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Tolerance to heavy metals in *Littorina saxatilis* from a metal contaminated estuary in the Isle of Man

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Population differences were measured in the tolerance of *Littorina saxatilis* from sites around the Isle of Man, to acute exposure to zinc, lead, copper and cadmium. Animals from a site influenced by disused mine run-off in Laxey estuary (high zinc) were compared with animals from less contaminated estuaries (Peel-high lead, but lower zinc), and the relatively uncontaminated Castletown and Ramsey estuaries, plus the open coast near Derbyhaven. Median lethal times (LT_{50}) were estimated for each test concentration (5, 10, 20 mg l^{-1} Zn; 5, 10 mg l^{-1} Pb; 0.5, 1.0, 2.0 mg l^{-1} Cu and Cd) except for those that did not produce sufficient mortalities. Individuals from Laxey estuary showed significantly higher tolerances to zinc (10 mg l^{-1}) and lead (5 mg l^{-1}) than animals from the unpolluted sites. No co-tolerance to copper or cadmium was apparent. Population tolerance to zinc was correlated with reduced accumulation rates. Lead tolerance may result from the ability of the tolerant individuals to sequester the metal and detoxify it in their tissues; the littorinids from Laxey had significantly higher rates of lead accumulation.

INTRODUCTION

Tolerance to heavy metals resulting from exposure of organisms to sub-lethal contamination has been known for a long time (e.g. terrestrial plants, Bradshaw, 1952; some terrestrial invertebrates, Posthuma & Vanstraalen, 1993). For marine organisms, the presence of enhanced tolerance has been reported in a variety of species (e.g. of Zn and Cu by *Nereis diversicolor*, *Carcinus maenas* and the nematode *Tripyloides marinus*; of Cu by *Nephthys hombergi*, *Corophium volutator* and *Scrobicularia plana*) from the highly contaminated Restronguet Creek in comparison with nearby estuaries (Bryan & Gibbs, 1983; Grant et al., 1989; Millward & Grant, 1995).

Tolerance to acute metal levels may result from genetic selection (genotypic) which might be expressed phenotypically; or through an adjustment of biochemical or physiological mechanisms (phenotypic) without a genetic basis (Lam, 1996). Irrespective of the basis, mechanisms of metal tolerance in aquatic invertebrates may involve reduced uptake or increased excretion leading to low accumulation (e.g. Bryan & Gibbs, 1983) or storage in inert forms which may be coupled with enhanced uptake (e.g. Brown, 1978; see Depledge & Rainbow, 1990 for general reviews). For tolerance to be genetically determined, local selection must occur. For many marine animals this is not possible due to broadcast larval stages. It is only in organisms with direct development or limited larval dispersal that such selection can occur. We selected *Littorina saxatilis* (Olivi) as a convenient directly developing organism for such work.

The Isle of Man has a long history of mining, with some catchments severely contaminated whilst others are largely uncontaminated (Southgate et al., 1983) allowing testing of local differences in tolerance. On the basis of tissue zinc concentrations in *L. saxatilis* (details below) it was hypothesized that the metal may be present in concentrations capable of inducing tolerance to zinc in animals from Laxey estuary in comparison with those from Peel, Castletown, and Ramsey estuaries and from the open coast of Castletown Bay near Derbyhaven. Tolerance was tested by means of long duration acute toxicity tests with lead, copper and cadmium being similarly assessed to examine if co-tolerance to these metals exists. Population differences in the accumulation of zinc and lead were examined to elucidate the mechanisms of tolerance.

MATERIALS AND METHODS

Study sites

The earliest record of mining dates back to the 13th Century (Garrod et al., 1972). Large scale exploitation started in the 18th Century with peak production in the mid 19th Century, with the last mines closing between 1919 and 1929. The distribution of mineral veins and mines (see Figure 1) suggests that zinc, lead, copper and iron are present in small amounts throughout the Manx slate series but the two main groups of mines were at Laxey (zinc) and Foxdale (lead). The freshwater input to the estuaries at Laxey and Peel comes from rivers draining the main former mining areas of Laxey and Foxdale respectively. Metal levels in sediment and biomonitors from these estuaries are higher than those from Castletown and Ramsey. The open coast site near Derbyhaven can be considered virtually uncontaminated (Southgate et al., 1983; Gibb et al., 1996; Daka et al., 2003; see Summary Table 1).

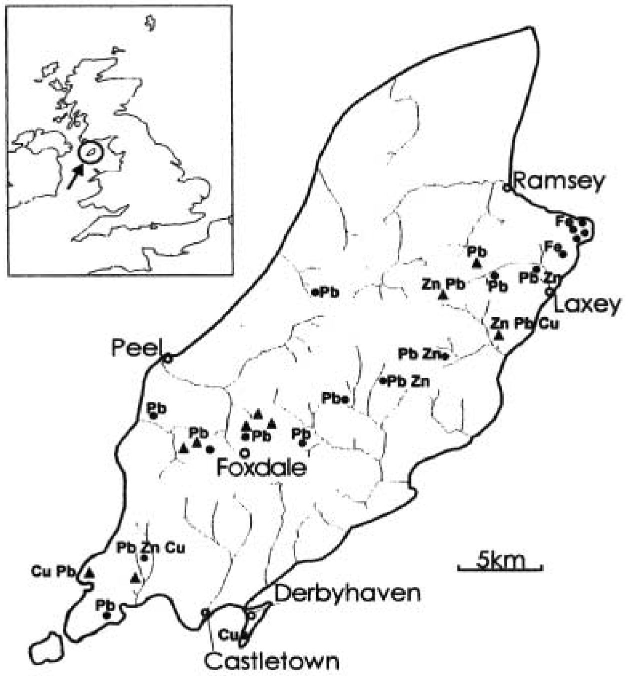

Figure 1. Locations of sampling sites (open circles) around the Isle of Man and past mining activities (modified from Southgate et al., 1983). Filled triangles and circles represent major and minor producing mines respectively with an indication of the order of importance of the ores produced (Cu, copper; Fe, iron; Pb, lead; Zn, zinc). Inset: The British Isles showing the geographical location of the Isle of Man.

