Sensitivity of freshwater snails to aquatic contaminants: Survival and growth of endangered snail species and surrogates in 28-day exposures to copper, ammonia and pentachlorophenol

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Abstract – Water quality degradation may be an important factor affecting declining populations of freshwater snails, many of which are listed as endangered or threatened under the U.S. Endangered Species Act. Toxicity data for snails used to develop U.S. national recommended water quality criteria include mainly results of acute tests with pulmonate (air-breathing) snails, rather than the non-pulmonate snail taxa that are more frequently endangered. Pulmonate pond snails (*Lymnaea stagnalis*) were obtained from established laboratory cultures and four taxa of non-pulmonate taxa from field collections. Field-collected snails included two endangered species from the Snake River valley of Idaho -- Idaho springsnail (*Pyrgulopsis idahoensis;* which has since been de-listed) and Bliss Rapids snail (*Taylorconcha serpenticola*) -- and two non-listed taxa, a pebblesnail (*Fluminicola* sp.) collected from the Snake River and Ozark springsnail (*Fontigens aldrichi*) from southern Missouri. Cultures were maintained using simple static-renewal systems, with adults removed periodically from aquaria to allow isolation of neonates for long-term toxicity testing. This method was successful for pond snails and Idaho springsnails, less successful with pebblesnails, and unsuccessful for Bliss Rapids snail or Ozark springsnail. Long-term (28-d) toxicity tests were conducted with copper, ammonia, and pentachlorophenol in hard reconstituted water (hardness 160-180 mg/L as $CaCO_3$). Juvenile Idaho springsnails (5-7 weeks post-hatch) generally were more sensitive than neonate (<7 days post-hatch) pondsnails in survival and growth tests with copper, ammonia, and pentachlorophenol. In 28-d lethality tests with older snails (from field collections or laboratory cultures), copper sensitivity was similar among the four non-pulmonate taxa, but ammonia sensitivity differed widely, with Ozark springsnail the most sensitive taxon to ammonia. For all three chemicals, toxicity values for one or more taxa of non-pulmonate taxa were among the lowest reported in the literature, whereas toxicity values for pond snails were in the middle or

upper end of species sensitivity distributions. The ambient water quality criterion for copper, based on the Biotic Ligand Model, was protective for the snail taxa we tested, but one or more snail taxa had toxicity values less than criteria for pentachlorophenol or ammonia. These results indicate that inclusion of chronic toxicity data for freshwater snails during the development or revision of water quality criteria for protection of aquatic life can increase the ability of these criteria to protect aquatic taxa – including endangered and threatened snail taxa – from adverse effects of chemicals.

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INTRODUCTION

The suitability of U.S. Environmental Protection Agency (USEPA) ambient water quality criteria (WQC) for protection of aquatic species listed under the U.S. Endangered Species Act (ESA) has been the topic of ongoing consultations between USEPA, the U.S. Fish and Wildlife Service (USFWS) and states. Studies with ESA-listed freshwater fish species determined that these species were not consistently more sensitive to acute or sublethal effects of toxic chemicals than common surrogates (Dwyer et al. 2005; Besser et al. 2005), but recent studies indicate that freshwater mussels, including listed species, may be more sensitive than most fish and invertebrate species to toxic effects of some chemicals (e.g., ammonia and copper; Wang et al. 2007; Augspurger et al. 2007).

Many species of freshwater snails are ESA-listed, including six species from the Snake River region of Idaho (USFWS 1995), but there are relatively few data on the sensitivity of freshwater snails to toxic effects of contaminants. Most published toxicity tests with snails have been acute lethality tests conducted with field-collected snails, although greater sensitivity is likely in chronic tests with juvenile test organisms and sublethal endpoints (e.g., Gomot 1998, Duft et al. 2003, Grosell et al. 2006, Pounds et al. 2008). Air-breathing snails of the subclass Pulmonata (e.g., the families Physidae, Lymnaeidae, and Planorbidae) have been most widely used for laboratory toxicity tests, and their rapid growth, short generation times, and high reproductive output make them easy to use in toxicity tests, including chronic tests with sensitive, sublethal endpoints. Non-pulmonate snails (formerly included in subclass Prosobranchia) are more taxonomically diverse, and their physiology (inability to breathe atmospheric oxygen) and life history (slow growth and low reproductive rate) may make them both subject to endangerment and difficult to culture and test in the laboratory.

This report summarizes research conducted to help USEPA and USFWS to determine if WQC developed under the Clean Water Act are protective of ESA-listed snails in Idaho. Although some of the snail species and chemicals tested have special relevance to aquatic habitats in the Snake River valley of Idaho, the toxicity data generated during this study should prove to be useful for other consultations throughout the United States.

The specific objectives of this study were:

(1) to develop methods for laboratory culture and chronic toxicity testing (28-d survival and growth tests) with freshwater snails, including ESA-listed snails from Idaho and surrogate species; and

(2) to conduct toxicity tests with several chemicals that may pose risks of toxicity in snail habitats and to compare chronic toxicity values for these chemicals among snail species and between snails and other aquatic taxa used to develop water quality criteria.

METHODS

Snail Collection and Culture

Four species of non-pulmonate snails were collected from the field and shipped to the Columbia Environmental Research Center in Columbia Missouri for laboratory culture. Three species of non-pulmonate snails were collected by the USFWS from habitats in the Snake River valley of Idaho in fall 2005 and fall 2006: Idaho springsnail (SS; *Pyrgulopsis idahoensis*) and Bliss Rapids snail (BR; *Taylorconcha serpenticola*), and a species of pebblesnail (PS; *Fluminicola* sp.). Both BR and SS were listed under the Endangered Species Act at the time of these collections, although SS has since been de-listed, because it is now considered to be a

population of a species with wider distribution, the Jackson Lake springsnail (*P. robusta*). Another non-pulmonate species, Ozark springsnail (OZ; *Fontigens aldrichi*) was collected from southern Missouri in fall 2006. We also obtained a culture of the pulmonate pondsnail (*Lymnaea stagnalis*; LS) from Dr. Marie-Noële Croteau, (USGS, Menlo Park CA).

Snails were held in 40-L aquaria with 35-L of well water (hardness 280 mg/L as $CaCO_3$) at 18 ºC. Aquaria were aerated and one-half of the water was replaced twice per week. Photoperiod was maintained at 16 hours light: 8 hours dark, with one-half of the tanks covered with shading material. Stainless steel tiles were offered as substrate. Snails were fed ad libitum a combination of Instant Algae and Shellfish Diet (Reed Mariculture, Campbell, CA). These algae suspensions were painted onto stainless steel substrates, which were air-dried before they were placed in the culture tanks. Additional foods offered to snail cultures included Wardley algae disks (Hartz Mountain Corp., Secaucus, NJ), and small portions of organically-grown carrots and iceberg lettuce.

Even-aged cohorts of non-pulmonate snails were obtained by transferring adults to new tanks monthly and rearing neonates that hatched from deposited eggs. To collect neonates, 90% of the water was siphoned from the tank and the sides and the bottom of the tank were sprayed gently with well water to loosen snails and detritus. Water containing neonates and detritus was rinsed through a 150-µm stainless steel sieve and sieve contents were examined with a 3.5X dissecting microscope. Neonate snails were collected using a capillary tube syringe system. This syringe system consists of a glass capillary tube (1.17-mm inner diameter) connected by vinyl tubing (1.0-mm inner diameter) to a 2.5-cm, 1-mL syringe with 16-gauge needle (ASTM 2008a). After collection, the young snails were placed into a 4-L beaker containing 2 L of well water. These beakers were aerated gently , a small amount of food was offered, and the snails were

acclimated to the hard reconstituted test water (ASTM 2008b) over a 48-hr period. Only SS produced cohorts large enough for conducting toxicity tests with uniform-age groups, with more than 2500 offspring produced from an initial cohort of 300 snails between October 2005 and October 2006. Reproduction of this species predominantly occurred in winter and spring 2006, within nine months of collection. PS from the fall 2006 collection produced about 200 neonates between July and October 2006 and these animals were combined over several months to produce mixed-age groups for testing in summer 2007. BR and OZ did not reproduce in our cultures and were tested only in mixed-age field-collected groups. LS produced abundant egg masses, which were collected as needed to provide neonates (<7 d after hatching) for testing.

Preliminary evaluation of test conditions

Two preliminary studies were conducted to evaluate the performance of two snail species, SS and LS, under potential test conditions for 28-d toxicity tests. The first of these studies evaluated survival and growth of SS and LS fed three different food types during a 21-d period. Diets in this test included: an algae mixture (1 ml of *Nannochloropsis* and 2 ml Shellfish Diet in 1800 ml of well water; Wang et al. 2007) used for tests with juvenile freshwater mussels (ASTM 2008a); the standard yeast-cereal leaf-trout food (YCT) diet (1.8 mg/ml); and a suspension of Tetrafin® flake fish food (6 mg/ml). Each diet was fed at low and high feeding rates (1 ml and 2 ml per chamber). General test conditions are summarized in Table 1. Tests were conducted with four replicates of LS (<7 d post hatch; 10 snails per chamber) for each combination of diet and feeding rate. Because of the limited availability of SS, single replicates (10 snails per chamber) were tested with seven of eight treatment combinations, excluding the high feeding rate of the YCT diet.

The second preliminary study investigated possible causes for high mortality of SS in controls in the first two 28-d toxicity tests with copper (Test A) and PCP (Test B). This 28-d study compared survival and growth of SS and LS in treatment combinations that differed in water type (hard reconstituted water, described below, vs. well water), carrier solvent (hard reconstituted water with and without 0.26 ml/L triethylene glycol, the carrier solvent used in the PCP exposures), and feeding rate (flake food suspensions; low vs. high). Feeding rates in the 'low' treatment were 0.5 ml/chamber on Monday-Wednesday-Friday for SS and 1.0 ml/chamber daily for LS. Feeding rates in the 'high' treatment were increased after day 13 to 0.5 ml daily for SS and 2 ml daily for LS. Each of the eight treatment combinations was tested with both SS and LS (3 replicates per species with 10 animals in each replicate).

Toxicity tests

Test conditions for 28-d toxicity tests with freshwater snails were similar to methods for toxicity testing with freshwater mussels (ASTM 2008a; Wang et al. 2007). All tests were conducted in hard reconstituted water (170 mg/L hardness as CaCO3; ASTM 2008b) at 20 °C. Test chambers were 300 ml beakers with 200 ml water volume, with 3.75 volume additions per day. Test chambers for tests with LS were covered with screened caps (initially designed for tests with flying adult midges; USEPA 2000) to prevent these air-breathing snails from escaping from exposure chambers). Additional details of test conditions are presented in Table 1.

Toxicity tests were conducted with copper (copper sulfate), pentachlorophenol (PCP; stock prepared in triethylene glycol), and ammonia (ammonium chloride). These chemicals were selected from the list of candidate chemicals with differing modes of toxic action suggested by Dwyer et al. (2005), based on their potential importance in snail habitats in the Snake River

valley (copper from aquaculture, ammonia from livestock) and sensitivity of snails in acute range-finding tests with copper, ammonia, PCP and carbaryl. In 96-hr range-finding tests, SS mortality was observed in exposures to copper (above 31 µg/L), pentachlorophenol (above 250 µg/L) and ammonia (above 8 mg N/L), but not carbaryl (no mortality at 10 mg/L; all concentrations nominal).

