Lab 3: Allometry and Temperature in Crickets

Please Read and Bring With You to Lab

What you should bring:

This handout

What you will be provided:

Live crickets of various ages (instars)
Vials for holding crickets
Electronic balance
Stopwatches
Beakers (250-500 ml)
An ice bath
Small paint brushes or needle probes
Thermometers

Objectives:

- To collect and analyze quantitative data using linear regression.
- To create predictions based on hypotheses and experimentally test them.
- To understand the allometric relationship between body size and other variables such as surface area.

Preparation:

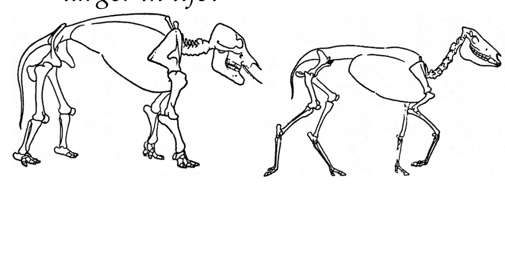
Students should read this handout and review Chapter 5 in Molles.

Introduction to Allometry

Everything about the biology of an animal is influenced by its body size, including its physiology (e.g., heart rate, respiratory rate, metabolic rate, growth rate), anatomy (e.g., organ mass, blood volume, surface area), and ecology (e.g., diet, home range size, life span, population density). As organisms increase in size, either during individual development or in the evolution of a species, the size and function of different parts typically grow at different rates. Such a difference in shape or function associated with changes in size is called **allometry**. The opposite of allometry is **isometry**, where changes in size produce no changes in shape or proportions. Scale models of animals would, if accurate, show an isometric relationship with the actual animal. On the other hand, animal growth is almost always allometric: juvenile vertebrates tend to have proportionately larger heads and eyes and shorter limbs than adults, for instance.

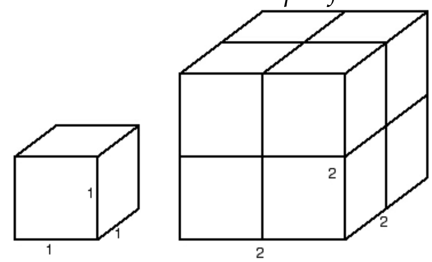
Similarly, species tend to show allometric variation as well. Compare the skeletons of two prehistoric mammals (Figure 1). Both skeletons are drawn to the same size, but it should be immediately obvious that the left skeleton represents a much larger animal due to its proportions such as thicker bones.

Figure 1. *Two different-sized mammals drawn at the same size. Which is larger in life?*



One of the principle driving factors for allometric growth and variation is geometry. As a three dimensional object increases in length, so that its shape remains the same, the surface area will increase with the square of the length increase and the volume and mass with increase with the cube of the length increase. Thus, a doubling of length will quadruple the surface area and increase the volume 8x (Figure 2). This will have profound biological effects. For example, the amount of body heat than an animal generates is roughly proportional to its mass, but the ability to retain or shed that heat is proportional to its surface area. Large animals have less surface area in proportion to their body mass and thus have more difficulty getting rid of excess heat. This is why large animals such as elephants and rhinos are often hairless (they have proportionately little surface area and so have little trouble conserving heat but difficulty getting rid of it). To compensate for the effects of scaling, animals may often evolve different shapes at different sizes. Elephants have large ears to increase surface area and shed heat, while small animals in cold climates will have very short ears to help decrease surface area.

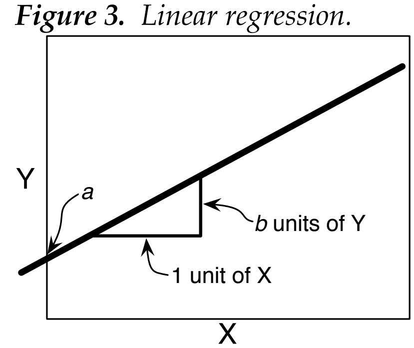
Figure 2. *Length, area, and volume relationships of a cube*



Overall, the study of allometry is the study of how various characteristics of organisms change relative to one another, particularly in response to overall size. To understand allometric relationships, we need to go beyond simply determining if there is a positive or negative relationship between two variables to determining the exact mathematical relationship between variables.

