Responses of polychaete *Hydroides elegans* life stages to copper stress

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ABSTRACT: Two experiments were performed to examine the sensitivity of *Hydroides elegans* to copper stress. The first experiment tested the development of 4 distinct early stages in copper solutions from 10 to 250 µg l^{-1}. Copper significantly reduced survivorship of the newly released oocyte and trochophore stages at concentrations $\geq$10 µg l^{-1}, and of the 2-cell and blastula stages at $\geq$25 µg l^{-1}. The EC_{50} values were 47, 50, 71 and 29 µg Cu l^{-1} for the newly released oocytes, 2-cell embryos, blastulae and trochophores, respectively. After 2 h into the bioassay, where *Isochrysis galbana* was used as food for the trochophores, algal sorption resulted in a reduction in copper concentration from 32% in the 10 µg l^{-1} treatment to 6% in the 250 µg l^{-1} treatment. Duration of development did not significantly differ among the treatments, except in the oocyte to 2-cell stage where higher copper concentration resulted in longer developmental time. In the second experiment, adult *H. elegans* were exposed to copper concentrations from 125 to 4000 µg l^{-1} for 48 h. Significant mortality occurred only at copper concentrations $\geq$500 µg l^{-1}. This study thus shows that the embryogenesis and larval development of *H. elegans* are sensitive to copper stress, and that algal sorption can substantially reduce soluble copper concentration and may affect the larval toxicity result.

KEY WORDS: *Hydroides* · Polychaete · Copper · Toxicity · Life cycle

INTRODUCTION

Although copper (Cu) is essential for the metabolism of marine animals, it can become toxic at elevated concentrations (White & Rainbow 1985). Concentrations of Cu are usually low in natural ecosystems (0.05 to 0.25 µg l^{-1}), but can be very high in polluted coastal waters (810 to 1000 µg l^{-1}) (Soegianto et al. 1999). In recent years, due to rigid control of tributyltin-containing antifouling paints in many countries, the use of Cu-based antifouling paints has been increasing, leading to concerns of elevated Cu concentrations in coastal waters (Seligman & Zirino 1998, Lorenzo et al. 2002) and animals (Claisse & Alzieu 1993).

In order to determine the toxicity of environmental samples, many bioassays have been developed using oysters, mussels, sea urchins, sand dollars, fishes and mysids as test organisms (Chapman et al. 1995, Nipper et al. 1997, Simon & Laginestra 1997, King & Riddle 2001, Carr & Nipper 2003). Such bioassays usually employ the embryos or larvae because they are sensitive to contaminants (Pechenik 1987, Rand et al. 1995), presumably due to their relatively small size, high surface area to volume ratio and undeveloped metabolic regulation mechanisms. Polychaetes have also been widely used in bioassays of chemicals and environmental samples since the 1960s, but the test organisms have been restricted to the juveniles and adults (reviews by Reish & Gerlinger 1997, Nipper & Carr 2003). In a recent study, Ross & Bidwell (2001) explored the use of embryos of the serpulid polychaete *Galeolaria caespitosa* in a toxicity test. The results

have shown that the embryogenesis of *G. caespitosa* is much more sensitive to copper (with a 48 h EC_{50} of 16 to 40 µg l^{-1}) than the benthic stages of most other polychaetes, which typically have an EC_{50} or LC_{50} of 100 to 1000 µg l^{-1}, with few exceptions (Reish & Gerlinger 1997). The sensitivity of the early development of other polychaetes, however, has not been reported.

Hydroides elegans (Haswell), a tube-building serpulid polychaete common in tropical and subtropical coastal waters (Huang & Cai 1984), reproduces in Hong Kong throughout the year (Qiu & Qian 1997). This species forms aggregates in shallow water and thus, is easy to collect. It is also easy to maintain in the laboratory and can be readily induced to spawn. However, how sensitive this species is to toxicants has not been assessed. Whether this species is under copper stress in some heavily polluted areas in Hong Kong is unknown, although high concentrations of copper have been reported in both the water column (up to 24.6 µg l^{-1}) (Wong et al. 2001) and sediment (up to 6250 mg kg^{-1}) (Blackmore 1998). In this study, we compared the sensitivity of different developmental stages of *H. elegans* to copper stress. The data will help us determine the copper level that may adversely affect the wild population and the suitability of this species for testing the toxicity of chemicals and environmental samples.

