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Sensitivity Comparison of Laboratory-Cultured and Field-Collected Amphipod *Corophium multisetosum* in Toxicity Tests

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Abstract The feasibility of using lab-cultured amphipods *Corophium multisetosum* (Stock 1952) to evaluate the toxicity of contaminants present within marine sediments was studied. This was done by comparing the sensitivity of lab-cultured amphipods in a cadmium toxicity test and to toxic sediment samples, during a 10-days bioassay, with field collected individuals. Different responses were observed between field and cultured individuals. Cadmium test indicated high temporal variability in the LC_{50} values of field amphipods (2.40–6.55 mg L^{-1}). Sensitivity of cultured amphipods was within the seasonal range of the field individuals (5.81 mg L^{-1}, LC_{50}). However, culture amphipods showed much lower sensitivity in toxic sediment samples. Our results indicate that sensitivity should be determined using a sediment matrix, if the assessment of toxicity is based upon bioassays performed with cultured burrower-amphipods.

Keywords Bioassay · Cadmium · *Corophium multisetosum* · Culture

Bioassays based upon the mortality of amphipods are frequently performed, to evaluate the toxicity of the contaminants present within marine sediments (ASTM 1990; USEPA 2001; OSPAR Commission 2005). Examples are sediments collected from ports (Casado-Martínez et al. 2007), from estuaries (Riba et al. 2004) and from coastal zones used as smelting dumps (Borja et al. 2008). Amphipods have been widely used to characterize the marine environment, due to their abundance and ecological importance in soft-bottom estuarine and marine benthic communities, their worldwide distribution and their sensitivity to toxicants (Casado-Martinez et al. 2007). *C. multisetosum* is probably one of the most appropriate marine amphipods to be used in sediment quality assessments in the Cantabrian Coast (North of Spain) due to their high abundance in the intertidal muddy and sandy flats (Pérez 2006).

Nevertheless, amphipod sampling in order to carry out the bioassays, can encounter certain problems. Firstly, depending upon the season, organisms might not be available in the field, due to their biological cycle. *C. multisetosum* is a burrow- or tube-dweller species, with bivoltine reproduction (two generations/year). Females begin incubating in spring and finish in winter when the water temperature is low (Cunha et al. 2000). According to experience gained from sampling in the Bidasoa Estuary (Northern Spain) since 2005, amphipods are available with a higher probability from April to September in the field, However, unpredictable environmental variables, such as heavy rain, or changes in the morphology of the sediments substrate also affect the availability of these organisms for any season. Furthermore, the sensitivity of field amphipods varies throughout the year, depending upon the biological cycle (Pérez-Landa et al. 2008).

In order to solve the aforementioned shortcomings, amphipods were cultured in laboratory conditions. The purpose of this study was to validate the use of the cultured amphipods for sediment toxicity testing, by comparing their responses to those of field amphipods.

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Materials and Methods

Laboratory tasks included culture maintenance, the performance of toxicity tests with cadmium (as a reference compound) and the accomplishment of bioassays, using sediments with different toxicity levels.

Corophium multisetosum was collected by hand in the intertidal muddy and sandy flats of the Bidasoa Estuary (43°20.570N, 1°45.880W) following sieving of the sediments (1 mm mesh). Cultures were maintained, under controlled conditions, over approximately one year (from 27-Jun-07 to 2-Aug-08). Following the EPA guidance protocol (USEPA 2001), 200 to 300 individuals, comprising all the living stages (from planktonic phases, to 9 mm adults), were located in each of 3 trays (18 cm width × 33 cm length). The bottoms of the trays were covered to a depth of 1.5–2 cm by sieved sediment (<1 mm), collected from the amphipods natural habitat. The sediment had previously been frozen, in order to eliminate organisms that could act as pathogens or competitors. Filtered (Whatman GF/C) sea water was poured into the trays, reaching a height of 4 cm above the sediment bed. Amphipods were nourished 3 days a week by MicroGram (SERA; 0.09 g/tray); this was enriched by one drop of a vitamin complex, FishTamin (SERA). Leaf litter from the estuary of the Bidasoa (previously frozen, dried at 60°C and ground) was sprinkled over the trays once weekly. Cultures were maintained at a constant temperature of 20°C and with a salinity of 25 psu. Three days a week, dead individuals were removed and the overlying water was partially renewed, by filtered sea water. The sediment within the trays was completely removed, every 2 months, and replaced by fresh sediment.

