Seeing is Believing: Developing Methods to Assess the Effects of Pollutants on Vision in *Pimephales promelas*

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Background:
- Several emerging contaminants (ECs) are known to modulate thyroid hormone signaling, which affects various aspects of growth, including eye development.
- Fathead minnows (*Pimephales promelas*) are a model organism used to study the effects of aquatic ECs.

Hypothesis: Exposure to thyroid-disrupting ECs alters eye development and function

Objective: Develop and refine methods for assessing visual function in the fathead minnow

Methods:
- Fathead minnow larvae were subjected to an optomotor assay at 144 and 240 hours post fertilization to determine at what age larvae elicit a stronger optomotor response. The effects of acclimation period lengths of 120 seconds and 240 seconds on optomotor response were similarly evaluated.
- Methods for assessing the prey capture ability of larvae were also assessed.

Optomotor Assay

Gauges a larvae's ability to respond to perceived movement by tracking their ability to follow moving stripes (Figure 1).

Results
- No significant differences were observed between acclimation period or age groups for the number of laps traveled with or against rotation (Figures 2A & 3A).
- No significant differences were observed between acclimation period or age groups for the latency period, measured as length of time before larvae began moving in the same direction as rotation once it had begun (Figures 2B & 3B).

However, from a practical perspective…
- Larvae tested with a longer acclimation period were:
  - more likely to move with rotation and less likely to move against rotation
  - more likely to have a longer latency period
- Older larvae:
  - spent more time traveling with rather than against rotation than younger larvae
  - were more likely to have a shorter latency period

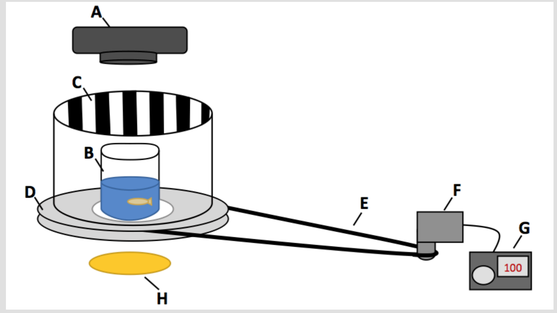


Figure 1. Components of the optomotor assay test chamber. A. GoPro Camera B. Larvae in beaker C. Stripes surrounding larvae D. Lazy Susan for stripe rotation E. Belt connecting Lazy Susan to motor F. Motor G. Speed setting H. Light source. Diagram created by Julie Krzykwa.

The adjacent QR code links to a video clip demonstrating a positive optomotor response, in which a larvae responds to movement of the chamber and swims along with the rotation of the surrounding stripes.

Effect of Acclimation Period on Optomotor Response

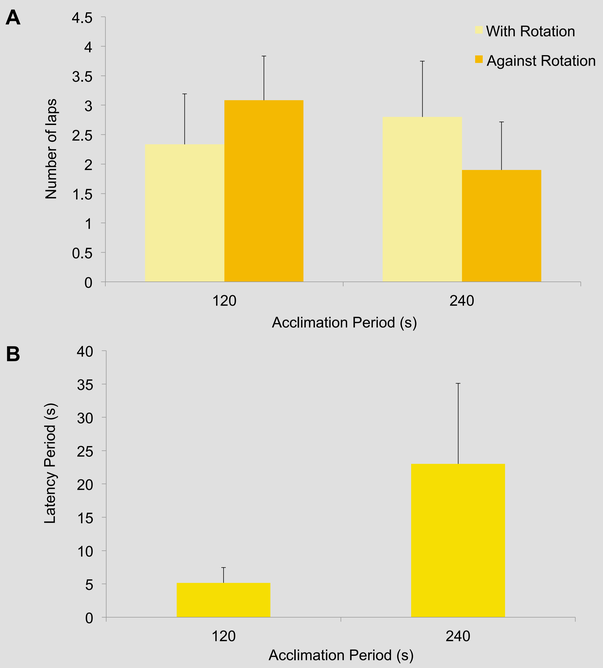


Figure 2. After an acclimation period of 120 seconds (n=6) or 240 seconds (n=5), larvae were assessed for **A.** The number of laps traveled around the optomotor chamber, both with and against the chamber rotation and **B.** Latency period, or seconds before a larvae begins moving with the rotation of the chamber after the rotation has begun. Error bars represent mean standard error.

