Water Quality Constituents Relevant to Freshwater Mussels: Progress and State of the Science

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NC STATE UNIVERSITY

Outline

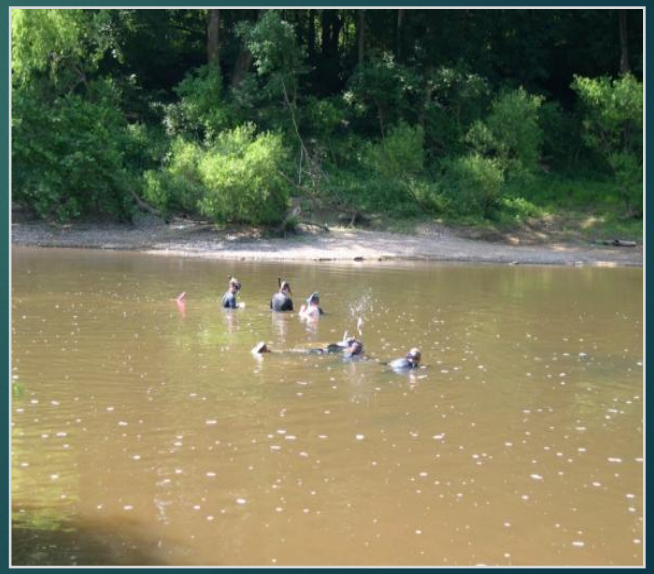
History—Evolution of Research Focus

- Highlights of scientific progress
  - Research: Standardization of toxicity test methods
  - Regulation: Protective water quality criteria
  - Freshwater mussel sensitivities
  - Improved mechanistic field methods
- State of the science
- Recent NCSU research
- Future directions

History Pre-1990s

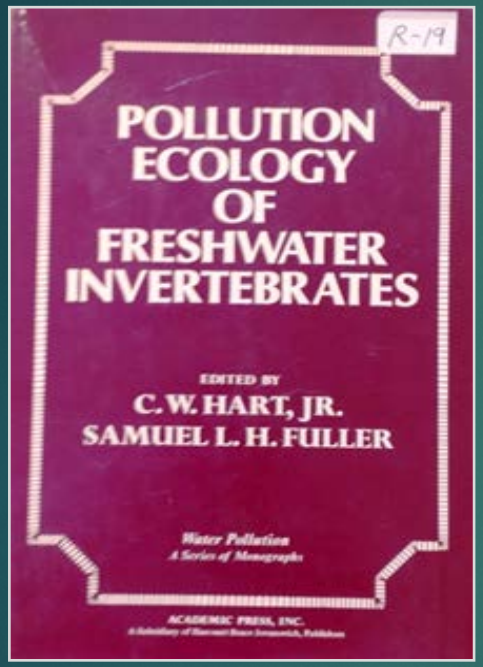
Descriptive Field-Based Studies

- Contaminant monitoring and bioaccumulation
- Habitat contamination and assemblage surveys

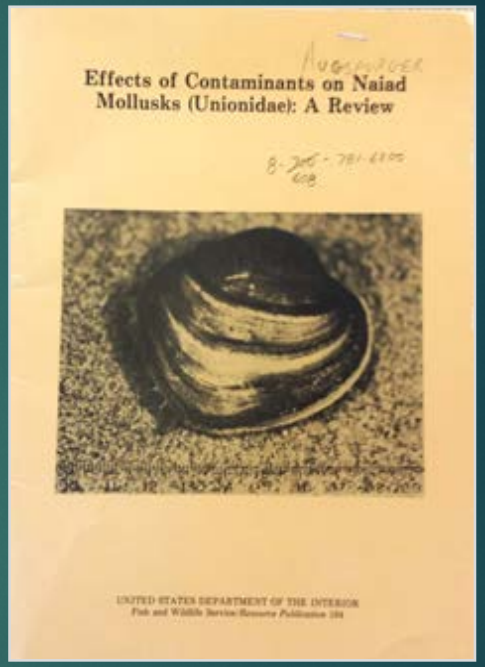


History Pre-1990s

Sources of Information—Limited



Fuller, S. L. H. 1974. Clams and mussels (Mollusca: Bivalvia). In: Pollution Ecology of Freshwater Invertebrates, (C. W. Hart, Jr. & S. L. H. Fuller, eds), pp. 215-273. Academic Press, New York.



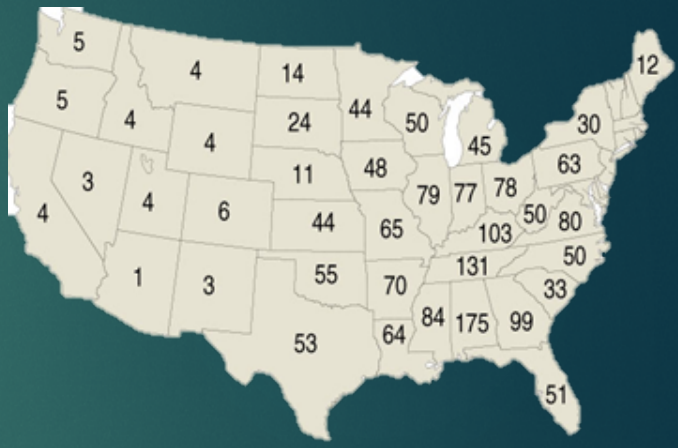
Havlik, M. E. and L. L. Marking. 1987. Effects of contaminants on naiad mollusks (Unionidae): a review. Resource Publ. 164, U. S. Department of the Interior, Fish and Wildlife Service, Washington, DC, 20pp.

Problem

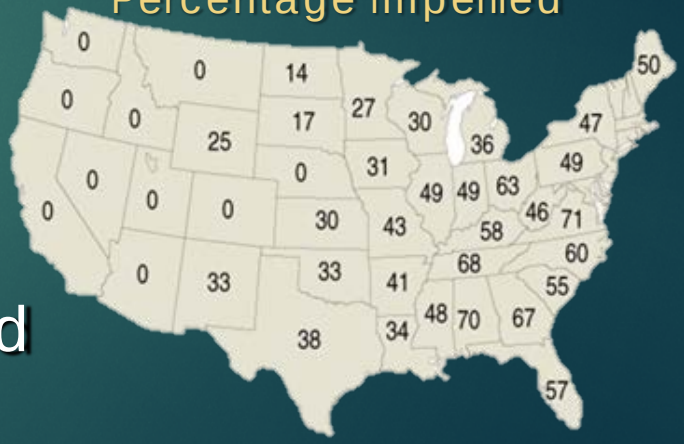
Declining Mussel Populations

- Pollution and water quality degradation
- Mussel data had never been used in establishing protective USEPA National Ambient Water Quality Criteria (WQC)
- What to do? Parallel the developments in other areas of aquatic toxicology
  - Standardized tests
  - Organism availability (culture and propagation)

Total Number of mussel species



Percentage Imperiled



Williams and Neves 1995

Research Progress

Toxicity Test Standardization

- Mussel toxicity data never used in establishing WQC
  - Data were available for some chemicals, life stages
  - No established standard method – EPA uncertainty
- How do we proceed in a regulatory framework?
  - FWS, EPA, University scientists summarized high quality data
  - Example: data suggested 1999 Ammonia WQC not protective of mussels

Research Progress

Toxicity Test Standardization

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Environmental Toxicology and Chemistry, Vol. 22, No. 11, pp. 2569–2575, 2003
Printed in the USA
0730-7268/04 $12.00 + .00

2003 Key Publication:

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

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(*Received* 18 *July* 2002; *Accepted* 3 *February* 2003)

Research Progress

Toxicity Test Standardization

Designation: E 2455 – 06

Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels[1]

This standard is issued under the fixed designation E 2455; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This standard guide describes methods for conducting laboratory toxicity tests with early life stages of freshwater mussels including glochidia and juvenile mussels in water-only exposures (Annex A1). Future revisions to this standard may describe methods for conducting toxicity tests with *(1)* adult freshwater mussels and *(2)* contaminated sediments using various life stages of freshwater mussels.

