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Use of the multispecies freshwater biomonitor to assess behavioral changes of *Corophium volutator* (Pallas, 1766) (Crustacea, Amphipoda) in response to toxicant exposure in sediment

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Abstract

Automated sediment toxicity testing and biomonitoring has grown rapidly. This study tested the suitability of the marine amphipod *Corophium volutator* (Pallas, 1766) for sediment biomonitoring using the Multispecies Freshwater Biomonitor (MFB). Two experiments were undertaken to (1) characterize individual behaviors of *C. volutator* using the MFB and (2) examine behavioral changes in response to sediment spiked with the pesticide Bioban. Four behaviors were visually identified (walking, swimming, grooming and falling) and characterized in the MFB as different patterns of locomotor activity (0–2 Hz range). Ventilation was not visually observed but was detected by the MFB (2–8 Hz). No clear diel activity patterns were detected. The MFB detected an overall increase in *C. volutator* locomotor activity after Bioban addition to the sediments (56, 100, 121 mg kg^{-1}). *C. volutator* was more active (both locomotion and ventilation) in the water column than the spiked sediment. *C. volutator* appears a sensitive and appropriate species for behavioral sediment toxicity assessment and biomonitoring.

Keywords: *Corophium volutator*; Bioban; Behavior; Sediment toxicity testing; Multispecies Freshwater Biomonitor

1. Introduction

Coastal biomonitoring has received high priority within the European Union due to the economic value of marine fisheries, aquaculture, and tourism in coastal zones (Kramer, 1994). However, as such zones are subject to elevated rates of human population growth and industrial development, associated discharges of waste and pollutants pose an increasing threat to ecosystem health. This risk is heightened by pollutants that are transported via large river systems such that the sea and coastal zones often act as final depositional zones (Kramer, 1994).

As coastal sediments can act as a sink for toxicants and concentrate substances, a key objective is determining levels that are lethal to sediment-dwelling benthic organisms through standard toxicity tests (Ingersoll, 1995). A wide variety of organisms have been used in saltwater sediment toxicity testing including the amphipod *Corophium volutator* and the polychaete *Arenicola marina* (Bat and Raffaelli, 1998). Often pore water, extracts, or eluent have been used instead of natural whole sediment, in part due to the lack of methods developed with appropriate benthic species to quantitatively measure biological fitness parameters (Gerhardt and Schmidt, 2002; but see Briggs et al., 2003). However, by failing to incorporate representative

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habitat simulations such methods may fail to accurately assess sediment toxicant effects. Furthermore, most standard sediment toxicity tests employ a test period which may last up to 10 days. Not only does this limit the rapidity of response to pollutants but it also fails to address sublethal effects on organisms under moderate levels of pollution (Allan and Maguire, 1995; Ingersoll, 1995).

Until now, no automated method has been used for recording behavior of benthic organisms in their natural sediment apart from behavioral recordings of the freshwater invertebrates *Hydropsyche angustipennis* (Gerhardt and Schmidt, 2002) and *Caenorhabditis elegans* (Gerhardt et al., 2002) with the Multispecies Freshwater Biomonitor (MFB; LimCo International, Germany). These studies showed that the measurement principle of the MFB, based on quadropole impedance conversion technology, can record behavioral signals of invertebrates in the sediment with accuracy similar to that in water. However, the utility of this system in monitoring behavioral responses of sediment-dwelling marine species is unknown. In this study, we assess the ability of the MFB to record behavioral responses of *C. volutator* and its potential for sediment toxicity assessment and biomonitoring in marine environments.

The aims of this study were (1) to identify and characterize individual behaviors and diel activity of *C. volutator* using the MFB and (2) to examine behavioral changes in response to sediment spiked with the pesticide Bioban.

