SUBLETHAL EFFECTS OF SILVER IN ZOOPLANKTON: IMPORTANCE OF EXPOSURE PATHWAYS AND IMPLICATIONS FOR TOXICITY TESTING

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Abstract—In aquatic environments, organisms are exposed to contaminants via direct uptake from water and by trophic transfer. However, most toxicity tests only examine uptake via the dissolved phase. We compared the response of marine and freshwater crustacean zooplankton to silver following dissolved and food exposure. Silver, like other metals, concentrates in aquatic food chains and may exert toxicity. In standard solute exposure toxicity tests, Ag is toxic to zooplankton at concentrations of 400 nM for marine copepods and 100 nM for freshwater cladocerans, concentrations far greater than those in most waters. However, if Ag is accumulated from algal food, reproductive success decreases by >50% when algae are exposed to only 1 nM Ag in copepods and 0.5 nM Ag in cladocerans. These concentrations are within an order of magnitude of those found in contaminated estuaries. Following dietary exposure, decreased egg production and viability occur when tissue Ag concentrations increase three- to fourfold to 0.3 ppm in cladocerans and 0.5 ppm in copepods. Assimilated Ag depresses egg production by reducing yolk protein deposition and ovarian development. Our results indicate that ecologically relevant toxicity tests should consider sublethal effects of contaminants obtained from food since these effects cannot be predicted from exposures to only dissolved contaminants.

Keywords—Silver  Copepods  Cladocerans  Toxicity  Egg production

INTRODUCTION

Many aquatic toxicity tests involve protocols in which an organism’s health is measured as a response to dissolved contaminants over a range of concentrations and do not assess the impact of contaminants accumulated from food [1,2]. This approach is clearly valid for aquatic plants but is questionable for animals, which can also accumulate contaminants through their food. For marine herbivores, dietary uptake can be a dominant route of entry for a variety of metals [3–10]. Further, the tissue distribution of a metal within an animal is dependent on the route of uptake [7,11–15]. These studies have generally shown that metals accumulated from the dissolved phase are more likely to deposit in gill and external tissues and metals taken up from food are deposited in internal tissues. Since metals taken up from food are accumulated in different tissues than metals taken up from water, it is likely that they would exert toxicity via different mechanisms. However, the toxicity of metals accumulated in aquatic invertebrates through their diet has received attention in only a few studies [16], and the mechanisms of toxicity remain largely unknown.

Toxicity tests frequently measure toxicity as a function of ambient metal concentrations and often do not account for the bioavailability of that metal. The bioavailability of a metal is not constant and instead can vary with water-column chemistry [17]. Certain metals may therefore exert greater toxicity in bodies of water with low concentrations of organic complexing agents [18,19]. Since organisms do not respond directly to ambient contaminants but only to contaminants associated with them [19,20], metal toxicity tests should examine the effects of metal body burdens or, if possible, the metal concentration in a sensitive tissue. Expressing toxicity as a function of the metal concentration of a sensitive tissue would allow for the integration of bioavailability of all species of the metal in question, account for the exposure history of the organism, and connect effect to the site of toxic action within the animal.

Moreover, toxicity tests often examine only lethal toxic effects. Consequently, metal toxicity tests commonly employ metal concentrations greatly in excess (frequently by several orders of magnitude) of concentrations in even the most polluted bodies of waters [1]. A safety factor (e.g., 1/10 the LC50 concentration, the dissolved contaminant concentration at which half the test animals die over a specified period of time) is sometimes applied in the absence of appropriate test data to protect for chronic sublethal effects [2]. Implicit in this approach is that chronic effects have the same underlying mechanism of toxicity [21]. However, if metals accumulated from different sources localize in different tissues and exert differing degrees of toxicity through different mechanisms, the application of a safety factor to an LC50 value based on solute uptake could be inappropriate.

We hypothesized that metals accumulated through dietary uptake would exert toxicity at different concentrations and by a different mechanism than metals accumulated from the dissolved phase because the tissues in which the metals are distributed is dependent on the uptake pathway. Consequently, the toxicity of ingested metals would not be predicted well from dissolved exposure toxicity tests.

Here we describe a series of experiments in which the toxicity and bioaccumulation of a potentially toxic metal, silver, were measured in freshwater cladocerans and marine copepods following accumulation from water or from food. Sublethal effects were measured, and the concentrations at which the effects were observed were compared to LC50 values. We focused on sublethal effects because sublethal endpoints like reproductive success may be more sensitive than adult survival and can be critical for the health of a population [22]. The tissue concentrations of Ag at which sublethal effects occurred were determined and were compared with background levels.