1.2 Many factors are cited as potentially contributing to the decline of freshwater mussel populations in North America. Of the nearly 300 taxa of freshwater mussels in North America, 70 species (23 %) are listed as endangered or threatened and another 40 species (14 %) are candidates for possible listing (Williams et al 1993 **(1)**; Neves 1997, 2004 **(2, 3)**).[2] Habitat alteration, introduction of exotic species, over-utilization, disease, predation and pollution are considered causal or contributing factors in many areas of the United States (Neves et al 1997) **(4)**. Over the past decade, there have been over 75 published studies conducted that have evaluated the role of contaminants in the decline of populations of freshwater mussels (Kernaghan et al 2005) **(5)**. In these studies, early life stages of mussels of several species are highly sensitive to some metals and ammonia in water exposures when compared to many of the most sensitive species of other invertebrates, fish, or amphibians that are commonly used to establish U.S. Environmental Protection Agency Water Quality Criteria (WQC; Augspurger et al 2003 **(6)**, Keller et al 2005 **(7)**, Kernaghan et al 2005 **(5)**; USGS (2005a,b) **(8, 9)** section 1.5). Importantly, results of these previous studies indicate WQC for individual chemicals established for the protection of aquatic organisms may not be adequately protective of sensitive stages of freshwater mussels.

1.3 *Summary of Life History of Freshwater Mussels*:

1.3.1 Freshwater mussels are bivalve mollusks belonging to the family Unionidae or Margaritiferidae (section 10.1). Adults are sedentary animals, spending their entire lives partially or completely burrowed in the bottoms of streams, rivers, or lakes. Adult mussels are filter feeders, using their gills to remove suspended particles from the water column. The microscopic, juvenile stage uses foot (pedal) feeding to some degree for the first several months of their lives, feeding on depositional materials in pore water of sediment, including bacteria, algae, and detritus. Freshwater mussels have an unusual and complex mode of reproduction, which includes a brief, obligatory parasitic stage on fish or other host organisms called glochidia (Fig. 1).

1.3.2 The successful transfer of mature glochidia to a suitable host constitutes a critical event in the life cycle of most freshwater mussels. Once the glochidia are released from the female, the glochidia need to attach to the gills or the fins of an appropriate fish host and encyst to complete development. Although glochidia may survive for months during brooding in the female mussel, glochidia typically survive for only a few days after release unless the glochidia reach a compatible host. Encystment on the host occurs by overgrowth of host tissue. Metamorphosis of juvenile mussels on the fish host occurs within days or weeks, depending on species and temperature. Host fish specificity varies among mussels. While some mussel species appear to require a single host organism, other species can transform their glochidia into juvenile mussels on several species of host fish. Following proper host infestation, glochidia transform into microscopic juveniles and excyst (drop off) and settle into suitable habitat to survive. The transformation of glochidia to juveniles results in the development of internal organs necessary for self-sustained existence as a benthic organism.

1.3.3 Newly-transformed juvenile mussels have a life style different from adult mussels. Transformed juvenile mussels may be at the sediment-water interface or may burrow several centimeters into sediment and rely on water percolating between substrate particles of sediment for food and oxygen. Newly-transformed juvenile mussels feed using ciliary currents on the foot and mantle. Older juvenile and adult mussels likely use different food types when living in different microenvironments. Given that glochidia and juvenile mussels are ecologically and physiologically different from adult mussels, protection of habitat quality of adult life stages may not be protective

[1] This guide is under the jurisdiction of ASTM Committee E47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.03 on Sediment Assessment and Toxicology.

Current edition approved April 1, 2006 Published May 2006. Originally approved in 2005. Last previous edition approved in 2005 as E 2455-05.

[2] The boldface numbers in parentheses refer to the list of references at the end of this standard.

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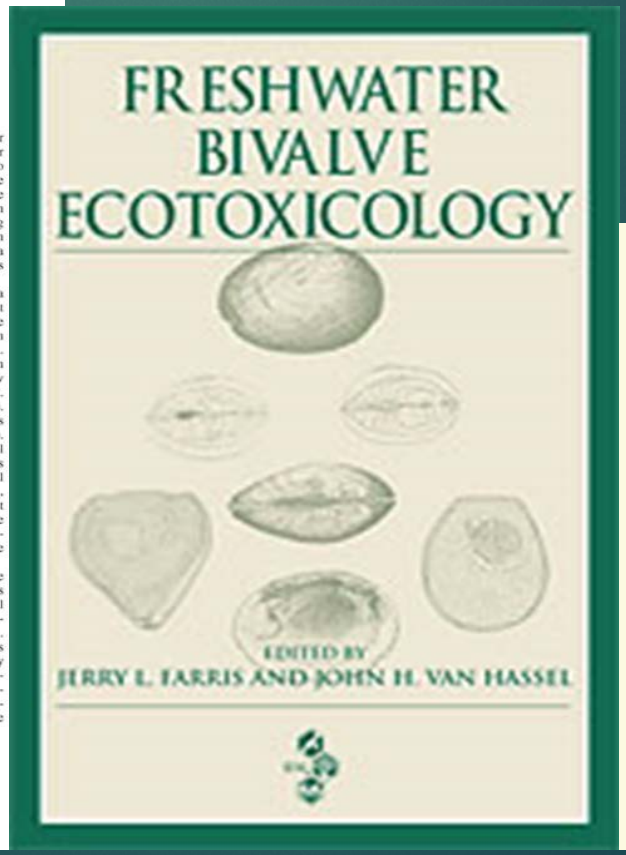

2005 2006 2007

Regulation Progress

Water Quality Criteria

United States Environmental Protection Agency | Office of Water 4304T | EPA-822-D-09-001 December 2009

EPA

DRAFT 2009 UPDATE AQUATIC LIFE AMBIENT WATER QUALITY CRITERIA FOR AMMONIA - FRESHWATER

- Success in 2009
- But….Included only juvenile mussel data; EPA still uncertain about use of glochidia

Research Progress

Toxicity Test Standardization

- Addressed remaining questions
  - Determine if the viability of glochidia is an ecologically relevant endpoint
  - Define appropriate test duration
- Major advance – Glochidia Included!