2. Materials and methods

2.1. The Multispecies Freshwater Biomonitor

The MFB, suitable for both invertebrates and vertebrates, is based on quadropole impedance conversion (Gerhardt et al., 1994, 1998; Gerhardt, 1999, 2000). MFB channels monitor individual chambers formed of cylindrical transparent plexiglass containing two pairs of opposing stainless-steel electrodes. A high-frequency alternating current signal (100 kHz) is sent by one electrode pair across the chamber, while the second electrode pair (not current carrying) detects changes in impedance, that is the electrical field change due to organisms' movements. Different types of movement can be characterized by their amplitude and frequency, such as locomotion (band 1: 0–2 Hz) and ventilation (band 2: >2 Hz). Chambers (2 cm diameter, 3 cm length) containing one individual were connected to the MFB, and settings were made for channel information, noise level (50 mV), and threshold value (Gerhardt and Schmidt, 2002) to obtain response data for the analysis of signal frequencies using the fast Fourier transformation method (Zonst, 2000). Lids of nylon mesh covered the chamber ends, permitting the circulation of water when submerged. Recording occurred over a 4 min duration with a 6 min interval between recording periods.

2.2. Test species

The study species *C. volutator* is a tube-dwelling marine amphipod found in high densities in estuarine and intertidal marine sediments, covering the west European and the northeast American coasts (Ciarelli et al., 1997). They inhabit small U-shaped burrows and are selective deposit feeders (Ciarelli et al., 1997) obtaining food from bacteria, algae, and diatoms adsorbed onto the surface of sediment particles (Meadows and Reid, 1966). *C. volutator* are suitable candidates for sediment toxicity tests as they are available throughout the year and are continuously exposed to toxicants in the sediment, which they ingest when they feed (Bat and Raffaelli, 1998).

2.3. Test substance

Bioban is a lipophilic biocide and has active ingredients that are likely to adsorb to particles and surfaces (Bjornestad et al., 1993; McKenna, 2001). Bioban P-1487 consists of 80% 4-(2-nitrobutyl)morpholine with 10% 4,4-(2-ethyl-2-nitrotrimethylene)dimorpholine, 5% 1-nitropropane, and 5% unrelated inactive material from the production stages. It has been used as a reference toxicant to evaluate the success of new organisms used for toxicity testing and has been widely used in ring tests to compare results of different laboratories. For example, in the evaluation of *Platynereis dumerilii* (Polychaeta: Nereidae), Bioban was selected as a reference toxicant to assess the response of this species in its different life stages to a toxicant (Hutchinson et al., 1995). In the present study, the highest concentration of Bioban used, 121 mg/kg (trimmed Spearman–Karber 95% CI 104.1–139.2), was equal to the 10-day LC_{50} as indicated by separate experiments (McKenna, 2001).

2.4. Experimental design

2.4.1. Preparation

C. volutator were collected, in July and October 2000, from sediment samples taken from the mid-shore of Horse Island, Co. Down, Northern Ireland (Lat 54°28′15N, Long 5°33′0W). Immediately prior to use, sediment was sieved through a 500-μm mesh to remove the amphipods. Sediment was saved for use within the experiments. Large individuals (>10 mm and thus easier to observe) were placed into dishes (9 cm diameter) of sediment and seawater (15 °C). Seawater was filtered and irradiated at an industrial laboratory (IRTU, Lisburn, Northern Ireland). All experiments were performed at 15 ± 1 °C and 12 h:12 h light:dark regime.

2.4.2. Behavioral patterns of C. volutator in water and sediment

MFB chambers, with one end of each enclosed with mesh and the other with a microscope slide, were placed in a shallow aquarium (1.5 L) with seawater (1 L). Each chamber contained a shallow layer (2–3 mm) of sediment to reduce the stress to the individual but not sufficiently deep to burrow out of sight. Additionally, behavior was recorded manually using a Psion II hand-held computer (Psion PLC, London, England) programmed for four visually predefined behaviors: walking, swimming, grooming, and falling (Kirkpatrick, 2001). Eight *C. volutator* were observed individually while the MFB was simultaneously recording.

