Public Comment
Dominguez Channel/LA/Long Beach
Waters Toxic Pollutants TMDL
Deadline: 10/28/11 by 12:00 noon

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION IX
75 Hawthorne Street
San Francisco, CA 94105

RECEIVED
10-28-11
SWRCB Clerk

Charles Hoppin
Board Chair
State Water Resources Control Board
PO BOX 100
Sacramento, CA 95812-0100

Dear Mr. Hoppin,

The U.S. Environmental Protection Agency (EPA) supports the Los Angeles Regional Water Quality Control Board's proposed Basin Plan amendment to establish Total Maximum Daily Loads (TMDLs) for many toxicants in Dominguez Channel and greater Los Angeles/Long Beach Harbor waters. We concur with the technical approach to restore beneficial uses for aquatic life and fish consumption via pollutant load reductions from upstream sources as well as existing bed sediments. We find the proposed TMDLs meet all federal regulatory requirements.

TMDL numeric targets for water are consistent with numeric criteria in the California Toxics Rule (CTR). Numeric targets are also identified for sediment and fish tissue, consistent with EPA guidance for addressing narrative water quality standards. The Regional Board has selected sediment quality guidelines based on Effects Range-Low values (ERLs) to protect benthic organisms living within contaminated sediments. Scientific studies defend this approach based on sediment mixtures of copper, DDT and pyrene (a PAH compound) and the adverse effects on benthic community structure (Balthis et al, 2010).

The TMDL includes a hydrodynamic and water quality model that builds upon existing watershed information as well as expanding into the estuarine and marine waters. The model specifically incorporated the following monitoring results: freshwater inputs from Dominguez Channel, Los Angeles River and San Gabriel River from 1995-2005, physical sediment parameters and transport information from 1998 to 2005, sediment chemistry results from 2000-2006 including those generated by Port of Los Angeles and Port of Long Beach monitoring project in 2006. Model specifications and results were reviewed by and generated comments from technical advisory group of stakeholders. Furthermore, in response to TMDL development, the Ports have utilized this publically available model (as opposed to previous ACOE models) as part of their Water Resources Action Plan for investigating future pollutant load reduction strategies.

The TMDL acknowledges the Montrose facility within the Dominguez Channel watershed. EPA's Superfund program has made considerable progress on controlling exposures from DDT in soils: a temporary cap was installed over the DDT-contaminated soils at the former Montrose plant property, and EPA removed contaminated soils from some areas within the stormwater pathway, which flows into Kenwood Drain, through Torrance Lateral and into Dominguez Channel estuary. Monitoring results to date show low DDT concentrations passing thru Torrance Lateral; nonetheless, the TMDL establishes additional monitoring – to further characterize this pollutant pathway – if higher DDT levels are observed in the stormwater pathway from routine monitoring.

The implementation plan provides adequate description of requirements and expectations for all concerned stakeholders.

We urge the State Board to approve the TMDLs to meet California's TMDL commitments and to enable EPA to meet its requirements under the consent decree (*Heal the Bay v. Browner, C. 98-48 25 SBA*, March 22, 1999). If you have any questions, please call me at (213) 244-1803.

Sincerely,

Cindy Lin

10-28-2011

Cindy Lin
TMDL coordinator, Water Division

Attachments

cc: Sam Unger, Los Angeles RWQCB

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Effects of chemically spiked sediments on estuarine benthic communities: a controlled mesocosm study

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Abstract Ambient sediments were collected from a reference site in the North Edisto River, SC and transferred to a laboratory facility to investigate effects of chemical contaminants on estuarine infaunal communities under controlled mesocosm conditions. Sediment contaminant slurries were prepared using dried sediments collected from the reference site and spiked with a metal (copper), a polycyclic aromatic hydrocarbon (pyrene), and a pesticide (4,4′-dichlorodiphenyltrichloroethane) to yield nominal mean effects range–median (ERM) quotients of <0.01 (no addition), 0.1, and 1.0 and applied to control, low dose (TRT A), and high dose (TRT B) treatment groups, respectively. Sediment samples for contaminant and benthic analyses were collected at the start of the experiment, 1 month after dosing, and 3 months after dosing. Near-nominal mean ERM quotients of 0.001, 0.075, and 0.818 were measured initially after dosing and remained fairly constant throughout the experiment. Measures of benthic condition, diversity, and richness were significantly reduced in both treatment groups relative to the control 1 month after dosing and persisted in TRT B at 3 months. The results demonstrate that benthic community effects can be observed at mean ERM quotients that are about an order of magnitude lower than levels that have been shown to be associated with significant toxicity in acute laboratory bioassays with single species (e.g., amphipods) in other studies.

Keywords Benthic mesocosm · Macroinfauna · Chemically spiked sediment · Sediment quality guidelines · Effects range median

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Introduction

The problem of chemical contamination of marine and estuarine sediments has been recognized as an environmental issue of national importance (National Research Council 1989). While chemical analysis of sediments can provide quantitative measures of the degree of contamination, assessing the ecological significance of mixtures of chemical contaminants in sediments is less straightforward. The results of laboratory bioassays alone can serve as indicators of degraded conditions (Long 2000), but they do not allow one to predict the likelihood of toxicity based on chemical contamination. Numerical sediment quality guidelines (SQGs) for individual chemicals have been developed for such purposes using a variety of both theoretical, mechanistic methods that rely upon equilibrium partitioning models and empirical methods that rely upon analyses of matching, field-collected chemistry and biological effects data (Long et al. 2006).

Values for one such widely used SQG, the effects range median (ERM), have been developed for nine trace metals, total polychlorinated biphenyls (PCBs), two pesticides, 13 polycyclic aromatic hydrocarbons (PAHs), and three classes of PAHs by defining concentration ranges that were frequently associated with adverse effects from various studies in the literature (Long et al. 1995). In addition, the mean ERM quotient (mERMQ), calculated by taking the mean of the ratios of each analyte concentration and its corresponding ERM, is often used as a means of assessing the significance of complex mixtures of potentially toxic chemicals present in sediments at varying concentrations (Long et al. 2006). Long and MacDonald (1998) reported a relatively high incidence (>70%) of toxicity in sediment samples with a mERMQ > 1.5, based on matching sediment chemistry and toxicity data from multiple surveys of sediment quality in coastal areas of the US. Toxicity was based on standard, 10-day amphipod survival tests using adult *Ampelisca abdita* or *Rhepoxynius abronius*. Hyland et al. (1999, 2003), in comparison, found a high incidence of benthic community-level effects in ambient sediments from southeastern estuaries at mERMQs > 0.058, well below those associated with toxicity in the above laboratory toxicity studies. They hypothesized that benthic community-level responses can be observed at lower chemical concentration ranges because they reflect the varying sensitivities of multiple species and life stages to longer-term exposures (Hyland et al. 1999, 2003). Other investigators have also reported that the response of benthic assemblages to chemical contamination in sediments may occur at lower concentrations than those observed in laboratory toxicity tests based on survival of amphipods (Ingersoll et al. 2005).

Correlative field observations of benthic–contaminant relationships offer the advantages of documenting in situ effects under natural and realistic exposure conditions, but they suffer from the drawback of numerous uncontrolled confounding factors (Word et al. 2005). Colonization studies using spiked sediments have been used to evaluate the impacts of contaminated sediments and to field-validate mechanistic and empirical SQGs, while controlling or accounting for sediment and habitat characteristics (Word et al. 2005). Word et al. (2005) recommended that mesocosm studies should be used to validate further the ability of SQGs to predict impacts on benthic communities. A number of studies have suggested that impacts on estuarine benthic communities may occur at chemical concentrations that are an order of magnitude lower than those identified in 10-day laboratory toxicity tests, indicating that sensitivity to contaminants may vary with different receptors, endpoints, and exposure regimes (Word et al. 2005). This approximate tenfold difference in sensitivity between benthic community-level responses and results of acute toxicity tests conducted with adult amphipods is consistent with acute–chronic ratios from aqueous-phase toxicity tests (Ingersoll et al. 2005).

As a follow-up to these earlier studies, the present study was conducted to characterize benthic community-level responses to varying concentrations of chemical contaminant mixtures in sediments using a spiked-sediment approach under controlled mesocosm conditions. We compare observed effect and no-effect concentrations in controlled mesocosm exposures to previously published benthic-based sediment quality targets derived from correlative field observations of benthic–contaminant relationships (Hyland et al. 1999, 2003) and to other related sediment quality targets based on sediment toxicity from short-term (10-day) laboratory assays with single species (amphipods; Long and MacDonald 1998; Long et al. 1998a). A mixture of chemical contaminants from three different chemical classes (metals, PAHs, pesticides), chosen for their known toxicity and observed prevalence in southeastern estuaries (Hyland et al. 1998, 2000), was used in a controlled mesocosm study using spiked sediments to test the hypothesis that an increase in chemical contamination will correspond to a decrease in the quality of benthic communities and that the observed effects will occur at mERMQs that are markedly lower than those observed in short-term amphipod toxicity tests from prior published studies. Sediment bioassays were not included in the present study.

Methods

Experimental design

The mesocosm design for this study followed that of Lauth et al. (1996) and incorporated the modifications of Pennington et al. (2004). The basic design consists of a series of replicated modular tanks fed by a closed seawater system and maintained within a temperature-controlled greenhouse facility. However, a further modification in the present study was to model an estuarine subtidal habitat (Fig. 1) rather than an intertidal habitat (with marsh grasses) as in prior applications. Twelve sampling units (mesocosm tanks) were used and each contained four rectangular high density polyethylene (116 cm long × 49 cm wide × 20 cm deep) subtidal sediment trays rather than the intertidal trays described by Lauth et al. (1996). The total volume of seawater in each mesocosm tank was 300 L. The total mass of sediment in each tray was 15.9 kg.

The mesocosm tanks were operated in an intermittent recirculating mode. Using a multi-event timer, they received eight regularly spaced (every 3 h) partial seawater exchanges in each 24-h period. Every 2.5 h, the timer switched the circulation pump off, allowing the seawater to drain by gravity into the lower seawater reservoir. After 30 min, mixed seawater from the lower reservoir was then pumped back into the upper tank.

The experiment consisted of three treatments (contaminant exposure levels; Table 1), with four replicates each, and four time intervals (start, 1, 3, and 6 months after dosing). While the system was maintained for 6 months, data from the 6-month sampling were not included in the analysis presented here due to heavy organic buildup (large algal and bacterial mats) leading to adverse changes even in the control treatments. The treatments and replicates were randomized spatially within the greenhouse to account for potential variation in air flow and solar irradiation. The mesocosm setup utilized a replicated design for each treatment as illustrated in Fig. 2.

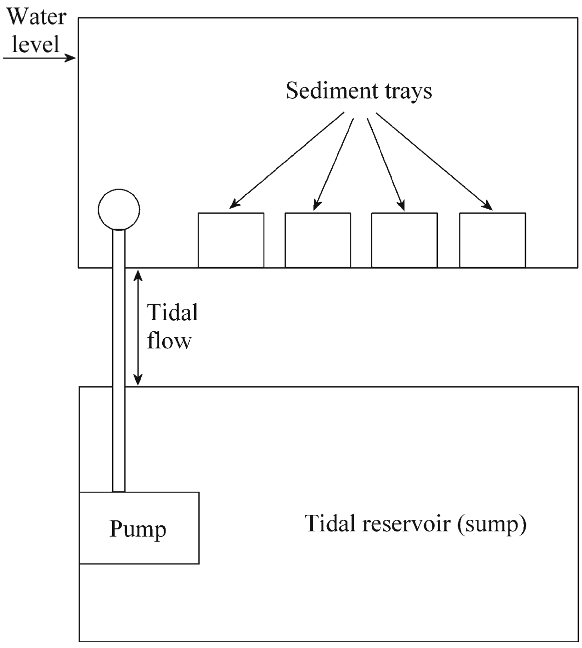

Fig. 1 Mesocosm design, modified from Lauth et al. (1996)

Seawater and sediment collections

Seawater was collected from Cherry Point Boat Ramp on Wadmalaw Island, SC (32°35′53″ N, 80°10′58″ W) on four occasions in 2004 (May 12, 17, 18, and 25) at high tide. The seawater was pumped into an 850-L container, transported back to the National Oceanic and Atmospheric Administration Center for Coastal Environmental Health and Biomolecular Research (CCEHBR) facility in Charleston, SC, and pumped into a series of large seawater storage tanks (1,110 L) located in the greenhouse. After homogenization with a pump overnight, 300 L of seawater was randomly dispensed into each mesocosm. The salinity at the collection site averaged 29.58 psu (±0.951 [standard deviation]) over the four collection events.

Sediments were collected from the North Edisto River, SC at the mouth of Leadenwah Creek (32°36′24″ N, 80°13′37″ W). Samples were collected by boat using a Young-modified Van Veen grab sampler (0.04 m^2). This site (RO99323)

Table 1 Target (nominal) concentrations of Cu, DDT, and pyrene for each spiked sediment treatment and resulting combined mERMQs

Treatment	Cu (mg/kg) dry weight	DDT (μg/kg) dry weight	Pyrene (μg/kg) dry weight	mERMQ[b]
CTL	–	–	–	< 0.01
TRT A	27.0	4.61	260.0	0.10
TRTB	270[a]	46.1[a]	2,600[a]	1.00

mERMQ mean effects range–median quotients, *CTL* control, *TRT A* low dose treatment group, *TRT B* high dose treatment group

[a]ERM

[b]Calculation of mERMQ based on three spiked contaminants

was previously characterized during the South Carolina Estuarine and Coastal Assessment Program (SCECAP; Van Dolah et al. 2002) and was found to have very low background contamination. During the course of the SCECAP study, the mERMQ was found to be 0.012 and the Benthic Index of Biotic Integrity (B-IBI; Van Dolah et al. 1999) was 4.0 (indicating very healthy benthic conditions). A previous study also found low levels of contamination: None of the nine samples collected in Leadenwah Creek were toxic in the amphipod tests (Long et al. 1998b). Sediments for the present study were collected during four sampling events in May and June of 2004 (May 11, 12, 27, and June 1). Twenty six individual grabs were collected at each sampling event for a total of 104 grabs. The total mass collected was in excess of 768 kg wet weight.

