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ABSTRACT

In situ-based testing using aquatic organisms has been widely reported, but is often limited in scope and practical usefulness in making decisions on ecological risk and remediation. To provide this capability, an integrated deployment system, the Sediment Ecotoxicity Assessment (SEA) Ring was developed, which incorporates rapid *in situ* hydrological, chemical, bioaccumulation, and toxicological Lines-of-Evidence (LoE) for assessing sediment and overlying water contamination. The SEA Ring system allows for diver-assisted, or diverless, deployment of multiple species of ecologically relevant and indigenous organisms in three different exposures (overlying water, sediment–water interface, and bulk sediment) for periods ranging from two days to three weeks, in a range of water systems. Measured endpoints were both sublethal and lethal effects as well as bioaccumulation. In addition, integrated passive sampling devices for detecting nonpolar organics (solid phase micro-extraction fibers) and metals (diffusive gradients in thin films) provided gradient measures in overlying waters and surficial sediments.

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1. Introduction

Traditional approaches for assessment of contaminated sediments and verification of remedial measures generally rely on laboratory-based exposures for toxicity and bioaccumulation coupled with bulk sediment chemical characterization and benthic community structure characterization (Wenning et al., 2005). While these laboratory methods provide a high degree of experimental control, often times this comes with a significant loss of representativeness due to excessive manipulation and loss of the natural conditions and integrity of the samples and exposures (Adams et al., 2005). In addition, strong linkages between these lines-of-evidence are often absent and so uncertainty often arises as a result of conflicting results (Burton et al., 2002). These issues are especially important where the exposure pathway cannot be adequately reproduced in the laboratory, when the exposure is transient in time, or where an *in situ* remedy is utilized. Examples of these instances include sites where groundwater discharge or tidal pumping of

porewater are present, sites where stormwater or transient resuspension events occur, and sites where reactive amendments or caps are utilized. *In situ* exposure approaches offer one alternative to these traditional strategies. *In situ* methods have been demonstrated for sediment assessment for a wide variety of organisms, endpoints and environmental settings (Crane et al., 2000; Geffard et al., 2001; Greenberg et al., 2002; Ringwood and Keppler, 2002; Anderson et al., 2004; Phillips et al., 2004; Adams et al., 2005; Burton et al., 2005). In certain situations, *in situ* methods are the only viable alternative to characterize transient exposures. Nevertheless *in situ* methods have been slow to gain acceptance in regulatory programs primarily due to perceived lack of experimental control, the complexity and high degree of expertise associated with the methods, and the more challenging aspects of the logistics for the field-based assessment methods as compared to laboratory-based methods. In addition, *in situ* toxicity or bioaccumulation methods have lacked an inherent integration with other chemical lines-of-evidence. To improve this situation, systems are needed that improve the level of experimental control for *in situ* methods, simplify and standardize the methods to the degree possible, and provide the ability to integrate chemical and biological lines-of-

evidence. Here we present the description and initial proof of concept for such an integrated device termed the Sediment Ecotoxicity Assessment (SEA) Ring. Part II of this series provides additional evidence of the utility of the device and other physicochemical tools resulting in an integrated multiple line of evidence approach at a contaminated sediment site where time-varying stressors were of potential concern (Rosen et al., 2012).

2. Materials and methods

2.1. Preliminary testing

2.1.1. Equilibration of in situ chamber water

A dye study was conducted using chambers described by Burton et al. (2005). Exposure chambers varied in screen size, ranging from 80 to 300 μm. Smaller (25 μm mesh) "drum" style chambers, constructed according to Phillips et al. (2004), were also placed into a subset of the 80 μm chambers to investigate water exchange with the external environment for use with particularly small and fragile (i.e., mussel embryos) organisms. All 80–300 μm exposure chambers were filled with a solution containing 1 mL red dye food coloring and 500 mL deionized (DI) water. The chambers were placed into a 17 gallon, high density polyethylene (HDPE) tub filled with DI water. An aquarium pump maintained flow at a rate of 100 gallons per hour (GPH). Two replicates for each mesh size were removed from the tub at each time interval and the absorbance of dye remaining was measured using a HACH DR/2400 spectrophotometer.

Separately, an alternative embryo-scale exposure chamber design using glass scintillation vials was evaluated. These chambers are typically used in laboratory embryo-larval development tests (USEPA, 1995), and can be examined directly on an inverted microscope, thus eliminating the need for transfer steps at the conclusion of the test. The solid plastic screw caps from these chambers were modified with 25 μm Nitex mesh screen covering a drilled out opening with a diameter of approximately 1.5 cm. This design was successful for laboratory-based microcosm exposures with Mytilus galloprovincialis embryos (Rosen and Lotufo, 2007, 2010). To examine site water equilibration of this chamber design, a 20 L polycarbonate tank was filled with natural seawater (33‰). Subsequently, 27 exposure chambers were filled with deionized water (0‰) and placed in the tank. Three replicate vials were removed at 9 different sampling points over a period of approximately 24 h, and salinity of the contents measured. The experiment was conducted under two flow conditions: static and under a continuous flow rate of 100 GPH using a MarineLand Bio-Wheel Pro30 aquarium filter.

2.1.2. Exposure chamber shaking

Species-specific sensitivity to physical stress potentially encountered during transport to the field or while deployed was evaluated for 48 h in laboratory chamber shaking experiments. Experiments were conducted using shakers set at three different speeds: 0 (control), 100, and 150 RPM. In situ chambers (Burton et al., 2005) were securely held (vertically) in 400 mL glass beakers containing 200 ml uncontaminated, filtered (0.45 μm) natural seawater (30‰), held at a temperature of 20 °C. Chambers were open on top, therefore, it is expected that this exposure scenario represented worst-case conditions, as significant water motion and splashing would be reduced in

capped, air tight, deployed chambers. Concurrent to the shaking experiments, additional in situ chambers (with both ends capped) were deployed at a depth of 1m off of the SSC Pacific research pier in San Diego Bay (average temperature of 16 °C and salinity of 33‰ during deployment), which receives moderate levels of small boat and ship traffic. Three test species were employed in these experiments: 5 mm cultured amphipods (Leptocheirus plumulosus), cultured juvenile (3 day old) mysid shrimp (Americamysis bahia), and <4 h old mussel (M galloprovincialis) embryos obtained from gravid adults using standard methods (USEPA, 1995). Amphipod and mysid chambers each held 10 organisms, while mussel chambers held approximately 200 embryos. A minimum of three replicate chambers were used for each treatment.

