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Behavioural responses of *Venerupis decussata* (Linnaeus, 1758) and *Venerupis pullastra* (Montagu, 1803) to copper-spiked marine sediments

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ABSTRACT

The burrowing behaviour of two marine clam species, *Venerupis decussata* (Linnaeus, 1758) and *Venerupis pullastra* (Montagu, 1803), was assayed in laboratory tests. By standardising abiotic and biotic factors, a routine static-renewal biotest for laboratory assays with *V. decussata* and *V. pullastra* was conducted, using burial as the principal sublethal endpoint. Measured burial times in marine sediments with different textures showed that both clam species are generalist burrowers for a wide range of medium-to-fine sand sediments with up to 40 % silt content. Sandy sediment was spiked with copper to examine clam response to heavy-metal contamination. Slowing of burial was observed in relation to added copper. After 24 h clams were observed to re-emerge from sediment. By counting re-emerged clams on the surface 4 days after starting the assay, a relation between re-emergence behaviour and spiked copper was observed. Experimental data suggest that porewater Cu concentrations, rather than sediment Cu concentrations, were responsible for both burial delay and re-emergence. Whereas burial delay cannot be viewed as a directed behavioural response, re-emergence might be part of a survival strategy to avoid toxic sediment conditions. The LC_{50} in a 10-day exposure period compared to significant levels of the burial endpoint, while re-emergence and reburial ability after 10-day exposure were considerably more sensitive. Since the observed threshold concentrations are in naturally occurring ranges, an application of the assay, especially with the endpoint of re-emergence, to examine contamination in the field seems possible.

Key words: Bioassay, sublethal endpoints, behavioural responses, copper, marine bivalves, *Venerupis*.

RESUMEN

***Respuestas de comportamiento de* Venerupis decussata *(Linnaeus, 1758) y de* Venerupis pullastra *(Montagu, 1803) a sedimentos marinos contaminados con cobre con el método de* spiking

El comportamiento de enterramiento de dos especies de bivalvos marinos, Venerupis decussata *(Linnaeus, 1758) y* Venerupis pullastra *(Montagu, 1803), se estudió en ensayos de laboratorio. La estandarización de los factores bióticos y abióticos permitió poner a punto un biotest tipo* static-renewal *para ensayos de laboratorio usando el parámetro de enterramiento. En ensayos con sedimentos marinos de textura variable,* V. decussata *y* V. pullastra *se demostraron como generalistas en un rango amplio de texturas de arena media y fina hasta un contenido de 40 % de fango. A un sedimento arenoso se añadió cobre para examinar la respuesta de los bivalvos a una contaminación de metales pesados. Se observó una reducción en la velocidad de enterramiento con relación a la cantidad de cobre añadido. Después de 24 horas se observó a las almejas reemergiendo del sedimento. Al contar los bivalvos en la superficie cuatro días después del inicio del ensayo, se pudo establecer también una relación entre el rechazo al sedimento y el cobre añadido. Los datos*

experimentales sugieren que eran los niveles de cobre en el agua intersticial los que causaron el enterramiento ralentizado y el rechazo al sedimento, y no el cobre encontrado en el sedimento. Según estos ensayos, el entierro ralentizado no se puede considerar una respuesta de comportamiento directa, mientras el rechazo puede ser parte de una estrategia de supervivencia para esquivar condiciones tóxicas. El LC_{50} de una exposición de 10 días es parecido a los niveles significativos de enterramiento, mientras el rechazo y la capacidad de reenterramiento después de una exposición de diez días son más sensibles. El margen de las concentraciones se encuentra dentro del rango de concentraciones encontradas en el campo. Por eso, también parece posible la aplicación del bioensayo para examinar la contaminación de sedimentos naturales.

Palabras clave: *Bioensayo, parámetros de toxicidad subletales, respuestas de comportamiento, cobre, bivalvos marinos,* Venerupis.

INTRODUCTION

Sediments in marine and limnic environments accumulate contaminants, such as heavy metals and organic substances, thus constituting a direct source of these substances to benthic biota. Biological assays are used to estimate the risks that sediments with high levels of contaminants pose to the living world. These assays are based on species that are naturally sensitive to contaminants and/or have developed means to detect contamination as a survival strategy. Even though many ecotoxicological studies have relied on the endpoint of mortality in chronic toxicity tests, it was possible to detect toxicity at much lower levels with bioassays that use sublethal responses, which can range from behavioural changes to physiological adaptations or abnormalities in cellular functions (Swartz, 1989).

Bivalves are especially useful as bioindicators, since they have a sedentary nature and many live buried in the sediment, thus continuously exposed to possible contaminants in the sediment or dissolved in the interstitial water. Copper was applied the as contaminant in the present study because it is known to be very toxic to marine life, especially to molluscs (Clark, 1989), and precedents for toxicity for many species can be found in the scientific literature (e.g. Mance, 1987). Copper is also a common contaminant in coastal waters, resulting from various anthropogenic sources and urban runoff (Clark, 1989).

In the present paper a static-renewal whole-sediment laboratory assay was conducted with two *Venerupis* species to examine the effects of toxicants under controlled conditions similar to the standard test with amphipods described by ASTM (Anon., 1993). Sediments of varying compositions were tested to see for what ranges of grain-size assays could be performed with the test species. Using a standardised set-up, behavioural differences between the test species were observed in relation to the added toxicant (Cu concentrations). Inherent clam sensitivity to copper for the two assayed species was determined, and the application of behaviour changes as sublethal endpoints was examined.

MATERIALS AND METHODS

Test species

The clams *Venerupis decussata* (Linnaeus, 1758) and *Venerupis pullastra* (Montagu, 1803) were obtained from a marine rearing centre in Ribadeo, Spain. They had been raised to an age of 9-12 months in flowing seawater containers under ambient conditions, without exposure to sediment, and fed with separately-raised phytoplankton. Young clams were used because they were accessible in large numbers, convenient to handle, and have often proved to be more susceptible to stress (Bamber, 1987).