Test organisms

The taxonomy of the *Littorina saxatilis* complex has been largely resolved (see review by Reid, 1996, pp. 287–292). The two oviparous species *L. arcana* and *L. compressa* (i.e. *L. nigrolineata*) are clearly distinct and their taxonomic status is no longer questioned. All ovoviviparous *Littorina* can be considered to belong to a single species *Littorina saxatilis*, although there is still some controversy about the status of ecotypes of *L. saxatilis* such as '*neglecta*' and '*tenebrosa*' (Grahame et al., 1995). The littorinids sampled for this work were large and ovoviviparous, so they would have all been *Littorina saxatilis*. To reduce size effects only animals of a similar size ($\sim$10 $\pm$2 mm shell height) were used.

Animals were collected during winter 1994 and summer 1995 from five sites around the Isle of Man (Figure 1): the estuary mouths at Laxey, Peel, Castletown and Ramsey, and the open shore in Castletown Bay near Derbyhaven. Tissue concentrations of zinc, lead, copper and cadmium were measured by atomic absorption spectrophotometry of nitric acid digests of dry tissue (Harper et al., 1989) (see Table 1). Reference material of *Mytilus edulis* (European Communities Bureau of Reference—BCR ref. material 278) was included in the analysis, and metal concentrations measured were within $\pm$10% of certified values. Zinc values were significantly higher in the samples from Laxey in comparison with all other sites (analysis of variance [ANOVA], Tukey tests, $P<0.01$). The tissue concentrations of lead in the winkles from Peel and Laxey were also significantly higher than those of the three other sites. The animals from Laxey represent a

Table 1. *Mean concentrations* $\pm$*SE (N=5) of zinc, lead, copper and cadmium in sediment,* Mytilus edulis, Fucus serratus *and* Littorina saxatilis *(N=10 except lead, N=5) from sites around the Isle of Man in June 1995. All values are in* $\mu g\, g^{-1}$ *dry weight (sediment* $<500\,\mu m$*) or tissue.* Mytilus edulis *and* F. serratus *samples were collected from the mouths of the estuaries. Sediment values are for locations close to the area of collection of* L. saxatilis *for tolerance studies; NB different values (Daka et al., 2003) were obtained at other points along the estuaries, but the ranking of contamination among sites was the same.*

Metal	Castletown	Derbyhaven	Laxey	Peel	Ramsey
		Sediment			
Zinc	104 ±24	n.p.	879 ±27	554 ±12	99 ±20
Lead	52 ±2	n.p.	181 ±13	264 ±8	81 ±17
Copper	19 ±1	n.p.	83 ±14	37 ±1	17 ±0
Cadmium	3 ±0	n.p.	2 ±0	3 ±0	1 ±0
		Mytilus edulis			
Zinc	124 ±13*	n.p.	257 ±25	339 ±71	99 ±10
Lead	n.d.*	n.p.	79 ±4	309 ±4	16 ±2
Copper	10 1.6*	n.p.	13 ±2	9 ±1	10 ±1
Cadmium	n.d.*	n.p.	3 ±0	3 ±0	2 ±0
		Fucus serratus			
Zinc	80 ±7	76 ±7	2333 ±219	628 ±118	120 ±10
Lead	6 ±0	6 ±0	20 ±2	38 ±9	6 ±0
Copper	3 ±0	3 ±0	8 ±1	4 ±0	4 ±0
Cadmium	2 ±0	2 ±0	4 ±0	2 ±0	2 ±0
		Littorina saxatilis			
Zinc	111.3 ±4.3	96.0 ±3.2	470.7 ±14.9	142.6 ±6.4	141.2 ±2.9
Lead	11.5 ±1.1	2.3 ±0.4	16.6 ±0.3	16.6 ±1.0	7.6 ±0.8
Copper	91.5 ±11.2	30.6 ±2.1	79.7 ±6.3	71.4 ±2.2	126.9 ±7.4
Cadmium	2.8 ±0.2	1.8 ±0.0	3.8 ±0.1	2.1 ±0.1	1.7 ±0.1

n.p., sediment and mussels not present in sufficient quantities for analysis; *, a very few mussels were found at the start of the study in Castletown and values for zinc and copper are given from preliminary work in April 1994; n.d., reliable data were not collected for lead and copper during this pilot survey.