2.2. Deployment approach

The SEA Ring (Patent No. US 8,011,239 BI. 2011) consists of a circular carousel capable of housing an array of in situ toxicity and bioaccumulation chambers, passive sampling devices, and water quality sensing devices (Fig. 1). The main platform is a carousel of ½" acrylic. The base and top of the carousel are circular in shape with diameters of 17 and 13″, respectively. The base portion has 14 circular cutouts. A 5½"-long cylindrical chamber holder is glued into each cutout, and serves as a means of housing the individual exposure chambers. Each chamber holder has 12 vertically oriented cutouts approximately 3″ long by ½" wide so as to maximize water flow across the mesh covered exposure chambers while maintaining structural rigidity of the holder.

The exposure chambers were designed for conducting water column (WC), sediment–water interface (SWI), or surficial sediment (SED) exposures (Fig. 2). The WC and SWI exposure chambers are 5″ long, while the SED chambers are 10″ long. Exposure chambers were modified designs of Anderson et al. (2004) and Burton et al. (2005). The WC and SWI chambers were maintained above the sediment surface with acrylic stops that were glued onto the bottom inside lip of the chamber holders, while the SED chambers extended approximately 5″ below the base portion of the SEA Ring in the sediment. Exposure chambers were made of cellulose acetate butyrate cylindrical tubing. Chambers used for housing smaller organisms such as amphipods, polychaetes, mysids, or bivalve embryos each possess two mesh cutouts, approximately $2^3/4$" tall by $1^3/4$" wide. Mesh pore size was typically 250–500 μm, except for bivalve embryos (25 μm mesh). Mesh was fastened to the cutouts with aquarium grade silicone glue. Exposure chambers housing larger organisms (i.e., adult bivalves) typically utilized chambers to which 1/8″ holes were drilled approximately every $3/4$" around and down the tube to maximize water flow, and did not require mesh. Exposure chamber contents were enclosed using polyethylene end caps ($2^3/4$" diameter). The bottom cap on SWI chambers was modified by placing a circular mesh covered acrylic ring inside an end cap to which a 2 ½" circular cutout was made to allow for exposure to potential contaminant flux at the sediment–water interface. The SED chambers housing smaller organisms were open at the bottom, and capped from the bottom prior to recovery by divers, while the chambers for larger organisms were fitted with a coarse ½" stainless steel mesh over the bottom.

Custom holders were designed for the SED chambers that allowed DGT probes to be deployed within SEA Rings. The holders position the DGT vertically so that the majority of the device is buried in the sediment during deployment, with ∼2 cm remaining in the water column to provide both a shallow porewater and overlying water measurement. The SPME samplers were deployed adjacent to the SEA Ring.
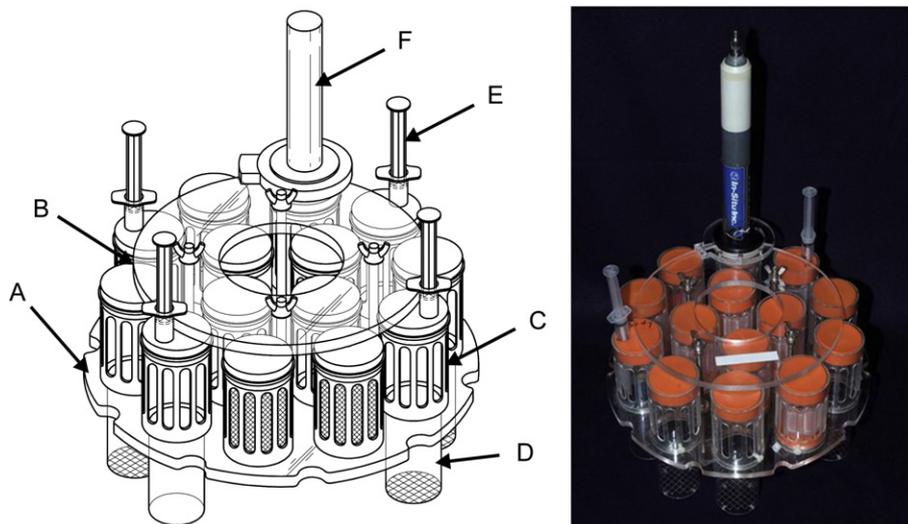

Fig. 1. Schematic (left) and photograph (right) of the Sediment Ecotoxicity Assessment Rings (SEA Ring) system showing (A) base plate; (B) top plate; (C) chamber holder; (D) exposure chamber; (E) syringe for dispensing sediment dwelling organisms; and (F) in situ water quality sensor.
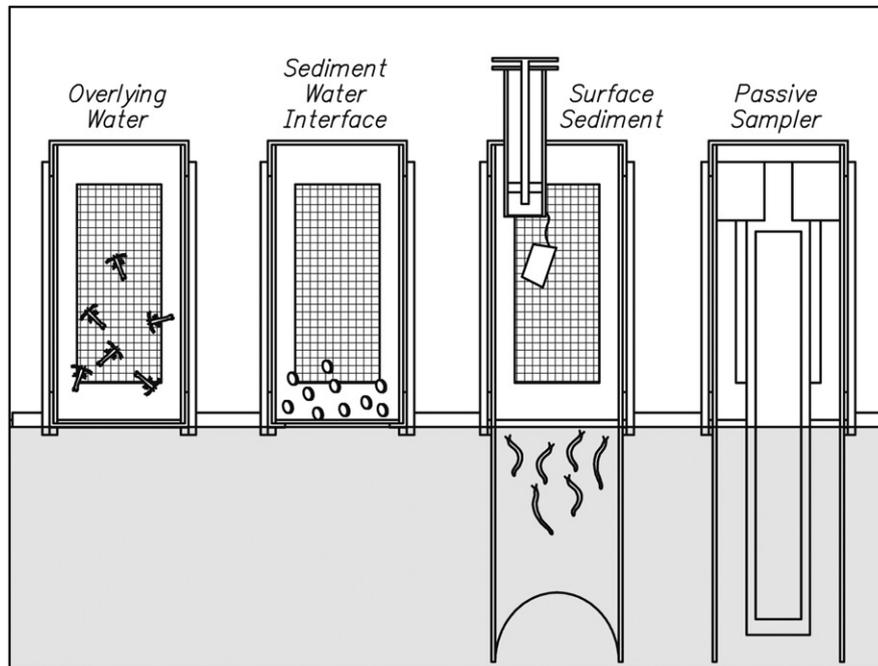
Fig. 2. Side view of the SEA Ring exposure chambers, including options for overlying water (WC), sediment–water interface (SWI), or surficial sediment (SED) exposures. Passive samplers are also integrated into chambers, as shown for DGT.

