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Comparison of Methods for Conducting Marine and Estuarine Sediment Porewater Toxicity Tests—Extraction, Storage, and Handling Techniques

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Abstract. A series of studies was conducted to compare different porewater extraction techniques and to evaluate the effects of sediment and porewater storage conditions on the toxicity of pore water, using assays with the sea urchin *Arbacia punctulata*. If care is taken in the selection of materials, several different porewater extraction techniques (pressurized squeezing, centrifugation, vacuum) yield samples with similar toxicity. Where the primary contaminants of concern are highly hydrophobic organic compounds, centrifugation is the method of choice for minimizing the loss of contaminants during the extraction procedure. No difference was found in the toxicity of pore water obtained with the Teflon® and polyvinyl chloride pressurized extraction devices. Different types of filters in the squeeze extraction devices apparently adsorbed soluble contaminants to varying degrees. The amount of fine suspended particulate material remaining in the pore water after the initial extraction varied among the methods. For most of the sediments tested, freezing and thawing did not affect the toxicity of porewater samples obtained by the pressurized squeeze extraction method. Pore water obtained by other methods (centrifugation, vacuum) and frozen without additional removal of suspended particulates by centrifugation may exhibit increased toxicity compared with the unfrozen sample.

The toxicity of pore water extracted from refrigerated (4°C) sediments exhibited substantial short-term (days, weeks) changes. Similarly, sediment pore water extracted over time from a simulated amphipod solid-phase toxicity test changed substantially in toxicity. For the sediments tested, the direction and magnitude of change in toxicity of pore water extracted from both refrigerated and solid-phase test sediments was unpredictable.

The feasibility of conducting sediment porewater toxicity tests with marine and estuarine sediments has been demonstrated (Carr *et al.* 1989; Long *et al.* 1990). The conclusion from these initial studies was that porewater toxicity testing is a promising approach for assessing sediment quality but requires further evaluation to optimize and standardize the methodology. In these initial studies, a pressurized Teflon® squeeze extraction device was used to obtain the porewater samples (Carr *et al.* 1989). The life-cycle test with the polychaete *Dinophilus gyrociliatus* (Carr *et al.* 1986) was used to test the toxicity of the porewater samples. The two primary issues recommended for further evaluation involved (1) the porewater extraction procedure and subsequent handling and storage of the pore water and (2) the test species and end points used to assess the toxicity of the porewater sample (Carr 1988). The objective of our study was to compare the effects of different porewater extraction techniques and sediment and porewater storage and handling variables on the toxicity of porewater samples. Studies addressing the use of alternative species and end points for use in porewater testing will be described in a companion paper (Carr and Chapman in review). The sea urchin fertilization and embryological development tests with *Arbacia punctulata* have been found to be suitable for assessing the toxicity of porewater samples (Carr 1993; USFWS 1992; NBS 1993, 1994a, 1994b) and were used in the present study.

Several different methods have been employed for extracting sediment pore (interstitial) water, including centrifugation (Edmunds and Bath 1976; Landrum *et al.* 1987; Giesy *et al.*, 1988), pressurized (squeezing) extraction (Reeburgh 1967; Bender *et al.* 1987; Jahnke 1988; Carr and Chapman 1992; Carr 1993), vacuum (suction) methods (Knezovich and Harrison 1987; Winger *et al.* 1991), and equilibration methods using dialysis membranes or fritted glass samplers (Hesslin 1976; Mayer 1976; Bottomley and Bayly 1984; Di Toro *et al.* 1990). Because the extraction time and sample size required for the equilibration methods are not compatible with the limitations imposed by most sediment quality assessment studies, only the centrifugation, pressurized and vacuum extraction methods were included in our study. In addition to the extraction, storage, and handling experiments, data from some complementary studies have also been included. As different extraction techniques yielded pore waters with varying amounts of suspended particulate material, studies were conducted to assess the effect of suspended particulates on the sea urchin fertilization assay. The utility of the porewater approach for detecting short-term changes in sediment quality was demonstrating by monitoring

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changes in the toxicity of sediments during the course of a simulated solid-phase exposure. The effect of freezing and thawing on the toxicity of porewater samples was also evaluated.

Materials and Methods

Extraction Techniques

The pressurized squeeze extraction device used in our initial studies was composed of a Teflon® cylinder and end plates that were made air tight with Teflon® O-rings (Carr *et al.* 1989). Inside the bottom end plate was a porous (~50 μm) Teflon® filter support plate. The device was pressurized with compressed air supplied by a second-stage regulator from a standard SCUBA tank. A GF/F glass fiber filter (0.7 μm) was used in the extraction device during our initial evaluations. More recent studies have demonstrated that hydrophobic organic compounds such as DDT may be preferentially adsorbed to GF/F glass fiber filters during filtration (Word *et al.* 1987). In the present study, a number of different filter materials including fluorocarbon (74 μm mesh opening, Spectrum Medical Industries Inc), nylon (8 μm mesh opening, Spectrum Medical Industries Inc), polyester (8 μm mesh opening, Spectrum Medical Industries Inc), polycarbonate (1 μm mesh opening, Poretics Corp), and GF/F glass fiber (0.74 μm mesh opening, Whatman) were evaluated. All of the filters were soaked in deionized water for ~24 h before use (except the GF/F glass fiber filters, which were not sturdy enough to withstand this treatment).

