ABSTRACT

Title of Dissertation:	BIOENERGETIC, REPRODUCTIVE, AND POPULATION-LEVEL EFFECTS OF DISSOLVED COPPER AND CADMIUM ON THE GRASS SHRIMP, *PALAEMONETES PUGIO*
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The grass shrimp, *Palaemonetes pugio*, was exposed to dissolved copper or cadmium in a series of laboratory experiments to determine effects on bioenergetics, reproduction, and population growth. In 14-day exposures, adults were exposed to either copper or cadmium to quantify bioenergetic effects. Both metals caused a decline in oxygen consumption (lowest observed effects concentrations [LOECs] = 7.5 μg Cu^{2+}/L and 6.6 μg Cd^{2+}/L) and growth rate (LOECs = 27 μg Cu^{2+}/L and 6.2 μg Cd^{2+}/L). Effects of copper on growth were more severe than those of cadmium, resulting in weight loss during the exposure. Reductions in oxygen consumption and growth, in combination with declines in reproduction observed in longer exposures, suggest that both copper and cadmium reduce energy allocation to respiration and production pathways.

In eight-month exposures, *P. pugio* were exposed to either copper or cadmium for a full life cycle, allowing larvae to attain maturation and reproduce. While survival was

little affected by exposure to cadmium, brood size and the percentage of ovigerous females were significantly reduced (LOECs = 1.5 and 2.5 µg Cd^{2+}/L, respectively). Population growth of *P. pugio* exposed to cadmium was projected using a stage-based matrix model and a z-transformed life cycle graph analysis. Both models projected a decrease in population growth rate (LOEC = 1.5 µg Cd^{2+}/L), although population growth remained positive. Decomposition analysis indicated that cadmium-induced declines in population growth could be attributed mainly to contributions from reproductive effects. In the eight-month exposure to copper, no lethal effects on larvae, juveniles, or adults were observed, but larval development was significantly delayed (LOEC = 9 µg Cu^{2+}/L). Upon reaching maturation, females exposed to copper were able to produce embryos, but the embryos did not hatch, precluding completion of the life cycle (LOEC = 9 µg Cu^{2+}/L). The results from subsequent experiments, which further examined reproductive effects, suggested that copper may inhibit larval recruitment via a combination of effects on hatching success, parental bioenergetics, and processes before or during spawning and/or fertilization. In conclusion, both copper and cadmium may have negative impacts on the sustainability of natural populations of *P. pugio* in contaminated habitats.

BIOENERGETIC, REPRODUCTIVE, AND POPULATION-LEVEL EFFECTS OF

DISSOLVED COPPER AND CADMIUM ON THE GRASS SHRIMP,

PALAEMONETES PUGIO

by

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PREFACE

The goal of this research project was to investigate various ecotoxicological effects of aqueous copper and cadmium on *Palaemonetes pugio* (grass shrimp) using a series of laboratory experiments. I examined bioenergetic effects on respiration, growth, consumption, and energy storage in several two-week exposures of adult shrimp to either copper or cadmium. To examine life stage-specific effects of copper and cadmium on survival, development, and reproduction, I conducted eight-month exposures that spanned the entire life cycle of the grass shrimp. Based upon results from these exposures, I performed two additional experiments to further examine effects of copper on reproduction. Herein, I present the results from the entire research project in a series of manuscript-formatted chapters that either have been or will be submitted for publication in peer-reviewed journals, with my graduate advisor (Christopher Rowe) as a co-author. Chapter 3 has already been published with the following citation: "Manyin, T., Rowe, C.L., 2008. Modeling effects of cadmium on population growth of *Palaemonetes pugio*: results of a full life cycle exposure. Aquat. Toxicol. 88, 111-120." With the exception of editorial revisions suggested by my advisor and committee members, all contents of this dissertation were written by me, Teresa Manyin.

In Chapter 1, I provide an overview of the effects of aqueous copper and cadmium on bioenergetics, reproduction, and population growth of aquatic invertebrates. This chapter serves as an introduction to the research project, summarizing the literature that has been published on this topic. In Chapter 2, I present results from the two-week exposures, quantifying effects of copper and cadmium on respiration, growth,

consumption, and energy storage. To evaluate toxicological effects of copper and cadmium on the energetic budget of *P. pugio*, I converted respiration and growth rates to their energetic equivalents. In Chapter 3, I report the effects of cadmium observed during the full life cycle exposure, including effects on survival and duration of each life stage, as well as effects on reproductive output. I used these individual-level effects to project effects of cadmium on population growth of *P. pugio* by employing two mathematical models: a stage-based matrix model and a z-transformed life cycle graph analysis. I conducted perturbation analyses to decompose population-level effects of cadmium into individual-level contributions and to determine the sensitivity of population growth to changes in individual-level parameters. In addition, I projected the ability of a cadmium-exposed population to withstand predation pressure. In Chapter 4, I present the effects of copper observed during the full life cycle exposure, including effects on survival and duration of each life stage, as well as reproduction. Because exposure to copper prevented larval production, and therefore prevented completion of the life cycle, it would not have been useful to model the effects of copper on population growth at the experimental concentrations. Instead, I further examined effects on reproduction by varying the timing of exposure relative to oviposition.

In summary, this dissertation expands the toxicological knowledge of effects of copper and cadmium. Chronic, individual-level effects from laboratory experiments were used to project effects at the population level. The impact of bioenergetic effects on population dynamics is a recurring theme, providing an ecological perspective on the toxicological effects of copper and cadmium.

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assistance and late-night dinners at the lab, but most importantly, always helping me to

maintain my perspective on life through it all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1:

AN OVERVIEW OF THE EFFECTS OF AQUEOUS COPPER AND CADMIUM ON BIOENERGETICS, REPRODUCTION, AND POPULATION GROWTH OF AQUATIC INVERTEBRATES

Introduction

Exposure to toxicants may result in wide-ranging bioenergetic effects, potentially impacting multiple aspects of an individual's energetic budget. A basic bioenergetic budget for an animal may be expressed by the equation, $C = R + P + E$, where C is consumption, R is respiration, P is production, and E is excretion and egestion. The total amount of energy available to an animal to support physiological processes is dependent upon the portion of energy that is assimilated from food. Assimilated energy is equal to the energy consumed minus energy lost due to excretion and egestion. An organism's ability to assimilate energy from food is determined by its assimilation efficiency, which is calculated as the amount of energy assimilated divided by the total energy consumed. Toxicants may alter the extent of an individual's bioenergetic budget by affecting its rates of consumption, excretion, and/or egestion, as well as its assimilation efficiency.

Physiological stress may be induced by exposure to toxicants, often manifesting as bioenergetic effects in which allocation of assimilated energy to the competing pathways of respiration and production are altered (Calow, 1991). Respiratory expenditures include maintenance (measured as standard metabolic rate), specific dynamic action (energy used for digestion and absorption of nutrients), and activity. Production pathways include somatic growth and energy storage, production of gametes

(i.e., reproductive investment), secretions (such as mucus), and eliminated tissues (such as shed exoskeletons).

Although many effects of toxicants on reproduction may be linked to reduced energetic investment in offspring by parents, toxicants may cause additional effects on reproduction that are not bioenergetic in nature. Frequently, it is difficult to distinguish between these two mechanisms of effects on reproduction. For example, a decrease in hatching success may be mediated by reduced energy allocation to egg formation or by direct effects of toxicants on survival of embryos. Regardless of the mechanism of effects, the end result of exposure is often a decline in growth and reproduction. When summed together, these effects on individuals may ultimately lead to a decline in population growth rate. This literature review summarizes the current body of knowledge regarding the effects of aqueous copper and cadmium on bioenergetics, reproduction, and population growth of aquatic invertebrates.

Concentrations of copper and cadmium are often elevated in aquatic habitats, especially estuaries and coastal regions, due to nearby human activities (Wright, 1986; Hall et al., 1998; Yang and Sañudo-Wilhelmy, 1998; Monbet, 2004; Munari and Mistri, 2007). Sources of both copper and cadmium to aquatic environments include mine drainage, wastewater discharge from metal smelting, runoff of agricultural fertilizers, and atmospheric fallout from fossil fuel combustion and refuse incineration; additionally, copper may be introduced to aquatic habitats through application of antifouling paint and algicides (WHO, 1992; WHO, 1998).

Copper

Essentiality and Mechanisms of Toxicity

Copper is an essential trace element in animals, functioning as a cofactor for many enzymes involved in oxidation/reduction reactions, such as oxidases, oxygenases, and superoxide dismutases (Stohs and Bagchi, 1995; WHO, 1998). As a component of hemocyanin, copper is also required for oxygen transport in the circulatory system of arthropods. Copper is actively accumulated; bioavailability of aqueous copper is largely dependent upon the concentration of the free divalent cation, Cu^{2+} (Zamuda and Sunda, 1982), but accumulation may also be affected by abiotic conditions such as salinity (Wright and Zamuda, 1987; Bidwell and Gorrie, 2006) and temperature (Mubiana and Blust, 2007). This review describes effects induced by the uptake of copper from solution only, although copper may also be accumulated via uptake from contaminated food (Weeks and Rainbow, 1993).

When present at concentrations exceeding those that are essential, copper may induce toxicological stress by binding to sulfhydryl, carboxylate, and imidazole sites on proteins and DNA, impairing protein function and resulting in errors in transcribed RNA (WHO, 1998). Copper can also catalyze the production of reactive oxygen species, resulting in lipid peroxidation, DNA and organelle damage, and ATP depletion (Stohs and Bagchi, 1995; WHO, 1998).

Effects on Bioenergetics, Reproduction, and Population Growth

Exposure to copper has been found to affect the rate of respiration (measured as oxygen consumption) in many aquatic invertebrates (Table 1.1). Most often, copper exposure leads to reduced respiration. Of the 18 species listed in Table 1.1, 14 displayed

a decrease in oxygen consumption and 3 exhibited an increase in response to copper. In one species, the crab *Potamonautes warreni*, the respiratory response was dependent upon the length of exposure; a shorter exposure (7 to 14 days) resulted in elevated respiration, but continued exposure ($\geq$ 21 days) subsequently reduced respiration (Vosloo et al., 2002). The authors proposed that the initial increase in respiration reflected an increased metabolic demand, while the subsequent decrease in respiration could be attributed to copper-induced damage to gill tissues (Vosloo et al., 2002).

It is therefore evident that copper may influence respiration rate via several mechanisms. Copper can inhibit several enzymes involved in metabolic pathways such as glycolysis (Strydom et al., 2006), potentially resulting in a decline in oxygen consumption. In addition, exposure to copper has been found to cause gill necrosis, as reported for *Carcinus maenas* (shore crab; Nonnotte et al., 1993) and *Penaeus japonicus* (Kuruma prawn; Soegianto et al., 1999a), which may result in direct inhibition of oxygen consumption. Furthermore, copper exposure can induce synthesis of metallothioneins or other metal-binding proteins (White and Rainbow, 1986; Moraga et al., 2005), which can sequester metals and protect against toxic effects, as well as heat shock proteins (Sanders et al., 1991; Moraga et al., 2005), which stabilize or repair damaged proteins. Induction of protein synthesis may result in elevated metabolic costs (Hawkins, 1991; Hawkins and Day, 1996) and consequently an increase in oxygen consumption. Therefore, copper has the potential to either reduce or elevate respiratory rates, depending upon the dominant mechanisms through which it acts. In the species surveyed, no taxonomic trends were evident in the respiratory responses to copper.

Exposure to copper has frequently been observed to reduce rates of growth in aquatic invertebrates, regardless of the metric employed (dry weight, wet weight, or body length; Table 1.2). Copper has also been found to reduce scope for growth, calculated as the amount of energy assimilated minus energy allocated to respiration (Table 1.2). Development from one life stage to the next is often delayed by copper exposure; copper has been found to slow development of embryos, larvae, and juveniles of several species (Table 1.2). However, Greco et al. (2001) reported faster development in *Tunicotheres moseri* (pea crab) postlarvae exposed to copper. Given that exposure to copper has been observed to reduce rates of growth and development in most of the studies reviewed, the majority of evidence suggests that energy allocated to production declines in response to copper.

Reproduction is also often negatively impacted by copper exposure. Effects of copper on reproduction, which may be driven by bioenergetic mechanisms, include delayed maturation and declines in brood size as well as the number of young produced by a female over her lifetime (Table 1.3). The observed declines in offspring production suggest that copper exposure decreases the energy available for reproductive investment. Additional effects of copper on reproductive measures, which may or may not be linked to bioenergetic effects, include reduced fertilization success, increased abortion rates, and reduced embryo hatching success (Table 1.3). Taken as a whole, the effects of copper on reproduction may have pronounced effects on the sustainability of populations in copper-polluted habitats.

Rates of food consumption have been observed to decrease in response to copper exposure in several species of aquatic invertebrates (Table 1.4), reducing the total amount

of energy available to the individual. Assuming that assimilation efficiency remains constant, a decline in consumption will result in a decrease in the amount of energy assimilated and thus available to support respiration and production. Copper-induced reductions in food consumption may be compounded by a decrease in assimilation efficiency (Table 1.5), further reducing the amount of energy assimilated. Copper may also affect rates of excretion and elimination (Table 1.5), influencing the rate of assimilation. Exposure to copper may cause excretion to increase (e.g., in the green mussel, *Perna viridis* [Krishnakumar et al., 1990]) or decrease (e.g., in the crab, *P. warreni* [Vosloo et al., 2002]). Copper has been observed to reduce the rate of elimination in *P. viridis*, although organic content of feces was elevated (Sze and Lee, 2000). The overall impact of excretion and/or elimination effects on the energy budget cannot be predicted without additional information on consumption and energetic expenditures for a particular species.

Other bioenergetic effects of copper include reductions in activity, clearance rate, and molting rate (Table 1.5), further suggesting decreases in respiration and production. The majority of bioenergetic evidence, including reductions in respiration, consumption, growth, and reproduction, suggests that exposure to copper results in an overall metabolic depression, as illustrated in *P. viridis* (Sze and Lee, 2000). Copper-induced declines in growth and reproduction at the level of the individual may be expressed at the population level as a decrease in population growth, as has been observed in several species (Table 1.6). Therefore, copper pollution in natural habitats may potentially result in a decline in populations of aquatic invertebrates.

Cadmium

Mechanisms of Toxicity

Cadmium is not an essential element, but may be accumulated via pathways for calcium uptake (Wright, 1995). Bioaccumulation of cadmium from solution is correlated with the free divalent cation concentration, Cd^{2+} (Sunda et al., 1978), but may also be influenced by abiotic factors such as salinity (De Lisle and Roberts, 1988), temperature (Mubiana and Blust, 2007), and calcium concentration (Wright and Frain, 1981; De Lisle and Roberts, 1994). As with copper, cadmium may also be accumulated via uptake from contaminated food (Khalil et al., 1995); however, this review describes only effects induced by the uptake of cadmium from solution.

Cadmium may cause toxic effects by binding to sulfhydryl, sulfate, and carbonyl sites on proteins and DNA (Furst et al., 1998), inhibiting their function. Cadmium may also interfere with calcium uptake and calcium channels via competition for binding sites (Furst et al., 1998; Strydom et al., 2006). In addition, cadmium may cause lipid peroxidation (Stohs and Bagchi, 1995) and inhibit DNA repair (Furst et al., 1998).

Effects on Bioenergetics, Reproduction, and Population Growth

Many researchers have observed a significant effect of cadmium exposure on respiration in aquatic invertebrates (Table 1.7). Exposure to cadmium often results in a decrease in respiration, although an increase in respiration sometimes occurs. Of the 22 species included in Table 1.7, 16 exhibited a decline in oxygen consumption in response to cadmium and 2 displayed an increase. In the remaining 4 species, the effect of cadmium on oxygen consumption varied, depending upon exposure conditions. Taxonomic trends were not evident in the effects of cadmium on respiration.

Conditions that may influence an individual's respiratory response to cadmium include duration of exposure, cadmium concentration, and/or salinity (Table 1.7). In *Daphnia magna*, De Coen and Janssen (2003) reported an increase in oxygen consumption at a low cadmium concentration (4 µg/L), and a decrease in oxygen consumption, relative to controls, at higher cadmium concentrations ($\geq$ 14 µg/L), but an explanation was not provided for these results. In the snail *Murex trunculus*, reduced oxygen consumption was observed in a short-term (60 h) exposure to a high concentration of cadmium (5000 µg/L), but oxygen consumption was elevated in a longer (34 d) exposure to a low concentration of cadmium (50 µg/L; Dalla Via et al., 1989). The authors proposed that the decrease in oxygen consumption was due to severe metabolic disturbances that impeded respiration at high cadmium concentrations, and that the increase in oxygen consumption at low cadmium concentrations reflected the induction of detoxification mechanisms, such as metallothionein synthesis (Dalla Via et al., 1989). The field crab, *Barytelphusa guerini*, exhibited an increase in oxygen consumption in shorter (6-24 h) exposures, but a decline in oxygen consumption in longer (4-15 d) exposures; the initial increase in respiration was proposed to be the result of defense mechanisms against cadmium toxicity, while the subsequent decline in respiration was attributed to irreparable tissue injury (Reddy and Venugopal, 1993). Vernberg et al. (1977) observed a salinity-dependent response in the grass shrimp, *Palaemonetes pugio*; at a lower salinity (5 ppt), cadmium exposure resulted in a decrease in oxygen consumption, but at a higher salinity (15 ppt), cadmium caused an increase in respiration. The authors were unwilling to speculate as to the cause of the seemingly contradictory responses in respiration due to the high degree of variability among

individuals, perhaps due to variation in activity levels (Vernberg et al., 1977).
Conversely, Hutcheson et al. (1985) reported a decrease in oxygen consumption in
P. pugio at a salinity of 15 ppt, although the exposure was shorter and the cadmium
concentration was higher than those employed by Vernberg et al. (1977).

Effects of cadmium on respiration may be mediated by several mechanisms. For
example, cadmium has been found to inhibit several enzymes involved in metabolic
pathways, including glycolysis, the Krebs cycle, gluconeogenesis, and oxidative
phosphorylation (Furst et al., 1998; Strydom et al., 2006), which may cause a decrease in
metabolic rate. In addition, exposure to cadmium may result in gill necrosis, as reported
for the dogwhelk *Nucella lapillus* (Leung et al., 2000), and several species of shrimp,
including *Penaeus duorarum* and *Palaemonetes vulgaris* (Nimmo et al., 1977a) and
P. japonicus (Soegianoto et al., 1999b). Such damage to gills may directly inhibit
oxygen consumption. Furthermore, cadmium exposure has been observed to cause a
decrease in the number of mitochondria per unit cell volume in the swan mussel,
Anodonta cygnea (Hemelraad et al., 1990) and eastern oyster, *Crassostrea virginica*
(Cherkasov et al., 2006), potentially leading to a reduction in the capacity for aerobic
respiration. However, cadmium-induced synthesis of protective proteins may cause an
increase in respiration; similar to copper, exposure to cadmium has been found to induce
synthesis of heat shock proteins (Werner and Nagel, 1997; Brown et al., 1995) and
metallothioneins or similar metal-binding proteins (e.g., Howard and Hacker, 1990;
Moraga et al., 2005). Hence, specific effects of cadmium at cellular and subcellular
levels may impact oxygen consumption differently; the net effect on respiration is
expected to be a synthesis of such lower level effects.

Reduced growth rates have been observed in many aquatic invertebrates in response to cadmium exposure (Table 1.8). Effects of cadmium on dry weight (total, tissue, or shell), wet weight, body length, shell length and area, accumulation of proteins and lipovitellin, and scope for growth have been reported for several species (Table 1.8). Exposure to cadmium has also been found to delay development of larval and juvenile stages (Table 1.8). Overall, the effects on growth and development suggest that cadmium exposure results in a decline in energy allocated to production pathways.

Reproduction is also commonly inhibited by cadmium exposure. Reproductive effects that may be driven by bioenergetic mechanisms include reduced brood size, a decline in the percentage of adults producing offspring, reduced offspring size, a delay in the release of young, and a reduction in the total number of young per female (Table 1.9). These effects suggest that exposure to cadmium reduces the energy allocated to reproductive investment in offspring. Less often, cadmium exposure has been observed to result in positive effects on reproductive measures (Table 1.9); examples include an increase in fecundity in the snail, *Biomphalaria glabrata* (Salice and Miller, 2003), and cladoceran, *D. magna* (Bodar et al., 1988b; Guan and Wang, 2006), an increase in the size of offspring in the sea urchin, *Anthocidaris crassispina* (Au et al., 2000), and accelerated maturation in the brine shrimp, *Artemia parthenogenetica* (Sarabia et al., 2003). Nonetheless, the majority of evidence suggests that cadmium exposure typically decreases the amount of energy allocated toward reproduction. Additional effects of cadmium on reproductive measures, which may or may not be linked to bioenergetic effects, include reduced hatching success, decreased sperm motility, and a decline in

fertilization success (Table 1.9). In sum, exposure to cadmium may have many negative

impacts on reproduction, although positive effects have occasionally been observed.

Effects of cadmium exposure on ingestion rates are usually negative (Table 1.10),

reducing the input to an animal's energy budget. However, Brown and Pascoe (1989)

observed an increase in consumption by the amphipod *Gammarus pulex* at a low

cadmium concentration (2.1 µg/L), whereas consumption decreased at a higher cadmium

concentration (6.0 µg/L). Researchers have also reported declines in the amount of

energy assimilated in response to cadmium exposure (Table 1.11). Assimilation

efficiency may either increase or decrease in cadmium exposures (Table 1.11). An

increase in assimilation efficiency, such as that observed in *D. magna*, might allow

organisms to compensate for a reduction in consumption (Guan and Wang, 2006).

Conversely, a decrease in assimilation efficiency, as observed in combination with

reduced ingestion in the mysid *Leptomysis lingvura*, could further reduce the amount of

energy available to the energetic budget (Gaudy et al., 1991). Increases in lipid

utilization in response to cadmium exposure (Table 1.11) suggest that organisms are

unable to satisfy their energetic needs via consumption and must resort to energy

reserves. Other bioenergetic effects include reduced activity, increased mucus

production, and mixed effects on rates of excretion and molting (Table 1.11). Although

cadmium appears to result in complex effects on the bioenergetics of aquatic

invertebrates, exposure to cadmium most often causes a decline in consumption and

production, resulting in a shortage in energy available to maintain standard rates of

growth and reproduction. These declines at the level of the individual may be expressed

as a decrease in population growth rate, as observed in several aquatic invertebrates (Table 1.12).

Conclusion

Copper and cadmium often have similar effects on rates of consumption, growth, development, and reproduction in aquatic invertebrates; exposure typically reduces consumption and assimilation, as well as energy allocation to production. Effects on respiration may vary depending upon metal concentration, duration of exposure, salinity, and species studied. Variable responses in respiration are not unexpected due to the complex set of physiological mechanisms that may affect oxygen consumption. The majority of evidence suggests that both copper and cadmium may reduce energy allocation to production pathways. Even at sublethal concentrations, a decrease in production may ultimately result in population declines in contaminated habitats, with unknown effects at the ecosystem level.

