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Contaminant Sensitivity of Freshwater Mussels

ACUTE TOXICITY OF COPPER, AMMONIA, AND CHLORINE TO GLOCHIDIA AND JUVENILES OF FRESHWATER MUSSELS (UNIONIDAE)

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Abstract—The objective of the present study was to determine acute toxicity of copper, ammonia, or chlorine to larval (glochidia) and juvenile mussels using the recently published American Society for Testing and Materials (ASTM) *Standard guide for conducting laboratory toxicity tests with freshwater mussels.* Toxicity tests were conducted with glochidia (24- to 48-h exposures) and juveniles (96-h exposures) of up to 11 mussel species in reconstituted ASTM hard water using copper, ammonia, or chlorine as a toxicant. Copper and ammonia tests also were conducted with five commonly tested species, including cladocerans (*Daphnia magna* and *Ceriodaphnia dubia*; 48-h exposures), amphipod (*Hyalella azteca*; 48-h exposures), rainbow trout (*Oncorhynchus mykiss*; 96-h exposures), and fathead minnow (*Pimephales promelas*; 96-h exposures). Median effective concentrations (EC50s) for commonly tested species were >58 μg Cu/L (except 15 μg Cu/L for *C. dubia*) and >13 mg total ammonia N/L, whereas the EC50s for mussels in most cases were <45 μg Cu/L or <12 mg N/L and were often at or below the final acute values (FAVs) used to derive the U.S. Environmental Protection Agency 1996 acute water quality criterion (WQC) for copper and 1999 acute WQC for ammonia. However, the chlorine EC50s for mussels generally were >40 μg/L and above the FAV in the WQC for chlorine. The results indicate that the early life stages of mussels generally were more sensitive to copper and ammonia than other organisms and that, including mussel toxicity data in a revision to the WQC, would lower the WQC for copper or ammonia. Furthermore, including additional mussel data in 2007 WQC for copper based on biotic ligand model would further lower the WQC.

Keywords—Freshwater mussels Glochidia Juvenile mussels Acute toxicity Water quality criteria

INTRODUCTION

Most freshwater mussels have a complex reproductive cycle involving a parasitic stage on fish. Sperm released by a male enters a female through the incurrent siphon, and fertilized eggs develop to larvae called glochidia that mature in specialized chambers (marsupia) of the female's gills. Glochidia are released into the water and must attach to the gills or fins of a suitable host fish. After one to several weeks of the parasitic stage, glochidia transform to juvenile mussels, detach from the fish, and drop to the stream or lake bottom to begin the free-living juvenile stage. Freshwater mussels are the most imperiled group of animals in the United States, with about 70% of species listed as endangered, threatened, or of special concern [1,2]. Environmental contamination is considered one of the causal or contributing factors [2,3]. Previous studies indicate that glochidia and juvenile mussels are more sensitive to some chemicals when compared to commonly tested aquatic organisms. For example, Keller and Zam [4] evaluated acute toxicity of six metals to juvenile paper pondshell (*Utterbackia imbecilis*) and found the juvenile mussels were more sensitive than commonly tested fish and aquatic invertebrates. Augspurger et al. [5] summarized ammonia toxicity data for glochidia and juvenile mussels and found that genus mean acute values for mussels were uniformly at the sensitive end of the range of the genus mean acute values for other aquatic species tested in the database used to derive the U.S. Environmental Protection Agency (U.S. EPA) water quality criteria (WQC) for ammonia. Nevertheless, the U.S. EPA has not used routinely the toxicity data generated from freshwater mussels in the derivation of WQC, mainly due to a historic lack of standardized guidance for conducting toxicity tests with freshwater mussels [6].

A series of studies was undertaken to refine methods for conducting acute and chronic toxicity tests with early life stages of mussels [6–10]. Based on these studies and previous literature, the American Society for Testing and Materials (ASTM) recently published a standard guide for conducting laboratory toxicity tests with freshwater mussels [11]. As one of a series of papers developed to assess contaminant sensitivity of early life stages of freshwater mussels, the present paper summarizes the results of acute toxicity tests conducted during the process of developing the ASTM standard. Specifically, the objectives of the present study were to evaluate the acute toxicity of copper, ammonia, or chlorine to glochidia and juvenile mussels and compare the sensitivity of early life

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stages of freshwater mussels with commonly tested cladoceran, amphipod, and fish species. Additionally, the exposure periods of some tests conducted with juvenile mussels were continued for 10 d to evaluate longer-term effects of copper, ammonia, or chlorine on survival or growth of juvenile mussels. Companion papers in this issue evaluate intra- and interlaboratory variability in acute toxicity tests with freshwater mussels [9] and evaluate the chronic toxicity of copper or ammonia to early life stages of mussels [10]. Copper, ammonia, and chlorine were selected as toxicants because of the following considerations: They occur widely in contaminated aquatic environments, limited chlorine toxicity data are available for mussels [12], and early life stages of mussels have been demonstrated to be sensitive to copper and ammonia [5,13]. Other toxicants, such as pesticides, were evaluated in two of the companion papers [7,8]. The toxicity data generated from these studies have been included in datasets used to assess the sensitivity of mussels to these chemicals [13,14].

MATERIALS AND METHODS

Test conditions and procedures for conducting toxicity tests in the present study followed the guidance provided in ASTM [11] and are described in more detail in Wang et al. [9].

Toxicity test with glochidia

Gravid females of 11 mussel species were collected from streams or rivers in New Hampshire (dwarf wedgemussel, *Alasmidonta heterodon*), Virginia (rainbow mussel, *Villosa iris*; wavy-rayed lampmussel, *Lampsilis fasciola*), Tennessee (oyster mussel, *Epioblasma capsaeformis*), and Missouri, USA (ellipse, *Venustaconcha ellipsiformis*; mucket, *Actinonaias ligamentina*; fatmucket, *L. siliquoidea*; Neosho mucket, *L. rafinesqueana*; pink mucket, *L. abrupta*; pink papershell, *Potamilus ohiensis*, and scaleshell, *Leptodea leptodon*) between March and June from 2003 to 2005 (common and scientific names of mussels are referred from Turgeon et al. [15]). The mussels were brought to or shipped by overnight mail to the Columbia Environmental Research Center (CERC), U.S. Geological Survey ([USGS], Columbia, MO), and held in a 600-L flow-through fiberglass tank with well water (hardness 280 mg/L as $CaCO_3$, alkalinity 250 mg/L as $CaCO_3$, pH 7.8) at a flow rate of about 2 L/min. Water was aerated and maintained at 10 to 13°C. Methods for holding and feeding the female mussels were as described in Wang et al. [9].

The viability of glochidia isolated from each female mussel was determined before starting a toxicity test. Glochidia were flushed gently from the marsupium of a mussel into a 300-ml crystallizing dish using a 1-mm needle and 35-ml syringe filled with culture water. Three subsamples of 100 to 200 glochidia were impartially taken from the dish using a 2-mm wide-bore pipette and transferred to each of three wells of a 24-well polystyrene tissue-culture plate filled with about 2 ml of well water. Glochidia in each well were examined with a dissecting microscope, and the number of closed glochidia was recorded. After adding one drop of saturated NaCl solution to each well, open and closed glochidia were counted within 1 min. Valve closure is an ecologically relevant endpoint that is critical for glochidia to successfully transform on the host [11]. Glochidia that closed in response to NaCl were categorized as alive (or viable), and glochidia that were closed before the addition of NaCl or that remained open after the addition of NaCl were categorized as dead (nonviable; [9]). Survival (viability) of glochidia was calculated as

Survival (%)

= 100 × (Number of closed glochidia after adding NaCl solution − Number of closed glochidia before adding NaCl solution)

÷ (Total number of open and closed glochidia after adding NaCl solution)

If the survival of glochidia from an individual mussel was >90%, the remaining glochidia from that mussel were used for toxicity testing. Glochidia isolated from three to six mussels were pooled and mixed in a Pyrex® glass baking dish for a test. Glochidia were acclimated to a mixture of 50% culture water (well water) and 50% dilution water (reconstituted ASTM hard water [16]) that was adjusted gradually to the test temperature over 2 h before the start of a toxicity test.

