1

Running head: Ammonia toxicity to freshwater mussels

Corresponding author:

Tom Augspurger

U. S. Fish and Wildlife Service

Ecological Services

P.O. Box 33726

Raleigh, North Carolina 27636-3726 USA

Phone: (919) 856-4520 ext. 21

Fax: (919) 856-4556

email: tom_augspurger@fws.gov

Total words in text, references, figure and table legends:

EVALUATION OF WATER QUALITY CRITERIA FOR PROTECTION OF FRESHWATER MUSSELS (UNIONIDAE) FROM ACUTE AMMONIA EXPOSURE

Tom Augspurger,*† Anne E. Keller,‡ Marsha C. Black,§ W. Gregory Cope,_ and F. James Dwyer#

†U.S. Fish and Wildlife Service, P.O. Box 33726, Raleigh, North Carolina 27636-3726

‡U.S. Environmental Protection Agency, Science and Ecosystem Support Division, 980 College

Station Road, Athens, Georgia 30605

§Department of Environmental Health Science, University of Georgia, Athens, Georgia 30602

_North Carolina State University, Department of Environmental and Molecular Toxicology,
Raleigh, North Carolina 27695

#U.S. Fish and Wildlife Service, 608 East Cherry Street, Columbia, Missouri 65201

* To whom correspondence may be addressed (tom_augspurger@fws.gov)

Abstract — Ammonia toxicity data for freshwater mussels (Unionidae), a significantly imperilled taxa, were compiled and used in a re-calculation of national water quality criteria for this common pollutant of natural waters. Twenty-two ≤96 h LC50s, covering nine species in eight unionid genera were retrieved Genus mean acute values (GMAVs) for these data ranged from 4.24 to 8.88 mg/l total ammonia as N, normalized to pH 8. These are uniformly at the sensitive end of the range of GMAVs in the national database used to derive the criteria maximum concentration (CMC). Re-calculated CMCs employing acute data for all freshwater mussel lifestages (2.46 mg/l total ammonia as N, normalized to pH 8) and employing only data for adult and juvenile mussel lifestages (3.22 mg/l total ammonia as N, normalized to pH 8) were approximately 60 % and 40% less than the CMC used for the current acute water quality criteria. There were no chronic ammonia exposure data for unionids and no assessments which incorporated sublethal endpoints. Use of two acute-chronic ratios yielded approximations of a criteria continuous concentration (CCC) from 0.31 to 0.56 mg/l total ammonia as N, normalized to pH 8, approximately 65% less than the CCC for pH 8 and 25°C in the revised national criteria. The current numerical criteria may not be protective of this taxa whose nearly 300 species are widespread in eastern North America.

Keywords — Ammonia Unionidae Freshwater mussels Water quality standards

INTRODUCTION

Many factors are cited in the decline of freshwater mussel (Family 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, over-

utilization, disease, predation, and pollution are considered causal or contributing factors in many areas of the United States [3-5]. Impaired water quality is a reasonable hypothesis to test as a limiting factor in the recovery of imperilled freshwater mussels. Toxic substances were among the stressors most 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], there are also published toxicological data indicating that early lifestages of freshwater mussels are among the most sensitive aquatic organisms yet 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 such a common pollutant of natural waters. Ammonia is a natural degradation product of nitrogenous organic matter: significant sources of enrichment include industrial waste, municipal waste water treatment plants, and agricultural run-off (animal wastes as well as chemical fertilizers). Sediment pore water concentrations of ammonia typically exceed those of overlying surface water [13], so freshwater mussels' anchorage in the substrate places them in the environmental compartment where ammonia concentrations are expected to be among the greatest. For these reasons, ammonia merits detailed attention among the myriad chemicals to evaluate for effects on mussels.

We undertook an evaluation of the ammonia toxicity data for freshwater mussels with two goals: 1) to derive estimates of the concentrations that would not be harmful in acute and chronic exposures; and 2) to compare those concentrations to water quality criteria and standards in order to assess the appropriateness of existing regulations and guidance.

METHODS

Development of the ammonia toxicity database

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

Because there are no U.S. EPA or American Society of Testing and Materials standard methods for freshwater mussel bioassays, we evaluated data from all sources for acceptability using guidance modified from the U.S. EPA [15]. Studies used demonstrated acceptable survival in control treatments (\geq 80 % for assays with glochidia and \geq 90 % for assays with juvenile and adults), used measured rather than nominal values for ammonia test concentrations, and documented test water pH and temperature to allow calculation of total and unionized ammonia concentrations.

