POREWATER TOXICITY TESTING: A NOVEL APPROACH FOR ASSESSING CONTAMINANT IMPACTS IN THE VICINITY OF CORAL REEFS

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ABSTRACT

Coral reef communities can be deleteriously affected by exposure to low levels of anthropogenic contaminants. Sediments in the vicinity of coral reefs serve as a sink (and when resuspended, as a source) for contaminants and provide an integrative measure of low and intermittent exposure. Sediment porewater toxicity tests using gametes and embryos of the sea urchin Arbacia punctulata were employed to provide a measure of the presence of bioavailable contaminants. As sediments are patchy and composed predominantly of sand in reef areas, sediment samples were collected by divers using hand cores. The utility of this approach was assessed at coral reef sites in Hawaii and southeastern Mexico. Toxicity was observed at several reef sites off Waikiki Beach in Honolulu, Hawaii and at the Sian Ka’an Biosphere Reserve in Quintana Roo, Mexico. The results of these studies have demonstrated that porewater toxicity tests are sensitive enough to differentiate among sediments with low to moderate levels of contamination and would be a valuable tool for assessing and monitoring contaminant exposure in coral reef ecosystems.

Regional anthropogenic impacts on coral reefs, exacerbated by global climatic changes, can increase rates of local species extinction (Chadwick-Furman, 1996). Coral bleaching events have been associated with chemical contamination in addition to the well-studied bleaching effects resulting from temperature increases, UV radiation, and sedimentation (Meehan and Ostrander, 1997). Domestic and industrial effluents, fertilizers, oil pollution, drilling muds, sedimentation, and disease have been identified as some of the main problems affecting coral populations (Goenaga, 1988; Fishelson, 1995; Richardson, 1998; Dustan, 1999; Harvell et al., 1999). Several authors (Brown and Howard, 1985; Dubinsky and Stambler, 1996; Meehan and Ostrander, 1997; Dustan, 1999), have reviewed the effects of stress and of marine pollution on coral reefs. Richardson (1998) emphasized that an important aspect of coral disease and overall reef degradation is the effect of anthropogenic influence, and possibly of terrestrial runoff, suggesting that this area demands focused research. Dustan (1999) reported that in the Florida Keys coral diseases seem to be prevalent in areas close to centers of human habitation, and the potential influence of pollutants on coral reefs as facilitators of disease outbreaks was discussed by Harvell et al. (1999).

Different methods have been used to assess the presence and impacts of contaminants in coral reef areas. Chemical measurements were made to identify the presence of a variety of contaminants in coral annual growth bands (Law et al., 1994; Deslarzes et al., 1995; Guzman and Jarvis, 1996; Readman et al., 1996; Buesseler, 1997; Scott and Davies, 1997), in tissues of soft corals (Denton and Burdon-Jones, 1986), hard corals (Snedaker et al., 1999), and in sediments collected from the vicinity of coral reefs (Reichelt and Jones, 1994; Snedaker et al., 1995). Field assessments showed significant reduction in biodiversity in reefs subject to land-based pollution (sewage, industrial effluent, agricultural and aquacultural runoff and sedimentation) relative to unpolluted coral reefs (Edinger...
et al., 1998). Toxicity tests with different life stages of coral species, and with other phyla from the coral reef community, have also been developed (Kusmaro et al., 1994; Davies, 1995; Peachey and Crosby, 1995; Te, 1998). The assessment of bioindicator organisms, e.g., stomatopod and amphipod crustaceans, butterfly fish, and sea urchins, has been suggested for the analysis of the health of coral reefs (Erdmann, 1998; Thomas, 1993; Risk, 1994; Reese, 1995).

