



The above image shows the order in which the landmarks must be digitized. *It is very important to maintain this order!*

1. Open tpsDig232.
2. Choose File > Input Source > File. The file browser opens up. Navigate to Kaija_data/BurstSpeed2019/2019_PostResp_photos/Exposure_Edit/for_landmarks and choose the tps file that you're working on.
 - a. There are 20 "sets" of images: set_001 to set_020.
3. The set of images will load into tpsDig2. At the bottom of the screen, you will see the name of the current image file, as well as a box that shows how many images are in the set.
 - a. Each set contains 25 images ($n = 25$), except for set_020, which contains 17 images ($n = 17$).
4. Expand the tpsDig window and zoom way in on the tadpole so you can see as much detail as possible. Use the +/- buttons in the top toolbar or the mouse wheel to zoom.
5. Set the scale
 - a. Choose Options > Image Tools. A dialog will open up.
 - b. Choose 'Measure'.
 - c. Check to make sure that the Reference length is set to 1 centimeter.
 - d. Click 'Set scale'.
 - e. Click and drag along the ruler in the image to create a line 1 centimeter long.
 - f. Click 'OK'
 - g. Keep the Image Tools dialog open so you won't have to reopen it when you navigate to the next image.
6. Click the blue crosshairs button in the top toolbar to begin digitizing landmarks.
7. Click landmarks *in the order shown above*. If you need to undo a landmark, right click on it and delete it. *If you delete a landmark other than the last one, you will lose the order*

and will have to delete all the subsequent landmarks one by one and start over. This is a huge pain! There's no easy "undo" function, nor can you modify multiple landmarks at once.

8. The landmarks are as follows
 1. Frontmost point of the snout, not necessarily where the line intersects.
 2. Upper lip.
 3. Lower lip.
 4. Center of the eye.
 5. Edge of the head directly above the eye.
 6. Edge of the head directly below the eye.
 7. Juncture between tail fin and dorsal edge of the body.
 8. Juncture between tail fin and ventral edge of the body (7 and 8 do not need to be collinear)
 9. Dorsal insertion of the leg at the body. This point should fall on the first pink vertical line.
 10. Ventral insertion of the leg at the body. *This point does NOT need to fall on the vertical line, although it might.*
 11. Tail tip
 - 12-15. Intersection of the vertical lines with the dorsal edge of the tail fin.
 - 16-19. Intersection of the vertical lines with the ventral edge of the tail fin.
 - 20-23. Intersection of the vertical lines with the dorsal edge of the tail muscle.
 - 24-26. Intersection of three of the vertical lines with the ventral edge of the tail fin.
9. When you have finished digitizing landmarks for a single image, click the red arrow in the top toolbar to advance to the next image.
10. When you are ready to save your work, choose File > Save Data. The file default will be to overwrite the same tps file you started with. Accept this default--choose Save > Overwrite.