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Finally, the mechanism behind observed toxic effects was examined. Silver is a common aquatic contaminant resulting from sewage discharge [23–26], has been shown to be toxic to some aquatic organisms at low concentrations, and may be of concern in some waters [1,27].

Crustacean zooplankton are important components of marine and freshwater food webs since they are the primary grazers in many ecosystems and are often the major food source for developing larvae and fish. Contaminant impacts on these animals are of interest since they can affect food web structure by altering the grazing on phytoplankton communities and by affecting the food supply of predators. Cladocerans are commonly used in toxicity tests because they are easily handled and sensitive to many contaminants. While cladocerans, specifically *Ceriodaphnia* and *Daphnia* species, are among the most commonly used test organisms, there is a limited data base on the impacts of metals at environmentally realistic concentrations. Further, few studies have measured the effects of low, environmentally realistic concentrations of metals on copepods, particularly planktonic calanoid copepods, which can dominate plankton assemblages.

**MATERIALS AND METHODS**

_Simocephalus* sp. was collected April through November from a pond on the campus of SUNY, Stony Brook (Stony Brook, NY, USA). *Ceriodaphnia dubia* was obtained from Aquatic Bio Systems (Fort Collins, CO, USA), and marine copepods were collected year round from Stony Brook Harbor at high tide using a 110-μm mesh net. Animals were acclimated to laboratory conditions for at least 24 h prior to their use in experiments. No stress effects were observed in survival or egg production of control zooplankton in different seasons, and no systematic differences were noted in response to Ag for animals acclimated for periods ranging from 24 to 72 h. Adult animals of the intended species were separated from the rest of the plankton prior to use with the aide of a dissecting microscope. Experiments were conducted at 15°C with the marine calanoid copepods *Acartia tonsa* and *A. hudsonica* and at 20°C with the cladocerans *Simocephalus* sp. and *C. dubia*.

Experimental animals were exposed to a range of Ag concentrations in food or in solution. The sublethal toxicity of Ag to zooplankton obtained from the dissolved phase was assessed with animals exposed to Ag concentrations of 0.5, 1, 2, and 5 nM for copepods and 0.125, 0.25, 0.5, and 1 nM for cladocerans; these concentrations were in addition to background Ag levels estimated at 0.01 nM [28] (1 nM Ag = 108 ng/L). Ag-containing solutions were prepared 1 d prior to addition of animals to allow the Ag to equilibrate between inorganic and organic ligands naturally present in the water [29]. Zooplankton were exposed to solutions containing dissolved Ag for 12 h, after which animals were transferred into filtered water without Ag additions and fed uncontaminated food. The uncontaminated food consisted of a mixture of *Chlorella autotrophica* and *Daphnia* pellets (from Carolina Biological Supply, Burlington, NC, USA) for the cladocerans or cells of the algae *Isochrysis galbana*, *Rhodomonas salina*, *Thalassiosira pseudonana*, and *Prorocentrum minimum* for the copepods. This combination of algal species promotes fecundity in marine copepods [30]. Sublethal effects were measured 24 h after their transfer out of Ag-containing solution. To determine lethal toxicity, animals were continuously exposed to Ag solutions ranging from 0.1 to 1,000 nM for 48 h following ASTM protocols [31]. Surviving animals were counted at 6, 24, and 48 h. Sublethal exposure periods were held to 12 h (for dissolved Ag exposures) to eliminate starvation as a possible complicating factor. Lethal toxicity tests were run for up to 48 h to facilitate comparisons with standardized protocols [31]; after 48 h, control animals began dying of starvation. Dissolved exposure solutions were made using either 0.2-μm filtered Southampton (NY, USA) surface seawater (collected 8 km offshore) for copepods or deionized water with WCL-1 salts [32] added, adjusted to pH 7, for cladocerans. The dissolved organic carbon concentrations, measured with a Shimadzu TOC 5000 analyzer (Shimadzu, Tokyo, Japan) were < 0.2 mg/L for freshwater and ~1 mg/L in seawater. The hardness of the WCL-1 medium was 16 mg CaCO₃/L.

