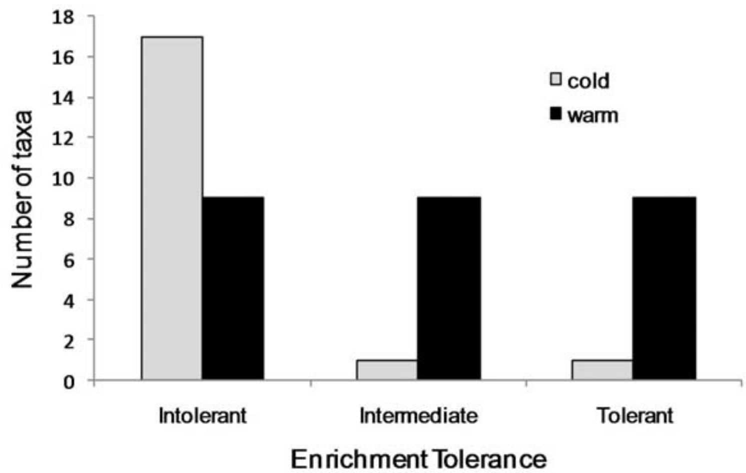


Figure 3-4. Relationship between Maine cold and warm-water-preference taxa and Maine enrichment tolerance scores. Taxa with enrichment tolerance scores of 0-3 were categorized as Intolerant, those with scores of 4-6 were Intermediate and those with scores of 7-10 were Tolerant.

Additional vulnerabilities of station quality classification are illustrated in aspects of the BCG (Gerritsen and Craig, 2008) as applied in New England (USEPA, 2007), even though the BCG is not a component of the Maine discriminant model classification scheme, or of the MMIs applied in other northeastern states. The BCG provides a more refined and explicit approach for defining and classifying condition, and includes five BCG attribute levels in New England: 2=highly sensitive taxa, 3=intermediate sensitive taxa, 4=taxa of intermediate tolerance, 5=tolerant taxa, 6=non-native or intentionally introduced taxa. Stations with communities composed of more sensitive taxa (2 or 3) generally receive better BCG-level assignments, while stations that have more tolerant taxa (5 or 6) are more likely to be classified in lower BCG levels. Twenty of the Maine cold-water-preference taxa are considered to be sensitive taxa (2 or 3), and two are considered to be tolerant (5) (Appendix E). Ten of the warm-water-preference taxa are considered to be tolerant (5 or 6) and 7 are considered to be sensitive (2 or 3) (Appendix E). If sensitive cold-water taxa are replaced by warm-water taxa that have higher BCG attribute assignments, then samples may receive lower tier assignments as temperatures increase. This can alter the assessment and rating of condition of a location based on biological composition over time.

Maine defines seven "Class A indicator taxa" to separate Class A and B sites. This study defines two of the seven taxa, *Eurylophella* and *Glossosoma*, as cold-water-preference taxa, and three, *Paragnetina*, *Serratella* and *Leucrocuta*, as warm-water-preference taxa (Table 3-3). *Brachycentrus* was initially classified as a warm-water-preference taxon. However, variation in temperature preferences among species within this genus cause this designation to be dropped, even though weighted average modeling (Stamp et al., 2010; USEPA, 2011) shows that *Brachycentrus* tends to occur more at warmer sites. The fairly even split between temperature preferences among Maine's Class A indicator taxa suggests that increasing temperatures may have contradictory effects on components of this metric, and lead to variable results.

Table 3-3. Temperature trait information for Class A Indicator taxa. Temperature optima (°C) and tolerance values are based on instantaneous water-temperature measurements and occurrences of organisms. The values were derived from weighted average modeling, using the guidelines of Yuan (2006). The rankings (Temp Rank_Opt = optima ranking; Temp Rank_Tol=tolerance ranking) range from 1 to 7 and are based on percentiles within each data set.

Class A Indicator Taxa	Temp Indicator	Temp Optima	Temp Tol	Temp Rank_Opt	Temp Rank_Tol	Comments
Eurylophella	cold	17.4	3.2	2	4	
Glossosoma	cold	18.7	4.8	3	7	
Psilotreta		18.8	3.0	3	4	Occurred at one of the warm water case study sites, otherwise would have been on the cold water list.
Paragnetina	warm	20.7	3.6	5	6	
Serratella	warm	20.8	3.8	5	6	
Leucrocuta	warm	21.2	3.3	6	5	
Brachycentrus		21.5	3.4	6	5	VT gave this a 'no' for warm-water-preference taxa due to variation among species within this genus

At Station 56817 (Sheepscot), the Class A indicator taxa metric was significantly correlated with several precipitation metrics and was higher in wet years compared to dry or normal years (see Section 2 and Appendix E), showing that potential future changes in precipitation may influence the Maine Class A Indicator metric at this and comparable locations. As shown with respect to temperature-driven responses, the projection for slight increases in precipitation in the northeast raises the possibility that Class A indicator taxa may fare better in the future and contribute to better station ratings due to climate change, independent of any actual change in environmental quality. Projected changes in precipitation are small, and seasonal patterns of precipitation (e.g., projected increases during winter and spring with possible decreases in summer) must be considered in concert with the season during which biomonitoring occurs. Therefore, the magnitude of vulnerability of this metric to changes in precipitation, and through this metric to station ratings, is probably small in the short term.

3.2. NORTH CAROLINA AND THE SOUTHEAST

North Carolina classifies sites as Excellent (5), Good (4), Good/Fair (3), Fair (2) or Poor (1) using EPT richness and the North Carolina Biotic Index (NCBI). Different scoring criteria are used for each major ecoregion (Mountain, Piedmont, Coastal). Details of each of these two indices and how they are combined for final scoring can be found in Appendix G.

Several analytical approaches contribute information to the potential vulnerabilities of the North Carolina MMI (the EPT richness metric and the NCBI) and bioclassification procedures. In one scenario we removed all cold-water-preference taxa from three reference Mountain sites (on average the Mountain sites have more cold-water taxa, see Appendix G). NCBI, EPT richness and bioclassification scores were recalculated to evaluate effects on site scores. In a second scenario we replaced taxa that typically inhabit Mountain sites with assemblages more typical of the Piedmont ecoregion. This was accomplished by applying Mountain scoring criteria to data from two Piedmont reference sites and evaluating by how much the scores changed (see Appendix C3 for site descriptions).

We also explored relationships between temperature preference taxa, pollution tolerance values, the biotic index, and climate-related variables. Applicable correlation analyses include:

1. temperature optima values vs. tolerance values;

2. temperature indicator metrics at selected Mountain and Piedmont reference sites (percent cold- and warm-water-preference individuals and number of cold- and warm-water-preference taxa) vs. BI scores; and
3. BI values vs. PRISM mean annual air temperature and precipitation.

The correlation analyses were performed on datasets that used genus-level tolerance values. Tolerance values can vary within some genera, and therefore these BI scores may vary somewhat from NCBI scores (though they are generally close).

We performed additional analyses relevant to other southeastern states that may not use EPT taxa richness or the NCBI. Metrics commonly used in other southeastern states include total taxa, EPT taxa, Ephemeroptera taxa, Plecoptera taxa, Trichoptera taxa, HBI, an assortment of functional feeding group and habit metrics, and percent dominant taxon. Other metrics evaluated include: metrics reflecting temperature preferences/ tolerances (developed using maximum likelihood modeling on the NC dataset); trait metrics that reflect sensitivity to changes in hydrologic regime; and metrics that incorporate combinations of traits that are most likely to be favorable or unfavorable if changes to climate occur as projected by NCAR models (warmer with a very slight increase in precipitation in North Carolina). Appendix G and Stamp et al. (2010) contain the full list of metrics evaluated, of cold- and warm-water-preference taxa, and calculated temperature-tolerance values.

3.2.1. Vulnerability of the North Carolina Bioclassification - Simulation of taxa replacement

Many predictions and observations of biological trends in response to climate change include shifts in ranges of sensitive taxa, often involving movements north and/or higher in elevation, such that northerly or higher elevation communities tend to become more similar to lower elevation or more southerly neighbors (e.g., Bonada et al., 2007b). Replacing higher elevation, more cold-sensitive North Carolina Mountain communities with lower elevation, more warm-tolerant Piedmont taxa, is a reasonable approximation of this type of biological response. This sets a boundary on the range of vulnerability of North Carolina’s bioclassification indices. The first scenario tests whether the Mountain biotic index as currently formulated will still accurately classify Mountain benthic communities that in the future may become increasingly like Piedmont benthos in composition. The results, however, show that at the most extreme (i.e.

at the point of complete community replacement) classifications would decrease by one level (Figure 3-5).

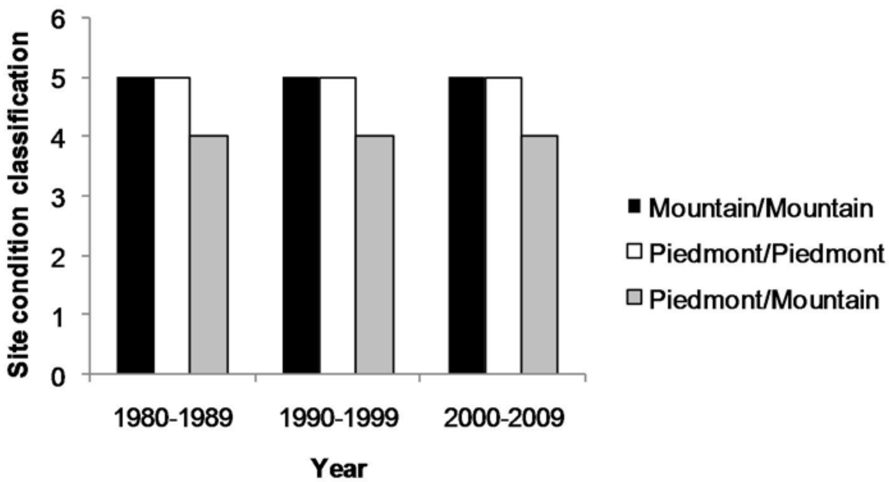


Figure 3-5. Site-condition classification scores at three reference Mountain sites (Station NC0109 (New), Station NC0207 (Nantahala) and Station NC0209 (Cataloochee)) and two reference Piedmont sites (Station NC0075 (Little River) and Station NC0248 (Barnes Creek)) averaged across three 10-year periods. The black bars represent average scores at Mountain sites when Mountain criteria are applied; the white bars represent average scores at Piedmont sites when Piedmont criteria are applied; the gray bars represent average scores at Piedmont sites when Mountain criteria are applied.

This second scenario is an upper bound of index vulnerability. It is unlikely that complete community replacement will occur, and certainly not in the near term. Current biological trends in the NC data are fairly weak and/or spatially inconsistent (see Section 2 and Appendix G). This, in part, reflects the relative paucity of long-term data adequate to define climate-related trends given high natural variability. Still, some sensitive taxa (e.g., EPT taxa) and trait groups (e.g., cold-sensitive taxa) do exhibit trends in North Carolina, especially in response to precipitation and in the Mountain region. Therefore, the nature of this vulnerability is valid, although the actual magnitude of vulnerability is probably modest, especially in the near term.

Table 3-4. Final bioclassification scores at 3 reference Mountain sites (NC0109 - New River, NC0209- Cataloochee and NC0207/2554 - Nantahala) before and after all cold-water-preference taxa are dropped from the sites

Site	Year	Before	After	Difference

		# cold-water-preference Taxa	Final Score	# cold-water-preference Taxa	Final Score	Final Scores
NC0109	1983	6	5	0	4	- 1
NC0109	1984	5	5	0	4	- 1
NC0109	1985	4	4	0	4	0
NC0109	1986	3	4	0	4	0
NC0109	1987	4	4	0	4	0
NC0109	1988	3	4	0	4	0
NC0109	1989	6	4	0	4	0
NC0109	1990	4	4	0	4	0
NC0109	1993	4	5	0	4	- 1
NC0109	1998	5	4	0	4	0
NC0109	2003	7	5	0	5	0
Site	**Year**	**Before**		**After**		**Difference**
		# cold-water-preference Taxa	**Final Score**	**# cold-water-preference Taxa**	**Final Score**	**Final Scores**
NC0209	1984	18	5	0	4	- 1
NC0209	1986	19	5	0	4	- 1
NC0209	1989	20	5	0	5	0
NC0209	1990	19	5	0	4	- 1
NC0209	1991	21	5	0	4	- 1
NC0209	1992	18	5	0	4	- 1
NC0209	1997	23	5	0	5	0
Site	**Year**	**Before**		**After**		**Difference**
		# cold-water-preference Taxa	**Final Score**	**# cold-water-preference Taxa**	**Final Score**	**Final Scores**
NC0207	1984	14	5	0	4	- 1
NC0207	1986	15	5	0	4	- 1
NC0207	1988	17	5	0	5	0
NC0207	1990	17	5	0	5	0
NC0207	1991	20	5	0	5	0
NC0207	1994	19	5	0	4	- 1
NC0207	1999	17	5	0	4	- 1
NC2554	2004	19	5	0	4	- 1

A similar response of the bioclassification results is observed if the cold-water-preference taxa are eliminated from the biotic assemblages at three references sites in the Mountain ecoregion. The maximum drop in station classification score is one bioclassification level (from Excellent to Good); this occurred for 3 of the 11 years at Site NC0109, 5 of the 7 years at Site NC0209, and 5 of the 8 years at Site NC0207/NC2554 (Table 3-4).

3.2.2. EPT Taxa Richness Metric

EPT richness is one of the two components of the North Carolina bioclassification scheme. EPT metrics also are used in other southeastern states. EPT metrics appear to be particularly vulnerable because they include many cold-water taxa. In North Carolina, 20 of the 31 cold-water-preference taxa (genus-level OTUs) are EPT taxa (Table 3-5). There are substantially fewer (5) EPT taxa on the warm-water-preference list (Table 3-6). Within the EPT genera on the cold-water-preference list, there are 53 species that could potentially be counted towards the EPT richness metric used in the bioclassification of sites in North Carolina, while only 5 species could be potentially counted from the warm-water-preference list.

Losses of cold-water-preference taxa and/or replacement by warmer water taxa in response to increasing temperatures may include loss of EPT taxa, potentially lowering bioclassification scores. At high quality sites, a loss of 3 (Coastal sites) or 4 (Mountain or Piedmont sites) EPT species would lower the EPT richness score by a full level, from a 5 (Excellent) to a 4 (Good) (see Appendix G). A greater loss, of 10 EPT taxa at Mountain sites, 8 taxa at Piedmont sites, or 7 at Coastal sites, would be needed to decrease scores by one level at sites of lesser condition (currently rated Good (4) or lower).

Table 3-5. Number of North Carolina cold-water-preference taxa in each order. EPT orders are *italicized*

Order	Total
Diptera	10
Plecoptera	*8*
Ephemeroptera	*6*
Trichoptera	*6*
Coleoptera	1
Odonata	1

The greatest effect of removing cold-water-preference EPT taxa from benthic assemblages was observed for three Mountain ecoregion reference stations, because cold-water-preference taxa comprise the greatest percentage of the benthic communities at higher elevations. In these cases, removal of cold-water taxa resulted in the loss of 9 to 14 EPT taxa, and decreases in EPT richness scores ranging from 0.4 to 1.2 (see Table 3-7 and Appendix G). The third Blue

Ridge reference site (NC0109) has fewer cold-water taxa in the assemblage, and the removal of cold-water taxa resulted in a loss of 4 species, and a decrease in EPT richness score of 0.6.

Table 3-6. Number of North Carolina warm-water-preference taxa in each order. EPT orders are *italicized*

Order	Total
Odonata	7
Diptera	5
Trichoptera	*4*
Coleoptera	2
Rhynchobdellida	2
Arhynchobdellida	1
Basommatophora	1
Decapoda	1
Ephemeroptera	*1*
Hemiptera	1
Isopoda	1
Unionoida	1

Table 3-7. EPT species richness values (EPT_S) and scores at 3 reference Mountain sites (NC0109 - New River, NC0209- Cataloochee and NC0207/2554 - Nantahala) before and after all cold-water-preference taxa are dropped from the sites

Site	Year	Before		After		Difference	
		EPT_S	EPT_S Score	EPT_S	EPT_S Score	EPT_S	EPT_S Score
NC0109	1983	50	5	47	5	- 3	0
NC0109	1984	45	5	42	4.6	- 3	-0.4
NC0109	1985	45	5	44	5	- 1	0
NC0109	1986	43	4.6	41	4.4	- 2	-0.2
NC0109	1987	41	4.4	38	4	- 3	-0.4
NC0109	1988	42	4.6	40	4.4	- 2	-0.2
NC0109	1989	43	4.6	39	4	- 4	-0.6
NC0109	1990	49	5	46	5	- 3	0
NC0109	1993	47	5	46	5	- 1	0
NC0109	1998	37	4	34	4	- 3	0
NC0109	2003	51	5	47	5	- 4	0

Site	Year	Before	After	Difference

		EPT_S	EPT_S Score	EPT_S	EPT_S Score	EPT_S	EPT_S Score
NC0209	1984	42	4.6	32	3.6	- 10	1
NC0209	1986	47	5	35	4	- 12	1
NC0209	1989	53	5	42	4.6	- 11	0.4
NC0209	1990	51	5	39	4	- 12	1
NC0209	1991	48	5	34	4	- 14	1
NC0209	1992	42	4.6	31	3.4	- 11	1.2
NC0209	1997	50	5	37	4	- 13	1

Site	Year	Before		After		Difference	
		EPT_S	EPT_S Score	EPT_S	EPT_S Score	EPT_S	EPT_S Score
NC0207	1984	45	5	36	4	- 9	1
NC0207	1986	48	5	40	4.4	- 8	0.6
NC0207	1988	49	5	38	4	- 11	1
NC0207	1990	53	5	43	4.6	- 10	0.4
NC0207	1991	54	5	41	4.4	- 13	0.6
NC0207	1994	48	5	36	4	- 12	1
NC0207	1999	49	5	39	4	- 10	1
NC2554	2004	49	5	37	4	- 12	1

3.2.3. The North Carolina Biotic Index (NCBI)

The second component of the North Carolina bioclassification scheme is the NCBI. This is North Carolina's version of the HBI, which is commonly used in site assessments in other states. The HBI documents the contribution of pollution tolerant taxa to the composition of the community (Hillsenhoff, 1987). Taxa are assigned pollution tolerance values ranging from 1 (most sensitive) to 10 (most tolerant). The higher the HBI, the more strongly the community is dominated by taxa tolerant of organic pollution, and the more impaired the site is considered.

Vulnerability of the NCBI (and HBI) is largely due to the high association of cold-water taxa with low tolerance to organic pollution (Figure 3-6). Taxa that show preferences for lower temperatures tend to have lower tolerance values and those that tend to occur more in warmer water habitats tend to have higher tolerance values. Most (22 of the 30) of the North Carolina cold-water-preference taxa for which the tolerance value is known have low tolerance values ($\leq$ 3) (Figure 3-6). Only one of the cold-water-preference taxa (the chironomid *Diamesa*) has a tolerance value $\geq$ 7. In contrast, most of the warm-water taxa have higher tolerance values.

Twelve of the warm-water-preference taxa that have been assigned tolerance values have tolerance values > 7. Only one of the warm-water taxa, *Chimarra*, has a tolerance value < 3. Based on this information alone, it is likely that a loss of cold water taxa and an increase in warmer water taxa would result in higher BI scores, which would contribute to lower bioclassification scores. An increase in BI scores of 0.1 can lower the classification of an Excellent site a full level from 5 to 4. At lower quality sites (those rated Good (4) or lower), it would take a greater increase in BI scores (by at least 0.6) to lower bioclassification levels a full level (i.e. go from a classification of 4 to 3, 3 to 2, or 2 to 1).

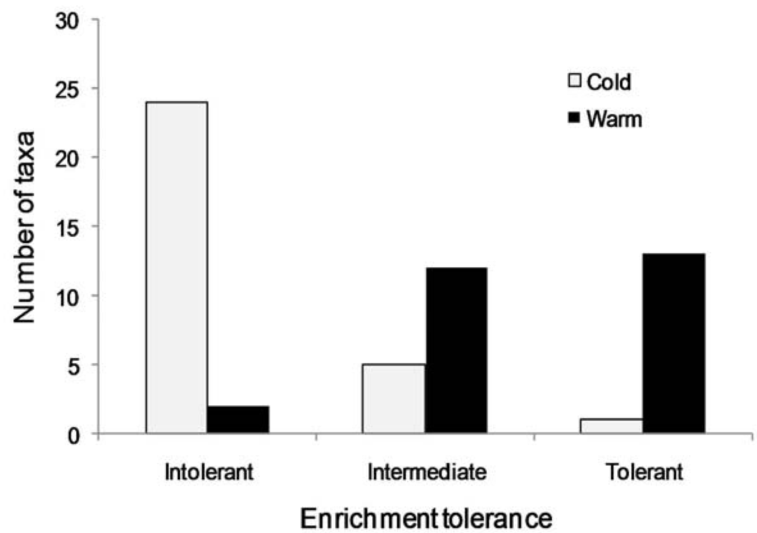


Figure 3-6. Relationship between North Carolina cold- and warm-water-preference taxa and North Carolina enrichment tolerance scores. Taxa with enrichment tolerance scores of 0-3 were categorized as Intolerant, those with scores of 4-6 were Intermediate and those with scores of 7-10 were Tolerant.

Again, three Mountain ecoregion reference sites with the greatest percentage composition of cold-water taxa were most vulnerable in terms of NCBI scores. In these cases, removal of cold-water taxa resulted in an increase in BI values ranging from 0.45 to 0.86 and decreases in NCBI scores ranging from 0 to 1 (Table 3-8 and Appendix G). At the Blue Ridge reference site (NC0109) with fewer cold-water-preference taxa, the loss of cold-preference taxa resulted in a maximum increase in NCBI value of 0.24, maximum decrease in NCBI score of 0.2.

Table 3-8. NCBI values and scores at 3 reference Mountain sites (NC0109 - New River, NC0209- Cataloochee and NC0207/2554 - Nantahala) before and after all cold-water-preference taxa are dropped from the sites

Site	Year	Before		After		Difference	
		BI	BI Score	BI	BI Score	BI	BI Score
NC0109	1983	4.60	4	4.67	4	+ 0.07	0
NC0109	1984	4.33	4	4.44	4	+ 0.11	0
NC0109	1985	5.48	3	5.51	3	+ 0.03	0
NC0109	1986	5.43	3	5.55	3	+ 0.12	0
NC0109	1987	4.87	3.6	4.93	3.4	+ 0.06	-0.2
NC0109	1988	5.37	3	5.46	3	+ 0.09	0
NC0109	1989	4.21	4	4.28	4	+ 0.07	0
NC0109	1990	4.87	3.6	4.91	3.4	+ 0.04	-0.2
NC0109	1993	4.70	4	4.74	4	+ 0.04	0
NC0109	1998	4.40	4	4.49	4	+ 0.09	0
NC0109	2003	3.61	5	3.85	5	+ 0.24	0

Site	Year	Before		After		Difference	
		BI	BI Score	BI	BI Score	BI	BI Score
NC0209	1984	3.32	5	3.90	5	+ 0.58	0
NC0209	1986	3.46	5	4.29	4	+ 0.83	-1
NC0209	1989	2.98	5	3.68	5	+ 0.70	0
NC0209	1990	3.12	5	3.88	5	+ 0.76	0
NC0209	1991	2.67	5	3.51	5	+ 0.84	0
NC0209	1992	3.00	5	3.86	5	+ 0.86	0
NC0209	1997	2.69	5	3.29	5	+ 0.60	0

Site	Year	Before		After		Difference	
		BI	BI Score	BI	BI Score	BI	BI Score
NC0207	1984	3.77	5	4.43	4	+ 0.66	-1
NC0207	1986	3.61	5	4.15	4	+ 0.54	-1
NC0207	1988	3.41	5	3.89	5	+ 0.48	0
NC0207	1990	3.00	5	3.47	5	+ 0.47	0
NC0207	1991	2.39	5	3.04	5	+ 0.65	0
NC0207	1994	2.60	5	3.13	5	+ 0.53	0
NC0207	1999	3.38	5	3.83	5	+ 0.45	0
NC2554	2004	3.19	5	3.79	5	+ 0.60	0

Further evidence of the potential vulnerability of the NCBI to climate change effects is that relative abundance of cold-water-preference taxa was significantly negatively correlated with NCBI values at all 3 Mountain sites (NC0109 r^2=0.46, p=.021; NC0207 r^2=0.66, p=.007;

NC0209 r^2=.85, p=.004) (Table 3-9 and Appendix G). The abundances of cold-water-preference taxa were lower at sites that had higher NCBI scores. At one of the Mountain sites, the warm-water metrics also were positively correlated with NCBI values. Replacement of colder water preference taxa with warmer preference taxa would likely contribute to a site receiving a higher NCBI score and therefore a poorer rating; this will most likely affect sites in the Mountain ecoregion.

Table 3-9. Correlations of benthic taxa grouped by temperature traits with BI at North Carolina Mountain and Piedmont reference stations. Significant correlations in bold text.

Temperature Metric	Mountain			Piedmont	
	NC0109	**NC0207**	**NC0209**	**NC0075**	**NC0248**
Cold-water-preference taxa – relative abundance	***-0.68*** ***N=11*** ***p=.021***	***-0.81*** ***N=9*** ***p=.007***	***-0.92*** ***N=7*** ***p=.004***	-0.25 N=7 p=.587	-0.11 N=7 p=.821
Warm-water-preference taxa – relative abundance	***0.66*** ***N=11*** ***p=.026***	0.12 N=9 p=.766	0.63 N=7 p=.127	-0.16 N=7 p=.726	0.19 N=7 p=.679
Cold-water-preference Taxa - richness	***-0.81*** ***N=11*** ***p=.003***	-0.46 N=9 p=.208	-0.57 N=7 p=.182	-0.37 N=7 p=.416	0.17 N=7 p=.708
Warm-water-preference Taxa - richness	***0.77*** ***N=11*** ***p=.006***	0.17 N=9 p=.664	0.65 N=7 p=.116	-0.01 N=7 p=.991	-0.51 N=7 p=.239

3.3. OHIO AND THE MIDWEST

Ohio also uses MMIs, the Index of Biotic Integrity (IBI) for fish communities, and the Invertebrate Community Index (ICI) for macroinvertebrates. Evaluations are separated by stream size categories and by level 3 ecoregions. Evaluations for Ohio were integrated with analyses of reference location re-sampling conducted to determine whether biological reference condition has changed since 1980 and as a foundation for the recalibration of Ohio biocriteria (Rankin 2008). The examination of trends in biological condition at reference sites and the exploration of potential causes was an essential component of this effort. Although climate change effects may be a contributing component to observed trends, there is evidence that other environmental changes may be responsible.

Based on approximately 30 years of watershed assessments in Ohio, there have been a variety of environmental changes identified that are associated with shifts in biological condition at assemblage and taxon levels (Rankin, 2008; Yoder et al., 2005). Environmental factors that have been identified as main contributors to these changes include reduction in point source loadings; changes in land uses (e.g., increased urbanization); altered pollutant loadings from agricultural lands (e.g., reductions in sediments and nutrients in response to increased conservation tillage); loss of habitat quality due to agricultural drainage practices and suburbanization; and localized improvement in habitat quality due to stream restoration. These environmental changes make it difficult to detect responses to climate-related changes in temperature and/or hydrology. The lack of readily available long-term data for temperature, flow and biology needed to define and separate such effects compounds the problem.

To examine long-term trends, the Ohio IBI and ICI were recalculated based on data from early and late sampling cycles, with an average of 14-16 years between data sets (Appendix H). This analysis corrected for any changes in taxonomic resolution over time. Values of the ICIs and IBIs for the most recent time period for each stream size and ecoregion category were almost always higher than or similar to the original values (i.e., the direction of change was either positive or neutral). This shows a strong pattern of environmental improvement reflected in the condition of biological communities. Although the overall pattern is compelling, none of the MMI differences are outside the range of natural variation for each index (Appendix H).

These effects, shown in changes over time in the Ohio MMIs, reflect, in some part, reductions in pollutant loadings and habitat degradation (Rankin, 2008). They could incorporate a climate change signal that is confounded or swamped by these apparent responses to improved environmental management. Given the probable confounding factors, two possibilities exist. One is that the actual improvement in environmental condition do to better management practices is greater than that reflected in the magnitude of MMI improvement. The implied suppression of the MMI response could result from climate change-related reductions in cold-water-preference taxa that are also pollution sensitive, and/or from increases in warm-water taxa that are pollution tolerant. This possibility would have the effect of reducing the MMI, leading to an underestimate of the magnitude of improvement. Other scenarios also are possible. For example, climate-related changes in precipitation and flow could have increased cold-sensitive taxa, as has been observed in other states (Section 2). Cold-sensitive taxa are often pollution sensitive (earlier sections of this Chapter); their increases would appear as an improvement in the MMI, leading to an over-estimate of improvements attributable to management practices. Some preliminary evidence of range extensions of flow-sensitive taxa into headwater streams in Ohio is presented in Appendix H. Thus it is possible that climate change has augmented, or contributed to the apparent improvements in environmental quality reflected in ICI and IBI improvements.

The direction of climate-related changes in BI scores could be positive or negative. The expectation for the more likely direction is, in part, informed by apparent relationships between temperature and/or hydrologic sensitivities of the Ohio fish and macroinvertebrate taxa and their associated pollution tolerance values. Figures 3-7 and 3-8 show a general concordance between these two taxon traits. With regard to macroinvertebrate temperature preferences, Figure 3-7 (upper and lower left graphs) shows that for both stream sizes plotted, many, though not all, of the pollution tolerant taxa (shown with red dots) have higher temperature preferences. The lowest temperature preferences are exhibited by taxa with “moderately intolerant” to “intolerant” (i.e., sensitive) pollution designations. These figures also show substantial variation. A few pollution-tolerant taxa exhibit relatively low temperature preferences, and there is a broad span of temperature preferences exhibited by taxa with moderate pollution sensitivity. A slightly clearer association is seen for hydrologic preferences and pollution tolerance, especially for fish (Figure 3-8). In this case, fish with the greatest pollution tolerance could tolerate lower flows, while the most pollution-sensitive fish had preference for higher flows.

In Ohio and other central states, climate change projections are for warmer temperatures and slight increases in precipitation (Appendix H). There is an associated expectation for increasing stream temperatures, although the expectation for changes in flow are more uncertain, being affected by both increasing precipitation, which may increase flows, and increasing temperatures, which can also increase evapotranspiration and contribute to decreasing flows at least seasonally. Community composition also will contribute to determining how climate change effects on component taxa will be reflected in the MMIs. For example, in the macroinvertebrate community, the balance in composition between cold- and warm-water-preference taxa will influence net response, as has been illustrated for other states. Results suggest that because of the general concordance between temperature and/or hydrologic sensitivity and pollution-tolerance sensitivity, it is plausible to expect the loss of sensitive taxa due to climate change (Appendix H). This may occur through replacements by or increases in occurrence and abundance of more tolerant taxa, with an associated apparent increase in the calculated pollution tolerance of the community. Decreasing ICI or IBI scores would make station conditions look more impaired, due only to climate change, or would mask detection of a portion of environmental improvement.

Because most, if not all, of Ohio's reference stations are considered "best available" (sensu Stoddard et al., 2006), conditions at reference locations in Ohio are changing, and mostly improving, in response to management and pollution control efforts. The detection of improvement is occurring in spite of potential climate change impacts. The ability to partition these responses is hampered by the lack of reference locations unaffected by pollution or land alterations. In addition, most stations are sampled on a regionally rotating basis, so that even over two or more decades of sampling, many locations have relatively few data points to support definition of trends. Without "natural" reference sites as an anchor, the definition of a gradient of site conditions using the BCG (Davies and Jackson, 2006) would provide a basis for selecting and sampling stations along a gradient of effects, which serve as an alternative approach for separating climate change from more conventional pollution responses.

Stressor identification and related processes contribute to elucidating causes of impairment through analysis of MMI and component responses. These same techniques are useful to identify sources of improvement in environmental condition. This does not by itself offer a mechanism for partitioning climate change responses from other causes, but would be valuable in tandem with gradient sampling along a BCG to support such an effort.

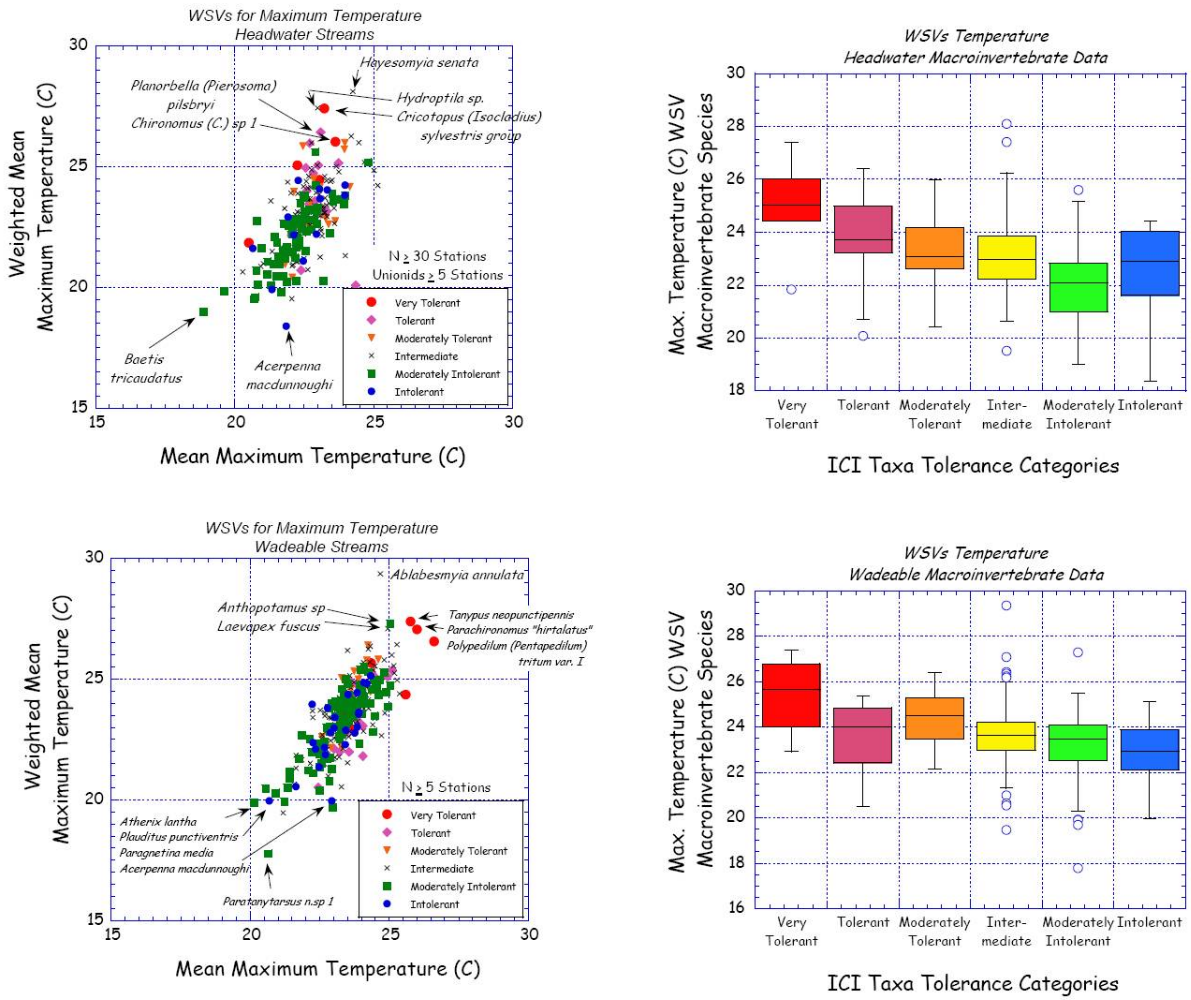


Figure 3-7. Plots of macroinvertebrate taxa maximum temperature Weighted Stressor Values (WSVs) vs mean maximum values for taxa for headwater streams (upper left) and wadeable streams (lower left), and box and whisker plots of maximum temperature by Ohio EPA macroinvertebrate tolerance values (derived for the ICI) for headwater streams (upper right) and wadeable streams (lower right). Data for taxa represent data from artificial substrates where at least 5 samples were represented for each stream size category.

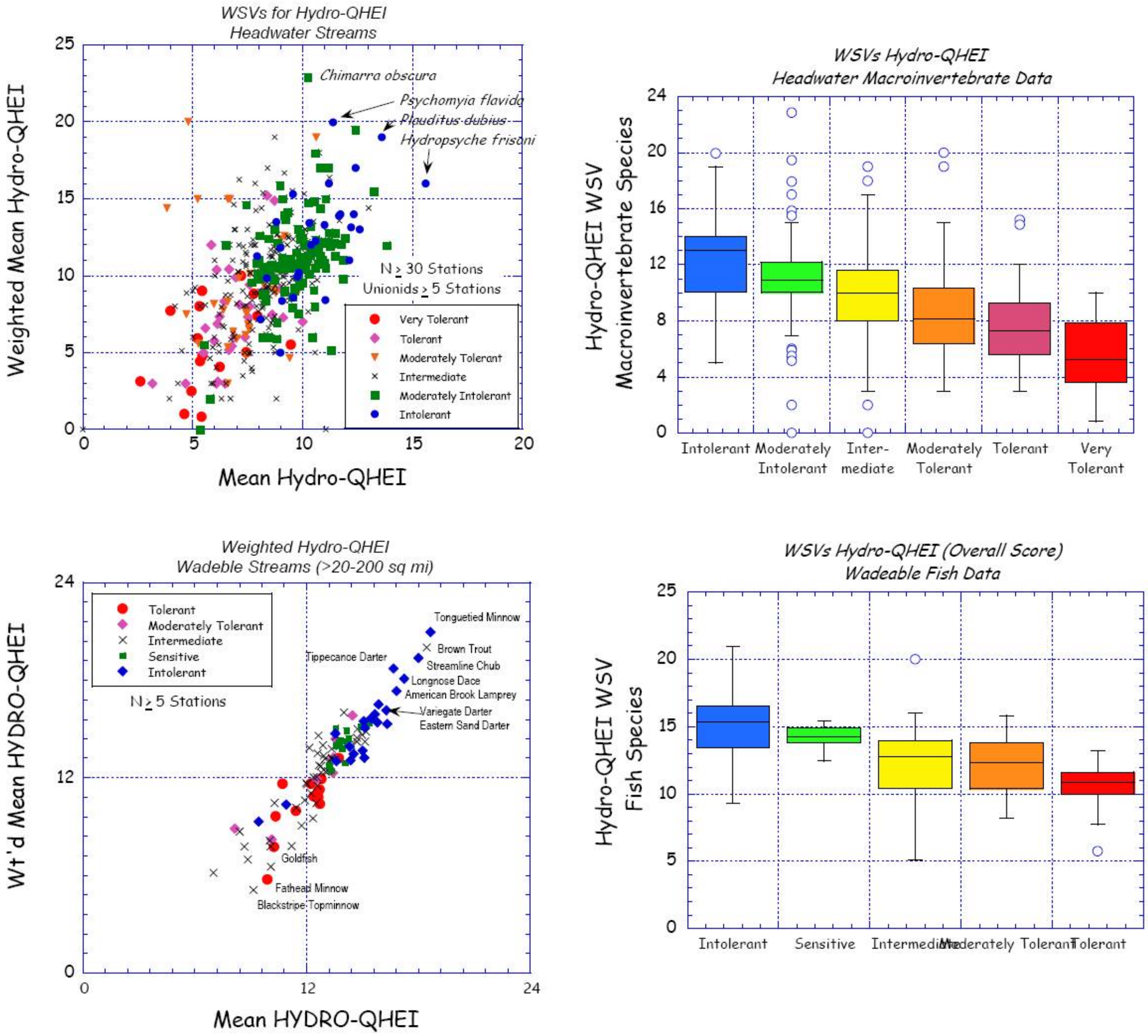


Figure 3-8. Scatter plots of taxa/species Hydro-QHEI (Qualitative Habitat Evaluation Index (QHEI) based only on hydrologic variables) WSVs vs mean Hydro-QHEI values for macroinvertebrate taxa for headwater streams (upper left) and wadeable streams (lower left), and box and whiskers plots of macroinvertebrates (upper right) and fish (lower right) WSVs for Hydro-QHEI for these waters. Data from Ohio EPA.

3.4. UTAH AND THE SOUTHWEST

Utah rates its sites with a RIVPACS model, in which data from reference sites are used to establish expected (E) macroinvertebrate assemblages and to which observed (O) assemblages at sites are compared (Appendix F). The ratio of these values (O/E) can be interpreted as a measure of taxonomic completeness. Values of O/E near 1 (one) suggest that the site is comparable to reference, whereas values that vary substantially from 1 suggest that the site is degraded (Yuan, 2006a).

3.4.1. Approach

Utah DEQ developed two different RIVPACS models: one for fall samples and the other for all seasons. They currently use the fall model for bioassessments, consistent with a focus on fall as their primary sampling period. The model has 15 predictor variables, 7 of which are related to climate (e.g., temperature, precipitation, freeze dates). Two fundamentally different approaches were used to evaluate possible vulnerabilities of RIVPACS assessments to climate change responses. One approach manipulates climate-related predictor variables within the model, within ranges informed by the magnitude of climate change projections for the southwest in temperature and precipitation. Half of the predictor variables included in the Utah fall RIVPACS model are climate related, showing that some climate factors expected to change in the future are important in controlling stream macroinvertebrate community composition across regions in Utah. Alteration of these variables at existing reference locations is intended to illustrate the range of model responses that might be expected over time due only to climate change, and thus be a measure of vulnerability of model-based decisions. A related analysis involved running the Utah fall RIVPACS model using only the climate-related predictor variables, assuming this would maximize their influence on definition of the expected community and thus, if possible, isolate components of the community most sensitive to climate variables. Details of the model runs are summarized in Appendix F.

The other analysis used extremes in existing data as proxies for future climate conditions, by partitioning data at long-term reference stations into years characterized by hottest (>75th percentile of the long-term temperature distribution), coldest (<25th percentile of temperature), and normal (25th to 75th percentile) average annual temperatures. Using similar thresholds, years were partitioned based on average annual precipitation into wettest, driest, and normal years (Appendix D). Examination of RIVPACS model responses between year groups was used as an indication of the direction and magnitude of responses in the RIVPACS O/E outcomes that might result from climate change. An assumption is that these temperature and precipitation differences drive responses in benthic communities that are reasonable proxies for the types of community changes that can be expected over the long term with climate change.

3.4.2. RIVPACS Responses – Utah Decision Vulnerabilities

Comparison of RIVPACS model outputs among hotter, colder and normal years provides evidence of potential vulnerabilities to climate change. Figure 3-9 shows results from two reference sites in the Colorado Plateaus ecoregion (site 4951200 (Virgin) and site 4936750 (Duchesne)) where mean O/E values from hottest year samples were significantly higher than mean O/E scores from coldest and normal year samples. These differences are in the range of differences relevant to the Utah DEQ decision matrix for determining whether a test location should be characterized as not supporting beneficial uses (i.e., classified as impaired). In this matrix, an O/E score <0.74 represents the first threshold of impairment, with another at O/E <0.54 (Appendix Attachment F6). The magnitude of average annual temperature differences between the "hottest" and "coldest" year samples is about 2 ^{o}C, comparable to long-term climate change projections for temperature increases in the Utah region by about 2050[11]. Therefore, this result is directly relevant to impairment decisions that Utah may make in the future, because it will introduce a range of variation among reference locations similar to the impairment decision threshold, and thus may be more difficult to determine impairment as temperatures increase. One peculiarity is that the median O/E scores at sites 4951200 (Virgin) and 4936750 (Duchesne)) are significantly higher (closer to 1) in hottest year samples. This means that the observed community in hottest years is closer to the expected community. A similar pattern occurred at site 4929750 (Weber) but this result was not statistically significant. These patterns might be partially explained by the fact that Utah DEQ calibrated their RIVPACS model based on data collected from 1999-2005, which happens to be a period during which some of the hottest and driest conditions occurred, in some cases in consecutive years (Appendix Section F5). Although it is possible that other confounding factors might have also contributed to O/E trends at these sites, results suggest that climatic variables likely influenced these changes in community composition.

[11] See NCAR website: http://rcpm.ucar.edu

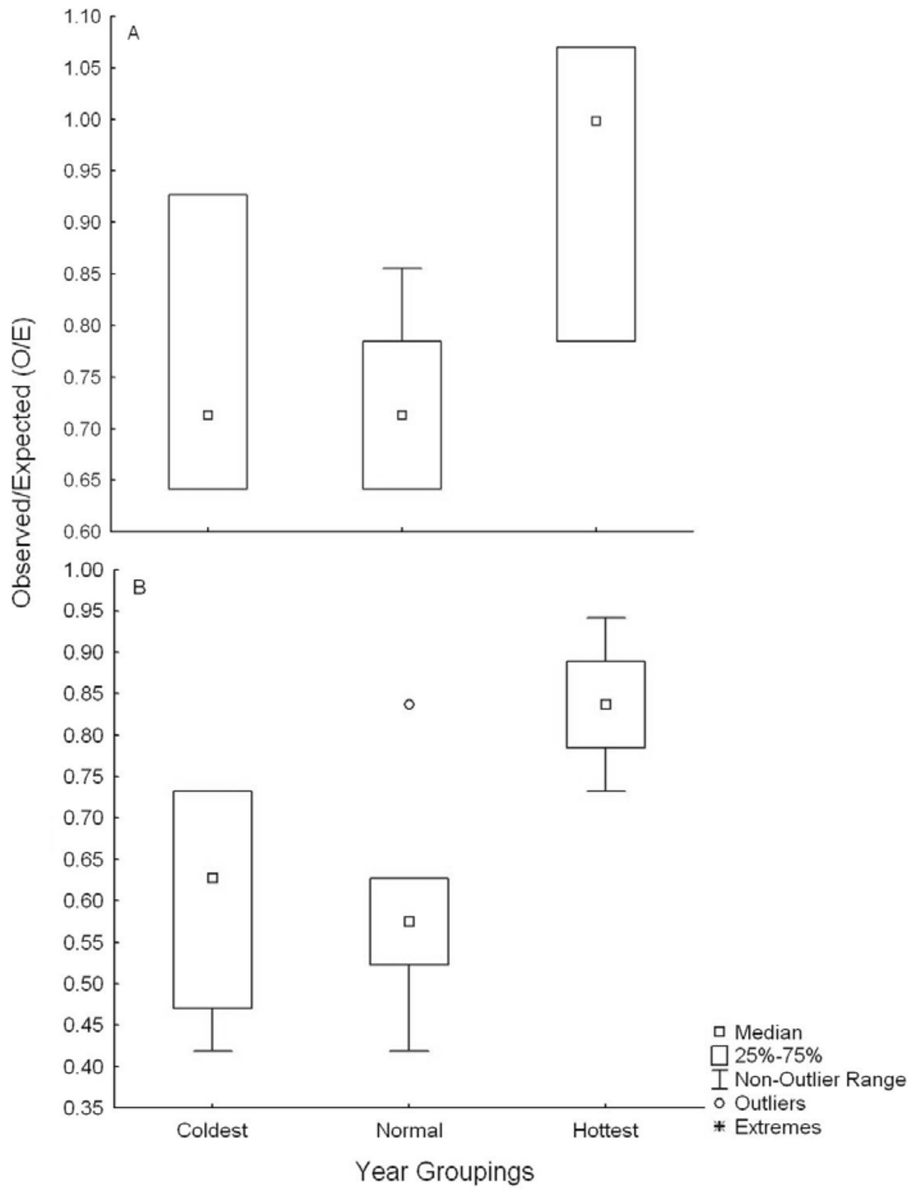


Figure 3-9. Distributions of observed/expected (O/E) values in coldest-, normal-, and hottest-year samples at Utah sites 4936750 (Duchesne) (A) and 4951200 (Virgin) (B). Year groupings are based on Parameter-elevation Regressions on Independent Slopes Model (PRISM) mean annual air temperatures from each site during time periods for which biological data were available. Average temperatures in hottest-year samples were 1.1 to 2.7˚C higher than coldest year samples. At both sites, O/E values were significantly higher in hottest year samples than in coldest and normal year samples. Data used in these analyses were limited to autumn (September–November) kick-method samples.

In addition to the O/E trend analysis, we also performed several exploratory analyses in which we manipulated the climate-related predictor variables that are used in the Utah fall

RIVPACS models. When climate-related predictor variables were altered, there was very little effect on O/E values (Figure 3-10). This occurred in both the original model (with probability of capture (Pc) set at 0.5, as used by Utah DEQ) and the model re-run with Pc <0.1 which allowed for inclusion of rare taxa. The greatest change in O/E occurred in the scenario in which all climate-related predictor variables were altered by the greatest amounts (‘scenario 5 in Figure 3-11) (for more information on model manipulations, see Appendix F). This amounted to a change in O/E of 0.03, which is within the range of natural variability (Appendix F). There also was little effect on O/E values when ‘unrealistic’ changes were made to climate-related variables to investigate possible thresholds (i.e. doubling temperature, halving precipitation variables), with O/E values never varying by more than one standard deviation (Appendix E).

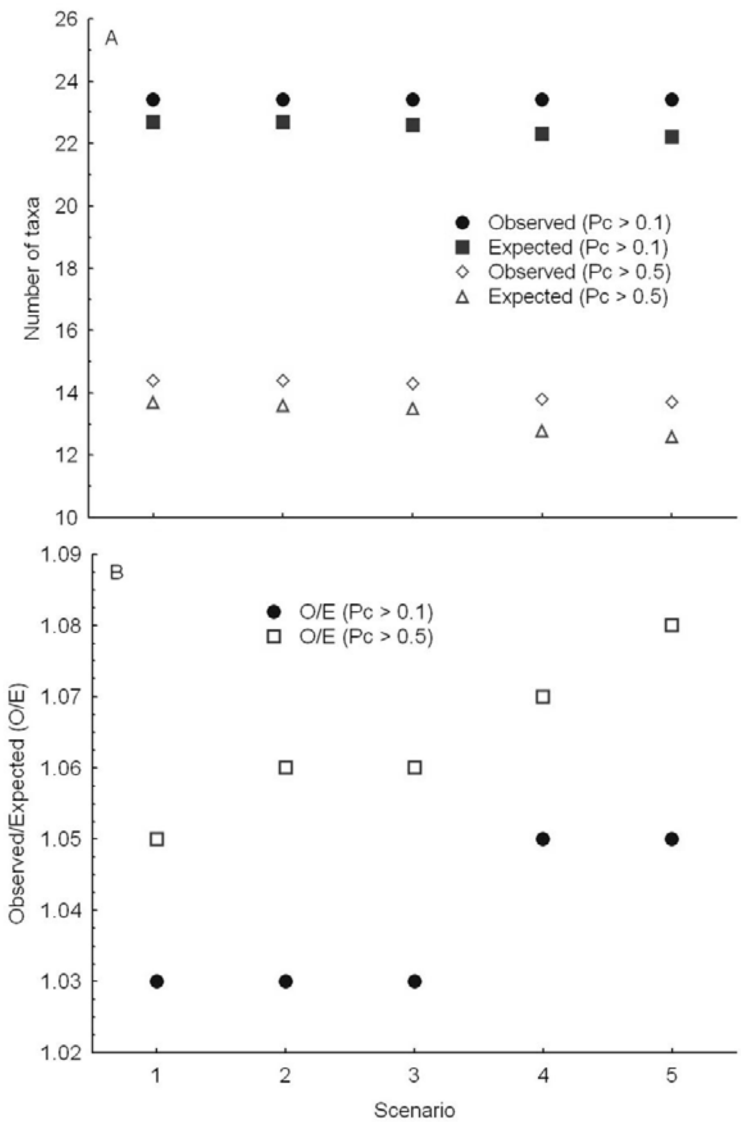


Figure 3-10. Exploration of how observed (O), expected (E) and observed/expected (O/E) values from the Utah Fall RIVPACS model may change as climate-related predictor variables change. Plot (A) shows changes in O and E and (B) shows changes in O/E at two different probabilities of capture (Pc) (0.1 and 0.5) under 5 different scenarios: 1=baseline; 2=temperature predictor variable values + 2, precipitation predictor variable values – 0.05; 3=temperature predictor variable values + 4, precipitation predictor variable values – 0.1; 4= temperature predictor variable values + 1, precipitation predictor variable values – 1, day of last freeze - 1, day of first freeze + 1; 5= temperature predictor variable values + 2, precipitation predictor variable values – 2, day of last freeze - 2, day of first freeze + 2.

There are a number of possible reasons why the alterations to the climate-related predictor variables resulted in small changes to O/E values. One is the fact that the analyses were based on reference site data. Reference sites are typically more stable than test sites. Another potentially important factor was that we disregarded elevation in the model manipulations. It would be worthwhile to explore how manipulations to the elevation-related predictor variables affect O/E values, especially since elevation and temperature are linked. Other potential factors relate to model development. The fall Utah RIVPACS model is comprised of 15 predictor variables. Recent analyses suggest that models with fewer predictor variables may have better performance (pers. Comm. Chuck Hawkins). Also, the Utah model is unique in that it uses a Random Forest model (Breiman and Cutler 2009) instead of discriminant analysis to predict site group membership. It is possible that using the random forest may make the RIVPACS model more robust to CC effects.

These results illustrate several important points in considering how RIVPACS models may be affected by future climate change effects. One is the importance of the calibration data used in model development. Ideally models are calibrated using data that encompass a full range of natural variability. Unfortunately, these types of long-term data sets are rare. However, it is something to consider and strive for as biomonitoring programs gather more data and recalibrate their models over time. Another important consideration has to do with the assumption that climate-related predictor variables, which are typically based on long-term (30-year) averages, are relatively invariant over ecologically-relevant time. If climate change is going to be an important factor in years to come, it would be interesting to develop a second RIVPACS model that includes predictor variables based on current climate (not just the historic benchmark climate) and to compare O/E values across these models over time. In theory, this would allow for partitioning of climate change effects over time.

3.5 CONCLUSIONS ACROSS PILOT STUDY STATES

There are a variety of regional differences in biological responses evident from this study. More and stronger trends and responses were found in Utah, largely related to temperature changes. Fewer significant trends were found in North Carolina and more were related to precipitation (see also Section 2). There is much spatial variation in these patterns, in part due to ecoregional, geographic, and climatological variations, and in part attributable to limitations of the available data. The results point to several conclusions. One is the importance of categorizing taxa based on ecological traits, especially temperature sensitivities, in order to evaluate responses to climate change variables and to estimate future vulnerabilities to climate change. It is a relatively consistent finding that biological metrics and indices used by states and tribes are either composites of cold-water and warm-water-preference taxa, or are dominated by one or the other. This composition defines the nature of responses, and therefore, the vulnerability of the metric or index to climate change effects. The richness of cold-water-preference taxa is a metric that was fairly consistently responsive, especially at higher elevations, because high-elevation communities tend to have more cold-water-preference taxa. Metrics using cold-water-preference taxa will help identify climate change 'sensitive' or vulnerable areas. Such information would assist in detecting climate change effects and in identifying sites to monitor these changes.

Another widespread and related finding is the moderate but significant relationship between temperature sensitivity and sensitivity to organic pollution. Metrics selected because the composite taxa were considered to be generally sensitive, such as EPT taxa, or generally tolerant, such as Diptera taxa, or to represent responses to conventional pollutants (e.g., organic pollution as in the HBI), also have demonstrable sensitivities to climate-related changes in temperature and flow conditions. We have shown these sensitivities to be related, at least in part, to the predominance of cold- (and/or warm-) water preference taxa at a location. Assemblage composition by cold and warm-water-preference taxa may be related to ecoregion, latitude, watershed size, and/or stream order, and is also clearly affected by elevation. This association between temperature and pollution sensitivities will affect how indices are interpreted with regard to the conventional stressors for which the indices were originally developed.

From more limited evidence it also appears that the ability to categorize taxa according to flow preferences and requirements could be useful. However, there are generally fewer data available for this analysis. We augmented the approach of grouping taxa by traits responsive to one climate variable (temperature) through consideration of a suite of traits. This was useful in some cases, though it produced fewer significant results. This was probably due to the fact that fewer taxa were included when categorized by a suite of several traits, resulting in more limited and/or more variable data and smaller sample sizes with which to test responses. Still, this is potentially a useful approach to apply as more data become available.

3.6 RECOMMENDATIONS FOR MODIFYING METRICS

In general, biological metrics (indicators) are selected for their diagnostic value (Verdonschot and Moog 2006). However, the effects of global climate changes in temperature and precipitation on biological metrics have, until now, been largely untested, because climate change was not considered a "stressor of concern" until recently (Hamilton et al., 2010a). Given our demonstrations of the vulnerabilities of traditional metrics to climate change, and associated impacts to the classification of station conditions, it is important that state and tribal biomonitoring programs consider adopting modified metrics with the purpose of tracking climate-associated changes in MMI outputs (Hamilton et al. 2010a). This will support making inferences about cause, helping differentiate climate change from other stressors as part of a weight of evidence evaluation. It will allow resource managers to more effectively make management and regulatory decisions on the basis of biomonitoring results in the face of climate change impacts (Hamilton et al. 2010b).

Our initial focus here is on the relative contribution of cold- and warm-water-preference ecological trait groups to the composition of traditional metrics. Our general recommendation is that the cold- and warm-preference components of traditional metrics be documented and tracked separately. A recommended approach for incorporating modified metrics into a biomonitoring data analysis regime is to continue calculating the traditional metric (e.g., EPT richness, HBI), while adding new cold- and warm-water-preference metrics. Proportional changes in cold- and warm-water-preference taxa would provide a basis for estimating how much of the difference in the total (traditional) metric can be accounted for by changes in temperature trait groups. This then becomes evidence for comparing potential climate change effects to those of other stressors in a weight of evidence assessment. Comparisons could be made over time, among locations, and/or groups of sites (both reference and non-reference). An option for tracking climate-related changes is to put traditional and modified metrics on the same plot and compare their trends over time (i.e. Figure 3-13). Another option that requires further testing is to track the ratio of the cold- or warm-modified metric to the original (total) metric. For example, separate tracking of cold-to-total EPT and warm-to-total EPT richness metrics was able to account for trends in total EPT richness over time in circumstances where changes in total EPT richness were caused by losses of cold-water-preference taxa, and where changes include both losses of cold-water-preference taxa plus gains of warm-water-preference taxa (i.e., taxon replacements) (Hamilton et al. 2010a).

We examined evidence in this study for the value of adopting temperature-modified metrics for diversity and total taxa richness metrics; for EPT-related metrics; and for pollution tolerance metrics, such as the HBI or related indices. However, the principle of partitioning metrics to separate component taxa based on cold or warm-water-preferences should be considered for other biological metrics (Hamilton et al. 2010a). These could include trait metrics related to functional feeding groups (e.g., predators, collector-filterers) or life history habits (e.g., swimmers, climbers). Such metric modification should be considered on a state or region-specific basis, in particular for climate-vulnerable regions (e.g., high elevations, low order streams, small watersheds). In addition, an OCH taxa metric may be valuable to track taxa that are robust to warmer conditions and/or more intermittent flows. This may be especially valuable in regions at lower elevations, where temperature increases may be large, and/or where summer flow conditions are likely to be especially vulnerable to climate change effects.

We cannot yet make strong suggestions for metrics related to hydrologic sensitivity, in part because the lack of flow data corresponding to biological collections has limited ability to calculate flow metric preferences by taxon (see Appendix K). However, hydrology-related trait characterizations can be based on known life history traits coupled with regional observations and literature information, as with the intermittent taxa metric used in North Carolina. A metric that accounts for tolerance to intermittent flows, requirement for perennial flows, or some similar hydrologic-preference metric, may become valuable as changes in flow conditions are more evident. Such a metric would have to be calibrated by region.

Calculation of modified metrics for incorporation into biomonitoring data evaluation will require designation of cold-and warm-water-preference ecological trait groups. Cold-and warm-water-preference taxa lists must be developed on a state- or region-specific basis, which is a substantial undertaking. The efforts initiated in this study, including the process of applying weighted average or maximum likelihood modeling in concert with literature information and best professional judgment to estimate temperature preferences by taxon from biomonitoring data, and the development of a traits database that documents the temperature preferences and tolerance results calculated for the 3 states analyzed in this study (see Stamp et al., 2010; USEPA, 2011) can be used as a starting point for future state efforts.

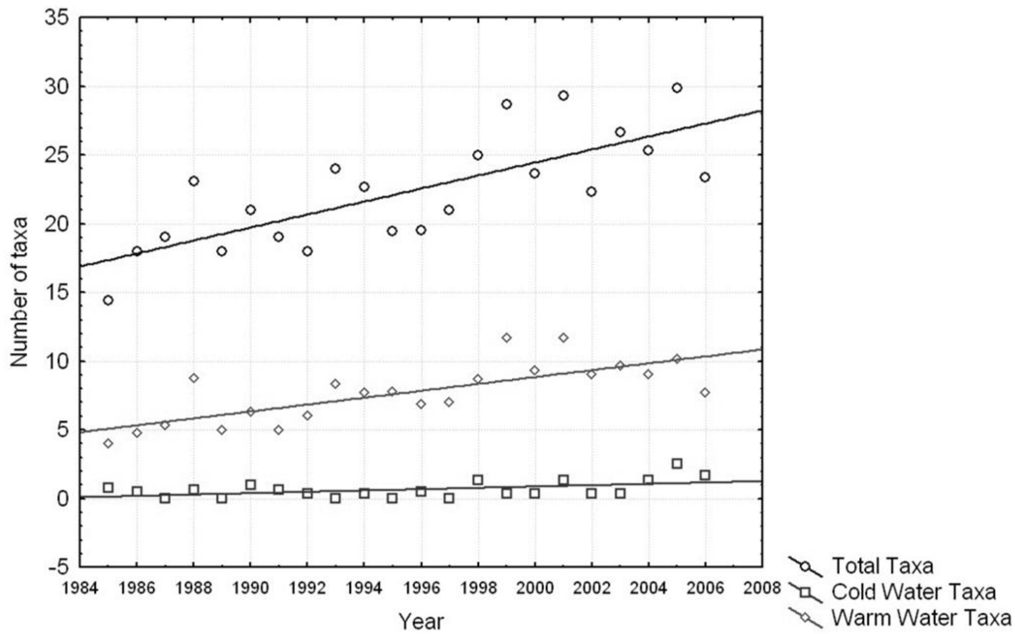


Figure 3-13. Method for tracking changes in cold- and warm-water-preference taxa and commonly used metrics (in this case, total number of taxa at Maine site 56817 (Sheepscot) over time.

4. REFERENCE STATION VULNERABILITIES

While partitioning indices and revising predictive models are important steps in assuring that the bioassessment design will continue to meet program needs under changing climatic conditions, several other program elements also need to be considered. The use of reference stations and comparison to reference conditions are central to bioassessment. Therefore, this study also examined potential vulnerabilities of the sampling design, the process to determine reference condition, and the location of reference sites.

4.1. VULNERABILITIES IN THE REFERENCE STATION SAMPLING DESIGN

State and tribal bioassessment programs establish reference stations across their jurisdictions for reference-based comparisons to assess condition, detect impairment, and identify causes. The main objectives of these programs focus on spatial comparisons, and program design elements reflect this. Assessment designs generally include random sampling within a stream reach or watershed, or a combination of random plus some targeted sampling. Random sampling tends to maximize spatial sources of variation. Rotating basin sampling designs are often used, which typically include sampling once every 5 years. Collections are usually of one sample per location per year, with measurements of few covariates.

In contrast to the original spatial objectives of biomonitoring designs, detection of climate change requires evaluation of trends over time, whether at a specific location or for a defined area or stratum. There are some commonly observed limitations of many existing biomonitoring programs with regard to assessment of trends. Despite the relatively large number of reference stations in the biomonitoring data sets analyzed, there are very few with long-term data, and typically few if any replication of long-term reference sites within a particular region (Table 4-1). In addition, samples are not collected from the same sites every year (Table 4-1), so many data sets have discontinuities, which make analyzing and detecting trends difficult. This limits the adequacy of many biomonitoring programs for detecting climate change effects. Continued accounting for climate change effects is desirable within the framework of state and tribal biomonitoring programs, to increase the robustness of their program assessments to the confounding effects of climate change. Modifying existing sampling design, potentially including establishing a sentinel monitoring network specifically to detect climate change effects would contribute to this objective.

Table 4-1. Time periods for which biological data were available at the long-term monitoring sites in Utah (UT), Maine (ME), and North Carolina (NC). Data used in these analyses were limited to autumn (September–November) kick-method samples in the Utah data set, summer (July–September) rock-basket samples in the Maine data set, and summer (July–August) standard qualitative samples in the North Carolina data set.

Station ID	Water body	Number of years of data analyzed	Years
UT 4927250	Weber	17	1985–1995, 1998, 2000, 2001, 2003–2005
UT 4951200	Virgin	14	1985–1993, 1996, 2000–2002, 2004
UT 4936750	Duchesne	12	1985–1993, 1995, 2000, 2001
UT 5940440	Beaver	9	1996–1998, 2000–2005
ME 56817	Sheepscot	22	1985–2006
ME 57011	W. Br. Sheepscot	12	1995–2006
ME 57065	Duck	9	1997–2005
NC 0109	New	11	1983–1990, 1993, 1998, 2003

4.2. VULNERABILITIES IN ASSESSING REFERENCE CONDITION

Reference station comparisons are central to bioassessment. Both in the United States (Clean Water Act) and in Europe (Water Framework Directive) the determination of ecological status and integrity is based on a comparative approach ("reference based comparisons") requiring reference locations that can be used to set expectations for "natural" conditions and associated variability (Barbour and Gerritsen, 2006; Stoddard et al., 2006; Verdonschot, 2006; Nijboer et al., 2004; Walin et al. 2003). Impairment in the regulatory context is representative of an unacceptable level of departure from this "expected condition" defined based on selected reference stations. The vulnerabilities of reference locations to climate change, as well as to ongoing changes in land use, is a significant issue that will impact the continued viability of bioassessment approaches as currently applied.

Under ideal circumstances, reference conditions are found in locations unaffected by human influences and thus represent natural, undisturbed conditions. At such sites, only natural determinants of environmental conditions should influence biological communities. In the absence of other stressors, long-term patterns in climate-related variables and associated biological responses could be attributed to climate change (with the caveat that multi-decadal climatic cycles also influence these communities). But "pristine" reference sites are seldom available. It is more often the case that reference stations represent "best available" or "minimally disturbed" conditions (Stoddard et al., 2006; Baily et al., 2004). Human influences of agriculture and development are both widespread and long term in their effects (Allan, 2004; Paul and Meyers, 2001), and many states have determined that they have no pristine or unaffected reference conditions existing (see, for instance, Snook et al., 2007; Appendix H). There also is variation among states in how reference stations are defined and selected. Some states apply land use criteria, or at least document land use conditions, for selection of reference stations. However, in many cases the selection of reference conditions are determined *post facto* using biological sampling results, or are based on best professional judgment. In these cases, the distribution of urban and agricultural land uses, or other factors affecting condition, can be less than ideal, and often are not documented.

4.2.1. Reference Stations Used in this Study

We focused analyses in this study on reference stations to minimize the potential influence of confounding factors. Given the variability in approaches for reference station selection, and in the information documented within each bioassessment data base, a set of criteria was established for selecting appropriate reference locations for analyses in this study. Land use distribution was the primary consideration, based on the assumption that there is a reasonable correspondence between extent and intensity of urban and agricultural land uses surrounding a station and the level of non-point as well as point source influences on the stream (e.g., Paul and Meyer, 2001; Arnold and Gibbons, 1996). Other factors considered in selecting reference stations for analysis were the length of the data set, the presence of dams upstream of the station, the occurrence of sewage treatment plant discharges, and consistent application of appropriate sampling methods (see Appendix C).

Land use composition among major categories such as urban, agricultural, forest, wetland, and barren, were obtained for a defined buffer area (1-km radius) around each sampling site using a Geographic Information System (GIS) (see Appendix C). Stations for all states were initially screened at 1%, 2%, and 5% urban, and 5% and 10% agricultural land use levels. Final levels applied were 5% urban/10% agricultural in Maine and North Carolina, and 2% urban/10% agricultural in Utah. These values were selected in part based on practical considerations, specifically the need to not eliminate all stations with data that could be used for long-term analyses. These criteria are more conservative than those used in several southeastern states. Georgia, Alabama, and South Carolina apply land use criteria for selecting reference stations of <15% urban/<20% agricultural for high gradient streams, and <15% urban/<30% agricultural in low gradient streams (Barbour and Gerritsen, 2006). It will take additional analysis to determine on a more objective level whether these criteria are adequate to minimize confounding of climate change effects.

It is reasonable and sometimes necessary to use less than "natural" conditions as a baseline for spatial comparisons. For example, accessibility of a site for frequent (e.g., annual) long-term sampling can be an important practical consideration. For example, the longest term reference station in Maine, 56817 (Sheepscot) is generally (though not always) categorized as an "A" station by MDEQ, but is surrounded by about 16% urban and 23% agricultural land uses (see Appendix C for characteristics of reference stations used in analyses for each state). Though higher than would be considered ideal for "unconfounded" analyses, the level of urban land use was stable over time (at about 16%), although forested conditions decreased from 84% to 57%, while the agricultural land use increased 0% to 23%. At Maine's Station 57065, there was an increase from 0% to 16% urban land use, but a decrease from 4% to 0% agricultural land use. At Maine's Station 57011, urban land use increased from about 4% to 9%, and agricultural use from 0% to 18.5% with the changes coming from both forested and wetland uses. It is possible that such land use changes may have contributed to trends observed at these sites (Appendices C and E). It is recommended for all sampling stations, but especially for reference stations, that quantification of land-use categories be documented. This will support tracking changes in land uses over time (although land-use data are often only available at infrequent intervals), which will aid in separating this from degradation due to climate change effects (and other stressors).

To supplement the spatial coverage of trend analyses from the limited number of individual long-term reference stations available in each state, we grouped other reference stations to form long-term data sets that could support climate change analyses. The intent was to subset regions based on major factors considered important in driving natural, and therefore predictable, variation in aquatic habitat characteristics. Typical factors include climate zone, elevation, geology, and topography and are often considered well represented if areas follow level 3 ecoregions. We therefore tested groupings within level 3 ecoregions and at a more refined stage of level 4 ecoregions. We also defined regions for station groupings by physiographic province in some cases. We screened candidate stations within each defined area according to land use, absence of dams, and as having at least two or more years of data. We combined data from all stations within a group for trend analyses. Ordinations and correlation analyses for station groups in each state showed that samples within each station (different annual collections) tended to cluster more closely than stations within the group. Site differences were often greater than climate-related trends, and drove observed temporal trends if some sites in a group were sampled early in the period and some later.

Within-group variation is an important result to consider, because in the biomonitoring context, reference conditions are often established not based on a single reference station, but on a population of reference locations that together reflect the range of natural variability for a region (Barbour and Gerritsen, 2006). Combining reference stations across major physiographic, geomorphic or climatological regions inflates the range of measured variation in biological parameters from predictable, natural sources (Barbour and Gerritsen, 2006). It is thus important to account for predictable, natural sources of variation. This will affect how many reference stations within a defined area must be sampled, how frequently they must be sampled, and the sampling duration needed to have the power detect climate change response trends. In the current study, groups of reference stations analyzed were typically not of sufficient duration to define statistically significant trends within the context of natural spatial and interannual variation. On the other hand, transfer of results on trends and other biological responses defined from individual long-term reference sites to the corresponding regions or states may be problematic, in that without sufficient spatial replication it is difficult to know whether the observed trends are representative of the region as a whole.

4.2.1. Climate Change Vulnerabilities of Reference Stations

Climate change influences reference station vulnerability through changes in biological communities at these stations. This study documents climate changes that have the potential of degrading reference station biological status in a manner that will make existing reference stations more similar to non-reference stations, at least in some vulnerable regions (e.g., high elevation sites, head-water or low order streams). In addition, at non-reference stations, effects of climate change may be additive with other stressors, or interactions between climate change and other stressors may augment or ameliorate responses (Figure 4-1).

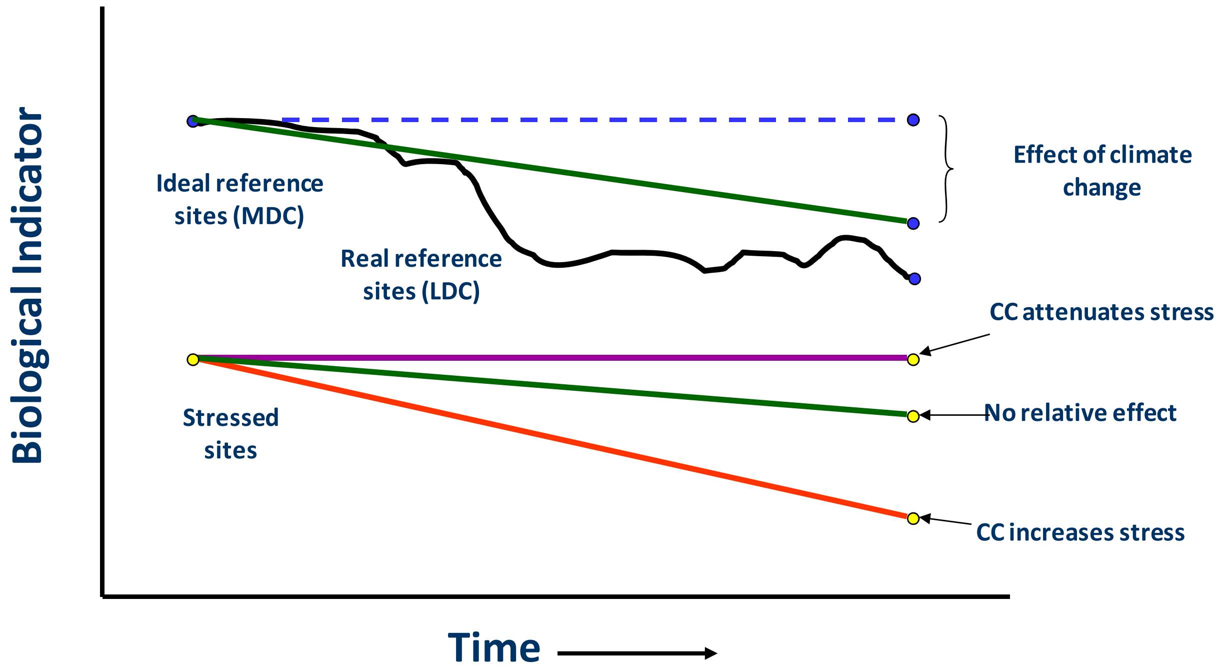


Figure 4-1. Conceptual model showing relationship between climate change trends and reference and stressed sites with an overlay of temporal variation on the trend (black line). "MDC" = minimally disturbed condition; "LDC" = least disturbed condition.

With regard to documenting reference station condition and establishing a framework within which changes in condition can be judged, BCG (Davies and Jackson, 2006) captures a more subtle range of biological conditions that have regulatory significance and value compared to an "impaired/not impaired" decision approach. The associated levels provide a uniform framework within which the degree of degradation attributable to climate change can be characterized. The BCG also provides a more meaningful basis for characterizing existing reference conditions. The more numerous, subtle and well defined levels captured in the BCG delineate a meaningful and scaled framework within which the degree of degradation attributable to climate change can be characterized. Figure 4-2 shows that as cold-water-preference taxa are lost from North Carolina biomonitoring stations, the percentage of stations that are characterized as excellent or good decreases. This climate change degradation of reference conditions will impact the stability of reference baselines and associated comparisons upon which management and regulatory decisions are base. A BCG would allow reference stations to be more accurately characterized, would support evaluation of reference station condition or drift over time, and would similarly support characterization of non-reference station changes over time (Figure 4-2). This affects the interpretation of the scope of response of reference communities to both climate change and conventional stressors and the interpretation of vulnerability of existing reference conditions to climate change.

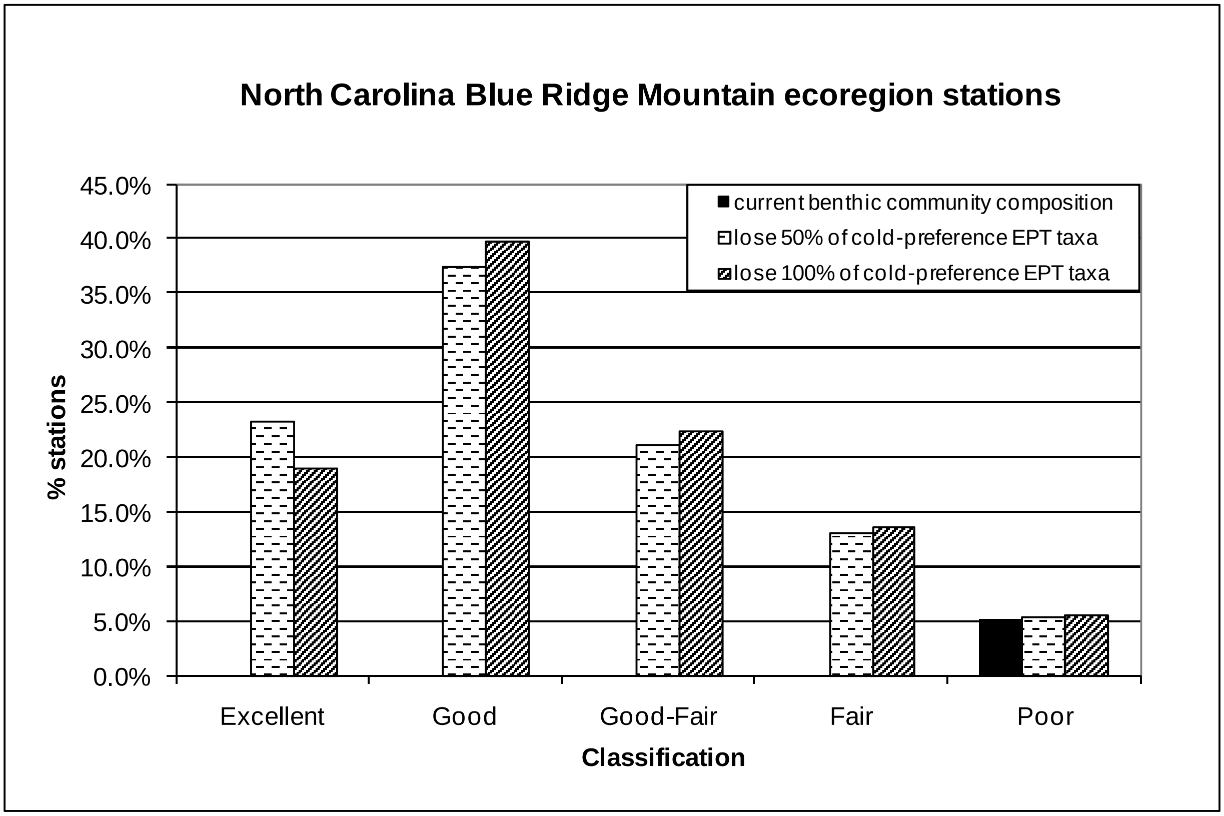


Figure 4-2. Reference station drift (degradation of assessed site condition) over time at Blue Ridge Mountain ecoregion stations as cold-preference taxa are lost over time due to climate change.

4.3. SYNERGISTIC EFFECTS BETWEEN CLIMATE CHANGE AND LAND USE

Though slightly different in geographic scale, both climate and land-use change can be considered large-scale impacts (Hamilton et al. 2010b). Global climate change drivers are well described (IPCC, 2007). Land-use change is generally considered a landscape-scale stressor, but is driven by global population growth (Nakićenović et al., 2000). Land-use changes, such as urban/suburban land development, have encroached on and impaired reference stations across the US. However, documentation of such problems has been sparse and likely has been handled on a local, case-by-case basis.

The successful use of biomonitoring data for evaluating pollution impairment in the context of climate change is in part related to understanding synergistic effects between climate change and conventional stressors, and how they can be separated. These synergistic effects can impact approaches used for attributing causes through the stressor identification process (see USEPA, 2000). Synergistic effects between climate change and other stressors are increasingly documented (Clement et al., 2008; Collier, 2008; Kaushal et al., 2008).

We examined the relative responses to climate change compared to land-use change (urbanization) through analyses of existing biomonitoring data (Appendix J). Hydrologic response variables play important roles in defining habitat conditions and structuring aquatic communities (e.g., Poff et al., 1997) and are responsive to both climate change and urbanization. Results show differences in the types of hydrologic variables (IHA, sensu Richter et al., 1996) that are likely to be most responsive to either climate change or urbanization effects. High flow metrics, such as flashiness, high-pulse-count duration, one-day maximum flow, and others, tend to strongly reflect urbanization, swamping inputs from climate change effects. In comparison, several low-flow metrics, such as 1-, 3- and 7-day minimum flows and low-pulse count, show responses to climate change effects more so than to land use (Appendix J). Where future climate change effects are small compared to land use, expectations are for more frequent, shorter, higher flows in urban-affected streams. Where future climate change effects are large compared to land-use effects, expectations are for more frequent, longer, lower flows. Accordingly, low-flow parameters should be selected as sensitive climate change indicators, and low-flow effects on biota are correspondingly expected to be most influential.

We further evaluated the relative effects of climate change and urbanization on stream condition through benthic invertebrate responses, using the sampling results from the Piedmont regions of Maryland and North Carolina as a test case. EPT taxa were evaluated as the responding biological metric (see Section 2). EPT taxa respond to both high flow metrics (flashiness) and to low flow metrics. For example, extreme increases in frequency of low-flow pulses (>20/y) are associated with EPT taxa loss, though low-pulse count did not differ much between the natural and urban streams in this analysis (Appendix J). There was a strong association of decreasing richness of EPT taxa with increasing flashiness (Figure 4-3, Appendix J), as well as confirmation of the greater flashiness of urban streams.

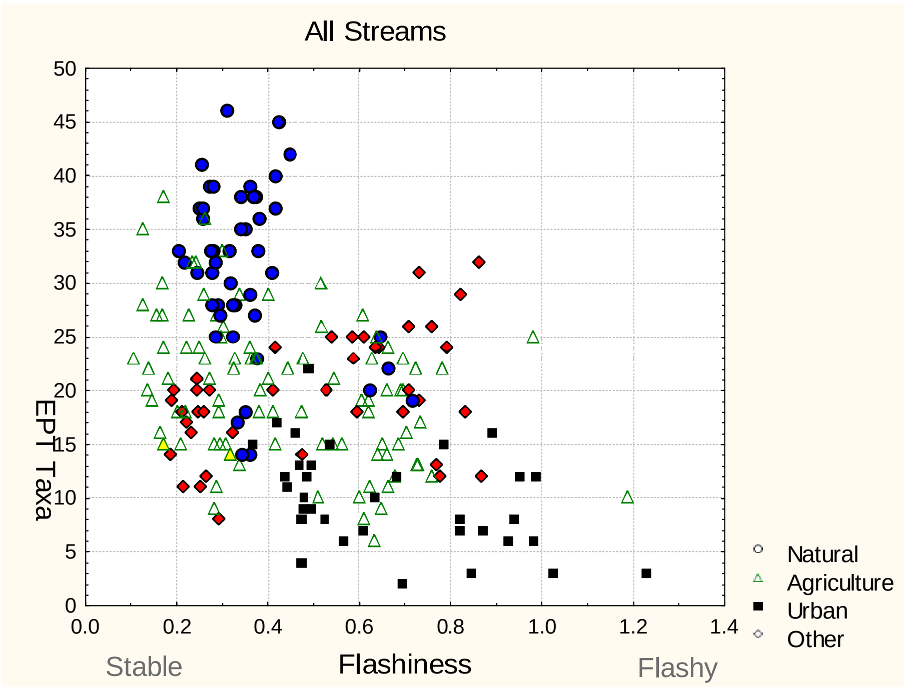


Figure 4-3. Relationship between richness of EPT taxa and flashiness (Baker's index) of the stream for stream types in the North Carolina Piedmont.

There is an apparent threshold response below minimum flows of about 15% in natural streams, where richness of EPT taxa is lower and less variable compared to higher flows, (Appendix J). In this component of the study, urban conditions were compared with natural stream conditions, and flow minima were more extreme in the urban streams. These results suggest that natural streams are more resilient to hydrologic changes within the range of recent past climate. Large changes in minimum or low flows may take much longer to become biologically meaningful, and in the shorter term, temperature effects may be more important.

4.4. FUTURE VULNERABILITIES OF REFERENCE STATIONS TO LAND USE

References stations are vulnerable to human-induced changes to the surrounding landscape. We evaluated current and future vulnerabilities of existing reference stations to urban/suburban development for three study states (Maine, Utah, and North Carolina), as well as for Florida as a case study (Appendix J) representing a high level of population growth. Data on current and future land uses comes from the Integrated Climate and Land Use Scenarios (ICLUS) project (Bierwagen et al. 2010). Future land-use scenarios are consistent with IPCC Special Report on Emissions Scenarios (SRES) social, economic, and demographic storylines used in global climate models (USEPA, 2009a; Nakicenovic and Swart, 2000). The ICLUS scenarios consider different levels of population growth, with different assumptions about development patterns (USEPA, 2009a). The two most extreme scenarios are: A2, which has high population growth rates and business-as-usual development patterns; and B1, which has low population growth rates and compact development patterns. We used a total of 248 reference sites compiled from Maine, Utah, and North Carolina to examine their vulnerability to current and future land use. The number and distribution of reference stations for these states are discussed in earlier sections of this report and in Appendices E, F, and G. Florida DEP has about 308 sampling locations, with 58 reference sites designated as "exceptional" (Figure 4-4).

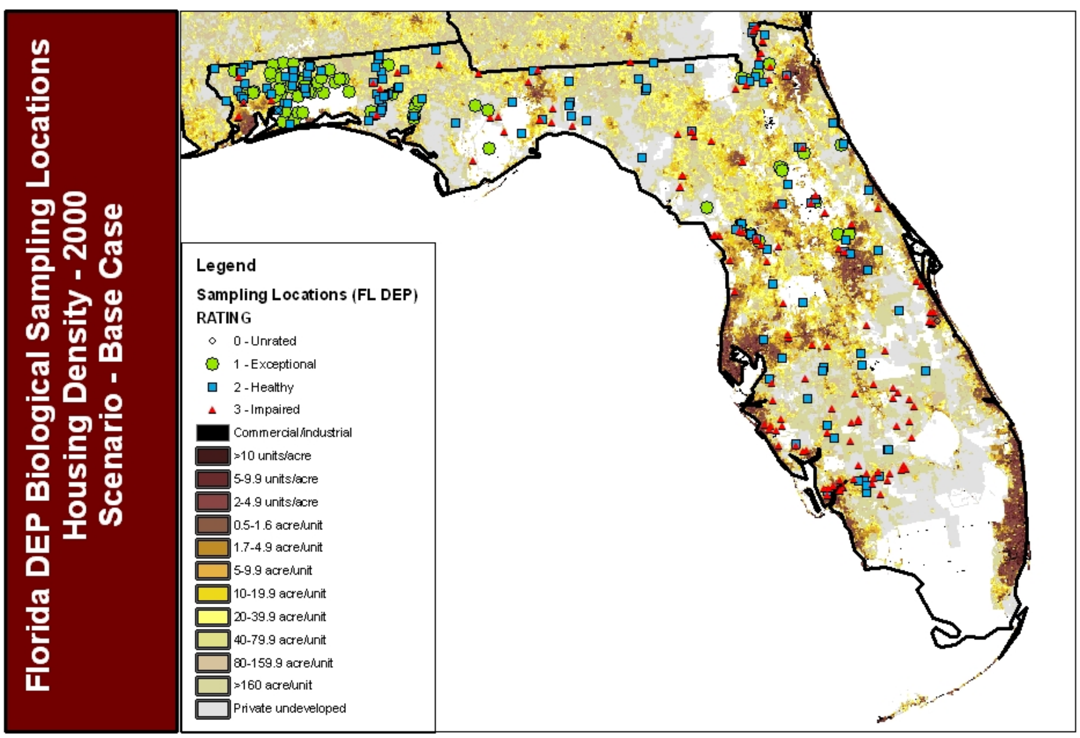


Figure 4-4. Florida's biomonitoring sampling stations, including "exceptional" reference locations (light green dots), show in relation to current land use.

Urbanization affects stream conditions through alterations in hydrology and geomorphology, with typically increased loading of nutrients, metals, pesticides, and other contaminants; these effects are associated with increases in impervious surface (Paul and Meyer, 2001). For the Florida case study, results of a broad spatial analysis in New England states of the relationship between human population density and Ephemeroptera (mayfly) taxon richness were used to estimate the degree of urbanization representing a threshold of impairment (Figure 4-5) (Snook et al., 2007). At low population densities, up to approximately 50 persons (~25 houses) per square mile, there are few detectable biological responses. From 50-500 people (25-250 houses) per square mile corresponds to a degradation gradient, and above 500 people (250 houses) per square mile, New England streams are degraded. Therefore, a threshold of housing density >25 houses per square mile was selected to indicate potential degradation. Using the land use composition within a 1-km (0.62-mi) radius buffer around each reference station, vulnerability was defined as ≥20% of the buffer with a land use at or above the threshold of housing density.

For the analysis conducted for Maine, Utah, and North Carolina, urban and suburban (>0.6 units/acre, or about 384 per square mile) was used. However, a threshold of 10% of development within a 1-km buffer was used to reflect expectations for impacts to the biological communities from urbanization (Schueler 1994, Booth and Jackson 1997, Wang et al. 2001). These differences in thresholds may account for some of the differences in results between the evaluation of the 3 study state reference stations and the Florida case study. Given the low threshold of development used and the high population growth rates for Florida, we take the Florida results to represent a worst-case scenario.

This analysis was done for several ICLUS scenarios to bracket a range of future projections, including the base case that approximates the current condition; the A2 scenario, that essentially represents a high estimate of population growth and development expansion; and the B1 scenario that represents a minimized estimate of population growth and compact development (USEPA, 2009a).

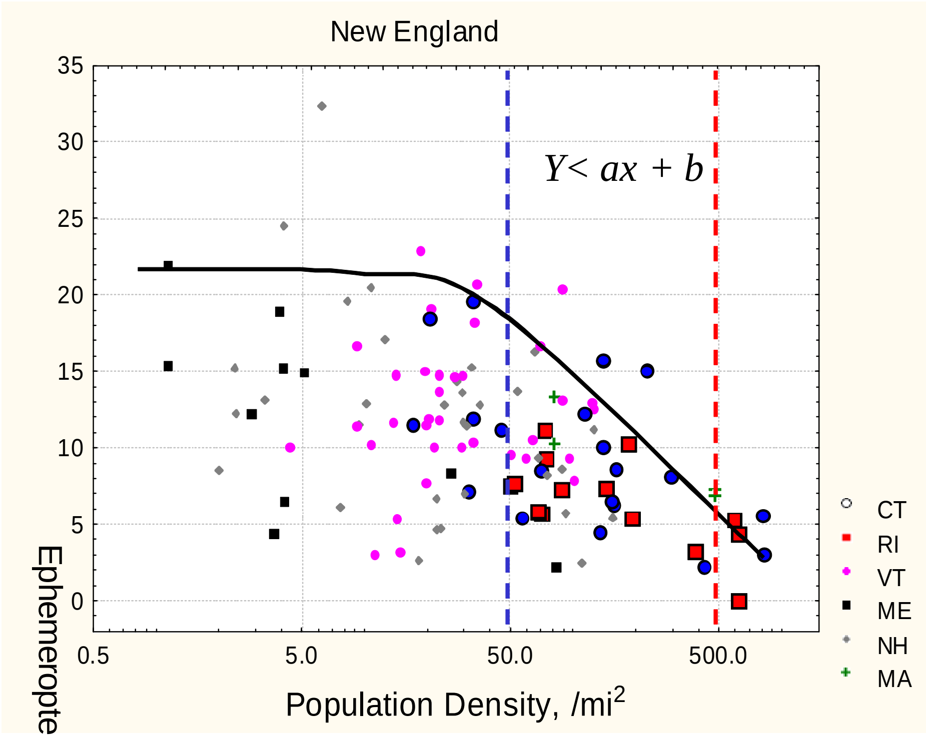


Figure 4-5. Relationship between human population density (i.e., degree of urban development) and Ephemeroptera (mayfly) taxon richness among six New England states (from Snook et al., 2007).

Among the 58 "exceptional"-grade reference stations in Florida under year 2000 conditions, 19% of the stations can be classified as vulnerable to land-use impacts (Table 4-2). That is, nearly 1/5 of Florida reference stations may already exhibit impacts from urbanization. Within the next two decades, more than one third of existing reference stations will be vulnerable, and by 2100, nearly half of current reference stations may be impacted by urbanization under the base case and A2 scenario. This level of vulnerability is significant. Figures 4-6 and 4-7 illustrate the distribution of this reference station land use vulnerability for the current base case, and for future (2100) projections of the base case, A2 and B1 scenarios. The spatial distribution of this vulnerability is broad. In Florida, most sampling stations are in the northern half of the state. Future projections of urbanization generally follow current patterns of development, with particularly dense future development projected for the northern half of the Florida peninsula (Figures 4-6 and 4-7). Sampling stations in these areas become vulnerable to future development, especially in the high population growth (A2) scenario (Figure 4-3, left panel), compared to the cluster of reference stations in northwestern Florida. The only reference locations that appear to be protected from future land development are those largely surrounded by water, and/or those within government-owned or protected lands that cannot be developed. In Florida this represents about 17% of existing reference locations.

Table 4-2. Percent of existing Florida reference stations (N=58, classified as "exceptional"), that have >20% developed land use (with 25 houses per square mile (9.65 houses per square kilometer) or more, categories 5-12 in the ICLUS data set) within a 1-km buffer surrounding the station, for current and decadal time periods through 2100.

	Scenario		
Year	BC	A2	B1
2000	19.0%	19.0%	19.0%
2010	36.2%	34.5%	36.2%
2020	36.2%	36.2%	36.2%
2030	37.9%	37.9%	36.2%
2040	41.4%	39.7%	36.2%
2050	44.8%	44.8%	36.2%
2060	44.8%	44.8%	36.2%
2070	44.8%	44.8%	36.2%
2080	44.8%	44.8%	36.2%
2090	44.8%	44.8%	36.2%
2100	44.8%	48.3%	36.2%

The results for Maine, North Carolina, and Utah show a somewhat lesser degree of vulnerability. Under current (2000) conditions, 22% reference locations in these three states have greater than 10% urban/suburban densities within a 1-km^2 neighborhood (Table 4-3). Under the worst case (A2) scenario, future housing development increased that to 34% by 2100. The maximum amount of suburban and urban development within the 1-km^2 neighborhood in 2000 was 58%; this increased to 99% by 2050. The average amount of development increased from 22% in 2000 to 28% in 2050 and 34% in 2100 using A2 scenario, while it leveled off at 26% using a lower population growth and higher development density scenario (B1) (Table 4-3). The results for Utah are difficult to interpret, and the projections not very meaningful, as the number of reference sites falling within the 10% development threshold as calculated for a 1-km^2 neighborhood was very small.

Table 4-3. Percent urban and suburban development within a 1 km^2 area surrounding reference sites, for all sites and for sites at or above the impact threshold of 10%. Number of sites is shown in parentheses. Scenario A2 has high population growth and business-as-

usual development pattern; scenario B1 has low population growth and compact development pattern (USEPA 2009).

	Area	2000	A2 2050	A2 2100	B1 2050	B1 2100
Mean of reference sites (≥10% threshold)	Combined	22% (35)	28% (37)	34% (45)	26% (37)	26% (37)
	Maine	23% (26)	24% (26)	30% (32)	23% (26)	23% (26)
	North Carolina	20% (9)	27% (9)	40% (10)	24% (9)	24% (9)
	Utah	0% (0)	87% (2)	64% (3)	77% (2)	77% (2)

The specific patterns of reference station distribution and vulnerability to land development will vary among states, although there are widely applicable lessons from these results. The high level of current vulnerability to urbanization (about 20% in all states tested except Utah) highlights the difficulties in siting reference locations in many areas and the probability of encountering substantial existing urban influences, which impact baseline (reference) conditions. This evidence suggests that protection of reference stations is of substantial importance. Options for protection may differ regionally and include zoning changes, limitations to development within buffer zones of selected stream reaches, incorporation into land protection programs (USEPA, 2009b), or other sociological, economic, and/or political solutions. If alternatives for protecting reference locations are limited or costly, it may be that reference stations in already protected areas, such as national parks, other government lands, or in otherwise inaccessible areas may represent the only “protected” references. This is likely to leave many watersheds and regional ecotypes without good reference conditions for comparison. In Florida, this would reduce the ratio of reference sites to total sampling sites from 19% to 3%. If reference sites are too scarce, they will be unrepresentative.

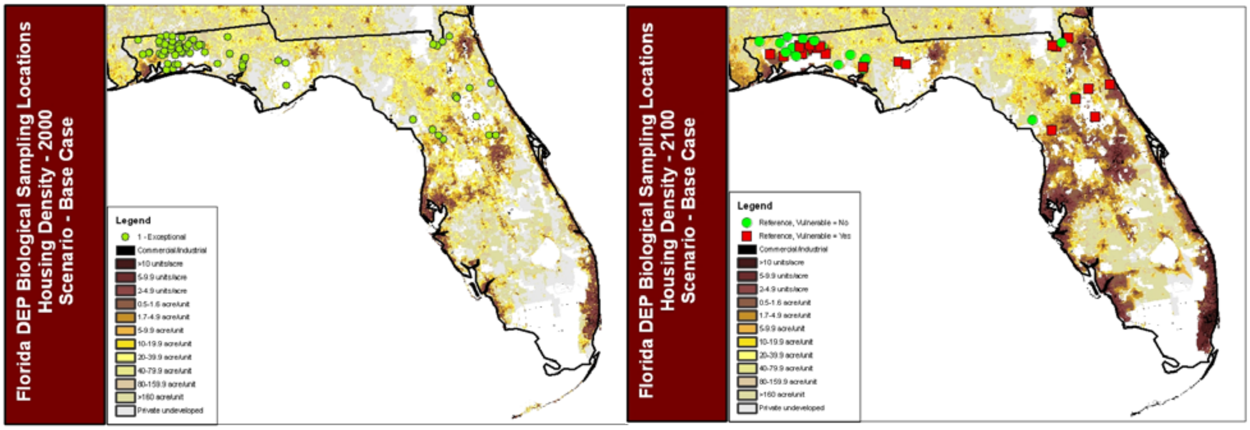


Figure 4-6. Distribution of Florida reference stations (N=58, classified as "exceptional"), plus categories of developed land use from ICLUS. Red squares highlight current reference stations that in the future with scenario-associated land use change projected to 2100, will have ≥20% developed land use (with 25 houses per square mile or more, categories 5-12 in the ICLUS data set) within a 1-km buffer surrounding the station. Left panel is current (2000) land use distribution; right panel is the base case scenario in 2100.

The need to protect reference locations is an important issue for the future of bioassessment. If reference stations become urbanized, the ability to detect climate change, and separate climate responses from conventional stressors in order to continue to manage resources, set permit limits, and meet CWA requires, may be hampered. It may become important to consider and promote more broad-based alternatives than just local or state-specific protections, such as regional cooperation in the establishment and monitoring of long-term fixed "sentinel" locations. The shifting baseline of reference condition demonstrates that both communication and understanding are immensely improved by measuring biological condition in comparison to pristine, undisturbed condition instead of to present-day reference.

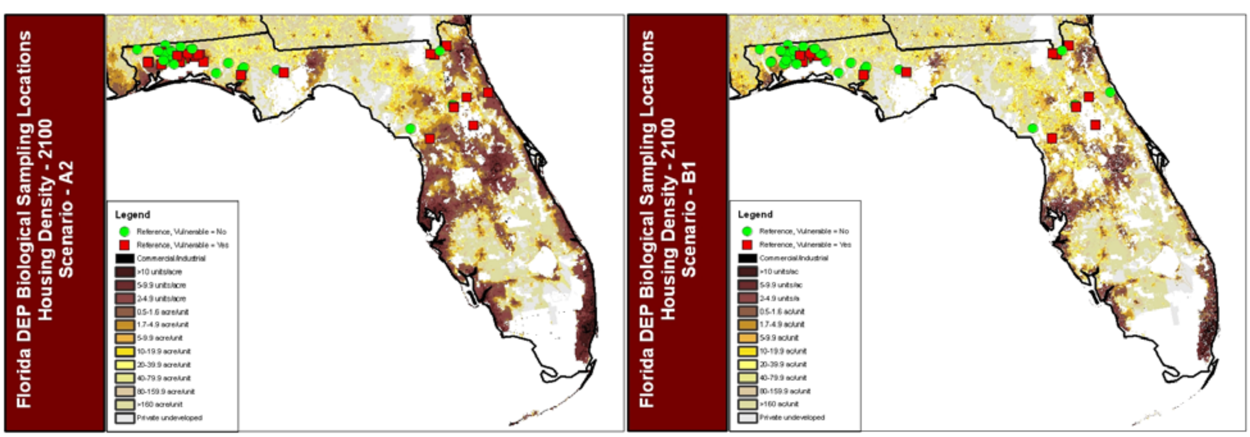


Figure 4-7. Distribution of Florida reference stations (N=58, classified as "exceptional"), plus categories of developed land use from ICLUS. Red squares highlight current reference stations that in the future with scenario-associated land use change projected to 2100, will have $\geq$20% developed land use (with 25 houses per square mile or more, categories 5-12 in the ICLUS data set) within a 1-km buffer surrounding the station. Left panel is the A2 scenario in 2100; right panel is the B1 scenario in 2100. See Figure 4-3 for the current (2000) condition for comparison.

4.5. SENTINEL MONITORING NETWORK

Results of this study have demonstrated the importance of accounting for climate change effects in order to maintain sound bioassessment decision making. The next step is to consider possibilities for augmenting existing programs to address this need. Section 5 discusses many of the typical characteristics of biomonitoring program and their inherent limitations with regard to detecting trends that might be associated with climate change. Approaches to address some of those limitations are discussed here.

A monitoring network designed to detect climate-related changes needs to account for regional variations in climate, geology (including soils), topography, elevation, latitude, vegetation, etc. Such conditions often cross state and tribal boundaries. Therefore, this kind of monitoring network may require collaboration among states and tribes with regard to technical considerations (e.g., site selection, sampling methods) and funding. Regional and national support may be important to facilitate this process.

Thorough coverage across ecoregions and other environmental variants would require a large network of sites. A modest initial effort for sentinel site monitoring could focus on highly vulnerable areas and watershed types. Since not all watersheds or community types would be represented by such selective establishment of a sentinel site monitoring network, the classification of conditions and transferability of bioassessment results will be integral for extrapolation to other areas (e.g., Allan et al., 1997; Gerritsen et al., 2000; Wu and Li, 2006).

In order to separate climate change effects from other stressors, both reference and some portion of impaired sites should be measured over time; thus, sentinel sites should be established along the BCG and be anchored in reference conditions. This would support an analysis approach in which temporal trends at reference sites could be compared to temporal trends at impaired sites, in order to differentiate between climate effects and conventional stressors.

Different levels of stressor effects could also be compared and synergistic effects considered. It is possible that in a monitoring context, as opposed to a controlled study, synergisms between climate change and conventional stressor responses could not be fully partitioned. Inference using literature studies, especially through use of CADDIS and the stressor identification process (Suter et al., 2002; USEPA, 2000) would contribute to data interpretation in a weight of evidence approach. The efficacy of conducting long-term sampling along the BCG should be considered through interactions with state and tribal biomonitoring managers, consideration of avenues of funding support, and finally, through practical evaluation of existing opportunities for establishing such a sentinel site monitoring network in representative and vulnerable regions.

If a sentinel site monitoring network along the BCG is infeasible, a less resource-intensive alternative would be to establish long-term sentinel sites only at high-quality reference locations. Lack of trend data from non-reference sentinel locations would present some limitations to separating climate change from other stressors responses. Selection of such locations would face some of the same difficulties as any reference selection effort conducted by individual states. However, the larger spatial scale and regional perspective necessary for implementation would offer opportunities to search for and select least-affected locations from a larger area and share results across jurisdictional boundaries.

While typical bioassessment approaches include sampling watersheds on a rotating, often 5-year basis, biomonitoring at sentinel sites should be considered on a regular, repeating basis, annually if possible. With less frequent data, temporal variations from interannual and cyclic climatic sources would greatly extend the time frame needed to describe climate change responses.

Another component of sentinel site monitoring for climate change is the recommendation for continued monitoring at targeted locations, even if initial site selection is probability-based, rather than application of a probability-based sampling approach in which all sites are reselected each year. Probability sampling has important strengths in capturing the (often large) range of variability within a defined stratum, such as low-order stream reaches (Barbour and Gerritsen, 2006; Hughes et al., 2000); it also provides valuable data about the status of our nation's waters at any given time (Hughes et al., 2000; Paulsen et al., 1998). This is important for defining the range of condition within the stratum at any one time, but it requires replication (multiple reference sites) within the stratum. There is also a high likelihood of never sampling the same location again. We found high among-site variability within ecoregions despite expectation that partitioning by ecoregion should control major predictable sources of variation. This maximizes the effects of “natural” site (spatial) variability on detection of temporal trends, and greatly extends the time it will take to discern climate change effects. This suggests a trade-off between gaining knowledge about regional status and knowledge about long-term trends. There is a valid consideration of whether detection of climate change patterns at a fixed location has meaning if it does not incorporate the real range of conditions that defines the stratum. However, replication of targeted locations within a region or stratum accounts for natural spatial variability. Combining some fixed with random sites in a pre-determined sampling pattern may be the most likely design that accomplishes both trend detection and representation (Urquhart et al. 1998).

Many different groups are considering, or have already started, monitoring for climate change effects. If possible, collaboration among at least some groups, particularly among bordering states, would have many potential benefits. Some duplication of effort could be avoided, results could be integrated in a more meaningful way, and resources could potentially be saved. Collaboration would foster consistency across groups in types of data collected, as well as potential use of a common database. Efforts to discuss and establish a sentinel monitoring network might facilitate collaboration among existing efforts. A common vision of sampling and agreement on types of data that could be incorporated into a common database related to a potential climate change monitoring network could have a better chance of success.

5. CHARACTERISTICS OF EXISTING BIOASSESSMENT PROGRAMS RELEVANT TO DISCERNING CLIMATE CHANGE TRENDS

There are some inherent qualities of biomonitoring data that limit the ability to define long-term trends. Limitations on the long-term trend analysis approach should be understood in the context of the nature of the data being analyzed, and the types of information needed about climate change responses in order to assess how state and tribal biomonitoring and biocriteria programs are likely to be affected in the future.

5.1 SUFFICIENCY AND LIMITATIONS OF DATA TO DEFINE AND PARTITION LONG-TERM TRENDS

One significant limitation is the small number of long-term monitoring sites available to support temporal analyses. As discussed in Section 2, the small number and limited distribution of long-term reference stations reduces the ability (1) to confirm regional trends, (2) assert the strength of any trends discerned, and (3) to compare biological responses between regions. Essentially, the very low number of stations with sufficient long-term data limits replication for testing climate change effects.

The small number of long-term stations is largely a product of the focus of most state and tribal biological monitoring programs. Objectives of these programs typically include assessing the status, health, and integrity of aquatic ecosystems in response to Clean Water Act (CWA) requirements (Barbour et al., 2000). The basis for such assessments is the comparison of test locations to reference locations to detect community differences concurrently. Temporal patterns seldom figure into these spatial comparisons. Figures 5-1 to 5-3, show the spatial distribution of biomonitoring locations in Maine, North Carolina, and Utah, and illustrate that spatial coverage using all sampling sites can be relatively extensive. Total spatial coverage of stations represents the composite of the stations periodically re-sampled across major watersheds to assess condition of state-wide aquatic resources and list impaired stream reaches, plus occasional additional spatial efforts that may arise for evaluation of a particular discharge or other local impact.

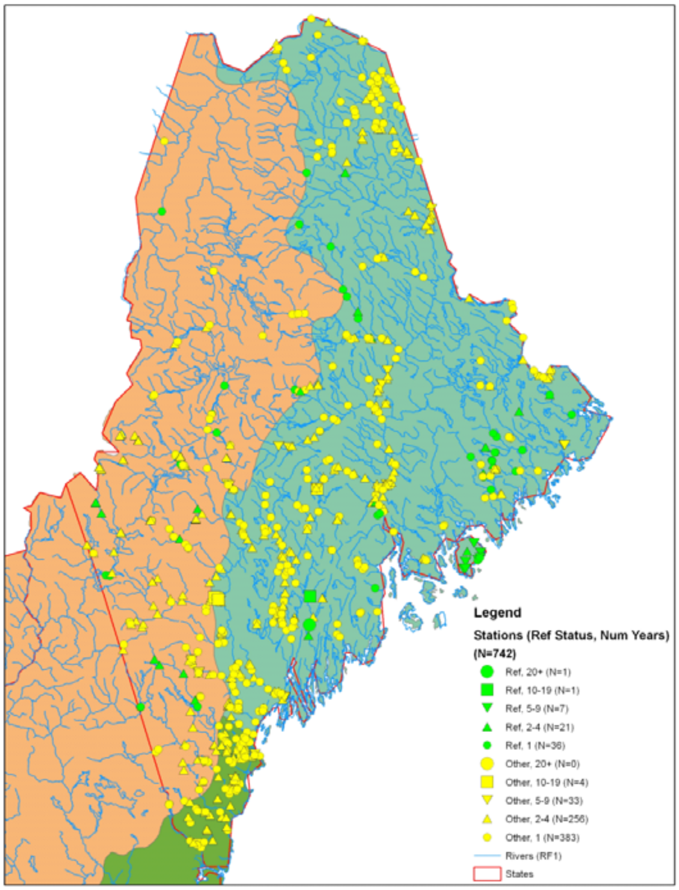
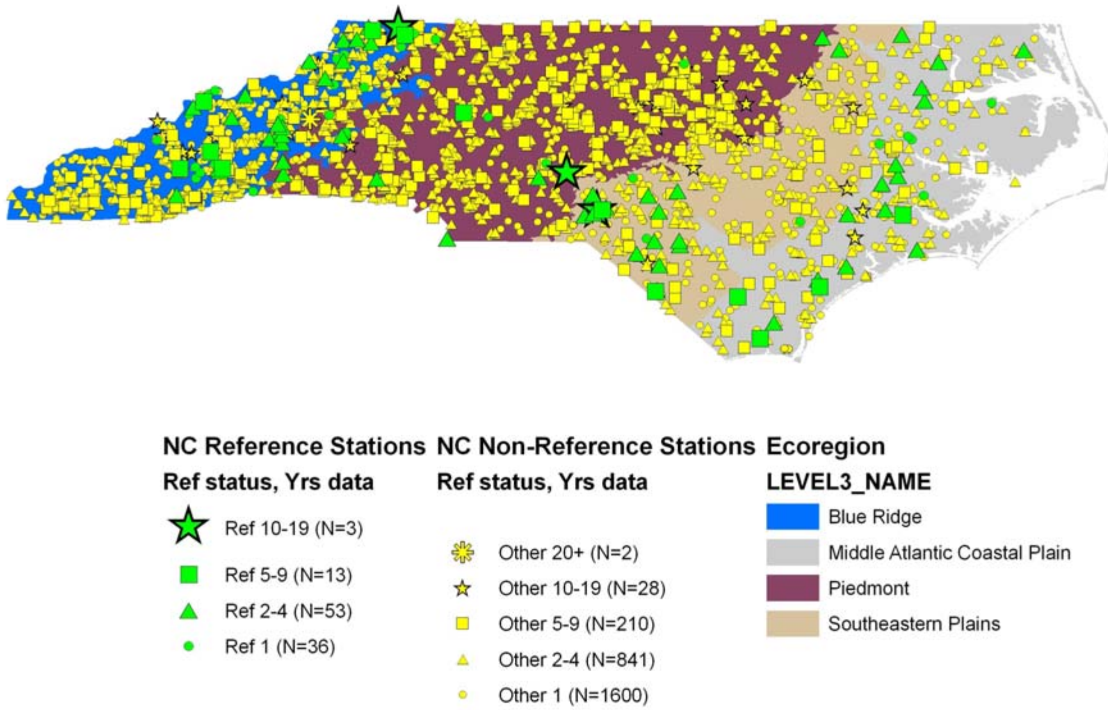


Figure 5-1. Maine biomonitoring stations, with data durations by reference and non-reference locations.



1239

1240 **Figure 5-2. North Carolina biomonitoring stations, with data durations by reference and**
1241 **non-reference locations.**

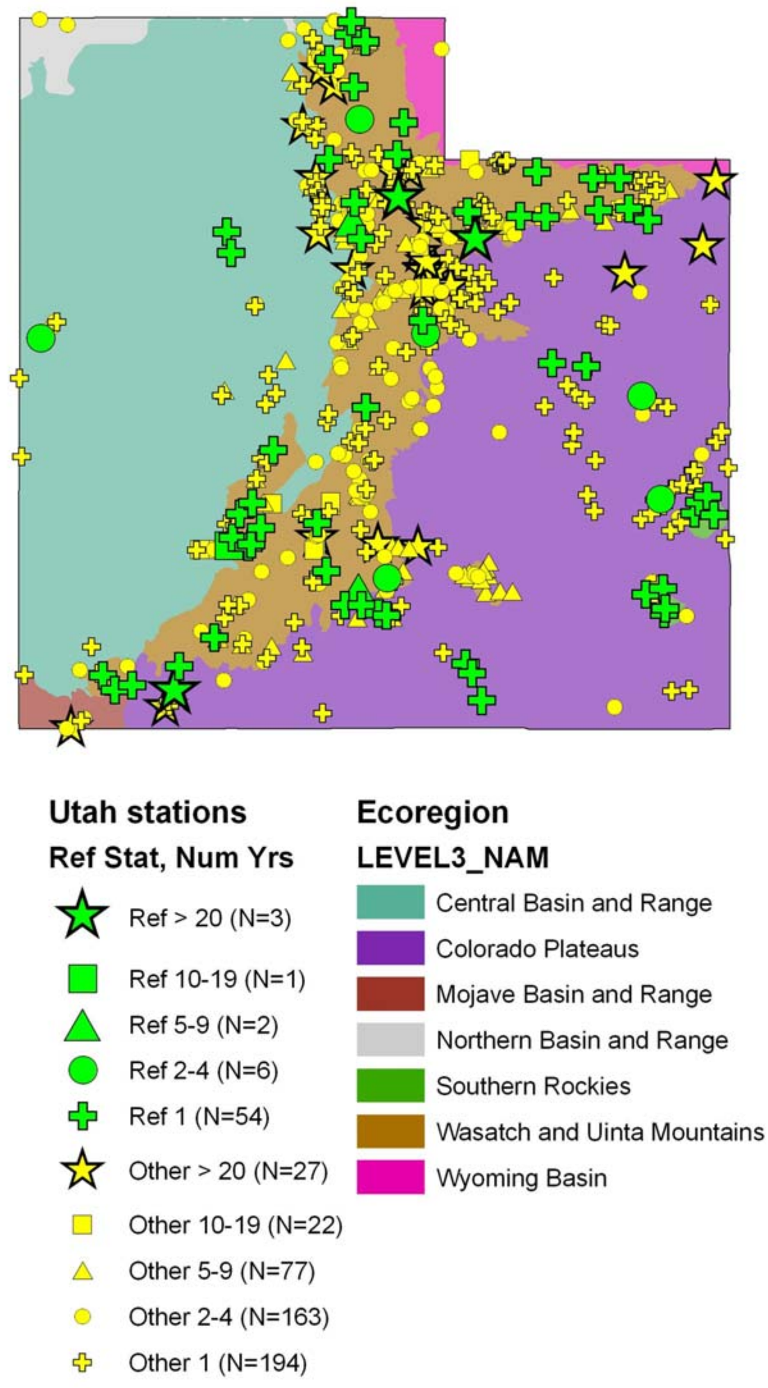


Figure 5-3. Utah biomonitoring stations, with reference condition and non-reference locations.

Despite the large number of stations, very few are sampled in more than one or a few years over the entire period of record. For example, Maine has at least 742 stations, but only 66 classified as reference locations (<10%) (Table 5-1). Only two of these have been sampled for more than a decade (Table 5-1), and only one of these for more than two decades. North Carolina's biomonitoring program has been operating for two decades; still, only one reference station exists with more than 10 years of data (Table 5-1). The small number of reference locations with long-term data is a surprising but important finding that likely applies to many other biomonitoring data sets.

Table 5-1. Average distribution of reference and total stations by state, categorized by duration of sampling.

Years Sampled	Maine		North Carolina		Utah		Average		
	Ref	Total	Ref	Total	Ref	Total	Ref	Total	% Ref
1 to 4	57	696	89	2530	61	482	207	3708	5.6
5 to 9	7	40	13	223	1	41	21	304	6.9
≥ 10	2	6	3	33	4	26	9	65	13.8
Total	66	742	105	2786	66	549	237	4077	5.8

Another limiting factor for long-term analyses is that data for trend analysis must be selected from reference data sets to minimize contributions from conventional stressors (see Section 4). Only qualified reference locations should be used. In addition, climate change responses differ among regions (see Section 3). Partitioning by ecoregion often means there are few (or no) individual long-term stations available for analyses.

A related factor is the actual length of the "long-term" data record. Reference locations in this study yielded some valuable results, but also many non-significant patterns. This suggests that the duration and data density from these stations borders on data sufficiency. As examples, the longest-term reference station in North Carolina, NC0109, had 11 years of data over a 21-year time span (1983-2003); the longest-term reference station in Maine had 23 years of data over a 23-year time span (1984-2006); and three long-term reference stations in Utah had 19 years of data over a 21-year span (1985-2005, station 4927250 - Weber), 15 years of data over a 20-year span (1985-2004, station 4951200 - Virgin), and 14 years of data over an 18-year span (1985-2002, station 4936750 - Duchesne). The sufficiency of data duration in combination with number of stations sampled and frequency of sampling is being further explored in subsequent work.

Data durations of about 15-20 years also appear in the literature as an apparent minimum. For example, analyzing an 18-year data set from a large number of streams in the UK, Durance and Ormerod (2008) found significantly increasing temperature trends and significant correlations of some invertebrate variables with temperature, although they concluded that water quality improvements confounded interpretation of results. Chessman (2009) found significant climate change trends in benthic invertebrate taxomonic families and trait groups within a 13-year data record in New South Wales, Australia. Daufresne et al. (2003) defined aquatic community trends in the Rhone River based on data durations of 20 (macroinvertebrates) to 21 (fish) years. Although Daufresne et al. (2003) found several meaningful community patterns and showed statistically significant trends in temperature, trends related to flow parameters were generally not found to be significant based on the same duration of data. Two possibilities are 1) in the Rhone River there were no temporal trends in flow and/or no relationships between flow and invertebrate or fish communities; or 2) given the typically high variability of hydrologic variables, the 20 to 21-year duration of data was not sufficient to detect any trends. Murphy et al. (2007) examined relationships between climate variables and benthic invertebrate responses in England based on about 20 years of data, indicating that while multi-decadal data sets required to define climate-driven trends were rarely available for rivers, potential responses of biota to climate forcing can be estimated based on relationships between climate variables and biological indicators using past data. Even with a long-term data set, Durance and Ormerod (2008) discounted stream benthic assemblage changes that were correlated with long-term (18 years) temperature increases at sites in southern England because some of the faunal changes included taxa with traits (e.g., preferences for high flows, high dissolved oxygen) that were contrary to expected responses to climate-driven increases in stream temperatures. The existence of trends, by themselves, is insufficient to assert climate change impacts, but must be interpreted based on consistency with expectations for biological responses to climate change.

One observation that stands out regarding the Maine, North Carolina, and Utah reference locations is that most of these have more frequent annual sampling than would be the case if they were only sampled on a “rotating basin” basis. Utah adopted a rotating basin sampling scheme as well as a probability-based station selection approach within the last decade (Utah DEQ, 2006). However, they maintain regular annual sampling at a small number of fixed locations with long-term historic records. Whether by formal decision or historic happenstance, some other states also have regularly sampled stations outside of rotating and/or probabilistic designs.

5.2. OTHER BIOMONITORING METHODS CONSIDERATIONS

Each of the states analyzed in this study use different collection methods. Utah collects a quantitative sample from riffle habitats during a September/October index period using the Environmental Monitoring and Assessment Program (EMAP) kick method (note: prior to 2006, samples were collected using the Hess method) (Utah DEQ, 2006). Maine uses artificial substrates (rock bags or baskets) to collect quantitative samples during late summer, low flow periods (July 1 to September 30) (Maine DEP, 2002). North Carolina uses several different collection methods, but for this study we focused on the standard qualitative, or ‘full-scale’, method. It is comprised of 2 kicks, 3 sweeps, 1 leaf pack sample, 2 fine mesh rock and/or log wash samples, 1 sand sample and visual collections (NCDENR, 2006). Abundance data is recorded as rare=1 (1-2 specimens), common=3 (3-9 specimens) or abundant (≥10 specimens). Ohio uses a modified Hester-Dendy multiplate artificial substrate sampler that is placed in-stream to colonize for six weeks between mid-June and late September (DeShonn, 1995).

Some methods are likely to be more effective than others for certain applications (e.g., Flotemersch et al., 2006). Artificial substrates specifically placed to remain wetted for the entire colonization period may be less sensitive to shifts in hydrology. In Maine, rock baskets are placed in run habitats that will have sufficient water for the entire deployment period. If there are drought-like conditions that cause a loss of edge habitat, the rock baskets are less likely to reveal the potential loss of edge taxa. Even protocols that sample only riffles may be less likely to collect edge-specialized fauna. However, the multiple habitat protocol used in North Carolina is more likely to detect such shifts.

It is difficult to define which sampling protocol is best suited for detecting climate change effects. Use of artificial substrates were favored for pollution detection on the premise that application of a uniform substrate eliminates the substrate variation among stations as a variable that would confound detection of community responses to a pollution discharge or other disturbance (e.g., Barbour et al., 1999; Cairns, 1982). At least in some regions, long-term changes in climate variables are expected to contribute to responses that can include drought or flood-related changes in flows and associated changes in nutrient loadings, sediment loadings, habitat availability, and other inter-related factors. Given these considerations, the ability to examine the full spectrum of naturally occurring biological community components may be advantageous. In-stream, multi-habitat sampling may be more likely to provide realistic estimates of abundance or richness of particular indicator taxa. On the other hand, there is a significant disadvantage to changes in sampling methods, due to the disruption it causes in temporal patterns that might otherwise be observed. Because of this, any consideration of changing sampling methods should at least be accompanied by a period of time in which both methods are applied simultaneously in order to develop translation models. It should be noted that such translational models may not always be effective or overcome inherent sampling biases. For example, if rock baskets almost never effectively collect edge taxa, then no factor can be defined that would translate multiple years of near-zero results into meaningful estimates of abundance.

Because of considerations such as these that bear on the consistency of results, states have a vested interest in continued use of their own methods to assure that new data are meaningful to their program. Additional sampling might be considered in representative and/or especially vulnerable regions as an adjunct to standard biomonitoring methods. For instance, in streams with a high likelihood of transitioning from perennial to intermittent status, collection of samples from edge habitats could be considered.

Another potential hindrance to effective detection of climate change trends is relatively low sampling effort and the lack of replication in station sampling. In most biomonitoring programs the concept of collection of replicate samples is relinquished in favor of collecting single composite samples. The composites can be either of multiple artificial substrates (e.g., in Ohio, 5 Hester-Dendy samples per station are composited and processed as a single unit (DeShon 1995)); or a single sample unit can be a composite of collections made in multiple representative habitats (NCDENR, 2006). In general, increasing the number of samples collected and composited for a site has been found to decrease variance among ‘replicate’ (similar) sites and increase the precision of characterizing the assemblage at the site (Cao et al., 2003; Diamond et al., 1996). Multi-habitat sampling, applied in many biomonitoring programs (e.g., Utah, North Carolina) is considered to yield representative, and therefore precise, samples (Barbour et al., 2006; Hering, 2004). Though replication is considered necessary to determine the precision of the sampling method (Barbour et al., 2000), it is often only accomplished on about 10% of collections (e.g., Stribling et al., 2008; Barbour et al., 2006; Flotemersch et al., 2006). However, with regard to understanding the significance and implications of climate change temporal trends, knowledge of spatial variation within a station (or stream reach), and between similar sites within a watershed or ecoregion, may be valuable.