The sensitivity of both amphipods and echinoids to chemical contamination is well known, particularly for sediment and porewater toxicity assessments (Chapman, 1998; Carr and Chapman, 1992, 1995; Carr et al., 1996a,b; Nipper et al., 1998). Several studies have been conducted integrating pollution in sediments with uptake or impact on the coral reef biota. In Panama, concentrations of hydrocarbons in sediments were significantly correlated with coral injury and reduced growth up to 5 yrs after a major oil spill (Guzman et al., 1994). The mobilization of metals, caused by dredging activities in the vicinity of coral reefs in Australia, was analyzed by Reichelt and Jones (1994). High uranium concentrations were found not only in sediments, but also in corals, algae and seagrass of polluted sites from the Gulf of Aqaba (Abu-Hilal, 1994). Also in Florida, pesticides could be measured in sediments and in coral reef biota (Glynn et al., 1995). Sea-surface microlayer samples from the Florida Keys region exhibited adverse effects in toxicity tests with sea urchin (*Lytechinus variegatus*) and spotted seatrout (*Cynoscion nebulosus*) embryos (Rumbold and Snedaker, 1999). Likewise, the use of sensitive toxicity tests, e.g., echinoid early-life stage tests, with sediments from the vicinity of coral reefs could be applied to assess the presence of contaminants in toxic amounts in coral reef areas. Sediments serve as a sink (and when resuspended, as a source) for contaminants and provide an integrative measure of low and intermittent exposure. It has been suggested that the bioavailable fraction of contaminants in sediments is primarily associated with the porewater, and is controlled by a variety of factors which include total organic carbon (TOC) and acid volatile sulfides (AVS) (DiToro et al., 1991; Ankley et al., 1996). Sandy sediments typically contain low levels of organic carbon and therefore contaminants contained in them would be readily bioavailable for the benthic biota and would be easily dispersed into the water column in events of sediment resuspension by both, natural phenomena and dredging activities. Sediment toxicity can be assessed by a variety of methods, among which solid phase and porewater tests seem to be the most commonly employed. Porewater toxicity tests with sensitive life stages of marine species, e.g., echinoid gametes and embryos, were shown to be significantly more sensitive that the most common solid phase test using amphipods (Carr and Chapman, 1992; Carr et al., 1996a,b). In the current study, sediment porewater toxicity tests using gametes and embryos of the sea urchin *Arbacia punctulata* were employed with the objective of providing a measure of the presence of bioavailable contaminants. The use of sensitive life stages of echinoids for toxicity tests is considered particularly relevant in coral reef studies, given the importance of the sea urchin *Diadema* for the maintenance of healthy coral reef systems. The utility of this approach was assessed at coral reef sites in Hawaii and southeastern Mexico. The sampled areas were subject to contaminant inputs from sewage outfalls, and urban and agricultural storm water runoff.
MATERIALS AND METHODS

SEDIMENT SAMPLING.—Surficial sediment samples were collected from six stations (M1–M6) in the Sian Ka’an Biosphere Reserve, Quintana Roo, Mexico (Fig. 1), in May 1998, and from 14 stations in Hawaii (H1–H14); 12 stations were located in Honolulu, Oahu, and two on the Kona coast, Island of Hawaii (Fig. 2), during June 1998. Site H14, on the Kona coast, was selected as a reference site on an apparently pristine location, while site 13 was adjacent to the Kona Airport.

Sediment cores (10 cm deep) were collected by divers using polycarbonate corers (6 cm diameter × 20 cm length), placed in presoaked half-gallon high density polyethylene containers, chilled, and shipped to the U. S. Geological Survey (USGS) Marine Ecotoxicology Research Station (MERS) in Corpus Christi, Texas, in insulated coolers with blue ice. Samples were received by the USGS in Corpus Christi, Texas, 2 d after shipment and were processed immediately upon receipt.

SEDIMENT POREWATER EXTRACTION PROCEDURE AND QUALITY MEASUREMENTS.—Pore water was extracted from the sediments using a pneumatic extraction device (Carr, 1998). This extractor is made of polyvinyl chloride (PVC) and uses a 5 mm polyester filter, and has been used successfully in numerous previous sediment quality assessment surveys (Carr and Chapman, 1992, 1995; Carr et al., 1996a,b). After extraction, the porewater samples were centrifuged in polycarbonate bottles at 1200 \( \times g \) for 20 min to remove any suspended particulate material; the supernatant was collected and frozen at \(-20^\circ C\).

Prior to toxicity tests, the samples were thawed in a tepid (20°C) water bath. Sample salinity was measured with a Reichert® refractometer and, if necessary, adjusted to 30 ± 1 ‰ using purified deionized water or concentrated brine prepared by slow evaporation of seawater. Temperature and dissolved oxygen (D.O.) were measured with YSI® meters, and D.O. was adjusted by gently stirring the sample if the measured concentration was <80% saturation. The water quality adjusted samples were used to prepare further test dilutions. Sulfide (as S\(^{-2}\)), total ammonia (expressed as nitrogen), and pH were measured with Orion® meters and their respective probes. Un-ionized ammonia (expressed as nitrogen) concentrations were calculated for each sample based on the respective pH and total ammonia values, and on test salinity (30‰) and temperature (20°C).

