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Reproductive and Life Stage-Specific Effects of Aqueous Copper on the Grass Shrimp, *Palaemonetes pugio*

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1 Reproductive and Life Stage-Specific Effects of Aqueous Copper on the Grass Shrimp,
2 *Palaemonetes pugio*

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Abstract

Palaemonetes pugio exposed to copper (9 or 26 $\mu\text{g Cu}^{2+}/\text{L}$) for a full life cycle were unable to produce viable embryos, precluding completion of the life cycle, despite no lethal effects on larval, juvenile, or adult life stages. Exposure to copper also resulted in a significant delay in larval development. When adults raised in copper were transferred to control seawater, they produced viable embryos, but larval output per clutch was reduced by 43% compared to clutches from females that had never been exposed to copper, suggesting reduced energetic allocation to reproduction. In acute exposures, adults exposed to copper for as little as three days were unable to produce viable embryos. Furthermore, exposure of control embryos to copper reduced hatching success by 74%. The reduction in hatching success and energetic allocation to reproduction are unlikely to be completely responsible for the lack of larval production observed in pre-spawning exposures to copper, suggesting that copper may also inhibit processes before or during spawning and fertilization.

Keywords: Copper; Ecotoxicology; Embryos; Fertilization; Hatching Success; Larvae;

Palaemonetes pugio; Reproduction; Sublethal Effects

1. Introduction

Exposure to contaminants for an entire life cycle can provide valuable insight into chronic effects at the individual and population level (Daniels and Allan, 1981; Pesch et al., 1991; Manyin and Rowe, 2006). For species with complex life cycles, it is important to quantify effects of contaminants on all life stages, because sensitivity to toxicants may vary substantially among different life stages (Munns et al., 1997; McGee et al., 1998; Salice and Roesijadi, 2002). The integration of effects on each life stage may reveal significant effects on population dynamics, even at sublethal concentrations (Levin et al., 1996). Such effects cannot be assessed using traditional acute toxicity exposures.

Copper is a widespread contaminant in coastal and estuarine ecosystems (e.g., Wright, 1986; Hall et al., 1998; Munari and Mistri, 2007). Sources of copper to aquatic habitats include application of antifouling paint and algicides, runoff of agricultural fertilizers, mine drainage, wastewater from metal smelting, and atmospheric fallout from fossil fuel combustion and refuse incineration (WHO, 1998). Bioavailability of copper is largely dependent upon the concentration of the free divalent cation, Cu^{2+} (Zamuda and Sunda, 1982), but bioaccumulation of copper can also be influenced by salinity (Wright and Zamuda, 1987; Bidwell and Gorrie, 2006). Copper is an essential trace element, functioning as a cofactor for enzymes involved in oxidation/reduction reactions (Stohs and Bagchi, 1995; WHO, 1998). Copper is also a component of hemocyanin, the molecule responsible for oxygen transport in the hemolymph of arthropods. However, copper can have toxic effects at concentrations above those that are essential (e.g., Burton and Fisher, 1990).

Mechanisms of copper toxicity include binding to proteins and DNA at sulfhydryl, carboxylate, and imidazole sites and catalyzing the production of reactive oxygen species (Stohs and Bagchi, 1995; WHO, 1998). Therefore, copper can result directly in impairment of protein function, peroxidation of lipids, damage to DNA and organelles, and depletion of ATP (Stohs and Bagchi, 1995; WHO, 1998). Toxic effects of copper can be expressed as reduced survival and delayed development in aquatic invertebrates (e.g., Wong et al., 1995). Copper may also inhibit reproduction in aquatic invertebrates by delaying sexual maturation (e.g., Koivisto and Ketola, 1995), reducing fertilization success (e.g., Reichelt-Brushett and Harrison, 2005), decreasing fecundity (e.g., Garnacho et al., 2001), and reducing the proportion of reproductive females in a population (e.g., Eriksson and Weeks, 1994).