An exposure chamber adapter was also developed to allow continuous monitoring of water quality onboard the SEA Ring using a portable Troll® 9500 (In Situ, Inc.) multi-parameter water quality monitoring and logging instrument. This allowed for monitoring of various water quality parameters (i.e., pH, temperature, salinity, dissolved oxygen, ORP, and depth) not only on the sea floor, but specifically inside a chamber that represented conditions encountered by the test organisms.

2.3. SEA Ring preparation and deployment

Organisms used in WC and SWI exposures were loaded into chambers in the laboratory. This step was conducted in circular 17 gallon HDPE chambers (ChemTainer), which were also used for transportation of the SEA Rings to the field site. Smaller organisms used in SED exposures were loaded into modified 20 or 30 mL plastics syringes. The luer-lock portion of the syringe was removed and silicone stoppers retained the organisms in clean seawater until deployment, based on a similar design reported by Anderson et al. (2004). The loaded syringes were transported to the test site in coolers filled with seawater at the test site temperature and salinity. Travel controls were utilized to ensure there was no stress from transport in the syringes, and results indicated there was none. Immediately prior to deployment, the syringes were inserted into previously drilled holes through the top end caps. Water sampling was conducted with a 40 mL plastic syringe that attached to the tubing, with the sample measured immediately by field crew.

Once on site, the containers were gently lowered into the water, and the SEA Rings removed from the transport containers by divers, who placed them on the sea floor. At each station, multiple SEA Rings were deployed to account for differing exposure periods (2–21 d). For SED exposure chambers, syringe stoppers were popped by depressing the plungers, thus releasing the organisms.

2.4. Recovery approach

Deployments were recovered by divers by first assessing the overall condition of the exposure chambers and removing overlying water quality samples through top cap sampling ports for immediate measurements of pH, temperature, salinity, DO and ORP. The open (bottom) end of SED chambers were capped by gently covering with polyethylene (PE) end caps with the SEA Ring still in place. The SEA Rings were placed into the appropriate ChemTainer and brought back up to the boat, then transferred back to the laboratory for processing.

2.5. Summary of the San Diego bay deployment as a proof-of-concept

2.5.1. Test site

Located on San Diego Bay, CA, several pier areas at Naval Base San Diego (NBSD) have been listed as potentially at risk for aquatic life impacts. A transect between piers 5 and 6 was selected based on historical data for evaluation of integrated *in situ* assessment tools including short-term toxicity and bioaccumulation testing (Table 1, Fig. 3).

2.5.2. Bulk sediment analysis

Sediments captured in the SED chambers were analyzed for physical and chemical characteristics. Grain size analysis of sediment samples for gravel, sand, silt and clay fractions were determined using combined sieve and sedimentation techniques. Sediment samples were analyzed for total organic carbon (TOC). Bulk sediments were analyzed for a suite of metals, PAHs, PCBs, and chlorinated pesticides using low-level detection EPA methods. For metals, samples were digested using a mixed acid digestion technique for total metals based on EPA method 200.2. All metals, except mercury and selenium, were analyzed by either inductively coupled plasma mass spectrometry (ICP-MS) following EPA Method 200.8 or by inductively coupled plasma optical emission spectroscopy (ICP-OES) following EPA Method 200.7. Mercury was analyzed by cold vapor atomic absorption following modified EPA Method 7471.A. Selenium was analyzed by flow injection atomic absorption based on EPA Method SW846, 7000 series. Sediment samples were simultaneously extracted for PAHs, PCBs, and chlorinated pesticides, and splits were subsequently made for analysis. The sample extracts were analyzed for PAHs by a modified version of EPA's SW-846 Method 8270. The PCB congener analysis method used a modified version of EPA's SW-846 Method 8081 using dual, dissimilar columns and dual detectors.

2.5.3. Test organisms

A number of species representing different phyla, feeding habits, exposure routes, and endpoint sensitivities were utilized for toxicity and bioaccumulation exposures. The following organisms, and starting size or age-class, were used for toxicity tests: amphipod (*Eohaustorius estuarius*) survival (3–5 mm), mysid shrimp (*A. bahia*) survival (3–5 days old), polychaete (*Neanthes arenaceodentata*) survival and post exposure feeding rate (6 week old), and mussel (*M. galloprovincialis*) embryo-larval

Table 1
Assessment methods (Lines-of-Evidence) used at the Naval Base San Diego.

