



Explanations for the disconnection between the laboratory and field in understanding the effects of metals on aquatic insects



David Buchwalter, Associate Professor  
Department of Biological Sciences  
North Carolina State University, USA

# Overview – major themes

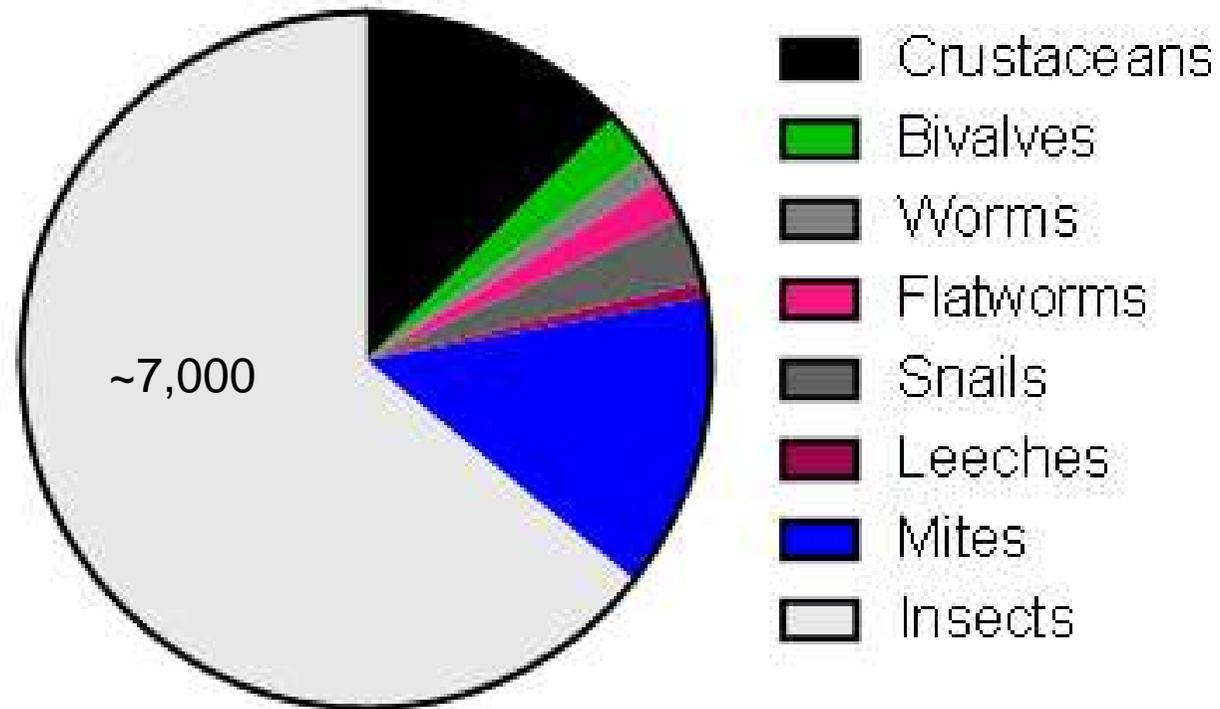


1. Representation of taxa
2. Adequacy of testing approaches
3. Potential paths forward

## Minimum requirements – freshwater – 8 families

- Salmonidae (Class Osteichthyes)
- A second family (Class Osteichthyes) (preferably commercially or recreationally important warm water species)
- A third family in the phylum Chordata
- A planktonic crustacean
- A benthic crustacean
- An insect
- A family in a phylum other than Arthropoda or Chordata
- A family in any order of insect or any phylum not already represented

## Invertebrate Freshwater Diversity in North America



Total=11,000 - 15,000

## In practice.....

- Species (3) from the genus *Chironomus* have been the “go-to” test organism to represent ~7,000 insects
  - Based on ease of lab culture
  - Generally extremely tolerant
  - Inadequately protective of sensitive groups (e.g. mayflies)
  - *Daphnia* more often represent “sensitive” inverts in species sensitivity distributions



# Two Sides of the Clean Water Act Coin

Develop WQC to protect communities



Monitor communities in nature

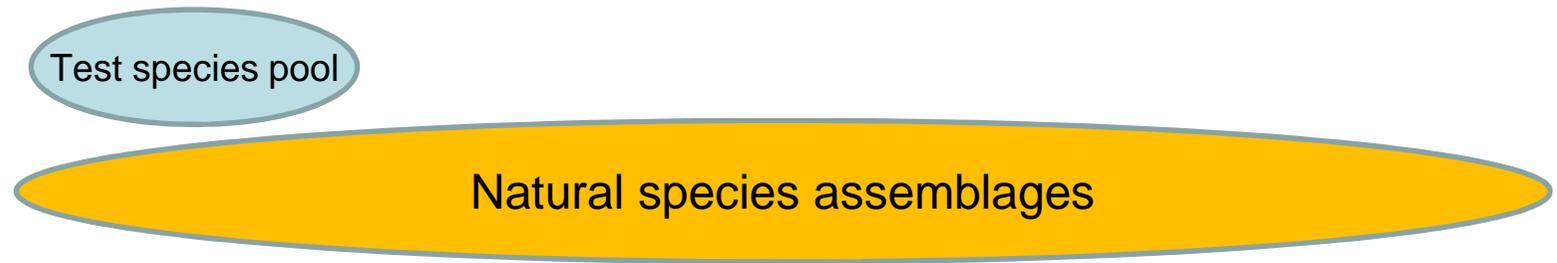
Test species pool

Natural species assemblages





# For metals.....



.....there is a fair amount of toxicity data for insects

Species	Common name	Hardness (CaCO <sub>3</sub> ) (mg/L)	LC <sub>50</sub> (ug/L)	References
<i>Arctopsyche sp.</i>	Caddisfly	30	<b>467</b>	Windward, 2002
<i>Aedes aegypti</i>	Mosquito	38	<b>16,500</b>	Rayms-Keller et al., 1998.
<i>Baetis tricaudatus</i>	Mayfly	156	<b>1,160</b>	Irving et al. 2003
<i>Baetis rhodani</i>	Mayfly	50	<b>2,500</b> (pH = 7.0)	Gerhart, 1992
<i>Baetis rhodani</i>	Mayfly	50	<b>1,000</b> (pH = 5.0)	Gerhart, 1992
<i>Chironomus riparius</i> (2 <sup>nd</sup> instar)	Midge	105	<b>13,000</b>	Williams et al., 1986
<i>Chironomus riparius</i> (4 <sup>th</sup> instar)	Midge	152	<b>300,000</b>	Williams et al. 1986
<i>Chironomus riparius</i> (4 <sup>th</sup> instar)	Midge	124	<b>140,000</b>	Pascoe et al., 1990
<i>Chironomus tentans</i>	Midge	17	<b>8,000</b>	Suedal et al., 1997
<i>Ephemerella grandis</i>	Mayfly	44	<b>2,000</b>	Warnick & Bell, 1969
<i>Ephemerella grandis</i>	Mayfly	NA	<b>28,000</b>	Clubb et al., 1975
<i>Leptophlebia marginata</i>	Mayfly	50	<b>4,400</b> (pH = 7.0)	Gerhart, 1992
<i>Leptophlebia marginata</i>	Mayfly	50	<b>3,600</b> (pH = 5.0)	Gerhart, 1992
<i>Pteronarcella badia</i>	Stonefly	NA	<b>18,000</b>	Clubb et al., 1975
<i>Perlodidae</i>	Stonefly	30	<b>5,130</b>	Evs Environment, 1996
<i>Rhithrogena hageni</i>	Mayfly	40-50	<b>10,500</b>	Brinkman & Johnson, 2008
<i>Rhithrogena sp</i>	Mayfly	21	<b>50</b>	Windward, 2002

# Insects are quite responsive to metals in nature



Will Clements

# Unfortunately.....the lab and field tell us different things

Review

The sensitivity of aquatic insects to divalent metals: A comparative analysis of laboratory and field data

Kevin V. Brix <sup>a,b,\*</sup>, David K. DeForest <sup>c</sup>, William J. Adams <sup>d</sup>

<sup>a</sup> EcoTox 575 Crandon Blvd., #703 Key Biscayne, FL 33149, United States

<sup>b</sup> RSMAS, University of Miami 4600 Rickenback Cswy, Miami, FL 33149, United States

<sup>c</sup> Windward Environmental 200 West Mercer Street, Suite 401 Seattle, WA 98119, United States

<sup>d</sup> Rio Tinto 8315 West 3595 South Magna, UT 84044, United States

## Using Biodynamic Models to Reconcile Differences Between Laboratory Toxicity Tests and Field Biomonitoring with Aquatic Insects

