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Toxicity of Silver, Zinc, Copper, and Nickel to the Copepod *Acartia tonsa* Exposed via a Phytoplankton Diet

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Toxicity tests were conducted with the marine copepod *Acartia tonsa* to assess the effects of dietary metal exposure. The diatom *Thalassiosira pseudonana* was cultured with Ag, Zn, Cu, or Ni and used as diets for adult *A. tonsa* over a 7-d exposure, and copepod survival and reproduction were measured throughout the exposure period. For all metals, reproduction was the most sensitive endpoint, with 20% effect concentrations (EC_{20}s) corresponding to exposures of *T. pseudonana* to 0.64, 0.3, 1.2, and 2.4 μg/L for Ag, Zn, Cu, and Ni, respectively. The corresponding metal concentrations in the algae added to copepod test solutions (EC_{20}s) were 5.44, 0.55, 22.3, and 15.3 μg/g for Ag, Zn, Cu, and Ni, respectively. None of the applied metal concentrations influenced algal growth. The results of this study have potential implications for water quality criteria considering that the estimated EC_{20}s fall below the current criteria of 3, 86, 3, and 8.3 μg/L for Ag, Zn, Cu, and Ni, respectively.

Introduction

Historically, Ambient Water Quality Criteria (AWQC) have been considered protective of metal exposure to aquatic organisms (*1*−*4*). However, for most metals, the toxicity tests used to derive AWQC only consider waterborne exposure, generally overlooking potential dietary exposure. Several researchers have reported effects on invertebrates (*5*−*9*) from dietary metals exposure associated with waterborne metal concentrations at or below proposed or existing AWQC. Unlike waterborne metal exposure, the mechanism of dietary toxicity is not well characterized. In addition, metal-laden diets vary in toxicity depending on whether the diet was artificially prepared, or biologically incorporated metal (*5*, *6*−*8*, *10*).

Copepods are known to be sensitive to acute waterborne Ag (*6*−*8*), Zn (*11*, *12*), and Cu exposures (*12*−*15*), although chronic sensitivity through dietary exposure is not as well evaluated. Existing literature pertaining to dietary metal toxicity to copepods is sparse, but studies indicate sensitivity of zooplankton to this exposure route (*6*−*8*, *16*) and potential assimilation of metals from algae (*17*, *18*).

The zooplankton *Ceriodaphnia dubia* in freshwater and a mix of *Acartia tonsa* and *Acartia hudsonica* in saltwater were pulse-exposed (4 h) with a single metal-enriched alga followed by 6 days of continuous feeding with a mixed diet of metal-free algae in studies conducted by Hook and Fisher (*6*−*8*). Decreased reproduction due to metal-laden diets was reported in zooplankton at metal concentrations below those which caused effects through waterborne exposure (*6*−*8*). The implications of this study were significant, indicating dietary metal exposure may affect sublethal responses at levels below AWQC. However, some uncertainties exist with the Hook and Fisher studies regarding the pulse exposure regime and the mixture of copepod species used, in that differences in sensitivities may exist between species.

The present study aimed to address the above-mentioned uncertainties by using a single copepod species (*A. tonsa*) and two different dietary metal exposure regimes (pulse or continuous). Ultimately, the goal of this research was to determine whether AWQC are protective of dietary metal exposures to *A. tonsa*.

Experimental Section

Algae Culture. *Thalassiosira pseudonana*, *Isochrysis galbana*, *Rhodomonas lens*, *Skeletonema costatum*, and *Tetraselmis suecica* were continuously cultured for copepod diets, following APHA guidelines (*19*). The algal medium consisted of filtered (0.22 μm) natural seawater (salinity = 30−32 g/L) and F/2 nutrients, which was autoclaved prior to inoculation (*20*). Algae were incubated under cool white fluorescent lights (14:10 light/darkn) at 20 °C with continuous aeration for 5−7 d. Algal densities were measured by hemocytometer (Hausser Scientific, Horsham, PA).