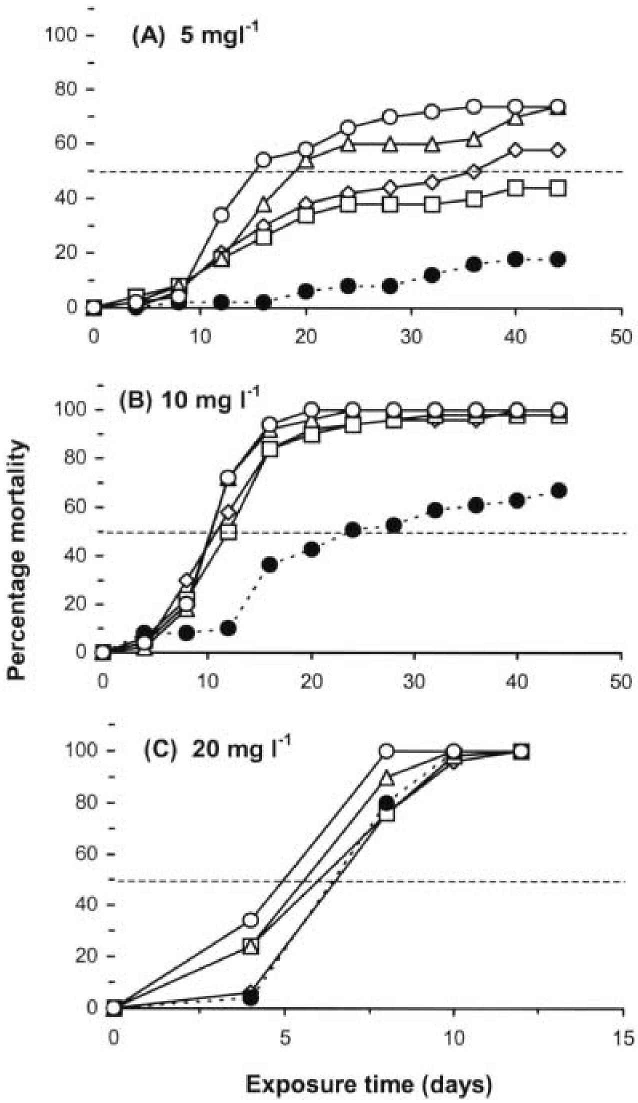

Figure 2. Mortality of *Littorina saxatilis* from sites in the Isle of Man exposed to zinc. (A) 5 mg l^{-1}; (B) 10 mg l^{-1}; (C) 20 mg l^{-1} added Zn. The plotted values are means of five replicates (NB differences in exposure time). Dotted lines represent 50% mortality. Negligible control mortality (maximum=4%) not plotted. ◇, Castletown; □, Derbyhaven; ●, Laxey; △, Peel; ○, Ramsey.

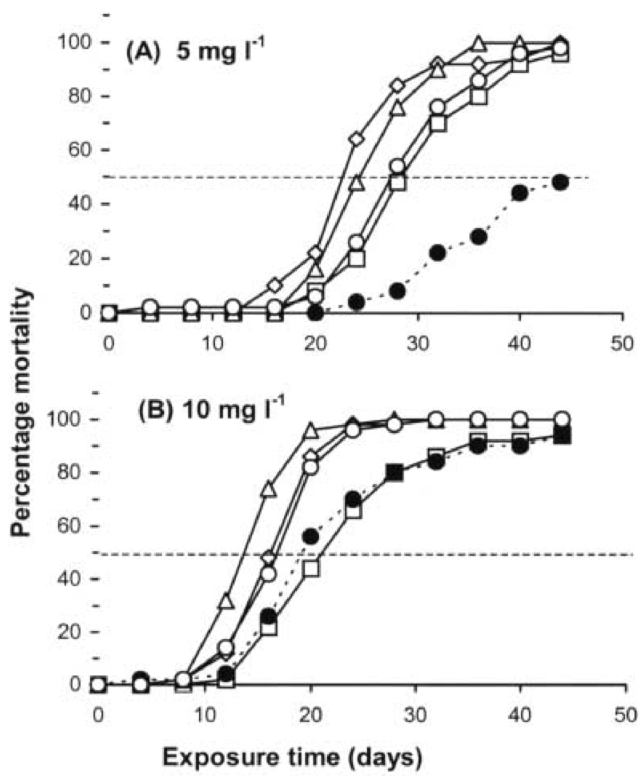

Figure 3. Mortality of *Littorina saxatilis* from sites in the Isle of Man exposed to lead. (A) 5 mg l^{-1}; (B) 10 mg l^{-1} added Pb (essentially a saturated solution). The plotted values are means of five replicates. Dotted lines represent 50% mortality. Control mortality for all sites=0%, not plotted. ◇, Castletown; □, Derbyhaven; ●, Laxey; △, Peel; ○, Ramsey.

highly contaminated population—especially with zinc. Animals from other sites provide comparisons with various degrees of contamination including essentially uncontaminated control sites.

Metal tolerance experiments

Littorina saxatilis were collected and acclimatized for seven days to laboratory conditions (constant temperature room of 10±1°C, salinity ~34 psu) in tanks containing aerated seawater. They were subsequently transferred to test chambers (Parrish, 1985), where the test animals were suspended in labelled nylon mesh bags. Test chambers consisted of 2-l conical flasks aerated via a hollow glass tube through the stopper.

After acclimatization the animals were placed in test solutions for up to 44 days. Test concentrations were prepared by diluting a freshly prepared stock solution (1000 mg l^{-1} in distilled water) of the respective metal salts with filtered seawater equilibrated to the test temperature. Metal salts used were zinc sulphate ($ZnSO_4.7H_2O$), copper sulphate ($CuSO_4.5H_2O$), cadmium sulphate ($3CdSO_4.8H_2O$), and lead nitrate $Pb(NO_3)_2$. The test concentrations selected were strong enough to elicit acute effects (cf. Mance, 1987): 5.0, 10.0 and 20.0 mg l^{-1} added zinc and added lead; 0.5, 1.0 and 2.0 mg l^{-1} added copper and added cadmium. Five replicate treatments of each concentration and controls (no metal added) were used. Replication was achieved by a randomized complete block design, with each block (test flask) containing bagged individuals (ten per bag) from each of the five sites. All tanks, flasks and bags were acid-washed before use. Test solutions were replaced every other day and individuals were examined for mortality every four days in clean seawater. An individual was considered dead if it failed to withdraw when touched. The test animals were not fed before or during the experiment, but since no control mortality was observed (except 4% in just one case) starvation caused no significant mortality.

