

WATER QUALITY GUIDANCE FOR PROTECTION OF FRESHWATER MUSSELS  
(UNIONIDAE) FROM AMMONIA EXPOSURE

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(Received 18 July 2002; Accepted 3 February 2003)

**Abstract**—Ammonia toxicity data for freshwater mussels (Unionidae), a significantly imperiled taxa, were used to derive estimates of concentrations that would not likely be harmful in acute and chronic exposures and to assess the protectiveness of current U.S. Environmental Protection Agency (U.S. EPA) water quality criteria to this family of organisms. Thirty acute (24–96-h) median lethal concentrations (LC50s), covering 10 species in eight unionid genera, were used to calculate genus mean acute values (GMAVs) ranging from 2.56 to 8.97 mg/L total ammonia as N at pH 8. Freshwater mussels are at the sensitive end of the range when added to the GMAVs from the database used to derive the U.S. EPA criteria maximum concentration (CMC). We derived two estimates of acute exposure water quality guidance for the protection of freshwater mussels (CMC<sub>FM</sub>) by a recalculation of the CMC after adding freshwater mussel GMAVs to the U.S. EPA data set. The CMC<sub>FM</sub>s of 1.75 and 2.50 mg/L total ammonia as N at pH 8 average 60% less than the U.S. EPA CMC of 5.62 mg/L total ammonia as N at pH 8 for application when salmonids are present. These values average about 75% less than the CMC for application when salmonids are absent. No chronic ammonia exposure data existed for unionids. Thus, we applied a range of estimated acute:chronic ratios to the acute toxicity data set, expanded with the freshwater mussel GMAVs, to estimate continuous ammonia concentrations that may be protective of freshwater mussels. These estimates ranged from 0.3 to 1.0 mg/L total ammonia as N at pH 8, about 20 to 75% less than the U.S. EPA criteria continuous concentration (CCC) of 1.24 mg/L total ammonia as N at pH 8 and 25°C. The current numeric criteria for ammonia may not be protective of mussels, more than half of whose nearly 300 species are in decline in North America. While the CMC<sub>FM</sub> and CCC<sub>FM</sub> are not equivalent to revised U.S. EPA criteria, they are offered as interim guidance for the protection of freshwater mussels.

**Keywords**—Ammonia Unionidae Freshwater mussels Water quality criteria

## INTRODUCTION

Many factors are cited in the decline of freshwater mussel (Unionidae) populations in North America and for the listing of greater than 70% of native unionids as endangered, threatened, or of special concern [1,2]. Habitat alteration, introduction of exotic species, overutilization, disease, predation, and pollution are considered causal or contributing factors to the decline of mussel populations in many areas of the United States [3–5]. Toxic substances were among the stressors frequently cited as limiting factors for freshwater mussels in a recent survey of experts for this taxa [6]. While mussels appear relatively tolerant to some organic solvents and pesticides [7,8], published toxicological data also indicate that early life stages of freshwater mussels are among the most sensitive aquatic organisms tested for impacts of inorganic chemicals, including chlorine [9], metals [10,11], and ammonia [9,12].

In addition to freshwater mussels' apparent sensitivity to ammonia, this compound is of particular interest as a potential limiting factor in their survival and recovery because it is a common pollutant. Ammonia is a natural degradation product of nitrogenous organic matter; significant sources of enrichment include industrial waste, municipal wastewater treatment plants, and agricultural runoff (animal wastes as well as chemical fertilizers). Sediment pore-water concentrations of am-

monia typically exceed those of overlying surface water [13], thereby placing the infaunal (burrowed in sediment) freshwater mussels in the environmental compartment where ammonia concentrations are frequently elevated. For these reasons, ammonia merits priority attention among the many chemicals to evaluate for effects on mussels. The U.S. EPA water quality criteria for ammonia were derived from a toxicity database created prior to the relatively recent availability of data for freshwater mussels. We undertook an evaluation of ammonia toxicity data for freshwater mussels with two goals: to derive estimates of the concentrations that would not be harmful in acute and chronic exposures and to compare those concentrations to U.S. EPA water quality criteria [14] to assess the protectiveness of existing guidance.

## METHODS

*Development of the ammonia toxicity database*

To compile available ammonia toxicity data for freshwater mussels, we reviewed the data set used in the recently revised U.S. EPA water quality criteria document for ammonia [14], searched the Toxline® (U.S. National Library of Medicine, Bethesda, MD) and AQUIRE (U.S. Environmental Protection Agency, Duluth, MN) databases, and queried researchers familiar to us with experience in mussel toxicity testing. Data from our own laboratories were also used.

