The Effects of Temperature and Body Size on Crayfish Metabolism

Objectives

- To demonstrate the relationship between body size and oxygen consumption in the aquatic poikioltherm (*Procambarus clarkii*).
- To observe the effects of temperature on metabolic processes in poikiothermic organisms using their Q_{10} indexes.
- To understand the concept of the Q_{10} effect.

Introduction

Biological activity is based on metabolic reactions that are influenced by temperature. The body temperature of active animals ranges from -2°C to +50°C, although some animals may survive at lower or higher temperatures. Many terms have been used to describe the heat exchange between organism and their environments. The terms "warm-blooded" and "cold-blooded" are vague in their attributes, because there is no distinction as to at what body temperature differentiates the two. The terms homiotherm and poikilotherm relate to warm-blooded and cold-blood respectively. A homiotherm has a constant body temperature (from the Greek "homoios," which means similar). Poikioltherms, on the other hand, have variable body temperatures (from the Greek "poikilos," which means changeable).

Temperature is an important physical property of the environment; it measures the motion and kinetic energy of molecules. The average kinetic energy of molecules is proportional to the absolute temperature by the equation: $E=1.5kT$, where k is the Boltzmann constant and T is the temperature measured in Kelvin. At a certain temperature, a fraction of the molecules have kinetic energies that exceed the energy of activation and the molecules collide. Using the Arrhenius equation, a specific rate constant, k, can be determined. The reaction rates can then be related to temperature. The ratio of k_1/k_2 for a 10° difference in temperature is referred to as the Q_{10}. The Q_{10} should decrease at increasing temperatures and increase with activation energy. In this lab exercise, k_1 and k_2 are metabolic rates (oxygen consumption) at temperatures t+10 and t, respectively.

In aerobic oxidation , the amount of heat produced is related to the quantity of oxygen consumed. Thus measurements of oxygen uptake can be used to calculate metabolic rate. In closed system respirometry, an animal is confined to a closed, water or air-filled chamber in which the amount of oxygen consumed is measured over designated periods of time. Oxygen consumption is revealed by successive determinations of the decreasing mount of oxygen dissolved in the water (or present in the air, for air-chambers). The measurements can be obtained using an oxygen electrode or by performing a chemical titration.

Aside from the influence of temperature on metabolic rate, another important determinant is the body weight of the organism. Oxygen consumption per individual increases indirectly as a function of its
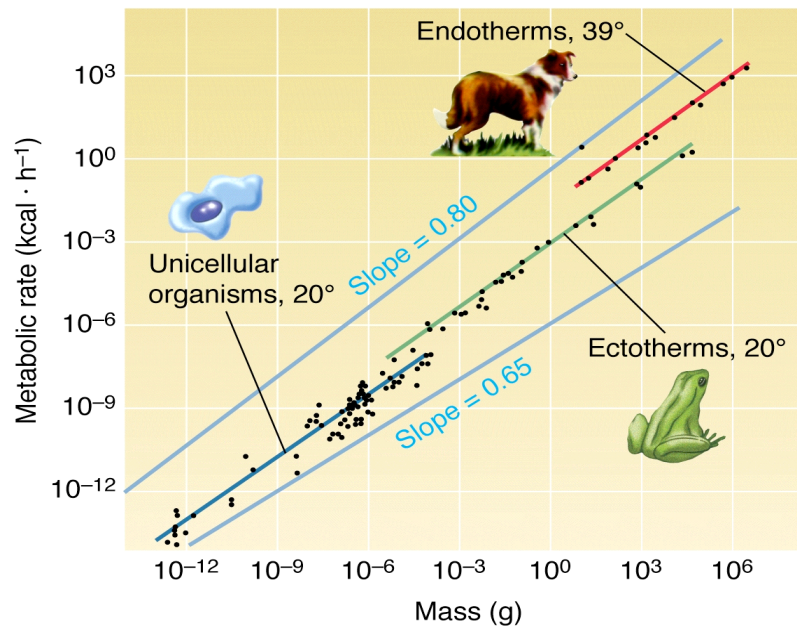
weight, ($W^{0.75}$). A log-log plot of body surface area (cm^2) against body weight of animals yields a straight line with a slope of approximately 0.67, in other words surface area is also an exponential function of body weight ($W^{0.67}$). Therefore, biologists have suggested that the metabolic demands of an animal are a direct function of their surface area.