Zooplankton were exposed to Ag via food by first preparing phytoplankton cells enriched with varying concentrations of Ag in culture. Phytoplankton prey (the diatom *Thalassiosira pseudonana* for copepods) were grown for 4 d (at least six cell divisions) in f/2 medium [32] prepared with filtered Southampton seawater but with no added ethylendiaminetetraacetic acid, Cu, or Zn [33]. For cladocerans, the chlorophyte *Chlorella vulgaris* was cultured in WCL-1 [32] medium prepared with deionized water. Thus, the concentrations of dissolved organic carbon and major cations and the pH of solutions were identical for food-exposure and solute-exposure treatments, and consequently the Ag speciation in algal media and zooplankton solute-exposure waters should have been similar. The algal growth media were prepared with a range of Ag concentrations of 0, 0.125, 0.25, 0.5, or 1 nM added Ag for freshwater cells and 0, 0.5, 1, 2, and 5 nM added Ag for marine cells. The Ag concentrations ≥5 nM prevented growth of the algae. The Ag additions were from a stock solution in 0.1 N HNO₃ and were followed by additions of 0.1 N NaOH to readjust the pH. After growth and Ag uptake, log-phase cells were filtered onto a 1-μm Nuclepore filter, resuspended into filtered seawater or WCL-1 without added Ag, and fed to the zooplankters at algal concentrations of 2 mg dry weight/L, as described previously [34]. The animals were allowed to feed for 4 h and were then fed uncontaminated algae (noted above) to purge unassimilated boluses of Ag-containing matter out of their guts. Feeding periods were held to 4 h to minimize recycling of Ag from fecal material during feeding.

Parallel experiments were designed to measure the accumulation of Ag in the zooplankton so that toxicity could be expressed as a function of the Ag body burden in the animals. Uptake of ¹¹⁰mAg by zooplankton in two replicate solutions was examined over a 4-h period and the Ag uptake rate constant (L/g wt animal/d), normalized for the ambient water concentration, was calculated following the protocols of Wang and Fisher [7]. The assimilation of ¹¹⁰mAg by zooplankters from radiolabeled algal food was also determined following established protocols [3,34]. Assimilated Ag is that which crosses the gut lining and gets incorporated into the animal’s tissues. To determine assimilation efficiency, cells of *T. pseudonana* (marine) or *C. vulgaris* (freshwater) were uniformly radiolabeled with ¹¹⁰mAg, resuspended, and fed to the animals as described for the nonradioactive cells. To prepare radiolabeled algal cells, microliter quantities of ¹¹⁰mAg, dissolved in 0.1 N HNO₃, were added to cell suspensions, the pH of the cultures was adjusted to 8.0 (marine) or 7.0 (freshwater), and cells were incubated for 3 to 4 d [33]. Zooplankters were fed for 4 h on cell suspensions of 1 × 10⁶ cells/ml, after which aliquots of animals were collected on a 210-μm mesh, rinsed with unlabeled water, and their radioactivity counted. Other radiola-
beled zooplankters were transferred to unlabeled algal suspensions to purge their guts of undigested radiolabeled food, and assimilation efficiencies were determined as described elsewhere [3,6]. Zooplankton uptake of $^{110m}$Ag that had desorbed from the radiolabeled cells during the 4-h feeding period was checked following procedures described elsewhere [6] and found to be negligible.

Body burdens of Ag (nmol/g dry wt animal) obtained from food were determined as [Ag concentration in algal cells]×[cells ingested]×[assimilation efficiency of ingested Ag]/[dry weight of the animal]. The measured dry weight of the copepods was 4 μg/individual and of the cladocerans 3 μg/individual. Concentrations of metal gained during the experiment were added to the animal’s background concentration. Background Ag concentration in the animals was estimated as 1.3 nmol/g dry weight [28]. The radioactivity of $^{110m}$Ag in all samples was determined, together with appropriate standards, using a NaI(Tl) gamma counter by measuring gamma emissions at 658 keV. Propagated counting errors were typically <5%.

Mortality, growth rate, respiration rate, feeding rate, egg production, and viability and behavior were toxic endpoints considered over a 7- to 10-d period following either exposure to dissolved Ag or feeding on food enriched with Ag. Egg production was measured by separating eggs from adults using gravity filtration with a 210-μm Nitex (Sefar, Heiden, Switzerland) mesh to catch adults and a 60-μm Nitex mesh to catch eggs. Eggs were sorted from fecal pellets by pipet and counted using a dissecting microscope. Hatching rates were determined by placing eggs into individual wells of a multiwell culture plate and visually inspecting for hatching. Degree of ovarian maturation was also considered in assessing reproductive capacity. Copepod sex was determined by microscopic examination of antennae morphology, and ovarian development was determined by microscopic inspection of formalin-fixed females [35].