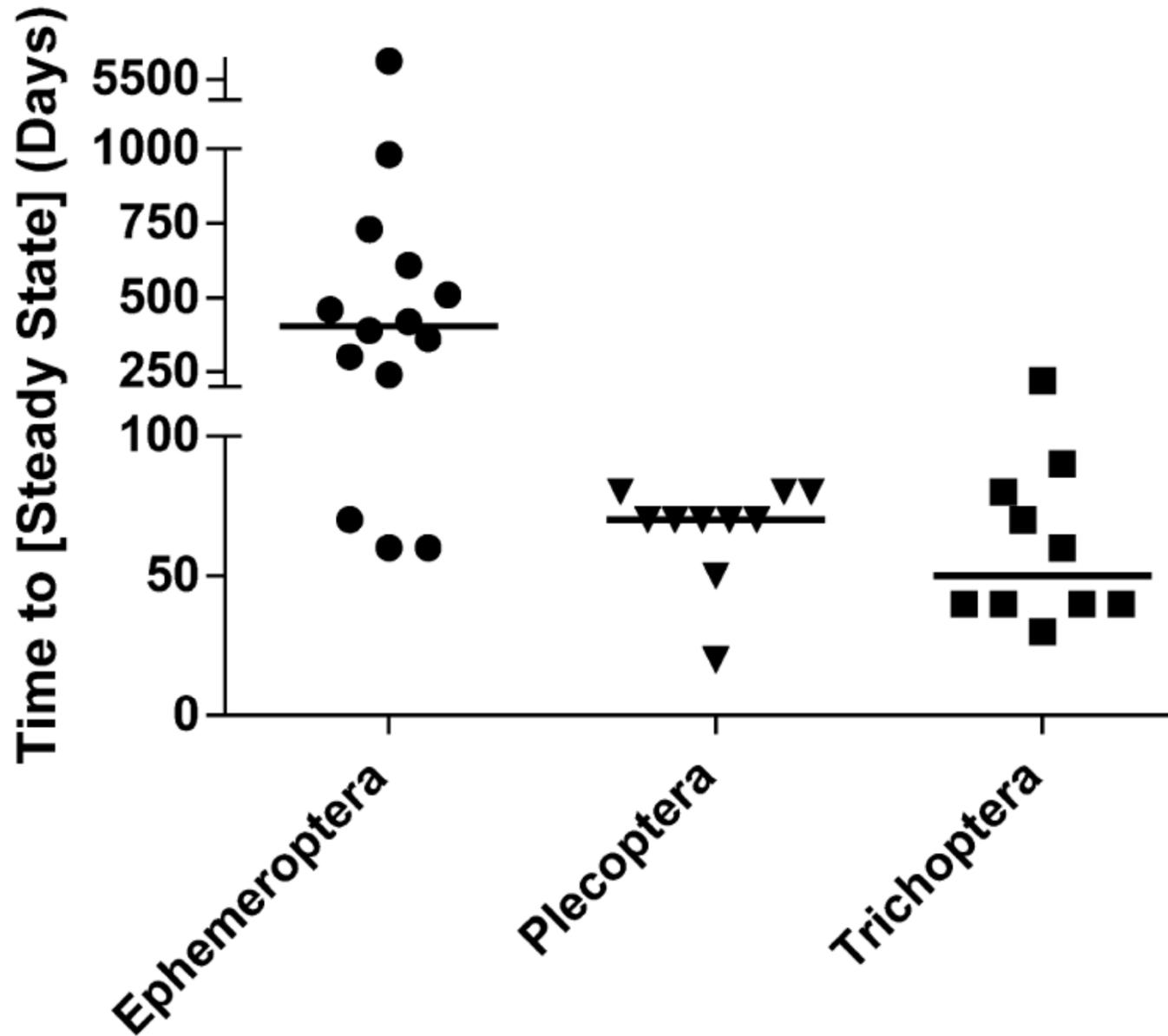
D. B. BUCHWALTER,<sup>\*,†</sup> D. J. CAIN,<sup>‡</sup>  
W. H. CLEMENTS,<sup>§</sup> AND S. N. LUOMA<sup>‡</sup>  
*Department of Environmental and Molecular Toxicology,  
North Carolina State University, Raleigh, North Carolina  
27695, Water Resources Division, U.S. Geological Survey,  
Menlo Park, California 94025, and Department of Fish,  
Wildlife and Conservation Biology, Colorado State University,  
Fort Collins Colorado 80523*

## **Four Reasons Why Traditional Metal Toxicity Testing with Aquatic Insects Is Irrelevant**

Monica D. Poteat and David B. Buchwalter\*

Environmental and Molecular Toxicology Program, Department of Biological Sciences, North Carolina State University, Raleigh, North Carolina 27695, United States

