

Local adaptation and maternal effects in two species of marine gastropod (genus *Crepidula*) that differ in dispersal potential

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ABSTRACT: Adaptation to local conditions should be less likely in species with wide dispersal since periodic immigration from populations not under selection would counteract the short-term results of local selection. To test this hypothesis, we used 2 species of sympatric marine gastropods of the genus *Crepidula* that have vastly different early life dispersal stages. Offspring of *C. fornicata* emerge as long-lived free-swimming larvae, whereas offspring of *C. convexa* emerge as benthic juveniles. We investigated the local adaptation of each species at 2 locations by assessing their tolerance to the toxic metal copper in the laboratory. We conducted 96 h acute copper toxicity tests with juveniles of *C. convexa* and larvae of *C. fornicata* released in the laboratory by adults collected from a reference site (Lynn Harbor, Nahant, Massachusetts, USA) or a polluted site (New Bedford Harbor, Fairhaven, Massachusetts). To test for maternal effects, we also raised *C. fornicata* released in the laboratory (F_1 generation) to sexual maturity and tested for differences in copper tolerance of their offspring (F_2). As expected, juveniles of *C. convexa* from the polluted site were significantly more copper tolerant than juveniles from the reference site ($p \leq 0.05$), suggesting local adaptation at the polluted site. Contrary to expectations, F_1 larvae of *C. fornicata* from the polluted site were not equally copper tolerant, but less copper tolerant than F_1 larvae from the reference site. However, copper tolerance did not differ for F_2 larvae of *C. fornicata* from the 2 sites. Our results suggest that populations of *C. convexa* can adapt to locally high metal concentrations, while populations of *C. fornicata* cannot, probably because of the periodic input of *C. fornicata* larvae from distant populations that do not experience high metal stress. Also, long-term exposure of *C. fornicata* adults to pollutants apparently decreased the copper tolerance of their offspring (F_1 generation), an effect not seen in the laboratory-propagated F_2 generation.

KEY WORDS: *Crepidula* · Local adaptation · Dispersal · Copper · Maternal effects · Pollution · Metals

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INTRODUCTION

Genetic adaptation to environmental stress through selection is the basis for evolution, but for many species this occurs over time scales too long to study directly. Local adaptation of a species across a heterogeneous environment allows the study of the evolution of traits across space instead of across time. Whether a species will adapt to local conditions depends partly on the amount of gene flow between populations under different degrees of selective pressure (Kawecki &

Ebert 2004). High gene flow between areas differing in the magnitude of a particular environmental stress will offset local adaptation, while limited gene flow between such areas will tend to maintain it (Kawecki & Ebert 2004). The strength of the selection and the consistency of its spatial heterogeneity over time will also affect whether a given species will become locally adapted (Kawecki & Ebert 2004).

Metals are persistent in marine sediments and some, like copper, are essential trace elements. Selection should therefore act strongly on an organism's ability

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to control the uptake, metabolism, and detoxification of such essential metals. Yet there have been few studies demonstrating evolved tolerance to metals in marine invertebrates (reviewed by Morgan et al. 2007) despite good evidence for the ability to evolve metal tolerance in freshwater invertebrates (Klerks & Levinton 1989, Ward & Robinson 2005) and vertebrates (Xie & Klerks 2004a). The role that dispersal may play in the ability of benthic marine invertebrates to adapt to localized sources of pollution has been considered in only 1 study, in which 2 species of crab and an amphipod were compared (Rainbow et al. 1999), and has never been examined for congeners. Marine invertebrates are particularly well suited for such studies, due to their diverse reproductive modes and the corresponding differences in dispersal potential.

In the present study we asked whether sister species in the genus *Crepidula* have evolved tolerance to copper at a highly polluted site in Massachusetts and whether the ability to evolve increased tolerance to copper has been limited in 1 species by dispersal during development. Unlike most gastropods, the 2 species in this study, *C. fornicata* and *C. convexa*, are suspension feeders: they have modified gills with unusually large surface areas that are efficient in particle capture (Fretter & Graham 1994) and may also, therefore, enhance uptake of water-borne pollutants. Females of *C. fornicata* release veliger larvae that remain planktonic for at least 3 to 4 wk (Pechenik 1984); thus, there is considerable potential for dispersal during development, which should limit the potential for local adaptation (Bohonak 1999, Kawecki & Ebert 2004). In contrast, females of *C. convexa* release fully developed juveniles (Conklin 1897) that have a much lower potential for dispersal during development. Although high dispersal potential does not guarantee high gene flow (e.g. Burton & Feldman 1981, and reviewed in Bohonak 1999), Collin (2001) showed through analyses of mitochondrial DNA that *C. convexa* along the Atlantic and Gulf coasts of North America had a much higher level of population differentiation than *C. fornicata*, a finding that is consistent with the hypothesized differences in realized dispersal and gene flow. We predicted, therefore, that local adaptation to high metal stress should be more likely for populations of *C. convexa* than for populations of *C. fornicata*.