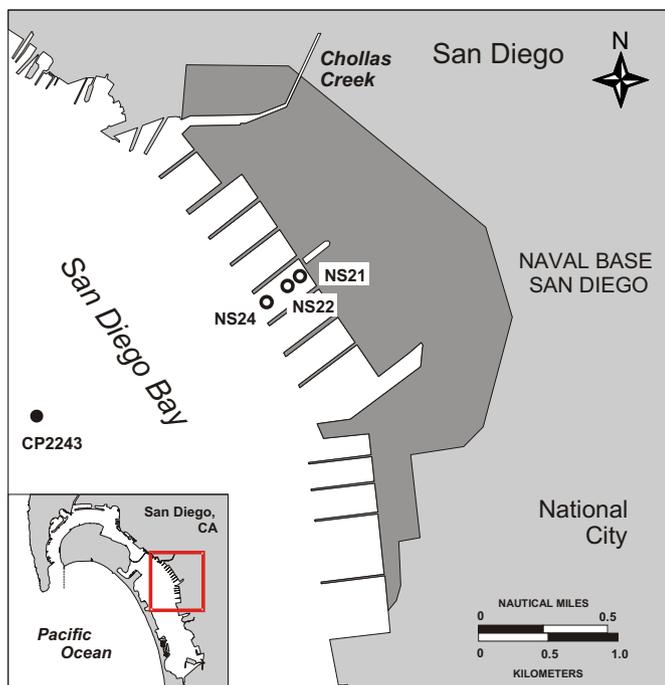| SEA ring supported lines-of-evidence	Lines-of-evidence used for Naval Base San Diego
Toxicity	*E. estuarius* (SED)
	N. arenaceodentata (SED)
	M. galloprovincialis (SWI)
	A. bahia (SWI, WC)
Bioaccumulation	*M. senhousia* (SED)
	N. arenaceodentata (SED)
Passive Samplers	SPME
	DGT
Bulk Sediment Chemistry	metals, PAHs, PCBs, pesticides, grain size, total organic carbon
Water quality	Continuous monitoring with *In Situ*, Inc. Troll® 9500

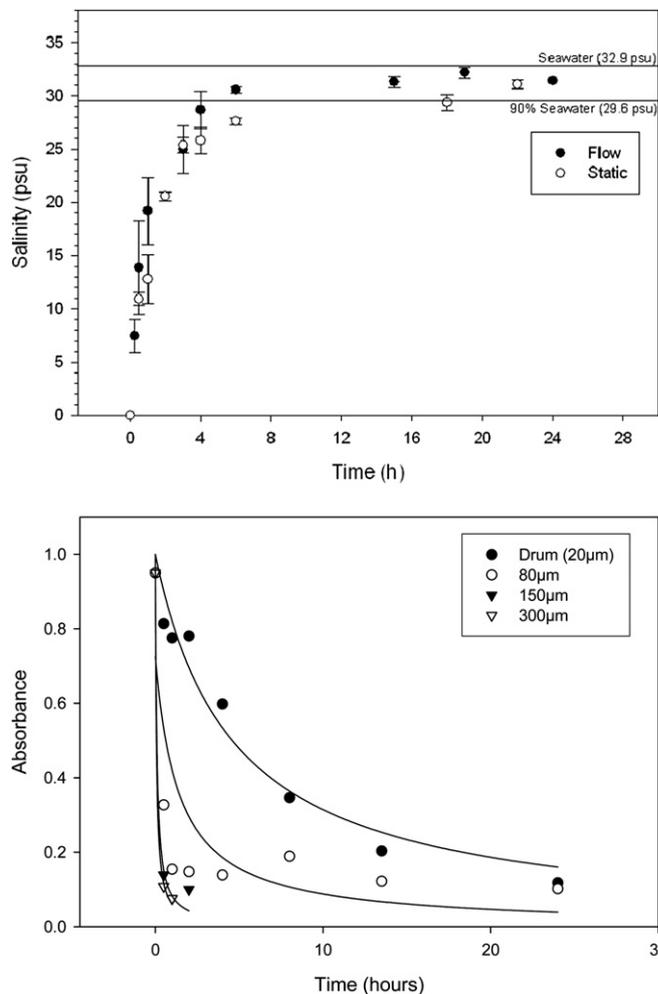**Fig. 3.** Study sites for the San Diego Bay deployments.

Fig. 4. Exposure chamber equilibration experiment results from multiple screen types using dye (bottom) and a prototype small mesh (20 μm) scintillation vial based chamber using salinity (top) to assess time to equilibrate with external environment.

development. Bioaccumulation chambers included infaunal mussels *Musculista senhousia* (2 cm) and 6 week old polychaetes (*N. arenaceodentata*). All test organisms were shipped overnight to the relevant laboratory and acclimated in uncontaminated seawater adjusted to the approximate site conditions for 24–48 h prior to deployment. All organisms were provided by commercial suppliers, and with the exception of the mysid shrimp and polychaete, were field collected.

2.5.4. Controls and reference sites

Laboratory toxicity tests included control sediment, which consisted of the home sediment for *E. estuarius* (Yaquina Bay, OR). Laboratory control and dilution water was 0.45 μm filtered seawater from the research piers at Scripps Institution of Oceanography (La Jolla, CA) or SSC Pacific (near the mouth of San Diego Bay). *In situ* exposures typically included both a laboratory and a travel control. Travel controls were treated the same as *in situ* deployed organisms in that they were caged and transported to the site, but instead of being deployed, they were held in the laboratory exposures for the duration of the field exposures. A single reference station was used (CP2243) where low chemistry and toxicity and healthy benthic communities had been observed in historical data (SCCWRP and SSC San Diego, 2005).

2.5.5. Toxicity exposures

In situ toxicity test durations included 2-day (amphipod, polychaete, mysid and bivalve) and 10-day (amphipod) exposures. Endpoints included amphipod survival, mysid survival, bivalve embryo-larval development, and polychaete survival and post exposure feeding rate. The feeding rate assay using *N. arenaceodentata* was developed as a relevant short-term sublethal endpoint using a standardized test organism (Rosen and Miller, 2011). The endpoint is consumption rate of *Artemia* sp. nauplii per hourm, assessed in the lab, following a field exposure. Toxicity assessment for all tests was conducted immediately upon return to the laboratory (within 30 min of diver SEA Ring retrieval). Survivors were enumerated immediately upon recovery from chamber or sieves. The post exposure feeding rate assessment was initiated following a 1 h acclimation period to laboratory conditions in clean seawater, while mussel larvae were preserved in buffered formalin for later microscopic examination.

