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TRANSGENERATIONAL PLASTICITY IN THE SEA: CONTEXT-DEPENDENT MATERNAL EFFECTS ACROSS THE LIFE HISTORY

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Abstract. Maternal effects can have dramatic influences on the phenotype of offspring. Maternal effects can act as a conduit by which the maternal environment negatively affects offspring fitness, but they can also buffer offspring from environmental change by altering the phenotype of offspring according to local environmental conditions and as such, are a form of transgenerational plasticity. The benefits of maternal effects can be highly context dependent, increasing performance in one life-history stage but reducing it in another. While maternal effects are increasingly well understood in terrestrial systems, studies in the marine environment are typically restricted to a single, early life-history stage. Here, I examine the role of maternal effects across the life history of the bryozoan *Bugula neritina*. I exposed maternal colonies to a common pollution stress (copper) in the laboratory and then placed them in the field for one week to brood offspring. I then examined the resistance of offspring to copper from toxicant-exposed and toxicant-naïve mothers and found that offspring from toxicant-exposed mothers were larger, more dispersive, and more resistant to copper stress than offspring from naïve mothers. However, maternal exposure history had pervasive, negative effects on the post-metamorphic performance (particularly survival) of offspring: offspring from toxicant-exposed mothers had poorer performance after six weeks in the field, especially when facing high levels of intraspecific competition. Maternal experience can have complex effects on offspring phenotype, enhancing performance in one life-history stage while decreasing performance in another. The context-dependent costs and benefits associated with maternally derived pollution resistance may account for why such resistance is induced rather than continually expressed: mothers must balance the benefits of producing pollution-resistant larvae with the costs of producing poorer performing adults (in the absence of pollution).

Key words: Brisbane, Australia; Bugula neritina; *egg size; offspring size; phenotypic plasticity; pollution resistance.*

INTRODUCTION

Maternal effects are maternally derived, nongenetic components of an offspring's phenotype, and their ecological and evolutionary importance is increasingly being recognized (Mousseau and Fox 1998, Agrawal et al. 1999). Maternal effects can dramatically alter the phenotype of offspring and in some species, maternal effects can be the most important determinant of offspring performance (Wade 1998). The maternal phenotype (as determined by the mother's environment and genotype) can affect the performance of offspring through differential provisioning of offspring, or the timing/site of birth (Mousseau and Fox 1998) and as such, maternal effects can be viewed as a conduit by which the maternal environment can affect offspring phenotype. Thus maternal effects can link the phenotype of offspring in one generation to changes in the environment in the previous generation (for a review of nongenetic, environmentally inherited effects, see Rossiter 1996). However, maternal effects can also buffer offspring from environmental change in the maternal environment and in this regard, maternal effects can be viewed as a form of transgenerational plasticity (Mousseau and Fox 1998, Agrawal 2001).

When the maternal environment is a good predictor of the environment that offspring will experience, mothers might be expected to alter the phenotype of their progeny to increase their fitness (Mousseau and Fox 1998, Agrawal 2002). Mothers can change their per offspring investment according to local conditions (Lack 1947, Fox et al. 1999, Shine and Downes 1999, Hendrickx et al. 2003), but it also appears that mothers can manipulate the expression of the offspring genome to maximize offspring fitness (Matzke and Matzke 1993, Agrawal et al. 1999, Agrawal 2001, 2002). For example, Agrawal et al. (1999) showed (in both plants and animals) that mothers exposed to natural enemies produce offspring with greater resistance to predation than offspring from naïve mothers. However, the manipulation of the offspring phenotype can have complex repercussions, enhancing performance in one life-history stage but reducing it in another (Strauss et al. 1996). For example, larval exposure to predators results in predator-resistant tadpoles that become lower-"quality" frogs (Relyea 2001, 2003). Thus maternal effects are complex and

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pervasive, with the potential to dramatically alter the phenotype of offspring across life-history stages making it difficult to predict their overall effect on offspring performance.

While the role of maternal effects in evolution (Kirkpatrick and Lande 1989, Mousseau and Fox 1998) and population dynamics (e.g., Ginzburg 1998) is generally well recognized, their importance in the marine environment is less well understood. For marine taxa, empirical examinations of maternal effects are scarce, and field tests almost nonexistent. In a rare example, McCormick (2006) showed that *Pomacentrus amboinensis* larvae from mothers kept at higher densities were smaller than larvae from isolated pairs. Our current view of maternal effects in marine organisms is largely restricted to individual, typically early, life-history stages. Given that maternal effects can have dramatically different influences across different life-history stages in other taxa (Strauss et al. 1996, Relyea 2000, 2001, 2003), similar effects seem likely in marine species. However, no study has yet examined the role of maternal effects across multiple life-history stages in a marine organism. Here, I examine the role of a maternal effect in a marine invertebrate (*Bugula neritina*) across both the larval and adult stages. Importantly, I examine the adult stage under field conditions, a crucial factor in determining the importance of maternal effects in nature (Fox 2000, Galloway 2001). Marine invertebrates with non-feeding larvae, such as *B. neritina*, are excellent candidates for examining maternal effects and their propagation through the life history; previous studies have shown strong phenotypic links between life-history stages within generations (Pechenik et al. 1998, Marshall et al. 2003*a*, Marshall 2005, Pechenik 2006). My study determines whether these phenotypic links also extend across generations.

