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CAN SEDIMENT TOTAL ORGANIC CARBON AND GRAIN SIZE BE USED TO DIAGNOSE ORGANIC ENRICHMENT IN ESTUARIES?

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Abstract—Eutrophication (i.e., nutrient enrichment, organic enrichment, and oxygen depletion) is one of the most common sources of impairment in Clean Water Act 303(d)-listed waters in the United States. Although eutrophication can eventually cause adverse effects to the benthos, it may be difficult to diagnose. Sediment organic carbon (OC) content has been used as an indicator of enrichment in sediments, but the amount of surface area available for carbon adsorption must be considered. We investigated the utility of the relationship between OC and sediment grain size as an indicator of eutrophication. Data from the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program was used to test this relationship. However, anthropogenic contaminants are also capable of causing adverse effects to the benthos and often co-occur with elevated levels of OC. Contaminant analysis and toxicity tests were not consistently related to enrichment status as defined by relationship between total OC and grain size. Although variability in response occurred, reflecting the variance in the water column factors (dissolved oxygen, chlorophyll *a*, and nutrients) and limited sample sizes, the data supported the hypothesis that sites designated as enriched were eutrophied. Dissolved oxygen levels were reduced at enriched sites, whereas chlorophyll *a* and nutrients were higher at enriched sites. This suggests that the relationship of OC to grain size can be used as a screening tool to diagnose eutrophication. Environ. Toxicol. Chem. 2011;30:538–547. © 2010 SETAC

Keywords—Total maximum daily load Benthos Sediment Eutrophication Organic carbon

INTRODUCTION

Estuaries are dynamic systems occupying the interface between land and sea. These areas are often highly populated and impacted by a variety of environmental stressors, including chemical contamination, habitat loss, and eutrophication. The U.S. Clean Water Act requires that the condition of estuaries be maintained and restored to meet their designated use as fishable and swimmable waters.

If these designated uses are not met, the system is deemed impaired, and the total maximum daily load (TMDL) process must be implemented for the pollutants causing impairment [1]. This process requires that the stressors responsible for causing the impairment be reduced to allow recovery; however, it is often difficult to diagnose which agent is primarily responsible for the observed adverse effects. Eutrophication (nutrient enrichment, organic enrichment, and oxygen depletion) is one of the most common sources of impairment in 303(d)-listed waters (i.e., waters that require TMDL development). The National Eutrophication Assessment [2] indicated that a majority of U.S. estuaries were moderately or highly eutrophied. Unfortunately, unlike other common impairments such as chemical contaminants or pathogens, eutrophication may be harder to diagnose.

Eutrophication has been defined as ''an increase in the rate of supply of organic matter to an ecosystem'' [3]. Eutrophied systems often show a record of this enrichment in the sediments as revealed by the presence of particulate organic carbon (OC). Because of this, OC has been used as an indicator of enrichment in sediments [4,5], but not specifically as a measure of eutrophication. Enhanced OC levels can be both beneficial and harmful to benthic organisms. As first conceptualized by Pearson and Rosenberg [6], the initial increase in OC provides food to the benthos, increasing both biomass and numbers of species. With higher loads, there are adverse effects, causing the benthos to be dominated by smaller, more abundant, opportunistic species. Eventually, organic loads can become high enough that only bacterial mats are present. These negative biological changes are driven by corresponding oxygen depletion and increasing ammonia and sulfide byproducts [5]. Anthropogenic contaminants are also capable of causing these biological changes and often co-occur with excess OC [6,7].

The organic content of marine and estuarine sediments is determined by multiple factors, including the sediment grain size, OC source, sedimentation rate, and preservation rate [8]. Often this carbon is derived from multiple sources, and its inflow may be enhanced by anthropogenic materials (e.g., municipal sewage or industrial wastewater), nutrient inputs that stimulate production, or human disturbance (e.g., increased impervious surfaces) that can cause increased sedimentation. Preservation rate is influenced by sedimentation rate, composition of the organic matter, extent of bioturbation, and average exposure to oxic conditions [8,9]. In the absence of marked anthropogenic perturbation of carbon sources, extreme sedimentation rates, or prolonged low oxygen levels, OC in sediments should be controlled largely by the sediment grain size [8,10]. This suggests that the relationship between OC and sediment grain size may be used as an indicator of eutrophication.

Eutrophication is often, but not always, linked to nutrient enrichment [2]. In coastal marine environments, nitrogen is

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most often the limiting nutrient and a major contributor to eutrophication [11]. With increasing nitrogen, rapidly growing producers such as algae (both phytoplankton and macroalgae) are favored, whereas slower growing forms such as seagrass decline. This can lead to high organic inputs to sediments, anoxia, and potentially adverse effects to fish and the benthos [2,12–14]. Therefore, although eutrophication is defined as excess carbon input into the system, more generally we can measure nutrients, which spur algal growth, chlorophyll *a* production (increased carbon), and dissolved oxygen depletion (as a result of excessive carbon sedimentation). We further expect that eutrophied systems would have high total organic carbon (TOC) levels in the sediments. Although we expect increased OC to be indicative of eutrophied conditions, chemical contaminants are also often associated with high carbon sediments [7–9]. Many chemical contaminants readily absorb to organic matter and its associated carbon constituents [15,16]. These suspended materials settle to the bottom either through flocculation at the head of the estuary [17,18] or eventual settlement after many cycles of bed load movement and resuspension [19,20]. Thus, although we expect that an elevated TOC to grain size ratio will be associated with eutrophication, it may also be an indicator of chemical contaminant effects.

To test whether the relationship between OC and grain size is a useful predictor of eutrophication, we developed relationships between TOC and grain size using unimpacted sites from the estuaries of three biogeographic provinces along the U.S. East Coast (Fig. 1) using monitoring data collected by the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP). Deviations from these reference relationships were used to identify enriched sites. We assessed whether the relationship would be expected to indicate adverse environmental impacts to estuarine benthos by comparing benthic indices between reference and enriched sites. We also wanted to examine whether the relationship could be used specifically as an indicator of eutrophication rather than a more generalized indicator of an impacted benthos resulting from multiple stressors, such as chemical contamination or enrichment). To do this, we compared sediment contamination and toxicity between reference and enriched sites as well as common eutrophication measures such as dissolved oxygen, chlorophyll *a*, and nutrients.