MATERIALS AND METHODS

Animal collection and care. We chose to work at 2 sites in Massachusetts—New Bedford Harbor in Fairhaven and Lynn Harbor in Nahant—where both species co-exist. New Bedford Harbor has been listed

on the USEPA National Priorities List, also known as the Superfund list, since 1983 (for site description and history, see www.epa.gov/ne/nbh/history.html) because of the high concentrations of polychlorinated biphenyl (PCB) contamination of the sediments, and the accompanying high concentrations of various metals (e.g. Cu, Cd, Ni) and polycyclic aromatic hydrocarbons (PAHs) (Pruell et al. 1990). We chose our reference site, Nahant, based on much lower concentrations of metals in the soft tissue of *Mytilus edulis* (Robinson et al. 1990).

We collected adult *Crepidula fornicata* and *C. convexa* from both sites. All animals (adults and their offspring) were kept in the laboratory in artificial seawater (Instant Ocean, 30‰ salinity) at room temperature (approximately 23°C). Adults were fed daily with either *Isochrysis galbana* (clone T-ISO) or *Dunaliella tertiolecta* (clone DUN) at an initial concentration of approximately 20×10^4 cells ml⁻¹. Larvae or juveniles were separated from adults after emergence, and fed daily with T-ISO or DUN for 5 to 26 d. This feeding period was intended to give all offspring sufficient energy reserves to minimize the effects of starvation during the acute toxicity tests. Since the length of this feeding period differed among tests, we checked for an effect of offspring age on copper tolerance (LC₅₀) by linear regression. The offspring were starved for 3 to 5 d before the toxicity tests to avoid fecal contamination; metals, including copper, are known to bind to organic matter (Rand 1995) and differences in fecal matter among replicates could therefore lead to differences in dissolved copper exposure concentrations. Most offspring were released by their mothers in the laboratory, and had never been in the field (offspring in 3 initial experiments were brooded in the field and released by their mothers in the laboratory).

96 h acute copper toxicity tests. Juveniles of *Crepidula convexa* and larvae of *C. fornicata* from both sites were tested for their copper tolerance by exposing them to dissolved copper in the laboratory for 96 h (20°C, 30‰ salinity). We used these developmental stages because they are the first free-living stages for each species, and their copper tolerance could be measured without previous exposure of the offspring to copper. The copper tolerance of these offspring should therefore reflect heritable genetic differences in copper tolerance, rather than physiological acclimation. These tests were not intended to model selection, but rather to determine whether the population from the polluted site showed increased copper tolerance. Acute toxicity tests necessarily expose individuals to higher toxicant concentrations than would be needed to exert selective pressure under conditions of chronic exposure. Each test was performed using offspring released on the same day from a unique

female. Procedures for both species were the same except where noted.

For initial experiments, we used the first offspring released from adults for toxicity tests. Later, the first offspring were not used unless they were released at least 1 mo after the adults had been brought into the laboratory to ensure that the offspring had not been exposed to field concentrations of metals as embryos. All offspring were visually inspected at 8 to 20 \times magnification, and any that were morphologically abnormal or inactive were excluded from the tests.

We made all copper solutions by serial dilution from a 10 mg l⁻¹ copper stock solution (CuCl₂) that was prepared no more than 24 h before starting each experiment. All solutions were changed daily. Offspring were not fed during the tests, as the presence of phytoplankton can influence the availability of dissolved copper to the snails (Xie et al. 2005). We made solutions on the day they were used with aerated 0.22 μ m filtered autoclaved artificial seawater (Instant Ocean) containing 50 mg l⁻¹ streptomycin and 40 mg l⁻¹ penicillin (according to Lord 1986) to limit bacterial growth; higher mortality in some replicate containers could otherwise have triggered bacterial growth, which itself could have led to additional mortality not directly caused by exposure to copper. Offspring were haphazardly selected from a single group of offspring and transferred by pipette to a bath of the appropriate copper concentration (to prevent dilution of the test solutions), and then transferred from the bath to each test container (3 to 4 replicates, each with 3 ml of solution in 1 well of an acid-washed 12-well plastic tissue culture plate).