Because no U.S. EPA or American Society for Testing and Materials standard methods exist that have specifically been

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developed for freshwater mussel toxicity tests, we evaluated data from all sources for acceptability using guidance modified from the U.S. EPA [15]. Studies that demonstrated acceptable survival in control treatments ( $\geq 80\%$ ) used measured rather than nominal values for ammonia test concentrations, and documented test water pH and temperature to allow calculation of total and un-ionized ammonia concentrations were deemed acceptable and were used in our analysis.

Data generated by our laboratories generally followed protocols published by us for mussel toxicity tests with other compounds [8]. Static toxicity tests were 24- to 96-h exposures with glochidia or juvenile stages of mussels. Tests were conducted in soft or moderately hard reconstituted water [16]. Exposures consisted of five ammonium chloride concentrations tested in replicates of three to six, with 50 to 100 glochidia or 10 to 20 juvenile mussels per replicate, depending on availability of organisms. A dilution water control was always included for each species. Photoperiod was 16:8 h light:dark; test organisms were not fed during the exposures. Dissolved oxygen, temperature, and pH were measured on each batch of reconstituted water before the start of the tests. Median lethal concentrations were calculated for all toxicity tests with the trimmed Spearman-Kärber method [16]. We measured total ammonia with an ion specific electrode using U.S. EPA Method 350.3 [17], with a lower detection level of 0.1 mg/L and an accuracy of  $\pm 20\%$  over the concentration ranges reported.

#### Summary of ammonia toxicity to freshwater mussels

The toxicity of ammonia varies with temperature and pH (which influence the fraction of total ammonia that exists in the ionized and more toxic un-ionized states). Recommended water quality criteria for ammonia have been presented as un-ionized ammonia ( $\text{NH}_3$ ) [18] and as total ammonia as nitrogen ( $\text{NH}_3 + \text{NH}_4^+ - \text{N}$ ) [14]. We used the original studies' reported total ammonia LC50s, if available. All reported un-ionized ammonia LC50s were converted to total ammonia as nitrogen using the reported temperature and pH data and a published pK relationship [19]; these were also normalized to pH 8 using the equations in appendix 3 of the U.S. EPA revised ammonia criteria document [14]. Concentrations for acute exposures are correspondingly reported as mg/L total ammonia as N at pH 8.

Acute toxicity data were summarized by the methodology described in U.S. EPA numeric water quality criteria guidelines [15]. In the process, available toxicity data are critically reviewed, and geometric mean LC50s for each genus (genus mean acute values [GMAVs]) are calculated. We calculated GMAVs in two ways: combined data for all mussel life stages within the genus (results from toxicity tests with glochidia, juvenile, and adult combined) and combined adult and juvenile data only (excluding the toxicity tests that used glochidia, the appropriateness of which has been questioned in toxicity tests to support water quality criteria development). The GMAVs are used in the demonstration of the sensitivity of mussels to ammonia relative to other invertebrates and fishes in the acute database of the current U.S. EPA ammonia criteria [14].

National water quality criteria in the United States generally consist of two estimated values designed to protect aquatic organisms; these are commonly referred to as the acute and chronic water quality criteria, but, more specifically, they are the criteria maximum concentration (CMC) and criteria continuous concentration (CCC), respectively. The CMC is an

estimate of the highest 1-h average concentration that should not result in unacceptable adverse effects to aquatic organisms; the number is derived from acute toxicity tests (generally 48–96-h exposures) that use lethality or immobilization as the measured endpoints. In deriving the CMC, the GMAVs are ranked from highest (most tolerant) to lowest (most sensitive). A cumulative probability is assigned on the basis of those ranks, and a final acute value (FAV) is derived as the fifth percentile of the GMAVs using an equation that gives equal weight to the GMAVs of the four genera with percentile ranks closest to 0.05. The CMC is calculated by dividing the FAV by two and results in a concentration that should not severely adversely affect too many individuals within the taxa (taxon) that were used for deriving the FAV [15]. Evaluation of acute toxicity data has generally shown that dividing an LC50 or median effective concentration by two provides a concentration equal to a very low effect or no-effect concentration. The process, by definition, is designed to protect populations of 95% of the species tested from adverse effects of short-term exposures to nonbioaccumulative chemicals.