Table 1.1. Effects of copper on rate of oxygen consumption.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Total Copper Concentration	Observed Effect	Reference
Dreissena polymorpha (zebra mussel)	adults	FW	NR	24 h	100 µg Cu/L	reduced oxygen consumption	Rao and Khan, 2000
Mytilis edulis (blue mussel)	adults	33 ppt	8.1	30-60 m	200 µg Cu/L	reduced oxygen consumption in intact and "propped open" individuals	Manley, 1983
					500 µg Cu/L	reduced oxygen consumption in individuals with posterior adductor muscle severed	
Perna viridis (green mussel)	adults	34 ppt	8.2-8.4	24 h	60 µg Cu/L	reduced oxygen consumption	Prabhudeva and Menon, 1986
Perna viridis (green mussel)	adults	SW	NR	84 d	50 µg Cu/L	reduced oxygen consumption	Sze and Lee, 2000
Ruditapes decussatus (clam)	adults	35.6 ppt	NR	2 d	10 µg Cu/L	increased oxygen consumption	Sobral and Widdows, 1997
Tapes philippinarum (clam)	adults	26-28 ppt	8.0-8.2	14 d	10 µg Cu/L	increased oxygen consumption	Munari and Mistri, 2007
Balanus amphitrite (barnacle) *Balanus tintinnabulum* (barnacle)	adults	SW	NR	2 h	255 µg Cu/L	reduced oxygen consumption	Rao et al., 1986
Gammarus pulex (amphipod)	adults	108 mg/L	8.0	10 d	10.8 µg Cu/L	increased oxygen consumption	Kedwards et al., 1996
Caridina rajadhari (shrimp)	adults	FW	NR	30 m	300 µg Cu/L	reduced oxygen consumption	Chinnayya, 1971
Farfantepenaeus paulensis (pink shrimp)	postlarvae	25 ppt	NR	15 m	17 µg Cu/L	reduced oxygen consumption	Santos et al., 2000

13

Table 1.1, continued.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Total Copper Concentration	Observed Effect	Reference
Orconectes immunis (crayfish)	juveniles	139 mg/L	6.9	5 d	160 µg Cu/L	reduced oxygen consumption	Khan et al., 2006
Carcinus maenas (shore crab)	adults	SW	NR	2 h	10000 µg Cu/L	reduced oxygen consumption	Depledge, 1984
Portunus pelagicus (blue swimming crab)	adults	18 ppt 27 ppt 35 ppt 44 ppt	NR	96 h	1500 µg Cu/L 750 µg Cu/L 3000 µg Cu/L 750 µg Cu/L	reduced oxygen consumption	Ketpadung and Tangkrock-olan, 2006
Potamonautes warreni (crab)	adults	FW	NR	7-14 d 21 d	1000 µg Cu/L	increased oxygen consumption reduced oxygen consumption	Vosloo et al., 2002
Scylla serrata (crab)	adults	SW	NR	96 h	10000 µg Cu/L	reduced oxygen consumption	Ahmed et al., 1997
Uca annulipes (fiddler crab)	adults	20 ppt	NR	48 h 96 h	870 µg Cu/L 500 µg Cu/L	reduced oxygen consumption	Devi and Rao, 1989
Uca triangularis (fiddler crab)				48 h 96 h	3410 µg Cu/L 4670 µg Cu/L		

NR = not reported
[1] Life stage with which exposure was initiated
[2] Water hardness is given in mg $CaCO_3$/L, salinity in ppt; FW = freshwater (hardness not reported); SW = saltwater (salinity not reported)

Table 1.2. Effects of copper on growth and development. The metric used to quantify growth rate is supplied in parentheses.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Total Copper Concentration	Observed Effect	Reference
Watersipora subtorquata (bryozoan)	larvae	SW	NR	6 h	100 µg Cu/L	reduced growth (diameter) of colonies started from exposed larvae, delayed metamorphosis of larvae	Ng and Keough, 2003
Lymnaea stagnalis (snail)	eggs	FW	NR	28 d	25 µg Cu/L	reduced growth (wet weight and shell size)	Girling et al., 2000
Physa integra (snail)	adults	45 mg/L	7.7	42 d	14.8 µg Cu/L	reduced growth (shell length)	Arthur and Leonard, 1970
Mytilus edulis (blue mussel)	adults	SW	NR	3 d	10 µg Cu/L	reduced growth (shell length)	Manley et al., 1984
Mytilus edulis (blue mussel)	adults	SW	NR	10 d / 20 d	4.6 µg Cu/L / 1.4 µg Cu/L	reduced growth (shell length)	Redpath, 1985
Mytilus edulis (blue mussel)	adults	30 ppt	NR	7 d	32 µg Cu/L	reduced scope for growth	Sanders et al., 1991
Perna viridis (green mussel)	adults	33.5 ppt	8.1	14 d	25 µg Cu/L	reduced scope for growth	Krishnakumar et al., 1990
Perna viridis (green mussel)	adults	SW	NR	84 d	50 µg Cu/L	reduced growth (shell length, dry weight of tissue, and condition index)	Sze and Lee, 2000
Ruditapes decussatus (clam)	adults	35.6 ppt	NR	2 d	10 µg Cu/L	reduced scope for growth	Sobral and Widdows, 1997
Tapes philippinarum (clam)	adults	26-28 ppt	8.0-8.2	5 d	10 µg Cu/L	reduced scope for growth	Munari and Mistri, 2007
Isognomon californicum (oyster)	larvae	SW	NR	4 d	5 µg Cu/L	reduced growth (dry weight)	Ringwood, 1992a

Table 1.2, continued.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Total Copper Concentration	Observed Effect	Reference
Bosmina longirostris (cladoceran)	neonates	FW	6.9-7.1	13-17 d	18 µg Cu/L	reduced growth (carapace length)	Koivisto and Ketola, 1995
Daphnia magna (cladoceran)	neonates	45.3 mg/L	7.74	21 d	40 µg Cu/L	reduced growth (dry weight)	Biesinger and Christensen, 1972
Tigriopus japonicus (copepod)	gravid females	30 ppt	NR	48 d	64.2 µg Cu/L	delayed development from nauplial to adult stage	D'Agostino and Finney, 1974
Gammarus pseudolimnaeus (amphipod)	adults	45 mg/L	7.7	42 d	14.8 µg Cu/L	reduced growth (body length)	Arthur and Leonard, 1970
Gammarus pulex (amphipod)	mixture of all stages	103 mg/L	7.9	100 d	11 µg Cu/L	reduced growth (increase in number of molts required to reach a given body length)	Maund et al., 1992
Gammarus pulex (amphipod)	neonates	FW	6-7	28 d	7 µg Cu/L	reduced growth (wet weight)	Girling et al., 2000
Farfantepenaeus paulensis (pink shrimp)	postlarvae	25 ppt	NR	35 d	43 µg Cu/L	reduced growth (wet weight)	Santos et al., 2000
					85 µg Cu/L	reduced growth (total length and dry weight)	
Metapenaeus ensis (shrimp)	larvae	30 ppt	7.9-8.1	10 d	60 µg Cu/L	delayed development of larvae	Wong et al., 1995
	postlarvae			8 d	80 µg Cu/L	reduced growth (body length)	
Palaemonetes pugio (grass shrimp)	embryos	20 ppt	7.0-7.8	7 d	1000 µg Cu/L	reduced growth (embryo length)	Rayburn and Fisher, 1995
				12 d	3000 µg Cu/L	delayed development of embryos	
Penaeus monodon (tiger shrimp)	juveniles	25 ppt	8.13	15 d	4500 µg Cu/L	reduced growth (total length and wet weight)	Chen and Lin, 2001
				30 d	900 µg Cu/L		
Tunicotheres moseri (pea crab)	larvae	37 ppt	NR	96 h	100 µg Cu/L	increased duration of first larval stage	Greco et al., 2001
	postlarvae				10 µg Cu/L	decreased duration of postlarval stage	

16

Table 1.2, continued.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Total Copper Concentration	Observed Effect	Reference
Chironomus riparius (midge)	larvae, 2nd instar	FW	6-7	10 d	25 µg Cu/L	reduced growth (wet weight)	Girling et al., 2000
Paracentrotus lividus (sea urchin)	embryos	34 ppt	8.0	48 h	32.9 µg Cu/L	reduced growth (length)	Lorenzo et al., 2002
					41.1 µg Cu/L	inhibition of development of embryos into larvae	

NR = not reported
[1] Life stage with which exposure was initiated
[2] Water hardness is given in mg $CaCO_3$/L, salinity in ppt; FW = freshwater (hardness not reported); SW = saltwater (salinity not reported)

Table 1.3. Effects of copper on reproduction.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Total Copper Concentration	Observed Effect	Reference
Acropora longicyathus (coral)	gametes	SW	NR	30 m	23.6 µg Cu/L	reduced fertilization success	Reichelt-Brushett and Harrison, 2005
Acropora tenuis (coral)					41.9 µg Cu/L		
Goniastrea aspera (coral)					20.4 µg Cu/L		
Goniastrea retiformis (coral)					20 µg Cu/L		
Goniastrea aspera (coral)	gametes	SW	NR	30 m	20 µg Cu/L	reduced fertilization success	Reichelt-Brushett and Harrison, 1999
Lobophytum compactum (coral)	gametes	SW	NR	30 m	69 µg Cu/L	reduced fertilization success	Reichelt-Brushett and Michaelek-Wagner, 2005
Mytilus edulis (blue mussel)	sperm	SW	7.91	20 m	6355 µg Cu/L	reduced sperm motility	Earnshaw et al., 1986
Isognomon californicum (oyster)	sperm	SW	NR	60 m	50 µg Cu/L	reduced fertilization success	Ringwood, 1992a
Bosmina longirostris (cladoceran)	neonates	FW	6.9-7.1	13-17 d	14 µg Cu/L	delayed maturation (first appearance of eggs in brood pouch), increased age at first reproduction	Koivisto and Ketola, 1995
					16 µg Cu/L	fewer young per brood	
					18 µg Cu/L	fewer young per female	
Daphnia pulex (cladoceran)					26 µg Cu/L	delayed maturation (first appearance of eggs in brood pouch)	

Table 1.3, continued.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Total Copper Concentration	Observed Effect	Reference
Daphnia ambigua (cladoceran)	neonates	130-160 mg/L	8.2-9.5	~130 d	60 µg Cu/L	decreased brood size	Winner and Farrell, 1976
Daphnia parvula (cladoceran)					80 µg Cu/L		
Daphnia pulex (cladoceran)					80 µg Cu/L		
Daphnia magna (cladoceran)	neonates	45.3 mg/L	7.74	21 d	22 µg Cu/L	fewer young per adult	Biesinger and Christensen, 1972
Praunus flexuosus (mysid shrimp)	adults	30 ppt	8	8 d	5 µg Cu/L	reduced percentage of brooding females, decreased brood size, increased percentage of abortions	Garnacho et al., 2001
Corophium volutator (amphipod)	adults	25 ppt	NR	14 d	100 µg Cu/L	reduced percentage of brooding females	Eriksson and Weeks, 1994
Gammarus pseudolimnaeus (amphipod)	adults	45 mg/L	7.7	42 d	8 µg Cu/L	fewer young per adult	Arthur and Leonard, 1970
Palaemonetes pugio (grass shrimp)	embryos	20 ppt	7.0-7.8	4 d	3000 µg Cu/L	reduced hatching success	Rayburn and Fisher, 1999
Callinectes sapidus (blue crab)	embryos	28 ppt	NR	6-8 d	1 µg Cu/L	reduced hatching success	Lee et al., 1996
Chironomus riparius (midge)	embryos	FW	6-7	time to hatch	1100 µg Cu/L	reduced hatching success	Girling et al., 2000
Echinometra mathaei (sea urchin)	sperm	SW	NR	60 m	10 µg Cu/L	reduced fertilization success	Ringwood, 1992a

Table 1.3, continued.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Total Copper Concentration	Observed Effect	Reference
Ciona intestinalis (ascidian)	gametes	33.3 ppt	8.06	20 h	32 μg Cu/L	reduced hatching success	Bellas et al., 2004

NR = not reported
[1] Life stage with which exposure was initiated
[2] Water hardness is given in mg $CaCO_3$/L, salinity in ppt; FW = freshwater (hardness not reported); SW = saltwater (salinity not reported)

Table 1.4. Effects of copper on ingestion rate.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Total Copper Concentration	Observed Effect	Reference
Campeloma decisum (snail)	adults	45 mg/L	7.7	42 d	14.8 µg Cu/L	reduced ingestion	Arthur and Leonard, 1970
Saduria entomon (isopod)	adults	6 ppt	7.6	7 d	10000 µg Cu/L	reduced ingestion	Pynnonen, 1996
Gammarus pulex (amphipod)	juveniles	158 mg/L	7.7	96 h	12.1 µg Cu/L	reduced ingestion	Blockwell et al., 1998
Farfantepenaeus paulensis (pink shrimp)	postlarvae	25 ppt	NR	30 m	43 µg Cu/L	reduced ingestion	Santos et al., 2000
Metapenaeus ensis (shrimp)	protozoea, 3rd instar	30-34 ppt	8.7	2 h	250 µg Cu/L	reduced ingestion	Wong et al., 1993
	postlarvae, 3rd instar			24 h	2000 µg Cu/L		
Penaeus monodon (tiger shrimp)	juveniles	25 ppt	8.13	1.5 h	5000 µg Cu/L	reduced ingestion	Chen and Lin, 2001

NR = not reported
[1] Life stage with which exposure was initiated
[2] Water hardness is given in mg $CaCO_3$/L, salinity in ppt; FW = freshwater (hardness not reported); SW = saltwater (salinity not reported)

Table 1.5. Other bioenergetic effects of copper.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Total Copper Concentration	Observed Effect	Reference
Mytilis edulis (blue mussel)	adults	33 ppt	8.1	30-60 m	10.1 µg Cu/L	reduced clearance rate	Manley, 1983
Mytilus edulis (blue mussel)	adults	30 ppt	NR	7 d	32 µg Cu/L	reduced clearance rate, decreased assimilation efficiency	Sanders et al., 1991
Perna viridis (green mussel)	adults	33.5 ppt	8.1	14 d	25 µg Cu/L	increased rate of ammonia excretion, reduced clearance rate	Krishnakumar et al., 1990
Perna viridis (green mussel)	adults	SW	NR	84 d	50 µg Cu/L	decreased assimilation efficiency, reduced clearance rate, reduced fecal production, increased organic content of feces, increased rate of ammonia excretion	Sze and Lee, 2000
Ruditapes decussatus (clam)	adults	35.6 ppt	NR	2 d	10 µg Cu/L	reduced clearance rate	Sobral and Widdows, 1997
Tapes philippinarum (clam)	adults	26-28 ppt	8.0-8.2	5 d / 9 d	10 µg Cu/L	reduced clearance rate / decreased assimilation efficiency	Munari and Mistri, 2007
Daphnia magna (cladoceran)	neonates	FW	NR	9 h / 13 h / 14 h	30 µg Cu/L / 20 µg Cu/L / 10 µg Cu/L	decreased average swimming velocity	Untersteiner et al., 2003
Gammarus duebeni (amphipod)	adults	15.5 ppt	NR	7 d	45 µg Cu/L	reduced swimming endurance (stamina)	Lawrence and Poulter, 1998
Penaeus monodon (tiger shrimp)	juveniles	25 ppt	8.13	75 d	900 µg Cu/L	inhibition of molting	Chen and Lin, 2001
Portunus pelagicus (sand crab)	larvae, 3rd instar	33 ppt	NR	72 h	10 µg Cu/L	inhibition of molting	Mortimer and Miller, 1994

Table 1.5, continued.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Total Copper Concentration	Observed Effect	Reference
Potamonautes warreni (crab)	adults	FW	NR	14 d	1000 μg Cu/L	decreased rate of ammonia excretion	Vosloo et al., 2002
				7 d		increased lipid metabolism	
Tunicotheres moseri (pea crab)	larvae, 1st instar	37 ppt	NR	96 h	0.5 μg Cu/L	inhibition of molting	Greco et al., 2001
	larvae, 2nd instar				1000 μg Cu/L		
	postlarvae				1000 μg Cu/L		

NR = not reported
[1] Life stage with which exposure was initiated
[2] Water hardness is given in mg $CaCO_3$/L, salinity in ppt; FW = freshwater (hardness not reported); SW = saltwater (salinity not reported)

Table 1.6. Effects of copper on population growth.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Total Copper Concentration	Observed Effect	Reference
Brachionus calyciflorus (rotifer)	neonates	FW	6-7	72 h	5 µg Cu/L	decreased population growth rate	Girling et al., 2000
Brachionus plicatilis (rotifer)	adults	34-36 ppt	8.5	10 d	125 µg Cu/L	decreased population growth rate at low food ration	Luna-Andrade et al., 2002
					250 µg Cu/L	decreased population growth rate at high food ration	
Daphnia ambigua (cladoceran)	neonates	130-160 mg/L	8.2-9.5	~130 d	60 µg Cu/L	decreased population growth rate	Winner and Farrell, 1976
Daphnia magna (cladoceran)					80 µg Cu/L		
Daphnia parvula (cladoceran)					60 µg Cu/L		
Daphnia pulex (cladoceran)					60 µg Cu/L		
Bosmina longirostris (cladoceran)	neonates	FW	6.9-7.1	13-17 d	18 µg Cu/L	decreased population growth rate	Koivisto and Ketola, 1995
Gammarus pulex (amphipod)	mixture of all stages	103 mg/L	7.9	100 d	14.6 µg Cu/L	decreased population growth rate	Maund et al., 1992

NR = not reported
[1] Life stage with which exposure was initiated
[2] Water hardness is given in mg $CaCO_3$/L, salinity in ppt; FW = freshwater (hardness not reported); SW = saltwater (salinity not reported)

Table 1.7. Effects of cadmium on rate of oxygen consumption.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Cadmium Concentration[3]	Observed Effect	Reference
Murex trunculus (snail)	adults	29-34 ppt	NR	60 h	5000 µg Cd/L	reduced oxygen consumption	Dalla Via et al., 1989
				34 d	50 µg Cd/L	increased oxygen consumption	
Nucella lapillus (dogwhelk)	adults	35 ppt	NR	20 d	500 µg Cd/L	reduced oxygen consumption	Leung et al., 2000
Donax trunculus (mussel)	adults	40 ppt	NR	24 h	100 µg Cd/L	reduced oxygen consumption, perhaps due to shell closure	Neuberger-Cywiak et al., 2005
Lampsilis ventricosa (pocketbook mussel)	adults	165 mg/L	8.1	28 d	22 µg Cd/L	reduced oxygen consumption	Naimo et al., 1992
Meretrix casta (clam)	adults	10-25 ppt	NR	4 d	1000 µg Cd/L	increased oxygen consumption	Kumarasamy and Karthikeyan, 1999
Crassostrea virginica (eastern oyster)	adults	SW	NR	21 d	25 µg Cd/L	reduced oxygen consumption in isolated mitochondria	Sokolova et al., 2005
Daphnia magna (cladoceran)	juveniles	FW	NR	96 h	4 µg Cd/L	increased oxygen consumption	De Coen and Janssen, 2003
					$\geq$ 14 µg Cd/L	reduced oxygen consumption as measured by electron transport activity	
Leptomysis lingvura (mysid)	adults	SW	NR	18-27 h	100 µg Cd/L	reduced oxygen consumption	Gaudy et al., 1991
Elasmopus rapax (amphipod)	adults	30 ppt	8.1	24 h	112 µg Cd/L	reduced oxygen consumption	Zanders and Rojas, 1992
Gammarus duebeni (amphipod)	adults	4-24 ppt	NR	6 h	1000 µg Cd/L	reduced oxygen consumption	Tedengren et al., 1988
Gammarus oceanicus (amphipod)							
Gammarus pulex (amphipod)	adults	FW	7.5	4 h	1000 µg Cd/L	reduced oxygen consumption	Aronsson and Ekelund, 2005
				24 h	200 µg Cd/L	reduced oxygen consumption	

Table 1.7, continued.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Cadmium Concentration[3]	Observed Effect	Reference
Litopenaeus schmitti (white shrimp)	juveniles	15 ppt	7.15-7.87	24 h	3000 µg Cd/L	reduced oxygen consumption	Wu and Chen, 2004
Litopenaeus schmitti (white shrimp)	larvae	36 ppt	8.2	3 h	500 µg Cd/L	reduced oxygen consumption	Barbieri, 2007
Palaemonetes pugio (grass shrimp)	adults	5 ppt 15 ppt	NR	7 d	50 µg Cd/L	reduced oxygen consumption increased oxygen consumption	Vernberg et al., 1977
Palaemonetes pugio (grass shrimp)	adults	15 ppt	NR	48 h	100 µg Cd/L	reduced oxygen consumption	Hutcheson et al., 1985
Orconectes immunis (crayfish)	juveniles	139 mg/L	6.9	5 d	160 µg Cd/L	reduced oxygen consumption	Khan et al., 2006
Homarus americanus (American lobster)	adults	24-26 ppt	NR	30 d	3 µg Cd/L	increased oxygen consumption	Thurberg et al., 1977
Barytelphusa guerini (field crab)	adults	FW	7.4	6-24 h 4-15 d	600 µg Cd/L	increased oxygen consumption reduced oxygen consumption	Reddy and Venugopal, 1993
Cancer irroratus (rock crab)	adults	17-32 ppt	8.0	48 h	120 µg Cd/L	reduced oxygen consumption	Thurberg et al., 1973
Carcinus maenas (green crab)	adults	17-32 ppt	8.0	48 h	500 µg Cd/L	reduced oxygen consumption	Thurberg et al., 1973
Eurypanopeus depressus (mud crab)	adults	25 ppt	7.0	72 h	7000 µg Cd/L	reduced oxygen consumption in excised gill tissue	Collier et al., 1973
Uca annulipes (fiddler crab)	adults	20 ppt	NR	48 h 96 h	4300 µg Cd/L 3330 µg Cd/L	reduced oxygen consumption	Devi and Rao, 1989

Table 1.7, continued.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Cadmium Concentration[3]	Observed Effect	Reference
Uca triangularis (fiddler crab)	adults	20 ppt	NR	48 h	1460 μg Cd/L	reduced oxygen consumption	Devi and Rao, 1989
				96 h	30 μg Cd/L		

NR = not reported
[1]Life stage with which exposure was initiated
[2]Water hardness is given in mg $CaCO_3$/L, salinity in ppt; FW = freshwater (hardness not reported); SW = saltwater (salinity not reported)
[3]Total cadmium concentration unless noted as μg Cd^{2+}/L, which refers to the free divalent cadmium ion concentration

Table 1.8. Effects of cadmium on growth and development. The metric used to quantify growth rate is supplied in parentheses.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Cadmium Concentration[3]	Observed Effect	Reference
Brotia hainanensis (snail)	juveniles	FW	7.4	4 d	800 µg Cd/L	reduced scope for growth	Lam, 1996
Hydrobia ulvae (mudsnail)	juveniles	13 or 23 ppt	NR	21 d	100 µg Cd/L	reduced growth (shell length)	Forbes and Depledge, 1992
Hydrobia ventrosa (snail)	juveniles	23 ppt	NR	21 d	200 µg Cd/L	reduced growth (shell length)	Forbes, 1991
				28 d	100 µg Cd/L		
		33 ppt		21 d	100 µg Cd/L		
Argopecten irradians (bay scallop)	juveniles	27.4-31.5 ppt	NR	42 d	60 µg Cd/L	reduced growth (shell length)	Pesch and Stewart, 1980
Isognomon californicum (oyster)	larvae	SW	NR	4 d	1 µg Cd/L	reduced growth (dry weight)	Ringwood, 1992a
Isognomon californicum (oyster)	larvae	34 ppt	NR	4 d	2 µg Cd/L	reduced growth (shell DW and tissue DW)	Ringwood, 1992b
					50 µg Cd/L	reduced growth (shell area)	
				14 d	10 µg Cd/L	reduced growth (shell length)	
Nephelopsis obscura (leech)	adults	FW	NR	91 d	50 µg Cd/L	reduced growth (wet weight)	Wicklum and Davies, 1996
Daphnia magna (cladoceran)	neonates	45.3 mg/L	7.74	21 d	1 µg Cd/L	reduced growth (dry weight)	Biesinger and Christensen, 1972
Daphnia magna (cladoceran)	neonates	150 mg/L	8.4	14 d	1 µg Cd/L	reduced growth (dry weight)	Bodar et al., 1988a
Daphnia magna (cladoceran)	adults	FW	NR	3-4 d (1 instar)	2 µg Cd/L	reduced growth (dry weight)	Barata and Baird, 2000
Daphnia magna (cladoceran)	neonates	FW	NR	8 d	8.4 µg Cd/L	reduced growth (carapace length)	Knops et al., 2001
				17 d	5.6 µg Cd/L		

Table 1.8, continued.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Cadmium Concentration[3]	Observed Effect	Reference
Daphnia magna (cladoceran)	neonates	FW	NR	21 d	4 µg Cd/L	reduced growth (carapace length)	De Coen and Janssen, 2003
Daphnia magna (cladoceran)	adults	FW	8.0	14 d	2.2 µg Cd^{2+}/L	reduced growth (body length)	Baillieul et al., 2005
					1.1 µg Cd^{2+}/L	reduced scope for growth	
Daphnia magna (cladoceran)	adults	FW	8.0	14 d	2.8 µg Cd^{2+}/L	reduced growth (carapace length, only at high food ration)	Smolders et al., 2005
Daphnia magna (cladoceran)	neonates	FW	8.2	72 d (6 generations)	3 µg Cd/L	reduced growth (wet weight) in all generations	Guan and Wang, 2006
Tigriopus japonicus (copepod)	gravid females	30 ppt	NR	48 d	43.8 µg Cd/L	delayed development from nauplial to adult stage	D'Agostino and Finney, 1974
Mysidopsis bahia (mysid)	juveniles	30 ppt	NR	18 d	4 µg Cd/L	reduced growth (dry weight)	Carr et al., 1985
Siriella armata (mysid)	juveniles	38 ppt	NR	5 d	1 µg Cd/L	reduced growth (wet weight)	Birmelin et al., 1995
Gammarus pulex (amphipod)	adults	FW	NR	6 d	20.63 µg Cd/L	reduced scope for growth	Stuhlbacher and Maltby, 1992
Litopenaeus vannamei (white shrimp)	postlarvae	15 ppt	7.15-7.87	7 d	400 µg Cd/L	reduced growth (wet weight)	Wu and Chen, 2005
				14 d	200 µg Cd/L	reduced growth (wet weight and body length)	
				28 d	100 µg Cd/L		
Callinectes sapidus (blue crab)	juveniles	2.5 ppt	NR	21 d	50 µg Cd/L	reduced scope for growth	Guerin and Stickle, 1995
Callinectes sapidus (blue crab)	oocytes	28 ppt	NR	4 d	20 µg Cd/L	reduced growth (protein and lipovitellin accumulation)	Lee et al., 1996