MATERIALS AND METHODS

Adult tubeworms were collected between July and December 2003 from PVC panels (100 cm^2 surface area) previously submerged into the water of Long Bay, Hong Kong (22° 27' N, 114° 23' W). Judging from the growth rate in the laboratory (Qiu & Qian 1998) and the time between panel deployment and retrieval, the tube worms were 1.5 to 2 mo old. They were brought to the laboratory at The Hong Kong University of Science and Technology and kept in seawater aquaria. These worms were used in 2 experiments, both conducted at 24°C in artificial seawater (ASW) (34 psu) prepared according to a recipe in Lorenzo et al. (2002). The background Cu content, as measured by differential pulse anodic stripping voltammetry (DPASV) using the standard addition method (Harvey 2000) was 0.81 ± 0.12 µg l^{-1} (n = 3).

Expt 1: early development under copper stress. The early development of *Hydroides elegans* (gamete to newly settled juveniles) can be divided into 4 distinct periods (gamete to 2-cell stage, 2-cell stage to blastula, blastula to trochophore and trochophore to newly-settled juvenile). This experiment consisted of 4 bioassays, each following the development of one early developmental stage to the subsequent stage. The procedures for obtaining organisms at these developmental stages were the same as in Qiu & Qian (1997). Briefly, mature adults were induced to spawn by breaking their tubes. Sperm and oocytes from 3 males and 4 females were mixed, respectively. In Expt 1A, newly released oocytes were transferred to empty petri dishes. Care was taken not to introduce more than 100 µl of water into the dishes when transferring the oocytes. When this was not practical, excessive water was removed using a pipette. Ten ml of ASW containing different concentrations of Cu were then added to the petri dishes. Five µl of ASW containing sperm were pipetted into the dishes. Meanwhile, other newly released oocytes were transferred into a beaker containing 600 ml ASW and 1 ml of ASW containing sperm was pipetted into the beaker to fertilize the oocytes. When the zygotes reached the 2-cell embryos, blastulae and trochophores, they were used in Expts 1B, 1C and 1D, respectively. Embryos and larvae at each of these stages were added to 7 Cu treatments (control seawater, 10, 25, 50, 100, 125 and 250 µg Cu l^{-1} as $CuSO_4$). In all of the 4 developmental stages, each treatment had 5 replicates and each replicate contained 20 to 35 individuals. In Expt 1A, embryonic development was checked every 30 min after sperm and oocytes were mixed, until the oocytes had undergone fertilization and cleavage or disintegrated in 6 h. The data were used to calculate overall survivorship and duration of development. In Expt 1B, cultures were examined at 3, 4, 5, 6, 7, 8, 10 and 12 h after the start of the experiment, until the 2-cell embryos had developed into blastulae or disintegrated. In Expt 1C, cultures were checked at 8, 10, 12 and 24 h after the onset of the experiment, until the blastulae had developed into trochophores or disintegrated. Expt 1D was designed to examine the effects of Cu concentration on the survivorship, duration of development of trochophores and percentage trochophores settled. The chrysophyte *Isochrysis galbana* (Clone T-ISO), cultured with a modified f/2 medium (Guillard 1975), was offered as food to the trochophores at 10^5 cells ml^{-1}. Before use, the algae were harvested by repeated centrifugation at $2800 \times g$ for 5 min and resuspension in ASW to remove algal culture medium that contained EDTA. The trochophores were transferred to fresh exposure media daily for a total of 8 d. At each transfer, the numbers of surviving larvae and new-settlers were counted.