The grass shrimp, *Palaemonetes pugio*, is abundant in estuaries and coastal habitats along the Atlantic and Gulf coasts of the United States (Gosner, 1971; Anderson, 1985), where it is an important prey item for many fish species (Nixon and Oviatt, 1973; Clark et al., 2003). The grass shrimp's life cycle consists of four life stages: embryo, larva, juvenile (postlarva), and adult. Fertilized embryos are carried by females in an external brood pouch until they hatch and larvae are released. Larvae pass through 3 to 11 sub-stages (Anderson, 1985) before metamorphosis to the juvenile stage, which is morphologically similar to the adult stage. Upon reaching sexual maturity, the male transfers a spermatophore to the female, where it is retained until oviposition (Anderson, 1985). Within seven hours of copulation, eggs are extruded by the female and fertilized externally as sperm are released from the spermatophore; after fertilization, the embryos are manipulated toward the brood pouch (Anderson, 1985). The entire life cycle can be completed in as little as two and a half months in natural habitats (Anderson, 1985) or four months in the laboratory (Manyin and Rowe, 2008), although rates of growth and development may vary considerably due to seasonal variations in temperature and food supply (Wood, 1967).

To determine the effects of aqueous copper on each life stage of *P. pugio*, we conducted a full life cycle exposure. The exposure was initiated with freshly hatched larvae, which were allowed to attain maturation and reproduce over a period of eight months. Effects of copper on the survival and duration of each life stage were measured, as well as the effects on reproduction. Due to the substantial effects observed on reproduction during the life cycle exposure, we conducted two subsequent experiments to examine reproductive effects in relation to the timing of exposure. In the "reciprocal cross" experiment, adults from the life cycle exposure were transferred from control conditions to copper exposure conditions and vice versa. The purpose of the reciprocal cross experiment was to determine the acute reproductive effects of exposure to copper prior to oviposition and to test for the potential recovery of reproductive performance following cessation of exposure to copper. We also conducted a post-spawning exposure to determine the effect of copper exposure on the viability of embryos that had been spawned under control conditions.

2. Materials and Methods

2.1. General exposure conditions

All exposures were conducted at a water temperature of 25 °C, photoperiod of 16:8 hours light to dark, and salinity of 10 ppt using Instant Ocean (Aquarium Systems, Mentor, OH) sea salts mixed with tap water that had been filtered by reverse osmosis (RO). The pH was adjusted to 7.8 or 7.9 with 1 M NaOH. To compensate for water lost due to evaporation, RO water was added daily to maintain water levels. A complete water change was conducted every 4 days for larval exposures and every 7 or 8 days for juvenile and adult exposures. Survival was measured during water changes. During water changes, test organisms were transferred to temporary holding chambers containing exposure water. All life stages were fed brine shrimp (*Artemia*) nauplii daily; the diet for juveniles and adults was supplemented with coarsely ground dry food (1:1 mixture of Wardley [Secaucus, NJ] Shrimp Pellets and Wardley Pond Ten Floating Fish Stix).

The concentration of free copper ions (Cu^{2+}) was buffered by adding nitrilotriacetic acid (NTA), a metal chelator, at 5×10^{-5} M (Perrin and Dempsey, 1974). Copper was added as CuCl_2 to achieve target free copper ion concentrations; the total metal concentration required was calculated using the chemical equilibrium program MINEQL+ v. 4.5 (Environmental Research Software, Hallowell, ME), given the target Cu^{2+} concentration, composition of Instant Ocean (Atkinson and Bingman, 1998), pH, and concentration of NTA. Control seawater contained no added CuCl_2 .