In addition to the Teflon® squeeze extraction device, the use of a similar device composed of polyvinyl chloride (PVC, Figure 1) was evaluated. The PVC extraction device was constructed from a compression coupling (Lascotite®) for 4-in. (i.d.) PVC pipe. These couplings are available commercially and consist of a cylinder with threaded ends and threaded open compression nuts. The compression coupling was fitted with 0.5-in. thick PVC end plates held in place by the threaded end pieces on the compression coupling. A silicon O-ring provided a better seal than the Teflon® O-rings in the PVC extractors. Like the Teflon® device, the bottom end plate had several interconnected concentric grooves to facilitate passage of the pore water to the central exit port. Both devices were fitted with pressure relief safety valves (50 psi) and quick disconnect fittings for attaching the compressed air line (Figure 2). The PVC devices were much simpler to use than the Teflon® extractors because the bottom end caps could be tightened in place before the sediment sample was loaded. A strap wrench and a wrench made of 0.5-in. PVC sheet, fashioned to fit the knurled end caps, were used to tighten or dismantle the end plates on the PVC devices. An experienced technician can keep a dozen or more PVC extractors operating simultaneously, which is not possible with the Teflon® extractors. The non-metal surfaces of the PVC extractors were soaked in a seawater bath with numerous water changes for several days before use and were acid-washed between uses.

Two other porewater extraction methods (vacuum and centrifugation) were compared with the pressurized extraction devices described above. Sediment pore water can be extracted from sediment by vacuum. A vacuum-operated extraction device described by Winger and Lasier (1991), consisting of a fused-glass aquarium air stone (Jungle Laboratories Corp, Cibolo TX) attached with aquarium airline tubing to a 50-cc polypropylene syringe was used. After inserting the air stone into the sediment, a vacuum was applied by retracting and bracing the syringe plunger. All tubing and syringes were washed prior to use. Pore water was also obtained by centrifuging sediment in 250-ml polycarbonate bottles at 4,200 g for 30 min. The resulting supernatant was carefully pipetted off and used without additional filtration.

For our extraction comparison studies, sufficient numbers of 5- to 10-cm-depth sediment cores from produced water (petroleum brine) discharge sites were collected with a PVC coring device to obtain ~16 L of sediment. The composite sample was homogenized with a stainless steel spoon and ~2 L aliquots of homogenized sample were placed in Ziploc® storage bags and stored on blue ice or held refrigerated at 4°C until processed. All porewater samples for a particular study were extracted within 30 h of collection. In general, porewater samples were frozen immediately after extraction. Samples were stored frozen until the day before the test, when they were thawed at room temperature or in a tepid water bath. After the samples were thawed, water quality measurements (dissolved oxygen, pH, salinity, and ammonia) were made and the salinity adjusted to 30‰ by the addition of Milli-Q® water or hypersaline brine. The samples were then centrifuged at 4,200 × *g* for 20 min and the supernatant collected. The samples were refrigerated (4°C) overnight and slowly brought back to room temperature the next day before the start of the test. The freezing and thawing process had no effect on any of the water quality parameters.

Porewater Storage and Handling Experiments

Preliminary studies indicated that freezing and thawing had no effect on the toxicity of porewater samples (Carr *et al.* 1989). The effect of freezing and thawing was evaluated further in a series of tests with both squeeze extracted (PVC extractor with polyester filters as described above) and centrifuged samples. Aliquots of pore water obtained by the two extraction methods (using subsamples from the same sediments) were frozen in a conventional freezer overnight, while the rest of the samples were refrigerated at 4°C. Prior to testing, the frozen aliquots were thawed in a refrigerator, and then all of the treatments were tested together on the following day.

Varying amounts of suspended particulate matter were present in porewater samples obtained by the different extraction methods for the same sediment. To quantify this observation, the amount of particulate matter remaining in the different porewater samples was determined by weighing the amount of material retained on a millipore (0.45 μm) filter (after drying for 24 h at 60°C) for a known volume of pore water. These determinations were made for pore water obtained by four different methods: PVC squeeze extractor with a polyester filter (8 μm), PVC squeeze extractor with a GF/F filter (0.7 μm), vacuum extraction, and centrifugation for 10 min at 4,500 g. The amount of suspended particulate material was then determined for freshly extracted, synoptically collected porewater samples after (1) resuspending the material by shaking and swirling the sample container just prior to taking the sample, (2) the sample had been allowed to settle for 90 min, and (3) the sample had been centrifuged for 10 min at 4,500 × *g*. A sandy sediment was used for this experiment, as this type of sediment had been observed to produce more turbid pore water by some of the extraction methods than more fine-grained sediments. Sediment grain size was determined by the ASTM (1963) bouyoucous hydrometer method.

