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The Effect of Seasonality and Body Size on the Sensitivity of Marine Amphipods to Toxicants

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Abstract Two factors, seasonality and body size, were studied to determine their influence on the sensitivity of the amphipods *Corophium urdaibaiense* and *Corophium multisetosum* to toxicants. Seasonality was studied by comparing LC50 values for cadmium and ammonia toxicity to both species over a year. Body size effect was studied by comparing LC50 values of ammonia in three size categories of *C. urdaibaiense*. Except for the case of *C. urdaibaiense* with ammonia as a toxicant, the sensitivity was maximum during summer and minimum during winter. Furthermore, differences in sensitivities were found among the three body size groups studied.

Keywords Toxicity tests · *Corophium* · Cadmium · Ammonium

One of the groups of organisms most widely used in sediment toxicity studies are the amphipods, due to their sensitivity to toxicants, their ecological relevance, and worldwide distribution (ASTM 2002). However, in many areas where amphipods are abundant, there is no information about their suitability for toxicological studies. This usually implies that, when performing toxicity tests with these organisms, preliminary work must be done to study the main factors that could potentially affect their sensitivity and, consequently, the toxicity results.

Numerous studies have investigated the influence of temperature and salinity on the response of amphipods and other crustaceans to toxicants (e.g. McGee et al. 1998). These studies have confirmed that, for multiple contaminants, the toxicity decreases when salinity increases and temperature decreases. Biological factors are also an important source of uncertainty. McGee et al. (1998) selected a group of the biological factors with more influence on the sensitivity of crustaceans to toxicants: life cycle (McCahon and Pascoe 1988), body size, sex, and reproductive stage (McGee et al. 1998; Kater et al. 2000).

The amphipod *Corophium urdaibaiense*, recently described as a new species (Marquiegui and Perez 2006), together with *Corophium multisetusum*, are probably one of the most appropriate marine species to be used to evaluate sediment toxicity in the North coast of Spain (Pérez 2006). This species inhabits several estuaries in the Cantabrian Coast (S. Bay of Biscay), in the high intertidal mudflats (Pérez et al. 2007), principally those composed by muddy sand. A study of a population dynamics during a 1-year period, established, for the first time, some biological patterns of *C. urdaibaiense* (Pérez et al. 2007). No toxicological studies have as yet been reported.

In order to provide the toxicological information that can support the use of *C. urdaibaienese* in routine monitoring programs in the study area, two factors that can affect the sensitivity to toxicants, namely seasonality and body size, have been studied. The toxicants used in this study are cadmium and total ammonia, both widely used as reference toxicants in amphipod protocols (Kohn et al. 1994; Schipper et al. 1999; Kater et al. 2000).

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Materials and Methods

Corophium urdaibaiense was collected from an intertidal mudflat of the Urdaibai Estuary (N 43°22.69′ W 2°40.70′) during the period November 2002–February 2004 and September 2004. *Corophium multisetosum* was collected in the Bidasoa estuary (N 43°20.57′ W 1°45.88′) during the period April 2004–January 2005. The organisms were collected by hand, by carefully sieving the sediments (1 mm mesh sieve). The collected amphipods were rinsed with seawater into polyethylene containers, which were then half-filled with seawater. Once in the laboratory, the amphipods (of a size range of 4–7 mm) were transferred to 4 L containers (30 × 20 × 8 cm) with sediment (1:4 sediment:overlying water volume ratio) collected from the same locations, and then stored in a climatic chamber at 15°C, with constant aeration.

A 72-h LC50 water-only test, described in Schipper et al. (1999), was used to expose the amphipods to the toxicants. The toxicants used were total ammonia ($NH_3 + NH_4^+$) (prepared from an analytical reagent grade NH_4Cl stock solution) and dissolved cadmium (prepared from an analytical reagent grade $CdCl_2$ stock solution). For *Corophium urdaibaiense*, the ammonia toxicity was tested in November 2002; in March, May, July, August and December 2003; and February 2004. For this species, two cadmium toxicity tests were performed in February and September of 2004. With *C. multisetosum*, the ammonia tests were carried out in April, June, July, September and October 2004, and January 2005. The cadmium tests for *C. multisetosum* were performed on the same occasions.

