Burrowing responses of the short-neck clam *Ruditapes philippinarum* to sediment contaminants

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**Abstract**

The burrowing responses of a common tropical bivalve, the short-neck clam *Ruditapes philippinarum*, to cadmium (Cd)-spiked sediment, variations of sediment grain size and natural sediments collected from 15 locations in Hong Kong’s inshore waters were investigated through a series of laboratory tests. Results showed that the burrowing response exhibited a negative relationship with an increase in Cd concentration in the spiked sediments. The level of Cd was also found to be directly proportional to the percent mortality of the clam. However, there was no significant difference (*p > 0.05*) in the time elapsed for the clam to burrow into sediments with different grain size composition. The elapsed time for 50% of the test clams to burrow into the sediment (ET50) over a period of 48 h was calculated for the sediment samples collected from the 15 locations. Results of ANOVA showed significant difference (*p < 0.05*) among the sediment samples. Tukey’s multiple comparison test revealed two groups of sediments: group 1 containing 3 sediment samples collected from Victoria Harbour and group 2 containing 12 samples obtained from other coastal areas of Hong Kong. The ET50 value for group 1 sediments was found to be greater than 2880 min whilst the ET50 for group 2 sediments had a mean of 173.9 min. Agglomerative classification of the sediment samples, based on metal content (As, Cd, Cr, Cu, Ni, Pb, Zn), also showed two similar groups, suggesting that the ET50 values were correlated with the metal level of the sediment samples. Group 1 sediments collected from Victoria Harbour had greater concentrations of Cd, Cr, Cu, Ni and Zn as compared to that in group 2. The present study demonstrated that high metal content in the sediment tends to inhibit the burrowing responses of the clam and that there is potential to develop the burrowing responses of *R. philippinarum* as a sublethal sediment toxicity test.

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**Keywords:** Bivalves; Burrowing responses; Metal; Sediment toxicity

**1. Introduction**

Sediment toxicity testing is an important step in assessment of the quality of dredged materials prior to their disposal at sea. Regulatory authorities need to determine the limits of acceptable biological responses to chemical contamination as part of the assessment process. In this regard, many ecotoxicological methods are described in the literature, using various test species, which may be applied in evaluating if an observed impact on benthic organisms is due to chemical contamination (e.g., Chapman and Morgan, 1983; Environment Canada, 1992; Dillon et al., 1993; ASTM, 1995a,b; Hooten and Carr, 1998; Moraes et al., 2000). Bivalves have been used as one of the common test animals in sediment toxicity testing and various end points have been investigated including mortality (Cheung and Wong, 1993; Naimo et al., 2000), bioaccumulation of contaminants (Thompson et al., 2000), larval development (Chapman et al., 1992; van den Hurk, 1994), histopathological changes (Bowmer et al., 1994) and behavioural responses (Olla and Bejda, 1983; Phelps, 1989; Roper and Hickey, 1994; Ruiz et al., 1994). In Hong Kong, sediment toxicity testing is still in its infancy and the requirements of biological testing for dredged sediments prior to their disposal at sea were only promulgated in last year (EPD, 2000). At present, there is no standard sediment toxicity testing protocol using local species as the test organisms in Hong Kong, and biological testing largely relies on the use of species imported from overseas. This is in contrast to the current trend in ecotoxicological research in which one of the main aims is to assess the most appropriate species for specific areas of concern by using indigenous species (Connell et al., 1999).

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The short-neck clam, *Ruditapes philippinarum* (Adams & Reeve), is widely distributed intertidally and subtidally in the Indo-Pacific region (Huang, 1994). In Hong Kong, this clam can be found in large abundance (> 200 m⁻²) on sandy shores (Lee, 1996) and in muddy sand bottom (Shin, unpublished data). Apart from its dominance in tropical waters, *R. philippinarum* is amenable to laboratory conditions (Cheung and Wong, 1993), making it an ideal species in biological testing. The purpose of this study is to investigate the burrowing responses of *R. philippinarum* in response to metal contamination and explore the potential of developing such an end point as a sublethal sediment toxicity test.