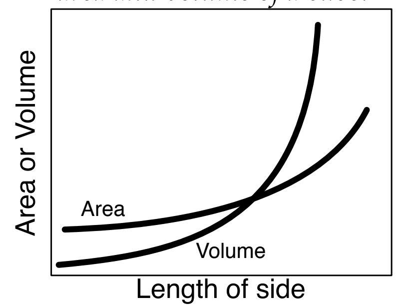
To describe a linear relationship, we can use **linear regression**. This analysis determines if the relationship between the independent variable X and dependent variable Y can be described as a straight line. It can be used to predict values of Y for a given value of X using the equation: $Y = a + bX$, where b is the slope of the line (the units of increase [or decrease if negative] in Y for every unit increase in X) and a is the Y-intercept (where it crosses the Y axis) (Figure 3). This equation describes an *isometric* relationship.

Figure 3. *Linear regression.*



In allometry, the relationship between X and Y may not be linear, but instead curved. Most commonly, that relatioship will be a power function, where X is raised to some power other than 1. The general equation for such allometric relationships is: $Y = aX^z$. The letter a indicates a constant that essentially converts X to Y (it can be used to convert among different units of measurement, for example). The exponent z is the **scaling factor**: it indicates how much faster (or slower) one variable (Y) changes in response to changes in the other (X). Unless the exponent z is 1 (which indicates isometry), the relationship between X and Y will a curved line instead of a straight line. For example, the relationship between the length of a cube (X) and its surface area (Y) is $Y = 6X^2$, while the relationship between the length and volume of a cube is $Y = 1X^3$ (Figure 4).

Figure 4. *Allometric relationships between length and area and volume of a cube.*



Since curved lines are more difficult to describe and visualize mathematically, we can alter the equation to form a line by taking the base-10 logarithm of both sides.[1] Doing that to our general allometry equation, $Y = aX^z$, we get:

$$\log Y = z \bullet \log X + \log a$$

Note that log a is still a constant (the intercept of the line), so we can replace it with another letter, such as m. If we plot log Y against log X, we will obtain a straight line[2] with slope z, the scaling factor. Alternatively, instead of taking logarithms, we can plot X and Y on a logarithmic scale (which can be done in software such as *Microsoft Excel*).

Body Size and Temperature

Temperature is a critical aspect of the environment for all organisms. If body temperatures are too low, metabolic processes are slowed and the organism may not be able to respond quickly enough. Too high, and enzymes can be denatured and stop working. Thus, most species must keep their body temperatures within relatively narrow limits in order to function.

Endothermic animals (primarily birds and mammals) can use metabolic heat (endothermy) to maintain fairly constant body temperatures (homeothermy). Most organisms are **ectothermic**, however, deriving body heat from external sources. For most small animals, this also means that their body temperature varies with environmental temperature, that is, they are **poikilothermic**.

Body size is a limiting feature in determining the response of a poikilothermic organism to changes in environmental temperature. Small organisms have relatively large surface areas relative to mass compared to large organisms, and thus gain or lose heat more rapidly. This, in turn, affects how quickly organisms can respond to changes in environmental temperature. Specifically, we would expect surface area-volume relationship of organisms to be the primary determinant of how it responds to changes in temperature. The amount of heat to be lost is directly related to the mass, and thus, volume, of the organism, while the rate at which it can exchange heat is related to its surface area. Since area increases with the square of length, and volume/mass with the cube of length, the relationship between surface area and mass should be:

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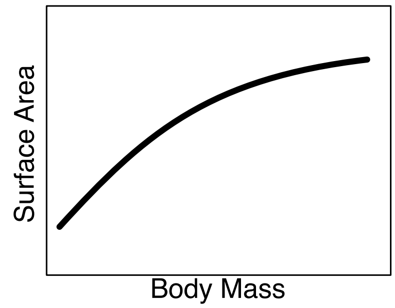
[1] Recall that taking the log of two values that are multiplied is the same as the sum of the logs of each variable added together: thus, the $\log(A \times B) = \log A + \log B$. Similarly, the log of a variable with an exponent is equal to the exponent times the log of the variable: $\log(A^B) = B \times \log(A)$.

[2] The line may not be straight if the exponent is not constant but actually varies across values of X.

