

# Investigation of the Edwards Protocol on Dreissenid Mussels

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# Edwards Protocol

- Developed in the Great Lakes region to limit risks of transfer of veligers during fish stocking
- Used KCl and Formalin with limited risk to fish
  - 750 mg/L KCL for 1 h
  - Add 25 mg/L formalin for 2 h
  - 100% mortality to zebra mussels in bench and limited field trials



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## Prevention of the Spread of Zebra Mussels during Fish Hatchery and Aquaculture Activities

WILLIAM J. EDWARDS, LISA BABCOCK-JACKSON, AND DAVID A. CULVER\*

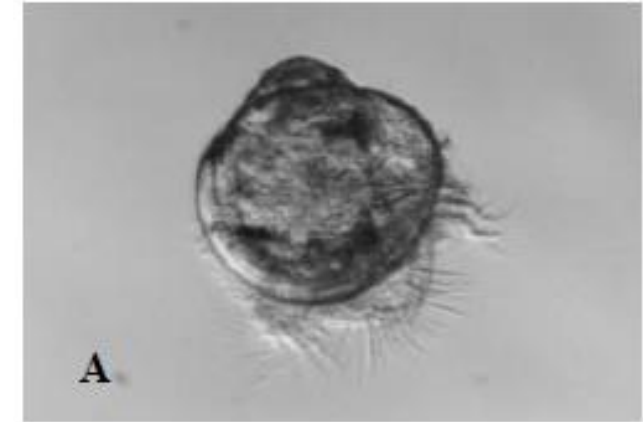
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## Field Testing of Protocols to Prevent the Spread of Zebra Mussels *Dreissena polymorpha* during Fish Hatchery and Aquaculture Activities

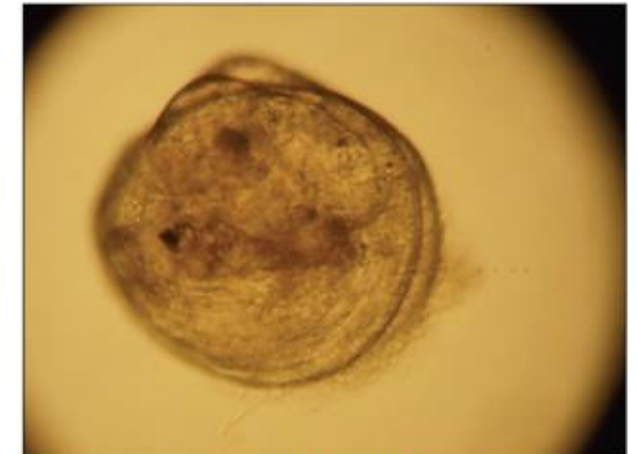
WILLIAM J. EDWARDS,\* LISA BABCOCK-JACKSON,<sup>1</sup> AND DAVID A. CULVER

# Trials with Quagga Mussels at Colorado River Hatcheries Not Successful

- Willow Beach NFH, AZ
  - Low veliger mortality even at  $>2,000$  mg/L KCl (Sykes 2009)
  - Veligers counted dead initially but in recovery alive
- At Lake Mead FH, NV
  - Low mortality (Pucherelli et al. 2014)
  - Used Pueblo, CO well and reservoir water & achieved higher mortality