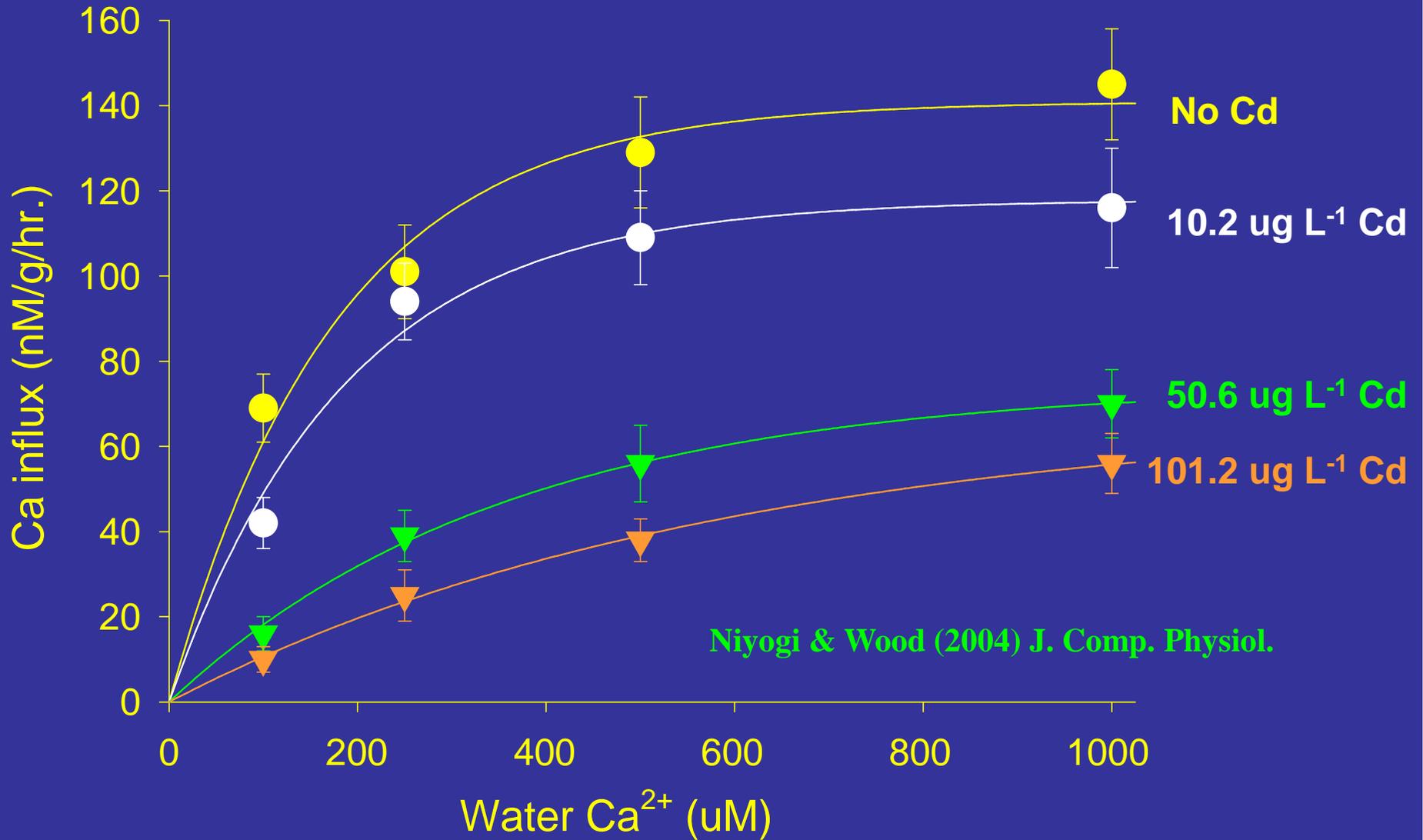
# Reason 1: Time

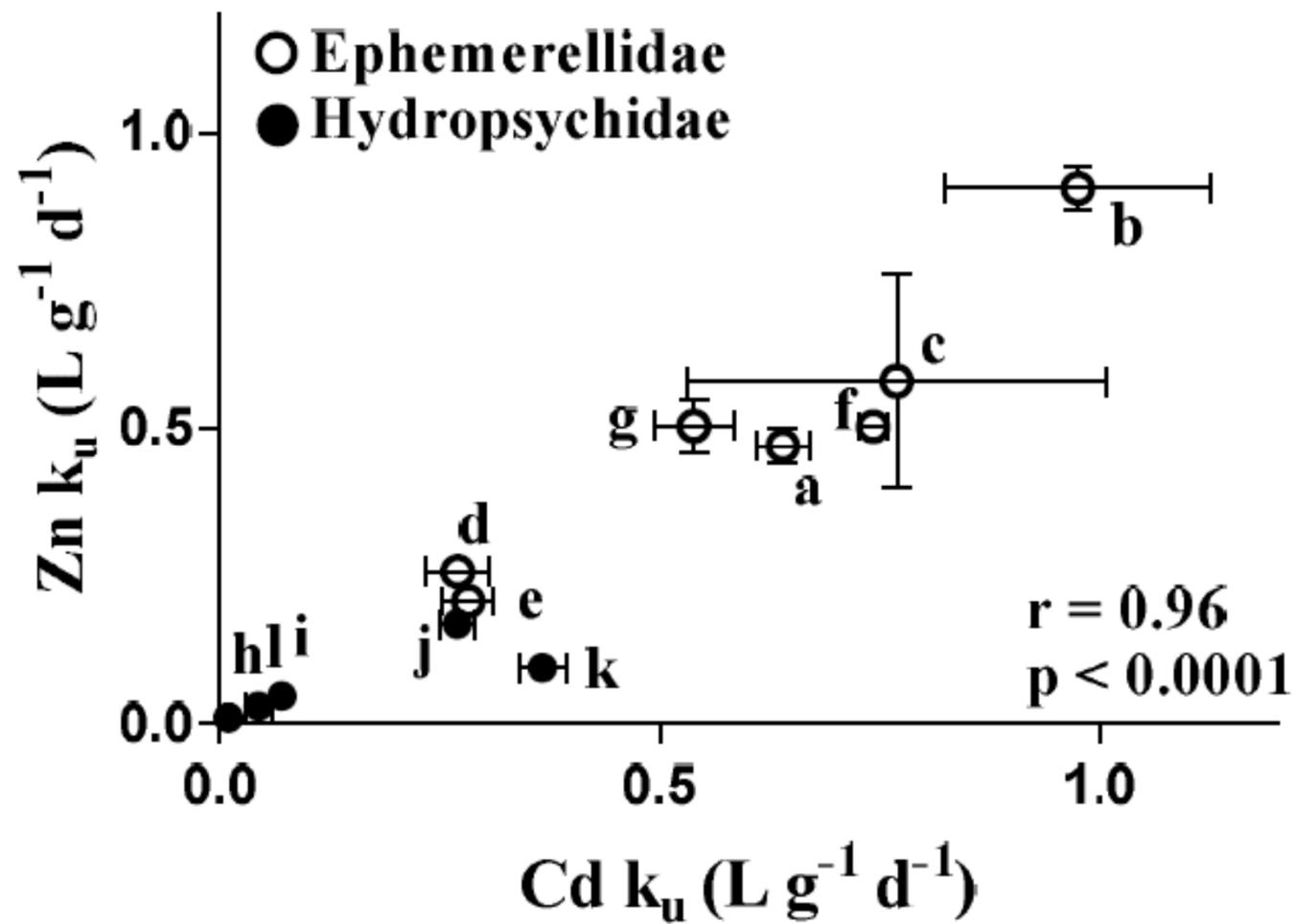


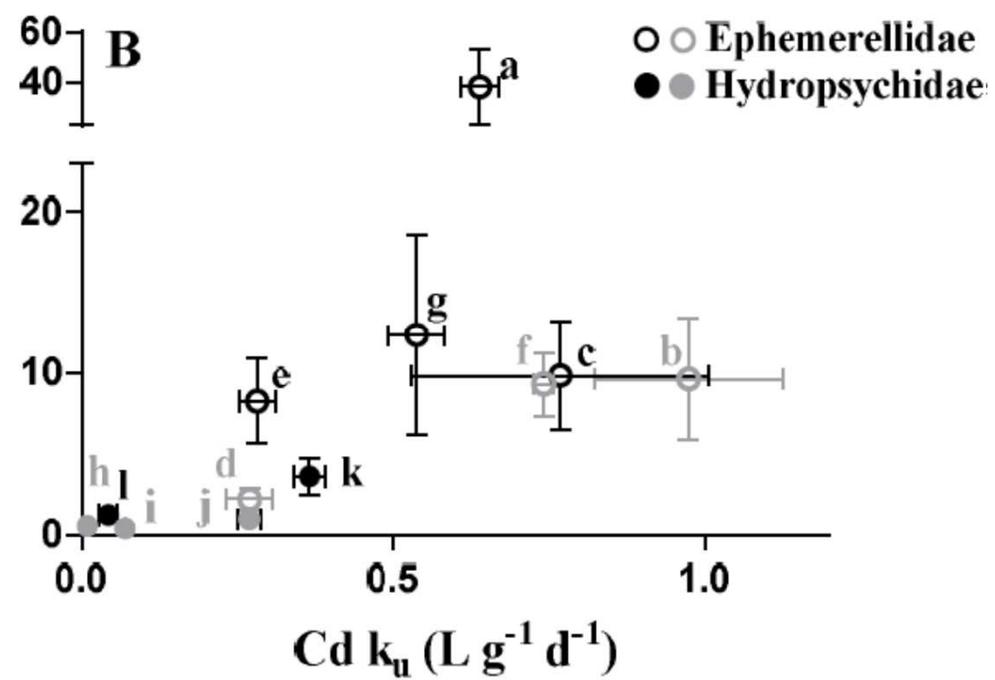
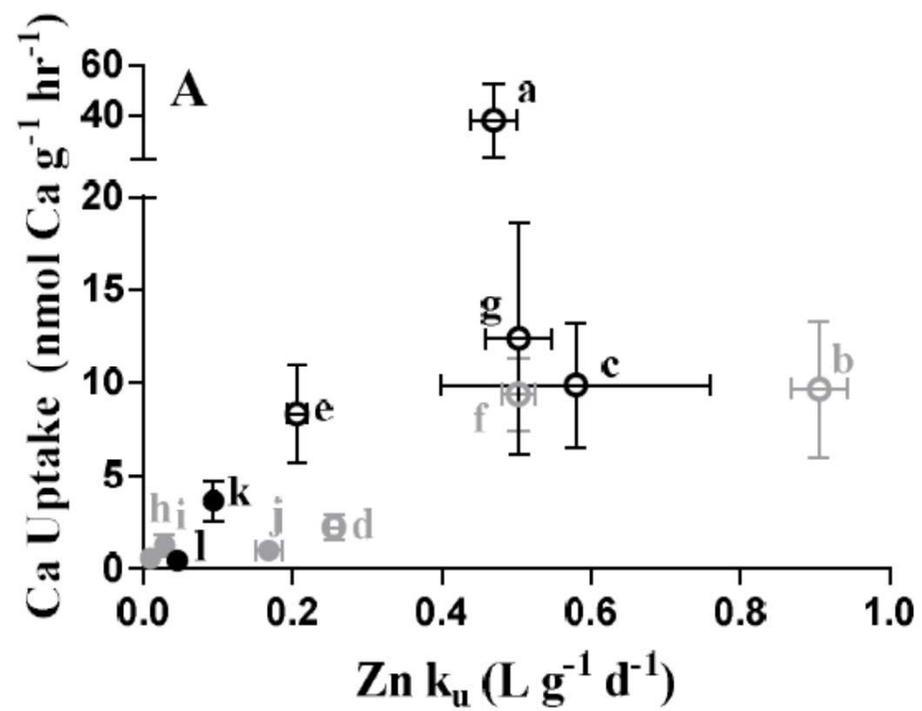
Reason 2: Mechanisms of toxicity:  
(limited evidence for metals causing  
ionoregulatory disturbance in insects)

- In fish and crustaceans, there is good evidence that metals target the transport of physiologically important ions.
- Calcium transport: affected by Zn, Cd, Pb
- Sodium transport: affected by Cu and Ag

