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Survival and Growth of *Corophium volutator* in Organically Enriched Sediment: A Comparison of Laboratory and Field Experiments

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Abstract: In this study, the amphipod *Corophium volutator* (Pallas) was evaluated as test organisms for use in sediment toxicity tests by adapting standard protocols for conducting 10-day and 28-day sediment toxicity tests. Combine laboratory and field bioassays showed that *Corophium* can survive in organically enriched sediment if they have no alternative, suggesting that *Corophium* is relatively tolerant of organically enriched sediment. Neither were there effects on emergence or reburying behaviour. Therefore this bioassay is considered inappropriate for estimating the quality of organically enricment sediment.

Key Words: *Corophium volutator,* toxicity, emergence, rebury

Corophium volutator'un Organikce Zengin Sedimentlerde Yaşama ve Büyümesi: Laboratuvar ve Arazi Deneylerinin Karşılaştırılması

Özet: Bu çalışmada amfipod *Corophium volutator* (Pallas) standard protokolden adapte edilerek 10 ve 28 günlük sediment toksisti deneylerinde bir test organizması olarak kullanılmıştır. Laboratuvar ve arazi çalışmaları *Corophium*'un eğer bir alternativi yoksa organikce zengin olan sedimentlerde yaşayabileceğini göstermiş ve buda *Corophium*'un organikce zengin sedimentlerde karşı tahammül edebileceği önerisini getirmiştir. Bu yüzden bu biyolojik deneyin organikce zengin sedimentlerin kalitesi ölçümlerinde uygun olmadığı görüşüne varılmıştır.

Anahtar Sözcükler: *Corophium volutator,* toksisiti, çıkma, tekrar gömülmek.

Introduction

The results of earlier works (1-3) have demonstrated the potential of the bioassay in the laboratory, but a potential problem of laboratory bioassays is that the test species might behave or react differently under field conditions. In other words, the actual effects of pollutants in the field may be different from the potential effects observed in the laboratory (4). Moreover, laboratory experiments are in many ways unrealistic in that they cannot simulate all the environmental influences and changes that continually occur in the field. Therefore, field bioassays may be more useful for predicting the potential effects of pollutants on the aquatic environments. However, there are many limitations of field bioassays (5), the chief one being that environmental variables are not held steady, so that field bioassay is difficult to monitor and often impossible to repeat at different times. Ideally, data on pollutants and mixtures should be obtained from a combination of laboratory and field bioassays, so that the combined data can provide more complete information about the potential impact of pollutants on the aquatic environment (5). For instance, Lenihan *et al.* (6) conducted a series of field and laboratory experiments with benthic invertebrates exposed to sediments contaminated with petroleum hydrocarbons and to sewage from McMurdo Station, Antarctica. Both experiments showed similar patterns of increased avoidance of sediment and decreased survival in sediments near to the source of contamination, Winter Qurters Bay in particular by the phoxocephalid amphipod *Heterophoxus videns.* This study describes how the *Corophium* bioassay approach might be similarly used in the field and laboratory to evaluate sediment quality. We therefore took the opportunity to evaluate the test's potential for assessing the quality of sediment with respect to organic enrichment.

Organic input into any freshwater or marine benthic area from natural or antropogenic sources, results in changes in a complex of chemical, physical and micro-biological factors which in turn have direct or indirect effect

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effects on the fauna inhabiting the area (7). These authors (7) have described dramatic and consistent changes in community composition with increasing distance from the source of contamination. Sediments closest to the point of enrichment may be so hostile in their physico-chemistry that they contain no macrofauna at all: As enrichment decreases slightly a few, very tolerant or opportunistic species occur in large abundance, thought to be due to the lack of competition and a rich food supply. With less enrichment, there is a lower abundance of organisms with more species and areas unaffected by the organic enrichment contain a relatively high diversity of species (Fig. 1).

There have beer several studies on the direct or indirect effects of organic enrichment on *Corophium volutator* (8-11) and *Corophium* has often been considered tolerant of enrichment (12; see also review by O'Sullivan (13). In the Ythan there are both direct and indirect effects of enrichment. Rafaelli *et al.* (8) have argued that levels of nutrients (nitrogen and phosphorus) mainly from agricultural run-off and but also locally from domestic sewage discharges, are likely to be responsible for the observed eutrophication in the Ythan since early 1960s. This is reflected by enhanced growth of opportunistic green macro-algae, such as *Enteromorpha* and *Ulva* spp. (8). Rafaelli *et al.* (9) have shown that *Corophium*