We added the unionid GMAVs to the acute data set for ammonia toxicity in the current U.S. EPA criteria document [14] and used equations from the U.S. EPA water quality criteria methodology [15] to derive acute exposure water quality guidance for protection of freshwater mussels (a recalculation of the FAV and CMC following inclusion of the freshwater mussel GMAVs). We defined outputs from this process as a freshwater mussel FAV ( $\text{FAV}_{\text{FM}}$ ) and a freshwater mussel criteria maximum concentration ( $\text{CMC}_{\text{FM}}$ ). Each of these values was derived in two ways: recalculation with addition of freshwater mussel GMAVs from tests with all mussel life stages and recalculation with addition of GMAVs from tests with adult and juvenile mussels only. Our use of the U.S. EPA water quality criteria equations to derive the  $\text{CMC}_{\text{FM}}$  is not intended to imply the outcome of a recalculated U.S. EPA acute ammonia criteria (which would likely include adding other data generated since the 1985 criteria document); it is intended to suggest general guidance for protection of freshwater mussels from short-term ammonia exposure.

No chronic ammonia exposure data existed for freshwater mussels. Therefore, no ammonia acute:chronic ratios (ACR) for freshwater mussels can be calculated. Without these data, a definitive estimate of the continuous ammonia concentration that would not be harmful to mussels could not be calculated. To evaluate the protectiveness of the current U.S. EPA CCC, we estimated the upper and lower bounds of ACRs (defined here as estimated ACRs, or eACRs) that could be applied to our two  $\text{FAV}_{\text{FM}}$ s. The current U.S. EPA ammonia criteria document [14] reports seven genus mean ACRs for fish and aquatic invertebrates ranging from 1.9 to 10.9, and the maximum value from that range defined our upper-bound eACR. Our lower-bound eACR was derived by evaluating two subchronic freshwater mussel ammonia tests. In juvenile *Lasmigona subviridis* exposures, a geometric mean 4-d LC50 of 3.83 mg/L total ammonia as N at pH 8 and a 15-d LC50 of 0.57 mg/L total ammonia as N at pH 8 have been reported [20]; the ratio of these two LC50s is 6.7. In juvenile *Utterbackia imbecillis* studies [21], a 4-d LC50 of 10 mg/L total ammonia as N at pH 8 and a 9-d no-observed-effect concentration of 2.6 mg/L total ammonia as N at pH 8 can be estimated; the ratio of these concentrations is 3.8. The geometric mean of these two acute to subchronic ratios (5.0) defined our lower-bound eACR.

The two  $FAV_{FM}$ s were each divided by the two eACRs to yield an estimated range of criteria continuous ammonia concentrations that may be protective of freshwater mussels (defined here as a  $CCC_{FM}$ ). These are then compared to the U.S. EPA ammonia CCCs. The estimated  $CCC_{FM}$ s are intended to frame a range of continuous ammonia concentrations that may be protective of freshwater mussels; however, these values are not intended to imply the outcome of a recalculated national CCC because the U.S. EPA did not use ACRs, relying instead on toxicity data from chronic tests.

## RESULTS

Thirty LC50s for unionid acute (24–96-h) ammonia exposures were identified that included 10 species in eight genera (Table 1). Additional relevant ammonia toxicity data were retrieved by our search for the genera *Amblema*, *Utterbackia*, *Cyrtonaias*, and *Toxolasma* [12,21], but acute ( $\leq 96$ -h) LC50s were not reported in these studies, and they were not used in our acute data calculations. Two longer-term (9–15-d) LC50s were also identified (Table 1); these also were not used in our acute data calculations but are included for reference and the calculation of our lower-bound eACR. No chronic ammonia exposure data existed for unionids, and no assessments that incorporated sublethal endpoints were identified by our search.

### *Sensitivity of freshwater mussels to ammonia*

Genus mean acute values for freshwater mussels ranged from 2.56 to 8.97 mg/L total ammonia as N, normalized to pH 8 (Table 2). These values are uniformly at the sensitive end of the range of GMAVs when added to the database used to calculate the U.S. EPA water quality criteria for ammonia [14]. Generally, glochidia were about two to four times more sensitive than juveniles in the three species for which acute data were available for both life stages (Table 1). Excluding tests with glochidia from the GMAV calculations changed their ranks but did not appreciably change the overall apparent ammonia sensitivity of mussels relative to other taxa represented in the database (Table 2).