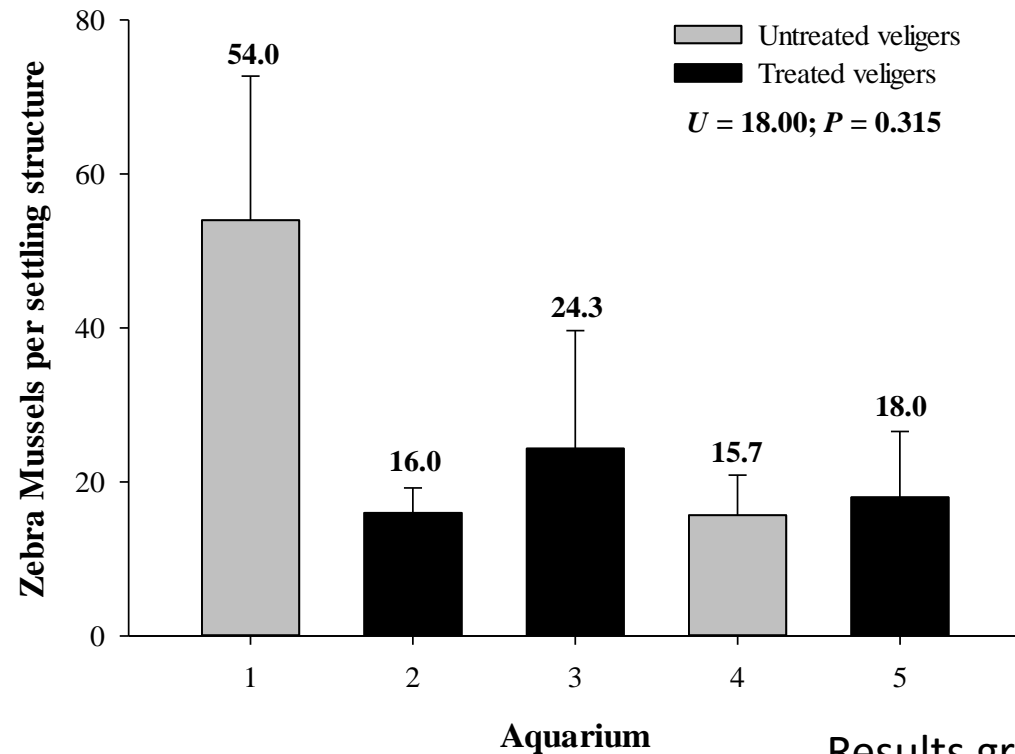
Recovered live veliger (Sykes 2009)



Dead Veliger (Pucherelli et al. 2014)

# Zebra Veligers & Settlement

- Kansas DNR
  - Filled 200 gal tanks with reservoir water
  - Edwards protocol treatment at 23°C
  - Zebra veligers taken out of tank and put into aquarium
  - After 305 days settlement not sig. different from control treatment



Results graph from KDNR report

# Discrepancies: Potential Hypotheses

Hypothesis	Edwards et al. (2000 & 2002)	Sykes (2009)	Pucherelli et al. (2014)	Kansas DNR (2015)
<b>Recovery Time</b>	None	2-6 h	24+ h	10 months settlement
<b>Species</b>	Zebra	Quagga	Quagga	Zebra
<b>Water Temp. (°C)</b>	20 & 27.6	18	13	23
<b>Water Chemistry</b> <b>Sodium Conc.</b> (Moffitt et al. 2016)	Great Lakes Region Low to Mid (0.2-0.5 mS/cm)	Colorado River High (1.0 mS/cm)	Colorado River High (1.0 mS/cm)	Kansas Mid (0.5 mS/cm)
<b>Testing Vessel</b>	Bench & Hatchery Truck	Bench	Bench	Hatchery Truck
<b>Fish Present</b>	No & Yes (6 g/L)	No	No	No
<b>Purity of Reagents</b>	Analytical & Industrial	Analytical	Analytical	Industrial

# Objectives – Edwards Protocol

- Bench Trials: Pursue the effects of temperature on the toxicity response with both zebra and quagga veligers
- Bench Trials: Evaluate the efficacy in relation to water quality (conductivity and Na<sup>+</sup>)
- Bench Trials: Compare the toxicity response using different grades of potassium chloride
- Field Trials: Evaluate the efficacy in replicated tests with a transport truck at typical low and high fish hauling densities (0.8 and 0.3 lbs/gal)