Metal accumulation experiments

The accumulation of zinc was determined at fixed concentrations of zinc with increasing time, while that of lead was with increased concentration in a fixed time. Collection and acclimatization procedures were as described above, but for zinc accumulation studies, acclimatization to the test chambers involved three replicates of 30 individuals for each site. Before exposure to

Table 2. *Median lethal times—LT_{50}(days) of* Littorina saxatilis *from various sites in the Isle of Man exposed to different concentrations of zinc, lead, copper and cadmium (N=5, mean (x) ±SE).*

Metal	Concentration ($mg\ l^{-1}$)	Castletown	Derbyhaven	Laxey	Peel	Ramsey
Zinc	10	11.0 ±0.8	11.1 ±1.1	23.2 ±2.7	10.7 ±0.5	10.4 ±1.0
	20	6.5 ±0.3	6.0 ±0.3	6.5 ±0.3	5.4 ±0.5	4.6 ±0.5
Lead	5	22.5 ±1.0	27.9 ±1.2	39.9 ±2.4	24.3 ±0.8	27.9 ±1.4
	10	16.4 ±0.7	21.7 ±1.7	19.8 ±1.2	13.6 ±0.8	16.8 ±0.9
Copper	0.5	23.0 ±1.5	19.4 ±1.0	24.9 ±1.4	18.4 ±1.0	15.0 ±0.8
	1	14.7 ±0.8	12.8 ±0.8	15.8 ±1.0	14.4 ±0.9	11.6 ±0.4
	2	11.8 ±0.6	12.3 ±0.5	12.5 ±0.8	9.4 ±0.9	7.3 ±0.5
Cadmium	1	21.0 ±1.2	23.5 ±1.1	21.3 ±0.9	20.5 ±1.4	21.1 ±1.4
	2	11.3 ±0.6	13.1 ±1.1	14.4 ±1.3	10.2 ±0.3	14.3 ±1.8

Table 3. *Analysis of variance (randomized-block design) on LT_{50} values (logx+1 transformed) for* Littorina saxatilis *from sites in the Isle of Man exposed to different concentrations of zinc, lead, copper and cadmium. Conclusions of Tukey tests for pair-wise significant differences between sites are shown. Sites that are not significantly different at* P *<0.05 are underlined.*

Metal	Concentration $mg\ l^{-1}$	df	MS	F	*P*	Tukey test inferences (sites in rank order)
Zinc	10.0	4	0.093	13.99	<0.001	L C D P R
	20.0	4	0.016	4.87	<0.01	L C D P R
Lead	5.0	4	0.042	45.50	<0.001	L R D P C
	10.0	4	0.027	8.04	<0.001	D L R C P
Copper	0.5	4	0.033	12.53	<0.001	L C D P R
	1.0	4	0.012	8.42	<0.001	L C P D R
	2.0	4	0.041	12.31	<0.001	L D C P R
Cadmium	1.0	4	0.003	2.92	>0.05	D L R C P
	2.0	4	0.016	7.40	<0.001	L R D C P

Sites: L, Laxey; C, Castletown; D, Derbyhaven; P, Peel; R, Ramsey.

metals, four individuals were removed from each bag for the assessment of initial metal concentration. The rest were transferred into three replicates of the nominal exposure concentrations of 2.5 $mg\,l^{-1}$ and 5 $mg\,l^{-1}$ zinc. Four individuals providing a pooled sample for metal analysis were collected every two days (with the exposure solutions replaced) up to eight days. For lead accumulation, sets of ten individuals per bag were exposed in triplicate to the appropriate metal concentrations (0.1, 0.5, 1.0 and 2.0 $mg\,l^{-1}$ lead) after acclimatization to the test chambers. The solutions were changed every two days and the experiments were terminated after six days when test animals were also collected for metal analysis as described earlier.

Data analysis

Median lethal times (LT_{50}) were obtained by graphical interpolations (see Rand & Petrocelli, 1985). Analysis of variance (randomized block design) of LT_{50} values (after log (x+1) transformation) followed by Tukey honestly significant difference multiple comparisons (Zar, 1984) tested differences in the tolerance of animals from the various sites. Correlation tests were made between LT_{50} values and the tissue burdens of the respective metals from the various sites. Tissue metal concentrations were plotted against time (zinc) or exposure concentrations (lead) and the slopes of the regressions were compared by analysis of covariance (ANCOVA) using MINITAB for Windows.

RESULTS

Metal tolerance

The time–mortality curves for *Littorina saxatilis* from different sites in the Isle of Man, exposed to various concentrations of zinc showed that the animals from Laxey were clearly less susceptible to zinc at 10 $mg\,l^{-1}$ than those from Derbyhaven, Castletown, Peel and Ramsey which all showed similar responses (Figure 2B). The animals from Laxey had significantly higher mean LT_{50} than animals from all other sites (ANOVAs: Tables 2 & 3), and the LT_{50} values were highly correlated with tissue zinc burdens ($N=5$, $r=0.99$, $P<0.001$). At the lowest concentration (5 $mg\,l^{-1}$) mortality was obviously much lower at Laxey than the other sites. Because they did not reach 50% mortality, ANOVA on LT_{50} values was not made but Friedman's test on median time to death (TTD_m—see Hoare & Davenport, 1994), showed significant differences between sites ($S_4=14.32$, $P<0.05$). When tested at 20 $mg\,l^{-1}$ zinc, the response curves did not show the marked differences typical at lower concentrations.

