Characterization of metallothionein in two ancient fishes exposed to metals: white sturgeon and lake sturgeon

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Sturgeon

24 species of sturgeon

White Sturgeon
(A. transmontanus)

Shovelnose Sturgeon
(S. platorynchus)

Russian Sturgeon
(A. gueldenstaedtii)
Sturgeon

All species of sturgeon are likely threatened

Hypotheses for declines in populations:
- Alterations to habitat
- Over-fishing
- Predation/competition from introduced species
- Exposure to pathogens
- Pollution
Sturgeon

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Hypothesis for declines in populations:

- Alterations to habitat
- Over-fishing
- Predation/competition from introduced species
- Exposure to pathogens
- Pollution
  - Metals
Metal Exposure

Exposure to metals has been implicated as a cause for declines in populations of sturgeon

Sources:
- Mines
- Metallurgical facilities
- Pulp and paper mills
- Industrial and municipal input sources

CEW, Saskatoon, SK

October 5, 2015
Species Sensitivity Distribution

Cadmium

Zinc

Copper

Vardy et al., 2013, 2014
Question

Why are sturgeon among the most sensitive species to these metals?

• Do they fail to cope with the exposure?

Metallothionein
Metallothionein (MT)

- A cysteine rich protein that binds metals for storage, transport or detoxification
- Has been shown to be inducible upon metal exposure
- A correlation exists between the ability of an organism to induce MT and their sensitivity
Questions

Why are sturgeon among the most sensitive species to these metals?

Does MT have a role in the observed sensitivity of sturgeon to Cd, Zn and Cu?

- Can MT in sturgeon bind metals?
- How responsive and inducible is MT in sturgeon?
Species of Interest

White Sturgeon (*A. transmontanus*)

-- Among the most sensitive species to Cd, Zn and Cu

Lake Sturgeon (*A. fulvescens*)

-- Sensitivity unknown

October 5, 2015
Metallothionein Sequence

White Sturgeon

<table>
<thead>
<tr>
<th>ATG GAT CCG CAA TCT TGC ACG ACT CAG GCT GGC TCG TGC AGC TGT GGT GAT</th>
<th>M D P Q S C T C T Q A G S C S C G D</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAC TGC AAG TGC ACA GAC TGC AAA TGC AAA ACT TGC AAG AAA AGC TGC TGC TCC</td>
<td>N C K C T D C K C K T C K K S C C S</td>
</tr>
<tr>
<td>TGT GTC CCC ACC GGC TGC AGC AAG TGT GCC CAG GGC TGC GCC TGC AAA GGG GGA</td>
<td>C C P T G C S K C A Q G C A C K G G</td>
</tr>
<tr>
<td>GCC ACC TGC GAC ACC GGC TGC TGC AAG TGA</td>
<td>A T C D T G C C K *</td>
</tr>
</tbody>
</table>

Lake Sturgeon

<table>
<thead>
<tr>
<th>ATG GAT CCG CAA TCT TGC ACG TGC ACC CAG GGT GGT TCG TGC AGC TGT GGT GAT</th>
<th>M D P Q S C T C T Q G G S C S C G D</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAC TGC AAG TGC ACA GAC TGC AAA TGC AAA ACT TGC AAG AAA AGC TGC TGC TCC</td>
<td>N C K C T D C K C K T C K K S C C S</td>
</tr>
<tr>
<td>TGT GTC CCC ACA GAC TGC AGC AAG TGT GCC CAG GGC TGC GCC TGC AAA GGG GGA</td>
<td>C C P T D C S K C A Q G C A C K G G</td>
</tr>
<tr>
<td>GCC ACC TGC GAC ACC GGC TGC TGC AAG TGA</td>
<td>A T C D T G C C K *</td>
</tr>
</tbody>
</table>

Similarities to fishes:
- CXXXCC motif is characteristic of MT in fishes
- 20 C residues is characteristic of all MT

Differences:
- Longer than any other MT in teleost fish
- Same length as MT in birds
Questions

Why are sturgeon among the most sensitive species to these metals?

Does MT have a role in the observed sensitivity of sturgeon to Cd, Zn and Cu?

• Can MT in sturgeon bind metals?
• How responsive and inducible is MT in sturgeon?
Challenges Associated With Studying Wild Fish Species

- Difficult to culture, especially larger species
- Labour, time and resource intensive
- Ethical concerns regarding use of live animals (especially in context with endangered or threatened species)
Alternative: *In Vitro* Tissue Explant Approach

- Smaller population size required
- Multiple species, compounds and concentrations are tested with ease
- Lower time and cost commitment
- While some *in vivo* properties are lost (e.g. feedback among different tissues), primary cultures often maintain some realistic properties such as paracrine interactions
**In vitro liver explant exposure**

**Excise liver tissue, slice into 1 mm³ pieces, rinse and place in supplemented L-15 media**

**Add tissue slices and chemical to a 24-well plate, incubate at 15⁰C for 24h**

**Upon termination, collect tissue and quantify transcript abundance using RT-PCR**
Results – Cadmium

White sturgeon

Lake sturgeon

Increase in MT transcript abundance

No change in MT transcript abundance

CEW, Saskatoon, SK

October 5, 2015
Results - Zinc

- White sturgeon: Increase in MT transcript abundance
- Lake sturgeon: No change in MT transcript abundance
Results - Copper

White sturgeon
Decrease in MT transcript abundance

Lake sturgeon
No change in MT transcript abundance
Summary of Results

MT transcript abundance when exposed to Cd and Zn
- Low magnitude of response

MT transcript abundance when exposed to Cu

No statistical change in MT transcript abundance when exposed to Cd, Zn, or Cu
Conclusions

1. The sensitivity of white sturgeon to metals such as Cd, Zn and Cu might be due to the lesser maximal response of MT following exposure

2. If the capacity of responses of MT to metals is important to sensitivity:
   - Lake sturgeon might be more sensitive than white sturgeon to Cd and Zn
   - White sturgeon might be more sensitive than lake sturgeon to Cu
Future Research

1. Identify if the low or lack of MT induction *in vitro* corresponds to an *in vivo* exposure
2. Investigate life-stage specific differences in responses of MT to metal exposure
   - Early life stages of white sturgeon (15-40 dph) show the greatest sensitivity to Cu
3. Characterize metal responsive transcription factor
4. Investigate other metal compensatory responses to identify the molecular mechanism for the greater sensitivity to these metals at early life stages
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