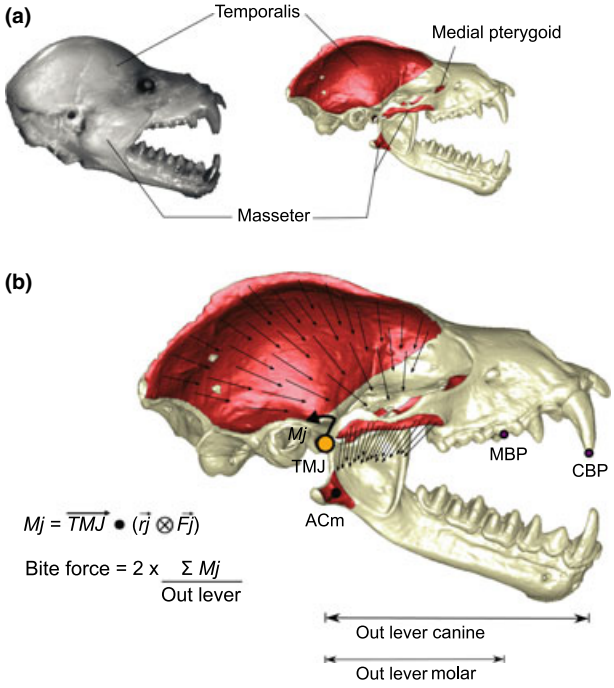
Data Collection. Bite Force from Bat Skulls: Body Size, Diet, & Phylogeny

Purpose:

An animal's features are shaped by it's ecology. We can see this clearly in the morphology of the feeding apparatus. We've seen in lab, for example, how herbivore skulls and teeth differ from carnivores. The species we've seen in those dietary categories are distantly related. It's interesting to see how diet and morphology shape each other in a more controlled context. We'll use the super diverse radation of batd to learn more about different species of bats by looking at their skull morphology and using images of their skulls to calculate their maximum estimated bite force relative to other information like diet, size, and phylogeny. The paper *Mechanics of bite force production and its relationship to diet in bats* by Sharlene Santana & colleagues (doi: 10.1111/j.1365-2435.2010.01703.x) is a good guide for what we'll be doing.



An animal's bite force is the sum of the forces that their temporalis, masseter and pterygoid muscle place around the temporomandibular joint. In the figure above from Santana et al (2010) we see these forces as the straight lines emanating from the origin of the muscle toward its insertion. We'll estimate these forces from scaled 2D photos. It's a pretty good approximation of reality, but there are much better methods that can very accurately account for the size of the muscle and the orientation of the muscle fibers.

Materials:

- Computer with Internet Access and ability to download programs (These instructions were created using a Mac).
- Data entry into the common Google Sheet: https://docs.google.com/spreadsheets/d/1fduU1fxpfDr8oxmjNs6l3b86-WE9w-aDnK-S0teltMM/edit#gid=0
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- RStudio & Markdown for analysis

Procedure/Instructions:

Introduction:

1) Begin by downloading Image J (Image J is a free program in the public domain)
    a. Go the following link: http://rsbweb.nih.gov/ij/download.html
    b. Download the version of Image J appropriate for your computer/operating system.

2) Create a Quaardvark account or login to your existing account by clicking *Login* or *Register* at the left side of the page at the following link: https://animaldiversity.ummz.umich.edu/quaardvark/
    a. It will be good (but not essential) to download your search results. **Make sure you register for the course SAMPLE_999**.

3) Instructions on how to use Quaardvark can be found on the site by clicking on *How to Use Quaardvark* on the left side of the page.

4) For this activity, you will be using Quaardvark to search for data & images of bat skulls.

5) Image J will then be used to measure the skulls for calculating the Maximum Estimated Bite Force (MEBF).