The concentrations used in each test are shown in Table 1. The cadmium concentrations for *C. multisetosum tests* were changed successively in June and July 2004 after observing in previous tests that LC50 values could be over 24 mg L^{-1}. Two replicates of 1 L glass vessels containing 0.5 L of the corresponding concentrations were prepared. For all the toxicants, only nominal concentrations were used to calculate the LC50 values. Ten amphipods, in all cases larger than 4 mm, were randomly selected and put into each vessel. Salinity, temperature, pH and dissolved oxygen were controlled at the beginning and end of each test. The test vessels were incubated in a climatic chamber at 15°C, with constant aeration. Finally, after 72 h, the content of the test vessels was sieved and survivors counted.

The body size experiments, consisted of collecting *C. urdaibaiense* and acclimatizing them, as described above. They were then classified into three arbitrary groups: large (2–4 mm), medium (1–2 mm) and short (0.5–1 mm), as determined by sieving. In each group, the individuals significantly larger or smaller than the rest were removed in order to increase the intra-group size homogeneity. Finally, each individual was measured with a dissecting microscope, equipped with a micrometer, from the head (without the antenna) to the urosome, in order to establish the size range of each group.

The organisms were used to run a 72-h water-only test (described above) in order to provide an LC50 value for each size group. In this case, the toxicant used was total ammonium.

All the LC50 values were calculated using the statistical package Statgraphics 2000®. The differences between LC50 values were calculated using the Fisher test.

Results and Discussion

The results of the seasonal LC50 values for both species are shown in Fig. 1. Figure 1a presents the annual variability of LC50 (72 h) for total ammonia ($NH_3 + NH_4^+$) toxicity to *C. urdaibaiense*. The minimum value was found in May (spring) (27.1 mg L^{-1}), i.e. this is the month when amphipods were more sensitive to ammonia. By contrast, the maximum value, and the lowest sensitivity, was obtained in December (Autumn) (110 mg L^{-1}). In August, the LC50 value dropped to a value of 61 mg L^{-1}, indicating an increase in the sensitivity during this period. For this species (*C. urdaibaiense*), the LC50 (72 h) values for Cd were 2.29 mg L^{-1} in February (Winter) and 0.88 mg L^{-1} in September (Summer).

Figure 1b shows the seasonal variability of LC50 for total ammonia and cadmium toxicity to *C. multisetosum*. For both toxicants, the minimum values were obtained in September (Summer) (25.6 mg L^{-1} total ammonium and 0.63 mg L^{-1} cadmium), and the maximum values in January (Winter) (115 mg l^{-1} total ammonia and 31.5 mg L^{-1} cadmium).

Table 1 Nominal concentrations of ammonia and cadmium used in the LC50 tests for each species and toxicant

Species	Ammonia concentrations (mg L^{-1})	Cadmium concentrations (mg L^{-1})			
		February-02 and September-02	Apr-04	Jun-04	July, Sep, Oct 04 and Jan-05
Corophium urdaibaiense	0, 36, 72, 110, 180, 360	0, 1, 2, 4, 8, 12			
Corophium multisetosum			0, 1, 2, 4, 8, 12	0, 1, 2, 4, 16, 24	0, 2, 4, 8, 16, 32