2.2. Life cycle exposure

Palaemonetes pugio larvae were exposed to copper at nominal free divalent cation concentrations of 9 and 26 $\mu\text{g Cu}^{2+}/\text{L}$. Water samples were taken after each water change, filtered through a 0.22 μm nitrocellulose membrane, and acidified to $\text{pH} < 2$ with HNO_3 . Samples were diluted 1:10 to reduce saltwater interference and analyzed for total dissolved copper using an Agilent (Santa Clara, CA, formerly Hewlett-Packard) HP4500 inductively coupled plasma mass spectrometer (ICP-MS). Due to variations in the ICP-MS measurements, the copper measurements were repeated using a PerkinElmer (Waltham, MA) AAnalyst 800 atomic absorption spectrometer (AA). On average, the measured total copper concentrations from both the ICP-MS and AA data sets were within ~5% of nominal concentrations. However, due to the addition of NTA to the exposure water, the total copper concentrations were very

1 similar for the two treatments (3200 and 3270 $\mu\text{g Cu/L}$) and as a result of variation in samples
2 and limits of analytical resolution, concentrations in the two treatments could not be reliably
3 distinguished using either ICP-MS or AA. Therefore, only the nominal free ion concentrations
4 are reported. The pH was ~ 7.8 throughout the life cycle exposure.

5 Larval *P. pugio* were derived from laboratory-bred stocks of wild-caught adults collected
6 from the Patuxent River, MD, USA. Newly hatched larvae (0- or 1-day post-hatch) were placed
7 in 1.5-L beakers, filled with 1 L of exposure water. Each treatment was replicated four times,
8 and each replicate began with 110 larvae. Sets of replicates, consisting of one replicate of each
9 treatment, were staggered in time, no more than two days apart. During larval and juvenile
10 stages, containers were arranged in a randomized complete block design; each replicate set was
11 blocked temporally, physically (by shelf height), and maternally (i.e., each set was initiated with
12 a subsample of larvae from a given group of females). Beyond Day 60 (due to the increased size
13 of tanks), the physical arrangement of tanks was altered to a randomized incomplete block
14 design, in which the number of blocks was increased and each block contained only a fraction of
15 the treatments (Anderson and McLean, 1974).

16 Between Day 11 and 46, individuals that had metamorphosed to the juvenile stage were
17 removed daily and transferred to another set of beakers until the juvenile exposure began. To
18 reduce variation in age, only the middle 80% of individuals to metamorphose were retained for
19 the juvenile exposure, discarding the initial 10% and final 10% to undergo metamorphosis. The
20 juvenile exposure was initiated on Day 28 by transferring 55 individuals per replicate to 8-L
21 tanks containing 6 L of exposure water. On Day 60, juveniles were transferred to 38-L tanks
22 filled with 17 L of exposure water.

23 Shrimp began to produce clutches on Day 75. Gravid females were removed daily to
24 individual 0.95-L jars filled with 0.6 L of exposure water. The females' chelae were trimmed to
25 prevent cannibalism of eggs (Little, 1968). While isolated, the females were fed *Artemia* only.
26 The water was changed after eight days and females were placed in a 3 mm-mesh cage,
27 suspended at the top of the jar. After the females' eggs hatched, larvae were counted and the
28 females were returned to their original tanks. If a female did not retain its eggs until hatching, it
29 was returned to its tank when it was no longer carrying eggs. The exposure continued for a total
30 of 240 days.

At the end of the exposure, several non-gravid adults (7-10 per tank) were allowed to depurate in clean seawater for three hours and preserved at -80 °C for subsequent analysis of total copper concentration. Tissue samples were freeze-dried, crushed, and homogenized. A subsample (~100 mg) was digested with 2 mL concentrated HNO₃ at 60 °C overnight. The digested sample was diluted and analyzed for copper by ICP-MS.

2.3. *Reciprocal cross exposure*

This experiment utilized adults from the life cycle exposure's control and 26 µg Cu²⁺/L treatment. After the life cycle exposure ended, half of the surviving adults were retained in their original exposure conditions and half were transferred to the alternate treatment, resulting in four possible combinations of primary and secondary exposure conditions. No gravid females were transferred to the alternate treatment. There were four replicates per treatment combination, arranged in a randomized complete block design, blocked by shelf height.

The exposure was conducted in 38-L tanks containing 10 L of exposure water, and each tank contained 13-16 adults. The pH was 7.8. As females became gravid, they were removed to individual containers, as described above, to obtain counts of hatched larvae. The exposure lasted for 21 days; any females gravid at this time were held until their eggs hatched or until they were no longer carrying eggs.