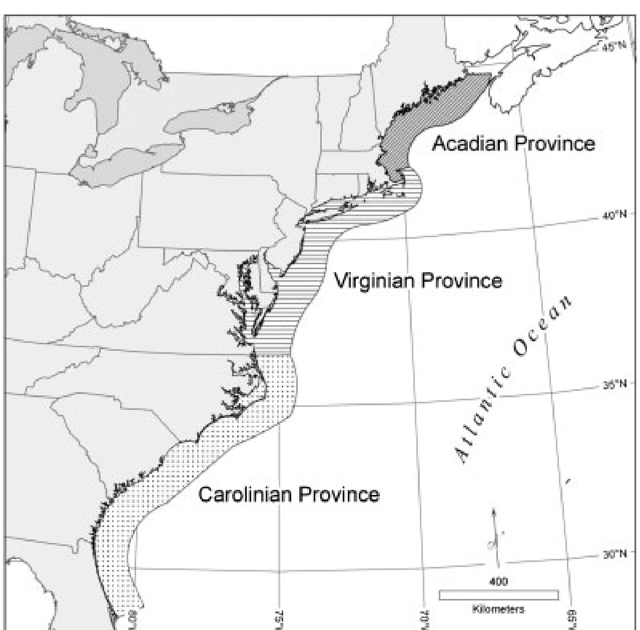

Fig. 1. Map of biogeographic provinces along the East Coast of the United States.

MATERIALS AND METHODS

Data sources

Data for the present study were obtained from EMAP (www.epa.gov/emap). In the Acadian province (Fig. 1), data from 220 stations were assembled from the 2000 to 2002 National Coastal Assessment-Northeast. In the Virginian Biogeographic province (Fig. 1), data from 998 stations were assembled from three data sets: 1990 to 1993 EMAP, Virginian province, 1997 to 1998 Mid-Atlantic Integrated Assessment, and 2000 to 2001 National Coastal Assessment-Northeast. For the Carolinian province (Fig. 1), data from 1,124 stations were assembled from three projects: 1993 to 1997 EMAP, Carolinian province, 1997 to 1998 Mid-Atlantic Integrated Assessment, and 2000 to 2004 National Coastal Assessment-Southeast. Data included percentage silt-clay, TOC, bottom dissolved oxygen, chemical contaminants in sediment, and measures of biological impairment (benthic indices and toxicity tests). When available, surface (within the top 1.5 m of the water column) nutrient and chlorophyll data were also obtained. All data were collected during a summer index period, May to early October, with most samples taken from July to September.

Data were collected and analyzed using common protocols (EMAP metadata; www.epa.gov/emap). Briefly, grain size was determined by weight using sieves and pipets. Sediments were acidified, and TOC was measured with a CHN (carbon, hydrogen, nitrogen) analyzer. Single measurements of dissolved oxygen were obtained using a conductivity, temperature, depth-deployed instrument in bottom waters during daylight hours. Water column nutrients including total dissolved nitrogen (TDN), particulate organic nitrogen, ammonium (NH_4), nitrate + nitrite (NO_2NO_3), nitrate (NO_3), and nitrite (NO_2) were measured with a nutrient analyzer. Chlorophyll *a* was measured by fluorometry. Organic contaminants were solvent extracted from sediments and analyzed using a gas chromatography–electron capture detector or gas chromatography–mass spectroscopy. These organic contaminants included polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and selected pesticides. Metal contaminants such as Ag, Cd, Cr, Cu, Ni, Pb, and Zn in sediments were acid extracted and analyzed using inductively coupled plasma–atomic emission spectroscopy or graphite furnace atomic absorption. Potential for sediment contaminants to cause adverse biological effects was evaluated by using effects range median quotients (ERMQ) [21] and equilibrium sediment benchmarks (ESBs) [22–24]. The ERMQ is calculated by dividing each individual chemical concentration by its associated effects range median (ER-M) value and then averaging across all chemicals, resulting in a unitless value. The ERMQ risk levels were calculated empirically based on percentiles [25]. The ESBs calculate the bioavailable fraction by accounting for sediment binding factors: acid volatile sulfide (AVS) for metals and TOC for organics. The ESB for metals is calculated as the difference between the molar concentration of sediment-associated metals and AVS. If this difference exceeds zero, the sediments may be toxic because of bioavailable metals; if the difference is less than or equal to zero, metals are not expected to cause toxicity. The organic ESB estimates the dissolved concentration in the sediment interstitial waters as a function of the concentration associated with the TOC (i.e., not bioavailable). The dissolved concentration is then divided by a known effects concentration, expressed as toxic units (TU). The ERMQ assesses whether toxic effects are likely to be seen, and the ESBs indicate whether observed effects can be attributed to certain classes of contaminants, such as metals or organics. When insufficient AVS data were available to assess metal ESBs, sediment contaminant levels were compared with ER-Ms and effects range low (ER-L) [26] to predict whether the levels of contaminants would be expected to cause adverse biological impact. Ten-day sediment toxicity tests were conducted using the amphipod *Ampelisca abdita* [27]. Benthic indices were calculated based on benthic invertebrate abundance measures and calibrated for each province [4,28,29]. The Acadian province benthic index [29] is calculated using logistic regression with Shannon–Weiner diversity, Rosenberg's species pollution tolerance measure, and the percentage of capitellid polychaetes. This index ranges from 0 to 10. The threshold for impairment is suggested to be identified using signal detection theory based on management objectives. The Virginian province benthic index [4] is calculated using discriminant function analysis with salinity-normalized Gleason's diversity, spionid polychaete abundance, and salinity-normalized tubificid oligochate abundance. Values less than zero are considered impaired. The Carolinian benthic index [28] is a multimetric index subdivided by salinity zone and latitude. The index is composed of abundance, species richness, dominance of two most abundant taxa, and percentage of pollution-sensitive taxa. The index uses scores from 0 to 5, with scores <3 being impaired. The benthic invertebrates used to develop these indices were collected on a 0.5-mm screen and identified to species, where feasible.

Development of TOC and grain size relationship

The initial relationship between TOC and grain size was developed separately for each province using reference data. Reference data were utilized to ensure that the relationship between TOC and grain size reflected, as much as possible, conditions in the absence of anthropogenic disturbance. It was assumed that OC loading and processing would vary with latitude, so the provinces might respond differently. Reference stations were identified as those with low toxicity (amphipod survival $\geq$80%), low levels of chemical contamination ($\leq$3 ER-L exceedences and no ER-M exceedences), and bottom dissolved oxygen concentrations $\geq$5 mg/L. A regression was performed on the square root of TOC on the percentage silt-clay. The square root transformation was appropriate because the radius of a sphere (related to grain size) is squared to yield surface area. This transformation also equalized variance across the range of silt-clay and made the distribution more closely approximate normality. Values above or below the 99% confidence intervals were removed as outliers. The regression was repeated iteratively until there were no outliers, generating the final relationship and its 99% confidence intervals. This is similar to the approach applied by Calabretta and Oviatt [30] to develop a relationship between metals and iron to allow diagnosis of metal enrichment in sediments.