Automated proportional diluters prepared and delivered five test concentrations in a 50 percent dilution series, plus a control. The control water for PCP tests included 0.26 ml/L of triethylene glycol at the same concentration of triethylene glycol in the highest PCP treatment. Stock solutions and test waters for ammonia tests were adjusted to pH 8.3. The pH of ammonium chloride stock solutions was increased by adding dilute sodium hydroxide and the pH of test water was decreased by titration with dilute hydrochloric acid using automatic pH controllers (Wang et al. 2008).

Water samples were collected every two weeks for measurement of copper or PCP and weekly for analysis of ammonia. Samples for analysis of copper or PCP were filtered (a polypropylene syringe filter; 0.45-µm pore diameter) and samples were analyzed without concentration or cleanup steps. Copper concentrations were determined by inductively-coupled plasma-mass spectroscopy (May et al. 1997). PCP concentrations were analyzed by high-performance liquid chromatography using a C18 column, a mobile phase of acetonitrile/water, and UV detection (Orazio et al. 1983). Total ammonia concentrations were determined on unfiltered samples by ion-selective electrode.

Different age/size groups of snails were used for toxicity tests, depending on the availability of various species (Table 2). All tests with LS were started with cohorts of neonates less than 7 d post-hatch. The first two 28-d toxicity tests with copper (Test A) and PCP (Test B)

were conducted with the youngest SS that could be handled routinely without mortality: (about 3-6 weeks after egg deposition or 2-3 weeks post-hatch). Endpoints of these studies were survival (both species) and growth (wet weight; LS only). Because of high control mortality in these tests (5 to 60% survival at Day 28), subsequent survival and growth tests with all three chemicals (Tests 1-3) were conducted with juvenile SS (5-13 weeks post-hatch) and neonate LS (<7 d post-hatch), with three or four replicate groups per treatment (depending on availability of organisms). Additional survival tests (Tests 4 and 5) were conducted with mixed-age snails from field collections (BR and OZ) or with older, mixed-age snails from cultures (SS and PS), with two replicates per treatment. Field-collected snails were held for periods from four months (OZ) to six months (BR) -- in attempts to initiate laboratory cultures -- before they were used in toxicity tests. Older, laboratory-produced groups included animals of widely-varying ages: roughly 4-8 months old for SS and 6-12 months old for PS. All toxicity tests were conducted for 28 d, with endpoints of survival (determined by observation of movement using microscopic examination) and growth. For preliminary tests A and B, growth of LS was determined by measurement of aggregate wet weights for each rep, and no growth data was collected for SS, which had problems with control survival. For subsequent survival and growth tests with SS and LS (Tests 1-3), growth of both species was evaluated by digital measurement of maximum shell diameter. Growth was not measured at the end of the survival tests conducted with the mixed-age snails (Tests 4 and 5).

Toxicity data were rank-transformed and analyzed by one-way ANOVA (Conover and Iman 1981), with significant differences from controls determined by Dunnett's test (SAS/STAT software, version 9.1; SAS Institute, Cary NC). ANOVA results were used to determine the no-observed-effect concentration (NOEC), the highest exposure concentration that did not differ

significantly from the control, and the lowest-observed-effect concentration (LOEC), the lowest exposure concentration above the NOEC. Concentration-response curves were analyzed to determine median lethal concentrations (LC50s) and concentrations associated with 20 percent reductions in survival and growth (EC20), with concentrations log-transformed for all analyses. For tests with well-defined concentration-mortality curves (typically, tests with two or more treatments with partial mortalities), LC50s and EC20s for reduced survival were estimated by logistic regression using the Toxicity Response Analysis Program (TRAP, Version 1.0; R. Erickson, USEPA-MED, Duluth MN). For other tests, LC50s were estimated by the trimmed Spearman-Karber method (Hamilton et al. 1977) and EC20s for survival and growth were estimated using linear interpolation (Inhibition Concentration or ICp method; TOXSTAT software, version 3.5, WEST Inc., Cheyenne WY). For each species and chemical, the lowest EC20 value for single tests (or the geometric means of lowest EC20s for multiple tests) was used as the 'species chronic value' (SCV) for inter-species comparisons.

RESULTS

Evaluation of test conditions

Results of the study of experimental diets indicated that LS had highest survival and growth when fed the flake diet (Figure 1), whereas SS had 100 percent survival in all diet treatments and had greater growth when fed either flake or YCT diets, compared to the algae diet. These results were the basis for selecting feeding rates of 0.5 ml/d of the flake diet (3 mg/d) for test chambers with neonate SS (5-13 weeks post-hatch) and 1 ml/d of the flake diet (6 mg/d) for chambers with neonate LS or with larger, mixed-age groups of other snail species.

Results of the second evaluation of test conditions indicated no consistent differences in survival (≥90% in all treatments) or growth (Figure 2) among water, carrier solvent, or feeding rate treatments. We concluded from this study that poor survival of SS in Test A and Test B was not caused by excessive feeding, inappropriate test water, or the presence of the carrier solvent. We concluded that problems with low SS survival in these tests was attributable to starting tests with very young snails (about 3-6 weeks after egg deposition). Subsequent toxicity tests were conducted with older SS (at least 5 weeks post-hatch; Table 2), with a reduced initial feeding rate (0.5 ml/d flake food on Monday, Wednesday, and Friday for the first 14 d of the 28-d exposures) and had no further problems with control survival.

Exposure concentrations and water quality

Exposure concentrations for toxicity tests generally were close to nominal concentrations. Measured copper concentrations generally were within 10% of nominal concentrations for all three tests (Table 3), except the highest exposure concentration in Test 1 averaged 146% of the nominal concentration, apparently caused by a short-term diluter malfunction. Measured copper concentrations in the lowest exposure levels were also slightly greater than nominal in all three tests, reflecting a low but consistent background copper concentration in our test systems (average for controls: 0.87 µg Cu/L). Measured concentrations of PCP in Test B were consistent with nominal concentrations except for losses, presumably attributable to sorption of PCP, at lower exposure levels (Table 4). In contrast, PCP concentrations in Test 2 averaged only about 50% of nominal concentrations. The cause of this problem was never fully determined, but the data suggest a problem with the injection volume from the toxicant delivery system. Despite this

problem, means of five measured PCP concentrations for each exposure level were consistent within treatments, and standard deviations were less than 10 percent of means. Mean ammonia concentrations were close to nominal (Table 5), although some losses were evident in Test 5 (i.e., measured concentrations as low as 83-84% of the nominal concentrations), probably indicating increased nitrification by the microbial community in the test system.

Water quality of test waters remained within acceptable ranges throughout tests. Mean hardness (169 mg/L as $CaCO_3$) and alkalinity (121 mg/L as $CaCO_3$) measured in nine batches of test water delivered to the proportional diluters were within acceptable ranges (ASTM 2008a,b; data not shown). The mean pH of incoming test water (8.41) was above the specified range of 7.8 to 8.0 for this water, but was within the normal range for freshly-prepared hard reconstituted waters prepared at CERC (e.g., Dwyer et al. 2005). Average major ion content of test waters (means of 7 analyses, in mg/L) were chloride, 4.9; sulfate 167; calcium, 30, magnesium, 22; potassium, 4.0; sodium, 50; and iron, 0.033 (Table 6). Dissolved organic carbon (DOC) was below the detection limit (<1.0 mg/L) for two analyses of incoming test water and subsequent analyses of ASTM hard water resulted in a measured DOC concentration of about 0.3 mg/L (Ning Wang, USGS, Columbia, MO, unpublished data). DOC also was below detection in seven of 14 analyses of water from test chambers (Table 6). Of the seven samples with detectable DOC, the four highest concentrations (59-100 mg/L) were collected during the PCP tests and reflect the presence of the triethylene glycol carrier solvent, which had a nominal maximum concentration of 137 mg/L in the high treatment and the solvent control. Three samples from tests without carrier solvent had low (<2 mg/L) but detectable concentrations.

Concentrations of other water quality measured during tests (Table 7) were similar to those in the incoming water supply, except mean pH in these equilibrated samples fell within a

slightly lower range (8.0 - 8.4). Test waters from ammonia tests had mean pH within a narrow range (8.22-8.29) and had slightly lower alkalinity, reflecting the influence of additions of small volumes of acid by the automatic pH control system used in the ammonia exposures. Dissolved oxygen in test chambers remained above 5.4 mg/L in all samples. Total ammonia concentrations remained low in test chambers (except in ammonia tests), with mean concentrations of 1.2 mg N/L or lower. Mean temperatures of all tests remained within the nominal range (19-21 ºC) although some individual measurements exceeded 22 ºC, apparently reflecting warming of samples that were removed from test chambers.

Toxicity data

Snails generally adapted well to test conditions during toxicity tests. Snails in the controls and in the low-chemical treatments moved throughout the test beakers and fed actively (as evidenced by removal of food and biofilm). Snails in high-chemical treatments tended to be less active, often remaining on the bottoms of the test chambers, allowing food and biofilm to accumulate. Pondsnails (LS) in all treatments sometimes crawled above the water level; this behavior was most common in high-copper treatments. Control survival was acceptable (90-100%) in all exposures except the first two SS exposures (Test A and Test B). In Test A (Table 8), SS survival was only 60% in the control, but survival in copper treatments followed trends similar to subsequent copper tests. In Test B (Table 9), SS had low survival (0-25%) in all treatments. The failure of these two tests, and the results of the second test-conditions study (Figure 2), led to the decision to use older SS for subsequent tests at a lower initial feeding rate, which resulted in acceptable control survival of SS in all subsequent tests. Snails in the LS control groups in Tests 1, 2, and 3 (started with <7-d old neonates) grew rapidly and consistently,

with mean shell length increasing by more than 300 percent by day 28. Growth of juvenile SS in these tests (started with animals 5-12 weeks post-hatch) was slower and more variable among tests, with mean shell diameter increasing by 32 to 99 percent by the end of tests. Control groups of SS in Tests 4 and 5, which were started with older snails, had greater increases in mean shell length (85-86%). Control groups of mixed-age BR, OZ, and PS had little change in mean shell length (maximum increase of 30%) during Tests 4 and 5.

Results of 28-d toxicity tests with copper, PCP, and ammonia are summarized in Tables 10 through 14 and Figures 3 through 5. Statistical analyses of data indicated significant reductions in survival and growth, relative to controls (one-way rank ANOVA; $P<0.05$) for most tests and endpoints. Exceptions were the survival data for LS and growth data for SS in Test 3 with ammonia, which had little variation among treatment means (Table 12), and survival data for PS in Test 5 with ammonia, which had a wide range of treatment means (0-100%), but also had extreme variation between replicates in two of the high-ammonia treatments (Table 14). Dunnett's test indicated that one or more treatment means were significantly less than controls for all tests that had significant ANOVA results, allowing estimation of NOEC and LOEC.