2.4. *Post-spawning exposure*

In this experiment, embryos that had been spawned under control conditions were exposed to copper. A mixture of wild-caught and laboratory-bred adults (84 total) was bred in control seawater. Gravid females were removed daily and each female was placed in an individual jar, containing either control seawater or copper-spiked seawater (26 µg Cu²⁺/L) at a pH of 7.9. Larvae were counted upon hatching. Because clutch size has been found to be correlated with size of the spawning female (Wood, 1967), the carapace length of the female was measured from the eye socket to the rear of the carapace. The experiment lasted 25 days, during which time nine shrimp were used in each treatment.

2.5. *Statistics*

The distribution of larval stage duration values, measured as the length of time from hatching until metamorphosis to the juvenile stage, was highly skewed to the right. To determine the average duration of the larval stage, we used failure time analysis ("distribution

analysis” for right-censored data in Minitab v. 13.31 statistical software [State College, PA]). The data fit a loglogistic distribution using maximum likelihood estimation (Anderson-Darling statistic = 14.81). Applying this distribution, the time at which 50% of the larvae had metamorphosed was calculated for each replicate to estimate the duration of the larval stage.

Because *P. pugio* exhibits no apparent morphological difference upon transition from the juvenile to adult stage, the presence of gravid females was used as an indicator of this transition. The duration of the juvenile stage was estimated by the length of time from metamorphosis until 5% of the females in a replicate were gravid, at which time all individuals in the replicate were assumed to be reproductively mature adults.

The General Linear Model (GLM) routine in Minitab was used to test for differences among treatments. Experimental block was included as a factor in statistical tests for the life cycle exposure and reciprocal cross exposure. Data were tested for normality and homogeneity of variance and were transformed as necessary. Pairwise comparisons were performed using the Tukey method. P-values resulting from pairwise comparisons are reported in the results. An *a priori* Type I error rate (α) of 0.05 was used to assess significance of all statistical tests.

To test for differences in larval production in the reciprocal cross exposure, a GLM was employed using the following factors: primary exposure conditions, secondary exposure conditions, the interaction between primary and secondary exposure conditions, and experimental block. For the post-spawning exposure, carapace length was included as a covariate in the GLM used to test for effects on larval production because the shrimp used in this exposure were more variable in size.

3. Results

3.1. Life cycle exposure

During the full life cycle exposure, there were no significant effects of copper on survival of larval, juvenile, and adult life stages (Fig. 1, $P \geq 0.76$). Survival averaged > 95% for each life stage. At the end of the eight-month exposure, the number of surviving shrimp was similar for all treatments.

Exposure to copper significantly increased the length of the larval stage ($P < 0.01$ and $P < 0.001$ for 9 and 26 $\mu\text{g Cu}^{2+}/\text{L}$, respectively). Average duration of the larval stage increased by two days when shrimp were exposed to 9 $\mu\text{g Cu}^{2+}/\text{L}$ and by three days when exposed to

26 $\mu\text{g Cu}^{2+}/\text{L}$, compared to control individuals (Fig. 2). However, the duration of the juvenile stage was not significantly affected by copper exposure (Fig. 2, $P \geq 0.67$).

Shrimp raised under control conditions produced $48 (\pm 5)$ larvae per clutch, with an average of 39 clutches spawned per control tank during the final five months of the exposure period. Although many shrimp in the copper treatments became gravid during the course of the life cycle experiment (producing ~ 27 clutches per replicate at $9 \mu\text{g Cu}^{2+}/\text{L}$ and ~ 20 clutches per replicate at $26 \mu\text{g Cu}^{2+}/\text{L}$), no larvae were produced from any of the clutches. Females often dropped all of their eggs from their brood pouch within two days after spawning. Hence, larval production was prevented by exposure to copper at either 9 or $26 \mu\text{g Cu}^{2+}/\text{L}$.

