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Sediment quality assessment in tidal salt marshes in northern California, USA: An evaluation of multiple lines of evidence approach

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HIGHLIGHTS

- Salt marsh sediment quality was assessed with expanded multiple lines of evidence.
- Sublethal responses in higher trophic level resident species are useful indicators.
- Susceptibility to natural confounding factors should be considered.
- Integration of molecular to population level responses supports accurate assessment.

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ABSTRACT

The objective of this study was to evaluate the efficacy of integrating a traditional sediment quality triad approach with selected sublethal chronic indicators in resident species in assessing sediment quality in four salt marshes in northern California, USA. These included the highly contaminated (Stege Marsh) and relatively clean (China Camp) marshes in San Francisco Bay and two reference marshes in Tomales Bay. Toxicity potential of contaminants and benthic macroinvertebrate survey showed significant differences between contaminated and reference marshes. Sublethal responses (e.g., apoptotic DNA fragmentation, lipid accumulation, and glycogen depletion) in livers of longjaw mudsucker (*Gillichthys mirabilis*) and embryo abnormality in lined shore crab (*Pachygrapsus crassipes*) also clearly distinguished contaminated and reference marshes, while other responses (e.g., cytochrome P450, metallothionein) did not. This study demonstrates that additional chronic sublethal responses in resident species under field exposure conditions can be readily combined with sediment quality triads for an expanded multiple lines of evidence approach. This confirmatory step may be warranted in environments like salt marshes in which natural variables may affect interpretation of toxicity test data. Qualitative and quantitative integration of the portfolio of responses in resident species and traditional approach can support a more comprehensive and informative sediment quality assessment in salt marshes and possibly other habitat types as well.

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1. Introduction

Bedded sediments are both a sink and a source for organic contaminants and trace metals. Toxic chemicals accumulated in sediments can

be released back into the adjacent ecosystem and consequently degrade the quality of ecological resources. Sediment contamination, especially in bays and estuaries, is a major environmental concern (Long, 2000) and many regional and national monitoring studies measure various toxic chemicals and biological indicators to collect information on sediment quality that is required to protect, manage, and sustain ecological resources (Barnett et al., 2008; U.S. EPA, 2004; Van Dolah et al., 2008).

Among many approaches, the sediment quality triad (SQT) is one of the most commonly applied approaches to assess sediment quality. The SQT measures three characteristics of sediments (concentrations

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of toxic chemicals, toxicity, and benthic invertebrate community structure) as a weight of evidence approach. It was developed in the mid-1980s (Chapman, 1986; Long and Chapman, 1985) and has been widely used successfully (Carr et al., 1996; Hunt et al., 2001; Sorensen et al., 2007). Various types of toxicity tests (e.g., sea urchin embryo tests, amphipod survival tests) that have been used for the SQT approach typically measure short-term responses in laboratory-incubated embryos or small fauna exposed under laboratory conditions; so, they have limitations in linking the observed responses to the ecology of diverse resident species such as fish and crabs. In addition, the laboratory toxicity tests are subject to interferences from ammonia and sulfides that may be elevated in many estuarine systems (Carr et al., 2006; Phillips et al., 1997), including the salt marshes that were a focus of the present study.

Since environmental managers are often concerned about the impacts of mixtures of toxic substances on higher trophic-level resident species and also may need to confirm findings of toxicity in more realistic environmental exposures, it may be useful to assess sediment quality using a series of selected responses in field exposed resident species that could provide integrated, practical and accurate site-specific information. Over the past decades, various techniques have been developed, enhanced, and successfully applied to environmental quality assessment programs (Belfiore and Anderson, 2001; Handy et al., 2003; Itow et al., 1998; Rice et al., 2000). Environmental managers, however, rarely adopt these methods due to lack of consensus about what methods could be applied and how observed responses could be interpreted. To encourage the inclusion of these updated methods as complementary and/or alternative approaches in environmental quality assessment programs, these methods need to be evaluated in well-designed field validation studies in targeted estuarine ecosystems.

Due to regulations on the use and discharge of toxic chemicals, it is becoming less common to find sites containing toxic chemicals at concentrations high enough to cause acute toxicity. As the environmental concerns have been shifting to chronic sublethal responses caused by long-term low-level exposure, it is desirable to add more tests with resident species that can reveal the long-term effects of contaminants under field-exposure conditions. The objective of the present study was to evaluate the efficacy of various indicators, including traditional triad components (e.g., sediment/porewater chemistry, porewater toxicity, invertebrate communities) and cost-effective sublethal chronic indicators (e.g., embryo abnormality, apoptotic DNA fragmentation, and glycogen depletion) in resident species, as an expanded multiple lines of evidence approach in assessing the impacts of sedimentary anthropogenic contaminants on the ecological health of salt marshes in California. This portfolio of indicators was developed by the Pacific Estuarine Ecosystem Indicator Research (PEEIR) consortium in the course of multi-year studies at fixed locations in several California salt marshes (Anderson et al., 2006). The overarching objective of the PEEIR program was to develop an approach for integrating indicators at multiple levels of biological organization for improved assessment of salt marsh health and integrity. The PEEIR program selected resident lined shore crab (*Pachygrapsus crassipes*) and longjaw mudsucker (*Gillichthys mirabilis*) as representative indicator species. Wide distribution of these species in salt marsh ecosystems of the west coast of the U.S. and sedentary, bottom-dwelling lifestyle make them suitable for the assessment of sediment quality on both small and large scales (Anderson et al., 2006). In addition, Morgan et al. (2006) determined that foraging ranges of lined shore crabs were restricted for juvenile and adult phases and Brooks (1999) demonstrated that longjaw mudsuckers can be caged for extended periods in the field for sediment quality assessment. Some other studies have used resident species as indicators of the condition of salt marsh ecosystems (Novak et al., 2006; Perez and Wallace, 2004; Wall et al., 2001) but they have focused on a limited set of responses in single species or multiple lower trophic level-species.

2. Material and methods

2.1. Study areas and sampling

Study areas (Fig. 1) were selected to validate the applicability of various indicators for highly contaminated and less disturbed marshes in northern California. Two marshes, Stege Marsh (ST: latitude-37.908487°, longitude-122.330425°) and China Camp Marsh (CC: latitude-38.013141°, longitude-122.494147°), are located in San Francisco Bay (SFB), which is the largest estuary on the west coast of the United States. Stege Marsh, which was designated as a Superfund site by U.S. Environmental Protection Agency (USEPA), was selected as a positive control marsh, while CC was selected as a relatively clean control marsh for SFB because CC is much less contaminated by human activities. China Camp Marsh is located in the northwest of central SFB and adjacent to the forested hills of China Camp State Park. Historically, CC was a shrimp village in the late 1800s and became a part of a state park in 1972. There is a decommissioned military base (Hamilton Army Airfield) approximately 3 km north of CC. Although this military base has been contaminated by various toxic chemicals, their impact on CC is negligible as indicated by sedimentary chemistry data (Hwang et al., 2006a, 2006b). The other two marshes, Tom's Point (TP: latitude-38.219353°, longitude-122.948830°) and Walker Creek Delta (WC: latitude-38.212430°, longitude-122.928300°), are situated in Tomales Bay (TB) that is located about 64 km northwest of SFB. Tomales Bay is relatively undisturbed by human activities compared with SFB. Thus, these two marshes were selected as reference marshes for this study.