In today's laboratory exercise, we will be using a closed system respiratory chamber and the Winkler reagent testing system to measure oxygen consumption of crayfish and determine their metabolic rate. 250-mL Erlenmeyer flasks fitted with watch glasses will serve as respiratory chambers. The crayfish tank will be continuously aerated, so that the water will be saturated with O_2. Remember to take temperature readings of the tank water.

Procedure

Setting-up the Respiratory Chambers

1. Fill two 250-mL Erlenmeyer flasks half full with tank water. Please use the beaker next to the tank to fill your chambers. Do not dip your flasks into the tank.
2. Select two crayfish, of varying sizes, from the tank. Place one in each chamber.
3. Line the rim of the flask with petroleum jelly and fill the chamber to the top with tank water. You want the water to come up over the lip (adhesion and cohesion properties of water).
4. Slide the flat side of the watch glass over the lip of the flask. Try not to get any air bubbles.
5. Keep the crayfish in their chambers for thirty minutes.

6. At the end of the test period, remove 60-mL from the respiratory chamber and put it in a biological oxygen demand bottle. When pouring water from one container to another, carefully decant the water down the side of the vessel to avoid mixing air with the sample.
7. Determine the O_2 concentration with the outline protocol below.
8. Make sure that steps six and seven are done for the large crayfish and the smaller one, as well as a control from the water tank. The control serves as your low temperature control.

Re-setting Chambers and Temperature Exercise

1. Replenish the water in the respiratory chambers by refilling with tank water. Place the chambers in the water bath.
2. Fill a third flask with tank water and use this as the control. Place this flask in the water bath as well.
3. Allow the water in all three chambers to equilibrate with the water bath (about fifteen minutes), aerating all three chambers continuously.
4. Once the temperature stabilizes, close the two test chambers as described earlier. Note the time.
5. Allow the test to run for 30 minutes.
6. Remove 60-mL from the control flask and put it in a biological oxygen demand bottle. Determine the O_2 concentration with the outlined protocol below. This sample serves as the high temperature control.
7. At the end of the test period, remove 60-mL from the respiratory chamber and put it in a biological oxygen demand bottle.
8. Determine the O_2 concentration for both the large crayfish and small crayfish at the higher temperature.
9. Using the balance in the lab, measure the weight of each crayfish to the nearest 0.01 gram.

Protocol to Measure Dissolved Oxygen

1. Make sure the biological oxygen demand bottle is filled with 60-mL of sample.
2. Empty the contents of a Dissolved Oxygen 1 packet into the bottle and a Dissolved Oxygen 2 packet into the bottle.
3. Put the stopper into the bottle, being careful not to trap any air. It works best by dropping the stopper from two inches above.
4. Invert the bottle a few times to mix the chemicals. Place the bottle on the lab bench and allow the brown precipitate to settle to the bottom of the bottle.
5. Remove the stopper and add the contents of one Dissolved Oxygen 3 packet to the bottle. Please be careful not to get this on your clothing and or skin.
6. Replace the stopper and slowly invert the bottle. The precipitate will dissolve, leaving a transparent yellow solution.
7. Pour 40-mL of this prepared sample into a clean 100-mL beaker.
8. While swirling the beaker to mix its contents, slowly add the sodium thiosulfate from the buret in a drop-wise fashion.
9. Note the amount of sodium thiosulfate used to titrate the solution from yellow to colorless. For the fully aerated low temperature control, you may need 3 or more -mLs.