Sediment Storage and Handling Studies

The toxicity of sediments has been shown to change considerably after varying lengths of storage (Malueg *et al.* 1986; Othoudt *et al.* 1991) and a maximum period of 2 weeks at 4°C has been recommended from the time of collection until the start of a test (ASTM 1992). Many sediment testing studies, however, are conducted with samples that are held considerably longer than the recommended 2-week period. In our laboratory, porewater samples are extracted as soon as possible (usually within 24 h) after the sediments are collected or received. In order to assess whether longer holding times affect the toxicity of the sediments, we conducted a time-series experiment with sediment collected near three different produced water discharges in local Texas estuaries. The primary contaminants of concern in these samples were metals and polycyclic aromatic hydrocarbons (PAHs). The sediments were refrigerated at 4°C in acid-washed, high-density polyethylene containers with no overlying water, and pore water was extracted (PVC extractor

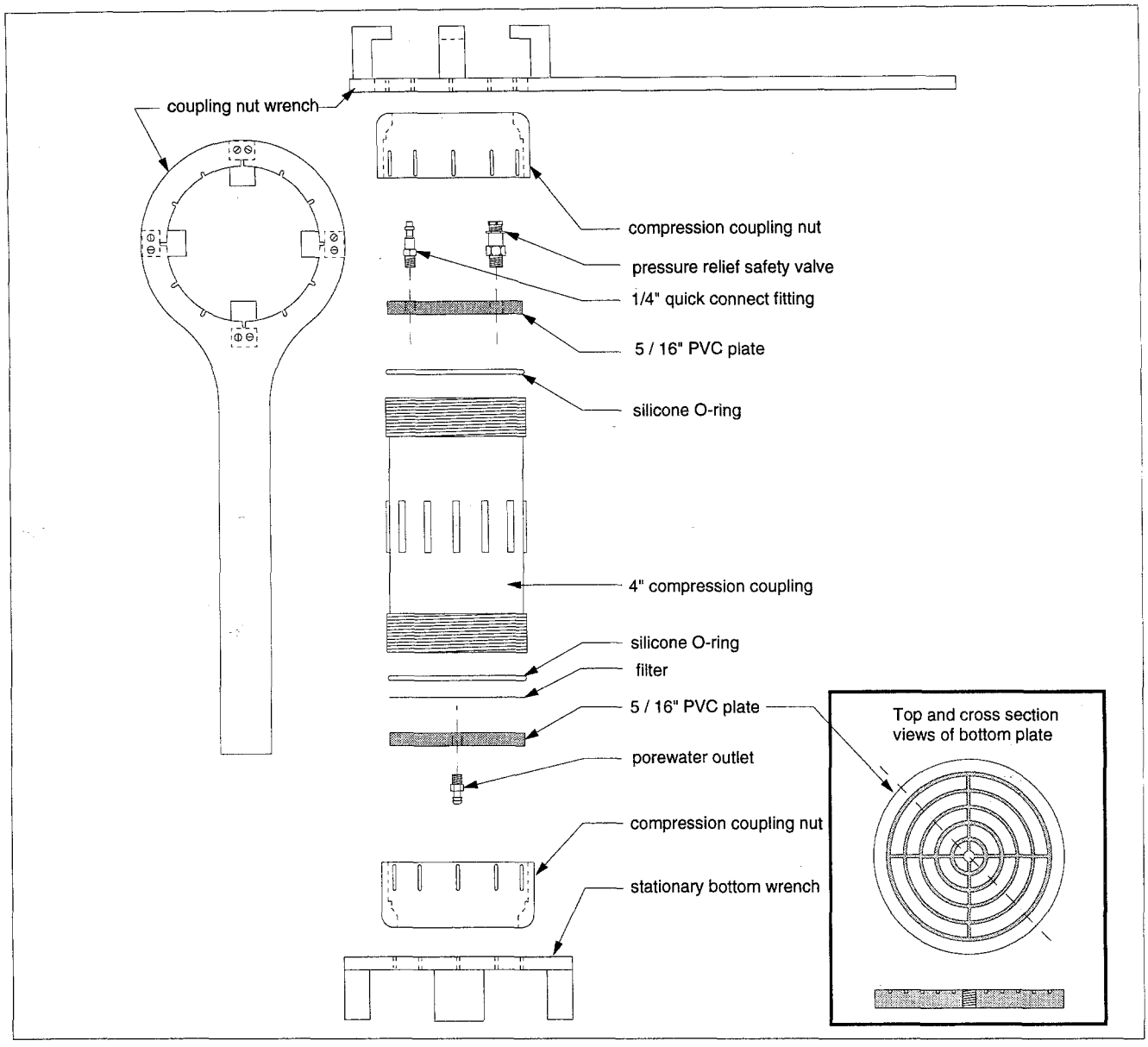

Fig. 1. Schematic diagram of porewater pressurized extraction device and accessories

with polyester filters) at approximately 1-week intervals over a 29-day period and stored frozen. The sediment samples were re-homogenized with a Teflon® spatula prior to obtaining an aliquot for porewater extraction at each sampling period. This experiment was conducted initially with one sample over a 14-day period and repeated with three different samples over a 29-day period.