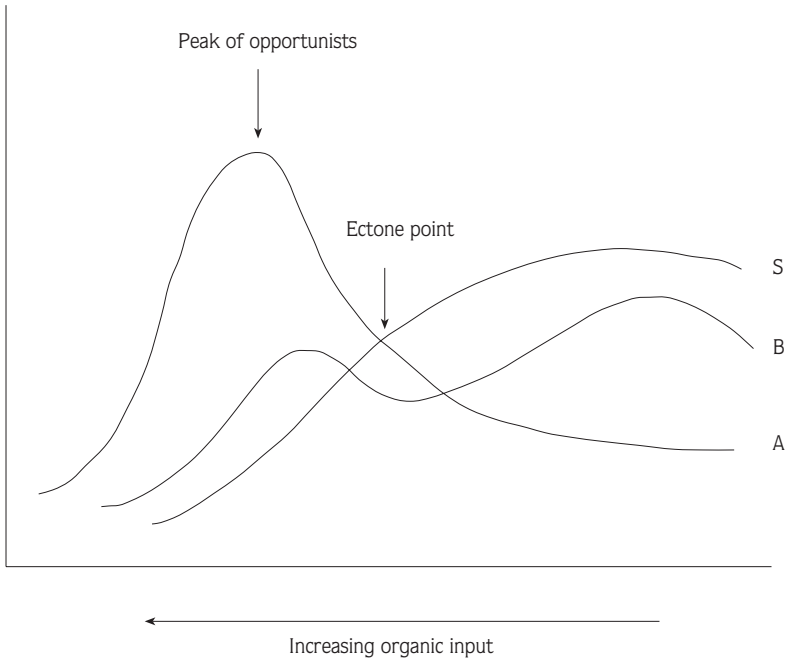

Figure 1. Generalised SAB diagram of shanges along a gradient of organic enrichment. (From Pearson and Rosenberg, 1978).
S=Species numbers
A=Total abundance
B=Total biomas

declines dramatically in abundance under macro-algal mats, mostly due to the phsical effects of the mats, although decaying weed also increases the BOD and generates a hostile physico-chemical environment in the sediment. The South Quay mudflat of the Ythan estuary (Fig. 2) is patchilly covered by macro-algae in summer in a predictable way, such that areas receiving high inputs of organic material are easily recognisable from year to year. *Corophium* can only recolonise these organically enriched patches after the weed has become buried or dispersed in September/October (8). In addition to the weed patches this mudflat is adjacent to Newburgh's domestic sewer (primary treatment only) and a series of surveys (*e.g.* 14, 15) have consistently identified areas of the mudflat differing in their ecology due to this enrichment. Both laboratory and field bioassays were therefore carried out on these sediments from the Newburgh South Quay mudflat.

Materials and Methods

The basic designs of two laboratory and field experiments are shown in Fig. 3 (see details below). Field experiments were carried out on the mudfalt (Fig. 2) at two sites (Fig. 4). Site A was close to the outfall which also has extensive macro-algal mats in summer but these were not evident at the time of the experiment (April). No *Corophium* were found here at all whereas Site B supported *Corophium* and is much less enriched.

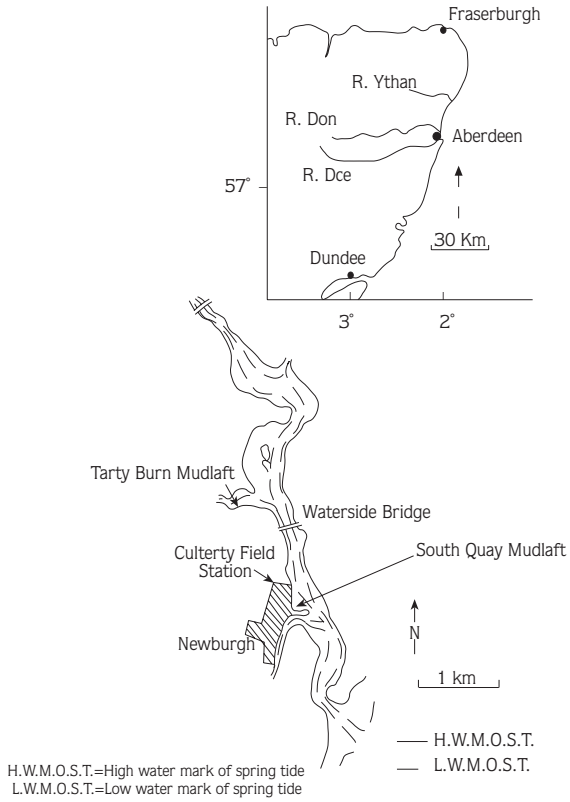

Figure 2. The Ythan estuary, showing locations for collection of sediment and the site of the field experiment.

Animal collection

All *Corophium* were collected grom the mudflat area opposite Culterty Field Station and well away from any effects of enrichment at the Quay. Animals were then sorted into two size classes: 4-7 mm (adults) and 2.5-4 mm (juveniles) for use in 10-and 28-day sediment bioassays, respectively. The 28-day bioassay with juvenile *Corophium* was devised by Ciarelli and Vonck (16) for developing a chronic toxicity test using growth as sublethal endpoint.