The reconstituted ASTM hard water was used as dilution water in all tests. Copper sulfate ($CuSO_4$, 99.9% purity; JT Baker, Phillipsburg, NJ, USA), ammonium chloride (NH_4Cl, 99.5% purity; Fisher Scientific, Houston, TX, USA), and sodium hypochlorite (NaOCl, available chlorine 10–13%; Aldrich, Milwaukee, WI, USA) were used as toxicants. Each test consisted of three replicates at each of five concentrations of a toxicant in a 50% serial dilution, plus a control. The nominal concentrations were 0, 6.25, 12.5, 25, 50, and 100 μg Cu/L for copper tests; 0, 1.0, 2.0, 4.0, 8.0, and 16 mg N/L for total ammonia tests; and 0, 6.25, 12.5, 25, 50, and 100 μg/L for total residual chlorine tests (except one early test at 0, 3.125, 6.25, 12.5, 25, and 50 μg/L). Static copper and ammonia tests were conducted in 200-ml glass crystallizing dishes (75-mm diameter, 35-mm high). Because chlorine is highly volatile, chlorine toxicity tests were conducted in a flow-through diluter system that provided about 120 ml of water to each 300-ml beaker every 20 min to maintain stable chlorine concentrations [9]. An inline flow splitter was attached to each delivery line to partition the water flow evenly to each of replicate beakers. Each beaker had a 2.5-cm hole in the side covered with 50 mesh (279-μm opening) stainless-steel screen and contained 200 ml of water. The diluter system delivered five concentrations with a dilution factor of 0.5, plus a control. Chlorine stock solution was held in a 600-ml plastic blood pack (Baxter Healthcare, Deerfield, IL, USA) to reduce volatilization of chlorine from the stock solution and was delivered with each cycle of the diluter by a Hamilton syringe pump (Hamilton Company, Reno, NV, USA).

At the beginning of a toxicity test, about 1,000 glochidia (about 2 h old after isolation from female mussels) were transferred impartially from the pooled sample of glochidia into each of 18 dishes containing about 100 ml of test solution (copper and ammonia tests) or into each of eighteen 300-ml beakers in the diluter system (chlorine tests). Test chambers were held in temperature-controlled water baths at 20 ± 1°C. Ambient laboratory light of about 200 lux was used with 16:8 h light:dark photoperiod. Dissolved oxygen, pH, conductivity, hardness, and alkalinity were measured on pooled replicate test solutions collected from the control, medium-, and high-exposure concentrations at the beginning and the end of each test using standard methods [17].

Survival of glochidia was determined at 6, 24, and 48 h during each 48-h exposure. A subsample of about 100 individuals with 2 ml of dilution water was taken from each replicate test chamber using a 2-mm wide-bore pipette and transferred into one well of a clean 24-well tissue-culture plate. One drop of the NaCl solution was added into the well, and

the response of glochidia (valve closure) within 1 min was recorded. The initial survival of glochidia for a toxicity test was estimated by determining the viability of three replicate controls at the beginning of the test. The mean viability value was used to adjust the survival of glochidia after 6, 24, or 48 h of exposure. For example, if the mean viability in the control was 90% at 0 h and an observed viability of glochidia in a replicate test chamber at 24 h was 85%, then the adjusted survival at 24 h of exposure was 94% (85/90). Adjusted survival values were used for the calculation of acute effect concentrations and control survival during exposure periods [9]. The acceptability criterion for a toxicity test was ≥90% control survival [11].

Toxicity test with juvenile mussels

Juveniles of seven mussel species (rainbow mussel, wavy-rayed lampmussel, oyster mussel, fatmucket, Neosho mucket, pink mucket, and scaleshell) were obtained from laboratory cultures at Virginia Polytechnic Institute and State University (Blacksburg, VA, USA), or from Missouri State University (Springfield, MO, USA), where glochidia isolated from at least three females of each species were pooled for production of juvenile mussels with host fish [9]. Newly transformed juvenile mussels, which were derived from a single collection day during the peak of drop-off (excystment) from host fish, were shipped overnight to the CERC for testing. Juveniles of the rainbow mussel, fatmucket, and pink mucket also were reared in the laboratories for two months with live algae (*Neochloris oleoabundans*) either in a recirculating aquaculture system containing fine sediment [18] or in a compact system [19] before shipping to the CERC for testing. The compact recirculating system consisted of two nested buckets that partitioned a volume of 18 L of culture water into an upper and lower compartment. A small submersible pump was used to move water from the lower compartment to the upper compartment, and the water then returned to the lower compartments through cylindrical screen-capped chambers that contained juvenile mussels [19]. Once the newly transformed (<5-d-old) or two-month-old juvenile mussels were received, temperature of the holding water was adjusted gradually to the test temperature (<3°C/h) by placing containers containing mussels at room temperature or into a water bath at 20°C. About 50% of water in the containers then was replaced with dilution water three or four times over a 24- or 48-h acclimation period. Gentle aeration of water was provided to the containers through a glass pipette to maintain an acceptable concentration of dissolved oxygen (>4.0 mg/L [11]). The juveniles were fed live algae (*N. oleoabundans*) at a feeding rate of about 20,000 cell/ml or a food mixture of nonviable algae [10] at a rate of adding 1 ml of the algal mixture into 200 ml of dilution water once or twice daily during the acclimation period.

Water temperature, light quality, photoperiod, dilution water, toxicants, dilution factor, nominal exposure concentrations, and the collection and measurements of water quality characteristics in the juvenile toxicity tests were the same for the glochidia toxicity tests, except that the duration of juvenile toxicity tests was 96 h or 10 d. Each test consisted of four replicates at each of five exposure concentrations, plus a control. Static-renewal copper and ammonia tests were conducted in 50-ml glass beakers containing 30 ml of test solution, and test solutions were renewed every other day by replacing about 75% of water volume. Chlorine tests were conducted in a flow-through diluter system as described for the glochidia tests. Five juvenile mussels exhibiting foot movement were transferred impartially into each of 24 glass beakers in each test using a 1-ml syringe with a 2.5-cm-long, 16-gauge needle connected to a 60-cm-long Tygon® tubing (1.0-mm inner diameter; Saint-Gobain Performance Plastics, Akron, OH, USA) with a glass capillary tube (1.17-mm inner diameter) at the end [11]. Juveniles were not fed during the tests. Survival of juvenile mussels was determined at 48 and 96 h in the 96-h exposures and also on day 10 in the 10-d exposures. Juvenile mussels that exhibited foot movement within a 5-min observation period were classified as alive using a dissecting microscope [11]. The acceptability criterion was ≥90% control survival for a 96-h toxicity test and ≥80% control survival for a 10-d toxicity test [11].

