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Population responses to acute and chronic cadmium exposure in sexual and asexual estuarine gastropods

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The acute lethal and chronic sublethal responses of sexual (*Hydrobia ventrosa*) and asexual (*Potamopyrgus antipodarum*) gastropod populations to cadmium exposure were examined. Three questions were addressed: (i) are there differences in responses of sexual and asexual populations; (ii) are the response patterns similar in lethal and sublethal exposure conditions; and (iii) how does pre-exposure to cadmium influence these responses. No differences between the two species in mean acute tolerance (LC_{50}) could be detected, but a significant difference was found between the slopes of the concentration-response curves. The steeper slope for *P. antipodarum* indicated a more uniform response for the asexual species than for *H. ventrosa*. In the sublethal experiment there was a significant difference in mean growth rate where, in general, *P. antipodarum* grew faster than *H. ventrosa*. *P. antipodarum* was more affected by cadmium at the low cadmium exposure, whereas growth rates were reduced equally for the two species at the high cadmium concentration. Pre-exposure to cadmium did not increase the tolerance to chronic exposure in either of the species, but *P. antipodarum* exhibited a tendency toward increased tolerance to acute cadmium stress after pre-exposure.

The results show that there are differences between these closely related sexual and asexual species in response to cadmium, and that these differences are more pronounced under chronic sublethal exposure conditions.

Keywords: mudsnails; population response; cadmium toxicity; LC_{50}; growth.

Introduction

A general problem in the assessment of chemical toxicity is the difficulty of achieving reproducibility in ecotoxicological studies. The aim of toxicity testing, i.e. to compare the toxicity of various chemicals and relate the effects found in the laboratory to impacts in the environment, relies heavily on the assumption that species will respond in the same way when exposed to a chemical. To enhance reproducibility in ecotoxicological tests, conditions are standardized, and clonal organisms or highly inbred strains are frequently employed as test species because they are thought to be phenotypically, as well as genetically, uniform (Gaddum, 1933; Shick and Dowse, 1985; Maynard Smith, 1989). Questions arise, however, as to whether standard laboratory conditions are relevant for field situations, and whether clones or inbred strains respond in the same way as the sexual organisms which predominate in natural ecosystems.

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Physiological variability in asexual populations is likely to differ from that in sexual populations (Forbes and Depledge, 1992). In toxicology, it is widely assumed that asexual clones have very steep dose-response curves (i.e. mortality plotted against exposure concentration) for lethal impacts indicating a uniform response. If sexually outcrossed populations are in fact more variable in their tolerance to pollutants, no-effect levels based on clonal test organisms may either underestimate or overestimate pollutant impacts, depending on the severity of the imposed stress.

Acute LC_{50} tests are the most widely used toxicity tests. These do not, however, provide information on life-cycle characters (e.g. long-term survival, growth or reproduction) that are likely to be more sensitive to toxic exposure. Differences in susceptibility among clones are often clear with acute toxic stress, while differences in susceptibility under chronic stress are less pronounced (Baird *et al.*, 1990; Koivisto *et al.*, 1992). The same pattern for acute versus chronic stress has been observed for sexual strains (Cuvin-Aralar, 1994). In the present study, responses are compared for a sexual and an asexual gastropod population to both acute and chronic toxic exposure.

The potential differences between clonal and sexual organisms and the implications of such for toxicity testing, are further complicated by the different mechanisms behind the acquisition of resistance to toxicants in sexual and clonal organisms. Resistance to pollutants may be acquired either by genetic selection (Klerks and Weis, 1987) or by physiological acclimation (Bryan and Hummerstone, 1971, 1973; Fraser, 1980; Chapman, 1985; Wright, 1986; Stuhlbacher and Maltby, 1992). In sexual populations, the presence of genetic variability makes it possible to select for increased resistance, as well as for individuals to acquire tolerance through physiological acclimation. Within clones, the lack of genetic variability does not preclude physiological acclimation, and hence the development of increased population tolerance. Lack of genetic variation in clones, however, precludes genetic selection for tolerant genotypes. Depending on the relative importance of the two mechanisms, there might be substantial differences in the ability of sexual and asexual populations to develop resistance to toxicants.