There is also some question concerning changes in the toxicity of sediments during the course of 10-day or longer solid-phase tests. To evaluate this phenomenon, a test was conducted in which sediment was placed in 3.8-L acid-washed polyethylene containers with overlying water in a sediment:water ratio similar to the standard test design for conducting solid-phase tests with amphipods (ASTM 1992). Gentle aeration was supplied to each test container to provide circulation of the overlying water as required in the amphipod test procedure. After varying lengths of time (0, 3, 7, 14, and 21 days), containers were sampled, the overlying water was carefully siphoned off, and the pore water extracted (using the PVC extractor with the polyester filters) and stored frozen. After all the porewater samples were collected, the toxicity was determined using the sea urchin ferilization and embryological development assays. The sediments used in this experiment were subsamples of the sediments from the produced water discharge sites used in the sediment storage experiments described previously.

Toxicity Test Methods

The sea urchin sperm cell (fertilization) test and the embryological development assay were used to test the toxicity of the pore water samples in this study. Adult sea urchins *Arbacia punctulata* were collected from the ship channel jetties in Port Aransas, Texas. The sperm cell test followed the standardized Environmental Protection Agency (EPA) method (Weber *et al.* 1988) with several modifications which have been described previously (Carr and Chapman 1992). Unlike the USEPA method, sperm were collected dry, and a pre-test was conducted prior to the actual test. The pre-test consisted of a

