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Genotype-dependent recovery from acute exposure to heavy metal contamination in the freshwater clam *Sphaerium novaezelandiae*

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ABSTRACT

The ability to recover from environmental perturbations is essential for the sustainability of ecological systems. Variation in the ability of individual organisms to recover from stressors influences overall resilience at higher levels of biological organisation. Such variation is likely to be genetically based. To investigate this hypothesis we examined the genetic basis of both resistance to and recovery from zinc, a common stormwater contaminant, in the New Zealand freshwater clam *Sphaerium novaezelandiae*. We undertook a 4-day toxicity test using zinc exposure concentrations ranging from 0.31 to 5.00 mg/L. These concentrations are consistent with levels recorded in urban streams during the first flush of storms. As our response measures we recorded mortality at the end of the 4-day period, as well as reburial rate (time to rebury in sediment) following the 4-day exposure ("exposure") and then again following a 24 h period of recovery ("recovery"). Genotypic composition was determined using allozyme electrophoresis, focusing on the enzyme Pgm (phosphoglucomutase). Overall, a significant effect on mortality was observed, with an average value of 78.6% (±7.9) at 5.00 mg/L zinc, compared with only 3.8% (±3.8) mortality at 0.31 mg/L zinc. An inhibition concentration (IC_{50}) of 1.16 mg/L was recorded, when considered regardless of genotypes. There was no significant genotype-specific differences in mortality. There was a significant difference in reburial rates across all genotypes at the end of the exposure period with an average reburial time of 83.0 ± 3.6 min at 5.00 mg/L (22.8 ± 2.9 min at 0.31 mg/L). There was a near-significant ($p = 0.058$) difference in time taken to rebury when comparing between genotypes at the "exposure" stage for any concentration. Significant differences in reburial rates across all genotypes were also observed following 24 h recovery. When individual genotypes were compared at this stage, genotype 33 reburied on average significantly faster (24.0 ± 4.5 min) than other genotypes at the highest exposure concentration and was also significantly faster than genotype 44 at 1.25 mg/L. Studies investigating the genetic basis to recovery from stressors at an individual level are limited. This study has shown that populations of organisms display genetically-based variation in their ability to recover from zinc exposure in the laboratory and that such variation is linked to a physiological trait (reburial). The potential effects on other life history traits (e.g. feeding), possible physiological trade-offs and the implications for such variation on ecosystem resilience requires further investigation.

1. Introduction

Variation in the ability of individual organisms to recover from a stressor is likely to influence the resilience capacity of populations and communities. Costanza and Mageau (1999) define a "healthy system" as one that can maintain its function (vigour) and structure (organisation) over time in the presence of external stress and which is therefore resilient. Resilience can be defined in terms of the magnitude of stress from which a system can recover (Holling, 1973) or as the time required for a system to recover from stress (Pimm, 1984). Variation in recovery rates is likely to reflect adaptation, suggesting a genetic basis, yet few studies directly investigate genotypic variation in recovery characteristics within populations (Ehlers et al., 2008; Reusch and Wood, 2007; Yan et al., 1996). Rather, the focus of many studies is on defining limits of tolerance, with survivorship as the measureable endpoint (Amiard-Triquet et al., 2009; Gale et al., 2003; Rocha-Olivares et al., 2004; Virgilio et al., 2005).

Populations may undergo rapid evolutionary adaptation as a response to stress, through selection for or against specific genotypes (e.g. Klerks and Weis, 1987; Lavie and Nevo, 1986; Levinton et al., 2003). One way in which this selection may be manifested is as a change in genetic diversity. A reduction in genetic diversity can result in genetic "bottlenecks", with a subsequent reduction

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in the ability of populations to adapt to changing environments (Belfiore and Anderson, 2001; Hoffman and Daborn, 2007). In contrast, greater genetic diversity may infer increased adaptability. For example, Ehlers et al. (2008) found that genotypic diversity enhanced resistance to temperature stress in eelgrass *Zostera marina*. In another study, Gamfeldt et al. (2005) observed increasing larval settlement associated with increasing genetic diversity in the barnacle *Balanus improvisus*. Genotypic diversity may be critical for maintaining ecosystem functioning, as well as providing the basis for adaptation to environmental change. Understanding the ecosystem consequences of such loss of genetic variation is therefore important, particularly for conservation and restoration efforts (Gardestrom et al., 2008).