Sediments containing ambient benthic fauna were placed into covered plastic bins and transported back to CCEHBR. Upon arrival, large portions of the collected sediments (538 kg) were placed directly into the subtidal mesocosm sediment trays to a sediment depth of 7 cm (approximately 11.2 kg sediment wet weight per tray). Mesocosm seawater flow was initiated immediately to allow live organisms within the sediments to begin acclimating to greenhouse (mesocosm) conditions.

The remaining 230 kg were set aside for the spiked sediment layer to be added after acclimation. They were placed in open plastic bins in the greenhouse and dried thoroughly for 4 to 5 weeks. The dried sediment was then sieved through a 3-mm screen to remove dead organisms and shell material. Prior to dosing, the dried sediments were

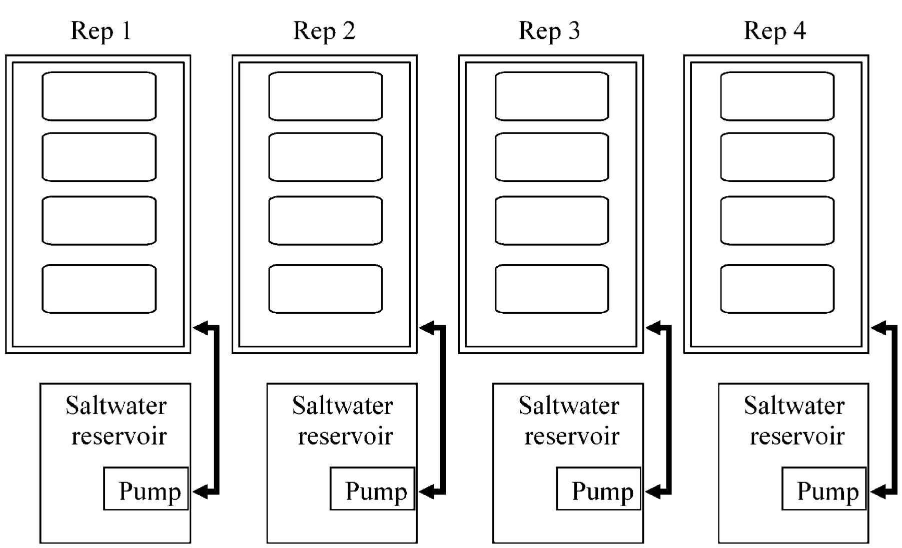

Fig. 2 Diagrammatic illustration of the replicated mesocosm design utilized for each treatment

divided into three aliquots, one for each treatment (30.84 kg dry weight each). Sterile seawater (30 psu) was added to each drum (35 L) to reconstitute the sediment and obtain a sediment slurry. The drums were sealed and rotated on a horizontal drum rotator for 20 min to produce the sediment slurry.

Preparation of spiked sediments

Three chemicals—copper, pyrene, and 4,4′-dichlorodiphenyltrichloroethane (DDT)—were selected to form the contaminant mixture based on their known toxicity, representation of three broad contaminant classes (metals, PAHs, and pesticides), and their prevalence in southeastern estuaries (Hyland et al. 1998, 2000). DDT is one of the best-known and most studied chlorinated hydrocarbon contaminants and has been shown to be toxic to aquatic invertebrates (USEPA 1980; Lotufo et al. 2000). Observations made by Reish (1988) indicate that the relative toxicity of metals to polychaetes in decreasing order is copper (Cu), mercury (Hg), zinc (Zn), arsenic (As), chromium (Cr), and cadmium (Cd; Mendez and Green-Ruiz 2005). Bat et al. (2000) found that Cu was more toxic to *Gammarus pulex pulex*, followed by Zn and lead (Pb). Pyrene has been shown to be toxic to microcrustacean invertebrates and is one of the 16 priority PAHs listed by the USEPA (Clément et. al 2005). Stock solutions for sediment spikes were prepared in 100% acetone for DDT and pyrene and deionized water for copper. Each of three 30.84-kg portions was dosed on a dry weight basis with appropriate amounts of copper, DDT, and pyrene to achieve a control (CTL) group (mERMQ $<$ 0.01) and two treatment groups (TRT A and TRT B; mERMQ $=$ 0.1 and 1.0, respectively). Target mERMQs were calculated based on the three contaminants used to spike sediments (copper, pyrene, DDT). Carrier solvent concentrations were normalized across all treatments and control. After spiking the sediment, the drums were then rotated for 2 h to facilitate homogenization. The mixed sediments were then allowed to settle for 2 days. At the start of the experiment on 12 July 2004, each drum was rotated again for 1 h to rehomogenize the sediments.

The seawater in the mesocosms was drained into the lower reservoirs. Any residual seawater on the surface of the sediment trays was siphoned off. The spiked sediments (4.79 kg) were then overlayed onto the existing live sediments to achieve a total sediment depth of 10 cm (15.9 kg) for CTL, TRT A, and TRT B mesocosms.

Sample collection and processing

Samples for identification and enumeration of benthic macrofauna were collected just prior to dosing, 1 month after dosing, and 3 months after dosing. To collect the infaunal and chemistry samples, the mesocosm water level was drawn down below the top edge of the sediment trays. One sediment tray was then removed from each system and placed on a table and the remaining overlying water in the sediment tray was siphoned off. A 0.04-m^2 high-density polyethylene form (27 $\times$ 15 $\times$ 10 cm), open on the top and bottom, was pushed into the sediment such that it was at least 2–3 cm from any side (to avoid edge effects). The entire content from within the form was sampled (4,050 cm^3) for infauna. Samples were sieved through a 0.5-mm mesh screen and preserved in 10% buffered formalin with rose bengal. All infaunal samples were transferred to 70% ethanol in the laboratory. Animals were sorted from sample debris under a dissecting microscope and identified to the lowest practical taxon, usually to species.

Sediment samples for chemical analysis were collected by scraping the top 2–3 cm of sediment from an undisturbed area of the sediment tray (outside of the form), using a small plastic scoop, from each of the four benthic sediment trays for each time–dose combination. The sediments for chemistry were composited and homogenized across replicates in an acetone-rinsed stainless steel bucket, yielding one composited sample for each time-dose combination. Sediment sample jars for chemistry were filled from the composited sediment and frozen at −30°C until analyzed.

The three targeted chemicals used to spike sediments were analyzed at all sampling intervals. A more expanded suite of contaminants, including the 24 (see Hyland et al. 1999, 2003) or 25

Table 2 Concentrations of copper, pyrene, and total DDT measured in spiked sediment slurries just prior to application to mesocosm sediments (ERM quotients are shown in parentheses)

Treatment	Copper (μg/g)	Pyrene (ng/g)	Total DDT (ng/g)[a]	Mean ERM quotient[b]
CTL	0 (0.0000)	8.59 (0.0033)	0 (0.0000)	0.0011
TRT A	25.9 (0.0959)	178 (0.0685)	2.789 (0.0605)	0.0750
TRT B	271 (1.0037)	2025 (0.7788)	30.992 (0.6723)	0.8183

[a]Sediments were spiked with 4,4′-DDT; calculation of total DDT included measured concentrations of 2,4′-dichlorodiphenyldichloroethane (DDD), 2,4′-dichlorodiphenyldichloroethylene (DDE), 2,4′-DDT, 4,4′-DDD, 4,4′-DDE, and 4,4′-DDT

[b]Calculated based on three ERMs: copper 270, pyrene 2600, and total DDT 46.1

(see Long et al. 1995, 2000) chemicals typically used in the calculation of mERMQs, also were measured at the beginning and end of the study. Sample preparation and analytical procedures for metals and organics were similar to those described by Sanders (1995), Fortner et al. (1996), Kucklick et al. (1997), and Fulton et al. (2007). Samples were extracted with CH_2CL_2 using accelerated solvent extraction, concentrated by nitrogen blow down, and cleaned by gel permeation chromatography. Chlorinated pesticides (DDTs) and PCBs were analyzed using gas chromatography/mass spectrometry with negative chemical ionization and electron impact ionization sources. An ion-trap mass spectrometer equipped with a gas chromatograph was used for analysis of PAHs (including the spiked pyrene). Sample preparation and analysis procedures for metals followed those described by Fortner et al. (1996). Trace metal analyses were performed using inductively coupled plasma mass spectrometry for the following suite of metals: Al, Cr, Cu, Fe, Mn, Ni, Sn, and Zn. Additional trace metals (Ag, As, Cd, Pb, Se) were analyzed using graphite furnace atomic absorption. Total mercury was quantified using direct mercury analysis (Milestone DMA-80 Mercury Analyzer). Quality control samples (blanks, spikes, duplicates, and SRMs) were analyzed for each group of samples for each analytical method.

Data analysis

The effects of treatment (CTL, TRT A, TRT B) and time (0, 1, and 3 months) on benthic measures of biotic integrity (B-IBI), diversity (Shannon H′), number of taxa (S), and infaunal density (m^{-2}) were assessed using two-way analysis of variance (ANOVA) models containing treatment, time, and a treatment × time interaction term. Separate one-way ANOVAs for each time point also were performed. Where the ANOVA result was significant, a multiple comparisons test of all treatments versus a control was carried out using Dunnett's (1955) test.

Patterns in the distribution of benthic infauna were examined using normal (Q-mode) cluster analysis (Boesch 1977). An unweighted pair-group method using arithmetic averages (Sneath and Sokal 1973) was used as the clustering method and Bray-Curtis similarity (Bray and Curtis 1957) was used as the resemblance measure. Analyses were run on double-square-root transformed

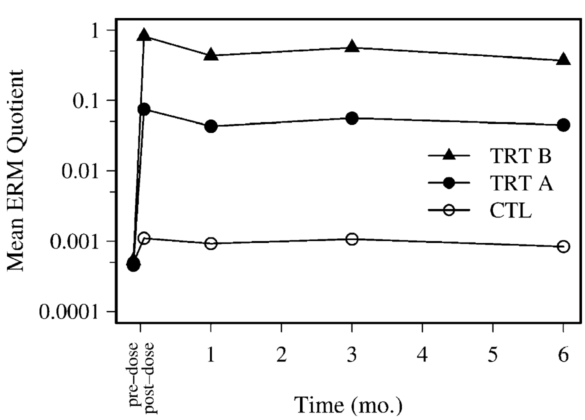

Fig. 3 Mesocosm sediment contaminant levels, expressed as mean ERM quotients, at 0, 1, 3, and 6 months after dosing in control (*CTL*), low (*TRT A*), and high (*TRT B*) mean ERM quotient treatment groups. Month 0 postdose samples were collected from spiked sediment slurries just prior to application. Mean ERM quotients were calculated based on the three contaminants used to spike sediments

Table 3 Diversity (Shannon H′), richness (number of taxa, S), abundance and density of benthic infauna, benthic index (B-IBI), and mERMQ in sediments from the benthic mesocosm study

Treatment	Time (months)	H′	S	Abundance ($0.04\ m^{-2}$)	Density (m^{-2})	B-IBI[a]	mERMQ[b]
CTL	0	2.3	12.5	82.5	2062.5	2.9	0.0005[c]
TRT A	0	2.1	8.3	54.8	1368.8	2.9	0.0005[c]
TRT B	0	3.3	15.8	40.5	1012.5	3.2	0.0005[c]
CTL	1	3.2	13.0	34.5	862.5	3.5	0.0009
TRT A	1	1.1	4.3	12.0	300.0	1.5	0.0428
TRT B	1	1.1	3.3	16.8	418.8	1.6	0.4323
CTL	3	2.9	12.5	50.5	1262.5	3.1	0.0011
TRT A	3	2.1	9.0	74.0	1850.0	2.6	0.0558
TRT B	3	1.7	5.8	32.0	800.0	2.4	0.5640

Following mesocosm setup and equilibration, samples from CTL, TRT A, and TRT B treatment groups were collected 0 (just prior to dosing), 1, and 3 months after dosing. Note: Subsequent organic buildup (algal/bacterial mats) in the mesocosms precluded the use of the 6-month sampling data

[a] Van Dolah et al. (1999)

[b] Mean ERM quotients were calculated based on the three contaminants used to spike sediments

[c] Month 0 samples for chemistry were collected just prior to application of spiked sediment slurries

abundances, combined over replicates within a treatment, using R statistical software (R Development Core Team 2006). Nonmetric multidimensional scaling (NMDS) also was used to confirm the site groupings obtained through cluster analysis.

Results

Concentrations of copper, pyrene, and total DDTs achieved in spiked sediment slurries are shown in Table 2. Mean ERM quotients, calculated as the mean of the individual ERM quotients for these three contaminants, were close to the targeted values of <0.01, 0.1, and 1.0 for the CTL, TRT A, and TRT B, respectively.

Sediment contaminant levels, expressed as mean ERM quotients, before and after dosing, 1, 3, and 6 months after dosing are shown in Fig. 3. Mean ERM quotients for CTL, TRT A, and TRT B all were <0.001 prior to dosing, based on the three contaminants used to spike sediments. Near-nominal mean ERM quotients of 0.001, 0.075, and 0.818 were achieved following application of the sediment slurries prepared for each treatment. Samples collected 1, 3, and 6 months after dosing demonstrate that sediment contaminant concentrations remained fairly constant following the initial application of sediment slurries.