The aspect of the maternal environment I chose to manipulate as a source of maternal effects was the presence of ecologically relevant concentrations (Teasdale et al. 2003) of the common pollutant, copper. I chose to examine the effect of copper for two reasons. First, anthropogenic pollution is one of the most powerful agents of selection acting on aquatic organisms today (Levinton et al. 2003), and many species, including *B. neritina*, show local adaptation to increased pollutant loads (e.g., Hoare et al. 1995, Martinez and Levinton 1996, Wallace et al. 1998, Levinton et al. 2003, Mouneyrac et al. 2003, Daka and Hawkins 2004, Rainbow et al. 2004, Piola and Johnston 2006) but the role of maternal effects in local adaptation is unknown. Second, some aquatic organisms exhibit transgenerational plasticity in response to pollution exposure. Mothers that have been exposed to a toxicant can produce offspring that are (at least initially) more resistant themselves (Munkittrick and Dixon 1988, Lin et al. 2000, Vidal and Horne 2003) suggesting that maternal pollution exposure may induce an adaptive maternal effect. However, maternal exposure to pollution can also constitute a stress that results in mothers reducing their investment in their offspring, decreasing offspring quality (Cox and Ward 2002). Finally, pollution resistance can carry a fitness cost in pollution-free conditions in marine invertebrates (Levinton et al. 2003). These lines of evidence suggest that the influence of pollution-mediated maternal effects is likely to differ among life-history stages and be context dependent. I examined the effects of maternal exposure to copper on the tolerance of their offspring to that same toxicant (at the larval stage, which is typically the most sensitive). I then examined the performance of offspring in the field from mothers that did or did not have a history of exposure.

Materials and Methods

Study site and species

I collected adults and conducted all of the experiments at Manly Boat Harbour, Moreton Bay, Brisbane, Australia in the austral winter of 2006. The site is sheltered from prevailing weather by a large breakwater and public access is restricted. *Bugula neritina* is an arborescent bryozoan that grows by asexual budding of zooids and broods its larvae in specialized zooids called ovicells. Larvae are brooded for approximately one week, in which time they grow as they receive nutrients from the colony via a placenta-like transport system (Woolocott and Zimmer 1972). After release, larvae spend only a short time (minutes to hours) in the plankton before permanently attaching to a suitable substrate and metamorphosing (Marshall and Keough 2003).

Equipment preparation

All experimental equipment was acid washed in 5% nitric acid for >24 hours and twice rinsed in MilliQ water prior to use. Analytical-reagent grade $CuSO_4$ (copper II sulfate anhydrous; Sigma Chemicals, Balcatta, Perth, Western Australia) was used as the reference toxicant for all experiments. For the laboratory experiments, a 1000 μg Cu/L solution was prepared at the beginning of each experimental run from a refrigerated stock solution and diluted with 0.45-μm filtered seawater (FSW) to make the nominal concentrations of 100 or 300 μg Cu/L solution. Experimental containers were presoaked in the appropriate treatment concentration for 12 hours prior to each run to minimize the reduction in copper via chelation to experimental containers (Batley et al. 1999).

General methods: manipulation of maternal exposure history

Reproductively mature colonies were collected from the field, maintained in light-proof boxes for 24 hours in the laboratory, and then exposed to bright light. This exposure caused colonies to release the larvae they were brooding so as to ensure later effects were not due to larval exposure to toxicant while in the ovicells. I then