## Effect of Cd on Branchial Ca Influx in Rainbow Trout





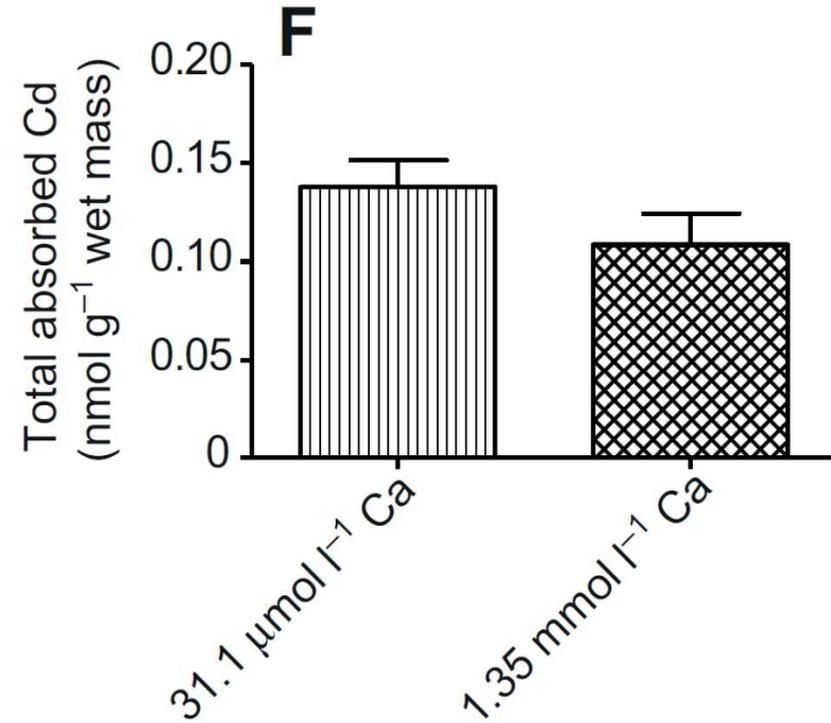
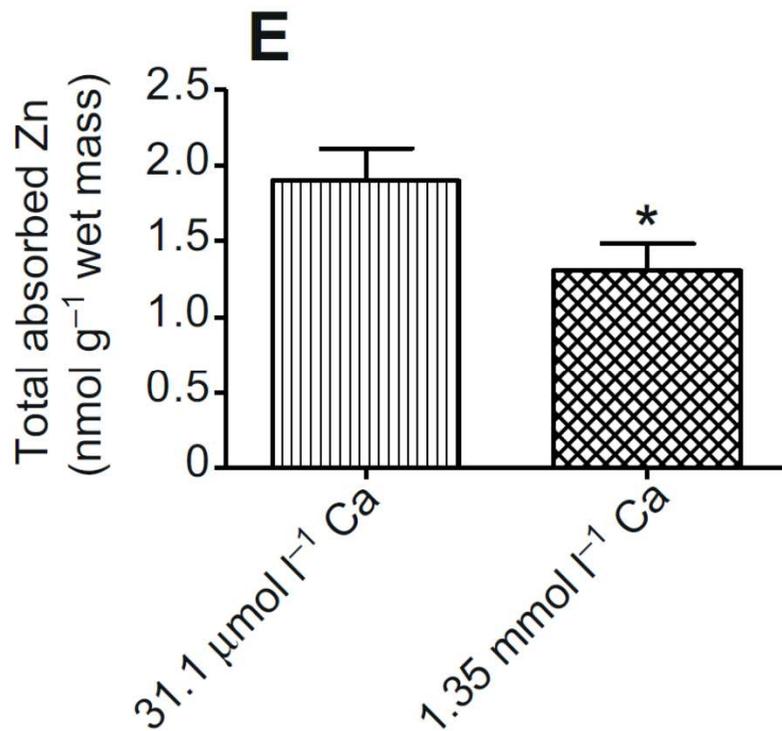


## RESEARCH ARTICLE

### Divalent metal (Ca, Cd, Mn, Zn) uptake and interactions in the aquatic insect *Hydropsyche sparna*

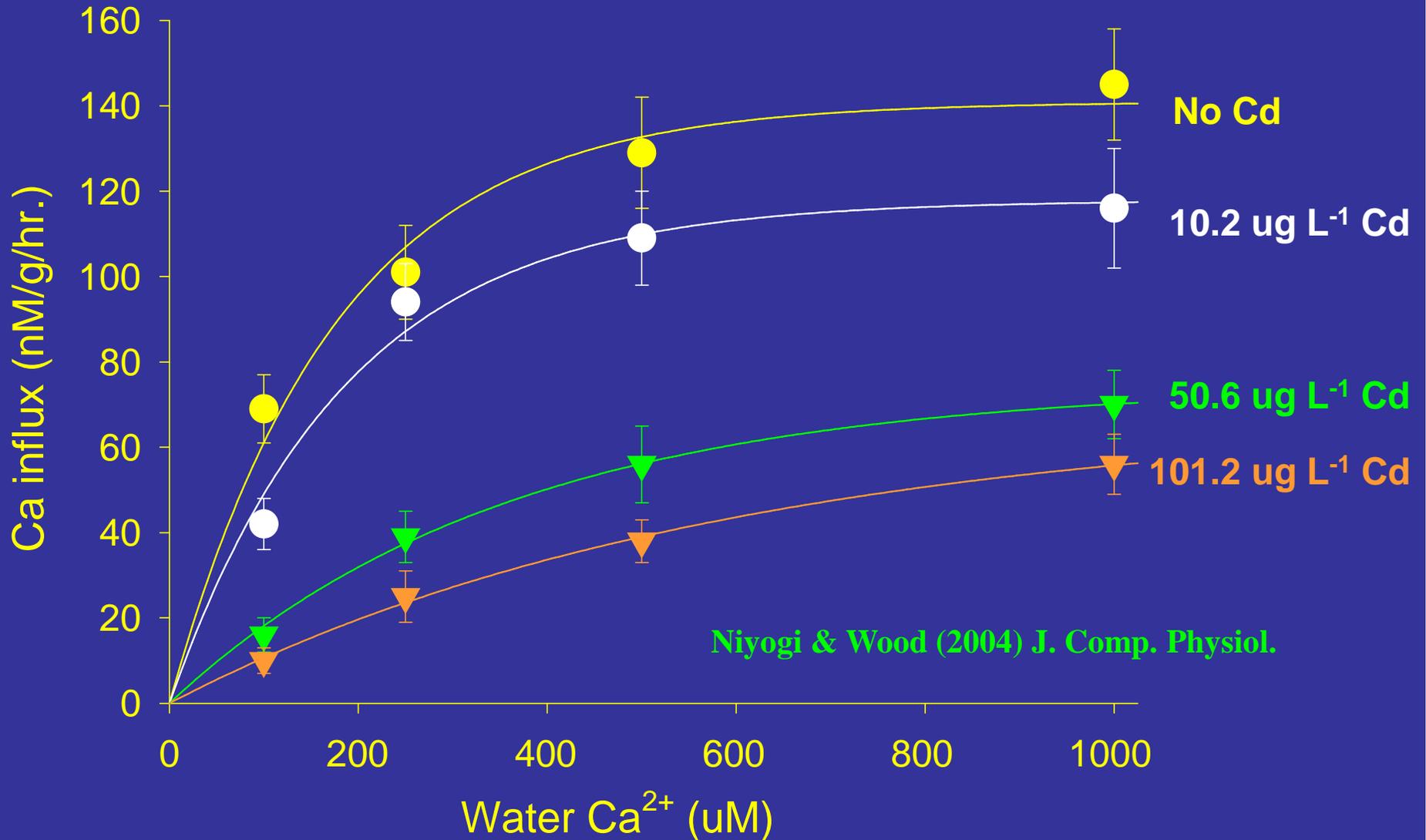
Monica D. Poteat<sup>1</sup>, Mauricio Díaz-Jaramillo<sup>2</sup> and David B. Buchwalter<sup>1,\*</sup>

The Journal of Experimental Biology 215, 1575-1583



A 43-fold increase in calcium concentration had modest (Zn) to no (Cd) effects on metal uptake rates

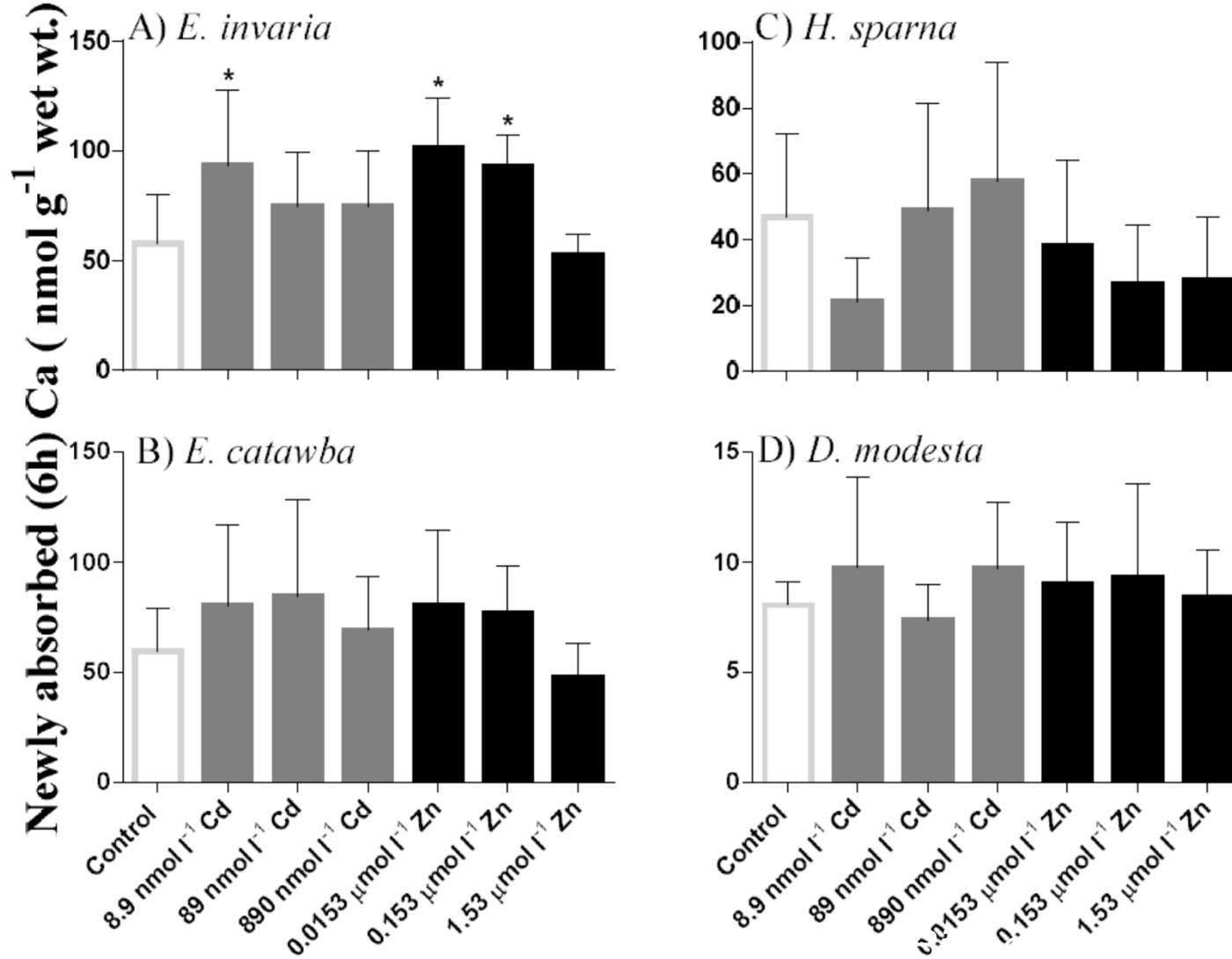
## Effect of Cd on Branchial Ca Influx in Rainbow Trout