The traces as recorded by the MFB were visually compared with the Psion data and this permitted clear identification of the specific behaviors in the MFB signals. The mean frequency and amplitude for each of the four behaviors was calculated from the MFB graphs. Eight *C. volutator* were exposed in chambers with ($n = 4$) or without ($n = 4$) sediment layer for a period of 48 h to study diel activity patterns.

2.4.3. Behavioral responses of C. volutator to Bioban

A stock solution of Bioban (Brenntag, Belgium) was prepared (1 g Bioban into 100 ml of filtered seawater), from which the required concentrations (56, 100, and 121 mg kg^{-1}) were made. The sediment was spiked by placing 500 g of sediment into a 2-L bottle, plus the appropriate concentration of Bioban, followed by another 500 g of sediment. The bottle was placed on a roller for 3 h to mix the two components, after which the sediment was ready to use.

Two MFB chambers were attached to each other to form a joined pair using rubber tubing (Fig. 1a). Four such pairs were constructed, with both upper and lower chambers attached to the MFB. Each pair was placed upright in an individual 1-L dish of seawater (15 ± 2 °C) and the lower chamber pipetted full with Bioban spiked sediment (Fig. 1b). An individual *C. volutator* was placed within each pair of chambers and given 20 min to settle, and MFB recordings were made over a 1-h period. There were four experimental groups ($n = 10$ each): (1) 0 (clean sediment control), (2) 56 mg kg^{-1}, (3) 100 mg kg^{-1}, and (4) 121 mg kg^{-1}.

2.5. Statistical analysis

Data on percentage activity time (arcsine transformed from proportional values (see Sokal and Rohlf, 1995)) were examined with respect to concentration (0, 56, 100, and 121 mg kg^{-1}), medium (sediment or overlying water), and activity type (band 1 or band 2) in a three-factor ANOVA, with the latter two factors as repeated measures. Figures show raw mean percentage of time spent in bands 1 and 2.

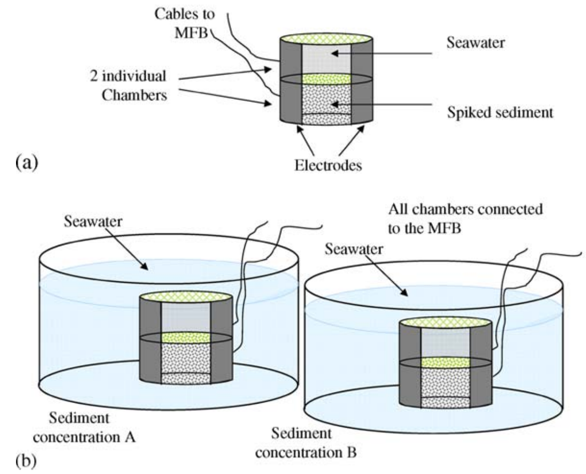

Fig. 1. Experimental setup. (a) The two MFB chambers joined together (pair chamber) connected to the MFB and PC, the lower one containing Bioban spiked sediment and the upper one containing the overlaying water. (b) The paired chambers with the same concentration of spiked sediment in each aquarium (two of four replicates shown), each pair containing one *C. volutator* for MFB observation.

3. Results

3.1. Behavioral patterns of C. volutator in water and sediment

Four behaviors were characterized for *C. volutator* (Figs. 2a–d). Mean frequencies for walking and swimming were 2 Hz, with amplitudes of 0.3 and 2.5 V, respectively (Figs. 2a and b). Mean frequencies for falling and grooming were 1 Hz, with amplitudes of 2.5 and 0.7 V, respectively (Figs. 2c and d). Falling typically followed a period of movement upward, by walking or swimming in the water column, after which the individual fell through the water (sank) back to the sediment. The frequencies correspond with the MFB predefined band 1 (0–2 Hz) or activity measure. Band 2 behavior (above 2 Hz) usually describes high-frequency behavior such as ventilation in amphipods (Gerhardt et al., 1994; Gerhardt, 1995), although this was not visually observed in *C. volutator*. However, ventilation appeared to be recorded and calculated by the MFB. There were no clear diel activity changes in *C. volutator* in water or sediment (Figs. 3a and b); however, activity was clearly higher in the absence of sediment (Figs. 3a and b).