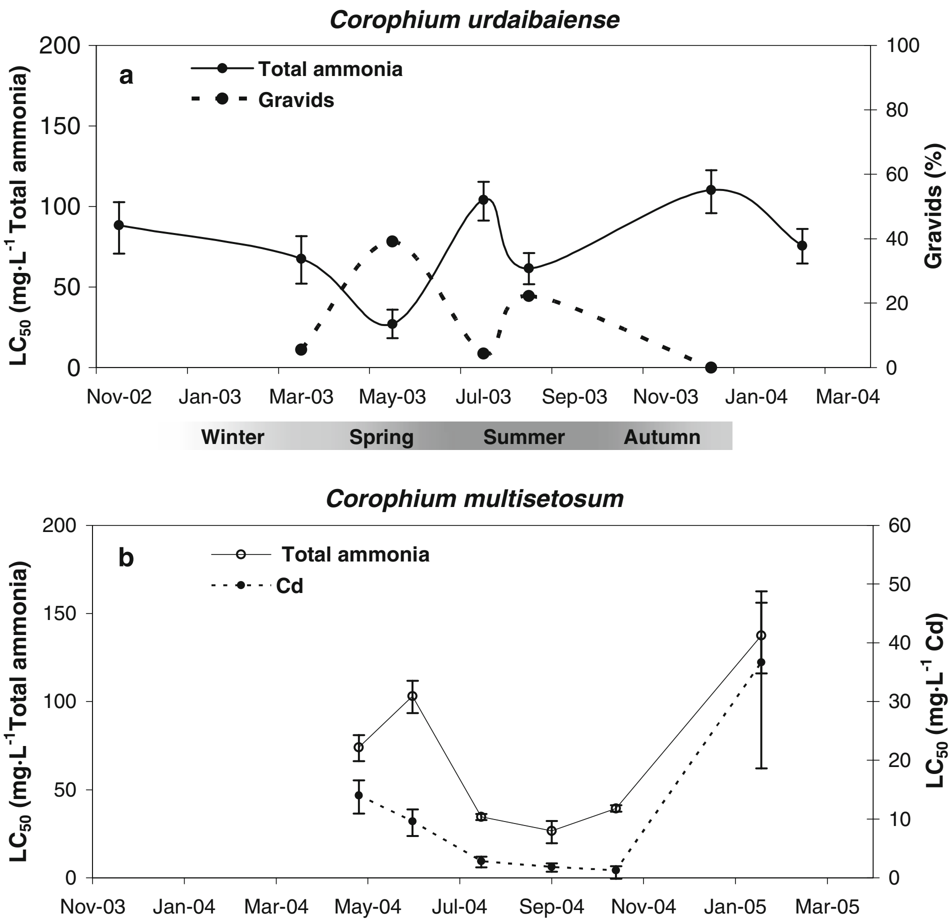

Fig. 1 (**a**) Total ammonia LC50 (72 h) values and 95% confidence intervals (represented as error barrs) for *C. urdaibaiense* during 2002–2004. The percentage of gravids, in relation to the total number of females obtained during the same period from Pérez et al. (2007), is also presented; (**b**) Total ammonia and cadmium LC50 (72 h) values and 95% confidence intervals (represented as error barrs) for *C. multisetosum* during 2004–2005

Several authors have discussed the factors affecting the seasonal variability in the sensitivity of marine amphipods to toxicants. Moulting and reproduction patterns can be highlighted among these factors. Wright and Welbourn (1994) established a relationship between crustaceans' moulting and cadmium intake. The intake of calcium during the moulting implies that, if present, cadmium is also incorporated. The frequency of moulting, which is related to the growth rate, increases during the summer. As a result, it can be concluded that during the summer period, in the presence of the same concentration, the organisms probably incorporate more cadmium than during the winter period, and consequently are more affected by cadmium. This appears to be consistent with the results of the present study in the species *C. multisetosum*, which showed a higher sensitivity to cadmium in summer. However, this explanation could only be true if the organisms experienced the same moulting frequency in the collection period than in the acclimation period, where the temperature was the same (15°C) in all tests. In addition, the seasonal patterns of *C. multisetosum* sensitivity to both ammonia and cadmium are very similar, indicating that the factor that explains this pattern might not be related specifically to cadmium.

The results for *C. urdaibaiense* show that during the spring, the LC50 values are the lowest of the year. By contrast, the LC50 values during the autumn and winter are the highest, together with value of July. Pérez et al. (2007) studied the population patterns during the same year as these experiments. Those studies included monthly measurements of abundance, biomass, juvenile density, male density, gravid female density, non-gravid female density, and the percentage of gravid females in relation to the total females. To investigate relationships between the changes in the sensitivity and biological patterns, the LC50 values obtained in this work were compared with all of the biological variables mentioned above (Pearson's correlation test). The results showed that only the percentage of gravid females in relation to the total of females represented in Fig. 1a, was significantly correlated (inversely) with the LC50 values (p-value < 0.05). In addition, the reproduction peaks, reported in that study, occurred on May (Spring) and during the August–October (Summer) period, suggesting two breeding periods a year.