Getting Data from Quaardvark:

1) Click on *Query & Report.*
2) You will first find skull and jaw images of species in the Canidae family.
    a. Click **Edit** and change the Animal Group to "Chiroptera" and click **Save.**
    b. Click **Add more data** at the first box under Report.
    c. Click Family under taxonomic ranks. Click **Save**
    d. Click **Add more data**
    e. Click Physical Description. Click Mass, make sure Average is checked, then click Save.
    f. Click Food Habits > Primary Diet. Make sure the first radio button is selected (List keywords under a column **Primary Diet). Click Save**
    g.
    h. Click **Media Assets: Specimens.**
    i. Click **Skull.**
    j. Either check the "Skull" box at the top of the list OR check the following boxes: "Dorsal View", "Lateral View", and "Ventral View". These three pictures are what you will need to proceed with the calculations. Make sure to check the box to only include specimens that have these data.
    k. Click **Save Changes.**
    l. Click **Add more data.**
    m. Click **Media Assets: Specimens.**
    n. Click **Lower Jaw.**
    o. Check the "Lateral View" box.
    p. Click **Save Changes.**
    q. To finalize you Query & Report, click **Submit** in the bottom right corner.
3) You should now have a table of 103 species with both skull and lower jaw images compiled from Animal Diversity Web.

4) To save your Query & Report, click **Save to Backpack** at the top of the page. To access this query again later, click **Show Backpack**.

Using Image J to Collect Data:

1) Create folder on your computer to place all of the pictures that you will be using for calculations. If you download your table form Quaardvark, it will only give you the numerical values. We will have to manually select, right click, and download the photos we need.

2) In these instructions, the giant golden-crowned flying fox (*Acerodon jubatus*) is used as an example. The data Dr. O'Mara collected are entered into the Google Sheet for you. Create a new entry with your own data. Enter the diet data as either: frugivore, nectarivore, omnivore, insectivore, carnivore (if it eats any vertebrates), sanguivore.

3) To make the images from Quaardvark available in Image J, click on the Dorsal View image in the first column under the *Acerodon jubatus*.

4) Right click on the expanded image and click **Save Image As.**

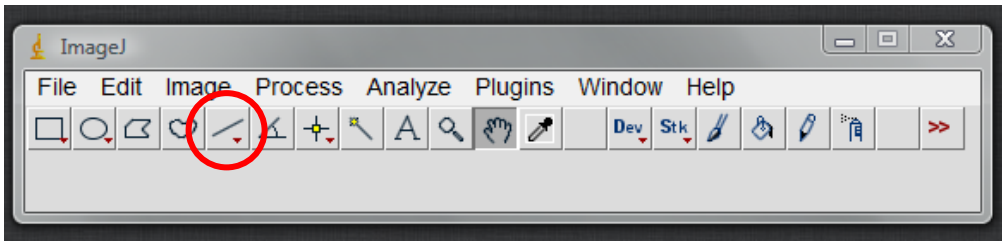
5) Locate your folder for you images, rename the picture to "AcjubDorsal" or something similar, and then click **Save.** Keeping a consistent name will help you

6) Repeat steps 3-5 for the Lateral View, Ventral View, and Lower Jaw Lateral View images of *Acerodon jubatus*.

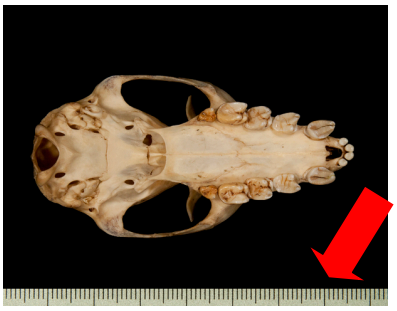
7) Once you have saved all of the pictures in your folder, open Image J.

8) Click **File** then **Open** and locate the Ventral View image of the flying fox skull in your folder.