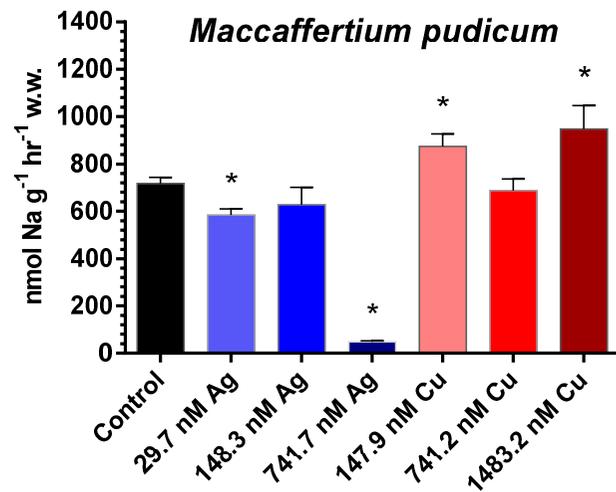
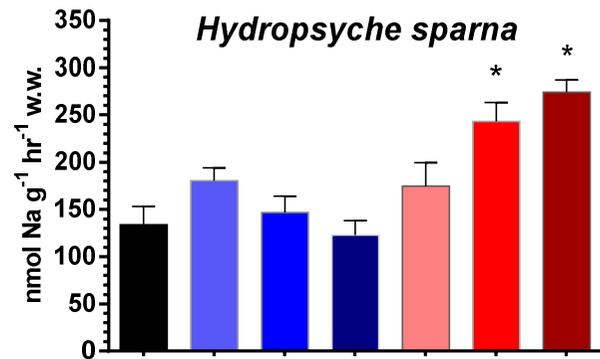
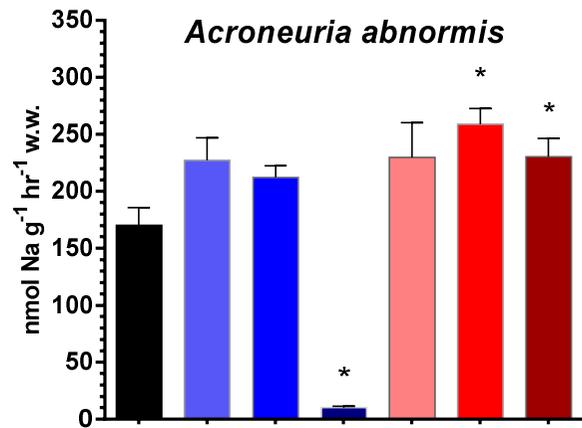


RESEARCH ARTICLE

# Calcium uptake in aquatic insects: influences of phylogeny and metals (Cd and Zn)

Monica D. Poteat and David B. Buchwalter\*





# Silver and copper effects on Na uptake rates

## Reason 3: Dietary exposures are very important

- Aquatic insects may receive the majority of their tissue metal burdens from their food
  - (Martin and Buchwalter, ES&T, 2007).

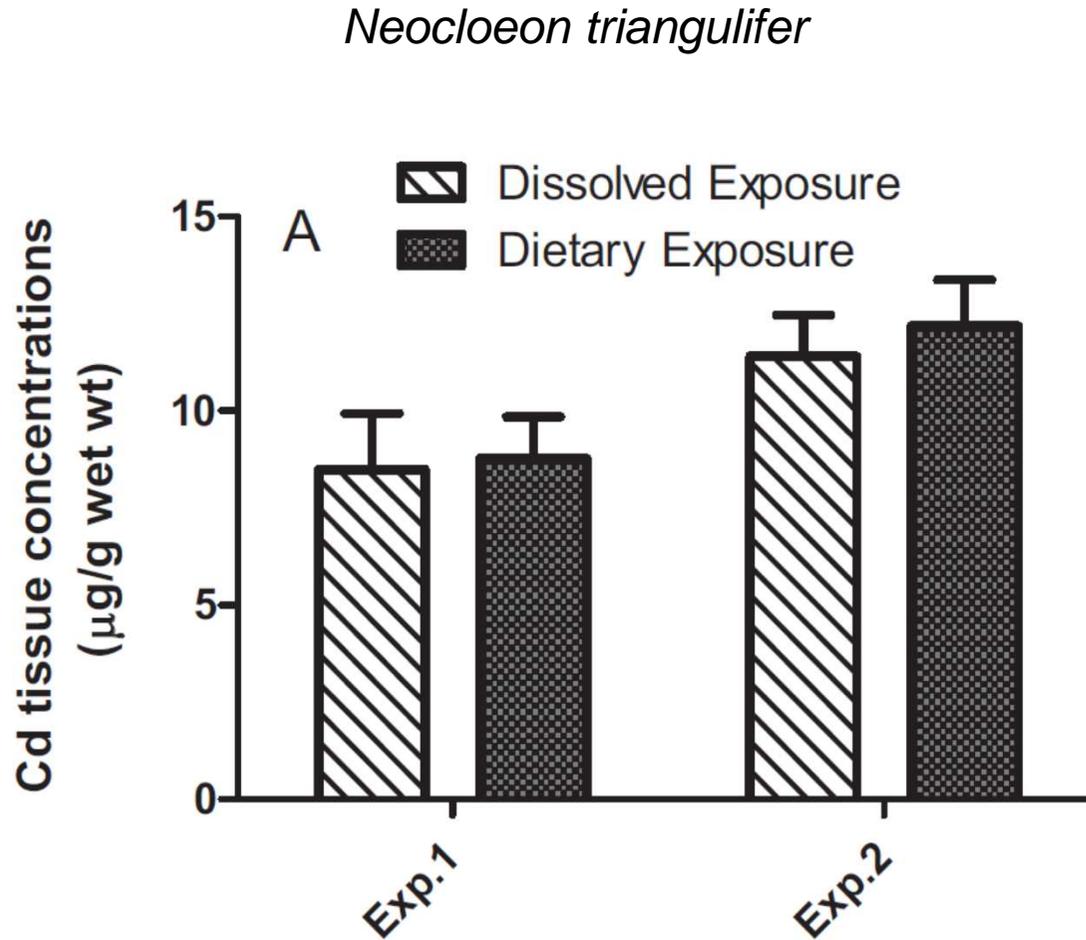
# Periphyton is a major sink for many metals