For all of the previously unpublished data used from our laboratories, protocols generally followed those published previously for mussel toxicity tests with other compounds [8]. These were 24 to 96 h static toxicity tests with glochidia or juvenile stages of mussels. Test water was reconstituted soft or moderately hard [16], and exposure series consisted of five concentrations tested in replicates of three to six depending on availability of organisms. A dilution water control was included for each species. Photoperiod was 16 h light, 8 h dark; test organisms were not fed. Dissolved oxygen, temperature, and pH were measured on each batch of reconstituted water before the start of the exposures. LC50s were calculated for all toxicity tests using Trimmed Spearman-Karber [16].

Summary of ammonia toxicity to freshwater mussels

The toxicity of ammonia varies with temperature and pH (which influence the fraction of total ammonia which exists in the ionized and more toxic un-ionized states). Recommended water quality criteria for ammonia have been presented on an un-ionized ammonia basis (NH₃) [17] and total ammonia as nitrogen basis (NH₃ + NH₄⁺ - 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 [18]; these were also normalized to pH 8 using the equations in appendix 3 of the U.S. EPA revised ammonia criteria document [14]. Concentrations are correspondingly reported as mg/l total ammonia as N, normalized to pH 8.

Acute data are summarized by the methodology of the national numeric water quality criteria guidelines [15]. Species mean acute values (SMAVs) and genus mean acute values (GMAVs) were calculated in two ways: 1) combined data for all mussel lifestages within the species or genus (i.e., results from assays with glochidia, juvenile, and adult combined); and, 2) combined adult and juvenile data only (i.e., excluding the assays which used the glochidia lifestage, for which the appropriateness of use in bioassays to support water quality criteria development has been questioned by some regulators). The GMAVs are used in the demonstration of the unionids' sensitivity to ammonia relative to other invertebrates and fishes in the existing national database [14]. National water quality criteria 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. We used the unionid GMAVs in a re-calculation of the CMC based on the national methodology [14,15]. Although CMC in the national ammonia criteria document are in the form of equations to account for effects of temperature and pH, they are based on a Final Acute Value (FAV) which served as the basis for our comparison. We defined the resulting re-calculations as freshwater mussel FAV guidelines (FAV_{FM}) and freshwater mussel criteria maximum concentration guidelines (CMC_{FM})

There were insufficient chronic ammonia exposure and sublethal effects data for unionids to re-calculate the CCC under the approach used in the national criteria. To derive an estimate of the chronic ammonia concentration that would not be harmful to mussels, we divided the FAV_{FM}S by the acute-chronic ratio, an approach allowed by the guidelines for derivation of water quality criteria [15]. Two approximations of freshwater mussel criteria continuous concentration guidelines (CCC_{FM}) were derived. The first was calculated by diving the FAV_{FM} by the overall acute-chronic ratio of 11.6 from page 78 of the revised ammonia criteria document (calculated by dividing the revised CMC by the revised CCC and representing the cumulative datasets that went into derivation of each of these parameters). As a second approximation, we used an acute-chronic ratio for a benthic bivalve, the fingernail clam (*Musculium transversum*); its acute-chronic ratio of 15.8 was derived by dividing the GMAV for this species (35.65 mg/l total ammonia as N, normalized to pH 8) [14] by its genus mean chronic value of 2.26 mg/l total ammonia as N, normalized to pH 8 [14].

RESULTS

Twenty-two LC50s for unionid acute ammonia exposures were retrieved; these covered

nine species in eight genera (Table 1). Additional relevant ammonia toxicity data were available for the genera *Amblema*, *Utterbackia*, *Cyrtonaias*, and *Toxolasma* [12, 19], but no acute (≤ 96 h) LC50s were reported in these studies. Two subacute (9 to 15 d) LC50s were retrieved (Table 1). There were no chronic ammonia exposure data for unionids and no assessments which incorporated sublethal endpoints; there were, correspondingly, no applicable acute-chronic ratios for ammonia impacts to freshwater mussels.