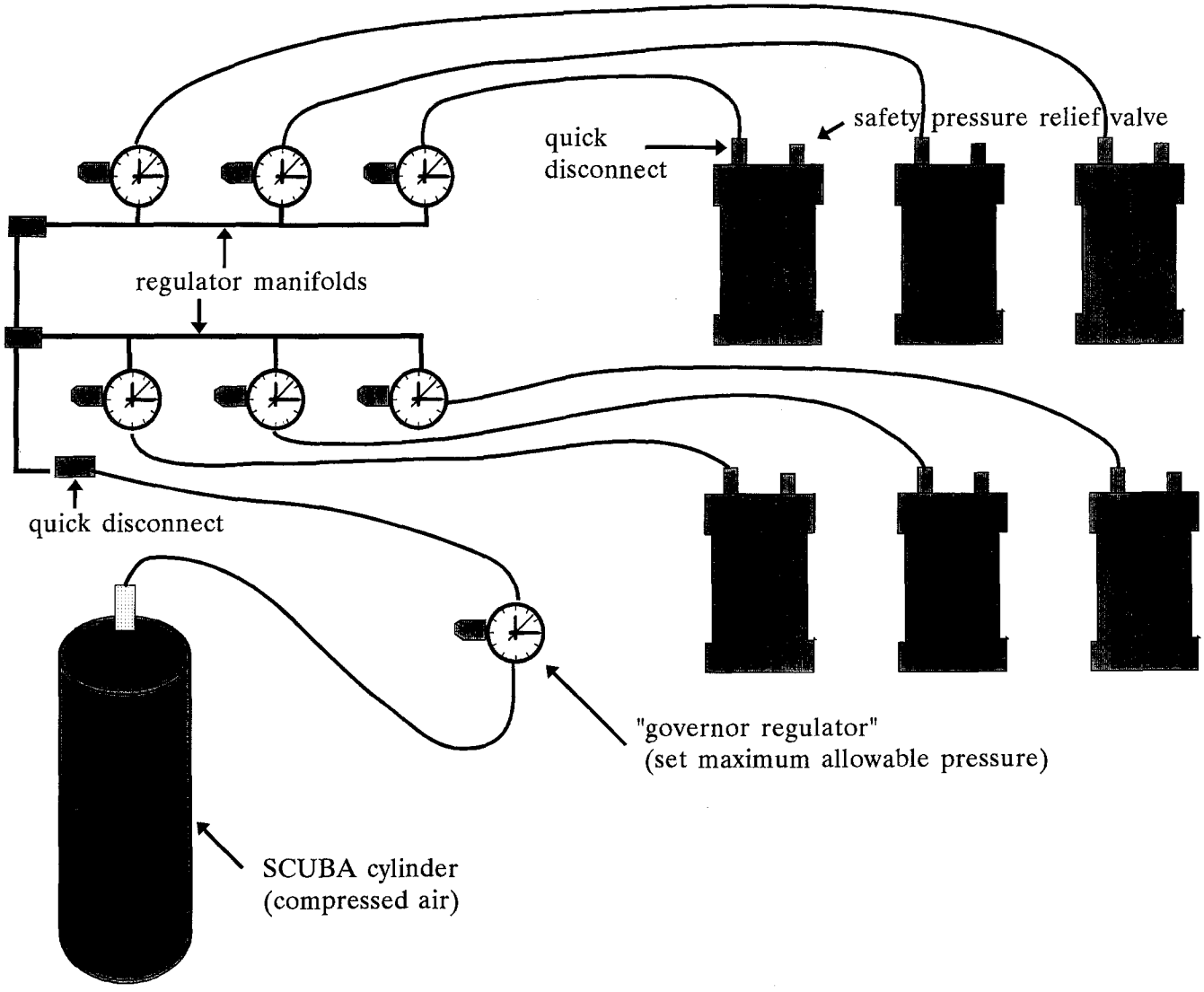

Fig. 2. Schematic diagram of porewater pressure extraction system

matrix of gametes from at least two females and two males with a minimum of three different sperm dilutions per male. This pre-test allowed us to pre-select a sperm:egg combination and ratio to optimize the sensitivity of the test. Sperm were exposed to the test treatments (5 replicates/treatment) for 30 min followed by the addition of a predetermined number of eggs. After an additional 30 min incubation period, the test was terminated by the addition of 10% buffered formalin and the percentage of eggs fertilized determined.

The sea urchin embryological development assay (Oshida *et al.* 1981; Pagano *et al.* 1982; Carr and Chapman 1992) was done in conjunction with the sperm cell test. The same sperm:egg ratio determined to be optimum in the pre-test was used in the embryological development test. The eggs were added first, followed as quickly as possible by the sperm. The embryos were exposed to the test treatments for 48 h at 20°C, at which time the test was terminated by the addition of 10% buffered formalin. Aliquots from each of the five replicates per treatment were examined microscopically to determine the percentage of embryos that had developed normally. A reference toxicant (sodium dodecyl sulfate) was included with each series of tests as a measure of gamete viability.

Statistical comparisons among treatments were made using ANOVA and Dunnett's one-tailed t-test (which controls the experimentwise error rate) on the arcsine square-root-transformed data with the aid of Statistical Analysis System (SAS 1989). The trimmed Spearman-Karber method with Abbott's correction was used to calculate EC_{50} and LC_{50} values for dilution series tests (Hamilton *et al.* 1977).

Results and Discussion

Extraction Techniques

The toxicity of pore water obtained with the Teflon® extractor using different filters and by centrifugation from subsamples of the same metal and PAH-contaminated sediment showed considerable difference among the various methods (Table 1). The centrifugation method and the use of the nylon filter (8 μm) produced the most toxic samples. This result was initially interpreted as indicating that the nylon filter was adsorbing less of the soluble contaminants than the other filter/filter-support plate combinations. Additional experiments (data not reported here) indicated that 24 h was not a sufficient period of time to remove all of the leachable toxicants from the nylon filters,

Table 1. Results of sea urchin fertilization test with *Arbacia punctulata* comparing the toxicity of pore water from a contaminated sediment (sand:silt:clay, 53:23:24) extracted with different filter types using the Teflon® extractor (including the Teflon® filter support plate unless designated otherwise) and a sample prepared by centrifugation

Filter type or treatment	Filter porosity (μm) relative to control	Percent fertilization (± SD)
Fluorocarbon filter	74	71.2 ± 9.4
Glass fiber filter (GF/F)	0.7	67.2 ± 14.0
Polyester filter	8	66.8 ± 14.9
Polyester filter without Teflon® filter support plate	8	52.0 ± 14.3
Teflon® filter support plate without any filter	—	46.4 ± 15.2
Centrifugation	—	38.8 ± 7.1
Nylon filter	8	25.2 ± 7.8

which were contributing to the higher toxicity of the nylon-filtered pore water in the comparison study. By soaking the nylon filter for 48 h in deionized water with a minimum of three water changes, the residual toxicity could be eliminated, and there was virtually no difference between the porewater samples obtained with the nylon and polyester filters. Therefore, the polyester (8 μm) filter was used for all of the other pressurized extractions in this study, except where noted otherwise. The higher toxicity of the centrifuged sample compared with polyester filter treatment may have resulted from the loss of soluble contaminants on the filter or may have been due to the higher suspended particulate content of the sample before it was frozen.

Because the initial prototype extractors constructed of Teflon® were awkward to use and expensive to construct, the use of extractors of a similar design constructed with PVC components was evaluated. A comparison between the Teflon® and PVC extractors indicated no difference in the toxicity of pore water obtained from an uncontaminated reference sediment (Table 2), indicating that no toxic material was leached from the PVC extractors during the extraction process. The use of GF/F filters without a filter support plate allowed glass fibers to be introduced into the porewater sample. The glass fibers (which were visible in the sample under the microscope) apparently produced artifactual toxicity in the sea urchin embryological development test (Table 2). The advantages and disadvantages of the various extraction techniques are summarized in Table 3.

