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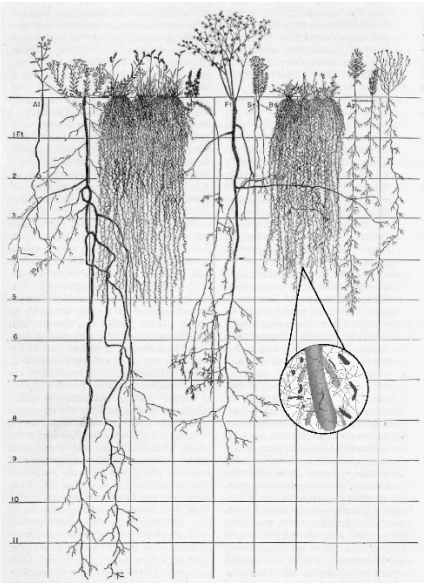
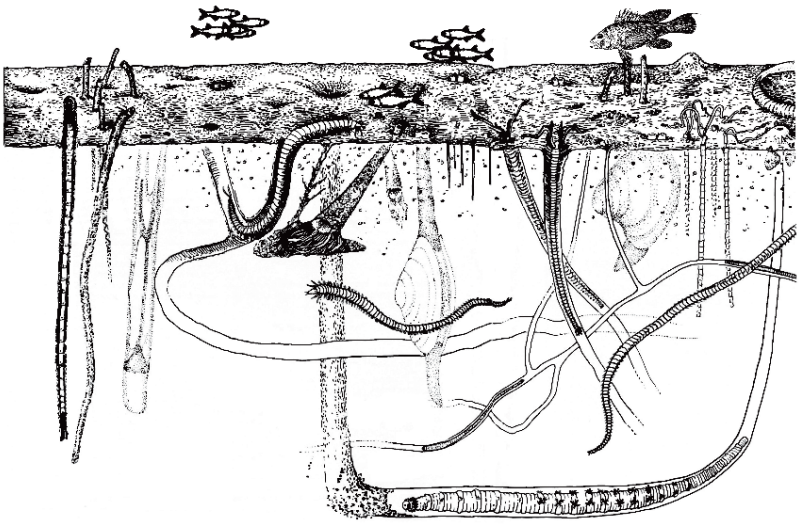
DETERMINATION OF THE BIOLOGICALLY RELEVANT SAMPLING DEPTH FOR TERRESTRIAL AND AQUATIC ECOLOGICAL RISK ASSESSMENTS





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National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
Eh	redox potential
EPA	U.S. Environmental Protection Agency
ERA	ecological risk assessment
ERAF	Ecological Risk Assessment Forum
ERASC	Ecological Risk Assessment Support Center
IT	intertidal
LCL	lower confidence limit
NCEA	National Center for Environmental Assessment
NOAA	National Oceanographic and Atmospheric Administration
NRCS	Natural Resources Conservation Service
ORD	Office of Research and Development
PLFA	phospholipid fatty acids
SD	standard deviation
ST	subtidal
UCL	upper confidence limit
USDA	U.S. Department of Agriculture

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EXECUTIVE SUMMARY

Ecological risk assessors are frequently faced with the challenge of defining the biologically active zone, or "biotic zone," in soils and sediments during the design and interpretation of soil and sediment sampling programs. Knowledge of the biotic zone is necessary when evaluating sediment/soil concentrations, calculating risks to ecological receptors, and attempting to delineate the relevant depth for remediation at sites where an action is needed. As current practice with regards to determining the biotic zone is quite varied, EPA's Ecological Risk Assessment Forum (ERAF) submitted a request to Office of Research and Development (ORD)'s Ecological Risk Assessment Support Center (ERASC) to develop a scientifically defensible definition for the depth of the biotic zone in soils and sediments (see Appendix). In response to the ERAF request, the present document attempts to provide defensible approximations for what the depth of the biotic zone is within certain environments. Actual sampling depths may be modified by the assessor based on the purpose of the assessment. The primary audience for this document is Superfund staff and contractors, and ecological risk assessors, though general ecologists should find the information useful as well. The methods used in this study differ somewhat between Part 1 (Terrestrial Biotic Zone) and Part 2 (Aquatic Biotic Zone). In Part 1, biological activity was quantified in forests and grasslands as a function of depth across selected metrics. In Part 2, the biotic zone(s) in various habitats was based on the 80th percentile of abundance or biomass depth distributions. Part 1 has also been summarized in Anderson et al., (2010).

Part 1 (Terrestrial Biotic Zone) of this study uses a meta-analysis approach to quantify the zone of highest biological activity for soil-dwelling ecological receptors commonly utilized in ecological risk assessments (ERAs). Endpoints evaluated include: invertebrate density, microbial biomass carbon (C), microbial density, mycelium production, root biomass, root production and total phospholipid fatty acids (PLFA). Results suggest sampling strategies should be adaptive allowing for variable depths. If constant depths *are* utilized, our results suggest that samples should be collected to a depth of approximately 25−30 cm.

Part 2 (Aquatic Biotic Zone) explores data from a wide realm of habitat types in an attempt to develop habitat-specific practical default values for the depth of the biotic zone, where most organism-substrate interactions occur. We recommend that the depth of the biotic zone be based upon the 80th percentile of abundance or biomass depth distributions. The biotic zone, based on benthic abundance, in most estuarine and tidal freshwater environments is 10 or 15 cm. Exceptions are oligohaline and polyhaline mud (5 cm) and oligohaline sand (5 cm). In marine muds (both coastal and offshore), the biotic zone is 15 cm. In other marine substrates it is 10 cm (marine coastal mixed and marine offshore sand) or 5 cm (marine coastal sand). In lentic

environments, the biotic zone is 15 cm. The biotic zone tends to be deeper when biomass is taken into account. The biotic zone in lotic systems varies from 15 to 35 cm depending upon water/habitat type. In areas populated by a high density of deep dwelling organisms such as the examples provided, the biotic zone may be somewhat deeper than our recommended values.

1. PART 1. TERRESTRIAL BIOTIC ZONE

1.1. INTRODUCTION

Risk assessors are frequently faced with the challenge of defining the biologically relevant sampling depth or "biotic zone" in soils and sediments during the design and interpretation of ecological studies. This may have important implications when evaluating ecological risk and/or designing a remediation scenario. For example, contamination occurring in layers deeper than the zone where most organisms live or feed may not be relevant to assessing ecological risk. In essence, spatial and vertical co-occurrence of soil contamination and ecological receptors need to be considered to estimate risks. While methodologies have been proposed that focus on optimizing the spatial scale of sampling efforts (Hathaway et al., 2008; Taylor and Ramsey, 2005), sampling depths for ERAs are usually dictated by the vertical distribution of soil contamination (Singh et al., 2008) or default to a generic value. These approaches may not adequately reflect site-specific exposures to soil biota. The default sampling depth for estimating exposure of plants, as well as earthworms, to contaminants has been reported as the top 30 cm (Suter, 2007); the top 12 cm has also been recommended as a default sampling depth for estimating exposure of plants to metals (U.S. EPA, 2005). The purpose of this study is to use a meta-analysis of ecological literature to quantify the zone of highest biological activity for soil-dwelling ecological receptors, and to determine whether or not a default value for the biologically relevant (soil) sampling depth can be supported.

1.2. METHODS

1.2.1. DATA EXTRACTION

Nonagricultural literature was searched using the Academic Search Complete database. Journal articles were limited to primarily 2000 through 2009. An exception was made in the case of a recent summary paper that cites earlier studies (Briones et al., 2007). There were no restrictions on publication sources so long as they were peer-reviewed. The database was searched with iterative combinations of (1) the keyword "soil" (2) keywords to locate studies containing appropriate biological metrics and (3) keywords to locate studies examining the metrics at stratified depths. Literature searches were restricted to soil invertebrate, plant, and microbial endpoints. Specifically, endpoints evaluated include: invertebrate density, microbial biomass carbon (C), microbial density, mycelium production, root biomass, root production and total PLFA. Studies were further restricted to those with data extractable from a table or a readable graph, reporting the depth for the top and bottom of each sample observation.

A categorical variable that refers to the dominant matrix vegetation at each site was defined and referred to as the "environment type" (e.g., forest, grassland, desert, shrubland, etc.)

and was extracted via site descriptions in the articles. However, sufficient data ($n > 10$) only existed for forests and grasslands. Consequently, only data from forests and grasslands were included in the analyses and are summarized in Table 1, which includes the biological metric, environment type, and number of depth intervals for each study. Admittedly, grouping sites into categories defined by generalized classes of vegetation is an oversimplification of the complexity of natural systems. However, we default to broad scale patterns in ecological organization necessary for meta-analyses of biological processes using studies with highly variable environment conditions (Levin, 2005).

An additional categorical variable that refers to the climate at each study site was also determined and included in analyses. Climate type was determined in a Geographic Information System. First, the geographic locations of study sites were extracted via site descriptions in the articles. Each site was then mapped with the Köppen-Geiger climate classification data (Kottek et al., 2006) and assigned a climate type based on its placement on the map. The broadest Köppen-Geiger categories (e.g., tropical humid [equatorial], dry [arid], mild mid-latitude [warm temperate], severe mid-latitude [snow], and polar) were used.

1.2.2. STATISTICAL ANALYSIS

Primary objectives of data analyses were to quantify biological activity as a function of depth for the selected metrics. To facilitate these objectives, paired data were necessary. Consequently, the midpoint of each depth interval was calculated to relate to the corresponding metric value reported from that particular depth interval. Relationships between midpoint depths and biological metric values were subsequently evaluated.

Relationships were evaluated collectively across metrics. However, it was first necessary to scale observations. First, all data within a metric were converted to a standard unit. Standard units were determined as the unit that was most frequently reported within a metric. Subsequently, all data within a metric were standardized to a standard normal variable (mean = 0, standard deviation [SD] = 1) across depths, environment types, and climates because each metric produced values with unique units or a completely different range of values for the same unit. Standard normal variables are simply computed by subtracting off the mean and dividing by the standard deviation. The idea being that data from similar depths would produce similar standardized metric values (i.e., z scores) that fall reasonably close to one another on the standard normal probability distribution allowing observations to be evaluated for depth, environment type, and climate effects across metrics.

Trends between standardized metric values and midpoint depths followed an exponential decay pattern. Consequently, nonlinear regression with an exponential decay function was used

to model relationships. Because standardized metric values contained both positive and negative values, a three parameter exponential decay function was utilized of the form:

$$y = (A + C)e^{Bx} + C \qquad \text{(Eq. 1)}$$

where y is standardized metric value, A is the y-intercept, B is a slope parameter, and C is a scale parameter necessary because metric values contained both negative and positive values.

Preliminary analyses indicated that significantly ($\alpha = 0.05$) different trends occurred between grasslands and forests as determined by contrasting residual sums of squares for full (both forests and grasslands) and nested reduced (forests and grasslands separately) models (see Equation 1). Consequently, Equation 1 was fitted to data from forests and grasslands separately. Unique parameters were estimated for each environment type. Climate effects were subsequently evaluated by testing the residuals from Equation 1 for differences across climate types within each vegetation class by analysis of variance (ANOVA). Nonlinear regression was performed using PROC NLIN and ANOVA was performed using PROC GLM in SAS Version 9.2 for Windows.

1.3. RESULTS AND DISCUSSION

Common soil-dwelling receptor groups evaluated during ERAs consist of plants and invertebrates (U.S. EPA, 2005). Microbial endpoints can be impacted by environmental contaminants (Giller et al., 1998), but they are often considered too variable to provide utility as a basis for chemical-specific soil screening levels (U.S. EPA, 2005). However, abundance of microbial communities is tightly coupled with the quality (i.e., carbon:nitrogen ratio) of substrates and regulates essential nutrient (e.g., nitrogen) availability in soils (Friedel and Gabel, 2001). Thus, microbial endpoints affect other higher order endpoints through feedback loops and were considered essential to our objectives.

1.3.1. META-ANALYSIS RESULTS

Relationships between the standardized metric meta-data and their corresponding midpoint sampling depths are presented in Figure 1. Three-parameter exponential decay functions (see Equation 1) were fitted to meta-data for grassland and forests separately. Climate was not significant ($\alpha = 0.05$) and did not influence relationships. Parameter estimates and approximate confidence intervals are presented in Table 2. Both models were highly significant ($p < 0.0001$). Grasslands produced an exponential decay function with higher standard normal

scores and a steeper slope indicating relatively higher values for each common metric (i.e., invertebrate density, mycelium production, and root biomass; see Table 1) and a faster rate of decline. However, both functions resulted in an asymptotic plateau at roughly 27 cm (see Figure 1).

Grassland soils contain greater amounts of organic matter than forest soils because of higher primary production rates at steady state with decomposition (Zak et al., 1994). In general, matrix vegetation in grasslands consists of perennial herbaceous plants with high root densities and receive relatively less precipitation (Saviozzi et al., 2001). This greatly suppresses microbial decomposition and allows for the accumulation of organic matter, which produces soils with darker surface horizons relative to forest soils (NRCS, 2003). As a result, soil biota are usually more productive in grasslands because they experience less carbon limitation (Zak et al., 1994), which is consistent with Figure 1.

1.3.2. RECOMMENDATION OF SAMPLING DEPTH

Soils are highly heterogeneous mixtures of inorganic and organic constituents. Complex, multi-trophic assemblages of organisms comprise the soil biology and inextricably interact with and feed back to the soil organic matter resulting in a zone of interdependent biological processes referred to as the rhizosphere. Microorganisms are essential to the rhizosphere through the development of stable organic compounds (i.e., humic substances) and the hierarchical structure of soil aggregates (Kandeler et al., 2001). Soil organic matter is responsible for giving the rhizosphere its characteristic darker color, which in general soil classification terms is referred to as the A horizon (NRCS, 2003). Soil organic matter provides a source of energy for microbial respiration, which in turn regulates essential plant nutrients (Luxhoi et al., 2006). Consequently, the A horizon, via the rhizosphere, provides the foundation to the food web for soil ecosystems and should contain the vast majority of biological activity.

