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DIAGNOSIS OF POTENTIAL STRESSORS ADVERSELY AFFECTING BENTHIC INVERTEBRATE COMMUNITIES IN GREENWICH BAY, RHODE ISLAND, USA

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Abstract

Greenwich Bay is an urbanized embayment of Narragansett Bay potentially impacted by multiple stressors. The present study identified the important stressors affecting Greenwich Bay benthic fauna. First, existing data and information were used to confirm that the waterbody was impaired. Second, the presence of source, stressor, and effect were established. Then linkages between source, stressor, and effect were developed. This allows identification of probable stressors adversely affecting the waterbody. Three pollutant categories were assessed: chemicals, nutrients, and suspended sediments. This weight of evidence approach indicated that Greenwich Bay was primarily impacted by eutrophication-related stressors. The sediments of Greenwich Bay were carbon enriched and low dissolved oxygen concentrations were commonly seen, especially in the western portions of Greenwich Bay. The benthic community was depauperate, as would be expected under oxygen stress. Although our analysis indicated that contaminant loads in Greenwich Bay were at concentrations where adverse effects might be expected, no toxicity was observed, due to high levels of organic carbon in these sediments reducing contaminant bioavailability. Our analysis also indicated that suspended sediment impacts were likely nonexistent for much of the Bay. This analysis demonstrates that the diagnostic procedure was useful to organize and assess the potential stressors impacting the ecological well-being of

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Data Availability: The reference data are available at <http://www.epa.gov/emap>. Some of the other data are available through links provided in the present study. The chemistry and toxicology data are provided in the Supplemental Data tables. Any remaining data can be requested from the authors (pelletier.peg@epa.gov).

Greenwich Bay. This diagnostic procedure is useful for management of waterbodies impacted by multiple stressors.

Keywords

Stressors; Water quality; Benthic invertebrates; Multivariate statistics; Toxicant identification

INTRODUCTION

Estuaries in the United States, especially those on the East Coast, have been impacted by anthropogenic stress for hundreds of years. As European colonization intensified, rivers were dammed, coastal marshes filled, and coastal birds, fish, and invertebrates overfished, and/or extirpated [1]. Narragansett Bay (Rhode Island, USA) is no exception. Many of the low lying mudflats and marshes surrounding major cities and towns around Narragansett Bay were filled in with sand, soil, and rock from surrounding areas to create solid land on which to build for commercial purposes. For example, much of the current developed downtown area of Providence, the largest city on Narragansett Bay, was originally mudflats, open waters, or marsh [2]. The industrial revolution brought population increases with corresponding inputs of sewage and chemicals to Narragansett Bay. In recent years, with the implementation of environmental regulations, steps have been taken to reduce the releases of pathogens, chemicals, and nutrients to Narragansett Bay through point source control and wastewater treatment upgrades; however, these historic stressors may still be causing adverse ecological effects.

Greenwich Bay is a small, shallow, urbanized embayment of Narragansett Bay (Figure 1). The embayment is spatially heterogeneous with the entire estuary impacted to a greater or lesser extent by stressors. The watershed is primarily residential. There is 1 wastewater treatment plant (WWTP) that discharges into Greenwich Cove, and many of the residential homes are serviced by septic systems and cesspools [3]. Greenwich Bay has long been impacted by organic enrichment and eutrophication [4,5]. Further, chemicals (legacy contaminants and contaminants of emerging concern) have historically been discharged into Greenwich Bay [6,7] and are still detected in the water column [8,9]. In addition, Greenwich Bay is known to be influenced by stressors originating from other parts of Narragansett Bay, especially from the Providence area [7,10]. A large and well publicized fish kill in Greenwich Bay in 2003 focused public and scientific effort on stressors impacting the bay. This event was due to anoxia caused by a combination of multiple environmental factors [11]. Finally, Greenwich Bay waters are listed on the 2002 303(d) list of impaired waters as being impacted by nutrients and low dissolved oxygen [12].

Due to the comingling of potential stressors multiple lines of evidence were needed to understand which stressors were primarily impacting benthic fauna. For the present investigation, we applied a diagnostic procedure [13], derived from the stressor identification and causal analysis decision information system [14], to identify the important stressors in Greenwich Bay affecting benthic fauna. This diagnostic procedure has 3 phases. First, existing data and information are used to confirm the waterbody impairment. Second, the

presence of source, stressor, and effect are established. Then linkages between source and stressor and between stressor and effect are developed. This allows identification of probable stressors adversely affecting the waterbody (Figure 2). For the present study, 3 pollutant categories were assessed: chemicals, nutrients, and suspended sediments, all of which are found in Greenwich Bay. Suspended sediment was included because it is 1 of the most common stressors listed in total maximum daily loads [15]. Benthic invertebrates were used to assess biological condition within the bay, and causative stressors were then diagnosed through a systematic assessment of the sources and stressors, and likelihood of effects of these stressors on the biological community. The procedure uses a weight of evidence approach to accurately diagnosis stressors in Greenwich Bay and to avoid incorrectly identifying stressors not impacting the waterbody.

MATERIALS AND METHODS

Study location and sampling

Greenwich Bay is found on the western side of Narragansett Bay, RI, within the Virginian biogeographic province (Figure 1). This embayment has 5 coves. Of these, Apponaug Cove, Greenwich Cove, and Warwick Cove are easily accessible by boat (Figure 3). The estuary is polyhaline and there is good tidal exchange with the larger Narragansett Bay system. Approximately 63% of the freshwater input comes from brooks entering Apponaug Cove and Greenwich Cove. The remainder of the freshwater input comes from surface runoff, ground water flow, the WWTP in Greenwich Cove, and smaller unmonitored streams [6].