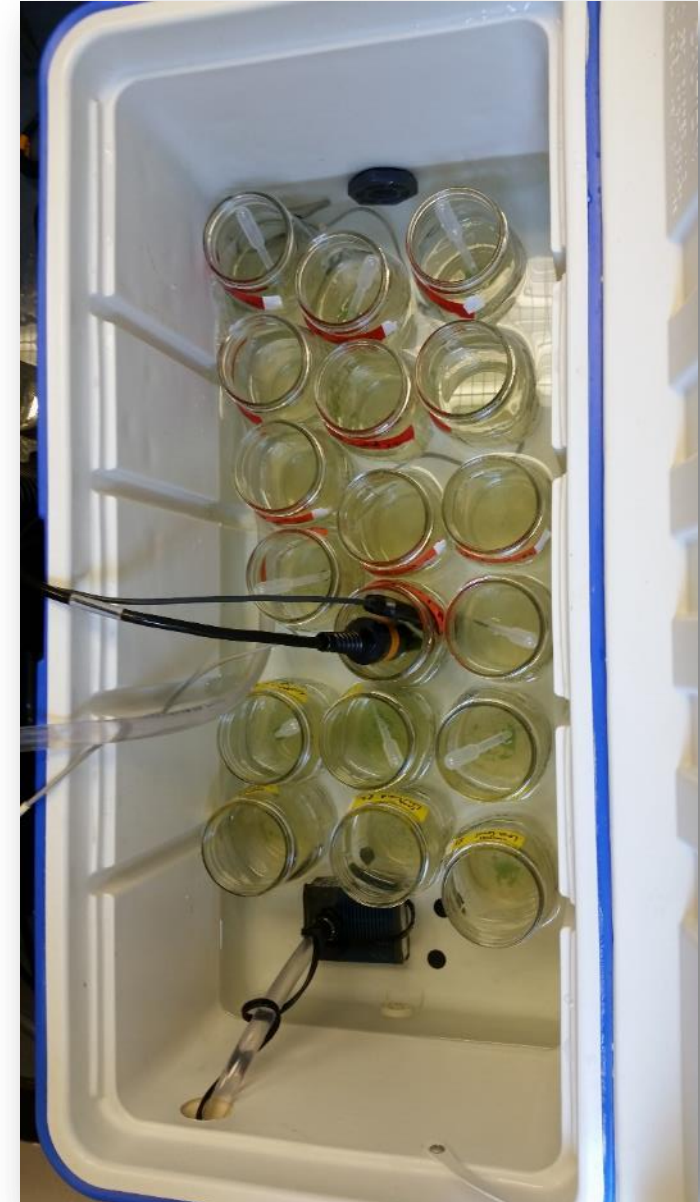
# Location of Study- Zebra Mussels

- Fairport State Fish Hatchery, Fairport, IA
- Mississippi River Water Source (August 2016)
  - Water Temperature:  $26^{\circ}\text{C}$  ( $\pm 1^{\circ}\text{C}$ )
  - Water Conductivity:  $0.37\text{ mS/cm}$
- Zebra mussel veligers
  - Morphological assessment
  - DNA analysis (BOR ROLES lab)
  - Assessment of adults