Once the final reference relationships were developed, the upper 99% confidence interval was used to identify stations that had higher than expected TOC-grain size values (enriched) as well as stations that were within the expected range of TOC-grain size (reference). Preliminary analysis indicated that those stations with TOC-grain size values below what was expected based on the relationship were not significantly different from reference values for any of the variables analyzed, so they were combined with the reference stations for future comparisons between reference and enriched sites. To address potential circularity (e.g., some variables compared between reference and enriched sites were also used to identify reference sites), screening variables were removed one by one and multiple reference relationships developed. Then, those variables not used in reference screening were compared using *t* tests. The results of these comparisons were similar to the original results, so the reference relationships developed using toxicity, chemical contamination, and dissolved oxygen were utilized. The slopes of the relationships developed for each province were compared by using ANOVA in SPSS version 16, $\alpha = 0.05$.

Comparison of reference and enriched sites

Dissolved oxygen, nutrients, chlorophyll *a*, amphipod toxicity, and benthic indices were examined for differences between reference and enriched sites using *t* tests in SPSS version 16, $\alpha = 0.05$. Amphipod toxicity, ERMQ, ESBs, chlorophyll *a*, and nutrient data were highly skewed and were therefore transformed by taking the log or square root prior to analysis. Benthic indices, which were expected to be higher at reference sites, were assessed using one-tailed tests. Amphipod toxicity, ERMQ, ESBs, and metals were assessed using two-tailed tests. Dissolved oxygen was expected to decline with enrichment and was also assessed by using a one-tailed test. Nutrients and chlorophyll *a* were assessed using one-tailed tests, with the expectation that these variables would be elevated at enriched sites. Results are presented as mean $\pm$ stan-standard error.

For the Virginian province, estuarine subsets were examined to determine whether the patterns observed over a large geographic range would also be evident at smaller scales. The relationship between TOC and grain size developed for the entire Virginian province was used to classify sites as being either reference or enriched. Only Narragansett Bay, Long Island Sound, Barnegat Bay, Delaware Bay, and Severn River (Fig. 2) had enough stations of each type (reference or enriched) to perform further analysis. Comparisons between reference and enriched sites were conducted for all the variables identified above for these five estuaries in SPSS. Results are presented as mean $\pm$ standard error.

RESULTS

TOC/Grain size relationship development

The relationship between TOC and grain size was significant in all provinces ($p < 0.0005$). In the Acadian province, 65.6% of the variation in TOC in reference sites was explained by percentage silt-clay (i.e., grain size). The regression relationship was developed based on 105 reference sites. The final relationship was

$$\text{Square root(TOC)} = 0.011 \cdot (\%\text{silt-clay}) + 0.556 \tag{1}$$

In the Virginian province, 85.5% of the variation in TOC in reference sites was explained by percentage silt-clay. The regression relationship was developed based on 359 reference sites. The final relationship was

$$\text{Square root(TOC)} = 0.012 \cdot (\%\text{silt-clay}) + 0.402 \tag{2}$$

In the Carolinian province, 84.6% of the variation in TOC in reference sites was explained by percentage silt-clay. The

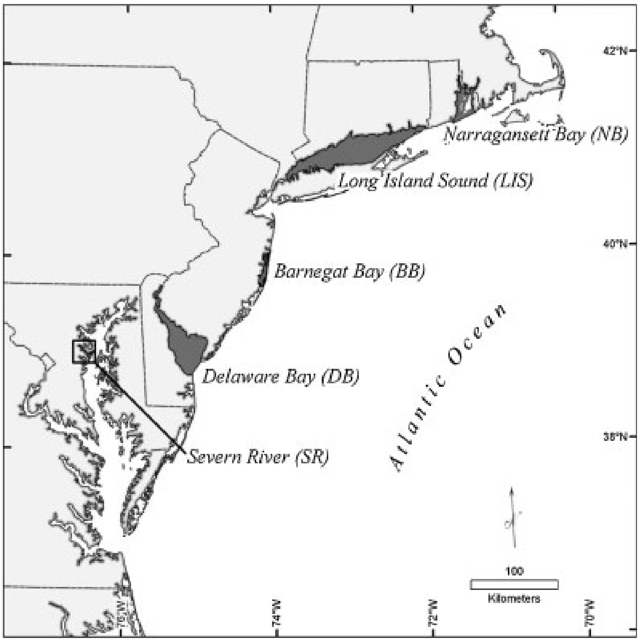

Fig. 2. Map of individual estuaries in the Virginian biogeographic province (USA) used for subset analysis.

regression relationship was developed based on 446 reference sites. The final relationship was

$$\text{Square root (TOC)} = 0.018 \cdot (\%\,\text{silt-clay}) + 0.345 \qquad (3)$$

The 99% upper confidence intervals were used to separate enriched from reference sites (Fig. 3). Significant differences were observed in slopes among the three provinces ($p = 0.0003$).

Acadian province

Significant differences were observed in the Acadian province benthic index between reference and enriched sites ($p < 0.0005$; Fig. 4a). Although no set threshold defines impairment for this index [29], separation between reference and enriched sites was clear.

A significant difference ($p = 0.002$) was noted in ERMQ between reference (0.094 ± 0.006) and enriched (0.212 ± 0.084) sites. Based on the work of Long et al. [31], this would correspond to high amphipod toxicity 11.6% of the time in reference sites and 31.9% of the time in enriched sites. No AVS data were available, so sediment metal concentrations were examined directly. All metal concentrations were significantly different between enrichment categories, with the exception of Mn and Ni (Supplemental Data, Table S1). No ER-Ls were exceeded at the reference sites. At the enriched sites, the ER-L, but not the ER-M was exceeded for Cr, Hg, and Pb, meaning that adverse effects were possible but not probable (Supplemental Data, Table S1). The ESBs for organic contaminants indicated no significant differences between reference and enriched sites for PAHs ($p = 0.570$), PCBs ($p = 0.073$), or all organics ($p = 0.443$). In fact, all ESBs had slightly higher levels at reference sites (PAH $= 2.919 \pm 0.913$ TU, PCB $= 0.003 \pm 0.001$ TU, All $= 3.776 \pm 1.0500$ TU) than at enriched sites (PAH $= 1.477 \pm 0.486$ TU, PCB $= 0.002 \pm 0.001$ ± 0.001 TU, All $= 1.736 \pm 0.579$ TU). Amphipod survival was high, with no significant difference ($p = 0.937$) seen between reference sites (93.9%) and enriched sites (94.5%).