Effect of Age on Optomotor Response

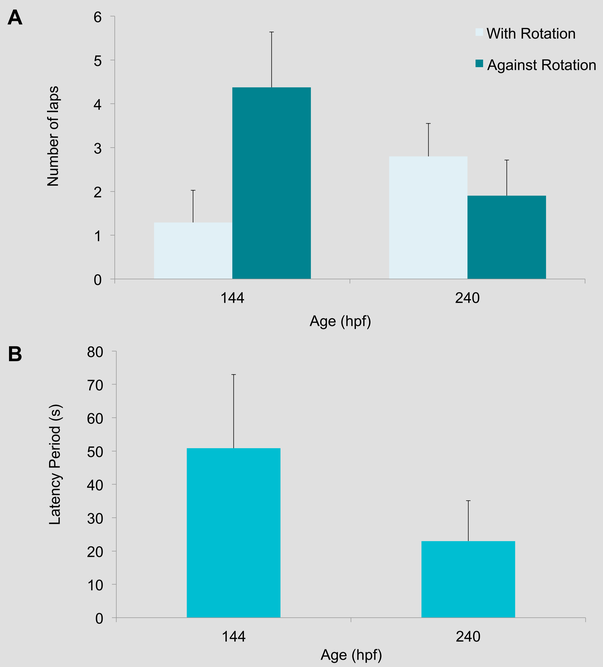


Figure 3. At 144 hours post-fertilization (n=6) and 240 hours post fertilization (n=5), larvae were assessed for **A.** Number of laps traveled around the optomotor chamber, both with and against the chamber rotation and **B.** Latency period, or seconds before a larvae begins moving with the rotation of the chamber after rotation has begun. Error bars represent mean standard error.

Feeding Assay

Gauges a larvae's ability to perceive prey movement by determining the number of prey consumed in a given period of time (Figure 4).

Results
- *Artemia nauplii* were placed into a test chamber at a density of 0.03 g *Artemia*/mL. Counting every specimen was unfeasible, but estimation using water samples proved to be a viable option.
- Over the course of one hour, a test dish of 10 larvae consumed approximately 310 *Artemia nauplii*, indicating a rate of approximately 31 prey per larvae in one hour.

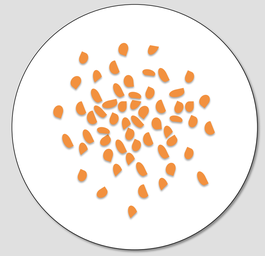
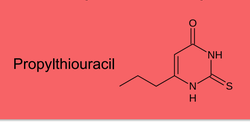


Figure 4. At the end of a one hour time period the remaining prey, *Artemia nauplii,* were collected on filter paper and counted using a dissecting microscope.

Future Directions

- While the feeding assay provided promising preliminary results, further testing with larger samples will help determine the sensitivity of this metric for detecting changes in visual function.
- The optomotor assay has the potential to be a compelling measure of both visual and neurological function, but it is also a complex enough test that further testing and development is needed for it to be a reliable source of information.
- Development of morphometric endpoints such as eye index, a calculation of the 2D single eye area divided by snout to vent length, will clarify the relationship between biological form and visual function.

- Gene expression analysis of eye development markers will establish whether changes are occurring on a molecular level, even if no significant differences can be detected in visual assays or morphology.
- Our goal is to develop these visual assays so that they can ultimately be used to test our hypothesis in future exposure experiments involving Propylthiouracil (PTU), a model thyroid disruptor, and perchlorate, an environmentally-relevant thyroidal EC.



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