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 33, No. 1, pp. 199–207, 2014
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ASSESSMENT OF TOXICITY TEST ENDPOINTS FOR FRESHWATER MUSSEL LARVAE (GLOCHIDIA)

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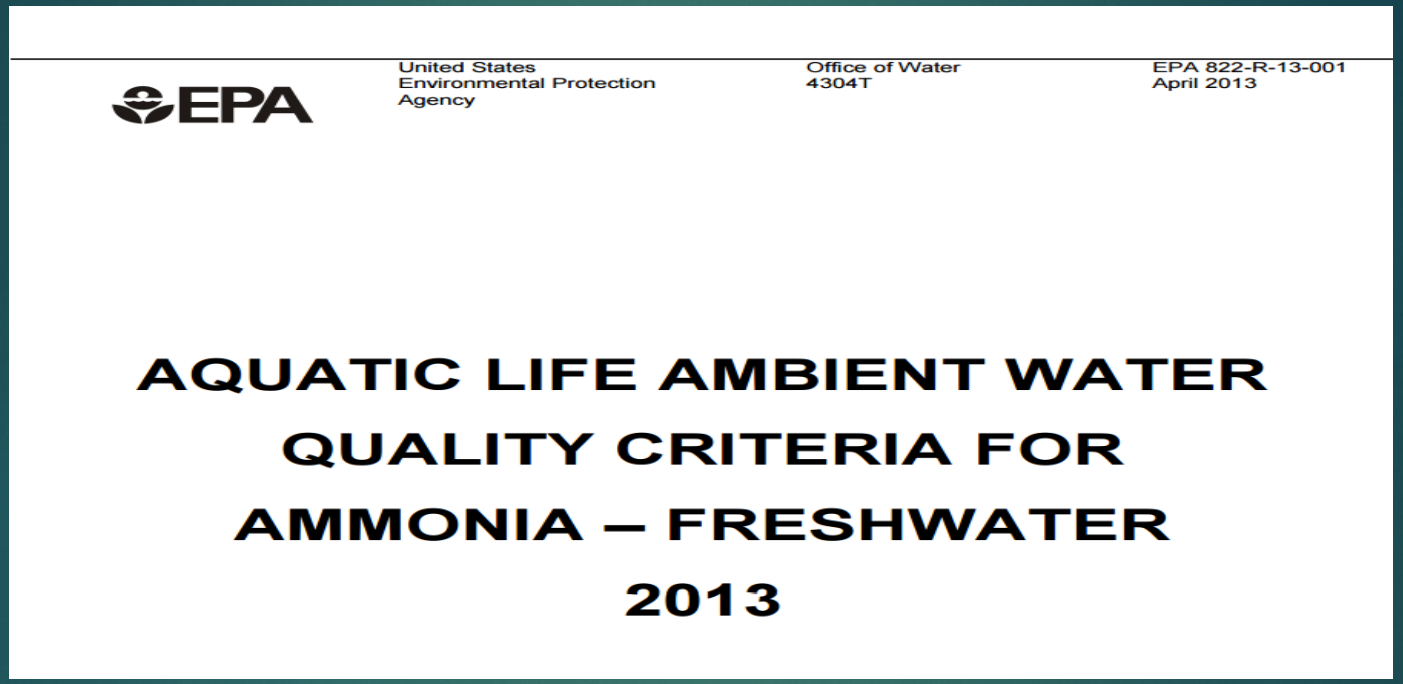
||US Environmental Protection Agency, Chicago, Illinois, USA

(Submitted 8 June 2013; Returned for Revision 23 July 2013; Accepted 27 September 2013)

Regulation Progress

Water Quality Criteria



EPA

United States
Environmental Protection
Agency

Office of Water
4304T

EPA 822-R-13-001
April 2013

AQUATIC LIFE AMBIENT WATER
QUALITY CRITERIA FOR
AMMONIA – FRESHWATER
2013

- Mission accomplished in 2013!?
- Over 10 years; multiple students, investigators, agencies, and organizations

Regulation Progress

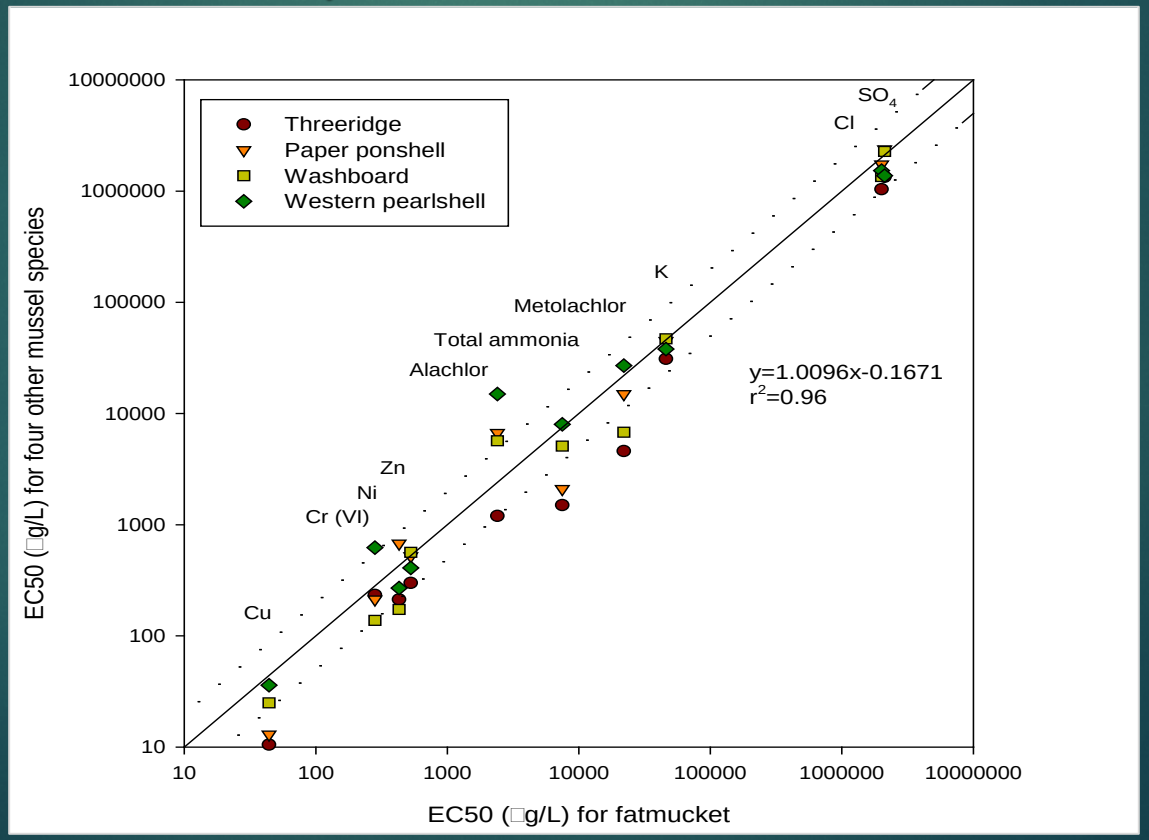
Water Quality Criteria

- Good news…Mussel toxicity data meets federal data quality objectives can be used in future criteria derivation
- Bad news…Outdated national WQC important to protection of mussels
  - Chloride, nickel, zinc
- Bad news…Lacking a national WQC that are important to protection of mussels
  - Potassium, sulfate, alachlor
- Good news…2007-2016 revisions to 4 national WQC contain at least some mussel data
  - Cadmium, copper, selenium, carbaryl

Freshwater Mussel Sensitivity

Is a Mussel a Mussel?