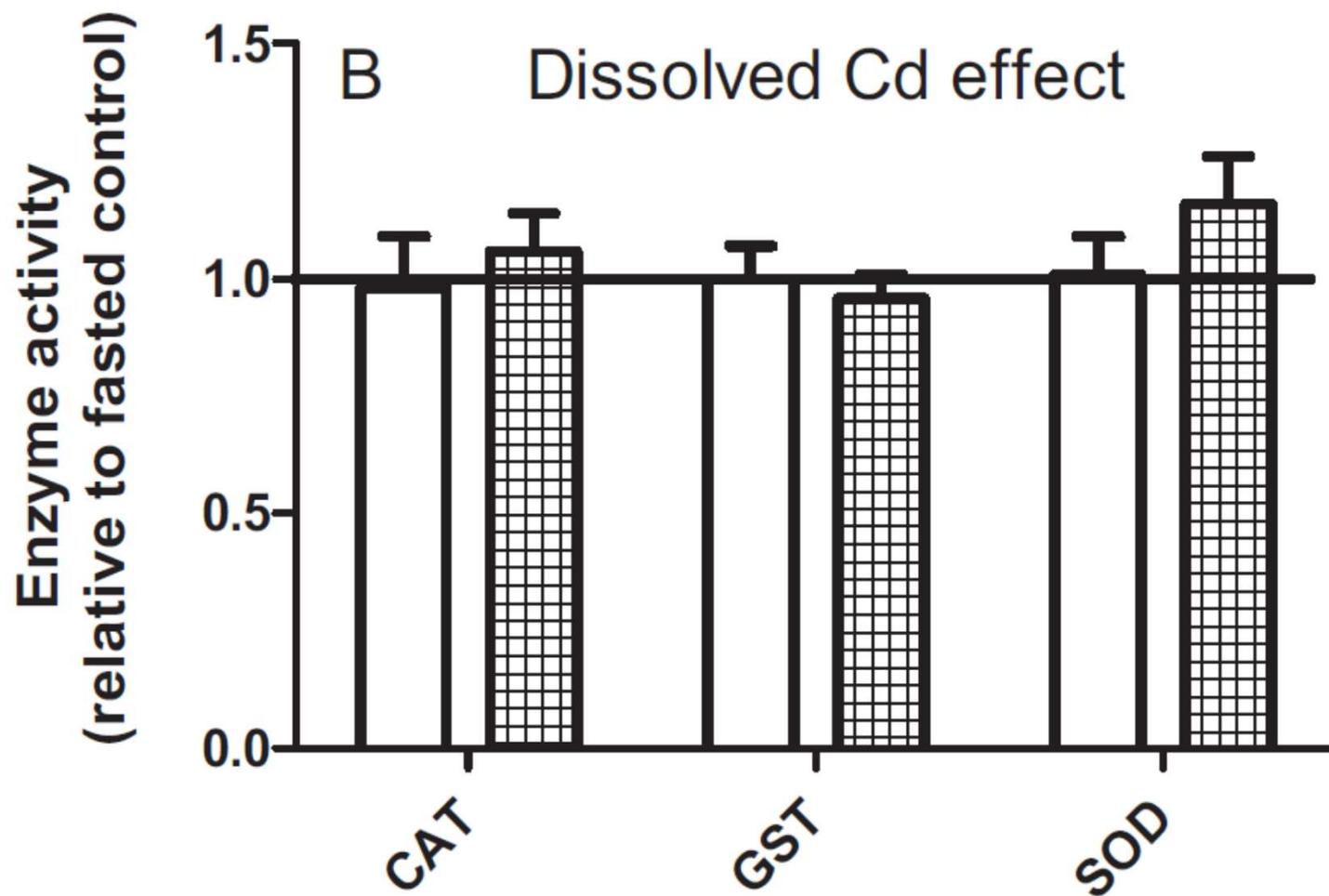
- Cd: Xie et al, Environmental Pollution, 2010
- Zn: Kim et al, Ecotoxicology, 2012
- Se: Conley et al papers

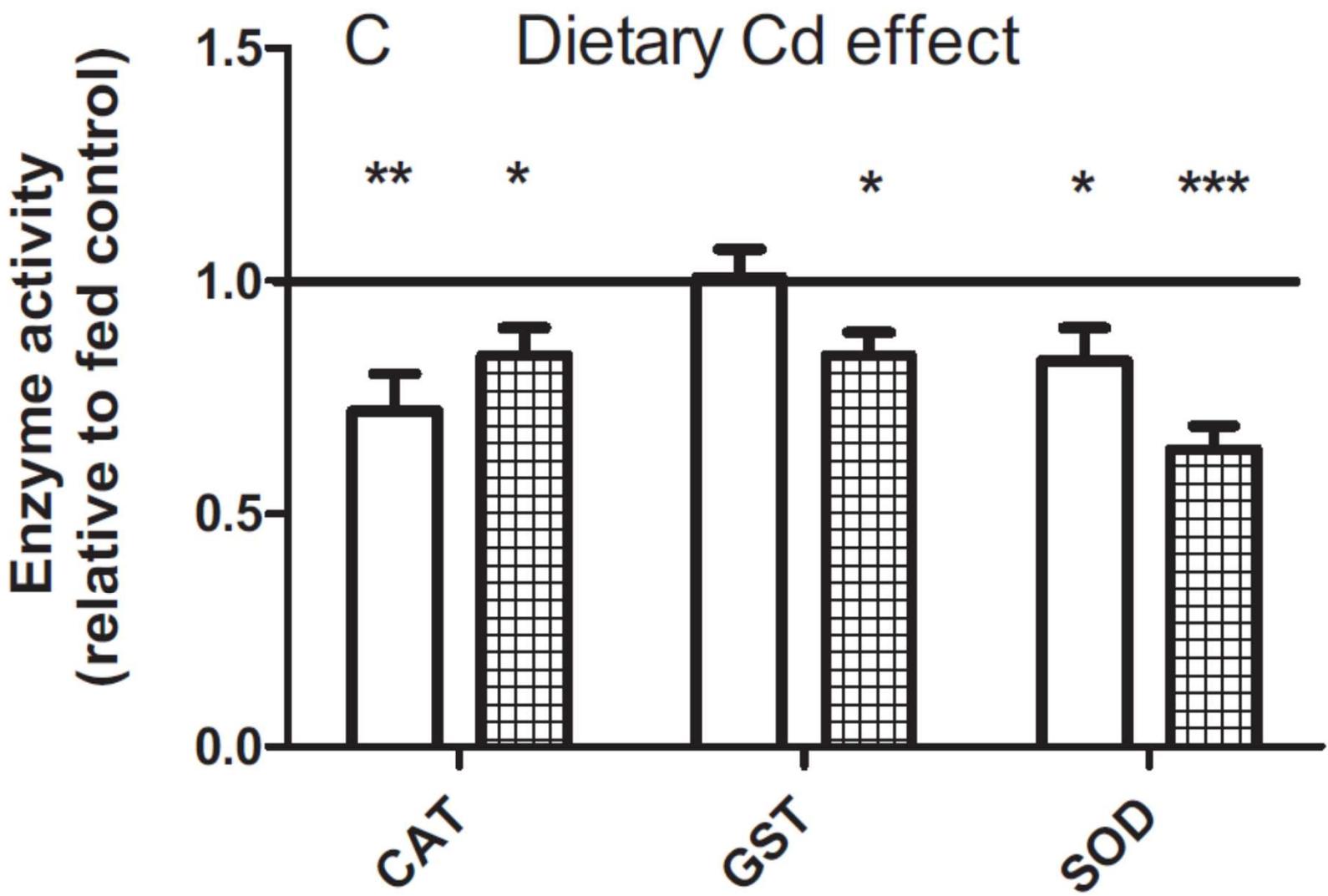
There is scientific consensus: Dietary metal exposures are extremely important in invertebrates

- Luoma
- Fisher
- Rainbow
- Wang
- Cain
- Croteau
- Hare

## 4. Reason: Dietary Exposures are Challenging







# Why traditional toxicity tests fail:

- Test durations are insufficient
- Assumptions of mechanisms of dissolved exposures are not supported
- Dietary routes of exposure are ignored
- Dietary exposures may be more challenging than aqueous exposures to aquatic insects

# Paths forward



# Developing a laboratory model: *Neocloeon triangulifer*



- Parthenogenetic – relatively easy to culture
- Non-diapausing eggs – clonal offspring
- Highly fecund – (temperature and nutrition dependent)

Genetic (gene sequence data and qPCR tools being developed)