There are some environmental variables that are, or can be, measured along with biological samples to aid in interpretation of results. For example, a detailed assessment of substrate and related habitat condition, as is used in EMAP (Lazorchak et al., 1998), is valuable in differentiating habitat disturbance from other stressors. If and when biomonitoring programs consider climate change as an additional stressor, it becomes valuable to have good information on water temperatures and flows from biological collection sites. Existing sampling protocols usually include concurrent point measurements of temperature, and sometimes also of pH, dissolved oxygen, and conductivity, as these values are relatively easy to obtain with portable sondes. However, the analyses conducted in this study illustrate that point measurements of temperature are not a good measure of the stream conditions to which an aquatic community is exposed. They tend to include a large amount of variation from time of day as well as date during the seasonal index period when that measurement happened to be taken.

In this study, the lack of long-term, site specific temperature and flow data impaired the ability to conduct weighted average modeling (or use of related approaches) to determine temperature or flow parameter preferences for many taxa. It also made it difficult to conduct simple trend and correlation analyses (see Sections 2 and 3). It would be beneficial to consider deploying *in situ* equipment to obtain continuous water temperature and flow measurements at as many climate change monitoring sites as possible. Though such equipment is widely available and much less expensive than it used to be, the sometimes severe resource limitations experienced by states and tribes may limit the extent to which this recommendation can be applied. Priorities could be set based on regional assessments of relative vulnerability to climate change. For example, a limited number of deployments could be done at reference locations in higher elevations, and/or in lower order streams. Such deployments also could be coordinated with implementation of monitoring at sentinel sites (Section 4). There is also high value in continued operation of USGS long-term flow and temperature gages.

6. CLIMATE CHANGE IMPLICATIONS FOR ENVIRONMENTAL MANAGEMENT

6.1 IMPAIRMENT LISTINGS AND TMDLS

6.1.1. Overview of impacts on impairment listings and TMDL development

One of the central objectives of state programs for establishing a reference condition baseline and conducting ongoing biomonitoring at reference and non-reference locations is to detect locations, or stream reaches, that are sufficiently different from the established baseline to be considered impaired. The approach and specific criteria used to make impairment decisions are established by states and tribes, and vary among regions to reflect the appropriate range of natural variability (Barbour and Gerritsen, 2006). But the assumptions inherent in the almost universally applied reference comparison approach include that the stressors likely to impair streams and rivers within a region are accounted for within the sampling and analysis scheme applied, and that if a real impairment exists, it can be detected with a reasonable level of confidence. The concept that all stressors must be accounted for presents an unusual problem with regard to climate change effects, because climate change effects are "global", so reference stations are equally at risk. This threatens the reference comparison paradigm.

Results of this study reveal changes in biological indicators and within specific ecological traits groups that are reasonably attributable to climate change effects and are likely to interfere with impairment determinations. Sections 2 and 3 document changes in cold- and warm-water-preference taxa at reference stations due to climate-change-related trends in temperature and precipitation. These trends result in corresponding changes in biological metrics used by states, such as EPT taxa richness or abundance in the HBI index. The observed and projected changes in biological metrics are sufficient to downgrade reference station condition (Section 4). Degradation of reference station condition is essentially causing references stations to become more similar to non-reference stations, and diminishes the ability to detect impairment (Section 4). Previous analyses presented preliminary evidence for this (USEPA, 2008). These findings imply that unless metrics are modified so that climate effects can be tracked and thresholds for defining impairment re-evaluated, degraded reference conditions will cause fewer stream reaches to be defined as impaired, at least in the most climate-vulnerable watersheds. This will lead to less corrective action and greater long-term degradation of stream conditions (see also Hamilton et al. 2010b).

When a stream segment is found to be impaired, total maximum daily loads (TMDLs) of pollutants are developed by states, and the cause(s) of the impairment are identified through the stressor identification process (USEPA, 2000; Suter et al., 2002). In permitting (e.g., the National Pollutant Discharge Elimination System (NPDES)), discharge limits must be set considering any existing TMDLs. Beyond the possibility of under-protection with fewer impairment listings and fewer requirements for TMDLs, there may be other direct climate change implications to TMDL development. Climate change scenarios show greater variability in runoff and flow, which may result in greater uncertainty in loadings expected from non-point sources. Critical low flows also drive TMDLs, and these may become uncertain and more difficult to predict. The identification of culpable stressors is also complicated by the effects of climate change on biological indicators.

6.1.2. Approaches to evaluate impairment listings and TMDL development in the context of climate change

The main approaches pertinent to preserving the ability to detect impairment concern climate change-related modifications of biological metrics, associated re-evaluation of impairment thresholds, and reference station classification and protection. These actions are directed at improving the ability to track effects of climate variables, compare these between reference and non-reference locations, and thus increase the information brought to bear on differentiating climate change from other stressors and detecting conventional stressor impairment. The stressor identification process, tailored to include detailed climate change information, would facilitate partitioning biological responses between climate change and other stressors. The paradigm for conventional stressor identification is based on spatial (reference/non-reference) comparisons, combined with weight-of-evidence evaluation of potential causes, augmented by research and other literature-based knowledge of major cause-effect expectations (Suter et al., 2002; USEPA, 2000). The need to partition climate change effects could add a relatively extensive time component to this framework if the process were to rely primarily on site-specific, long-term field data. However, it is impractical and undesirable from a decision-maker's point of view to obtain this degree of detailed, long-term sampling for every case of impairment assessment. From a practical perspective, it also is likely to be outside of the level of resources available to most states or tribes for routine bioassessment sampling. An alternative approach includes monitoring a more limited network of sentinel sites (Section 4.5). Documentation of trends for monitoring data, other aspects of weight-of-evidence evaluation of potential causes, and an expanded knowledge data base on biological responses to climate change could be included in an expanded stressor identification process.

With regard to other vulnerabilities in the TMDL development process, there is a need for watershed-specific modeling to predict how flow dynamics change with climate, to provide support for estimating future changes in low flows, and to modify loading calculations and limitations accordingly.

6.2. WATER QUALITY STANDARDS AND BIOCRITERIA

6.2.1. Overview of impacts on the development of water quality standards and biocriteria

Biological responses to climate change will likely impact water quality standards and biocriteria through shifts in baseline conditions. This study illustrates several avenues through which climate change is affecting stream communities in ways that have implications for biocriteria programs. Details are presented in Section 2, which discusses how trait groups, taxonomic groups, and to some extent, individual taxa appear to be responding over time to climate drivers, responding in ways that are predicable, and responding in ways that are consistent with expectations relative to climate change. Section 3 discusses implications of these changes to various MMIs and predictive models. The cascading effects of climate change-related trends in temperature and precipitation on watershed conditions, water quality, and aquatic biological communities, will lead to shifting, most often degrading, baseline conditions. Decreases in mean abundances and/or species richness of cold-preference or other sensitive taxa and trait groups, increases in warm-preference or other tolerant taxa and groups, and also increases in variability of these indicators drive reference sites to greater similarity with non-reference areas, as well as greater difficulty in establishing statistical differentiation (USEPA, 2008). As a result, reference-based standards will be liable to progressive under-protection.

By itself, climate change can be expected to alter some uses and their attainability, especially in vulnerable streams or regions. For example, some cold-water streams could take on cool-water characteristics, with declining abundances and/or richness of sensitive cold-water taxa, possible increases in warm-water taxa, and other changes potentially related to altered hydrologic patterns. Regulated parameters such as temperature, dissolved oxygen, and ammonia, may also be sensitive to climate change effects, and their values may need to be adjusted relative to revised designated uses.

6.2.2. Approaches to modify the development of water quality standards and biocriteria in the context of climate change

There are numerous criteria, both biological and chemical, that are addressed in water quality standards, and which may be affected by climate change (Table 6-1). Biocriteria are of particular interest, as they tie closely to the indices and thresholds used to determine condition and impairment. The climate-related causes of drifting (degrading) baseline conditions cannot be directly controlled, but can be assessed, at least to the degree resources allow. The concepts that support this include clear documentation of reference conditions, tracking of changes in reference conditions over time, and to the extent possible, protection of reference conditions from other encroaching impacts, particularly land-use changes (Section 4). This may be extended to include repetitive regional monitoring of sentinel sites, carefully chosen to represent the best conditions of the most vulnerable regional watersheds (Section 4). Further efforts to address climate change impacts to standards would require examination of which water quality standards are resilient to climate change impacts and will remain protective, and identification of susceptible standards that may need adjustment.

For watersheds that are found to be particularly vulnerable to climate change effects, including those that are characterized by particularly vulnerable trait groups, more refined aquatic life uses should be considered for application. Refinement of aquatic life uses can be applied to guard against lowering of water quality protective standards. Uses are designated for a stream segment based on conditions at similar reference stream segments, using information on habitat characteristic and associated biological communities, and potentially also consideration of economics and human-related conditions. Criteria are set to protect designated uses, and often differ between use levels. Application of refined aquatic uses could provide a greater number of more narrowly defined categories, which could accommodate potentially "irreversible" changes (e.g., increased temperatures driven by long-term climate change), but with sufficient scope to maintain protection, and also support anti-degradation from regulated causes.

Table 6-1. Variables addressed in criteria and pathways through which they may be affected by climate change (from Hamilton et al. 2009)

Criteria	Climate change impacts
Pathogens	Increased heavy precipitation and warming water temperatures may require the evaluation of potential pathogen viability, growth, and migration.
Sediments	Changing runoff patterns and more intense precipitation events will alter sediment transport by potentially increasing erosion and runoff.
Temperature	Warming water temperatures from warming air temperatures may directly threaten the thermal tolerances of temperature-sensitive aquatic life and result in the emergence of harmful algal blooms (HABs), invasion of exotic species, and habitat alteration.
Nutrients	Warming temperatures may enhance the deleterious effects of nutrients by decreasing oxygen levels through eutrophication (hypoxia), intensified stratification, and extended growing seasons.
Chemical	Some pollutants (e.g., ammonia) are made more toxic by higher temperatures, and also by pH, which may change as a result of climate change.
Biological	Climate changes such as temperature increases may impact species distribution and population abundance, especially of sensitive and cold-water species in favor of warm-tolerant species including invasive species. This could have cascading effects throughout the ecosystem.
Flow	Changing flow patterns from altered precipitation regimes is projected to increase erosion, sediment and nutrient loads, pathogen transport, and stress infrastructure. Depending on region it is also projected to change flood patterns and/or drought and associated habitat disturbance.
Salinity	Sea level rise will inundate natural and manmade systems resulting in alteration and/or loss of coastal and estuarine wetland, decreased storm buffering capacity, greater shoreline erosion, and loss of habitat of high value aquatic resources such as coral reefs and barrier islands. Salt water intrusion may also affect groundwater.
pH	Ocean pH levels have risen from increased atmospheric CO_2, resulting in deleterious effects on calcium formation of marine organisms and dependent communities and may also reverse calcification of coral skeletons.

Climate change effects that contribute to degradation of water quality and biological resource condition bring into question how antidegradation policies can be managed considering the additional influences of climate change. High quality water bodies may be most vulnerable to climate change degradation, making application of antidegradation policies in vulnerable water bodies important. Management approaches and special considerations for implementation of antidegradation policies may need attention. In addition, the application of use attainability analyses (UAA) on vulnerable water bodies may be pertinent for characterizing climate change effects.

7. CONCLUSIONS

Climate change will affect many of the components of bioassessment programs, including assessment design, implementation, and environmental management. Implementing the recommendations derived from the results in this study can improve the resilience of bioassessment programs and ensure that management goals can be met under changing climatic conditions. These steps can help manage the risks associated with not meeting goals, even though the magnitude and timing of climate change effects on aquatic resources is uncertain.

There are four main sets of recommendations from this study specific to adaptations of biomonitoring programs:

1. Multi-metric indices should be revised to reflect the sensitivity of taxa and trait groups to climate change effects; predictive models should also reflect these changes in indicators and periodically revise the expected community composition used in the analysis. At present, the most accessible information relates to temperature sensitivities and preferences; however, sensitivities to changing hydrologic conditions should be pursued in the future.
2. A monitoring network to detect climate change effects should be set up, at least for the most climate-vulnerable regions. This network will need to be more comprehensive spatially and sampled more frequently than current bioassessment sites. Detecting climate change at these monitoring sites requires that they are protected from other stressors.
3. Abiotic data needs to be collected more frequently and at more sites; a monitoring network to detect climate change effects should incorporate abiotic data collection as well, including water temperature and flow. The value of better water temperature and flow data is great, and consideration should be given to deploying *in situ* temperature and flow meters.
4. TMDLs and water quality standards should be examined to ensure that these remain protective of aquatic life uses under changing climatic conditions.

We have some additional recommendations for further study and collaboration that would enhance our ability to track climate change effects and separate these from other stressor responses in the context of biomonitoring:

1. The use of thermal-preference metrics for detecting climate-related trends should be further explored. Monitoring of thermal-preference metrics will increase the probability of detecting community responses to warming trends and reduce the likelihood that they will be obscured by taxonomic variability.
2. The listing lists of cold- and warm-water-preference taxa developed in this study should be refined and extended to more states and regions. Refinements can be made by using continuous water-temperature data instead of instantaneous water-temperature data, by calculating propensity scores to help improve the robustness of the analyses (Yuan 2010), and by using species-level OTUs for genera in which differences in which species-level thermal preferences are known to occur.
3. Continue to further our knowledge of traits and how they relate to climate change. More information is needed about which traits are most important in the context of climate change, the influence of each trait on an organism's ability to adapt, and which combinations of traits are most adaptive to particular environmental conditions (Stamp et al. 2010). A key component of furthering the traits-based framework will be expansion and unification of existing trait databases (Statzner and Beche, 2010).

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APPENDIX A

Basic Evidence for Climate Change Effects: Long-term Trends in Annual Air Temperature, Precipitation, and Water temperatures

There is reasonable evidence of climate change effects on both terrestrial and aquatic biological assemblages at various levels, including changes in ecosystem process, community composition, phenology of populations, number of reproductive cycles, evolutionary adaptations, and genetic selection (e.g., Parmesan and Galbraith, 2004; Root et al., 2003; Poff et al., 2002; Walther et al., 2002). More recently, there are also documented responses in freshwater ecosystems (Chessman, 2009; Buisson et al., 2008, Hiddink and Hofstede, 2008; Collier, 2008; Durance and Ormerod, 2007; Daufresne and Boet, 2007).

Stream water temperature regimes will be altered by air temperature increases and modified by other influences (Cassie et al., 2006; Mohseni et al., 2003; Daufresne et al., 2003; Hawkins et al., 1997). Temperature regimes determine the distribution and abundance of aquatic species through temperature tolerances and evolutionary adaptations, along with competitive interactions, effects on food supply, and other factors (e.g., Matthews, 1998; Hawkins et al., 1997; Vannote and Sweeney, 1980; Sweeney and Vannote, 1978). Changes in prevailing temperature regime, as well as climate change-associated increases in variability of temperature, may have various biological effects.

Evidence for climate change effects can be pursued within both abiotic (e.g., temperature, precipitation, flow) and biotic components of the environment (Figure A-1). Examples from each category (climate change projections, stream changes, ecological responses) were examined for existing evidence of climate change effects.

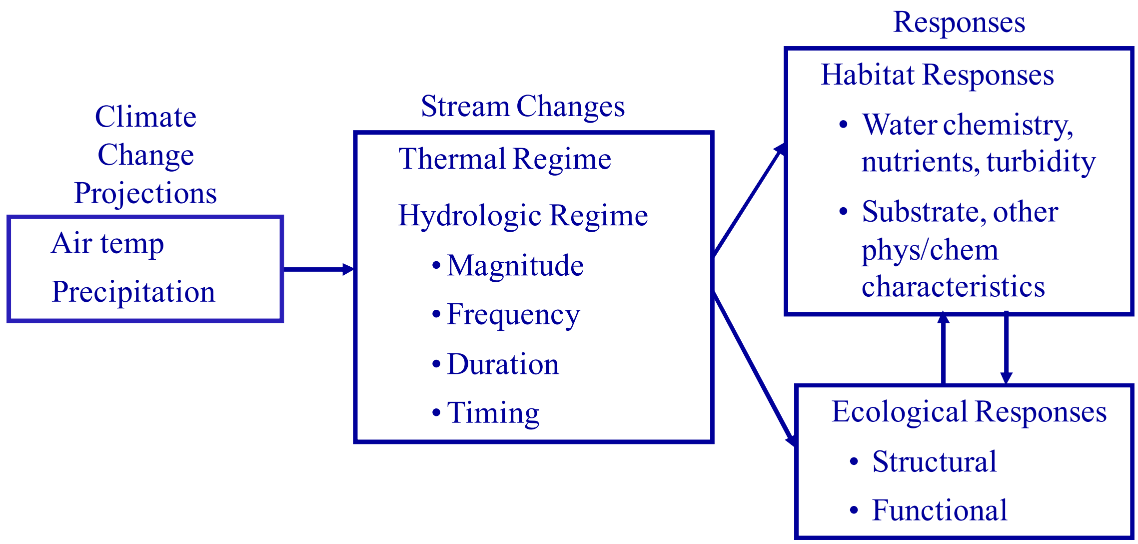


Figure A-1. Mechanisms linking climate changes with streams.

A.1 Trends and Variability in Annual Air Temperature and Precipitation using PRISM

One way to detect whether climatic changes have occurred in a region that could have implications for aquatic organisms is to examine air temperatures. There is a general correspondence between air and stream temperatures, though the magnitude and seasonal patterns of changes in stream water temperatures are likely to vary regionally, due to factors including water source influences, watershed characteristics, and season (Caissie, 2006; Daufresne et al., 2003). Stephan and Preudhomme (1993) estimated a linear relationship (factor of 0.86 in °C) between weekly average water and air temperatures for eleven streams in the Mississippi River Basin; and a similar linear relationship has been applied by others (e.g., Eaton and Scheller, 1996). However, Mohseni et al. (2003) suggest the relationship between air and water temperatures is better explained by an S-curve, such that at higher air temperatures, stream temperature increases level off due to evaporative cooling.

Below are plots of Parameter-elevation Regressions on Independent Slopes Model (PRISM) (PRISM Climate Group, Oregon State University, Corvallis, Oregon; http://www.prismclimate.org, data) mean annual air temperature values[1] at biological sampling sites in each of the three states examined (Maine, Utah, and North Carolina). PRISM uses a

[1] maximum and minimum air temperature values were averaged to derive what we refer to as mean annual air temperature.

digital elevation model and point measurements of climate data to generate estimates of annual, monthly, and event-based climatic elements.

Annual air temperatures in these states have increased gradually from 1974 and 2006. Stations in Utah showed the strongest trend and experienced the greatest increase in air temperature (about 2 ^{o}C, r^2=0.42) (Figure A-1b), with Maine and North Carolina showing weaker trends (about 1 ^{o}C, r^2=0.15 and r^2=0.11, respectively) (Figure A-2a and A-2c). Absolute air temperatures within each state differed by ecoregion, but the change in air temperatures (e.g., the increasing trends) were similar across ecoregions. Maine had the greatest difference, where the Northeastern Highlands ecoregion had a stronger upward trend than the other two ecoregions (r^2=0.23 versus r^2=0.12 and 0.13) (Figure A-2a). This ecoregion is at a higher elevation than the other two as well.

Plots of annual precipitation patterns from PRISM data are also displayed below. Trends in those patterns were highly variable and were not significantly correlated with year in any of the states (r^2 values ranging from 0.004 to .01; see figure A-3). The amount of annual precipitation across ecoregions within a state often varied quite a bit. For instance, the mountain ecoregions in both Utah and North Carolina had higher annual precipitation than the plateau or coastal regions (true also for Maine, but to a lesser extent) (Figure A-4). However, mean annual precipitation values in all ecoregions were highly variable over the 30 years analyzed (Figure A-5).

From 1974 to 2006, fluctuations between years in temperature and precipitation have also been highly variable. However, in Utah, the differences between consecutive years (i.e., current year minus previous year) in both air temperature and precipitation have declined. Precipitation showed a stronger trend in this than temperature (r^2=0.12 temperature, r^2=0.28 precipitation) (Figures A-6b and A-7b). Unlike Utah, the trends in inter-annual climate variability in Maine and North Carolina showed almost no trend for both annual air temperature and precipitation.

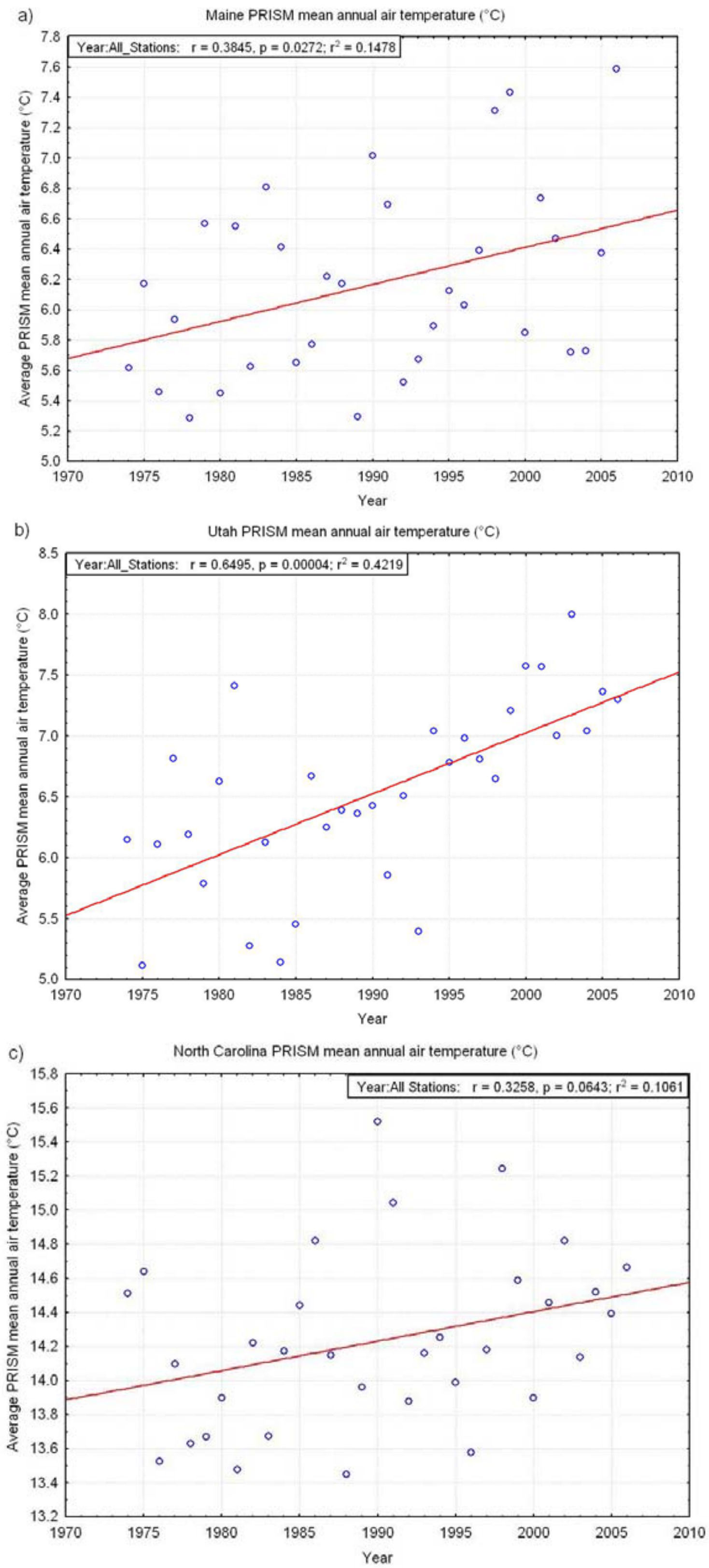
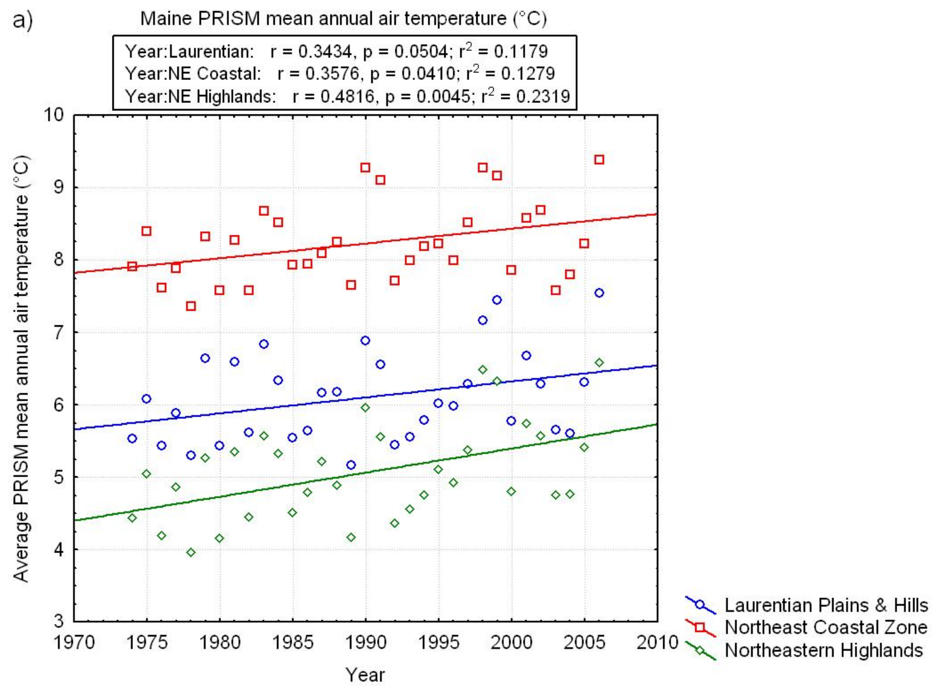
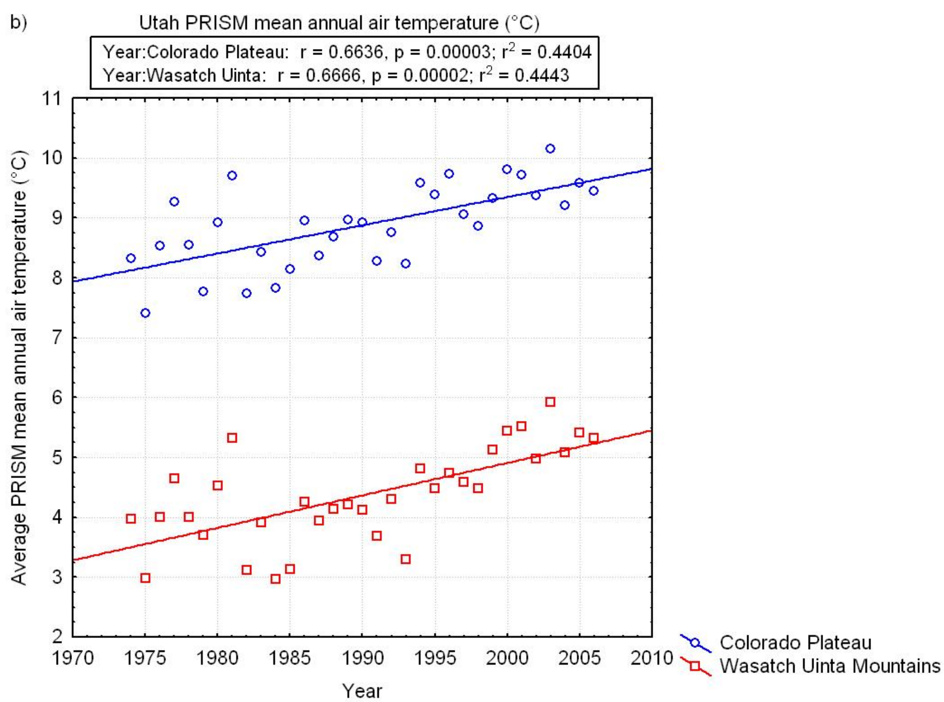


Figure A-2. Plots of PRISM mean annual air temperature (°C) values (averaged across all stations) for Maine (a), Utah (b) and North Carolina (c).


a)
Maine PRISM mean annual air temperature (°C)
Year:Laurentian: r = 0.3434, p = 0.0504; r2 = 0.1179
Year:NE Coastal: r = 0.3576, p = 0.0410; r2 = 0.1279
Year:NE Highlands: r = 0.4816, p = 0.0045; r2 = 0.2319
Average PRISM mean annual air temperature (°C)
Year
Laurentian Plains & Hills
Northeast Coastal Zone
Northeastern Highlands


b)
Utah PRISM mean annual air temperature (°C)
Year:Colorado Plateau: r = 0.6636, p = 0.00003; r2 = 0.4404
Year:Wasatch Uinta: r = 0.6666, p = 0.00002; r2 = 0.4443
Average PRISM mean annual air temperature (°C)
Year
Colorado Plateau
Wasatch Uinta Mountains

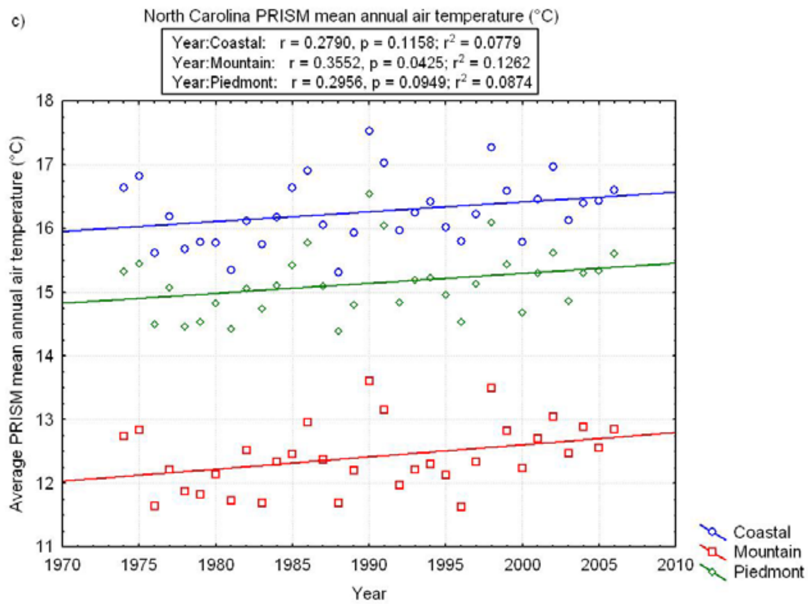


Figure A-3. Plots of PRISM mean annual air temperature (°C) values (averaged across each major ecoregion) for Maine (a), Utah (b) and North Carolina (c).

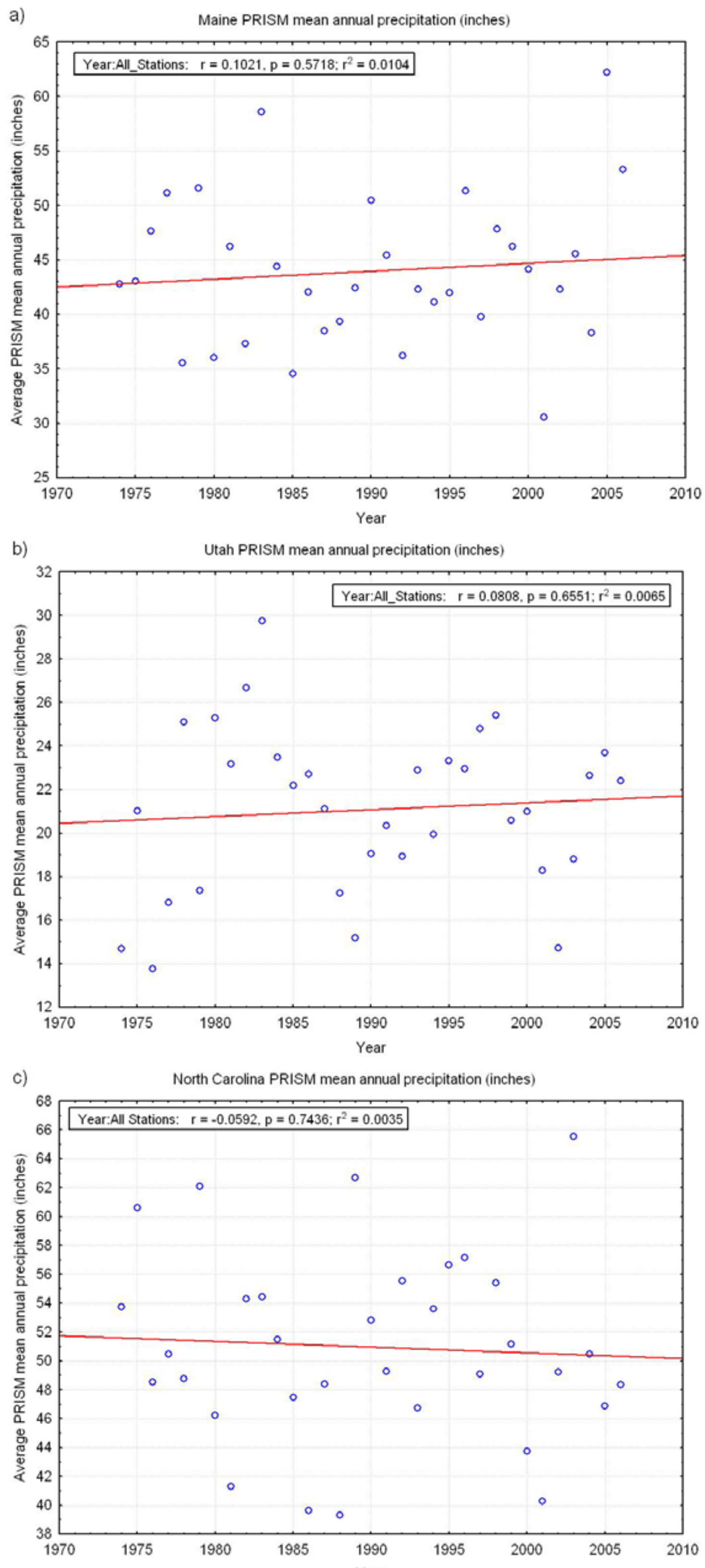
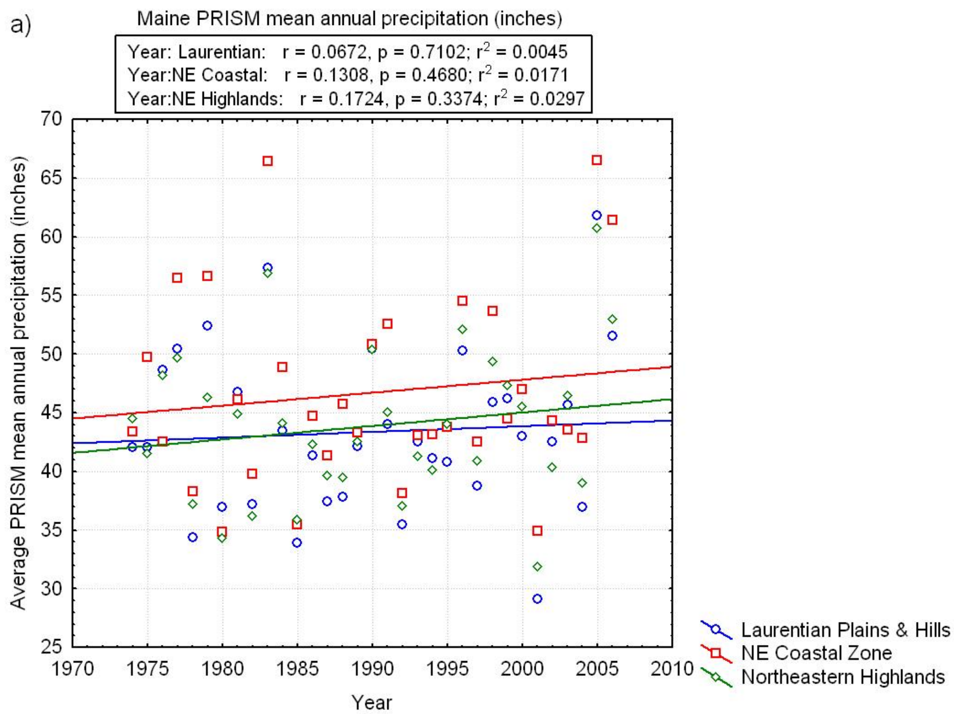
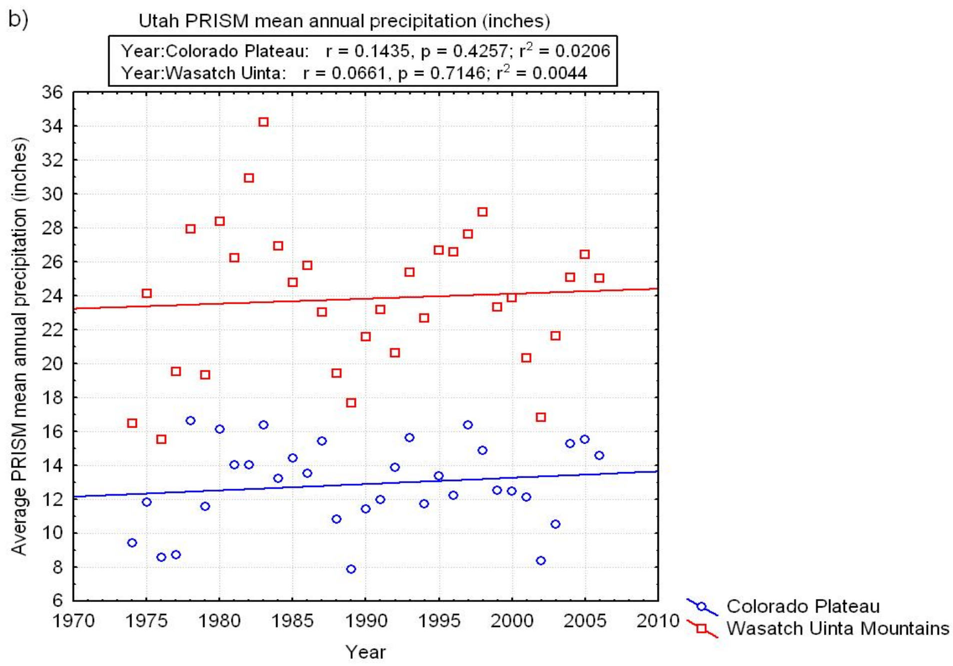


Figure A-4. Plots of PRISM mean annual precipitation (inches) values (averaged across all stations) for Maine (a), Utah (b)_and North Carolina (c).


a)
Maine PRISM mean annual precipitation (inches)
Year: Laurentian: r = 0.0672, p = 0.7102; r2 = 0.0045
Year:NE Coastal: r = 0.1308, p = 0.4680; r2 = 0.0171
Year:NE Highlands: r = 0.1724, p = 0.3374; r2 = 0.0297
Average PRISM mean annual precipitation (inches)
Year
Laurentian Plains & Hills
NE Coastal Zone
Northeastern Highlands


b)
Utah PRISM mean annual precipitation (inches)
Year:Colorado Plateau: r = 0.1435, p = 0.4257; r2 = 0.0206
Year:Wasatch Uinta: r = 0.0661, p = 0.7146; r2 = 0.0044
Average PRISM mean annual precipitation (inches)
Year
Colorado Plateau
Wasatch Uinta Mountains

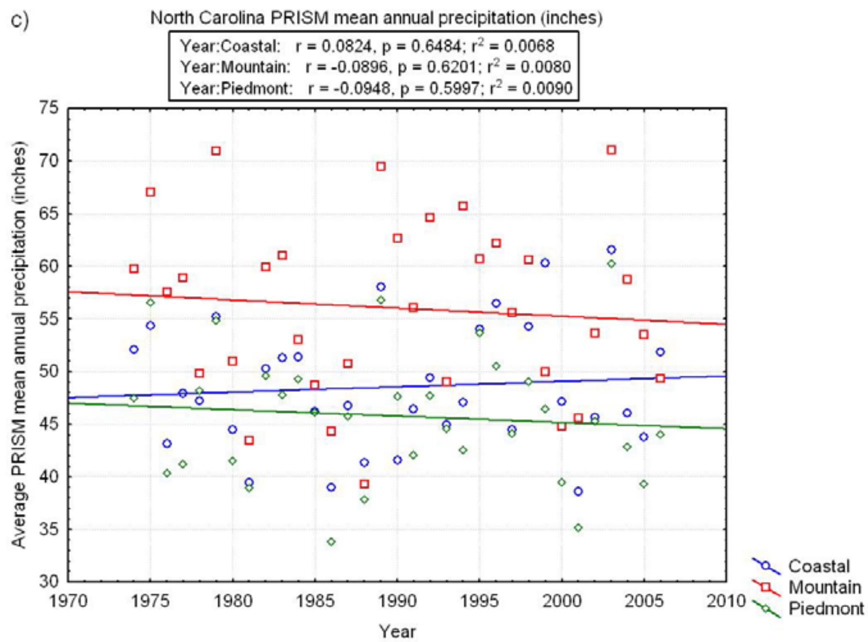


Figure A-5. Plots of PRISM mean annual precipitation (inches) values (averaged across each major ecoregion) for Maine (a), Utah (b) and North Carolina (c).

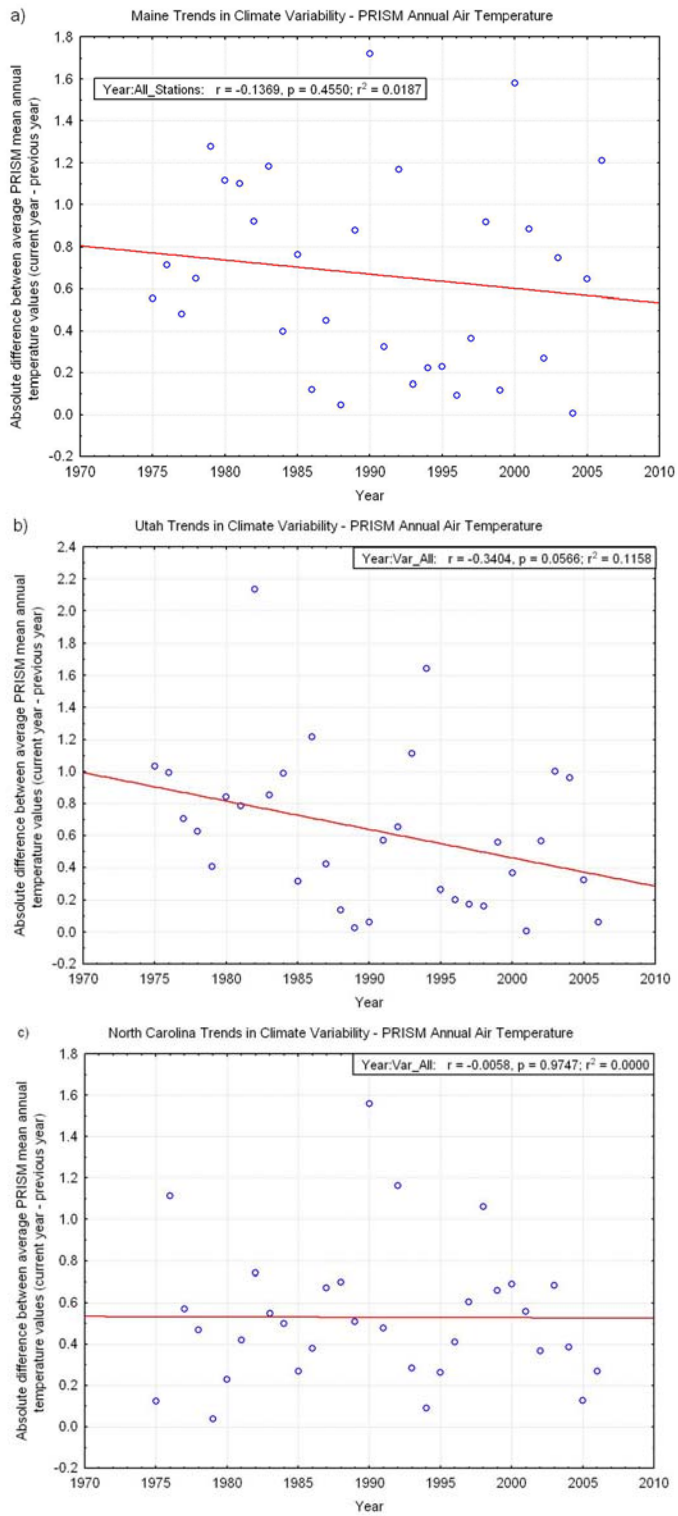


Figure A-6. Trends in Climate Variability - PRISM Annual Air Temperature. Values represent the absolute difference between average PRISM mean annual temperature values (current year - previous year) for all stations in Maine (a), Utah (b) and North Carolina (c).

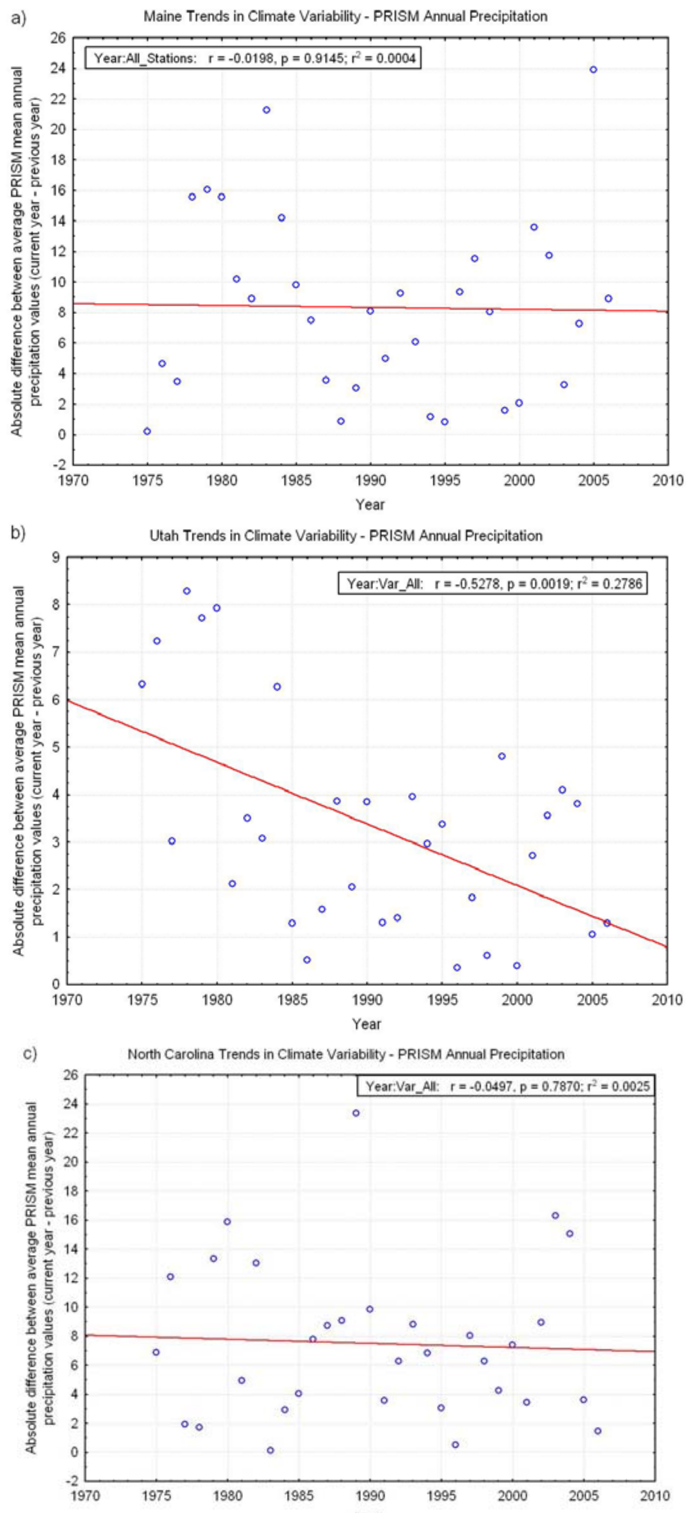


Figure A-7. Trends in Climate Variability - PRISM Annual Precipitation. Values represent the absolute difference between average PRISM mean annual precipitation values (current year - previous year) for all stations in Maine (a), Utah (b) and North Carolina (c).

A.2. Long-term water temperature trends at USGS gage stations

Data from USGS gages with long-term water temperature records (30 years) were compiled. Initially a screening process, outlined in Figure A-8, was applied to minimize the likelihood of confounding effects (e.g., sewage treatment plant discharges, heavy urban/suburban development, effects of dam releases), or temporal discontinuities from methods or data quality issues. However, screening criteria had to be relaxed in certain regions because stations were not meeting all the criteria. To expand stream site coverage nationwide, sites that did not meet all of these criteria had to be added to the list (i.e. Colorado River sites were used even though they are higher order and have dams, but this was the best data available for this region). Data were downloaded from the USGS real-time water data website: http://waterdata.usgs.gov/nwis/rt. About 25 stations were evaluated for trends. Plots of seasonal means, minimum, and maximum temperatures were developed to partition seasonal variation when checking for long-term patterns. Summer temperatures generally showed greater trends, and were used to evaluate rates of temperature change per 10-year period at 23 of the stations (Table A-1).

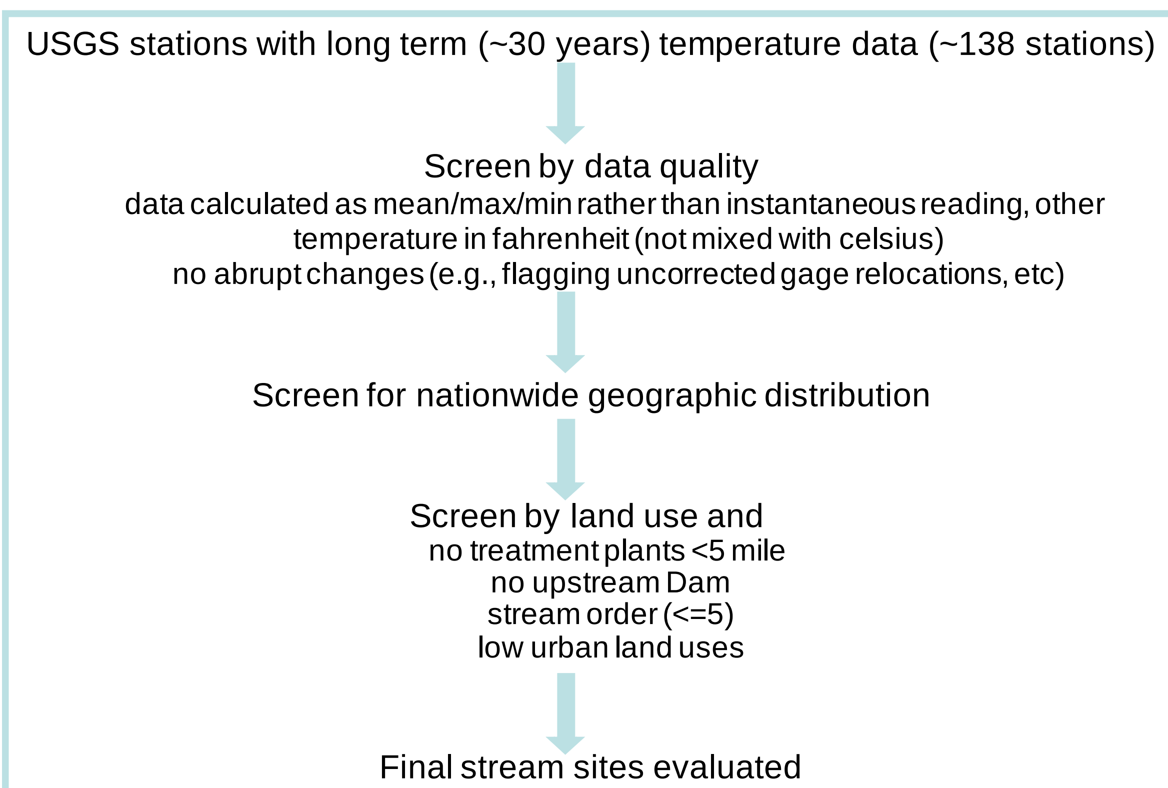


Figure A-8. Flow chart showing the screening process that was followed when determining which USGS stations to use in the water temperature trend analyses.

Table A-1. Summary of results from water temperature trend analyses at 23 USGS stations that met the screening criteria. Rates of temperature (°C) change per 10-year period were evaluated at 23 of the stations.

Site #	Stream Name	Stream Order	NPDES	Land Use	State	TempΔ/ 10year	R^2
2423130	Cahaba River	3	no	FOR/AG (URB)	AL	0.73	0.024
10339400	Martis Creek	3	no	FOR	CA	0.28	0.02
7086000	Cache Creek	2	no	FOR	CO	1.48	0.151
9169500	Dolores River	5	no		CO	0.93	0.05
2266300	Reedy Creek	3	no	URB	FL	0.3	0.081
5474000	Skunk River	6	no	FOR	IA	0.25	0.006
13340600	Beaver Creek	4	no		ID	0.4	0.032
3354000	White River	5	no	AG	IN	0.32	0.017
1600000	North Branch Potomac River	5	no		MD	0.5	0.013
1021050	Saint Croix River	6	no	URB/FOR	ME	0.39	0.02
12363000	Flathead River	6	no	AG (URB)	MT	1.36	0.17
2077200	Hyco Creek	3	no	FOR	NC	0.7	0.192
6338490	Missouri River	1	no	GRASSLAND	ND	5.09	0.508
5056000	Sheyenne River	4	no	GRASSLAND	ND	0.41	0.013
5058700	Sheyenne River	1	no	GRASSLAND	ND	0.43	0.018
1466500	McDonalds Branch	1	no	FOR	NJ	0.33	0.03

1428500	Delaware River	6	no	FOR	NY	0.42	0.019
14138870	Fir Creek	2	no		OR	0.38	0.059
14372300	Rogue River	6	no	FOR	OR	0.16	0.011
2160700	Enoree River	5	no	FOR (urb)	SC	0.5	0.04
8123800	Beals Creek	5	no	Shrub	TX	0.46	0.018
8181500	Medina River	5	no	AG	TX	0.7	0.095
408000000	Middle Branch Embarrass River	3	no	AG	WI	0.96	0.03

Stations in Utah, Maine and North Carolina were of particular interest because biological data from these states were analyzed for climate change effects. Summer temperatures have increased gradually at stations in each of these three states (Figures A-9 to A-11). Hyco Creek in North Carolina has shown the greatest increase in daily maximum temperature (from about 23 to 25°C over about a 40 year period, r^2=0.192). This may be influenced by stream size, as Hyco Creek is a 3rd order stream, while the St. Croix in Maine and the Colorado in Utah are 6th order or higher.

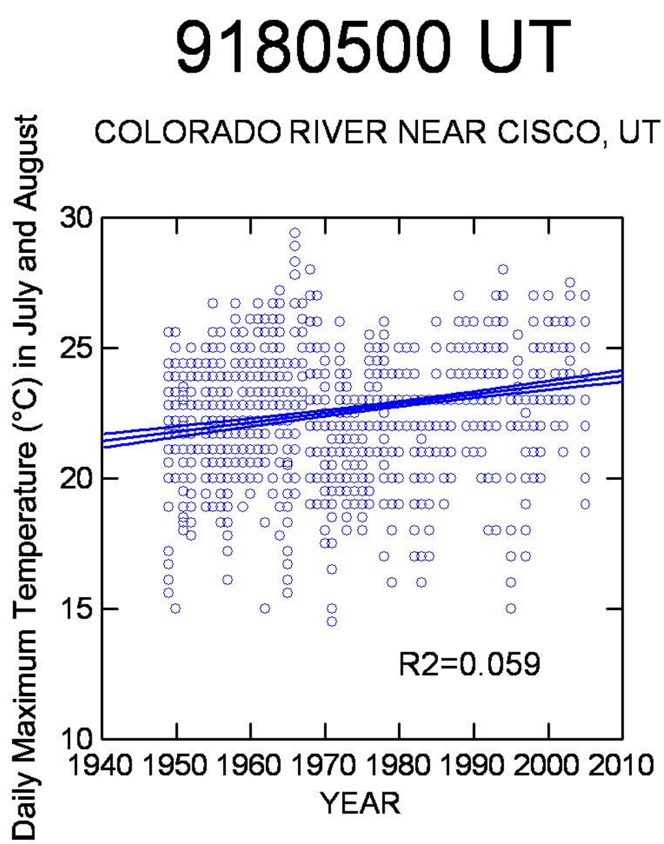


Figure A-9. Summary of daily maximum temperature trends for July and August data from USGS Gage 9180500 on the Colorado River near Cisco, Utah.

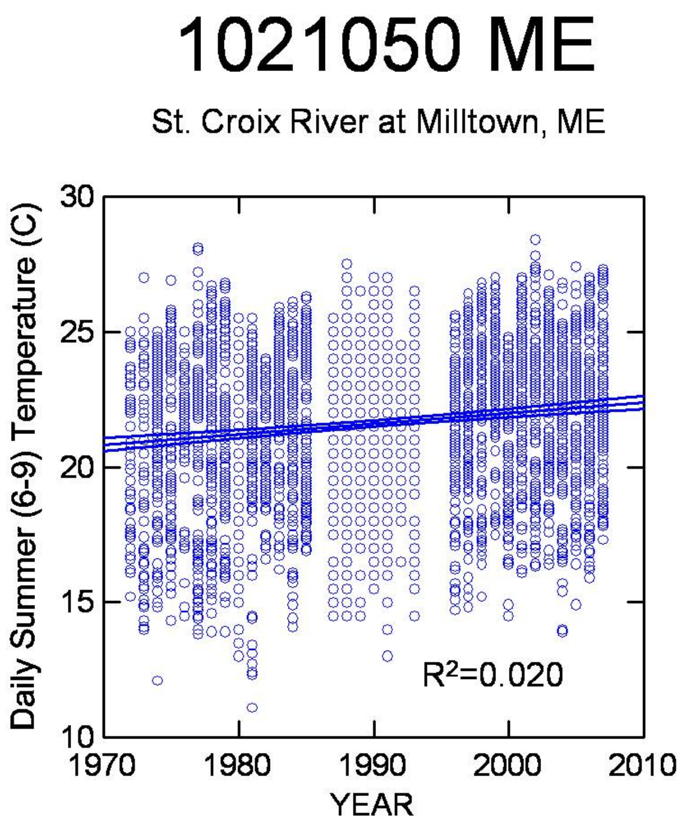


Figure A-10. Summary of daily maximum temperature trend for summer data from USGS Gage 1021050 on the St. Croix River at Milltown, Maine.

Around the country, temperature responses are quite variable, though long-term increasing water temperature trends are observable in many rivers and streams (Table A-1). Rates of temperature change per 10-year period for 23 stations in 18 different states range from 5.09 degrees in 10 years in a 1st order reach of the Missouri River, North Dakota ($r^2 = 0.5$), to 0.25 degrees in 10 years in a 6th order reach of the Skunk River, Iowa ($r^2 = 0.006$). Results varied across stations. The average rate of increase per 10-year period was 0.76 degrees. Similar increases in stream and river water temperatures over recent decades have been documented across the US (Kaushal et al. 2010) and in Europe (e.g., Webb and Nobilis (2007).

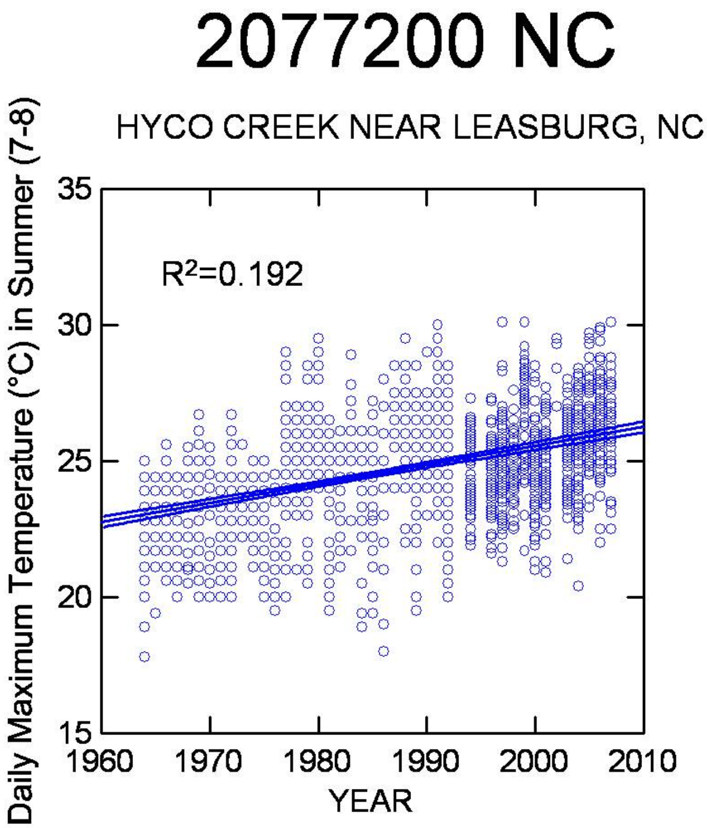


Figure A-11. Summary of daily maximum temperature trend for summer data from USGS Gage 2077200 on Hyco Creek, North Carolina.

A.3 Benthic Macroinvertebrate Inferred Temperature

Annual water temperature values for selected sites were inferred based on relative abundance and temperature optima data for macroinvertebrate taxa that occurred at each site. The temperature optima values used in these calculations were derived from weighted averaging or maximum likelihood modeling on appropriate subsets of the state biomonitoring data (Appendix D). The "benthic inferred temperature" for a station is then calculated as another weighted average, taking model results of temperature optima for each taxon occurring at a station, multiplied by the abundance of that taxon, with those products summed over all taxa at the station, and divided by the sum of taxa abundances. Questions that were addressed using this approach include whether benthic communities reflect water temperatures at the time of collection; and whether long-term changes in inferred temperatures provide evidence of benthic community changes over time related to temperature.

Most of the long-term stations within ecoregions that were tested showed slight to distinct increasing trends in benthic inferred temperatures over time, though not all the trends were significant. In Maine, inferred temperatures for Station 56817, a long-term but low elevation station in the Laurentian Hill and Plains ecoregion, showed a gradual upward trend since 1984 (Figure A-12). A steeper upward trend was evident at the selected Maine East Coast region reference sites, which included some higher elevation locations (Northeast Highlands ecoregion) (Figure A-13). There is no real pattern for the group of relatively low elevation sites in the Maine Central Interior biophysical region (Figure A-14). The greater inferred temperature responses are evidence of climate change increases in temperature, with greater apparent responsiveness in higher elevation locations. This is consistent with findings of greater climate change effects at higher elevation areas based on other biologic metrics (Section 2).

The plot of inferred temperatures for multiple stations across all three ecoregions in North Carolina (excluding the coastal plain) showed a gradual temperature increase since 1994 (Figure A-15), though the trend with year was not significant. The benthic inferred temperature trend at three reference stations in Utah (sampled in October-November) showed a gradual, but statistically significant, increase (Figure A-16). The rate of increase is equivalent to approximately 3^{o} C in 25 years. In the plots in which multiple sites were grouped together, site-specific differences were often evident. In all of these cases, the close relationship between the benthic inferred temperatures and the field-measured water temperatures shows that the approach of using benthic invertebrate occurrence and abundance coupled with temperature preferences is a reliable means of estimating water temperature at the time of collection. More importantly, it provides evidence of benthic community changes over time related to long-term changes in temperature.

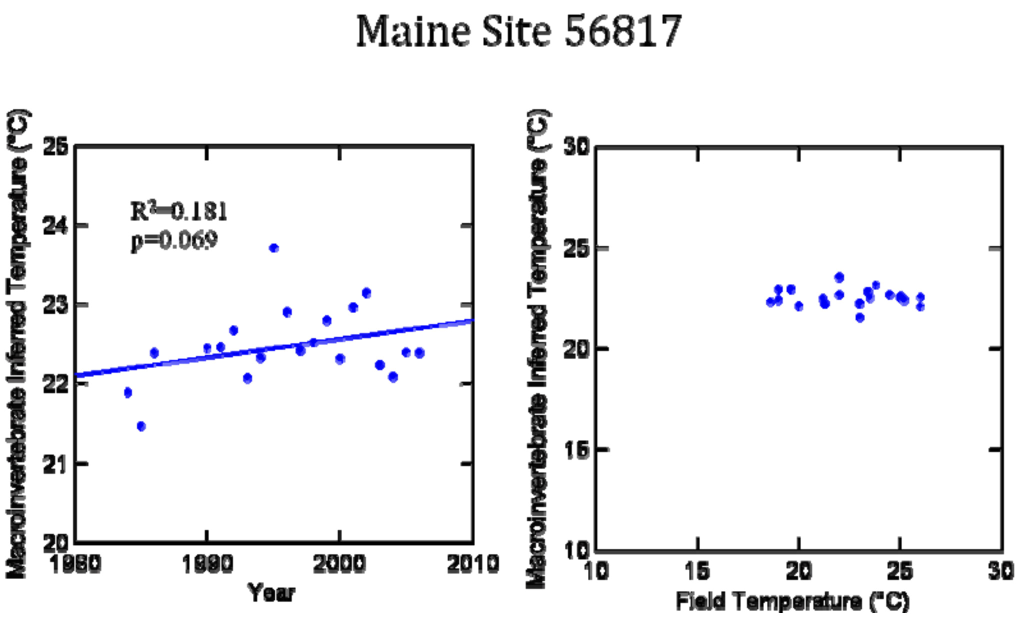


Figure A-12. Benthic macroinvertebrate inferred temperature trend for Maine Site 56817.

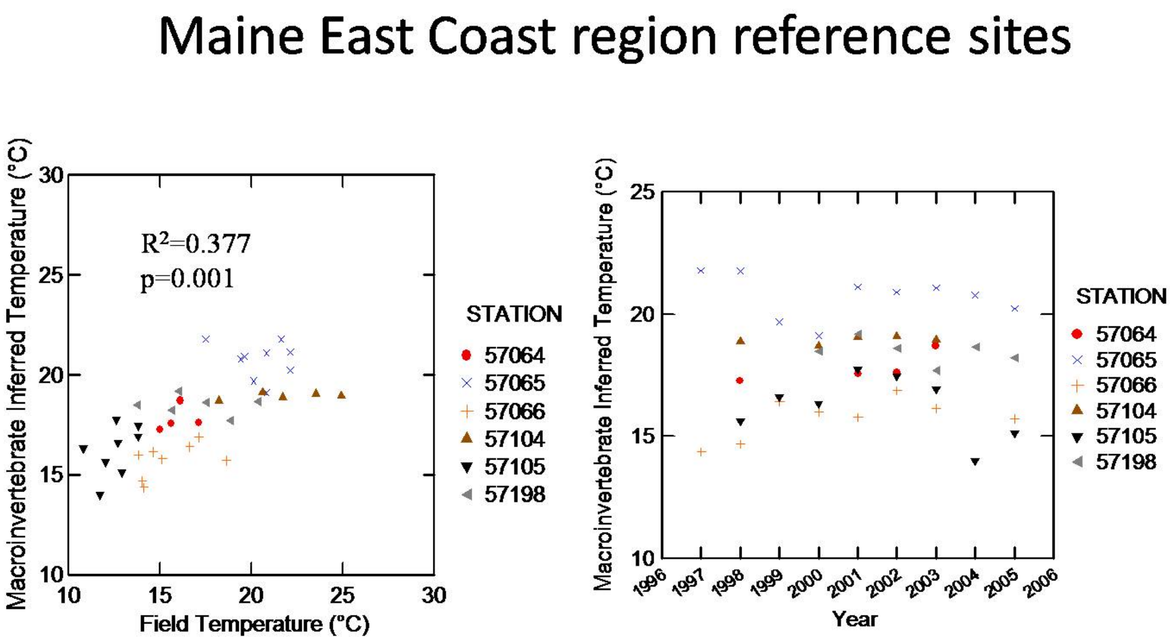


Figure A-13. Benthic macroinvertebrate inferred temperature trend for selected reference sites in the Maine East Coast region.

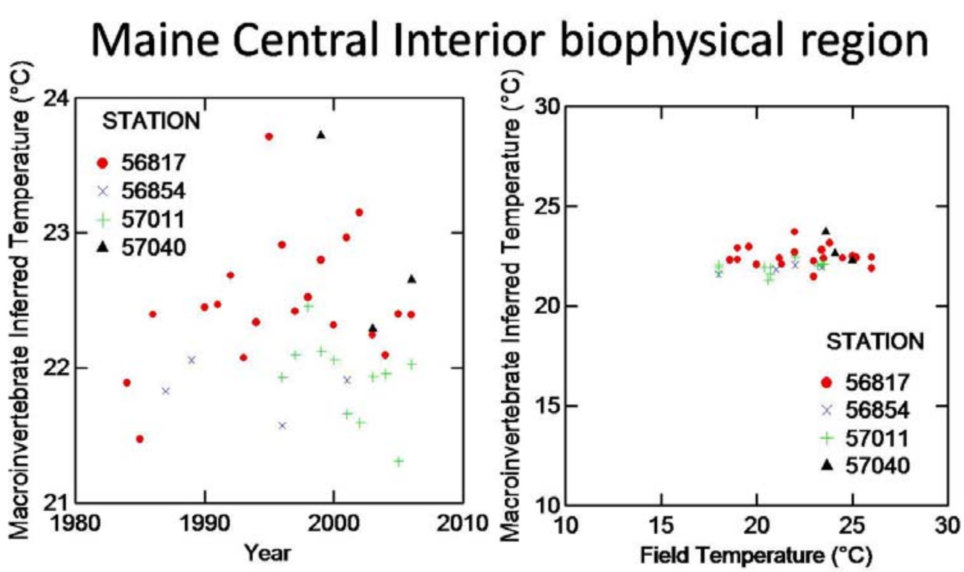


Figure A-14. Benthic macroinvertebrate inferred temperature trend for selected sites in the Maine Central Interior biophysical region. Note that Station 57040 has a statutory class of AA but its use in this analysis is questionable because of its proximity to a Superfund site.

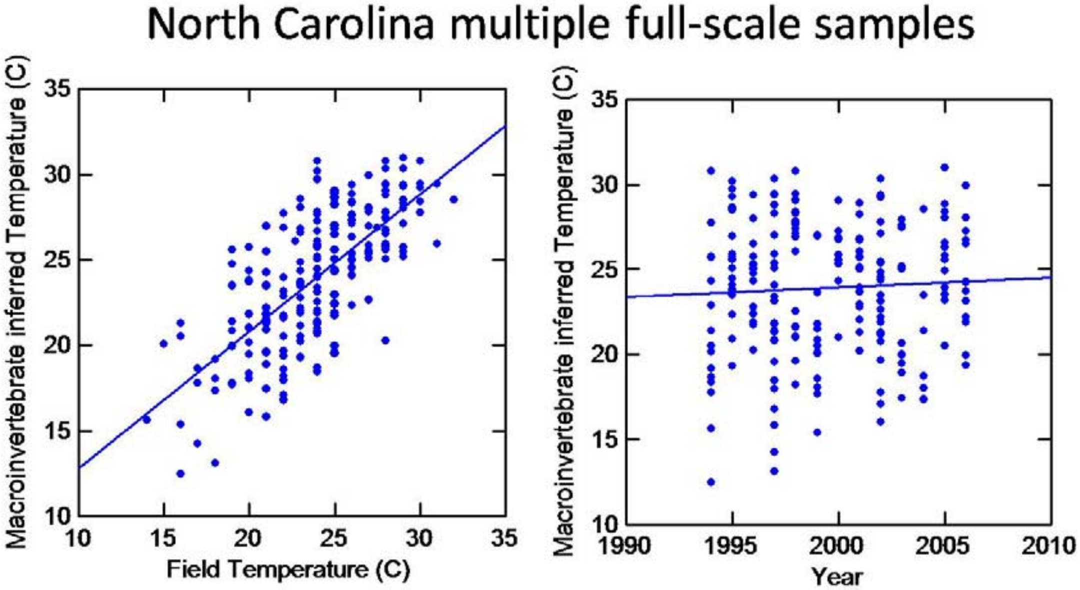


Figure A-15. Temperature inference model for multiple full-scale samples in North Carolina.

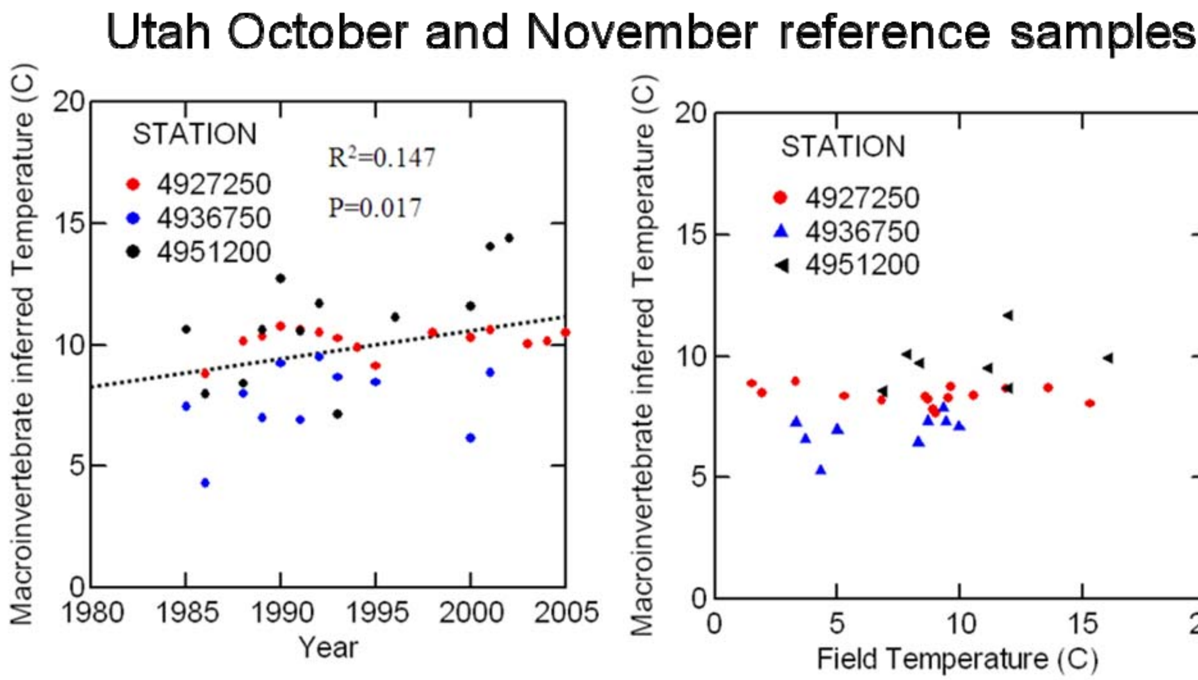


Figure A-16. Benthic macroinvertebrate inferred temperature trend for selected reference sites in Utah. Only samples collected in October and November were used in these calculations.

APPENDIX B

Data preparation and management

The purpose of this appendix is to provide detailed information on how the state databases were selected, what collection and assessment methods are used by each of the states, and how the data for each of the states were prepared for analysis.

B1. Selection of the Four State Databases: Maine, North Carolina, Ohio and Utah

Four state benthic macroinvertebrate and/or fish databases were selected for the regional climate change pilot studies. Overall criteria for selection of existing state data sets were to include representatives of a distribution of regions around the country that would reflect different climatic, geographic, and ecological zones, as well as different ranges of future climate change projections. We considered state programs that have been well-established for the longest times and would have long-term data bases with consistent methods and strong quality control (QC). Additional rationale for the final selections include:

Maine. Maine has a benthic macroinvertebrate dataset that is long-term with consistent methods, and with repeat sampling at some locations (i.e. one site has over 20 years of data). It is in an area with regional climate change modeling and is expected to show sensitive responses to climate change given its northerly location.

North Carolina. North Carolina captures the unique expectations for climate responses in the southeastern region. The North Carolina invertebrate data set is long-term, with consistent methods and good quality control (QC).

Utah. Both the Utah and New Mexico datasets were strongly considered for analyses in the western/southwestern region of the country. Utah was selected because it had more long-term repeat sampling (up to 19 years of data over a 21 year time span) and had a better distribution of sampling locations. A shortcoming of the Utah data was that (unlike the New Mexico data) most of the historic data set (i.e., older than about the last 8 years) had only recently been entered into an electronic format from hard-copy data sheets and had not been QC'd or previously analyzed as a unified long-term data set.

Ohio. Both the Ohio and Wisconsin datasets were strongly considered for analyses in the Midwestern region of the country because they both have long-term fish data in addition to benthic invertebrate data. Wisconsin is expected to show shifts between cold- and cool- or warm-water fauna. However, it was not clear that collection and reporting methods for the Wisconsin fish data were standardized. Because Ohio has a long-term fisheries dataset with standard methods, and had long-term benthic data as well that was already being analyzed for long-term trends, it was selected.

B2. State Collection Methods

Each of the four states uses different benthic macroinvertebrate collection methods. Utah collects a quantitative sample from riffle habitats during a September/October index period using the EMAP kick method (note: prior to 2006, samples were collected using the Hess method). Maine uses artificial substrates (rock bags or baskets) to collect quantitative samples during late summer, low flow periods (July 1 to September 30). North Carolina uses several different collection methods and collects samples throughout the year, but for this study the focus was on summer (June through September) samples collected using the standard qualitative ('full-scale') method, which is comprised of 2 kicks, 3 sweeps, 1 leaf pack sample, 2 fine mesh rock and/or log wash samples, 1 sand sample and visual collections. Ohio collects quantitative macroinvertebrate samples using a modified multiple-plate Hester Dendy artificial substrate sampler. A routine sample consists of a composite of five samplers that are colonized for a 6 week period that normally falls between June 15 and September 30. In addition to the artificial substrate, a qualitative sample is taken from all available natural habitats within the reach. When sampling for fish, Ohio uses pulsed direct current electrofishing techniques. Depending on stream size, crews either use headwater, wading or boat site protocols.

B3. Database Preparation

Biomonitoring data from Maine, North Carolina, Utah and Ohio were compiled into Ecological Data Application System (EDAS) databases, which are custom database applications that are used with MS Access. The data from Maine were taken from Maine's existing Oracle/Access database (EGAD). North Carolina data were provided in various formats (MS Excel and MS Access). Data for Utah were obtained from STORET. For Ohio, data were originally obtained from STORET; however, interactions with Ohio EPA revealed that data generation, data base development and management, as well as ongoing analyses for Ohio are conducted by Ed Rankin and Chris Yoder of Midwest Biodiversity Institute (MBI). Therefore, the additional data manipulation and analyses needed for this study were conducted by MBI under subcontract to Tetra Tech.

B3.1 Data screening

Data were screened to minimize chances of detecting false trends. Preliminary iterative data summary and screening procedures included:

1. Tabulating numbers of samples by station (e.g., station name, station ID number, and/or sample ID number) and date. Results were examined for consistent number of samples by station/date and for breaks in sample collection at stations across years. Problems discovered through this approach included changes over time in collection and/or reporting of replicates; and errors or changes in station naming that resulted in data for the same location appearing under different station names. It also helped identify locations with long-term data records.
2. Tabulating total abundance and total number of taxa by station and collection date. Results were examined for discontinuities in magnitude or trends in values between stations and across dates. Problems discovered through this approach included changes in reporting of abundances (e.g., from number per sample to number per square meter; whether replicates were averaged, summed, or reported separately); and changes or errors in whether sub-sampling was applied during sample analysis and how it was accounted for in the data.
3. Tabulating taxa (at the lowest levels reported) by collection date. For these, either taxa abundance or occurrence was tabulated, and these were either averaged over all stations within the state, or within each ecoregion and/or other appropriate subset (e.g., river basin or watershed). For this purpose, the tabulations of taxa were placed in phylogenetic order, and some higher-level phylogenetic structure (e.g., order and family names, or others as needed) was included for reference. Results were examined for many types of patterns, including:
    a. changes in taxonomic naming over time (e.g., changes in genus or higher level names, changes in placement within families). This not only revealed changes in systematics over time, but also caught changes in taxonomists and/or labs used to analyze samples.

   b. changes in level of attribution over time (e.g., increasing use of species names in recent years where individuals were typically left at the genus or family level in earlier samples);
   c. changes in other types of naming conventions (e.g., changes in level of placement for taxa such as water mites).

   Problems identified through these procedures included extensive changes in taxonomic knowledge and systematics over the decades of sample analysis. For illustration, one example is changes in the mayfly genus *Ephemerella*, including changes in the inclusion of various species of *Ephemerella* between *Ephemerella* and *Drunella*. In addition, we found many instances of changes in the higher-level groups under which various taxa would be reported, so that in the data base the same genus (or species, or family) would appear in more than one place. The effect of this was that these would act like separate taxa when a taxa ID name or number was invoked for trend analysis. Many associated corrections were applied to the phylogenetic structuring and naming conventions in the data bases. In many cases, changes in taxonomic naming of genera and/or species, or greater prevalence of species identifications in recent years, required standards to be set for summing species back to the genus level (or similar procedures at other levels), or for combining two or more genera that cannot always be reliably separated. This type of correction falls into the category of developing ‘Operational Taxonomic Units” (OTUs), and is discussed in more detail below.

4. Tabulations of station descriptive data, to identify reference locations and any data documented in support of reference station status.
5. Tabulations of ‘ancillary’ environmental data, such as temperature, water chemistry, substrate characteristics, habitat characteristics, by station over time. These results were compared for concordance with biological data.
6. Data also were screened for changes in sampling methods over time and/or between stations.

We used Non-metric Multidimensional Scaling (NMDS) to evaluated whether the database ‘fixes’, and in particular the taxonomic corrections and application of OTU rules, were effective in minimizing changes over time due to taxonomic identification procedures rather than actual community changes. NMDS is an ordination that takes the taxa in the samples and shows in ordination space how closely related the samples and stations are based on their species composition. Grouping variables (i.e. year, month, collection method, taxonomy lab, ecoregion, watershed, etc.) can be overlaid to look for trends. The NMDS ordinations were performed only on reference sites in order to eliminate differences due to other disturbances. The NMDS ordinations were run before and after generating genus level OTUs. Patterns were examined for distinct shifts that might indicate changes in taxonomists or labs during the sampling period of record, as well as ineffective OTU procedures (see results below).

B3.2 Development of operational taxonomic units (OTUs)

The intent of OTUs is to exclude ambiguous taxa from analyses (e.g., Cuffney et al., 2007) and include only distinct/unique taxa. Since a complete and correct master taxa list is required before OTUs can be established, the master taxa lists in each of the databases were first verified through several iterative procedures (see above). Next, three levels of OTUs were established: lowest taxonomic unit (generally species), genus and family. Rules were developed based on a general procedure of Remove Parent / Merge Children (RPMC) (retain the Child taxa (finer level of detail) and remove the Parent taxon or merge the Child taxa into the Parent taxon). According to Cuffney et al. (2007), this appears to be the most robust method for retaining taxa richness and abundance information for further analysis. All decisions were data set dependant. Rules were created on the dataset as a whole and then applied to individual samples prior to analysis. The last step in the process was to manually review the list of OTU designations and make final corrections where necessary.

B3.2 Utah

Data for Utah were obtained from STORET and compiled. The process was less efficient than originally hoped, in that data had to be gathered in sections by data type and pieced back together. This was largely due to limitations placed on data downloads from the STORET website. Jeff Ostermiller from the Utah Department of Environmental Quality (UT DEQ) was the contact person for data interactions.

Through examination of results in the Utah data base, it was determined that genus-level OTUs were most appropriate for the long-term analyses. One of the more noteworthy OTU ‘fixes’ that had to be made was that all midges had to be grouped to the family level (Chironomidae), as subfamily and/or genus level identifications only occurred in later years in the Utah data. As another example, changes also had to be made to OTU assignments for *Ephemerella* and *Drunella*.

To check for trends in the Utah dataset, pre- and post-OTU NMDS analyses were performed using the following grouping variables: taxonomy lab (pre- and post-1989), level 3 ecoregion, reference status, and HUC04 hydrologic basins. Trends related to latitude and longitude were also evaluated in reference status plots. An obvious trend appeared in the pre-OTU plot that used taxonomy lab as the grouping variable (**Figure B3-1a**). This was due to the change in taxonomy lab that occurred in 1989. The OTU sufficiently corrected for this change, as can be seen in the post-OTU taxonomy lab plot (**Figure B3-1b**). Results from the other NMDS ordinations can be found in **Figures B3-2–B3-6**.

Another issue that arose with the Utah data was that there was some uncertainty as to the consistency of how abundance data was recorded over the years. These questions related to whether the recorded abundances were corrected for subsampling in the laboratory, area sampled, and/or replication. These questions could not be fully resolved based on institutional knowledge of Utah DEQ scientists or from extant database metadata or other documentation. Because of this uncertainty, relative abundances were used in analyses. We also found that although Utah reports using a late-summer to fall index period for sample collection, the Utah database includes samples collected throughout the year. For most analyses, only fall samples were used to minimize variation associated with seasonal differences in taxonomic composition.

B3.4 Maine

Data for Maine were obtained from Maine’s Oracle/Access database (EGAD) as output in an Access database, and compiled. Susanne Meidel from the Maine Department of Environmental Protection (ME DEP) was the contact person for data interactions.

As for Utah, it was determined based on evaluation of the Maine data that genus-level OTUs were appropriate for the long-term analyses. To check for trends at the genus-level OTU in the Maine dataset, NMDS analyses were performed using reference status, level 3 ecoregion, year (in 5, 10 and 20-year increments) and taxonomy lab as grouping variables. Trends related to latitude and longitude were also evaluated in the reference status plots. Unlike the Utah data, there were no defined breakpoints (**Figures B3-7–B3-14**). Rather there were small breaks in the data in 1990-91 and 1999, along with a subtle shift towards finer taxonomic resolution from the early 1980's to the present (as one would assume due to improved taxonomic keys, etc.). The improved resolution is evident in plots that show the average number of species and genus-level identifications per year (**Figure B3-15**).

The break in the data that was detected in 1990-91 resulted from an increase in species-level identifications that were recorded for a number of different Orders. This was particularly evident for the order Trombidiformes (water mites). Water mites were identified to the suborder level (Prostigmata) prior to 1991, but from 1991 onwards, there were 28 different identifications associated with the water mites, with some to the species-level. This was accounted for by an OTU correction in which all taxa from the Order Trombidiformes were grouped into the suborder Prostigmata. An increase in taxonomic resolution for Chironomidae also tracks with the 1990-91 break in the data (**Figure B3-16**). We considered grouping all Chironomidae to the family-level, but decided that this would result in the loss of too much information, and that the trends were not consistent enough to warrant the change. The second more subtle break in the data occurs in 1999. This is likely due to variability among the taxonomic labs, since four new labs started doing taxonomic identifications for Maine in 1999.

The genus-level OTU procedures resolved most of these observed differences, as can be seen in the post-OTU NMDS plots. Other possible refinements were problematic, because of the multitude of taxonomy labs that were used over the years. In the 26 year period over which data were collected, sixteen different taxonomy labs did identifications (Table B3-1) (NOTE – this list is revised from that which appears in the Maine database, based on personal communication with Leon Tsomides of Maine DEP). Seven of the labs did 10 or fewer samples, while 4 did 100 or more samples. Once sample size is factored in, there is not a clear difference in the distribution of total taxa among labs (**Figure B3-17**). The NMDS plots that use taxonomy lab as the grouping variable also failed to reveal any clear or consistent patterns.

Another issue that arose with the Maine data involved differences in collection methods. Maine DEP typically collects samples using rock baskets and rock cones that are deployed for about a month. During some years, Maine DEP experimented with different collection methods (i.e. qualitative methods). To minimize variability due to collection method, we only used rock basket and rock cone samples in their analyses. Another factor that was taken into account when doing the analyses was temporal differences. The majority of samples were collected during the summer and fall. However, some samples were collected in the winter and spring. Seasonal variability was accounted for by limiting its analyses to samples that were collected from June through November. Differences in subsampling were also investigated (mainly for effects on richness metrics; abundances had already been adjusted for subsampling). In the Maine DEP database, subsampling information is recorded in a field titled 'Dilution factor' (a value of 1 means that the entire sample was analyzed, a value of 2 means half the sample was analyzed, a value of 4 means that a quarter of the sample was analyzed, etc.). This field had limited worth because many entries were blank. However, for analyses in which long-term trends in generic richness were investigated at specific sites, subsampling information was noted when available. Due to the inconsistency in whether subsampling information was included for samples in the data base, no corrections to taxa richness information were actually applied.

Abundance information appeared to be recorded in a consistent manner in the Maine data. Maine DEP typically deploys three rock baskets or cones per site. Each rock basket is considered to be a replicate. For purposes of the analyses, the replicates from each site were grouped into a single 'BenSamp' and subsampling of the data was done to 200 organisms (± 20%) [160 - 240]. Relative abundances of the taxa were calculated for all the BenSamps.

B3.5 North Carolina

Data for North Carolina were compiled from into a database from the raw data provided by Trish MacPherson from North Carolina Department of Water Quality (NCDWQ). We found that North Carolina records data by water body name, location description, latitude and longitude, and date, but does not assign unique Station IDs to its sampling sites. We therefore had difficulty determining whether some stations represented the same or different sites. Some samples have similar water body names but with slightly different spellings (for example, 'Creek' might be spelled out in one sample record and abbreviated as 'Cr' in another). Samples with similar water body names and location descriptions might have had slightly different latitudes and longitudes. Some sites had the same water body name but slightly different location descriptions. To address this issue, we created unique identifiers for sites (Station IDs) based on matching a combination of water body name, location, and latitude-longitude, for the individual stations that were analyzed for long-term trends.

As with the other states, evaluation of the North Carolina data confirmed that genus-level OTUs were appropriate for the long-term analyses. To check for trends at the genus-level OTU in the North Carolina dataset, NMDS analyses were performed using collection method, reference status, level 3 ecoregion, and year (in 5-year increments) as grouping variables. Because the same people in the North Carolina biomonitoring program have done all the taxonomic identifications for the last 25-30 years, we felt it was unnecessary to include taxonomy lab as a grouping variable. Any inconsistencies in taxonomic identifications over the years are most likely due to changes in taxonomic keys.

An obvious trend occurred in the NMDS plot that used collection method as the grouping variable (**Figure B3-18**). Samples that were collected using different collection methods generally formed different groups. EPT samples in particular formed a very distinct group. Another noticeable pattern in the data occurred in 1998, when there was a spike in the total number of taxa identified, despite fact that the number of stations sampled in 1998 was only slightly higher than in previous years (**Figure B3-19a**). Many of these taxa only occur in the database during 1998. Upon further investigation, we found that a large number of estuarine sites were sampled in 1998. Many of these sites were not sampled prior to 1998 and have not been sampled since. By limiting the samples to full-scale method only, these trends were eliminated (**Figure B3-19b**). Based on these results, only full-scale collection method samples were used in analyses. This resulted in the loss of 4 years of data (1978-1981) and reduced the overall number of taxa in the database, but was a necessary and effective step in minimizing the chances of detecting false trends in the biological data (**Figure B3-20**).

The NMDS plots that used reference status as the grouping variable did not show a clear or consistent pattern (**Figure B3-21**), but plots with samples grouped by level 3 ecoregion did (**Figure B3-22**). Samples generally grouped together by ecoregion (both pre- and post-OTU).

We account for this by performing most analyses, as appropriate, on subsets of data specific to particular ecoregions (an exception is the maximum likelihood temperature optima and tolerance calculations, for which sample size was an issue and having a wide range of temperatures is needed and appropriate). Temporal differences also had to be accounted. NCDWQ collects samples throughout the year. Some taxa, such as the winter stoneflies, are strongly seasonal. To minimize such predictable variation associated with seasonal differences in taxonomic composition, the datasets used in most of the analyses were limited to samples collected from June through November.

Abundance information appeared to be recorded in a consistent manner in the North Carolina data. NCDWQ records its abundance data as categorical variables, 1=rare (1-2 specimens), 3=common (3-9 specimens), and 10=abundant (10 or more specimens), which limits the type of analyses that can be performed. Data were converted to presence-absence and/or relative abundance (calculated using the categorical variables (1, 3 and 10)) when performing analyses.

B3.6 Ohio

As mentioned above in Section B.3, data manipulation and analyses needed for this study were conducted by MBI under subcontract to Tetra Tech. This included taxonomic comparisons, OTU development, and analyses such as NMDS applied to assess the effectiveness of these data management efforts (see Appendix H, especially H.3).

B4 Discussion

Preparing the data for the analyses was a very time consuming yet necessary step. It is essential that proper quality assurance procedures are followed to ensure the validity of the analyses. For this project in particular, the detection of false trends in the long-term data was a major concern.

Factors that were shown to contribute to changes that had to be accounted for prior to trend analysis include collection method (Maine and North Carolina), sample collection dates (all three databases), and taxonomic labs (Utah). Although one cannot entirely eliminate these issues, by selecting appropriate subsets of data and establishing appropriate OTUs, chances of detecting false trends in the biological data can be minimized.

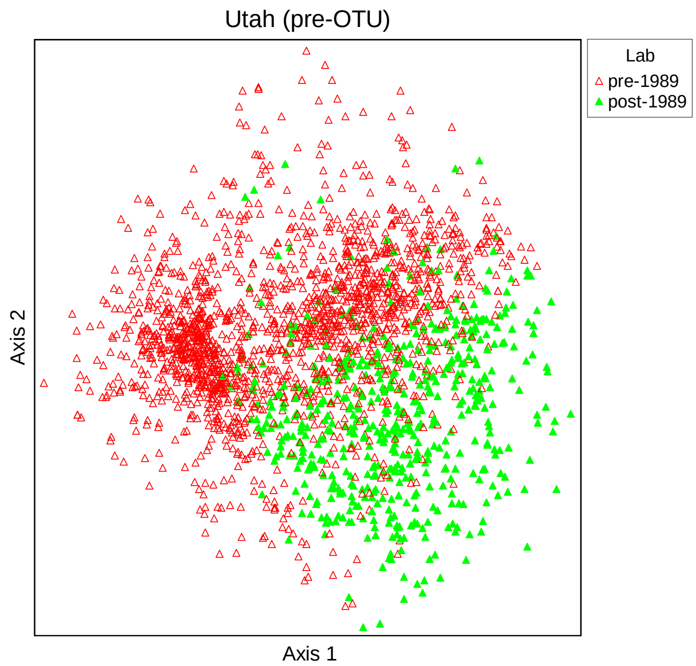


Figure B3-1a. Pre-OTU (genus) NMDS plot when lab is used as the grouping variable.

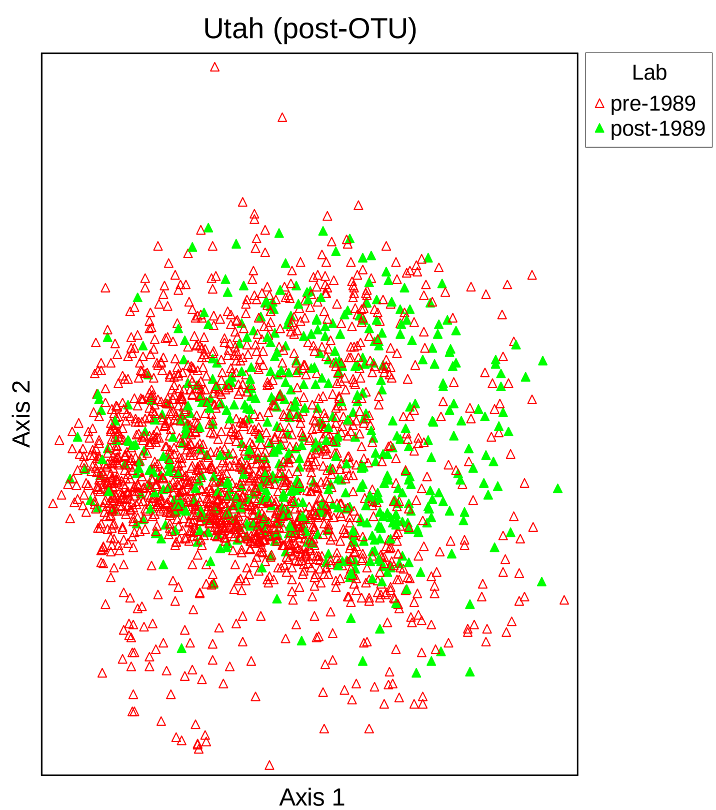
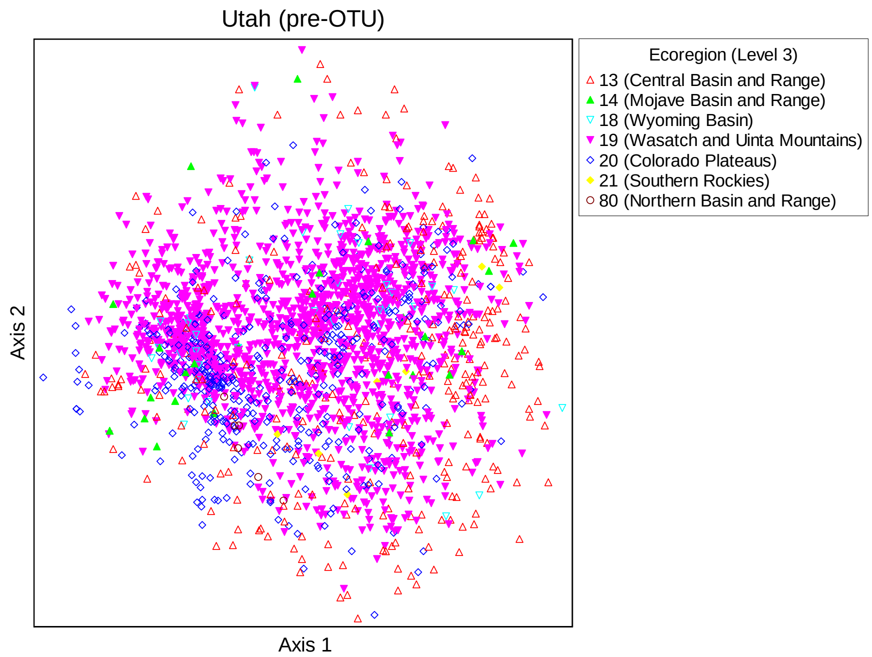
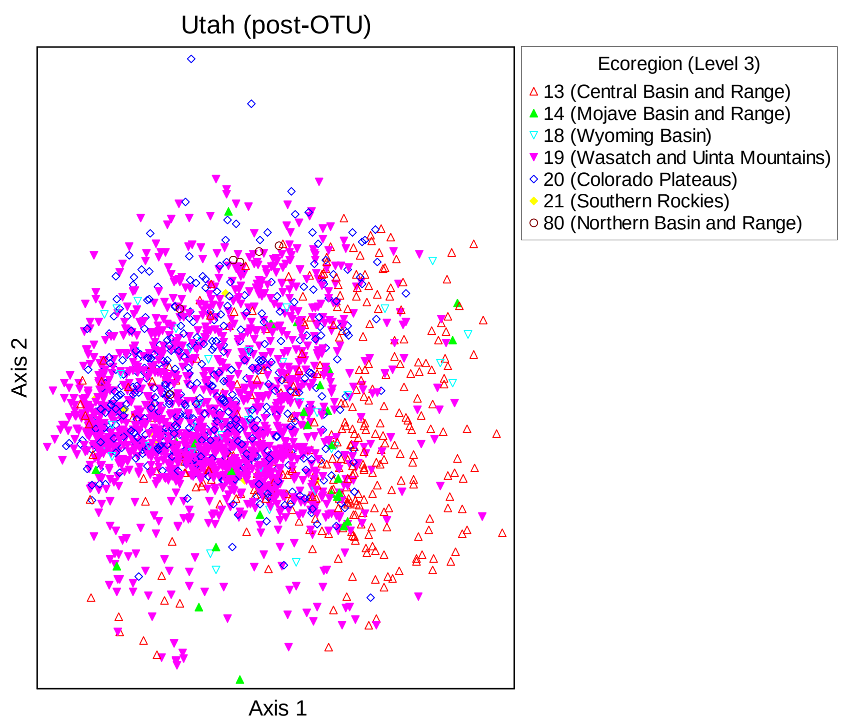


Figure B3-1b. Post-OTU (genus) NMDS plot when lab is used as the grouping variable.



320

321 **Figure B3-2a. Pre-OTU (genus) NMDS plot when level 3 ecoregion is used as the grouping**
322 **variable.**



323

324 **Figure B3-2b. Post-OTU (genus) NMDS plot when level 3 ecoregion is used as the grouping**
325 **variable.**

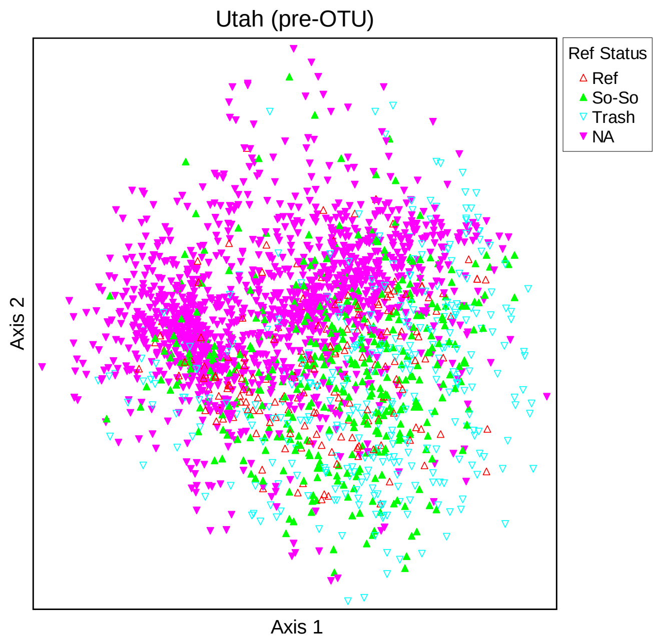


Figure B3-3a. Pre-OTU (genus) NMDS plot when reference status is used as the grouping variable.

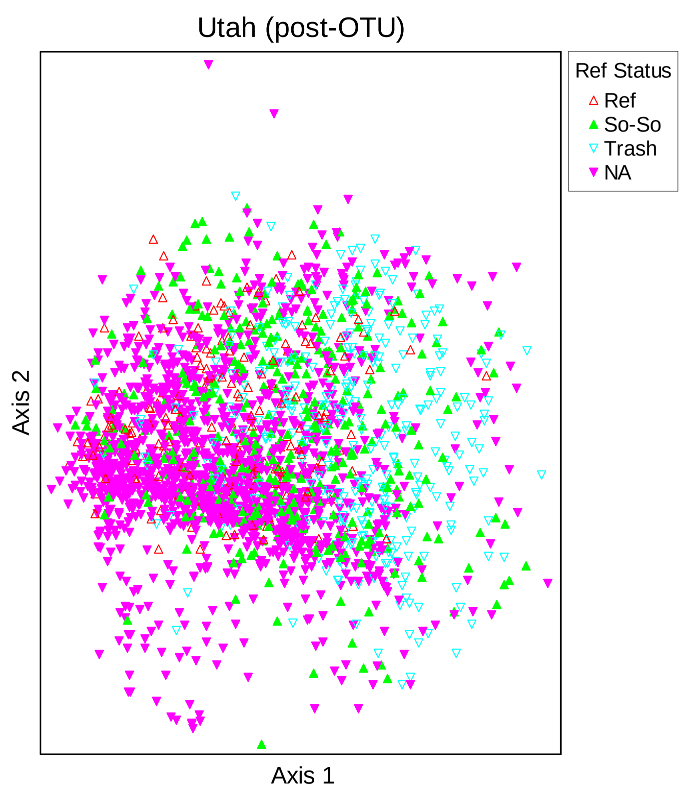


Figure B3-3b. Post-OTU (genus) NMDS plot when reference status is used as the grouping variable.

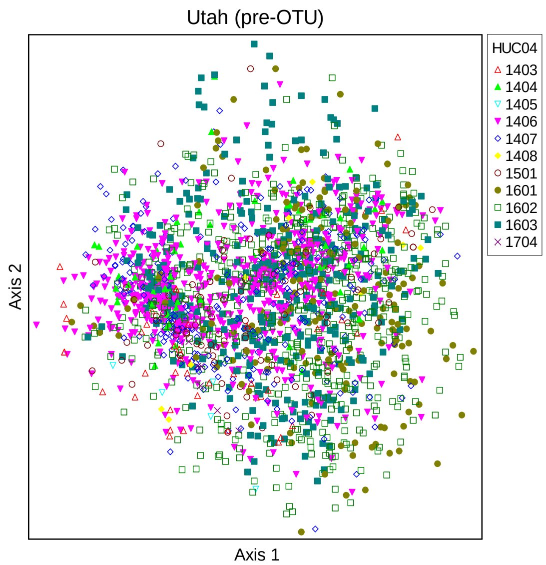


Figure B3-4a. Pre-OTU (genus) NMDS plot when HUC04 is used as the grouping variable.

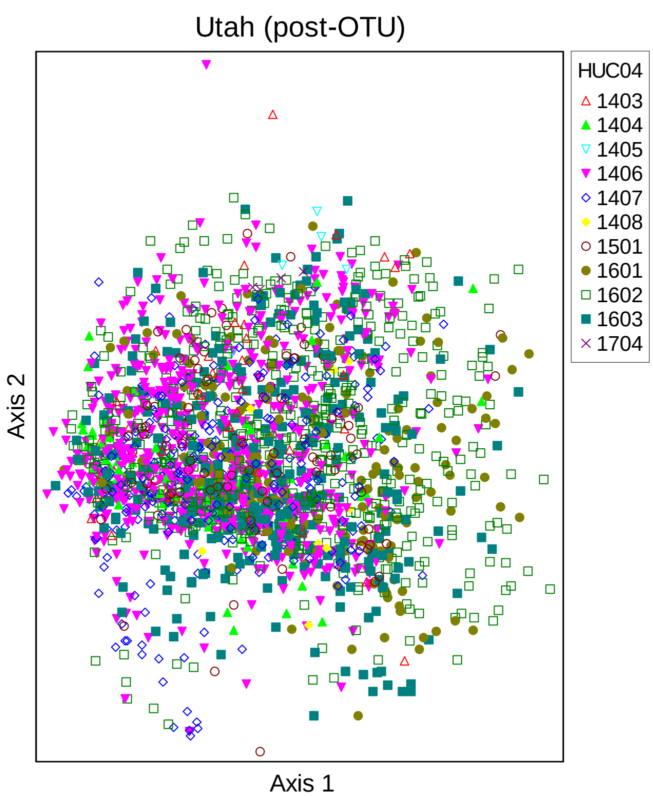


Figure B3-4b. Post-OTU (genus) NMDS plot when HUC04 is used as the grouping variable.

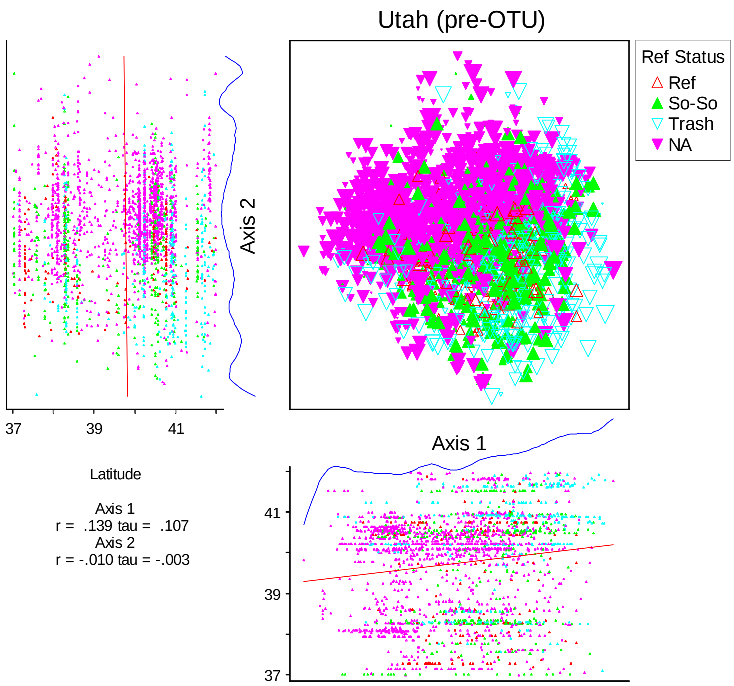


Figure B3-5a. Pre-OTU (genus) NMDS plot when reference status is used as the grouping variable. Trends related to latitude are also evaluated.

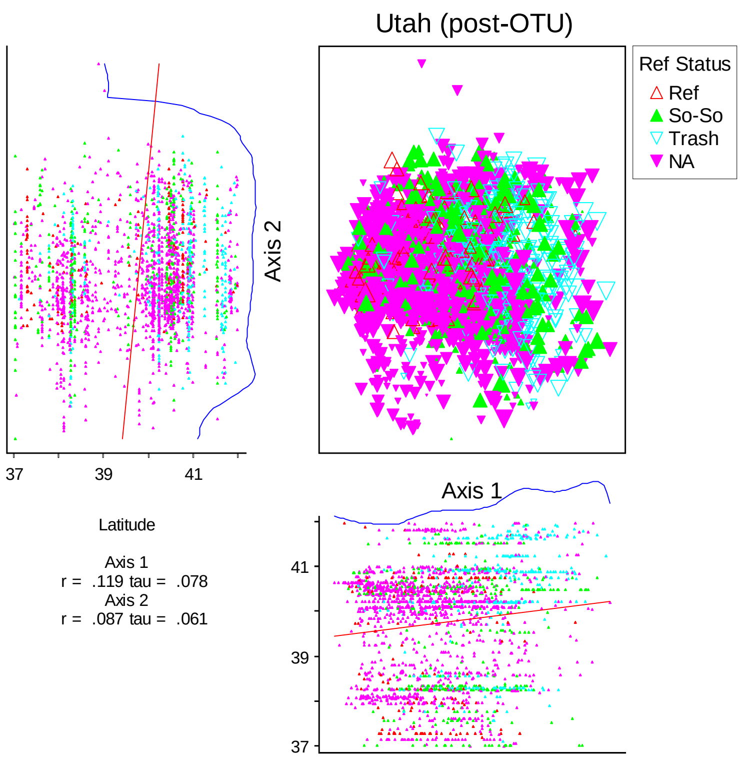


Figure B3-5b. Post-OTU (genus) NMDS plot when reference status is used as the grouping variable. Trends related to latitude are also evaluated.

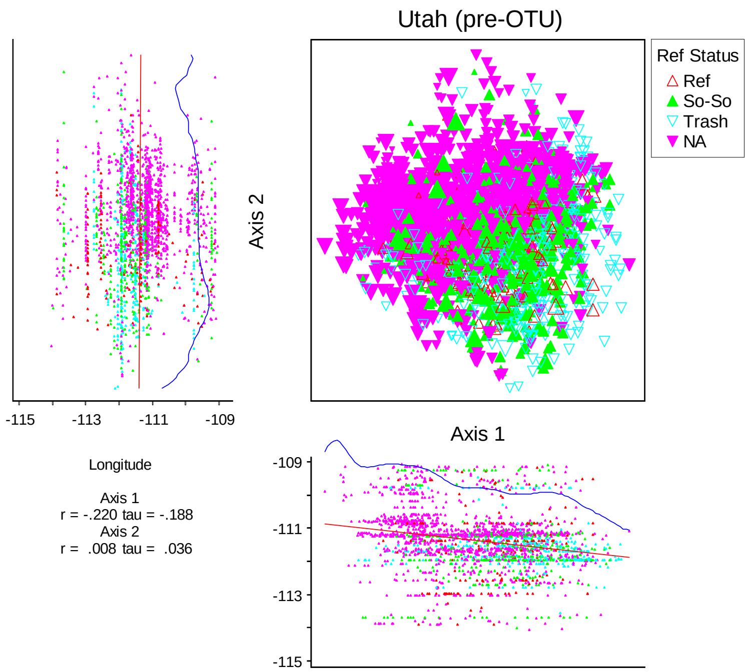


Figure B3-6a. **Pre-OTU (genus) NMDS plot when reference status is used as the grouping variable. Trends related to longitude are also evaluated.**

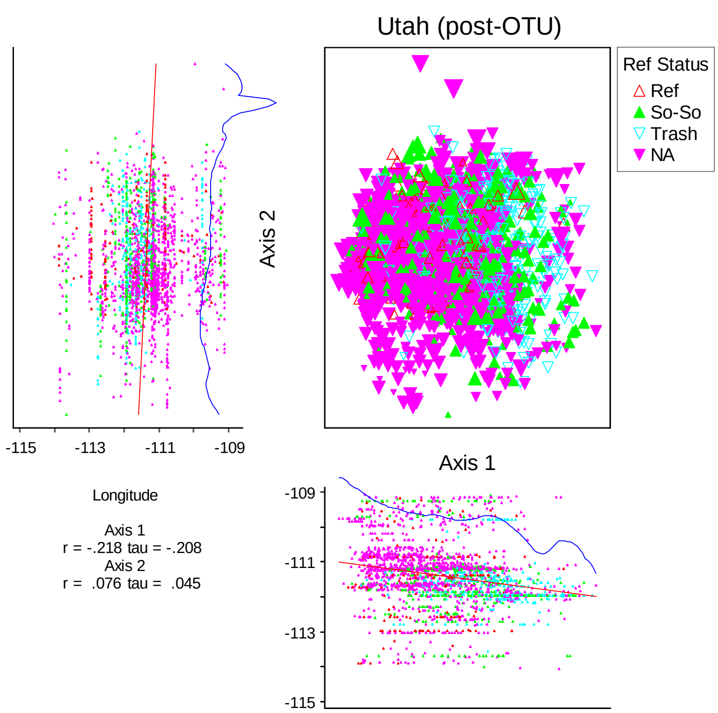


Figure B3-6b. **Post-OTU (genus) NMDS plot when reference status is used as the grouping variable. Trends related to longitude are also evaluated.**

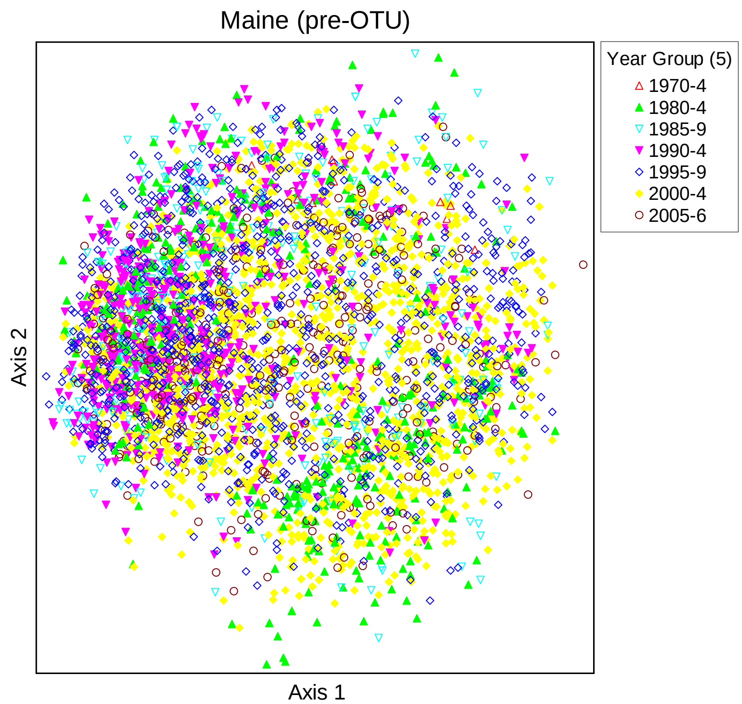


Figure B3-7a. **Pre-OTU (genus) NMDS plot using sample years (5-year increments) as the grouping variable.**

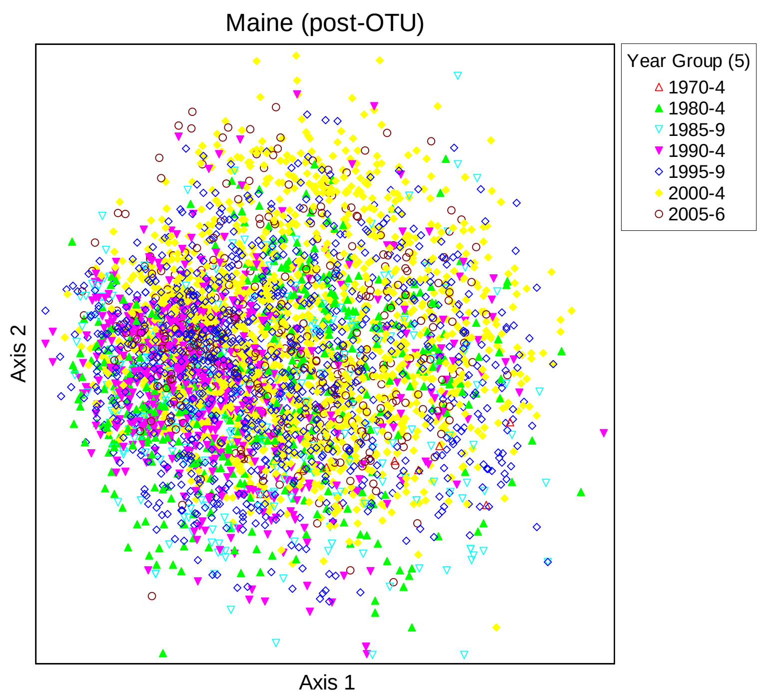


Figure B3-7b. Post-OTU (genus) NMDS plot using sample years (5-year increments) as the grouping variable.

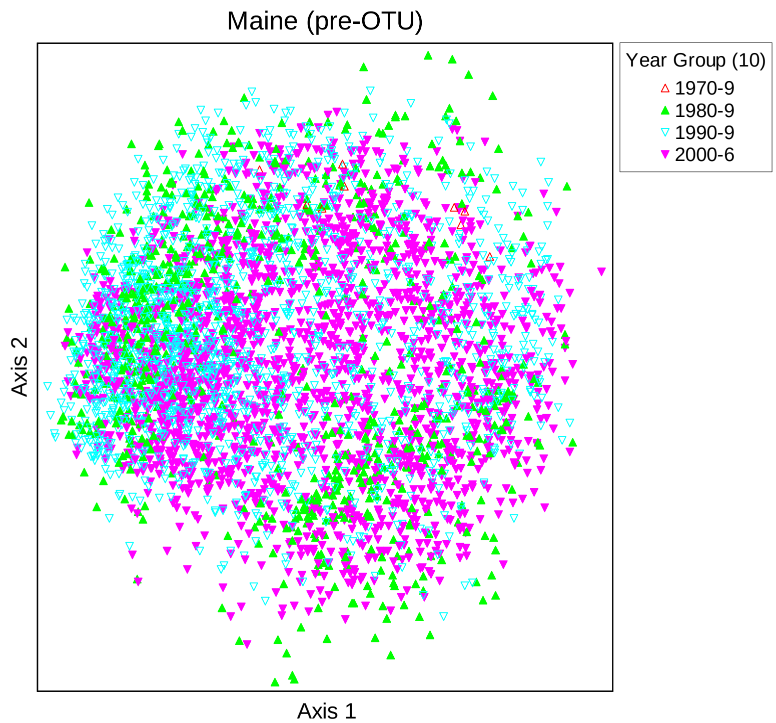


Figure B3-8a. **Pre-OTU (genus) NMDS plot using sample years (10-year increments) as the grouping variable.**

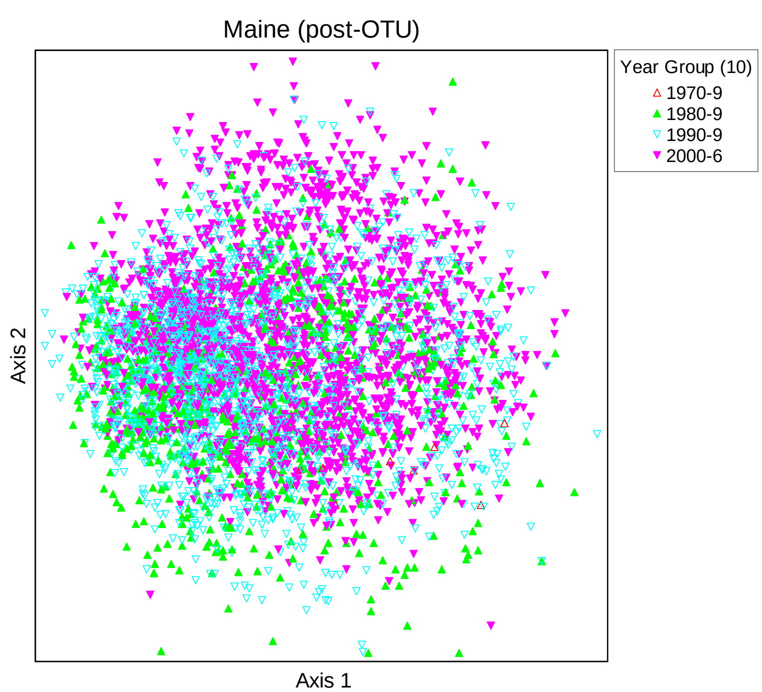


Figure B3-8b. Post-OTU (genus) NMDS plot using sample years (10-year increments) as the grouping variable.

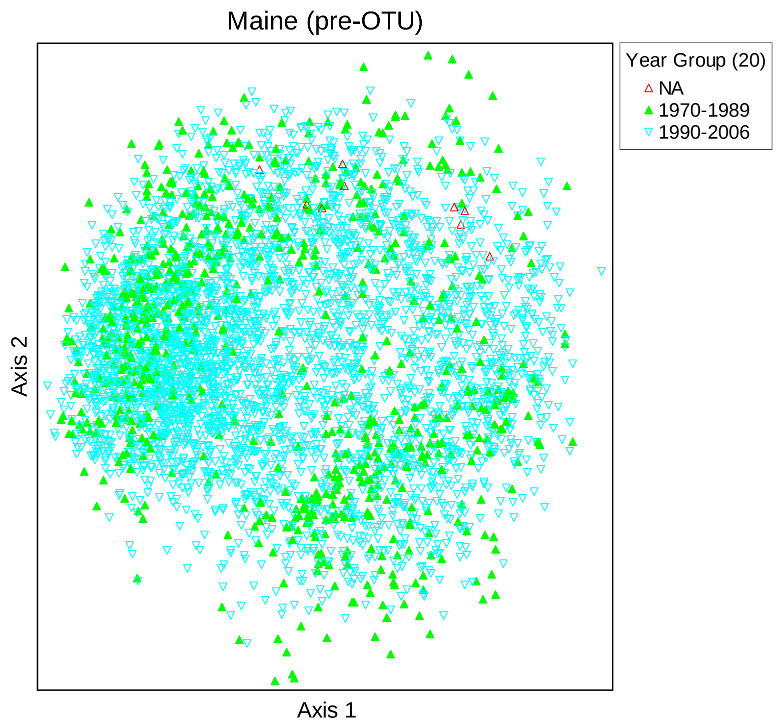


Figure B3-9a. Pre-OTU (genus) NMDS plot using sample years (20-year increments) as the grouping variable.

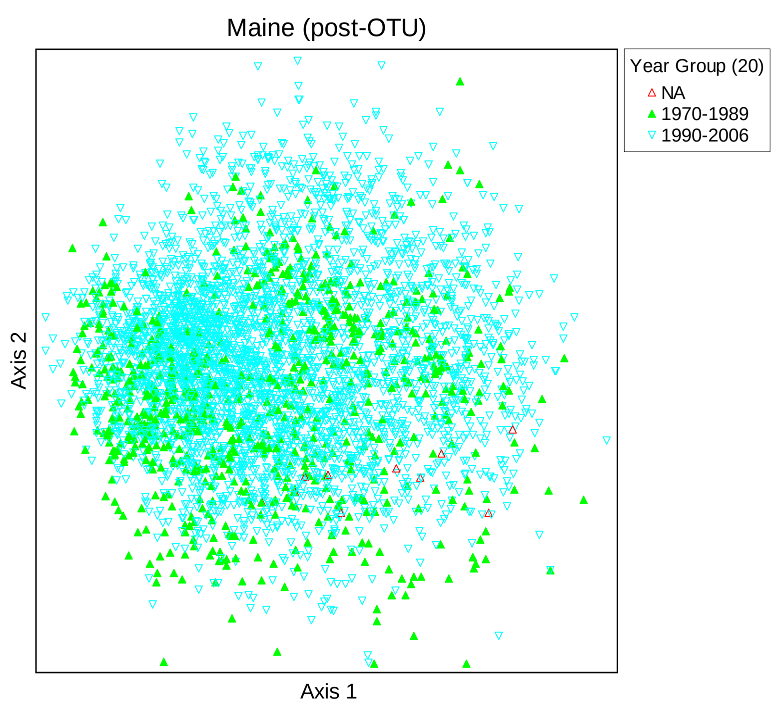


Figure B3-9b. Post-OTU (genus) NMDS plot using sample years (20-year increments) as the grouping variable.