Chronic toxicity values (NOEC and LOEC) were determined for all endpoints with significant ANOVAs (Table 15). Median lethal concentrations (LC50s) were determined for all exposures, except two exposures that had survival greater than 50 percent in all treatments: LS exposure to PCP in test B (Table 9), and LS exposure to ammonia in Test 3 (Table 12. The LC50 for PCP toxicity to LS in Test 2 was estimated by the inhibition concentration method (IC50) because this test did not have any treatments with zero survival (Table 11).

Survival and growth EC20s (Table 15) were defined for all exposures except for ammonia exposures in Test 3 (Table 12). In this test, survival and growth of LS and growth of

SS did not have 20% reductions relative to controls (EC20 >8.0 mg N/L). In contrast, survival of SS had greater than 20% reductions in survival in all treatments except the control (EC20 < 0.48 mg N/L). Species chronic values (SCV) were estimated as the lower of EC20s for survival or growth for each chemical in single tests for a species, or as the geometric mean of lowest EC20s from multiple tests with the same species.

Differences in toxicity values among snail species

Copper. Analysis of variance (ANOVA) demonstrated significant reductions in survival with increased copper concentrations for all eight exposures (Test A, Test 1, and Test 4; Figure 3). LOECs for survival in copper exposures ranged from 13 µg/L, for all species in Test 4 (SS, BR, PS, and OZ), to 87 µg/L for LS in Test 1 (Tables 10 and 13). Median lethal concentrations (LC50s) in copper tests indicated a narrower range of sensitivity among species and between tests (Table 15). The ranges of LC50s for repeated tests with LS and SS overlapped, but survival EC20s for LS (16.9 and 22.4 µg/L) were greater than those for SS or any other non-pulmonate taxa (range: 8.58-16.8 µg/L). Analyses of the growth response for LS and SS were somewhat inconclusive, with LOECs and EC20s indicating somewhat different trends for Test A and Test 1 (Tables 8, 10, 15). Overall, copper EC20s differed substantially among repeated tests and endpoints for LS (6.15-22.4 µg/L) and SS (7.4-13.4 µg/L), but SCVs for copper fell within a narrow range (9.55-16.8 µg/L) for all five snail taxa tested (Table 15).

Pentachlorophenol. Responses of SS and LS to PCP exposure were quite similar in Test 2 (Figure 4). Survival of SS and LS was reduced significantly only at the highest PCP concentrations tested (>200 µg/L), but growth of SS was affected significantly at much lower concentrations. Survival of LS was even less affected by PCP in Test B, but comparisons to SS

are not available for this test because of the failure of the SS exposure. In contrast, growth of both species was affected by PCP exposure – in Test B for LS and in Test 2 for SS (Table 15). The apparently greater sensitivity of LS growth in Test A compared to Test 2 suggests that the one-dimensional measure of shell diameter (Table 11) did not fully express changes that were evident in wet weight (Table 11). As a result of between-test differences in growth EC20, SCVs for PCP were lower for SS than for LS, even though the lowest growth EC20s for the two species were similar (Table 15).

Ammonia. Sensitivity to ammonia toxicity varied widely among the snail taxa (Figure 5). LS was least sensitive to ammonia effects on survival, with no significant reductions at the highest concentrations tested (nominal 8.0 mg N/L as total ammonia), and OZ was most sensitive, with a LOEC 0.83 mg N/L (Tables 12 and 14). Although ANOVA indicated no significant differences among ammonia concentrations for PS in Test 5, due to wide variation between the two replicates in several treatments, the LC50 and survival EC20 for PS were the second-lowest of the species tested (Table 15). For SS, LC50s and survival EC20s for ammonia toxicity differed widely between Test 3 and Test 5, perhaps reflecting differences in sensitivity between juveniles and adults. Growth responses to ammonia exposure in Test 3 were not significant for SS, but reductions in growth were small but significant for LS. Although the growth LOEC for LS was low (1.8 mg N/L), the growth EC20 was not defined because the magnitude of growth reductions were small (from 6% at 1.8 mg N/L to 16% at 8.0 mg N/L). As a result, the SCV for ammonia toxicity to LS was much greater than those for the four non-pulmonate species.

DISCUSSION

Sensitivity of snails relative to other aquatic taxa

Copper. All five snail taxa tested were sensitive to copper exposure, relative to SCVs reported by USEPA (2007) and results from other recent studies at CERC (Figure 6a). After SCVs were adjusted to a water hardness of 85 mg/L (USEPA 2004), only five of 25 values – including three chronic values for freshwater mussels (Wang et al. 2007) – were lower than those for several snail taxa tested in this study (SS, PS, and LS). All five species tested were more sensitive than the only other snail species in the USEPA chronic copper toxicity database, the pulmonate species, *Campeloma decisum* (Arthur and Leonard 1970; Figure 6). The chronic WQC for copper derived using the Biotic Ligand Model (USEPA 2007; calculated for ASTM reconstituted water with hardness of 85 mg/L and DOC of 0.5 mg/L) appears to provide adequate protection for the snail taxa tested. These comparisons do not attempt to model the influence of DOC in various toxicity test waters, because DOC data were not reported for many of the studies reported by USEPA (2007). Most measured DOC concentrations in test chamber were below detection (<1.0 mg/L), with a few detectable concentrations as high as 1.8 mg/L (Table 6).

Pentachlorophenol. The two snail species tested, LS and SS, had widely differing sensitivity to PCP (Figure 6b). The SCV for SS, based on an EC20 for growth of 16.6 µg/L, is the lowest chronic value reported for aquatic taxa exposed to PCP, and is lower than the current chronic water criterion (24 µg/L at pH 8.25). In contrast, the SCV for LS ranked near the middle of the species sensitivity distribution, and another pulmonate snail, *Physa gyrina*, was among the least sensitive aquatic taxa tested (Hedke et al. 1986; Figure 6). The fact that two of 11 available SCVs – for SS (this study) and the fountain darter (Besser et al. 2005) – fall at or below the

current chronic WQC suggests that this criterion may not adequately protect snails or other aquatic taxa from toxic effects of PCP.

Ammonia. Tests with five taxa indicated that freshwater snails are among the aquatic taxa most sensitive to ammonia toxicity (Figure 6). Three of the five snail species we tested had SCVs (OZ, PS, and SS) close to or less than the lowest SCV reported by USEPA (1999). The SCV for OZ was lower than the current chronic WQC for ammonia, and was lower than chronic values for other aquatic taxa, except for two freshwater mussel taxa tested by Wang et al. (2007). For SS, both the survival EC20 and LC50 from Test 3 were even lower than the SCVs for OZ, although the SCV for SS was increased by the substantially higher survival EC20 from Test 5. Although the survival data for SS in Test 3 were highly variable, the contrast between 100 percent control survival and mean survival of 60 percent or less in all ammonia treatments suggests that the juvenile SS in this test were substantially more sensitive than the cohort of older SS exposed to ammonia in Test 5.

Relative sensitivity of snail taxa and endpoints

Results of the chronic toxicity tests indicated that LS was less sensitive than the non-pulmonate snail species for two of the three chemicals tested. This trend is less evident in results of previous acute tests with these same chemicals, using a variety of pulmonate and non-pulmonate snail taxa (Table 16). For ammonia and PCP, 96-hr LC50s for pulmonate taxa (*Lymnaea* spp. and *Physa* spp.) overlapped broadly with those for non-pulmonate taxa (*Potamopyrgus* spp*. and Gillia altilis)*. For copper, the 96-hr LC50 for *Lymnaea luteola* was lower than 96-hr LC50s reported for the non-pulmonate species, *Potamopyrgus jenkinsi.*

Evaluating differences in sensitivity among taxa is complicated by the influence of various test conditions. Acute toxicity of PCP under ambient conditions in Mississippi River water was substantially greater during summer than during winter (Hedke et al. 1986) and PCP was more acutely toxic in flow-through tests than in tests conducted under static conditions (Stuart and Robertson 1985). Acute LC50s for *Potamopyrgus jenkinsi* were lower for juveniles than adults for ammonia (by a factor of 2.6) and copper (by 50%; Watton and Hawkes 1984). Our 28-d tests with SS also indicated greater sensitivity of younger animals. In tests with both copper and ammonia, survival EC20s and LC50s for young SS (2-13 weeks old, in Test A, Test 1, and Test 3) consistently were lower than values determined from tests with older, mixed-age cohorts (Test 4 and Test 5; Table 15)

Comparison of chronic sensitivity of pulmonate and non-pulmonate snails also may be affected by differences in the endpoints that are feasible for laboratory chronic tests. Several chronic toxicity studies with *Physa* spp. and *Lymnaea* spp. have reported sensitive sublethal effects on reproduction (e.g., Hedke et al. 1986, Gomot 1998), and/or growth (e.g., Grosell et al. 2006, De Schamphelaere et al. 2008) in these pulmonate taxa, which have rapid growth and short generation times. Fewer chronic studies have reported sensitive sublethal responses in non-pulmonate snails, but egg production was reported to be more sensitive than survival in 28-d water-only tests with *Bithynia tentaculata* (van Wijngaarden et al. 1998) and in spiked sediment tests with the New Zealand mudsnail, *Potamopyrgus antipodarum* (Duft et al. 2003). Characteristics of *P. antipodarum*, notably the occurrence of populations that are parthenogenic and ovoviviparous, have led to proposals for its use in reproductive toxicity studies (Duft et al. 2007). Tests reporting reproductive endpoints of either pulmonate or non-pulmonate snails require long test duration (e.g., 5-6 weeks; Hedtke et al. 1986, Arthur and Leonard 1970) or tests

started with adult- or sub-adult snails. No reproduction of LS was observed in the 28-d tests, which started with early juveniles (<7 d post-hatch), but some egg production of PS in 28-d was observed in tests started with mixed-age groups (Test 5). There also were not consistent differences in sensitivity between growth and mortality endpoints in tests with LS or SS. Of seven tests with survival and growth endpoints, EC20s for growth were substantially lower in two tests (copper Test A for LS, PCP Test 2 for SS), the EC20 for survival was lower in one test (ammonia Test 3 for SS), and the two endpoints had similar EC20s in the remaining four tests (Table 15).

Summary

Objective 1: Develop methods for laboratory culture and chronic toxicity testing (28-d survival and growth tests) with freshwater snails, including ESA-listed snails from Idaho and surrogate species.

Chronic (28-d) toxicity tests were completed successfully with two to five taxa of freshwater snails exposed to copper (7 tests), PCP (3 tests), and ammonia (6 tests). With the exception of the first two tests with SS, toxicity tests with five species of freshwater snails, conducted under routine flow-through test methods, had good control survival and produced reliable estimates of toxicity thresholds. Simple static culture methods produced neonates of two species (LS and SS) in numbers adequate for toxicity testing, and also produced lesser numbers of a third species (PS). Tests with laboratory-reared and field-collected snails produced useful data on snail survival in 28-d toxicity tests. Tests conducted with uniform-aged groups generated low-variability estimates of growth

based on digital measurement of shell diameter, and toxicity values for growth were lower than those for survival for two of seven exposures.

Objective 2: Conduct toxicity tests with several chemicals that may pose risks of toxicity in snail habitats and to compare chronic toxicity values for these chemicals among snail species and between snails and other aquatic taxa used to develop water quality criteria.