Table 1.8, continued.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Cadmium Concentration[3]	Observed Effect	Reference
Rhithropanopeus harrisii (mud crab)	larvae	20 ppt	7.6	96 h	0.2 µg Cd^{2+}/L	reduced growth (dry weight)	Thorpe and Costlow, 1989
Chironomus riparius (midge)	larvae, 1st instar	98 mg/L	7.6	14 d / 26 d	150 µg Cd/L	delayed development of larvae / delayed adult emergence	Pascoe et al., 1989
Glyptotendipes pallens (midge)	larvae, 4th instar	150 mg/L	7.44-8.25	96 h	1000 µg Cd/L	reduced growth (dry weight)	Heinis et al., 1990

NR = not reported
[1]Life stage with which exposure was initiated
[2]Water hardness is given in mg CaCO$_3$/L, salinity in ppt; FW = freshwater (hardness not reported); SW = saltwater (salinity not reported)
[3]Total cadmium concentration unless noted as µg Cd^{2+}/L, which refers to the free divalent cadmium ion concentration

Table 1.9. Effects of cadmium on reproduction.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Cadmium Concentration[3]	Observed Effect	Reference
Goniastrea retiformis (coral)	gametes	SW	NR	30 m	5000 µg Cd/L	reduced fertilization success	Reichelt-Brushett and Harrison, 2005
Biomphalaria glabrata (snail)	embryos	FW	NR	14 d	22.5 µg Cd/L	increased time to hatch	Salice and Roesijadi, 2002
Biomphalaria glabrata (snail)	embryos	FW	NR	> 60 d	2.8 µg Cd/L	reduced hatching success and increased fecundity in a wild-bred parasite-resistant strain	Salice and Miller, 2003
					5.6 µg Cd/L	decreased fecundity in a laboratory-bred parasite-susceptible strain	
					11.2 µg Cd/L	reduced hatching success in a laboratory-bred parasite-susceptible strain	
Neanthes arenaceodentata (polychaete)	adults	35 ppt	NR	77 d	1.1 µg Cd/L	inhibition of egg production	Jenkins and Mason, 1988
Daphnia magna (cladoceran)	neonates	45.3 mg/L	7.74	21 d	0.17 µg Cd/L	fewer young per adult	Biesinger and Christensen, 1972
Daphnia magna (cladoceran)	neonates	240 mg/L	8.0	14 d	7.5 µg Cd/L	fewer young per adult	Elnabarawy et al., 1986
Daphnia magna (cladoceran)	neonates	150 mg/L	8.4	25 d	0.5 µg Cd/L	more neonates per female	Bodar et al., 1988b
					10 µg Cd/L	fewer neonates per female	
					0.5 µg Cd/L	reduced offspring size (length)	
Daphnia magna (cladoceran)	adults	FW	NR	3-4 d (1 instar)	0.4 µg Cd/L	fewer young per brood, decreased brood mass	Barata and Baird, 2000

Table 1.9, continued.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Cadmium Concentration[3]	Observed Effect	Reference
Daphnia magna (cladoceran)	neonates	FW	NR	21 d	4 µg Cd/L	fewer young per female, fewer young per brood	De Coen and Janssen, 2003
Daphnia magna (cladoceran)	adults	FW	8.0	14 d	2.2 µg Cd^{2+}/L	fewer young per female	Baillieul et al., 2005
Daphnia magna (cladoceran)	adults	FW	8.0	14 d	2.8 µg Cd^{2+}/L	fewer young per female (only at high food ration)	Smolders et al., 2005
Daphnia magna (cladoceran)	adults	FW	8.0	14 d	2.2 µg Cd^{2+}/L	fewer young per female	Baillieul et al., 2005
Daphnia magna (cladoceran)	neonates	FW	8.2	72 d (6 generations)	3 µg Cd/L	more neonates per adult in generations 2 through 5	Guan and Wang, 2006
Daphnia pulex (cladoceran)	neonates	FW	7.7	24 d	1 µg Cd/L	fewer young per adult, fewer young per brood, fewer broods per adult	Bertram and Hart, 1979
				88 d	10 µg Cd/L	reduced percentage of adults producing young	
Daphnia pulex (cladoceran)	neonates	240 mg/L	8.0	14 d	25 µg Cd/L	fewer young per adult	Elnabarawy et al., 1986
Ceriodaphnia reticulata (cladoceran)				7 d	0.75 µg Cd/L		
Artemia parthenogenetica (brine shrimp)	nauplii	SW	NR	~100 d (2 generations)	80 µg Cd/L	reduced age-specific fecundity, shortened pre-reproductive period	Sarabia et al., 2003
Acartia tonsa (copepod)	adults	17 ppt	NR	4 d	40 µg Cd/L	fewer eggs produced per unit adult body mass	Toudal and Riisgard, 1987
Mysidopsis bahia (mysid)	juveniles	10-27 ppt	NR	17 d	6.4 µg Cd/L	delayed formation of brood pouches, delayed release of young, fewer young per female	Nimmo et al., 1977b

Table 1.9, continued.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Cadmium Concentration[3]	Observed Effect	Reference
Mysidopsis bahia (mysid)	juveniles	15-25 ppt	NR	23 d	6.4 µg Cd/L	fewer young per female	Nimmo et al., 1978
					10.6 µg Cd/L	delayed release of young	
Callinectes sapidus (blue crab)	embryos	28 ppt	NR	6-8 d	0.1 µg Cd/L	reduced hatching success	Lee et al., 1996
Anthocidaris crassispina (sea urchin)	adults	30 ppt	NR	28 d	10 µg Cd/L	reduced sperm motility (including percentage of motile sperm and percentage of sperm with normal trajectory), delayed cleavage rate in embryo	Au et al., 2000
					100 µg Cd/L	reduced sperm velocity, reduced sperm quality (fertilization success), larger eggs (volume)	
Echinometra mathaei (sea urchin)	sperm	SW	NR	60 m	20 µg Cd/L	reduced fertilization success	Ringwood, 1992b
Ciona intestinalis (ascidian)	gametes	33.3 ppt	8.06	20 h	512 µg Cd/L	reduced hatching success	Bellas et al., 2004

NR = not reported
[1]Life stage with which exposure was initiated
[2]Water hardness is given in mg $CaCO_3$/L, salinity in ppt; FW = freshwater (hardness not reported); SW = saltwater (salinity not reported)
[3]Total cadmium concentration unless noted as µg Cd^{2+}/L, which refers to the free divalent cadmium ion concentration

Table 1.10. Effects of cadmium on ingestion rate.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Cadmium Concentration[3]	Observed Effect	Reference
Brotia hainanensis (snail)	juveniles	FW	7.4	4 d	800 µg Cd/L	reduced ingestion	Lam, 1996
Hydrobia ulvae (mudsnail)	juveniles	13 ppt	NR	15 m	200 µg Cd/L	reduced ingestion	Forbes and Depledge, 1992
Nephelopsis obscura (leech)	adults	FW	NR	91 d	50 µg Cd/L	reduced ingestion	Wicklum and Davies, 1996
Daphnia magna (cladoceran)	neonates	150 mg/L	8.4	14 d	5 µg Cd/L	reduced ingestion	Bodar et al., 1988a
Daphnia magna (cladoceran)	adults	FW	NR	3-4 d (1 instar)	0.4 µg Cd/L	reduced ingestion	Barata and Baird, 2000
Daphnia magna (cladoceran)	neonates	FW	8.2	72 d (6 generations)	3 µg Cd/L	reduced ingestion in all generations	Guan and Wang, 2006
Acartia tonsa (copepod)	adults	17 ppt	NR	4 d	40 µg Cd/L	reduced ingestion	Toudal and Riisgard, 1987
Leptomysis lingvura (mysid)	adults	SW	NR	18-27 h	100 µg Cd/L	reduced ingestion	Gaudy et al., 1991
Echinogammarus meridionalis (amphipod)	adults	263 mg/L	7.92	6 d	6.53 µg Cd/L	reduced ingestion	Pestana et al., 2007
Gammarus pulex (amphipod)	adults	129 mg/L	7.38	1 d	2.1 µg Cd/L	increased ingestion	Brown and Pascoe, 1989
					6.0 µg Cd/L	reduced ingestion	
Atyaephyra desmarestii (shrimp)	adults	263 mg/L	7.92	6 d	6.53 µg Cd/L	reduced ingestion	Pestana et al., 2007
Litopenaeus vannamei (white shrimp)	postlarvae	15 ppt	7.15-7.87	28 d	200 µg Cd/L	reduced ingestion	Wu and Chen, 2005

Table 1.10, continued.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Cadmium Concentration[3]	Observed Effect	Reference
Callinectes sapidus (blue crab)	juveniles	2.5 ppt	NR	21 d	50 µg Cd/L	reduced ingestion	Guerin and Stickle, 1995

NR = not reported
[1]Life stage with which exposure was initiated
[2]Water hardness is given in mg CaCO$_3$/L, salinity in ppt; FW = freshwater (hardness not reported); SW = saltwater (salinity not reported)
[3]Total cadmium concentration unless noted as µg Cd^{2+}/L, which refers to the free divalent cadmium ion concentration

Table 1.11. Other bioenergetic effects of cadmium.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Cadmium Concentration[3]	Observed Effect	Reference
Brotia hainanensis (snail)	juveniles	FW	7.4	4 d	800 µg Cd/L	decreased amount of energy assimilated	Lam, 1996
Nucella lapillus (dogwhelk)	adults	35 ppt	NR	20 d	500 µg Cd/L	decreased activity (recovery time from upside-down posture), increased mucus secretion	Leung et al., 2000
Donax trunculus (mussel)	adults	40 ppt	NR	24 h	10000 µg Cd/L	decreased rate of ammonia excretion	Neuberger-Cywiak et al., 2005
Meretrix casta (clam)	adults	10-33 ppt	NR	4 d	1000 µg Cd/L	reduced filtration rate (each salinity tested separately)	Kumarasamy and Karthikeyan, 1999
Nephelopsis obscura (leech)	adults	FW	NR	91 d / 7 d	50 µg Cd/L	decreased activity / increased mucus production	Wicklum and Davies, 1996
Daphnia magna (cladoceran)	neonates	150 mg/L	8.4	14 d	5 µg Cd/L	decreased amount of energy assimilated	Bodar et al., 1988a
Daphnia magna (cladoceran)	juveniles	FW	NR	48 h / 96 h	0.4 µg Cd/L / 4 µg Cd/L	reduced lipid reserves	De Coen and Janssen, 2003
Daphnia magna (cladoceran)	adults	FW	8.0	14 d	1.1 µg Cd^{2+}/L	decreased amount of energy assimilated	Baillieul et al., 2005
Daphnia magna (cladoceran)	neonates	FW	8.2	72 d (6 generations)	3 µg Cd/L	increased assimilation efficiency in generations 2 and 3, reduced assimilation efficiency in generation 6	Guan and Wang, 2006
Leptomysis lingvura (mysid)	adults	SW	NR	18-27 h	100 µg Cd/L	reduced assimilation efficiency, decreased rate of ammonia excretion	Gaudy et al., 1991
Mysidopsis bahia (mysid)	juveniles	30 ppt	NR	4 d	4 µg Cd/L	increased utilization of lipids and carbohydrates	Carr et al., 1985

Table 1.11, continued.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Cadmium Concentration[3]	Observed Effect	Reference
Gammarus pulex (amphipod)	adults	FW	NR	6 d	20.63 µg Cd/L	decreased amount of energy assimilated	Stuhlbacher and Maltby, 1992
Hippolyte inermis (shrimp)	adults	36 ppt	NR	immediate response	3500 µg Cd/L	reduced swimming velocity	Untersteiner et al., 2005
				1 h	2000 µg Cd/L		
				3 h	1000 µg Cd/L		
Litopenaeus schmitti (white shrimp)	juveniles	15 ppt	7.15-7.87	24 h	3000 µg Cd/L	increased rate of ammonia excretion	Wu and Chen, 2004
Litopenaeus schmitti (white shrimp)	larvae	36 ppt	8.2	3 h	500 µg Cd/L	increased rate of ammonia excretion	Barbieri, 2007
Palaemonetes pugio (grass shrimp)	adults	10 ppt	NR	7 d	50 µg Cd/L	increased molting frequency	Vernberg et al., 1977
Palaemonetes pugio (grass shrimp)	adults	15 ppt	NR	48 h	560 µg Cd/L	decreased activity	Hutcheson et al., 1985
Rhithropanopeus harrisii (mud crab)	larvae	20 ppt	7.6	96 h	0.2 µg Cd^{2+}/L	inhibition of molting	Thorpe and Costlow, 1989
Glyptotendipes pallens (midge)	larvae, 4th instar	150 mg/L	7.44-8.25	96 h	2500 µg Cd/L	decreased activity	Heinis et al., 1990

NR = not reported
[1]Life stage with which exposure was initiated
[2]Water hardness is given in mg $CaCO_3$/L, salinity in ppt; FW = freshwater (hardness not reported); SW = saltwater (salinity not reported)
[3]Total cadmium concentration unless noted as µg Cd^{2+}/L, which refers to the free divalent cadmium ion concentration

Table 1.12. Effects of cadmium on population growth.

Species	Initial Life Stage[1]	Water Hardness or Salinity[2]	pH	Duration of Exposure	Cadmium Concentration[3]	Observed Effect	Reference
Biomphalaria glabrata (snail)	embryos	FW	NR	> 60 d	2.8 µg Cd/L	decreased population growth rate in a wild-bred parasite-resistant strain	Salice and Miller, 2003
					5.6 µg Cd/L	decreased population growth rate in a laboratory-bred parasite-susceptible strain	
Aeolosoma headleyi (oligochaete)	adults	168 mg/L	NR	14 d	56 µg Cd/L	decreased population growth rate	Niederlehner et al., 1984
		60 mg/L		12 d	40 µg Cd/L		
Daphnia magna (cladoceran)	neonates	FW	NR	21 d	4 µg Cd/L	decreased population growth rate	De Coen and Janssen, 2003
Daphnia pulex (cladoceran)	neonates	FW	7.7	27 d	5 µg Cd/L	decreased population growth rate	Bertram and Hart, 1979

NR = not reported
[1]Life stage with which exposure was initiated
[2]Water hardness is given in mg $CaCO_3$/L, salinity in ppt; FW = freshwater (hardness not reported); SW = saltwater (salinity not reported)
[3]Total cadmium concentration unless noted as µg Cd^{2+}/L, which refers to the free divalent cadmium ion concentration

CHAPTER 2:

BIOENERGETIC EFFECTS OF AQUEOUS COPPER AND CADMIUM ON THE

GRASS SHRIMP, *PALAEMONETES PUGIO*

Abstract

Adult grass shrimp (*Palaemonetes pugio*) were exposed to either aqueous copper (ranging from 7.5 to 41 μg Cu^{2+}/L) or cadmium (2.5 to 6.6 μg Cd^{2+}/L) for 14 days in laboratory experiments to quantify effects on survival and bioenergetic processes, including respiration, somatic growth, energy (lipid) storage, and food consumption. The lowest observed effect concentrations for mortality were 41 μg Cu^{2+}/L or 6.6 μg Cd^{2+}/L, expressed as free metal ion concentrations. Both copper and cadmium caused a decrease in respiration rate at concentrations of 7.5 to 41 μg Cu^{2+}/L or 6.6 μg Cd^{2+}/L. Exposure to copper ($\geq$ 27 μg Cu^{2+}/L) resulted in negative somatic growth (i.e., weight loss); cadmium exposure (6.2 μg Cd^{2+}/L) caused a decrease in growth rate, relative to the control, but growth remained positive. Nonpolar lipid content and food consumption were not significantly affected by exposure to either copper or cadmium. Overall, the results suggest that both copper and cadmium result in a metabolic depression, decreasing energy allocation to both maintenance and production.

Introduction

Copper and cadmium are common contaminants in coastal habitats (Wright, 1986; Hall et al., 1998; Yang and Sañudo-Wilhelmy, 1998; Monbet, 2004; Munari and Mistri, 2007). Sources of copper and cadmium include mine drainage, wastewater from

metal smelting, runoff of agricultural fertilizers, and atmospheric fallout from fossil fuel combustion and refuse incineration; copper is also released into aquatic environments through application of antifouling paint and algicides (WHO, 1992; WHO, 1998). Elevated concentrations of copper and cadmium may exert toxic effects on aquatic organisms, including crustaceans such as the grass shrimp, *Palaemonetes pugio* (Burton and Fisher, 1990), a common inhabitant of estuaries and coastal systems in eastern North America and the Gulf of Mexico.

Copper is an essential trace element, functioning as a cofactor for enzymes such as oxidases (e.g., cytochrome c), oxygenases, and superoxide dismutase (Stohs and Bagchi, 1995; WHO, 1998). In arthropods, copper is also required as a component of hemocyanin, the oxygen-transport molecule circulating in hemolymph. Bioaccumulation of copper is largely dependent upon the concentration of the free divalent cation, Cu^{2+} (Zamuda and Sunda, 1982), but may also be affected by salinity (Bidwell and Gorrie, 2006) and temperature (Mubiana and Blust, 2007). When present at concentrations above those that are essential, exposure to copper may result in toxic effects. For example, copper can alter the structure of proteins and DNA by binding at sulfhydryl, carboxylate, and imidazole sites, impairing protein function and causing DNA to be misread (WHO, 1998). Copper also serves as a catalyst for the production of reactive oxygen species, resulting in peroxidation of lipids, DNA and organelle damage, and ATP depletion (Stohs and Bagchi, 1995; WHO, 1998).

In contrast, cadmium is not an essential element, having no known biological function. The bioavailable form of cadmium is the free divalent cation, Cd^{2+} (Sunda et al., 1978), but bioaccumulation may also be influenced by salinity (De Lisle and Roberts,

1988), temperature (Mubiana and Blust, 2007), and calcium concentration (De Lisle and Roberts, 1994). Cadmium may exert toxic effects by binding to proteins and DNA at sulfhydryl, sulfate, and carbonyl sites (Furst et al., 1998). Cadmium inhibits calcium uptake and calcium channels by competing with calcium for binding sites (Furst et al., 1998; Strydom et al., 2006). Cadmium can also inhibit DNA repair (Furst et al., 1998) and cause lipid peroxidation (Stohs and Bagchi, 1995).

Exposure to toxic chemicals often results in bioenergetic consequences (e.g., Rowe et al., 1998; Rowe et al., 2001). A basic bioenergetic budget for an animal can be expressed as

$$C = P + R + E$$

where C is energy consumed, P represents energy invested in production (including somatic growth, reproduction, energy storage, secretions, and eliminated tissues), R is respiratory expenditure (i.e., metabolic rate), and E is energy loss to excretion and egestion. Toxic stress can induce energetic costs, which alter allocation of energy among these bioenergetic pathways (Calow, 1991). Such costs can originate from active transport and excretion of chemicals (e.g., Rainbow and White, 1989), synthesis of proteins to metabolize or form complexes with toxins (i.e., enzymes or protective proteins; e.g., Howard and Hacker, 1990), increased mucus secretion (e.g., Leung et al., 2000), and repair of damaged tissue (e.g., Soegianto et al., 1999a; Calow, 1991). Protein synthesis is particularly costly (Hawkins, 1991; Hawkins and Day, 1996), and both copper and cadmium are known to induce production of metal-binding proteins, such as metallothioneins, which serve to regulate free concentrations of essential metals and sequester nonessential metals to protect against toxic effects (Roesijadi, 1992). Heat

shock proteins, which stabilize or repair proteins damaged by contaminant exposure, can also be induced in response to cadmium exposure (Stohs and Bagchi, 1995). Regardless of the specific mechanism by which they originate, metabolic costs resulting from exposure to metals may modify energy budgets, compromising growth, reproduction, and/or energy storage by individuals. Direct effects of copper and cadmium on metabolic rates and production pathways have been reported in many aquatic invertebrates (e.g., Wu and Chen, 2004; Munari and Mistri, 2007).

In the present study, I investigated the bioenergetic effects of chronic (14-day) exposure to aqueous copper and cadmium on adult *P. pugio*. To quantify effects on allocation among energetic pathways, I measured effects on respiration rate, somatic growth, food consumption, and energy (lipid) storage. I hypothesized that toxic effects would alter metabolic processes and/or food consumption, ultimately detracting from energy investment in production.

Methods

Test Species

The grass shrimp, *Palaemonetes pugio* is an estuarine, epibenthic, decapod crustacean with a wide distribution along the Atlantic and Gulf coasts of North America (Gosner, 1971; Anderson, 1985). Found in the littoral zone to a depth of 9 m (Gosner, 1971), *P. pugio* is an omnivore, consuming detritus, algae, and small invertebrates (Anderson, 1985). *Palaemonetes pugio* is an important prey item for many fish species (Clark et al., 2003), thereby providing a key link between the benthic and pelagic portions of the food web. Furthermore, the grass shrimp has been used as a model

aquatic invertebrate test species in toxicological studies, largely due to its tolerance of handling stress and a broad range of environmental conditions (e.g., salinity and temperature).

Exposure Conditions

Adult *P. pugio* were collected from the lower and middle Patuxent River, Maryland, USA, and were maintained in flow-through laboratory tanks, receiving ambient water from the lower Patuxent River, until experiments were initiated. Exposures were conducted in the laboratory in 38-L tanks, containing 20 L of artificial seawater. Tanks were arranged on shelves in a temperature-controlled room, maintaining a constant water temperature of 25 °C. Municipal water, filtered by reverse osmosis (RO), was mixed with Instant Ocean sea salt (Aquarium Systems, Mentor, OH) to a salinity of 10 ppt. The pH was adjusted to approximately 8 by adding 20% HCl. The light regime was 16 hours light: 8 hours dark. Before exposures began, *P. pugio* were acclimated to the temperature and salinity used during exposures for at least 21 hours.

For each experiment, *P. pugio* were exposed to either copper or cadmium. One copper and two cadmium experiments were conducted. The cadmium experiments were performed twice due to slight temperature fluctuations during the initial experiment, which appeared to affect shrimp growth. The experimental design was a randomized complete block design with four treatment levels per experiment (three different concentrations of metal and a control), replicated four times. For the copper experiment, the treatments are referred to as Cu0, Cu1, Cu2, and Cu3, and for the cadmium experiments, as Cd0, Cd1, Cd2, and Cd3, where "0" is the control and the concentration of metal increases from "1" to "3". Tanks were arranged in two blocks defined by shelf

height, due to possible temperature variations between shelves. Each block contained two replicates of each treatment. Sets of replicates were staggered in time (no more than 3 days apart) for logistical purposes.

Copper and cadmium were added to exposure water as metal chlorides; controls contained no added metals. To buffer the free metal ion concentration, NTA (nitrilotriacetic acid) was added at 5×10^{-5} M (Perrin and Dempsey, 1974). The target free metal ion concentrations ranged from 24 to 47 µg Cu^{2+}/L for copper and from 2.2 to 5.6 µg Cd^{2+}/L for cadmium (Table 2.1). The concentrations of metal chlorides necessary to achieve target free ion concentrations were estimated using the chemical equilibrium program MINEQL+ v. 4.5 (Environmental Research Software, Hallowell, ME), assuming a constant composition of Instant Ocean in exposure water (Atkinson and Bingman, 1988).

Experiments were initiated with 20 adult *P. pugio* per tank, with the exception of the second cadmium experiment, which began with 15 individuals per tank. The volume of water was also reduced to 15 L for the second cadmium experiment. Average mass of individual shrimp at the initiation of exposure was 155 mg per individual for the copper experiment and 105 and 127 mg for the first and second cadmium experiments, respectively. The duration of each experiment was 14 days. A complete water change was performed after the first seven days, during which exposure conditions were maintained by transferring test organisms to temporary holding chambers containing exposure water. Water lost due to evaporation was replaced with RO water every other day to reduce variation in salinity and metal concentrations. During exposures, shrimp were fed a ration of coarsely ground dry food, consisting of a 1:1 mixture of Wardley

Shrimp Pellets (Secaucus, NJ) and Wardley Pond Ten Floating Fish Stix. The ration was 0.2 g (dry weight) food per g (wet weight) shrimp every two days, based on the initial average weight per shrimp and accounting for mortality during the exposure period. The ration was previously determined to be sufficient to promote growth (personal observation).