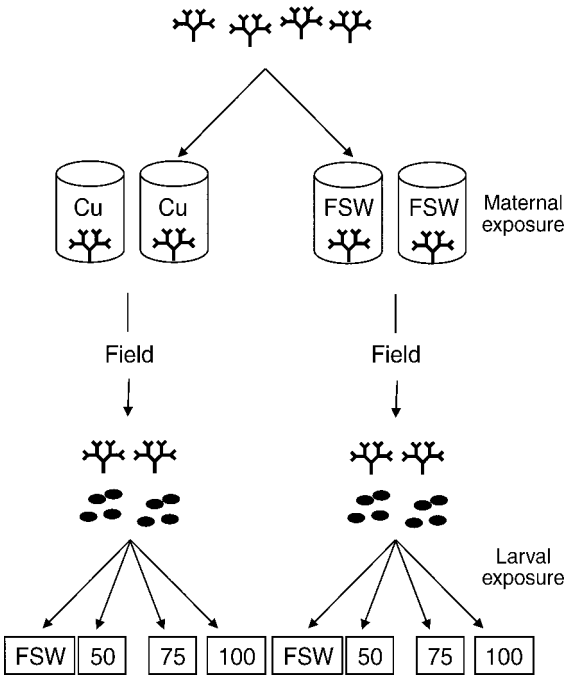

FIG. 1. Schematic showing experimental design for the exposure of *Bugula neritina* colonies to a toxicant stress (300 μg Cu/L, labeled Cu in the figure) or filtered seawater (FSW in the figure) before being transplanted into the field for one week to produce offspring. The bryozoan colonies were then returned to the laboratory and spawned. The larvae from toxicant-exposed and naïve colonies were then exposed to a range of toxicant concentrations (in boxes; μg Cu/L).

placed individual colonies in their own glass beakers with 200 mL of toxicant solution (100 or 300 μg Cu/L) or FSW, and colonies were left in the solution for six hours. No colonies died during this exposure and when transplanted back into the field, there was no difference in the survival of colonies from the different treatments even after three weeks in the field (only one colony died in each of the treatments after three weeks; D. J. Marshall, *unpublished data*). After exposure, I deployed the colonies back into the field for a week. I first attached the colonies to artificial substrata (PVC panels, $500 \times 500 \times 6$ mm) using the method outlined in Keough (1989). Toxicant-treated and control colonies were interspersed on panels and depending on the experiment, I deployed 2–4 panels into the field. The panels were hung with the colonies facing down at a depth of 1 m below the surface on floating docks at Manly Boat Harbour. I left the colonies in the field for a week and then returned them to the laboratory where they were again placed in light proof containers for 24 hours. I then spawned the colonies as before to collect larvae that had been produced after the exposure of colonies to toxicant or control solutions, and these were the offspring that were the focus of my investigations. Importantly, there was no difference between toxicant-exposed and toxicant-naïve colonies in terms of whether they released larvae upon return to the laboratory. Therefore I did not artificially select for intrinsically copper-resistant colonies because copper-treated colonies survived and spawned as well as toxicant-naïve colonies.

General methods: field experiments

To examine the performance of offspring in the field from mothers with different histories of exposure, I exposed mothers and spawned their offspring a week later (as described previously). I then settled the spawned larvae on roughened petri dishes and marked the position of individual settlers; any larvae that had not settled within one hour of release were discarded. The settlers were then left to metamorphose for 24 hours at a constant temperature of 20°C. To deploy the settlers into the field, I drilled a 6-mm hole in the dishes and affixed the dishes to PVC panels (dimensions as before) with stainless steel bolts before suspending the panels face down from the floating dock at Manly Boat Harbour. The assignment of petri dishes was haphazard but care was taken to ensure that at least one of each treatment was on each panel. After six weeks in the field, I returned the colonies to the laboratory and measured colony survival and colony size (colony size was measured as the number of bifurcations in the longest branch, an excellent predictor of fecundity; Marshall et al. 2003*a*). Thus I define "performance" here as post-metamorphic survival and colony size in the field and in light of previous studies on *Bugula* (Marshall et al. 2003*a*, Marshall and Keough 2006), I am confident this is a good proxy for fitness.

Experiment 1: effect of maternal history on offspring dispersal potential

I examined the size and dispersal potential (the length of the planktonic phase) of larvae from naïve and toxicant-exposed colonies. To measure the size of larvae, I used standard methods as described in Marshall et al. (2003*a*) to measure larvae from 20 colonies that had either been exposed to FSW or 300 μg Cu/L as described previously. To estimate the length of the planktonic phase, I placed 10 larvae in roughened Petri dishes and recorded how many larvae had settled within three hours of release from the colony ($n = 30$ colonies). I repeated this experiment but instead of measuring settlement after three hours, I monitored the settlement of individual larvae every 15 minutes for three hours.

Experiment 2: effect of maternal history on offspring resistance

In this experiment, I was interested in whether larvae from toxicant-exposed colonies were more resistant to the same stress themselves. Here, I define "resistance" as the ability of offspring to survive an exposure to copper during the larval phase and complete metamorphosis. I used an orthogonal design whereby larvae from stressed and unstressed colonies were exposed to increasing levels

of stress (Fig. 1). Larvae from 34 colonies (17 that had been exposed to 300 μg Cu/L a week earlier and 17 control colonies) were placed in petri dishes containing solutions of either FSW, 50, 75, or 100 μg Cu/L. I used lower copper concentrations for the larval exposures because exposure to 300 μg Cu/L during the larval phase was fatal regardless of maternal exposure history (D. J. Marshall, *unpublished data*). For each larval treatment/colony treatment combination, I had a single group of 10 larvae from a colony placed in their own petri dish and this was the unit of replication. The larvae were allowed to settle for three hours, after which, any unsettled larvae were removed and the seawater solutions were replaced with FSW. I marked the position of each settler on the petri dish with a pencil. I placed the developing settlers in a constant temperature cabinet (at 20°C) and allowed them to metamorphose for 48 hours. I then assessed settler survival whereby settlers were classed as "alive" if they were present and had a normally developed lophophore: settlers were classed as "dead" if they were absent or their lophophore was deformed or absent.