The test organisms employed here, *Potamopyrgus antipodarum* and *Hydrobia ventrosa* (Gastropoda; Hydrobiidae), are closely related species with different reproductive strategies. This renders them suitable for comparing the response of asexual (*P. antipodarum*) and sexual (*H. ventrosa*) reproductive modes to toxicants. *P. antipodarum* reproduces by apomictic parthenogenesis, and isoenzyme electrophoresis (Møller, 1993) as well as morphological and DNA fingerprint analysis (Jacobsen *et al.*, submitted) suggest that this population consists of a single clone. In this paper, both sublethal and lethal responses to cadmium are investigated, for *P. antipodarum* and *H. ventrosa* populations from a shallow Danish fjord in which these two species co-exist.

Materials and methods

ACUTE EXPOSURE

The LC_{50} experiment

P. antipodarum and *H. ventrosa* were collected from Odense Fjord, Funen, Denmark, in June 1992. An initial LC_{50} experiment was carried out after acclimating the animals for four days in the laboratory at 15 °C in 15 ‰S artificial seawater (Marinemix: Wiegandt GMBH & Co., D-4150 Krefeld, Germany). Three replicate groups of 15 snails of each

species were placed in acid-washed 100 ml pyrex beakers at five cadmium exposure concentrations (4, 6, 8, 10 and 12 mg $Cd\,l^{-1}$) plus a control (artificial seawater). Cadmium was added to the water from stock solutions made from $CdCl_2\ 2H_2O$ (Merck, Darmstadt, Germany). The snails used (2–3 mm shell length) were assigned randomly to the different treatments. Following an exposure period of 48 h, the snails were transferred to clean water for 24 h, after which mortality was assessed. Death was defined as a failure to respond to probing with forceps.

Pre-exposure experiment

Stocks of experimental animals were maintained in 2.5 litre plastic aquaria containing natural sea water (15 ‰S) and natural sediment that had been sieved (500 μm) and frozen to eliminate living infauna. They were held at 15 °C under full-spectrum light in a 12 h:12 h light:dark regime. Two pre-exposure groups of snails from each species were maintained, one held in such conditions as a control, and the other with cadmium (0.2 mg $Cd\,l^{-1}$) added to the water. These pre-exposure groups were maintained for two months. Water was changed and fresh sediment added every two weeks.

Subsequent exposure

After pre-exposure periods of one and two months, mortality during exposure to 8 mg $Cd\,l^{-1}$ (approximately the LC_{50} value from the initial LC_{50} experiment) was assessed. Snails of each species and pre-exposure group were assigned randomly to three replicate groups of 20 animals each. Following a 48 h exposure period, the snails were transferred to clean water for 24 h, and mortality was assessed as previously described.

CHRONIC EXPOSURE

Pre-exposure experiment

Individual juvenile snails of similar size (0.8–1.5 mm shell length) were placed in 2.5 cm in diameter (12 ml) plastic chambers. Each chamber contained a 0.5 mm layer of natural sediment from the collection site, treated as above. The chambers were fitted with a mesh lid (180 μm pore diameter) and 32 chambers were attached to a glass plate, and placed in a 10 l plastic aquarium containing aerated artificial seawater of 15 ‰S. There were 2 replicate aquaria for each of the pre-exposure treatments: control, 0.1 mg $Cd\,l^{-1}$ and 0.2 mg $Cd\,l^{-1}$. The experiments were performed at 15 °C (± 1 °C) under full-spectrum light with a 12 h:12 h light:dark cycle, to permit growth of the snails' algal food source. The shell lengths of the snails were measured before and after the pre-exposure period of 43 days, using a dissecting microscope fitted with an ocular micrometer.

Subsequent exposure

After the pre-exposure period, all of the surviving snails except one of the control groups for each species, were transferred to 0.2 mg $Cd\,l^{-1}$. The remaining control groups were maintained as before (Table 1 outlines the experimental design). Water and sediment were changed in the aquaria at the same time. After a second exposure period of 30 days, shell lengths were measured to assess growth.

Table 1. The design of the growth experiment. Abbreviations used in the following are C: control, Cd: cadmium (subscripts: L: low (0.1 mg Cd per l), H: high (0.2 mg Cd per l) level). The first code gives the pre-exposure conditions and the second the treatments during exposure (C: control or Cd: cadmium (0.2 mg Cd per l))

Treatment group	Pre-exposure	Exposure	Replicates
C/C	control	control	1
C/Cd	control	0.2 mg Cd per l	1
Cd_L/Cd	0.1 mg Cd per l	0.2 mg Cd per l	2
Cd_H/Cd	0.2 mg Cd per l	0.2 mg Cd per l	2

Throughout all experiments, cadmium concentration, salinity and temperature were measured weekly. Cadmium concentrations were measured by atomic absorption spectrophotometry (Perkin Elmer 2380, Norwalk, CT, USA).