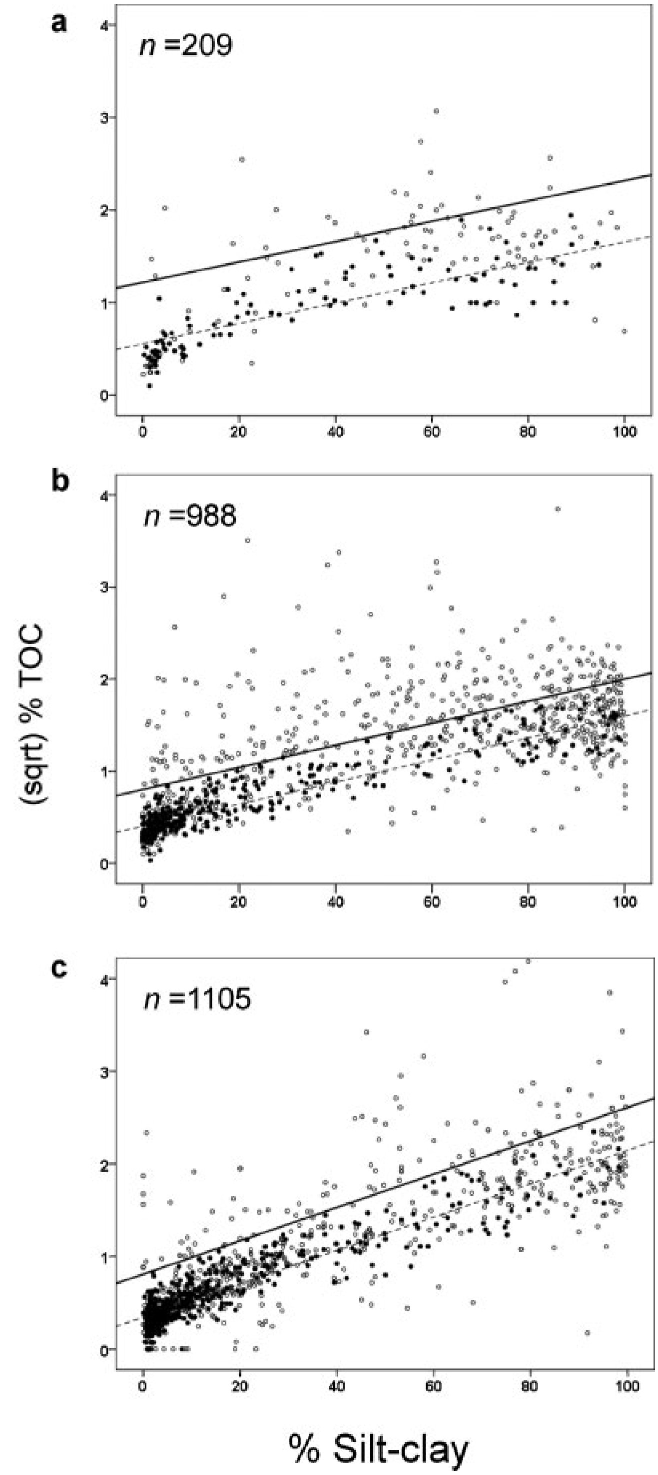

Fig. 3. Graphs of data used in this analysis. The solid circles show those stations used to develop the relationship between organic carbon and grain size. The dotted line represents the reference relationship. The solid line is the upper 99% confidence interval. Points above these lines indicate enriched sites. (**a**) Acadian province. (**b**) Virginian province. (**c**) Carolinian province (USA). TOC = total organic carbon; sqrt = square root.

Dissolved oxygen was significantly lower at enriched sites than at reference sites (Table 1). Chlorophyll *a* levels were also significantly higher at enriched sites relative to reference sites (Table 1). All measured nutrients were elevated at enriched sites relative to reference sites, but this difference was statistically significant only for nitrate + nitrite (NO_2NO_3; Table 1).

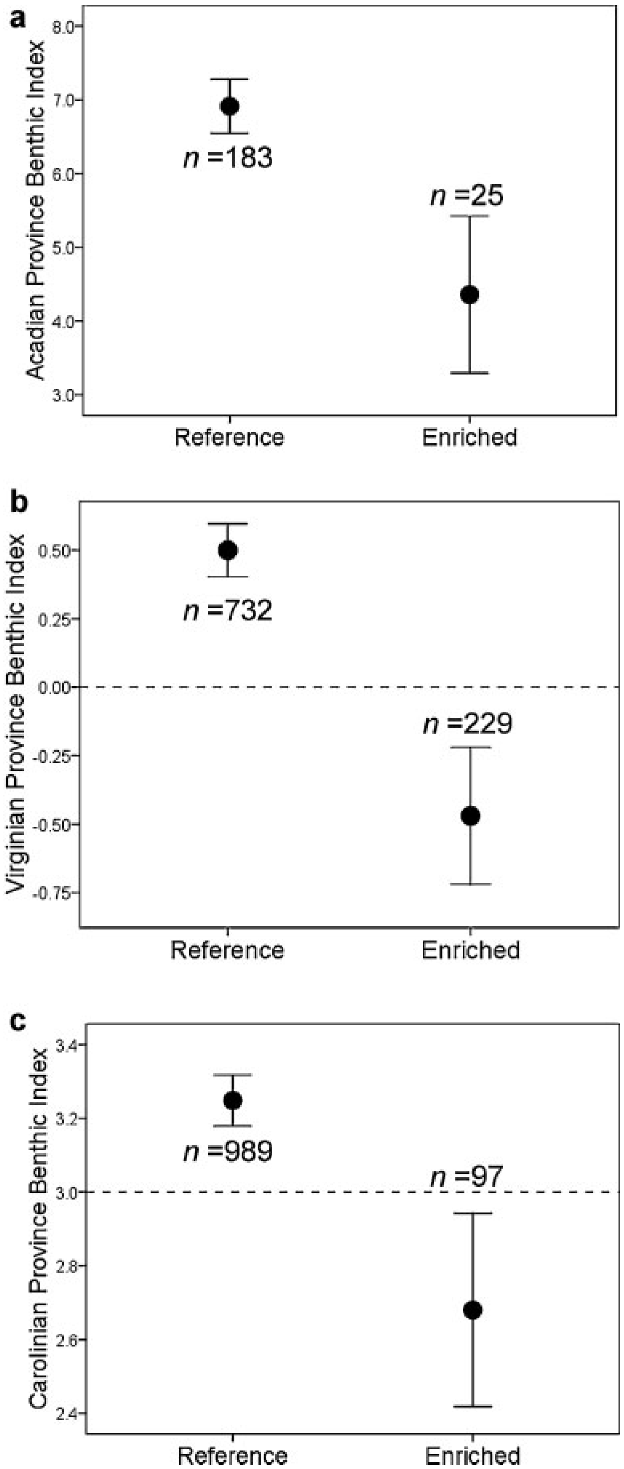

Fig. 4. Benthic indices for all three provinces (mean ± standard error). The dotted line indicates the impaired–unimpaired threshold. There is no set threshold for the Acadian province (USA). (**a**) Acadian province (index ranges from 0 to 10). (**b**) Virginian province (values ≥0 are unimpaired). (**c**) Carolinian province (index ranges from 0 to 5, values ≥3 are unimpaired).