The initial tests consisted of 4 replicates of 4 tested copper concentrations (0 to 1000 μ g l⁻¹) with 8 juveniles of *Crepidula convexa* or 20 larvae of *C. fornicata* per replicate. Later tests included only 3 replicates but tested 7 copper concentrations (0 to 1000 μ g l⁻¹) with either 4 to 8 juveniles of *C. convexa* (depending on the number of juveniles available) or 10 larvae of *C. fornicata*.

After 96 h, we checked all offspring at 32 to 50 \times magnification for evidence of activity, including swimming, muscular movement (either spontaneous or in response to quick changes in lighting), or a heartbeat (visible through the shell, only for larvae). Any offspring lacking all of these were determined to be dead.

Maternal effects on copper tolerance. Although all offspring were kept in controlled conditions in the laboratory, their parents were collected in the field, and so parents from different sites experienced different conditions before they were collected. These differences may have caused differences in the hardness of their offspring (Bayne et al. 1975). Snails collected from the polluted site may not have provided for

their offspring as well as those that grew up at the less-polluted reference site. To test this hypothesis, adults of both species were collected from both sites (P generation) and their offspring (F₁) were raised to sexual maturity in controlled conditions in the laboratory. We then paired the F₁ juveniles with non-siblings, reared them to adulthood in the laboratory, and performed 96 h acute copper toxicity tests on their offspring (F₂) as described above. Time from hatching to sexual maturity was approximately 5 to 7 mo for each generation of the 2 species. Unfortunately, an incubator malfunction resulted in the death of the entire *Crepidula convexa* F₁ generation before they produced offspring. Fortunately, data for the F₂ generation were critical for interpreting our results for *C. fornicata*, but not for *C. convexa*.

Data analysis. We calculated the median lethal concentration (LC₅₀) for each group of offspring by either the Spearman-Kärber method when mortalities in the lowest and highest concentrations were 0 and 100%, respectively, or the trimmed Spearman-Kärber method when the above conditions were not met (US Environmental Protection Agency 2002). We used software originally developed at Montana State University and obtained from the website of the US Environmental Protection Agency (www.epa.gov/ceampubl/fchain/lc50/). We performed linear regressions of data to assess whether offspring age affected their tolerance of copper. Since we tested a different developmental stage for each species, no comparisons of metal tolerance were made directly between species; we tested only for intraspecific differences in copper tolerance between sites or generations. Differences between LC₅₀ values for *Crepidula convexa* from both sites were analyzed using a pooled variances *t*-test. Differences between LC₅₀ values for both generations (F₁ and F₂) of *C. fornicata* from both sites were analyzed using a 2-way Model I ANOVA. Bonferroni post hoc comparisons (4 comparisons, so $\alpha = 0.0125$) were performed to test for differences between sites for each generation (F₁ and F₂) and between generations for each site (reference and polluted). All statistical analyses were performed according to Zar (1999) using the statistical software Systat v.10 or by hand (Bonferroni post hoc comparisons). Since the results from the initial experiments did not appear to deviate from results for later tests (see Fig. 2a,b), they were not excluded from statistical analysis.

RESULTS

Offspring age (and therefore the length of the feeding period before the test) had no effect on the copper

tolerance for either species. Age did not affect the copper tolerance (LC_{50}) of juveniles of *Crepidula convexa* (Fig. 1a, linear regressions, reference site: $F_{1,2} = 0.24$, $p = 0.68$; polluted site: $F_{1,1} = 8.93$, $p = 0.21$; data from both populations analyzed together: $F_{1,5} = 4.38$, $p = 0.09$). Similarly, age did not affect the copper tolerance of F_1 generation larvae of *C. fornicata* from either population (Fig. 1b, linear regressions, reference site: $F_{1,2} = 0.01$, $p = 0.92$; polluted site: $F_{1,3} = 0.09$, $p = 0.79$; both populations: $F_{1,7} = 1.23$, $p = 0.31$), nor the copper tolerance of F_2 generation larvae (Fig. 1c, linear regressions, reference site: $F_{1,5} = 0.94$, $p = 0.38$; polluted site: $F_{1,1} = 2.52$, $p = 0.36$; both populations: $F_{1,8} = 2.72$, $p = 0.14$).