Results from Figure 1 were compared to the average depth of soil horizons. Accordingly, a regional data set was obtained from the U.S. Department of Agriculture (USDA) National Resource Conservation Service (NRCS) Cooperative Soil Survey Program. Depths of dominant soil horizons (O, A, B, and C) were utilized, which were measured from 636 soil pedons (i.e., the smallest volume of material that can be called "soil") from around the conterminous United States. The database is freely available and can be accessed online at http://soils.usda.gov/survey/geochemistry/index.html. Only data from Alfisol (characteristic forest soil) and Mollisol (characteristic grassland soil) soil orders (i.e., the highest level of USDA classification) were evaluated (NRCS, 2003).

Figure 2 illustrates the average biologically relevant sampling depth. Mean horizon depths for both Mollisol and Alfisol soil orders are shown overlaid on the first derivatives of

Figure 1. Mollisol and Alfisol soil orders are characterized with mean A horizons that extend to 19.3 and 20.2 cm, respectively. First derivatives from Figure 1 reach an approximate minimum, on an absolute scale, at 27 cm, roughly consistent with the mean depth of A horizons, although depths associated with derivative values are midpoints of a sampling interval. However, standard deviations for mean A horizon depths for Mollisol and Alfisol soil orders are 19.4 and 53.6, respectively, suggesting the minimum (on an absolute scale) derivative value of 27 cm falls within error limits of the A horizon for both soil orders. Thus, a definitive conclusion of this study is that A horizons, although not necessarily all inclusive, represent the average biologically active zone, at least for the metrics evaluated. Hence, capturing the A horizon is paramount to accurately evaluating potential exposure of environmental contaminants to soil biota.

Soil development is rarely uniform and processes such as erosion and deposition can influence the vertical distribution of biological activity across landscapes. Sampling strategies where a constant depth is collected may not accurately reflect site-specific exposures of environmental contamination to the soil biota. Samples that either fail to capture the extent of, or exceed, the A horizon may not accurately represent contaminant exposure to soil biota, resulting in inaccurate risk estimates. The depth of horizontal soil horizons can vary across the landscape (Luxhoi et al., 2006), which may also confound ERAs that utilize a constant depth. Consequently, sampling strategies should be adaptive allowing for A horizons with variable depths. If constant depths *are* utilized, our results suggest that samples should be collected to a depth of approximately 25-30 cm (see Figure 2) as opposed to shallower depths.

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Table 1. Summary of Data Used in Meta-analysis

Reference	Biological Metric	Environment Type	*N*[a]
Allison et al. (2007)	total PLFA	grassland	6
Altesor et al. (2006)	root biomass	grassland	5
Borken et al. (2007)	root biomass	forest	24
Briones et al. (2007) (Review Article)			
Abrahamsen and Thompson (1979)	invertebrate density	forest	1
Chalupsky (1986)	invertebrate density	forest	1
Chiba et al. (1976)	invertebrate density	forest	1
Hutha (1984)	invertebrate density	forest	1
Kairesalo (1978)	invertebrate density	forest	1
Lundkvist (1982)	invertebrate density	forest	1
Lundkvist (1983)	invertebrate density	forest	1
Makulec (1983)	invertebrate density	forest	1
Nurminen (1967)	invertebrate density	forest	1
Phillipson et al. (1979)	invertebrate density	forest	1
Thambi and Dash (1973)	invertebrate density	grassland	1
Yeates (1986)	invertebrate density	grassland	1
Claus and George (2005)	root biomass	forest	33
Davis et al. (2007)	root biomass	grassland	5
Davis et al. (2007)	root biomass	forest	6
Kemmitt et al. (2008)	root biomass	grassland	1
Kemmitt et al. (2008)	root biomass	forest	6
Steinaker and Wilson (2008)	invertebrate density	grassland	5
Steinaker and Wilson (2008)	mycelium production	grassland	5
Steinaker and Wilson (2008)	root production	grassland	5
Steinaker and Wilson (2008)	invertebrate density	forest	5
Steinaker and Wilson (2008)	mycelium production	forest	4
Steinaker and Wilson (2008)	root production	forest	5
Tsai et al. (2007)	microbial density	forest	90
Zheng et al. (2005)	microbial biomass C	forest	7

[a]Number of observations. Each observation represents a discrete depth interval.

Table 2. Parameter Estimates and 95% Lower and Upper Confidence Intervals[a] (LCL and UCL, Respectively) for the Nonlinear Function (See Equation 1) Fit to Standardized Data for Both Forests and Grassland Environment Types

Environment Type	Parameter	95% LCL	Estimate	95% UCL
Forest	A	0.873	1.56	2.26
	B	−0.185	−0.0919	0.00127
	C	−0.527	−0.303	−0.0783
Grassland	A	2.32	4.89	7.47
	B	−0.295	−0.160	−0.024
	C	−1.12	−0.641	−0.162

[a]Confidence intervals for nonlinear functions are only approximate (Kutner et al., 2004).

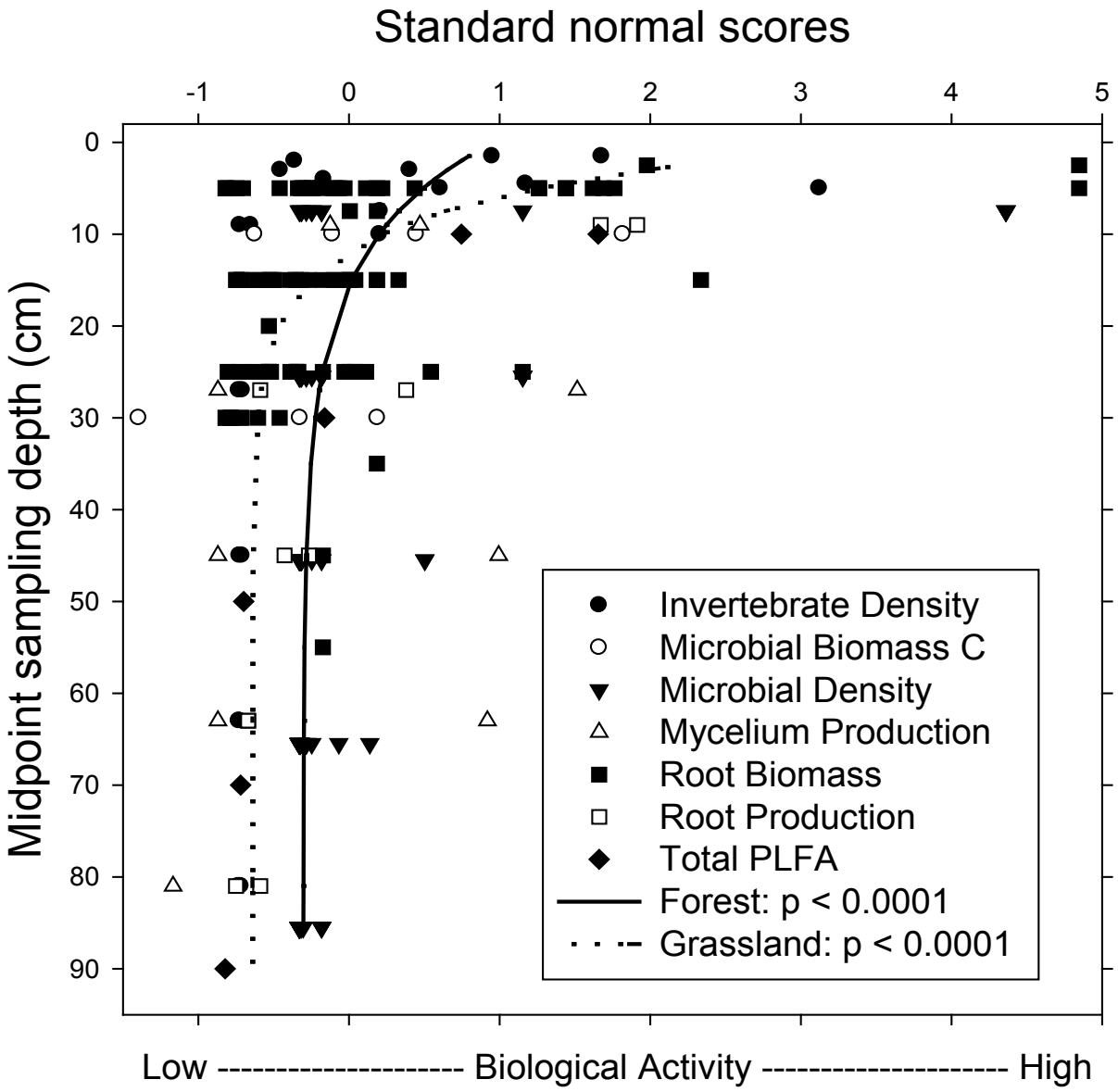

Figure 1. Nonlinear (see Equation 1) Relationships Between Standardized (mean = 0; SD = 1) Biological Metrics and Their Midpoint Sampling Depths for Forests and Grasslands.

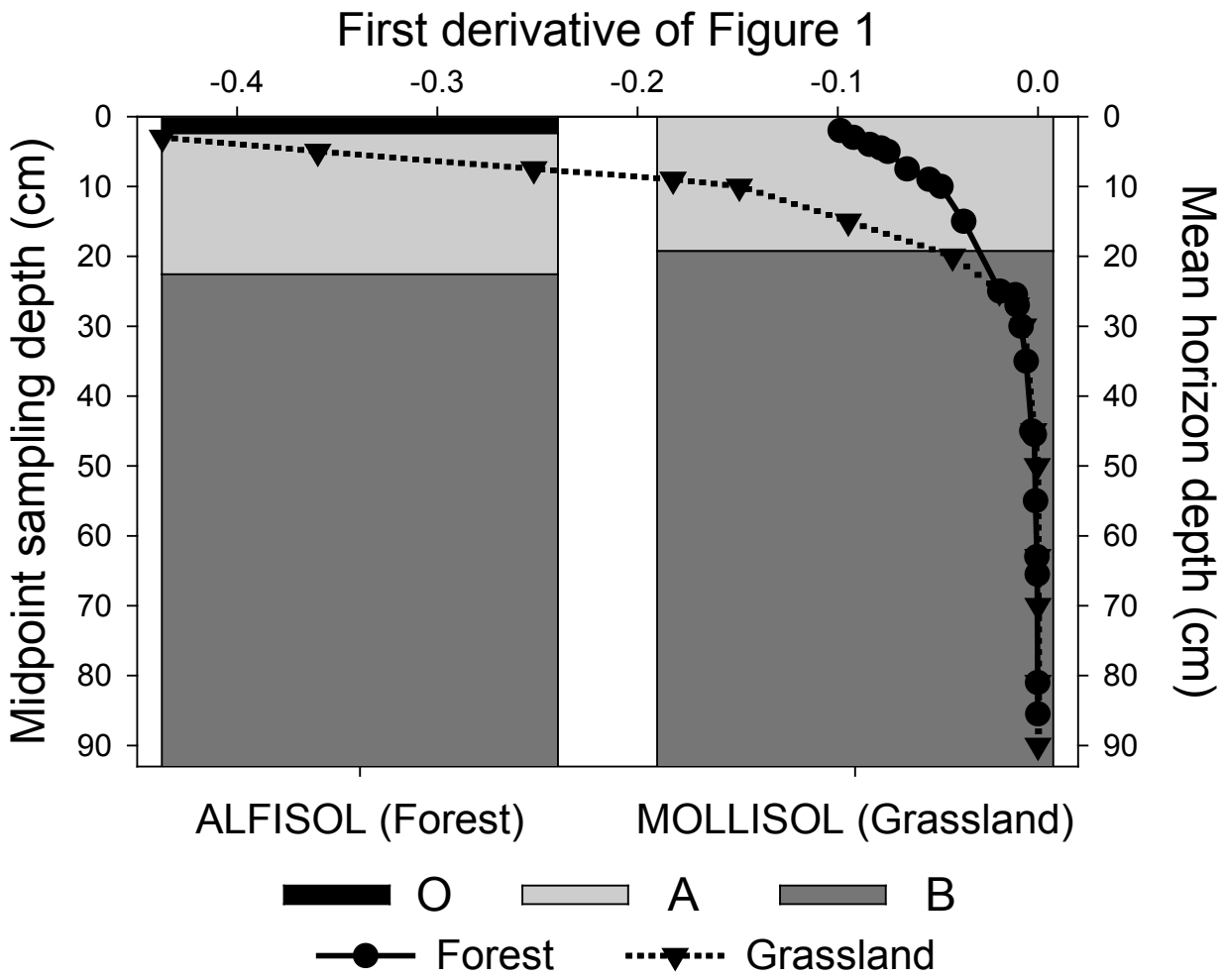

Figure 2. Illustration of the Average Biologically Relevant Sampling Depth. Mean soil horizon (O, A, and B) depths (determined from available data produced by the USDA/NRCS Cooperative Soil Survey) are summarized by the vertical bars. The first derivatives from the nonlinear functions, illustrated in Figure 1, reach a minimum (on an absolute scale) value (i.e., constant biological activity) at approximately the transition between the A and B soil horizons for the depths evaluated.