In addition to changes in overall genetic diversity, selection for or against specific genotypes may result in a modification of key life history traits present within a population, particularly through effects on resource allocation (Bubliy and Loeschcke, 2005; Gardestrom et al., 2008; Hendrickx et al., 2008; Ketola and Kotiaho, 2009; Shirley and Sibly, 1999). For example, Jeyasingh et al. (2009) observed greater phosphorus efficiency use in phosphoglucose isomerase (Pgi) homozygotes in *Daphnia pulicaria* clones exposed to green alga (*Scendesmus obliquus*) with varying phosphorus:carbon (P:C) ratios. While the selection of tolerant genotypes may appear beneficial at first, it may result in increased susceptibility to other selection pressures due to reduced diversity (Gillespie and Guttman, 1993). It may also reduce overall fitness of a population. For example, Watt (1992) found that individuals with the most heat-tolerant genotypes in female insects (*Colia* sp.) were also the least fecund. This result has implications, for example, for prediction of population demographic structure under various climate change scenarios.

In urban streams, aquatic organisms are exposed to a complex array of factors which lead to degradation of stream function and ecological values (Walsh, 2000). In addition to diffuse and point sources of contaminants, habitat alterations, including changes in stream bed characteristics, hydrological regime, and loss of riparian vegetation can all alter biotic community structure and function (Walsh et al., 2005). In extreme situations this can result in changes to fundamental life-support functions (e.g. availability of dissolved oxygen). In such cases, contamination effects, such as those that may be associated with stormwater, are likely to be secondary. However, the more common situation in urban streams is where stream function is only partially impacted and biotic communities retain some measure of intactness. How resilient are such communities to long-term low level stressors, for example, those associated with sediment metal contamination? Metals are often present at relatively low levels in the water column or can accumulate in sediment-bound form, from which they may be intermittently released to the water column through changes in sediment chemistry (Chapman et al., 1998). There is considerable evidence indicating the significant role that heavy metals play in selection for or against specific genetic variants, resulting in a modification of genetic diversity levels (see review in Belfiore and Anderson, 2001). In many cases, a reduction in genetic diversity is observed (Gardestrom et al., 2008). However, in some areas where pollution is highly mutagenic (for example, in association with radiation), increases in genetic diversity have been observed (Theodorakis and Shugart, 1997).

The endemic New Zealand freshwater clam *Sphaerium novaezelandiae* has been the subject of a number of toxicological investigations (e.g. Hickey and Martin, 1995, 1999; Hickey and Vickers, 1994; Quinn et al., 1994; Roa, 1997). This species displays relatively high sensitivity to a range of stressors, especially ammonia (Hickey and Martin, 1999). Genetic studies of this species are limited to phylogenetic analyses of higher taxonomic groupings (e.g. Cooley and Foighil, 2000), although other species within the genus have received more attention (e.g. *Sphaerium striatum*, Hornbach et al., 1980). *S. novaezelandiae* possesses several characteristics which make it particularly desirable as a study organism for ecotoxicological and genetic studies. It is relatively easy to maintain in the laboratory and has an ovoviviparous reproductive mode, producing live young without a larval phase (Kuiper, 1983), making it amendable to studies utilising fecundity and other reproductive endpoints. In addition, it starts reproducing at around 3 months of age (Roa, 1997), so that it is feasible to undertake multigenerational studies within 1 or 2 years, with the lack of larval phase making it a useful species for genetic analysis of selection effects between generations.

