Justification for Idaho's Recalculated Ammonia Criteria for Cold Water Aquatic Life

State of Idaho

Department of Environmental Quality

July 2026

Prepared by
Idaho Department of Environmental Quality
Water Quality Division
1410 N. Hilton St.
Boise, ID 83706

Accessibility Services: The Idaho Department of Environmental Quality will provide reasonable language access services and/or disability services for documents at no charge. To request an accommodation under Title VI of the Civil Rights Act of 1964 or Americans with Disabilities Act, contact DEQ's nondiscrimination coordinator at (208) 373-0271 or *accessibility@deq.idaho.gov*. Para obtener información en español, visite *https://www.deq.idaho.gov/about-us/accessibility/*.

DEQ, July 2026, Cost codes: 24530014.245029.22503. Costs associated with this publication are available from the State of Idaho Department of Environmental Quality in accordance with Idaho Code § 60-202.

Table of Contents

1 Introduction 1
2 Determining Idaho's Resident Species 2
2.1 Unionid Occurrence and Habitat 2
2.2 Other Sensitive Species 4
2.3 Idaho's Resident Species List 4
3 Toxicity Studies for Resident Unionids 4
4 Acute Criteria Recalculation Process 5
4.1 Acute Dataset Deletion Process 6
4.2 Idaho-Specific Acute Toxicity Dataset 9
4.3 Recalculation of the Acute Criteria 12
4.4 Recalculation of the Chronic Criteria 13
5 Protectiveness of Recalculated Ammonia Criterion 16
5.1 Resident Species 16
5.2 Downstream Waters 17
5.3 Beneficial Uses 17
References 19
Appendix A. Resident Fishes, Mollusks, and Amphibians of Idaho 21
Appendix B. Acute Criterial at Selected pH and Temperature Conditions 28
Appendix C. Chronic Criteria at Selected pH and Temperature Conditions 29
Appendix D. Toxicity Studies on Western Unionids 30

List of Tables

Table 1. Acute data added to EPA ammonia toxicity database. 5
Table 2. Acute toxicity data removed by deletion process. 7
Table 3. Idaho-specific genus mean acute values (normalized to pH 7 and 20 °C). 10
Table 4. Idaho-specific FAV and CMC. 12
Table 5. Resident Unionid GMAVs and Surrogate GMCVs 14
Table 6. Chronic toxicity data removed by deletion process. 14
Table 7. Idaho-specific genus mean chronic values (normalized to pH 7 and 20 °C). 15
Table 8. Idaho-specific FCV and CCC. 15

List of Figures

Figure 1. Idaho 4th-field HUCs (HUC4), with and without recorded unionid mussel observations. 3

List of Equations

Equation 1. Acute Value pH Conversion Equation. 12
Equation 2. Acute Value Temperature Conversion Equation. 13
Equation 3. Acute Criteria Equation for Toxicity Dataset. 13
Equation 4. Criteria Equation for Protection of Adult Rainbow Trout. 13
Equation 5. Acute Criteria Equation for Protection of All Idaho Species 13
Equation 6. Chronic Criteria Equation for Protection of All Idaho Species. 16
Equation 7. Simplified Chronic Criteria Equation for Protection of All Idaho Species. 16

Abbreviations, Acronyms, and Symbols

ACR	acute-chronic ratio
CCC	criterion continuous concentration
CMC	criterion maximum concentration
CV	chronic value
DEQ	Idaho Department of Environmental Quality
EPA	United States Environmental Protection Agency
FAV	final acute value
FCV	final chronic value
GMAV	genus mean acute value
GMCV	genus mean chronic value
HUC	hydrologic unit code
IDFG	Idaho Department of Fish and Game
ID-SC	Idaho-specific criteria
mg/L	milligrams per liter
SMAV	species mean acute value
SMCV	species mean chronic value
SSC	site-specific criterion
TAN	total ammonia nitrogen

1 Introduction

This document provides the scientific justification for developing - recalculated criteria equations for ammonia in Idaho Administrative Code (IDAPA 58.01.02.250.02.d) for waters in the state of Idaho designated for cold water aquatic life.

These Idaho-specific criteria (ID-SC) was developed according to IDAPA 58.01.02.275.01.h. These procedures allow aquatic life criteria to be derived using scientifically justifiable approaches consistent with the assumptions and rationale in United States Environmental Protection Agency (EPA) guidance. Specifically, the Idaho Department of Environmental Quality (DEQ) derived acute and chronic criteria using current EPA guidance on site-specific species deletion (EPA 2013a) and criterion development (EPA 2010) to account for differences in ammonia sensitivity between resident mussel species in Idaho and those species used in deriving the recommended nationwide criterion.

Ammonia is a highly toxic chemical of concern in surface waters due to releases from wastewater plants, fertilizer products, and industrial processes. Effects on aquatic life include inhibition of nitrification in bacteria, reversal of diffusion gradients in fish species, and overall reduced function in bivalves (Russo 1985). These effects depend on pH and temperature conditions, increasing in severity with increases in either measure (EPA 2013b).

EPA's 2013 criteria recommendations incorporate data from new studies that show the extreme sensitivity of mussels in the order unionida to effects from ammonia. Several of these sensitive species are listed as endangered in eastern US habitats and merit a high level of protection. The criteria provided by EPA are protective of these sensitive species and were assumed to be protective of other, related species of mussels.

However, new toxicity data from studies on western unionid mussel species (Wang 2017; PER 2018a,b,c) found a significant difference in their sensitivity to ammonia as compared to the species included in the EPA's 2013 criteria documents. This difference in sensitivity indicated that protective criteria for Idaho's resident mussels could be achieved at higher ammonia levels. These higher thresholds for western unionids mean that conditions for healthy populations of Idaho's resident aquatic life can be achieved with less restrictive water quality standards, lessening the burden on monitoring and treating water that enters waters of the state.

Consistent with EPA's recommended national criterion (EPA 2013b), the proposed recalculated criteria consists of two criterion equations that depend on pH and temperature. They include a criterion maximum concentration (CMC) equation for protection of organisms from exposures to high concentrations for short periods of time (acute effects), and a criterion continuous concentration (CCC) equation that provides protection from exposures to lower concentrations over longer time periods (chronic effects). The equations maintain the established invertebrate and vertebrate toxicity dependance on pH and temperature, consistent with Idaho's current criteria and EPA's recommendations.

DEQ's ID-SC elements are protective of Idaho's entire aquatic community, including fish, amphibians, and invertebrates. The criterion equations provide context for relative toxicity risk to resident aquatic species, including the increased toxicity to invertebrates at high temperatures, and the increased toxicity to most species at increased pH. The criteria equations also maintain protection to sensitive adult Rainbow Trout, which are the most sensitive species at low temperatures (Thurston and Russo 1983; EPA 2013b).

DEQ's proposed acute criterion equation is based on the four taxa closest to the 95th-percentile of the state-specific acute toxicity dataset (*Gonidea angulata*, *Margaritifera falcata*, *Fluminicola sp., and Notemigonus crysoleucus*) and are protective of the most sensitive species (*Prosopium williamsoni*). DEQ's proposed chronic criterion equation is based on the four most sensitive genera in the state-specific chronic toxicity dataset (*Gonidea*, *Margaritifera*, *Anodonta*, and *Lepomis*).

These equations were developed using EPA's deletion process and water quality criteria guideline documents (EPA 2010, 2013a).

The core steps for developing the proposed ID-SC include the following:

- Determine the resident species that occur in Idaho.
- Collect toxicity data relevant to resident species.
- Add toxicity data for resident species and deleting nonresidents from the state-specific toxicity dataset.
- Develop criteria equations based on the state-specific dataset and established. relationships between toxicity and environmental conditions.
- Evaluate protectiveness of the SSC to resident species expected to be present in Idaho.

2 Determining Idaho's Resident Species

To identify whether EPA's 2013 recommendations for ammonia criteria were appropriate for adoption into Idaho's water quality standards, DEQ conducted an assessment of Idaho's resident aquatic species, particularly for the presence of unionid mussels.

2.1 Unionid Occurrence and Habitat

Unionid mussel presence has been historically documented throughout Idaho. Many species were a natural resource and food source to Native Americans and were harvested for button manufacturing throughout the 1800s (Nedeau et al. 2009). Due to their cryptic nature and relatively small size, records of specific mussel populations are inconsistent and typically limited to small geographic areas.

The Idaho Department of Fish and Game (IDFG) provided DEQ with scientific surveys, targeted inventories, and incidental observations (IDFG 2024). DEQ combined this database with the Western Mussel Atlas data from the Xerces Conservation Society (Xerces 2024) to construct a robust dataset of mussel observations and eDNA detections in the state. These records were

used to document the mussels within each of Idaho's 4th-field hydrologic unit codes (HUC4), shown in Figure 1.

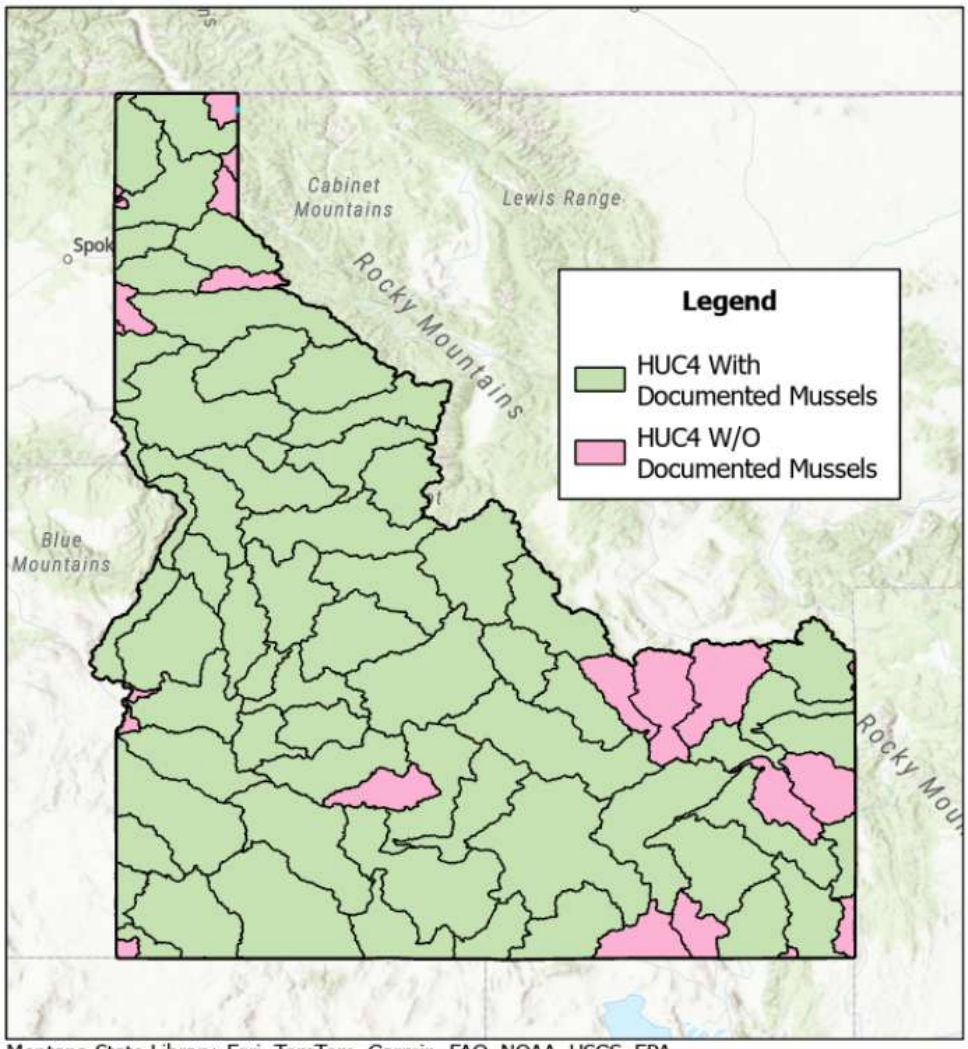


Figure 1. Idaho 4th-field HUCs (HUC4), with and without recorded unionid mussel observations.

Considering the prevalence of unionid mussel populations throughout the state, and the difficulty performing statewide surveys would present, DEQ chose to pursue one set of ammonia criteria that would be protective of Idaho's resident unionids. EPA provided information on conducting and reviewing freshwater mussel surveys to support developing unionid-absent site-specific criteria (EPA 2013c). DEQ will use the information to develop guidance for stakeholders to reference when applying for waterbody- and site-specific criteria. Work towards establishing standard procedures and best practices for this guidance began in 2024 and is expected to continue through 2027.

2.2 Other Sensitive Species

Though the 2013 EPA criteria update is specifically targeted at protecting unionid mussels, DEQ also assessed the resident status of other sensitive species in the EPA toxicity databases. These include the Mountain Whitefish, the most sensitive fish species in the database, the Lost River Sucker, an endangered species found only in Oregon and California, and the pebblesnail, the most sensitive non-unionid invertebrate. This review found Mountain Whitefish throughout the state, no reports or records of the Lost River Sucker, and several recorded pebblesnails. This confirmed that the toxicity dataset provided by EPA may not represent Idaho's resident species, and there may be toxicity data for species residing in Idaho that may better represent Idaho's aquatic life.

2.3 Idaho's Resident Species List

DEQ compiled a resident species list, based on best available knowledge, for all practical categories of species. The list was created using sources considered authoritative on their respective topics, with taxonomy updated to reflect Integrated Taxonomic Information System (ITIS 2025) per EPA recommendations. Resources used for resident species list compilation include the following:

- Fishes of Idaho: A Natural History Survey (Sigler and Zaroban 2018)
- Idaho DEQ 2023 Stream Benthos Taxa Report (EcoAnalysts 2023)
- Idaho Amphibian List (R. Dixon, IDFG, email to G. Hilborn, DEQ. "Amphibians of ID." October 27, 2025)
- Freshwater Mussels of the Pacific Northwest (Nedeau et al. 2009)

In situations where a taxon was not identified down to a species level, IDFG's species catalogue (IDFG 2025) was used to annotate lower-level taxonomic groupings that may be present. The species catalogue was not used as a primary source due to its inclusion of nonreviewed, citizen observations, and the inclusion of some species as a historical reference despite their extirpation or extinction.

Nonmollusk invertebrate species data was provided as a part of the Stream Benthos Taxa report, but was not reviewed or edited, due to the number of species included, and the relative ubiquity of the genus and species of this category included in EPA's toxicity dataset. As a result, DEQ retained all nonmollusk invertebrates in the toxicity dataset, to provide protection to Idaho residents of this type.

The resident fish, mollusk, and amphibian species list is included in Appendix A.

3 Toxicity Studies for Resident Unionids

With a list of resident unionids for the state completed, DEQ noted that Idaho only contained two families and three genera in the order unionida. These genera are not represented in the national toxicity datasets and are instead provided protection via taxonomic relation to the

tested unionids in the criteria dataset. If toxicity values are available for more closely related species, or for the resident species themselves, those values could be substituted for the nonresident unionids in EPA's dataset via EPA's criteria recalculation process (EPA 2013a). The recalculation procedure is a method used to adjust national aquatic life water quality criteria, so they better reflect the biological and taxonomic conditions of a specific body of water.

Since the release of EPA's 2013 criteria recommendations, several studies on the toxicity of ammonia to western unionids have been completed and were used for criteria recalculation in California's Central Valley (CVCWA 2020). Western pearlshell mussels (*Margaritifera margaritifera*) were studied in Wang et al. (2017), California floaters (*Anodonta californiensis*), Oregon floaters (*Anodonta oregonensis*) and western ridged mussels (*Gonidea Angulata*) were studied by Pacific EcoRisk in 2018 (PER 2018a, b, c). These studies were reviewed by DEQ and determined to be of high quality and suitable for criteria derivation purposes.

The species mean acute values (SMAVs) and genus mean acute values (GMAVs) derived from these studies demonstrated that western unionids are significantly less sensitive than the eastern unionids used in EPA's 2013 criteria recommendations. The acute values (adjusted to pH 7 and 20 °C) and their associated species are listed in Table 1.

Table 1. Acute data added to EPA ammonia toxicity database.

Scientific Name	Species	SMAV (mg/L)	GMAV (mg/L)	Reference
Anodonta oregonensis	Oregon floater	63.67	81.65	PER 2018a
Anodonta californiensis	California floater	104.7	81.65	PER 2018b
Margaritifera falcata	Western pearlshell	61.23	61.23	Wang et al. 2017
Gonidea angulata	Western ridged mussel	59.81	59.81	PER 2018c

Note: milligram per liter (mg/L)

4 Acute Criteria Recalculation Process

EPA developed a recalculation procedure (EPA 2013a) for creating a revised toxicity dataset for development of aquatic life criteria where residents differ from the respective EPA dataset. The procedure provides guidance on modifying the national toxicity dataset for ammonia by correcting, adding, and/or deleting test results for species not relevant to the site. Deletion is based on taxonomic composition of the site; tested species most closely related to those occurring at the site are retained as surrogates.

According to the recalculation procedure, a species included in the national toxicity dataset for the pollutant under consideration must be retained in the dataset and used to develop the final criterion if the species is a resident of the site, state, or region. However, if a species in the national toxicity dataset is not a resident and does not serve as a surrogate for another species, it may be deleted from the dataset used to calculate the criterion. Further, if data is available for resident species that are currently represented by a surrogate species, the surrogate toxicity values may be replaced by resident values, provided they no longer stand as a surrogate for another species.

4.1 Acute Dataset Deletion Process

EPA summarizes the revised deletion process as follows (EPA 2013a).

1. Looking within a genus, are all of its resident species tested? If so, delete the nonresident tested species in that genus. If not, retain them as surrogates.
2. Moving up to the family level, does every resident genus in a family contain at least one tested species? If so, delete the tested species in the family's nonresident genera. If not, retain them.
3. Moving up each subsequent level, to order, class, and phylum, the concept remains parallel. Does every resident family in an order contain at least one tested species? Does every resident order in a class contain at least one tested species? Does every resident class in a phylum contain at least one tested species? In each case, if so, delete the nonresident. If not, retain as surrogates.

Adding the western unionid toxicity values noted in Section 3 and then following the deletion process resulted in removing the species listed in Table 2 from the acute criteria dataset. These deletions retain resident species that are in the dataset and protect other residents with the most closely related surrogate species possible. This results in the most accurately protective criteria, without the inclusion of sensitive nonresidents that may cause the criteria to be overly restrictive. Most of the deleted species were nonresident unionids, replaced with toxicity values for resident species that directly represent Idaho's resident species, per EPA's recalculation procedure.

More precise site-specific criteria could be developed by conducting EPA's recalculation procedure for an individual waterbody or a selection of waterbodies. This procedure would be appropriate in waterbodies without salmonids or unionids. DEQ opted not to adopt tiered criteria based on presence or absence of sensitive residents due to the difficulty in performing large-scale surveys for unionids, the ubiquity of adult Rainbow Trout (the most sensitive species at low temperatures), and for simplicity in assessing use attainment and developing permits. DEQ will provide technical guidance to stakeholders seeking more localized site-specific criteria for ammonia. DEQ developed survey methods for determining unionid mussel presence/absence based on EPA's 2013 recommendations and existing survey techniques. The methods are undergoing revision and pilot testing in 2026.

Table 2. Acute toxicity data removed by deletion process.

Rank	Genus	Species	Common Name	GMAV (mg/L TAN)	Notes
37	Salmo	salar	Atlantic **sa**lmon	136.7	Extirpated in Idaho - Salmo genus is represented by Brown Trout
36	Morone	americana	White **pe**rch	134.8	No moronidae species in ID - all families in Perciformes are represented in dataset
36	Morone	chrysops	White **b**ass	134.8	No moronidae species in ID - all families in Perciformes are represented in dataset
36	Morone	saxatilis	Stripe**d ba**ss	134.8	No moronidae species in ID - all families in Perciformes are represented in dataset
36	Morone	saxatilis x chrysops	Sunshine **ba**ss	134.8	No moronidae species in ID - all families in Perciformes are represented in dataset
29	Alasmidonta	heterodon (LS)	Dwarf wedgemussel	>109.0	All ID unionids have toxicity values available at genus level or lower
28	Potamilis	ohiensis	Pink papershell	>109.0	All ID unionids have toxicity values available at genus level or lower
21	Micropterus	treculii	Guadalupe **b**ass	89.06	All other species in Micropterus genus are in toxicity database
15	Actinonaias	ligamentina	Mucket	71.25	All ID unionids have toxicity values available at genus level or lower
15	Actinonaias	pectorosa	Pheasantshell	71.25	All ID unionids have toxicity values available at genus level or lower
14	Pyganodon	grandis	Giant floater mussel	70.73	All ID unionids have toxicity values available at genus level or lower
9	Deltistes	luxatus (LS)	Lost River Sucker	56.62	All ID unionids have toxicity values available at genus level or lower
7	Fusconaia	masoni	Atlantic pigtoe	47.4	All ID unionids have toxicity values available at genus level or lower
6	Utterbackia	imbecillis	Pondshell mussel	46.93	All ID unionids have toxicity values available at genus level or lower
5	Lampsilis	abrupta (LS)	Pink Mucket	46.63	All ID unionids have toxicity values available at genus level or lower
5	Lampsilis	cardium	Plain pocketbook	46.63	All ID unionids have toxicity values available at genus level or lower
5	Lampsilis	fasciola	Wavy-rayed lampmussel	46.63	All ID unionids have toxicity values available at genus level or lower
5	Lampsilis	higginsii (LS)	Higgin's eye	46.63	All ID unionids have toxicity values available at genus level or lower
5	Lampsilis	rafinesqueana (LS)	Neosho Mucket	46.63	All ID unionids have toxicity values available at genus level or lower
5	Lampsilis	siliquoidea	Fatmucket	46.63	All ID unionids have toxicity values available at genus level or lower
4	Villosa (Cambarunio)	Iris	Rainbow Mussel	34.23	All ID unionids have toxicity values available at genus level or lower

Rank	Genus	Species	Common Name	GMAV (mg/L TAN)	Notes
3	Epioblasma	capsaeformis (LS)	Oyster Mussel	31.14	All ID unionids have toxicity values available at genus level or lower
2	Lasmigona	subviridis	Green Floater	23.41	All ID unionids have toxicity values available at genus level or lower
1	Venustaconcha	ellipsiformis	Ellipse	23.12	All ID unionids have toxicity values available at genus level or lower

Notes: milligram per liter (mg/liter); total ammonia nitrogen (TAN)

4.2 Idaho-Specific Acute Toxicity Dataset

Completing the deletion process resulted in an Idaho-specific acute toxicity dataset used to develop protective ammonia criteria. This dataset is still required to meet EPA's minimum requirements for developing water quality criteria (EPA 2010), including at least one family of the following types:

- Family Salmonidae in the class Osteichthyes
- Second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species
- Third family in the phylum cordata (may be in the Class Osteichthyes or may be an amphibian)
- Planktonic crustacean
- Benthic crustacean
- Insect
- Family in phylum other than Arthropoda or Chordata (e.g., Mollusca)
- Family in any order of insect or any phylum not already represented

DEQ reviewed the requirements and determined that the Idaho-specific dataset meets EPA's minimum thresholds for all eight family types. The dataset is presented in Table 3.

Per the criteria development guidelines, since there are more than 59 GMAVs in the dataset, the final acute value (FAV) is calculated from the four GMAVs closest to the 5th-percentile of the distribution. For the ID-specific dataset, this means that GMAVs ranked 2–5 are used in this calculation. These four species are the golden shiner, pebblesnail, western pearlshell, and western ridged mussel. Two of the four species used to calculate the FAV are resident unionids, confirming the orders' sensitivity to ammonia, and ensuring that the criteria will be protective of these residents. Note that the FAV calculation does not include the most sensitive species, Mountain Whitefish; although, the criteria derivation process accounts for this and provides criteria below the GMAV for Mountain Whitefish.

Table 3. Idaho-specific genus mean acute values (normalized to pH 7 and 20 °C).

Rank	Genus	Species	Common Name	GMAV (mg/L TAN)
60	Erythromma	Najas	Insect	2515
59	Philarctus	Quaeris	Caddisfly	994.5
58	Stenelmis	Sexlineata	Beetle	735.9
57	Orconectes (Faxonius)	Immunis	Crayfish	686.2
	Orconectes (Faxonius)	Nais	Crayfish	686.2
56	Chironomus	riparius	Midge	681.8
	Chironomus	Tentans	Midge	681.8
55	Drunella	Grandis	Mayfly	442.4
54	Caecidotea	Racovitzai	Aquatic sowbug	387
53	Asellus	Aquaticus	Isopod	378.2
52	Gasterosteus	Aculeatus	Threespine stickleback	281.5
51	Callibaetis	Skokianus	Mayfly	246.5
	Callibaetis	sp.	Mayfly	246.5
50	Pachydiplax	Longipennis	Blue Dasher	233
49	Cottus	Bairdii	Mottled sculpin	222.2
48	Gambusia	Affinis	Western mosquitofish	219.3
47	Lumbriculus	Variegatus	Oligochaete worm	218.7
46	Tubifex	Tubifex	Tubificid worm	216.5
45	Planorbella	Trivolvis	Marsh ramshorn snail	211.6
44	Hyalella	Azteca	Scud	192.6
43	Skwala	americana	Stonefly	192.4
42	Oreochromis	Mossambicus	Mozambique tilapia	185.2
41	Crangonyx	Pseudogracilis	Amphipod	181.8
	Crangonyx	sp.	Amphipod	181.8
40	Limnodrilus	Hoffmeisteri	Tubificid worm	170.2
39	Physa	Gyrina	Pouch snail	164.5
38	Enallagma	sp.	Damselfly	164
37	Chydorus	Sphaericus	Water flea	162.6
36	Pimephales	Promelas	Fathead minnow	159.2
35	Salvelinus	Fontinalis	Brook trout	157.8
	Salvelinus	Namaycush	Lake trout	157.8
34	Acipenser	brevirostrum (LS)	Shortnose sturgeon	156.7
33	Catostomus	Commersonii	White sucker	157.5
32	Pantosteus	Platyrhynchus	Mountain sucker	136.2
31	Ceriodaphnia	Acanthine	Water flea	143.9
	Ceriodaphnia	Dubia	Water flea	143.9
30	Simocephalus	Vetulus	Water flea	142.9
29	Ictalurus	Punctatus	Channel catfish	142.4
28	Procambarus	Clarkii	Red swamp crayfish	138
27	Salmo	Trutta	Brown trout	136.7
26	Daphnia	Magna	Water flea	125

Rank	Genus	Species	Common Name	GMAV (mg/L TAN)
	Daphnia	Pulicaria	Water flea	125
25	Xenopus	Laevis	Clawed toad	122.5
24	Dendrocoelum	Lacteum	Flatworm	119.5
23	Sander	Vitreus	Walleye	117.1
22	Campostoma	Anomalum	Central stoneroller	115.9
21	Micropterus	Dolomieu	Smallmouth bass	89.06
	Micropterus	Salmoides	Largemouth bass	89.06
20	Cyprinella	Lutrensis	Rainbow dace	110
	Cyprinella	Spiloptera	Spotfin shiner	110
	Cyprinella	Whipplei	Steelcolor shiner	110
19	Lepomis	Cyanellus	Green sunfish	106.9
	Lepomis	Gibbosus	Pumpkinseed	106.9
	Lepomis	Macrochirus	Bluegill	106.9
18	Cyprinus	Carpio	Common carp	106.3
17	Onchorhynchus	Aguabonita	Golden trout	99.15
	Onchorhynchus	Clarkii	Cutthroat trout	99.15
	Onchorhynchus	Gorbuscha	Pink salmon	99.15
	Onchorhynchus	kisutch (LS)	Coho salmon	99.15
	Onchorhynchus	mykiss (LS)	Rainbow trout	99.15
	Onchorhynchus	tsawytscha (LS)	Chinook salmon	99.15
16	Notropis	Topeka	Topeka shiner	96.72
15	Lithobates	Pipiens	Leopard frog	96.38
14	Musculium	Transversum	Long fingernailclam	89.36
13	Lymnaea	Stagnalis	Great pond snail	88.62
12	Anodonta	Californiensis	California Floater	81.65
	Anodonta	Oregonensis	Oregon Floater	81.65
11	Poecilia	Reticulata	Guppy	74.66
10	Etheostoma	Nigrum	Johnny darter	74.25
	Etheostoma	Spectabile	Orangethroat darter	74.25
9	Hybognathus	Amarus	Rio Grande silvery minnow	72.55
8	Pseudacris	Crucifer	Spring peeper	71.56
	Pseudacris	Regilla	Pacific tree frog	71.56
7	Chasmistes	Brevirostris	Shortnose sucker	69.36
6	Pleurocera	Uncialis	Pagoda hornsnail	68.54
5	Notemigonus	Crysoleucas	Golden shiner	63.02
4	Fluminicola	sp.	Pebblesnail	62.15
3	Margaritifera	Falcata	Western Pearlshell	61.23
2	Gonidea	Angulata	Western-Ridged Mussel	59.81
1	Prosopium	Williamsoni	Mountain Whitefish	51.93

Notes: milligram per liter (mg/liter); total ammonia nitrogen (TAN)

4.3 Recalculation of the Acute Criteria

Recalculation of the acute criteria began with derivation of an FAV according to the process described in the 2010 guidelines. The FAV, CMC, and species used to derive both numbers are presented in Table 4.

Table 4. Idaho-specific FAV and CMC.

Rank	GMAV (mgTAN/L)	Genus	Species	Common Name
5	63.02	*Notemigonus*	*crysoleucas*	Golden shiner
4	62.15	*Fluminicola*	*sp.*	Pebblesnail
3	61.23	*Margaritifera*	*falcata*	Western pearlshell
2	59.81	*Gonidea*	*angulata*	Western ridged mussel
1	51.93	*Prosopium*	*williamsoni*	Mountain Whitefish
FAV	61.16			
CMC	30.58			

Notes: milligram per liter (mg/L); total ammonia nitrogen (TAN)

The CMC shown in Table 4 is presumed to be protective of residents at a pH of 7 and 20 °C; however, the final criteria must be protective at all likely pH and temperature values. Since the most sensitive species include both invertebrates and vertebrates, the criteria must include the established pH and temperature dependance relationships (EPA 2013b). The pH dependency is captured in Equation 1 and the temperature dependency in Equation 2. The criteria must also protect for adult Rainbow Trout, which are the most sensitive species at low temperatures (Thurston and Russo 1983). DEQ followed EPA's 2013 recommendations to develop a criteria equation that reflects this.

Derivation of the criteria equation was done via the following process:

1. The FAV is 61.16, based on the rank 2–5 species in the dataset. To derive a protective CMC at pH 7 and 20 °C, the FAV is divided by two. Thus, the CMC is 30.58 mg TAN/L.
2. To control for effects of 5th-percentile extrapolation over the full range of pH and temperature conditions, the CMC is divided by the lowest GMAV in the ID-specific dataset, which is 51.93 mg TAN/L for Mountain Whitefish. This results in a conversion factor of 0.5888.
3. DEQ uses the CMC, the 5th-percentile protection factor, and the known pH and temperature relationship equations (Equation 1 and Equation 2, respectively), and the lowest GMAVs for invertebrates (59.81) and fish (51.93), to derive an acute criteria equation that is protective of Idaho's most sensitive species (Equation 3).

$$AV_{t,7} = {AV_t}\Big/\left(\frac{0.0114}{1+10^{7.204-pH}}+\frac{1.6181}{1+10^{pH-7.204}}\right)$$

Equation 1. Acute value pH conversion equation.

Where AV_t is the acute value at temperature t, and $AV_{t,7}$ is the acute value at temperature t and pH 7.

$$\log(AV_{t,7,20}) = log(AV_{t,7}) - [-0.036(T - 20)]$$

Equation 2. Acute value temperature conversion equation.

Where $AV_{t,7,20}$ is the acute value at 20 °C and pH 7.

$$CMC = 0.5888 \times \left(\frac{0.0114}{1 + 10^{7.204-pH}} + \frac{1.6181}{1 + 10^{pH-7.204}}\right) \times MIN(51.93, \left(59.81 \times 10^{0.036\times(20-T)}\right)$$

Equation 3. Acute criteria equation for toxicity dataset.

4. Although the GMAV for Mountain Whitefish is the lowest in the dataset, adult Rainbow Trout (*Oncorhynchus mykiss*) have a known sensitivity to ammonia at low temperatures that exceeds that of other life stages. The acute value for adult Rainbow Trout is 48.21 mg TAN/L, resulting in a CMC of 24.10. Incorporating this CMC with the pH relationship equation produces Equation 4, which produces criteria that are protective of adult Rainbow Trout.

$$CMC = 24.10 \times \left(\frac{0.0114}{1 + 10^{7.204-pH}} + \frac{1.6181}{1 + 10^{pH-7.204}}\right)$$

Equation 4. Criteria equation for protection of adult rainbow trout.

5. Combining Equation 3 and Equation 4 and simplifying provides a criteria equation that is protective of Idaho's resident invertebrates and Idaho's resident fish and protects for adult Rainbow Trout at low temperatures (Equation 5).

$$CMC = MIN\left(\begin{array}{c}\left(\frac{0.275}{1 + 10^{7.204-pH}} + \frac{39.0}{1 + 10^{pH-7.204}}\right), \\ \left(\frac{0.4015}{1 + 10^{7.204-pH}} + \frac{56.98}{1 + 10^{pH-7.204}}\right) \times 10^{0.036\times(20-T)}\end{array}\right)$$

Equation 5. Acute criteria equation for protection of all Idaho species

Notably, Equation 5 produces criteria values equivalent to Idaho's current ammonia standards at temperatures less than or equal to 24 °C, as adult Rainbow Trout continue to be the most sensitive species under these conditions. When temperatures exceed these conditions, however, the sensitivity of resident unionids and other invertebrates is increased such that their protection requires lower levels of ammonia than is required to protect adult Rainbow Trout.

4.4 Recalculation of the Chronic Criteria

DEQ reviewed several methods for calculating chronic toxicity values from the studies cited in the acute recalculation, including those cited in California's Central Valley Ammonia Criteria Recalculation report (CVCWA 2020). In that report, an EPA comment provides a chronic criteria

recalculation that translates GMAVs from the acute dataset into chronic toxicity values for each genus of unionid in Idaho using a literature-derived acute-to-chronic ratio (ACR) of 15.52. This translation provided surrogate genus mean chronic values (GMCVs) for the three genera of unionid present in Idaho, shown in Table 5.

Table 5. Resident unionid GMAVs and surrogate GMCVs.

Genus	GMAV (mg TAN/L)	Unionid Family ACR	Surrogate GMCV (mg TAN/L)
Anodonta	81.65	15.52	5.261
Margaritifera	61.23	15.52	3.945
Gonidea	59.81	15.52	3.854

Notes: milligram per liter (mg/liter); total ammonia nitrogen (TAN)

These surrogate GMCVs can be used to replace the nonresident unionid GMCVs in EPA's chronic toxicity dataset. The chronic toxicity dataset included three species of unionids that are not residents of Idaho (i.e., rainbow mussel, fatmucket, and wavy-rayed lamp mussel). All other species in the chronic dataset are either residents of Idaho, or they represent a resident species. The species deletion and toxicity data replacement needed was determined to solely relate to the newly available toxicity data for Idaho's resident unionids. The chronic toxicity values removed by deletion are shown in Table 6.

Table 6. Chronic toxicity data removed by deletion process.

Rank	Genus	Species	Common Name	SMCV (mg/L TAN)	GMCV (mg/L TAN)	Notes
2	*Villosa*	*iris*	Rainbow mussel	3.501	3.501	GMAVs available for resident unionids, converted to GMCV's using unionid ACR
1	*Lampsilis*	*siliquoidea*	Fatmucket	3.211	2.126	GMAVs available for resident unionids, converted to GMCV's using unionid ACR
1	*Lampsilis*	*fasciola*	Wavy-rayed lamp mussel	1.408	2.126	GMAVs available for resident unionids, converted to GMCV's using unionid ACR

Notes: milligram per liter (mg/liter); total ammonia nitrogen (TAN)

Addition of the surrogate GMCVs and deletion of nonresident unionids from EPA's chronic toxicity dataset results in an Idaho-specific chronic toxicity dataset, shown in Table 7. This chronic toxicity dataset satisfies EPA's 1985 minimum data requirements and can be used to develop a chronic criteria equation.

Since the Idaho-specific chronic toxicity dataset contains only 17 GMCVs, the four most sensitive values are used to calculate the final chronic value (FCV) and CCC (at pH 7 and 20 °C) shown in Table 8.

Table 7. Idaho-specific genus mean chronic values (normalized to pH 7 and 20 °C).

Rank	Genus	Species	Common Name	GMCV (mg TAN/L)
17	*Pteronarcella*	*Badia*	Stonefly	73.74
16	*Ceriodaphnia*	*Acanthina*	Water Flea	53.75
	Ceriodaphnia	*Dubia*	Water Flea	
15	*Daphnia*	*Magna*	Water Flea	41.46
14	*Hyalella*	*Azteca*	Amphipod	29.17
13	*Ictalurus*	*Punctatus*	Channel Catfish	21.36
12	*Esox*	*Lucius*	Northern Pike	20.38
11	*Cyprinus*	*Carpio*	Common Carp	16.53
10	*Oncorhynchus*	*Clarkii henshawi (LS)*	Lahontan Cutthroat Trout	12.02
	Oncorhynchus	*Mykiss (LS)*	Rainbow Trout	
	Oncorhynchus	*Nerka (LS)*	Sockeye Salmon	
9	*Catostomus*	*Commersonii*	White Sucker	11.62
8	*Micropterus*	*Dolomieu*	Smallmouth Bass	11.07
7	*Pimephales*	*Promelas*	Fathead Minnow	9.187
6	*Fluminicola*	*sp.*	Pebblesnail	7.828
5	*Musculium*	*Transversum*	Long Fingernail Clam	7.547
4	*Lepomis*	*Cyanellus*	Green Sunfish	6.92
	Leopomis	*Macrochirus*	Bluegill	
3	*Anodonata*	*Californiensis*	California Floater	5.261
	Anodonata	*Oregonensis*	Oregon Floater	
2	*Margaritifera*	*Falcata*	Western Pearlshell	3.945
1	*Gonidea*	*Angulata*	Western-Ridged Mussel	3.854

Notes: milligram per liter (mg/liter); total ammonia nitrogen (TAN)

Table 8. Idaho-specific FCV and CCC.