The surviving juveniles at the end of 10-d exposures were preserved in 70% ethanol for growth measurement. The maximum shell length of each juvenile mussel was measured to the nearest 0.001 mm with a digitizing system using video micrometer software (Image Caliper, Resolution Technology, Dublin, OH, USA).

The standard guide in ASTM [11] suggests that it is desirable to determine if juvenile mussels are able to avoid exposure to a chemical in 96-h toxicity tests by closing their valves. At the end of two 96-h copper toxicity tests conducted with newly transformed juveniles and with two-month-old juvenile fatmucket, all test organisms were transferred at 96 h into clean test chambers with dilution water and held for an additional 24 h to determine if either of these life stages were able to avoid exposure to copper. Test conditions were the same as those for the 96-h toxicity tests except for no addition of copper to the dilution water. Survival (foot movement) of juvenile mussels was determined 24 h after the end of the initial 96-h exposure.

Toxicity tests with commonly tested species

Acute copper and ammonia tests were conducted with five commonly tested aquatic species, including cladocerans (*Daphnia magna* and *Ceriodaphnia dubia*), an amphipod (*Hyalella azteca*), fathead minnow (*Pimephales promelas*), and rainbow trout (*Oncorhynchus mykiss*). These species are relatively sensitive to copper or ammonia [20–22]. Test organisms were obtained from laboratory cultures at CERC with the exception of fathead minnows, which were obtained from Aquatic BioSystems (Fort Collins, CO, USA). Tests were conducted in accordance with the recommended test conditions outlined in ASTM [16] and U.S. EPA [23] standard methods. The age of test organisms was <24 h for cladocerans, ~8 d for amphipod, 2 d for fathead minnow, and ~20 d for rainbow trout. Tests with cladocerans and amphipods were conducted for 48 h at 20°C with four replicates per exposure concentration. Five individuals of cladocerans or amphipods were transferred impartially into each of twenty-four 50-ml glass beakers with 30 ml of test solution. Fish tests were conducted for 96 h at 20°C for fathead minnows and 12°C for rainbow trout, with two replicates per concentration. Ten fish were impartially transferred into 1,000-ml glass beakers with 500 ml of test solution (fathead minnows) or 4,000-ml glass beakers with 2,000 ml of test solution (rainbow trout). Test solution was renewed after 48 h by replacing about 75% of water volume. Other test conditions, such as light quality, photoperiod, dilution water, number of exposure concentrations, dilution factor, nominal exposure concentrations (except for the ammonia

Table 1. Water quality characteristics for toxicity tests conducted with glochidia and juveniles of up to 11 mussel species and five commonly tested crustacean and fish species. Values are means (± standard deviation) for multiple tests with each toxicant

Test organism	Exposure time	Toxicant	No. of tests	pH	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Conductivity (μS/cm)
Glochidia	2 d	Copper	21[a]	8.4 ± 0.2	177 ± 11	121 ± 5.3	589 ± 29
		Ammonia	19	8.3 ± 0.2	175 ± 10	117 ± 4.5	633 ± 30
		Chlorine	17	8.4 ± 0.1	169 ± 8.0	115 ± 4.0	560 ± 25
Juvenile mussels	4 d	Copper	19	8.5 ± 0.1	177 ± 10	126 ± 7.1	604 ± 24
		Ammonia	11	8.3 ± 0.1	179 ± 7.6	119 ± 6.5	653 ± 14
		Chlorine	8	8.3 ± 0.1	178 ± 8.7	125 ± 5.7	600 ± 24
Juvenile mussels	10 d	Copper	13	8.4 ± 0.1	187 ± 6.6	131 ± 5.8	631 ± 15
		Ammonia	10	8.4 ± 0.1	184 ± 6.7	120 ± 5.8	660 ± 13
		Chlorine	7	8.4 ± 0.1	176 ± 8.5	123 ± 4.6	593 ± 23
Commonly tested species	2 or 4 d	Copper	5	8.4 ± 0.1	175 ± 6.7	127 ± 2.4	608 ± 17
		Ammonia	5	8.4 ± 0.1	173 ± 2.6	125 ± 4.1	647 ± 70

[a] No water quality measurement for one test with scaleshell glochidia.

test with rainbow trout at 0, 2, 4, 6, 8, 16, and 32 mg N/L), and the collection and determination of water quality characteristics, were as described for the tests with glochidia and juvenile mussels. Mortality was determined at the end of each test. The criterion for death was lack of reaction to gentle prodding. The acceptability criterion for a toxicity test was ≥90% control survival [16,23].

Chemical analysis and data analysis

Water samples for analysis of toxicants were collected at the beginning and end of each test, except for five preliminary copper tests with glochidia. Total ammonia nitrogen of all concentrations in each ammonia test was measured at the beginning and end of the test within 1 h of sample collection using an Orion Ammonia Electrode and Orion EA940 meter (Thermo Electron, Beverly, MA, USA). The meter was calibrated each time before measuring samples with 1 and 10 mg N/L independent calibration verification standards. The percent recovery of the standards ranged from 90 to 100%. For total ammonia nitrogen concentrations in water samples, a minimum reporting limit of 0.1 mg N/L, was selected based on the method detection limit of 0.02 mg N/L and method quantitation limits of 0.06 mg N/L. Water samples for chlorine measurement were analyzed immediately after sample collection. Total residual chlorine was measured at the highest chlorine concentrations (50 or 100 μg/L) with a Hach spectrophotometer (model DR/2000, Hach, Loveland, CO, USA) using *N,N*-diethyl-*p*-phenylenediamine method 8370 for clean water (http://www.hach.com). The lower concentrations (<50 μg/L) could not be detected consistently using this meter and therefore were not measured. The meter for chlorine analysis was calibrated each time before measuring samples following procedures recommended by the manufacturer.

Water samples were acidified to 1% (v/v) ultrapure nitric acid for copper analysis. Water samples, collected at the beginning of the 48-h glochidia tests, at the beginning and end of the 96-h juvenile mussel tests, and on days 0, 4, or 10 for the 10-d juvenile mussel tests, were analyzed for dissolved copper at the control, low-, medium-, and high-exposure concentrations. Water samples collected at the end of five glochidia tests also were analyzed to determine the consistency of copper concentrations during the 48-h exposure period. Copper concentrations were determined by inductively coupled plasma–mass spectrometry (PE/SCIEX ELAN 6000, PerkinElmer, Norwalk, CT, USA). Samples were automatically delivered to the inductively coupled plasma–mass spectrometry by means of a software-controlled CETAC ASX-500/ADX-100 autosampler/autodiluter system (CETAC Technologies, Omaha, NE, USA) [9]. Analytical precision for quantitative inductively coupled plasma–mass spectrometry was determined by analyzing copper samples in duplicate during the instrumental run and determining the relative percent differences, which ranged from 0.1 to 7.9% for all analysis duplicates. Recoveries of copper spiked into water samples and analyzed by quantitative inductively coupled plasma–mass spectrometry ranged from 85 to 107%. Measured copper concentrations were not adjusted for recovery efficiency. Instrumental detection limit was <0.016 μg/L, and the method detection limit was <0.2 μg/L.