Sensitivity of freshwater mussels to ammonia

Genus mean acute values for freshwater mussels ranged from 4.24 to 8.88 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 reported in the database used to calculate the national water quality criteria for ammonia [14]. In general, glochidia were approximately 1.5 to 3.5 times more sensitive than juveniles in the three species for which acute data are available for both lifestages (Table 1). Excluding tests with glochidia from the GMAV calculations changed their ranks, but it did not appreciably change the overall apparent sensitivity of unionids relative to other taxa represented in the database (Table 3).

Derivation of water quality criteria

Addition of GMAVs for the eight unionid genera for which acute ammonia toxicity data are available allowed a re-calculation of the national water quality criteria to determine the influence of the additional data. The revised FAV_{FM} and corresponding CMC_{FM} employing data from assays with all freshwater mussel lifestages were approximately 60 % less than the FAV and

CMC used in the calculation of the current acute water quality criteria, and the revised FAV_{FM} and CMC_{FM} employing data from assays with only juvenile and adult stages of freshwater mussels was approximately 40 % less than the FAV and CMC from the criteria document (Table 4).

No chronic exposure data for freshwater mussels were retrieved. There were no applicable acute-chronic ratios for sublethal ammonia impacts to freshwater mussels. Employing the acute-chronic ratio of 11.6 (the ratio of the national CMC to the CCC at pH 8 and 25°C) yielded CCC_{FM}'s of 0.42 to 0.56 mg/l total ammonia as N, normalized to pH 8 when applied to our FAV_{FM}s. Employing the acute-chronic ratio of 15.8 (from the fingernail clam) yielded CCC_{FM}'s of 0.31 to 0.41 mg/l total ammonia as N, normalized to pH 8 when applied to our FAV_{FM}s. The average of these four CCC_{FM}s (0.42 mg/l total ammonia as N, normalized to pH 8) is approximately 65% less than the CCC for pH 8 and 25°C in the revised national criteria (Table 5).

DISCUSSION

Freshwater mussel data are not included in the current database for calculation of the federal water quality criteria for ammonia. Recently available data for this family includes eight GMAVs which are less than those used to derive the CMC in the criteria document and two subacute LC50s which are less than the final CCC, supporting the contention that the tested mussel species are sensitive to ammonia relative to other invertebrates and fishes. The current numerical criteria may not be protective of this taxa whose nearly 300 species are widespread in eastern North America.

Issues related to the 1999 revision to the ammonia criteria

National water quality criteria generally consist of two estimated values designed to protect aquatic organisms, the CMC and CCC, along with guidance on their implementation. The CMC is an estimate of the highest one-hour average concentration that should not result in unacceptable adverse effects to aquatic organisms; the number is derived from acute toxicity tests (generally 48 to 96 h exposures) that use lethality or immobilization as the measured biological endpoints. Available toxicity data are critically reviewed, and geometric mean LC50s or EC50s for each genus represented in the review are ranked from highest (most tolerant) to lowest (most sensitive). A cumulative probability is assigned based on those ranks, and an FAV is derived via an equation which gives equal weight to the LC50s or EC50s of the four genera closest to the 0.05 probability (more sensitive). The CMC is calculated by dividing the FAV by 2 and results in a concentration that should not severely adversely affect too many individuals within the taxa (taxon) which were used for deriving the FAV [15]. Evaluation of acute toxicity data has generally shown that dividing an LC50 or EC50 by 2 provides a concentration equal to a very low effect or no effect concentration (i.e., an LC1 or LC0). The process, by definition, is designed to protect populations of 95% of the species tested from adverse effects of short term exposures to non-bioaccumulative chemicals.

The scope of the 1999 revision to the national 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 for the 1985 criteria [17]. The 1985 database was relatively large with 34 genera represented. This significantly exceeds the minimum database requirements for derivation of numeric criteria [15], and it was thought that additional data would have little influence on the four lowest GMAVs which drive the criteria calculations [14]. Our assessment

notes the value of incorporating all acute toxicity data which meet the data quality requirements during criteria calculation or revision. Several of the references we used were available at the time of the revision of the national criteria [9, 19-21] and two [9, 19] were cited in that document as among the more recent data which would not be used because of the reliance on the 1985 database [14]. Because they fall uniformly at the sensitive end of the distribution of FAVs used to calculate the CMC, the additional data for unionids drive the re-calculation of the acute criteria, indicating that unionids may be under-protected by the existing CMC.