Considering that the lowest LC50 values occurred in May and, after a peak in July, decreased again in September, coinciding with the reproduction peaks (Fig. 1a), the sensitivity changes of *C. urdaibaiense* to ammonium

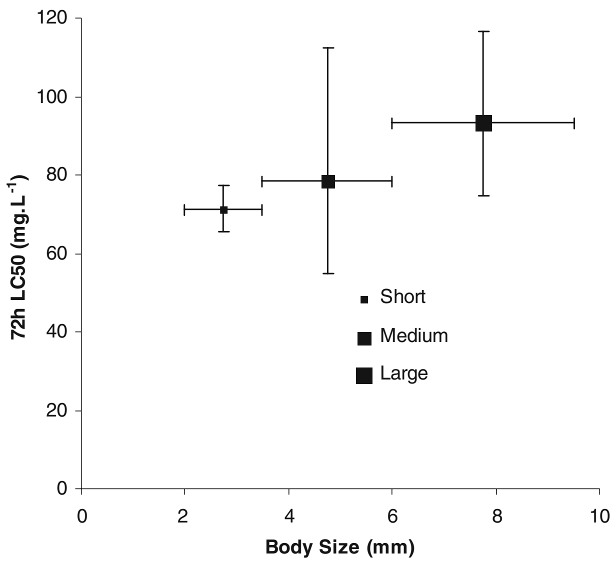

Fig. 2 Results of 72-h LC50 values for total ammonium toxicity and the confidence range for each size group (short, medium and large) of *C. urdaibaiense*; the size ranges and mean values for each group are also shown. LC50 differences between large and small amphipods were significant (Fisher test, $p < 0.05$)

could be related to the reproduction pattern. A possible explanation could be that, during the reproduction period, although only non-gravid females were used in the toxicity test, an important number of post-incubating females could have been included in the test. After releasing the eggs, the females could be more sensitive to toxicants and, therefore, decrease the LC50 values.

The effects of the body size on LC50 values are presented in Fig. 2. Larger amphipods showed the highest LC50 values, and there was a positive correlation between body size and LC50, i.e., a negative correlation between body size and sensitivity. However, the differences in sensitivity are only statistically significant between the "large" and "small" groups.

The maximum body size observed in a juvenile and the minimum size of an adult of the species *C. urdaibaiense* are 3.96 and 3.66 mm, respectively (Pérez 2006). This implies that the "small" group (size range 2–3.5 mm, see Fig. 2), is composed almost exclusively of juveniles and the "large" group (size range 6–9.5 mm) of adults. The "medium" group (size range 3.5–6 mm) is composed of a mixture of adults and juveniles.

Several studies have found higher sensitivities to toxicants in juvenile aquatic species compared to adults. McGee et al. (1998) observed that LC50 values for cadmium toxicity to the amphipod *Leptocheirus plumulosus* increased significantly when a larger body size was considered. King et al. (2005) studied the sensitivity of several amphipod species to zinc and copper, and found that juveniles were more sensitive than adults. Similarly, a recent study (Spadaro et al. 2008) has shown that 4-day LC50 values for copper toxicity to the amphipod *Melita plumulosa* increased linearly with the body length. Similar results have been obtained in studies using ammonia as the toxicant (including the present work).

Several reasons have been proposed to explain the negative relationship between sensitivity and body size. McGee et al. (1998) proposed that the distinctive crustacean moulting behaviour could modify their sensitivity to cadmium, i.e., the younger stages moult more frequently than the older ones. In addition, Wright and Welbourn (1994) related crustaceans moulting with cadmium intake (see above), so the juveniles are more exposed to cadmium than adults for the same concentrations. Verriopoulos and Moraitou-Apostolopoulou (1982) proposed three principal reasons: (1) the ratio of surface/volume is higher in juveniles than in adults, and therefore, juveniles are more exposed to toxicant intake; (2) the exoskeleton is thinner in juveniles than in adults, so the toxicants can be incorporated more easily; and (c) juveniles do not have well-developed detoxifying mechanisms. Other authors mention metabolic activity, higher in juveniles than in adults, to explain their higher sensitivity to toxicants (Moore et al. 1997). The gills are the principal pathway of ammonia intake, and the higher metabolic activity of juveniles implies higher ammonia incorporation.

From the results of this study, it can be concluded that when performing toxicity tests with these species of marine amphipods, the changes in sensitivity must be taken into account. It is crucial that LC50 estimates are performed each time a toxicity test is carried out in order to know whether the sensitivity of the organisms is within the "normal" range. Also, in order to avoid extreme values of sensitivity and, consequently, the risk of failing to fulfil the requirements of the established protocols, toxicity tests should be performed outside the breeding period. For routine testing, it is recommended that mixtures of juveniles and adults not be used.

Further studies on the factors affecting the sensitivity of test organisms are required, however, especially for those species that have been incorporated in toxicity tests.

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