Biochemical analysis was performed on eggs. Eggs were collected with a pipet, filtered onto a glass-fiber filter homogenized using a bead beater, then centrifuged at 14,600 g for 15 min. The supernatant was collected and analyzed for total protein content [36]. An SDS-PAGE analysis [37] was performed to identify the dominant proteins in the copepod eggs. Respiration rate was determined by measuring O$_2$ consumption using a micro-Winkler technique [38]. Feeding rates were calculated from cell count measurements before and after feeding; cell counts were performed with a Coulter Multisizer particle counter (Coulter Electronics, Luton, UK) and were confirmed with microscopic examinations. Animal growth rate was determined by measuring changes in the length of animals and determining naupliar instar stage after a fixed time from hatching. For assessing effects on behavior, animals were videotaped using a fixed-frame, laser-illuminated video imaging system, as described elsewhere [39]. Zooplankton were filmed in the dark for normal response, with a light source for a phototactic response, and following addition of a drop of water to the test chamber for an escape response. Movements were analyzed using the computer program Optimus to determine if swimming speed or directional response to stimuli (e.g., light, vibrations) changed with Ag exposure.

Data were analyzed using analysis of variance, goodness of fit, and linear regression [40]. All experiments were performed two to four times to check for seasonal variations (which were negligible). Toxicological results were based on data from five replicates per treatment, and bioaccumulation results were based on three replicates per treatment. Because no temporal differences were noted, data from replicate experiments were pooled, resulting in replications ranging from 7 to 25 per treatment.

RESULTS AND DISCUSSION

Silver exerted both sublethal and lethal effects on the zooplankters. Copepods and cladocerans displayed no sensitivity to Ag obtained from the dissolved phase at concentrations below 100 nM (Figs. 1 and 2). Following exposure to dissolved Ag, the 48-h LC50 of Ag was 250 nM for cladocerans and 400 nM for copepods, concentrations that are more than four orders of magnitude greater than concentrations in seawater [41] and two to four orders of magnitude above concentrations in most bays, estuaries, and rivers [25]. The cladoceran LC50 was slightly lower than a reported 48-h LC50 of 324 nM Ag for another cladoceran, Daphnia magna, in river water [42].

Sublethal effects of Ag were only evident after Ag exposure following assimilation from food, the most prominent effect being depressed egg production, which decreased by up to 50% (Fig. 1). Copepods were impacted when their algal food was exposed to 1 nM Ag (Fig. 1); cladocerans (both C. dubia and Simocephalus sp.) were affected when their algal food was exposed to 0.5 nM Ag (Fig. 1), and no sensitivity differences were noted between the cladoceran species. Comparable levels of Ag in the dissolved phase had no effect on egg production (Fig. 1). The Ag concentrations in algal food that affected egg production were about 37 nmol/g dry weight for marine cells (approximately 90 times greater than typical values observed in the field [28]) and about 19 nmol/g dry weight for freshwater cells (approximately 2–40 times greater than freshwater particulate matter in diverse rivers and lakes [43]) (Table 1). No other endpoint, including mortality, was altered by exposure to Ag via food. The other sublethal endpoints, including respiration rate, feeding rate, growth rate, and behavior, were unaffected by Ag exposure (dissolved or in food) at environmentally realistic concentrations (data not shown).

Silver was accumulated by cladocerans and copepods from the dissolved phase and from food, with body burdens in whole animals being generally higher following solute exposure to Ag than dietary exposure (Table 2). Silver accumulated from the dissolved phase in cladocerans reached concentrations of 7 nmol/g dry weight (= 0.76 ppm dry wt) following the 12-h exposure to 1 nM Ag, and copepods accumulated Ag from the dissolved phase to a concentration of 31.9 nmol/g dry weight (= 3.4 ppm dry weight) following exposure to 5 nM Ag. Nevertheless, these concentrations, which are up to 24 times greater than concentrations of Ag in coastal marine copepods [28], had no observable toxic effect. Body burdens of Ag resulting from dietary exposure that were considerably lower (3.1 nmol/g dry wt in cladocerans, 4.4 nmol/g dry wt in copepods: Table 2) did have significant toxic effects on the animals. To compare Ag uptake results to previous studies, bioaccumulation uptake parameters were calculated from measured Ag concentrations in the zooplankton. The Ag uptake rate constant in copepods was 12.4 L/g/d, slightly higher than the value of 10.4 L/g/d measured by Wang and Fisher [7] for a larger calanoid copepod, Temora longicornis. Assimilation efficiencies of ingested Ag were 15% in copepods and 16% in cladocerans, comparable to previous findings [3,7]. The Ag uptake data were used to evaluate toxicity as a function of Ag dose in the animals.