Experiment 3a: effect of maternal history on offspring performance in the field

In the first experiment, I examined the performance of offspring from mothers that had been exposed to either 300 μg Cu/L (16 colonies) or FSW (15 colonies). For each colony, I settled larvae on two replicate petri dishes. The average number of settlers per petri dish was 4.4 (range 3–7). The 62 petri dishes were deployed across four panels in the field.

Experiment 3b: effect of different levels of maternal exposure

The maternal dose (300 μg Cu/L) that I used probably represents the upper extreme of copper concentrations during pulse pollution events that *Bugula neritina* would experience in the field (Teasdale et al. 2003) and so I examined whether lower concentrations of toxicant had similar effects on subsequent performance. Thus in this experiment, I exposed colonies to either a control, 100, or 300 μg Cu/L solution using the methods outlined previously (27, 20, and 21 colonies exposed to each of the solutions, respectively). I then spawned and settled larvae from these colonies a week after exposure using the methods described previously and examined the performance of these larvae as colonies for six weeks in the field (68 petri dishes across five panels).

Experiment 3c: effect of maternal history on offspring performance at different densities

The density of conspecifics can strongly modify the impact of maternal effects (Marshall et al. 2006) so in this experiment I manipulated maternal exposure history as before, but I also varied the density of settlers on the petri dishes that were deployed into the field. I settled larvae at different densities, high (19.77 ± 3.144 settlers per dish) and low (4.04 ± 0.147 settlers per dish), which represented the range of settler densities observed at Manly Boat Harbour and at the higher densities, growth is typically lower (*personal observation*; see also *Results*). For the high density treatment, despite some dishes containing >20 settlers, only 20 settlers (randomly chosen) were marked and followed. For this experiment, I used larvae from 23 colonies (12 exposed to FSW and 11 exposed to 300 μg Cu/L) and two replicate petri dishes for each colony/density combination yielding a total of 92 petri dishes across seven backing panels.

Data analysis

To examine the effect of maternal exposure history on the size of larvae that were subsequently produced, I analyzed larval size with a nested ANOVA where colony identity was nested within treatment. To examine the effect of maternal exposure history on larval settlement, I again used nested ANOVA. For the effect of maternal exposure history on larval settlement time, I used nested ANOVA but because I had found that larvae from exposed mothers were larger and swam for longer in a previous experiment (see *Results*), I used a one-tailed test. To examine the effect of maternal exposure history on offspring resistance, I used partly nested ANOVA.

To examine the effect of maternal exposure history on subsequent offspring performance in the field for all three experiments, I used a mixed-model ANOVA where backing panel was a random factor and maternal exposure history was a fixed factor. In all of the field experiments, I used several metrics to estimate the effect of maternal exposure on offspring phenotype: post-metamorphic survival (proportion of colonies that survived), mean colony size, and performance, which was the product of these two metrics. This measure of performance was used because it appeared that the treatment of interest often had influenced either survival or growth on any one petri dish but that these results were not independent. For example, in some instances where there was high early mortality, the remaining colonies had higher growth rates due to a reduction in competition so I wanted a metric that reflected both survival and growth simultaneously. As colony size is an excellent predictor of colony fecundity (Marshall et al. 2003*a*), my measure of performance is probably a good proxy for fitness.

For the first and third field experiments, I used quasi-F ratios (following Quinn and Keough 2002) to calculate the effects of maternal environment and density (where appropriate) because I had two replicate petri dishes from each colony. For the second field experiment, I used only a single petri dish per colony. For this analysis, I found a significant overall difference in the performance of offspring from mothers with different exposure histories so I used planned comparisons to first test whether there was any difference in performance of offspring that had come from exposed (either 100 or 300 μg Cu/L) mothers; there was none. Following Quinn