Prior to dividing by 2, the FAV can be lowered if the SMAV for a recreationally or commercially important. species is less than (more sensitive) than the calculated FAV [15]. This approach has been applied and was used in the national ammonia criteria [14] to lower the FAV to the SMAV for rainbow trout (*Oncorhynchus mykiss*) prior to deriving the CMC. The applicability of this provision to freshwater mussels has not, to our knowledge, been evaluated. Some species of mussels have commercial importance in the cultured pearl and jewelry industries [5] which could provide justification for lowering the FAV to the mussel SMAV for criteria development. We also suggest that the apparent ammonia sensitivity of taxa within this widely distributed family merits criteria revision for reasons of ecological integrity.

Our approach did not consider additional margins of safety that could be recommended for protection of endangered species where information is specifically. Because no threatened or endangered mussels have been tested for ammonia effects, additional approaches may be required.

Freshwater mussel toxicity data

The 9 species for which we found acute ammonia exposure data represent approximately

3% of the unionid species known from North America, and the 8 genera represent approximately 20% of the genera within this family [1]. More acute toxicity data would be beneficial for these species, however there appears to be sufficient data to support establishment of State or local water quality standards for acute exposure. Chronic exposure data and sublethal endpoints assessments are generally lacking and should be initiated. Until these long-term exposure and sublethal effects data are produced, it will be difficult to generate definitive protective State or site-specific standards or national criteria for chronic exposure. Until that time, the use of the CCC derived here using the ammonia acute-chronic ratio for the fingernail clam may be appropriate.

There is a need to work toward standardizing the bioassays for sensitive lifestages of freshwater mussels in order to overcome these problems. 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 embrace unionid ammonia toxicity data that have been available for nearly a decade [9, 19] and more recently [20, 21]. While there is merit in standardizing freshwater mussel bioassays, our results indicate good agreement among the results for nine species, generated by six investigators and three mussel lifestages. These data should be useful in establishment of State or local water quality standards.

Ammonia as a potential limiting factor in unionid survival and recovery

Ammonia may be a significant limiting factor for unionids given that ambient concentrations well-above the guidelines we derived have been documented. Also, most ambient surface water ammonia data are for the top portion of the water column. Sediment pore water

concentrations of ammonia typically exceed those of overlying surface water [13], so freshwater mussels' anchorage in the substrate places them in the environmental compartment where ammonia concentrations are expected to be among the greatest. Unionids' feeding strategies include filtration of interstitial water and sediment-associated fine particles [22] increasing their exposure. As a widespread pollutant to which unionids appear to be sensitive, ammonia should be considered among the factors which may be limiting survival and recovery at individual locations. Site-specific concentrations of ammonia could be evaluated against the guidelines developed in this evaluation until chronic exposure data, evaluating important sublethal effects (such as biomass and reproduction) are available.

Acknowledgement — reviewers

REFERENCES

- 1. Williams JD, Warren Jr. ML, Cummings KS, Harris JL, Neves RJ. 1993. Conservation
- 2. status of freshwater mussels of the United States and Canada. Fisheries 18:6-22.
- 3. Neves RJ, Bogan AE, Williams JD, Ahlstedt SA, Hartfield PW. 1997. Status of mollusks in the southeastern United States: A downward spiral of diversity. In Benz GW, Collins DE, eds, *Aquatic Fauna in Peril: The Southeastern Perspective*. Special Publication 1, Southeast Aquatic Research Institute, Lenz Designs and Communications, Decatur, GA, USA. pp 31-85.
- Fuller SLH. 1974. Clams and mussels (Mollusca: bivalvia). In Hart Jr. CW, Fuller SLH, eds, Pollution Ecology of Freshwater Invertebrates. Academic Press, New York, NY, USA. pp 215-273.