Area = (constant)xMass$^{2/3}$ (Fig. 5)

We will examine how size and thermoregulation interact in a poikilothermic organism, the common house cricket, *Acheta domestica*. If allometric changes in the surface-volume ratio is the main factor determining the rate at which crickets lose body heat, then the scaling factor for the relationship between the rate of heat loss and body mass should be ⅔ (0.67). If the scaling factor deviates significantly from this value it suggests that crickets may be changing shape or physiology as they grow.

Figure 5. *The allometric relationship between body mass and surface area (z = 0.67).*



Procedure

1. You will work in groups of 3 for this lab. You can organize how you will gather the data as a group, but you will need to ensure all members of your group have the data (and may need to record the data on a class computer.
2. Colonies of commercially purchased crickets of varying sizes (instars) will be provided in the lab.Retrieve your crickets from the colonies as you need them by trapping them in the provided vials.
3. Select a cricket and weigh the vial plus cricket on the balance.
4. Place the cricket in a beaker that has been placed in an ice bath on you lab bench. Immediately note the time or start a timer.
5. While the cricket is in the beaker, one member of your group should weigh the vial without the cricket. Calculate the cricket weight by subtracting the vial weight from this total weight.
6. Continue to observe your cricket until it stops moving.
7. When the cricket has not moved for 30 seconds, gently touch the cricket with a small paintbrush or probe, especially at the paired sensory structures, the cerci, that are located at the rear.
8. If the cricket moves when touched, continue to observe the cricket until it no longer moves.
9. If the cricket did not respond to the touch of the probe, note the time, and touch the cricket again 30 seconds later. If it moves at this point, continue observations as before. If the cricket does not move again, wait another 30 seconds to try again.
10. If the cricket has not moved for 90 seconds even when being touched by the probe, record the time until it stopped moving (excluding the last 90 seconds) and return the cricket to its vial or other container as indicated by your instructor.

11. Repeat the procedure with as many additional crickets as time will allow. Remember: more data mean less chance of making a Type II error.

12. Your instructor will determine if you will be analyzing only your own data or the class data. If the latter, record the data on the laptop in class.

Analysis

You should examine the untransformed data first (time and mass) to determine if there is a relationship between the two variables, and if that relationship appears linear or curved. If linear, you can calculate the linear regression to determine the intercept and the slope of the relationship. The regression analysis will determine if the slope is significanly different from 0.

If the data appear curved, you should apply a log-transformation to the data to obtain the power function. Take the base-10 logarithms [in *Excel*, =LOG10(array)] of both the cricket mass and the time to stop moving. Then use these log-transformed values in the regression analysis. The slope of the log-log equation will give the scaling factor exponent.

Use scatterplots to best represent your data. You have several options for how to present your data:

- Use the original data on linear axes without transformation. Use the power trendline option (or use a linear trendline, but only if you are confident that the relationship is isometric [the scaling factor is close to 1.0]).
- Plot the original data, but use log-scaled axes on the graph (In *Excel*, click on an axis and check the "logarithmic scale" box). Use the power trendline.
- Plot the log-transformed data (do not use log-scaled axes if you are plotting data that has already been log-transformed). Use the linear trendline. Remember that with this option, your axes should be labeled "$\log_{10}$(mass)" rather than just "mass."

If your regression analyses are statistically significant, add a best-fit line to your graph. (In *Excel*, under the Chart Layout tab, click on the "Trendline" option and select either "Linear Trendline" or "Trendline Options." For the latter, then select the "Power Trendline" (under Type). Which of these you choose depends on what data you used to generate the graph, as indicated above.

Assignment:

For this lab you will write up detailed "Methods" and "Results" sections as if you were writing up a complete scientific paper. Follow the guidelines in Chapters 4 (especially pages 71-81) and Chapters 7-8 (especially pages 155-177) of *Writing Papers in the Biological Sciences*. See also the handout from the first lab for presenting statistical results in a concise format. Include any figures (such as scatterplots) that you feel will help illustrate your findings. This assignment will be due at the start of the next lab.

Cricket #	Vial + cricket weight	Vial weight	Cricket weight[1]	Time until stopped moving
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
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25				
26				
27				
28				
29				
30				

1 = (Vial + cricket weight) - (Vial weight)