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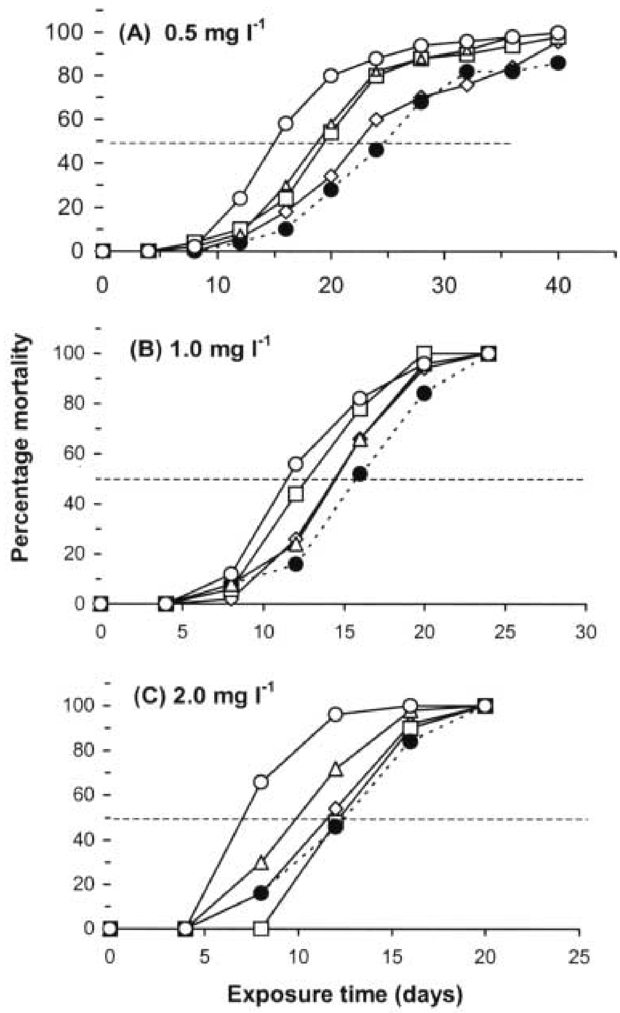

Figure 4. Mortality of *Littorina saxatilis* from sites in the Isle of Man exposed to copper. (A) 0.5 mg l^{-1}; (B) 1.0 mg l^{-1}; (C) 2.0 mg l^{-1} added Cu. The plotted values are means of five replicates. Dotted lines represent 50% mortality. Control mortality=0%, not plotted. ◇, Castletown; □, Derbyhaven; ●, Laxey; △, Peel; ○, Ramsey.

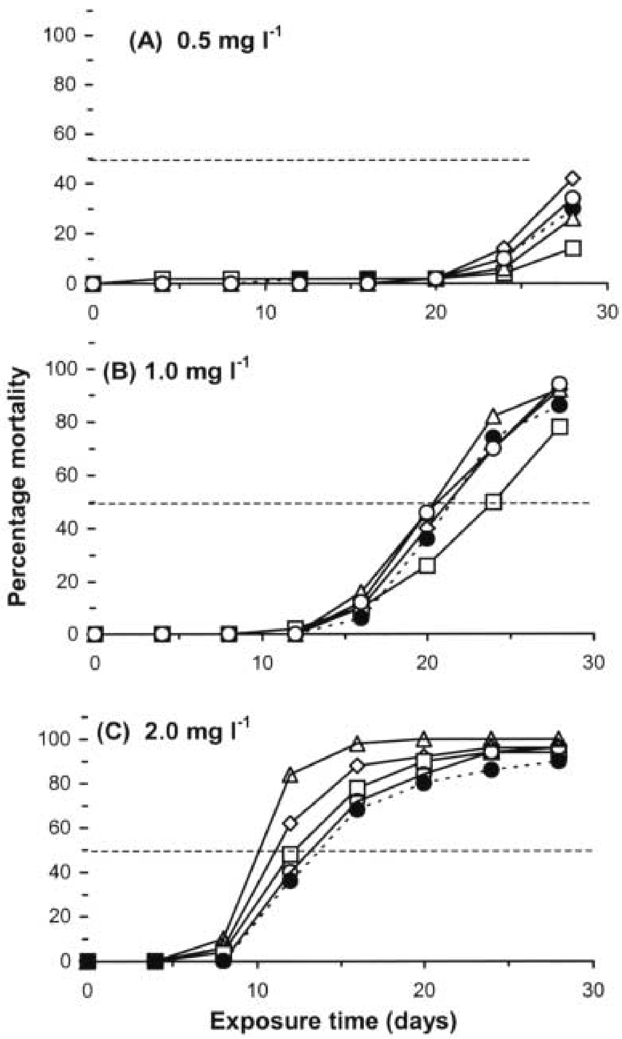

Figure 5. Mortality of *Littorina saxatilis* from sites in the Isle of Man exposed to cadmium. (A) 0.5 mg l^{-1}; (B) 1.0 mg l^{-1}; (C) 2.0 mg l^{-1} added Cd. The plotted values are means of five replicates. Dotted lines represent 50% mortality. Control mortality=0%, not plotted. ◇, Castletown; □, Derbyhaven; ●, Laxey; △, Peel; ○, Ramsey.