Virginian province

Significant differences were observed in the Virginian province benthic index between reference and enriched sites ($p < 0.0005$; Fig. 4b). The reference sites had values generally ≥0, indicating a lack of benthic impairment [4], whereas enriched sites were generally <0 (impaired).

A significant difference was noted in ERMQ ($p < 0.0005$) between the reference (0.111 ± 0.008) and enriched (0.244 ± 0.015) sites, indicating that both categories had a high risk of degraded benthos resulting from chemical contamination [25]. The ESB for metals (SEM-AVS) [23] was not significantly different ($p = 0.739$) between reference (-1.597 ± 0.084 μmol/g) and enriched (-1.661 ± 0.156 μmol/g) sites. Both categories had negative values, indicating that impairment was not due to metals. The ESBs for organic contaminants indicated no significant differences between reference and enriched sites for PAHs and all organics (PAH $p = 0.348$, All $p = 0.236$), and neither class of site had toxic units high enough to cause toxicity. A significant difference was observed in PCB ESB ($p = 0.014$), but levels were higher at reference sites (0.014 ± 0.004 TU) than at enriched sites (0.006 ± 0.001 TU). In fact, ESBs also had slightly higher levels at reference sites than at enriched sites for PAHs (reference $= 2.901 \pm 1.214$ TU, enriched $= 2.876 \pm 1.073$ TU) and all organics (reference $= 4.616 \pm 1.445$ TU, enriched $= 3.946 \pm 1.145$ TU). Although amphipod survival was significantly higher ($p = 0.024$) at reference sites (94.4%) than at enriched sites (90.5%), the difference is not considered biologically significant, insofar as both categories had had survival greater than 80%.

Dissolved oxygen was significantly lower at enriched sites than at reference sites (Table 2). Chlorophyll *a* levels were also significantly higher at enriched sites relative to reference sites (Table 2). Total dissolved nitrogen (TDN), ammonium (NH_4), nitrate + nitrite (NO_2NO_3), nitrate (NO_3), and nitrite (NO_2) were also significantly elevated at enriched sites (Table 2).

Carolinian province

Significant differences were noted in the Carolinian province benthic index between reference and enriched sites ($p < 0.0005$; Fig. 4c). The reference sites had values generally ≥3, indicating a lack of benthic impairment [28], while enriched sites were generally <3 (impaired).

A significant difference was observed in ERMQ between the reference (0.039 ± 0.002) and enriched (0.154 ± 0.041) sites ($p < 0.0005$). Reference sites were expected to have a medium risk of degraded benthos, whereas enriched sites were expected to be at high risk [25]. The ESB for metals (SEM–AVS) [23] was not significantly different ($p = 0.784$) between reference (-15.613 ± 4.457 μmol/g) and enriched (-13.163 ± 3.722 μmol/g) sites. Negative SEM–AVS indicate that metals were not bioavailable and therefore did not cause impairment. The ESBs for organics indicated no significant differences between reference and enriched sites for PAHs ($p = 0.293$) or all organics ($p = 0.065$). A significant difference in PCB ESB ($p = 0.019$) was noted, but levels were higher at reference sites (0.002 ± 0.000 TU) than at enriched sites (0.001 ± 0.000 TU). As in the other provinces, all ESBs had slightly higher levels at reference sites (PAH $= 0.340 \pm 0.047$ TU, all $= 1.878 \pm 0.702$ TU) than at enriched sites (PAH $= 0.255 \pm 0.057$ TU, all $= 0.554 \pm 0.126$ TU). Amphipod survival was not significantly different ($p = 0.072$) between reference (96.2%) and enriched (92.9%) sites and is not biologically significant, being greater than 80%.

Dissolved oxygen and chlorophyll *a* were not significantly different between reference and enriched sites (Table 3). Among all the measured nutrients, only nitrate + nitrite (NO_2NO_3) was statistically higher at enriched sites relative to reference sites (Table 3).

Virginian province subsets

Significant differences were observed in the Virginian province benthic index [4] between reference and enriched sites in Narragansett Bay (NB; $p < 0.0005$), Barnegat Bay (BB;

Table 1. Dissolved oxygen, chlorophyll *a,* and nutrient data from the Acadian province (USA)

	Units	Reference n	Reference Mean ± SD	Enriched n	Enriched Mean ± SD	p Value	Significant?
Dissolved oxygen	mg/L	172	7.91 ± 0.079	22	7.31 ± 0.283	0.008	Yes
Chlorophyll *a*	μg/L	165	2.59 ± 0.188	22	3.97 ± 0.724	0.004	Yes
NH_4	mg/L as N	121	0.079 ± 0.019	19	0.082 ± 0.015	0.474	No
NO_2NO_3	mg/L as N	113	0.038 ± 0.005	16	0.094 ± 0.032	0.009	Yes
NO_3	mg/L as N	78	0.041 ± 0.006	9	0.084 ± 0.048	0.295	No
NO_2	mg/L as N	48	0.004 ± 0.000	10	0.005 ± 0.001	0.286	No

Table 2. Dissolved oxygen, chlorophyll *a*, and nutrient data from the Virginian province (USA)

	Units	Reference n	Reference Mean ± SD	Enriched n	Enriched Mean ± SD	p Value	Significant?
Dissolved oxygen	mg/L	709	6.24 ± 0.073	223	5.65 ± 0.154	0.0005	Yes
Chlorophyll *a*	μg/L	337	10.8 ± 0.57	143	15.9 ± 1.56	0.002	Yes
Particulate organic nitrogen	mg/L as N	164	0.354 ± 0.014	45	0.357 ± 0.045	0.277	No
Total dissolved nitrogen	mg/L as N	157	0.587 ± 0.037	41	0.918 ± 0.127	0.0165	Yes
NH_4	mg/L as N	334	0.047 ± 0.004	144	0.069 ± 0.008	0.003	Yes
NO_2NO_3	mg/L as N	329	0.183 ± 0.022	145	0.434 ± 0.063	<0.0005	Yes
NO_3	mg/L as N	64	0.294 ± 0.052	42	0.764 ± 0.159	0.0075	Yes
NO_2	mg/L as N	274	0.009 ± 0.001	128	0.014 ± 0.002	0.0005	Yes

$p = 0.035$), Delaware Bay (DB; $p < 0.0005$), and Severn River (SR; $p = 0.003$; Fig. 5). Overall, the reference sites had values generally >0, indicating a lack of benthic impairment, while enriched sites were generally <0 (impaired). No significant difference ($p = 0.406$) was noted in the Virginian province benthic index between reference sites (0.123) and enriched sites (0.042) in Long Island Sound (LIS).