Sediment collection

Control sediment (not affected by organic enrichment) was collected from Tarty Burn (Fig. 2). This area is known to be unaffected by sewage and supports a healthy population of *Corophium* (Rafaelli, personal observations), but has sediment characteristics similar to those of South Quay (S. Way, unpublished data). Test sediments were collected from the two sites at South Quay (described above). All sediments were passed through a 250 μm mesh to remove any associated macrofauna, including *Corophium.* These sediments were used in laboratory bioassay experiments. Tarty Burn sediment was also used as a control in the field experiments. Additional sediment samples were taken from South Quay and Tarty

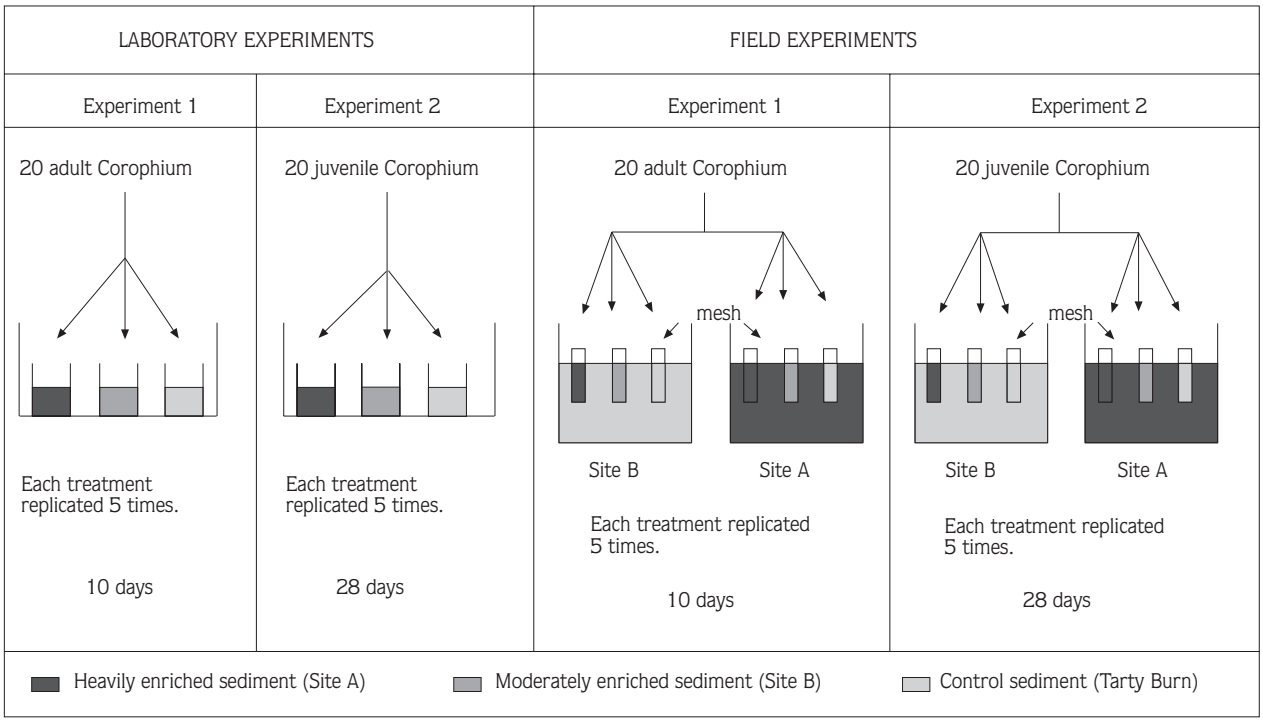

Figure 3. Diagram showing experimental design of two laboratory and two field experiments.