Sensitivity to the toxic effects of three test chemicals varied widely among the five snail species tested. The pulmonate pondsnail (LS) was less sensitive to PCP than the non-pulmonate Idaho springsnail (SS) and less sensitive to ammonia than all four non-pulmonate taxa. All five snail taxa had similar sensitivity to copper. The sensitivity of the two ESA-listed species, SS (which is no longer listed) and BR, to ammonia was similar to or less than that of the non-listed snail taxa. Overall, study results indicate that freshwater snails, especially non-pulmonate snails, are very sensitive to toxic effects of some aquatic contaminants. The WQC derived from datasets that do not represent snail taxa, such as the current ammonia criteria (USEPA 1999), may not adequately protect freshwater mollusks. The de-listing of SS and the limited testing conducted with the federally-endangered species, BR (survival data only, from tests with field-collected animals) leaves some uncertainty about the relative sensitivity of listed vs. unlisted snail species. Ongoing research in our laboratory is focused on development of improved culture methods for BR, with the goal of producing even-age cohorts that can be tested using the 28-d survival and growth test method presented here.

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Table 1. Summary of test conditions for toxicity studies with freshwater snails in accordance to ASTM (2008a,b,c).

1. Test organisms	See Table 2
2. Test type	Flow-through toxicity test
3. Test Duration	28 d
4. Toxicants	Copper (copper sulfate) Pentachlorophenol (stock prepared in triethylene glycol) Ammonia (Ammonium chloride; pH of stock adjusted to 8.3 with sodium hydroxide)
5. Dilution series	Control and five concentrations in 50% dilution series (see Tables 3-5 for nominal and measured concentrations)
6. Temperature	20±1°C
7. Lighting	Ambient laboratory light (about 200 lux, 16 hr light:8 hr dark)
8. Aeration	None
9. Feeding	Homogenized flake fish food suspension (Tetrafin®, 6 mg/ml): Pondsnails (and adults of other species), 1 ml/beaker/d; Idaho springsnails, 0.5 ml /beaker on Monday, Wednesday, and Friday (daily starting on day 14).
10. Test water	Hard reconstituted water (hardness 160-180 mg/L as $CaCO_3$; ASTM 2008b). pH in ammonia tests maintained at 8.3 by automated titration with dilute HCl (Wang et al. 2008)
11. Water addition	3.8 volume-additions/day (125 ml/chamber every 4 hr))
12. Test chamber	300 ml screened beaker (200 ml water). Beakers with pond snails had screened lids (midge emergence caps; USEPA 2000).
13. Age of test organisms	See Table 2
14. Organisms/chamber	10
15. Replication	2 to 4 chambers per exposure level (depending on snail availability)
16. Water quality	Temperature, dissolved oxygen, pH, alkalinity, hardness, ammonia (weekly); dissolved organic carbon (DOC) and major ions (day 14)
17. Toxicant analyses	Dissolved (filtered <0.45 µm) copper and PCP on days 1, 14, 27; total ammonia at least weekly
18. Endpoints	Survival, days 4, 7, 14, 21, and 28; growth (shell diameter), day 28

Table 2. Species, age, and size of snails used in 28-d toxicity tests. [SD=standard deviation, N=number of animals measured.]

Species (ID)	Test	Chemical	Approximate age (weeks post-hatch)	Starting shell diameter (mm)		
				Mean	SD	N
Pondsnail, *Lymnaea stagnalis* (LS)	A	Cu	<1	2.41	0.46	22
		PCP	<1	1.33	0.58	26
		Cu	<1	1.65	0.17	10
		PCP	<1	1.83	0.26	11
	3	NH3	<1	1.74	0.25	10
Idaho springsnail, *Pyrgulopsis idahoensis* (SS)	A	Cu	2-3	0.463	0.040	23
		PCP	2-3	0.589	0.089	27
		Cu	5-7	0.492	0.101	13
		PCP	6-9	0.934	0.102	10
		NH3	11-13 [b]	1.108	0.306	8
		NH3	7-9 [b]	0.940	0.123	10
	4, 5	Cu, NH3	Mixed	2.000	0.660	15
Bliss Rapids Snail, *Taylorconcha serpenticola* (BR) [a]	4, 5	Cu, NH3	Mixed	1.981	0.348	32
Pebblesnail, *Fluminicola* sp. (PS)	4, 5	Cu, NH3	Mixed	2.685	0.861	12
Ozark springsnail, *Fontigens aldrichi* (OZ)	4, 5	Cu, NH3	Mixed	1.311	0.288	12

[a] E=Endangered species; [b] Larger cohort tested in replicates 1 and 2; smaller cohort tested in replicates 3 and 4.

Table 3. Nominal and measured concentrations of copper (Cu) in toxicity tests with freshwater snails. All samples filtered through polypropylene filter cartridges (0.45-µm pore diameter). [SD = standard deviation; N=number of analyses.]

Test	Nominal Cu (µg/L)	Measured Cu (µg/L)					
		N	Mean	SD	Minimum	Maximum	Percent of nominal
A	0	1	0.6	--	--	--	--
	1.9	1	2.6	--	--	--	135
	3.8	1	3.9	--	--	--	103
	7.5	1	6.1	--	--	--	81
	15	1	15	--	--	--	100
	30	1	33	--	--	--	109
1	0	4	1.1	0.5	0.8	1.9	--
	3.8	4	4.6	0.6	4.0	5.2	122
	7.5	4	8.3	1.4	7.0	9.6	111
	15	4	17	3.1	14	20.5	112
	30	4	32	4.0	27	36.6	107
	60	4	87	9.3	75.6	97.1	146
4	0	4	0.7	0.2	0.5	0.9	--
	3.8	4	4.3	0.7	3.4	5.0	113
	7.5	4	6.6	0.4	6.2	7.1	88
	15	4	13	1.2	12.2	14.7	88
	30	4	26	1.9	24.5	28.8	88
	60	4	62	9.2	54.9	75.0	103

Table 4. Nominal and measured concentrations of pentachlorophenol (PCP) in toxicity tests with freshwater snails. All samples filtered through polypropylene filter cartridges (0.45-µm pore diameter). [SD = standard deviation; N=number of analyses.]

Study	Nominal PCP (µg/L)	Measured PCP (µg/L)					
		N	Mean	SD	Minimum	Maximum	Percent of Nominal
B	0	4	3	2	1	6	--
	25	4	19	4	13	22	76
	50	4	41	5	37	47	83
	100	4	88	6	82	95	88
	200	4	178	5	170	182	89
	400	4	377	10	370	391	94
2	0	5	0	0.3	0.03	0.8	--
	25	5	11	2	9	13	43
	50	5	24	4	20	28	49
	100	5	50	5	45	54	50
	200	5	97	3	92	100	49
	400	5	224	22	200	240	56

Table 5. Nominal and measured concentrations of total ammonia (NH_3) in toxicity tests with freshwater snails. SD [SD = standard deviation; N=number of analyses.]

Study	Nominal NH_3 (mg N/L)	Measured NH_3 (mg N/L)					
		N	Mean	SD	Minimum	Maximum	Percent of nominal
3	0	7	0.14	0.09	0.04	0.29	--
	0.5	7	0.48	0.08	0.36	0.61	97
	1	7	1.0	0.1	0.89	1.3	101
	2	7	1.8	0.3	1.4	2.3	91
	4	7	3.6	0.6	2.8	4.6	91
	8	7	8.0	0.8	6.6	9.3	100
5	0	7	0.05	0.02	0.03	0.08	--
	0.5	7	0.45	0.09	0.27	0.55	90
	1	7	0.83	0.19	0.57	1.1	83
	2	7	1.7	0.3	1.1	2.1	84
	4	7	3.6	0.7	2.5	4.7	91
	8	6	7.9	0.5	7.3	8.4	99

Table 6. Major ions and dissolved carbon in test waters during snail toxicity tests. [DOC=dissolved organic carbon; DIC=dissolved inorganic carbon; feed=diluter water delivery line; -- indicates no data]

Test	Day	Source	Concentration (mg/L)								
			DOC	DIC	Chloride	Sulfate	Calcium	Magnesium	Potassium	Sodium	Iron
A	21	Feed	--	--	6.2	188	31	21	4.3	47	0.03
A	21	LS	1.8	24	--	--	--	--	--	--	--
A	21	SS	<1.0	22	--	--	--	--	--	--	--
A	28	Feed	--	--	4.5	188	31	21	4.5	51	0.01
A	28	LS	<1.0	25	--	--	--	--	--	--	--
A	28	SS	1.2	20	--	--	--	--	--	--	--
B	28	LS	100*	--	--	--	--	--	--	--	--
B	28	SS	59*	--	--	--	--	--	--	--	--
B	28	Feed	--	--	7.9	189	30	23	4.4	48	0.06
1	14	LS	<1.0	--	--	--		--	--	--	--
1	14	SS	1.1	--	--	--	--	--	--	--	--
1	14	Feed	--	--	5.6	136	34	23	3.4	51	<.01
2	28	LS	81*	--	--	--	--	--	--	--	--
2	28	SS	68*	--	--	--	--	--	--	--	--
2	28	Feed	--	--	5.2	166	28	23	4.2	57	ND
4	21	BR	<1.0	--	--	--		--	--	--	--
4	21	OZ	<1.0	--	--	--		--	--	--	--
4	21	PS	<1.0	--	--	--	--	--	--	--	--
4	21	SS	<1.0	--	--	--	--	--	--	--	--
4	21	Feed	<1.0	--	3.4	148	28	21.5	3.8	49	--
5	21	Feed	<1.0	--	1.7	155	28	22.2	3.6	48	--

*DOC measurements from PCP tests reflect presence of carrier solvent.

Table 7. Water quality in exposure chambers during toxicity tests with freshwater snails. [SD = Standard deviation. Treatment codes: for feeding study, A1/A2=algae diet (1 or 2 ml/d), F1/F2=flake diet (1 or 2 ml/d), Y1/Y2=YCT diet (1 or 2 ml/d); for test conditions study, A=ASTM hard water, W=well water, C=control (no solvent), S=solvent (triethylene glycol), L/H=low/high feeding rates.]

