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Population differences in susceptibility to stress in *Littorina saxatilis* from metal contaminated and uncontaminated sites in the Isle of Man

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Abstract: The population of the intertidal gastropod *Littorina saxatilis* from Laxey estuary in the Isle of Man is exposed to mine-related contamination of Zn (and is tolerant to Zn and Pb) compared to those of Castletown, Derbyhaven, Peel and Ramsey. Tolerances to salinity and desiccation stress were assayed to test how metal tolerance could affect tolerance to general stress. The winkles were experimentally exposed to salinities of 0, 17 and 60 practical salinity units (psu) with 34 psu as control. Desiccation stress was determined by aerial exposure of the winkles at 24℃. No control mortalities were recorded and mortalities in 17 psu were less than 50% for all populations over a 24-day exposure period. LT_{50} values (mean $\pm SD$, n=5) at 0 psu ranged from 6.6 ± 0.6 to 7.5 ± 1.3 d for winkles from Ramsey and Laxey respectively. The values for 60 psu ranged from 7.0 ± 0.7 to 8.4 ± 0.9 d. No significant differences in LT_{50} values were obtained for the salinity exposures(ANOVA, P>0.05) but there was a significant difference in tolerance to desiccation (P<0.01). The winkles from Laxey showed very high susceptibility to desiccation stress probably indicating a physiological trade-off to metal tolerance.

Keywords: tolerance; salinity; desiccation; winkles; Isle of Man

Introduction

Pollution-induced stress indices at various levels of organization, from sub-cellular to organismal level, have been investigated as a means of identifying and monitoring environmental contamination. Since changes elicited by toxic materials must occur at the biochemical, cellular and tissue levels of organization before effects would be observed at the organismal level, such measures have been suggested as potential short-term, functional indices which can be used to predict the effects of chronic exposures at higher levels of organization (Giesy and Graney, 1989). An organismal level response, the "scope for growth" (SFG) response, is thought to combine both quick response and ecological relevance(Bayne *et al.*, 1979). The SFG assay evaluates the effects of stress at the level of individual energy budgets and reflects the balance between energy acquisition (feeding and digestion) and energy expenditure (metabolism and excretion). Thus it provides an instantaneous measure of the energy status of an animal (Maltby and Naylor, 1990; Widdows *et al.*, 1995).

Viarengo *et al.*(1995) proposed the use of stress on stress (SOS) response (in mussels) as an index of general stress at the organismal level, which can be applied as a monitoring tool for the assessment of contaminated coastal areas. The population of *Littorina saxatilis* from the metal contaminated Laxey estuary in the Isle of Man has been shown to be tolerant to zinc and lead owing to mine-related contamination by heavy metals (Daka and Hawkins, 2004). The stress on stress response has been extended to evaluate physiological trade-off to metal tolerance by studying the differences in salinity and desiccation tolerance of *L. saxatilis* from metal contaminated and uncontaminated sites in the Isle of Man.

1 Materials and methods

1.1 Test organism and study sites

The taxonomy of the *Littorina saxatilis* complex has been largely resolved(Reid, 1996). The littorinids sampled for this work were large and ovoviviparous, so they would have all been *L. saxatilis* (Daka and Hawkins, 2004). Animals were collected from five sites around the Isle of Man(Fig.1): the estuary mouths at Laxey, Peel, Castletown and Ramsey, and the open shore in Castletown Bay near Derbyhaven. The animals from Laxey (tolerant to zinc and lead) represent a highly contaminated population, especially with zinc, while those from other sites provide comparisons with various degrees of contamination including essentially uncontaminated control sites (Daka and Hawkins, 2004). To reduce size effects, animals of a similar size (about 10 mm shell height) were used.

1.2 Experimentation

L. saxatilis were collected from various sites as indicated above and transported to the laboratory in moist seaweed. Individuals of a standard size(as stated above) were acclimatized to test conditions (10 ± 1℃) initially in tanks containing aerated seawater and subsequently to the test chambers (Mance, 1987). The test chambers consisted of 2 L conical flasks with rubber stoppers that were opened for the passage of air delivery tubes. The acclimatization period lasted for a week.