# Methods- Bench Studies

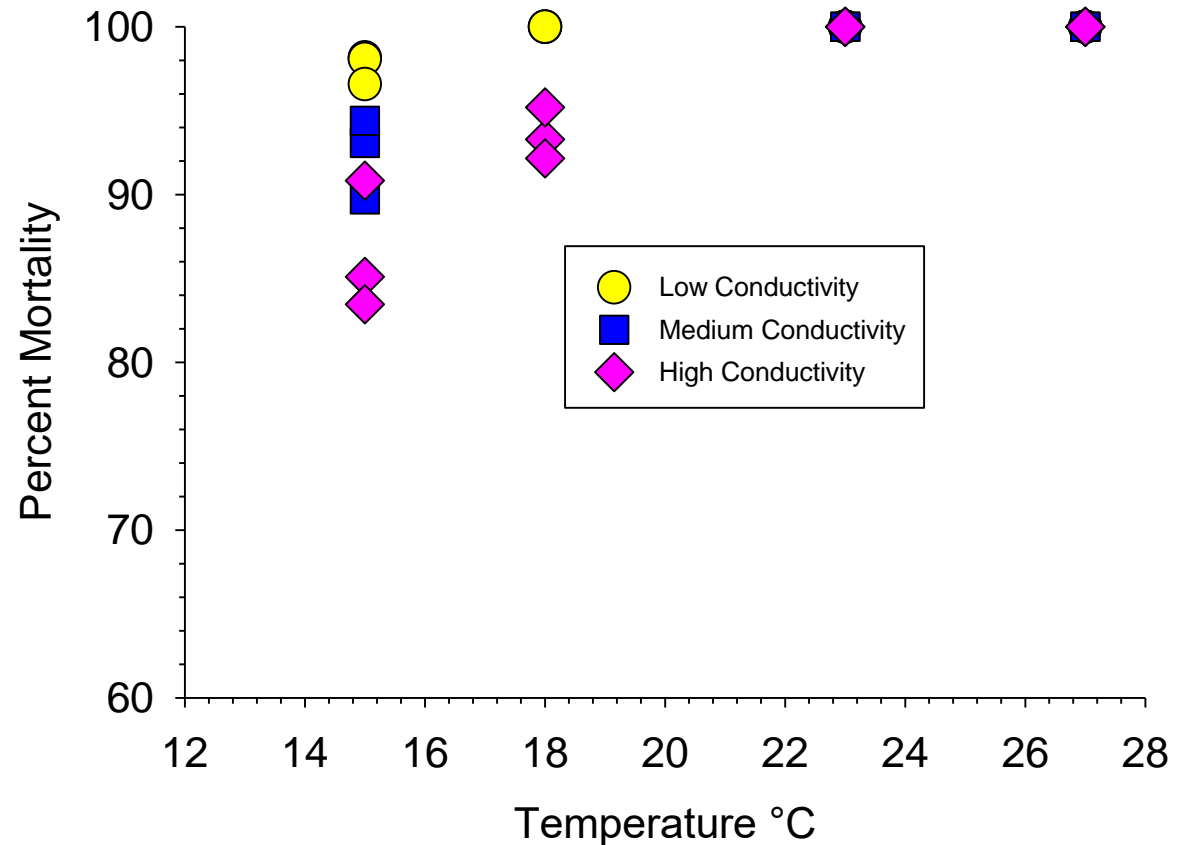
- Conductivities
  - Low (0.37 mS/cm): no NaCl addition
  - Medium (0.5 mS/cm): addition of NaCl
  - High (1.0 mS/cm): addition of NaCl (*level of Colorado River*)
- 4 Temperatures: 27, 23, 18, 15°C
- Muriate of potash, Parasite-S formalin
- Rinsed and used Fast Green stain for status
- Assessed mortality and compared results with chi-squared analysis





# Zebra Veliger Bench Study

- Confirms toxicity related to conductivity ( $\text{Na}^+$ ) and showed a temperature effect
- Low temperatures had veliger survival regardless of conductivity of water
- Temperature and mortality increased together and **achieved 100% mortality**
- As conductivity increased mortality decreased



# Fish Hauling Truck Study

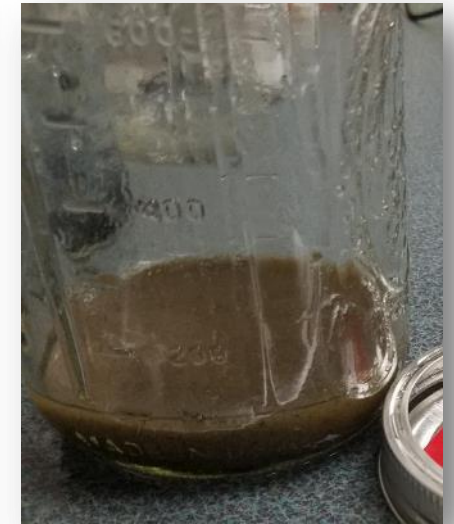
- Chemicals: KCl, formalin
- Filled trucks at night, added KCl
- Fish loaded into tank in morning
- Concentrated veligers add to all tanks to start trials
- Aerators with O<sub>2</sub>
- Subsample of 2/3 of water analyzed
- Replicated over 3 days



Chemicals no Fish	LD Fish Chemicals	HD Fish Chemicals	HD Fish Control
			Control

# Fish Hatchery Truck Results

- 100% veliger mortality in treated without fish
- Sample **counts low in fish treatments** due to precipitating mucous and organics from water and made analysis difficult
- All low fish density treatments had no surviving veligers. One live veliger was found in a high fish density treatment with **well** water not **river** water