Bioenergetic Measurements

Somatic growth rate was measured as the change in average wet weight per individual over the 14-day exposure; average wet weight was calculated as the total weight of shrimp in each tank, divided by the number of individuals. Respiration was measured as oxygen consumption rate at the end of the exposure, using a computer-controlled Micro OxyMax respirometer (Columbus Instruments, Columbus, OH). The methodology is described in detail by Rowe et al. (2001) and only summarized here. Briefly, individuals used for respiration measurements were removed from their tanks after 13 days of exposure and held unfed (under exposure conditions) for one day to avoid postprandial elevations in respiration rate. Oxygen consumption was measured for three shrimp per replicate, each in an individual 100-mL glass respiratory chamber containing 60 mL of exposure water. The chambers were maintained in the dark at 25 °C. The oxygen concentration in the overlying headspace was measured every two hours for a total of ~16 hours, resulting in eight measurements per individual. An empty chamber was used as a blank. A reference standard, consisting of an 8.4 V battery (DA146 Procell zinc air medical battery, Duracell, Bethel, CT) in a separate chamber, was used to monitor performance of the oxygen sensor.

Energy storage was measured as total nonpolar lipid content, quantified gravimetrically by Soxhlet extraction (Sawicka-Kapusta, 1975). At the end of each exposure, tissue samples were preserved at -80 °C for subsequent analysis. Each sample, consisting of a group of three shrimp per tank, was freeze-dried, crushed, extracted with boiling petroleum ether for approximately seven hours, and re-dried. Lipid content was calculated as the change in dry weight resulting from the extraction.

Food consumption was measured at the end of the exposures. Three shrimp per tank (if available) were fed canned chopped clams (Snow's/Doxsee, Cape May, NJ) for 16 hours in 2 L of exposure water at 25 °C. Clams had previously been allowed to equilibrate osmotically with exposure water for ~20 hours. Consumption was measured as the change in wet weight of food over the test period. Food consumption was not measured in the first cadmium experiment because there were not enough surviving individuals at the end of the exposure to perform all of the bioenergetic measurements.

Due to minor temperature fluctuations (1-2 °C) during the first cadmium experiment, the growth data were considered unreliable, given that they were closely correlated with temperature. Survival and respiration rate are reported for the first experiment, because survival did not appear to be affected by the small temperature fluctuations and respiration rate was measured in a temperature-controlled incubator after the exposure ended. The cadmium experiment was repeated on a smaller scale to provide data for growth under conditions in which temperature was less variable. Respiration was not measured in the second cadmium experiment because the measurements from the first cadmium experiment were deemed to be acceptable; instead of respiration, food consumption was measured in the second cadmium experiment.

Chemical Analyses

Water samples were collected at the initiation of the exposures and immediately after water changes to ascertain whether target metal concentrations were achieved. Samples were also taken before water changes and at the termination of exposure to assess the change in metal concentrations during the exposure period. Water was filtered through a 0.22-μm nitrocellulose membrane, acidified to pH < 2 with HNO_3, diluted 1:10 to reduce saltwater interference, and analyzed for copper and cadmium using an HP4500 (Agilent, formerly Hewlett-Packard, Santa Clara, CA) inductively coupled plasma mass spectrometer (ICP-MS). Free metal ion concentrations were calculated using MINEQL+ v. 4.5 software (Environmental Research Software, Hallowell, ME).

Bioaccumulation of metals was quantified at the end of the exposure. Shrimp (3-14 per tank, as available) were depurated for three hours in control seawater and frozen at -80 °C for later analysis. Samples were subsequently freeze-dried, crushed, and a subsample (~100 mg) of the homogenized sample was digested with 2 mL concentrated HNO_3 at 60 °C overnight. After digestion, the sample was diluted and analyzed for copper or cadmium by ICP-MS.

Statistical Analysis

Comparisons among treatments were made using the General Linear Model (GLM) routine in Minitab v. 13.31 statistical software (Minitab Inc., State College, PA). Data from each experiment were analyzed separately. Data were tested for normality and homogeneity of variance and were transformed as necessary. Experimental block (shelf height) was included as a factor in all models, except the analysis of metal concentrations in exposure water. In GLMs that included block as a factor, the maximum number of

degrees of freedom for error was 11. Block was significant in only one statistical test, the GLM which analyzed effects on growth in the copper experiment ($F_{1,11}$ = 5.8; P = 0.035), suggesting that subtle temperature differences between blocks influenced growth rate. An *a priori* Type I error rate (α) of 0.05 was used to assess significance of all tests. The Tukey method of pairwise comparisons was employed for all analyses to identify specific treatments that differed. Correlations between variables were analyzed using Pearson's product moment correlation coefficient (r).

Growth was calculated as the percent change in wet weight ([final-initial]/initial) during the exposure period. During the second cadmium experiment, the tanks nearest to the door were found to be slightly cooler (~24 °C) than the other tanks (~25 °C); the cooler tanks were removed from data analysis because growth of *P. pugio* was found to be strongly correlated with temperature in the first cadmium experiment.

The rate of oxygen consumption corresponding to the standard metabolic rate (the metabolic rate of a post-absorptive organism at rest) was calculated for each individual by taking the lower quartile of the measurements for that individual (Rowe, 2002), adjusted for the blank. The lower quartile was calculated as the mean between the second-lowest and third-lowest of the eight measurements for a given individual. Use of the lower quartile rather than the average was intended to exclude observations that were elevated due to periods of activity (Rowe, 2002). Oxygen consumption for each individual was corrected for mass by dividing the rate of oxygen consumption (μL O_2/min) by the individual's wet weight, raised to an empirically-derived allometric exponent (Packard and Boardman, 1987; Manyin and Rowe, 2006). The rate of oxygen consumption for each replicate was calculated by averaging the mass-corrected values

from the three shrimp for that replicate. Oxygen consumption is reported on a per gram basis (μL O_2/min·g) for ease of interpretation and comparison with previously reported values.

Food consumption data were analyzed using a GLM with shrimp mass as a covariate (Beaupre and Dunham, 1995). Lipid content, expressed as % dry weight, was analyzed by a GLM. Bioenergetic measurements were converted to units of energy (Joules) using the mathematical relationships and bioenergetic constants for *P. pugio* defined in Vernberg and Piyatiratitivorakul (1998), including the relationship between wet weight and dry weight of tissue, the oxycaloric coefficient for metabolic rate (0.0203 J/μL O_2), and average caloric value of grass shrimp (17.06 J/ mg dry weight).

Results

Metal Concentrations in Water and Shrimp Tissue

Concentrations of metals in exposure water are shown in Table 2.1. In the cadmium experiments, all treatment levels were significantly different (1st Cd experiment: $F_{3,12} = 3.4$ x 10^3, $P < 0.001$; 2nd Cd experiment: $F_{3,12} = 3.1$ x 10^4, $P < 0.001$). There were only slight differences in cadmium concentrations between the two cadmium experiments. During the course of the experiments, free cadmium ion concentration decreased an average of 23% between water changes. In the copper experiment, variation in metal data was higher than in the cadmium experiment, possibly due to saltwater interference with copper detection during the ICP-MS analysis. There was an overall difference in free copper ion concentrations ($F_{3,12} = 13.5$, $P < 0.001$) among treatment levels, but not all pairwise comparisons were statistically significant

(Table 2.1). Loss of free copper ion between water changes was impossible to estimate precisely due to high variability in measured concentrations.

Concentrations of copper and cadmium in shrimp tissue varied among treatment levels (Table 2.1; Cu: $F_{3,11} = 42.3$, $P < 0.001$ 1st Cd experiment: $F_{3,11} = 63.4$, $P < 0.001$; 2nd Cd experiment: $F_{3,11} = 56.2$, $P < 0.001$;). Bioaccumulation of metals was closely correlated with free metal ion concentration in exposure water (Cu: $r = 0.71$, $P = 0.002$; 1st Cd experiment: $r = 0.93$, $P < 0.001$; 2nd Cd experiment: $r = 0.95$, $P < 0.001$). During the second cadmium experiment, bioaccumulation of cadmium at each treatment level was slightly lower than in the first cadmium experiment, although aqueous cadmium concentrations were quite similar (Table 2.1).

Survival and Bioenergetics

Exposure to copper significantly affected survival of *P. pugio* ($F_{3,11} = 5.8$, $P = 0.013$). A significant reduction in survival was observed only at the highest copper concentration, yet average survival for this treatment still exceeded 65% (Fig. 2.1). Significant lethal effects were also observed in the first cadmium experiment ($F_{3,11} = 4.6$, $P = 0.025$); mortality was significant only at the highest cadmium concentration, at which survival averaged 64%. In the second cadmium experiment, cadmium exposure did not reduce survival relative to the control ($F_{3,11} = 2.5$, $P = 0.117$). In those experiments where survival was significantly affected by metal exposure, survival was inversely correlated with metal bioaccumulation (Cu: $r = -0.59$, $P = 0.017$; 1st Cd experiment: $r = -0.70$, $P = 0.002$; 2nd Cd experiment: $r = -0.30$, $P = 0.267$).

Copper and cadmium each resulted in a decrease in oxygen consumption (Fig. 2.2; Cu: $F_{3,11} = 6.3$, $P = 0.010$, Cd: $F_{3,11} = 3.7$, $P = 0.045$). The decline in respiration

was approximately equal in all copper-exposed shrimp, regardless of exposure concentration. In contrast, a stepwise decrease was observed as cadmium concentration increased.

Somatic growth rate was significantly affected by exposure to copper (Fig. 2.3; $F_{3,11} = 12.4$, P = 0.001); all copper treatment levels resulted in a negative average growth rate (i.e., weight loss). Growth rates at the Cu2 and Cu3 treatment levels were significantly different from the control. At the highest copper concentration, shrimp lost weight at a rate twice as great as the rate of weight gain in control shrimp. Cadmium exposure resulted in a decrease in growth rate relative to the control ($F_{3,7} = 4.6$, P = 0.044), but the average growth rate was positive, even at the highest cadmium concentration (Fig. 2.3).

The rate of food consumption was not significantly affected by either copper or cadmium exposure (Fig. 2.4; Cu: $F_{3,8} = 3.4$, P = 0.075; Cd: $F_{3,10} = 0.3$, P = 0.843). Neither copper nor cadmium exposure affected nonpolar lipid content of shrimp tissue (Cu: $F_{3,11} = 1.1$, P = 0.410; 1st Cd experiment: $F_{3,11} = 0.2$, P = 0.865, 2nd Cd experiment: $F_{3,11} = 1.0$, P = 0.413). Lipid content averaged 2-3% of dry body weight regardless of treatment level (Table 2.1).

Energetic investments in growth and respiration are shown in Fig. 2.5. Respiration comprised a considerably larger portion of the shrimp's energy budget than growth. The sum of growth and respiration, an approximation of energy assimilated from food, decreased as the concentration of copper or cadmium increased.

Discussion

Exposure to copper and cadmium resulted in a decrease in energy allocation to both maintenance and production pathways. Both metals resulted in a decrease in oxygen consumption of *P. pugio*, indicating a reduction in standard metabolic rate. The cadmium-induced decline in respiration was concentration-dependent, whereas the effects of copper appeared to be independent of exposure concentration. A decline in respiration is consistent with numerous studies that have demonstrated a significant decrease in oxygen consumption of aquatic invertebrates exposed to copper (e.g., *Mytilus edulis* [Brown and Newell, 1972], *Farfantepenaeus paulensis* [Santos et al., 2000]) or cadmium (e.g., *Nucella lapillus* [Leung et al., 2000], *Litopenaeus schmitti* [Barbieri, 2007]). Less frequently, an increase in oxygen consumption has been observed in response to copper (e.g., Cu: *Gammarus pulex* [Kedwards et al., 1996], *Tapes philippinarum* [Munari and Mistri, 2007]) or cadmium exposure (e.g., *Homarus americanus* [Thurberg et al., 1977], *Meretrix casta* [Kumarasamy and Karthikeyan, 1999]).

The declines in respiration that I observed may reflect effects of copper and cadmium at tissue, cellular, and biochemical levels. Gill necrosis, which would directly inhibit oxygen consumption, has been observed in crustaceans exposed to copper (*Carcinus maenas* [Nonnette et al., 1993], *Penaeus japonicus* [Soegianto et al., 1999a]) or cadmium (*Penaeus duorarum* and *Palaemonetes vulgaris* [Nimmo et al., 1977a], *P. japonicus* [Soegianto et al., 1999b]). Cadmium has also been shown to decrease the number of mitochondria per unit cell volume in *Anodonta cygnea* (Hemelraad et al., 1990) and *Crassostrea virginica* (Cherkasov et al., 2006), leading to a reduction in the

capacity for aerobic respiration. Furthermore, copper and cadmium can inhibit enzymes involved in metabolic pathways, including glycolysis (both Cu and Cd), the Krebs cycle (Cd), gluconeogenesis (Cd), and oxidative phosphorylation (Cd; Furst et al., 1998; Strydom et al., 2006), potentially resulting in a decline in oxygen consumption. It is unknown which of these processes, if any, were responsible for the metal-induced decline in respiration that I observed. Regardless of the mechanism(s) responsible, the expression of toxic effects as reduced respiration rate suggests that copper and cadmium altered energy allocation to metabolic processes required for maintenance and survival.

Growth of *P. pugio* exhibited a concentration-dependent decrease during exposure to either copper or cadmium. Copper effects were more severe, resulting in a weight loss of 11% at the highest concentration. A decline in somatic growth rate is consistent with many previous studies that have reported a decline in growth in response to copper (e.g., *Perna viridis* [Sze and Lee, 2000], *Penaeus monodon* [Chen and Lin, 2001]) or cadmium exposure (e.g., *Hydrobia ventrosa* [Forbes, 1991], *Litopenaeus vannamei* [Wu and Chen, 2005]). A decrease in growth suggests a reduction in energy allocation to the production pathway, which also supports reproduction and energy storage. Although reproduction did not occur during the current study (in either control or exposed *P. pugio*), copper and cadmium have been shown to reduce reproduction in several aquatic species (e.g., Cu: *Gammarus pseudolimnaeus* [Arthur and Leonard, 1970], *Bosmina longirostris* [Koivisto and Ketola, 1995]; Cd: *Daphnia magna*, *D. pulex*, and *Ceriodaphnia reticulata* [Elnabarawy et al., 1986], *P. pugio* [Manyin and Rowe, 2008]), which may reflect reduced allocation to the production pathway as a whole.

Neither copper nor cadmium had a significant effect on nonpolar lipid content, an estimate of energy storage, in *P. pugio*. Lipid content averaged 2-3% of dry tissue mass regardless of treatment, as compared to 7-15% in field-collected shrimp during late spring, when resources are very abundant (Rowe, unpublished data). The low lipid content in the laboratory individuals, as compared to field-collected individuals, is likely due to a comparatively limited food supply in the former, precluding large investments in energy stores even under control conditions. However, cadmium has been found to significantly reduce lipid stores in *D. magna* (De Coen and Janssen, 2003) and copper has been observed to increase lipid metabolism in *Potamonautes warreni* (Vosloo et al., 2002). Therefore, both copper and cadmium appear to be capable of reducing energy storage as lipids in some situations, although I did not observe such an effect.

Neither copper nor cadmium exposure affected the rate of food consumption by *P. pugio*, although both metals have been shown to reduce ingestion rates in other crustaceans (e.g., Cu: *F. paulensis* [Santos et al., 2000], *D. magna* [Knops et al., 2001]; Cd: *Callinectes sapidus* [Guerin and Stickle, 1995], *L. vannamei* [Wu and Chen, 2005]). It is possible that the metal concentrations used in the current study were not great enough to elicit an effect on consumption or that the effects of copper and cadmium are species-specific.

Bioaccumulation of copper and cadmium by *P. pugio* was proportional to the free ion concentration in the exposure water. Furthermore, in the experiments where metal exposure significantly affected survival, mortality was correlated with metal accumulation. In the first cadmium experiment, bioaccumulation was greater than in the second experiment, possibly due to differences in shrimp size; shrimp used in the first

experiment were smaller in mass. Bioaccumulation of metals can be related to body mass by a power function (Boyden, 1974), similar to functions relating metabolic processes with mass, causing smaller organisms to have higher concentrations of metals than larger organisms of the same species (Cubadda et al., 2001; Bjerregaard and Depledge, 2002). Consequently, accumulation of cadmium could have been disproportionately higher in the smaller shrimp, potentially accounting for higher mortality observed in the first cadmium experiment. Cadmium exposure did not cause significant mortality in the second experiment, corresponding with lower metal bioaccumulation.

When respiration and growth are examined with respect to energy use or investment (Fig. 2.5), it is apparent that respiration comprises a much larger portion of *P. pugio*'s energy budget than does growth, as was also demonstrated in an energy budget developed for *P. pugio* by Vernberg and Piyatiratitivorakul (1998). Assuming that energy allocated to other bioenergetic pathways, such as activity and reproduction, is minimal (e.g., shrimp were not reproductive during exposures), the amount of energy assimilated from food can be estimated by respiration plus growth (R+G, Fig. 2.5). Both copper and cadmium exposures resulted in a concentration-dependent decrease in R+G, reflecting a decline in the energy allocated to both metabolic and production pathways. Neither copper nor cadmium affected food consumption; therefore the dose-dependent decrease in R+G suggests that metal exposure may have caused a decrease in assimilation efficiency, as was reported for *Tapes philippinarum* exposed to copper (Munari and Mistri, 2007) and for *Leptomysis lingvura* exposed to cadmium (Gaudy et al., 1991).

Conclusion

Exposure of *P. pugio* to either copper or cadmium resulted in a significant decrease in respiration rate and growth. Both metals caused an overall metabolic depression, which reduced the amount of energy allocated to all bioenergetic pathways. A similar metabolic depression has been reported for copper in *P. viridis* (Sze and Lee, 2000) and for cadmium in *N. lapillus* (Leung et al., 2000). Due to *P. pugio*'s ecological importance in estuarine systems, a metal-induced metabolic depression could ultimately affect the efficiency of energy flow through benthic and epibenthic communities in contaminated habitats.

Table 2.1. Chemistry data from 14-day exposures (means, SE in parentheses): dissolved free metal ion concentrations in exposure water (µg/L), metal concentrations in shrimp tissue (µg/g dry weight), and lipid content in shrimp tissue (% dry weight). Free metal ion concentration was calculated from the measured total metal concentration using the chemical equilibrium program MINEQL+ v. 4.5 (Environmental Research Software, Hallowell, ME). Different superscript letters indicate significant differences among treatments within a given experiment. M = metal used in experiment (Cu or Cd).

	Treatment	Target $[M^{2+}]_{(aq)}$	Measured $[M^{2+}]_{(aq)}$	Tissue [M]	Tissue [Lipids]
Cu Experiment	Cu0	0	< 0.01[a] (< 0.01)	148.37[a] (10.89)	1.93 (0.14)
	Cu1	24	7.54[ab] (2.15)	286.65[b] (10.89)	1.70 (0.10)
	Cu2	35	27.03[bc] (8.61)	327.95[b] (19.96)	1.67 (0.17)
	Cu3	47	41.29[c] (5.04)	440.60[c] (25.95)	1.99 (0.19)
First Cd Experiment	Cd0	0	0.07[a] (< 0.01)	1.19[a] (0.11)	2.98 (0.12)
	Cd1	2.2	2.48[b] (0.03)	42.06[b] (3.86)	3.30 (0.42)
	Cd2	3.9	4.65[c] (0.04)	64.06[c] (5.42)	3.02 (0.37)
	Cd3	5.6	6.55[d] (0.08)	67.52[c] (3.15)	2.98 (0.19)
Second Cd Experiment	Cd0	0	0.07[a] (< 0.01)	0.90[a] (0.12)	2.17 (0.14)
	Cd1	2.2	2.55[b] (0.01)	28.99[b] (2.70)	1.80 (0.19)
	Cd2	3.9	4.49[c] (0.02)	37.17[b] (2.15)	1.79 (0.19)
	Cd3	5.6	6.17[d] (0.02)	52.02[c] (4.55)	2.01 (0.16)
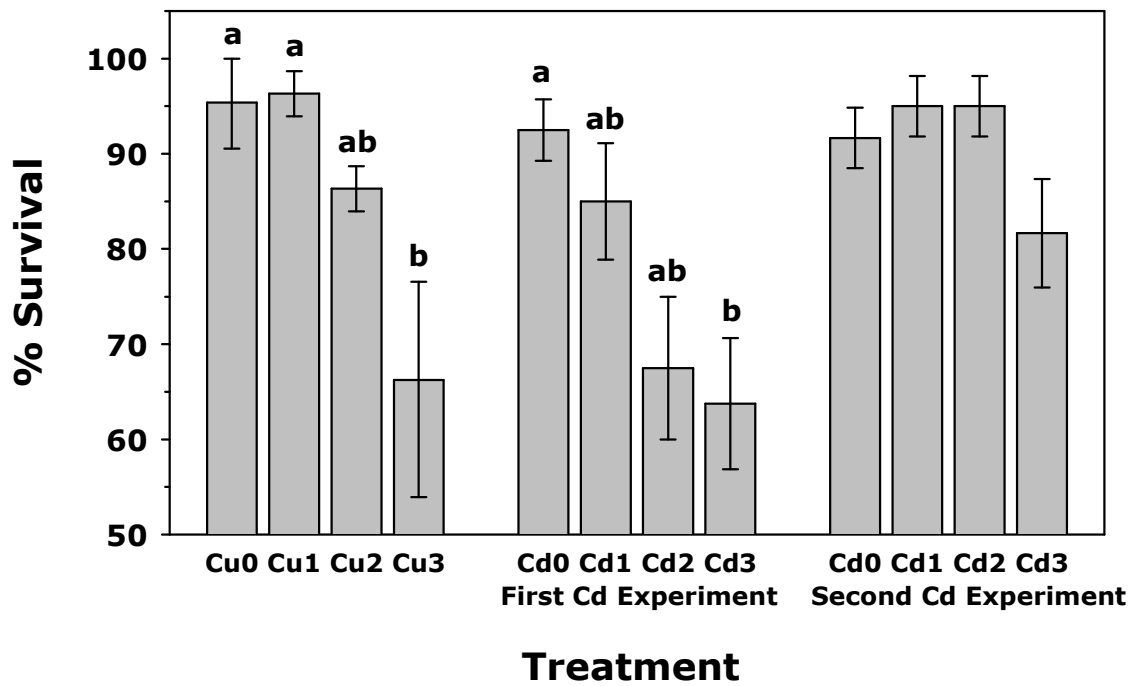
Fig. 2.1. Survival of *P. pugio* after 14 days of exposure to either copper or cadmium. Treatments are denoted by the metal used in the exposure (Cu or Cd) and a number ranging from 0 to 3, where 0 indicates the control and 3 is the highest metal concentration. Actual copper and cadmium concentrations are provided in Table 2.1. Different letters above bars represent significant differences. There were no significant effects on survival in the second cadmium experiment.
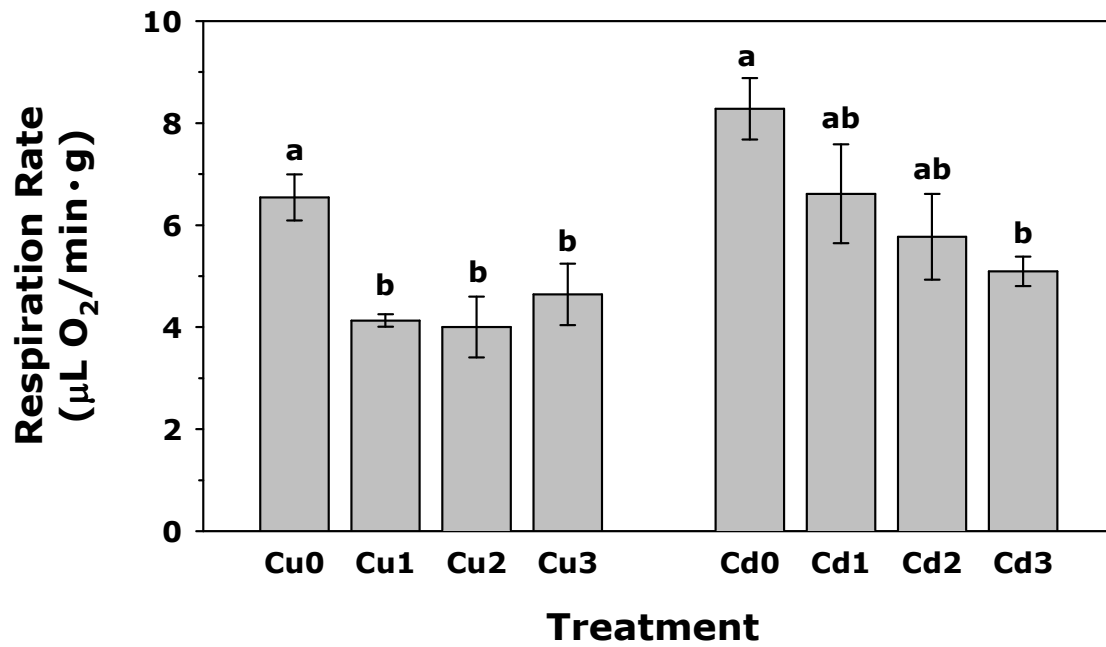
Fig. 2.2. Respiration (standard metabolic rate) of *P. pugio* after a 14-day exposure to either copper or cadmium, expressed as oxygen consumption (μL/min) per g (wet weight) shrimp tissue. Copper and cadmium exposure concentrations are provided in Table 2.1. Different letters above bars represent significant differences.