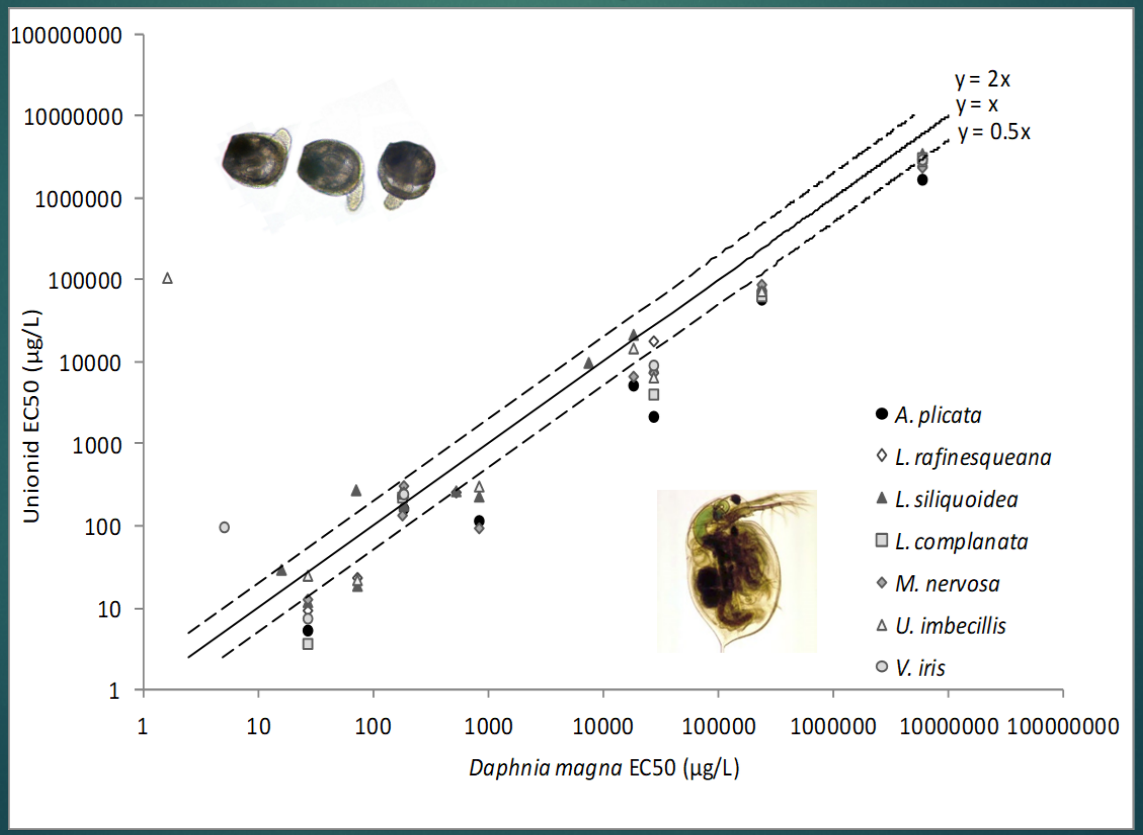
- Comparison of a commonly tested mussel (Fatmucket, *L. siliquoidea*) and other 4 mussel species (Wang et al. 2017)



Freshwater Mussel Sensitivity

Is a Mussel a Crustacian?

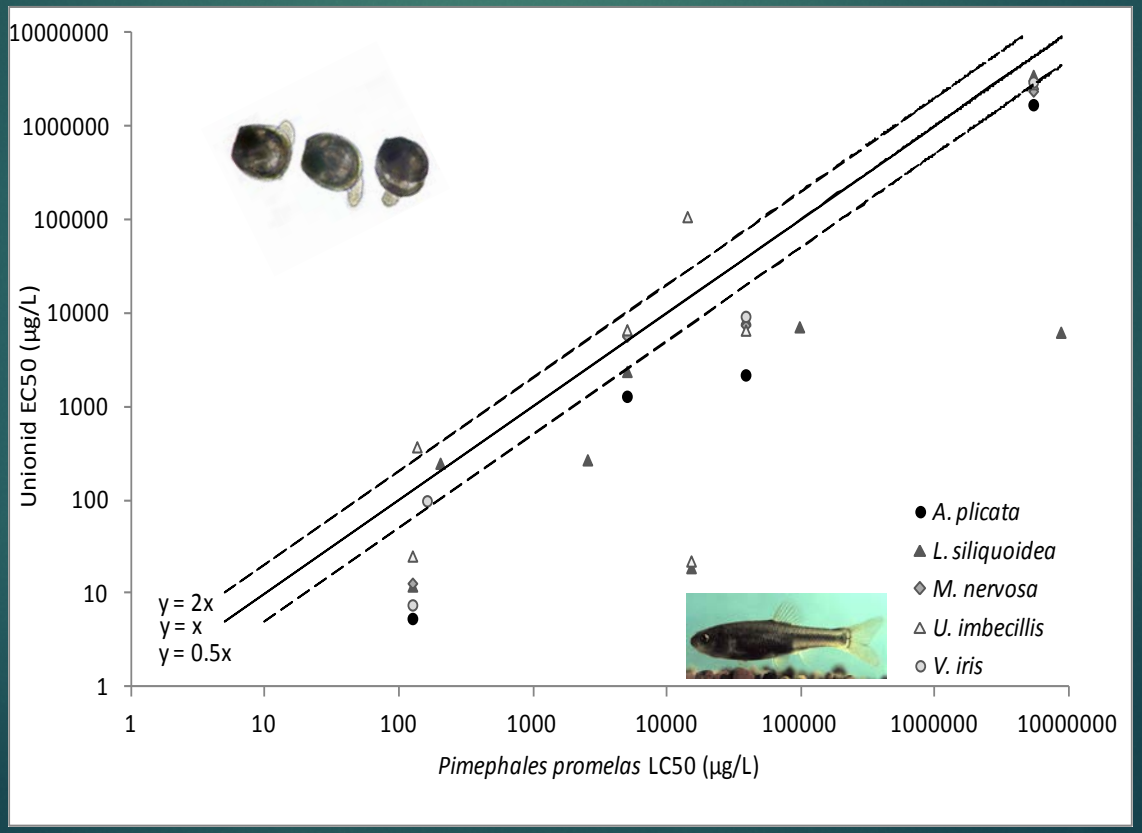
- Comparison of a commonly tested cladoceran (*Daphnia magna*) to 7 mussel species in acute exposures to 10 chemicals (Raimondo et al. 2016)



Freshwater Mussel Sensitivity

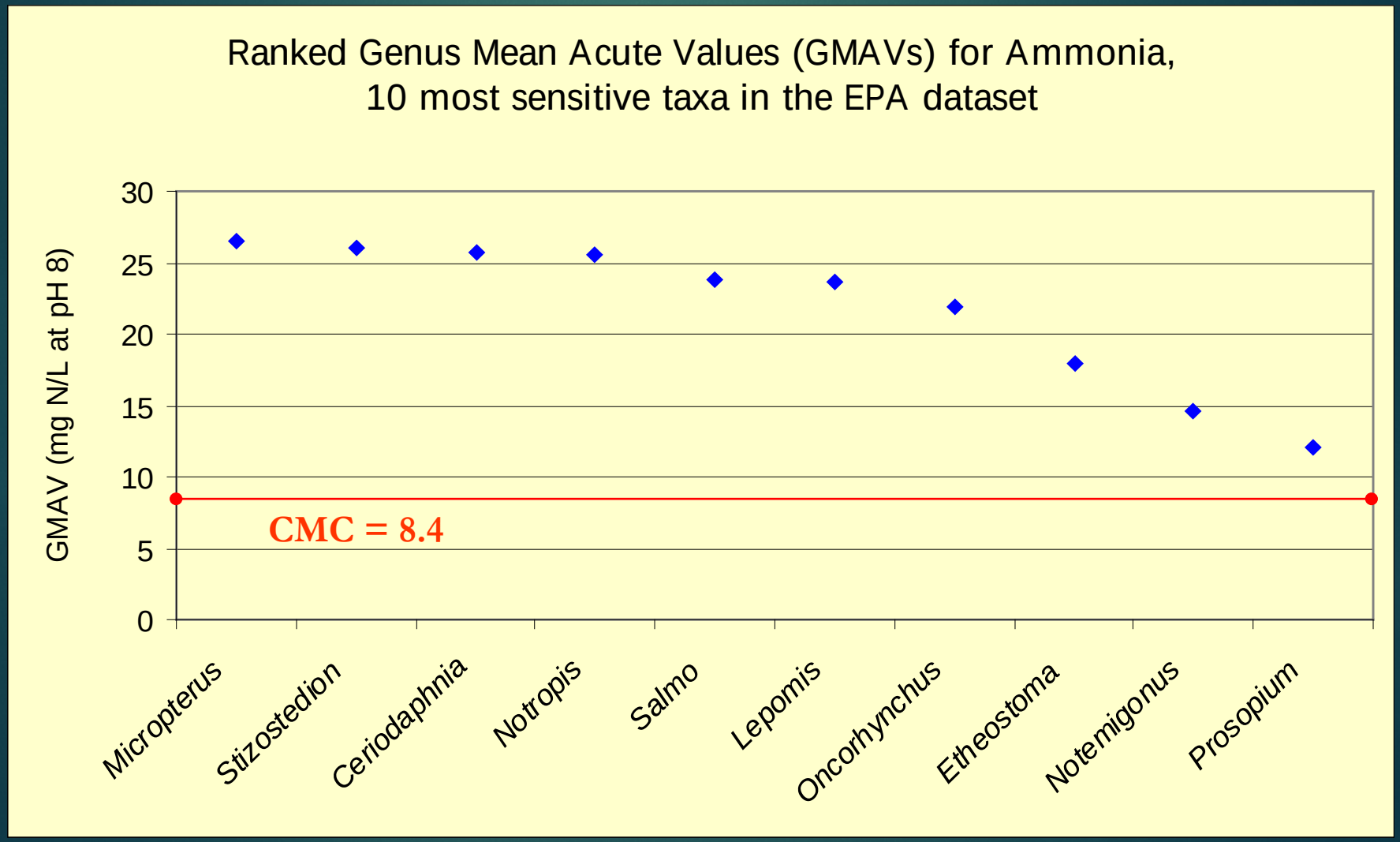
Is a Mussel a Fish?