### *Derivation of water quality guidance for freshwater mussels*

Addition of unionid GMAVs to the acute data set for ammonia toxicity in the current U.S. EPA criteria [14] and use of equations from the U.S. EPA water quality criteria methodology [15] allowed us to recalculate water quality guidance with a data set in which mussels are well represented. Adding data from toxicity tests with all freshwater mussel life stages, the  $FAV_{FM}$  (3.50 mg/L total ammonia as N at pH 8) and corresponding  $CMC_{FM}$  (1.75 mg/L total ammonia as N at pH 8) were about 70% less than the FAV (11.23 mg/L total ammonia as N at pH 8 for use when salmonids are present) and CMC (5.62 mg/L total ammonia as N at pH 8 for use when salmonids are present) used in the calculation of the current acute water quality criteria (Table 3). The  $FAV_{FM}$  and  $CMC_{FM}$  using data from assays with only juvenile and adult freshwater mussels were about 60% less than the FAV and CMC (for use when salmonids are present) from the criteria document (Table 3). The  $CMC_{FM}$ s are even further below (about 75% on average) the CMC for application when salmonids are absent (8.40 mg/L total ammonia as N at pH 8).

The lower- and upper-bound eACRs (5.0 and 10.9, respectively) yielded estimates of  $CCC_{FM}$ s from 0.3 to 0.7 mg/L total ammonia as N at pH 8 and 25°C when applied to our  $FAV_{FM}$

calculated with toxicity data for all mussel life stages. The range increased slightly (0.5–1.0 mg/L total ammonia as N at pH 8 and 25°C) when applied to our  $FAV_{FM}$  calculated following addition of toxicity data from tests with only juvenile and adult mussels. The range of  $CCC_{FM}$ s are between 20 and 75% less than the U.S. EPA CCC of 1.24 mg/L total ammonia as N at pH 8 and 25°C.

## DISCUSSION

Freshwater mussel data are not included in the current database for calculation of the U.S. EPA water quality criteria for ammonia. Recently available data for this family includes eight GMAVs that are less than the GMAVs used to derive the CMC in the U.S. EPA ammonia criteria document, 14 acute LC50s that are less than the CMC, and two LC50s that are less than the final CCC. These data indicate that freshwater mussels are sensitive to ammonia relative to other invertebrates and fishes and that the current numeric criteria may not be protective of mussels, many of whose nearly 300 species are in widespread decline in North America.

### *Issues related to the 1999 revision to the ammonia criteria*

The scope of the 1999 revision to the U.S. EPA water quality criteria for ammonia did not include a comprehensive literature search and review of the most recent acute toxicological data [14], relying instead on the database from the 1985 criteria [18]. The 1985 database was relatively large with 34 genera represented, and this number significantly exceeded the minimum database requirements for derivation of numeric criteria [15]. Also, that revision was initiated in 1996, when much of the data reviewed here [20,22–24] were not available. Our analyses indicate that mussels would rank at the sensitive end of the distribution of GMAVs used to calculate the CMC. The additional data for mussels could drive the derivation of acute criteria, indicating that unionids may be underprotected by the existing CMC. A recent review of methods for deriving numeric water quality criteria notes the importance of determining whether one particular type of organism is more sensitive to a particular pollutant than other kinds of animals [25]. This information helps guide minimum database requirements. While incorporation of additional data requires expenditure of limited resources, our assessment indicates that freshwater mussels should be represented in the criteria database for ammonia. Also, the apparent ammonia sensitivity of genera within this widely distributed family may merit acute criteria revision for reasons of ecological integrity.

Prior to dividing by two to generate the CMC, the FAV can be lowered if the species mean acute value for a recreationally or commercially important species is less (more sensitive) than the calculated FAV [15]. This approach was used in the U.S. EPA ammonia criteria [14] to lower the FAV to the species mean acute value for rainbow trout (*Oncorhynchus mykiss*) prior to deriving the CMC. The applicability of this provision for freshwater mussel protection has not, to our knowledge, been evaluated. Some species of freshwater mussels have commercial importance in the cultured pearl and jewelry industries [5], and this could provide justification for lowering the FAV to a mussel species mean acute value for criteria development. That procedure could result in even lower estimates of acute criteria than the approach we used.