Allozyme electrophoresis has been widely used in the study of genetic responses to stressors and is considered an effective and practical tool for investigating selection effects, especially as many genetic markers are considered selectively neutral (Martins et al., 2007; USEPA, 1989; Virgilio et al., 2006; Yap and Tan, 2007). Despite the traditional view that allozyme variation is not selectively adaptive (Kimura, 1991), there is an increasing number of studies that provide contradictory evidence for this hypothesis (see Belfiore and Anderson, 2001 for a review). Differences in allozyme function can be linked with differences in fitness and therefore it is potentially possible to establish links with ecologically-relevant responses to contaminants using this relatively simple method (Watt, 1994). Changes in allele frequencies have been documented in contaminated environments, including at loci associated with survival or reproduction (Bickham et al., 2000). Such selection can act on the loci themselves, or on genes that regulate allozyme expression. Genetic "hitchhiking", whereby allozymes are linked to other loci under selection, can also then result in an allozyme locus appearing to be under selection (Baker, 1982).

In this paper we investigated the genetic basis for both tolerance (the ability to cope with stressor effects but not necessarily return to the original state) and resilience (the ability to recover from a stressor effect by returning to the pre-stressed state) to heavy metal contamination, reflected as variability in fitness (reburial rate) of individuals of the freshwater clam *S. novaezelandiae*. We hypothesised that genotype-dependent differences in response would be reflected in ability to tolerate and to recover from contaminant exposure.

2. Materials and methods

2.1. Study animals

All clams used in this experiment were sourced from Hamurana, Lake Rotorua, New Zealand (176°15.509′E longitude, 38°02.078′S latitude, WGS84/NZGD2000). Animals were collected by scooping approximately the top 2 cm of sediment from the lake bed (lake depth 0.5–1.0 m) with a plastic scoop. Samples were returned to shore and subsequently passed through a 1 mm sieve. The sediment remaining in the sieve was then visually searched for *S. novaezelandiae*, with individuals (approximately 2 mm shell length) being collected into a small (2 L) bucket filled with lake water. Individuals were transported in aerated buckets to the laboratory and acclimatised to laboratory conditions (20 °C) for at least 24 h prior to testing, thereby also allowing for clearance of the digestive system. Identification as *S. novaezelandiae* was confirmed by examination of a random sub-sample of collected animals. Roa (1997) found only *S. novaezelandiae* at this site.

2.2. Toxicity tests

We undertook an acute (96 h) static toxicity test with zinc as our test contaminant, using an adaptation of the protocol outlined

in Hickey and Martin (1995). A total of 5 test solutions (1 control and 4 treatments) were used, with 3 replicates per test solution and 10 individual clams per replicate. Individuals were placed in clean 40 ml sterile plastic cups to which prepared test solutions had been added. No sediment was added to the test containers. An initial range finding study was undertaken to determine a suitable test concentration range. Nominal test solution concentrations of 0, 0.31, 0.63, 1.25 and 5.00 mg/L zinc were prepared from a stock solution of 100 mg/L $ZnSO_4 \cdot 7H_2O$ (chemically validated as 102 mg/L). The zinc concentrations used were consistent with zinc levels recorded from urban Auckland streams during the first flush of storms (Mills and Williamson, 2008). Raw water sourced from the Waikato River (hardness = 30 mg $CaCO_3$/L) was used for control and dilution waters (particle (<1 μm) and carbon filtered, held in bulk aerated storage at 15 °C). Dissolved oxygen and pH measures were recorded in 3 randomly selected replicates of each treatment at the beginning and end of the experiment, with an average reduction in value of 1.2% and 0.1 pH units, respectively, over the duration of the test. Animals were not fed prior to or during the test, in accordance with the test protocol (Hickey and Martin, 1995).

