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Effects of Exposure to Water in Agricultural Drainage Ditches on Vitellogenin in Male Fishes

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**Effects of Exposure to Water from Agricultural Drainage Ditches on Vitellogenin in Male Fishes**

Michael Zijlstra and Dr. Bob Gillespie, Department of Biology

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**Introduction**

Agriculture is the most extensive land use activity in Indiana and Ohio. As a result, agricultural chemicals are common contaminants in headwater streams. Atrazine, a commonly-detected herbicide in surface water, has been shown to cause feminization of male gonads in frogs and zebrafish in laboratory exposures (Hayes et al. 2011). Vitellogenin, produced in the liver, is a precursor to yolk proteins in ovaries. Because Vtg is induced by estrogen, it is a useful biological marker for feminization in males (Hiramatsu, 2006). Induction of vitellogenin (Vtg) has been associated with feminization in male frogs and fishes. The objective of this research was to measure the concentrations of Vtg in livers of male fishes that inhabit agricultural ditches (field studies) and fishes that were experimentally-exposed to ditch water (bioassays) from drainage ditches.

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**Methods**

Fish were collected from drainage ditches and reference sites during 2011 at two study areas in Indiana and Ohio. Additionally, streamside bioassays were conducted at three ditches and a laboratory reference in Indiana (Figure 1).

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**Results and Discussion**

We hypothesized that Vtg in male fish from ditch sites would be greater than that in male fish from reference sites. However, the results did not support this hypothesis. There were no significant differences in Vtg of creek chubs between ditch sites and reference sites (Table 1), or between fatheads exposed to ditch water and those exposed to reference water (Table 2). The apparent lack of effect of agrochemicals on Vtg in chubs and fatheads may have resulted from 1) lack of sensitivity to agrochemicals, 2) concentrations of chemicals below the threshold that causes endocrine disruption, 3) lack of estrogenic activity of chemical exposure. Atrazine concentrations in ditches rarely exceeded 8 µg/L during 2011 (Figure 2). Concentrations within this range were not associated with induction of Vtg in male fatheads (Table 3). A search of published research literature found only one lab study that reported an association between induction of Vtg in males and exposure to atrazine in rainbow trout. Although exposure to agrochemicals did not result in increased Vtg in male fish, there may be other negative effects to reproduction. Future studies should include more sensitive species and assess the impacts of agrochemicals on reproductive output in females.

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**Table 1. Concentrations of vitellogenin (Vtg) in livers of male creek chubs from six ditch sites and two reference sites in the Upper Big Walnut Creek and Cedar Creek Watersheds. No significant difference (t-test; p>0.05) in Vtg between ditches and reference sites.**

<table>
<thead>
<tr>
<th>Site</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ditches</td>
<td>35</td>
<td>16.27</td>
<td>23.62</td>
</tr>
<tr>
<td>Reference</td>
<td>14</td>
<td>12.14</td>
<td>12.25</td>
</tr>
<tr>
<td>Female chubs</td>
<td>154</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Concentrations of vitellogenin (Vtg) in livers of male fathead minnows exposed to ditch water from three sites in the Cedar Creek Watershed and to reference water in the lab. No significant difference (t-test; p>0.05) in Vtg between ditches and reference water.**

<table>
<thead>
<tr>
<th>Site</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ditches</td>
<td>28</td>
<td>4.82</td>
<td>8.60</td>
</tr>
<tr>
<td>Reference</td>
<td>9</td>
<td>1.63</td>
<td>1.47</td>
</tr>
<tr>
<td>Three female fatheads averaged</td>
<td>172; ranged</td>
<td>80-250</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Results from lab and field studies that exposed fishes to Atrazine.**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Site</th>
<th>Species</th>
<th>Males</th>
<th>Females</th>
<th>Atrazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knight, et al. 2013</td>
<td>Field Minnow</td>
<td>No Effect</td>
<td>Defeminization</td>
<td>0.2-5 ug/L</td>
<td></td>
</tr>
<tr>
<td>Salaberria, et al. 2009</td>
<td>Lab</td>
<td>Induced Vtg</td>
<td>Decreased</td>
<td>2, 200 ug/L</td>
<td></td>
</tr>
</tbody>
</table>

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**Acknowledgements**

I would like to thank Dr. Gillespie for giving me the opportunity to help work on his research project and giving his time to help explain things to me along the way. I also want to thank Mrs. Boatright for allowing me to help her out on her project as well as being patient with me with all my questions. I also want to thank Dr. Blumenthal for allowing us to work in his lab.

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**Literature Cited**