- Comparison of fathead minnow to 5 mussel species in acute exposures to 10 chemicals (Raimondo et al. 2016)



Freshwater Mussel Sensitivity

Is a Mussel a Fish?



Freshwater Mussel Sensitivity

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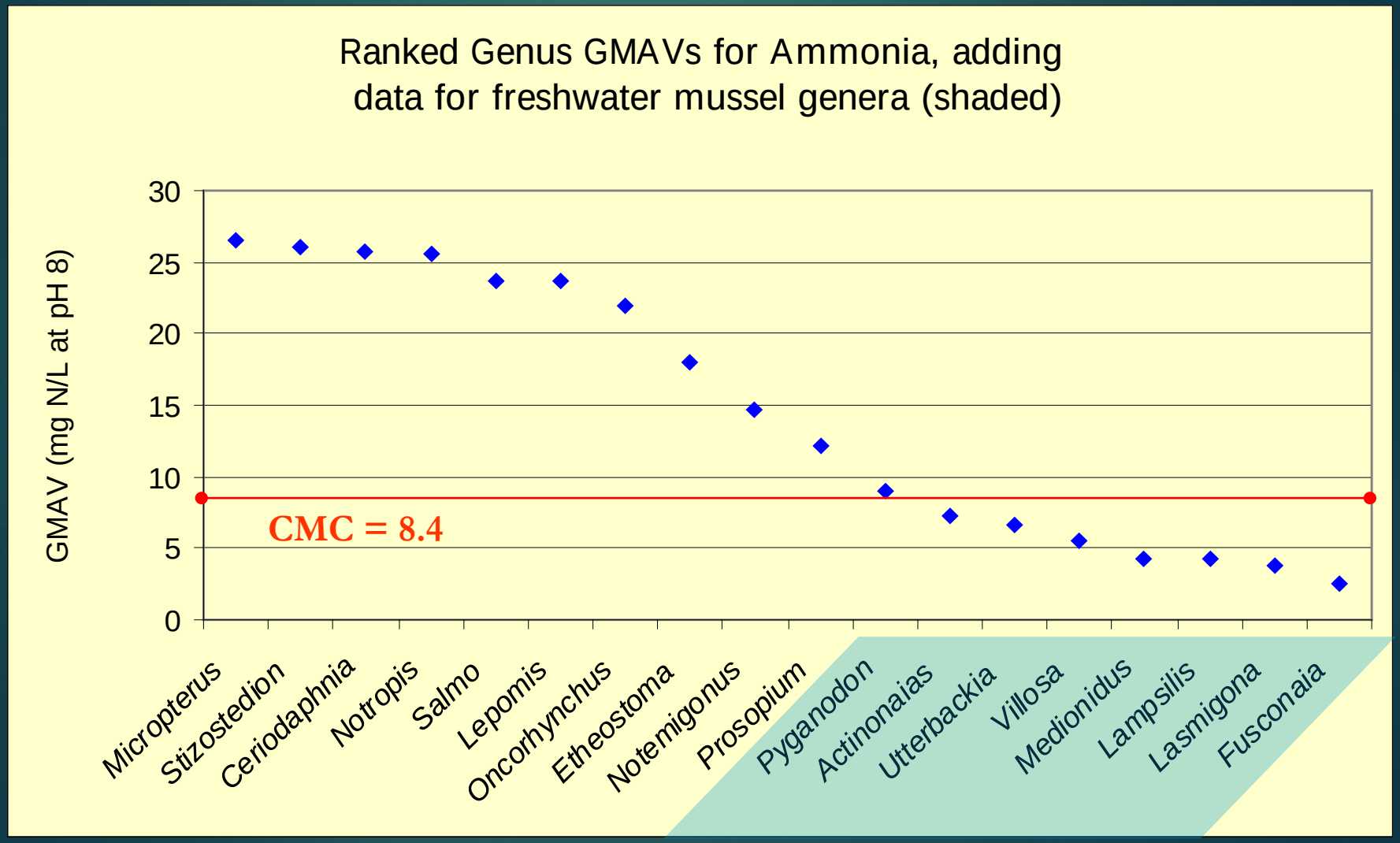


Freshwater Mussel Sensitivity

WQC GMAV Summary

Criteria	GMAVs in WQC	GMAVs w/Mussels	Lowest Mussel Rank (1 = Most Sensitive)
Ammonia	34	42	1 (1, 2, 3, 4)
Atrazine (draft)	17	21	15
Chlorine	28	35	2 (2, 5, 9, 14)
Chlorpyrifos	15	16	13
Copper	43	57	1 (1, 2, 3, 5)
Diazinon	20	21	21
Mercury	29	30	10
Nonylphenol	15	21	13
PCP	32	37	17

State of the Science

Improving Benchmarks

- What does it mean?