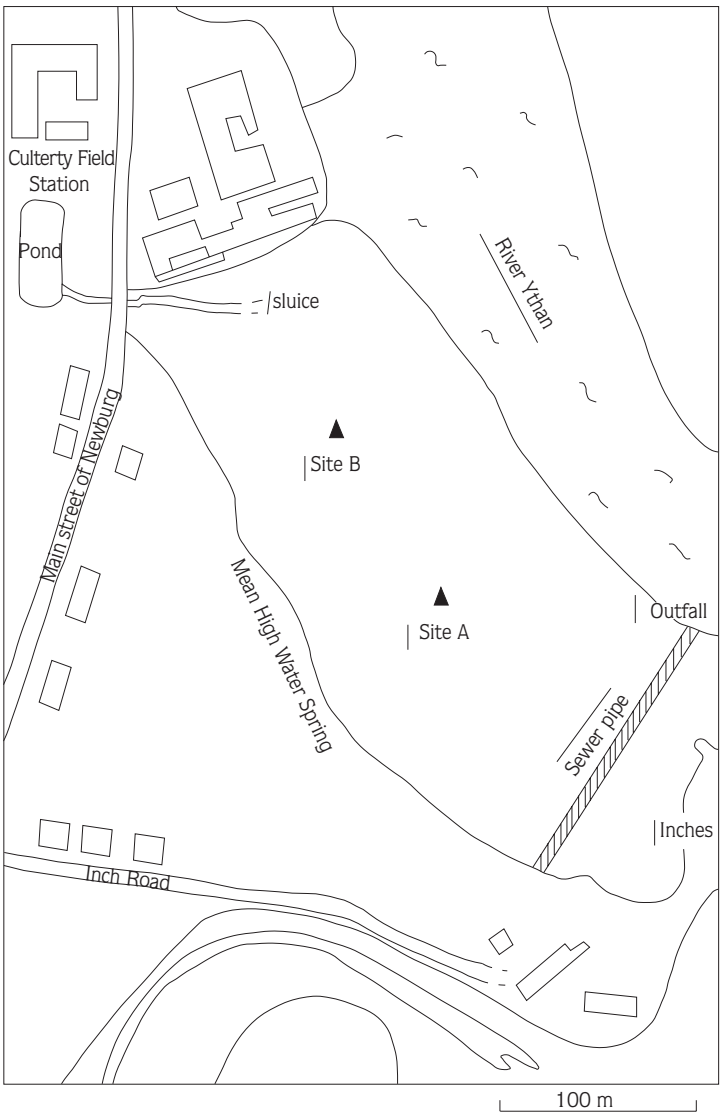

Figure 4. The location of the sites chosen in the Newburgh South Quay mudflat.

Burn for analysis of copper, cadmium, zinc, lead, chromium, nickel and arsenic. These metals were chosen because their toxicity to *Corophium* and other amphipods is well documented (1-3, 17-23).

Samples for total sediment organic carbon analysis were dried at 60°C in an oven for 48 h. A five gram sample was then treated with hydrochloric acid vapour overnight in a desiccating jar to convert any calcium carbonate to chlorides. Weighed, dired samples were then placed in a muffle furnace at 600°C for four hours and the loss on ignition taken as the organic carbon content of the sediment (24).

Experimental design

Laboratory experiments

Corophium were placed in containers containing static clean seawater and a 2 cm layer of sediment collected from Tarty Burn (control sediment) and sites A and B from South Quay (test sediments). Survival, emergence from sediment and reburial were recorded for a 10-day sediment bioassay, and growth was measured in a 28-day sediment bioassay. Five replicates of the control and test sediments with 20 amphipods (adults for 10-day and juveniles for 28-day) per container were used in all bioassays.

The number of dead and emerged amphipods was recorded daily. After days any live *Corophium* were

allowed to rebury in clean Tarty Burn sediment, as described by Bat (3). At the end of the 28-day bioassay, the number of survining amphipods in each container was noted, individuals with clean seawater, depurted in clean seawater for 48 h and dried at 70°C for 48 h.

Tests were run at ambient seawater temperature to ensure comparability of the field and laboratory bioassays.

Field experiments

For the field experiment, Tarty Burn (control) sediment was passed through a 250 mm mesh to remove any associated macrofauna, including *Corophium* and this material transferred to both sites (Site A and Site B) at South Quay (Fig. 5). At both sites the top 2 cm layer of original sediment was gently removed to eliminate any associated benthic organisms. Sediment from Site A was then transferred to Site B and vice-versa (Fig. 5). It should be noted that if enrichment is primarily a surface effect, then this may have lessened any differences between Site and Site B with respest to sediment quality. The implications of this are discussed later. In first field experiment, 15 corers (8.5 cm in diameter and 14.5 cm deep) were sunk 10 cm into the sediment (5 of each sediment type) at each site and 20 adult amphipods were added to each corer. Immediately after adding animals a 500 μm mesh was secured over the top of each corer which was then depressed a further 2 cm into the sediment. Corers were open at top and bottom to permit free drainage. After 10 days, corers were collected, returned to the laboratory and amphipods removed by passing sediments through a 500 μm mesh. For each corer the number of survivors was recorded. Any live *Corophium* were allowed to rebury in the Tarty (clean) sediment.

In the second experiment, only juvenile *Corophium* were used. The methodology was the same as the 10-day field experiment but this time each core was covered with a smaller (250 mm) mesh as described above and the experiment ran for 28 days. At the end of the experiment, survivors in each core were counted, washed with clean seawater, depurated in clean seawater for 48 h and then dried immediately at 70°C. After 48 hours, they were weighed.

The mean numbers of animals survining in the laboratory and field experiments were compared with ANOVA. The Tukey test was used for multiple comparisons between means (25).