2.5.6. Bioaccumulation exposures

Exposure durations were evaluated at two days for *N. arenaceodentata* and 21 days for *M. senhousia*. Organisms exposed in situ were kept in clean seawater 4–18 h to clear gut contents yet prevent excessive elimination or transformation of lighter weight contaminants, and frozen for extraction and analysis. A micro-scale technique (Jones et al., 2006) was used to analyze the relatively small tissue masses generated in the in situ exposure (0.3–1 g) for polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) total body residue.

2.5.7. Passive sampler – SPME

Solid phase microextraction devices (SPMEs) utilizing polydimethyl siloxane (PDMS) coated glass fibers were included as an indicator of relative biaoavialbility for organic contaminants. The PDMS fibers used in this study were FG 230/210 fibers

and had a 210 μm core with a 10 μm PDMS coating resulting in an outer diameter of 230 μm. The fibers were housed in small diameter (∼0.25 inch) stainless steel tubes with micro-slots along their length to allow communication with the porewater and surface water. For the *in situ* assessment, they were deployed in tandem with the SEA Rings, positioned around perimeter within close (∼1–2 inches) proximity to the bioaccumulation exposure chambers. SPME deployment periods were two and 21 days. Upon retrieval, the PDMS fibers were immediately cleaned, processed into solvent in 5-cm intervals, and analyzed for PAHs. PAH analysis of the PDMS material was performed at the University of Texas at Austin (UT) using high performance liquid chromatography for separation with fluorescence detection (HPLC/FD) for quantification, in accordance with EPA Method 8310 using a Waters 2795 Separations Module. The total organic carbon (foc) of sediment samples was determined by elemental analysis on a Carlo-Erba 1108 (i.e., overnight vapor acidification with a hydrochloric acid atmosphere to remove inorganic carbon from samples).

2.5.8. Passive sampler- DGT

Diffusive gradients in thin film (DGT) accumulate a variety of dissolved "labile" substances, including metals (DGT Research Ltd.). DGT removes dissolved components from porewater in a similar fashion to uptake by biological organisms, and the concentration at the porewater-DGT interface (C_{DGT}) provides a relative measure of the uptake of bioavailable metals that is a more accurate measure of sediment metal toxicity than sequential extraction methods. Commercially available DGT probes consisting of a diffusive gel protected by a plastic housing (Zhang et al., 2001) were used. Custom holders were fitted to SED chambers that allowed DGT probes to be deployed within SEA Rings (Fig. 2) at each of the four stations. They were inserted into sediments such that about two thirds of the device was within the sediment and the top third was within the water column. Exposure duration was two days. Upon recovery of the SEA Rings, DGTs were removed from their holders, and rinsed thoroughly in deionized water to remove all traces of sediment. Acidified water samples were shipped to an outside laboratory (Alloway Laboratory, Lima, OH) and
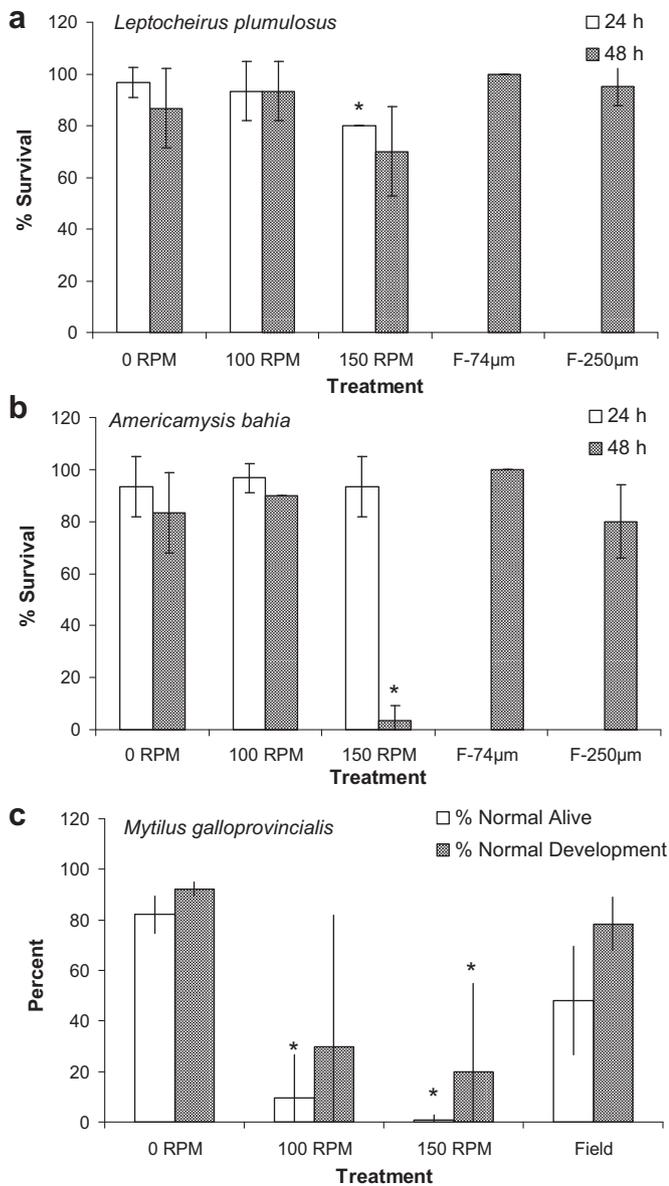
Fig. 5. Shaker study results for *Leptocheirus plumulosus* (a), *Americamysis bahia* (b), and *Mytilus galloprovincialis* (c). F-74 μm and F-250 μm represent Field exposure in exposure chamber with 74 and 250 μm mesh windows, respectively, which were only recorded at 48 h. All *M. galloprovincialis* exposures were conducted with 20 μm mesh windows.

analyzed for Cu, Zn, Ni, Pb, and Cd using EPA method 200.8 (USEPA, 1994b). Dissolved metal concentrations were ultimately converted to DGT concentrations (C_{DGT}) using temperature specific diffusion coefficients provided by the DGT manufacturers.

