Vol. 257: 77–85, 2003 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published August 7

Delayed effects of larval exposure to Cu in the bryozoan *Watersipora subtorquata*

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ABSTRACT: Larval experience affects early post-metamorphic performance of a range of marine invertebrates, and even brief events during the larval stage can be important. One of the important larval stresses for organisms living in urban coastal environments is exposure to toxicants. Larvae may pass through patches of toxicants in their dispersal stage with the potential to affect their post-metamorphic performance. However, most studies on embryonic or larval tolerance to pollutants end at, or before, metamorphosis, have little follow-up and almost all are done under laboratory conditions. In this study, we tested whether short-term larval exposure to copper has short- and long-term carry-over effects in the encrusting bryozoan *Watersipora subtorquata* by following settlement and metamorphosis in the laboratory, then transplanting colonies into the field, in 2 seasons and at 2 sites in SE Australia. Cu at 100 µg l^{-1} accelerated larval attachment in winter and summer, but inhibited metamorphosis. When transplanted to the field, juveniles survived and grew well, and effects on survival and growth did not appear until several weeks or months after settlement. Larval exposure to Cu reduced survival of colonies to about 2/3 that of control colonies in summer and at 1 site, reduced survival to about 20% that of controls in winter. The fall in survivorship occurred abruptly after 3 to 5 wk in summer and 14 wk in winter. The surviving colonies grew more slowly than the controls. In general, there was little temporal variation in Cu effects, but there was spatial variation in effects on survival and growth of colonies within and between sites.

KEY WORDS: Cu · Carry-over effects · Attachment · Metamorphosis · Post-metamorphic survival · Post-metamorphic growth · Spatial variations · Temporal variations · Bryozoan

INTRODUCTION

Larval and post-metamorphic processes have, in the past, largely been treated as independent events. Only recently have biologists found evidence of the impact of larval experience on post-metamorphic performance of marine invertebrates (reviewed in Pechenik et al. 1998), and even short-term larval experiences can carry over to future life history stages of development. Most studies to date are on impacts of larval starvation or delaying metamorphosis with the effects usually appearing in the days following metamorphosis.

One important larval stress for organisms living in urban coastal environment is exposure to toxicants. During their dispersal stage, larvae may pass through patches of toxicants, such as areas of industrial discharge or stormwater runoff, and near surfaces of ships coated by antifouling paint. Embryos or larvae are generally more sensitive to pollutants than are juveniles or adults (Connor 1972, Calabrese et al. 1973, Moore & Dwyer 1974); hence, early larval experience of environmental contaminants may affect post-metamorphic performance. Pechenik et al. (1998) predicted that exposing larvae to sublethal pollutant stress would cause similar impacts to those of starvation on juvenile survival, growth rates,

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competitive ability, time to reach reproductive maturity or fecundity. However, so far, laboratory studies on embryonic or larval tolerance to pollutants end at, or before, metamorphosis, have little follow-up and most are done under laboratory conditions. Even when carry-over effects of prolonged swimming or larval nutrition have been examined, they have generally not extended very far beyond the completion of metamorphosis, leaving open the possibility that some effects may only become apparent after a considerable time.

In a previous study, we found there were impacts of brief larval exposure to Cu on performance of the arborescent bryozoan *Bugula neritina* (T. Y.-T. Ng & M. J. Keough unpubl. data). Larval photopositive behaviour was affected and the metamorphosis was delayed or inhibited. Post-metamorphic survival and growth of the colonies were reduced, although the actual combination of effects varied between different sites. Here, we extend this approach to another bryozoan and test whether brief larval stresses have effects on attachment and metamorphosis of larvae as well as on juvenile and adult colonies. To understand the consistency of any effects, we transplanted colonies to 2 field sites in SE Australia during the summer field season and repeated the experiment at 1 site in winter.

Watersipora subtorquata is an encrusting bryozoan that is widely distributed in protected temperate and subtropical waters. Some older laboratory data exist on its sensitivity to metals. Wisely & Blick (1967) compared the toxicity of Cu on larvae of several fouling species, including bryozoans, molluscs, polychaetes and crustaceans. *Watersipora* was the most sensitive species and the LC_{50} of Cu (citrate form) over a 2 h exposure was about 600 µg l^{-1}. *Watersipora* larvae swam less freely and sank after experiencing a high Cu dose (Wisely 1962a). Although the larval survival and behaviour were sensitive to Cu, attachment was more resistant (Wisely 1958). Juveniles of *Watersipora* could grow over Cu-based antifouling paints of moderate toxicity or be found in Cu-polluted water in marinas (Weiss & Ketchum 1945, Weiss 1947, Wisely 1958, Turner et al. 1997). The fate of colonies after larval experience of Cu is unclear because previous studies were of short durations.

Specific questions asked in this study are: (1) does a short-term larval exposure of *Watersipora subtorquata* to Cu affect the larval attachment and metamorphosis? or (2) the post-metamorphic survival and growth of field-transplanted colonies? and (3) do these effects vary with seasons or sites? We answered these questions by both laboratory and field investigations. In the laboratory, we looked at the effects on larval attachment and metamorphosis in summer and winter. Then we transplanted the settled juveniles to 2 field sites to measure their subsequent survival and growth.

MATERIALS AND METHODS

Sample collection. Mature colonies of *Watersipora subtorquata* for the winter experiment were collected at Breakwater Pier, Williamstown, in Port Philip Bay, SE Australia, by snorkelling. For the summer experiment, we used those left-over colonies of the control after the winter experiment had been finished. Some other reproductive colonies were recruited from the plates hung from the pier before the start of summer at St Kilda Pier, St Kilda, in Port Philip Bay. Colonies were transported back to the laboratory and put in a recirculating seawater system. They were kept in darkness overnight and induced to spawn under bright light the next day (Wisely 1958).