Mean values of dissolved oxygen, pH, conductivity, hardness, and alkalinity for each toxicity test were calculated based on measures of the control, medium-, and high-exposure concentrations at the beginning and the end of the 48- and 96-h tests, or on days 0, 4, and 10 of the 10-d tests. Percentage of the nominal concentration for each measured concentration in a toxicity test was calculated. An arithmetic mean for each test was calculated by averaging all percentages of nominal concentrations for all measured concentrations in the test. Median effective concentrations (EC50s) were calculated based on nominal concentrations, using a Probit model when appropriate and either a Spearman-Karber or trimmed Spearman-Karber method otherwise [23] with TOXSTAT software [24]. Growth effects in 10-d juvenile tests were determined by calculating the lowest-observed-effect concentration for growth with analysis of variance and Dunnett's *t*-test [24,25]. The level of statistical significance was set at $p \leq 0.05$. To identify the effects on growth that occurred at concentrations less than those affecting survival, concentrations above the EC50 for survival were excluded from statistical analysis for growth of juvenile mussels in 10-d exposures.

RESULTS AND DISCUSSION

Water quality

Mean water quality characteristics for copper, ammonia, or chlorine tests with glochidia, juvenile mussels, or the commonly tested species are summarized in Table 1 (individual measurements for each test are reported in a USGS quarterly project summary, USGS, unpublished data). Means of hardness and alkalinity of the dilution water typically were within

Table 2. Percent nominal concentrations of measured toxicant concentrations on exposure days 0, 2, 4, or 10 in toxicity tests with glochidia, juvenile mussels, and commonly tested species. Values are means (± standard deviation) for multiple tests with each toxicant

Test organism	Toxicant	Percentage of nominal toxicant concentration			
		0 d	2 d	4 d	10 d
Glochidia	Copper	109 ± 17 (17)[a]	99 ± 10 (5)	—[b]	—
	Ammonia	104 ± 11 (19)	44 ± 16 (18)	—	—
	Chlorine	103 ± 6.8 (17)	96 ± 27 (16)	—	—
Juvenile mussels	Copper	107 ± 14 (18)	—	107 ± 13 (17)	124 ± 19 (13)
	Ammonia	104 ± 12 (11)	—	63 ± 7.1 (11)	63 ± 14 (9)
	Chlorine	103 ± 7.3 (8)	—	101 ± 13 (8)	107 ± 7.8 (7)
Commonly tested species	Copper	106 ± 18 (5)	—	—	—
	Ammonia	98 ± 2.9 (5)	38 ± 10 (3)[c]	84 ± 10 (2)[d]	—

[a] Total number of tests in parentheses.
[b] Not applicable.
[c] Values of three 48-h tests with *Ceriodaphnia dubia*, *Daphnia magna*, and *Hyalella azteca*.
[d] Values of two 96-h tests with fathead minnow and rainbow trout.

10% of the range of values for ASTM hard water (hardness 160–180 mg/L as $CaCO_3$ and alkalinity 110–120 mg/L as $CaCO_3$; [16]), whereas mean pH values ranged from 8.3 to 8.5 and were above the listed range of 7.8 to 8.0 in ASTM [16]. Higher pH values of the ASTM reconstituted hard water also have been reported in previous studies [9,10,22,26]. Because the range of water quality values among tests for a single toxicant was relatively low (Table 1), the effects of water hardness or pH on the toxicity results were likely negligible. Dissolved oxygen was above 7.0 mg/L during all tests.

Measured exposure concentrations

Individual measured exposure concentration and the percent nominal concentration of measured concentrations at different concentrations of copper, ammonia, or chlorine for over 100 toxicity tests with glochidia, juvenile mussels, and the commonly tested species are reported in the above-mentioned USGS quarterly project summary (USGS, unpublished data). The percentages of nominal concentrations at different exposure concentrations within a test were similar (within 20% difference) except that some higher percentages of nominal concentrations were observed at the low-test concentrations in some copper or ammonia tests.

Measured copper concentrations were similar to nominal concentrations in the 48-h static exposures and in the 4- or 10-d renewal exposures. Mean percentage of nominal concentration for measured copper concentrations at the beginning of tests was 109% for glochidia tests, 107% for juvenile mussel tests, and 106% for commonly tested species tests (Table 2). Mean percentage of nominal copper concentration was 99% at the end of 48-h glochidia tests and was 107% on day 4 and 124% on day 10 of the juvenile mussel tests (Table 2). The higher percentage of nominal concentrations at the end of the 10-d tests was consistent with higher values of hardness, alkalinity, and conductivity of dilution water measured in copper tests at the end of 10-d tests (Table 1), which might be the results of evaporation of some of the water from the small 50-ml beakers during 10-d exposures despite renewal of about 75% of the test water every other day. Covering test chambers might have reduced evaporation during these 10-d exposures.

Measured total ammonia concentrations were similar to nominal concentrations at the beginning of tests but decreased during exposure periods. At the beginning of the tests, mean percentages of nominal concentration for measured total ammonia concentrations was 104% for both glochidia and juvenile mussel tests and 98% for the tests with commonly tested species (Table 2). The mean percentage of nominal concentrations decreased to 44% in glochidia tests and 38% in commonly tested invertebrate tests at the end of 48-h exposures, and decreased to 63% in juvenile mussel tests on test days 4 and 10 (Table 2). However, the mean percentage of nominal concentration was 84% in two fish tests at the end of 96-h exposures. These results indicate that the renewal of test solution at intervals of 48 h may not be sufficient to maintain total ammonia concentrations in small test chambers with only 30 ml of test solution in tests with mussels and commonly tested invertebrates, but may maintain more constant total ammonia concentrations in larger chambers with about 500 to 2,000 ml of test solution in fish tests. Perhaps this was due to less volatilization of ammonia because of a lower surface-to-volume ratio in the larger chambers.

Measured concentrations of chlorine were nearly equal to the nominal concentrations throughout 2-, 4-, and 10-d exposure periods. Mean percentage of nominal concentrations ranged from 96 to 107% (Table 2).

These results indicate that measured concentrations of copper and chlorine generally were constant and similar to the nominal concentrations throughout the exposures, whereas total ammonia concentrations were near the nominal concentrations at the beginning of tests, but declined over exposure time. Therefore, using the nominal concentrations to calculate EC50s of copper and chlorine in this study was appropriate, whereas using nominal total ammonia concentrations to calculate EC50s might underestimate ammonia toxicity. The EC50s calculated from nominal ammonia concentrations could be adjusted to measured ammonia concentrations based on mean percentage of nominal concentrations at different exposure times (Table 2). However, Wang et al. [10] observed similar 96-h EC50s in ammonia toxicity tests conducted concurrently with two-month-old juvenile mussels under static-renewal conditions (i.e., fluctuating total ammonia concentration) and flow-through conditions (i.e., relatively constant ammonia concentrations). Hence, the decline in total ammonia concentrations in test chambers over the 96-h exposures might not have substantially influenced test results and expressing the EC50s based on the starting ammonia concentrations may reasonably represent the effect concentrations observed in the 96-h static exposures compared to the EC50s based on an average exposure concentration measured at the beginning and the end of the 96-h exposures.