Test	Treat-ment	N	Temperature (ºC)		Dissolved oxygen (mg/L)		Ammonia (mg N/L)		pH		Hardness (mg/L)		Alkalinity (mg/L)	
			Mean	Max	Mean	Min	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Feeding study	A1	3	20.1	20.1	9.2	9.0	0.1	0.0	8.43	0.07	159	1	120	8
	A2	3	20.1	20.1	9.2	8.9	0.0	0.0	8.39	0.10	165	7	120	8
	F1	3	19.8	20.0	8.7	8.5	0.3	0.1	8.08	0.13	162	3	120	0
	F2	3	19.8	20.0	7.6	6.6	0.3	0.1	8.03	0.07	161	1	122	3
	Y1	3	19.9	20.2	9.0	8.6	0.1	0.1	8.40	0.11	161	1	121	7
	Y2	3	20.1	20.1	8.9	8.6	0.2	0.1	8.40	0.17	160	14	113	4
Test conditions study	ACH	3	20.1	20.4	7.6	6.9	0.9	1.4	8.27	0.44	171	9	123	6
	ACL	1	19.6	19.6	8.4	8.4	0.1	--	8.64	--	180	--	130	--
	ASH	3	20.0	20.2	7.6	6.9	0.2	0.1	8.21	0.37	174	7	127	12
	ASL	1	19.7	19.7	8.2		0.1		8.70	--	180	--	124	--
	WCH	3	19.4	19.7	8.0		0.2		8.03	0.11	239	68	244	8
	WCL	1	18.9	18.9	8.3	8.2	0.1	--	8.44	--	280	--	230	--
	WSH	3	19.7	19.8	7.6	7.8	0.2	0.1	7.98	0.10	280	2	237	6
	WSL	1	19.6	19.6	8.3	8.3	0.1	--	8.10	--	280	--	234	--
Test A: Copper, (µg/L)	0	5	20.9	23.5	8.2	7.6	0.2	0.1	8.26	0.23	169	12	122	6
	2	5	20.9	23.5	8.4	8.0	0.2	0.1	8.28	0.14	171	8	122	4
	4	5	20.8	23.5	8.5		0.2		8.33	0.14	167	10	123	5
	7.5	5	20.8	23.5	8.3		0.2		8.30	0.15	171	10	124	5
	15	5	20.9	23.5	8.4	8.1	0.2	0.1	8.35	0.15	171	8	124	4
	30	5	20.8	23.5	8.4	8.0	0.1	0.1	8.36	0.13	173	9	124	3
Test 1: Copper (µg/L)	0	4	20.8	21.4	7.7	8.0	0.1	0.1	8.26	0.14	166	5	122	4
	3.75	3	20.4	20.7	7.4	7.3	0.1	0.1	8.20	0.08	167	7	121	2
	7.5	3	20.5	20.9	7.2		0.2		8.17	0.05	172	7	123	2
	15	4	20.6	21.2	7.3		0.2		8.22	0.14	169	7	124	3
	30	3	20.4	20.6	7.1	6.9	0.2	0.1	8.21	0.12	169	5	125	4
	60	3	20.5	20.7	6.9	6.4 6.7	0.2	0.0 0.0	8.22	0.15	169	5	125	4

Table 7 (continued).

Test	Treat-ment	N	Temperature (ºC)		Dissolved oxygen (mg/L)		Ammonia (mg N/L)		pH		Hardness (mg/L)		Alkalinity (mg/L)	
			Mean	Max	Mean	Min	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Test 4: Copper (µg/L)	0	5	20.2	20.8	7.9	5.9	0.2	0.2	8.39	0.11	168	8	121	3
	3.75	6	20.5	21.5	7.7	6.0	0.2	0.1	8.38	0.10	171	6	121	3
	7.5		20.4	21.5	7.7	6.8	0.4	0.5	8.35	0.14	169	8	121	3
	15		20.2	21.4	7.7	5.9	0.3	0.5	8.44	0.08	162	21	122	3
	30	5	20.4	21.4	7.5	6.0	0.7	1.1	8.43	0.11	160	23	120	4
	60	6	20.3	21.4	7.8	5.9	1.2	2.6	8.44	0.13	169	6	123	3
Test B: PCP (µg/L)	0	5	21.0	22.3	7.9	6.1	0.2	0.1	8.42	0.30	170	7	127	3
	25	5	20.8	21.7	8.4	6.5	0.2	0.1	8.46	0.17	172	2	126	5
	20		21.3	22.3	8.0	5.4	0.1	0.0	8.38	0.20	174	4	127	4
	100		21.2	22.1	8.0	7.3	0.1	0.1	8.48	0.10	170	7	127	4
	200	5	21.2	22.2	8.1	6.7	0.1	0.1	8.42	0.16	172	4	126	5
	400	5	21.0	22.3	8.0	6.7	0.1	0.1	8.43	0.16	171	7	128	6
Test 2: PCP (µg/L)	0	4	20.8	23.4	8.1	7.5	0.2	0.1	8.47	0.12	191	13	127	3
	25	4	20.5	21.2	8.1	7.4	0.1	0.1	8.47	0.13	190	8	126	1
	50	4	20.1	20.8	7.8	6.5	0.1	0.1	8.39	0.21	189	7	123	2
	100	4	20.2	20.8	7.6	5.9	0.1	0.1	8.37	0.26	187	10	128	9
	200	4	20.4	21.0	7.9	7.0	0.1	0.1	8.42	0.15	189	8	128	6
	400	4	20.4	21.0	7.8	6.4	0.2	0.1	8.35	0.30	189	8	126	4
Test 3: Ammonia (mg N/L)	0	8	20.3	21.6	7.7	6.0	(See Table 5)		8.26	0.04	170	5	117	6
	0.5		20.3	21.7	8.1	7.0			8.27	0.06	168	6	119	4
	1	7	20.0	21.3	7.8	5.9			8.22	0.04	168	5	119	3
	2	7	20.0	21.1	7.7	6.0			8.24	0.04	169	6	117	6
	4	7	19.8	21.1	7.6	6.1			8.26	0.07	169	7	120	3
	8	7	20.0	21.0	7.9	5.9			8.25	0.04	170	8	116	5
Test 5: Ammonia (mg N/L)	0	5	20.9	22.0	8.4	7.7	(See Table 5)		8.29	0.13	166	8	119	6
	0.5	5	21.0	22.3	8.3	7.8			8.23	0.07	166	6	120	7
	1	5	20.8	21.9	8.3	7.6			8.28	0.04	166	7	119	4
	2	5	20.5	21.8	8.2	7.5			8.27	0.06	166	8	119	4
	4	5	21.0	22.0	8.5	7.8			8.25	0.08	167	8	119	5
	8	5	20.8	22.5	8.4	7.8			8.25	0.06	167	8	119	5

Table 8. Treatment means and analysis of variance for 28-d exposures of freshwater snails to copper (Test A). For each species and endpoint, p-values indicate significance of ANOVA for differences among treatments. Asterisks indicate means that are significantly less than control (Dunnett's test). Cell borders indicate NOEC and LOEC. [LS=pondsnail, SS=Idaho springsnail. SE = Standard error.]

Species	Copper (µg/L)	N	Survival (percent)				Copper (µg/L)	Wet weight (mg)			
			Mean	SE	Dunnett	p-value		N	Mean	SE	Dunnett	p-value
LS	0.63	4	92.5	4.8		0.0186	0.63	4	27.7	0.4		<0.0001
LS	2.6	4	92.5	2.5			2.6	4	20.6	0.6	*	
LS	3.9	4	87.5	7.5			3.9	4	20.7	1.8	*	
LS	6.1	4	92.5	2.5			6.1	4	25.8	0.7		
LS	15	4	87.5	2.5			15	4	6.8	1.0	*	
LS	33	4	0	0	*		33	0	--			
SS	0.63	4	60	10		<0.0001						
SS	2.6	4	80	8.2								
SS	3.9	4	82.5	8.5								
SS	6.1	4	60	7.1								
SS	15	4	30	10.8								
SS	33	4	0	0	*							

Table 9. Treatment means and analysis of variance for 28-d exposures of freshwater snails to pentachlorophenol (PCP; Test B). For each species and endpoint, p-values indicate significance of ANOVA for differences among treatments. Asterisks indicate means that are significantly less than control (Dunnett's test). Cell borders indicate NOEC and LOEC. [LS=pondsnail, SS=Idaho springsnail. SE = Standard error.]

Species	PCP (µg/L)	Survival (percent)					PCP (µg/L)	Wet weight (mg)				
		N	Mean	SE	Dunnett	p-value		N	Mean	SE	Dunnett	p-value
LS	2.8	4	97.5	2.5		0.0029	2.8	4	44.3	2.1		<0.0001
LS	19	4	102.5	2.5			19	4	35.4	1.7		
LS	41	4	87.5	4.8			41	4	33.6	4.9	*	
LS	88	4	97.5	2.5			88	3	23.5	2.3	*	
LS	178	4	95	2.9			178	4	27.0	2.7	*	
LS	377	4	52.5	18.9	*		377	4	5.3	0.4	*	
SS	2.8	4	5	5		0.0007						
SS	19	4	7.5	4.8								
SS	41	4	25	6.5								
SS	88	4	0	0								
SS	178	4	0	0								
SS	377	4	0	0								

Table 10. Treatment means and analysis of variance for 28-d exposures of freshwater snails to copper (Test 1). For each species and endpoint, p-values indicate significance of ANOVA for differences among treatments. Asterisks indicate means that are significantly less than control (Dunnett's test). Cell borders indicate NOEC and LOEC. [LS=pondsnail, SS=Idaho springsnail. SE=standard error.]

Species	Copper. (µg/L)	Survival (percent)					Copper (µg/L)	Shell diameter (mm)				
		N	Mean	SE	Dunnett	p-value		N	Mean	SE	Dunnett	p-value
LS	1.1	4	95.0	5.0		0.0009	1.1	4	7.74	026		0.0058
LS	4.6	4	92.5	7.5			4.6	4	7.18	0.14		
LS	8.3	4	97.5	2.5			8.3	4	7.58	0.16		
LS	17	4	97.5	2.5			17	4	7.49	0.19		
LS	32	4	52.5	18.9			32	4	4.20	0.95	*	
LS	87	4	0.0	0.0	*		87		--			
SS	1.1	4	97.5	2.5		<0.0001	1.1	4	0.83	0.05		<0.0001
SS	4.6	4	100.0	0.0			4.6	4	1.14	0.06		
SS	8.3	4	100.0	0.0			8.3	4	0.74	0.02		
SS	17	4	17.5	8.5	*		17	3	0.62	0.03	*	
SS	32	4	0.0	0.0	*		32		--			
SS	87	4	0.0	0.0	*		87		--			

Table 11. Treatment means and analysis of variance for 28-d exposures of freshwater snails to pentachlorophenol (PCP; Test 2). For each species and endpoint, p-values indicate significance of ANOVA for differences among treatments. Asterisks indicate means that are significantly less than control (Dunnett's test). Cell borders indicate NOEC and LOEC. [LS=pondsnail, SS=Idaho springsnail. SE = standard error.]

Species	PCP (µg/L)	Survival (percent)					PCP (µg/L)	Shell diameter (mm)				
		N	Mean	SE.	Dunnett	p-value		N	Mean	SE	Dunnett	p-value
LS	0.33	4	100.0	0.0		<0.0001	0.33	4	7.77	0.17		0.0115
LS	11	4	100.0	0.0			11	4	7.38	0.17		
LS	24	4	100.0	0.0			24	4	7.21	0.13		
LS	50	4	100.0	0.0			50	4	7.04	0.27		
LS	97	4	100.0	0.0			97	4	6.95	0.36		
LS	224	4	25.0	18.5	*		224	3	3.88	0.68	*	
SS	0.33	4	100.0	0.0		0.0014	0.33	4	1.86	0.10		0.0023
SS	11	3	100.0	0.0			11	3	1.56	0.08		
SS	24	3	86.7	13.3			24	3	1.33	0.08	*	
SS	50	3	100.0	0.0			50	3	1.50	0.12	*	
SS	97	3	93.3	6.7			97	2	1.24	0.10	*	
SS	224	3	0.0	0.0	*		224					

Table 12. Treatment means and analysis of variance for 28-d exposures of freshwater snails to ammonia (Test 3). For each species and endpoint, p-values indicate significance of ANOVA for differences among treatments. Asterisks indicate treatment means that are significantly less than control (Dunnett's test). Cell borders indicate NOEC and LOEC. [LS=pondsnail, SS=Idaho springsnail.SE = standard error.]