Table 4 Results of two-way ANOVA of the spiked-sediment benthic data

Model	*df*	*F*	Pr >*F*	Effect	*df*	*F*	Pr > *F*
H′ = treatment + time + (treatment × time)	8	3.16	0.0118	Treatment	2	3.77	0.0361
				Time	2	2.22	0.1277
				Treatment × time	4	3.32	0.0247
No. of taxa = treatment + time + (treatment × time)	8	2.63	0.0288	Treatment	2	3.53	0.0436
				Time	2	2.98	0.0678
				Treatment × time	4	2.00	0.1233
Density = treatment + time + (treatment × time)	8	0.97	0.4821	Treatment	2	0.90	0.4203
				Time	2	2.10	0.1421
				Treatment × time	4	0.44	0.7820
B-IBI = treatment + time + (treatment × time)	8	3.28	0.0096	Treatment	2	4.30	0.0239
				Time	2	3.27	0.0534
				Treatment × time	4	2.78	0.0472

Table 5 Results of separate one-way ANOVAs, by time of sampling, of the spiked-sediment benthic data

Effect	Time (months)	*df*	*F*	*Pr* > *F*	Significant differences
H′	0	2	1.34	0.3098	
	1	2	5.44	0.0282	TRT B vs. CTL[a]; TRT A vs. CTL[a]
	3	2	5.75	0.0246	TRT B vs. CTL[a]
No. of taxa	0	2	1.17	0.3544	
	1	2	7.53	0.0120	TRT B vs. CTL[a]; TRT A vs. CTL[a]
	3	2	1.99	0.1919	
Density	0	2	0.42	0.6678	
	1	2	1.81	0.2189	
	3	2	0.73	0.5096	
B-IBI	0	2	0.24	0.7939	
	1	2	10.79	0.0041	TRT B vs. CTL[a]; TRT A vs. CTL[a]
	3	2	1.15	0.3589	

[a]Differences significant at $\alpha = 0.05$, based on Dunnett's test for differences in all treatments relative to a control

Sampling of benthic infauna yielded similar values for abundance, diversity (Shannon H′), number of taxa (S), and benthic index (B-IBI) among CTL, TRT A, and TRT B treatment groups at time 0 (Table 3). None of the parameters for dosed treatments at time 0 were significantly different from control. Two-way ANOVA of the benthic data demonstrates significant differences in H′, S, and B-IBI among CTL, TRT A, and TRT B (Table 4). While treatment had the strongest effect on these parameters, some influence of time also is apparent, particularly the interaction of treatment × time (differences among treatments were more pronounced at 1 and 3 months).

Additional insight can be gained by conducting separate one-way ANOVAs among treatments at each time point (Table 5). Neither spiked treatment was significantly different from CTL at time 0 for H′, S, density, or B-IBI. However, H′, S, and B-IBI were significantly reduced in both treatments, relative to the control, 1 month after dosing. Diversity still was significantly lower in TRT B relative to CTL after 3 months. Number of taxa in TRT B also remained at a much lower level at 3 months in comparison to the predose period.

While there were significant dose-related reductions in H′, S, and B-IBI, among-treatment

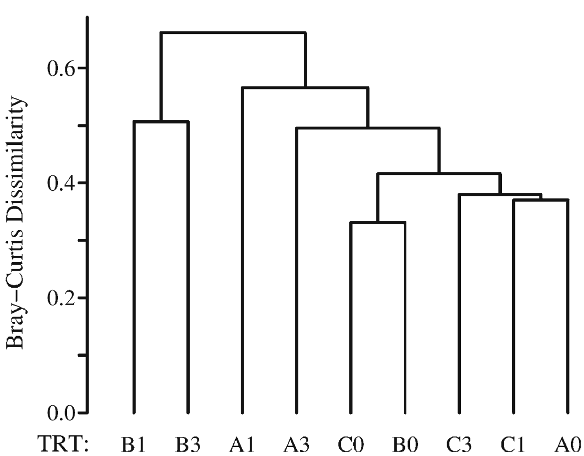

Fig. 4 Dendrogram summarizing results of hierarchical clustering of pooled benthic replicates (summed abundances) for control (*C*), low dose (*A*), and high dose (*B*) treatment groups at 0, 1, and 3 months after dosing

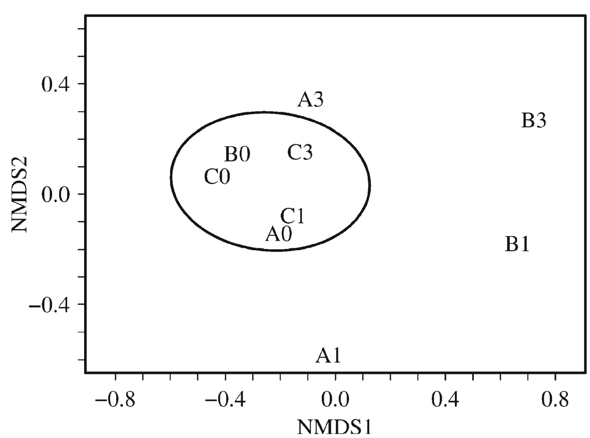

Fig. 5 Results of nonmetric multidimensional scaling (*NMDS*) of pooled benthic replicates (summed abundances) from the benthic mesocosm study. Labels represent controls (*C*), low dose treatments (*A*), and high dose treatments (*B*) at 0, 1, and 3 months after dosing. Bounded area represents a 90% confidence ellipse for the control treatment group

Table 6 Visual observations of benthic mesocosm replicate trays at 1, 3, and 6 months after dosing

Treatment	Time (after dosing)		
	1 month	3 months	6 months
CTL	Sediment grayish-brown in appearance, similar to predose condition. No strong odor. Good amount of shell hash. Obvious signs of bioturbation: burrows, polychaete mounds with fecal material, and tubes extending above sediment surface. Bivalves present. Sediment appears well-mixed, with no distinct line between dosed sediment and original 7 cm of mud	Sediment dark grayish-brown. No odor. Healthy looking communities. Polychaetes, large molluscs (bivalves, gastropods), and ophiuroids (brittlestars) present. Signs of active bioturbation of upper sediment layer across all trays. Sediment well-mixed throughout each tray. Many brittle star arms extended out of sediment and waving. Lots of algae covering nearly all trays	Sediment black. Weak sulfur odor. Very few signs of bioturbation. Sediment no longer appeared to be well-mixed. Some small polychaetes, bivalves, and brittle stars present. No signs of life in some replicate trays
TRT A	Sediment dark gray. Slight, but not strong, odor. Good amount of shell hash. Algae in sheets, not as bright green as CTL. Evidence of bioturbation. Polychaetes, worm tubes, and bivalves present. Sediment appears well-mixed, with no distinct dividing line between dosed layer and original sediments	Sediment dark brown to black. Strong sulfur odor from one replicate tray. Fair amount of algae, though not as extensive as in CTL. Observed some polychaetes, few gastropods, and brittle stars. No live molluscs observed. Fewer polychaetes and brittlestars than in CTL treatments. Some distinction between upper and lower sediment layers in some trays	Sediment grayish-black. Strong sulfur odor. Little evidence of bioturbation, sediment compacted, not well-mixed. Some polychaetes (*Pectinaria* sp.) present. Some empty polychaete tubes observed. One replicate tray with a single brittlestar living along the side of the tray
TRT B	Sediment black. Strong sulfur odor. Algae in this tank were mostly brown. Surface of sediment flat, no relief or other signs of bioturbation. Did not observe any signs of living biota when sieving the samples. Most replicate trays had black anoxic sediment below a thin layer of surface sediment. Several trays had a distinct line between the dosed sediment and original 7 cm of sediment	Sediment black. Strong sulfur odor. Some polychaetes in one replicate tray (single large burrow and polychaete tube). No bioturbation observed in other 3 replicates. No algae present. Distinct difference between upper (black) sediment layer and underlying (gray) sediment. Ophiuroids (brittlestars) have abandoned sediment trays and are living in the bottom of the tank	Sediment black. Sulfur odor. Little to no evidence of bioturbation. Sediment not well-mixed. Few signs of life, except one replicate tray with large population of amphipods (*Gammarus mucronatus*) living on sediment surface. Observed one polychaete (*Pectinaria* sp.). Dead, broken brittlestars present

differences in total faunal density were not significant (at $\alpha = 0.05$) for any period (Table 5). Dominant taxa (five most abundant among the three treatments) were the polychaetes *Polydora cornuta*, *Streblospio benedicti*, and *Cirrophorus* spp.; tubificid oligochaetes; insect larvae including unidentified Chironomidae and *Raphium* spp. (Dolichopodidae); the bivalve *Nucula aegeensis*; and the amphipod *Gammarus mucronatus*. Similar to the insignificant differences in total faunal density, densities of most of these individual dominant taxa did not show consistently clear dose-related patterns. However, the insect larvae, tubificid oligochaetes, and the amphipod *G. mucronatus* were particularly abundant in the spiked treatments. Also, the bivalve *N. aegeensis* did not occur in TRT B sediments after the initial dose.

Hierarchical clustering of the benthic data further illustrates overall community-level effects of the spiked sediment treatments (Fig. 4). Essentially, the various treatment groups clustered according to the level of sediment contamination. The treatments that were the most similar (lowest dissimilarity values) included CTL at all time points, TRT A at time 0 (TRT A-0), and TRT B at time 0 (TRT B-0). The low treatment group after dosing (TRT A-3 and TRT A-1) separates from this group at dissimilarity values of 0.50 and 0.58, respectively. The high treatment group at 1 and 3 months after dosing (TRT B-1 and TRT B-3) forms a separate, distinct cluster at dissimilarity = 0.67. NMDS of these data provides a good summary of the overall results: CTL at all time periods and TRT A and TRT B at time 0 were most similar; TRT A and TRT B at 1 month separate from the "all controls/time 0" group, with the separation being more pronounced for TRT B; some degree of recovery is evident at 3 months, with TRT A being less separated from the "all controls/time 0" group and TRT B still separated from the other treatments at 3 months (Fig. 5).

Water quality parameters were monitored for all treatments throughout the duration of the experiment. Daily ($n = 81$) mean values [± standard deviation] for CTL, TRT A, and TRT B were 27.4°C [±2.1°C], 27.3°C [±2.1°C], and 26.3°C [±2.1°C] for temperature; 33.0 [±2.4], 32.6 [±6.9], and 34.5 [±3.0] psu for salinity; 8.1 [±0.2], 8.3 [±0.2], and 8.3 [±0.1] for pH; and 4.8 [±0.9], 7.0 [±1.0], and 6.6 [±1.2] mg/L for dissolved oxygen, respectively.

Visual observations of animal behavior and general condition of the exposure chambers were recorded at 1, 3, and 6 months after dosing (Table 6). Reference (CTL) sediments appeared to contain healthy benthic assemblages at 1 and 3 months with obvious signs of rigorous biological activity including bioturbation, burrows, fecal mounds, and filter feeding by ophiuroids (*Hemipholis elongata*). Healthy ophiuroids were observed with their arms extended out of their burrows and actively waving in the water column. Reductions in such biological activity, along with visible signs of deteriorating sediment conditions (dark gray to black color, strong sulfur odor), were especially evident in TRT B even at 1 month. By 6 months, even the CTL mesocosms appeared to show significant signs of degradation due to organic buildup, as noted above in the "Methods" section.

Discussion

The replicated, modular estuarine mesocosm design has been used successfully to test water-borne exposures of various organisms to pesticides such as azinphosmethyl (Lauth et al. 1996), endosulfan (Pennington et al. 2004), fipronil (Wirth et al. 2004), and atrazine (Bejarano et al. 2005). The present modification to accommodate the application of spiked sediment slurries to field-collected sediments demonstrates the feasibility of such an approach and its suitability for incorporating a benthic phase. In the current study, benthic infaunal assemblages were found to have significantly lower B-IBI scores, diversity (Shannon H′), and numbers of taxa relative to controls 1 month after application of spiked-sediment slurries dosed with a mixture of three chemical contaminants (copper, pyrene, DDT) at nominal concentrations of 0.1 to 1 times their respective ERMs (thus, mERMQs of 0.1 to 1.0). Furthermore, effects on diversity persisted 3 months after dosing at the higher dose level (mERMQ = 1.0).

These results serve to validate the applicability of SQGs such as ERMs in assessing the potential toxicity of chemical contaminant mixtures on benthic fauna. Moreover, the finding that diversity and number of taxa were significantly reduced in the moderately contaminated treatment group (mERMQ = 0.1; TRT A) relative to CTL demonstrates that adverse effects on infaunal assemblages can occur at low levels of contamination below an average ERMQ of 1.0. Impacts on benthic fauna in a similar low range, but based on correlative field data, have been observed in estuaries of the southeastern US (mERMQ > 0.058; Hyland et al. 1999) and throughout US Gulf of Mexico, southeastern US, and northeastern US estuaries combined (mERMQ > 0.361; Hyland et al. 2003). Long et al. (2006) reviewed several case studies of exposure/response relationships using mean SQG quotients and found that data from some of those studies (Long et al. 1999, 2003) also agreed with the observations of relatively greater sensitivity of the benthos to lower chemical concentrations as compared to that of the 10-day single-species (amphipod) survival tests. While the previous benthic studies cited above found a high incidence of benthic impacts associated with mERMQs in the range of 0.06–0.4, based on correlative field data, the present study represents a confirmation of such impacts in a manipulative experiment using field-collected estuarine sediments under controlled mesocosm conditions (i.e., a direct cause-and-effect demonstration). As suggested by Hyland et al. (2003), the benthic community-level responses observed at these lower levels of chemical contamination may reflect the varying sensitivities of multiple species and life stages to longer-term exposures persisting over several generations. Evaluating a different set of toxicological indicators (e.g., those based on sublethal endpoints, longer-term exposures, earlier life stages) in relation to mERMQs may produce toxicity thresholds that are closer to the benthic community-level responses (Ingersoll et al. 2005).

