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Evidence for sedimenting particles as the origin of the microbial community in a coastal marine sediment

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ABSTRACT: The origin of the microbial community in a coastal marine sediment was investigated by examining the physical, chemical, and microbial characteristics of the sedimenting particles in Halifax Harbor, Canada. Particles were collected using particle interceptor traps placed on the sediment surface. The measured rate of particle accumulation compared to the sediment accumulation rate indicates that over 85% of the dry weight of the particles is removed (including resuspension) or solubilized before deposition as sediment. Active decomposition is facilitated by the large microbial community present. The particles are heavily colonized by both bacteria (10^9 g^{-1}) and protozoa (10^6 g^{-1}) before they reach the sediment such that the microbial communities of the sinking particles and sediment-water interface are nearly indistinguishable. The particles and sediment are also similar with respect to community growth rates and carbon, nitrogen, and ATP content. Artificial sediment cores inserted into the sediment and left in place for 1 yr were colonized only to a depth of 4 cm. The data indicate that an extensive microbial community is already established on particles before they become sediment and that in situ sediment colonization is not necessary for the establishment of the sediment microbial community.

INTRODUCTION

It is a well established fact that the surface layers of both marine and freshwater sediments are habitats for large microbial communities. Indeed, populations in excess of 10^{10} bacteria per g of sediment have been reported. Several aspects of these communities have been investigated: distribution (Schroder & Van Es 1980, Rublee 1982), metabolic activity (Novitsky 1983a), biomass (Craven et al. 1986, Novitsky & Karl 1986), enumeration of metabolically-active cells (Douglas et al. 1987), growth rate (Novitsky 1987, Karl & Novitsky 1988), productivity (Newell & Fallon 1982, Burns et al. 1984), degradation of microbial biomass (Novitsky 1986), and the relationship between microbiology and sediment chemistry (Meyer-Reil et al. 1980). However, to date, no study has addressed the question of the origin of the sediment microbial community. Is it a specific community that develops in or on the sediment, or is it the accumulation of microbes that have sedimented from the water column either as free cells or as cells attached to particles? Is the sediment merely a depository or graveyard for pelagic microbes? In a previous publication (Novitsky 1986) I examined the fate, but not the origin, of microbial biomass in marine sediments. That study indicated that dead microbial biomass is quickly degraded, suggesting that the large sediment community is alive (but not necessarily metabolically active). Furthermore, an additional study (Novitsky 1987) has shown that most of the microbial community is not growing. If this microbial community originates in, and actively colonizes the sediment as it is deposited, nearly 100% of the population would be expected to be actively growing. Putting these points and observations together, it seems reasonable to hypothesize that the sediment microbial community does not originate in the sediment but is deposited from some other source. After sedimentation, the sediment environment then selects the microbes best suited for growth under the present conditions. The nongrowing portion, active prior to sedimentation, now remains dormant (possibly moribund) until conditions again become favorable for growth. The present study was designed to test these hypotheses.

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MATERIALS AND METHODS

Sample collection. All samples were collected from the Eastern Passage area of Halifax Harbor, Canada, a site used in previous studies (Novitsky 1983a,b, 1987). All samples were collected by hand by SCUBA divers. Water samples were collected in clean, 1 l plastic bottles. Sediment samples were collected in plastic core tubes carefully inserted into the sediment (Novitsky 1983b). Sediment-water interface samples were removed from intact, undisturbed cores by careful aspiration using a large syringe fitted with a 16 g teflon canula (Novitsky 1983b). Sedimented particles were collected in particle interceptor traps based on a design described by Knauer et al. (1979). Each trap was composed of a plastic tube 6 × 60 cm closed at the bottom end with a rubber stopper and fitted on the top end with a collar containing a nylon screen (square, 1.96 mm openings). Eight traps were fitted into a weighted, sturdy plastic box that could be set on the sediment surface with the traps in a vertical position. Before deployment, each trap was filled with the appropriate solution and capped. Once deployed and stable, SCUBA divers would carefully remove the caps from the tubes to begin collection. Traps were routinely deployed for a period of 2 wk except for growth rate and DNA synthesis determinations. These 'live' collections were for the minimum period needed to collect an adequate amount of material, 4 to 6 d. For recovery, divers would gently descend, recap the tubes, and attach a recovery line to the box. For recovery of fresh material, traps were filled with filtered (Millipore, 0.45 µm membrane) seawater. For the collection and preservation of cells for direct microscopic counts, traps were filled with 1.5 l of a high density formalin solution containing: 40 g artificial sea-salts (Rila Marine Mix, Rila Marine Products, Teaneck, NJ) and 2% (final concentration) formalin in 1 l seawater. For the in situ extraction of ATP, traps were filled with 1.5 l of a high density acid solution containing: 132 g NaCl, 51 ml concentrated phosphoric acid, and distilled water to make 1.5 l. The high density solutions were filtered (Millipore, 0.45 µm membrane) and added to the traps. The remaining trap volume was carefully filled with filtered seawater.