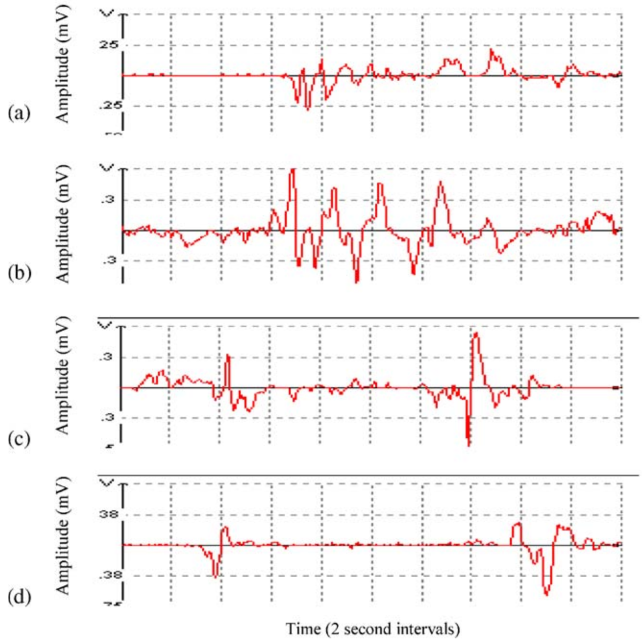

Fig. 2. MFB traces showing the characteristic waveforms of *C. volutator* for behaviors observed: (a) walking, (b) swimming, (c) falling, and (d) grooming.

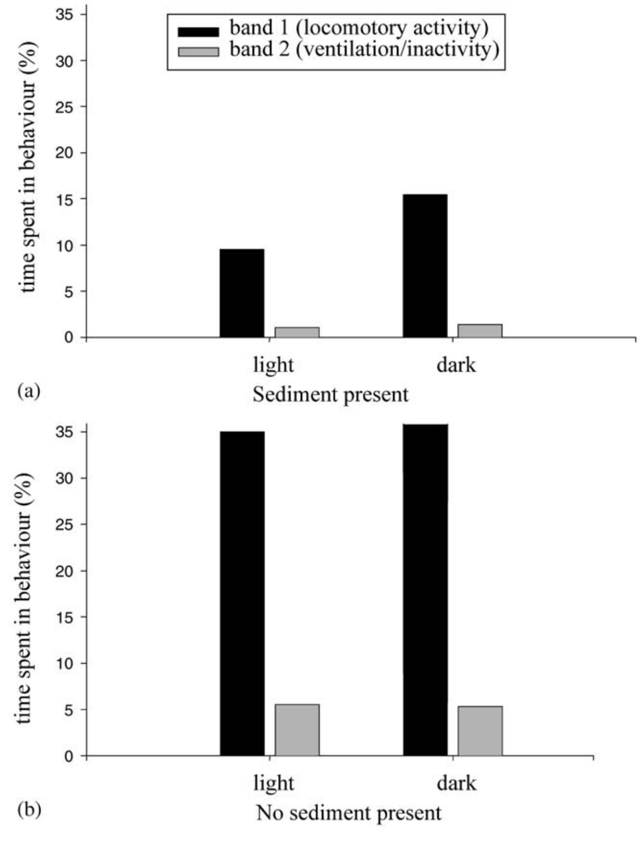

Fig. 3. Activity of *C. volutator* in seawater, showing band 1 (locomotory activity) and band 2 (ventilation/inactivity) in MFB chambers with sediment (a) and without sediment (b).