Identification of reference stations

It was not possible to use any areas of Greenwich Bay as reference sites for the present study because the entire Greenwich Bay estuary is impacted to a greater or lesser extent by stressors. It was also not possible to use sites in unimpacted areas of Narragansett Bay because the physical conditions of those areas were not similar to those of the Greenwich Bay estuary. For these reasons, Greenwich Bay stations were compared with reference stations from the Virginia biogeographic province (Figure 1). All reference stations had similar depth, salinity, and percentage of silt to clay composition as the stations within Greenwich Bay (Figure 3), and were compiled using data from the US Environmental Protection Agency's Environmental Monitoring and Assessment program [16]. These stations were classified as reference using concurrently collected sediment contaminant data screened using the effect range median (ER-M) and effect range low (ER-L) thresholds [17,18], amphipod toxicity, total organic carbon (TOC), and dissolved oxygen concentrations [19,20]. The ER-M indicates a sediment concentration of a contaminant that will generally, but not always, be associated with adverse effects whereas the ER-L represents sediment contaminant concentrations below which adverse effects are unlikely to be observed [18]. All of the comparative reference stations had no ER-M and less than 4 ER-L exceedances, amphipod survival greater than 80% relative to the control, TOC less than or equal to 2%, and bottom dissolved oxygen greater than or equal to 5 mg/L.

Sample processing and analysis

TSSs.—Filters were dried at 60 °C for 24 h to 48 h, and the TSS in the water column determined by subtracting the initial weight of the filter from the final weight of the dried filter with sample.

Sediment TOC and grain size.—Sediment samples were dried at 60 °C, ground, homogenized, and acidified with 1 M HCl to remove carbonates. Following acidification the samples were dried at 60 °C, cooled, and stored in a desiccator until analysis. The samples were analyzed using a Carlo Erba model EA 1110 elemental analyzer (Rodano). Sediment grain size was determined by laser diffraction using a Malvern Mastersizer 2000. Samples were run in duplicate and each replicate subsampled 3 times. The percentage of clay (particles between 0–3.9 μm), percentage of silt (particles between 3.91–62.5 μm), and percentage of sand (particles between 62.51–2000 μm) were calculated using the Udden-Wentworth scale [21].

Sediment polycyclic aromatic hydrocarbons.—Wet sediment (4–5 g) was placed into a centrifuge tube and spiked with deuterated polycyclic aromatic hydrocarbons (PAH) internal standards (Supelco) to correct for losses during preparation. The samples were sonicated at 20 °C for 60 min with 50 mL of 50:50 acetone:hexane and centrifuged at 2200 rpm. The overlying solvent was removed and the extraction was repeated 2 more times. The solvent extracts were combined and dried with sodium sulfate, volume reduced, and exchanged to hexane under a nitrogen stream, then eluted through Waters Sep-Pak Plus silica cartridges using 90:10 hexane:dichloromethane.

The samples were analyzed for PAHs using an Agilent 5973 gas chromatograph mass spectrometer fitted with a 30 m (0.25 mm $\times$ 0.25 μm) DB5-MS column operating in splitless mode (Agilent). The mass selective detector was operated in select ion monitoring mode and PAHs quantified using a multipoint (6 level) internal standard calibration curve with a minimum r^2 value of 0.99 for each compound. Quality control of the extraction and quantification procedures was evaluated using the National Institute for Standards and Technology's (NIST) Standard Reference Materials 1944 for sediment. Further details on PAH analysis can be found in Cantwell et al. [22].

Sediment metals.—Sediments sampled for metals were processed using a CEM MDS 2000 microwave oven and Teflon digestion vessels. A combination of concentrated acids, 3 mL hydrofluoric, 2 mL hydrochloric, and 5 mL nitric, were added to each sample and microwaved. Metals were analyzed on an Applied Research Laboratories model 3410 inductively coupled plasma atomic emission spectrometer. Ten point calibrations were performed using NIST traceable calibration standards with linear calibration curves yielding an r^2 of 0.99 or better. Calibration verification standards were run every 10 samples with a minimum recovery of 90%. Samples were analyzed utilizing blank and duplicate samples to assess precision and accuracy. Duplicate samples were within 5% relative percent deviation, whereas blanks were below the detection limit. A standard reference material, MESS-2 (National Research Council, Canada), an estuarine sediment with certified metal values was

digested and analyzed along with the samples. The standard reference materials recoveries for the metals in the present study ranged between 80% and 115%.

Sediment toxicity.—Seven day acute survival tests were conducted using the amphipod *Ampelisca abdita* (field-collected 0.7–1.0 mm) and the mysid *Americamysis bahia* (48 h old) using methods described in Ho et al. [23]). Briefly, tests were conducted in triplicate in 120-mL glass chambers containing 20 g of wet sediment and 60 mL of filtered seawater (30‰). Chambers were gently aerated and maintained at 20 °C on a 16:8-h light:dark cycle. After 24 h to allow for sediment settling, 10 organisms of each species were added to each chamber and the mysids were fed a small quantity of brine shrimp (*Artemia*) daily. Long Island Sound reference sediment was used as a negative control and survival was required to be at least 80% in the controls.