Stege Marsh is on the western margin of central SFB and located near the city of Richmond. This marsh has become heavily contaminated over the last 100 years by the adjacent historic industrial activities, including facilities that manufactured agrochemicals (e.g., pesticides) until recently. Western ST has been highly contaminated by polychlorinated biphenyls (PCBs), which were mainly released from a utility company's former operation site. This area is also highly contaminated by mercury, which was released from a manufacturer of mercury fulminate for blasting caps. Remediation of this site was initiated in 2001 (URS, 2003) to reduce the potential for adverse effects of these contaminants on humans and wildlife, especially endangered species such as the California clapper rail. This marsh was used as a city landfill during the 1940s through the 1960s. During this period, various filling materials, including broken concrete, asphalt, various earth materials and substantial quantities of lead-containing crushed battery casings were deposited at this site (URS, 2003). Stormwater runoff entering through Carson Creek and Meeker Creek from adjacent urban areas and highways also contributes to contamination in ST. Due to high concentrations of contaminants, the Bay Protection and Toxic Cleanup Program, which was established under the San Francisco Bay Regional Water Quality Control Board (SFBRWQCB), included inner ST in the list of ten high-priority toxic hot spots and issued Cleanup and Abatement Orders to facilitate its cleanup (SFBRWQCB, 1999). Although concentrations of toxic chemicals in ST sediments have declined, they are still high enough to possibly cause toxic effects (Hwang et al., 2008).

Walker Creek Delta is located on the western margin of northern TB. Walker Creek has been listed as an impaired waterbody due to contamination by mercury from two historic mercury mines (Gambonini and Soule Mine) within the watershed. Total mercury concentrations in surface sediments in TB ranged from 0.05 to 3.1 µg/g, with the most elevated concentrations in the Walker Creek Delta (Johnson et al., 2009) and lower concentrations (0.05–0.5 µg/g) in other areas.

Concentrations of toxic chemicals, such as trace metals (e.g., Pb, Cu, Zn) and hydrophobic organic compounds (e.g., PCBs, PAHs), in sediment do not usually change abruptly and thus chemical measurements and the toxic responses measured in fish and crabs for the present study are not sensitive to short-term variation in sampling time. However, naturally-occurring toxicants, such as unionized ammonia, in pore

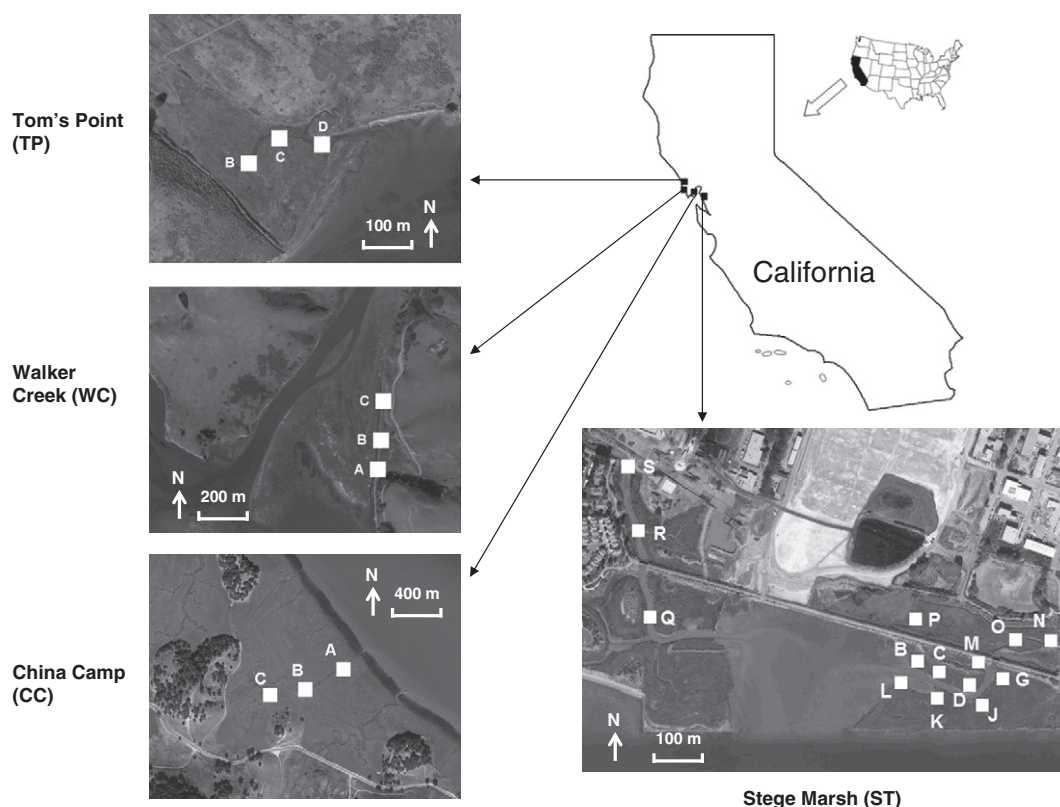


Fig. 1. Study area map showing locations of stations in each marsh.

water may change markedly from one day to the next due to tidal flushing, salinity changes, and hypoxia so the timing of sampling is a critical factor in conducting porewater toxicity tests and surveying benthic invertebrate communities. It is important to measure these natural variables to better interpret the results of toxicity tests (Carr et al., 2006). To minimize such artifacts, a total of 22 stations in intertidal areas were sampled in the four marshes between August 11 and 15, 2003. In each marsh, surface (0–5 cm) sediments were sampled at multiple fixed stations on the banks of major tidal creeks (Fig. 1).

2.2. Toxicity potential of contaminants in sediments

Sediment samples were analyzed for organic contaminants (PAHs, PCBs, DDTs, chlordanes) and trace elements (As, Ag, Cd, Cr, Cu, Ni, Pb, Zn). Detailed information on the geographical distribution and concentrations of these chemicals were reported in previous publications (Hwang et al., 2006a, 2006b). Measured chemical concentrations were converted into mean PELQs (probable effects level quotients) that represent the relative toxicity potential of measured contaminants (MacDonald et al., 1996; Long et al., 2006). PELQ was computed by normalizing the concentration of each contaminant with the corresponding PEL value and the PELQs of each contaminant were averaged to obtain mean PELQ (mPELQ) for each station. Toxicity potential of sediments was ranked in the order of mPELQ values (Hwang et al., 2008). Fairey et al. (2001) evaluated the ability of mPELQ to correctly predict toxicity of contaminants in sediments by comparing sediment chemistry and toxicology data (survival of amphipod; *Eohaustorius estuaries* and *Rhepoxynius abronius*) from the State of California's Bay Protection and Toxic Cleanup Program. They showed that the incidence of toxicity was higher than 50% when mPELQ is greater than 0.5, which is the value used as a threshold for the present study.