The clams were transported in small freezer boxes with cooling aggregates, and arrived within 1 day. The holding containers in our laboratory consisted of aquaria filled with 3 cm of clean, sandy sediment and at least 10 cm of filtered, constantly aerated seawater. Marine saltwater was field-collected (salinity: 46-50 mS/cm and pH: 7.7-7.9) and passed through a paper filter with pores of approx. 0.240 mm. Holding tanks and assay chambers were located in a temperature-controlled room set at 16 °C with a photoperiod of 12:12 hours. Salinity of the water was kept at approx. 33 ‰ by adding distilled water as necessary. Under these conditions, animals could be kept without external feeding for

many weeks without any apparent effect on burrowing behaviour: mortality in the holding containers was zero over a period of 2 months. Any individuals that did not achieve complete burial (e.g. no part of the shell visible) in the holding tanks within the first hour were considered unfit and removed. The period for acclimatisation to laboratory conditions was a minimum of 3 days.

Sediments

Sediments of different textures, ranging from mud to sand, were collected from the intertidal zones of the Noia and Betanzos Rias, in Galicia (northwest Spain). At low tide, sediments were collected in plastic bags with a polyethylene spatula, and for the finer sediments care was taken not to include the lower anoxic layers. The sediments were taken to the laboratory in a freezer box with cooling aggregates and, upon arrival, stored in a refrigerator. For assays, sediments were thawed and used directly, whereas samples for sediment texture analysis were dried by exposure to sunlight. Larger objects, like shells or stones, were removed by hand if encountered.

Sediment texture was determined by the method of discontinued sedimentation with a Robinson pipette and wet sieving for larger particles (> 50 μm). Organic matter was determined as weight loss upon ignition. Metal content (Cu, Cd, Cr, Zn, Pb) was determined with a strong acid attack on sediment sieved to 63 μm with concentrated HNO_3, and a weak acid attack with 1N Hcl (extractable metal) following formulae given in Real, Barreiro and Carballeira (1994). Extracts were measured with a Perkin Elmer 2100 atomic absorption spectrometer with flame absorption technique and an air/acetylene flame. The efficiency of the extraction method was examined by using the standard marine sediment reference material MESS-2, provided by the Institute for Environmental Research and Technology of the National Research Council, Canada.

Spiking procedure

We thoroughly mixed 650 ml of wet sediment with 1 litre of a $CuSO_4$ solution (concentration between 1 and 200 mg/l Cu added to marine saltwater, according to desired Cu contamination level) in closed plastic containers on a turning wheel for 15 min and then left it to stabilise overnight at 4 °C. The solution was decanted (which led to a small loss of finer particles) and clean marine saltwater added instead (to wash out excess Cu, not absorbed by sediment), mixed for 15 min on the turning wheel and again decanted. Then 650 ml of sediment was transferred with a large Teflon spoon to 15 × 15 cm^2 plastic Tupperware boxes, with about 3 cm of sediment in every box. Minimising the disturbance of sediment, 1.2 l of seawater was added carefully. After installing the aeration system the experimental chamber was left overnight to stabilise, and bioassays were conducted the next day (14 + hours later).

Bioassays

At the start of an assay, subsamples of sediment were taken for the analysis of Cu content (total metal and extractable metal) in every experimental chamber. A sample of the overlying water and of the interstitial water were similarly retained to be analysed for Cu content. The porewater was obtained with a needle attached to a filter mounted on a plastic syringe. This method made taking interstitial water samples during the course of the assay possible. Interstitial and overlying water samples from the assays were stabilised with a drop of concentrated nitric acid and left to evaporate on a hot plate (80 °C). The remaining solids were dissolved in concentrated nitric acid and diluted with distilled water to be measured with flame absorption spectophotometry. The lowest reliable concentrations were 0.010 mg Cu/l.

In preliminary assays, it became obvious that a minimum of 30 clams per box should be used to minimise differences between replicates. Three replicates for a specific metal concentration or sediment type were used. Burial assays were always started at the same time of day, 2 h after the start of the 12-h light period, since length of light period had been observed to affect burrowing speed. The 30 clams were placed on the sediment, and in the first 30 min the number of animals remaining on the surface was recorded every 2-3 min, up to a maximum of 8 h. A clam was considered buried when no part of the shell was visible. The number of clams on the surface was recorded daily up to 10 days af-

ter the start of the experiment. About 90 % of the overlying water was changed on the first day after completion of the burial assay, and then every third day, to avoid build-up of copper concentrations in the water. This change was done carefully, by scooping out the 'old' water with a plastic cup then placing a plastic tray on the sediment to absorb the impact of the 'new' water being filled in, so that resuspension of sediment was largely avoided.

The sediment in the experimental chambers was left to settle and equilibrate overnight after the clams were dug up at the end of the first 10-day assay, and tested again with new clams in a follow-up assay. In this second assay, initial burial time and re-emergence (number of clams reappearing on the surface or number of clams on the surface at a certain time) were measured as described above.

Data analysis

Numerical data derived from burial monitoring were used to calculate the ET_{50}, the effective time for 50 % of the population to bury itself, with the help of probit approximation. Probit analysis was carried out with the SSPS statistical package for Microsoft Windows. The logit option within probit analysis was used for the parameters number of animals buried against the decadic logarithm of the time, which yielded the best approximations. The Pearson goodness-of-fit ψ^2-test was used to evaluate the validity of the approximation. If this criterion is not fulfilled, the SPSS program used a heterogeneity factor in its calculations; values that fell into this category were marked with an asterisk throughout this study. If the calculated 95 % fiducial limits of two ET_{50}-values did not overlap, then these values were considered statistically different. LC_{50} (10 days) (concentration lethal to 50 % of the population in a 10-day exposure period) also were calculated with probit.

Surfacing, or re-emergence of clams on the surface, was characterised by the arithmetic mean of the clams encountered on the surface 4 days after the start of the assay. Statistical significance from controls was examined with one-factorial ANOVA performed using Microsoft Excel software.