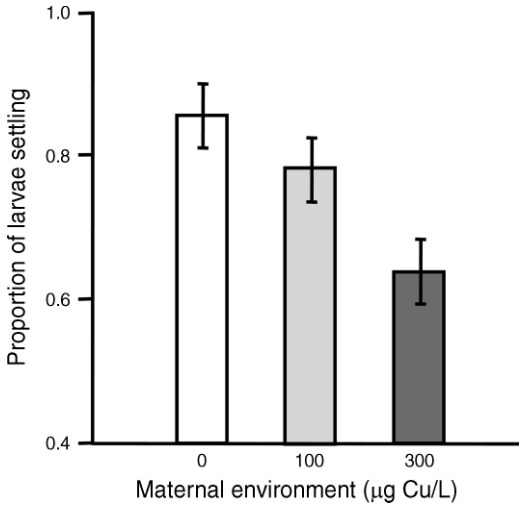

FIG. 2. Effect of maternal exposure history on the dispersal potential of *Bugula neritina* larvae. Bars show the proportion of larvae (mean ± SE) that settled after three hours. The open bar indicates larvae from toxicant-naïve mothers (0 = control), the light-shaded bar indicates larvae from mothers exposed to 100 μg Cu/L a week before spawning, and the dark-shaded bar indicates larvae from mothers exposed to 300 μg Cu/L a week before spawning.

and Keough (2002), I then pooled the exposed groups and compared them to the control.

In the third field experiment, I included an additional factor, settler density. Because there was mortality over the course of the experiment, not every treatment/density was represented on every panel, and thus I could not get an estimate of the maternal environment × density × backing panel interaction for all the backing plates. Therefore my approach was to analyze a reduced data set with two backing panels removed, including the three-way interaction, and as this was not significant ($F_{4,51} = 1.09$, $P = 0.371$), I then re-ran the analysis on the full data set omitting the three-way interaction.

RESULTS

Experiment 1: effect of maternal history on offspring dispersal potential

Offspring that came from colonies that had been exposed to a toxicant stress were larger than offspring that came from control colonies (maternal environment, $F_{1,23} = 14.07$, $P = 0.001$; colony(maternal environment), $F_{23,104} = 2.07$, $P = 0.007$). The average size of larvae from exposed colonies was 12% greater than that from unexposed colonies (46 800 ± 679 and 41 487 ± 687 μm^2, respectively, mean ± SE). After three hours of swimming, larvae from unexposed colonies showed more settlement than larvae from exposed colonies with larvae from mothers exposed to 100 μg Cu/L having intermediate settlement to mothers exposed to FSW and 300 μg Cu/L ($F_{2,27} = 6.07$, $P = 0.007$; Fig. 2). After five hours, all the larvae had settled, regardless of maternal exposure history. In a second experiment, larvae from exposed mothers swam for ~25% longer than larvae from naïve mothers ($F_{1,26} = 2.92$, $P = 0.049$).

Experiment 2: effect of maternal history on offspring resistance

There was a strong interaction between maternal exposure history and the larval environment on the subsequent resistance of offspring to the toxicant. Offspring from toxicant-exposed mothers were more resistant to exposure to high levels of that toxicant themselves. While the survival of settlers from mothers with different exposure histories was relatively similar in 0, 50, and 75 μg Cu/L larval environments, the survival of larvae in 100 μg Cu/L strongly depended on the exposure history of their mothers (between subjects, maternal environment, $F_{1,32} = 1.51$, $P = 0.223$; within subjects, larval environment, $F_{3,93} = 1.42$, $P = 0.242$; larval environment × maternal environment, $F_{3,93} = 4.49$, $P = 0.005$; Fig. 3).

Experiment 3a: effect of maternal history on offspring performance in the field

The effects of maternal exposure history on subsequent offspring performance were variable in the first experiment. Across three backing panels, offspring from mothers that had been exposed to the toxicant stress performed poorly relative to offspring from control mothers. However, on the fourth backing plate (which, by chance had a 25% lower density of settlers at the outset), there was little effect of maternal exposure history on offspring performance as indicated by a significant panel × maternal environment interaction (Table 1). This effect was driven largely by an

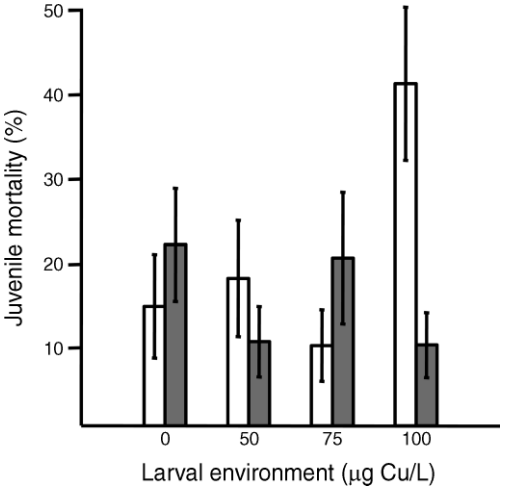

FIG. 3. Effect of maternal exposure history and larval environment on the mortality (mean ± SE) of *Bugula neritina* juveniles after 48 hours in the laboratory. Open bars indicate mortality of juveniles from toxicant-naïve mothers, and closed bars indicate mortality of juveniles from mothers exposed to 300 μg Cu/L a week before spawning. On the *x*-axis are the different environments that juveniles were exposed to as larvae (0 = control).