Analytical procedures. For ATP extractions from fresh sediment trap material and interface samples, quintuplicate, 1 ml slurry samples were injected into 5 ml boiling, 60 mM phosphate buffer and allowed to boil for 5 min. For water samples, duplicate, 250 ml samples were passed through Whatman GF/F filters which, after filtration was complete, were immediately placed into boiling phosphate buffer. For in situ-extracted ATP from sediment traps, ten 50 ml subsamples from each trap were processed as described previously (Novitsky & Karl 1985). After extraction, for all samples, ATP was measured using the firefly bioluminescence reaction (Karl & Craven 1980). To correct for apparent and real losses of extractable ATP, internal standards were added to quintuplicate samples from all sites and processed in an identical manner.

The organic carbon content and the carbon/nitrogen ratio of the sediment trap material and the sediment-water interface were determined by filtering 10 ml of a sediment slurry onto silver filters (0.8 µm Hytrex membranes, Osmonics, Inc., Minnetonka, MN). After drying the filters were exposed to fuming acid and then analyzed for carbon and nitrogen using a carbon-hydrogen-nitrogen analyzer (Hewlett-Packard 185B).

DNA synthesis rates were measured using ^{3}H-adenine as described by Karl & Novitsky (1988) and Novitsky & Karl (1986). Interface or sediment trap slurries used in this study contained ca 5 to 10 mg dry wt sediment ml^{-1}

Samples for dissolved oxygen determinations were removed from the sediment traps immediately upon recovery. Samples were removed from various depths within the traps using a siphon of small-bore plastic tubing. Oxygen was determined by the Winkler method as modified and described by Strickland & Parsons (1972).

Direct microscopic counts. Interface and fresh sediment trap samples were fixed with 2% formalin. All samples were handled and mixed very gently to protect the protozoa. Five to 10 ml portions of fixed samples or appropriate dilutions were filtered with minimum vacuum onto prestained (overnight in a 1:15 000 solution of Sudan black B in 50% ethanol) 0.2 µm Nuclepore filters and washed once with 2 ml Trizma HCl (Sigma Chemical Co.) buffer (0.1 M, pH 4). The cells were stained with 2 ml of primulin stain (25 mg primulin in 100 ml Trizma buffer) for 5 min followed by a 2 ml Trizma buffer wash. Air-dried filters were mounted in immersion oil and examined with a Wild-Leitz epifluorescence microscope. For direct counts, at least 40 fields from one edge, through the center, to the opposite edge of the filter were counted. Cells with characteristic protozoan morphology without red-orange chlorophyll autofluorescence were counted as protozoa. Cells showing autofluorescence, regardless of morphology, were counted as autotrophs. After the protozoa were enumerated the samples were homogenized in a blender (3 × 30 s) to increase the precision of the bacteria counts. Bacteria were enumerated after staining with acridine orange as previously described (Novitsky 1983a).

Growth rate determinations. Two methods were used to determine the growth rate of the microbial community. For both methods interface or sediment trap slurries were prepared containing ca 5 to 10 mg

dry wt sediment ml^{-1}. Adenine nucleotide (AN) pool turnover rate measurements were performed according to the procedure of Karl & Bossard (1985) using ^{3}H-adenine (Karl et al. 1987). Growth rates were calculated from the AN pool turnover times assuming that the pool turns over 40 × $generation^{-1}$ (Karl & Bossard 1985). Growth rates were also determined using ^{3}H-adenine and the pulse labeling technique of LaRock et al. (1988). For interface and sediment trap material ^{3}H-adenine pulses of 105 min followed by acid precipitation of nucleic acids and isolation and purification of ^{3}H-DNA (Novitsky & Karl 1986) were carried out at 1 h intervals over a 6 h period. For water samples, ^{3}H-adenine pulses of 30 min were carried out at 1 h intervals over a 6 h period. After each pulse, triplicate 100 ml aliquots were filtered through Whatman GF/F filters and the nucleic acids were precipitated with cold, 10 % trichloroacetic acid. After precipitation, the filters were washed with 95 % ethanol (3 × 10 ml) and dried. The radioactivity on the filters was determined by liquid scintillation counting.