Cu exposure. Analytical grade $CuCl_2 \cdot 2H_2O$ was the reference toxicant used to provide Cu^{2+}, which is the most toxic form of Cu (Andrew et al. 1977). Marine Biological Laboratory artificial seawater (Cavanaugh 1956) was used as the dilution medium instead of natural seawater in order to minimise any variation in effects arising from complexation of Cu with organic components from natural seawater. Complexation can reduce the amount of Cu^{2+} in solution. A pilot study showed that larvae behaved and settled normally in the artificial seawater.

In all experiments, the exposure period was set to 6 h, to represent a brief but substantial part of the larval period of *Watersipora* spp. (e.g. *Watersipora cucullata*: Wisely 1958). Artificial seawater was used as the control and the Cu treatment was at a concentration of 100 µg l^{-1}. This concentration was selected because it is well below the LC_{50} for the bryozoan larvae (M. J. Keough unpubl. data) and therefore sublethal, but high enough to provide some stress. All the apparatus for toxicity tests was soaked in 5% nitric acid overnight and rinsed with distilled water before use (Greensberg et al. 1994).

Attachment and metamorphosis. Winter and summer laboratory experiments were conducted in May 1999 at room temperature (ca. 15°C) and November 1999 (ca. 22°C), respectively.

Bottoms of plastic Petri dishes (90 mm in diameter) were roughened by sand paper to maximise attachment. We put 15 larvae into each Petri dish containing control or Cu solution with 10 replicates for each treatment. All samples were kept in darkness. Numbers of larvae swimming and attached were observed at 3, 6, 24 and 48 h in the winter experiment. Since a saturation of most responses was found after 24 h in the winter experiment, the summer observation times were 3, 6, 9 and 24 h. After 6 h, the control or Cu solution was replaced with natural seawater. As *Watersipora subtorquata* larvae undergo metamorphosis, their orifices begin to appear and initial stages of calcification are

indicated by the development of ridges. We began recording the development of a clear orifice and ridge after 24 h, at daily intervals until 144 h.

Field study. The winter field study was conducted between late May and early October 1999 (18 wk) with average seawater temperature around 11°C in Melbourne. The summer study was conducted between late December 1999 and late March 2000 (12 wk) with average seawater temperature around 20°C.

Field sites: In winter, as a limited number of larvae could be obtained, the field experiment was conducted at St Kilda pier only. In summer, experiments were conducted at 2 sites, St Kilda Pier and Breakwater Pier. Both sites are located in Port Philip Bay, a large (2000 km^2) embayment in Victoria, in SE Australia. Breakwater Pier is situated 7 km southwest of Melbourne. The pier extends 300 m from the shore and includes a rocky breakwater on the northern side. St Kilda Pier is situated about 4.5 km away from the Breakwater Pier and also includes a rocky breakwater. The sessile assemblages of bryozoans, ascidians, sponges, hydroids and polychaetes have been described elsewhere (Russ 1977, Klemke 1993, Todd & Keough 1994, 1998, Keough & Raimondi 1995). The background dissolved Cu concentrations in both sites are less than 3 µg l^{-1} (Fabris et al. 1999, Webb & Keough 2002).

Survival and growth: After the attachment and metamorphosis experiment, settled juveniles were transplanted to the field to measure their post-metamorphic performance. Juveniles on the edge of Petri dishes or that did not develop orifices were scraped away. The size of ancestrulae was measured from the anterior (tip of orifice) to the posterior end before transplanting. Positions of juveniles were marked on the Petri dishes before transplanting, so that any newly recruited colonies could be identified and removed at each field visit. Dishes with the juveniles were bolted on the underside of PVC backing panels (80 × 35 × 1 cm) to minimise light and sedimentation. There were 10 replicate dishes for each treatment with about 8 settled juveniles each. Petri dishes from the control and Cu treatments were bolted alternately on the backing panels in order to ensure that any micro-environmental variation could not bias the outcome of the experiment. There were 3 backing panels that were hung horizontally and separately, 2 m apart and 1.5 m below the low water mark.

Backing plates were removed from the water and placed into a holding tank at weekly intervals. Survival of colonies was recorded, and pale colonies with empty zooids were defined as dead. New recruits or colonies that overlapped the neighbouring colonies were scraped off *in situ* in order to prevent cessation of growth at the contact edge of overlapping colonies (Gappa 1989, Barnes & Rothery 1996). Colonies were also photographed with a 1 cm grid-marked perspex plate placed underneath the dish. Since *Watersipora subtorquata* colonies are generally circular in shape and only produce buds at the outer edge, diameter of the colony was used as a measurement of their area or size (Bak et al. 1981, Bathgate 1994). Size of colonies was measured from the projected slide images on a white paper, with the scale taken from a perspex plate. Three diameters were measured from different edges of colonies (Bak et al. 1981) when colonies were growing irregularly, and the geometric mean of these diameters was used as the size of each colony. The winter and summer experiments ended at Weeks 18 and 11, respectively.

Statistical analyses. We tested for the normality and homogeneity of our data by looking at boxplots of the data before analysis. We did not apply the arcsine transformations to our data analyses. Most of our percentage data in the samples fell into 2 types—all intermediates (~50 to 70%) or all extremes (~0 or ~100%). The intermediate data were generally normally distributed, so they did not need transformations. For the data at extreme range, an arcsine transformation did not improve the normality.

Repeated measures analysis of variance was used to test for variation in Cu effects over time, with Cu treatment as the between-subjects effect and time as the repeated effect. *T*-tests were used to identify times affected by Cu when there was a significant Time × Cu effect. For the field experiments, we analysed the variation in Cu effects arising from different backing panels by doing a 3-factor (Cu and panels as the between subjects factors) repeated measures analysis of variance. When we found significant interactions between Cu and panels, we analysed the effects of Cu on each panel separately. When there was no Cu × Panel effect, we omitted the panel factor and re-ran the model.