2. PART 2. AQUATIC BIOTIC ZONE

2.1. INTRODUCTION

Benthic organisms alter the fluxes of particulate and dissolved chemical species through their burrowing, ingestion and excretion, tube-building, and biodeposition activities (Thoms et al., 1995). Hence, the zone or area of the substrate where these organisms reside is important as a site of exchange for nutrients and contaminants, especially with overlying waters. The vertical extent of this zone is often referred to as the depth of bioturbation, or mixed layer. Thoms et al. (1995) compiled data on the depth of the mixed layer, mainly from radio-isotope tracer studies. Mixing depths ranged from less than 1 cm (Amazon continental shelf) to greater than 35 cm (e.g., deep Puget Sound). Based on radio-isotope tracer profiles from a large number of studies, Boudreau (1994) determined the mean (± SD) mixing depth worldwide to be 9.8 ± 4.5 cm. Based on tracer profiles, as well as sediment profile imaging literature and surveys, Teal et al. (2008) estimated the global mean (± SD) mixing depth to be 5.75 ± 5.67 cm. Other studies have utilized cores to determine the depth distribution of benthic invertebrates from specific locations around the world. Ecological risk assessors should consider the depth of this "biotic zone" in the design and interpretation of sediment sampling programs, as this is where exposure to contaminants or other stressors will occur. This zone is also the source of prey for benthic-feeding fishes (and shore birds in the intertidal) and, potentially, trophic transfer of pollutants.

Knowledge of the biotic zone is necessary when attempting to delineate the relevant depth for remediation at sites where an action is needed. When evaluating remedial alternatives in cases where contaminant hotspots extend deep within the sediment, it may not be prudent (for environmental and cost reasons) to consider zones deeper than where the bulk of organisms reside. In the case where contaminated sediments are capped with clean substrate, the thickness of the cap should exceed the depth to which infauna burrow, or the depth of the biotic zone, in order to avoid infiltration of contaminants through the cap and into the water column. The present paper explores data from a wide realm of habitat types in an attempt to develop habitat-specific practical default values for the depth of the biotic zone, where most organism-substrate interactions occur. We use the 80th percentile of abundance or biomass depth distributions as a common measure for comparison among samples. In our judgement, use of the "80th percentile" strikes a balance by including most of the organisms, but without going to depths where the biota are very sparse. We acknowledge a degree of subjectivity in choosing this value, but note that a number of assessment programs (National Oceanographic and Atmospheric Administration Status and Trends Program; EPA Environmental Monitoring and Assessment Program) use a 20 percent effects level (i.e. 80% nonaffected) as a threshold of ecological significance (Long, 2000).

2.2. BENTHIC ORGANISMS AND THEIR ENVIRONMENT

For benthic organisms, the nature of their interaction with the sediment is determined by the manner in which food is obtained (trophic type), where their activities are carried on (life position) and their mobility (Fisher, 1982). Feeding types for benthos that are applicable to fresh water are presented in Fisher (1982; after Walker and Bambach, 1974). Feeding types applicable to marine waters are presented in Lee and Swartz (1980). The majority of suspension feeders are located near the sediment-water interface, while suspension-feeding bivalves with siphon tubes, and deposit feeders may burrow deeper. Examples of deep-burrowing species are presented in Table 3.

Among environmental determinants of the type of organisms, and, hence, benthic community structure of an area, sediment grain size is very important because it reflects the hydrodynamic regime and the quantity and quality of organic carbon. High proportions of fines are representative of depositional environments and provide a greater surface area (compared to coarse-grained sediments) for sorption of organic carbon and contaminants.

The microbial degradation of labile organic matter largely determines the redox potential (Eh) and pH observed at various depths in the sediment and is responsible for a variety of secondary reactions involving metals (e.g., desorption, release to pore water, formation of sulfide and associated fixation of trace metals) (Batley et al., 2005). Because the flux of labile organic matter to the sediment is usually much faster than the diffusive flux of oxygen across the sediment water interface, it is commonly observed that oxygen concentrations in sediments become anaerobic close to the sediment-water interface (Batley et al., 2005). The oxic zone may vary in thickness from a few millimeters in silty sediments to several cm in coarser riverine and estuarine sands and is underlain by a suboxic and an anoxic area. This oxygen gradient, along with other reactions described above, leads to vertical zonation in sediments and pore waters of pH, Eh and various chemical species, including Pb and Mn, and trace metals (Batley et al., 2005).

A number of macroinvertebrates can span both oxic and anoxic layers of sediment. Some that ingest particles at depth and egest them upon the sediment surface—the 'head-down' conveyor-belt species of Rhoads (1974)—are major agents of sediment reworking in many benthic communities. These species, some of which are included in Table 3, dominate late successional stage equilibrium assemblages associated with a deeply oxygenated sediment surface where the redox zone commonly reaches depths of over 10 cm (Rhoads and Germano, 1986). Tubificid oligochaetes can feed in anoxic sediment layers while waving their tails in the water column for the purpose of respiration (McCall and Tevesz, 1982). During feeding, material ingested from several centimeters beneath the sediment surface is deposited at the

sediment-water interface, resulting in the rapid burial of components originally deposited at the sediment surface as well as the upward transport of subsurface material (including pollutants) (Krezoski and Robbins, 1985). Many marine bivalves use siphon tubes to inspire overlying water, while physically residing in deeper anoxic sediment (Batley et al., 2005).

The benthic community in marine sediments has great taxonomic diversity, including a number of species that burrow to depths greater than 20 cm (see Table 3; Matisoff, 1995). Freshwater sediments are inhabited by a variety of macrobenthos, principally arthropods (insects and amphipods), annelids (oligochaetes and leeches), and mollusks (bivalves and gastropods) (Fisher, 1982). Along with chironomids, tubificid oligochaete worms are usually the dominant macrofauna in lake profundal regions (McCall and Tevesz, 1982). Populations of a few score to a few thousand worms per square meter occur commonly, with higher populations in organically rich environments (Davis, 1974).

2.3. BENTHIC HABITAT TYPES

Chapman et al. (2005) summarize environmental characteristics of five types of water bodies as follows:

> Lacustrine: low-energy environment; generally depositional; groundwater interaction decreasing away from shore; organic matter decreasing with distance from shore; often fine-grained sediment
>
> Riverine: low- to high-energy environment; depositional or erosional; potential for significant groundwater interaction; significant variability in flow and sediment characteristics within and between rivers.
>
> Estuarine: generally low- or moderate-energy environment; generally depositional; generally fine-grained sediment grading to coarse sediment at ocean boundary.
>
> Estuaries are dynamic, complex, and unique systems that can have strong physical-chemical gradients, particularly of salinity, dissolved oxygen, pH, nutrients, sediment grain size, and organic matter content. Estuarine systems are divided into a number of categories based on salinity (see Boesch, 1977). Estuarine sediments can come from inland and/or the sea, depending on the freshwater sediment load and the estuarine circulation patterns. Due to the dynamic nature of sediments in estuaries with strong flows or currents, the stability of estuarine benthic environments can vary and should be taken into account in any ecological assessment. Sediment total organic carbon, which typically varies with fine sediment particles, provides a good overall index of organic loading and composition. It integrates carbon enrichment from multiple sources, including land-based inputs, detritus, and algal and microbial metabolism.

Coastal Marine: relatively high-energy environment, decreasing with depth and distance from shore; often coarse sediments.

Offshore Marine: generally low-energy environment; generally depositional; generally fine-grained sediment.

Benthic communities in marine environments are typically below the photosynthetic zone, other than along the coastal margins. Consequently, benthic food chains are typically built on organic materials carried into the system; thus, the food chain is primarily allochthonous. Materials such as phytoplankton may be filtered from the water, or deposits may provide organic material for bacterial growth, which can then be harvested by filtering or grazing organisms.

2.3.1. LOTIC VERSUS LENTIC ENVIRONMENTS

Lotic environments (include rivers and streams) may be either depositional or erosional. High-gradient streams and other erosional environments differ significantly from lentic systems in terms of major physical processes, factors that limit primary production, nutrient dynamics, types of primary producers, and the relative importance of autochthonous versus allochthonous energy sources (Chapman et al., 2005). The defining feature of lotic environments is the unidirectional flow of water, responsible for the downstream transport of biotic and abiotic materials, including sediments, and the biota (downstream colonization). The potential for movement of sediments is much greater in lotic than lentic environments. Due to greater energy levels and greater potential for sediment transport, grain size is larger, and organic carbon content is generally lower in lotic erosional environments than in lotic depositional or lentic environments. Unlike depositional habitats, fine-grained sediments in erosional environments are highly mobile. Materials such as nutrients, sediments, and contaminants are transported downstream, deposited in slower moving sections of the river, and then resuspended during periods of high discharge. Because the velocity of water flow decreases downstream, mean particle size will generally decrease, and amounts of organic carbon increase, from headwater reaches to downstream reaches (Chapman et al., 2005).

2.3.2. HYPORHEIC ZONE

The hyporheic zone of rivers and streams is the spatially fluctuating ecotone between the surface water body and the deep groundwater where exchanges of water, nutrients, and organic matter occur in response to variations in discharge and bed topography and porosity (Boulton et al., 1998). The interstitial spaces among sediment particles in the hyporheic zone are occupied by a diverse array of aquatic invertebrates, termed the “hyporheos.” The hyporheos includes many types of crustaceans, segmented worms, flatworms, rotifers, water mites, and juvenile stages of aquatic insects (Boulton et al., 1998). The organisms inhabiting the hyporheic zone

may be either epigean or hypogean depending upon their affinities for surface or subsurface habitat, respectively. Though many insect larvae and epigean crustaceans colonize the superficial benthos of running waters, epigean species can also penetrate deeper where water circulates freely through the sediments and much organic matter and oxygen are available (Ward et al., 1998). The present paper does not cover fauna that live strictly in groundwater zones that can be located 2 – 3 km from river channels (noted in Stanford and Ward, 1993).

The composition of the hyporheos represents a complex response to interstitial water velocity, sediment composition (particularly the amount of fine sediments), sediment pore size, organic matter content, dissolved oxygen concentration, vertical hydrological exchange, and other environmental parameters as well as biological interactions (Boulton, 2007; Dole-Olivier and Marmonier, 1992; Olsen and Townsend, 2003). The deeper layers of the hyporheic zone can serve as a refuge from environmental perturbations such as flooding and drought, or from predation (Griffith and Perry, 1993; Angradi et al., 2001).

2.4. METHODS

Literature relevant to the biotic zone was obtained by searching on the keyword combinations (1) "sediment" AND "biotic zone" OR "bioturbation zone," (2) "sediment" AND "invertebrates" AND "vertical distribution," and (3) "sediment" AND "invertebrates" AND "vertical distribution" AND "sediment type." We searched the literature from 1985 to present but included a number of key references that were older. Data on organism abundance or biomass with depth in the sediment were extracted from tables or graphs. Data from sites that were acknowledged by the study authors to be impacted by a local pollution source were not included. The data available consist of 234 datasets, each consisting of one or more cores from a particular habitat type (see Table 4) that detail the depth distribution of organisms by abundance or biomass. A publication may contain more than one dataset for a habitat type if sets of cores were taken from different locations (within that habitat type) or at different times. The data were summarized by first computing for each dataset an 80th percentile depth. This was determined as the midpoint of the stratum containing the 80th percentile of the abundance or biomass distribution from the sediment surface to depth. Where data were presented on a volume instead of areal basis and the strata were of unequal thickness (e.g., 0−2, 2−5, 5−10 cm), the values were weighted to account for the fact that thicker strata contain a greater volume of sediment.

Based on the 80th percentile of depth distributions, we developed practical default values for the depth of the biotic zone (i.e., biologically relevant sediment depth) in various habitats for decisions related to ecological assessment or remediation. We calculated and graphed the mean 80th percentile depths (for abundance or biomass) for each habitat type; the maximum 80th

percentile depth for each habitat type was also graphed. Each mean 80th percentile depth was rounded to the next (deeper) 5-cm boundary (i.e. 5, 10, 15, etc.) to determine the biologically relevant sampling depth or biotic zone for the respective habitat type. Where the maximum 80th percentile depth for a habitat type exceeded the mean 80th percentile depth by more than 5 cm, we added 5 cm to the mean and rounded to the next boundary to arrive at the biotic zone for that category.

Habitat types were classified by salinity (within estuarine habitats) and sediment type within seven broad categories: estuarine intertidal, tidal freshwater, estuarine subtidal, lentic, lotic, marine coastal, and marine offshore (see Table 4). The lotic category comprised (1) stream coarse grained/sand, (2) stream coarse grained/sand with fines, and (3) river coarse grained/sand with fines, where "fines" denote grain sizes <2 mm in substantial quantity (approximately 20% or more by weight). Sediment types were taken directly from the respective papers or designated using the classification of Shepard (1954). The "mixed" category refers to muddy sand or sandy mud, where mud = silt + clay.

2.5. RESULTS—BENTHIC BIOTIC ZONE: ABUNDANCE AND BIOMASS

The mean and maximum 80th percentile of benthic abundance depth distributions in various habitats are shown in Figure 3. A number of organisms can burrow significantly deeper than the 80th percentile depth distribution (see Table 3 for examples). Nonetheless, in performing ecological assessments related to sediment contaminants, it is important to identify the zone of greatest organism-substrate interaction, i.e., the biotic zone. We developed practical default values for the depth of the biotic zone in various habitats based on the 80th percentile of depth distributions. First we summarize these distributions.

In terms of benthic abundance depth distribution, the mean 80th percentile in estuarine intertidal, tidal freshwater, most estuarine subtidal, and lentic habitats extends to 5–10 cm (see Figure 3). Exceptions are oligohaline and polyhaline mud, and oligohaline sand, where the mean 80th percentile is less than 5 cm. Overall depth distributions within estuarine habitats tend to be deepest in mixed substrates and in sand (except oligohaline sand). The maximum 80th percentiles in estuarine intertidal sand, oligohaline mixed substrates, and polyhaline sand extend to 15–20 cm. The maximum 80th percentile in lakes (profundal mud) extends to 20–25 cm (see Figure 3).

In most marine coastal and offshore habitats, the mean 80th percentile of abundance depth distributions extends to 5–10 cm. Exceptions are marine coastal sand, and marine offshore mixed substrates, where the mean 80th percentile is less than 5 cm. (Note however that only one data set was available for the latter habitat type.) Overall depth distributions in marine coastal

and offshore muds tend to be deeper than in other marine substrates, with the maximum 80th percentile for marine coastal mud extending to 15–20 cm.

