Development of tools to control filter-feeding aquatic invasive species including Asian carps and dreissenid mussels.

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USGS Upper Midwest Environmental Sciences Center

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U.S. Geological Survey
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Mission – UMESC provides the scientific information needed by managers, decision makers, and the public to protect, enhance and restore the ecosystems in the Upper Mississippi River Basin, the Midwest and worldwide.
UMESC Research and Monitoring Themes

1. Large River Ecosystems
2. Geospatial Sciences and Decision Support System Development
3. Invasive Species Control
4. Fisheries Management Chemical and Drug Development and Registration
5. Endangered Species
6. Contaminant Effects on Wildlife
USGS Invasive Species Research

- Prevention of AIS introduction
- Early detection / rapid assessment of AIS
- Monitoring & forecasting of AIS distribution
- Effects of AIS on native species
- Control / management of AIS
- Information dissemination
Dreissenid mussel distribution
Asian carp distribution

Silver carp

Bighead carp
Asian carp distribution
AIS control challenges

Four current biocides
- antimycin
- rotenone
- 3-trifluoromethyl-4-nitrophenol (TFM)
- niclosamide

Minimal specificity
- TFM & niclosamide for sea lamprey
- Non-target effects
Success for AIS control tools

1. Selective
   - Limited effect on native species

2. Scaleable
   - Mussel beds to lake trout spawning beds
   - Backwaters to large rivers

3. Economics
   - Application $ << Resource value $
AIS research goals

- Develop microparticle formulations for selective delivery of control agents to filter-feeding aquatic invasive species
- Identify and evaluate potential selective biocides for bighead and silver carp and dreissenid mussels
- Evaluate physical methods to limit Asian carp and dreissenid mussel populations.
- Evaluate current molecular surveillance techniques and develop next-generation molecular surveillance techniques
- Evaluate the potential of ZEQUANOX® to control dreissenid mussels in open water.
AIS research goal

Develop microparticle formulations for selective delivery of control agents to filter-feeding aquatic invasive species
Control agent-laden microparticles

1. ‘Hold’ the agent
2. Appropriately sized
3. Readily consumed
4. Targeted release
5. Scaled production

Black sandshell | Zebra mussel

Biobullets® on zebra mussel gill

Biobullet®-laden pseudofeces expelled from native mussel
Potential application to dreissenid mussels

Enzymatic release of control agent in targeted species
Potential application to Asian carp

Video courtesy
Dr. Ed Little,
USGS-CERC
Developing microparticles

1. Feeding characteristics
   • Filtration rate
   • Size selectivity

2. Digestive processes
   • pH / Digestive enzymes
   • Temporal changes

3. Control agent
   • Selection
   • Loading

4. Registration
Developing species-specific microparticles for Asian carps or dreissenid mussels

Current research:
- Compare digestive enzyme activity in AIS versus native fish
- Evaluate particle retention by AIS
- Determine lethal control agent levels for AIS vs native fish or mussels

Future research:
- Evaluate microparticles to control Asian carp or dreissenid mussels and non-target effects on native fish
  - Lab, field, and environmental fate
- Register microparticles with EPA
Digestive physiology

• Mussels
  - Mississippi River (Winona, MN)
    1. Zebra mussel *Dreissena polymorpha*
    2. Threeridge *Amblema plicata*
    3. Pocketbook *Lampsilis cardium*

• Fish
  - Illinois R. (IL), Wabash R. (IN), Jim R. (SD)
    1. Bighead carp *Hypophthalmichthys nobilis*
    2. Silver carp *H. molitrix*
    3. Gizzard shad *Dorosoma cepedianum*
    4. Bigmouth buffalo *Ictiobus cyprinellus*

• 2010-2012 (bi-monthly depending on flow)
• Digestive system enzymes assayed
• Expanding to larval aquatic insects
Enzyme assays – AIS vs native species

19 digestive enzymes
Normalized to equal amounts of protein
Zebra mussels vs. two native mussel species
Silver carp vs. two native planktivorous fish

Gizzard shad
Bigmouth buffalo
Silver carp

Easy to visualize
>intensity = >enzyme

Optical Density

Alkaline phosphatase  Esterase  Esterase lipase  Lipase  Leucine aminopeptidase  Cystine aminopeptidase  Trypsin  Alpha-aminophosphatase  Naphthol-AS-Bl...  Alpha-galactosidase  Beta-galactosidase  Alpha-glucosidase  Beta-glucosidase  N-acetyl-beta-mannosidase  Fucosidase
Change in mussel enzyme activity