RESULTS

In the burial assay, the number of totally buried clams was plotted against the elapsed time, and sigmoidal curves were obtained as displayed for burial of *V. pullastra* on sandy sediment (N2) in the upper diagram of figure 1. For practical purposes this curve could best be described by calculation of ET50s with 95 % fiducial limits by logit transformation (lower diagram). In the case shown, the burial time was approximately 15.6 min (= $10^{1.193}$ min) and calculated fiducial limits were 14.3-16.9 min.

In preliminary experiments, we found that clam size greatly influenced burial time, with larger individuals burying themselves faster. However, a size-window of 10-14 mm ensured homogeneous burial times, and hence only clams fitting that condition were used for all performed burial assays.

Burial assays with *V. decussata* were repeated on sandy sediment on consecutive days with the same sample population and with different populations to see whether burial time, under the established assay conditions, was reproducible (refer to table I). Differences between calculated ET_{50} values proved to be small, with burial time ranging from 9.1 min to 12.5 min, with 95 % fiducial limits overlapping for the most part.

When testing clam burial on marine field-collected sediments, the texture proved to be a dominating factor. The texture and total metal content of sediments used in the assays are shown in table II. Both species had their shortest burial times in fine-to-medium sands, between mean grain sizes of 94-214 μm (see figure 2). Sediments with a mean grain size outside this range caused exponential elongation of burial times, which was more apparent in the case of *V. decussata*. In the fine silt/sand sediment B1, with a mean grain size of 60 μm, very long burial times were observed for both species, due to individually differing burial times and a refusal of part of the population to bury itself completely. Since total metal levels of the tested sediments were at or just slightly above background levels, they are not believed to have had an effect on burial times. Also, acid-washing of sediment B1 did not result in significantly different burial times compared with burial times in untreated B1.

Burial assays with both species performed in copper-spiked sandy sediment showed a distinct slowing of burial with increasing Cu levels. At high levels of added Cu, a large part of the population refused to bury itself and closed up, making the determination of an ET_{50} impossible. A repeat of burial assays on aged sediments (10 and 20 days) gave significant slowing of burial only at much

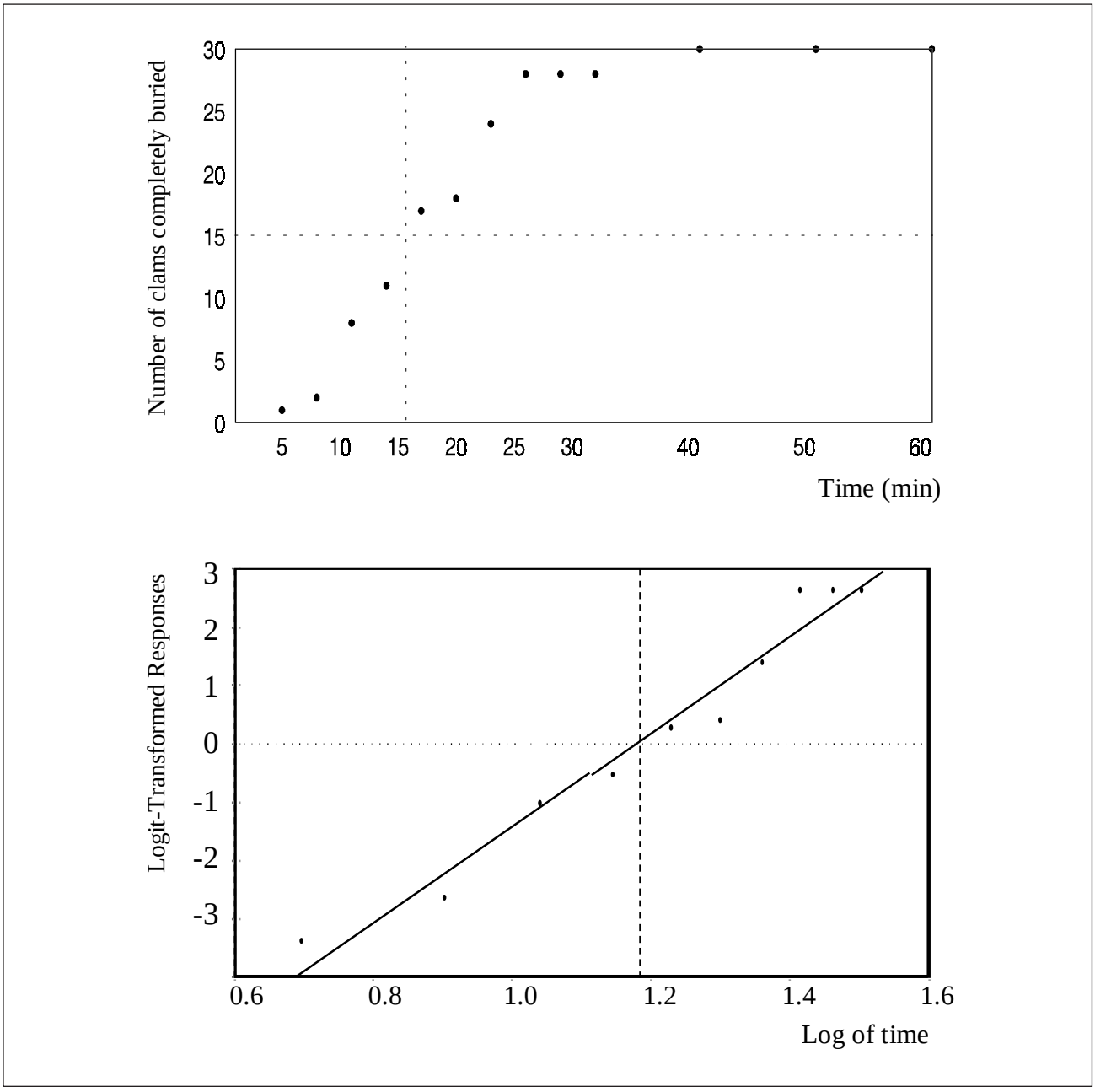

Figure 1. In the upper diagram, completely buried clams are plotted against the elapsed time. The lower diagram plots logit-calculated values against the decadic logarithm of time in minutes. The ET_{50} is determined as the time corresponding to a logit-value of 0

higher levels of added copper, compared with the original assay. Cu sediment concentrations in aged sediment were practically unchanged from the original sediment (losses in 10-day period were never > 7 %). On the other hand, Cu concentrations in the porewater fell drastically in the same period. A plot of burrowing times from both initial and aged sediments against corresponding porewater Cu shows an exponential relation between the two factors (figure 3). The two species generally reacted similarly to the addition of Cu with slowing of burial, although *V. pullastra* was more sensitive: porewater concentrations of 95 µg/l caused significantly slower burial for *V. pullastra*, whereas 75 µg Cu/l did not significantly affect burial behaviour (non-effective concentration, or NOEC). For *V. decussata*, the determined NOEC was 166 µg/l.