Batley et al. (2005) have suggested that, because they are based on co-occurrence, empirical SQGs for individual chemicals have little to do with the effects of individual chemicals. Rather, they are more indicative of the toxic effects of the entire mixture of chemicals present in the sediment. Moreover, while the toxicity of individual contaminants may be additive (Swartz et al. 1988, 1997; Xie et al. 2006; Evans and Nipper 2007), their corresponding SQGs may not be (also see Swartz 1999). Hence, summed SQG quotients (e.g., summed ERMQs) are likely to be inappropriate for predicting sediment toxicity for complex mixtures of contaminants, while other methods of expressing SQG-normalized sediment concentrations, such as a mERMQ, may be more appropriate. In this study, mERMQs of 0.001, 0.056, and 0.564 were observed 3 months after dosing for CTL, TRT A, and TRT B, respectively, based on the three spiked contaminants, with effects on benthic communities observed during the course of the experiment at both the moderate and high treatment levels (i.e., in the range 0.056–0.56). This is well below the range (>1.0) associated with a high incidence of mortality in short-term laboratory toxicity tests with amphipods (e.g., Long and MacDonald 1998). However, in order to make direct comparisons with these previous field-derived effect levels, we also present mERMQs based on this same set of 24 contaminants. These results (mERMQ = 0.011, 0.018, and 0.059 at the end of the experiment for CTL, TRT A, and TRT B, respectively) suggest effects on benthic assemblages at slightly lower levels consistent with the earlier field-derived data. As pointed out by Batley et al. (2005), however, the use of a mERMQ can "dilute" the predicted impact of a dominant toxicant (such as the three spiked compounds) when other contaminants are present at low concentrations, as was the case in this study.

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recommends or endorses any proprietary product mentioned herein, or which has as its purpose an interest to cause the advertised product to be used or purchased because of this publication.

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INCIDENCE OF STRESS IN BENTHIC COMMUNITIES ALONG THE U.S. ATLANTIC AND GULF OF MEXICO COASTS WITHIN DIFFERENT RANGES OF SEDIMENT CONTAMINATION FROM CHEMICAL MIXTURES

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Abstract: Synoptic data on concentrations of sediment-associated chemical contaminants and benthic macroinfaunal community structure were collected from 1,389 stations in estuaries along the U.S. Atlantic and Gulf of Mexico coasts as part of the nationwide Environmental Monitoring and Assessment Program (EMAP). These data were used to develop an empirical framework for evaluating risks of benthic community-level effects within different ranges of sediment contamination from mixtures of multiple chemicals present at varying concentrations. Sediment contamination was expressed as the mean ratio of individual chemical concentrations relative to corresponding sediment quality guidelines (SQGs), including Effects Range-Median (ERM) and Probable Effects Level (PEL) values. Benthic condition was assessed using diagnostic, multi-metric indices developed for each of three EMAP provinces (Virginian, Carolinian, and Louisianian). Cumulative percentages of stations with a degraded benthic community were plotted against ascending values of the mean ERM and PEL quotients. Based on the observed relationships, mean SQG quotients were divided into four ranges corresponding to either a low, moderate, high, or very high incidence of degraded benthic condition. Results showed that condition of the ambient benthic community provides a reliable and sensitive indicator for evaluating the biological significance of sediment-associated stressors. Mean SQG quotients marking the beginning of the contaminant range associated with the highest incidence of benthic impacts (73-100% of samples, depending on the province and type of SQG) were well below those linked to high risks of sediment toxicity as determined by short-term toxicity tests with single species. Measures of the ambient benthic community reflect the sensitivities of multiple species and life stages to persistent exposures under actual field conditions. Similar results were obtained with preliminary data from the west coast (Puget Sound).

Keywords: Benthic indicators, chemical contaminants, sediment quality, predicting benthic stress, U.S. Atlantic and Gulf of Mexico estuaries.

1. Introduction

Although concentrations of some chemical contaminants have shown decreasing trends in estuarine sediments in recent years (Valette-Silver, 1992), potential biological impacts from sediment contamination remain a key environmental concern in many coastal areas around the U.S. Numerical sediment quality guidelines (SQGs) have been developed to provide interpretive tools for assessing the biological significance of individual chemical contaminants in sediments (e.g., see review by Chapman, 1989). Combining (e.g., by averaging) the ratios of individual chemical concentrations in a sample relative to corresponding SQG values

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also has been used to quantify potentially harmful mixtures of chemical contaminants present at varying concentrations (Long *et al.*, 1998).

An empirical framework for estimating the probability of observing sediment toxicity within different ranges of mean SQG quotients was introduced recently, based on results of amphipod toxicity tests conducted with samples from estuaries nationwide (Long *et al.*, 1998, 2000; Long and MacDonald, 1998). These analyses demonstrated a relatively low incidence of toxicity in samples with the lowest amount of chemical contamination (mean SQG quotients < 0.1) and an increasing incidence of toxicity with increasing contamination. The incidence of highly toxic samples (i.e., mean survival $< 80\%$ of controls, and significantly different at $p < 0.05$) was highest at mean ERM quotients > 1.5 and mean PEL quotients > 2.3 (Long and MacDonald, 1998). Hyland *et al.* (1999) applied a similar approach in efforts to develop a basis for evaluating risks of benthic community-level effects in relation to sediment contamination for estuaries of the southeastern U.S. This latter study demonstrated that condition of the ambient benthic community can serve as a reliable and sensitive indicator of potential disturbances resulting from these chemical stressors. Mean SQG quotients associated with a high incidence of benthic impacts (i.e., mean ERM quotients > 0.058 and mean PEL quotients > 0.096) were well below those associated with a similar high incidence of sediment toxicity in the prior laboratory studies.

In the present study, we extend the initial analysis of benthic-contaminant relationships in southeastern estuaries by Hyland *et al.* (1999) to additional data sets from mid-Atlantic and Gulf of Mexico estuaries. The purposes of the study were to determine bioeffect ranges for evaluating risks of chemical contaminant effects on benthic communities in these other coastal regions, and ultimately to expand our understanding of the relationships between chemical exposure levels and risks of adverse effects on resident fauna over broader spatial scales. The use of the term "risk" here means the likelihood of an effect occurring based on its observed incidence (percentage of occurrence) in the sample population.

2. Methods

Data were collected as part of the estuaries component of the U.S. EPA's Environmental Monitoring and Assessment Program (EMAP), conducted in collaboration with the National Status and Trends (NS&T) program of NOAA and multiple other federal, state, academic, and private research institutions. The data were derived from sediment samples collected at 1,389 stations sampled throughout three EMAP estuarine provinces (Figure 1): (1) the Virginian Province (Cape Cod to mouth of Chesapeake Bay) represented by 511 stations sampled during summers 1990–93; (2) Carolinian Province (mouth of Chesapeake Bay to St. Lucie Inlet, FL) represented by 290 stations sampled during summers 1994–97; and (3)

Louisianian Province (Anclote Key, FL to Rio Grande, TX) represented by 588 stations sampled during summers 1991–94. Though many environmental variables were sampled as part of EMAP (Holland, 1990), the present analysis was performed using data on concentrations of chemical contaminants in sediments and condition of benthic macroinfaunal communities measured synoptically at each site.

Concentrations of organic chemicals and metals were measured in a single sample of composited surface sediment (upper 2–3 cm) collected at each station with a 0.04-m^2 Young grab sampler. Typically, 16 inorganic elements, four butyltins, 27 aliphatic hydrocarbons, 44 polynuclear aromatic hydrocarbons (PAHs), 20 polychlorinated biphenyl (PCB) congeners, and 24 pesticides were measured at each station using methods consistent with general EMAP and NS&T protocols (U.S. EPA, 1995; Lauenstein and Cantillo, 1993). Specific methods for the Virginian, Carolinian, and Louisianian Provinces are summarized in Paul *et al.* (1999), Hyland *et al.* (1999), and Summers *et al.* (1992), respectively.

Total chemical contamination in each sediment sample was expressed as the mean of the ratios of individual chemical concentrations relative to corresponding SQG values. Thus, of the total number of chemical contaminants that were measured, only those for which SQGs have been developed were included in this computation (Table 1). These "concentration-to-SQG quotients" were computed using two different types of SQGs: Effects Range-Median (ERM) values (Long *et*

Figure 1. Map of EMAP-Estuaries Provinces showing location of the three provinces included in the present study (larger bolded font). Data supporting this study were from 1,389 stations sampled as follows: 511 stations in the Virginian Province from 1990–93; 290 stations in the Carolinian Province from 1994–97; and 588 stations in the Louisianian Province from 1991–94.

al., 1995) and Probable Effects Level (PEL) values (MacDonald *et al*., 1996). ERM and PEL values both represent mid-range concentrations of chemicals above which adverse effects on a wide variety of benthic organisms are likely to occur. Both types of guidelines are listed in Table 1 by each corresponding chemical contaminant. Methods used here to compute mean ERM and PEL quotients are the same as those used by Long *et al*. (1998; 2000) and Long and MacDonald (1998) to evaluate the incidence of sediment toxicity within different chemical contaminant ranges. Concentrations of chemical contaminants below analytical detection limits were treated here as zeros and included in the computations.

The condition of benthic macroinfaunal communities was assessed using diagnostic, multi-metric benthic indices specific for each region:

- Virginian Province: EMAP Multivariate Benthic Index (Paul *et al*., 1999). Component variables are the Gleason diversity index and abundances of tubificid oligochaetes and spionid polychaetes.
- Louisianian Province: EMAP Multivariate Benthic Index (Engle and Summers, 1999). Component variables are Shannon-Wiener diversity (H'); abundances of tubificids; and proportions of capitellid polychaetes, bivalves, and amphipods.

Table 1. Chemicals and corresponding sediment quality guideline (SQG) values used in the calculation of mean SQG Quotients. NA = Not Available.

Chemical	*ERM*[a]	*PEL*[b]
Metals (µg/g or ppm)		
Arsenic	70	41.6
Cadmium	9.6	4.21
Chromium	370	160
Copper	270	108
Lead	218	112
Mercury	0.71	0.7
Silver	3.7	1.77
Zinc	410	271
Pesticides (ng/g or ppb)		
4,4'-DDD (p,p'-DDD)	NA	7.81
4,4'-DDE (p,p'-DDE)	27	374
4,4'-DDT (p,p'-DDT)	NA	4.77
Dieldrin	NA	4.3
Gamma BHC (Lindane)	NA	0.99
Total Chlordane[c]	NA	4.79
Total DDTs[d]	46.1	51.7
PAHs (ng/g or ppb)		
Acenaphthene	500	88.9
Acenaphthylene	640	128
Anthracene	1100	245
Benzo[a]anthracene	1600	693
Benzo[a]pyrene	1600	763
Chrysene	2800	846
Dibenz[a,h]anthracene	260	135
Fluoranthene	5100	1494
Fluorene	540	144
2-Methylnaphthalene	670	201
Naphthalene	2100	391
Phenanthrene	1500	544
Pyrene	2600	1398
PCBs (ng/g or ppb)		
Total PCBs	180	189
Total No. SQGs Used	24	29

[a]From Long *et al*. (1995)
[b]From MacDonald *et al*. (1996)
[c]Includes alpha-, gamma-, and oxychlordane
[d]Includes 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, 2,4'-DDD, 2,4'-DDE, and 2,4'-DDT

- Carolinian Province: Multi-metric Benthic Index of Biotic Integrity, B-IBI (Van Dolah *et al*., 1999). Component variables are species richness, total faunal abundance, dominance, and % abundance of pollution-sensitive taxa.

Different approaches were used to develop the three benthic indices and each is based on a different combination of biological attributes as indicated above. However, they are all similar in that multiple, benthic attributes were combined into a single measure designed to maximize the ability to distinguish between degraded versus non-degraded benthic condition. Also, all three indices were developed with methods included to help account for biological variability attributable to key, natural controlling factors (e.g., latitude, salinity, sediment particle size). Efforts to account for such variability focused on factors shown, through multivariate statistical analysis, to have the strongest influence on benthic distributions under non-degraded (reference) conditions within each specific province (e.g., latitude and salinity in the case of the Carolinian Province). Further details on these procedures are included in the references cited above.

Data used in the calculation of benthic indices were derived from replicate macroinfaunal samples collected at each station with a 0.04-m^2 Young grab sampler. Benthic and chemistry samples were collected at each station from separate grabs on the same day (usually < 2 hours apart) and within close proximity to one another. Because sampling was conducted from an anchored boat, both types of samples generally were taken within a 10-m radius of the station coordinates (i.e., within the anchor swing). Benthic samples were collected to a maximum sediment depth of 10 cm (the penetration limit of the grab) and were rejected if < 5 cm (a precautionary measure used to prevent significant loss of fauna concentrated in this upper sediment layer). Contents of the grabs were live-sieved in the field with a 0.5-mm mesh screen, fixed in 10%-buffered formalin with rose bengal, and transferred to the laboratory for further processing. Animals were sorted from sample debris under a dissecting microscope and identified to the lowest possible taxon (usually to species).