Expt 2: survival of adults under copper stress. The sensitivity of adults to copper stress was tested using non-reproductive individuals. The tubeworms were detached from a worm aggregate into individuals or groups of 2 to 3 individuals with attached tubes and were allowed to acclimate for 2 d in ASW. Only healthy individuals were used in the experiment. The experiment consisted of the following 8 Cu treatments:

control seawater, 125, 250, 500, 1000, 2000, 3000 and 4000 µg Cu l^{-1} as $CuSO_4$. Each treatment had 5 replicates, and each replicate had 10 individuals in 20 ml solution. The experiment was run for 48 h, during which no food was offered to the worms but the exposure medium was renewed after 24 h. At the end of exposure, the survivors were counted and the data were used to calculate survivorship.

Chemical analysis. Cu concentration in a separate set of dishes containing the exposure media was measured by differential pulse anodic stripping voltammetry (DPASV). Measurements were taken with a Metrohm 693 VA processor coupled with a Metrohm 694 VA Stand. A hanging mercury drop electrode was used as working electrode. The potentials were measured with an Ag/AgCl reference electrode and an auxiliary platinum electrode. Samples were stirred at 3000 rpm and degassed by nitrogen for 5 min before measurement. The potential was −170 mV ± 35 mV and the deposition time was 60 s.

ASW was spiked with a $CuSO_4$ stock solution to different Cu concentrations. In Expts 1A–C, the solution was acidified to pH 1.5 with nitric acid 2 and 24 h after the start of the bioassay. In Expt 1D, *Isochrysis galbana* was added into the culture vessels before the addition of $CuSO_4$ solution to reach a final concentration of 10^5 cells ml^{-1}. After 2 and 24 h, the samples were centrifuged at 3500 rpm for 10 min to precipitate the algae. The supernatant was poured into another container, acidified to pH 1.5 with nitric acid and analyzed after 3 h of stabilization.

Statistical analysis. Relationship between nominal Cu concentration and measured Cu concentration was assessed with linear correlation. Data of survivorship, percentage reaching settlement and duration of development were assessed for normality with the Shapiro & Wilk Test (Zar 1999). Since none of the data met the normality assumption for parametric analysis, they were analyzed using nonparametric statistics. This was done by transforming the values to ranks and then applying parametric statistics on the ranks, as described in Zar (1999) and SAS (1988). Effects of Cu concentration on the survivorship, duration and percentage settlement were analyzed with a single-factor analysis of variance (ANOVA), followed by the Tukey multiple comparisons (Zar 1999). The EC_{50} for inhibition of embryogenesis and larval development, and LC_{50} for adults were calculated using the Trimmed Spearman-Karber method (Hamilton et al. 1977).

RESULTS

Chemical analysis

Table 1 shows the nominal and measured Cu concentrations in the exposure vessels. In Expts 1A–C and Expt 2, where the exposure media did not include algae, the measured Cu concentration ranged from 82 to 110% of the nominal concentration. However, the percentage was not dependent on the nominal Cu concentration. There was generally a slight increase in Cu concentration from the beginning of the experiment (Hour 2) to the time of exposure medium renewal (Hour 24). Since the measured Cu concentrations did not deviate much from the nominal concentrations, we calculated Cu toxicity based on the nominal concentrations. When algae were used to support trochophore development (Expt 1D), algal sorption resulted in a significant decline in Cu concentration. After 2 h, Cu reduction ranged from 32% in the 10 µg l^{-1} treatment to 6% in the 250 µg l^{-1} treatment. In general, the percentage of reduction was inversely correlated with nominal Cu concentration (reduction % = −0.098 Cu concentration + 26.030, $r^2 = 0.74$, p < 0.05). Except in the 25 µg Cu l^{-1} treatment, there was a further reduction (2.4 to 11.7%) in Cu concentration from Hour 2 to Hour 24. Since the values were not stable during the course of the experiment, we were unable to express Cu toxicity using measured concentrations.