STATISTICAL ANALYSIS

The results from the LC_{50} experiment were analysed by probit transformation of the proportional mortality data (Finney, 1971). The lines resulting from a plot of probit values versus log cadmium concentrations were fitted by linear regression and the residual variance term checked for a lack of fit (i.e. deviations from linearity; Zar, 1984). A *t*-test was performed to test whether the slopes of the lines for the two species were significantly different from each other (Zar, 1984). LC_{50} values and their 95% confidence intervals were calculated by inverse prediction (Zar, 1984), and the LC_{50} values were considered to be different if the 95% confidence limits did not overlap.

Differences in mortality for snails exposed to 8 mg Cd l^{-1} (after pre-exposure for different periods) were compared by analysis of variance (ANOVA) and Bonferroni's adjusted pairwise comparisons (Kirby, 1993), after arcsine transformation. Homogeneity of group variances and normality were checked with Bartlett's test and with plots of residuals.

Differences in shell length and growth rate (percent increase per day) between groups of snails were first analysed by analysis of covariance. The initial shell length was significant as a covariate, but there were also significant interactions between the covariate and the main factors indicating different slopes in the different treatment groups. Therefore, conventional factorial ANOVAs (without the covariate) were performed, followed by Bonferroni's adjusted pairwise comparisons. Proportional mortality in the pre-exposure groups was also analysed by ANOVA (after the proportional mortality data were arcsine transformed). Normality and homogeneity of group variances was checked as above. In cases where data did not meet the assumptions required for ANOVA, non-parametric ANOVAs (Kruskal-Wallis) were performed (Zar, 1984), followed by nonparametric multiple comparisons (Zar, 1984). Statistical significance was defined as $p < 0.05$. Statistical analyses were performed with the aid of Systat (Wilkinson, 1990).

Results

ACUTE EXPOSURE

The LC_{50} experiment

Mortality versus cadmium concentration for the initial LC_{50} experiment is plotted in Fig. 1a for the two species. *P. antipodarum* showed the conventional s-shaped dose-response curve, with a very steep slope at intermediate concentrations. In contrast, *H. ventrosa* exhibited a more gradual increase in mortality with increasing cadmium concentration. The same data are shown in Fig. 1b as lines in a probit versus log Cd plot; statistical data for the two lines are given in Table 2. The lack of fit term was insignificant for both species, indicating a linear relationship for the probit/log Cd plot in both cases. The slopes were significantly different, but no difference in the LC_{50} values (Table 2) was detected, as the lines crossed close to the LC_{50} values.

Subsequent exposure

Results from the subsequent acute exposure experiment are shown in Fig. 2. There were no significant differences in mortality for *H. ventrosa* when exposed to 8 mg Cd l^{-1} after pre-exposure to either no cadmium (control) or 0.2 mg Cd l^{-1}. For *P. antipodarum*, mortality in 8 mg Cd l^{-1} decreased over time both for the control and pre-exposed groups. In the ANOVA, there were no significant main effects of species or pre-exposure concentration on mortality, but there was a significant species-pre-exposure interaction. There was also a significant effect of time of pre-exposure and a time-species interaction. The interactions were due to the fact that for *H. ventrosa*, mortality was constant over time and did not change with pre-exposure, but for *P. antipodarum*, mortality decreased with both time and pre-exposure to cadmium.

CHRONIC EXPOSURE

An ANOVA of initial shell length (data not shown) indicated that there was a significant difference between species in starting size (mean $\pm$ SE values were *H. ventrosa* 1.184 $\pm$ 0.014 mm; *P. antipodarum* 1.103 $\pm$ 0.013 mm). This difference was, however, very small compared to the later differences between the species in growth rates and therefore did not play an important role in the final results. The initial shell lengths within species replicates and treatment groups were not significantly different.

Pre-exposure experiment

Growth rates during the pre-exposure period are shown in Fig. 3. Species, cadmium treatment and species-treatment interaction had significant effects on growth. Overall, *P. antipodarum* grew faster than *H. ventrosa*. Multiple comparisons showed that for *P. antipodarum* the two exposed groups were not significantly different, but both grew slower than the control group. For *H. ventrosa*, only control (C) and the high cadmium concentration (Cd_H) were significantly different. Thus, cadmium reduced growth in a different manner for the two species.