In order to determine fitness we measured individual clam reburial into standard sediment. This behavioural-response endpoint has been found to be an appropriate measure of response for shellfish species (Hickey and Martin, 1995, 1999; Hickey and Golding, 1998). Approximately 2 cm of clean Lake Rotorua sediment, that had been passed through a 1 mm mesh sieve and rinsed well with the standard test water, was placed into sterile 40 ml plastic cups and filled with clean water. Individual clams were then transferred from the test solution to the clean containers. Reburial was recorded initially in 5 min intervals for the first 15 min and then every 15 min for up to 60 min. Individuals not reburied after 60 min were deemed to be "not reburied". "Initial", "exposure" and "recovery" reburial measures were recorded prior to, immediately after and 24 h after exposure, respectively. The recovery phase involved transferring individuals from test containers into containers with clean water (without sediment) for a period of 24 h. We also measured percentage mortality/moribund at the end of the exposure phase. All individuals that were not buried at the end of the experiment were considered to be moribund.

All tests were undertaken in a constant room temperature (20 ± 0.2 °C) with a light:dark cycle of 16:8 h, while reburial tests had continuous light (ca. 50 μE/m^2/s). Test and recovery solutions were gently aerated throughout the experimental period. Aeration was not used during the reburial measurements. Following completion of the toxicity tests, all animals were frozen for subsequent genetic analysis.

2.3. Genetic analyses

We used allozyme electrophoresis to determine the genetic identity of individual clams. Whole animals were homogenised in a grinding solution comprised of 50 μl of Tris–HCl pH 8.0 (Hebert and Luiker, 1996). Once homogenised, the samples were centrifuged at 10,000 rpm (9558 × *g*) for 1 min and 10 μl of each sample was immediately transferred to the sample gels for electrophoretic analysis. Allozymes were separated by cellulose acetate electrophoresis (Helena Super Z-12 applicator kit, 76 mm × 76 mm Titan III Cellulose Acetate plates) using techniques described in Hebert and Luiker (1996). Initial screening was undertaken for 5 enzymes, these being: isocitrate dehydrogenase (Idh, EC 1.1.1.42), phosphoglucomutase (Pgm, EC 5.4.2.2), malic enzyme (ME, EC 1.1.1.40), malate dehydrogenase (Mdh, EC 1.1.1.37) and glucose-6-phosphate isomerase (Gpi, EC 5.3.1.9). All but Pgm were found to be monomorphic at the most common allele (0.95 criterion) (N Phillips, unpublished data). For each resolvable locus, the most commonly occurring allele was designated as 3, with sequentially faster or slower migrating alleles designated relative to this. Lineup gels (*sensu* Richardson et al., 1986) were employed to compare the relative mobility of alleles recorded on different gels.

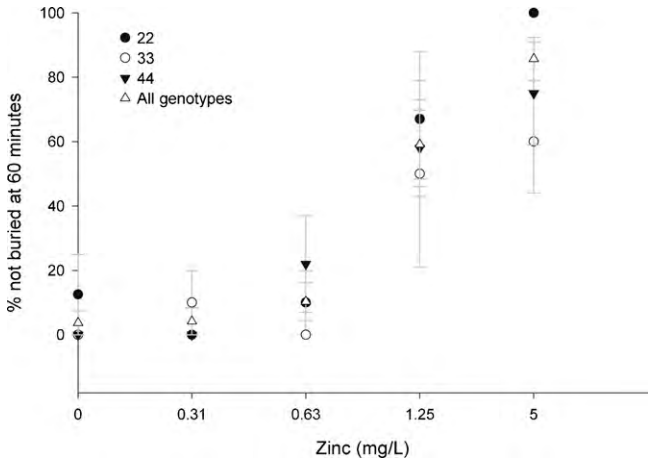

Fig. 1. Percent mortality and morbidity (±1 SE) for the three most common genotypes at the Pgm locus.