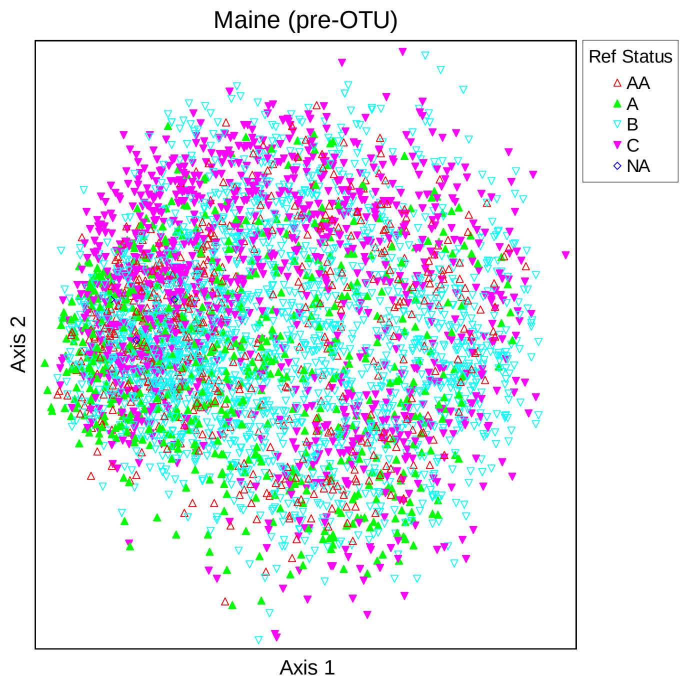


Figure B3-10a. Pre-OTU (genus) NMDS plot when reference status is used as the grouping variable.

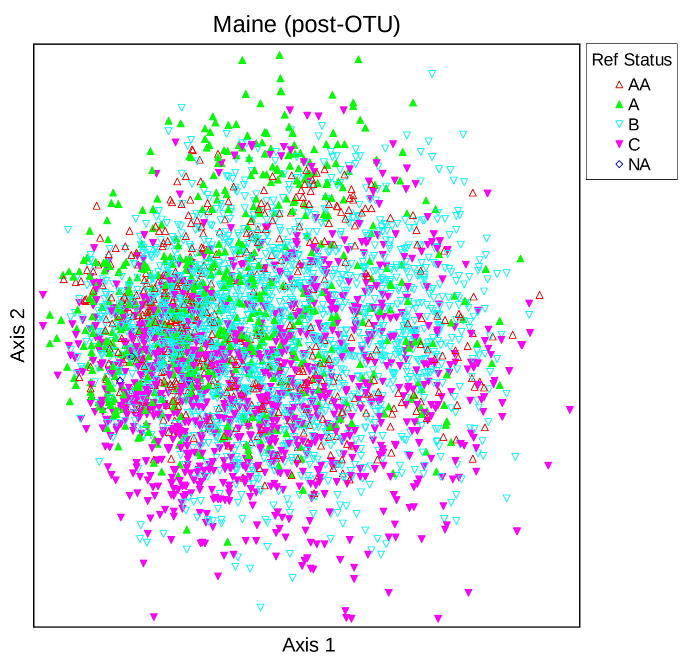


Figure B3-10b. Post-OTU (genus) NMDS plot when reference status is used as the grouping variable.

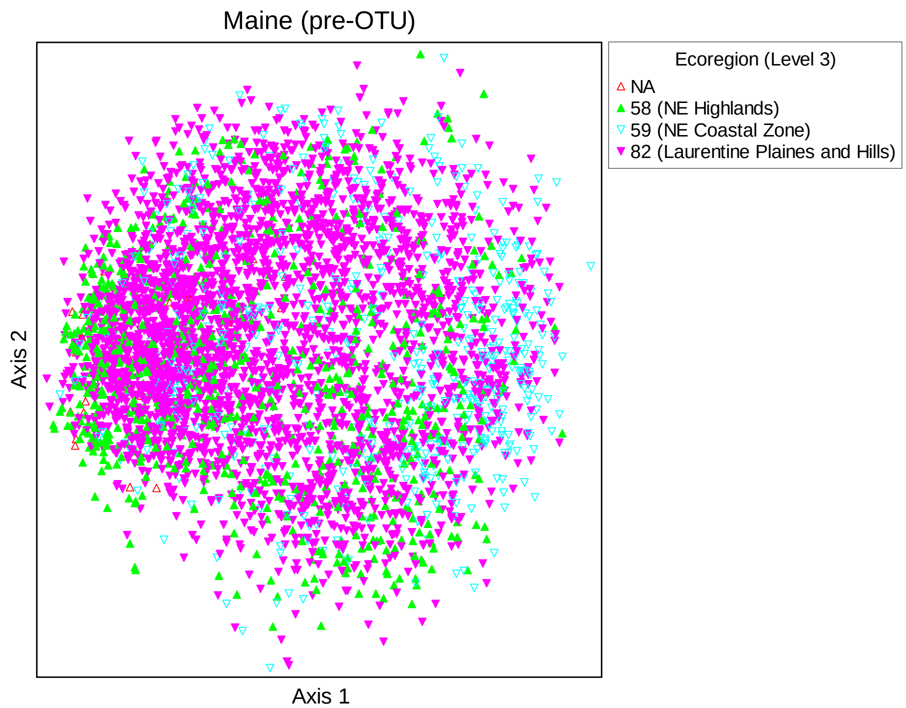


Figure B3-11a. Pre-OTU (genus) NMDS plot when level 3 ecoregion is used as the grouping variable.

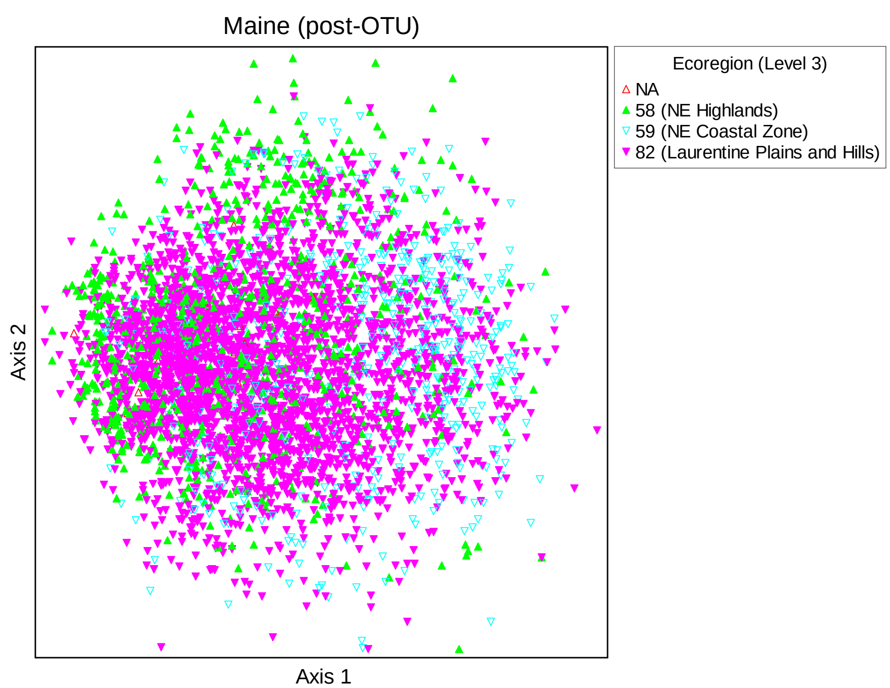


Figure B3-11b. Post-OTU (genus) NMDS plot when level 3 ecoregion is used as the grouping variable.

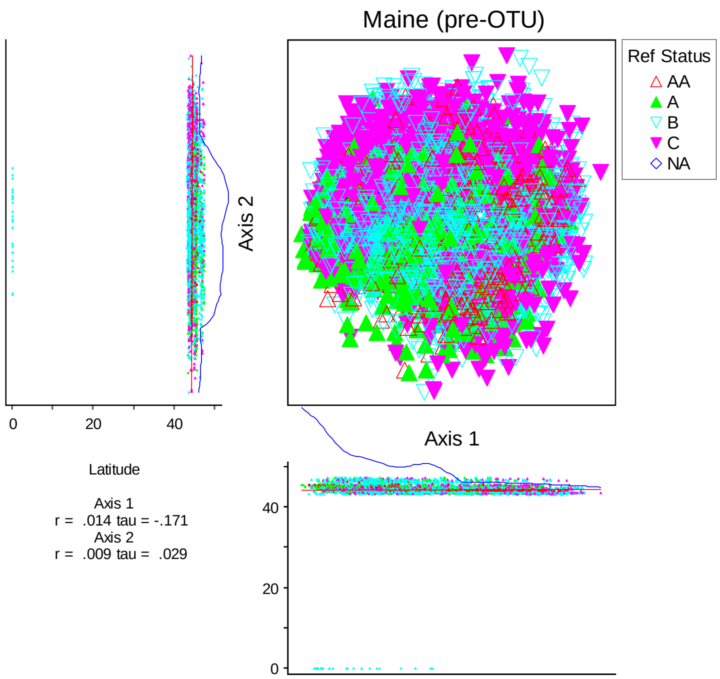


Figure B3-12a. Pre-OTU (genus) NMDS plot when reference status is used as the grouping variable. Trends related to latitude are also evaluated.

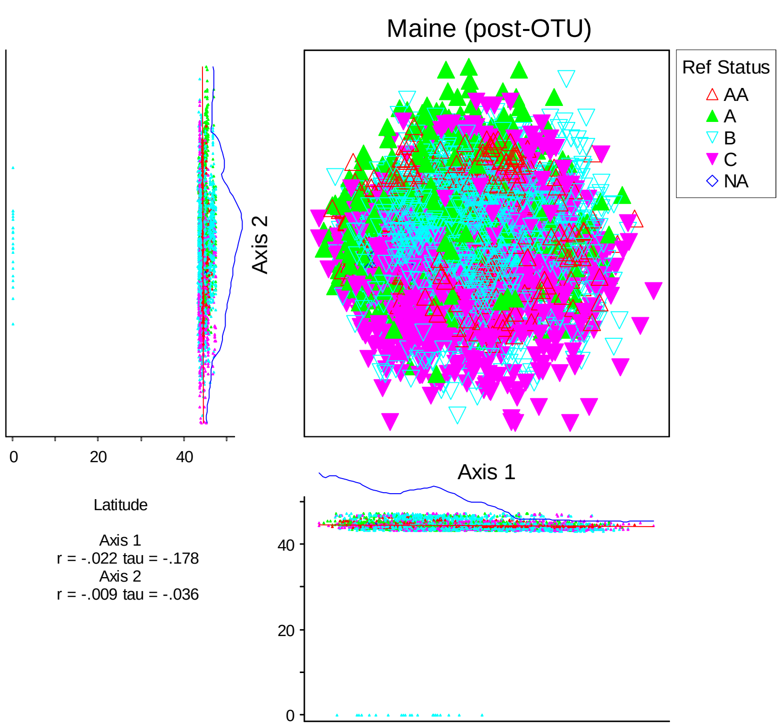


Figure B3-12b. Post-OTU (genus) NMDS plot when reference status is used as the grouping variable. Trends related to latitude are also evaluated.

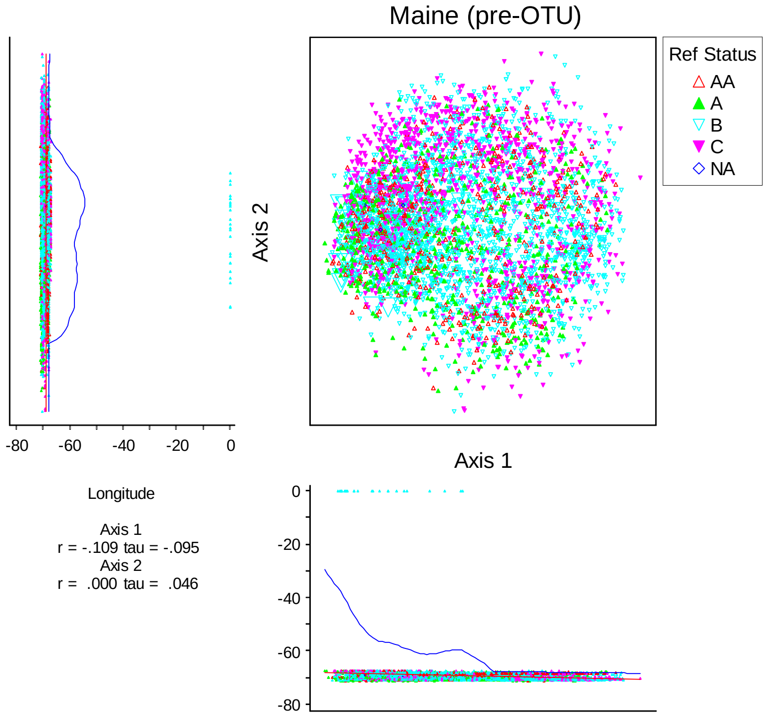


Figure B3-13a. Pre-OTU (genus) NMDS plot when reference status is used as the grouping variable. Trends related to longitude are also evaluated.

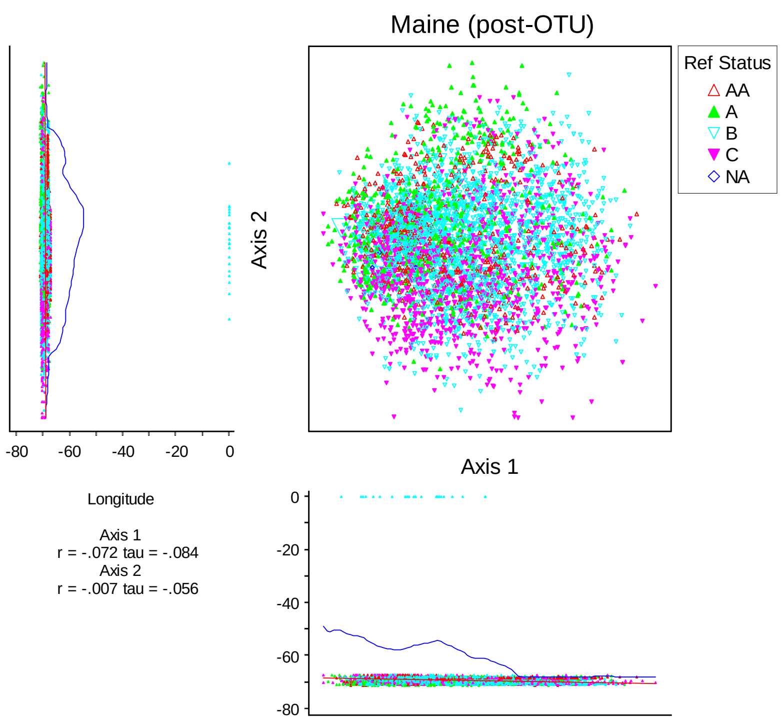


Figure B3-13b. Post-OTU (genus) NMDS plot when reference status is used as the grouping variable. Trends related to longitude are also evaluated.

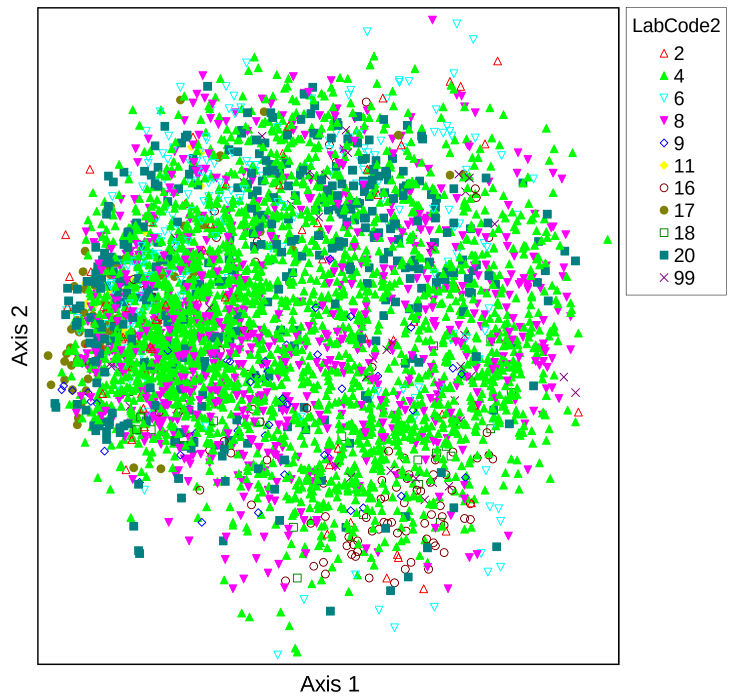


Figure B3-14a. Pre-OTU (genus) NMDS plot for Maine data when lab is used as the grouping variable.

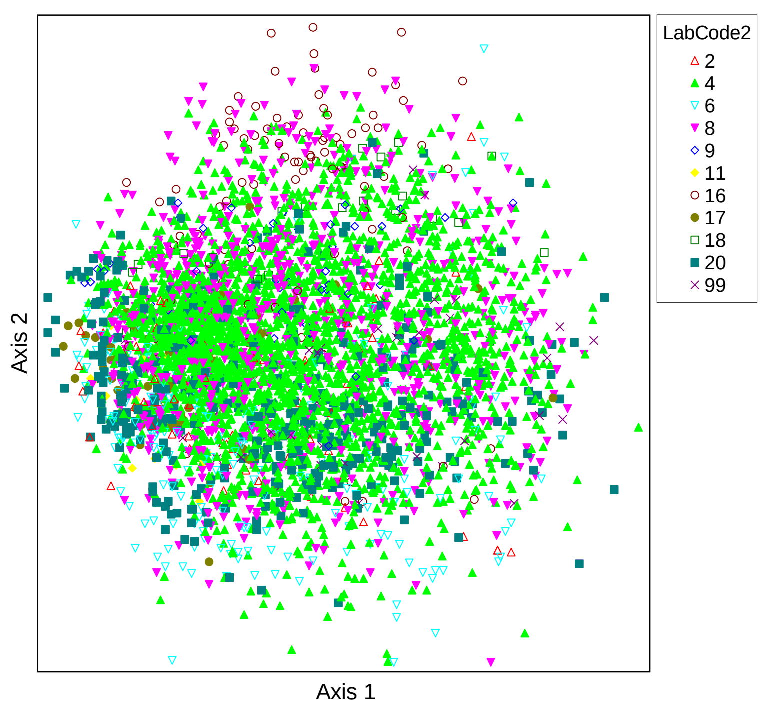


Figure B3-14b. Post-OTU (genus) NMDS plot for Maine data when lab is used as the grouping variable.

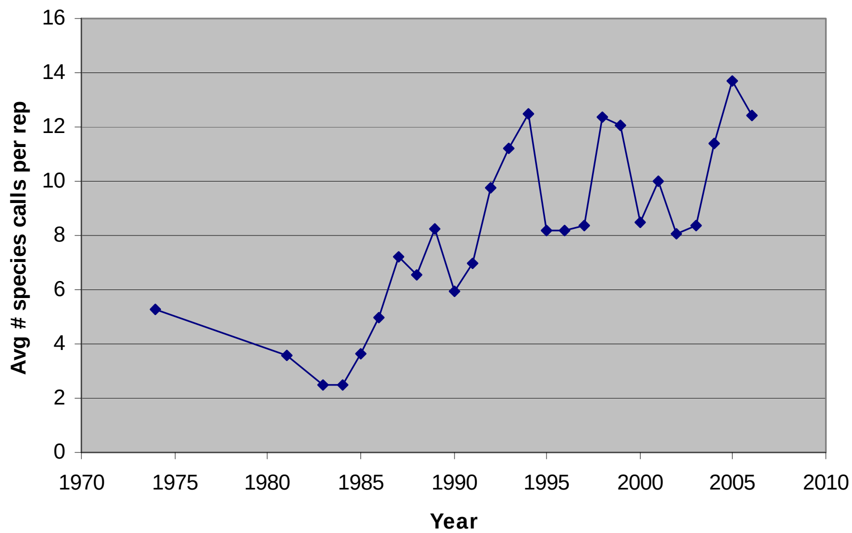


Figure B3-15a. Average number of species-level identifications per replicate sample per year in the Maine database (using original data (not adjusted for OTUs).

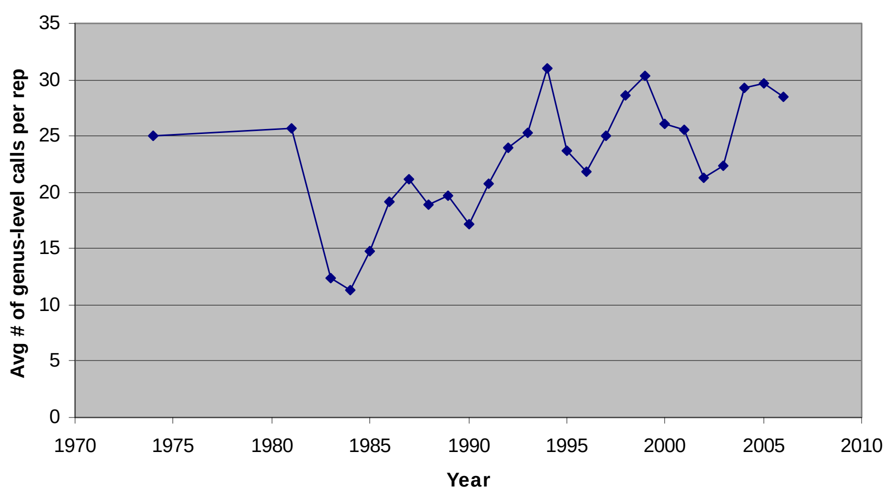


Figure B3-15b. Average number of genus-level identifications per replicate sample per year in the Maine database (using original data (not adjusted for OTUs).

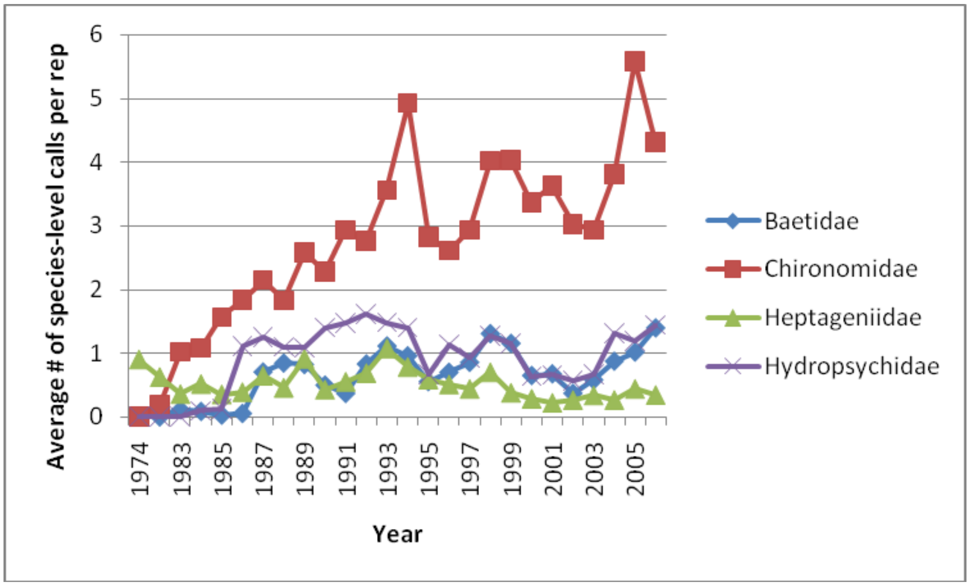


Figure B3-16a. Average number of species-level identifications per replicate sample per year for selected families in the Maine database (using original data (not adjusted for OTUs).

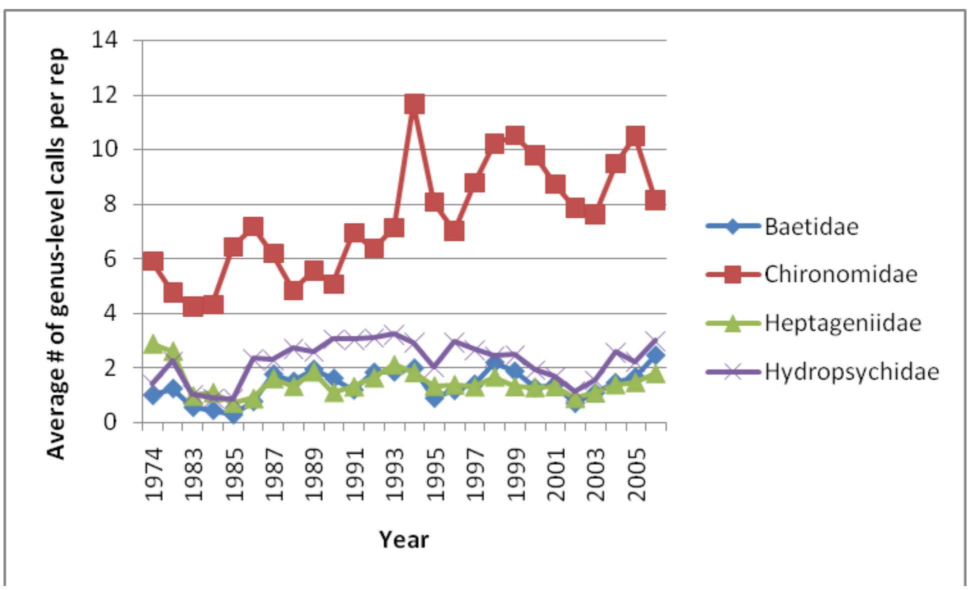


Figure B3-16b. Average number of genus-level identifications per replicate sample per year for selected families in the Maine database (using original data (not adjusted for OTUs).

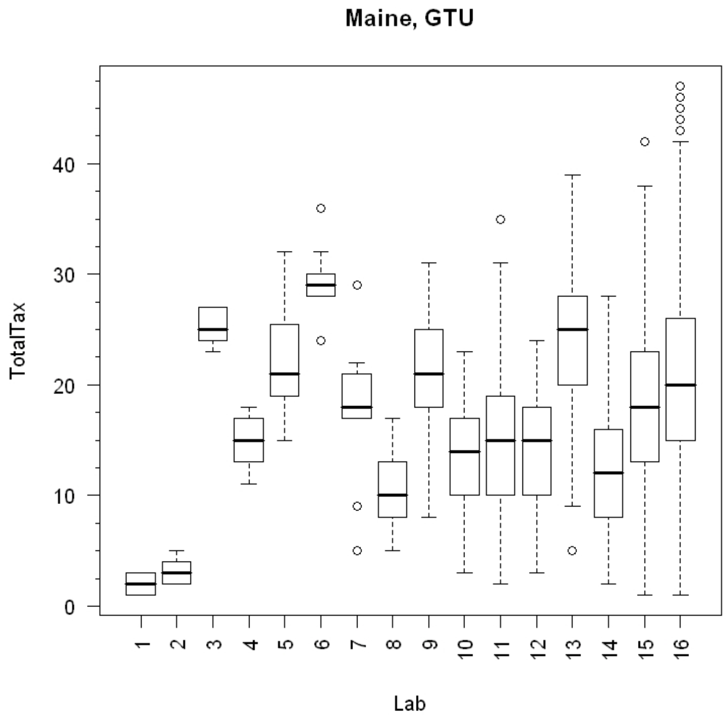


Figure B3-17. Distribution of the total number of taxa (average per replicate) among laboratories.

Table B3-1. Per communication with Leon Tsomides Maine DEP some adjustments were made

Lab	Year_Min	Year_Max	#Samp	LabNum
BILLIE BESSIE	1996	1996	2	1
DAVID COURTEMANCH	1983	1983	5	2
B.A.R ENVIRONM	1994	1994	6	3
WOODWARD CLYDE	1981	1981	6	4
unknown	1995	1995	7	5
BBL SCIENCES	2004	2004	9	6
CF RABENI	1974	1974	10	7
QST ENVIRONMENTAL (BOWATER)	1994	1996	20	8
CHRIS PINNUTO	2000	2000	22	9
NORMANDEAU	1989	1999	45	10
SUSAN DAVIES	1981	1989	74	11
NEW BRUNSWICK	1999	2001	84	12
IDAHO ECOANALYSTS	1999	2005	100	13
TERRY MINGO	1983	1987	254	14
LOTIC	1988	2006	743	15
MICHAEL WINNELL	1983	2006	2509	16

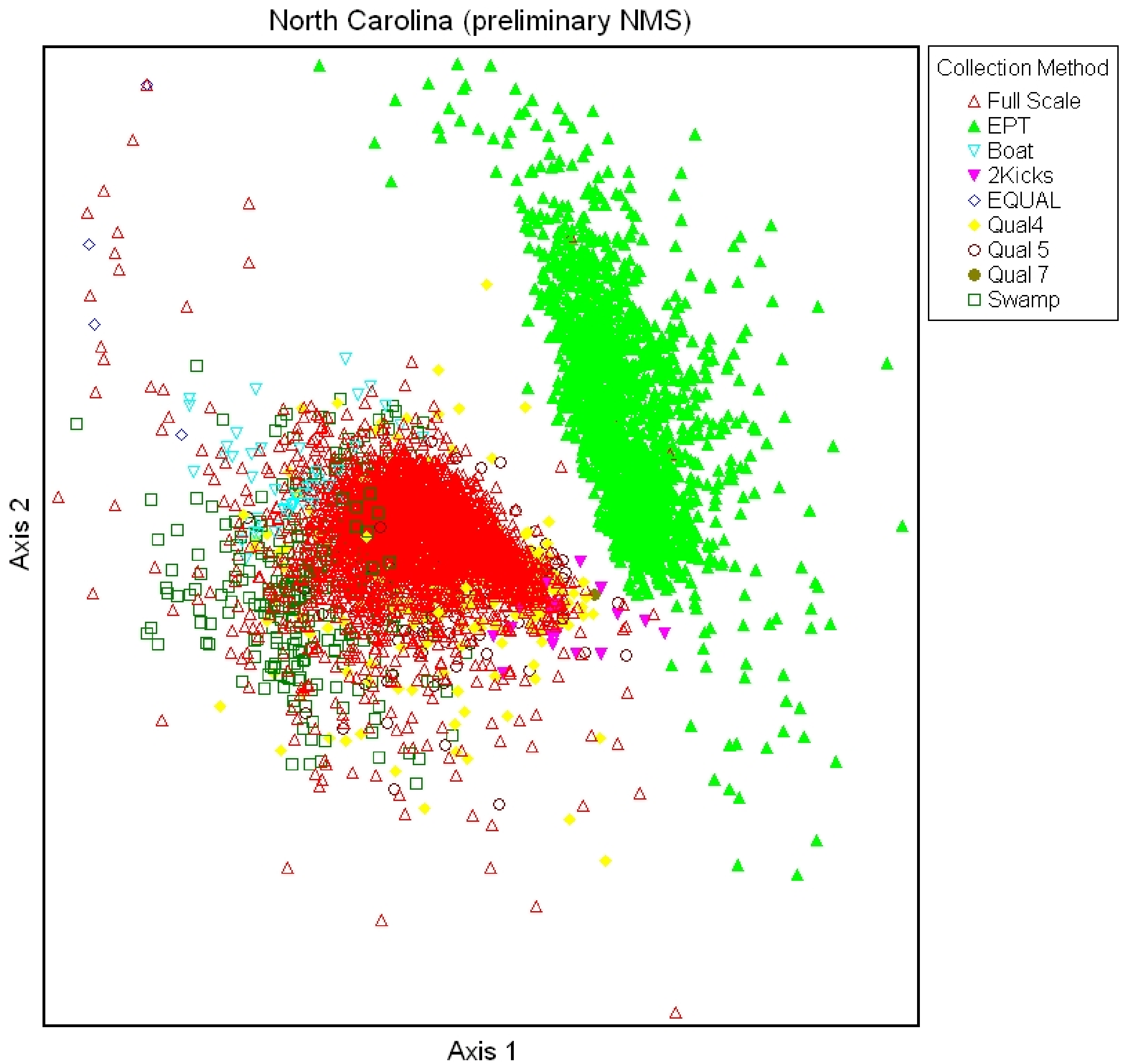


Figure B3-18. Preliminary NMDS plot (genus-level OTU) using collection method as the grouping variable.

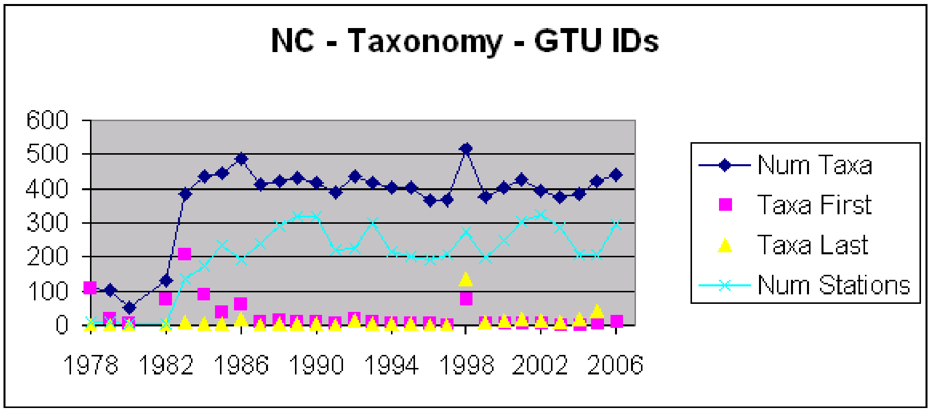


Figure B3-19a. North Carolina (genus-level OTU or GTU) data using all collection methods. "Num Taxa" refers to the total number of taxa recorded in a particular year; "Taxa First" refers to the number of taxa that appear in the database for the first time in a particular year; "Taxa Last" refers to the number of taxa that appear in the database for the last time in a particular year; "Num Stations" refers to the number of stations sampled in a particular year.

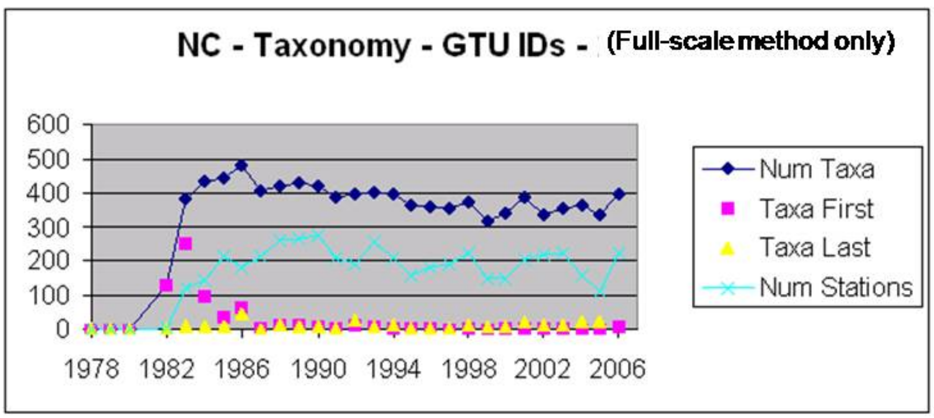


Figure B3-19b. North Carolina (genus-level OTU or GTU) using data from only the Full-scale collection method. "Num Taxa" refers to the total number of taxa recorded in a particular year; "Taxa First" refers to the number of taxa that appear in the database for the first time in a particular year; "Taxa Last" refers to the number of taxa that appear in the database for the last time in a particular year; "Num Stations" refers to the number of stations sampled in a particular year.

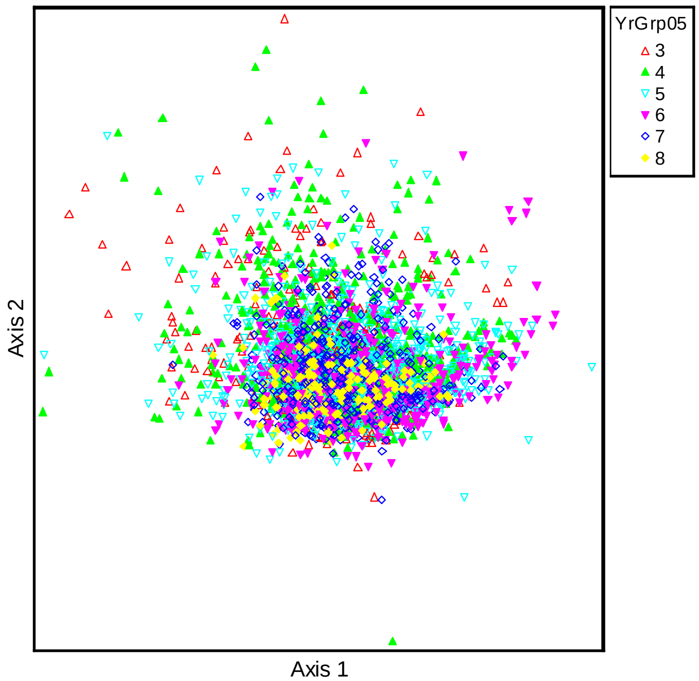


Figure B3-20a. **Pre-OTU (genus) NMDS plot for North Carolina data when year (5-year increments) is used as the grouping variable and only full-scale collection method data is used.**

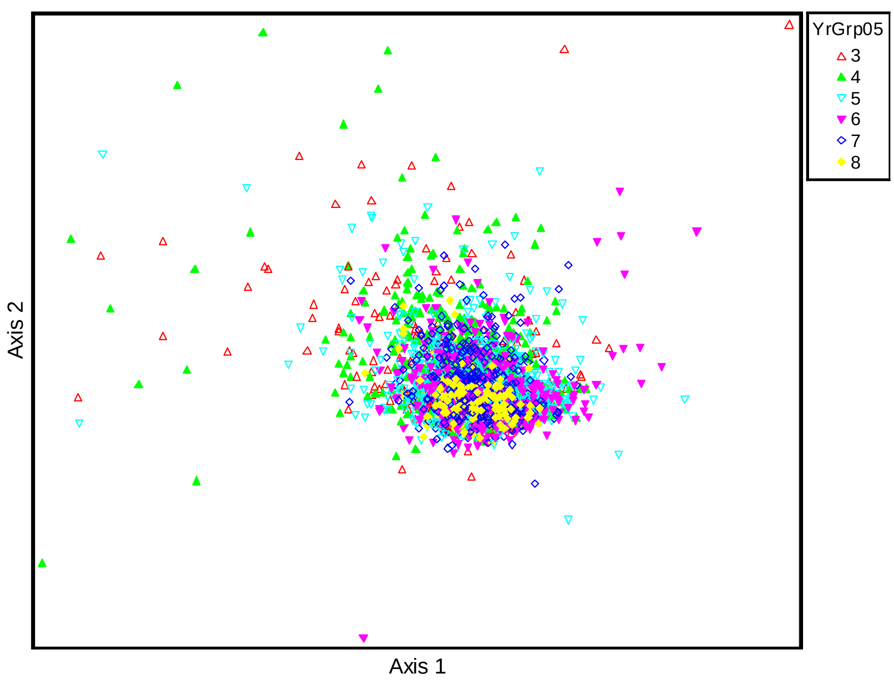


Figure B3-20b. Post-OTU (genus) NMDS plot for North Carolina data when year (5-year increments) is used as the grouping variable and only full-scale collection method data is used.

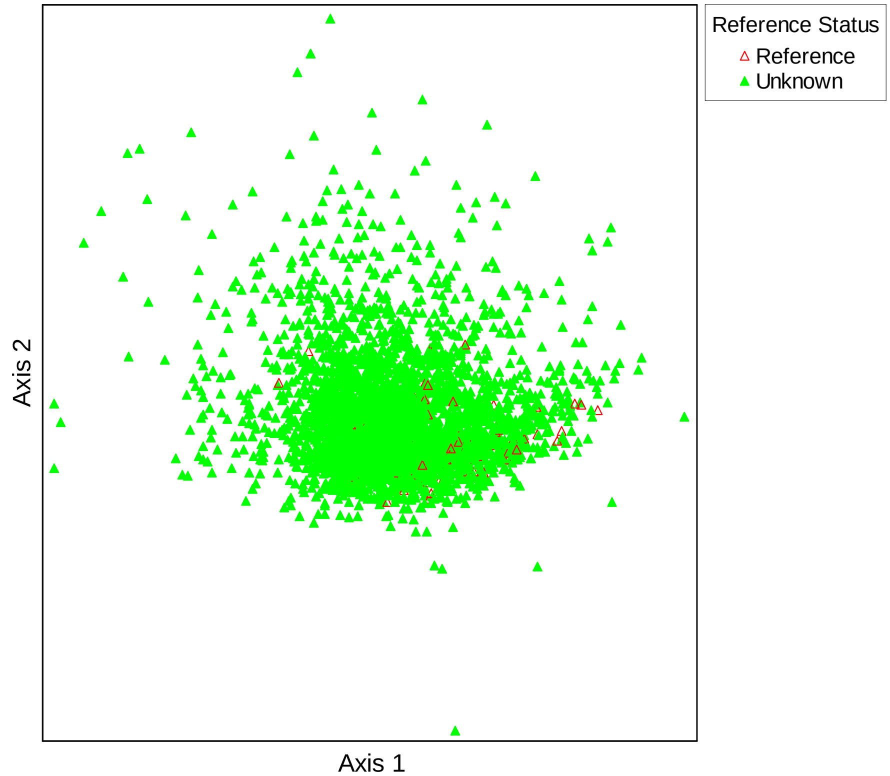


Figure B3-21a. Pre-OTU (genus) NMDS plot for North Carolina data using reference status as the grouping variable and only full-scale collection method data is used.

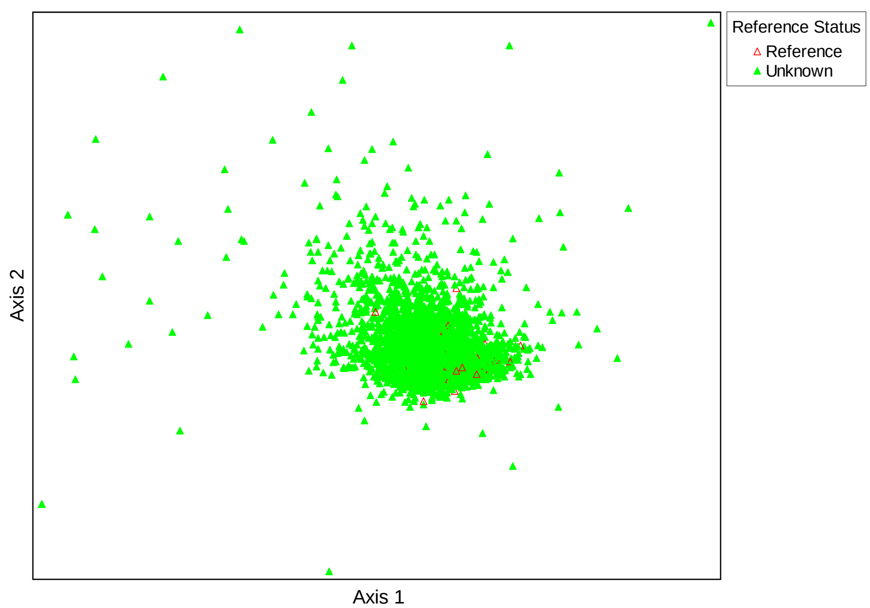


Figure B3-21b. Post-OTU (genus) NMDS plot for North Carolina data using reference status as the grouping variable and only full-scale collection method data is used.

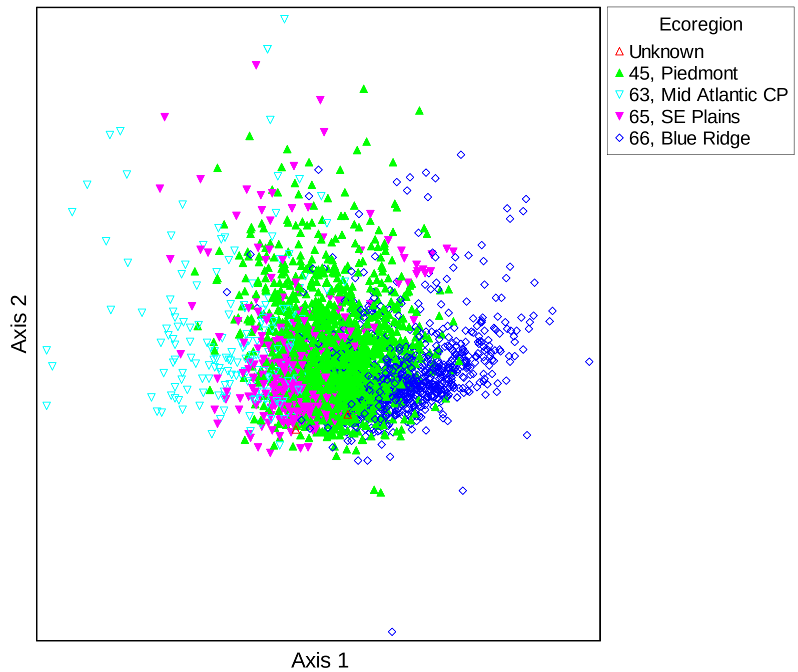


Figure B3-22a. Pre-OTU (genus) NMDS plot for North Carolina data using level 3 ecoregion as the grouping variable and only full-scale collection method data is used.

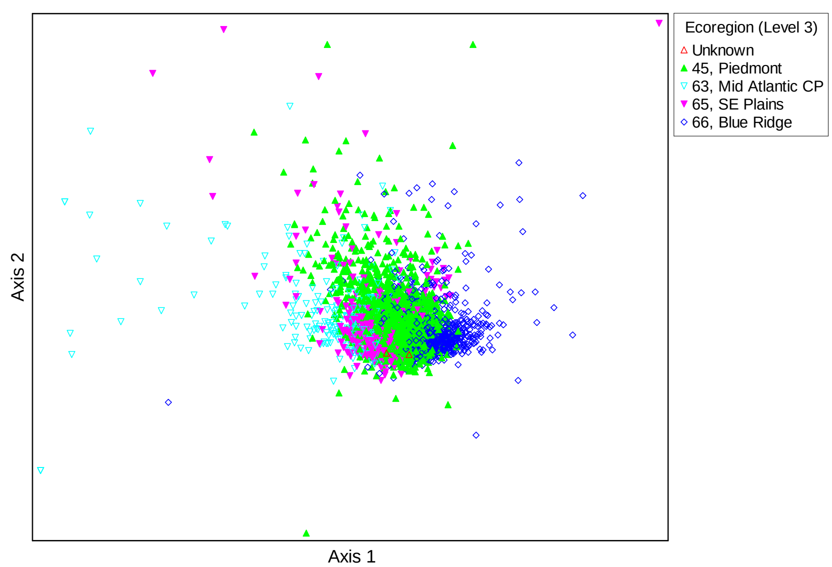


Figure B3-22b. Post-OTU (genus) NMDS plot for North Carolina data using level 3 ecoregion as the grouping variable and only full-scale collection method data is used.

APPENDIX C

Site selections and site groupings

The purpose of this appendix is to provide comprehensive and detailed information on individual biological sampling sites and groups of sites that were selected for long-term trend analyses in Maine, Utah and North Carolina.

C1. MAINE

C1.1 Individual Station Selection

In this study we refer to Class A and AA stations (as determined by Maine DEP, based on biological attainment) as reference stations[1]. Reference sites with the longest-term biological data were identified for analysis of long-term trends. There were two reference stations in Maine that had 10 or more years of data (**Table C1-1**). These 2 stations plus the reference station with the next longest data record (9 years) were included in the individual station analyses. Locations of all the reference stations are shown in **Figure C1-1** and locations of the 3 individual stations that were closely examined are shown in **Figure C1-2**. Brief descriptions of the 3 stations are given below and are summarized in **Table C1-2**. Additional information (i.e. aerial photos) is available upon request.

Table C1-1. Summary of how many years of data were available for the different classes of Maine stations

# Years Sampled	Reference Stations (A & AA)	B & C Stations	Not Attaining (NA)
10-19	2	4	0
5-9	10	30	0
2-4	94	183	0
1	116	302	1

[1] Class A sites are not necessarily designated as reference sites by Maine DEP (Maine DEP is in the process of developing strict reference criteria; considerations are not based on biology and include land use land cover and proximity to NPDES discharges).

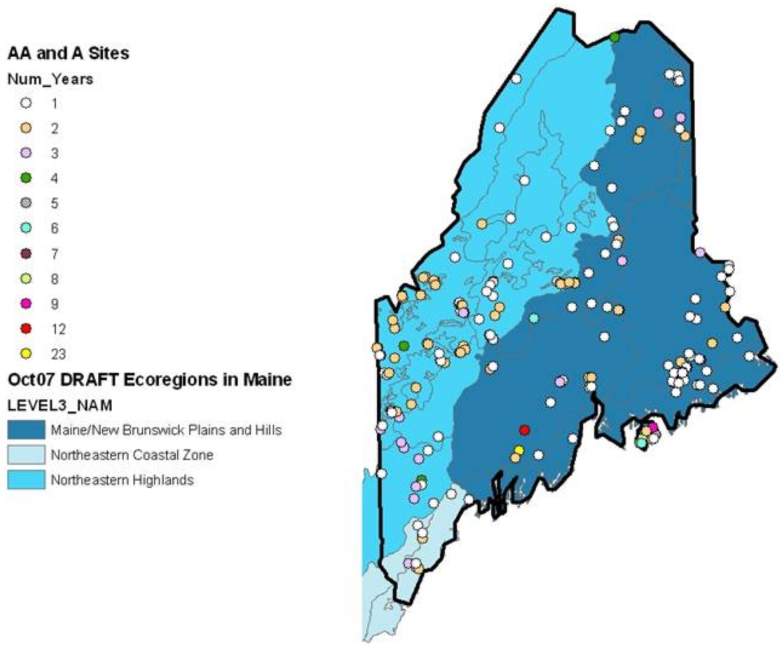


Figure C1-1. Distributions of reference (Class A & AA) sites in Maine among the different level 3 ecoregions (the Maine/New Brunswick Plains and Hills was formerly called the Laurentian Plains and Hills). The number of years of data available for each station is also shown.

StationID 56817 (Latitude 44.22319, Longitude -69.59334). The station is located on the Sheepscot River, Maine DEP Station 74, in the town of Whitefield. It is in the Laurentian Plains and Hills (which has recently been updated to Maine/New Brunswick Plains and Hills) level 3 ecoregion and Central Interior Biophysical Region at an elevation of 104 feet. This station is located on a 4th Strahler order reach and has a drainage area of 145 square miles. It is classified as ‘AA’ but (per communication with Maine DEP) has been influenced by non-point source pollution and has occasionally received ‘B’ ratings. The station has been monitored on an annual basis since 1984. Long-term USGS gage flow data are available for this station.

StationID 57011 (Latitude 44.36791, Longitude -69.53129). The station is located on the West Branch Sheepscot River, Maine DEP Station 268, in the town of China. It is in the Laurentian Plains and Hills level 3 ecoregion and Central Interior Biophysical Region at an elevation of 230 feet. This station is located on a 3rd Strahler order reach and appears (based on aerial photographs and land use land cover information) to be influenced by human activities. Twelve years of continuous data (1995-2006) are available for this station.

StationID 57065 (Latitude 44.3934, Longitude -68.23461). This station is located on Duck Brook, Maine DEP Station 322, in the town of Bar Harbor. It is in the Laurentian Plains and Hills level 3 ecoregion and East Coastal Region Biophysical Region at an elevation of 179 feet. This station is located on a 1^{st} Strahler order reach and has 9 years of continuous data (1997 to 2005). Forest is the dominant surrounding land use.

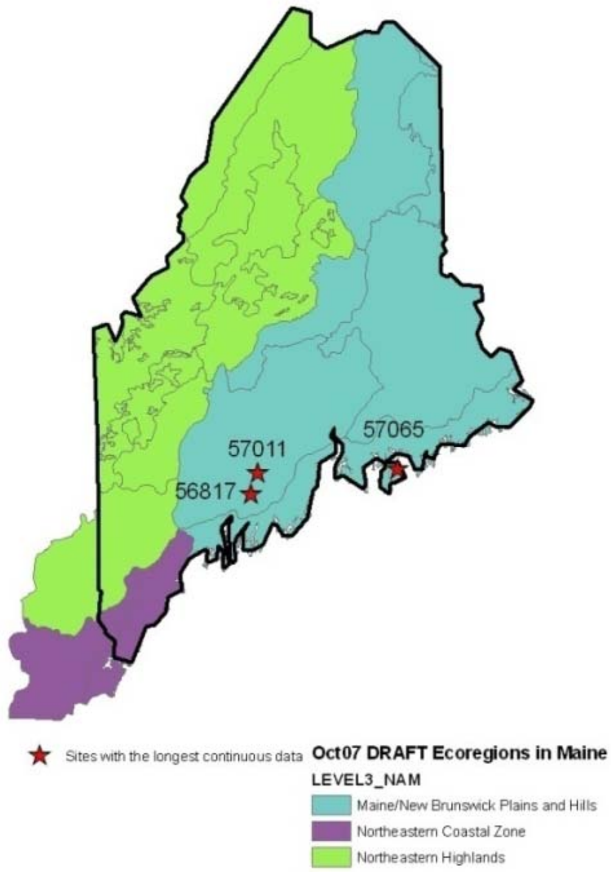


Figure C1-2. Locations of the 3 reference sites in Maine that have the longest term biological data.

Table C1-2. Station information for the 3 Maine reference sites with the longest-term biological data. # years of data refers to June-September samples only. Eco_L3 is level 3 ecoregion. Reference status (Class A & AA) was designated by Maine DEP. % land use refers to the area within a 1 km buffer of the station (NLCD 2001).

Station	WaterbodyName	# Yrs of Data	Eco_L3	Biophysical	Order	Elev_ft	Ref Status	%URB	%AGR	%BAR	%FOR	%WET
56817	SHEEPSCOT RIVER - ME STATION 74	23	LAURENTIAN PLAINS AND HILLS	CENTRAL INTERIOR	4	103.8	AA	16.4	23	0	56.8	3.8
57011	WEST BRANCH SHEEPSCOT RIVER - ME STATION 268	12	LAURENTIAN PLAINS AND HILLS	CENTRAL INTERIOR	3	229.9	AA	9.1	18.5	0	68.3	4
57065	DUCK BROOK - ME STATION 322	9	LAURENTIAN PLAINS AND HILLS	EAST COASTAL REGION	1	179.1	AA	15.9	0	0	75	8.9

C3.2 Site Group Selection

Due to the limited number of individual sites with long-term data, sites were grouped together to obtain more long-term biological datasets to analyze. Our initial approach to identify appropriate station groupings involved cluster analyses to determine which reference stations had similar assemblages and could be grouped together. In one dendrogram, stations were coded by biophysical region (**Figure C1-3**) and in another, by level 3 ecoregion (**Figure C1-4**). Results show fairly strong site-specific differences among assemblages, which does not support grouping them for trend analyses. The stations that showed the greatest similarities were more closely examined, but did not have enough continuous data among them to make analyses worthwhile.

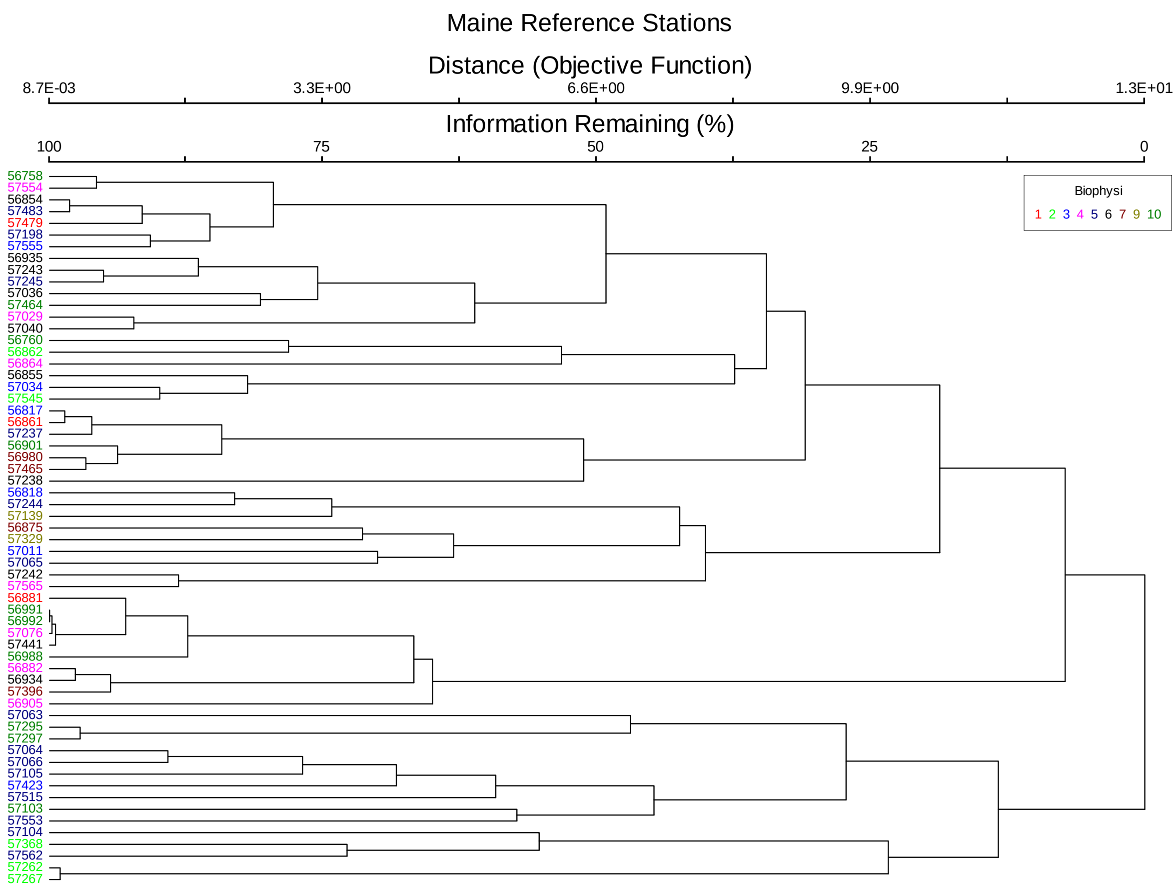


Figure C1-3. Dendrogram of Maine reference stations color-coded by biophysical region. Biophysical region 1=Aroostook Hills, 2=Mixed, 3=Central Interior, 4=Central Mountains, 5=East Coastal Region, 6=Eastern Interior, 7=Eastern Lowlands, 8=Southwest Interior, 9=Western Foothills, 10=Western Mountains.

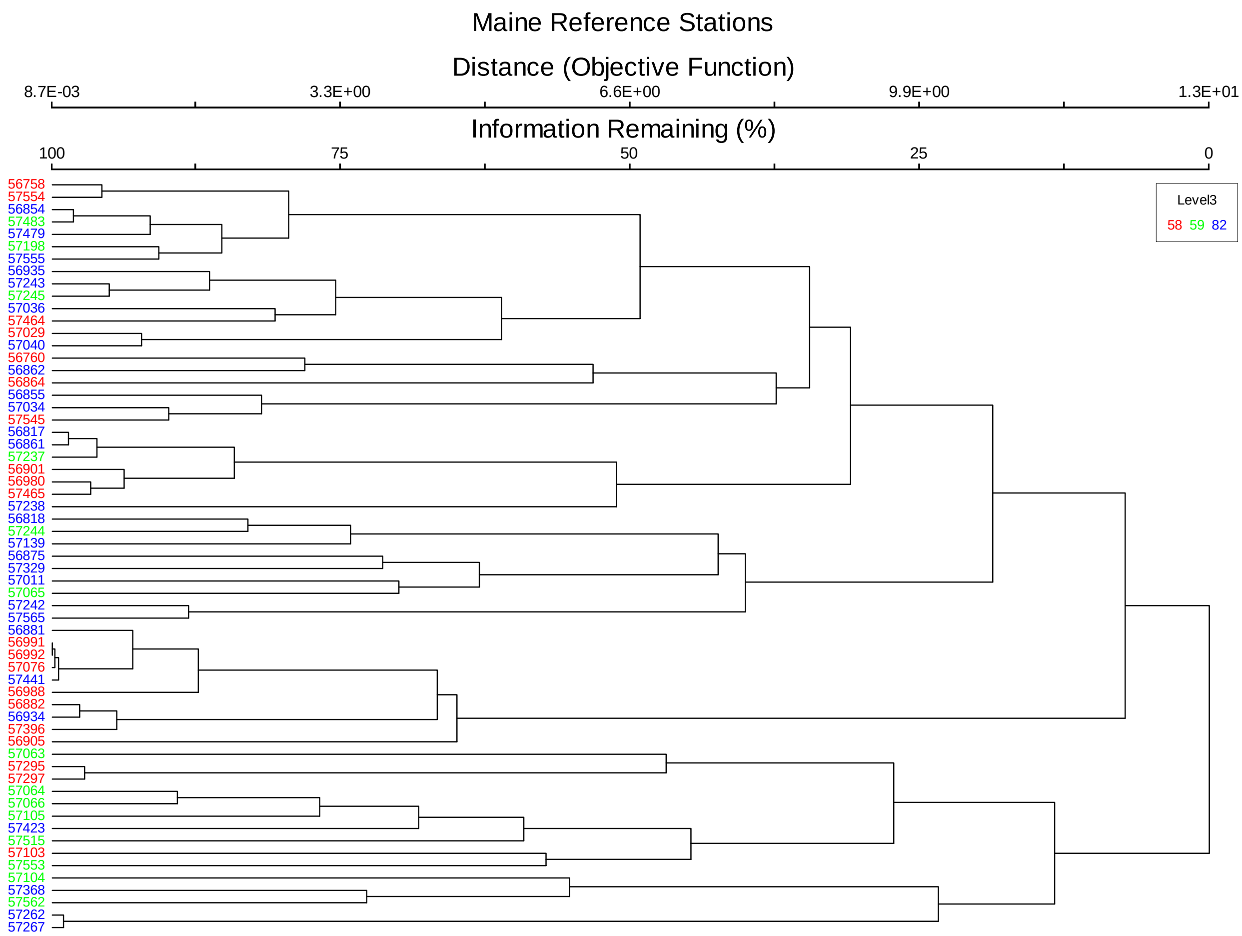


Figure C1-4. Dendrogram of Maine reference stations color-coded by level 3 ecoregion. Level 3 Code 58=Northeastern Highlands, 59=Northeast Coastal Zone and 82=Laurentian Plains and Hills.

In addition to the cluster analysis, other site grouping options were explored. None of these analyses proved any more successful in forming site groupings. Information on these other options is available upon request.

C2 UTAH

C2.1 Individual Station Selection

Reference stations (as designated by Utah DWQ[2]) with the longest-term biological data were identified and analyzed for long-term trends. There were four stations in Utah that had 10 or more years of data (**Table C2-1**). Locations of these stations are shown in **Figure C2-1.** Brief descriptions of the 4 sites are given below and are summarized in **Table C2-2**. Additional information (i.e. aerial photos) is available upon request.

Table C2-1. Summary of how many years the reference and unclassified stations in Utah had been sampled

# Years Sampled	Reference Stations	Unclassified Stations
10-19	4	3
5-9	4	29
2-4	7	178
1	54	300

StationID 4927250 (Latitude 40.7529444, Longitude -111.3735833). This station is located on the Weber River about 0.5 miles above Rockport Reservoir in Summit County. It is in the Wasatch Uinta Mountains/Mountain Valleys ecoregion at an elevation of 6059 feet. This station has 19 years of data, ranging from 1985 to 2005. Samples were taken in the spring, summer and fall. When limited to only fall samples, 17 years of data are available. Based on aerial photographs, this station appears to be influenced by human activities.

StationID 5940440 (Latitude 38.28, Longitude -112.5671111). This station is located on the Beaver River above a USGS gage in Beaver County. It is in the Wasatch Uinta Mountains/Semiarid Foothills ecoregion at an elevation of 6249 feet. This station has 11 years of data, ranging from 1994-2005. It has a mix of spring and fall samples. When limited to only fall samples, 9 years of data are available. Examination of aerial photographs reveals that is located near a road (Hwy 153), but there does not appear to be any other obvious confounding factors.

[2] reference criteria are based on a combination of a reference scoring sheet (multiple lines of scoring, i.e., habitat, land use, chemistry) and independent ranking of sites from field crew/scientists.

StationID 4951200 (Latitude 37.2848333, Longitude -112.9480833). This station is located on the Virgin River below Zion Narrows in Washington County. It is in the Colorado Plateaus/ Escarpments ecoregion at an elevation of 4492 feet. This station has 15 years of data, ranging from 1985-2004. It is in close proximity to Zion National Park. The aerial photographs that were examined did not provide much information because they were of poor quality.

StationID 4936750 (Latitude 40.4613889, Longitude -110.83). This station is located in Duchesne County. It is in the Colorado Plateaus/Semiarid Benchlands and Canyonlands ecoregion at an elevation of 6967 feet. This station has 14 years of data, ranging from 1985-2002. When limited to only fall samples, 12 years of data are available. Examination of aerial photographs shows that the station is surrounded by roads, is located in a valley, and that there is agricultural land in the upstream catchment area.

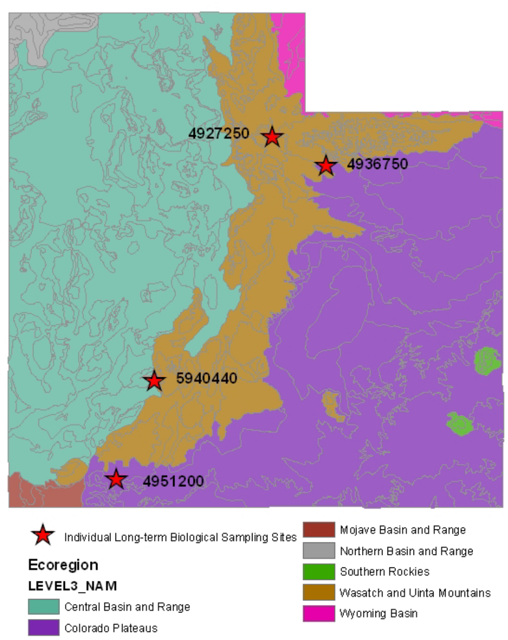


Figure C2-1. Locations of the 4 reference sites in Utah that have the longest term biological data.

Table C2-2. Station information for the 4 Utah reference sites with the longest-term biological data. # years of data refers to fall samples only. Eco_L3 is level 3 ecoregion and Eco_L4 is level 4 ecoregion. Reference status was designated by Utah DWQ. % land use refers to the area within a 1 km buffer of the station (NLCD 2001).

StationID	# Years of Data	Eco_L3	Eco_L4	UT Watershed Group	Elev_ft	Ref Status	%URB	%AGR	%BAR	%FOR
4951200	15	Colorado Plateaus	Escarpments	Sevier/Virgin/Beaver	4492	REF	3.4	0.5	28.8	67.2
5940440	9	Wasatch and Uinta Mountains	Semiarid Foothills	Sevier/Virgin/Beaver	6249.3	REF	3.9	0	0	96.1
4927250	17	Wasatch and Uinta Mountains	Mountain Valleys	Bear/Weber	6058.5	REF	4.5	21.1	0	67.1
4936750	12	Colorado Plateaus	Semiarid Benchlands and Canyonlands	Uinta Basin	6967	REF	4.8	10.3	1.1	83.7

C2.2 Site Group Selection

Due to the limited number of individual sites with long-term data, sites were grouped together to obtain more long-term biological datasets to analyze. Efforts were focused in the Wasatch Uinta Mountains and Colorado Plateaus ecoregions, where most of the stations are located. One limitation of the reference stations that were used in the individual station analyses was that % urban and % agricultural land uses within a 1 km buffer of the stations was higher than desired. Because of this, different reference criteria were used to screen for stations to include in the site groups. Initially stations in the Wasatch and Uinta Mountains that had <1% urban and <10% agricultural land uses were selected, but not enough stations met this criteria, so the criteria was changed to <2% urban and <10% agricultural land use. This resulted in a site group consisting of 150 sites with data from 1983-2005. Jeff Ostermiller of Utah DWQ recommended that groups be further refined because there is a lot of variation among sites within the level 3 ecoregions (particularly between mountain and valley sites). When the 150 sites were divided into level 4 ecoregions, two of the resulting site groups had enough stations to work with: Mid-elevation Uinta Mountains (39 sites) and Semiarid Foothills (62 sites). These datasets were further refined so that they only contained stations with 2 or more years of data. Efforts were also made to limit the number of basins or watershed groups within which stations were located, because NMDS ordinations of the preliminary data showed that stations tended to cluster together based on basin/watershed group. The Mid-elevation Uinta Mountains site group was limited to sites within the Ashley-Brush and Duchesne basins and the Semiarid Foothills group excluded sites in the Colorado Watershed Group.

A similar process was followed when selecting stations within the Colorado Plateaus level 3 ecoregion. Stations that had <2% urban and <10% agricultural land use within the 1 km buffer area were selected. The resulting group consisted of 60 stations. The best option was to divide the groups into level 4 ecoregions, the Semiarid Benchlands and Canyonlands, with 21 stations. Because of the small sample size, all stations were included in the group regardless of basin or number of years of data.

Locations of the stations in each of the 3 site groups are shown in **Figure C2-2.** Site information for the stations in the 3 site groups is summarized in **Table C2-3**, and the lists of stations comprising each site group and the years for which data were available for each station are shown in **Tables C2-4, -5 and -6.**

A number of different analyses were performed on the site group datasets. Preliminary ordinations showed that stations generally clustered together by watershed/basin groups. Attempts were made to reduce the differences among stations but results still need to be interpreted with caution because site specific differences were still evident in the ordinations that were performed on the revised datasets, as well as in results from the correlation analyses. In some instances, trends were detected but they were due to the presence/absence of certain taxa at certain sites that were sampled during certain years, rather than due to differences associated with changes in climatic variables. Selected results from the NMDS ordinations are shown in **Figures C2-3**, **-4** and **-5**. More results from these ordinations and also from the correlation analyses are available upon request.

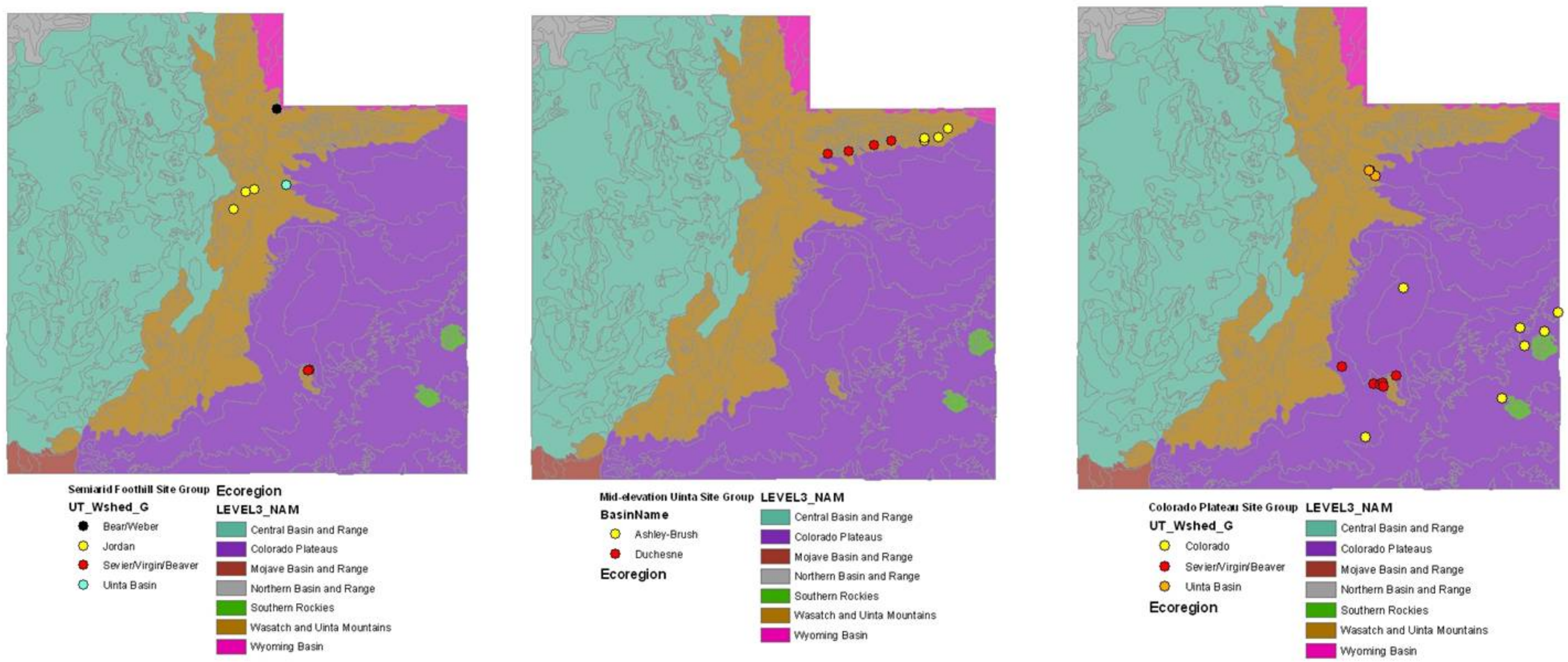


Figure C2-2. Locations of stations in the 3 Utah site groups.

Table C2-3. Summary of site information for the 3 Utah site groups. WU_SF refers to the Semiarid Foothills site group, WU_ME refers to the Mid-elevation Uinta Mountains site group and CP refers to the Colorado Plateaus Semiarid Benchlands and Canyonlands site group. % land use refers to the area within a 1 km buffer of the station (NLCD 2001).

SiteGroup	# Sites	# Yrs of Data	Samples Used	Eco_L3	Eco_L4	Elev_ft	%URB	%AGR	%BAR	%FOR	%WET
WU_SF	8	20	June-November	Wasatch and Uinta Mountains	Semiarid Foothills	5164 to 8048	0 to 1.7	0 to 0.1	0 to 2.7	97 to 100	0 to 2
WU_ME	9	12	June-November	Wasatch and Uinta Mountains	Mid-elevation Uinta Mountains	7200 to 9776	0 to 1.9	0	0 to 5	86 to 100	0 to 3.9
CP	16	14	June-November	Colorado Plateaus	Semiarid Benchlands and Canyonlands	4126 to 7479	0 to 1.1	0 to 8.9	0 to 33	62 to 99.9	0 to 5.6

Table C2-4. Semi-arid Foothill stations and the years during which they were sampled

Year	StationID							
	4936660	**4926370**	**5988610**	**4954450**	**4954230**	**4995710**	**4995820**	**4954440**
1983					x			
1984								
1985								
1986								
1987				x	x			x
1988				x	x			x
1989			x	x	x	x		x
1990	x		x				x	
1991	x							
1992	x						x	
1993	x					x		
1994			x					
1995	x							
1996	x							
1997	x						x	
1998	x	x						
1999		x						
2000		x						
2001		x						
2002		x						
2003		x						
2004		x						
2005		x				x		

Table C2-5. Mid-elevation stations and the years during which they were sampled

Year	StationID								
	Ashley-Brush				Duchesne				
	5987290	5987000	5987350	5987230	4936840	5987530	5987610	5987700	5987870
1983						x			
1984									
1985									
1986									
1987	x					x		x	x
1988	x						x	x	x
1989	x	x				x	x		x
1990	x						x	x	
1991	x	x						x	x
1992								x	x
1993			x	x					
1994									
1995									
1996	x		x	x					
1997		x	x	x					x
1998									
1999									
2000									
2001									
2002					x				
2003					x				
2004									
2005									

Table C2-6. Colorado Plateaus stations and the years during which they were sampled

Year	StationID															
	Colorado								Sevier/Virgin/Beaver					Uinta Basin		
	4930340	4954196	4954460	4955820	4956400	4958032	4958730	4958755	4954220	4954090	4954110	4954140	4954180	5987860	5987880	4936200
1977					x		x									
1983																
1984												x	x			
1985												x	x			
1986												x	x			
1987									x	x	x	x	x			
1988			x						x		x	x	x			
1989			x						x		x		x			
1990																x
1992			x													
1994																x
1996																
1997	x				x									x	x	
1998	x				x											
2002																
2003		x														
2004				x												
2005						x		x								

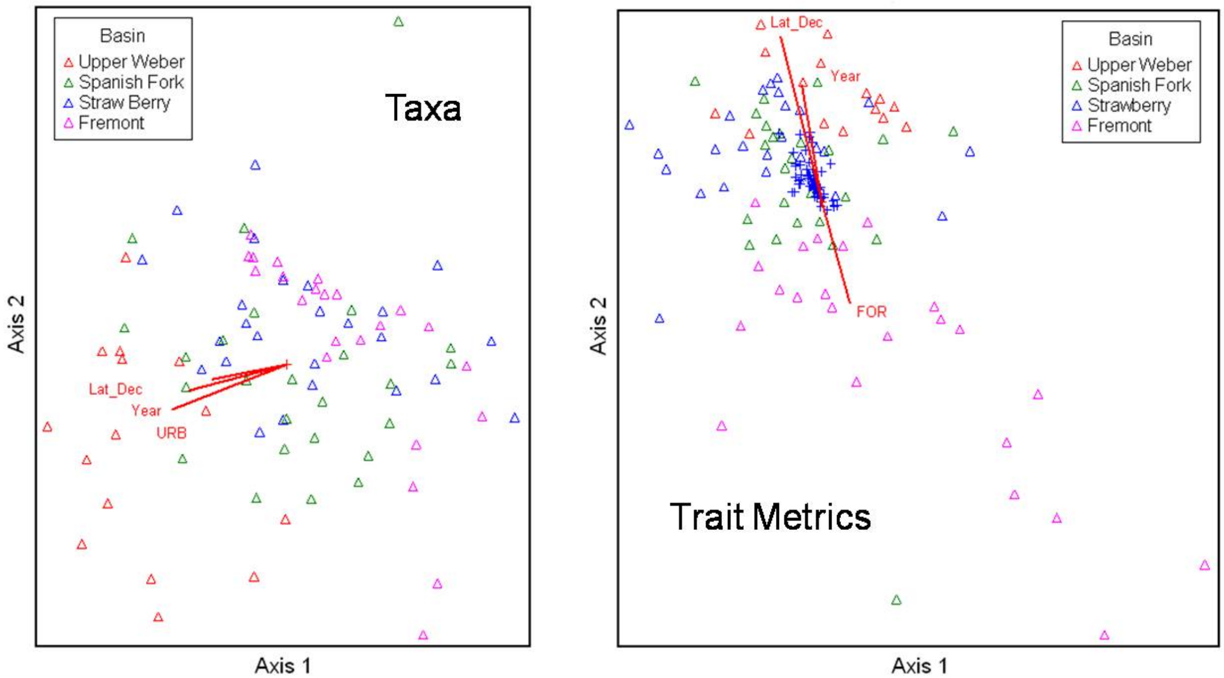


Figure C2-3. Plots of Utah Semi-arid Foothills Site Group NMDS ordinations based on taxonomic composition and selected trait metrics.

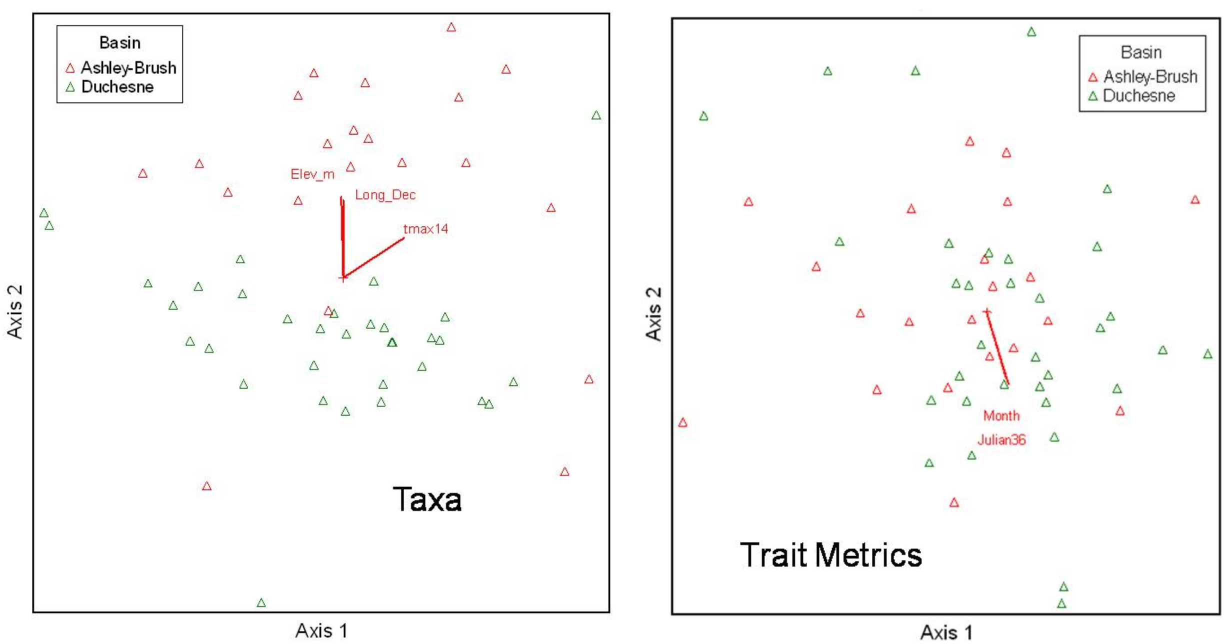


Figure C2-4. Plots of Utah Mid-Elevation Site Group NMDS ordinations based on taxonomic composition and selected trait metrics.

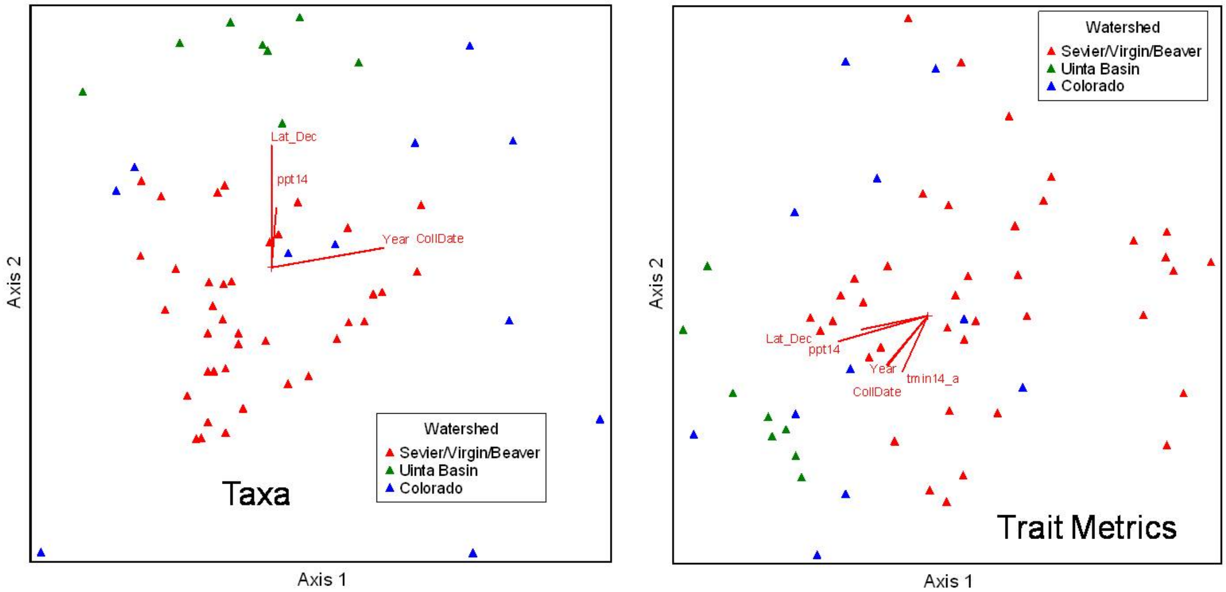


Figure C2-5. **Plots of Colorado Plateaus Site Group NMDS ordinations based on taxonomic composition and selected trait metrics.**

C3. NORTH CAROLINA

C3.1 Individual Station Selection

Reference stations[3] (as designated by NCDENR) with the longest-term biological data were identified and analyzed for long-term trends. There was one reference station (sampled using the standard qualitative/full-scale collection method) in North Carolina that had 10 or more years of data (**Table C3-1**). This station plus four other reference stations were included in the individual station analyses. Locations of these reference stations are shown in **Figure C3-1**. Brief descriptions of the five stations are given below and are summarized in **Table C3-2**. Additional information (i.e. aerial photos) is available upon request.

Table C3-1. Summary of how many years of data were available for the reference and unclassified biological sampling stations in North Carolina. These numbers apply only to stations that were sampled using the standard qualitative (full-scale) collection method.

# Years Sampled	Reference Stations	Unclassified Stations
10 +	1	8
5 to 9	2	146
3 to 4	4	182
2	8	237
1	12	933

StationID NC0109 (Latitude 36.5522, Longitude -81.1833). This station is located on the New River at SR 1345 in Alleghany County. It is in the Blue Ridge EPA level 3 ecoregion and Mountain NCDENR ecoregion. This station has the most number of years of biological data (11 years: 1983-1990, 1993, 1998 & 2003). Land use/land cover within the 1 km buffer is 44% forest, 44% agricultural (of this, 99.6% is pasture hay) and 3% urban.

StationID NC0207 (Latitude 35.126944, Longitude -83.61916). This station is located on the Nantahala River at FSR 437 in Macon County. It is in the Blue Ridge EPA level 3 ecoregion and Mountain NCDENR ecoregion. This station has 9 years of biological data: 1984,

[3] Land use/land cover in the upstream catchment area was a major consideration in reference site selection. These sites were recommended by Trish MacPherson (formerly NCDENR).

1986, 1988, 1990, 1991, 1993, 1994, 1999 & 2004. Land use/land cover within the 1 km buffer is 96% forest and 2.6% urban. Much of the upstream watershed is located in the Nantahala National Forest. A USGS gage is located at this site (USGS 03504000).

StationID NC0209 (Latitude 35.66722, Longitude -83.07277). This station is located on Cataloochee Creek at SR 1395 in Haywood County. It is in the Blue Ridge EPA level 3 ecoregion and Mountain NCDENR ecoregion. This station has 8 years of biological data: 1984, 1986, 1989, 1990, 1991, 1992, 1997 & 2002. It is located in the Great Smokey Mountains National Park. Land use/land cover within the 1 km buffer is 97% forest and 3% urban. Based on aerial photography, the urban land use is comprised of a campground, a park road and some park buildings. A USGS gage is located at this site (USGS 03460000).

StationID NC0075 (Latitude 35.38638, Longitude -79.8322). This station is located on Little River at SR 1340 in Montgomery County. It is in the Piedmont ecoregion. This station has 8 years of data: 1983, 1985, 1988, 1989, 1995, 1996, 2001 & 2006. Land use/land cover within the 1 km buffer is 1% urban, 19% shrub and grassland, and 80% forest. A USGS gage is located at this site (USGS 02128000).

StationID NC0248 (Latitude 35.43861, Longitude -80.00055). This station is located on Barnes Creek at SR 1303 in Montgomery County. It is in the Piedmont ecoregion. This station has 7 years of data: 1984, 1985, 1987, 1989, 1996, 2001 & 2006. Land use/land cover within the 1 km buffer it is 0.6% urban,5 % agricultural and 88% forest. There is a nearby road. This site is located in the Uwharrie National Forest, but there are more agricultural lands in this watershed than at some of the other sites. Trish MacPherson identified it as an interesting site because there are a few mountain taxa still hanging on in the Uwharrie Mountains, but these are old, eroded mountains that don't look anything like the western mountains and are actually in the middle of the Piedmont. She believes this could be an area where cold-water taxa such as *Epeorus* might disappear first as temperatures rise. Trish considers this a "relict population" site that is fairly undisturbed.

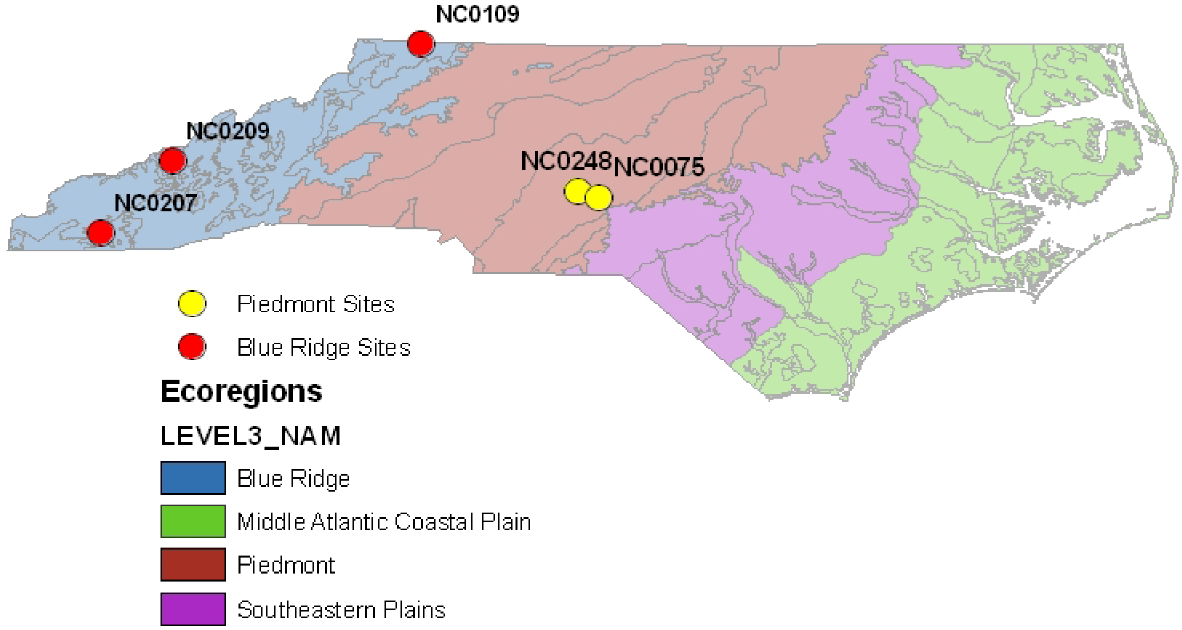


Figure C3-1. Locations of the 5 reference sites in North Carolina that were examined for long-term trends.

Table C3-2. Station information for the 5 North Carolina reference sites with the longest-term biological data. # years of data refers to standard qualitative/full-scale collection method samples only. Eco_L3 is level 3 EPA ecoregion and Eco_L4 is level 4 EPA ecoregion. Reference status was designated by NCDENR. % land use refers to the area within a 1 km buffer of the station (MLRC 2001).

StationID	WaterbodyName	# Yrs of Full Scale Data	Eco_L3	Eco4_Name	Elev_ft	%URB	%AGR	%BAR	%FOR	%WET	Sample Months Used
NC0109	NEW R - SR 1345	11	Blue Ridge	New River Plateau	2341.3	3.3	44*	0	44.1	0.2	July & Aug
NC0207	NANTAHALA R - FS RD 437	9	Blue Ridge	Southern Crystaline Ridges and Mountains	6162.4	2.6	0.4	0	96	0	July, Aug and Nov
NC0209	CATALOOCHEE CR - SR 1395	7	Blue Ridge	Southern Metasedimentary Mountains	2483.3	3	0	0	97	0	July & Aug
NC0248	BARNES CR - SR 1303	7	Piedmont	Carolina Slate Belt	350.1	0.6	5.4	0.2	87.5	1	May, July, Aug, Sept and Oct
NC0075	LITTLE R - SR 1340**	7	Piedmont	Carolina Slate Belt	489.8	1.4	0.1	0	79.7	0.1	July, Aug and Nov

*99.6% pasture/hay
*18.7% shrub and grasslands

C3.2 Site Group Selection

Due to the limited number of individual sites with long-term data, we also tried performing an analysis in the Blue Ridge ecoregion in which sites were grouped together to obtain more long-term biological datasets. The dataset that was used for this analysis was derived from reference sites (as designated by NCDENR) that were: 1. sampled from June-September; and 2. sampled using the standard qualitative/full-scale collection method. The 15 sites that comprised the dataset are listed in **Table C3-3** Locations of these sites are shown in **Figure C3-2** The years during which each of the sites was sampled are listed in **Table C3-4**. Eighteen years of (non-continuous) data are available from 1983 through 2006. A genus-level OTU was used to derive the taxa list, and relative abundance was used. The raw data from which the relative abundances were calculated is categorical (1=rare (1-2 specimens), 3=common (3-9 species) and 10=abundant (10 or more species). When multiple sites were sampled in a year, the mean value was calculated so that there was only one value for each trait or taxa per year (i.e. in 1983, Sites NC0107 and NC0109 were sampled. The one value that was used for 1983 was the average value from those two sites).

Two main types of analyses were performed. One involved looking for trends among individual taxa and the other involved searching for trends among traits. First, correlation analyses were performed to see whether any taxa or traits were significantly correlated with year. Next, correlation analyses were performed to see whether any taxa or traits were significantly correlated with PRISM air temperature or precipitation data. To briefly summarize the results, 41 taxa (19 EPT, 22 non-EPT) were significantly correlated with year, 27 (13 EPT, 14 non-EPT) were significantly correlated with at least one of the PRISM air temperature variables (minimum, maximum or mean) and 19 (4 EPT, 15 non-EPT) were significantly correlated with PRISM annual precipitation. Thirteen of the % individual trait metrics were significantly correlated with year and 6 were significantly correlated with a PRISM variable. Seventeen of the taxa richness (# of taxa) variables were significantly correlated with year and 8 were significantly correlated with a PRISM variable. Plots and summary tables of the significant correlations are available upon request. Results from this analysis are not included or used anywhere else in this report.

Table C3-3. List of Blue Ridge reference stations (as designated by NCDENR)

StationID	# Years Sampled	WaterbodyName	Location	Lat_Dec	Long_Dec
NC0107	7	N FK NEW R	NC 16	36.50389	-81.39028
NC0109	11	NEW R	SR 1345	36.55222	-81.18333
NC0200	7	MILLS R	SR 1337	35.39861	-82.59500
NC0366	3	N FK MILLS R	SR 1341	35.39667	-82.62472
NC0806	2	THREE TOP CR	SR 1100	36.42806	-81.62389
NC0812	3	LITTLE R	SR 1128	36.46778	-81.13333
NC1006	1	W FK FRENCH BROAD R	OFF NC 281	35.18583	-82.95889
NC1285	2	CROOKED CR	SR 1135	35.60556	-82.11694
NC1289	1	S HOMINY CR	NC 151	35.53444	-82.69222
NC1438	2	S FK MILLS R	SR 1340	35.37583	-82.61500
NC1540	1	MILL CR	SR 1400	35.63667	-82.21861
NC1573	3	BOONE FK	SR 1561	36.12306	-81.77000
NC1591	1	BEECH CR	US 321	36.26111	-81.89667
NC1827	1	MACKEY CR	BE US 70	35.66972	-82.11417
NC2757	1	E FK PIGEON R	US 276	35.41056	-82.81000

Table C3-4. Years during which the Blue Ridge reference stations were sampled

Year	# of Sites Sampled	Sites					
1983	2	NC0107	NC0109				
1984	2	NC0109	NC0200				
1985	3	NC0107	NC0109	NC0366			
1986	2	NC0109	NC0200				
1987	2	NC0107	NC0109				
1988	2	NC0109	NC0200				
1989	2	NC0107	NC0109				
1990	3	NC0109	NC0200	NC1006			
1992	1	NC0200					
1993	6	NC0107	NC0109	NC0366	NC0806	NC0812	NC1438
1994	3	NC1540	NC1573	NC1591			
1997	3	NC0200	NC1285	NC1289			
1998	4	NC0107	NC0109	NC0806	NC0812		
1999	1	NC1573					
2002	5	NC0200	NC0366	NC1285	NC1438	NC1827	
2003	3	NC0107	NC0109	NC0812			
2004	1	NC1573					
2006	1	NC2757					

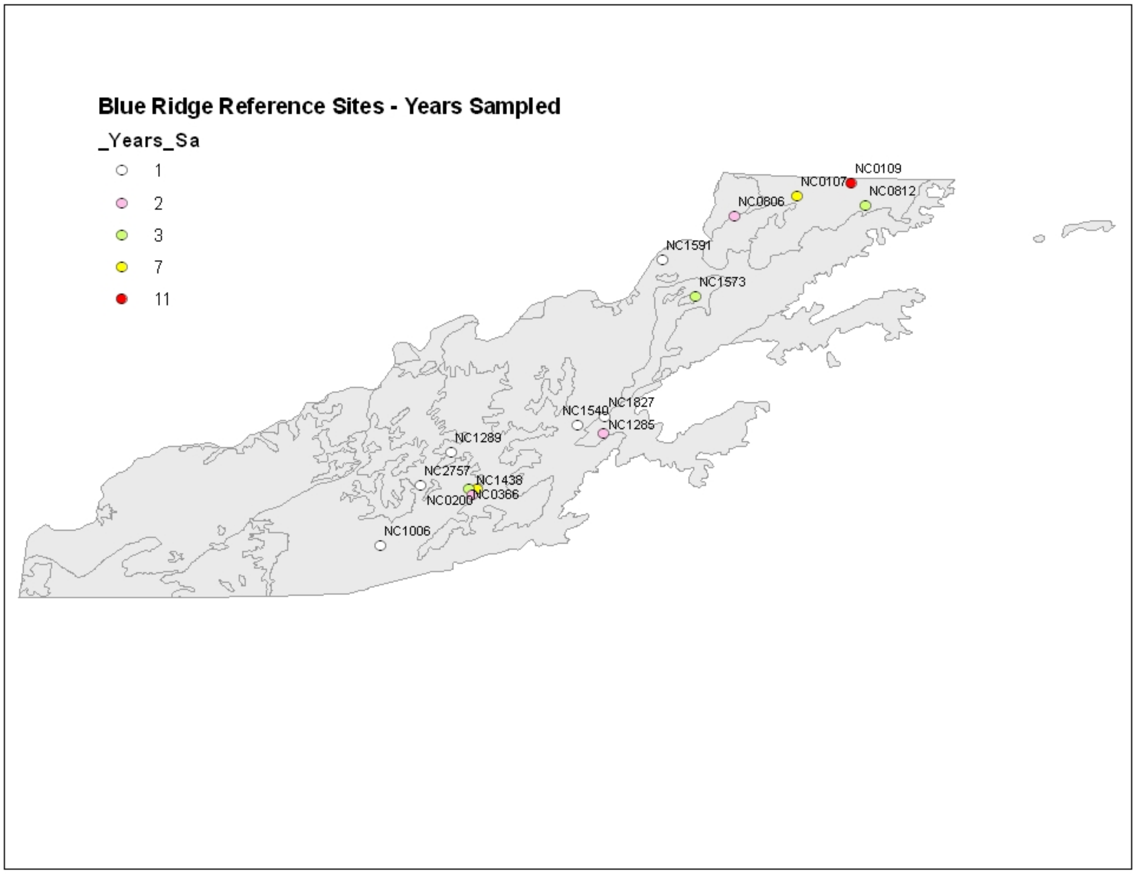


Figure C3-2. **Locations of Blue Ridge reference sites that were used in this analysis.**

APPENDIX D

Data Analyses Methods

The intent of this appendix is to present more comprehensive descriptions of the analytical approaches and methods applied to evaluate the selected state biomonitoring data sets. Each major question or approach is presented separately, with common methods described first, and then any state-specific variations.

D1. GENERATION OF TEMPERATURE-PREFERENCE AND TOLERANCE DATA

Temperature is an environmental parameter of particular interest in this project. We therefore attempted to gather as much existing relevant temperature-preference and tolerance information as possible and to use analyses of the state data sets to generate temperature preference and tolerance information for as many taxa, defined by generic-level operational taxonomic units (OTUs), as possible. The specific sources and types of existing temperature-preference and tolerance information gathered in this study and their application in categorizing temperature traits of OTUs are described in U.S. EPA (2011).

We used weighted average modeling or related approaches (e.g., maximum likelihood estimates, general linear modeling) to estimate the optima values and ranges of occurrence (tolerances) for temperature, and in some cases flow parameters, for each OTU from each state that had a sufficient distribution and number of observations to support the analysis. The methods described in Yuan (2006) were applied to derive temperature and flow optima and tolerance values.

Weighted averaging is a simple, robust approach for estimating the central tendencies of different taxa, or in our case, temperature optima and tolerance values (ter Braak and Looman, 1986). The basic approach is a straightforward weighted average--the temperature at each site in a state at which the species is observed, multiplied by the relative abundance of the species at that site, with the sum over all sites of the weighted temperatures divided by the sum of the abundances of that species from all sites. This mean temperature is taken as the preferred temperature for the taxon, and the breadth of the distribution (size of the standard deviation or other measure of spread) represents an estimate of the tolerance or sensitivity of the taxon. The approached is illustrated in Table D-1 and Figure D-1.

Table D-1. Example to illustrate the derivation of a weighted average model temperature optimum (weighted mean) estimate

Species A Temperature preference			
StationID	**Relative Abundance (RA)**	**Observed Temperature (Temp)**	**RA * Temp**
A	0.10	22	2.20
B	0.02	33	0.66
C	0.02	12	0.24
D	0.04	14	0.56
SUM	***0.18***		***3.66***

Weighted Average = 3.66 ÷ 0.18 = 20.3333

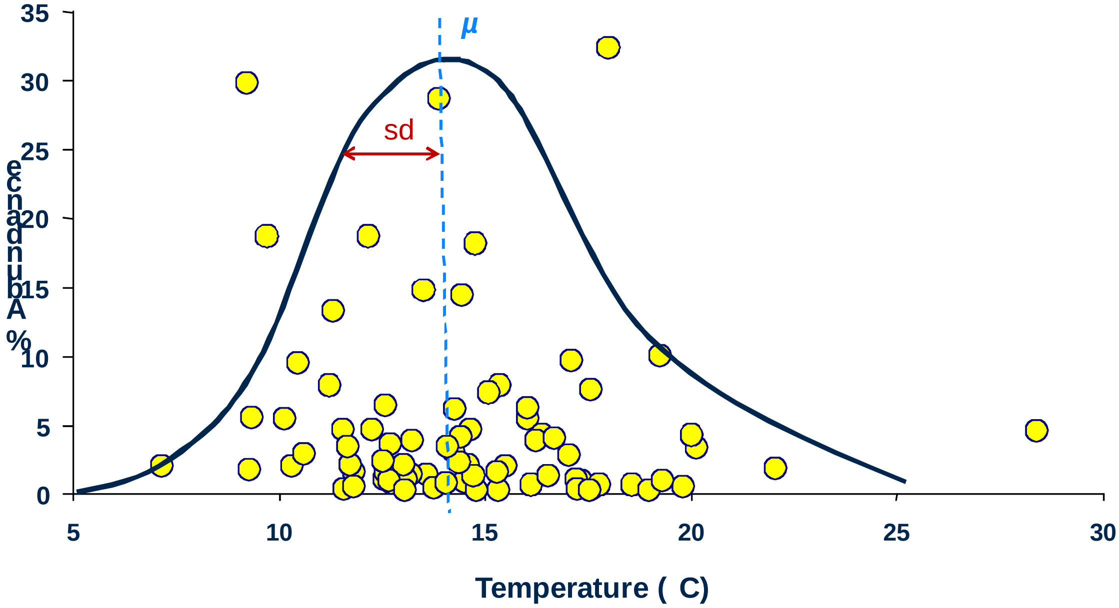


Figure D-1. Illustration of weighted average temperature distribution, where the weighted average mean (u) is taken as the temperature optimum (preference) for the taxon, and the magnitude of standard deviation (sd) is taken as an estimate of the temperature sensitivity or tolerance.

When using weighted averages, a wide distribution of samples across the environmental gradient results in a more robust estimate of temperatures of occurrence, and therefore, of inferred preference. For a given state data set, weighted average tolerance values for each OTU are computed using the same set of environmental data; therefore, any bias arising from an uneven distribution of data will be the same for all OTU, and their relative placement along the temperature gradient will generally be preserved.

The generalized linear model is also used to estimate taxon-environment relationships for each combination of taxon and environmental variable. In addition to providing a means of computing tolerance values, regression estimates of the taxon-environment relationship quantify the strength of the association between a given environmental gradient and changes in the occurrence probability or abundance of a taxon. In the case of presence/absence data, the response variable is modeled as a binomial distribution; in the case of abundance data, a negative binomial distribution is often assumed (maximum likelihood estimates).

In our analyses, weighted average calculations were used for the states that had absolute (non-categorical) abundance data by taxon (Maine, Utah and Ohio). If only presence/absence (categorical or qualitative abundance) data were available, a generalized linear model was used (North Carolina). Calculations were made separately for each state. Since use of the widest range of temperature variation available is desired in this type of analysis, all stations within each state across all ecoregions were retained in each state analysis. However, data were subset to account for seasonal variation (when needed), as well as for variation associated with different sampling methods. For example, in Utah, only samples collected during the fall index period were used. In North Carolina, only samples collected by the full collection method were analyzed.

Several statistical models were run (using R and C2 software), and model performance was compared for possible improvement of the weighted average model, weighted average partial least square regression (WA-PLSR), and maximum likelihood (ML). The WA-PLSR model result was difficult to interpret and only slightly improved the WA model. The ML model had similar performance to the WA model; therefore the WA model was used when sample sizes were sufficiently large (>500 samples). Only taxa occurring at more than 9 sites were included in the WA analyses. Low sample size affects the regression model and biases the optima and breadth values for rare taxa, especially under extreme conditions.

Based on the derived optima and tolerance values for each OTU analyzed for each state, we defined optima and tolerance rankings to support relative comparisons among taxa and regions. For example, relative rankings of taxa as cold or warm preference can be compared between Utah, where most samples were collected in the fall, and Maine, where most were collected in the summer, which might otherwise result in differences in absolute temperature values. It also allowed comparison of the Utah results from this study to be compared to results from other western datasets, which were generally based on summer samples and therefore had noticeable differences in ranges of absolute temperatures used as thresholds for designating cold- and warm-preference taxa (Herbst and Silldorff, 2007; Brandt, 2001). Comparison to these results was used to support final designation of taxa membership in cold-preference and warm-preference groups (U.S. EPA 2011).

Ranks were defined separately for temperature optima and tolerances using a scoring system. Both the temperature optima for all taxa in a state and the standard deviations were divided into the following percentiles: 0.1, 0.25, 0.4, 0.6, 0.75, 0.9, 1. Taxa associated with the lowest temperature optima and those with the smallest standard deviations (narrowest tolerance ranges), i.e., those in the lowest 10th percentile, received scores of 1. Those in the next percentile category (>0.1 up to the 25th percentile) received a score of 2, and so on, up to the highest temperature optima and widest tolerance ranges (the 90th percentile or greater) which received a score of 7 (Figure D2-2). Lower ranks for temperature optima reflect preference for colder water, and higher ranks reflect preference for warmer water. It was a relatively arbitrary judgment to include taxa with optima rankings of 1, 2 or 3 as cold water taxa and those with rankings of 5, 6 or 7 as warm water taxa. Similarly, standard deviation ranks of 1, 2 or 3 were considered sensitive (e.g., stenothermal), while ranks of 5, 6, or 7 were considered tolerant (eurythermal).

Percentile	Optimum	Breadth	Rank	
0	4.57029	2.02959	1	
0.1	6.847701	2.770389	2	Cold/Stenotherms
0.25	7.722833	3.317888	3	
0.4	8.411832	3.600784	4	
0.6	9.188384	3.812142	5	
0.75	9.689138	3.997378	6	Warm/Eurytherms
0.9	10.5325	4.442977	7	
1	15.7144	5.06721		

Figure D2-2. Example taken from Utah analysis results to illustrate development of ranking for temperature (or other environmental parameter) preference and tolerance rankings from weighted average or GLM temperature distribution results.

D2. EVALUATION OF BIOLOGICAL RESPONSES TO CLIMATE VARIABLES

D2.1 Characterization of Years as Proxy for Future Climate Conditions

To evaluate responses of a variety of biological metrics, trait and taxonomic groups, as well as indices and predictive-model results to differing climatic conditions that could be expected, we used extremes in climate variables among existing data as proxies for future climate conditions. We partitioned data at long-term reference stations in each state into years characterized by hotter (>75th percentile of the temperature distribution during years of biological collections), colder (<25th percentile of temperature), and normal (25th to 75th percentile) average annual air temperatures. Using similar thresholds, years were partitioned based on average annual precipitation into wetter, drier, and normal years. When flow data were available, a similar partitioning of high and low flow years was applied. An assumption is that these temperature, precipitation, and flow differences drive responses in benthic communities that are reasonable proxies for the types of community changes that can be expected over the long term with climate change. Another assumption is that PRISM air temperature is a reasonable surrogate for water temperature, and PRISM precipitation for flow (see Appendix A, Section A.1 for substantiation and references). Table D2-1 summarizes how these categories were grouped and designated for ANOVAs at long-term references stations among the three states analyzed.

Table D2-1. **Descriptions of the temperature, precipitation, and flow (IHA parameters) categories that were used in ANOVA analyses for long-term reference stations in all states. Note that flow was only available for the Maine long-term station 56817.**

Variable	Description

Cat1_Temp	Temperature categories: 1=coldest years (defined as years when the PRISM mean annual air temperature was < 25th percentile of the overall temperature values); 2=normal years (25th-75th percentile), 3=hottest years (>75th percentile).
Cat1_Precip	Precipitation categories: 1=driest years (defined as years when the PRISM mean annual precipitation was < 25th percentile of the overall precipitation values) 2=normal precip year (25th-75th percentile), 3=wettest years (>75th percentile).
Cat2_Temp	Temperature categories: 1=coldest years & normal years (defined as years when the PRISM mean annual air temperature was ≤ 75th percentile of the overall temperature values); 2=hottest years (>75th percentile).
Cat2_Precip	Precipitation categories: 1=driest years (defined as years when the PRISM mean annual precipitation was < 25th percentile of the overall precipitation values); 2=normal years and wettest years (≥ 25th percentile).
Cat_Flow	IHA median monthly flows averaged across July-September: 1=years with the lowest flow (<25th percentile; 2=years with normal flow (25th-75th percentile), 3=years with the highest flow (>75th percentile).

D2.2 Reference Stations and Seasonal Data used in Analyses

For Maine, the various ANOVA and correlation analyses described in Section D2.3 were conducted at stations 56817, 57011, and 57065 (see Appendix C1 for details). These 3 sites, all located in the Laurentian Plains and Hills ecoregion, are reference sites (rated as Class AA by the Maine DEQ) that have the longest-term biological data. Only rock basket samples that were collected from June-November were used in the analysis.

For Utah, the various ANOVA and correlation analyses were conducted at stations 4927250 and 5940440 in the Wasatch and Uinta Mountains and at stations 4951200 and 4936750 in the Colorado Plateau (see Appendix C2 for details). These represent reference locations with the longest-term biological data records available. Many of the analyses also were performed on reference stations grouped into three site groups: the Wasatch and Uinta Semi-arid Foothills, the Wasatch and Uinta Mid-elevation Mountains, and the Colorado Plateau. Only samples collected during the fall season were used in analyses.

For North Carolina, analyses were conducted at five reference sites--Stations NC0109, NC0207, NC0209, NC0075 and NC0248; although only one, NC0109, had relatively long-term data (>10 years) (see Appendix C3 for details). Two site groups were used--the Blue Ridge and Piedmont EPA level 3 ecoregions, which are very similar to the NCDENR Mountain and Piedmont ecoregions. All samples in the NC database that were collected using the standard qualitative method during the summer index period (June-September) were used in this analysis.

D2.3 ANOVA

One-way ANOVA tests were used to evaluate whether significant differences exist among various mean metric values from samples collected at the selected long-term reference sites for each state during hot, cold, wet, dry, and normal years. Numerous biological metrics were tested for all states (Table D2-2). The O/E metric was also tested in Utah. The ecological trait groups of cold-water and warm-water-preference taxa were tested for differences among hot, cold, wet, dry and normal years. If the p-levels from the Tukey honest significant difference (HSD) test for unequal sample size (N) (Spjotvoll/Stoline) were less than 0.05, the differences in metric values among the different temperature and precipitation groups were considered to be significant.

We examined the distributions of cold- and warm-water-temperature indicator taxa to try to identify areas that are more likely to be 'vulnerable' to the effects of climate change, in particular the increase in temperature. One-way ANOVAs were used to determine whether significant differences exist between the number of cold- and warm-water taxa between ecoregions and between elevations. All samples in each state data base were used in these (spatial) analyses. Ecoregions included for each state were:

- Maine—Laurentian Plains and Hills, Northeastern Highlands and Northeast Coastal Zone.
- Utah—Wasatch and Uinta Mountains and Colorado Plateaus level 3 ecoregions.
- North Carolina— Mountain, Piedmont and Coastal ecoregions.

Specific elevation categories varied among states:

- Maine—sites < 150 m and > 150 m.
- Utah-- sites < 2000 m and > 2000 m.
- North Carolina—sites < 500 m and > 500 m.

D2.3.1 Maine ANOVAs

The analyses using Maine's data used ANOVA to explore the relative importance of the various input metrics used in the linear discriminant models for classification of station condition and to relate these to any effects on the metrics due to climate change. These analyses also examined (1) how model input values differ among the different station classifications; (2) how much metric values have to change for a sample to change classification, e.g. from Class A to Class B (or B to C, etc.); (3) whether certain metrics are more important than others in contributing to classification changes; and (4) whether certain metrics are more likely to be affected by climate change than others, and if so, how they are affected, and how this affects overall classification. Understanding these aspects of the data is difficult, because Maine's classification models look at multiple variables simultaneously, and because there are no firm thresholds or metric values at which a sample goes from being a Class A to Class B, etc.

Instead, ANOVA was used on all the samples in the Maine database to see how mean metric values differed among the different classes. At Station 56817, which has the longest-term biological data and is considered by Maine DEP to be a reference site, 9 of the 22 annual samples collected from 1985 to 2006 were classified as Class B, while all the others were Class A. We used one-way ANOVA to determine which model input metrics had significantly different mean values between the Class A and Class B samples.

To determine which of Maine's station classification discriminant model metrics are affected by climate-related variables (temperature, precipitation and flow), one-way ANOVA tests were used to evaluate whether significant differences exist between mean model input metric values from samples collected during hot, cold, wet, dry, low flow, high flow, and normal years.

Because Maine's linear discriminant models are not used in other northeastern states, we performed ANOVAs on metrics that are commonly used to assess streams in northeastern states and determine if significant differences occurred between hot, cold, wet, dry, and normal years. Among the most commonly-used metrics are total taxa, EPT taxa, Ephemeroptera taxa, Plecoptera taxa, Trichoptera taxa, Hilsenhoff Biotic Index (HBI), an assortment of functional feeding group and habit metrics, percent dominant taxon and the Shannon Wiener diversity index. In addition, a variety of other biological metrics were evaluated, as listed in **Table D2-2**.

It should be noted that richness values are affected by the operational taxonomic unit (OTU) that is used in the analysis. A mostly genus-level OTU was used in this analysis because this taxonomic level was found to be most appropriate for the long-term Maine dataset.

Table D2-2. List of biological metrics that were evaluated in Maine, Utah, and North Carolina.

Metric	Descriptions
Total taxa	# of Total taxa
Ephemeroptera taxa	# of Ephemeroptera taxa
Trichoptera taxa	# of Trichoptera taxa
Plecoptera taxa	# of Plecoptera taxa
EPT Taxa	# of Ephemeroptera, Plecoptera and Trichoptera taxa
Percent Plecoptera	Percent individuals in the Order Plecoptera
Percent EPT	Percent individuals - Ephemeroptera, Plecoptera and Trichoptera
HBI	Hilsenhoff Biotic Index (calculated using New Mexico tolerance values)
Clinger Taxa	Habit - number of clinger taxa
Swimmer Taxa	Habit - number of swimmer taxa
Burrower Taxa	Habit - number of burrower taxa
Climber Taxa	Habit - number of climber taxa
Sprawler Taxa	Habit - number of sprawler taxa
Percent Clinger	Habit - percent clinger individuals
Percent Swimmer	Habit - percent swimmer individuals
Percent Burrower	Habit - percent burrower individuals
Percent Climber	Habit - percent climber individuals
Percent Sprawler	Habit - percent sprawler individuals
Collector-gatherer Taxa	Functional Feeding group - number of collector-gatherer taxa
Collector-filterer Taxa	Functional Feeding group - number of collector-filterer taxa
Shredder Taxa	Functional Feeding group - number of shredder taxa
Herbivore/Scraper Taxa	Functional Feeding group - number of herbivore/scraper taxa
Predator Taxa	Functional Feeding group - number of predator taxa
Percent Collector-gatherer	Functional Feeding group - percent collector-gatherer individuals
Percent Collector-filterer	Functional Feeding group - percent collector-filterer individuals
Percent Shredder	Functional Feeding group - percent shredder individuals
Percent Herbivore/Scraper	Functional Feeding group - percent herbivore/scraper individuals
Percent Predator	Functional Feeding group - percent predator individuals

Shannon Wiener DI	Shannon Wiener Diversity Index (log2)
% Dominant01 taxa	Percent dominant taxon individuals
Temp_CoreColdPct	Thermal Preference and Tolerance -Percent cold water individuals
Temp_CoreWarmPct	Thermal Preference and Tolerance -Percent warm water individuals
Temp_CoreCold_Tax	Thermal Preference and Tolerance -Number of cold water taxa
Temp_CoreWarm_Tax	Thermal Preference and Tolerance -Number of warm water taxa
PerennialPct	Percent perennial stream individuals (these taxa require water for their entire life cycle).
IntermitPct	Percent intermittent stream individuals (these taxa are found in perennial streams but tend to be more dominant in numbers in intermittent conditions).
Drought_Pct	Percent individuals that possess at least one of the following traits: ability to survive desiccation, adult ability to exit, respiration plastron/spiracle
Drier_WinPct	Percent individuals that possess the most number of traits states that are predicted or have been shown to be most favorable in a drier climate scenario
Drier_LoserPct	Percent individuals that have the fewest favorable trait states and the most number of unfavorable trit states in a drier climate scenario
WarmDrier_LoserPct	Percent individuals that have the fewest favorable trait states and the most number of unfavorable trait states in a warmer drier climate scenario
OCH_Pct	Percent individuals - Odonata, Coleoptera and Hemiptera

Table D2-2. Continued

Metric	Descriptions
PerennialTax	Number of perennial stream taxa (these taxa require water for their entire life cycle)
IntermitTax	Number of intermittent stream taxa (these taxa are found in perennial streams but tend to be more dominant in numbers in intermittent conditions).
DroughtTax	Number of taxa that possess at least one of the following traits: ability to survive desiccation, adult ability to exit, respiration plastron/spiracle
WarmDrier_LoserTax	Number of taxa that have the fewest favorable trait states and the most number of unfavorable trait states in a warmer drier climate scenario
Drier_LoserTax	Number of taxa that have the fewest favorable trait states and the most number of unfavorable trait states in a drier climate scenario
OCHTax	Number of Odonata, Coleoptera and Hemiptera taxa

D2.3.2 Utah ANOVAs

Biological metrics that are commonly used to assess streams in southwestern states were selected for this analysis, for example from Idaho, New Mexico, Colorado, Nevada, Wyoming, Montana and Arizona. The list of metrics that were evaluated is shown in **Table D2-2** (O/E was also analyzed). Richness values are affected by the OTU that is used in the analysis. A mostly genus-level OTU was used in this analysis because this taxonomic level was found to be most appropriate for the long-term Utah dataset. Some taxa, such as Chironomidae, were grouped to family level and higher.

O/E scores were evaluated to provide information on the sensitivity of O/E scores to changes in annual temperature and precipitation. Scores used in the ANOVAs were calculated for Stations 4927250, 4951200, 4936750 and 5940440 using the fall Utah RIVPACS model. These calculations involved changing O and keeping predictor variables, which are long-term averages, constant. The OTUs used by the Utah DEQ in model construction were retained, so that taxa lists would be consistent across years. Because the model was developed using a subset of more recent data (about 5 years worth), it may not perform as well on older datasets.

Therefore, these OTUs may not be as appropriate for the longer-term datasets that were analyzed in this exercise.

D2.3.3 North Carolina ANOVAs

In Maine and Utah we were able to perform one-way ANOVA tests to evaluate whether significant differences exist between mean metric values from samples collected at selected sites during hot, cold, wet, dry, and normal years. For the North Carolina dataset, we only had sufficient data to conduct this type of analysis on site NC0109 (11 yrs data). Correlation analyses were also used to evaluate relationships between the selected metric values and mean annual air temperature and precipitation variables at NC0109 as well as at the other sites (see Section D2.4).

D2.4 Correlation Analyses

Correlation analyses were performed in all states to test the relationships between the biological metrics listed in **Table D2-2** and year to test for temporal trends; or annual average air temperature or precipitation to examine basic relationships to climate variables. In Utah correlation analyses were also performed to explore relationships between O/E values and climatic variables for each site and sampling year.

In North Carolina, to further explore the relationship between temperature indicator taxa, tolerance values, the NCBI and climate-related variables, three different correlation analyses were performed: 1. correlation analysis of temperature optima values vs. tolerance values; 2. correlation analysis of temperature-indicator metrics at selected Mountain and Piedmont reference sites (percent cold- and warm-water-indicator individuals and number of cold- and warm-water-indicator taxa) vs. NCBI scores; and 3. correlation analysis of BI values and PRISM mean annual air temperature and PRISM mean annual precipitation. The correlation analyses were performed on datasets that used genus-level tolerance values. Tolerance values can vary within some genera, and therefore, these NCBI scores may vary somewhat from NCDENR BI scores (but they are generally close).

Because not all southeastern states use EPT taxa richness and the NCBI to rate biological sampling sites, we performed additional analyses on metrics listed in **Table D2-2**. Richness values are affected by the OTU that is used in the analysis. A mostly genus-level OTU was used in this analysis because this taxonomic level was found to be most appropriate for the long-term NC dataset.

D2.5 NMDS Ordinations

Non-metric Multidimensional Scaling (NMDS) ordinations were performed on data from selected reference stations with sufficient long-term data:

- Maine--Station 56817;
- Utah-- Stations 4927250 and 4951200;
- North Carolina—Insufficient data .

NMDS is an ordination that takes the taxa in the samples and shows in ordination space how closely related the samples are based on their species composition. NMDS was performed using PCOrd (McCune & Mefford, 1999), a Sorensen distance measure, and a maximum of 3 axes. Annual samples were categorized based on hot/cold/normal, and wet/dry/normal years to assess patterns. We also used the environmental variables described in **Table D2-3** to group the data while looking for trends.