Rank	GMCV (mg TAN/L)	Genus	Species	Common Name
4	6.920	*Lepomis*	*cyanellus*	Green sunfish
			macrochyrus	Bluegill
3	5.261	*Anodonta*	*sp.*	Floater surrogate
2	3.945	*Margaritifera*	*falcata*	Western pearlshell surrogate
1	3.854	*Gonidea*	*angulata*	Western ridged mussel surrogate
FCV	3.327			
CCC	3.3			

Notes: milligram per liter (mg/L); total ammonia nitrogen (TAN)

The FCV is combined with the known chronic invertebrate relationships to pH and temperature, as well as the species mean chronic value (SMCV) for adult Rainbow Trout, which continues to be the most sensitive species at low temperatures. This chronic criteria in Equation 6 provides for protection of Idaho resident species at all temperatures and pH conditions.

$$CCC = 0.8634 \times \left(\frac{0.0278}{1 + 10^{7.688-pH}} + \frac{1.1994}{1 + 10^{pH-7.688}}\right) \times MIN\left(6.663, 3.854 \times 10^{0.028\times(20-T)}\right)$$

Equation 6. Chronic criteria equation for protection of all Idaho species.

The results of this equation are criteria that are more protective than Idaho's current criteria. For example, at pH 7 and 20 °C, the chronic criterion decreases from 4.9 mg TAN/L to 3.3 mg TAN/L. As temperatures decrease, invertebrate sensitivity decreases until the sensitivity of Rainbow Trout exceeds that of resident unionids.

Equation 6 is simplified to an equivalent form in Equation 7.

$$CCC = \left(\frac{0.0240}{1 + 10^{7.688-pH}} + \frac{1.0356}{1 + 10^{pH-7.688}}\right) \times MIN\left(6.663, 3.854 \times 10^{0.028\times(20-T)}\right)$$

Equation 7. Simplified chronic criteria equation for protection of all Idaho species.

DEQ notes that this deletion process, and the resulting FCV, and CCC equation, were all developed by EPA and proposed for use by CVCWA instead of using one singular unionid surrogate value, to more closely resemble the development of GMCVs for each resident unionid genus (CVCWA 2020). The species included in the state-specific chronic toxicity database are representative of both the Central Valley and Idaho's resident species; therefore, DEQ decided to adopt EPA's alternative recommendation without any modifications, other than mathematical simplification.

5 Protectiveness of Recalculated Ammonia Criterion

5.1 Resident Species

The criteria equations that DEQ developed are believed to be protective of Idaho's resident species due to the quality of toxicity studies available for Idaho's residents, the use of known relationships between pH and temperature conditions for fish and invertebrates, and DEQ's adherence to EPA-approved criteria derivation and recalculation methods.

EPA's deletion process maintains representation of resident species even when not included in the GMAV/GMCV datasets. This representation ensures criteria development continues with a conservative approach by maintaining surrogate species that are most closely related to the residents. The use of criteria equations, instead of single number criteria, maintains EPA and DEQ's approach to the changing toxicity of ammonia to aquatic organisms under different pH and temperature conditions. The relationship equations for pH and temperature and acute to chronic ratios used to develop GMCVs were taken from EPA's 2013 criteria recommendations and reflect the latest science on ammonia toxicity.

As discussed in the acute and chronic derivation sections, there are no conditions under which the proposed criteria are less protective than DEQ's current ammonia criteria. Incorporating the equation that accounts for the sensitivity of adult Rainbow Trout ensures continued protection of this important resident species and maintains consistency with DEQ's existing criteria. DEQ is not aware of any new information that indicates the existence of resident species not represented in the GMAV and GMCV datasets or evidence suggesting that resident species would not be adequately protected from adverse effects where the proposed criteria are met.

5.2 Downstream Waters

Idaho water quality standards for aquatic life criteria must be met in all Idaho waters and at the pour points of upstream waters into Idaho waterbodies. If a waterbody does not meet an aquatic life criterion, additional tools are employed to identify the source of the pollutant and address the issue (e.g., total maximum daily loads, source identification, point-source permit limits) so that aquatic life are protected within the waterbody.

Protecting downstream waters is required in IDAPA 58.01.02.070.08, which states that all waters must maintain a level of water quality at their pour point into downstream waters that provides for the attainment and maintenance of the water quality standards of those downstream waters, including waters of another state or tribe. States that neighbor Idaho have varying ammonia standards that must be met at the pour point; however, if multiple standards are applicable in a water, the most restrictive criteria apply.

5.3 Beneficial Uses

Under IDAPA 58.01.02, the waterbodies within a site have the following designated or presumed beneficial uses:

- Cold water—water quality appropriate for the protection and maintenance of a viable aquatic life community for cold water species.
- Salmonid spawning—a subset of cold water aquatic life beneficial uses, these waters provide or could provide a habitat for active self-propagating populations of salmonid fishes.
- Warm water—water quality appropriate for the protection and maintenance of a viable aquatic life community for warm water species.
- Modified—water quality appropriate for an aquatic life community that is limited due to one or more conditions stated in 40 CFR 131.10(g), which preclude attainment of reference streams or conditions.

The proposed ammonia criteria would apply to waters designated for cold water aquatic life use, inclusive of salmonid spawning waters (as no water is designated for salmonid spawning without also being designated for cold water aquatic life). Warm water criteria remain unchanged by this rulemaking. DEQ also intends to remove the site-specific criteria for the Spokane River (IDAPA 58.01.02.283) as these site-specific criteria were developed prior to DEQ adopting EPA's 1999 recommended criteria and are identical to DEQ's current statewide

criteria. The criteria were not adopted due to the unique character of the Spokane River or its resident species but to implement the updated criteria within that geographic area before the statewide adoption of EPA's 1999 recommendations. As a result, the site-specific criteria are now redundant and no longer necessary.

References

CVCWA (Central Valley Clean Water Association). 2020. *Phase IIc Freshwater Mussel Collaborative Study for Wastewater Treatment Plants: Ammonia Criteria Recalculation Final Report*. Fairfield, CA: Pacific EcoRisk, Robertson-Brayn Inc, Larry Walker Associates.

EcoAnalysts. 2023. *Idaho DEQ 2023 Stream Benthos Taxa Report*. Moscow, ID: EcoAnalysts.

EPA (US Environmental Protection Agency). 1999. *1999 Update of Ambient Water Quality Criteria for Ammonia - Freshwater*. Washington, D.C.: Office of Water. EPA-822-R-99-014.

EPA (US Environmental Protection Agency). 2010. *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*. Duluth, MN: Office of Research and Development. PB85-227049.

EPA (US Environmental Protection Agency). 2013a. *Revised Deletion Process for the Site-Specific Recalculation Procedure for Aquatic Life Criteria*. Washington, D.C.: Office of Water, Office of Science and Technology. EPA-823-R-13-001.

EPA (US Environmental Protection Agency). 2013b. *Aquatic Life Ambient Water Quality Criterion for Ammonia – Freshwater 2013*. Washington, D.C.: Office of Water. Office of Science and Technology. EPA 822-R-18-002.

EPA (US Environmental Protection Agency). 2013c. *Technical Support Document for Conducting and Reviewing Freshwater Mussel Occurrence Surveys for the Development of Site-specific Water Quality Criteria for Ammonia*. Washington, D.C.: Office of Water. Office of Science and Technology. EPA 800-R-13-003.

IDAPA. 2025. "Water Quality Standards." Idaho Administrative Code. IDAPA 58.01.02

Nedeau, E.J., A.K. Smith, J. Stone, and S. Jepsen. 2009. *Freshwater Mussels of the Pacific Northwest.* 2nd ed. Portland, OR: Xerces Society.

IDFG (Idaho Department of Fish and Game). 2024. *IDFG Mussel Survey Summary for IDEQ.*

IDFG (Idaho Department of Fish and Game). 2025. *The Idaho Species Catalog*. *https://idfg.idaho.gov/species/*.

ITIS (Integrated Taxonomic Information System). 2025. “Integrated Taxonomic Information System on-line database.” *itis.gov*.

PER (Pacific EcoRisk). 2018a. *Acute Sensitivity of the Freshwater Mussel Anodonta oregonensis to Ammonia*. Fairfield, CA: Pacific EcoRisk.

PER (Pacific EcoRisk). 2018b. *Acute Sensitivity of the Freshwater Mussel Anodonta californiensis to Ammonia. Fairfield*, CA: Pacific EcoRisk.

PER (Pacific EcoRisk). 2018c. *Acute Sensitivity of the Freshwater Mussel Gonidea angulata to Ammonia*. Fairfield, CA: Pacific EcoRisk.

Russo, R.C. 1985. “Ammonia, Nitrite, and Nitrate.” In: *Fundamentals of Aquatic Toxicology and Chemistry*. Rand, G.M. and S.R. Petrocelli, eds. Hemisphere Publishing Corp., Washington, D.C.

Sigler, J.W. and D.W. Zaroban. 2018. *Fishes of Idaho: A Natural History Survey*. Caldwell, ID: Caxton Press.

Thurston, R.V. and R.C. Russo. 1983. “Acute Toxicity of Ammonia to Rainbow Trout.” *Transactions of the American Fisheries Society*. 112: 696–704.

Wang, N., C.D. Ivey, C.G. Ingersoll, W.G. Brumbaugh, D. Alvarez, E.J. Hammer, C.R. Bauer, T. Augsourger, S. Raimondo, M.C. Barngart. 2017. "Acute Sensitivity of a Broad Range of Freshwater Mussels to Chemicals with Different Modes of Toxic Action." *Environmental Toxicology and Chemistry*. 36(3): 786–796.

Xerces (Xerces Society for Invertebrate Conservation and the Confederated Tribes of the Umatilla Indian Reservation Mussel Project). 2024. *Western Freshwater Mussel Database.*

Appendix A. Resident Fishes, Mollusks, and Amphibians of Idaho

Class	Order	Family	Genus	Species	Common name	Still Resident?	Note
Amphibia	Anura	Ascaphidae	Ascaphus	Montanus	Rocky Mountain Tailed Frog	Yes	
Amphibia	Anura	Bufonidae	Anaxyrus	Boreas	Western Toad	Yes	
Amphibia	Anura	Bufonidae	Anaxyrus	Woodhousii	Woodhouse's Toad	Yes	
Amphibia	Anura	Hylidae	Pseudacris	Maculata	Boreal Chorus Frog	Yes	
Amphibia	Anura	Hylidae	Pseudacris	Regilla	Pacific Treefrog	Yes	
Amphibia	Anura	Ranidae	Amerana	Luteiventris	Columbia Spotted Frog	Yes	
Amphibia	Anura	Ranidae	Aquarana	Catesbeiana	North American Bullfrog	Yes	
Amphibia	Anura	Ranidae	Boreorana	Sylvatica	Wood Frog	Unknown	1
Amphibia	Anura	Ranidae	Lithobates	Pipiens	Northern Leopard Frog	Yes	
Amphibia	Anura	Scaphiopodidae	Spea	Intermontana	Great Basin Spadefoot	Yes	
Amphibia	Caduata	Ambystomatidae	Ambystoma	macrodactylum	Long Toed Salamander	Yes	
Amphibia	Caduata	Ambystomatidae	Ambystoma	Mavortium	Western Tiger Salamander	Yes	
Amphibia	Caduata	Ambystomatidae	Dicamptodon	Aterrimus	Idaho Giant Salamander	Yes	
Amphibia	Caduata	Plethodontidae	Plethodon	Idahoensis	Coeur d'Alene Salamander	Yes	
Amphibia	Caduata	Salamandridae	Taricha	Granulosa	Rough Skinned Newt	Yes	
Bivalvia	Sphaeriida	Sphaeriidae	Musculium		Musculium sp.	Yes	2
Bivalvia	Sphaeriida	Sphaeriidae	Pisidium	casertanum	Ubiquitous Peaclam	Yes	
Bivalvia	Sphaeriida	Sphaeriidae	Pisidium	compressum	Ridged-Beak Peaclam	Yes	
Bivalvia	Sphaeriida	Sphaeriidae	Pisidium	idahoense	Giant Northern Peaclam	Yes	
Bivalvia	Sphaeriida	Sphaeriidae	Pisidium		Pisidium sp.	Yes	2
Bivalvia	Sphaeriida	Sphaeriidae	Sphaerium	patella	Rocky Mountain Fingernail Clam	Yes	
Bivalvia	Sphaeriida	Sphaeriidae	Sphaerium	striatinum	Striated Fingernail Clam	Yes	
Bivalvia	Sphaeriida	Sphaeriidae	Sphaerium		Sphaerium sp.	Yes	2
Bivalvia	Unionida	Margaritiferidae	Margaritifera	falcata	Western Pearlshell	Yes	
Bivalvia	Unionida	Margaritiferidae	Margaritifera	margaritifera	Eastern Pearlshell	Yes	
Bivalvia	Unionida	Margaritiferidae	Margaritifera		Margaritifera sp.	Yes	2

Class	Order	Family	Genus	Species	Common name	Still Resident?	Note
Bivalvia	Unionida	Unionidae	Anodonta	nuttalliana	Winged Floater	Yes	
Bivalvia	Unionida	Unionidae	Anodonta		Anodonta sp.	Yes	2
Bivalvia	Unionida	Unionidae	Gonidea	angulata	Western Ridged Mussel	Yes	
Bivalvia	Veneroida	Cyrenidae	Corbicula	fluminea	Asian Clam	Yes	
Bivalvia	Veneroida	Cyrenidae	Corbicula		Corbicula sp.	Yes	2
Cephalaspidimorphi	Petromyzontiformes	Petromyzontidae	Entosphenus	tridentatus	Pacific Lamprey	Yes	
Chondrostei	Acipenceriformes	Acipenseridae	Acipenser	transmontanus	White Sturgeon	Yes	
Gastropoda	Cerithiida	Thiaridae			Thiaridae	Yes	2
Gastropoda	Ectobranchia	Valvatidae	Valvata	humeralis	Glossy Valvata	Yes	
Gastropoda	Ectobranchia	Valvatidae	Valvata	utahensis	Desert Valvata	Yes	
Gastropoda	Ectobranchia	Valvatidae	Valvata		Valvata sp.	Yes	2
Gastropoda	Littorinida	Amnicolidae	Amnicola		Amnicola sp.	Yes	2
Gastropoda	Littorinida	Hydrobiidae	Pyrgulopsis	robusta	Jackson Lake Springsnail	Yes	
Gastropoda	Littorinida	Hydrobiidae	Pyrgulopsis		Pyrgulopsis sp.	Yes	2
Gastropoda	Littorinida	Hydrobiidae	Prionoxystus		Prionoxystus sp.	Yes	2
Gastropoda	Littorinida	Hydrobiidae			Hydrobiidae	Yes	2
Gastropoda	Littorinida	Lithoglyphidae	Fluminicola	fuscus	Ashy Pebblesnail	Yes	
Gastropoda	Littorinida	Lithoglyphidae	Fluminicola		Fluminicola sp.	Yes	2
Gastropoda	Littorinida	Lithoglyphidae	Pristinicola	hemphilli	Pristine Pyrg	Yes	
Gastropoda	Littorinida	Lithoglyphidae	Pristinicola		Pristinicola sp.	Yes	2
Gastropoda	Littorinida	Tateidae	Potamopyrgus	antipodarum	New Zealand mud snail	Yes	
Gastropoda	Littorinida	Tateidae	Potamopyrgus		Potamopyrgus sp.	Yes	2
Gastropoda	Lymnaeida	Lymnaeidae	Fisherola	nuttalli	Shortface Lanx	Yes	
Gastropoda	Lymnaeida	Lymnaeidae	Galba		Galba sp.	Yes	2
Gastropoda	Lymnaeida	Lymnaeidae	Idaholanx	fresti	Banbury Springs Lanx	Yes	
Gastropoda	Lymnaeida	Lymnaeidae	Lanx		Lanx sp.	Yes	2
Gastropoda	Lymnaeida	Lymnaeidae	Lymnaea	stagnalis	Swamp Lymnaea	Yes	
Gastropoda	Lymnaeida	Lymnaeidae	Lymnaea		Lymnaea sp.	Yes	2
Gastropoda	Lymnaeida	Lymnaeidae	Radix	auricularia	Big-Earred Radix	Yes	

Class	Order	Family	Genus	Species	Common name	Still Resident?	Note
Gastropoda	Lymnaeida	Lymnaeidae	Stagnicola		Stagnicola sp.	Yes	2
Gastropoda	Lymnaeida	Lymnaeidae			Lymnaeidae	Yes	2
Gastropoda	Lymnaeida	Physidae	Physa		Physa (Physella) sp.	Yes	2
Gastropoda	Lymnaeida	Physidae			Physidae	Yes	2
Gastropoda	Lymnaeida	Planorbidae	Ferrissia	rivularis	Creeping Ancylid	Yes	
Gastropoda	Lymnaeida	Planorbidae	Ferrissia		Ferrissia sp.	Yes	2
Gastropoda	Lymnaeida	Planorbidae	Gyraulus		Gyraulus sp.	Yes	2
Gastropoda	Lymnaeida	Planorbidae	Helisoma		Helisoma sp.	Yes	2
Gastropoda	Lymnaeida	Planorbidae	Menetus	opercularis	Button Sprite	Yes	
Gastropoda	Lymnaeida	Planorbidae	Planorbella		Planorbella sp.	Yes	2
Gastropoda	Lymnaeida	Planorbidae	Promenetus	umbilicatellus	Umbilicate Sprite	Yes	
Gastropoda	Lymnaeida	Planorbidae	Promenetus		Promenetus sp.	Yes	2
Gastropoda	Lymnaeida	Planorbidae	Vorticifex	effusa	Artemesian Ramshorn	Yes	
Gastropoda	Lymnaeida	Planorbidae	Vorticifex		Vorticifex sp.	Yes	2
Gastropoda	Lymnaeida	Planorbidae			Planorbidae	Yes	2
Gastropoda	Lymnaeida	Semisulocospiridae	Juga		Juga sp.	Yes	2
Gastropoda	Lymnaeida	Pleuroceridae			Pleuroceridae	Yes	2
Teleostei	Clupeiformes	Clupeidae	Alosa	sapidissima	American Shad	Yes	
Teleostei	Cypriniformes	Catostomidae	Catostomus	ardens	Utah Sucker	Yes	
Teleostei	Cypriniformes	Catostomidae	Catostomus	catostomus	Longnose Sucker	Yes	
Teleostei	Cypriniformes	Catostomidae	Catostomus	columbianus	Bridgelip Sucker	Yes	
Teleostei	Cypriniformes	Catostomidae	Catostomus	macrocheilus	Largescale Sucker	Yes	
Teleostei	Cypriniformes	Catostomidae	Chasmistes	muriei	Snake River Sucker	No	3
Teleostei	Cypriniformes	Catostomidae	Pantosteus	bondi	Cascadian Sucker	Yes	
Teleostei	Cypriniformes	Catostomidae	Pantosteus	platyrhynchus	Mountain Sucker	Yes	
Teleostei	Cypriniformes	Catostomidae	Pantosteus	virescens	Green Sucker	Yes	
Teleostei	Cypriniformes	Cobitidae	Misgurnus	anguillicaudatus	Oriental Weatherfish	Yes	
Teleostei	Cypriniformes	Cyprinidae	Carassius	auratus	Goldfish	Yes	
Teleostei	Cypriniformes	Cyprinidae	Ctenopharyngodon	idella	Grass Carp	Yes	

Class	Order	Family	Genus	Species	Common name	Still Resident?	Note
Teleostei	Cypriniformes	Cyprinidae	Cyprinus	carpio	Common Carp	Yes	
Teleostei	Cypriniformes	Cyprinidae	Tinca	tinca	Tench	Yes	
Teleostei	Cypriniformes	Leuciscidae	Couesius	plumbeus	Lake Chub	Yes	
Teleostei	Cypriniformes	Leuciscidae	Gila	alutacea	Chiselmouth	Yes	
Teleostei	Cypriniformes	Leuciscidae	Gila	atraria	Utah Chub	Yes	
Teleostei	Cypriniformes	Leuciscidae	Lepidomeda	copei	Northern Leatherside Chub	Yes	
Teleostei	Cypriniformes	Leuciscidae	Mylocheilus	caurinus	Peamouth	Yes	
Teleostei	Cypriniformes	Leuciscidae	Notemigonus	crysoleucus	Golden Shiner	Yes	
Teleostei	Cypriniformes	Leuciscidae	Notropis	hudsonius	Spottail Shiner	Yes	
Teleostei	Cypriniformes	Leuciscidae	Pimephales	promelas	Fathead Minnow	Yes	
Teleostei	Cypriniformes	Leuciscidae	Ptychocheilus	oregonensis	Northern Pikeminnow	Yes	
Teleostei	Cypriniformes	Leuciscidae	Rhinichthys	cataractae	Longnose Dace	Yes	
Teleostei	Cypriniformes	Leuciscidae	Rhinichthys	falcatus	Leopard Dace	Yes	
Teleostei	Cypriniformes	Leuciscidae	Rhinichthys	osculus	Speckled Dace	Yes	
Teleostei	Cypriniformes	Leuciscidae	Rhinichthys	umatilla	Umatilla Dace	Yes	
Teleostei	Cypriniformes	Leuciscidae	Richardsonius	balteatus	Redside Shiner	Yes	
Teleostei	Cypriniformes	Leuciscidae	Siphateles	bicolor	Tui Chub	Yes	
Teleostei	Cyprinodontiformes	Fundulidae	Fundulus	diaphanus	Banded Killfish	Yes	
Teleostei	Cyprinodontiformes	Poecillidae	Gambusia	affinis	Western Mosquitofish	Yes	
Teleostei	Cyprinodontiformes	Poecillidae	Poecilia	mexicana	Shortfin Molly	Yes	
Teleostei	Cyprinodontiformes	Poecillidae	Poecilia	reticulata	Guppy	Yes	
Teleostei	Cyprinodontiformes	Poecillidae	Xiphophorus	hellerii	Green Swordtail	Yes	
Teleostei	Cyprinodontiformes	Poecillidae	Xiphophorus		Platy sp.	Yes	
Teleostei	Esociformes	Esocidae	Esox	lucius	Northern Pike	Yes	
Teleostei	Gadiformes	Gadidae	Lota	lota	Burbot	Yes	
Teleostei	Osmeriformes	Osmeridae	Osmerus	mordax	Rainbow Smelt	No	3
Teleostei	Perciformes	Centrarchidae	Ambloplites	rupestris	Rock Bass	No	3
Teleostei	Perciformes	Centrarchidae	Lepomis	cyanellus	Green Sunfish	Yes	
Teleostei	Perciformes	Centrarchidae	Lepomis	gibbosus	Pumpkinseed	Yes	

Class	Order	Family	Genus	Species	Common name	Still Resident?	Note
Teleostei	Perciformes	Centrarchidae	Lepomis	gulosus	Warmouth	Yes	
Teleostei	Perciformes	Centrarchidae	Lepomis	humilis	Orangespotted Sunfish	No	3
Teleostei	Perciformes	Centrarchidae	Lepomis	macrochirus	Bluegill	Yes	
Teleostei	Perciformes	Centrarchidae	Micropterus	dolomieu	Smallmouth Bass	Yes	
Teleostei	Perciformes	Centrarchidae	Micropterus	salmoides	Largemouth Bass	Yes	
Teleostei	Perciformes	Centrarchidae	Pomoxis	annularis	White Crappie	Yes	
Teleostei	Perciformes	Centrarchidae	Pomoxis	nigromaculatus	Black Crappie	Yes	
Teleostei	Perciformes	Cichlidae	Archocenctrus	nigrofasciata	Convict Cichlid	Yes	
Teleostei	Perciformes	Cichlidae	Astronotus	ocellatus	Oscar	Yes	
Teleostei	Perciformes	Cichlidae	Oreochromis	aureus	Blue Tilapia	Yes	
Teleostei	Perciformes	Cichlidae	Oreochromis	mossambicus	Mozambique Tilapia	Yes	
Teleostei	Perciformes	Cichlidae	Tilapia	zillii	Redbelly Tilapia	Yes	
Teleostei	Perciformes	Percidae	Perca	flavescens	Yellow Perch	Yes	
Teleostei	Perciformes	Percidae	Sander	canadensis	Sauger	No	3
Teleostei	Perciformes	Percidae	Sander	vitreus	Walleye	Yes	
Teleostei	Percopsiformes	Percopsidae	Percopsis	transmontana	Sand Roller	Yes	
Teleostei	Salmoniformes	Salmonidae	Coregonus	clupeaformis	Lake Whitefish	Yes	
Teleostei	Salmoniformes	Salmonidae	Oncorhynchus	aguabonita	Golden Trout	Yes	
Teleostei	Salmoniformes	Salmonidae	Oncorhynchus	clarkii	Cutthroat Trout	Yes	
Teleostei	Salmoniformes	Salmonidae	Oncorhynchus	clarkii behnkei	Snake River Fine-Spotted Cutthroat Trout	Yes	
Teleostei	Salmoniformes	Salmonidae	Oncorhynchus	clarkii bouvieri	Yellowstone Cutthroat Trout	Yes	
Teleostei	Salmoniformes	Salmonidae	Oncorhynchus	clarkii lewisi	Westslope Cutthroat Trout	Yes	
Teleostei	Salmoniformes	Salmonidae	Oncorhynchus	clarkii utah	Bonneville Cutthroat Trout	Yes	
Teleostei	Salmoniformes	Salmonidae	Oncorhynchus	keta	Chum Salmon	No	4
Teleostei	Salmoniformes	Salmonidae	Oncorhynchus	kisutch	Coho Salmon	Yes	
Teleostei	Salmoniformes	Salmonidae	Oncorhynchus	mykiss	Rainbow Trout/Steelhead	Yes	
Teleostei	Salmoniformes	Salmonidae	Oncorhynchus	mykiss	Redband Trout	Yes	
Teleostei	Salmoniformes	Salmonidae	Oncorhynchus	mykiss	Steelhead Trout	Yes	

Class	Order	Family	Genus	Species	Common name	Still Resident?	Note
Teleostei	Salmoniformes	Salmonidae	Oncorhynchus	nerka	Kokanee Salmon	Yes	
Teleostei	Salmoniformes	Salmonidae	Oncorhynchus	nerka	Sockeye Salmon	Yes	
Teleostei	Salmoniformes	Salmonidae	Oncorhynchus	tshawytscha	Chinook Salmon	Yes	
Teleostei	Salmoniformes	Salmonidae	Prosopium	abyssicola	Bear Lake Whitefish	Yes	
Teleostei	Salmoniformes	Salmonidae	Prosopium	coulterii	Pygmy Whitefish	Yes	
Teleostei	Salmoniformes	Salmonidae	Prosopium	gemmifer	Bonneville Cisco	Yes	
Teleostei	Salmoniformes	Salmonidae	Prosopium	spilonotus	Bonneville Whitefish	Yes	
Teleostei	Salmoniformes	Salmonidae	Prosopium	williamsoni	Mountain Whitefish	Yes	
Teleostei	Salmoniformes	Salmonidae	Salmo	salar	Atlantic Salmon	No	4
Teleostei	Salmoniformes	Salmonidae	Salmo	trutta	Brown Trout	Yes	
Teleostei	Salmoniformes	Salmonidae	Salvelinus	alpinus oquassa	Sunapee Trout	Yes	
Teleostei	Salmoniformes	Salmonidae	Salvelinus	confluentus	Bull Trout	Yes	
Teleostei	Salmoniformes	Salmonidae	Salvelinus	fontinalus	Brook Trout	Yes	
Teleostei	Salmoniformes	Salmonidae	Salvelinus	namaycush	Lake Trout	Yes	
Teleostei	Salmoniformes	Salmonidae	Thymallus	arcticus	Arctic Grayling	Yes	
Teleostei	Scorpaeniformes	Cottidae	Cottus	bairdii complex	Mottled Sculpin Complex	Yes	
Teleostei	Scorpaeniformes	Cottidae	Cottus	beldingii	Paiute Sculpin	Yes	
Teleostei	Scorpaeniformes	Cottidae	Cottus	cognatus	Slimy Sculpin	Yes	
Teleostei	Scorpaeniformes	Cottidae	Cottus	confusus	Shorthead Sculpin	Yes	
Teleostei	Scorpaeniformes	Cottidae	Cottus	extensus	Bear Lake Sculpin	Yes	
Teleostei	Scorpaeniformes	Cottidae	Cottus	greenei	Shoshone Sculpin	Yes	
Teleostei	Scorpaeniformes	Cottidae	Cottus	hubbsi	Columbia Sculpin	Yes	
Teleostei	Scorpaeniformes	Cottidae	Cottus	leiopomus	Wood River Sculpin	Yes	
Teleostei	Scorpaeniformes	Cottidae	Cottus	rhotheus	Torrent Sculpin	Yes	
Teleostei	Scorpaeniformes	Cottidae	Cottus	schitsuumsh	Cedar Sculpin	Yes	
Teleostei	Siluriformes	Ictaluridae	Ameiurus	melas	Black Bullhead	Yes	
Teleostei	Siluriformes	Ictaluridae	Ameiurus	natalis	Yellow Bullhead	Yes	
Teleostei	Siluriformes	Ictaluridae	Ameiurus	nebulosus	Brown Bullhead	Yes	
Teleostei	Siluriformes	Ictaluridae	Ictalurus	furcatus	Blue Catfish	Yes	

Class	Order	Family	Genus	Species	Common name	Still Resident?	Note
Teleostei	Siluriformes	Ictaluridae	Ictalurus	punctatus	Channel Catfish	Yes	
Teleostei	Siluriformes	Ictaluridae	Noturus	gyrinus	Tadpole Madtom	Yes	
Teleostei	Siluriformes	Ictaluridae	Pylodictus	olivaris	Flathead Catfish	Yes	

Notes: 1 – Resident status unknown, 2 – Identified only to indicated taxonomic level, 3 – Extinct species, 4 – Extirpated from Idaho waters

Appendix B. Acute Criteria at Selected pH and Temperature Conditions

	Temperature (°C)								
pH	**0-22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
6.5	33	33	33	31	29	27	25	23	21
6.6	31	31	31	30	28	26	24	22	20
6.7	30	30	30	29	26	24	22	21	19
6.8	28	28	28	27	25	23	21	19	18
6.9	26	26	26	25	23	21	20	18	17
7.0	24	24	24	23	21	20	18	17	15
7.1	22	22	22	21	20	18	17	15	14
7.2	20	20	20	19	18	16	15	14	13
7.3	18	18	18	17	16	14	13	12	11
7.4	15	15	15	15	14	13	12	11	10
7.5	13	13	13	13	12	11	10	9.2	8.5
7.6	11	11	11	11	10	9.3	8.6	7.9	7.3
7.7	10	10	10	9.3	8.6	7.9	7.3	6.7	6.2
7.8	8.1	8.1	8.1	7.8	7.2	6.6	6.1	5.6	5.2
7.9	6.8	6.8	6.8	6.5	6.0	5.5	5.1	4.7	4.3
8.0	5.6	5.6	5.6	5.4	5.0	4.6	4.2	3.9	3.6
8.1	4.6	4.6	4.6	4.5	4.1	3.8	3.5	3.2	3.0
8.2	3.8	3.8	3.8	3.7	3.4	3.1	2.9	2.7	2.4
8.3	3.1	3.1	3.1	3.0	2.8	2.6	2.4	2.2	2.0
8.4	2.6	2.6	2.6	2.5	2.3	2.1	2.0	1.8	1.7
8.5	2.1	2.1	2.1	2.1	1.9	1.7	1.6	1.5	1.4
8.6	1.8	1.8	1.8	1.7	1.6	1.4	1.3	1.2	1.1
8.7	1.5	1.5	1.5	1.4	1.3	1.2	1.1	1.0	0.94
8.8	1.2	1.2	1.2	1.2	1.1	1.0	0.93	0.85	0.79
8.9	1.0	1.0	1.0	1.0	0.92	0.85	0.78	0.72	0.66
9.0	0.88	0.88	0.88	0.85	0.79	0.72	0.67	0.61	0.56

Notes: Criteria expressed as mg TAN/L. The 1-hour average concentration of a waterbody is not to exceed the criterion value more than once every 3 years.

Appendix C. Chronic Criteria at Selected pH and Temperature Conditions

		Temperature (°C)																			
pH	**0-10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
6.5	6.5	6.5	6.3	5.9	5.5	5.2	4.9	4.6	4.3	4.0	3.8	3.5	3.3	3.1	2.9	2.7	2.5	2.4	2.2	2.1	2.0
6.6	6.4	6.4	6.2	5.8	5.4	5.1	4.8	4.5	4.2	3.9	3.7	3.5	3.2	3.0	2.9	2.7	2.5	2.4	2.2	2.1	1.9
6.7	6.3	6.3	6.1	5.7	5.3	5.0	4.7	4.4	4.1	3.9	3.6	3.4	3.2	3.0	2.8	2.6	2.5	2.3	2.2	2.0	1.9
6.8	6.1	6.1	5.9	5.6	5.2	4.9	4.6	4.3	4.0	3.8	3.5	3.3	3.1	2.9	2.7	2.6	2.4	2.3	2.1	2.0	1.9
6.9	6.0	6.0	5.8	5.4	5.1	4.8	4.5	4.2	3.9	3.7	3.4	3.2	3.0	2.8	2.7	2.5	2.3	2.2	2.1	1.9	1.8
7.0	5.8	5.8	5.6	5.2	4.9	4.6	4.3	4.0	3.8	3.5	3.3	3.1	2.9	2.7	2.6	2.4	2.3	2.1	2.0	1.9	1.7
7.1	5.5	5.5	5.3	5.0	4.7	4.4	4.1	3.9	3.6	3.4	3.2	3.0	2.8	2.6	2.5	2.3	2.2	2.0	1.9	1.8	1.7
7.2	5.2	5.2	5.1	4.8	4.5	4.2	3.9	3.7	3.5	3.2	3.0	2.8	2.7	2.5	2.3	2.2	2.1	1.9	1.8	1.7	1.6
7.3	4.9	4.9	4.8	4.5	4.2	3.9	3.7	3.5	3.3	3.0	2.9	2.7	2.5	2.4	2.2	2.1	1.9	1.8	1.7	1.6	1.5
7.4	4.6	4.6	4.5	4.2	3.9	3.7	3.4	3.2	3.0	2.8	2.7	2.5	2.3	2.2	2.1	1.9	1.8	1.7	1.6	1.5	1.4
7.5	4.2	4.2	4.1	3.9	3.6	3.4	3.2	3.0	2.8	2.6	2.5	2.3	2.2	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3
7.6	3.9	3.9	3.7	3.5	3.3	3.1	2.9	2.7	2.5	2.4	2.2	2.1	2.0	1.8	1.7	1.6	1.5	1.4	1.3	1.3	1.2
7.7	3.5	3.5	3.4	3.2	3.0	2.8	2.6	2.4	2.3	2.1	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.1
7.8	3.1	3.1	3.0	2.8	2.6	2.5	2.3	2.2	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.1	1.0	0.94
7.9	2.7	2.7	2.6	2.5	2.3	2.2	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.1	1.0	0.94	0.88	0.83
8.0	2.4	2.4	2.3	2.2	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.1	1.0	0.93	0.87	0.82	0.77	0.72
8.1	2.0	2.0	2.0	1.9	1.7	1.6	1.5	1.4	1.3	1.3	1.2	1.1	1.0	1.0	0.91	0.86	0.80	0.75	0.70	0.66	0.62
8.2	1.7	1.7	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.1	1.0	0.95	0.89	0.83	0.78	0.73	0.69	0.64	0.60	0.57	0.53
8.3	1.5	1.5	1.4	1.3	1.3	1.2	1.1	1.0	1.0	0.92	0.86	0.80	0.75	0.71	0.66	0.62	0.58	0.55	0.51	0.48	0.45
8.4	1.3	1.3	1.2	1.1	1.1	1.0	0.94	0.88	0.83	0.77	0.73	0.68	0.64	0.60	0.56	0.53	0.49	0.46	0.43	0.41	0.38
8.5	1.1	1.1	1.0	1.0	0.90	0.85	0.79	0.74	0.70	0.65	0.61	0.57	0.54	0.51	0.47	0.44	0.42	0.39	0.37	0.34	0.32
8.6	0.90	0.90	0.87	0.81	0.76	0.71	0.67	0.63	0.59	0.55	0.52	0.49	0.46	0.43	0.40	0.38	0.35	0.33	0.31	0.29	0.27
8.7	0.76	0.76	0.73	0.69	0.65	0.60	0.57	0.53	0.50	0.47	0.44	0.41	0.39	0.36	0.34	0.32	0.30	0.28	0.26	0.25	0.23
8.8	0.64	0.64	0.62	0.58	0.55	0.51	0.48	0.45	0.42	0.40	0.37	0.35	0.33	0.31	0.29	0.27	0.25	0.24	0.22	0.21	0.20
8.9	0.55	0.55	0.53	0.50	0.47	0.44	0.41	0.39	0.36	0.34	0.32	0.30	0.28	0.26	0.25	0.23	0.22	0.20	0.19	0.18	0.17
9.0	0.47	0.47	0.46	0.43	0.40	0.38	0.35	0.33	0.31	0.29	0.27	0.26	0.24	0.23	0.21	0.20	0.19	0.17	0.16	0.15	0.14

Notes: Criteria expressed as mg TAN/L. The 30-day average concentration of a waterbody is not to exceed the criterion value more than once every 3 years. The highest 4-day average within 30 days is not to exceed 2.5 times the criteria.

Appendix D. Toxicity Studies on Western Unionids

Continued on next page.