At the end of the life cycle exposure, copper concentrations in adult shrimp tissue were significantly greater in shrimp exposed to copper ($P < 0.01$ and $P = 0.05$ for 9 and $26 \mu\text{g Cu}^{2+}/\text{L}$, respectively), but did not differ between the two copper concentrations (Table 1).

3.2. Reciprocal cross exposure

Shrimp that had been raised under control conditions and then transferred to copper-spiked seawater ($26 \mu\text{g Cu}^{2+}/\text{L}$) were able to spawn, but no embryos hatched (Table 2). The first shrimp spawned three days after transfer from control to copper conditions. Secondary exposure of shrimp to copper prevented production of larvae, regardless of the primary exposure conditions. Females exposed to copper usually dropped all of their eggs within three days after spawning.

Shrimp that had been exposed to copper ($26 \mu\text{g Cu}^{2+}/\text{L}$) during the full life cycle exposure successfully produced larvae when transferred to control seawater (Table 2). The first females to produce viable embryos had been in control conditions for only two days. (No embryos were produced during the first day of this experiment.) Although shrimp transferred from copper to control conditions succeeded in producing larvae, they produced 43% fewer larvae per clutch, on average, than shrimp that had been exposed to control conditions continuously (Table 2, $P = 0.04$). The duration of the embryonic stage averaged 13 days, regardless of exposure conditions.

During the exposure, at least six females became gravid in reciprocal treatment combinations (treatments in which the primary and secondary exposure conditions differed). Only two females became gravid in the copper “positive control”, in which shrimp had been

exposed to copper during both the life cycle exposure and the reciprocal cross exposure. No adult mortality occurred during the reciprocal cross exposure.

3.3. *Post-spawning effects*

When gravid shrimp that had spawned in control seawater were transferred to copper-spiked seawater ($26 \mu\text{g Cu}^{2+}/\text{L}$), 7 out of 9 clutches successfully produced larvae. On average, gravid shrimp transferred from control to copper conditions produced about 26% as many larvae as shrimp maintained under control conditions (Fig. 3, $P < 0.01$). The duration of the embryonic stage averaged 13 days, regardless of exposure conditions.

4. Discussion

During the life cycle exposure, females exposed to copper could readily produce eggs, but the eggs were not viable at free copper ion concentrations as low as $9 \mu\text{g Cu}^{2+}/\text{L}$. In the absence of organic ligands, the free ion test concentrations of 9 and $26 \mu\text{g Cu}^{2+}/\text{L}$ would correspond to total copper concentrations of 33 and $95 \mu\text{g Cu}_{\text{Total}}/\text{L}$, respectively, at the experimental pH and salinity. Similar copper concentrations, as high as $72 \mu\text{g Cu}_{\text{Total}}/\text{L}$, have been observed in the tributaries of the Chesapeake Bay, USA (Hall et al., 1998), a region where *P. pugio* are abundant. Although lethal effects might not be evident at these field concentrations, disruption of the shrimp's life cycle would be expected to occur due to the absence of successful larval recruitment. Areas with consistently high copper concentrations could therefore act as habitat sinks for grass shrimp.

The acute effects of copper exposure (prior to oviposition) on larval production appeared to be identical to those observed in the life cycle exposure. Adults exposed to copper during the reciprocal cross exposure were unable to produce viable eggs, regardless of prior exposure conditions. Because eggs that were spawned in control conditions and then transferred to the copper exposure regime were sometimes able to hatch successfully in the post-spawning exposure, inhibitory effects of copper on reproduction were not solely due to reduced hatching success. Therefore, a secondary effect of copper on reproduction appears to occur either before or during spawning and fertilization. While effects of copper on the process of fertilization would be difficult to observe directly in grass shrimp, the combination of experiments employed in this study have yielded results that indirectly demonstrate an effect of copper on spawning and/or fertilization.