Results

Table 1: Low Temperature Results

	Volume of Water in Flask (mL)	Weight of the Crayfish (grams)	Temperature (C)	Volume of Titer (mL)
Low Temperature Control				
Crayfish 1 Chamber				
Crayfish 2 Chamber				

Table 2: High Temperature Results

	Volume of Water in Flask (mL)	Weight of the Crayfish (grams)	Temperature (C)	Volume of Titer (mL)
High Temperature Control				
Crayfish 1 Chamber				
Crayfish 2 Chamber				

Calculations: Total Available O₂ in the Respiratory Chamber

Since crayfish are mostly water, 1-mL of water is equal to 1 gram of crayfish.

Volume of Crayfish 1 =

Volume of Crayfish 2 =

Calculate the volume of the chamber using the following equation:

$$Volume_{(Chamber\ in\ liters)} = Volume_{(Flask\ in\ liters)} - Volume_{(Crayf\ is\hbar\ in\ liters)}$$

Respiratory Chamber 1 (low temperature)=

Respiratory Chamber 2 (low temperature)=

Respiratory Chamber 1 (high temperature) =

Respiratory Chamber 2 (high temperature)=

Calculate the Concentration of O$_2$

Use the following equation to calculate the Concentration of O$_2$:

$$\frac{mg\ O_2}{liter} = \frac{8000 \times (volume\ of\ titer) \times (normality\ of\ sodium\ thiosulfate)}{(volume\ of\ sample\ titrated)}$$

In our exercises the normality of the sodium thiosulfate is 0.01 N and the volume of sample titrated is 40 -mL

[O$_2$] in the Low Temperature Control=

[O$_2$] in the Low Temperature Respiratory Chamber 1=

[O$_2$] in the Low Temperature Respiratory Chamber 2=

[O$_2$] in the High Temperature Control=

[O$_2$] in the High Temperature Respiratory Chamber 1=

[O$_2$] in the High Temperature Respiratory Chamber 2=

Calculate the Quantity of O$_2$ Consumed by the Crayfish

Use the following equation to calculate the quantity of O$_2$ Consumed by the Crayfish:

$$mg\ O_2\ consumed = \left[O_2\right]_{(Chamber\ Control)} - \left[O_2\right]_{(Chamber\ Flask)} \times Volume_{(Chamber\ in\ liters)}$$

Low Temperature Crayfish 1 =

Low Temperature Crayfish 2=

High Temperature Crayfish 1=

High Temperature Crayfish 2=

Determine the Metabolic Rate for the Crayfish

Use the following equation to calculate the metabolic rate of each crayfish:

$$\frac{mL\ O_2}{grams/hour} = \frac{(mg\ O_2\ consumed) \times (60\ \min)}{(1.43) \times (wt\ of\ crayfish) \times (duration\ of\ test)}$$

Metabolic rate of low temperature Crayfish 1=

Metabolic rate of low temperature Crayfish 2=

Metabolic rate of high temperature Crayfish 1=

Metabolic rate of high temperature Crayfish 2=

Calculate the Q_{10} for both Crayfish

Use the following equation to calculate the Q_{10} for each Crayfish:

$$Q_{10} = \left(\frac{k_1}{k_2}\right)^{10/(t_1 - t_2)}$$

Where k_1 and k_2 represent metabolic rate.

Q_{10} of Crayfish 1=

Q_{10} of Crayfish 2=

Group Data and Discussion

1. Make a graph to display the relationship between metabolic rate and body mass of the Crayfish. Plot the metabolic rate on the y- axis and mass on the x- axis.
   a. Are there any correlations between body mass and metabolic rate? If so, what are they?

	Large Crayfish Metabolic Rate		Small Crayfish Metabolic Rate		Large Crayfish Q_{10}	Small Crayfish Q_{10}
	Low Temperature	High Temperature	Low Temperature	High Temperature		
Group 1						
Group 2						
Group 3						
Group 4						
Group 5						
Group 6						
Group 7						
Group 8						
Group 9						
Group 10						

2. Define the terms thermoregulator and thermoconformer. Based on the data collected in this lab, are Crayfish thermoregulators or thermoconformers?

3. Analyze the Q_{10} values from the class. What can you conclude about this data?