Assessing the Acute Sensitivity of Juvenile *Anodonta californiensis* to Ammonia

Prepared for

Central Valley CleanWaterAssociation
700 R Street,Suite200
Sacramento,CA95811

Prepared by

Pacific EcoRisk
2250 CordeliaRoad
Fairfield,CA94534

July 2018

PACIFIC ECORISK
ENVIRONMENTAL CONSULTING & TESTING

Assessing the Acute Sensitivity ofJuvenile *Anodonta californiensis* to Ammonia

Table of Contents

Page

1. INTRODUCTION 1
2. METHODS 1
   2.1 Test Organism Source, Culture Methods, and Acclimation 1
   2.2 Toxicity Test Procedures 2
      2.2.1 Preparation of Test Solutions 3
      2.2.2 Statistical Determination of Toxicity Point Estimates 3
   2.3 Measurement of Ammonia Test Concentrations 4
3. RESULTS 5
   3.1 Toxicity of Ammonia to *Anodonta californiensis*: Test 1 5
   3.2 Toxicity of Ammonia to *Anodonta californiensis*: Test 2 5
4. QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) 6
   4.1 Toxicity Test Acceptability Requirements 6
      4.1.1 Negative Control 6
      4.1.2 Test Conditions 6
   4.2 Total Ammonia Measurement QA/QC Assessment 6
5. SUMMARY 8
6. REFERENCES 8

Appendices

Appendix A Data Sheets and Summary of Statistical Analysis for Determination of Ammonia LC_{50} Values with Juvenile *Anodonta californiensis*: Test 1

Appendix B Data Sheets and Summary of Statistical Analysis for Determination of Ammonia LC_{50} Values with Juvenile *Anodonta californiensis*: Test 2

Appendix C Temperature Control Charts

1. INTRODUCTION

The Central ValleyCleanWaterAssociation(CVCWA)isintheprocessofconductinga collaborative studyamong42agenciesrepresenting47CentralValleyPublicallyOwned Treatment Works(POTWs)titled: *Freshwater Mussel Collaborative Study for Wastewater Treatment Plants*.Thisstudyisbeingimplementedonbehalfof CVCWAbytheconsultantteam of Robertson-Bryan,Inc.,LarryWalkerAssociates,andPacificEcoRisk.PhaseIIcofthisstudy, led by Pacific EcoRisk,isfocusedonacutetoxicitytestingwithCentralValleyresident freshwater musselstodeterminetheirsensitivitytoammonia.Thetoxicitydatageneratedwill then be used by thisteam,workinginclosecoordinationwiththeCentralValleyRegionalWater Quality Control Board(CentralValleyWaterBoard)andtheUnitedStatesEnvironmental Protection Agency(U.S.EPA)staff,toderivesite-specificammoniaobjectivesfortheCentral Valley of California,usingU.S.EPA'sRecalculationProcedure.Oncederived,theCentral Valley Water Boardintendstoadoptthesite-specificammoniaobjectivesintoitsWaterQuality Control Plan for theSacramentoandSanJoaquinRiverBasins(Region5BasinPlan).

This report presentstheresultsfromtestingtheacutesensitivityof *Anodonta californiensis* (California floater)toammonia.Inconductingthiswork,PacificEcoRiskwasspecifically responsible for:

- acclimationandholdingofjuvenile *A. californiensis*;
- preparationofammoniaspikedtestsolutions;
- performanceofacutetoxicitytestswith *A. californiensis*;and
- calculationbenchmarktoxicityvalues(e.g.,LC_{50} pointestimates).

werefieldcollectedbythePresidioTrustandharvested Gravid adult female *A. californiensis* glochidia were metamorphosedtojuvenilesbyDr.ChristopherBarnhartofMissouriState University. This reportdescribesandsummarizestheperformanceandresultsoftheaquatic toxicity testing performedinsupportofthisevaluation.

2. METHODS

Testing and study design closely followed the guidance documents listed below:

- *Standard GuideforConducting Laboratory Toxicity TestswithFreshwater Mussels,* ASTM E2455-06 (ASTM 2013); and
- *Phase IIc Freshwater Mussel Collaborative Studyfor WastewaterTreatment Plants: Study Work Plan for the Acute Toxicity Testing with Resident Freshwater Mussels and Ammonia Criteria Recalculation* (PER et al., 2018).

2.1 Test Organism Source, Culture Methods, and Acclimation

Ten feral and gravid adult *A. californiensis* were collected from Laguna Creek (Elk Grove, CA) by the Presidio Trust (San Francisco, CA). Adult organisms were shipped by overnight courier to Dr. Christopher Barnhart, Department of Biology at Missouri State University (Springfield, MO). Mature glochidia were extracted and metamorphosed by the *in vitro* method.

Upon completion of metamorphosis, approximately 500 transformed juveniles were collected and shipped by overnight courier to Pacific EcoRisk. Upon receipt, juveniles were acclimated to U.S. EPA synthetic hard water (EPA 2002) per the acclimation specifications summarized in Table 1. Juveniles were fed *Raphidocelis subcapitata* (formally *Selenastrum capricornutum*) during this pre-test acclimation period (N. Wang, pers. com., April 10, 2018).

Table 1. Summary of organism acclimation conditions.

1.	Acclimation period	≥24 hours
2.	Acclimation regime (water)	50:50 (culture water:dilution medium) for 2 hours, then 25:75 for 2 hours, then 0:100 for 20+ hours
3.	Acclimation regime (temperature)	Not more than 3°C/hour

2.2 Toxicity Test Procedures

Testing followed the standardtests conditions detailed in ASTM E2455-06, as summarized in Table 2. Two tests were completed, with the first initiated on April 15, 2018 and the second initiated April 16, 2018.

Table 2. Summary of test conditions.

1.	Test type	Static Renewal 20°C Fluorescent 100-1000 lux 16L:8D 50 mL glass beaker 30 mL At 48 hours Approximately 75% <5 days 5 4 7 treatments plus control (8 total treatments) ≥0.5 None None None, unless DO falls <4 mg/L, then aerate all treatments at approximately 1 bubble/second from a glass pipette EPAH (EPA Hard Water) 96 hours Survival, measured as presence of foot/cillia movement in a 5 minute observation period, or observation of heartbeat
2.	Temperature	
3.	Light quality	
4.	Light intensity	
5.	Photoperiod	
6.	Test chamber size	
7.	Test solution volume	
8.	Renewal frequency	
9.	Renewal volume	
10.	Age of organisms	
11.	Organisms per chamber	
12.	Replicates per treatment	
13.	Number of treatments	
14.	Dilution factor	
15.	Feeding regime	
16.	Cleaning	
17.	Aeration	
18.	Dilution water	
19.	Test duration	
20.	Endpoint	

Test solutions were prepared as described in Section 2.2.1. Water quality characteristics (pH, D.O., and conductivity) were measured for each test solution immediately prior to use in these tests. Alkalinity and hardness were measured in the Lab Water Control, the middle, and high concentration of the dilution series at test initiation and test termination.

There were 4 replicates for each test treatment, with each replicate consisting of 30 mL of test solution in a 50-mL glass beaker. The tests were initiated by randomly allocating 5 juvenile *A. californiensis* (<5 days old and within 24 hours age range) into each replicate beaker. The replicate beakers were placed in a temperature-controlled room at 20˚C, under cool-white fluorescent lighting on a 16L:8D photoperiod with a light intensity of 100-1000 lux.

Each replicate beaker was examined after 48 hours exposure, and the number of live juveniles in each beaker was recorded. Fresh test solutions were prepared at this time and were characterized as before. That same day, approximately 75% of the old media in each replicate container was carefully poured out and replaced with the fresh test solution. "Old" water quality characteristics (pH, D.O., and conductivity) were measured at 48 and 96 hours for the old test solutions that had been discarded from all replicates of each treatment. At 96 hours, the test was terminated and the number of live juveniles in each replicate was determined.

2.2.1PreparationofTest Solutions

The Lab Water Control medium consisted of EPA synthetic hard water (EPA 2002), which was prepared by the addition of reagent grade chemicals to Type 1 water (reverse-osmosis, de-ionized water).

A 50 mg/L total ammonia nitrogen (TAN) stock solution was prepared by the addition of ACS reagent grade ammonium chloride (NH_4Cl, Sigma Aldrich, ³99.5%) to Lab Water Control medium. The stock ammonia solution was analytically verified prior to use in testing. The ammonia stock solution was subsequently diluted by the addition of unspiked Lab Control Medium in order to obtain the test concentrations listed in Table 3. The range of nominal ammonia test concentrations was selected assuming similar ammonia sensitivity to that of other unionid mussel species as published in the scientific literature.

Table 3. Nominal ammonia (TAN) test concentrations.

Testing Test	Test Concentrations (mg/L TAN)
Test 1: April 15	0, 2.0, 4.0, 8.0, 16, 24, 36, 50
Test 2: April 16	0, 2.0, 4.0, 8.0, 16, 24, 36, 50

2.2.2 Statistical Determination of Definitive Toxicity Point Estimates

Determination of TAN LC_{50} point estimates and 95% confidence intervals (CI) were made using CETIS® (TidePool Scientific Software, McKinleyville, CA). Point estimates were determined using the mean of test initiation, test renewal (old and new solutions), and test completion measurements of TAN. Because test methods specified a 75% renewal, the renewal solution

were calculated as a 3:1 ratio (new:old) of the new and old solution TAN measurements at 48 hours. Point estimates were determined by the Spearman-Kärber method.

2.3 Measurement of Ammonia Test Concentrations

Total ammonia-nitrogen was measured for each treatment at test initiation, test renewal (old and new solutions), and test completion via spectrophotometric method (HACH TNTplus, EPA 350.1 equivalent). Ongoing precision and recovery was demonstrated and documented prior to each batch of ammonia analyses through the analysis of a 10 mg/L TAN standard and comparison of results to corresponding limits for recovery and precision (i.e., 90-110% recovery and maximum relative standard deviation of 10%).

3. RESULTS

3.1 Toxicity of Ammonia to *Anodonta californiensis*:Test 1

The results of this test are presented in Table 4. The test data and summary of statistical analyses for this test are presented in Appendix A.

Table 4. Acute toxicity of ammonia to *A. californiensis*: Test 1.

Nominal (mg/L TAN)	Measured Ammonia (mg/L TAN)					% Survival				
	T0 New	T_{48} New[a]	T_{48} Old	T_{96} Old	Test Mean	Rep A	Rep B	Rep C	Rep D	**Mean**
0	<1.0	<1.0	<1.0	<1.0	<1.0	100	100	100	100	**100**
2.0	2.1	2.0	1.7	1.4	1.8	100	100	100	100	**100**
4.0	4.0	3.5	2.6	2.3	3.1	100	100	100	100	**100**
8.0	8.1	7.6	5.9	5.9	6.9	100	100	100	100	**100**
16	16	15	12	11	14	100	100	100	100	**100**
24	24	23	20	17	21	20	100	40	80	**60**
36	36	35	30	29	33	0	0	0	0	**0**
50	50	50	48	42	48	0	0	0	0	**0**
Summary of Statistics										
LC_{50} (95% CI) =						22 (20-24)				

a – reported TAN values represents a 3:1 weighted concentration of new and old solutions at 48 hours.

3.2 Toxicity of Ammonia to *Anodonta californiensis*:Test 2

The results of this test are presented in Table 5. The test data and summary of statistical analyses for this test are presented in Appendix B.

Table 5. Acute toxicity of ammonia to *A. californiensis*: Test 2.

Nominal (mg/L TAN)	Measured Ammonia (mg/L TAN)					% Survival				
	T0 New	T_{48} New[a]	T_{48} Old	T_{96} Old	Test Mean	Rep A	Rep B	Rep C	Rep D	**Mean**
0	<1.0	<1.0	<1.0	<1.0	<1.0	100	100	100	100	**100**
2.0	2.2	2.0	1.5	1.5	1.8	100	100	100	100	**100**
4.0	4.3	4.0	3.0	3.2	3.6	100	100	100	100	**100**
8.0	8.3	7.4	5.9	6.1	6.9	100	100	100	100	**100**
16	16	15	12	13	14	100	100	100	100	**100**
24	25	24	19	21	22	60	60	40	40	**50**
36	38	36	29	31	33	0	0	0	0	**0**
50	51	49	42	44	46	0	0	0	0	**0**
Summary of Statistics										
LC_{50} (95% CI) =						22 (20-24)				

a– reported TAN values represents a 3:1 weighted concentration of new and old solutions at 48 hours.

4. QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

Testing was subject to the QA/QC procedures of ASTM E2455-06 (ASTM 2013). A QA/QC review is provided below.

4.1 Toxicity Test Acceptability Requirements

Toxicity testing incorporated standard QA/QCprocedures to ensure that the test results were valid, including the use of a negative control.These QA/QC procedures were consistent with the test acceptability requirements for toxicity testsconducted with freshwater juvenile mussels outlined in ASTM E2455-06.

4.1.1Negative Control

The biological response in the negative Control treatments were within test acceptability limits of ≥90% survival.

4.1.2Test Conditions

All test conditions were within acceptable limits. Continuously measured test temperatures was within +/- 1˚C of the target test temperature of 20˚C (Appendix C) and the difference between the maximum and minimum test temperature across treatments was less than 2˚C (Table 6). Test treatment pH, hardness, and alkalinity varied less than 10% over the duration of the test (Table 7 and Table 8), and test dissolved oxygen (DO) was maintained above 4 mg/L (Table 9).

4.2 Total Ammonia Measurement QA/QC Assessment

Measurement of total ammonia incorporated standard QA/QC procedures to ensure that the test results were valid, including measurement of laboratory control spike recovery and precision. Spike recovery and relative standard deviation were within specified limits (Table 10).

Table 6. Summary of test temperature (˚C).

Test Treatment	Test 1				Test 2			
	Mean	Min	Max	D (˚C)	Mean	Min	Max	D (˚C)
0	19.7	19.3	20.1	0.8	20.4	19.7	20.6	0.9
2.0	19.9	19.6	20.2	0.6	20.5	19.8	20.9	1.1
4.0	19.9	19.6	20.2	0.6	20.5	19.9	20.9	1.0
8.0	19.8	19.5	20.1	0.6	20.6	19.9	20.9	1.0
16	19.9	19.6	20.1	0.5	20.6	19.9	20.8	0.9
24	19.9	19.6	20.2	0.6	20.5	19.9	20.7	0.8
36	19.9	19.5	20.2	0.7	20.3	19.9	20.6	0.7
50	19.7	19.2	20.1	0.9	20.0	19.9	20.2	0.3

Table 7. Summary of test pH.								
Test Treatment	Test 1				Test 2			
	Mean	Min	Max	D (%)	Mean	Min	Max	D (%)
0	8.23	8.19	8.27	0.98	8.17	8.10	8.24	1.7
2.0	8.21	8.19	8.23	0.49	8.16	8.13	8.23	1.2
4.0	8.18	8.15	8.20	0.61	8.14	8.11	8.21	1.2
8.0	8.13	8.10	8.15	0.62	8.11	8.07	8.18	1.4
16	8.06	8.05	8.07	0.25	8.06	8.01	8.13	1.5
24	8.01	7.99	8.05	0.75	8.01	7.94	8.10	2.0
36	7.94	7.88	7.98	1.3	7.94	7.88	7.98	1.3
50	7.84	7.80	7.90	1.3	7.86	7.81	7.90	1.2

Table 8. Summary of test hardness and alkalinity (mg/L $CaCO_3$).												
Test Treatment	Test 1						Test 2					
	Hardness			Alkalinity			Hardness			Alkalinity		
	Initial	Final	D (%)	Initial	Final	D (%)	Initial	Final	D (%)	Initial	Final	D (%)
0	160	160	0.0	110	100	9.1	160	170	6.3	100	100	0.0
16	160	170	6.3	110	110	0.0	160	170	6.3	110	110	0.0
50	170	-	[a]	110	-	[a]	170	-	[a]	110	-	[a]

a – Final concentrationnotmeasureddue to completemortalityinthistreatment.

Table 9. Summary of test dissolved oxygen (mg/L).								
Test Treatment	Test 1				Test 2			
	Mean	Min	Max	D (mg/L)	Mean	Min	Max	D (mg/L)
0	8.6	7.2	9.1	1.9	7.6	5.7	8.8	3.1
2.0	8.8	7.8	9.1	1.3	8.1	7.1	8.9	1.8
4.0	8.7	7.6	9.2	1.6	8.0	7.1	8.9	1.8
8.0	8.7	7.6	9.2	1.6	7.9	6.6	8.9	2.3
16	8.7	7.6	9.1	1.5	7.9	6.5	8.9	2.4
24	8.6	7.2	9.2	2.0	8.0	6.7	8.9	2.2
36	8.6	7.3	9.1	1.8	8.0	6.8	8.9	2.1
50	8.6	7.2	9.1	2.0	8.0	6.6	8.8	2.2

Table 10. Summary of daily batch ammonia recovery and precision.		
Date	Mean Recovery (%)	RSD (%)
4/15/18	106	0.91
4/16/18	99.8	0.35
4/17/18	100	1.1
4/18/18	100	0.78
4/19/18	103	0.69
4/20/18	97.9	0.94

5. SUMMARY

Total ammonia LC_{50} values (and accompanying 95% CI)for juvenile *Anodontacalifornienses* are presented in Table 11. All results were calculated asdescribed in this report.

Table 11. Total Ammonia LC_{50} for juvenile *Anodonta californiensis.*

Test No.	Total Ammonia LC_{50} value (mg/L TAN)	95% Confidence Interval (mg/L TAN)
Test 1	22	20-24
Test 2	22	20-24

6. REFERENCES

ASTM (ASTM International). 2013. Standard guide for conducting laboratory toxicity tests with freshwater mussels. E2455-06 (2013). West Conshohocken, PA. 2013.

EPA (2002). Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. EPA 821-R-02-012. US Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN. October 2002.

Pacific EcoRisk, Robertson-Bryan, Inc, Larry Walker Associates. 2018. Phase IIc Freshwater Mussel Collaborative Study for Wastewater Treatment Plants: Study Work Plan for the Acute Toxicity Testing with Resident Freshwater Mussels and Ammonia Criteria Recalculation. Prepared for Central Valley Clean Water Association. February 2018.

Appendix A

Data Sheets and Summaryof Statistical Analysis for Determinationof AmmoniaLC_{50} Values with Juvenile *Anodonta californiensis*: Test 1

CETIS Summary Report

Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

Batch ID:	01-1906-9301	**Test Type:**	Survival (96h)	**Analyst:**	Stevi Vasquez
Start Date:	15 Apr-18 11:32	**Protocol:**	ASTM E2455-06	**Diluent:**	Hard Synthetic Water
Ending Date:	19 Apr-18 11:31	**Species:**	Anodonta californiensis	**Brine:**	Not Applicable
Duration:	96h	**Source:**	Missouri State University	**Age:**	4

Sample ID:	12-1233-6913	**Code:**	NH4Cl	**Client:**	Reference Toxicant
Sample Date:	15 Apr-18 11:32	**Material:**	Ammonia (as NH3-N)	**Project:**	28761
Receipt Date:	15 Apr-18 11:32	**Source:**	Reference Toxicant		
Sample Age:	n/a (20.1 °C)	**Station:**	In House		

Comments:
Mean measured ammonia values

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	NOEL	LOEL	TOEL	TU	PMSD ✓
11-8738-5373	96h Survival Rate	Steel Many-One Rank Sum Test	21	> 21	n/a		23.2%

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	Level	mg/L NH3	95% LCL	95% UCL	TU ✓
11-7141-1202	96h Survival Rate	Spearman-Kärber	EC50	22.2	20.2	24.4	

96h Survival Rate Summary

Conc-mg/L NH3-N	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LW	4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
1.8		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
3.1		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
6.9		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
14		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
21		4	0.600	0.019	1.000	0.200	1.000	0.183	0.365	60.86%	40.00%
33		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100.00%
48		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100.00%

96h Survival Rate Detail

Conc-mg/L NH3-N	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LW	1.000	1.000	1.000	1.000
1.8		1.000	1.000	1.000	1.000
3.1		1.000	1.000	1.000	1.000
6.9		1.000	1.000	1.000	1.000
14		1.000	1.000	1.000	1.000
21		0.200	1.000	0.400	0.800
33		0.000	0.000	0.000	0.000
48		0.000	0.000	0.000	0.000

96h Survival Rate Binomials

Conc-mg/L NH3-N	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LW	5/5	5/5	5/5	5/5
1.8		5/5	5/5	5/5	5/5
3.1		5/5	5/5	5/5	5/5
6.9		5/5	5/5	5/5	5/5
14		5/5	5/5	5/5	5/5
21		1/5	5/5	2/5	4/5
33		0/5	0/5	0/5	0/5
48		0/5	0/5	0/5	0/5

Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

Analysis ID:	11-8738-5373	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.9.2
Analyzed:	11 Jun-18 16:44	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	C > T	21	> 21	n/a		23.23%

Steel Many-One Rank Sum Test

Control	vs	Conc-mg/L N	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Lab Water Contr		1.8	18	10	1	6	Asymp	0.8333	Non-Significant Effect
		3.1	18	10	1	6	Asymp	0.8333	Non-Significant Effect
		6.9	18	10	1	6	Asymp	0.8333	Non-Significant Effect
		14	18	10	1	6	Asymp	0.8333	Non-Significant Effect
		21	12	10	1	6	Asymp	0.1424	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.660331	0.132066	5	4.97	0.0049	Significant Effect
Error	0.477937	0.026552	18			
Total	1.13827		23			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Levene Equality of Variance Test	24.2	4.25	2.1E-07	Unequal Variances
Variances	Mod Levene Equality of Variance Test	24.1	4.25	2.1E-07	Unequal Variances
Distribution	Shapiro-Wilk W Normality Test	0.581	0.884	3.8E-07	Non-Normal Distribution

96h Survival Rate Summary

Conc-mg/L NH3-N	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LW	4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
1.8		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
3.1		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
6.9		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
14		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
21		4	0.600	0.019	1.000	0.600	0.200	1.000	0.183	60.86%	40.00%

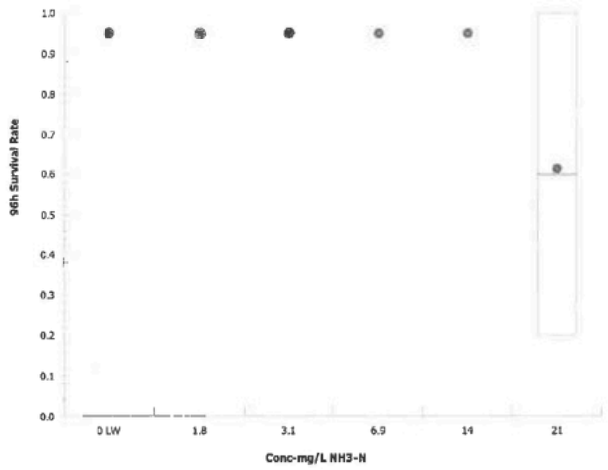
Angular (Corrected) Transformed Summary

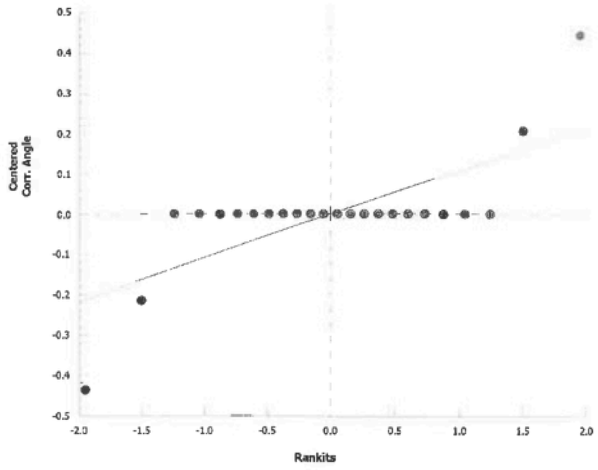
Conc-mg/L NH3-N	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LW	4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
1.8		4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
3.1		4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
6.9		4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
14		4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
21		4	0.9	0.265	1.54	0.896	0.464	1.35	0.2	44.34%	33.08%

Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

Analysis ID:	11-8738-5373	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.9.2
Analyzed:	11 Jun-18 16:44	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Acute Juvenile Freshwater Mussel Survival — Pacific EcoRisk

Analysis ID:	11-7141-1202	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.9.2
Analyzed:	11 Jun-18 16:44	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

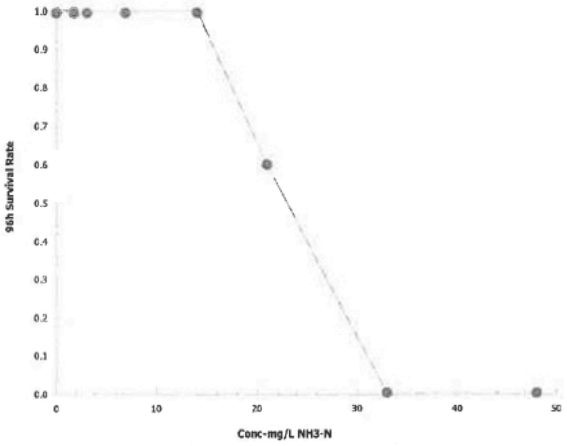
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	1.35	0.0204	22.2	20.2	24.4

96h Survival Rate Summary

			Calculated Variate(A/B)								
Conc-mg/L NH3-N	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	LW	4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
1.8		4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
3.1		4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
6.9		4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
14		4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
21		4	0.600	0.200	1.000	0.183	0.365	60.90%	40.0%	12	20
33		4	0.000	0.000	0.000	0.000	0.000		100.0%	0	20
48		4	0.000	0.000	0.000	0.000	0.000		100.0%	0	20

Graphics



96 Hour Acute *Anodonta californiensis* Toxicity Test Data

Client: **Pacific EcoRisk** Test Date: 4/15/18

Test Material: Ammonia (as NH_3 - N) Control/Diluent: EPA Hard Water

Test ID#: 78027 Project # 28761 Control Water Batch: 324

Randomization: 8.4.2 Organism Source: 10910

Treatment (mg/L NH3 - N)	Temp (°C)	Room Temp (°C) Max/Min	pH New	pH Old	D.O. New	D.O. Old	Conductivity (µS/cm)	# Live Animals A	B	C	D	Sign-Off
Lab Water Control	20.1	19.5/19.5	8.19		9.0		587	5	5	5	5	Date: 4/15/18
2	20.1		8.19		9.0		603	5	5	5	5	Sample ID: —
4	20.0		8.15		9.0		627	5	5	5	5	Test Solution Prep: JL
8	20.0		8.10		9.0		666	5	5	5	5	New WQ: NL
16	20.0		8.07		9.0		745	5	5	5	5	Initiation Time: 1132
24	20.1		8.05		9.0		822	5	5	5	5	Initiation Signoff: NL
36	20.1		7.98		9.0		941	5	5	5	5	
50	20.1		7.90		9.0		1079	5	5	5	5	
Meter ID	110A	110A	PH21		RD10		EC10					
Lab Water Control	19.8	19.1/19.4										Date: 4/16/18
2	19.6											Temp Time: 1129
4	19.6											Temp Signoff: NL
8	19.7											
16	19.6											
24	19.7											
36	19.5											
50	19.8											
Meter ID	110A	110A										
Lab Water Control	19.3	19.2/19.7	8.24	8.23	9.0	9.1	588	5	5	5	5	Date: 4/17/18
2	19.6		8.23	8.21	9.1	9.1	603	5	5	5	5	Sample ID: —
4	19.6		8.19	8.20	9.2	9.0	622	5	5	5	5	Test Solution Prep: JL
8	19.5		8.15	8.14	9.2	9.0	662	5	5	5	5	New WQ: NL
16	19.7		8.06	8.05	9.1	9.1	736	5	5	5	5	Count Time: 1135
24	19.6		8.01	8.00	9.2	9.0	813	3	5	5	5	Count Signoff: NL
36	19.6		7.94	7.98	9.1	9.1	929	3	2	2	2	Old WQ: NL
50	19.2		7.87	7.80	9.2	9.1	1069	1	1	0	1	
Meter ID	110A	110A	PH21	PH21	RD10	RD10	EC10					

96 Hour Acute *Anodonta californiensis* Toxicity Test Data

Client: **Pacific EcoRisk** Test Date: 4/15/18

Test Material: Ammonia (as NH_3 - N) Control/Diluent: EPA Hard Water

Test ID#: 78027 Project # 28761 Control Water Batch: 324

Randomization: 8.4.2 Organism Source: 10910

Treatment (mg/L NH3 - N)	Temp (°C)	Room Temp (°C) Max/Min	pH New	pH Old	D.O. New	D.O. Old	Conductivity (µS/cm)	# Live Animals A	B	C	D	Sign-Off
Lab Water Control	19.7	19.3/19.7										Date: 4/18/18
2	20.0											Temp Time: 1202
4	19.9											Temp Signoff: MS
8	19.8											
16	19.9											
24	19.9											
36	19.9											
50	19.7											
Meter ID	110A	110A										
Lab Water Control	19.7	19.1/19.7		8.27		7.2 ~~6.95~~	675	5	5	5	5	Date: 4/19/18
2	20.2			8.22		7.8	679	5				Termination Time: 1131
4	20.2			8.18		7.6	708	5				Termination Signoff: NL
8	20.1			8.12		7.6	719	5				Old WQ: NL
16	20.1			8.05		7.4	798	5	5	5	5	
24	20.2			7.99		7.2	885	1	5	2	4	
36	20.2			7.88		7.3	971	0	0	0	0	
50	19.9			7.80		7.2	1121	0	0	—	0	
Meter ID	110A	110A		PH21		RD13	EC10					

Ancillary Water Chemistries	Initial (mg/L) Alkalinity *	Initial (mg/L) Hardness*	Final Alkalinity*	Final Hardness*
Control	108	160	100	160
16 mg/L	113	165	110	170
50 mg/L	107	168	—	—

*Measure in each test treatment at test initiation and termination as long as live organisms are present.

Appendix B

Data Sheets and Summaryof Statistical Analysis for Determination of AmmoniaLC_{50} Values with Juvenile *Anodonta californiensis*: Test 2

CETIS Summary Report

Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

Batch ID:	13-7125-6416	**Test Type:**	Survival (96h)	**Analyst:**	Stevi Vasquez
Start Date:	16 Apr-18 11:15	**Protocol:**	ASTM E2455-06	**Diluent:**	Hard Synthetic Water
Ending Date:	20 Apr-18 11:20	**Species:**	Anodonta californiensis	**Brine:**	Not Applicable
Duration:	4d 0h	**Source:**	Missouri State University	**Age:**	5

Sample ID:	17-7215-5195	**Code:**	NH4Cl	**Client:**	Reference Toxicant
Sample Date:	16 Apr-18 11:15	**Material:**	Ammonia (as NH3-N)	**Project:**	28762
Receipt Date:	16 Apr-18 11:15	**Source:**	Reference Toxicant		
Sample Age:	n/a (19.7 °C)	**Station:**	In House		

Comments:
Mean measured ammonia values

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	NOEL	LOEL	TOEL	TU	PMSD ✓
17-8043-2695	96h Survival Rate	Dunnett Multiple Comparison Test	14	22	17.55		9.09%

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	Level	mg/L NH3	95% LCL	95% UCL	TU ✓
18-2311-5802	96h Survival Rate	Spearman-Kärber	EC50	21.7	19.8	23.9	

96h Survival Rate Summary

Conc-mg/L NH3-N	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LW	4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
1.8		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
3.6		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
6.9		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
14		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
22		4	0.500	0.316	0.684	0.400	0.600	0.058	0.115	23.09%	50.00%
33		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100.00%
46		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100.00%

96h Survival Rate Detail

Conc-mg/L NH3-N	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LW	1.000	1.000	1.000	1.000
1.8		1.000	1.000	1.000	1.000
3.6		1.000	1.000	1.000	1.000
6.9		1.000	1.000	1.000	1.000
14		1.000	1.000	1.000	1.000
22		0.600	0.600	0.400	0.400
33		0.000	0.000	0.000	0.000
46		0.000	0.000	0.000	0.000

96h Survival Rate Binomials

Conc-mg/L NH3-N	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LW	5/5	5/5	5/5	5/5
1.8		5/5	5/5	5/5	5/5
3.6		5/5	5/5	5/5	5/5
6.9		5/5	5/5	5/5	5/5
14		5/5	5/5	5/5	5/5
22		3/5	3/5	2/5	2/5
33		0/5	0/5	0/5	0/5
46		0/5	0/5	0/5	0/5

Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

Analysis ID: 17-8043-2695 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.9.2
Analyzed: 11 Jun-18 16:47 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	C > T	14	22	17.55		9.09%

Dunnett Multiple Comparison Test

Control	vs	Conc-mg/L	N	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Lab Water Contr		1.8		0	2.41	0.081	6	CDF	0.8333	Non-Significant Effect
		3.6		0	2.41	0.081	6	CDF	0.8333	Non-Significant Effect
		6.9		0	2.41	0.081	6	CDF	0.8333	Non-Significant Effect
		14		0	2.41	0.081	6	CDF	0.8333	Non-Significant Effect
		22*		16.7	2.41	0.081	6	CDF	2.7E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.0449	0.208981	5	92.8	<1.0E-37	Significant Effect
Error	0.040545	0.0022525	18			
Total	1.08545		23			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Distribution	Shapiro-Wilk W Normality Test	0.578	0.884	3.6E-07	Non-Normal Distribution

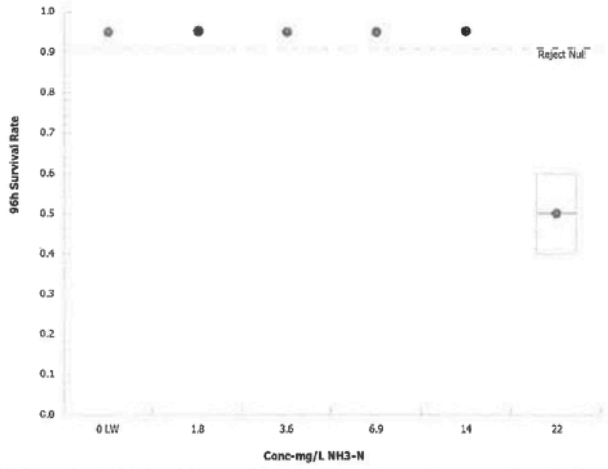
96h Survival Rate Summary

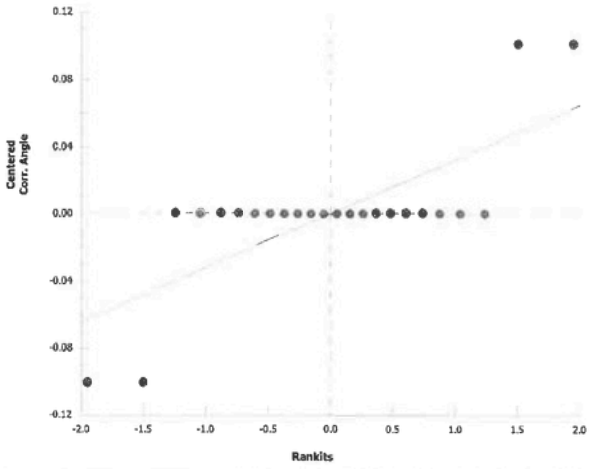
Conc-mg/L NH3-N	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LW	4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
1.8		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
3.6		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
6.9		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
14		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
22		4	0.500	0.316	0.684	0.500	0.400	0.600	0.058	23.09%	50.00%

Angular (Corrected) Transformed Summary

Conc-mg/L NH3-N	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LW	4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
1.8		4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
3.6		4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
6.9		4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
14		4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
22		4	0.785	0.6	0.97	0.785	0.685	0.886	0.0581	14.80%	41.62%

Graphics





CETIS Analytical Report

Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

Analysis ID:	18-2311-5802	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.9.2
Analyzed:	11 Jun-18 16:47	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

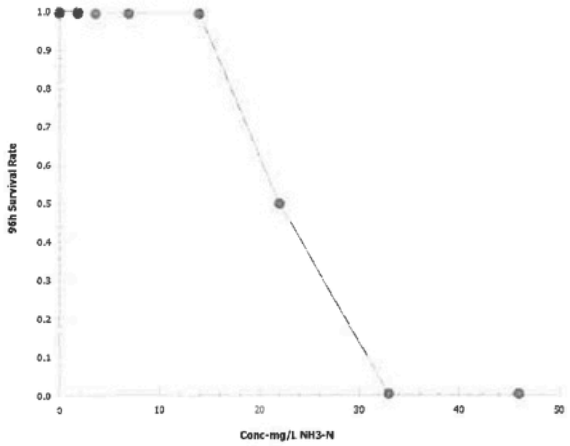
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	1.34	0.0208	21.7	19.8	23.9

96h Survival Rate Summary — Calculated Variate(A/B)

Conc-mg/L NH3-N	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	LW	4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
1.8		4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
3.6		4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
6.9		4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
14		4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
22		4	0.500	0.400	0.600	0.058	0.115	23.10%	50.0%	10	20
33		4	0.000	0.000	0.000	0.000	0.000		100.0%	0	20
46		4	0.000	0.000	0.000	0.000	0.000		100.0%	0	20