The cumulative frequencies (percentages) of stations with degraded vs. non-degraded benthic communities were plotted in relation to ascending values of the two mean SQG quotients. Plots for the Carolinian Province are included here for illustration purposes (Figure 2). For each individual region, 10th-, 50th-, and 90th-percentile points from the cumulative-frequency plots of degraded stations were used as critical points to separate mean SQG quotients into four ranges: those associated with either a low incidence of benthic impacts (values below the corresponding 10th-percentile point), moderate incidence of impacts (values between the 10th- and 50th-percentile points), high incidence of impacts (values between the corresponding 50th- and 90th-percentile points), or very high incidence of impacts (values above the 90th-percentile point).

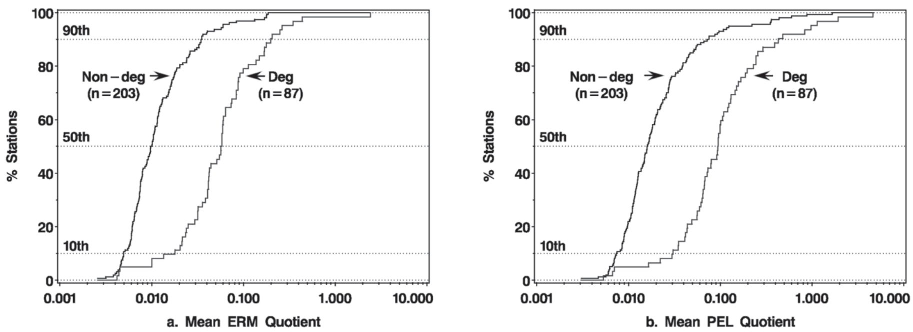

Figure 2. Cumulative percentage of stations with degraded vs. non-degraded benthic assemblages plotted in relation to (a) mean ERM and (b) mean PEL quotients for the EMAP Carolinian Province. Similar plots were generated for the other EMAP provinces (though not shown here).

Data also were merged across provinces to support the derivation of a single set of critical points for all three provinces combined. For this larger combined data set, 5th- and 95th-percentile points were used in place of the 10th and 90th to enhance predictability in the lower and upper contaminant ranges. With the larger merged data set, these narrower limits could be used while still retaining sufficient sample sizes at either end.

In the present analysis, stations with low dissolved oxygen (DO) were excluded to reduce the chance of misinterpreting any benthic responses that may have been influenced by this factor. Stations were excluded if DO concentrations were < 2 mg/L. This range is often associated with a high risk of adverse effects on benthic fauna (Diaz and Rosenberg, 1995). Although this precaution was taken, it is important to recognize the possibility that some of the remaining stations included in the analysis may have experienced low-DO conditions prior to the sampling date. Similarly, confounding effects of other unmeasured stressors (e.g., high concentrations of ammonia or sulfide in sediments), although unknown, may have contributed to the condition of benthic communities at some of these stations as well.

3. Results and Discussion

3.1 Chemical-Contaminant Bioeffect Ranges for the Benthos

Relationships between the incidence of degraded benthic communities and increasing chemical contamination are illustrated in Figure 2. Values of mean SQG quotients along the x-axis were always higher for degraded than non-degraded cases at corresponding cumulative-frequency points along the y-axis. For example, 10% of Carolinian Province stations with a degraded benthos had mean ERM quotients ≤ 0.018, while 10% of those with a healthy benthos had values ≤ 0.005 (Figure 2a). Fifty percent of the stations with a degraded benthos had mean ERM quotients ≤ 0.057, while 50% of those with a healthy benthos had values ≤ 0.010. Hence, the proportion of degraded to non-degraded benthic communities increased with increasing chemical contamination.

Similar patterns were observed for mean PEL quotients (Figure 2b), though individual values of the two different SQG quotients varied for any given sample. Mean ERM quotients were always less than mean PEL quotients (typically within a factor of two). This difference is due to the fact that PEL guidelines are lower than corresponding ERM guidelines for most chemicals (Table 1). Thus, concentration-to-SQG quotients based on ERM values (as a denominator) will be lower than those based on PEL values.

Lowest risks of benthic impacts were observed at mean SQG quotients below the 10th-percentile critical points (Tables 2 and 3). Six to 32% of stations in these lower ranges, depending on the location or type of SQG, had degraded benthic communities. Higher probabilities occurred at mean SQG quotients between the 50th- and 90th-percentile points, with about 54-86% of stations in these ranges having degraded benthic communities. The incidence of degraded benthic communities was highest (73-100%) at stations with quotients above the 90th-percentile points.

Critical points separating chemical contamination into the four concentration ranges varied from region to region (Tables 2 and 3). Such differences were fairly small (generally within a factor of 2) for critical points separating the first two ranges and the largest (up to a factor of 8) for those defining the beginning of the uppermost range. Generally, the critical points were the lowest for the Louisianian Province, highest for the Virginian Province, and in-between for the Carolinian Province. Reasons for these differences are not known but may be related to variations in magnitude of sediment contamination, susceptibility of benthic fauna, or confounding effects of other unmeasured environmental factors across the provinces. Nevertheless, all three provinces showed a consistent response of a very high incidence of degraded benthic condition (73–100% of samples, depending on the location or type of SQG) beginning at relatively moderate chemical-contaminant concentrations (upper ERM critical points of 0.062 to 0.473, and upper PEL critical points of 0.118 to 0.969).

Similar results were obtained when data from all provinces were merged (Table 4). The incidence of degraded benthic communities was lowest (18%) at mean SQG quotients below the 5th-percentile critical points (mean ERM and PEL quotients < about 0.01). The incidence of these effects increased with increasing chemical contamination. For example, a much higher incidence of degraded benthic communities (62–63%) occurred at mean SQG quotients between the 50th and 95th percentile points (i.e., mean ERM quotients of 0.044 to 0.361, and mean PEL quotients of 0.076 to 0.781). The highest incidence of degraded benthic communities (74–77%) occurred at mean SQG quotients above the 95th-percentile points (i.e., mean ERM quotients > 0.361 and mean PEL quotients > 0.781).

Table 2. Benthic risk levels and the corresponding observed incidence of effects within different ranges of mean ERM quotients. VA = EMAP Virginian Province; CAR = EMAP Carolinian Province; LA = EMAP Louisianian Province.

Risk Level	*EMAP Province*	*Mean ERM Quotient Range*	*Number of Stations*	*% Stations with Degraded Benthos*
Low	VA	≤ 0.022	148	9%
	CAR	≤ 0.018	158	7%
	LA	≤ 0.013	118	30%
Medium	VA	> 0.022–0.098	220	31%
	CAR	> 0.018–0.057	70	43%
	LA	> 0.013–0.036	267	52%
High	VA	> 0.098–0.473	123	54%
	CAR	> 0.057–0.196	51	69%
	LA	> 0.036–0.062	166	86%
Very High	VA	> 0.473	20	85%
	CAR	> 0.196	11	100%
	LA	> 0.062	37	92%

Table 3. Benthic risk levels and the corresponding observed incidence of effects within different ranges of mean PEL quotients. VA = EMAP Virginian Province; CAR = EMAP Carolinian Province; LA = EMAP Louisianian Province.

Risk Level	*EMAP Province*	*Mean PEL Quotient Range*	*Number of Stations*	*% Stations with Degraded Benthos*
Low	VA	≤ 0.040	160	10%
	CAR	≤ 0.030	155	6%
	LA	≤ 0.022	119	32%
Medium	VA	> 0.040–0.179	209	32%
	CAR	> 0.030–0.094	69	43%
	LA	> 0.022–0.062	265	52%
High	VA	> 0.179–0.969	120	55%
	CAR	> 0.094–0.420	51	71%
	LA	> 0.062–0.118	166	85%
Very High	VA	> 0.969	22	77%
	CAR	> 0.420	15	73%
	LA	> 0.118	38	95%

Table 4. Benthic risk levels and the corresponding observed incidence of effects within different ranges of mean SQG quotients for all three EMAP provinces combined.

SQG Type	*Risk Level*	*Percentile*	*Mean SQG Quotient Range*	*Number of Stations*	*% Stations with Degraded Benthos*
ERM	Low	≤ 5th	≤ 0.007	165	18
	Medium	5th – 50th	> 0.007–0.044	752	36
	High	50th – 95th	> 0.044–0.361	430	63
	Very High	> 95th	> 0.361	42	74
PEL	Low	≤ 5th	≤ 0.012	177	18
	Medium	5th – 50th	> 0.012–0.076	737	37
	High	50th – 95th	> 0.076–0.781	435	62
	Very High	> 95th	> 0.781	40	77

3.2 COMPARISON TO CHEMICAL CONTAMINANT RANGES ASSOCIATED WITH SEDIMENT TOXICITY

Long and MacDonald (1998) reported a relatively high probability of observing sediment toxicity in samples with mean ERM quotients > 1.5 and mean PEL quotients > 2.3. These predictions were based on matching sediment chemistry and toxicity data from multiple surveys of sediment quality performed in coastal areas nationwide. Toxicity was based on standard, 10-day, survival tests conducted with adult amphipods (*Ampelisca abdita* and *Rhepoxynius abronius*). The incidence of highly toxic samples (i.e., mean survival in sample < 80% of control, and significantly different at $p < 0.05$) in these upper, chemical contamination ranges was 74% and 76% for mean ERM and PEL quotients, respectively.

Probable, chemical-contaminant bioeffect ranges observed here for benthic communities were well below the chemical contamination ranges associated with an equivalent high incidence of sediment toxicity in the above laboratory toxicity studies. This pattern was repeated across all three EMAP provinces. We hypothesize that benthic community-level responses should be observed at lower, chemical concentration ranges because they reflect the varying sensitivities of multiple species and life stages to longer-term exposures that may be persisting over several generations. Such measures provide a realistic snapshot of how the ambient benthic ecosystem is responding to chemical exposure.

These comparisons are not meant to imply that the laboratory toxicity data are invalid or of lesser value. Rather, they help to show that the biological significance of chemical contamination varies with different receptors, endpoints, and exposure regimes. If other toxicological indicators (e.g., those based on sublethal endpoints, longer-term chronic exposures, earlier life stages) were evaluated in relation to mean SQG quotients, one might find toxicity thresholds that are much closer to these benthic community-level responses. For example, Chung (1999) found 50% mortality in young juvenile clams (*Mercenaria mercenaria*) at a mean ERM quotient of about 0.4, which is very similar to the mean-ERM-quotient, upper-range critical point of 0.36 reported here for the merged benthic data set (Table 4). Also, acute:chronic ratios from results of aqueous-phase toxicity tests are typically within a factor of 10 (sometimes up to 100 or more) for many chemicals, which is similar to the magnitude of variation observed here between benthic community-level responses and results of acute toxicity tests conducted with adult amphipods.

3.3 Preliminary Comparison with Benthic Data from other Regions

Preliminary data from a recent survey of Puget Sound conducted by NOAA and the State of Washington provided an additional opportunity to see if similar patterns of benthic response extended to other regions. Figure 3 shows the relationship between the mean values (and 95% confidence intervals) of two benthic variables, H' diversity (Shannon and Weaver, 1949) and Swartz Dominance Index (Swartz *et al.*, 1985), across different ranges in mean ERM quotients, based on data from 297 stations sampled throughout Puget Sound, from 1997–99.

Note that for these data, unlike the East Coast and Gulf of Mexico data sets, we did not have the benefit of a diagnostic benthic index that could be used to classify samples as having degraded vs. non-degraded benthic condition, and which was designed to account for the influence of natural controlling factors on benthic distributions. Thus, these data could not be subjected to the same kind of analysis used to determine chemical-contaminant bioeffect ranges for Atlantic and Gulf of Mexico benthic communities. Nevertheless, Figure 3 shows clear reductions in both benthic indicators at mean ERM quotients beginning at about the 0.1 to 0.2 range. This is the same range in which we observed a high incidence of benthic stress for the Atlantic and Gulf of Mexico coasts and again well below the range associated with an equivalent high incidence of sediment toxicity. Similarly, Long

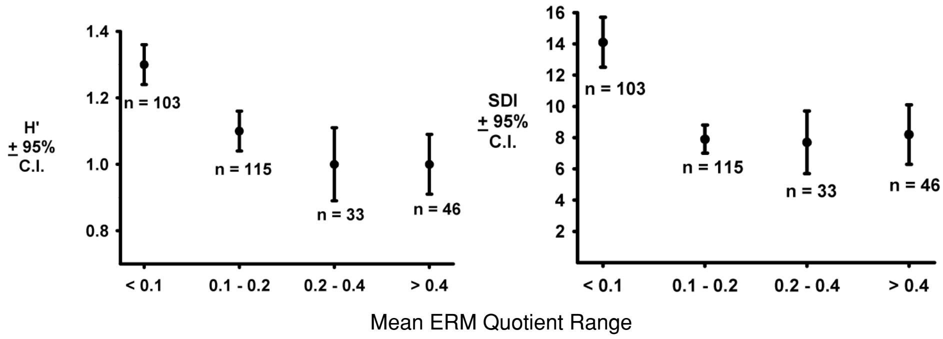

Figure 3. Plots of mean values of H' and Swartz Dominance Index (± 95% confidence intervals) within different ranges of mean ERM quotients at 297 stations sampled throughout Puget Sound from 1997–99.

et al. (in review) found evidence of benthic impacts in Biscayne Bay, FL at mean ERM quotients beginning in this same range (0.1-0.2), and that were lower than those associated with acute toxicity in laboratory survival tests performed on samples from the same locations (i.e., mean ERM quotients > 1.25).