Biomass carbon production. DNA synthesis rates were measured using ^{3}H-adenine as described by Novitsky & Karl (1986) using the ATP integrated specific activity method of Winn & Karl (1984). Carbon production was extrapolated from rates of DNA synthesis by assuming that 1 pmol of DNA is equivalent to 1236 pg of DNA produced and that DNA accounts for 2 % of cell carbon.

Artificial sediment studies. Small glass beads with a nominal diameter of 5 µm (size MS-XLX, Waldron Co., Inc., North Haven, CT) were used as an artificial sediment medium to study sediment colonization. Prior to deployment, the beads were soaked for 2 h in detergent, washed 10 × with distilled water, soaked overnight in 10 % HCl, washed 10 × with distilled water, and then washed twice with filtered seawater. The slurry was then added to 6 × 29 cm plastic tubes fitted with 44 µm mesh screens near the bottom to retain the beads. Slurry was added and allowed to settle until a column of ca 20 cm was added. The bottom of each tube was capped with a rubber stopper, the head space filled with filtered seawater, and the top capped with a rubber stopper. Eight such tubes were lowered in a box to a depth of 10 m at which time divers removed the top and bottom stoppers. The tubes were taken to the bottom and inserted vertically into the sediment so that the artificial and natural sediment surfaces were approximately even. For recovery, a diver would recap the tube, remove it from the sediment, restopper the bottom, and bring it to the surface. In the laboratory, any collected natural sediment was carefully removed from the surface of the artificial sediment before the artificial sediment was extruded from the tube. Horizons, 0.5 to 1 cm, were cut from the sediment core, placed in a beaker and mixed thoroughly. Quintuplicate subsamples were then assayed for ATP as follows: ca 1 cm^3 was loaded into a plastic, cut-off syringe barrel and then injected into 5 ml of boiling phosphate buffer to extract ATP. After the ATP analysis, the tubes containing the sediment and buffer were centrifuged and the remaining buffer was aspirated. The tube and sediment were dried at 80 °C and weighed. The sediment was then discarded and the tubes reweighed. The dry weight of the sediment sample was then calculated by difference.

Table 1. Chemical comparison of sedimented particles and the sediment-water interface of Halifax Harbor, Canada

Parameter	Particles	Interface
ATP [µg (g dry wt)$^{-1}$]		
Extracted upon recovery[a]	10.74 (n=18)	25.50 (n=6)
Extracted in situ	24.66 (n=6)	
Organic carbon (% dry wt)	1.49	1.76
Percent living carbon[b]	41.65[c]	36.13
C:N (molar ratio)	6.08	5.69

[a] Deployments of 12 to 14 d
[b] (ATP × 250/organic carbon) × 100
[c] In situ-extracted ATP used in calculation

RESULTS AND DISCUSSION

The sedimentation of particles was measured over a 6 mo period from May to October, 1988. The traps in this study were placed directly on the sediment surface to collect particles as close (temporally and spatially) as possible to the sediment-water interface. While this positioning increased the possibility of collecting resuspended material, the design of the traps and comparison of inter- and intratrap data lead me to conclude that this was not a significant problem in the relatively short collection periods used in this study. The particulate matter collected in the sediment traps ranged from 26.2 (May) to 55.2 g dry wt m^{-2} d^{-1} (July). Due to weather conditions it was impossible to deploy the traps during the winter so a conservative figure of 8000 g dry wt m^{-2} yr^{-1} was estimated. The dry weight of 1 m^2 of the top 1 cm of sediment at this location was determined to be 4530 g while the sediment accumulation rate at this site was previously measured at 0.25 cm (1133 g) yr^{-1} (Kepkay & Novitsky 1980). Using these estimates and measurements, over 85 % of the sedimenting material is solubilized, mineralized, resuspended, or otherwise removed before final sediment deposition. There is no doubt that the microbial community is responsible for mineralization of some of the sedimenting carbon; however, since the organic carbon represents only 1.5 % of the dry weight of the particles (Table 1) it is

doubtful that microbial metabolism accounts for a significant portion of the material removed. The most likely possibility is that winter storms resuspend and redistribute surface sediments within the harbor or actually transport material out to sea. Such significant transport will have to be considered and studied more carefully when considering coastal sediment diagenesis.