After acclimatization, the animals were intro-

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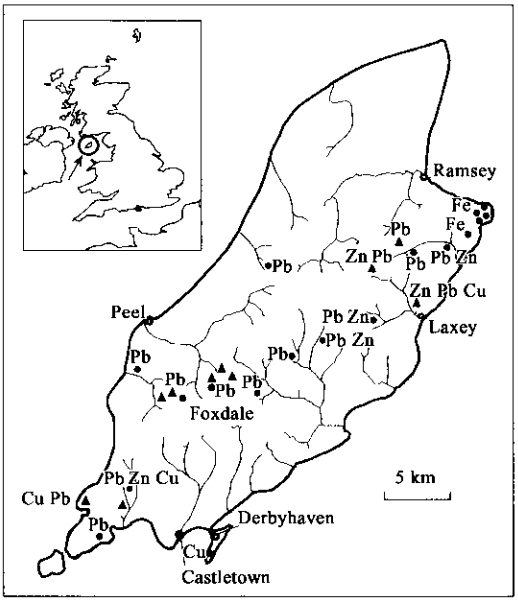

Fig.1 Locations of sampling sites (open circles) around the Isle of Man and past mining activities; filled triangles and circles represent major and minor producing mines respectively (Cu; Fe; Pb; Zn); the British Isles showing the geographical location of the Isle of Man

duced to the appropriate treatment of hypo/hyper saline solutions. Salinities tested were 0, 17 and 60 practical salinity unit (psu); and 100% sea water(34 psu) was used as control. Zero psu was obtained from double distilled water, 17 psu was prepared as a 1 : 1 dilution of sea water in double distilled water and 60 psu was prepared by the addition of a commercial aquarium salt (Instant Ocean, Aquarium Systems, France) in sea water. The tests were run in five replicates for each salinity. Replication was achieved by a randomized complete block design, with each block(test chamber) containing bagged individuals(ten individuals per bag) from each of the five sites. Aeration was maintained throughout the experiment with filtered air and the animals were not fed before or during the experiment. Mortality was examined every 4 d. At 0 and 60 psu the experiments were terminated on total mortality, obtained on the day 12 but the 17 psu treatment and controls were monitored for a further 12 d.

Tolerance to desiccation was tested by aerial exposure of five replicates of 10 bagged individuals (per replicate) at 24℃ (variations of up to 3℃ were recorded). Replicates were placed in a completely random order in a controlled-temperature room and the experiment was run for 44 d. Mortalities were examined every 4 d by placing animals in sea water for 30 min. Live animals usually emerged from the shell or showed opercular response to gentle mechanical stimulation.

1.3 Data analysis

Median lethal times (LT_{50}s) were obtained by graphical interpolations (Parrish, 1985). Analysis of variance was performed on the LT_{50} values (after $\log_{10}(x+1)$ transformation) to test for differences in tolerance of animals from various sites using MINITAB for Windows R14. Where significant differences occurred, Tukey HSD multiple comparisons were applied to test the differences between individual means between pairs of sites(Zar, 1984). Relationships between desiccation and metal tolerance (Daka and Hawkins, 2004) were assessed by simple correlations between the LT_{50} values.

2 Results and discussion

No control mortalities were observed throughout the study period and the mortalities at 50% seawater (17 psu) were also very low with the highest average mortality of only 14% at the termination of the experiments after 24 d exposure period (Fig.2a). Time-mortality curves for exposures to 0 psu and 60 psu show that in both extreme salinity treatments, no mortalities were observed on the day 4 but huge mortalities were recorded on the day 8, and by the day 12 there was total mortality in most cases (Fig.2b and 2c). The order of tolerance was also similar in both 0 psu and 60 psu with an indication that *L. saxatilis* from Laxey were the most tolerant and those from Ramsey the least. The order of tolerance of animals from the other sites was Derbyhaven, Castletown, Peel. The median lethal times (LT_{50}s) were generally higher at 0 psu than 60 psu (Table 1) and these confirm the interpopulation variation of tolerance indicated by the time-response curves. However, analysis of variance (ANOVA) did not show any significant difference in mean LT_{50}s between sites at any salinity exposure (Table 1).