Algal Bioconcentration Experiments. Radiolabeled Ag (^{110m}Ag) as $AgNO_3$ (0−6 μg/L), radiolabeled Zn (^{65}Zn) as $ZnCl_2$ (0−4 μg/L), radiolabeled Ni (^{63}Ni) as $NiCl_2$ (0−28 μg/L), or Cu as $CuSO_4$ (0−6 μg/L) was added to 1-L Erlenmeyer flasks containing the algae medium (750 mL) and equilibrated for 24 h (see below for specific radioactivity). A 1-L control flask (in which no metal was added) was also equilibrated for 24 h. Metal concentrations were chosen to bracket AWQC with the lower end of the concentration ranges being environmentally relevant. Background metal concentrations in the filtered seawater were <0.20 μg/L Zn (3 nM), <1 μg/L Cu (16 nM), and <0.18 μg/L Ni (3 nM). Water samples were then taken to measure metal content in the medium. *T. pseudonana* was cultured (in the presence of metals) as described above with the exceptions of no aeration and no additional ethylenediaminetetraacetic acid (EDTA), Cu, or Zn in the culture medium. The algae were exposed throughout log phase growth and harvested at the end of this period (6−7 days). The cultures were stirred by swirling once/day. Throughout the exposure, samples were collected to measure cell density and metal content in both media and cells. At day 7, algae were filtered (1 μm), re-suspended in filtered seawater, cell density counted, then refrigerated for use as copepod food. Algal cell density was measured throughout the copepod studies to verify constant cell count. At harvest, algal cell density approximated 1 000 000 cells/mL and was concentrated to approximately 10 000 000 cells/mL. Algal dry weights were determined using methods of Xu and Wang (*21*). In brief, algal cells were filtered onto a preweighed filter, rinsed with 0.5 M ammonium formate, and dried at 80 °C for 1 d.

Copepod Culture. *Acartia tonsa* were obtained from Guernsey Sea Farms (Parc Lane, Vale, Guernsey, U.K.). The copepods were held in 30-L aquaria, containing 20 L natural filtered seawater (30 g/L salinity), with continuous low aeration under cool white fluorescent lighting. The culture

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was maintained at a density <19 copepods per 1 L of medium, according to standard methods (*19*, *22*). Copepods were fed once per day a mixed algal diet ($2.0-6.0 \times 10^7$ total cells/L) consisting of four of the following algae (or diatoms): *T. pseudonana*, *I. galbana*, *R. lens*, *S. costatum*, and *T. suecica*.

Copepod Experiments. All experiments were conducted at a salinity of 30 g/L and a temperature of 15 °C. In one experiment, copepods were pulse-exposed to Ag-enriched *T. pseudonana* (200 000 cells/mL) for 4 h then transferred to new medium and fed 200 000 cells/mL/d of a noncontaminated mixed algal diet (*T. pseudonana*, *I. galbana*, and *S. costatum*) for 7 d. In all subsequent experiments, copepods were continuously fed 200 000 cells/mL/d metal-enriched *T. pseudonana* (Ag, Zn, Cu, or Ni) for 7 d. There were 5 replicates (100-mL beakers with 80 mL of filtered seawater) per treatment, each with 5 copepods per replicate. Approximately 90% of the medium was renewed and eggs were collected on days 3 and 5. Survival was recorded on days 3 and 7. At the end of exposures, adults were fixed in 10% formaldehyde and sexed using an inverted microscope. Eggs were incubated in filtered seawater for 3 days after collection to allow for hatching. Nauplii were offered *I. galbana* daily. After incubation, remaining eggs and nauplii were then fixed in 10% formaldehyde solution, stained with rose Bengal and counted for reproductive output. Unhatched eggs and nauplii were counted in the Ag pulse exposure test to determine total number of eggs produced and to assess whether this was a more sensitive endpoint. All other reproductive output is reported as nauplii per adult female at test initiation.