The mean and maximum 80th percentile of abundance depth distributions in lotic habitats is deeper than that in the other habitats. The three lotic habitats covered here are stream coarse grained/sand, stream coarse grained/sand with fines, and river coarse grained/sand with fines. The mean 80th percentile for these habitats extends to 25–30, 15–20, and 10–15 cm respectively. The maximum 80th percentiles extend to 35–40 cm, 30 cm, and 15 cm respectively (see Figure 3).

In most habitats where data are available, the 80th percentile of depth distributions based on biomass exceeds respective distributions based on abundance. Oligohaline mixed substrates are an exception to this trend (see Figures 3 and 4). The biomass-based depth distribution for lake profundal muds exceeds that for abundance, but this represents an artifact in that biomass data were only available for the profundal area of a shallow lake in Japan, where the fauna (oligochaetes) burrowed deeper than in other localities.

Based on the 80th percentile of depth distributions, and using the procedure outlined in the Methods section, we developed practical default values for the depth of the biotic zone in various habitats. These values, shown in Table 5, may be used for decisions related to ecological assessment or remediation in aquatic scenarios. The biotic zone, based on benthic abundance, in most estuarine and tidal freshwater environments is 10 or 15 cm. Exceptions are oligohaline and polyhaline mud (5 cm) and oligohaline sand (5 cm). In marine muds (both coastal and offshore), the biotic zone is 15 cm. In other marine substrates it is 10 cm (marine coastal mixed and marine offshore sand) or 5 cm (marine coastal sand). In lentic environments, the biotic zone is 15 cm. The biotic zone tends to be deeper when biomass is taken into account. The biotic zone in lotic systems varies from 15 to 35 cm depending upon water/habitat type. In areas populated by a high density of deep dwelling organisms such as those listed in Table 3, the biotic zone may be somewhat deeper than our recommended values.

2.6. DISCUSSION

Organisms in aerobic, sand or mixed (sand and mud) sediments of estuaries tend to penetrate deeper into the substrate than those in mud habitats (Dauer et al., 1987; Nilsen et al., 1982). Deep-dwelling species that exist in mud habitats either have a direct connection to the surface via a tube or permanent burrow, or are tolerant of high sulfide low oxygen conditions. In the present synthesis, in terms of benthic abundance, the practical default values for the biotic zone in estuarine muds range from 5 cm (oligohaline and polyhaline mud) to 10 cm (mesohaline mud), whereas in estuarine sands and estuarine mixed substrates the values range from 5 cm

(oligohaline sand) to 15 cm (polyhaline sand and oligohaline mixed substrate) (see Table 5). For most habitat types, the practical default values for the biotic zone are usually deeper when biomass is taken into account. For example, in mesohaline mud, the biotic zone in terms of biomass (25 cm) is relatively deep compared to the biotic zone in terms of abundance (10 cm) (see Table 5). This is largely due to the presence of bivalves such as *Macoma balthica*.

In our synthesis, the general trend of deeper penetration by the benthos in estuarine sands or mixed substrates versus mud is not evident in coastal and offshore environments. In coastal and offshore environments, factors in addition to sediment type may play an important role in determining faunal depth distributions. As one proceeds seaward into the marine coastal environment, the rate of deposition has a controlling effect on the depth distribution of the benthos, with depth penetration increasing with reduced deposition (Rhoads et al., 1985). Areas of the seafloor where sedimentation rates are $<< 4$ cm y^{-1} and where the frequency of physical resuspension or bedload transport is low, display sedimentary fabrics dominated by relatively large equilibrium species that commonly feed 'head down' at depth within the sediment (Rhoads et al., 1985).

With respect to lotic systems, a number of variables are of great importance in determining the depth of the biotic zone. These include dissolved oxygen, quantity of fines (less than 1-2 mm-size grains), and porosity. The lack of pore space at depth can be a barrier to penetration of the sediment by benthos Where fines are of sufficient quantity, they can reduce pore space and lead to clogging of the interstices, or, colmation (Meidl and Schönborn, 2004; Weigelhofer and Waringer, 2003). This makes the sediment too dense to provide living space or to support necessary water exchange between the channel and the hyporheic zone and between the groundwater and the hyporheic zone (Findlay, 1995). In the current synthesis, the greater depth of penetration of benthos in *stream coarse grained/sand without fines*—versus *with substantial* quantities *of fines*—is probably due in part to greater porosity in the former. A similar pattern of greater depth penetration in porous habitats has been noted by McElravy and Resh (1991) and Maridet et al. (1992). It is interesting to note that the more porous *coarse grained/sand without fines* category in our synthesis is comprised mainly of higher order reaches (see Table 4).

2.7. RECOMMENDATION

Ideally, to determine the depth of the biotic zone at a specific location, it is best to use data derived from sampling that area. The depth of bioturbation and the degree of contact between biota and sediment/pore water is influenced by the life habits of the resident organisms (e.g., degree of motility, creation of temporary versus permanent burrows, whether tubiculous or

not), and their local environment. Clarke et al. (2001) noted that in making site-specific bioturbation depth estimates, it is advisable to obtain the opinions of local experts in benthic ecology. Where data/expertise are not available, the recommendations in this paper (see Table 5) can serve as guidelines for determining the depth of the biotic zone. When considering the biotic zone depth in the design of a cap for isolating contaminated sediments from the overlying water column, the thickness of the cap should exceed the depth of the biotic zone by a safety margin (sensu Brannon et al., 1986). In areas populated by a high density of deep-dwelling organisms such as those in Table 3, the biotic zone may be somewhat deeper than the values shown in Table 5.

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Table 3. Examples of Deep-Burrowing and/or Feeding Benthos

Faunal Group/Species	Sediment Depth (cm)	Reference	Comments[a]
Annelids (Polychaetes)			
Clymenella torquata	to 30	Rhoads (1967); Mangum (1964); Mach et al. (2012); Nilsen et al. (1982)	Atlantic and Gulf coasts of North America; introduced to coasts of British Columbia (Canada), Washington (USA) and United Kingdom; muddy sand to sand; IT, ST[a]
Clymenella mucosa	to 15-20	Mangum (1964)	North Carolina to Florida (USA); Gulf of Mexico; Caribbean Sea; prefers fine to medium sands; IT, ST
Macroclymene zonalis (formerly in genus *Clymenella*)	to 25	Dauer et al. (1987); Moretzsohn et al. (2015); Mangum (1964)	Maine to Florida, USA; Gulf of Mexico; medium to fine sand; ST
Axiothella rubrocincta	to 30	Kudenov (1978)	British Columbia, Canada south to Mexico and Gulf of California; IT, ST
Sabaco elongatus (formerly *Asychis elongata*)	to 50	Caffrey (1995); Light (1974); Nichols (1979); Read (2015)	Maine to Florida, USA; Gulf of Mexico; Belize; introduced to San Francisco Bay, California (USA), where can occur in dense patches; mud and sandy mud; IT, ST
Maldane sarsi	to 21-25	Blanchard and Knowlton (2013); WoRMS (2015a)	cosmopolitan; IT, ST
Paraonis fulgens	to 20	D'Andrea et al. (2004); Gaston et al. (1992); WoRMS (2015b)	widely distributed in N Atlantic; marine, estuarine; sand; IT, ST
Heteromastus filiformis	to 20-35	Nilsen et al. (1982); Hines and Comtois (1985); Frey (1970); Cadee (1979)	cosmopolitan; marine, estuarine (polyhaline, mesohaline); mud to muddy sand; IT, ST
Notomastus tenuis	to 26	Johnson (1967); Garcia-Garza et al. (2012)	eastern N Pacific from California through Washington, USA; bays, estuaries; IT, shallow ST
Notomastus latericeus	to 20	Swift (1993); Mayhew (2005)	cosmopolitan; sand or muddy sand; low IT to deep ST
Arenicola marina	to 20-40	Cadee (1976); Luttikhuizen and Dekker (2010); Longbottom (1970); Tyler-Walters (2008)	western N Atlantic (Greenland, Bay of Fundy to Long Island); eastern N Atlantic; estuarine, marine; common in fine sand or muddy sand; predominantly IT
Arenicola cristata	to 30+	Lippson and Lippson (2006); Kaplan (1988)	western N Atlantic from Cape Cod to Florida (USA), Gulf of Mexico, Caribbean Sea; marine, estuarine (polyhaline, mesohaline); IT
Arenicola defodiens	to 40-70	Cadman (1997); Luttikhuizen and Dekker (2010)	eastern N Atlantic: British Isles; western Wadden Sea, North Sea; Skagerrak; high-energy low IT and ST

Table 3. Examples of Deep-Burrowing and/or Feeding Benthos (continued)

Faunal Group/Species	Sediment Depth (cm)	Reference	Comments
Abarenicola pacifica	to 20	Krager and Woodin (1993); Rudy and Rudy (1983); Hobson (1967)	N Pacific: Alaska to N California (USA); Japan; muddy sand of coastal bays; predominantly IT
Abarenicola claparedi vagabunda	to 30	Healy and Wells (1959)	Eastern N Pacific: Washington (USA); loose clean sand; low IT
Amphitrite ornata	to 30	Aller and Yingst (1978); WoRMS (2015c); Lippson and Lippson (2006)	western N Atlantic, including Cobscook Bay and Gulf of Maine; marine, estuarine (polyhaline); IT, ST
Lanice conchilega (sand mason)	to 20+	Van Hoey et al. (2006); Ager (2008); de Kluijver et al. (2000a)	Arctic to Mediterranean, Persian Gulf; Pacific; marine, estuarine (polyhaline); sand or muddy sand; IT,ST
Thoracophelia mucronata (formerly in genus *Euzonus*)	to 20	Kozloff (1993); Dales (1952)	Vancouver Island, BC, Canada to Baja California (Punta Banda region), Mexico; sand beaches experiencing fairly heavy surf; IT
Bhawania heteroseta (formerly in genus *Paleanotus*)	to 20	Dauer et al. (1987); Perkins (1985)	W Atlantic from Virginia, USA to Gulf of Mexico; sandy estuarine and marine; ST
Cirriformia moorei	to 22	Ronan et al. (1981) (as *C. spirabrancha*); Light and Carlton (2007)	California, USA; mudflats of estuaries and bays, often associated with eelgrass beds; low IT, ST
Scoletoma zonata (formerly in genus *Lumbrineris*)	to 22	Johnson (1967); Rudy and Rudy (1983)	Alaska to W Mexico; marine, estuarine; IT, ST
Glycera americana	to 40	Nilsen et al. (1982)	prefers mud mesohaline to polyhaline
Glycera dibranchiata	to 40	Nilsen et al. (1982)	wide range of sediments, mesohaline to polyhaline
Nereis succinea	to 40	Nilsen et al. (1982)	wide range of sediments and salinities
Alitta virens (formerly in genus *Nereis*)	to 40	Andersen and Kristensen (1991); Creaser et al. (1983); Glasby (2015)	western N Atlantic: Gulf of St. Lawrence, Canada to Virginia, USA; Iceland; eastern N Atlantic: Norway, North Sea, France, Ireland; White Sea, Russia; IT, ST
Hediste diversicolor (formerly in genus *Nereis*)	to 15-20	Reise (1981); Budd (2008)	Widespread along eastern N Atlantic including Baltic Sea, North Sea, Mediterranean Sea; euryhaline; IT
Chaetopterus cf. *variopedatus* (formerly *C. pergamentaceus*)	to 15+	Thompson and Schaffner (2000, 2001)	W Atlantic from NE USA to Florida; marine, estuarine; IT, ST
Spiochaetopterus costarum oculatus	to 15+	Woodin (1981); Bhaud (1998); Barnes (1964)	W Atlantic from Massachusetts, USA to Gulf of Mexico; IT, ST

Table 3. Examples of Deep-Burrowing and/or Feeding Benthos (continued)

Faunal Group/Species	Sediment Depth (cm)	Reference	Comments
Mesochaetopterus taylori	to 30	Sendall et al. (1995)	eastern N Pacific from British Columbia, Canada to Mexico; muddy sand and among roots of eel grass; IT
Marenzelleria neglecta	to 35	Zettler et al. (1995) (as *M. viridis*); Sikorski and Bick (2004); Bastrop et al. (1998)	Baltic Sea; North Sea (Elbe estuary); Arctic (Northwest Territories, Canada); western N Atlantic from Chesapeake Bay to Georgia, US; predominantly oligohaline to mesohaline; ST
Marenzelleria viridis (formerly in genus *Scolecolepides*)	to 30	Essink and Kleef (1988); Sikorski and Bick (2004); Blank et al. (2008)	North Sea; Baltic Sea; western N Atlantic from Nova Scotia, Canada to Cape Henlopen, Delaware and Chesapeake Bay, US; predominantly mesohaline to polyhaline; IT, ST
Pseudeurythoe ambigua	to 40	Nilsen et al. (1982)	wide range of sediments, mesohaline to polyhaline
Sigambra tentaculata	to 30	Nilsen et al. (1982)	muddy sands mesohaline to polyhaline
Diopatra cuprea	to 50-60	Mangum et al. (1968)	U.S. Atlantic and Gulf of Mexico coasts; IT; builds sand and mucous tube
Onuphis microcephala	to 45	Frey and Howard (1969)	low IT, shallow ST
Scalibregma inflatum	to 30-60	Ashworth (1901)	cosmopolitan; ST
Annelids (Tubificid oligochaetes)			
Various	to 20	McCall and Tevesz (1982)	mainly freshwater
Various	to 30	Reinharz and O'Connell (1983)	estuarine
Tubificoides spp.	to 25	Hines and Comtois (1985)	estuarine/marine
Phoronids			
Phoronopsis harmeri	to 20	Johnson (1967)	mostly intertidal, in tubes
Phoronis spp.	to 20	Nilsen et al. (1982)	sand polyhaline
Nemertea (ribbon worms)			
Cerebratulus lacteus	to 50	Nilsen et al. (1982); Frey (1970)	prefers mud mesohaline to polyhaline; IT, shallow ST
Bivalves (Unionid, or freshwater mussels)			
Elliptio complanata	to 20	Amyot and Downing (1991); Fisher & Tevesz (1976)	Eastern North America lotic and lentic systems; abundant in shallow (< 3 m) waters; those at depth in sediment are significantly smaller than those that are epibenthic
Unio tumidus	to 20	Schwalb and Pusch (2007); Van Damme (2011a)	Europe (widely distributed); lowland fresh waters