2.3. Sediment toxicity tests and toxicity identification evaluations

2.3.1. Sediment porewater toxicity test

Sediment porewater samples were collected in situ from 10 of 13 stations at ST on August 11, 2003 and 3 CC stations on August 14, 2003 using vacuum-extraction devices consisting of ground-glass aquarium air stones as the filtering medium attached to pre-cleaned 60-mL high density polyethylene syringes (Winger and Lasier, 1991). Eight to ten syringes were deployed in the creek bank of each inter-tidal station during low tide periods. The porewater samples were transferred to acid washed 300 mL polycarbonate centrifuge bottles and shipped on blue ice by overnight express mail to the Marine Ecotoxicology Research Station (MERS) in Corpus Christi, Texas. Upon arrival at the laboratory, samples were centrifuged to remove suspended particulates and the supernatant was subdivided into pre-cleaned amber glass bottles and frozen (-20°C). Samples were thawed to room temperature and salinity was measured and adjusted to $30 \pm 1\%$, if necessary, prior to testing. Dissolved oxygen, pH, hydrogen sulfide and ammonium concentrations were also measured before the toxicity tests. After the measurement of these parameters, any samples containing less than 80% dissolved oxygen saturation were gently aerated by stirring the sample on a magnetic stir plate. Although this stirring step may influence the concentrations of sulfide in the porewater samples, it is necessary to provide adequate oxygenation.

Toxicity of the sediment pore waters was measured using sea urchins (*Arbacia punctulata*) that were obtained from Woods Hole Biological Marine Laboratory. Porewater toxicity was determined using the relative percentage of embryos that fertilized or developed normally compared to those observed in reference tests (Carr and Chapman, 1995). A reference porewater sample collected from Aransas Bay, Texas and a reference toxicant, sodium dodecyl sulfate, were included in each toxicity test as a negative control and a positive control, respectively. Each of

the 13 porewater samples was tested in a dilution series design at 100, 50, and 25% pore water with five replicates per treatment. Dilutions were made with 0.45- μm filtered seawater collected from Aransas Bay, Texas. Statistical comparisons between treatments and controls were made using analysis of variance (ANOVA) and Dunnett's one-tailed t-test (which controls the experiment-wise error rate) on the arcsine square root transformed percent response data with the aid of SAS®. Detectable significance criteria (DSC), which were developed to determine the 95% confidence values based on power analysis of similar tests performed at MERS, were also applied to minimize false positives (Carr and Biedenbach, 1999).

2.3.2. Toxicity identification evaluation (TIE)

Samples found to be toxic in the sea urchin fertilization tests were fractionated for phase I TIE studies. Details on the procedures can be found in USEPA's marine toxicity identification evaluation, phase I guidance document (USEPA, 1996). TIE phase I manipulations were designed to identify the category of contaminants causing the observed toxicity. Each sample was manipulated sequentially by addition of EDTA (ethylenediaminetetraacetic acid) to remove metals by complexing, solid-phase extraction with Bakerbond C₁₈ Speed Disk or Millipore Sep-pak C₁₈ cartridge to remove nonpolar organic contaminants, and filtration with Whatman 0.45 μm nylon filters to remove any suspended particles.

2.3.3. Sediment elutriate toxicity test

Sediment elutriate toxicity tests were conducted using the USEPA method (USEPA, 1995) at Pacific EcoRisk in Martinez, California. Surface (0–5 cm) sediment samples (~500 g) were collected from the same 13 ST stations (August 12, 2003) and three CC stations (August 14, 2003) where the pore water samples were collected. Samples were not collected from TB and WC. At each station, samples were collected on the tidal-channel creek bank and marsh edge using a Teflon piston pipe in August 2003. Upon arrival at the laboratory, 100 g of each sample was transferred into a 500-mL glass Erlenmeyer flask and 200 mL of filtered seawater was added to each flask. Flasks were sealed with silicon stoppers and shaken for 30 min with a wrist-action shaker. After mixing, the contents of the flasks were allowed to settle for 15 min and the overlaying water was decanted into precleaned 250-mL Teflon centrifuge bottles. After 30 min of centrifugation at 2500 $\times g$, the supernatants were transferred into 250-mL glass beakers. These solutions comprised the 100% elutriate for each sediment sample. To make 10% elutriate, 100% elutriate was diluted with clean filtered sea water. Toxicity tests were performed with both 100% and 10% elutriate solutions for each station.

An aliquot (10 mL) of elutriates and approximately 200 fertilized sand dollar (echinoid: *Dendraster excentricus*) embryos were added to 30-mL glass vials. Three replicate vials for each sample were placed in a water bath (15 °C) with a 16 h:8 h light:dark photoperiod. After 72 h, the larval echinoderms were preserved with the addition of 0.5 mL of 5% glutaraldehyde solution. The echinoderm larvae were examined microscopically and the number of echinoderms that had successfully developed to the normal pluteus stage and the number of abnormal larvae were counted. Seawater from Bodega Bay, California was filtered with 0.2- μm polycarbonate filters and used as control water. To validate the response of *D. excentricus* embryos, one set of positive control samples spiked with copper (as CuSO₄·5H₂O) was tested concurrently with each set of sediment elutriate tests. The sample results were statistically analyzed and compared to the control treatment results using ANOVA (CETIS, Tidepool Scientific Software, McKinleyville, CA).

2.4. Benthic community assessment

Five to eight surface sediment core samples (5 cm diameter \times 5 cm deep) were collected using a polyvinyl chloride pipe from the same stations where the surface sediment samples were collected for chemical

analysis at ST, CC, WC, and TP between August 11 and 15, 2003 (Horne et al., 1999). Sediment samples were immediately transported to the laboratory on ice. Upon arrival at the laboratory, each sample was preserved in 10% buffered formalin and sieved through a 0.5-mm screen. Organisms from multiple samples were combined for each station, placed in deionized water for 24 h, split into two fractions for the measurement by two different staff, and stored in a 70% ethanol solution. All macroinvertebrates were sorted and identified under a dissecting microscope to the lowest taxonomic level possible, typically to species. Total abundance, species diversity (Shannon *H'*; Shannon and Weaver, 1949), and species richness of SFB marshes were compared with those of reference marshes and differences were determined using ANOVA. Pearson's correlation was used to demonstrate relationships between benthic survey data and toxicity potential of sedimentary contaminants (mPELQ). Mean values of each variable in each marsh were used for the Pearson's correlation.

2.5. Crab embryo abnormal development

Embryo abnormalities were measured in lined shore crabs (*P. crassipes*) that were collected from stations B, D, G, J, and M in ST ($n=79$), A and B in TP ($n=29$), and B and C in WC ($n=31$) in August 2003 (Fig. 1). Frequency of abnormalities was measured in embryos in the internal and external portions of each egg clutch using a dissection microscope. For each crab, about 200 embryos each were counted for internal and external portions of broods. Embryo abnormality was recorded as the percentage of embryos that failed to develop and that developed slowly or abnormally.