2. Materials and methods

Individual adults of *R. philippinarum* of 3–4 cm shell length were collected from the lower shore of a sandy beach and acclimated in the laboratory for 3 d prior to being used for the following experiments.

2.1. Test on dose-response

A 48-h test on the mortality and burrowing response of the short-neck clam was carried out using cadmium-spiked sediment. Prior to the experiment, sediment from the field where the clams were collected was sieved through a 2 mm mesh, to remove large shells or particles. Solution of cadmium chloride was added onto the sieved sediment with nominal concentrations of 0.56, 1.80, 5.60, 18.0, 36.0 mg Cd kg⁻¹ sediment (wet weight) and mixed thoroughly using an electric blender. A series of three replicates of test tanks (25 × 14 × 14 cm³) were set up for each Cd concentration, each containing 5-cm layer of the cadmium-spiked sediment overlying with 2 l of aerated seawater. In each replicate, 10 *R. philippinarum* were placed on the surface of the sediment and the number of the clams that completely burrowed into the sediment was recorded according to the following scheme modified from Phelps et al. (1983): 0–1 h at 10 min interval, 2–3 h at 30 min interval, 4–9 h at 90 min interval, and at 1440 and 2880 min, respectively. Mortality of the test clams was also recorded at the same time. In addition to the cadmium-spiked sediment, a negative control with only natural sediment was established. The experiment was conducted in an environmental chamber with temperature at 20 °C, salinity 33‰, dissolved oxygen 6.5 mg l⁻¹, and 12:12 h L/D cycle. The actual concentration of the cadmium in the natural and spiked sediments was analyzed by digesting 2 g each of freeze-dried sediment samples in a mixture (2:1) of concentrated nitric and hydrochloric acids at 100 °C for 1 h. The digested mixture was then filtered and diluted to 50 ml for metal determination using Plasma Emission Spectrometry (Perkin–Elmer model 1000/2000). All analyses were carried out with three replicates of each sediment sample. Certified reference sediment (Buffalo River Sediment SRM2704 from National Institute of Standards and Technology, USA) was used for quality control of the metal analysis.

2.2. Test on grain size variation

A 48-h test of the burrowing response of the short-neck clam in sediments with different grain size composition was studied. In this experiment, sediment from the field where *R. philippinarum* were collected was air-dried for 2 weeks and separated into different size fractions by sieving: coarse sand (2000–500 µm), medium sand (500–250 µm), fine sand (250–62 µm) and silt (< 62 µm). A series of 4 test sediments with different grain size composition was produced by mixing the above sediment fractions in appropriate proportions (Table 1). These test sediments plus the control (natural sediment) were used in the burrowing response experiment similar to the Dose-Response test. Experimental conditions were also kept constant.

2.3. Test on field sediments

Sediments sampled from 15 locations in Hong Kong coastal waters (Fig. 1) were used in a 48-h test on the burrowing response of the short-neck clam. The experimental procedures were similar to the Dose-Response Test. A control was also set up using the natural sediment where *R. philippinarum* were collected. The elapsed time (ET₅₀) for 50% of the test clams completely burrowed in the sediments was recorded. In addition, the level of seven trace metals (As, Cd, Cr, Cu, Ni, Pb and Zn) in the field sediments was analyzed using acid digestion and Plasma Emission Spectrometry at the start and end of the experiment. Quality control of the metal analyses was similar to that in the Dose-Response Test.