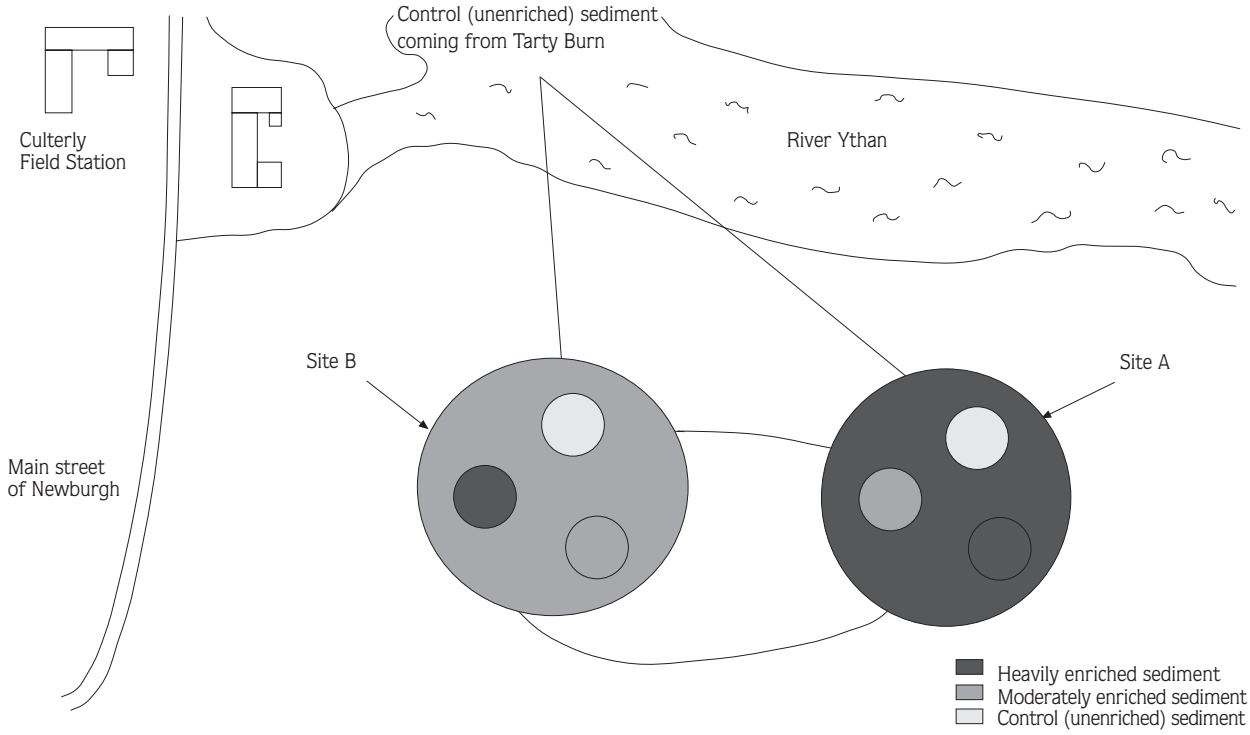

Figure 5. Design of the field experiments on South Quay

Results and Discussion

Despite expectations, no differences were found in the organic content of the sediments from sites A and B, but they differed the controls in this respect (Fig. 6) (ANOVA;$F_{2, 6}$=6.09, p=0.036). However the average organic content values for the top 2 cm was 12.28% and 10.17% in Site A and B, respectively and this difference was just statistically significant (t=4.801, p=0.041), confirming that the areas were originally different in this respect and that removal of the top 2 cm (see Methods) may have obscured this difference.

The concentrations of Cd, Pb and Ni in all of the sediments were under the limit of detection, whereas the concentrations of Cu, Cr and As were <1 ppm and Zn <6 ppm. From previous works (1-3), it is unlikely that such low metal concentrations would have any effect on amphipod survival, emergence, growth and ability to rebury.

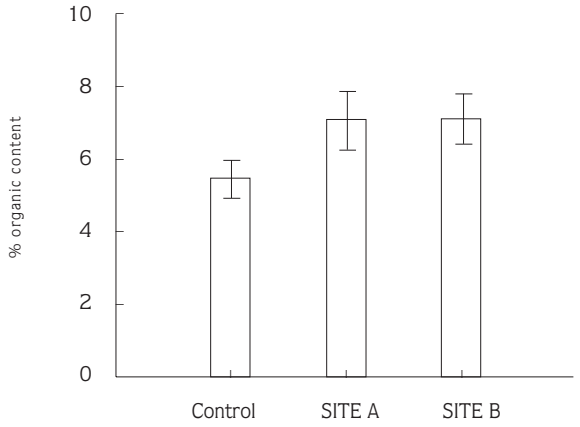

Figure 6. Percentage of organic content in the three sediments. (CONTROL=unenriched sediment from Tarty Burn, SITE A=highly enriched sediment from South Ouay, and SITE B=moderately enriched sediment from South Quay). (Error bars=SE).

Laboratory experiments

The mean temperature for the 10-day experimental period in all bioassays was 11°C±1, dissolved oxygen was 89%±4, salinity was 31%±1 and pH was 7.25±0.12. In the 10-day laboratory bioassay, survival of *Corophium* was high (Fig. 7) and not significantly different for all the sediments tested (Table 1). Control survival ranged from 99% to 100% across all the experiments. Only 1.7% of amphipods that were exposed to organically enriched sediment died.

Only a mean of one *Corophium* emerged from the control sediments under laboratory bioassay conditions, whereas marginally more emerged from Site A and B sediments (Fig. 8). Although the difference was small, it was significantly different (Table 2). All live *Corophium* were able to rebury in celan sediment within 1 hour at the end