TABLE 1. Effect of maternal exposure history on subsequent performance of *Bugula neritina* colonies after six weeks in the field.

Source	df	MS	*F*	*P*
Maternal environment	1	1.391	0.08	0.800
Panel	3	6.722	0.98	0.507
Maternal environment × panel	3	6.879	3.51	0.030
Colony(maternal environment)	29	1.958	0.99	0.505
Error	25	1.960		

Notes: In this experiment, mothers were exposed to either filtered seawater (FSW) or 300 μg Cu/L a week before reproduction. Performance was the product of the proportion of offspring that survived to six weeks and average colony size.

interaction between maternal environment and panel on subsequent colony survival (analysis for survival alone, maternal environment × panel, $F_{3,25} = 2.49$, $P = 0.083$; analysis for size alone, maternal environment × panel, $F_{3,15} = 0.71$, $P = 0.561$).

Experiment 3b: effect of different levels of maternal exposure

The second field experiment showed that the exposure of mothers to 100 μg Cu/L had similar effects on subsequent offspring performance to exposure to 300 μg Cu/L. Overall, the performance of offspring from control mothers after six weeks in the field was more than twice that of offspring from mothers that had been exposed to either the high or low concentration of copper (maternal environment, $F_{2,8} = 5.11$, $P = 0.037$; panel, $F_{4,62} = 3.99$, $P = 0.006$; maternal environment × panel, $F_{8,62} = 0.59$, $P = 0.78$; planned comparisons, 100 μg Cu/L vs. 300 μg Cu/L, $P = 0.55$; 100 μg Cu/L + 300 μg Cu/L vs. control, $P = 0.03$; Fig. 4). This effect was driven by a strong effect of maternal exposure history on subsequent survival (pooled analysis for survival alone, $F_{1,4} = 21.48$, $P = 0.01$; pooled analysis for size alone, $F_{1,4} = 0.06$, $P = 0.8$; see Appendix: Figs. A1 and A2 for separate effects).

Experiment 3c: effect of maternal history on offspring performance at different densities

In the third field experiment, maternal exposure history affected offspring performance but these effects were strongest when offspring were in the field at high densities (Table 2). The density of colonies affected performance overall with colonies at high density having lower performance on average than colonies at low density (Fig. 5). After six weeks in the field at high densities, colonies from control mothers had 300% greater performance than colonies from exposed mothers but after the same period at low densities, colonies from control mothers had similar performance to colonies from exposed mothers (Fig. 5). Again, this effect was driven by differences in colony survival (analysis for survival alone, maternal environment × competition, $F_{1,45} = 8.9$, $P = 0.005$; analysis for size alone, maternal environment × competition, $F_{1,45} = 0.886$, $P = 0.351$; see Appendix: Figs. A3 and A4 for separate effects).

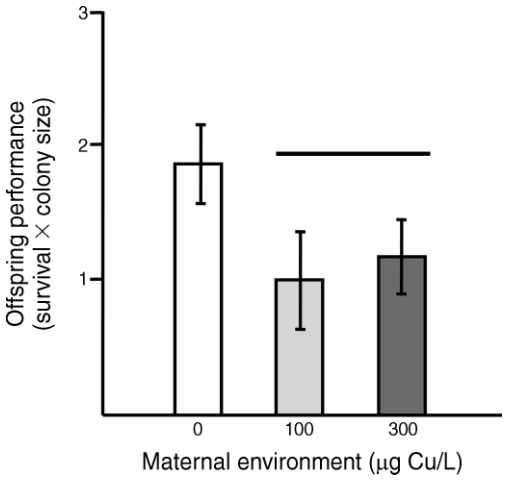

FIG. 4. Effect of maternal exposure history on the performance of *Bugula neritina* colonies after six weeks in the field. Bars represent performance (mean ± SE) of offspring from toxicant-naïve mothers (as indicated by open bars) and offspring from mothers that were exposed to either 100 (light-shaded bar) or 300 μg Cu/L (dark-shaded bar) a week before spawning. The horizontal bar indicates no significant difference between 100 and 300 μg Cu/L exposure histories using planned comparisons.

DISCUSSION

Transgenerational toxicant resistance

Maternal exposure to the toxicant copper altered the phenotype of offspring in *Bugula neritina*. The performance of the maternal brood colonies appeared to be similar (at least for three weeks following exposure) but remarkably, the effects of exposure permeated through to affect offspring phenotype. Larvae from toxicant-exposed colonies had greater resistance to higher copper concentrations themselves than larvae from toxicant-naïve colonies. The mechanism for increased resistance to the toxicant is unclear. There was no difference in

TABLE 2. Effect of maternal exposure history and intraspecific competition on the performance of *Bugula neritina* colonies after six weeks in the field.