Fig. 2.3. Somatic growth (% change in wet weight) of *P. pugio* during a 14-day exposure to either copper or cadmium. Copper and cadmium exposure concentrations are provided in Table 2.1. Different letters above bars represent significant differences.
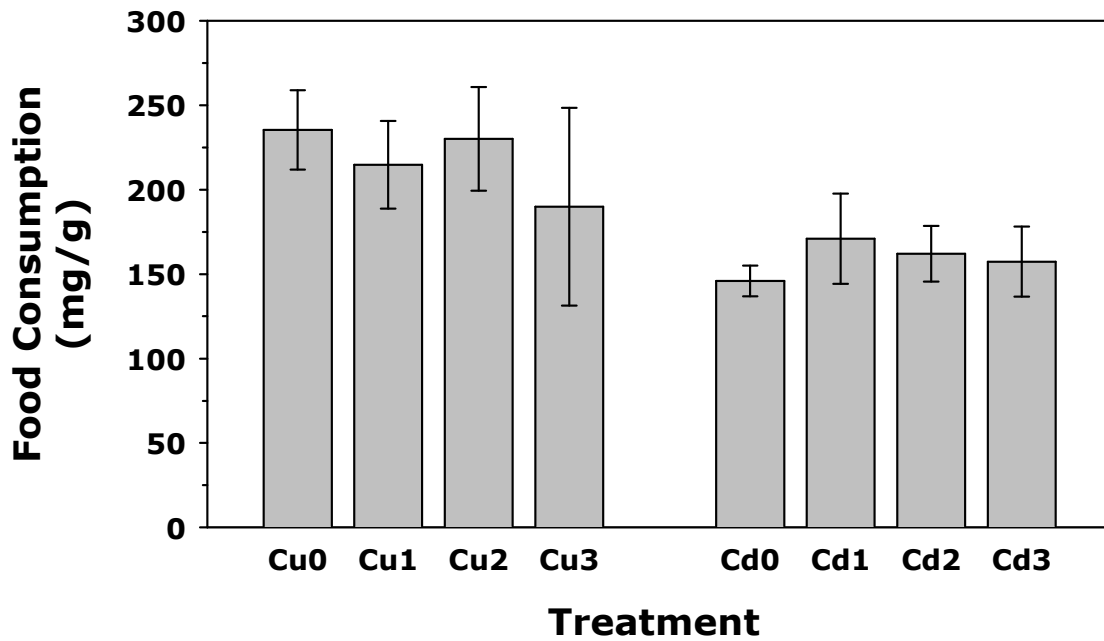
Fig. 2.4. Consumption of food (mg [wet weight] clams per g [wet weight] shrimp) over 16 hours after a 14-day exposure to either copper or cadmium. Copper and cadmium exposure concentrations are provided in Table 2.1. There were no significant differences among treatments.
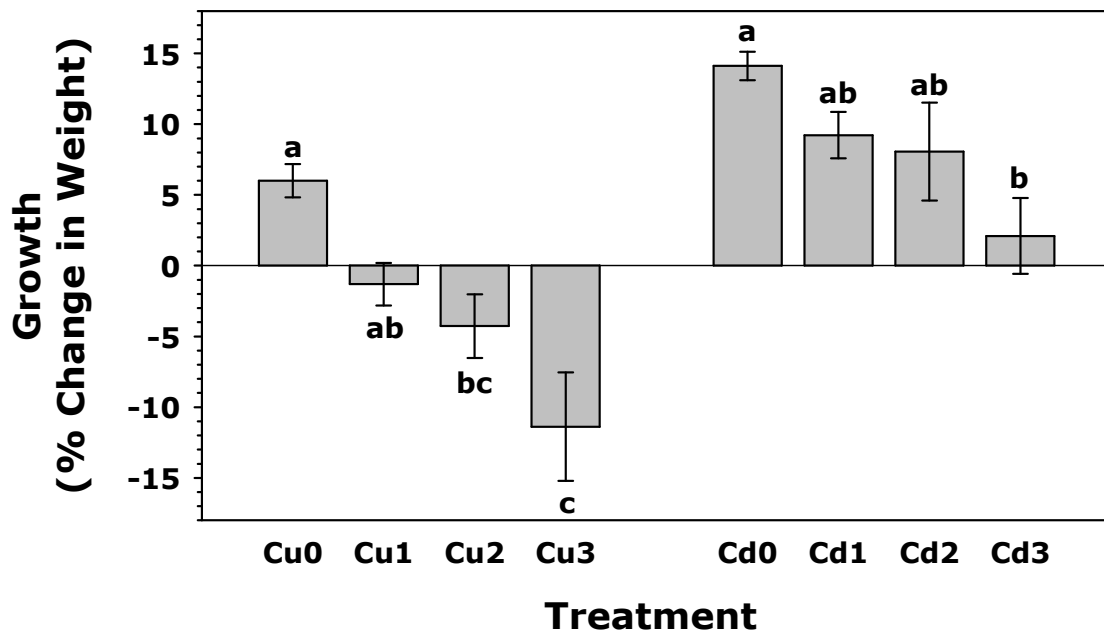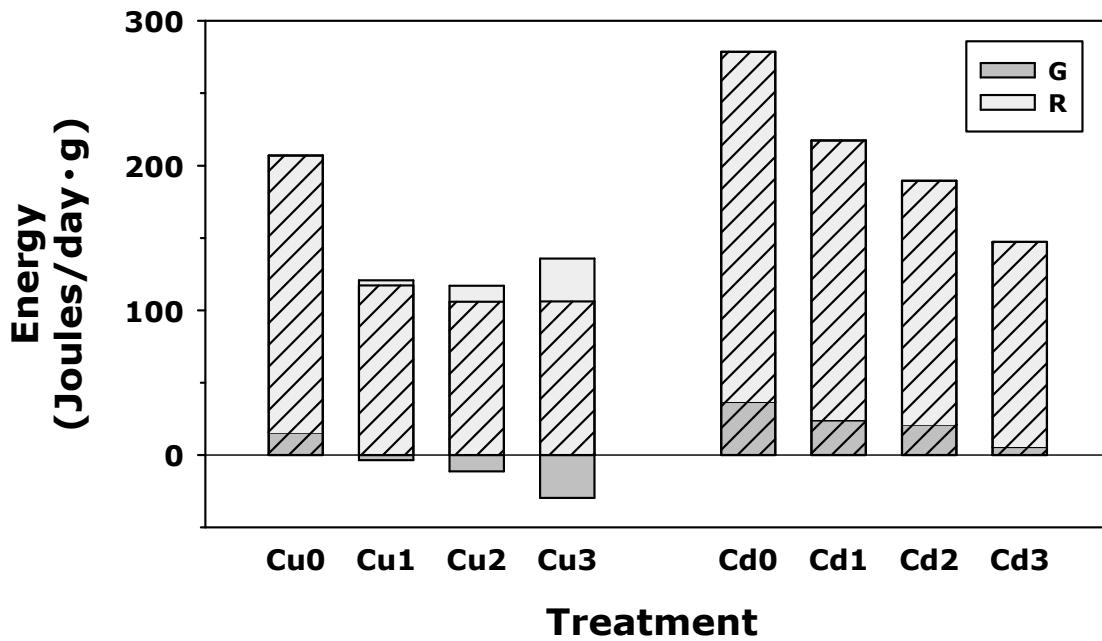
Fig. 2.5. Energetic values for somatic growth (G, dark grey background) and respiration
(R, light grey background). Bioenergetic parameters have been converted to their daily
energetic equivalents per gram (wet weight) of shrimp tissue. The cross-hatched portion
represents G + R, which is an approximation of the energy assimilated from food. The
area of R that is not cross-hatched is energy derived from the loss of somatic tissue.
Copper and cadmium exposure concentrations are provided in Table 2.1.

MODELING EFFECTS OF CADMIUM ON POPULATION GROWTH OF

PALAEMONETES PUGIO: RESULTS OF A FULL LIFE CYCLE EXPOSURE[1]

Abstract

In an eight-month laboratory experiment, *Palaemonetes pugio* (grass shrimp) were exposed to aqueous cadmium (free cadmium ion concentrations of 1.51 or 2.51 μg Cd^{2+}/L) for an entire life cycle, from larva to reproductive adult and through to production of second-generation larva. Individual-level effects on survival, life stage duration, and reproduction were measured, and population growth was projected using two models: a stage-based matrix model and a z-transformed life cycle graph analysis. Adult survival was significantly reduced at 2.51 μg Cd^{2+}/L, but cadmium exposure had no effect on survival or stage duration of embryos, larvae, or juveniles. Survival of second-generation larvae was unaffected by maternal exposure. Brood size was reduced by 27% at 1.51 μg Cd^{2+}/L and by 36% at 2.51 μg Cd^{2+}/L. The percent of females in the population that was gravid was approximately 50% lower at 2.51 μg Cd^{2+}/L, compared to controls. Both population models projected a dose-dependent decrease in population growth rate (λ), up to a 12% reduction at 2.51 μg Cd^{2+}/L, which can be attributed mainly to contributions from reproductive effects. Elasticity analysis revealed that population growth rate was most sensitive to changes in survival of juveniles and adults. However, lethal effects of cadmium made only a small contribution to the effect on population growth rate. Even though both models project positive growth (λ > 1) of grass shrimp

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populations exposed to low concentrations of cadmium, the ability of populations to withstand predation pressure would be compromised.

Introduction

Mathematical modeling is a valuable tool for the prediction of population-level effects resulting from chronic exposure to sublethal concentrations of contaminants (e.g., Daniels and Allan, 1981; Munns et al., 1997; Hansen et al., 1999; Salice and Miller, 2003; Raimondo and McKenney, 2006). Population growth rate has been frequently used as a measure of population performance (Caswell, 1996), and is believed to be a more appropriate measure of toxicological effects than individual-level responses, because it integrates the interactions among individual-level effects into a single measure of ecological impact (Forbes and Calow, 1999). Species with complex life cycles will often exhibit stage-specific responses to contaminants (e.g., McGee et al., 1993; Munns et al., 1997; Jensen et al., 2001). Effects on each life stage must be considered, in concert, to obtain an accurate projection of chronic effects at the population level. Population modeling allows the integration of individual-level effects on each life stage to project chronic effects at the population level.

Cadmium is a relatively rare, naturally occurring element that is not biologically essential. Cadmium serves many technological uses, such as in the protective plating of steel, as a stabilizer for polyvinyl chloride, and as electrode material in nickel-cadmium batteries (WHO, 1992). Concentrations of cadmium in coastal habitats are often elevated due to nearby human activities (e.g., Hall et al., 1998; Yang and Sañudo-Wilhelmy, 1998; Chiffoleau et al., 2001). Aquatic sources of cadmium include drainage from metal mines

and smelters, runoff of agricultural fertilizers, and atmospheric fallout of byproducts from combustion of fossil fuels and refuse incineration (WHO, 1992).

Although cadmium is not an essential element, organisms may accumulate it via pathways used for calcium uptake (Wright, 1995). The bioavailable, toxic form of cadmium is generally accepted to be the divalent free metal ion, Cd^{2+} (e.g., Sunda et al., 1978), but salinity and calcium concentration may also influence cadmium toxicity (Wright and Frain, 1981; De Lisle and Roberts, 1988). General mechanisms of cadmium toxicity include binding to proteins and DNA at sulfhydryl, sulfate, and carbonyl sites (Furst et al., 1998), resulting in the impairment of protein and DNA function, and leading to wide-ranging physiological effects. Cadmium can also inhibit calcium uptake and calcium channels, inhibit DNA repair, and cause lipid peroxidation (Stohs and Bagchi, 1995; Furst et al., 1998; Strydom et al., 2006). Sublethal effects of cadmium on population growth rate have been described in various species of aquatic invertebrates, including *Daphnia magna* (Van Leeuwen et al., 1987), *Potamopyrgus antipodarum* (Jensen et al., 2001), *Moinodaphnia macleayi* (Barata et al., 2002), and *Biomphalaria glabrata* (Salice and Miller, 2003).

Palaemonetes pugio is an abundant and widely distributed estuarine inhabitant of the Atlantic and Gulf coasts of the United States (Gosner, 1971; Anderson, 1985), where it serves as an important prey item for numerous fish species, including commercially important species (Wood, 1967). The life cycle of *P. pugio* consists of four stages: embryo, larva, juvenile, and adult. Embryos are carried in an external brood pouch by the adult female until they hatch and larvae are released. Larvae pass through seven or eight sub-stages (Little, 1968) prior to metamorphosis to the juvenile life stage, when

they become morphologically similar to adults. Individuals are defined as adults once they reach sexual maturity. Under optimal conditions in the laboratory, embryos can mature to adulthood within four months (Wood, 1967; Little, 1968). Sexually mature females may produce a total of four to six broods per year in the laboratory (Poole, 1987); in Texas, near the southern end of its range, *P. pugio* was found to have two peak spawning seasons per year (Wood, 1967). The lifespan of *P. pugio* may be as long as two years in the wild (Poole, 1987), but is often much shorter due to intense predation pressures by fish (Nixon and Oviatt, 1973; Clark et al., 2003).

In this study, *P. pugio* were exposed to cadmium for an entire life cycle, allowing me to quantify effects on all life stages. Exposures were conducted for eight months, starting with freshly hatched larvae and continuing through to production of second-generation larvae. Effects of cadmium on survival and duration of each life stage were measured. Reproduction was measured as fecundity and frequency of broods. Two types of models were applied to the individual-level data to project population-level effects of cadmium on *P. pugio*: a stage-based matrix model and a z-transformed life cycle graph analysis (Caswell, 2001). Each model provides an independent estimate of population growth rate (λ), decomposes population-level effects of cadmium into individual-level contributions, and predicts the vital rate that has the greatest influence on λ through sensitivity analysis. Given that the two models utilize different variables to describe population dynamics, each produces a different set of sensitivities and contributions. Finally, the effect of predation on growth of cadmium-exposed populations was projected.

Methods

Experimental Organisms

      Adult *P. pugio* were collected from the Patuxent River, MD, USA and maintained in flow-through laboratory stock tanks, which received ambient water from the lower Patuxent River. Breeding was encouraged by increasing water temperature to 25 °C and providing a generous food supply, consisting of a combination of brine shrimp (*Artemia*) nauplii and dry food (1:1 mixture of Wardley [Secaucus, NJ] Shrimp Pellets and Wardley Pond Ten Floating Fish Stix, both coarsely ground). When females became gravid, they were isolated in individual 1-L beakers, their chelae were trimmed to reduce cannibalism of eggs (Little, 1968), and they were suspended in a 3 mm-mesh cage at the top of the beaker. As the embryos hatched, the larvae passed through the mesh to the bottom of the beaker. Cadmium exposures were initiated with freshly-hatched larvae (0- or 1-day post-hatch); larvae from thirty-one clutches were divided approximately equally among treatments to reduce confounding effects due to clutch-specific differences in genetics and/or maternal effects on offspring fitness.

General Exposure Conditions

      The exposures occurred in the laboratory in a temperature-controlled room at a constant water temperature of 25 °C, which falls within the optimal temperature ranges for spawning, embryonic development, larval development, and overall survival of *P. pugio* (Wood, 1967; Poole, 1987). Exposure water consisted of Instant Ocean artificial seawater (Aquarium Systems, Mentor, OH), diluted to a salinity of 10 ppt with tap water that had been filtered by reverse osmosis (RO). Nitrilotriacetic acid (NTA), a metal chelator, was added to the exposure media at a concentration of 5×10^{-5} M, in order to

buffer the concentration of free metal ion (Perrin and Dempsey, 1974). The pH was adjusted to ~7.8 with NaOH. Salinity was maintained by replacing water lost to evaporation with RO water daily. A complete water change was conducted every four days during the larval stage and every eight days during the juvenile and adult stages. During water changes, exposure conditions were maintained by transferring test organisms to temporary holding chambers containing exposure water. All life stages were fed a generous ration of *Artemia* nauplii daily, which was supplemented with dry food for juveniles and adults, as described above. The light regime consisted of a 16:8 hour ratio of light to dark; long photoperiods have been found to induce breeding in *P. pugio* (Little, 1968; Rayburn and Fisher, 1999).

Cadmium was added as $CdCl_2$ to achieve target free ion concentrations of either 1.36 μg Cd^{2+}/L (Cd-low) or 2.27 μg Cd^{2+}/L (Cd-high). The amount of metal required to achieve the target free ion concentration of cadmium was calculated using the chemical equilibrium program MINEQL+ v. 4.5 (Environmental Research Software, Hallowell, ME), given the target free ion concentration, composition of Instant Ocean (Atkinson and Bingman, 1998), and concentration of NTA. The control contained no added $CdCl_2$. Each treatment was replicated four times; sets of replicates (each set consisting of one replicate of each treatment) were staggered in time, no more than two days apart, for logistical purposes. Replicates were blocked by shelf height to account for effects of small temperature variations (< 1 °C) observed among shelves. Each block consisted of one set of replicates. During larval and juvenile stages, a randomized complete block design was employed, with each block (shelf) containing one replicate of each treatment.

During the adult stage, a randomized incomplete block design (Anderson and McLean, 1974) was utilized due to space restrictions.

Life Cycle Exposure

Exposures began with 110 larvae per replicate in 1.5-L beakers, filled with 1 L of exposure water. Survival was measured every four days, coinciding with water changes. Larvae were surveyed daily for metamorphosis to the juvenile stage. Upon metamorphosis, juveniles were removed to another set of beakers, in which they were held prior to initiating the juvenile exposures. In order to reduce variation in the age of individuals, only the middle 80% of larvae to metamorphose were retained; approximately the initial 10% and final 10% to metamorphose were discarded, removing the extremes from the population.

After metamorphosis (on day 28), juveniles were transferred to 8-L tanks filled with 6 L of exposure water, and the number of individuals per replicate was reduced to 55 to prevent overcrowding. Juveniles derived from each replicate during the larval exposure were placed in a corresponding replicate for the juvenile exposure. From this point onward, survival was measured every 8 days, again coinciding with water changes. On day 60, juveniles were transferred to 38-L tanks filled with 17 L of exposure water.

On day 86, the first female became gravid, after which each replicate was checked daily for the presence of gravid females. Gravid females were removed, their chelae were trimmed, and they were transferred to individual 0.95-L mason jars filled with 0.6 L of exposure water. While isolated, females were fed *Artemia* only. After 8 days, the water was changed, and females were suspended in a 3 mm-mesh cage at the top of the jar. Upon hatching (13 days after eggs were laid, on average), larvae were counted and

the adult female was returned to its original tank. Survival and reproduction continued to be measured until the experiment was terminated on day 240; any females gravid at this time were held until their eggs hatched.

Hatching success was measured by removing embryos from gravid females on the third day after being laid, when the embryos had reached the "tissue cap" stage of development (Rayburn and Fisher, 1999) and could be easily separated from each other without damage. Forty embryos per female were removed (3-5 females per replicate) and placed in individual wells of polypropylene microcentrifuge tube racks with 2 mL of exposure water and incubated at 25 °C until they hatched.

Second-Generation Effects

To determine effects of parental exposure on offspring fitness, survival of larvae collected from the control and Cd-high treatment females was measured. Thirty, second-generation (F_2) larvae from each of three females per replicate were transferred to 1.5-L beakers filled with 1 L of exposure water. Fifteen larvae were placed in control exposure water and fifteen in Cd-high exposure water. Larval survival was measured after 8 days. Survival measurements were not continued beyond this point due to personnel and space limitations.

Metals Analysis

Water samples for cadmium analysis were taken from the first generation tanks (one replicate per treatment) immediately after each water change. Immediately before every third water change, water samples were taken to assess the change in cadmium concentration between water changes. Samples were filtered through a 0.22 μm nitrocellulose membrane, acidified to pH < 2 with HNO_3, diluted 1:10 to reduce saltwater

interference, and analyzed using an Agilent (Santa Clara, CA, formerly Hewlett-Packard) HP4500 inductively coupled plasma mass spectrometer (ICP-MS). Free cadmium ion concentrations were calculated using MINEQL+ v. 4.5.

At the end of the exposure (day 240), several non-gravid adults (7-10 per replicate) were allowed to depurate in clean seawater for three hours and were then preserved for tissue analysis by freezing at -80 °C. Tissue samples were later freeze-dried and crushed in a plastic bag with a pestle. A subsample (~100 mg) of the homogenized sample was digested by adding 2 mL concentrated HNO_3 and heating in a 60 °C oven overnight. The digested sample was diluted and analyzed for cadmium by ICP-MS.

Matrix Model

To project population growth, a stage-classified matrix model was constructed, using the general principles explained by Caswell (2001). Matrix calculations were performed using PopTools (Hood, 2003), an add-in for Microsoft Excel. The life cycle graph (Fig. 3.1A) illustrates the transitions from one life stage to the next. G, P, and F comprise the vital rates of the population. G_i is the probability that an individual in stage i will survive and graduate to the next life stage over one time step and P_i is the probability that an individual in stage i will survive and remain in the same life stage. F_i is the fertility of an individual in stage i, that is, the average number of surviving female offspring produced over one time step per individual in stage i at the beginning of the time step. Juveniles may mature to adulthood and reproduce within one time step, using the birth-flow model, and therefore they have an associated F term. A time step of 13 days was used, which was the average duration of the embryonic stage across all

treatments. The model follows only females and female offspring, and assumes a 1:1 sex ratio in the population.

G, P, and F values were used to construct a population projection matrix, **A**, such that n(t+1) = **A**n(t), where n(t) is the vector of abundances at time t, and n(t+1) is the vector of abundances of each life stage at time t+1, one time step later. The projection matrix contains F values in the first row, P values along the diagonal, and G values along the subdiagonal. The dominant eigenvalue of **A** was calculated to obtain an estimate of the finite rate of population growth, λ.

$$
A = \begin{pmatrix} P_1 & 0 & F_3 & F_4 \\ G_1 & P_2 & 0 & 0 \\ 0 & G_2 & P_3 & 0 \\ 0 & 0 & G_3 & P_4 \end{pmatrix}
$$

Calculations of G, P, and F were performed using the methods for stage-based matrices described by Caswell (2001). The probability of survival of an individual in stage i over one time step, σ_i, was estimated by changing the time scale of the experimental survival data to the 13-day time step:

$$
\sigma_{i,t=13days} = \sigma_{i,t=n}^{13/n} \tag{1}
$$

where n = the number of days between survival measurements. The probability of growth from stage i to stage i+1 over one time step, γ_i, was estimated by:

$$
\gamma_i = \frac{(\frac{\sigma_i}{\lambda})^{T_i} - (\frac{\sigma_i}{\lambda})^{T_i-1}}{(\frac{\sigma_i}{\lambda})^{T_i} - 1} \tag{2}
$$

where T_i is the duration of stage i in number of time steps, measured during the full life cycle exposure. The value of λ was estimated iteratively by solving for the dominant

eigenvalue of the projection matrix (the elements of which are dependent upon γ_i) and reinserting this value into Eq. 2 until a stable value of λ was reached.