3.2. Behavioral responses of *C. volutator* to bioban

There was a significant effect of concentration on overall percentage activity duration ($F_{3,36} = 4.38$, $P < 0.01$; Figs. 4a and b), with Fisher's protected least significant difference tests indicating that percentage activity duration at $56\,\mathrm{mg\,kg^{-1}}$ was significantly lower than those at 0 and $100\,\mathrm{mg\,kg^{-1}}$ (both $P < 0.05$) and $121\,\mathrm{mg\,kg^{-1}}$ ($P < 0.01$). There was also a significant effect of medium, with greater overall activity in the water than in the sediment ($F_{1,36} = 7.69$, $P < 0.01$; Figs. 4a and b). There was an overall concentration × activity type interaction effect ($F_{3,36} = 2.81$, $P = 0.05$), reflecting an increase in percentage of time spent in band 1 compared to that in band 2 with increasing concentration of Bioban.

4. Discussion

4.1. Behavioral patterns of *C. volutator* in water and sediment

Field studies have described *C. volutator* as having low mobility and traveling only short distances, generally crawling rather than swimming over the

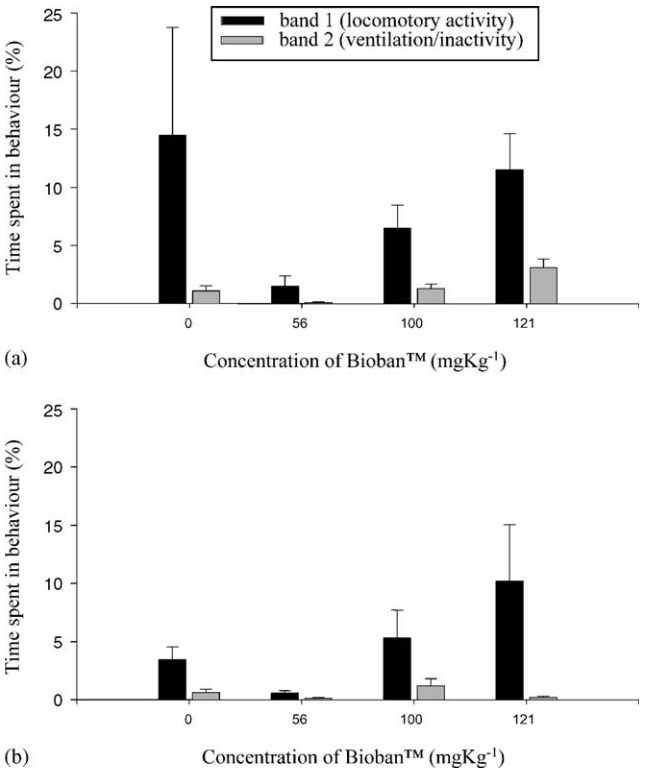

Fig. 4. Percentage time spent (a) in the water column and (b) in the sediment column during 1 h MFB recording of *C. volutator* on locomotory activity and ventilation/inactivity in sediment spiked with three Bioban concentrations (56, 100, $121\,\mathrm{mg\,kg^{-1}}$).

sediment (Lawrie and Raffaelli, 1998). However, we identified four locomotor behaviors of *C. volutator*, each of which could be characterized in the MFB signals. Similarly, behavioral components such as swimming and walking were previously reported and characterized by the MFB for the freshwater amphipods *Crangonyx pseudogracilis* (A.J. Kirkpatrick et al., unpublished) and *Gammarus pulex* (Gerhardt, 1995; Gerhardt et al., 1998). Analysis of each behavioral component by fast Fourier transformation, which considers signal frequency as opposed to amplitude and pattern, assigned all four behaviors to band 1, representing activity. This provided a useful summary of overall locomotory behavior since, for example, falling, which was observed as part of swimming behavior, occurred infrequently and varied among individuals, while walking alternated often with swimming.