Table D2-3. Summary of the environmental variables that were used to group data and look for trends in the NMDS

Variable	Description
Cat_Temp	Temperature categories: 1=coldest years (defined as years when the PRISM mean annual air temperature was < 25th percentile of the overall temperature values); 2=normal years (25th-75th percentile), 3=hottest years (>75th percentile).
Cat_Precip	Precipitation categories: 1=driest years (defined as years when the PRISM mean annual precipitation was < 25th percentile of the overall precipitation values) 2=normal precip year (25th-75th percentile), 3=wettest years (>75th percentile).
tmean14	PRISM mean annual air temperature
ppt14	PRISM mean annual precipitation
PrevYr_tmean14	PRISM mean annual air temperature from the previous year (lag effects)
PrevYr_ppt14	PRISM mean annual precipitation from the previous year (lag effects_
tmean14_absdifc	Absolute difference between the PRISM mean annual air temperature from the sampling year and the previous year
ppt14_absdifc	Absolute difference between the PRISM mean annual precipitation from the sampling year and the previous year
In addition, for Maine:	
MonthMed	IHA - Average of median flow values from July, August and September
Flash	R-B Flashiness Index
1d_min	IHA - 1 day minimum flow
3d_min	IHA - d day minimum flow
1d_max	IHA - 1 day maximum flow
3d_max	IHA - 3 day maximum flow

D3 EFFECTS OF TEMPERATURE-SENSITIVITY TRAITS GROUP COMPOSITION ON VARIOUS STATE METRICS AND INDICES

D3.1 How cold- and warm-water-indicator taxa may affect EPT metrics, HBIs and BCG tier assignments and how these effects may vary across different ecoregions in Maine

Because annual air temperature is predicted to increase as a result of climate change, temperature-preference and tolerance traits are of particular interest. We examined how changes in species composition resulting from replacement of cold-water-indicator taxa may affect state assessment methods. Specifically, we examined potential effects on EPT metrics and the HBI because these are commonly used in assessments in many states. In addition, in Maine, we evaluated Biological Condition Gradient (BCG) attribute levels that were assigned to the temperature indicator taxa during The New England Wadeable Stream Survey (NEWS) (US EPA 2007); Class A indicator taxa temperature preferences and tolerances; and the distribution of the temperature indicator across the different level 3 ecoregions to see whether some ecoregions are more likely to be more vulnerable to climate change effects than others.

D3.2 How cold- and warm-water-indicator taxa may affect indices in North Carolina

We evaluated relationships between temperature indicator taxa and EPT taxa richness, the NCBI and final bioclassification scores. This involved looking at the number of temperature-indicator taxa that are EPT taxa and the tolerance values of the temperature-indicator taxa. In addition, we evaluated two different scenarios: 1. a ‘worst case’ scenario in which all the cold-water-temperature-indicator taxa at selected Mountain reference sites were dropped; 2. a scenario in which Mountain criteria were applied to biotic assemblages at selected reference Piedmont sites (this simulated a scenario in which taxa that are typically found in Mountain sites are replaced by taxa that typically inhabit Piedmont sites). For both scenarios, we evaluated how this affected the EPT richness, NCBI and final bioclassification scores.

APPENDIX E

Detailed Results for Maine

The intent of this appendix is to provide more comprehensive and detailed information on the large number of analyses that were performed on the Maine data. Some of the analyses that are covered in this appendix are also referenced (generally in less detail) in the main body of the report. When this occurred, attempts were made to reduce any overlap or duplication in the reporting of results.

E1 OVERVIEW OF MAINE'S LINEAR DISCRIMINANT MODEL

Information in this section was provided by Maine DEP (see Davies, SP; Tsomides, L. 2002. Methods for Biological Sampling and Analysis of Maine's Rivers and Streams. DEP LW0387-B2002. Prepared for the State of Maine Department of Environmental Protection. http://www.maine.gov/dep/blwq/docmonitoring/biomonitoring/materials/finlmeth1.pdf).

Maine DEP rates sites using aquatic life decision models. These are four statistical models that use 30 variables of the macroinvertebrate community to determine the strength of association of a sample community to Maine's water quality classes. The first stage model acts as a screen and gives the strength of association of the sample to each of the different water quality classes. This model provides four initial probabilities that a given site attains one of three classes (A, B, or C) or is in nonattainment (NA) of the minimum criteria for any class. Association values are computed for each classification using one four-way model and three two-way models. These probabilities have a possible range from 0.0 to 1.0 and are used, after transformation, as variables in each of the three subsequent second stage or final decision models. Each of the four linear discriminant models uses different variables, providing independent estimates of class membership. The same criterion is applied to all sites. A flow chart depicting decision criteria is shown in **Figure E1-1**. The protocol is outlined in the Maine DEP methods manual (Davies and Tsomides 2002).

Process for Determining Attainment Class Using Association Values

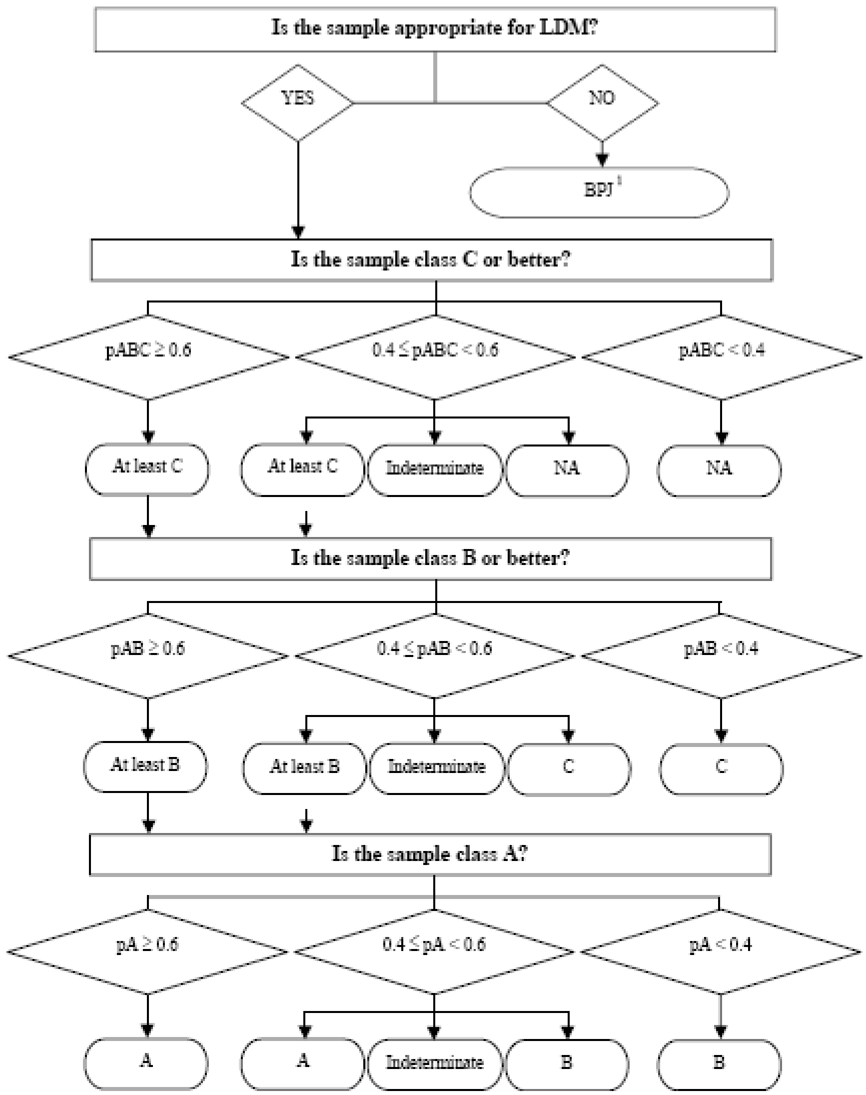


Figure E1-1. Flow chart that outlines the process that Maine DEP uses for determining attainment class using association values from its 4 linear discriminant models (chart by Thomas J. Danielson, taken from ME DEP 2002 monitoring manual).

The variables used in the first stage model are variables important to the evaluation of all classes. Of the nine variables used in the first modeling stage, 5 measure abundance, 2 measure richness, and 2 variables are biotic indices involving tolerance to pollution and abundance. The first stage model uses the following nine variables: total abundance, generic richness, Plecoptera and Ephemeroptera abundance, Shannon-Wiener Generic Diversity Index, Hilsenhoff Biotic Index (HBI), Relative Abundance Chironomidae, Relative Richness Diptera and Hydropsyche Abundance. A list of all the model input metrics can be seen in **Table E1-1**.

The final decision models (the three, two-way models- C or Better Model, B or Better Model, or A Model.) are designed to distinguish between a given class and any higher classes as one group and any lower classes as another group (e.g. Classes A+B+C vs NA; Classes A+B vs Class C+NA; Class A vs Classes B+C+NA). The equations for the final decision models use the predictor variables relevant to the class being tested. The process of determining attainment class using association values is outlined in Appendix F of the ME DEP methods manual (Davies and Tsomides 2002). Application of the three second-stage models or two-group tests is hierarchical.

Table E1-1. Metrics that are used in Maine's Linear Discriminant Models

#	Metric	Model
1	Total Abundance	First Stage Model
2	Generic Richness	First Stage Model
3	Plecoptera Abundance	First Stage Model
4	Ephemeroptera Abundance	First Stage Model
5	Shannon-Wiener Generic Diversity	First Stage Model
6	Hilsenhoff Biotic Index	First Stage Model
7	Relative Abundance Chironomidae	First Stage Model
8	Relative Richness Diptera	First Stage Model
9	Hydropsyche Abundance	First Stage Model
10	Probability (A+B+C) from First Stage Model	
11	Cheumatopsyche Abundance	C or Better Model
12	EPT Generic Richness Divided by Diptera Generic Richness	C or Better Model
12	Relative Abundance Oligochaeta	C or Better Model
13	Perlidae Abundance	B or Better Model
14	Tanypodinae Abundance	B or Better Model
15	Chironomini Abundance	B or Better Model
16	Relative Abundance Ephemeroptera	B or Better Model
17	EPT Generic Richness	B or Better Model
18	Summed Abundance's of: Dicrotendipes (warm), Micropsectra, Parachironomus and Helobdella	B or Better Model
19	Relative Generic Richness Plecoptera	A Model
20	Summed Abundances of: Cheumatopsyche, Cricotopus, Tanytarsus and Ablabesmyia	A Model
21	Summed Abundances of: Acroneuria, Maccaffertium and Stenonema	A Model
22	EP Generic Richness/14	A Model
23	Class A Indicator Taxa/7	A Model

E2 MAINE ECOREGION DESCRIPTIONS

Northeastern Highlands. This is a relatively sparsely populated region located in the western part of Maine. It is characterized by hills and mountains, a mostly forested land cover, nutrient-poor frigid and cryic soils (mostly Spodosols), and numerous high-gradient streams and glacial lakes. Typical forest types include northern hardwoods (maple-beech-birch), northern hardwoods/spruce, and northeastern spruce-fir forests. Recreation, tourism, and forestry are primary land uses (Hellyer draft Ecoregion descriptions 2007). On average, biological sampling sites in this ecoregion are located at higher elevations (average of 829 feet) and have lower urban and agricultural land use within 1 km of the sites (averages of 12% and 7%, respectively). Unfortunately, sites that met our selection criteria (<5% urban and <10% agricultural land use within a 1 km buffer) lacked long-term data (note: the most number of years of data at Northeastern Highland sites was 9 years, and these sites were classified as 'C'). At one of the selected sites, there were 3 years of data. The remaining selected sites only had 1 or 2 years of data, and this data had mostly been collected from 2000 onwards. When we attempted to search for long-term trends in a limited dataset comprised of a group of Northeastern Highland sites, there was not enough data to effectively work with and the site groups did not work well (see Appendix C). If trends were observed, they appeared to be due to site-specific differences rather than climate-related changes.

Northeastern Coastal Zone. This region is located in the southeastern corner of Maine. This ecoregion contains much greater concentrations of human population than the Northeastern Highlands (including the city of Portland). Current land use mainly consists of forests, woodlands, and urban and suburban development, with only some minor areas of pasture and cropland. Forests are mostly white, red, and jack pine and oak-hickory, and the soils are generally Inceptisols and Entisols (Hellyer draft Ecoregion descriptions 2007). Sites in this ecoregion are located at lower elevations (average of 97 ft) and have higher urban land use within 1 km of the sites (average of 44%). Only one site in this ecoregion met our selection criteria and it only had 2 years of data, so no attempts were made to perform trend analyses on Northeastern Coastal Zone sites.

Laurentian Plains and Hills (or Maine/New Brunswick Plains and Hills). This is a mostly forested region located in the eastern part of Maine. It has dense concentrations of continental glacial lakes, is less rugged than the Northeastern Highlands, and is considerably less populated than the Northeastern Coastal Zone. Vegetation is mostly spruce-fir with some patches of maple, beech, and birch. The majority of biological sampling sites are located in this ecoregion. The average elevation of sites is 214 ft and average land use is 23% urban and 13% agricultural.

E3 RESULTS

E3.1 ANOVA – Comparison of mean model input metric values among the different classifications using all the samples in the Maine database

Detailed results of the ANOVA can be found in **Attachment E1**. There were significant differences between mean model input metric values among many of the classes. The amounts that the mean metric values changed between the different classes varied and are therefore difficult to summarize. Also, looking at each metric individually has limited value because the linear discriminant models look at multiple variables simultaneously. Results from the Station 56817 analyses were used to identify which of the 24 metrics were most likely to be influenced by climate-related changes. Those results are summarized below.

E3.2 ANOVA – Comparison of mean model input metric values between Class A and Class B samples at Station 56817

Comparison of Class A versus Class B samples at Station 56817 showed that the only model input metric that had a significantly different mean value between the Class A and Class B samples was the Hilsenhoff Biotic Index (HBI). Mean HBI values were significantly higher in the Class B samples (**Figure E3-1**). Because the HBI model input metric was not significantly related to climatic variables (as shown in the correlation analyses with PRISM mean annual air temperature and precipitation and by the ANOVA analyses comparing mean values across hot/cold/wet/dry/normal years), it is likely that non-climatic factors, such as non-point source pollution, contributed to the change in classification.

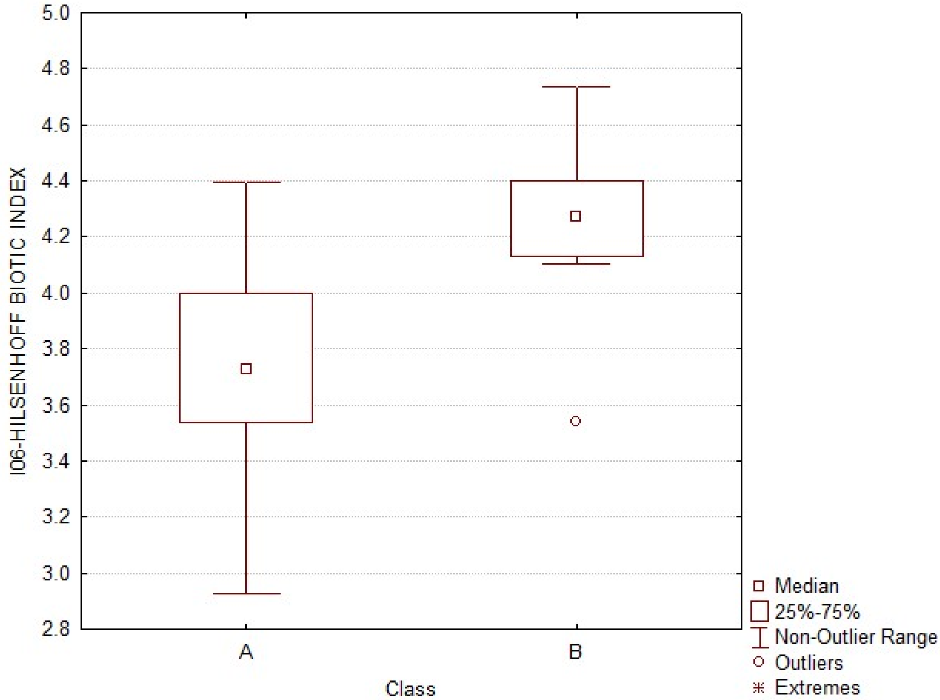


Figure E3-1. **Box and whisker plots for the HBI model input metric at Maine Station 56817 for samples that received different classifications (Class A versus Class B).**

E3.3 ANOVA - Station 56817- hot/cold/wet/dry/normal years

There were differences in some of the model input metric values from samples collected at Station 56817 during hot, cold, wet, dry, low-flow, high-flow and normal years, but none of them were significant when tested with the Tukey honest significant difference (HSD) test for unequal sample size (N) (Spjotvoll/Stoline). There were, however, significant correlations between six of the metrics and precipitation or flow variables (**Table E3-1**).

The Class A indicator taxa metric (which equals the number of Class A indicator taxa divided by 7) was significantly correlated with both mean annual precipitation (ppt14) and the categorical precipitation variable (1=dry years; 2=normal years; 3=wet years). Class A indicator taxa include: *Brachycentrus* (Trichoptera: Brachycentridae), *Serratella* (Ephemeroptera: Ephemerellidae), *Leucrocuta* (Ephemeroptera: Heptageniidae), *Glossosoma* (Trichoptera: Glossosomatidae), *Paragnetina* (Plecoptera: Perlidae), *Eurylophella* (Ephemeroptera: Ephemerellidae), and *Psilotreta* (Trichoptera: Odontoceridae). At Station 56817, on average, more Class A indicator taxa were present during wetter years (Figure 2-25 in main report). The relative abundance of collector-gatherers was higher during higher flow years (**Figure E3-2**)

Table E3-1. Summary of results of the correlation analysis using data from Station 56817. Only the significant correlations are shown

	Climate-related Variables			
Model Input Metric	**Cat1_Precip**	**ppt14**	**Cat_Flow**	**Avg Median Flow**
I30-PRESENCE OF A INDICATOR TAXA	r=.4686 N=22 p=.028	r=.5407 N=22 p=.009		
I08-RELATIVE DIPTERA RICHNESS			r=-.4316 N=22 p=.045	
I12-EPT GENERIC RICHNESS DIVIDED BY DIPTERA RICHNESS			r=.4350 N=22 p=.043	r=.6214 N=22 p=.002
I16-TANYPODINAE ABUNDANCE			r=-.5049 N=22 p=.017	
I31-EPT GENERIC RICHNESS RELATIVE TO EPT PLUS DIP			r=.4505 N=22 p=.035	r=.5831 N=22 p=.004
I33-COLLECTOR-GATHERERS RELATIVE ABUNDANCE			r=.4346 N=22 p=.043	

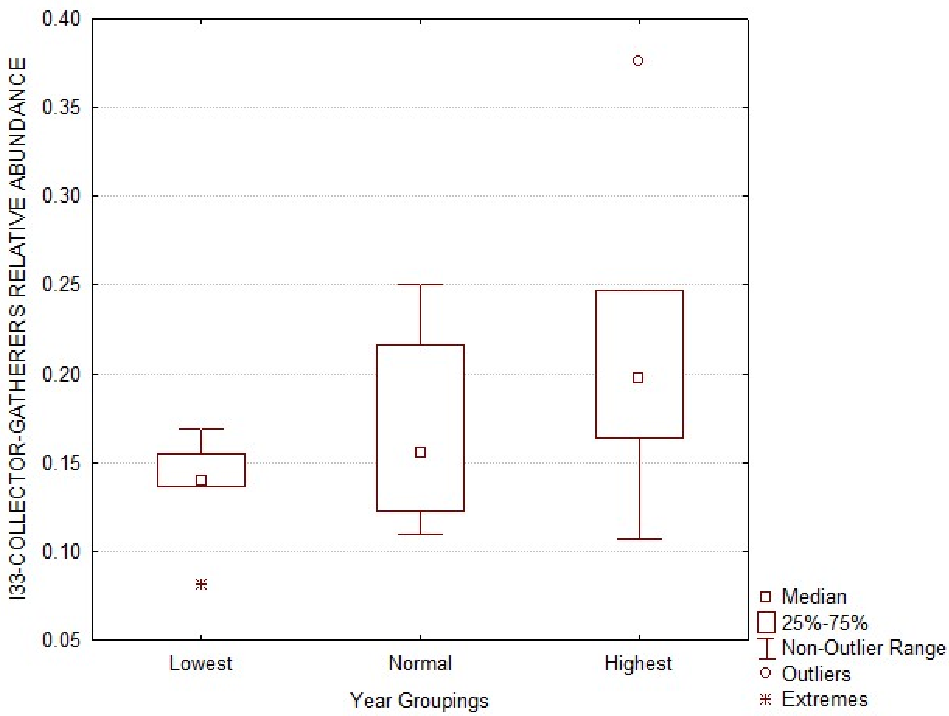


Figure E3-2. **Box and whisker plot for the Collector-gatherers relative abundance metric. Samples were grouped by the following flow categories (Station 56817): 1=low flow years, 2= normal years, 3=high flow years.**

E3.4 NMDS ordination - Station 56817 - hot/cold/wet/dry/normal years

Results from the NMDS ordination show that samples from Station 56817 do not form distinct clusters when grouped by hot/cold/wet/dry/normal years, so species composition did not change in a consistent way when the climate-related variables changed (Figure 2-24 in main body of report and **E3-3**). The plots that are shown are for the 2nd and 3rd axes because these axes explained the greatest amount of variance (Axis 3 in particular). The environmental variable that is most highly correlated with Axis 3 is the absolute difference between the PRISM mean annual precipitation from the sampling year and the previous year (r=-0.377). This variable is also the most highly correlated variable with Axis 2. The 2 minimum flow IHA parameters (1-day and 3-day minimum flow) have the next strongest correlations with Axis 3 (r=0.35 for both). There is an outlying sample in the plots. In 2005, mean annual precipitation was much higher than normal (and was much higher than the previous year) but minimum annual flows and median flows during the sampling months were relatively low (a lot of the rain occurred in October).

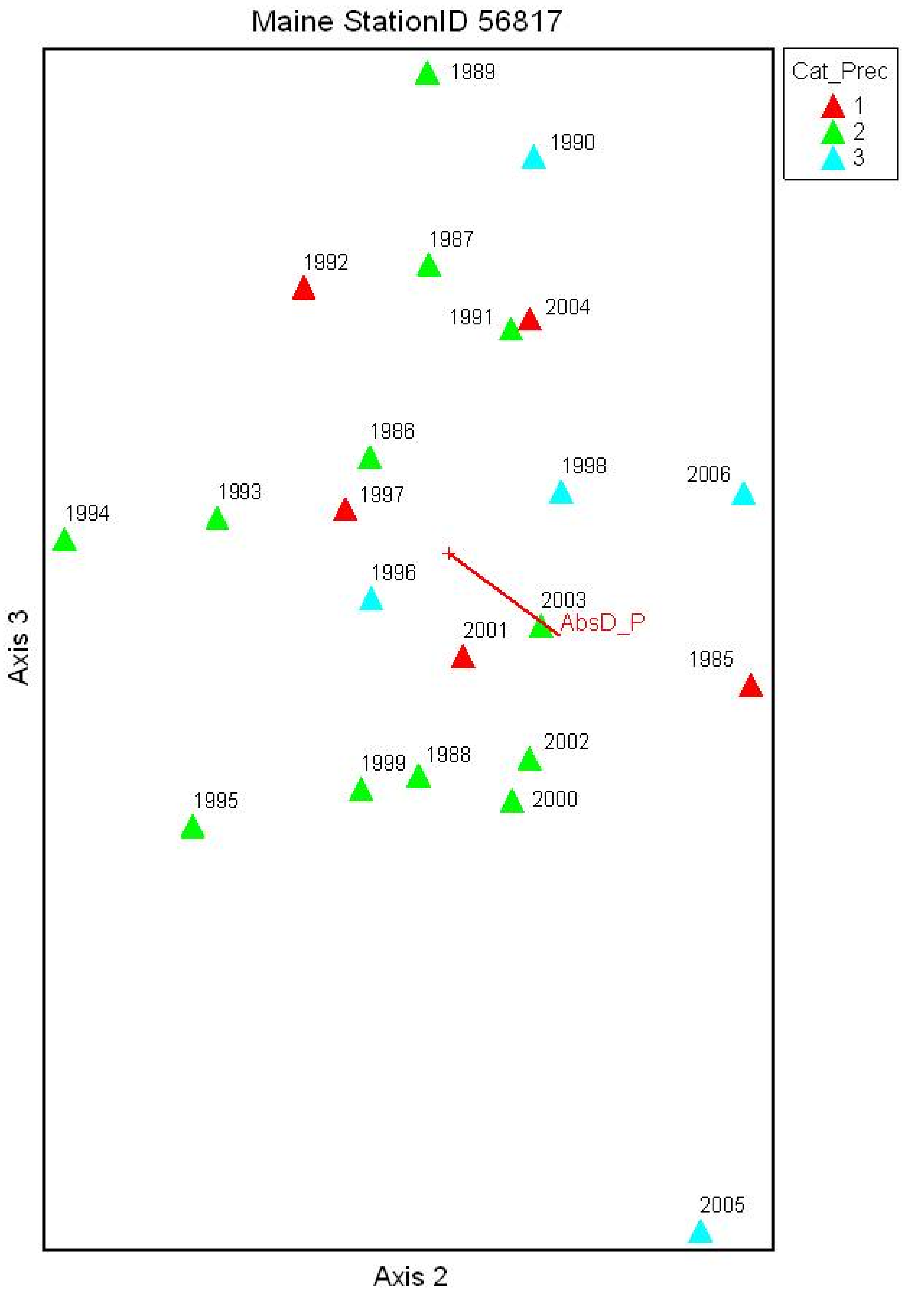


Figure E3-3. **NMDS plot (Axis 3-2). Cat_Prec refers to the precipitation categories, which are: 1=dry years; 2=normal years; 3=wet years. Samples are labeled by collection year. Absolute difference between the PRISM mean annual precipitation from the sampling year and the previous year (AbsD_P) is the most strongly correlated environmental variable with Axes 2 & 3.**

Figure E3-4 shows which taxa are the strongest drivers along the 2^{nd} and 3^{rd} axes. Tricorythodes, Oecetis and Ablabesmyia have the strongest negative correlations with Axis 3, and Pseudocloeon has the strongest positive correlation with Axis 3. Closer examination of these taxa plotted in ordination space shows that none of them occur exclusively in a particular temperature or precipitation category, although Ablabesmyia, Tricorythodes and Pseudocloeon did occur more often in samples collected during normal precipitation years (**Figure E3-5**).

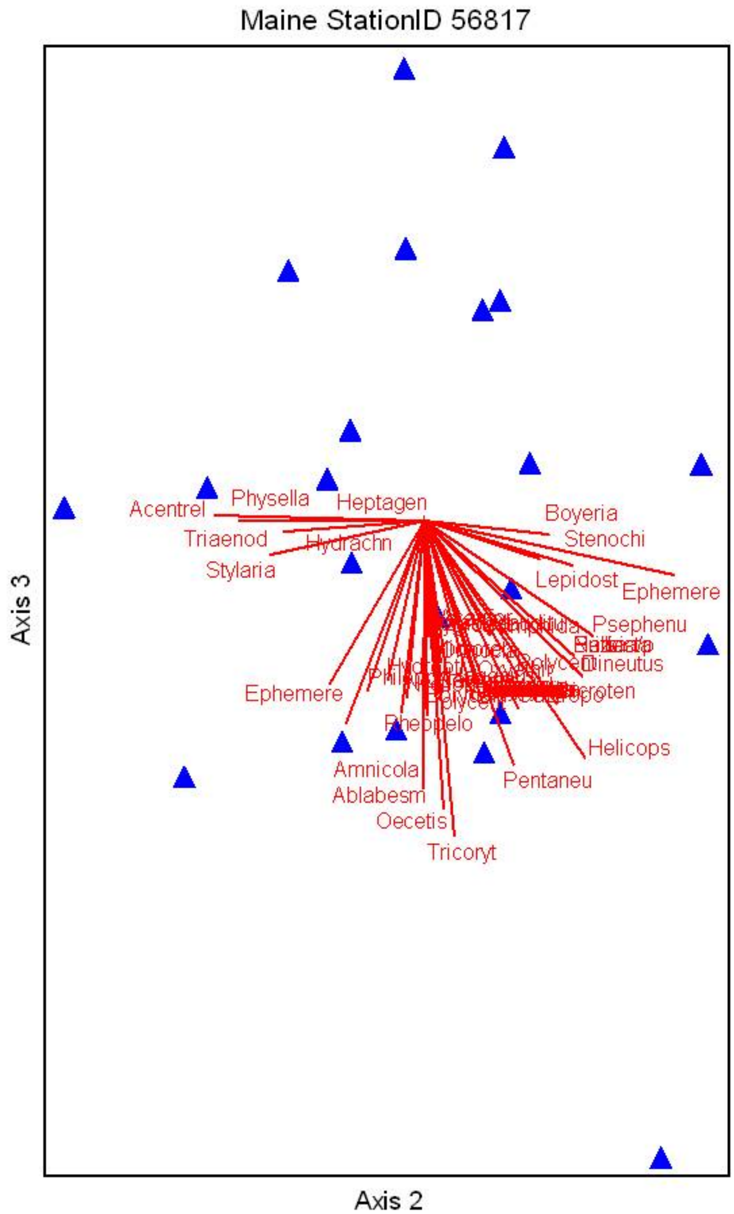


Figure E3-4. **NMDS plot (Axis 3-2) that shows which taxa are most highly correlated with each axis.**

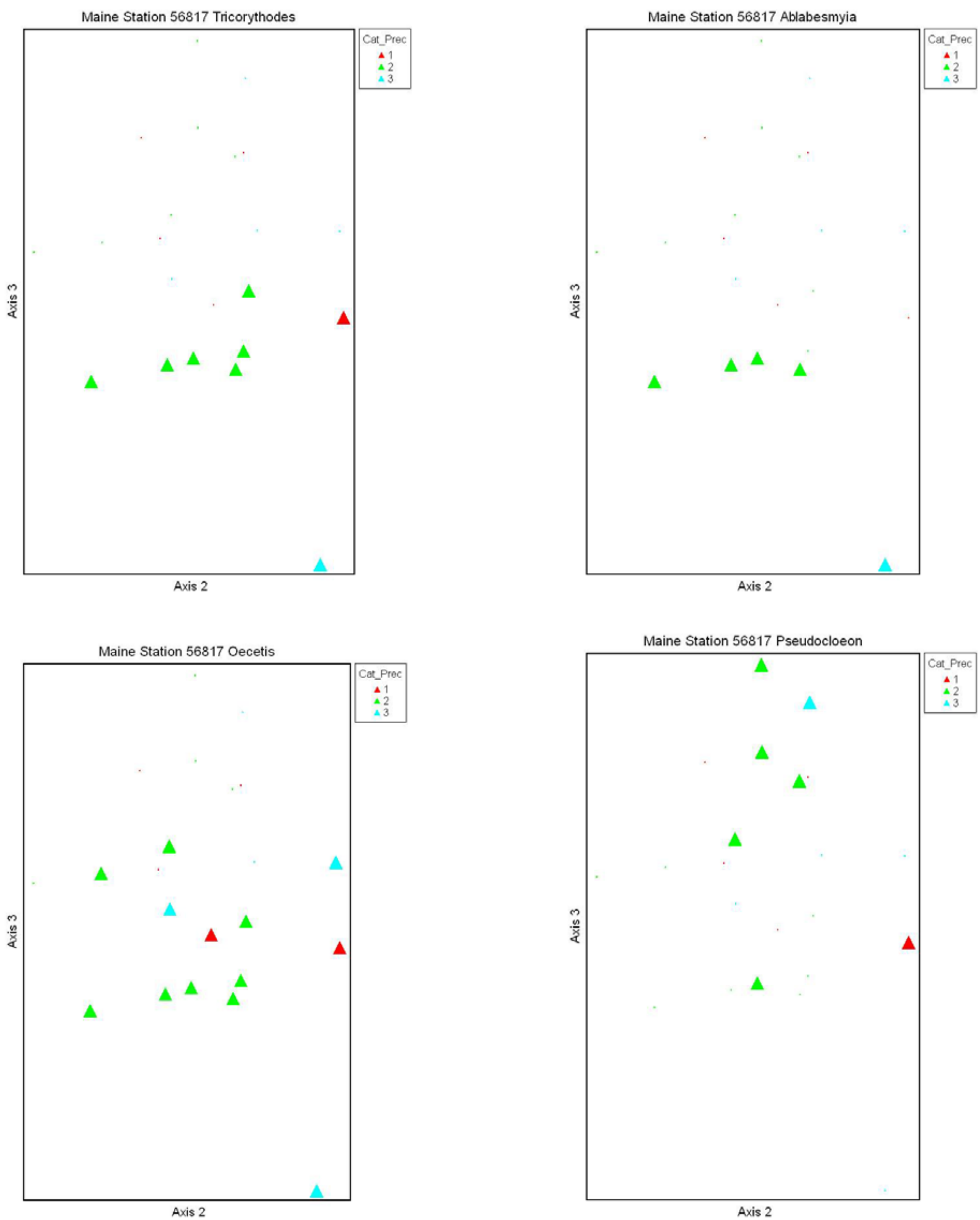


Figure E3-5. NMDS plots of the taxa that have the strongest correlations with Axis 3 (Tricorythodes, Oecetis, Ablabesmyia and Pseudocloeon). Cat_Precip refers to the precipitation categories, which are: 1=dry years; 2=normal years; 3=wet years.

E3.5 ANOVA - commonly used metrics in northeastern states - hot/cold/wet/dry/normal years at 3 Maine reference stations with the most years of biological data (Station 56817, 57011 and 57065).

Metrics that had at least one significant difference when one-way analysis of variance was done to evaluate differences in samples grouped by coldest, normal, and hottest or driest, normal and wettest years are shown in **Tables E3-2** and **E3-3**. These tables do not include results for thermal preference metrics, which are shown in **Table 2-2** of the main report. Additional results are available upon request.

Table E3-2. These metrics had at least one significant difference when one-way analysis of variance was done to evaluate differences in samples grouped by coldest, normal, and hottest years. Year groups were based on Parameter-elevation Regressions on Independent Slopes Model (PRISM) mean annual air temperature values at each site. Groups with the same superscripts are not significantly different ($p < 0.05$).

Site	Metric	Coldest	Normal	Hottest
56817 (Sheepscot)	% Swimmer individuals	5.2 ± 3.6^{A}	5.7 ± 2.4^{AB}	10.6 ± 5.0^{B}
57011 (W.Br. Sheepscot)	% EPT individuals	23.3 ± 9.4^{A}	58 ± 6.8^{B}	63.6 ± 18.7^{B}
	Hilsenshoff Biotic Index	$5.4 \pm .01^{A}$	$3.9 \pm .07^{B}$	$4.5 \pm .04^{A}$
	% Collector-filterer individuals	68.2 ± 11.7^{A}	16.6 ± 6.0^{B}	28.2 ± 27.0^{AB}
	Shannon-Wiener diversity index	2.6 ± 0.6^{A}	3.7 ± 0.4^{B}	3.5 ± 0.4^{AB}
	% Most dominant individuals	59.7 ± 12.3^{A}	23.9 ± 6.23^{B}	32.1 ± 3.8^{B}
	% Perennial individuals	19.9 ± 3.6^{A}	57.1 ± 17.5^{B}	64.8 ± 16.7^{B}
	% Intermittent individuals	67.4 ± 9.3^{A}	22.0 ± 7.2^{B}	24.4 ± 16.9^{B}
	% Drier vulnerable individuals	10.5 ± 2.6^{A}	29.9 ± 10.3^{AB}	39.2 ± 10.3^{B}
	% OCH individuals	4.6 ± 2.4^{A}	17.2 ± 6.2^{B}	8.4 ± 2.6^{AB}

Table E3-3. These metrics had at least one significant difference when one-way analysis of variance was done to evaluate differences in samples grouped by driest, normal, and wettest years. Year groups were based on Parameter-elevation Regressions on Independent Slopes Model (PRISM) mean annual precipitation values at each site. Groups with the same superscripts are not significantly different ($p < 0.05$).

Site	Metric	Driest	Normal	Wettest
56817 (Sheepscot)	% Warmer-drier vulnerable individuals	0.43 ± 0.6^{AB}	0.04 ± 0.1^{A}	1.3 ± 1.3^{B}
	# Warmer-drier vulnerable taxa	0.32 ± 0.4^{AB}	0.05 ± 0.1^{A}	0.7 ± 0.4^{B}
57065 (Duck)	% Climber individuals	8.2 ± 0.9^{AB}	10.7 ± 3.4^{A}	3.9 ± 2.2^{B}

E3.6 How cold- and warm-water-indicator taxa may affect EPT metrics and HBIs, how they relate to BCG tier assignments and Class A indicator taxa and how changes in temperature indicator taxa may vary across different ecoregions in Maine

Attachment E3 contains tables with lists of the temperature-indicator taxa, temperature optima and tolerance values that were calculated from the weighted average modeling, the tolerance values assigned by Maine DEP (which are used to calculate the HBI) and BCG attribute levels assigned to each taxa during the New England Wadeable Streams (NEWS) project (US EPA, 2007).

E3.7 Distribution of cold and warm-water temperature indicator taxa

Additional results are reported below (**Tables E3-6, E3-7, E3-8, E3-9, E3-10** and **E3-11).**

Tables E3-10 and **E3-11** summarize distribution and abundance information for the Maine temperature-indicator taxa at the 3 sites (Stations 56817, 57011 and 57065) and 2 site groups that were analyzed for long-term trends. Boyeria and Eurylophella appear to be two of the strongest cold-water indicators because they occurred at all or most of the sites and generally had higher mean relative abundances than the other taxa. Nigronia, Pagastia, and Leuctra also occurred at most of the sites. Overall, the cold-water taxa are not well-represented at the 3 individual stations, but have a greater presence among the site groups, especially the Northeastern Highlands. The warm-water-indicator taxa show a different pattern. They are well-represented at the individual sites and are poorly represented in the site groups, especially among the Laurentian Plains and Hills site group. Stenonema and Stenelmis appear to be two of the strongest warm-water indicators because they occur at all the sites and site groups and are present in higher numbers than the other taxa. Acroneuria, Ceraclea, Hydra, Neureclipsis, Nilotanypus and Oecetis are present at 4 of the 5 sites/site groups, and Leucrocuta also occurs in relatively high abundances.

Table E3-10. Summary of distribution and abundance information for the *cold-water* temperature indicator taxa at the 3 sites (Stations 56817, 57011 and 57065) and 2 site groups (Laur = Laurentian Plains and Hills, NEHigh = Northeastern Highlands). #Sites refers to the number of sites or site groups at which the taxa occurs. A=absent. P=present (highlighted in grey). Relative abundance codes: L=low (<0.01), M=medium (0.01-0.1), H=high (>0.1) (M or H are in bold type). Guide to interpretation: P-1L = present, occurred during1 year, low relative abundance (RA), P-11M = present, occurred during 11 years, medium RA, etc.

FinalID	#Sites	ME56817	ME57011	ME57065	Laur	NEHigh
Ameletus	1	A	A	A	A	P-4L
Apatania	1	A	A	A	A	P-2L
Boyeria	5	P-11L	**P-11M**	**P-9M**	**P-8M**	**P-3M**
Capnia	0	A	A	A	A	A
Diplectrona	2	A	A	P-1L	A	P-1L
Epeorus	2	P-10L	A	A	A	**P-4M**
Eurylophella	4	A	P-1L	**P-9M**	**P-7M**	**P-7M**
Glossosoma	3	P-1L	A	A	P-4L	P-2L
Heterotrissocladius	2	A	A	P-1L	A	P-3L
Hydatophylax	3	A	P-1L	A	P-2L	P-1L
Lanthus	1	A	A	A	A	P-1L
Larsia	1	A	A	A	A	**P-3M**

Table E3-10. Continued

FinalID	#Sites	ME56817	ME57011	ME57065	Laur	NEHigh
Leuctra	4	A	P-4L	P-1L	P-5L	**P-6M**
Limnephilus	1	A	A	A	**P-4M**	A
Macropelopia	1	A	A	A	A	P-2L
Malirekus	0	A	A	A	A	A
Micrasema	2	P-9L	A	A	A	P-1L
Natarsia	1	A	A	A	A	P-1L
Nemoura	0	A	A	A	A	A
Nigronia	4	P-1L	P-6L	P-5L	P-1L	A
Oligostomis	2	A	A	A	**P-6M**	P-2L
Oulimnius	2	A	A	A	P-2L	P-4L
Pagastia	4	P-1L	P-1L	A	**P-6M**	**P-5M**
Palaeagapetus	0	A	A	A	A	A
Paracapnia	2	A	P-1L	A	P-5L	A
Paranemoura	0	A	A	A	A	A
Parapsyche	1	A	A	A	A	P-3L
Peltoperla	1	A	A	A	A	P-1L

Perlodidae	3	P-2L	A	A	**P-4M**	**P-7M**
Prodiamesa	0	A	A	A	A	A
Prostoia	0	A	A	A	A	A
Pseudodiamesa	1	A	A	A	A	P-1L
Psychoglypha	2	A	A	A	P-4L	P-1L
Pteronarcys	1	A	A	A	A	**P-6M**
Rhithrogena	1	A	A	A	A	**P-2M**
Sweltsa	3	A	A	P-1L	P-4L	**P-6M**
Taenionema	0	A	A	A	A	A
Tallaperla	1	A	A	A	A	**P-4M**
Utacapnia	0	A	A	A	A	A
Utaperla	0	A	A	A	A	A
Zapada	0	A	A	A	A	A

Table E3-11. Summary of distribution and abundance information for the *warm-water* temperature indicator taxa at the 3 sites (Stations 56817, 57011 and 57065) and 2 site groups (Laur = Laurentian Plains and Hills, NEHigh = Northeastern Highlands). #Sites refers to the number of sites or site groups at which the taxa occurs. A=absent. P=present (highlighted in grey). Relative abundance codes: L=low (<0.01), M=medium (0.01-0.1), H=high (>0.1) (M or H are in bold type). Guide to interpretation: P-1L = present, occurred during1 year, low relative abundance (RA), P-11M = present, occurred during 11 years, medium RA, etc.

FinalID	#Sites	ME56817	ME57011	ME57065	Laur	NEHigh
Acroneuria	4	**P-23M**	**P-12M**	**P-9M**	A	**P-3M**
Amnicola	3	P-12L	P-2L	P-5L	A	A
Argia	3	P-7L	P-6L	P-1L	A	A
Attaneuria	1	P-3L	A	A	A	A
Caenis	1	A	P-3L	A	A	A
Cardiocladius	1	P-1L	A	A	A	A
Ceraclea	4	P-5L	P-3L	P-2L	A	P-1L
Chaetogaster	3	P-1L	A	P-2L	P-1L	A
Dicrotendipes	3	P-2L	P-2L	P-2L	A	A
Erpobdella	1	A	A	A	A	P-1L
Ferrissia	2	P-5L	P-2L	A	A	A
Helicopsyche	3	P-7L	**P-8M**	A	A	P-1L
Helisoma	1	A	A	P-2L	A	A
Hemerodromia	3	P-6L	**P-11M**	P-3L	A	A
Hydra	4	P-1L	P-1L	P-3L	P-2L	A
Hydroptila	3	**P-14M**	P-3L	A	P-1L	A
Isonychia	2	**P-22M**	P-3L	A	A	A
Labrundinia	2	P-2L	P-2L	A	A	A
Leucrocuta	3	**P-19M**	**P-11M**	**P-6M**	A	A
Macrostemum	2	**P-16M**	P-3L	A	A	A
Neureclipsis	4	**P-22M**	P-2L	P-1L	A	P-2L
Nilotanypus	4	P-5L	P-2L	P-1L	A	P-2L
Oecetis	4	P-8L	**P-9M**	**P-8M**	A	P-3L
Orconectes	1	P-1L	A	A	A	A
Parachironomus	0	A	A	A	A	A
Paragnetina	2	P-2L	P-1L	A	A	A
Pentaneura	2	P-13L	P-1L	A	A	A
Physa	2	A	A	**P-4M**	P-3L	A
Physella	2	P-8L	A	**P-5M**	A	A
Plauditus	3	P-6L	P-1L	A	A	P-1L
Prostoma	2	P-1L	P-1L	A	A	A
Psectrocladius	3	P-1L	A	**P-8M**	P-1L	A
Pseudocloeon	1	P-7L	A	A	A	A
Rheopelopia	3	P-8L	P-5L	P-1L	A	A
Serratella	3	**P-15M**	P-2L	A	A	**P-1M**
Stenacron	2	A	P-1L	**P-9M**	A	A
Stenelmis	5	**P-19M**	**P-10M**	P-1L	**P-6M**	P-4L
Stenonema	5	**P-23M**	**P-12M**	**P-9M**	**P-7M**	**P-6M**
Tribelos	1	A	A	A	P-1L	A
Tricorythodes	2	P-6L	**P-11M**	A	A	A

E3.8 Summary

- In general, samples with the following characteristics received better ratings (=higher classifications):
    - High generic richness
    - High richness and abundance of EPT taxa
    - High Shannon-Wiener diversity index values
    - Low HBI scores
    - Low Chironomidae abundances
    - Low relative Diptera richness
    - Low relative Oligochaeta abundance
    - Greater presence of Class A indicator taxa
    - Greater scraper relative abundance

- Results from the NMDS ordination show that samples from Station 56817 do not form distinct clusters when grouped by hot/cold/wet/dry/normal years, so species composition did not change in a consistent way when the climate-related variables changed. None of the taxa that were the strongest drivers in the analysis occurred exclusively in a particular temperature or precipitation category.
- Although there were significant differences among certain metrics at certain sites, the only 'consistent' pattern (=one that occurred at more than one site) was that the percentage of swimmers was higher during the warmer years at 2 sites. The other differences appeared to be site-specific.
- At Station 56817, precipitation appeared to have a greater influence on the biotic assemblage than temperature. At Station 57011, temperature had a greater influence on metric values than precipitation.
- At Station 56817, the mean richness and abundance of cold-water taxa was higher during the wet years, as were the richness and abundance of taxa that are predicted to be more vulnerable in a warmer drier climate scenario.
- At Station 57011, samples collected during cold years had higher percent dominant taxon individuals, higher percent collector-filterer individuals, higher HBI scores, lower Shannon-Wiener diversity index scores, higher intermittent taxa individuals, lower EPT percent individuals, lower percent perennial taxa individuals, lower percent Odonata/Coleoptera/Hemiptera individuals, lower percent warm-water individuals and lower percent 'drier vulnerable individuals.
- Only one metric at Station 57065 had significant differences among the temperature and precipitation categories. At that site, the number of collector-filterer taxa was higher in samples collected during the normal/wet years, but this should be interpreted with caution due to a low sample size.

- Many of the cold-water-indicator taxa in Maine are EPT taxa: 16 of the cold-water taxa are Plecopterans, 10 are Trichopterans and 3 are Ephemeropterans. There are also a relatively high number of EPT taxa on the warm-water indicator list: 9 of the warm-water taxa are Ephemeropterans, 6 are Trichopterans and 3 are Plecopterans.
- Eight of Maine's model input metrics are related in some way to EPT taxa. Two of the model input metrics are specifically related to Ephemeropterans: Ephemeroptera Abundance and Relative Abundance Ephemeroptera. Three model input metrics are specifically related to Plecopterans: Plecoptera abundance, Perlidae abundance and relative Plecoptera richness. Two of the model input metrics are specifically related to Trichopterans: Hydropsyche abundance and Cheumatopsyche abundance.
- Seven of the cold-water taxa are Dipterans from the family Chironomidae, and ten of the warm-water taxa are Dipterans. Several model input metrics are specifically related to Dipterans.
- All but two of the tolerance values of the cold-water indicator taxa are low (≤ 3). Nine of the warm-water taxa have tolerance values ≥ 7, but it should be noted that 10 of the warm-water taxa have low tolerance values (≤ 3), so there is a mix.
- Tolerance values had a weak but significant correlation (r=0.29, p=001) with temperature optima values.
- When BCG attributes of temperature indicator taxa are examined, twenty of the cold-water taxa are considered to be sensitive taxa (2 or 3) and two of the taxa are considered to be tolerant (5).
- Two of the Class A indicator taxa, Eurylophella and Glossosoma, are on the cold-water list and three, Paragnetina, Serratella and Leucrocuta, are on the warm-water list. Brachycentrus was initially on the warm-water list but was removed due to variation in temperature preferences among species within this genus.
- At Station 56817, on average, more Class A indicator taxa were present during wetter years
- The Northeastern Highlands sites have the highest mean number of cold-water indicator taxa (followed closely by the Northeastern Coastal Zone sites). It should be noted that the number of cold-water taxa in all the ecoregions is low (values generally range from 1 to 2 taxa). The mean number of ***warm-water*** indicator taxa at sites in the Laurentian Plains and Hills is significantly higher than at sites in the other ecoregions, while the Northeastern Highlands sites have the lowest mean number of warm-water indicator taxa.
- On average, there are more ***cold-water*** indicator taxa at higher elevation ($\geq$ 500 ft) sites and more ***warm-water*** indicator taxa at lower elevation (< 500 ft) sites.
- At Station 56817, 5 model metrics were significantly correlated with flow category. The general pattern was that Dipteran Richness and Tanypodinae abundance decreased during higher flow years, while the EPT:Diptera ratio metrics increased. The relative abundance of collector-gatherers was higher during higher flow years.
- Bottom lines: it is tough to predict effects on Maine's classification models because they look at multiple variables simultaneously. There are no firm thresholds. We did the best we could with what we had, but predictions at this point are only speculative.
- Unfortunately we lack long-term data for Northeastern Highlands sites, which seems to be the area where we would have been most likely to see a trend or detect a climate-related shift in the biology.

Attachment E1

Results of the ANOVA analysis in which mean model input metric values were compared across the different classifications

This attachment contains a table and box plots that summarize mean model input metric values for Class A, B, C and NA samples.

Table E1-1. Results of the one-way ANOVA that was performed on all the samples in the Maine database to see how mean metric values varied among the different classes. Mean metric values, sample sizes (N) and standard deviations (Std. Dev.) are shown. P-levels from the Tukey honest significant difference (HSD) test for unequal sample size (N) (Spjotvoll/Stoline) are shown if they are significant (<0.5). Note: I35-PIERCERS RELATIVE ABUNDANCE and I38-PARASITES RELATIVE ABUNDANCE had low sample sizes and were therefore excluded from this analysis.

	CLASS												Significance level (Tukey p-level)					
	A			B			C			NA								
Metric	**N**	**Mean**	**Std. Dev.**	**N**	**Mean**	**Std. Dev.**	**N**	**Mean**	**Std. Dev.**	**N**	**Mean**	**Std. Dev.**	**A-B**	**A-C**	**A-NA**	**B-C**	**B-NA**	**C-NA**
I01-TOTAL ABUNDANCE	577	460.5	548.8	448	909.8	948	353	1132.1	1800.2	227	598.8	1128.7	0	0		0.04	0.02	0
I02-GENERIC RICHNESS	577	42.9	14.4	448	42.4	13.2	353	36.3	13.3	227	28.1	11.9		0	0	0	0	0
I03-PLECOPTERA ABUNDANCE	577	21.1	38.7	445	11.5	15.6	170	6.3	11.4	19	5.6	15.7	0	0				
I04-EPHEMEROPTERA ABUNDANCE	576	99.5	132.9	448	144.8	163.9	353	90.7	155.7	142	10	31.7	0		0	0	0	0
I05-SHANNON-WEINER GENERIC DIVERSITY	577	3.8	0.7	448	3.4	0.7	353	3.2	0.8	227	2.7	0.9	0	0	0	0	0	0
I06-HILSENHOFF BIOTIC INDEX	577	3.7	0.8	448	4.7	0.7	353	5.3	1	227	5.8	1.2	0	0	0	0	0	0
I07-RELATIVE CHIRONOMIDAE ABUNDANCE	574	0.2	0.2	448	0.3	0.2	353	0.3	0.2	222	0.3	0.3		0	0	0	0.02	
I08-RELATIVE DIPTERA RICHNESS	576	0.4	0.1	448	0.4	0.1	353	0.4	0.1	224	0.4	0.2		0	0	0.01	0	
I09-HYDROPSYCHE ABUNDANCE	472	103	154.4	395	216.4	301.1	263	343	600.2	138	134.8	618.6	0	0		0		0
I11-CHEUMATOPSYCHE ABUNDANCE	362	22.4	48.5	383	98.8	283.8	264	175.5	433.4	124	118.2	371.7	0	0		0.01		
I12-EPT GENERIC RICHNESS DIVIDED BY DIPTERA RICHNESS	576	1.5	1	448	1.3	0.6	353	1	0.6	224	0.8	0.9	0	0	0	0	0	0.02

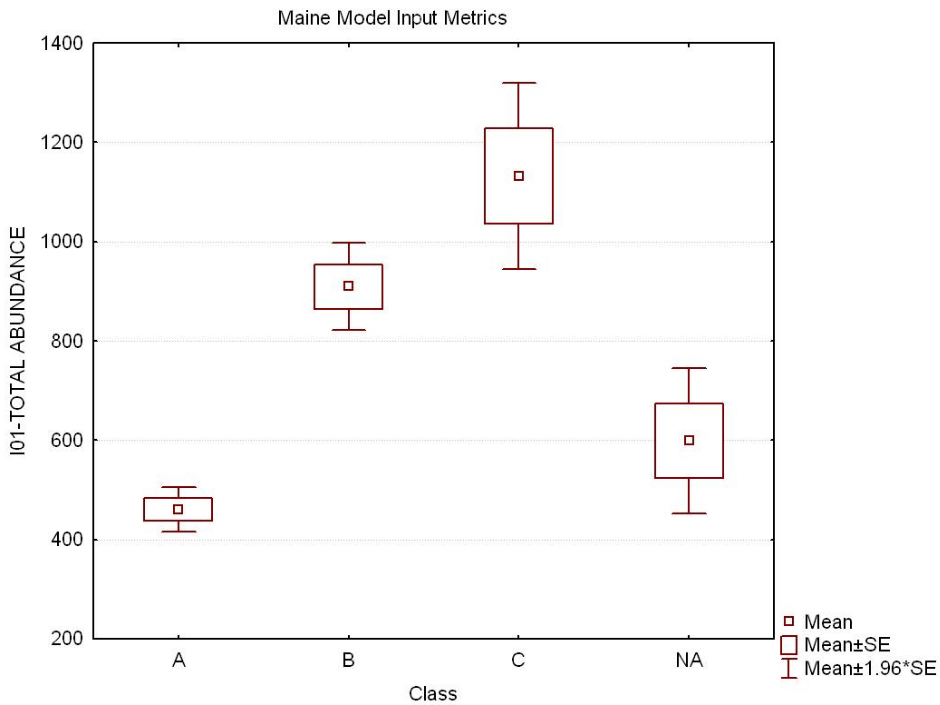
361 **Table E1-1. Continued**

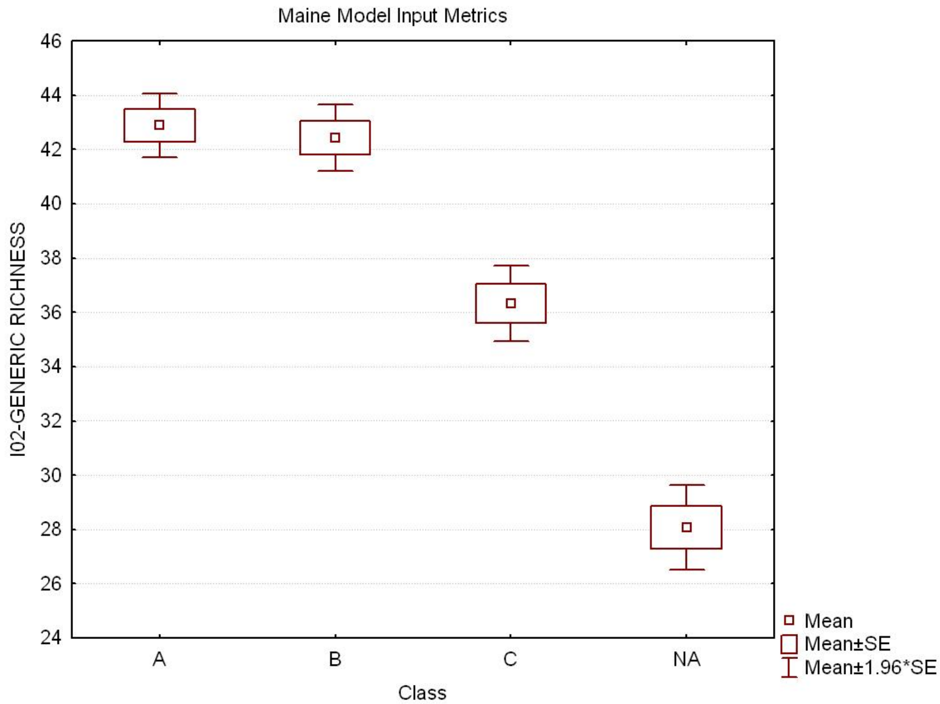
	CLASS												Significance level (Tukey p-level)					
	A			B			C			NA								
Metric	**N**	**Mean**	**Std. Dev.**	**N**	**Mean**	**Std. Dev.**	**N**	**Mean**	**Std. Dev.**	**N**	**Mean**	**Std. Dev.**	**A-B**	**A-C**	**A-NA**	**B-C**	**B-NA**	**C-NA**
I13-RELATIVE OLIGOCHAETA ABUNDANCE	277	0	0	221	0	0.1	218	0	0.1	168	0.1	0.2			0		0	0
I15-PERLIDAE ABUNDANCE	401	10.2	8.9	376	9.7	12.3	104	6.2	11	10	5.5	9.7		0.04				
I16-TANYPODINAE ABUNDANCE	465	8.2	14.7	405	15.2	25	316	25.4	48.5	190	21.3	43	0.01	0	0	0		
I17-CHIRONOMINI ABUNDANCE	511	27.7	70.7	432	52.3	122.9	331	109.5	323.9	187	68	192.2		0		0		
I18-RELATIVE EPHEMEROPTERA ABUNDANCE	576	0.3	0.2	448	0.2	0.2	353	0.1	0.2	142	0	0.1	0	0	0	0	0	0
I19-EPT GENERIC RICHNESS	577	19.3	5.7	448	17.3	4.6	353	11.5	4	216	5.9	3.3	0	0	0	0	0	0
I21-SUMMED ABUNDANCE OF DICROTENDIPES, MICROPSECTRA, PARACHIRONO	302	9.5	22	231	17.2	88.8	231	36.6	132	149	55.3	193		0.05	0		0.02	
I23-RELATIVE PLECOPTERA RICHNESS	577	0.1	0.1	445	0.1	0	170	0	0	19	0.1	0	0	0				
I25-SUMMED ABUNDANCE OF CHEUMATOPSYCHE, CRICOTOPUS, TANYTARSUS A	518	28.1	60.7	446	121.1	275.8	347	183.5	403.3	213	96.7	305.2	0	0	0.04	0.01		0.01
I26-SUMMED ABUNDANCE OF ACRONEURIA, STENONEMA AND MACCAFFERTIUM	463	32	37.1	417	51.7	59.2	262	37.6	64.7	60	6.4	19.9	0		0.03	0.01	0	0.01

Table E1-1. Continued

	CLASS												Significance level (Tukey p-level)					
	A			B			C			NA								
Metric	**N**	**Mean**	**Std. Dev.**	**N**	**Mean**	**Std. Dev.**	**N**	**Mean**	**Std. Dev.**	**N**	**Mean**	**Std. Dev.**	**A-B**	**A-C**	**A-NA**	**B-C**	**B-NA**	**C-NA**
I28-EP GENERIC RICHNESS DIVIDED BY 14	577	0.8	0.3	448	0.6	0.2	353	0.4	0.2	149	0.2	0.1	0	0	0	0	0	0
I30-PRESENCE OF A INDICATOR TAXA	522	0.4	0.2	348	0.2	0.1	152	0.2	0.1	43	0.2	0.1	0	0	0	0.04		
I31-EPT GENERIC REICHNESS RELATIVE TO EPT PLUS DIPTERA	576	0.6	0.1	448	0.5	0.1	353	0.5	0.1	214	0.4	0.2	0	0	0	0	0	0
I32-COLLECTOR-FILTERERS RELATIVE ABUNDANCE	577	0.4	0.2	448	0.5	0.3	353	0.4	0.3	214	0.3	0.3	0	0.01	0.04	0	0	0
I33-COLLECTOR-GATHERERS RELATIVE ABUNDANCE	577	0.2	0.1	448	0.2	0.1	352	0.2	0.2	218	0.2	0.2	0	0	0			
I34-PREDATORS RELATIVE ABUNDANCE	577	0.1	0.1	447	0.1	0.1	351	0.1	0.1	223	0.1	0.2				0	0.01	
I36-SHREDDERS RELATIVE ABUNDANCE	567	0.1	0.1	433	0.1	0.1	336	0.1	0.1	200	0.2	0.2	0.03		0		0	0
I37-SCRAPERS RELATIVE ABUNDANCE	565	0.1	0.1	444	0.1	0.1	344	0.1	0.1	194	0.1	0.2						
I39-ETO GENERIC RICHNESS	577	17.5	6.1	448	17.1	5	353	12.6	4.5	222	7	3.5		0	0	0	0	0

The next 32 graphs are categorical box and whisker plots for the Maine model input metrics. They show the mean metric values for Class A, B, C and NA samples that are currently in the Maine database. These plots were examined to gain more insight into the following questions: how do model input metric values differ among the different classifications? Do certain metrics appear to be more important than others in contributing to classification changes?

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46
44
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38
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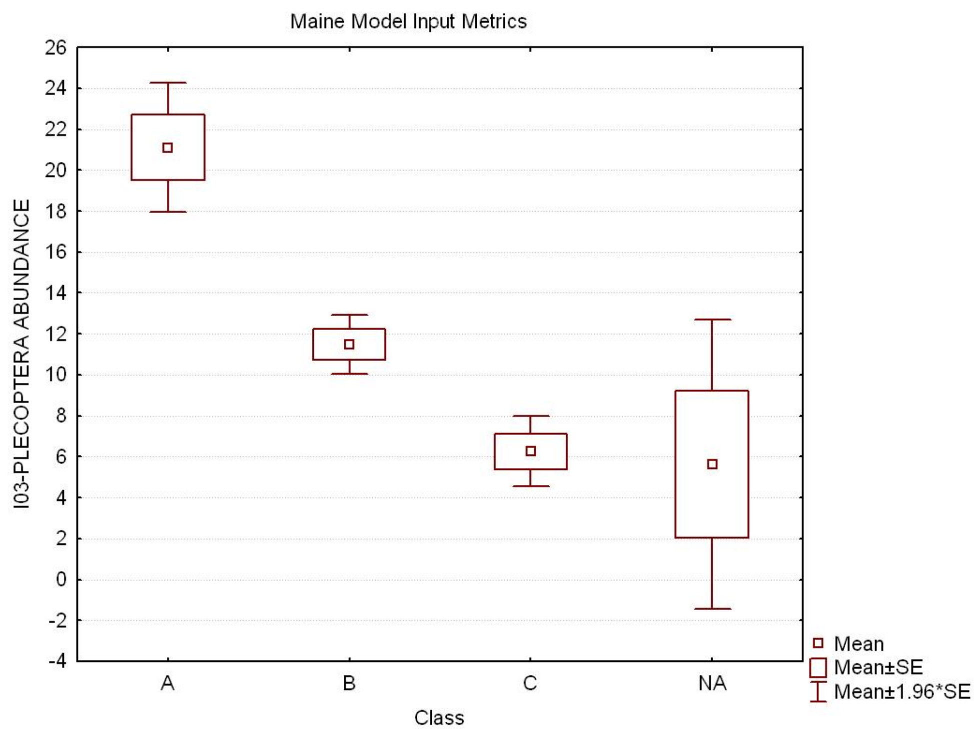
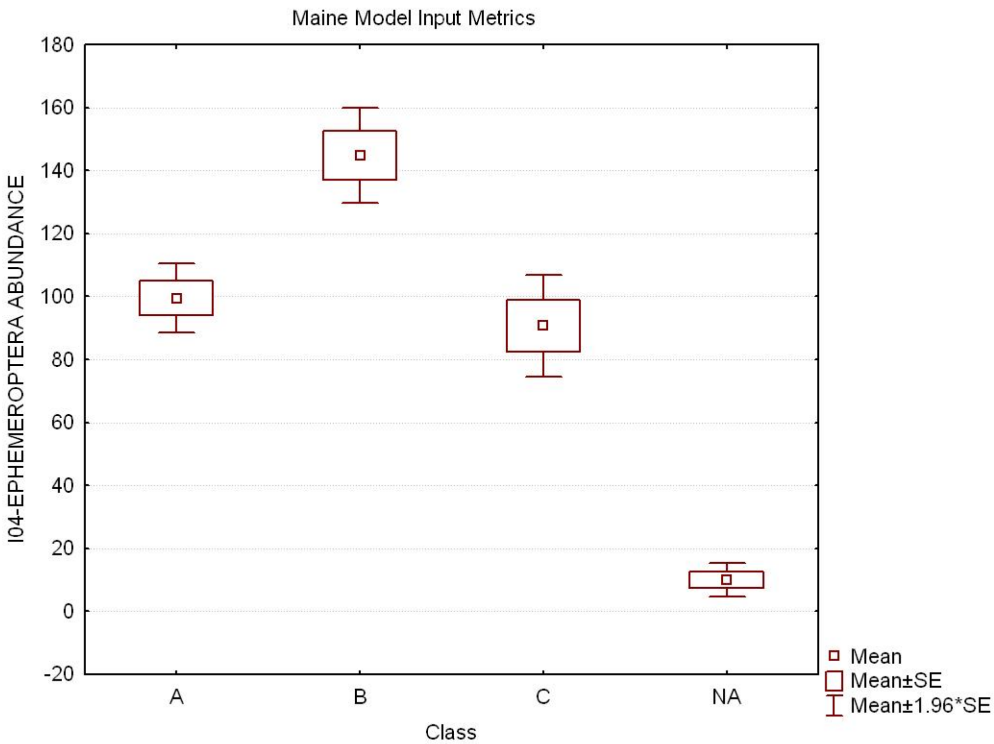
34
32
30
28
26
24
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE


Maine Model Input Metrics
I03-PLECOPTERA ABUNDANCE
26
24
22
20
18
16
14
12
10
8
6
4
2
0
-2
-4
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE


Maine Model Input Metrics
I04-EPHEMEROPTERA ABUNDANCE
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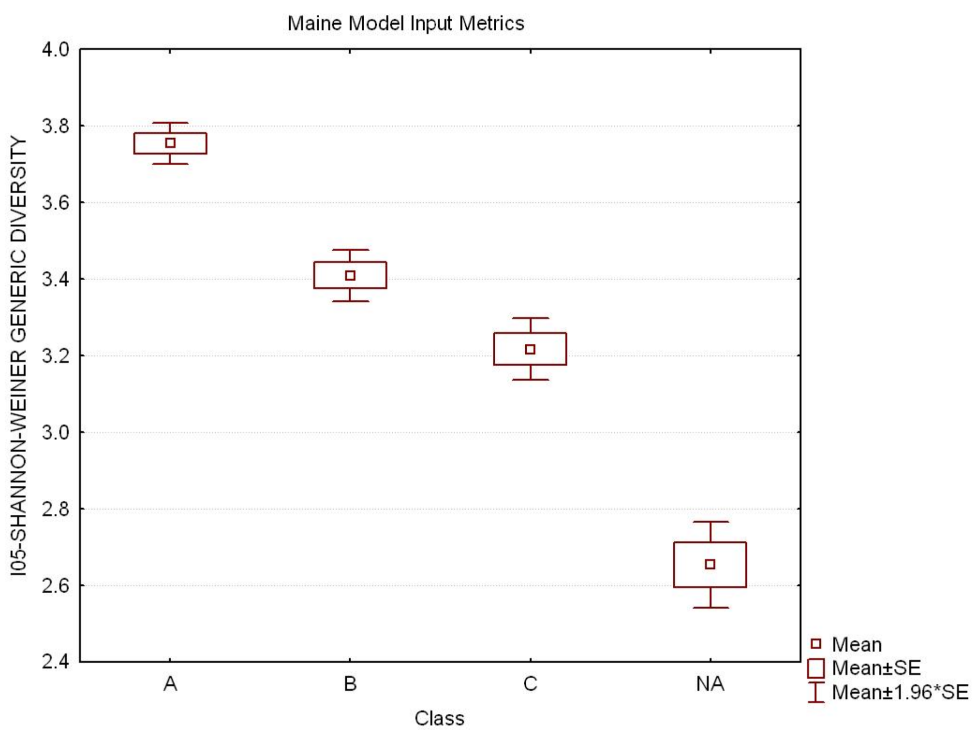
160
140
120
100
80
60
40
20
0
-20
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE


Maine Model Input Metrics
I05-SHANNON-WEINER GENERIC DIVERSITY
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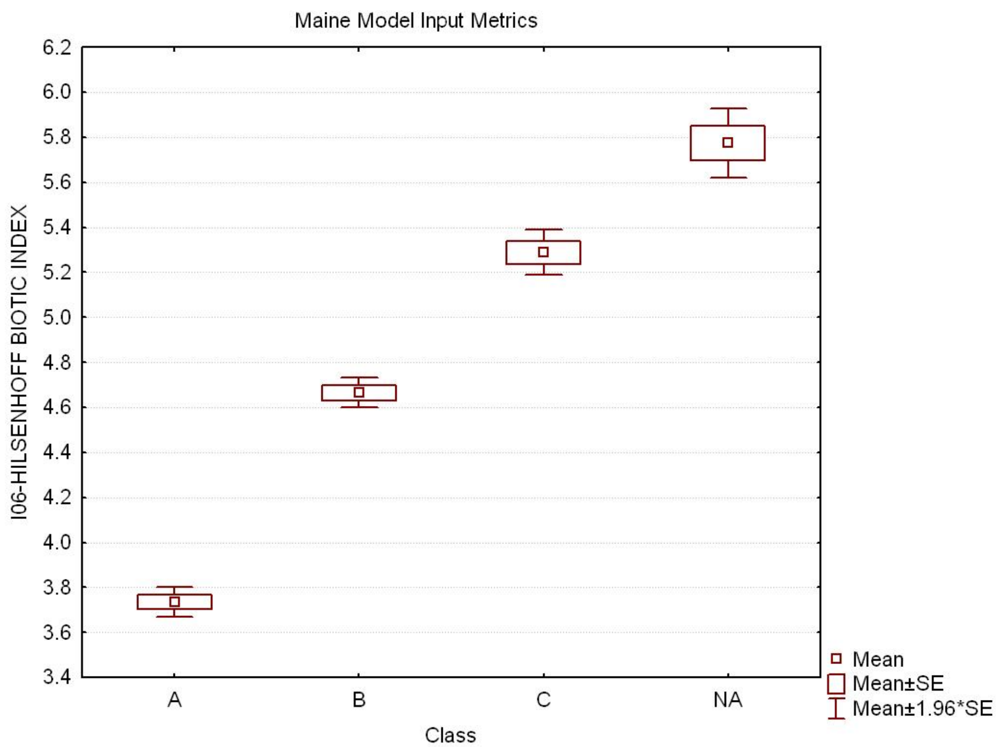
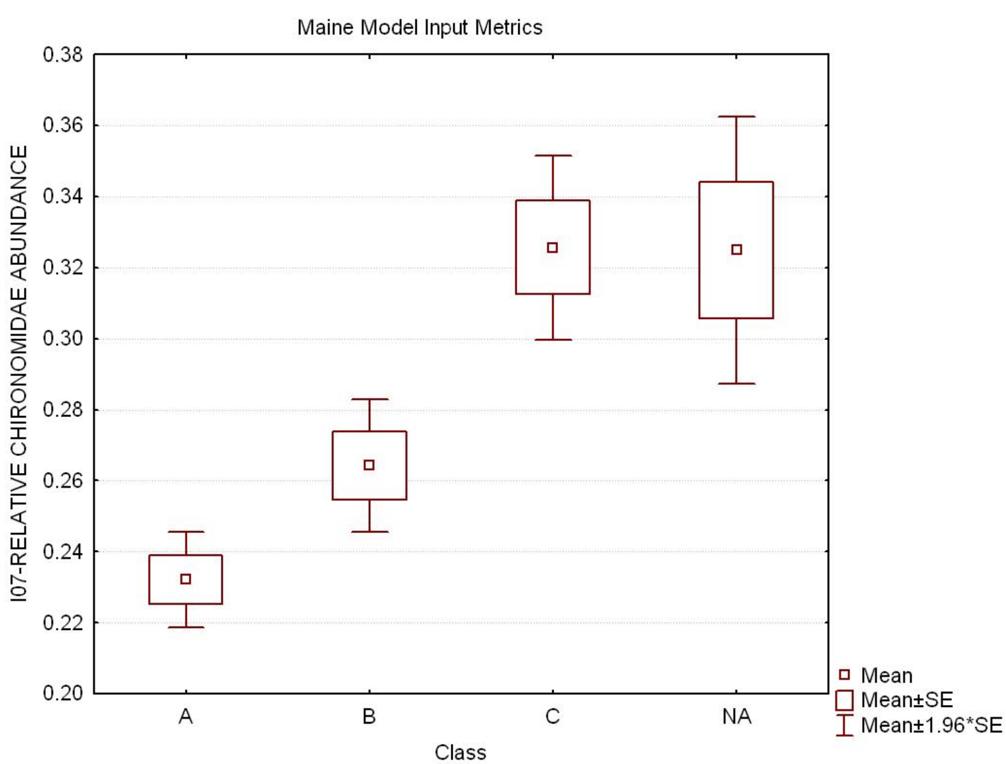
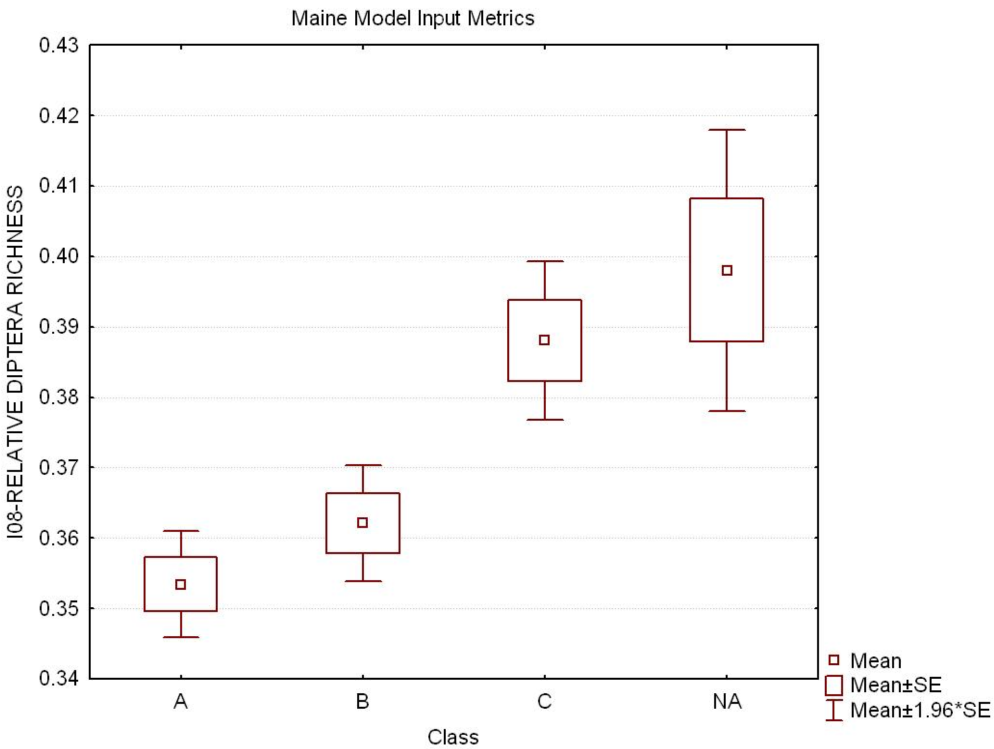
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE


Maine Model Input Metrics
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
I06-HILSENHOFF BIOTIC INDEX
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE


Maine Model Input Metrics
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
I07-RELATIVE CHIRONOMIDAE ABUNDANCE
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE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I08-RELATIVE DIPTERA RICHNESS
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0.42
0.41
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0.39
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0.35
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A
B
C
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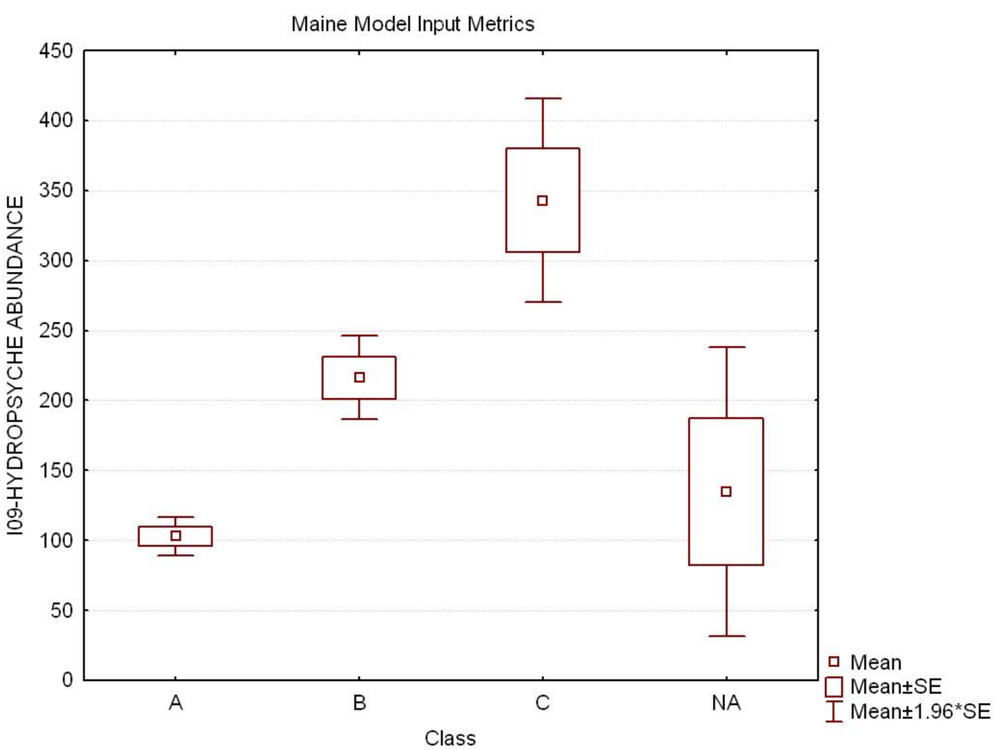
Class
Mean
Mean±SE
Mean±1.96*SE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I09-HYDROPSYCHE ABUNDANCE
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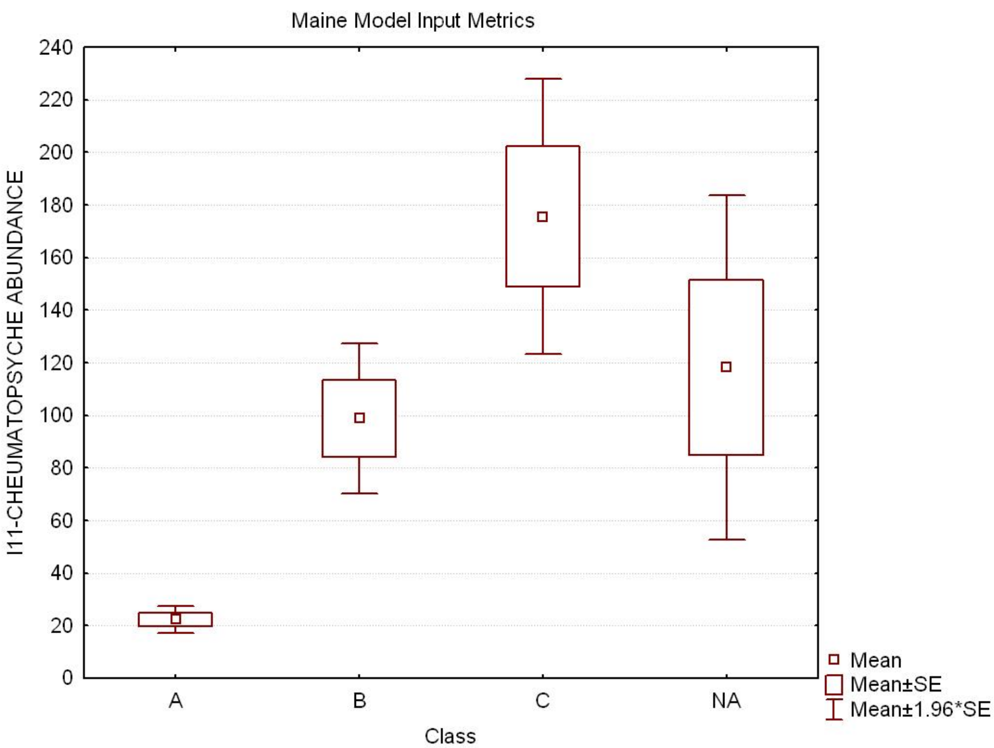
200
150
100
50
0
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE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I11-CHEUMATOPSYCHE ABUNDANCE
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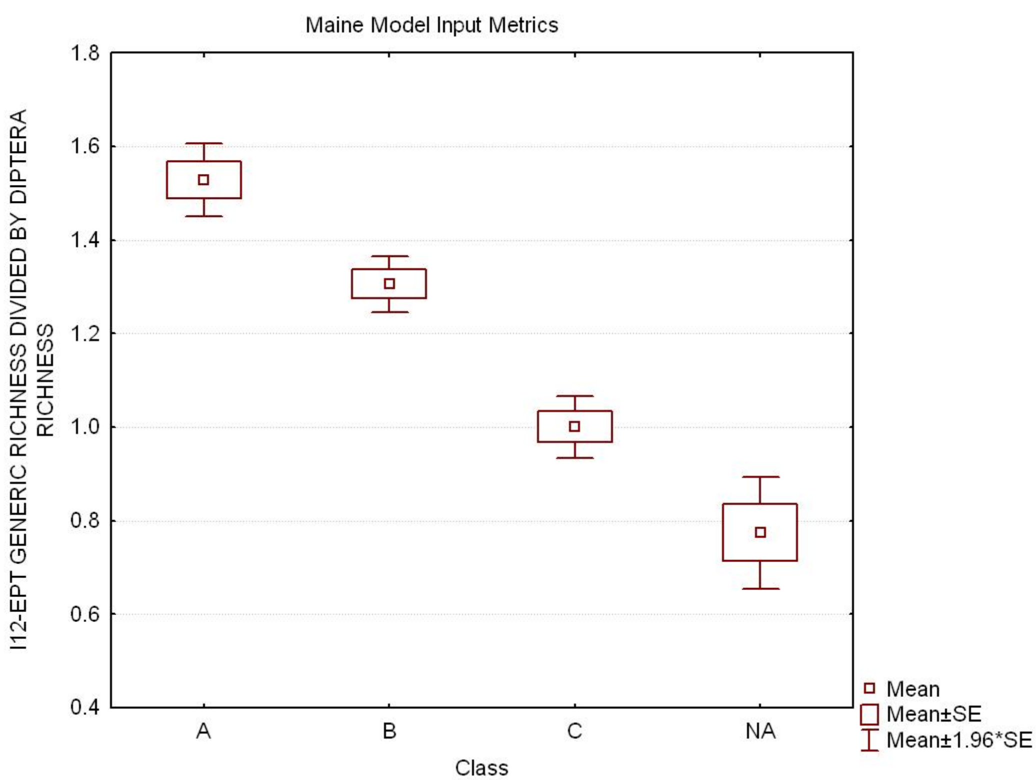
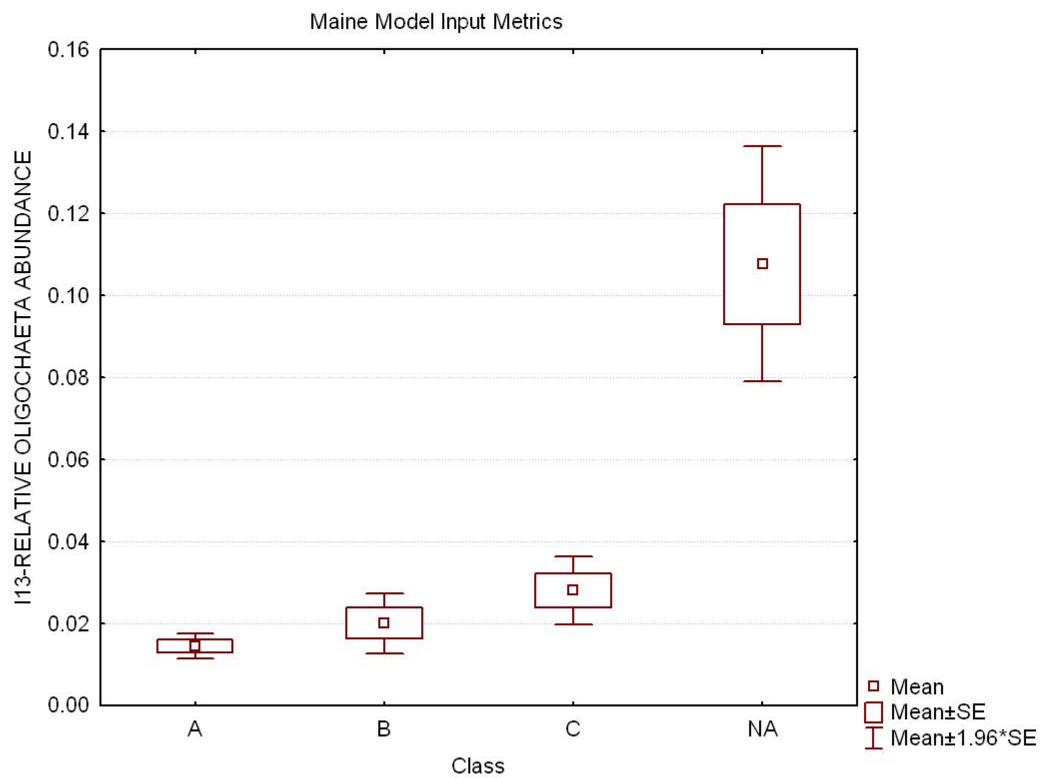
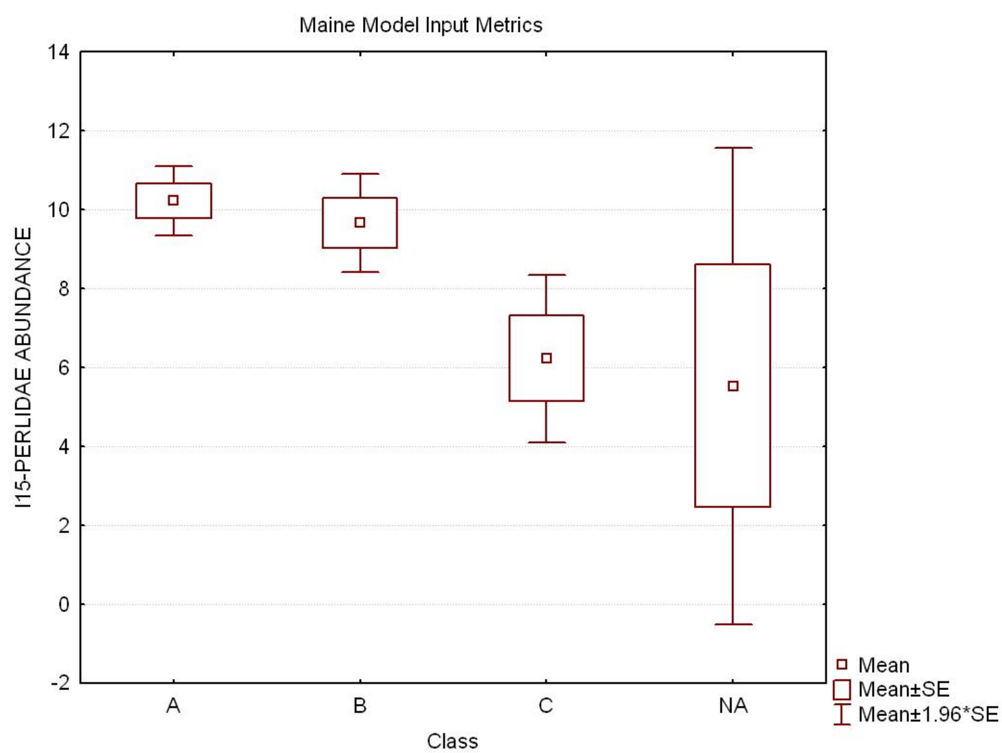
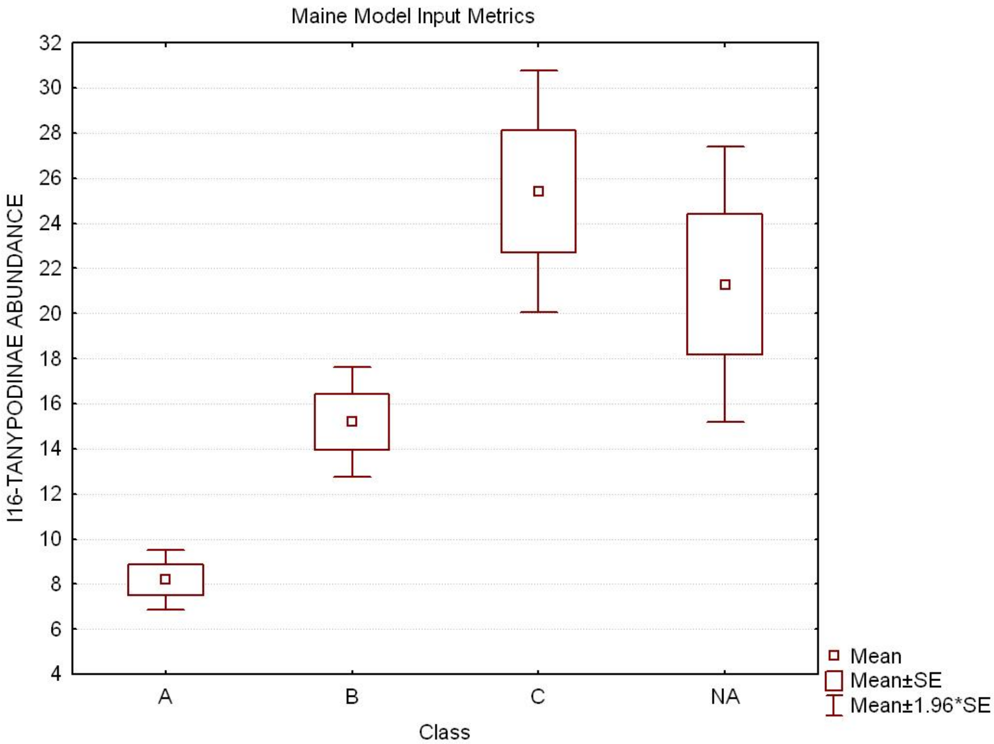
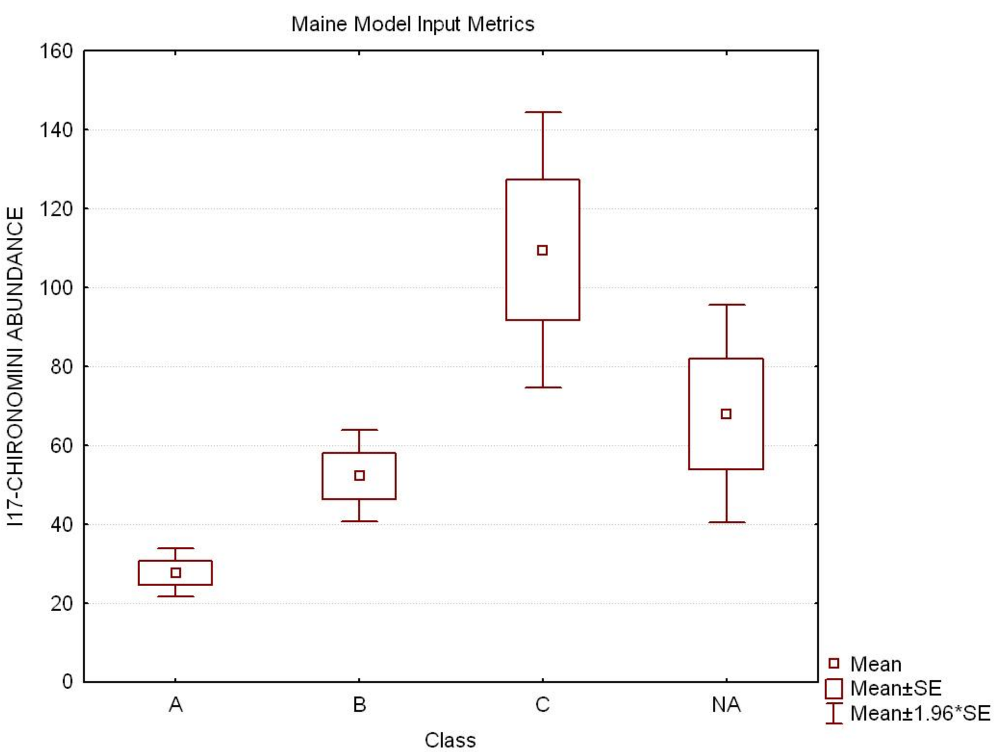
220
200
180
160
140
120
100
80
60
40
20
0
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE


Maine Model Input Metrics
I12-EPT GENERIC RICHNESS DIVIDED BY DIPTERA RICHNESS
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE


Maine Model Input Metrics
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
I13-RELATIVE OLIGOCHAETA ABUNDANCE
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE


Maine Model Input Metrics
14
12
10
8
6
4
2
0
-2
I15-PERLIDAE ABUNDANCE
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE


Maine Model Input Metrics
I16-TANYPODINAE ABUNDANCE
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE


Maine Model Input Metrics
I17-CHIRONOMINI ABUNDANCE
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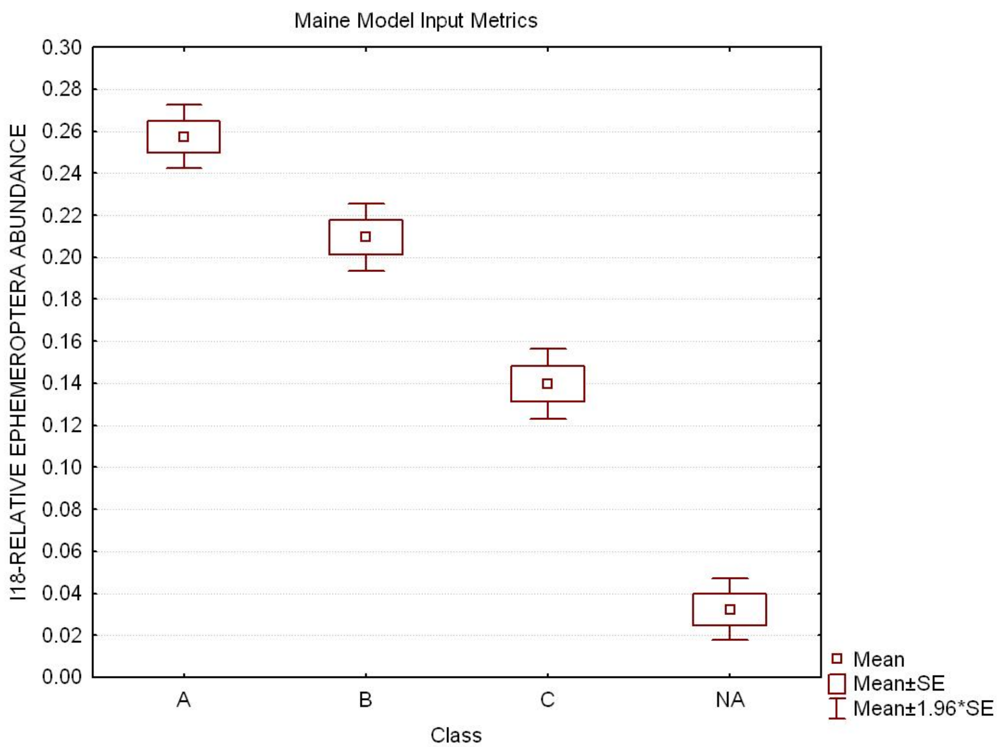
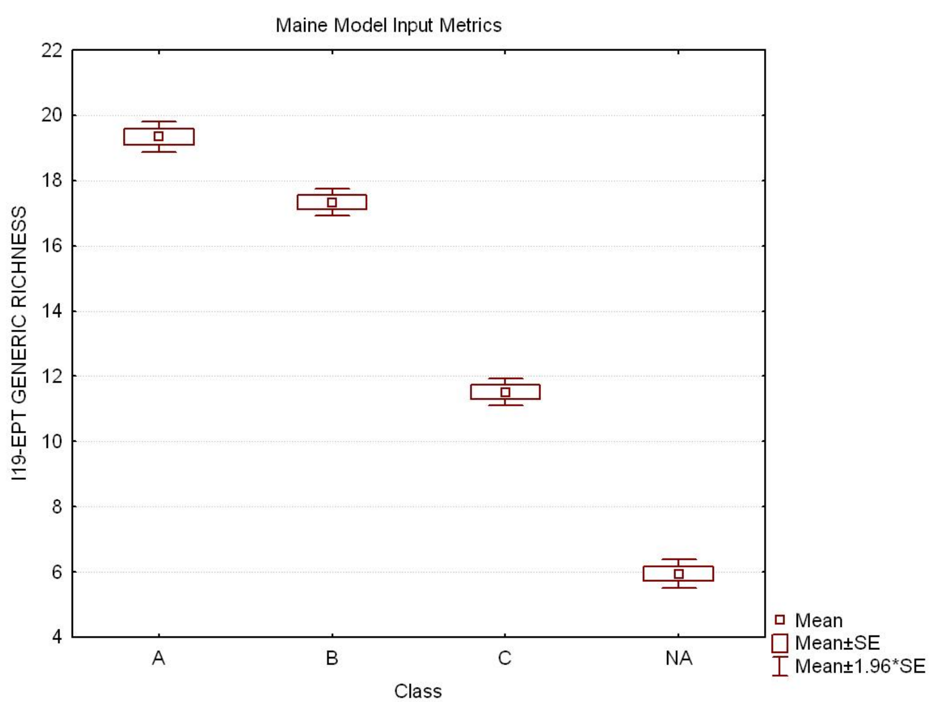
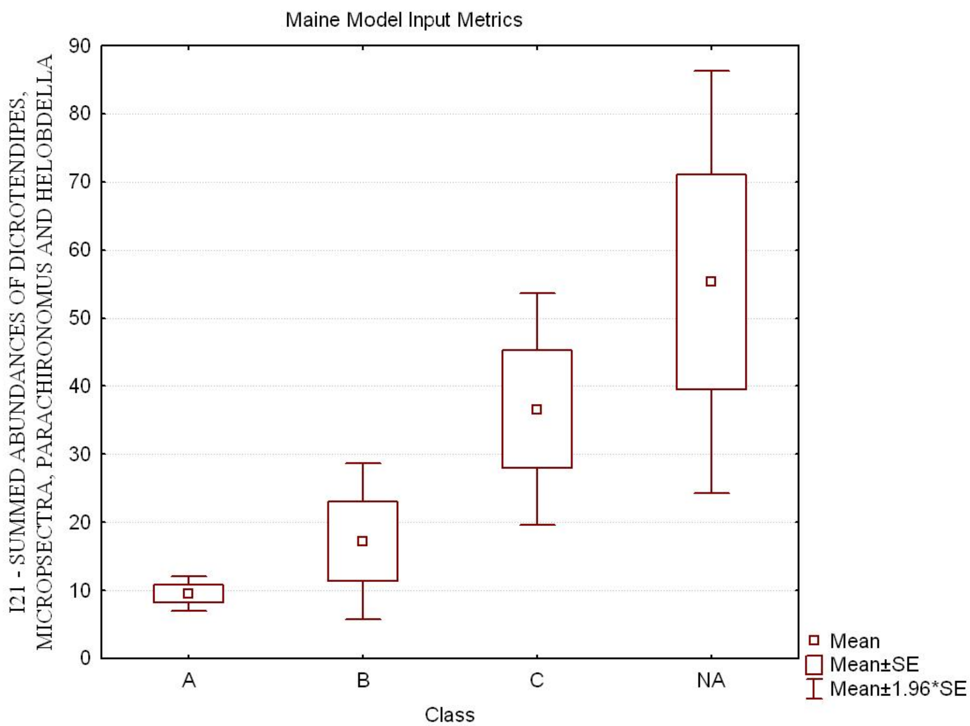
140
120
100
80
60
40
20
0
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE

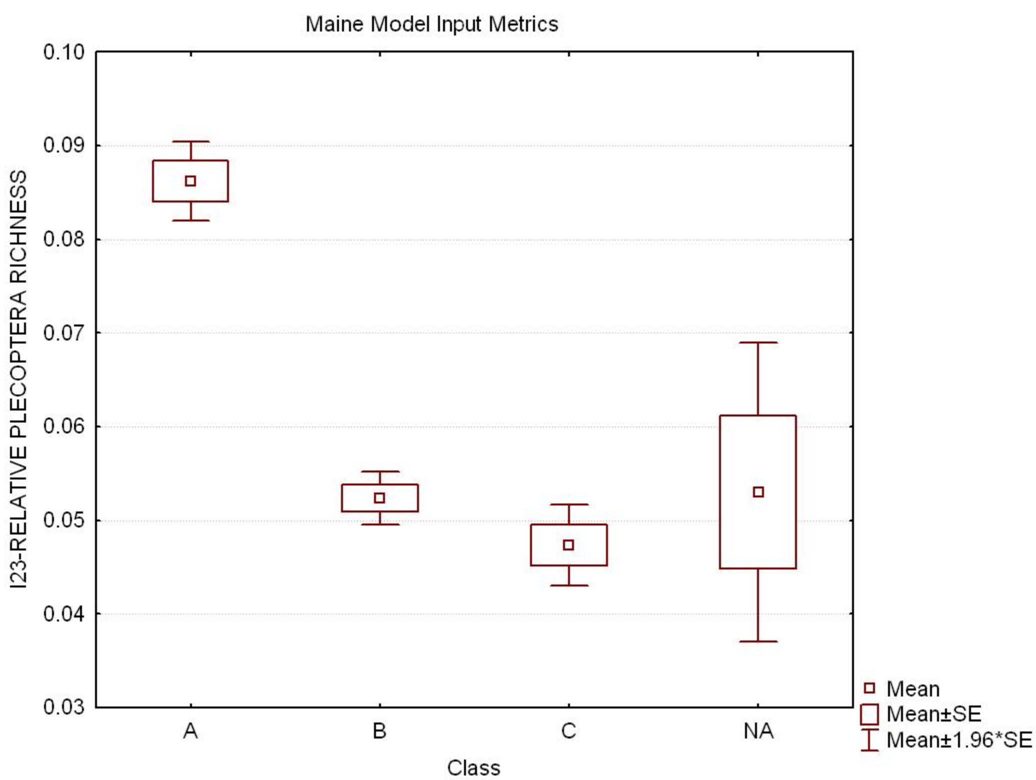


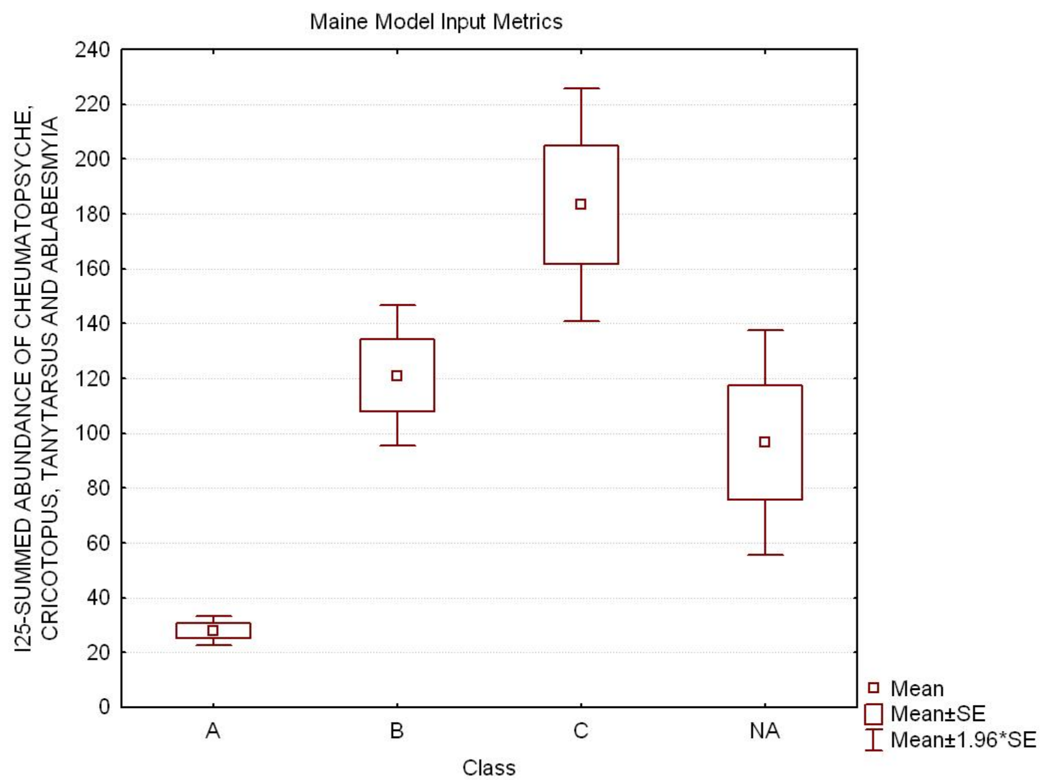
Maine Model Input Metrics
I18-RELATIVE EPHEMEROPTERA ABUNDANCE
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE

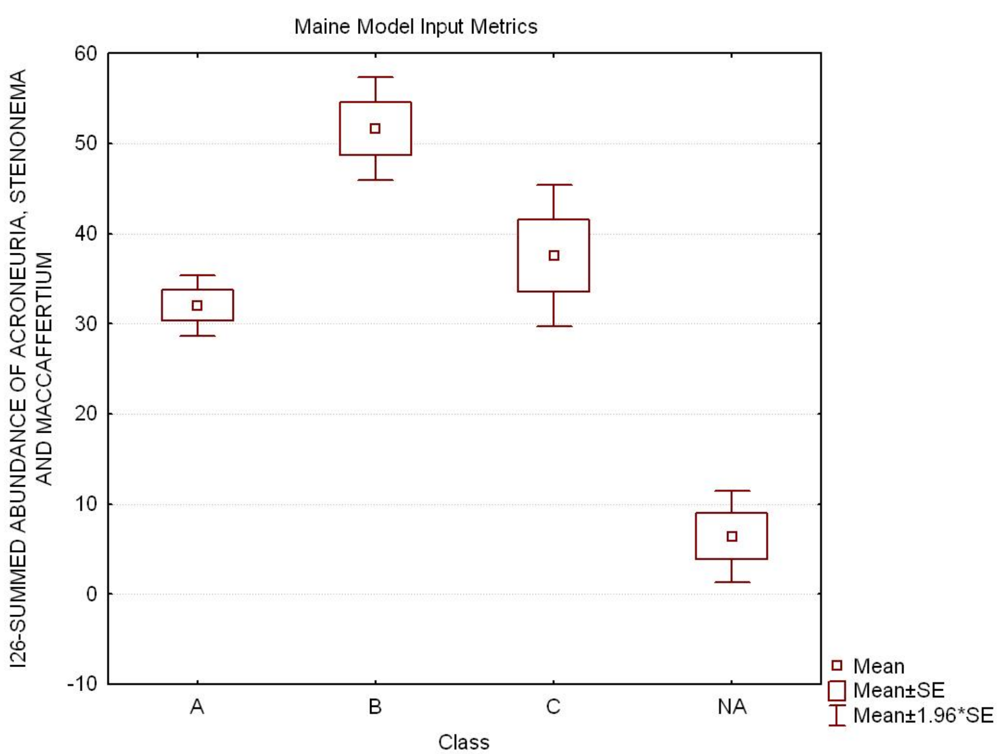


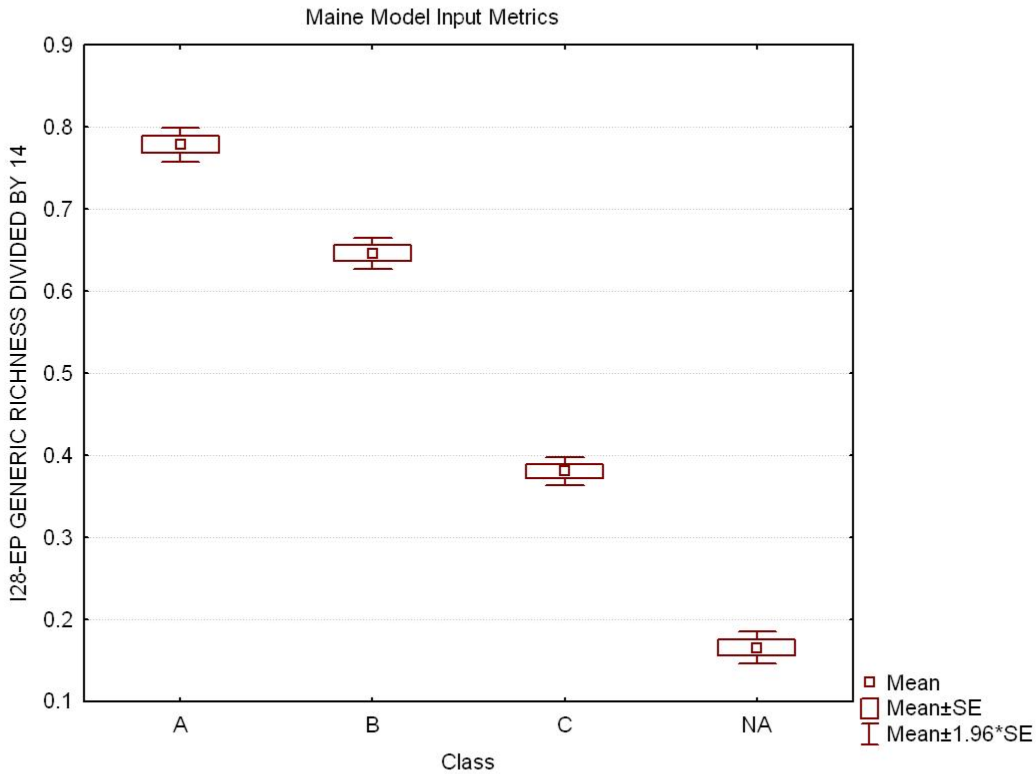
Maine Model Input Metrics
I19-EPT GENERIC RICHNESS
22
20
18
16
14
12
10
8
6
4
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE


Maine Model Input Metrics
I21 - SUMMED ABUNDANCES OF DICROTENDIPES, MICROPSECTRA, PARACHIRONOMUS AND HELOBDELLA
90
80
70
60
50
40
30
20
10
0
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE

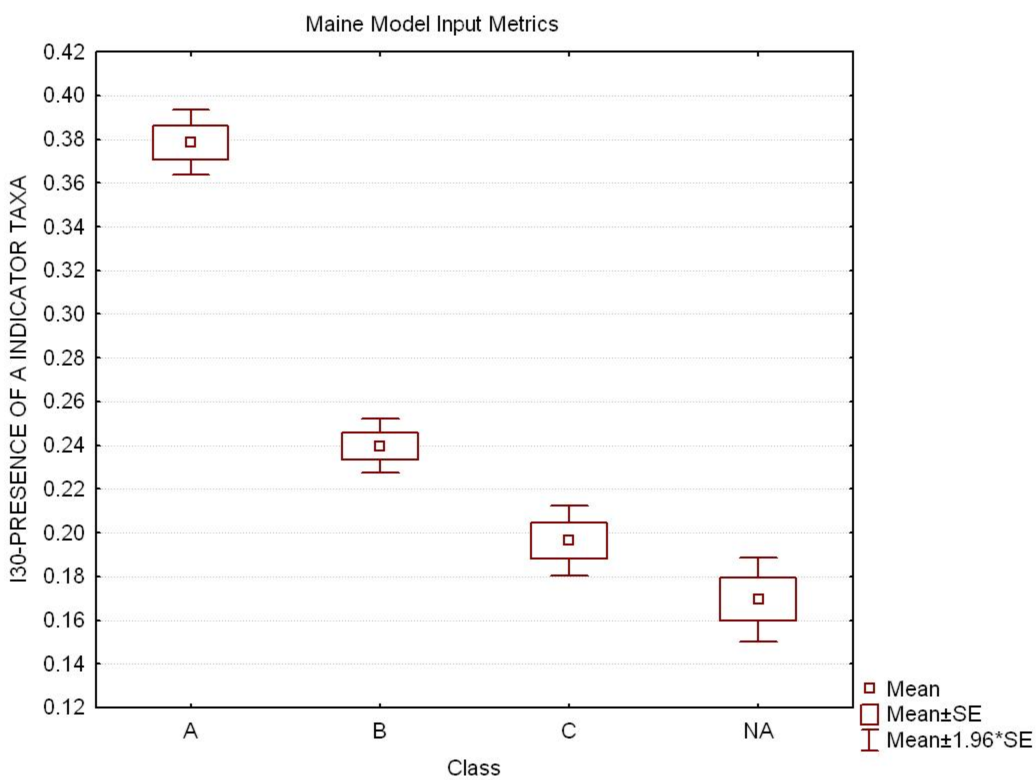

Maine Model Input Metrics
I23-RELATIVE PLECOPTERA RICHNESS
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE


Maine Model Input Metrics
I25-SUMMED ABUNDANCE OF CHEUMATOPSYCHE, CRICOTOPUS, TANYTARSUS AND ABLABESMYIA
0
20
40
60
80
100
120
140
160
180
200
220
240
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE

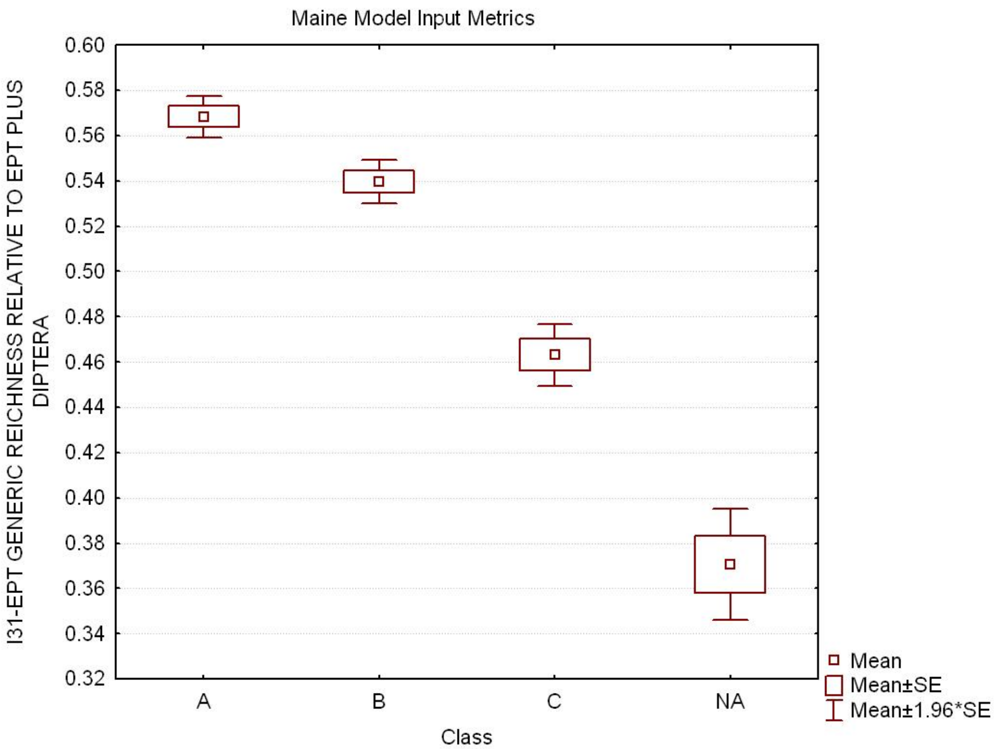

Maine Model Input Metrics
I26-SUMMED ABUNDANCE OF ACRONEURIA, STENONEMA AND MACCAFFERTIUM
-10
0
10
20
30
40
50
60
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE

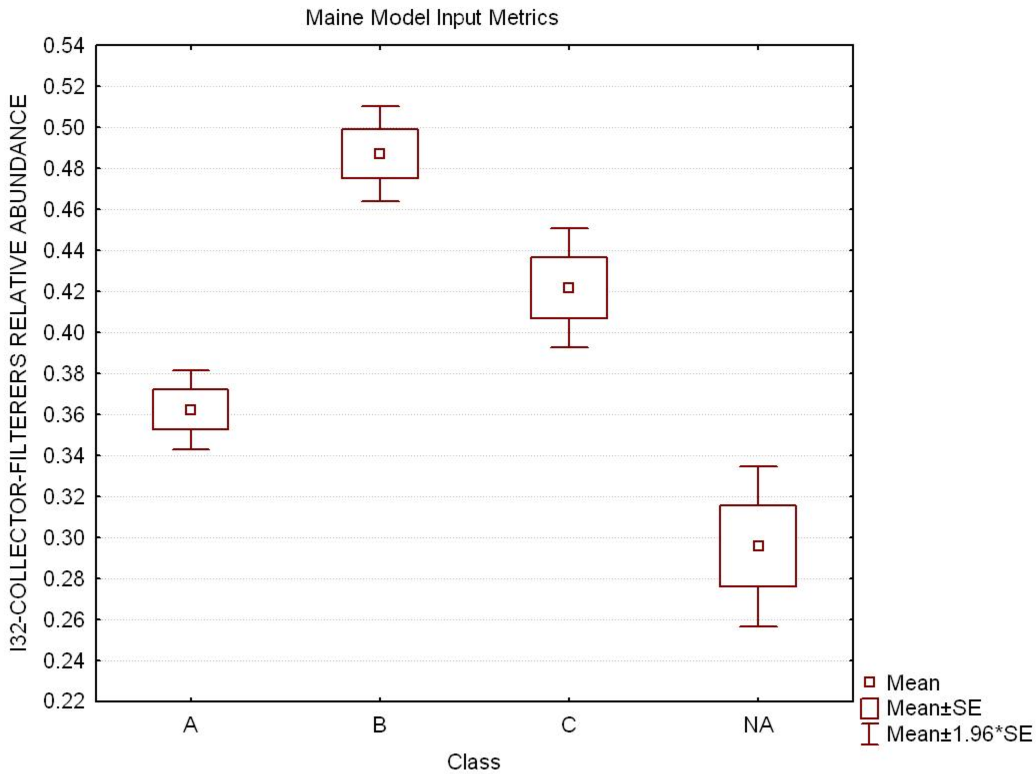


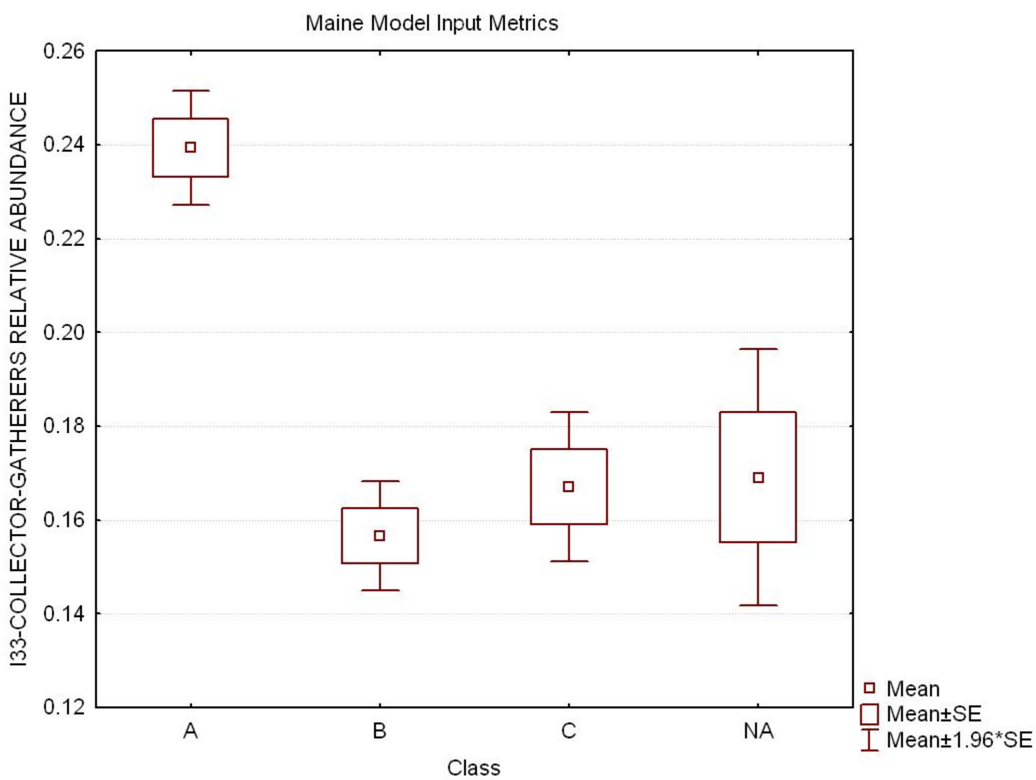
Maine Model Input Metrics
I28-EP GENERIC RICHNESS DIVIDED BY 14
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE

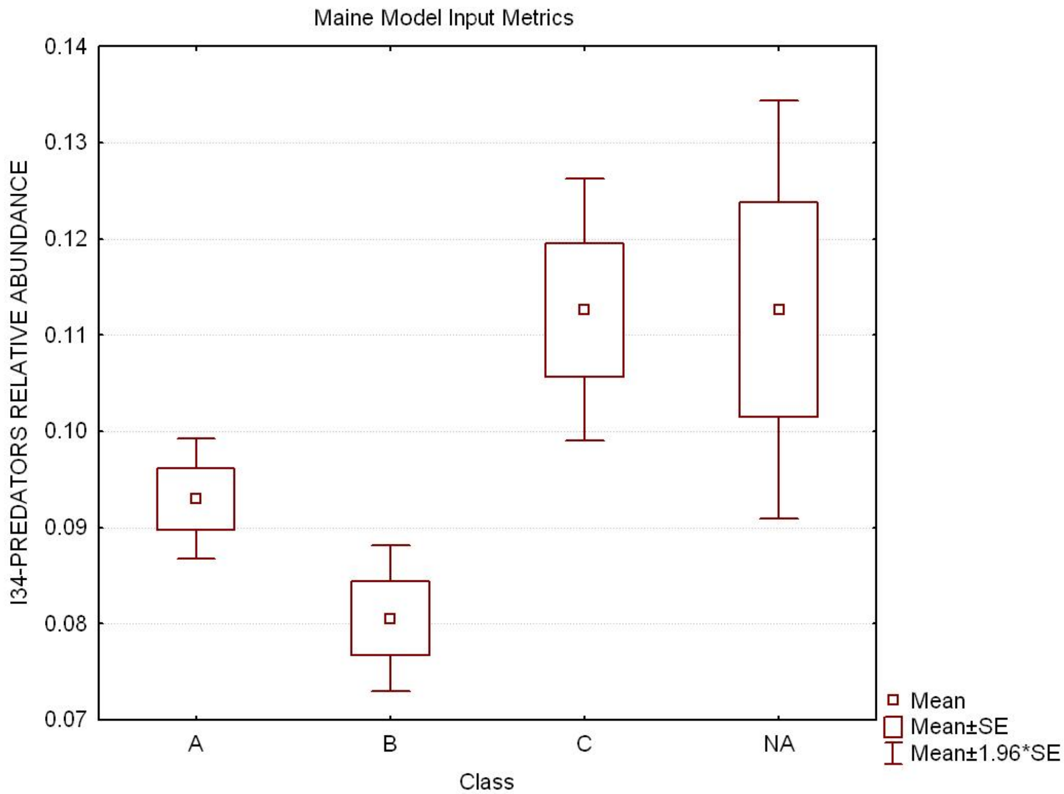


Maine Model Input Metrics
I30-PRESENCE OF A INDICATOR TAXA
0.42
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE

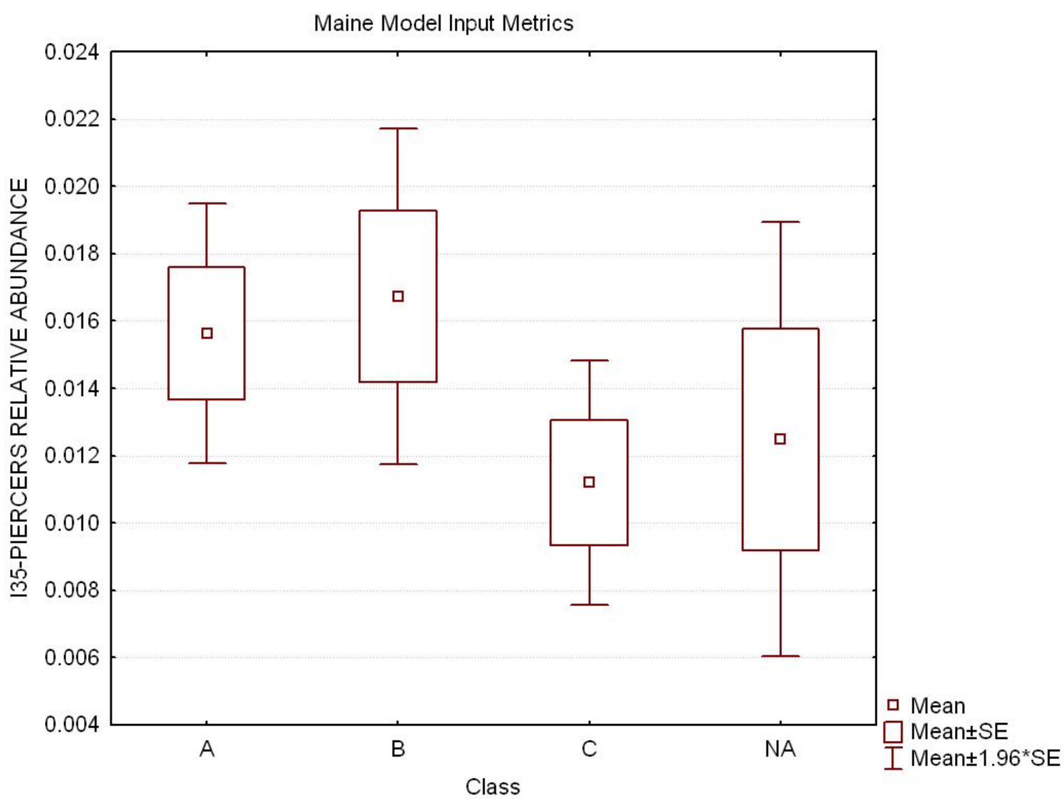

Maine Model Input Metrics
I31-EPT GENERIC REICHNESS RELATIVE TO EPT PLUS DIPTERA
0.60
0.58
0.56
0.54
0.52
0.50
0.48
0.46
0.44
0.42
0.40
0.38
0.36
0.34
0.32
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE


Maine Model Input Metrics
I32-COLLECTOR-FILTERERS RELATIVE ABUNDANCE
0.54
0.52
0.50
0.48
0.46
0.44
0.42
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE

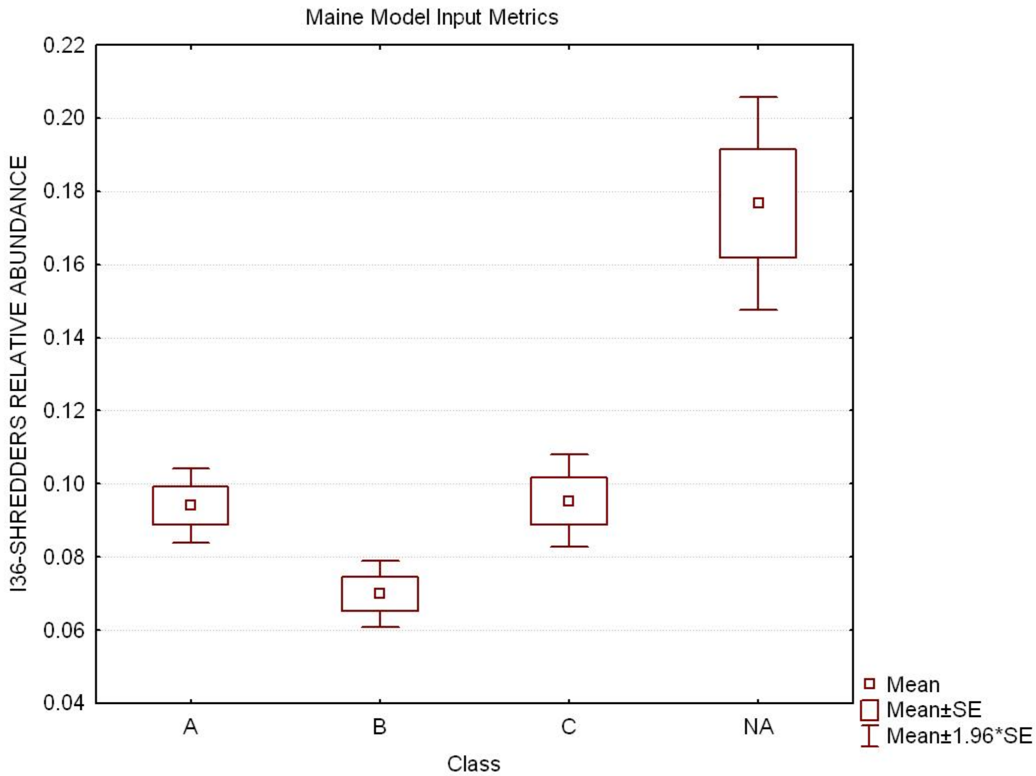

Maine Model Input Metrics
I33-COLLECTOR-GATHERERS RELATIVE ABUNDANCE
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE


Maine Model Input Metrics
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
I34-PREDATORS RELATIVE ABUNDANCE
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE

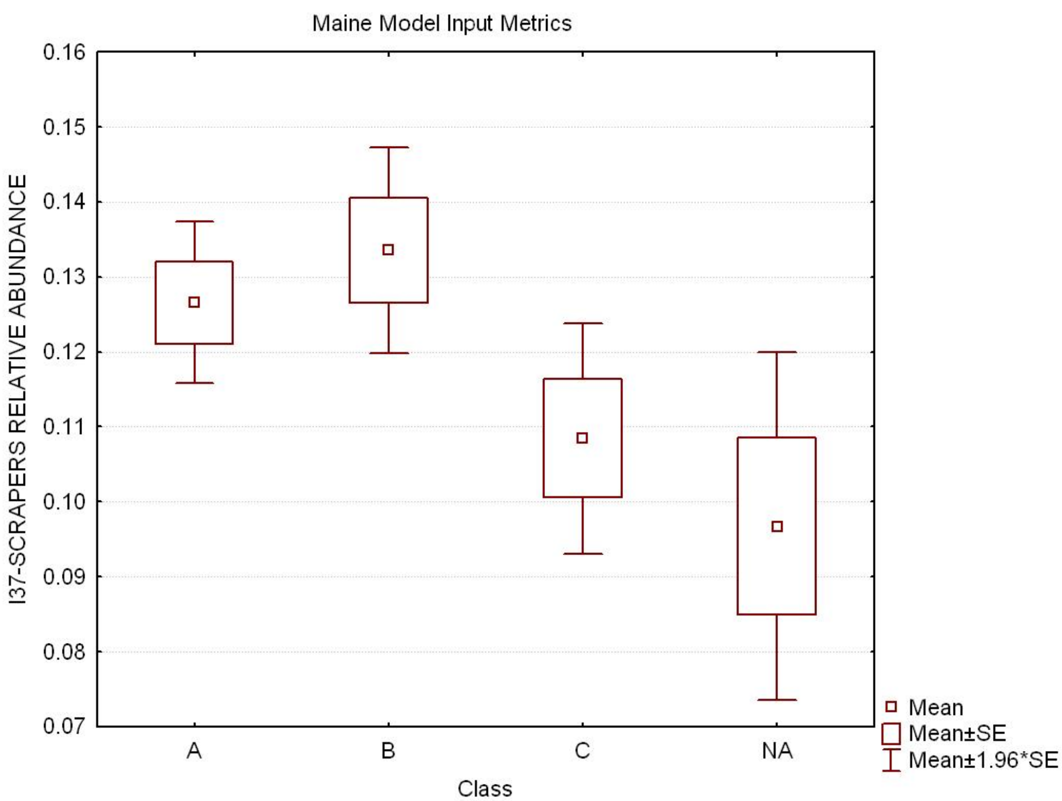
403


Maine Model Input Metrics
0.024
0.022
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
I35-PIERCERS RELATIVE ABUNDANCE
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE

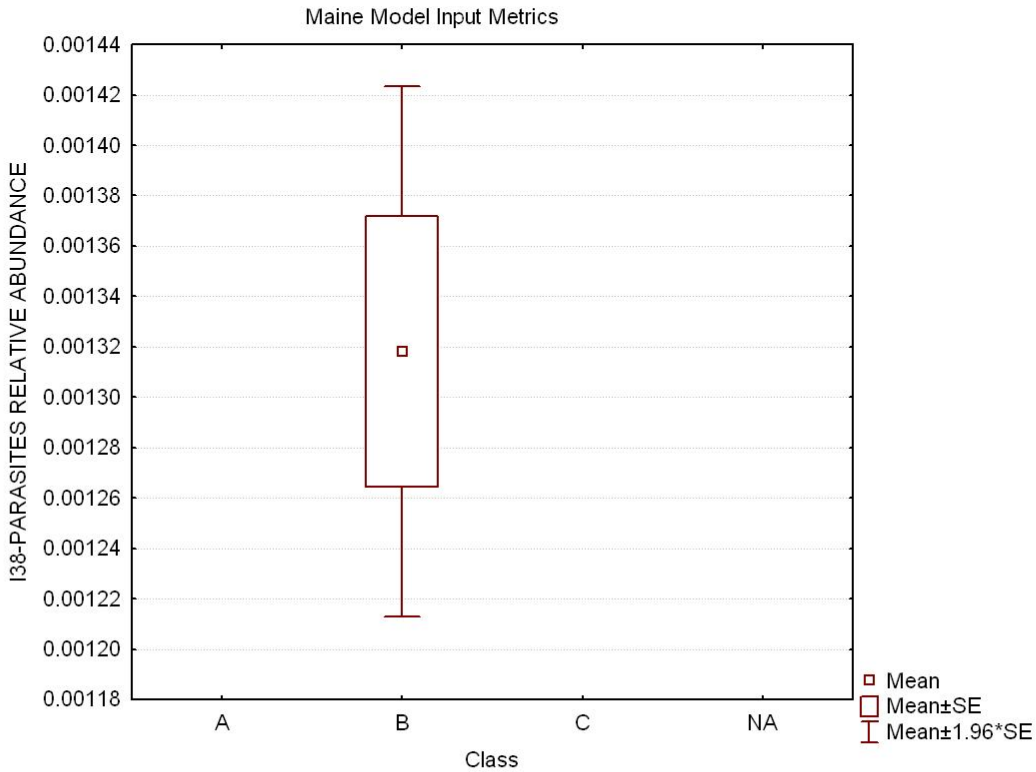
404

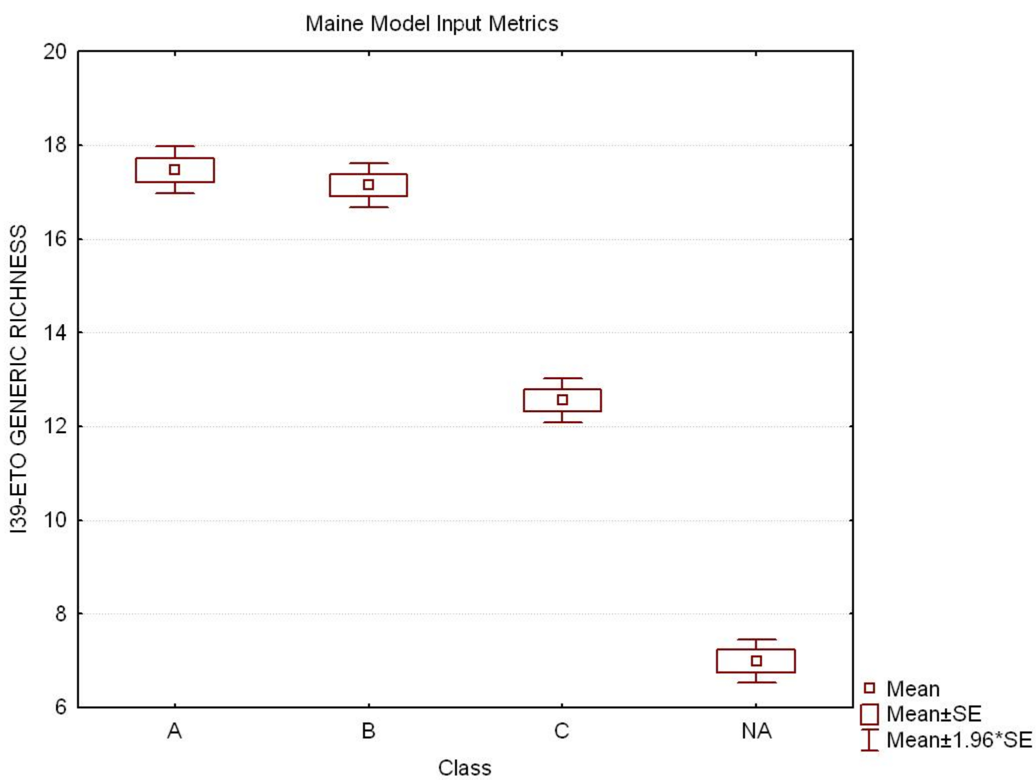

Maine Model Input Metrics
I36-SHREDDERS RELATIVE ABUNDANCE
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE

405


Maine Model Input Metrics
I37-SCRAPERS RELATIVE ABUNDANCE
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE

406


Maine Model Input Metrics
I38-PARASITES RELATIVE ABUNDANCE
0.00144
0.00142
0.00140
0.00138
0.00136
0.00134
0.00132
0.00130
0.00128
0.00126
0.00124
0.00122
0.00120
0.00118
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE


Maine Model Input Metrics
I39-ETO GENERIC RICHNESS
20
18
16
14
12
10
8
6
A
B
C
NA
Class
Mean
Mean±SE
Mean±1.96*SE

Attachment E2

Maine Temperature-Indicator Taxa

This attachment contains tables with lists of the Maine temperature-indicator taxa and describes the process that we followed to develop these lists.