Porewater Storage and Handling Experiments

The effect of freezing and thawing on the toxicity of pore water from 12 different sites with varying degrees and types of contamination was assessed for centrifuged (30 min at 4,200 × *g*) and pressurized extraction (PVC extractors with polyester filters) samples (Table 4). There was no appreciable difference for any of the water quality parameters between the fresh and frozen/thawed samples. For one of the 14 samples, the fresh-squeezed sample was more toxic than the frozen and thawed sample, and one squeezed frozen then thawed sample was statistically more toxic than the fresh sample. For four of the 14 centrifuged samples, freezing and thawing significantly increased the toxicity of the

Table 2. Comparison among different combinations of extractors and filter types for an uncontaminated reference sediment using the sea urchin embryological development assay with *Arbacia punctulata* to determine the toxicity of the various porewater samples

Extractor	Filter	Percent normal development (± SD)
Polyvinyl chloride (PVC)	Nylon	93.2 ± 4.7
Teflon®	Nylon	91.8 ± 4.4
Teflon®	GF/F with Teflon® filter support plate	80.4 ± 13.6
PVC	Nylon and GF/F without Teflon® filter support plate	29.4 ± 7.2

sample. Ten of 14 samples were significantly different in toxicity between the squeezing and centrifugation extraction methods. In nine of the ten samples where there was a significant difference between the centrifuged and squeezed samples, the centrifuged sample was more toxic. For sample #4, which exhibited the greatest difference between the fresh-squeezed and centrifuged pore water, the increased toxicity in the centrifuged sample was probably due to the presence of oil in the sediment, which is concentrated in the supernatant (emulsified oil droplets were visible) and is not removed by additional centrifugation.

The statistically significant increase in toxicity after freezing and thawing that was observed in four of the 14 centrifuged samples but in only one of squeezed sample is likely due to the considerably higher amount of particulate material remaining in the pore water extracted by centrifugation as compared with the squeezed sample (Figure 3). Higher amounts of particulate material were also observed in the pore water extracted from sandy sediments with the vacuum extraction method compared with the pressurized extraction technique (Figure 3). This fine suspended material cannot be reduced substantially by increasing the duration of the centrifugation during the initial extraction. It is likely that this phenomenon is due to the electrostatic repulsion of similarly charged particles. After recentrifuging the supernatant (or pore water obtained from squeezing or vacuum), the amount of suspended material retained on a 0.45-μm filter was negligible because the pellet of similarly charged particles was not present to keep the fine particulates in suspension. The samples used in these studies were estuarine (salinities ranged between 22‰ and 32‰), and electrostatically stabilized suspensions are more stable in solutions with low ionic strength (Everett 1988). Complete removal of this size of suspended particle (>0.45 μm) from freshwater samples may not be possible with similar centrifugation speeds. Rees *et al.* (1991) found that an increased electrostatic charge of colloidal particles decreased centrifugation efficiency. When the suspended material containing contaminant-sorbed particles is frozen, apparently some contaminants can be released back into solution after thawing. This would explain the increased toxicity in some of the frozen then thawed samples (Table 4), especially the centrifuged samples which had a higher suspended particulate concentration. (The frozen porewater samples were centrifuged after thawing, regardless of the original extraction method, so that this result is not related to the suspended particulate toxicity artifact discussed below.)

The effect of suspended particulates on the sea urchin fertilization test was demonstrated by a toxicity test comparison

Table 3. Pros and cons of different porewater extraction techniques

Extractor type	Pros	Cons
Centrifuge	1. Minimizes contact with surfaces, compared to other methods thereby minimizing adsorptive loss of contaminants 2. Majority of pore water can be extracted from clay sediments 3. By recentrifuging the supernatant (or by centrifuging pore water extracted by other methods), most suspended solid particles of greater than 0.45 μm may be removed from marine/estuarine pore water	1. Very little pore water can be collected from sandy sediments because sand does not compact easily. 2. Cleaning centrifuge tubes after porewater extraction from clay sediments is difficult and time consuming. Large centrifuge tubes are too expensive to be considered disposable. 3. Cannot easily be performed in the field. 4. Most expensive technique, in terms of initial equipment cost.
Vacuum	1. Least expensive method, in terms of equipment cost 2. All materials are disposable, which minimizes cleanup costs and reduces possibility of cross sample contamination 3. Can easily be performed in the field	1. Was least successful technique for removing particles of greater than 0.45 μm from pore water. 2. Extraction of pore water from clay samples may be prohibitively time consuming.
Pressure extraction	1. Can extract majority of the pore water from sediment, regardless of grain size. Best method for maximizing the quantity of pore water obtained from a limited quantity of sediment for studies with a wide variety of sediment textures 2. Was most successful method for removal of suspended solid particles of greater than 0.45 μm 3. Can easily be performed in the field	1. Extraction of pore water from clay sediments takes longer than with the centrifugation method. 2. Requires more initial start-up costs than the suction method but in the long run may be most cost effective for large scale programs. 3. More hydrophobic contaminants may be adsorbed by the filter and other surfaces as compared with the centrifugation methods.