Graphics



96 Hour Acute *Anodonta californiensis* Toxicity Test Data

Client: **Pacific EcoRisk** Test Date: 4/16/18

Test Material: Ammonia (as NH_3 - N) Control/Diluent: EPA Hard Water

Test ID#: 78028 Project # 28762 Control Water Batch: 324

Randomization: 4, 8, 2 Organism Source: 10910

Treatment (mg/L NH3 - N)	Temp (°C)	Room Temp (°C) Max/Min	pH New	pH Old	D.O. New	D.O. Old	Conductivity (µS/cm)	# Live Animals A	B	C	D	Sign-Off
Lab Water Control	19.7	19.0/19.9	8.20		8.6		589	5	5	5	5	Date: 4/16/18
2	19.8		8.16		8.8		608	5	5	5	5	Sample ID: —
4	19.9		8.12		8.7		629	5	5	5	5	Test Solution Prep: CO
8	19.9		8.10		8.7		666	5	5	5	5	New WQ: JZ
16	19.9		8.05		8.6		741	5	5	5	5	Initiation Time: 1115
24	19.9		8.03		8.7		823	5	5	5	5	Initiation Signoff: CO
36	19.9		7.97		8.6		943	5	5	5	5	
50	19.9		7.90		8.7		1084	5	5	5	5	
Meter ID	99A	110A	pH15		RD13		EC11					
Lab Water Control	20.6	19.2/19.7										Date: 4/17/18
2	20.9											Temp Time: 0926
4	20.9											Temp Signoff: JZ
8	20.9											
16	20.8											
24	20.5											
36	20.3											
50	19.9											
Meter ID	110A	110A										
Lab Water Control	20.5	M.3/M.7	8.15	8.10	8.8	7.2	590	5	5	5	5	Date: 4/18/18
2	20.8		8.14	8.13	8.9	7.4	610	5	5	5	5	Sample ID: —
4	20.9		8.12	8.11	8.9	7.3	629	5	5	5	5	Test Solution Prep: MS
8	20.8		8.07	8.09	8.9	7.5	668	5	5	5	5	New WQ: MS
16	20.7		8.01	8.04	8.9	7.6	745	5	5	5	5	Count Time: 1255
24	20.7		7.94	8.00	8.9	7.6	825	5	5	5	5	Count Signoff: MS
36	20.4		7.88	7.92	8.9	7.6	947	5	3	3	3	Old WQ: MS
50	19.9		7.82	7.81	8.8	7.7	1085	1	0	0	1	
Meter ID	110A	110A	pH15	pH15	RD13	RD13	EC11					

96 Hour Acute *Anodonta californiensis* Toxicity Test Data

Client: **Pacific EcoRisk** Test Date: 4/16/18

Test Material: Ammonia (as NH_3 - N) Control/Diluent: EPA Hard Water

Test ID#: 78028 Project # 28762 Control Water Batch: 324

Randomization: 4, 8, 2 Organism Source: 10910

Treatment (mg/L NH3 - N)	Temp (°C)	Room Temp (°C) Max/Min	pH New	pH Old	D.O. New	D.O. Old	Conductivity (µS/cm)	# Live Animals A	B	C	D	Sign-Off
Lab Water Control	20.0	19.1/19.7										Date: 4/19/18
2	20.1											Temp Time: 1050
4	20.1											Temp Signoff: BV
8	20.4											
16	20.6											
24	20.6											
36	20.6											
50	20.2											
Meter ID	110A	110A										
Lab Water Control	20.6	19.4/19.6		8.21		5.7	687	5	5	5	5	Date: 4/20/18
2	20.8			8.23		7.1	668	5	5	5	5	Termination Time: 1120
4	20.8			8.21		7.1	674	5	5	5	5	Termination Signoff: CO
8	20.8			8.18		6.6	702	5	5	5	5	Old WQ: MS
16	20.8			8.13		6.5	741	5	5	5	5	
24	20.7			8.10		6.7	848	3	3	2	2	
36	20.3			7.98		6.8	987	0	0	0	0	
50	20.0			7.90		6.6	1157	0	-	-	0	
Meter ID	110A	110A		PH21		RD13	EC11					

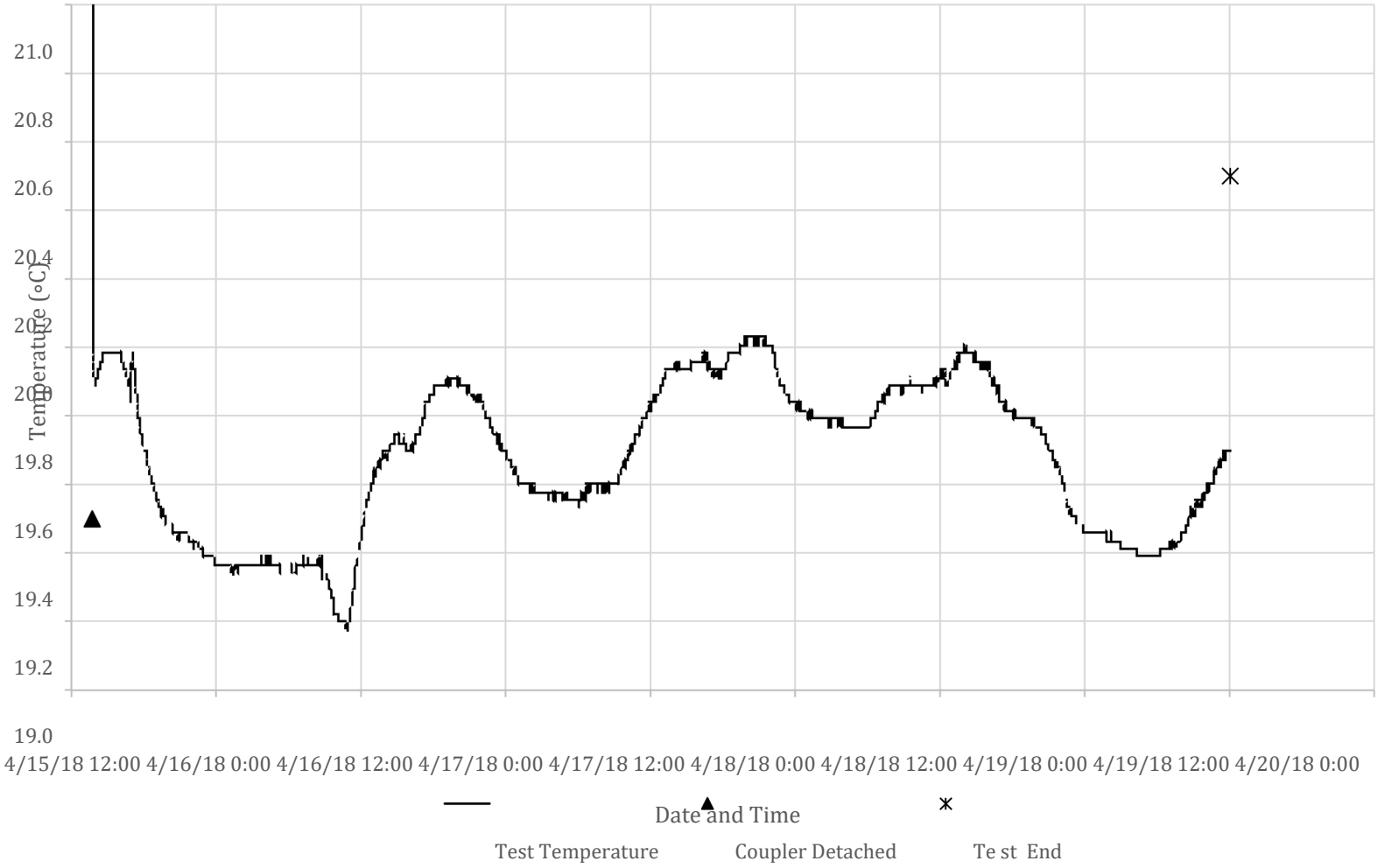
Ancillary Water Chemistries	Initial (mg/L) Alkalinity *	Initial (mg/L) Hardness*	Final Alkalinity*	Final Hardness*
Control	101 ✓	161 ✓	105 ✓	170 ✓
16 mg/L	109 ✓	163 ✓	115 ✓	175 ✓
50 mg/L	115 ✓	166 ✓	- ✓	- ✓

*Measure in each test treatment at test initiation and termination as long as live organisms are present.

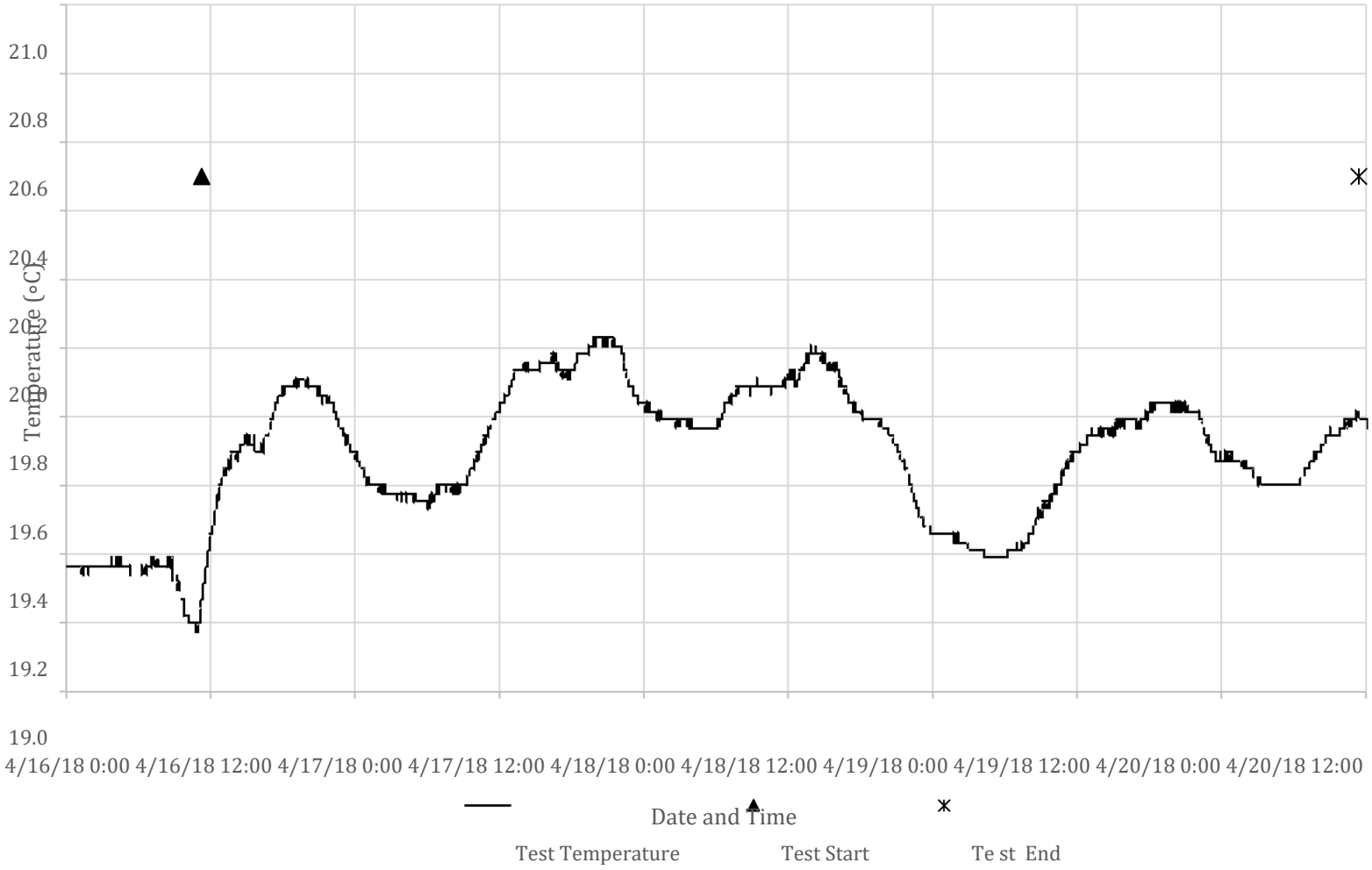
Appendix C

TemperatureControl Charts

Test 1: *Anodonta californiensis* (April 15, 2018)



Test 2: *Anodonta californiensis (*April 16, 2018)



Debbie Webster
Central Valley Clean Water Association
700 R Street
Sacramento, CA 95811

March 15, 2019

Debbie:

I have enclosed our report "Assessing the Acute Sensitivity of Juvenile *Gonidea angulata* to Ammonia" for the testing performed in July 2018, and originally reported in August 2018.

If you have any questions regarding the performance and interpretation of these test, please feel free to contact me at (707) 207-7760.

Regards,

Brant Jorgenson, Ph.D.
Vice President/Special Projects Director

Pacific EcoRisk is accredited in accordance with NELAP (ORELAP ID 4043). Pacific EcoRisk certifies that the test results reported herein conform to the most current NELAP requirements for parameters for which accreditation is required and available. Any exceptions to NELAP requirements are noted, where applicable, in the body of the report. This report shall not be reproduced, except in full, without the written consent of Pacific EcoRisk. This testing was performed under Lab Order 29183 and 29184.

Assessing the Acute Sensitivity of Juvenile *Gonidea angulata* to Ammonia

Prepared for

Central Valley Clean Water Association
700 R Street, Suite 200
Sacramento, CA 95811

Prepared by

Pacific EcoRisk
2250 Cordelia Road
Fairfield, CA 94534

August 2018

PACIFIC ECORISK
ENVIRONMENTAL CONSULTING & TESTING

Assessing the Acute Sensitivityof Juvenile *Gonidea angulata* to Ammonia

Table of Contents

Page

1. INTRODUCTION 1
2. METHODS 1
   2.1 Test Organism Source, Culture Methods, and Acclimation 1
   2.2 Toxicity Test Procedures 2
      2.2.1 Preparation of Test Solutions 3
      2.2.2 Statistical Determination of Toxicity Point Estimates 4
   2.3 Measurement of Ammonia Test Concentrations 4
3. RESULTS 5
   3.1 Toxicity of Ammonia to *Gonidea angulata*: Test 1 5
   3.2 Toxicity of Ammonia to *Gonidea angulata*: Test 2 5
4. QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) 6
   4.1 Toxicity Test Acceptability Requirements 6
      4.1.1 Negative Control 6
      4.1.2 Test Conditions 6
   4.2 Total Ammonia Measurement QA/QC Assessment 6
5. SUMMARY 8
6. REFERENCES 8

Appendices

Appendix A Data Sheets and Summary of Statistical Analysis for Determination of Ammonia LC_{50} Values with Juvenile *Gonidea angulata*: Test 1

Appendix B Data Sheets and Summary of Statistical Analysis for Determination of Ammonia LC_{50} Values with Juvenile *Gonidea angulata*: Test 2

Appendix C Temperature Control Charts

1. INTRODUCTION

The Central ValleyCleanWaterAssociation(CVCWA)isintheprocess of conducting a collaborative studyamong42agenciesrepresenting47CentralValley Publicly Owned Treatment Works(POTWs)titled:*FreshwaterMusselCollaborativeStudy for Wastewater Treatment Plants*.Thisstudyisbeingimplementedonbehalfof CVCWA by the consultant team of Robertson-Bryan,Inc.,LarryWalkerAssociates,andPacificEcoRisk. Phase IIc of this study, led by Pacific EcoRisk,isfocusedonacutetoxicitytestingwithCentral Valley resident freshwater musselstodeterminetheirsensitivitytoammonia.Thetoxicity data generated will then be used by thisteam,workinginclosecoordinationwiththeCentral Valley Regional Water Quality Control Board(CentralValleyWaterBoard)andtheUnitedStates Environmental Protection Agency(U.S.EPA)staff,toderivesite-specificammoniaobjectives for the Central Valley of California,usingU.S.EPA'sRecalculationProcedure.Once derived, the Central Valley Water Boardintendstoadoptthesite-specificammoniaobjectives into its Water Quality Control Plan for theSacramentoandSanJoaquinRiverBasins(Region 5 Basin Plan).

This report presentstheresultsfromtestingtheacutesensitivityof*Gonidea angulata* (Western Ridged Mussel) toammonia.Inconductingthiswork,PacificEcoRisk was specifically responsible for:

- acclimationandholdingofjuvenile *G. angulata*;
- preparationofammoniaspikedtestsolutions;
- performanceofacutetoxicitytestswith *G. angulata*;and
- calculationbenchmarktoxicityvalues(e.g.,LC_{50} pointestimates).

Gravid adult female *G. angulata* werefieldcollectedbyPacificEcoRisk, with assistance from Spring Rivers EcologicalSciences,andharvestedglochidiaweremetamorphosed to juveniles by Dr. Christopher BarnhartofMissouriStateUniversity.Thisreportdescribes and summarizes the performance and resultsoftheaquatictoxicitytestingperformedinsupport of this evaluation.

2. METHODS

Testing and study design closely followed the guidance documents listed below:

- *Standard GuideforConducting Laboratory Toxicity TestswithFreshwater Mussels,* ASTM E2455-06 (ASTM 2013); and
- *Phase IIc Freshwater Mussel Collaborative Studyfor WastewaterTreatment Plants: Study Work Plan for the Acute Toxicity Testing with Resident Freshwater Mussels and Ammonia Criteria Recalculation* (Pacific EcoRisk et al., 2018).

2.1 Test Organism Source, Culture Methods, and Acclimation

Ten feral and gravid adult *G. angulata* were collected from the PitRiver (Burney, CA) by the Pacific EcoRisk (Fairfield, CA), with assistance from Spring RiversEcological Sciences (Cassel,

CA). Adult organisms were shipped by overnight courier to Dr. Christopher Barnhart, Department of Biology at Missouri State University (Springfield, MO). Mature glochidia were extracted and metamorphosed on host fish (*Cottus* sp.).

At ten days post-inoculation, 500 transformed juveniles were collected and shipped by overnight courier to Pacific EcoRisk. Upon receipt, juveniles were acclimated to U.S. EPA synthetic hard water (EPA 2002) per the acclimation specifications summarized in Table 1. Juveniles were fed *Raphidocelis subcapitata* (formally *Selenastrum capricornutum*) during this pre-test acclimation period (N. Wang, pers.com., April 10, 2018).

Table 1. Summary of organism acclimation conditions.

1.	Acclimation period	≥24hours
2.	Acclimation regime (water)	50:50 (culture water:dilution medium) for 2 hours, then 25:75 for 2 hours, then 0:100 for 20+ hours
3.	Acclimation regime (temperature)	Not more than 3°C/hour

2.2 Toxicity Test Procedures

Testing followed the standardtests conditions detailed in ASTM E2455-06, as summarized in Table 2. Two tests were completed, with the first initiated on July 25, 2018 and the second initiated on July 26, 2018.

Test solutions were prepared as described in Section 2.2.1. Water quality characteristics (pH, D.O., and conductivity) were measured for each test solution immediately prior to use in these tests. Alkalinity and hardness were measured in the Lab Water Control, the middle, and high concentration of the dilution series at test initiation and test termination.

There were 4 replicates for each test treatment, with each replicate consisting of 30 mL of test solution in a 50-mL glass beaker. The tests were initiated by randomly allocating 5 juvenile *G. angulata* (<5 days old and within 24 hours age range) into each replicate beaker. The replicate beakers were placed in a temperature-controlled room at 20˚C, under cool-white fluorescent lighting on a 16L:8D photoperiod with a light intensity of 100-1000 lux.

Each replicate beaker was examined after 48 hours exposure, and the number of live juveniles in each beaker was recorded. Fresh test solutions were prepared at this time and were characterized as before. That same day, approximately 75% of the old media in each replicate container was carefully poured out and replaced with the fresh test solution. “Old” water quality characteristics (pH, D.O., and conductivity) were measured at 48 and 96 hours for the old test solutions that had been discarded from all replicates of each treatment. At 96 hours, the test was terminated and the number of live juveniles in each replicate was determined.

Table 2. Summary of test conditions.		
1.	Test type	Static Renewal 20°C Fluorescent 100-1000 lux 16L:8D 50 mL glass beaker
2.	Temperature	30 mL At 48 hours Approximately 75% <5 days 5 4 7 treatments plus
3.	Light quality	control (8 total treatments) ≥0.5 None None None, unless DO falls <4 mg/L,
4.	Light intensity	then aerate all treatments at approximately
5.	Photoperiod	1 bubble/second from a glass pipette
6.	Test chamber size	EPAH (EPA Hard Water)
7.	Test solution volume	96 hours
8.	Renewal frequency	Survival, measured as presence of foot/cillia movement in a 5 minute observation period, or observation of heartbeat
9.	Renewal volume	
10.	Age of organisms	
11.	Organisms per chamber	
12.	Replicates per treatment	
13.	Number of treatments	
14.	Dilution factor	
15.	Feeding regime	
16.	Cleaning	
17.	Aeration	
18.	Dilution water	
19.	Test duration	
20.	Endpoint	

2.2.1PreparationofTest Solutions

The Lab Water Control medium consisted of EPA synthetic hard water (EPA 2002), which was prepared by the addition of reagent grade chemicals to Type 1 water (reverse-osmosis, de-ionized water).

A 50 mg/L total ammonia nitrogen (TAN) stock solution was prepared by the addition of ACS reagent grade ammonium chloride (NH_4Cl, Sigma Aldrich, ³99.5%) to Lab Water Control medium. The stock ammonia solution was analytically verified prior to use in testing. The ammonia stock solution was subsequently diluted by the addition of unspiked Lab Control Medium in order to obtain the test concentrations listed in Table 3. The range of nominal ammonia test concentrations was selected assuming similar ammonia sensitivity to that of other unionid mussel species as published in the scientific literature.

Table 3. Nominal ammonia (TAN) test concentrations.	
Testing Dates	Test Concentrations (mg/L TAN)
Test 1: July 25	0, 2.0, 4.0, 8.0, 16, 24, 36, 50
Test 2: July 26	0, 2.0, 4.0, 8.0, 16, 24, 36, 50

2.2.2 Statistical Determination of Definitive Toxicity Point Estimates

Determination of TAN LC_{50} point estimates and 95% confidence intervals (CI) were made using CETIS® (TidePool Scientific Software, McKinleyville, CA). Point estimates were determined using the mean of test initiation, test renewal (old and new solutions), and test completion measurements of TAN. Because test methods specified a 75% renewal, the renewal solution were calculated as a 3:1 ratio (new:old) of the new and old solution TAN measurements at 48 hours. Point estimates were determined by the Spearman-Kärber method.

2.3 Measurement of Ammonia Test Concentrations

Total ammonia-nitrogen was measured for each treatment at test initiation, test renewal (old and new solutions), and test completion via spectrophotometric method (HACH TNTplus, EPA 350.1 equivalent). Ongoing precision and recovery was demonstrated and documented prior to each batch of ammonia analyses through the analysis of a 10 mg/L TAN standard and comparison of results to corresponding limits for recovery and precision (i.e., 90-110% recovery and maximum relative standard deviation of 10%).

3. RESULTS

3.1 Toxicity of Ammonia to *Gonidea angulata*:Test 1

The results of this test are presented in Table 4. The testdata and summary of statistical analyses for this test are presented in Appendix A.

Table 4. Acute toxicity of ammonia to *G. angulata* : Test 1.

Nominal (mg/L TAN)	Measured Ammonia (mg/L TAN)					%Survival				
	T0 New	T_{48} New[a]	T_{48} Old	T_{96} Old	Test Mean	Rep A	Rep B	Rep C	Rep D	**Mean**
0	<1.0	<1.0	<1.0	<1.0	<1.0	100	100	100	100	**100**
2.0	2.0	1.6	0.53J	0.60J	1.2	100	100	100	100	**100**
4.0	4.0	3.2	1.0	1.8	2.5	100	100	100	100	**100**
8.0	8.0	7.0	4.4	5.5	6.2	100	100	100	80	**95**
16	16	14	11	11	13	100	80	80	60	**80**
24	25	22	17	19	21	0	0	0	0	**0**
36	37	34	27	29	32	0	0	0	0	**0**
50	51	49	44	39	46	0	0	0	0	**0**
Summary of Statistics										
LC_{50} (95% CI) =						14 (12-16)				

J: estimated value.
a – reported TAN values represents a 3:1 weighted concentration of new and old solutions at 48 hours.

3.2 Toxicity of Ammonia to *Gonidea angulata*:Test 2

The results of this test are presented in Table 5. The testdata and summary of statistical analyses for this test are presented in Appendix B.

Table 5. Acute toxicity of ammonia to *G. angulata* : Test 2.

Nominal (mg/L TAN)	Measured Ammonia (mg/L TAN)					%Survival				
	T0 New	T_{48} New[a]	T_{48} Old	T_{96} Old	Test Mean	Rep A	Rep B	Rep C	Rep D	**Mean**
0	<1.0	<1.0	<1.0	<1.0	<1.0	100	100	100	100	**100**
2.0	1.9	1.8	1.3	1.1	1.5	100	100	100	100	**100**
4.0	3.8	3.6	3.1	2.9	3.3	100	100	100	100	**100**
8.0	7.9	7.4	6.1	5.7	6.8	100	100	100	100	**100**
16	16	15	12	12	14	40	20	60	20	**35**
24	24	23	21	20	22	0	0	0	0	**0**
36	37	35	30	30	33	0	0	0	0	**0**
50	51	49	44	44	47	0	0	0	0	**0**
Summary of Statistics										
LC_{50} (95% CI) =						12 (11-14)				

a – reported TAN values represents a 3:1 weighted concentration of new and old solutions at 48 hours.

4. QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

Testing was subject to the QA/QC procedures of ASTM E2455-06 (ASTM 2013). A QA/QC review is provided below.

4.1 Toxicity Test Acceptability Requirements

Toxicity testing incorporated standard QA/QCprocedures to ensure that the test results were valid, including the use of a negative control.These QA/QC procedures were consistent with the test acceptability requirements for toxicity testsconducted with freshwater juvenile mussels outlined in ASTM E2455-06.

4.1.1Negative Control

The biological response in the negative Control treatments were within test acceptability limits of ≥90% survival.

4.1.2Test Conditions

All test conditions were within acceptable limits. Continuously measured test temperatures were within +/- 1˚C of the target test temperature of 20˚C (Appendix C) and the difference between the maximum and minimum test temperature across treatments was less than 2˚C (Table 6). Test treatment pH, hardness, and alkalinity varied less than 10% over the duration of the test (Table 7 and Table 8), and test dissolved oxygen (DO) was maintained above 4 mg/L (Table 9).

4.2 Total Ammonia Measurement QA/QC Assessment

Measurement of total ammonia incorporated standard QA/QC procedures to ensure that the test results were valid, including measurement of laboratory control spike recovery and precision. Spike recovery and relative standard deviation were within specified limits (Table 10).

Table 6. Summary of test temperature (˚C).

Test Treatment	Test 1				Test 2			
	Mean	Min	Max	D (˚C)	Mean	Min	Max	D (˚C)
0	20.1	19.9	21.0	1.1	20.1	19.9	20.3	0.4
2.0	20.2	19.9	21.0	1.1	20.2	20.0	20.5	0.5
4.0	20.2	19.9	21.0	1.1	20.3	19.9	20.5	0.6
8.0	20.3	20.1	20.9	0.8	20.3	19.9	20.4	0.5
16	20.4	20.2	21.0	0.8	20.3	19.9	20.5	0.6
24	20.4	20.1	20.9	0.8	20.3	19.9	20.5	0.6
36	20.3	20.0	21.0	1.0	20.3	20.0	20.5	0.5
50	20.3	20.0	20.9	0.9	20.3	20.0	20.4	0.4

Table 7. Summary of test pH.								
Test Treatment	Test 1				Test 2			
	Mean	Min	Max	D (%)	Mean	Min	Max	D (%)
0	8.22	8.20	8.26	0.7%	8.20	8.16	8.27	1.3%
2.0	8.16	8.10	8.23	1.6%	8.18	8.10	8.26	2.0%
4.0	8.14	8.09	8.18	1.1%	8.15	8.12	8.24	1.5%
8.0	8.09	8.03	8.14	1.4%	8.11	8.07	8.23	2.0%
16	8.03	7.97	8.06	1.1%	8.04	7.98	8.11	1.6%
24	7.96	7.93	8.01	1.0%	7.99	7.91	8.05	1.8%
36	7.89	7.86	7.90	0.5%	7.90	7.84	7.98	1.8%
50	7.82	7.80	7.85	0.6%	7.83	7.80	7.88	1.0%

Table 8. Summary of test hardness and alkalinity (mg/L $CaCO_3$).												
Test Treatment	Test 1						Test 2					
	Hardness			Alkalinity			Hardness			Alkalinity		
	Initial	Final	D (%)	Initial	Final	D (%)	Initial	Final	D (%)	Initial	Final	D (%)
0	170	170	0.0	120	130	8.3	160	170	6.3	120	130	8.3
16	160	170	6.3	110	120	9.1	170	170	0.0	110	110	0.0
50	170	-	[a]	110	-	[a]	160	-	[a]	110	-	[a]

a – Final concentrationnotmeasureddue to completemortalityinthistreatment.

Table 9. Summary of test dissolved oxygen (mg/L).								
Test Treatment	Test 1				Test 2			
	Mean	Min	Max	D (mg/L)	Mean	Min	Max	D (mg/L)
0	8.0	7.2	8.5	1.3	8.2	7.6	8.8	1.2
2.0	7.7	6.2	8.5	2.3	8.3	7.8	8.7	0.9
4.0	7.8	6.4	8.5	2.1	8.3	8.0	8.6	0.6
8.0	8.0	6.5	8.6	2.1	8.3	8.0	8.6	0.6
16	7.9	6.4	8.6	2.2	8.3	7.8	8.5	0.7
24	7.9	6.0	8.7	2.7	8.4	8.0	8.6	0.6
36	8.1	6.8	8.7	1.9	8.4	8.1	8.6	0.5
50	8.0	6.8	8.5	1.7	8.4	8.0	8.6	0.6

Table 10. Summary of daily batch ammonia recovery and precision.		
Date	Mean Recovery (%)	RSD (%)
7/25/18	99.7	1.2
7/26/18	101	1.9
7/27/18	102	2.6
7/28/18	99.4	1.9
7/29/18	103	1.4
7/30/18	99.0	0.84

5. SUMMARY

Total ammonia LC_{50} values (and accompanying 95% CI)for juvenile *G. angulata* are presented in Table 11. All results were calculated as described in this report.

Table 11. Total Ammonia LC_{50} for juvenile *G. angulata.*

Test No.	Total Ammonia LC_{50} value (mg/L TAN)	95% Confidence Interval (mg/L TAN)
Test 1	14	12-16
Test 2	12	11-14

6. REFERENCES

ASTM (ASTM International). 2013. Standard guide for conducting laboratory toxicity tests with freshwater mussels. E2455-06 (2013). West Conshohocken, PA. 2013.

EPA (2002). Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. EPA 821-R-02-012. US Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN. October 2002.

Pacific EcoRisk, Robertson-Bryan, Inc, Larry Walker Associates. 2018. Phase IIc Freshwater Mussel Collaborative Study for Wastewater Treatment Plants: Study Work Plan for the Acute Toxicity Testing with Resident Freshwater Mussels and Ammonia Criteria Recalculation. Prepared for Central Valley Clean Water Association. February 2018.

Appendix A

Data Sheets and Summaryof Statistical Analysis for Determinationof AmmoniaLC_{50} Values with Juvenile *Gonidea angulata*: Test 1

CETIS Summary Report

Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

Batch ID:	07-3346-4689	**Test Type:**	Survival (96h)	**Analyst:**	Charlene Derheim
Start Date:	25 Jul-18 10:45	**Protocol:**	ASTM E2455-06	**Diluent:**	Hard Synthetic Water
Ending Date:	29 Jul-18 10:45	**Species:**	Gonidea angulata	**Brine:**	Not Applicable
Duration:	96h	**Source:**	Missouri State University	**Age:**	<5

Sample ID:	04-2658-0712	**Code:**	NH4Cl	**Client:**	Reference Toxicant
Sample Date:	25 Jul-18 10:45	**Material:**	Ammonia (as NH3-N)	**Project:**	29183
Receipt Date:	25 Jul-18 10:45	**Source:**	Reference Toxicant		
Sample Age:	n/a (21 °C)	**Station:**	In House		

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	NOEL	LOEL	TOEL	TU	PMSD ✓
13-7939-8594	96h Survival Rate	Steel Many-One Rank Sum Test	13	> 13	n/a		14.5%

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	Level	mg/L NH3	95% LCL	95% UCL	TU ✓
19-7832-5058	96h Survival Rate	Spearman-Kärber	EC50	14	12.3	16.1	

96h Survival Rate Summary

Conc-mg/L NH3-N	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LW	4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
1.2		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
2.5		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
6.2		4	0.950	0.791	1.000	0.800	1.000	0.050	0.100	10.53%	5.00%
13		4	0.800	0.540	1.000	0.600	1.000	0.082	0.163	20.41%	20.00%
21		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100.00%
32		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100.00%
46		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100.00%

96h Survival Rate Detail

Conc-mg/L NH3-N	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LW	1.000	1.000	1.000	1.000
1.2		1.000	1.000	1.000	1.000
2.5		1.000	1.000	1.000	1.000
6.2		1.000	1.000	1.000	0.800
13		1.000	0.800	0.800	0.600
21		0.000	0.000	0.000	0.000
32		0.000	0.000	0.000	0.000
46		0.000	0.000	0.000	0.000

96h Survival Rate Binomials

Conc-mg/L NH3-N	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LW	5/5	5/5	5/5	5/5
1.2		5/5	5/5	5/5	5/5
2.5		5/5	5/5	5/5	5/5
6.2		5/5	5/5	5/5	4/5
13		5/5	4/5	4/5	3/5
21		0/5	0/5	0/5	0/5
32		0/5	0/5	0/5	0/5
46		0/5	0/5	0/5	0/5

Acute Juvenile Freshwater Mussel Survival — Pacific EcoRisk

Analysis ID:	13-7939-8594	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.9.2
Analyzed:	31 Jul-18 9:57	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	C > T	13	> 13	n/a		14.53%

Steel Many-One Rank Sum Test

Control	vs	Conc-mg/L N	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Lab Water Contr		1.2	18	10	1	6	Asymp	0.8000	Non-Significant Effect
		2.5	18	10	1	6	Asymp	0.8000	Non-Significant Effect
		6.2	16	10	1	6	Asymp	0.5661	Non-Significant Effect
		13	12	10	1	6	Asymp	0.1228	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.164087	0.0410218	4	4.16	0.0184	Significant Effect
Error	0.148039	0.0098693	15			
Total	0.312126		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Levene Equality of Variance Test	3.21	4.89	0.0432	Equal Variances
Variances	Mod Levene Equality of Variance Test	1.67	4.89	0.2081	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.707	0.866	4.8E-05	Non-Normal Distribution

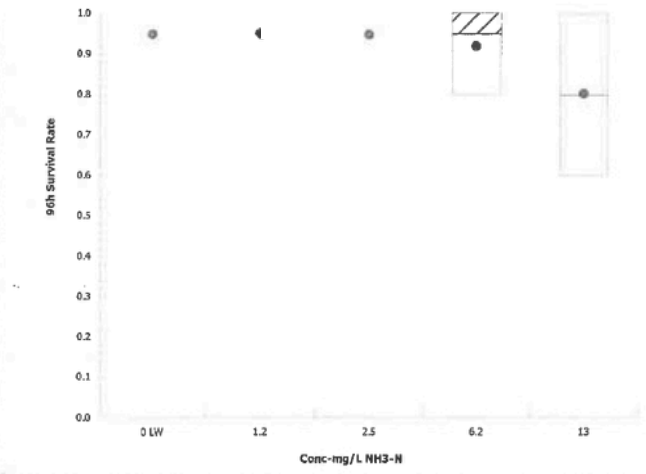
96h Survival Rate Summary

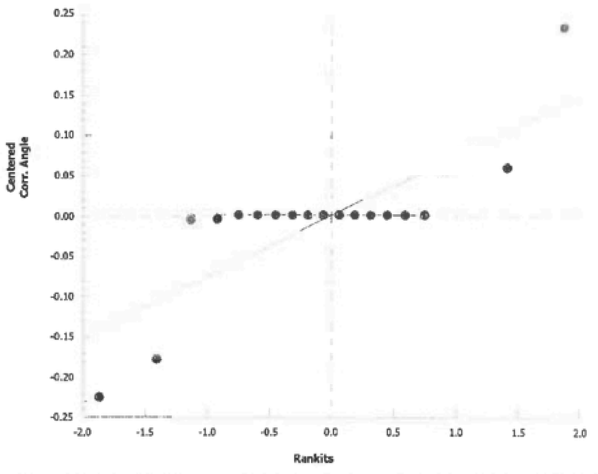
Conc-mg/L NH3-N	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LW	4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
1.2		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
2.5		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
6.2		4	0.950	0.791	1.000	1.000	0.800	1.000	0.050	10.53%	5.00%
13		4	0.800	0.540	1.000	0.800	0.600	1.000	0.082	20.41%	20.00%

Angular (Corrected) Transformed Summary

Conc-mg/L NH3-N	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LW	4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
1.2		4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
2.5		4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
6.2		4	1.29	1.1	1.48	1.35	1.11	1.35	0.0595	9.26%	4.43%
13		4	1.11	0.813	1.41	1.11	0.886	1.35	0.0938	16.87%	17.38%

Graphics





CETIS Analytical Report

Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

Analysis ID:	19-7832-5058	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.9.2
Analyzed:	31 Jul-18 10:24	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

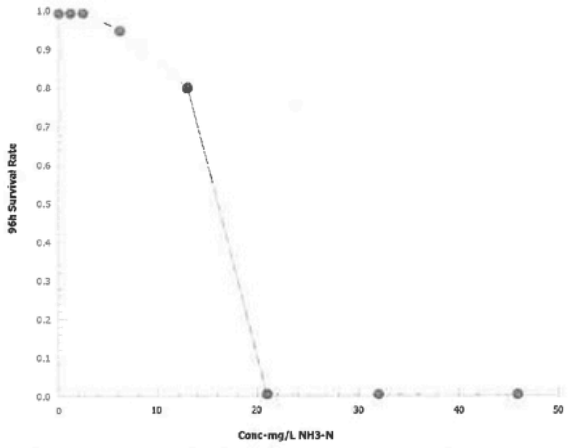
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	1.15	0.0294	14	12.3	16.1

96h Survival Rate Summary

			Calculated Variate(A/B)								
Conc-mg/L NH3-N	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	LW	4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
1.2		4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
2.5		4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
6.2		4	0.950	0.800	1.000	0.050	0.100	10.50%	5.0%	19	20
13		4	0.800	0.600	1.000	0.082	0.163	20.40%	20.0%	16	20
21		4	0.000	0.000	0.000	0.000	0.000		100.0%	0	20
32		4	0.000	0.000	0.000	0.000	0.000		100.0%	0	20
46		4	0.000	0.000	0.000	0.000	0.000		100.0%	0	20

Graphics



96 Hour Acute *Gonidea angulata* Toxicity Test Data

Client: **Pacific EcoRisk** Test Date: 7/25/18

Test Material: Ammonia (as NH3 - N) Control/Diluent: EPA Hard Water

Test ID#: 79217 Project # 29183 Control Water Batch: 334

Randomization: 4, 8, 1 Organism Source: Pit River

Treatment (mg/L NH3 - N)	Temp (°C)	Room Temp (°C) Max/Min	pH New	pH Old	D.O. New	D.O. Old	Conductivity (µS/cm)	# Live Animals A	B	C	D	Sign-Off
Lab Water Control	21.0	19.7/21.0	8.20		8.5		583	5	5	5	5	Date: 7/25/18
2	21.0		8.20		8.5		614	5	5	5	5	Sample ID: —
4	21.0		8.18		8.5		623	5	5	5	5	Test Solution Prep: JZ
8	20.9		8.14		8.6		664	5	5	5	5	New WQ: JZ
16	21.0		8.05		8.5		740	5	5	5	5	Initiation Time: 1045
24	20.9		7.98		8.5		819	5	5	5	5	Initiation Signoff: CO
36	21.0		7.90		8.5		938	5	5	5	5	
50	20.9		7.83		8.4		1075	5	5	5	5	
Meter ID	100A	85A	pH15		RD11		EC11					
Lab Water Control	19.9	19.7/21.0										Date: 7/26/18
2	20.1											Temp Time: 0910
4	20.1											Temp Signoff: CO
8	20.2											
16	20.2											
24	20.3											
36	20.3											
50	20.4											
Meter ID	100A	85A										
Lab Water Control	19.9	19.6/19.9	8.26	8.20	8.3	7.2	587	5	5	5	5	Date: 7/27/18
2	19.9		8.23	8.10	8.5	6.2	611	5	5	5	5	Sample ID: —
4	20.0		8.18	8.11	8.3	6.4	620	5	5	5	5	Test Solution Prep: NB
8	20.1		8.12	8.03	8.6	6.5	664	5	5	5	4	New WQ: NB
16	20.2		8.06	7.97	8.6	6.4	733	5	5	5	4	Count Time: 1205
24	20.1		7.94	7.93	8.7	6.0	816	5	2	1	1	Count Signoff: CO
36	20.1		7.90	7.86	8.7	6.8	933	1	0	1	2	Old WQ: BT
50	20.1		7.85	7.80	8.5	6.8	1055	0	0	0	0	
Meter ID	85A	85A	pH15	pH24	RD11	RD13	EC11					

96 Hour Acute *Gonidea angulata* Toxicity Test Data

Client: **Pacific EcoRisk** Test Date: 7/25/18

Test Material: Ammonia (as NH_3 - N) Control/Diluent: EPA Hard Water

Test ID#: 79217 Project # 29183 Control Water Batch: 334

Randomization: 4.8.1 Organism Source: Pit River

Treatment (mg/L NH3 - N)	Temp (°C)	Room Temp (°C) Max/Min	pH		D.O.		Conductivity (µS/cm)	# Live Animals				Sign-Off
			New	Old	New	Old		A	B	C	D	
Lab Water Control	19.9	19.5/19.8										Date: 7/28/18
2	20.0											Temp Time: 0905
4	19.9											Temp Signoff: SZ
8	20.2											
16	20.2											
24	20.2											
36	20.0											
50	20.1											
Meter ID	85A	85A										
Lab Water Control	19.9	19.6/19.9		8.22		7.8	665	5	5	5	5	Date: 7/29/18
2	20.0			8.12		7.6	672	5	5	5	5	Termination Time: 1045
4	20.0			8.09		7.8	689	4	6	5	5	Termination Signoff: SZ
8	20.2			8.07		8.1	688	5	5	5	4	Old WQ: BS
16	20.2			8.03		8.1	772	5	4	4	3	
24	20.3			8.01		8.3	825	0	0	0	0	
36	20.2			7.90		8.2	947	0	–	0	0	
50	20.0			7.80		8.2	1093	–	–	–	–	
Meter ID	85A	85A		PH25		12013	EC11					

Ancillary Water Chemistries	Initial (mg/L)		Final	
	Alkalinity *	Hardness*	Alkalinity*	Hardness*
Control	117.6	168	126.8	174.4
16 mg/L	111	164	116.4	173.2
50 mg/L	111	168	–	–

*Measure in each test treatment at test initiation and termination as long as live organisms are present.