Mean shell lengths at the end of the pre-exposure period are shown in Table 3, together with the percent reduction in shell length in exposed groups, compared to control groups. These calculations confirm the results from the above analysis: a minor effect occurred in the low cadmium (Cd_L) group for *H. ventrosa* (5% reduction) and a

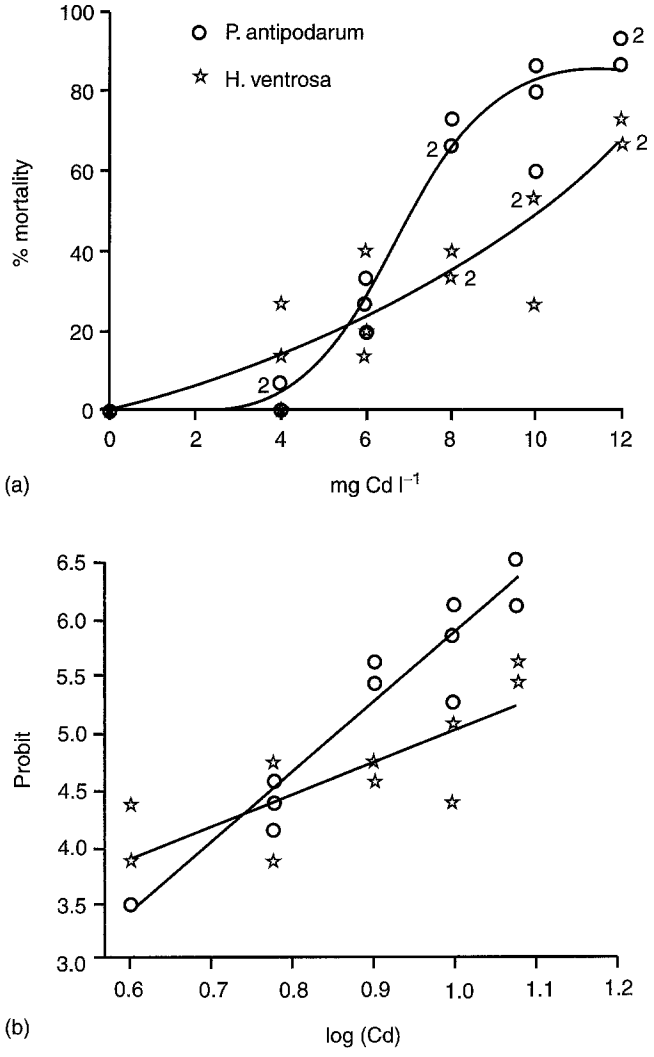

Fig. 1. (a) Proportional mortality for *H. ventrosa* and *P. antipodarum*, plotted as function of cadmium concentration. Symbols with a 2 beside them indicate that two data points are plotted as one symbol. (b) The same mortality data plotted as probits versus log Cd concentration. Lines were fitted by linear regression.

larger (23%) reduction was noted for the Cd_H group. Shell length was reduced similarly (by 16–20%) at both cadmium concentrations for *P. antipodarum*.

Mortality during pre-exposure

Figure 4 shows the percentage of snails that survived the pre-exposure period. Mortality was generally higher for the group pre-exposed to the highest cadmium concentrations. Analysis of variance revealed a significant effect of pre-exposure on mortality during pre-exposure, and a significant interaction between pre-exposure and species. The interaction

Table 2. Statistical data for the LC_{50} tests

	b	b_0	r^2	LC_{50}	95% conf. limits
P. antipodarum	6.08	−0.21	0.937	7.20	[5.70; 9.07]
H. ventrosa	2.79	2.22	0.665	9.88	[5.59; 19.67]

b = slope, b_0 = y-intercept, r^2 = coefficient of determination, LC_{50} values and 95% confidence limits are given in mg Cd per l