Our use of the U.S. EPA water quality criteria equations [15] to derive the  $CMC_{FM}$  is not intended to imply the outcome of a recalculated U.S. EPA acute ammonia criteria. Modifi-

Table 1. Toxicity data for ammonia and freshwater mussels. Median lethal concentrations (LC50s) reported in original references have been converted to mg/L total ammonia as N, normalized to pH 8

Species Life stage	Duration	Temp.	pH	LC50	Reference
<b>Rainbow</b>					
<i>(Villosa iris)</i>					
Glochidia	24 h	22	8.1	5.17	[9]
Glochidia	24 h	20	7.9	2.42	[22]
Juvenile	96 h	25	8.2	9.09	[22]
Juvenile	96 h	25	8.2	8.21	[22]
Juvenile	96 h	25	8.1	5.64	[22]
Juvenile	96 h	12	7.3	6.60	[24]
Juvenile	96 h	21	7.4	4.23	[24]
<b>Paper pondshell</b>					
<i>(Utterbackia imbecillis)</i>					
Glochidia	48 h	25	8.0	10.42	[20]
Glochidia	48 h	25	8.0	2.38	[20]
Glochidia	48 h	25	8.0	3.15	[20]
Glochidia	48 h	25	8.1	7.46	Manuscript in preparation <sup>a</sup>
Juvenile	96 h	25	8.0	2.73	[20]
Juvenile	96 h	25	8.3	15.46	[20]
Juvenile	96 h	25	8.2	8.00	[20]
Juvenile	96 h	25	8.2	7.13	[20]
Juvenile	96 h	25	8	19.67	Manuscript in preparation <sup>a</sup>
<b>Giant floater</b>					
<i>(Pyganodon grandis)</i>					
Adult	96 h	25	7.5	8.69	[22]
Adult	96 h	25	7.7	9.26	[22]
<b>Green floater</b>					
<i>(Lasmigona subviridis)</i>					
Juvenile	96 h	24	7.7	4.05	[20]
Juvenile	96 h	24	7.7	4.05	[20]
Juvenile	96 h	25	7.9	3.42	[20]
<b>Atlantic pigtoe</b>					
<i>(Fusconaia masoni)</i>					
Glochidia	24 h	25	7.6	2.56	[20]
<b>Pheasantshell</b>					
<i>(Actinonaias pectorosa)</i>					
Glochidia	48 h	25	8	3.76	Manuscript in preparation <sup>a</sup>
Juvenile	96 h	25	8	14.05	Manuscript in preparation <sup>a</sup>
<b>Cumberland moccasinshell</b>					
<i>(Medionidus conradicus)</i>					
Glochidia	48 h	25	8	4.24	Manuscript in preparation <sup>a</sup>
<b>Fatmucket</b>					
<i>(Lampsilis siliquoidea)</i>					
Juvenile	96 h	24	8.3	0.74	[23]
Juvenile	96 h	24	8.3	2.27	[23]
<b>Plain pocketbook</b>					
<i>(Lampsilis cardium)</i>					
Juvenile	96 h	20	8.5	9.97	Personal communication <sup>b</sup>
<b>Wavy-rayed lampmussel</b>					
<i>(Lampsilis fasciola)</i>					
Juvenile	96 h	12	7.8	10.88	[24]
Juvenile	96 h	21	8.0	7.18	[24]
<b>Longer-term tests</b>					
<i>Utterbackia imbecillis</i>					
Juvenile	9 d	24	7.8	3.05	[21]
<i>Lasmigona subviridis</i>					
Juvenile	15 d	22	8.0	0.57	[20]

<sup>a</sup> A. Keller, U.S. Environmental Protection Agency (Jacksonville, FL).

<sup>b</sup> T.J. Newton, (U.S. Geological Survey, Upper Midwest Environmental Sciences Center, LaCrosse, WI, personal communication) (water-only exposure).

cation to the national criteria would likely not be done by adding data solely from a particular family but would rely on all data generated since the 1985 criteria document. In addition, the U.S. EPA may apply additional data quality objectives in evaluating the suitability of data for inclusion in their database. Finally, the addition of eight new genera all in one family and largely occurring in the eastern United States would need to

be evaluated for how this might inappropriately skew a database used to derive a national criteria.