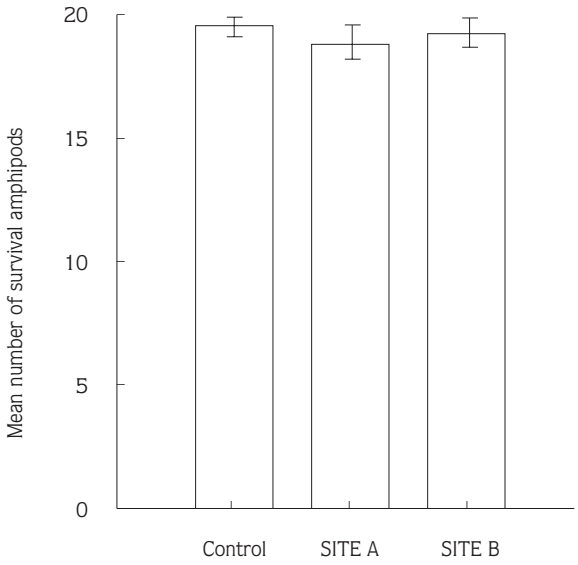

Figure 7. Survival of *Corophium* after 10 days of exposure to control and organically enriched sediments in the laboratory experiment. Each value included 5 peplicates of 20 amphipods. (CONTROL=unenriched sediment from Tarty Burn, SITE A=highly enriched sediment from South Quay, and SITE B=moderately enriched sediment from South Quay). (Error bars=SE).

Table 1. One way ANOVA of survival of Corophium exposed to organically enriched sediment in a 10-day laboratory bioassay.

Source	df	SS	MS	F	P	Homogenous subsets		
Site	2	0.933	0.467	0.87	0.442	Siet A	Site B	Control
Error	12	6.400	0.533					
Total	14	7.333						

of the 10-day period. The mean temperature for the 28-day experimental period in all bioassays was 11°C±2, dissolved oxygen was 85%±5, salinity was 31%±2 and pH was 7.25±0.18. In the 28-day laboratory bioassay mean survival was high in all sediments (Fig. 9), but mortality was significantly higher at the most enriched sediment (Site A) compared to moderaterly enriched (Site B) and control sediment (Table 3). The final mean weights of amphipods in all sediments tested are shown in Fig. 10. There were no significant differences between sites (Table 4).

Field experiments

Survival of *Corophium* in the control (Tarty Burn) sediment in the 10-day field bioassay was high and similar to that in the two South Quay sediments (Fig. 11: Table 5 and 6). Although survival was always slightly less and in original Site A sediment, there were no significant differences between sites (Tables 5-6).

All live animals were able to rebury at the end of the 10 days. Survival of animals in the 28-day field bioassay was also high (Fig. 12). Again there were no significant

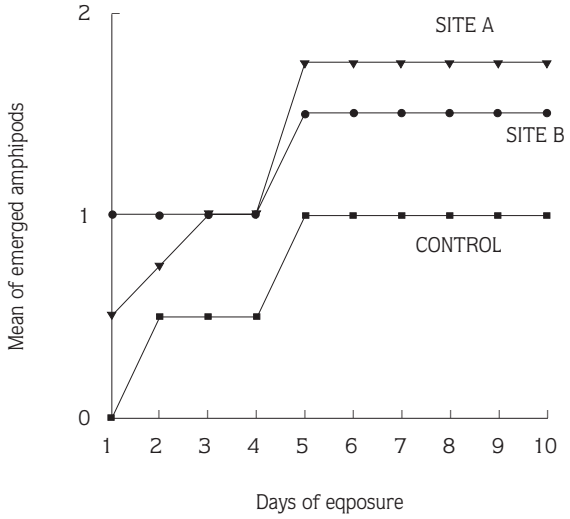

Figure 8. Emergence of *Corophium* fron control sediment and from organically enriched sediments in the 10-day of in the laboratory experiment. Each value included 5 replicates of 20 amphipods.
(CONTROL=unenriched sediment from Tarty Burn,
SITE A=highly enriched sediment from South Quay, and
SITE B=moderately enriched sediment from South Quay).
(Error bars=SE).

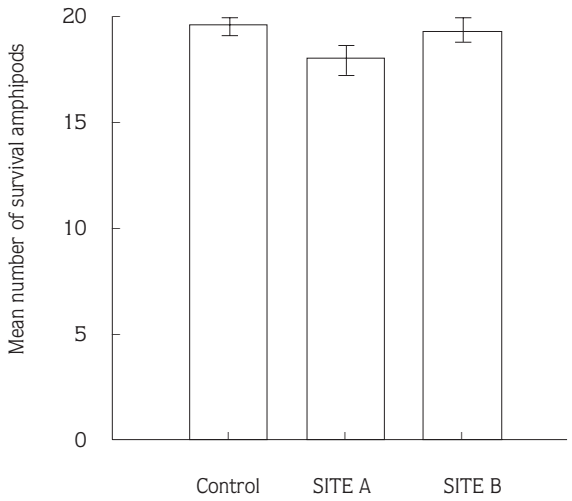

Figure 9. Survival of *Corophium* after 28 days of exposure to control and organically enriched sediments in the laboratory experiment. Each value included 5 replicates of 20 amphipods.
(CONTROL=unenriched sediment from Tarty Burn,
SITE A=highly enriched sediment from South Quay, and
SITE B=moderately enriched sediment from South Quay).
(Error bars=SE).

Table 2. One way ANOVA of emerged *Corophium* exposed to organically enriched sediment in a 10-day laboratory bioassay.