Table 4. Summary of sediment extraction and storage experiments with contaminated sediments. Significant differences determined by a pair-wise t-test on the arc sine square root transformed data. Porewater samples were not centrifuged prior to freezing after the initial extraction

		Centrifuged		Squeezed	
Sample no.	Endpoint	Fresh	Frozen	Fresh	Frozen
1	% Fertilization (100% PW)	81.6[a]	87.6	93.2	90.8
2	% Fertilization (100% PW)	98.4[a]	82.0[b]	59.4[b]	96.0
3	% Fertilization (25% PW)	34.8[a]	16.8[a]	97.6	98.0
4	% Fertilization (100% PW)	0[a]	0[a]	97.0	93.8
5	% Fertilization (100% PW)	54.4[a]	35.6[a]	98.6	97.8
6	% Fertilization (100% PW)	2.2[a]	1.4[a]	26.2	16.8
7	% Normal development (25% PW)	0	0	0	0
8	% Normal development (100% PW)	98.0	93.6	98.0	97.6
9	% Normal development (100% PW)	95.4	90.0[a]	97.4	98.0
10	% Normal development (100% PW)	98.6	86.6[a,b]	99.0	97.8
11	% Normal development (100% PW)	96.0	96.8	98.2	97.0
12	% Normal development (100% PW)	88.0	35.0[a,b]	98.6	98.0
13	% Normal development (100% PW)	98.6	30.6[a,b]	99.0	83.6[b]
14	EC_{50} (% Normal development)	51.8	30.0	41.4	48.6

[a] Statistically significant difference between centrifuged vs. squeezed for either the fresh or frozen samples compared independently ($\alpha < 0.01$)
[b] Statistically significant difference between fresh vs. frozen for either the centrifuged or squeezed samples compared independently ($\alpha < 0.01$)

between fresh pore water extracted by the vacuum method before and after an additional centrifugation (Table 5). For four different non-toxic sediments, sea urchin fertilization success was significantly reduced in the uncentrifuged sample containing higher concentrations of suspended particulates.

Sediment Storage and Handling Studies

The results of several time-series studies indicate that the toxicity of pore water extracted from sediment stored at 4°C can change significantly during the recommended 2-week holding period (Table 6). This phenomenon has been observed previously with copper-spiked freshwater sediments (Malueg *et al.* 1986) and during longer holding periods with contaminated Great Lakes and river sediments (Othoudt *et al.* 1992). Othoudt *et al.* (1992), using reproduction in *Daphnia magna* as an end point, observed significant decreases in toxicity after sediments were stored refrigerated for 28 and 56 days (compared with the results after 14 days of storage), followed by a return to 14-day toxicity levels after 112 days of storage for several different sediments. They concluded that there was no significant trend due to holding time on the sediment toxicity because the data did not follow their preconceived expectations of linearity.

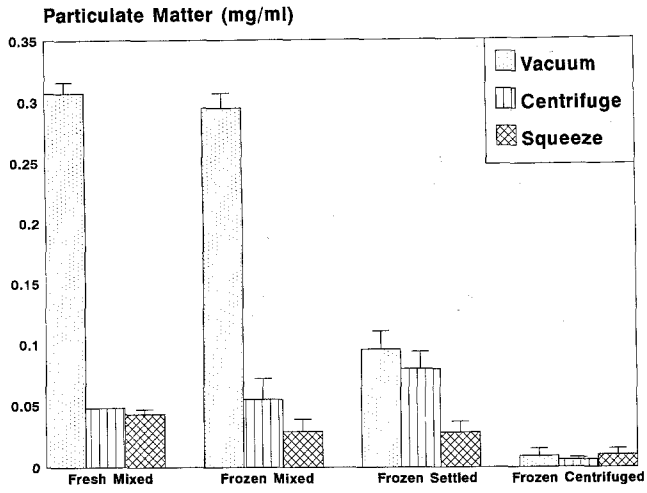

Fig. 3. Mean (±SD, n = 4) weight of particulate matter (≥0.45 μm) remaining in pore water (22‰ salinity) from sample B(sand:silt:clay, 52:17:31) after different extraction methods and manipulations

Table 5. Results of sea urchin fertilization tests with freshly extracted (by vacuum method) pore water containing different concentrations of suspended particulates before and after centrifugation. Asterisk denotes significant difference ($\alpha \leq 0.01$) as determined by Dunnett's t-test

Sample	Sand:silt:clay	Suspended particulates (mg/ml)	% fertilization
A	28:28:44	0.253	82.2*
A-cent.		0.003	97.0
B	52:17:31	0.307	58.4*
B-cent.		0.009	96.4
C	52:23:24	0.241	57.4*
C-cent.		0.002	98.0
D	26:51:23	0.235	21.4*
D-cent.		0.006	94.0

Table 6. Results of sea urchin fertilization test with *Arbacia punctulata* comparing the toxicity of pore water extracted from subsamples of the same sediment that was held at 4°C for varying lengths of time before the pore water was extracted