Among the 297 stations sampled in Puget Sound, only one had sediments that were highly toxic in amphipod assays (mean survival in sample < 80% of control, and significantly different at $p < 0.05$), and this site had no chemical contaminants present at concentrations above the SQGs. Thus, an important observation is that adverse effects in these benthic communities showed a higher concordance with increases in chemical contamination (and possibly other unmeasured stressors) than did sediment toxicity based on the amphipod assay.

The Puget Sound data appear to show patterns consistent with those observed in the other EMAP provinces on the Atlantic and Gulf of Mexico coasts. However, these results must be regarded as preliminary until the data can be subjected to more rigorous analyses, including efforts to determine what natural controlling factors may be influencing benthic responses. It would be helpful as well to have the ability to apply a benthic condition index, or some other diagnostic method for classifying samples as degraded versus non-degraded, and which takes into account the influence of these other controlling factors on benthic responses.

3.4 DATA UNCERTAINTIES

We have attempted here to provide an empirical framework for predicting relative risks of benthic community-level effects within different ranges of sediment contamination by chemical mixtures. Predictive ability within these ranges, though high, was not perfect. As shown in Tables 2–4, it is possible that some sites in the lowest risk range will have degraded benthic communities and that some sites in the highest risk range will have non-degraded ones. A variety of factors may contribute to such uncertainty. A degraded benthic community might occur in sediments with low mean SQG quotients (false positives) due to potential effects from: (1) unmeasured chemical contaminants and other stressors (e.g., ammonia, sulfide); (2) measured chemical contaminants not included in the computation of mean SQG quotients (due to lack of bioeffect guidelines); or (3) other physical and biological sources of disturbance (e.g., sediment erosion and deposition due to bottom currents and storm events; predation and other biological interactions). Possible explanations for observing a non-degraded benthic community in sediments with high mean SQG quotients (false negatives) include: (1) contaminants were present at high concentrations but not in bioavailable forms (e.g., due to binding with sediments and organic complexes); (2) the fauna were not exposed to toxic concentrations of contaminants due to small-scale spatial variations; or (3) actual bioeffect levels for individual chemical contaminants present at the site are higher than the corresponding SQGs used to compute the concentration-to-SQG quotients.

Future research should focus on reducing such uncertainties. Of particular importance is the need to differentiate between the relative contributions of measured chemical contaminants to observed benthic effects vs. other possible stressors (e.g., ammonia, sulfide, unmeasured contaminants) that may be co-varying in relation to common environmental factors (such as % sediment fines). Condition of the ambient benthic community appears to be a sensitive indicator of the biological significance of sediment contamination. However, the incidence of a degraded benthic community at the high chemical-contamination range does not preclude the possibility that the observed bioeffects were due partly to other co-varying stressors. Where co-occurrences of high sediment contamination and degraded benthic-community condition are observed, follow-up studies are recommended to determine exact causes and stressor sources.

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PREDICTING STRESS IN BENTHIC COMMUNITIES OF SOUTHEASTERN U.S. ESTUARIES IN RELATION TO CHEMICAL CONTAMINATION OF SEDIMENTS

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Abstract—Matching data on sediment contaminants and macroinfaunal community structure from 231 subtidal stations in southeastern U.S. estuaries were used to develop a framework for evaluating risks of benthic impacts from multiple-contaminant exposure. Sediment contamination was expressed as the mean ratio of individual contaminant concentrations relative to corresponding sediment quality guidelines, that is, to effects range–median (ERM) values, probable effects level (PEL) values, or an aggregate of the two. The probability of a degraded benthos was relatively low in samples with mean ERM quotients $\leq$0.020, PEL quotients $\leq$0.035, or combined ERM/PEL quotients $\leq$0.024. Only 5% of stations within these ranges had degraded benthic assemblages, while 95% had healthy assemblages. A higher probability of benthic impacts was observed in samples with mean ERM quotients $>$0.058, PEL quotients $>$0.096, or ERM/PEL quotients $>$0.077. Seventy-three to 78% of stations with values in these upper ranges had degraded benthic assemblages, while 22 to 27% had healthy assemblages. Only four stations (three with degraded, one with healthy assemblages) had mean ERM or PEL quotients $>$1.0, which is the beginning of the range associated with a high probability of mortality in short-term laboratory toxicity tests using amphipods.

Keywords—Benthos Sediment contamination Sediment quality guidelines Southeastern estuaries Environmental Monitoring and Assessment Program

INTRODUCTION

In 1993, the U.S. Environmental Protection Agency (U.S. EPA) and the National Oceanic and Atmospheric Administration (NOAA) initiated a joint study of the quality of southeastern estuaries by coordinating two nationwide environmental monitoring efforts, the U.S. EPA Environmental Monitoring and Assessment Program (EMAP) and the NOAA National Status and Trends program (NS&T). The study region, known as the Carolinian Province, extends from Cape Henry, Virginia, USA, through the southern end of Indian River Lagoon on the east coast of Florida, USA (Fig. 1). Estuaries in this region encompass an estimated 11,622 km^2 of the coastal zone and contain natural resources of both high economic and ecological value. There has been an increasing need for effective management of these systems given the enormous influx of people and businesses predicted to occur in southeastern coastal states over the next decade [1] and the ensuing pressures of human activities in these areas.

The Carolinian Province study was initiated to help support such management needs by providing year-to-year estimates of estuarine condition based on a variety of biological, chemical, toxicological, and aesthetic indicators. Estimates of the overall quality of these estuaries using various indicators have been reported elsewhere [2–5]. In this paper, we use selected Carolinian Province data on sediment contamination and macroinfaunal composition to examine relationships between the incidence of a degraded benthos and variations in the magnitude of combined contaminant concentrations. The purpose of this analysis is to develop a framework for interpreting the biological significance of multiple-contaminant exposures in future assessments based on field-derived data.

A variety of numerical sediment quality guidelines (SQGs) has been developed to provide interpretive tools for assessing the biological significance of concentrations of individual chemicals (see [6–13] for reviews). Combining the ratios of individual contaminant concentrations in a sample relative to respective SQG values (either by averaging or summing) has appeared in the recent ecotoxicology literature as a method of quantifying potentially harmful mixtures of contaminants present in varying concentrations [2,14–17]. Long et al. [17] also provided a useful framework for judging the relative probability of detecting adverse bioeffects within different ranges of mean SQG quotients based on the relationship between sediment chemistry and toxicity in laboratory tests with samples collected from estuaries nationwide. In the present study, we have expanded upon this approach in an effort to develop a basis for evaluating risks of benthic impacts in relation to measures of multiple-contaminant concentrations in sediments of southeastern estuaries. A unique feature of the present analysis is that the basis for judging biological significance in relation to sediment contaminant concentrations is derived from observations of benthic community-level responses in field samples rather than results of acute toxicity tests with single species. To our knowledge, this is the first effort to provide estimates of bioeffect thresholds for mixtures of contaminants based on ambient benthic-contaminant relationships.

MATERIALS AND METHODS

Data were derived from sediment samples collected at 231 subtidal stations sampled during the summers of 1994 to 1996

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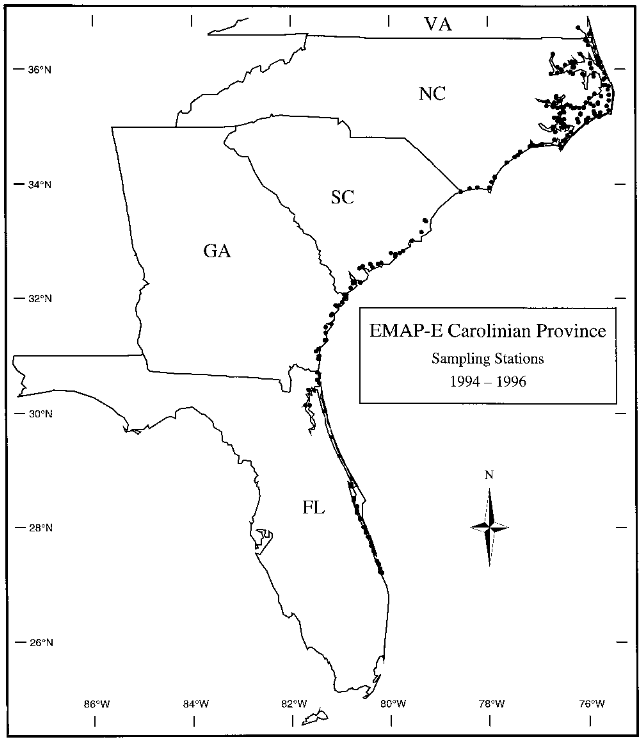

Fig. 1. Study area.

in estuaries from Cape Henry, Virginia, USA, to St. Lucie Inlet, Florida, USA (Fig. 1). Samples were collected as part of the joint EMAP/NS&T monitoring program conducted in the Carolinian Province. Though many environmental variables were sampled as part of this program [3–5], the present analysis was limited to data on sediment contamination and macroinfaunal composition measured synoptically at each site. Other relevant data on sediment bioeffects (not used in the present analysis) included estimates of sediment toxicity based on amphipod (*Ampelisca abdita* and *A. verrilli*), juvenile clam (*Mercenaria mercenaria*), and Microtox® assays (Azur, Carlsbad, CA, USA). Such data can be obtained from the literature [3,4] or by contacting the senior author directly.

Organic and metal contaminants were measured in subsamples of composited surface sediment (upper 2–3 cm) collected at each station with a 0.04-m^2 Young grab sampler deployed from an anchored research vessel. A single composited sample was analyzed at most stations; duplicates were run at ~10% of the stations for quality-control purposes. A total of 16 inorganic metals, four butyltins, 27 aliphatic hydrocarbons, 44 polynuclear aromatic hydrocarbons (PAHs), 20 polychlorinated biphenyls (PCBs), and 24 pesticides were measured at each station. Table 1 summarizes the measurement units, detection limits, analytical methods, and protocol references for various analytes or chemical groups.

Three to four replicate macroinfaunal samples also were collected from each station with a 0.04-m^2 Young grab sampler. Because sampling was conducted from an anchored boat, replicates generally were taken within a 10-m radius of the station coordinates (i.e., within the anchor swing). Samples were collected to a maximum sediment depth of 10 cm and were rejected if <5 cm. Contents of the grabs were live-sieved in the field with a 0.5-mm mesh screen, fixed in 10% buffered formalin with rose bengal, and transferred to the laboratory for further processing. Two of the replicate samples from each

Table 1. Summary of analytical methods for the analyses of contaminants in sediments

Analyte	Target detection limits[a]	Units (dry wt)	Method[b]	Reference
Si	10,000	μg/g	FAA	[25]
Al	1,500	μg/g	FAA	[25]
Fe	500	μg/g	INAA	[25]
Cr	5.0	μg/g	INAA	[25]
Zn	2.0	μg/g	FAA	[25]
Mn	1.0	μg/g	FAA	[25]
Cu	5.0	μg/g	GFAA	[25]
As	1.5	μg/g	INAA	[25]
Ni	1.0	μg/g	GFAA	[25]
Pb	1.0	μg/g	GFAA	[25]
Sb	0.2	μg/g	INAA	[25]
Se, Sn	0.1	μg/g	GFAA	[25]
Cd	0.05	μg/g	GFAA	[25]
Ag	0.01	μg/g	GFAA	[25]
Hg	0.01	μg/g	CVAA	[25]
Butyltins[c]	1.0	ng Sn/g	GC/FPD	[26]
PAHs[d]	5.0	ng/g	GC/MS-SIM	[27]
Aliphatics[e]	25	ng/g	GC/FID	[28]
Pesticides[f]	0.1	ng/g	GC/ECD	[27]
PCBs[g]	0.1	ng/g	GC/ECD	[27]

[a] Based on sample size of 0.2 g for metals and 15 g for organics.
[b] CVAA = cold vapor atomic absorption; GC/ECD = gas chromatography/electron capture detection; GC/MS-SIM = GC/mass spectroscopy-selective ion monitoring mode; GC/FID = GC/flame ionization detection; FAA = flame atomic absorption; GC/FPD = GC/flame photometric detection; GFAA = graphite furnace atomic absorption; INAA = instrumental neutron activation analysis.
[c] Butyltins: mono, di, tri, tetra.
[d] PAHs = polynuclear aromatic hydrocarbons: 44 parent compounds and alkylated homologues, total PAHs.
[e] Aliphatics: C10–C34 alkanes, total Alkanes, pristane, phytane.
[f] Pesticides: dichlorodiphenyldichloroethane (DDD; 2,4′ and 4,4′), dichlorodiphenyldichloroethylene (DDE; 2,4′ and 4,4′), DDT (2,4′ and 4,4′), total DDD/DDE/DDT, aldrin, chlordane (alpha-, gamma-, oxy-), dieldrin, heptachlor, heptachlor epoxide, hexachlorobenzene, benzenehexachloride (BHC) (or hexachlorocyclohexane [HCH]; alpha-, beta-, gamma-, delta-), mirex, trans- and cis-nonachlor, endrin, endosulfan, toxaphene.
[g] PCBs: congeners 8, 18, 28, 44, 52, 66, 101, 105, 188/108/149, 128, 138, 153, 170, 180, 187/182/159, 195, 206, 209, total PCBs.

station were processed to characterize the infaunal assemblages, and the remaining samples were archived. Animals were sorted from sample debris under a dissecting microscope and identified to the lowest possible taxon (usually to species). The data were used to compute numbers of species and individuals; H′ diversity [18]; percent abundance of key taxonomic groups; and a combined benthic index of biotic integrity (B-IBI), developed during this program as a tool for assessing habitat quality in estuaries of the southeastern United States [4,19].