POREWATER TOXICITY TESTING WITH SEA URCHINS.—Porewater toxicity was assessed using the sea urchin (Arbacia punctulata) fertilization and embryo development tests, following the methodology described in Carr and Chapman (1992) and Carr et al. (1996a).

The sea urchin fertilization test involved exposure of the sperm to test media 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 fertilized eggs was determined. The sea urchin embryological development test was executed concurrently with the fertilization test. Eggs were pre-fertilized and then inserted into the exposure vials. The embryos were exposed to the test solutions for 48 h, after which time the test was terminated by the addition of 10% buffered formalin. Aliquots from each of the five replicates were examined microscopically to determine the percentage of embryos that developed normally to the echinopluteus stage.

A. punctulata urchins used in this study were obtained from Gulf Specimen Company, Inc. (Pana- cea, Florida). Each of the porewater samples was tested in a dilution series design at 100, 50, and 25%, with five replicates per treatment. Dilutions were made with 0.45 μm filtered seawater. A reference porewater sample collected from Redfish Bay, Texas, which had been handled identically to the test samples, was included with each toxicity test as a negative control. This site is far removed from any known sources of contamination and has been used previously as a reference site (Carr and Chapman, 1992, 1995; Carr et al., 1996a,b). In addition, a dilution series test with sodium dodecyl sulfate (SDS) was included as a positive control.

SEA URCHIN TOXICITY TESTING DATA ANALYSIS.—Statistical comparisons among treatments were made using ANOVA and Dunnett’s one-tailed t-test (which controls the experiment-wise error rate) on the arcsine square root transformed data with the aid of SAS® (SAS, 1989). Prior to statistical analysis, the transformed data sets were screened for outliers (SAS, 1992). Outliers were detected...
Figure 1. Sample sites in Mexico. Color and pattern differentiation of symbol indicates that toxic effects in those stations were significantly different from the reference in the sea urchin (*Arbacia punctulata*) fertilization and embryological development tests (Dunnett’s *t*-test, $\alpha \leq 0.05$ and $\alpha \leq 0.01$, and detectable significance criteria applied).
by comparing the Studentized residuals to a critical value from a t-distribution chosen using a Bonferroni-type adjustment. The adjustment is based on the number of observations, n, so that the overall probability of a type I error is at most 5%. The critical value, cv, is given by the following equation: 

\[ cv = t(df_{error}, 0.05/(2 \times n)) \]

After omitting outliers but prior to further analysis, the transformed data sets were tested for normality and for homogeneity of variance using SAS/LAB® Software (SAS, 1992).

A second criterion was also used to compare test means to reference means. Detectable significance criteria (DSC) were developed to determine the 95% confidence values based on power analysis of all similar tests performed by our lab (Carr and Biedenbach, 1999). This value is the percent minimum significant difference from the reference that is necessary to accurately detect a difference from the reference. The DSC value for the sea urchin fertilization assay is 15.5% at \( \alpha = 0.05 \), and 19% at \( \alpha = 0.01 \). For the embryo development assay, the DSC value is 16.4% at \( \alpha = 0.05 \), and 20.6% at \( \alpha = 0.01 \). Only results that were significantly different from the reference sample and were below DSC were considered significantly toxic.

The EC_{50} (effective concentration to 50% of the organisms) of the reference toxicant (SDS) tests was calculated using the trimmed Spearman-Karber method (Hamilton et al., 1977) with Abbott’s correction (Morgan, 1992).

**RESULTS**

**POREWATER QUALITY AND SEDIMENT CHARACTERIZATION.—**Porewater quality measurements are presented on Table 1. Salinity of the pore water from the stations in Southeastern Mexico was 38‰ in the coral reef samples (M4–M6) (Fig. 1), and ranged from 22 to 30‰ in the lagoon samples (M1–M3). Stations M1 and M2 had low initial porewater D.O. concentrations (67.8 and 51.6%, respectively) and relatively high sulfide (1.4 and 3.5 mg L^{-1}, respectively). These samples were gently stirred until D.O. concentration reached >80% saturation prior to toxicity testing. The other four samples (M3–M6) had D.O. above 80% and sulfide concentration below detection limit (<0.01 mg L^{-1}). Values for pH in the six samples ranged from 7.02 to 7.61, being higher (8.04) in the reference pore water from Redfish Bay, TX. Total ammonia concentrations ranged from 0.34 to 5.02 mg L^{-1}, and un-ionized ammonia ranged from 3.64 to 38.06 \( \mu g \) L^{-1}.