Toxicity tests with glochidia

Among 58 tests conducted with glochidia from nine mussel species, the majority of tests were completed with >90% control survival over a 48-h exposure period. However, over 90% control survival was observed only at 6 h in tests with oyster mussel and scaleshell (two federally endangered species; control survival of glochidia of the two species was below 90% after 24 h). Therefore, only 6-h EC50s were reported for the two species with a relatively short longevity (Table 3) as recommended by ASTM [11]. The 24- or 48-h EC50s for copper, total ammonia, or chlorine were generally similar among the species, with some exceptions (Table 3). The glochidia of ellipse typically had lower EC50s for all three toxicants, whereas the glochidia of wavy-rayed lampmussel had lower EC50s for copper and total ammonia but not chlorine (Table 3). In contrast, the glochidia of dwarf wedgemussel, a federally endangered species, always had the highest EC50s for all three toxicants among the species (Table 3). Results indicate that the sensitivity of glochidia was similar among most species tested, although some species were more sensitive to all three toxicants and other species were more sensitive to one toxicant but less sensitive to another.

The EC50s in each test typically decreased over the exposure periods of 6, 24, and 48 h (Table 3). The mean 48-h EC50s (copper 23 μg/L, total ammonia 7.8 mg N/L, chorine 63 μg/L; Table 3) were significantly lower than the mean 24-h EC50s (copper 39 μg/L, total ammonia 10 mg N/L, and chlorine 83 μg/L; paired *t*-test, $p < 0.001$), indicating that a longer exposure (e.g., from 24–48 h) resulted in lower EC50s.

Glochidia tests generally have been conducted for 24 or 48 h in previous studies [6]. The ASTM [11] recommended that the duration of a toxicity test conducted with glochidia should be up to 24 h because the time between the release of glochidia from females of some species to attachment on the gill of a host fish may be only a few hours. However, many anodontinae species release glochidia into the water column, and the released glochidia can remain viable for days before infesting host fish [6,11]. For these species, a prolonged glochidia test would have ecological relevance, which has been recognized in ASTM [11]. The results of the present study provide evidence of the feasibility and sensitivity of toxicity tests conducted with glochidia for 48 h.

Toxicity tests with juvenile mussels

The EC50s for copper and total ammonia in each test with newly transformed juveniles generally decreased as exposure periods increased (2, 4, and 10 d; Table 4), and average EC50s for copper or ammonia for various species were significantly different among the three exposure periods (Tukey's test, $p < 0.05$). The EC50s for chlorine were greater than the highest exposure concentrations of 100 μg/L on test days 2 and 4, but were substantially lower on day 10 (Table 4). The 10-d EC50s for the three toxicants generally decreased more than 50%, compared to 96-h EC50s (Table 4). These results indicate that a 96-h exposure duration is necessary to estimate acute toxicity of a toxicant to newly transformed juvenile mussels, and that a 10-d exposure duration may provide useful information on longer-term effects of copper, ammonia, or chlorine. Chronic 28-d toxicity tests with juvenile fatmucket and rainbow mussels have been completed at our laboratory using copper and ammonia as toxicants [10]. Compared to the 28-d chronic effect concentrations for survival and growth of juvenile fatmucket (8.5 μg Cu/L and 0.37 mg total ammonia N/L [10]) and rainbow mussels (5.1 μg Cu/L and <0.40 mg total ammonia N/L [10]), the 10-d EC50s for survival of the two species in the present study were equal or up to three times higher in copper tests (8.1–32 μg/L for fatmucket and 8.6–13 μg/L for rainbow mussel; Table 4), and were 2 to 10 times higher in the ammonia tests (1.2–2.7 mg N/L for fatmucket and 0.8–4.3 mg N/L for rainbow mussel; Table 4). Although evaluating survival of juvenile mussels in 10-d nonfeeding toxicity tests may provide some information on longer-term effects, a prolonged test period (e.g., 28 d) and measurement of sublethal endpoints (e.g., growth) are needed to better estimate chronic toxicity of copper or ammonia to juvenile mussels [10].

A similar pattern of EC50s decreasing with increasing exposure times was observed in two-month-old juvenile mussels (Table 4). However, the 4-d copper EC50s for the two-month-old juveniles were higher than those for newly transformed juveniles. For example, the 96-h copper EC50s for two-month-old fatmucket (32 and 60 μg/L) and rainbow mussels (24 and 33 μg/L) were higher than those for newly transformed fatmucket (18–25 μg/L) and rainbow mussels (17 μg/L), indicating that older juvenile mussels may be somewhat less sensitive to copper. In contrast, the total ammonia EC50s for two-month-old juveniles were equal to or lower than those for newly transformed juveniles (Table 4).

In two copper toxicity tests, test organisms were held in clean water for 24 h after the initial 96-h copper exposure. The EC50s for the newly transformed mussels did not change after 24 h in clean water, whereas EC50s for the two-month-old mussels increased at least threefold (Fig. 1). The results indicate that the older juveniles may have avoided exposure to copper in the 96-h tests by temporarily closing their valves. However, similar ammonia EC50s between newly transformed and two-month-old mussels suggests that the older juveniles may not avoid exposure to ammonia. Additional studies are needed to determine why the mussels avoid copper but not ammonia, and further evidence is required to support the hypothesis of avoidance behavior of juvenile mussels. Importantly, acute effect concentrations on juvenile mussels should be expressed as an EC50 rather than as a median lethal concentration given the uncertainty if test organisms are affected behaviorally (i.e., an EC50) or if test organisms are dead (i.e., a median lethal concentration) in acute 96-h exposures. If it is suspected that juvenile mussels are avoiding exposure to a chemical in a toxicity test, it is desirable to conduct a recovery test by placing test mussels into dilution water that does not contain any test chemical for 1 to 2 d after the toxicity test to determine whether these test organisms are alive or dead [11].

Mean EC50s for copper and total ammonia were similar between 48-h tests with glochidia (23 μg Cu/L, 7.8 mg N/L; Table 3) and 96-h tests with newly transformed juvenile mussels (22 μg Cu/L, 9.3 mg N/L; Table 4). However, 48-h EC50s for chlorine in glochidia tests (Table 3) were generally lower than 96-h EC50s in juvenile mussel tests (Table 4). The results indicate that glochidia and newly transformed juvenile mussels had similar sensitivities to copper and total ammonia, whereas glochidia were more sensitive to chlorine than juvenile mussels. When it is difficult to obtain juvenile mussels for toxicity testing due to limited number of female mussels available (such as endangered species) or due to the lack of techniques for culturing juvenile mussels of a particular species (such as a host fish is unknown), a 48-h glochidia test may be useful to assess the relative sensitivity of a particular species to copper, ammonia, or chlorine.

Table 3. Median effective concentrations (EC50s) for survival of glochidia in copper, ammonia, or chlorine toxicity tests. Numbers in parentheses represent 95% confidence intervals