Table 1. Nominal and measured Cu concentrations (µg l^{-1}) in the exposure vessels 2 and 24 h after setting up the experiment. Data are the mean of 3 measurements ±SD. In Expts 1A–C and Expt 2, no algae were added to the exposure vessels. In Expt 1D, the concentration of *Isochrysis galbana* was 10^5 cells ml^{-1}

| Nominal	Expts 1A–C Measured		Expt 1D Measured		Nominal	Expt 2 Measured	
	Hour 2	Hour 24	Hour 2	Hour 24		Hour 2	Hour 24
10	8.2 ± 0.4	10.3 ± 0.2	6.8 ± 0.4	5.6 ± 0.7	250	253.5 ± 35.6	260.1 ± 3.7
25	21.1 ± 1.8	22.8 ± 0.5	19.1 ± 0.1	20.3 ± 0.3	500	470.4 ± 12.3	499.7 ± 14.5
50	45.8 ± 3.3	46.6 ± 0.2	39.7 ± 0.6	38.5 ± 0.1	1000	999.2 ± 31.3	1097.9 ± 27.3
100	88.6 ± 2.2	99.3 ± 2.5	89.5 ± 0.4	81.3 ± 0.6	2000	1928.7 ± 14.3	2026.4 ± 70.3
125	117.6 ± 0.6	129.1 ± 0.8	114.4 ± 0.9	103.4 ± 1.1	3000	3140.4 ± 65.3	3227.8 ± 42.6
250	253.5 ± 35.6	260.1 ± 3.7	234.0 ± 3.7	211.8 ± 0.8	4000	3885.0 ± 16.9	4126.3 ± 24.6
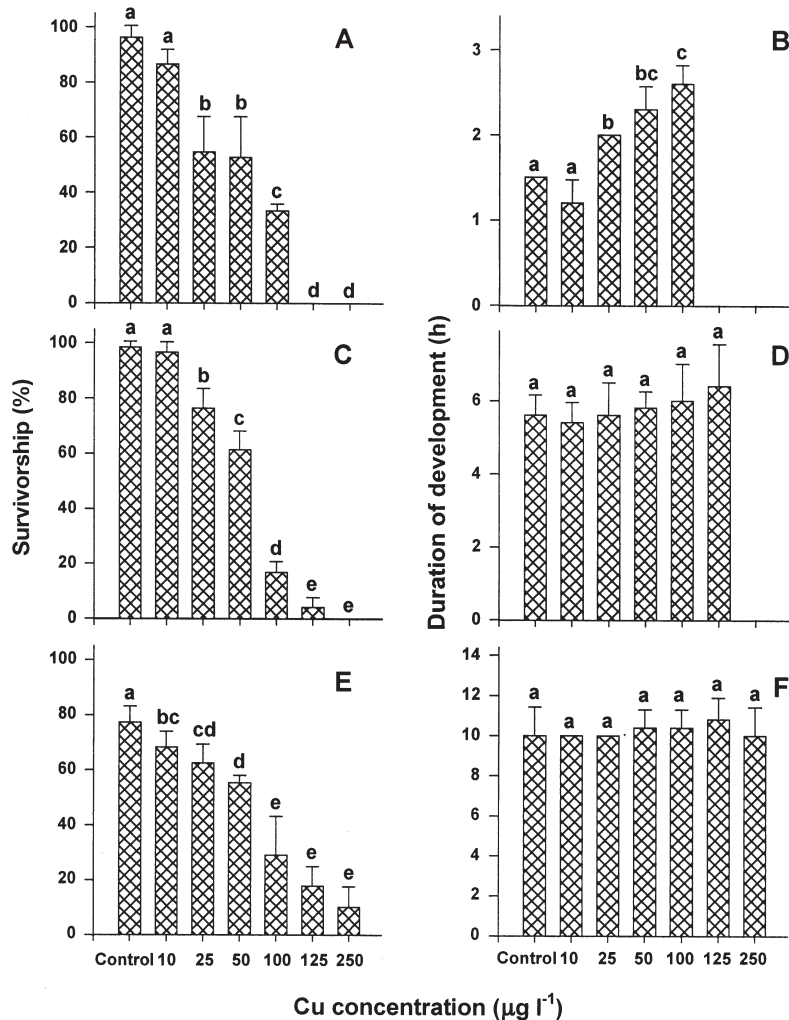
Fig. 1. *Hydroides elegans*. Effects of Cu concentration on embryonic development from (A,B) newly released oocyte to the 2-cell stage, (C,D) the 2-cell stage to blastula and (E,F) blastula to trochophore. The survivorship and duration of development data are mean + SD of 5 replicate cultures of 20 to 35 individuals. Bars for a certain developmental stage having the same lower case letter do not differ significantly from each other at the 0.05 level

ferent between the 2 treatments; it increased with increasing Cu concentration from 2 h in the 25 µg l^{-1} treatment to 2.6 h in the 100 µg l^{-1} treatment.

