

Procedure for examination of trematodes on mudsnails

Before you go:

Check the tides! To collect the mudsnails you need to go within a couple hours on either side of low tide. Tide charts can be accessed at:

http://tidesandcurrents.noaa.gov/tide_predictions.html

Materials needed:

Three 5 gallon buckets (one with the bottom cut out), a metal sieve, extra smaller bucket or plastic container, datasheets and pencil, mm ruler or calipers (preferable), magnifying glass or magnifying glasses (preferable) and camera if available. Remember to bring sunscreen and proper clothing (including boots or shoes that can get muddy). The bottomless bucket should be marked with a waterproof pen 3cm from the cut end.

1. Leave everything except one bucket in a safe spot away from the water's edge
2. Collect snails along a transect: with your bucket, go near the water's edge and mark your start point, then walk parallel to the shoreline.
 - a. Mark your path as you go or note it, you will need to find it again in step 12 below.
 - b. Approximately every 1-3 meters (approximately every 2-5 strides) stop and collect 5-10 snails until you have about 100 snails or more. The point here is to collect a variety of snails from across your chosen locality.
 - c. Estimate the length of the shoreline that you sampled by counting strides as you go.
3. When you're done with your transect, collect some seawater to cover over the snails you collected. You need approximately 15-30 cm of seawater in the bucket.
4. Make a local estimate of the number of snails, following the Coffin et al. (2008) method:
 - a. Use a random number generator to select three random points along your entire transect
 - b. At each point, press the cut end of the bottomless 5 gallon bucket 3 centimeters into the sand and scrape up the sand 3 cm deep into the sieve
 - c. Wash the sand out of the sieve to expose the snails
 - d. Count the snails in the sieve, record the 3 counts on data sheet
5. Return to your empty bucket and equipment. Turn the empty bucket over and sit down for examination of the snails.
6. Pick up a single snail from the bucket and using the metric ruler or calipers measure the total length and maximum aperture length (opening through which the foot extends) of the snail. Record the data (in mm, not cm, and definitely not inches!).
7. Using the magnifying glass (or glasses) examine foot and note if operculum is present or absent. Record data (0=absent, 1=present).
8. Using the magnifying glass (or glasses) carefully examine the shell and operculum for the presence of the metacercarial cysts. The cysts are about 1mm in diameter and appear as pearly white spheres on the surface of the snail (see Fig. 1). Record data (number of cysts present. Remember to include all snails with and without cysts in your counts. NOTE: If there is sand adhering to the snails you may want to dip them in the seawater in order to remove the sand and make it easier to observe the cysts.
9. If possible, take close-up pictures of snails that have metacercarial cysts.
10. Place the snails in the extra smaller bucket or plastic container (for release at the end of the data collection).

11. Repeat the process for the rest of the snails. If you get to 100 and found no cysts, collect as many more snails as possible to verify that the locality does not have *Pleurogonius malaclemys*.
12. Release all snails at the back at the water's edge.
13. Make sure your data sheets are complete (with name, date, and locality). Submit datasheets and feel proud of the excellent work done!

Note: Examination of snails for cysts can of course take place in a lab, using a dissecting microscope. Educators or students that are interested in examining snails for internal stages of this parasite (and other trematodes) should feel free to contact us for additional information and resources.