Analytical Chemistry. Silver, Zn, and Ni concentrations were measured by ^{110m}Ag, ^{65}Zn, or ^{63}Ni radioisotopic dilution. Graphite furnace atomic absorption spectrophotometry (GFAAS; Varian 220FS Mulgrave, Victoria, Australia) was used to measure the metal concentration of the stock solutions nominally containing 10 mg/L Ag with 0.25 μCi/mL of ^{110m}Ag; 10 mg/L Zn with 0.065 μCi/mL of ^{65}Zn; and 10 mg/L Ni with 1.6 μCi/mL ^{63}Ni. A gamma counter (Tm Analytic) was used to measure radioactivity of ^{110m}Ag and ^{65}Zn and a beta counter (Tm Analytic) was used to measure radioactivity of ^{63}Ni. Total Ag, Zn, or Ni concentration in each treatment was calculated from sample activity and measured specific activity of the stock solution. This method was also used to measure Ag, Zn, and Ni concentrations in algal samples. The detection limits for Ag, Zn, and Ni were 0.25, 0.50, and 0.10 μg/L, respectively. Copper concentrations in water were measured by coprecipitating with Fe (*23*) followed by GFAAS. As quality control, spiked samples were coprecipitated and measured for metal content. Algal Cu concentrations were determined by digesting in concentrated HNO_3^- and analyzing by GFAAS. The detection limit using this method was ≥0.20 μg/L.

Data Analysis. Statistical differences between treatments ($\alpha = 0.05$), no observable effects concentrations (NOECs), and lowest observable effects concentrations (LOECs) were calculated using Dunnett's Test (*24*). Concentrations at which 50% and 20% of the organisms were affected, LC_{50}s/EC_{50}s and EC_{20}s, respectively, were calculated by Probit method or Linear Interpolation method when appropriate (*25*). All statistical analyses were performed using ToxCalc v5.0 (Tidepool Scientific, McKinleyville, CA).

Results

Algae Metal Uptake. Aqueous metal accumulation by *T. pseudonana* primarily occurred within 24−48 h. Metal concentration per algal cell then decreased as a function of increasing algal density and stabilized as algal growth reached a plateau. Cell growth patterns were similar for all metals tested (data not shown). *T. pseudonana* cultured with metals grew as well as controls or better in most cases (data not shown), consistent with previous findings (*6*, *26*, *27*). The dry mass of *T. pseudonana* was 2.89×10^{-11} g/cell.

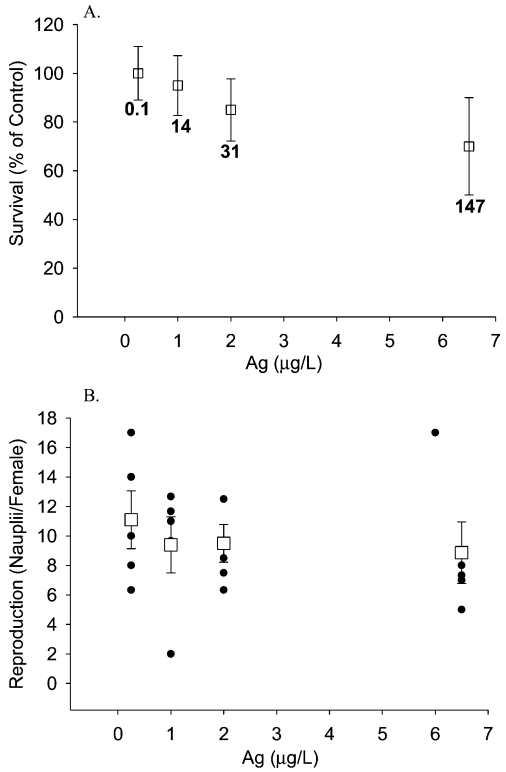


FIGURE 1. *A. tonsa* survival (A) and reproduction (B) after a 4-h pulse feeding of Ag-laden *T. pseudonana* followed by 6 days continuous feeding of Ag-free mixed algal diet. *X*-axis represents Ag concentration. Boxes and error bars represent mean and standard error ($n = 5$). Circles represent individual measurements. Numbers represent [Ag] in algae (μg/g dry mass).

Ag and *A. tonsa*. Silver was not detected (detection limit = 0.25 μg/L) in the copepod media during the 7-d exposure suggesting that the majority stayed bound to algae. No statistically significant differences were detected in survival between treatments pulse exposed to Ag-laden *T. pseudonana* and controls (Figure 1A). Consequently, the EC_{20} for survival was >147 μg Ag/g algal dry mass (>5.50 μg Ag/L in algal medium), the highest concentration tested. Survival of control copepods was 80%. As seen for survival, no statistically significant differences in reproduction (total number of eggs or nauplii) were detected during pulse exposure (Figure 1B). The total number of eggs produced was not a more sensitive endpoint than the number of nauplii produced in this experiment (data not shown). Consequently only the total number of nauplii was scored in following tests. Number of nauplii produced was approximately 70−85% of the total eggs produced.

