THE INFLUENCE OF TEMPERATURE ON THE HEART RATE OF CRAYFISH

PURPOSE
This is one of the few labs in which we will explicitly outline the purpose for you. There are actually three purposes behind this lab. They are to:

- give you an opportunity to experiment with a physical parameter that has a TREMENDOUS impact on virtually all aspects of physiology: Ambient Temperature.
- introduce you to PowerLab hardware and software.
- begin refining your lab note taking skills

To help with the first point, we have assigned some background reading in your text. In terms of the second point, you just did this in the Intro to PowerLab exercise. To help with the third point, we have included some notes within the text to remind you to include specific points within your lab notebook. These reminders are preceded by a *

* Include a sketch of the equipment set up in your lab book that is of sufficient quality that you could use it to set up the same experiment at a later date

BACKGROUND READING

ANIMALS & EQUIPMENT

Living material
The crayfish, Procambarus clarkii.

Equipment
Macintosh computer; PowerLab virtual chart recorder; impedance converter; pin electrodes; BNC cables and other assorted cable connections; glass dishes; ice bath; digital thermometer; crayfish restraining devise (you will come up with the exact set-up that works). (*Include a sketch of the equipment set up in your lab book that is of sufficient quality that you could use it to set up the same experiment at a later date)

STANDARD EXPERIMENT

*Remember to also make comments on the screen with PowerLab while collecting data
1. Set up the equipment so that output from the impedance converter feeds into Channel 1 of PowerLab. Since you will only be using one channel, adjust the screen such that only Channel 1 is showing.
2. Impedance Converter: The controls
   • ON-OFF-TEST: Turns the instrument on and off and tests the quality of the battery.
   • BALANCE: This control is a precision, 10-turn potentiometer which adjusts the IMPEDANCE CONVERTER oscillator for proper operation. For proper operation, the needle must hover around zero. The instrument must be re-balanced to accommodate any major change in impedance (which can occur if the electrodes get moved). It is normal to have to re-balance the instrument during a physiological experiment (especially when the subject is uncooperative) so do not be hesitant to repeatedly re-adjust the balance.
   • AC-LONG/SHORT: You will mostly be using the AC output in this class.
   • AC-LONG: Provides a coupling constant of 1 sec (i.e. cuts off all signals with frequencies longer than 1 sec).
   • AC-SHORT: Provides a coupling constant of 0.1 sec (i.e. cuts off all signals with frequencies longer than 0.1 sec).
   • SIZE: This knob adjusts the amplitude (= gain) of the signal.
   • OUTPUTS: This instrument uses BNC connectors.
   • INPUT: The source impedance (i.e. biological specimen) is connected by electrodes + wires to the green binding posts on the back of the instruments.
   • CALIB: This switch is connected to a 0.25 ohm resistor. Depressing this switch shorts out the resistor, resulting in a 0.25 ohm change. Normal physiological impedance changes range from 0.2 ohm (cardiograph) to 5 ohm (respiration).

3. Impedance Converter: How to use one
   • Connect the output of the Impedance Converter to a recording device.
   • Attach electrode cables to the green input binding posts.
   • Place the electrodes on either side of the biological structure of which you are measuring impedance. Try to make them as secure as possible without harming your subject.
   • Adjust the balance to hover around zero and begin recording data.

4. Obtain a crayfish and place it in dish containing fresh water to a depth of about 1 inch. Determine the temperature of the water. (* record the sex of the crayfish and
the temperature of the water. It would also be a good idea to make a note of the animal's behavior during this initial phase of the experiment.)

5. When the crayfish has calmed down a bit, insert the pin electrodes on either side of the heart (see the figure below and also Figure 23.22 in your text).

6. Begin collecting data, adjusting the chart speed and Y-axis scale until the display is satisfactory. (* write down these settings for later reference) Collect data for 2 - 3 min. (* carefully record the behavior of the crayfish during this time because, as you know, behavior will influence heart rate; why is it better to record behavioral observations directly on the computer in the Chart program as you collect data than in your notebook?)

7. Carefully pack the animal in ice and let rest for about 10 min. (To maximize efficiency, go to #9 while the animal is cooling).

8. Insert the temperature probe and record the temperature. Collect data for another 2 - 3 min.

9. Obtain another crayfish and repeat steps 4 - 8 for a sample size of at least 2.
PRE-LAB QUESTIONS  
(Due at the start of the lab)

1. In general, how do physiological rate processes (e.g., heart rate, breathing rate, etc.) change with changes in temperature? Be as complete as possible.

2. Why might a physiologist be interested in measuring an animal's heart rate? Hint: think about what heart rate could reflect.

3. What are three factors other than temperature that could influence an animal's heart rate?

4. What is a typical habitat for crayfish? Given this type of environment, do you think that crayfish are likely to experience a wide range of temperatures on a daily and/or annual basis?

POST-LAB  
(Due next week in lab)

1. Generate one graph that summarizes the main result of your study on how temperature influences crayfish heart rate.
   ➢ The graph must be computer generated and made as "pretty" as possible (i.e., don't just slap something together in StatView and print it out).
   ➢ Don't forget to label the axes and include units.

2. Articulate the main results shown in the graph in a short paragraph that could serve as the basis for a figure legend (you'll learn more about the elements that need to be included in a real figure legend later).

3. Calculate a $Q_{10}$ value for heart rate for each animal used in your study (this calculation is introduced in your reading and will be covered in lecture).
**NOTE:** If you are going to write this paper up for credit, you need to incorporate data from additional lab groups that performed the same experiments. This will give you a larger sample size and make your analysis much more meaningful and fun. Your professor will facilitate data exchange among lab groups.

The first step in turning this study into a research paper (or even deciding if you want to go through the trouble) is determining what your data are telling you and how to best present them. Some questions to consider when thinking about how to analyze and present these results are:

- Is it important to show raw data? Why or why not?
- What is the best way to show the trends that I want to emphasize? For example, if you think that your data show that size or temperature influences the metabolic rate of *P. clarkii*, how would you best illustrate this to convince your readers? Look in your assigned reading for some effective ways to present these types of data.
- How should I deal with any outliers in my data set?
- You should have enough data points to analyze your data statistically. What aspects of your data would it be logical to compare? What test(s) will you use? How will you present the statistical results?

Some questions to consider when thinking about how to pitch your study to readers.

- Is the crayfish typical of an ectotherm in terms of responses to cold? In terms of effects of body size?
- Are the conditions to which we subjected the animals similar to those that the animals might encounter in the wild? If not, why have we done this experiment?

Discussions should definitely include a comparison with previous studies. Another element you could bring into the discussion concerns the experimental design. What do you think your results would look like if the animals were *acclimated to* or *raised in* the different temperatures rather than acutely exposed?