Specimens of both clam species that had originally buried themselves were observed to re-

Table I. Day-to-day variations and variations between sample groups in burial time. Data are ET_{50} in minutes with calculated 95 % fiducial limits in parentheses. For data with asterisk, Pearson goodness-of-fit ψ^2-value is higher than degrees of freedom. Each sample group represents 3 × 30 clams

	Repeat 1	Repeat 2	Repeat 3
Sample group 1	12.3 (11.1-13.5)	12.4* (8.4-16.1)	11.4 (9.8-12.9)
Sample group 2	9.1 (7.8-10.4)	11.5 (9.9-13.0)	9.2 (8.5-9.8)
Sample group 3	10.0 (8.1-12.0)	11.8 (10.1-13.6)	12.5 (9.9-15.1)

Table II. Particle distribution, organic matter, and total heavy-metal content of field-collected sediments B1, N1, B2, N2, N3 and sediment mixtures B and A. Values from metal analysis are arithmetic means of n = 3. Background values for metals from other authors: (1): Barreiro (1991); and (2): Carral (1992)

	µm	B1	B	N1	A	B2	N2	N3	Background value
% particles	> 500	0	0	0	1	2	1	29	
	> 200	2	1	1	3	5	36	38	
	> 50	28	58	87	89	92	63	31	
	> 20	38	24	10	5	0	0	0	
	> 2	29	15	2	2	0	0	0	
	2	3	2	0	0	1	0	2	
Mean grain size	(µm)	59	94	115	136	158	214	535	
% OM		10.0	7.8	3.6	2.3	1.0	0.3	1.3	
ppm Cd		1.5		1.7		2.0	2.0	2.1	
ppm Cr		79		60		66	35	54	25(1), 32(2)
ppm Cu		27		41		35	24	40	25(1), 28(2)
ppm Pb		42		55		46	37	43	25(1), 53(2)
ppm Zn		110		107		195	160	109	100(1), 122(2)

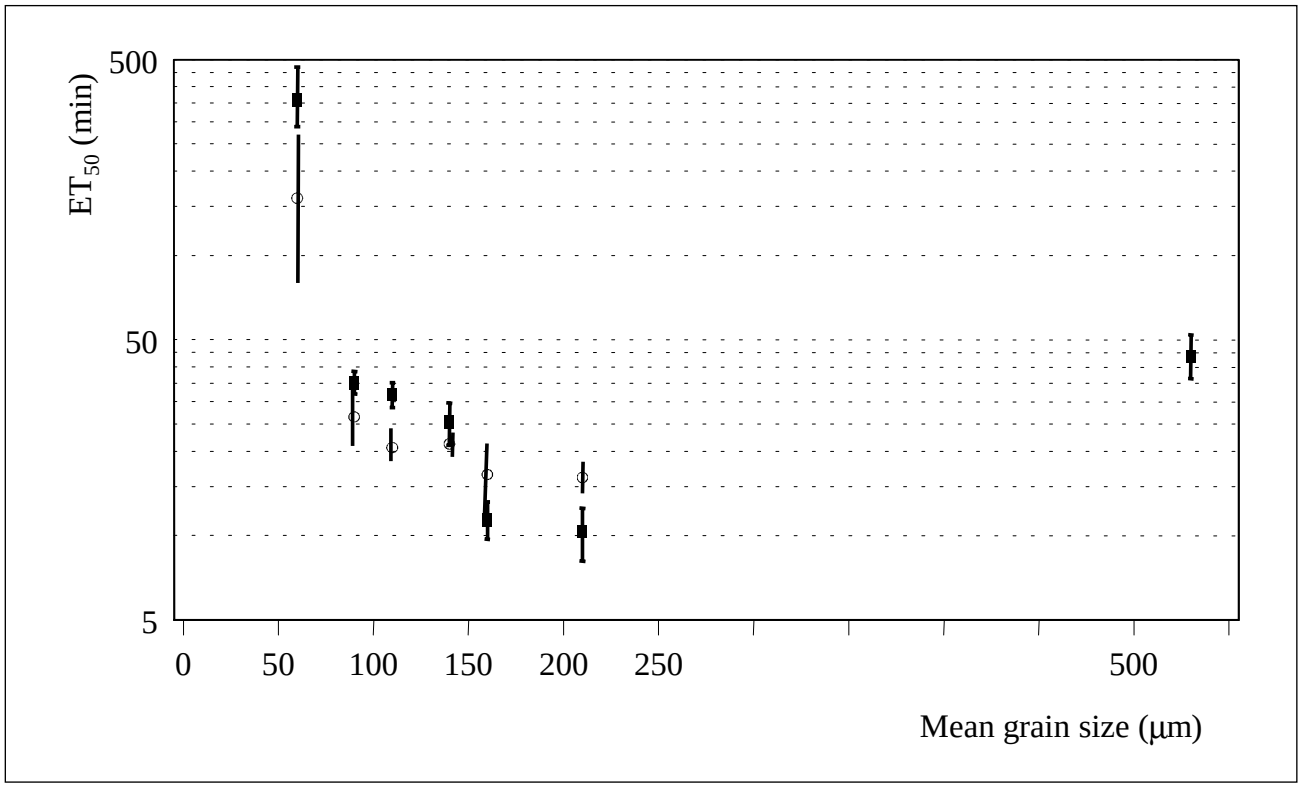

Figure 2. Burial times logarithmically displayed against calculated mean grain size for field-collected marine sediments and sediment mixtures listed in table II. Each ET_{50}-value represents 3 repetitions of 30 clams, and is shown with calculated 95 % fiducial limits. Squares represent values for *V. decussata* and circles for *V. pullastra*