3. Results and discussion

3.1. Preliminary testing

The dye study showed rapid equilibration between the inside of the exposure chambers and the external environment with only 10% of the dye remaining after 30 min for the 150 and 300 μm screen sizes, and 1 h for the 80 μm screen size (Fig. 4). The 25 μm drum chamber housed inside an 80 μm screen chamber took the longest to equilibrate requiring between 13.5 and 24 h to achieve the same degree of equilibration. The overall rapid equilibration observed suggests that conditions inside the *in situ* exposure chambers mimics the external environmental well, even over short exposure durations.

Similarly, the modified scintillation vial chamber design considered for small (25–80 μm) organisms such as mussel embryos showed rapid equilibration using salinity as a surrogate for a contaminant (Fig. 4). Within 4 h, salinity within the vials was 79 and 88% of the salinity in the external environment for static and flow through conditions, respectively (Fig. 4). Steady-state (90%) conditions were achieved within 6 h under the flow conditions, and by the 18 h time-point under static conditions. Rapid equilibration is particularly important for short (i.e. two day) exposures.

Results of the chamber shaking experiments showed *L. plumulosus* and *A. bahia* were unaffected at 100 RPM after 48 h, but some reduced survival was observed (at 48 h only) for both species at 150 RPM (Fig. 5). The 48 h reduced survival at 150 RPM was particularly apparent for *A. bahia* (mean survival = $3.3 \pm 5.8\%$), suggesting that they are less tolerant than *L. plumulosus* (mean survival = $70 \pm 17\%$) to physical stress. *L. plumulosus*, which are considerably larger than *A. bahia*, concentrated near the bottom of the containers during exposure. It should be noted, however, that the controlled shaking studies may have been overly stressful due to the fact that concurrent 48 h pierside *in situ* deployments in an area with reasonable high boat traffic yielded high survival for both species at both time points ($\geq 80\%$; Fig. 5).

Mussel (*M. galloprovincialis*) embryo-larval development shaking results generally showed a substantial reduction in the number of normally developed larvae relative to the initial number of embryos added (% normal alive) and the percent normally developed of those counted (% normal development) (Fig. 5), with the former being a somewhat more sensitive endpoint to physical stress. The pierside *in situ* deployments yielded higher embryo-larval development than

Table 2
Bulk sediment concentrations summary at Naval Base San Diego.

Analyte	Units	Site/Station					
		ERL	ERM	NS21	NS22	NS24	CP2243[a]
Cadmium	mg/kg	1.2	9.6	0.354	0.267	0.669	0.136
Chromium	mg/kg	81	370	81.1	94.5	79.7	48.0
Copper	mg/kg	34	270	277	316	197	79.8
Mercury	mg/kg	0.15	0.71	0.76	0.91	0.79	0.35
Lead	mg/kg	46.7	218	73.9	75.4	63.2	33.3
Zinc	mg/kg	150	410	342	338	308	159
Total Chlordane	μg/kg			0.600	0.420	0.820	0.04
Total DDTs	μg/kg	1.58	46.1	6.32	6.62	7.41	0.88
Total PAH	μg/kg	4022	44,792	5214	5105	2924	415
Total PCB	μg/kg	22.7	180	234	172	207	21.4
Silt/Clay	%			72.5	85.9	67.1	40.9
TOC	%			2.01	2.14	1.58	0.710

ERL = effects-range-low; ERM = effects-range-median (MacDonald et al. 1996)
No data shown where not measured or not applicable.
 [a] Reference station.

shaking, which was not significantly different from laboratory controls. The experimental shaking exposure scenario (open top, with high turbulence) is unlikely in closed field-deployed exposures. Greater variability among replicates with caged *M. galloprovincialis* embryos relative to standard static laboratory exposures was also observed in microcosm experiments by Rosen and Lotufo (2010).

3.2. Proof-of-concept – San Diego deployment results

Multiple lines-of-evidence were investigated in this study in this first major deployment of the SEA Rings. Linkage of exposure and effects was assessed. The second part of this paper series (Rosen et al., 2012) elaborates on the importance of these linkages at a site where multiple contaminant pathways of exposure were possible. Bulk sediment concentrations for sediments captured in the SEA Rings are summarized in Table 2, and are compared to sediment quality guidelines i.e., Effects Range Low [ERL] and Effects Range Median [ERM] parameters; MacDonald et al. (1996). The results show a general increasing trend toward the shoreline for PAHs, but overall, similar values exist among stations for the other chemicals of concern. Highest concentrations of Ch, Pb, Zn, chlordane, total DDTs and total PAHs generally were between the ERL and ERM values. The highest concentrations of Cu, Hg and PCBs generally exceeded the ERM and Cd was below the ERL. These screening-level thresholds suggest a reasonable potential for toxicity. In contrast, concentrations at the reference station (CP2243) were always lower by a factor of two to one order of magnitude and were below ERMs.

Toxicity from field exposures was lower than expected based on bulk sediment contaminant concentrations and historical data at the site (SCCWRP and SSC San Diego, 2005). Two-day *in situ* exposures resulted in ≥90% survival at the reference site (CP2243) for both *E. estuarius* and *A. bahia*, while *N. arenaceodentata* mean survival was 75% (Table 3).

No toxicity (survival ≥97.5%) was observed at any station in *A. bahia* exposures of the water column (Table 3), indicating relative robustness of this test organism for use *in situ* and the sediments as the more likely adverse exposure route. Clark et al. (1987) also reported successful use of *A. bahia* in field exposures.

On the contrary, *M. galloprovincialis* larval recovery from *in situ* SWI exposures was low and variable for all stations including the reference (Table 3). Water quality from the Troll 9500 sensors are shown in Table 4, and show acceptable conditions for all organisms used in this study. The water quality data, however, are representative of conditions at the SWI in SED chambers with a 250 μm mesh size. Based on discrete water quality measurements made on individual *M. galloprovincialis* chambers, which utilized a 25 μm screen, D.O. concentration was markedly reduced (<1 mg/L) and contents of some of those chambers smelled strongly of sulfides. It is likely that flow through the mussel chambers was inhibited due to clogging by fine sediment particles. In addition to water quality related artifacts, microscopic assessment of the mussel samples was extremely difficult due to aggregated particulate matter of similar size to the larvae. Therefore, non-contaminant related artifacts likely contributed to the heightened sensitivity of the field-deployed mussel embryos.