RESULTS

Attachment and metamorphosis

In winter, fewer larvae swam in the Cu treatment over time (Table 1), with an obvious effect at 24 h (t = test: $p < 0.001$; Fig. 1a). In summer, the effect of Cu was consistent, appearing after 3 h, with only about $^1/_2$ of the larvae swimming in the Cu treatment (Fig. 1b). Attachment of larvae during the first 9 h was higher in summer than winter (Fig. 1c,d). In winter, Cu accelerated the attachment of larvae (Table 1), with the greatest effect at 24 h (t-test: $p = 0.008$; Fig. 1c). In summer, Cu had similar effects (Table 1, Fig. 1d), but large effects were apparent by 3 h.

Table 1. *Watersipora subtorquata*. Repeated measures analyses results for experiments on larval exposure to Cu. p-values in bold indicate significant differences at α = 0.050. p = 0.000 denotes value <0.0005

Experiment	Cu			Time × Cu (× Panel)		
	df	p	MS_{error}	df	p	MS_{error}
Winter laboratory study						
Free-swimming	1	**0.010**	532.574	3	**0.000**	104.802
Attachment	1	0.088	801.800	3	**0.000**	124.315
Ridge development	1	**0.022**	1053.896	3	0.361	254.367
Orifice development	1	0.982	1473.949	2	0.085	475.732
Summer laboratory study						
Free-swimming	1	**0.001**	739.550	3	0.071	156.414
Attachment	1	**0.026**	928.717	3	**0.024**	142.983
Ridge development	1	**0.017**	178.666	4	**0.000**	32.267
Orifice development	1	**0.012**	236.344	4	0.165	113.391
Winter field study						
Survival	1	**0.041**	2622.010	18	**0.000**	224.703
Size	1	0.049	38.913	18	**0.003**	4.467
Summer field study						
Survival – St Kilda						
Pooled panels	1	0.092	4060.645	12	**0.007**	201.008
Panel 1	1	0.577	5685.121	12	0.999	337.042
Panel 2	1	**0.033**	2044.613	12	**0.000**	85.761
Panel 3	1	0.229	3639.973	12	**0.034**	112.204
Survival – Williamstown	1	0.384	1234.378	9	**0.002**	60.817
Size – St Kilda						
Pooled panels	1	0.075	173.092	12	**0.005**	14.352
Panel 1	1	**0.043**	232.457	12	**0.000**	16.609
Panel 2	1	0.377	146.897	12	**0.009**	13.934
Panel 3	1	0.778	273.521	12	0.963	21.069
Size – Williamstown	1	0.333	59.750	9	0.070	7.858

All attached larvae developed ridges faster in summer than winter (Fig. 1e,f). In winter, Cu inhibited ridge development consistently (Table 1). In summer, Cu also inhibited the ridge development (Table 1), but the effect was apparent only at 48 h (*t*-test: $p < 0.001$; Fig. 1f) with similar rates as the control after that. Development of the orifice in summer was generally faster than in winter (Fig. 1g,h). In winter, Cu did not affect the orifice development but it had consistent effects in summer (Table 1).

Field study

In winter, the effect of Cu was consistent across panels (repeated measures analyses of survival, $p = 0.958$; size, $p = 0.932$), so panels were omitted from the statistical model. Survival of colonies in the field remained very high, and only after a long period (11 wk) did it start to drop in both treatments (Fig. 2a). Survival of colonies was lower in the Cu treatment (Table 1) and the effect appeared after Week 14 (*t*-tests: $p < 0.050$ at Week 14; $p < 0.010$ between Weeks 15 and 18). In summer, a strong interaction was found between panel and treatment at St Kilda (Table 1), so colonies on each panel were analysed separately. No effect of Cu was detected on Panel 1 (Table 1, Fig. 3a) while there were significant effects on Panels 2 and 3 (Table 1, Fig. 3b,c). The effects appeared about 5 wk after transplant. At Williamstown, no significant variation caused by panels was found, and Cu reduced the survival of colonies after Week 3 (Table 1, Fig. 2c).

In winter, growth was only analysed up to Week 12 because there were insufficient colonies in the Cu and control treatment in the later weeks. After transplant, starting from Week 3 (*t*-tests: $p < 0.050$), colonies in the Cu treatment grew more slowly and were smaller than colonies in the control (Table 1, Fig. 4a). In summer, at St Kilda, different panels had different effects of Cu over time (Table 1). Both Panels 1 and 2 had larger colonies in the Cu treatment (Fig. 5a,b), while Panel 3 had no significant difference in the size of colonies in both treatments (Fig. 5c). At Williamstown, no strong interaction between panels and effects of Cu was found. Colonies in the Cu treatment were similar in size to the colonies in the control (Table 1, Fig. 4c).