MAINE TEMPERATURE-INDICATOR TAXA

Sources. The Maine cold- and warm-water taxa lists were developed using several different sources: 1. weighted average calculations based on a subset of the Maine biomonitoring database (using site average temperature values (July, August, and September) from 616 sites); 2. the thermal preference trait from the Poff et al. (2006) traits matrix; 3. the thermal-preference trait from the USGS traits database (Vieira et al., 2006); 4. the thermal-preference trait from the compilation of EPA Environmental Requirements and Pollution Tolerance series from the late 1970's (Beck et al., 1977; Harris et al., 1978; Hubbard et al., 1978; Surdick et al., 1978); and 5. best professional judgment of the New England Climate Change traits feedback group[1].

Designation as cold-water taxa. Taxa were placed on the Maine cold-water taxa list if they met the following criteria: 1. They received a rank temperature optima value of 1 or 2 or 3 (the rank optima value is based on percentiles of the dataset; for these taxa, the weighted average optima value was less than the 0.4 percentile value of the dataset it was derived from); or 2. the thermal preference in the Poff et al. 2006 traits matrix was 'cold_cool'; or 3. The thermal preference in the USGS traits database (Vieira et al., 2006) was 'cold stenothermal' or 'cold-cool eurythermal' (temperature preference of less than 15°C); or 4. The thermal preferences in the EPA Environmental Requirements and Pollution Tolerance series were 'oligothermal' or 'stenothermal' or 'metathermal' (temperature preference of less than 15°C); or 5. If anyone in the New England Climate Change feedback group felt a taxa should be added to this list.

Designation as warm-water taxa. Taxa were placed on the Maine warm-water taxa list if they met the following criteria: 1. They received a rank temperature optima value of 5 or 6 or 7 (the rank optima value is based on percentiles of the dataset; for these taxa, the weighted average optima value was greater than the 0.6 percentile value of the dataset it was derived from); or 2. the thermal preference in the Poff et al. 2006 traits matrix was 'warm'; or 3. The thermal preference in the USGS traits database (Vieira et al., 2006) was 'hot euthermal' or 'warm eurythermal' (temperature preference of greater than 15°C); or 4. The thermal preferences in the

[1] New England Climate Change group: Maine DEP (Leon Tsomides, Tom Danielson, Dave Courtemanche, Susan Davies), Vermont DEC (Doug Burnham, Steve Fiske, Jim Kellogg, Rich Langdon), New Hampshire (Dave Neils – NH DES, Don Chandler- UNH), Mike Winnell (professional taxonomist who works on a lot of Maine samples).

EPA Environmental Requirements and Pollution Tolerance series were ‘euthermal’ or ‘eurythermal’ or ‘mesothermal’ (temperature preference of greater than 15°C); or 5. If anyone in the New England Climate Change feedback group felt a taxa should be added to this list.

Limitations. These lists were developed using the best information available, but it should be noted that the available information is limited. The weighted average calculations are based on instantaneous water temperature measurements that were taken at the time of the sampling event. Ideally, continuous water temperature data should be used, since this provides more information about the thermal regime, especially during times of greatest thermal stress (i.e. summer baseflow conditions). However, these data are generally unavailable. The weighted average calculations also have limitations. One of the main concerns is that the analysis does not take into account the confounding factors (‘noise’) that are not related to temperature. However, the theory is that with a sufficient amount of data, the noise essentially cancels itself out. Another limitation is that the operational taxonomic unit that was most appropriate for this analysis is at the genus-level (in some instances, family-level was most appropriate). Within certain genera in particular, the thermal preference among species varies, so the assigned thermal preference may not be appropriate for all species within a genera. Attempts were made to note these genera (see ‘species-variation’ column in the worksheets).

We want to reiterate that when we developed these lists, we did the best we could with the data that was available. These lists should be viewed as a first step, not a final product. It would be very helpful if future research included a combination of short- and long-term field and experimental studies designed to better evaluate climate change effects on freshwater ecosystems.

Initial Results. Initially there were 106 taxa on the cold-water list and 82 taxa on the warm-water list. These lists were based on weighted average calculations and literature. These lists were further refined through the evaluation of additional evidence. This evidence included analyses of other datasets, case studies, and best professional judgment. Taxa with the greatest amount of evidence were designated as temperature-indicator taxa. More detailed information about the steps that were used to develop the temperature indicator taxa lists is summarized below:

Considerations

A. Results from weighted average or maximum likelihood thermal optima and tolerance calculations were a major consideration. Results from the following eight analyses were used:

- California (taken from 'Herbst_CABW.2007_Sierra.climate.change.ppt')
- Idaho (taken from 'Temperature Preferences and Tolerances for 137 Common Idaho Macroinvertebrate Taxa. Darren Brandt. Idaho DEQ. November 2001.')
- Maine (based on site average temperature values (July- September) from 616 sites in the Maine biomonitoring database)
- North Carolina (based on maximum likelihood calculations for the North Carolina biomonitoring database, full-scale collection method only)
- Ohio (Ed Rankin, these are PRELIMINARY, and are based on average mean temperature values)
- Oregon (Shannon Hubler (2007), based on the Oregon DEQ database)
- Utah (based on 572 fall samples from the Utah biomonitoring database)
- Yuan 2006 (Estimation and Application of Macroinvertebrate Tolerance Values. Report No. EPA/600/P-04/116F, based on Western EMAP data).

A scoring system was developed to summarize results from the eight different analyses. It takes into account thermal preference, thermal tolerance and sample size. Scores were assigned (for each of the eight analyses) as follows:

COLD-WATER TAXA

- 2=cold stenotherm (rank optima of 1 or 2 or 3 and rank tolerance of 1 or 2 or 3), adequate sample size (20 or more counts)
- 1=cold preference (rank optima of 1 or 2 or 3), adequate sample size (20 or more counts)
- 1=cold stenotherm (rank optima of 1 or 2 or 3 and rank tolerance of 1 or 2 or 3), low sample size (less than 20 counts)
- 0.5=cold preference (rank optima of 1 or 2 or 3), low sample size (less than 20 counts)

WARM-WATER TAXA

- 2=warm eurythermal (rank optima of 5 or 6 or 7 and rank tolerance of 5 or 6 or 7), adequate sample size (20 or more counts)

- 1=warm preference (rank optima of 5 or 6 or 7), adequate sample size (20 or more counts)
- 1= warm eurythermal (rank optima of 5 or 6 or 7 and rank tolerance of 5 or 6 or 7), low sample size (less than 20 counts)
- 0.5=warm preference (rank optima of 5 or 6 or 7), low sample size (less than 20 counts)

In addition to the weighted average and maximum likelihood results, information on thermal preferences was also derived from literature. The taxon received a score of 1 if it was cited as a cold- or warm-water taxon in at least one of the following sources: Poff et al. 2006 traits matrix; or USGS traits database (Vieira et al., 2006); or EPA Environmental Requirements and Pollution Tolerance series from the late 1970's (Beck et al., 1977; Harris et al., 1978; Hubbard et al., 1978; Surdick et al., 1978). If the weighted-average results showed the taxon to have a preference for cold- or warm-water but the literature showed conflicting results (i.e. based on the weighted-average results, the taxon was a cold-water taxa, but the literature showed it to be a warm-water taxa), then the taxon was not included on the temperature indicator list.

After scores were assigned as described above, they were summed so that each taxon received a total score. The higher the total score, the more evidence there was in the eight analyses and the literature that supported the designation of the taxon as a temperature indicator taxon.

B. Several 'case studies' were performed to see whether the cold- or warm-water taxa occurred at sites in Maine and Vermont that had the warmest or coldest summer water temperatures. The following case studies were performed:

a. **Cold-Water Case Study #1**. Vermont provided us with taxa lists from two sites that they regard as cold-water habitat. They are located below a dam that does profundal releases, and the water temperature remains around 8°C year round. The dam is a confounding factor (although a study by the VT DEC indicates minor impacts on the macroinvertebrate community from the whitewater releases), but temperature is regarded as a major factor influencing community composition at these sites.

b. **Cold-Water Case Study #2**. Taxa lists from the following 3 sites in Maine: Station 57514 (Cold Brook (Dead River) – Maine DEP Station 772), Station 57513 (Cold Brook (Dead River) – Maine DEP Station 771) and Station 57512 (Cold Brook (Dead

River) – Maine DEP Station 770). These sites were selected for the following reasons: 1. Water temperature readings at these 3 sites were among the lowest in the database, ranging from 7.8 to 13.9°C (these were July-Sept readings); based on the surrounding land use land cover (1km buffer), these sites appear to have few confounding factors (0-1% urban, 0% agricultural). Wetlands may influence the biota at these sites, especially Station 57512 (23% wetland), but temperature is believed to be a factor influencing community composition at these sites.

c. **Warm-Water Case Study #1**. Taxa lists from two sites in Maine with the warmest average water temperatures: Station 56834 (Mattanawcook Stream – Maine DEP Station 91, below Lincoln Pulp and Paper (cooling water), which had an average summer water temperature of 31°C; and Site 57055 (Birch Stream (Bangor) – Maine DEP Station 312), which had an average summer water temperature of 30°C. These are not reference sites. Within the 1 km buffer, Station 56834 is 24% urban and Station 57055 is 60% urban.

d. **Warm-Water Case Study #2**. Taxa lists from 5 sites in Maine with the warmest average water temperatures that were <5% urban and <10% agricultural within a 1 km buffer. Average water temperatures ranged from 26-27°C. Sites included: Station 57560 (West Seboeis Stream – Maine DEP Station 818), Station 56871 (Penobscot River - Maine DEP Station 128), Station 56953 (Dead River - Maine DEP Station 210), Station 57228 (Pollard Brook - Maine DEP Station 485) and Station 56952 (Dead River - Maine DEP Station 209).

C. In addition to the case studies, best professional judgment from the New England Climate Change group was taken into account.

Development of the Temperature Indicator Cold-Water Taxa List. Taxa were placed on the cold-water list if the following criteria were met:

1. The taxon was NOT present at the warm-water case study sites.
2. The taxon was present at one or more of the cold-water case study sites and/or the New England Climate Change feedback group believed that it should be on the list.

Development of the Temperature Indicator Warm-Water List. Taxa were placed on the warm-water list if the following criteria were met:

1. The taxon was NOT present at the cold-water case study sites.
2. The taxon was present at one or more of the warm-water case study sites and/or the New England Climate Change feedback group believed that it should be on the list.

Temperature Indicator Lists. The cold-water taxa list was comprised of 41 taxa and the warm-water taxa list was comprised of 40 taxa. Temperature indicator taxa lists can be found in **Tables E2-1** and **E2-2**.

Important Notes – variation within genera. Some noteworthy genera were left off the Maine warm-water taxa list. These included Brachycentrus, Hydropsyche, and Ceratopsyche. Genera left off the Maine cold-water list included Eukiefferiella and Rhyacophila. The reason they were not included is because there is variation in temperature preferences among species within these genera, and this was noted by the New England Climate Change feedback group or in the literature (Vermont DEC suggested a list of species to include on the lists – see **Tables E2-6** and **E2-7**).

It is also worth noting the absence of two other genera from the cold-water list – Antocha and Dicranota. In the weighted average and maximum likelihood analyses, these two taxa were often listed as cold-water taxa. However, in the case studies, it became apparent that these genera were widespread and occurred at sites at which cold and warm temperatures had been recorded.

Dispersal Ability. If temperature is a major factor influencing community composition, then taxa that are able to adapt to warming temperatures or that are able to disperse to more favorable habitats (generally believed to be upstream or to higher elevations) have a better chance of surviving. Five mobility traits were examined for the taxa on the Maine temperature indicator lists: dispersal (adult), adult flying strength, occurrence in drift, maximum crawling rate and swimming ability. More information on these traits can be found in **Table E2-3**. Dispersal (adult) and adult flying strength received the greatest amount of consideration. Because movement is most likely to be upstream, taxa that are strong fliers are likely to have a better chance of success. It will be difficult for taxa that disperse via occurrence in drift to migrate upstream, and taxa that disperse via crawling or swimming are likely to have difficulty moving the distances required to find more favorable habitats.

Two of the 41 taxa on the Maine temperature indicator cold-water taxa list (for which w had trait information), Boyeria and Pteronarcys, are considered to have high dispersal ability and strong adult flying strength. Another taxon, Lanthus, is categorized as having strong flying ability but low adult dispersal ability. Eleven of the 40 taxa on the warm-water list are categorized as having high adult dispersal ability. Four of these taxa are considered to be strong fliers.

Abundance and Distribution. In addition to dispersal ability, abundance and distribution are also important considerations. Those taxa that are widespread and common are likely to have greater genetic diversity and greater chance of adapting than rare taxa that only occur in isolated, localized populations (Sweeney et al. 1992). Moreover, the more abundant taxa are more likely to affect the state biomonitoring assessments. Abundance and distribution information for the temperature indicator taxa can be found in **Tables E2-1** and **E2-2**.

The most abundant cold-water-temperature-indicator taxa are Leuctra (Plecopteran), Epeorus (Ephemeropteran), Eurylophella (Ephemeropteran), Perlodidae (Plecopteran) and Boyeria (Odonata). These taxa comprise only 0.3 to 0.4% of the total individuals in the Maine database. Thirty-one of the cold-water taxa have overall abundances of less than 0.1%. Stenonema and Neureclipsis are the most abundant warm-water taxa, with overall abundances of 5.2 and 2.6%, respectively. Nine of the warm-water taxa have overall abundances of less than 0.1%. Of the cold-water taxa, Boyeria occurs at the largest percentage of sites (38%), followed by a Plecopteran, Perlodidae, which occurs at 25% of the sites. Thirty-one of the taxa occur at less than 10% of the sites. Among the warm-water taxa, Stenonema occurs at the highest percentage of sites (63%), followed by Acroneuria (39%) and Neureclipsis (38%). Eight of the warm-water taxa occur at less than 10% of the sites.

Additional information – Cold-Water Taxa. Sixteen of the cold-water taxa are Plecopterans, ten are Trichopterans, seven are Dipterans, and three are Ephemeropterans. The rest are Coleopterans, Odonates and Megalopterans. The families with the most number of taxa on the cold-water list are Chironomidae and Nemouridae (**Table E2-4**). It should be noted that two of the taxa on the cold-water list, Malirekus and Taenionema, do not occur in the Maine database. They were added per best professional judgment of the Vermont DEC.

Additional information – Warm-Water Taxa. Ten of the warm-water taxa are Dipterans, nine are Ephemeropterans and six are Trichopterans. The families with the most number of taxa on the warm-water list are Chironomidae and Perlidae (**Table E2-5**).

Table E2-1. List of Maine cold-water temperature indicator taxa. Distribution and abundance information is also included. Sum_Individuals=the total number of individuals from that taxon in the Maine database; Pct_Abund=percent of total individuals in the database comprised of that taxon; Num_Stations=number of stations in the database that the taxon occurred at; Pct_Stations=percent of stations in the database at which the taxon occurred

Type	Order	Family	FinalID	Sum_Individs	Pct_Abund	Num_Stations	Pct_Stations
cold	Ephemeroptera	Ameletidae	Ameletus	63	0.01	26	3.06
cold	Trichoptera	Apataniidae	Apatania	48	0.01	23	2.71
cold	Odonata	Aeshnidae	Boyeria	1761	0.3	321	37.81
cold	Plecoptera	Capniidae	Capnia	71	0.01	5	0.59
cold	Trichoptera	Hydropsychidae	Diplectrona	1137	0.19	47	5.54
cold	Ephemeroptera	Heptageniidae	Epeorus	2132	0.36	172	20.26
cold	Ephemeroptera	Ephemerellidae	Eurylophella	1785	0.3	170	20.02
cold	Trichoptera	Glossosomatidae	Glossosoma	945	0.16	119	14.02
cold	Diptera	Chironomidae	Heterotrissocladius	447	0.08	73	8.6
cold	Trichoptera	Limnephilidae	Hydatophylax	114	0.02	49	5.77
cold	Odonata	Gomphidae	Lanthus	36	0.01	11	1.3
cold	Diptera	Chironomidae	Larsia	269	0.05	58	6.83
cold	Plecoptera	Leuctridae	Leuctra	2407	0.4	142	16.73
cold	Trichoptera	Limnephilidae	Limnephilus	889	0.15	62	7.3
cold	Diptera	Chironomidae	Macropelopia	322	0.05	43	5.06
cold	Plecoptera	Perlodidae	Malirekus	0	0	0	0
cold	Trichoptera	Brachycentridae	Micrasema	405	0.07	87	10.25
cold	Diptera	Chironomidae	Natarsia	430	0.07	65	7.66
cold	Plecoptera	Nemouridae	Nemoura	17	0	4	0.47
cold	Megaloptera	Corydalidae	Nigronia	713	0.12	170	20.02
cold	Trichoptera	Phryganeidae	Oligostomis	485	0.08	87	10.25
cold	Coleoptera	Elmidae	Oulimnius	237	0.04	37	4.36
cold	Diptera	Chironomidae	Pagastia	420	0.07	96	11.31
cold	Trichoptera	Hydroptilidae	Palaeagapetus	1	0	1	0.12
cold	Plecoptera	Capniidae	Paracapnia	52	0.01	17	2

Table E2-1. Continued

Type	Order	Family	FinalID	Sum_Individs	Pct_Abund	Num_Stations	Pct_Stations
cold	Plecoptera	Nemouridae	Paranemoura	3	0	3	0.35
cold	Trichoptera	Hydropsychidae	Parapsyche	398	0.07	27	3.18
cold	Plecoptera	Peltoperlidae	Peltoperla	9	0	4	0.47
cold	Plecoptera	Perlodidae	Perlodidae	1775	0.3	212	24.97
cold	Diptera	Chironomidae	Prodiamesa	392	0.07	28	3.3
cold	Plecoptera	Nemouridae	Prostoia	6	0	1	0.12
cold	Diptera	Chironomidae	Pseudodiamesa	139	0.02	12	1.41
cold	Trichoptera	Limnephilidae	Psychoglypha	329	0.06	37	4.36
cold	Plecoptera	Pteronarcyidae	Pteronarcys	248	0.04	80	9.42
cold	Ephemeroptera	Heptageniidae	Rhithrogena	193	0.03	23	2.71
cold	Plecoptera	Chloroperlidae	Sweltsa	640	0.11	66	7.77
cold	Plecoptera	Taeniopterygidae	Taenionema	0	0	0	0
cold	Plecoptera	Peltoperlidae	Tallaperla	126	0.02	12	1.41
cold	Plecoptera	Capniidae	Utacapnia	71	0.01	3	0.35
cold	Plecoptera	Chloroperlidae	Utaperla	2	0	2	0.24
cold	Plecoptera	Nemouridae	Zapada	2	0	1	0.12

Table E2-2. **List of Maine warm-water temperature indicator taxa. Distribution and abundance information is also included. Sum_Individuals=the total number of individuals from that taxon in the Maine database; Pct_Abund=percent of total individuals in the database comprised of that taxon; Num_Stations=number of stations in the database that the taxon occurred at; Pct_Stations=percent of stations in the database at which the taxon occurred**

Type	Order	Family	FinalID	Sum_Individs	Pct_Abund	Num_Stations	Pct_Stations
warm	Plecoptera	Perlidae	Acroneuria	4857	0.82	331	38.99
warm	Mesogastropoda	Hydrobiidae	Amnicola	4589	0.77	160	18.85
warm	Odonata	Coenagrionidae	Argia	869	0.15	137	16.14
warm	Plecoptera	Perlidae	Attaneuria	172	0.03	36	4.24
warm	Ephemeroptera	Caenidae	Caenis	1783	0.3	169	19.91
warm	Diptera	Chironomidae	Cardiocladius	200	0.03	52	6.12
warm	Trichoptera	Leptoceridae	Ceraclea	876	0.15	152	17.9
warm	Haplotaxida	Naididae	Chaetogaster	342	0.06	70	8.24
warm	Diptera	Chironomidae	Dicrotendipes	1978	0.33	169	19.91
warm	Arhynchobdellida	Erpobdellidae	Erpobdella	265	0.04	65	7.66
warm	Basommatophora	Ancylidae	Ferrissia	594	0.1	102	12.01
warm	Trichoptera	Helicopsychidae	Helicopsyche	2563	0.43	104	12.25
warm	Basommatophora	Planorbidae	Helisoma	716	0.12	66	7.77
warm	Diptera	Empididae	Hemerodromia	1764	0.3	260	30.62
warm	Hydroida	Hydridae	Hydra	483	0.08	113	13.31
warm	Trichoptera	Hydroptilidae	Hydroptila	1799	0.3	189	22.26
warm	Ephemeroptera	Isonychiidae	Isonychia	5413	0.91	225	26.5
warm	Diptera	Chironomidae	Labrundinia	618	0.1	137	16.14
warm	Ephemeroptera	Heptageniidae	Leucrocuta	3320	0.56	208	24.5
warm	Trichoptera	Hydropsychidae	Macrostemum	4557	0.77	168	19.79
warm	Trichoptera	Polycentropodidae	Neureclipsis	15523	2.61	320	37.69
warm	Diptera	Chironomidae	Nilotanypus	413	0.07	133	15.67
warm	Trichoptera	Leptoceridae	Oecetis	3390	0.57	306	36.04
warm	Decapoda	Cambaridae	Orconectes	381	0.06	99	11.66

Table E2-2. Continued

Type	Order	Family	FinalID	Sum_Individs	Pct_Abund	Num_Stations	Pct_Stations
warm	Diptera	Chironomidae	Parachironomus	946	0.16	83	9.78
warm	Plecoptera	Perlidae	Paragnetina	625	0.11	103	12.13
warm	Diptera	Chironomidae	Pentaneura	881	0.15	139	16.37
warm	Basommatophora	Physidae	Physa	1373	0.23	115	13.55
warm	Basommatophora	Physidae	Physella	1681	0.28	155	18.26
warm	Ephemeroptera	Baetidae	Plauditus	1285	0.22	125	14.72
warm	Hoplonemertea	Tetrastemmatidae	Prostoma	267	0.04	61	7.18
warm	Diptera	Chironomidae	Psectrocladius	1693	0.28	161	18.96
warm	Ephemeroptera	Baetidae	Pseudocloeon	1147	0.19	113	13.31
warm	Diptera	Chironomidae	Rheopelopia	729	0.12	144	16.96
warm	Ephemeroptera	Ephemerellidae	Serratella	2534	0.43	191	22.5
warm	Ephemeroptera	Heptageniidae	Stenacron	6503	1.09	196	23.09
warm	Coleoptera	Elmidae	Stenelmis	2638	0.44	280	32.98
warm	Ephemeroptera	Heptagenidae	Stenonema	30768	5.18	536	63.13
warm	Diptera	Chironomidae	Tribelos	1781	0.3	78	9.19
warm	Ephemeroptera	Leptohyphidae	Tricorythodes	2655	0.45	205	24.15

Table E2-3. Mobility traits that were evaluated. The source of most of this information was the Poff et al. 2006 traits matrix. Some also came from the USGS traits database (Vieira et al. 2006)

Mobility Trait	Trait States
Dispersal (adult)	low (<1 km flight before laying eggs), high (>1 km flight before laying eggs)
Adult flying strength	weak (e.g. cannot fly into light breeze), strong
Occurrence in drift	rare (catastrophic only), common (typically observed), abundant (dominant in drift samples)
Maximum crawling rate	very low (<10 cm/h), low (<100 cm/h), high (>100 cm/h)
Swimming ability	none, weak, strong

Table E2-4. Number of cold-water taxa in each family

Family	Total
Chironomidae	7
Nemouridae	4
Capniidae	3
Limnephilidae	3
Chloroperlidae	2
Elmidae	2
Hydropsychidae	2
Peltoperlidae	2
Perlodidae	2
Aeshnidae	1
Ameletidae	1
Apataniidae	1
Brachycentridae	1
Corydalidae	1
Ephemerellidae	1
Glossosomatidae	1
Gomphidae	1
Heptageniidae	1
Hydroptilidae	1
Leuctridae	1
Phryganeidae	1
Pteronarcyidae	1
Taeniopterygidae	1

Table E2-5. Number of warm-water taxa in each family

Family	Total
Chironomidae	9
Perlidae	3
Physidae	2
Leptoceridae	2
Heptageniidae	2
Baetidae	2
Tetrastemmatidae	1
Polycentropodidae	1
Planorbidae	1
Naididae	1
Leptohyphidae	1
Isonychiidae	1
Hydroptilidae	1
Hydropsychidae	1
Hydrobiidae	1
Hydridae	1
Heptagenidae	1
Helicopsychidae	1
Erpobdellidae	1
Ephemerellidae	1
Empididae	1
Elmidae	1
Coenagrionidae	1
Cambaridae	1
Caenidae	1
Ancylidae	1

Table E2-6. Potential cold-water *species* (per recommendation by Vermont DEC)

Order	Genus	Species
Diptera	Polypedilum	aviceps
Diptera	Neostempellina	reissi
Diptera	Tvetenia	bavarica
Ephemeroptera	Rhithrogena	sp
Ephemeroptera	Ameletus	sp
Trichoptera	Arctopsyche	sp
Trichoptera	Arctopsyche	ladogensis
Trichoptera	Rhyacophila	carolina
Trichoptera	Rhyacophila	torva
Trichoptera	Rhyacophila	nigrita
Trichoptera	Rhyacophila	invaria
Trichoptera	Rhyacophila	acutiloba
Plecoptera	Peltoperla	sp
Plecoptera	Tallaperla	sp
Plecoptera	Taenionema	sp
Decapoda	Cambarus	Cambarus bartoni
Trichoptera	Palaeagapetus	sp
Diptera	Eukiefferella	brevicalar, brehmi, and tirolensis
Coleoptera	Oulimnius	latiusculus
Coleoptera	Promoresia	tardella

Table E2-7. Potential warm-water *species* (per recommendation by Vermont DEC)

Order	Genus	Species
Diptera	Eukiefferella	claripennis
Diptera	Polypedilum	flavum
Diptera	Tvetenia	discoloripes
Trichoptera	Leucotrichia	sp
Trichoptera	Rhyacophila	mainensis
Trichoptera	Rhyacophila	manistee
Trichoptera	Rhyacophila	minora
Plecoptera	Neoperla	sp
Plecoptera	Taeniopteryx	sp

Attachment E3

Tolerance values and BCG attribute levels of the cold and warm-water temperature indicator taxa

This attachment contains tables with lists of the temperature indicator taxa, temperature optima and tolerance values that were calculated from the weighted average modeling, the tolerance values assigned by Maine DEP (which are used to calculate the HBI) and BCG attribute levels assigned to each taxa during the New England Wadeable Streams (NEWS) project (US EPA 2007). These tables were used to examine whether temperature indicator taxa were considered to be sensitive or tolerant taxa.

Table E4-1. ***Cold-water*** **temperature indicator taxa. Temp_Opt is the temperature optima (°C) and Temp_Tol is the temperature tolerance calculated during the weighted average modeling. TolVal_ME is the tolerance value that was assigned by Maine DEP and that is used in the calculation of the HBI. BCG_NEWS is the BCG attribute level assigned to each taxa during the New England Wadeable Streams project and BCG_Cat is the category associated with the BCG_NEWS attribute levels (2=highly sensitive taxa, 3=intermediate sensitive taxa, 4=taxa of intermediate tolerance, 5=tolerant taxa).**

Order	Family	FinalID	Temp_Opt	Temp_Tol	TolVal_ME	BCG_NEWS
Coleoptera	Elmidae	Oulimnius				3
Diptera	Chironomidae	Heterotrissocladius	16.3	2.8	0	3
Diptera	Chironomidae	Larsia	17.5	3.6	6	4
Diptera	Chironomidae	Macropelopia	15.5	1.9		5
Diptera	Chironomidae	Natarsia	16.6	2.5	8	5
Diptera	Chironomidae	Pagastia	17.1	3.7	1	4
Diptera	Chironomidae	Prodiamesa	15.6	2	3	2
Diptera	Chironomidae	Pseudodiamesa				
Ephemeroptera	Ameletidae	Ameletus			0	2
Ephemeroptera	Heptageniidae	Epeorus	19.9	4.9	0	2
Ephemeroptera	Ephemerellidae	Eurylophella	17.4	3.2	3	3
Ephemeroptera	Heptageniidae	Rhithrogena			0	2
Megaloptera	Corydalidae	Nigronia	20.4	2.8	0	3
Odonata	Aeshnidae	Boyeria	20.4	2.9	2	4
Odonata	Gomphidae	Lanthus				3
Plecoptera	Capniidae	Capnia			1	
Plecoptera	Leuctridae	Leuctra	16.3	3	0	2
Plecoptera	Nemouridae	Nemoura			1	
Plecoptera	Capniidae	Paracapnia			1	3
Plecoptera	Nemouridae	Paranemoura				
Plecoptera	Peltoperlidae	Peltoperla				
Plecoptera	Perlodidae	Perlodidae	17.3	4.4		3

Table E4-1. Continued

Order	Family	FinalID	Temp_Opt	Temp_Tol	TolVal_ME	BCG_NEWS
Plecoptera	Nemouridae	Prostoia				
Plecoptera	Pteronarcyidae	Pteronarcys	19.1	3.9	0	2
Plecoptera	Chloroperlidae	Sweltsa	14.9	3.5		3
Plecoptera	Peltoperlidae	Tallaperla				2
Plecoptera	Capniidae	Utacapnia				
Plecoptera	Chloroperlidae	Utaperla				
Plecoptera	Nemouridae	Zapada				
Trichoptera	Apataniidae	Apatania				3
Trichoptera	Hydropsychidae	Diplectrona	16.8	2.5	0	4
Trichoptera	Glossosomatidae	Glossosoma	18.7	4.8	0	3
Trichoptera	Limnephilidae	Hydatophylax	17.7	3.2	2	3
Trichoptera	Limnephilidae	Limnephilus	17.4	2.5	3	
Trichoptera	Brachycentridae	Micrasema	18.6	5.3	2	3
Trichoptera	Phryganeidae	Oligostomis	16.6	2.8	2	2
Trichoptera	Hydroptilidae	Palaeagapetus				
Trichoptera	Hydropsychidae	Parapsyche	12.9	2.2	0	
Trichoptera	Limnephilidae	Psychoglypha	15.3	1.7	0	

Table E4-2. ***Warm-water*** **temperature indicator taxa. Temp_Opt is the temperature optima (°C) and Temp_Tol is the temperature tolerance calculated during the weighted average modeling. TolVal_ME is the tolerance value that was assigned by Maine DEP and that is used in the calculation of the HBI. BCG_NEWS is the BCG attribute level assigned to each taxa during the New England Wadeable Streams project and BCG_Cat is the category associated with the BCG_NEWS attribute levels (2=highly sensitive taxa, 3=intermediate sensitive taxa, 4=taxa of intermediate tolerance, 5=tolerant taxa, 6=non native or intentionally introduced taxa).**

Order	Family	FinalID	Temp_Opt	Temp_Tol	TolVal_ME	BCG_NEWS
Arhynchobdellida	Erpobdellidae	Erpobdella	20.7	3.2		6
Basommatophora	Ancylidae	Ferrissia	21.8	2.9		4
Basommatophora	Planorbidae	Helisoma	21	2.7		5
Basommatophora	Physidae	Physa	21.6	3.3		4
Basommatophora	Physidae	Physella	20.5	3.3		4
Coleoptera	Elmidae	Stenelmis	21.6	2.5	5	4
Decapoda	Cambaridae	Orconectes	22.4	2.6		5
Diptera	Chironomidae	Cardiocladius	21.8	2.4	5	6
Diptera	Chironomidae	Dicrotendipes	21.2	3.3	8	6
Diptera	Empididae	Hemerodromia	20.8	3.1	3	5
Diptera	Chironomidae	Labrundinia	21.5	3.2	7	5
Diptera	Chironomidae	Nilotanypus	22.2	2.2	6	4
Diptera	Chironomidae	Parachironomus	21.7	2.2	10	5
Diptera	Chironomidae	Pentaneura	22.4	2.8	6	4
Diptera	Chironomidae	Psectrocladius	21.6	3.3	8	4
Diptera	Chironomidae	Rheopelopia	20.8	3.1		
Diptera	Chironomidae	Tribelos	20.4	2.7	5	4
Ephemeroptera	Caenidae	Caenis	21.4	3.5	7	4
Ephemeroptera	Isonychiidae	Isonychia	22	2.8	2	3
Ephemeroptera	Heptageniidae	Leucrocuta	21.2	3.3	1	3
Ephemeroptera	Baetidae	Plauditus	21.2	2.8		3
Ephemeroptera	Baetidae	Pseudocloeon	21.4	3.2	4	4

Table E4-2. Continued

Order	Family	FinalID	Temp_Opt	Temp_Tol	TolVal_ME	BCG_NEWS
Ephemeroptera	Ephemerellidae	Serratella	20.8	3.8	2	3
Ephemeroptera	Heptageniidae	Stenacron	21.6	2.8	7	4
Ephemeroptera	Heptagenidae	Stenonema	21	3.1	4	4
Ephemeroptera	Leptohyphidae	Tricorythodes	22.1	2.1	4	4
Haplotaxida	Naididae	Chaetogaster	20.5	1.9		5
Hoplonemertea	Tetrastemmatidae	Prostoma	23.1	2.4		4
Hydroida	Hydridae	Hydra	20.5	3.5		
Mesogastropoda	Hydrobiidae	Amnicola	22.7	2.4		5
Odonata	Coenagrionidae	Argia	22.7	3	7	4
Plecoptera	Perlidae	Acroneuria	21.6	2.9	0	3
Plecoptera	Perlidae	Attaneuria			1	
Plecoptera	Perlidae	Paragnetina	20.7	3.6	1	3
Trichoptera	Leptoceridae	Ceraclea	21.2	3	3	2
Trichoptera	Helicopsychidae	Helicopsyche	22	2.3	3	4
Trichoptera	Hydroptilidae	Hydroptila	20.4	4.2	6	4
Trichoptera	Hydropsychidae	Macrostemum	22.7	2	3	4
Trichoptera	Polycentropodidae	Neureclipsis	22.1	2.7	7	4
Trichoptera	Leptoceridae	Oecetis	21.5	2.7	8	4

APPENDIX F

Detailed Results for Utah

The intent of this appendix is to provide more comprehensive and detailed information on the large number of analyses that were performed on the Utah data. Some of the analyses that are covered in this appendix are also referenced in the main body of the report. When this occurred, attempts were made to reduce any overlap or duplication in the reporting of results.

F1. OVERVIEW OF THE UTAH RIVPACS MODEL

A number of states use a predictive bioassessment approach called River InVertebrate Prediction And Classification System (RIVPACS) to assess stream condition (Wright, 2000). In the RIVPACS model, data from reference sites are used to establish expected (E) macroinvertebrate assemblages, and the observed (O) assemblages at sites are compared to these expected assemblages. The ratio of these values (O/E) can be interpreted as a measure of taxonomic completeness. Values of O/E that are near 1 at a test site suggest that the site is comparable to reference sites, whereas values that differ substantially from 1 suggest that the site is degraded (Yuan, 2006a).

Utah recently started using a RIVPACS model (fall samples) to rate its sites (Ostermiller, unpublished presentation titled 'Development of a biological assessment framework'). Sites are scored based on the ratio of O to E assemblages (expected assemblages are established based on reference site data). Differences in site characteristics are taken into account when sites are scored. Flow charts depicting the criteria and decision-making process that go into rating sites are shown in **Figures F1-1** and **F1-2**.

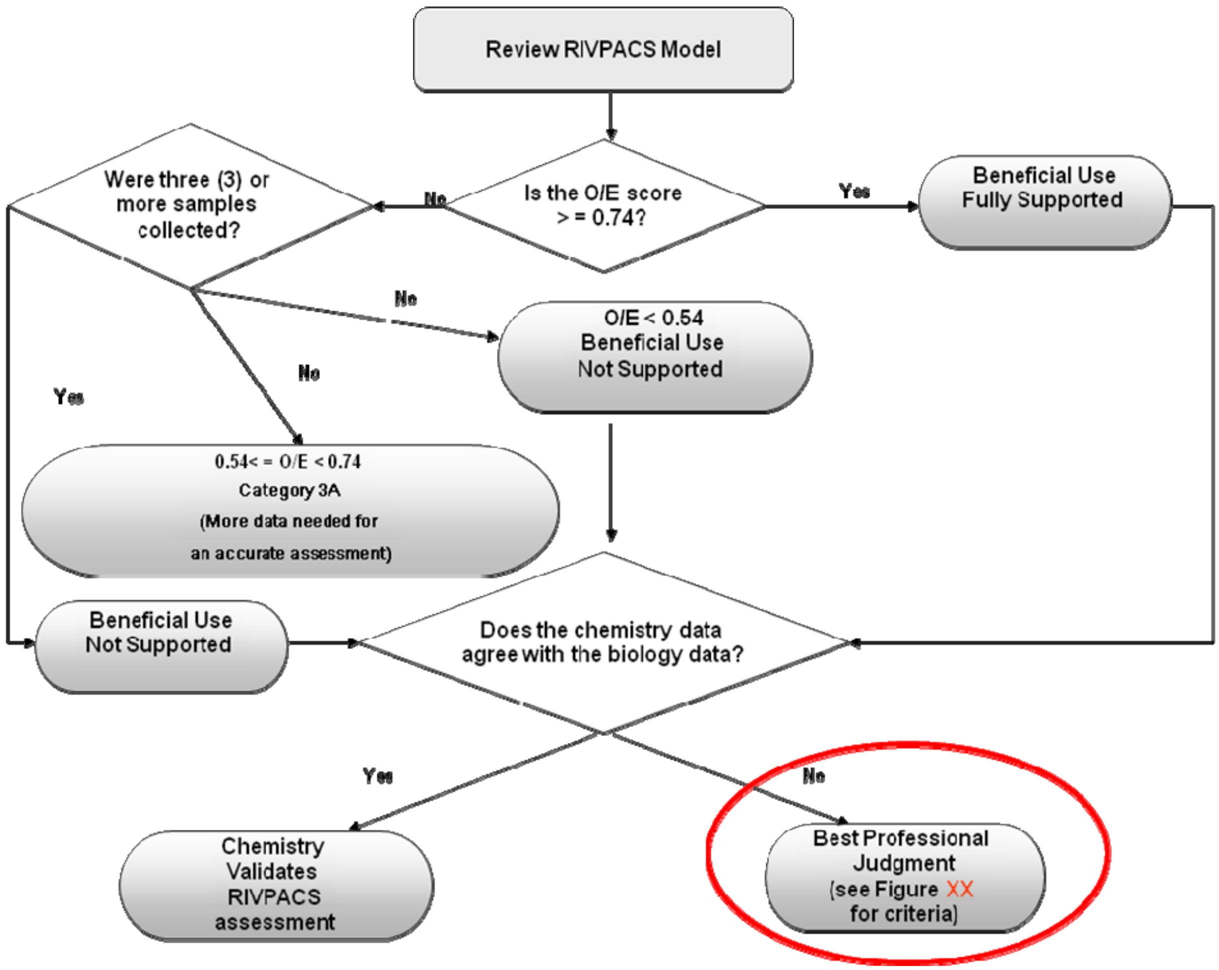


Figure F1-1. Summary of the decision-making process and criteria that go into rating sites using the Utah fall RIVPACS model.

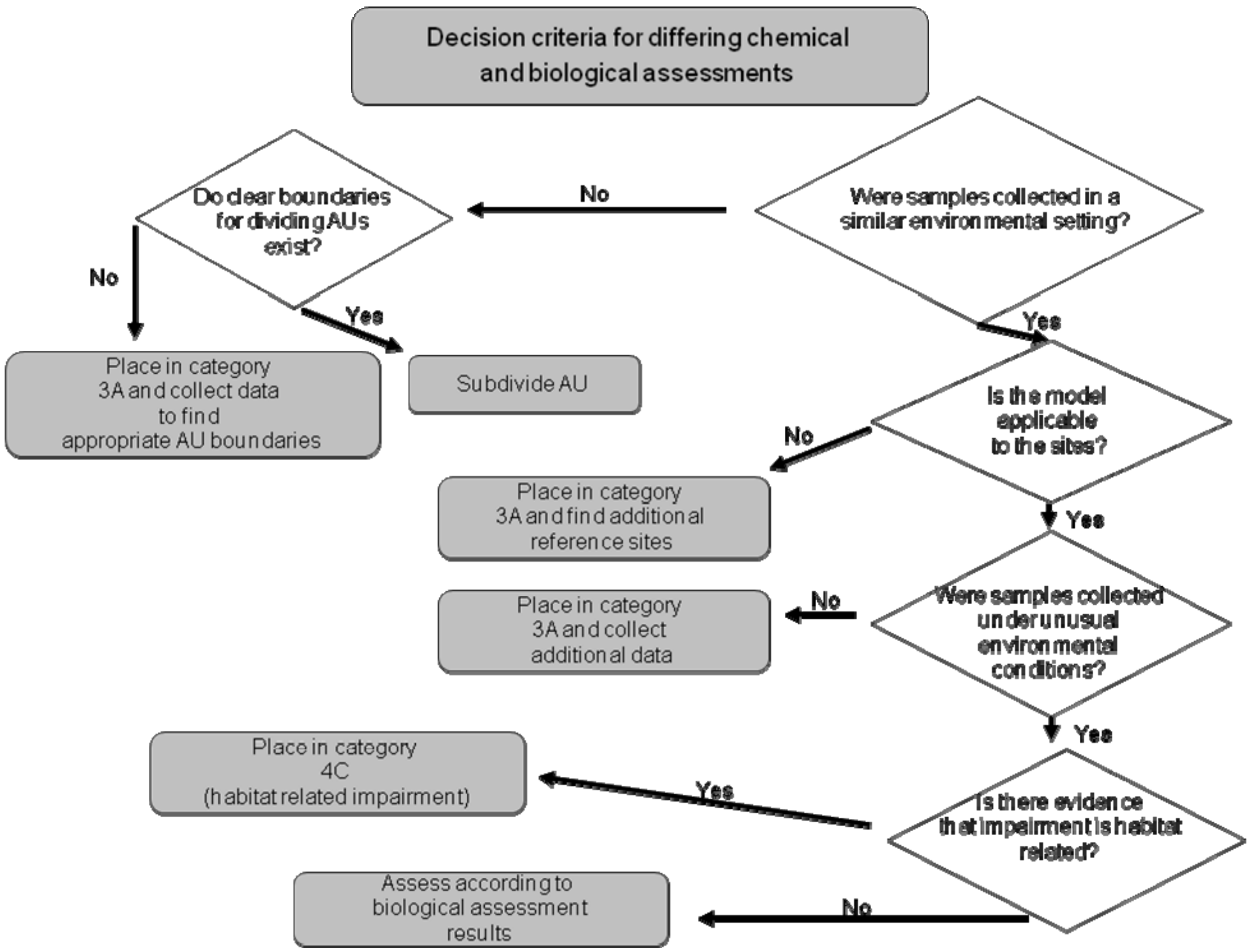


Figure F12-2. Summary of the decision criteria that is used for the Utah fall RIVPACS model when results of chemical and biological assessments differ.

RIVPACS models are built using predictor variables that are minimally affected by human disturbance and that are considered to be relatively invariant over ecologically relevant time (Tetra Tech, 2008; Wright et al., 1984; Hawkins et al., 2000; Wright, 2000; Utah State University, 2009). Variables that are typically used include those related to geographic position (i.e. latitude, longitude, elevation), watershed area, climate, and surficial geology (Utah State University, 2009). If alterable variables were used (i.e., nutrient concentrations, conductivity, forest cover), it would be difficult to discriminate the natural gradient from that caused by human activity, and confident prediction of an expected community in the absence of human disturbance for a test site would be impossible (Tetra Tech, 2008).

The development of RIVPACS models requires several steps: 1) group reference sites into clusters with similar biological assemblages; 2) examine how natural factors vary within the clusters of reference sites; 3) for each test site, use natural factors to predict the clusters in which the site would most likely be grouped; 4) the expected biological composition of the test site is predicted to be the same as that observed in the reference cluster (this is expressed as the capture probability for each taxon); and 5) compare the observed taxa list to the expected taxa list, as expressed by the O/E ratio (Yuan, 2006b). To elaborate further on the expected taxa list, it is conceptually a weighted average of taxa frequencies found across all reference sites, where the weights are the probability a site is in a particular group of reference sites; average taxa frequencies from reference sites that are physically very similar to a test site are weighted most (Tetra Tech, 2009). The expected taxa list can be set to different thresholds (e.g., to exclude rare taxa, the threshold can be set to 50%).

Utah DEQ uses a RIVPACS model for assessing wadeable streams. During model development, the random forests method was used to select predictor variables that best discriminated among the site groups (Breiman and Cutler, 2009) (NOTE: this is oftentimes accomplished using a discriminant model). A major benefit of using the random forests method is that the calculations are done in a way that prevents the model from being overfit. Another valuable feature is that it gives estimates of what variables are important in the classification, both overall and within each site group (Breiman and Cutler, 2009).

For this assessment, we explored how climate-related shifts in macroinvertebrate assemblages may affect Utah's predictive bioassessment approach. RIVPACS analyses were run using a number of different scenarios in which climate-related predictor variables were altered. Questions we examined include: How does site class (membership probability) shift with changes to the climate influenced variables? Are the climate-related predictor variables being changed "enough" to cause any shift in the site class (membership probability) and thus a change in E? Do the climate predictive variables have enough predictive power to change the O/E score? Which of the climate predictor variables are most important, both overall and among site groups?

F2. RIVPACS MODEL MANIPULATION ANALYSES

The fall model (and not the ‘all seasons’ model) was evaluated in this analysis, because the fall model is the one that Utah DEQ currently uses to assess wadeable stream sites. Jeff Ostermiller of Utah DEQ provided the R Code and data input files for the model; it is available upon request. The data input files contained information on the 88 reference sites that were used in the construction of the fall model. The files contain data on taxa, predictor variables and site groups (these are available upon request). **Table F2-1** contains a list of the predictor variables that are used in the model.

RUN 1. This approach examined model performance under different climate change scenarios. Associated questions were: how much do O, E and O/E values change in each of the different scenarios? Is the change in O/E greater than the natural variability among reference scores (which equals 0.13=1 Standard Deviation)? Which of the climate-related predictor variables were most important overall? Which predictor variables were most important in each of the different site groups? We included several different approaches for this analysis. In the first approach, we changed combinations of climate predictor variables while keeping the observed (O) values constant (i.e., we kept the biology, which was based on about 5 years of reference data, the same) and the probability of capture (Pc) limit at > 0.5 (this is the Pc value that Utah uses when running the model).

We used the NCAR[1] projections for the southwestern US for 2050 and 2090 as guidance for how much to alter the climate- predictor variables. We also ran two scenarios in which the freeze date and the temperature and precipitation variables (i.e., all the climate-related predictor variables) were altered simultaneously. Since we did not have information on how much freeze dates are likely to change, we used best professional judgment and long-term averages of freeze dates (minimum, average, maximum) from reporting stations that were closest to the 4 sites to estimate the numbers. There were 5 different ‘alteration scenarios’ that were used in the analyses. These are summarized in **Table F2-2**. Compared to annual climatic variations, the alteration increments may seem small, but they are realistic for purposes of this analysis because

[1] Regional Climate-Change Projections from Multi-Model Ensembles (RCPM) data and analysis were provided by the Institute for the Study of Society and Environment (ISSE) at the National Center for Atmospheric Research (NCAR), based on model data from the World Climate Research Programme's Coupled Model Intercomparison Project phase 3 (WCRP CMIP3) multi-model dataset. More information about the RCPM analysis can be found at http://rcpm.ucar.edu. © 2006 University Corporation for Atmospheric Research. All Rights Reserved."

the predictor variables that are used in the RIVPACS models are long-term averages (i.e. 1971-2000).

RUN 2. One limitation of the approach described above is that by only including taxa that have Pc >.5, we may be missing an important piece of the climate change picture, which is: what is happening to the rare taxa that are at the edges of their ranges? Are their distributions shifting, but the model is not detecting these changes because the Pc is set to > 0.5? To address this question, we re-ran the approach described above with the Pc set to > 0.1 to evaluate how things changed.

RUN 3. Another question of interest was how well the RIVPACS model would perform with only climate-related predictor variables. How much variation would climate variables alone explain? Which of the 7 climate-related predictor variables are the key drivers? To examine this, we ran the random forests method (Breiman and Cutler, 2009) with only the climate-related predictor variables and evaluated performance by calculating SD and RMSE of the reference site O/E scores.

Table F2-1. Predictor variables that are used in the fall model. Climate-related variables are in red italicized print

Predictor Variables	Description
MINWD.WS	Watershed average of the annual minimum of the predicted mean monthly number of days with measurable precipitation (days) derived from PRISM data. Each watershed grid cell calculated as MIN[Xi], where Xi = the predicted minimum mean number of days with me
BDH.AVE	Watershed mean high values of soil bulk density of soils types within the basin (grams per cubic centimeter) from State Soil Geographic (STATSGO) Database.
G.PH.STD	Predicted physical activity based on lithology from state geology maps and estimated physical weathering rates based on known rock hardness. Ordinal ranking from low activity (1, granitic, gneiss, limestone) to high activity (5, siltstone, shale).
AWCH.AVE	Watershed mean high values of available water capacity of soils (fraction) from State Soil Geographic (STATSGO) Database.
GPT.VOLC	Dummy Variable indicating dominant geology (1=yes; 2=N0)
ELEV.MAX	Maximum watershed elevation (meters) from National Elevation Dataset

135 **Table F2-1. Continued**

Predictor Variables	Description
FST32AVE	Watershed average of the mean day of year (1-365) of the first freeze derived from the PRISM data.
MEANP.PT	Annual mean of the predicted mean monthly precipitation (mm) derived from the PRISM datas for the sampling site. Calculated as sum Xi/12, where Xi = the predicted mean precipitation for month i (1-12) derived from 29 years of record (1961-1990).
SQ.KM	Watershed area in square kilometers.
TMEAN.WS	Watershed average of the annual mean of the predicted mean monthly air temperature (tenths of degree Celsius) derived from PRISM data. Each watershed grid cell calculated as sum Xi/12, where Xi = the predicted mean air temperature for month i (1-12) deriv
MINP.PT	Annual minimum of predicted mean monthly precipitation (mm) derived from the PRISM data for the sampling site. Calculated as MIN[Xi], where Xi = the predicted minimum mean precipitatioin for month i (1-12) derived from 29 years of record (1961-1990).
ELEV.WS	Mean watershed elevation (meters) from National Elevation Dataset.
SLOPE.GIS	Average slope calculated from GIS
LST32AVE	Watershed average of the mean day of year (1-365) of the last freeze derived from the PRISM data.
TMEANNET	Stream network average of the annual mean of the predicted mean monthly air temperature (tenths of degree Celsius) derived from PRISM data. Each stream network grid cell calculated as sum Xi/12, where Xi = the predicted mean air temperature for month i

Table F2-2. Descriptions of how the climate-related predictor variables were altered in each of the 5 runs of the RIVPACS model

Scenario	Category	Altered Predictor variables	Rationale
1	Baseline	None - used original values	Obtain baseline values
2	Temperature & Precipitation	TMEAN.WS + 2 & TMEAN.NET + 2 & MEANP.PT - .05	NCAR annual temperature and precipitation predictions (2050)
3		TMEAN.WS + 4 & TMEAN.NET + 4 & MEANP.PT - .1	NCAR annual temperature and precipitation predictions (2090)
4	All	LST32AVE-1, MINP.PT-1, MEANP.PT-1, TMEAN.NET+1, TMEAN.WS+1, FST32AVE+1, MINWD.WS-1	Best professional judgment
5		LST32AVE-2, MINP.PT-2, MEANP.PT-2, TMEAN.NET+2, TMEAN.WS+2, FST32AVE+2, MINWD.WS-1	Best professional judgment

F3. RESULTS - RIVPACS MODEL MANIPULATION ANALYSES

RUN 1. Overall, both mean O and mean E decreased in each of the scenarios, with E decreasing by a greater amount than O (**Tables F3-1** and **F3-2**). The greatest changes in O and E values (-0.70 and -1.12, respectively) occurred in Scenario ‘All 2.’ O/E scores increased by very small amounts in each scenario. The maximum change in the O/E score occurred in Scenario ‘All 2’ but this was only an increase of 0.03 from the baseline O/E score. The output of results from all sites is available upon request.

NOTE: We also ran some ‘extreme’ scenarios (i.e. doubling temperature, dividing precipitation values by two, changing freeze dates by 30 days, etc.) to satisfy our curiosity about how much alteration it would take in order to result in a substantial change to O/E scores. Even with these extremes, the O/E scores never varied by more than one standard deviation (0.13) and were therefore still within the realm of natural variability. Results are shown in **Attachment F1**.

The overall importance of the 15 predictor variables used in the Utah fall RIVPACS model was also evaluated. **Table F3-3** and **Figure F3-1** list the variables in order of highest overall variable importance to lowest (this is measured by Mean Decrease Accuracy, see Breiman and Cutler (2009) for more information). By importance, we are referring to how important the variable is in predicting the class correctly. The 5 most important overall variables are annual minimum of predicted mean monthly precipitation (MINP.PT), average slope calculated from GIS (SLOPE.GIS), watershed average of the mean day of year of the last freeze (LST32AVE), mean watershed elevation (ELEV.WS) and stream network average of the annual mean of the predicted mean monthly air temperature (TMEANNET). Six of the top ten most important variables are climate-related.

In addition to evaluating the overall dataset, results within the 8 different site groups were examined. O and E values did vary among the site groups (**Table F3-4**). For example, Site Group 4 had higher O and E values than the other groups and Site Group 6 had lower values. However, O/E scores were very similar across all site groups and if O/E scores changed, they only changed by small amounts. Differences from baseline O/E scores ranged from 0 to 0.10, with the greatest changes generally occurring in the ‘All 1’ and ‘All 2’ Scenarios (**Table F3-5**). Mean O/E scores in Site Group 4 changed the most, but were still within the range of natural variation (=1 StDev, 0.13). The 6 most important predictor variables in Site Group 4 are climate-related, which may be part of the reason for the bigger change (**Table F3-6**). However, this is not clear because the 3 most important predictor variables in Site Group 8 were climate-related, and Site Group 8 O/E values were the same as the baseline values in each scenario. The small sample size (5 sites) may be a contributing factor.

Table F3-1. Mean O, E and O/E values for each scenario

Scenario	O	E	O/E	Altered predictor variables
Baseline	***14.40***	***13.70***	***1.05***	***used original values***
Temp/Precip 1	14.36	13.56	1.06	TMEAN.WS + 2 & TMEAN.NET + 2 & MEANP.PT - .05
Temp/Precip 2	14.33	13.49	1.06	TMEAN.WS + 4 & TMEAN.NET + 4 & MEANP.PT - .1
All 1	13.83	12.80	1.07	LST32AVE-1, MINP.PT-1, MEANP.PT-1, TMEAN.NET+1, TMEAN.WS+1, FST32AVE+1, MINWD.WS-1
All 2	13.69	12.59	1.08	LST32AVE-2, MINP.PT-2, MEANP.PT-2, TMEAN.NET+2, TMEAN.WS+2, FST32AVE+2, MINWD.WS-1

Table F3-2. Mean difference between baseline O, E and O/E values and mean O, E and O/E values for each scenario

	Mean Difference from Baseline Values			
Scenario	**O**	**E**	**O/E**	**Altered predictor variables**
Temp/Precip 1	-0.03	-0.15	0.01	TMEAN.WS + 2 & TMEAN.NET + 2 & MEANP.PT - .05
Temp/Precip 2	-0.07	-0.21	0.01	TMEAN.WS + 4 & TMEAN.NET + 4 & MEANP.PT - .1
All 1	-0.57	-0.91	0.03	LST32AVE-1, MINP.PT-1, MEANP.PT-1, TMEAN.NET+1, TMEAN.WS+1, FST32AVE+1, MINWD.WS-1
All 2	-0.70	-1.12	0.03	LST32AVE-2, MINP.PT-2, MEANP.PT-2, TMEAN.NET+2, TMEAN.WS+2, FST32AVE+2, MINWD.WS-1

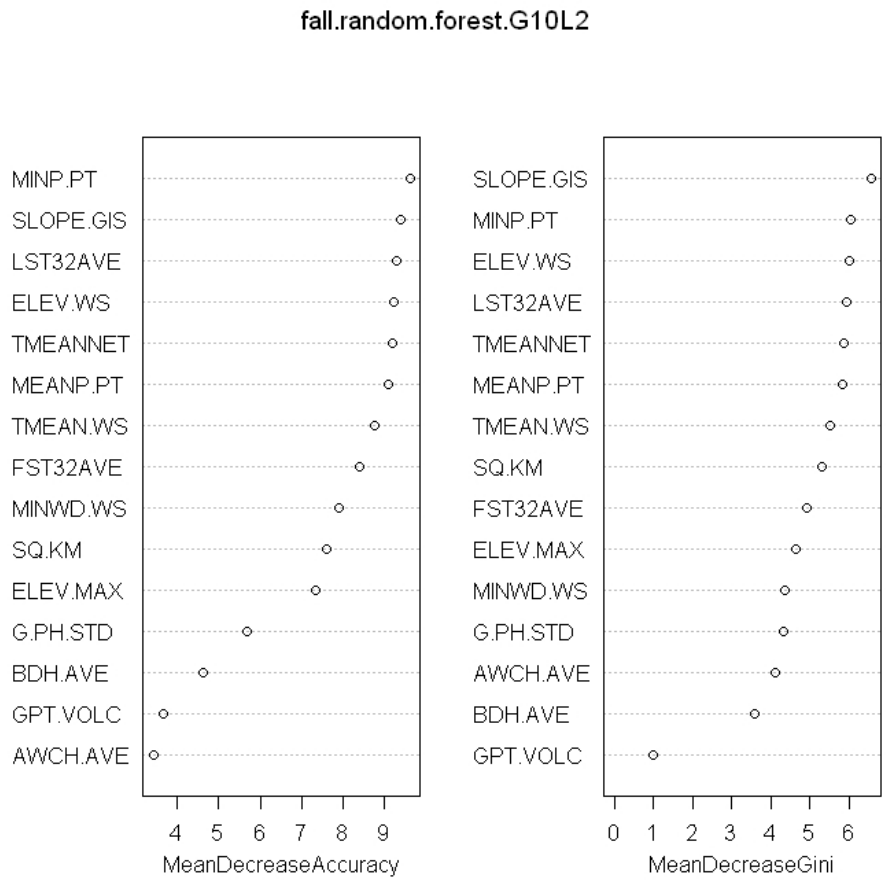


Figure F3-1. **Plot summarizing importance of the predictor variables (more important variables have higher Mean Decrease Accuracy and Mean Decrease Gini scores). The following R code command was used to obtain this table: varImpPlot(fall.random.forest.G10L2).**

Table F3-3. Predictor variables are listed in order of highest overall variable importance to lowest (as measured by Mean Decrease Accuracy). The importance of the predictor variables varies among site groups. The values under each site group give the importance of the variable for predicting that class correctly. The Mean Decrease Gini calculation is performed by adding up the Gini decreases for each individual variable over all trees in the forest. This gives a fast variable importance that is often very consistent with the permutation importance measure. The following R code command was used to obtain this table: importance(fall.random.forest.G10L2).

	Site Group									
Predictor Variable	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean Decrease Accuracy**	**Mean Decrease Gini**
MINP.PT	4.9	2.5	5.7	31.5	11.0	8.0	22.0	28.2	9.62	6.04
SLOPE.GIS	2.3	0.1	8.9	14.3	20.6	19.1	23.2	8.8	9.39	6.55
LST32AVE	8.9	11.7	2.1	33.6	3.7	4.8	18.0	27.9	9.28	5.95
ELEV.WS	6.3	8.7	6.7	16.1	10.1	4.7	27.7	29.1	9.24	5.99
TMEANNET	5.1	5.8	2.2	25.1	6.4	5.5	22.7	33.3	9.18	5.86
MEANP.PT	1.1	9.9	2.9	21.3	14.3	21.8	14.9	26.5	9.08	5.82
TMEAN.WS	4.0	7.4	2.7	21.8	7.4	1.2	22.7	33.1	8.75	5.50
FST32AVE	7.9	9.9	1.7	13.6	0.6	3.0	28.6	33.0	8.39	4.90
MINWD.WS	3.6	0.2	-0.9	25.9	4.7	0.5	24.2	23.8	7.89	4.36
SQ.KM	5.6	-2.7	6.2	9.6	11.5	18.6	16.1	21.5	7.60	5.30
ELEV.MAX	4.9	7.8	-1.4	18.3	7.9	7.9	4.4	25.2	7.34	4.65
G.PH.STD	1.5	-0.5	9.0	11.4	10.8	5.8	3.2	-1.8	5.68	4.30
BDH.AVE	2.3	13.3	-0.4	7.7	-2.0	3.9	5.3	21.0	4.62	3.57
GPT.VOLC	10.8	5.0	-3.4	4.9	-3.1	7.5	5.7	4.8	3.67	1.01
AWCH.AVE	-2.4	16.0	0.1	5.2	3.2	8.8	-3.7	8.0	3.43	4.12

Table F3-4. For each scenario and each site group, O, E and O/E values were calculated. N=number of sites in the dataset that are assigned each site group. See Tables F3-1 or F3-2 for descriptions on how variables were altered in the Temp/Precip 1, Temp/Precip 2, All 1 and All 2 scenarios.

		Baseline			Temp/ Precip 1			Temp/Precip 2			All 1			All 2		
Site Group	**N**	**O**	**E**	**O/E**	**O**	**E**	**O/E**	**O**	**E**	**O/E**	**O**	**E**	**O/E**	**O**	**E**	**O/E**
1	16	**15.1**	**14.2**	**1.1**	15.2	14.2	1.1	15.1	14.1	1.1	14.5	13.2	1.1	14.5	13.0	1.1
2	10	**15.9**	**14.8**	**1.1**	16.0	14.8	1.1	16.0	14.7	1.1	15.2	13.7	1.1	15.1	13.5	1.1
3	16	**17.2**	**16.3**	**1.1**	16.9	15.9	1.1	16.9	15.9	1.1	16.4	14.9	1.1	15.8	14.4	1.1
4	7	**24.3**	**23.2**	**1.0**	24.1	22.7	1.1	24.1	22.4	1.1	22.6	20.0	1.1	22.6	19.7	1.1
5	19	**12.0**	**11.7**	**1.0**	12.0	11.5	1.0	12.1	11.6	1.0	11.6	11.3	1.0	11.6	11.3	1.0
6	9	**8.3**	**8.1**	**1.0**	8.3	8.1	1.0	8.0	7.9	1.0	8.0	7.8	1.0	7.8	7.7	1.0
7	6	**10.2**	**9.7**	**1.1**	10.2	9.6	1.1	10.2	9.6	1.1	10.5	9.8	1.1	10.5	9.7	1.1
8	5	**11.4**	**11.0**	**1.0**	11.4	11.1	1.0	11.4	11.1	1.0	11.4	11.1	1.0	11.4	11.1	1.0

Table F3-5. **For each scenario and each site group, differences between mean O/E values and mean baseline O/E values were calculated. N=number of sites in the dataset that are assigned each site group. See Tables F3-1 or F3-2 for descriptions on how variables were altered in the Temp/Precip 1, Temp/Precip 2, All 1 and All 2 scenarios.**

			Mean Difference from Baseline O/E			
Site Group	**N**	**Baseline**	**Temp/ Precip 1**	**Temp/ Precip 2**	**All 1**	**All 2**
1	16	**1.06**	0.01	0.01	0.03	0.05
2	10	**1.07**	0.01	0.02	0.03	0.05
3	16	**1.06**	0.01	0.01	0.04	0.04
4	7	**1.05**	0.02	0.03	0.08	0.10
5	19	**1.03**	0.01	0.01	0.00	0.00
6	9	**1.03**	0.00	-0.03	0.00	-0.02
7	6	**1.05**	0.00	0.00	0.02	0.03
8	5	**1.03**	0.00	0.00	0.00	0.00

Table F3-6. Predictor variables are listed in order of highest variable importance to lowest within each site group (this refers to the importance of the variable for predicting class correctly). These results are derived from the Baseline scenario. Variable importance was also evaluated for the other scenarios, but it was determined that results (at least for the 5 most important variables in each site group) either did not change or varied only slightly and are therefore not reported.

Site Group								Overall
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
GPT.VOLC	AWCH.AVE	G.PH.STD	**LST32AVE**	SLOPE.GIS	**MEANP.PT**	**FST32AVE**	**TMEANNET**	**MINP.PT**
LST32AVE	BDH.AVE	SLOPE.GIS	**MINP.PT**	**MEANP.PT**	SLOPE.GIS	ELEV.WS	**TMEAN.WS**	SLOPE.GIS
FST32AVE	**LST32AVE**	ELEV.WS	**MINWD.WS**	SQ.KM	SQ.KM	**MINWD.WS**	**FST32AVE**	**LST32AVE**
ELEV.WS	**MEANP.PT**	SQ.KM	**TMEANNET**	**MINP.PT**	AWCH.AVE	SLOPE.GIS	ELEV.WS	ELEV.WS
SQ.KM	**FST32AVE**	**MINP.PT**	**TMEAN.WS**	G.PH.STD	**MINP.PT**	**TMEAN.WS**	**MINP.PT**	**TMEANNET**
TMEANNET	ELEV.WS	**MEANP.PT**	**MEANP.PT**	ELEV.WS	ELEV.MAX	**TMEANNET**	**LST32AVE**	**MEANP.PT**
ELEV.MAX	ELEV.MAX	**TMEAN.WS**	ELEV.MAX	ELEV.MAX	GPT.VOLC	**MINP.PT**	**MEANP.PT**	**TMEAN.WS**
MINP.PT	**TMEAN.WS**	**TMEANNET**	ELEV.WS	**TMEAN.WS**	G.PH.STD	**LST32AVE**	ELEV.MAX	**FST32AVE**
TMEAN.WS	**TMEANNET**	**LST32AVE**	SLOPE.GIS	**TMEANNET**	**TMEANNET**	SQ.KM	**MINWD.WS**	**MINWD.WS**
MINWD.WS	GPT.VOLC	**FST32AVE**	**FST32AVE**	**MINWD.WS**	**LST32AVE**	**MEANP.PT**	SQ.KM	SQ.KM
SLOPE.GIS	**MINP.PT**	AWCH.AVE	G.PH.STD	**LST32AVE**	ELEV.WS	GPT.VOLC	BDH.AVE	ELEV.MAX
BDH.AVE	**MINWD.WS**	BDH.AVE	SQ.KM	AWCH.AVE	BDH.AVE	BDH.AVE	SLOPE.GIS	G.PH.STD
G.PH.STD	SLOPE.GIS	**MINWD.WS**	BDH.AVE	**FST32AVE**	**FST32AVE**	ELEV.MAX	AWCH.AVE	BDH.AVE
MEANP.PT	G.PH.STD	ELEV.MAX	AWCH.AVE	BDH.AVE	**TMEAN.WS**	G.PH.STD	GPT.VOLC	GPT.VOLC
AWCH.AVE	SQ.KM	GPT.VOLC	GPT.VOLC	GPT.VOLC	**MINWD.WS**	AWCH.AVE	G.PH.STD	AWCH.AVE

RUN 2. Normally the Utah fall RIVPACS model is run with the probability of capture (Pc) limit set at > 0.5. To evaluate model performance when rare taxa are included (i.e. taxa that occur at the edges of their ranges and are likely to be more sensitive than others to climate change), the model was run with the Pc set to > 0.1. This model did not perform as well. The standard deviation of O/E scores for the Pc > 0.1 run was 0.18 versus 0.13 for the Pc > 0.5 run (**Table F3-7)**. The mean O/E score for the Pc > 0.1 run was slightly lower (a difference of 0.02). When the two sets of O/E values are fitted with a linear regression line, the r^2 value = 0.51 (**Figure** F**3-2**).

When mean O, E and O/E values are further compared among the Pc > 0.1 and Pc > 0.5 datasets, as expected, the mean O and mean E values in the Pc > 0.1 dataset are higher (by about 10 taxa) than those in the Pc > 0.5 but differences in mean O/E values are very small, ranging from 0.02 to 0.03 (**Tables F3-8 and F3-9**). The alteration of the climate-related predictor variables had little if any effect on mean O and mean E values in the Pc > 0.1 dataset, as well as on O/E values. Only the 'All1' and 'All 2' scenarios resulted in changes to the O/E values, and they only increased by 0.02. A comparison of mean differences from baseline values shows that the alteration of predictor variables generally caused a greater change in mean O, E and O/E values in the Pc > 0.5 dataset. Mean E values changed the most in both datasets; the maximum change in mean E values was -1.12 in the Pc > 0.5 dataset, whereas in the Pc > 0.1 dataset, the maximum change was -0.55. This occurred in the 'All 2' Scenario.

Table F3-7. Comparison of the mean O/E scores and standard deviations that were derived from the original Pc > 0.5 model versus the Pc > 0.1 model

Model	Mean O/E	St Dev
Pc > 0.1	1.03	0.18
Pc > 0.5	1.05	0.13

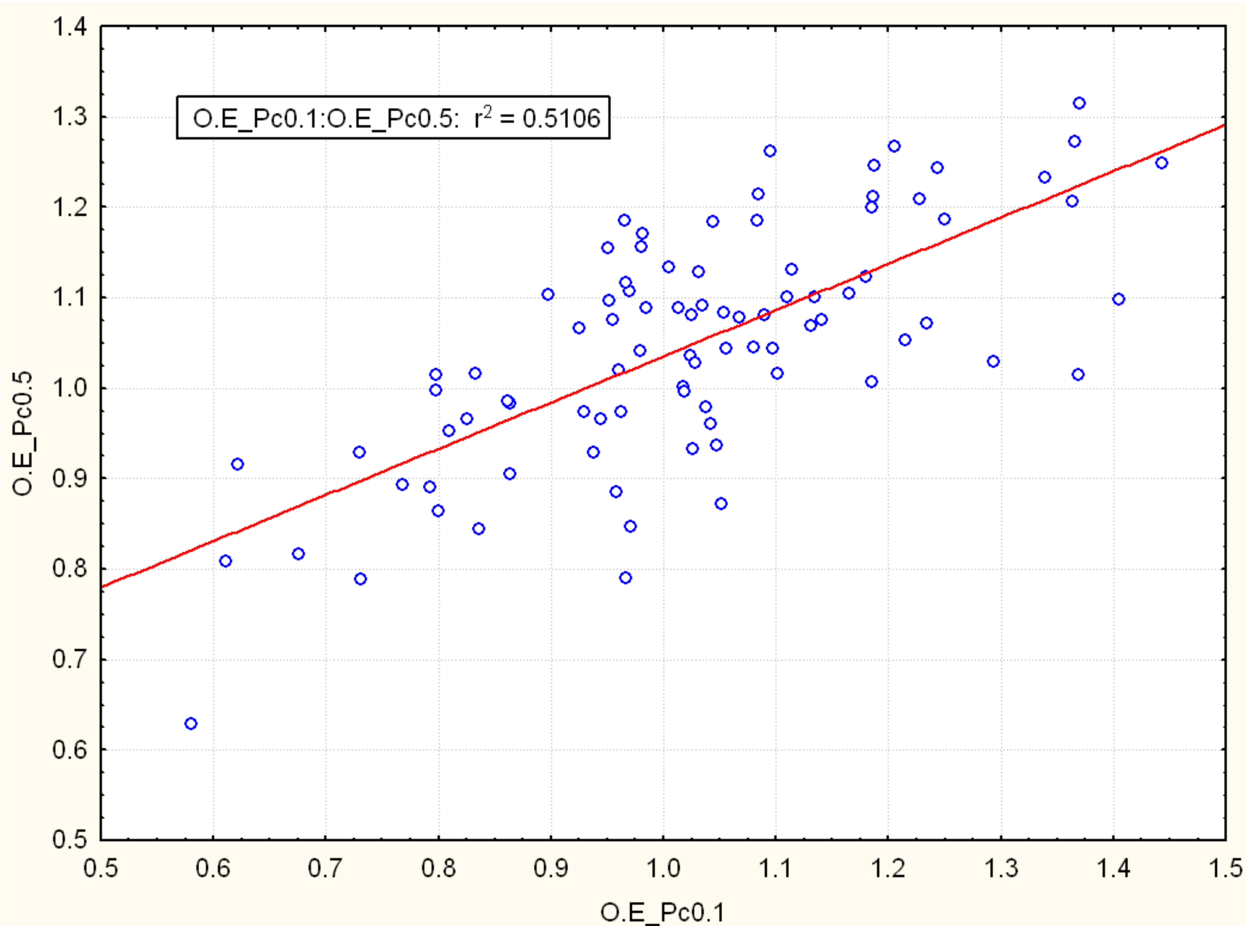


Figure F3-2. Plot of O/E scores when the probability of capture (Pc) limit is set at > 0.5 versus O/E scores when the probability of capture (Pc) limit is set at > 0.1.

Table F3-8. Mean O, E and O/E values for each scenario for both the Pc > 0.1 and Pc > 0.5 runs

	Pc > 0.1			Pc > 0.5			
Scenario	**O**	**E**	**O/E**	**O**	**E**	**O/E**	**Altered predictor variables**
Baseline	***23.4***	***22.7***	***1.03***	***14.4***	***13.7***	***1.05***	***used original values***
Temp/Precip 1	23.4	22.7	1.03	14.4	13.6	1.06	TMEAN.WS + 2 & TMEAN.NET + 2 & MEANP.PT - .05
Temp/Precip 2	23.4	22.6	1.03	14.3	13.5	1.06	TMEAN.WS + 4 & TMEAN.NET + 4 & MEANP.PT - .1
All 1	23.4	22.3	1.05	13.8	12.8	1.07	LST32AVE-1, MINP.PT-1, MEANP.PT-1, TMEAN.NET+1, TMEAN.WS+1, FST32AVE+1, MINWD.WS-1
All 2	23.4	22.2	1.05	13.7	12.6	1.08	LST32AVE-2, MINP.PT-2, MEANP.PT-2, TMEAN.NET+2, TMEAN.WS+2, FST32AVE+2, MINWD.WS-1

Table F3-9. Mean differences between baseline O, E and O/E values and mean O, E and O/E values for each scenario for both the Pc > 0.1 and Pc > 0.5 runs

	Mean Differences from Baseline Values						
	Pc > 0.1			**Pc > 0.5**			
Scenario	**O**	**E**	**O/E**	**O**	**E**	**O/E**	**Altered predictor variables**
Temp/Precip 1	0.01	-0.03	0.00	-0.03	-0.15	0.01	TMEAN.WS + 2 & TMEAN.NET + 2 & MEANP.PT - .05
Temp/Precip 2	0.02	-0.09	0.00	-0.07	-0.21	0.01	TMEAN.WS + 4 & TMEAN.NET + 4 & MEANP.PT - .1
All 1	0.06	-0.45	0.02	-0.57	-0.91	0.03	LST32AVE-1, MINP.PT-1, MEANP.PT-1, TMEAN.NET+1, TMEAN.WS+1, FST32AVE+1, MINWD.WS-1
All 2	-0.03	-0.55	0.02	-0.70	-1.12	0.03	LST32AVE-2, MINP.PT-2, MEANP.PT-2, TMEAN.NET+2, TMEAN.WS+2, FST32AVE+2, MINWD.WS-1

RUN 3. The performance of the RIVPACS model with only the climate-related predictor variables was evaluated and compared to original model results. Model performance was evaluated by looking at the standard deviations of the reference site O/E scores. When limited to climate-related predictor variables only, the model performs well. The standard deviation of the O/E scores using the original model is 0.13 versus 0.14 with the climate-related variables only model (**Table F3-10**). Also, when the two sets of O/E values are plotted against one another, there is a tight fit ($r^2 = 0.91$) (**Figure F3-3**). The most important predictor variables are annual minimum of predicted mean monthly precipitation (MINP.PT) and watershed average of the mean day of year of the last freeze (LST32AVE) (**Table F3-11**).

Table F3-10. Comparison of the mean O/E scores and standard deviations that were derived from the original model versus the model with only the climate-related variables

Model	Mean O/E	St Dev
Original	1.05	0.13
Climate-related Only	1.05	0.14

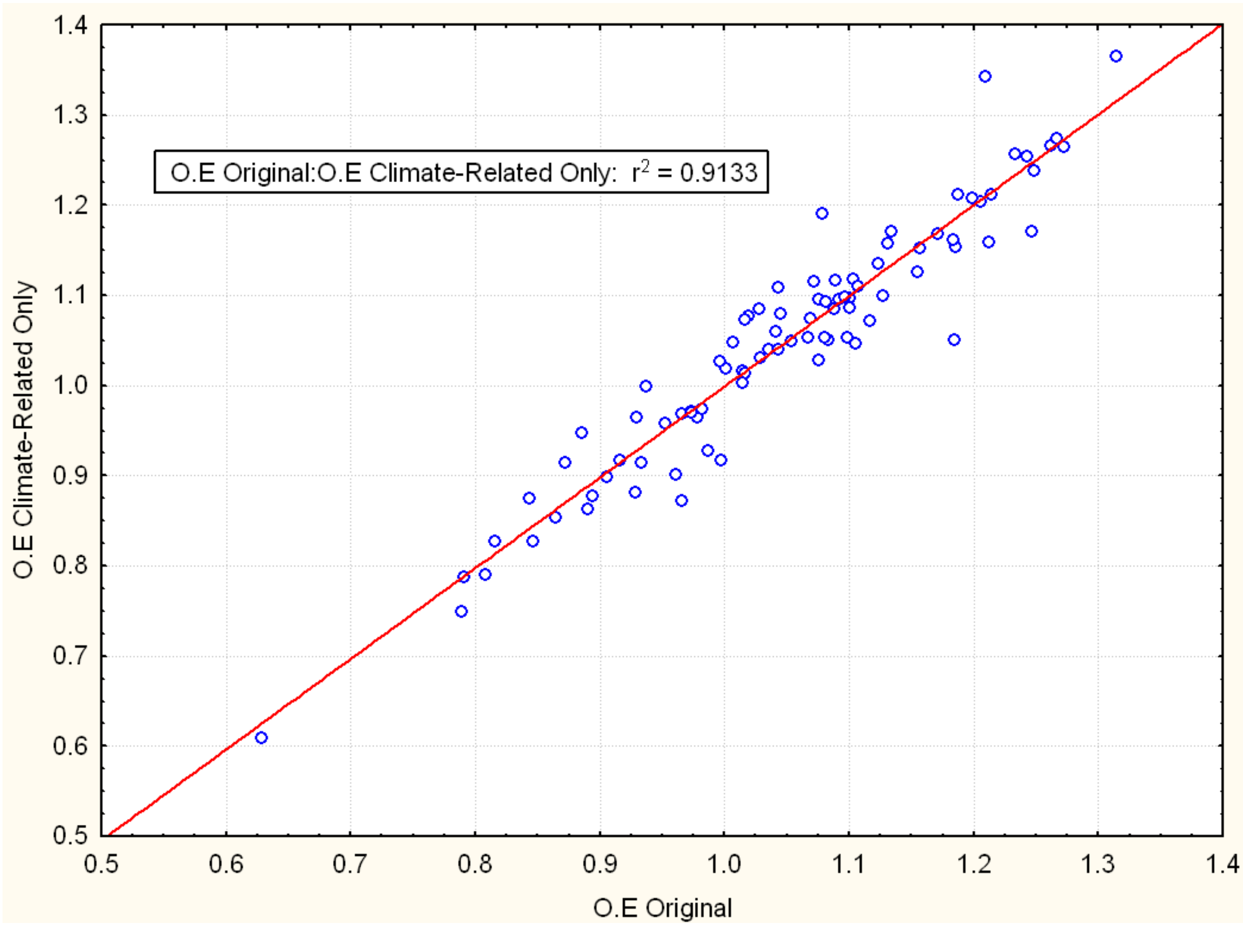


Figure F3-3. Plot of O/E scores from the original model versus O/E scores from the climate-related predictor variables only model.

Table F3-11. Predictor variables are listed in order of highest overall variable importance to lowest (as measured by Mean Decrease Accuracy). The importance of the predictor variables varies among site groups. The values under each site group give the importance of the variable for predicting that class correctly. The Mean Decrease Gini calculation is performed by adding up the Gini decreases for each individual variable over all trees in the forest. This gives a fast variable importance that is often very consistent with the permutation importance measure. The following R code command was used to obtain this table: importance(fall.random.forest.G10L2).

	Site Group									
Predictor Variable	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean Decrease Accuracy**	**Mean Decrease Gini**
MINP.PT	8.17	2.34	6.69	36.10	18.63	14.73	32.57	35.95	12.31	11.53
LST32AVE	12.88	15.40	0.94	50.80	6.89	3.03	18.58	32.54	12.07	11.89
TMEANNET	6.32	8.75	2.83	27.01	7.52	0.84	24.11	42.99	10.43	10.37
MEANP.PT	-2.79	9.85	2.45	25.92	18.09	26.67	14.86	33.50	10.30	11.54
TMEAN.WS	5.68	8.18	3.27	20.70	11.82	-2.56	24.17	41.54	10.24	9.96
FST32AVE	9.68	11.30	6.41	11.07	-2.89	-3.85	39.83	44.08	10.22	10.74
MINWD.WS	5.09	-2.36	0.78	27.29	4.33	10.02	31.69	28.09	9.22	7.89

SUMMARY OF RIVPACS MODEL MANIPULATION RESULTS

- Overall, altering the climate-related predictor variables had very little effect on O/E values. The greatest change occurred in the ‘All 2’ scenario but this only amounted to a change of 0.03, which is within the realm of natural variability (-0.13, 1 st dev) (see main report section 3.4.2 for discussion on possible reasons for the small change).
- There was also little effect on O/E values when ‘unrealistic’ changes were made to climate-related variables (i.e. doubling temperature, halving precipitation variables). O/E values never varied by more than one standard deviation (see main report section 3.4.2 for discussion on possible reasons for the small change).
- In the Utah fall RIVPACS model, the (overall) most important climate-related variables are annual minimum of predicted mean monthly precipitation (MINP.PT), watershed average of the mean day of year of the last freeze (LST32AVE) and stream network average of the annual mean of the predicted mean monthly air temperature (TMEANNET). Six of the top ten most important variables are climate-related.
- When O/E values were evaluated within the 8 different site groups, scores were very similar across all site groups and if O/E scores changed, they only changed by small amounts. Mean O/E scores in Site Group 4 changed the most, but were still within the range of natural variation. The 6 most important predictor variables in Site Group 4 are climate-related, which may be part of the reason for the bigger change within this site group.
- When the probability of capture (Pc) limit was changed from Pc > 0.5 to > 0.1, the Utah fall RIVPACS model did not perform as well (st dev of 0.18 versus 0.13). The alteration of the climate-related predictor variables had little if any effect on O/E values. (in fact, alteration of climate-related variables generally had less of an effect in the Pc < 0.1 run). Only the ‘All1’ and ‘All 2’ Scenarios resulted in changes to the O/E values, and they only increased by 0.02.
- When run with climate-related predictor variables only, the Utah RIVPACS model performed very well. The standard deviation of the O/E scores using the original model is 0.13 versus 0.14 with the climate-related variables only model. When the two sets of O/E values are plotted against one another, there is a tight fit ($r^2 = 0.91$).
- See Section 3 of the report for discussion of possible explanations on why the RIVPACS model appears to be insensitive to climate change effect, at least based on results from our analyses. To briefly summarize, the long-term averages of the climate predictor variables in the model capture major spatial differences between the regions (classes), which at this point are probably bigger than long-term climate change difference are.

F4. UTAH ECOREGION DESCRIPTIONS

Wasatch and Uinta Mountains. This ecoregion is composed of a core area of high, precipitous mountains with narrow crests and valleys flanked in some areas by dissected plateaus and open high mountains. The elevational banding pattern of vegetation is similar to that of the Southern Rockies except that aspen, chaparral, and juniper-pinyon and oak are more common at middle elevations. This characteristic, along with a far lesser extent of lodgepole pine and greater use of the region for grazing livestock in the summer months, distinguish the Wasatch and Uinta Mountains ecoregion from the more northerly Middle Rockies (US EPA 2002).

Colorado Plateaus. Rugged tableland topography is typical of the Colorado Plateau ecoregion. Precipitous side-walls mark abrupt changes in local relief, often from 300 to 600 meters. The region is more elevated than the Wyoming Basin to the north and therefore contains a far greater extent of pinyon-juniper woodlands. However, the region also has large low lying areas containing saltbrush-greasewood (typical of hotter drier areas), which are generally not found in the higher Arizona/New Mexico Plateau to the south where grasslands are common (US EPA 2002).

Tables F4-1 and F4-2 summarize distribution and abundance information for the Utah temperature indicator taxa at the 4 sites (Stations 4927250, 4951200, 4936750 and 5940440) and 3 site groups that were analyzed for long-term trends. Ephemerella seems to be the strongest indicator because it occurred at all the sites and generally had higher mean relative abundances than the other taxa. Chelifera, Chloroperlidae, Cinygmula, Lepidostoma and Rhitrogena also occurred at all the sites, although generally in lower abundances. Overall, the cold-water taxa are well-represented at most of the sites and site groups. Station 5940440 has the least number of cold-water taxa, but nevertheless has moderate abundances of Chloroperlidae and Rhitrogena. Leptohyphidae appears to be the strongest indicator among the warm-water taxa[2] because it occurred at 5 sites and generally had higher mean relative abundances than the other taxa. The next strongest warm-water indicators appear to be Oecetis and Cheumatopsyche, which are present at 6 sites but occurred in lower abundances.

Table F4-1. Summary of distribution and abundance information for the *cold-water* temperature indicator taxa at the 4 sites (Stations 4927250, 4951200, 4936750 and 5940440) and 3 site groups (WU_SF=Wasatch and Uinta Semi-arid Foothills, WU_ ME= Wasatch and Uinta Mid-elevation Mountains, CP=Colorado Plateaus). #Sites refers to the number of sites or site groups at which the taxa occurs. A=absent. P=present (highlighted in grey). Relative abundance codes: L=low (<0.01), M=medium (0.01-0.1), H=high (>0.1) (M or H are in bold type). Guide to interpretation: P-1L = present, occurred during 1 year, low relative abundance (RA), P-11M = present, occurred during 11 years, medium RA, etc.

FinalID	#Sites	4927250	4936750	4951200	5940440	WU_SF	WU_ME	CP
Ameletus	5	A	P-1L	P-1L	A	P-3L	P-9L	P-3L
Anagapetus	0	A	A	A	A	A	A	A
Apatania	3	A	**P-11M**	P-2L	A	A	P-2L	A
Bezzia	6	P-2L	P-2L	P-4L	A	P-11L	P-6L	P-5L
Bibiocephala	1	A	A	A	A	A	P-2L	A
Capniidae	6	P-1L	A	P-1L	P-2L	P-9L	**P-10M**	P-5L
Chelifera	7	P-6L	P-6L	P-2L	P-1L	**P-11M**	P-8L	P-3L
Chloroperlidae	7	P-6L	P-10L	P-1L	**P-9M**	**P-19M**	**P-12M**	P-7L
Cinygma	1	A	A	P-2L	A	A	A	A

[2] There are noticeably fewer warm-water indicator taxa in Utah (when compared to cold-water taxa and also when compared to warm-water indicator taxa lists in Maine and North Carolina). The most likely explanation appears to be the fact that our OTU caused Chironomidae to be grouped to the family-level (Number of warm-water taxa that are in the Chironomidae family in Maine=9 and in North Carolina=5). There may be several other contributing factors as well.

Cinygmula	7	P-2L	P-4L	P-5L	P-1L	**P-14M**	**P-10M**	**P-8M**
Cultus	6	P-5L	P-1L	A	P-1L	P-8L	P-6L	P-3L
Dicranota	5	A	P-1L	P-3L	A	P-11L	P-5L	P-5L
Ecclisomyia	0	A	A	A	A	A	A	A
Ephemerella	7	**P-13M**	**P-10M**	**P-11M**	P-2L	**P-16M**	**P-10M**	**P-6M**
Glutops	1	A	P-1L	A	A	A	A	A
Heterlimnius	0	A	A	A	A	A	A	A
Ironodes	0	A	A	A	A	A	A	A
Kogotus	1	A	A	A	A	P-1L	A	A
Lepidostoma	7	P-8L	**P-8M**	P-2L	P-6L	P-11L	**P-8M**	**P-4M**
Leuctridae	3	A	A	A	A	P-7L	P-6L	P-2L
Megarcys	2	A	A	A	A	P-1L	P-2L	A
Nematoda	6	P-7L	**P-9M**	**P-8M**	A	**P-13M**	P-9L	P-7L
Neothremma	4	A	P-2L	A	A	P-4L	**P-8M**	P-3L
Oligophlebodes	3	A	**P-1M**	A	A	A	**P-5M**	P-2L
Oreogeton	2	A	A	A	A	P-2L	P-1L	A

Table F4-1. Continued

FinalID	#Sites	4927250	4936750	4951200	5940440	WU_SF	WU_ME	CP
Parapsyche	2	A	A	A	A	P-1L	P-2L	A
Pericoma	6	P-1L	P-1L	P-3L	A	**P-15M**	P-6L	P-3L
Rhabdomastix	0	A	A	A	A	A	A	A
Rhithrogena	7	P-5L	**P-6M**	P-2L	**P-9M**	**P-13M**	P-8L	P-6L
Taenionema	5	P-1L	A	**P-5M**	A	P-3L	P-3L	P-2L
Visoka	0	A	A	A	A	A	A	A
Wiedemannia	3	A	A	P-1L	P-1L	P-1L	A	A
Yoraperla	0	A	A	A	A	A	A	A

Table F4-2. Summary of distribution and abundance information for the *warm-water* temperature indicator taxa at the 4 sites (Stations 4927250, 4951200, 4936750 and 5940440) and 3 site groups (WU_SF=Wasatch and Uinta Semi-arid Foothills, WU_ ME= Wasatch and Uinta Mid-elevation Mountains, CP=Colorado Plateaus). #Sites refers to the number of sites or site groups at which the taxa occurs. A=absent. P=present (highlighted in grey). Relative abundance codes: L=low (<0.01), M=medium (0.01-0.1), H=high (>0.1) (M or H are in bold type). Guide to interpretation: P-1L = present, occurred during1 year, low relative abundance (RA), P-11M = present, occurred during 11 years, medium RA, etc.

FinalID	#Sites	4927250	4936750	4951200	5940440	WU_SF	WU_ME	CP
Ambrysus	2	A	A	P-1L	A	A	A	P-4L
Asellidae	3	P-1L	A	P-1L	A	P-1L	A	A
Caenis	1	A	A	A	A	P-1L	A	A
Calineuria	1	A	A	A	A	A	P-1L	A
Caloparyphus	2	A	A	P-2L	A	P-3L	A	A
Cheumatopsyche	6	P-4L	P-3L	P-3L	A	P-2L	P-1L	P-1L
Coenagrionidae	3	A	A	P-3L	A	P-1L	A	P-4L
Leptohyphidae	5	P-9L	P-3L	**P-15H**	A	P-8L	A	**P-6M**
Maruina	2	A	A	A	A	P-1L	A	P-1L
Microcylloepus	2	A	A	**P-4M**	A	A	A	**P-2M**
Nectopsyche	0	A	A	A	A	A	A	A
Ochrotrichia	3	P-1L	A	P-1L	A	P-1L	A	A
Oecetis	6	P-7L	P-1L	P-1L	A	P-9L	P-2L	P-1L
Ordobrevia	0	A	A	A	A	A	A	A
Psephenus	0	A	A	A	A	A	A	A
Tinodes	1	P-5L	A	A	A	A	A	A

F5. NMDS Ordination and ANOVA analyses

Results from the NMDS ordinations show that hottest year samples at Stations 4927250 (Weber) and 4951200 (Virgin) form distinct clusters when grouped by hot/cold/normal years, and coldest and normal samples are generally mixed together (Figures 2-17 and 2-18, respectively, in main body of report). The following environmental variables were most strongly correlated with Axes 1 and 2, which are the axes that explained the most variance: PRISM mean annual air temperature from the year the biological sample was collected; PRISM mean annual air temperature from the previous year; absolute difference between collection year and previous year PRISM mean annual precipitation. It should be noted that the hottest years occurred sequentially (2000-2005). When grouped by precipitation category, samples at Stations 4927250 (Weber) and 4951200 (Virgin) do not form distinct clusters (Figures F5-1 and F5-2, respectively).

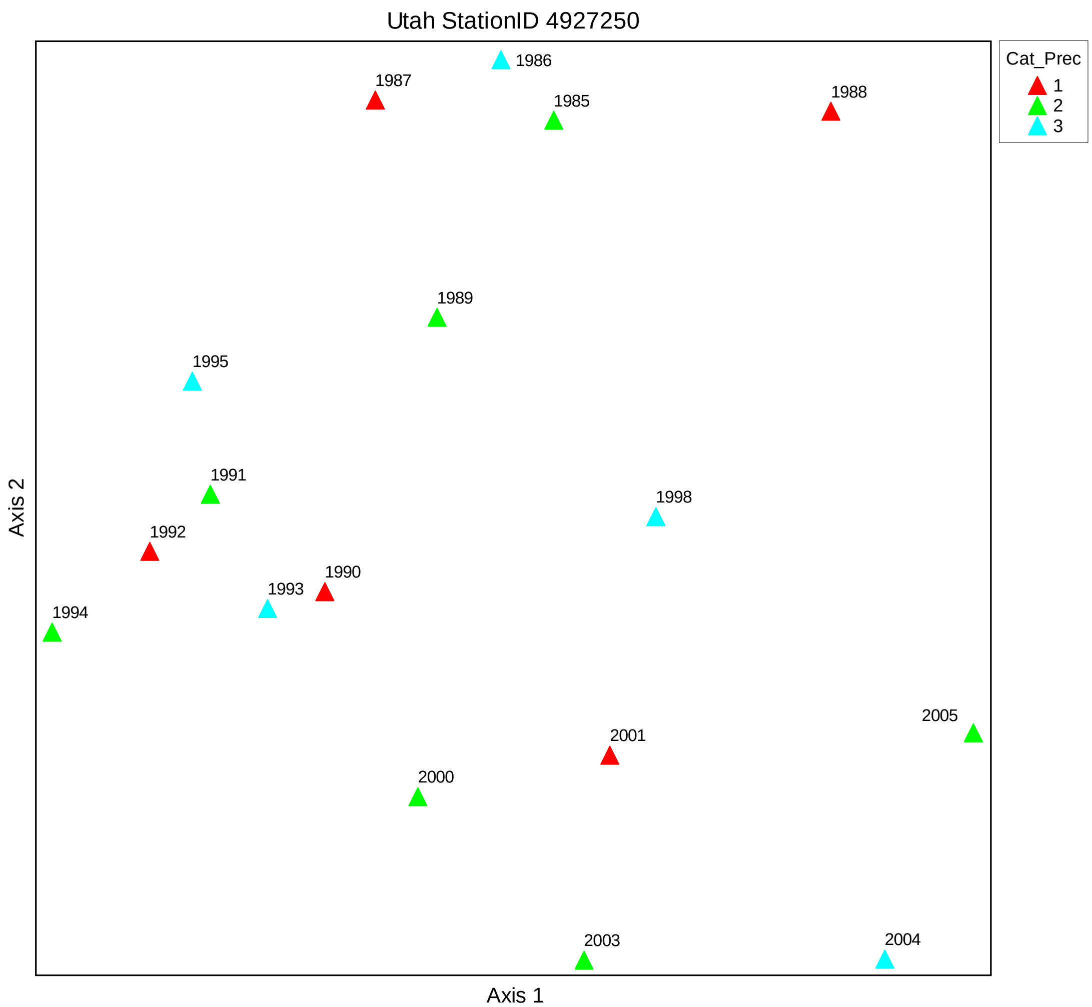


Figure F5-1. NMDS plot (Axis 1-2). Cat_Prec refers to the precipitation categories, which are: 1=dry years; 2=normal years; 3=wet years. Samples are labeled by collection year.

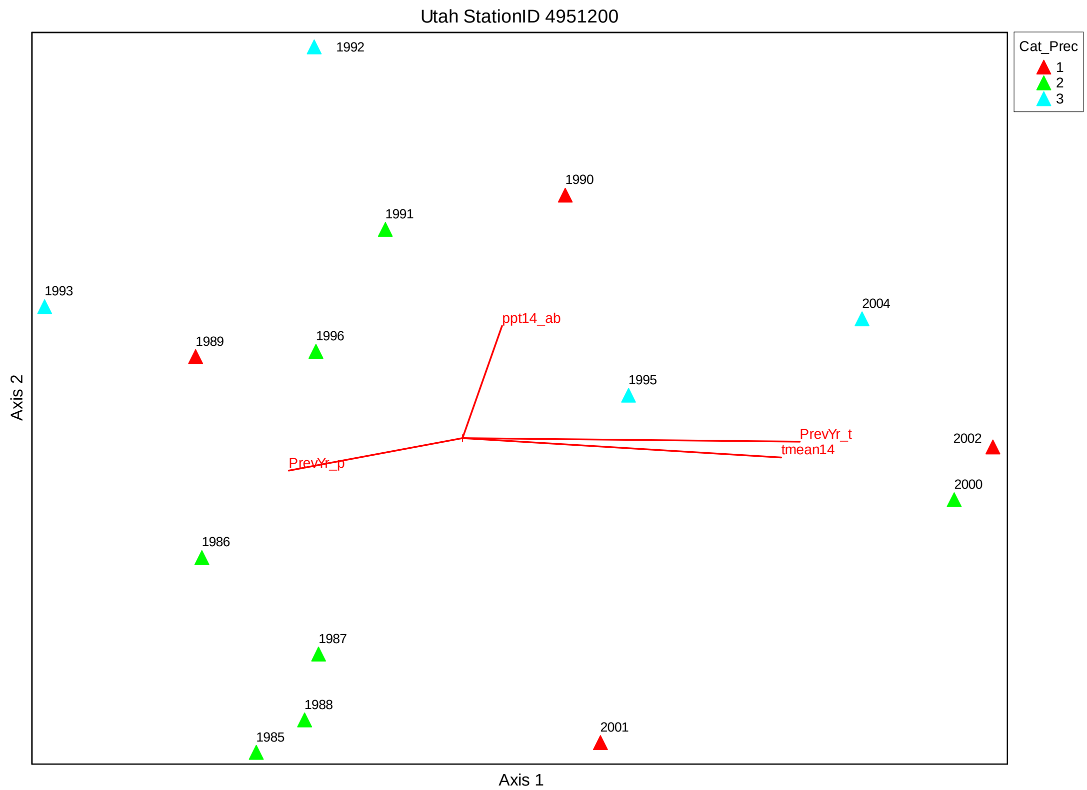


Figure F5-2. NMDS plot (Axis 1-2). Cat_Prec refers to the precipitation categories, which are: 1=dry years; 2=normal years; 3=wet years. Samples are labeled by collection year.

In addition, for the NMDS ordinations on coldest/normal/hottest year samples, we evaluated which taxa were most strongly correlated with each axis. As shown in Figure 2-19 in the main report, at Station 4927250 (Weber), Pteronarcys, Chloroperlidae and Ephemerella have the strongest positive correlations with Axis 2, and Optioservus, Lepidostoma and Hyallela have the strongest negative correlations with Axis 2. Closer examination of those taxa plotted in ordination space shows that Chloroperlidae and Pteronarcys are absent from the hottest year samples and Ephemerella is present in all the coldest and normal year samples and in only one hottest year sample. Some additional taxa that occurred during multiple years that were not found in hottest year samples include Rhithrogena, Nematoda, and Tubificidae. NMDS plots of Optioservus, Lepidostoma and Hyallela show these taxa to be present in at least 4 of the 5 hottest year samples. These taxa are also present in coldest and normal year samples. These plots and associated information are available upon request.

As shown in Figure 2-20 in the main report, at Station 4951200 (Virgin), Ephemerella, Nematoda and Heptagenia have the strongest negative correlations with Axis 1, and Forcipomyia/Probezzia, Microcylloepus, Caloparyphus and Chimarra have the strongest positive correlations with Axis 1. Closer examination of those taxa plotted in ordination space shows that Nematoda is absent from the hottest year samples. Ephemerella and Heptagenia are present in all coldest year samples, 6 of the 7 normal year samples and only 1 of the hottest year samples. Forcipomyia/Probezzia, Microcylloepus, Caloparyphus and Chimarra are present in at least 2 of the 4 hottest year samples. These taxa are not present in coldest and/or normal year samples. These plots and associated information are available upon request.

One-way ANOVA analyses were performed to evaluate differences in mean values of commonly-used, ecological trait and scenario metrics when samples were grouped by coldest, normal, and hottest or driest, normal and wettest years. Results varied by site. Two stations (4927250 (Weber - Wasatch Uinta) and 4951200 (Virgin - Colorado Plateau)) showed relatively strong temperature patterns, while one site (5940440 - Beaver) showed no patterns at all. The greatest differences generally occurred between hottest and coldest year samples, while coldest and normal year samples tended to be similar. Metrics that had at least one significant difference between coldest, normal, and hottest or driest, normal and wettest years are shown in **Tables F5-1** and **F5-2**. These tables do not include results for thermal-preference metrics, which are shown in **Table 2-2** of the main report. Additional results are available upon request.

Table F5-1. These metrics had at least one significant difference when one-way analysis of variance was done to evaluate differences in samples grouped by coldest, normal, and hottest years. Year groups were based on Parameter-elevation Regressions on Independent Slopes Model (PRISM) mean annual air temperature values at each site. Groups with the same superscripts are not significantly different ($p < 0.05$).

Station	Metric	Coldest	Normal	Hottest
4927250 (Weber)	# Ephemeroptera taxa	7.1 ± 1.2^{A}	4.9 ± 2.1^{AB}	2.6 ± 0.9^{B}
	# EPT taxa	17.4 ± 2.1^{A}	13.6 ± 4.9^{AB}	8.8 ± 2.2^{B}
	% Collector-filterer individuals	13.4 ± 7.6^{A}	32.1 ± 15.4^{AB}	40.1 ± 17.3^{B}
	% Collector-gatherer individuals	69.9 ± 16.9^{A}	50.9 ± 23.6^{AB}	33.5 ± 19.7^{B}
	# Collector-gatherer taxa	8.2 ± 0.8^{A}	6.3 ± 2.3^{AB}	4.0 ± 1.4^{B}
	# Predator taxa	7.3 ± 1.6^{A}	5.9 ± 2^{AB}	3.8 ± 1.3^{B}
	# Clinger taxa	17.8 ± 1.3^{A}	14 ± 4.8^{AB}	9 ± 1.2^{B}

	# Plecoptera taxa	3.2 ± 0.8^{A}	3.1 ± 1.5^{AB}	0.8 ± 0.4^{B}
	# Total taxa	27.5 ± 3.5^{A}	21.5 ± 7.8^{AB}	17.2 ± 3.3^{B}
	# Warmer-drier vulnerable taxa	4.0 ± 1.2^{A}	2.7 ± 1.1^{AB}	1.0 ± 0.7^{B}
	# Drier vulnerable taxa	12.6 ± 0.9^{A}	9.0 ± 2.9^{AB}	5.4 ± 1.8^{B}
4951200 (Virgin)	# Ephemeroptera taxa	6.8 ± 2.2^{A}	4.8 ± 0.8^{AB}	2.5 ± 0.6^{B}
	# EPT taxa	12.3 ± 3.9^{A}	9.5 ± 2.6^{AB}	5.3 ± 1.5^{B}
	# Herbivore taxa	5.3 ± 1.5^{A}	4.2 ± 1.6^{A}	1.5 ± 0.6^{B}
	# Burrower taxa	1 ± 0^{AB}	1.5 ± 0.5^{A}	0.3 ± 0.5^{B}
	% Swimmer individuals	13.2 ± 3.7^{A}	14.8 ± 8.4^{A}	33 ± 10.9^{B}
	Shannon-Wiener diversity index	2.8 ± 0.4^{AB}	2.9 ± 0.2^{A}	2.2 ± 0.4^{B}
	# Total taxa	22.8 ± 6.6^{A}	19.8 ± 3.2^{AB}	14.5 ± 1.9^{B}
	% Drought-resistant individuals	45.8 ± 17.9^{A}	40 ± 20.3^{AB}	13.1 ± 4.4^{B}
	# Perennial taxa	10.3 ± 2.2^{A}	7.7 ± 2.2^{AB}	6 ± 0.8^{B}

Table F5-2. These metrics had at least one significant difference when one-way analysis of variance was done to evaluate differences in samples grouped by driest, normal, and wettest years. Year groups were based on Parameter-elevation Regressions on Independent Slopes Model (PRISM) mean annual precipitation values at each site. Groups with the same superscripts are not significantly different ($p < 0.05$).

Station	Metric	Driest	Normal	Wettest
4927250 (Weber)	# Intermittent taxa	1.4 ± 0.5^{A}	1.7 ± 0.5^{AB}	2.6 ± 0.9^{B}
4951200 (Virgin)	% Collector-gatherer individuals	47.9 ± 9.6^{A}	73.0 ± 15.7^{B}	75.9 ± 0.3^{B}

Mean O/E values at the two Colorado Plateau stations (4951200-Virgin and 4936750-Duchesne) were significantly different between hot year and cold/normal year samples (see **Section 3** of the report). O/E values at the 2 Wasatch Uinta reference sites were not significantly different when grouped by temperature categories, but O/E values at Station 4927250 (Weber) were higher during hot years. These results suggest that climate change effects on O/E values will vary spatially and may result in classifications improving. There are no significant differences in mean O/E values at any of the sites when the data are grouped into precipitation categories.

Results of the correlation analyses show that O/E scores are only significantly correlated with one or two of the climatic variables at 2 of the sites. The significant correlations occur at Stations 4951200 (Virgin) and 5940440 (Beaver) (**Table F5-9**). At site 4951200 (Virgin), O/E values are positively correlated with two of the PRISM mean annual air temperature variables (sample year and prior year). At Site 5940440 (Beaver), O/E values are negatively correlated with the prior year mean annual precipitation variable.

It should be noted that there are other potential confounding factors that may be influencing trends in O/E values at the sites. For example, at Station 4951200 (Virgin), pH is significantly correlated with O/E values (r=0.77, p=.03) (pH ranges from 7.95 to 8.5 at this site). Therefore results should be interpreted with caution. See **Section 2** of the report for more information on potential confounding factors.

NOTE: Additional NMDS and/or ANOVA results for each station (4927250, 4951200, 4936750 and 5940440) are available upon request.

Table F5-9. Results of the correlation analyses between O/E values and climatic variables. R and p-level values that are significantly correlated are in red bold print.

Climatic Variables	Station 4927250 (N=17)	4936750 (N=12)	4951200 (N=14)	5940440 (N=9)
PRISM mean annual air temperature	-0.17 p=.517	0.35 p=.265	**0.70** **p=.006**	0.27 p=.486
PRISM mean annual precipitation	0.18 p=.496	0.00 p=.998	-0.14 p=.628	-0.23 p=.551
Previous year PRISM mean annual air temperature	0.10 p=.714	0.49 p=.103	**0.72** **p=.004**	-0.25 p=.518
Previous year PRISM mean annual precipitation	0.08 p=.768	0.12 p=.720	0.02 p=.940	**-0.79** **p=.012**
Absolute difference between the PRISM mean annual air temperature from the sampling year and the previous year	-0.02 p=.936	-0.38 p=.225	-0.26 p=.361	0.28 p=.472
Absolute difference between the PRISM mean annual precipitation from the sampling year and the previous year	0.16 p=.550	-0.03 p=.918	0.27 p=.348	0.00 p=.997

F6. TREND PLOTS

Trends in two commonly-used metrics (# of total taxa and # of EPT taxa) were plotted over time at the 4 Utah stations. Figures F6-1 through F6-8 show how trends in these metrics related to trends in PRISM mean annual air temperature and PRISM mean annual precipitation over time.

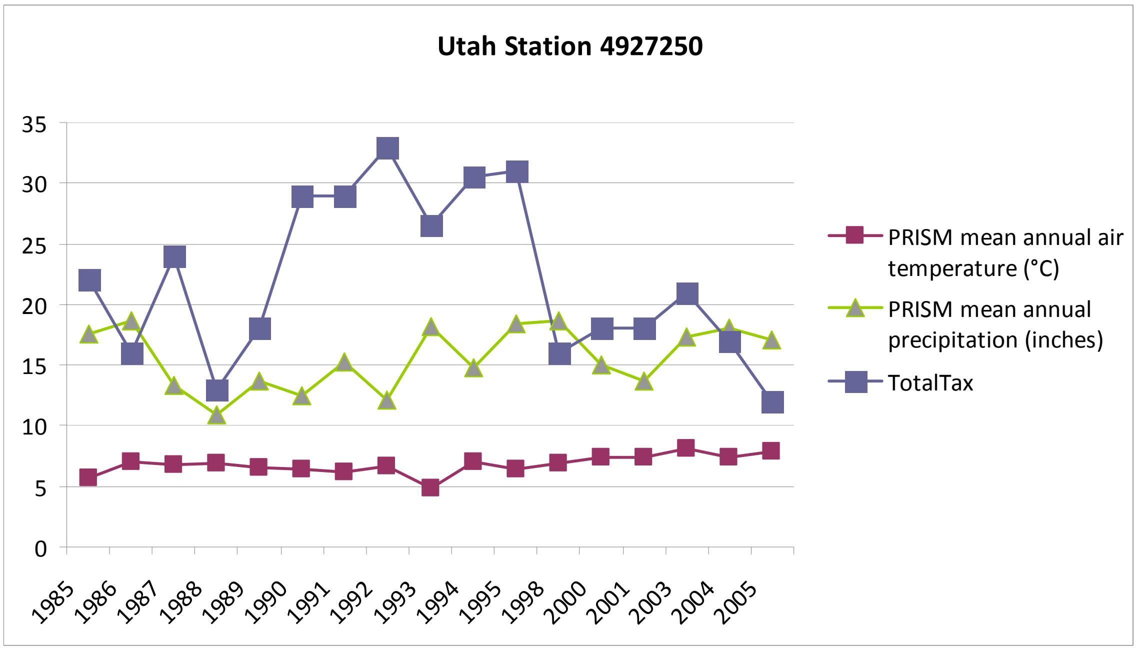


Figure F6-1. Trends in number of total taxa, PRISM mean annual air temperature and precipitation at Station 4927250 (Weber).