HD Treated Sample Sludge



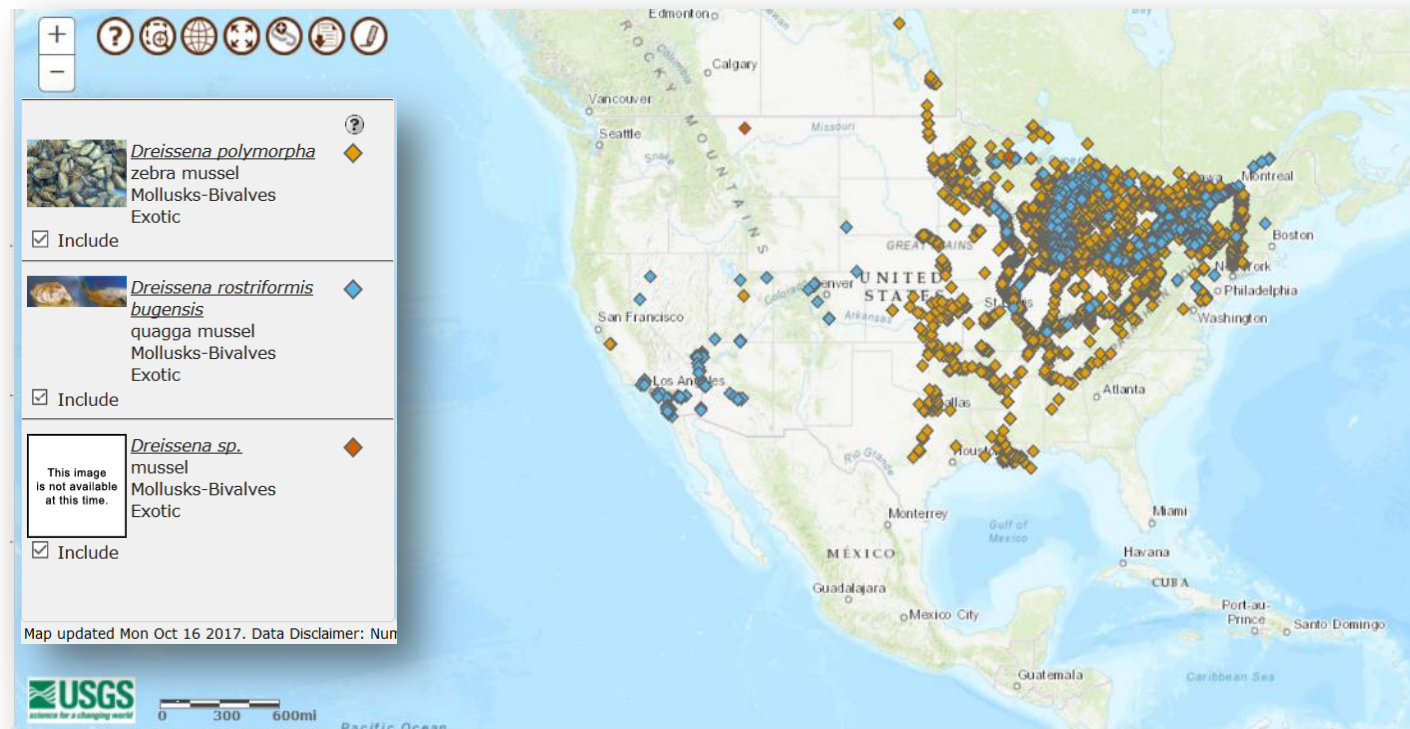
# Fish Truck Water Quality

Water Source	Rep	Temp (°C)	pH	Sp. Cond (mS/cm)	Salinity (ppt)	TDS (mg/L)	Sodium (mg/L)
Filtered River	1	27.69	8.34	0.347	0.162	0.235	<10
Filtered River	2	27.79	8.21	0.355	0.17	0.231	<10
Filtered River	3	25.03	8.79	0.312	0.15	0.203	<10
Well	3	24.2	8.03	0.612	0.3	0.398	33



