

## BIO 315 – Invertebrate Biology: Laboratory & Field Manual

### Exercise 6: *Daphnia* Behavior and Physiology

#### Introduction

*Daphnia* sp. are common freshwater cladocerans, often classified with other tiny crustaceans as “microcrustaceans.” Cladocerans are commonly known as water fleas. Most species in the Order Cladocera are freshwater species, although there are some marine species. The classification of Cladocera is as an order within the Subclass Diplostraca within the Class Branchiopoda within the Subphylum Crustacea. *Daphnia magna* and *D. pulex* are both widely used experimental organisms in zoology, ecology, and toxicology. Ecological studies have determined their important role in aquatic food webs – they graze on phytoplankton and, in so doing, increase phytoplankton rate of production. At the same time, many insects and fish consume zooplankton, and the microcrustaceans are thus a key source of food for organisms at higher trophic levels in many aquatic habitats.

#### Anatomy and Behavior

Obtain an individual *Daphnia* using a pipet to extract one from the culture. For observation of anatomy, place the individual in a depression slide. For observation of behavior and locomotion, place one or a few individuals in a small watch glass. There may also be some prepared slides available. Consult your textbook and Figure 1 to review the anatomy of *Daphnia pulex*. Our species may or may not be the same, but the general anatomy is very similar among *Daphnia* species. Observe foraging behavior and swimming. Introduce a minor disturbance in the colony of *Daphnia* and observe anti-predator behavior.

From observations and review of your textbook answer the following questions:

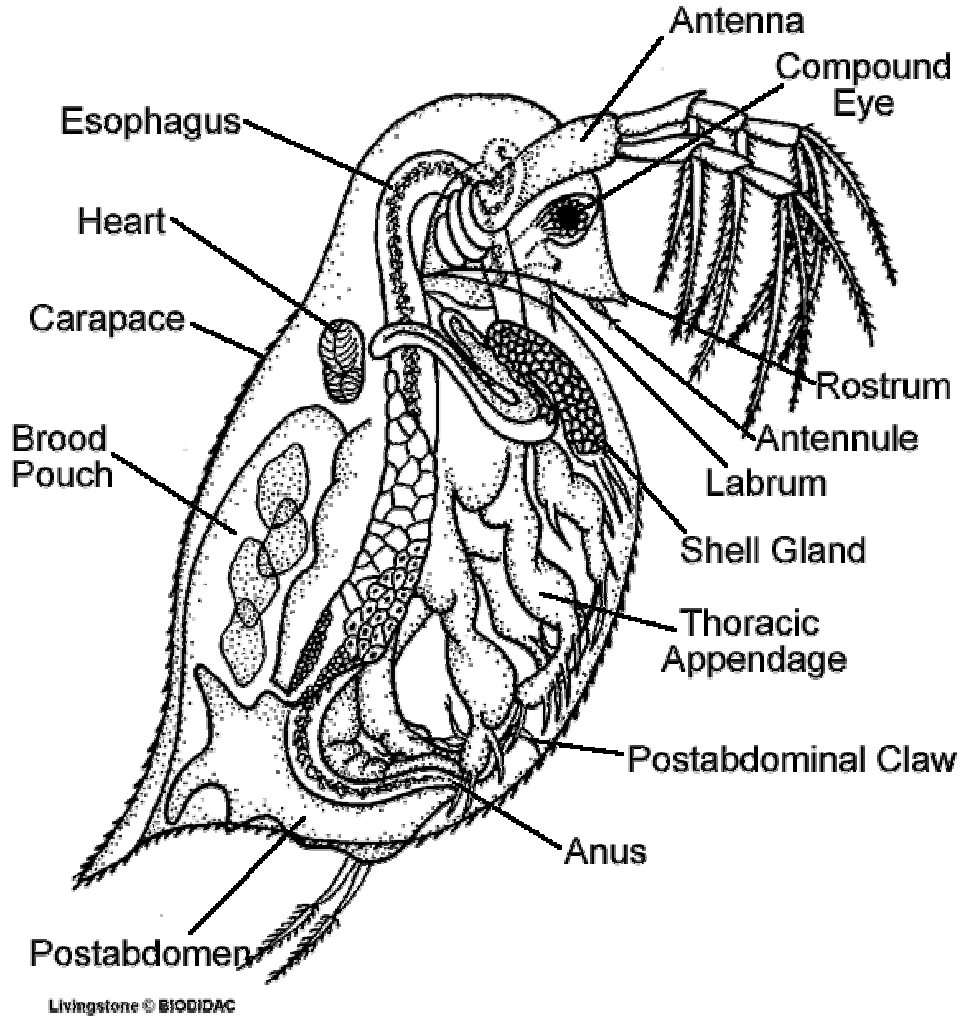
1. How do *Daphnia* swim and what appendages are used for locomotion?
2. How do they feed and on what do they feed?
3. What internal organs do you recognize?
4. Were you able to see any individuals with young? Describe if observed.
5. What anti-predator defense mechanisms (morphology or behavior) did you observe?