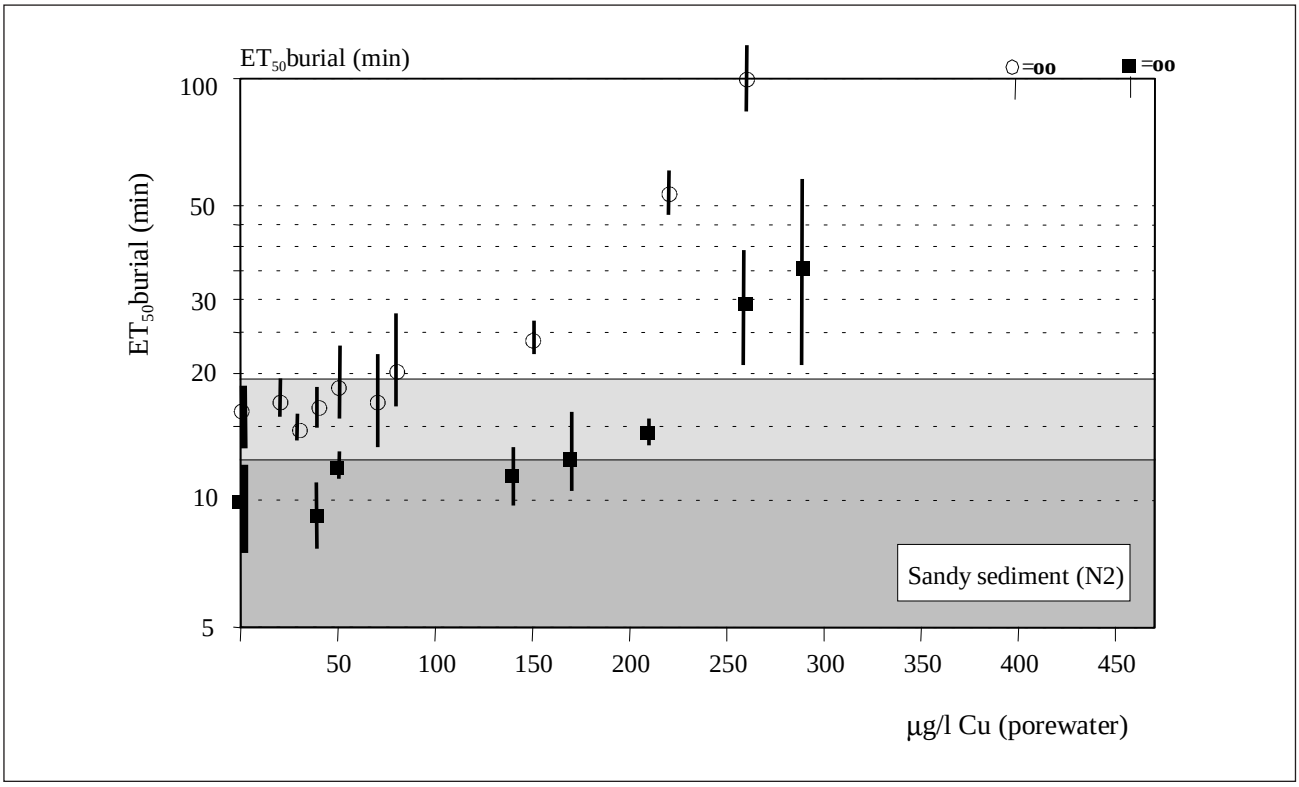

Figure 3. Burial times (ET_{50}) for both clam species logarithmically displayed against porewater copper concentrations on spiked sandy sediment N2. White circles represent data for *V. decussata* and black squares data for *V. pullastra*. Coloured areas indicate range for which burial times are not significantly different from controls: light grey for *V. decussata*, dark grey for *V. pullastra*

emerge to the surface 24-120 h after the start of the assay. This behaviour was practically absent in the controls, and increased with the amount of Cu contamination (figure 4). Furthermore, higher Cu concentrations usually led to faster re-emergence behaviour. The only exception to this observation, at a copper-spiking level of 100 mg/l in figure 4, may be a result of clam impairment due to the high Cu level, so clams were unable to surface. Higher Cu levels already caused shell-closing reactions (90 % of clams on the surface). Counting the clams on the surface at day 4 (96 h), a good relation of surfaced clams to the respective Cu contamination level is obtained. Figure 5 plots clams on the surface at day 4 (96 h) against the actual extractable Cu sediment concentrations.

The surfacing behaviour was less pronounced in aged sediment with almost identical sediment Cu concentrations. However, similar to observations for changes in burial times, lower porewater concentrations could explain the re-emergence behavioural pattern. On the other hand, the fact that porewater Cu levels are not stable, but decline over the time range during which re-emergence was observed, complicates the interpretation. Based on porewater Cu levels, the data suggest in this case a higher sensitivity of *V. decussata;* levels between 22 and 51 µg/l of Cu caused significant surfacing behaviour. *V. pullastra* was observed to re-emerge at 56-84 µg/l, whereas 19-79 µg/l did not cause surfacing.

Copper concentrations that caused burials delays and re-emergence were also observed to seriously affect clam health over the assay period of 10 days. Copper toxicities for the two species appear to be very similar: the LC_{50} (10 days) for *V. decussata* was determined as 100* (34-307) µg/l Cu in the porewater on assays with sandy sediment N2. An LC_{50} (10 days) of 88* (64-125) µg/l Cu was determined for *V. pullastra* in the same sediment. At lower Cu concentrations, clams were alive, but showed impaired burrowing behaviour. Reburial assays with clams from 10-day assay exposure gave NOECs of 22-51 µg/l Cu for *V. decussata* and 63-75 µg/l porewater Cu for *V. pullastra*. Higher Cu levels led to distinct slowing of reburial of exposed clams on clean sandy sediment.