No grain size analyses were conducted with the samples from Mexico, but visual and tactile observation revealed that sediments varied from very soft mud inside the lagoon system, to coarse white sand in the vicinity of the coral reef.

The salinity of all samples from Hawaii (Fig. 2), presented in Table 1, was 36‰, and salinity adjustments were necessary to satisfy the test salinity requirement of 30 ± 1‰. Sulfide concentrations were below the detection limit of 0.01 mg L^{-1} in all samples (H1–H14), and D.O. concentrations were high in all samples, ranging from 93.4 to 105.8%. Values for pH ranged from 7.57 to 7.86, slightly lower than in the reference pore water (8.04). Total ammonia concentrations ranged from 0.07 to 1.29 mg L^{-1}, and un-ionized ammonia ranged from 0.86 to 24.84 \( \mu g \) L^{-1}.

The un-ionized ammonia lowest observed effect concentration (LOEC) values for the *A. punctulata* sea urchin fertilization test (800 \( \mu g \) L^{-1}) and embryo development test (90 \( \mu g \) L^{-1}) (Carr et al., 1996b) were not exceeded in any of the samples from Mexico or Hawaii. Therefore, ammonia was not considered to be responsible for any of the observed toxic effects.

Grain size analyses done with the samples from Hawaii revealed sandy sediments (>99.5% sand), except for the samples from Honolulu Harbor (H1 and H2) and from the vicinity of the coral reef.
Table 1. Pore water quality parameters and grain size distribution for samples from Mexico and Hawaii. D.O., dissolved oxygen; NH$_4^+$ + NH$_3$, total ammonia; NH$_3^-$, unionized ammonia; Ref., reference porewater from Redfish Bay, Texas; ND, not determined.