Although behavioral assessment may be used as a sublethal alternative to acute toxicity testing, such methods can prove difficult for sediment-dwelling organisms (Briggs et al., 2003). Indeed, the four types of behavior were visually observed using the Psion II in water-filled chambers and so could have varied greatly from behavioral responses occurring within sediments. Although the MFB demonstrated that *C. volutator* exhibited reduced activity in the sediment in comparison to that in the water column, its ability to record similar activities in both habitat types supported its potential as an additional method for behavioral recording in marine sediments.

4.2. Behavioral responses of *C. volutator* to Bioban

In a field situation, benthic organisms can be exposed to toxicants via three possible routes: overlying water, pore water, or through contact with or ingestion of contaminated sediment (Conrad et al., 1999). An inherent difficulty is establishing whether the effects arise from the water or the sediment portion (Reynoldson, 1987). For example, in a study examining the effects of the insecticide permethrin on the midge *Chironomus riparius*, Conrad et al. (1999) compared laboratory spiked sediment tests with a field study where permethrin was applied to the water. The results from the sediment toxicity tests underestimated the toxic effect shown in the field study; however, the results from the laboratory water-only acute tests were found to predict the toxicity, as found in the field study, more accurately (Conrad et al., 1999). Current sediment toxicity testing using standardized methods assumes that equilibrium has been reached between the toxicant and its sediment-binding phase so that the test organisms receive their primary exposure through contaminated sediment particles or pore water (Conrad et al., 1999), with the exposure from the overlying water demonstrated to be negligible. In the current study, Bioban was spiked to the sediment only. *C. volutator* exposed to sediment-bound Bioban for 1 h spent an increasing amount of time on locomotory activity in a concentration-dependent way, with the highest concentration corresponding to the 10-day LC_{50} for *C. volutator* exposed to Bioban (McKenna, 2001). However, our results additionally showed a rapid behavioral response at sublethal concentrations of Bioban. The increase in locomotory activity exhibited in both the toxic sediment and the nontoxic overlaying water appears to represent avoidance and escape behavior and corresponds to the first step of behavioral responses in the stepwise stress model (Gerhardt, 1999). A second response, an increase in ventilation while inactive (band 2: >2 Hz), was observed at 121 mg kg^{-1} in the water compartment and might serve as a mechanism to counteract gill disturbance by increasing the water flow across the gills (Gerhardt, 1995).

This study represents the first automated method to quantify behavioral responses of a sediment-dwelling marine invertebrate to a toxicant. Although turbidity has been used previously as a surrogate of *C. volutator* activity responses to toxicants (see Briggs et al., 2003), such methods were unable to characterize specific behaviors while also failing to partition effects of turbidity on behavior and mortality. Furthermore, Briggs et al. (2003) acknowledged that large particle sizes might preclude assessment of sediment toxicity of sites when activity by corophids is insufficient to cause particle resuspension. This study suggests that the MFB may overcome such problems and those of previous studies (see Gerhardt and Schmidt, 2002) by allowing an unambiguous and thus more realistic assessment of the toxicity of marine sediments. Furthermore, the lengthy period and lethal concentration doses employed in most standard sediment toxicity tests (see Allan and Maguire, 1995) preclude rapid detection of pollutants. As we observed a rapid behavioral response to moderate concentrations of Bioban, the MFB may provide a less retrospective assessment method and offer an advantage as an early warning system in coastal ecosystems. To fully evaluate the potential of the MFB in monitoring and prevention of sediment pollutants, testing of a wider range of toxicants is required.

5. Conclusions

(1) The MFB quantified locomotory and ventilatory activity of *C. volutator* in sediment-filled chambers where visual methods failed.
(2) *C. volutator* reacted to sublethal concentrations of Bioban within 1 h of exposure, exhibiting escape behavior (sediment, water) followed by increased ventilation in the water compartment.

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