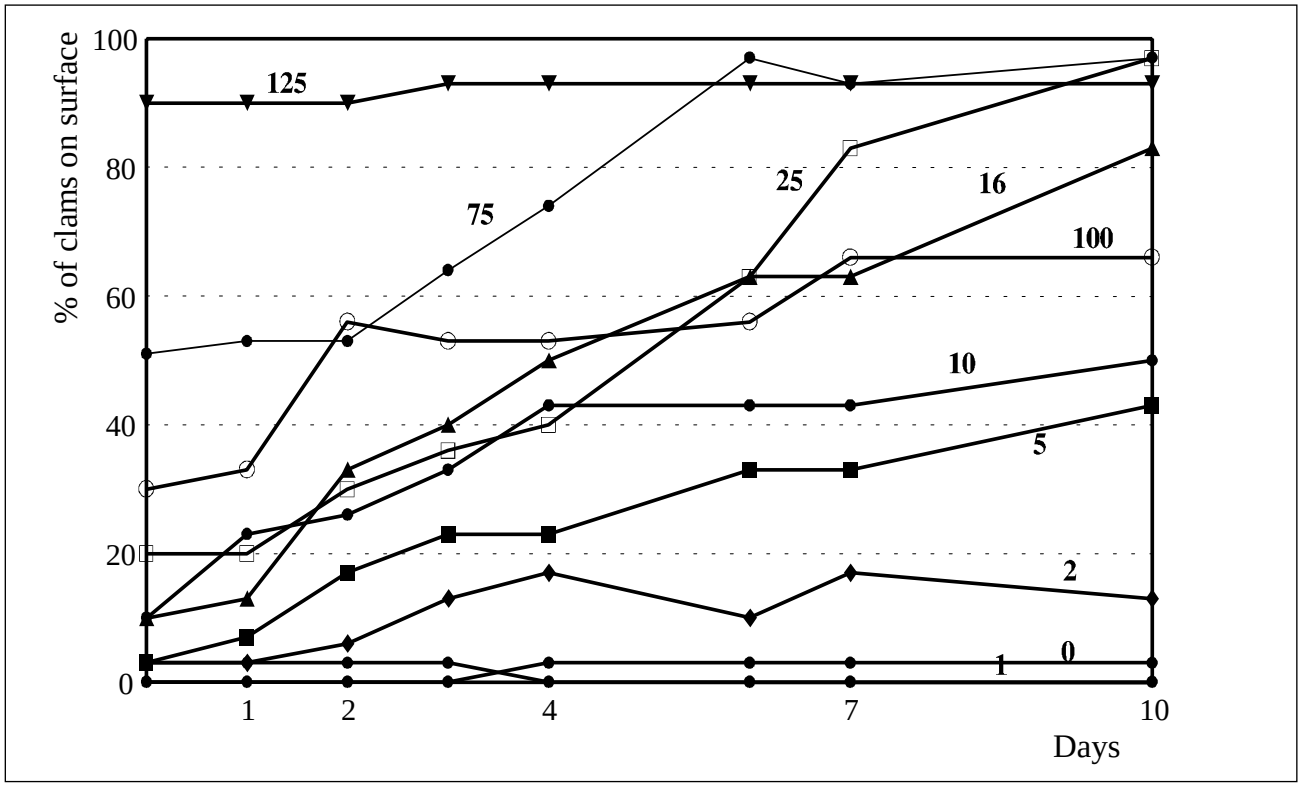

Figure 4. Re-emergence of *V. decussata* from sediment in 10-day period. Numerals labelling surfacing curves are concentrations of copper in spiking solutions (in mg/l) with which sediments were treated prior to running the bioassay. Numbers of clams on surface are average values from three repetitions (of 30 clams); standard deviations are not shown

DISCUSSION

A static-renewal biotest for laboratory assays with *V. decussata* and *V. pullastra* using burial as principal sublethal endpoint was conducted. Similar to findings of other authors with other clam species (Phelps *et al.*, 1983; Emerson, Grant and Rowell, 1990), size was observed to increase the burial times. Narrowing the size window and controlling abiotic factors, constant values for average burial time could be obtained for the two species in sandy sediments.

Understanding how benthic organisms respond to different sedimentary environments is important in the interpretation of the responses of benthic populations and communities to environmental stress, including pollution (DeWitt, Ditsworth and Swartz, 1988). Similar to the findings of Alexander, Stantom and Dodd (1993) with artificially prepared sediments of defined grain size, *V. decussata* and *V. pullastra* were observed to be generalist burrowers over a wide range of medium/fine sand sediments with up to 40 % silt content. This is the suggested range for biotesting with the current scenario. *V. decussata* is naturally encountered in clean sands, while *V. pullastra* is also encountered in finer sands and sand/silt mixtures (Vilala, 1950; Pérez Camacho, 1980). This is coherent with the observed higher burrowing facility of *V. pullastra* in finer sediments, compared with *V. decussata* in the burial assays. Adaptations to their habitat, such as shell forms and shell surface, are possible reasons for this observation. Grain-size effects in general are considered to be due to problems of anchorage, particle cohesiveness, and traction (Emerson, Grant and Rowell, 1990; Alexander, Stanton and Dodd, 1993).