2.4. Data analyses

For data analysis purposes, those individuals not burying within 60 min were allocated a burial time of 90 min, to differentiate from those that had buried at 60 min (the maximum measured time).

Mortality point estimates were calculated using linear interpolation (IC_p method, USEPA, 1989), which is the preferred method for quantitative data. Bootstrapping was also applied to this method to allow derivation of 95% confidence limits. IC_{50} values (the exposure concentration at which 50% inhibition is observed) were calculated across all commonly occurring genotypes and then separately for each commonly occurring genotype. The software package ToxCalc V5.0.22A (Tidepool Scientific Software, 1994) was used for these calculations.

Significance of differences in mortality and reburial rates was tested using factorial Analysis of Variance (ANOVA) within Statistica v7.1 (Statsoft Inc, Tulsa, Oklahoma), with time (initial, exposure or recovery), treatment (0–5.00 mg/L) and genotype (22, 33, 44) as fixed factors. One-way ANOVAs were used to test for differences between treatments across all genotypes. All data were log-transformed to satisfy normality requirements. Tukey's post-hoc tests were subsequently used.

3. Results

3.1. Allele and genotype frequencies

Three alleles were recorded for PGM (designated as 2, 3 and 4), occurring at relatively equal frequency within the study population (0.308, 0.338 and 0.354, respectively). Three equally common genotypes (22, frequency = 0.285; 33, frequency = 0.323 and 44, frequency = 0.346) were recorded, along with 2 rarer genotypes (23, frequency = 0.031 and 24, frequency = 0.015).

3.2. Genotype-dependent responses

3.2.1. Mortality

Across all genotypes, there was a significant effect of treatment ($F = 32.05$, $p < 0.001$). On average, mortality was 78.6% (±7.9) at 5.00 mg/L, compared with only 3.8% (±3.8) at 0.31 mg/L zinc (Fig. 1). There was no significant interaction between treatment and genotype ($F = 0.67$, $p = 0.71$), indicating that mortality rates were not

Table 1
Inhibition concentrations (IC_{50}) and 95% confidence limits for mortality and morbidity.

Genotype	IC_{50} (mg/L)	95% confidence limits	
		Lower	Upper
All	1.16	0.94	1.28
22	1.10	0.36	1.11
33	2.40	0.00	4.79
44	1.25	0.00	1.25

genotype-specific ($F = 0.94$, $p = 0.39$). An inhibition concentration (IC_{50}) of 1.16 mg/L was recorded across all genotypes. Pgm genotype 33 recorded an IC_{50} of 2.40 mg/L, almost twice that of the two other common genotypes (Table 1). However, confidence limits overlapped between all 3 genotypes, resulting in no significant difference.

3.2.2. Reburial

3.2.2.1. Initial reburial rates. There was no significant difference in initial reburial rates of clams allocated randomly to the treatments when examined across all genotypes ($p = 0.71$) or between genotypes ($p = 0.253$, Table 2a).

(a) Exposure—immediately after 96 h exposure

Across all genotypes, there was a significant difference in time to reburial between treatments ($p < 0.001$) (Fig. 2), with an average reburial time of 83.0 (±3.6) min at 5.00 mg/L, compared with 22.8 (±2.9) min at 0.31 mg/L. Across all treatments, there was a near-significant interaction between genotype and treatment ($p = 0.058$) (Fig. 2, Table 2b). In this case, the treatment effect (effect size = 0.534) was much larger than the genotype effect (effect size = 0.032), with significant differences between treatments ($p < 0.001$) but not genotype.