Although one goal of this work was to develop a rapid on-site assessment protocol, *E. estuarius* were also exposed for 10 days *in situ*, which is the standard duration for acute exposures in the laboratory (USEPA, 1994a). Recoveries of *E. estuarius* after 10 days of exposure were relatively poor (averaging <40%; Table 3), but still showed a greater response at station NS21, consistent with the 2-day data and increased risk associated with other lines-of-evidence. Temperatures during the 10-day exposure reached 24 °C, which likely were in excess of that tolerated by *E. estuarius*, traditionally

Table 3
In situ toxicity test results for multiple species and endpoints at Naval Base San Diego.

Species	E. estuarius		E. estuarius		N. arenaceodentata		N. arenaceodentata		A. bahia		M. galloprovincialis	
Duration (days)	2		10		2		2		2		2	
Exposure Type	SED		SED		SED		SED		WC		SWI	
Endpoint	% Survival		% Survival		% Survival		Feeding Rate[a]		% Survival		%Normal Alive	
Sample ID	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lab Control	96	9.0	88	8.4	100	0	157	5.8	100	0	91	8.4
Travel Control	93	5.0	93	5.0	100	0	135	23	100	0		
NS21	67	27.5	**1.3**	**2.5**	88	15	**101**	**19**	100	0	**11**	**9.7**
NS22	79	14.9	**40**	**41**	**75**	**13**	129	19	100	0	**6**	**5.2**
NS24	70	21.6	30	21	**75**	**17**	**105**	**19**	100	0	**17**	**3.5**
CP2243	90	7.1	24	30	75	17	136	7.8	98	5	**10**	**7.9**

Bold indicates statistically lower than associated Lab or Travel Control using unequal variance *t*-tests (*p* < 0.05).
SED = surficial sediment; WC = water column; SWI = sediment–water interface
[a] Number of brine shrimp nauplii consumed in 1 h following a 2 day sediment exposure.

Table 4

Water quality parameters expressed as mean (minimum/maximum) from Naval Base San Diego. Water quality was measured inside a representative sediment chamber just above the sediment−water interface at 30 s intervals for first 48 h of deployment. Dashes indicate the sensor did not collect data for that parameter. D.O. = dissolved oxygen. ORP = oxidation−reduction potential.

Station	Temperature (°C)	Depth (m)	D.O. (mg/L)	pH	Salinity (‰)	Conductivity (mS/cm)	ORP (mV)
NS21	22.3 (21.4/22.8)	10.5 (9.4/11.6)	5.7 (4.0/7.1)	6.8 (6.8/7.0)	35.7 (35.1/36.1)	51.2 (49.7/52.1)	84 (−156/164)
NS22	22.2 (21.3/22.8)	10.2 (9.1/11.3)	4.9 (3.1/7.3)	7.6 (7.3/7.9)	33.1 (32.7/33.3)	47.8 (46.4/48.5)	194 (−72/254)
NS24	22.6 (21.7/23.3)	11.2 (10.0/12.3)	5.6 (4.3/6.4)	7.7 (7.4/7.9)	34.0 (33.4/34.4)	49.3 (47.7/50.5)	159 (−84/234)
CP2243[a]	23.1 (22.5/23.7)	4.7 (3.5/5.8)	7.1 (6.5/8.2)	7.9 (7.7/8.0)	33.4 (31.7/36.0)	49.0 (46.8/52.6)	−

[a] Reference station.

tested at 15 °C (USEPA, 1994a). Results for the 2-day bivalve larvae SWI and 10-day amphipod SED exposures suggest these tests require more refinement to achieve reliable results.

Other lines-of-evidence to assess bioavailability included the passive samplers and polychaete and mussel bioaccumulation. DGT-data in the top 5 cm of the sediment (Fig. 6) generated predicted porewater concentrations (C_{DGT}) ranging from 1.1 to 3.1 mg/m^3 for copper and 4.2−16.3 mg/m^3 for zinc. While the spatial trend was generally consistent with the observed gradients in bulk sediment and toxicity, the concentrations are generally well below dissolved metal levels that would be expected to cause toxicity to *E. estuarius* or *N. arenaceodentata* (McPherson and Chapman 2000; Rosen and Miller, 2011). A relationship between C_{DGT} and toxicity for these metals was not apparent.

The 21-day deployment of the infaunal mussel *M. senhousia*, and *N. arenaceodentata*, yielded satisfactory to relatively poor survival (74 and 42% overall, respectively) for body burden determinations. Metal bioaccumulation for *in situ* exposures of mussels showed tissue concentrations ranging from about 3 to 12 μg/g dw for copper and 8−13 μg/g for zinc, with lowest concentrations measured at the reference station, CP2243. Together, these lines-of-evidence for metal bioavailability indicate limited metals bioaccumulation consistent with observed contamination gradients and limited toxicity.

Porewater concentrations of PAHs from the *in situ* SPME samplers were evaluated in relation to bioaccumulation in mussels (Fig. 7). Both lines-of-evidence showed a consistent pattern with higher porewater and tissue concentrations at stations NS21 and NS24, lower levels at NS22, and lowest levels at the reference station
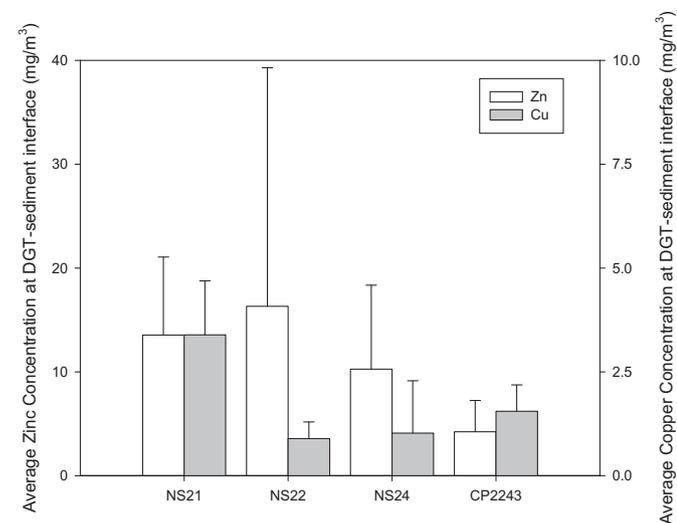
CP2243. This bioavailability evidence, when compared to the acute toxicity to *E. estuarius* and *N. arenaceodetnata*, provides a different perspective from the bulk sediment chemistry, which showed a monotonic increase toward the shoreline. The relatively higher bioavailability at NS21 might help explain the increase in toxicity observed at this station, while the overall absence of toxicity at NS22 is supported by relatively low bioavailability measures including the *in situ* tissue and passive sampler measurements, particularly with respect to PAHs.