Specifically, Laxey was not an outlier at the higher concentration (Figure 2C; Table 2) showing similar tolerance to Castletown both of which differed from the other sites. Some significant differences in tolerance between populations were, however, still apparent (Table 3). There was also no significant correlation between LT_{50} values and the tissue concentrations of zinc (N=5, r=0.05, $P>0.05$).

On exposure to 5 mg l^{-1} lead, the animals from Laxey were less susceptible than those from all other sites (Figure 3A), having significantly higher LT_{50} values (Tables 2 & 3). No significant correlation occurred between LT_{50} values and tissue lead burdens found in samples from the field (N=5, r=0.42, $P>0.05$). Rather, there was a significant correlation between tissue zinc concentrations and the LT_{50} values for 5 mg l^{-1} lead (N=5, r=0.93, P=0.01) indicating co-tolerance (see Discussion). The 10 mg l^{-1} added lead solutions were essentially saturated solutions because of the insolubility of lead nitrate above 5 mg l^{-1} in seawater (see Mance, 1987), and some precipitation was observed to occur in the test solutions. However, mortalities were higher in this treatment than at 5 mg l^{-1} lead (Figure 3B).

In all test concentrations of copper, animals from Laxey were the most tolerant whilst those from Ramsey showed the least tolerance. Individuals from other sites did not show any consistent pattern (Figure 4, Tables 2 & 3). All correlations between LT_{50} values of the various populations for exposure to copper with tissue burdens were not significant (0.5 mg l^{-1}: N=5, r=−0.29, $P>0.05$; 1 mg l^{-1}: N=5, r=−0.22, $P>0.05$) although at the highest concentration there were indications of a negative effect (2 mg l^{-1}: N=5, r=−0.69, P=0.10). Mortalities at 0.5 mg l^{-1} cadmium were low (Figure 5A) and only a few replicates showed up to 50% mortality. At 1 mg l^{-1} cadmium the animals from Derbyhaven appeared to be the least susceptible (Figure 5B); but this was not significant (Tables 2 & 3). Susceptibilities to cadmium at 2 mg l^{-1} (Figure 5C) were significantly different between some sites (Laxey=Ramsey=Derbyhaven > Peel, Table 3). No significant correlation was found between LT_{50} values for cadmium exposures and tissue cadmium concentrations (1 mg l^{-1} Cd: N=5, r=−0.25 $P>0.05$; 2 mg l^{-1} Cd: N=5, r=0.21, $P>0.05$).

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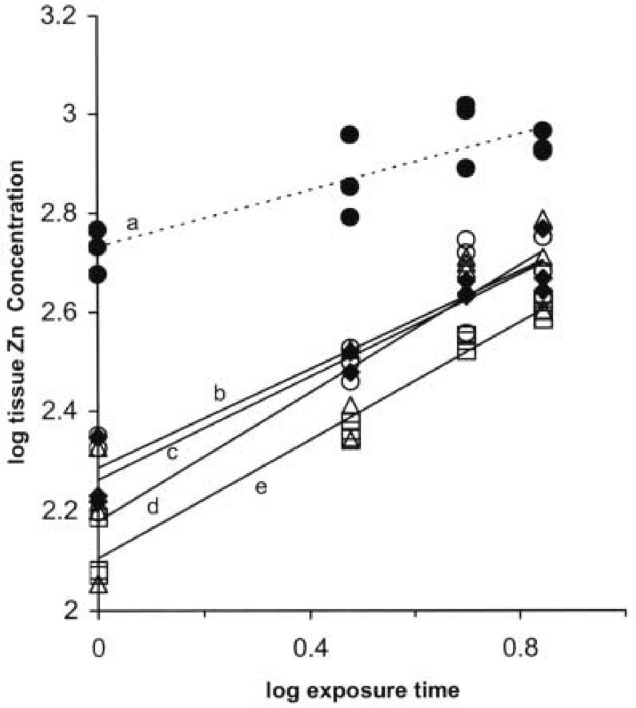

Figure 6. Regressions of zinc accumulation with time in *Littorina saxatilis* from five sites in the Isle of Man exposed to 2.5 mg l^{-1} Zn. Tissue Zn concentrations (μg g^{-1} dry weight) and exposure time (days) were log(x+1) transformed. a, Laxey: y=2.73+0.285x; b, Ramsey: y=2.29+0.496x; c, Castletown: y=2.26+0.519x; d, Peel: y=2.18+0.644x; e, Derbyhaven: y=2.11+0.593x. ◆, Castletown; □, Derbyhaven; ●, Laxey; △, Peel; ○, Ramsey.