2.6. Sublethal responses in fish liver

Up to 12 longjaw mudsuckers (*G. mirabilis*) were collected from the same 22 stations where sediment samples were collected using minnow traps and beach seines in August 2003 and transported live to the laboratory. Livers were surgically removed and processed immediately to measure apoptotic DNA fragmentation for genotoxicity through terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay and histopathological analysis through lipid accumulation and glycogen depletion. Detailed procedures of the TUNEL assay were published in Rose (2005) and Rose et al. (2006). Briefly, liver samples were fixed in 10% methanol-free formalin for 48 h. The samples were dehydrated, imbedded in paraffin, and sectioned according to standard protocol at Central Histology Services (Sacramento, CA). The sectioned liver samples were deparaffinized and rehydrated and incubated sequentially with proteinase K for 30 min at 37 °C, terminal deoxynucleotidyl transferase (TdT) equilibration buffer for 10 min at room temperature, and a nucleotide mix with fluorescein-12-dUTP for 1 h at 37 °C. The slides were counterstained with Hoechst 33258 solution for and the percent of TUNEL positive nuclei was determined using an Olympus BX50WI fluorescence microscope. For histopathological analysis, liver tissues were stained with hematoxylin and eosin (H&E) and the severity of glycogen depletion and lipid accumulation was examined using a light microscope (Teh et al., 1997). To measure cytochrome P4501A (CYP1A) proteins, a small portion of the removed liver tissues (~25 mg) was homogenized with Tris-HEPES EDTA buffer with sucrose (THE sucrose) and protease inhibitor. Homogenates diluted in Laemmli sample buffer were boiled for 3 min and then separated using electrophoresis on 10% SDS-PAGE gels. Proteins were transferred to nitrocellulose membranes and P4501A was detected using a rabbit anti-P4501A IgG primary antibody and a goat anti-rabbit HRP secondary antibody. Quantification was performed using an UVP Epi-Chemi Darkroom (UVP, Upland, CA) that measures optical density (OD) of sample bands. Levels of CYP1A are reported as OD/mg tissue. ANOVA was used to examine differences of these responses among marshes.

Table 1

Concentrations of ammonia in porewater, sea urchin (*Arbacia punctulata*) embryological development and fertilization tests, and sand dollar (*Dendraster excentricus*) embryo–larval development test measured in tidal salt marshes in northern California.

Site	Station	TAN	UAN	Sea urchin ^a		Sand dollar
		mg/L	µg/L	Normal (%)	Fertilization (%)	Normal development (%)
ST	B	3.82	93	2.6	71.0	96.2
ST	C	2.92	80.8	70.0	90.2	96.6
ST	D	10.0	211	0.0	65.0	95.7
ST	G	2.20	54.4	90.2	89.8	98.1
ST	J	1.96	32.2	94.4	92.4	96.4
ST	K	8.98	226	0.0	74.6	96.6
ST	M	2.67	66.2	50.6	87.8	96.7
ST	N	NM	NM	NM	NM	97.0
ST	O	2.78	89.9	13.6	78.4	98.1
ST	P	6.63	117	0.0	33.4	97.2
ST	Q	NM	NM	NM	NM	96.5
ST	R	3.03	114	13.8	82.4	95.9
ST	S	NM	NM	NM	NM	97.8
CC	A	3.75	108	0.0	67.0	97.9
CC	B	3.96	90.9	13.0	74.0	98.6
CC	C	3.11	22.4	87.3	33.0	96.5
TP	A	NM	NM	NM	NM	NM
TP	B	NM	NM	NM	NM	NM
TP	C	NM	NM	NM	NM	NM
WC	B	NM	NM	NM	NM	NM
WC	C	NM	NM	NM	NM	NM
WC	D	NM	NM	NM	NM	NM

ST: Stege Marsh, CC: China Camp, WC: Walker Creek, TP: Tom's Point.

TAN: total ammonia as nitrogen.

UAN: unionized ammonia as nitrogen (higher than LOEC for embryo development endpoint is in bold).

NM: not measured.

^a Sea urchin test responses that were significantly lower than controls are in bold.

2.7. Sediment grain size

Three to five surface sediment core samples (5 cm diameter × 5 cm deep) were collected from each station when samples for benthic

community assessment were collected. Wet sediment was sieved through 500-µm and 63-µm mesh sieves and sediments remaining on the sieves were dried and weighed as coarse and fine sand, respectively. Silt (2–63-µm) and clay (<2-µm) fractions in the sieved portion were analyzed using a hydrometer method (Gee and Bauder, 1979).

3. Results and discussion

3.1. Toxicity potential and bioavailability of contaminants in sediments

Overall toxicity potential of sedimentary contaminants between ST and reference marshes clearly differed (Table 1). Among the four marshes, ST had the highest concentrations of organic contaminants and trace metals. The lowest concentrations were found in the reference marshes (TP and WC). However, without a linkage to adverse biological effects, chemical concentration alone is less meaningful in assessing the risk of contaminants to aquatic organisms. When chemistry data are converted to toxicological impact information, they can better predict the health risk of contaminants and can increase their validity as one of the major components of this weight of evidence approach.

The magnitude of overall toxicity potential of these mixtures of contaminants was expressed as mPELQs (Hwang et al., 2008). Although the concentrations of toxic chemicals in ST sediments have declined significantly since the 1970s (Hwang et al., 2008), surface sediments from all 13 stations in this marsh still had at least one chemical exceeding a PEL and mean PELQs were greater than 0.5 (Table 2). Mean PELQs in sediments from CC, TP, and WC were lower than 0.5 at all sampling stations and no measured chemicals exceeded PELs in these samples (Table 2). Detailed information on the occurrence and sources of toxic chemicals in sediments and their toxicity potential and bioavailability was reported previously (Hwang et al., 2006a, 2006b, 2008). Mercury was not measured for the present study, however, other studies indicate that mercury concentrations might be higher than the PEL in ST samples and possibly WC samples as well (Johnson et al., 2009; SFBRWQCB, 1999). According to Johnson et al. (2009), total mercury concentrations in almost all surface sediments from the Walker Creek Delta were higher than PEL (0.486 µg/g), with higher concentrations (> 1 µg/g) in

Table 2

Toxic potential of contaminants in sediments from tidal salt marshes in northern California.

Adapted from Hwang et al. (2008).