United States Environmental Protection Agency | Office of Water 4304T | EPA-822-R-07-001 February 2007

EPA

AQUATIC LIFE AMBIENT FRESHWATER QUALITY CRITERIA - COPPER

2007 Revision

United States Environmental Protection Agency | Office of Water 4304T | EPA-820-R-12-007 April 2012

EPA

AQUATIC LIFE AMBIENT WATER QUALITY CRITERIA FOR CARBARYL - 2012

EPA | United States Environmental Protection Agency | Office of Water 4304T | EPA 822-R-13-001 April 2013

AQUATIC LIFE AMBIENT WATER QUALITY CRITERIA FOR AMMONIA – FRESHWATER 2013

EPA | United States Environmental Protection Agency | Office of Water 4304T | EPA 822-R-16-006 June 2016

Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016

EPA | United States Environmental Protection Agency | Office of Water 4304T | EPA-820-R-16-002 March 2016

AQUATIC LIFE AMBIENT WATER QUALITY CRITERIA CADMIUM - 2016

- More work needed

Problem—Multiple Factors

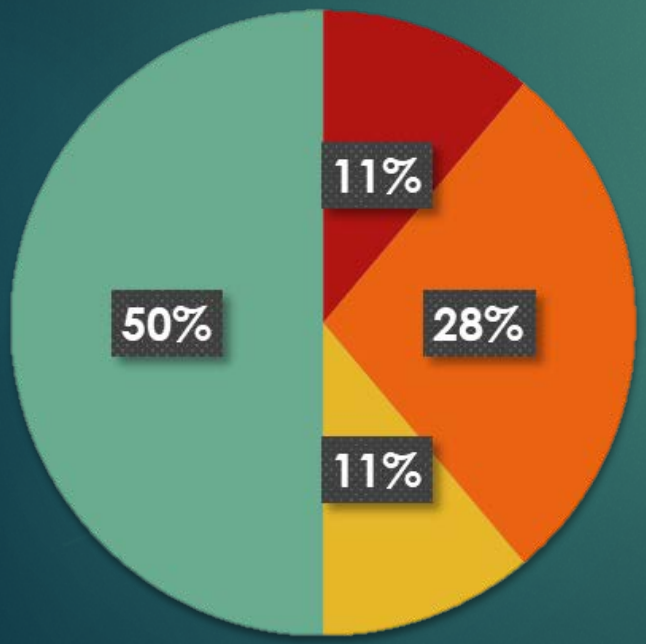
Frequency of Explanation for Unionacean Declines Postulated by Authors of 45 Published Articles (From Strayer and co-workers, 2004)

Cause	Percentage
Pollution, water quality degradation	47
Habitat destruction, alteration	47
Damming and impoundment	33
Introduction of exotic species	29
Hydrologic change	20
Exploitation and harvesting	18
Recruitment failure, lack of fish hosts	13
Watershed alterations	13
Riparian alterations	07
Predation	07