Although significant differences were noted in ERMQ between reference and enriched sites in all estuaries, the response varied (Supplemental Data, Table S2). In NB, BB, and DB, reference sites would be expected to have a medium risk of degraded benthos, whereas, at enriched sites, this risk would be expected to be high [25]. In LIS and SR, both reference and enriched sites were considered at high risk for degraded benthos [25].

Only DB and SR had sufficient SEM and AVS data to assess the ESB for metals. In both estuaries, there were no significant differences (DB $p = 0.084$, SR $p = 0.714$) between reference (DB = − 0.817 ± 0.224 μmol/g, SR = − 1.771 ± 0.286 μmol/g) and enriched (DB = − 1.676 ± 0.432 μmol/g, SR = − 1.620 ± 0.264 μmol/g) sites. Because all values were negative, impairment was not due to metals. In the remaining estuaries, the sediment metal values were compared with ER-L and ER-M values [26]. In NB, all metals were significantly different between enrichment categories (Supplemental Data, Table S3). However, the ER-L was not exceeded by any metal at the reference sites. At enriched sites, Cr, Cu, Hg, Pb, and Zn exceeded the ER-L but not the ER-M, indicating that adverse effects were possible but not probable. Silver exceeded both the ER-L and the ER-M, indicating that adverse effects were probable. However, even assuming a very low AVS level comparable to that found in sand (1 μmol/g) [32], all Ag would be bound and not bioavailable, so no adverse effects would be expected. In LIS, there were significant differences between reference and enriched sites for Ag, As, Cd, Cu, Pb, Se, Sn, and Zn (Supplemental Data, Table S4). The ER-L for Ag was exceeded at both reference and enriched sites, whereas the ER-M was only exceeded at enriched sites. As described above, assuming a very low AVS value of 1 μmol/g, silver would not be bioavailable. For Hg and Pb, the ER-L, but not ER-M, was exceeded at both reference and enriched sites. The ER-L, but not ER-M, was exceeded at enriched sites only for Cu and Zn. This indicates that adverse effects were possible but not probable. In BB, all metals, with the exception of Ag, Al, and Mn, were significantly different between reference and enriched sites (Supplemental Data, Table S5). No metals exceeded the

Table 3. Dissolved oxygen, chlorophyll *a*, and nutrient data from the Carolinian province (USA)

	Units	Reference n	Reference Mean ± SD	Enriched n	Enriched Mean ± SD	p Value	Significant?
Dissolved oxygen	mg/L	983	5.32 ± 0.05	94	5.24 ± 0.21 + I4	0.727	No
Chlorophyll *a*	μg/L	701	10.25 ± 0.28	58	12.61 ± 1.97	0.1895	No
Total dissolved nitrogen	mg/L as N	36	0.446 ± 0.047	10	0.501 ± 0.035	0.1755	No
Total nitrogen	mg/L as N	238	0.643 ± 0.017	29	0.674 ± 0.059	0.283	No
NH_4	mg/L as N	698	0.049 ± 0.002	59	0.032 ± 0.006	>0.9995	No
NO_2NO_3	mg/L as N	699	0.065 ± 0.005	58	0.099 ± 0.018	0.0285	Yes
NO_3	mg/L as N	301	0.057 ± 0.008	26	0.100 ± 0.031	0.0895	No
NO_2	mg/L as N	392	0.025 ± 0.002	33	0.023 ± 0.009	0.8745	No

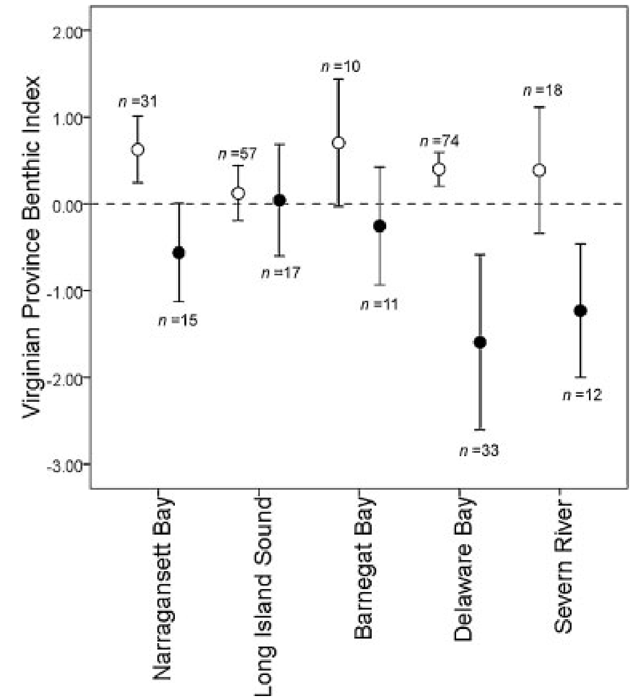

Fig. 5. Benthic indices for individual estuaries in the Virginian province (USA; mean ± standard error). Open circles are reference sites; solid circles are enriched sites. The dotted line indicates the impaired–unimpaired threshold.

ER-L at the reference sites. At the enriched sites, only Hg and Pb were above the ER-L, and both were below the ER-M, indicating that adverse effects were possible but not probable. No significant differences were noted in ESBs for PAHs, PCBs, or all organics between reference and enriched sites in any estuary, although the patterns varied (Supplemental Data, Table S6).

Amphipod survival was high in all subset estuaries regardless of enrichment category (Supplemental Data, Table S7). In NB and LIS significant differences were noted between reference and enriched sites. Survival was greater than 80%, so these differences were not considered to be biologically significant. In BB, DB, and SR, no statistical differences between reference and enriched sites were observed, and survival was always greater than 80%.

Mean bottom dissolved oxygen levels were significantly lower at enriched sites in NB ($p = 0.005$), DB ($p = 0.0005$), and SR ($p = 0.003$) but were not significantly different in LIS or BB (Table 4). Chlorophyll *a* levels were significantly higher at enriched sites relative to reference sites in NB ($p = 0.021$) but were not significantly different in any other estuary (Table 4). Nutrient concentrations varied by estuary (Table 4). In NB, LIS, and SR, no significant differences were observed between reference and enriched sites for any of the measure nutrients (Table 4). In BB, NH_4 was significantly higher ($p = 0.048$) at enriched sites than at reference sites. In DB, TDN ($p = 0.005$) and NH_4 ($p = 0.045$) were significantly higher at enriched sites relative to reference sites.

DISCUSSION

The present study was conducted to determine whether the relationship between TOC and grain size could be used to indicate adverse environmental impact to the benthos and to diagnose eutrophication as the cause. The Pearson and Rosenberg [6] model proposes that, as OC loading increases, there are predictable adverse changes in benthic communities. However, these changes have also been associated with chemical contamination [33]. Because these responses can be attributed to either eutrophication or pollutant chemical loading, it is important to be able to distinguish between them.