2.4. Data treatment

In the Dose-Response Test, mortality of the short-neck clam in the presence of Cd was expressed as the LC₅₀ (lethal concentration killing 50% of the exposed organisms) whereas inhibition of the burrowing behaviour of the clam was expressed as the IC₅₀ (inhibition concentration preventing 50% of the exposed organisms

<table>
<thead>
<tr>
<th>Sediment (%)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coarse sand</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>70</td>
<td>60</td>
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<td>20</td>
</tr>
<tr>
<td>Fine sand</td>
<td>10</td>
<td>70</td>
<td>10</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Silt</td>
<td>70</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>
to burrow) using probit analysis by the software ToxCalc (TidePool, 1996). In the Grain Size Variation Test, individuals completely burrowed within 48 h were expressed as percentage of the total test organisms and difference among the sediments was analyzed using one-way ANOVA. The percent data were arcsine transformed prior to the analysis. In the Field Sediments Test, the difference in ET₅₀ for the field sediments was analyzed using one-way ANOVA followed by Tukey’s multiple comparison test (Zar, 1996). The trace metal content in the field sediments was also analyzed by classification technique (Tabachnick and Fidell, 1996), using Euclidean distance as a correlation measure of the trace metal content and unweighted pair-group average sorting method by the software STATISTICA (StatSoft, 1995).

### 3. Results

In the Dose-Response Test, the mean measured Cd concentration for the cadmium-spiked sediments analyzed at the start and end of the experiment was 0.41, 1.31, 3.13, 4.87 and 7.24 mg Cd kg⁻¹ dw (dry weight), respectively. There was no cadmium level found in the controlsedimentcollectedfromthefieldwhere *R. philippinarum* were used for the test. The percent recovery of Cd from the certified reference material was 105.1 ± 0.7.

Figs. 2 and 3 show the percent burrowing clams and mortality at the end of the test versus the measured Cd concentration in the spiked sediments. Results of the probit analysis showed that the IC₅₀ value was estimated at 1.26 mg Cd kg⁻¹ dw with the 95% fiducial
limits at 0.38 and 2.38 mg Cd kg\(^{-1}\) dw, whereas the \(\text{LC}_{50}\) value was 4.52 mg Cd kg\(^{-1}\) dw with the 95% fiducial limits at 2.46 and 7.25 mg Cd kg\(^{-1}\) dw.

Mean percent burrowing clams observed in the Grain Size Variation Test are presented (Fig. 4). The burrowing clams ranged from 86.7% in sediment A with a grain size composition of 10% coarse sand, 10% medium sand, 10% fine sand and 70% silt to 100% in sediment D with 70% coarse sand, 10% medium sand, 10% fine sand and 10% silt. There was no statistical difference of percent burrowing clams in the test and control sediments (ANOVA, \(p > 0.05\)).

\(\text{ET}_{50}\) values for \textit{R. philippinarum} exposed to 15 sediments in the Field Sediments Test are presented (Table 2). The mean \(\text{ET}_{50}\) value of three sediments (VS2, VS3, VS5) was recorded \(>2880\) min as less than 50% of the test clams was found completely burrowing into the sediment at the end of the 48-h test period. In analysis of the \(\text{ET}_{50}\) data using ANOVA, the \(\text{ET}_{50}\) value of these three sediments was assumed at 2880 min. Results of ANOVA indicated significant difference \((p < 0.05)\) in \(\text{ET}_{50}\) among the 15 sediment samples. Tukey’s multiple comparison test revealed two major groups of sediments: Group 1 containing 3 sediment samples (VS2, VS3, VS5) whereas Group 2 containing the remaining 12 sediment samples (SS1–4, WS2, MS8, VS6, ES1–2, CW, TS5, REF). The mean \(\text{ET}_{50}\) for Group 1 samples was found to be \(>2880\) min whilst the \(\text{ET}_{50}\) for Group 2 sediments had a mean of 173.9 min. Results of the mean \(\text{ET}_{50}\) for \textit{R. philippinarum} exposed to the control sediment in the Dose-Response Test, Grain Size Variation Test and Field Sediments Test ranged from 50 to 53.3 min and there was no significant difference among the tests (ANOVA, \(p > 0.05\)). Mean values of the seven trace metals analyzed in the 15 sediments are given (Table 3). The percent recoveries of these metals from the certified reference material were: As (98.2 ± 0.9), Cd (108.0 ± 1.3), Cr (97.4 ± 1.7), Cu (101.2 ± 0.6), Ni (106.7 ± 0.4), Pb (98.8 ± 2.3) and Zn (96.3 ± 1.4). The results of classification of the sediment samples based on metal content are given (Fig. 5). Two distinct groups of sediments were delineated with similar grouping as from the Tukey’s multiple comparison test. Group 1 sediments (VS2, VS3, VS5) had higher levels of Cd, Cr, Cu, Ni and Zn than Group 2 sediments (Table 3).