(b) Recovery—following 24 h in clean water

Across all genotypes, there was a significant treatment effect ($p < 0.001$) (Fig. 3), with individuals at 1.25 and 5.00 mg/L reburying slower (55.2 ± 6.3 min) than those at lower exposure concentrations (36.3 ± 6.57 min at 0.31 mg/L). There was a significant interaction between genotype and treatment ($p < 0.001$). Both treatment ($p < 0.001$) and genotype ($p < 0.001$) contributed significantly to the overall differences (effects sizes of 0.226 and 0.121, respectively) (Table 2c). Post-hoc tests indicated that there was a significant difference between genotypes at 5.00 mg/L ($p = 0.004$), with genotype 33 showing significantly faster recovery (24.0 ± 4.5 min) than both 44 ($p = 0.001$) (78.2 ± 7.9 min) and 22 ($p = 0.016$) (67.5 ± 9.8 min) (Fig. 3). Individuals with genotype 33 also recovered faster than genotype 44 at the 1.25 mg/L treatment level. There were no significant differences in reburial rates between individuals of genotype 22 and 44 at any treatment level.

Table 2
Results of analysis of variance (ANOVA) to test for differences in genotype-specific reburial rates (a) initially, (b) following 96 h exposure to zinc (exposure) and (c) following 24 h recovery. DF = degrees of freedom, MS = mean square value, ES = effect size (partial eta squared). Relationships satisfying the significance criterion ($p < 0.05$) are in bold text.

	DF	MS	F	p	ES
(a) Initial					
Intercept	1	81776.71	112.6	**<0.001**	0.49
Genotype	2	526.38	0.73	0.486	0.01
Treatment	4	414.85	0.57	0.683	0.02
Genotype × treatment	8	939.21	1.29	0.253	0.08
Error	115	725.64			
(b) Exposure					
Intercept	1	252462.81	531.06	**<0.001**	0.82
Genotype	2	909.13	1.91	0.152	0.03
Treatment	4	15710.25	33.05	**<0.001**	0.53
Genotype × treatment	8	929.01	1.95	0.058	0.12
Error	115	475.48			
(c) Recovery					
Intercept	1	189336.04	295.01	**<0.001**	0.72
Genotype	2	5112.82	7.97	**0.001**	0.12
Treatment	4	5402.91	8.42	**<0.001**	0.23
Genotype × treatment	8	1431.48	2.23	**0.029**	0.13
Error	115	641.85			

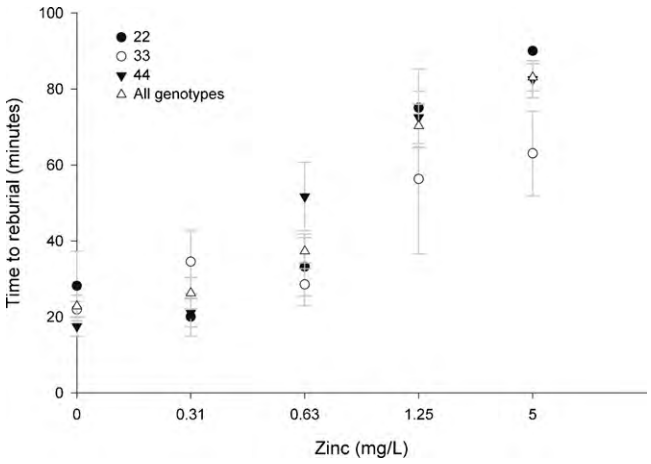

Fig. 2. Mean time to reburial (±1 SE) at the completion of exposure to zinc in solution (exposure) for the three most common genotypes.

4. Discussion

4.1. Genotype-specific tolerance

Genotype-dependent tolerance to aquatic contamination has been widely reported in a range of organisms, including amphipods (Chung et al., 2008); gastropods (Lavie and Nevo, 1982); fish (Lewis et al., 2001; Mulvey et al., 2003); mussels and barnacles (Ma et al., 2000) and cladocera (Martins et al., 2007). These studies provide strong evidence for a selection effect from exposure. Few investigations have been undertaken on bivalves within the Sphaeriidae. Sloss et al. (1998) reported elevated frequencies of a pollution-tolerant genotype at the Gpi locus in populations of the fingernail clam *Musculium transversum*. In our study, we have shown differential recovery characteristics associated with one Pgm genotype. Pgm has commonly been reported to have alleles under selection