9) To collect values that can be used in your calculations, you must first determine the scale of the picture (number of pixels in 1 cm).
   a. Click the Straight Segment Tool

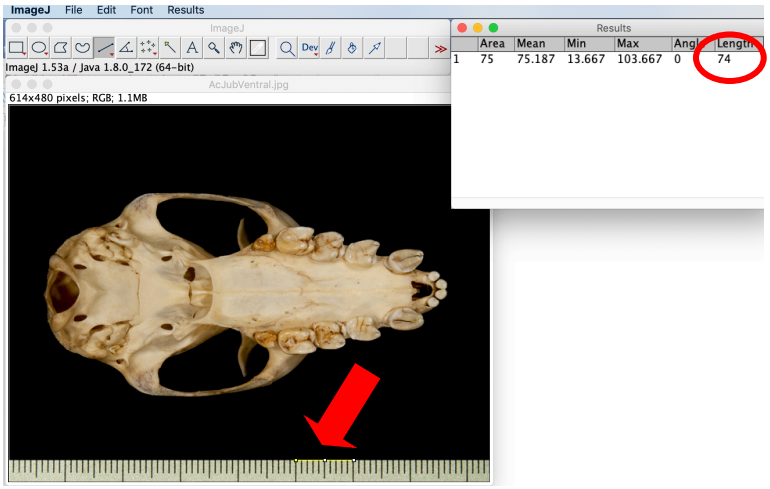


   b. Use the tool to measure the length of the scale in the image by right clicking, holding it down, and dragging the cursor across the scale from the mark on one end to the other mark on the other end.



As you measure, the length of your line in pixels will show under the tool bar. Warning: this number will also show along the scale at the top, but when you move your cursor it will change. Keep track of the number before you move your cursor around. Alternatively, Press Command-m (Mac) or Control-m (PC). This will record the length of your line in a data table that will pop up. Best to save the length into a data table (you could save this if you wanted). Record this number in

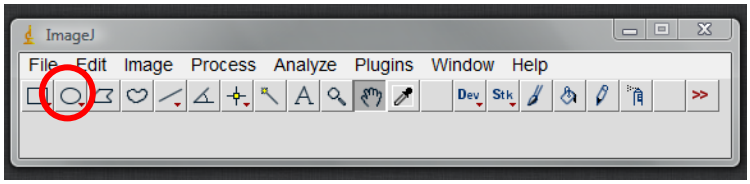
- ScaleVentral_Px.1cm (your number may be different from the example shown).



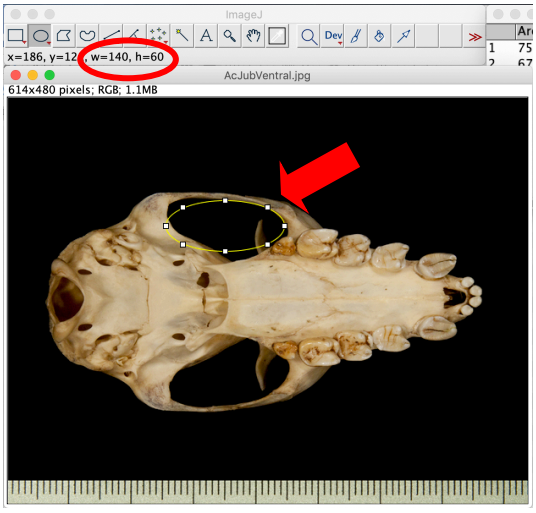
IMPORTANT: At this point, you may NOT adjust the size of the image because it will cause the scaling to be inaccurate. You must measure the scale and the skull in the image WITHOUT changing the size in between measurements to be accurate.

10) <u>Cross section of the masseter/pterygodial muscles.</u>

a. Click the Oval tool.