In July 2008, following the protocol described by the RIKZ (Public Dutch Institution for Marine Research) and by Schipper et al. (1999), a 72-h liquid phase toxicity test was carried out on the field organisms (Fig. 1). Cadmium chloride was used as the reference compound, in order to specify the seasonal sensitivity ranges. Dissolved cadmium was prepared from an analytical reagent grade $CdCl_2$ stock solution.This exploratory approach helped to refine the experimental design of subsequent toxicity tests, in terms of cadmium concentrations and replicates.

Considering the number of cultured amphipods, a cadmium toxicity test and a sediment phase bioassay were performed. In this way, in August 2008, a 72-h toxicity test was conducted, this time with organisms from the cultures and from the field. The nominal concentrations were 0, 2, 5, 10 and 20 mg L^{-1} of cadmium, with two replicates per concentration and with 15 individuals per replicate. Amphipods were maintained at 15°C, 32 psu salinity, and exposed to a 16/8 h light/dark photoperiod. Temperature, salinity, dissolved oxygen, pH and the actual Cd concentrations were checked at the beginning and end of each of the tests. The last determination was performed using atomic absorption flame determination AAS 800 (Perkin Elmer). Before each analysis, a patron sample (Cd 1 mg L^{-1}) was prepared in order to ensure the accuracy of the Cd measurements. After 72 h, surviving organisms were counted, to calculate the mortality percentage. The Lethal mean concentration (LC_{50}) calculation was derived from an adjustment, by a Probit analysis to a log-normal class mortality curve (Pérez-Landa et al. 2008). Sensitivity differences between cultured and field organisms were compared, by means of the following statistical test (1) (Environment Canada 2002):

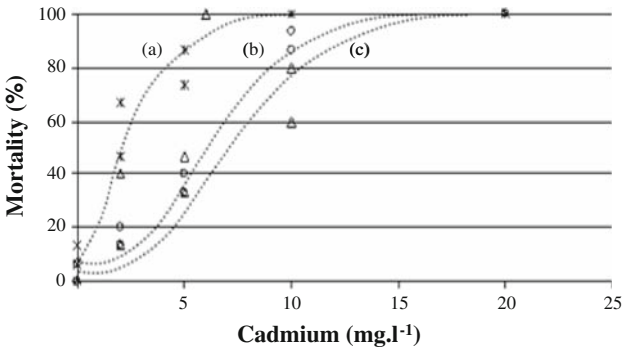

Fig. 1 Mortality (%) of cultured and field amphipods (*C. multisetosum*) related to cadmium concentration (mg L^{-1}). Key: **a** *x*: field individuals (August 2008), $LC_{50} = 2.40$ mg L^{-1}; **b** *o*: cultured individuals (August 2008), $LC_{50} = 5.81$ mg L^{-1} and **c** *Δ*: field individuals (July 2008), $LC_{50} = 6.55$ mg L^{-1}

$$f_1 = UCL_1/LC_{50} \quad (1)$$

$$f_2 = UCL_2/LC_{50}$$

$$f_{1,2} = \text{antilog SQRT } (\log f_1)^2 + (\log f_2)^2$$

where UCL = Upper Confidence Limit.

If $f_1/f_2 < f_{1,2}$ there are not any significant differences between the two LC_{50} values ($^*p < 0.05$).

If $f_1/f_2 > f_{1,2}$ there are significant differences between the two LC_{50} values ($^*p < 0.05$).

In addition, in August 2008 a 10-days sediment bioassay following the protocol described in Casado-Martínez et al. (2007), was carried out with field and cultured amphipods, using 3 marine sediment samples (<1 mm grain size). These sediment samples are classified according to a toxicity gradient: high toxicity ("High-T"); moderate toxicity ("Mod-T"); and absence of toxicity ("Non-T"). This toxicity gradient is shown in Table 1 and had been established on the basis of previous tests (June 2008), using Microtox® (Environment Canada 2002), sea urchin larvae (Bellas et al. 2005) and field amphipods (OSPAR Commission 2005).

Table 1 Sediment samples toxicity (Microtox®, sea urchins and amphipods) and metal concentrations (Cu, Zn)

Sample	Microtox (LC50)	Sea urchin (% embryo- success)	Amphipod (% mortality)	Cu (mg kg^{-1})	Zn (mg kg^{-1})
"Non- T"	2168	81	15	81	216
"Mod- T"	476	80	50	122	414
"High- T"	353	59	83	715	1252

"High-T" high toxicity; *"Mod-T"* moderate toxicity; *"Non-T"* absence of toxicity

Sediment toxicity was related to high concentrations of Cu and Zn. Three replicates were prepared for each sample as well as for the control sediment, which was collected from the Bidasoa Estuary. After 10 days, the mortality percentage of each replicate was determined; this was normalised, using angular transformation. ANOVA and Tukey tests were applied, using the statistical package Statgraphics® Plus 5.0, to identify significant homogeneous samples ($*p < 0.05$).