Table 3. Examples of Deep-Burrowing and/or Feeding Benthos (continued)

Faunal Group/Species	Sediment Depth (cm)	Reference	Comments
Unio pictorum	to 20	Schwalb and Pusch (2007); Van Damme (2011b)	Widely distributed throughout Europe and Russia; lowland fresh waters
Unio crassus	to 30-35	Schwalb and Pusch (2007); Schultes (2010)	Europe except Iberian Peninsula and British Isles, to Black Sea region and Iraq; sandy and stony substrate of lowland clean rivers and smaller running waters
Anodonta anatina	to 20	Schwalb and Pusch (2007); Lopes-Lima (2014)	N Europe and Asia, below 65 degrees, to Sicily and Turkey; sandy and gravel substrate of lotic and lentic systems
Bivalves (other)			
Macoma balthica	to 30	Hines and Comtois (1985); Schaffner et al. (1987)	important at mesohaline mud and sandy mud sites; burrowing depth varies with shell size
Macoma mitchelli	to 20	Reinharz and O'Connell (1983)	mesohaline, all sediment types
Macoma nasuta	to 10-20	Ricketts et al. (1985)	Eastern N Pacific; IT
Solecurtus strigilatus	to 27	Dworschak (1987a)	Adriatic Sea; eastern N Atlantic from Portugal to Senegal; IT, ST
Tagelus plebeius	to 40+	Frey (1968); Frey (1970); Lippson and Lippson (2006)	Massachusetts to S Florida (USA); Gulf of Mexico; marine, estuarine (polyhaline, mesohaline); mixed mud-sand; IT, ST
Tagelus divisus	to 30	Frey (1968); Lippson and Lippson (2006)	Massachusetts to S Florida (USA); Gulf of Mexico; Caribbean; marine, estuarine (polyhaline); prefers sand or muddy sand; shallow ST
Tagelus californianus	to 50	Ricketts et al. (1985); Morris et al. (1980)	Eastern N Pacific: Humboldt Bay, CA (USA) to Panama; IT
Zirfaea pilsbryi	to 50	Morris et al. (1980)	Alaska to Baja California, Mexico; bays, estuaries, occasionally open coast; heavy mud, sticky clay, soft shale; low IT, ST
Ensis directus	to 20	Nilsen et al. (1982); Gollasch, et al. (2015)	western N Atlantic: Labrador, Canada to South Carolina, USA; eastern N Atlantic (introduced): Spain to Norway, including UK, and western Baltic; marine, estuarine (polyhaline); prefers fine-medium sand; IT, ST
Ensis ensis	to 54	Keegan and Konnecker (1973) (as *Solen ensis*); Von Cosel (1990); de Kluijver et al. (2000b)	eastern N Atlantic: North Sea and British Isles to Portugal and Mediterranean; sand; IT, ST
Ensis siliqua	to 60	Gaspar et al. (1998); de Kluijver et al. (2000c)	eastern N Atlantic: Norway to the Mediterranean; sand; IT, ST

Table 3. Examples of Deep-Burrowing and/or Feeding Benthos (continued)

Faunal Group/Species	Sediment Depth (cm)	Reference	Comments
Solen rostriformis	to 30	Morris et al. (1980) (as *S. rosaceus*); Light and Carlton (2007)	eastern N Pacific from Morro Bay, California (USA) to Mazatlan, Mexico; protected bays; sandy mud; low IT
Solen sicarius	to 30-35	Morris et al. (1980)	eastern N Pacific from Vancouver Island BC, Canada to Baja California, Mexico; sheltered bays, especially in beds of eelgrass; low IT, shallow ST
Mya arenaria (soft-shelled clam)	to 30-40	Hines and Comtois (1985); Zwarts and Wanink (1989); Kondo (1987)	eastern N Pacific; both sides of Atlantic; burrowing depth varies with shell size; marine, estuarine (polyhaline, mesohaline) soft sediments; IT, ST
Lucinoma borealis	to 20	Dando et al. (1986)	NE Atlantic; Mediterranean Sea; low IT, ST
Nuttallia nuttallii	to 30-40	Morris et al. (1980)	eastern N Pacific from Bodega Bay Harbor, California (USA) to Baja California Sur, Mexico; outer coast and in bays with strong tidal currents; sand or gravel; low IT
Nuttallia obscurata	to 30	Fofonoff et al. (2003)	western N Pacific (native): Russia, Japan, China; eastern N Pacific (introduced): Strait of Georgia (Canada) to Puget Sound, Willapa Bay and Coos Bay, Oregon (USA); prefers estuaries (mesohaline, polyhaline) but also marine; IT, shallow ST
Saxidomus gigantea (butter clam)	to 35	Cowles (2005a); Cheney and Mumford (1986)	eastern N Pacific: Aleutian Islands and SE Bering Sea, Alaska to San Francisco Bay; prefers sandy or gravelly substrate with mixed shell; IT, ST
Tresus nuttallii	to 100	Ricketts et al. (1985)	eastern N Pacific; IT
Tresus capax	to 100	Cowles (2005b)	eastern N Pacific from Kodiak Island, Alaska to central California USA; bays, occasionally open coast; mud; IT, ST
Panopea generosa (geoduck)	to 30-100	Willner (2006); Goodwin and Pease (1989); Gosling (2015)	N Pacific: Alaska to Baja California, Mexico; Japan; very abundant in Puget Sound, Washington and British Columbia; burrowing depth is age-dependent (1-yr to 30 cm depth; 10-yr to 90 cm); sand or sand-mud substrates; ST, IT
Panopea zelandica	to 30-45	Ministry for Primary Industries (2013)	New Zealand: North, South and Stewart Islands; ST
Cyrtopleura costata (angel wing)	to 60+	Schaffner et al. (2001); Gustafson et al. (1991); Lippson and Lippson (2006)	western Atlantic from Massachusetts, USA to Brazil; marine, estuarine (polyhaline, mesohaline); sandy mud; low IT, shallow ST

Table 3. Examples of Deep-Burrowing and/or Feeding Benthos (continued)

Faunal Group/Species	Sediment Depth (cm)	Reference	Comments
Insects (Chironomid larvae)			
Chironomus plumosus	to 15	McCall and Tevesz (1982)	lakes
Insects (mayfly larvae)			
Hexagenia limbata	to 20	Matisoff and Wang (1998)	lakes
Insects (beetle)			
Bledius spp	to 40	Wyatt and Foster (1991)	intertidal salt marshes; around lakes/salt lakes and in river banks
Crustaceans (Thalassinidean shrimp)			
Callianassa subterranea	to 86+	Nickell and Atkinson (1995)	North Sea; ST
Callianassa truncata	to 60-70	Kristensen and Kostka (2005); Ziebis et al. (1996)	Mediterranean Sea; sandy sediments; ST
Callichirus major[*]	to 215	Griffis and Suchanek (1991); Heard et al. (2007)	SE USA; Gulf of Mexico; Brazil; open beaches; primarily IT, but also shallow ST
Callichirus islagrande[*]	to 50	Felder and Griffis (1994)	N Gulf of Mexico; sandy beaches facing higher salinity (≥ 15 ppt) embayments and the Gulf; IT, shallow ST
Callichirus kraussi[*]	to 30+	Griffis and Suchanek (1991)	S Africa; IT, ST
Callichirus laurae (formerly in genus *Glypturus*)	to 150	Whitehead et al. (1988); Griffis and Suchanek (1991)	Red Sea; sand or coral sand, sometimes with seagrass cover; IT, ST
Neocallichirus grandimana[*] (formerly *Callianassa branneri*)	to 36	Dworschak and Ott (1993)	W Atlantic from Florida, USA to Brazil; protected back-reef sands; IT, shallow ST
Neocallichirus rathbunae[*]	to 150	Griffis and Suchanek (1991); Abed-Navandi (2000)	subtropical and tropical western Atlantic; carbonate sediments; ST, IT
Neocallichirus jousseaumei[*]	to 90	Griffis and Suchanek (1991); Dworschak (2011)	widely distributed in Indo-W Pacific; coral rubble covered by fine sand; IT, ST
Trypaea australiensis[*]	to 100+	Webb and Eyre (2004)	E and SE Australian estuaries; prefers sand flats; IT, ST
Neotrypaea californiensis[*]	to 75	Hornig et al. (1989); Campos et al. (2009)	Alaska, USA to W coast Baja California Sur, Mexico; prefers sand; IT
Neotrypaea gigas[*]	to 40	Griffis and Suchanek (1991); Campos et al. (2009)	Vancouver Island, Canada to W coast Baja California Sur, Mexico; prefers muddy sand; IT
Sergio guassutinga (formerly in genus *Neocallichirus*)	to 60	Griffis and Suchanek (1991); Manning and Felder (1995)	Brazil; IT

Table 3. Examples of Deep-Burrowing and/or Feeding Benthos (continued)

Faunal Group/Species	Sediment Depth (cm)	Reference	Comments
Sergio trilobata[*]	to 90	Dobbs and Guckert (1988); Manning and Lemaitre (1993)	Gulf coast of Florida, USA; IT, ST
Pestarella tyrrhena[*]	to 62	Dworschak (1987b, 2004)	Adriatic Sea; eastern N Atlantic; IT, shallow subtidal
Pestarella candida[*]	to 65	Dworschak (2002)	Adriatic Sea; IT, ST
Pestarella whitei[*]	to 28+	Dworschak (2002)	Adriatic Sea; coarse sand or mud under stones; IT, shallow ST
Lepidophthalmus louisianensis[*]	to 250	Griffis and Suchanek (1991); Felder and Griffis (1994)	N Gulf of Mexico; muddy shorelines of low salinity (10-15 ppt) estuaries; IT, shallow ST
Lepidophthalmus sinuensis	to 50	Felder and Griffis (1994); Nates & Felder (1999)	estuaries on Caribbean coast of Colombia; IT, ST
Biffarius filholi[*]	to 45	Griffis and Suchanek (1991); Berkenbusch and Rowden (2000)	New Zealand; IT and shallow ST
Biffarius arenosus[*]	to 58	Bird and Poore (1999)	E and SE Australia; sand and mud flats; IT, ST
Corallianassa longiventris	to 150	Griffis and Suchanek (1991); Dworschak et al. (2006)	W Atlantic from Bermuda to Brazil; back-reef sediments near seagrass beds; ST
Corallianassa coutierei[*]	to 69	Kneer et al (2008); Sepahvand et al. (2013)	Indo-W Pacific; carbonate sand and coral rubble; IT, ST
Nihonotrypaea japonica[*]	to 65	Tamaki and Ueno (1998); Tamaki et al. (1999)	Japan; polyhaline, extensive sandflats of medium-fine sands; IT
Nihonotrypaea harmandi[*]	to 36+	Tamaki and Ueno (1998); Tamaki et al. (1999)	Japan; euhaline, small to medium sandflats and beaches of medium-fine sands; IT
Glypturus acanthochirus	to 160	Griffis and Suchanek (1991); Dworschak and Ott (1993)	Florida, Virgin Islands, Belize; bare sediments of mangrove channels and back-reef subtidal sediments; IT, ST
Glypturus armatus	to 150	Griffis and Suchanek (1991)	S Pacific; Aldabra; Seychelles; sheltered reef sediments; IT, ST
Calocaris macandreae	to 22	Nash et al. (1984)	North Sea; ST
Neaxius acanthus	to 50	Kneer et al. (2008)	Indo-W Pacific; carbonate sand and coral rubble with seagrass cover; ST
Upogebia affinis	to 50	Heard et al. (2007)	Massachusetts to S Texas, USA; firm mud or mud-sand substrates; IT, ST
Upogebia deltaura	to 65	Tunberg (1986); Christiansen (2000)	eastern N Atlantic; North Sea; ST
Upogebia pugettensis	to 90	Griffis and Suchanek (1991); Campos et al. (2009)	Alaska to Morro Bay California, USA; IT
Upogebia stellata	to 26.5	Nickell and Atkinson (1995)	North Sea; ST

Table 3. Examples of Deep-Burrowing and/or Feeding Benthos (continued)

Faunal Group/Species	Sediment Depth (cm)	Reference	Comments
Upogebia pusilla	to 80	Dworschak (1987b, 2004)	Mediterranean Sea; eastern N Atlantic; IT, ST
Upogebia africana	to 60	Griffis and Suchanek (1991)	S Africa; IT, ST
Upogebia tipica	to 40	Griffis and Suchanek (1991)	Adriatic Sea; ST
Upogebia macginitieorum	to 60	Griffis and Suchanek (1991); Campos et al., (2009)	S California, USA to Baja California Sur, Mexico
Upogebia major	to 208	Kinoshita (2002)	Japan; IT
Jaxea nocturna	to 92	Nickell and Atkinson (1995); Pervesler and Dworschak (1985)	North Sea; Adriatic Sea; ST
Axiopsis serratifrons	to 100	Griffis and Suchanek (1991); Kensley (1980)	Circumtropical; back-reef areas; ST
Axianassa australis	to 130	Dworschak and Rodrigues (1997); Felder et al. (2009)	western Atlantic from Florida USA to Brazil, including Gulf of Mexico and Colombia; muddy sand or mud near mangroves; IT
Crustaceans (snapping shrimp)			
Alpheus heterochaelis	to 100	Howard and Frey (1975); McClure (1995)	widespread throughout temperate and tropical W Atlantic; bays and quiet waters; IT, shallow ST
Alpheus floridanus (a species complex)	to 36	Dworschak and Ott (1993); Soledatde and Almeida (2013)	W Atlantic: S Florida USA, Bahamas, Mexico, West Indies, Brazil; IT, ST
Crustaceans (mantis shrimp)			
Squilla empusa	to 15-50	Myers (1979); Mead and Minshall (2012); Lippson and Lippson (2006)	winter burrows up to 410 cm depth; western N Atlantic from Cape Cod to Gulf of Mexico; silty substrates; low IT, ST
Squilla mantis	to 31	Atkinson and Froglia (1999); Ragonese et al. (2012)	Mediterranean Sea and eastern Atlantic from Gulf of Cadiz to Angola; soft substrates; ST
Lysiosquilla scabricauda	to 150	Bieler and Mikkelsen (1988); Foster et al. (2004)	W Atlantic, from South Carolina USA to S Brazil, including Gulf of Mexico, Caribbean, Bahamas, Bermuda; IT, ST
Crustaceans (ghost crabs)			
Ocypode quadrata	to 100+	Pombo and Turra (2013); Knott (2010)	W Atlantic from Rhode Island USA to Brazil, including Gulf of Mexico and Caribbean; upper intertidal to fore dunes of sandy beaches