<table>
<thead>
<tr>
<th>Station</th>
<th>Salinity (%)</th>
<th>D.O. (%)</th>
<th>pH</th>
<th>NH$_4^+$ + NH$_3$ (mg L$^{-1}$)</th>
<th>NH$_3^-$ (μg L$^{-1}$)</th>
<th>Sulfide (mg L$^{-1}$)</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref.</td>
<td>26</td>
<td>92.0</td>
<td>8.04</td>
<td>0.542</td>
<td>18.70</td>
<td>&lt;0.01</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mexico</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>29</td>
<td>67.8</td>
<td>7.37</td>
<td>5.020</td>
<td>38.06</td>
<td>1.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M2</td>
<td>30</td>
<td>51.6</td>
<td>7.33</td>
<td>0.956</td>
<td>6.61</td>
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<td>M3</td>
<td>22</td>
<td>97.4</td>
<td>7.57</td>
<td>1.840</td>
<td>22.01</td>
<td>&lt;0.01</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M4</td>
<td>38</td>
<td>94.8</td>
<td>7.53</td>
<td>0.884</td>
<td>9.65</td>
<td>&lt;0.01</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>M5</td>
<td>38</td>
<td>101.0</td>
<td>7.61</td>
<td>0.341</td>
<td>4.47</td>
<td>&lt;0.01</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M6</td>
<td>38</td>
<td>89.1</td>
<td>7.02</td>
<td>1.070</td>
<td>3.64</td>
<td>&lt;0.01</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Hawaii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1</td>
<td>36</td>
<td>99.2</td>
<td>7.78</td>
<td>1.290</td>
<td>24.84</td>
<td>&lt;0.01</td>
<td>22.9</td>
<td>32.7</td>
<td>44.4</td>
</tr>
<tr>
<td>H2</td>
<td>36</td>
<td>102.4</td>
<td>7.79</td>
<td>0.968</td>
<td>19.07</td>
<td>&lt;0.01</td>
<td>58.3</td>
<td>30.1</td>
<td>11.6</td>
</tr>
<tr>
<td>H3</td>
<td>36</td>
<td>101.0</td>
<td>7.68</td>
<td>0.490</td>
<td>7.53</td>
<td>&lt;0.01</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H4</td>
<td>36</td>
<td>94.9</td>
<td>7.82</td>
<td>0.113</td>
<td>2.38</td>
<td>&lt;0.01</td>
<td>99.7</td>
<td>0.1</td>
<td>0.2</td>
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<tr>
<td>H5</td>
<td>36</td>
<td>99.8</td>
<td>7.86</td>
<td>0.641</td>
<td>14.78</td>
<td>&lt;0.01</td>
<td>99.5</td>
<td>1.2</td>
<td>0.3</td>
</tr>
<tr>
<td>H6</td>
<td>36</td>
<td>103.1</td>
<td>7.60</td>
<td>1.150</td>
<td>14.73</td>
<td>&lt;0.01</td>
<td>99.2</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>H7</td>
<td>36</td>
<td>93.4</td>
<td>7.83</td>
<td>0.109</td>
<td>2.35</td>
<td>&lt;0.01</td>
<td>99.6</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>H8</td>
<td>36</td>
<td>94.5</td>
<td>7.58</td>
<td>0.445</td>
<td>5.45</td>
<td>&lt;0.01</td>
<td>36.3</td>
<td>45.9</td>
<td>17.8</td>
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<tr>
<td>H9</td>
<td>36</td>
<td>99.9</td>
<td>7.57</td>
<td>1.290</td>
<td>15.43</td>
<td>&lt;0.01</td>
<td>99.7</td>
<td>1.1</td>
<td>0.2</td>
</tr>
<tr>
<td>H10</td>
<td>36</td>
<td>101.8</td>
<td>7.70</td>
<td>0.074</td>
<td>1.19</td>
<td>&lt;0.01</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H11</td>
<td>36</td>
<td>95.9</td>
<td>7.75</td>
<td>0.228</td>
<td>4.10</td>
<td>&lt;0.01</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H12</td>
<td>36</td>
<td>97.8</td>
<td>7.58</td>
<td>0.070</td>
<td>0.86</td>
<td>&lt;0.01</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H13</td>
<td>36</td>
<td>105.8</td>
<td>7.78</td>
<td>0.237</td>
<td>4.56</td>
<td>&lt;0.01</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H14</td>
<td>36</td>
<td>103.3</td>
<td>7.71</td>
<td>0.929</td>
<td>15.27</td>
<td>&lt;0.01</td>
<td>99.9</td>
<td>0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Ala Wai canal (H8) (Table 1), which ranged from 22.9 (H1) to 58.3% sand (H2), with total silt and clay up to 77.1% (H1).

**SEA URCHIN TOXICITY TESTING.**—Toxicity test results with samples from Mexico are summarized in Figure 1. None of the samples was toxic in the urchin fertilization test. In the more sensitive embryological development test, toxicity was exhibited in the undiluted porewater of all samples except M5. Samples M1, M2, and M6 were also toxic in a 50% dilution of the pore water. The most toxic sample was from station M2, still showing significant toxicity at a 25% concentration.

Toxicity test results for the samples from Hawaii are summarized in Figure 2. Station H14, selected as a reference station, was not toxic. The samples from stations H1, H2, and H13 were significantly toxic in the fertilization test at 100% sample. Data for station H1 and H13 were below the DSC at a $\alpha \leq 0.05$ and for station H2 it was below the DSC at a $\alpha \leq 0.01$. In the embryological development test, the samples from stations H1, H5, H9, H11, and H12 were significantly toxic (below DSC at a $\alpha \leq 0.01$) at 100% sample. Station H12 was the most toxic, still exhibiting significant toxicity (below DSC at a $\alpha \leq 0.01$) in the 50% dilution of the sample.
Figure 2. Sample sites in Honolulu. Color and pattern differentiation of symbols indicates that toxic effects in those stations were significantly different from the reference in the sea urchin (Arbacia punctulata) fertilization and embryological development tests (Dunnett’s t-test, α = 0.01, and detectable significance criteria applied).
The mean fertilization rate and mean percent normal developed embryos in the reference sample from Redfish Bay, Texas, was 94 and 93.7%, respectively. Control reference toxicant (sodium dodecyl sulfate) results showed that the sensitivity of the batch of organisms used for these tests was within the norm, with EC$_{50}$ values of 6.01 mg L$^{-1}$ (5.66–6.38) in the fertilization test and 4.93 mg L$^{-1}$ (4.56–5.33) in the embryo development test.