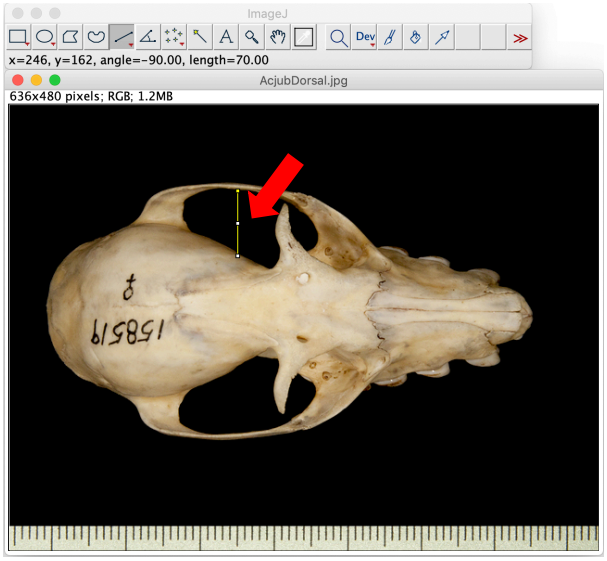


b. Right click and hold to drag your figure over the area occupied by the masseter and pterygodial muscles.

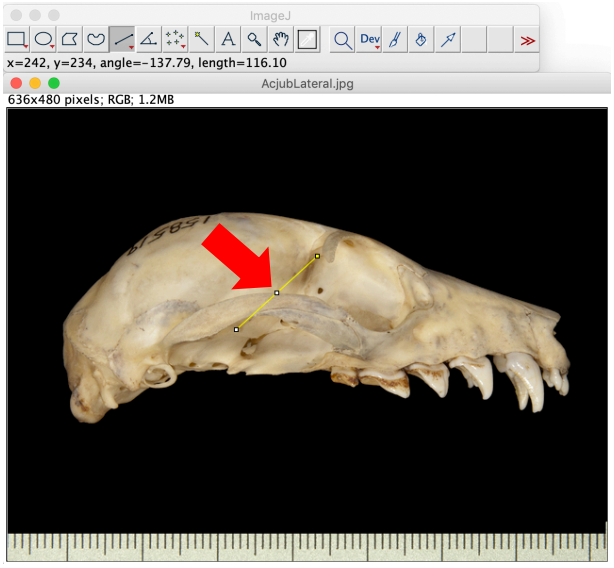


c. As you measure the opening, the dimensions of the figure will show under the tool bar. Record these measurements in pixels in the sheet. You can't save these into the data table, unfortunately!
   - Measure1.Masseter_Pterygoidal_Width_px
   - Measure2.Masseter_Pterygoidal_Height_px

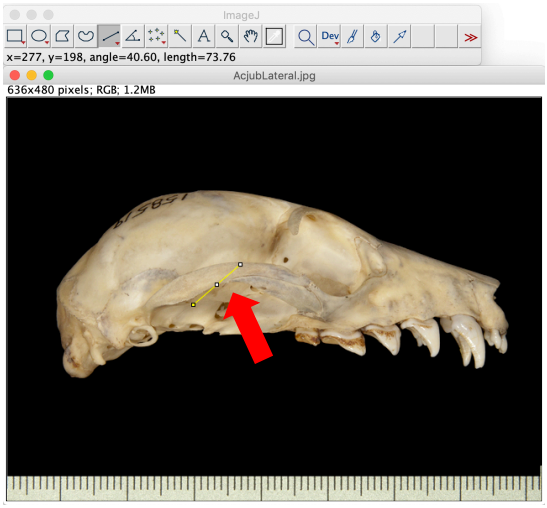
11) Cross section of the temporalis muscle. You will need to do two separate measurements for this area, one on the Dorsal View and one on the Lateral View.
   a. Click **File** then **Open** and locate the Dorsal View image of the coyote skull in your folder.
   b. Repeat step 9 to scale the image and record the number of pixels in 1 cm in Table C under Ventral.
   c. Use the Straight Segment Tool to measure the width of the opening for the temporalis muscle. Record this as:
      - Measure3.Temporalis_Width_px