Table 3. Examples of Deep-Burrowing and/or Feeding Benthos (continued)

Faunal Group/Species	Sediment Depth (cm)	Reference	Comments
Crustaceans (fiddler crabs)			
Uca pugilator (Atlantic sand fiddler)	to 34	Christy (1982)	Massachusetts to Texas, USA; sandy upper intertidal and supratidal substrates in tidal marshes, bays and sounds
Uca pugnax (Atlantic marsh fiddler)	to 15-25	Montague (1980); Bergey and Weis (2008)	Massachusetts to Florida, USA; muddy intertidal substrates in salt marshes in sheltered bays and estuaries
Uca minax (red-jointed fiddler)	to 30-65	Montague (1980); Powers (1977)	Massachusetts to NE Florida, USA; Gulf of Mexico; freshwater or brackish water tidal marshes, often supratidal
Crustaceans (other crabs)			
Helice tridens	to 40	Takeda and Kurihara (1987)	Japan; salt marsh
Neohelice granulata (formerly in genus *Chasmagnathus*)	to 33	Iribarne et al. (1997)	SW Atlantic; mud flats and marshes (deepest burrows in vegetated marshes)
Sesarma reticulatum (marsh crab)	to 30+	Koretsky et al. (2002); Abele (1992)	eastern North America and Gulf of Mexico salt and brackish marshes; IT
Eurytium limosum	to 30	Koretsky et al. (2002); Felder et al. (2009)	W Atlantic from New York, USA to Brazil; Gulf of Mexico; Caribbean Sea; vegetated and unvegetated salt marshes; IT
Crustaceans (lobsters)			
Nephrops norvegicus (Norway Lobster)	to 25	Rice and Chapman (1971)	
Homarus americanus (American lobster)	to 60-80	Cooper and Uzmann (1980)	western N Atlantic from Labrador, Canada to North Carolina, USA; ST
Crustaceans (crayfish)			
Cambarus diogenes (devil crawfish)	to 457	Hobbs and Hart (1959); Hobbs (1989); Cordeiro et al. (2010)	widespread east of the Rockies and south of Great Lakes, except peninsular Florida and the Alleghenies (USA); Ontario, Canada; ponds and streams in spring season; burrows in banks of streams
Procambarus clarkii (red swamp crayfish)	to 70	Oluoch (1990); Hobbs (1989); FAO (2007)	N Mexico to Escambia County Florida, and north to S Illinois and Ohio; widely introduced elsewhere; sluggish waters of lentic and lotic habitats
Crustaceans (amphipods)			
Pseudohaustorius caroliniensis	to 20-30	D'Andrea et al. (2004)	IT

Table 3. Examples of Deep-Burrowing and/or Feeding Benthos (continued)

Faunal Group/Species	Sediment Depth (cm)	Reference	Comments
Echinoderms (Holothurians or sea cucumbers)			
Pseudocucumis mixta	to 15-25	Konnecker and Keegan (1973)	W coast Ireland
Holothuria arenicola	to 15-20	Mosher (1980)	circumtropical
Molpadia oolitica	to 20	Rhoads and Young (1971); Pawson et al. (2010)	western N Atlantic from Massachusetts to Florida (USA); Gulf of Mexico; mud; ST
Molpadia intermedia	to 35	Lambert (1997)	eastern N Pacific from Kodiak Island, Alaska to Gulf of Panama; mud; ST
Echinoderms (heart urchins)			
Echinocardium cordatum	to 15-20	Rees and Dare (1993); Kroh (2015)	cosmopolitan; typically sand or muddy sand; mainly ST
Cnidarians (anthozoans)			
Ceriantheopsis americanus	to 60+	Nilsen et al. (1982); Frey (1970)	IT, shallow ST
Pachycerianthus fimbriatus	to 100	Light and Carlton (2007); Cowles (2010)	S Alaska to Baja California, Mexico; predominantly in very soft mud; ST, rarely IT
Sipunculids (peanut worms)			
Golfingia elongata	to 40	Keegan (1974); Cutler (1994); de Kluijver et al. (2000d)	widespread: western and eastern N Atlantic, including Mediterranean; Pacific (East and South China Seas); muddy sand or gravel; low IT, ST
Golfingia vulgaris	to 30-50	Swift (1993); de Kluijver et al. (2000e)	widespread but patchy distribution: N Atlantic from Greenland and northern Norway to W Africa and eastern Mediterranean; Indo-West Pacific region; Antarctic; muddy sand or gravel; low IT to several hundred meters
Sipunculus nudus (a species complex)	to 15-35	Volkel and Grieshaber (1992); Kawauchi and Giribet (2014); de Kluijver et al. (2000f)	cosmopolitan; low IT, ST
Echiuran worms			
Maxmuelleria lankesteri	to 80	Hughes et al. (1996)	widespread around British and Irish coasts, most commonly in fine muds
Urechis caupo (fat innkeeper worm)	to 36+	Julian et al. (2001); Arp et al. (1992)	California, USA; mudflats; IT, ST
Echiurus echiurus	to 50	Anker et al. (2005); Pilger and Murina (2015); Ricketts et al. (1985)	widely distributed in the arctic, both in northern part of N. Atlantic and in N. Pacific, as far south as 45° N Latitude; IT, ST

Table 3. Examples of Deep-Burrowing and/or Feeding Benthos (continued)

Faunal Group/Species	Sediment Depth (cm)	Reference	Comments
Enteropneusts (acorn worms)			
Balanoglossus gigas	to 30	Bjornberg (1959); van der Land (2015)	W Atlantic from Georgia, USA to SE Brazil, Gulf of Mexico; Greater Antilles; IT
Balanoglossus aurantiaca (= *B. aurantiacus*)	to 60	Duncan (1987); Frey 1970); Konikoff et al. (2015)	W North Atlantic; IT, shallow ST
Balanoglossus clavigerus	to 60	Bromley (1996)	Mediterranean Sea; British Isles; IT
Balanoglossus australiensis	to 20-25	Morton (1950); Konikoff and van der Land (2015)	Gulf of Carpentaria; New Zealand; New South Wales, Australia; Solomon Sea, Great Barrier Reef; fine sand; IT, ST
Saccoglossus kowalevskii	to 25-40	Carey and Farrington (1989); Smith et al. (2003)	Georgia to Maine, USA; IT, shallow ST
Saccoglossus horsti	to 10-20	Burden-Jones (1951)	The Solent, UK; IT
Saccoglossus ruber (synonymised with *S. cambrensis*)	to 5-25	Knight-Jones (1953); Burdon-Jones and Patil (1960)	Welsh coast; W coast Ireland; IT

[a]Intertidal and subtidal represented by IT and ST, respectively.

[*]formerly in genus *Callianasssa*

Table 4. Data Sources and Information (Realms/Ecoregions after Spalding et al., 2007 [marine] and Abell et al., 2008 [freshwater]) used to Determine 80th Percentile of Benthic Abundance (see Figure 3) and Benthic Biomass (see Figure 4) Depth Distributions. Abundance and biomass data denoted by A and B, respectively. *N* = number of datasets. (The total number of cores comprising datasets from each habitat type/reference pair is noted in parentheses.)

Habitat Type	Reference	*N* (Total Cores)	Location	Sampler; Sample Area and Depth; Sieve Size	Realm/ Ecoregion(s)
Estuarine Intertidal					
Intertidal Mixed	Mermillod-Blondin et al. (2003) (A)	1(3)	St. Lawrence Estuary, Canada	Cylindrical tube; 78.5 cm^2 by 20 cm; 0.5 mm	Temperate N. Atlantic/Gulf of St. Lawrence-Eastern Scotian Shelf
Intertidal Sand	Johnson (1967) (A)	4(32)	White Gulch and Lawsons Flat, Tomales Bay, California, USA	Brass coring tube; 25 cm^2 by 25 cm; core dissected	Temperate N. Pacific/ Northern California
	Rodil et al. (2008) (A)	18(54)	Sheltered beach on inner part of Ria of Arousa on NW coast of Iberian Peninsula, Spain	Metal core; 188.7 cm^2 by 25 cm; 1 mm	Temperate N. Atlantic/ South European Atlantic Shelf
	D'Andrea et al. (2004) (A)	4(12)	Debidue Flat, South Carolina, USA	Core; 38.5 cm^2 by 30 cm; 0.5 mm	Temperate N. Atlantic/ Carolinean
Intertidal Poikilohaline Mixed	Mucha et al. (2004) (A)	1(3)	Douro Estuary, Portugal	Core sampler; 35 cm^2 by 15 cm; 0.5 mm	Temperate N. Atlantic/ South European Atlantic Shelf
Intertidal Poikilohaline Sand	Mucha et al. (2004) (A)	4(12)	Douro Estuary, Portugal	Core sampler; 35 cm^2 by 15 cm; 0.5 mm	Temperate N. Atlantic/ South European Atlantic Shelf
Tidal Freshwater					
Tidal Freshwater Mixed	Dauer et al. (1987) (A,B)	1(3)	Lower Chesapeake Bay tributaries (James, York and Rappahanock rivers), USA	Box corer; 184 cm^2 by 25 cm; 0.5 mm	Temperate N. Atlantic/ Virginian
	Schaffner et al. (1987) (A,B)	3(3)	James River Estuary (Chesapeake Bay Tributary), USA	Spade box corer; 600 cm^2 by 50 cm; 0.5 mm	Temperate N. Atlantic/ Virginian

Table 4. Data Sources and Information (Realms/Ecoregions after Spalding et al., 2007 [marine] and Abell et al., 2008 [freshwater]) used to Determine 80th Percentile of Benthic Abundance (see Figure 3) and Benthic Biomass (see Figure 4) Depth Distributions (continued).

Habitat Type	Reference	*N* (Total Cores)	Location	Sampler; Sample Area and Depth; Sieve Size	Realm/ Ecoregion(s)
Estuarine Subtidal					
Oligohaline Mixed	Schaffner et al. (1987) (A,B)	2(2)	James River Estuary (Chesapeake Bay Tributary), USA	Spade box corer; 600 cm^2 by 50 cm; 0.5 mm	Temperate N. Atlantic/Virginian
	Reinharz and O'Connell (1983) (A,B)	2(4)	Upper Chesapeake Bay	Spade box corer; 630 cm^2 by up to 60 cm; 0.5 mm	Temperate N. Atlantic/Virginian
Oligohaline Mud	Schaffner et al. (1987) (A,B)	1(1)	James River Estuary (Chesapeake Bay Tributary), USA	Spade box corer; 600 cm^2 by 50 cm; 0.5 mm	Temperate N. Atlantic/Virginian
	Reinharz and O'Connell (1983) (A,B)	1(3)	Upper Chesapeake Bay	Spade box corer; 630 cm^2 by up to 60 cm; 0.5 mm	Temperate N. Atlantic/Virginian
Oligohaline Sand	Reinharz and O'Connell (1983) (A,B)	2(3)	Upper Chesapeake Bay	Spade box corer; 630 cm^2 by up to 60 cm; 0.5 mm	Temperate N. Atlantic/Virginian
Mesohaline Mixed	Schaffner et al. (1987) (A,B)	2(2)	James River Estuary (Chesapeake Bay Tributary), USA	Spade box corer; 600 cm^2 by 50 cm; 0.5 mm	Temperate N. Atlantic/Virginian
	Reinharz and O'Connell (1983) (A,B)	2(8)	Central Chesapeake Bay	Spade box corer; 630 cm^2 by up to 60 cm; 0.5 mm	Temperate N. Atlantic/Virginian
Mesohaline Mud	Dauer et al. (1987) (A,B)	2(6)	Lower Chesapeake Bay tributaries (James, York and Rappahanock rivers), USA	Box corer; 184 cm^2 by 25 cm; 0.5 mm	Temperate N. Atlantic/Virginian
	Hines and Comtois (1985) (A,B)	1(10)	Mouth of Rhode River, Chesapeake Bay, USA	Scuba-collected cores; 80 cm^2 by 35 cm within 900 m^2 area; 0.5 mm	Temperate N. Atlantic/Virginian

Table 4. Data Sources and Information (Realms/Ecoregions after Spalding et al., 2007 [marine] and Abell et al., 2008 [freshwater]) used to Determine 80th Percentile of Benthic Abundance (see Figure 3) and Benthic Biomass (see Figure 4) Depth Distributions (continued).