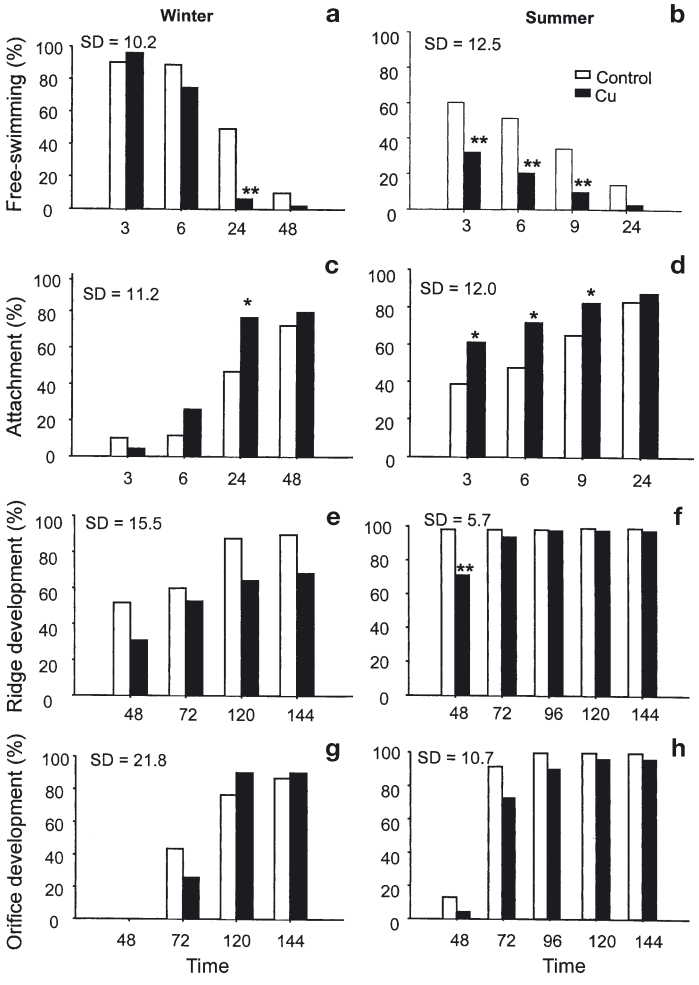

Fig. 1. *Watersipora subtorquata*. Effects of Cu on attachment and metamorphosis in winter and summer. (a) Free-swimming (%), winter; (b) free-swimming (%), summer; (c) attachment (%), winter; (d) attachment (%), summer; (e) ridge development (%), winter; (f) ridge development (%), summer; (g) orifice development (%), winter; (h) orifice development (%), summer. Each bar represents the mean value and SD is the square-root of MS_{error} of the Larvae × Time effects, which are used to assess the differences between treatments through time. *p < 0.05, **p < 0.01, n = 10

DISCUSSION

Cu accelerated the attachment and inhibited the metamorphosis of *Watersipora subtorquata* larvae in summer and winter. These effects were also observed in past studies with a higher Cu dose and longer exposure on bryozoans (Miller 1946, Wisely 1958, 1962a,b). Our earlier study (unpubl.) showed that exposure to Cu (100 µg l^{-1}) for 6 h did not affect the attachment of the arborescent bryozoan *Bugula neritina*, but it deterred the development of lophophores. This study showed that Cu affected both the attachment and metamorphosis of *W. subtorquata*. The reason for this difference between species is unclear, although one potential cause, a difference in length of the larval stage, appears unlikely. In Port Phillip Bay, *W. subtorquata* larvae have a swimming period that is 8 to 20 h longer than that of *B. neritina*. However, extending the swimming period in *B. neritina* did not alter the effects of Cu (T. Y.-T. Ng & M. J. Keough unpubl. data).

There was variation in the effects of Cu on attachment and metamorphosis between seasons. Cu inhibited the development more in summer than winter, as it deterred both ridge and orifice development in summer but only ridge development in winter. In winter, Cu effects developed through time, while they were apparent almost immediately in summer. This pattern may be explained by the different larval activities in

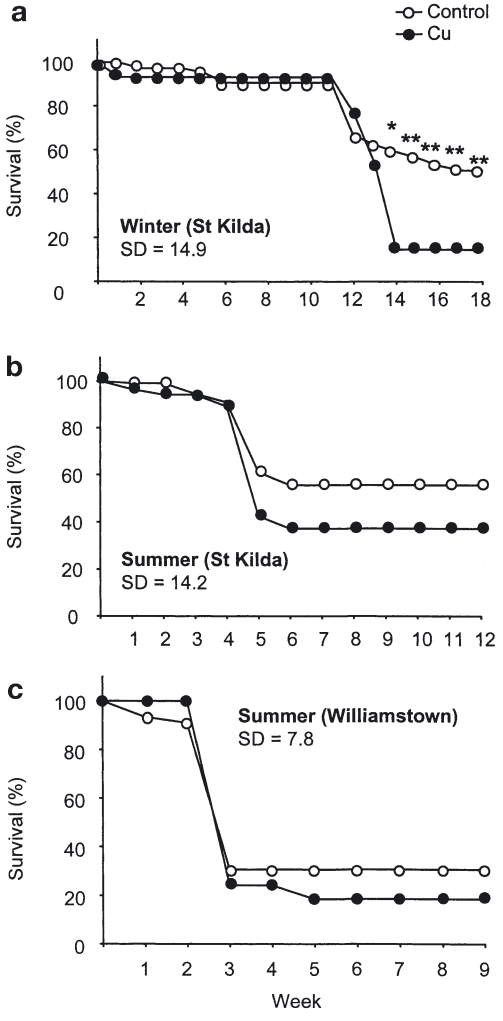

Fig. 2. *Watersipora subtorquata*. Effects of Cu on survival of field-transplanted colonies in winter and summer. (a) Winter, St Kilda, n = 9; (b) summer, St Kilda, n = 10; (c) summer, Williamstown, n = 6. The data are mean values and SD is the pooled SD (see Fig. 1). SD for St Kilda was calculated from repeated measures analyses (Cu × Panel). *p < 0.05, **p < 0.01