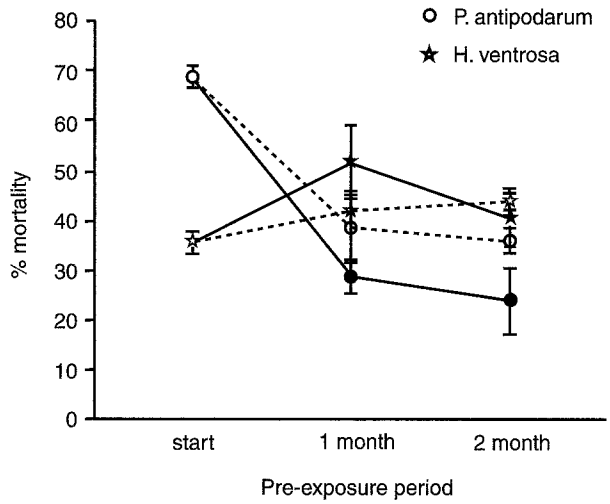

Fig. 2. Proportional mortality in 8 mg Cd per l, plotted versus the length of the pre-exposure period (start: mortality results from the LC_{50} experiment). Filled symbols and solid lines represent individuals pre-exposed to 0.2 mg Cd per l, while open symbols and broken lines are individuals held in control conditions for the pre-exposure period. Error bars are standard errors of the means (SE).

was due to higher mortality for *H. ventrosa* than *P. antipodarum* in the group pre-exposed to 0.1 mg Cd l^{-1} (Cd_L). There was no difference in mortality between species in either the control or the 0.2 mg Cd l^{-1} treatments. Furthermore, there was no effect of initial size on mortality (data not shown).

Subsequent exposure

Growth rates during the subsequent chronic exposure period are shown in Fig. 5. Growth rates differed between species and exposure groups, and there was also an interaction between species and exposure groups. Bonferroni's adjusted comparisons showed that for both species, the C/Cd and Cd_L/Cd groups were not significantly different, but both grew slower than the control groups and exhibited higher growth rates than the Cd_H/Cd groups. In all cases but Cd_H/Cd, *P. antipodarum* grew faster than *H. ventrosa*, resulting in the interaction between species and exposure groups.

The percentage reduction in growth rate in exposed groups compared to control groups was calculated, to compare the effects of cadmium on the two species (Table 4). Since sizes were different in the various groups after pre-exposure, shell length could not be compared directly, as in the pre-exposure analysis. The C/Cd (28–29% reduction

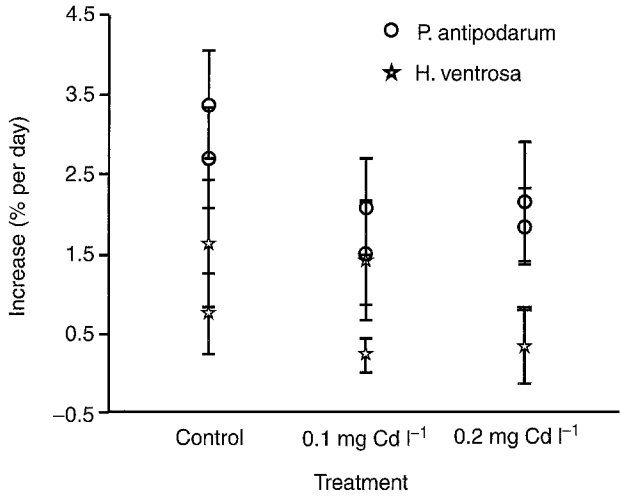

Fig. 3. Growth rates (% increase per day) for the different pre-exposure conditions. Data are the means ± standard errors of the means (SE) for 2 replicate aquaria.

Table 3. Shell length in the different treatments after pre-exposure (mean ± SD), and the percent reduction in these due to cadmium exposure relative to control

	H. ventrosa		*P. antipodarum*	
Treatment	Shell length mm	Reduction	Shell length mm	Reduction
C	1.69 ± 0.33		2.35 ± 0.73	
Cd_L	1.59 ± 0.41	5%	1.87 ± 0.32	20%
Cd_H	1.31 ± 0.29	23%	1.97 ± 0.38	16%

in growth rate) and the Cd_H/Cd (65–72% reduction) groups were affected similarly by cadmium in both species. For *P. antipodarum*, growth of the Cd_L/Cd group (26% reduction) was not further reduced compared to that of the C/Cd group, but in *H. ventrosa* there was a 46% reduction in growth rate in the Cd_L/Cd group compared to only 29% in the C/Cd group.