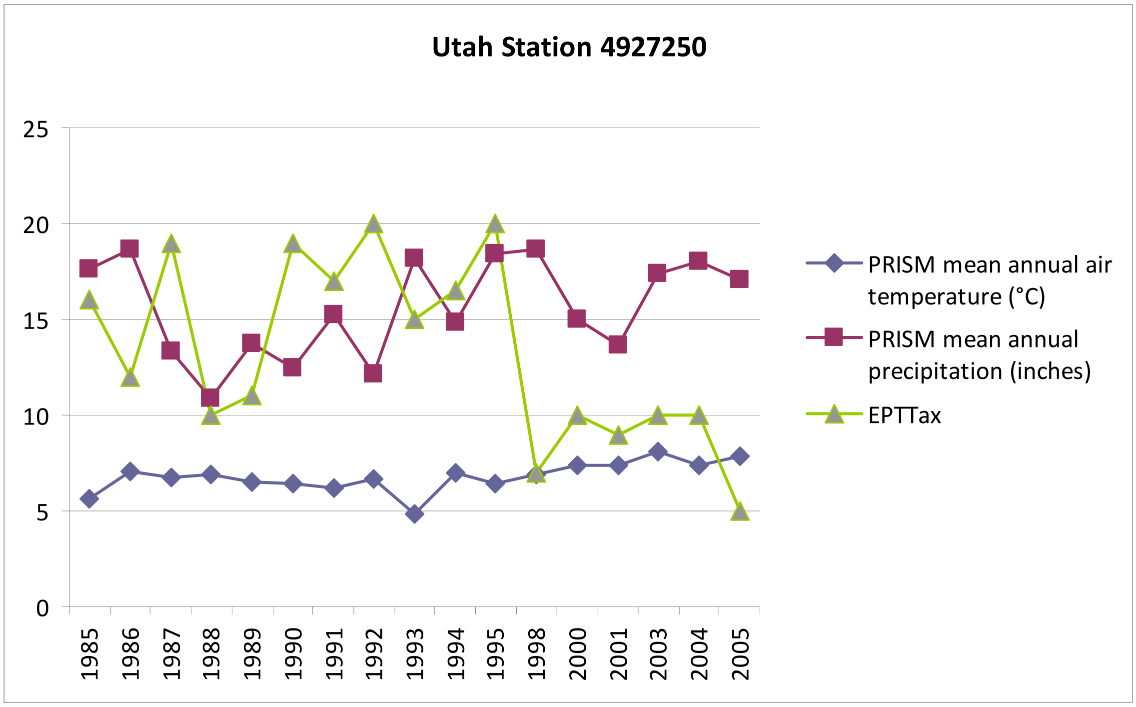


Figure F6-2. Trends in number of EPT taxa, PRISM mean annual air temperature and precipitation at Station 4927250 (Weber).

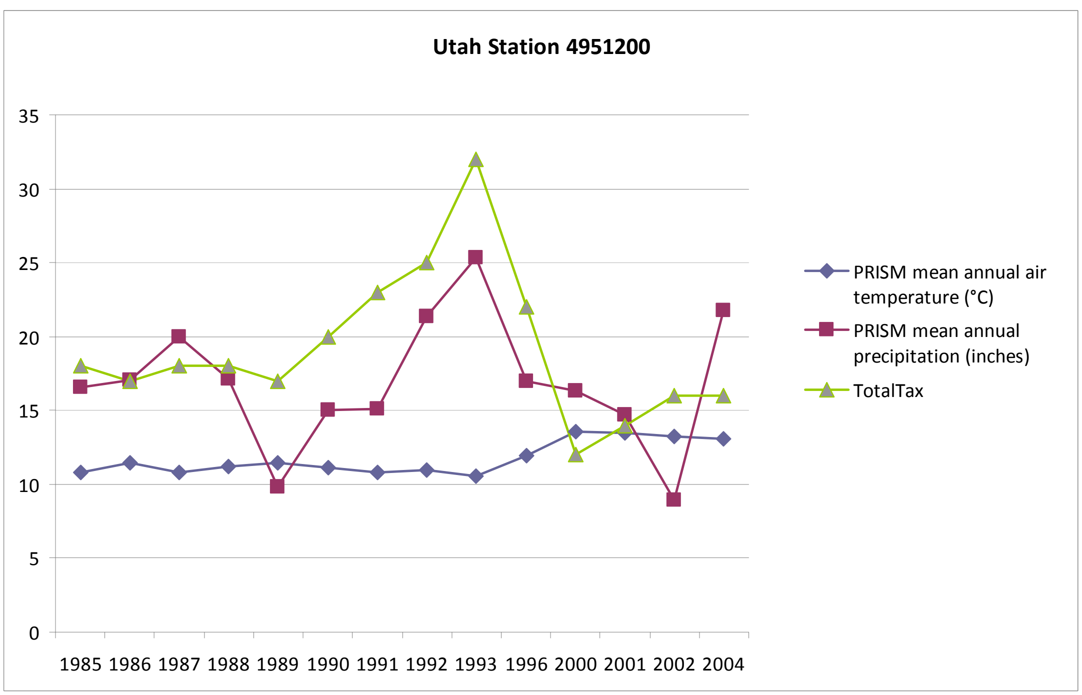


Figure F6-3. Trends in number of total taxa, PRISM mean annual air temperature and precipitation at Station 4951200 (Virgin).

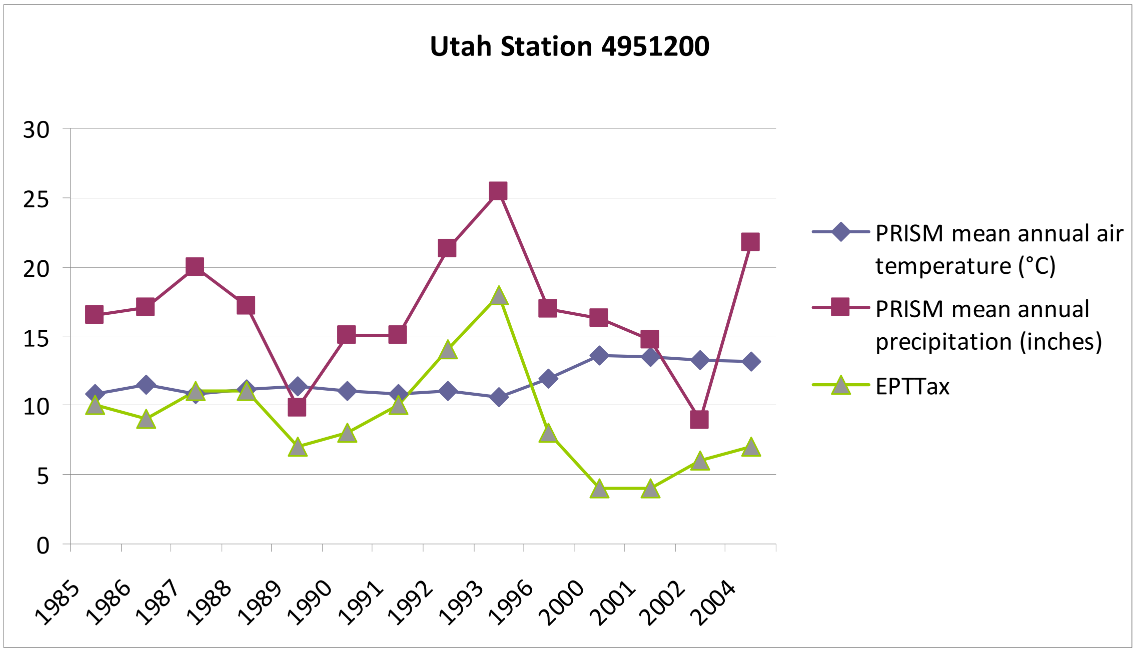


Figure F6-4. Trends in number of EPT taxa, PRISM mean annual air temperature and precipitation at Station 4951200 (Virgin).

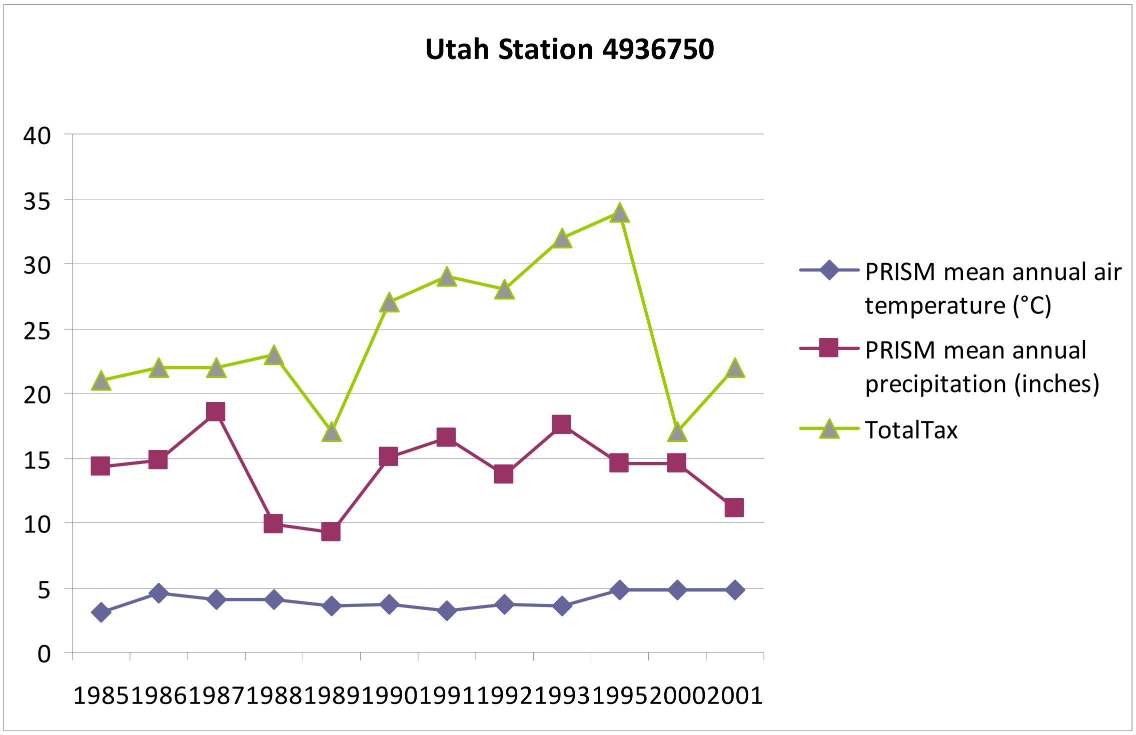


Figure F6-5. Trends in number of total taxa, PRISM mean annual air temperature and precipitation at Station 4936750 (Duchesne).

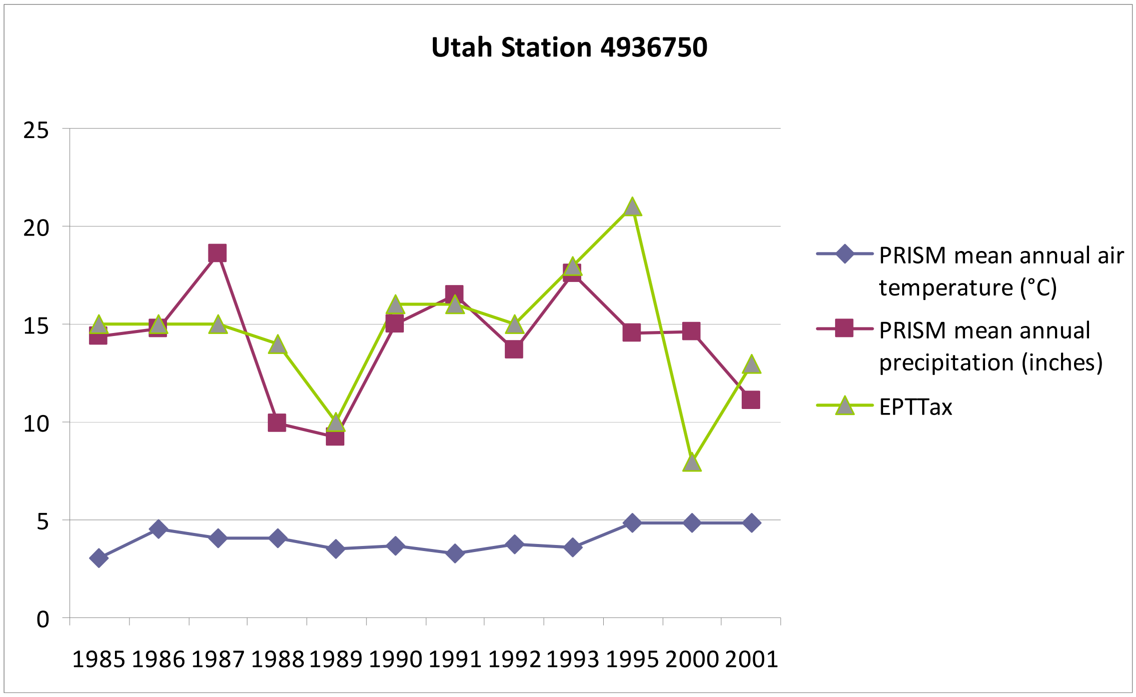


Figure F6-6. Trends in number of EPT taxa, PRISM mean annual air temperature and precipitation at Station 4936750 (Duchesne).

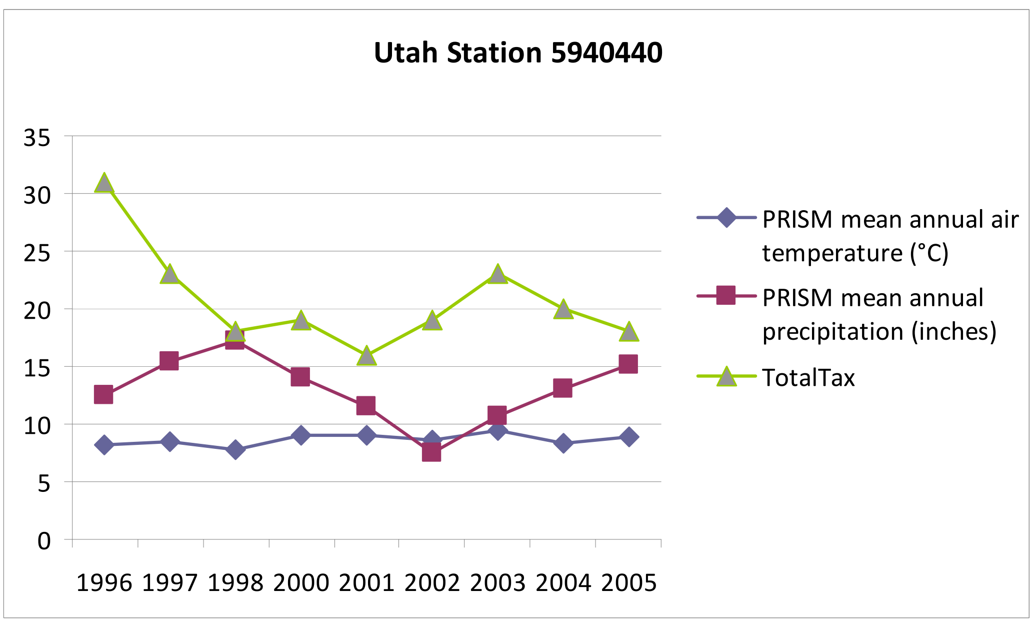


Figure F6-7. Trends in number of total taxa, PRISM mean annual air temperature and precipitation at Station 5940440 (Beaver).

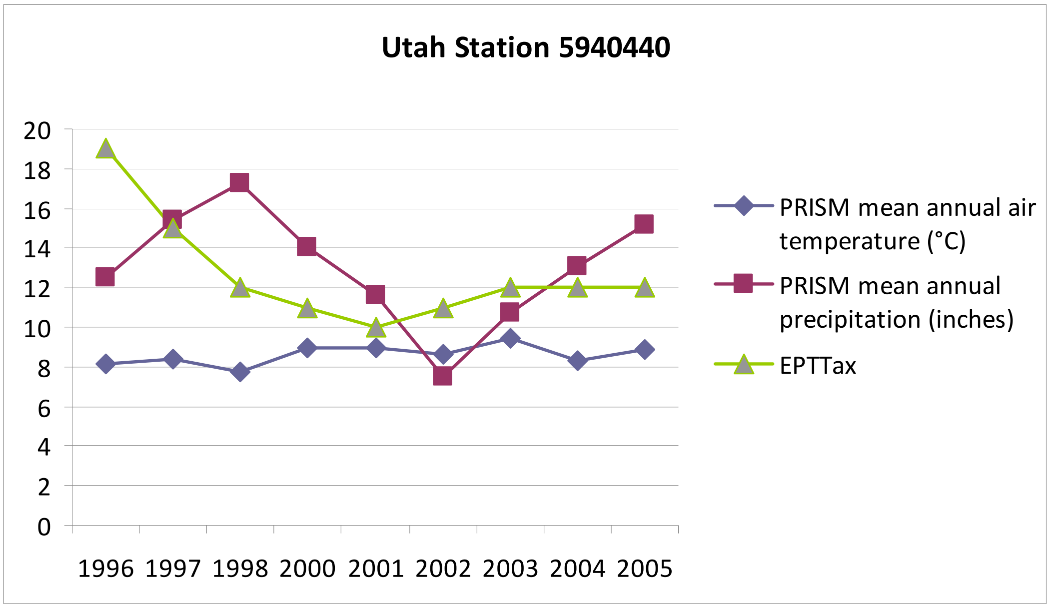


Figure F6-8. Trends in number of EPT taxa, PRISM mean annual air temperature and precipitation at Station 5940440 (Beaver).

SUMMARY OF RESULTS

- Site-specific: two sites (4927250 and 4951200) showed stronger patterns than the others when data were grouped into temperature categories. One site, 5940440, showed no patterns at all.
- Temperature appears to be a more important influence than precipitation: more significant differences occurred when samples were grouped by temperature categories vs. precipitation categories.
- When patterns occurred, the greatest differences were between hot- vs. cold-year samples: in the ANOVA analyses, the greatest number of significant differences occurred between hot and cold year samples. In the NMDS ordination, hot-year samples formed distinct clusters from the other samples when data from sites 4927250 and 4951200 were grouped by temperature categories.
- Not much difference between cold and normal year samples: In the ANOVA analyses, no significant differences occurred between cold and normal year samples, and in the ordinations, cold and normal year samples were generally mixed together.

- The metrics that had the most number of significant differences between hot-year and cold- or normal-year samples are total taxa, Ephemeroptera taxa, Plecoptera taxa, EPT taxa, cold-water taxa and herbivore/scraper taxa.
- Not many metrics had significant differences when grouped by precipitation categories: only 3 metrics were significantly different when grouped by precipitation categories, and these occurred at single sites. The 3 metrics are: % collector-gatherer, % herbivore/scraper and # of intermittent taxa.
- The temperature metric that performed ‘best’ is # of cold-water taxa. The other temperature metrics (except for % warm-water individuals) performed fairly well at the 2 sites where patterns occurred (meaning mean values were significantly different between hot and cold and/or normal year samples at least at one site).
- Are certain taxa consistently driving trends? The ordinations showed that Ephemerella, which was considered to be a cold-water taxon in these analyses, was a key player at both sites 4927250 and 4951200. Nematoda, also considered a cold-water taxon, was present at these two sites. Other taxa (i.e. warm-water taxa) were important at one site but not the other (i.e. Forcipomyia/Probezzia, Microcylloepus, Caloparyphus and Chimarra)
- ‘Hydrologic’ metrics (i.e. perennial, intermittent, drier scenario, etc.) did not show any significant associations with the climate-related variables.
- Results from the ANOVA analyses on the data from the 4 sites show that there are significant differences in O/E values between hot year and cold/normal year samples at 2 of the 4 sites (4951200 and 4936750). At both sites, mean O/E scores from the hot year samples are significantly higher than mean O/E scores from cold and normal year samples**.**
- Results from the correlation analyses on the data from the 4 sites show that O/E scores are only significantly correlated with one or two of the climatic variables at 2 of the sites. Patterns are not consistent between the 2 sites.
- See Section 6 of the report for information on potential confounding factors that may have influenced trends at these sites.

Attachment F1

‘Extreme’ alterations of Utah fall RIVPACS model climate-related predictor variable values

As referenced on page F-7 in this Appendix, we also ran some ‘extreme’ scenarios (i.e. doubling temperature, dividing precipitation values by two, changing freeze dates by 30 days, etc.) to further explore how much the climate-related predictor variables would have to change in order to result in substantial changes to O/E scores. The tables in this attachment show which scenarios were run and what the results were.

Table F1-1. Descriptions of how the climate-related predictor variables were altered in the ‘extreme alteration’ RIVPACS analyses

Run#	Category	Altered Predictor variables	Rationale
1	Baseline	None - used original values	get baseline values and QC
2		TMEAN.WS + 2 & TMEAN.NET + 2	NCAR annual temperature predictions (2050)
3		TMEAN.WS + 4 & TMEAN.NET + 4	NCAR annual temperature predictions (2090)
4		TMEAN.WS + 10 & TMEAN.NET + 10	curiosity
5	Temperature	TMEAN.WS + 20 & TMEAN.NET + 20	curiosity
6		MEANP.PT - .05	NCAR annual precipitation predictions (2050)
7		MEANP.PT - .1	NCAR annual precipitation predictions (2090)
8		MEANP.PT - Minimum PRISM ppt14	based on PRISM ppt14 minimum values (1975-2006)
9		MEANP.PT/2	curiosity
10		MINP.PT/2	curiosity
11		MEANP.PT/2 & MINP.PT/2	curiosity
12	Precipitation		curiosity
13		TMEAN.WS + 2 & TMEAN.NET + 2 & MEANP.PT - .05	NCAR annual temperature and precipitation predictions (2050)
14	Temperature & Precipitation	TMEAN.WS + 4 & TMEAN.NET + 4 & MEANP.PT - .1	NCAR annual temperature and precipitation predictions (2090)
15		LST32AVE - 2	best professional judgment
16		LST32AVE - 5	best professional judgment
17		FST32AVE + 5	best professional judgment
18	Freeze Date	LST32AVE - 5 & FST32AVE + 5	best professional judgment
19		LST32AVE - 10	curiosity
20MINWD.WS/2		FST32AVE + 10	curiosity
21		LST32AVE - 10 & FST32AVE + 10	curiosity
22		LST32AVE - 15	curiosity

Table F1-1. Continued.

Run#	Category	Altered Predictor variables	Rationale
23	Freeze Date	LST32AVE - 15 & FST32AVE + 15	curiosity
24		LST32AVE-1, MINP.PT-1, MEANP.PT-1, TMEAN.NET+1, TMEAN.WS+1, FST32AVE+1, MINWD.WS-1	best professional judgment
25	Combine All	LST32AVE-2, MINP.PT-2, MEANP.PT-2, TMEAN.NET+2, TMEAN.WS+2, FST32AVE+2, MINWD.WS-1	best professional judgment

Table F1-2. Results for the scenarios in which temperature predictor variables were altered

			Baseline (original)			TMEAN.WS + 2 & TMEAN.NET + 2			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	14	14.92	0.94	0.01
7	4951200	120184	10	9.58	1.04	10	9.56	1.05	0
1	4936750	118524	15	14.04	1.07	15	14	1.07	0
6	4927250	127718	8	8.74	0.92	8	8.74	0.92	0

			Baseline (original)			TMEAN.WS + 4 & TMEAN.NET + 4			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	14	14.8	0.95	0.02
7	4951200	120184	10	9.58	1.04	10	9.6	1.04	0
1	4936750	118524	15	14.04	1.07	15	14	1.07	0
6	4927250	127718	8	8.74	0.92	7	8.25	0.85	-0.07

			Baseline (original)			TMEAN.WS + 10 & TMEAN.NET + 10			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	14	14.65	0.96	0.03
7	4951200	120184	10	9.58	1.04	10	9.61	1.04	0
1	4936750	118524	15	14.04	1.07	15	13.89	1.08	0.01
6	4927250	127718	8	8.74	0.92	7	8.24	0.85	-0.07

			Baseline (original)			TMEAN.WS + 20 & TMEAN.NET + 20			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	13	14.08	0.92	0
7	4951200	120184	10	9.58	1.04	10	9.63	1.04	-0.01
1	4936750	118524	15	14.04	1.07	15	13.44	1.12	0.05
6	4927250	127718	8	8.74	0.92	7	8.24	0.85	-0.07

Table F1-3. Results for the scenarios in which precipitation predictor variables were altered

			Baseline (original)			MEANP.PT - .05			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	14	15.1	0.93	0
7	4951200	120184	10	9.58	1.04	10	9.59	1.04	0
1	4936750	118524	15	14.04	1.07	15	14	1.07	0
6	4927250	127718	8	8.74	0.92	8	8.75	0.91	0

			Baseline (original)			MEANP.PT - .1			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	14	15.08	0.93	0
7	4951200	120184	10	9.58	1.04	10	9.58	1.04	0
1	4936750	118524	15	14.04	1.07	15	14.01	1.07	0
6	4927250	127718	8	8.74	0.92	8	8.74	0.92	0

			Baseline (original)			MEANP.PT - Min ppt14 PRISM			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	14	14.78	0.95	0.02
7	4951200	120184	10	9.58	1.04	10	9.51	1.05	0.01
1	4936750	118524	15	14.04	1.07	15	13.79	1.09	0.02
6	4927250	127718	8	8.74	0.92	8	8.71	0.92	0

			Baseline (original)			MEANP.PT/2			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	14	14.79	0.95	0.02
7	4951200	120184	10	9.58	1.04	10	9.43	1.06	0.02
1	4936750	118524	15	14.04	1.07	15	13.8	1.09	0.02
6	4927250	127718	8	8.74	0.92	8	8.68	0.92	0.01

			Baseline (original)			MINP.PT/2			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	13	13.92	0.93	0.01
7	4951200	120184	10	9.58	1.04	10	9.46	1.06	0.01
1	4936750	118524	15	14.04	1.07	15	13.58	1.1	0.04
6	4927250	127718	8	8.74	0.92	8	8.69	0.92	0.01

Table F1-3. Continued

			Baseline (original)			MEANP.PT/2 & MINP.PT/2			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	13	13.69	0.95	0.02
7	4951200	120184	10	9.58	1.04	10	9.33	1.07	0.03
1	4936750	118524	15	14.04	1.07	15	13.38	1.12	0.05
6	4927250	127718	8	8.74	0.92	8	8.16	0.98	0.07

			Baseline (original)			MINWD.WS/2			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	13	13.81	0.94	0.01
7	4951200	120184	10	9.58	1.04	10	9.53	1.05	0.01
1	4936750	118524	15	14.04	1.07	15	13.47	1.11	0.05
6	4927250	127718	8	8.74	0.92	7	7.63	0.92	0

Table F1-4. Results for the scenarios in which both temperature and precipitation predictor variables were altered

			Baseline (original)			TMEAN.WS + 2 & TMEAN.NET + 2 & MEANP.PT - .05			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	14	14.93	0.94	0.01
7	4951200	120184	10	9.58	1.04	10	9.56	1.05	0
1	4936750	118524	15	14.04	1.07	15	14.01	1.07	0
6	4927250	127718	8	8.74	0.92	7	8.24	0.85	-0.07

			Baseline (original)			TMEAN.WS + 4 & TMEAN.NET + 4 & MEANP.PT - .1			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	14	14.83	0.94	0.02
7	4951200	120184	10	9.58	1.04	10	9.58	1.04	0
1	4936750	118524	15	14.04	1.07	15	14.02	1.07	0
6	4927250	127718	8	8.74	0.92	7	8.26	0.85	-0.07

Table F1-5. Results for the scenarios in which freeze date predictor variables were altered

			Baseline (original)			LST32AVE - 2			
GROUP	**SITE**	**SAMPLE**	**O**	**E**	**O.E**	**O**	**E**	**O.E**	**Dif'ce O.E**
1	5940440	127636	14	15.09	0.93	14	15.05	0.93	0
7	4951200	120184	10	9.58	1.04	10	9.58	1.04	0
1	4936750	118524	15	14.04	1.07	15	14.01	1.07	0
6	4927250	127718	8	8.74	0.92	7	8.25	0.85	-0.07

			Baseline (original)			LST32AVE - 5			
GROUP	**SITE**	**SAMPLE**	**O**	**E**	**O.E**	**O**	**E**	**O.E**	**Dif'ce O.E**
1	5940440	127636	14	15.09	0.93	14	14.733	0.95	0.02
7	4951200	120184	10	9.58	1.04	10	9.5648	1.05	0
1	4936750	118524	15	14.04	1.07	15	13.999	1.07	0
6	4927250	127718	8	8.74	0.92	7	8.2433	0.85	-0.07

			Baseline (original)			FST32AVE + 5			
GROUP	**SITE**	**SAMPLE**	**O**	**E**	**O.E**	**O**	**E**	**O.E**	**Dif'ce O.E**
1	5940440	127636	14	15.09	0.93	15	15.374	0.98	0.05
7	4951200	120184	10	9.58	1.04	10	9.5875	1.04	0
1	4936750	118524	15	14.04	1.07	15	14.028	1.07	0
6	4927250	127718	8	8.74	0.92	8	8.7184	0.92	0

			Baseline (original)			LST32AVE - 5 & FST32AVE + 5			
GROUP	**SITE**	**SAMPLE**	**O**	**E**	**O.E**	**O**	**E**	**O.E**	**Dif'ce O.E**
1	5940440	127636	14	15.09	0.93	13	14.128	0.92	-0.01
7	4951200	120184	10	9.58	1.04	10	9.5647	1.05	0
1	4936750	118524	15	14.04	1.07	15	13.992	1.07	0
6	4927250	127718	8	8.74	0.92	7	8.224	0.85	-0.06

			Baseline (original)			LST32AVE - 10			
GROUP	**SITE**	**SAMPLE**	**O**	**E**	**O.E**	**O**	**E**	**O.E**	**Dif'ce O.E**
1	5940440	127636	14	15.09	0.93	13	14.02	0.93	0
7	4951200	120184	10	9.58	1.04	10	9.56	1.05	0
1	4936750	118524	15	14.04	1.07	15	13.7	1.09	0.03
6	4927250	127718	8	8.74	0.92	7	8.23	0.85	-0.07

Table F1-5. Continued

			Baseline (original)			FST32AVE + 10			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	14	14.713	0.95	0.02
7	4951200	120184	10	9.58	1.04	10	9.6097	1.04	0
1	4936750	118524	15	14.04	1.07	15	13.797	1.09	0.02
6	4927250	127718	8	8.74	0.92	7	8.1843	0.86	-0.06

			Baseline (original)			LST32AVE - 10 & FST32AVE + 10			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	13	13.743	0.95	0.02
7	4951200	120184	10	9.58	1.04	10	9.6115	1.04	0
1	4936750	118524	15	14.04	1.07	15	13.532	1.11	0.04
6	4927250	127718	8	8.74	0.92	7	8.1706	0.86	-0.06

			Baseline (original)			LST32AVE - 15			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	13	13.945	0.93	0
7	4951200	120184	10	9.58	1.04	10	9.5818	1.04	0
1	4936750	118524	15	14.04	1.07	15	13.454	1.11	0.05
6	4927250	127718	8	8.74	0.92	7	8.2214	0.85	-0.06

			Baseline (original)			LST32AVE - 15 & FST32AVE + 15			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	13	13.415	0.97	0.04
7	4951200	120184	10	9.58	1.04	10	9.6052	1.04	0
1	4936750	118524	15	14.04	1.07	14	12.787	1.09	0.03
6	4927250	127718	8	8.74	0.92	7	8.1713	0.86	-0.06

Table F1-6. Results for scenarios in which combinations of all climate-related predictor variables were altered simultaneously

			Baseline (original)			Changed by 1			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	13	14.04	0.93	0
7	4951200	120184	10	9.58	1.04	10	9.51	1.05	0.01
1	4936750	118524	15	14.04	1.07	15	14.03	1.07	0
6	4927250	127718	8	8.74	0.92	8	8.71	0.92	0

			Baseline (original)			Changed by 2			
GROUP	SITE	SAMPLE	O	E	O.E	O	E	O.E	Dif'ce O.E
1	5940440	127636	14	15.09	0.93	13	13.81	0.94	0.01
7	4951200	120184	10	9.58	1.04	10	9.49	1.05	0.01
1	4936750	118524	15	14.04	1.07	15	14.03	1.07	0
6	4927250	127718	8	8.74	0.92	7	8.23	0.85	-0.06

Attachment F2

Utah Temperature-Indicator Taxa

This attachment contains tables with lists of the Utah temperature-indicator taxa and describes the process that we followed to develop these lists.

F2. UTAH TEMPERATURE-INDICATOR TAXA

Sources. The Utah cold- and warm-water taxa lists were developed using several different sources: 1. weighted-average calculations based on a subset of the Utah biomonitoring database (using fall samples (sample size=572)); 2. the thermal-preference trait from the Poff et al. 2006 traits matrix; 3. the thermal-preference trait from the USGS traits database (Vieira et al., 2006); 4. the thermal-preference trait from the compilation of EPA Environmental Requirements and Pollution Tolerance series from the late 1970's (Beck et al., 1977; Harris et al., 1978; Hubbard et al., 1978; Surdick et al., 1978); and 5. best professional judgment of the Utah Climate Change feedback group[3].

Many of the same general criteria that were used to designate cold- and warm-water indicator taxa in Maine were also used in Utah (see Attachment F2). Also, see Attachment F2 for general limitations of the weighted averaging, as well as for information on general considerations that were taken into account.

Initial Results. Initially there were 76 taxa on the cold-water list and 53 taxa on the warm-water list. These lists were based on weighted-average calculations and literature. These lists were further refined through the evaluation of additional evidence. This evidence included analyses of other datasets, case studies, and best professional judgment. Taxa with the greatest amount of evidence were designated as temperature indicator taxa. More detailed information about the steps that were used to develop the temperature indicator taxa lists is summarized below:

Considerations (unique to Utah)

In addition to Considerations A and C in Attachment E2, a subset of the scores that included only the western states (California, Oregon, Idaho, Utah, Yuan Western EMAP) was also taken into account when developing the lists. The reasoning behind this is that the data from these states is more similar and therefore more comparable to Utah than data from Ohio, North Carolina and Maine. Therefore it was given more weight in the consideration process. Taxa that

[3] Utah Climate Change group: Utah DWQ (Jeff Ostermiller), Utah State University Bug Lab (Mark Vinson), Eric Dinger (formerly of the USU Bug Lab), David Herbst (California Sierra Nevada), Wyoming (Eric Hargett), Pyramid Lake Paiute Tribe (Dan Mosely), and Shann Stringer (formerly New Mexico).

received higher scores had more evidence supporting their inclusion on the temperature indicator taxa list. Cold- and warm-water taxa lists from these western states were also evaluated for conflicting evidence. If a taxon showed a preference for cold- or warm-water in Utah but was shown to have the opposite preference in the California, Oregon, Idaho, or Yuan Western EMAP analyses (i.e. cold-water taxon in Utah was listed as a warm-water taxon in Oregon), it was not included on the temperature indicator list.

Several 'case studies' were performed to see whether the cold- or warm-water taxa occurred at sites in Utah that had the warmest- or coldest-water temperatures (June-September). The following case studies were performed:

Cold-water Case Study #1. Taxa lists from 4 sites in the Wasatch and Uinta Mountains level 3 ecoregion that had the coldest average water temperatures (using June-September samples) and that had <2% urban and <10% agricultural land use/land cover within a 1 km buffer were evaluated. Sites include: Station 4938910 (avg temp 5.75°C, 0% urban, 0% agricultural), Station 4936700 (avg temp 9.1°C, 0.74% urban, 0% agricultural), Station 4935970 (avg temp 9.5°C, 0% urban, 0% agricultural), and Station 4995830 (avg temp 9.6°C, 0% urban, 0% agricultural).

Cold-water Case Study #2. Taxa lists from 4 sites in the Colorado Plateaus level 3 ecoregion that had the coldest average water temperatures (using June-September samples) and that had <2% urban and <10% agricultural land use/land cover within a 1 km buffer were evaluated. Sites include: Station 4937720 (avg temp 10.9°C, 0.17% urban, 1.4% agricultural), Station 4936200 (avg temp 12.5°C, 0.11% urban, 0% agricultural), Station 4954140 (avg temp 14.1°C, 0% urban, 0% agricultural), and Station 4956480 (avg temp 14.2°C, 0% urban, 0% agricultural).

Warm-water Case Study #1. Taxa lists from two sites in the Colorado Plateaus level 3 ecoregion that had the warmest average water temperatures (using June-September samples) and that had <2% urban and <10% agricultural land use/land cover within a 1 km buffer were evaluated. Sites include: Station 4933120 (avg temp 32°C, 1.6% urban, 3.4% agricultural) and Station 4950790 (avg temp 26.2°C, 0% urban, 0% agricultural).

Development of the Temperature Indicator Cold-water Taxa List. Taxa were placed on the cold-water list if the following criteria were met:

1. The taxon was NOT present at the warm-water case study site.
2. The taxon (and no species within the genera) was NOT on the warm-water lists derived from the California, Oregon, Idaho, and Yuan Western EMAP datasets.
3. The Utah Climate Change feedback group did not specify that they did not think the taxon should be on the list (based on best professional judgment).
4. The taxon had to be on the cold-water taxa list in at least two of the western datasets, or if it was only listed in one dataset, it also had to be present at one or more of the cold-water case study sites.

Development of the Temperature-Indicator Warm-Water List. Taxa were placed on the warm-water list if the following criteria were met:

1. The taxon was NOT present at the cold-water case study sites.
2. The taxon (and no species within the genera) was NOT on the cold-water lists derived from the California, Oregon, Idaho, and Yuan Western EMAP datasets.
3. The Utah Climate Change feedback group did not specify that they did not think the taxon should be on the list (based on best professional judgment).
4. The taxon had to be on the warm-water taxa list in at least two of the western datasets, or if it was only listed in one dataset, it also had to be present at the warm-water case study sites.

Temperature-Indicator Lists. The cold-water taxa list was comprised of 33 taxa and the warm-water taxa list was comprised of 16 taxa. Temperature indicator taxa lists can be found in **Tables F2-1** and **F2-2**.

Important Notes – variation within genera. Some noteworthy genera were left off the Utah cold-water taxa list. These include Zapada, Epeorus, Drunella, Brachycentrus and Rhyacophila. The reason they were not included is because there is variation in temperature preferences among species within these genera. For example, *Zapada cinctipes* is on the warm-water taxa lists in the Oregon and Idaho datasets, but the other species within this genus are listed as cold-water taxa. *Epeorus albertae* is on the warm-water list in the Oregon dataset, but other species within this genus are generally listed as cold-water taxa. *Drunella grandis* is listed as a warm-water taxa (barely – it received a rank optima score of 5) in the Oregon and Idaho datasets, but other species within this genus are generally listed as cold-water taxa. Within the family Rhyacophilidae, there are a few taxa that are listed as warm-water taxa and several that are listed as cold-water taxa. There is similar variation within the genus Brachycentrus.

Dispersal Ability. If temperature is a major factor influencing community composition, then taxa that are able to adapt to warming temperatures and/or that are able to disperse to more favorable habitats (generally believed to be upstream or to higher elevations) have a better chance of surviving. Five mobility traits were examined for the taxa on the Utah temperature indicator lists: dispersal (adult), adult flying strength, occurrence in drift, maximum crawling rate and swimming ability. More information on these traits can be found in **Table F2-3**.

Dispersal (adult) and adult flying strength received the greatest amount of consideration. Because movement is most likely to be upstream, taxa that are strong fliers are likely to have a better chance of success. It will be difficult for taxa that disperse via occurrence in drift to migrate upstream, and taxa that disperse via crawling or swimming are likely to have difficulty moving the distances required to find more favorable habitats.

All of the taxa on the Utah temperature indicator cold-water taxa list (that we had trait information for) are considered to have low dispersal ability and weak adult flying strength. Six of the taxa on the temperature indicator warm-water taxa list (that we had trait information for) are considered to have high dispersal ability (Cheumatopsyche, Microcylloepus, Ochrotrichia, Oecetis, Calineuria and Nectopsyche). Two of these are categorized as strong fliers (Cheumatopsyche and Calineuria).

Abundance and Distribution. In addition to dispersal ability, abundance and distribution are also important considerations. Those taxa that are widespread and common are likely to have greater genetic diversity and greater chance of adapting than rare taxa that only occur in isolated, localized populations (Sweeney et al., 1992). Moreover, the more abundant taxa are more likely to affect the state biomonitoring assessments.

Abundance and distribution information for the temperature-indicator taxa can be found in **Tables F2-1** and **F2-2**. The most abundant cold-water-temperature-indicator taxa are two Ephemeropterans, *Ephemerella* and *Cinygmula*, which comprise 1.85 and 1.03 percent of the total individuals, respectively. Twenty of the cold-water taxa have overall abundances of less than 0.1%. Asellidae and Leptohyphidae are the most abundant warm-water taxa, with overall abundances of 3.12 and 1.42%. Eleven of the warm-water taxa have overall abundances of less than 0.1%. Of the cold-water taxa, Chloroperlidae occurs at the highest percentage of sites (49%), followed by two Ephemeropterans (*Ephemerella* and *Cinygmula*), which occur at 44 and 46% of the sites, respectively. Fifteen of the cold-water taxa occur at less than 10% of the sites. Among the warm-water taxa, Leptohyphidae occurs at the highest percentage of sites (31%), followed by Coenagrionidae (18%) and *Cheumatopsyche* (17%). Eleven of the warm-water taxa occur at less than 10% of the sites.

Additional information – Cold-water Taxa. Ten of the cold-water taxa are Plecopterans, eight are Dipterans, seven are Trichopterans and six are Ephemeropterans (**Table F2-4a**). The families with the most number of taxa on the cold-water list are Heptageniidae, Empididae and Perlodidae (**Table F2-4b**).

Additional information – Warm-water Taxa. Five of the warm-water taxa are Trichopterans, three are Coleopterans, and two are Dipterans and Ephemeropterans (**Table F2-5a**). The families with the most number of taxa on the warm-water list are Elmidae and Leptoceridae (**Table F2-5b**).

Table F2-1. List of Utah cold-water temperature indicator taxa. Distribution and abundance information is also included. Sum_Individuals=the total number of individuals from that taxon in the Utah database; Pct_Abund=percent of total individuals in the database comprised of that taxon; Num_Stations=number of stations in the database that the taxon occurred at; Pct_Stations=percent of stations in the database at which the taxon occurred.

Type	Order	Family	FinalID	Sum_Individs	Pct_Abund	Num_Stations	Pct_Stations
cold	Ephemeroptera	Ameletidae	Ameletus	13157.6	0.03	137	21.57
cold	Trichoptera	Glossosomatidae	Anagapetus	42	0	2	0.31
cold	Trichoptera	Apataniidae	Apatania	20154.3	0.04	39	6.14
cold	Diptera	Ceratopogonidae	Bezzia	109267.1	0.23	232	36.54
cold	Diptera	Blephariceridae	Bibiocephala	2257	0	15	2.36
cold	Plecoptera	Capniidae	Capniidae	113578.8	0.24	228	35.91
cold	Diptera	Empididae	Chelifera	94014.1	0.2	261	41.1
cold	Plecoptera	Chloroperlidae	Chloroperlidae	203579.9	0.44	309	48.66
cold	Ephemeroptera	Heptageniidae	Cinygma	606.2	0	6	0.94
cold	Ephemeroptera	Heptageniidae	Cinygmula	479866.5	1.03	278	43.78
cold	Plecoptera	Perlodidae	Cultus	20419.7	0.04	97	15.28
cold	Diptera	Tipulidae	Dicranota	35439.2	0.08	220	34.65
cold	Trichoptera	Limnephilidae	Ecclisomyia	1262.8	0	14	2.2
cold	Ephemeroptera	Ephemerellidae	Ephemerella	859335.8	1.85	292	45.98
cold	Plecoptera	Pelecorhynchidae	Glutops	91	0	4	0.63
cold	Coleoptera	Elmidae	Heterlimnius	16463	0.04	50	7.87
cold	Ephemeroptera	Heptageniidae	Ironodes	551.6	0	6	0.94
cold	Plecoptera	Perlodidae	Kogotus	1288.7	0	14	2.2
cold	Trichoptera	Lepidostomatidae	Lepidostoma	353679.8	0.76	240	37.8
cold	Plecoptera	Leuctridae	Leuctridae	21176.5	0.05	106	16.69
cold	Plecoptera	Perlodidae	Megarcys	7129.9	0.02	65	10.24
cold	Dorylaimida	Dorylaimidae	Nematoda	141425.3	0.3	249	39.21
cold	Trichoptera	Uenoidae	Neothremma	129853.8	0.28	100	15.75
cold	Trichoptera	Uenoidae	Oligophlebodes	147256.9	0.32	101	15.91
cold	Diptera	Empididae	Oreogeton	228.5	0	13	2.05

Table F2-1. Continued

Type	Order	Family	FinalID	Sum_Individs	Pct_Abund	Num_Stations	Pct_Stations
cold	Trichoptera	Hydropsychidae	Parapsyche	3552.5	0.01	40	6.3
cold	Diptera	Psychodidae	Pericoma	145582.7	0.31	210	33.07
cold	Diptera	Tipulidae	Rhabdomastix	8	0	1	0.16
cold	Ephemeroptera	Heptageniidae	Rhithrogena	198501.8	0.43	243	38.27
cold	Plecoptera	Taeniopterygidae	Taenionema	79949.8	0.17	87	13.7
cold	Plecoptera	Nemouridae	Visoka	50	0	1	0.16
cold	Diptera	Empididae	Wiedemannia	458	0	13	2.05
cold	Plecoptera	Peltoperlidae	Yoraperla	72.7	0	5	0.79

Table F2-2. List of Utah warm-water temperature indicator taxa. Distribution and abundance information is also included. Sum_Individuals=the total number of individuals from that taxon in the Utah database; Pct_Abund=percent of total individuals in the database comprised of that taxon; Num_Stations=number of stations in the database that the taxon occurred at; Pct_Stations=percent of stations in the database at which the taxon occurred.

Type	Order	Family	FinalID	Sum_Individs	Pct_Abund	Num_Stations	Pct_Stations
warm	Hemiptera	Naucoridae	Ambrysus	25879.7	0.06	39	6.14
warm	Isopoda	Asellidae	Asellidae	1450840.4	3.12	81	12.76
warm	Ephemeroptera	Caenidae	Caenis	567	0	11	1.73
warm	Plecoptera	Perlidae	Calineuria	245	0	9	1.42
warm	Diptera	Stratiomyidae	Caloparyphus	9652	0.02	26	4.09
warm	Trichoptera	Hydropsychidae	Cheumatopsyche	172233.9	0.37	105	16.54
warm	Odonata	Coenagrionidae	Coenagrionidae	45144.1	0.1	117	18.43
warm	Ephemeroptera	Leptohyphidae	Leptohyphidae	659670.3	1.42	197	31.02
warm	Diptera	Psychodidae	Maruina	1140.2	0	16	2.52
warm	Coleoptera	Elmidae	Microcylloepus	114016	0.24	50	7.87
warm	Trichoptera	Leptoceridae	Nectopsyche	8434.7	0.02	35	5.51
warm	Trichoptera	Hydroptilidae	Ochrotrichia	6768.2	0.01	29	4.57
warm	Trichoptera	Leptoceridae	Oecetis	28993.3	0.06	90	14.17
warm	Coleoptera	Elmidae	Ordobrevia	360	0	5	0.79
warm	Coleoptera	Psephenidae	Psephenus	65.8	0	4	0.63
warm	Trichoptera	Psychomyiidae	Tinodes	12774.6	0.03	34	5.35

Table F2-3. Mobility traits that were evaluated. The source of most of this information was the Poff et al. 2006 traits matrix. Some also came from the USGS traits database (Vieira et al., 2006).

Mobility Trait	Trait States
Dispersal (adult)	low (<1 km flight before laying eggs), high (>1 km flight before laying eggs)
Adult flying strength	weak (e.g. cannot fly into light breeze), strong
Occurrence in drift	rare (catastrophic only), common (typically observed), abundant (dominant in drift samples)
Maximum crawling rate	very low (<10 cm/h), low (<100 cm/h), high (>100 cm/h)
Swimming ability	none, weak, strong

Table F2-4a. Number of cold-water taxa in each order

Order	Total
Plecoptera	10
Diptera	8
Trichoptera	7
Ephemeroptera	6
Coleoptera	1
Dorylaimida	1

Table F2-4b. Number of cold-water taxa in each family

Family	Total
Heptageniidae	4
Empididae	3
Perlodidae	3
Tipulidae	2
Uenoidae	2
Ameletidae	1
Apataniidae	1
Blephariceridae	1
Capniidae	1
Ceratopogonidae	1
Chloroperlidae	1
Dorylaimidae	1
Elmidae	1
Ephemerellidae	1
Glossosomatidae	1
Hydropsychidae	1
Lepidostomatidae	1
Leuctridae	1
Limnephilidae	1

Table F2-4b. Continued

Family	Total
Nemouridae	1
Pelecorhynchidae	
Peltoperlidae	1
Psychodidae	1
Taeniopterygidae	1
	1

Table F2-5a. Number of warm-water taxa in each order

Order	Total
Trichoptera	5
Coleoptera	3
Diptera	2
Ephemeroptera	2
Hemiptera	1
Isopoda	1
Odonata	1
Plecoptera	1

Table F2-5b. Number of warm-water taxa in each family

Family	Total
Elmidae	2
Leptoceridae	2
Asellidae	1
Caenidae	1
Coenagrionidae	1
Hydropsychidae	1
Hydroptilidae	1
Leptohyphidae	1
Naucoridae	1
Perlidae	1
Psephenidae	1
Psychodidae	1
Psychomyiidae	1
Stratiomyidae	1

APPENDIX G

Detailed Results North Carolina

The intent of this appendix is to provide more comprehensive and detailed information on the large number of analyses that were performed on the North Carolina data. Some of the analyses that are covered in this appendix are also referenced in the main body of the APM report. When this occurred, attempts were made to reduce any overlap or duplication in the reporting of results.

G1. Overview

North Carolina’s Biological Assessment Unit rates sites as Excellent (5), Good (4), Good/Fair (3), Fair (2) or Poor (1). Historically bioclassifications had been assigned based on EPT richness alone or in combination with total taxa richness. However, interpretations were sometimes troublesome because criteria often needed to be adjusted to account for differences in factors like collection method, stream size, seasonal changes and ecoregion, so the North Carolina Biotic Index (NCBI) was developed as an independent method of rating water quality. The NCBI uses tolerance values that are derived from the NC database. The NCBI is a summary measure of the tolerance values of organisms found in the sample relative to their abundance (see NC Standard Operating Procedures (SOP) 2006 for more details). NCBI scores, which range from 0 (best) to 10 (worst), are calculated for samples collected using the standard qualitative (full-scale) or EPT collection methods.

For most sites, equal weight is given to both the NCBI value and EPT taxa richness when assigning bioclassifications. Exceptions are outlined in the NC SOP (2006) and include such things as pristine high altitude mountain streams, swamp streams, and Coastal B streams (see NC SOP 2006 for more details). Under normal circumstances NCBI and EPT taxa richness measures are averaged together. A rounding approach is used when the two scores differ by one bioclassification and produce a final score midway between two ratings (1.5, 2.5, 3.5 or 4.5). In this situation EPT abundance is taken into account when deciding whether to round up or round down. Abundance of organisms is recorded as rare=1 (1-2 specimens), common=3 (3-9 specimens) or abundant (≥10 specimens). Scoring criteria are outlined in **Figure G-1** (see NC SOP 2006 for more details). **Figure G-1** also shows that bioclassification criteria have been developed for three major ecoregions (as defined by NCDENR): Mountain (MT), Piedmont (P) and Coastal Plain (CA).

Score	BI Values Mt	BI Values P	BI Values CA	EPT Values MT	EPT Values P	EPT Values CA
5	<4.00	<5.14	<5.42	>43	>33	>29
4.6	4.00-4.04	5.14-5.18	5.42-5.46	42-43	32-33	28
4.4	4.05-4.09	5.19-5.23	5.47-5.51	40-41	30-31	27
4	4.10-4.83	5.24-5.73	5.52-6.00	34-39	26-29	22-26
3.6	4.84-4.88	5.74-5.78	6.01-6.05	32-33	24-25	21
3.4	4.89-4.93	5.79-5.83	6.06-6.10	30-31	22-23	20
3	4.94-5.69	5.84-6.43	6.11-6.67	24-29	18-21	15-19
2.6	5.70-5.74	6.44-6.48	6.68-6.72	22-23	16-17	14
2.4	5.75-5.79	6.49-6.53	6.73-6.77	20-21	14-15	13
2	5.80-6.95	6.54-7.43	6.78-7.68	14-19	10-13	8-12
1.6	6.96-7.00	7.44-7.48	7.69-7.73	12-13	8-9	7
1.4	7.01-7.05	7.49-7.53	7.74-7.79	10-11	6-7	6
1	>7.05	>7.53	>7.79	0-9	0-5	0-5

Biotic Index corrections for non-summer data:
Summer = Jun-Sep, Fall = Oct-Nov, Winter = Dec-Feb, Spring = Mar-May

	Fall	Winter	Spring
Mountain Correction	+0.4	+0.5	+0.5
Piedmont Correction	+0.1	+0.1	+0.2
Coastal A Correction	+0.2	+0.2	+0.3

Rounding Criteria: Round down if EPT N < criterion, otherwise round up.

Bioclassification (Score)	MT	P	CA
Excellent (5) vs. Good (4)	191	135	108
Good(4) vs. Good-Fair (3)	125	103	91
Good-Fair (3) vs. Fair (2)	85	71	46
Fair (2) vs. Poor (1)	45	38	18

Figure G-1. **These tables are used to determine the scores for EPT taxa richness values and NCBI values for all standard qualitative samples after seasonal corrections are made. EPT N refers to EPT abundance (NC SOP 2006).**

G2 North Carolina Ecoregion Description

The major ecoregions defined by NCDENR differ slightly from EPA Level 3 ecoregions. Sites in the NCDENR Mountain ecoregion generally fall within the Blue Ridge EPA Level 3 ecoregion, which runs along the western portion of the state. Sites in the NCDENR Piedmont ecoregion are generally in the Piedmont EPA Level 3 ecoregion, which runs through the central portion of North Carolina. The NCDENR Coastal ecoregion generally overlaps with the Southeastern Plains and Middle Atlantic Coastal Plain EPA Level 3 ecoregions, which are located in the eastern portion of the state.

The major ecoregions are quite different. Terrain in the Mountain ecoregion ranges from narrow ridges to hilly plateaus to more massive mountainous areas with high peaks. Elevations generally range from 305-1524 meters, with Mount Mitchell, the highest point in North Carolina, and highest in the U.S. east of the Mississippi River, reaching 2037 meters. There is a high diversity of flora and fauna with high gradient, cool, clear streams with rocks and boulders. Forest-related land uses occur along with some small areas of pasture, apple orchards, and Christmas tree farms. Low-density recreational activities in forested settings have also become a typical land-use. (Griffith et al., 2002)

The Piedmont ecoregion is a transitional area between the mostly mountainous ecological regions of the Appalachians and the relatively flat coastal plain. Several major land cover transformations have occurred in the Piedmont over the past 200 years, from forest to farm, back to forest, and now in many areas, spreading urban- and suburbanization. Once largely cultivated with crops such as cotton, corn, tobacco and wheat, most of the Piedmont soils were moderately to severely eroded (Trimble, 1974). Much of this region is now in planted pine or has reverted to successional pine and hardwood woodlands with some pasture in the land cover mosaic (Griffith, et al. 2002).

The Coastal ecoregion consists of low elevation, flat plains, with many swamps, marshes, and estuaries. Pine plantations for pulpwood and lumber are typical with some areas of cropland. In some areas there is a mix of cropland, pasture, woodland, and forest. Over the past three centuries, naval stores or pine tar production, logging, open range cattle and feral hog grazing, agriculture, and fire suppression removed almost all of the longleaf pine forests. Streams in this area are relatively low-gradient and sandy-bottomed (Griffith et al., 2002).

More biological sampling sites are located in the Mountain and Piedmont ecoregions (1185 and 1007, respectively) than in the Coastal ecoregion (365). As expected, average elevations of the Mountain sites are much higher than the other sites (637 meters in the mountains vs. 155 meters in the Piedmont and 37 meters in the Coastal Plain).

G3 Results

G3.1 Distributions of temperature-indicator taxa

Results are summarized in **Section 2** of the report. We used PRISM mean annual precipitation as a surrogate for flow at sites that did not have USGS gages. In the **Figure G-2** example, PRISM mean annual precipitation tracked fairly well with mean monthly July-August discharge (which corresponded to the collection period for benthic samples that we examined in our analyses)

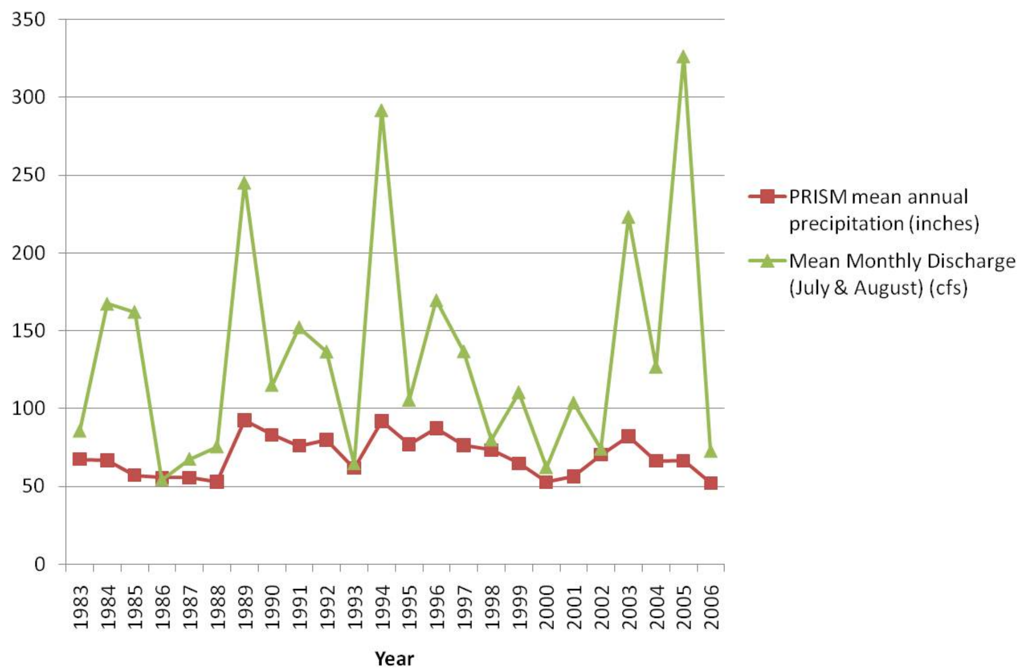


Figure G-2. Relationship between PRISM mean annual precipitation and mean monthly discharge over time at NC0207 (Nantahala River).

It was difficult to examine the relationship between flow and biology at most individual sites due to lack of flow data or discontinuities in the biological data at sites with USGS flow gages (**Figure G-3**). However, data from NC0109 (New River) showed significant relationships between PRISM mean annual precipitation and several biological metrics (**Figures G-4 and G-5**). This included thermal-preference richness metrics (# cold-water taxa $r=0.85$, $p<0.01$; # warm-water taxa $r= -0.65$, $p=0.03$).

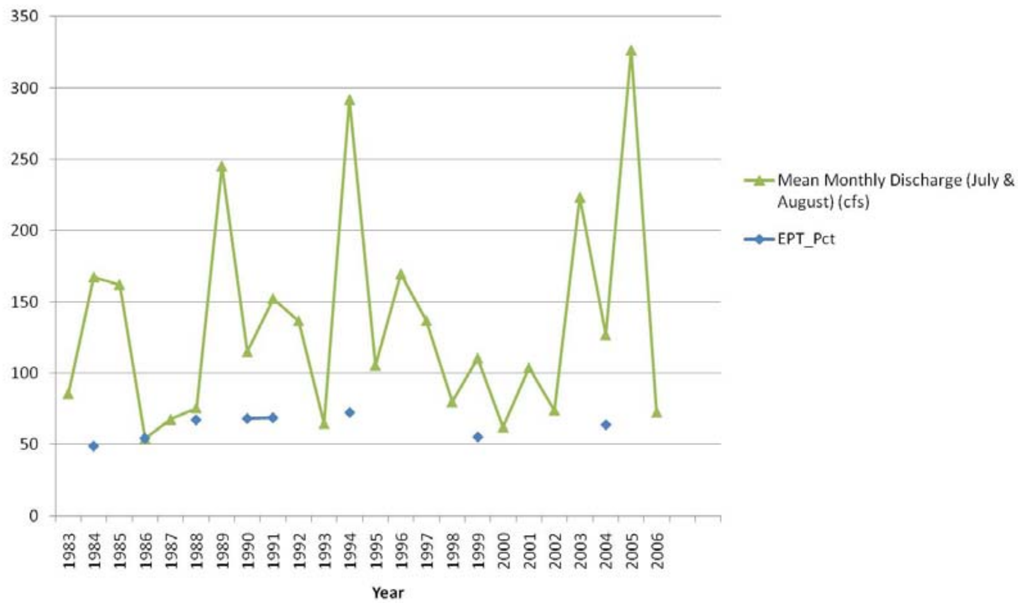


Figure G-3. Relationship between mean monthly discharge and % EPT individuals over time at NC0207 (Nantahala River).

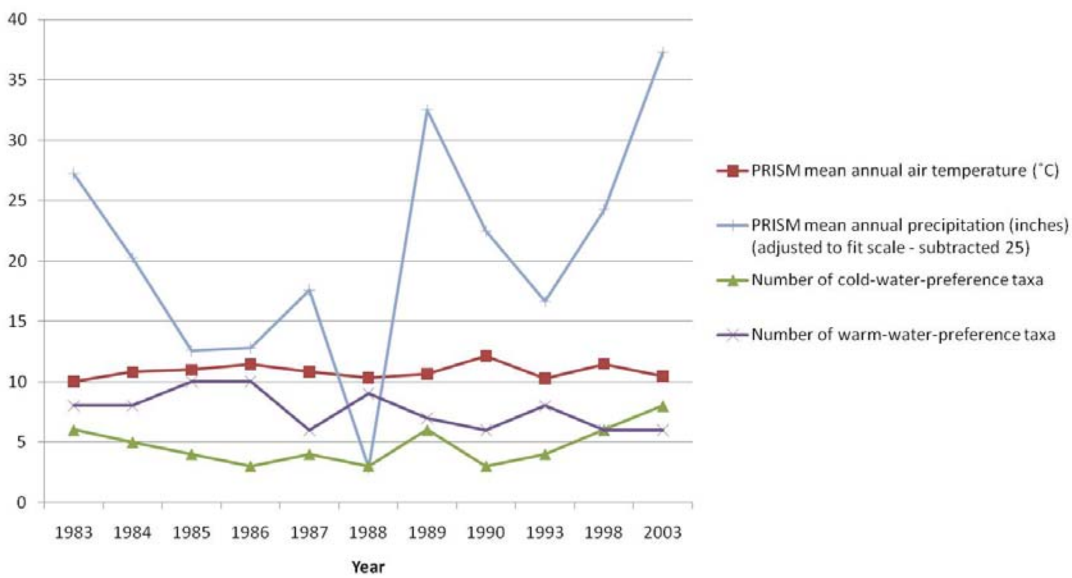


Figure G-4. Relationship between PRISM mean annual air temperature, PRISM mean annual precipitation and thermal preference richness metrics at site NC0109 (New River).

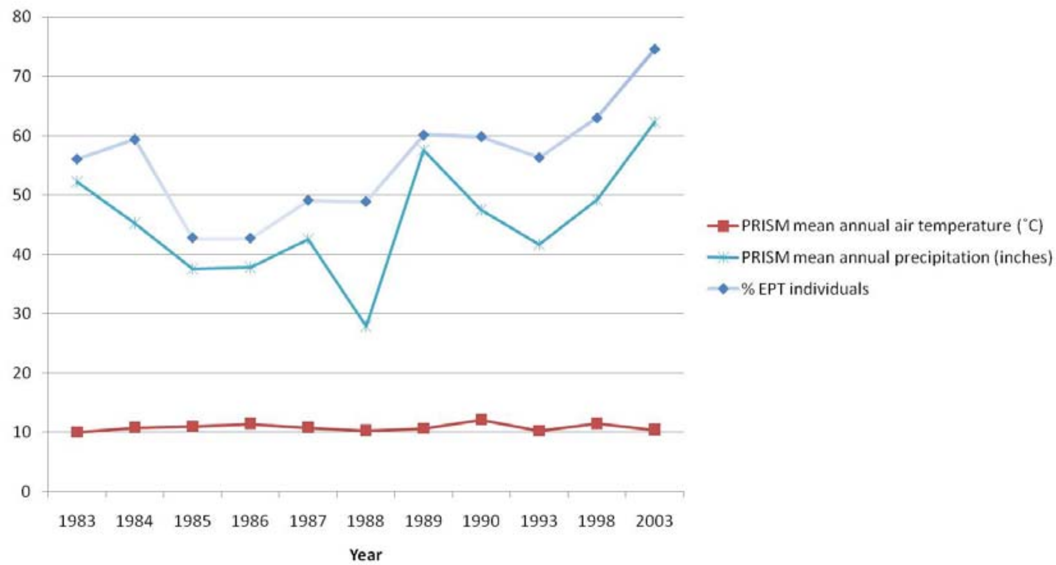


Figure G-5. Relationship between PRISM mean annual air temperature, PRISM mean annual precipitation and % EPT individuals at site NC0109 (New River).

Tables G-1 and **G-2** summarize distribution and abundance information for the North Carolina cold- and warm-water-preference taxa at the 5 sites (Stations NC0109 - New, NC0207 - Nantahala, NC0209 - Cataloochee, NC0075 - Little and NC0248 - Barnes) that were analyzed for long-term trends. At these stations, the most prevalent cold-water-preference taxa were Antocha and Promoresia which occurred at all the sites and in low to moderate abundances. Acentrella, Atherix, Dolophilodes, Epeorus, and Eukiefferiella occurred at 6 of the sites and therefore also appear to be stronger indicators. Procladius, Placobdella and Stenochironomus were the most prevalent warm-water-preference taxa. They occurred at 5 sites and generally had higher mean relative abundances than the other taxa. Chimarra and Macromia also appear to occur in higher abundances than most of the other warm-water-preference taxa.

Table G-1. Summary of distribution and abundance information for the *cold-water-preference taxa*at the 5 sites (Stations NC0109, NC0207, NC0209, NC0075 and NC0248). #Sites refers to the number of sites at which the taxa occurs. A=absent. P=present (highlighted in grey). Relative abundance codes: L=low (<0.01), M=medium (0.01-0.1), H=high (>0.1) (M or H are in bold type). Guide to interpretation: P-1L = present, occurred during1 year, low relative abundance (RA), P-11M = present, occurred during 11 years, medium RA, etc.

FinalID	#Sites	NC0109	NC0207	NC0209	NC0075	NC0248
Acentrella	6	P-3L	A	P-1L	P-1L	**P-5M**
Agapetus	3	A	P-3L	P-1L	A	A
Amphinemura	2	A	P-2L	A	A	A
Antocha	7	P-3L	**P-8M**	**P-7M**	P-3L	P-3L
Apatania	3	A	P-1L	P-1L	A	A
Arctopsyche	3	A	**P-6M**	**P-6M**	A	A
Atherix	6	A	P-7L	**P-7M**	**P-5M**	**P-6M**
Cardiocladius	5	P-5L	A	P-3L	A	P-1L
Cinygmula	0	A	A	A	A	A
Clioperla	3	A	A	A	P-1L	P-1L
Cultus	4	A	P-2L	P-1L	A	P-1L
Diamesa	3	A	P-1L	P-2L	A	A
Dicranota	3	A	**P-9M**	**P-7M**	A	A
Diploperla	3	A	P-1L	A	A	A
Dolophilodes	6	P-2L	**P-9M**	**P-7M**	A	P-2L
Drunella	4	P-8L	**P-8M**	**P-7M**	A	A
Epeorus	6	P-6L	**P-9M**	**P-7M**	A	**P-6M**
Eukiefferiella	6	P-3L	P-6L	**P-7M**	P-2L	A
Glossosoma	4	P-3L	**P-8M**	**P-7M**	A	A
Heleniella	2	A	P-1L	A	A	A
Isoperla	5	A	**P-6M**	**P-7M**	A	P-2L
Lanthus	5	A	**P-9M**	**P-7M**	A	P-1L
Malirekus	3	A	P-4L	**P-6M**	A	A
Nixe	3	A	P-1L	P-5L	A	A
Pagastia	4	P-1L	**P-6M**	P-4L	A	A
Parapsyche	2	A	A	P-1L	A	A
Potthastia	4	A	P-2L	P-1L	A	P-1L
Promoresia	7	P-10M	P-6L	**P-7M**	P-1L	P-3L
Rheopelopia	3	A	P-2L	P-2L	A	A
Rhithrogena	5	P-3L	P-4L	**P-7M**	A	A
Tallaperla	5	P-5L	**P-9M**	**P-7M**	A	A
Zapada	0	A	A	A	A	A

Table G-2. Summary of distribution and abundance information for the *warm-water-preference taxa* at the 5 sites (Stations NC0109, NC0207, NC0209, NC0075 and NC0248). #Sites refers to the number of sites at which the taxa occurs. A=absent. P=present (highlighted in grey). Relative abundance codes: L=low (<0.01), M=medium (0.01-0.1), H=high (>0.1) (M or H are in bold type). Guide to interpretation: P-1L = present, occurred during1 year, low relative abundance (RA), P-11M = present, occurred during 11 years, medium RA, etc.

FinalID	#Sites	NC0109	NC0207	NC0948	NC0075	NC0248
Belostoma	0	A	A	A	A	A
Berosus	1	P-1L	A	A	A	A
Caecidotea	3	A	A	P-1L	P-4L	A
Chimarra	4	**P-11M**	A	A	**P-6M**	**P-7M**
Elliptio	2	P-2L	A	A	A	A
Epicordulia	0	A	A	A	A	A
ERPOBDELLA/ MOOREOBDELLA	5	P-4L	A	P-1L	P-1L	P-2L
Helobdella	4	P-2L	A	A	P-2L	P-1L
Helocordulia	3	A	A	A	P-3L	P-1L
Hetaerina	1	P-3L	A	A	A	A
Ischnura	0	A	A	A	A	A
Lioporeus	1	A	A	A	P-1L	A
Macromia	5	P-7L	A	A	**P-7M**	**P-6M**
Macrostemum	2	P-6L	A	A	P-1L	A
Neureclipsis	2	P-7L	A	A	A	A
Neurocordulia	4	P-1L	A	A	P-5L	P-2L
Nilothauma	4	A	P-1L	A	A	P-2L
Palaemonetes	1	A	A	A	A	A
Parachironomus	0	A	A	A	A	A
Pentaneura	1	P-1L	A	A	A	A
Phylocentropus	5	A	A	A	P-1L	P-1L
Physella	4	**P-8M**	A	A	P-3L	P-4L
Placobdella	6	P-2L	P-1L	P-1L	P-2L	P-1L
Procladius	7	**P-10M**	P-2L	P-2L	P-2L	P-2L
Stenochironomus	6	**P-8M**	P-1L	P-2L	P-4L	P-3L
Tetragoneuria	1	A	A	A	P-1L	A
Tricorythodes	2	**P-11M**	A	A	A	A

G3,2 How cold- and warm-water indicator taxa may affect EPT taxa richness, the NCBI and final bioclassification levels

Attachment G2 contains tables with lists of the temperature indicator taxa, temperature optima values that were calculated from the maximum likelihood modeling, and the tolerance values assigned by NCDENR that are used to calculate the NCBI. It should be noted that the tolerance values are assigned at the species level by NCDENR. Because the maximum likelihood modeling was done at the genus-level, some of the tolerance values had to be averaged across species to get one value for each genus. The number of species within each genus that have been assigned tolerance values along with minimum and maximum tolerance values of species within each genus are also included in these tables. There is a fair amount of variation within some genera.

Results of the analyses that were performed to examine potential climate change effects on the EPT taxa richness metric are discussed in **Section 2** of the report. Potential effects on the NCBI are discussed in **Section 3** of the report (see also **Attachment G2).** One set of results that was not included in the report, but that are shown here, is from the correlation analysis of BI values and PRISM mean annual precipitation variables. Results show BI values[1] and PRISM mean annual precipitation variables to be significantly correlated at 3 of the sites (**Figure G-6**).

[1] For this particular analysis, we used the original BI values that were provided to us by NCDENR- this is important to note because, as mentioned earlier, NCDENR calculates the BI values based on species, while Tetra Tech calculated it based on genus-level OTUs.

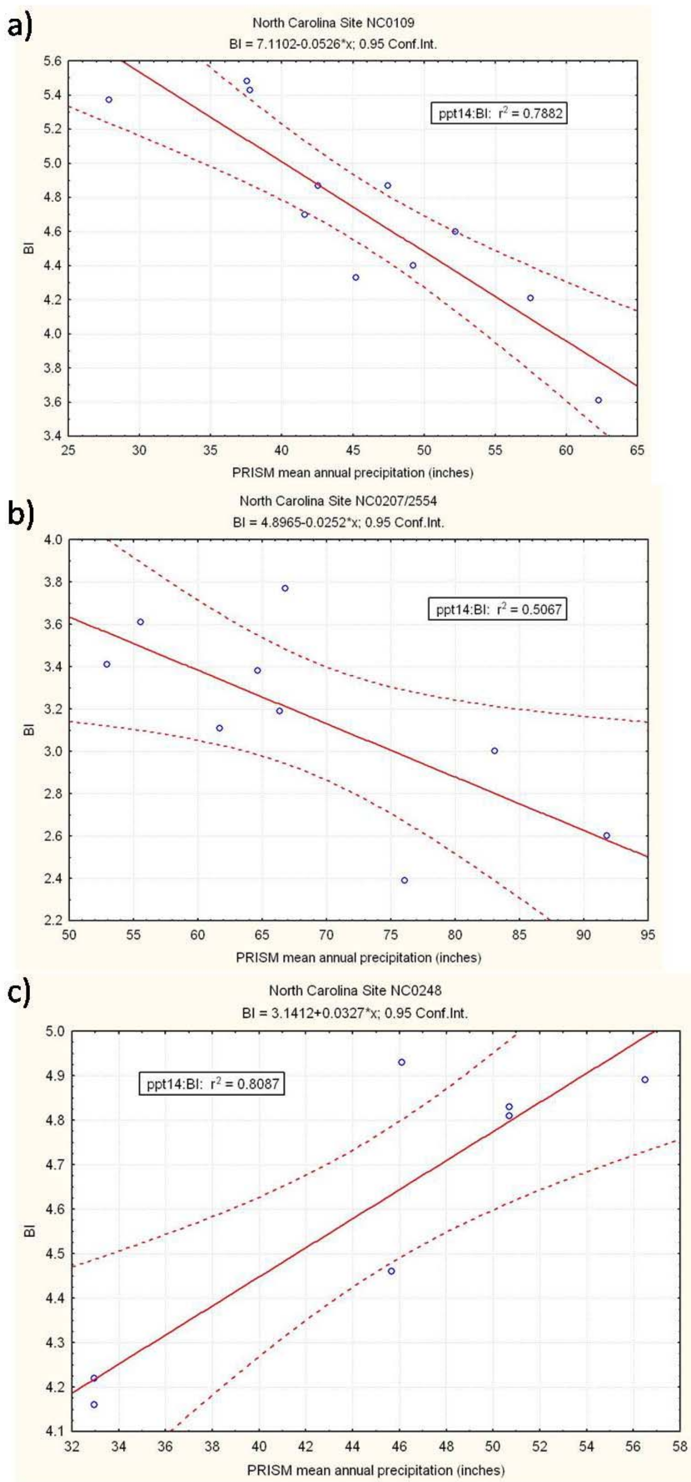


Figure G-6. BI values and PRISM mean annual precipitation variables were significantly correlated at 3 of the reference sites that were used in the correlation analyses: a) NC0109 - New, b) NC 0207 - Nantahala **and c) NC0248 -** Barnes**. BI values are original values provided to us by NCDENR.**

Two of the sites are Mountain sites and one is a Piedmont site. At the 2 Mountain sites, NC0207 (Nantahala) and NC0109 (New), the variables are significantly negatively correlated (r^2=0.51 and r^2=0.79, respectively), while at the Piedmont site, NC0248 (Barnes), the variables are positively correlated (r^2=0.81). This suggests that there are site-specific differences as well as differences among major ecoregions.

Results of the analysis simulating the effects of the loss of cold-water taxa on bioclassification scores for 3 Mountain reference sites are discussed in **Section 3** of the report, as are results of the analysis in which Mountain criteria were applied to biotic assemblages at selected reference Piedmont sites**.**

G3,3 Correlation analyses - commonly used metrics and climate-related variables

Metrics that are significantly correlated with the PRISM air temperature variables are summarized in **Table G-3**. There are not many strong or consistent relationships between the commonly used metrics and the temperature variables. Results appear to be mostly site-specific. Only two metrics were significantly correlated with a temperature variable at more than one site: the % climbers metric was negatively correlated with previous year PRISM mean annual air temperature at 2 Blue Ridge sites; and the % predators metric was positively correlated with PRISM mean annual air temperature at a Blue Ridge site and negatively correlated with it at a Piedmont site. The 2 Piedmont sites have the most number (5) of metric values significantly correlated with mean annual average air temperature (from the sampling year). Station NC0207 (Nantahala) has the most number (5) of significantly correlated metrics with the temperature difference (sampling year – previous year) variable.

Results of the correlation analyses using the PRISM mean annual precipitation variables are summarized in **Table G-4**. More metrics were significantly correlated with precipitation variables than with temperature variables. But as with the temperature variables, there are not many strong or consistent relationships and results appear to be mostly site-specific. Four metrics were significantly correlated with a precipitation variable at more than one site: the Hilsenhoff Biotic Index (HBI) (which used NC tolerance values, averaged at the genus-level) was negatively correlated with PRISM mean annual precipitation at 2 Blue Ridge sites; the % climber metric was negatively correlated with mean annual precipitation at 1 Blue Ridge and 1 Piedmont site; the % shredder metric was negatively correlated with ***previous year*** mean annual precipitation at 1 Blue Ridge and 1 Piedmont site; and the % burrower metric was negatively correlated with the precipitation difference (sampling year – previous year) variable at 1 Blue Ridge site and positively correlated at 1 Piedmont site. Station NC0109 has the most number (16) of metric values significantly correlated with PRISM mean annual precipitation values. Station NC0207 (Nantahala) has the most number (10) of significantly correlated metrics with the precipitation difference (sampling year – previous year) variable. Closer examination of the data shows that mean annual precipitation values increased by 30 inches from 1993-1994, which likely affected the biota.

Table G-3. Metric values that are significantly correlated with the selected *temperature* variables are shown. + means positively correlated; - means negatively correlated. Values are in bold print if they are significant at more than one site.

	Metric	**Blue Ridge**			**Piedmont**	
		NC0109 (New)	**NC0207** (Nantahala)	**NC0209** (Cataloochee)	**NC0075** (Little)	**NC0248** (Barnes)
PRISM mean annual average air temperature (tmean14)	% Burrowers				-	
	# Predator Taxa				-	
	% Predators		+		-	
	# Cold-water Indicator Taxa					-
	% Drier Losers					-

	Metric	**Blue Ridge**			**Piedmont**	
		NC0109	**NC0207**	**NC0209**	**NC0075**	**NC0248**
Previous year PRISM mean annual average air temperature	# Trichoptera Taxa	+				
	# Climber Taxa			-		
	% Swimmers			+		
	% Climbers		-	-		
	% Sprawlers				-	
	% Collector-gatherers		-			

Table G-3. Continued

	Metric	Blue Ridge			Piedmont	
		NC0109	NC0207	NC0209	NC0075	NC0248
Absolute difference between PRISM mean annual average air temperature from the sampling year and the previous year	# Total Taxa		+			
	# Plecoptera Taxa		+			
	# Swimmer Taxa		+			
	# Climber Taxa		+			
	% Clingers					-
	# Predator Taxa		+			
	% Shredders			-		
	% Cold-water Indicators					+
	# OCH Taxa					+

Table G-4. Metric values that are significantly correlated with the selected *precipitation* variables are shown. + means positively correlated; - means negatively correlated. Values are in bold italicized print if they are significant at more than one site.

		Blue Ridge			Piedmont	
	Metric	**NC0109** (New)	**NC0207** (Nantahala)	**NC0209** (Cataloochee)	**NC0075** (Little)	**NC0248** (Barnes)
PRISM mean annual precipitation (ppt14)	# Total Taxa	-				
	# Ephemeroptera Taxa					-
	# Plecoptera Taxa	+				
	% Plecoptera		+			
	% EPT	+				
	HBI	-	-			
	# Climber	-				
	% Clingers	+				
	% Climbers	-			-	
	# Herbivore Taxa	-				
	# Predator Taxa	-				
	% Predators	-				
	% Cold-water Indicators	+				
	# Cold-water Indicator Taxa	+				
	# Warm-water Indicator Taxa	-				
	% Perennial	+				
	% Drought Resistant	+				
	# of Intermittent Taxa	-				
		Blue Ridge			**Piedmont**	
	Metric	**NC0109**	**NC0207**	**NC0209**	**NC0075**	**NC0248**
Previous year PRISM mean annual precipitation	# EPT Taxa		+			
	% Plecoptera		+			
	# Burrower Taxa			-		
	# Collector-gatherer taxa					+
	% Shredders			-	-	
	% Drier Losers			+		

	% Warm Drier Losers			+		
	# OCH Taxa	-				

Table G-4. **Continued**

	Metric	Blue Ridge			Piedmont	
		NC0109	**NC0207**	**NC0209**	**NC0075**	**NC0248**
Absolute difference between PRISM mean annual precipitaton from the sampling year and the previous year	# Total Taxa		-			
	# Plecoptera Taxa					-
	# EPT Taxa			+		
	# Swimmer Taxa		-			
	# Burrower Tax		-			
	# Sprawler Taxa		-			
	% Burrowers	-				+
	# Collector-gatherer taxa		-			
	# Predator Taxa		-			
	% Collector-filterer	+				
	% Shredders		+			
	% Herbivores		-			
	% Predators					+
	% Perennial	+				
	% Intermittent					+
	# Perennial Taxa		-			
	# Intermittent Taxa		-			

G3.4 Summary

- The mean number of cold-water -preferencetaxa at sites in the Mountain ecoregion is significantly higher than the mean number of cold-water-preference taxa at sites in the other two ecoregions. The mean number of warm-water-preference taxa is significantly different between all 3 ecoregions with the highest number occurring in the Coastal ecoregion and the lowest number occurring in the Mountain ecoregion.
- Significantly more cold-water-preference taxa are present at higher elevation sites than at lower elevation sites. There is a significantly higher number of warm-water-preference taxa at lower elevation sites.
- Many of the cold-water-preference taxa in North Carolina are EPT taxa: 8 of the cold-water-preference taxa are Plecopterans, 6 are Trichopterans and 6 are Ephemeropterans. There are substantially fewer EPT taxa on the warm-water-preference list: 1 warm-water-preference taxais an Ephemeroptera, 4 are Trichopterans and none are Plecopterans.

- Within the EPT genera on the cold-water-preference list, there are 53 species that could potentially be counted towards the EPT richness metric that is used in the bioclassification of sites in NC, while there are only 5 species that could be potentially counted from the warm-water-preference list (therefore changes to the cold-water-preference taxa are likely to have a greater effect on EPT richness scores).
- EPT Richness: a loss of 3 (Coastal sites) or 4 (Mountain or Piedmont sites) EPT species can lower the EPT richness score at a high quality site a full level, from a 5 (Excellent) to a 4 (Good). To drop the EPT richness bioclassification by a full level at sites of lesser quality, it would take a loss of 10 taxa at Mountain sites, 8 taxa at Piedmont sites and 7 taxa at Coastal sites.
- NCBI: an increase in BI scores of 0.1 can lower the BI score at a high quality site a full level, from a 5 (Excellent) to a 4 (Good). To drop the NCBI bioclassification by a full level at sites of lesser quality, NCBI scores would have to increase by at least 0.6 (it varies by ecoregion and bioclassification level).
- When cold-water-preference taxa were eliminated from the biotic assemblages at 3 references sites in the Mountain ecoregion, the effects on EPT richness scores, NCBI scores and overall bioclassification levels were relatively small and site-dependent:
    - At Station NC0109, which had fewer cold-water-preference taxa than the other 2 sites, the loss of cold-water-preference taxa resulted in little if any change to EPT richness ratings (maximum loss of 4 species, maximum decrease in EPT_S score of 0.6), little if any change to BI values and scores (maximum increase in BI ***value*** of 0.24, maximum decrease in BI ***score*** of 0.2) and the maximum drop in overall score was 1 bioclassification level (from Excellent to Good), and this occurred 3 out of 11 years
    - At Stations NC0209 and NC0207/2554, removal of cold-water-preference taxa resulted in the loss of 9 to 14 EPT species decreases in EPT_S scores ranging from 0.4 to 1.2, an increase in BI values ranging from 0.45 to 0.86 and decreases in BI scores ranging from 0 to 1, and the maximum drop in score was one bioclassification level (from Excellent to Good), which occurred 5 out of 7 years at Site NC0209 and 5 out of 8 years at Site NC0207/NC2554.
- Effects at the other 2 sites were more noticeable because they have many more cold-water-preference taxa. Removal of cold-water-preference taxa resulted in the loss of 9 to 14 EPT species decreases in EPT_S scores ranging from 0.4 to 1.2.
- 22 of the 30 cold-water-preference taxa that have been assigned tolerance values have low tolerance values (< 3). Tolerance values for most of the warm-water-preference taxa are higher. Twelve of the warm-water-preference taxa that have been assigned tolerance values have tolerance values > 7.
- Temperature optima values are significantly and positively correlated with tolerance values (r=0.53, p=00), indicating that taxa that show preferences for lower temperatures tend to have lower tolerance values and those that tend to occur more in warmer water habitats tend to have higher tolerance values.
- Results from correlation analyses using thermal preference metrics and BI values suggest that replacement of colder water taxa with warmer water taxa would likely contribute to a site receiving a higher BI score and therefore a poorer rating, and that this is most likely to affect sites in the Mountain ecoregion.

- BI values (which were provided to us by NCDENR) and PRISM mean annual precipitation variables were significantly correlated at 3 of the sites. At the 2 Mountain sites, the variables are negatively correlated (r^2=0.51 and r^2=0.79, respectively), while at the Piedmont site, the variables are positively correlated.
- When Mountain bioclassification criteria was applied to the biotic assemblages at the 2 Piedmont sites to simulate how ratings may change if taxa that typically inhabit Mountain sites are replaced by assemblages that are more typical of the Piedmont ecoregion, bioclassifications consistently dropped by 1 level.
- There are not many strong or consistent relationships between the commonly used metrics and the temperature variables. Results appear to be mostly site-specific.
- Two metrics were significantly correlated with a temperature variable at more than one site: the % climbers metric was negatively correlated with previous year PRISM mean annual air temperature at 2 Blue Ridge sites; and the % predators metric was positively correlated with PRISM mean annual air temperature at a Blue Ridge site and negatively correlated with it at a Piedmont site.
- More metrics were significantly correlated with precipitation variables than with temperature variables. But as with the temperature variables, there are not many strong or consistent relationships and results appear to be mostly site-specific.
- Four metrics were significantly correlated with a precipitation variable at more than one site: the HBI (which used NC tolerance values, averaged at the genus-level) was negatively correlated with PRISM mean annual precipitation at 2 Blue Ridge sites; the % climber metric was negatively correlated with mean annual precipitation at 1 Blue Ridge and 1 Piedmont site; the % shredder metric was negatively correlated with ***previous year*** mean annual precipitation at 1 Blue Ridge and 1 Piedmont site; and the % burrower metric was negatively correlated with the precipitation difference (sampling year – previous year) variable at 1 Blue Ridge site and positively correlated at 1 Piedmont site.

Attachment G1

North Carolina Temperature Indicator Taxa

This attachment contains tables with lists of the North Carolina temperature-indicator taxa and describes the process that we followed to develop these lists.

ATTACHMENT G1. NORTH CAROLINA TEMPERATURE-INDICATOR TAXA

Sources. The North Carolina cold- and warm-water taxa lists were developed using several different sources: 1. maximum likelihood calculations based on a subset of the North Carolina biomonitoring database (using full-scale collection method data); 2. the thermal-preference trait from the Poff et al. (2006) traits matrix; 3. the thermal-preference trait from the USGS traits database (Vieira et al., 2006); 4. the thermal preference trait from the compilation of EPA Environmental Requirements and Pollution Tolerance series from the late 1970's (Beck et al., 1977; Harris et al., 1978; Hubbard et al., 1978, Surdick et al., 1978); 5. best professional judgment of the Southeast Climate Change traits feedback group[2].

The same general criteria and guidelines that were used to designate cold- and warm-water indicator taxa in Maine were also used in North Carolina (see Attachment D2). Also, see Attachment D2 for general limitations of the analyses.

Initial Results. Initially there were 126 taxa on the cold-water list and 112 taxa on the warm-water list. These lists were based on maximum likelihood calculations and literature. These lists were further refined through the evaluation of additional evidence. This evidence included analyses of other datasets, case studies, and best professional judgment. Taxa with the greatest amount of evidence were designated as temperature indicator taxa. More detailed information about the steps that were used to develop the temperature indicator taxa lists is summarized below:

Considerations (unique to North Carolina)

Several 'case studies' were performed to see whether the cold- or warm-water taxa occurred at sites in North Carolina that had the warmest or coldest summer water temperatures. The following case studies were performed:

a. **Cold-water Case Study #1**. Taxa lists from two Blue Ridge reference sites(NC1560-BEAR CR and NC1561-HAZEL CR) that have full-scale collection method data, have <5% urban and <10% agricultural land use within a 1 km buffer, and have the coldest recorded summer water temperatures (13-14° C in July). Note: there were a number of sites with temperature readings of 0°C; these readings seemed questionable so they were not used.

[2] North Carolina DWQ (Trish MacPherson), South Carolina (Jim Glover) and Tennessee (Debbie Arnwine)

b. **Cold-water Case Study #2**. The taxa list from the Piedmont site (NC0634-TOWN FORK CR) that has full-scale collection method data and has the coldest recorded Piedmont summer water temperature (9° C in August). This site has 4.3% urban and 11% agricultural land uses within a 1 km buffer.

c. **Cold-water Case Study #3**. Taxa lists from three Piedmont reference sites (NC0248-BARNES CR, NC0713-CATAWBA R, NC1607-MARLOWE CR) that have full-scale collection method data, have <5% urban and <10% agricultural land uses within a 1 km buffer, and have the coldest recorded summer water temperatures (16-17° C in August and September).

d. **Warm-water Case Study #1**. Taxa lists from the two warmest reference sites in the state (NC1466-CAPE FEAR R and NC1467-CAPE FEAR R) that have full-scale collection method data, have <5% urban and <10% agricultural land uses within a 1 km buffer, and have the warmest recorded summer water temperatures (30-32° C in July).

e. **Warm-water Case Study #2**. Taxa lists from the two Piedmont reference sites (NC0219-TAR R and NC0573-DEEP R) that have full-scale collection method data, have <5% urban and <10% agricultural land uses within a 1 km buffer, and have the warmest recorded summer water temperatures (28-29° C in July).

f. **Warm-water Case Study #3**. Taxa list from the warmest Blue Ridge reference site(NC1285-CROOKED CR) that has full-scale collection method data, has <5% urban and <10% agricultural land uses within a 1 km buffer, and has the warmest recorded summer water temperature (24° C in July).

Development of the Temperature-Indicator Cold-Water Taxa List. Taxa were placed on the cold-water list if the following criteria were met:

1. The taxon received a 'yes' per best professional judgment AND has been recorded at one or more of the cold-water case study sites AND has NOT been recorded at either of the two warm-water case study sites.

2. The taxon received a 'yes' per best professional judgment AND received a Total Score of 5 or more.

3. The taxon received a 'no comment' per best professional judgment AND has been recorded at two or more of the cold-water case study sites AND no species variation was noted.

Development of the Temperature-Indicator Warm-Water List. Taxa were placed on the warm-water list if the following criteria were met:

1. The taxon received a 'yes' per best professional judgment AND has been recorded at one or more of the warm-water case study sites AND has NOT been recorded at either of the two cold-water case study sites.
2. The taxon received a 'yes' per best professional judgment AND received a Total Score of 5 or more.
3. The taxon received a 'no comment' per best professional judgment AND has been recorded at two or more of the warm-water case study sites AND no species variation was noted.
4. The taxon received a 'no comment' per best professional judgment AND received a Total Score of 5 or more AND has been recorded at one or more warm-water case study sites AND NOT at any of the cold-water case study sites AND no species variation was noted.

Temperature Indicator Lists. The cold-water taxa list was comprised of 32 taxa and the warm-water taxa list was comprised of 27 taxa. **Tables G1-1** and **G1-2** show the temperature indicator taxa lists.

Important Notes – variation within genera. Some noteworthy genera were left off the North Carolina cold-water taxa list. These included Ephemerella, Neophylax, Rhyacophila, Goera, Eurylophella and Paragnetina. The reason they were not included is because there is variation in temperature preferences among species within these genera, and this was noted by the Southeast Climate Change feedback group. Genera that were left off the warm-water list due to species variation included Hydropsyche, Oecetis and Polypedilum.

Dispersal Ability. If temperature is a major factor influencing community composition, then taxa that are able to adapt to warming temperatures and/or that are able to disperse to more favorable habitats (generally believed to be upstream or to higher elevations) have a better chance of surviving. Five mobility traits were examined for the taxa on the North Carolina temperature indicator lists: dispersal (adult), adult flying strength, occurrence in drift, maximum crawling rate and swimming ability. **Table G1-3** lists more information on these traits.

Dispersal (adult) and adult flying strength received the greatest amount of consideration. Because movement is most likely to be upstream, taxa that are strong fliers are likely to have a better chance of success. It will be difficult for taxa that disperse via occurrence in drift to migrate upstream, and taxa that disperse via crawling or swimming are likely to have difficulty moving the distances required to find more favorable habitats.

One of the 32 taxa on the North Carolina temperature indicator cold-water taxa list (that we had trait information for), Clioperla, is categorized as having 'high' dispersal ability. Another taxon, Lanthus, is categorized as having strong flying ability but low adult dispersal ability. Nine of the 27 taxa on the warm-water list are categorized as having high adult dispersal ability. Six of these taxa are considered to be strong fliers.

Abundance and Distribution. In addition to dispersal ability, abundance and distribution are also important considerations. Those taxa that are widespread and common are likely to have greater genetic diversity and greater chance of adapting than rare taxa that only occur in isolated, localized populations (Sweeney et al., 1992). Moreover, the more abundant taxa are more likely to affect the state biomonitoring assessments. Abundance and distribution information for the temperature indicator taxa can be found in **Tables G1-1** and **G1-2**. It should be noted once again that the abundance data in the North Carolina dataset is categorical (1=rare (1-2 specimens), 3=common (3-9 species) and 10=abundant (10 or more species).

The most abundant cold-water temperature indicator taxa are Epeorus (Ephemeropteran), Antocha (Dipteran), Isoperla (Plecopteran) and Tallaperla (Plecopteran). These taxa comprise only 0.4 to 0.6% of the total individuals in the North Carolina database. Seventeen of the cold-water taxa have overall abundances of less than 0.1%. Physella (Basommatophora), Chimarra (Trichopteran) and Macromia (Odonata) are the most abundant warm-water taxa, with overall abundances ranging from 0.6 to 0.8%. Twelve of the warm-water taxa have overall abundances of less than 0.1%. Of the cold-water taxa, Antocha occurs at the largest percentage of sites (25%), followed by a Chironomidae, Eukiefferiella, and a Plecopteran, Isoperla, which occur at 18-19% of the sites. Eighteen of the cold-water taxa occur at less than 10% of the sites. Among the warm-water taxa, Physella occurs at the highest percentage of sites (30%), followed by Macromia (29%) and Stenochironomus (27%). Nineteen of the warm-water taxa occur at less than 10% of the sites.

Additional information – Cold-water Taxa.

Ten of the cold-water taxa are Dipterans, eight are Plecopterans, six are Ephemeropteran and six are Trichopterans. The rest are Coleopterans and Odonates. The families with the most number of taxa on the cold-water list are Chironomidae, Perlodidae and Heptageniidae (**Table G1-4**).

Additional information – Warm-water Taxa.

Seven of the warm-water taxa are Odonates, five are Dipterans and four are Trichopterans. The families with the most number of taxa on the warm-water list are Chironomidae and Corduliidae (**Table G1-5**).

Table G1-1. List of North Carolina cold-water temperature indicator taxa. Distribution and abundance information is also included. Sum_Individuals=the total number of individuals from that taxon in the North Carolina database; Pct_Abund=percent of total individuals in the database comprised of that taxon; Num_Stations=number of stations in the database that the taxon occurred at; Pct_Stations=percent of stations in the database at which the taxon occurred.

Type	Order	Family	FinalID	Sum_Individs	Pct_Abund	Num_Stations	Pct_Stations
cold	Ephemeroptera	Baetidae	Acentrella	2745	0.33	427	15.19
cold	Trichoptera	Glossosomatidae	Agapetus	247	0.03	53	1.89
cold	Plecoptera	Nemouridae	Amphinemura	1210	0.14	281	10
cold	Diptera	Tipulidae	Antocha	5103	0.61	711	25.29
cold	Trichoptera	Apataniidae	Apatania	339	0.04	47	1.67
cold	Trichoptera	Hydropsychidae	Arctopsyche	222	0.03	40	1.42
cold	Diptera	Athericidae	Atherix	1236	0.15	240	8.54
cold	Diptera	Chironomidae	Cardiocladius	2300	0.27	376	13.38
cold	Ephemeroptera	Heptagenidae	Cinygmula	247	0.03	40	1.42
cold	Plecoptera	Perlodidae	Clioperla	574	0.07	155	5.51
cold	Plecoptera	PERLODIDAE	Cultus	296	0.04	70	2.49
cold	Diptera	Chironomidae	Diamesa	734	0.09	185	6.58
cold	Diptera	Tipulidae	Dicranota	1384	0.16	284	10.1
cold	Plecoptera	Perlodidae	Diploperla	393	0.05	122	4.34
cold	Trichoptera	Philopotamidae	Dolophilodes	2905	0.35	316	11.24
cold	Ephemeroptera	EPHEMERELLIDAE	Drunella	2846	0.34	218	7.76
cold	Ephemeroptera	Heptageniidae	Epeorus	5226	0.62	403	14.34
cold	Diptera	CHIRONOMIDAE	Eukiefferiella	2974	0.35	533	18.96
cold	Trichoptera	Glossosomatidae	Glossosoma	1755	0.21	309	10.99
cold	Diptera	Chironomidae	Heleniella	95	0.01	50	1.78
cold	Plecoptera	PERLODIDAE	Isoperla	4556	0.54	498	17.72
cold	Odonata	Gomphidae	Lanthus	1174	0.14	300	10.67
cold	Plecoptera	Perlodidae	Malirekus	753	0.09	132	4.7
cold	Ephemeroptera	HEPTAGENIIDAE	Nixe	64	0.01	16	0.57
cold	Diptera	Chironomidae	Pagastia	751	0.09	157	5.59

Table G1-1. **Continued**

Type	Order	Family	FinalID	Sum_Individs	Pct_Abund	Num_Stations	Pct_Stations
cold	Trichoptera	Hydropsychidae	Parapsyche	280	0.03	52	1.85
cold	Diptera	CHIRONOMIDAE	Potthastia	757	0.09	292	10.39
cold	Coleoptera	Elmidae	Promoresia	3020	0.36	332	11.81
cold	Diptera	Chironomidae	Rheopelopia	135	0.02	64	2.28
cold	Ephemeroptera	Heptageniidae	Rhithrogena	725	0.09	152	5.41
cold	Plecoptera	Peltoperlidae	Tallaperla	3337	0.4	377	13.41
cold	Plecoptera	NEMOURIDAE	Zapada	3	0	3	0.11

Table G1-2. **List of North Carolina warm-water temperature indicator taxa. Distribution and abundance information is also included. Sum_Individuals=the total number of individuals from that taxon in the North Carolina database; Pct_Abund=percent of total individuals in the database comprised of that taxon; Num_Stations=number of stations in the database that the taxon occurred at; Pct_Stations=percent of stations in the database at which the taxon occurred.**

Type	Order	Family	FinalID	Sum_Individs	Pct_Abund	Num_Stations	Pct_Stations
warm	Hemiptera	Belostomatidae	Belostoma	173	0.02	99	3.52
warm	Coleoptera	Hydrophilidae	Berosus	1843	0.22	277	9.85
warm	Isopoda	ASELLIDAE	Caecidotea	3203	0.38	544	19.35
warm	Trichoptera	Philopotamidae	Chimarra	5178	0.62	554	19.71
warm	Unionoida	UNIONIDAE	Elliptio	1556	0.18	189	6.72
warm	Odonata	Corduliidae	Epicordulia	178	0.02	78	2.77
warm	Arhynchobdellida	ERPOBDELLIDAE	ERPOBDELLA/ MOOREOBDELLA	760	0.09	210	7.47
warm	Rhynchobdellida	Glossiphoniidae	Helobdella	835	0.1	225	8
warm	Odonata	Corduliidae	Helocordulia	188	0.02	95	3.38
warm	Odonata	Calopterygidae	Hetaerina	854	0.1	153	5.44
warm	Odonata	Coenagrionidae	Ischnura	318	0.04	101	3.59
warm	Coleoptera	Dytiscidae	Lioporeus	182	0.02	83	2.95

150 **Table G1-2. Continued**

Type	Order	Family	FinalID	Sum_Individs	Pct_Abund	Num_Stations	Pct_Stations
warm	Odonata	Corduliidae	Macromia	5064	0.6	813	28.92
warm	Trichoptera	Hydropsychidae	Macrostemum	1753	0.21	134	4.77
warm	Trichoptera	Polycentropodidae	Neureclipsis	2092	0.25	241	8.57
warm	Odonata	Corduliidae	Neurocordulia		0.18	278	9.89
warm	Diptera	Chironomidae	Nilothauma	180	0.02	124	4.41
warm	Decapoda	Palaemonidae	Palaemonetes	2262	0.27	271	9.64
warm	Diptera	Chironomidae	Parachironomus	395	0.05	128	4.55
warm	Diptera	Chironomidae	Pentaneura 1511	771	0.09	154	5.48
warm	Trichoptera	Dipseudopsidae	Phylocentropus	576	0.07	201	7.15
warm	Basommatophora	Physidae	Physella	6677	0.79	853	30.35
warm	Rhynchobdellida	Glossiphoniidae	Placobdella	677	0.08	339	12.06
warm	Diptera	Chironomidae	Procladius	3460	0.41	706	25.12
warm	Diptera	Chironomidae	Stenochironomus	3419	0.41	750	26.68
warm	Odonata	CORDULIIDAE	Tetragoneuria	687	0.08	202	7.19
warm	Ephemeroptera	Leptohyphidae	Tricorythodes	4939	0.59	363	12.91

Table G1-3. Mobility traits that were evaluated. The source of most of this information was the Poff et al. 2006 traits matrix. Some also came from the USGS traits database (Vieira et al., 2006).

Mobility Trait	Trait States
Dispersal (adult)	low (<1 km flight before laying eggs), high (>1 km flight before laying eggs)
Adult flying strength	weak (e.g. cannot fly into light breeze), strong
Occurrence in drift	rare (catastrophic only), common (typically observed), abundant (dominant in drift samples)
Maximum crawling rate	very low (<10 cm/h), low (<100 cm/h), high (>100 cm/h)
Swimming ability	none, weak, strong

Table G1-4. Number of cold-water taxa in each family

Family	Total
Chironomidae	7
Perlodidae	5
Heptageniidae	4
Glossosomatidae	2
Hydropsychidae	2
Nemouridae	2
Tipulidae	2
Apataniidae	1
Athericidae	1
Baetidae	1
Elmidae	1
Ephemerellidae	1
Gomphidae	1
Peltoperlidae	1
Philopotamidae	1

Table G1-5. **Number of warm-water taxa in each family**

Family	Total
Chironomidae	5
CORDULIIDAE	5
Glossiphoniidae	2
Asellidae	1
Belostomatidae	1
Calopterygidae	1
Coenagrionidae	1
Dipseudopsidae	1
Dytiscidae	1
ERPOBDELLIDAE	1
Hydrophilidae	1
Hydropsychidae	1
Leptohyphidae	1
Palaemonidae	1
Philopotamidae	1
Physidae	1
Polycentropodidae	1
Unionidae	1

Attachment G2

Tolerance values of the North Carolina temperature-indicator taxa

This attachment contains tables with lists of the temperature-indicator taxa, temperature optima and tolerance values that were calculated from the maximum likelihood modeling, and the tolerance values assigned by North Carolina DWQ (which are used to calculate the NCBI). These tables were used to examine whether temperature-indicator taxa were considered to be sensitive or tolerant taxa.

ATTACHMENT G2 TOLERANCE VALUES OF THE NORTH CAROLINA TEMPERATURE INDICATOR TAXA

Table G2-1. *Cold-water* temperature indicator taxa. Temp_Opt is the temperature optima (°C) calculated during the maximum likelihood modeling (temperature tolerance could not be calculated for this dataset). Avg_TolVal was calculated by taking the average of the tolerance values assigned to species within the genera. The number of species within each genus that have been assigned tolerance values (NumSpecies) along with minimum (Min_TolVal) and maximum tolerance values (Max_TolVal) of species within each genus are also included.

Order	Family	Genus	Temp_Opt	NumSpecies	Avg_TolVal	Min_TolVal	Max_TolVal
Coleoptera	Elmidae	Promoresia	10.6	3	1.5	0	2.4
Diptera	Athericidae	Atherix	9	2	2.1	2.1	2.1
Diptera	Chironomidae	Cardiocladius	13.2	1	5.9	5.9	5.9
Diptera	Chironomidae	Diamesa	15.8	1	8.1	8.1	8.1
Diptera	CHIRONOMIDAE	Eukiefferiella	9	6	3.4	2.2	5.6
Diptera	Chironomidae	Heleniella	13	1	0	0	0
Diptera	Chironomidae	Pagastia	15.3	1	1.8	1.8	1.8
Diptera	CHIRONOMIDAE	Potthastia	15.2	3	5	2	6.5
Diptera	Chironomidae	Rheopelopia	9				
Diptera	Tipulidae	Antocha	15.7	1	4.3	4.3	4.3
Diptera	Tipulidae	Dicranota	9	1	0	0	0
Ephemeroptera	Baetidae	Acentrella	16.9	4	4.3	3.6	5.5
Ephemeroptera	EPHEMERELLIDAE	Drunella	9	8	0.2	0	1
Ephemeroptera	Heptagenidae	Cinygmula		1	0	0	0
Ephemeroptera	Heptageniidae	Epeorus	9	4	1.3	1	1.8
Ephemeroptera	HEPTAGENIIDAE	Nixe	9	3	0.7	0	1
Ephemeroptera	Heptageniidae	Rhithrogena	14.6	5	0.3	0.3	0.3
Odonata	Gomphidae	Lanthus	9	3	1.8	1.8	1.8
Plecoptera	Nemouridae	Amphinemura	9	1	3.3	3.3	3.3
Plecoptera	NEMOURIDAE	Zapada					
Plecoptera	Peltoperlidae	Tallaperla	9	1	1.2	1.2	1.2
Plecoptera	Perlodidae	Clioperla		1	4.7	4.7	4.7

Table G2-1. Continued

Order	Family	Genus	Temp_Opt	NumSpecies	Avg_TolVal	Min_TolVal	Max_TolVal
Plecoptera	PERLODIDAE	Cultus		1	1.6	1.6	1.6
Plecoptera	Perlodidae	Diploperla		2	2.1	1.4	2.7
Plecoptera	PERLODIDAE	Isoperla	9	12	1.7	0	5.4
Plecoptera	Perlodidae	Malirekus		1	1.2	1.2	1.2
Trichoptera	Apataniidae	Apatania		1	0.6	0.6	0.6
Trichoptera	Glossosomatidae	Agapetus		1	0	0	0
Trichoptera	Glossosomatidae	Glossosoma	9	1	1.6	1.6	1.6
Trichoptera	Hydropsychidae	Arctopsyche		1	0	0	0
Trichoptera	Hydropsychidae	Parapsyche	13.1	1	0	0	0
Trichoptera	Philopotamidae	Dolophilodes	9	1	0.8	0.8	0.8

Table G2-2. *Warm-water* temperature indicator taxa. Temp_Opt is the temperature optima (°C) calculated during the maximum likelihood modeling (temperature tolerance could not be calculated for this dataset). Avg_TolVal was calculated by taking the average of the tolerance values assigned to species within the genera. The number of species within each genus that have been assigned tolerance values (NumSpecies) along with minimum (Min_TolVal) and maximum tolerance values (Max_TolVal) of species within each genus are also included.

Order	Family	FinalID	Temp_Opt	NumSpecies	Avg_TolVal	Min_TolVal	Max_TolVal
Arhynchobdellida	ERPOBDELLIDAE	ERPOBDELLA/ MOOREOBDELLA	29.4	1	8.3	8.3	8.3
Basommatophora	Physidae	Physella	32	1	8.8	8.8	8.8
Coleoptera	Dytiscidae	Lioporeus	32	2	3	3	3
Coleoptera	Hydrophilidae	Berosus	32	1	8.4	8.4	8.4
Decapoda	Palaemonidae	Palaemonetes	31.5	2	7.1	7.1	7.1
Diptera	Chironomidae	Nilothauma	32	1	5	5	5
Diptera	Chironomidae	Parachironomus	32	4	8.5	6.5	9.6
Diptera	Chironomidae	Pentaneura	32	1	4.7	4.7	4.7
Diptera	Chironomidae	Procladius	32	1	9.1	9.1	9.1

Table G2-2. Continued

Order	Family	FinalID	Temp_Opt	NumSpecies	Avg_TolVal	Min_TolVal	Max_TolVal
Diptera	Chironomidae	Stenochironomus	32	1	6.5	6.5	6.5
Ephemeroptera	Leptohyphidae	Tricorythodes	32	1	5.1	5.1	5.1
Hemiptera	Belostomatidae	Belostoma	29.5	1	9.8	9.8	9.8
Isopoda	ASELLIDAE	Caecidotea	26.1	1	9.1	9.1	9.1
Odonata	Calopterygidae	Hetaerina	28.1	1	5.6	5.6	5.6
Odonata	Coenagrionidae	Ischnura	32	1	9.5	9.5	9.5
Odonata	Corduliidae	Epicordulia	28.4	2	5.6	5.6	5.6
Odonata	Corduliidae	Helocordulia	27.8	2	4.9	4.8	4.9
Odonata	Corduliidae	Macromia	32	2	6.2	6.2	6.2
Odonata	Corduliidae	Neurocordulia	32	4	3.5	1.8	5.2
Odonata	CORDULIIDAE	Tetragoneuria	32	2	8.6	8.5	8.6
Rhynchobdellida	Glossiphoniidae	Helobdella	28.9	3	9.1	8.6	9.5
Rhynchobdellida	Glossiphoniidae	Placobdella	27	3	8.9	8.7	9
Trichoptera	Dipseudopsidae	Phylocentropus	32	1	6.2	6.2	6.2
Trichoptera	Hydropsychidae	Macrostemum	32	1	3.5	3.5	3.5
Trichoptera	Philopotamidae	Chimarra	32	1	2.8	2.8	2.8
Trichoptera	Polycentropodidae	Neureclipsis	32	2	4.2	4.2	4.2
Unionoida	UNIONIDAE	Elliptio	32	3	4.2	2.4	5.1

APPENDIX H

Temporal Change in Regional Reference Condition as a Potential Indicator of Global Climate Change: Analysis of the Ohio Regional Reference Condition Database (1980-2006)

Analyses on Ohio data conducted by

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The intent of this appendix is to provide more comprehensive and detailed information on the analyses that were performed on the Ohio data. Some of the analyses that are covered in this appendix are also referenced in the main body of the report. When this occurred, attempts were made to reduce any overlap or duplication in the reporting of results.

H1 Overview of Ohio Indices and Associated Assessment Approach

The State of Ohio Environmental Protection Agency (Ohio EPA) calculates an Invertebrate Community Index (ICI) to evaluate biological condition based on the benthic macroinvertebrate assemblage and an Index of Biotic Integrity (IBI) used to evaluate fish assemblages at wading sites, boat sites and headwaters stream sites. The metrics that go into the ICI and IBI are shown in **Figures H1-1** and **H1-2** (State of Ohio Environmental Protection Agency, Environmental Assessment Section, Division of Water Quality, Planning and Assessment. 1989 (last updated 2008). Biological Criteria for the Protection of Aquatic Life: Volume III: Standardized Biological Field Sampling and Laboratory Methods for Assessing Fish and Macroinvertebrate Communities. http://www.epa.state.oh.us/dsw/bioassess/BioCriteriaProtAqLife.html).

		Score			
	Metric	0	2	4	6
1.	Total Number of Taxa	Varies with drainage area (Fig. 5-1)			
2.	Total Number of Mayfly Taxa	Varies with drainage area (Fig. 5-2)			
3.	Total Number of Caddisfly Taxa	Varies with drainage area (Fig. 5-3)			
4.	Total Number of Dipteran Taxa	Varies with drainage area (Fig. 5-4)			
5.	Percent Mayfly Composition	0	>0,≤10	>10,≤25	>25
6.	Percent Caddisfly Composition	Varies with drainage area (Fig. 5-6)			
7.	Percent Tribe Tanytarsini Midge Composition	0	>0,≤10	>10,≤25	>25
8.	Percent Other Dipteran and Non-Insect Composition	Varies with drainage area (Fig. 5-8)			
9.	Percent Tolerant Organisms (from Table 5-2)	Varies with drainage area (Fig. 5-9)			
10.	Total Number of Qualitative EPT Taxa	Varies with drainage area (Fig. 5-10)			

Figure H1-1. Macroinvertebrate community metrics and criteria for calculating the Invertebrate Community Index (ICI) and ICI scores for evaluating biological condition in

Ohio. Taken from Table 5-1 in Ohio EPA's 'Standardized Biological Field Sampling and Laboratory Methods for Assessing Fish and Macroinvertebrate Communities' (1989).

IBI Metric	Headwaters Sites[1,2]	Wading Sites[2]	Boat Sites[3]
1. Total Number of Species[4]	X	X	X
2. Number of Darter Species	X[5]	X	
% Round-bodied Suckers[6]			X
3. Number of Sunfish Species		X	X
Number of Headwaters Species	X		
4. Number of Sucker Species		X	X
Number of Minnow Species	X		
5. Number of Intolerant Species		X	X
Number of Sensitive Species	X		
6. % Green sunfish			
% Tolerant Species	X	X	X
7. % Omnivores	X	X	X
8. % Insectivorous Cyprinids			
% Insectivorous Species	X	X	X
9. % Top Carnivores		X	X
% Pioneering Species	X		
10. Number of Individuals[7]	X	X	X
11. % Hybrids			
% Simple Lithophils		X	X
Number of Simple Lithophilic Species	X		
12. % Diseased Individuals			
% DELT Anomalies[8]	X	X	X

1 applies to sites with drainage areas less than 20 sq. mi.
2 these sites are sampled with wading methods; 3 these sites are sampled with boat methods; 4 excludes exotic species; 5 includes sculpins.
6 includes suckers in the genera Hypentelium, Moxostoma, Minytrema, and Erimyzon; excludes white sucker (Catostomus commersoni).
7 excludes species designated as tolerant, hybrids, and exotics.
8 includes deformities, eroded fins, lesions, and external tumors (DELT).

Figure H1-2. Index of Biotic Integrity metrics used to evaluate wading sites, boat sites and headwaters stream sites in Ohio. Original metrics from Karr (1981) are given first with substitute metrics following. Taken from Table 4-1 in Ohio EPA's 'Standardized Biological Field Sampling and Laboratory Methods for Assessing Fish and Macroinvertebrate Communities' (1989).

H2 OHIO EPA REGIONAL REFERENCE DATABASE – BACKGROUND

Ohio was one of the early states to systematically use biological assemblage data to determine aquatic life use designations and assess the condition of those uses dating back to the late 1970s. Ohio implemented standardized sampling methods for biological assessments early on (late 1970s) hence their data represent a nearly thirty year span of standardized biological data for two assemblage groups. From the late 1970s to 2006 the Ohio fish assemblage database represents >10,000 unique sites and >24,000 unique sampling events; macroinvertebrate assemblage data were also collected at most of these same sites. Qualitative Habitat Evaluation Index (QHEI) data has also been included at the fish sites (Ohio EPA 2006; Rankin 1995, 1989). While the QHEI is visually based, recent analyses have shown it to be as precise as a quantitative habitat assessment tool to which it was compared (Miltner et al., 2009; Rankin, in preparation). The purpose of analyses presented here is to analyze any changes in the reference dataset that could represent signal or lack of signal related to the effects of global climate change.

In the 1980s and with assistance from the U.S. EPA, Office of Research and Development, Ohio EPA began a focused sampling of least impacted reference sites in order to determine the efficacy of level III ecoregions (Omernik, 1987) as a way to account for and stratify natural variations in biological assemblages (Yoder ,1989; Ohio EPA, 1987a; Whittier et al., 1987). Ohio EPA used this and other sampling data to establish a network of “least impacted” regional reference sites that eventually supported the derivation of numerical biocriteria for Ohio streams and rivers. This was also accomplished across all practically sampleable stream and rivers from >1 mi^2 up to the largest inland rivers (~6000-8000 mi^2). This includes both wadeable and non-wadeable. Fish assemblage indices were stratified by three stream- and river-size strata; headwater streams (<20 mi^2), “wadeable” streams (20 -~300 mi^2), and “boatable” (i.e., non-wadeable) rivers (>~150-200 mi^2) (Yoder and Rankin, 1995). Macroinvertebrate assemblage indices were calibrated continuously across the entire range of stream and river sizes. The initial reference dataset was developed from a statewide network of about 300 reference sites that was sampled over a ten year period (1980-89; Table H2-1). That reference site network was maintained and expanded with the initial re-sampling during 1990-99 and a second re-sampling that will be completed at the end of 2009 (2000-09). Data on habitat quality (QHEI), water quality, and other physical data such as temperature were also collected and were based on multiple grab samples collected during “normal” seasonal flows within a summer-fall seasonal index period (mid-June through mid-October).

Table H2-1. Summary of Ohio EPA regional reference site network including original sites (1980-89) and updates via first (1990-99) and second round re-sampling (2000-06) that were used in data analyses.

Reference Network	Size Type	Fish: Latest (All Data)	Macroinvertebrates
Original Reference Sites: 1980-89 (Sites/Samples)	Headwaters	112/225	242
	Wadeable	166/399	
	Boatable	97/254	
New Reference Sites: 1990-2006 (Sites/Samples)	Headwaters	115/(149)/150 (296)	309 (525)
	Wadeable	184(231)/281(539)	
	Boatable	68(84)/127(278)	

H3 DATA ANALYSES

Our primary goal is to examine the Ohio reference database for trends and the entire dataset for candidate indicators of climate change. Important effects of climate change include changes in not only temperature, but also rainfall patterns and resulting hydrological regimes in Ohio streams and rivers thus we also explored the usefulness of using the QHEI as an indirect measure of hydrological change.

H3.1 Trends in Ohio Reference Sites

We conducted an initial exploration of Ohio's reference database to determine whether biological reference condition has changed over time since 1980. This analysis directly overlapped with an Ohio EPA-sponsored effort to conduct the initial data analysis steps for the recalibration of the Ohio biocriteria (Rankin, 2008). Of particular importance to our analyses is the examination of trends in biological condition at the reference sites and exploring the potential causes that are associated with the observed changes. As such it is essential to understand the environmental changes that have also occurred that could potentially confound any signals of climate change-related effects. Based on nearly thirty years of intensive watershed assessments Ohio EPA has identified a variety of environmental changes that are associated with shifts in biological condition at the assemblage and species/taxa levels. Such environmental changes include, 1) a reduction in point source loadings (particularly important in non-wadeable rivers where some reference sites are necessarily downstream of point sources), 2) changes in land uses (e.g., increased urbanization), 3) changing loadings of pollutants from agricultural lands (e.g., declining sediments and nutrients in response to increased conservation tillage), 4) habitat changes (e.g., loss of habitat quality from agricultural drainage practices [common], suburbanization [common], improved habitat quality resulting stream restoration [rare and localized]), and 5) potential climate change related influences from changes to the temperature and/or hydrological regimes. These latter changes may be the most difficult to detect due to the lack of readily available long-term data for temperature and flow and the indirect actions of any adverse impacts. It is first important to identify any methodological differences in data collection (environmental and biological) that could either confound or mask apparent trends. In the Ohio dataset this is most likely represented by taxonomic refinements from an improving resolution in the identification of macroinvertebrates over the past 30 years. Thus we included some initial explorations and recommendations related to this factor for the Ohio data set. We focused primarily on the mayflies because they are an important component of the Ohio ICI, taxonomic refinements are known to have occurred, and taxonomic refinements would be expected to influence multiple metrics (total taxa, mayfly taxa, qualitative EPT taxa, etc.).

H3.2 Taxonomic Analyses

We used the entire Ohio database to identify “earliest” and “latest” years for all taxa in order to extract a list of possible taxa that could affect ICI scoring via taxonomic refinement (splitting or lumping of taxa). We focused on the mayfly taxa at reference sites and identified taxa and sites that occurred in the original reference sites, but not the new sites and vice versa. Table H2-1 lists all mayfly taxa collected at the Ohio reference sites that appeared earlier and then “disappeared” (“earlier”) or those that “appeared” later, mostly at re-sampled reference sites (“later”). We then conferred with senior Ohio EPA taxonomists (Mike Bolton and Jack Freda, Ohio EPA) and determined whether any of these taxa are purely a result of taxonomic changes made in the intervening time. These taxa were identified (Table H3-1) and the ICI recalculated with the same taxon designations as for the original references sites in order to attribute any changes in the total taxa metric, the mayfly metric, and the qualitative EPT metric to observed changes in the ICI. This effort primarily consisted of “lumping” individual taxa designations of mayfly taxa back to “Baetis sp.” or “Pseudocloeon sp.” (Table H3-1).

Table H3-1. Mayfly taxa from reference sites in Ohio that abruptly appeared (Later) or disappeared (Earlier) in the Ohio dataset and explanation of change. Explanations were provided by Mike Bolton and Jack Freda of Ohio EPA.