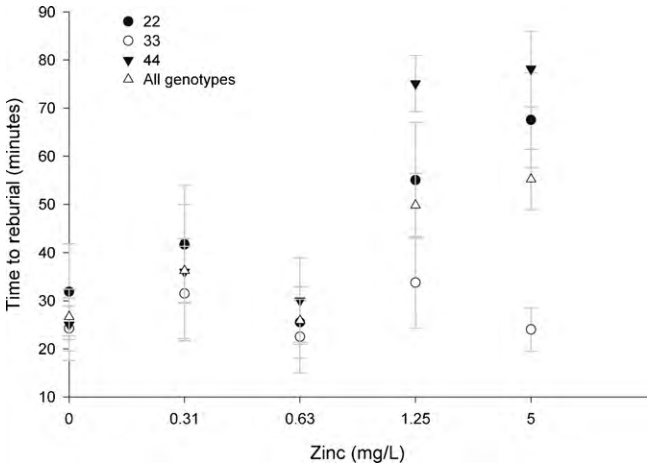

Fig. 3. Mean time to reburial (±1 SE) following 24 h recovery from zinc exposure (recovery) for the three most common genotypes.

by metals in a range of taxa (e.g. Gale et al., 2003; Laroche et al., 2002). One proposed mechanism of selection by metals for these enzymes is differential susceptibility to metal substitution of the cofactor Mg^{2+} (Milstein, 1961). The enzyme phosphoglucomutase (Pgm) catalyses the inter-conversion of glucose-1-phosphate and glucose-6-phosphate in the presence of glucose-1,6-diphosphate and Mg^{2+} and plays a central role in the synthesis and breakdown of glycogen (Ray and Roscelli, 1964). Variations in the activity of this enzyme could therefore contribute to the regulation of energy. Studies on the functional properties of PGM variants have shown correlations between Pgm polymorphism, glycogen content and Pgm activity (Leigh Brown, 1977). Tanguy et al. (2006) showed that Pgm mRNA expression could be modulated by some xenobiotics such as hydrocarbons or pesticides. The different enzyme kinetics, metabolic efficiency, and sensitivity to metals of Pgm allozymes (Nevo, 2001) may therefore provide increased fitness under stressful, energy-demanding conditions (Calow, 1991; Knapen et al., 2007, 2009). Reburial is an indirect functional measure of energy production and thus the results of this study, using allozyme electrophoresis, provide indirect support for a potential link between an organism's functional activity (i.e., reburial rate), the biochemical function of the enzymes and expression of different allozymes. Direct measurement of additional metabolic activity, such as respiration rate, pumping rate, heat production or electron transport rate would be required to establish genetic and functional linkages.

4.2. Genotype-specific recovery

In this study we found that the ability to recover from exposure to zinc was influenced by genotype, with individuals with one genotype displaying significantly greater ability to recover than other genotypes. Studies specifically addressing the genetic basis to an organism's ability to recover from stressors are limited. Reusch et al. (2005) found that rate of recovery of populations of eelgrass *Z. marina* to temperature extremes was with positively correlated with levels of genetic diversity. In a study following cleanup of a highly contaminated Superfund site, Levinton et al. (2003) estimated that recovery to pre-cadmium exposure genetic structure within populations of the oligochaete *Limnodrilus hoffmeisteri* was likely within 9–18 generations, highlighting the importance of environmental restoration in genetic recovery of populations.