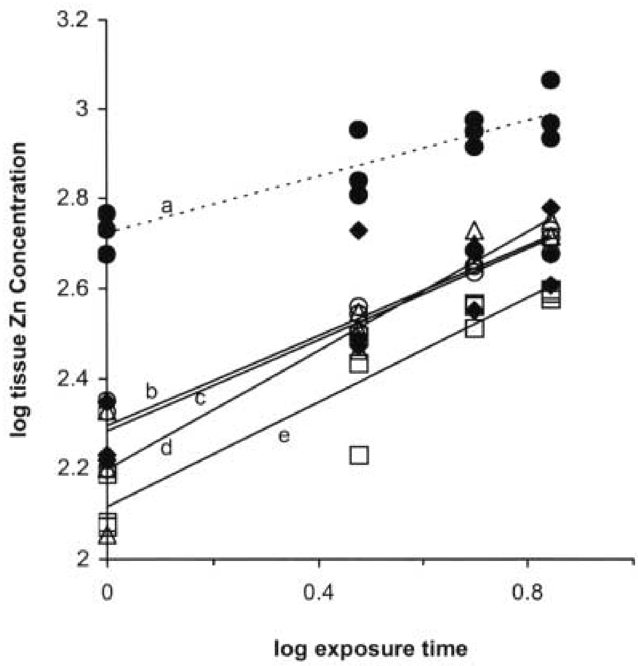

Figure 7. Regressions of zinc accumulation with time in *Littorina saxatilis* from five sites in the Isle of Man exposed to 5 mg l^{-1} Zn. Tissue Zn concentrations (μg g^{-1} dry weight) and exposure time (days) were log(x+1) transformed. a, Laxey: y=2.72+0.316x; b, Ramsey: y=2.29+0.502x; c, Castletown: y=2.28+0.508x; d, Peel: y=2.20+0.662x; e, Derbyhaven: y=2.11+0.582x. ◆, Castletown; □, Derbyhaven; ●, Laxey; △, Peel; ○, Ramsey.

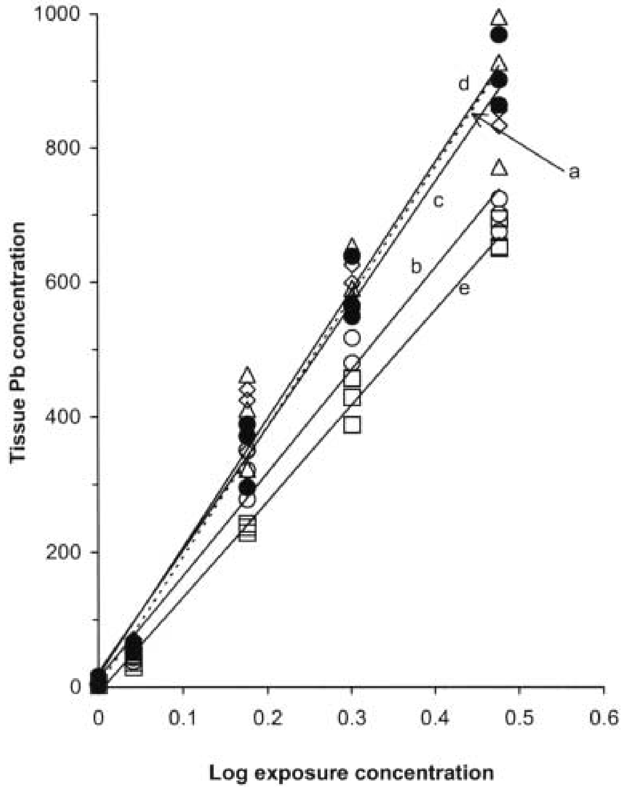

Figure 8. Regressions of tissue lead burdens (μg g^{-1} dry weight) with increasing exposure concentrations (mg l^{-1}) in *Littorina saxatilis* from five sites in the Isle of Man. Exposure concentrations were log(x+1) transformed. a, Laxey: y=3.10+1917x; b, Ramsey: y=13.2+1521x; c, Castletown: y=21.8+1817x; d, Peel: y=17.5+1901x; e, Derbyhaven: y=8.34+1416x. ◇, Castletown; □, Derbyhaven; ●, Laxey; △, Peel; ○, Ramsey.

Metal accumulation

The accumulation of zinc in *L. saxatilis* at 2.5 mg l^{-1} and 5 mg l^{-1} added zinc are given as log–log regressions of tissue metals against time (Figures 6 & 7). The animals from Laxey had a much lower rate of zinc accumulation than individuals from all other sites (ANCOVA: 2.5 mg l^{-1}, $F_{4,50}$=7.25, $P<0.001$; 5.0 mg l^{-1}, $F_{4,50}$=7.24, $P<0.001$).

The regressions of lead accumulation against lead concentration (Figure 8) gave significant differences between regression coefficients (ANCOVA, $F_{4,65}$=12.15, $P<0.001$). *Littorina saxatilis* from Laxey had the highest slopes (i.e. the highest rate of lead accumulation), and animals from other sites were in the order: Peel > Castletown > Ramsey > Derbyhaven.

Zinc tolerance in the Laxey population was coincident with low zinc accumulation and significant negative correlations were found (2.5 mg l^{-1} Zn, N=5, r=−0.90, $P<0.05$; 5 mg l^{-1} Zn, N=5, r=−0.86, $P<0.05$). There appears to be no relationship between lead accumulation and lead tolerance (N=5, r=0.19, $P>0.05$).

DISCUSSION

There was much variation in tolerance to metals among the populations studied. In the zinc and lead concentrations where tolerance was not unequivocally demonstrated and for those metals (Cd and Cu) where no population showed across-the-board tolerance, some significant differences were observed showing the presence

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of natural variation among the sites. This emphasizes the importance of using multiple control sites (as opposed to one control site of some authors e.g. Lam, 1996) to make valid conclusions about metal tolerance.

Tolerance

Our work shows that prior exposure to zinc has resulted in enhanced tolerance to lethal concentrations of zinc in *Littorina saxatilis*; animals from Laxey which had the highest zinc levels were significantly more tolerant on exposure to 10 mg l^{-1}, and to a lesser extent 5 mg l^{-1} zinc, than animals from other sites which showed similar levels of mortality. Toxic effects at 20 mg l^{-1} zinc, however, were not markedly different for animals from most sites. Roesijadi & Fellingham (1987) constructed a conceptual model of metal tolerance that separates the responses of individual organisms to exposure into four stages: (i) responses to natural background conditions and low level exposure, no discernible difference between control and exposed organisms; (ii) exposure results in bioaccumulation; protective systems are mobilized; protection against toxicity conferred; (iii) maximal participation of protective systems; upper limit of compensatory response; and (iv) severe exposure: acutely toxic, mobilization of detoxification systems not sufficient to protect essential metabolic pathways. The intraspecific responses to zinc obtained for *Littorina* in this study broadly conform to this model. The elevated zinc level in Laxey suggests animals from that estuary have protective systems mobilized at the time of sampling (Stage II). The concentration at which tolerance was demonstrated conformed to Stage III, but at the higher test concentration, Stage IV is reached whereby the protective systems are overwhelmed by the severity of exposure.