Source	df	MS	*F*	*P*
Maternal environment	1	7.720	4.62	0.0627
Competition	1	1.941	0.94	0.367
Panel	6	8.003	2.12	0.154
Colony(maternal environment)	25	0.839	1.06	0.426
Maternal environment × competition	1	6.820	6.985	0.005
Maternal environment × panel	6	2.265	2.725	0.033
Competition × panel	6	2.028	3.354	0.020
Error	45	0.796		

Note: Performance was the product of the proportion of offspring that survived to six weeks and average colony size.

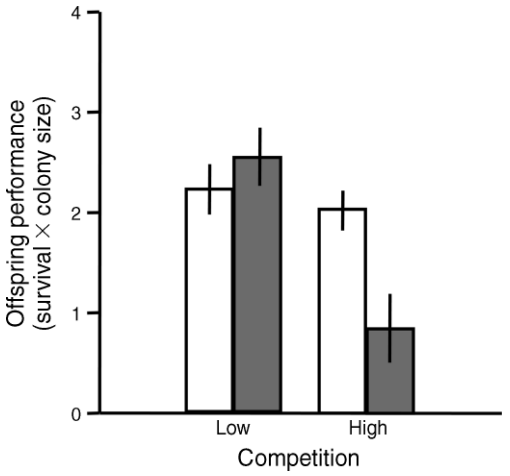

FIG. 5. Effect of maternal exposure history and intraspecific competition on the performance of *Bugula neritina* colonies after six weeks in the field. Bars represent the performance (mean ± SE) of offspring in the presence and absence of competition. Open bars indicate performance of offspring from toxicant-naïve mothers, and shaded bars indicate performance of offspring from mothers exposed to 300 μg Cu/L a week before spawning.

mortality between the exposed and unexposed colonies, and both groups released larvae so there was no artificial selection for copper-resistant lines. In other taxa, mothers increase the level of metallothionein-producing RNA in their offspring in response to heavy metal exposure, mitigating (at least temporarily) the damaging effects of the heavy metal (Lin et al. 2000). Larvae from exposed mothers were also larger than larvae from naïve mothers. While larger larvae have higher fitness generally in marine invertebrates, including *B. neritina*, I am not aware of any study showing that offspring size is correlated with toxicant resistance in marine invertebrates. Hendrickx et al. (2003) showed that populations of wolf spiders where there were higher heavy metal loads produced larger offspring than spiders from unpolluted populations, but it was unclear whether larger offspring have greater resistance to the toxicant per se. An interesting next step will be to examine whether resistance to copper exposure is correlated with larval size in *B. neritina*. Regardless of the mechanism underlying this maternal effect, *B. neritina* mothers produce larger, more resistant offspring following exposure to copper.

Bugula neritina mothers exposed to a toxicant produce offspring that are more resistant to that toxicant, and it is tempting to view this maternal effect as a form of transgenerational adaptive plasticity (Mousseau and Fox 1998). However, this change in offspring phenotype is only adaptive if maternal exposure to the toxicant is a good predictor of the likelihood of offspring exposure (Moran 1992). I have no information on the frequency and scale of pollution events in my study population of *B. neritina*, and so it is difficult to assess the likelihood of this scenario. It is important to note that the response of brood colonies to copper exposure does not necessarily mean that pollution events occur on a weekly cycle in my study population. It could be that any copper exposure experienced by mothers is a reasonable predictor that their offspring are more likely to experience copper exposure themselves. Importantly, one should not focus on whether a six-hour exposure is likely to be good predictor of subsequent exposure to copper or not: simply that this level of exposure is sufficient to induce a maternal response. Alternatively, the changes in offspring phenotype could simply represent a "reproductive bailout" whereby mothers that are in a stressful environment allocate more of their resources to their offspring (e.g., Hughes et al. 2003) but this seems unlikely. In a previous study on *B. neritina*, a different maternal stress (a simulated predation event) resulted in a decrease rather than an increase in offspring size (Marshall and Keough 2004). The nature of the maternal stress therefore seems important: when colonies suffer a simulated predation event, mothers benefit from investing less in offspring, diverting these resources to their own growth (Marshall and Keough 2004), perhaps in order to gain a size refuge from predation. In contrast, there may be no advantage to being a larger colony in the presence of copper and thus resources are better directed toward offspring.