When the toxic effects of ingested Ag are expressed on the
Silver effects in zooplankton

Fig. 1. Response (as % control) of zooplankton to Ag. Plots A and B show survival and egg production of cladocers (Ceriodaphnia dubia, Ceriodaphnia sp., and Simocephalus sp.) and copepods (Acartia tonsa, A. hudsonica) to varying concentrations of Ag to which animals were exposed directly (solute exposure). Plots C and D show survival and egg production (as % control) in cladocers and copepods to dietary exposure to Ag. Concentrations in plots C and D shown are the Ag concentrations to which food was exposed. Points denote means from replicate experiments (n = 7–25 replicates per treatment). Least squares regression lines were drawn through all data points. Dashed lines denote regression lines significantly different from other lines. Egg production declined (p < 0.05, ANOVA) when food was exposed to Ag concentrations ≥1 nM in copepods and ≥0.5 nM in cladocers. Significant increases in mortality occurred at Ag concentrations ≥400 nM for copepods and ≥100 nM for cladocers.

Fig. 2. Relative reproductive success (as % control) in copepods (Acartia tonsa and A. hudsonica) following exposure to Ag. Reproductive success is defined as the number of eggs produced per individual multiplied by the hatching frequency per egg. Plot A depicts toxicity as a function of added Ag concentration, plot B as a function of Ag body burden. ○, egg production following exposure to Ag from the dissolved phase; ●, egg production following exposure to Ag via food. Points on graph denote means of all experiments (n = 7–25 replicates per treatment). Least squares regression lines were drawn through all data points. Dashed lines denote regression lines significantly different from other lines. Significant declines (p < 0.01, Chi-square) in egg hatching rate occurred when food was exposed to Ag concentrations ≥2 nM and at zooplankton Ag body burdens ≥7.4 nmol/g dry weight following dietary exposure to Ag (see also Table 1). No decline in reproductive success was observed following solute exposure.

basis of zooplankton tissue concentration, rates of egg production were about half of control values in cladocers (from 2.3 ± 0.3 eggs/female/d, or 16.1 eggs/female/week, in controls to 0.9 ± 0.7 eggs/female/d) at Ag concentrations of 3.1 nmol/g dry weight and in copepods (from 16.2 ± 4.0 eggs/female/d in controls to 9.4 ± 3.1 eggs/female/d) at Ag concentrations of 4.4 nmol/g dry weight (Fig. 3). The Ag body burden that significantly affected egg production in copepods is about 3.3 times greater than concentrations of Ag in coastal marine copepods [28]. Since the copepods used in our experiments release their eggs into the water and the cladocers brood their young, egg production per individual is always greater for the
Table 1. Hatching rate, ovarian development, and protein content per egg in copepods (Acartia tonsa and A. hudsonica) following exposure to Ag via food; values shown are percent of control values. Also shown are Ag concentrations to which phytoplankton food was exposed and resulting Ag concentrations in food presented to the copepods. Hatching rate of control eggs was 66% (comparable to the 60–70% rate reported by Jonasdottir [30]), ovarian development in control females was 70%, and total protein content per egg in controls was 18 ± 1 ng. Values reported are means of five replicates ± 1 SD. For hatching rates and ovarian development, relative frequencies are reported. Chi-square goodness-of-fit tests were run for hatching rate and ovarian development data. ANOVA for protein content of eggs. Asterisks denote values significantly different (p < 0.01) from controls. ND, not determined

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copepods. Furthermore, the hatching rate of the eggs produced by adult copepods exposed to Ag in food was also affected, so that body burdens of Ag of about 9 nmol/g dry weight reduced the number of viable offspring to only 25% of control values (Fig. 2). Eggs collected from control copepods that were subsequently exposed to dissolved Ag at concentrations up to 5 nM had normal hatching success (data not shown). Reproductive success was not affected in copepods exposed to dissolved Ag, as no effects on either egg production or hatching rates were detected (Fig. 2).