Taxa Code	Taxon Name	Appear-ence	Explanation of Change
11010	Acentrella sp	Later	Improved taxonomy allow this taxa to be distinguished Pseudocloeon sp.
11014	Acentrella turbida	Later	Improved taxonomy allow this taxa to be distinguished from Pseudocloeon sp.
11015	Acerpenna sp	Later	Improved taxonomy allow this taxa to be distinguished from Baetidae sp.
11018	Acerpenna macdunnoughi	Later	Improved taxonomy allow this taxa to be distinguished from Baetidae sp.
11020	Acerpenna pygmaea	Later	Improved taxonomy allow this taxa to be distinguished from Baetidae sp.
11110	Acentrella parvula	Later	Improved taxonomy allow this taxa to be distinguished from Pseudocloeon sp. or was renamed from Pseudocloeon parvulum
11115	Baetis tricaudatus	Later	Improved taxonomy allow this taxa to be distinguished from Baetidae sp.
11118	Plauditus dubius	Later	Improved taxonomy allow this taxa to be distinguished Pseudocloeon sp.
11119	Plauditus dubius or P. virilis	Later	Improved taxonomy allow this taxa to be distinguished Pseudocloeon sp.
11120	Baetis flavistriga	Later	Improved taxonomy allow this taxa to be distinguished from Baetidae sp.
11125	Pseudocloeon frondale	Later	Improved taxonomy allow this taxa to be distinguished from Baetidae sp.
11130	Baetis intercalaris	Later	Improved taxonomy allow this taxa to be distinguished from Baetidae sp.
11150	Pseudocloeon propinquum	Later	Improved taxonomy allow this taxa to be distinguished from Baetidae sp.
11155	Plauditus punctiventris	Later	Improved taxonomy allow this taxa to be distinguished Pseudocloeon sp.
11175	Plauditus virilis	Later	Improved taxonomy allow this taxa to be distinguished Pseudocloeon sp.
11250	Centroptilum sp (w/o hindwing pads)	Later	Improved taxonomy allow this taxa to be distinguished Cloeon sp.
11400	Centroptilum sp or Procloeon sp (formerly in Cloeon	Earlier	Improved taxonomy allow this taxa to be distinguished Cloeon sp.
11430	Diphetor hageni	Later	Improved taxonomy allow this taxa to be distinguished from Baetidae sp.
11503	Heterocloeon curiosum	Later	Renamed Heterocloeon (H.) sp, Heterocloeon sp.
11600	Paracloeodes sp 1	Later	Improved taxonomy allow this taxa to be distinguished from Paracloeodes sp

Table H3-1. Continued

11625	Paracloeodes sp 3	Later	Improved taxonomy allow this taxa to be distinguished from Paracloeodes sp
11645	Procloeon sp	Later	Was earlier classified as Centroptilum sp or Cloeon sp
11650	Procloeon sp (w/ hindwing pads)	Later	Was earlier classified as Cloeon sp
11651	Procloeon sp (w/o hindwing pads)	Later	Was earlier classified as Centroptilum sp
11670	Procloeon irrubrum	Later	Improved taxonomy allow this taxa to be distinguished from Cloeon sp
11700	Acentrella sp or Plauditus sp (formerly in Pseudoc	Earlier	Renamed as Pseudocloeon sp
13010	Leucrocuta hebe	Earlier	Renamed as Heptagenia hebe
13030	Leucrocuta maculipennis	Earlier	Renamed as Heptagenia maculipennis
14501	Leptophlebiidae	Earlier	Now coded as Leptophlebia sp
14900	Leptophlebia sp	Later	Leptophlebia sp
14950	Leptophlebia sp or Paraleptophlebia sp	Later	Small specimens lumped

H3.3 Weighted Stressor Values (WSVs)

Candidate fish and macroinvertebrate taxa that could serve as indicators of climate change (sensitive to temperature or other measures such as hydrological stressors) were determined from weighted stressor values (WSVs) and “Taxa Indicator Values” (TIVs) for temperature and habitat measures that would be correlated with hydrological alterations. The WSVs were generated by relating historical taxa/species from sites in Ohio to chemical and habitat stressors and calculating weighted average values for each taxa/stressor combination where the weighting is the relative abundance of the taxa/species at a site. TIV values for taxa were then ranked from most to least sensitive for each of the pertinent parameters and converted to an ordinal scale of 1-10 where 1 is the most sensitive and 10 the most tolerant following the methodology of Meador and Carlisle (2007). WSVs were then plotted vs. a simple means code by Ohio taxa/species tolerance designations to identify the indicator taxa that occur at the extremes of the distributions.

H3-4 QHEI Data

QHEI includes the habitat attributes of substrate, cover, channel, riparian, pools, riffle, and stream gradient (Rankin 1995, 1989). Recent analyses of the QHEI shows it to be relatively precise (Miltner et al., 2009), and it has been collected by trained professionals since its inception by Ohio EPA. We used a subset of the metric components to create a sub-index (Hydro-QHEI) that extracts the habitat attributes that are responsive either directly (current speed components) or indirectly (stream depth measures) to alterations of the flow regime. Scoring calculations for the Hydro-QHEI are detailed in Table H3-2. Hydro-QHEI ranges from 0 to 25 and includes the two QHEI subcomponents most related to hydrology, current and depth. We used the Hydro-QHEI and its two subcomponents to detect any trends in these components over time as evidence for potential effects from hydrological alterations. We also calculated WSVs for these components to identify taxa/species that could be sensitive to hydrological changes in Ohio.

Table H3-2. Sub-components of the Ohio QHEI which were used to score a Hydro-QHEI and current and depth sub-scores

Current Metric		**Depth Metric**	
QHEI Current Attribute	Score	QHEI Depth Attribute	Score
Very Fast Current	+5	Deep Pools (Cover Metric)	+4
Fast Current	+3	Pool Depths > 1m	+4
Moderate Current	+2	Pool Depths 0.7 – 1.0 m	+3
Slow Current	+1	Pool Depths 0.4 – 0.7 m	+2
Eddies	+2	Pool Depths 0.2 – 0.4 m	+1
Very Deep Riffles	+3	Pool Depths < 0.20	-1
Moderate Depth Riffles	+1	Deep Riffles	+3
Interstitial Flow	-1	Moderate Riffles	+2
Intermittent Flow	-3	Shallow Riffles	+1
		Riffles Absent or Non-functional	-1

H4 RESULTS

H3.1 Potential Trends in Ohio Reference Sites

Some of the following analyses were conducted for Ohio EPA in an initial assessment towards re-calibrating Ohio EPA's biocriteria based on data after 1988 (Rankin, 2008). Ohio's original reference site data was collected between 1978 and 1988. Table H4-1 summarizes the ranges of years that represent the universe of original and re-sampled reference sites. For analyzing trends in reference sites we used the latest data available for calculating updated biocriteria statistics. On average the latest data period was 13-16 years after the mean of the original reference sample dates (Table H4-1).

Table H4-1. Average and range of years represented by original reference site data and re-sampled (latest) data by index and stream size category pertaining to fish samples

Index/Stream Size	Mean Year Sampled (Range)	
	Original Reference Sites	Re-Sampled Sites
ICI – All Sites	1984 (1980-1988)	2000 (1989-2007)
IBI - Headwaters	1984 (1978-1988)	2000 (1989-2006)
IBI - Wading	1984 (1979-1988)	2000 (1990-2006)
IBI - Boat	1984 (1979-1988)	1997 (1990-2005)

Table H4-1 reports the original biocriteria values and statistics, a re-calculation of those statistics using refined variables, and “new” biocriteria values based on the latest re-sampled reference sites. Because possible IBI or ICI scores based on single samples are always even values, calculated percentile values were rounded upwards (e.g., 41 to a 42). Discrepancies between the original calculations and our recalculations are highlighted in yellow. The original biocriteria statistics were re-calculated in the database because there are a few minor discrepancies related to uncertainties about the exact membership of the original reference sites and gradual changes made to the database since 1990 due to changing taxonomy and a more precise calculation of drainage area (Rankin, 2009).

The direction of change in the biocriteria between the original and latest reference site data was either positive (an increase) or neutral (no change) with only three instances where the new biocriteria were lower. These included: 1) the ICI biocriterion for the non-acidic mine drainage modified use (-4 pts; possible small sample size); 2) the IBI for WWH headwater site type in the EOLP ecoregion (-2 pts); and, 3) the IBI for WWH headwater site type in the WAP ecoregion (-2 pts). None of these changes are considered to be greater than the non-significant departure for each index.

Table H4-2. Original Ohio biocriteria (O), recalculated biocriteria (R) using similar sites, and new biocriteria (N) using the latest data from re-sampling of original reference sites. Sites with discrepancies between original and recalculated criteria are highlighted in yellow

Ecoregion	Modified Warmwater Habitat (MWH)									WWH			EWH		
	Channelized			Non-Acidic Mine Drainage			Impounded								
IBI – Headwater Site Type															
	O	**R**	**N**	**O**	**R**	**N**	**O**	**R**	**N**	**O**	**R**	**N**	**O**	**R**	**N**
HELP	**20**	**20**	**26**							**28**	**-**	**-**	**50**	**50**	**52**
IP	**24**	**24**	**26**							**40**	**40**	**40**			
EOLP										**40**	**38**	**36**			
WAP				**24**	**24**	**a**				**44**	**44**	**42**			
ECBP										**40**	**40**	**44**			
IBI – Wadeable Site Type															
HELP	**22**	**22**	**22**							**32**	**-**	**-**	**50**	**50**	**52**
IP	**24**	**24**	**30**							**40**	**40**	**44**			
EOLP	**24**	**24**	**30**							**38**	**38**	**42**			
WAP	**24**	**24**	**30**	**24**	**24**	**32**				**44**	**44**	**46**			
ECBP	**24**	**24**	**30**							**40**	**40**	**40**			
IBI – Boatable Site Type															
HELP	**20**	**20**	**20**				**22**	**22**	**26**	**34**	**30** **34**	**30** **34**	**48**	**48**	**52**
IP	**24**	**24**	**24**				**30**	**28**	**34**	**38**	**38**	**47**			
EOLP	**24**	**24**	**24**				**30**	**28**	**34**	**40**	**40**	**46**			
WAP	**24**	**24**	**24**	**24**	**24**	**26**	**30**	**28**	**34**	**40**	**40**	**40**			
ECBP	**24**	**24**	**24**				**30**	**28**	**34**	**42**	**42**	**42**			
MIwb – Wadeable Site Type															
HELP	**5.6**	**5.9**	**6.4**							**7.3**	**-**	**-**	**9.4**	**9.4**	**9.5**
IP	**6.2**	**6.4**								**8.1**	**8.1**	**8.1**			
EOLP	**6.2**	**6.4**								**7.9**	**7.9**	**8.2**			
WAP	**6.2**	**6.4**		**5.5**	**4.7**	**6.1**				**8.4**	**8.3**	**8.8**			
ECBP	**6.2**	**6.4**								**8.3**	**8.3**	**7.8**			
MIwb – Boatable Site Type															
HELP	**5.7**	**5.7**	**7.5**[a]				**5.7**	**5.7**	**7.4**	**8.6**	**-**	**-**	**9.6**	**9.6**	**10.2**
IP	**5.8**	**5.7**	**6.1**[a]				**6.6**	**7.0**	**7.5**	**8.7**	**8.7**	**9.6**			
EOLP	**5.8**	**5.7**	**6.1**[a]				**6.6**	**7.0**	**7.5**	**8.7**	**8.8**	**8.9**			
WAP	**5.8**	**5.7**	**6.1**[a]	**5.4**	**5.4**	**6.4**	**6.6**	**7.0**	**7.5**	**8.6**	**8.6**	**9.2**			
ECBP	**5.8**	**5.7**	**6.1**[a]				**6.6**	**7.0**	**7.5**	**8.5**	**8.5**	**9.7**			

Table H4-2. Continued

ICI – All Site Types Combined													
HELP	**22**	**22**	**24**					**34**	**34**	**42**			
IP	**22**	**22**	**24**					**30**	**30**	**38**			
EOLP	**22**	**22**	**24**					**34**	**34**	**44**	**46**	**46**	**50**
WAP	**22**	**22**	**24**	**30**	**30**	**26**		**36**	**36**	**40**			
ECBP	**22**	**22**	**24**					**36**	**36**	**42**			
a – Non-acidic mining influenced modified sites for headwaters combined with wading sites due to small sample size.													

The direction of climate-related changes in biological index scores could be in either direction. However, the most plausible expectation would be for a decline due to the immediate loss of highly intolerant species and taxa (i.e., temperature- and flow-sensitive taxa/species) and a co-occurring increase in intermediate, moderately, and/or highly tolerant taxa/species. Such expectations are supported by our analyses that identify a general concordance between intolerant and sensitive species as categorized for the IBI and ICI and species sensitive to temperature and habitat features indicative of altered flow conditions.

The largest positive changes in the biocriteria were in the WWH boatable fish sites (IBI and MIwb) and in the WWH ICI. The fish assemblage changes in large rivers are most attributable to reduced pollution from point sources, mostly due to municipal wastewater treatment plant upgrades after 1988 (Yoder et al., 2005). While it was necessary in the derivation of the original Ohio IBI for boatable sites to include reference sites located in effluent dominated rivers, the sites were positioned below known recovery points. Nevertheless, the lessening of secondary impacts from nutrient enrichment by the aforementioned controls had positive effects on the fish assemblages at these reference sites. Taxonomic changes in fish nomenclature did not influence IBI scores between these time periods nor did the fish sampling technology as the methodology and equipment was generally stable between these time periods.

H4.2 Influence of Taxonomic Changes on Trend Assessment in Ohio

The question concerning the relative contribution of taxonomic changes to the macroinvertebrate assemblage trends in the Ohio biocriteria values at reference sites was also examined during this phase of the data analysis. While fish data can be influenced by factors such as sampling efficiency, their taxonomy has been comparatively stable during the period over which the Ohio reference database was developed. As for sampling methodology, methods used by Ohio EPA for both fish and macroinvertebrates have been stable over the period of the Ohio reference database. However, there have been significant changes in macroinvertebrate taxonomy over this time period mostly in the form of improved discrimination within certain genera (e.g., Baetid mayflies) that could result in changes to the ICI "number of" metrics for mayflies and other taxonomic groups that are also identified to more refined taxonomic resolution.

We developed a program to scan the Ohio EPA database and identify taxa that may have been revealed by improved taxonomy which would result in two or more taxa in lieu of a single taxon. This program resulted in a listing of all taxa and the first and last occurrence of each taxon in the Ohio EPA database. We then focused on the taxonomic changes in mayflies to examine the quantitative contribution of the refined taxonomy on ICI scoring for three metrics; total taxa, mayfly taxa, and qualitative EPT taxa. We then recalculated the mean number of taxa for each metric as it now occurs in the database ("refined" taxonomy) and then again with the taxonomy "lumped" to match the level of taxonomy that was prevalent during the derivation of the original biocriteria (Table H4-2). We also recalculated the biocriteria statistics (25^{th} percentiles by ecoregion for WWH; 75^{th} percentiles statewide for EWH) based on the newly refined and lumped taxonomy (Table H4-3).

The recalculation of ICIs from all sites indicated a 5.9 point increase in the mean ICI score between the two time periods. When mayfly taxonomy was lumped between these time periods the increase was 5.0 showing that taxonomic refinement in mayflies accounted for 14% of the increase in the mean ICI between the two reference time periods (Table H4-3). Only two cases showed a change in the biocriteria the HELP WWH biocriterion (38.5 compared to 42) and the EOLP WWH biocriterion (42 compared to 44).

The changes in mayfly taxonomy reflect the greatest influence on ICI scoring in the Ohio database; other taxa would likely have a lesser impact compared to the impact on mayfly metrics (Jack Freda, personal communication). Future work should isolate all of the other taxonomic refinements that could confound trends in metrics and index scores. Comparisons of similarity of macroinvertebrate taxonomy in samples between European countries concluded that taxonomic adjustments prior to analyses of the separate data sets reduced species richness from 45 to 81% by country and 85% for all countries combined (Verdonschot and Nijboer, 2004). We are dealing with much smaller changes in the Ohio database.

Table H4-3. Changes in ICI and mayfly influence ICI metrics related to increasing taxonomic resolution over time in the Ohio EPA least impacted reference data set

Metric	Original Reference Sites		New Reference Sites (Latest Data)	
	Standard Taxonomy Mean Taxa (Mean Score)	Lumped Taxonomy Mean Taxa (Mean Score)	Standard Taxonomy Mean Taxa (Mean Score)	Lumped Taxonomy Mean Taxa (Mean Score)
Total Taxa	35.97 (4.89)	35.93 (4.89)	38.36 (5.18)	37.65 (5.04)
Number of Mayfly Taxa	6.95 (4.20)	6.90 (4.17)	7.42 (4.59)	6.59 (4.16)
QUAL EPT Taxa	11.29 (3.63)	11.24 (3.60)	15.16 (5.16)	14.23 (4.91)
ICI Score	39.59	39.53	45.35	44.56

Table H4-4. Table of original and recalibrated Ohio biocriteria with adjustments made to equilibrate taxonomic advances made in the later time period. Highlighted cells indicate where standardizing taxonomic resolution would have resulted in altered criteria.

<table>
<tr><th></th><th colspan="3">Warmwater Habitat</th><th colspan="3">Exceptional Warmwater Habitat</th></tr>
<tr><th>Ecoregion</th><th>Original Reference</th><th>Latest Reference</th><th>Latest Reference w/ Refined Taxonomy</th><th>Original Reference</th><th>Latest Reference</th><th>Latest Reference w/ Refined Taxonomy</th></tr>
<tr><td>HELP</td><td>34</td><td>42</td><td>38.5</td><td rowspan="5">46</td><td rowspan="5">50</td><td rowspan="5">50</td></tr>
<tr><td>IP</td><td>30</td><td>38</td><td>38</td></tr>
<tr><td>EOLP</td><td>34</td><td>44</td><td>42</td></tr>
<tr><td>WAP</td><td>36</td><td>40</td><td>40</td></tr>
<tr><td>ECBP</td><td>36</td><td>42</td><td>42</td></tr>
</table>

H4.3 Weighted Stressor Values (WSVs)

We calculated WSVs for maximum temperature and Hydro-QHEI variables separately for headwater streams (drainage area ≤20 mi.^{2}) and wadeable streams (drainage area >20 to 300 mi.^{2}). These data are ordered by WSV for each parameter to provide a sequential listing of sensitive species/taxa that can be used to detect trends in relation to temperature or flow alterations. It also provides a listing of tolerant species that might increase in predominance if temperature were to increase or the hydrological regime became increasingly variable.

H4.4. Temperature

We used the maximum temperature recorded from summer-fall grab samples collected during the same period within which the biological data were collected to calculate WSVs for headwater and wadeable streams. To visualize the distribution of these data with taxa sensitivities we plotted the means of these values vs. the weighted means (WSVs) color coded by the existing taxa tolerance rankings of Ohio EPA (Figure H4-1). Because the temperature indicator was derived from a small number of grab samples, the precision of these data could be rather low for a given site. However, when aggregated across the temporal and spatial extent of Ohio EPA database we expect that relationships between taxa relative abundance and maximum summer temperatures should be much more representative of taxa sensitivities. Figure H3 represents plots of WSVs based on maximum temperatures (°C) from grab samples at sites with macroinvertebrate taxa collected from artificial substrates in headwater and wadeable streams. The WSVs for maximum temperature generally track with the "general" tolerance categories assigned by Ohio EPA for each taxon for both headwater (Figure H4-1, upper right) and wadeable streams (Figure H4-1, lower right). A similar pattern was observed for fish species. WSVs for temperature can be confounded with WSVs for other stressors, particularly habitat. However, the extremes of these distributions can be useful for identifying possible indicator taxa for future applications.

It is interesting to note that selected Chironomidae taxa occurred at both extremes of the WSV for temperature. For example, *Paratanytarsus n.sp 1* had the lowest WSV for temperature at wadeable sites and *Parachironomus "hirtalatus"* and *Tanypus neopunctipennis* had among the highest WSVs (Figure H4-1, lower left). Additional analysis using environmental traits could help in determining the rare taxa that could exhibit some sensitive traits, but which may be too rare by themselves to serve as useful indicators.

H4.5 Hydro-QHEI

We generated WSVs for Hydro-QHEI variables separately for headwater and wadeable streams for both fish and macroinvertebrates. We plotted several examples of the WSVs for these variables vs. the simple means for these same variables (Figure H4-2) in order to reveal the distributions of tolerant and sensitive species along this gradient as we did for temperature. Fish and macroinvertebrate WSVs for Hydro-QHEI and its subcomponents tracked relatively closely to the Ohio EPA tolerance designations for macroinvertebrate taxa and fish species (Figure H4-2). Outlier points and variability are often associated with small sample sizes for a given species at a given stream size. Intolerant species are frequently rarer than "sensitive" species, especially so for fish, and as such may exhibit more variation than "sensitive" species where sample sizes are typically larger. As expected, tolerant species generally have wider sensitivity ranges.

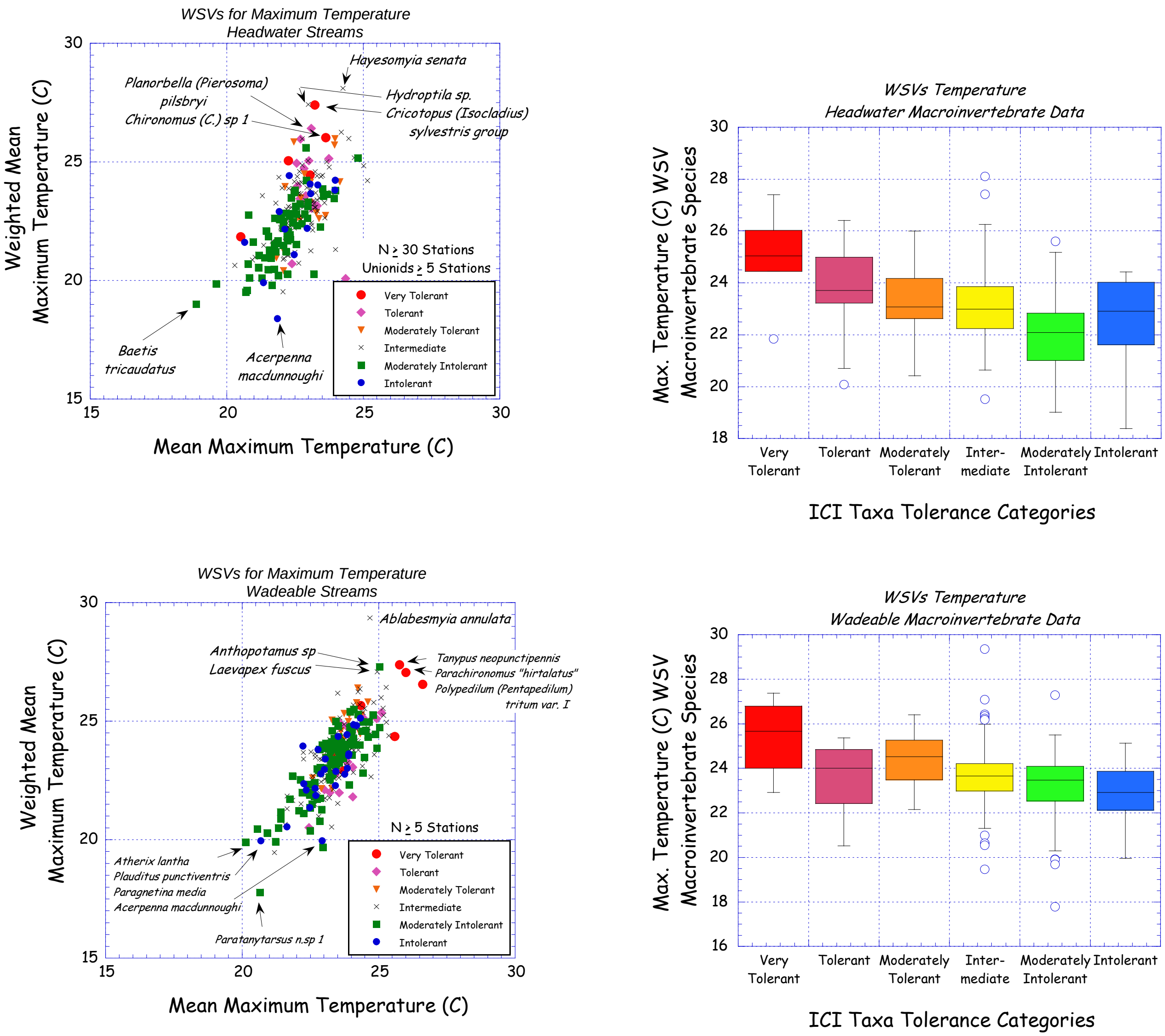

Figure H4-1. Plots of macroinvertebrate taxa maximum temperature WSV values vs. mean maximum values for taxa for headwater streams (upper left) and wadeable streams (lower left) and box and whisker plots of WSVs for maximum temperatures by Ohio EPA macroinvertebrate tolerance values (derived for the ICI) for headwater streams (upper right) and wadeable streams (lower right). Data for taxa represents data collected from artificial substrates where at least five samples were represented for each stream size category.

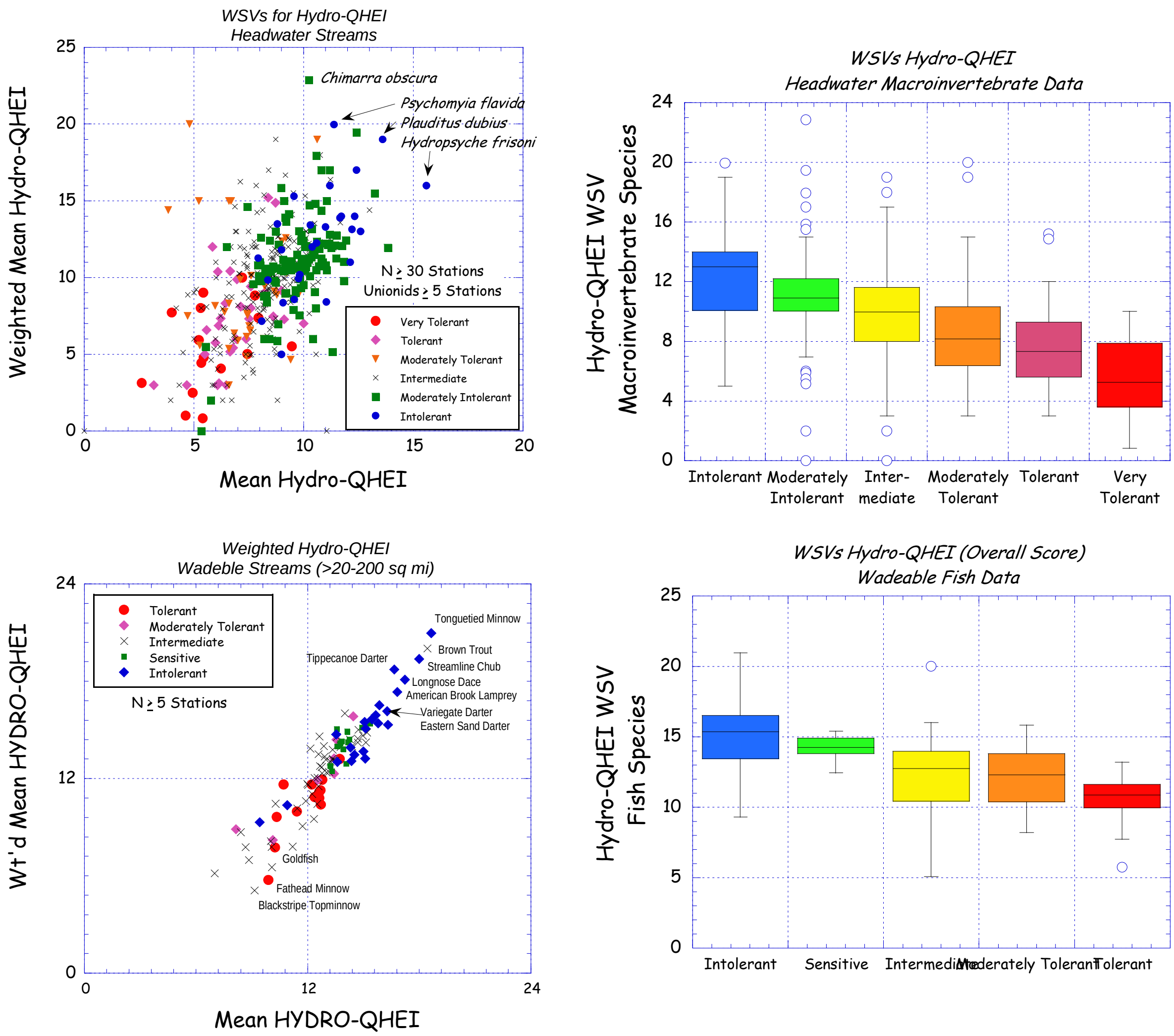

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Figure H4-2. Scatter plots of taxa/species Hydro-QHEI WSV values vs. mean Hydro-QHEI values for macroinvertebrates taxa for headwater streams (upper left) and for species in wadeable streams (lower left) and box and whisker plots of macroinvertebrate (upper right) and fish (lower right) WSVs for Hydro-QHEI for these waters. Data from Ohio EPA.

Although QHEI is a visual habitat tool, recent analyses of variation from sites with multiple QHEI values using signal/noise ratio analyses indicate the index is precise and the subcomponents are moderately precise to precise (Miltner et al., 2009 Draft; Rankin et al., in preparation). We chose Hydro-QHEI subcomponents that are expected to change in response to flow alterations. For example the presence of fast current or the presence of eddies is a characteristic of permanent summer base flows (QHEI assessments are generally conducted during summer-fall low flow periods). Habitat attributes related to depth (i.e., deep pool and deep runs) are also associated with permanent base flows. Thus the Hydro- QHEI is expected to reflect a gradient of base flow stability, one of the attributes that would be expected to change with changes in precipitation patterns as a result of climate change. Sensitive fish species and macroinvertebrate taxa were positively correlated with the Hydro-QHEI, thus it promises to be a useful tool for indicating hydrological changes that may be associated with climate change. These data are commonly collected by states throughout the Midwestern U.S.

H4.6 Species Distribution by Stream Size

The identification of certain intolerant fish species in headwater streams at the "sensitive" end of the Hydro-QHEI gradient suggests that the distribution of these species at the tails of their preferred stream size range may reflect the degree of base flow. Fish species such as streamline chub, variegate darter, river chub and stonecat madtom (all with high Hydro-QHEI WSVs) are generally found in larger wadeable streams and their presence in headwater streams is associated with high Hydro-QHEI scores that indicate more stable flow regimes. Year-to-year or long-term trends of these species in headwater streams could represent a response to climate-induced hydrologic changes. Thus we suggest that this could be an opportunity to explore whether the stream size "tails" of sensitivity distributions shift with hydrological change.

The Ohio database does contain a stream-size bias because headwater streams were less frequently sampled in the 1980s than in the 1990s and 2000s. With the knowledge of this bias as a test of the ability to detect species distribution changes at the edge of their distribution we divided the dataset into three time periods and examined whether a suite of sensitive species distributions along stream size was apparent through time. We recognized that the distribution of sites was different between these periods and we wanted to test whether it would be evident in low percentiles (1^{st}, 5^{th}, and 25th) for species distributions across all sites in Ohio. The results of this initial test showed that some bias between time periods exists for species distributions where nearly all selected sensitive species had distributions that extended further into small streams during the later (1998-2008) compared to the earliest (1978-1989) sampling periods (Table H8).

In this table species with a + “increased” their distribution in small streams sampled in the most recent years (Ohio EPA, 2002).

We then restricted this analysis to sites that had only been sampled in all three sampling periods so that the resulting distributions were not an artifact of stream size bias. The distribution of each species was then examined along a stream size gradient as measured by the same low percentiles (1st, 5th and 25th) (Table H4-5). There is still a possible bias in this initial analysis because some of these sites that were sampled across all three periods may have been sampled more frequently during some periods which could increase the probability of capture. However, as an initial exploratory analysis we were interested in whether any obvious trends were apparent.

The results (Table H4-6) do not indicate evidence of the same patterns similar to what was evident in Table H4-5 that were attributable to the sampling frequency among small streams. This analysis assumes, however, that some strong long-term shifts would have occurred during these time periods that would affect the tails of stream size distributions more than inter-annual flow variation. A more sensitive analysis would control or consider year-to-year variability in flow or temperature within each time periods that may confound the current analysis. We suggest that these distributional shifts could be a fruitful path for analysis when annual variation and regional variation in flows, which can be extracted from USGS flow data using IHA flow indicators, are incorporated into the analyses. The initial analyses conducted herein establish a basis for more detailed analyses.

Table H4-5. Analysis of frequency of species collections by stream size as measure by 1st, 5th and 25th percentiles of drainage area at sites with these species collected

		Sample Size		1st Percentile			5thPercentile			25th Percentile		
Species Code	Species Name	1978-1989	1998-2008	1978-1989	1998-2008	Trend	1978-1989	1998-2008	Trend	1978-1989	1998-2008	Trend
34-001	central mudminnow [T]	18	1	5.0	9.0	-	5.0	9.0	-	10.8	9.0	+
40-009	black redhorse [I]	211	153	10.1	28.2	+	35.6	69.8	+	105.3	140.8	+
40-010	golden redhorse [M]	507	353	7.8	8.0	+	27.6	24.5	+	111.3	122.0	+
40-015	northern hog sucker [M]	553	424	5.3	6.0	+	10.0	14.5	+	46.7	71.5	+
43-001	common carp [T]	562	287	11.5	8.4	+	28.4	19.3	+	207.0	130.0	+
43-004	hornyhead chub [I]	37	19	1.5	1.5	+	9.4	4.0	+	32.0	32.8	+
43-005	river chub [I]	90	73	6.7	41.0	+	33.0	47.9	+	80.0	105.0	+
43-007	bigeye chub [I]	16	20	15.0	16.9	+	15.6	16.9	+	34.0	56.7	+
43-012	longnose dace [R]	0	0	0.0	0.0	+	0.0	0.0	+	0.0	0.0	+
43-014	tonguetied minnow [S]	12	5	34.0	7.5	-	34.0	7.5	+	34.0	27.4	+
43-017	redside dace [I]	22	17	5.0	5.0	+	5.0	5.8	+	7.5	7.5	+
43-021	silver shiner [I]	191	148	10.7	13.9	+	32.0	16.5	+	74.0	45.0	+
43-022	rosyface shiner [I]	133	114	6.8	5.0	+	32.0	9.1	+	79.5	99.0	+
43-034	sand shiner [M]	111	262	5.9	7.5	+	16.9	16.7	+	51.4	77.0	+
43-042	fathead minnow [T]	53	51	0.8	0.8	-	2.8	2.5	+	16.0	7.4	+
43-043	bluntnose minnow [T]	646	449	1.5	1.5	+	7.4	6.8	+	37.0	29.0	+
47-004	yellow bullhead [T]	296	176	2.1	1.8	+	8.0	9.0	+	32.5	23.0	+
47-008	stonecat madtom [I]	115	68	20.5	10.9	+	33.0	69.0	+	101.0	105.0	+
47-012	brindled madtom [I]	42	24	19.7	19.7	+	19.7	29.7	+	32.0	89.0	+
77-004	smallmouth bass [M]	520	428	11.4	8.7	+	24.5	23.9	+	104.0	125.5	+
77-008	green sunfish [T]	673	396	2.5	1.5	+	8.0	6.9	+	44.5	29.5	+
77-011	longear sunfish [M]	510	339	8.6	6.3	+	28.2	15.2	+	132.0	122.0	+
80-004	dusky darter [M]	12	27	16.1	4.9	+	21.4	14.4	+	120.5	157.0	+
80-011	logperch [M]	138	246	1.5	14.3	+	28.5	32.0	+	74.0	151.0	+
80-011	logperch [M]	138	246	1.5	14.3	+	28.5	32.0	+	74.0	151.0	+
80-013	eastern sand darter [R]	2	3	286.0	174.0	+	286.0	174.0	+	286.0	202.0	+
80-015	greenside darter [M]	299	306	8.5	5.9	+	16.0	9.9	+	42.0	41.0	+
80-015	greenside darter [M]	299	306	8.5	5.9	+	16.0	9.9	+	42.0	41.0	+
80-022	rainbow darter [M]	267	253	2.5	2.6	+	6.8	8.2	+	28.2	26.5	+

Table H4-6. Analysis of frequency of species collections by stream size as measured by 1st, 5th and 25th percentiles of drainage area at sites with these species collected

		Sample Size		1st Percentile			5thPercentile			25th Percentile		
Species Code	Species Name	1978-1989	1998-2008	1978-1989	1998-2008	Trend	1978-1989	1998-2008	Trend	1978-1989	1998-2008	Trend
01-006	least brook lamprey [ ]	19	16	4.9	4.9	-	4.9	4.9	-	16.3	9.6	+
34-001	central mudminnow [T]	18	1	5.0	9.0	-	5.0	9.0	-	10.8	9.0	+
40-009	black redhorse [I]	211	153	10.1	28.2	-	35.6	69.8	-	105.3	140.8	-
40-010	golden redhorse [M]	507	353	7.8	8.0	-	27.6	24.5	+	111.3	122.0	-
40-015	northern hog sucker [M]	553	424	5.3	6.0	-	10.0	14.5	-	46.7	71.5	-
43-001	common carp [T]	562	287	11.5	8.4	+	28.4	19.3	+	207.0	130.0	+
43-004	hornyhead chub [I]	37	19	1.5	1.5	-	9.4	4.0	+	32.0	32.8	-
43-005	river chub [I]	90	73	6.7	41.0	-	33.0	47.9	-	80.0	105.0	-
43-007	bigeye chub [I]	16	20	15.0	16.9	-	15.6	16.9	-	34.0	56.7	-
43-012	longnose dace [R]	0	0	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
43-014	tonguetied minnow [S]	12	5	34.0	7.5	+	34.0	7.5	+	34.0	27.4	+
43-017	redside dace [I]	22	17	5.0	5.0	-	5.0	5.8	-	7.5	7.5	-
43-021	silver shiner [I]	191	148	10.7	13.9	-	32.0	16.5	+	74.0	45.0	+
43-022	rosyface shiner [I]	133	114	6.8	5.0	+	32.0	9.1	+	79.5	99.0	-
43-034	sand shiner [M]	111	262	5.9	7.5	-	16.9	16.7	+	51.4	77.0	-
43-042	fathead minnow [T]	53	51	0.8	0.8	-	2.8	2.5	+	16.0	7.4	+
43-043	bluntnose minnow [T]	646	449	1.5	1.5	-	7.4	6.8	+	37.0	29.0	+
47-004	yellow bullhead [T]	296	176	2.1	1.8	+	8.0	9.0	-	32.5	23.0	+
47-008	stonecat madtom [I]	115	68	20.5	10.9	+	33.0	69.0	-	101.0	105.0	-
47-012	brindled madtom [I]	42	24	19.7	19.7	-	19.7	29.7	-	32.0	89.0	-
54-002	blackstripe topminnow [	74	36	2.6	1.5	+	10.3	2.6	+	32.0	25.0	+
77-004	smallmouth bass [M]	520	428	11.4	8.7	+	24.5	23.9	+	104.0	125.5	-
77-008	green sunfish [T]	673	396	2.5	1.5	+	8.0	6.9	+	44.5	29.5	+
77-011	longear sunfish [M]	510	339	8.6	6.3	+	28.2	15.2	+	132.0	122.0	+
80-004	dusky darter [M]	12	27	16.1	4.9	+	21.4	14.4	+	120.5	157.0	-
80-011	logperch [M]	138	246	1.5	14.3	-	28.5	32.0	-	74.0	151.0	-
80-013	eastern sand darter [R]	2	3	286.0	174.0	+	286.0	174.0	+	286.0	202.0	+
80-015	greenside darter [M]	299	306	8.5	5.9	+	16.0	9.9	+	42.0	41.0	+
80-022	rainbow darter [M]	267	253	2.5	2.6	-	6.8	8.2	-	28.2	26.5	+
90-002	mottled sculpin [ ]	107	137	1.5	1.5	-	2.5	4.9	-	9.6	16.7	-
95-001	brook stickleback [ ]	17	10	7.4	7.4	-	8.6	7.4	+	21.0	8.0	+

Appendix I

Selected subsets of results from correlation analyses for Maine, Utah and North Carolina

The purpose of this Appendix is to show selected subsets of results from correlation analyses performed on data for Maine, Utah and North Carolina. Results are presented to allow for comparisons of trends both within and across states. Numerous traits variables and taxa were analyzed. The object was to identify biological variables that were significantly correlated with year, PRISM annual air temperature or PRISM annual precipitation (PRISM variables were typically the best site-specific climatic variables available). Metrics that are presented in this Appendix relate to temperature preferences and tolerances, EPT taxa, HBI, OCH taxa, hydrology and scenario metrics. Additional results are available upon request.

I1. OVERVIEW

Several approaches were used in analyzing data for long-term trends. To briefly summarize:

- We did correlation analyses and ordinations (i.e.Non-metric Multidimensional Scaling (NMDS) and Canonical Correlation Analyses (CCA)) on several of different subsets of data from each of the states.
- We evaluated subsets of data from individual long-term biological sampling sites and from groups of sites.
- We evaluated each site and site group for confounding factors (non-climate) that may be influencing trends. Examples of factors that we evaluated (availability varied) included: habitat (i.e. width, depth, visual substrate estimates), a variety of water chemistry parameters, land use/land cover, and organic enrichment (using HBI calculations as surrogates because long-term nutrient data were generally not available).
- We used a ‘two-pronged’ approach and evaluated both taxonomic composition (mainly using relative abundance) and traits metrics (percent individuals and number of taxa).

Table I1-1 contains metadata for the environmental and biological variables that were included in the correlation analyses. The climate variables used in the analyses are PRISM mean annual air temperature and PRISM mean annual precipitation. Variables associated with the North Atlantic Oscillation (NAO), Oceanic Niño Index (ONI) and Pacific Decadal Oscillation (PDO) were also analyzed (see **Attachment I1**). The procedure was automated to run in R software (the R code is available upon request – it produces a correlation matrix, a table with significant correlations (with option to set the p-value) and plots of the significantly-correlated variables).

Because there are so many results, we selected the most relevant subset of summary tables and plots to present in this Appendix (all the correlation matrices are available upon request). The summary tables include side-by-side results from Maine, Utah and North Carolina sites and site groups so that patterns can be compared across states and regions (see Table I-2 for a list of the sites and site groups). The Pearson product moment correlations were calculated using Statistica software (Version 8.0, Copyright StatSoft, Inc., 1984-2007).

The following groups of results are presented in Attachments I2 through I8:

I2. Climate Variable -Year Trends;
I3. Year & Climate Variable -Temperature Metric Trends;
I4. Year & Climate Variable -EPT Metric Trends;
I5. Year & Climate Variable -HBI Trends;
I6. Year & Climate Variable –OCH Metric Trends;
I7. Year & Climate Variable –Hydrologic Metric Trends;
I8. Year & Climate Variable –Scenario Metric Trends; and

There are a number of limitations that should be noted. Correlation analyses cannot establish unambiguous causal relationships between the environmental and biological variables. We tried to disentangle confounding factors from the climate change effects by using reference data, but some reference stations still are influenced by anthropogenic factors. In addition, significant correlations can sometimes be driven by outliers. We attempted to address this issue by reviewing plots of significantly-correlated variables. Another issue is that the climate variables used (PRISM mean annual air temperature and precipitation), while bearing relationships to in-stream conditions, are not direct measures of actual stream thermal and hydrologic regimes at the biological sampling sites. Ideally we would use continuous water temperature and flow data in the analyses.

Some limitation with the traits analyses include:

- Experimental evidence regarding which individual traits are most important in the context of climate change is still lacking, so that application of trait analyses was related to some published literature, but still requires some ‘best professional judgment’;
- Redundancy of information among traits has been cited as an issue (Poff et al., 2006). We also found a number of individual trait metrics to be correlated (r>0.8). Efforts to limit impacts of redundancy (Poff et al., 2006) were dataset-dependent, making broad generalizations about which trait metrics to exclude difficult.
- We calculated trait metrics (% individual and number of taxa) for about 30 different traits (which each had 2 to 5 trait states). There were a lot of significant correlations, but interpretation was difficult, since few showed consistent patterns across sites and states.

- In selecting which trait metrics to summarize, we used the following guidelines to the extent possible:
    - Focus on traits for which we have the most amount of information for the most number of taxa (i.e. functional feeding group (FFG) and habit)
    - Focus on ***groups*** of traits rather than individual traits, to the extent support by available literature information on trait characteristics in various trait categories by taxon. We approached consideration of combinations of traits by developing ‘scenario’ traits metrics, where scenarios represent projected future climate characteristics for a region (e.g., warmer and drier in Utah). Taxa were then grouped based on suites of traits expected to confer an advantage in surviving these projected future conditions. “Robust” were groups of taxa with the most number of favorable traits states for that scenario, while “vulnerable” were groups of taxa with the fewest favorable trait states and the most number of unfavorable trait states.

101 **Table I1-1**. Metadata for the environmental and biological variables that were used in the correlation analyses.

Category	**Variable**	**Description**
	StationID	self-explanatory
	Year	year sample was collected
	JulianDate	collection (Julian) date of biological sample
	Month	month sample was collected
PRISM	tmean14	PRISM mean annual air temperature (=avg of tmin and tmax) (°F)
PRISM	tmax14	PRISM mean annual maximum air temperature (=avg of tmin and tmax) (°F)
PRISM	tmin14	PRISM mean annual minimum air temperature (=avg of tmin and tmax) (°F)
PRISM	ppt14	PRISM mean annual precipitation (inches)
PRISM	tmean14_difc	difference between value from year of sampling event minus value from previous year. Calculation based on PRISM tmean14 data.
PRISM	tmax14_difc	difference between value from year of sampling event minus value from previous year. Calculation based on PRISM tmax14 data.
PRISM	tmin14_difc	difference between value from year of sampling event minus value from previous year. Calculation based on PRISM tmin14 data.
PRISM	ppt14_difc	difference between value from year of sampling event minus value from previous year. Calculation based on PRISM ppt14 data.
PRISM	tmean14_absdifc	absolute difference between value from year of sampling event minus value from previous year. Calculation based on PRISM tmean14 data.
PRISM	tmax14_absdifc	absolute difference between value from year of sampling event minus value from previous year. Calculation based on PRISM tmax14 data.
PRISM	tmin14_absdifc	absolute difference between value from year of sampling event minus value from previous year. Calculation based on PRISM tmin14 data.
PRISM	ppt14_absdifc	absolute difference between value from year of sampling event minus value from previous year. Calculation based on PRISM ppt14 data.
Taxa	Taxon	relative abundance of taxon
HBI	HBI_NM	Hilsenhoff Biotic Index calculated using the tolerance values in the New Mexico database (this was used in Utah only)
HBI	HBI	Hilsenhoff Biotic Index calculated using the tolerance values from the state being analyzed
Selected Metrics	PlecopPct	Percent individuals in the Order Plecoptera

102 Table 1.continued…

Category	Variable	Description
Selected Metrics	ChiroPct	Percent individuals in the Family Chironomidae
Selected Metrics	EPT_Pct	Percent individuals - Ephemeroptera, Plecoptera and Trichoptera
Selected Metrics	PlecopTax	Number of taxa in the Order Plecoptera
Selected Metrics	ChiroTax	Number of taxa in the Family Chironomidae
Selected Metrics	EPTTax	Number of Ephemeroptera, Plecoptera and Trichoptera taxa
FFG	FFG_CGPct	Functional Feeding group - percent collector-gatherer individuals
FFG	FFG_CFPct	Functional Feeding group - percent collector-filterer individuals
FFG	FFG_SHPct	Functional Feeding group - percent shredder individuals
FFG	FFG_HBPct	Functional Feeding group - percent herbivore individuals
FFG	FFG_PRPct	Functional Feeding group - percent predator individuals
FFG	FFG_CFTax	Functional Feeding group - number of collector-filterer taxa
FFG	FFG_CGTax	Functional Feeding group - number of collector-gatherer taxa
FFG	FFG_HBTax	Functional Feeding group - number of herbivore taxa
FFG	FFG_PRTax	Functional Feeding group - number of predator taxa
FFG	FFG_SHTax	Functional Feeding group - number of shredder taxa
Habit	Habit_CNPct	Habit - percent clinger individuals
Habit	Habit_SWPct	Habit - percent swimmer individuals
Habit	Habit_BUPct	Habit - percent burrower individuals
Habit	Habit_SKPct	Habit - percent skater individuals
Habit	Habit_CBPct	Habit - percent climber individuals
Habit	Habit_SPPct	Habit - percent sprawler individuals
Habit	Habit_CBTax	Habit - number of climber taxa
Habit	Habit_CNTax	Habit - number of clinger taxa
Habit	Habit_SPTax	Habit - number of sprawler taxa
Habit	Habit_BUTax	Habit - number of burrower taxa
Habit	Habit_SWTax	Habit - number of swimmer taxa

Table 1.continued…

Category	Variable	Description
Hydro	PerennialPct	Percent perennial stream individuals (list of perennial taxa was based on NC intermittent stream report and Del Rosario et al. (2000): includes taxa listed in Table 1 of NC report (except for Peltoperlidae, since those occurred only at the intermittent site in Del Rosario), plus gilled snails (Subclass Prosobranchia) and Simuliidae (Del Rosario et al. 2000). These taxa, which require water for their entire life cycle, should be found a later instar larvae. Some are indicators of perennial stream features.
Hydro	IntermitPct	Percent intermittent stream individuals (list based on NC intermittent stream report: amphipods, isopods, small elongate Dipteran larvae (Ceratopogonidae, Blephariceridae, Chironomidae, Deuterophlebiidae, Psychodidae) winter stoneflies (Capniidae, Taeniopterygidae), Dytiscidae. Unique to intermittent=Helichus larvae and Dasyhela (family Dolchopodidae). Rest are also found in perennial. They just tend to be more dominant in numbers in intermittent conditions (probably due to loss of predators).
Hydro	Drought_Pct	Percent individuals that possess at least one of the following traits: ability to survive desiccation, adult ability to exit, respiration plastron/spiracle
Hydro	PerennialTax	Number of perennial stream taxa (list of perennial taxa was based on NC intermittent stream report & Del Rosario et al. 2000 JNABS paper: includes taxa listed in Table 1 of NC report (except for Peltoperlidae, since those occurred only at the intermittent site in Del Rosario), plus gilled snails (Subclass Prosobranchia) and Simuliidae (Del Rosario et al. 2000). These taxa, which require water for their entire life cycle, should be found a later instar larvae. Some are indicators of perennial stream features.
Hydro	IntermitTax	Number of intermittent stream taxa (list based on NC intermittent stream report: amphipods, isopods, small elongate Dipteran larvae (Ceratopogonidae, Blephariceridae, Chironomidae, Deuterophlebiidae, Psychodidae) winter stoneflies (Capniidae, Taeniopterygidae), Dytiscidae. Unique to intermittent=Helichus larvae and Dasyhela (family Dolchopodidae). Rest are also found in perennial. They just tend to be more dominant in numbers in intermittent conditions (probably due to loss of predators).
Hydro	DroughtTax	Number of taxa that possess at least one of the following traits: ability to survive desiccation, adult ability to exit, respiration plastron/spiracle
Hydro	OCH_Pct	Percent individuals - Odonata, Coleoptera and Hemiptera
Hydro	OCHD_Pct	Percent individuals - Odonata, Coleoptera, Hemiptera and Diptera
Hydro	OCHTax	Number of Odonata, Coleoptera and Hemiptera taxa
Hydro	OCHDTax	Number of Odonata, Coleoptera, Hemiptera and Diptera taxa
Temp	Temp_ColdPct	Thermal Preference -Percent cold individuals (optima ranking of 1, 2 or 3)
Temp	Temp_ColdStenoPct	Thermal Preference and Tolerance -Percent cold stenotherm individuals (optima ranking of 1, 2 or 3 and tolerance ranking of 1, 2 or 3)

Table 1.continued…

Category	**Variable**	**Description**
Temp	Temp_InterPct	Thermal Preference -Percent intermediate individuals (optima ranking of 4)
Temp	Temp_WarmEuryPct	Thermal Preference and Tolerance -Percent warm eurythermal individuals (optima ranking of 5, 6 or 7 and tolerance ranking of 5, 6 or 7)
Temp	Temp_WarmPct	Thermal Preference -Percent warm individuals (optima ranking of 5, 6 or 7)
Temp	Temp_CoreColdPct	Thermal Preference and Tolerance -Percent core cold individuals (see temperature indicator writeups)
Temp	Temp_CoreWarmPct	Thermal Preference and Tolerance -Percent core warm individuals (see temperature indicator writeups)
Temp	Temp_ColdTax	Thermal Preference -Number of cold taxa (optima ranking of 1, 2 or 3)
Temp	Temp_ColdStenoTax	Thermal Preference and Tolerance -Number of cold stenotherm taxa (optima ranking of 1, 2 or 3 and tolerance ranking of 1, 2 or 3)
Temp	Temp_WarmTax	Thermal Preference -Number of warm taxa (optima ranking of 5, 6 or 7)
Temp	Temp_WarmEuryTax	Thermal Preference and Tolerance -Number of warm eurythermal taxa (optima ranking of 5, 6 or 7 and tolerance ranking of 5, 6 or 7)
Temp	Temp_InterTax	Thermal Preference -Number of intermediate taxa (optima ranking of 4)
Temp	Temp_CoreCold_Tax	Thermal Preference and Tolerance -Number of core cold taxa (see temperature indicator writeups)
Temp	Temp_CoreWarm_Tax	Thermal Preference and Tolerance -Number of core warm taxa (see temperature indicator writeups)
Scenario	Drier_WinPct	Percent individuals that possess the most number of traits states that are predicted or have been shown to be most favorable in a drier climate scenario
Scenario	Drier_VulnerablePct	Percent individuals that have the fewest favorable trait states and the most number of unfavorable trait states in a drier climate scenario
Scenario	WarmDrier_Vulnerable Pct	Percent individuals that have the fewest favorable trait states and the most number of unfavorable trait states in a warmer drier climate scenario
Scenario	WarmDrier_WinPct	Percent individuals that possess the most number of traits states that are predicted or have been shown to be most favorable in a warmer drier climate scenario
Scenario	Wet_WinPct	Percent individuals that possess the most number of traits states that are predicted or have been shown to be most favorable in a wetter climate scenario

Table 1.continued…

Category	Variable	Description
Scenario	Wet_LosPct	Percent individuals that have the fewest favorable trait states and the most number of unfavorable trait states in a wetter climate scenario
Scenario	WarmWet_LosPct	Percent individuals that have the fewest favorable trait states and the most number of unfavorable trait states in a warmer wetter climate scenario
Scenario	WarmWet_WinPct	Percent individuals that possess the most number of traits states that are predicted or have been shown to be most favorable in a warmer wetter climate scenario
Scenario	Drier_WinTax	Number of taxa that possess the most number of traits states that are predicted or have been shown to be most favorable in a drier climate scenario
Scenario	Drier_VulnerableTax	Number of taxa that have the fewest favorable trait states and the most number of unfavorable trait states in a drier climate scenario
Scenario	WarmDrier_VulnerableTax	Number of taxa that have the fewest favorable trait states and the most number of unfavorable trait states in a warmer drier climate scenario
Scenario	WarmDrier_WinTax	Number of taxa that possess the most number of traits states that are predicted or have been shown to be most favorable in a warmer drier climate scenario
Scenario	Wet_WinTax	Number of taxa that possess the most number of traits states that are predicted or have been shown to be most favorable in a wetter climate scenario
Scenario	Wet_LosTax	Number of taxa that have the fewest favorable trait states and the most number of unfavorable trait states in a wetter climate scenario
Scenario	WarmWet_LosTax	Number of taxa that have the fewest favorable trait states and the most number of unfavorable trait states in a warmer wetter climate scenario
Scenario	WarmWet_WinTax	Number of taxa that possess the most number of traits states that are predicted or have been shown to be most favorable in a warmer wetter climate scenario

Table I1-2. Results for these sites and site groups are presented in the summary tables in Attachments I2-I8.

State	Site/ Site Group
Maine	56817
	57011
	57065
	NE High (= Northeastern Highlands Site Group)
	Laur (= Laurentian Plains and Hills Site Group)
Utah	4927250
	4936750
	4951200
	5940440
	WU_SF (= Wasatch Uintas Semiarid Foothills Site Group)
	WU_ME (= Wasatch Uintas Mid-elevation Mountains Site Group)
	CP (= Colorado Plateaus Site Group)
North Carolina	NC0109 (BR)
	NC0207 (BR)
	NC0209 (BR)
	NC0075 (Pied)
	NC0248 (Pied)

Attachment I1

NAO/ONI/PDO

The purpose of this attachment is to provide background on the variables from the North Atlantic Oscillation (NAO), Oceanic Niño Index (ONI) and Pacific Decadal Oscillation (PDO) datasets that were used in the correlation analyses. Results from these analyses are available upon request.

ATTACHMENT I1. NAO/ONI/PDO

Data sources:

North Atlantic Oscillation (NAO): http://www.cgd.ucar.edu/cas/jhurrell/indices.html

Oceanic Niño Index (ONI):

http://www.cpc.noaa.gov/products/analysis_monitoring/ensostuff/ensoyears.shtml and

http://www.cdc.noaa.gov/data/climateindices/List/

Pacific Decadal Oscillation (PDO): http://jisao.washington.edu/pdo/PDO.latest

Correlation analyses were performed on NAO, ONI, and PDO datasets to evaluate whether these cyclic climate indices have shown significant trends over the last 32 years, and also to examine whether the indices are significantly associated with trends in biological data (i.e. do year-to-year changes in species composition track the NAO, ONI and PDO?). We used NAO data in the Maine and North Carolina analyses because the NAO affects the Eastern seaboard states (personal communication with James Hurrell (Email: jhurrell@ucar.edu)). The ONI and PDO indices have greater relevance in western states and were therefore used in the Utah analyses.

There are many different variables associated with the NAO, ONI and PDO datasets (e.g., monthly values, various averages). It was difficult to know which ones had the greatest relevance to our analyses, especially with the ONI and PDO datasets. This is because there is no unique or universally accepted way to define the spatial structure of these phenomena. More information seemed to be available on the NAO than the ONI and PDO. Bradley and Ormerod (2001) were used as guidance in selecting the following 2 NAO variables: NAO PC-Based Seasonal December-January-February-March (DJFM) index and the NAO PC-Based Annual index. We used the December-January-February-March (DJFM) seasonal index because the main "season" of the NAO is northern winter; this is when the atmosphere is most dynamically active (personal communication with James Hurrell (Email: jhurrell@ucar.edu). We chose the Principal Component (PC)-based time series data over the station-based indices because they were available for the appropriate timeframe, and they provide better representations of the full NAO spatial pattern than station-based indices. Station-based indices are limited because they are fixed in space and can therefore only adequately capture NAO variability for parts of the year.

Moreover, their pressures are significantly affected by small-scale and transient meteorological phenomena not related to the NAO and, thus, contain more noise.

We were unable to find references to help guide our selection of ONI and PDO indices for the Utah analyses, so we included all the variables. The ONI is based on sea surface temperature departures from average in the Niño 3.4 region, and is a principal measure for monitoring, assessing, and predicting the El Niño-Southern Oscillation (ENSO). ENSO is a combination of atmospheric and oceanic phenomena in the tropical Pacific Ocean. It is manifested in the atmosphere by changes in the pressure difference between Tahiti and Darwin, Australia and in the ocean by warming of surface waters of the tropical Eastern Pacific Ocean. NOAA's operational definitions of El Niño and La Niña are keyed to the ONI index. NOAA's Climate Prediction Center (CPC) considers El Niño or La Niña conditions to occur when the monthly Niño3.4 sea surface temperature departures meet or exceed +/-0.5°C along with consistent atmospheric features. These anomalies must also be forecasted to persist for 3 consecutive months[1]. The PDO is a long-lived El Niño-like pattern of Pacific climate variability. While the two climate oscillations have similar spatial climate fingerprints, they have very different behavior in time. Two main characteristics distinguish PDO from El Niño/Southern Oscillation (ENSO): first, 20th century PDO "events" persisted for 20-to-30 years, while typical ENSO events persisted for 6 to 18 months; second, the climatic fingerprints of the PDO are most visible in the North Pacific/North American sector, while secondary signatures exist in the tropics - the opposite is true for ENSO. More sources of information on the ONI and PDO are available upon request.

[1] For more information, see http://www.cpc.noaa.gov/products/analysis_monitoring/lanina/enso_evolution-status-fcsts-web.pdf.

Attachment I2

Climate Variable -Year Trends

In this Attachment, we summarize yearly trend results for PRISM mean, maximum and minimum annual air temperature and PRISM mean annual precipitation at Maine, Utah and North Carolina biological sampling sites and site groups.

ATTACHMENT I2. CLIMATE VARIABLE-YEAR TRENDS

We examined yearly trends in PRISM mean, maximum and minimum annual air temperature and PRISM mean annual precipitation at Maine, Utah and North Carolina biological sampling sites and site groups. Results are summarized in **Table I2-1**. Utah had the most number of sites and site groups that showed significant yearly temperature trends across the most number of temperature variables; year is significantly correlated with mean and minimum annual air temperature at 6 of the 7 sites/ site groups. Three of the 4 Maine sites/site groups were significantly and positively correlated with mean and minimum annual air temperature, and 6 of the 7 North Carolina sites/site groups were significantly correlated with minimum annual air temperature. None of the sites/site groups had significant yearly trends in mean annual precipitation (which tends to be highly variable).

Table I2-1. Pearson product moment correlations of PRISM mean, maximum and minimum annual air temperature (tmean14, tmax14 and tmin14) and PRISM mean annual precipitation (ppt14) versus ***year*** for individual sites and site groups in Maine, Utah and North Carolina. Correlations with NAO and ONI variables were also included (see Attachment I1 for more details on these variables). Highlighted correlations were significant (p<0.05).

PRISM/NAO/ONI - YEAR														
State	**Site/ Site Group**	**N**	**tmean14**		**tmax14**		**tmin14**		**ppt14**		**NAO_DJFM_PC**			
			r	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**N**	**p**	
Maine	56817	32	0.24	p=.188	0.09	p=.620	0.33	p=.065	0.04	p=.820	-0.15	N=23	p=.506	
	57011	32	0.09	p=.635	-0.03	p=.888	0.17	p=.351	0.08	p=.674	-0.22	N=12	p=.502	
	57065	32	**0.42**	**p=.018**	0.28	p=.123	**0.46**	**p=.008**	-0.16	p=.373	-0.23	N=9	p=.549	
	NE High	32	**0.52**	**p=.002**	**0.41**	**p=.019**	**0.55**	**p=.001**	0.22	p=.236	-0.35	N=8	p=.391	
	Laur	32	**0.41**	**p=.019**	0.28	p=.119	**0.46**	**p=.009**	-0.13	p=.494	-0.15	N=8	p=.731	
State	**Site/ Site Group**	**N**	**tmean14**		**tmax14**		**tmin14**		**ppt14**		**ONI_DJF**			
			r	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**N**	**p**	
Utah	4927250	32	**0.57**	**p=.001**	**0.60**	**p=.000**	0.35	p=.051	-0.04	p=.824	0.18	N=17	p=.494	
	4936750	32	**0.48**	**p=.005**	0.14	p=.443	**0.70**	**p=.000**	0.11	p=.540	-0.14	N=12	p=.656	
	4951200	32	**0.74**	**p=.000**	**0.71**	**p=.000**	**0.71**	**p=.000**	-0.08	p=.674	-0.09	N=15	p=.736	
	5940440	32	**0.71**	**p=.000**	**0.55**	**p=.001**	**0.74**	**p=.000**	-0.13	p=.483	0.22	N=9	p=.567	
	WU_SF	32	**0.77**	**p=.000**	**0.52**	**p=.002**	**0.83**	**p=.000**	-0.20	p=.262	-0.25	N=20	p=.284	
	WU_ME	32	0.30	p=.093	0.18	p=.321	**0.36**	**p=.042**	0.04	p=.846	-0.28	N=12	p=.383	
	CP	32	**0.65**	**p=.000**	**0.45**	**p=.009**	**0.75**	**p=.000**	0.06	p=.724	0.27	N=14	p=.359	
State	**Site/ Site Group**	**N**	**tmean14**		**tmax14**		**tmin14**		**ppt14**		**NAO_DJFM_PC**			
			r	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**N**	**p**	
North Carolina	NC0109 (BR)	32	0.15	p=.412	0.19	p=.302	0.07	p=.688	0.04	p=.820	0.02	N=11	p=.954	
	NC0207 (BR)	32	**0.73**	**p=.000**	**0.58**	**p=.001**	**0.77**	**p=.000**	-0.12	p=.497	-0.02	N=9	p=.969	
	NC0209 (BR)	32	**0.68**	**p=.000**	**0.53**	**p=.002**	**0.69**	**p=.000**	0.19	p=.292	0.09	N=32	p=.637	
	NC0075 (Pied)	32	0.29	p=.107	-0.09	p=.627	**0.54**	**p=.001**	-0.17	p=.348	-0.46	N=7	p=.294	
	NC0248 (Pied)	32	0.37	p=.039	0.01	p=.942	**0.57**	**p=.001**	-0.12	p=.527	-0.41	N=7	p=.365	

Attachment I3

Climate Variable -Temperature Metric Trends

In this Attachment, we show results for a selected subset of temperature-sensitive metrics, which were examined for yearly trends and trends related to PRISM air temperature and precipitation variables.

ATTACHMENT I3.CLIMATE VARIABLE-TEMPERATURE METRIC TRENDS

Results for a selected subset of temperature-sensitive metrics are shown in **Tables I3-1** through **I3-6**. Overall, there were few consistent trends among the metrics (or taxa) that occurred across the site/site groups and across states. In addition, some of the significant correlations were found to be driven by outliers. Some may also have been influenced by confounding factors (see **Section 2** of the report for more information).

There are some notable regional differences. In Utah, the temperature metric trends seemed more evident (maybe because Utah has experienced a more noticeable temperature increase than the other states). This may have been because of the availability of at least some long-term reference data from sites/site groups in Utah at higher elevations. In Maine, the ecoregion with higher elevations (Northeastern Highlands) had no sites with sufficient long term data detect such trends.

For temperature preference trait groups in each state, 'long-list' metrics were based on the original list of cold- or warm-water taxa (which were derived from weighted average and maximum likelihood calculations and literature – see Appendix K for more information). The 'short-list' metrics were based on a subset of the 'long-list' taxa that are referred to (in other sections of this report) as temperature indicator taxa. Data from other states, case studies (i.e. evaluation of taxa lists at the coldest and warmest sites), and best professional judgment from the regional climate change working groups were taken into account when developing the temperature indicator lists (see Appendices E, F and G for more information on the working groups; and Attachments to these appendices for information on temperature indicator taxa for each state). The 'short-list' metrics were developed because it was believed that they would have a greater chance of showing trends.

Overall, results from the 'short-list' and 'long-list' metrics were similar (Tables I3-1 to 6). We examined what taxa might be driving the differences at certain sites. In Utah, *Ephemerella* (designated as a cold-water taxon) was influential over trends at two of four long-term reference sites. There are also a few taxa that were excluded from the 'short-list', but showed trends (although some trends were counter-intuitive trends and must be related to factors other than climate or discounted). Nevertheless, in Utah, these taxa are worth further consideration: Rhyacophilidae, *Drunella*, and *Brachycentrus*. In North Carolina, there were a few taxa on the warm-water list that showed counter-intuitive trends (i.e. decreased as PRISM mean annual air temperature increased). These were: *Chimarra*, *Dromogomphus* and *Gomphus*. This suggests what has been noted in other sections of this report: that the cold- and warm-water taxa lists and the derived biological temperature metrics are not final, that they do not necessarily capture all relevant considerations. Although not presented here, another result worth noting is that the 'intermediate' taxa did not show strong or consistent trends.

Table I3-1. Pearson product moment correlations of temperature-sensitive ***richness*** metrics versus ***year*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant (p<0.05). The 'long-list' metrics were based on the original list of cold and warm-water taxa (which were derived from weighted average and maximum likelihood calculations and literature – see Appendix K for more information). The 'short-list' metrics were based on a subset of the 'long-list' taxa that are referred to (in other sections of this report) as temperature indicator taxa. Data from other states, case studies (i.e. evaluation of taxa lists at the coldest and warmest sites), and best professional judgment from the regional climate change working groups were taken into account when developing the temperature indicator lists (see Attachments in Appendices E, F and G for more information on temperature indicator taxa for each state). The 'short-list' metrics were developed because it was believed that they would have a greater chance of showing trends.

Temperature Richness Metrics - YEAR													
State	**Site/ Site Group**	# Cold-water Taxa (***Short-list***)			# Warm-water Taxa (***Short-list***)			# Cold-water Taxa (***Long-list***)			# Warm-water Taxa (***Long-list***)		
		r	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**
Maine	56817	**0.49**	**N=23**	**p=.017**	**0.78**	**N=23**	**p=.000**	**0.41**	**N=23**	**p=.049**	**0.73**	**N=23**	**p=.000**
	57011	0.04	N=12	p=.896	**0.65**	**N=12**	**p=.023**	**0.66**	**N=12**	**p=.019**	**0.77**	**N=12**	**p=.003**
	57065	0.54	N=9	p=.133	0.58	N=9	p=.101	0.57	N=9	p=.111	0.56	N=9	p=.115
	Laur	**0.82**	**N=8**	**p=.012**	0.28	N=8	p=.494	0.43	N=8	p=.292	0.57	N=8	p=.141
	NEHigh	-0.25	N=8	p=.554	-0.05	N=8	p=.900	0.26	N=8	p=.537	-0.13	N=8	p=.761
Utah	4927250	**-0.71**	**N=17**	**p=.002**	-0.21	N=17	p=.416	**-0.59**	**N=17**	**p=.012**	0.08	N=17	p=.762
	4936750	-0.38	N=12	p=.227	0.38	N=12	p=.222	-0.32	N=12	p=.313	0.23	N=12	p=.468
	4951200	**-0.60**	**N=15**	**p=.017**	**0.81**	**N=15**	**p=.000**	**-0.64**	**N=15**	**p=.009**	**0.60**	**N=15**	**p=.019**
	5940440	-0.64	N=9	p=.065	0.00	N=9	p=1.00	**-0.68**	**N=9**	**p=.043**	-0.26	N=9	p=.502
	WU_SF	0.10	N=20	p=.677	**0.67**	**N=20**	**p=.001**	0.37	N=20	p=.113	0.30	N=20	p=.206
	WU_ME	-0.27	N=12	p=.400	**0.72**	**N=12**	**p=.009**	-0.42	N=12	p=.169	0.52	N=12	p=.080
	CP	0.13	N=14	p=.662	0.50	N=14	p=.067	0.29	N=14	p=.321	**0.61**	**N=14**	**p=.021**
North Carolina	NC0109 (BR)	0.55	N=11	p=.080	-0.58	N=11	p=.059	0.14	N=11	p=.682	-0.56	N=11	p=.074
	NC0207 (BR)	0.38	N=9	p=.316	-0.04	N=9	p=.914	-0.21	N=9	p=.593	-0.52	N=9	p=.151
	NC0209 (BR)	**0.82**	**N=7**	**p=.024**	-0.53	N=7	p=.219	0.27	N=7	p=.562	**-0.86**	**N=7**	**p=.013**
	NC0075 (P)	-0.39	N=7	p=.393	0.60	N=7	p=.157	0.35	N=7	p=.441	0.45	N=7	p=.316
	NC0248 (P)	0.28	N=7	p=.542	0.14	N=7	p=.772	0.57	N=7	p=.177	-0.50	N=7	p=.253

Table I3-2. Pearson product moment correlations of temperature-sensitive ***% individual*** metrics versus ***year*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant (p<0.05). The 'long-list' metrics were based on the original list of cold and warm-water taxa (which were derived from weighted average and maximum likelihood calculations and literature – see Appendix K for more information). The 'short-list' metrics were based on a subset of the 'long-list' taxa that are referred to (in other sections of this report) as temperature indicator taxa. Data from other states, case studies (i.e. evaluation of taxa lists at the coldest and warmest sites), and best professional judgment from the regional climate change working groups were taken into account when developing the temperature indicator lists (see Attachments in Appendices E, F and G for more information on temperature indicator taxa for each state). The 'short-list' metrics were developed because it was believed that they would have a greater chance of showing trends.

Temperature % Individual Metrics - YEAR													
State	**Site/ Site Group**	% Cold-water Individs (***Short-list***)			% Warn Water Individs (***Short-list***)			% Cold-water Individs (***Long-list***)			% Warm-water Individs (***Long-list***)		
		r	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**
Maine	56817	**0.47**	**N=23**	**p=.025**	**0.55**	**N=23**	**p=.006**	0.11	N=23	p=.612	-0.06	N=23	p=.794
	57011	**-0.67**	**N=12**	**p=.017**	**-0.59**	**N=12**	**p=.043**	0.13	N=12	p=.687	0.02	N=12	p=.947
	57065	0.45	N=9	p=.226	-0.36	N=9	p=.336	0.62	N=9	p=.076	-0.56	N=9	p=.121
	Laur	-0.01	N=8	p=.990	-0.38	N=8	p=.347	-0.14	N=8	p=.745	0.23	N=8	p=.589
	NEHigh	-0.45	N=8	p=.258	-0.02	N=8	p=.958	0.01	N=8	p=.986	0.05	N=8	p=.905
Utah	4927250	**-0.72**	**N=17**	**p=.001**	-0.21	N=17	p=.416	0.03	N=17	p=.918	-0.27	N=17	p=.291
	4936750	-0.15	N=12	p=.635	0.42	N=12	p=.174	-0.42	N=12	p=.173	0.08	N=12	p=.801
	4951200	**-0.63**	**N=15**	**p=.013**	0.40	N=15	p=.140	**-0.63**	**N=15**	**p=.011**	0.21	N=15	p=.460
	5940440	-0.12	N=9	p=.764	0.00	N=9	p=1.00	0.27	N=9	p=.487	-0.33	N=9	p=.388
	WU_SF	-0.12	N=20	p=.603	**0.60**	**N=20**	**p=.005**	**0.58**	**N=20**	**p=.008**	-0.12	N=20	p=.610
	WU_ME	**0.64**	**N=12**	**p=.026**	**0.63**	**N=12**	**p=.028**	0.30	N=12	p=.346	-0.28	N=12	p=.375
	CP	-0.02	N=14	p=.951	0.48	N=14	p=.084	0.20	N=14	p=.503	0.43	N=14	p=.127
North Carolina	NC0109 (BR)	0.57	N=11	p=.067	-0.04	N=11	p=.904	0.36	N=11	p=.273	-0.37	N=11	p=.256
	NC0207 (BR)	0.33	N=9	p=.391	0.09	N=9	p=.824	0.46	N=9	p=.212	-0.28	N=9	p=.462
	NC0209 (BR)	0.29	N=7	p=.522	-0.33	N=7	p=.469	0.48	N=7	p=.277	-0.46	N=7	p=.300
	NC0075 (P)	-0.02	N=7	p=.969	-0.73	N=7	p=.060	-0.26	N=7	p=.567	0.66	N=7	p=.107
	NC0248 (P)	-0.14	N=7	p=.760	-0.55	N=7	p=.202	0.19	N=7	p=.685	-0.31	N=7	p=.494

Table I3-3. Pearson product moment correlations of temperature-sensitive ***richness*** metrics versus ***PRISM mean annual air temperature*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant (p<0.05). The 'long-list' metrics were based on the original list of cold and warm-water taxa (which were derived from weighted average and maximum likelihood calculations and literature – see Appendix K for more information). The 'short-list' metrics were based on a subset of the 'long-list' taxa that are referred to (in other sections of this report) as temperature indicator taxa. Data from other states, case studies (i.e. evaluation of taxa lists at the coldest and warmest sites), and best professional judgment from the regional climate change working groups were taken into account when developing the temperature indicator lists (see Attachments in Appendices E, F and G for more information on temperature indicator taxa for each state). The 'short-list' metrics were developed because it was believed that they would have a greater chance of showing trends.

Temperature Richness Metrics - PRISM mean annual air temperature													
State	**Site/ Site Group**	**# Cold-water Taxa (*Short-list*)**			**# Warm-water Taxa (*Short-list*)**			**# Cold-water Taxa (*Long-list*)**			**# Warm-water Taxa (*Long-list*)**		
		r	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**
Maine	56817	0.31	N=23	p=.147	0.21	N=23	p=.341	0.08	N=23	p=.709	0.18	N=23	p=.423
	57011	0.02	N=12	p=.947	0.27	N=12	p=.388	-0.09	N=12	p=.772	0.18	N=12	p=.586
	57065	-0.58	N=9	p=.103	**-0.73**	**N=9**	**p=.024**	-0.62	N=9	p=.078	-0.53	N=9	p=.143
	Laur	**-0.71**	**N=8**	**p=.049**	-0.18	N=8	p=.675	-0.56	N=8	p=.151	-0.56	N=8	p=.150
	NEHigh	-0.50	N=8	p=.203	0.54	N=8	p=.165	-0.54	N=8	p=.169	0.48	N=8	p=.227
Utah	4927250	**-0.63**	**N=17**	**p=.007**	-0.44	N=17	p=.076	**-0.61**	**N=17**	**p=.009**	-0.23	N=17	p=.374
	4936750	-0.08	N=12	p=.815	-0.03	N=12	p=.929	-0.04	N=12	p=.913	-0.13	N=12	p=.694
	4951200	**-0.65**	**N=15**	**p=.009**	**0.75**	**N=15**	**p=.001**	**-0.72**	**N=15**	**p=.003**	0.35	N=15	p=.207
	5940440	-0.14	N=9	p=.726	0.00	N=9	p=1.00	-0.32	N=9	p=.405	0.16	N=9	p=.682
	WU_SF	0.11	N=20	p=.639	**0.53**	**N=20**	**p=.016**	0.34	N=20	p=.147	0.01	N=20	p=.953
	WU_ME	**-0.66**	**N=12**	**p=.018**	**0.65**	**N=12**	**p=.023**	**-0.74**	**N=12**	**p=.006**	0.23	N=12	p=.469
	CP	0.15	N=14	p=.619	0.51	N=14	p=.063	0.30	N=14	p=.302	0.37	N=14	p=.196
North Carolina	NC0109 (BR)	-0.38	N=11	p=.246	-0.18	N=11	p=.592	0.17	N=11	p=.614	-0.08	N=11	p=.825
	NC0207 (BR)	0.43	N=9	p=.245	0.27	N=9	p=.478	0.36	N=9	p=.341	-0.19	N=9	p=.626
	NC0209 (BR)	0.00	N=7	p=.993	-0.48	N=7	p=.276	0.13	N=7	p=.784	-0.23	N=7	p=.617
	NC0075 (P)	-0.09	N=7	p=.841	0.56	N=7	p=.194	-0.15	N=7	p=.743	-0.08	N=7	p=.863
	NC0248 (P)	-0.20	N=7	p=.667	-0.47	N=7	p=.291	0.27	N=7	p=.562	**-0.92**	**N=7**	**p=.004**

Table I3-4. Pearson product moment correlations of temperature-sensitive ***% individual*** metrics versus ***PRISM mean annual air temperature*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant ($p<0.05$). The 'long-list' metrics were based on the original list of cold and warm-water taxa (which were derived from weighted average and maximum likelihood calculations and literature – see Appendix K for more information). The 'short-list' metrics were based on a subset of the 'long-list' taxa that are referred to (in other sections of this report) as temperature indicator taxa. Data from other states, case studies (i.e. evaluation of taxa lists at the coldest and warmest sites), and best professional judgment from the regional climate change working groups were taken into account when developing the temperature indicator lists (see Attachments in Appendices E, F and G for more information on temperature indicator taxa for each state). The 'short-list' metrics were developed because it was believed that they would have a greater chance of showing trends

Temperature Metrics - PRISM mean annual air temperature													
State	**Site/ Site Group**	**% Cold-water Individs (*Short-list*)**			**% Warn Water Individs (*Short-list*)**			**% Cold-water Individs (*Long-list*)**			**% Warm-water Individs (*Long-list*)**		
		r	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**
Maine	56817	0.15	N=23	p=.506	0.13	N=23	p=.546	-0.25	N=23	p=.258	-0.09	N=23	p=.687
	57011	-0.16	N=12	p=.617	0.37	N=12	p=.232	0.03	N=12	p=.921	-0.07	N=12	p=.834
	57065	-0.27	N=9	p=.480	0.05	N=9	p=.903	-0.23	N=9	p=.546	0.17	N=9	p=.666
	Laur	0.42	N=8	p=.295	0.38	N=8	p=.358	0.66	N=8	p=.076	-0.21	N=8	p=.612
	NEHigh	0.46	N=8	p=.250	0.20	N=8	p=.642	-0.56	N=8	p=.152	0.46	N=8	p=.252
Utah	4927250	-0.30	N=17	p=.236	-0.35	N=17	p=.174	0.26	N=17	p=.313	-0.05	N=17	p=.848
	4936750	-0.20	N=12	p=.534	0.01	N=12	p=.981	-0.10	N=12	p=.754	0.14	N=12	p=.664
	4951200	**-0.53**	**N=15**	**p=.044**	**0.62**	**N=15**	**p=.014**	**-0.54**	**N=15**	**p=.037**	0.17	N=15	p=.547
	5940440	-0.29	N=9	p=.455	0.00	N=9	p=1.00	-0.37	N=9	p=.326	-0.08	N=9	p=.846
	WU_SF	0.05	N=20	p=.826	**0.44**	**N=20**	**p=.050**	**0.70**	**N=20**	**p=.001**	-0.24	N=20	p=.307
	WU_ME	0.31	N=12	p=.324	**0.75**	**N=12**	**p=.005**	0.02	N=12	p=.945	-0.38	N=12	p=.225
	CP	0.04	N=14	p=.898	**0.65**	**N=14**	**p=.012**	0.26	N=14	p=.362	0.36	N=14	p=.205
North Carolina	NC0109 (BR)	-0.32	N=11	p=.344	0.00	N=11	p=.998	-0.13	N=11	p=.697	0.10	N=11	p=.781
	NC0207 (BR)	0.17	N=9	p=.665	0.26	N=9	p=.491	0.08	N=9	p=.848	-0.24	N=9	p=.537
	NC0209 (BR)	0.07	N=7	p=.883	-0.45	N=7	p=.310	0.32	N=7	p=.491	-0.71	N=7	p=.073
	NC0075 (P)	-0.10	N=7	p=.838	-0.16	N=7	p=.738	-0.05	N=7	p=.915	0.28	N=7	p=.545
	NC0248 (P)	-0.10	N=7	p=.837	-0.54	N=7	p=.215	0.43	N=7	p=.331	**-0.82**	**N=7**	**p=.024**

Table I3-5. Pearson product moment correlations of temperature-sensitive ***richness*** metrics versus ***PRISM mean annual precipitation*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant ($p<0.05$). The 'long-list' metrics were based on the original list of cold and warm-water taxa (which were derived from weighted average and maximum likelihood calculations and literature – see Appendix K for more information). The 'short-list' metrics were based on a subset of the 'long-list' taxa that are referred to (in other sections of this report) as temperature indicator taxa. Data from other states, case studies (i.e. evaluation of taxa lists at the coldest and warmest sites), and best professional judgment from the regional climate change working groups were taken into account when developing the temperature indicator lists (see Attachments in Appendices E, F and G for more information on temperature indicator taxa for each state). The 'short-list' metrics were developed because it was believed that they would have a greater chance of showing trends.

Temperature Richness Metrics - PRISM mean annual precipitation													
State	**Site/ Site Group**	# Cold-water Taxa (***Short-list***)			# Warm-water Taxa (***Short-list***)			# Cold-water Taxa (***Long-list***)			# Warm-water Taxa (***Long-list***)		
		r	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**
Maine	56817	0.44	N=23	p=.035	0.07	N=23	p=.751	0.32	N=23	p=.130	0.05	N=23	p=.829
	57011	0.18	N=12	p=.585	-0.04	N=12	p=.909	0.19	N=12	p=.561	0.01	N=12	p=.975
	57065	-0.51	N=9	p=.161	-0.13	N=9	p=.733	-0.12	N=9	p=.765	0.00	N=9	p=.993
	Laur	-0.23	N=8	p=.581	-0.15	N=8	p=.725	0.03	N=8	p=.935	-0.16	N=8	p=.714
	NEHigh	-0.19	N=8	p=.654	-0.03	N=8	p=.936	0.09	N=8	p=.832	0.14	N=8	p=.741
Utah	4927250	-0.11	N=17	p=.678	-0.05	N=17	p=.835	-0.07	N=17	p=.794	-0.11	N=17	p=.687
	4936750	0.42	N=12	p=.169	0.21	N=12	p=.504	0.46	N=12	p=.135	0.29	N=12	p=.363
	4951200	0.21	N=15	p=.452	-0.25	N=15	p=.361	0.26	N=15	p=.353	-0.18	N=15	p=.517
	5940440	0.01	N=9	p=.975	0.00	N=9	p=1.00	0.25	N=9	p=.512	-0.14	N=9	p=.723
	WU_SF	0.06	N=20	p=.803	-0.32	N=20	p=.171	0.03	N=20	p=.890	-0.19	N=20	p=.426
	WU_ME	0.40	N=12	p=.201	-0.50	N=12	p=.097	0.18	N=12	p=.584	-0.70	N=12	p=.010
	CP	0.00	N=14	p=.996	-0.04	N=14	p=.896	0.00	N=14	p=.991	0.11	N=14	p=.703
North Carolina	NC0109 (BR)	0.85	N=11	p=.001	-0.65	N=11	p=.029	0.63	N=11	p=.036	-0.83	N=11	p=.002
	NC0207 (BR)	0.39	N=9	p=.305	-0.29	N=9	p=.445	-0.04	N=9	p=.921	-0.78	N=9	p=.013
	NC0209 (BR)	0.31	N=7	p=.498	-0.56	N=7	p=.189	0.30	N=7	p=.518	-0.75	N=7	p=.053
	NC0075 (P)	0.34	N=7	p=.457	0.20	N=7	p=.663	0.40	N=7	p=.369	0.03	N=7	p=.942
	NC0248 (P)	0.00	N=7	p=.992	-0.43	N=7	p=.333	-0.60	N=7	p=.154	0.37	N=7	p=.408

Table I3-6. Pearson product moment correlations of temperature-sensitive ***% individual*** metrics versus ***PRISM mean annual precipitation*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant ($p<0.05$). The 'long-list' metrics were based on the original list of cold and warm-water taxa (which were derived from weighted average and maximum likelihood calculations and literature – see Appendix K for more information). The 'short-list' metrics were based on a subset of the 'long-list' taxa that are referred to (in other sections of this report) as temperature indicator taxa. Data from other states, case studies (i.e. evaluation of taxa lists at the coldest and warmest sites), and best professional judgment from the regional climate change working groups were taken into account when developing the temperature indicator lists (see Attachments in Appendices E, F and G for more information on temperature indicator taxa for each state). The 'short-list' metrics were developed because it was believed that they would have a greater chance of showing trends.

Temperature % Individual Metrics - PRISM mean annual precipitation													
State	**Site/ Site Group**	% Cold-water Individs (***Short-list***)			% Warn Water Individs (***Short-list***)			% Cold-water Individs (***Long-list***)			% Warm-water Individs (***Long-list***)		
		r	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**
Maine	56817	**0.58**	**N=23**	**p=.003**	0.04	N=23	p=.852	0.27	N=23	p=.218	-0.25	N=23	p=.247
	57011	0.03	N=12	p=.932	-0.10	N=12	p=.764	-0.44	N=12	p=.154	-0.18	N=12	p=.585
	57065	-0.02	N=9	p=.968	-0.44	N=9	p=.234	0.26	N=9	p=.497	-0.39	N=9	p=.298
	Laur	0.13	N=8	p=.756	0.00	N=8	p=.996	-0.24	N=8	p=.566	-0.32	N=8	p=.446
	NEHigh	0.36	N=8	p=.381	-0.43	N=8	p=.286	0.12	N=8	p=.768	-0.08	N=8	p=.846
Utah	4927250	0.08	N=17	p=.748	-0.14	N=17	p=.596	0.17	N=17	p=.524	0.05	N=17	p=.849
	4936750	0.30	N=12	p=.345	0.33	N=12	p=.290	0.40	N=12	p=.203	-0.54	N=12	p=.072
	4951200	0.10	N=15	p=.720	-0.34	N=15	p=.210	0.11	N=15	p=.701	0.01	N=15	p=.968
	5940440	0.54	N=9	p=.137	0.00	N=9	p=1.00	0.40	N=9	p=.291	-0.41	N=9	p=.275
	WU_SF	**-0.46**	**N=20**	**p=.042**	-0.17	N=20	p=.485	-0.27	N=20	p=.241	0.15	N=20	p=.529
	WU_ME	-0.20	N=12	p=.529	-0.24	N=12	p=.446	**-0.60**	**N=12**	**p=.039**	0.15	N=12	p=.635
	CP	-0.09	N=14	p=.763	-0.16	N=14	p=.586	-0.09	N=14	p=.761	0.21	N=14	p=.481
North Carolina	NC0109 (BR)	0.63	N=11	p=.038	-0.57	N=11	p=.065	**0.82**	**N=11**	**p=.002**	**-0.83**	**N=11**	**p=.001**
	NC0207 (BR)	**0.71**	**N=9**	**p=.032**	-0.32	N=9	p=.402	0.75	N=9	p=.020	-0.41	N=9	p=.267
	NC0209 (BR)	0.54	N=7	p=.209	-0.58	N=7	p=.168	0.55	N=7	p=.202	-0.27	N=7	p=.552
	NC0075 (P)	0.64	N=7	p=.119	0.10	N=7	p=.824	0.54	N=7	p=.214	-0.52	N=7	p=.229
	NC0248 (P)	-0.29	N=7	p=.527	0.25	N=7	p=.581	**-0.79**	**N=7**	**p=.035**	0.56	N=7	p=.194

Attachment I4

Climate Variable -EPT Metric Trends

In this Attachment, we show results for a selected subset of EPT metrics, which were examined for yearly trends and trends related to PRISM air temperature and precipitation variables.

ATTACHMENT I4. CLIMATE VARIABLE-EPT METRIC TRENDS

Results for Ephemeroptera/Plecoptera/Trichoptera (EPT) metrics (richness and percent individuals) are shown in Tables I4-1 through I4-3. There were a few significant associations between EPT metrics, year, and PRISM mean annual air temperature and annual precipitation. At 3 of the Maine sites, EPT richness was positively (and significantly) correlated with year. At 3 of the Utah sites, EPT richness was negatively (and significantly) correlated with PRISM mean annual air temperature. Two of the North Carolina stations were significantly correlated with PRISM mean annual precipitation (one was positive, the other negative). Overall there was a lack of consistent patterns, which makes it difficult to project how EPT metrics may change as a result of climate change. Developing EPT metrics that are geared more specifically towards detecting climate change effects may be worth exploring (i.e. one that detects replacement of cold-water EPT taxa with warm-water EPT taxa).

Table I4-1. Pearson product moment correlations of EPT richness (EPT_Tax) and % individual (EPT_Pct) metrics versus ***year*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant (p<0.05).

EPT Metrics - Year							
State	**Site/ Site Group**	**EPT_Pct**			**EPT_Tax**		
		r	**N**	**p**	**r**	**N**	**p**
Maine	56817	0.06	N=23	p=.801	**0.75**	**N=23**	**p=.000**
	57011	-0.52	N=12	p=.082	**0.76**	**N=12**	**p=.004**
	57065	-0.36	N=9	p=.342	0.51	N=9	p=.156
	Laur	0.39	N=8	p=.337	**0.71**	**N=8**	**p=.050**
	NEHigh	-0.67	N=8	p=.067	-0.60	N=8	p=.117
Utah	4927250	0.06	N=17	p=.812	**-0.59**	**N=17**	**p=.014**
	4936750	-0.26	N=12	p=.416	-0.21	N=12	p=.520
	4951200	0.00	N=15	p=.992	-0.49	N=15	p=.066
	5940440	0.44	N=9	p=.232	-0.65	N=9	p=.058
	WU_SF	0.14	N=20	p=.570	0.42	N=20	p=.068
	WU_ME	-0.57	N=12	p=.052	-0.34	N=12	p=.273
	CP	-0.49	N=14	p=.077	0.30	N=14	p=.303
North Carolina	NC0109 (BR)	**0.74**	**N=11**	**p=.010**	0.30	N=11	p=.364
	NC0207 (BR)	0.27	N=9	p=.485	-0.18	N=9	p=.651
	NC0209 (BR)	0.60	N=7	p=.157	0.16	N=7	p=.724
	NC0075 (P)	-0.60	N=7	p=.153	0.15	N=7	p=.747
	NC0248 (P)	0.36	N=7	p=.434	0.54	N=7	p=.210

Table I4-2. Pearson product moment correlations of EPT richness (EPT_Tax) and % individual (EPT_Pct) metrics versus ***PRISM mean annual air temperature*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant (p<0.05).

EPT Metrics - PRISM mean annual air temperature							
State	**Site/ Site Group**	**EPT_Pct**			**EPT_Tax**		
		r	**N**	**p**	**r**	**N**	**p**
Maine	56817	0.08	N=23	p=.714	0.17	N=23	p=.444
	57011	**0.64**	**N=12**	**p=.025**	0.25	N=12	p=.428
	57065	-0.07	N=9	p=.868	-0.64	N=9	p=.062
	Laur	0.16	N=8	p=.698	-0.50	N=8	p=.211
	NEHigh	0.58	N=8	p=.135	0.29	N=8	p=.493
Utah	4927250	0.03	N=17	p=.912	**-0.57**	**N=17**	**p=.017**
	4936750	0.04	N=12	p=.899	-0.09	N=12	p=.772
	4951200	0.27	N=15	p=.335	**-0.73**	**N=15**	**p=.002**
	5940440	0.07	N=9	p=.864	-0.43	N=9	p=.248
	WU_SF	0.18	N=20	p=.449	0.31	N=20	p=.186
	WU_ME	-0.27	N=12	p=.396	**-0.77**	**N=12**	**p=.004**
	CP	-0.43	N=14	p=.125	0.37	N=14	p=.187
North Carolina	NC0109 (BR)	-0.09	N=11	p=.796	0.00	N=11	p=.988
	NC0207 (BR)	-0.22	N=9	p=.574	0.35	N=9	p=.359
	NC0209 (BR)	0.03	N=7	p=.943	0.39	N=7	p=.381
	NC0075 (P)	0.14	N=7	p=.764	-0.15	N=7	p=.754
	NC0248 (P)	0.24	N=7	p=.610	0.41	N=7	p=.365

Table I4-3. Pearson product moment correlations of EPT richness (EPT_Tax) and % individual (EPT_Pct) metrics versus ***PRISM mean annual precipitation*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant ($p<0.05$).

EPT Metrics - PRISM mean annual precipitation							
State	**Site/ Site Group**	**EPT_Pct**			**EPT_Tax**		
		r	**N**	**p**	**r**	**N**	**p**
Maine	56817	0.01	N=23	p=.973	0.20	N=23	p=.365
	57011	-0.05	N=12	p=.884	0.24	N=12	p=.449
	57065	0.17	N=9	p=.662	-0.12	N=9	p=.756
	Laur	-0.45	N=8	p=.262	-0.34	N=8	p=.411
	NEHigh	0.06	N=8	p=.895	0.34	N=8	p=.416
Utah	4927250	-0.29	N=17	p=.265	-0.245	N=17	p=.343
	4936750	0.32	N=12	p=.303	0.4497	N=12	p=.142
	4951200	-0.18	N=15	p=.527	0.4483	N=15	p=.094
	5940440	0.32	N=9	p=.396	0.1728	N=9	p=.657
	WU_SF	-0.03	N=20	p=.909	-0.0528	N=20	p=.825
	WU_ME	0.43	N=12	p=.164	-0.2622	N=12	p=.410
	CP	-0.19	N=14	p=.521	-0.1404	N=14	p=.632
North Carolina	NC0109 (BR)	**0.82**	**N=11**	**p=.002**	0.36	N=11	p=.275
	NC0207 (BR)	0.44	N=9	p=.234	0.26	N=9	p=.502
	NC0209 (BR)	0.59	N=7	p=.162	0.54	N=7	p=.213
	NC0075 (P)	0.15	N=7	p=.743	0.39	N=7	p=.382
	NC0248 (P)	-0.70	N=7	p=.081	**-0.80**	**N=7**	**p=.033**

Attachment I5

Climate Variable -HBI Trends

In this Attachment, we show results for the HBI metric, which we examined for yearly trends and trends related to PRISM air temperature and precipitation variables.

ATTACHMENT I5. CLIMATE VARIABLE-HBI TRENDS

Results for the Hilsenhoff Biotic Index (HBI) are shown in **Tables I5-1**through **I5-3**. We evaluated long-term HBI trends because HBIs are used as part of water quality assessments in many states (including Maine and North Carolina, analyzed in this study). It was also valuable because we lacked long-term nutrient data for most sites, and the HBI provided us with some insight as to whether or not a site had been influenced by organic enrichment.

There were a few significant associations between HBI values, year, PRISM mean annual air temperature, and PRISM annual precipitation. At Maine Station 57011, HBI values were positively (and significantly) correlated with year, which suggests that long-term trends at this site may have been influenced by organic enrichment (higher HBI scores suggest greater organic enrichment). One site in Utah and 1 site in North Carolina were negatively (and significantly) correlated with year. HBI values at one of the Utah site groups (Wasatch Uinta Semiarid Foothills) was negatively (and significantly) correlated with PRISM mean annual air temperature. Two of the Blue Ridge North Carolina sites had strong negative correlations between HBI values and PRISM mean annual precipitation. Overall there was a lack of consistent patterns, which makes it difficult to project how HBI values may change as a result of climate change.

It should be noted that the tolerance values that were used in the HBI calculations for Maine and North Carolina sites/site groups were derived from their respective state datasets. Utah HBIs were calculated using tolerance values from the New Mexico traits database because we did not have access to state-specific ones at the time of the analyses.

Table I5-1. Pearson product moment correlations of HBI versus ***year*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant (p<0.05).

HBI - Year				
State	**Site/ Site Group**	**r**	**N**	**p**
Maine	56817	-0.13	N=23	p=.544
	57011	**0.75**	**N=12**	**p=.005**
	57065	0.00	N=9	p=.992
	Laur	-0.03	N=8	p=.951
	NEHigh	0.37	N=8	p=.363
Utah	4927250	-0.19	N=17	p=.466
	4936750	0.32	N=12	p=.313
	4951200	0.27	N=15	p=.329
	5940440	-0.46	N=9	p=.213
	WU_SF	**-0.64**	**N=20**	**p=.002**
	WU_ME	-0.32	N=12	p=.312
	CP	-0.06	N=14	p=.841
North Carolina	NC0109 (BR)	**-0.63**	**N=11**	**p=.038**
	NC0207 (BR)	-0.34	N=9	p=.374
	NC0209 (BR)	-0.50	N=7	p=.251
	NC0075 (P)	0.23	N=7	p=.614
	NC0248 (P)	-0.17	N=7	p=.710

Table I5-2. Pearson product moment correlations of HBI versus ***PRISM mean annual air temperature*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant ($p<0.05$).

HBI - PRISM mean annual air temperature				
State	**Site/ Site Group**	**r**	**N**	**p**
Maine	56817	-0.07	N=23	p=.760
	57011	-0.21	N=12	p=.512
	57065	0.12	N=9	p=.761
	Laur	-0.44	N=8	p=.275
	NEHigh	-0.15	N=8	p=.725
Utah	4927250	-0.32	N=17	p=.208
	4936750	0.09	N=12	p=.773
	4951200	0.00	N=15	p=.993
	5940440	0.09	N=9	p=.816
	WU_SF	**-0.69**	**N=20**	**p=.001**
	WU_ME	-0.38	N=12	p=.227
	CP	-0.26	N=14	p=.376
North Carolina	NC0109 (BR)	0.13	N=11	p=.705
	NC0207 (BR)	-0.07	N=9	p=.855
	NC0209 (BR)	-0.12	N=7	p=.790
	NC0075 (P)	-0.56	N=7	p=.193
	NC0248 (P)	0.13	N=7	p=.789

Table I5-3. Pearson product moment correlations of HBI versus ***PRISM mean annual precipitation*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant (p<0.05).

HBI - PRISM mean annual precipitation				
State	**Site/ Site Group**	**r**	**N**	**p**
Maine	56817	-0.22	N=23	p=.309
	57011	0.25	N=12	p=.434
	57065	-0.51	N=9	p=.165
	Laur	0.42	N=8	p=.299
	NEHigh	-0.47	N=8	p=.235
Utah	4927250	0.16	N=17	p=.533
	4936750	-0.55	N=12	p=.064
	4951200	0.32	N=15	p=.246
	5940440	-0.37	N=9	p=.322
	WU_SF	0.18	N=20	p=.442
	WU_ME	**0.68**	**N=12**	**p=.016**
	CP	0.38	N=14	p=.176
North Carolina	NC0109 (BR)	**-0.86**	**N=11**	**p=.001**
	NC0207 (BR)	**-0.72**	**N=9**	**p=.030**
	NC0209 (BR)	-0.57	N=7	p=.179
	NC0075 (P)	-0.14	N=7	p=.770
	NC0248 (P)	0.60	N=7	p=.154

Attachment I6

Climate Variable –OCH Metric Trends

In this Attachment, we show results for a selected subset of OCH metrics, which were examined for yearly trends and trends related to PRISM air temperature and precipitation variables.

ATTACHMENT I6. CLIMATE VARIABLE-OCH METRIC TRENDS

Results for Odonata/Coleoptera/Hemiptera (OCH) trait metrics (richness and percent individuals) are shown in **Tables I6-1** through **I6-3**. OCH metrics may be useful as 'hydrologic indicator' metrics because these Orders are generally expected to do better during drier, more intermittent conditions (Bonada et al. 2007a). Results in Maine, North Carolina and Utah show that there were 4 significant correlations between OCH metrics and PRISM mean annual air temperature, and all of them were positive and all occurred at site groups (Maine Laurentian Plains and Hills site group and all 3 Utah site groups). None of the OCH metrics were significantly correlated with PRISM mean annual precipitation. Most of the significant correlations were with year and occurred at the Utah sites and site groups. None of the OCH metrics at the North Carolina sites/site groups showed significant trends.

It should be noted that the lack of consistent patterns may be due in part to sampling methods. There are probably not many state biomonitoring programs that target Hemipterans for capture or that record data on them consistently. Collection methods are likely also a factor in the capture of Odonata. There tends to be greater Odonate abundance and diversity in edge habitats, and many state biomonitoring programs target riffle habitats only.

Table I6-1. Pearson product moment correlations of OCH (Odonata/Coleoptera/Hemiptera) richness (OCH_Tax) and % individual metrics (OCH_Pct) versus ***year*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant (p<0.05).

OCH Metrics - Year							
State	**Site/ Site Group**	**OCH_Pct**			**OCH_Tax**		
		r	**N**	**p**	**r**	**N**	**p**
Maine	56817	0.28	N=23	p=.204	**0.43**	**N=23**	**p=.038**
	57011	-0.52	N=12	p=.087	0.43	N=12	p=.162
	57065	0.37	N=9	p=.329	0.28	N=9	p=.468
	Laur	**-0.80**	**N=8**	**p=.016**	-0.16	N=8	p=.709
	NEHigh	0.06	N=8	p=.895	0.34	N=8	p=.416
Utah	4927250	**0.66**	**N=17**	**p=.004**	**0.61**	**N=17**	**p=.010**
	4936750	0.32	N=12	p=.313	**0.83**	**N=12**	**p=.001**
	4951200	0.38	N=15	p=.160	0.45	N=15	p=.091
	5940440	-0.59	N=9	p=.097	0.28	N=9	p=.471
	WU_SF	0.30	N=20	p=.201	**0.80**	**N=20**	**p=.000**
	WU_ME	**0.75**	**N=12**	**p=.005**	**0.88**	**N=12**	**p=.000**
	CP	**0.84**	**N=14**	**p=.000**	**0.66**	**N=14**	**p=.010**
North Carolina	NC0109 (BR)	0.14	N=11	p=.676	0.10	N=11	p=.775
	NC0207 (BR)	-0.59	N=9	p=.091	-0.25	N=9	p=.509
	NC0209 (BR)	-0.07	N=7	p=.875	0.06	N=7	p=.902
	NC0075 (P)	0.69	N=7	p=.083	0.48	N=7	p=.272
	NC0248 (P)	-0.27	N=7	p=.560	-0.40	N=7	p=.375

Table I6-2. Pearson product moment correlations of OCH (Odonata/Coleoptera/Hemiptera) richness (OCH_Tax) and % individual metrics (OCH_Pct) versus ***PRISM mean annual air temperature*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant (p<0.05).

OCH Metrics - PRISM mean annual air temperature							
State	**Site/ Site Group**	**OCH_Pct**			**OCH_Tax**		
		r	**N**	**p**	**r**	**N**	**p**
Maine	56817	0.01	N=23	p=.977	0.13	N=23	p=.541
	57011	-0.09	N=12	p=.787	0.35	N=12	p=.259
	57065	-0.33	N=9	p=.379	-0.10	N=9	p=.802
	Laur	**0.89**	**N=8**	**p=.003**	0.55	N=8	p=.160
	NEHigh	0.17	N=8	p=.679	0.38	N=8	p=.349
Utah	4927250	0.44	N=17	p=.074	0.11	N=17	p=.684
	4936750	-0.11	N=12	p=.741	0.27	N=12	p=.395
	4951200	0.27	N=15	p=.328	0.13	N=15	p=.656
	5940440	-0.01	N=9	p=.981	0.59	N=9	p=.092
	WU_SF	-0.06	N=20	p=.812	**0.64**	**N=20**	**p=.003**
	WU_ME	0.51	N=12	p=.087	**0.58**	**N=12**	**p=.047**
	CP	**0.71**	**N=14**	**p=.005**	0.35	N=14	p=.219
North Carolina	NC0109 (BR)	0.30	N=11	p=.369	0.14	N=11	p=.679
	NC0207 (BR)	0.05	N=9	p=.891	-0.03	N=9	p=.942
	NC0209 (BR)	0.28	N=7	p=.543	-0.11	N=7	p=.818
	NC0075 (P)	0.18	N=7	p=.696	0.16	N=7	p=.728
	NC0248 (P)	0.09	N=7	p=.855	-0.42	N=7	p=.344

Table I6-3. Pearson product moment correlations of OCH (Odonata/Coleoptera/Hemiptera) richness (OCH_Tax) and % individual metrics (OCH_Pct) versus ***PRISM mean annual precipitation*** for individual sites and site groups in Maine, Utah and North Carolina. No correlations were significant (p<0.05).

OCH Metrics - PRISM mean annual precipitation							
State	**Site/ Site Group**	**OCH_Pct**			**OCH_Tax**		
		r	**N**	**p**	**r**	**N**	**p**
Maine	56817	0.25	N=23	p=.244	0.28	N=23	p=.198
	57011	0.29	N=12	p=.359	0.07	N=12	p=.819
	57065	-0.33	N=9	p=.390	-0.44	N=9	p=.237
	Laur	0.17	N=8	p=.692	-0.31	N=8	p=.455
	NEHigh	-0.29	N=8	p=.493	-0.25	N=8	p=.546
Utah	4927250	-0.04	N=17	p=.886	0.46	N=17	p=.063
	4936750	0.25	N=12	p=.430	0.15	N=12	p=.640
	4951200	-0.33	N=15	p=.230	0.23	N=15	p=.415
	5940440	-0.28	N=9	p=.470	-0.30	N=9	p=.440
	WU_SF	0.23	N=20	p=.328	-0.23	N=20	p=.336
	WU_ME	-0.45	N=12	p=.143	-0.27	N=12	p=.392
	CP	0.08	N=14	p=.783	0.28	N=14	p=.328
North Carolina	NC0109 (BR)	-0.22	N=11	p=.520	-0.06	N=11	p=.861
	NC0207 (BR)	-0.23	N=9	p=.551	-0.50	N=9	p=.169
	NC0209 (BR)	-0.48	N=7	p=.274	0.52	N=7	p=.236
	NC0075 (P)	-0.24	N=7	p=.597	0.09	N=7	p=.853
	NC0248 (P)	0.26	N=7	p=.572	-0.13	N=7	p=.783

Attachment I7

Climate Variable –'Hydrologic' Metric Trends

In this Attachment, we show results for a selected subset of hydrologic metrics, which were examined for yearly trends and trends related to PRISM air temperature and precipitation variables.

ATTACHMENT I7. CLIMATE VARIABLE-HYDROLOGIC METRIC TRENDS

The perennial and intermittent 'hydrologic indicator' metrics are based on literature (NCDWQ, 2005; Del Rosario and Resh, 2000). If these taxa, which require water for their entire life cycle, are found at a site in a later instar larval stage, they are considered indicators of perennial stream features. The list of intermittent taxa was based on interpretation of NCDWQ (2005) and includes amphipods, isopods, small elongate Dipteran larvae (Ceratopogonidae, Blephariceridae, Chironomidae, Deuterophlebiidae, Psychodidae) winter stoneflies (Capniidae, Taeniopterygidae), Dytiscidae, Helichus larvae and Dasyhela (family Dolchopodidae). These taxa tend to be more dominant in numbers in intermittent conditions (probably due in part to loss of predators), but are (aside from Helichus larvae and Dasyhela) also found in perennial streams.

Results for the perennial and intermittent metrics (richness and percent individuals) are shown in **Tables I7-1** through **I7-3**. There were a few significant associations between these metrics, year, PRISM mean annual air temperature, and PRISM mean annual precipitation in each of the states. All of the significant correlations with PRISM mean annual air temperature occurred at the Utah sites/site groups: at 3 sites/site groups, the perennial richness metric was negatively correlated with annual air temperature, and at one of the site groups (Wasatch Uinta Semiarid Foothills), the intermittent richness metrics was positively correlated with annual air temperature. The intermittent metrics were significantly correlated with PRISM mean annual precipitation at 4 sites/site groups (3 in Utah, 1 in North Carolina), while % perennial individuals was positively correlated with annual precipitation at 1 of the North Carolina sites (Station NC0109). In terms of yearly trends, all 4 metrics were significantly correlated with year at Maine Station 57011. The metrics also showed various yearly trends (more with richness metrics than with % individuals) at 3 sites/site groups in Utah and 1 site in North Carolina (Station NC0109).

Table I7-1. Pearson product moment correlations of 'hydrologic' richness (_Tax) and % individual (_Pct) metrics versus ***year*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant (p<0.05). The perennial taxa require water for their entire life cycle and the intermittent taxa tend to be more dominant in numbers in intermittent conditions. Perennial and intermittent taxa lists were derived from NCDWQ 2005 and Del Rosario et al. 2000.

Perennial/Intermittent Metrics - YEAR													
State	**Site/ Site Group**	**Perennial_Pct**			**Intermit_Pct**			**Perennial_Tax**			**Intermit_Tax**		
		r	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**
Maine	56817	0.16	N=23	p=.466	-0.22	N=23	p=.323	0.75	N=23	p=.000	0.62	N=23	p=.001
	57011	**-0.68**	**N=12**	**p=.016**	**0.58**	**N=12**	**p=.046**	**0.66**	**N=12**	**p=.020**	**0.71**	**N=12**	**p=.010**
	57065	-0.38	N=9	p=.314	0.06	N=9	p=.877	0.53	N=9	p=.146	0.26	N=9	p=.493
	Laur	-0.09	N=8	p=.841	0.18	N=8	p=.677	0.70	N=8	p=.054	0.16	N=8	p=.706
	NEHigh	-0.56	N=8	p=.145	0.46	N=8	p=.252	-0.69	N=8	p=.058	0.46	N=8	p=.248
Utah	4927250	0.18	N=17	p=.499	-0.39	N=17	p=.119	-0.26	N=17	p=.319	0.41	N=17	p=.105
	4936750	0.26	N=12	p=.411	0.07	N=12	p=.833	0.11	N=12	p=.734	0.48	N=12	p=.115
	4951200	-0.11	N=15	p=.688	-0.30	N=15	p=.277	-0.43	N=15	p=.114	0.15	N=15	p=.601
	5940440	0.31	N=9	p=.424	-0.42	N=9	p=.255	-0.55	N=9	p=.123	-0.73	N=9	p=.027
	WU_SF	-0.04	N=20	p=.860	-0.39	N=20	p=.094	**0.48**	**N=20**	**p=.033**	**0.69**	**N=20**	**p=.001**
	WU_ME	0.34	N=12	p=.281	-0.29	N=12	p=.362	-0.21	N=12	p=.508	**0.58**	**N=12**	**p=.050**
	CP	0.03	N=14	p=.922	0.08	N=14	p=.778	0.46	N=14	p=.098	**0.67**	**N=14**	**p=.009**
North Carolina	NC0109 (BR)	**0.68**	**N=11**	**p=.023**	**-0.70**	**N=11**	**p=.016**	-0.01	N=11	p=.968	**-0.78**	**N=11**	**p=.005**
	NC0207 (BR)	0.39	N=9	p=.296	-0.35	N=9	p=.349	-0.47	N=9	p=.199	-0.44	N=9	p=.238
	NC0209 (BR)	0.60	N=7	p=.157	-0.70	N=7	p=.083	0.46	N=7	p=.300	-0.29	N=7	p=.527
	NC0075 (P)	-0.48	N=7	p=.279	0.34	N=7	p=.462	-0.10	N=7	p=.830	0.06	N=7	p=.893
	NC0248 (P)	-0.07	N=7	p=.886	0.49	N=7	p=.261	-0.34	N=7	p=.450	0.58	N=7	p=.175

Table I7-2. Pearson product moment correlations of 'hydrologic' richness (_Tax) and % individual (_Pct) metrics versus ***PRISM mean annual air temperature*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant (p<0.05). The perennial taxa require water for their entire life cycle and the intermittent taxa tend to be more dominant in numbers in intermittent conditions. Perennial and intermittent taxa lists were derived from NCDWQ 2005 and Del Rosario et al. 2000.

Perennial/Intermittent Metrics - PRISM mean annual air temperature													
State	**Site/ Site Group**	**Perennial_Pct**			**Intermit_Pct**			**Perennial_Tax**			**Intermit_Tax**		
		r	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**
Maine	56817	0.08	N=23	p=.726	-0.11	N=23	p=.604	0.11	N=23	p=.620	0.36	N=23	p=.087
	57011	0.52	N=12	p=.084	-0.52	N=12	p=.082	0.45	N=12	p=.143	-0.09	N=12	p=.774
	57065	-0.11	N=9	p=.768	0.23	N=9	p=.547	-0.54	N=9	p=.135	-0.27	N=9	p=.479
	Laur	0.61	N=8	p=.106	-0.69	N=8	p=.056	-0.28	N=8	p=.498	-0.58	N=8	p=.134
	NEHigh	0.61	N=8	p=.108	-0.57	N=8	p=.139	0.32	N=8	p=.437	-0.47	N=8	p=.240
Utah	4927250	-0.09	N=17	p=.741	-0.20	N=17	p=.430	**-0.52**	**N=17**	**p=.034**	0.09	N=17	p=.721
	4936750	-0.12	N=12	p=.709	0.13	N=12	p=.695	-0.05	N=12	p=.869	0.14	N=12	p=.668
	4951200	-0.12	N=15	p=.666	-0.42	N=15	p=.121	**-0.68**	**N=15**	**p=.005**	-0.13	N=15	p=.656
	5940440	0.20	N=9	p=.600	-0.10	N=9	p=.799	-0.21	N=9	p=.591	-0.39	N=9	p=.304
	WU_SF	-0.28	N=20	p=.235	-0.29	N=20	p=.217	0.35	N=20	p=.129	**0.51**	**N=20**	**p=.023**
	WU_ME	0.34	N=12	p=.275	-0.29	N=12	p=.353	**-0.68**	**N=12**	**p=.014**	0.23	N=12	p=.467
	CP	-0.36	N=14	p=.211	0.22	N=14	p=.456	0.29	N=14	p=.312	0.12	N=14	p=.684
North Carolina	NC0109 (BR)	0.07	N=11	p=.845	-0.21	N=11	p=.543	0.24	N=11	p=.473	-0.08	N=11	p=.809
	NC0207 (BR)	-0.07	N=9	p=.862	0.14	N=9	p=.724	0.05	N=9	p=.892	0.24	N=9	p=.534
	NC0209 (BR)	0.29	N=7	p=.522	-0.25	N=7	p=.593	0.41	N=7	p=.360	-0.30	N=7	p=.511
	NC0075 (P)	0.08	N=7	p=.869	-0.26	N=7	p=.566	-0.11	N=7	p=.819	-0.19	N=7	p=.687
	NC0248 (P)	-0.07	N=7	p=.887	0.30	N=7	p=.518	-0.27	N=7	p=.565	0.21	N=7	p=.656

Table I7-3. Pearson product moment correlations of 'hydrologic' richness (_Tax) and % individual (_Pct) metrics versus ***PRISM mean annual precipitation*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant ($p<0.05$). The perennial taxa require water for their entire life cycle and the intermittent taxa tend to be more dominant in numbers in intermittent conditions. Perennial and intermittent taxa lists were derived from NCDWQ 2005 and Del Rosario et al. 2000.

Perennial/Intermittent Metrics - PRISM mean annual precipitation													
State	**Site/ Site Group**	**Perennial_Pct**			**Intermit_Pct**			**Perennial_Tax**			**Intermit_Tax**		
		r	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**
Maine	56817	0.02	N=23	p=.939	-0.01	N=23	p=.950	0.22	N=23	p=.324	-0.01	N=23	p=.978
	57011	0.21	N=12	p=.506	-0.03	N=12	p=.918	0.41	N=12	p=.188	0.13	N=12	p=.696
	57065	0.05	N=9	p=.891	-0.17	N=9	p=.654	-0.38	N=9	p=.317	-0.30	N=9	p=.440
	Laur	-0.36	N=8	p=.382	0.22	N=8	p=.597	-0.42	N=8	p=.305	0.07	N=8	p=.874
	NEHigh	0.06	N=8	p=.886	-0.26	N=8	p=.530	0.06	N=8	p=.893	-0.09	N=8	p=.831
Utah	4927250	-0.32	N=17	p=.204	0.33	N=17	p=.198	-0.11	N=17	p=.679	**0.59**	**N=17**	**p=.013**
	4936750	0.34	N=12	p=.281	-0.53	N=12	p=.075	0.37	N=12	p=.234	0.43	N=12	p=.167
	4951200	0.06	N=15	p=.845	**0.63**	**N=15**	**p=.012**	0.30	N=15	p=.272	0.15	N=15	p=.590
	5940440	0.22	N=9	p=.562	-0.34	N=9	p=.366	0.16	N=9	p=.687	0.19	N=9	p=.616
	WU_SF	0.35	N=20	p=.134	0.12	N=20	p=.609	0.04	N=20	p=.856	-0.08	N=20	p=.748
	WU_ME	-0.14	N=12	p=.654	0.39	N=12	p=.206	-0.34	N=12	p=.278	-0.07	N=12	p=.819
	CP	0.09	N=14	p=.753	0.03	N=14	p=.918	0.08	N=14	p=.784	**0.54**	**N=14**	**p=.045**
North Carolina	NC0109 (BR)	**0.66**	**N=11**	**p=.026**	-0.39	N=11	p=.242	0.03	N=11	p=.941	**-0.76**	**N=11**	**p=.006**
	NC0207 (BR)	0.35	N=9	p=.354	-0.48	N=9	p=.187	-0.13	N=9	p=.746	-0.44	N=9	p=.237
	NC0209 (BR)	0.31	N=7	p=.495	-0.44	N=7	p=.326	0.60	N=7	p=.159	-0.36	N=7	p=.431
	NC0075 (P)	-0.31	N=7	p=.495	0.29	N=7	p=.529	0.34	N=7	p=.451	0.50	N=7	p=.252
	NC0248 (P)	-0.30	N=7	p=.510	-0.02	N=7	p=.974	-0.29	N=7	p=.524	-0.05	N=7	p=.907

Attachment I8

Climate Variable –Scenario Metric Trends

In this Attachment, we show results for a selected subset of ‘scenario’ metrics, which were examined for yearly trends and trends related to PRISM air temperature and precipitation variables.

ATTACHMENT I8. CLIMATE VARIABLE-SCENARIO METRIC TRENDS

In addition to looking at individual trait metrics, we also developed metrics that were based on combinations of traits. The first step was to select traits and trait states likely to be "functionally" linked to the projected changes in temperature and precipitation associated with climate change. We used the available information (some literature, some best professional judgment) to develop composite lists of favorable and unfavorable traits and trait states under two different generalized scenarios (thus we termed these metrics 'scenario' metrics): 1. conditions become drier and warmer (i.e. interrupted flows, more pool-like conditions, maybe some perennial streams become intermittent, conditions become more unpredictable and organisms experience more disturbances); and 2.conditions become warmer and wetter (i.e. more frequent and severe flood events, more winter rains (instead of snow), more high flows, conditions become more unpredictable and organisms experience more disturbances). Lists of the traits and trait states that were deemed favorable and unfavorable are shown in **Tables I8-1** (drier-warmer scenario) and **I8-2** (wetter-warmer scenario). Taxa that possessed the most number of favorable traits states formed the basis of the 'robust' metrics. Those that had the most number of unfavorable trait states formed the basis of the 'vulnerable' metrics.

There are too many results to show and easily summarize, but all are available upon request. Results for the warmer-drier-vulnerable, drier-vulnerable and drier-robust scenario metrics (richness and % individuals) are shown in **Tables I8-3** through **I8-8**. The drier scenario metrics were chosen because it seems likely that drier conditions will impact the biota more than wetter conditions. There was at least one significant association between at least one of these metrics, year and PRISM mean annual air temperature and annual precipitation in each of the states. As expected, the drier-vulnerable/warmer-drier-vulnerable metrics tended to follow similar patterns. Drier-vulnerable ***richness*** metrics were significantly and negatively correlated with PRISM mean annual air temperature at Maine Station 57065 and at 3 Utah sites/site groups. The drier-robust richness metric was negatively correlated with annual air temperature at the Utah Colorado Plateau site group. The drier-vulnerable ***% individuals*** metrics did not show consistent patterns across sites within or across states (i.e. warmer-drier-vulnerable was negatively correlated with annual air temperature at Maine Laurentian Plains and Hills site group and positively correlated at the Utah Wasatch Uinta Mid-elevation Mountain site group). Only a few of the metrics were significantly correlated with PRISM mean annual precipitation and these metrics sometimes showed unexpected patterns (i.e. % drier-vulnerable individuals was negatively correlated with mean annual precipitation with a Utah site group). Both the richness and % individual metrics showed some significant but mixed yearly trends in each state, with the most number of significant correlations occurring in Utah and the least number in North Carolina.

There are limitations associated with the scenario metrics:

- These metrics are essentially exploratory. It was difficult to know which groups of traits to use in the metrics (see Appendix K for more information on traits and trait selections), and these results should be viewed as a first step that motivates additional investigation. As more information becomes available about which combinations of traits and trait states are most strongly linked to climate change effects, these metrics should be further refined. More experimental data would be very helpful.
- Some traits are likely more important than others and should probably be weighted differently. However, we had insufficient information on which to base such decisions at this time.
- The different scenarios are not mutually exclusive (i.e., there could be both wetter (more flood events) and drier (drought or more frequent and severe low flow events) conditions occurring in the same year in some regions.
- Climate models consistently project that temperatures will increase but there is more uncertainty regarding the potential changes to hydrologic regimes. It is tough to make generalizations about favorable/unfavorable trait states because the characteristics of the hydrologic events can vary so much (severity, timing, duration and frequency).
- Intuitively, it would seem that taxa that are best suited to surviving unpredictable conditions/more frequent disturbance will fare better (i.e. can reproduce quickly, develop quickly, small size). More experimental data on which taxa do best under disturbance conditions also would be very helpful.

Table I8-1. SCENARIO: Drier Warmer, Drier Warmer Conditions (more pool-like, maybe some perennials go intermittent, unpredictable, more disturbance)

Traits	**Favorable**	**Unfavorable**
Life history		
Voltinism	bi_multi	semi (<1 generation/yr
Development		
Synchronization of emergence		
Adult life span		
Adult ability to exit	present	absent
Ability to survive desiccation	present	absent
Mobility		
Dispersal (adult)	high	low
Adult flying strength	strong	weak
Occurrence in drift	rare	abundant
Maximum crawling rate		
Swimming ability	strong	none
Morphology		
Attachment		
Armoring	good	none
Shape	not_stream	
Respiration	plastron_spir	tegument
Size at maturity	small	
Resource acquisition/preference		
Rheophily	depo	eros
Habit (primary)	SK, SW	
Functional feeding group (primary)	CG	CF, SH
Temperature Indicator	warm	cold

Table I8-2. SCENARIO: Wetter Warmer , More frequent and severe flood events, more winter rains (vs. snow) (more high flows, unpredictable, more disturbance)

Traits	Favorable	Unfavorable
Life history		
Voltinism	bi_multi	semi
Development		
Synchronization of emergence		
Adult life span		
Adult ability to exit	present	absent
Ability to survive desiccation		
Mobility		
Dispersal (adult)	high	low
Adult flying strength		
Occurrence in drift	abundant	
Maximum crawling rate	high	very low
Swimming ability		
Morphology		
Attachment		
Armoring		
Shape	stream	not_stream
Respiration		
Size at maturity	small	large
Resource acquisition/preference		
Rheophily	eros	depo
Habit (primary)		SK, SW
Functional feeding group (primary)	CF, SH	CG
Temperature Indicator	warm	cold

Table I8-3. Pearson product moment correlations of drier scenario ***richness*** metrics versus ***year*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant (p<0.05).