Results and Discussion

The nominal and measured concentrations at the beginning ($t = 0$ h) and at the end ($t = 72$ h) of the test are shown in Table 2.

The measured concentrations followed the nominal values closely, ranging from 80.0% to 108.2% at the beginning of the assay and from 76.5% to 113.7% at the end of the tests. Variation of Cd concentration in solutions during the assay was generally negligible.

The relationship between the total cadmium concentration and field and cultured amphipod (*C. multisetosum*) mortality is shown in Fig. 1. The results plotted represent two tests: the first performed only with field amphipods (an exploratory experiment) in July 08; and the second performed with field and cultured amphipods, in August 08. In all cases a Probit function fitted significantly ($**p < 0.01$). In August, the LC_{50} values obtained were 2.40 mg L^{-1} (1.64–3.19 mg L^{-1}, 95% confidence limit) and 5.81 mg L^{-1} (4.84–6.98 mg L^{-1}, 95% confidence limit), for the field and cultured amphipods, respectively. The statistical test (1) (Environment Canada 2002) featured significant differences between both LC_{50} values ($*p < 0.05$). Sensitivity of field amphipods to toxicants showed large variability according to the month of sampling. As can be observed in Fig. 1, the LC_{50} value obtained in July (6.55 mg L^{-1}) was higher than the LC_{50} value obtained in August, with the field amphipods (2.40 mg L^{-1}) ($p < 0.05$). Elsewhere, Pérez-Landa et al. (2008) also observed high temporal variability in the LC_{50} of field amphipods. The LC_{50} (Cd) values calculated in this study, both for field and cultured amphipods, lie within the range of those obtained previously by Pérez-Landa et al. (2008), in June and July 2004 (9.24 and 2.68 mg L^{-1}, respectively), with the *C. multisetosum* population of the Bidasoa Estuary. A comparable sensitivity to cadmium has also been recorded in different field amphipod populations, such as, *C. urdaibaiense* (LC_{50}: 1.6 mg L^{-1}) (Pérez 2006); *C. volutator* (LC_{50}: 2–10.3 mg L^{-1}) (Stronkhorst et al. 2003); *Rhepoxynius abronius* (LC_{50}: 0.76 mg L^{-1}) (DeWitt et al. 1989).

Table 2 Nominal and measured concentrations of Cd (average ± standard deviation), and the percentage of the nominal concentration

Nominal	Measured 0 h	%	Measured 72 h	%
Concentration (mg L^{-1})				
0	0.02 ± 0.00		0.03 ± 0.00	
2	1.60 ± 0.02	80.0	1.53 ± 0.01	76.5
5	5.41 ± 0.34	108.2	5.49 ± 0.42	109.8
10	10.44 ± 1.48	104.4	11.37 ± 1.06	113.7
20	19.55 ± 0.96	97.8	21.06 ± 2.75	105.3

The results obtained from the 10-days bioassays on the sediment samples showed different trends to those observed from the 3-days cadmium tests, performed on the liquid phase. In the sediment bioassays, the survival percentage in the control samples was above the maximum guideline for test acceptability (90%) validating these bioassays. Mortality values were not significantly different between the culture and the field control samples (Fig. 2). "High-T" and "Mod-T" samples were toxic for the field individuals, both in June and August bioassays. This is indicated by statistically significant differences ($*p < 0.05$) and by the high mortalities observed in the samples, in relation to the controls (more than 25%). In contrast, the cultured organisms did not respond to the toxic samples and the observed mortality was not significantly different to the control sediment. In this way, the cultured amphipods showed much lower mortality than the field amphipods; therefore, much lower sensitivity to toxicity, when tested with sediment samples. As expected, "Non-T" was not toxic to any of the three amphipod classes. Independently, the burial tendency of the organisms was examined resulting in a significantly lower burial percentage of the cultured amphipods in "High-T" ($*p < 0.05$), i.e. the most toxic sample. In "Mod-T" and "Non-T" samples, a high