Expt 1B: 2-cell stage to blastula

Cu concentration significantly affected the survivorship (Fig. 1C). In the control and the 10 µg Cu l^{-1} treatment, over 95% of the 2-cell embryos developed into blastulae. From 25 to 125 µg Cu l^{-1}, progressively lower percentages (71 to 4%) of the 2-cell embryos developed into blastulae. All individuals died in the 250 µg Cu l^{-1} treatment. At 100 and 125 µg Cu l^{-1}, the surviving blastulae swam very sluggishly and near the bottom of the dish. In other treatments, the blastulae swam actively and randomly in the dish. The EC_{50} was 50 µg Cu l^{-1}. The duration of development ranged from 4.8 to 5.5 h and was not significantly different among the Cu treatments (Fig. 1D).

Expt 1C: blastula to trochophore

Survivorship decreased with increasing Cu concentration (Fig. 1E). In the control, 77% of the blastulae developed into trochophores. From 10 to 250 µg Cu l^{-1}, progressively lower percentages (68 to 10%) of the blastulae developed into trochophores. At 50 µg l^{-1} and lower Cu concentrations, the trochophores swam actively and randomly in the dish. In the higher Cu concentration treatments, the trochophores swam sluggishly and near the bottom of the dish. The EC_{50} was 71 µg Cu l^{-1}. The duration of development ranged from 10.4 to 10.8 h and was not significantly different among the Cu treatments (Fig. 1F).

Expt 1A: newly released oocyte to 2-cell stage

Survivorship and duration of development significantly differed among the Cu treatments (Fig. 1A,B). In the control and the 10 µg Cu l^{-1} treatment, 96 and 87% of oocytes successfully developed into 2-cell embryos, respectively. Progressively lower percentage of oocytes reached the 2-cell stage with increasing Cu concentration from 25 to 100 µg l^{-1}. In both 125 and 250 µg Cu l^{-1} treatments, all oocytes had disintegrated by the end of the 6 h exposure. The EC_{50} for embryogenesis inhibition was 47 µg Cu l^{-1}. The duration of development was 1.5 and 1.2 h in the control and the 10 µg Cu l^{-1} treatment, respectively, and was not significantly dif-