Appendix B

Data Sheets and Summaryof Statistical Analysis for Determination of AmmoniaLC_{50} Values with Juvenile *Gonidea angulata*: Test 2

CETIS Summary Report

Report Date: 31 Jul-18 10:25 (p 1 of 1)
Test Code: 79218 | 00-6331-1082

Acute Juvenile Freshwater Mussel Survival — Pacific EcoRisk

Batch ID:	17-3055-5104	**Test Type:**	Survival (96h)	**Analyst:**	Charlene Derheim
Start Date:	26 Jul-18 09:30	**Protocol:**	ASTM E2455-06	**Diluent:**	Hard Synthetic Water
Ending Date:	30 Jul-18 09:30	**Species:**	Gonidea angulata	**Brine:**	Not Applicable
Duration:	96h	**Source:**	Missouri State University	**Age:**	<5

Sample ID:	11-8570-7187	**Code:**	NH4Cl	**Client:**	Reference Toxicant
Sample Date:	26 Jul-18 09:30	**Material:**	Ammonia (as NH3-N)	**Project:**	29183
Receipt Date:	26 Jul-18 09:30	**Source:**	Reference Toxicant		
Sample Age:	n/a (20 °C)	**Station:**	In House		

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	NOEL	LOEL	TOEL	TU	PMSD ✓
09-6778-1605	96h Survival Rate	Steel Many-One Rank Sum Test	6.8	14	9.757		13.5%

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	Level	mg/L NH3	95% LCL	95% UCL	TU ✓
17-9226-6294	96h Survival Rate	Spearman-Kärber	EC50	12	10.6	13.6	

96h Survival Rate Summary

Conc-mg/L NH3-N	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LW	4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
1.5		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
3.3		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
6.8		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
14		4	0.350	0.045	0.655	0.200	0.600	0.096	0.191	54.71%	65.00%
22		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100.00%
33		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100.00%
47		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100.00%

96h Survival Rate Detail

Conc-mg/L NH3-N	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LW	1.000	1.000	1.000	1.000
1.5		1.000	1.000	1.000	1.000
3.3		1.000	1.000	1.000	1.000
6.8		1.000	1.000	1.000	1.000
14		0.400	0.200	0.600	0.200
22		0.000	0.000	0.000	0.000
33		0.000	0.000	0.000	0.000
47		0.000	0.000	0.000	0.000

96h Survival Rate Binomials

Conc-mg/L NH3-N	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LW	5/5	5/5	5/5	5/5
1.5		5/5	5/5	5/5	5/5
3.3		5/5	5/5	5/5	5/5
6.8		5/5	5/5	5/5	5/5
14		2/5	1/5	3/5	1/5
22		0/5	0/5	0/5	0/5
33		0/5	0/5	0/5	0/5
47		0/5	0/5	0/5	0/5

Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

Analysis ID:	09-6778-1605	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.9.2
Analyzed:	31 Jul-18 10:02	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	C > T	6.8	14	9.757		13.54%

Steel Many-One Rank Sum Test

Control	vs	Conc-mg/L N	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Lab Water Contr		1.5	18	10	1	6	Asymp	0.8000	Non-Significant Effect
		3.3	18	10	1	6	Asymp	0.8000	Non-Significant Effect
		6.8	18	10	1	6	Asymp	0.8000	Non-Significant Effect
		14*	10	10	0	6	Asymp	0.0350	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.66238	0.415596	4	50.4	<1.0E-37	Significant Effect
Error	0.123796	0.0082531	15			
Total	1.78618		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Levene Equality of Variance Test	15.3	4.89	3.5E-05	Unequal Variances
Variances	Mod Levene Equality of Variance Test	10.2	4.89	3.4E-04	Unequal Variances
Distribution	Shapiro-Wilk W Normality Test	0.594	0.866	2.5E-06	Non-Normal Distribution

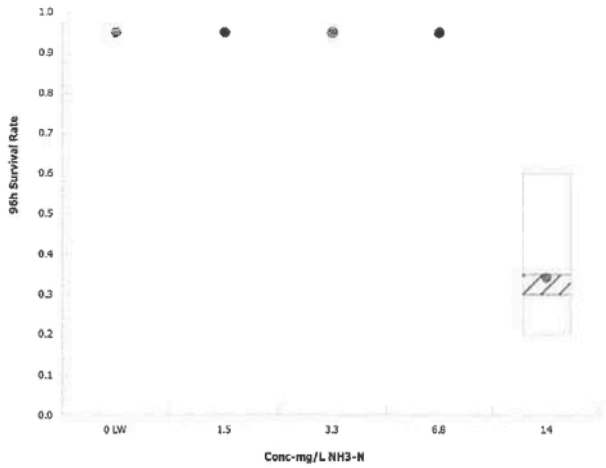
96h Survival Rate Summary

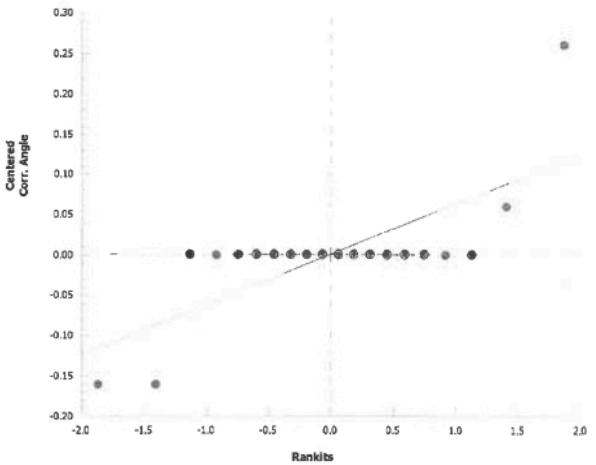
Conc-mg/L NH3-N	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LW	4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
1.5		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
3.3		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
6.8		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
14		4	0.350	0.045	0.655	0.300	0.200	0.600	0.096	54.71%	65.00%

Angular (Corrected) Transformed Summary

Conc-mg/L NH3-N	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LW	4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
1.5		4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
3.3		4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
6.8		4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
14		4	0.625	0.301	0.948	0.574	0.464	0.886	0.102	32.53%	53.58%

Graphics





CETIS Analytical Report

Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

Analysis ID:	17-9226-6294	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.9.2
Analyzed:	31 Jul-18 10:25	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

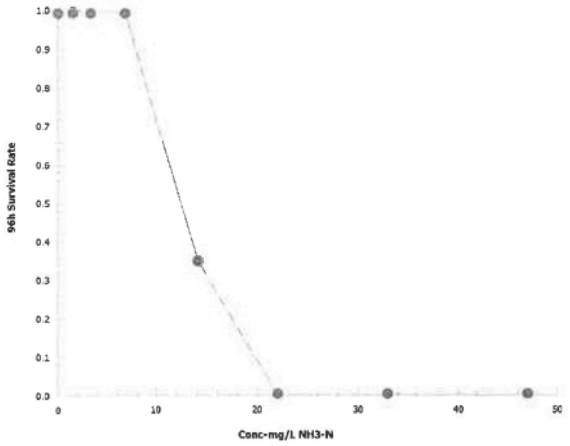
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	1.08	0.0272	12	10.6	13.6

96h Survival Rate Summary — Calculated Variate(A/B)

Conc-mg/L NH3-N	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	LW	4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
1.5		4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
3.3		4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
6.8		4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
14		4	0.350	0.200	0.600	0.096	0.191	54.70%	65.0%	7	20
22		4	0.000	0.000	0.000	0.000	0.000		100.0%	0	20
33		4	0.000	0.000	0.000	0.000	0.000		100.0%	0	20
47		4	0.000	0.000	0.000	0.000	0.000		100.0%	0	20

Graphics



96 Hour Acute *Gonidea angulata* Toxicity Test Data

Client: **Pacific EcoRisk** Test Date: 7/26/18

Test Material: Ammonia (as NH_3 - N) Control/Diluent: EPA Hard Water

Test ID#: 79218 Project #: 29183 Control Water Batch: 334

Randomization: 4.8.2 Organism Source: Pit River

Treatment (mg/L NH3 - N)	Temp (°C)	Room Temp (°C) Max/Min	pH New	pH Old	D.O. New	D.O. Old	Conductivity (µS/cm)	# Live Animals A	B	C	D	Sign-Off
Lab Water Control	20.0	19.7/21.0	8.27		8.8		580	5	5	5	5	Date: 7/26/18
2	20.0		8.26		8.7		600	5	5	5	5	Sample ID: -
4	19.9		8.24		8.6		619	5	5	5	5	Test Solution Prep: JL
8	19.9		8.23		8.6		658	5	5	5	5	New WQ: JL
16	19.9		8.11		8.5		736	5	5	5	5	Initiation Time: 0930
24	19.9		8.03		8.6		815	5	5	5	5	Initiation Signoff: JL
36	20.0		7.94		8.5		931	5	5	5	5	
50	20.0		7.86		8.5		1070	5	5	5	5	
Meter ID	107A	85A	pH15		RD11		EC13					
Lab Water Control	20.0	19.6/19.9										Date: 7/27/18
2	20.2											Temp Time: 1200
4	20.3											Temp Signoff: CD
8	20.4											
16	20.4											
24	20.5											
36	20.5											
50	20.4											
Meter ID	85A	85A										
Lab Water Control	20.2	19.5/19.8	8.16	8.16	8.6	7.8	576	5	5	5	5	Date: 7/28/18
2	20.3		8.18	8.17	8.5	8.1	597	5	5	5	5	Sample ID: —
4	20.4		8.13	8.12	8.4	8.0	615	5	5	5	5	Test Solution Prep: JL
8	20.4		8.07	8.07	8.4	8.0	653	5	5	5	5	New WQ: JL
16	20.4		7.98	7.99	8.4	7.8	728	5	5	5	5	Count Time: 1034
24	20.4		7.91	7.97	8.4	8.0	805	1	1	0	4	Count Signoff: JL
36	20.3		7.84	7.87	8.4	8.1	921	0	0	0	0	Old WQ: JL
50	20.3		7.80	7.80	8.4	8.0	1055	0	0	0	0	
Meter ID	85A	85A	pH15	pH15	RD10	RD10	EC10					

96 Hour Acute *Gonidea angulata* Toxicity Test Data

Client: **Pacific EcoRisk** Test Date: 7/26/18

Test Material: Ammonia (as NH_3 - N) Control/Diluent: EPA Hard Water

Test ID#: 79218 Project #: 29184 Control Water Batch: 334

Randomization: 4.8.2 Organism Source: Pit River

Treatment (mg/L NH3 - N)	Temp (°C)	Room Temp (°C) Max/Min	pH New	pH Old	D.O. New	D.O. Old	Conductivity (µS/cm)	# Live Animals A	# Live Animals B	# Live Animals C	# Live Animals D	Sign-Off
Lab Water Control	20.3	19.6/19.9										Date: 7/29/18
2	20.5											Temp Time: 0923
4	20.5											Temp Signoff: JL
8	20.4											
16	20.5											
24	20.4											
36	20.4											
50	20.4											
Meter ID	85A	85A										
Lab Water Control	19.9	19.2/19.8		8.20		7.6	628	5	5	5	5	Date: 7/30/18
2	20.2			8.10		7.8	643	5	5	5	5	Termination Time: 0930
4	20.3			8.12		8.0	647	5	5	5	5	Termination Signoff: JL
8	20.3			8.08		8.1	685	5	5	5	5	Old WQ: SL
16	20.3			8.08		8.3	748	2	1	3	1	
24	20.4			8.05		8.4	817	0	0	—	0	
36	20.4			7.98		8.6	919	—	—	—	—	
50	20.3			7.98		8.6	1069	—	—	—	—	
Meter ID	85A	85A		pH24		RD10	EC10					

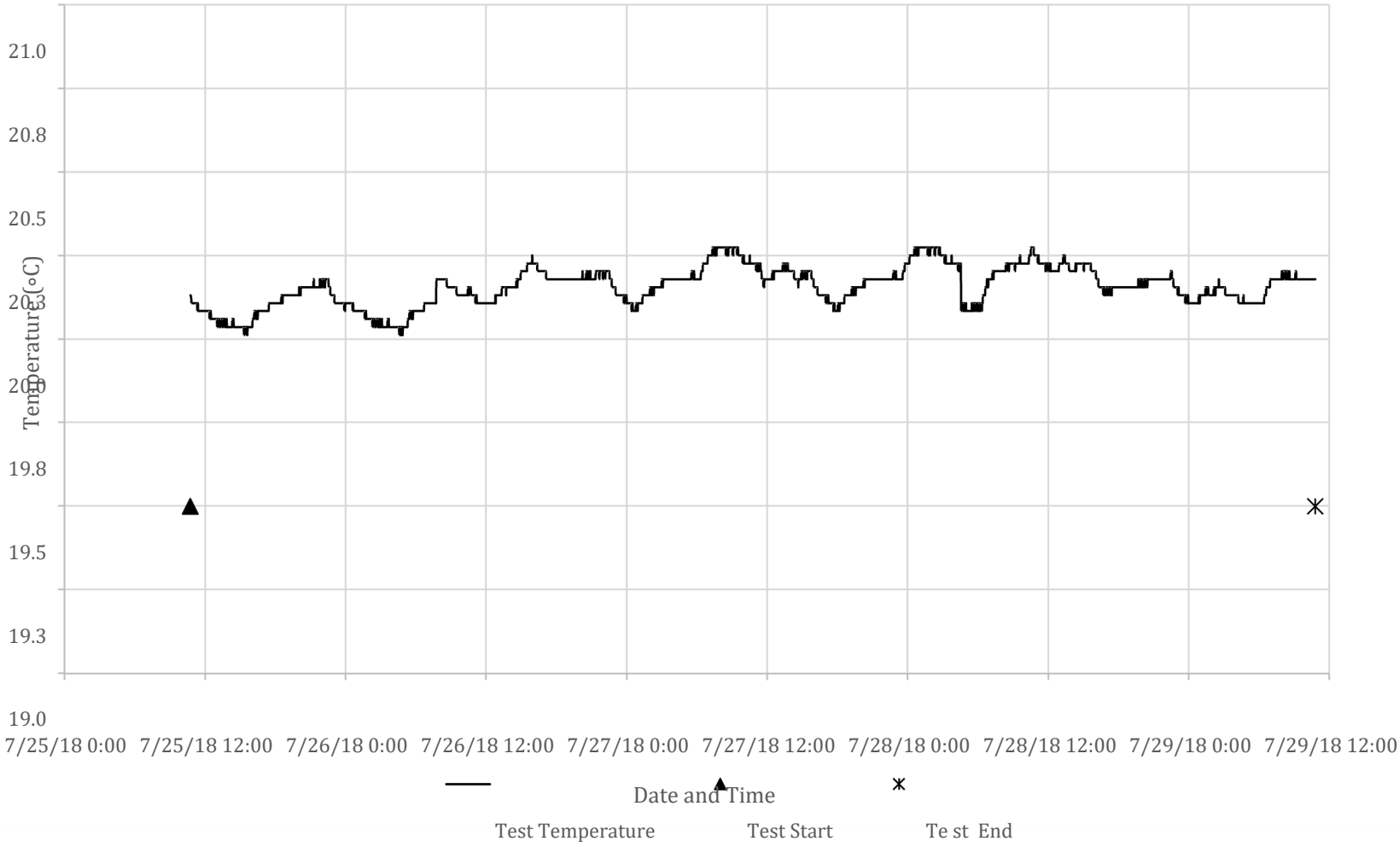
Ancillary Water Chemistries	Initial (mg/L) Alkalinity *	Initial (mg/L) Hardness*	Final Alkalinity*	Final Hardness*
Control	116	162	126	168.4
16 mg/L	113	168	110.4	165.6
50 mg/L	112	164	—	—

*Measure in each test treatment at test initiation and termination as long as live organisms are present.

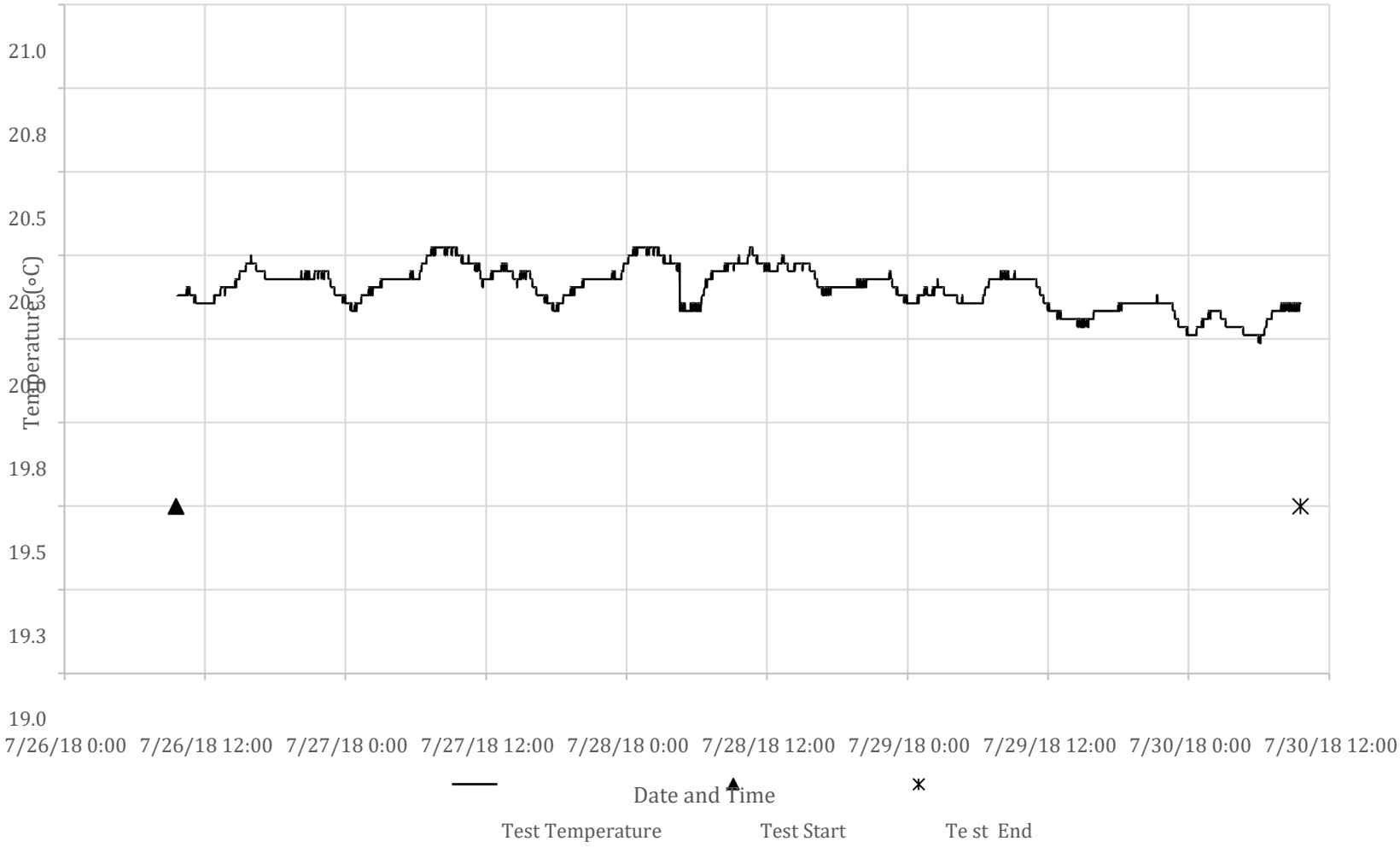
Appendix C

TemperatureControl Charts

Test 1: *Gonidea angulata* (July 25, 2018)



Test 2: *Gonidea angulata* (July 26, 2018)



Debbie Webster
Central Valley Clean Water Association
700 R Street
Sacramento, CA 95811

March 15, 2019

Debbie:

I have enclosed our report "Assessing the Acute Sensitivity of Juvenile *Anodonta oregonensis* to Ammonia" for the testing performed in July 2016, and originally reported in September 2018.

If you have any questions regarding the performance and interpretation of these test, please feel free to contact me at (707) 207-7760.

Regards,

Brant Jorgenson, Ph.D.
Vice President/Special Projects Director

Pacific EcoRisk is accredited in accordance with NELAP (ORELAP ID 4043). Pacific EcoRisk certifies that the test results reported herein conform to the most current NELAP requirements for parameters for which accreditation is required and available. Any exceptions to NELAP requirements are noted, where applicable, in the body of the report. This report shall not be reproduced, except in full, without the written consent of Pacific EcoRisk. This testing was performed under Lab Order 26007 and 26014.

Assessing the Acute Sensitivity of Juvenile *Anodonta oregonensis* to Ammonia

Prepared for

Central Valley Clean Water Association
700 R Street, Suite 200
Sacramento, CA 95811

Prepared by

Pacific EcoRisk
2250 Cordelia Road
Fairfield, CA 94534

September 2018

PACIFIC ECORISK
ENVIRONMENTAL CONSULTING & TESTING

Assessing the Acute Sensitivity of Juvenile *Anodonta oregonensis* to Ammonia

Table of Contents

Page

1. INTRODUCTION 1
2. METHODS 1
   2.1 Test Organism Source, Culture Methods, and Acclimation 1
   2.2 Toxicity Test Procedures 2
      2.2.1 Preparation of Test Solutions 3
      2.2.2 Statistical Determination of Toxicity Point Estimates 4
   2.3 Measurement of Ammonia Test Concentrations 4
3. RESULTS 5
   3.1 Toxicity of Ammonia to *Anodonta oregonensis*: Test 1 5
   3.2 Toxicity of Ammonia to *Anodonta oregonensis*: Test 2 5
4. QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) 6
   4.1 Toxicity Test Acceptability Requirements 6
      4.1.1 Negative Control 6
      4.1.2 Test Conditions 6
5. SUMMARY 8
6. REFERENCES 8

Appendices

Appendix A Data Sheets and Summary of Statistical Analysis for Determination of Ammonia LC_{50} Values with Juvenile *Anodonta oregonensis*: Test 1

Appendix B Data Sheets and Summary of Statistical Analysis for Determination of Ammonia LC_{50} Values with Juvenile *Anodonta oregonensis*: Test 2

Appendix C Temperature Control Charts

1. INTRODUCTION

The Central Valley Clean Water Association (CVCWA) is in the process of conducting a collaborative study among 42 agencies representing 47 Central Valley Publicly Owned Treatment Works (POTWs) titled: *Freshwater Mussel Collaborative Study for Wastewater Treatment Plants*. This study is being implemented on behalf of CVCWA by the consultant team of Robertson-Bryan, Inc., Larry Walker Associates, and Pacific EcoRisk. Phase IIc of this study, led by Pacific EcoRisk, is focused on acute toxicity testing with Central Valley resident freshwater mussels to determine their sensitivity to ammonia. The toxicity data generated will then be used by this team, working in close coordination with the Central Valley Regional Water Quality Control Board (Central Valley Water Board) and the United States Environmental Protection Agency (U.S. EPA) staff, to derive site-specific ammonia objectives for the Central Valley of California, using U.S. EPA's Recalculation Procedure. Once derived, the Central Valley Water Board intends to adopt the site-specific ammonia objectives into its Water Quality Control Plan for the Sacramento and San Joaquin River Basins (Region 5 Basin Plan).

This report presents the results from testing the acute sensitivity of *Anodonta oregonensis* (Oregon Floater) to ammonia. In conducting this work, Pacific EcoRisk was specifically responsible for:

- acclimation and holding of juvenile *A. oregonensis*;
- preparation of ammonia spiked test solutions;
- performance of acute toxicity tests with *A. oregonensis*; and
- calculation benchmark toxicity values (e.g., LC_{50} point estimates).

Gravid adult female *A. oregonensis* were field collected by Julie Campbell-Hansen of the United States Fish and Wildlife Service, and harvested glochidia were metamorphosed to juveniles by Dr. Christopher Barnhart of Missouri State University. This report describes and summarizes the performance and results of the aquatic toxicity testing performed in support of this evaluation.

2. METHODS

Testing and study design closely followed the guidance documents listed below:

- *Standard GuideforConducting Laboratory Toxicity TestswithFreshwater Mussels,* ASTM E2455-06 (ASTM 2013); and
- *Phase IIc Freshwater Mussel Collaborative Study for Wastewater Treatment Plants Study Work Plan for the Acute Toxicity Testing with Resident Freshwater Mussels and Ammonia Criteria Recalculation* (Pacific EcoRisk et al., 2018).

2.1 Test Organism Source, Culture Methods, and Acclimation

Ten feral and gravid adult *A. oregonensis* were collected from Hayes Island on the Upper Columbia River (Harter, WA) by Julie Campbell-Hansen of the United States Fish and Wildlife

Service. Adult organisms were delivered to Dr. Christopher Barnhart, Department of Biology at Missouri State University (Springfield, MO). Mature glochidia were extracted and metamorphosed by the *in vitro* method

Upon completion of metamorphosis, approximately 500 transformed juveniles were collected and shipped by overnight courier to Pacific EcoRisk. Upon receipt, juveniles were acclimated to U.S. EPA synthetic hard water (EPA 2002) per the acclimation specifications summarized in Table 1.

Table 1. Summary of organism acclimation conditions

1.	Acclimation period	≥24 hours
2.	Acclimation regime (water)	50:50 (culture water:dilution medium) for 2 hours, then 25:75 for 2 hours, then 0:100 for 20+ hours
3.	Acclimation regime (temperature)	Not more than 3°C/hour

2.2 ToxicityTestProcedures

Testing followedthestandardtestsconditionsdetailedinASTME2455-06,assummarizedin Table 2. Twotestswerecompleted,withthefirstinitiatedonJuly7,2016andthesecond initiated July8,2016.

Test solutionswerepreparedasdescribedinSection2.2.1.Waterqualitycharacteristics(pH, D.O., and conductivity)weremeasuredforeachtestsolutionimmediatelypriortouseinthese tests. AlkalinityandhardnesswеremeasuredintheLabWaterControl,themiddle,andhigh concentration of the dilution series at test initiation and test termination.

There were4replicatesforeachtesttreatment,witheachreplicateconsistingof30mLoftest solution ina50-mLglassbeaker.Thetestswereinitiatedbyrandomlyallocating5juvenile *A. oregonensis*(<5daysoldandwithin24hoursagerange)intoeachreplicatebeaker.The replicate beakerswereplacedinatemperature-controlledroomat20˚C,undercool-white fluorescent lighting on a 16L:8D photoperiod with a light intensity of 100-1000 lux.

Each replicatebeakerwasexaminedafter48hoursexposure,andthenumberoflivejuvenilesin each beaker wasrecorded.Freshtestsolutionswerepreparedatthistimeandwerecharacterized as before. Thatsameday,approximately75%oftheoldmediaineachreplicatecontainerwas carefully pouredoutandreplacedwiththefreshtestsolution.“Old”waterqualitycharacteristics (pH, D.O., andconductivity)weremeasuredat48and96hoursfortheoldtestsolutionsthathad been discardedfromallreplicatesofeachtreatment.At96hours,thetestwasterminatedandthe number of livejuvenilesineachreplicatewasdetermined.

Table 2. Summary of test conditions.		
1.	Test type	Static Renewal 20°C Fluorescent 100-1000 lux 16L:8D 50 mL glass beaker
2.	Temperature	30 mL At 48 hours Approximately 75% <5 days 5 4 7 treatments plus
3.	Light quality	control (8 total treatments) ≥0.5 None None None, unless DO falls <4 mg/L,
4.	Light intensity	then aerate all treatments at approximately
5.	Photoperiod	1 bubble/second from a glass pipette
6.	Test chamber size	EPAH (EPA Hard Water)
7.	Test solution volume	96 hours Survival, measured as presence of foot/cillia movement in a 5 minute
8.	Renewal frequency	observation period, or observation of heartbeat
9.	Renewal volume	
10.	Age of organisms	
11.	Organisms per chamber	
12.	Replicates per treatment	
13.	Number of treatments	
14.	Dilution factor	
15.	Feeding regime	
16.	Cleaning	
17.	Aeration	
18.	Dilution water	
19.	Test duration	
20.	Endpoint	

2.2.1 Preparation of Test Solutions

The Lab Water Control medium consisted of EPA synthetic hard water (EPA 2002), which was prepared by the addition of reagent grade chemicals to Type 1 water (reverse-osmosis, de-ionized water).

A 50 mg/L total ammonia nitrogen (TAN) stock solution was prepared by the addition of ACS reagent grade ammonium chloride (NH_4Cl, Sigma Aldrich, ³99.5%) to Lab Water Control medium. The stock ammonia solution was analytically verified prior to use in testing. The ammonia stock solution was subsequently diluted by the addition of unspiked Lab Control Medium in order to obtain the test concentrations listed in Table 3. The range of nominal ammonia test concentrations was selected assuming similar ammonia sensitivity to that of other unionid mussel species as published in the scientific literature.

Table 3. Nominal ammonia (TAN) test concentrations.	
Testing Test	Test Concentrations (mg/L TAN)
Test 1: July 7	0, 2.0, 4.0, 8.0, 16, 24, 36, 50
Test 2: July 8	0, 2.0, 4.0, 8.0, 16, 24, 36, 50

2.2.2 Statistical Determination of Definitive Toxicity Point Estimates

Determination of TAN LC_{50} point estimates and 95% confidence intervals (CI) were made using CETIS® (TidePool Scientific Software, McKinleyville, CA). Point estimates were determined using the mean of test initiation, test renewal (old and new solutions), and test completion measurements of TAN. Because test methods specified a 75% renewal, the renewal solution were calculated as a 3:1 ratio (new:old) of the new and old solution TAN measurements at 48 hours. Point estimates were determined by the Spearman-Kärber method.

2.3 Measurement of Ammonia Test Concentrations

Total ammonia-nitrogen was measured for each treatment at test initiation, test renewal (old and new solutions), and test completion via spectrophotometric method (HACH TNTplus, EPA 350.1 equivalent). Formal ongoing precision and recovery demonstration was not undertaken at the time of this testing.

3. RESULTS

3.1 Toxicity of Ammonia to *Anodonta oregonensis*:Test 1

The results of this test are presented in Table 4. The test dataand summary of statistical analyses for this test are presented in Appendix A.

Table 4. Acute toxicity of ammonia to *A. oregonensis*: Test 1.

Nominal (mg/L TAN)	Measured Ammonia (mg/L TAN)					% Survival				
	T0 New	T_{48} New[a]	T_{48} Old	T_{96} Old	Test Mean	Rep A	Rep B	Rep C	Rep D	**Mean**
0	<1.0	<1.0	<1.0	<1.0	<1.0	100	100	100	100	**100**
2.0	1.7	1.7	1.6	1.4	1.6	100	100	100	100	**100**
4.0	3.6	3.7	3.1	3.4	3.5	100	100	100	100	**100**
8.0	7.8	7.8	7.2	6.6	7.4	100	100	100	100	**100**
16	16	16	14	13	15	60	40	60	20	**45**
24	23	24	22	23	23	0	0	0	0	**0**
36	35	36	35	34	35	0	0	0	0	**0**
50	52	49	45	45	48	0	0	0	0	**0**
Summary of Statistics										
LC_{50} (95% CI) =						14 (12-15)				

J: estimated value.
a – reported TAN values represents a 3:1 weighted concentration of new and old solutions at 48 hours.

3.2 Toxicity of Ammonia to *Anodonta oregonensis*:Test 2

The results of this test are presented in Table 5. The test dataand summary of statistical analyses for this test are presented in Appendix B.

Table 5. Acute toxicity of ammonia to *A. oregonensis*: Test 2.

Nominal (mg/L TAN)	Measured Ammonia (mg/L TAN)					% Survival				
	T0 New	T_{48} New[a]	T_{48} Old	T_{96} Old	Test Mean	Rep A	Rep B	Rep C	Rep D	**Mean**
0	<1.0	<1.0	<1.0	<1.0	<1.0	100	100	100	100	**100**
2.0	1.9	1.9	1.7	1.8	1.8	100	100	100	100	**100**
4.0	4.1	3.9	3.6	3.7	3.8	100	100	80	100	**95**
8.0	8.4	7.9	7.3	7.5	7.8	80	80	80	100	**85**
16	17	17	15	15	16	40	40	40	0	**30**
24	25	24	22	24	24	0	0	0	0	**0**
36	38	36	34	36	36	0	0	0	0	**0**
50	51	49	45	47	48	0	0	0	0	**0**
Summary of Statistics										
LC_{50} (95% CI) =						11 (10-14)				

a – reported TAN values represents a 3:1 weighted concentration of new and old solutions at 48 hours.

4. QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

Testing was subject to the QA/QC procedures of ASTM E2455-06 (ASTM 2013). A QA/QC review is provided below.

4.1 Toxicity Test Acceptability Requirements

Toxicity testing incorporated standard QA/QCprocedures to ensure that the test results were valid, including the use of a negative control.These QA/QC procedures were consistent with the test acceptability requirements for toxicity testsconducted with freshwater juvenile mussels outlined in ASTM E2455-06.

4.1.1Negative Control

The biological response in the negative Control treatments were within test acceptability limits of ≥90% survival.

4.1.2Test Conditions

All test conditions were within acceptable limits. Continuously measured test temperatures were within +/- 1˚C of the target test temperature of 20˚C (Appendix C) and the difference between the maximum and minimum test temperature across treatments was less than 2˚C (Table 6). Test treatment pH, hardness, and alkalinity varied less than 10% over the duration of the test (Table 7 and Table 8), and test dissolved oxygen (DO) was maintained above 4 mg/L (Table 9).

4.2 Total Ammonia Measurement QA/QC Assessment

Formal ongoing precision and recovery demonstration wasnot undertaken at the time of this testing.

Table 6. Summary of test temperature (˚C).