Source	df	SS	MS	F	P	Homogenous subsets		
Site	2	2.33	1.165	7.85	0.002	Siet A	Site B	Control
Error	27	4.01	0.148					
Total	29	6.34						

Table 3. One way ANOVA of survival of *Corophium* exposed to organically einriched sediment in the 28-day laboratory bioassay.

Source	df	SS	MS	F	P	Homogenous subsets		
Site	2	7.60	3.8	10.36	0.02	Siet A	Site B	Control
Error	12	4.40	0.367					
Total	14	12.0						

Table 4. One way ANOVA of final weights of *Corophium* exposed to organically enriched sediment in the 28-day laboratory bioassay.

Source	df	SS	MS	F	P	Homogenous subsets		
Site	2	0.0085	0.0043	0.40	0.679	Siet A	Site B	Control
Error	12	0.1280	0.0107					
Total	14	0.1365						

Table 5. One way ANOVA of survival of *Corophium* exposed to organically enriched sediment at Site A of South Quay in the 10-day field bioassay.

Source	df	SS	MS	F	P	Homogenous subsets		
Site	2	2.8	1.400	1.5	0.262	Siet A	Site B	Control
Error	12	11.2	0.933					
Total	14	14						

Table 6. One way ANOVA of survival of *Corophium* exposed to organically enriched sediment at Site B of South Quay in a 10-day field bioassay.

Source	df	SS	MS	F	P	Homogenous subsets		
Site	2	1.733	0.867	2	0.178	Siet A	Site B	Control
Error	12	5.200	0.433					
Total	14	6.933						

Table 7. One way ANOVA of survival of *Corophium* exposed to organically enriched sediment at Site A of South Quay in the 28-day field bioassay.

Source	df	SS	MS	F	P	Homogenous subsets		
Site	2	1.733	0.867	1.73	0.218	Siet A	Site B	Control
Error	12	6.000	0.5					
Total	14	7.733						

Table 8. One way ANOVA of survival of *Corophium* exposed to organically enriched sediment at Site B of South Quay in the 28-day field bioassay.

Source	df	SS	MS	F	P	Homogenous subsets		
Site	2	0.933	0.467	0.93	0.420	Siet A	Site B	Control
Error	12	6.000	0.5					
Total	14	6.933						

Table 9. One way ANOVA of growth of *Corophium* exposed to organically enriched sediment at Site A of South Quay in the 28-day field bioassay.

Source	df	SS	MS	F	P	Homogenous subsets		
Site	2	0.0382	0.0191	0.42	0.669	Siet A	Site B	Control
Error	12	0.5505	0.0459					
Total	14	0.5886						

differences in mean survival among transplanted sediments, although that in the highly enriched sediment was marginally lower (Tables 7 and 8).

The mean weights of amphipods after 28 days in the different treatments ade shown Fig. 13. There were no significant differences between the sites (Tables 9 and 10).

Table 10. One way ANOVA of growth of *Corophium* exposed to organically enriched sediment at Site B of South Quay in the 28-day field bioassay.

Source	df	SS	MS	F	P	Homogenous subsets		
Site	2	0.0168	0.0084	0.31	0.741	Siet A	Site B	Control
Error	12	0.3280	0.0273					
Total	14	0.3448						

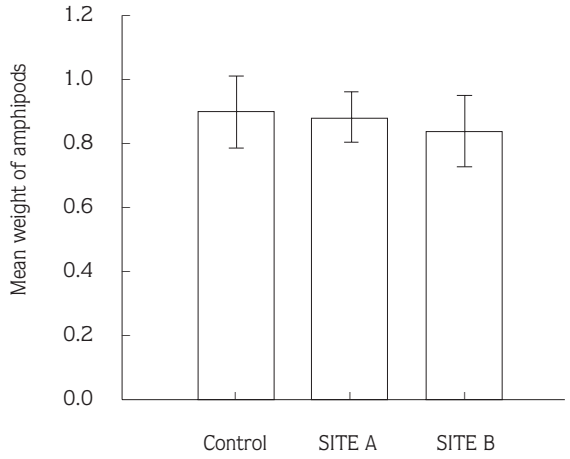

Figure 10. Mean weight of *Corophium* after 28 days of exposure to control and organically enriched sediments in the laboratory experiment. Each value included 5 replicates of 20 amphipods.
(CONTROL)=unenriched sediment from Tarty Burn,
SITE A= highly enriched sediment from South Quay, and
SITE B=moderately enriched sediment from South Quay).
(Error bars=SE).