The heterogeneity and patchiness of the interface and the collected particles continue to be problems. Interface samples taken within centimeters of each other, or individual cores divided into 4 portions, yielded quite different results, especially for growth rate determinations. Different portions of homogenized individual, or pooled multiple samples yielded results that were within experimental error indicating that the methods themselves are precise and reproducible. Visual examination of the samples confirm the heterogeneity although no distinct differences in microbial communities could be discerned by microscopic examination. Any study of these sediments and particles must therefore take this patchiness into account as a fact of life. Growth rates (Table 2) were reported as the range of values recorded rather than an average.

The 2 methods used to determine the microbial community growth rate produced strikingly different results even when the sediment patchiness was taken into account. The pulse label (PL) technique (LaRock et al. 1988) consistently produced a range of rates that were much faster for both the particles and the interface than did the adenine nucleotide turnover (AN) technique (Karl et al. 1987). Which is correct? Without an absolute standard it is impossible to determine the accuracy of the methods. However, based on the designs of the techniques and what is known about the sediment communities, I have formed the following hypothesis. The AN technique measures ATP pool labeling over a period of 12 (or more) h until the pool reaches isotopic equilibrium. A small number of relatively fast-growing bacteria will equilibrate their pools quickly but, because they are few in number, their ATP pool and its radioactivity will be small and hence masked by the larger pool and higher radioactivity of the larger, but slower growing remainder of the population. Therefore, over the longer incubation times used in the AN technique, the 'average' (weighted toward the slower growing portion) community growth rate is measured. On the other hand, the PL technique employs a number of 1 h (or less) incubations favoring the fast-growing portion of the population. Since the slower growing portion cannot take up significant radiolabel in 1 h, its signal cannot mask the smaller signal generated by the fast growers. The result is a growth rate that represents those members of the population that can metabolize a labelled precursor in a short period of time: the active, fast-growing (most likely) bacteria. According to this hypothesis, both methods should yield identical results for populations composed of members with approximately equal growth rates. Indeed, this may have been the case for the water samples studied by LaRock et al. (1988). The results of these 2 methods, as far as the present study is concerned, indicate heterogeneity even within the microbial communities. The particle community appears to be composed of members that can double in as little as 8 h whereas the entire community will double approximately every 36 h. The interface community, on the other hand, also doubles approximately every 36 h but lacks the fast growing members associated with the particles. This may indicate that as the particles age and become sediment, the community changes as the readily-utilizable growth substrates are depleted.

Table 2. Growth rates and carbon production by microbial communities associated with sedimented particles and the sediment-water interface of Halifax Harbor, Canada. ND: not determined

Parameter	Particles	Interface
Community doubling time (h)		
Pulse label technique[a]	8–15	17–24
Adenine nucleotide turnover technique[b]	34–39	23–46
Carbon production[c] [µg (g dry wt)$^{-1}$ h^{-1}]		
Measured upon recovery	16–29	45–90
Measured in situ[d]	14–21	ND

[a] LaRock et al. (1988) – ^{3}H-adenine used as substrate, ^{3}H-DNA isolated and measured
[b] Karl et al. (1987). Doubling time is extrapolated from adenine nucleotide pool turnover time assuming that the pool turns over 40 × generation^{-1} (Karl & Bossard 1985)
[c] Carbon production was extrapolated from rates of DNA synthesis by assuming that 1 pmol of DNA is equivalent to 1236 pg of DNA produced and that DNA accounts for 2 % of total cell carbon
[d] ^{3}H-adenine was added to the traps before depolyment. Upon recovery after a 13 d deployment, the specific activity of the ATP pool was determined, and the ^{3}H-DNA was isolated and measured