In contrast, using a continuous exposure regime, a dose−response relationship was observed for adult copepods exposed to dietary Ag. Survival decreased by 73% in copepods continuously fed Ag-laden algae at 120 μg Ag/g algal dry mass as compared to controls (Figure 2A). The EC_{20} for survival was 1.60 μg Ag/g algal dry mass corresponding to a waterborne concentration 0.46 μg Ag/L in the algal media (Table 1). Survival of control copepods was 88%. Reproduction was affected at the same Ag concentration that caused decreased survival (Figure 2B). The NOEC for both survival and reproduction was 24.0 μg Ag/g algal dry mass corresponding to 1.39 μg Ag/L in the algal medium (Table 1). The EC_{20} for reproduction was 5.44 μg Ag/g algal dry mass (0.64 μg Ag/L in the algal media; Table 1).

Zn and *A. tonsa*. Limited leaching from algae into the copepod medium occurred in the three highest dietary Zn concentrations. The detection limit was 0.50 μg Zn/L. The

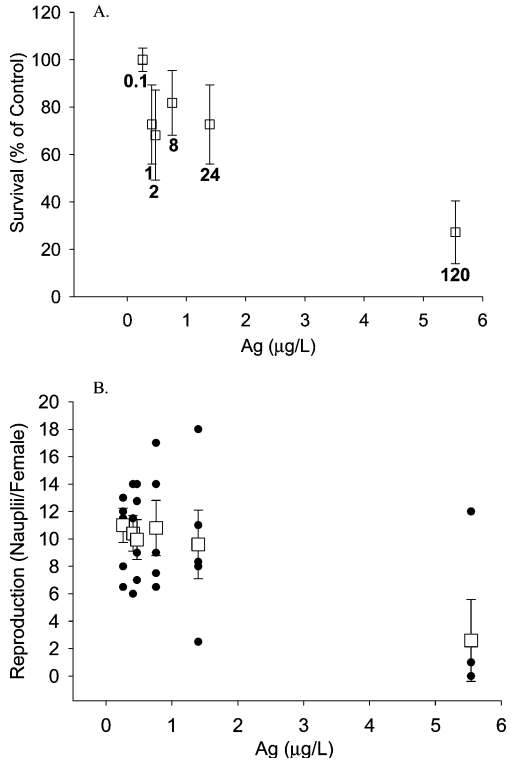


FIGURE 2. *A. tonsa* survival (A) and reproduction (B) after continuous feeding of Ag-laden *T. pseudonana* for 7 d. *X*-axis represents Ag concentration. Boxes and error bars represent mean and standard error ($n = 5$). Circles represent individual measurements. Numbers represent [Ag] in algae (μg/g).

waterborne Zn concentrations in those treatments were 0.50, 1.40, and 3.00 μg Zn/L by day 7. No differences in survival were detected during the first 3 days of exposure. However, by day 7, survival was significantly reduced compared to controls ($p < 0.05$) in all Zn exposures except the lowest concentration tested (Figure 3A). Percent survival of control copepods was 92%. The NOEC for survival in Zn-exposed copepods was 3.05 μg Zn/g algal dry mass (0.40 μg Zn/L in the algal medium), which was the lowest concentration tested (Table 1). Reproduction was significantly reduced in all Zn-exposed copepods (Figure 3B). The estimated EC_{20}s for survival and reproduction were 1.86 and 0.55 μg Zn/g algal dry mass (0.54 and 0.30 μg Zn/L), respectively (Table 1).

Cu and *A. tonsa*. Survival of copepods exposed to dietary Cu was not significantly reduced (Figure 4A). Survival in the control group was 72%. Reproduction, however, was significantly reduced in all Cu treatments (Figure 4B). The estimated EC_{20} value for reproduction was 22.3 μg Cu/g algal dry mass (1.20 μg/L in the algal medium; Table 1). The NOEC for reproduction was <31.9 μg Cu/g (<1.50 μg Cu/L in the algal media; Table 1). Copper concentrations in the copepod media were not elevated above background levels (1 μg/L) indicating that the majority of Cu stayed bound to algae. The detection limit for Cu was ≥0.20 μg/L.