Differences in Ag toxicity in the zooplankters following solute exposure and ingestion of Ag-enriched food were not attributable to different Ag body burdens in whole animals with different uptake pathways (Fig. 2 and Table 2). Kinetic modeling based on laboratory Ag bioaccumulation experiments indicates that, under conditions typically prevailing in nature, 50 to 80% of a copepod’s Ag content is taken up from the dissolved phase, and much of this metal associates with the chitinous exoskeleton of these animals [7]. The association of Ag with the exoskeleton may explain our finding that accumulation of Ag from the dissolved phase, while contributing to the overall body burden of Ag in these animals, has no effect on these animals at environmentally realistic concentrations. Since metals accumulated from food and water are distributed in different tissues in aquatic invertebrates [7,11–14], they would also have different sites of toxic action. Consequently, analysis of a contaminant’s concentration in a whole animal would not necessarily correlate well with the toxic response. The metal concentrations of sensitive internal tissues are a more direct measure for predicting toxicological effects than ambient concentrations of total or free ionic metal.

Reproductive impairment in copepods exposed to Ag via food was also evident from histological and biochemical examination. Although eggs produced by exposed copepods were the same size as eggs produced by controls, the total protein concentration per egg following dietary exposure to Ag decreased significantly with increasing Ag concentration (Table 1). For example, when algal food was exposed to 1 nM Ag (yielding cells with 39 nmol Ag/g dry wt), protein content per egg decreased by 22%; doubling the Ag exposure resulted in a decrease of 44% of egg protein. Similarly, the percentage of females with developed ovaries decreased with exposure to ingested Ag (Table 1). Neither egg protein content nor ovarian development was altered in copepods exposed to dissolved Ag (data not shown).

When Ag is accumulated from food, we hypothesize that reproduction is affected in these animals due to decreased accumulation of yolk proteins (vitellogenesis) in the ovary. The SDS-PAGE analyses indicated that the dominant proteins in the eggs were similar in molecular weight to lipovitellin isolated from grass shrimp [44], so the protein content of the egg was presumed to be primarily yolk protein. Since lipovitellin is the dominant soluble protein in many crustacean eggs [44] and accumulation of lipovitellin is the focal point of ovarian development [45], the decrease observed in egg protein and ovarian development may be due to alterations in production or processing of lipovitellin. This process may also account for the extreme sensitivity of copepod egg production to Zn [16], suggesting that Ag is not unique among metals in its effects on these animals. Egg production in other crustaceans may also be impacted by low metal concentrations. For example, vitellogenesis in blue crabs is interrupted following exposure to sublethal concentrations of Cd through food [46]. It is unlikely that the observed decrease in reproductive capacity is due to a general stress response since the other sublethal endpoints, such as respiration rate and behavior, were unaffected.

Sublethal toxic effects on egg production in copepods occurred when food was exposed to 1 nM Ag, a concentration that is only about four times higher than the ∼250 pM levels

Table 2. Body burdens of Ag in zooplankton following exposure to dissolved Ag at different concentrations or to algal food that had been exposed to dissolved Ag. Values shown (nmol/g dry wt) reflect Ag accumulated from experimental exposures plus background Ag concentrations in animals (1.3 nmol/g dry wt) [28]. ND, not determined

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reported for both the Hudson River Estuary (New York, USA) and South San Francisco Bay (CA, USA) [23–26]. Further, body burdens of Ag that depressed egg production in copepods are only three to four times the Ag body burdens in calanoid copepods in the coastal Mediterranean [28]; we are unaware of any published Ag measurements in freshwater zooplankton samples. Egg production in cladocerans was impacted when algal food was exposed to Ag in the 0.25 to 0.5 nM range, a range that has been observed in some contaminated bodies of freshwater [23–26,41,47]. It thus appears that, as for other metals [16], planktonic animals live in waters with concentrations of Ag that are not far below toxic levels and may occasionally be exposed to higher levels. Clearly, impacts of any contaminant on the reproductive success of a species could affect the population dynamics of that species and possibly other species through selective grazing or predation.

These results demonstrate the importance of measuring sublethal effects and examining different exposure pathways when evaluating the toxicity of aquatic contaminants. The sublethal effects demonstrated in this work would be missed by standard acute toxicity tests, which only expose test organisms to dissolved metals and which overlook sublethal effects entirely. Given that the total Ag concentration at which egg production was impacted is 1/500 of the LC50 in cladocerans and 1/400 of the LC50 in copepods, it is clear that LC50s or even the application of a typical safety factor to LC50s based on solute exposures would be inadequate in protecting zooplankton, and possibly other animals, in surface waters.

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