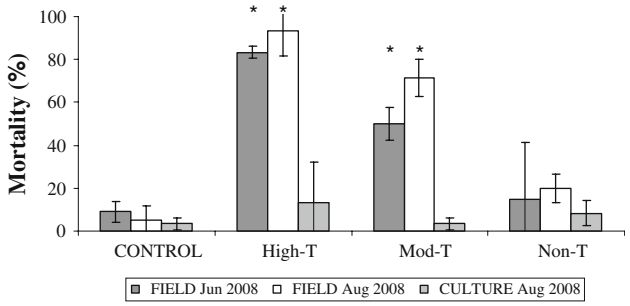

Fig. 2 Mortality percentage of *C. multisetosum* (field individuals collected in June and August 2008, together with cultured individuals over the same season), for a selection of sediment samples with different toxicity levels: high ("High-T"), moderate ("Mod-T") and in the absence of toxicity ("Non-T"). Key: *asterisk* significant differences in mortality, between amphipods in the samples and in the control (Tukey test; $*p < 0.05$). *Bars*: ± standard deviation

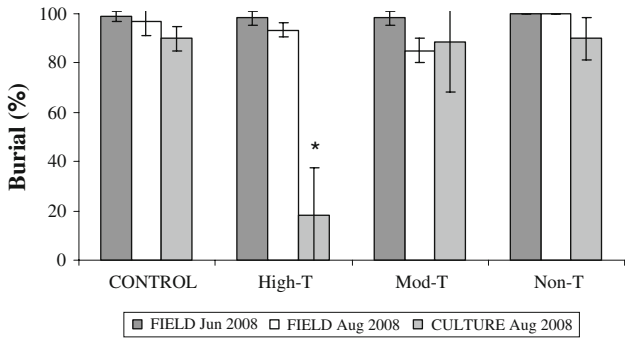

Fig. 3 Burial percentage of *C. multisetosum* (field individuals collected in June and August 2008, together with cultured individuals over the same season), for a selection of sediment samples with different toxicity levels: high ("High-T"); moderate ("Mod-T"); and in the absence of toxicity ("Non-T"). Key: *asterisk* significant differences in burial tendency, between amphipods in the samples and in the control (Tukey test; $*p < 0.05$). *Bars*: ± standard deviation

burial tendency was detected, both in the cultured and field amphipods (Fig. 3).

The effects of culture conditions on the sensitivity of marine animals to pollution have rarely been examined. For example, Robinson et al. (1988) found that cultured amphipods (*R. abronius*) were more sensitive to cadmium, than freshly collected individuals. In contrast, other studies found that the sensitivity of cultures of *Ampelisca abdita* and *C. multisetosum* amphipods to cadmium and *C. volutator* to ammonia was comparable to that of the field-collected animals (Redmond et al. 1994; Peters and Ahlf 2005; Ré et al. 2009). However, our results agree with those of Ciarelli et al. (1997), McGee et al. (1998), Kater et al. (2000) and Schipper et al. (2008), who concluded that the field populations were typically more sensitive.

Several hypotheses could be considered to explain the differences in sensitivity between amphipods from the field and from the culture. On one hand, cultured amphipods were maintained in sediments enriched with MicroGram and vitamins, before the initiation of the bioassay. This procedure may have prevented amphipods from feeding on toxic sediments (according to the low burial percentage of cultured amphipods, in the most toxic sample). This fact could explain the low mortality percentage of the cultured amphipods in the bioassay. On the other hand, the increased sensitivity of field amphipods in some of the toxicity tests performed in this study may be related to multiple environmental stressors affecting their overall performance; this has been observed in other invertebrate species (Schipper et al. 2008). Another hypothesis is that amphipods from the culture could have been genetically selected, favouring the survival of the most resistant individuals. This observation could explain their reduced sensitivity to cadmium and their high survival percentage in highly toxic sediment. Nonetheless, the LC_{50} value calculated for the cultured amphipods was within the range of the seasonal variation observed for field collected individuals from the same estuary (Pérez-Landa et al. 2008; this study). Thus, due to the influence of seasonality on the sensitivity of the field amphipods, the sensitivity to cadmium of the cultured animals could be comparable to that of field-collected animals for certain months. As a result, the first hypothesis (food enrichment, during the culture) may be more plausible for explaining the mortality differences between cultured and field collected amphipods in the sediment bioassays.

Due to the different responses of the amphipods from the culture and the field to the polluted sediments, it may be concluded at this time that amphipods obtained under laboratory conditions should not be used to evaluate the quality of the marine sediments. However, taking into account the strong seasonal variability in the sensitivity of this species to cadmium, being more sensitive in summer (Pérez-Landa et al. 2008), together with the difference in the enrichment of sediment during the maintenance phase, further research is needed in order to establish the influence of both factors (seasonality in field populations and sediment enrichment in culture populations) upon the mortality responses in 10-day bioassays.

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