Habitat Type	Reference	*N* (Total Cores)	Location	Sampler; Sample Area and Depth; Sieve Size	Realm/ Ecoregion(s)
	Schaffner et al. (1987) (A,B)	3(3)	James River Estuary (Chesapeake Bay Tributary), USA	Spade box corer; 600 cm^2 by 50 cm; 0.5 mm	Temperate N. Atlantic/Virginian
	Reinharz and O'Connell (1983) (A,B)	2(20)	Central Chesapeake Bay	Spade box corer; 630 cm^2 by up to 60 cm; 0.5 mm	Temperate N. Atlantic/Virginian
Mesohaline Sand	Hines and Comtois (1985) (A,B)	1(10)	Mouth of Rhode River, Chesapeake Bay, USA	Scuba-collected cores: 80 cm^2 by 35 cm within 900 m^2 area; 0.5 mm	Temperate N. Atlantic/Virginian
	Reinharz and O'Connell (1983) (A,B)	1(2)	Central Chesapeake Bay	Spade box corer; 630 cm^2 by up to 60 cm; 0.5 mm	Temperate N. Atlantic/Virginian
Polyhaline Mixed	Dauer et al. (1987) (A,B)	2(5)	Lower Chesapeake Bay tributaries and mainstem, USA	Box corer; 184 cm^2 by 25 cm; 0.5 mm	Temperate N. Atlantic/Virginian
	Nilsen et al. (1982)[a] (A)	6(6)	Lower Chesapeake Bay, USA	Spade box corer; 620 cm^2 by up to 50 cm; 0.5 mm	Temperate N. Atlantic/Virginian
Polyhaline Mud	Dauer et al. (1987) (A,B)	1(2)	Lower Chesapeake Bay Mainstem, USA	Box corer; 184 cm^2 by 25 cm; 0.5 mm	Temperate N. Atlantic/Virginian
	Nilsen et al. (1982)[b] (A)	3(3)	Lower Chesapeake Bay, USA	Spade box corer; 620 cm^2 by up to 50 cm; 0.5 mm	Temperate N. Atlantic/Virginian
Polyhaline Sand	Nilsen et al. (1982)[a] (A)	6(6)	Lower Chesapeake Bay, USA	Spade box corer; 620 cm^2 by up to 50 cm; 0.5 mm	Temperate N. Atlantic/Virginian
Lentic					
Lake Profundal Mud	Fukuhara et al. (1987) (A,B)	4 (8)	Profundal region of shallow lake (Suwa), Central Japan; tubificid oligochaetes (*Limnodrilus*)	Lenz grab; 225 cm^2 by 33 cm; 0.2 mm	Palearctic/Biwa Ko[c]

Table 4. Data Sources and Information (Realms/Ecoregions after Spalding et al., 2007 [marine] and Abell et al., 2008 [freshwater]) used to Determine 80th Percentile of Benthic Abundance (see Figure 3) and Benthic Biomass (see Figure 4) Depth Distributions (continued).

Habitat Type	Reference	*N* (Total Cores)	Location	Sampler; Sample Area and Depth; Sieve Size	Realm/ Ecoregion(s)
	Newrkia and Wijegoonawardana (1987) (A)	2 (14)	prealpine lake (Mondsee), Upper Austria; oligochaetes	Modified Kajak corer; 19.6 cm^2 by 20 cm; 0.2 mm	Palearctic/Upper Danube
	Cole (1953) (A)	2(90)	Douglas Lake, Michigan, USA; tubificid oligochaetes (*Limnodrilus*)	Small vertical core sampler; 3.8 cm^2 by 24 cm; 0.18 mm (upper 10 cm) – 0.21 mm (below 10 cm)	Nearctic/ Laurentian Great Lakes
	Milbrink (1973) (A)	4(15)	Lake Malaren and Lake Erken, Sweden; tubificid oligochaetes	Microstratification sampler; 167 cm^2 by up to 19 cm; 0.3 mm	Palearctic/N. Baltic Drainages
	Boyer and Whitlatch (1989) (A)	1(16)	Caribou Island Basin of Lake Superior; oligochaetes	Modified 225 cm^2 Eckman box corer; subcores 13.7 cm^2 by up to 16 cm; 0.3 mm	Nearctic/ Laurentian Great Lakes
	Sarkka and Paasivirta (1972) (A)	1(35)	Lake Paijanne, Finland; tubificid and lumbriculid oligochaetes	Lenz sampler; 260 cm^2 by 30 cm; 0.8 mm	Palearctic/N. Baltic Drainages
Lotic					
Stream Coarse Grained/Sand	James et al. (2008) (A)	6(24)	Three small streams, southern North Island, New Zealand	Hyporheic colonization chambers; 78.5 cm^2 by 40 cm; 0.5 mm	Australasia/New Zealand
	Omesová and Helešic (2007) (A)	1(10)	Loucka River, 4th-order stream, Czech Republic	Liquid nitrogen freeze cores; 19.6 cm^2 by 20 cm; 0.1 mm	Palearctic/Upper Danube
	Olsen and Townsend (2005) (A)	1(14)	Kye Burn, 4th-order stream, South Island, New Zealand	Liquid nitrogen freeze cores; 9.6 cm^2 by 50 cm; 0.25 mm	Australasia/New Zealand
	Olsen et al. (2001) (A)	3(18)	Kye Burn, South Island, New Zealand	Liquid nitrogen freeze cores; 9.6 cm^2 by 50 cm; 0.25 mm	Australasia/New Zealand

Table 4. Data Sources and Information (Realms/Ecoregions after Spalding et al., 2007 [marine] and Abell et al., 2008 [freshwater]) used to Determine 80th Percentile of Benthic Abundance (see Figure 3) and Benthic Biomass (see Figure 4) Depth Distributions (continued).

Habitat Type	Reference	*N* (Total Cores)	Location	Sampler; Sample Area and Depth; Sieve Size	Realm/ Ecoregion(s)
	Maridet et al. (1992) (A)	3(4)	Loire River (5th-order reach), Galaure (3rd-order reach) and Drac (alpine torrential stream, 3rd-order reach), France	Liquid nitrogen freeze cores with in situ electro-positioning; 19.6 cm^2 by 60 cm; macroinvertebrates separated by elutriation	Palearctic/Central and Western Europe
	Angradi et al. (2001) (A)	3(90)	2nd, 3rd and 4th-order reaches of Elklick Run at Fernow Experimental Forest, West Virginia, USA	Multilevel colonization samplers; 95 cm^2 by 30 cm; 0.25 mm	Nearctic/Teays-Old Ohio
Stream Coarse Grained/Sand with Fines	Angradi et al. (2001) (A)	1(30)	1st-order reach of Elklick Run at Fernow Experimental Forest, West Virginia, USA	Multilevel colonization samplers; 95 cm^2 by 30 cm; 0.25 mm	Nearctic/Teays-Old Ohio
	Strommer and Smock (1989) (A)	1(415)	1st-order stream in Blackwater River watershed, Virginia, USA	Cores frozen on dry ice; 18.1 cm^2 by up to 40 cm; 0.053 mm	Nearctic/ Appalachian Piedmont
	Winkelmann et al. (2003) (A)	2(12)	Two small 2nd-order mountain streams, Gauernitzbach and Tannichtgrundbach, that drain into the River Elbe, Germany	Liquid nitrogen freeze cores; 19.6 cm^2 by 30 cm; macroinvertebrates separated by hand-picking and elutriation	Palearctic/ Central and Western Europe
	Adkins and Winterbourn (1999) (A)	2(40)	Two upland streams, Middle Bush and Grasmere, South Island, New Zealand	Dry ice freeze cores; 9.6 cm^2 by 30 cm; 0.12 mm	Australasia/New Zealand
	Meidl and Schönborn (2004) (A)	4(20)	Schwarza Brook, low mountain stream in Thuringian Slate Mountains, Germany	Liquid nitrogen freeze cores with in situ electro-positioning; 19.6 cm^2 by 60 cm; macroinvertebrates separated by sorting	Palearctic/ Central and Western Europe

Table 4. Data Sources and Information (Realms/Ecoregions after Spalding et al., 2007 [marine] and Abell et al., 2008 [freshwater]) used to Determine 80th Percentile of Benthic Abundance (see Figure 3) and Benthic Biomass (see Figure 4) Depth Distributions (continued).

Habitat Type	Reference	*N* (Total Cores)	Location	Sampler; Sample Area and Depth; Sieve Size	Realm/ Ecoregion(s)
	Varricchione et al. (2005) (A)	4(54)	Glaciated stream sites (Montana; 2 data sets), and unglaciated stream sites (Idaho; 2 data sets), USA	Liquid nitrogen freeze cores with in-situ electro-positioning; 19.6 cm^2 by 50 cm; 0.063 mm	Glaciated: Nearctic/ Columbia Glaciated; Upper Missouri Unglaciated: Nearctic/ Columbia Unglaciated; Upper Snake; Bonneville
	McElravy and Resh (1991) (A)	5(40)	2nd-order reach of Big Canyon Creek, northern California Coast Range, USA	Substrate colonization samplers; 44.2 cm^2 by 35 cm; 0.063 mm	Nearctic/Sacramento-San Joaquin
	Maridet et al. (1996) (A)	3(35)	Three streams (Vianon, Ozange, Triouzoune) in French granitic Massif Central mountains, France	Liquid nitrogen freeze cores with in situ electro-positioning; 19.6 cm^2 by 60 cm; 0.5 mm	Palearctic/Cantabric Coast-Languedoc
	Weigelhofer and Waringer (2003) (A)	2(66)	3rd-order reach of the Weidlingbach, a tributary of the Danube, northwest of Vienna, Austria	Liquid nitrogen freeze cores with in situ electro-positioning; 19.6 cm^2 by 60 cm; 0.1 mm	Palearctic/Upper Danube
	Marchant (1988) (A)	1(17)	Thomson River, 10 km downstream of Thomson Dam, Victoria, Australia	Dry ice freeze cores; 9.6 cm^2 by 30 cm; 0.15 mm	Australasia/Bass Strait Drainages
	Poole and Stewart (1976) (A)	5(10)	Brazos River, Texas, USA	Vertical stratification colonization sampler; 201.1 cm^2 by 40 cm; 0.5 mm	Nearctic/East Texas Gulf
	Marchant (1995) (A)	6(30)	Acheron River, Victoria, Australia	Dry ice freeze cores; 9.6 cm^2 by 30 cm; invertebrates separated by floatation	Australasia/ Murray-Darling
Marine Coastal					
Marine Coastal Mixed	Dauwe et al. (1998) (A,B)	2(7)	Frisian Front and German Bight, North Sea	Cylindrical Reineck type box corer; 754.8 cm^2 by up to 50 cm; 0.5 mm	Temperate N. Atlantic/ North Sea
	Rhoads et al. (1985) (A,B)	1(?)[d]	East China Sea off Changjiang	0.25 m^2 spade box corer; 181.5 cm^2 by up to 43 cm; 0.5 mm	Temperate N. Pacific/ East China Sea

Table 4. Data Sources and Information (Realms/Ecoregions after Spalding et al., 2007 [marine] and Abell et al., 2008 [freshwater]) used to Determine 80th Percentile of Benthic Abundance (see Figure 3) and Benthic Biomass (see Figure 4) Depth Distributions (continued).

Habitat Type	Reference	*N* (Total Cores)	Location	Sampler; Sample Area and Depth; Sieve Size	Realm/ Ecoregion(s)
Marine Coastal Mud	Simonini et al. (2004) (A,B)	2(48)	Off of Po and Adige-Brenta river deltas, North Adriatic Sea	Box corer; 200 cm^2 by 20 cm; 0.5 mm	Temperate N. Atlantic/ Adriatic Sea
	Hayashi (1988) (A,B)	1(5)	Sado Strait, Sea of Japan	0.1 m^2 box corer; 225 cm^2 by 25 cm (2 or 3 per box core); 0.5 mm	Temperate N. Pacific/ Sea of Japan
	Moodley et al. (1998) (A,B)	6(12)	Adriatic Sea, northern basin	Large box corer; 283.5 cm^2 by 20 cm perspex cores (2 per box core); 0.5 mm	Temperate N. Atlantic/ Adriatic Sea
	Moodley et al. (2000) (A,B)	2(4)	Adriatic Sea, northern and middle basins	Large box corer; 283.5 cm^2 by 20 cm perspex cores (2 per box core); 0.5 mm	Temperate N. Atlantic/ Adriatic Sea
	Rhoads et al. (1985) (A,B)	2(?)[d]	East China Sea off Changjiang	0.25 m^2 spade box corer; 181.5 cm^2 by up to 43 cm; 0.5 mm	Temperate N. Pacific/ East China Sea
Marine Coastal Sand	Dauwe et al. (1998) (A,B)	1(3)	Broad Fourteens, North Sea	Cylindrical Reineck type box corer; 754.8 cm^2 by up to 50 cm; 0.5 mm	Temperate N. Atlantic/ North Sea
	Spies and Davis (1979) (A)	1(5)	Santa Barbara Channel, California, USA	Tin core samplers; 73.9 cm^2 by up to 35 cm; 0.5 mm	Temperate N. Pacific/ S. California Bight
	Oliver et al. (1980) (A)	1(10)	Monterey Bay, California, USA	Diver-operated corer; 180 cm^2 by up to 60 cm; 0.5 mm	Temperate N. Pacific/ N. California
	Oliver et al. (1980) (B)	1(4)	Monterey Bay, California, USA	Hydraulic suction dredge; 0.25 m^2 cylinder; 1.0 mm mesh bags	Temperate N. Pacific/ N. California
Marine Offshore					
Marine Offshore Mixed	Rhoads et al. (1985) (A,B)	1(?)[d]	East China Sea off Changjiang	0.25 m^2 spade box corer; 181.5 cm^2 by up to 43 cm; 0.5 mm	Temperate N. Pacific/ East China Sea
Marine Offshore Mud	Stull et al. (1996) (A,B)	1(3)	Palos Verdes Shelf, California, USA	Gray-O'Hara box corer; 500 cm^2 by up to 50 cm; 1.0 mm	Temperate N. Pacific/ S. California Bight

Table 4. Data Sources and Information (Realms/Ecoregions after Spalding et al., 2007 [marine] and Abell et al., 2008 [freshwater]) used to Determine 80th Percentile of Benthic Abundance (see Figure 3) and Benthic Biomass (see Figure 4) Depth Distributions (continued).