Effects of copper exposure on fertilization in aquatic invertebrates *in vitro* have been well-documented in species with gametes that are easily isolated (in contrast to *P. pugio*). Copper exposure has been observed to reduce fertilization success in several coral species (Reichelt-Brushett and Harrison, 1999; Reichelt-Brushett and Harrison, 2005; Reichelt-Brushett and Michalek-Wagner, 2005), oysters (*Isognomon californicum* [Ringwood, 1992]), and sea urchins (*Echinometra mathaei* [Ringwood, 1992]) at copper concentrations ranging from 10 to 50 $\mu\text{g}_{\text{Total}}/\text{L}$. In addition, reduced sperm motility has been observed in mussels (*Mytilus edulis*) exposed to 6 mg $\text{Cu}_{\text{Total}}/\text{L}$ (Earnshaw et al., 1986). Therefore, it is likely that a decrease in fertilization success contributed to the prevention of larval production observed in pre-spawning exposures of *P. pugio* to copper. The effect of copper on larval production of *P. pugio* appeared to be more extreme than that observed on fertilization success in other aquatic invertebrates. In contrast to the 0% egg viability observed in *P. pugio*, fertilization success rates as high as ~ 75-80% have been reported in corals at 69 $\mu\text{g}_{\text{Total}}/\text{L}$ (Reichelt-Brushett and Michalek-Wagner, 2005) and in oysters at ~ 48 $\mu\text{g}_{\text{Total}}/\text{L}$ (Ringwood, 1992); these total copper concentrations correspond approximately to the range of test concentrations employed in the current experiment. Furthermore, the complete inhibition of larval production in the absence of lethal effects on any life stage of *P. pugio* (other than embryos) indicates that fertilization and/or hatching success are profoundly more sensitive to copper than would be indicated by survival rates of larvae, juveniles, and adults. This underscores the need for reproductive measures in the determination of aquatic toxicity of contaminants.

In the post-spawning exposure, eggs that had been spawned in control seawater were sometimes viable in seawater containing copper, but larval production was significantly reduced, indicating that copper exposure reduces the hatching success of *P. pugio* embryos. Exposure to a much higher concentration of copper (3 mg $\text{Cu}_{\text{Total}}/\text{L}$) was reported to reduce hatching success of *P. pugio* embryos *in vitro* (Rayburn and Fisher, 1999). Furthermore, copper-induced decreases in hatching success rates have been observed in other crustaceans such as crabs (*Callinectes sapidus* [Lee et al., 1996]) and mysid shrimp (*Praunus flexuosus* [Garnacho et al., 2001]). Although there was a decrease in the number of larvae per brood in the post-spawning exposure to copper, a moderate percentage of embryos nonetheless hatched successfully, indicating that the lack of larval production observed in the life cycle exposure was not solely due to effects of copper on hatching success.

In the reciprocal cross exposure, females that had been exposed to copper for eight months were able to produce larvae successfully when transferred to control seawater prior to oviposition, demonstrating that the effect on larval production was reversible. This suggests that shrimp populations experiencing periodic exposures to high copper concentrations (e.g., during stormwater events) would be able to recover the ability to produce viable eggs quickly. However, chronic exposure of shrimp to copper was found to significantly reduce the number of larvae produced after transfer to control conditions, which may be due to a carryover of bioenergetic effects on the parental generation. In subchronic exposures of *P. pugio* to copper, bioenergetic measurements revealed a decrease in energy allocated toward production pathways (Manyin and Rowe, 2009). The decrease in larval output observed in the current study may partially reflect a decrease in the energy available for production. Exposure to copper has also been found to reduce the amount of energy allocated to production pathways in other aquatic invertebrates, including mussels (e.g., *Perna viridis* [Sze and Lee, 2000]), clams (e.g., *Tapes philippinarum* [Munari and Mistri, 2007]), cladocera (e.g., *Daphnia pulex* [Winner and Farrell, 1976]), amphipods (e.g., *Gammarus pulex* [Maund et al., 1992]), and shrimp (e.g., *Farfantepenaeus paulensis* [Santos et al., 2000]). The exposure of *P. pugio* to copper for a full life cycle thus may have resulted in cumulative bioenergetic effects, reducing energy allocation to reproduction even after transfer to control conditions and resulting in a decrease in larval production.