One potential problem with comparing sediment measures to water column measures is the lack of fit between spatial and temporal scales. Although the sediment record and benthic invertebrates integrate conditions over months to years at a given location, water column measures are more ephemeral and spatially patchy. Nutrient and chlorophyll *a* measures are particularly problematic, in that nutrients are constantly cycled through the ecosystem, especially during the growing season [34,35]. Identifying anthropogenic impacts of eutrophication might be difficult because of the natural variability in nutrient concentrations and related processes [13]. Chlorophyll levels

Table 4. Dissolved oxygen, chlorophyll *a*, and nutrient data from estuary subsets in the Virginian province (USA)

		Narragansett Bay		Long Island Sound		Barnegat Bay		Delaware Bay		Severn River	
		n	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD
Dissolved	Reference	31	6.20 ± 0.271[a]	62	5.41 ± 0.224	11	7.45 ± 0.404	66	6.82 ± 0.148[a]	17	4.88 ± 0.676[a]
oxygen (mg/L)	Enriched	16	4.67 ± 0.578[a]	12	5.16 ± 0.483	11	6.87 ± 0.561	35	5.70 ± 0.371[a]	12	1.80 ± 0.694[a]
Chlorophyll *a*	Reference	27	8.15 ± 2.52[a]	15	3.58 ± 0.625	7	5.40 ± 1.66	51	8.21 ± 0.852	17	18.4 ± 1.78
(μg/L)	Enriched	15	20.2 ± 6.54[a]	8	1.82 ± 0.613	8	9.31 ± 2.34	32	13.3 ± 3.26	12	14.0 ± 1.95
Total dissolved nitrogen	Reference							24	0.891 ± 0.122[a]	17	0.355 ± 0.021
(mg/L as N)	Enriched							11	1.73 ± 0.270[a]	11	0.309 ± 0.016
Particulate organic nitrogen	Reference							25	0.318 ± 0.030	17	0.471 ± 0.032
(mg/L as N)	Enriched							12	0.484 ± 0.152	12	0.343 ± 0.035
NH_4	Reference	27	0.084 ± 0.027	17	0.055 ± 0.028	8	0.014 ± 0.005[a]	50	0.063 ± 0.008[a]	17	0.034 ± 0.011
(mg/L as N)	Enriched	14	0.038 ± 0.012	8	0.018 ± 0.013	8	0.029 ± 0.007[a]	32	0.129 ± 0.022[a]	11	0.023 ± 0.006
NO_2NO_3	Reference	27	0.082 ± 0.041	17	0.083 ± 0.029	8	0.013 ± 0.001	50	0.483 ± 0.065	17	0.009 ± 0.001
(mg/L as N)	Enriched	14	0.052 ± 0.028	8	0.057 ± 0.041	8	0.046 ± 0.034	32	0.800 ± 0.135	12	0.016 ± 0.007
NO_3	Reference							21	0.442 ± 0.063		
(mg/L as N)	Enriched							12	0.650 ± 0.191		
NO_2	Reference	27	0.003 ± 0.001			8	0.002 ± 0.001	50	0.019 ± 0.002	17	0.002 ± 0.000
(mg/L as N)	Enriched	14	0.003 ± 0.001			8	0.002 ± 0.000	32	0.029 ± 0.005	12	0.002 ± 0.000

[a] Significantly different between reference and enriched sites.

tend to be similarly variable, insofar as blooms are episodic (days to weeks) and quickly dissipate through grazing, advection, or settlement [14]. Using the large EMAP data set that spanned both temporal and spatial scales increased our ability to distinguish underlying patterns despite the inherent variability in the data.

The relationship between TOC and grain size was developed separately for each province. The difference in slopes seen among the provinces likely reflects their different geographic settings. The Acadian province is relatively undeveloped, with rocky coasts and extensive oceanic influence. In contrast, the Carolinian province is characterized by relatively flat topography with extensive tidal marshes and relatively small tidal ranges. The Virginian province is more highly urbanized than the Acadian province, but does not have extensive tidal marshes as found in the Carolinian province. These differences likely result in different OC sources, quality, and processing.

The relationship between TOC and grain size developed with reference sites in the Acadian, Virginian, and Carolinian provinces appears to be a useful tool to identify eutrophied sites. The enriched sites had significantly impaired benthic invertebrate communities as measured by benthic indices, whereas reference sites were unimpaired. This held true for all of the broader areas (the entire Acadian, Virginian, or Carolinian province) and most of the individual estuaries (Narragansett Bay, Barnegat Bay, Delaware Bay, and Severn River) but not Long Island Sound.

Although benthos can be impaired as a result of elevated contaminant levels and organic enrichment, our analysis indicated that contaminants did not appear to be strongly related to enrichment as defined by the relationship between TOC and silt-clay. In all three provinces, the ERMQ was significantly higher in enriched sites relative to reference sites and generally indicated a potential risk for degraded benthos. However, no consistent pattern indicating higher risk at enriched sites was observed. In contrast, ESBs for metals and organics were either not statistically different between enrichment categories or were higher at reference sites. It is not too surprising that bioavailable metals and organic contaminants would be lower at enriched sites: the higher OC is available to bind organics and some metal fractions. Furthermore, organically enriched sites can also be areas of high sulfides [5], which bind many cationic metals. In the Acadian province, metal ESBs could not be calculated, but no ER-Ms were exceeded for any metal, indicating that effects were not probable. This lack of correspondence between ERMQ and ESBs has been seen previously [36]. Burgess et al. [36] suggested that the accuracy of the different approaches be assessed via sediment toxicity testing. In the present study, toxicity testing indicated that amphipod survival was always high, with no biologically significant differences seen, more closely matching the ESB predictions and the direct examination of ER-Ls and ER-Ms. The individual estuaries examined here showed patterns similar to the pattrerns seen in the entire Virginian province. The ERMQ was significantly higher at enriched sites relative to reference sites for all estuaries and, generally, indicated a potential risk for degraded benthos, but again no consistent pattern indicating elevated risk in enriched sites was noted. In contrast, ESBs for metals and organics either were not statistically significant between enrichment categories or were higher at reference sites. In two estuaries (NB and LIS), Ag was above the ER-M level, indicating that adverse effects were probable. However, even at very low AVS levels, no Ag would be bioavailable. No other metal exceeded the ER-M in any estuary, although the ER-L was sometimes exceeded, indicating that adverse effects were possible. Over the entire Virginian province, amphipod survival was always greater than 80%.