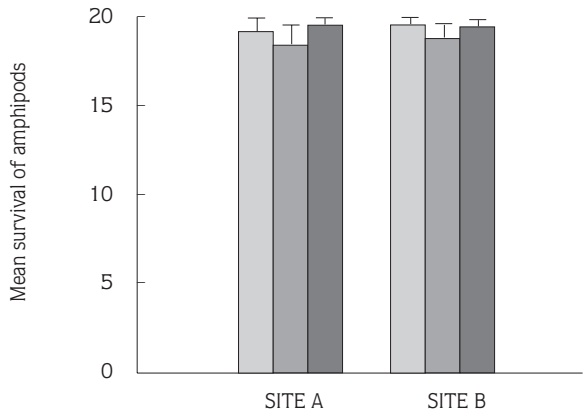

Figure 11. Survival of *Corophium* after 10 days of exposure to control and organically enriched sediments in the field experiment at two sites of South Quay. Each value is based on 5 replicates of 20 amphipods. (Error bars=SE).
Control (unenriched sediment)
Highly enriched sediment
Moderately enriched sediment

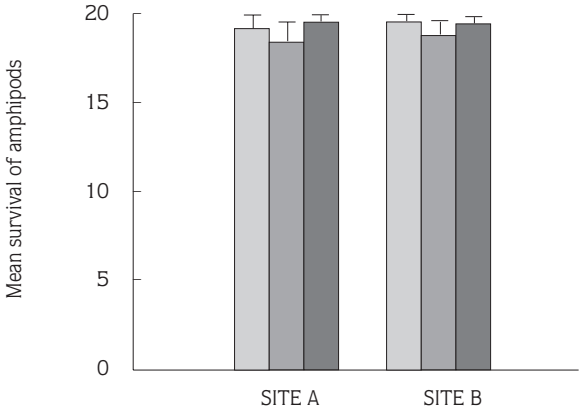

Figure 12. Survival of *Corophium* after 10 days of exposure to control and organically enriched sediments in the field experiment at two sites of South Quay. Each value is based on 5 replicates of 20 amphipods. (Error bars=SE).

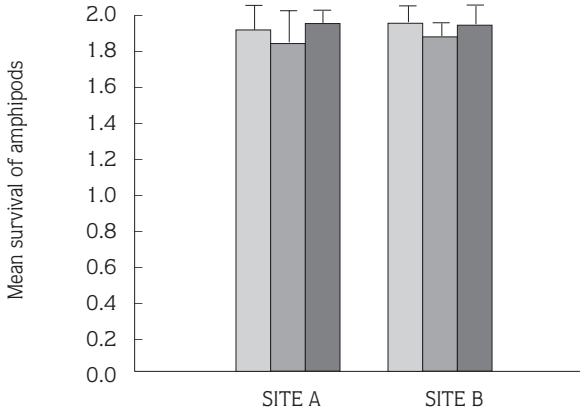

Figure 13. Survival of *Corophium* after 10 days of exposure to control and organically enriched sediments in the field experiment at two sites of South Quay. Each value is based on 5 replicates of 20 amphipods. (Error bars=SE).

Discussion

Clearly, there were no significant differences in survival, emergence, reburial ability and the mean size of individuals in different sediments in either the laboratory or the field experiments. However, if the overall size of amphipods in the laboratory experiments is compared with that in the field experiments, there was a highly significant difference (ANOVA; $F_{2, 42}$=54.46, p=0.001). Clearly, the animals did not grow as well in the laboratory compared to the field. This could be due to an artifact in handling the sediment. Deans *et al.* (26) showed that the collection, handling and treatment of sediments for microbiological and sediment collection experiments often caused marked changes in physical, chemical and microbiological properties. They found that storage of sediment in the laboratory at 10°C for 3 days caused only a small change in the number of heterotrophic bacteria and in the chlorophyll *a* level, but the redox potential and penetrability significantly decreased. They also found that sieving caused a considerable reduction in the number of heterotrophic bacteria or in the chlorophyll *a* level and a loss of sulphide and organic carbon, but a marked increase in redox potential. In addition penetrability decreased and both shear strength and permeability increased (26). Water content and particle size did not change significantly during collection. In the present study, sediments were collected, sieved and transferred to containers. Clean seawater then was added to each container and the sediment and the water were allowed to equilibrate for 48 hours before adding any *Corophium.* These procedures may have changed the properties of the sediment as discussed above, although since they were the same for all sediments, between treatment comparisons remain valid. Furthermore, amphipods exposed to the artificial conditions of a bioassay are likely to be stressed, and in the laboratory experiments, the overlying water was static and not changed during the course of the experiment. This makes it difficult direct comparisons of the results of laboratory and field experiments, but both kind of experiments should be seen as complementary.

The lack of significant differences between sediments at South Quay and Tarty Burn is rather unexpected because the density of *Corophium* in South Quay is very low compared to other sites and its organic content is high (9, 11). The significant difference between South Quay sites was detected in the upper 2 cm and removal of this may well have lessened the changes of finding effects using this bioassay. However, it would not have been possible to carry out the bioassay without removing this layer since it contained numerous macrofuana and, at Site B, many *Corophium.* Celarly, this limits the use of the bioassay for assessing the toxicity of sediments already supporting some *Corophium.* Furthermore, the results of the present study show that *Corophium* can survive and behave normally in organically enriched sediment if they have no alternative, suggesting that *Corophium* is relatively tolerant of such sediments. Although the present study illustrates well the advantages and disadvantages of a combined laboratory and field bioassay approach, it would appear that the *Corophium* bioassay is inappropriate for the estimating the quality of organically enrichment sediments.

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