Drier Scenario Richness Metrics - YEAR										
State	**Site/ Site Group**	**WarmDrier_VulnerableTax**			**Drier_VulnerableTax**			**Drier_WinTax**		
		r	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**
Maine	56817	**0.42**	**N=23**	**p=.047**	**0.71**	**N=23**	**p=.000**	0.10	N=23	p=.659
	57011	0.29	N=12	p=.352	**0.60**	**N=12**	**p=.040**	0.25	N=12	p=.443
	57065	0.38	N=9	p=.313	0.43	N=9	p=.253	-0.19	N=9	p=.618
	Laur	0.39	N=8	p=.345	0.58	N=8	p=.135	**-0.78**	**N=8**	**p=.021**
	NEHigh	-0.37	N=8	p=.373	-0.33	N=8	p=.422	0.24	N=8	p=.574
Utah	4927250	**-0.64**	**N=17**	**p=.006**	**-0.57**	**N=17**	**p=.018**	**0.66**	**N=17**	**p=.004**
	4936750	-0.08	N=12	p=.815	-0.01	N=12	p=.964	0.00	N=12	p=1.00
	4951200	**-0.62**	**N=15**	**p=.014**	-0.35	N=15	p=.205	-0.05	N=15	p=.858
	5940440	-0.51	N=9	p=.163	-0.58	N=9	p=.104	-0.47	N=9	p=.197
	WU_SF	0.39	N=20	p=.087	0.42	N=20	p=.062	0.35	N=20	p=.128
	WU_ME	-0.50	N=12	p=.098	-0.45	N=12	p=.147	0.23	N=12	p=.468
	CP	0.15	N=14	p=.607	0.26	N=14	p=.367	-0.13	N=14	p=.665
North Carolina	NC0109 (BR)	-0.16	N=11	p=.637	-0.19	N=11	p=.573	0.34	N=11	p=.313
	NC0207 (BR)	-0.21	N=9	p=.579	-0.94	N=9	p=.000	0.00	N=9	p=1.00
	NC0209 (BR)	0.12	N=7	p=.799	-0.26	N=7	p=.567	0.00	N=7	p=1.00
	NC0075 (P)	-0.09	N=7	p=.854	-0.08	N=7	p=.871	0.41	N=7	p=.356
	NC0248 (P)	-0.32	N=7	p=.481	-0.65	N=7	p=.116	-0.06	N=7	p=.901

Table I8-4. Pearson product moment correlations of drier scenario ***% individual*** metrics versus ***year*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant (p<0.05).

% Drier Scenario Metrics - YEAR										
State	**Site/ Site Group**	**Drier_WinPct**			**Drier_VulnerablePct**			**WarmDrier_VulnerablePct**		
		r	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**
Maine	56817	0.16	N=23	p=.476	0.19	N=23	p=.389	0.49	N=23	p=.018
	57011	0.19	N=12	p=.561	-0.53	N=12	p=.078	-0.35	N=12	p=.270
	57065	-0.22	N=9	p=.566	-0.17	N=9	p=.659	0.36	N=9	p=.336
	Laur	-0.51	N=8	p=.199	0.62	N=8	p=.101	**0.82**	**N=8**	**p=.012**
	NEHigh	-0.04	N=8	p=.930	-0.54	N=8	p=.167	-0.20	N=8	p=.635
Utah	4927250	**0.60**	**N=17**	**p=.011**	0.09	N=17	p=.719	**-0.72**	**N=17**	**p=.001**
	4936750	0.00	N=12	p=1.00	-0.46	N=12	p=.137	0.01	N=12	p=.985
	4951200	0.07	N=15	p=.793	-0.17	N=15	p=.534	**-0.58**	**N=15**	**p=.023**
	5940440	-0.06	N=9	p=.879	0.30	N=9	p=.426	-0.10	N=9	p=.790
	WU_SF	0.34	N=20	p=.143	0.15	N=20	p=.533	-0.16	N=20	p=.488
	WU_ME	0.23	N=12	p=.468	0.45	N=12	p=.145	**0.79**	**N=12**	**p=.002**
	CP	-0.35	N=14	p=.220	-0.16	N=14	p=.583	-0.02	N=14	p=.957
North Carolina	NC0109 (BR)	0.33	N=11	p=.317	**0.77**	**N=11**	**p=.005**	**0.65**	**N=11**	**p=.030**
	NC0207 (BR)	0.00	N=9	p=1.00	0.04	N=9	p=.921	0.20	N=9	p=.605
	NC0209 (BR)	0.00	N=7	p=1.00	0.31	N=7	p=.493	0.26	N=7	p=.568
	NC0075 (P)	0.09	N=7	p=.853	-0.54	N=7	p=.208	0.13	N=7	p=.783
	NC0248 (P)	-0.22	N=7	p=.635	-0.60	N=7	p=.153	-0.09	N=7	p=.845

Table I8-5. Pearson product moment correlations of drier scenario ***richness*** metrics versus ***PRISM mean annual air temperature*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant (p<0.05).

Drier Scenario Richness Metrics - PRISM mean annual air temperature										
State	**Site/ Site Group**	**WarmDrier_VulnerableTax**			**Drier_VulnerableTax**			**Drier_WinTax**		
		r	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**
Maine	56817	0.30	N=23	p=.159	0.13	N=23	p=.547	0.21	N=23	p=.329
	57011	-0.01	N=12	p=.975	0.29	N=12	p=.354	-0.11	N=12	p=.738
	57065	**-0.71**	**N=9**	**p=.033**	**-0.73**	**N=9**	**p=.026**	0.02	N=9	p=.962
	Laur	-0.70	N=8	p=.054	-0.48	N=8	p=.231	0.70	N=8	p=.051
	NEHigh	-0.06	N=8	p=.883	0.09	N=8	p=.826	-0.64	N=8	p=.090
Utah	4927250	**-0.56**	**N=17**	**p=.019**	**-0.66**	**N=17**	**p=.004**	0.16	N=17	p=.537
	4936750	0.01	N=12	p=.973	-0.08	N=12	p=.810	0.00	N=12	p=1.00
	4951200	**-0.62**	**N=15**	**p=.013**	**-0.61**	**N=15**	**p=.016**	-0.32	N=15	p=.239
	5940440	0.09	N=9	p=.820	-0.08	N=9	p=.833	0.09	N=9	p=.828
	WU_SF	0.43	N=20	p=.058	0.38	N=20	p=.095	0.12	N=20	p=.626
	WU_ME	-0.40	N=12	p=.198	**-0.60**	**N=12**	**p=.040**	0.18	N=12	p=.580
	CP	0.14	N=14	p=.624	0.27	N=14	p=.359	**-0.57**	**N=14**	**p=.032**
North Carolina	NC0109 (BR)	-0.23	N=11	p=.501	-0.06	N=11	p=.853	0.26	N=11	p=.449
	NC0207 (BR)	-0.41	N=9	p=.279	-0.47	N=9	p=.205	0.00	N=9	p=1.00
	NC0209 (BR)	0.30	N=7	p=.519	-0.27	N=7	p=.560	0.00	N=7	p=1.00
	NC0075 (P)	-0.24	N=7	p=.601	0.15	N=7	p=.750	-0.44	N=7	p=.323
	NC0248 (P)	-0.02	N=7	p=.975	-0.29	N=7	p=.522	-0.71	N=7	p=.072

Table I8-6. Pearson product moment correlations of drier scenario ***% individual*** metrics versus ***PRISM mean annual air temperature*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant (p<0.05).

% Drier Scenario Metrics - PRISM mean annual air temperature										
State	**Site/ Site Group**	**Drier_WinPct**			**Drier_VulnerablePct**			**WarmDrier_VulnerablePct**		
		r	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**
Maine	56817	0.17	N=23	p=.425	0.18	N=23	p=.407	0.17	N=23	p=.428
	57011	-0.05	N=12	p=.876	**0.64**	**N=12**	**p=.026**	-0.14	N=12	p=.661
	57065	0.29	N=9	p=.447	-0.29	N=9	p=.442	-0.37	N=9	p=.329
	Laur	0.68	N=8	p=.062	-0.12	N=8	p=.781	**-0.71**	**N=8**	**p=.050**
	NEHigh	-0.13	N=8	p=.755	0.30	N=8	p=.472	0.16	N=8	p=.707
Utah	4927250	**0.52**	**N=17**	**p=.032**	0.31	N=17	p=.231	-0.30	N=17	p=.243
	4936750	0.00	N=12	p=1.00	-0.06	N=12	p=.862	-0.29	N=12	p=.357
	4951200	-0.06	N=15	p=.819	-0.17	N=15	p=.538	-0.48	N=15	p=.071
	5940440	0.38	N=9	p=.311	-0.38	N=9	p=.314	-0.27	N=9	p=.481
	WU_SF	0.19	N=20	p=.433	0.11	N=20	p=.647	0.03	N=20	p=.914
	WU_ME	0.18	N=12	p=.580	0.27	N=12	p=.394	**0.58**	**N=12**	**p=.048**
	CP	**-0.68**	**N=14**	**p=.007**	-0.37	N=14	p=.189	0.04	N=14	p=.891
North Carolina	NC0109 (BR)	0.01	N=11	p=.984	0.04	N=11	p=.910	-0.24	N=11	p=.476
	NC0207 (BR)	0.00	N=9	p=1.00	-0.40	N=9	p=.284	0.03	N=9	p=.936
	NC0209 (BR)	0.00	N=7	p=1.00	0.20	N=7	p=.675	0.44	N=7	p=.325
	NC0075 (P)	-0.51	N=7	p=.244	0.17	N=7	p=.719	-0.13	N=7	p=.786
	NC0248 (P)	-0.49	N=7	p=.262	-0.75	N=7	p=.052	0.28	N=7	p=.549

Table I8-7. Pearson product moment correlations of drier scenario ***richness*** metrics versus ***PRISM mean annual precipitation*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant ($p<0.05$).

Drier Scenario Richness Metrics - PRISM mean annual precipitation										
State	**Site/ Site Group**	**WarmDrier_VulnerableTax**			**Drier_VulnerableTax**			**Drier_WinTax**		
		r	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**
Maine	56817	**0.45**	**N=23**	**p=.032**	0.27	N=23	p=.217	-0.04	N=23	p=.865
	57011	0.07	N=12	p=.833	0.49	N=12	p=.105	0.37	N=12	p=.237
	57065	-0.51	N=9	p=.160	-0.49	N=9	p=.181	-0.08	N=9	p=.848
	Laur	-0.52	N=8	p=.183	-0.42	N=8	p=.298	-0.08	N=8	p=.853
	NEHigh	0.09	N=8	p=.832	0.27	N=8	p=.513	-0.49	N=8	p=.217
Utah	4927250	-0.19	N=17	p=.474	-0.12	N=17	p=.656	0.42	N=17	p=.090
	4936750	**0.60**	**N=12**	**p=.037**	0.31	N=12	p=.334	0.00	N=12	p=1.00
	4951200	0.24	N=15	p=.392	0.24	N=15	p=.384	0.47	N=15	p=.078
	5940440	-0.11	N=9	p=.785	0.22	N=9	p=.578	-0.04	N=9	p=.928
	WU_SF	-0.12	N=20	p=.622	0.03	N=20	p=.885	0.10	N=20	p=.676
	WU_ME	0.16	N=12	p=.631	-0.18	N=12	p=.569	0.31	N=12	p=.331
	CP	-0.01	N=14	p=.972	-0.03	N=14	p=.910	0.45	N=14	p=.103
North Carolina	NC0109 (BR)	0.24	N=11	p=.477	-0.53	N=11	p=.091	-0.33	N=11	p=.324
	NC0207 (BR)	0.04	N=9	p=.920	-0.22	N=9	p=.577	0.00	N=9	p=1.00
	NC0209 (BR)	0.10	N=7	p=.838	-0.02	N=7	p=.963	0.00	N=7	p=1.00
	NC0075 (P)	0.01	N=7	p=.977	0.18	N=7	p=.692	0.19	N=7	p=.690
	NC0248 (P)	0.07	N=7	p=.876	0.23	N=7	p=.618	0.15	N=7	p=.744

Table I8-8. Pearson product moment correlations of drier scenario ***% individual*** metrics versus ***PRISM mean annual precipitation*** for individual sites and site groups in Maine, Utah and North Carolina. Highlighted correlations were significant (p<0.05). The Blue Ridge Drier_WinPct entry was NA (=not available) because of a low sample size/lack of drier-robust individuals in this site group.

% Drier Scenario Metrics - PRISM mean annual precipitation										
State	**Site/ Site Group**	**Drier_WinPct**			**Drier_VulnerablePct**			**WarmDrier_VulnerablePct**		
		r	**N**	**p**	**r**	**N**	**p**	**r**	**N**	**p**
Maine	56817	-0.03	N=23	p=.877	0.06	N=23	p=.780	**0.59**	**N=23**	**p=.003**
	57011	0.48	N=12	p=.115	0.31	N=12	p=.322	-0.21	N=12	p=.517
	57065	0.05	N=9	p=.904	-0.01	N=9	p=.979	-0.06	N=9	p=.869
	Laur	0.09	N=8	p=.832	0.16	N=8	p=.703	-0.35	N=8	p=.390
	NEHigh	-0.63	N=8	p=.095	-0.01	N=8	p=.987	0.39	N=8	p=.338
Utah	4927250	0.33	N=17	p=.199	0.23	N=17	p=.372	0.08	N=17	p=.763
	4936750	0.00	N=12	p=1.00	0.52	N=12	p=.082	0.23	N=12	p=.465
	4951200	0.42	N=15	p=.119	-0.46	N=15	p=.085	0.13	N=15	p=.634
	5940440	-0.28	N=9	p=.463	0.28	N=9	p=.460	0.53	N=9	p=.141
	WU_SF	0.04	N=20	p=.853	-0.03	N=20	p=.909	-0.27	N=20	p=.242
	WU_ME	0.31	N=12	p=.331	**-0.62**	**N=12**	**p=.030**	-0.19	N=12	p=.563
	CP	0.26	N=14	p=.379	0.13	N=14	p=.657	-0.09	N=14	p=.747
North Carolina	NC0109 (BR)	-0.11	N=11	p=.748	0.16	N=11	p=.639	0.57	N=11	p=.067
	NC0207 (BR)	0.00	N=9	p=1.00	0.10	N=9	p=.806	-0.20	N=9	p=.614
	NC0209 (BR)	0.00	N=7	p=1.00	0.55	N=7	p=.196	0.45	N=7	p=.310
	NC0075 (P)	0.23	N=7	p=.624	-0.22	N=7	p=.636	0.47	N=7	p=.283
	NC0248 (P)	0.16	N=7	p=.727	0.40	N=7	p=.368	-0.32	N=7	p=.479

APPENDIX J

Case Studies

Part of this project involved doing 3 case studies. One was a case study on the combined effects of climate change and urbanization on stream condition in the North Carolina Piedmont physiographic region. In the second study, we compared hydrologic response to fluctuating climate with land use effects in the Mid-Atlantic region. In the third, data from Florida reference sites were analyzed to assess the vulnerability of reference condition and biological monitoring to climate change and increasing population densities. This Appendix contains summaries of each of the case studies.

J1. Combined effects of climate change and urbanization on stream condition (North Carolina Piedmont physiographic region)
J2. Another face of the changing climate: comparing hydrologic response to fluctuating climate with land use effects (Mid-Atlantic region)
J3. Shifting Baselines of Perception: Vulnerability of Reference Condition to Climate and Land Use Change (Florida Reference Sites)

J1. Combined Effects of Climate Change and Urbanization on Stream Condition

Data from the North Carolina Piedmont physiographic region were used in this case study. Locations of the sampling sites are shown in Figure J**1-1**. The study area has undergone rapid population growth and urbanization since 1945, which has contributed to flashier streams and altered habitat. Data preparation for the study involved developing operational taxonomic units (OTUs), calculating taxa richness-based metrics, calculating Indicator of Hydrologic Alteration (IHA) parameters (Richter et al., 1996) and Baker's Flashiness Index (Baker et al., 2004) for 67 biological sampling sites that were associated with USGS gage stations, and dividing the sites into natural, urban, agricultural and other land-use categories based on quick examination of the watersheds in Google Earth.

The main objective of this study was to assess the response of macroinvertebrates in urban and non-urban streams to hydrologic changes. We used number of EPT taxa as the principal response metric and flashiness (sum of daily flow changes divided by total flow), low pulse count (number of events per year where flow is below the 25^{th} percentile) and 1-day minimum flow as the hydrologic indicators. Flashiness is predicted to increase with urbanization but not with climate change, while low pulse count and 1-day minimum flow are predicted to increase with climate change.

Results showed that the number of EPT taxa was strongly associated with flashiness. As expected, the urban streams were flashier than the non-urban streams (**Figures J1-2** through **J1-4**). The flashiest urban streams had poorer condition than the moderately flashy urban streams (**Figure J1-4**). In the plots it appears that there may be a possible threshold at 0.5 (sites that had flashiness values of less the 0.5 generally showed no relationship, while sites with flashiness values greater than 0.5 generally showed strong relationships).

Natural and urban streams did not differ greatly in low pulse count, although the Smith River is an important exception. This site is dominated by natural land cover but has extremely high low pulse counts (28-44 per year) because it is regulated by a peaking hydropower dam. Overall results show that there was not a strong relationship between low pulse count and number of EPT taxa (**Figures J1-5** through **J1-7**). Low pulse count was most strongly associated with EPT taxa loss when there was an extreme increase in frequency of low pulses (>20 per year), which occurred at the Smith River site as mentioned above.

The urban streams had lower 1-day minimum flows than natural streams (**Figures J1-8** through **J1-10**). However, within the urban sites, there was no association between number of EPT taxa and minimum flow. In the plots, it appears that there may be a possible threshold at 15%, but this is confounded by the association of minimum flows with flashiness.

There were several conclusions that were drawn from this study, and also several questions that remained unanswered. We are aware the flow regime is a causal link that changes habitat, but we are uncertain as to whether or not it is a direct stressor. In this study, intermediate-term changes in flow were not associated with taxa change within streams, but this analysis had low power. The biological responses that were seen indicate that natural stream communities are highly resilient within the range of natural hydrologic variability. Because of this resilience, we may be unlikely to see effects from hydrologic changes associated with climate change unless these changes are truly extreme, such as those that occurred in the regulated river in this study, or occur in concert with rising temperatures that cross biological thresholds. Future climatic changes are likely to be beyond the variability observed in the recent past. Therefore, historic patterns are likely not as extreme as projected variability, and this makes it difficult to predict future impacts. A powerpoint presentation of this case study is available upon request.

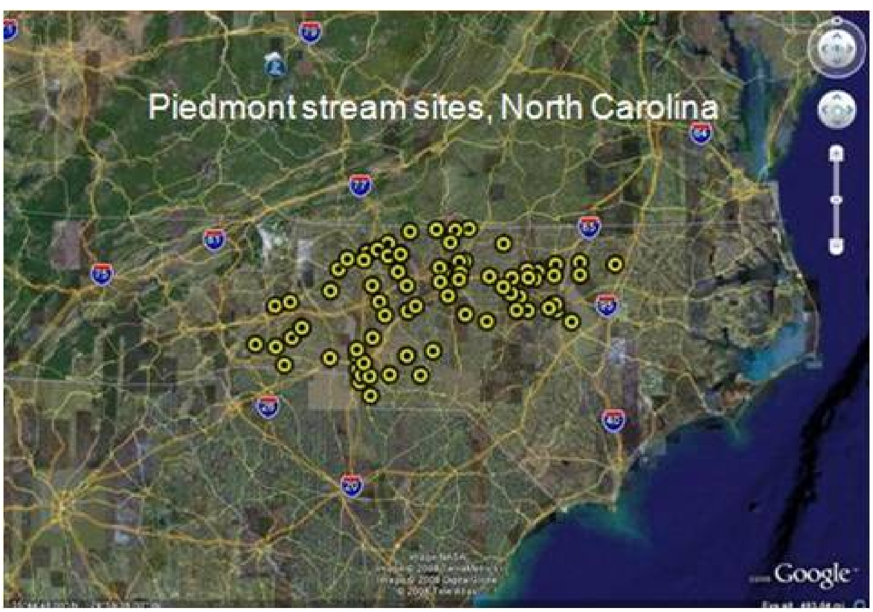


Figure J1-1. Locations of the North Carolina Piedmont stream sites that were used in the case study.

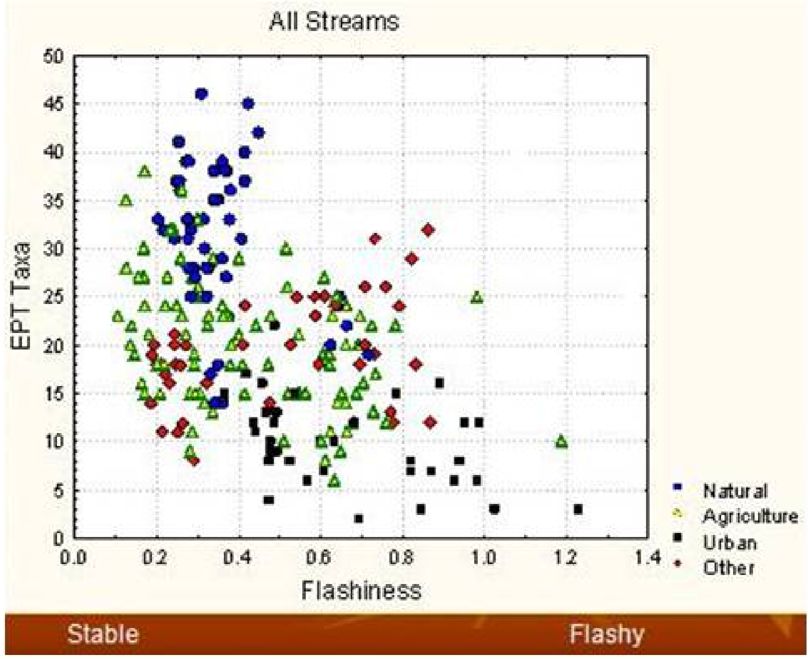


Figure J1-2. Number of EPT taxa is negatively associated with flashiness.

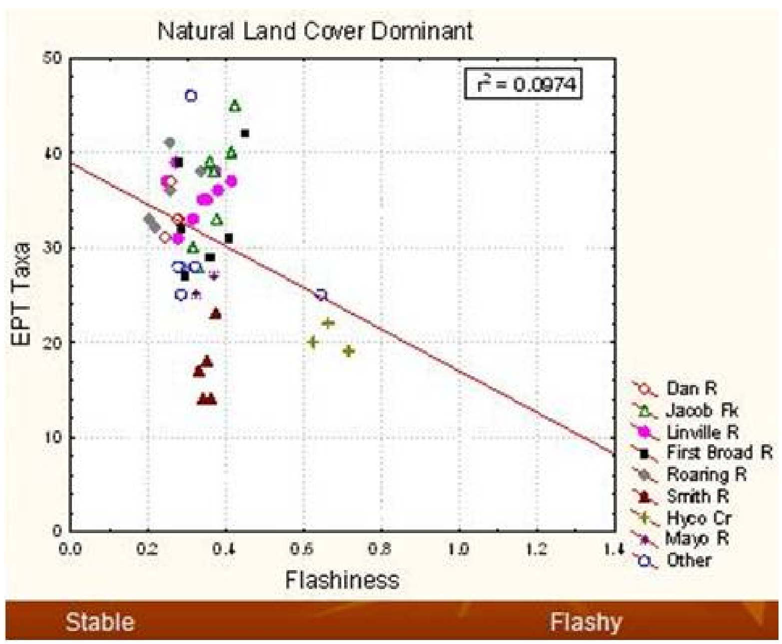


Figure J1-3. Association between number of EPT taxa and flashiness at sites dominated by natural land cover.

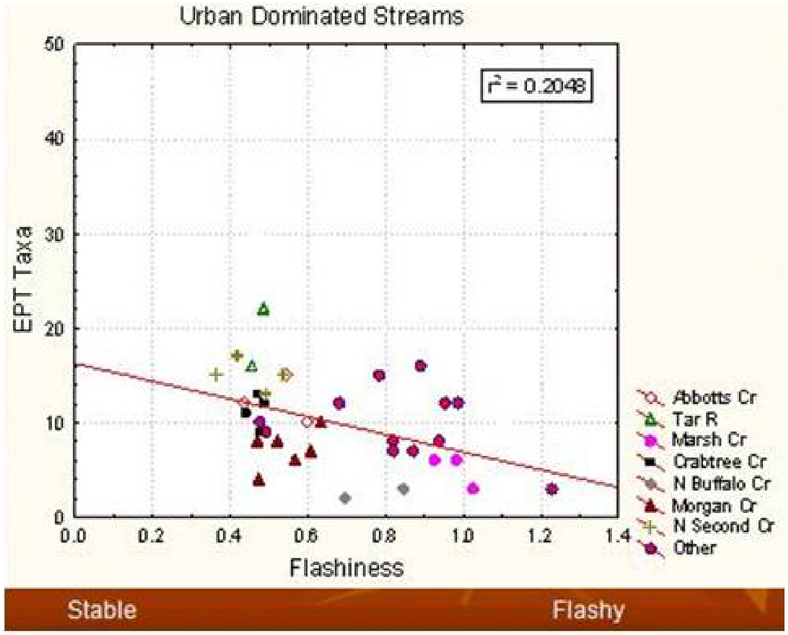


Figure J1-4. Association between number of EPT taxa and flashiness at sites dominated by urban land cover.

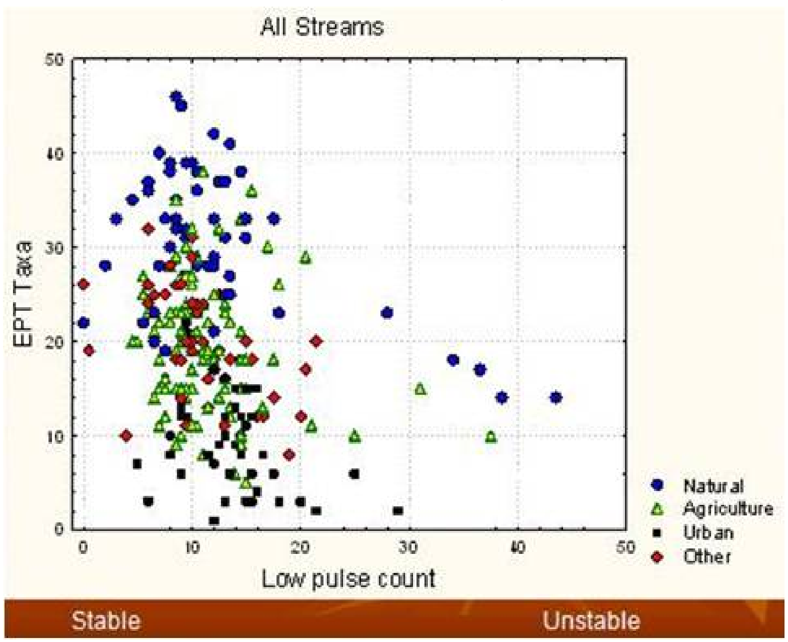


Figure J1-5. Association between number of EPT taxa and low pulse count at all streams.

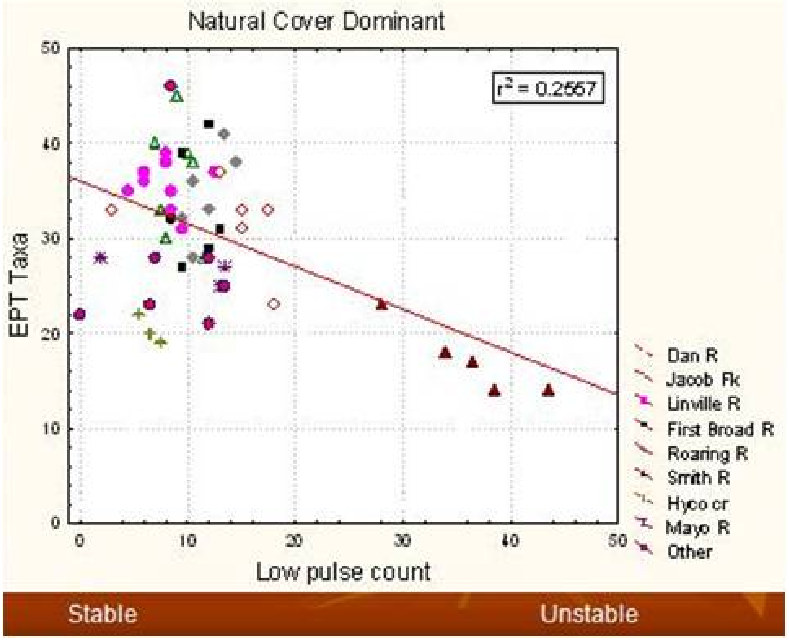


Figure J1-6. Association between number of EPT taxa and low pulse count at sites dominated by natural land cover.

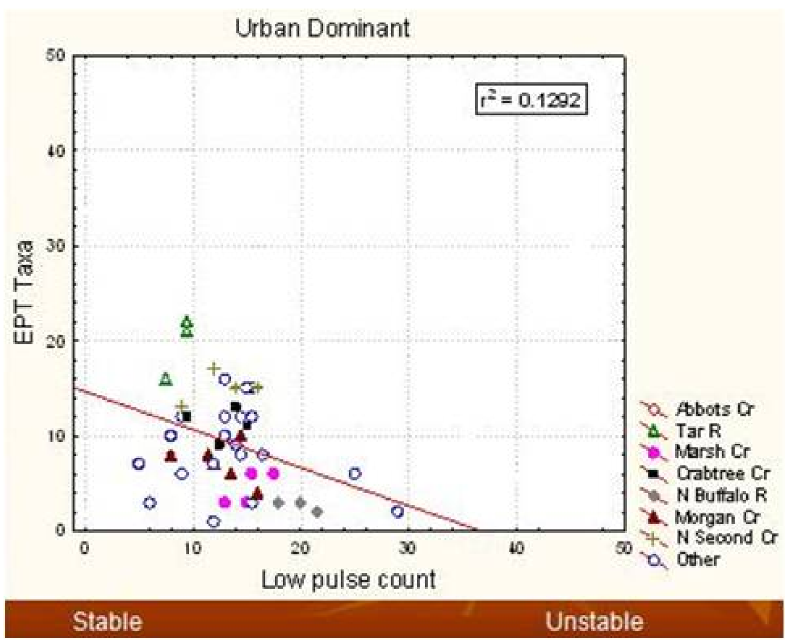


Figure J1-7. Association between number of EPT taxa and low pulse count at sites dominated by urban land cover.

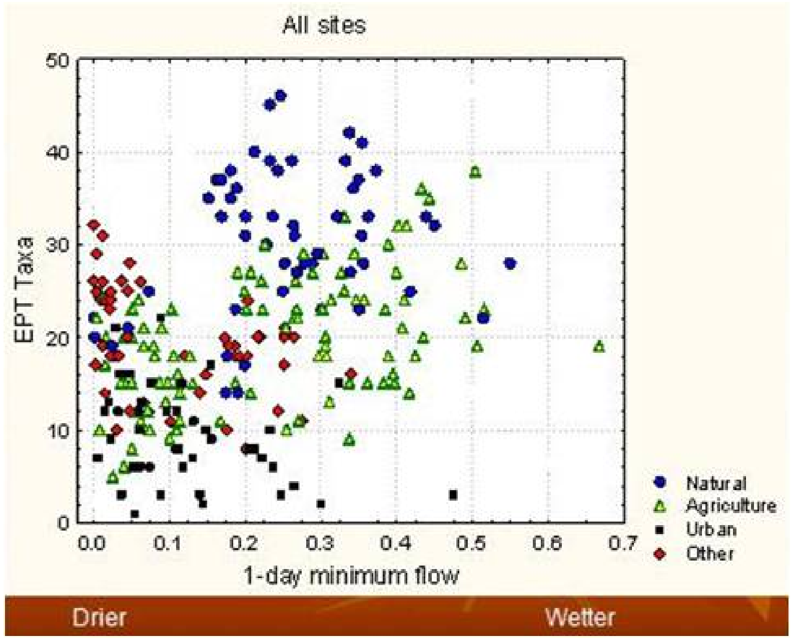


Figure J1-8. Association between number of EPT taxa and 1-day minimum flow at all streams.

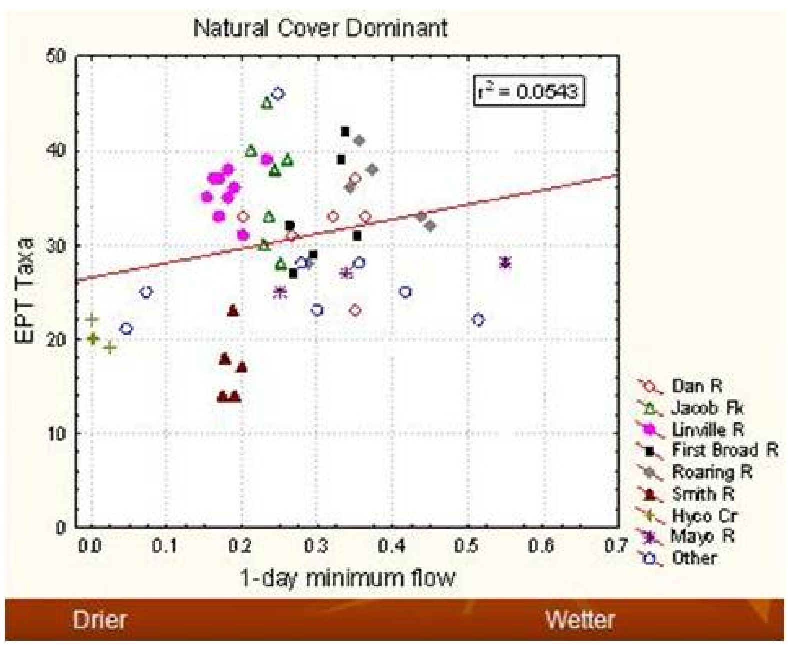


Figure J1-9. Association between number of EPT taxa and 1-day minimum flow at sites dominated by natural land cover.

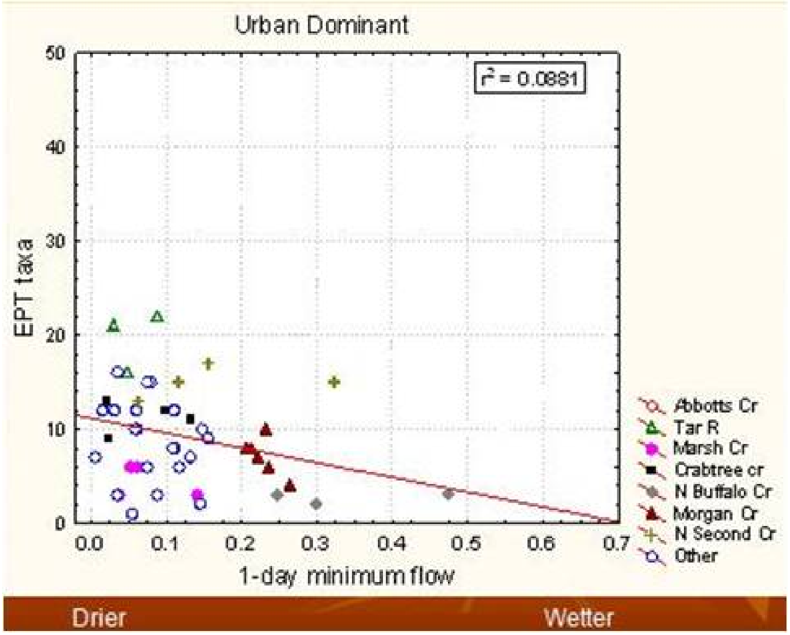
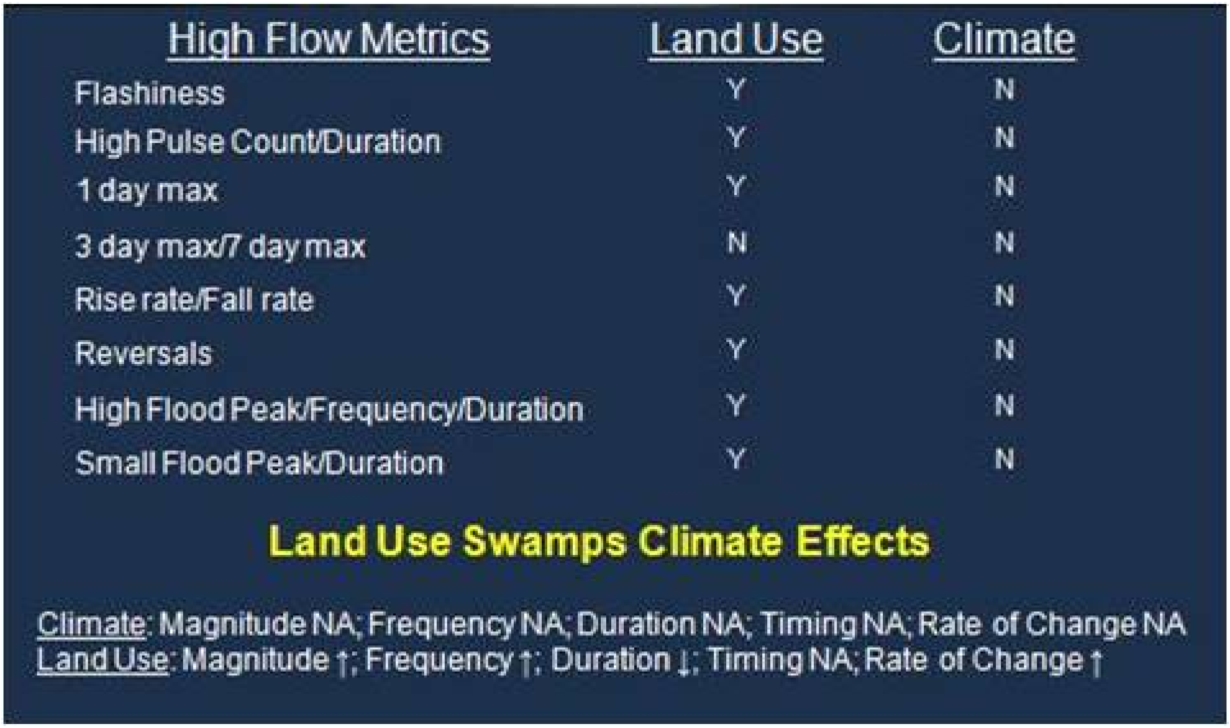


Figure J1-10. Association between number of EPT taxa and 1-day minimum flow at sites dominated by urban land cover.

J2. Comparing Hydrologic Respone to Fluctuating Climate with Land-Use Effects

Flow data from USGS gages in the Baltimore-Washington D.C. area (Mid-Atlantic region) were used in this case study. The main question that was addressed was how hydrologic response to climatic change in the Mid-Atlantic would compare with land-use impacts. Data preparation involved gathering historical flow and precipitation data for urban and forested sites, calculating Baker's Flashiness Index (Baker et al., 2004) and IHA parameters for these sites, and identifying which historical years of data had conditions that most resembled those that are projected to occur in the future. Data were analyzed using ANOVA analyses.

Results are summarized in **Figures J2-1** and **J2-2**. They show that for high flow metrics, climate effects were small relative to land use change, while for low flow metrics, climate change effects were large relative to land use. Plots of the ANOVA results for some of the IHA parameters are also included. **Figure J2-3** provides guidance on how to interpret these plots, **Figures J2-4** through **J2-7** show results for high flow IHA parameters and **Figures J2-8** through **J2-13** show results for low flow IHA parameters. Overall conclusions were that climate will affect stream flow. This will be happening over an ongoing dramatic change in land use, and the effects of climate change will be felt to differing degrees relative to land use change. A powerpoint presentation of this case study is available upon request.

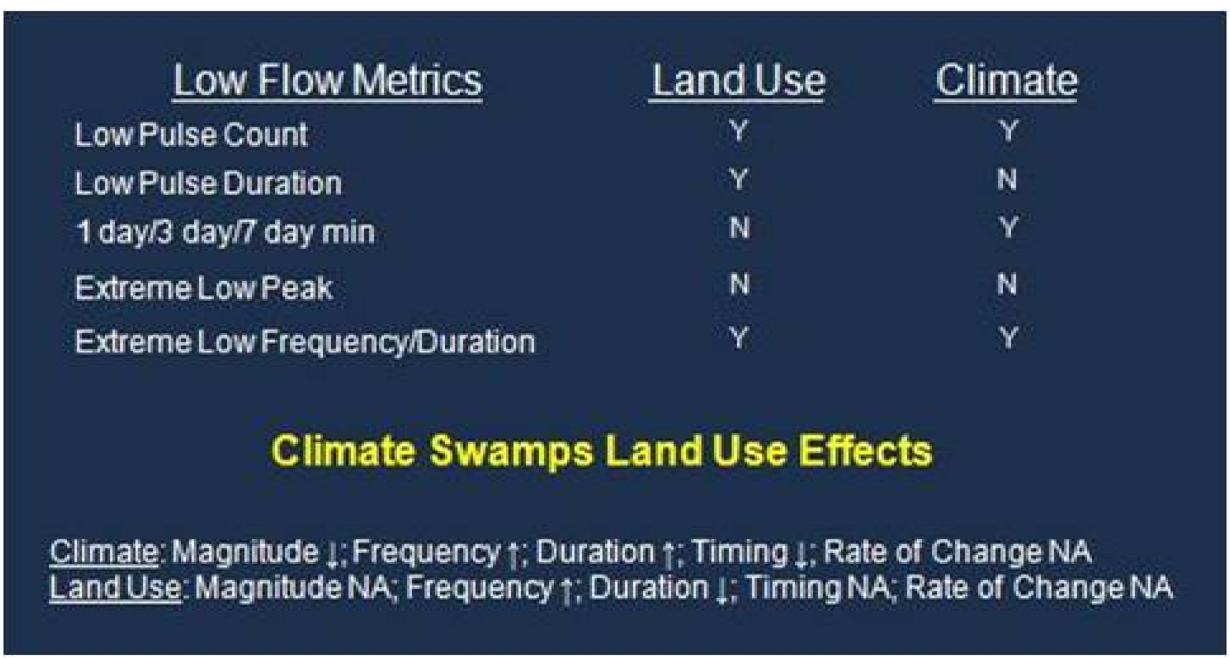


High Flow Metrics	Land Use	Climate
Flashiness	Y	N
High Pulse Count/Duration	Y	N
1 day max	Y	N
3 day max/7 day max	N	N
Rise rate/Fall rate	Y	N
Reversals	Y	N
High Flood Peak/Frequency/Duration	Y	N
Small Flood Peak/Duration	Y	N

Land Use Swamps Climate Effects

Climate: Magnitude NA; Frequency NA; Duration NA; Timing NA; Rate of Change NA
Land Use: Magnitude ↑; Frequency ↑; Duration ↓; Timing NA; Rate of Change ↑

Figure J2-1. Summary of ANOVA results for high flow IHA metrics.



Low Flow Metrics	Land Use	Climate
Low Pulse Count	Y	Y
Low Pulse Duration	Y	N
1 day/3 day/7 day min	N	Y
Extreme Low Peak	N	N
Extreme Low Frequency/Duration	Y	Y

Climate Swamps Land Use Effects

Climate: Magnitude ↓; Frequency ↑; Duration ↑; Timing ↓; Rate of Change NA
Land Use: Magnitude NA; Frequency ↑; Duration ↓; Timing NA; Rate of Change NA

Figure J2-2. Summary of ANOVA results for low flow IHA metrics.

Figure J2-3. Aid for interpreting the ANOVA plots.

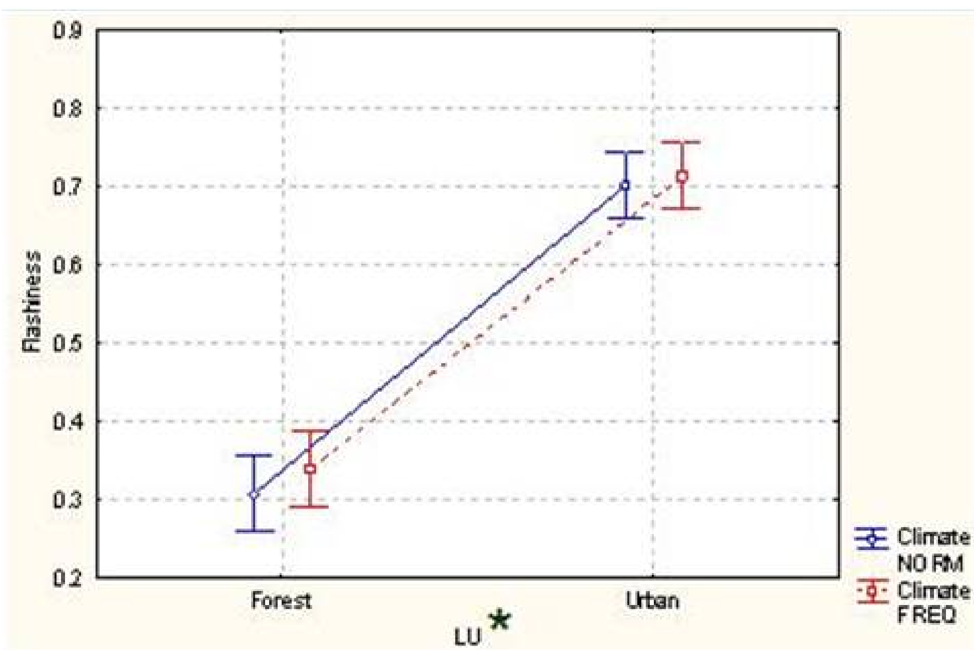


Figure J2-4. ANOVA results for flashiness at forested and urban sites.

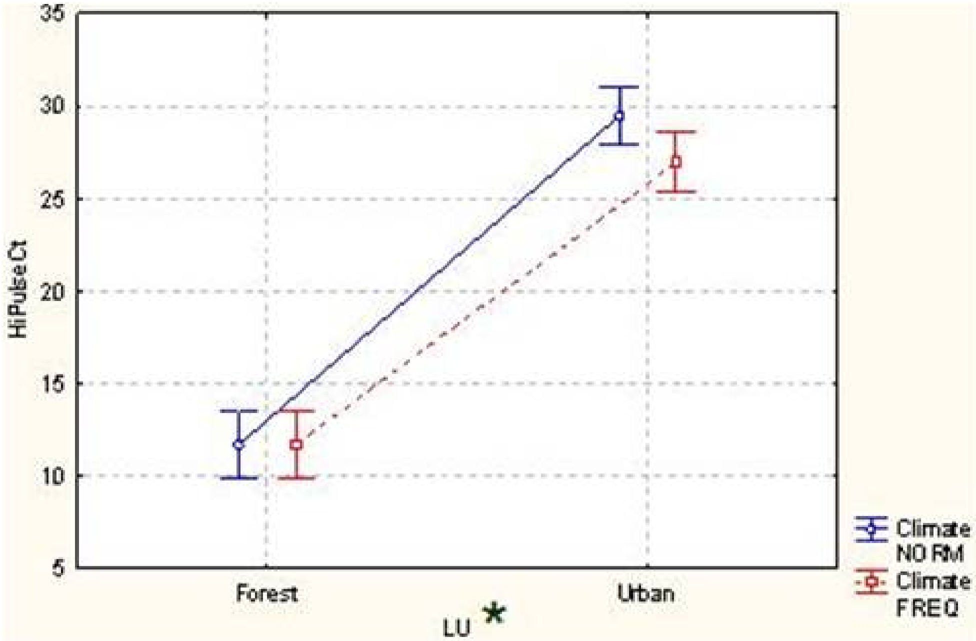


Figure J2-5. ANOVA results for high pulse count at forested and urban sites.

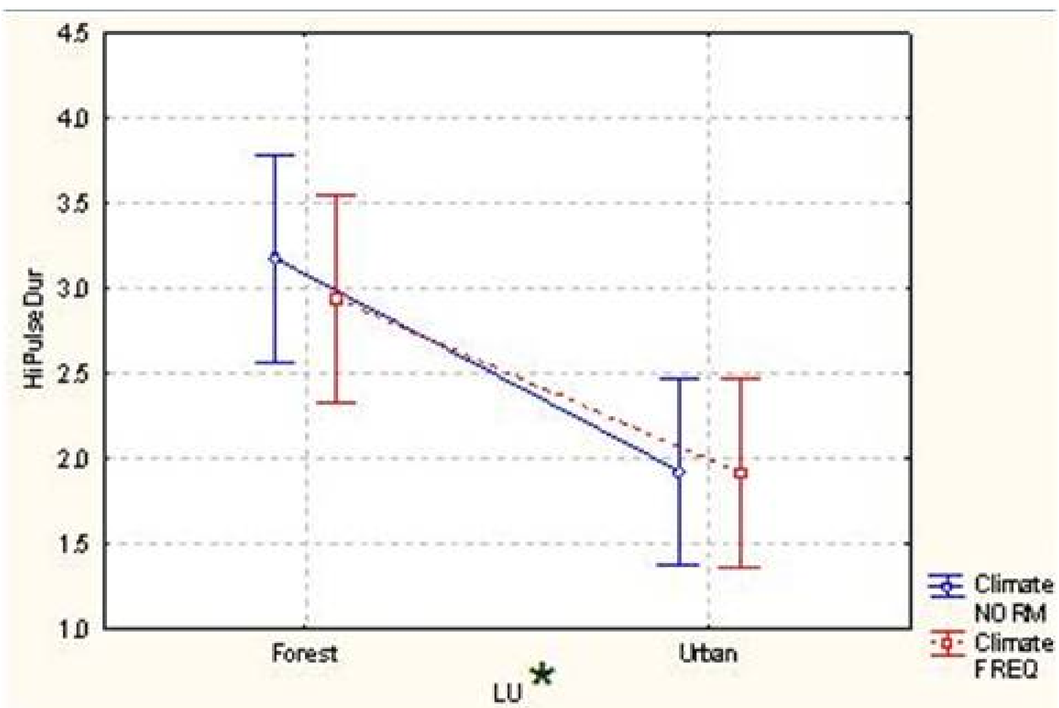


Figure J2-6. ANOVA results for high pulse duration at forested and urban sites.

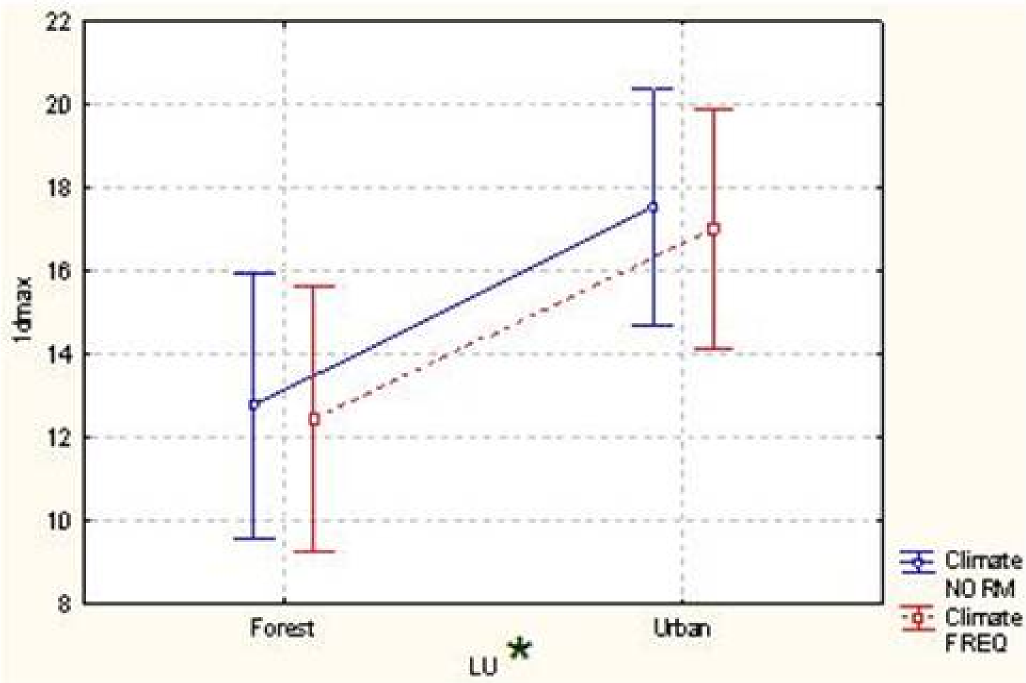


Figure J2-7. ANOVA results for 1-day maximum flow at forested and urban sites.

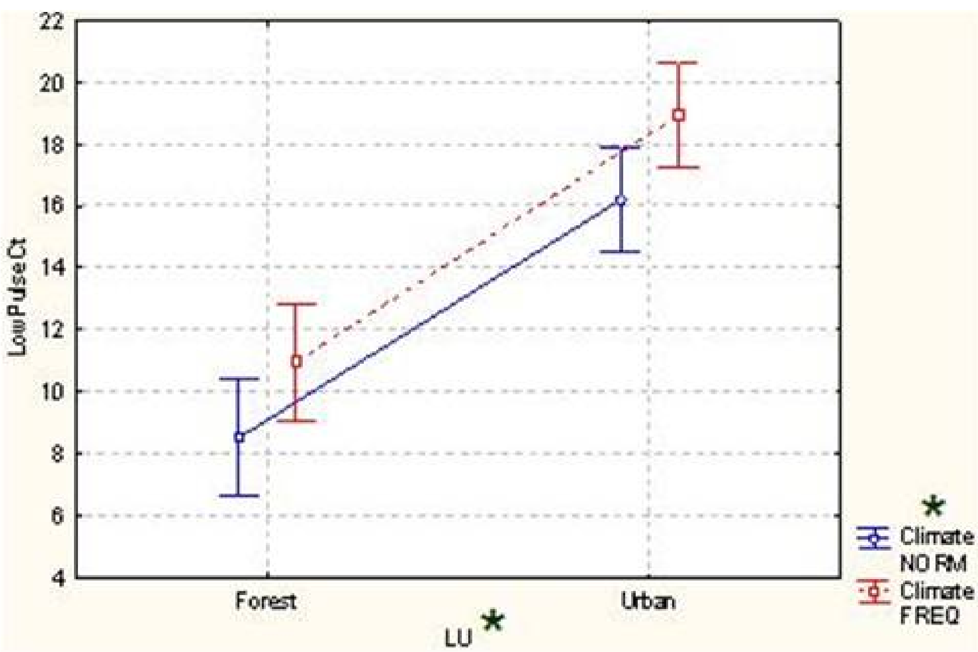


Figure J2-8. ANOVA results for low pulse count at forested and urban sites.

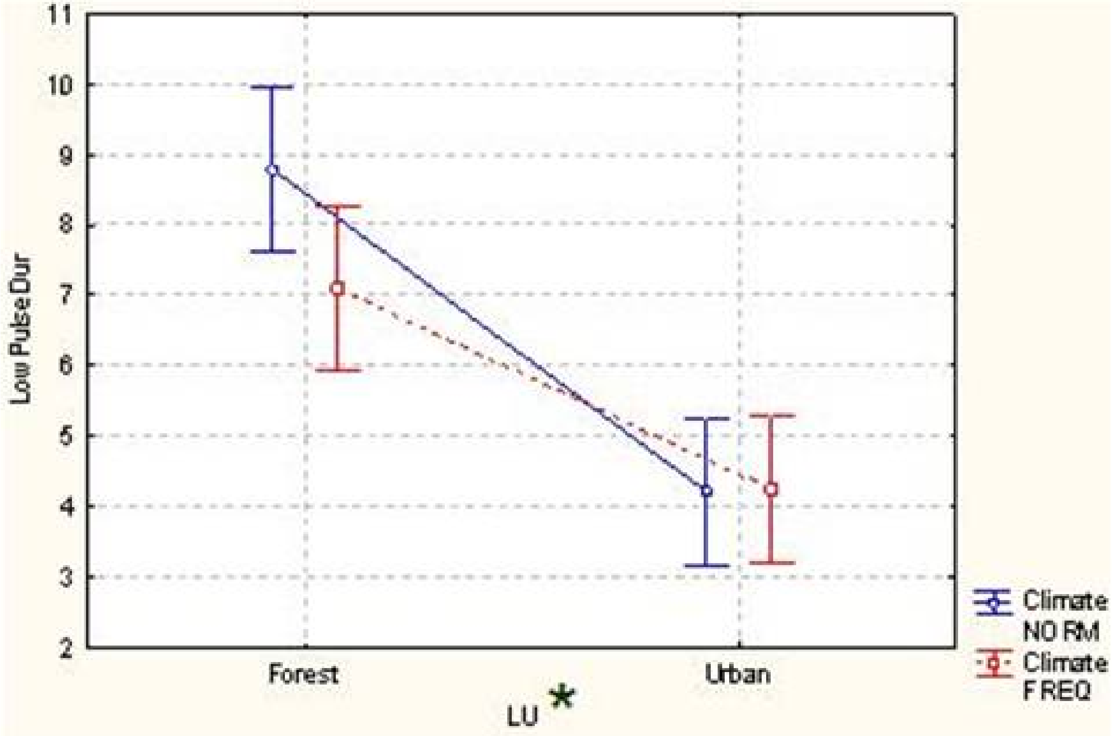


Figure J2-9. ANOVA results for low pulse duration at forested and urban sites.

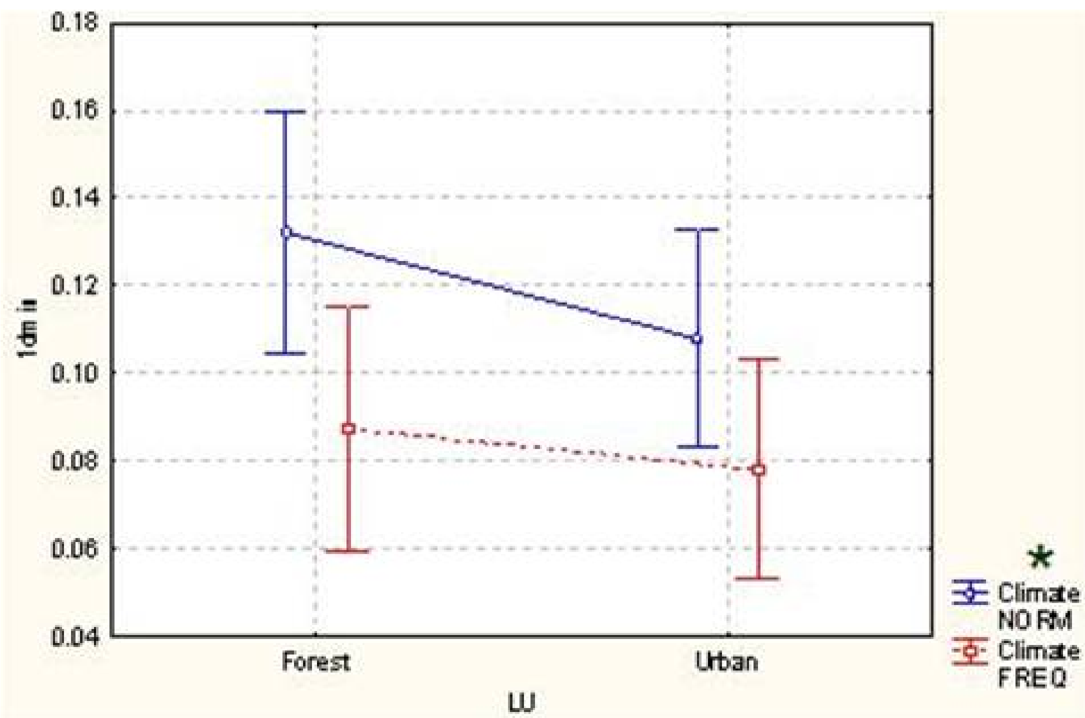


Figure J2-10. ANOVA results for 1-day minimum flow at forested and urban sites.

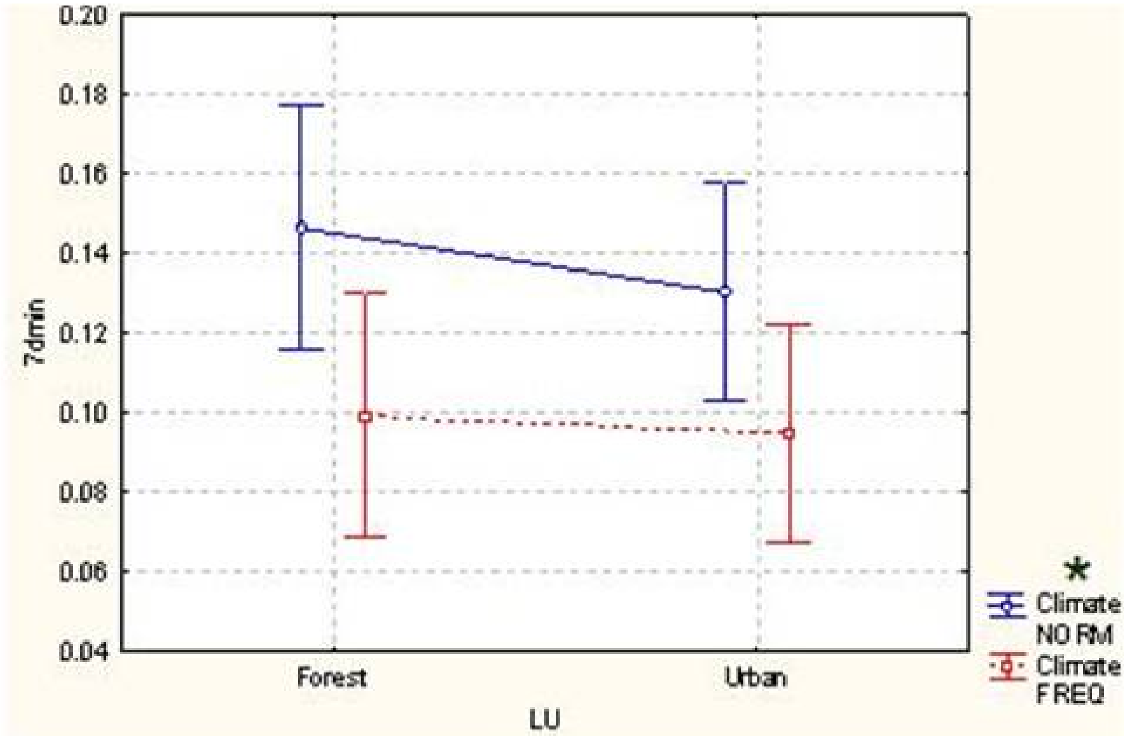


Figure J2-11. ANOVA results for 7-day minimum flow at forested and urban sites.

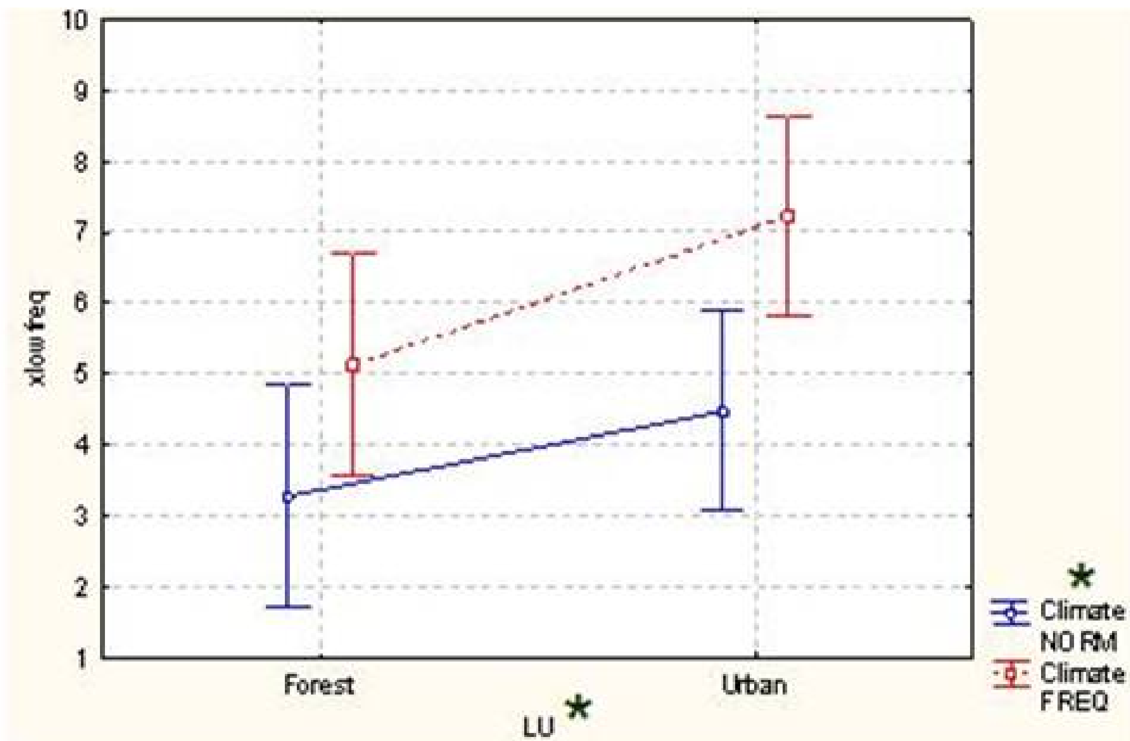


Figure J2-12. ANOVA results for extreme low flow frequency at forested and urban sites.

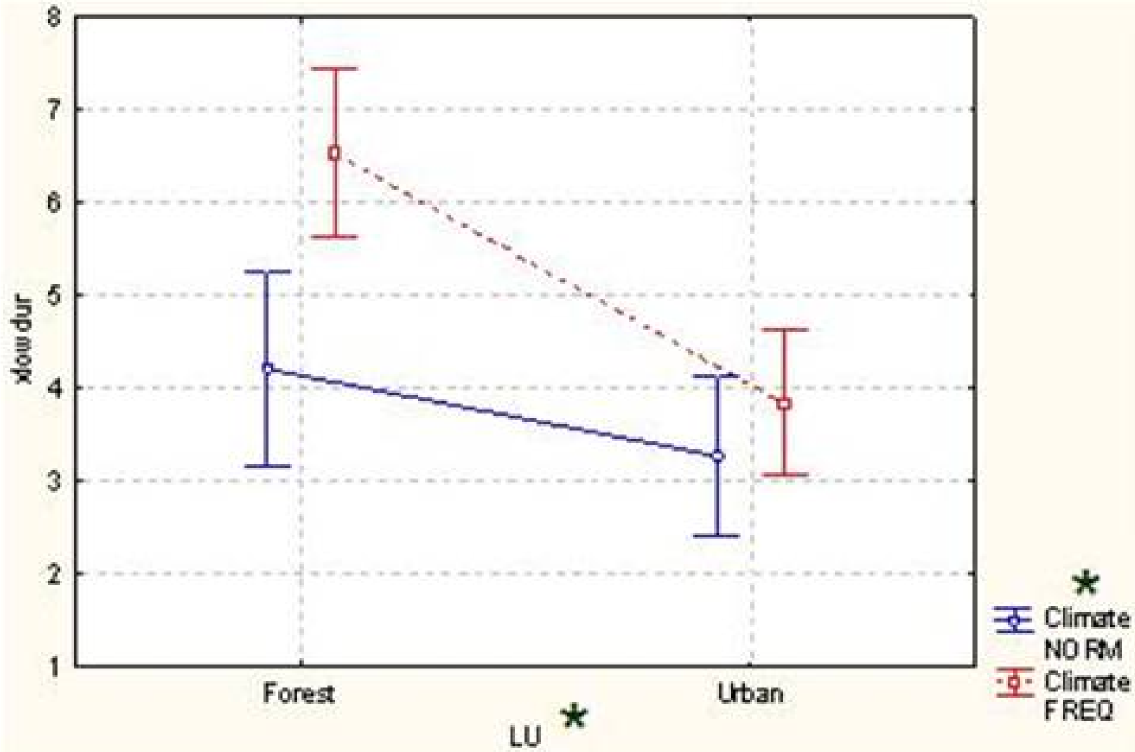


Figure J2-13. ANOVA results for extreme low flow duration at forested and urban sites.

J3. Shifting Baselines of Perception: Vulnerability of Reference Condition to Climate and Land Use Change

The vulnerability of reference locations to climate change and the need to protect reference locations is recognized as an important issue for the future of bioassessment. Our concept of the natural condition of streams is based on these reference locations, yet we also recognize that they have been subject to industrial anthropogenic influences and disturbance for up to 2 centuries. While reference sites may often be located in remote and less developed regions, they are nevertheless vulnerable to human development and urbanization.

We examined actual and potential reference sites for aquatic biological monitoring, and examined both their regional vulnerability to future climate changes, as well as vulnerability to land-use changes. Where possible, we also examined the degree of change from pre-European settlement in North America to current reference condition. Florida was chosen for this case study because its historical pollution has been less than in other eastern states, but its current-era growth and urbanization has been extraordinary.

In this study we examined 54 Florida reference sites under future growth scenarios: A1 (IPCC) (rapid global economic growth and lower population growth), A2 (IPCC) (slower economic growth and higher population growth) and Base (closest to current conditions) (Nakicenovic and Swart, 2000; USEPA, 2009a). Projections for population growth under each scenario are shown in **Figure J3-1**. To assess vulnerability, land use within a 1 km buffer around each reference site was calculated, land use for each decade was projected from the A2 and Base Case scenario outputs, and the fraction of buffer in categories of increasing housing density were estimated.

The link between population density and biota has been previously examined in New England, as shown in **Figure J3-2**. Results showed that effects begin but are not universal or severe when densities reach 50 people per square mile (25 houses). A degradation gradient becomes evident at densities of 50-500 people per square mile (25-250 houses). Once densities exceed 500 people per square mile (>250 houses), streams in New England are generally degraded.

Results of the housing density and fraction suburban projections for sites in Florida are shown in **Figures J3-3** through **J3-5**. Based on the New England results, the average site (statewide) in Florida will approach the 'complete degradation' point by 2100. The average reference site will exceed the 'effects threshold' around 2020 but will not reach the 'complete degradation' point. Seventeen percent of the reference sites appear to be protected in that they are surrounded by government land or water and approximately 25% of the reference sites appear to be completely unprotected from development. A PowerPoint presentation of this case study is available upon request.

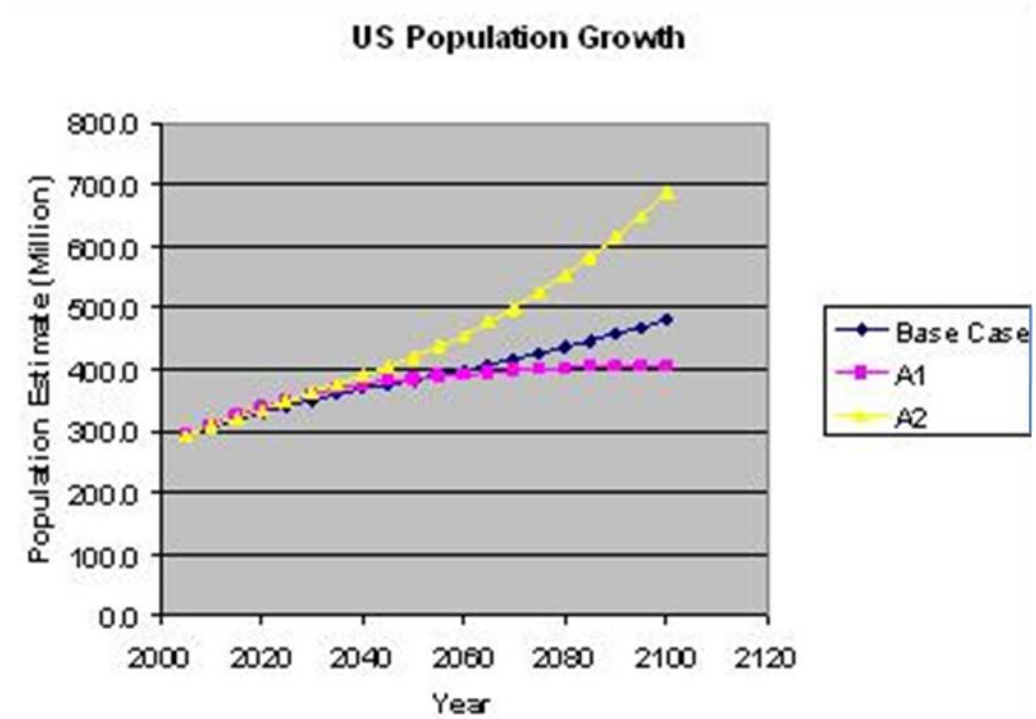


Figure J3-1. Projections of future population growth under the 3 scenarios: A1, A2 and Base.

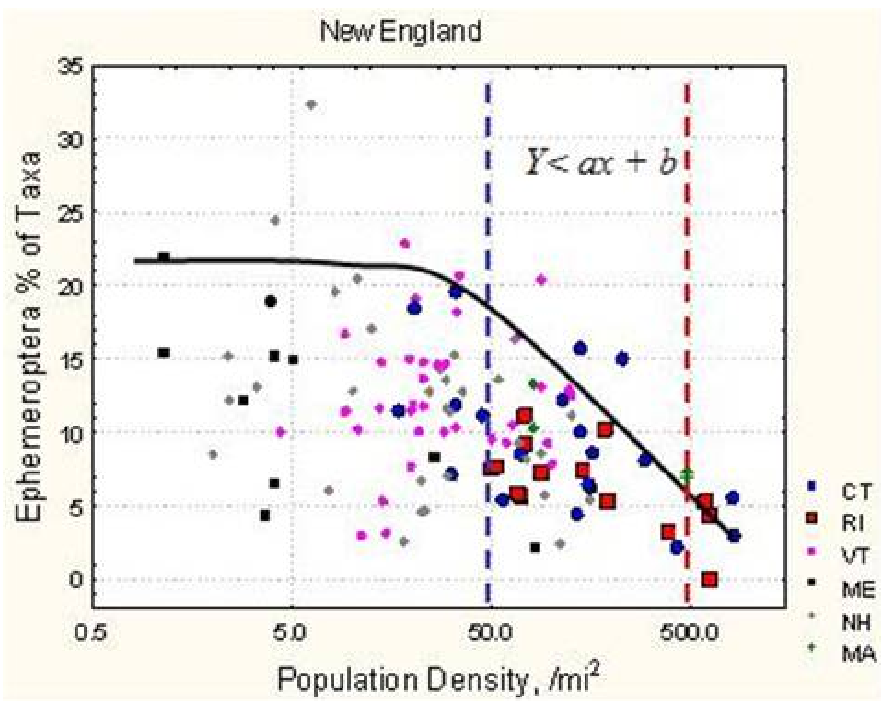


Figure J3-2. The link between population density and biota in New England.

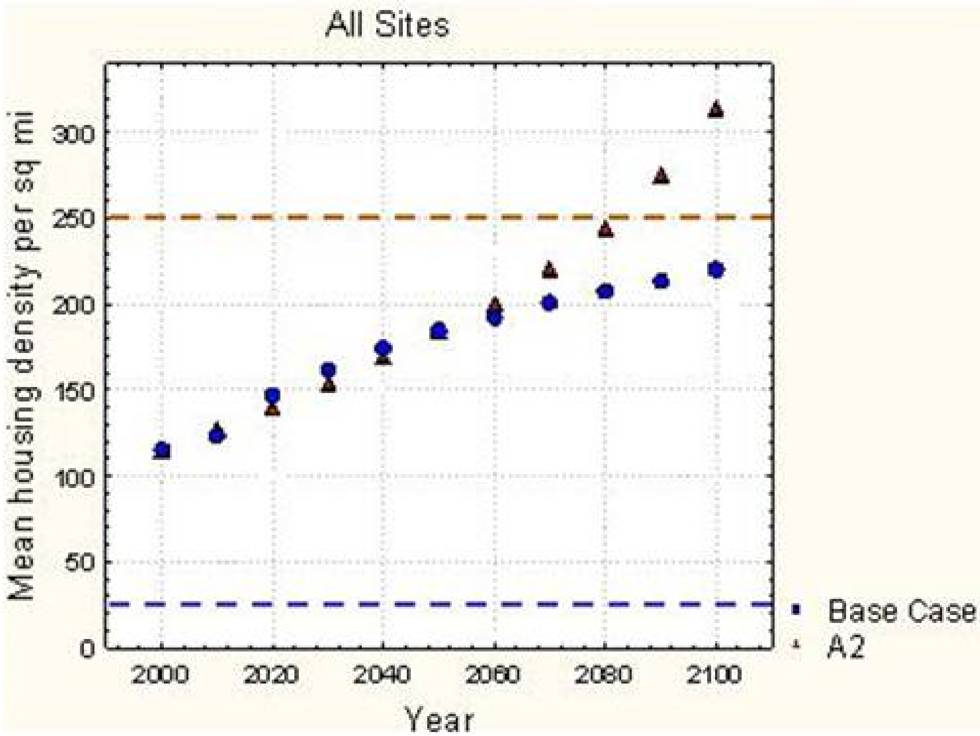


Figure J3-3. A2 and Base Case projections for mean housing density per square mile at all Florida sites.

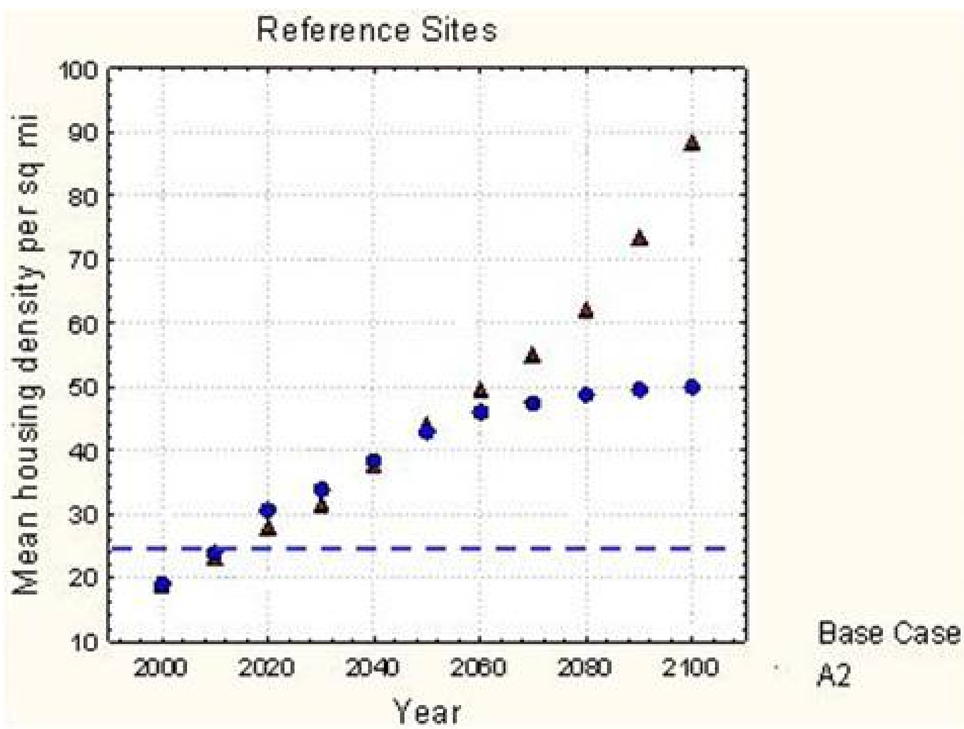
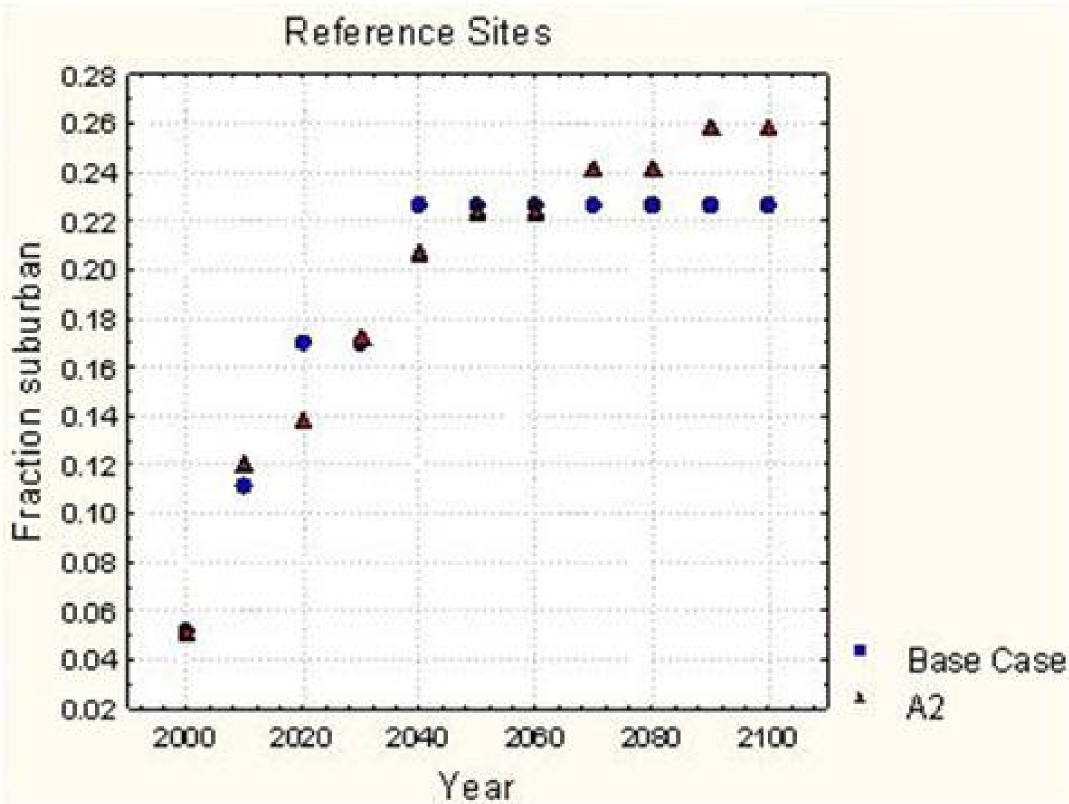


Figure J3-4. A2 and Base Case projections for mean housing density per square mile at Florida reference sites.



J3-5. A2 and Base Case projections for fraction suburban land use at Florida reference sites.

APPENDIX K

Explorations of relationships between hydrological and biological data

The intent of this appendix is to provide more comprehensive and detailed information on the analyses that were performed on the Utah and North Carolina hydrological data. Some of the analyses that are covered in this appendix are also referenced in the main body of the report. When this occurred, attempts were made to reduce any overlap or duplication in the reporting of results.

K1 Overview

Changes in hydrology are projected to occur as a result of climate change. In this study, we attempted to gain a better understanding of the link between hydrology and biology by creating and analyzing datasets comprised of paired hydrological and biological data. To derive these datasets, we used the following criteria to match USGS gages with biological sampling sites:

- If a biological sampling site fell within 500-meters of the gage, the gage was retained and matched with the biological sampling site (this was done using ArcGIS).
- Stream gages were excluded if they were not on the same stream reach as the biological sampling site, or if tributaries entered between the gage and site
- All available data from the following time period was downloaded: 1940-01-01 to 2007-12-31

Hydrologic data for the matched stations were downloaded from the USGS real-time flow data website: http://waterdata.usgs.gov/nwis/rt. Indicators of Hydrologic Alteration (IHA) software (version 7.0.4.0) was then used to calculate a suite of IHA parameters for each site. The Richards-Baker Flashiness Index (RBI) (Baker et al., 2004) was also analyzed (the R code that was used to calculate the RBI is available upon request). The RBI uses flow data to quantify the frequency and rapidity of short-term changes in stream flow. The IHA and RBI data was then matched with the biological data from the site. These merged datasets were then used in our analyses. Descriptions of the analyses that were performed on the Utah and North Carolina datasets are described in Sections L2 and L3[1]. Only the subset of IHA parameters that were believed to have greatest relevance to this study was used in our analyses. A list of these parameters is shown in Table K1-1.

[1] Similar types of analyses were attempted in Maine, but there were not enough USGS gages associated with biological sampling sites to make weighted average and ordination analyses worthwhile.

Table K1-1. Summary of IHA parameters used in biological analyses

Annual IHA parameters	Description	Conversion (to standardize)
monthly	median discharge (cfs)	divided by median value for entire period of gage data
1-day min	annual minima, 1-day mean (cfs)	divided value for each year by mean annual flow
3-day min	annual minima, 3-day means (cfs)	divided value for each year by mean annual flow
1-day max	annual maxima, 1-day mean (cfs)	divided value for each year by mean annual flow
3-day max	annual maxima, 3-day means (cfs)	divided value for each year by mean annual flow
Date min	Julian date of each annual 1-day minimum	none
Date max	Julian date of each annual 1-day maximum	none
Lo pulse #	Number of low pulses within each water year	none
Lo pulse L	Median duration of low pulses (days)	none
Hi pulse #	Number of high pulses within each water year	none
Hi pulse L	Median duration of high pulses (days)	none
Environmental Flow Components (EFC)		
Xlow1 peak	minimum ('peak') flow (cfs) during extreme low flow event (within each year)	divided value for each year by mean annual flow
Xlow1 dur	duration of extreme low flow event (days)	none
Xlow1 time	Julian date of peak flow	none
Xlow1 freq	frequency of extreme low flows during water year	none

High1 peak	maximum ('peak') flow (cfs) during extreme high flow event (within each year)	divided value for each year by mean annual flow
High1 dur	duration of extreme high flow event (days)	none
High1 time	Julian date of peak flow	none
High1 freq	frequency of extreme high flows during water year	none
Baseflow index	7-day minimum flow/mean flow for year	none
Number of reversals:	Number of hydrological reversals	none

Definitions-

high flow events	*All flows above the 75th percentile of all flows are classified as high flow events*
low flow events	*All flows less than or equal to the 50th percentile of all flows are classified as low flow events*
extreme low flow	*10th percentile of all low flows*

Hydrographs were also generated for selected reference sites in Maine (see Attachment K1), Utah (see Attachment K2) and North Carolina (see Attachment K3). The R code that was used to create these plots is available upon request. The hydrographs were used in the initial phases of our analyses to gain a better understanding of the natural flow regimes in each of the 3 states. In addition, they can provide information on how strong the groundwater influence is at a site. During discussions at the 2008 Workshop on Bioindicators and Climate Change in Crystal City, VA,[2] the importance of learning more about groundwater influence came up on several occasions, so we attempted to gather groundwater data and incorporate it into our analyses. Unfortunately we could not find the type of data that we needed. However, we were able to find a number of valuable resources (i.e., NCDENR, 2004, NCDWQ, 2005, NCDENR, 2005, Borwick et al., 2006, Douglas, 2006). Another potential lead, which was suggested to us by Maine DEP, was to use water temperature data. If summer low flow temperatures were less than 20°C, there were generally believed to be at least some groundwater influence.

K2. Utah

A number of different analyses were run on a subset of Utah IHA-biological data that was derived from 43 biological sampling sites (locations of these stations are shown in **Figure K2-1**). The dataset was somewhat limited by sample size and by the fact that some sites had many more years of data than others (i.e. one site had 19 years of data, others had 1 year of data). One analysis involved examining taxonomical trends using Canonical Correspondence Analysis (CCA) and Nonmetric Multidimensional Scaling (NMDS). In another analysis, a subset of data that only had sites with multiple years of data was evaluated. A third analysis involved calculating weighted average (WA) indicator values for the parameters that showed the strongest influence on taxonomic composition. For the final analysis, correlation analyses were performed on data from the 7 Utah stations that had the most number of years of biological-hydrological data.

[2] A report on the workshop is available online at: oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=486153. Additional information on the workshop can be found at: http://www.epa.gov/ncea/workshop/.

Results of the CCA are shown in Section 2 of the main report. Additional CCA results (species trends) are shown in **Figure K2-2.** Results of the NMDS analyses are shown in **Figure K2-3**. Both analyses indicated that year had the strongest influence on taxonomic composition. However, when only data from sites with multiple years of data were used, year showed a weaker effect in the NMDS analysis. Results from the WA model are shown in **Tables K2-1** and **K2-2**. Of all the models tested, most low-flow parameters performed better than high-flow parameters. The WA model for year had very strong performance (r^2=0.6). The next best parameter was the IHA parameter for annual minima, 3-day means. Optima and tolerance results for taxa that had more than 20 occurrences in the dataset (which is generally regarded as an adequate sample size) show that Leuctridae, Asellidae and Zapada had the lowest values, while Hyalella and Helicopsyche had the highest. Leuctridae and Zapada had relatively low tolerance ranges, while Hyalella and Helicopysche had large tolerance ranges. These results suggest that Leuctridae and Zapada are better adapted (perhaps partly due to their smaller sizes) to lower flow conditions than other taxa.

There are too many results from the correlation analyses to show in this report, but results are available upon request. There were a number of significant correlations, but none of the taxa, trait metrics or IHA parameters showed consistent patterns across the 7 sites, which makes the results very difficult to summarize. Site information for the sites is summarized in **Table K2-3**.

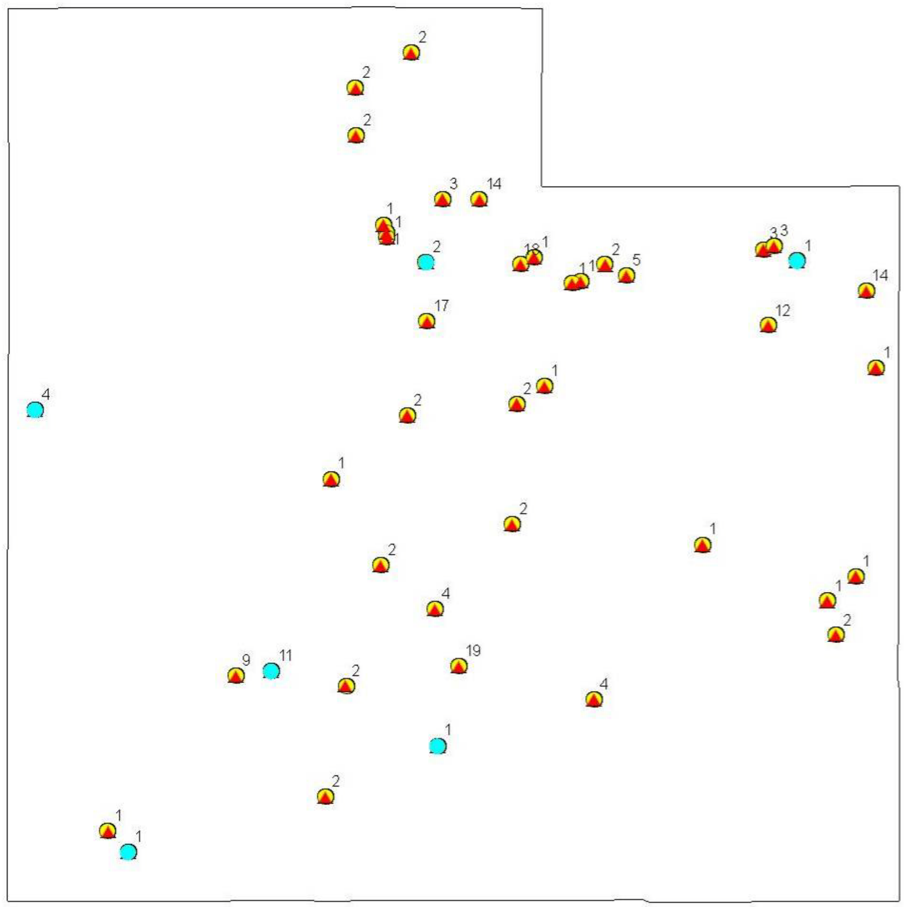


Figure K2-1. Locations of the 43 Utah biological sampling stations (red triangles) and associated USGS stream gages (yellow circles). Stations that are highlighted in blue are classified as reference sites by Utah DWQ. The numbers next to the sites are the number of years of data that were available for each station.

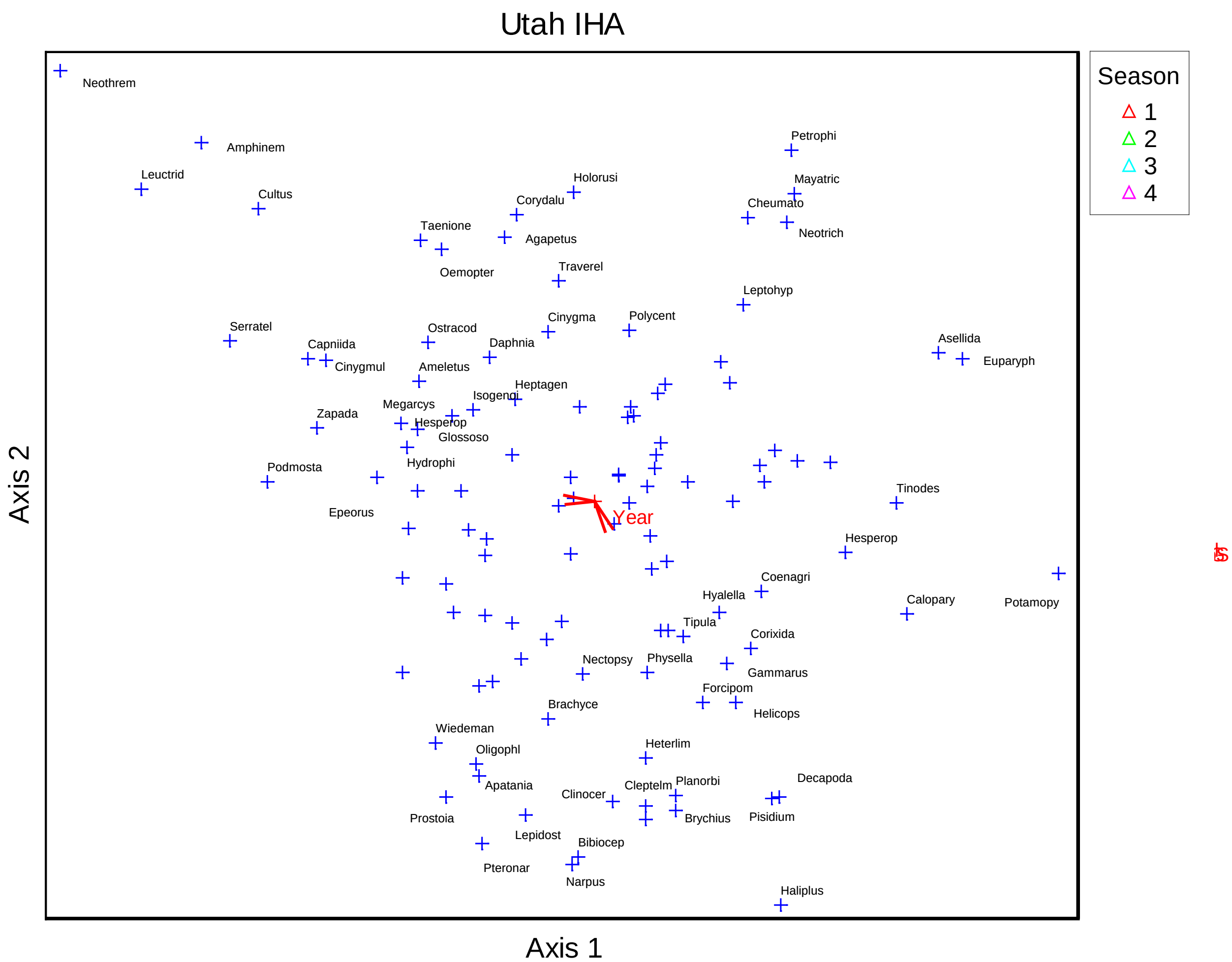


Figure K2-2. **Species trends along year. These were derived from the CCA analysis.**

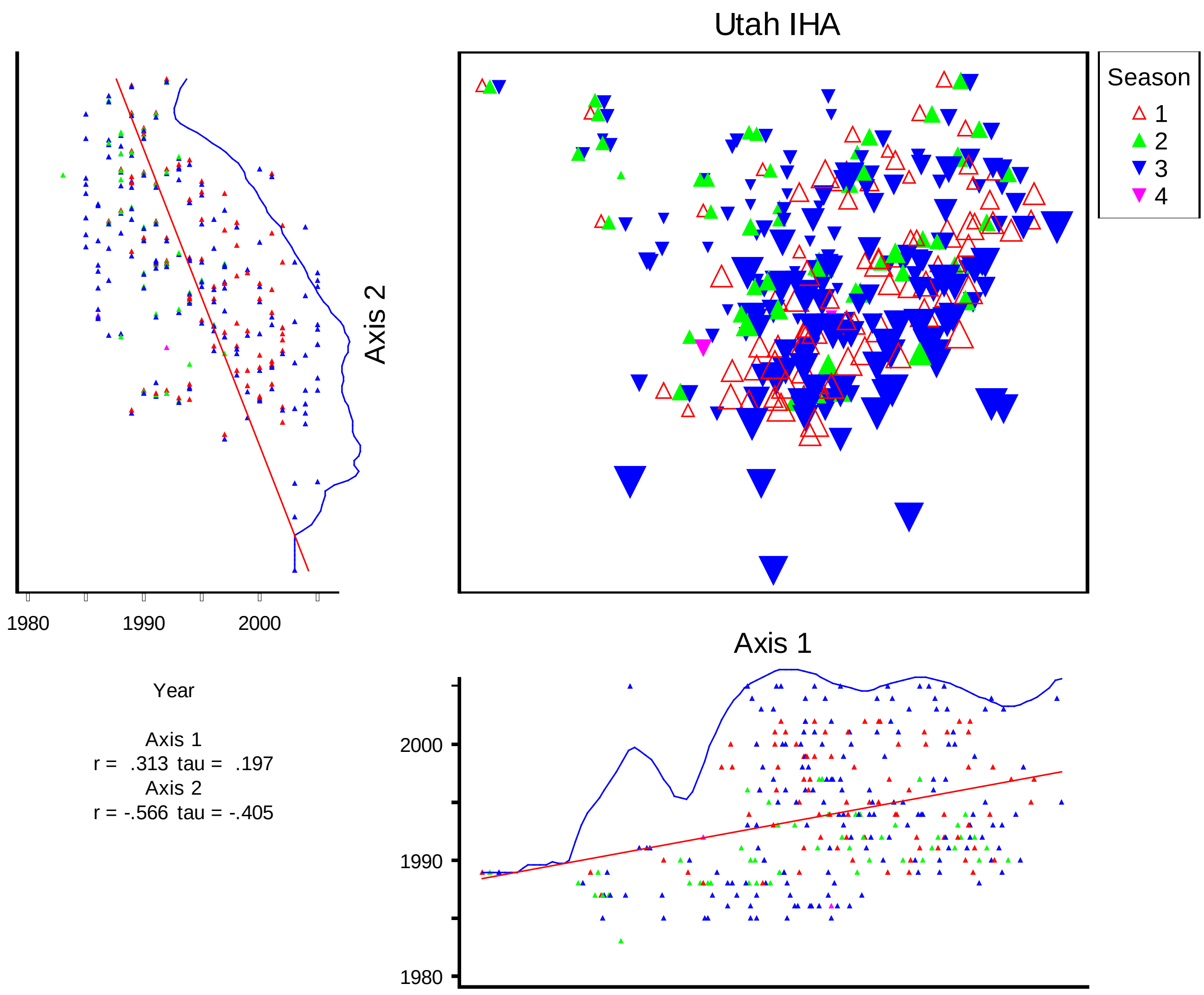


Figure K2-3. Taxonomical trends in the Utah dataset were examined using Nonmetric Multidimensional Scaling (NMDS). Year had the strongest influence on taxonomical composition. However, when NMDS ordinations were run on a selected subset of data that only contained data from sites with multiple years of samples, the year trend was not as strong.

Table K2-1. Weighted average indicator values for year, which had very strong performance (r^2=0.6). Sorted by optimum values. Ranks are on a scale of 1 to 7 and are based on the following percentiles: 0, 0.1, 0.25, 0.4, 0.6, 0.75, 0.9 and 1.

YEAR					
Taxa	**Optimum**	**Tolerance**	**Rank_Opt**	**Rank_Tol**	**Count**
Traverella	1989.4	3.59	1	2	10
Taenionema	1989.5	4.38	1	3	29
Cultus	1989.8	3.66	1	2	20
Leuctridae	1990.1	4.50	1	4	24
Zapada	1990.2	4.55	1	4	35
Planaria	1990.9	3.11	1	2	90
Apatania	1991.3	3.71	1	2	20
Serratella	1991.4	4.70	1	4	11
Nematoda	1991.5	3.44	1	2	125
Hesperoperla	1991.7	5.08	2	5	33
Ostracoda	1991.7	4.20	2	3	96
Cinygmula	1991.8	4.62	2	4	90
Copepoda	1991.9	3.46	2	2	35
Pelecypoda	1992.0	3.02	2	2	44
Capniidae	1992.0	5.28	2	5	38
Ameletus	1992.1	6.25	2	7	26
Mayatrichia/Neotrichia	1992.2	1.89	2	1	16
Alisotrichia/Leucotricia	1992.2	2.66	2	2	32
Heptagenia	1992.2	2.67	2	2	58
Neotrichia	1992.2	2.04	2	1	12
Micrasema	1992.3	5.61	2	6	55
Glossosoma	1992.4	4.13	3	3	60
Podmosta	1992.6	3.78	3	3	10
Dicranota	1992.6	5.57	3	6	32
Cheumatopsyche	1992.7	5.20	3	5	55
Agapetus/Culoptila/Protoptila	1992.7	3.15	3	2	12
Ephemerella	1992.7	4.53	3	4	149
Epeorus	1992.9	4.99	3	4	92
Dytiscidae	1993.0	6.80	3	7	10
Euparyphus	1993.1	2.43	3	1	12
Skwala	1993.2	5.27	3	5	31
Neothremma	1993.3	7.28	3	7	19
Leucotrichia	1993.4	1.99	3	1	23
Paraleptophlebia	1993.5	5.07	4	4	96
Pericoma	1993.6	5.41	4	6	47
Simuliidae	1993.6	5.13	4	5	234
Chloroperlidae	1993.6	5.82	4	7	105

66 **Table K2-1. Continued**

Taxa	Optimum	Tolerance	Rank_Opt	Rank_Tol	Count
Leptohyphidae	1993.7	4.89	4	4	133
Drunella	1993.8	5.61	4	6	119
Hemerodromia	1993.8	3.84	4	3	103
Atherix	1993.9	5.85	4	7	81
Baetidae	1994.0	5.27	4	5	277
Bezzia	1994.0	2.66	4	2	53
Pteronarcella	1994.0	5.73	4	6	91
Isoperla	1994.0	4.87	4	4	105
Isogenoides	1994.1	5.74	4	6	19
Hydroptila	1994.1	4.49	4	4	97
Physa	1994.1	5.00	4	4	54
Antocha	1994.2	5.27	4	5	126
Acarina	1994.3	5.47	4	6	268
Oligophlebodes	1994.3	4.80	5	4	35
Tubificidae	1994.4	2.16	5	1	107
Hydropsyche	1994.5	5.38	5	5	232
Planorbidae	1994.5	4.04	5	3	37
Lymnaea	1994.5	3.85	5	3	15
Chironomidae	1994.5	5.63	5	6	291
Rhyacophilidae	1994.5	5.41	5	6	98
Rhithrogena	1994.5	5.69	5	6	127
Petrophila	1994.6	4.04	5	3	36
Chelifera	1994.6	3.62	5	2	98
Oecetis	1994.7	4.72	5	4	45
Hirudinea	1994.7	5.14	5	5	75
Arctopsyche	1994.8	5.22	6	5	99
Hexatoma	1995.2	5.18	6	5	88
Brachycentrus	1995.3	5.39	6	6	145
Asellidae	1995.4	4.84	6	4	45
Hyalella	1995.4	4.39	6	3	62
Lepidostoma	1995.7	4.68	6	4	88
Ambrysus	1995.8	3.80	6	3	17
Helicopsyche	1995.9	4.33	6	3	68
Gammarus	1995.9	6.89	6	7	15
Claassenia	1996.0	6.40	6	7	12
Hesperophylax	1996.1	6.31	6	7	12
Coenagrionidae	1996.1	5.52	6	6	36
Bibiocephala	1996.3	5.31	7	5	17
Optioservus	1996.5	4.45	7	4	148
Zaitzevia	1996.6	4.46	7	4	97

Table K2-1. Continued

Taxa	Optimum	Tolerance	Rank_Opt	Rank_Tol	Count
Pteronarcys	1996.7	5.75	7	7	27
Tipula	1998.1	3.80	7	3	31
Physella	2000.5	1.55	7	1	13
Forcipomyia/Probezzia	2001.4	1.80	7	1	20
Microcylloepus	2001.6	2.32	7	1	10
Pisidium	2002.3	1.40	7	1	16

Table K2-2. Weighted average indicator values for annual minima, 3-day means, which had relatively strong performance

3-DAY ANNUAL MINIMA					
Taxa	**Optimum**	**Tolerance**	**Rank_Opt**	**Rank_Tol**	**Count**
Pisidium	0.030	0.04	1	2	16
Ambrysus	0.041	0.05	1	3	17
Mayatrichia/Neotrichia	0.045	0.03	1	2	16
Neotrichia	0.046	0.04	1	2	12
Leuctridae	0.049	0.03	1	1	24
Asellidae	0.050	0.06	1	4	45
Lymnaea	0.056	0.04	1	3	15
Zapada	0.057	0.04	1	3	35
Neothremma	0.059	0.04	1	3	19
Physella	0.060	0.06	2	5	13
Skwala	0.061	0.02	2	1	31
Petrophila	0.062	0.05	2	4	36
Coenagrionidae	0.064	0.07	2	6	36
Bibiocephala	0.065	0.01	2	1	17
Cultus	0.066	0.04	2	3	20
Serratella	0.067	0.04	2	2	11
Dytiscidae	0.068	0.04	2	2	10
Pelecypoda	0.069	0.06	2	5	44
Hesperoperla	0.069	0.05	2	4	33
Epeorus	0.070	0.04	2	2	92
Physa	0.071	0.06	2	5	54
Claassenia	0.072	0.03	3	1	12
Podmosta	0.072	0.03	3	1	10
Tipula	0.072	0.05	3	4	31
Capniidae	0.073	0.05	3	4	38
Apatania	0.073	0.02	3	1	20
Oecetis	0.073	0.04	3	2	45

73 **Table K2-2. Continued**

Taxa	Optimum	Tolerance	Rank_Opt	Rank_Tol	Count
Baetidae	0.073	0.06	3	6	277
Heptagenia	0.075	0.05	3	4	58
Pteronarcella	0.076	0.04	3	2	91
Ephemerella	0.076	0.05	3	4	149
Chloroperlidae	0.076	0.04	3	2	105
Hemerodromia	0.076	0.07	3	6	103
Antocha	0.077	0.05	4	3	126
Ostracoda	0.077	0.06	4	5	96
Lepidostoma	0.077	0.05	4	4	88
Paraleptophlebia	0.078	0.04	4	2	96
Arctopsyche	0.078	0.05	4	3	99
Rhithrogena	0.078	0.04	4	3	127
Simuliidae	0.079	0.06	4	5	234
Chelifera	0.079	0.06	4	5	98
Isoperla	0.080	0.04	4	3	105
Cheumatopsyche	0.080	0.07	4	6	55
Rhyacophilidae	0.080	0.05	4	4	98
Cinygmula	0.080	0.05	4	3	90
Optioservus	0.080	0.06	4	5	148
Glossosoma	0.081	0.05	4	4	60
Acarina	0.081	0.06	4	5	268
Zaitzevia	0.081	0.05	4	4	97
Planaria	0.082	0.07	4	7	90
Leptohyphidae	0.082	0.07	5	6	133
Ameletus	0.082	0.05	5	4	26
Hydroptila	0.082	0.06	5	6	97
Nematoda	0.082	0.06	5	6	125
Hexatoma	0.082	0.03	5	2	88
Hydropsyche	0.083	0.06	5	5	232
Taenionema	0.083	0.04	5	3	29
Copepoda	0.084	0.07	5	6	35
Microcylloepus	0.085	0.04	5	3	10
Leucotrichia	0.085	0.06	5	5	23
Chironomidae	0.085	0.07	5	6	291
Euparyphus	0.086	0.10	5	7	12
Isogenoides	0.086	0.04	6	2	19
Drunella	0.087	0.05	6	4	119
Dicranota	0.089	0.05	6	4	32
Tubificidae	0.090	0.06	6	5	107
Pteronarcys	0.090	0.03	6	1	27

Table K2-2. Continued

Taxa	Optimum	Tolerance	Rank_Opt	Rank_Tol	Count
Atherix	0.091	0.05	6	4	81
Planorbidae	0.091	0.08	6	7	37
Alisotrichia/Leucotricia	0.091	0.06	6	6	32
Micrasema	0.092	0.05	6	4	55
Brachycentrus	0.093	0.06	6	5	145
Hirudinea	0.094	0.09	6	7	75
Oligophlebodes	0.094	0.05	6	4	35
Forcipomyia/Probezzia	0.094	0.08	7	7	20
Agapetus/Culoptila/Protoptila	0.097	0.03	7	1	12
Pericoma	0.100	0.07	7	6	47
Bezzia	0.103	0.08	7	7	53
Helicopsyche	0.110	0.08	7	7	68
Hyalella	0.111	0.09	7	7	62
Traverella	0.116	0.03	7	1	10
Hesperophylax	0.159	0.08	7	7	12
Gammarus	0.170	0.07	7	6	15

Table K2-3. Data that was used in the Utah correlation analyses was gathered from these biological sampling stations/USGS gages. %URB = % urban, %AGR=% agricultural and %FOR=% forested land use within a 1 km buffer of the sites.

BioStationID	USGS gage	# Yrs of data	Elev_ft	Eco_L3	Eco_L4	Ref Status	%URB	%AGR	%FOR
4926350	10131000	14	5573.3	Wasatch and Uinta Mountains	Mountain Valleys	TRASH	32.5	27.9	30.2
4934100	9302000	12	4762.6	Colorado Plateaus	Uinta Basin Floor	UNKNOWN	3.9	18.4	24
4937900	9261000	14	4766.1	Colorado Plateaus	Uinta Basin Floor	SO-SO	0	20.3	65.1
4954380	9330000	19	6940.5	Wasatch and Uinta Mountains	Semiarid Foothills	TRASH	6.9	30.3	56
4996690	10163000	17	4521.3	Central Basin and Range	Moist Wasatch Front Footslopes	TRASH	73.2	15.8	5.3
4998400	10154200	18	6971.4	Wasatch and Uinta Mountains	Mid-elevation Uinta Mountains	SO-SO	5.7	0.7	93.6
5940440	10234500	11	6249.3	Wasatch and Uinta Mountains	Semiarid Foothills	REF	3.9	0	96.1

K3. North Carolina

A number of different analyses were run on a subset of North Carolina IHA-biological data.

One analysis involved examining taxonomical trends using NMDS. One set of results is shown in Section 2 of the report. Additional results are shown in **Figures K3-1 through K3-3.** They show that baseflow index (a parameter representing low-flow influence) had the strongest correlation with macroinvertebrate species composition, though this relationship may be mostly due to ecoregional distributions of taxa. A number of covariates, such as elevation, temperature, and other factors may co-affect the observed pattern. The second IHA parameter that related to taxonomical compositions was number of reversals, which is a measurement of flashiness. The RBI had weaker correlation with species axes. Other factors that showed correlations were low pulse and high pulse parameters. Selected results from the Pearson and Kendall Correlations with Ordination Axes are shown in **Table K1-1**.

IHA parameter inference models. According to the NMDS ordination, the most important parameters associated with species compositions are baseflow index, number of reversals, and RBI (which is much weaker compared to the previous two). Inference models were developed for these three parameters using both R and C2 (**Table K3-1**).

Additional analyses were performed on this dataset to generate species response curves for baseflow index (Attachment K4), number of reversals (Attachment K5) and RBI (Attachment KL6). These were derived from a generalized linear model (GLM) output (Yuan 2006). The y-axis shows the probability of capture for a single taxon, and the gradient of environmental variables is represented on the x-axis. The curve is the GLM fitting into the dataset.

Table K3-1. According to the NMDS ordination, the most important parameters associated with species compositions are baseflow index, number of reversals, and RBI (which is much weaker compared to the previous two). Inference models were developed for these three parameters using both R and C^2. The final reported indicator values were based on R results.

	R^2			RMSE		
	Baseflow index	Number of reversal	RBI	Baseflow index	Number of reversal	RBI
C2	0.556	0.413	0.437	0.149	0.135	0.227
Bootstrap	0.492	0.245	0.369	0.154	0.141	0.219

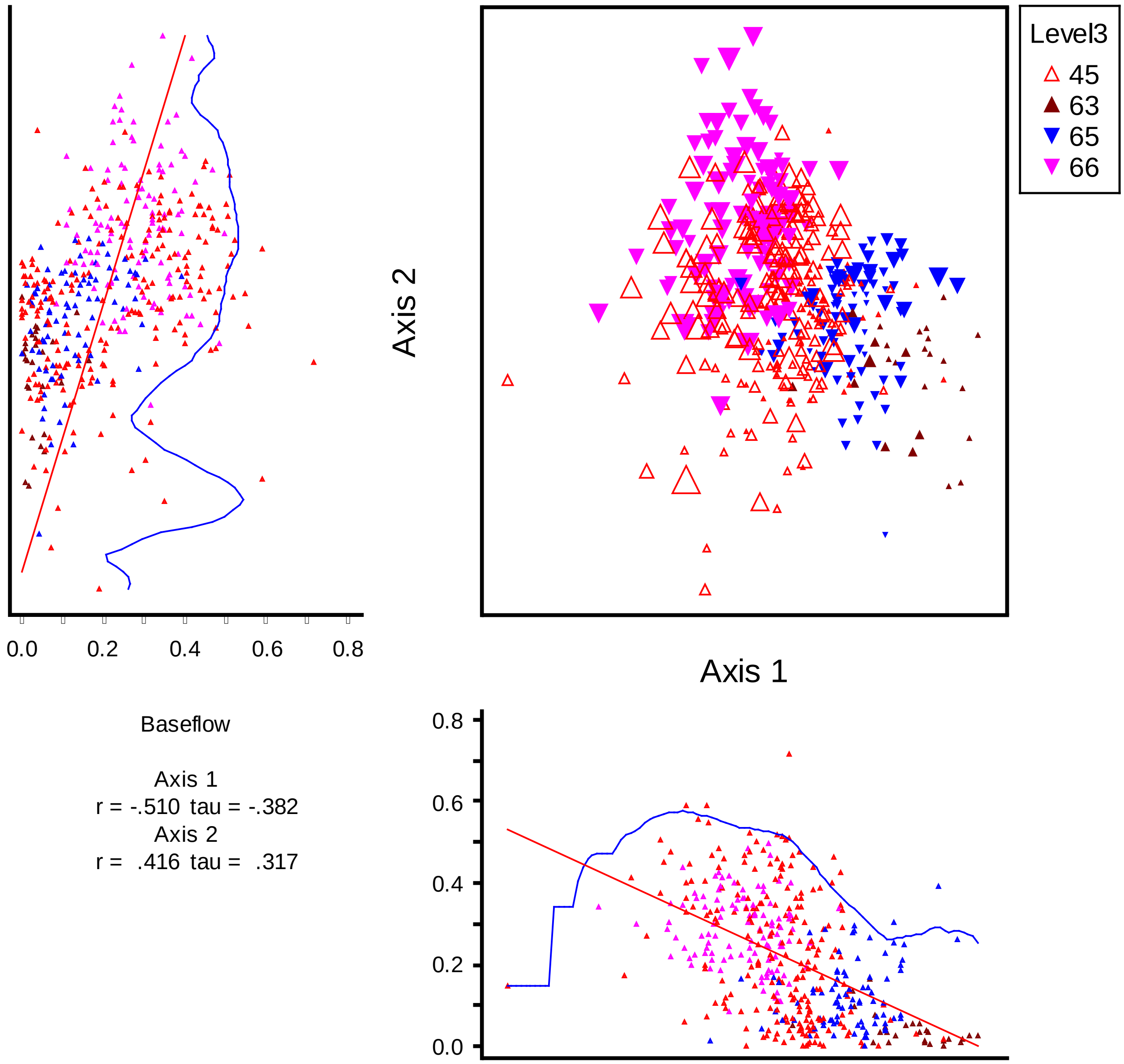


Figure K3-1. NMDS of macroinvertebrate taxonomical composition and its relationship with the baseflow index. Samples are grouped by level 3 ecoregion. Only samples collected using the standard qualitative/full-scale method were used in this analysis.

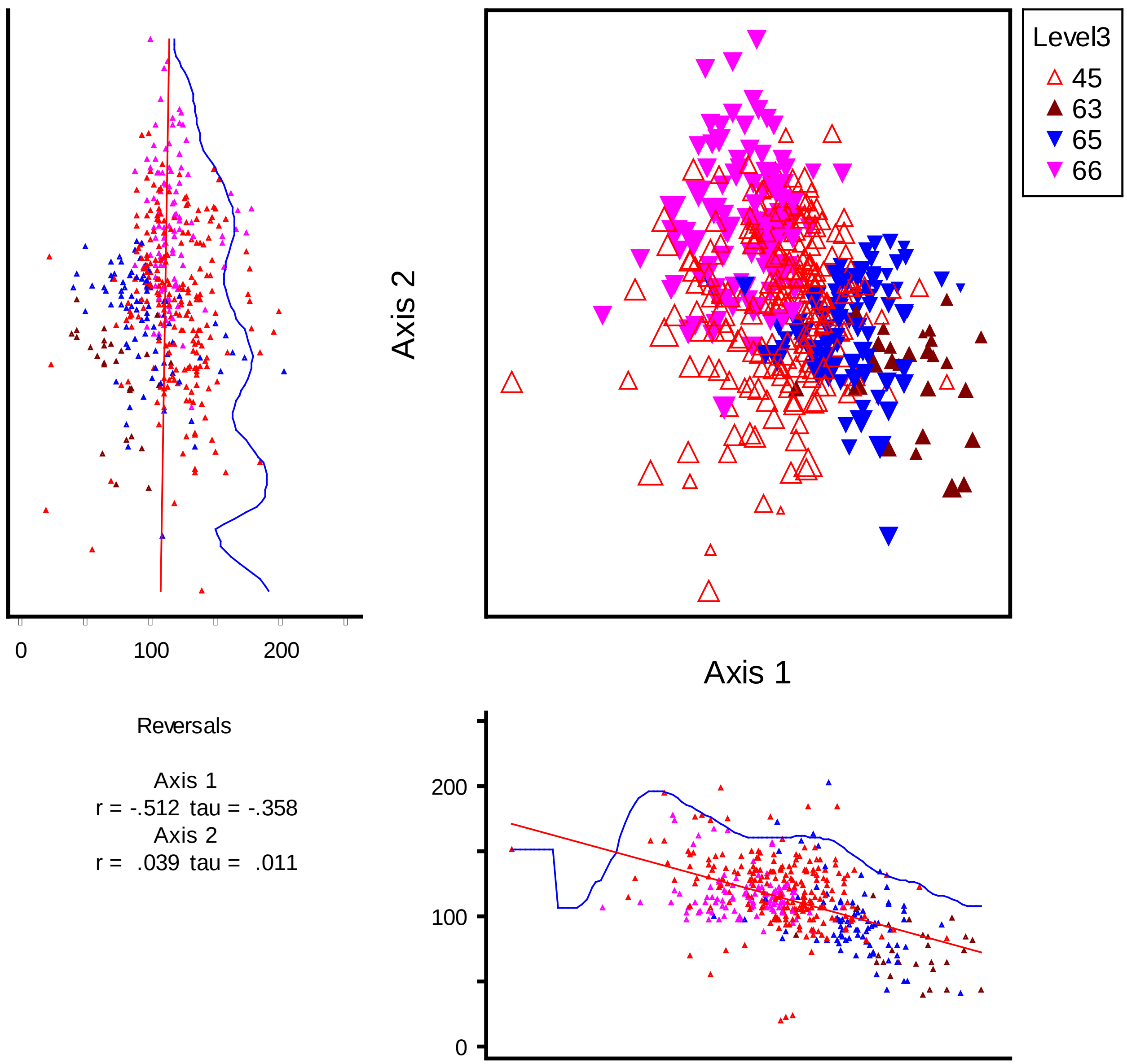


Figure K3-2. NMDS of macroinvertebrate taxonomical composition and its relationship with the number of reversals index. Samples are grouped by level 3 ecoregion. Only samples collected using the standard qualitative/full-scale method were used in this analysis.

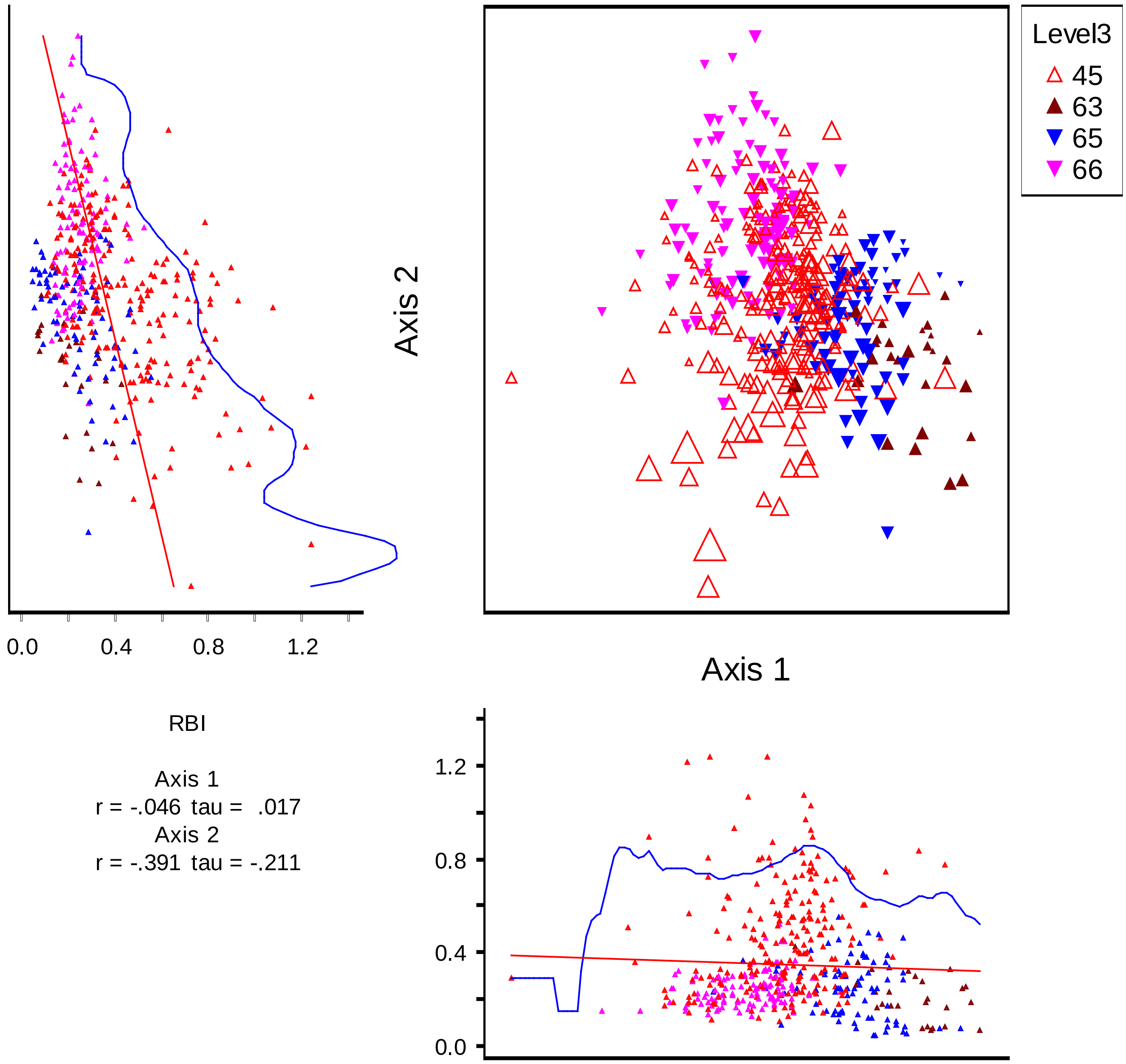


Figure K3-3. **NMDS of macroinvertebrate taxonomical composition and its relationship with the Richards-Baker Flashiness Index (R-B Flashiness Index). Samples are grouped by level 3 ecoregion. Only samples collected using the standard qualitative/full-scale method were used in this analysis.**