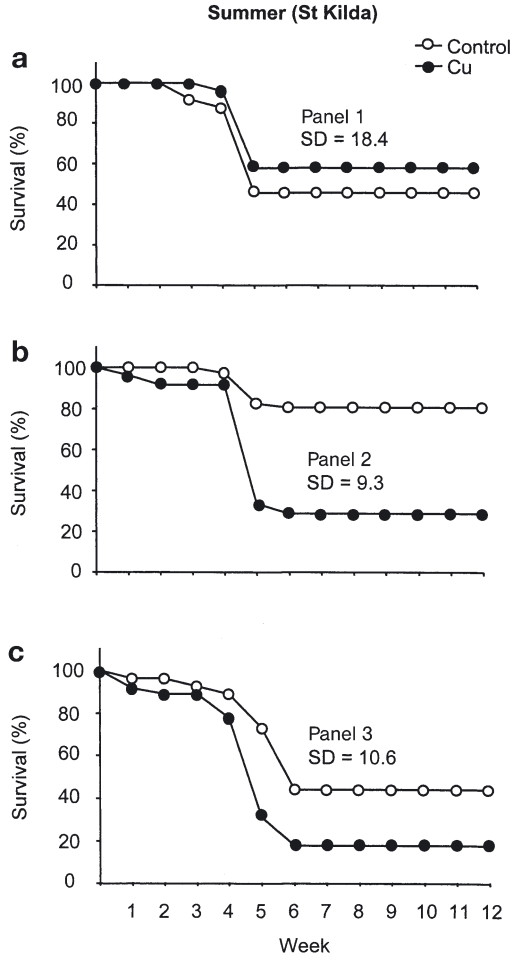

Fig. 3. *Watersipora subtorquata*. Effects of Cu on survival of field-transplanted colonies on different panels in summer at St Kilda. (a) Panel 1, n = 4; (b) Panel 2, n = 3; (c) Panel 3, n = 3. The data are mean values and SD is the pooled SD (see Fig. 1)

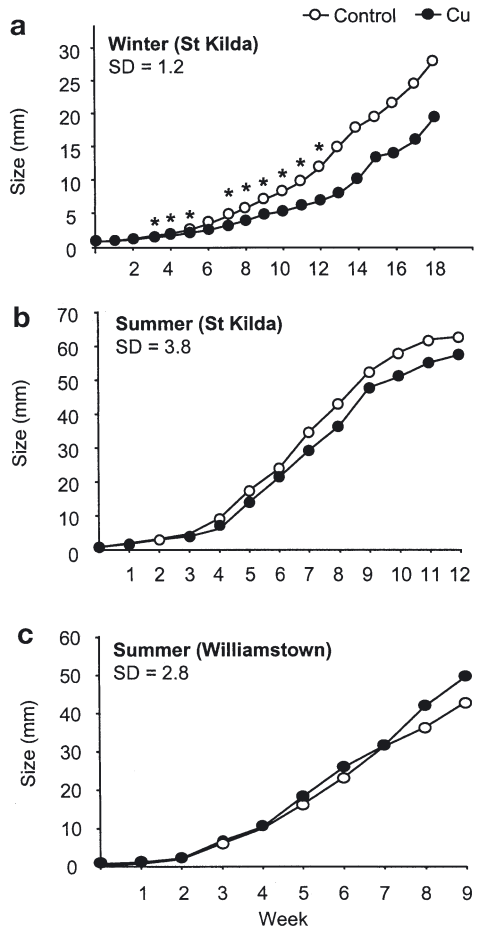

Fig. 4. *Watersipora subtorquata*. Effects of Cu on size of field-transplanted colonies in winter and summer. (a) Winter, St Kilda, n = 9; (b) summer, St Kilda, n = 10; (c) summer, Williamstown, n = 6. The data are mean values and SD is the pooled SD (see Fig. 1). SD for St Kilda was calculated from repeated measures analyses (Cu × Panel). *$p < 0.05$

different seasons. In summer, the temperature is higher and the metabolic activity of larvae is higher and hence, larvae started attachment or metamorphosis earlier than in winter, resulting in an early effect of Cu; an effect that persisted.

Although *Watersipora* larvae attached faster in a Cu-polluted environment, their metamorphosis was slower or incomplete. This delay could provide a time lag of 12 to 24 h in the commencement of feeding and result in a higher risk of predation or slower rate of colony development.

There are few studies following the fate of a colony after the larvae pass through the toxicant pulse. Here, we provide the first evidence of carry-over effects of a toxicant on the post-metamorphic performance of *Watersipora subtorquata*. A larval exposure to 6 h Cu had carry-over effects on survival and growth of *Watersipora* colonies, with the effects not appearing until weeks (summer) or months (winter) after exposure. This is far later than has been reported for other carry-over effects.

Watersipora colonies survived well for at least 5 wk after transplant in both seasons, contrasting to the survival drop at the beginning in *Bugula neritina* (Keough 1986, 1989, Keough & Chernoff 1987, T. Y.-T. Ng & M. J. Keough unpubl. data) and juveniles of most other marine invertebrates (Gosselin & Qian 1997, Hunt & Scheibling 1997). However, a dramatic drop in survival of *Watersipora* colonies occurred in the later period and far fewer colonies survived in the Cu treatment. The size of colonies was about 10 to 15 mm in diameter, corresponding to approximately 100 zooids, when the survival dropped significantly in both treatments. The colonies had undergone several cycles of budding at that time and it is unlikely that the larva could accumulate enough Cu in 6 h to affect all zooids.

Therefore, it is possible that the delayed effect on survival was caused by the damage to some biochemical pathways in the larvae after the exposure, which was passed on during budding. There is also a lot of evidence showing that stress experienced at larval stage impairs the performance of later stages of a range of marine invertebrates (Highsmith & Emlet 1986, Pechenik & Eyster 1989, Woollacott et al. 1989, Orellana & Cancino 1991, Pechenik & Cerulli 1991, Miller 1993, Pechenik et al. 1993, 1996a,b, Hoare et al. 1995, Qian & Pechenik 1998, Gebauer et al. 1999, Maldonado & Young 1999). Pechenik et al. (1998) explained these effects by suggesting that the magnitude and timing of gene transcription for future development of marine invertebrates may be interrupted in their early stage. Here, the effects of Cu on survival appeared 2 mo earlier in summer than in winter. This faster occurrence of Cu effects in summer was also found in the laboratory study of attachment and metamorphosis.