Equations to calculate G and P take the general form of Eqs. 3 and 4, respectively:

$$G_i = \sigma_i \gamma_i \tag{3}$$

$$P_i = \sigma_i (1 - \gamma_i) \tag{4}$$

Fertility, F, was calculated using the birth-flow model for continuously reproducing species, which assumes that adults reproduce halfway through the time step, requiring that offspring survive through half of a time step before being counted in the first stage class:

$$F_i = l(0.5)(\frac{(1 + P_i)m_i + G_i m_{i+1}}{2}) \tag{5}$$

where m_i (or maternity) is the average number of female offspring per female in stage i per time step (Eq. 6), and $l(0.5)$ is the probability of an embryo surviving to the midpoint of the next time step. Maternity was estimated by using fecundity and incorporating the average percentage of adult females that was gravid at any given time and also assuming a 1:1 sex ratio of offspring:

$$m_4 = (\%\ females\ gravid)(mean\ brood\ size)/2 \tag{6}$$

Specific calculations of G, P, and F are shown in the following equations:

$$G_1 = \sigma_1^{1/2} \sigma_4^{1/2} \gamma_1 \tag{7}$$

$$P_1 = \sigma_1^{1/2} \sigma_4^{1/2} (1 - \gamma_1) \tag{8}$$

$$G_2 = \sigma_2 \gamma_2 \tag{9}$$

$$P_2 = \sigma_2 (1 - \gamma_2) \tag{10}$$

$$G_3 = \sigma_3 \gamma_3 \tag{11}$$

$$P_3 = \sigma_3(1 - \gamma_3) \tag{12}$$

$$P_4 = \sigma_4 \tag{13}$$

$$F_3 = \sigma_1^{1/2} \sigma_4^{1/2} G_3 m_4 / 2 \tag{14}$$

$$F_4 = \sigma_1^{1/2} \sigma_4^{1/2} (1 + P_4) m_4 / 2 \tag{15}$$

Embryonic survival values ($\sigma_1^{1/2}$) in P_1 and G_1 correspond to only one half of a time step, in order to offset the inclusion of $\sigma_1^{1/2}$ in F (Brault and Caswell, 1993). Also, the survival term for adults (σ_4) is included in the formulae for P_1, G_1, and F, because survival through the embryonic period is contingent upon survival of the mother, who carries the embryos until they hatch; in a natural setting, the eggs cannot survive if the mother dies. This is a necessary adaptation of the generalized equations (Eqs. 3 and 4) for any species in which survival of the embryonic life stage depends upon survival of the adult.

Sensitivity analysis, or prospective perturbation analysis, was performed in order to measure the effect on the population growth rate, λ, of an infinitesimal change in a vital rate, while all other vital rates are held constant (De Kroon et al., 1986). Sensitivities are measured as a response of λ to equal absolute changes in each vital rate. For the matrix model, sensitivities were calculated using the dominant right and left eigenvectors (**w** and **v**, respectively) of the projection matrix, **A**. The sensitivity of λ to each matrix element, a_{ij}, is denoted by $\partial\lambda/\partial a_{ij}$, and was calculated in Eq. 16 (Caswell, 1978), where $<>$ indicates the scalar product of two vectors:

$$\frac{\partial\lambda}{\partial a_{ij}} = \frac{v_i w_j}{<w, v>} \tag{16}$$

To calculate elasticities, e_{ij}, sensitivities for each vital rate were all normalized to the same scale, as shown in Eq. 17 (De Kroon et al., 1986):

$$e_{ij} = \frac{a_{ij}}{\lambda} \frac{\partial \lambda}{\partial a_{ij}} \qquad (17)$$

The elasticities of λ to perturbations in G, P, and F sum to one, and measure the response of population growth rate to proportional perturbations of each vital rate (De Kroon et al., 1986). Elasticities, rather than sensitivities, are reported, because they may be compared amongst each other more easily than sensitivities.

Retrospective perturbation analysis was also performed in order to decompose treatment effects on λ into contributions from each stage-specific vital rate. The contribution, c_{ij}, of each matrix element was calculated by:

$$c_{ij} = (a_{ij}^{(b)} - a_{ij}^{(r)}) \frac{\partial \lambda}{\partial a_{ij}} \qquad (18)$$

where b refers to treatment values, r refers to reference or control values, and the sensitivity, $\partial \lambda / \partial a_{ij}$, is evaluated at the mean a_{ij} between the treatment and control, $(a_{ij}^{(b)} + a_{ij}^{(r)})/2$ (Caswell, 1996). The sum of the contributions of treatment effects on the matrix elements is approximately equal to the treatment effect on λ (Caswell, 1996):

$$\lambda^{(b)} - \lambda^{(r)} \approx \sum_{i,j} (a_{ij}^{(b)} - a_{ij}^{(r)}) \frac{\partial \lambda}{\partial a_{ij}} \qquad (19)$$

Z-Transformed Life Cycle Graph Analysis

Population growth was also modeled using a z-transformed life cycle graph analysis. This modeling method provides a second estimate of population growth rate and allows the calculation of sensitivities and contributions of λ to lower level parameters, as compared to the matrix model. The method is based on the z-transformed life cycle graph shown in Fig. 3.1B (derived from methods in Caswell, 1996 and Caswell, 2001, as illustrated in Salice and Miller, 2003), which is a modification of the life cycle

graph used for the matrix model. In the z-transformed life cycle graph, all vital rates are multiplied by λ raised to a negative exponent that is equivalent to the time required for the transition indicated by the arrow, and the F_3 term and self-loops have been removed, with the exception of the adult self-loop (Caswell, 1996).

In the z-transformed life cycle graph analysis, P_i is the probability of survival of an individual in stage i over the duration of the transition indicated in the z-transformed life cycle graph, as defined by the following equations:

$$P_1 = \sigma_1^{\delta_1} \sigma_4^{\delta_1} \tag{20}$$

$$P_2 = \sigma_2^{\delta_2} \tag{21}$$

$$P_3 = \sigma_3^{\delta_3} \tag{22}$$

$$P_4 = \sigma_4 \tag{23}$$

where δ_i is the duration of stage i in time steps, i.e., the number of time steps required to develop from stage i to stage i+1 (equivalent to the term T_i in the matrix model). Fertility, F, was calculated using a post-breeding birth pulse model (Caswell, 2001):

$$F = P_4 m_4 \tag{24}$$

A characteristic equation was derived from the z-transformed life cycle graph by setting the sum of all loops in the graph equal to 1 (Hubbell and Werner, 1979; Caswell, 2001) and simplifying:

$$\lambda^{\delta_1 + \delta_2 + \delta_3 + 1} - P_1 P_2 P_3 F - P_4 \lambda^{\delta_1 + \delta_2 + \delta_3} = 0 \tag{25}$$

The characteristic equation was then solved to obtain an estimate of λ.

The sensitivities of λ to the parameters in the z-transformed life cycle graph were determined by implicit differentiation of the characteristic equation (Caswell, 1996).

This method allows the calculation of sensitivities of λ to the underlying vital rates, σ_i and δ_i, in combination with fertility, F. The resulting sensitivities are shown in Eqs. 26-33, where $\partial\lambda/\partial x$ denotes the sensitivity of λ to x.

$$\frac{\partial \lambda}{\partial \sigma_1} = \frac{\delta_1 \sigma_1^{\delta_1-1} \sigma_2^{\delta_2} \sigma_3^{\delta_3} \sigma_4^{\delta_1} F}{(\delta_1 + \delta_2 + \delta_3 + 1)\lambda^{\delta_1+\delta_2+\delta_3} - (\delta_1 + \delta_2 + \delta_3)\sigma_4 \lambda^{\delta_1+\delta_2+\delta_3-1}} \tag{26}$$

$$\frac{\partial \lambda}{\partial \sigma_2} = \frac{\delta_2 \sigma_1^{\delta_1} \sigma_2^{\delta_2-1} \sigma_3^{\delta_3} \sigma_4^{\delta_1} F}{(\delta_1 + \delta_2 + \delta_3 + 1)\lambda^{\delta_1+\delta_2+\delta_3} - (\delta_1 + \delta_2 + \delta_3)\sigma_4 \lambda^{\delta_1+\delta_2+\delta_3-1}} \tag{27}$$

$$\frac{\partial \lambda}{\partial \sigma_3} = \frac{\delta_3 \sigma_1^{\delta_1} \sigma_2^{\delta_2} \sigma_3^{\delta_3-1} \sigma_4^{\delta_1} F}{(\delta_1 + \delta_2 + \delta_3 + 1)\lambda^{\delta_1+\delta_2+\delta_3} - (\delta_1 + \delta_2 + \delta_3)\sigma_4 \lambda^{\delta_1+\delta_2+\delta_3-1}} \tag{28}$$

$$\frac{\partial \lambda}{\partial \sigma_4} = \frac{\delta_1 \sigma_1^{\delta_1} \sigma_2^{\delta_2} \sigma_3^{\delta_3} \sigma_4^{\delta_1-1} F + \lambda^{\delta_1+\delta_2+\delta_3}}{(\delta_1 + \delta_2 + \delta_3 + 1)\lambda^{\delta_1+\delta_2+\delta_3} - (\delta_1 + \delta_2 + \delta_3)\sigma_4 \lambda^{\delta_1+\delta_2+\delta_3-1}} \tag{29}$$

$$\frac{\partial \lambda}{\partial \delta_1} = \frac{\sigma_1^{\delta_1} \sigma_2^{\delta_2} \sigma_3^{\delta_3} \sigma_4^{\delta_1} F \ln(\sigma_1 \sigma_4) + (\sigma_4 \lambda^{\delta_1+\delta_2+\delta_3} - \lambda^{\delta_1+\delta_2+\delta_3+1})\ln \lambda}{(\delta_1 + \delta_2 + \delta_3 + 1)\lambda^{\delta_1+\delta_2+\delta_3} - (\delta_1 + \delta_2 + \delta_3)\sigma_4 \lambda^{\delta_1+\delta_2+\delta_3-1}} \tag{30}$$

$$\frac{\partial \lambda}{\partial \delta_2} = \frac{\sigma_1^{\delta_1} \sigma_2^{\delta_2} \sigma_3^{\delta_3} \sigma_4^{\delta_1} F \ln \sigma_2 + (\sigma_4 \lambda^{\delta_1+\delta_2+\delta_3} - \lambda^{\delta_1+\delta_2+\delta_3+1})\ln \lambda}{(\delta_1 + \delta_2 + \delta_3 + 1)\lambda^{\delta_1+\delta_2+\delta_3} - (\delta_1 + \delta_2 + \delta_3)\sigma_4 \lambda^{\delta_1+\delta_2+\delta_3-1}} \tag{31}$$

$$\frac{\partial \lambda}{\partial \delta_3} = \frac{\sigma_1^{\delta_1} \sigma_2^{\delta_2} \sigma_3^{\delta_3} \sigma_4^{\delta_1} F \ln \sigma_3 + (\sigma_4 \lambda^{\delta_1+\delta_2+\delta_3} - \lambda^{\delta_1+\delta_2+\delta_3+1})\ln \lambda}{(\delta_1 + \delta_2 + \delta_3 + 1)\lambda^{\delta_1+\delta_2+\delta_3} - (\delta_1 + \delta_2 + \delta_3)\sigma_4 \lambda^{\delta_1+\delta_2+\delta_3-1}} \tag{32}$$

$$\frac{\partial \lambda}{\partial F} = \frac{\sigma_1^{\delta_1} \sigma_2^{\delta_2} \sigma_3^{\delta_3} \sigma_4^{\delta_1}}{(\delta_1 + \delta_2 + \delta_3 + 1)\lambda^{\delta_1+\delta_2+\delta_3} - (\delta_1 + \delta_2 + \delta_3)\sigma_4 \lambda^{\delta_1+\delta_2+\delta_3-1}} \tag{33}$$

Elasticities of λ to σ_i, δ_i, and F were calculated as in Eq. 17, by substituting σ_i, δ_i, or F for a_{ij}.

The contributions of parameters from the z-transformed life cycle graph to treatment effects on λ were calculated as in Eq. 18, again substituting σ_i, δ_i, or F for a_{ij}. The sum of the contributions of treatment effects on σ_i, δ_i, and F is approximately equal to the treatment effect on λ, as shown in Eq. 34 (Caswell, 1996):

$$\lambda^{(b)} - \lambda^{(r)} \approx \sum_i (\sigma_i^{(b)} - \sigma_i^{(r)}) \frac{\partial \lambda}{\partial \sigma_i} + \sum_i (\delta_i^{(b)} - \delta_i^{(r)}) \frac{\partial \lambda}{\partial \delta_i} + (F^{(b)} - F^{(r)}) \frac{\partial \lambda}{\partial F} \qquad (34)$$

Projected Effects of Predation

The effect of predation on population growth of cadmium-exposed populations was projected by applying a predation rate, which is the percent of the population removed by predators per time step, to the estimates of λ for each exposure concentration. The predation rate, between 0 and 25%, was subtracted from one, and the resulting number was multiplied by the estimate of λ from the matrix model projection to obtain a net population growth rate. The range of predation rates was chosen to target the transition from positive to negative population growth, after the value of λ had been determined for each treatment. The model assumes that predation pressure is equal across all life stages because actual predation rates in the field would be dependent upon the density and species of predators present, and to my knowledge, no information is available in the scientific literature comparing field predation rates for each life stage of *P. pugio*.

Statistical Analysis

Calculations of each individual-level model parameter (σ_i, δ_i, P, G, and F) were performed separately on each replicate, and the variance among replicates in each treatment was calculated for each model parameter (e.g., Rao and Sarma, 1986; Munns et al., 1997; Barata et al., 2002; Salice and Miller, 2003). Population growth rate, λ, was calculated and sensitivity analyses were performed separately for each replicate, treating each as a separate population. Since the decomposition of λ requires the evaluation of sensitivities at the mean between the control and treatment, contribution values do not

include an estimate of variance and differences in contributions among treatments could not be tested statistically.

Survival for each life stage, σ_i, was estimated by averaging the observed survival values for the duration of that stage. The duration of the embryonic stage, δ_1, was estimated by the length of time for eggs to hatch, starting from the day that the clutch appeared on the female. Larval duration, δ_2, was estimated by the time from hatching until metamorphosis to the juvenile stage. The distribution of larval duration values was highly skewed to the right, since the vast majority of the larvae metamorphosed within 20 days, but a small number remained in the larval stage as late as day 46. To accommodate the non-normal distribution, average larval duration was estimated by failure time analysis ("distribution analysis" in Minitab v. 13 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 estimated for each replicate and used for the value of δ_2.

Because *P. pugio* exhibits no apparent morphological difference upon maturation from juvenile to adult, the presence of gravid females was used as an indicator of sexual maturation. When the percent of gravid females reached 5%, all individuals were considered to be reproductively mature adults; this cut-off point was used to estimate the duration of the juvenile stage, δ_3.

Two-way analyses of variance (ANOVAs) were used to test for differences in vital rates among treatments, with treatment and replicate set as factors. Each set of replicates, consisting of one replicate of each treatment, belonged to the same temporal and physical block and was initiated with larvae from the same group of females. The

replicate set was included as a factor to account for variability due to clutch differences, temporal variation (because the replicates were staggered in time), and physical location (due to the blocked experimental design). Replicate set was a significant factor only in ANOVAs that tested for effects of cadmium exposure on reproduction and population growth rate, most likely due to the effects of small temperature differences between blocks. Pairwise comparisons were performed using the Tukey method (Neter et al., 1990). To test for differences in survival of second-generation larvae, a two-way ANOVA was employed, with factors consisting of parental and offspring treatments. A Type I error rate (α) of 0.05 was employed for all statistical analyses.

Results

Water and Tissue Chemistry

Free cadmium ion concentrations in freshly mixed exposure water were slightly higher than target concentrations (Table 3.1). Variation in cadmium concentration was low, and cadmium concentrations were distinctly different among treatments ($P < 0.001$). Between water changes, free cadmium ion concentration decreased, on average, by 4.4% for Cd-low and by 5.4% for Cd-high. Exposure resulted in substantial bioaccumulation of cadmium; cadmium concentrations in adult tissue were strongly dose-dependent ($P < 0.001$, Table 3.1).

Individual-Level Effects

Survival of adults was significantly reduced at the higher concentration of cadmium, relative to the control and lower cadmium concentration ($P < 0.001$, Fig. 3.2). There were no significant differences in survival of other life stages. Average survival of

larvae, juveniles, and adults was greater than 85% over each 13-day time step, regardless of cadmium concentration, while survival of embryos averaged approximately 60%.

Larval and juvenile life stage durations were not significantly affected by cadmium exposure (Fig. 3.3). Larval stage duration averaged 16 days post-hatch across treatments, while juvenile stage duration averaged 99 days. Duration of the embryonic stage was constant at 13 days for all treatments, resulting in a 13-day time step for the matrix model.

Cadmium exposure reduced the number of larvae per brood at both cadmium concentrations (P = 0.016, Fig. 3.4). Relative to the control, females had 27% fewer larvae per brood at the Cd-low exposure concentration and 36% fewer at Cd-high. The percent of females in the population that was gravid was about 50% lower at the Cd-high concentration (P = 0.031), but was not significantly affected at Cd-low (Fig. 3.4).

Maternal exposure had no effect on survival of larvae in second-generation exposures. Regardless of the exposure regime, survival of F_2 larvae averaged at least 95% over eight days, with little variability.

Projected Population Growth

Both population models projected a dose-dependent decrease in population growth rate (P < 0.001 for each model, Table 3.2). For Cd-low, both models estimate a 6% decrease in λ, as compared to the control. Exposure to the Cd-high concentration is estimated to reduce λ by 12% for the matrix model and 11% for the z-transformed life cycle graph analysis. All projected λ values are greater than one, indicating positive population growth.

Estimated vital rates for each model are shown in Table 3.3. Cadmium exposure resulted in significant decreases in fertility parameters for both models ($P \leq 0.034$) and survival parameters for adults ($P < 0.001$ for both models) and juveniles (matrix model only, $P = 0.01$).

Elasticity Analysis

For the matrix model, the parameters with the highest elasticities were P_3 and P_4, which are the probabilities of surviving and remaining in the juvenile and adult stages, respectively (Fig. 3.5A). The elasticities for P_3 and P_4 were approximately equal, and far exceeded the elasticity of any other parameter. The magnitude of elasticity for each parameter varied only slightly among treatments, and the overall pattern remained the same.

For the z-transformed life cycle graph analysis, elasticities were highest for σ_3 and σ_4, which are survival probabilities for juveniles and adults, respectively (Fig. 3.5B). In this model, juvenile survival had a higher elasticity than adult survival, but both were again much greater than the elasticity of other parameters. Elasticities of life stage duration parameters (δ) are negative, as expected, due to a decrease in λ as δ increases.

Contribution Analysis

Decomposition analysis revealed that the effect of cadmium on population growth rate can be attributed mostly to effects on fertility (Fig. 3.6). In the matrix model, F_4 (fertility of adults) had the greatest contribution to the change in λ (Fig. 3.6A); this contribution is negative, reflecting the decrease in F_4 resulting from cadmium exposure (Table 3.3). G_3 (the probability of juveniles surviving and graduating to adulthood) had a

relatively large positive contribution, corresponding to the increase in G_3 in cadmium-exposed shrimp (Table 3.3).

In the z-transformed life cycle graph analysis, the largest contribution to the effect of cadmium on λ comes from F (Fig. 3.6B). The next largest contributions, belonging to σ_1 and σ_4, are relatively small and can be traced back to an (insignificant) increase in embryonic survival and a decrease in survival of adults exposed to cadmium, respectively (Fig. 3.2).

Projected Predation Effects

The projected effect of predation on net population growth rate is shown in Fig. 3.7. Growth of control populations becomes negative ($\lambda < 1$) when the predation rate is greater than 21% of the population per time step. Cadmium-exposed populations exhibit negative growth when the predation rate exceeds 16% at Cd-low and 11% at Cd-high exposure concentrations.

Discussion

Water and Tissue Chemistry

Dissolved cadmium concentrations in the current study were relatively high, compared to concentrations normally found in estuaries. In the absence of NTA, the total cadmium concentration needed to achieve the free cadmium ion exposure concentrations would be approximately 10 μg Cd_T/L for Cd-low and 16 μg Cd_T/L for Cd-high at a salinity of 10 ppt. Similar total cadmium concentrations have been occasionally observed in the Chesapeake Bay region, for example, in the Potomac River, VA (Hall et al., 1998) and the Upper Chesapeake Bay (Hall et al., 1992).

Bioaccumulation of cadmium in *P. pugio* was clearly dose-dependent. The concentration of cadmium in adult tissues increased by 41% from Cd-low to Cd-high, corresponding to effects on adult survival, fertility, and population growth. Cadmium concentrations in tissues were considerably higher (> 5x) than those reported in shorter (14- to 21-day) exposures that resulted in sublethal effects on respiration and molting frequency (Vernberg et al., 1977; Rule and Alden, 1996). Tissue concentrations similar to the current study were reported in adult *P. pugio* exposed to higher concentrations of cadmium for six weeks, resulting in over 50% mortality (Pesch and Stewart, 1980). In the current eight-month exposure, it is possible that sequestration of accumulated cadmium by metallothionein or a similar metal-binding protein (Howard and Hacker, 1990) allowed the gradual accumulation of high concentrations of cadmium with relatively low effects on survival.

Effects on Survival

Cadmium exposure resulted in a decrease in survival of adult *P. pugio*, but had no significant effect on survival of other life stages. Adults may have a lower tolerance to cadmium than other life stages, or may have been more vulnerable due to cumulative effects over the 240-day exposure. A similar trend was found in the freshwater cladoceran *M. macleayi*, in which adults exhibited a greater sensitivity to cadmium than juveniles (Barata et al, 2002). However, juvenile aquatic invertebrates often have a lower tolerance to cadmium than adults, as observed in the freshwater amphipod *Gammarus pulex* (McCahon and Pascoe, 1988), the saltwater mysid *Siriella armata* (Birmelin et al., 1995), the estuarine amphipod *Leptocheirus plumulosus* (McGee et al., 1998), the

estuarine gastropod *Potamopyrgus antipodarum* (Jensen et al., 2001), and the freshwater snail *B. glabrata* (Salice and Roesijadi, 2002).

Survival of *Palaemonetes pugio* larvae was high, averaging over 85% per 13-day time step regardless of cadmium concentration. In contrast, Thorpe and Costlow (1989) reported complete mortality of *P. pugio* larvae during a 4-day exposure to 2.0 μg Cd^{2+}/L, which is halfway between the two cadmium concentrations in the current study. The vast disparity in survival may be due to the difference in temperature between the two studies; Thorpe and Costlow's (1989) exposure was conducted at 30 °C, while the temperature of the current study was 25 °C. Lethal effects of cadmium on *P. pugio* have been shown to increase dramatically with temperature (Howard and Hacker, 1990).

Embryonic survival averaged about 60%, regardless of cadmium concentration. Previous studies have reported a wide range of values for survival of *P. pugio* embryos that have been removed from gravid females, from > 90% (Rayburn and Fisher, 1999) to < 35% (Reinsel et al., 2001), with low survival possibly due to handling stress. Therefore, while embryonic survival was lower than survival of any other life stage in the current study, it still falls within the range of previously reported values.

Although maternal exposure to cadmium did not affect F_2 larval survival, it is possible that second-generation effects could emerge later in the life cycle or in future generations. Multigenerational effects of cadmium on survival, growth, and reproduction have been observed in *D. magna* (Muyssen and Janssen, 2004; Guan and Wang, 2006). The population models in the current study assume that vital rates of *P. pugio* will remain constant over successive generations of cadmium exposure.

Reproductive Effects

Cadmium exposure greatly reduced reproduction of *P. pugio*, resulting in effects on both brood size and the percent of females that was gravid at any given time. Fertility parameters (F) for both models were reduced by approximately 50% at Cd-low and 75% at Cd-high. In addition, fertility effects provided the greatest contribution to effects of cadmium on population growth rate. Cadmium-induced decreases in reproductive rate have also been observed in *D. pulex* (Bertram and Hart, 1979), *D. magna* (Barata and Baird, 2000; Smolders et al., 2005), *Potamopyrgus antipodarum* (Jensen et al., 2001), and *M. macleayi* (Barata et al., 2002). Effects on reproduction may reflect bioenergetic effects of cadmium, since reductions in scope for growth have been reported in *G. pulex* (Stuhlbacher and Maltby, 1992), *Callinectes sapidus* (Guerin and Stickle, 1995), and *D. magna* (Baillieul et al., 2005) following chronic exposure to cadmium. Reduced brood size may also be the result of a decrease in fertilization success, as observed in cadmium-exposed sea urchins (*Anthocidaris crassispina*), accompanied by a decline in sperm motility (Au et al., 2001).

Perturbation Analyses

Elasticity analysis indicated that *Palaemonetes pugio* population growth is most sensitive to changes in juvenile and adult survival. Although fertility parameters had relatively low elasticities, they provided the greatest contributions to the cadmium-induced decrease in population growth rate, reflecting the drastic effect of cadmium exposure on reproduction, as compared to effects on survival. Similarly, in *Potamopyrgus antipodarum* (Jensen et al., 2001) and *M. macleayi* (Barata et al., 2002), effects of cadmium on population growth were attributed mainly to reduced reproduction.