Species	Copper EC50 (μg/L)			Ammonia EC50 (mg N/L)			Chlorine EC50 (μg/L)		
	6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h
Dwarf wedgemussel[a]	>100	>100	86 (81–91)	>16	>16	15 (14–16)	>100	>100	>100
Ellipse	18 (17–19)	10 (9.3–11)	8.6 (8.3–8.9)	>16	5.0 (4.8–5.4)	2.7 (2.5–2.9)	>100	76 (74–78)	44 (42–46)
Fatmucket[b]	43 (42–45)	36 (34–38)	23 (21–24)	13 (13–14)	11 (11–12)	9.1 (8.8–9.5)	>100	47 (45–49)	34 (32–35)
Fatmucket[b]	55 (53–57)	29 (27–30)	15 (14–15)	16 (15–16)	9.8 (9.5–10)	8.0 (7.7–8.3)	88 (84–93)	81 (78–85)	60 (57–63)
Fatmucket	77 (71–82)	42 (40–45)	28 (27–30)	>16	15 (14–16)	12 (12–13)	>100	90 (82–99)	79 (75–84)
Fatmucket	42 (40–44)	31 (29–32)	20 (19–21)	>16	13 (13–14)	12 (11–12)	>100	67 (65–69)	57 (54–60)
Fatmucket	87 (80–93)	38 (36–40)	31 (30–32)	>16	>16	13 (12–13)	>100	>100	>100
Fatmucket	NV[c]	41 (39–43)	23 (22–24)	—[d]	—	—	—	—	—
Fatmucket	48 (46–50)	33 (32–35)	17 (16–18)	12 (12–13)	7.3 (7.0–7.7)	5.2 (4.9–5.4)	>100	82 (78–86)	70 (66–74)
Mucket[b]	>100	59 (57–62)	23 (22–24)	14 (13–15)	6.9 (6.7–7.2)	5.9 (5.7–6.1)	>100	74 (71–77)	40 (38–42)
Mucket	83 (79–88)	35 (34–36)	20 (19–21)	15 (14–16)	9.1 (8.7–9.6)	4.7 (4.5–4.9)	>100	>100	52 (48–55)
Mucket	62 (55–69)	66 (62–69)	32 (31–33)	12 (11–13)	5.7 (5.4–6.0)	3.4 (3.3–3.6)	>100	>100	48 (46–51)
Mucket	>100	53 (51–55)	31 (30–32)	15 (13–18)	10 (10–11)	7.6 (7.3–8.0)	—	—	—
Neosho mucket	>100	41 (38–44)	19 (18–20)	14 (13–14)	8.3 (7.9–8.7)	5.7 (5.5–6.0)	>100	>100	53 (50–57)
Oyster mussel[a]	47 (45–49)	NR[e]	NR	5.0 (4.5–5.4)	NR	NR	23 (20–26)	NR	NR
Oyster mussel[a]	22 (21–24)	NR	NR	3.4 (3.1–3.8)	NR	NR	—	—	—
Pink mucket[a]	>100	34 (32–35)	13 (12–13)	—	—	—	—	—	—
Pink papershell	65 (62–68)	14 (13–15)	13 (12–13)	>16	>16	7.3 (7.0–7.6)	>100	58 (55–61)	25 (24–27)
Rainbow mussel[b]	>100	37 (35–39)	22 (21–23)	14 (13–15)	12 (11–12)	9.2 (8.8–9.6)	>50	>50	43 (41–45)
Scaleshell[ab]	21 (20–22)	NR	—	—	—	—	—	—	—
Wavy-rayed lampmussel	>100	18 (17–19)	7.3 (6.9–7.6)	>16	8.7 (8.2–9.3)	6.3 (5.9–6.7)	>100	>100	>100
Wavy-rayed lampmussel	58 (56–61)	16 (15–16)	6.5 (5.9–7.1)	13 (12–13)	6.2 (5.8–6.6)	4.3 (4.0–4.7)	>100	>100	>100
Mean[f] ± standard deviation	68 ± 29	39 ± 21	23 ± 17	14 ± 3.6	10 ± 3.7	7.8 ± 3.6	92 ± 22	83 ± 19	63 ± 26

[a] Federally endangered species.
[b] No water sample was taken for copper analysis in this test.
[c] NV = No value because survival was not determined at that time.
[d] Not applicable.
[e] NR = Not report because control survival was below 90%.
[f] EC50 values greater than the highest exposure concentrations (e.g., >100 μg/L) are considered as estimated EC50s (e.g., 100 μg/L) for the calculation of means.

Table 4. Median effective concentrations (EC50s) for survival of juvenile mussels in copper, ammonia, or chlorine toxicity tests. Numbers in parentheses represent 95% confidence intervals. Asterisk (*) indicates the lowest-observed-effect concentration for shell length was below the EC50 for survival

	Copper EC50 (μg/L)			Ammonia EC50 (mg N/L)			Chlorine EC50 (μg/L)		
Species	2 d	4 d	10 d	2 d	4 d	10 d	2 d	4 d	10 d
Newly transformed juveniles									
Fatmucket	29 (23–36)	18 (15–22)	8.1 (7.3–8.9)	>16	10 (8.7–12)	2.7 (2.4–3.1)	>100	>100	56 (42–75)
Fatmucket	35 (30–42)	20 (18–24)	—[a]	—	—	—	—	—	—
Fatmucket	52 (37–72)	23 (19–27)	—	—	—	—	—	—	—
Fatmucket	34 (27–42)	21 (18–25)	—	—	—	—	—	—	—
Fatmucket	45 (37–55)	25 (20–30)	—	—	—	—	—	—	—
Neosho mucket	60 (49–72)	43 (31–61)	8.8 (6.3–12)*	12.1 (11–14)	11.1 (11–12)	3.6 (3.0–4.4)	—	—	—
Neosho mucket	37 (28–49)	23 (19–27)	8.7 (7.6–10)	>16	11 (8.1–14)	4.5 (3.8–5.3)	>100	>100	54 (45–65)
Oyster mussel[b]	19 (15–24)	17 (13–21)	15 (11–19)*	9.2 (7.9–11)	5.7 (4.5–7.2)	2.5 (2.1–2.9)*	>100	>100	29 (17–51)
Oyster mussel[b]	10 (8.0–13)	6.8 (4.8–9.7)	5.9 (4.5–7.7)	—	—	—	—	—	—
Rainbow mussel	37 (26–51)	17 (16–19)	12 (10–15)	12.9 (10–16)	6.3 (4.3–9.2)	4.3 (3.1–6.1)	>100	>100	16 (11–24)
Scaleshell[b]	29 (23–37)	22 (19–27)	14 (11–19)	—	—	—	—	—	—
Wavy-rayed lampmussel	56 (45–71)	21 (16–28)	6.7 (5.2–8.6)	—	—	—	—	—	—
Wavy-rayed lampmussel	73 (64–82)	25 (21–31)	4.8 (4.2–5.4)	>16	7.4 (5.5–9.9)	1.7 (1.1–2.7)	—	—	—
Mean[c] ± SD[d]	40 ± 17	22 ± 8.0	9.3 ± 3.6	14 ± 2.8	8.6 ± 2.4	3.2 ± 1.1	>100	>100	39 ± 19
2-month-old juveniles									
Fatmucket	23 (17–30)	32 (22–47)	—	—	—	—	—	—	—
Fatmucket	>100	60 (46–78)	32 (25–40)	8.1 (6.7–10)	4.9 (4.0–6.0)	1.5 (1.2–1.8)	>100	>100	97 (66–142)
Pink mucket[b]	38 (29–50)	37 (27–50)	14 (11–17)	6.1 (5.3–6.9)	2.3 (2.0–2.6)	1.2 (1.0–1.6)	—	—	—
Rainbow mussel	45 (33–60)	33 (24–45)	13 (10–16)	6.8 (5.4–8.5)	3.0 (2.6–3.4)	0.8 (0.7–0.9)	80 (62–100)	68 (58–79)	22 (13–38)
Rainbow mussel	20 (16–24)	24 (19–31)	8.6 (7.1–10)	9.5 (7.1–13)	10.7 (8.0–14)	1.3 (1.0–1.6)	>100	>100	>100
Mean ± SD	45 ± 32	37 ± 14	17 ± 10	7.6 ± 1.5	5.2 ± 3.8	1.2 ± 0.3	93 ± 12	89 ± 18	73 ± 44

[a] Not applicable.
[b] Federally endangered species.
[c] EC50 values greater than the highest exposure concentrations (e.g., >100 μg/L) are considered as estimated EC50s (e.g., 100 μg/L) for the calculation of means.
[d] SD = Standard deviation.