Test Treatment	Test 1				Test 2			
	Mean	Min	Max	D (˚C)	Mean	Min	Max	D (˚C)
0	20.2	20.1	20.3	0.2	20.2	20.1	20.3	0.2
2.0	20.2	20.1	20.4	0.3	20.2	20.1	20.3	0.2
4.0	20.3	20.1	20.4	0.3	20.2	20.1	20.4	0.3
8.0	20.2	20.1	20.4	0.3	20.2	20.1	20.3	0.2
16	20.2	20.1	20.4	0.3	20.2	20.1	20.3	0.2
24	20.3	20.1	20.5	0.4	20.2	20.1	20.4	0.3
36	20.2	20.1	20.5	0.4	20.2	20.1	20.4	0.3
50	20.2	20.1	20.3	0.2	20.2	20.1	20.4	0.3

Table 7. Summary of test pH.								
Test Treatment	Test 1				Test 2			
	Mean	Min	Max	D (%)	Mean	Min	Max	D (%)
0	8.10	8.05	8.13	1.0	8.05	7.98	8.08	1.3
2.0	8.14	8.13	8.16	0.4	8.11	8.07	8.16	1.1
4.0	8.17	8.13	8.21	1.0	8.15	8.13	8.17	0.5
8.0	8.17	8.13	8.22	1.1	8.16	8.14	8.19	0.6
16	8.21	8.12	8.20	1.0	8.12	8.06	8.17	1.4
24	8.06	8.02	8.14	1.5	8.08	8.04	8.17	1.6
36	8.03	7.98	8.08	1.3	8.01	7.93	8.09	2.0
50	7.95	7.92	8.02	1.3	7.94	7.87	8.03	2.0

Table 8. Summary of test hardness and alkalinity (mg/L $CaCO_3$).												
Test Treatment	Test 1						Test 2					
	Hardness			Alkalinity			Hardness			Alkalinity		
	Initial	Final	D (%)	Initial	Final	D (%)	Initial	Final	D (%)	Initial	Final	D (%)
0	160	160	0.0	120	120	0.0	160	170	6.3	110	120	8.3
16	160	160	0.0	120	110	8.3	160	170	6.3	110	110	0.0
50	160	-	[a]	120	-	[a]	160	-	[a]	120	-	[a]

a– Final concentrationnotmeasureddue to completemortalityinthistreatment.

Table 9. Summary of test dissolved oxygen (mg/L).								
Test Treatment	Test 1				Test 2			
	Mean	Min	Max	D (mg/L)	Mean	Min	Max	D (mg/L)
0	8.6	8.4	8.9	0.5	8.8	8.6	9.1	0.5
2.0	8.7	8.6	8.9	0.3	8.8	8.6	9.0	0.4
4.0	8.8	8.7	8.9	0.2	8.8	8.6	9.0	0.4
8.0	8.9	8.9	9.0	0.1	8.8	8.7	9.0	0.3
16	8.8	8.7	8.9	0.2	8.9	8.7	9.0	0.3
24	8.8	8.7	8.9	0.2	8.8	8.7	9.0	0.3
36	8.8	8.7	8.9	0.2	8.8	8.6	9.0	0.4
50	8.8	8.7	8.9	0.2	8.9	8.7	9.0	0.3

5. SUMMARY

Total ammonia LC_{50} values (and accompanying 95% CI)for juvenile *A. oregonensis* are presented in Table 10. All results were calculated as described in this report.

Table 10. Total Ammonia LC_{50} for juvenile *A. oregonensis*.

Test No.	Total Ammonia LC_{50} value (mg/L TAN)	95% Confidence Interval (mg/L TAN)
Test 1	14	12-15
Test 2	11	10-14

6. REFERENCES

ASTM (ASTM International). 2013. Standard guide for conducting laboratory toxicity tests with freshwater mussels. E2455-06 (2013). West Conshohocken, PA. 2013.

EPA (2002). Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. EPA 821-R-02-012. US Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN. October 2002.

Pacific EcoRisk, Robertson-Bryan, Inc, Larry Walker Associates. 2018. Phase IIc Freshwater Mussel Collaborative Study for Wastewater Treatment Plants: Study Work Plan for the Acute Toxicity Testing with Resident Freshwater Mussels and Ammonia Criteria Recalculation. Prepared for Central Valley Clean Water Association. February 2018.

Appendix A

Data Sheets and Summaryof Statistical Analysis for Determinationof AmmoniaLC_{50} Values with Juvenile *Anodonta oregonensis*: Test 1

CETIS Summary Report

Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

Batch ID:	06-8517-7768	**Test Type:**	Survival (96h)	**Analyst:**	Alison Briden
Start Date:	07 Jul-16 14:30	**Protocol:**	ASTM E2455-06	**Diluent:**	Hard Synthetic Water
Ending Date:	11 Jul-16 12:35	**Species:**	Anodonta oregonensis	**Brine:**	Not Applicable
Duration:	94h	**Source:**	Missouri State University	**Age:**	4
Sample ID:	01-5601-5780	**Code:**	NH4Cl	**Client:**	Reference Toxicant
Sample Date:	07 Jul-16 14:30	**Material:**	Ammonia (as NH3-N)	**Project:**	26007
Receive Date:	07 Jul-16 14:30	**Source:**	Reference Toxicant		
Sample Age:	NA (20.1 °C)	**Station:**	In House		

Batch Note: Mean measured ammonia values

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
10-8828-3675	96h Survival Rate	7.4	15	10.54	13.5%		Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	mg/L NH3	95% LCL	95% UCL	TU	Method
10-8618-1045	96h Survival Rate	EC50	13.6	12	15.4		Spearman-Kärber

96h Survival Rate Summary

C-mg/L NH3-	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	4	1	1	1	1	1	0	0	0.0%	0.0%
1.6		4	1	1	1	1	1	0	0	0.0%	0.0%
3.5		4	1	1	1	1	1	0	0	0.0%	0.0%
7.4		4	1	1	1	1	1	0	0	0.0%	0.0%
15		4	0.45	0.145	0.755	0.2	0.6	0.0957	0.191	42.6%	55.0%
23		4	0	0	0	0	0	0	0		100.0%
35		4	0	0	0	0	0	0	0		100.0%
48		4	0	0	0	0	0	0	0		100.0%

96h Survival Rate Detail

C-mg/L NH3-	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water Contr	1	1	1	1
1.6		1	1	1	1
3.5		1	1	1	1
7.4		1	1	1	1
15		0.6	0.4	0.6	0.2
23		0	0	0	0
35		0	0	0	0
48		0	0	0	0

96h Survival Rate Binomials

C-mg/L NH3-	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water Contr	5/5	5/5	5/5	5/5
1.6		5/5	5/5	5/5	5/5
3.5		5/5	5/5	5/5	5/5
7.4		5/5	5/5	5/5	5/5
15		3/5	2/5	3/5	1/5
23		0/5	0/5	0/5	0/5
35		0/5	0/5	0/5	0/5
48		0/5	0/5	0/5	0/5

Acute Juvenile Freshwater Mussel Survival — Pacific EcoRisk

Analysis ID:	10-8828-3675	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Jul-16 9:14	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	13.5%	7.4	15	10.54	

Steel Many-One Rank Sum Test

Control	vs	C-mg/L NH3-N	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Water Control		1.6	18	10	1	6	0.8000	Asymp	Non-Significant Effect
		3.5	18	10	1	6	0.8000	Asymp	Non-Significant Effect
		7.4	18	10	1	6	0.8000	Asymp	Non-Significant Effect
		15*	10	10	0	6	0.0350	Asymp	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.210921	0.3027302	4	37.3	<0.0001	Significant Effect
Error	0.121714	0.008114268	15			
Total	1.332635		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	7.96	4.89	0.0012	Unequal Variances
Variances	Levene Equality of Variance	11.9	4.89	0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.576	0.866	<0.0001	Non-normal Distribution

96h Survival Rate Summary

C-mg/L NH3-N	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Water Contr	4	1	1	1	1	1	1	0	0.0%	0.0%
1.6		4	1	1	1	1	1	1	0	0.0%	0.0%
3.5		4	1	1	1	1	1	1	0	0.0%	0.0%
7.4		4	1	1	1	1	1	1	0	0.0%	0.0%
15		4	0.45	0.145	0.755	0.5	0.2	0.6	0.0957	42.6%	55.0%
23		4	0	0	0	0	0	0	0		100.0%
35		4	0	0	0	0	0	0	0		100.0%
48		4	0	0	0	0	0	0	0		100.0%

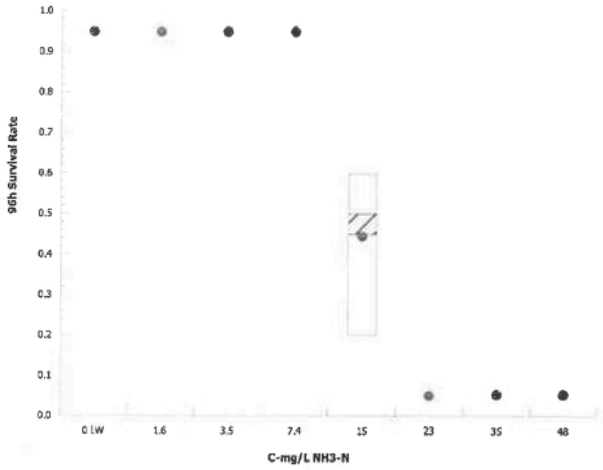
Angular (Corrected) Transformed Summary

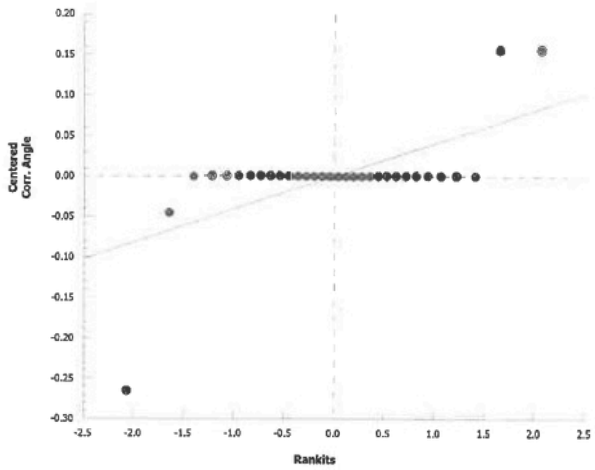
C-mg/L NH3-N	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Water Cont	4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.0%	0.0%
1.6		4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.0%	0.0%
3.5		4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.0%	0.0%
7.4		4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.0%	0.0%
15		4	0.73	0.41	1.05	0.785	0.464	0.886	0.101	27.6%	45.7%
23		4	0.226	0.225	0.226	0.226	0.226	0.226	0	0.0%	83.2%
35		4	0.226	0.225	0.226	0.226	0.226	0.226	0	0.0%	83.2%
48		4	0.226	0.225	0.226	0.226	0.226	0.226	0	0.0%	83.2%

Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

Analysis ID:	10-8828-3675	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Jul-16 9:14	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

Analysis ID:	10-8618-1045	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Jul-16 9:14	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

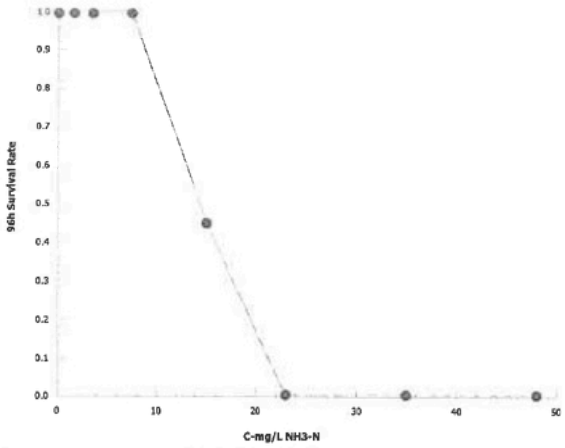
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	1.13	0.0274	13.6	12	15.4

96h Survival Rate Summary

			Calculated Variate(A/B)								
C-mg/L NH	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Water Contr	4	1	1	1	0	0	0.0%	0.0%	20	20
1.6		4	1	1	1	0	0	0.0%	0.0%	20	20
3.5		4	1	1	1	0	0	0.0%	0.0%	20	20
7.4		4	1	1	1	0	0	0.0%	0.0%	20	20
15		4	0.45	0.2	0.6	0.0957	0.191	42.6%	55.0%	9	20
23		4	0	0	0	0	0		100.0%	0	20
35		4	0	0	0	0	0		100.0%	0	20
48		4	0	0	0	0	0		100.0%	0	20

Graphics



96 Hour Acute *Anodonta oregonensis* Toxicity Test Data

Client: **Pacific EcoRisk** Test Date: 7/7/16

Test Material: Ammonia (as NH3 - N) Control/Diluent: EPA Hard Water

Test ID#: 68550 Project #: 26007 Control Water Batch: 259

Randomization: 4.8.8 Organism Source: 91668 Age: 4d

Treatment (mg/L NH3 - N)	Temp (°C)	Room Temp (°C) Max/Min	pH New	pH Old	D.O. New	D.O. Old	Conductivity (µS/cm)	# Live Animals A	B	C	D	Sign-Off
Lab Water Control	20.1	19.9/20.3	8.05		8.9		553	5	5	5	5	Date: 7/7/16
2	20.1		8.14		8.9		573	5	5	5	5	Sample ID: –
4	20.1		8.18		8.9		589	5	5	5	5	Test Solution Prep: CD
8	20.1		8.19		8.9		629	5	5	5	5	New WQ: CD
16	20.1		8.18		8.9		705	5	5	5	5	Initiation Time: 1430
24	20.1		8.14		8.9		780	5	5	5	5	Initiation Signoff: NL
36	20.1		8.08		8.9		897	5	5	5	5	
50	20.1		7.96		8.9		1033	5	5	5	5	
Meter ID	59A	59A	PH21		RD10		EC08					
Lab Water Control	20.2	20.2/20.3										Date: 7/8/16
2	20.3											Temp Time: 1455
4	20.4											Temp Signoff: CD
8	20.3											
16	20.3											
24	20.3											
36	20.2											
50	20.2											
Meter ID	59A	59A										
Lab Water Control	20.2	19.6/20.3	8.11	8.10	8.4	~~5839.0~~	558	5	5	5	5	Date: 7/9/16
2	20.2		8.13	8.15	8.6	~~5928.8~~	575	5	5	5	5	Sample ID: –
4	20.2		8.16	8.13	8.7	8.8	597	5	5	5	5	Test Solution Prep: NL
8	20.1		8.16	8.13	8.9	8.6	636	5	5	5	5	New WQ: CD
16	20.2		8.14	8.12	8.7	8.6	709	3	3	5	5	Count Time: 1243
24	20.2		8.02	8.07	8.7	8.7	786	3	2	5	4	Count Signoff: CD
36	20.2		7.98	8.02	8.7	8.6	914	2	0	0	0	Old WQ: NL
50	20.1		7.92	7.92	8.7	8.7	1035	0	0	0	0	
Meter ID	59A	59A	PH21	PH21	RD10	RD10	EC08					

96 Hour Acute *Anodonta oregonensis* Toxicity Test Data

Client: **Pacific EcoRisk** Test Date: 7/7/16

Test Material: Ammonia (as NH_3 - N) Control/Diluent: EPA Hard Water

Test ID#: 68550 Project # 26007 Control Water Batch: 259

Randomization: 4.8.8 Organism Source: 9668

Treatment (mg/L NH3 - N)	Temp (°C)	Room Temp (°C) Max/Min	pH		D.O.		Conductivity (µS/cm)	# Live Animals				Sign-Off
			New	Old	New	Old		A	B	C	D	
Lab Water Control	20.3	19.6/20.3										Date: 7/10/16
2	20.2											Temp Time: 0820
4	20.4											Temp Signoff: JBL
8	20.2											
16	20.2											
24	20.3											
36	20.2											
50	20.2											
Meter ID	59A	59A										
Lab Water Control	20.3	19.9/20.2		8.13		8.8	592	5	5	5	5	Date: 7/11/16
2	20.4			8.16		8.7	598	5	5	5	5	Termination Time: 1235
4	20.4			8.21		8.9	626	5	5	5	5	Termination Signoff: JBL
8	20.4			8.22		9.0	668	5	5	5	5	Old WQ: JBL
16	20.4			8.20		8.9	742	3	2	3	1	
24	20.5			8.02		8.9	806	0	0	0	0	
36	20.5			8.05		8.9	925	0	0	0	0	
50	20.3			8.02		8.9	1064	0	0	0	0	
Meter ID	59A	59A		pH21		RD10	EC08					

Ancillary Water Chemistries	Initial (mg/L)		Final	
	Alkalinity *	Hardness*	Alkalinity*	Hardness*
Control	138 116 7/7/16	160	118	160
16 mg/L	123	160	110	162
50 mg/L	118	162	86	163

*Measure in each test treatment at test initiation and termination as long as live organisms are present.

Appendix B

Data Sheets and Summaryof Statistical Analysis for Determination of AmmoniaLC_{50} Values with Juvenile *Anodonta oregonensis*: Test 2

Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

Batch ID:	05-7993-1914	**Test Type:**	Survival (96h)	**Analyst:**	Stevi Vasquez
Start Date:	08 Jul-16 13:15	**Protocol:**	ASTM E2455-06	**Diluent:**	Hard Synthetic Water
Ending Date:	12 Jul-16 12:10	**Species:**	Anodonta oregonensis	**Brine:**	Not Applicable
Duration:	95h	**Source:**	Missouri State University	**Age:**	5

Sample ID:	08-4290-0488	**Code:**	NH4Cl	**Client:**	Reference Toxicant
Sample Date:	08 Jul-16 13:15	**Material:**	Ammonia (as NH3-N)	**Project:**	26014
Receipt Date:	08 Jul-16 13:15	**Source:**	Reference Toxicant		
Sample Age:	n/a (20.1 °C)	**Station:**	In House		

Comments:
Mean measured ammonia values

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	NOEL	LOEL	TOEL	TU	PMSD ✓
10-2795-4501	96h Survival Rate	Dunnett Multiple Comparison Test	7.8	16	11.17		18.0%

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	Level	mg/L NH3	95% LCL	95% UCL	TU ✓
03-2592-5345	96h Survival Rate	Regression: Log-Normal (Probit)	EC5	6.67	4.03	8.55	
			EC10	7.62	4.96	9.5	
			EC15	8.34	5.7	10.2	
			EC20	8.96	6.35	10.8	
			EC25	9.52	6.97	11.4	
			EC40	11.1	8.72	13.1	
			EC50	12.2	9.89	14.4	
01-0779-2900	96h Survival Rate	Spearman-Kärber	EC50	11.4	9.58	13.7	

96h Survival Rate Summary

Conc-mg/L NH3-N	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LW	4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
1.8		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
3.8		4	0.950	0.791	1.000	0.800	1.000	0.050	0.100	10.53%	5.00%
7.8		4	0.850	0.691	1.000	0.800	1.000	0.050	0.100	11.76%	15.00%
16		4	0.300	0.000	0.618	0.000	0.400	0.100	0.200	66.67%	70.00%
24		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100.00%
36		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100.00%
48		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100.00%

96h Survival Rate Detail

Conc-mg/L NH3-N	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LW	1.000	1.000	1.000	1.000
1.8		1.000	1.000	1.000	1.000
3.8		1.000	1.000	0.800	1.000
7.8		0.800	0.800	0.800	1.000
16		0.400	0.400	0.400	0.000
24		0.000	0.000	0.000	0.000
36		0.000	0.000	0.000	0.000
48		0.000	0.000	0.000	0.000

Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

96h Survival Rate Binomials

Conc-mg/L NH3-N	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LW	5/5	5/5	5/5	5/5
1.8		5/5	5/5	4/4	5/5
3.8		5/5	5/5	4/5	5/5
7.8		4/5	4/5	4/5	5/5
16		2/5	2/5	2/5	0/5
24		0/5	0/5	0/5	0/5
36		0/5	0/5	0/5	0/5
48		0/5	0/5	0/5	0/5

Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

Analysis ID:	10-2795-4501	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.9.2
Analyzed:	11 Jun-18 16:40	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	C > T	7.8	16	11.17		17.98%

Dunnett Multiple Comparison Test

Control	vs	Conc-mg/L N	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Lab Water Contr		1.8	0.0753	2.36	0.212	6	CDF	0.7747	Non-Significant Effect
		3.8	0.66	2.36	0.212	6	CDF	0.5327	Non-Significant Effect
		7.8	1.98	2.36	0.212	6	CDF	0.0963	Non-Significant Effect
		16*	8.6	2.36	0.212	6	CDF	1.4E-06	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.71392	0.428481	4	26.4	1.2E-06	Significant Effect
Error	0.243768	0.0162512	15			
Total	1.95769		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Levene Equality of Variance Test	4.84	4.89	0.0105	Equal Variances
Variances	Mod Levene Equality of Variance Test	0.537	4.89	0.7107	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.87	0.866	0.0118	Normal Distribution

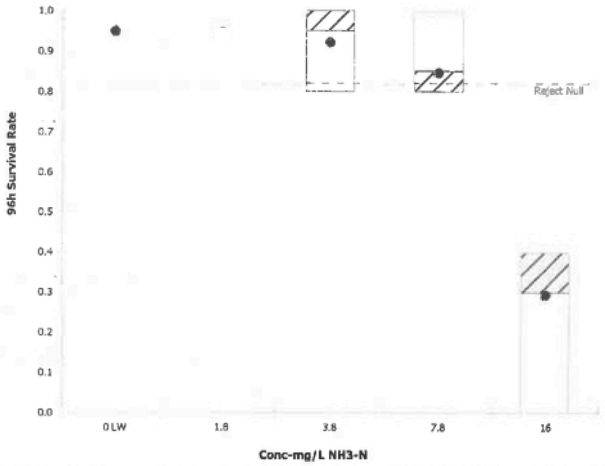
96h Survival Rate Summary

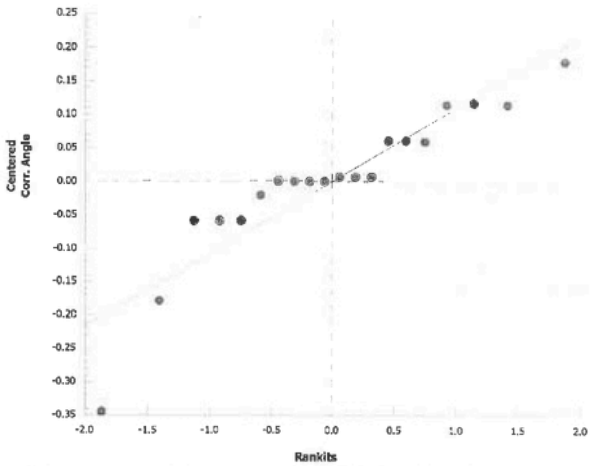
Conc-mg/L NH3-N	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LW	4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
1.8		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
3.8		4	0.950	0.791	1.000	1.000	0.800	1.000	0.050	10.53%	5.00%
7.8		4	0.850	0.691	1.000	0.800	0.800	1.000	0.050	11.76%	15.00%
16		4	0.300	0.000	0.618	0.400	0.000	0.400	0.100	66.67%	70.00%

Angular (Corrected) Transformed Summary

Conc-mg/L NH3-N	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LW	4	1.35	1.34	1.35	1.35	1.35	1.35	0	0.00%	0.00%
1.8		4	1.34	1.32	1.36	1.35	1.32	1.35	0.00679	1.01%	0.50%
3.8		4	1.29	1.1	1.48	1.35	1.11	1.35	0.0595	9.26%	4.43%
7.8		4	1.17	0.977	1.36	1.11	1.11	1.35	0.0595	10.21%	13.28%
16		4	0.57	0.205	0.935	0.685	0.226	0.685	0.115	40.29%	57.64%

Graphics





Acute Juvenile Freshwater Mussel Survival — Pacific EcoRisk

Analysis ID:	03-2592-5345	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.9.2
Analyzed:	11 Jun-18 16:40	**Analysis:**	Linear Regression (GLM)	**Official Results:**	Yes

Linear Regression Options

Model Name	Link Function	Threshold Option	Thresh	Optimized	Pooled	Het Corr	Weighted
Log-Normal (Probit)	η=inv Φ[π]	Control Threshold	0.000001	Yes	No	No	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
27	-13.4	33.6	37.2	1.09	0.16	0.99	0.234	2.62	0.9440	Non-Significant Lack of Fit

Point Estimates

Level	mg/L NH3	95% LCL	95% UCL
EC5	6.67	4.03	8.55
EC10	7.62	4.96	9.5
EC15	8.34	5.7	10.2
EC20	8.96	6.35	10.8
EC25	9.52	6.97	11.4
EC40	11.1	8.72	13.1
EC50	12.2	9.89	14.4

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Threshold	0.0169	0.017	-0.0164	0.0503	0.996	0.3276	Non-Significant Parameter
Slope	6.26	1.22	3.87	8.65	5.13	1.7E-05	Significant Parameter
Intercept	-6.8	1.41	-9.57	-4.04	-4.82	4.2E-05	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	1860	928	2	1610	<1.0E-37	Significant
Lack of Fit	0.775	0.155	5	0.234	0.9438	Non-Significant
Pure Error	15.9	0.663	24			
Residual	16.7	0.575	29			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF Test	16.7	42.6	0.9668	Non-Significant Heterogeneity
	Likelihood Ratio GOF Test	13.3	42.6	0.9945	Non-Significant Heterogeneity
Variances	Mod Levene Equality of Variance	0.762	2.42	0.6239	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.77	0.934	1.2E-05	Non-Normal Distribution
	Anderson-Darling A2 Normality Te	2.06	2.49	<1.0E-37	Non-Normal Distribution

96h Survival Rate Summary — Calculated Variate(A/B)

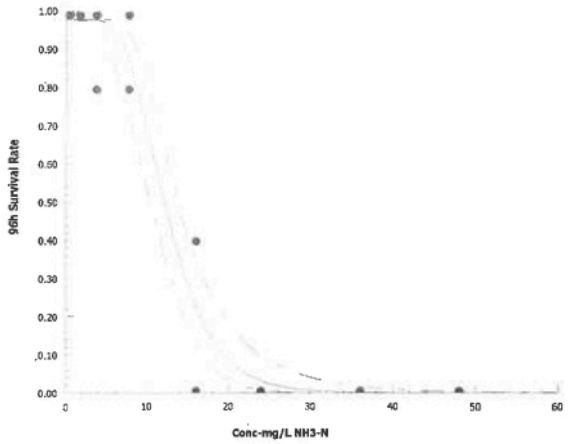
Conc-mg/L NH3-N	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	LW	4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
1.8		4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	19	19
3.8		4	0.950	0.800	1.000	0.050	0.100	10.50%	5.0%	19	20
7.8		4	0.850	0.800	1.000	0.050	0.100	11.80%	15.0%	17	20
16		4	0.300	0.000	0.400	0.100	0.200	66.70%	70.0%	6	20
24		4	0.000	0.000	0.000	0.000	0.000		100.0%	0	20
36		4	0.000	0.000	0.000	0.000	0.000		100.0%	0	20
48		4	0.000	0.000	0.000	0.000	0.000		100.0%	0	20

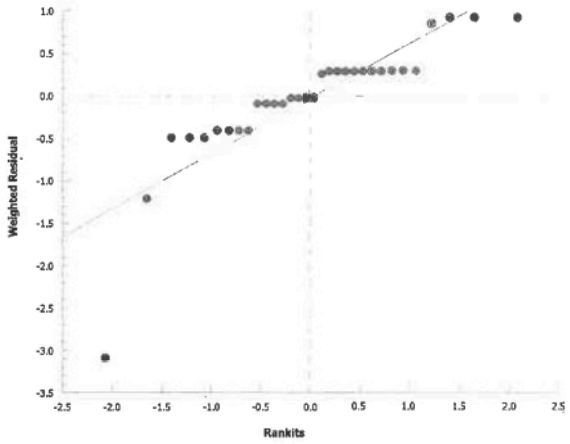
Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

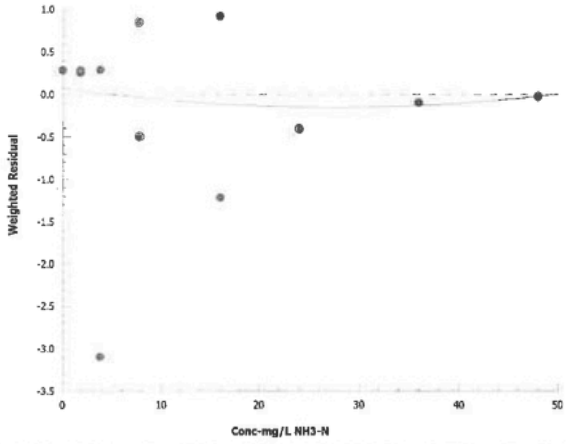
Analysis ID:	03-2592-5345	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.9.2
Analyzed:	11 Jun-18 16:40	**Analysis:**	Linear Regression (GLM)	**Official Results:**	Yes

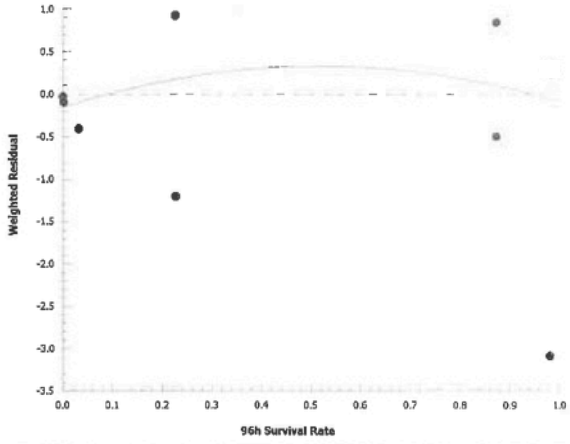
Graphics

Log-Normal: inv Φ[π]=α+β·log[x]









Acute Juvenile Freshwater Mussel Survival — **Pacific EcoRisk**

Analysis ID:	01-0779-2900	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.9.2
Analyzed:	11 Jun-18 16:40	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

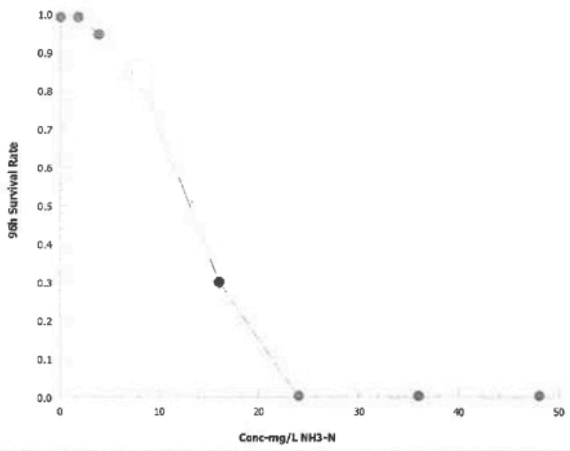
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	1.06	0.0386	11.4	9.58	13.7

96h Survival Rate Summary — Calculated Variate(A/B)

Conc-mg/L NH3-N	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	LW	4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	20	20
1.8		4	1.000	1.000	1.000	0.000	0.000	0.00%	0.0%	19	19
3.8		4	0.950	0.800	1.000	0.050	0.100	10.50%	5.0%	19	20
7.8		4	0.850	0.800	1.000	0.050	0.100	11.80%	15.0%	17	20
16		4	0.300	0.000	0.400	0.100	0.200	66.70%	70.0%	6	20
24		4	0.000	0.000	0.000	0.000	0.000		100.0%	0	20
36		4	0.000	0.000	0.000	0.000	0.000		100.0%	0	20
48		4	0.000	0.000	0.000	0.000	0.000		100.0%	0	20

Graphics



96 Hour Acute *Anodonta oregonensis* Toxicity Test Data

Client: **Pacific EcoRisk** Test Date: 7/8/16

Test Material: Ammonia (as NH_3 - N) Control/Diluent: EPA Hard Water

Test ID#: 68551 Project #: 26014 Control Water Batch: 259

Randomization: 4.8.2 Organism Source: 9668 Age: <5days

Treatment (mg/L NH3 - N)	Temp (°C)	Room Temp (°C) Max/Min	pH New	pH Old	D.O. New	D.O. Old	Conductivity (µS/cm)	# Live Animals A	B	C	D	Sign-Off
Lab Water Control	20.1	20.2/20.3	8.08		8.6		555	5	5	5	5	Date: 7/8/16
2	20.1		8.11		8.6		574	5	5	5	5	Sample ID: –
4	20.1		8.14		8.6		599	5	5	5	5	Test Solution Prep: M
8	20.1		8.14		8.7		653	5	5	5	5	New WQ: CO
16	20.1		8.11		8.8		714	5	5	5	5	Initiation Time: 1315
24	20.1		8.04		8.7		786	5	5	5	5	Initiation Signoff: CO
36	20.1		7.93		8.6		899	5	5	5	5	
50	20.1		7.87		8.7		1035	5	5	5	5	
Meter ID	59A	59A	PH21		RD10		EC08					
Lab Water Control	20.1	19.6/20.3										Date: 7/9/16
2	20.1											Temp Time: 1530
4	20.4											Temp Signoff: NL
8	20.3											
16	20.3											
24	20.3											
36	20.4											
50	20.4											
Meter ID	59A	59A										
Lab Water Control	20.2	19.6/20.3	8.06	8.07	8.7	9.1	572	5	5	5	5	Date: 7/10/16
2	20.3		8.16	8.07	8.7	9.0	574	5	5	4*	5	Sample ID: –
4	20.3		8.17	8.15	8.7	9.0	615	5	5	5	5	Test Solution Prep: JBL
8	20.3		8.17	8.19	8.7	9.0	652	5	5	4	5	New WQ: JBL
16	20.3		8.13	8.06	8.7	9.0	724	5	5	5	5	Count Time: 1315
24	20.4		8.07	8.05	8.7	9.0	804	2	1	3	0	Count Signoff: JBL
36	20.3		8.00	8.03	8.7	9.0	922	0	0	0	0	Old WQ: JBL
50	20.3		7.93	7.93	8.8	9.0	1050	0	0	0	0	
Meter ID	59A	59A	PH21	PH21	~~8.8~~ 7/10 RD10	~~9.0~~ 7/10 RD10	EC08					

* Only 4 organisms observed. Statistics corrected.

96 Hour Acute *Anodonta oregonensis* Toxicity Test Data

Client: **Pacific EcoRisk** Test Date: 7/8/16

Test Material: Ammonia (as NH3 - N) Control/Diluent: EPA Hard Water

Test ID#: 68551 Project # 26014 Control Water Batch: 259

Randomization: 4.8.2 Organism Source: 9668 Age: <5days

Treatment (mg/L NH3 - N)	Temp (°C)	Room Temp (°C) Max/Min	pH New	pH Old	D.O. New	D.O. Old	Conductivity (µS/cm)	# Live Animals A	B	C	D	Sign-Off
Lab Water Control	20.3	19.9/20.0										Date: 7/11/16
2	20.2											Temp Time: 0845
4	20.3											Temp Signoff: JBL
8	20.3											
16	20.2											
24	20.3											
36	20.3											
50	20.3											
Meter ID	59A	59A										
Lab Water Control	20.2	19.8/20.0		7.98		8.9	579	5	5	5	5	Date: 7/12/16
2	20.2			8.11		8.9	595	5	5	4	5	Termination Time: 1210
4	20.1			8.13		8.9	654	5	5	4	5	Termination Signoff: JBL
8	20.1			8.16		8.9	685	4	4	4	5	Old WQ: JBL
16	20.1			8.17		8.9	760	2	2	2	0	
24	20.1			8.17		8.9	838	~~2~~ 0	~~2~~ 0	~~2~~ 0	0	
36	20.1			8.09		8.9	946	0	0	0	0	
50	20.1			8.03		8.9	1076	0	0	0	0	
Meter ID	59A	59A		pH21		RD10	EC08					

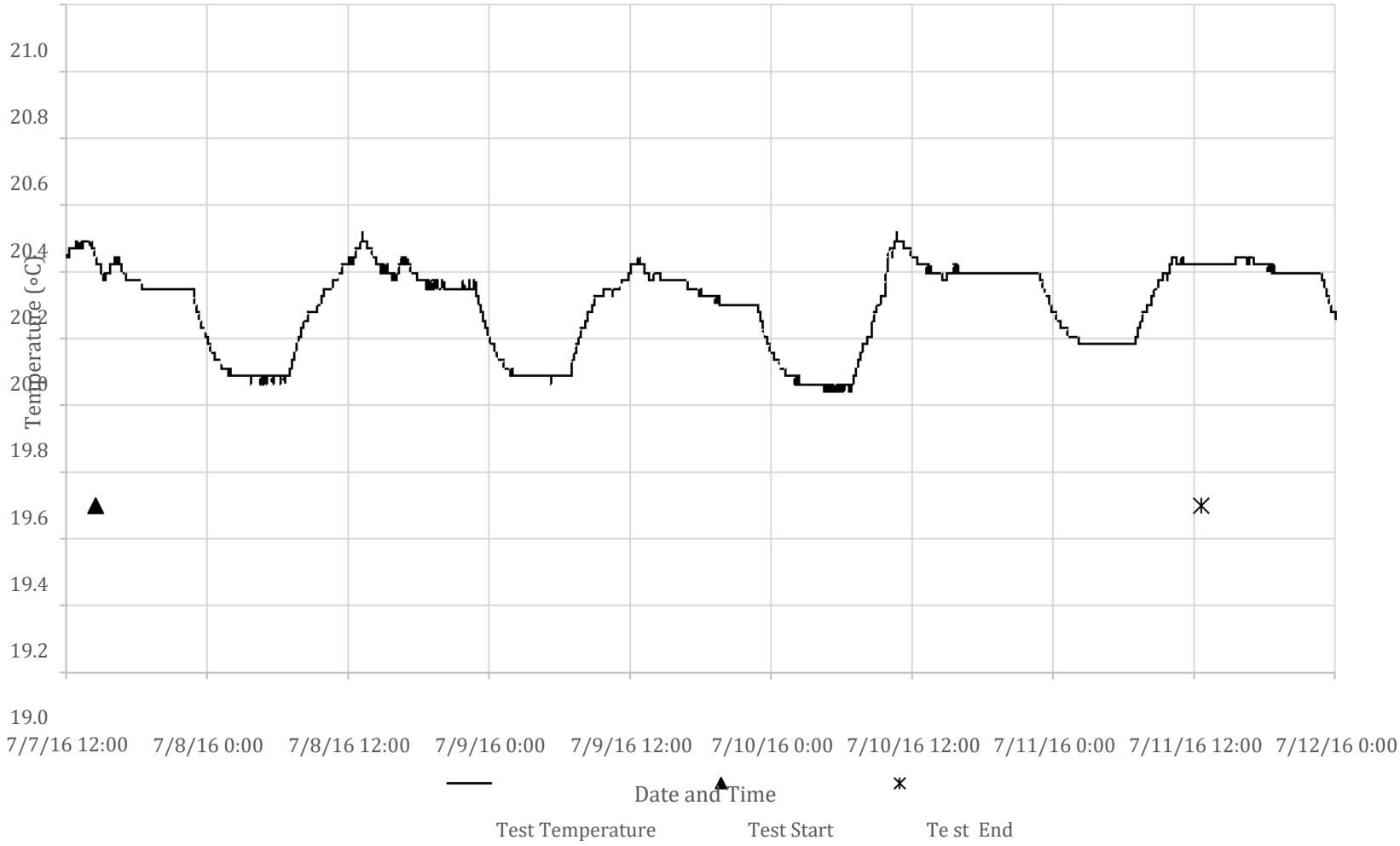
Ancillary Water Chemistries	Initial (mg/L) Alkalinity *	Initial (mg/L) Hardness*	Final Alkalinity*	Final Hardness*
Control	109	158	124	166
16 mg/L	114	161	114	166
50 mg/L	116	161	NL 7/12/16 ~~75~~ 95	176

*Measure in each test treatment at test initiation and termination as long as live organisms are present.