In contrast, the greatest contribution to reduced population growth in cadmium-exposed *B. glabrata* resulted from a decline in either embryonic or juvenile survival, depending upon the strain of snail tested (Salice and Miller, 2003). Therefore, the relative importance of individual-level effects in cadmium-exposed populations may vary among and within species.

Population Growth and Effects of Predation

Both the matrix model and z-transformed life cycle graph analysis projected a dose-dependent decrease in population growth, but growth remains positive ($\lambda > 1$), even at the higher cadmium concentration, in the absence of predation. However, the models suggest that populations exposed to cadmium would be much more vulnerable to a predator-induced decline. Compared to populations exposed to the Cd-high concentration, model predictions suggest that control populations are able to withstand a predation rate that is almost twice as great (21% over 13 days) before population growth becomes negative ($\lambda < 1$).

Palaemonetes pugio is an important prey item for many fish species, including *Fundulus heteroclitus* (mummichog), *Micropogonias undulatus* (Atlantic croaker), *Morone americana* (white perch), *M. saxatilis* (striped bass), and *Anguilla rostrata* (American eel; Nixon and Oviatt, 1973; Clark et al., 2003). In experimental settings, extremely high predation rates on adult *P. pugio* have been observed. For example, the predation rate on tethered *P. pugio* in shallow depths of the Rhode River, MD averaged ~12% over only 30 minutes (Clark et al., 2003). In a structured artificial habitat, striped bass consumed ~24% of grass shrimp present within only 22 hours, while mummichogs consumed ~8% (Davis et al., 2003). Predation was likely overestimated in these studies

because behavior of the prey was constrained; in the field, *P. pugio* are likely to exhibit predator-avoidance behavior (Dorn et al., 2006). In a more natural setting, small mummichogs exhibited an average predation rate of 30% on *P. pugio* over two weeks in large enclosures in an intertidal marsh (Kneib, 1988). This estimate is much more similar to the predation rates that could be sustained by the modeled populations in the current study. Predation rates on neonate and juvenile *P. pugio* appear to be largely unstudied. It is likely that neonates and juveniles experience pressures from a variety of non-piscine predators that specifically feed on small prey sizes. In natural populations, many factors can influence predation rate, including predator density, prey density, predator type, and habitat structure.

Comparison of Two Population Models

The two population growth models resulted in very similar projections of λ, and both models predict a dose-dependent effect of cadmium on λ. The estimates of λ from the life cycle graph analysis were all slightly lower than those from the matrix model. Elasticity analysis results varied only slightly, with the life cycle graph analysis resulting in a greater elasticity for juvenile survival, while the matrix model results suggest similar elasticities for juvenile and adult survival.

Decomposition analysis for the two models revealed similar patterns of contributions. For both models, fertility provided the greatest contribution to the cadmium-induced decrease in λ. However, the matrix model analysis revealed a fairly large, positive contribution of G_3 to population growth, suggesting that cadmium exposure actually provides an advantage to juveniles by increasing the probability that a juvenile will survive and graduate to adulthood. The positive contribution of G_3 to λ can

be traced to a small (non-significant) increase in G_3 in cadmium-exposed *P. pugio*, which appears to be an artifact of slight changes in juvenile survival and duration. However, the contribution of G_3 becomes inflated due to the high sensitivity of λ to juvenile survival. In contrast, juvenile survival and duration have very small contributions in the z-transformed life cycle graph analysis. Decomposition analysis for the life cycle graph analysis does not suggest an advantage of cadmium exposure for juveniles, because this model allows the calculation of contributions of base-level parameters, rather than complex functions of these parameters (Caswell, 1996). Sensitivities and contributions resulting from matrix model calculations can, therefore, muddle information from various parameters, so that it is difficult to determine the exact source of the toxicological effect on population growth.

Conclusion

Both the matrix model and the z-transformed life cycle graph analysis projected a dose-dependent decline in population growth rate of *P. pugio* chronically exposed to dissolved cadmium, driven mainly by a reduction in brood size and the proportion of gravid females. Lethal effects were only observed in the adult life stage, and did not contribute greatly to the decline in population growth. Even though population growth remained positive in populations exposed to free cadmium ion concentrations ≤ 2.51 µg Cd^{2+}/L, these populations are more vulnerable to a predator-induced decline. A comparison of decomposition analyses between the two models suggests that the z-transformed life cycle graph analysis provides a more clear explanation of the source of contributions from individual-level responses to population growth rate.

Table 3.1. Cadmium concentrations (mean ± SE) in exposure water (μg Cd^{2+}/L) and shrimp tissue (μg Cd/g dry mass). Free cadmium ion concentration was estimated from the measured total cadmium concentration using the chemical equilibrium program MINEQL+ v. 4.5. Values with different superscripts are significantly different ($P \leq 0.05$).

Treatment	Target $[Cd^{2+}]_{(aq)}$	Measured $[Cd^{2+}]_{(aq)}$	[Cd] in Tissue
Control	0	0.03^a +/- 0.002	0.64^a +/- 0.05
Cd-low	1.36	1.51^b +/- 0.01	56.96^b +/- 3.41
Cd-high	2.27	2.51^c +/- 0.01	80.40^c +/- 8.80

Table 3.2. Population growth rate (λ) estimates (mean ± SE) for each model. Values with different superscripts are significantly different (P ≤ 0.05).

Treatment	Matrix Model λ	Life Cycle Graph Analysis λ
Control	1.28[a] +/- 0.02	1.26[a] +/- 0.02
Cd-low	1.20[b] +/- 0.02	1.19[b] +/- 0.02
Cd-high	1.13[c] +/- 0.03	1.12[c] +/- 0.03

Table 3.3. Model parameter estimates (mean, SE) for the matrix model and z-transformed life cycle graph analysis. The value of the P_1 parameter for the matrix model was zero for all treatments, because the duration of the first life stage is equal to the time step. Values with different superscripts are significantly different ($P \leq 0.05$).

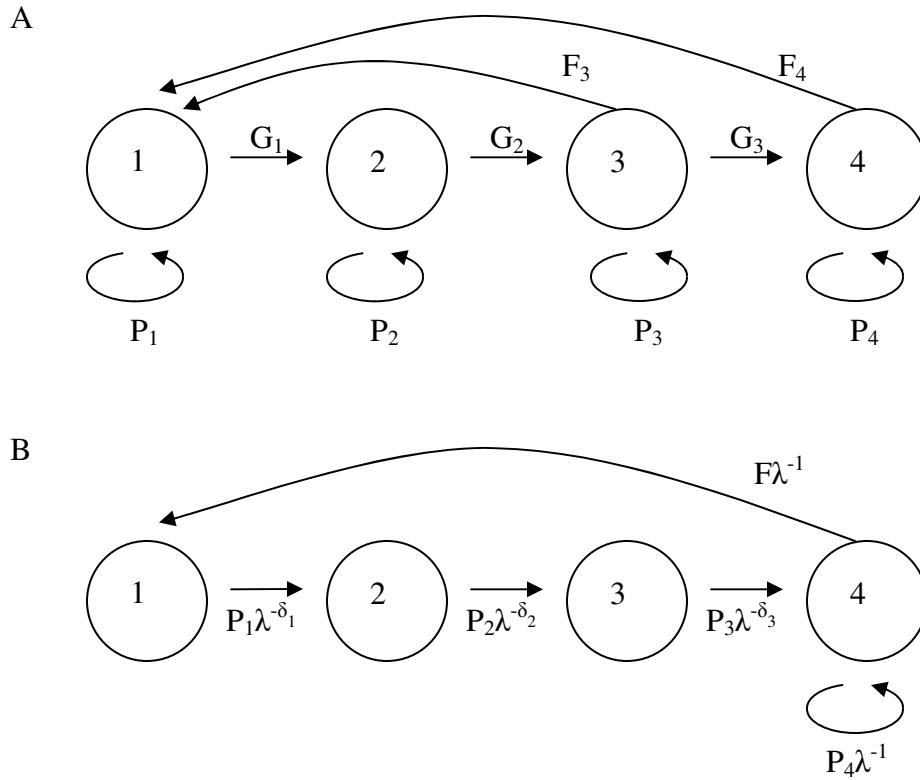| Treatment	Matrix Model								Z-Transformed Life Cycle Graph Analysis				
	F_3	F_4	G_1	G_2	G_3	P_2	P_3	P_4	F	P_1	P_2	P_3	P_4
Control	0.12^a	5.23^a	0.72	0.68	0.05	0.21	0.94^a	0.99^a	7.51^a	0.53	0.87	0.94	0.99^a
	(0.02)	(1.31)	(0.04)	(0.02)	(0.004)	(0.01)	(0.004)	(0.001)	(2.25)	(0.05)	(0.02)	(0.01)	(0.001)
Cd-low	0.07^b	2.36^b	0.76	0.67	0.07	0.23	0.92^{ab}	0.98^a	3.29^{ab}	0.58	0.87	0.94	0.98^a
	(0.01)	(0.84)	(0.04)	(0.02)	(0.01)	(0.02)	(0.01)	(0.01)	(1.36)	(0.06)	(0.03)	(0.03)	(0.01)
Cd-high	0.05^b	1.45^b	0.77	0.64	0.08	0.23	0.90^b	0.93^b	1.79^b	0.6	0.83	0.86	0.93^b
	(0.01)	(0.54)	(0.05)	(0.01)	(0.02)	(0.01)	(0.01)	(0.01)	(0.66)	(0.08)	(0.03)	(0.04)	(0.01)

Fig. 3.1. Life cycle graphs for *P. pugio* used for the stage-based matrix model (A) and z-transformed life cycle graph analysis (B). Stage 1 = embryos, stage 2 = larvae, stage 3 = juveniles, and stage 4 = adults. In the matrix model, G is the probability of survival and graduation to the next life stage, P is the probability of surviving and remaining in the same life stage, and F is fertility. In the z-transformed life cycle graph, P is the probability of survival of a given life stage, δ is the duration of the life stage in time steps, and F is fertility.
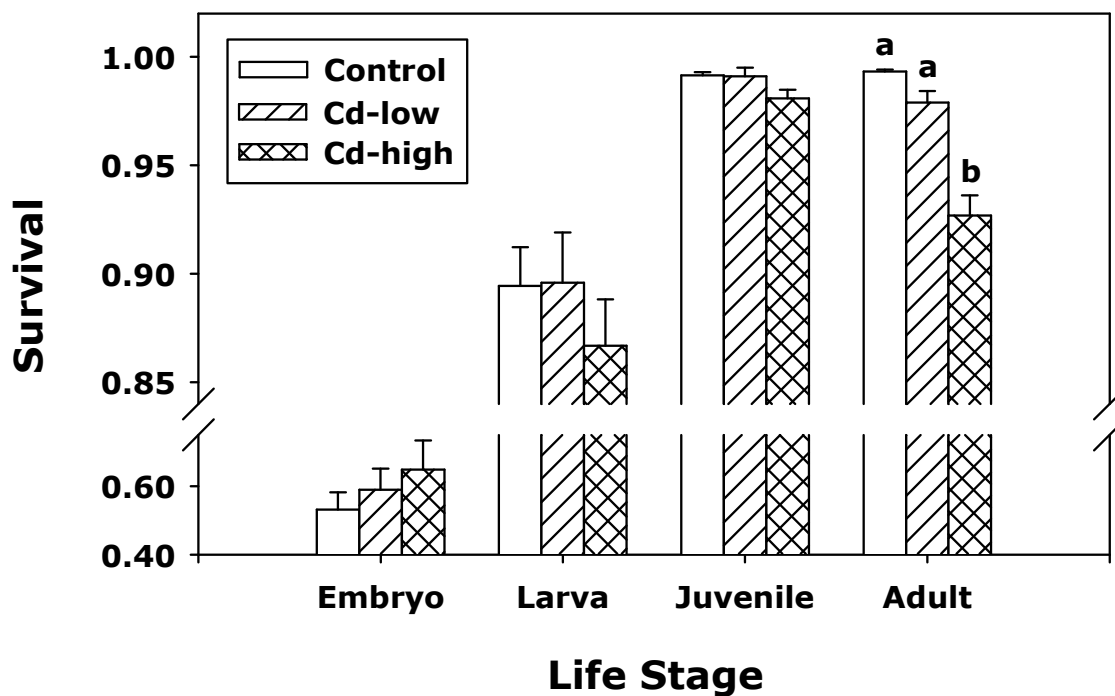
Fig. 3.2. Effect of cadmium on survival (σ) of each life stage of *P. pugio* (mean $\pm$ SE).

Survival values have been adjusted to one time step (13 days). Bars labeled with

different letters are significantly different (P $\leq$ 0.05).
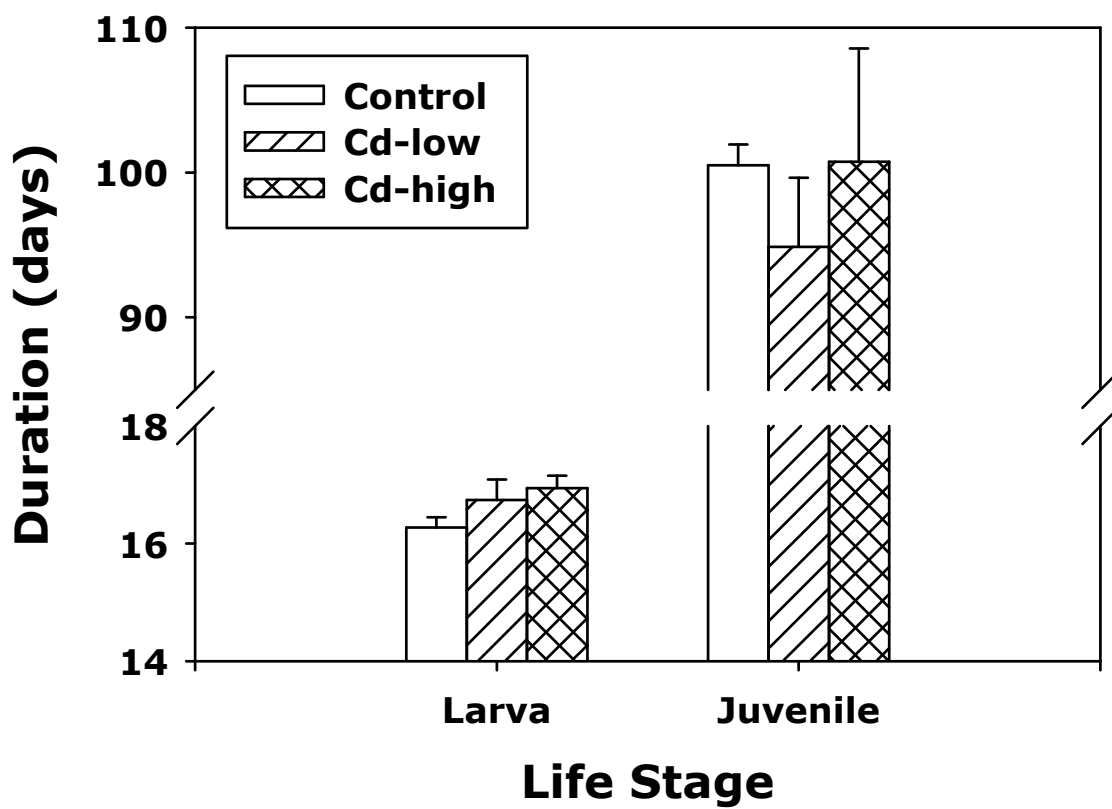
Fig. 3.3. Effect of cadmium on duration of larval and juvenile stages (mean ± SE). There were no significant differences among treatments. Embryonic duration was constant across treatments (13 days).
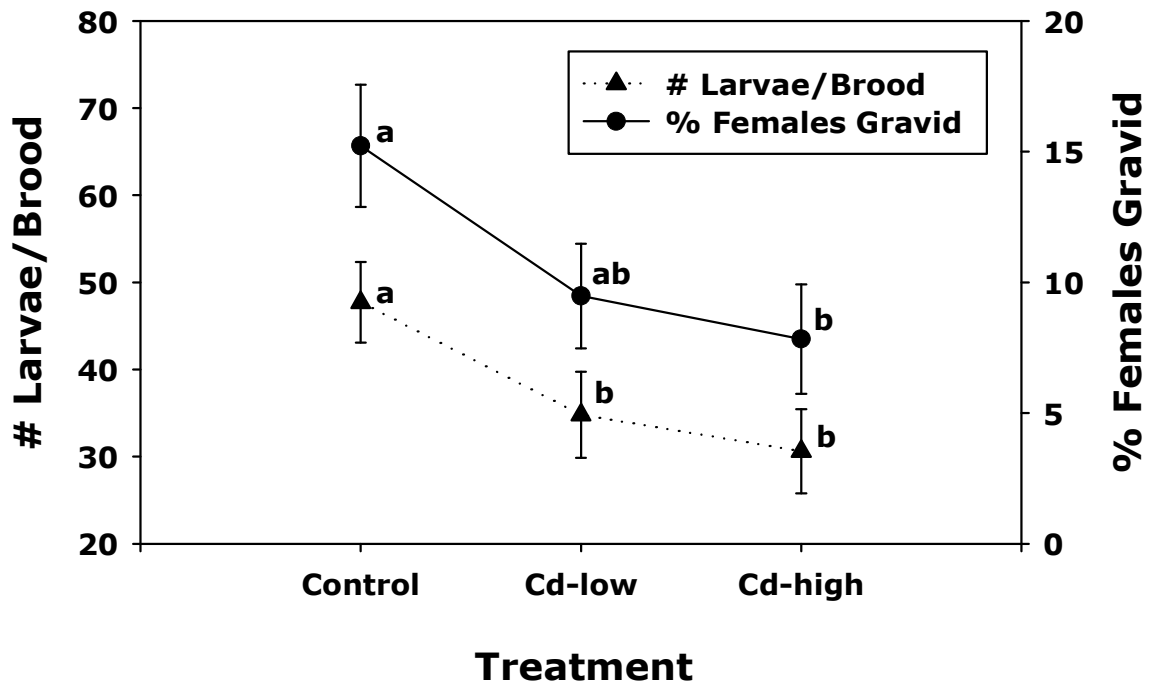
Fig. 3.4. Reproductive effects of lifetime exposure to cadmium: number of larvae per brood and the percent of adult females that was gravid, on average, at any given time (mean ± SE). Different letters indicate significant differences (P ≤ 0.05).
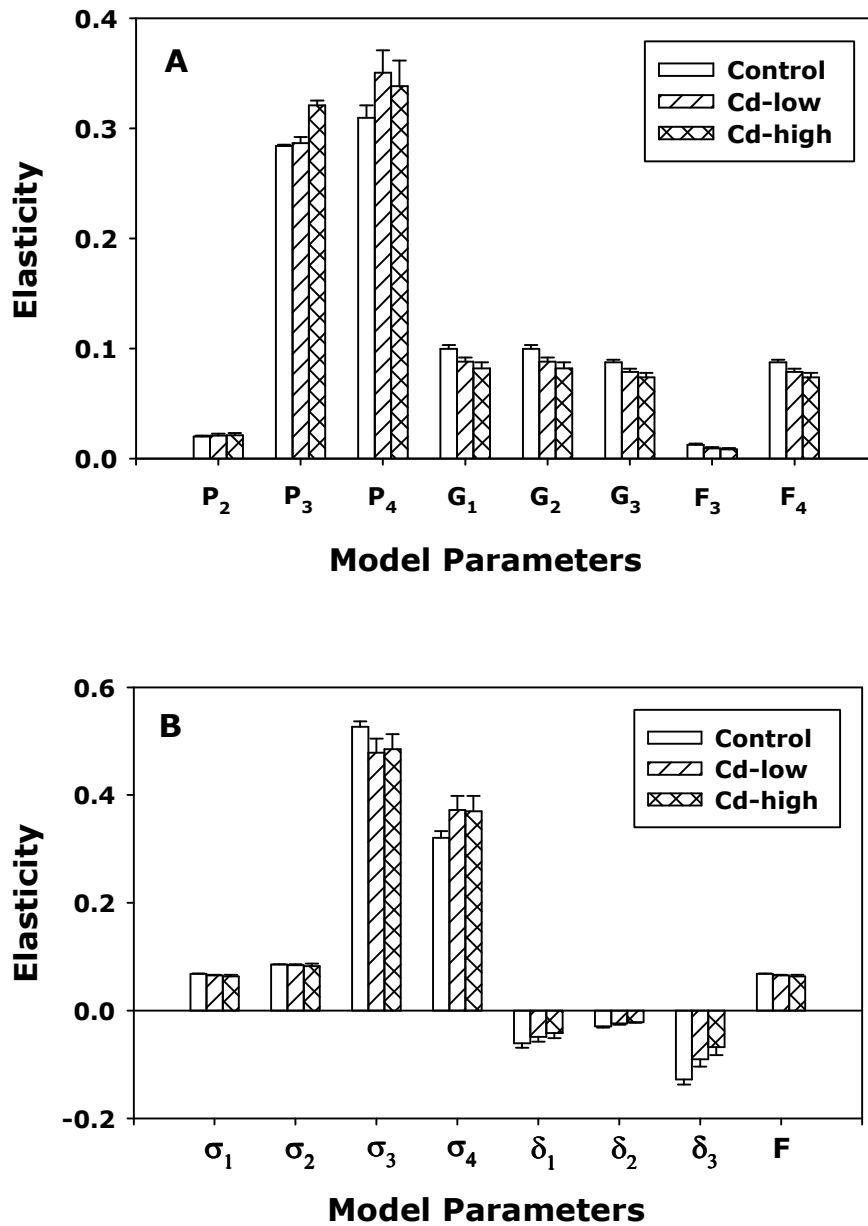
Fig. 3.5. Elasticity (mean ± SE) of λ to each model parameter for the matrix model (A)

and z-transformed life cycle graph analysis (B).
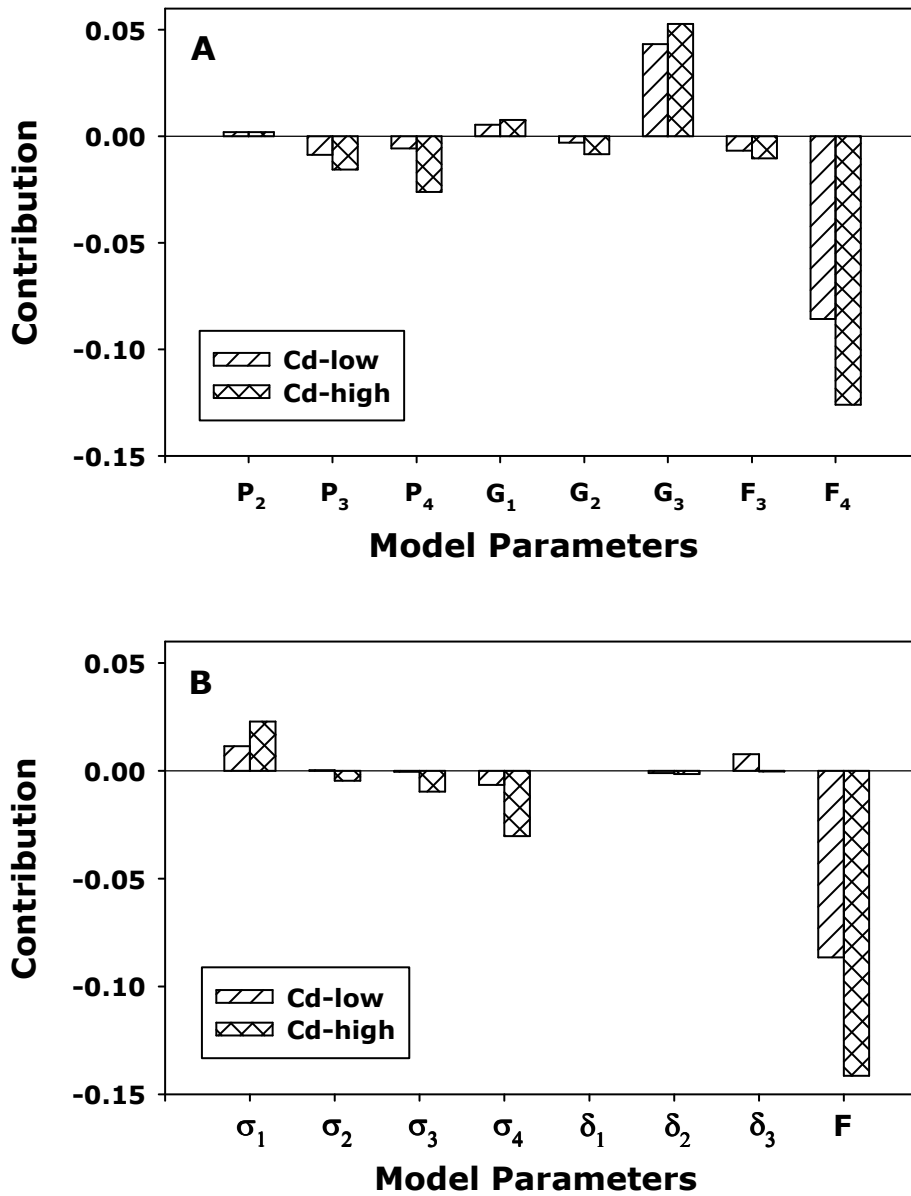
Fig. 3.6. Contribution of each model parameter to the effect of cadmium on λ for the

matrix model (A) and z-transformed life cycle graph analysis (B).