Table 1 Median lethal times-LT_{50} of *Littorina saxatillis* from five sites in the Isle of Man exposed to extreme salinity (0 psu and 60 psu) and desiccation stress*

	LT_{50}(mean ± SD, n = 5)		
Site	0 psu	60 psu	Desiccation
Castletown	6.9±0.4	7.8±1.0	35.7^{1}±3.5
Derbyhaven	7.1±0.6	7.9±0.9	$25.2^{1,2}$±9.8
Laxey	7.5±1.3	8.4±0.9	10.4^{3}±4.3
Peel	6.8±0.4	7.7±0.9	$14.4^{2,3}$±7.1
Ramsey	6.6±0.6	7.0±0.7	10.1^{3}±3.3
ANOVA	$F_{4,16}$=2.978, P>0.05	$F_{4,16}$=1.749,P>0.05	$F_{4,20}$=13.49,P>0.01

Notes: Superscripts with different numbers along a column show significant difference(Tukey tests, P<0.05); * no LT_{50} values for control (34 psu—no mortality) and 17 psu (low mortalities)

Fig.3 shows that there were clear inter-site differences in the tolerance of *L. saxatilis* subjected to desiccation stress (aerial exposure at 24℃). Animals from Laxey which suffered the least mortalities at extreme salinities were amongst the most susceptible

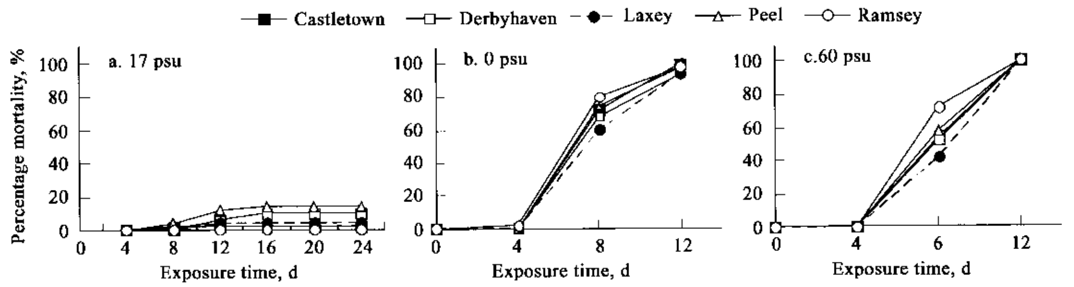

Fig.2 Time-mortality curves for *Littorina saxatilis* from five sites in the Isle of Man exposed to 0 psu, 17 psu and 60 psu salinity Values plotted are means of five replicates(N B. differences in exposure time), control mortality(0%) not plotted

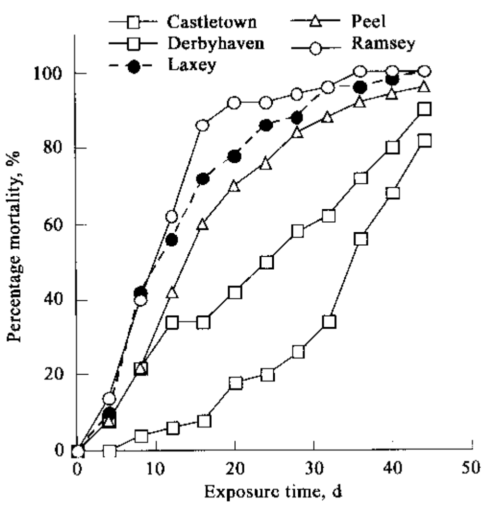

Fig.3 Time mortality curves for *Littorina saxatilis* from five sites in the Isle of Man exposed to desiccation stress (aerial exposure at 24℃), values plotted are means of five replicates