- Havlik ME, Marking LL. 1987. Effects of contaminants on Naiad mollusks (Unionidae): A review. Resource Publication 164. U.S. Fish and Wildlife Service, Washington, DC, USA.
- 6. National Native Mussel Conservation Committee. 1998. National strategy for the conservation of native freshwater mussels. *J Shellfish Res* 17:1419-1428.
- 7. Richter BD, Braun DP, Mendelson MA, Master LL. 1997. Threats to imperiled freshwater fauna. *Conserv Biol* 11:1081-1093.
- 8. Keller AE. 1993. Acute toxicity of several pesticides, organic compounds, and a wastewater effluent to the freshwater mussel, *Anodonta imbecilis*, *Ceriodaphnia dubia*, and *Pimephales promelas*. *Bull Environ Contam Toxicol* 51:696-702.
- 8. Keller AE, Ruessler DS. 1997. The toxicity of malathion to unionid mussels: Relationship to expected environmental concentrations. *Environ Toxicol Chem* 16:1028-1033.
- 9. Goudreau SE, Neves RJ, Sheehan RJ. 1993. Effects of wastewater treatment plant effluents on freshwater mollusks in the upper Clinch River, Virginia, USA. *Hydrobiologia* 252:211-230.
- Keller AE, Zam SG. 1991. The acute toxicity of selected metals to the freshwater mussel,
 Anodonta imbecilis. Environ Toxicol Chem 10:539-546.
- Jacobson PJ, Farris JL, Cherry DS, Neves RJ. 1993. Juvenile freshwater mussel (bivalvia: Unionidae) responses to acute toxicity testing with copper. *Environ Toxicol Chem* 20:879-833.
- Horne FR, McIntosh S. 1979. Factors influencing distribution of mussels in the Blanco
 River of central Texas. *Nautilus* 94:119-133.

- 13. Frazier BE, Naimo TJ, Sandheinrich MB. 1996. Temporal and vertical distribution of total ammonia nitrogen and un-ionized ammonia nitrogen in sediment pore water from the upper Mississippi River. *Environ Toxicol Chem* 15:92-99.
- U.S. Environmental Protection Agency 1999. 1999 Update of ambient water quality
 criteria for ammonia.. EPA 822-R-99-014. Office of Water, Washington, DC, USA.
- Stephan CE., Mount DI, Hansen DJ, Gentile JH, Chapman GA, Brungs WA. 1985.
 Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. U.S. Environmental Protection Agency, Office of Research and Development Washington, DC, USA.
- U.S. Environmental Protection Agency. 1993. Methods for estimating the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA/600/4-90/027F. Office of Research and Development, Washington, DC, USA.
- 17. U.S. Environmental protection Agency. 1985. Ambient water quality criteria for ammonia.
 EPA 440/5-85-001. Office of Water Regulations and Standards, Criteria and Standards
 Division, Washington, DC, USA.
- 18. Emerson K, Russo RC, Lund RE, Thurston RV. 1975. Aqueous ammonia equilibrium calculations: Effect of pH and temperature. *J Fish Res Bd Can* 32:2379-2383.
 - 19. Wade DC. 1992. Definitive evaluation of Wheeler Reservoir sediments toxicity using
 - juvenile freshwater mussels (*Anodonta imbecillis* Say). TVA/WR-92/25. Tennessee
 Valley Authority, Water Resources Division, Muscle Shoals, AL, USA.
- 20. Scheller JL. 1997. The effect of dieoffs of Asian clams (*Corbicula fluminea*) on native freshwater mussels (Unionidae). Masters thesis. Virginia Polytechnic Institute and State

- University, Blacksburg, VA, USA.
- 21. Myers-Kinzie ML. 1998. Factors affecting survival and recruitment of Unionid mussels in small midwestern streams. PhD thesis. Purdue University, West Lafayette, IN, USA...
- 22. Yeager MM, Cherry DS, Neves RJ. 1994. Feeding and burrowing behaviors of juvenile rainbow mussels, *Villosa iris* (Bivalvia: Unionidae). *JN Am Benthol Soc* 13: 217-222.

Table 1. Acute and subacute toxicity data for ammonia and freshwater mussels. LC50s reported in original reference have been converted to total ammonia as N, normalized to pH 8.