Discussion

ACUTE EXPOSURE

Although the LC_{50} values were not significantly different for the two species, the shape of the lethal concentration-response curves for *Hydrobia ventrosa* and *Potamopyrgus antipodarum* differed. The steeper slope of the curve for *P. antipodarum* supports the hypothesis that clonal organisms exhibit a more uniform response when tolerance limits are exceeded (Shick and Lamb, 1977; Shick and Dowse, 1985; Hughes, 1989; Forbes and Depledge, 1992). Thus, most individuals in the clonal population responded in a similar fashion to lethal cadmium stress, whereas greater variability in the response among individuals in the sexual population resulted in a more gradual increase in mortality.

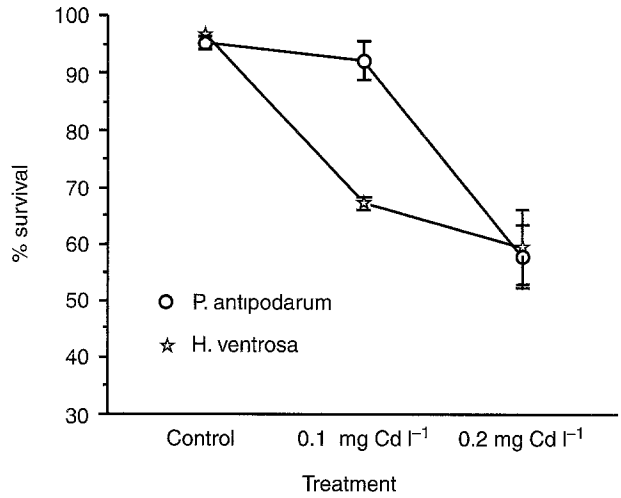

Fig. 4. Percentage survival after pre-exposure to three levels of cadmium. Error bars are given as standard errors of the means (SE).

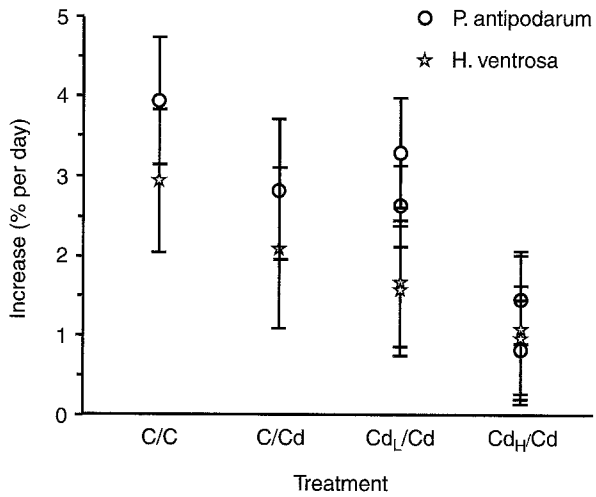

Fig. 5. Growth rates (% increase per day) for four pre-exposure/exposure combinations. Data are the means ± standard errors of the means (SE) for 2 replicate aquaria.

Table 4. Growth rates (% increase per day) in the different treatments after exposure (mean ± SD) and the percent reduction in these due to cadmium exposure, relative to the control

	H. ventrosa		*P. antipodarum*	
Treatment	Growth rate	Reduction	Growth rate	Reduction
C/C	2.95 ± 0.81		3.95 ± 0.79	
C/Cd	2.09 ± 1.01	29%	2.83 ± 0.87	28%
Cd_L/Cd	1.59 ± 0.80	46%	2.93 ± 0.68	26%
Cd_H/Cd	1.02 ± 0.85	65%	1.09 ± 0.67	72%

The LC_{50} values noted in this study are consistent with the general range of 0.1–10 mg Cd l^{-1} for aquatic invertebrates reported by McLusky *et al.* (1986) and Hong and Reish (1987), but their data refer to 96 h values, by contrast to the 48 h values reported here. Eisler (1971) reported 48 h LC_{50} values for crustaceans between 0.5–17 mg Cd l^{-1}, while his values for molluscs are much higher (28–165 mg Cd l^{-1}, at 20 °C and 20 ‰S). In particular, the 48 h LC_{50} for the eastern mud snail (*Nassarius obsoletus*) was 125 mg Cd l^{-1}, whereas the 96 h value was only 10.5 mg l^{-1}. The two species tested in the present study were as sensitive to cadmium as many other invertebrates, whereas the molluscs reported by Eisler (1971) were extremely tolerant, with 48 h LC_{50} values an order of magnitude higher. The slopes measured by Eisler (1971) for *N. obsoletus* and here for *H. ventrosa* (both sexual species) were similar (3.5 for *Nassarius obsoletus* and 2.79 for *H. ventrosa*) and were lower than that for the asexual population of *P. antipodarum* (slope = 6.08). Previous LC_{50} experiments with a freshwater population of *P. antipodarum* gave LC_{50} values between 1–4 mg Cd l^{-1} depending on temperature (Møller *et al.*, 1994). The slightly higher LC_{50} value found in this study (7 mg Cd l^{-1}) is consistent with expectations of a lower bioavailability of cadmium in waters of higher salinity.