Expt 1D: trochopore to newly settled juvenile

Percentage survival differed significantly among the Cu treatments (Fig. 2). At the end of the experiment, approximately 75% of the individuals in the control remained alive. Percentage survival progressively decreased from 64% in the 10 µg Cu l^{-1} treatment to 49% in the 50 µg Cu l^{-1} treatment. The trochophores in the 100 µg Cu l^{-1} and higher Cu concentration treatments
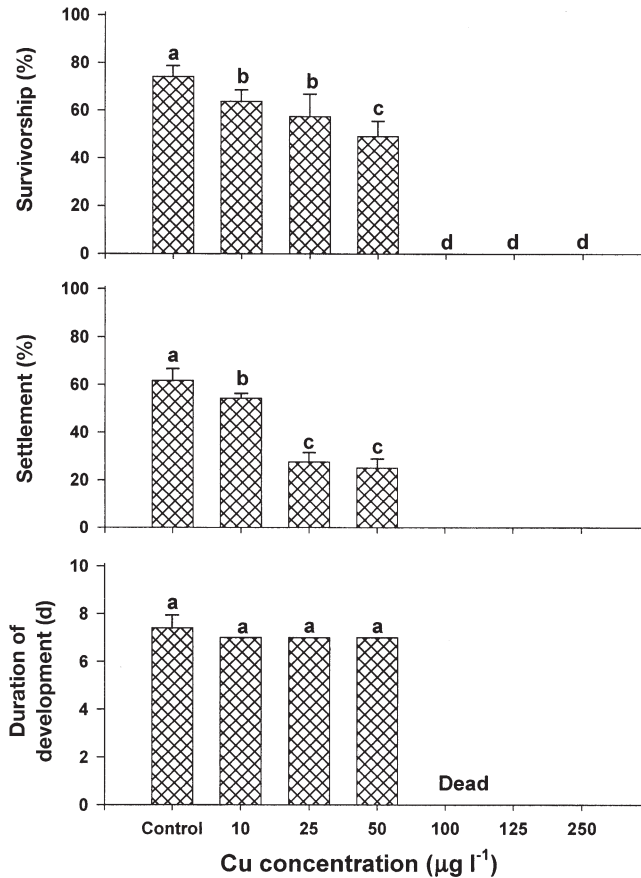
Fig. 2. *Hydroides elegans.* Expt 1D: effects of Cu concentration on survivorship, percentage reaching settlement and duration of development from trochophore to newly settled juvenile. Trochophores all died in the 100, 125 and 250 µg Cu l^{-1} treatments. Data are mean + SD of 5 replicate cultures of 20 to 35 individuals. Bars having the same lower case letter do not differ significantly from each other at the 0.05 level
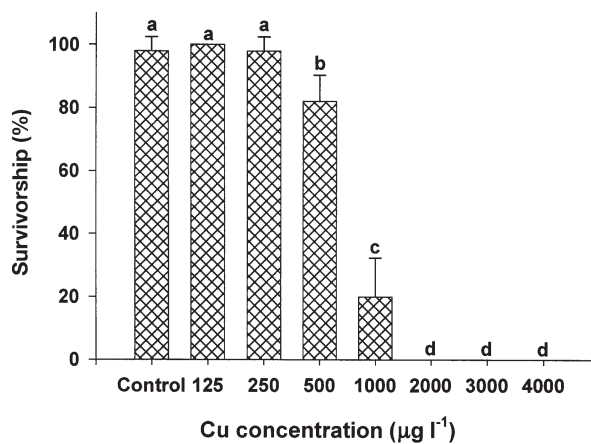

Fig. 3. *Hydroides elegans.* Expt 2: effects of Cu concentration on adult survival. Adults all died in the 2000 µg l^{-1} and higher Cu concentration treatments. Data are mean + SD of 5 replicate cultures of 10 individuals. Bars having the same lower case letter do not differ significantly from each other at the 0.05 level

did not survive through the 8 d experiment. The LC_{50} for survival was 52 µg Cu l^{-1}. Percentage reaching settlement also significantly differed among Cu treatments (Fig. 2). In the control and 10 µg Cu l^{-1} treatment, over 50% of the trochophores successfully settled and metamorphosed into juveniles. In the 25 and 50 µg Cu l^{-1} treatments, only approximately 25% of the larvae developed into juveniles. These juveniles, however, appeared to have normal body and tentacular structures, and exhibited normal feeding activities. The EC_{50} for settlement inhibition was 29 µg Cu l^{-1}. The development from trochophore to newly settled juvenile took 7.0 to 7.4 d and was not significantly different among the Cu treatments.

Expt 2: survival of adults

Cu concentration significantly affected survival of the adults during the 48 h exposure period (Fig. 3). In the control and Cu concentration treatments up to 250 µg l^{-1}, the survivorship was high (>98%), and the difference among these 3 treatments was insignificant. From 500 to 1000 µg Cu l^{-1}, the survivorship declined sharply from 82 to 20%. No surviving worms were found at Cu concentrations higher than 1000 µg l^{-1}. The LC_{50} was 715 µg Cu l^{-1}.