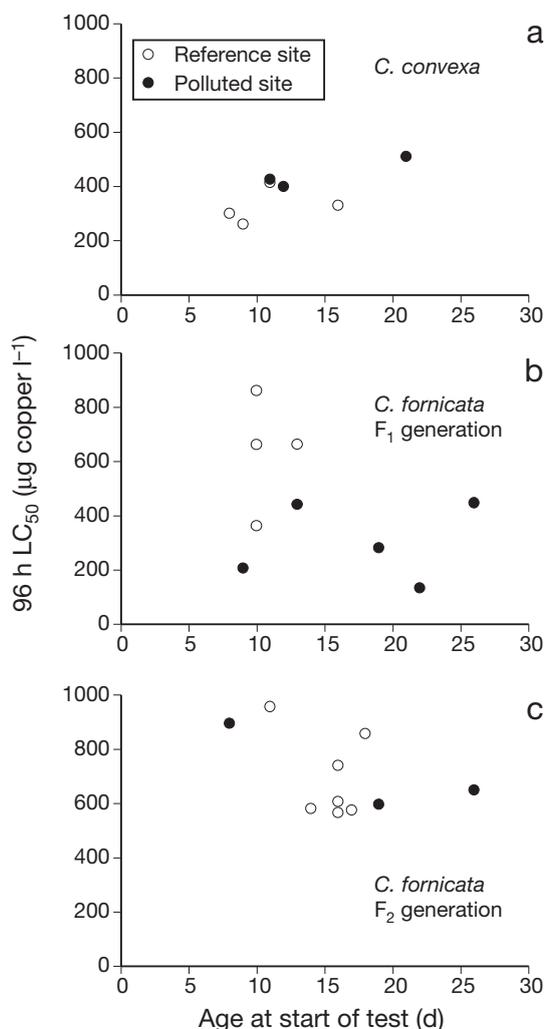


Fig. 1. *Crepidula convexa* and *C. fornicata*. Age and median lethal concentration (LC_{50}) of copper for (a) juveniles of *C. convexa* and (b, c) larvae of *C. fornicata*. Each point represents one 96 h acute copper toxicity test with offspring from a unique set of parents. Each test consisted of 3 or 4 replicates with 4 to 8 juveniles or 10 to 20 larvae at each of 4 to 7 copper concentrations (0 to 1000 $\mu\text{g l}^{-1}$). We tested for an effect of the age of the offspring on their copper tolerance (LC_{50}) by linear regression

Mortalities for juveniles of *Crepidula convexa* in 96 h acute copper toxicity tests are shown in Fig. 2a (note that the results of initial tests were very similar to the results of later tests from the same site). Juve-