Stressor identification

Sources.—Basemap imagery was obtained from the Rhode Island Geographic Information System [24]. Residential development and associated population growth around Greenwich Bay increased in the 1920s through the 1970s and had stabilized by the 1980s [6]. There was little to no land use change in the watershed between 2003 and 2011, [25] so the basemap imagery was applicable to the 2004 study despite not being an exact temporal match. Our data and imagery included the watershed outline from the 1990 Rhode Island water drainage basins and subbasins, roads from the 2011 NAVTEQ streets for the United States, railroads from the 2010 Rhode Island rights of way, the 2010 1:5000 Rhode Island lakes and ponds, the 2001 1:5000 Rhode Island rivers and streams, 2011 Rhode Island land use and land cover, and the sewage treatment plant location from the 2000 Rhode Island point discharge elimination system sanitary waste sites. Data were also digitized from the Greenwich Bay Special Area Management Plan [26]. This included location of combined sewer overflows and location of marinas, docks, and mooring fields. Dissolved oxygen data were interpolated using inverse distance weighting in a geographic information system to create a smoothed surface of oxygen in Greenwich Bay.

Stressors.—Concentrations of PAHs and metals in sediments were used to assess the contaminant load of Greenwich Bay. These contaminants have historically been discharged to the bay and there may have been current inputs from runoff and marinas. Sediment polychlorinated biphenyl (PCB) concentrations were not measured in the bay as previous studies had indicated the PCBs in sediment cores were below the ER-L [27,28]. A recent core study examining Greenwich Bay sediments confirmed that with the exception of metals and PAHs, legacy contaminants (metals, PAHs, PCBs, and pesticides) were below ER-L (M. Cantwell, unpublished data). Although contaminants of emerging concern including antimicrobial compounds and pharmaceuticals are present in Greenwich Bay [7–9], they are well below predicted no effect concentrations (M. Cantwell, personal communication). Sediment contaminant threshold values ER-M and ER-L [17,18] were used to assess the potential for toxic effects in Greenwich Bay sediments.

Water column nutrients were not measured directly in the present study as rapid scavenging and turnover of nutrients by organisms makes direct nutrient measure an unreliable indicator

of eutrophication. Instead, nutrient and eutrophication status were assessed using levels of dissolved oxygen concentrations and elevated concentrations of TOC normalized to grain size [29]. In the absence of eutrophication, the amount of carbon in sediments is a function of surface area (i.e., grain size). The square root of TOC is graphed against the percentage of silt to clay and then compared against a reference line developed for the appropriate biogeographic province [29]. Values above the reference line indicate enriched sites. Bottom DOCs were also measured in the present study. To ensure that the oxygen levels seen in 2004 were representative, we assembled oxygen data for multiple years [30,31]. These data were collected in several locations in Greenwich Bay during neap low tides, when oxygen concentrations were projected to be at their lowest values. The data from Prell et al. [30] was from nighttime surveys. The data were instantaneous but well correlated with continuous dissolved oxygen measurements from moored buoys [32]. The data from Prell et al. [31] were from daytime surveys, as 5 yr of monitoring had shown that the spatial distribution of low oxygen was not dominated by diurnal effects (D. Murray, Brown University, Providence, RI, USA, personal communication). Oxygen data were classified into bins based on Rhode Island's water quality regulations. Values at or below 4.8 mg/L are classified as suboxic and values at or below 2.9 mg/L are classified as hypoxic [33]. The TOC normalized to grain size concentrations from Greenwich Bay were compared with the Virginian province reference stations [16].

Suspended sediments were also compared with the reference stations. The mean of TSS values from the reference areas was used to define background concentrations. Total suspended solids are often compared to background concentrations, however Environment Canada guidelines also suggest that TSS not exceed 25 mg/L [34].

Effects.—The benthic invertebrate community was used to assess condition of the estuary. The Greenwich Bay benthic community data from 2004 was compared to the Virginian province reference stations to assess overall condition. Stations were visually examined using multidimensional scaling (ordination) using square root transformation and Bray Curtis similarity in PRIMER 6.0. The procedure ANOSIM (PRIMER 6.0) was used to assess differences between the groups (Virginian province reference and Greenwich Bay) and SIMPER (PRIMER 6.0) was used to examine which species defined the differences between groups. Univariate measures of community structure were compared between Virginian province reference stations and Greenwich Bay stations using *t*-tests (SPSS, Ver 20). Comparisons were also made between stressed and unstressed sites within Greenwich Bay using ANOSIM and SIMPER. Stressed sites were defined broadly as those where sediment measures of contaminants exceeded ER-Ls, TOC normalized to grain size indicated enriched conditions, or where TSS concentrations exceeded background concentrations. Species that were shown to be most important in defining the differences between stressed and unstressed sites within Greenwich Bay were correlated (Spearman's rho, SPSS, Ver 20) with the stressors within the bay. The benthic community data were also compared with environmental data using the BEST program in PRIMER 6.0. The BEST procedure looks at correlations between the benthic community matrix and the matrix of environmental variables and indicates which combination of environmental variables is most closely associated with the community data. Prior to running this procedure, correlated

environmental data ($r > 0.80$) were deleted and, if needed, environmental variables transformed to approximate a normal distribution. Enriched and unenriched sites, as defined by TOC normalized to grain size exceedances, were compared using t -tests (SPSS, Ver 20). Sediment toxicity tests were also used to assess the effects of sediment contaminants on invertebrate communities.

RESULTS

Sources

The Greenwich Bay watershed is highly developed and primarily residential (Figure 4). These residences may be contributing nutrients to Greenwich Bay through the groundwater because many of the houses are not connected to public sewers [3]. The Greenwich Bay Special Area Management Plan [26] lists septic systems, cesspools, and discharge from the WWTP (Figure 4) as major sources of nutrients to Greenwich Bay. In addition, there is the potential for lawn fertilizer runoff to end up in the bay. The high density of roads and storm drains (Figure 5A) provides a direct conduit for contaminants such as metals and petroleum products to be washed into the bay. In addition, loose sediment can also be washed into the bay. Greenwich Bay is also home to many marinas and mooring areas (Figure 5B). These areas are potential sources of organic compounds through oil and gas spills. The large density of recreational boats has the potential to produce elevated concentrations of metals associated with antifouling bottom paints in the water column [35]. Finally, boat wakes, washes, and propellers can suspend sediments into the water column.