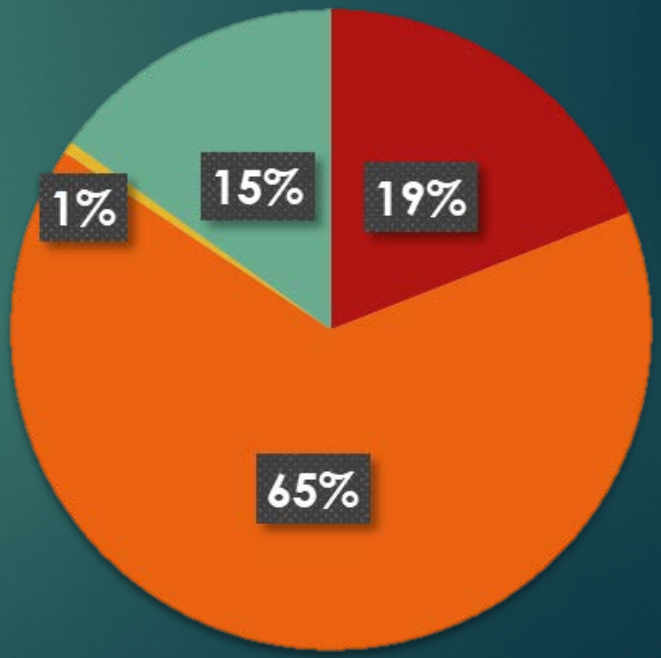
State of the Science

Overall Database Analysis Results: 30 References; 18 Chemicals

Number of Chemicals



Number of Results

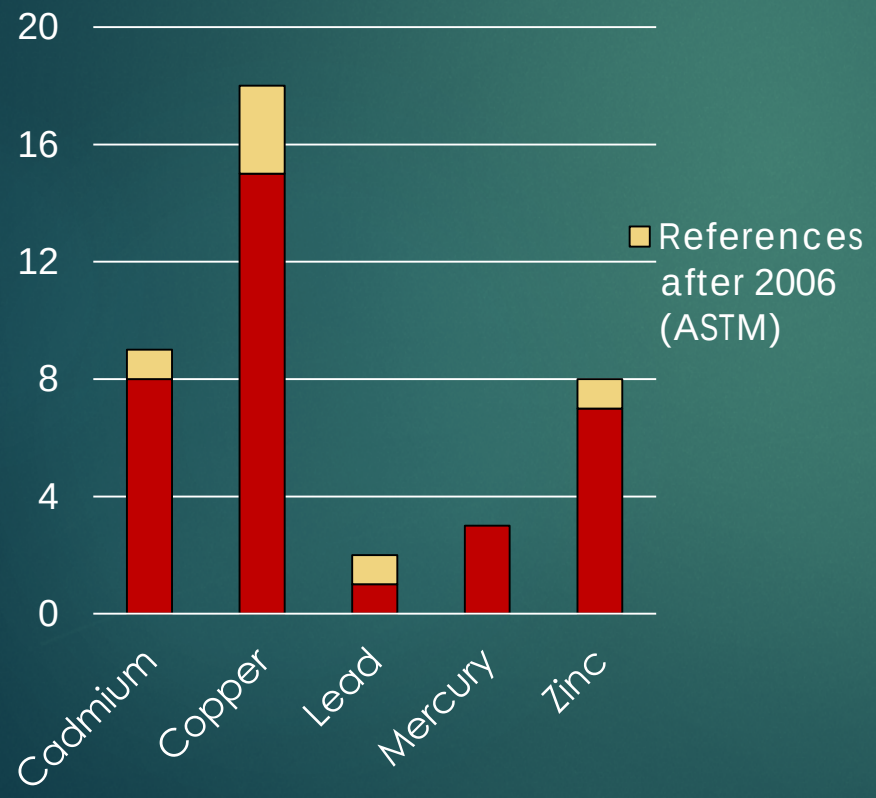


- Toxicants
- Metals
- Ions
- Pesticides

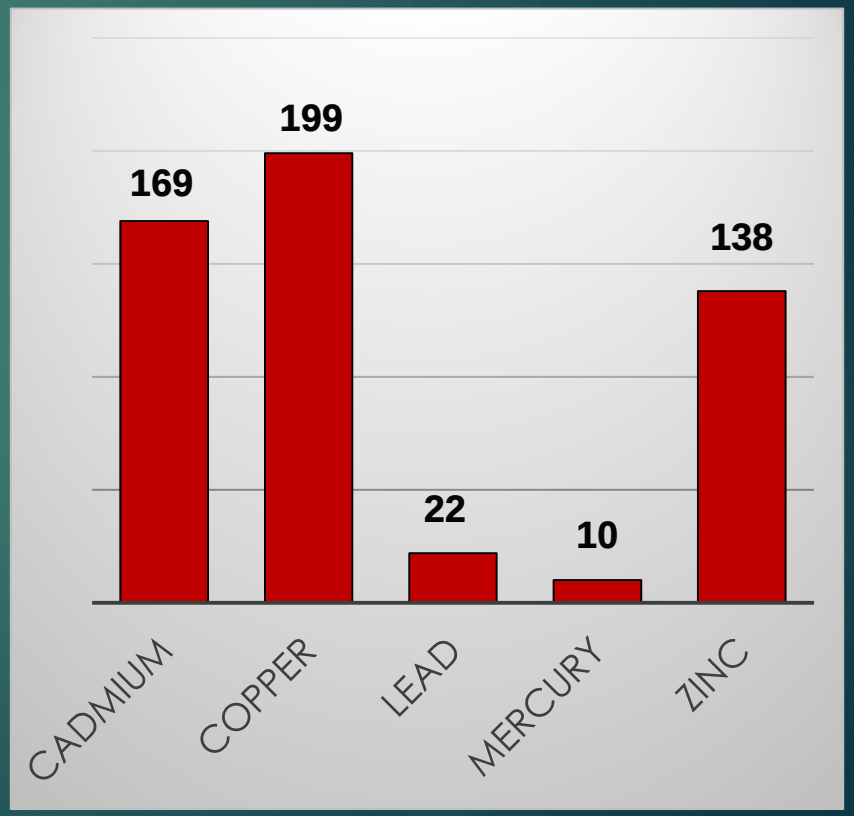
State of the Science

Delving Deeper: Database Results for Metals

Number of References: Metals



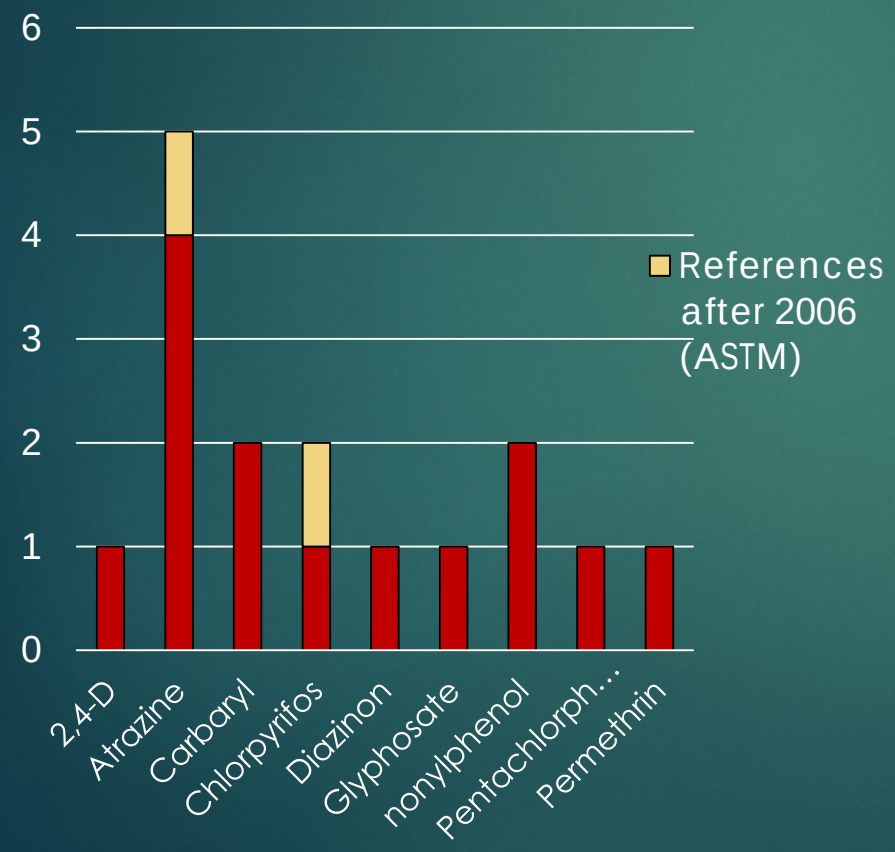
Number of Database Results Per Metal



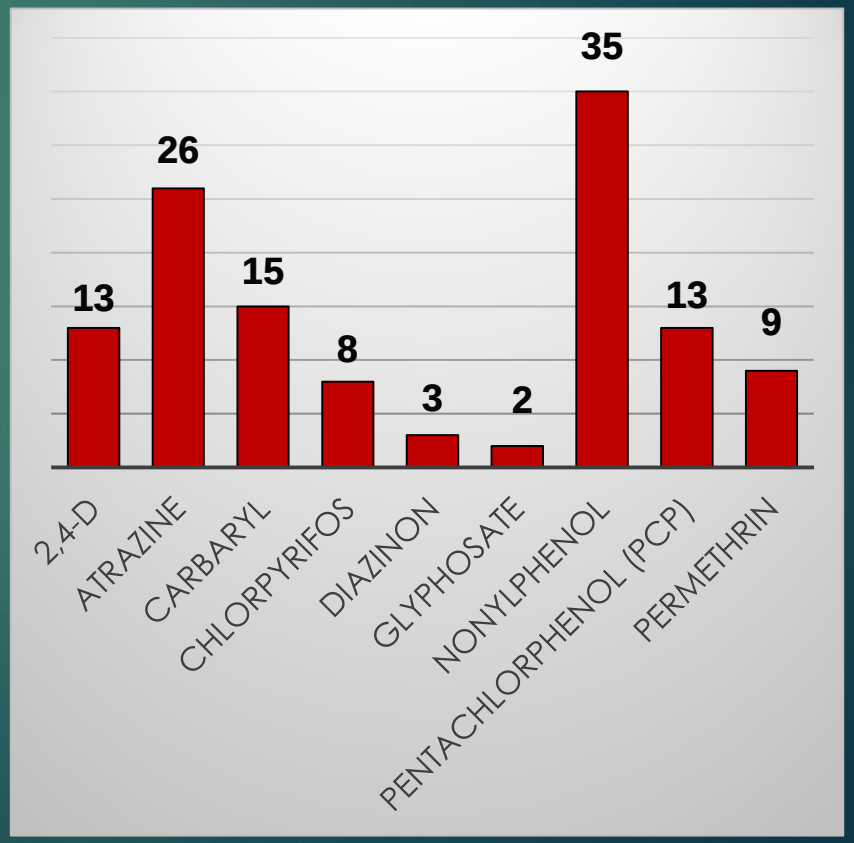
State of the Science

Delving Deeper: Database Results for Pesticides

Number of References: Pesticides



Number of Database Results Per Pesticides



State of the Science

Database Recommendations

- Toxicity results from tests with emerging contaminants need additional representation
  - Major ions
  - Pharmaceuticals (PPCP)
  - Chemicals from pollution events in freshwater
- Toxicity tests should follow ASTM guidelines, and quantitative results are needed
- Funding is needed to maintain mussel toxicity database
  - Raimondo *et al*. 2016 DOI: 10.1002/etc.3245
  - USEPA-Gulf Breeze, FL
  - NCSU

Problem—Multiple Factors

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Recent Mussel Toxicology

Habitat Destruction, Alteration, and Sedimentation

- Erosion and turbidity control (2009 USEPA 280 NTU)

Recent Mussel Toxicology

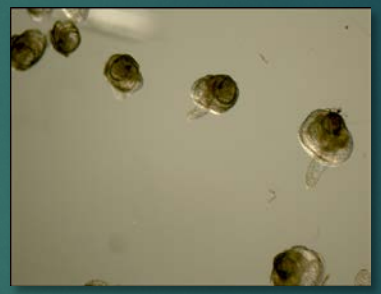
Pollution, water quality degradation

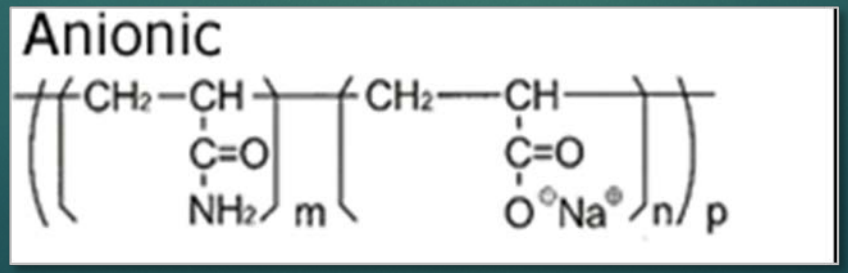
- Anionic polyacrylamide toxicity
  - Reduces turbidity by as much as 90%
  - Flocculating agent
  - Unregulated chemical
  - Formed through polymerization of acrylamide