to aerial exposure, whereas Castletown samples showed the highest tolerance. Mean LT_{50} values for the various sites are presented in Table 1 and these were shown by ANOVA to be significantly different ($P < 0.001$). Multiple comparisons by Tukey tests indicate that animals from both Castletown and Derbyhaven were significantly more tolerant to desiccation than those from Laxey and Ramsey (Table 1). Individuals from Castletown also had significantly higher LT_{50} values than those from Peel ($P<0.005$); but no significant difference was found between the Derbyhaven and Castletown populations. *L. saxatilis* from all sites were less susceptible to desiccation stress than exposures to 0 psu and 60 psu salinities (Table 1).

There were negative correlations between LT_{50} values for desiccation with 10 mg/L Zn and 5 mg/L Pb (Table 2), the concentrations at which clear tolerance was demonstrated for these metals by animals from Laxey Estuary (Daka and Hawkins, 2004). All correlations between 0 psu and 60 psu and all metal-concentration combinations were positive, but most were not significant (Table 2). As indicated above, no significant differences were found in tolerance to extreme salinity stress in *L. saxatilis* from sites in the Isle of Man. However, significant differences were obtained on exposure to desiccation stress, with animals from Laxey which showed enhanced tolerance to Zn and Pb being amongst the most susceptible to desiccation. This implies that the detection of any physiological trade-off or "cost" of metal tolerance would depend on the stressor applied. The desiccation experiment detected an apparent "cost" of tolerance to metals as the LT_{50} values for desiccation were negatively correlated with LT_{50} values for exposure to 10 mg/L Zn and 5 mg/L Pb, the concentrations at which unequivocal tolerance was demonstrated for these metals by the animals from Laxey (Daka and Hawkins, 2004). Also, animals from the relatively uncontaminated sites, Castletown and Derbyhaven, were significantly more tolerant to desiccation than those from Laxey. The pattern of intraspecific tolerance to extreme salinity exposure did not detect any physiological trade-off in animals from Laxey. In fact, animals from Laxey nominally showed the least susceptibilities at both salinity concentrations. However, these responses would have to be examined in the light of uncontrollable spatial heterogeneity of conditions at the sites from which the

Table 2 Correlations(r) between LT_{50} of *Littorina saxatillis* exposed to general stressors (salinity and aerial exposures) and LT_{50} for exposure to different heavy metals

Metals	Conc., mg/L	Desication	LT_{50}	
			0 psu	60 psu
Zinc	10	-0.403	0.881*	0.738
Zinc	20	0.528	0.772	0.909*
Lead	5	-0.587	0.797	0.552
Lead	10	0.091	0.614	0.441
Lead	20	0.308	0.838	0.795
Copper	0.5	0.321	0.822	0.916*
Copper	1	0.125	0.701	0.848
Copper	2	0.485	0.820	0.915*
Cadmium	1	0.283	0.302	0.219
Cadmium	2	-0.459	0.331	0.002

Note: * Significant correlations($P<0.05$, $n=5$)

animals were collected.

Differences in salinity and emersion regimes, as well as exposure of habitat and shore height between the sites of collection could affect tolerance to salinity. Arnold (1972) observed differences in response to reduced salinity in littorinids from different sites. The lowest mortalities and highest activity scores were obtained at salinities approaching those prevailing at the sites of collection. *L. saxatilis* from shores in Sweden were found by Sundell (1985) to be more tolerant to reduced salinities than animals from sheltered habitats. Tolerance to low salinity has been found to be greatest in populations collected from sites subjected to reduced salinity. Rosenberg and Rosenberg (1973) reported that *L. littorea* in three Scandinavian populations showed varying tolerance to reduced salinity with the population from the area of lowest salinity showing the least susceptibility. Intraspecific differences in response to reduced salinity also occur with shore height (Arnold, 1972). The animals from Laxey were found further up the estuary than those from all other sites and were likely to experience higher variations in salinity. It was therefore not surprising that animals from Laxey would be more tolerant than those from other sites. It is difficult to assume that they would have had a significantly higher tolerance if they were not exposed to metal stress.