Stressors

Contaminants.—Metal concentrations were elevated within Greenwich Bay (Table 1). Effect range median exceedances for metals were seen in Apponaug and Greenwich Coves whereas ER-L exceedances for metals were seen in the coves and western portions of Greenwich Bay (Figure 6A). There were only 2 ER-M exceedances for individual PAHs in Greenwich Bay, but numerous individual PAH ER-L exceedances (Supplemental Data, Table S1). For total PAH values, the only ER-L exceedances were seen in Apponaug and Greenwich Coves and at the westernmost station within Greenwich Bay adjacent to a marina (Figure 6B).

Nutrients.—Bottom dissolved oxygen concentrations were generally, but not always below the Rhode Island suboxic criteria level of 4.8 mg/L throughout Greenwich Bay, and in some areas below the Rhode Island hypoxic criteria level of 2.9 mg/L (Figure 7A). This pattern was also seen over multiple years (2003–2005) from a survey designed to detect low dissolved oxygen events [30,31] (Figure 7B). Most of the stations in Greenwich Bay had elevated concentrations of TOC normalized to grain size relative to the Virginian province reference stations whereas most of the Virginian province reference stations were unenriched (Figure 8A). All but 3 of the enriched Greenwich Bay stations were found in the coves and western portion of Greenwich Bay (Figure 8B).

Suspended Sediments.—The background TSS level (average TSS level in the reference stations) was 20 mg/L. Only 5 stations had total suspended sediment concentrations above

background levels (Figure 9). Four of these stations were above the Environment Canada guideline of 25 mg/L, and 1 station was very close to this value. These stations were in Apponaug Cove near the WWTP outfall within Greenwich Cove, and at the westernmost station within Greenwich Bay.

Effects

The benthic community within Greenwich Bay was significantly different from the communities found in the reference stations (Figure 10; $p = 0.001$, $R = 0.667$). Specifically, Greenwich Bay had higher average numbers of the tube-building amphipod *A. abdita* (250 per square meter), the polychaete *Streblospio benedicti* (173 per square meter), and the amphipod *Monocorophium tuberculatum* (161 per square meter) than were found in the reference areas (36 per square meter, 84 per square meter, and 5 per square meter, respectively). In contrast, the polychaete *Mediomastus ambesita* had higher average numbers in the reference areas (100 per square meter) than in Greenwich Bay (36 per square meter). The Greenwich Bay stations had significantly ($p < 0.001$) lower species richness (19.7 ± 7.6) and Shannon's diversity (2.5 ± 0.5) than the reference stations (40.7 ± 15.3 and 3.3 ± 0.3 , respectively). Within Greenwich Bay the stressed communities were significantly different from unstressed communities ($p = 0.011$, $R = 0.352$). There was considerable overlap in species compositions between the groups, however the unstressed group was dominated by the bivalve *Gemma gemma*, the polychaetes *Tharyx* and *Exogone dispar*, and the gastropod *Nassarius trivittatus*. In contrast, the stressed sites were dominated by amphipods (various *Ampelisca* species, *Microdeutopus* species, and *Monocorophium* species), the polychaetes *S. benedicti*, *Capitella capitata*, *Polydora cornuta*, *Leitoscoloplos robustus*, *Eumida sanguinea*, and oligochaetes. There were no significant differences in species richness or diversity between stressed and unstressed sites. All of the stations in Greenwich Bay had toxicity tests survival greater than 80% (Supplemental Data, Table S2). All *A. bahia* test survival was >95%, whereas all *A. abdita* test survival was >80%.

Linking source, stressor, and effects

Contaminants.—Sources of contaminants include runoff from developed land and roads, as well as from marinas and boats. There were metal ER-M exceedances in Apponaug and Greenwich Coves, which indicate that metals could cause adverse effects in these areas. These exceedances were found in the same areas as marinas and mooring areas with high boat density. The exceedances decrease with distance from the marinas, which spatially links the metals source with the stressor. Metal ER-L exceedances were seen in Warwick Cove and in western Greenwich Bay, indicating that metals might cause adverse effects in this area. There were only 2 ER-M exceedances for PAHs, but more ER-L exceedances in Apponaug and Greenwich Cove and at 1 station in western Greenwich Bay, indicating that PAHs might cause adverse effects to Greenwich Bay biota. Species that SIMPER identified as distinguishing between stressed and unstressed sites were correlated with concentrations of sediment contaminants at those sites (Supplemental Data, Table S3). *Tharx* was significantly correlated with nickel (Ni; $r = -0.800$), lead (Pb; $r = -0.782$), zinc (Zn; $r = -0.658$), and total PAH ($r = -0.807$). *Ampelisca vadorum* was significantly correlated with cadmium (Cd; $r = -0.597$). *Streblospio benedicti* was significantly correlated with Ni ($r = 0.442$). *Polydora cornuta* was significantly correlated with copper (Cu; $r = 0.400$), Ni ($r =$

0.368), and Cd ($r = 0.383$). *Leitoscoloplos robustus* was significantly correlated with Cu ($r = 0.393$), Ni ($r = 0.571$), and Zn ($r = 0.731$). The BEST procedure indicated that Cd was significantly correlated with the benthic community (Supplemental Data, Table S4). In 3 out of 10 of these models Pb was also a significant variable. Total PAH was only a significant variable in 1 model. Copper, Ni, Zn, and iron were significantly correlated with TOC and not included in the model (Supplemental Data, Table S5). Although the benthic community was impacted in Greenwich Bay, the toxicity tests showed no statistically significant impact from contaminants. There was a link between source and stressor for sediment contaminants. Benthic impairment was observed in Greenwich Bay, when compared with reference benthic communities outside the bay. The BEST model showed correlation between selected contaminants and the benthic community. Correlation between contaminants and selected invertebrate species was also observed. Selected contaminants were above the ER-M level but there was no significant toxic effect on survival. This indicates a tentative link between stressor and effect (Table 2).