Habitat Type	Reference	*N* (Total Cores)	Location	Sampler; Sample Area and Depth; Sieve Size	Realm/ Ecoregion(s)
	Dauwe et al. (1998) (A,B)	1(2)	Skagerrak, North Sea	Cylindrical Reineck type box corer; 754.8 cm^2 by up to 50 cm; 0.5 mm	Temperate N. Atlantic/ North Sea
	Rhoads et al. (1985) (A,B)	1(?)[d]	East China Sea off Changjiang	0.25 m^2 spade box corer; 181.5 cm^2 by up to 43 cm; 0.5 mm	Temperate N. Pacific/ East China Sea
	Hayashi (1988) (A,B)	2(10)	Sado Strait, Sea of Japan	0.1 m^2 box corer; 225 cm^2 by 25 cm (2 or 3 per box core); 0.5 mm	Temperate N. Pacific/Sea of Japan
	Moodley et al. (2000) (A,B)	1(2)	Adriatic Sea, northern and middle basins	Large box corer; 283.5 cm^2 by 20 cm perspex cores (2 per box core); 0.5 mm	Temperate N. Atlantic/Adriatic Sea
	Josefson (1981) (A)	2(30)	Skagerrak, North Sea	0.1 m^2 box corer; 500 cm^2 by 28 cm (1 per box core); 1.0 mm	Temperate N. Atlantic/ North Sea
Marine Offshore Sand	Simonini et al. (2004) (A,B)	1(24)	North Adriatic Sea, offshore	Box corer; 200 cm^2 by 20 cm; 0.5 mm	Temperate N. Atlantic/ Adriatic Sea
	Oliver et al. (1980) (A)	2(14)	Monterey Bay, California, USA	Diver-operated corer; 180 cm^2 by up to 60 cm; 0.5 mm	Temperate N. Pacific/ N. California
	Oliver et al. (1980) (B)	1(4)	Monterey Bay, California, USA	Hydraulic suction dredge; 0.25 m^2 cylinder; 1.0 mm mesh bags	Temperate N. Pacific/ N. California

[a]Includes data sets from meso-polyhaline (2) and poly-euhaline (2) transition zones.
[b]Includes two data sets from meso-polyhaline transition zone.
[c]The ecoregion Biwa Ko is described as one consisting of *large lakes* habitat. Lake Suwa, the location for our data, is a *small* lake near Lake Biwa Ko.
[d]Number of subcores representing a box core is not specified.

Table 5. Biologically Relevant Sediment Depths—Biotic Zones—for Decisions Related to Ecological Assessment or Remediation. The biotic zone noted in column 2 is based on benthic abundance. The biotic zone shown in column 3 is based on benthic biomass (where information was available). Note that the biotic zone tends to be deeper when biomass is taken into account.

Habitat Type	Biotic Zone (cm)	Biotic zone (cm) (Considering Biomass)
Estuarine Intertidal		
Estuarine Intertidal Sand	15	
Estuarine Intertidal (Other Substrates)	*	
Estuarine Intertidal Poikilohaline	10	
Tidal Freshwater		
Tidal Freshwater Mixed Substrate	10	15
Estuarine Subtidal		
Oligohaline Sand	5	10
Mesohaline Sand	10	20
Polyhaline Sand	15	
Oligohaline Mud	5	5
Mesohaline Mud	10	25
Polyhaline Mud	5	*
Oligohaline Mixed Substrate	15	15
Mesohaline Mixed Substrate	10	30
Polyhaline Mixed Substrate	10	15
Lentic		
Lake Profundal Mud[a]	15	20
Lotic		
Stream Coarse Grained/Sand	35	
Stream Coarse Grained/Sand with Fines[b]	25	
River Coarse Grained/Sand with Fines[b]	15	
Marine Coastal		
Sand	5	20
Mud	15	15
Mixed Substrate	10	15
Marine Offshore		
Sand	10	20
Mud	15	20
Mixed Substrate	*	*

[*]Biotic zone not estimated because based on only one data set.
[a]Biotic zones for this category are based on oligochaetes.
[b]Fines denote grain sizes <2 mm in substantial quantity (approximately 20% or more by weight).

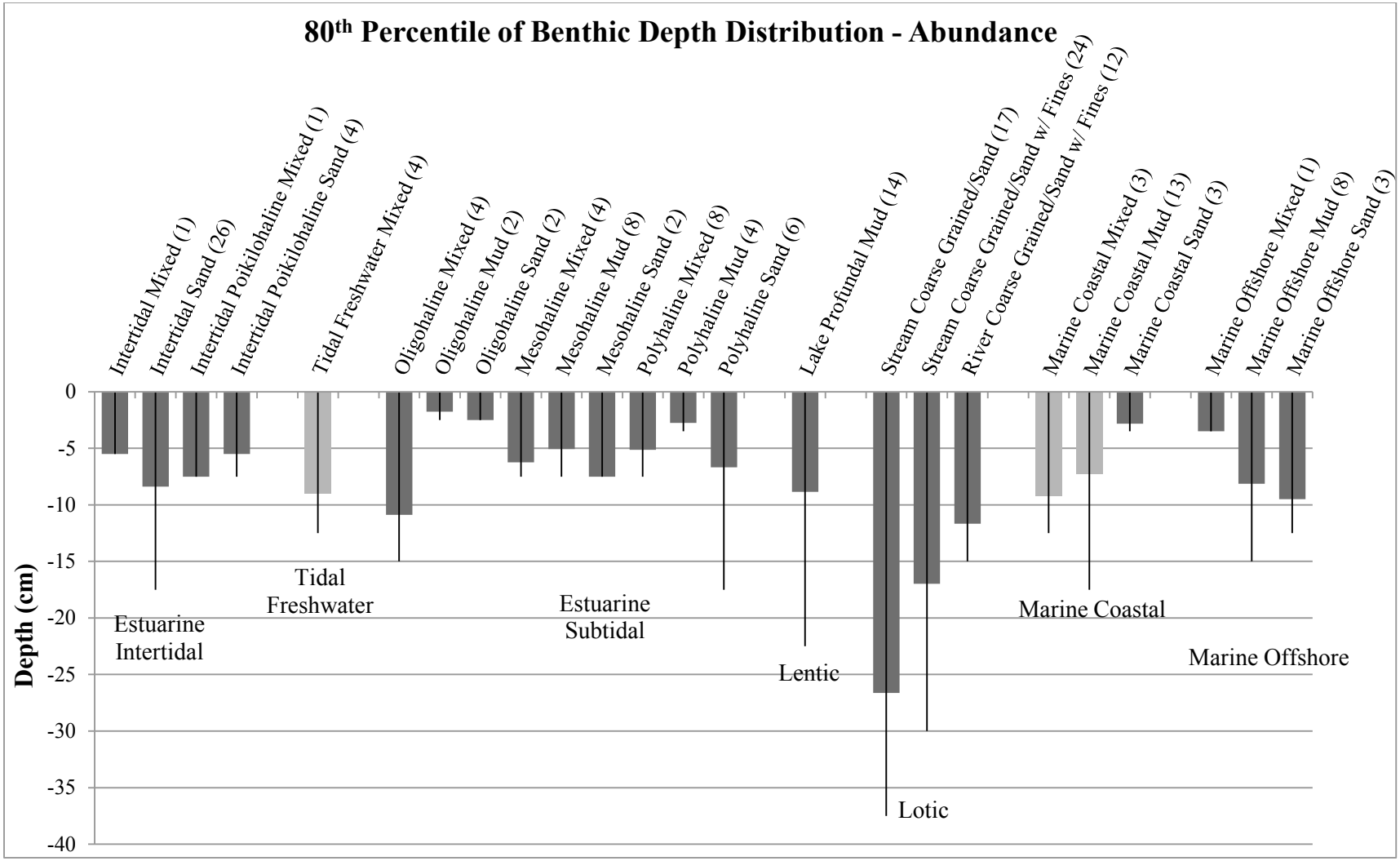

Figure 3. Mean 80th Percentile of Benthic Abundance Depth Distribution (+ Maximum 80th Percentile) in Various Habitats. Number of data sets in parentheses (the number of cores comprising data sets from each habitat type is noted in Table 4). Also see Table 4 for data locations.

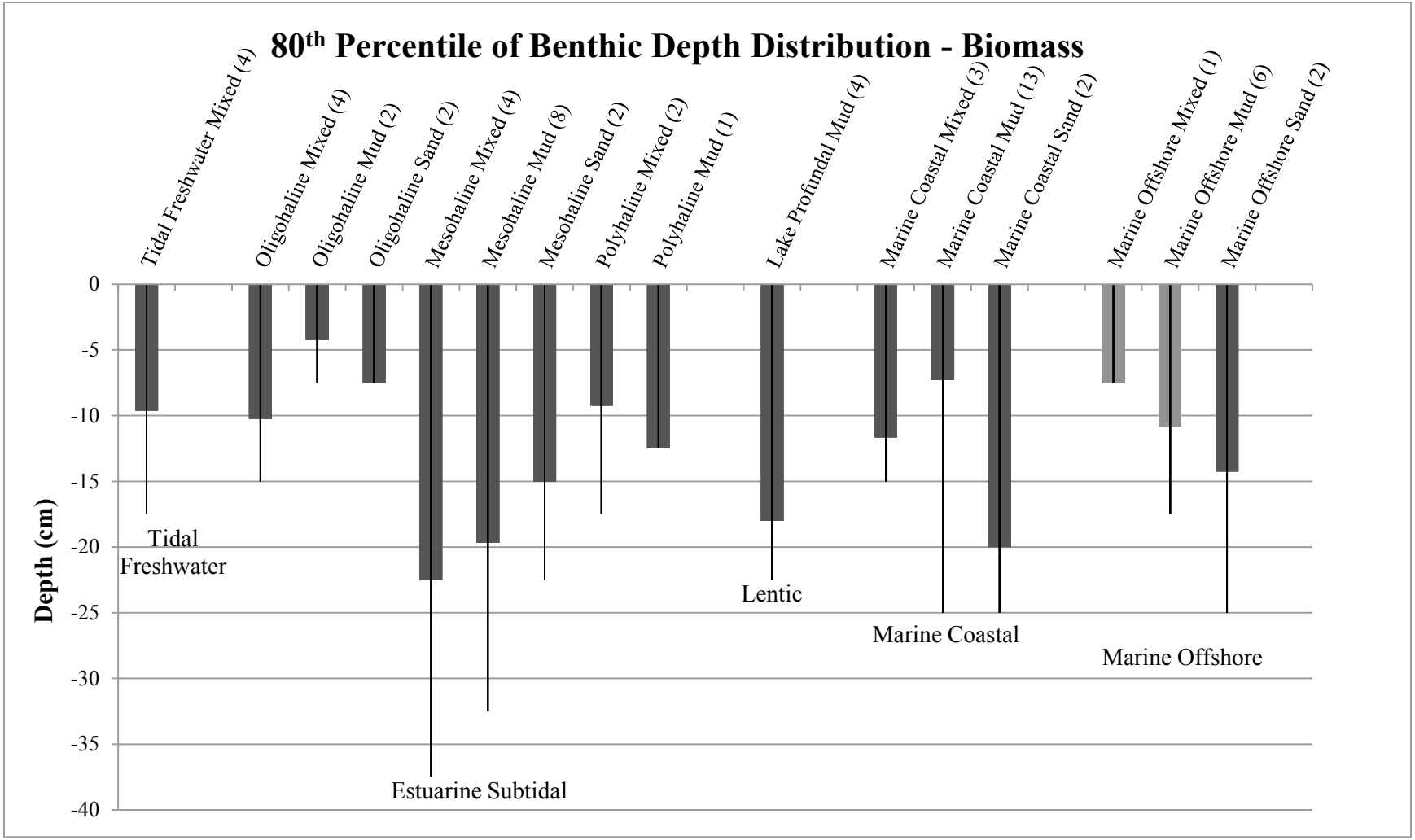

Figure 4. Mean 80th Percentile of Benthic Biomass Depth Distribution (+ Maximum 80th Percentile) in Various Habitats. Number of data sets in parentheses (the number of cores comprising data sets from each habitat type is noted in Table 4). Also see Table 4 for data locations.

APPENDIX

ECOLOGICAL RISK ASSESSMENT SUPPORT CENTER REQUEST FORM

ERASC Request No. 0015
Requestor: Marc Greenberg, Environmental Response Team
Problem Statement: What is a scientifically defensible definition for the depth of the biotic zone in soils and sediments?
Background: We are frequently faced with the challenge of defining the "biotic zone" in soils and sediments during the design and interpretation of soil and sediment sampling programs. This may pose challenges later when we evaluate sediment concentrations (e.g., depth-integrated, mass per unit area, surface-weighting, etc.), calculate or model current and future risks to ecological receptors and humans, and attempt to delineate the relevant depth for remediation at sites where an action is needed. This can have large implications on the cost, protectiveness, and effectiveness of a selected remedy (e.g., capping, dredging, monitored natural recovery, excavation, etc.). Other terms used to describe the biotic zone include "ecologically-relevant zone," "biologically-active zone" and "bioturbation zone."
Expected Outcome: The ERASC should develop a document that will provide a defensible approximation or a range of reasonable approximations for what the depth of the biotic zone is within certain environments. For example, there are those who assume that 4 cm is adequate to define the biotic zone for sediment benthos. Others would argue that 0-2 cm, 10 cm (6 in.) or even as far as 12 in. are reasonable. We need some clarity.
Additional Comments: For sediments, this question should be answered with a primary focus on benthic macroinvertebrates (e.g., bugs and bivalves) and their distribution among various sediment microhabitats. The reason for focusing on benthic macroinvertebrates is because they are measurement endpoints that provide decision-oriented data. The document should provide general explanations of the biotic zone in various aquatic habitats (e.g., stream, river, lake, coastal, estuarine environments) where a remediation may occur. For soils, the focus should be on both invertebrates and vertebrate receptors.