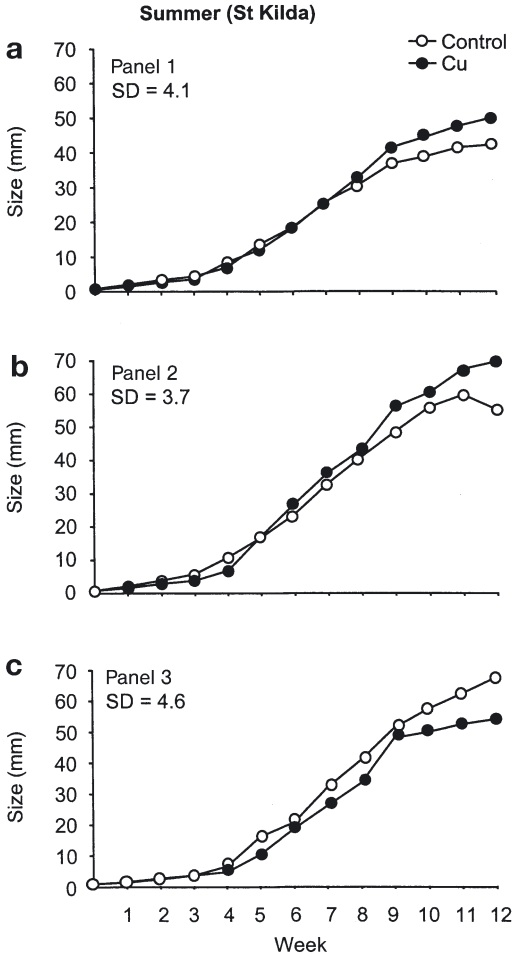

Fig. 5. *Watersipora subtorquata*. Effects of Cu on size of field-transplanted colonies on different panels in summer at St Kilda. (a) Panel 1, n = 4; (b) Panel 2, n = 3; (c) Panel 3, n = 3. The data are mean values and SD is the pooled SD (see Fig. 1)

The carry-over effect on colony growth happened as soon as the colony started to grow rapidly. Cu reduced the growth of colonies in both seasons, but more dramatically in winter and only on 2 of 3 panels at 1 site in summer. Hoare et al. (1995) suggested that the exposure to Cu could alter the larval physiology, reducing the lipid reserve that is essential for larval metamorphosis and growth. While reduced lipid reserves might account for smaller ancestrulae, it is surprising that such effects persist through several cycles of budding, when budding is fuelled by feeding of many zooids. However, larvae of the ascidian *Diplosoma listerianum* with energy reserves presumably depleted by prolonged swimming, produced zooids with smaller feeding structures (Marshall et al. 2003). Colonies continued to produce such zooids for several weeks after metamorphosis.

When the survival and growth profiles of colonies were compared, we found that the survival of colonies in both treatments decreased in temporal coincidence with the increase of growth rates in both seasons. In addition, in summer, embryogenesis might also have started when the survival of the colonies decreased, as the reproductive zooids were first observed 2 wk later. Therefore, it is possible that the mortality rate rose with a shift in colony activity. In bryozoans, successive zooids are linked by a strand of nutritive and nervous tissues known as the funiculus, through which translocation of metabolites is believed to occur (Bobin 1977, Lutaud 1977, Best & Thorpe 1985). One study of fragmentation suggested that resources may not be distributed evenly for growth to every live zooid in the *Watersipora subtorquata* colonies, and that there is more growth in the younger zooids than the older zooids (Hart 2001). The responsibility for growth is mainly with the few zooids behind the growing edge and that resources in zooids further back may contribute more to reproduction (Hart 2001). In addition, the translocation of resources for growth has been demonstrated from the central to peripheral zooids in the bryozoan *Membranipora membranacea* (Best & Thorpe 1985). Therefore, a faster growth or embryogenesis of *Watersipora* may reflect a change in resource allocation in the colonies and increased demands of fast growth or embryogenesis may affect any colonies in poorer condition.

In the field, the effects of Cu on survival of *Watersipora subtorquata* did not appear for weeks or months; however, in contrast, the effects on *Bugula neritina* colonies occurred shortly after colonies were transplanted (T. Y.-T. Ng & M. J. Keough unpubl. data). The differences between species may relate to the difference in growth profiles—*B. neritina* grew faster at the beginning, then slowed down, while *W. subtorquata* grew slowly just after metamorphosis, accelerating later.

Effects of Cu on survival and size of colonies also varied between sites or among panels in summer. The small-scale variation among panels was small at Williamstown but large at St Kilda. At St Kilda, the variation among panels in colony growth was more than in survival. The distribution of field experimental panels may explain the small-scale variation of Cu effects. Panels 2 and 3 were hung 1 m apart along the pier pilings, and Panel 1 was hung from another pier piling, which was 5 m away. There may have been more microhabitat differences between Panel 1 and the other panels, which caused the spatial difference in response by colonies on different panels. In fact, small- and large-scale variation of the performance of the arborescent bryozoan *Bugula neritina* has already been reported in our earlier study (unpubl.) as well as others (Keough 1986, 1989, Keough & Chernoff 1987). Environmental variables, e.g. salinity, temperature, water flow, sedimentation and food, can affect the growth or zooid morphology of bryozoan colonies (Hughes & Hughes 1986, Hughes 1992, Okamura 1992, Keough & Black 1996, O'Dea & Okamura 1999). St Kilda Pier has more food, a higher water flow and more sedimentation than Breakwater Pier (T. Y.-T. Ng unpubl. data). These variations may have caused the differences in the response of bryozoan colonies between sites.