Nutrients.—The residential development of the watershed results in nutrient loading to Greenwich Bay that can contribute to eutrophication. The bottom dissolved oxygen measurements taken in 2004 indicate the western portion of the bay was either suboxic or hypoxic. Species that SIMPER identified as distinguishing between stressed and unstressed sites were correlated with eutrophication-related stressors (Supplemental Data, Table S3). *Streblospio benedicti* was significantly correlated with dissolved oxygen ($r = -0.484$). Data collected between 2003 and 2005 show that Apponaug Cove and the area just outside the cove was hypoxic, whereas much of Greenwich Bay was suboxic. There were significant positive correlations between dissolved oxygen and *A. abdita* ($r = 0.624$) and *Microdeutopus gryllotalpa anomalous* ($r = 0.583$) and negative correlations with *L. robustus* ($r = -0.460$). Concentrations of TOC normalized to grain size, a proxy for excess nutrients and eutrophication, indicated that Greenwich Bay sediments were enriched, and that most of this enrichment occurred in the western portion of Greenwich Bay. The density of *L. robustus* was significantly higher ($p = 0.012$) in enriched sites (17 ± 13 individuals) than in unenriched sites (7 ± 7 individuals). Species that SIMPER identified as distinguishing between stressed and unstressed sites were correlated with TOC (Supplemental Data, Table S3). There were negative correlations between TOC and *Tharx* ($r = -0.738$), *Nassarius trivittatus* ($r = -0.524$), and *A. abdita* ($r = -0.520$). Positive correlations were seen between TOC and *P. cornuta* ($r = 0.399$) and TOC and *L. robustus* ($r = 0.559$). Low dissolved oxygen concentrations found in areas where nutrient sources were more likely, such as the WWTP in Greenwich Cove and residential sources in the western portions of the bay spatially link eutrophication and nutrient sources. However, many of these sources existed throughout Greenwich Bay (Figures 3 and 4) and were likely responsible for low dissolved oxygen concentrations observed throughout the bay. The benthic community of Greenwich Bay was statistically different from reference locations and was dominated by organisms indicative of pollution (see *Discussion*). In addition, species that distinguished between stressed and unstressed sites were correlated with eutrophication-related stressors (Supplemental Data, Table S3). All BEST models included a combination of eutrophication related indicators (Supplemental Data, Table S4): bottom dissolved oxygen and chlorophyll a. Total organic carbon was a significant variable in 3 out of 10 of these models. In regards to nutrients, there

was a link between source and stressor and a probable link between stressor and effect (Table 2).

Suspended Sediments.—The developed land and associated roads surrounding the bay are a potential source of suspended sediments to the bay. Marinas and moorings also contribute to sediment loads through dredging or resuspension. Finally, boat traffic and the use of propellers can also cause sediment resuspension. Elevated suspended sediments were only seen in the shallow western coves (Apponaug and Greenwich Coves) and just outside a marina on the western shore of Greenwich Bay. The elevated TSS adjacent to this marina links the sediment source to the measured stressor. Although there is no definitive assay for the effects of suspended sediments, changes in the benthic community may occur, and the benthic community in Greenwich Bay was changed relative to reference sites. *Ampelisca vadorum*, 1 of the species identified by SIMPER as distinguishing stressed and unstressed sites within the bay (Supplemental Data, Table S3) was significantly correlated with TSS ($r = -0.625$). The BEST procedure indicated that TSS was correlated with the benthic community in some cases (Supplemental Data, Table S4). There was a link between source and stressor (TSS) and a possible link between stressor (TSS) and effect (Table 2).

DISCUSSION

Greenwich Bay is potentially impacted by multiple stressors. In the present study, we identified the important stressors affecting Greenwich Bay benthic fauna. Because all of the stressors co-occur, especially in the more western portions of Greenwich Bay, the relative importance of each stressor needs to be addressed through a weight of evidence approach. The benthic fauna in Greenwich Bay were shown to be adversely impacted by stressors resulting in lower diversity when compared with reference areas outside of the bay. Greenwich Bay was dominated by the tube-building amphipod *A. abdita* and the polychaete *S. benedicti*. *Ampelisca abdita* is an opportunist well adapted to exploit carbon-enriched sediments [36] and *S. benedicti* is a well-known indicator of pollution [37]. The presence of both of these species may indicate impacts of eutrophication/low oxygen or contaminants. Within Greenwich Bay, sites that did not appear to be impacted by enrichment, contaminants, or suspended sediments were dominated by the bivalve *G. gemma*. Although adult bivalves can be highly tolerant to hypoxia [38] and metals [39], their larvae are, while juveniles and larvae, considerably more sensitive [40,41]. Bivalves have also been shown to be adversely impacted by suspended sediments [42]. Larvae seem to be most impacted, with decreased survival and development between 100 mg/L and 1000 mg/L of suspended sediment [42], which is within the observed range of 2 of the affected 4 stations in Greenwich Bay.