The manner in which physiological modifications occur in the animals in response to salinity and aerial exposure may also explain the differences in response to the two stressors. Marine molluscs are generally considered to be poikilosmotic animals with no power of extracellular osmotic regulation and such is the case with littorindis(Mayes, 1962; Avens and Sleigh, 1965; Taylor and Andrews, 1988). However, behavioural responses, notably shell closure may enable animals in some cases to temporarily isolate themselves from the external medium and reduce the amplitude of osmotic shock. Avens and Sleigh (1965) observed that *L. saxatilis* maintained a hyperosmotic state for up to 3 d in low salinity solutions using a "shell closing" mechanism. They found that on exposure to low salinity, *L. saxatilis* does not behave as a perfect osmometer, but rather as a leaky one allowing some ionic movements. On transfer from 100% to 50% seawater, an inflow of water resulting in a rapid increase in weight was accompanied by a loss of salts, but the final weight at 50% seawater reached a steady level above that in 100% seawater. Mayes (1962) similarly found about 10% gain in wet tissue weight after 36 h of transfer of *L. saxatilis* from 100% to 50% seawater. Immersion in hypersaline water (200% seawater), on the contrary, resulted in a decrease in weight. Up to 45% loss of weight was recorded after a week of aerial exposure of *L. saxatilis* at 10—15℃; and exposure for up to 12 d(beyond which no animals survived) resulted in about 100% increase in blood urine concentration(Avens and Sleigh, 1965).

There is considerable difference in the survival capacity of *L. saxatilis* to extreme salinity and aerial exposures reported by different authors. The median lethal times obtained in this study (6.5—7.5 d) for exposure of *L. saxatilis* to 0 psu salinity are comparable with those of Cannon and Hughes(1992) for animals collected from Cable Bay, Anglesey(ca. 5 d). Sundell(1985) reported a mean survival time of 14 to 18 d for animals from the Swedish coast. The results of this study also differ considerably from those of Webb (1990) for animals from sites in the Isle of Man. Firstly, he found that animals from Castletown were much more tolerant at 0 psu salinity than those from Laxey with LT_{50} of ca. 10 and 5 d respectively. The values obtained in this study were ca. 7 d for Castletown and 7.5 d for Laxey. Secondly at 50% seawater, minimal mortalities were found in animals from all sites over a 24-d exposure period while Webb (1990) found LT_{50} values of ca. 14 d in animals from Laxey and 10 d in those from Peel, but for individuals from Castletown < 50 % mortality was obtained at the termination of his experiments after 20 d.

The nature of experimental treatments may affect the response of animals to stress. When animals previously exposed to hyposaline and hypersaline solutions were re-introduced into 100% seawater, a reversal in the weight changes resulted (Mayes, 1962; Avens and Sleigh, 1965). Similarly, a rapid rehydration and weight gain ensured when animals exposed to desiccation are immersed in seawater (Avens and Sleigh, 1965). The short periods of immersion in 100% seawater during the examination of individuals for mortality may have resulted in increased osmotic shock in my salinity experiments. This may partly account for the huge mortalities obtained on the day 8 (second examination) while no mortalities were obtained on the day 4 in both 0 psu and 60 psu exposures. However, for the desiccation experiment, the rehydration achieved during examination may have resulted in longer survival times. Avens and Sleigh (1965) recorded total mortalities in *L. saxatilis* within 12 d in experiments where animals were exposed to desiccation in jars and mortality examined by prodding. In contrast the animals from the least tolerant site in this study did not record 100% mortality until the day 36.

3 Conclusions

In conclusion, it appears that metal tolerance may be exerting an effect on the ability of the population of *L. saxatilis* from Laxey to withstand basic intertidal stress, especially desiccation. This is probably a physiological trade-off in function but ecological differences between sites may partly account for the differences observed.

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