The B-IBI was derived from a modification of methods used by Weisberg et al. [20] for the development of a similar benthic index for the Chesapeake Bay. It is a multimetric index that reflects the degree to which component measures of key biological attributes at a station deviate from corresponding optimum values expected under undisturbed conditions, based on the distribution of values at pristine or best available reference sites. The Carolinian benthic index is an average of the scores for the following four metrics: numbers of species, total faunal abundance, dominance, and percent abundance of pollution-sensitive taxa (ampeliscid and haustoriid amphipods, lucinid and tellinid bivalves, hesionid and cirratulid polychaetes, and the isopod *Cyathura burbanki*). This specific combination of metrics was shown to be the most efficient at discriminating between nondegraded and degraded sites classified on the basis of dissolved oxygen (DO), sediment chemistry, and sediment toxicity results [4,19].

The first step in computing the B-IBI was to produce a single-station mean for each component metric by averaging the values for all replicate samples from the same station. The mean value of each metric was then scored relative to reference conditions established for similar habitat types (e.g., similar latitudes and salinity zones) to help partition out biological variations attributable to natural abiotic factors. Scoring thresholds for various metric–habitat combinations are given in Van Dolah et al. [19]. Last, the individual metric scores were averaged to yield a single combined score for the station. The index ranges from one to five. Values $\leq$1.5 are indicative of a highly degraded benthos, values $\geq$3 are indicative of a healthy benthos, and transitional values between 1.5 and 3 reflect partial symptoms of stress. Stations in the present study were classified as having a degraded benthos if the B-IBI score was $<$3.

In the present analysis, stations with low DO were excluded from the database to prevent misinterpreting any benthic responses that may have been influenced by this factor. Stations were considered to have low DO if any one of the following conditions occurred: DO $<$ 0.3 mg/L at any time throughout a 24-h time series (measurements at 30-s intervals), DO $<$ 2.0 mg/L for more than 20% of the time series, or DO $<$ 5.0 mg/L throughout the entire time series [4]. Linear regressions also were run to test for the dependence of benthic index scores on other abiotic factors (salinity, percent silt-clay, total organic carbon, and depth) also known to affect benthic distributions.

The incidence of a degraded benthos was examined in relation to total sediment contamination expressed as the mean of the ratios of individual contaminant concentrations in a sample relative to corresponding SQG values. These concentration-to-SQG quotients were computed using three different types of SQGs: effects range–median (ERM) values [7,21], probable effects level (PEL) values [12,22], and a combination of the two. The ERM and PEL values both represent midrange concentrations of chemicals above which adverse effects on a wide variety of benthic organisms are likely to occur. MacDonald et al. [22] provide a general overview and comparison of the approaches used to derive individual ERM and PEL values. A list of the chemicals and corresponding guideline values used here to compute the three different types of mean SQG quotients is given in Table 2. Mean ERM and PEL quotients were computed with the same methods and chemicals used by Long et al. [17]. The combined ERM/PEL measure is based on the same 24 ERM values used to compute the mean ERM quotient but makes use of additional available ERM values for antimony, nickel, and total PAHs and additional PEL values for 4,4′-dichlorodiphenyldichloroethane (DDD), 4,4′-DDT, dieldrin, lindane, and total chlordane.

The cumulative frequencies of stations with degraded and nondegraded benthic assemblages were plotted in relation to ascending values of the three mean SQG quotients. Separate curves were generated for degraded versus nondegraded assemblages. Tenth- and 50th-percentile points from the cumulative frequency plots of degraded stations were used as thresholds to separate mean SQG quotients into three ranges: those associated with a relatively low incidence of benthic impacts (values below the corresponding 10th-percentile point), those associated with a relatively moderate incidence of impacts (values between the 10th- and 50th-percentile points), and those associated with a relatively high incidence of impacts (values above the corresponding 50th-percentile point).

Table 2. Chemicals and corresponding sediment quality guideline (SQG) values used in the calculation of SQG quotients

Chemical	ERM[a]	PEL[b]	Combined ERM/PEL
Metals (µg/g)			
Antimony	NA[c]	NA	25[d]
Arsenic	70	41.6	70
Cadmium	9.6	4.21	9.6
Chromium	370	160	370
Copper	270	108	270
Lead	218	112	218
Mercury	0.71	0.7	0.71
Nickel	NU	NU	51.6[a]
Silver	3.7	1.77	3.7
Zinc	410	271	410
PAHs (ng/g)			
Acenaphthene	500	88.9	500
Acenaphthylene	640	128	640
Anthracene	1,100	245	1,100
Benzo[*a*]anthracene	1,600	693	1,600
Benzo[*a*]pyrene	1,600	763	1,600
Chrysene	2,800	846	2,800
Dibenz[*a*,*h*]anthracene	260	135	260
Fluoranthene	5,100	1,494	5,100
Fluorene	540	144	540
2-Methylnaphthalene	670	201	670
Naphthalene	2,100	391	2,100
Phenanthrene	1,500	544	1,500
Pyrene	2,600	1,398	2,600
Total PAHs[e]	NU	NU	44,792[a]
PCBs (ng/g)			
Total PCBs	180	189	180
Pesticides (ng/g)			
4,4′-DDD[c] (p,p′-DDD)	NA	7.81	7.81
4,4′-DDE[c] (p,p′-DDE)	27	374	27
4,4′-DDT (p,p′-DDT)	NA	4.77	4.77
Dieldrin	NA	4.3	4.3
Gamma BHC (lindane)	NA	0.99	0.99
Total chlordane[f]	NA	4.79	4.79
Total DDTs[g]	46.1	51.7	46.1
Total no. of SQGs used	24	29	32

[a] ERM = effects range–median; data from Long et al. [21].
[b] PEL, probable effects level; data from MacDonald [12], MacDonald et al. [22].
[c] NA = not applicable; NU = not used; DDD = dichlorodiphenyldichloroethane; DDE = dichlorodiphenyldichloroethylene.
[d] [7].
[e] Excluding perylene.
[f] Includes alpha-, gamma-, and oxychlordane.
[g] Includes 4,4′-DDD, 4,4′-DDE, 4,4′-DDT, 2,4′-DDD, 2,4′-DDE, and 2,4′-DDT.

RESULTS

General environmental characteristics

A summary of biological, chemical, and physical characteristics of stations with degraded versus nondegraded benthic assemblages is given in Table 3. Sites with a degraded benthos generally had fewer species, lower abundances, lower diversity, and lower benthic index scores than sites with a healthy

Table 3. Summary statistics for selected biological, chemical, and physical characteristics at stations with a nondegraded versus degraded benthos

	Nondegraded benthos					Degraded benthos				
Parameter	*n*	Range	Mean	Median	SD	*n*	Range	Mean	Median	SD
Biological										
Number of species	163	4.0–77.0	19.0	15.0	12.3	76	0.0–28.0	4.9	4.0	4.6
Abundance	163	6.5–1,577.5	168.5	112.5	205.0	76	0.0–398.0	41.1	15.0	66.6
H′	163	0.75–4.78	2.89	2.86	0.83	76	0.00–3.76	1.33	1.27	0.86
B-IBI score	163	3.0–5.0	3.9	4.0	0.6	76	1.0–2.5	1.7	1.5	0.6
Chemical										
ERM quotient	162	0.00–0.19	0.02	0.01	0.03	76	0.00–2.41	0.11	0.06	0.28
PEL quotient	162	0.00–1.66	0.05	0.02	0.16	76	0.01–4.62	0.24	0.10	0.58
ERM/PEL quotient	162	0.00–1.54	0.04	0.01	0.15	76	0.00–1.92	0.16	0.08	0.34
Physical										
Bottom salinity (‰)	163	0.1–39.1	21.3	22.9	10.3	76	0.2–34.1	15.5	17.1	8.7
Depth (m)	159	0.0–12.7	2.8	2.0	2.5	66	0.0–10.6	4.0	4.0	2.1
Silt-clay (%)	163	0.3–99.6	15.8	5.8	23.8	76	0.7–99.6	69.2	88.4	34.2
TOC (%dry wt)	160	0.0–6.8	0.6	0.3	0.9	74	0.1–14.8	3.1	3.1	2.4
Bottom DO (mg/L)	160	1.7–10.2	6.4	6.3	1.4	66	0.3–12.5	5.0	5.6	2.6

[a] SD = standard deviation; B-IBI = benthic index of biotic integrity; ERM = effects range–median; PEL = probable effects level; TOC = total organic carbon; DO = dissolved oxygen.

benthos (as illustrated by comparison of means and medians between groups). As a group, sites with a degraded benthos also tended to have higher contaminant levels (larger mean SQG quotients), lower salinity, muddier sediments, higher sediment total organic carbon (TOC) levels, and slightly greater depths.

Both categories of stations were dominated by annelids (polychaetes and tubificid oligochaetes) and arthropods (mostly peracarid crustaceans and chironomid insect larvae) (Table 4). In general (with the exception of arthropods at degraded sites), these two taxonomic groups accounted for the majority of both abundance and numbers of species. Mollusks were more abundant than arthropods at sites with degraded benthic assemblages. *Mediomastus* spp. (Polychaeta), unidentified oligochaetes, and *Streblospio benedicti* (Polychaeta) were the three most numerically dominant taxa at both categories of stations (Table 5). There was a stronger dominance of these taxa at degraded than nondegraded sites (55% vs 22% of the cumulative percentile abundance, respectively). Abundance was much more evenly distributed among species at sites with a healthy benthic assemblage. Further details on these fauna (e.g., a quantitative species list) can be obtained from the senior author.

Benthic-contaminant relationships

Figure 2 provides plots of the cumulative frequencies of stations with degraded and nondegraded benthic assemblages in relation to ascending values of the three mean SQG quotients. Of the 231 stations, 70 showed evidence of a degraded benthos and 161 appeared to have a healthy benthos based on the B-IBI. Mean ERM quotients ranged from 0.004 to 2.41 at stations with degraded benthic assemblages and from 0.003 to 0.186 at stations with healthy assemblages. Values of mean ERM quotients along the *x*-axis were always higher for degraded than nondegraded cases at corresponding cumulative-frequency points along the *y*-axis. For example, 10% of stations with a degraded benthos had mean ERM quotients ≤0.020, while 10% of those with a healthy benthos had values ≤0.005. Fifty percent of stations with a degraded benthos had mean ERM quotients ≤0.058, while 50% of those with a healthy benthos had values ≤0.010. Hence, the proportion of degraded to nondegraded benthic assemblages increased with increasing contaminant concentration.

Similar patterns were observed for the other two measures of chemical contamination, though individual values of the three different measures varied for any given sample. Mean ERM quotients were always less than mean PEL quotients. In a few samples, the mean PEL quotient exceeded the mean ERM quotient by up to a factor of 15. However, for most samples (87%), the mean PEL quotient was only one to two times higher than the mean ERM quotient. Mean ERM/PEL quotients more closely resembled the mean ERM quotients given that most of the SQGs used to compute this combined measure were ERMs (Table 2). Ninety-two percent of the samples had mean ERM and mean combined ERM/PEL quotients that differed by less than a factor of two.

Mean PEL quotients ranged from 0.005 to 4.62 at stations with a degraded benthos and from 0.003 to 1.66 at stations with a healthy benthos (Fig. 2b). Mean combined ERM/PEL quotients ranged from 0.004 to 1.92 at stations with a degraded benthos and from 0.002 to 1.54 at stations with a healthy benthos (Fig. 2c). As noted for mean ERM quotients, both of these other chemical measures had values along the *x*-axis that were always higher for degraded than nondegraded cases at corresponding cumulative-frequency points along the *y*-axis.