DISCUSSION

Polychaetes have been used in testing the toxicity of pure chemicals and sediment samples since the 1960s. According to Reish & Gerlinger 1997, 48 species in 20 families of polychaetes have been used as test organisms, with members of families Capitellidae, Dinophilidae, Dorvilleidae and Nereididae being the most frequently used species. The majority of these bioassays used juvenile or adult stages, whereas the sensitivity of the embryonic and larval stages of polychaetes remains largely unknown. In this study, we reported the ontogenetic changes in tolerance to Cu in a serpulid polychaete, *Hydroides elegans.* The results showed that the EC_{50} values were 47, 50, 71 and 29 µg Cu l^{-1} for the development of the newly released oocyte, 2-cell stage, blastula and trochophore, respectively. The 48 h LC_{50} for the adults was 715 µg Cu l^{-1}. A comparison between these toxicity values with the highest reported Cu concentration in Hong Kong waters (24.6 µg l^{-1}, in Wong et al. 2001) indicates that the wild *H. elegans* population is unlikely to be under copper stress. The results also indicate that the embryos and larvae of *H. elegans* were 10 to 25 times as sensitive as the adults to Cu stress. The EC_{50} values for the early developmental stages of *H. elegans* were

comparable with the result of Ross & Bidwell (2001) on the embryos of *G. caespitosa*, suggesting that the early development of both serpulid polychaetes is of similar sensitivity to Cu stress. It should also be noted that the experimental durations for the 4 early stages were different. The experiment starting with oocytes, 2-cells and blastulae, lasting for 6, 12 and 24 h, respectively, resulted in similar EC_{50} values (around 50 µg Cu l^{-1}). Compared with these values, the EC_{50} from trochophores was lower (29 µg Cu l^{-1}), indicating that trochophores are more sensitive to copper stress than the other 3 embryonic stages. However, the experimental duration was much longer (10 d), which should be taken into account when selecting a developmental stage for toxicity testing.

Sensitivity to pollutants depends on the type of organisms and the stage of development used. The embryos and larvae of bivalves, such as mussels and oysters, have been widely used in toxicity tests, partly because of their high sensitivity. His et al. (1999) conducted a comprehensive review of the assessment of marine pollution using bivalve embryos and larvae as testing organisms. They found that the sensitivity was comparable among different bivalve species, but varied dramatically with developmental stage in a given species. Among the early developmental stages of a species, embryos were usually more sensitive than larvae. The average EC_{50} for inhibition of mollusk embryogenesis was 39.8 µg l^{-1} and the average LC_{50} for larval mortality was 86.5 µg l^{-1}. The embryos of sea urchins were also very sensitive to Cu. For example, the 48 h EC_{50} for inhibition of embryogenesis was 32 to 100 µg l^{-1} in *Paracentrotus lividus* (Kobayashi 1981, Pagano et al. 1986, Fernández & Beiras 2001, Lorenzo et al. 2002), 31 to 44 µg l^{-1} in *Arbacia punctulata* (Carr 1996) and 43 µg l^{-1} in *Diadema setosum* (Ramachandran et al. 1997). A comparison of the above EC_{50} values with those of *Hydroides elegans* showed that the polychaete embryos and larvae have comparable levels of sensitivity to Cu stress.

In this study, we explored the sensitivity of the early development of *Hydroides elegans* using the following endpoints: mortality, duration of a developmental stage and percentage larval settlement. These endpoints were easy to quantify, but not all of them were suitable for use in routine testing. Significant mortality of the oocytes and trochophores occurred in the ≥10 µg Cu l^{-1} treatments, and of the 2-cell embryos and blastulae in the ≥25 µg Cu l^{-1} treatments. The dose-response curves were gradual over a relatively wide range of Cu concentrations, resulting in the EC_{50} of 47, 50, 71 and 29 µg Cu l^{-1} for the newly released oocytes, 2-cell embryos, blastulae and trochophores, respectively. Mortality is, thus, sensitive and can be used as an endpoint in toxicity tests. However, it is

inappropriate to use duration of development as an endpoint to assess the toxicity of Cu in this species. The duration of development was not significantly different among the Cu treatments, except in Expt 1A, where the duration of development lengthened with increasing Cu concentration. But in Expt 1A, the toxicological effect had to be observed in a short period of time due to the brief developmental time (1.2 to 2.5 h) from oocyte to 2-cell embryo.