Regardless of minor differences in overall community growth rates, both the particles and the interface are heavily colonized by both bacteria and protozoa (Table 3). By the time particles reach the bottom, the sizes of the microbial communities of the particles and the sediment-water interface are virtually identical. With 10^{10} bacteria and 10^6 protozoa per g, particles appear to be an excellent habitat for microbial growth [although autotrophs are present, their low relative numbers and the fact that light intensity at the sediment-water interface is $< 1.0\%$ of the intensity at the water surface (Novitsky 1983b), their growth and impact on this environment would be minimal]. As already discussed, community doubling times measured for the microbial community associated with the particles are no greater than 40 h (a relatively fast growth rate at 2 °C), comparable to doubling times measured for interface communities (Table 2). The biomass carbon production was higher for the interface than for the particles on both an absolute and per unit (ATP) biomass basis. The reason(s) for this is unclear especially in light of the similar growth rates. One possibility is that the cells in the interface are larger and hence produce more biomass with the same doubling time. While possible, this is doubtful since it was impossible to show a statistical difference in cell size between the two communities. In addition, both habitats have similar ATP and organic carbon content as well as similar C:N ratios (Table 1).

The results of this study that indicate the sedimenting particles are a good habitat for microbial growth contradict the conclusion reached by Karl et al. (1988) for sinking particles in the north Pacific. They based their conclusion, in part, on the limited utilization of particulate organic carbon (POC) and particulate organic nitrogen (PON) by live traps compared to formalin-killed controls and the difference between ATP extracted in situ and ATP extracted upon recovery (shipboard ATP). Since the shipboard/in situ ratio was low (usually < 0.25), they concluded that the microbial community of the particles was in a state of decline and therefore that the particles were a poor habitat for microbial colonization. I have also measured a low recovery/in situ ATP ratio (0.44, Table 1) and agree that the microbes in the traps are dying; however, I propose a hypothesis for the cause of this death that was not discussed by Karl et al. (1988). The in situ-extracted ATP content of the particles in this study is virtually the same as that measured for the interface. Since the particles were collected just above the sediment surface, the interface should represent 'aged' particles similar to those caught in the traps. Why is the ATP content of these 'aged' particles different from the ATP content of the interface? A possibility is that during the period of particle collection [12 to 14 d, this study; 6 to 33 d, Karl et al. (1988)] the microbial communities in the traps utilize the oxygen present and quickly create anaerobic conditions, thus committing microbial suicide. Since the traps are specifically designed to prevent mixing of the contents with the surrounding seawater, oxygen could only be replenished by diffusion. To test this hypothesis I measured dissolved oxygen in a recovered trap that had been deployed for 14 d. Oxygen measured in the water at the mouth of the trap was 9.7 mg l^{-1}; in the middle (30 cm from the top) 6.7 mg l^{-1}; and only 5.2 mg l^{-1} near the bottom (50 cm from the top). In addition, although they didn't report oxygen concentrations, Knauer et al. (1979) reported the presence of H_2S in unpreserved trap material. Certainly these depleted oxygen conditions must have an effect on the microbes. Accordingly, the more organic material collected and the longer the deployment, the greater depletion of oxygen that will occur Although Karl et al. (1988) deployed traps for up to 33 d, the amount of POC collected per trap was low (ca 1 to 3 mg C $trap^{-1}$ compared to 50 mg C $trap^{-1}$ in this study). With this small amount of carbon collected it seems unlikely that any corresponding small decrease in oxygen would cause a significant decrease in microbial biomass or activity. Nevertheless, until this hypothesis is examined more fully, care must be taken when interpreting results from these in situ 'live' incubations. If live material must be collected, the collection time should be as short as possible. Apparently unaffected by the in situ incubation is the total number of microbes trapped (Table 3). Although 50% of the biomass is dead, the

Table 3. Protozoa and bacteria (cells, per g dry wt) associated with sedimenting particles and the sediment-water interface of Halifax Habor, Canada