To summarise, this study demonstrates that *Watersipora subtorquata* larvae exposed to Cu for 6 h attached faster in winter and summer, but metamorphosis was either delayed or totally inhibited. Well-developed colonies in the Cu treatment started to die and grow more slowly later in the field. There was generally no temporal difference in response to Cu except that the time of occurrence of effects was earlier in summer than winter. However, in summer, effects were very dependent on different spatial scales, within and between sites.

The results for *Watersipora subtorquata* and *Bugula neritina* have several important implications. In a practical sense, they reinforce other calls for caution in use and interpretation of laboratory toxicity studies. Larvae that were exposed to toxicants for a short period, much less than the conventional 48 and 96 h, survived and settled, but they subsequently grew slowly or died sooner. This delayed mortality of apparently healthy individuals must be incorporated into any interpretation of laboratory studies. A more positive result from this work is that the technique used here, of laboratory exposure, followed by transplantation to the field, is a useful tool for investigating subtle effects of toxicants on individual performance. Individual performance can be measured in a more natural and ecologically relevant way. Finally, this unexpected and fascinating result for survival of *W. subtorquata* shows that larval stresses can have prolonged or delayed carry-over effects. This result extends the links between larval experience and adult performance factors further into organisms' life cycles. Identifying the underlying mechanism will be a challenge.

Acknowledgements. We are grateful to F. Ng for her regular assistance in the field, V. Barmby for the thoughtful discussions and assistance in the initial design of experiment, D. Marshall for the snorkelling assistance in collecting animals, D. Paul for technical advice in taking field photos, and St Kilda Royal Melbourne Yacht Squadron and Breakwater Pier Environmental Office for giving access to piers for doing experiments. This work was supported by an Overseas Postgraduate Research Scholarship and the David Hay Postgraduate Writing-up Award from the University of Melbourne, Australia.

LITERATURE CITED

Andrew RW, Biesinger KE, Glass GE (1977) Effects of inorganic complexing on the toxicity of copper to *Daphnia magna*. Water Res 11:309–315

Bak RPM, Sybesma J, Van-Duyl FC (1981) The ecology of the tropical compound ascidian *Trididemnum solidum*. II. Abundance, growth and survival. Mar Ecol Prog Ser 6: 43–52

Barnes DKA, Rothery P (1996) Competition in encrusting Antarctic bryozoan assemblages: outcomes, influences and implications. J Exp Mar Biol Ecol 196:267–284

Bathgate R (1994) The role of larval behaviour and post-settlement mortality in recruitment of sessile marine invertebrates. Honours thesis, University of Melbourne

Best MA, Thorpe JP (1985) Autoradiographic study of feeding and the colonial transport of metabolites in the marine bryozoan *Membranipora membranacea*. Mar Biol 84:295–300

Bobin G (1977) Interzooecial communications and the funicular system. In: Woollacott RM, Zimmer RL (eds) Biology of bryozoans. Academic Press, New York, p 307–333

Calabrese A, Collier R, Nelson DA, Maclnnes J (1973) The toxicity of heavy metals to embryos of the American oyster *Crassostrea virginica*. Mar Biol 18:162–166

Cavanaugh GM (ed) (1956) Formula and methods IV of the marine biological laboratory chemical room. Marine Biological Laboratory, Woods Hole, MA

Connor PM (1972) Acute toxicity of heavy metals to some marine larvae. Mar Pollut Bull 3:190–192

Fabris GJ, Monahan CA, Batley GE (1999) Heavy metals in waters and sediments of Port Philip Bay, Australia. Mar Freshw Res 50:503–513

Gappa JJL (1989) Overgrowth competition in an assemblage of encrusting bryozoans settled on artificial substrata. Mar Ecol Prog Ser 51:121–131

Gebauer P, Paschke K, Anger K (1999) Costs of delayed metamorphosis: reduced growth and survival in early juveniles of an estuarine grapsid crab, *Chasmagnathus granulata*. J Exp Mar Biol Ecol 238:271–281

Gosselin LA, Qian PY (1997) Juvenile mortality in benthic marine invertebrates. Mar Ecol Prog Ser 146:195–197

Greensberg AE, Clesceri LS, Eaton AD (eds) (1994) Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC

Hart SP (2001) The consequences of fragmentation in the encrusting bryozoan *Watersipora subtorquata*. Honours thesis, University of Melbourne

Highsmith RC, Emlet RB (1986) Delayed metamorphosis: effect on growth and survival of juvenile sand dollars (Echinoidea: Clypeasteroida). Bull Mar Sci 39:347–361

Hoare K, Davenport J, Beaumont AR (1995) Effects of exposure and previous exposure to copper on growth of veliger larvae and survivorship of *Mytilus edulis* juveniles. Mar Ecol Prog Ser 120:163–168

Hughes DJ (1992) Genotype-environment interactions and relative clonal fitness in a marine bryozoan. Ecology 61: 291–306

Hughes DJ, Hughes RN (1986) Life history variation in *Celleporella hyalina* (Bryozoa). Proc R Soc Lond Ser B 228:127–132

Hunt HL, Scheibling RE (1997) Role of early post-settlement mortality in recruitment of benthic marine invertebrates. Mar Ecol Prog Ser 155:269–301

Keough MJ (1986) The distribution of a bryozoan on seagrass blades: settlement, growth, and mortality. Ecology 67: 846–857

Keough MJ (1989) Variation in growth rate and reproduction of the bryozoan *Bugula neritina*. Biol Bull Mar Biol Lab (Woods Hole) 177:277–286

Keough MJ (1998) Responses of settling invertebrate larvae to the presence of established recruits. J Exp Mar Biol Ecol 231:1–19

Keough MJ, Black KP (1996) Predicting the scale of marine impacts: understanding planktonic links between populations. In: Schmitt RJ, Osenberg CW (eds) Detecting ecological impacts: concepts and applications in coastal habitats. Academic Press, San Diego, p 199–234