Site	Station	TELs ^a exceeded	PELs ^b exceeded	Mean TELQ	Total PELQ		Mean PELQ	Contaminants of concern
					Organics	Metals		
ST	B	9	5	4.74	5.44	5.90	1.03	PCBs, DDTs, Chlordanes, Cu, Pb, Zn
ST	C	10	6	7.07	16.0	6.13	2.02	PCBs, DDTs, Chlordanes, Cu, Pb, Zn
ST	D	8	2	3.31	8.61	1.52	0.92	PCBs, Chlordanes
ST	G	10	3	4.58	12.5	4.07	1.51	PCBs, DDTs, Chlordanes
ST	J	10	6	7.42	17.8	7.21	2.27	PCBs, DDTs, Chlordanes, Cu, Pb, Zn
ST	K	9	3	3.80	7.56	4.24	1.07	PCBs, Chlordanes, Zn
ST	M	8	1	2.82	5.18	2.57	0.70	Chlordanes
ST	N	8	2	3.73	6.85	1.73	0.78	DDTs, Chlordanes
ST	O	8	4	4.79	7.12	4.89	1.09	DDTs, Chlordanes, Cu, Zn
ST	P	8	4	6.11	6.27	6.97	1.20	DDTs, Chlordanes, Cu, Zn
ST	Q	9	3	4.37	7.50	3.24	0.98	PCBs, Chlordanes, Zn
ST	R	10	6	47.3	70.5	5.78	6.94	PCBs, DDTs, Chlordanes, Cu, Pb, Zn
ST	S	9	2	5.09	10.2	2.13	1.12	PCBs, Chlordanes
CC	A	7	0	1.68	0.40	3.53	0.36	None
CC	B	6	0	1.31	0.44	2.49	0.27	None
CC	C	0	0	0.42	0.20	0.86	0.10	None
TP	A	0	0	0.30	0.05	0.72	0.07	None
TP	B	0	0	0.23	0.05	0.53	0.05	None
TP	C	0	0	0.35	0.03	0.89	0.08	None
WC	B	3	0	0.50	0.04	1.28	0.12	None
WC	C	0	0	0.30	0.00	1.28	0.12	None
WC	D	0	0	0.39	0.08	0.90	0.09	None

ST: Stege Marsh, CC: China Camp, WC: Walker Creek, TP: Tom's Point.

^a Number of chemicals exceeding TELs.

^b Number of chemicals exceeding PELs.

sediments observed near the mouth of the creek and lower concentrations ($<1 \mu\text{g/g}$) in sediments from northeast shoals, which are close to the sampling sites of the present study. Because the sediment samples for the present study were collected from the bank of a small tidal channel located in the eastern part of Walker Creek Delta having the lowest mercury deposition rate, mercury concentrations in the samples might be lower or slightly higher than the PEL. And thus, mean PELQs are likely to be almost the same as those presented in Table 2 even if mercury is included in calculating mean PELQs. Although Ni concentrations were higher than PEL in all samples, Ni was not included in calculating mean PELQs because, in northern California, background Ni concentrations can be high even in remote areas due to input from weathering of Ni-enriched natural geological source rocks (Topping and Kuwabara, 2003).

A previous 60-day transplantation study (Hwang et al., 2008) showed tissue body burdens of DDTs, PCBs, and trace elements in fish (longjaw mudsucker; *G. mirabilis*) elevated up to 127 times of the concentrations found in pre-transplant fish, demonstrating that organic contaminants and trace metals in ST were bioavailable. Tissue body burdens of DDTs and PCBs in these fish exceeded ecological screening levels reported by U.S. EAP (1997) and Canadian Council of Ministers of the Environment (CCME, 2001) for protection of high trophic level organisms such as fish-eating birds, suggesting that toxic chemicals in ST sediment could also affect the health of birds.

3.2. Sediment pore water toxicity tests

Dissolved oxygen ($>90\%$ saturation), salinity ($30 \pm 1\%$), and pH (7.9 ± 0.1) in pore water were within acceptable ranges during testing (data not shown). Hydrogen sulfide (H_2S) concentrations were below the detection limit (0.01 mg/L) in all porewater samples. Total ammonium as nitrogen ranged from 1.96 to 10 mg/L (Table 1). Concentrations of unionized ammonia, which is the most toxic fraction of ammonia, ranged from 22.4 to $226 \mu\text{g/L}$, falling within a range typically observed in field-collected porewater samples (Carr et al., 2006).

The sea urchin embryo development (ED) test, which is often the more sensitive of the two porewater tests conducted in the present study, exhibited a significant decrease at 10 of 13 stations ($p < 0.01$; Table 1) compared to those in control. However, seven of these toxic samples had concentrations of unionized ammonia exceeding $90 \mu\text{g/L}$, the lowest observed effect concentration (LOEC) for the more ammonia sensitive ED endpoint (Carr et al., 2006). As expected, when the LOEC for unionized ammonia was exceeded, significant toxicity was observed for the ED test (Fig. 2). Due to the confounding influence of ammonia, it is difficult to determine whether the reduction in embryological

development was related to other anthropogenic contaminants without conducting additional TIE studies.

The sea urchin fertilization test exhibited a statistically significant reduction at seven of 13 stations ($p < 0.01$; Table 1) compared to those in control. The concentrations of unionized ammonia were lower than the no-observed-effect concentration (NOEC: $398 \mu\text{g/L}$) and LOEC ($800 \mu\text{g/L}$) for the fertilization test (Carr et al., 2006) in all samples and thus unionized ammonia was not likely the major contributor to the observed toxicity. Fertilization reduction was not correlated ($r^2 = 0.12$) with the toxicity potential of chemicals measured in the present study (results not shown), but showed a negative correlation ($r^2 = 0.45$) with porewater unionized ammonia concentrations (Fig. 2). This apparent correlation could be because the increase of ammonia occurred as a result of reduced bioturbation by benthic organisms (Carr et al., 2006) that might be linked to the increased concentrations of the unmeasured contaminants that likely inhibited the fertilization of sea urchin embryos.

Samples found to be toxic for the fertilization test were used in phase I TIE studies. The addition of EDTA to the samples did not decrease toxicity for any of these samples (data not shown), indicating that metals did not contribute to the observed reduction in fertilization. Reduced toxicity was observed in all samples after C_{18} solid-phase extraction (data not shown); this indicates that organic contaminants, which were retained on the C_{18} resin, were likely responsible for the observed reduction in fertilization. While particulates were visible in one CC sample, there were no visible particulates in the other samples. Concentrations of organic contaminants (Hwang et al., 2006a) and current use pesticides, such as biphenthrin, permethrin, and molinate (Kuivila, unpublished data) were not correlated with the toxicity test results and thus other organic contaminants that were not measured were possibly responsible for the observed toxicity.

Sea urchin fertilization and ED tests were not significantly different between the more anthropogenically contaminated marsh (ST) and the less contaminated marsh (CC). The responses of porewater toxicity tests exhibited no correlation ($r^2 < 0.15$) with benthic community indices. Unionized ammonia concentrations in the porewater samples were not significantly different between ST and CC. For salt marsh sediment samples containing high concentrations of unionized ammonia, alternative tests such as the zoospore (*Ulva fasciata*) test, which is tolerant to ammonia but sensitive to other contaminants, particularly herbicides, may offer an alternative option for assessing the degradation of sediment (Hooten and Carr, 1998).