Species	Life stage	Duration Temp.	Temp.	Hd	LC50ª	Reference	
Villosa iris	glochidia	24 h	22	8.1	5.18	[6]	THE PROPERTY AND ADDRESS ASSESSMENT ASSESSME
	glochidia	24 h	20	7.9	2.25	[20]	
	juvenile	96 h	25	8.2	9.44	[20]	
	juvenile	96 h	25	8.2	8.54	[20]	
	juvenile	H 96	25	8.1	5.50	[20]	
Utterbackia imbecillis glochidia	glochidia	48 h	25	∞	5.12	Unpublished ^b	
	glochidia	48 h	25	8.1	7.46	Unpublished	
	juvenile	Ч 96	25	«	5.79	Unpublished ^b	
	juvenile	96 h	25	∞	19.67	Unpublished	
	juvenile	ч 96	25	∞	68.9	${ m Unpublished}^{ m c}$	
Pyganodon grandis	adult	96 h	25	7.5	8.74	[20]	
	adult	96 h	25	7.7	9.03	[20]	
Lasmigona subviridis	juvenile	24 h	25	∞	6.61	Unpublished ⁶	
	juvenile	24 h	25	∞	6.61	Unpublished ^b	
	juvenile	24 h	25	∞	5.19	Unpublished ^b	
Fusconaia masoni	glochidia	24 h	25	∞	5.18	Unpublished ^b	
Fusconaia masoni	glochidia	24 h	25	∞		5.18	

	ţ
48 h	77 6.
sa glochidia	
reunonaias pectorosa glocl	
reunona)	

$Unpublished^\circ$ $Unpublished^\circ$	$^{ m Unpublished^c}$ [21]	$Unpublished^d$	[19] Unpublished ^b	
25 8 3.76 25 8 14.05 25 8 4.24	24 8.3 0.73 24 8.3 2.26 19 8.3	0.2 21.64	2 8 0.57	lized to pH 8
Medionidus conradicus glochidia 48 h Lampsilis siliquoidea juvenile 96 h	juvenile cardium juvenile 9	Subacute Utterbackia imbecillis juvenile Lasmigona subviridis .		o M. Black and M. Barfield, unpublished 3.

b M. Black and M. Barfield, unpublished data. University of Georgia, Athens, GA. 2000.

d T. J. Newton, preliminary data, Upper Midwest Environmental Sciences Center, LaCrosse, WI. 2000. c A. Keller, unpublished data. USEPA, Athens, GA. 1999.

Table 2. Comparison of freshwater mussel (all lifestages) species mean acute values (SMAVs) and genus mean acute values (GMAVs) for ammonia toxicity to other sensitive taxa from the national database, listed in order of increasing sensitivity. Freshwater mussel GMAVs are in bold type. All GMAVs are in mg/l total ammonia as N, normalized to pH 8.

Rank	Genus	Species Used in GMAV Deri	vation	SMAV	GMAV
12	Oncorhynchus	Golden trout		26.10	21.95
		Cutthroat trout		25.80	
		Pink salmon		42.07	
		Coho salmon		20.26	
		Rainbow trout		11.23	
		Chinook salmon		17.34	
11	Etheostoma	Orangethroat darter		17.96	17.96
10	Notemigonus	Golden shiner	14.67		14.67
9	Prosopium	Mountain whitefish		12.11	12.11
8	Pyganodon	Giant floater		8.88	8.88
7	Utterbackia	Paper pondshell		7.86	7.86
6	Actinonaias	Pheasantshell	7.27		7.27
5	Lasmigona	Green floater		6.10	6.10
4	Villosa Rai	nbow	5.53		5.53
3	Lampsilis	Plain pocketbook		21.64	5.26
		Fatmucket		1.28	
2	Fusconaia	Atlantic pigtoe		5.18	5.18
1	Medionidus	Cumberland moccasinshell		4.24	4.24

Table 3. Comparison of freshwater mussel (juvenile and adult lifestages only) species mean acute values (SMAVs) and genus mean acute values (GMAVs) for ammonia toxicity to other sensitive 19 taxa from the national database, listed in order of increasing sensitivity. Freshwater mussel-GMAVs are in bold type. All GMAVs are in mg/l total ammonia as N, normalized to pH 8.