Keough MJ, Chernoff H (1987) Dispersal and population variation in the bryozoan *Bugula neritina*. Ecology 68:199–210

Keough MJ, Raimondi PT (1995) Responses of settling invertebrate larvae to bioorganic films: effects of different films. J Exp Mar Biol Ecol 185:235–253

Klemke JE (1993) Life history variation in the bryozoan *Mucropetraliella ellerii* (Macgillivray). PhD thesis, University of Melbourne

Lutaud G (1977) The bryozoan nervous system. In: Woollacott RM, Zimmer RL (eds) Biology of bryozoans. Academic Press, New York, p 377–410

Maldonado M, Young CM (1999) Effects of the duration of larval life on postlarval stages of the demonsponge. J Exp Mar Biol Ecol 232:9–21

Marshall DJ, Pechenik JA, Keough MJ (2003) Larval activity levels and delayed metamorphosis affect post-larval performance in the colonial ascidian *Diplosoma listerianum*. Mar Ecol Prog Ser 246:153–162

Miller MA (1946) Toxic effects of copper on attachment and growth of *Bugula neritina*. Biol Bull Mar Biol Lab (Woods Hole) 90:122–140

Miller SE (1993) Larval period and its influence on post-larval life history: comparison of lecithotrophy and facultative planktotrophy in the aeolid nudibranch *Phestilla sibogae*. Mar Biol 117:635–645

Moore SF, Dwyer RI (1974) Effects of oil on marine organisms: a critical assessment of published data. Water Res 8: 819–827

O'Dea A, Okamura B (1999) Influence of seasonal variation in temperature, salinity and food availability on module size and colony growth of the estuarine bryozoan *Conopeum seurati*. Mar Biol 135:581–588

Okamura B (1992) Microhabitat variation and patterns of colony growth and feeding in a marine bryozoan. Ecology 73:1502–1513

Orellana MC, Cancino JM (1991) Effects of delaying settlement on metamorphosis and early colonial growth in *Celleporella hyalina* (Bryozoa: Cheilostomata). In: Bigey FP (ed) Bryozoa living and fossil. Saint-Herblain Publisher, Nantes, p 309–316

Pechenik JA, Cerulli TR (1991) Influence of delayed metamorphosis on survival, growth, and reproduction of the marine polychaete *Capitella* sp. I. J Exp Mar Biol Ecol 151: 17–27

Pechenik JA, Eyster LS (1989) Influence of delayed metamorphosis on the growth and metabolism of young *Crepidula fornicata* (Gastropoda) juveniles. Biol Bull Mar Biol Lab (Woods Hole) 176:14–24

Pechenik JA, Rittschof D, Schmidt AR (1993) Influence of delayed metamorphosis on survival and growth of juvenile barnacles *Balanus amphitrite*. Mar Biol 115:287–294

Pechenik JA, Estrella MS, Hammer K (1996a) Food limitation stimulates metamorphosis of competent larvae and alters postmetamorphic growth rate in the marine prosobranch gastropod *Crepidula fornicata*. Mar Biol 127:267–275

Pechenik JA, Hammer K, Weise C (1996b) The effect of starvation on acquisition of competence and post-metamorphic performance in the marine prosobranch gastropod *Crepidula fornicata* (L.). J Exp Mar Biol Ecol 199:137–152

Pechenik JA, Wendt DE, Jarrett JN (1998) Metamorphosis is not a new beginning—larval experience influences juvenile performance. BioScience 48:901–910

Qian PY, Pechenik JA (1998) Effects of larval starvation and delayed metamorphosis on juvenile survival and growth of the tube-dwelling polychaete *Hydroides elegans* (Haswell). J Exp Mar Biol Ecol 227:169–185

Russ GR (1977) A comparison of the marine fouling occurring at the 2 principal Australian dockyards; report no. MRL-R-688. Department of Defence, Victoria

Todd CD, Keough MJ (1994) Larval settlement in hard substratum epifaunal assemblages: a manipulative field study of the effects of substratum filming and the presence of incumbents. J Exp Mar Biol Ecol 181:159–187

Turner SJ, Thrush SF, Cummings VJ, Hewitt JE, Wilkinson MR, Williamson RB, Lee DJ (1997) Changes in epifaunal assemblages in response to marina operations and boating activities. Mar Environ Res 43:181–199

Webb JA, Keough MJ (2002) Measurement of environmental trace-metal levels with transplanted mussels and diffusive gradients in thin films (DGT): a comparison of techniques. Mar Pollut Bull 44:222–229

Weiss CM (1947) The comparative tolerances of some fouling organisms to copper and mercury. Biol Bull Mar Biol Lab (Woods Hole) 93:56–63

Weiss CM, Ketchum BH (1945) Service test applications of antifouling paints to wood bottom vessels in the Miami region; report no. 12. Woods Hole Oceanographic Institute to the Bureau of Ships, Miami

Wisely B (1958) The settling and some experimental reactions of a bryozoan larva, *Watersipora cucullata* (Busk). Aust J Mar Freshw Res 9:362–371

Wisely B (1962a) Effect of an antifouling paint on a bryozoan larva. Nature 195:543–544

Wisely B (1962b) Effects of antifouling paints on settling larvae of the bryozoan *Bugula neritina* L. Aust J Mar Freshw Res 14:44–59

Wisely B, Blick AP (1967) Mortality of marine invertebrate larvae in mercury, copper, and zinc solutions. Aust J Mar Freshw Res 18:63–72

Woollacott RM, Pechenik JA, Imbalzano KM (1989) Effects of duration of larval swimming period on early colony development in *Bugula stolonifera* (Bryozoa: Cheilostomata). Mar Biol 102:57–63

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: August 12, 2002; Accepted: January 13, 2003
Proofs received from author(s): July 17, 2003