CHRONIC EXPOSURE

In the sublethal experiment, the stress imposed by the low level pre-exposure to cadmium resulted in significant mortality and reduced growth in both species. Exposure of juvenile organisms to concentrations of 0.03–0.2 mg Cd l^{-1} has been shown to decrease growth in a range of species, e.g. fish (Borgmann and Ralph, 1986), shrimp (Liao and Hsieh, 1988; Thorpe and Costlow, 1989) and isopods (Giudici and Guarino, 1989). Moreover, *P. antipodarum* and *H. ventrosa* have previously exhibited reduced growth at these concentrations (Forbes, 1991; Møller, 1991; Forbes *et al.*, 1995).

In general, *P. antipodarum* grew faster than *H. ventrosa*. This might be explained by a higher degree of heterozygosity in *P. antipodarum*, as the reproductive mode (ameiotic parthenogenesis) of this species is thought to lead to increased heterozygosity (Phillips and Lambert, 1990). A significant correlation between heterozygosity and growth has been demonstrated in a variety of plant and animal phyla (Bayne, 1987).

The low growth rate observed for *H. ventrosa* may have been due to salinity stress. Although populations of *H. ventrosa* have been found at 5 ‰S (Muus, 1967), growth studies suggest that the optimal salinity for this species is approximately 20 ‰S (Hylleberg, 1975; Forbes, 1991). Given that *H. ventrosa* and *P. antipodarum* co-exist in natural environments at the salinity used in this study (15 ‰S), it is likely that *H. ventrosa* can grow reasonably well at this salinity. Furthermore, other environmental factors (e.g. temperature, water turbulence, tolerance to anoxia and desiccation) influence the natural distribution of hydrobiid species (Fenchel, 1975) and might favour *H. ventrosa* at this site.

The large difference in mean growth rates in control conditions between the two species complicates comparisons of the effect of cadmium on growth. Therefore, the percent reduction in shell length was calculated for cadmium-exposed groups compared to control groups. In *H. ventrosa*, there was a greater reduction in size (23%) at the higher cadmium concentration than in the low cadmium exposure (5%), whereas for *P. antipodarum* there was essentially no difference in the reduction in size (20 and 16%) between the two cadmium concentrations relative to control. Thus,

P. antipodarum was more affected by cadmium than *H. ventrosa* in the low cadmium concentration, but less affected in the high concentration. The opposite pattern was found for mortality during pre-exposure, where *H. ventrosa* exhibited a higher mortality in the low cadmium concentration. This could explain the growth results, in that the most sensitive *H. ventrosa* would have died in the low cadmium concentration, whereas almost all *P. antipodarum* survived, but exhibited a reduced overall growth rate.

The observed increase in shell length of approximately 1 mm per month for *P. antipodarum* in control conditions agrees well with growth rates observed for a freshwater population of *P. antipodarum* (Møller, 1991). The reduction in growth due to cadmium in the present experiment (20% and 16% in 0.1 and 0.2 mg Cd l^{-1}, respectively) was higher for the low-cadmium treatment but similar for the high-cadmium treatment compared to the fresh water population (10 and 17%, at the same Cd concentrations). This suggests that the brackish water population studied here was either more sensitive to chronic cadmium exposure (despite the fact that the bio-availability of cadmium is lower in seawater) or that salinity stress augmented the effect of cadmium.

A growth rate for *H. ventrosa* in control conditions of 0.5 mm per month at 15 ‰S is within the expected range based on previous studies. Forbes (1991) measured growth rates of 0 and 1.4 mm during 3 weeks in 13 and 23 ‰S, respectively, for control snails from the same site. The final shell lengths noted by Forbes (1991) were reduced by 10 and 20% in 0.1 and 0.2 mg Cd l^{-1}, respectively, in 23 ‰S, which is similar to the reduction in shell length observed in this experiment (5 and 23%, respectively).