Overall, however, results from the *in situ* tests indicate a weak gradient of response corresponding roughly with the increasing concentration gradient toward the shoreline. This suggests contaminants at the site have a low degree of bioavailability.

3.3. Refinements

Several ongoing refinements to the prototype SEA Rings were made following lessons learned from this initial site deployment. This included incorporation of a re-circulating water pump system and several modifications to improve reliability and reduce dependence on diver support. Initial deployments, particularly those with small mesh and/or extended exposure duration, indicated that at more biologically productive sites, screens fouled and reduced water flow and water quality. Subsequent exposures included an integrated water circulation system to increase surface water flow through the chambers and thereby reduce the likelihood of dissolved oxygen sags within the chambers. A small (2 watt) submersible pump was

Fig. 6. Concentrations of metals measured using diffusive gradient thin films (DGT) probes at the porewater-DGT interface (C_{DGT}; mg/m^3). Reported concentrations from DGT probes are averaged across the top 5 cm of the sediment. Error bars = range values in one cm slices of 5 cm. Open bars are zinc concentrations; solid bars are copper concentrations.
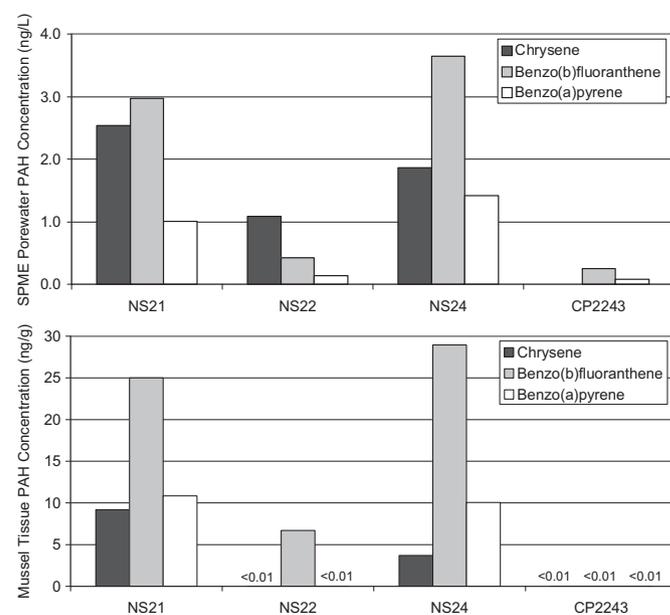

Fig. 7. Porewater concentrations of PAHs from the SPME samplers (above), and bioaccumulation in 21-day mussel exposures (below). Reported porewater concentrations from PAHs are averages across the top 7 cm of the sediment.

attached to a custom-built waterproof battery housing. Tygon tubing gently sprayed site water through small holes in the tubing placed external but adjacent to exposure chamber windows on the interior of the SEA Ring. Reduced diver dependence was achieved by incorporating a bracket to the top portion of the SEA Ring to which a series of poles could be inserted for deployment from a boat. Once adequately positioned on the sediment surface, a pin attached by a line was pulled allowing the pole to be pushed to a second tier, which triggered release of SED test organisms housed in the modified plastic syringes. Recovery of open-bottomed SED chambers was addressed by modifying the PE end caps with a series of cross-sectional slices (kept open with an acrylic ring during deployment) that served much like a core catcher, trapping sediment and test organisms when pulled from the sediment, but preserving the stratification of the sediment during the field exposure

4. Conclusions

The scale and scope of sediment-related risk assessment projects is often enormous and there is a tendency to use overly simplistic bulk chemistry analysis and comparisons to sediment quality guidelines that do not consider factors affecting bioavailability and do not account for co-occurrence issues. This practice is not supported scientifically and should only be used as a crude Tier 1 assessment, followed by an assessment based on multiple lines-of-evidence (e.g., habitat, laboratory and in situ-based toxicity, indigenous biota) (Adams et al., 2005; Wenning et al., 2005). The SEA Ring approach described here reduces the uncertainty around sediment risk assessments and remedial decision-making, because it provides for simultaneous analysis of chemical exposure (including mixtures) and biological responses in situ. Additionally, this approach has the potential to assess impacts of non-contaminant stressors, which can be important to consider in understanding causality and evaluating remedial options.

The SEA Ring platform allowed for simultaneous deployment of multiple marine and estuarine species exposed via three compartments (overlying water, sediment–water interface, and bulk sediment) for toxicity and bioavailability measurements, along with passive samplers as indicators for metal (DGT) and non-polar organic (SPME) bioavailability, and the collection of collocated bulk sediment samples. The SEA Ring also incorporates sensors to continuously monitor water quality parameters from within the in situ exposure cages, thereby providing meaningful ancillary data with which to interpret the in situ results. The overarching goal of the approach was to improve the accuracy when assessing ecological risk and remediation efficacy at contaminated sediment sites by improving the linkage between exposure and effects, particularly in scenarios where traditional laboratory-based methods alone are inadequate to make informed management decisions, such as the assessment of in place sediment remedy effectiveness, and time-varying stressors such as storm water discharges, ground-water surface water interactions, oil spills, and unexploded ordnance assessments. The approach is rapid, and should prove cost-effective, with many of the measurements being made on-site in less than one week.

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