4. Discussion

The results of the present study demonstrate that the short-neck clam, \textit{Ruditapes philippinarum}, exhibits a clear dose-response when adult individuals were exposed to sediments spiked with cadmium. The low \(\text{IC}_{50}\) value as compared to \(\text{LC}_{50}\) also suggests that the burrowing response of \textit{R. philippinarum} can serve as a more sensitive end point than mortality when the clams were exposed to the cadmium-spiked sediments. Similar burrowing behaviour was observed in other marine bivalves exposed to sediment contaminants. Roper and Hickey (1994) reported that in copper-dosed sediment, burial of the bivalve \textit{Macomona liliana} was slowed at 15 mg Cu kg\(^{-1}\) dw. In another experiment using the same bivalve species, Roper et al. (1995) observed a slow burial response when \textit{M. liliana} were exposed to sediments spiked with 25 mg Cu kg\(^{-1}\) or 80 mg Zn kg\(^{-1}\) dw. Using the littleneck clam \textit{Protothaca staminea} as the test species, Phelps et al. (1983) estimated that above a threshold of 5.8 mg Cu kg\(^{-1}\) dw, the \(\text{ET}_{50}\) value for \textit{P. staminea} to burrow increased logarithmically with increasing sediment copper concentration. Byrne and O’Halloran (1999) observed that the burrowing activity of \textit{Tapes semidecussatus} (= \textit{Ruditapes philippinarum}) was inhibited in sediments containing 5.8–19.8 mg Cu kg\(^{-1}\) dw. Our \(\text{IC}_{50}\) for \textit{R. philippinarum} was calculated at 1.26 mg Cd kg\(^{-1}\) dw. Whilst data are not directly comparable, the low \(\text{IC}_{50}\) value obtained for the cadmium-spiked sediments may be due to the relatively higher toxicity of cadmium than copper or zinc to marine life (Clark, 1989).
**R. philippinarum** appears to adapt well in a wide range of sediments, as demonstrated from its similar burrowing response to test sediments with different grain size composition (ANOVA, \( p > 0.05 \)). Roper et al. (1995) also showed that the burial of *M. liliana* was not correlated with both grain size and sediment organic carbon. Such adaptability to a larger range of sediment types is an important factor for using the burrowing response of bivalves as an endpoint in sediment bioassays, as many marine organisms exhibit distinct burial rate and behaviour dependent on grain size (McFarland, 1981; Pinto et al., 1984; Nel et al., 1999).

The ET\(_{50}\) values estimated from the 48-h burrowing response test of *R. philippinarum* on 15 field sediments were successfully used to separate the test sediments into two main groups, which were largely related to metal content in the sediments as demonstrated from the classification analysis. A delay in burrowing by the short-neck clam was evident when *R. philippinarum* was exposed to more toxic sediments (as expressed by high trace metal levels). In the present study, ET\(_{50}\) of three sediments could not be accurately estimated as the percent test clam showed complete burial was less than 50% even at the end of the 48-h test. This suggests that a longer time should be used as the test duration. These three sediments (VS2, VS3, VS5) were collected in Victoria Harbour between Hong Kong Island and Kowloon (Fig. 1). The harbour receives preliminarily treated sewage from some 3.5 million people and various industries and is considered grossly polluted.