# Quagga Veliger Bench Study at Willow Beach NFH

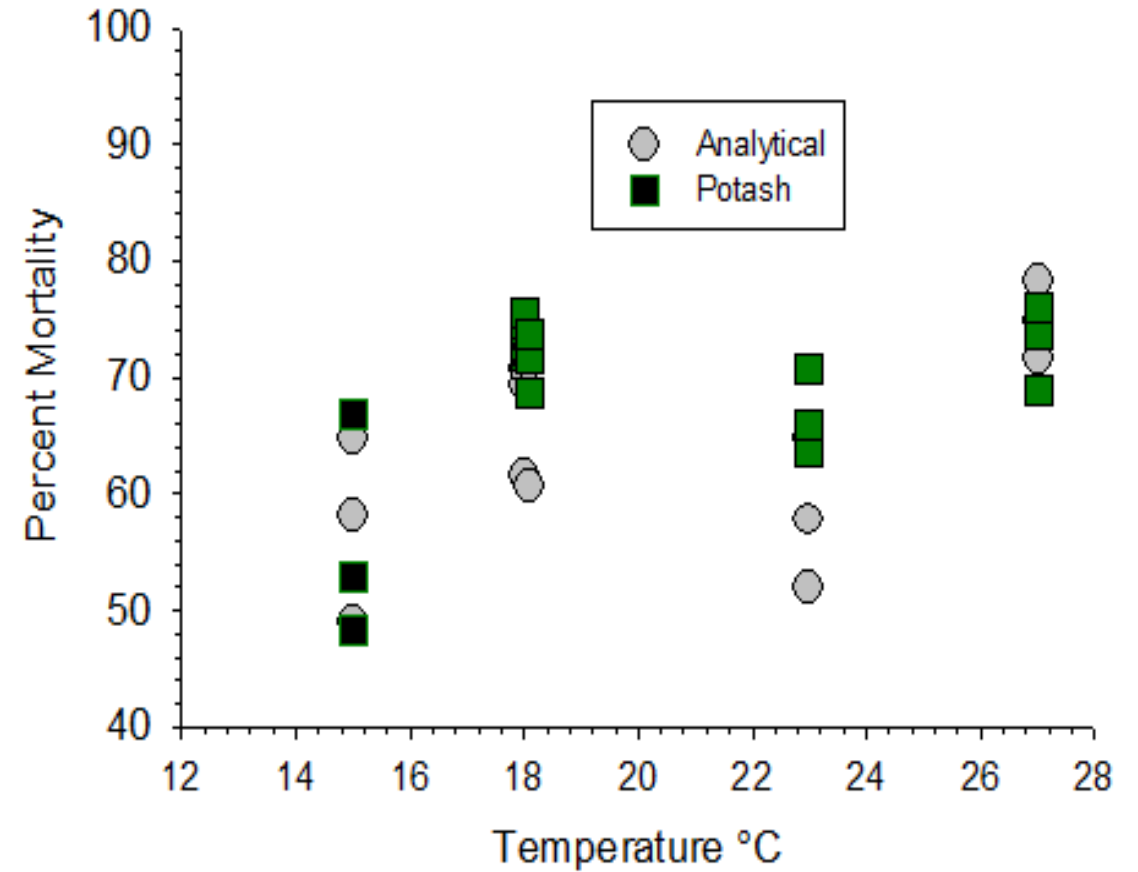
- 1.0 mS/cm conductivity Colorado River water
- 4 temperatures: 27, 23, 18, 15°C
- Analytical vs muriate of potash KCl





# Quagga Veliger Bench Study Results

- Mortality between 50 and 80% in all treatments
- **No trend** showing that analytical KCl is different from potash KCl