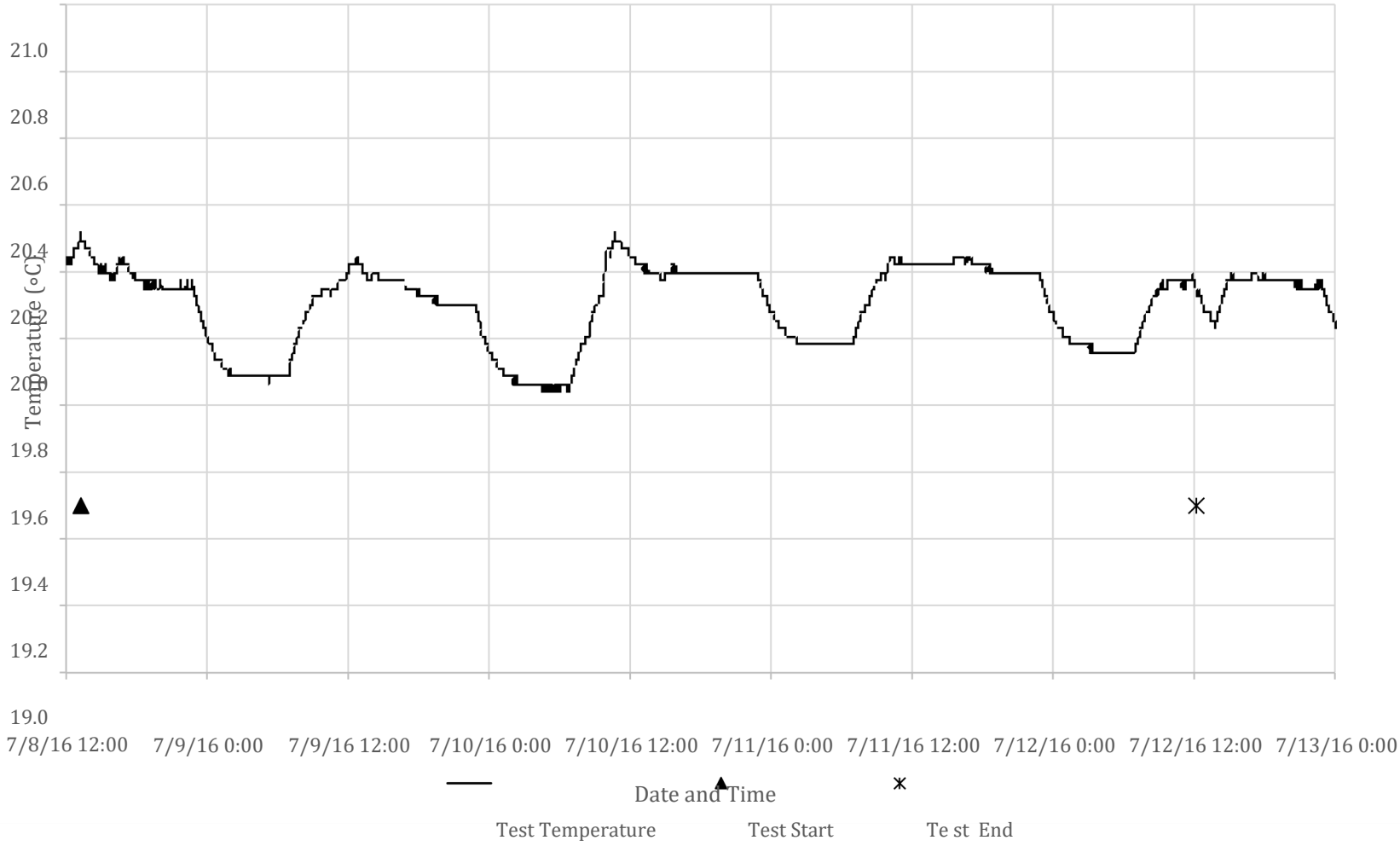
Appendix C

TemperatureControl Charts

Test 1: *Anodonta oregonensis* (July 7, 2018)



Test 1: *Anodonta oregonensis* (July 8, 2018)



EPA Public Access
Author manuscript
Environ Toxicol Chem. Author manuscript; available in PMC 2021 June 23.
About author manuscripts | Submit a manuscript

Published in final edited form as:
Environ Toxicol Chem. 2017 March ; 36(3): 786–796. doi:10.1002/etc.3642.

Acute sensitivity of a broad range of freshwater mussels to chemicals with different modes of toxic action

Ning Wang[1], **Christopher D Ivey**[1], **Christopher G Ingersoll**[1], **William G Brumbaugh**[1], **David Alvarez**[1], **Edward J Hammer**[2], **Candice R Bauer**[2], **Tom Augspurger**[3], **Sandy Raimondo**[4], **M Christopher Barnhart**[5]

[1]US Geological Survey, Columbia Environmental Research Center, Columbia, Missouri, USA.

[2]US Environmental Protection Agency, Water Quality Branch, Chicago, Illinois, USA.

[3]US Fish and Wildlife Service, Raleigh, North Carolina, USA.

[4]US Environmental Protection Agency, Gulf Ecology Division, Gulf Breeze, Florida, USA.

[5]Department of Biology, Missouri State University, Springfield, Missouri, USA.

Abstract

Freshwater mussels, one of the most imperiled groups of animals in the world, are generally underrepresented in toxicity databases used for the development of ambient water quality criteria and other environmental guidance values. Acute 96-h toxicity tests were conducted to evaluate the sensitivity of 5 species of juvenile mussels from 2 families and 4 tribes to 10 chemicals (ammonia, metals, major ions, and organic compounds) and to screen 10 additional chemicals (mainly organic compounds) with a commonly tested mussel species, fatmucket (Lampsilis siliquoidea). In the multi-species study, median effect concentrations (EC50s) among the 5 species differed by a factor of ≤2 for chloride, potassium, sulfate, and zinc; a factor of ≤5 for ammonia, chromium, copper, and nickel; and factors of 6 and 12 for metolachlor and alachlor, respectively, indicating that mussels representing different families or tribes had similar sensitivity to most of the tested chemicals, regardless of modes of action. There was a strong linear relationship between EC50s for fatmucket and the other 4 mussel species across the 10 chemicals (r^2 = 0.97, slope close to 1.0), indicating that fatmucket was similar to other mussel species; thus, this commonly tested species can be a good surrogate for protecting other mussels in acute exposures. The sensitivity of juvenile fatmucket among different populations or cultured from larvae of wild adults and captive-cultured adults was also similar in acute exposures to copper or chloride, indicating captive-cultured adult mussels can reliably be used to reproduce juveniles for toxicity testing. In compiled databases for all freshwater species, 1 or more mussel species were among the 4 most sensitive species for alachlor, ammonia, chloride, potassium, sulfate, copper, nickel, and zinc; therefore, the development of water quality criteria and other environmental guidance values for these chemicals

Supplemental Data

Publisher's Disclaimer: Disclaimer

The views expressed herein do not necessarily represent the views of the US Environmental Protection Agency and the US Fish and Wildlife Service. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

Data Availability

Data, associated metadata, and calculation tools are available from the corresponding author (nwang@usgs.gov).

EPA Author Manuscript
EPA Author Manuscript
EPA Author Manuscript

should reflect the sensitivity of mussels. In contrast, the EC50s of fatmucket tested in the single-species study were in the high percentiles (>75th) of species sensitivity distributions for 6 of 7 organic chemicals, indicating mussels might be relatively insensitive to organic chemicals in acute exposures.

Keywords

Acute toxicity; Juvenile mussels; Species sensitivity distribution; Water quality criteria; Water quality guidelines

INTRODUCTION

Freshwater mussels (order Unionoida) are one of the most imperiled groups of animals, and environmental contamination has been identified as a causal or contributing factor to the decline of mussel populations 1–4. Previous studies indicate that freshwater mussels are more sensitive than commonly tested organisms to some chemicals (e.g., copper, ammonia, and chloride 5–11). However, mussels are generally under-represented in toxicity databases used for the development of water quality criteria, standards, and other environmental guidance values 6, 7, 12, 13. In addition, the limited mussel data available have been generated mostly from toxicity tests with a few species of the taxonomic tribe Lampsilini 5–16. Studies with broader phylogenetic representation are needed to understand the range of sensitivity among freshwater mussels, compare the overall sensitivity of mussels to other freshwater species, and evaluate the degree to which existing or proposed environmental guidance values are protective of mussels.

Freshwater mussels are a taxonomically diverse group of bivalve mollusks with a complex reproductive cycle. Approximately 300 species in the order Unionoida historically occur in North America. These species are classified in 2 families, with 5 in the family Margaritiferidae and the rest in the family Unionidae. Unionidae include 2 subfamilies, the Unioninae and the Ambleminae. Subfamily Unioninae includes the tribe Anodontini, and subfamily Ambleminae includes tribes Lampsilini, Amblemini, Pleurobemini, Quadrulini, and Gonideini 17. Ambleminae includes 250 North American species and 37 genera, which represent 85% of North American species and 75% of North American genera of Unionoida 17, 18. Most freshwater mussels have a 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, which mature in specialized chambers (marsupia) of the mussel gills. Glochidia are released into the water and must attach to the gills or fins of a suitable host fish. After 1 wk 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.

The primary objectives of the present study were, first, to compare the sensitivity of 5 phylogenetically diverse species of mussels in acute exposures to 10 chemicals with different modes of toxic action (multi-species study) and, second, to screen 10 additional chemicals with a commonly tested mussel species (single-species study). For the multi-species study, the family Margaritiferidae was represented by western pearlshell

EPA Author Manuscript EPA Author Manuscript EPA Author Manuscript

EPA Author Manuscript EPA Author Manuscript EPA Author Manuscript

(*Margaritifera falcata*), which is native to the western United States, and the family Unionidae was represented by threeridge (*Amblema plicata*; Amblemini), paper pondshell (*Utterbackia imbecillis*; Anodontini), fatmucket (*Lampsilis siliquoidea*; Lampsilini), and washboard (*Megalonaias nervosa*; Quadrulini), which are distributed widely in the Midwest and the Southeast of the United States. These mussels include 2 life-history strategies 4: 2 short-term brooders and 3 long-term brooders (Table 1). Fatmucket was selected for the single-species study with 10 additional chemicals because it has been used widely in previous toxicity tests in the United States and Canada 5–7, 11, 13, 14. In addition, the sensitivity of the commonly tested fatmucket among different populations was evaluated in acute exposures to 2 commonly used reference toxicants (copper and sodium chloride).

The 10 chemicals selected for the multi-species study were 2 organic compounds used as herbicides (alachlor and metolachlor), ammonia, 3 major ions (potassium, chloride, and sulfate), and 4 metals (chromium [VI], copper, nickel, and zinc; Table 2). These chemicals were selected based on interest of the US Environmental Protection Agency (USEPA) in developing or updating ambient water quality criteria, the availability of toxicity data for non-mollusk species (e.g., at least 10 species) in the USEPA Interspecies Correlation Estimation (ICE) database 19, 20, the high sensitivity of mollusks that are not mussels, different chemical classes and modes of toxicity action 21, and environmental relevance. The 10 chemicals for the single-species study (Table 2) were selected based on the same approach for chemical selection in the multi-species study except that no or few toxicity data from mussels were available for these chemicals. The chemicals used in the single-species study included 7 organic compounds used as pesticides (2,4-dichlorophenoxyacetic acid, 4-nonylphenol, azoxystrobin, bifenthrin, carbaryl, malathion, and molinate), arsenic (V), calcium chloride, and aluminum.

Two air-breathing freshwater snail species (*Physa gyrina* and *Lymnaea stagnalis*) and 3 commonly tested invertebrates (amphipod *Hyalella azteca*, and cladocerans *Ceriodaphnia dubia* and *Daphnia magna*) were also tested in acute exposures with the 10 chemicals used in the multi-species study following ASTM International standard methods 22. The methods and results for the acute exposures with the snails and other invertebrates are reported in a companion paper Ivey et al. 23 in this issue. However, the toxicity data from these non-mussel species were included in toxicity databases compiled in the present study to compare the mussel sensitivity with that of other freshwater species.

MATERIALS AND METHODS

Acute 96-h toxicity tests with newly transformed (6 d to 10 d old) juvenile mussels were conducted following ASTM International standard methods 24. Conditions for conducting the toxicity tests are summarized in Supplemental Data, Table S1.

Test organisms

Gravid female mussels brooding mature glochidia (larval mussels) were collected during early season of glochidia release between 2012 and 2015 from Missouri, USA (fatmucket, paper pondshell); Wisconsin, USA (threeridge, washboard); and California, USA (western pearlshell; Table 1). Mussels at all collection sites were abundant and apparently healthy,

EPA Author Manuscript EPA Author Manuscript EPA Author Manuscript

and reproduction and recent recruitment were evident. For toxicity tests to evaluate the sensitivity among different populations of fatmucket, adult mussels were obtained from 2 wild populations and 1 captive population in May 2014: a population from Silver Fork of Perche Creek, Boone County, Missouri (this population has been used routinely in previous toxicity studies 6–10); a population from the Bourbeuse River, Gasconade County, Missouri; and a captive population at the Kansas City Zoo (Kansas City, MO, USA). Mussels in the captive population were 4 yr old and were derived from glochidia obtained from 6 females collected from the Silver Fork of Perche Creek.

The collected female paper pondshell, fatmucket, and western pearlshell were transferred to culture facilities at Missouri State University (Springfield, MO, USA), and female threeridge and washboard were transferred to Genoa National Fish Hatchery (Genoa, WI, USA). The adult mussels were held at 10 °C to 12 °C and fed commercially available cultured algae (*Nannochloropsis* and Shellfish Diet; Reed Mariculture). The culture water used at Missouri State University was filtered (10 μm) river water collected from James River, Greene County, Missouri, with a hardness 160 mg/L as $CaCO_3$ and pH 8.1; the culture water used in the Genoa National Fish Hatchery was well water with a hardness 200 mg/L as $CaCO_3$ and pH 7.5. To culture juvenile mussels, roughly equal numbers of glochidia were removed from each of at least 3 adult mussels (Table 1). The viability of glochidia isolated from each adult mussel was tested with a subsample using the closing response to sodium chloride 24. The viability of glochidia from all samples exceeded 90%. The glochidia isolated from the adult mussels were pooled and placed on hatchery-reared fish for metamorphosis. The number of adult mussels used for glochidia sampling and the viability of glochidia met the recommendations in ASTM International methods 24. The host fish (Table 1) were maintained at 20 °C to 22 °C (except for western pearlshell at 13 °C to 15 °C) in a recirculating system equipped for recovery of juveniles. Juvenile mussels were recovered 2 wk (5 wk for western pearlshell) after fish infestation. Juvenile mussels recovered from the host fish during the 2-d peak of recovery were shipped overnight to the US Geological Survey, Columbia Environmental Research Center (Columbia, MO) for toxicity testing.

The newly transformed juvenile mussels (generally <5 d old) were acclimated to test water and temperature in 1-L glass beakers with gentle aeration through a glass pipette or in a recirculating bucket 25 for at least 2 d before the start of a test. Approximately 25% of the water in the containers was gradually replaced with test water twice daily. The juvenile mussels were fed an algal mixture (*Nannochloropsis* concentrate and Shellfish Diet; Reed Mariculture 7) during the acclimation period twice daily in the morning and afternoon, with an algal density of 5 nL cell volume/mL to 10 nL cell volume/mL after each feeding.

Test water and exposure concentrations

Test water (control water) was prepared by diluting Columbia Environmental Research Center well water of hardness 300 mg/L as $CaCO_3$ with deionized water to a hardness of 100 mg/L as $CaCO_3$. The formula, purity, source, and modes of toxic action of test chemicals are listed in Table 2. For each chemical, 5 concentrations plus a control were tested. Test concentrations were chosen based on results of previous acute toxicity tests with juvenile mussels. When a prediction of toxicity for a particular chemical was not available

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

for mussels, an initial 96-h range-finding test was first conducted with a limited number of mussels (5 to 10) and replicates (1 or 2) in a control and 5 concentrations of the test chemical that differed by a dilution factor of up to 10.

Toxicity tests were conducted under static-renewal conditions, with the exception that ammonia toxicity tests were conducted under flow-through conditions to maintain constant concentrations over 96-h exposures 8. For static-renewal toxicity tests, a solution of the highest exposure concentration was prepared 4 h to 24 h before the start of a test by adding either a certain amount of chemical (e.g., NaCl, KCl, and Na_2SO_4) or a certain volume of stock solution (metals and organic chemicals) into 1000 mL or 2000 mL of control water in a glass jar. One-half of the solution was then used for 50% dilutions to create other solutions of lower concentrations. The control water and solutions were held in the dark at 4 °C and warmed to test temperature in water baths for use at the beginning of a test and for water renewal. Triethylene glycol (Sigma-Aldrich) was used as solvent to prepare stock solutions of organic chemicals, with the exceptions of carbaryl and bifenthrin, for which acetone (Sigma-Aldrich) was used. The solvent concentration in test solutions was kept to a minimum and did not exceed 0.5 mL/L 24. A dilution-water control and a solvent control containing the highest concentration of the solvent in test solutions were included in tests with organic chemicals. Ammonia toxicity tests were conducted in an intermittent flow-through proportional diluter system 8. The diluter delivered 5 ammonia concentrations with a 50% dilution series plus a control. Ammonium chloride (NH_4Cl; Table 2) was used to prepare an ammonia stock solution in a 2000-mL volumetric flask. The pH in the stock solution was adjusted to a pH of 8.0 (close to the pH in the control water) by adding <1 mL of a solution of ammonium hydroxide (NH_4OH; 30% purity; Fisher Scientific). The stock solution was delivered with each cycle of the diluter system by Hamilton syringe pump (Hamilton).

Toxicity testing

At the beginning of each static-renewal test, 5 juvenile mussels were impartially transferred into each of 4 separate 50-mL replicate glass beakers containing 30 mL of water. Test beakers were held in a plastic container (30 cm × 18 cm × 10 cm) with a cover to reduce evaporation. The containers were held in a water bath at 23 ± 1 °C (or 20 ± 1 °C for western pearlshell, a western US resident species that inhabits cooler water). Water temperature was monitored daily. Ambient laboratory light was 400 lux to 500 lux with 16:8-h light:dark photoperiod. Test organisms were not fed during 96-h exposures. Approximately 75% of the water in each replicate beaker was removed and renewed after 48 h. At the end of exposures, mussels in each beaker were examined under a dissecting microscope. The endpoint was mortality (empty shell with little or no tissue) plus immobility (no foot or shell movement within a 5-min observation period). The test acceptability criterion was ≥90% control survival 24.

For the flow-through ammonia toxicity tests conducted in the diluter, 10 mussels were impartially transferred into each of 4 replicate exposure units at the beginning of exposures. The exposure unit consisted of an inner chamber and an outer beaker 10. The inner chamber was a 160-mL glass tube with stainless-steel screen (254-μm opening) at the bottom, used to

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

facilitate retrieval of small juvenile mussels at the end of the acute exposure. Two glass rods were attached to the top of the inner chamber to keep the chamber suspended in a 300-mL glass outer beaker. The outer beaker had a 2.5-cm hole in the side covered with a stainless-steel screen (279-μm opening) and contained 200 mL of water. The diluter system delivered 125 mL of test solution into each inner chamber once every 4 h. The excess water flowed out through the side screen of the outer beaker. At the end of exposures, mussels in each inner chamber were rinsed into a glass tray with control water, and recovered and transferred into a 50-mL glass beaker containing 20 mL of the test solution; survival was determined under a dissecting microscope. Other test conditions and test acceptability criteria were the same as those described previously for the static-renewal tests.

Water quality and chemical analysis

Dissolved oxygen, pH, conductivity, hardness, and alkalinity were measured using standard methods 26 on composite water samples collected from the replicates in the control, medium, and high exposure concentrations at the beginning and the end of each test. Water samples for major cations (calcium, potassium, magnesium, and sodium), major anions (chloride and sulfate), and dissolved organic carbon (DOC) were periodically collected from control water. Major cations were analyzed by inductively coupled plasma–mass spectrometry (ELAN DRC-e; PerkinElmer) according to USEPA method 6020B 27, and major anions were analyzed by ion chromatography (ICS-1100; Dionex) according to USEPA method 9056A 28. Samples for the analysis of DOC were filtered through 0.45 μm polyethersulfone membranes and measured by a total organic carbon analyzer (Model TOC-L CSH; Shimadzu Scientific Instruments) according to USEPA method 415.3 29.

Water samples for the analyses of toxicants were collected in all exposure concentrations generally at the beginning of metal exposures, and at the beginning and the end of exposures to other chemicals for which the stability of concentration over 96-h exposure was uncertain (Supplemental Data, Tables S4 and S5). Water samples for metal analysis were drawn from mid-depth of each exposure chamber with an all-polypropylene syringe fitted with a tetra-fluoroethylene sipper straw. The sample was then dispensed through a 0.45-μm pore size polyethesulfone membrane filter into an acid-cleaned polyethylene bottle, except the sample for analysis of total aluminum, which was not filtered. Each 20-mL sample was stabilized within 24 h by adding 0.2 mL of concentrated nitric acid. Concentrations of the metals were determined by inductively coupled plasma–mass spectrometry according to USEPA method 6020B 27. Chloride and sulfate were analyzed by the ion chromatography described previously or by a Hach HQ440d benchtop dual input, multi-parameter meter (Hach). In 2 chloride toxicity tests conducted earlier with threeridge and washboard, chloride concentrations were not measured. Rather, salinity and conductivity were measured in all exposure concentrations at the beginning and the end of each test to confirm the chloride concentrations (Supplemental Data, Table S4–4). Total ammonia nitrogen (N) was determined by a Hach HQ440d benchtop meter with a PHC301 Re-fillable pH Electrode. The meter was calibrated before measuring samples with 1.0 mg N/L and 10 mg N/L calibration standards. The method detection limit was 0.03 mg N/L.

EPA Author Manuscript EPA Author Manuscript EPA Author Manuscript

Water samples for analysis of organic chemicals were extracted using Oasis HLB solid phase extraction cartridges (60 mg, 3 cc; Waters Corp). The cartridges were conditioned using ethyl acetate and methanol followed by deionized water prior to the drop-wise addition of the 10-mL water samples. The cartridges were then dried under nitrogen or by pulling laboratory air through for 5 min, placed in plastic bags and stored at −20 °C until extraction. Chemicals were eluted from the cartridges using 80:20 (v/v) dichloromethane:methyl-*tert*-butyl ether (alachlor, carbaryl, malathion, and metolachlor), methanol (2,4-dichlorophenoxyacetic acid and 4-nonylphenol), and ethyl acetate (azoxystrobin, bifenthrin, and molinate) prior to analysis. Analyses for alachlor, carbaryl, malathion, and metolachlor were conducted using an Agilent 6890 gas chromatograph with a 5973N mass selective detector. Analyses for 2,4-dichlorophenoxyacetic acid and 4-nonylphenol were conducted using liquid chromatography with photodiode array detection (Surveyor system; Thermo-Finnigan). Analyses for azoxystrobin, bifenthrin, and molinate were conducted using an Agilent 7890A gas chromatograph with a 5975C mass selective detector. Additional details of the extraction and analysis methods can be found in Alvarez et al. 30 and Hladik et al. 31.

Analyses of tested chemicals were performed by chemistry laboratories at the Columbia Environmental Research Center and US Geological Survey (Sacramento, CA), following internal standard operating procedures and quality assurance/quality control protocols. Established laboratory quality assurance/quality control procedures and sample types (second source calibration verification, laboratory spikes, duplicates, reference/laboratory control materials) were used to verify instrument performance, accuracy, and precision throughout the analyses. These established procedures were in place to ensure method performance and instrumental suitability. Results from each laboratory underwent data quality review prior to use in the present study.

Data analysis

Median effect concentrations (EC50s) based on mortality plus immobility of juvenile mussels were estimated with the Toxicity Response Relationship Analysis Program (TRAP; Ver 1.30a 32), using the tolerance distribution analysis with the Gaussian (normal) distribution model and with log-transformed exposure concentrations. When the data did not meet the requirements of the TRAP (least 2 partial responses), either a Spearman-Karber or a trimmed Spearman-Karber method was used following the flowchart for EC50 determination recommended by the USEPA 33 using TOXSTAT® software (Ver 3.5; Western EcoSystems Technology).

To compare the relative sensitivity of mussels with other freshwater species, toxicity databases were compiled by adding the mussel toxicity data from the present study to additional databases, including the USEPA ICE database 19, 20 for alachlor, metolachlor, chloride (as NaCl), potassium (as KCl), calcium chloride, 2,4-dichlorophenoxyacetic acid, 4-nonylpgenol, azoxystrobin, bifenthrin, malathion, molinate, chromium (VI), and nickel; the databases used to derive USEPA water quality criteria for arsenic (V) 34, ammonia 35, copper 36, and carbaryl 37; the database used to update water quality criteria for aluminum (D. Eignor, USEPA, Washington, DC, unpublished data); a zinc database 38; a sulfate

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

database representing a hardness range of 80 mg/L to 120 mg/L 16; and the toxicity data from the companion study with the 2 snails and 3 other invertebrates (Ivey et al. 23 in this issue) and unpublished toxicity data from acute toxicity tests conducted at the Columbia Environmental Research Center that met ASTM International test acceptability criteria, such as >90% control survival. Toxicity data for nickel and zinc were normalized to a hardness of 50 mg/L as $CaCO_3$ using the equations in the USEPA water quality criteria for nickel or zinc 34, and copper data were normalized using a biotic ligand model 36. Ammonia data were normalized to total ammonia-nitrogen at pH 7 and 20°C 35. Aluminum data in a pH range of 6.5 to 9.0 were normalized to a hardness of 100 mg/L as $CaCO_3$ (D. Eignor, USEPA, Washington, DC, draft update water quality criteria for aluminum). Because sodium chloride toxicity typically decreases with increasing water hardness 11, 39, 40, the chloride data were compiled in a hardness range of 80 mg/L to 180 mg/L.

RESULTS AND DISCUSSION

Water quality

Water quality characteristics are summarized in Supplemental Data, Tables S2 and S3. The concentrations of dissolved oxygen ranged from 7 mg/L to 9 mg/L. Mean measured water quality characteristics were similar to nominal values of the diluted well water with hardness of 100 mg/L as $CaCO_3$, alkalinity of 90 mg/L as $CaCO_3$, conductivity of 250 μS/cm, and pH of 8.2 (Supplemental Data, Tables S2 and S3), with a few exceptions. Higher alkalinity of 131 mg/L to 168 mg/L was observed in high exposure concentrations in the toxicity tests with 2,4-dichlorophenoxyacetic acid, arsenic (V), and aluminum, probably because of the addition of a large amount of the tested chemical or the counterion of chemicals (i.e., up to 800 mg/L 2,4-dichlorophenoxyacetic acid, 1020 mg/L sodium arsenate dibasic heptahydrate, or 360 mg/L aluminum chloride). In addition, as expected, the conductivity increased with increasing exposure concentrations of ammonium chloride, potassium chloride, sodium chloride, and sodium sulfate. Measured conductivity values at different exposure concentrations for these chemicals are presented in Supplemental Data, Tables S4–3 to S4–6. Mean major ions and DOC measured periodically in the control water were 26 mg Ca/L, 9.0 mg mg/L, 9.0 mg Na/L, 0.9 mg K/L, 11 mg Cl/L, 20 mg SO_4/L, and 0.5 mg/L DOC, which were similar to those reported in previous studies with the diluted well water 16, 41.

Chemical analysis

Mean measured concentrations of inorganic chemicals typically differed less than 20% from nominal concentrations, whereas measured concentrations of most organic chemicals were 20% to 50% lower than nominal concentrations (Supplemental Data, Tables S4 and S5). Measured concentrations of organic chemicals were generally consistent at the beginning and end of a test, except that the concentrations of carbaryl and malathion decreased substantially at the end of testing (Supplemental Data, Table S5). The EC50s were calculated based on measured concentrations or on nominal concentrations adjusted by measured concentrations in some of organic chemical tests where not all concentrations were measured (Supplemental Data, Tables S4 and S5). Nominal concentrations were used for the EC50 calculation in 2 chloride tests with threeridge and washboard where chloride concentrations were not measured. Mean measured salinity and conductivity values were

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

relatively consistent with the nominal NaCl concentrations (Supplemental Data, Table S4–4), confirming that the chloride concentrations were close to the nominal concentrations. Nominal concentrations were also used to calculate EC50s in 2 organic chemical tests (threeridge–alachlor test and fatmucket–carbaryl test) because the measured concentrations showed high, unexplained variability (1 measured concentration was similar to the nominal but another measured concentration was much different from the nominal; Supplemental Data, Tables S4–1 and S5) and the nominal concentrations represented our best estimate of actual exposure concentrations. Detailed information on the concentrations used for EC50 calculation is provided in Supplemental Data, Tables S4 and S5.

Toxicity tests with the 5 mussel species and 10 chemicals in the multi-species study

Toxicity data—In the multi-species study, 60 acute toxicity tests were completed with 5 mussels and 10 chemicals (Table 3). A test was repeated (if a new batch of mussels was available) when an EC50 could not be estimated because more than 50% mortality was observed in all exposure concentrations (fatmucket–potassium test and western pearlshell–potassium test; Table 3) or because there were no partial kills (fatmucket–nickel test). The EC50s for chloride and copper from the tests with fatmucket from different populations are also reported in Table 3 (test number 1 for the Silver Fork population, test number 2 for the Bourbeuse River population, and test number 3 for the Kansas City Zoo population). Ammonia toxicity was not tested with fatmucket in the present study because fatmucket has been intensively tested at the Columbia Environmental Research Center in previous ammonia studies 6, 8, 10. Two ammonia EC50 values for fatmucket, obtained previously with similar test conditions (e.g., flow-through testing) and with the same life stage (newly transformed mussels) are included in Table 3 for the purpose of comparison among the 5 species.

Survival was ≥90% in the dilution water controls in 56 of the 60 tests, and survival in the solvent controls was ≥90% in all organic chemical tests (Supplemental Data, Tables S4 and S5) and met the test acceptability criterion of ≥90% control survival 24. The control survival in 4 tests with threeridge (alachlor, metolachlor, sulfate, and chromium tests) ranged from 75% to 88% (Supplemental Data, Table S4–1,2,6,7) and did not meet the test acceptability criterion of ≥90% control survival. However, the control survival in the other 6 chemical tests, which were conducted concurrently with the alachlor, metolachlor, sulfate, and chromium tests using same batch of threeridge juveniles, was above 90%, ranging from 92% to 100% (Supplemental Data, Table S4–3,4,5,8,9,10), and an overall control survival for all 10 chemical tests was 90% (total of 44 control replicates, including solvent control). Furthermore, another copper toxicity test was conducted with a different batch of threeridge (Supplemental Data, Table S4–8), and the copper EC50s between the 2 threeridge tests were almost identical (Table 3). Therefore, the EC50s from the 4 tests with lower control survival were included in Table 3 with a footnote denoting that the control survival was less than 90%. When using the overall control survival data to recalculate the EC50s for the 4 tests, the EC50s would be slightly lower than those reported in Table 3: 1.1 mg/L (0.7–1.8 mg/L) alachlor, 4.4 mg/L (2.9–6.6 mg/L) metolachlor, 1017 mg/L (834–1241 mg/L) sulfate, and 206 μg/L (156–273 μg/L) chromium (VI).

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

Sensitivity comparisons among the 5 mussel species

The EC50s of the 5 mussel species ranged from 1.2 mg/L to 15 mg/L alachlor, 4.6 mg/L to 29 mg/L metolachlor, 1.5 mg/L to 8.0 mg/L total ammonia, 1038 mg/L to 2246 mg/L chloride, 31 mg/L to 48 mg/L potassium, 1338 mg/L to 2709 mg/L sulfate, 138 μg/L to 624 μg/L chromium, 10 μg/L to 55 μg/L copper, 173 μg/L to 676 μg/L nickel, and 299 μg/L to 576 μg/L zinc (Table 3). No single tested species had lower or higher EC50s than other species across all 10 chemicals. However, threeridge had the lowest EC50s for 8 of the 10 chemicals, and western pearlshell had the highest EC50s for 4 of the 10 chemicals. The EC50s for each chemical among the 5 species differed by a factor of ≤2 for chloride, potassium, sulfate, and zinc; a factor of ≤5 for ammonia, chromium, copper, and nickel; and factors of 6 and 12 for metolachlor and alachlor, respectively. In most cases, the 95% confidence limits for each chemical overlapped among the 5 mussel species. The results indicate that the mussels across different families or tribes had similar sensitivity to most of the tested chemicals, regardless of modes of action.

Sensitivity comparisons within a mussel species from the same population or from different populations

The EC50s for a single species in the repeated tests were similar (Table 3). Specifically, the EC50s for a species differed within a factor of 1.1 to 1.4 in the tests with 2 or 3 different batches of juveniles from the same population (i.e., fatmucket–chloride tests 1, 4 and 5; fatmucket–potassium test; threeridge–copper test; fatmucket–nickel test; Table 3). The low variability in EC50 among tests in a single laboratory was consistent with a previous study, in which the differences in EC50s in repeated copper tests with juvenile fatmucket from 1 population were within a factor of 1.4 42. The EC50s for chloride or copper among different populations of fatmucket were also similar, within a factor of 1.2 for chloride and 1.6 for copper (Table 3; test number 1 for fatmucket from the Silver Fork, test number 2 for fatmucket from the Bourbeuse River, and test number 3 for fatmucket from the Kansas City Zoo). The results indicate that the sensitivity of juvenile fatmucket among different populations was similar in acute exposures to copper or chloride. Importantly, the sensitivity of juvenile fatmucket cultured from larvae of wild adults was similar to the sensitivity of juveniles cultured from larvae of captive-cultured adults (Kansas City Zoo). This result shows that captive-cultured adult mussels can reliably be used to reproduce juveniles for toxicity testing.

Sensitivity comparisons between fatmucket and other mussels

The sensitivity of the commonly tested fatmucket was compared to the other 4 mussels across the 10 chemicals with a regression plot. A strong linear relationship was obtained between EC50s for fatmucket and the other mussel species ($r^2 = 0.97$), and the slope of the regression was close to 1.0 (Figure 1). Over 73% of EC50s for the other 4 species were within 2-fold of EC50s for fatmucket. The results indicate that the toxicity of these chemicals to different species of mussels can be predicted with fatmucket toxicity data. Raimondo et al. 20 developed a toxicity database, including preliminary data from the present study, to examine the variability in sensitivity among mussels, cladocerans, and fish to chemicals with different modes of action. Their study demonstrated that 71% to 79% of

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

EC50s for the most commonly tested mussel species (fatmucket, paper pondshell, or rainbow mussel *Villosa iris*) were within 2-fold of EC50s for up to 10 other mussel species; however, only 34% to 37% of EC50s for commonly tested cladocerans (*D. magna* and *C. dubia*) and 16% to 23% of commonly tested fish (*Oncorhynchus mykiss*, *Pimephales promelas*, and *Lepomis macrochirus*) were within 2-fold of EC50s for mussels 20.

Species sensitivity comparisons among mussels and other freshwater organisms

In the companion study (Ivey et al. 23 in this issue), the freshwater snail species (*P. gyrina* and *L. stagnalis*) and commonly tested amphipod (*H. azteca*) and cladocerans (*C. dubia* and *D. magna*) were tested in acute exposures to the 10 chemicals used in the multi-species study with the 5 mussels. Compared with the 5 mussels tested in the present study, the 2 snails had greater EC50s for ammonia, potassium, sulfate, and chromium but had similar EC50s for the other 6 chemicals. For the 10 chemicals, the EC50s for *C. dubia* were similar to or less than the EC50s for *D. magna, H. azteca*, and the 2 snails. However, *C. dubia* had substantially greater EC50s for ammonia and potassium compared with the 5 mussels. The results indicate that *C. dubia* may sufficiently represent sensitivity of the snails to the 10 chemicals but may not adequately represent the sensitivity of mussels to some chemicals. In the expanded database with more chemicals and mussel species, Raimondo et al. 20 found that, in general, *C. dubia* was less sensitive than mussels.