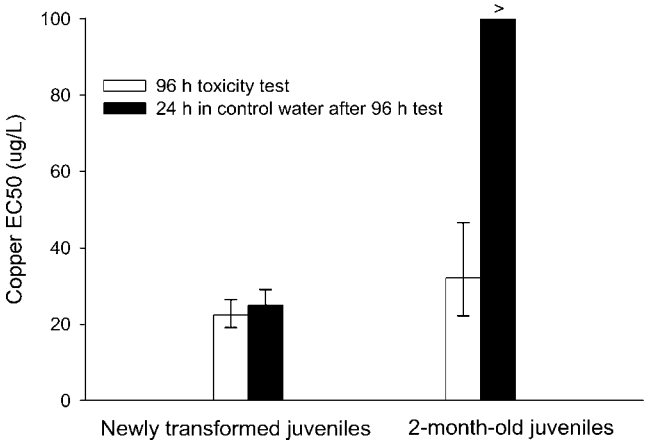

Fig. 1. Median effective concentrations (EC50s) of copper for newly transformed and two-month-old juvenile mussels (fatmucket, *Lampsilis siliquoidea*) at the end of 96-h toxicity tests and after a 24-h holding period in control water following the 96-h toxicity tests. Error bar represents 95% confidence intervals. The symbol (>) above a bar indicates that the EC50 value was greater than the highest test concentration.

Significant growth effects were observed in only three of thirty 10-d tests with copper, total ammonia, and chlorine (Table 4). The lowest-observed-effect concentration for growth in the three tests was 6.25 μg Cu/L for Neosho mucket (5.7% shell length reduction compared to control) and 12 μg Cu/L (14% shell length reduction) and 2.0 mg total ammonia N/L (8.1% shell length reduction) for oyster mussels. Although this present study was not specifically designed to estimate sublethal effect concentrations, results of these tests indicate that growth of juvenile mussels in the 10-d nonfeeding tests did not provide a consistent indicator of an effect beyond measuring survival alone. In 96-h and 10-d nonfeeding ammonia toxicity tests with juvenile mussels (plain pocketbook, *Lampsilis cardium* and Higgins eye, *L. higginsii*), Newton et al. [27] and Newton and Bartsch [28] also found that juvenile mussels grew poorly and did not exhibit a dose-response relation in water-only tests, whereas growth was a sensitive endpoint in sediment tests (i.e., EC50s for growth were lower than median lethal concentrations for survival). The juvenile mussels in the sediment tests might have received substantial benefit from the presence of sediment, which provides the food for juvenile mussels [28]. These results indicate that the growth of juvenile mussels in water-only, nonfeeding toxicity test may not be a sensitive endpoint, and that feeding is necessary in longer-term toxicity tests to evaluate the potential effect on growth.

Sensitivity comparison between early life stages of mussels and commonly tested species

Among the five commonly tested species, *C. dubia* and rainbow trout were more sensitive to copper, and fathead minnows were more sensitive to total ammonia (Fig. 2). The EC50s were within the range of EC50s reported by others [20–22].

Compared to EC50s for the three crustacean species (48-h exposure) and two fish species (96-h exposure), 24-h or 48-h EC50s for glochidia and 96-h EC50s for juvenile mussels were lower in most cases (Fig. 2), indicating that early life stages of mussels were more sensitive than the commonly tested species. Exceptions to this generalization included the relatively low copper EC50 for *C. dubia* and higher copper or ammonia EC50s for glochidia of dwarf wedgemussel. Furthermore, EC50s for glochidia and juvenile mussels in most cases were at or below the final acute value ([FAV], an estimate of the fifth percentile of the distribution of the genus mean acute values for all the tested genera) in the datasets used to derive the U.S. EPA 1996 hardness-dependent acute WQC for copper [20] or the 1999 pH-dependent WQC for ammonia [21] (Fig. 2).

These results indicate that acute copper and ammonia toxicity data for commonly tested species may not be protective of freshwater mussels, and that the U.S. EPA 1996 acute WQC for copper and 1999 acute WQC for ammonia based on a dataset that does not include mussel toxicity data may not adequately protect the early life stages of freshwater mussels from acute exposures to copper or ammonia. Therefore, mussel toxicity data generated in tests that meet ASTM criteria for acceptable toxicity tests with mussels [11] should be considered in a reversion to the WQC for copper and ammonia. The U.S. EPA published a 2007 revised WQC for copper [29], which is based on the biotic ligand model (BLM) and dependent on a number of water quality parameters (e.g., dissolved organic carbon, pH, temperature, major cations, and anions) and includes mussel toxicity data for only two species (pheasantshell, *Actinonaias pectorosa*, and paper pondshell, *Utterbackia imbecillis*). The BLM-based FAV is 12.5 μg/L for the reconstituted ASTM hard water used in the present study (20°C, pH 8.3, and the estimated water quality parameters based on the specified formulas [29]) and is about four times lower than the hardness-based copper criterion [24] in the water tested. March et al. [13] determined the BLM-normalized EC50s for mussels reported in the present study, added these data to the dataset used to update the 2007 copper criteria [29], and recalculated the FAV. March et al. [13] concluded that including the mussel toxicity data from the present study in the recalculation of the BLM-based FAV would lower the copper FAV. Additional studies are needed to determine how dissolved organic carbon and other water quality parameters, which are included in the calculation of BLM-based WQC, influence the acute toxicity of copper to mussels.

In contrast to copper and ammonia, the 24- or 48-h chlorine EC50s for glochidia and 96-h EC50s for juvenile mussels were above the FAV for chlorine (38 μg/L [20]; Tables 3 and 4). These chlorine EC50s are within the range of the acute effect concentrations for fish and cladocerans tested in other studies (e.g., 40–110 μg/L for rainbow trout, 82–130 μg/L for fathead minnows, and 7–45 μg/L for *D. magna* [30]). Thus, the commonly tested species and acute WQC likely would be protective of these mussel species tested in the acute exposures to chlorine.

Comparison of mussel toxicity data from the present and previous tests

Toxicity data generated from this study have been included in the mussel toxicity datasets used to derive estimates of chemical concentrations that likely would not be harmful in acute and chronic exposures and to assess the protectiveness of current U.S. EPA WQC to freshwater mussels [5,13,14]. Specifically, March et al. [13] summarized copper toxicity data from mussel tests including toxicity tests in the present study. When normalized to a water hardness of 50 mg/L as $CaCO_3$, the majority of the results of copper toxicity tests with freshwater mussels in the present study were within the range of