Species	Ammonia (mg N/L)	Survival (percent)					Ammonia (mg N/L)	Shell diameter (mm)				
		N	Mean	SE	Dunnett	p-value		N	Mean	SE	Dunnett	p-value
LS	0.14	4	97.5	2.5		0.8534	0.14	4	7.11	0.17		0.0007
LS	0.48	4	100.0	0.0			0.48	4	6.87	0.05		
LS	1.0	4	95.0	2.9			1.0	4	6.93	0.10		
LS	1.8	4	90.0	10.0			1.8	4	6.67	0.08	*	
LS	3.6	4	95.0	5.0			3.6	4	6.38	0.24	*	
LS	8.0	4	97.5	2.5			8.0	4	5.98	0.06	*	
SS	0.14	4	100.0	0.0		0.0011	0.14	4	1.35	0.02		0.3691
SS	0.48	4	44.4	20.3	*		0.48	4	1.47	0.10		
SS	1.0	4	27.5	10.3	*		1.0	3	1.29	0.08		
SS	1.8	4	62.5	16.5			1.8	4	1.52	0.11		
SS	3.6	4	20.0	14.1	*		3.6	2	1.55	0.20		
SS	8.0	4	2.5	2.5	*		8.0	1	1.10			

Table 13. Treatment means and analysis of variance for 28-d exposures of freshwater snails to copper (Test 4). For each species and endpoint, p-values indicate significance of ANOVA for differences among treatment means. Asterisks indicate means that are significantly less than control (Dunnett's test). Cell borders indicate NOEC and LOEC. [BR=Bliss Rapids snail; OZ=Ozark springsnail; PS=pebblesnail; SS=Idaho springsnail. SE = standard error.]

Species	Copper (µg/L)	Survival (percent)				
		N	Mean	SE	Dunnett	p-value
BR	0.70	2	100.0	0.0		<0.0001
BR	4.3	2	85.0	5.0	*	
BR	6.6	2	100.0	0.0		
BR	13	2	80.0	0.0	*	
BR	26	2	0.0	0.0	*	
BR	62	2	0.0	0.0	*	
OZ	0.70	2	100.0	0.0		0.0008
OZ	4.3	2	91.7	8.3		
OZ	6.6	2	100.0	0.0		
OZ	13	2	66.7	0.0	*	
OZ	26	2	58.3	8.3	*	
OZ	62	2	0.0	0.0	*	
PS	0.70	2	90.0	0.0		<0.0001
PS	4.3	2	100.0	0.0		
PS	6.6	2	100.0	0.0		
PS	13	2	50.0	10.0	*	
PS	26	2	0.0	0.0	*	
PS	62	2	0.0	0.0	*	
SS	0.70	2	100.0	0.0		<0.0001
SS	4.3	2	100.0	0.0		
SS	6.6	2	100.0	0.0		
SS	13	2	75.0	0.0	*	
SS	26	2	50.0	12.5	*	
SS	62	2	0.0	0.0	*	

Table 14. Treatment means and analysis of variance for 28-d exposures of freshwater snails to ammonia (Test 5). For each species and endpoint, p-values indicate significance of ANOVA for differences among means. Asterisks indicate treatment means that are significantly less than control (Dunnett's test). Cell borders indicate NOEC and LOEC. [BR=Bliss Rapids snail; OZ=Ozark springsnail; PS=pebblesnail; SS=Idaho springsnail.SE = standard error.]

Species	Ammonia (mg N/L)	Survival (percent)				
		N	Mean	SE	Dunnett	p-value
BR	0.046	2	100.0	0.0		0.0003
BR	0.45	2	100.0	0.0		
BR	0.83	2	100.0	0.0		
BR	1.7	2	85.0	5.0	*	
BR	3.6	2	80.0	10.0	*	
BR	7.9	2	30.0	0.0	*	
OZ	0.046	2	100.0	0.0		<0.0001
OZ	0.45	2	93.8	6.3		
OZ	0.83	2	50.0	25.0	*	
OZ	1.7	2	0.0	0.0	*	
OZ	3.6	2	0.0	0.0	*	
OZ	7.9	2	0.0	0.0	*	
PS	0.046	2	100.0	0.0		0.339
PS	0.45	2	90.0	10.0		
PS	0.83	2	90.0	10.0		
PS	1.7	2	45.0	45.0		
PS	3.6	2	50.0	50.0		
PS	7.9	2	0.0	0.0		
SS	0.046	2	100.0	0.0		0.0209
SS	0.45	2	100.0	0.0		
SS	0.83	2	100.0	0.0		
SS	1.7	2	100.0	0.0		
SS	3.6	2	75.0	25.0		
SS	7.9	2	12.5	12.5	*	

Table 15. Summary of 28-d toxicity values for freshwater snails. Species chronic value is lowest EC20 for a single test, or geometric mean of lowest survival or growth EC20s (**bold**) for multiple tests. Upper boundary value used for 'less than' values. [N=neonate, J=juvenile; M=mixed. LC50=median lethal concentration; EC20=20% effect concentration; conf=95% confidence interval.]

Chemical	Species	Age	Test	Survival				Growth		Species chronic value
				EC20	(conf)	LC50	(conf)	EC20	(conf)	
Copper	LS	N	A	16.9	(16-17)	21.7	(20-23)	***6.15***	(6.1-6.6)	
		N	1	22.4	(19-34)	36.2	(30-44)	***21.5***	(19-27)	11.5
		J	A	***8.58****	(1.7-43)	13.1*	(6.0-28)			
		J	1	9.89	(9.7-10.3)	12.9	(12-14)	***7.4***	(6.8-8.5)	
		M	4	13.7*	(7.1-26)	23.8*	(17-34)			9.55
	SS	M	4	***16.8***	(16.8-16.8)	20.8	(18-24)			16.8
		M	4	***14.0****	(4.1-47)	25.6*	(13-51)			14.0
		M	4	***11.1***	(10.6-12.1)	16.9	(14-20)			11.1
PCP	LS	N	B	>337	--	>337	--	***19.1***	(19-55)	
	OZ	N	2	121	(115-152)	170**	(154-191)	***118***	(100-135)	47.5
	PS	N	B	--						
		N	2	109	(96-114)	143	(138-151)	***16.6***	(11-69)	16.6
NH_3	LS	N	3	>7.9	--	>7.9	--	>7.9		>7.9
	SS	J	3	<***0.48***	--	0.609	(0.39-0.81)	>8.0		
		M	5	***3.24****	(4.4-5.3)	4.84*	(4.4-5.3)			1.25
		M	5	***3.42****	(2.0-5.7)	5.92*	(4.4-8.0)			3.42
	SS	M	5	***0.61***	(0.52-0.87)	0.963	(0.78-1.18)			0.61
		M	5	**1.02***	(0.12-8.6)	2.38*	(0.75-7.6)			1.02
	BR									
	OZ									
	PS									

*EC20 or LC50 estimated by Toxicity Response Analysis Program (TRAP, Version 1.0; R. Erickson, USEPA-MED, Duluth MN)..

**LC50 estimated by linear interpolation (IC50).

Table 16. Published toxicity values for toxicity of copper, pentachlorophenol, and ammonia to freshwater snails. [Endpoints: S=survival, G=growth, R=reproduction. Metrics: LC50=median lethal concentration; NOEC=no observed effect concentration; LOEC=lowest observed effect concentration; ChV=geometric mean of NOEC and LOEC.]

Species	Duration	Endpoint	Metric	Concentration	Notes	Reference
			Copper (ug/L)			
Lymnaea luteola	96h	S	LC50	27		Khangarot et al. 1988
Potamopyrgus jenkinsi	96 h	S	LC50	54	juveniles	Watton and Hawkes 1984
Potamopyrgus jenkinsi	96 h	S	LC50	77	adults	Watton and Hawkes 1984
Potamopyrgus jenkinsi	96 h	S	LC50	79	senescent	Watton and Hawkes 1984
Pomacea canaliculata	10 d	G	NOEC	68	juveniles	Pena and Pocsidio 2007
Pomacea canaliculata	50 d	G	NOEC	>68	juveniles	Pena and Pocsidio 2007
Physa integra	6 wk	SG	LOEC	14.8	adults	Arthur and Leonard 1970
Campeloma decisum	6 wk	SGR	LOEC	14.8	adults	Arthur and Leonard 1970
			Pentachlorophenol (ug/L)			
Lymnaea acuminata	96 h	S	LC50	160		Gupta and Rao 1982
Physa gyrina	96 h	S	LC50	220	summer	Hedtke et al. 1986
Physa gyrina	96 h	S	LC50	1380	winter	Hedtke et al. 1986
Physa gyrina	96 h	S	LC50	267	Lk. Superior	Hedtke et al. 1986
Gillia altilis	96 h	S	LC50	810	static	Stuart and Robertson 1985
Gillia altilis	96 h	S	LC50	300	flow-through	Stuart and Robertson 1985
Physa gyrina	96 h	S	ChV	150		Hedtke et al. 1986
Physa gyrina	36 d	G	ChV	74		Hedtke et al. 1986
Physa gyrina	21 d	R	ChV	<26		Hedtke et al. 1986

Table 16 (continued)

Species	Duration	Endpoint	Metric	Concentration	Notes	Reference
			Total Ammonia (mg N/L)			
Lymnaea stagnalis	96 h	S	LC50	0.825		Williams et al. 1985
Physa fontinalis	96 h	S	LC50	1.4		Williams et al. 1985
Potamopyrgus antipodarium	96 h	S	LC50	2.02		Alonso and Camargo 2003
Potamopyrgus antipodarium	96 h	S	LC50	0.31	lowest	Hickey and Vickers 1994
Potamopyrgus antipodarium	96 h	S	LC50	0.44	highest	Hickey and Vickers 1994
Potamopyrgus jenkinsi	96 h	S	LC50	0.315	juveniles	Watton and Hawkes 1984
Potamopyrgus jenkinsi	96 h	S	LC50	0.85	adults	Watton and Hawkes 1984
Potamopyrgus jenkinsi	96 h	S	LC50	0.49	senescent	Watton and Hawkes 1984