Rank Genus 10 Oncorhyr	Species Used in Goldon	MAVD	as N, no	rmalized to pH 8
· icornyr	Golden trout		SMAV	VGMAV
	Cutthroat trout		26.10	21.95
	Pink salmon		25.80	
	Coho salmon		42.07	
	Rainbow trout	4	20.26	
9 Fthan	Chinook salmon	1	1.23	
EtheostomaNotari	Orangethroat darter	17	⁷ .34	
Notemigomus Activity	Golden shiner	17	.96	17.96
Actinonaias Programme	Pheasantshell	14.67	14.6	
Prosopium Utta	Mountain whitefish	14.05	14.05	5
Utterbackia	Paper pondshell	12.1	1	12.11
Pyganodon Viu	Giant floater	9.22)	9.22
Villosa Ra	inbow	8.88		8.88
Lasmigona	Green floater	7.63	7.63	-100
Lampsilis	Plain pocketbook	6.10		6.10
	Fatmucket	21.64		5.26
		1.28	·	

Table 4. Comparison of ammonia Final Acute Values and Criteria Maximum Concentrations derived from the national database used to calculate the ammonia criteria to those derived with datasets expanded by adding freshwater mussel toxicity testing results. All data are in mg/l, total ammonia as N, normalized to pH 8.

Dataset	, normalized to pH 8.	3, 10	sting results.	All data are in mg/l, total
1999 Revision	Oncorhynchus	Calculated I		ed FAV ^b CMC ^c
Re-calculation wi all freshwater mus data	sel Lampsilis Fusconaia	14.32	11.23°	5.62
Re-calculation with tests using only adult and juvenile mussel data	Villosa Lasmigona	4.93	4.93	2.46
	Om equation on page 31	6.45	6.45	3.22

Final Acute Value from equation on page 31 of the U.S. EPA Guidelines for deriving

d all data from U.S. Environmental Protection Agency 1999. 1999 Update of ambient water quality criteria for ammonia.. EPA 822-R-99-014. Office of Water, Washington, DC, USA.

numerical national water quality criteria for the protection of aquatic organisms and their uses b Final Acute Value can be lowered to the Species Mean Acute Value for sensitive recreationally or commercially important species not protected by the calculated FAVc Criteria maximum Concentration (one-half of FAV)

e FAV lowered to the Species Mean Acute Value for rainbow trout

Table 5. Comparison of ammonia Criteria Continuous Concentrations (CCC) derived from the

national database used to calculate the ammonia criteria to those derived (CCC_{FM}) with datasets expanded by adding freshwater mussel acute toxicity testing results and acute-chronic ratios

(Judging treshwate	r mer to	those derived
$(ACR)_{A}$	Il data	ulussel acute toxicity	derived (CCC _{FM}) with
***************************************	ata are in mg/l, to	atal a	resulte and
		cai ammonia as N nor "	those derived (CCC _{FM}) with dat results and acute-chronic ratios
00-		, normalized	to pH & on 1 and
CCC	Figh .	r mussel acute toxicity testing tal ammonia as N, normalized	F11 8 and 25 °C.

CCC	as IV, nor	malized to pH 8 and 25 °C.
CCC	lifestages pro-	V.
	Fish early lifestages absent	1.24
$CCC_{FM}I$	ACR of the	1.24
$CCC_{FM}2$	ACR of 11.6 applied to FAV for adult and ACR of 11.6 applied to FAV for all musses	d juvenile mussel data
$CCC_{FM}3$	403C	ⁱ data
$CC_{FM}4$	ACR of 15.8 applied to The	0.42
	ACR of 15.8 applied to FAV for all mussel d	ata 0.41
		0.31

Subject: Apologia-L: Re: None

To: Multiple recipients of list Apologia-L <Apologia-L@transporter.com> Dear Kara, You asked:

...hey can any body help me on the sedevacantist position?...Teresa?

Sorry it took so long had a big gathering for our daughter's 1st Holy Communion this weekend. What is a 'Sedevacantist?'

(Sede Vacante: Latin for 'The Chair is empty'; in reference to the Chair of St. Peter.) Sedevacantists hold the belief that the Roman Catholic Church is without a "true" Pope. They believe (in short) that the "Vatican II" Popes lost their authority as Pope after teaching "heresy." They were started by Francis Shuckardt in Coeur d'Alene, Idaho. Shuckardt was a renegade Bishop consecrated by the schismatic 'North American Old Roman catholic' group. Schuckardt ended up being arrested for possesion of drugs and stolen property in California. SOME EXTRACTS FROM CORRESPONDENCE FROM SEDEVACANTISTS

PRAKASH JOHN M. a.k.a LUKE VIRGIL H. JOHN L. a.k.a HELEN L.

in response to my:

AN OPINION ON ALLEGATIONS REGARDING THE POPE RECEIVING THE "MARK OF SHIVA", AND HIS SEMI-NAKED MASS IN PAPUA NEW GUINEA Allegations In respect of Council Vatican II: "the phony Balaamistic reforms?