Exposure to copper during the life cycle exposure was found to delay the development of larvae to the juvenile stage by 2 to 3 days. Although this amount of time appears to be trivial relative to the length of the shrimp's life cycle, it results in a 12-18% increase in the duration of the larval stage, which may be the most vulnerable to predation due to its planktonic nature (Anderson, 1985). Additional examples of copper-induced delayed development in aquatic invertebrates include delayed embryonic development in *P. pugio* (Rayburn and Fisher, 1999), slower larval development in *Metapenaeus ensis* (shrimp; Wong et al., 1995) and *Watersipora subtorquata* (bryozoan; Ng and Keough, 2003), and delayed sexual maturation in *Tigriopus japonicus* (copepod; D'Agostino and Finney, 1974). Delays in metamorphosis may result in a greater impact of predators on population growth in areas where copper concentrations are elevated.

5. Conclusion

Exposure to copper at free ion concentrations of 9 or 26 $\mu\text{g Cu}^{2+}/\text{L}$ resulted in failure to complete the life cycle of *P. pugio*, given that no larvae were produced. Although females were able to spawn in copper exposures, they were unable to produce viable embryos. Adults that had been exposed to copper for a full life cycle were able to produce viable embryos after being transferred to control conditions, but the number of larvae per clutch was reduced in comparison to reference values, possibly due to bioenergetic effects on the parental generation that reduced allocation of energy to reproduction. Gravid shrimp exposed to copper after spawning in control conditions were able to produce a limited number of larvae per brood, which likely indicates reduced hatching success of embryos. The overall effect of copper exposure on larval production may be due to a combination of effects on hatching success, parental bioenergetics, and processes occurring before or during spawning and/or fertilization. Given that reproduction by *P. pugio* is severely constrained at copper concentrations that are otherwise sublethal, severe effects on population dynamics may occur in conditions that likely would not be deemed deleterious on the basis of short-term, lethality-based studies.

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Table 1. Copper concentrations (mean $\pm$ SE) in adult shrimp tissue ($\mu\text{g/g}$ dry mass) at the end of the full life cycle exposure. Values with different letters are significantly different ($P \leq 0.05$).

Nominal Treatment	[Cu] in Tissue
Control	95.8 ^a $\pm$ 12.6
9 $\mu\text{g Cu}^{2+}/\text{L}$	165.4 ^b $\pm$ 13.9
26 $\mu\text{g Cu}^{2+}/\text{L}$	140.5 ^b $\pm$ 9.6

Table 2. Number of larvae (mean $\pm$ SE) hatched per clutch in the reciprocal cross exposure. Adults were exposed to primary conditions for eight months and exposed to secondary conditions for up to 21 days. Values with different letters are significantly different ($P \leq 0.05$).

		Primary Exposure Conditions	
		Control	26 $\mu\text{g Cu}^{2+}/\text{L}$
Secondary Exposure Conditions	Control	95 ^a $\pm$ 15	54 ^b $\pm$ 1
	26 $\mu\text{g Cu}^{2+}/\text{L}$	0 ^c $\pm$ 0	0 ^c $\pm$ 0

Figure Captions

Fig. 1. Survival (mean $\pm$ SE) of each life stage of *P. pugio* in the full life cycle exposure to copper. Larval and juvenile survival was measured over 4-day intervals; adult survival was measured over 8-day intervals.

Fig. 2. Effects of copper exposure on duration (mean $\pm$ SE) of larval and juvenile stages. Bars labeled with different letters are significantly different ($P \leq 0.05$).

Fig. 3. Number of larvae (mean $\pm$ SE) hatched per clutch in post-spawning exposures. Shrimp that spawned in control seawater were either retained in control seawater or exposed to copper ($26 \mu\text{g Cu}^{2+}/\text{L}$) until their embryos hatched. Different letters indicate a significant difference ($P \leq 0.05$).

Figure 1

Figure 2

Figure 3