**DISCUSSION**

The relatively high levels of sulfide and low D.O. in the pore water from the soft lagoon sediments (stations M1 and M2) suggest a high organic load at those sites. The toxicity of sulfides to gametes and embryos of *A. punctulata* has not been assessed, but studies with *Strongylocentrotus purpuratus* resulted in an LOEC of 0.09 mg L$^{-1}$ total sulfides (Knezovich et al., 1996). The results with *S. purpuratus* suggest that the sulfide levels in samples M1 and M2 (1.4 and 3.5 mg L$^{-1}$, respectively, Table 1) may have contributed to their observed toxicity (Fig. 1) to *A. punctulata* embryos.

Some of the sediment properties that enhance sorption of contaminants include organic carbon content, particle size distribution, and clay type and content. Clay contributes significantly to sorption, therefore reducing contaminant bioavailability, and organic carbon is considered as a major factor because it can both sorb organic chemicals and complex metals (Landrum and Robbins, 1990). The desorption of sediment-sorbed organic chemicals is mediated by interstitial water (Knezovich et al., 1987), and the same would be expected to be true for other categories of contaminants. Based on these facts, it is generally believed that sandy sediments do not retain contaminants. However, even if they do not retain high amounts of contaminants, those chemicals that are retained are expected to readily desorb to the pore water of sandy sediments, due to their low level of clay and typically low organic carbon content.

The results from station M4–6 and H3–13 (Figs. 1 and 2) (except H8, which was a muddy sediment from Ala Wai canal yacht harbor, Table 1) showed toxicity of the pore water from several sandy sediments to sea urchin gametes and/or embryos, suggesting the presence of bioavailable contaminants. Sample H8 was not toxic, although the levels of contaminants in Ala Wai canal would be expected to be high based on past studies. Sediments from Ala Wai canal were described as rich in organic carbon (ca 1–8%) (Glenn et al., 1995), containing Pb, Cd, Cu and Zn from anthropogenic and natural sources (De Carlo and Spencer, 1995; Spencer et al., 1995), and elevated levels of mercury particularly in the Yacht Harbor, possibly from antifouling paints (0.054–2.810 µg g$^{-1}$) (Raine et al., 1995). Although experimental sediments from Ala Wai canal exhibited high concentration of mercury, the interstitial water did not contain elevated levels of biologically available mercury (Luoma, 1977). Based on these data contaminants present nowadays in the sediment from Ala Wai canal (H8) would not be expected to desorb into the pore water, explaining the lack of toxicity of that sample despite possible high contamination of the sediment. Most of the contaminants measured in past studies were described as entering Ala Wai canal via stormwater runoff. Contaminants entering the system with storm water could also be distributed by marine currents to areas farther from the coast, near the coral reef surroundings analyzed in this study, where they would be more readily bioavailable due to the characteristics of the sediments.

It was pointed out by Dubinsky and Stambler (1996) that the expansion and intensification of agriculture leads to the exposure of increasing areas of coral reefs to runoff con-
taining pesticides. Coral planulae have shown increased mortality when exposed to organochlorinated pesticides (Acevedo, 1991), and abnormalities and disease in coral colonies were attributed to the presence of pesticides and heavy metals (Glynn et al., 1989). Although chemical analyses of the sediment and porewater samples were not conducted as part of this study, marine sediments in the Sian Ka’an Biosphere Reserve, Mexico (Fig. 1), have been observed to be contaminated by organochlorinated pesticides (Gerardo Gold-Bouchot, Department of Marine Resources, Center for Research and Advanced Studies at Mérida, Yucatán, Mexico, pers. comm.).

Scott and Davies (1997) detected significantly elevated presence of heavy metals in corals collected in the vicinity of a small airstrip on Matan Island, Sele Straits, Indonesia, suggesting the input of such contaminants due to airport activities. This might help explaining the toxic effect from station H13, in the vicinity of the Kona airport (Fig. 2).