Sample	Cells (SD)					
	n	Bacteria	n	Protozoa	n	Autotrophs
Sediment traps (samples fixed after 2 wk deployment)	18	1.67 (1.52) × 10^{10}	18	4.74 (1.89) × 10^6	3	3.05 (0.87) × 10^5
Sediment traps (samples fixed in situ)	11	2.68 (2.73) × 10^{10}	11	3.72 (1.49) × 10^6	4	6.41 (1.27) × 10^5
Sediment-water interface	8	1.49 (0.35) × 10^{10}	8	4.30 (2.94) × 10^6	4	5.06 (3.58) × 10^5

cells are still discernable by microscopic examination. These results notwithstanding, the use of a preservative as recommended by Knauer et al. (1979) is the best way to assure unchanged samples [for a comprehensive discussion of sediment trap material preservatives see Knauer et al. (1984)]. Although the conclusions of Karl et al. (1988) and this study are contradictory, they are not inconsistent. Combining the results from both studies a working hypothesis can be formed. Sinking particles in surface (depth < 50 m), primarily coastal, waters are rich in labile organic matter and are good habitats for microbial growth and decomposition. In this situation, the microbial community associated with the particles plays an important role in the decomposition and flux of particulate organic matter (POM), and, as shown by this study, the diagenesis of coastal sediments. However, by the time particles reach deep (depth > 50 m), open-ocean waters, labile POM has been removed and the sinking particles are no longer a good habitat for microbial colonization, resulting in a generally moribund community. In this situation, as suggested by Karl et al. (1988), microbial communities that are free-living in the water column or associated with non-sinking particles are more important in active remineralization of organic matter.

To examine the colonization of deeper horizons of sediment by particles sedimenting onto the sediment surface, artificial sediment cores were deployed for periods of up to 1 yr. After 3 mo, a visible interface layer developed on top of the artificial sediment. This layer was identical in appearance and ATP content to interface material collected from real sediment cores. Below this interface, however, there was very little colonization of the artificial sediment. As can be seen in Fig. 1, ATP was detectable only in the top 4 cm and even in the 0 to 0.5 cm horizon, the ATP content of the artificial sediment was 2 orders of magnitude lower than would be found in normal surface sediment (Novitsky 1987). It is apparent that there is no extensive vertical movement either by the micro- or macro-biota in these sediments and that bioturbation is minimal. These observations suggest that the microbial community in the deeper horizons is initially deposited at the surface and becomes buried with continuous surface sediment accumulation. In addition, it is unlikely that deeper horizons are overturned to establish or re-establish surface sediment communities.

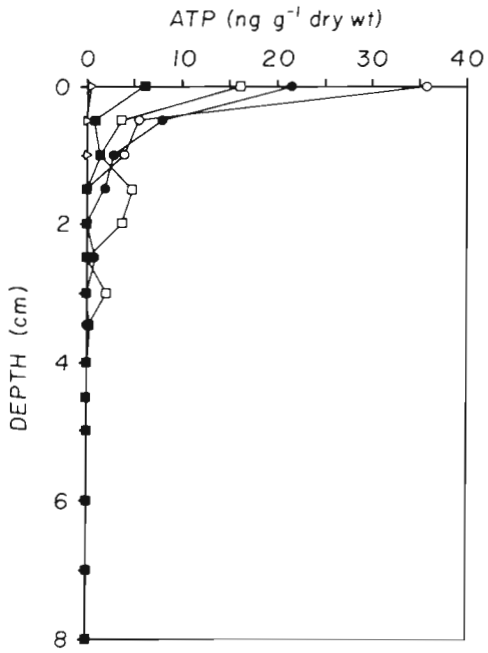

Fig. 1 Vertical profile of ATP biomass in artificial sediment cores deployed in Halifax Harbor, Canada, sediment. Length of deployment: ○, ● (2 cores), 1 mo; □, ■ (2 cores), 3 mo; △ (1 core), 1 yr

While it is impossible to rule out, at this time, the possibility of a specific microbial community developing in or on the sediment, the data suggest that the sediment community originates on sedimenting particles and is well established before the particles reach the sediment surface. Undoubtedly, some changes in the species composition of the population occurs, but the bulk of the microbial biomass sediments from the water column above. In addition, I have shown (Novitsky 1987) that most of the sediment microbial community is not actively growing. This conclusion raises the possibility that the nongrowing portion of the sediment community is composed of former particle-associated microbes that cannot grow in the sediment environment. Further studies are needed to identify the individual species comprising the water column, particle-associated and sediment-associated communities.

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