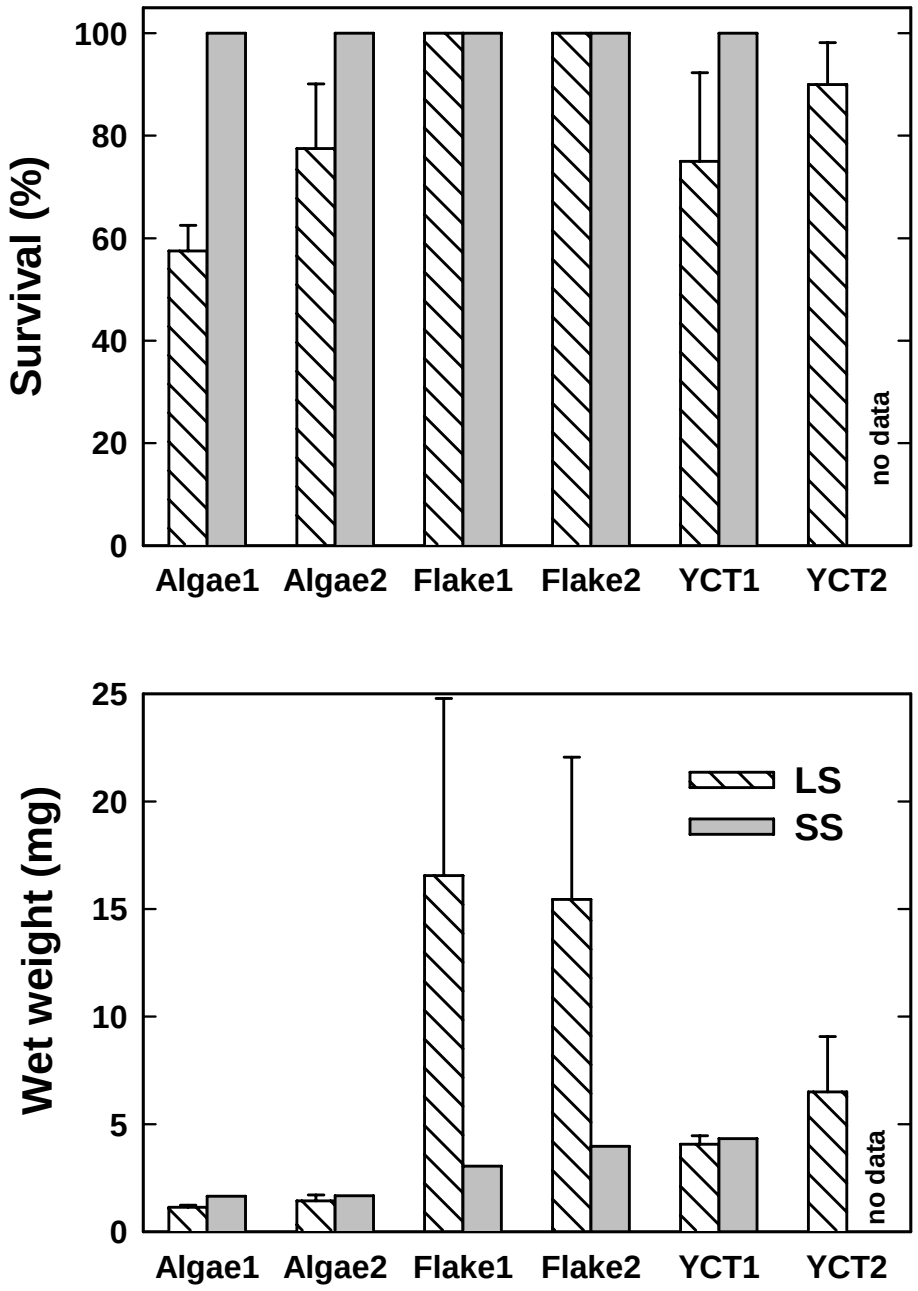

Figure 1. Survival and growth of pondsnail (LS) and Idaho springsnail (SS) in preliminary Study 1 (21-d). Results for SS are from individual exposure groups; results for LS are means of 4 replicates (with standard deviation). Labels that include '1' and '2' indicate low and high feeding rates, respectively, for each diet.

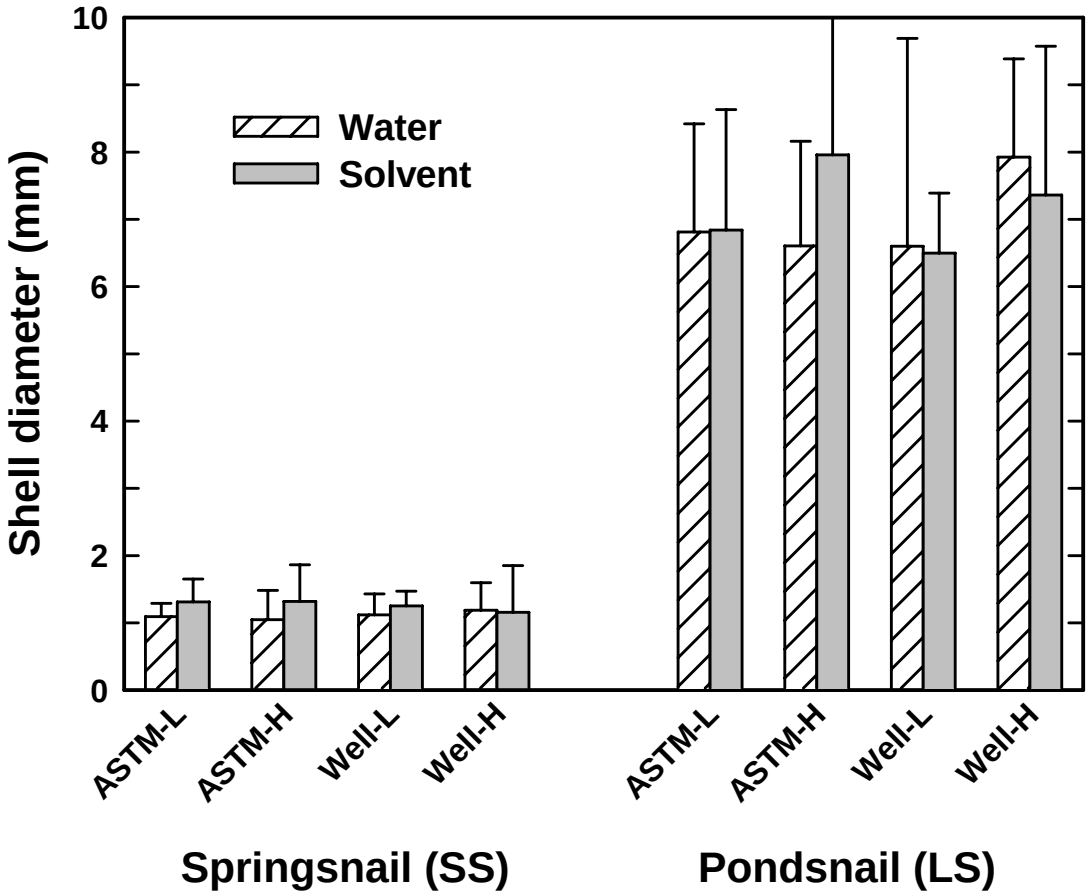

Figure 2. Growth of Idaho springsnail (SS) and pondsnail (LS) in preliminary Study 2. Bars are mean shell diameters for individual snails after 28 d, with standard deviation (n=9-10). Treatments are combinations of test water (ASTM hard vs. well water), solvent (control or triethylene glycol), and feeding rate (L=low; H=high).

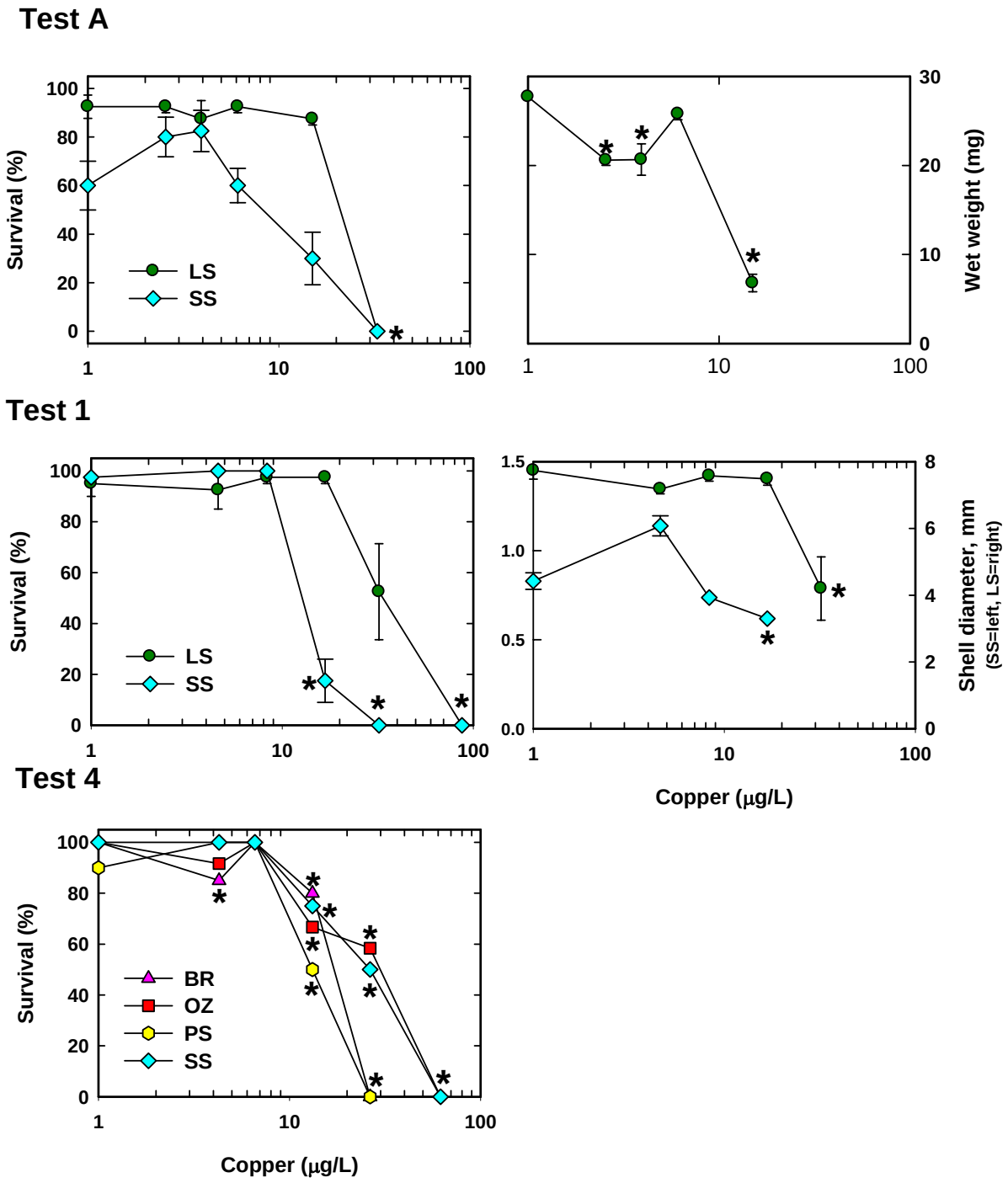

Figure 3. Toxicity of copper to freshwater snails. Survival and growth during 28-d exposures: means with standard errors (Test A and Test 1; n=4) or means only (Test 4; n=2). Asterisks indicate significant differences from controls (rank ANOVA with Dunnett's test).