The contaminant analysis and toxicity tests showed no consistent evidence that the benthic community impairment observed in enriched sites as defined by the relationship between TOC and silt-clay was strongly related to anthropogenic chemicals. In contrast, common eutrophication measures (dissolved oxygen, chlorophyll *a*, and nutrients) were more consistently related to enrichment. We expected that areas with elevated TOC sediments would have higher biological sediment demand, resulting in lower bottom dissolved oxygen levels [37,38]. Limited flushing may enhance carbon deposition, resulting in areas of enhanced hypoxia. Because of this, we examined water quality data to determine whether, as expected, dissolved oxygen was depressed. We also anticipated elevated chlorophyll *a* and nutrient values. With higher nutrient loading, there would be increased phytoplankton production [2,13]. Eventually this biomass or a portion of it would be delivered to the bottom of the estuary, resulting in higher TOC levels relative to sediment grain size.

In the Acadian and Virginian provinces, dissolved oxygen levels were lower at enriched sites than at reference sites, whereas dissolved oxygen was not significantly different between enrichment types in the Carolinian province. This matched results from the National Oceanic and Atmospheric Administration (NOAA) Estuarine Eutrophication Study [2], which showed that the South Atlantic Region (roughly, the Carolinian province) had moderate to high susceptibility to eutrophication (i.e., low to moderate flushing and dilution) and moderate to high nutrient loads. However, most estuaries had low to moderate problems with dissolved oxygen. In the Virginian province, dissolved oxygen levels were significantly lower at enriched sites in Narragansett Bay, Delaware Bay, and Severn River but the levels were not expected to drop below 2 mg/L, which would adversely affect the benthos [39]. Because water quality data were collected during the day, when dissolved oxygen levels would be expected to be relatively high [40], the values likely did not reflect average daily dissolved oxygen concentrations. In addition, measurements taken in the water column (even those collected in bottom water) indicate the oxygen conditions present at a particular time, whereas measurements taken from the sediments represent an integrative measure of conditions in the area. Although compiling a large number of daytime oxygen measures in an area is expected to provide a more representative picture of ambient conditions, it does not capture the lowest nighttime values, when respiration is dominant.

The premise of this research is that enriched sites are eutrophied. Thus, we expected and often observed that there would be higher chlorophyll *a* or elevated nutrients at enriched sites. In the Acadian province, chlorophyll *a* values and NO_2NO_3 were higher at enriched sites than at reference sites. In the Virginian province, chlorophyll *a*, TDN, NH_4, NO_2NO_3, NO_3, and NO_2 were significantly elevated at enriched sites relative to reference sites. In the Carolinian province, only NO_2NO_3 was higher at enriched sites relative to reference sites. The individual estuaries, which had limited data, showed a variable response. In Narragansett Bay, only chlorophyll *a* was significantly higher at enriched sites relative to reference sites, whereas nutrients were not significantly different between reference and enriched sites. In Barnegat Bay, NH_4 was significantly higher at enriched sites. In Delaware Bay, both TDN and NH_4 were significantly higher at enriched sites. In Long Island Sound and Severn River, there were no significant differences in chlorophyll *a* or nutrients between enrichment categories.

The Long Island Sound results were unexpected, with no significant differences observed in the benthic index at enriched and reference sites. This estuary has a highly developed watershed and summer stratification that leads to seasonal hypoxia [2]. However, the hypoxia varies spatially and temporally, with low dissolved oxygen conditions more frequently seen in the western portions of the Sound [38]. In fact, the enriched sites were found mostly in the western end of Long Island Sound. Spatial and temporal variability in oxygen conditions might have contributed to the lack of response we saw in the present study. With a higher number of samples, perhaps patterns would be more evident.

The variation seen among provinces and in individual estuaries was not surprising. It was similar to what was seen in NOAA's National Eutrophication Survey [2], with the Acadian province being the least impacted by eutrophication and the Virginian province being the most affected. For example, NO_2NO_3 was over four times higher in the Virginian province relative to the Acadian province. Dissolved oxygen levels were also very high in the Acadian province, despite being statistically different between enrichment types. Interestingly, nitrate + nitrite levels were similar in the Acadian and Carolinian province. This may be due to extensive marshes in the Carolinian province that are able to absorb higher amounts of nutrients [18] so that levels are more similar to the Acadian province, which has low nutrient loads and a predominantly rocky coast.

In the Virginian province, all the expected signs of eutrophication were seen in the water column corresponding to enriched sites. For example, dissolved oxygen levels were depressed; chlorophyll *a* and nutrient levels were elevated. In the Acadian province, chlorophyll *a* and NO_2NO_3 were elevated. In the Carolinian province, only nitrate + nitrite was significantly elevated at enriched sites, although other nutrients trended higher there. The subset of estuaries in the Virginian province all showed the expected differences in water column variables between eutrophication categories, but the significance of individual water column variables varied. For example, Narragansett Bay had lower dissolved oxygen and elevated chlorophyll *a* at enriched sites but no differences in nutrients. Delaware Bay had low dissolved oxygen and elevated nutrients but not high chlorophyll *a* at enriched sites. In Barnegat Bay, only nutrients were elevated at enriched sites. In Severn River, only dissolved oxygen was depressed at enriched sites. This variability reflects both smaller samples and, likely, the large variability in nutrient load and system dynamics in the different estuaries.

The relationship between TOC and grain size was used to identify enriched (eutrophied) sites along the East Coast of the United States. Enriched sites had impacted benthos, whereas reference sites were not impacted. The contaminant analysis and toxicity tests did not provide strong evidence that enrichment as defined by the relationship between TOC and grain size was related to sediment contaminants, such as metals, PCBs, PAHs, and pesticides. This does not imply that chemical contaminant effects are unimportant, just that the relationship between TOC and grain size does not seem to be diagnostic of contaminant effects. Although variability in response was noted, reflecting the variance in the water column variables (i.e., dissolved oxygen, chlorophyll *a* and nutrients) and limited sample sizes, the data supported the hypothesis that sites designated as enriched were eutrophied. Dissolved oxygen levels were reduced at enriched sites, but chlorophyll *a* and nutrients were higher at enriched sites. Thus, the evidence supports the hypothesis that the relationship of TOC to grain size is an indicator of eutrophication. We believe that this screening tool will be useful to diagnose this common impairment in estuaries in support of the TMDL process.

SUPPLEMENTAL DATA

Table S1. Metals data from the Acadian Province. (28 KB XLS)

Table S2. ERMQ data from estuary subsets in the Virginian Province. (25 KB XLS)

Table S3. Metals data from Narragansett Bay. (28 KB XLS)

Table S4. Metals data from Long Island Sound. (28 KB XLS)

Table S5. Metals data from Barnegat Bay. (28 KB XLS)

Table S6. Organic ESBs for Virginian Province subset estuaries. (27 KB XLS)

Table S7. Amphipod toxicity data from estuary subsets in the Virginian Province. (26 KB XLS)

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