Elevated suspended sediment concentrations were only seen in a few locations within Greenwich Bay. Most of these stations were also impacted by all other stressors, and were located in Apponaug Cove, a shallow subembayment characterized by repeated low dissolved oxygen, organic enrichment, and sediment contamination. Approximately half the cove is filled with boat moorings, so resuspension in this area might be expected to be common. Another station with elevated TSS is located close to the East Greenwich WWTP outfall and may reflect sediment resuspension due to effluent introduction to the Cove. The

BEST procedure showed a relationship between the benthic community and TSS for only 4 of 10 models, and the density of 1 benthic invertebrate, the amphipod *A. vadorum*, was negatively correlated with TSS. Although sediment resuspension may have caused some adverse impacts, its spatial distribution was limited and thus TSS likely contributed relatively less to changes in benthic community structure than the other stressors present.

Concentrations of some of the individual PAHs and total PAHs, as well as most of the metals, exceeded their ER-L but not ER-M concentrations, so adverse effects might be expected to occasionally occur. Based on the ER-M, there were 5 stations where Cu, Pb, and/or Zn had elevated concentrations that might be expected to cause adverse effects. The elevated concentrations of these 3 metals was not surprising given the high numbers of boats that are docked or moored in Greenwich Bay. The BEST procedure results indicated that Cd had the strongest relationship to the overall benthic community. When Cd concentrations were elevated, the amphipod *A. vadorum* abundance was lower, whereas the spionid polychaete *Polydora cornuda* abundance was higher. However, although Cd concentrations exceeded the ER-L, they never exceeded the ER-M. Copper and Zn were correlated with TOC, which had a low number of BEST models significantly related to the benthic community. Lead was related to the benthic community in 30% of the BEST models. The polychaetes *L. robustus* and *P. cornuda* were positively correlated with metal concentrations indicating no negative response to contaminants. In contrast, the density of the polychaete *Tharyx* was negatively correlated with elevated contaminant concentrations (Ni, Pb, Zn, total PAHs). Although the correlations between the benthic community and some metals and the correlation between some individual species and selected contaminants indicate that these contaminants could be affecting the benthos, the results of the amphipod and mysid sediment toxicity tests indicated that these contaminants were not causing the expected toxic effect.

The lack of expected sediment toxicity may be due to high organic carbon in the sediment. The sedimentary organic carbon in Greenwich Bay ranged from 0.2% to 8.0%. Concentrations of organic carbon exceeding 1% will affect the bioavailability of both organic and metal contaminants [43,44]. Consequently, the presence of elevated sedimentary organic carbon at several locations in Greenwich Bay likely made sediment contaminants not readily bioavailable. The toxic units associated with the total PAHs measured in the Greenwich Bay sediment can be calculated by dividing the organic carbon normalized sediment concentration of each PAH quantified ($\mu\text{g/Kg}$ organic carbon) by a known effects concentration for each PAH also expressed on an organic carbon normalized basis ($\mu\text{g/Kg}$ organic carbon) [44]. The ratio of these 2 values, by convention multiplied by 100, is the number of toxic units associated with a given PAH. A toxic units value of 1.0 is equivalent to the amount of PAH necessary to cause sublethal toxicity to 50% of the organisms exposed in a sediment toxicity test. Because PAH toxicity to invertebrates is caused primarily by a narcotic mode of action, the individual PAH toxic units can be added together to calculate a total number of PAH toxic units associated with a given sediment. For example, an analysis of the number of toxic units based on total measured PAH concentration indicated that none of the stations exceeded 0.3 toxic units anywhere in Greenwich Bay. Given there was insufficient PAH toxic units to cause sublethal toxicity in Greenwich Bay sediments, we would expect there would be far too few toxic units to cause any mortality (i.e., many more

toxic units are needed to cause acute effects like mortality), which agrees with our sediment toxicity testing results. Although not specifically examined in the present study, elevated sediment organic carbon is indicative of reduced conditions that are likely to result in sedimentary sulfides sequestering toxic divalent metals like Cu, Pb, and Zn [45], and metals were highly correlated with TOC (Supplemental Data, Table S6). This consideration of contaminant bioavailability likely explains the lack of acute sediment toxicity within Greenwich Bay despite the presence of chemicals (Table 2). This is further supported by examining the ordination of stations within Greenwich Bay (Figure 11). The stations with metal ER-M exceedances are not tightly grouped together and are intermixed with the other stations. There was no significant difference between the benthic community composition of stations with metal ER-M exceedances and those without metal ER-M exceedances ($p = 0.254$, $R = 0.078$). Overall, the data indicate that the elevated contaminant concentrations in Greenwich Bay sediments should not pose a toxicological threat to the benthos. Although toxicity was not observed in this round of testing, changes in environmental conditions such as sediments being resuspended by storms or boat traffic might cause contaminants to desorb from the sediment. This would increase bioavailability to water column organisms, including sensitive early life stage larvae.

The dissolved oxygen data indicated that much of Greenwich Bay was suboxic or hypoxic, with higher oxygen concentrations seen in the eastern portions, which is open to Narragansett Bay. This was seen both for data collected for the present study and longer-term oxygen data collected by others [5, 30–33, 46]. Similarly, the TOC normalized to grain size values indicated that Greenwich Bay was eutrophic, with most of the enriched sites located in the western portion of the bay. This is consistent with previous work in the bay. Granger et al. [46] reported low dissolved oxygen concentrations in Apponaug Cove and western Greenwich Bay between 1995 and 1997. Codiga et al. [33] attributed anoxia observed between 2003 and 2006 in western Greenwich Bay in part due to a gyre in the western portion of the bay. This gyre is driven by southeast winds and can increase water residence time from 9 d to 1 mo (JM Rogers, 2008, Master's thesis, University of Rhode Island, Kingston, RI, USA). These data indicate that nutrient loading and hydrodynamics predispose the western portion of Greenwich Bay to hypoxic conditions.