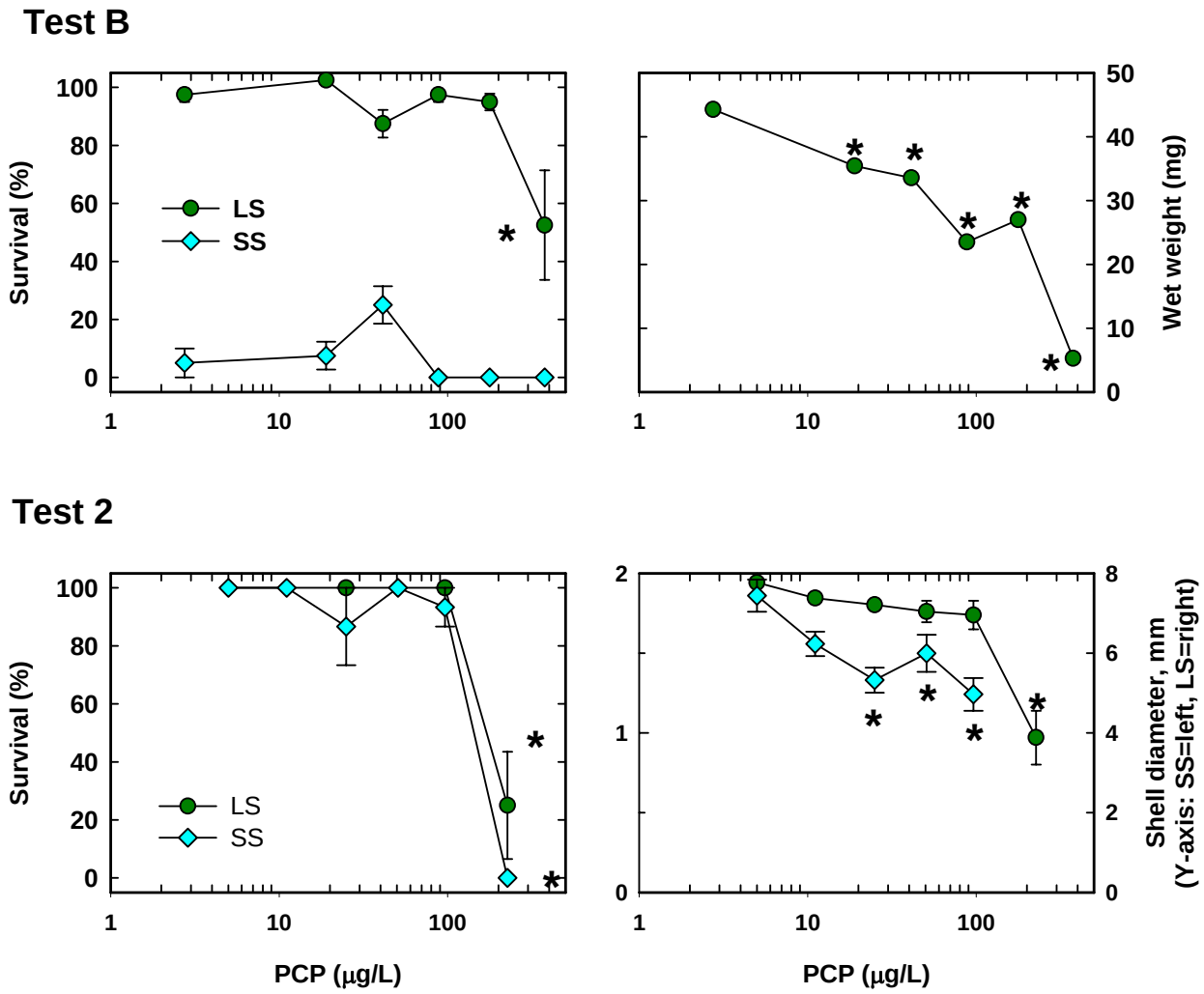

Figure 4. Toxicity of pentachlorophenol (PCP) to freshwater snails. Survival and growth during 28-d exposures (means with standard errors; n=4). Asterisks indicate significant differences from controls (rank ANOVA with Dunnett's test).

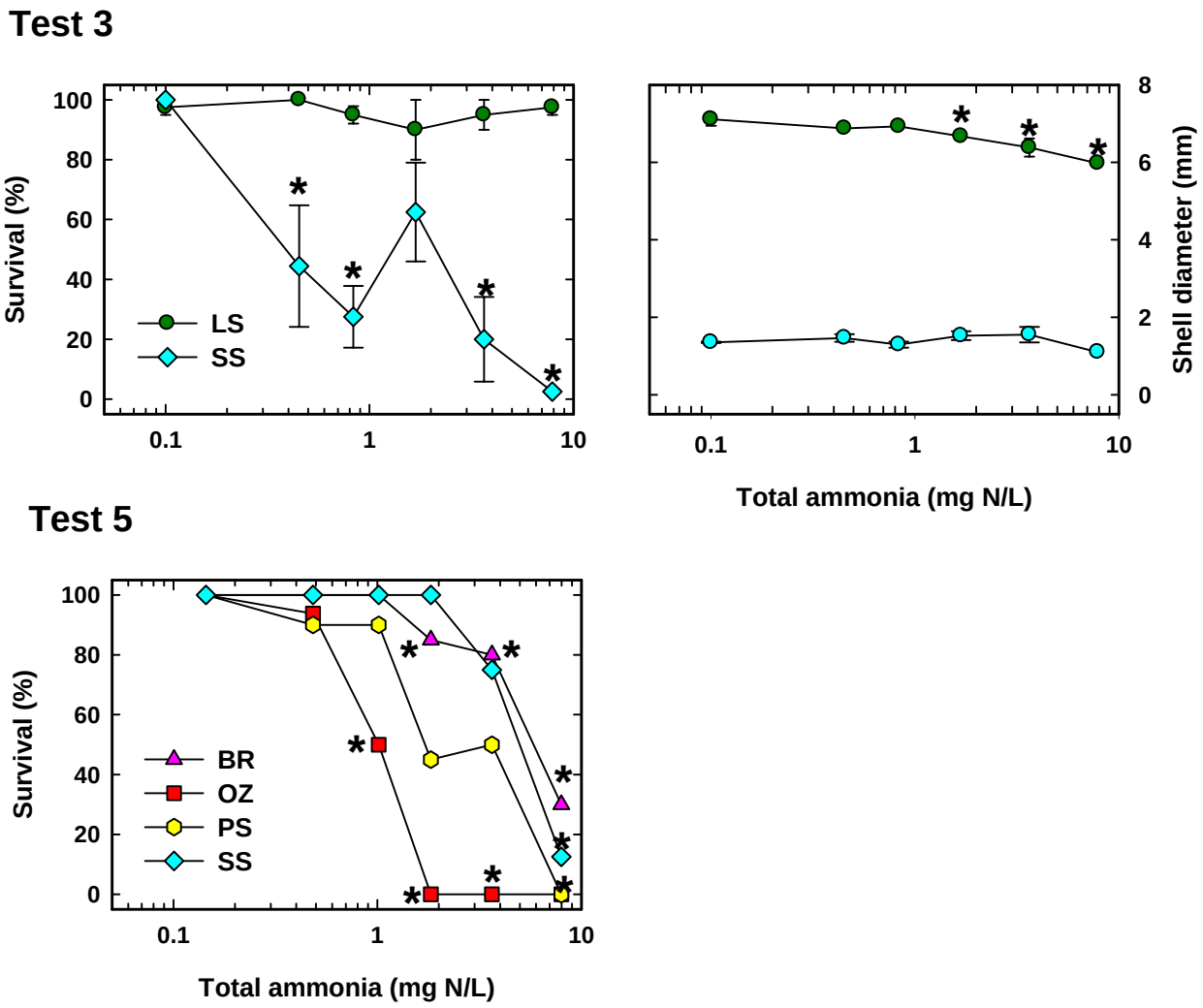

Figure 5. Toxicity of ammonia to freshwater snails. Survival and growth during 28-d exposures: means with standard errors (Test 3; n=4) or means (Test 5; n=2). Asterisks indicate significant differences from controls (rank ANOVA with Dunnett's test).

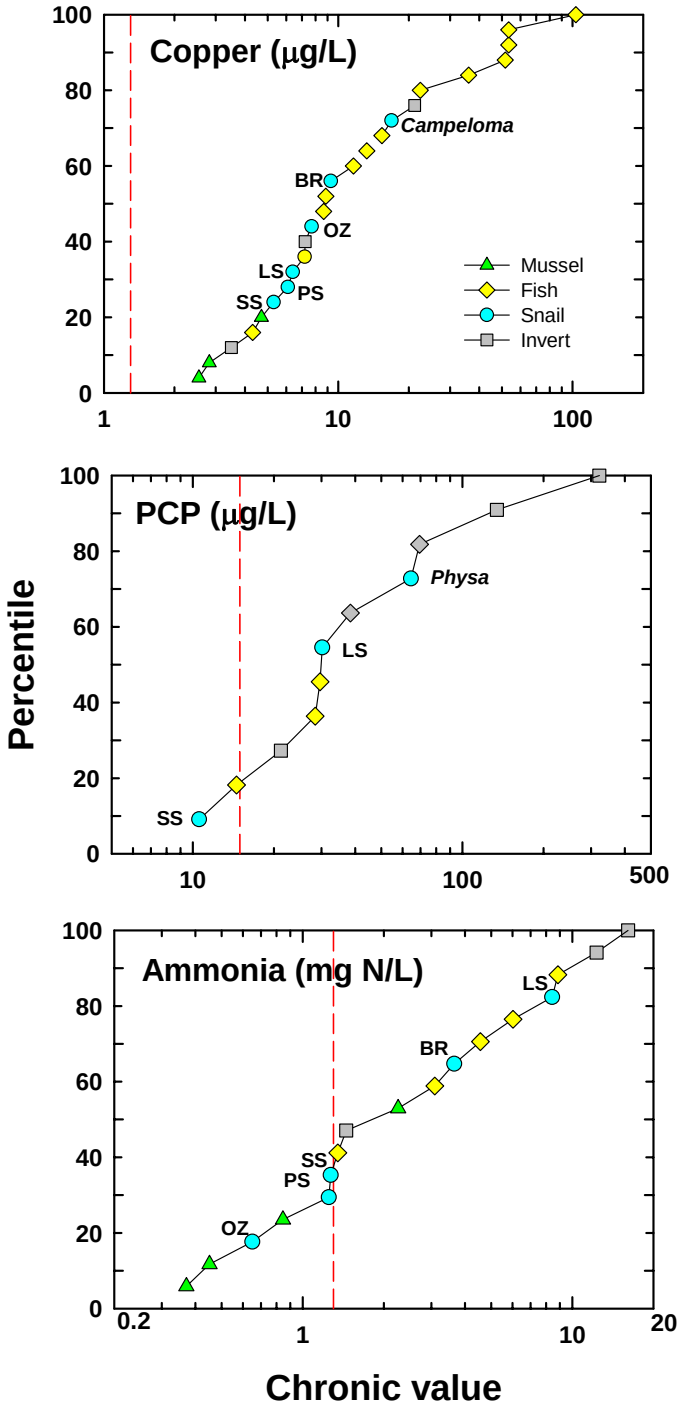

Figure 6. Species sensitivity distributions and chronic water quality criteria (dashed lines) for copper, PCP, and ammonia. Values were adjusted to common conditions for comparisons: 85 mg/L hardness for copper (USEPA 19995, 2007); pH 7.8 for PCP (USEPA 1996); and pH 8.0 and 25 ºC for ammonia (USEPA 1999). Toxicity values from Tables 15 and 16.