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**Table 3**

<table>
<thead>
<tr>
<th>Sediment</th>
<th>As (mg kg(^{-1}) dw)</th>
<th>Cd (mg kg(^{-1}) dw)</th>
<th>Cr (mg kg(^{-1}) dw)</th>
<th>Cu (mg kg(^{-1}) dw)</th>
<th>Ni (mg kg(^{-1}) dw)</th>
<th>Pb (mg kg(^{-1}) dw)</th>
<th>Zn (mg kg(^{-1}) dw)</th>
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<td>SS1</td>
<td>4.76</td>
<td>0.04</td>
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<td>6.01</td>
<td>43.07</td>
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<td>0.00</td>
<td>9.46</td>
<td>11.24</td>
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<td>48.89</td>
<td>36.44</td>
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<tr>
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<td>0.00</td>
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<td>21.84</td>
<td>6.14</td>
<td>48.15</td>
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<td>13.19</td>
<td>6.03</td>
<td>40.67</td>
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<td>0.00</td>
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<td>1223.85</td>
<td>41.44</td>
<td>44.97</td>
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<tr>
<td>VS6</td>
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<td>0.05</td>
<td>14.14</td>
<td>66.40</td>
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<td>4.91</td>
<td>35.18</td>
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![Fig. 5. Classification of field sediments using Euclidean distance as a correlation measure of trace metal content and unweighted pair-group average sorting method.](image-url)
(Wong and Tanner, 1997). McGreer (1979) found that sewage-contaminated sediments containing a range of metals slowed burrowing of the Baltic clam *Macoma balthica* at concentrations of 30 mg Cu kg\(^{-1}\), 73 mg Zn kg\(^{-1}\) and 18 mg Pb kg\(^{-1}\) dw. In a 21-d whole sediment toxicity experiment, Byrne and O’Halloran (1999) also observed significant variation in the burrowing behaviour of the bivalves *Tapes semidecussatus* (= *Ruditapes philippinarum*) and *Scrobicularia plana* exposed to sediments collected from different sites in Irish waters, with lower burial activity in sediments containing higher levels of Cu (58–198 mg kg\(^{-1}\)), Zn (242–7834 mg kg\(^{-1}\)), Hg (0.58–15.4 mg kg\(^{-1}\)) and Pb (301–2336 mg kg\(^{-1}\)). Comparable findings of slower burrowing response for *R. philippinarum* exposed to sediments with higher metal content were obtained in the present study.

In this study, the burrowing response of *R. philippinarum* has shown to be sensitive to marine sediments containing different levels of metal contaminants. Other chemical characteristics of test sediments, such as trace nutrients, mineralogical composition or naturally occurring toxic compounds, may also have contributed to the behavioural effect observed in whole sediment bioassays (Ankley et al., 1994). Changes in burial behaviour of several mollusc species have also been found in response to exposure to sediments contaminated with oil (Olla and Bejda, 1983; Leaver et al., 1987), pesticides (Møhlenberg and Kiørboe, 1983), and a range of organic and inorganic pollutants (Chapman et al., 1987; Phelps, 1990). All these studies suggest that burrowing responses can be a valid end point and sensitive means of assessing the sublethal toxicity of contaminants to marine bivalves. Apart from its adaptability to burrow in sediments with different grain size composition, *R. philippinarum* exhibited similar burial activity in the control sediment in all our tests indicating the reproducibility of test results. A larger variation in ET\(_{50}\) was noted, however, in replicates of some test sediments (Table 2). This could be related to the longer observation interval selected for recording the burial response especially at the later stage of the 48-h test. The use of a time-lapse video camera may be able to circumvent such a problem by recording exactly when the clams completely burrow into the sediment over the test period. *R. philippinarum* is also tolerant of a wide salinity range (Sakura et al., 1996), easy to maintain in the laboratory (Cheung and Wong, 1993), available all year round (Buikema et al., 1982), and ecologically relevant as the clam is predominately a deposit feeder directly ingesting sediment particles (Byrne and O’Halloran, 1999). *R. philippinarum* thus fulfills many of the criteria set down for species selection in toxicity testing (Connell et al., 1999) and the sensitivity of its burrowing response to sediment contaminants makes it a potential candidate for use in sublethal sediment toxicity test, especially in Hong Kong.

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References