- Samples collected August & September (2010)
- 7°C change between collections
- Expanded to more seasons, locations and aquatic insects

Bars = 10 individuals

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Silver carp vs native planktivores

**β-galactosidase**
Converting lactose to glucose and galactose

**Common source:** Plants
*Commonly used in molecular biology to screen bacteria*
Silver carp vs native planktivores

**Phosphatases**
Higher in SVC in August, September and March

Always higher in SVC compared to GIS
Silver carp vs native planktivores

Trypsin

- Optical density (enzyme activity)

<table>
<thead>
<tr>
<th>Month</th>
<th>Gizzard shad</th>
<th>Silver carp</th>
</tr>
</thead>
<tbody>
<tr>
<td>August</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>September</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>March</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>May</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>June</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

PROVISIONAL DATA

- % with food in gut (March)

<table>
<thead>
<tr>
<th>Species</th>
<th>% with food in gut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gizzard shad</td>
<td>0</td>
</tr>
<tr>
<td>Bigmouth buffalo</td>
<td>0</td>
</tr>
<tr>
<td>Silver carp</td>
<td>100</td>
</tr>
</tbody>
</table>
Mussel filtration

Flow through system (similar to Filgueira et al., 2006)

Fatmucket\textsuperscript{1} (\textit{Lampsilis siliquoidea})

Threeridge\textsuperscript{1} (\textit{Amblema plicata})

Zebra mussel\textsuperscript{2} (\textit{Dreissena polymorpha})

\textsuperscript{1}Photo source: IL NHS
\textsuperscript{2}Photo source: USGS
Mussel filtration

Points represent the mean of 3 independent trials

PROVISIONAL DATA
Mussel filtration

Algae removal (mL/h/g)

<table>
<thead>
<tr>
<th>10 mg/L</th>
<th>25 mg/L</th>
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<tbody>
<tr>
<td>Threeridge</td>
<td></td>
</tr>
<tr>
<td>Fatmucket</td>
<td></td>
</tr>
<tr>
<td>(large)</td>
<td></td>
</tr>
<tr>
<td>(small)</td>
<td></td>
</tr>
</tbody>
</table>

PROVISIONAL DATA
Mussel filtration

Specific gravity of algae cells ~ 1

• Removal
  - ~0.003 mL/h/g dry wt
  - = 3.0 mg/h/g dry wt

• Dose = mass x load x available
  - 3.0 mg/h/g x 5% x 75% = ~0.1 mg/g/h
Asian carp filtration

Gizzard shad gill

SVC gill
Asian carp filtration

- 5 g/90 L water
- Sample 1 = $3.6 \times 10^5$ particles/L (~355 particles/mL)
- Sample 2 = $1.7 \times 10^5$ particles/L (~172 particles/mL)
- SVC, BHC, Hybrid carp
  - 35-40 g

Sample 1
Mean 27.8 µm
Median 8.6 µm
6.4 x $10^6$ particles/g

Sample 2
Mean 67.6 µm
Median 76.8 µm
3.1 x $10^6$ particles/g

PROVISIONAL DATA
Asian carp filtration
Asian carp filtration

BHC – Sample 1

Red bars = 0-h
Blue bars = 6-h

USGS PROVISIONAL DATA
Asian carp filtration

Removal of microparticles

BHC gill

SVC gill
Asian carp filtration

Removal of microparticles

Particles retained in the GIT of a BHC

Particles retained in the GIT of a SVC
Asian carp filtration

Removal of suspended particles
(PROVISIONAL DATA)

Each point represents the mean of 4 independent experimental units
Planktivore particle overlap

PROVISIONAL DATA
Asian carp filtration

Microparticle retention

• Extract GI tract
• Rank (quartiles) foregut and mid-hindgut fullness
• Extract GI tract contents
  ▪ Foregut – most tests
  ▪ Mid/hindgut limited
  ▪ Entire GIT – most tests
# Asian carp microparticle retention

### Foregut contents

<table>
<thead>
<tr>
<th>Species/test</th>
<th>SVC S1</th>
<th>SVC S2</th>
<th>SVC</th>
<th>BHC T1</th>
<th>BHC T2</th>
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</thead>
<tbody>
<tr>
<td>Foregut content/BW (mg/g)</td>
<td>0.00</td>
<td>0.10</td>
<td>0.20</td>
<td>0.30</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>0.60</td>
<td>0.70</td>
<td>0.80</td>
<td>0.90</td>
</tr>
</tbody>
</table>

**PROVISIONAL DATA**

[Graph showing data for different species and tests]