Table 4. Relative percentages of major taxa at nondegraded and degraded benthic stations

	Nondegraded benthos		Degraded benthos	
	% Abundance	% Species	% Abundance	% Species
Annelida	53	38	73	42
Arthropoda	20	28	9	31
Mollusca	19	23	13	18
Other	8	11	5	9

Table 5. Dominant infaunal species (listed in decreasing order of abundance) at nondegraded and degraded benthic stations; mean abundance per grab (0.04 m^2) averaged over all stations, cumulative percent abundance, and frequency of occurrence (percent stations) are given; P = Polychaeta, O = Oligochaeta, B = Bivalvia, A = Amphipoda, T = Tanaidacea, C = Chironomidae insect larvae

Nondegraded benthos				Degraded benthos			
Species	Mean	Cumulative %	% Stations	Species	Mean	Cumulative %	% Stations
Mediomastus spp. (P)	15	9	77	*Mediomastus* spp. (P)	14	29	43
Unidentified Oligochaetes (O)	13	17	66	Unidentified Oligochaetes (O)	7	42	52
Streblospio benedicti (P)	9	22	59	*Streblospio benedicti* (P)	5	55	47
Halmyrapseudes bahamensis (T)	9	27	2	*Mulinia lateralis* (B)	4	63	25
Mulinia lateralis (B)	7	31	42	*Paraprionospio pinnata* (P)	2	68	35

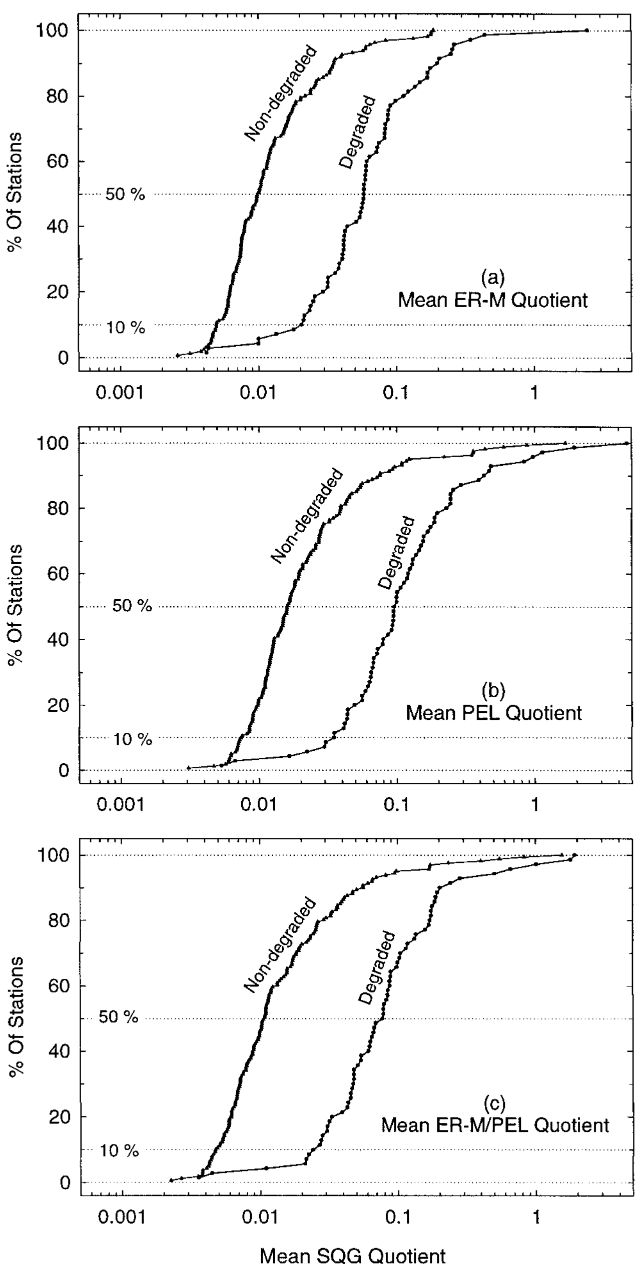

Fig. 2. Cumulative percentage of stations versus mean SQG quotients based on effects range–median (ERM) values (**a**), probable effects level (PEL) values (**b**), and combined ERM/PEL values (**c**). Plots for stations with both degraded and nondegraded benthos are shown.

As described in the methods section, 10th- and 50th-percentile points from the cumulative frequency plots of degraded stations were used to define three ranges in mean SQG quotients associated with low, moderate, or high probabilities of detecting adverse benthic conditions (Table 6). Based on this approach, the probability of observing a degraded benthos would be relatively low in samples with a mean SQG quotient below the corresponding 10th-percentile point: mean ERM quotients $\leq$0.020, mean PEL quotients $\leq$0.035, or mean combined ERM/PEL quotients $\leq$0.024. Regardless of the measure used, only 5% of stations in this lower risk zone had degraded benthic assemblages and 95% had healthy ones. Sediment contaminant levels at moderate risk to benthic assemblages would have mean SQG quotients between the 10th- and 50th-percentile points: mean ERM quotients $>$0.020 to 0.058, mean PEL quotients $>$0.035 to 0.096, and mean combined ERM/PEL quotients $>$0.024 to 0.077. Forty-eight to 55% of stations in this transitional range had degraded benthic assemblages and 45 to 51% had healthy ones. The probability of a degraded benthos would be relatively high in samples with a mean SQG quotient above the corresponding 50th-percentile point: mean ERM quotients $>$0.058, mean PEL quotients $>$0.096, or mean combined ERM/PEL quotients $>$0.077. Seventy-three to 78% of stations in this upper risk zone had degraded benthic assemblages, while only 22 to 27% had healthy ones.

The derivation of benthic risk levels from the above faunal–contaminant relationships did not appear to be influenced by additional natural environmental factors also known to affect benthic distributions. This point is supported by results of regression analysis (Table 7), which showed no significant influence (α = 0.05) of salinity, percent silt-clay, TOC, or depth on B-IBI scores among nonpolluted sites (stations with low sediment contamination and toxicity). These results reflect the fact that the B-IBI was designed to maximize detection of pollution impacts independent of the influence of such controlling abiotic factors [4,19]. There were significant inverse relationships between B-IBI scores and three of the four abiotic factors (depth, percent silt-clay, and %TOC) among polluted sites. We believe that these latter associations are due to a combination of the inverse relationship between benthic health and sediment contamination and the natural tendency of contaminants to accumulate at higher concentrations in deeper, muddier, and more organically enriched depositional areas.

Stations with low DO were excluded from the database to prevent the misinterpretation of any benthic responses that may have been influenced by this factor. It should be noted, however, that low DO did not appear to be a pervasive condition throughout these estuaries. Low DO was observed at only seven additional stations sampled during the 1994 to 1996

Table 6. Incidence of benthic impacts within three ranges of mean sediment quality guideline (SQG) quotients computed from effects–range median (ERM), probable effects level (PEL), and combined ERM/PEL values; cutpoints separating low from moderate risk and moderate from high risk are based on the 10 and 50% points, respectively, from the cumulative frequency plots of stations with a degraded benthos; benthic assemblages were classified as degraded if benthic index of biotic integrity (B-IBI) score <3.0

SQG type	Risk of benthic impacts	Mean SQG quotient range	Number of stations	% With degraded benthos	% With non-degraded benthos
ERM	Low	≤0.020	135	5	95
	Moderate	>0.020–0.058	51	55	45
	High	>0.058	45	78	22
PEL	Low	≤0.035	131	5	95
	Moderate	>0.035–0.096	52	54	46
	High	>0.096	48	73	27
ERM/PEL	Low	≤0.024	128	5	95
	Moderate	>0.024–0.077	56	48	51
	High	>0.077	47	77	23

study period. Of these seven stations, five also showed evidence of moderate to high sediment contamination and a degraded benthos, and one had low contamination and a healthy benthos. There was only one station where degraded benthic condition co-occurred with low DO alone. Thus, degraded condition of infaunal assemblages was more closely coupled with sediment contamination than with DO.

DISCUSSION

Threshold limits separating mean SQG quotients into the three benthic risk levels were higher for the mean PEL quotient compared to the mean ERM quotient. This difference is due to the fact that PEL guidelines are lower than corresponding ERM guidelines for most chemicals (Table 2). Thus, concentration-to-SQG quotients based on ERM values (as a denominator) tend to be lower than those based on PEL values for many of the chemicals included in the computation. As expected, lower and upper thresholds for the combined ERM/PEL quotient produce ranges that are between those derived from mean ERM and PEL quotients.

The above risk levels represent ranges in mean SQG quotients associated with low, moderate, or high probabilities of observing benthic impacts. As shown in Table 6, it is possible that some sites in the lower risk range will have degraded benthic assemblages and that some sites in the upper risk range will have healthy ones. A variety of factors may contribute to such scenarios. A degraded benthos might occur in sediments with low mean SQG quotients (false positives) due to potential effects from unmeasured contaminants or other physical and biological sources of disturbance (e.g., sediment erosion and deposition due to bottom currents and storm events, predation, and other biological interactions). Possible explanations for the appearance of a healthy benthos in sediments with high mean SQG quotients (false negatives) include (1) contaminants were not present in bioavailable forms (e.g., due to binding with sediments and organic complexes), (2) the fauna were not exposed to toxic levels of contaminants due to small-scale spatial variations in contaminant concentrations, or (3) actual bioeffect levels for individual contaminants present at the site are higher than the corresponding SQGs used to compute the concentration-to-SQG quotients. With respect to the latter point, Fulton et al. [23] found that ERM and PEL values for total DDT (0.0461 and 0.0517 μg/g, respectively) are at least 100 times below LC50 values for copepods (>10 μg/g), grass shrimp (4.5 μg/g), clams (5.8 μg/g), and amphipods (8.2–8.3 μg/g) in 10-d sediment exposures.

Long et al. [17] predicted a relatively high probability of observing toxicity in sediment samples with mean SQG quotients >1.0 and a relatively low probability in samples with mean SQG quotients <0.1. These predictions were based on matching sediment chemistry and amphipod (*Ampelisca abdita* and *Rhepoxynius abronius*) toxicity data from multiple surveys of sediment quality performed in estuaries along the Atlantic, Pacific, and Gulf of Mexico coasts. The probabilities of highly toxic and marginally toxic responses were 71 and 6%, respectively, for mean ERM quotients >1 and 56 and 9%, respectively, for mean PEL quotients >1. Probabilities decreased to 12 and 21%, respectively, for mean ERM quotients <0.1 and to 10 and 22%, respectively, for mean PEL quotients <0.1.

Our estimates of the ranges in mean SQG quotients associated with a high probability of observing adverse bioeffects are 10 to 20 times lower than those reported by Long et al. [17]. We suspect that a major cause of this variation is the difference in approaches used to assess biological significance. The assessment of adverse bioeffects in the present study was based on observations of benthic community-level responses in field samples rather than short-term (acute) laboratory toxicity tests with single species. Measures of ambient benthic community condition reflect the sensitivities of multiple component species to longer-term exposures and potential interactions with a variety of natural environmental factors. Thus,

Table 7. Tests for dependence of benthic condition (benthic index of biotic integrity [B-IBI]) on the abiotic variables salinity, percent silt-clay, total organic carbon (TOC), and depth; significant regressions (at $\alpha = 0.05$) are underscored

	Nonpolluted sites		Polluted sites	
	R^2	Probability > F	R^2	Probability > F
IBI versus salinity	0.075	0.475	0.108	0.389
IBI versus percent silt-clay	0.401	0.067	0.764	0.002
IBI versus TOC	0.318	0.114	0.724	0.004
IBI versus depth	0.350	0.093	0.612	0.013

in situ benthic condition may serve as a more sensitive indicator of the biological significance of observed contaminant levels.

Moreover, the laboratory toxicity data used by Long et al. [17] to assess bioeffects in relation to mean SQG quotients were based on the amphipod survival test with solid-phase (bulk) sediments. Though this is a standard toxicity test used in studies of sediment quality throughout North America, it has been shown to be a relatively insensitive indicator of toxicity in contaminated sediments from southeastern U.S. estuaries [3,4,24]. For example, toxicity tests with amphipods (*Ampelisca abdita* and *A. verrilli*) were performed during the Carolinian Province program on sediment samples from 198 of the 231 stations discussed in the present benthic analysis. Only 5 of the 198 samples (2.5%) were toxic to amphipods and, of these five samples, only two showed co-occurrences of high sediment contamination. Similarly, Long et al. [24] found that the spatial extent of sediment toxicity based on the amphipod test was restricted to a much smaller portion of U.S. estuaries (10.9%) in comparison to estimates based on tests with other species, sediment phases, and toxicity endpoints (e.g., 61% based on Microtox® tests with organic-solvent extracts of sediments, 43% based on sea urchin fertilization tests and mollusk embryo tests with undiluted sediment pore water).

Regional variations in the magnitude of sediment contamination may also explain some of the differences that we observed in bioeffect levels relative to those reported by Long et al. [17]. Only 4 of the 231 stations in the present study (three with degraded, one with healthy assemblages) had mean ERM or PEL quotients >1.0, the lower limit of the range that Long et al. [17] associated with high probabilities of observing sediment toxicity in samples collected nationwide. Most stations with degraded benthic assemblages in the present study had mean ERM and PEL quotients <0.1; Long et al. [17] observed toxicity in samples with mean SQG quotients in this lower range, though very infrequently. Thus, contaminant–bioeffect relationships were determined in the present study with data representing a much lower range in concentrations of sediment contaminants. Roughly one third of the samples used in the Long et al. [17] study were collected in specific bodies of water near urbanized centers, while our primary source of data consisted of samples from randomized sites among a widespread population of estuaries along the southeastern U.S. coastline, including many undeveloped areas.

We have focused on the use of mean SQG quotients as an approach to quantifying potentially harmful levels of mixtures of contaminants in a sample. One advantage to using mean SQG quotients is that they can be compared across studies in which varying numbers of contaminants have been analyzed. An alternative method of combining concentration-to-SQG ratios is by summing instead of averaging. Ringwood et al. [2] recently applied this method to EMAP Carolinian data as a means of quantifying overall sediment contamination and the potential for additive bioeffects from the individual components. Summed SQG quotients provide additional measures of the cumulative magnitude of individual contaminant concentrations relative to corresponding bioeffect levels and can be useful as a basis for ranking conditions among sites at which the same numbers of contaminants have been analyzed. The benthic risk levels presented in Table 6 can be converted to summed SQG quotients simply by multiplying the values for mean ERM, PEL, and combined ERM/PEL quotients by factors of 24, 29, and 32, respectively (i.e., the number of chemicals used in the averaging process, as indicated in Table 2). The relative risks of benthic impacts within the resulting three ranges of summed SQG quotients would have the same probabilities reported in Table 6 for mean SQG quotients.

The above ranges in the various mean SQG quotients provide a basis for evaluating risks of benthic impacts from exposure to contaminant mixtures in future studies of southeastern U.S. estuaries. Although the data were derived from a large number of sites throughout the region, our estimated benthic risk levels may not be applicable in other coastal areas. A similar approach should be attempted on a broader national scale, using measures of benthic condition appropriate for the various component regions. Our estimates also have been dependent on the availability and reliability of individual SQGs. The predictive ability of the benthic bioeffect ranges presented here could perhaps be increased with improvements to existing SQGs (e.g., as supporting databases expand) or the development of additional ones for chemicals not included in the analysis. Further research to refine our understanding of the relationships between contaminant exposure levels and in situ biotic responses should substantially improve our ability to predict potential environmental risks associated with increased human activities in the coastal zone.

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