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Daphnia as a Biosensor: Understanding the Sensory Biology of a Sentinel Species for Improved Toxicological Assessments

Overview

Goal

To describe the chemosensory capabilities of the sentinel species *Daphnia* for effective real time toxicological assessment of water born chemicals.

How?

- Use our knowledge of sensory biology to decipher the neurobiological properties of *Daphnia*.
- Link the effect different chemicals have on the feeding behavior of *Daphnia* to physiology and assess the effect these chemicals can have on the ecosystem as a whole.

Why?

In order to assess and predict the effects different chemicals can have in an aquatic system we need to develop a real time toxicological system that exploits an animals' ability to immediately respond to changing chemical conditions.

Main Hypothesis

Daphnia have chemoreceptors that allow them to respond immediately to changing chemical conditions, and that these immediate responses manifest themselves behaviorally.

Rationale

Since the early 1980's *Daphnia* has been used to assess chronic and acute effects of toxins in freshwater systems. To date little is known about its sensory capability and no one has linked behavioral response with chemosensory ability. The majority of procedures available for assessing chemical effects on an ecosystem take hours or days before the toxicity of a chemical becomes known. The end point for most of these tests is percent mortality after a set time period. Toxic events normally do not last for more than a few hours, and monitoring behavioral changes, such as feeding rate, can shed light on an animals' immediate response to the exposure rather than having to see if it survives it.

Daphnia are ideal candidates for toxicological research for the following reasons:

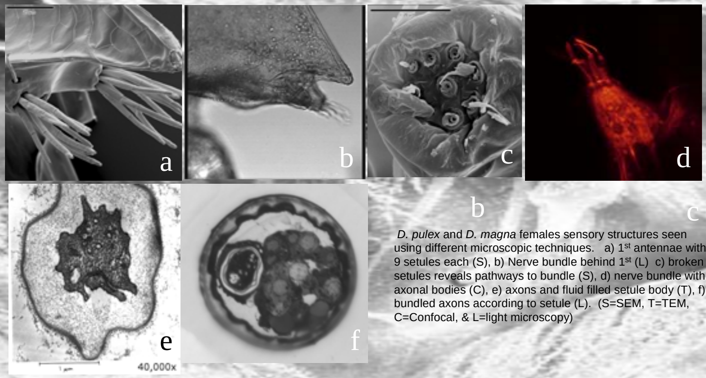
- Highly abundant in many freshwater ecosystems
- High sensitivity to chemicals – known as a sentinel species
- Can be cultured with ease in the laboratory
- Can asexually (clones itself) or sexually reproduce in the laboratory
- Abundant research has gone into describing its ecology
- The *Daphnia* Genome Consortium is working on making the genome available this year

Scientific Approach

Identification of Sensory Structures on *Daphnia*

Sensory structures of *Daphnia* will be investigated in *D. pulex* and *D. magna*. Both species are commonly used for toxicity assessments. *D. pulex* is a native North American species, and although *D. magna* is not, it has the advantage of being the largest known *Daphnia* species.

I hypothesize that most chemosensors must be located within the path of the feeding current, dictating the feeding rate of Daphnia.

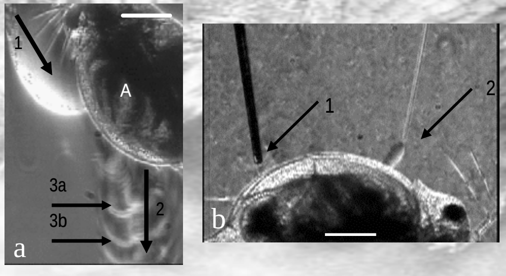


D. pulex and *D. magna* females sensory structures seen using different microscopic techniques. a) 1st antennae with 9 setules each (S), b) Nerve bundle behind 1st (L) c) broken setules reveals pathways to bundle (S), d) nerve bundle with axonal bodies (C), e) axons and fluid filled setule body (T), f) bundled axons according to setule (L). (S=SEM, T=TEM, C=Confocal, & L=light microscopy)

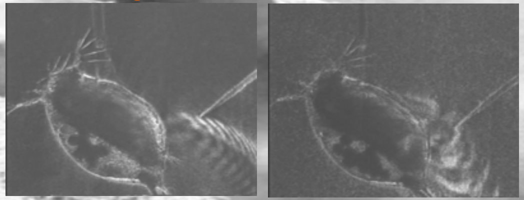
Development of Feeding Response Monitoring System

By measuring the rate at which *Daphnia* introduces and expels water into its feeding mechanism, we can assess the amount of exposure and behavioral response of the animal to the chemical. We measure this by means of a sensor placed in the outflow that detects each wave of water expulsed by the animal (see below).

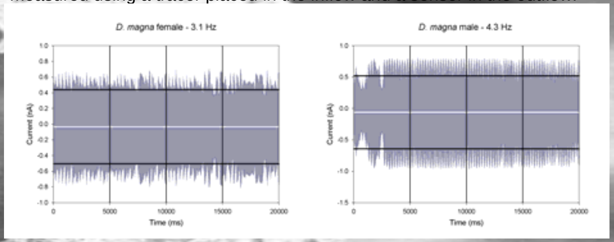
D. pulex tethered to a hair and viewed using the Schlieren system. Tracers delivered through a micropipette for visualization of the current created by the feeding appendages of *Daphnia*. Fig. a) Arrow 1- origin of laminar inflow, 2- direction of turbulent outflow, and 3a & b.- repetitive waves seen in outflow current. Bar = 0.2mm. Fig. b) Arrow 1 – microelectrode, Arrow 2 – micropipette.



Current Feeding Behavior Data



Differences in appendage beat frequency or feeding rate can be seen and measured using a tracer placed in the inflow and a sensor in the outflow.



Current data indicates that male daphnids have a higher feeding rate that females, these results are credible as males have a more active lifestyle and therefore a higher metabolic demand. The differences seen in these two graphs correspond to 72 more beats/minute for the male. (Penalva-Arana et al., in preparation)

Future Work

Application and Questions to be addressed:

-Link feeding behavior changes with exposure to different chemicals: Are emerging contaminants, such as pharmaceuticals, immediately detected and what effect do they have on *Daphnia*?

- Link chemosensory capabilities to genes in the *Daphnia* genome: Are genes for odor binding proteins present in the *Daphnia* genome and does their presence predict behavioral response?
- Map out the neuronal network of *Daphnia*, to make identify the location of sensors and their capabilities: Where are the sensors located and how quickly is the animal able to identify a chemical threat?
- Transfer the methodology of the feeding response monitoring system to other invertebrates with similar morphologies living in different environments: For example our system can be utilized to test the effect chemicals have on marine organisms, thus serve as a marine monitoring system.

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