#### *Freshwater mussel toxicity data and guidance*

The absence of standard toxicity testing methods for this taxa helps explain the lack of robust toxicity data and the hesitancy on the part of the U.S. EPA and others to utilize

Table 2. Comparison of freshwater mussel genus mean acute values (GMAVs) for ammonia toxicity to other sensitive taxa from the U.S. Environmental Protection Agency database, listed in order of increasing sensitivity. All GMAVs are in mg/L total ammonia as N, normalized to pH 8

Genus rank	All mussel life stages <sup>a</sup>		Juvenile and adult mussel life stages <sup>b</sup>	
	Genus	GMAV	Genus	GMAV
12	<i>Oncorhynchus</i>	21.95	<i>Salmo</i>	23.74
11	<i>Etheostoma</i>	17.96	<i>Lepomis</i>	23.61
10	<i>Notemigonus</i>	14.67	<i>Oncorhynchus</i>	21.95
9	<i>Prosopium</i>	12.11	<i>Etheostoma</i>	17.96
8	<i>Pyganodon</i> <sup>c</sup>	8.97	<i>Notemigonus</i>	14.67
7	<i>Actinonaias</i> <sup>c</sup>	7.27	<i>Actinonaias</i> <sup>c</sup>	14.05
6	<i>Utterbackia</i> <sup>c</sup>	6.71	<i>Prosopium</i>	12.11
5	<i>Villosa</i> <sup>c</sup>	5.47	<i>Pyganodon</i> <sup>c</sup>	8.97
4	<i>Medionidus</i> <sup>c</sup>	4.24	<i>Utterbackia</i> <sup>c</sup>	8.61
3	<i>Lampsilis</i> <sup>c</sup>	4.20	<i>Villosa</i> <sup>c</sup>	6.52
2	<i>Lasmigona</i> <sup>c</sup>	3.83	<i>Lampsilis</i> <sup>c</sup>	4.20
1	<i>Fusconaia</i> <sup>c</sup>	2.56	<i>Lasmigona</i> <sup>c</sup>	3.83

<sup>a</sup> Freshwater mussel GMAVs presented using data from toxicity tests with adult, juvenile, and glochidial mussels.

<sup>b</sup> Freshwater mussel GMAVs excluding data for toxicity tests with glochidia. Note that no data are available for juvenile or adult life stages of *Medionidus* or *Fusconaia*.

<sup>c</sup> Freshwater mussel genera.

unionid ammonia toxicity data that have been available for nearly a decade [9,21] and more recently [20,22–24]. A need exists to work toward standardizing the toxicity tests for early life stages of freshwater mussels. Challenges to using these organisms include difficulty in their laboratory culture, uncertainty over appropriate test durations relative to their long life span, lack of sensitive sublethal endpoints, and the potential importance of including sediment in laboratory exposures aimed at reproducing environmental conditions [9,26]. While a need exists for standard methods, our results indicate good agreement among the results for 10 species, which addressed

three mussel life stages and were generated by eight independent investigators. These data should be useful in establishment of ammonia criteria or state or local water quality standards.

The 10 species for which acute ammonia exposure data are available represent only about 3% of the unionid species known from North America, and the eight genera represent about 20% of the genera within this family [1]. Additional acute toxicity data would be beneficial for these species, although sufficient data seem to exist to support revision of the U.S. EPA acute ammonia criteria or establishment of state or local water quality standards for acute exposure. In the interim, our CMC<sub>FM</sub>s may be used as approximations of the water quality conditions that protect mussels from acute ammonia exposures.

Chronic exposure data and sublethal endpoints assessments are generally lacking for mussels and should be initiated. Our process for deriving the CCC<sub>FM</sub>s was not equivalent to the process used by U.S. EPA to derive the current ammonia CCC. The U.S. EPA did not use ACRs in deriving the CCC but rather relied directly on toxicity data from chronic tests. Adding another group with acute but no chronic data would not necessarily change the CCC under that procedure. Also, the lack of ACRs for mussels and ammonia is a hindrance. Our eACRs would likely not be used in a national criteria recalculation that would favor use of actual chronic data or at least an ACR derived specifically from a long-term test evaluating sublethal impacts to mussels from ammonia. Our lower-bound eACR of 5.0, derived from 9- to 15-d ammonia toxicity tests with mussels that measured lethality as the test endpoints, is only an initial approximation of a suitable ACR. Our upper-bound eACR of 10.9 is also uncertain; it is merely the highest of the seven genus mean ACRs reported in the current U.S. EPA ammonia criteria document [14], but individual species ACRs for fish and aquatic invertebrates ranged from 1.2 to 20.7 [14]. Until long-term ammonia exposure and sublethal effects data are produced for mussels, it will be difficult to