We do not have information on the levels of contaminants in the vicinity of Hawaiian coral reefs, but some of the samples that showed toxicity were collected near sewage outfalls and dredged channels (H5 and H9). Grigg (1995) reported that raw sewage discharges before 1977 had a serious but localized impact on the coral reefs of Mamala Bay, bordering the southern shore of Oahu and the city of Honolulu, Hawaii, but that the reef recovered from the sewage impact when the treatment was upgraded to an advanced primary system. Neither effects of point nor of non-point source pollution could be detected on coral calcification, growth, species composition, diversity or community structure in 1993–94 (Grigg, 1995). However, the porewater toxicity tests conducted in the same area resulted in toxic effects of the samples from stations H5, H9, H11, and H12 (Fig. 2), suggesting the presence of contaminants.

The use of echinoid gametes and embryos is of particular significance for coral reef studies, since sea urchins play an essential role in coral reef communities. The importance of the sea urchin *Diadema antillarum* for the regulation of coral reef communities is well known (Rivera and Vicente, 1977; Sammarco, 1980; Hughes et al., 1985, 1987; Van Stevenick and Bak, 1986; Carpenter, 1988). The reasons for observed mass mortalities of *D. antillarum* in several areas of the Caribbean Sea, e.g., Curaçao (Bak et al., 1984), U.S. Virgin Islands (Carpenter, 1990a,b), Jamaica (Liddell and Ohlhorst, 1986) are not fully understood. The mass mortality in Jamaica was attributed to disease (Liddell and Ohlhorst, 1986), and the event in Curaçao was reported to have spread from the mouth of the harbor advancing along the coast in the down-current direction (Bak et al., 1984). In the U.S. Virgin Islands, the recruitment of *D. antillarum* larvae was low over a period of 2 yrs after the mass mortality and population densities remained at 2 to 30% of their premortality levels (Carpenter, 1990a). Although disease has been suggested as the main cause of *D. antillarum* mass mortality, pollution should not be ruled out as a contributing factor, both by enhancing susceptibility of the urchins to disease, and by affecting their embryos and preventing larval recruitment. The occurrence of mortality down current of the harbor in Curaçao suggests that pollution could be a contributing factor.

Coral reefs in American Samoa are severely damaged as a result of both natural disasters and pollution. There is concern that human disturbances adversely impact the natural recovery processes of coral reefs affected by natural disasters (Craig et al., 1995). Risk (1994) suggested the use of low-tech methods for the assessment of health of coral reefs. The methods suggested include community surveys, including the assessment of indicator species such as polychaetes, tunicates, sponges, sea urchins and some species of fish, and determination of coral growth rates. The use of the butterfly fish as a bioindicator was
suggested for the development of an early warning system which would serve to detect low-level, sublethal changes and therefore act as an identifier of stress within the coral reef system (Reese, 1995). We hereby suggest that the use of porewater toxicity tests using sea-urchin gametes and embryos can serve as a non-invasive technique to be used as an early warning system for the assessment of contamination and potential pollution impacts in coral reef areas. Ideally, such studies should be conducted using gametes and embryos of a tropical sea-urchin inhabiting coral reef environments, although this should not be considered essential for these assessments. Gametes and embryos of several echi-noid species occurring from tropical areas of the Southern Atlantic, to temperate areas of the Northern Atlantic and Southern and Northern Pacific, showed similar sensitivity to a variety of contaminants (Nacci et al., 1986; Nipper et al., 1993, 1997). This indicates their usefulness for toxicity assessments worldwide.

The results from the present study have demonstrated that porewater toxicity tests are sensitive enough to differentiate among sandy sediments with low to moderate levels of contamination. It is also important to use more than one kind of test, since chemicals with different modes of action can affect different organisms and life stages in different manners. This became clear with the present data, where the embryo development was the most sensitive endpoint with most toxic samples, but two Hawaiian samples were toxic to fertilization only. Sediment-related effects are not necessarily restricted to the benthic biota, since pelagic and planktonic organisms may be affected by chemicals that desorb from sediments into the water column (Knezovich et al., 1987). Therefore, porewater toxicity tests with sediments from the vicinity of coral reefs would be a valuable tool for assessing and monitoring contaminant exposure in coral reef ecosystems.

**Conclusions**

The use of toxicity tests with pore water extracted from sand cores was an effective method of assessing the presence of sediment contamination in coral reef areas, and would be an adequate early-warning system for pollution assessments in such areas.

The toxic effects caused by pore water from sandy sediments indicates not only the high bioavailability of chemicals in sand, which would be expected, but also that contaminants are in fact retained in sandy bottoms.

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**Literature Cited**


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