RESPONSE TO PRE-EXPOSURE

In the acute exposure experiment, there was a tendency for increased tolerance after pre-exposure in the asexual species, but not in the sexual species. This supports the hypothesis that the asexual genotypes exhibit greater phenotypic plasticity (Shick and Lamb, 1977), which enables individuals to increase their tolerance to stress despite the lack of genetic variation. For *H. ventrosa*, there was no increase in tolerance, implying that neither physiological acclimation (possibly due to a more narrow range of phenotypic response) nor any measurable selection of tolerant genotypes occurred during this experiment.

In the growth experiment, the selection process (i.e. death of weaker individuals) during the pre-exposure period resulted in 35% mortality for *H. ventrosa* and 5% for *P. antipodarum* at 0.1 mg Cd l^{-1} (Cd_L), and 40% mortality for both species in 0.2 mg Cd l^{-1}. In *P. antipodarum*, the group with low mortality (Cd_L/Cd) showed no further reduction in growth rates than the C/Cd group. For both species, the decrease in growth rate for surviving snails in the treatments with high mortality (i.e. high selection pressure) showed that the ability to withstand the stress during pre-exposure was not associated with enhanced growth during subsequent exposure. Chapman (1985) reported that increased tolerance to lethal stress is sometimes associated with increased sublethal toxicity (measured as growth, reproduction etc.).

The acquisition of tolerance during pre-exposure to low levels of a toxicant is only possible over a restricted concentration range (Chapman, 1985). At the lower end of the range the toxicant concentration is too low to induce detoxification mechanisms, while at the upper end there is a latent lethal effect of pre-exposure in its own right. Chapman (1985) reported that this 'zone of acclimation' for fish was between 20–50% of the 96 h

LC_{50} values. However, for *Gammarus pulex*, acclimation concentrations 100–200 times lower than the 48 h LC_{50} gave the highest tolerance (Stuhlbacher and Maltby, 1992). The pre-exposure concentration used in this experiment was approximately 40 times lower than the 48 h LC_{50} values.

Considerable differences in development of tolerance to a toxicant with different durations of acclimation have been observed by others. Experiments on fish showed that tolerance was greatest after one week of acclimation, but had decreased to control levels after two weeks of acclimation (Chapman, 1985). Increased tolerance in lethal toxicity tests after short periods of pre-exposure (one to seven days) has also been shown for other species e.g. *Nereis diversicolor* to Cu (Bryan and Hummerstone, 1971, 1973); *Asellus aquaticus* to Pb (Fraser, 1980); *Marinogammarus marinus* to Pb, Cu and Zn (Wright, 1986); and *Gammarus pulex* to Cd (Stuhlbacher and Maltby, 1992). In contrast, studies with longer pre-exposure periods (over generations) with *Neanthes arenaceodentata* showed no adaptive changes in acute tolerance to Cr (Oshida, 1976). Given the different reproductive modes, the concentrations or durations of acclimation necessary for acquisition of tolerance are probably different for the two species used in the present experiments. It was not possible with the current experimental design to determine whether shorter acclimation time or higher pre-exposure concentrations could have increased tolerance in either or both of these species.

Conclusions

Comparisons of lethal and sublethal responses of *H. ventrosa* and *P. antipodarum* confirms that there are differences in the patterns of response between short-term, high-level exposure and long-term, low-level exposure. In contrast to most other studies (Baird *et al.*, 1990; Koivisto *et al.*, 1992; Cuvin-Aralar, 1994), the most significant difference between the two species in mean response in the present work was found in the sublethal exposure and not in the lethal exposure.

Although there was no difference between the two species in mean acute response (LC_{50}), there was a significant difference in the slopes of the dose-response curves, indicating a more uniform response for the asexual population. Differences between these sexual and asexual species in the variability of their response to acute as well as to chronic exposure, and the implications of such differences, are discussed elsewhere (Møller, Forbes and Depledge, in preparation).

Pre-exposure to cadmium increased the tolerance to lethal cadmium concentrations in *P. antipodarum*, but not in *H. ventrosa*. In the growth experiment, pre-exposure did not increase the sublethal tolerance to cadmium in either of the species, but resulted in a reduced difference between the two species in mean growth rates.

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