No substantial or toxicologically relevant reduction in normal sand dollar embryo-larval development was found in any of the sediment elutriate samples (Table 1). Sediment samples that exhibited reduction in sea urchin fertilization tests were not toxic for the elutriate test probably because the elutriate sand dollar embryo-larval development test is less sensitive to toxic chemicals present in the samples compared to the sea urchin test. A possible explanation for much less frequent detection of toxic responses in the elutriate test is dilution of toxic chemicals during the preparation of sediment elutriates. Ankley et al. (1991) and McDonald (2005) examined toxicity of porewater and elutriate samples concurrently with several different organisms such as amphipods, bivalve larvae, cladocerans, fathead minnows, and oligochaetes and found that pore waters were consistently much more toxic than elutriates because of reduced toxic chemical concentrations in elutriates, demonstrating that dilution is an important factor for interpretation of elutriate test results.

3.3. Benthic macroinvertebrates in sediments

A survey of abundance of benthic macroinvertebrates, their species diversity and richness, and presence and abundance of sensitive and pollution tolerant species can provide insights into the habitat quality of sediments (Bilyard, 1987; Hunt et al., 2001; Thompson and Lowe, 2004). Comparison of total abundance of individuals,

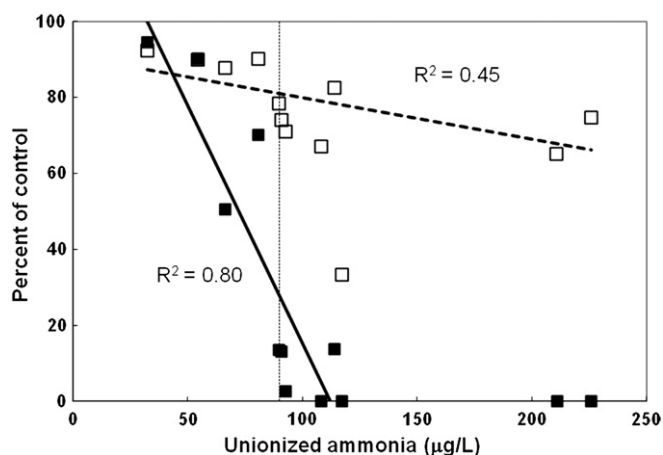


Fig. 2. Relationships of sea urchin porewater toxicity tests (embryological development: solid square and fertilization: open square) to unionized ammonia. Embryological fertilization and development are expressed as relative percentage of embryos that fertilized or developed normally compared to those observed in control tests with clean porewater.

Table 3

Benthic invertebrate community survey data and grain size composition and total organic carbon content of surface sediment from tidal salt marshes in northern California.

Site	Station	Benthic invertebrate community				Silt	Clay	Sand		TOC
		Total abundance ^a	Shannon H'	Richness	Enchytraeidae/total (%)	(%)	(%)	Fine (%)	Coarse (%)	
ST	B	27,962	1.97	19	53.3	40	24	37	0	2.49
ST	C	14,310	2.17	16	61.7	49	34	17	0	2.21
ST	D	18,259	1.76	17	79.1	49	39	12	0	3.22
ST	G	9469	1.61	13	82.5	41	41	18	0	1.81
ST	J	4501	1.79	12	39.6	40	41	19	0	1.91
ST	K	39,788	2.32	17	44.5	36	35	29	0	2.22
ST	M	33,121	1.94	14	38.5	44	42	14	0	1.44
ST	N	24,756	1.99	20	36.9	27	34	28	11	2.39
ST	O	28,832	1.44	14	67.5	63	21	16	0	2.75
ST	P	49,580	1.74	16	48.2	63	21	16	0	2.82
ST	Q	24,204	1.68	17	80.0	51	44	6	0	2.24
ST	R	14,820	1.49	13	73.1	46	38	15	0	2.47
ST	S	38,981	0.80	16	90.8	37	48	14	0	2.69
CC	A	6217	2.14	16	13.1	35	56	9	0	1.89
CC	B	6836	1.89	11	9.94	32	61	7	0	2.22
CC	C	7473	2.29	12	59.7	28	57	15	0	1.97
TP	A	55,223	3.27	18	9.00	18	22	39	21	1.59
TP	B	19,771	2.47	15	31.2	33	31	35	1	1.25
TP	C	15,396	2.32	20	24.4	35	28	31	6	1.97
WC	B	15,329	3.12	19	19.1	22	20	42	16	1.20
WC	C	8493	2.96	14	23.5	39	18	28	15	3.87
WC	D	16,348	2.93	20	14.3	49	26	25	0	2.57

ST: Stege Marsh, CC: China Camp, WC: Walker Creek, TP: Tom's Point.

TOC: total organic carbon (from Cordova et al., 2006).

^a Total abundance (individuals/m²).

species diversity (H'), and species richness with toxicity potentials of sedimentary chemicals (mPELQ) and porewater unionized ammonia concentrations can discern possible impacts of toxic chemicals on benthic macroinvertebrates.

Benthic macroinvertebrate survey results (Table 3) indicated that toxic chemicals detected in the sediment samples might be responsible for lower species diversity, but not for richness and total abundance. This finding is consistent with the results reported by other studies (Horne et al., 1999; Weis et al., 2004) that also observed the species diversity was more closely related to the degree of sediment contamination but total abundance was associated with the elevation of the sites relative to tidal levels. In the present study, mean H' values were significantly higher, which means greater diversity, in reference marshes (TP and WC) than in contaminated (ST). Marsh-wide mean H' values exhibited a negative correlation ($r^2 = 0.71$) with marsh-wide mean

mPELQ values (Fig. 3). When mPELQ values were above 0.5, mean H' values ranged from 0.80 to 2.31 with an average of 1.75 ± 0.41 , which were statistically ($p < 0.01$) significantly lower than H' values (1.89 to 3.27 with an average of 2.60 ± 0.48) in less contaminated sediments (mPELQ < 0.5). Lower species diversity observed in Stege Marsh was associated with a higher abundance of opportunistic species that can tolerate disturbed conditions. There were no significant correlations ($r^2 < 0.15$) between benthic macroinvertebrate survey data and porewater unionized ammonia concentrations.