Copper-spiking sandy sediment affected clam behaviour. When concentrations were very high, the shell closure reaction was observed. At lower concentrations, an elongation of burial times was observed. Longer burial times for marine bivalves in spiked sediments under laboratory conditions have been found by other authors (Phelps *et al.*, 1983; Phelps, Pearson and Hardy, 1985; Phelps, 1989; Phelps, 1990; Roper and Hickey, 1994; Roper *et al.*, 1995). Furthermore, slowing of burial was also observed in metal-contaminated, field-collected sediment (McGreer, 1979; DelValls *et al.*, 1994; Del

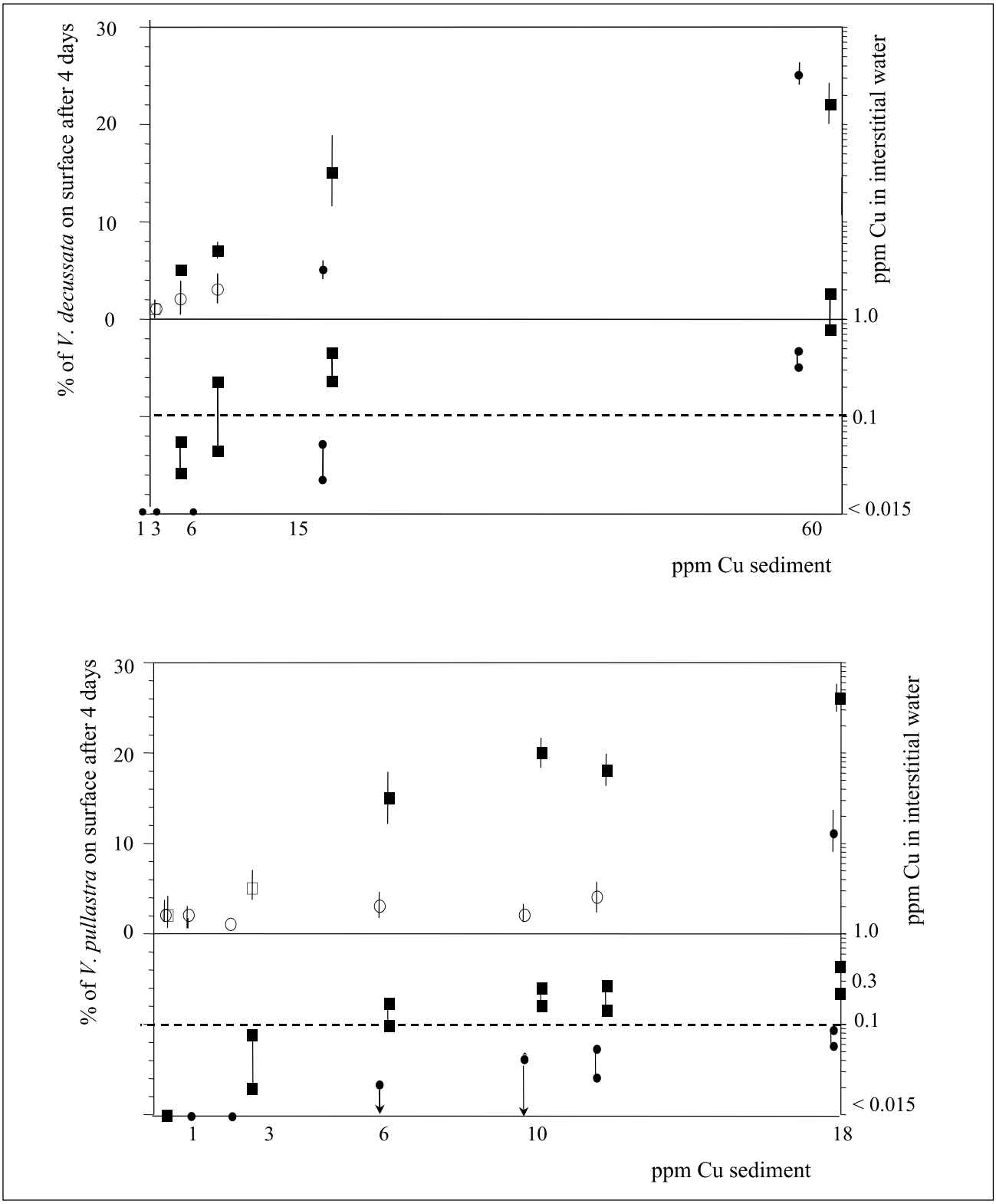

Figure 5. Sediment Cu and interstitial water Cu in relation to clam surfacing. Upper diagram refers to *V. decussata*, lower diagram to *V. pullastra*. Each diagram is divided into two parts: in the upper part, the number of surfaced clams after 4 days (with standard deviation) is plotted according to the left abscissa against extractable sediment Cu. Squares refer to results in original spiked sediment, and circles refer to aged sediment. Black squares and circles are significantly different from control; white symbols are not. On the lower part of each diagram, the range of porewater Cu levels, with minimum and maximum levels from day 1 to day 4, are plotted according to the abscissa to the right of the diagram. Squares are values for original sediment, circles for aged sediment. Note the different scales of the ordinates of the two diagrams

Valls, 1994). Other contaminants, such as oil pollutants and chlorine, have been shown to affect burial (Pearson *et al.*, 1981; Olla, Bejda and Pearson, 1983; Stenton-Dozey and Brown, 1994). Slowing of burial appears to be a common response for marine bivalves upon exposure to toxicants.

In the present study, both clam species were increasingly slowed in their burrowing speeds by increasing levels of Cu contamination, and an exponential relationship was observed. The lengthening of burrowing time was due to those clams that totally refused to bury themselves (especially at very high Cu levels), clams that began burrowing but only slowly proceed to bury themselves completely, and clams that remained for a very long time half-submersed in the sediment without continuing to burrow. Initially, the number of clams on the surface at a certain time was also measured to determine the LC_{50}, but this was unsatisfactory, because the arbitrary choice of the time endpoint could affect the results.

Initially, burial times were believed to be related to Cu found in sediment, since higher contamination levels resulted in longer burial times and other authors had presented changes of burial times in relation to added metal in spiked sediment (Roper and Hickey, 1994; Roper *et al.,* 1995). However, the results of the present assay obtained in aged sediment showed that, for almost identical Cu concentrations in the sediment, the impact on slowing of burial was much weaker. On the other hand, porewater Cu levels satisfactorily explained all observed burial time increases (in original and aged sediment) and similar Cu levels in the porewater always caused similar burial time values, even when corresponding sediment concentrations were different. Phelps *et al.* (1983) and Phelps, Pearson and Hardy (1985) also came to the conclusion in their spiking assays that porewater Cu caused the slowing of burial reaction. The experimental difficulty of distinguishing between effects caused by sediment metal and metal in porewater lies in the fact that porewater metal is inherently coupled to the sediment concentrations, and it is difficult to manipulate their concentrations separately (Kemp and Swartz, 1988). In the present study, advantage was taken of the fact that interstitial water Cu concentrations and sediment- bound Cu had not yet established an equilibrium at the beginning of the assay. Thus, the two factors could be examined separately by repeating the assay under the same conditions, using aged sediment.