Table 3. Comparison of ammonia final acute values (FAV) and criteria maximum concentrations derived from the U.S. Environmental Protection Agency (U.S. EPA) database used to calculate the current ammonia criteria to values recalculated with data sets expanded by adding freshwater mussel toxicity test results. All data are in mg/L, total ammonia as N, normalized to pH 8

Data set	Most sensitive genera	Calculated FAV <sup>a</sup>	Revised FAV <sup>b</sup>	CMC <sup>c</sup>
1999 revisions <sup>d</sup>	<i>Oncorhynchus</i> <i>Etheostoma</i> <i>Notemigonus</i> <i>Prosopium</i>	14.32	11.23 <sup>e</sup>	5.62 <sup>f</sup> 8.40 <sup>g</sup>
Recalculation adding data for all mussel life stages	<i>Medionidus</i> <i>Lampsilis</i> <i>Lasmigona</i> <i>Fusconaia</i>	3.50	3.50	1.75
Recalculation adding data for adult and juvenile mussels only	<i>Utterbackia</i> <i>Villosa</i> <i>Lampsilis</i> <i>Lasmigona</i>	5.00	5.00	2.50

<sup>a</sup> Final acute value from equation on page 31 of Stephan et al. [15].

<sup>b</sup> Final acute value can be lowered to the species mean acute value for sensitive recreationally or commercially important species not protected by the calculated FAV.

<sup>c</sup> Criteria maximum concentration (one-half of FAV).

<sup>d</sup> All data from U.S. EPA [14].

<sup>e</sup> FAV lowered to the species mean acute value for rainbow trout.

<sup>f</sup> CMC when salmonids are present.

<sup>g</sup> CMC when salmonids are absent.

generate definitive protective state- or site-specific standards or national criteria for chronic exposure. In the interim, use of the CCC<sub>FM</sub>s may be appropriate. The range we estimate by using data from all mussel life stages (0.3–0.7 mg/L total ammonia as N at pH 8) is similar to estimated safe ammonia concentrations for two unionid species [24] that are about 0.2 and 0.5 mg/L total ammonia as N when normalized to pH 8.

Because of ammonia's pH- and temperature-dependent ionization, the CMC varies with pH, and the CCC varies with pH and temperature. The criteria also vary, depending on the presence of salmonids (CMC) and fish early life stages (CCC), the sensitivity of which results in lower allowable ammonia concentrations. Our results suggest a need for criteria revision, but at least the more restrictive criteria (salmonids and sensitive life stages present) should apply until criteria are revised or state standards are adopted that incorporate the data reviewed here. Our CMC<sub>FM</sub>s and CCC<sub>FM</sub>s were calculated by normalizing all data to pH 8, and the CCC<sub>FM</sub> used data at about 25°C; the equations in the U.S. EPA criteria document [14] will need to be applied to adjust these values for other pH and temperature combinations. Also, it is emphasized that our CMC<sub>FM</sub> and CCC<sub>FM</sub> define the magnitude of concentrations that may protect freshwater mussels provided that exposure duration and frequency components of the CMC and CCC are still applied [14,15].

#### *Ammonia as a potential limiting factor in unionid survival and recovery*

Ammonia may be a significant limiting factor for unionids given that concentrations above the guidelines that we derived have been documented. Moreover, sediment pore-water concentrations of ammonia typically exceed those of overlying surface water [13], thereby placing the infaunal freshwater mussels in the environmental compartment where ammonia concentrations are frequently elevated. Unionids' feeding strategies include filtration of surface and pore water, suspended sediment, and sediment-associated fine particles [27], also potentially increasing their ammonia exposure. Relative to reference sites, low freshwater mussel diversity and abundance have been observed downstream of significant ammonia sources [9,12], but definitive cause-and-effect relationships have not been documented in these or other field investigations of this issue [28]. More work is needed to establish the extent of linkage between the field exposure and effects data.

As a common pollutant to which unionids appear to be sensitive, ammonia should be considered among the factors that may be limiting survival and recovery of freshwater mussels. Moreover, our calculations did not consider additional margins of safety that could be recommended for protection of threatened or endangered mussel species in instances where information is specifically lacking. Because threatened or endangered mussels have not been tested for sensitivity to ammonia, additional approaches may be required.

*Acknowledgement*—Chris Ingersoll, Russ Erickson, and Sara Ward provided helpful reviews of an earlier version of the manuscript; their expertise is appreciated. Two anonymous reviewers provided comments that also improved the paper.

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