Allegations That The Roman Catholic Church Is NOT The "TRUE" Church: "Msgr. Benny Aguiar, the former editor of the New Church diocese of Bombay's paper 'The Examiner'''

Allegations That The Vatican II and post-Vatican II Popes Are Heretics:

J.L.:8. "Anyway, this event (whatever it was) in no way impinges on the dogma of Papal Offallibility, which means that the Pope is incapable of teaching heresy as dogmatic truth, not lat he is incapable of sin, of scandal, or of exercising bad judgment. Furthermore, the burden Proof of any allegation rests on the party making the allegation - not upon the defender of

Poly: That is true. It would only show, that the 'pope' was stupid, confused or misguided by hosts - the local church. But it is not on this that Catholics base their rejection of this man ope, or even as a Christian. It is on the teachings of A.J. Roncalli (the antipope Balaam I) of the Latrocinium of the Vatican (1962-5) to which he adheres. His anti-Catholic acts here there, such as at Assisi, Canterbury, etc, etc, made ad nausem, are useful only to show for Dick Neves <mussel@vt ed...

•			
			:
			The second section of the second seco
	,		
			Transition and the second second

Subject: Re: ammonia and mussels

To: Paul_Hartfield@fws.gov, Bob_Butler@fws.gov

Cc: ahlstedt@usgs.gov, John_Schmerfeld@fws.gov, mussel@vt.edu

Hello -

Here's the freshwater mussel ammonia information Bob mentioned. It is a draft manuscript that I'm developing (with Anne Keller, Marsha Black, Jim Dwyer and Greg Cope) with all of the data from a poster which was presented a little over a year ago at the Society of Environmental Toxicology and Chemistry's annual meeting. I can send you the reduced poster if you want it, but this is better because it is easier to read (larger font), has more detail on the derivation of the numbers, and contains all the references.

I'm comfortable with the acute (short-term exposure...generally less than 96 hours) data and comparisons to other acute data. I'm not yet comfortable with the approach taken on the chronic (long-term exposure) data. ... long-tern exposure and effects data are pretty sparse for mussels making alternate approaches to deriving a protective concentration necessary for now (but hopefully a data gap we can fill in the next couple years).

All in all, the data indicate mussel are pretty sensitive to ammonia, a natural degradation product of nitrogenous organic matter in all waters which can certainly be elevated by other sources (run-off of residential or agricultural fertilizers, animal feedlot wastes, municipal wastewater effluents, etc). I do not have data for other nutrients.

Let me know if you have any questions or suggestions or if I can help further. Feel free to use this as a manuscript in preparation (December 2001 draft).

Take care,

Tom Augspurger

Phone: 919/856-4520 (x. 21)

Fax: 919/856-4556

email: tom_augspurger@fws.gov

(See attached file: ammoninams4.wpd)

Bob Butler

To:

Paul Hartfield/R4/FWS/DOI@FWS, Tom

04/10/2002 09:04

Augspurger/R4/FWS/DOI@FWS, John

Schmerfeld/R5/FWS/DOI@FWS,

ΑM

ahlstedt@usgs.gov, mussel@vt.edu

CC;

Subject: Re: Recovery Plan

Paul:

I have had conversations with Tom concerning ammonia, the highest concentrations being at the water/substrate interface, its lethality to mussels, and its prevalence below feedlots and sewage outfalls, but not with other, more typical, nutrients.

Gentlemen:

Do any of you have answers to Pauls questions below? If so, please pass on and cc: me if you would.

Thanks a bunch...

Bob.b

---- Forwarded by Bob Butler/R4/FWS/DOI on 04/10/02 08:54 AM ----

Paul Hartfield

To:

Bob Butler/R4/FWS/DOI@FWS

04/09/02 04:09 PM

CC:

Subject: Re: Recovery Plan(Document link: Bob Butler)

Bob: do you know of any other info on the effects of nutrients on mussels. Particularly as they are manifested in dense growth of filamentous algae. I've often associated the decline of mussels with presence of algal mats. Has anyone else? Anything written? Paul