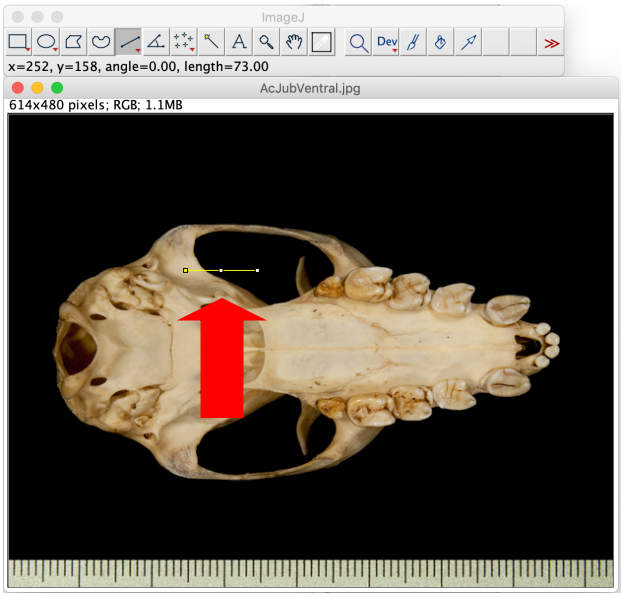
   d. Next, click **File** then **Open** and locate the Lateral View image of the flying fox skull.
   e. Repeat step 9 to scale the image and record the number of pixels in 1 cm as:
      - ScaleLateral_Px.1cm
   f. Use the Straight Segment Tool to measure the length of the opening for the temporalis muscle to achieve the other dimension. Record the measurement as
      - Measure4.Temporalis_Height_px.



12) The final three measurements for the calculations use the Straight Segment Tool on the Lateral,Ventral, and Lower Jaw Lateral views.
    a. Temporalis moment arm. With the Lateral View still open, measure the Temporalis moment arm. The moment arm is the length between a joint axis and the line of force acting on that joint. We'll measure this as the distance from the center of the maximum cross section area of the muscle to the joint where the muscle translates the force. Measure from the top of the temporomandibular joint to the midline of the muscle belly in the Lateral View. Record the value in
        - Measure5.Temporalis_Moment_Arm Length.

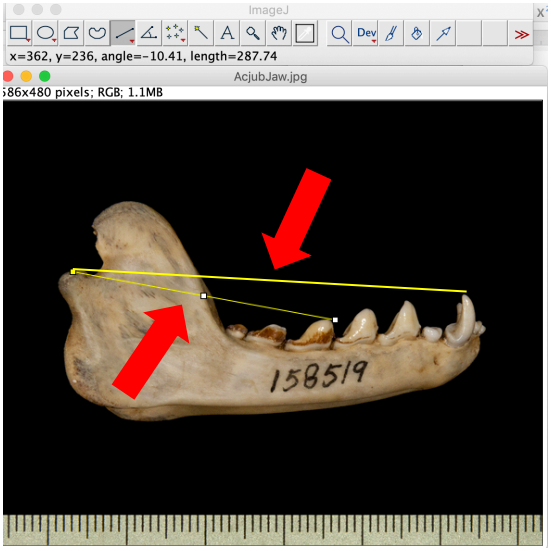


    b. Masseter moment arm. Measure the masseter moment mrm in the Ventral View. If your photo was still open, or if you are at the same zoom as for the first measurements you will not need to rescale the photo. Measure the moment arm from the temporomandibular joint to the middle of the temporal fossa. Record the value in
        - Measure6.Masseter_Moment_Arm



c. Open up the Lower Jaw Lateral View image, extract the scale and add it to
   - ScaleJaw_Px.1cm_Jaw.

d. Measure the length of the lower jaw to the M1 and to the canine in the Lower Jaw Lateral View and record the value as:
   - Measure7.Length_M1
   - Measure8.Length_C



13) That's it! Those are all of the measurements you need from the Quaardvark images. Now you can switch over to RStudio for the data calculations & analysis.

Assignment

Select 4 different bat species and collect estimated bite force data using the same procedures described above. Try to pick bats from different families, sizes, and dietary categories. Enter all of these data into the common Google sheet to be able to complete the data analysis assignment in RStudio.