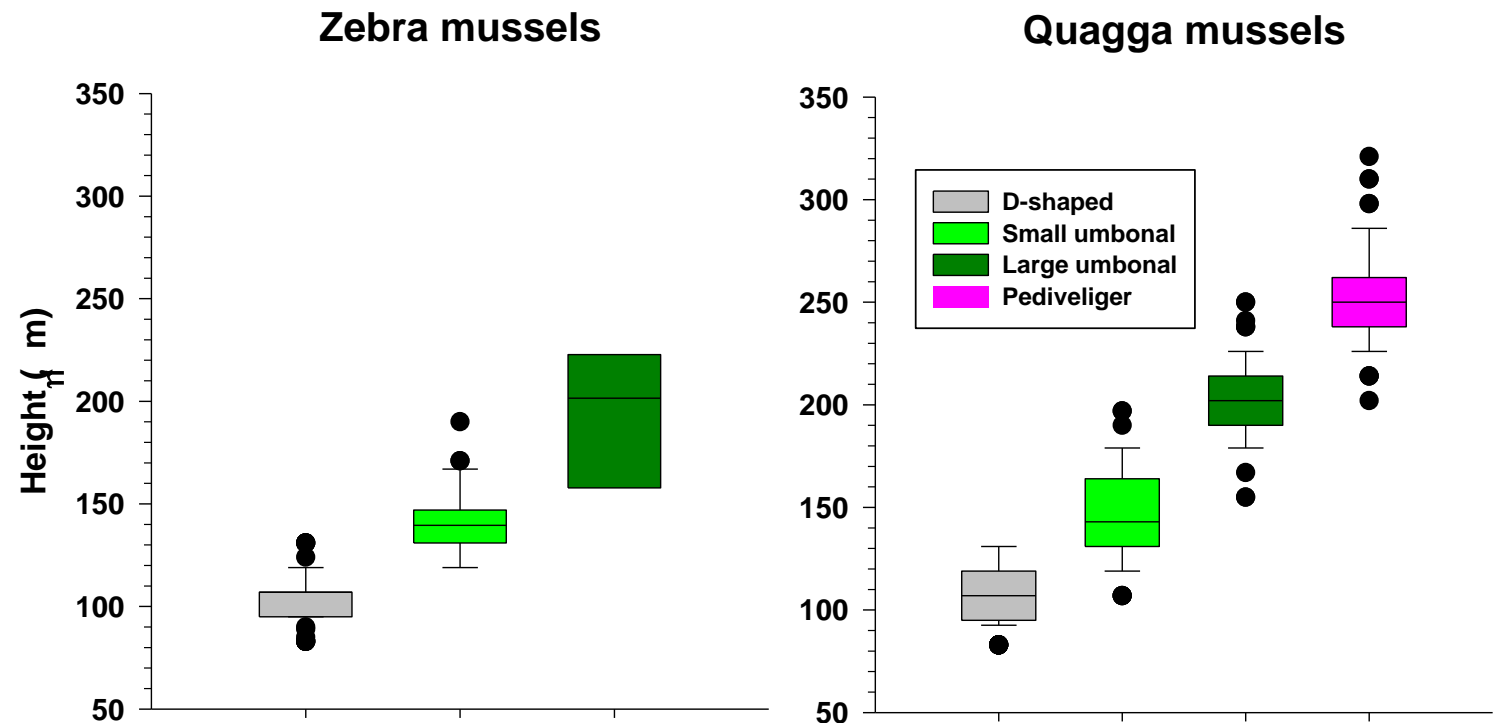


# Quagga vs. Zebra Response at 1.0 mS/cm

- Analysis of life stage

	D-shaped	Small Umbonal	Large Umbonal	Pediveligers
Zebra mussels (N=1031)	75.1%	19.5%	5.4%	0.0%
Quagga mussels (N=1881)	20.7%	23.1%	31.7%	24.5%
Quagga Mortality	100%	100%	79.2%	26.2%

- Zebra mostly D-shaped
- Quagga mixed
- Mortality highest in small life stages and higher temperatures