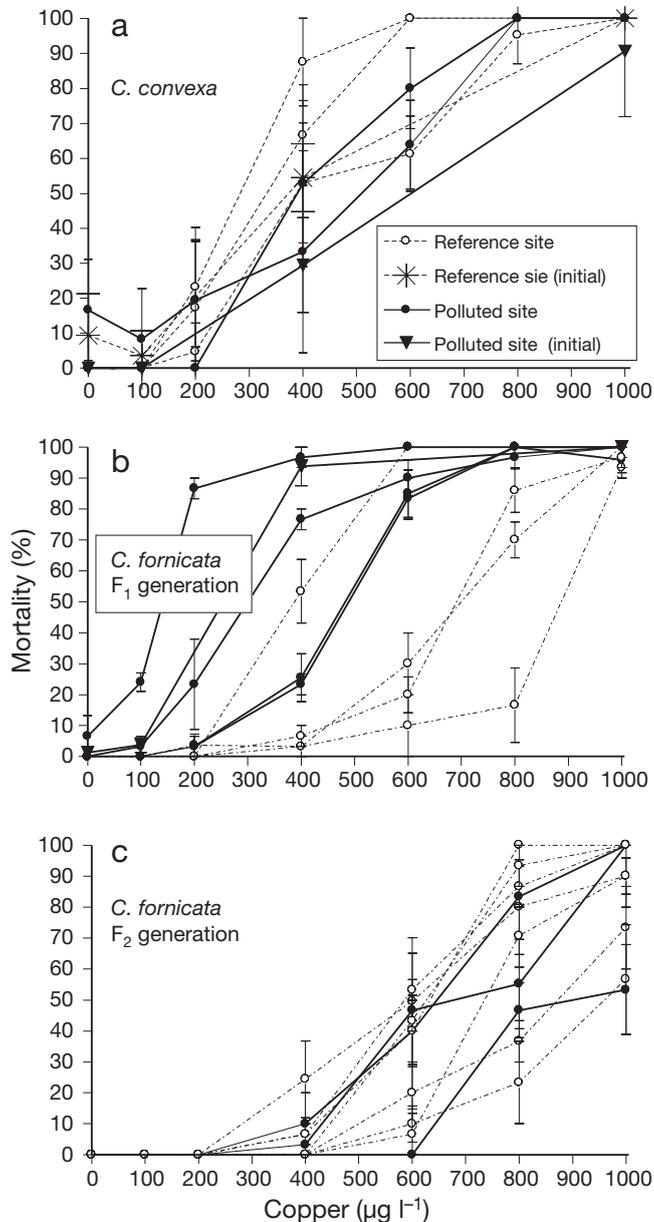


Fig. 2. *Crepidula convexa* and *C. fornicata*. Mortality for (a) juveniles of *C. convexa* and (b, c) larvae of *C. fornicata* exposed to copper for 96 h. Parents of *C. convexa* and F_1 *C. fornicata* were collected from a reference site (Lynn Harbor, Nahant, Massachusetts) or a polluted site (New Bedford Harbor, Fairhaven, Massachusetts) and kept in artificial seawater until offspring were released. F_2 larvae of *C. fornicata* are the offspring of laboratory-reared adults whose parents were collected from each site as indicated. Each line represents one 96 h acute copper toxicity test, while each point represents the mean (± 1 SEM) of 3 or 4 replicates with 4 to 8 juveniles or 10 to 20 larvae

niles of *C. convexa* from the polluted site population (mean $LC_{50} \pm 1 \text{ SEM} = 444.5 \pm 33.2 \mu\text{g Cu l}^{-1}$) were significantly more copper tolerant than juveniles from the reference site population (mean $LC_{50} \pm 1 \text{ SEM} = 325.0 \pm 32.5 \mu\text{g Cu l}^{-1}$) (Fig. 3a; pooled variances *t*-test, $t = 2.52$, $df = 5$, $p = 0.05$).

Mortalities for F_1 and F_2 generation larvae of *Crepidula fornicata* during 96 h acute copper toxicity tests are shown in Fig. 2b & c, respectively (note that the results of 1 initial test were very similar to the results of later tests from the same site). The effect of site was not significant, while the effect of generation and the interaction of these 2 factors were both significant (Table 1). Post hoc tests, however, reveal that all differences were driven by the copper sensitivity of F_1 larvae from the polluted site population. F_1 larvae of *C. fornicata* from the polluted site population were significantly more sensitive to copper (mean $LC_{50} \pm 1 \text{ SEM} = 301.1 \pm 62.5 \mu\text{g Cu l}^{-1}$) than F_1 larvae from the reference site population (mean $LC_{50} \pm 1 \text{ SEM} = 636.3 \pm 205.8 \mu\text{g Cu l}^{-1}$, Bonferroni, $\alpha = 0.0125$, $t = 3.04$, $df = 15$, $p = 0.01$) and also significantly more sensitive than F_2 larvae from the polluted site population (mean $LC_{50} \pm 1 \text{ SEM} = 712.9 \pm 92.0 \mu\text{g Cu l}^{-1}$, Bonferroni, $\alpha = 0.0125$, $t = -3.43$, $df = 15$, $p < 0.005$). Copper tolerance did not differ significantly between different generations of larvae from the reference site population (F_2 reference site mean $LC_{50} \pm 1 \text{ SEM} = 697.1 \pm 59.5 \mu\text{g Cu l}^{-1}$, Bonferroni, $\alpha = 0.0125$, $t = -0.59$, $df = 15$, $p = 0.56$; Fig. 3b), nor between F_2 larvae from the 2 sites (Bonferroni, $\alpha = 0.0125$, $t = -0.14$, $df = 15$, $p = 0.89$; Fig. 3b).