Ni and *A. tonsa*. A U-shaped dose−response relationship was observed for copepod survival in the Ni experiment, precluding a straightforward statistical analysis (Figure 5A). Control survival was 72%. In contrast, a dose-dependent decrease in copepod reproduction was observed (Figure 5B). The NOEC for reproduction was 23.4 μg/g (3.82 μg Ni/L in the algal media; Table 1). The estimated EC_{20} for reproduction was 15.3 μg Ni/g algal dry mass (2.43 μg Ni/L in the algal medium; Table 1). Nickel concentrations in the copepod media were below detection (detection limit = 0.10 μg/L) and below background, indicating that Ni predominantly stayed bound to the algae.

Discussion

Our data indicate that *A. tonsa* is very sensitive to Ag, Cu, Ni, and Zn when exposed via phytoplankton. These results partially confirm previous studies (*6*−*8*) on Ag and Zn, although there were differences with respect to pulse versus continuous exposure to Ag. Effect concentration values (EC_{20}s) were calculated because current AWQC are regulated by these values. However, there are several caveats concerning derivation of the EC_{20} values. First, there was consistent response of approximately 20% decreased survival as compared to controls in all of the treatments except the highest Ag concentration; therefore, the EC_{20} estimation was driven largely on the response of the highest treatment only and should be used with caution. Second, the calculated EC_{20}s for Zn and Ni were less than the lowest value tested. Since these values are extrapolated and also lower than the NOEC values, which were derived by hypothesis testing, they should be treated with caution when used as effect levels. Overall, our results suggest current or proposed water quality criteria for Cu, Ni, and Zn may not be protective of *A. tonsa* exposed through the diet. These issues and associated uncertainties are discussed in the following.

Comparison to Earlier Studies. One objective of our study was to validate the methods and results previously obtained by Hook and Fisher (*6*−*8*) for Ag and Zn. In their experiments, copepods pulse-exposed (4 h) to algae grown with metal (Ag, Cd, Zn) followed by exposure to algae grown without metal addition for the remaining exposure period (7 d) exhibited significant effects on reproduction. A second experiment (Zn only) in which copepods were exposed continuously to dietary Zn for 7 d at levels below those in the pulse exposures resulted in no significant effects on reproduction (*8*).

In contrast, our experiments indicated pulse exposure to dietary Ag had no significant effect on survival or reproduction; whereas continuous exposure to dietary Ag levels similar to those used in the pulse exposure did elicit effects at a waterborne Ag concentration approximately an order of magnitude higher than those reported by Hook and Fisher (*6*−*8*). We did not conduct a pulse exposure study with Zn; however, our continuous exposure experiment resulted in effects on reproduction at 0.62 μg/L total Zn, nearly the same waterborne Zn concentration (0.65 μg/L) at which Hook and Fisher (*6*−*8*) observed effects using a pulse exposure. Some leaching of Zn from the algae to the water occurred at concentrations of ≤3 μg/L Zn in the highest three treatments. Verriopoulos and Hardouvelis (*28*) reported a decrease in the number of egg sacs produced when the copepod *Tisbe holothuriae* was exposed to 10 μg/L waterborne Zn, which is more than 3 times the waterborne concentration in our highest treatment. Therefore, it is likely that the effects observed resulted from the dietary exposure only. The Zn concentration accumulated in the algae in our study was less than that reported by Hook and Fisher (*6*), possibly reflecting different time points at which the alga was harvested. Algal cell density influences metal concentration per cell. Therefore, differences in the time points at which the algae were harvested, possibly reflecting differences in algal cell density, may have impacted metal concentration per cell and possibly the metal distribution within algae. Conceivably, metal distribution within the algae might alter metal bioavailability to the copepods.