# Neocloeon triangulifer use is expanding



# Laboratory approaches

Full life cycle exposures

Ability to incorporate both dissolved and aqueous exposures





- Cadmium
- Zinc
- Selenium

Dietary transfer is very important

-Manganese – lost during molting

-Arsenic – apparently little trophic transfer from periphyton

# Crustaceans are not always good surrogates for insects



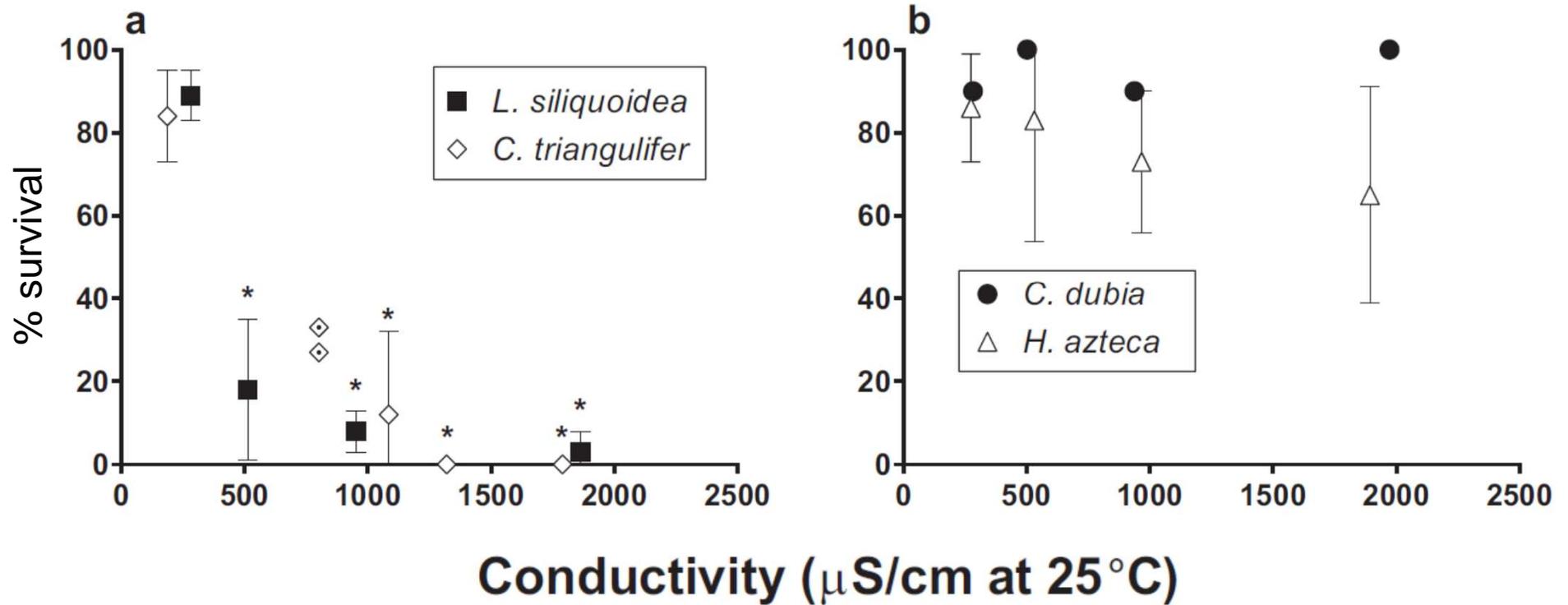
Insects are secondarily aquatic and have different biology/physiology than crustaceans and other aquatic forms with a more proximate marine origin

In some cases, insects may be more sensitive than the crustaceans thought to represent sensitive invertebrates

# Example: Total Dissolved Solids

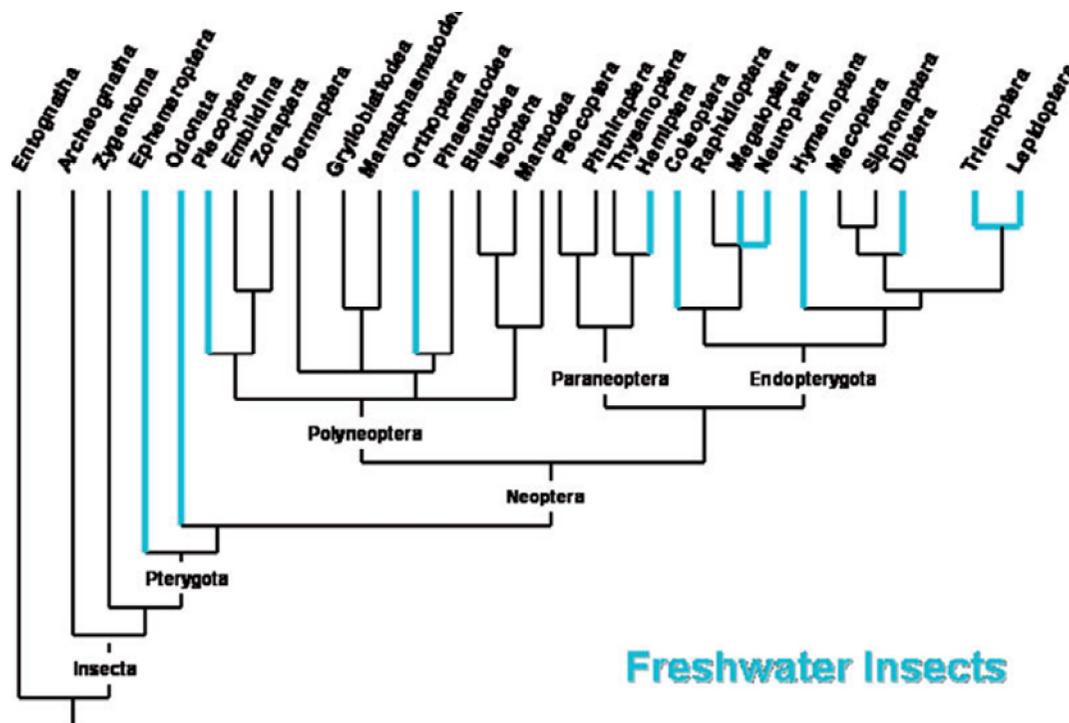
USE OF RECONSTITUTED WATERS TO EVALUATE EFFECTS OF ELEVATED MAJOR IONS ASSOCIATED WITH MOUNTAINTOP COAL MINING ON FRESHWATER INVERTEBRATES

JAMES L. KUNZ,<sup>\*†</sup> JUSTIN M. CONLEY,<sup>‡</sup> DAVID B. BUCHWALTER,<sup>‡</sup> TERESA J. NORBERG-KING,<sup>§</sup> NILE E. KEMBLE,<sup>†</sup>  
NING WANG,<sup>†</sup> and CHRISTOPHER G. INGERSOLL<sup>†</sup>