Table 1. *Crepidula fornicata*. Results of a 2-way Model I ANOVA of the 96 h copper LC_{50} values for 2 generations (F_1 and F_2) from reference and polluted site populations (Lynn Harbor, Nahant, Massachusetts, and New Bedford Harbor, Fairhaven, Massachusetts, respectively)

Parameter	df	MS	F	p
Site	1	110 159	4.08	0.06
Generation	1	241 113	8.94	0.01
Site \times generation	1	132 953	4.93	0.04
Residual	15	26 973		

DISCUSSION

We hypothesized that species with low dispersal would be better able to adapt to local conditions of persistently high metal concentration. Therefore, we expected that individuals of *Crepidula convexa* from the polluted site population would be more copper tolerant than individuals from the reference site population, but that individuals of the other species, *C. fornicata*, from the 2 sampled populations would have similar copper tolerance. The observed pattern for copper tolerance in juveniles of *C. convexa* met our expectations. While the observed pattern for *C. fornicata* was consistent with our hypothesis that this species would not evolve increased tolerance at the polluted site, the higher sensitivity of F_1 larvae from the polluted site compared to F_1 larvae from the reference site was unexpected. The observed pattern for F_2 larvae of *C. fornicata*, however, did meet our initial expectations;

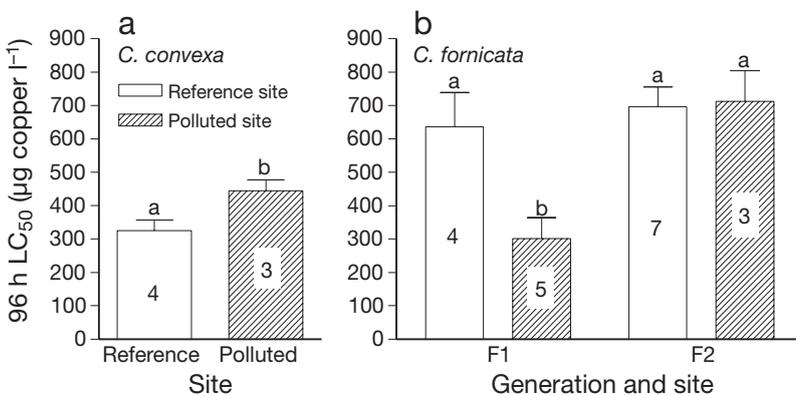


Fig. 3. *Crepidula convexa* and *C. fornicata*. Mean LC_{50} values for (a) juveniles of *C. convexa* and (b) larvae of *C. fornicata* from reference and polluted site populations. LC_{50} values determined by either conventional or trimmed Spearman-Kärber method using the LC_{50} software program from the US Environmental Protection Agency. Numbers in the columns = number of toxicity tests to calculate the mean ($\pm 1 \text{ SEM}$) represented by each column; letters over the bars: significantly different LC_{50} values at $\alpha = 0.05$ for *C. convexa* or $\alpha = 0.0125$ for *C. fornicata* (Bonferroni post hoc test with 4 comparisons, therefore $\alpha = 0.05 \div 4$)

the copper tolerances of F_2 larvae from the 2 sampled populations were essentially the same. Since the 2 generations of *C. fornicata* from the reference site population were also similar in copper tolerance, the low copper tolerance of larvae from the polluted site population in the F_1 generation probably reflected stress experienced by the parental generation at the polluted site, i.e. the greater sensitivity of F_1 larvae from the polluted site probably reflected negative maternal effects. If so, adult *C. convexa* at the polluted site would likely also have been stressed, since the 2 species are ecologically very similar (both are benthic suspension feeders). Therefore, the substantial difference between copper tolerances that we observed for F_1 generation juvenile *C. convexa* from the 2 sites may actually underestimate the difference we could expect for the F_2 generation juveniles.

Similarly, it could be that the greater copper tolerance of juvenile *Crepidula convexa* from the polluted site was caused by positive maternal effects (see Bernardo 1996 for a review of maternal effects in animals) rather than genetic adaptation. Such positive maternal effects may act through different mechanisms than the negative maternal effects discussed in the previous paragraph, offsetting or even counteracting them. The exposure of adults to contaminants at the polluted site might, for example, induce transcription of metallothionein mRNA during oogenesis, thereby increasing tolerance of offspring phenotypically rather than genotypically and counteracting negative maternal effects. For example, Lin et al. (2000) showed that increased cadmium tolerance for offspring of Cd-injected tilapia *Oreochromis mossambicus* was correlated with increased metallothionein mRNA in the adults' oocytes. In contrast, Tsui & Wang (2005) found that, although maternal exposure to mercury increased offspring tolerance in the freshwater cladoceran *Daphnia magna*, it did not affect the concentration of metallothionein-like proteins in the offspring of exposed adults. Differences in metallothionein mRNA and protein in the eggs of adult *C. convexa* and *C. fornicata* before and after copper exposure would indicate whether there is the potential for a non-genetic cross-generational increase in copper tolerance for these species, and whether the potential for this type of trans-generational plasticity differs for the 2 species. In theory, plasticity is more likely in a species with wide dispersal, such as *C. fornicata*, since dispersive offspring are more likely to experience unpredictable environmental stress than the offspring of a species with limited dispersal (Sultan & Spencer 2002). We would therefore predict greater plasticity of copper tolerance for *C. fornicata* than for *C. convexa*, which is the opposite of what we observed.