The benthic community data patterns supported the conclusion that eutrophication related stressors impacted Greenwich Bay. Eutrophication is expected to have widespread effects, which is what was seen. The benthic community in Greenwich Bay was significantly different from reference communities outside of the bay. In eutrophied systems, excess nutrients stimulate algal blooms, which settle to the bottom of the bay, increasing carbon loads to the sediment, resulting in hypoxia [47]. Within the bay, the species responsible for the differences between stressed and unstressed sites were all correlated with bottom dissolved oxygen concentrations or elevated TOC concentrations. Benthic macroinvertebrates have predictable responses to hypoxia [38]. Echinoderms and crustaceans are most sensitive, whereas polychaetes are least sensitive. Species richness is also expected to decline with declining oxygen concentrations [48]. Echinoderms were present in the Virginian province reference stations, but no echinoderms were present in Greenwich Bay. However there were many amphipods (crustaceans) present in Greenwich Bay. The amphipods are able to process the carbon rich sediments and feed on algal blooms

in the bay when water column conditions are adequate to support these communities [36]. Species richness was also significantly lower in Greenwich Bay than in comparable reference stations, as would be expected with decreasing oxygen conditions. The amphipods *A. abdita* and *M. gryllotalpa* declined with lower dissolved oxygen concentrations, whereas the polychaete *S. benedicti* abundance increased. Pearson and Rosenberg [48] first conceptualized how benthic communities respond to organic enrichment and pollution. They suggested that healthy macrobenthic communities would be diverse and characterized by larger organisms that would be able to deeply penetrate the sediment. With organic enrichment and pollution, oxygen penetration would diminish, and the community would become less diverse and dominated by smaller, more opportunistic taxa. Later researchers have expanded on this idea and categorized species as pollution tolerant or pollution sensitive [19,49], or used groups of benthic experts to categorize species by their pollution tolerance [37,50]. The amphipod *A. abdita*, mud snail *Nassarius tritritatus*, and polychaete *Tharyx* declined with increasing TOC, whereas the polychaetes *L. robustus* and *P. cornuda* increased. With the exception of *N. tritritatus*, all the species responsible for the differences for the differences in benthic community composition between sites were those expected to be stimulated or highly stimulated by organic enrichment [37]. The variance in species response reflects that although many of these species are stimulated by carbon enrichment, they have varying sensitivity to hypoxia [38,48]. All the BEST models included either bottom dissolved oxygen or chlorophyll a concentrations as important variables, which also provides evidence for eutrophication-related impacts.

Included in the BEST analysis were depth, salinity, and grain size. These are important environmental variables known to structure benthic communities. The BEST output included depth as an important variable in most of the models, whereas salinity was included in 3 of the 10 possible models. Grain size was not included in any of the models despite the presence of discrete sand and mud patches within the bay. This may reflect a homogenization of the community due to eutrophication, which reduced the effect of grain size as a factor structuring the benthic community. This has been seen in the Adriatic Sea [51]. When eutrophication was not prevalent, there were 2 historic assemblages representing a mud and a sand community. However, with increasing eutrophication, algal blooms, and hypoxia, a new and different community emerged that did not distinguish between sand and mud. This is analogous to what we saw in Greenwich Bay. The benthic community is distinct from the reference community, does not show distinct communities that are related to grain size, and some of the species that distinguish between stressed and unstressed areas within the bay are those known to respond to organic enrichment.

Although Greenwich Bay was impacted by multiple potential stressors, the evidence most strongly indicates that eutrophication related stressors and indicators (low dissolved oxygen, chlorophyll a, and elevated sediment TOC) are predominant. The sediments of Greenwich Bay were carbon enriched and suboxic dissolved oxygen concentrations were commonly seen, especially in the more western portions of Greenwich Bay. The benthic community was depauperate, as would be expected under oxygen stress, and dominated by opportunistic species. The contaminant loads in Greenwich Bay were at concentrations where adverse effects might be seen, but given that the contaminated stations are also highly carbon enriched, it is likely that the contaminants were not bioavailable much of the time. Any

contaminant related impacts are likely to be seen only in the water column due to storms and boat traffic that cause sediment resuspension and potential desorption of bound contaminants, along with direct runoff. The results of the present study indicate that suspended sediment impacts were likely to be minimal or nonexistent for much of the bay.

The analyses in the present study demonstrated that this diagnostic procedure [13,52] was useful for organizing and assessing the relative impacts of potential stressors impacting Greenwich Bay. Like many estuaries, Greenwich Bay is spatially heterogeneous with the entire estuary impacted to a greater or lesser extent by stressors. Thus there was not a reference area within the bay, and we were unable to use reference stations from the larger Narragansett Bay because the physical conditions in unimpacted areas were not similar to this embayment. Comparison of Greenwich Bay to less impacted reference stations within the same biogeographic province allowed assessment and interpretation of conditions within the bay. Due to the comingling of potential stressors, multiple lines of evidence were needed to understand which stressors were primarily responsible for degraded conditions. Estuaries throughout the world are commonly impacted by multiple comingled stressors. Our diagnostic procedure provides a mechanism to organize and interpret multiple lines of evidence to better manage other waterbodies impacted by multiple stressors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Map of study area showing the entire Virginian biogeographic province, with inset maps of Narragansett Bay and Greenwich Bay.

Figure 2.
Schematic of diagnostic procedure used in Greenwich Bay assessment.