Holding time prior to extraction (days)	% fertilization (x ± SD)		
Experiment 1	**Sample 1**		
0	61.4 ± 10.8		
1	63.8 ± 8.8		
4	94.0 ± 3.0		
7	93.2 ± 1.9		
14	54.6 ± 17		
Experiment 2	**Sample 2**	**Sample 3**	**Sample 4**
1	90 ± 3.1	8.2 ± 7.7	40.2 ± 4.3
2	98.4 ± 0.9	41.4 ± 8.9	—
8	96.2 ± 2.2	10.8 ± 3.6	—
15	96.2 ± 1.6	43.0 ± 6.4	6.8 ± 0.8
22	91.2 ± 4.1	3.0 ± 1.6	97.4 ± 0.9
29	92.2 ± 3.5	6.4 ± 2.2	15.0 ± 3.8

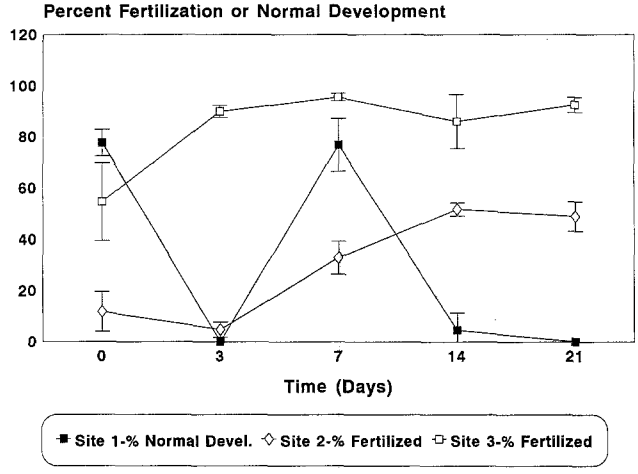

Fig. 4. Results of sea urchin fertilization tests with pore water extracted from sediments after varying lengths of time in a simulated amphipod solid-phase test

Although the toxicity of relatively uncontaminated sediments may not change significantly over time, it is not possible, at present, to predict the direction and magnitude of change over a period of weeks or even days for some sediments.

Related to these changes in the toxicity of sediment samples stored refrigerated is the issue of changes in the toxicity of sediments during the course of solid-phase toxicity tests. Pore water collected during a time-series experiment that simulated the experimental design of the standard amphipod sediment toxicity test (ASTM 1992) exhibited substantial changes in toxicity over a period of 3 weeks for the two more toxic sediments (Figure 4). One might predict that the toxicity of pore water, which is in the process of establishing a dynamic equilibrium with the sediment particles, microbial interactions, and overlying water, would change over time. As with the refrigerated storage experiment, the magnitude and direction of change for these sediments was unpredictable.

Conclusions and Recommendations

The results of our extraction method comparison studies indicate that the toxicity of porewater samples can be greatly influenced by the method used. If care is taken to minimize contact of the porewater sample with materials that strongly adsorb contaminants of concern, many different methods can be used with comparable results. No difference was found in the toxicity of pore water obtained with the Teflon® and PVC pressurized extraction devices. Where the primary contaminants of concern are highly hydrophobic organic compounds, centrifugation is the method of choice for maximizing the sensitivity of the test. Other researchers have reached similar conclusions (Word *et al.* 1987; Schults *et al.* 1992). Schults *et al.* (1992) have recently compared a variety of methods for collecting interstitial (pore) water for trace organic and metals analyses. Although the methods they evaluated were not directly comparable to the methods evaluated in the present study (except for centrifugation), they observed that the two hydrophobic organic compounds (fluoranthene and *p,p'*-DDE) showed the greatest variability and a significant loss during extraction for all the methods investigated.

Regardless of the method used for the initial extraction (be it pressurization, centrifugation, or vacuum), it is recommended that the sample be (re-) centrifuged prior to testing or freezing. If the sea urchin fertilization or embryological development toxicity tests is to be used, the samples should be centrifuged again after thawing to minimize artifactual responses resulting from precipitated suspended particulate matter in the sample. The presence of particulate material inhibits the ability of sperm to either locate or fertilize the eggs. If the above precautions are followed, the toxicity of the porewater samples will not be significantly affected by the freezing and thawing process.

Changes in the toxicity of contaminated sediments can occur during short-term (days, weeks) storage of sediments and during the course of a solid-phase toxicity tests. One of the advantages of the porewater test approach is that the pore water can be extracted and preserved frozen within a relatively short period after the sample is collected, thereby minimizing the effect of this variable when large numbers of samples collected over a period of weeks or months are being compared. The effects of sediment storage and sediment toxicity changes taking place during the course of a test need to be evaluated more thoroughly. The assumption that little or no change in the toxicity of the sediment takes place during the recommended maximum 2-week holding period, as currently practiced in the regulatory arena, warrants further scrutiny. Additional studies with spiked sediments and porewater chemical measurements in conjunction with toxicity tests are also warranted to further elucidate the differences among the various extraction methods.

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