Littorina saxatilis from Laxey were also more tolerant to lead at 5 mg l^{-1} lead than animals from all other sites. However, while tolerance to 10 mg l^{-1} zinc was significantly positively correlated with tissue burdens of zinc, lead tolerance at 5 mg l^{-1} was not significantly correlated with tissue lead levels. There was, nevertheless, a significant correlation between tissue zinc concentrations and the LT_{50} values for 5 mg l^{-1} lead ($N=5$, $r=0.93$, $P=0.01$). This suggests that there was co-tolerance between zinc and lead in the Laxey population, with presumably the presence of zinc tolerance predisposing the animals to lead tolerance. The populations from Laxey, Derbyhaven, Castletown and Peel showed similar levels of tolerance to copper at one concentration or the other (see Tables 2 & 3). There were no significant differences in tolerance to cadmium at 0.5 and 1.0 mg l^{-1} cadmium; at 2.0 mg l^{-1} cadmium the LT_{50} values for individuals from Derbyhaven, Laxey and Ramsey were also similar. The indications, therefore, were that no co-tolerance to copper or cadmium existed with tolerance to zinc in *L. saxatilis* from Laxey estuary.

Tolerance to more than one metal has been reported in other species; in some the tolerance between metals is linked but in others it is independent. *Nereis diversicolor* from Restronguet Creek have developed zinc and copper tolerance by separate means (see Bryan & Hummerstone, 1971, 1973; Grant et al., 1989). Brown (1978) reported co-tolerance to lead by a copper-tolerant population of *Asellus meridianus* from the River Hayle, Cornwall, but a lead-tolerant population did not, however, possess co-tolerance to copper.

Samples for this study were collected from essentially sheltered locations, stratified to exclude the effects of exposure and shore height. It is possible for inter-site differences in natural environmental conditions to affect metal tolerance (Cannon & Hughes, 1992). It is possible, but unlikely that some of the differences might reflect differences in taxonomic composition. The patterns of tolerance to metals observed were, however, largely the result of prior exposure to metals.

Mechanism

Tolerance to heavy metals has been shown by several workers to be coincidental with lowered net uptake rates of metals in aquatic organisms (e.g. zinc tolerance of the polychaete *Nereis diversicolor*, and the crab *Carcinus maenas*, from Restronguet Creek, Bryan & Gibbs, 1983). Similar results have been reported for copper in *Cerastoderma edule* from the Tamar estuary (Naylor, 1987) and littorinids (zinc and copper) from Dulas Bay, Anglesey, Wales (Webb, 1990). The metal accumulation studies have shown that the tolerances to zinc and lead observed for *L. saxatilis* in our study were associated with different mechanisms. While zinc tolerance was correlated with reduced net uptake, lead tolerance was correlated with higher lead accumulation in the animals from Laxey. Hence, tolerance to zinc could be due to reduced uptake, reduced sequestration or increased rate of excretion, or all three. Enhanced lead accumulation could reflect the opposite situation. Correlations between LT_{50} and lead accumulation values (excluding values for Laxey) showed that the putative tolerance of the Castletown and Derbyhaven populations of *L. saxatilis* can be attributed to lower accumulation. However, in the case of the Laxey population, enhanced tolerance was coincidental with higher lead accumulation, suggesting that lead was sequestered on an excess metal binding capacity in the tolerant winkles.

Detoxification mechanisms of metals in littorinids include changes in metabolically active sites by binding to proteins (Han et al., 2003), or sequestration in metal containing granules (e.g. Mason & Nott, 1981; Brough & White, 1990). Phosphate granules found in digestive glands of metal tolerant species might also play a role (Nott & Nicolaidu, 1989; Gibbs et al., 1998).

Environmental pollutants may exert strong selective pressures on natural populations (Kim et al., 2003). The prevalence of widely dispersed propagules or larval stages of marine organisms reduces the likelihood of localized selection occurring in many species. It is only likely in species with direct development or limited dispersal such as *Littorina saxatilis* (Johannesson et al., 1995). Evidence for inherited heavy metal tolerance has been shown for freshwater *Asellus meridianus* from the Gannel, Cornwall (Brown, 1976); and *Nereis diversicolor* from Restronguet Creek (Bryan & Hummerstone, 1971; Bryan & Gibbs, 1983), confirmed by more recent work by Grant et al. (1989). The historical extent of contamination of the Laxey estuary and the ovoviviparous mode of reproduction of *L. saxatilis* suggests the possibility that tolerance to zinc may be genotypic; but this requires confirmation by breeding studies.

CONCLUSION

The exposure of *Littorina saxatilis* in the Laxey estuary to high concentrations of zinc from a catchment that has been contaminated from past mining, has resulted in enhanced tolerance to toxic concentrations of zinc in comparison with less contaminated sites. There was also co-tolerance to lead in the population of winkles from Laxey estuary but no co-tolerance to copper or cadmium. Zinc tolerant animals appeared generally to have lower net uptake of zinc in solution, but tolerance to lead was associated with enhanced accumulation.

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