Recent Mussel Toxicology

Anionic Polyacrylamide Toxicity

- LC50s for freshwater species
  - Daphnia (48h LC50): 152 mg/L
  - Fathead minnow (96h LC50): > 100 mg/L
  - Mussels (24 – 96h LC50): 127 to >1000 mg/L

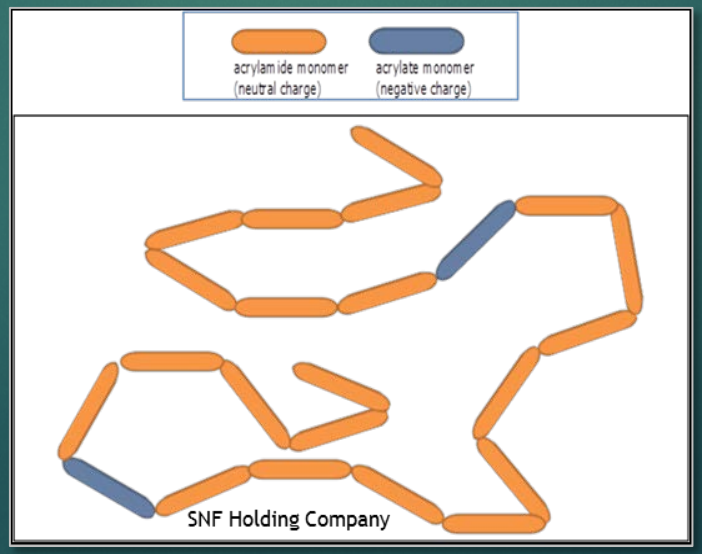




Recent Mussel Toxicology

Anionic Polyacrylamide Toxicity Summary

- 24 to 126- fold margin of safety
- Greater toxicity for PAM's with high charge density and low molecular weight
- Species sensitivity differences
- Life stage sensitivity differences



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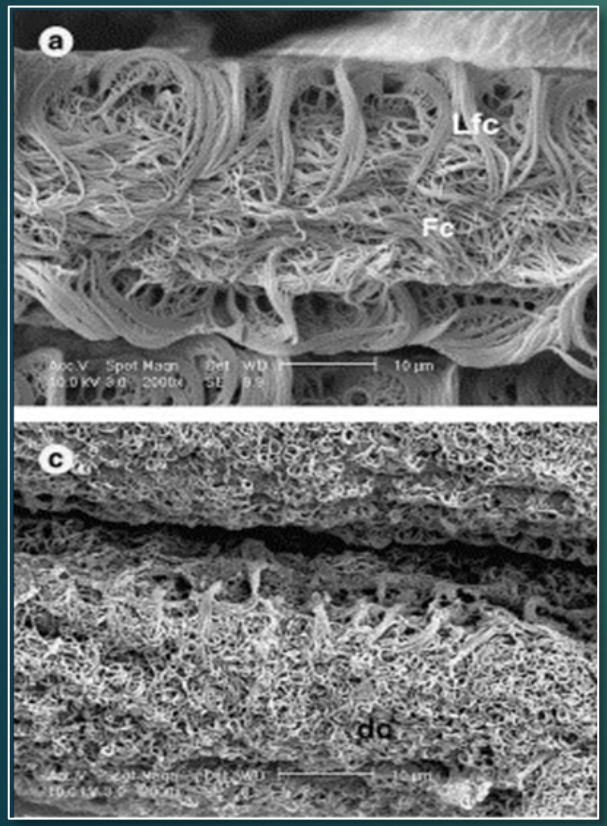
Recent Mussel Toxicology

Habitat destruction, alteration, and sedimentation

- Is the 280 NTU mandate protective?



Cheung and Shin 2005

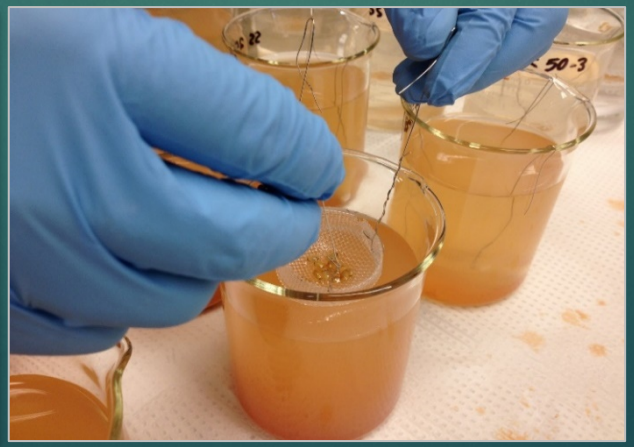
Recent Mussel Toxicology

Sediment Toxicity

- Sediment exposure
  - 96 h & 20 d exposure
  - 3 sediment conditions
  - 5 turbidity treatments

Settled Sediment

Suspended Sediment



Flocculated Sediment

Recent Mussel Toxicology

Sediment Toxicity Results

- No effect of turbidity treatment or exposure condition on survival
- Anionic PAM does not appear to elicit additional lethal or sub-lethal toxicity
- Improved management and regulatory decision

State of the Science

Laboratory Summary

- Developed standard toxicity testing methodology
- Data should be useful for water quality criteria and standards, permit limits, clean-up values, toxicity reference values
- Data show that mussels routinely rank among the more sensitive organisms, but not always
- Water quality benchmarks for mussels are scarce, but recent mussel toxicity data are reliable
- Need for a maintained toxicity database

- Bring what we have learned in lab back to field

Research Progress

Mechanistic Field-Based Studies

- Focus on habitat, causes of impairment
- Focus on growth, survival, physiology endpoints

Barnhart, M.C., T.B. Fobian, D.W. Whites, and C.G. Ingersoll, 2007. Mussel silos: Bernoulli Flow Devices for Caging Juvenile Mussels in Rivers. Proceedings of the 5th Biennial Symposium of the Freshwater Mollusk Conservation Society, Little Rock, AR, p. 107.

Research Progress

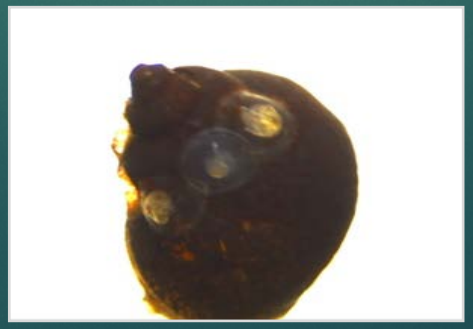
Contaminant Exposure Routes

- Water
  - Glochidia, encysted, juveniles and adults
- Sediments
  - Juveniles and adults
- Pore water
  - Juveniles and adults
- Fish hosts
  - glochidia, encysted
- Diet
  - juveniles and adults

Future Directions

Mollusk Toxicology Needs

- Develop ASTM standard for sediment and effluent tests
- Advance chronic and partial life-cycle test methods
- Refinement of gastropod toxicity test methods
- Advance health assessment tools and biomarkers
- Continue research on toxicants for WQC (e.g., Cl, SO4)
- Influence of temperature and climate change on chemical toxicity



Overall Summary

Water Quality Constituents Relevant to Freshwater Mussels: Progress and State of the Science

- Continuing to make progress in laboratory based toxicity testing
- Mechanistic field based studies
- Mitigation and management decision making based on toxicity data

- Need for a maintained toxicity database

Acknowledgements

- Tom Augspurger, M. Chris Barnhart, Robert B. Bringolf, Patricia L. Gillis, Teresa J. Newton, Ning Wang, Edward J. Hammer, Christopher G. Ingersoll
- NCSU database: Rebecca Bryan

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