#### Phototactic Behavior

Many animals, including freshwater zooplankton, move toward light. That behavior is termed a positive phototaxis. In fact, many species of zooplankton have a diurnal vertical migration pattern, where they move up and down in the water column at different times of the day. Generally, the zooplankton are found in shallower water during the night. Movement toward light might have some adaptive advantages, and in freshwater ecosystems it may equate to movement toward shallower, warmer water that is rich in algae and microbes that zooplankton consume. There is a potential trade-off, however, because in these habitats where they are feeding they may become more vulnerable to their own predators. This is likely the reason why they are often found in deeper waters during the day so as to escape detection by visually hunting predators. Natural selection might favor one type of response to light when zooplankton do not perceive predators (low predation risk), and a different response when predator risk is high and have been perceived to be present. This trade-off may play a role in vertical migration as well, depending on the zooplankton’s ability to detect predators.

*Objectives*

1. To examine phototactic behavior and detection of predators in *Daphnia magna*.
2. To test for behavioral trade-offs in *Daphnia magna*.



**Figure 1.** Anatomy of *Daphnia magna*. Figure obtained from BIODIDAC, <http://biodidac.bio.uottawa.ca/>.

*Materials*

*Daphnia magna* or *D. pulex*  
ring stands and clamps  
rubber stoppers

glass tubes  
wide mouth pipets  
grease pencils

microscope lights  
aquarium with fish

*Procedures*

1. Obtain a clean glass tube and two rubber stoppers. Place a stopper on one end of the tube.
2. Mark off three equal sections of the tube and fill the tube almost to the top with spring water.
3. Add 20 *Daphnia* to the tube and put on the second rubber stopper. Clamp the tube to the ring

stand so that it is horizontal. Place the apparatus in a dimly lit room with diffuse lighting. You don't want to place it near a directional source of light.

4. If you are in a very dark room you may turn on a distant light source for this next step. Every two minutes for the next 10 minutes record the positions of the twenty *Daphnia*. If it is hard to see where they are, count as many as you can in each section.
5. After 10 minutes, clamp a microscope light to the ring stand above and at least 5 cm away from the tube. Angle it so that it shines on one end of the tube. Record the distribution of *Daphnia* every two minutes for the next 10 minutes.
6. Repeat the entire experiment once more. Calculate the mean percentage of *Daphnia* in each third of the tube over time for dark and illuminated treatments. Average over both trials.
7. A third trial may be done with water from a fish tank. Chemical cues from fish might act as a signal to *Daphnia* that there is a predator around. Repeat steps a-e with aquarium water.
8. Finally, if *Daphnia* and other zooplankton do indeed move up and down in the water column, how do we know if it is response to light or gravity? What experimental manipulation can we try to help separate the effects of light and gravity? Try adjusting our procedure to test the effects of gravity, or gravity and light simultaneously.

### **Physiology: Heartbeat at different temperatures**

In ectothermic organisms that do not metabolically regulate body temperature, ambient temperature may have a great effect on physiological processes. Growth, reproduction, and response to stimuli may all be temperature-dependent.

#### *Objectives*

1. To experimentally determine the effects of water temperature on the heartbeat frequency of *Daphnia magna*.
2. To apply the results to general conclusions regarding the effects of temperature on physiological processes in ectotherms.

#### *Materials*

<i>Daphnia magna</i>	wide mouth pipets	Vaseline
watch glasses	stopwatches	dissecting microscopes
ice water	thermometers	water baths at different temperatures

#### *Procedures*

1. Place a small drop of Vaseline in the center of a watch glass. Fasten an individual *Daphnia* by one valve of its carapace to the Vaseline. Be careful that water circulates between the valves.
2. Observe the heartbeat at different temperatures.
3. Use multiple *Daphnia* at each temperature. Record the number of heartbeats in 15 seconds twice for each individual at each temperature, using degree differences of about 3°C, from 0°C to 30°C.
4. Perform a regression analysis of heartbeat at each temperature.

### **Preparing Your Report**

This will be your second scientific report. You will write up your results for the phototactic and temperature experiments. Include Introduction, Materials and Methods, Results, Discussion, and References. We will also go over the statistical procedures in class.