Legend:
- MM 1
- MM 2
- NC
- CONTROL
Asian carp GIT particle distribution

GIT contents (mg)
- Foregut
  - $19.7 \pm 7.1$ mg
  - $6.1 \times 10^4$ particles
- GIT
  - $131.4 \pm 20.5$ mg
  - $4.1 \times 10^5$ particles

Particle distribution
- Foregut ~ 16%
- Mid/hindgut ~ 84%

Particle mass/BW
- Foregut ~ 0.6 mg/kg BW
- GIT ~ 4500 mg/kg BW

6 SVC @ level 4 after exposure to Sample 2
Asian carp microparticles – agent release

PROVISIONAL DATA
Asian carp potential dose delivery

- Dose ($D$) = $M \times L \times A$
  - $M =$ Mass consumed
  - $L =$ % loading (5% w/w)
  - $A =$ % available (75%)

SVC

Foregut: $600 \text{ mg/kg} \times 5\% \times 75\% = \sim 23 \text{ mg/kg dose}$
Entire GIT: $4500 \text{ mg/kg} \times 5\% \times 75\% = \sim 170 \text{ mg/kg dose}$

PROVISIONAL DATA
Asian carp response to rotenone

- Active absorption
- Similar uptake and excretion
- Silver and bighead carp use different molecular processes to respond to rotenone exposure
Toxicity of rotenone microparticles

% BW of microparticles present in the foregut of silver carp during filtration studies

% BW of microparticle required to be consumed by silver carp to result in 60% mortality (20 mg/Kg)

% BW of microparticle required to be consumed by silver carp to result in 100% mortality (25 mg/Kg)

PROVISIONAL DATA
Toxicity of antimycin microparticles – RBT

% BW of microparticles present in the foregut of silver carp during filtration studies

- % BW of microparticle required to be consumed by silver carp to result in 60% mortality (1 mg/Kg)
- % BW of microparticle required to be consumed by silver carp to result in 100% mortality (2 mg/Kg)

Required consumption (% BW)

Antimycin concentration (mg/kg)

PROVISIONAL DATA
Microparticles characteristics

1. Particle degradation
   Measure particle size in water (20 °C) over time
   - microscopically
   - Coulter Counter

2. Particle Settling
   Measure particle settling in water (20 °C)

PROVISIONAL DATA
Microparticles characteristics

PROVISIONAL DATA
Microparticle settling

PROVISIONAL DATA
Summary – Zebra mussel

• Filtration rate >> natives
  Incorporate low levels of a toxicant?

• Minimal change in zebra mussels digestive enzymes with decreasing water temperature
  Potential seasonal application?
  Increased phosphatases and proteases - potential release target?

• Incorporate control agent into microparticles
  Initiate/complete lab exposure trials
Summary – Asian carp

• Asian carp retain preliminary microparticles
  Determine filtration and gut evacuation rates

• Activity of certain digestive enzymes are higher in Asian carp vs. native planktivores
  Trypsin, phosphatases – potential particle release
  Active feeding earlier than natives

• Incorporate control agent into microparticles
  Initiate/complete lab exposures
  Confirm dose
Registration of control agent in a microparticle

U.S. Environmental Protection Agency

- Existing control agents
  - likely considered a new "formulation"
  - shorter registration path
    (product chemistry, user safety, environmental fate, ecological effects)

- New control agents
  - full registration
Use of *Pseudomonas fluorescens* (*Pf*-CL145A; ZEQUANOX®) to control dreissenid mussels

**Current research:**
- Assess the safety of *Pf*-CL145A to three life stages (glochidia, juvenile, subadult) of 7 native mussel species
- Assess the safety of *Pf*-CL145A to 10 native fish species

**Future research:**
- Assess efficacy and potential non-target effects of *Pf*-CL145A used to control zebra mussels in open water around native mussel beds and propagation cages
- Assess efficacy of *Pf*-CL145A to control zebra mussel veligers in fish transport water

UMESC laboratory trials with Pf-CL145A

- Research according to Good Laboratory Practice regulations with controlled environmental conditions
- Designed to support EPA registration for open water uses
UMESC laboratory trials with *Pf*-CL145A

Glochidia viability

Juvenile viability

USGS PROVISIONAL DATA
UMESC laboratory trials with *Pf*-CL145A

Evaluate effects of *Pf*-CL145A on fish
- in progress
Controlled field trials with *Pf-CL145A*
Acknowledgements

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• More information
  • http://www.umesc.usgs.gov/

LOGOS
Questions?