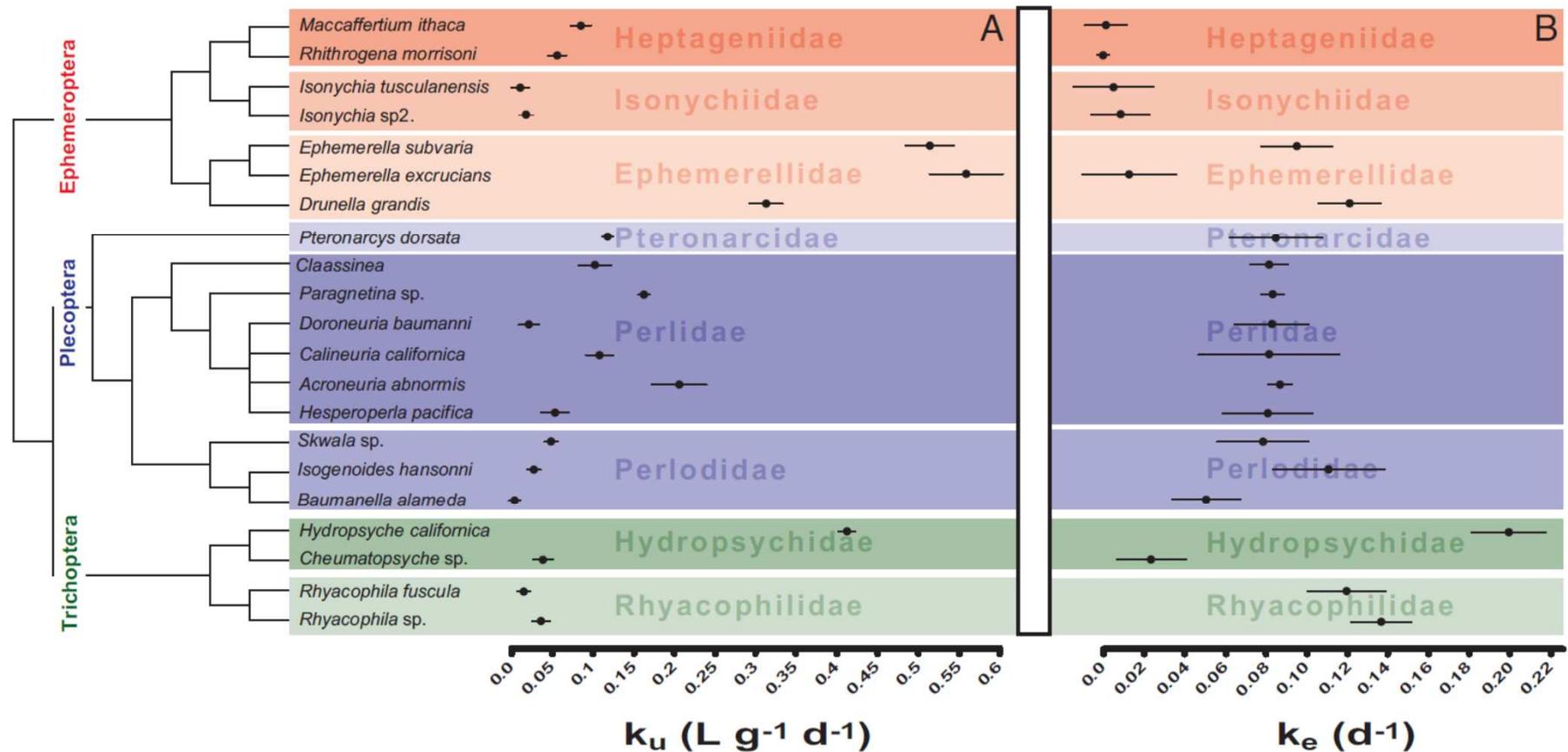
# Phylogenetic signal and extrapolation

Terrestrial ancestry vs marine ancestry



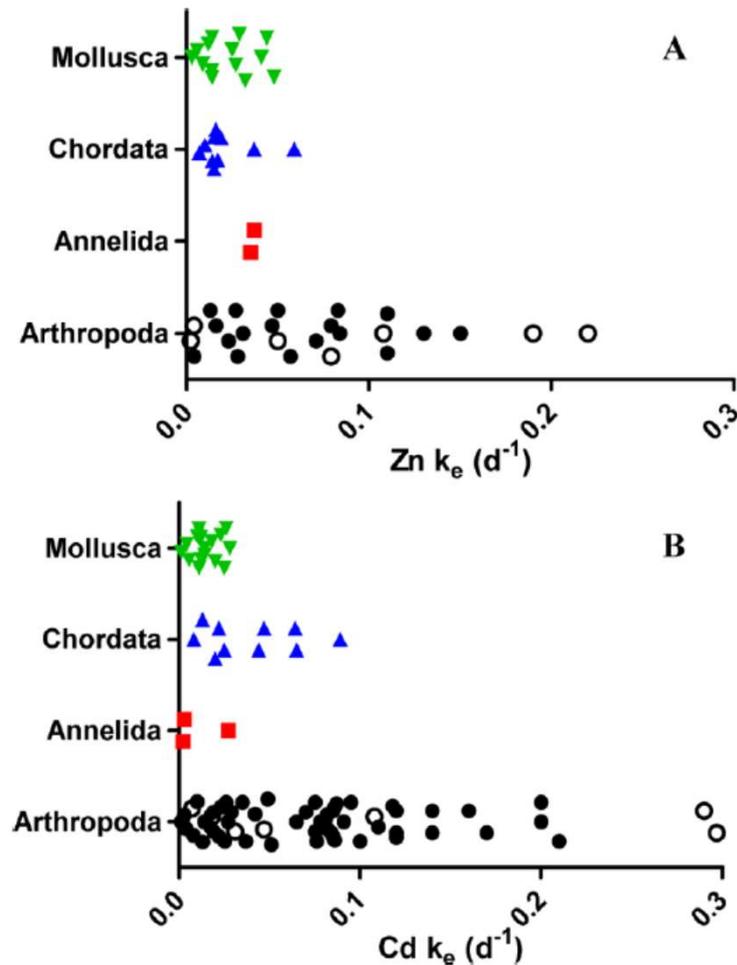
- Species groups have solved the problems of successful transitions from land to water differently
- Species groups have been aquatic for different lengths of time (~375M – mayflies)
- Rates of evolution and speciation vary among species groups
- Massive differences in the physiology of organisms living in the same place (a rock, riffle, stream reach....)

# Phylogenetic signal exists in bioaccumulation data



Aquatic insect ecophysiological traits reveal phylogenetically based differences in dissolved cadmium susceptibility

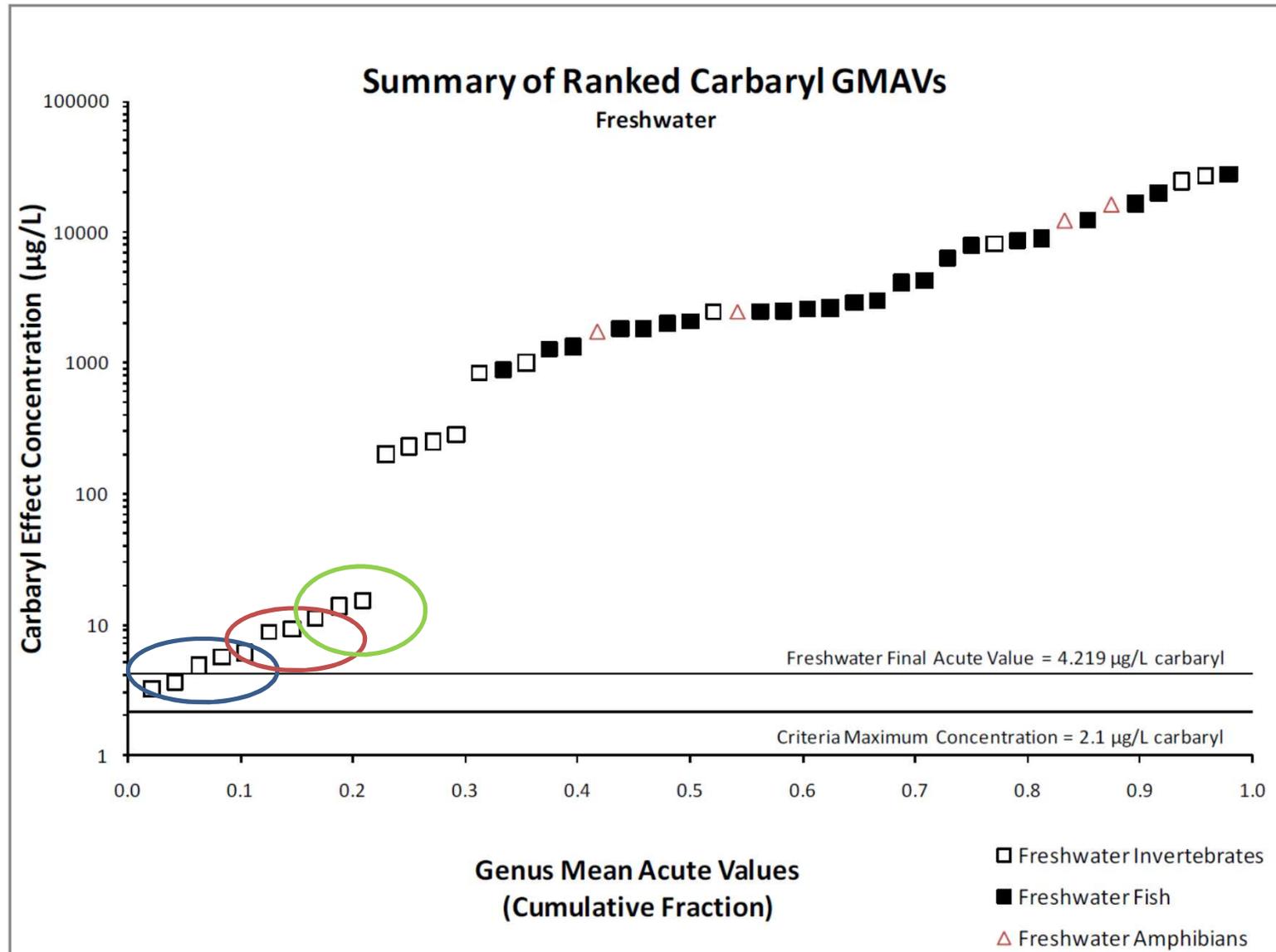
# Phylogenetic signal exists in bioaccumulation data



## Evolutionary Patterns in Trace Metal (Cd and Zn) Efflux Capacity in Aquatic Organisms

Monica D. Potat,<sup>†</sup> Theodore Garland, Jr.,<sup>‡</sup> Nicholas S. Fisher,<sup>§</sup> Wen-Xiong Wang,<sup>||</sup> and David B. Buchwalter<sup>\*†</sup>

# Phylogenetic signal exists in tox data



Can phylogenetics be used to predict or extrapolate toxicity?

Can extrapolations create SSDs that reflect the biodiversity of freshwater ecosystems?

A scenic view of a river flowing through a dense forest. The river is in the foreground, with water rippling over rocks. The banks are covered in lush green trees and vegetation. The sky is bright and clear. The text "Thank you!" is overlaid in the center of the image.

**Thank you!**

## North American Freshwater Biodiversity

- Fish: ~1,200
- Invertebrates: ~10,000 – 15,000
  - Crustacea: ~1384
    - Mysidacea: 4
    - Amphipoda: 150
    - Copepoda: 230
    - Decapoda: 350
    - Isopoda: 130
    - Ostracoda: 300
    - Cladocera: 150
    - Others: ~70

# North American freshwater invertebrates

from Thorp and Covich 1991

<b>Taxon</b>	<b>Common Name</b>	<b>No. of Known Species</b>
<b>Turbellaria</b>	<b>Flatworms</b>	<b>&gt;200</b>
<b>Gastropoda</b>	<b>Snails</b>	<b>~350</b>
<b>Bivalvia</b>	<b>Mussels and clams</b>	<b>&gt;250</b>
<b>Oligochaeta</b>	<b>Worms</b>	<b>~150</b>
<b>Hirudinea</b>	<b>Leeches</b>	<b>~80</b>
<b>Acari</b>	<b>Water mites</b>	<b>&gt;1500</b>
<b>Insecta</b> Ephemeroptera Odonata Plecoptera Heteroptera Coleoptera Trichoptera Diptera	Mayflies Dragonflies and damselflies Stoneflies True bugs Beetles Caddisflies True flies	~575 ~415 ~550 324 >1100 >1340 >2000 <sup>a</sup>
	<b>Total</b>	<b>~8834</b>

<sup>a</sup>Estimate is for the Nearctic region (Coffman and Ferrington 1996).