Figure 3. Greenwich Bay stations sampled for the present study in 2004. All stations were sampled once in July. Stations 2,7,10, 17, 18, 22, and 26 were sampled multiple times in July and August.

Figure 4.
Residential and commercial land in the Greenwich Bay watershed in 2011. There was no land use change in the watershed between 2003 and 2011.

Figure 5. Potential dischargers into Greenwich Bay: A) Roads, sewage treatment plants, and “other pipes” – primarily combined sewer overflows, and B) Marinas, docks, and mooring fields. WWTF = wastewater treatment facility.

Figure 6.
Sediment contaminant effect range low (ER-L) and effect range median (ER-M)
exceedances for A) individual metals and B) total polycyclic aromatic hydrocarbons (PAH).

Figure 7. Greenwich Bay bottom dissolved oxygen (DO). Categories are based on Rhode Island dissolved oxygen criteria thresholds. A) 2004 data (the present study); B) 2003–2005 survey [30,31].

Figure 8.

Total organic carbon (TOC) normalized to grain size for A) comparison of Greenwich Bay and Virginian province (VP) reference data to the Virginian province reference line (square root [TOC] = $0.012 \times \% \text{ silt to clay} + 0.402$). The reference line is the 99th confidence interval of the relationship between TOC and silt and clay from stations within the Virginian province that had low sediment toxicity, low chemical concentrations in the sediment, and high dissolved oxygen concentrations in bottom waters [29]. Values above the Virginian province reference line are enriched. B) Spatial distribution of enriched sites within Greenwich Bay.

Figure 9.
Concentration and spatial distribution of total suspended sediments (TSS) levels above reference background levels (20 mg/L).

Figure 10.
Ordination of benthic invertebrate data from Greenwich Bay compared with reference stations from the Virginian province (VP) that have similar depth, salinity, and grain size as the Greenwich Bay stations.

Figure 11. Ordination of benthic invertebrate data from within Greenwich Bay. The coves within the bay are represented by stars, diamonds, and triangles. The circled stations are those with metal effect range median exceedances.

Table 1.

Metal concentrations in Greenwich Bay sediments. Bold, boxed text indicates that the value exceeds the ER-M value. Bold Italics indicate that the value exceeds the ER-L value.

Station	mg/kg dry weight					
	Cu	Ni	Pb	Zn	Cd	Fe
1	73.13	8.57	83.89	129.13	0.40	16686
2	138.79	9.29	379.40	160.54	0.59	21030
3	334.65	35.37	224.22	474.29	3.38	37462
4	142.32	13.04	121.12	194.73	0.93	24404
5	369.67	32.33	283.33	354.33	1.33	37936
6	387.25	39.58	275.70	370.64	1.38	37911
7	310.49	30.80	236.66	494.84	1.59	36258
8	216.86	24.39	164.85	239.19	1.76	31232
9	31.39	2.77	23.87	64.49	0.00	9910
10	184.37	15.61	104.58	200.73	0.25	28753
11	257.87	18.99	105.68	223.74	0.28	30028
12	171.08	14.59	58.17	171.77	0.69	23963
13	200.86	27.79	151.23	233.31	2.55	30852
14	5.89	0.00	10.70	14.43	0.48	4849
15	7.22	1.80	9.28	24.49	0.26	7563
16	120.38	24.18	104.01	176.53	0.25	29566
17	5.50	0.45	14.04	12.69	0.34	4631
18	98.37	18.73	93.42	143.79	0.00	28386
19	137.49	31.55	112.30	195.17	1.18	34866
20	113.26	26.85	96.66	167.69	0.24	32282
21	15.39	2.95	19.57	35.33	0.12	9460
22	119.63	27.94	104.38	170.66	0.00	34442
23	4.49	0.00	11.48	15.09	0.00	6743
24	121.38	22.67	112.20	175.04	0.29	33261
25	6.37	1.14	8.19	19.91	0.00	6878
26	159.81	23.81	118.44	232.42	0.89	29548
27	3.30	0.00	5.32	12.03	0.32	5217
28	4.08	0.00	12.94	11.25	0.14	4373

Table 2.

Summary of Diagnostic Procedure for Greenwich Bay

Potential Impairment	Source	Stressor	Effect	Analysis	Conclusion
Contaminants	1. Boats	1. Sediment metals	1. Benthic Impairment	1. ER-M and ER-L guidelines exceeded at several stations	Not a major contributor to adverse benthic effects
	2. Runoff from land and/or roads	2. Sediment PAHs	2. Correlation between benthos and stressors	2. No significant toxicity was observed in sediment toxicity tests	
	3. Historical industrial inputs		2. Toxicity	3. Toxic unit analysis indicates PAHs are not bioavailable 4. High TOC values indicate metals are also not bioavailable 5. ANOSIM indicates no differences between metal impaired sites and other stations within Greenwich Bay	
Nutrients	1. Runoff from homes/lawns	1. Dissolved oxygen levels	1. Benthic Impairment	1. Much of Greenwich Bay classified as suboxic or hypoxic	Major contributor to adverse benthic effects
	2. Sewage Treatment Plants	2. Elevated TOC normalized to grain size	2. Correlation between benthos and stressors	2. TOC normalized to grain size above reference levels	
	3. Groundwater from septic systems 4. Contributions from upper Narragansett Bay			3. Dissolved oxygen impairments corresponded to patterns of benthic impairment	
Suspended Sediments	1. Boats/marinas	1. Total Suspended solids	1. Benthic Impairment	1. TSS above reference levels at several sites	Not a major contributor to adverse benthic effects
	2. Runoff from roads and/or land		2. Correlation between benthos and TSS		