The total abundance of individuals was not correlated with the concentrations of sedimentary toxic chemicals. The highest and lowest total abundance were found in ST ($25,300 \pm 13,000$ per m²) and in CC samples (6800 ± 600 per m²), respectively. Anthropogenic organic carbon and nutrient enrichment are also known to boost benthic macrofaunal production (Sarda et al., 1996). Organic carbon content in all sediment samples was similar, ranging from 1.3 to 3.9% (Cordova et al., 2006), and did not exhibit any correlations with the total abundance. Nutrient concentrations in the sediment samples were not measured but typically their concentrations correlate with organic carbon content so nutrients do not likely exhibit any correlation with the total abundance. Levin et al. (1998) reported that abundance of macroinvertebrates had a positive relationship with the percentage of fine sediment that may vary naturally. The total abundance of benthic invertebrates observed in the present study was also not correlated to silt and clay content. Almost all sediments were dominated by oligochaetes (*Enchytraeidae* and *Tubificidae*) and polychaetes (*Streblospio benedictii*). Two stations in CC and one station in TP were dominated by crustaceans. Higher total abundance observed in ST was because of proliferation of *Enchytraeidae* that accounted for 37% to 91% of total abundance. They accounted for 9% to 31% in the reference marshes (TP and WC). The percentage of *Enchytraeidae* exhibited a positive correlation with mPELQ. The higher percentage of *Enchytraeidae* is also associated with lower species diversity in more contaminated sediments (Fig. 4), indicating that percentage of *Enchytraeidae* could be a possible indicator for sediment quality. Insects were extremely rare or absent in most samples probably due to high salinity. Crustaceans in most samples accounted for up to 30% of the total abundance.

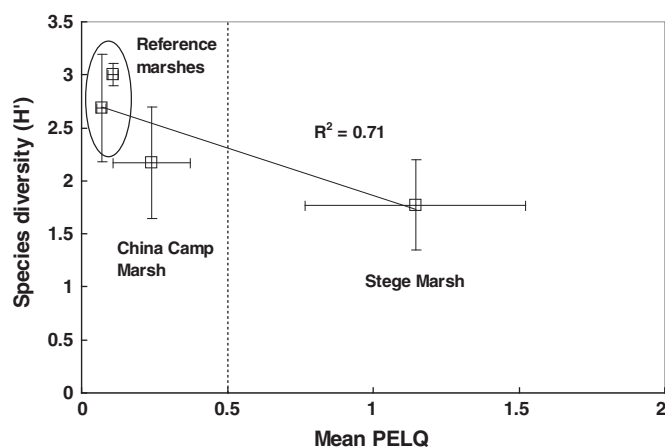


Fig. 3. Relationship of species diversity (Shannon H') of benthic macroinvertebrates to toxicity potential of sedimentary contaminants (mean PELQs). When mPELQ values are above 0.5, adverse biological effects are likely to be observed frequently. Error bars indicate standard deviation of mean PELQ and Shannon H' .

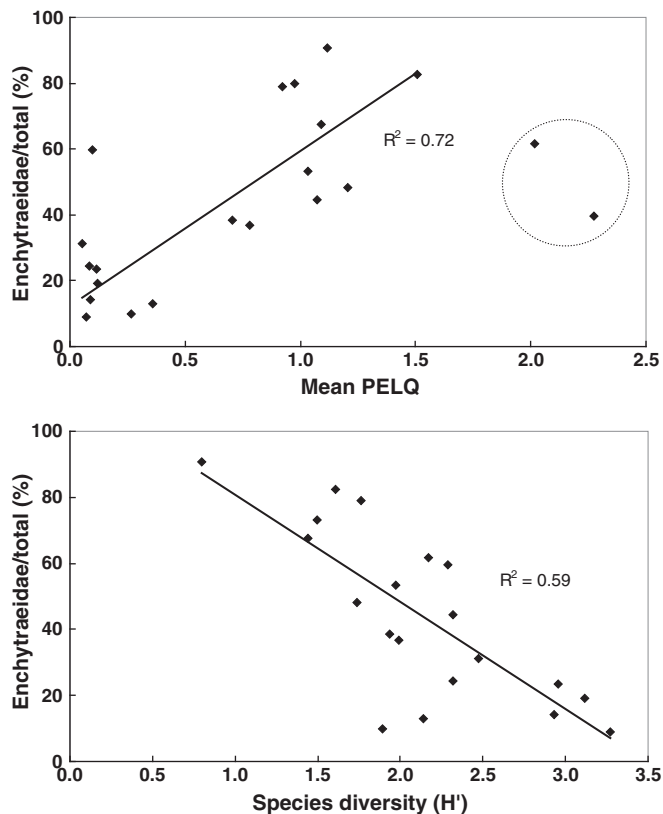


Fig. 4. Relationships of the percentage of Enchytraeidae to mean PELQs (top) and species diversity of benthic macroinvertebrates (bottom). Data points in circles are outliers (greater than average ± 2 standard deviation) that are not included in regression analysis.

3.4. Sublethal responses in resident fish and crab

Measuring toxic responses in field collected resident species can be more realistic than responses in laboratory-exposed species, because resident species can integrate bioavailability of multiple contaminants, their bioaccumulation over long periods of time, and other various site-specific conditions. Though there are some drawbacks to be considered such as natural acclimation of resident species to contaminants, use of resident species could provide more accurate and realistic site-specific information as well as confirmatory information where positive interferences are a concern. This is especially true in ecosystems like salt marshes where natural confounding factors such as ammonia and sulfides may be elevated (Eddy, 2005).

The multi-year PEEIR program applied an expanded multiple lines of evidence approach (Table 4), which integrates a diverse range of selected cost-effective indicators to validate their potential in assessing

sediment quality more rapidly and accurately and providing advanced early-warnings of environmental stresses in salt marsh ecosystems. Apoptotic DNA fragmentation, measured as TUNEL positive cells, in field-collected longjaw mudsucker liver was a sensitive and informative biomarker for environmental exposure and effect. Mean percent of TUNEL positive liver cells was statistically ($p < 0.05$) significantly higher in mudsuckers from ST than in those from the remote marshes (TP and WC). It is an indication that contaminants in ST include cytotoxic or genotoxic chemicals and the higher frequencies of apoptosis (cell death) in ST fish may lead to greater tumor prevalence. Histopathological effects, measured as lipid accumulation and glycogen depletion, which are signs of functional liver damage, were also statistically significantly ($p < 0.05$) higher in the livers of longjaw mudsuckers from ST than in those from reference marshes (Rose, 2005).

Levels of cytochrome P450 (CYP1A), which is known to be induced by PAHs and dioxin-like compounds (Oost and Vermeulen, 2003; Toyoshiba et al., 2004), in field-collected native longjaw mudsuckers were not significantly different among the marshes (Anderson et al., 2006; Rose, 2005), although ST contains much higher concentrations of PAHs and dioxin-like compounds than the other marshes. Metallothioneins, which are known to be induced by trace metals (Oost and Vermeulen, 2003), were also not different among the marshes. These results suggest that some functions like enzyme induction in resident fish in contaminated areas like ST have already acclimated to the toxic chemicals in their habitats. These biomarkers can be also influenced by natural factors such as age, reproductive stage, nutritional state, parasitism, and ambient temperature (Bucheli and Fent, 1995). And thus they are not useful to assess the impacts of contaminants at the sites assessed for the present study.