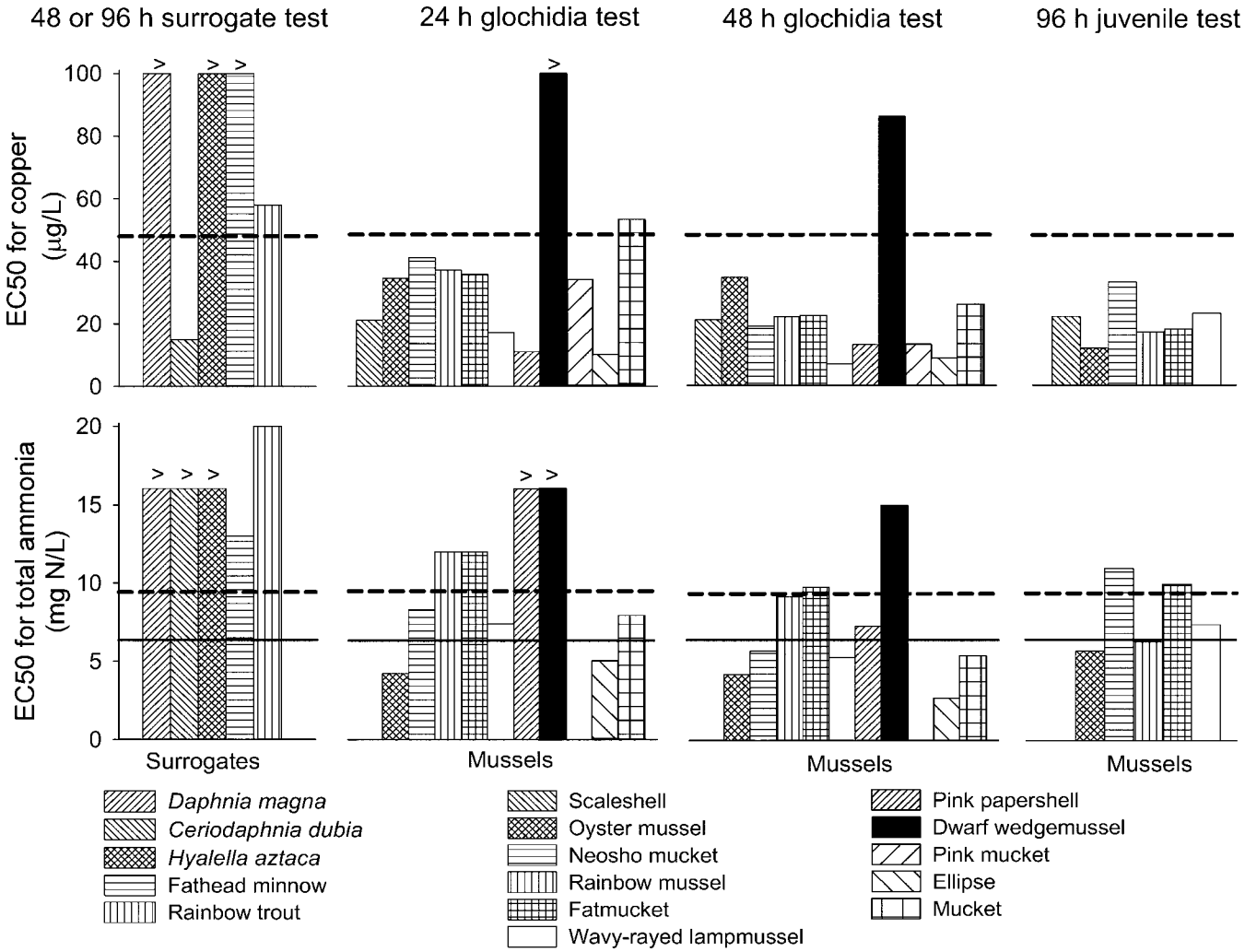

Fig. 2. Acute 48- or 96-h median effective concentrations (EC50s) for copper and total ammonia in tests with commonly tested crustacean and fish species, the 24- or 48-h EC50s for glochidia (except 6-h EC50s for scaleshell and oyster mussels because the control survival of these species was below 90% after 24-h exposures), and the 96-h EC50s for newly transformed juvenile mussels. The mean EC50 was used if multiple tests with one species were conducted. Dashed line represents the final acute value (FAV) used to derive the U.S. Environmental Protection Agency 1996 hardness-dependent acute water quality criterion (WQC) for copper at hardness 180 mg/L or the 1999 pH-dependent acute WQC for ammonia based on the dataset without salmonid fish at pH 8.3. Solid line represents the FAV in acute WQC for ammonia with data for salmonid fish. The symbol (>) above a bar indicates that the EC50 value was greater than the highest test concentration.

EC50s for mussels reported by others (Table 1 in March et al. [13]). However, three species (ellipse, pink papershell, and oyster mussels) tested in the present study are much more sensitive to copper (genus mean acute values ranging from 3.0–4.3 µg/L [13]) compared to the other 17 species (genus mean acute values ranging from 14–57 µg/L; [13]).

The toxicity of ammonia varies with pH, which influences the fraction of total ammonia that exists in the ionized, and more toxic, unionized states. Ammonia toxicity data for freshwater mussels have been expressed by others as unionized ammonia (NH_3), total ammonia as nitrogen ($NH_3 + NH_4^+$-N), and total ammonia as nitrogen normalized to a constant pH [21]. Tables 1, 3, and 4 provide the data to compare the results of the present study to those of other studies for each of these ways of expressing ammonia toxicity data. If ammonia concentrations are expressed as either unionized ammonia or as total ammonia, the results of the present study are typically within the previously reported ranges [5,27,28]. An exception was dwarf wedgemussel, which was the least sensitive mussel in the present study (acute EC50s were >27 mg N/L when normalized to pH 8.0) and was less sensitive than any other species tested by others (EC50s ranged from 0.74–20 mg N/L at pH 8.0 [5,27,28]). A study recently was conducted at our laboratory to evaluate the influence of pH on the toxicity of ammonia to juvenile fatmucket in 96-h exposures and demonstrated that the EC50s for total ammonia decreased with increasing pH, and the pH-ammonia relationship for the juvenile mussels was similar to the generic pH-ammonia relationship reported in U.S. EPA WQC for ammonia (N. Wang, unpublished data). Therefore, the generic relationship in the current WQC could be used to accurately predict acute ammonia toxicity to juvenile mussels across a broad range of pH.

Chlorine toxicity data for freshwater mussels are limited; few acute tests have been conducted with glochidia of mussels [12,31]. Compared to the 48-h EC50s for chlorine from these previous static-renewal tests (80–260 µg/L for five mussel species), the EC50s in the flow-through tests of the present study were lower (<80 µg/L in most cases; Table 3). Specifically, the 48-h EC50 for rainbow mussels was 43 µg/L in the flow-through test (Table 3), but was 260 µg/L in the static test

conducted by Valenti et al. [12]. Further study is needed to determine the influence of test conditions (static renewal vs. flow through) on chlorine EC50s.

CONCLUSION

In conclusion, the glochidia and newly transformed juvenile mussels tested in the present study, in most cases, were more sensitive to copper and total ammonia than the commonly tested cladoceran, amphipod, and fish species, and the EC50s of copper and total ammonia for the early life stages of mussels often were at or below the FAVs used to derive the U.S. EPA 1996 acute WQC for copper and 1999 acute WQC for ammonia, whereas chlorine EC50s for the mussel species generally were above the FAV in the acute WQC for chlorine. The results indicate that the early life-stages of freshwater mussels are acutely sensitive to copper and ammonia, but relatively tolerant to chlorine. The U.S. EPA 1996 acute WQC for copper and 1999 acute WQC for ammonia may not be adequately protective of the mussel species tested. For the water tested in the present study, the 2007 BLM-based acute WQC for copper is about four times lower than the 1996 hardness-based WQC. However, including additional mussel toxicity data in the recalculation of the new BLM-based WQC for copper would further lower the WQC.

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