Use of Environmental DNA to Detect the Genetic Presence of Bighead and Silver Carp

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eDNA Calibration Study (ECALS)

ECALS is an interagency study (USACE-USGS-USFWS) that will improve our understanding and interpretation of eDNA positive detections

- What does a positive eDNA detection in the CAWS mean?
  - Where did it come from?
  - How long has it been there?
  - Did more than one Asian carp contribute to the sample?
- Improve sampling and analytical efficiency
  - Reduce the TIME and COST
- Provide context and improve confidence in conclusions based on eDNA monitoring as an effective tool for resource managers in decision making

ECALS is funded through the GLRI, with three major tasks in the ACRCC Framework:

  Vectors
  Markers
  Calibration
Confirmed DNA Vectors

- Storm sewers
- Fishing boats and gear
- Fish-eating birds
- Barge carcasses
- Sediment
  - DNA sorption on sediments confirmed
  - Low-level, long term releases possible from undisturbed and re-suspended sediment
    - Based on 21 day study
  - Sediment DNA contribution to water samples likely minimal unless
    - Turbidity is high
    - Particulates captured on filter
Estimated Copies (CN) of Silver Carp eDNA in 1 ul of DNA Extraction
Elute from 50-ml Sample from 10 ft Length of Commercial Fishing
Net Rinsed in 4 Gallons of Water

Copy Number (CN) of Silver Carp eDNA Calculated for
Total Length (300 ft) of Commercial Fishing Net

- Mean CN Day 0
- Mean CN Month 1

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<tr>
<th>Net</th>
<th>Net 1</th>
<th>Net 2</th>
<th>Net 3</th>
<th>Net 4</th>
<th>Net 5</th>
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<tr>
<td>CN Day 0</td>
<td>10^5</td>
<td>10^6</td>
<td>10^7</td>
<td>10^8</td>
<td>10^9</td>
</tr>
<tr>
<td>CN Month 1</td>
<td>10^3</td>
<td>10^4</td>
<td>10^5</td>
<td>10^6</td>
<td>10^7</td>
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LOG AXIS -- Estimated Silver Carp eDNA Copy Number (CN) obtained from 25 cm² of Commercial Fishing Boat Hull in Morning, Following Fishing, and After DNA Removal Protocol
ECALS: Shedding and Degradation

Shedding/Loading Report

- More DNA is evidence of more fish
- Shedding rate was NOT affected by temperature
- Algae can mask detection of eDNA
- Lots of eDNA in sperm (detectable for 3 weeks)
- Fed fish excrete 10X as much DNA as non-fed fish

Degradation Report

- The majority of the DNA degraded either rapidly or very rapidly over a few days, but in all cases a small portion of DNA persisted beyond 2 to 4 weeks
- Both temperature and pH affected the rate of degradation
- Microbial loads (bacteria) and turbulence did not affect rates of degradation
Technology Transfer from ECALS to Monitoring: An approach to the validation and then implementation of newly developed markers for eDNA

- Collaboration among USGS-UMESC, USFWS-WGL, USACE-ERDC

- Reliable data is important in making management decisions. Validation of newly developed eDNA markers gives confidence in the reliability of data produced by the use of those markers and methods

- A document outlining the development and validation of new markers to be used in eDNA monitoring has been drafted and is in review among all three collaborating labs
DNA degradation over time and the ability of an eDNA marker to detect it.

Marker Target Region 2

Marker Target Region 3

Short Marker #1

Longer Marker #2

Longest Marker #3

Positive Detection?

Yes

Yes

Time since DNA was shed:

0 1 2 3 4 5 6
2014 Monitoring

• ERDC, UMESC, WGL validate several new markers so that a suite of markers can be selected for use
  – 7 qPCR markers
  – 5 cPCR markers

• Tested on six different environmental samples
  – 3 AC positive waters
  – 3 AC negative waters

• Complete the round-robin validation by May
  – If sampling has already begun, WGL will receive and extract samples
  – WGL will wait for new markers before analyzing extracts
Probabilistic Model (PM) Objective & Status

• The PM will estimate the probability that:
  • The potential sources of eDNA in a water body are, in fact, an actual source of the eDNA detected in monitoring samples; and
  • An Asian carp is present above the monitoring location.

• The PM will integrate various ECALS & ACRCC research efforts to provide a context for interpreting eDNA monitoring results.

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<th>#</th>
<th>Milestone Description</th>
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<td>3</td>
<td>Draft report on fully parameterized PM.</td>
<td>May 31, 2014</td>
<td>In progress</td>
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<tr>
<td>4</td>
<td>Final report: PM and analysis of historical monitoring data.</td>
<td>Sept. 31, 2014</td>
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ECALS is simulating hydrodynamics and eDNA fate and transport in the CAWS

- A large steady source of eDNA is required to produce concentrations with a consistently high probability of detection in the CAWS
- Fresh eDNA released by a primary source (live fish) would not travel far from the point of release
- About 85% of fresh eDNA degrades within five days
- A recalcitrant portion (15%) persists beyond five days, and (1%) may persist beyond 18 days.
ECALS is estimating eDNA loads from secondary sources, such as birds.

- Cormorants are the most common fish eating bird inhabiting the CAWS area.
- Thousands of cormorants nest near the CAWS during the breeding season.
- A large fraction of cormorants show evidence of having eaten carp.
- Birds may contribute billions of copies per day via fecal deposits.
- Figure shows an estimate for the breeding season assuming 100% Asian carp diet.
2014 eDNA Milestones

- Third Interim ECALS Report
- Finalize Regional eDNA plans
- USFWS eDNA Training
- Marker validation complete
- Gradient study for centrifuging
- Annual update/release of QAPP
- eDNA toolkit

www.asiancarp.us/ecals
www.fws.gov/midwest/fisheries/eDNA