Perhaps differences in Ag toxicity between our pulse-exposure study and the Hook and Fisher study (*7*) are due to the Ag body burden in the copepods. Even though Ag concentrations in algae were similar when fed to copepods, the fraction of bioavailable metal in algae may have differed, depending on the time at which it was harvested. It has been

TABLE 1. No Observable Effects Concentrations (NOECs), Lowest Observable Effect Concentrations (LOECs), and Concentrations Which Caused a 20% Effect (EC_{20}s) in Copepods Exposed to Dietary Ag, Zn, Cu, or Ni

endpoint	Ag (μg/L)[a]	Ag (μg/g)[b]	Zn (μg/L)[a]	Zn (μg/g)[b]	Cu (μg/L)[a]	Cu (μg/g)[b]	Ni (μg/L)[a]	Ni (μg/g)[b]
survival NOEC	1.39	24	0.40	3.05	5.61	158	***	***
survival LOEC	5.50	120	0.62	14.0	>5.61	>158	***	***
survival EC_{20}	0.46	1.6	0.54*	1.86*	**	**	***	***
reproduction NOEC	1.39	24	<0.40	<3.05	<1.50	<31.9	3.82	23.4
reproduction LOEC	5.50	120	0.40	3.05	1.50	31.9	7.60	58.1
reproduction EC_{20}	0.64	5.44	0.30*	0.55*	1.20	22.3	2.43*	15.3*

[a] Values represent dissolved metal concentration in the algal medium. Single asterisk indicates estimated EC_{20} is an extrapolated value below the lowest value tested. Double asterisks indicate no observed effect at the concentrations tested. Triple asterisks indicate that the value was unable to be calculated. [b] Values represent dissolved metal concentration in the algal medium.

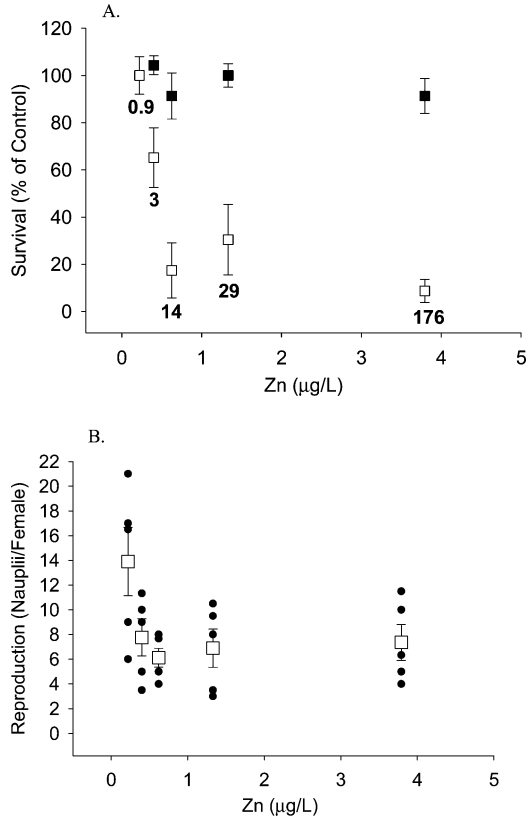


FIGURE 3. *A. tonsa* survival (A) and reproduction (B) after continuous feeding of Zn-laden *T. pseudonana* for 7 d. *X*-axis represents Zn concentration. Clear boxes and error bars represent mean and standard error at d 7 ($n = 5$). Solid boxes and error bars represent the mean and standard error at d 3. Circles represent individual measurements. Numbers represent [Zn] in algae (μg/g).