Reproductive performance, as measured as the frequency of abnormal embryos, in shore crab appears to be a quick, easy, cost-effective, nondestructive, and early-warning indicator for coastal habitat condition monitoring (Anderson et al., 2006). The frequency of developmental abnormalities was significantly ($p < 0.05$) higher in ST than in the reference marshes (TP and WC). Frequencies of abnormalities in embryos were greater in the external portion of broods than in the internal portion (Table 4) and were more strongly correlated with sedimentary heavy metal concentrations possibly due to the external portion being directly exposed to sediment. Measurement of embryo abnormality provided sensitive and accurate information, indicating that toxic chemicals in ST are bioavailable and can cause adverse effects on resident species. Crab population size, age-class structure, and sex ratios were not good indicators of toxic effects (S Morgan, University of California, Davis, CA, unpublished data).

3.5. Expanded multiple lines of weight of evidence approach

Multiple lines of evidence approach has been used for more than decades and become the most commonly used method in assessing sediment quality because it can significantly improve the discriminatory

Table 4
Responses of each component used to assess sediment quality in tidal salt marshes in northern California.

Metric	Metric measurement	San Francisco Bay		Tomales Bay (Reference marshes)
		Stege Marsh	China Camp	
Benthic macroinvertebrate	Total abundance (individual/m ²)	25,300 \pm 13,000	6,800 \pm 600	21,800 \pm 16,800
	Species diversity (H')	1.75 \pm 0.41	2.10 \pm 0.19	2.85 \pm 0.37
	Species richness	15.7 \pm 2.4	13.0 \pm 2.6	17.7 \pm 2.6
Fish genotoxicity	Liver cell apoptosis (%)	2.6 \pm 0.3	3.1 \pm 1.3	1.4 \pm 0.2
Fish liver damage	Lipid accumulation (severity level)	1.15 \pm 0.15	0.39 \pm 0.35	0.29 \pm 0.20
	Glycogen depletion (severity level)	0.89 \pm 0.16	0.39 \pm 0.35	0.25 \pm 0.18
Enzyme induction	Cytochrome P450 (CYP1A) (OD/mg liver wet wt.)	5.94 \pm 2.37	5.15 \pm 1.73	5.83 \pm 1.93
Crab reproduction	Embryo abnormality (internal) (%)	4.19 \pm 0.98	NM	1.69 \pm 1.20
	Embryo abnormality (external) (%)	6.30 \pm 4.04	NM	2.17 \pm 1.19
	Embryo abnormality (combined) (%)	5.25 \pm 2.28	NM	1.98 \pm 1.08

NM: not measured.

power compared to single line of evidence approach (Carr et al., 1996; Morales-Casseltes et al., 2009; Hall and Giddings, 2000; Long and Chapman, 1985). The present study added additional lines of evidence such as chronic sublethal responses in field-exposed resident species to expand traditional multiple lines of evidence approach, which is SQT. Individual lines of evidence can be interpreted qualitatively and/or quantitatively to derive conclusions regarding sediment quality. Quantitative evaluation typically integrates multiple lines of evidence using various methods such as indexing, ranking, scoring, and normalization (Wenning et al., 2005).

To quantify relative sediment-quality degradation, each line of evidence was scored 0 or 1 (Table 5). Each line of evidence was equally weighted because of the uncertainty of how each of these reflected the risk of sediment contamination to aquatic organism. The toxicity potential of chemicals in sediments of each marsh was scored 0 or 1 if mPELQ values are <0.5 or ≥ 0.5 , respectively. Other responses in ST and CC were scored 1 or 0 if they exhibit statistically-significant adverse effects when compared to the combined responses of the reference marshes. The sea urchin and sand dollar porewater tests are not included because no measurements were taken in reference marshes. Seven of 9 and three of 8 components are scored 1 in ST and in CC, respectively. The average score of ST is 0.67 (Table 5), indicating that sediment quality in ST is highly degraded and may not fully support the health of aquatic organisms. The average score of CC is 0.38, showing much less degradation than ST, and those in TP and WC are 0.00 and 0.00, respectively. These average scores imply that the indicators measured for the present study can differentiate contaminated areas from reference areas with a high degree of confidence.

Among the evidence summarized in Table 5, effects-based guideline (mPELQ), benthic macroinvertebrate species diversity, crab embryo abnormality, liver cell apoptosis, and lipid accumulation and glycogen depletion in fish liver appear to be more sensitive indicators than the others. To assess impacts of dioxin-like compounds and trace metals, different biomarkers, other than cytochrome P450 (CYP1A) and metallothioneins, need to be evaluated. It is also desirable to evaluate biomarkers to be used to identify the impacts of contaminants on the endocrine systems. To increase the power of these evidence in discriminating among impacted areas, the relative predictability of each evidence should be investigated in future studies. In other words, relative weights of these evidence need to be adjusted based on their sensitivity, selectivity, dose-response relationships, repeatability, and other factors through the accumulation of supporting datasets.

4. Conclusions

The present study demonstrates that chronic sublethal responses measured in resident species under field-exposed conditions can be

readily combined with traditional approaches for an expanded multiple lines of evidence approach in a sediment quality assessment. Toxic responses were found at the molecular and cellular levels as well as in population and community levels such as abnormal reproduction in crab embryos and benthic macroinvertebrate species diversity and richness, validating the usefulness of lower biological level responses in resident species as early warning indicators. Integration of the portfolio of responses can support a more comprehensive, accurate, and informative sediment quality assessment. The results of the present study highlight the importance of complementing traditional approaches with resident species indicators that could be a promising framework for the assessment of contamination in salt marsh ecosystems. Although some biomarkers evaluated in the present study proved to be effective in identifying contaminated sites, additional biomarkers need to be evaluated to find better combinations of biomarkers to be applicable to larger scale assessment programs. Susceptibility of biomarkers to natural confounding factors also should be considered to delineate the impacts of anthropogenic contaminants.

The present field case study confirms that both resident lined shore crab and longjaw mudsucker are excellent sentinel species that can be used for multiple lines of evidence approach to evaluate possible effects of contaminants in California salt marshes. The present study integrated molecular level to population-level toxic responses and the bioaccumulation of sedimentary contaminants. We believe that the present study will significantly help advance the application of a portfolio, including multiple responses in field-exposed resident species and traditional approach to salt marsh ecosystems and other habitat types for ecological risk assessments.

Conflict of interest

There is no conflict of interest.

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Table 5
Scores of components measured to assess sediment quality in tidal salt marshes in northern California.

Metric	Metric measurement	San Francisco Bay		Tomales Bay (reference marshes)	<i>p</i> level (less than)
		Stege Marsh	China Camp		
Toxic chemical level	mPELQ	1	0	0	NA
Benthic macroinvertebrate	Total abundance	0	0	0	0.05
	Species diversity (<i>H'</i>)	1	1	0	0.05
	Species richness	0	1	0	0.05
	Fish genotoxicity	1	1	0	0.05
Fish liver damage	Lipid accumulation	1	0	0	0.05
	Glycogen depletion	1	0	0	0.05
	Enzyme induction	0	0	0	0.05
Crab reproduction	Embryo abnormality (combined)	1	NM	0	0.05
Overall average score		0.67	0.38	0.00	

NA: not applicable.

NM: not measured.

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