The trochophores require feeding in order to complete larval development. In larval toxicity tests, algae not only serve as food, but also provide binding sites for toxicants. Although algae are routinely used in the culture of invertebrate larvae (His & Robert 1981, Watling 1982, Beaumont et al. 1987), the effect of metal sorption by algae on the toxicity result has not been assessed. Obviously, the extent of algal sorption depends on the algal concentration and the metal concentration. In this study, we fed the trochophores with *Isochrysis galbana* at 10^5 cells ml^{-1}. By Hour 2, the Cu concentration was reduced by 32% in the 10 µg l^{-1} treatment and 6% in the 250 µg l^{-1}. The reduction in Cu concentration in the aqueous phase might have affected the toxicity result because the reported EC_{50} (29 µg Cu l^{-1}) was calculated using nominal Cu concentrations. Should the measured Cu concentrations have been used, the EC_{50} value would have been approximately 30% lower (21.4 µg Cu l^{-1} based on the measured Cu concentrations at Hour 2, and 20.2 µg Cu l^{-1} based on the measured Cu concentrations at Hour 24). Although the measured Cu concentrations were unstable over time, the data indicate that when algae are used to feed animals in toxicity tests, algal sorption could result in a significant decline in toxicant concentration in the aqueous phase. When nominal toxicant concentrations are low, a large proportion of the toxicant might be bound to the algae, resulting in underestimation of the toxicity. Further studies should quantify such sorption effects with different algal species and algal concentrations.

In addition to sensitivity to toxicants, environmental relevance and ease of maintenance of an organism are also among the criteria for selecting species for routine bioassays. *Hydroides elegans* is widely distributed along the coasts of Australia, New Zealand, Singapore, Southern Japan, Southern China, Egypt, India, California, Hawaii, the English Channel and the Bristol Channel (ten Hove 1974, Huang & Cai 1984, Bastida-Zavala & ten Hove 2002). The ubiquitous distribution of *H. elegans* in tropical and subtropical waters indicates that it is a common receiver of pollutants in coastal waters. In Hong Kong, this species reproduces throughout the year, making it possible to obtain gametes for embryonic work in all

seasons. Furthermore, because this species is less than 2.5 cm in body length, hundreds of individuals can be cultured in a small aquarium. We maintained a population of *H. elegans* from 1997 to 1998 in a 20 l aquarium by feeding the worms with a mixture of *Isochrysis galbana* and *Chaetoceros muelleri*, during which the worms were sampled from time to time. Reproductive individuals approximated 25 to 50% at each sampling, offering sufficient gametes for conducting experiments. Compared with oysters and other bivalves, this species requires less laboratory space to keep.

Exposure duration is another criterion to consider when developing bioassays. Under optimal laboratory conditions, the whole life-cycle of *Hydroides elegans* can be completed in approximately 22 d, including less than 1 d for embryogenesis, 5 d for larval development and 16 d for growth and maturation (Qiu & Qian 1997, 1998). Although the duration of embryogenesis is also short in bivalves and sea urchins (several hours to 1 d), the duration of their larval development is quite long (usually a month or longer) and the duration from settlement to maturation is even longer (usually more than 1 yr) (Seed & Brown 1977, Strathmann 1987, Qiu et al. 2002). As a result of financial and labor constraints imposed by the chronic bioassay, most toxicity tests with bivalve larvae only lasted for 2 to 15 d (Table 10 in His et al. 1999), without even allowing the larvae to develop to the eyed-veliger stage. A similar situation occurs in bioassays using sea urchin larvae as the test organisms, where the larvae are generally maintained only for 3 to 4 d (Chapman et al. 1995), which is too short a time period in which to complete larval development. The short life-cycle in *H. elegans*, thus, offers the potential for testing of not only acute toxicity to embryonic and larval stages, but also sublethal growth and reproductive responses in the whole larval and juvenile stages within a relatively short period of time.

In summary, this study has shown that, in Hong Kong waters, environmental Cu levels may not negatively affect any of the developmental stages of *Hydroides elegans*. Within the life cycle, embryos and larvae are much more sensitive to Cu stress than adults. Among different biological responses measured, mortality provides a gradual dose-response relationship and can be used as an endpoint in a toxicity test. Duration of development, however, does not correlate well with Cu concentration and thus, is not a suitable toxicity endpoint.

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