Species mean acute values (calculated as geometric mean of EC50s for a test species) for all freshwater species in the compiled toxicity databases were ranked and plotted in cumulative distribution for the 10 chemicals tested in the present study (Figure 2). The ranges of ranked SMAVs for mussels in different families or tribes were relatively narrow, generally within the lower 50th percentile of the species sensitivity distributions for the tested chemicals, except for the 2 organic chemicals (alachlor and metolachlor; Figure 2). One or more mussel species were among the 4 most sensitive species in the databases for 8 of the 10 chemicals tested in the multi-species study. Of the 6 tested chemicals for which the USEPA has water quality criteria 43, most species mean acute values for chloride or nickel from mussels and some species mean acute values for ammonia, copper, or zinc from mussels were similar to or less than the final acute value used to derive the USEPA acute criterion (Figure 2). These results indicate that mussels representing different tribes or families had similar sensitivity to inorganic chemicals across different modes of toxic action whereas the sensitivity to organic chemicals was relatively variable among mussels, and the water quality criteria development for alachlor, chloride, potassium, sulfate, copper, nickel, and zinc should reflect the sensitivity of mussels to these chemicals. The USEPA uses the genus level sensitivity distribution approach to develop acute water quality criterion, which is typically based on laboratory toxicity data from a suite of aquatic organisms that are assumed to represent the sensitivity of untested species (i.e., minimum data requirement of 8 different families 44). Because of the high sensitivity of mussels to numerous chemicals across different modes of action, water quality criteria that better protect freshwater organisms may be obtained if the minimum data requirement for deriving water quality criteria were updated to include native mussels as a required family.

EPA Author Manuscript EPA Author Manuscript EPA Author Manuscript

The USEPA updated the ammonia water quality criteria in 2013, and the acute criterion is 1.4-fold lower than the previous acute criterion, primarily because of the inclusion of mussel toxicity data 35. In the 2013 water quality criteria, the 10 lowest species mean acute values, 7 lowest genus mean acute values, and 2 lowest species mean chronic values are for mussels. It is reasonable to expect some of the approximately 270 US native species of mussels that were not included in that dataset may be equally or more sensitive. A few mussel species, including threeridge from the present study, had ammonia EC50s below the 2013 water quality criteria final acute value (Figure 2). This and other information on mussel sensitivity to ammonia may be utilized to develop site-specific environmental guidance values and to provide enhanced conservation of especially vulnerable or important populations and/or communities of mussels. Another approach may be the development of taxon-specific criteria. Conceptually, a taxon-specific criterion could be derived to protect a species, genus, or family that is not adequately protected by general national aquatic life water quality criteria. Taxon-specific criteria would provide probabilistic estimates of hazard based on the subset of data most relevant to individual taxa of conservation concern. A mussel-specific ammonia criterion would complement the general national water quality criteria, which are derived to be protective of a large number of taxa but not meant to protect all species, by providing a technically sound risk management option for water quality managers' consideration when developing state or tribal water quality standards.

Toxicity tests with fatmucket and 10 additional chemicals in the single-species study

Ten additional chemicals were tested with fatmucket in the single-species study. Mean survival was >90% in the controls, including the solvent controls in organic chemical tests, and met the test acceptability criterion of ≥90% control survival 24. Survival was >80% in all treatments at the end of exposures to 2,4-dichlorophenoxyacetic acid, bifenthrin, carbaryl, and aluminum (Supplemental Data, Table S5), even though the high nominal concentrations of the 3 organic chemicals were close to solubility and a large amount of aluminum floc was observed on the bottom of test beakers in the high and medium-high concentrations.

The EC50s for the 10 chemicals tested in the single-species study with fatmucket and the percentiles of the species mean acute values for fatmucket in the species sensitivity distribution for all freshwater species are presented in Table 4. The EC50s for fatmucket were in the higher percentiles (≥45th) of the species sensitivity distribution, except for 4-nonylphenol in the 27th percentile. There were limited mussel data for chemicals tested in the single-species study. The EC50 of 23 mg/L malathion for fatmucket tested in the present study was close to the low range of 96-h EC50s from 24 mg/L to 219 mg/L for juveniles of 6 other mussel species tested in a previous study 45. Milam et al. 46 conducted acute toxicity tests with glochidia of 6 mussel species in 24-h exposures that included 3 organic chemicals tested in the present study with juvenile fatmucket. The EC50s of >311 mg/L 2,4-dichlorophenoxyacetic acid, 0.099 mg/L 4-nonylphenol, and >8.0 mg/L carbaryl for juvenile fatmucket tested in the present study were within the 24-h EC50 ranges of 82 mg/L to 437 mg/L 2,4- dichlorophenoxyacetic acid, 0.057 mg/L to 1.19 mg/L 4-nonylphenol, and 3.1 mg/L to 43 mg/L carbaryl for glochidia of the 6 mussel species 46, respectively. Of the 3 tested chemicals (carbaryl, arsenic [V], and aluminum) for which the USEPA has water

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

quality criteria, the species mean acute values for fatmucket tested in the present study and other mussel species tested in previous studies 45, 46 were far above the final acute values (Table 4). The results indicate that mussels tested were not sensitive to the chemicals tested in the single-species study, except 4-nonylphenol. Notably, 7 of the 10 chemicals tested in the single-species study were organic chemicals (Table 4). The mussels appeared to be less sensitive to organic chemicals. The relative (to other taxa) insensitivity of mussels to other organic contaminants has been reported previously 47.

The demonstrated sensitivity of mussels to a diversity of inorganic toxicants makes ensuring mussels are represented in water quality criteria development or other hazard assessments critical for the protection of mussels. As mentioned previously, direct testing of mussels is recommended when deriving estimates of protective concentrations for inorganic constituents because mussels have been demonstrated to be among the most sensitive forms of aquatic life to some metals and common ions. Where direct testing of the taxa is not feasible, extrapolation approaches such as the USEPA ICE models can provide estimated values that represent inherent taxa sensitivity. The ICE models are log-linear relationships of acute sensitivity between a surrogate species and predicted taxa of interest and can be used to estimate toxicity to the predicted taxa (species, genus, family) from measured toxicity of the surrogate 19. The ICE models rely on an existing database of diverse species and chemicals, such as that used to develop species sensitivity distributions in the present study. Previous versions of the USEPA web-based ICE application had limited predictions to freshwater mussels because of limited measured data. A recent study expanded the database for freshwater mussels and provided additional guidance on inclusion of the mussel data into ICE models 20.

CONCLUSIONS

The present study confirms findings from previous studies that mussels are generally sensitive to ammonia, metals, and ion constituents, but are not generally among the most sensitive organisms for organic chemicals (including various pesticides) in acute exposures. Furthermore, mussels representing different families or tribes had similar sensitivity to most tested chemicals regardless of modes of toxic action. The sensitivity of the commonly tested fatmucket was similar to other mussel species tested. In addition, the sensitivity of juvenile fatmucket among different populations or cultured from larvae of wild adults and captive-cultured adults was also similar in acute exposures to copper or chloride. Use of toxicity data from fatmucket, in conjunction with the available USEPA ICE models, should provide good estimates of risk to mussels regardless of their taxonomic classification for the purpose of deriving water quality criteria or other environmental guidance values and conducting risk assessments.

In compiled toxicity databases for freshwater organisms, mussels were among the more sensitive species to alachlor, ammonia, chloride, potassium, sulfate, copper, nickel, and zinc. Therefore, the development of water quality criteria and other environmental guidance values for these chemicals should reflect the sensitivity of mussels. Including a native mussel as a required family in the minimum data requirement for deriving water quality criteria 44 should be considered in any water quality criteria developments or updates.

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

Further studies are warranted to evaluate chronic sensitivity of mussels across phylogenetically diverse species in longer-term (e.g., 28 d to 90 d) exposures to chemicals with different modes of toxic action and to derive or update water quality criteria and other guidance to protect these long-lived mussels from long-term exposures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

We thank the staff of the Toxicology Branch and Environmental Chemistry Branch of US Geological Survey (Columbia, MO, USA) for technical assistance, E.A. Glidewell of Missouri State University (Springfield, MO, USA) and N. Eckert of Genoa National Fish Hatchery (Genoa, WI, USA) for providing juvenile mussels for testing, T. Divis of the Kansas City Zoo for mussel culture, C. Lilavois for assistance with the USEPA Web-Interspecies Correlation Estimation database, K.L. Smalling and M.L. Hladik of the US Geological Survey (Sacramento, CA, USA) for analyses of some organic chemicals, and 3 anonymous journal reviewers for their comments. Funding for the present study was provided in part by the Great Lakes Restoration Initiative.

References

1. Williams JD, Warren ML, Cummings KS, Harris JL, Neves RJ. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18: 6–12.
2. Lydeard C, Cowie RH, Ponder WF, Bogan AE, Bouchet P, Clark SA, Cummings KS, Frest TJ, Gargominy O, Herbert DG, Hershler R, Perez KE, Roth B, Seddon M, Strong EE, Thompson FG. 2004. The global decline of nonmarine mollusks. BioScience 54: 321–330.
3. Strayer DL, Downing JA, Haag WR, King TL, Layzer JB, Newton TJ, Nichols SJ. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. BioScience 54: 429–439.
4. Haag WR. 2012. North American Freshwater Mussels: Natural History, Ecology, and Conservation. Cambridge University Press, New York, NY, USA.
5. March FA, Dwyer FJ, Augspurger T, Ingersoll CG, Wang N, Mebane CA. 2007. An evaluation of freshwater mussel toxicity data in the derivation of water quality guidance and standards for copper. Environ Toxicol Chem 26: 2066–2074. [PubMed: 17867876]
6. Wang N, Ingersoll CG, Hardesty DK, Ivey CD, Kunz JL, May TW, Dwyer FJ, Roberts AD, Augspurger T, Kane CM, Neves RJ, Barnhart MC. 2007. Acute toxicity of copper, ammonia, and chlorine to glochidia and juveniles of freshwater mussels (Unionidae). Environ Toxicol Chem 26: 2036–2047. [PubMed: 17867873]
7. Wang N, Ingersoll CG, Greer IE, Hardesty DK, Ivey CD, Kunz JL, Dwyer FJ, Roberts AD, Augspurger T, Kane CM, Neves RJ, Barnhart MC. 2007. Chronic toxicity of copper and ammonia to juvenile freshwater mussels (Unionidae). Environ Toxicol Chem 26: 2048–2056. [PubMed: 17867874]
8. Wang N, Erickson RJ, Ingersoll CG, Ivey CD, Brunson EL, Augspurger T, Barnhart MC. 2008. Influence of pH on the acute toxicity of ammonia to juvenile freshwater mussels (fatmucket, Lamsilis siliquoidea). Environ Toxicol Chem 27: 1141–1146. [PubMed: 18419178]
9. Wang N, Mebane CA, Kunz JL, Ingersoll CG, Brumbaugh WG, Santore RC, Gorsuch JW, Arnold WR. 2011. Influence of dissolved organic carbon on toxicity of copper to a unionid mussel (Villosa iris) and a cladoceran (Ceriodaphnia dubia) in acute and chronic water exposures. Environ Toxicol Chem 30: 2115–2125. [PubMed: 21681812]
10. Miao J, Barnhart MC, Brunson EL, Hardesty DK, Ingersoll CG, Wang N. 2010. An evaluation of the influence of substrate on the response of juvenile freshwater mussels (fatmucket, Lampsilis siliquoidea) in acute water exposure to ammonia. Environ Toxicol Chem 29: 2112–2116. [PubMed: 20821670]

EPA Author Manuscript EPA Author Manuscript EPA Author Manuscript

11. Gillis PL. 2011. Assessing the toxicity of sodium chloride to the glochidia of freshwater mussels: Implications for salinization of surface waters. Environ Pollut 159: 1702–1708. [PubMed: 21429642]
12. Augspurger T, Dwyer FJ, Ingersoll CG, Kane CM. 2007. Advances and opportunities in assessing contaminant sensitivity of freshwater mussel (Unionidae) early life stages. Environ Toxicol Chem 26: 2025–2028. [PubMed: 17867884]
13. Wang N, Ingersoll CG, Ivey CD, Hardesty DK, May TW, Augspurger T, Roberts AD, Genderen EV, Barnhart MC. 2010. Sensitivity of early life stages of freshwater mussels (Unionidae) to acute and chronic toxicity of lead, cadmium, and zinc in water. Environ Toxicol Chem 29: 2053–2063. [PubMed: 20821663]
14. Bringolf RB, Cope WG, Eads CB, Lazaro PA, Barnhart MC, Shea D. 2007. Acute and chronic toxicity of technical grade pesticides to glochidia and juveniles of freshwater mussels (Unionidae). Environ Toxicol Chem 26: 2086–2093. [PubMed: 17867872]
15. Cope WG, Bringolf RB, Buchwalter DB, Newton TJ, Ingersoll CG, Wang N, Augspurger T, Dwyer FJ, Barnhart MC, Neves RJ, Hammer E. 2008. Differential exposure, duration, and sensitivity of unionoidean bivalve life stages to environmental contaminants. J N Am Benthol Soc 27: 451–462.
16. Wang N, Dorman RA, Ingersoll CG, Hardest DK, Brumbaugh WG, Hammer EJ, Bauer CR, Mount DR. 2016. Acute and chronic toxicity of sodium sulfate to four freshwater organisms in water-only exposures. Environ Toxicol Chem 35: 115–127. [PubMed: 26139383]
17. Graf DL, Cummings KS. 2007. Review of the systematics and global diversity of freshwater mussel species (Bivalvia: Unionoida). J Molluscan Studies 73: 291–314.
18. Campbell DC, Serb JM, Buhay JE, Roe KJ, Minton RL, Lydeard C. 2005. Phylogeny of North American amblemines (Bivalvia, Unionoida): Prodigious polyphyly proves pervasive across genera. Invertebrate Biol 124: 131–164.
19. Raimondo S, Jackson CR, Barron MG. 2010. Influence of taxonomic relatedness and chemical mode of action in acute interspecies estimation models for aquatic species. Environ Sci Technol 44: 7711–7716. [PubMed: 20795664]
20. Raimondo S, Lilavois C, Lee L, Augspurger T, Wang N, Ingersoll C, Bauer C, Hammer E, Barron M. 2016. Assessing variability in chemical acute toxicity of unionid mussels: Influence of intra- and inter-laboratory testing, life stage, and species. Environ Toxicol Chem 35: 750–758. [PubMed: 26369904]
21. Barron MG, Lilavois CR, Martin TM. 2015. A comprehensive mode of action and acute aquatic toxicity database for predictive model development. Aquat Toxicol 161: 102–107. [PubMed: 25700118]
22. ASTM International. 2015. ASTM International standard guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E729–96 (2007). Annual Book of ASTM International Standards, Vol 11.06. West Conshohocken, PA, USA.
23. Wang N, Ivey CD, Ingersoll CG, Brumbaugh WG, Alvarez D, Hammer EJ, Bauer CR, Augspurger T, Raimondo S, Barnhart MC. 2017. Acute sensitivity of a broad range of freshwater mussels to chemicals with different modes of toxic action. Environ Toxicol Chem 36: 786–796 (this issue). [PubMed: 27699830]
24. ASTM International. 2013. ASTM International standard guide for conducting laboratory toxicity tests with freshwater mussels. E2455–06 (2013). Annual Book of ASTM International Standards, Vol 11.06. West Conshohocken, PA, USA.
25. Barnhart MC. 2006. Bucket of muckets: A compact recirculating system for rearing juvenile freshwater mussels. Aquaculture 254: 227–233.
26. Eaton AD, Clesceri LS, Rice EW, Greenberg AE, eds. 2005. Standard Methods for the Examination of Water and Wastewater, 21st ed. American Public Health Association, Water Environment Federation, American Water Works Association, Washington, DC.
27. US Environmental Protection Agency, 2014. Method 6020B: Inductively coupled plasma-mass spectrometry. SW-846. [cited 2016 September 30]. Available from: https://www.epa.gov/sites/production/files/2015-12/documents/6020b.pdf

EPA Author Manuscript EPA Author Manuscript EPA Author Manuscript

28. US Environmental Protection Agency. 2007. Method 9056A: Determination of inorganic anions by ion chromatography. SW-846. [cited 2016 September 30]. Available from: https://www.epa.gov/sites/production/files/2015-12/documents/9056a.pdf
29. Potter BB, Wimsatt JC. 2005. Method 415.3: Measurement of total organic carbon, dissolved organic carbon, and specific UV absorbance at 254 nm in source water and drinking water. US Environmental Protection Agency, Washington, DC. [cited 2016 September 30]. Available from: https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=103917
30. Alvarez DA, Cranor WL, Perkins SD, Clark RC, Smith SB. 2008. Chemical and toxicological assessment of organic contaminants in surface water using passive samplers. J Environ Qual 37: 1024–1033. [PubMed: 18453425]
31. Hladik ML, Smalling KL, Kuivila KM. 2008. A multi-residue method for the analysis of pesticides and pesticide degradates in water using HLB solid-phase extraction and gas chromatography-ion trap mass spectrometry. Bull Environ Contam Toxicol 80: 139–144. [PubMed: 18196191]
32. Erickson RJ. 2015. Toxicity Relationship Analysis Program (TRAP), Ver 1.30a. EPA 600/C-11/002. US Environmental Protection Agency, Washington, DC.
33. US Environmental Protection Agency. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th ed. EPA 821/R-02/012. Washington, DC, USA.
34. US Environmental Protection Agency. 1995. 1995 updates: Water quality criteria documents for the protection of aquatic life in ambient water. EPA 820/B-96/001. Washington, DC.
35. US Environmental Protection Agency. 2013. Aquatic life ambient water quality criteria for ammonia—Freshwater. EPA 822/R-13/001. Washington, DC.
36. US Environmental Protection Agency. 2007. Aquatic life ambient freshwater quality criteria—Copper, 2007 revision. EPA 822/R-07/001. Washington, DC.
37. US Environmental Protection Agency. 2012. Final national recommended ambient water quality criteria for Carbaryl. EPA 820/F-12/004. Washington, DC.
38. DeForest DK, Van Gendere EJ. 2012. Application of U.S. EPA guidelines in a bioavailability-based assessment of ambient water quality criteria for zinc in freshwater. Environ Toxicol Chem 31: 1264–1272. [PubMed: 22447746]
39. Lasier PJ, Winger PV, Hardin IR. 2006. Effect of hardness and alkalinity in test waters on the reproduction of *Ceriodaphnia dubia*. Environ Toxicol Chem 25: 2781–2786. [PubMed: 17022421]
40. Elphick JR, Bergh KD, Bailey HC. 2011. Chronic toxicity of chloride to freshwater species: Effects of hardness and implications for water quality guidelines. Environ Toxicol Chem 30: 239–246. [PubMed: 20872898]
41. Wang N, Ingersoll CG, Dorman RA, Brumbaugh WG, Mebane CA, Kunz JL, Hardesty DK. 2014. Chronic sensitivity of white sturgeon (Acipenser transmontanus) and rainbow trout (Oncorhynchus mykiss) to cadmium, copper, lead, or zinc in laboratory water-only exposures. Environ Toxicol Chem 33: 2246–2258. [PubMed: 24862826]
42. Wang N, Augspurger T, Barnhart MC, Bidwell JR, Cope WG, Dwyer FJ, Geis S, Greer IE, Ingersoll CG, Kane CM, May TW, Neves RJ, Newton TJ, Roberts AD, Whites DW. 2007. Intra- and inter-laboratory variability in acute toxicity tests with glochidia and juveniles of freshwater mussels (Unionidae). Environ Toxicol Chem 26: 2029–2035. [PubMed: 17867871]
43. US Environmental Protection Agency. 2016. National Recommended Water Quality Criteria. [cited 2016 September 30]. Available from: http://water.epa.gov/scitech/swguidance/standards/criteria/current/index.cfm
44. Stephen 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. PB85–227049. National Technical Information Service, Springfield, VA, USA.
45. Keller AE, Ruessler DS. 1997. The toxicity of malathion to unionid mussels: Relationship to expected environmental concentrations. Environ Toxicol Chem 16: 1028–1033.
46. Milam CD, Farris JL, Dwyer FJ, Hardesty DK. 2005. Acute toxicity of six freshwater mussel species (glochidia) to six chemicals: Implications for daphnids and Utterbackia imbecillis as surrogates for protection of freshwater mussels (Unionidae). Arch Environ Contam Toxicol 48: 166–173. [PubMed: 15772883]

47. Keller A, Lydy M, Ruessler DS. 2007. Unionid mussel sensitivity to environmental contaminants. In Farris JL, Van Hassel JH, eds, Freshwater Bivalve Ecotoxicology. SETAC, Pensacola, FL, USA, pp 151–167.

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

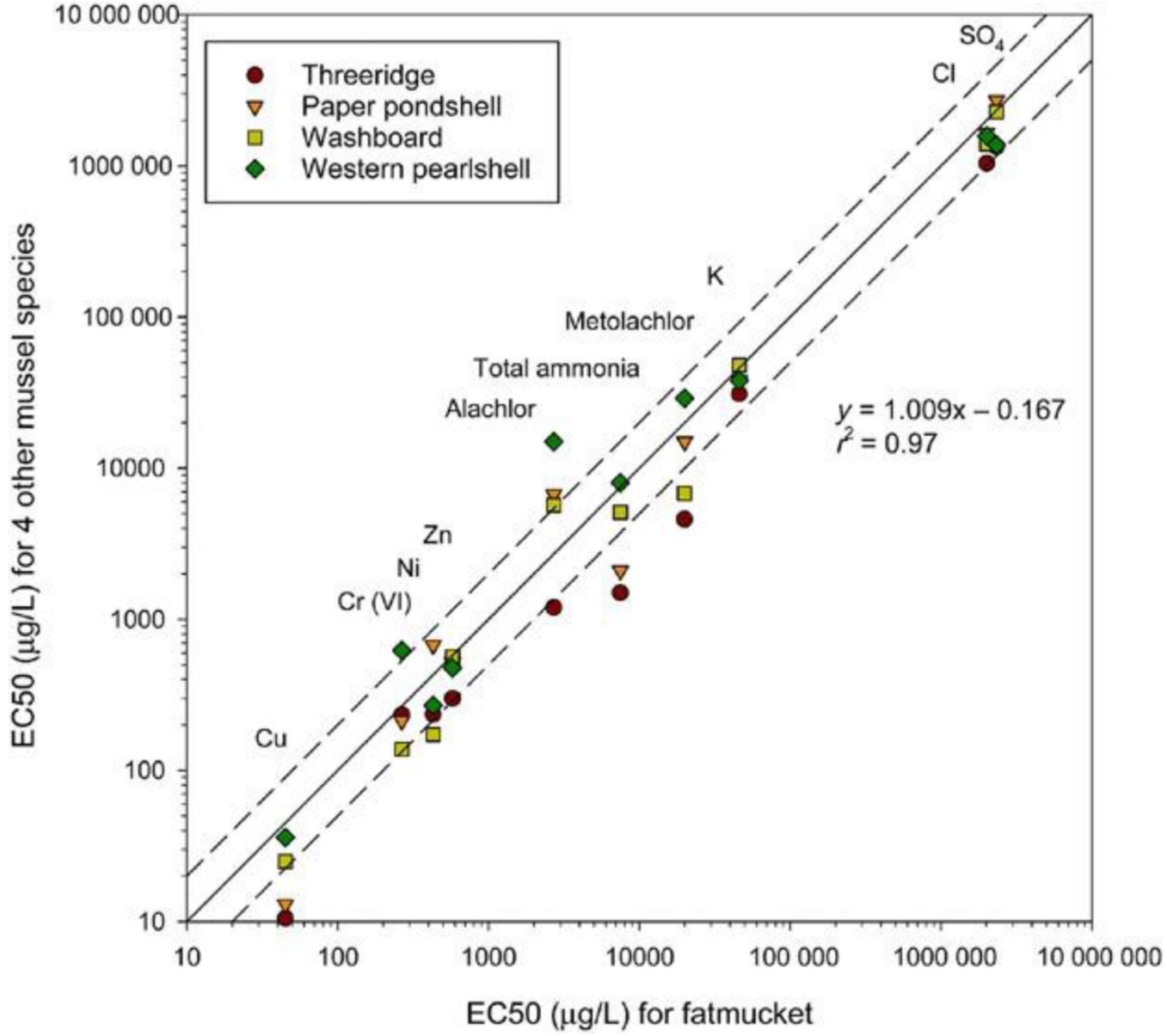


Fig 1.

Relationship between the median effect concentrations (EC50s) from a commonly tested mussel (fatmucket) and the EC50s from 4 other mussels in acute 96-h exposures with the 10 chemicals for the multi-species study. The solid line indicates the 1:1 line of perfect agreement, and dashed lines indicate 1:2 and 2:1 lines (i.e., bounds for EC50s from the 4 mussel species being within 2-fold of EC50s from fatmucket). For repeat tests with a chemical (Table 3), a geometric mean of EC50s was used. The results of linear regression analysis (SigmaPlot, Ver 13.0; Systat Software) are also shown.

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

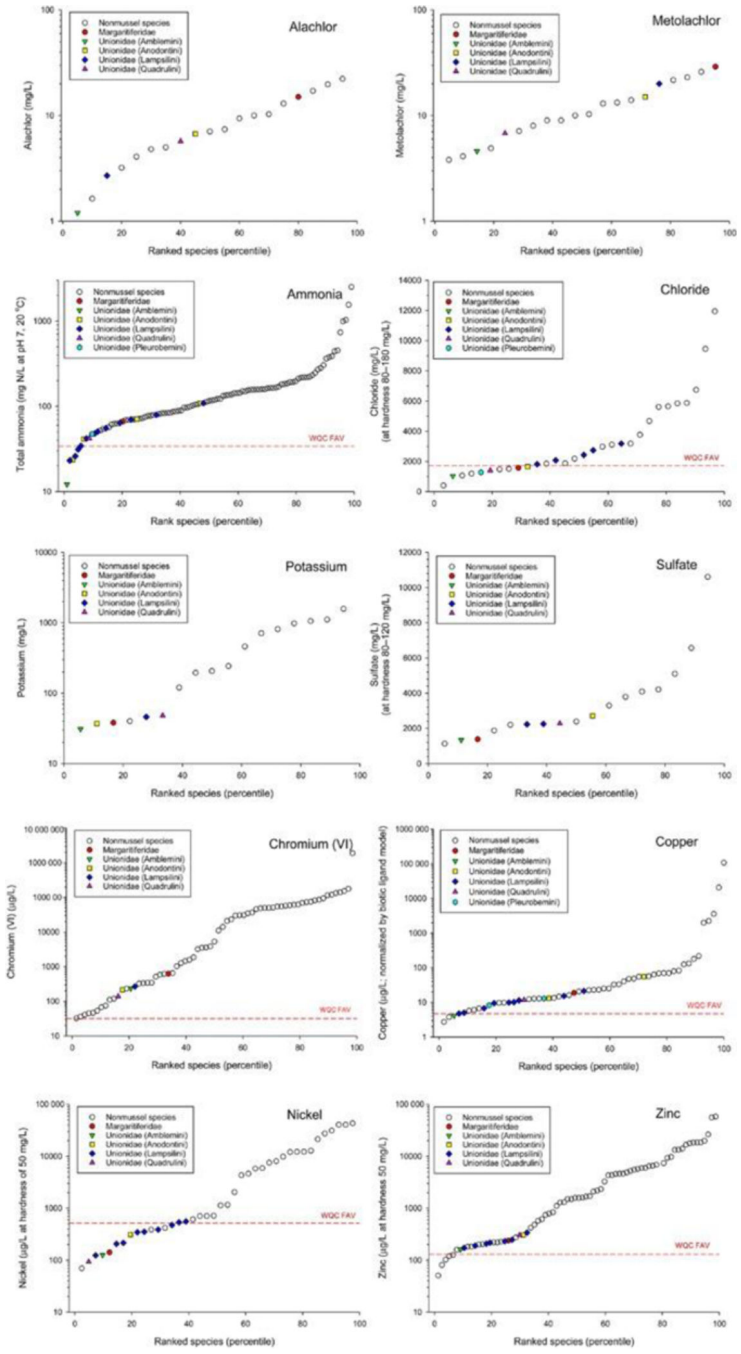


Fig 2.

Ranked freshwater species mean acute values in compiled databases, with addition of species mean acute values for freshwater mussels of different tribes or families tested in the present study. Dashed line indicates the final acute value (FAV) in the US Environmental Protection Agency ambient water quality criteria (WQC)

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

Table 1.

Gravid female mussels and host fish for juvenile mussel culture

Family (Tribe)	Species	Reproduction	Collection location	No. of mussels for a batch culture	Host fish
Unionidae (Amblemini)	Threeridge (*Amblema plicata*)	Short-term brooder	Mississippi River, Prairie du Chien, WI, USA	4 or 8	Largemouth bass (*Micropterus salmoides*)
Unionidae (Anodontini)	Paper pondshell (*Utterbackia imbecillis*)	Long-term brooder	Kansas City Zoo, Kansas City, MO, USA	3	Bluegill (*Lepomis macrochirus*)
Unionidae (Lampsilini)	Fatmucket (*Lampsilis siliquoidea*)	Long-term brooder	Perche Creek, Boone County, MO, USA[a]	3 or 5	Largemouth bass (*Micropterus salmoides*)
Unionidae (Quadrulini)	Washboard (*Megalonaias nervosa*)	Long-term brooder	Mississippi River, Crawford County, WI, USA	15	Channel catfish (*Ictalurus punctatus*)
Margaritiferidae	Western pearlshell (*Margaritifera falcata*)	Short-term brooder	South Fork Eel River, Mendocino County, CA, USA	7 or 8	Rainbow trout (*Oncorhynchus mykiss*)

[a]Two other populations of fatmucket were collected from Bourbeuse River (Gasconade County, MO, USA) and Kansas City Zoo (Kansas City, MO, USA) for reference toxicant (sodium chloride and copper) tests to determine the sensitivity among three populations (details in text).

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

Table 2.

Chemicals and modes of toxic action in two studies[a]

Toxicant	Chemical formula	Purity (%)	Broad MOA[b]	Specific MOA[b]
Multi-species study				
Alachlor	$C_{14}H_{20}ClNO_2$	99.2	Narcosis	Nonpolar
Metolachlor	$C_{15}H_{22}ClNO_2$	97.6	Narcosis	Nonpolar
Ammonia	NH_4Cl	99.5	Lono/Osmoregulatory/Circulatory impairment	Other osmoregulatory impairment
Potassium	KCl	99.7	Lono/Osmoregulatory/Circulatory impairment	Other osmoregulatory impairment
Chloride	NaCl	100	Lono/Osmoregulatory/Circulatory impairment	Other osmoregulatory impairment
Sulfate	Na_2SO_4	100	Lono/Osmoregulatory/Circulatory impairment	Other osmoregulatory impairment
Chromium (VI)	CrO_3	98	Lono/Osmoregulatory/Circulatory impairment	Metallic iono/osmoregulatory impairment
Copper	$CuSO_4 \cdot 5H_2O$	99	Lono/Osmoregulatory/Circulatory impairment	Metallic iono/osmoregulatory impairment
Nickel	$NiCl_2$	100	Lono/Osmoregulatory/Circulatory impairment	Metallic iono/osmoregulatory impairment
Zinc	$ZnCl_2$	98	Lono/Osmoregulatory/Circulatory impairment	Metallic iono/osmoregulatory impairment
Single-species study				
2,4-dichlorophenoxyacetic acid	$C_8H_6Cl_2O_3$	97	Narcosis	Nonpolar
4-nonylphenol	$C_{15}H_{24}O$	100	Narcosis	Polar
Azoxystrobin	$C_{22}H_{17}N_3O_5$	100	Narcosis	Ester
Bifenthrin	$C_{23}H_{22}ClF_3O_2$	100	Neurotoxicity	Pyrethroid sodium channel modulation
Carbaryl	$C_{12}H_{11}NO_2$	97	Acetylcholinesterase inhibition	Carbamate
Malathion	$C_{10}H_{19}O_6PS_2$	100	Acetylcholinesterase inhibition	Organophosphate
Molinate	$C_9H_{17}NOS$	100	Narcosis	Nonpolar
Arsenic (V)	$Na_2HAsO_4 \cdot 7H_2O$	98	Electron transport inhibition	Arsenical respiratory inhibition
Calcium chloride	$CaCl_2$	98	Uncertain	Uncertain
Aluminum	$AlCl_3$	99	Lono/Osmoregulatory/Circulatory impairment	Metallic iono/osmoregulatory impairment

[a] All chemicals were obtained from Sigma-Aldrich, except ammonia chloride and potassium chloride (obtained from Fisher Scientific).

[b] Mode of action (MOA) determined from Barron et al. 21.

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

Table 3.

Acute median effect concentrations (EC50s) and 95% confidence limits (CLs; in parenthesis) for 10 chemicals in the multi-species study with 5 species of mussels representing different tribes or families of freshwater mussels

		EC50 (95% CL)				
Toxicant (Test no.)	**Unit**	**Threeridge**	**Paper pondshell**	**Fatmucket**	**Washboard**	**Western pearlshell**
Alachlor	mg/L	1.2 (0.8–1.8)[a]	6.7 (5.6–7.9)	2.7 (2.5–2.8)	5.7 (5.0–6.4)[b]	15 (12–20)
Metolachlor	mg/L	4.6 (3.0–7.1)[a]	15 (12–18)	20 (16–26)	6.8 (6.6–7.1)	29 (27–31)
Ammonia (total)	mg/L	1.5 (1.1–2.0)	2.1 (1.4–3.0)	5.2c/11[c]	5.1 (4.7–5.5)	8.0 (7.7–8.4)
Chloride (1)[d]	mg/L	1038 (808–1333)	1657 (1346–2039)	1897 (1850–1945)	1398 (1169–1670)	1576 (1391–1786)
Chloride (2)	mg/L	__[e]	—	1944 (1773–2131)	—	—
Chloride (3)	mg/L	—	—	2246 (2211–2281)	—	—
Chloride (4)	mg/L	—	—	1893 (1751–2045)[f]	—	—
Chloride (5)	mg/L	—	—	2092 (1910–2290)[f]	—	—
Potassium (1)[d]	mg/L	31 (17–57)	38 (35–42)	<56	48 (41–56)	<56
Potassium (2)	mg/L	—	—	46 (38–54)	—	38 (37–39)
Sulfated	mg/L	1338 (no CL)[a, g]	2709 (2548–2880)	2325 (2176–2485)	2279 (2206–2355)	1378 (1335–1422)
Chromium (VI)	µg/L	233 (179–303)[a]	213 (187–242)	266 (224–316)	138 (132–144)	624 (603–647)
Copper (1)	µg/L	11 (10–12)	13 (11–15)	48 (40–57)	25 (24–26)	36 (35–38)
Copper (2)	µg/L	10 (8.2–13)	—	35 (30–42)	—	—
Copper (3)	µg/L	—	—	55 (47–64)	—	—
Nickel (1)	µg/L	234 (207–264)	676 (648–705)	506 [350–731][h]	173 (165–181)	269 (259–280)
Nickel (2)	µg/L	—	—	350 (298–411)	—	—
Nickel (3)	µg/L	—	—	445 (429–461)	—	—
Zinc	µg/L	299 (205–438)	520 (446–606)	576 (507–654)	566 (532–602)	447 (97–502)

[a]The EC50 should be used with caution due to low (<90%) control survival. See text for details.

[b]Only 45% mortality was observed in the highest test concentration (Supplemental Data, Table S4–1).

[c]Values for fatmucket were from a previous study at pH 8.1 and 20 °C 8 (see details in text).

[d]Chloride was tested as sodium chloride, potassium as potassium chloride, and sulfate as sodium sulfate (details in Table 2).

[e]A dash (—) indicates that the chemical was not tested with that species.

[f]Exposure solutions were prepared with a 30% series dilution (rather than 50% dilution in other tests).

[g]No CL could be calculated due to inadequate partial effects (Supplemental Data, Table S4–6).

[h]An EC50 could not be calculated due to no partial mortality (Supplemental Data, Table S4–9). The geometric mean of the bracketing concentrations with 0% and 100% mortality was calculated to obtain an estimated EC50. The 0% and 100% effect concentrations are provided in bracket as [0–100% effect concentration].

EPA Author Manuscript

EPA Author Manuscript

EPA Author Manuscript

Table 4.

Acute median effect concentrations (EC50s) and 95% confidence limits (CLs; in parentheses) for 10 chemicals in the single-species study with fatmucket (*Lampsilis siliquoidea*)[a]

Toxicant	EC50 (95% CL) (mg/L)	FAV (mg/L)	SSD percentile
2,4-dichlorophenoxyacetic acid	>311	NA	76 (*n* = 24)
4-nonylphenol	0.099 (0.096–0.102)	0.056	27 (*n* = 29)
Azoxystrobin	0.725 (0.697–0.754)	NA	83 (*n* = 5)
Bifenthrin	>0.0267	NA	86 (*n* = 6)
Carbaryl	>8.0	0.004	77 (*n* = 61)
Malathion	23 [16–33][b]	NA	82 (*n* = 75)
Molinate	53 (51–55)	NA	94 (*n* = 17)
Arsenic (V)	117 (113–122)[c]	0.68	62 (*n* = 12)
Calcium chloride	5383 [3862–7502][b]	NA	45 (*n* = 10)
Aluminum (total)	>54	1.5[d]	72 (*n* = 17)

[a]Final acute values (FAVs) in the US national ambient water quality criteria and a percentile of EC50 for fatmucket in the species sensitivity distribution (SSD) for all freshwater species are presented.

[b]An EC50 could not be calculated because of no partial mortality (Supplemental Data, Table S5). The geometric mean of the bracketing concentrations with 0% and 100% mortality was calculated to obtain an estimated EC50. The 0% and 100% effect concentrations are provided in bracket as [0–100% effect concentration].

[c]The effect concentration was calculated in terms of assayed arsenic concentration.

[d]The FAV in draft updated water quality criteria for aluminum at hardness 100 mg/L and pH range of 6.5 to 9.0 (D. Eignor, US Environmental Protection Agency, Washington, DC, unpublished data).

NA = not applicable.