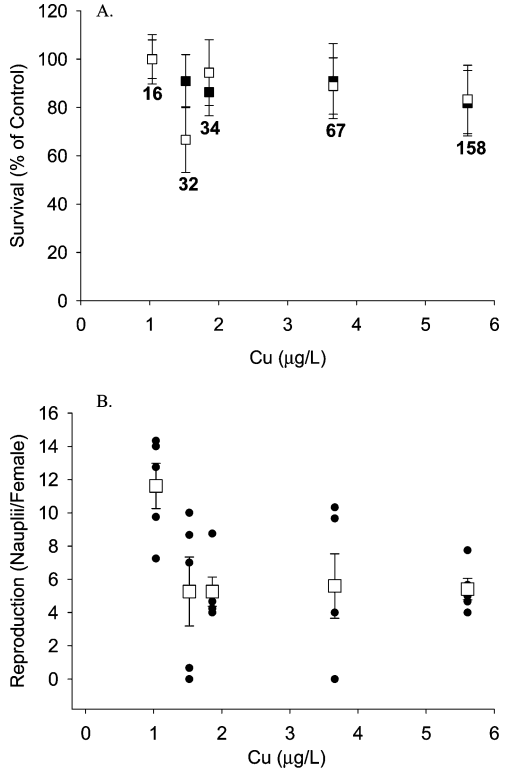


FIGURE 4. *A. tonsa* survival (A) and reproduction (B) after continuous feeding of Cu-laden *T. pseudonana* for 7 d. *X*-axis represents Cu concentration. Clear boxes and error bars represent mean and standard error at d 7 ($n = 5$). Solid boxes and error bars represent mean and standard error at d 3. Circles represent individual measurements. Numbers represent [Cu] in algae (μg/g).

demonstrated that the assimilation efficiency of dietary metals by *A. tonsa* is strongly related to the fraction of metal in algal cytoplasm (*17, 21, 29*); but may also be related to metals loosely bound to cell surfaces, which potentially become bioavailable during digestion. The possibility remains that as algae grows over time and metal distribution per algal cell changes, so does compartmentalization of metals. Clearly, additional studies on the subcellular distribution of metals in algae over time, at concentrations known to exert effects on copepods, are needed.

Metal Toxicity. The dose−response relationship for dietary Zn, Cu, and Ni was characterized by an approximately 50% decrease in copepod reproduction, and this effect did not substantially increase with metal concentrations >5.6 μg/L Cu and >3.8 μg/L Zn. Silver toxicity to copepods increased significantly with increasing dose and no attenuation of this effect was observed with concentrations up to 5.5 μg/L. It is also important to note that, unlike the other metals tested, Ag is not an essential nutrient. Our results are consistent with previous findings that copepod reproductive output is significantly impacted when fed diets previously exposed to low metal concentrations, however, the slope of the effect was contingent upon the metal tested.

Influence of Diet. It is also important to consider that *A. tonsa* adults were fed a single algal diet in these experiments to simplify characterization of metal transfer between two trophic levels. *A. tonsa* usually feed on a mixed algal diet in the wild; however, characterizing metal uptake in such a system would be very complex. There have been conflicting results making it unclear whether monoalgal diet is a confounding factor in this study; however, the success of copepods in the wild may in part be due to the selection of prey items, particularly avoidance of contaminated food (*30*−*34*). Feeding rate was not measured in our studies; however, the possibility remains that the elevated dietary metal may have reduced copepod feeding rates and contributed to

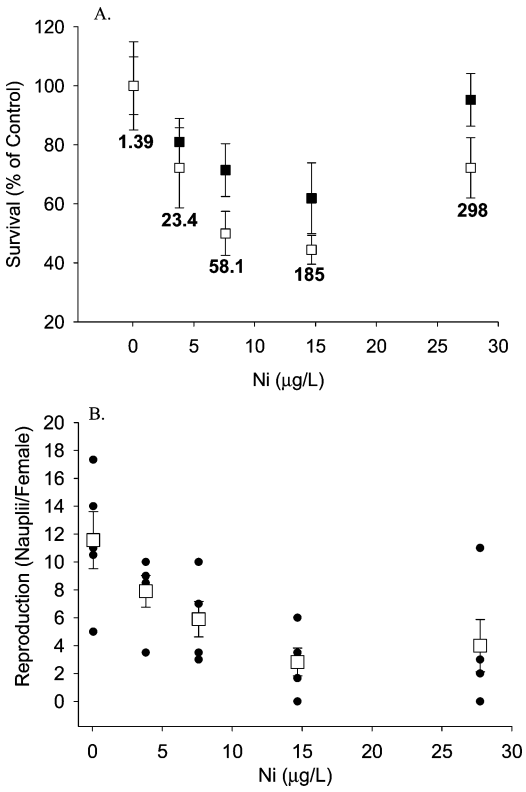


FIGURE 5. *A. tonsa* survival (A) and reproduction (B) after continuous feeding of Ni-laden *T. pseudonana* for 7 d. *X*-axis represents Ni concentration. Clear boxes and error bars represent the mean and standard error at d 7 ($n = 5$). Solid boxes and error bars represent the mean and standard error at d 3. Circles represent individual measurements. Numbers represent [Ni] in algae (μg/g).