### *Basic Guidelines*

1. Prepare summary tables and figures. Identify key points around which to structure your paper.
2. Save everything in more than one place and save any early hardcopies you prepare.
3. Prepare an outline. The more time you take to construct it carefully, the more logical your paper will be and the easier it will be to turn the outline into text.
4. Prepare at least two drafts of text. The second draft may be ready for your instructor.
5. Avoid jargon. State your meaning in a simple and concise manner. Reading aloud helps identify unnecessary words and awkward phrases. Concise, economical sentences drive their messages home forcefully.
6. Use the “spelling and grammar check” feature of your word processor! By removing errors, you help the reader focus on your message, rather than by distracting them with your errors.

### *The Form of a Scientific Report*

Both tradition and common sense support the fundamental structure of scientific report consisting of an introduction, materials and methods, results, discussion, and references cited. In preparing your reports, you may work together in your groups to compare and compute results, but each individual must write their own report. We'll go over preparation of tables and graphs.

#### *1. Introduction:*

As before, this is your statement of purpose. Introduce the problems and questions addressed in your experiment (why did you do it?). Make a statement of your hypothesis or question. For our purposes, an introduction of 1-2 paragraphs should be sufficient. Use information from class, the handout, and the text to provide background, including the test species and the protocol.

#### *2. Methods and Materials:*

For M & M, cite the handout for general methods, but not everything we did was in there, and we made some modifications. Provide a brief summary of the methods, perhaps with a list of highlights. Be very clear about the treatments and modifications. Describe in detail only materials and methods not included in the handout. Ask me if you have questions about any specifics.

The purpose of this section is to allow the reader to critique or repeat the experiment performed. Usually you would address the following questions: What did we do? How did we do it? Where did we do it? What organisms did we study? What equipment did we use? How did we analyze the data? How did you set up your experiment? How many experimental groups did you have? How did you measure the effect you studied? In a laboratory exercise, however, the methods will be presented in this manual or by the instructor. To repeat them in your report would only be busy work. I will ask you only to describe materials and methods **not** included above or in another handout. Anything that is different from methods stated in the manual must be explicitly stated. Ask me if you have questions about any specifics.

#### *3. Results:*

This section presents the key findings and experimental results. Show the data that relate to your stated hypotheses and questions and that make specific points. Each finding should be briefly discussed and perhaps related to other results, although you should save discussion of the

truly significant conclusions for the next section. The object here is to describe in a clear and logical way what you discovered. **You must provide text, not just tables and figures!**

Include at least two graphs (one from each experiment; see Appendix A). The graphs should show the average number or percentage (+/- s.d.) of *Daphnia* located in each section of the tube at any time point for each treatment, or heartbeat at any particular temperature. Present this in the form of line graphs or histograms, whichever you feel is most appropriate. When possible, calculate standard deviations and include them in the graphs. Review the instructions from the first laboratory report for presentation of your data.

Although we did not perform any statistical analyses, you should include a regression analysis for your heartbeat data, and you can do that in Excel. I will demonstrate in class. Our null hypothesis is that there is no effect of temperature, or no effect of light, depending on the experiment, and rejection of the null leads to acceptance of our alternative hypothesis, the hypothesis that there is a difference among treatments and control.

#### 4. *Discussion:*

This section should contain a detailed examination for the few (usually two to three) major issues illustrated by the experiments. Point out why the research was significant, what general conclusions can be drawn, and the relation between your findings and basic scientific principles and concepts. Consider the following questions: How do your results support our hypotheses? What new questions come to mind after examining the results? What does the study say about the ecology and behavior of *Daphnia* in ecosystems? Other issues that come to mind should be included. How would you perform the experiment differently to help eliminate error? What new questions come to mind after examining the results?

#### 5. *References:* Include complete citations of any works you cite. Use the standard format below (find the example that best fits your source).

Merritt, RW, Dadd, RH, & Walker, ED (1992) Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annual Review of Entomology* 37:349-376.

Power, ME, Parker, MS, & Wootton, JT (1995) Disturbance and food chain length in rivers. Pages 286-297 in G. A. Polis and K. O. Winemiller, editors. *Food Webs: Integration of Patterns and Dynamics*. Chapman and Hall, New York, New York, USA.

Carpenter, SR, & Kitchell, JF (1993) *The Trophic Cascade In Lakes*. Cambridge University Press, London, England.

Davidson College Department of Biology (1999) <http://www.bio.davidson.edu/index.html>.

Rith, J (1988) Plant succession on abandoned railways in rural New York State. Proceedings of the 73<sup>rd</sup> Annual Meeting of the Ecological Society of America, Davis, CA.