Changes in offspring dispersal potential

The changes in offspring phenotype after maternal exposure to copper were not limited to their resistance to toxicant exposure: larvae from toxicant-exposed mothers also swam for longer in the laboratory. This effect may be a simple consequence of toxicant-exposed mothers producing larger offspring: the larval swimming period is correlated with larval size in *B. neritina* (Marshall and Keough 2003). Nevertheless, larvae that swim for longer, are more likely to disperse further (Pechenik 1999, Shanks et al. 2003). In the case of *B. neritina*, larger larvae tend to be discriminating toward settlement surfaces and are therefore more likely to settle in higher-quality habitats (Marshall and Keough 2003). Thus mothers that were exposed to copper produce larvae that are more likely to disperse out of the maternal habitat. Again, this may be an adaptive maternal effect, but this strongly depends on the scale at which the toxicant occurs and the scale at which larvae disperse. It is interesting to note, however, that other taxa (including marine invertebrates) change the dispersive properties of their offspring when conditions in the maternal habitat degrade (Krug and Zimmer 2000, Krug 2001, Fowler 2005, but see Massot and Clobert 1995). At the very least, my study shows that the dispersal properties of offspring in *B. neritina*, as in other systems, are a polymorphic trait under maternal influence (Zerra and Denno 1997, Mandak and Pysek 1999, De Fraipont et al. 2000, Parciak 2002).

Negative transgenerational effects of toxicant exposure

The effects of maternal toxicant exposure on offspring phenotype were not exclusively positive; the post-metamorphic performance of offspring from exposed mothers was lower than that of offspring from toxicant-naïve mothers. This effect on performance was mediated principally through the lower survival of offspring from toxicant-exposed colonies but in the first experiment it was a combined, subtle effect on both survival and growth simultaneously. Interestingly, the effects of maternal exposure were greater when conditions were harsher (when survival and growth were lower) both within and among experiments. Within experiments 3a and c, when intraspecific competition was high (and thus performance was lower), maternal exposure history had much stronger effects than when competition was lower. Among experiments, maternal exposure history did not have an effect on colonies at a low density in experiment 3c but did have an effect at similar densities in experiment 3b. This among-run variation may be explained by the fact that overall performance was ~30% lower overall in experiment 3b compared to 3c, suggesting conditions overall were more benign during experiment 3c. This context-dependent effect of maternal exposure history suggests that whatever change in offspring phenotype is induced by maternal exposure, it results in the offspring having lower resistance to the general stresses associated with increased conspecific densities (e.g., lower food availability).

The fact that offspring from toxicant-exposed mothers were larger but had lower post-metamorphic performance than offspring from toxicant-naïve mothers is surprising. In previous studies, post-metamorphic performance was strongly correlated with offspring size in *B. neritina* (Marshall et al. 2003*a*, Marshall 2005), and in other species, the benefits of increased offspring size are increased under intraspecific competition (Marshall et al. 2006). This suggests that some aspect of larvae in addition to size differed between offspring from mothers with the different exposure histories but this awaits further testing.

Context-dependent benefits of transgenerational plasticity

If increased resistance to copper as larvae represents a significant drain on nutritional reserves around metamorphosis (for example due to the up-regulation of metallothionein production), this could also explain the lower post-metamorphic performance of offspring from exposed mothers. For colonial organisms such as *B. neritina*, small differences in initial performance can translate into larger differences over time (Wendt 1998, Marshall et al. 2003*b*, Podolsky and Moran 2006). In other species, pollution-adapted genotypes have lower performance overall (Shirley and Sibly 1999, Levinton et al. 2003), and it appears that similar costs occur when pollution resistance is induced rather than genetic.

The benefits of the resistant phenotype in *Bugula neritina* appear to be highly context dependent and are therefore likely to maintain high levels of plasticity within the population (Moran 1992). When copper pollution is present, offspring from exposed mothers are likely to have greater fitness than offspring from naïve mothers but when absent, offspring from exposed mothers will perform more poorly overall, particularly in the presence of intraspecific competition. Several models suggest that environmental variation and context-dependent benefits of changes in phenotype will result in strong selection for phenotypic plasticity (reviewed in Berrigan and Scheiner [2004]). It is unclear whether this level of plasticity is likely to favor the genetic evolution of pollution resistance (Price et al. 2003), but it is interesting that marine invertebrate populations can exhibit rapid shifts in their resistance to pollutants over small spatial scales (Levinton et al. 2003, Piola and Johnston 2006). It appears that mothers must balance the costs and benefits of producing pollution-resistant phenotype, and the costs may explain why pollution resistance is not permanently induced across generations. While a number of studies have demonstrated that costs are associated with the induction of defenses within generations (Strauss et al. 1996, Shirley and Sibly 1999, Stibor and Navarra 2000, Relyea 2001); this study represents one of the few to suggest there are costs associated with defense induction across generations. In marine invertebrates, there are strong phenotypic links between life-history stages (Pechenik et al. 1998); it appears that these links also extend across generations and this has interesting implications for the population dynamics of this group.

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APPENDIX

Figures showing the survival and growth of *Bugula neritina* colonies (*Ecological Archives* E089-023-A1).