observed reductions in fecundity. Copepods in this study were offered a higher cell density of *T. pseudonana* than that used in other similar studies (*6*, *7*, *21*). Xu and Wang (*21*) reported that physiological turnover rate was independent of food concentration and that a lower fraction of Ag was retained in the gut of *Acartia tonsa* fed a high algal cell concentration.

In addition, the algae used in this study, as well as those used by Hook and Fisher (*6*−*8*), were grown without the addition of Cu and Zn in the algal medium to better quantify exposure concentrations, and without the addition of EDTA, which is used in culture medium to enhance uptake of Fe and other micronutrients. The Cu and Zn, usually added to algal medium, are generally complexed by EDTA, reducing accumulation in the tissues and ultimately metal toxicity is reduced (*34*). Natural seawater was used in these experiments, therefore low levels of Cu and Zn were present in the algal medium. Copper and Zn are required nutrients and when present at low levels uptake may increase due to high affinity binding sites and/or upregulation of metal importers, which may have subsequently resulted in increased metal uptake during exposure to elevated metal concentrations.

Metals in the Environment. Finally, it is important to consider both the metal concentrations and their forms in coastal environments where *T. pseudonana* and *A. tonsa* are found. Metal concentrations in seawater generally range from 1 to 100 ng/L Ag (*35*), 0.003−16 μg/L Zn (*36*, *37*), 0.13−9.5 μg/L Cu (*38*), and from 0.2 to 130 μg/L Ni (*39*, *40*). The highest Zn, Cu, and Ni concentrations were measured in estuaries with significant anthropogenic inputs. However, in most cases the concentration of organic ligands, such as humic and fulvic substances, as well as the concentration of inorganic ligands in seawater exceed metal concentrations thereby forming complexes and rendering metals less bioavailable to aquatic organisms (*41*−*45*). Polluted marine environments may contain phytoplankton with metal levels as high as 58 μg/g Ag (*46*, *47*), 115 μg/g Zn (*48*), and 57 μg/g Cu (*46*). Metal concentrations causing reproductive effects in this study were 120 μg/g Ag, 3.05 μg/g Zn, and 31.9 μg/g Cu. Nickel concentrations in phytoplankton were not found. Although the Ag concentrations causing effects are 2-fold higher than reported values in the environment, both Zn and Cu may be problematic to *A. tonsa* in polluted environments.

Thus, in the present study, we demonstrate effects on copepods at naturally (or frequently) occurring metal concentrations found in near shore coastal environments. Copepods are generally abundant in these coastal areas, which could indicate artifactually low effect levels in this study, although it cannot be excluded that natural populations of copepods are impacted by existing metal concentrations. The seawater used in these experiments contained relatively low DOC concentration (0.1607 ± 0.0010 mM; *49*) compared to most coastal areas (DOC typically 0.05−0.83 mM) where the highest metal concentrations occur (*50*).

Significance and Use of Findings. In summary, when *T. pseudonana* was cultured with relatively low levels of Ag, Zn, Cu, or Ni and continuously fed to *A. tonsa* reproductive impairment occurred. Metal levels in algal medium which caused effects to *A. tonsa* were below existing AWQC for Zn, Cu, and Ni, and near the proposed AWQC for Ag, suggesting that the criteria may not always be protective. However, some uncertainty exists regarding the environmental realism of this exposure system with respect to feeding mono-algal diets to copepods and adequacy of essential metal levels, both of which may lead to unrealistic algae metal accumulation and reduced nutritional value. Finally, although we demonstrated effects at commonly occurring metal concentrations, typical of coastal environments, it should be kept in mind that tests were performed at low DOC concentrations using soluble forms of the four metals. Overall, our findings agree with earlier studies suggesting potential effects of dietary metals on copepods at waterborne concentrations below AWQC. Further research in these areas under different environmental exposure scenarios is recommended.

Acknowledgments

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