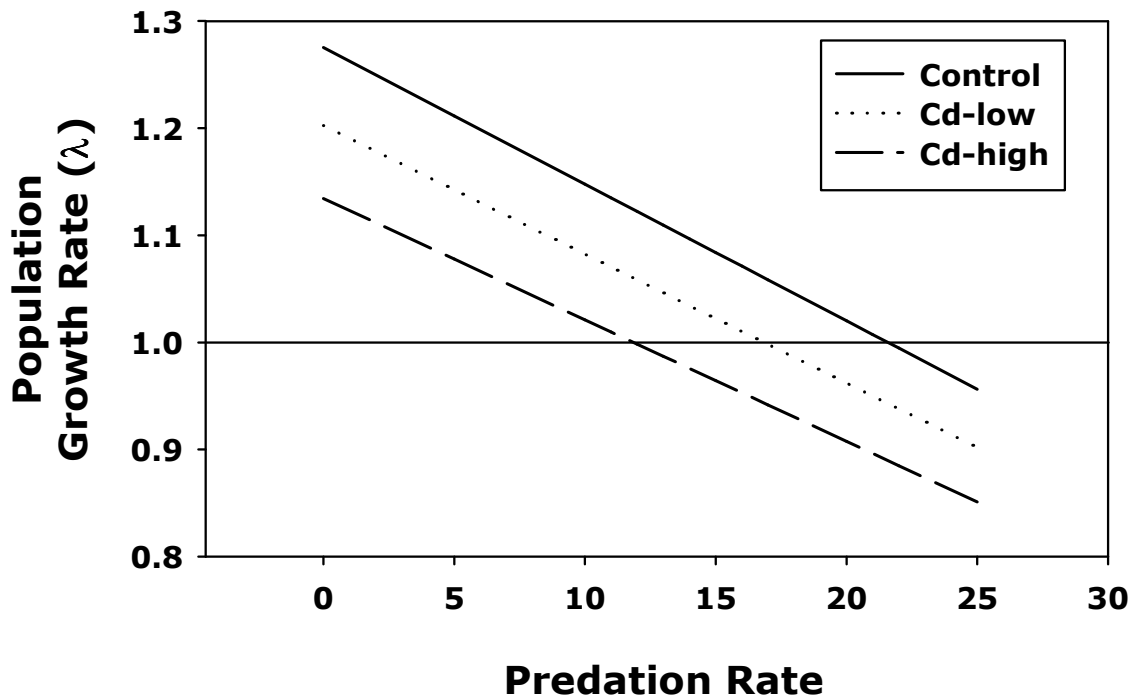Fig. 3.7. Predicted effect of predation pressure on population growth rate of cadmium-exposed populations, using estimates of λ from the matrix model. Predation rate is the percent of the population removed per time step. When $\lambda < 1$, population growth is negative.

CHAPTER 4:

REPRODUCTIVE AND LIFE STAGE-SPECIFIC EFFECTS OF AQUEOUS COPPER ON THE GRASS SHRIMP, *PALAEMONETES PUGIO*

Abstract

Palaemonetes pugio (grass shrimp) were exposed to aqueous copper for an entire life cycle, starting with larvae and allowing them to attain maturation and reproduce. During this 240-day exposure, effects of copper on the survival and duration of each life stage duration were measured, as well as effects on reproduction. Copper at free ion concentrations of 9 or 26 μg Cu^{2+}/L had no lethal effects on larval, juvenile, or adult stages. Larval development was significantly delayed at both 9 and 26 μg Cu^{2+}/L. Although females exposed to copper (9 or 26 μg Cu^{2+}/L) produced embryos during the life cycle exposure, the embryos did not hatch, precluding completion of the life cycle. In a subsequent experiment, adults from the life cycle exposure to copper (26 μg Cu^{2+}/L) that were transferred to control seawater produced viable embryos; however, the larval output per clutch was 43% less than in clutches from females that had never been exposed to copper, perhaps due to effects on the parental generation that reduced energetic allocation to reproduction. Females that were raised in control seawater and then transferred, prior to oviposition, to seawater containing 26 μg Cu^{2+}/L were unable to produce viable embryos after only three days of exposure. Hence, either acute or chronic exposure to 26 μg Cu^{2+}/L prevented production of larvae. To determine the post-spawning effects of copper exposure on larval production, gravid females that had spawned in control seawater were transferred to seawater containing 26 μg Cu^{2+}/L; some

embryos hatched successfully, although clutches produced only 26% as many larvae as those hatched in control seawater. This reduction in hatching success is unlikely to be completely responsible for the lack of larval recruitment observed in pre-spawning exposures to copper, suggesting that copper may also inhibit processes before or during spawning and fertilization. Given that reproduction by *P. pugio* was severely constrained at copper concentrations that were otherwise sublethal ($\geq$ 9 µg Cu^{2+}/L), severe effects on population dynamics may occur in conditions that would not likely be deemed deleterious on the basis of short-term, lethality-based studies.

Introduction

Exposure to contaminants for an entire life cycle can provide valuable insight into chronic effects at the individual and population level (Rowe, 2003; Salice and Miller, 2003; 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). *Palaemonetes pugio* (grass shrimp) were exposed to aqueous copper for an entire life cycle, starting with larvae and allowing them to attain maturation and reproduce.

To determine the effects of aqueous copper on each life stage of *P. pugio*, I conducted a full life cycle exposure, similar to the life cycle exposure to cadmium described in Chapter 3. 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. Although I originally intended to apply a population growth model to these individual-level measurements, as conducted for cadmium in Chapter 3, exposure to copper prevented larval production, thereby preventing population growth. Due to the substantial effects observed on reproduction during the life cycle exposure, I

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. I 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.

Methods

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. During water changes, test organisms were transferred to temporary holding chambers containing exposure water. Survival was measured during water changes. 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), and concentration of NTA. Control seawater contained no added $CuCl_2$.

Life Cycle Exposure

Palaemonetes pugio larvae were exposed to copper at nominal free divalent cation concentrations of 9 and 26 μg Cu^{2+}/L. Water samples were taken after each water change, filtered through a 0.22 μm nitrocellulose membrane, and acidified to 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 similar for the two treatments (3200 and 3270 μg Cu/L) and as a result of variation in samples and limits of analytical resolution, concentrations in the two treatments could not be reliably distinguished using either ICP-MS or AA. Therefore, only the nominal free ion concentrations are reported. The pH was ~7.8 throughout the life cycle exposure.

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

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

Shrimp began to produce clutches on Day 75. Gravid females were removed daily to individual 0.95-L jars filled with 0.6 L of exposure water. The females' chelae were trimmed to prevent cannibalism of eggs (Little, 1968). While isolated, the females were fed *Artemia* only. The water was changed after eight days and females were placed

in a 3 mm-mesh cage, suspended at the top of the jar. After the females' eggs hatched, larvae were counted and the females were returned to their original tanks. If a female did not retain its eggs until hatching, it was returned to its tank when it was no longer carrying eggs. The exposure continued for a total 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 HNO_3 at 60 °C overnight. The digested sample was diluted and analyzed for copper by ICP-MS.

Reciprocal Cross Exposure

This experiment utilized adults from the life cycle exposure's control and 26 μg Cu^{2+}/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.

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 Cu^{2+}/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.

Statistical Analysis

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, I 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 the 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. This was an arbitrary cutoff, which equated to more than one female in a replicate being gravid at the same time.

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, although block was not a significant factor in any of the GLM tests. 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.

Results

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. 4.1, 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 µg Cu^{2+}/L, respectively). Average duration of the larval stage increased by two days when shrimp were exposed to 9 µg Cu^{2+}/L and by three days when exposed to 26 µg Cu^{2+}/L, compared to control individuals (Fig. 4.2). However, the duration of the juvenile stage was not significantly affected by copper exposure (Fig. 4.2, $P \geq 0.67$).

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 µg Cu^{2+}/L and ~20 clutches per replicate at 26 µg Cu^{2+}/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 recruitment was prevented by exposure to copper at either 9 or 26 µg Cu^{2+}/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 µg Cu^{2+}/L, respectively), but did not differ between the two copper concentrations (Table 4.1).

Reciprocal Cross Exposure

Shrimp that had been raised under control conditions and then transferred to copper-spiked seawater (26 µg Cu^{2+}/L) were able to spawn, but no embryos hatched

(Table 4.2). 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 µg Cu^{2+}/L) during the full life cycle exposure successfully produced larvae when transferred to control seawater (Table 4.2). 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 4.2, $P = 0.04$). 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.

Post-Spawning Effects

When gravid shrimp that had spawned in control seawater were transferred to copper-spiked seawater (26 µg Cu^{2+}/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. 4.3, $P < 0.01$). The duration of the embryonic stage averaged 13 days, regardless of exposure conditions.

Discussion

Effects of Exposure Prior to Oviposition

During the life cycle exposure to copper, pronounced effects on reproduction by *P. pugio* were observed. Although females exposed to copper could readily produce eggs, no larvae were produced from any of the clutches. Therefore, subsequent completion of the shrimp's life cycle was precluded at either 9 or 26 µg Cu^{2+}/L, despite the absence of lethal effects on larvae, juveniles, and adults. In the absence of organic ligands, free ion concentrations of 9 and 26 µg Cu^{2+}/L would correspond to total copper concentrations of 33 and 95 µg Cu/L, respectively, at the experimental pH and salinity. Copper concentrations as high as 72 µg Cu/L have been observed in the tributaries of the Chesapeake Bay, USA (Hall et al., 1998), a region where *P. pugio* are abundant.

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 26 µg Cu^{2+}/L during the reciprocal cross exposure were unable to produce viable eggs, regardless of prior exposure conditions. Conversely, eggs that were spawned under control conditions and then transferred to 26 µg Cu^{2+}/L were sometimes able to hatch successfully in the post-spawning exposure, indicating that inhibitory effects of copper on reproduction are 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.

Effects of copper exposure on fertilization in aquatic invertebrates have been well-documented. 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, 1992a]), and sea urchins (*Echinometra mathaei* [Ringwood, 1992a]) at total copper concentrations ranging from 10 to 50 μg/L. In addition, reduced sperm motility has been observed in mussels (*Mytilus edulis*) exposed to 6 mg Cu_{Total}/L (Earnshaw et al., 1986). Therefore, it is possible that a decrease in fertilization success contributed to the prevention of larval production observed in pre-spawning exposures of *P. pugio*.

Effects of Exposure on Hatching Success

In the post-spawning exposure, clutches that had been spawned in control seawater were often able to produce larvae in seawater containing 26 μg Cu^{2+}/L. However, each clutch produced only 26% as many larvae, on average, as clutches that were not exposed to copper. Therefore, copper exposure appears to reduce the hatching success of *P. pugio* embryos. Exposure to a much higher concentration of copper (3 mg Cu_{Total}/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.

Potential Bioenergetic Effects on Reproduction

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. However, they produced 43% fewer larvae than shrimp that had been maintained continuously under control conditions. The observed decrease in larval production may be due to a carryover of bioenergetic effects on the parental generation. Exposure to copper has been found to reduce the amount of energy allocated to production pathways in 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] and *P. pugio* [see Chapter 2]). 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.

Effects on Duration of the Larval Stage

During the life cycle exposure, development of larvae to the juvenile stage was delayed by 2 to 3 days at copper concentrations of either 9 or 26 μg Cu^{2+}/L. 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).

Conclusion

Exposure to copper at free ion concentrations of 9 or 26 μg Cu^{2+}/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 before or during spawning and/or fertilization. The results from this study suggest that even acute exposure to sublethal concentrations of copper can prevent completion of *P. pugio*'s life cycle. Thus, copper pollution in natural habitats, even at relatively low concentrations, may have marked effects on the population dynamics of grass shrimp.

Table 4.1. Copper concentrations (mean ± SE) in adult shrimp tissue (µg/g dry mass) at the end of the full life cycle exposure. Values with different letters are significantly different (P ≤ 0.05).

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

Table 4.2. Number of larvae (mean ± 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 ≤ 0.05).

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

117
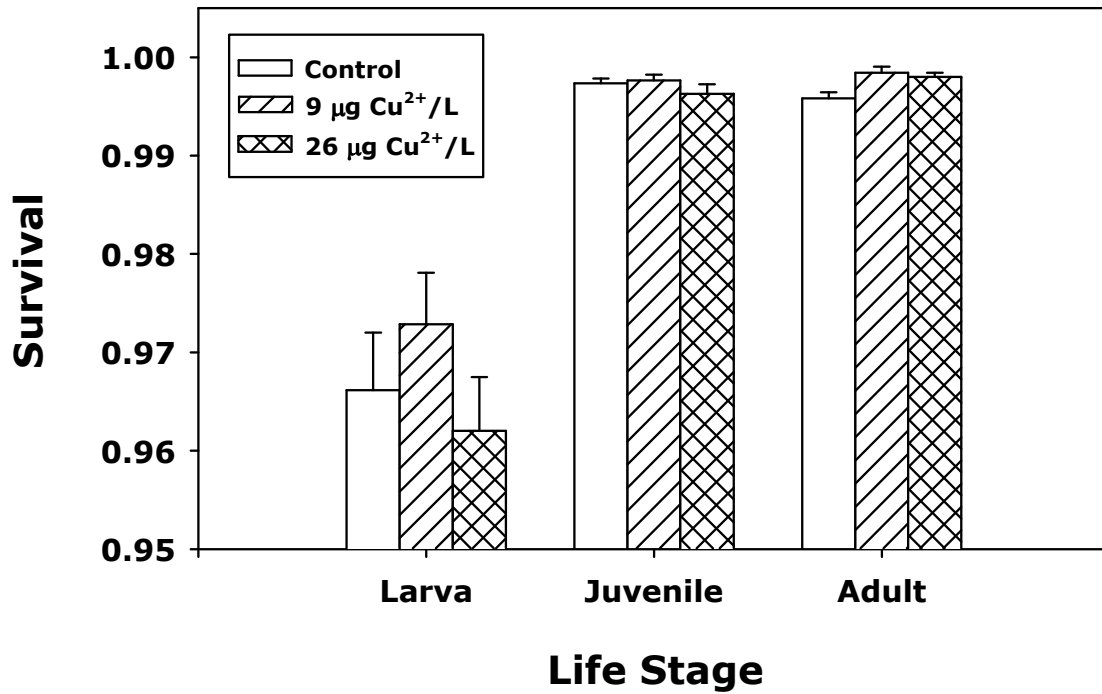
Fig. 4.1. Survival (mean ± SE) of each life stage of *P. pugio* in the full life cycle exposure to copper. Larval and juvenile survival were measured over 4-day intervals; adult survival was measured over 8-day intervals.
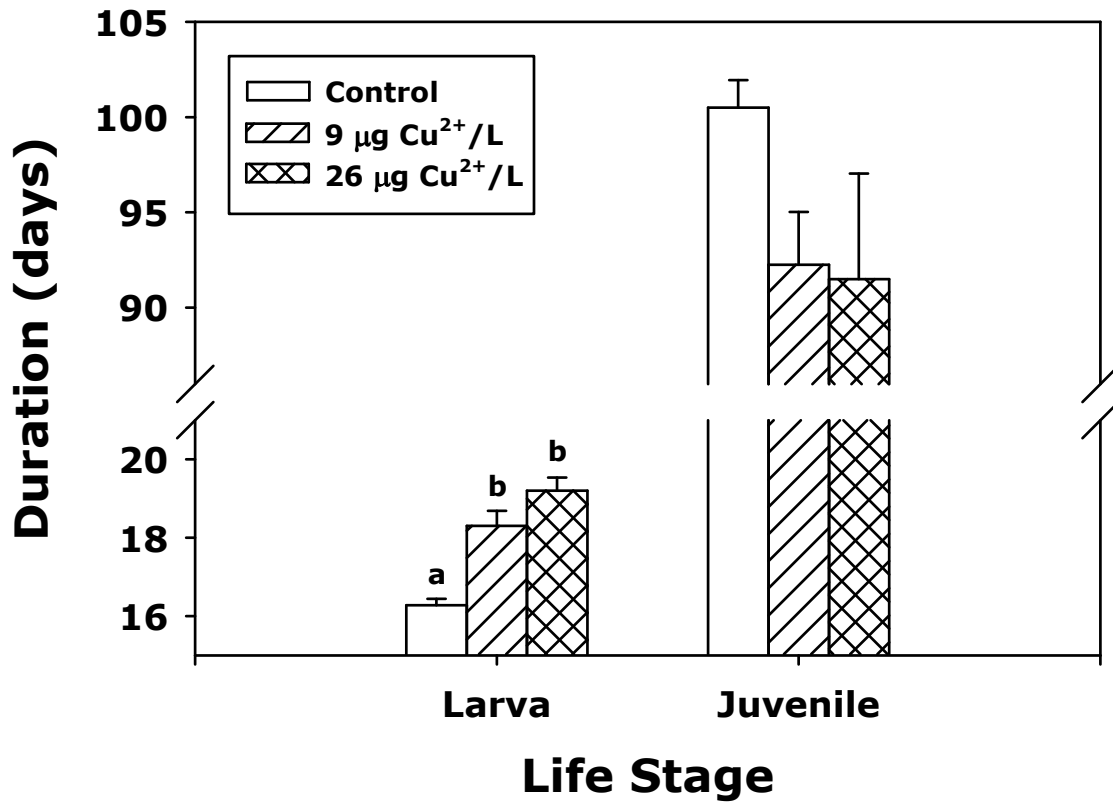
Fig. 4.2. Effects of copper exposure on duration (mean ± SE) of larval and juvenile

stages. Bars labeled with different letters are significantly different (P ≤ 0.05).
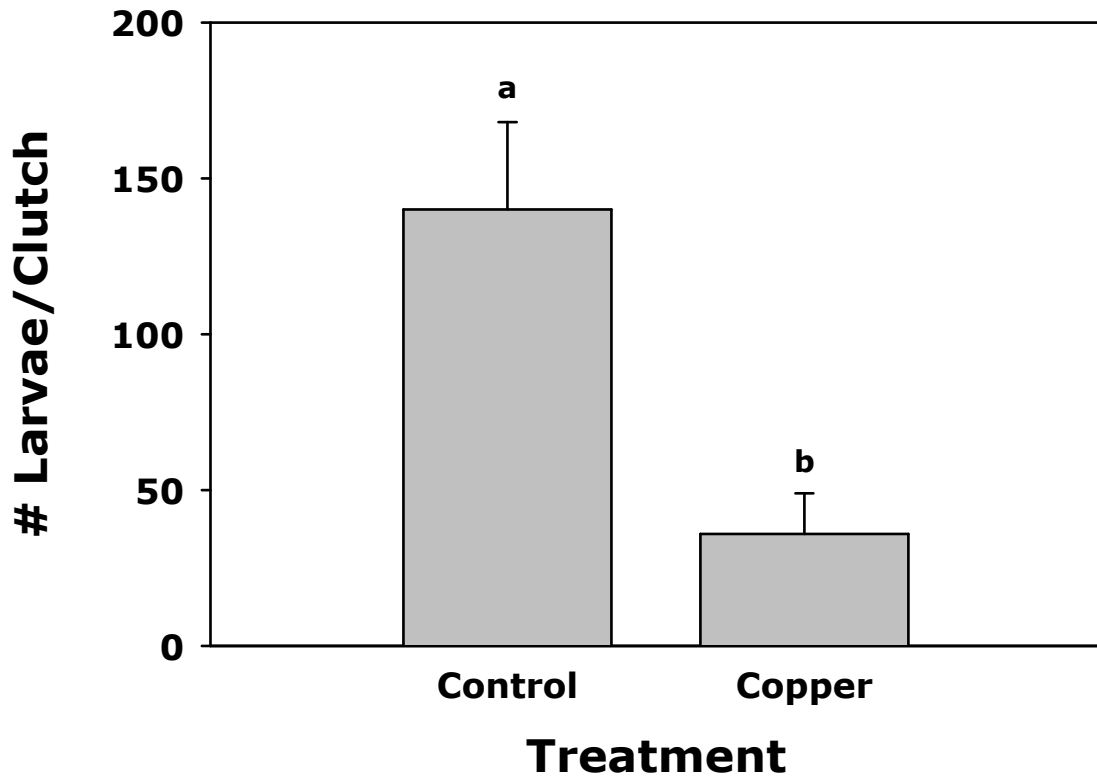
Fig. 4.3. Number of larvae (mean ± SE) hatched per clutch in post-spawning exposures.

Shrimp that spawned in control seawater were either retained in control seawater or

exposed to copper (26 μg Cu^{2+}/L) until their embryos hatched. Different letters indicate a

significant difference ($P \leq 0.05$).

CONCLUDING REMARKS

Bioenergetic effects of copper and cadmium on the grass shrimp, *Palaemonetes pugio* were observed in both the two-week exposures and in the eight-month, full life cycle exposures. Results from the two-week exposures, presented in Chapter 2, indicate that both copper and cadmium reduce the rates of standard metabolism and growth. The effect of copper on growth was more severe than that of cadmium; exposure to copper resulted in a negative growth rate (weight loss) even at a sublethal concentration. Food consumption and energy storage were not significantly affected by exposure to either metal. In the full life cycle exposure, cadmium caused a decrease in reproductive output by reducing brood size as well as the percentage of reproductive females in the population (Chapter 3). Although exposure to copper prevented larval production during the life cycle exposure, shrimp were able to successfully produce a limited number of larvae when transferred to control conditions (Chapter 4). The results suggested that parental energetic allocation to reproduction had been reduced by long-term exposure to copper. In sum, the bioenergetic effects of copper and cadmium on metabolic rate, growth, and reproduction suggest a decline in energy allocated to both respiration and production pathways, which is consistent with the majority of bioenergetic effects of copper and cadmium reported for aquatic invertebrates (reviewed in Chapter 1). Recommendations for future research include the measurement of effects of copper and cadmium on the assimilation efficiency, activity level, and molting rate of *P. pugio*.

Effects of cadmium on population dynamics, presented in Chapter 3, were explored using two methods of population modeling. Exposure to cadmium was

projected to reduce population growth of *P. pugio*, even at a concentration that was sublethal to each life stage. Results from decomposition analyses indicated that effects on population growth could be attributed mainly to effects of cadmium on reproduction. From these analyses, it is apparent that bioenergetic effects on reproduction at the individual level may have significant consequences at the population level. Even though both models projected positive population growth at the experimental concentrations, exposure to cadmium would reduce the ability of populations to withstand predation pressure. Given that *P. pugio* is an important prey item to several fish species, a reduction in the population growth rate of grass shrimp may have implications for their predators as well. Predator populations may potentially be affected by cadmium both indirectly, i.e., by a decline in prey availability, and directly, i.e., via toxic effects of cadmium on the predators themselves. Field surveys in cadmium-polluted areas are recommended to explore the effects of cadmium on both *P. pugio* and their predators.

The effects of copper on the life cycle of *P. pugio*, presented in Chapter 4, were particularly striking. Although the concentrations studied were sublethal to larvae, juveniles, and adults, females exposed to copper were unable to produce viable embryos, preventing completion of the life cycle. Subsequent experiments revealed that copper exposure may significantly reduce the energetic allocation to reproduction, as well as the hatching success of embryos, although these effects of copper are unlikely to be completely responsible for the lack of larval production observed during the life cycle exposure. Hence, it is likely that exposure to copper also inhibits processes that occur either before or during spawning and/or fertilization. In view of the fact that copper is known to reduce fertilization success in several species of aquatic invertebrates

(Chapter 1), this mechanism of inhibiting reproduction may very well have contributed to the prevention of larval production in *P. pugio*. Due to the severe nature of the effects of copper on reproduction in *P. pugio*, future research is recommended to examine effects of copper on the reproductive processes of other aquatic invertebrates, particularly closely related crustaceans with external fertilization.

In conclusion, effects of copper and cadmium on bioenergetics, reproduction, and population growth of *P. pugio* suggest that both metals may reduce the sustainability of grass shrimp populations in contaminated habitats, even at sublethal concentrations. Population declines of this widely distributed epibenthic invertebrate could have far-reaching implications at the level of the ecosystem, including effects on nutrient cycling and predator populations.

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