4.3. Genetic basis to trait responses

Adaptation to stress implies not only that selection for tolerant genotypes has resulted, but also that effects on traits associated with life history processes such as survival, growth and reproduction have occurred (Bubliy and Loeschcke, 2005; Shirley and Sibly, 1999). The genetically-based differences in reburial rates observed in our study suggest that exposure to zinc contamination may ultimately impact on key life history characteristics of exposed *S. novaezelandiae* populations. For example, increased exposure to predators may result if reburial rates are reduced. In addition, reduced reburial activity may result in reduced pedal feeding activity, which would normally allow sediments laden with organic detritus to be renewed continually at the foot surface. Hornbach and Wissing (1984) suggested that up to 65% of total organic carbon assimilated by *Sphaerium stratinum* was likely to come from sediment detrital sources.

As energy associated with many life history traits may be limited, resource allocation to one trait generally means that other traits are affected as a consequence, resulting in trade-offs (Reznick et al., 2000; van Noordwijk and de Jong, 1986). Such trade-offs have been reported between stress resistance and a number of life history traits. For example, Marchand et al. (2004) observed a reduction in fecundity, growth rate and condition factor in "tolerant" populations of the flounder *Platichthys flesus* from contaminated sites. Metal-resistant populations of the polychaete worm *Nereis diversicolor* displayed scope-for-growth that was 46–62% less than that of animals from non-resistant populations, as well as reduced lipid and carbohydrate levels, contributing to a reduction in mass-specific fecundity in resistant animals (Pook et al., 2009). Harshman et al. (1999) found that selection for starvation resistance in *Drosophila melanogaster* was correlated with slower development time. Hoffman and Parsons (1989) observed reduced activity and metabolic rate, as well as lower fecundities associated with greater desiccation tolerance in *D. melanogaster* lines. Such trade-offs are not always observed. For example, Knapen et al. (2009) demonstrated higher condition factor associated with Gpd genotypes carrying the most abundant allele in contaminated populations of gudgeon. While we did not specifically examine trait trade-offs in this study, recent investigations using multi-generational studies in *S. novaezelandiae* indicate reduced fecundity associated with increased tolerance (N. Phillips, unpublished data).

4.4. Implications for management

Heavy metals are often delivered to aquatic environments in pulses associated with stormwater, as well as from changes in metal availability associated with release of sediment-bound forms. Indirect exposure, for example, through prey consumption or through direct consumption of contaminated sediment, also represents a significant, intermittent source (Fleeger et al., 2003). Our study has shown that populations of organisms display variation in their ability (at an individual level) to recover from zinc exposure in the laboratory. These results provide insight into the resilience capacity of populations. While our work has focused at the individual organism level, it is possible that responses observed at this level may have direct and indirect effects at higher levels of organisation (population, community, ecosystem) (Medina et al., 2007; Parker et al., 1999). The extent of such responses will be influenced by the functional role that the species plays within its environment, as well as the nature and extent of contamination. An understanding of the variability of both tolerance to and recovery from contaminant exposure would enable more targeted management activities, as it would provide a greater ability to predict potential critical thresholds for overall ecosystem response (rather than simply predicting "tipping points" for mortality), as well as likely effectiveness of restoration activities. Medina et al. (2007) have suggested that, while there is some evidence to indicate that current approaches to the protection of aquatic ecosystems may account for genetic variation in tolerance, further investigation is required on the longer-term effects on higher levels of organization. Approaches utilizing multi-generational studies (e.g. Hendrickx et al., 2008), as well as those which examine variability of functional response traits (e.g. Medina et al., 2007) offer much promise in this regard.

5. Conclusion

Our study has demonstrated the role that major stormwater contaminants such as zinc can have in negatively affecting life history traits such as reburial activity. In addition, it has demonstrated the differential responses likely within a single population. Such differences may not be identified using traditional ecotoxicity endpoints which focus on the initial exposure response but often neglect ability to recover. It is this latter response measure which provides insight into the overall resilience capacity of the exposed population. Investigation of longer-term effects through multi-generational studies would greatly enhance our understanding of the impacts of stormwater contaminants on a range of life his-

tory traits. By combining with genetic measures, complex linkages between genetic selection processes and physiological responses could be investigated.

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