Selection for copper tolerance could only have occurred if genetic variation for this trait existed in the original population (Wright 1931), so it could be argued that the failure of *Crepidula fornicata* at the polluted site to adapt to local conditions may have been due to low genetic variation for the required trait. This seems unlikely, however, since Collin (2001) documented higher within-population genetic variation for mitochondrial DNA of *C. fornicata* compared to *C. convexa*. Laboratory selection for increased copper tolerance of *C. fornicata* coupled with genetic variation analysis (see Klerks & Levinton 1989, Ward & Robinson 2005) could test the hypothesis that this species does not have sufficient genetic variation in traits affecting copper tolerance to adapt to increased copper stress.

While the immediate benefits of increased tolerance to pollutants are clear for the individual organism, the long-term consequences for the population and community

are often less obvious. Xie & Klerks (2004b) reported many fitness costs, such as decreased fecundity, reduced size at birth, and shorter female life expectancy in a cadmium-resistant population of the least killifish *Heterandria formosa*. Such population-level declines in fitness can change community structure directly, since some species can adapt to increased pollution, while other species cannot (Blanck 2002). Pollutants can also change community structure indirectly by impacting parasite–host or predator–prey relationships (Lefcort et al. 2002) or by shifting the balance in competition for a limited resource (Johnston & Keough 2003). Another community-level impact of increased tolerance involves the trophic transfer of toxic elements, which would be exacerbated when the mechanisms for increased tolerance result in elevated tissue concentrations of the toxic elements (Rainbow et al. 2004). Further studies on fitness costs and mechanisms of increased copper tolerance in *Crepidula convexa*, the extent to which *C. convexa* competes for food and substrate with other species, and the relative impact of parasites and predators on metal-adapted populations are necessary to determine the wider ramifications of increased copper tolerance in populations of this species.

While our results support the hypothesized relationship between copper tolerance and species dispersal, our experiments were not designed to examine the underlying mechanism or mechanisms accounting for this difference. Rainbow et al. (1999) did not find a relationship between dispersal potential and metal uptake rates for an amphipod species that broods its young and 2 species of crab that release planktonic larvae, but it is possible that any or all of those species depend on mechanisms other than changes in metal uptake to tolerate high metal stress. Experiments testing for differences in cellular compartmentalization of copper, tissue-specific copper concentrations, and copper assimilation and elimination rates would help to reveal possible mechanisms for the increased tolerance we have documented for *C. convexa* living in highly polluted conditions (Lin et al. 2000, Rainbow et al. 2004, Xie & Klerks 2004a).

Our studies do not indicate that the increased copper tolerance that we documented for *Crepidula convexa* is caused by copper alone as the selective agent. New Bedford Harbor is contaminated with a variety of persistent pollutants, including PCBs, PAHs, and metals (Pruell et al. 1990). Selection for tolerance of *C. convexa* to another pollutant may have resulted in physiological mechanisms that were pre-adaptive for copper tolerance, and these possibilities should also be explored.

Our study is the first to support a connection between dispersal potential and metal tolerance in marine invertebrates. While we found that 1 species (*Crepidula convexa*) from a polluted site produced off-

spring with increased metal tolerance, we also found that a closely related species (*C. fornicata*) from the same environment produced offspring with reduced metal tolerance. Both of these effects were long term, since the parents had been kept in artificial seawater in the laboratory for several months before they released the offspring used in our studies. Adaptation to local conditions may help species with limited dispersal to counteract negative maternal effects, while species with greater dispersal fare poorly in the same stressful environment. Further experiments with other closely related species that differ primarily in dispersal potential (see Bohonak 1999 for review) are necessary to validate this apparent relationship between local adaptation, negative maternal effects, and dispersal potential across a range of taxonomic groups.

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