# Hypothesis Revisited

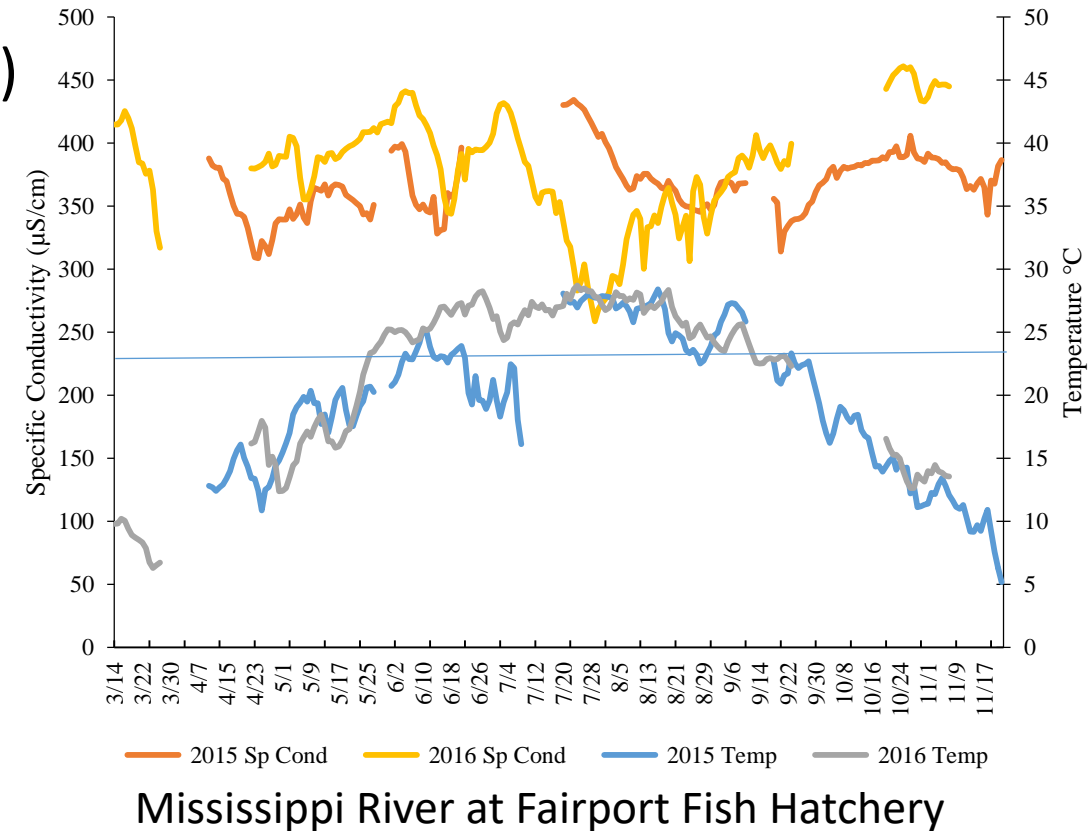
Discrepancy	Edwards et al. (2000 & 2002)	Sykes (2009)	Pucherelli et al. (2014)	Kansas DNR (2015)	This Study
<b>Recovery Time</b>	None	2-6 h	24+ h	10 months settlement	<b>Stained</b>
<b>Species</b>	Zebra	Quagga	Quagga	Zebra	<b>Zebra &amp; Quagga</b>
<b>Water Temp. (°C)</b>	20 & 27.6	18	13	23	<b>15, 18, 23, 27</b>
<b>Water Chemistry Sodium Conc.</b> (Moffitt et al. 2016)	Great Lakes Low to Mid (0.2-0.5 mS/cm)	Colorado River High (1.0 mS/cm)	Colorado River High (1.0 mS/cm)	Kansas Mid (0.5 mS/cm)	<b>Low, Med, High (0.37, 0.5, 1.0 mS/cm)</b>
<b>Testing Vessel</b>	Bench & Hatchery Truck	Bench	Bench	Hatchery Truck	<b>Bench &amp; Hatchery Truck</b>
<b>Fish Present</b>	No & Yes (6 g/L)	No	No	No	<b>No &amp; Yes (35 &amp; 95 g/L)</b>
<b>Purity of Reagents</b>	Analytical & Industrial	Analytical	Analytical	Industrial	<b>Analytical &amp; Industrial</b>
<b>Veliger Size</b>	D and post D- shaped	Mixture	Mixture	Mixture to Large	<b>D and Post D &amp; Mixture</b>

# Conclusions From Studies

- Temperature affects veliger mortality
  - Lower temperatures achieved lower mortality
- Higher conductivity, **especially sodium**, increases survival
- Edwards protocol successfully killed veligers in fish hauling tanks at **lower conductivity** (0.37 mS/cm)
- Trials with fish confounded assessment due to heavy mucus and organics in water
- No difference in response to type of KCl used in testing
- Quagga mussels lower mortality than zebra mussels at same conductivity but life stage dependent

# Edwards Protocol Still a Viable Method?

- The protocol should be used with prior knowledge of
  - Veliger life stage
  - Water temperature
  - Water conductivity (Sodium concentration)
- Optimal period of use at Fairport FH
  - June through August
  - Life stage needs to be known for fall
- At low temps, high conductivity, or large veliger life stages
  - Higher concentrations of KCl and formalin
  - Longer duration times





# Acknowledgements

- Funding: Mississippi River Basin Panel
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# Questions?

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