Surfacing of sediment-dwelling organisms due to contaminants has not been observed to the same extent as burrowing in the literature. As far as marine bivalves are concerned, only Olla *et al.* (1984) commented on surfacing due to oil-contaminated sand. In our assays re-emergence from sediment began for both clam species 24 h after the start of the bioassay and had concluded generally after 120 hours. A direct relationship between Cu contamination levels and the re-emergence reaction could be observed. For smaller concentration levels, only part of the clam population was observed to re-emerge, even though some impairment (inability to rebury) at these levels over the 10-day period did occur. This proportionality of the reaction would suppose an individually-differing threshold for toxic conditions. Swartz et al (1985) observed that never more than 60 % of an amphipod population showed emergence due to Cd levels in sediment.

For quantification of this behaviour as a sublethal endpoint parameter, we decided to examine the number of clams on the surface at 4 days. This is justified, since surfacing was observed predominantly in the first days of the assay and since characteristic differences to the control that could be observed in longer time periods (e.g. 10 days) had usually already been established by day 4. To increase the efficiency of the assay, it was important to limit the time factor.

Aging of spiked sediments reduced the surfacing phenomenon. The surfacing data for aged and freshly-spiked sediment are similar, however, when compared with the respective interstitial water concentrations. The decrease of toxicity in aged sandy sediment, as measured by the behavioural endpoint of surfacing, could therefore be largely explained by a dilution of the interstitial water in aged sediment, as was done in the burial assay. However, to establish a stringent relationship is quite difficult, since it should take into consideration a range of declining Cu concentrations (from day 1 to 4).

The question to be asked is whether the observed behavioural changes are indeed directed responses to contaminants. Olla, Bejda and Pearson (1983) and Phelps, Pearson and Hardy (1985) believe burial delay could be an adaptive strategy to avoid toxicants. Olla *et al.* (1984) give three criteria to justify labelling behaviour patterns as directed responses: the detection of the toxicant, recognition of the toxicant as inimical, and the appropriate response by the animal to avoid exposure to the toxicant. In

the present study, lower burial rates certainly did not keep the clams from being exposed to potentially toxic conditions and even increased the chance of predation. Hence, it seems that this behavioural change does not constitute an appropriate response. Burial refusal, however, in connection with shell closure effectively contributed to the survival of a part of the population, but was only observed at very high Cu levels. Valve closure is a common escape response in burrowing bivalves suddenly exposed to a chemical pollutant, as well as to drastic changes in salinity. Some ability to detect contaminants must be present in the clams, otherwise the clam closure reaction for very high Cu levels and the slowing of burial could not be explained satisfactorily, especially since impairment seems unlikely due to short exposure times. In this context, it is also important to note that the form of Cu responsible for the clam reaction was found in the water rather than in the sediment, being more detectable and accessible to chemotactile senses. Molluscs are known for their various mechano- and chemoreceptors (Purchon, 1977).

On the other hand, there are reasons to believe that the emergence behaviour of the two clam species was a direct behavioural response, according to the criteria cited above. Possibility of detection and recognition of the contaminant as toxic can be assumed from the existence of both the burial and the emergence responses, which show a clear correlation to contaminant levels, and the shell-closing reaction. In contrast to slower burial, the surfacing phenomenon is a more adequate response to the toxic sediment, since the clam actively tries to avoid the contaminant. Moreover, surfacing forces the clams to leave their habitat (the sediment), which provides food and protection against predation, and hence would only be expected to happen in extreme cases of danger (such as toxic contaminant levels). On the sediment surface, passive displacement by wave action could be part of a survival strategy, similar to the drifting observed by Roper *et al.* (1995).

That the re-emergence by *V. decussata* and *V. pullastra* is triggered by physiological impairment due to intoxication should also be considered, since the emergence behaviour in many cases did not save the clams from long-term debilitation (reduced reburial). However, it is also true that these effects would have been higher if no re-emergence had occurred, simply because of longer exposure times.

The two clam species generally behaved similar in the assays. *V. pullastra* was slightly more sensitive in burial assays: significant delays were observed in relation to porewater Cu levels at around 100 µg Cu/l. For *V. decussata,* 166 µg Cu/l had caused no burial delay, whereas concentrations of 203 µg Cu/l and higher in the interstitial water did cause significant burial delay. *Protothaca staminea* also slowed burial at porewater concentrations of 200 µg Cu/l (Phelps, Pearson and Hardy, 1985). Slowing of burial is observed at concentrations comparable to our results for 10-day chronic toxicity tests. Burial refusal occurred for both species at levels around 390-460 µg Cu/l in porewater. To adequately describe the behavioural pattern refusal to bury, concentrations in the water/sediment interface should be measured, which was not done in the current study. Re-emergence is a more sensitive toxicological endpoint measure than burial. Significant changes in behaviour were observed in aquaria that had caused no burial delay. Porewater Cu levels of 50 µg/l Cu at the beginning of the assay (porewater levels always declined) caused significant re-emergence. In this case, *V. decussata* appeared to be more sensitive. These values are very similar to significant concentrations obtained by reburial assays after a 10-day exposure to contaminants, thus directly related to thresholds for impairment.

In the natural environment, similar and higher Cu levels are encountered due to anthropogenic sources of metal, e.g. harbours, mining rivers, spills. In harbours from the New England coast, 60 and 100 µg/l of Cu were measured (Burgess *et al.*, 1993); Restronguet Creek showed interstitial water on the order of 100 µg/l Cu, whereas no more than a few ug/l are found in interstitial water from clean sediments (Bryan and Langston, 1992). To these levels, the very large concentrations of several other contaminants would contribute to the toxicity effect. Hence, the application of our bioassay to determine toxicity in the field or of field-collected sediments seems possible.

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