May 20, 2022

Duffy Lab Daphnia Field Sample Processing

Kira Monell[1], Meghan Duffy[1]

[1]University of Michigan - Ann Arbor

1

document .

Duffy Lab, EEB, University of Michigan

Kira Monell

This protocol is used to process field samples for *Daphnia* in the Duffy Lab. This protocol doesn't include species or parasite identification guides.

Kira Monell, Meghan Duffy 2022. Duffy Lab Daphnia Field Sample Processing . **protocols.io** https://protocols.io/view/duffy-lab-daphnia-field-sample-processing-b9p5r5q6

document ,

May 20, 2022

May 20, 2022

62941

This protocol is used to process field samples for *Daphnia* in the Duffy Lab. This protocol doesn't include species or parasite identification guides.

Duffy Lab *Daphnia* Field Sample Processing – May 2022

Materials needed

-field notebook
-labels for specimens being counted
-benchtop counter
-beaker (typically ~400-600 mL)
-plunger to mix sample (plunger is made by attaching a rubber stopper to a wooden dowel)
-ladle made to a known volume (ladle is made by cutting a falcon tube to the desired volume and attaching it to a dowel)
-mesh sieve (made by sandwiching mesh of your desired micron size to a small piece of PVC tube)

-embryo dish
-Pasteur and wide bore pipettes
-microscope slide
-something to manipulate the plankton with (in the Duffy lab we use an insect pin bent at a 90 degree angle and stuck into the eraser of a pencil)
-dissecting scope

Protocol

1. Before starting to process a sample, look in the field notebook for what Daphnia species and parasites are in the lake you are processing. Write labels for each of the combinations of *Daphnia*species, age of *Daphnia* (adult or juvenile), and parasite (ex: adult *dentifera* with *metsch*) that were in the lake from a previously processed sample and place them on a benchtop counter.
2. Place sample into a beaker and add water to dilute (usually total sample volume is ~300 mL)
    I. Record volume of sample in field notebook, this is important in measuring density of sample.

3. Using a plunger, carefully plunge the sample up and down. Be careful not to press down to the bottom of the beaker to avoid squishing the plankton. Continue mixing until the sample is homogenous.
4. Moving quickly to avoid the sample becoming stratified again, press ladle down to the bottom of the beaker then moving up quickly to collect a subset of the sample.
5. Pour subset of sample into a mesh sieve. Continue adding scoops into the mesh sieve until the amount of sample you want to count is in the filter. An ideal subset is around a few hundred Daphnia which is visually estimated.
6. Rinse out sample with water into an embryo dish.
7. Pipette sample with a wide bore pipette onto a slide.
8. Using the insect pin as a wall to hold plankton back, pipette out the water using a Pasteur pipette without touching the plankton. This must be done under the dissecting scope to see what you are pipetting.
9. Once enough water is removed so that the plankton are not swimming around but not so much that they are drying out, use the insect pin to carefully manipulate the plankton into lines on the slide to make counting easier.
10. Under the scope, look through each of the lines while using the benchtop counter to tally up the *Daphnia* species and parasites you see.
11. When the entire subset is counted, rinse out both the Pasteur and wide bore pipette with water into an embryo dish. Look under the scope to see if any *Daphnia* were stuck in the pipette. If yes, count these as well.
12. How to know when to stop processing the sample:
    I. Have you counted 200 total individuals (adults and juveniles together) for each of the *Daphnia* species you see?
        i. If yes, you are done processing

ii. If no, then move on to next step

II. For the *Daphnia* species you have counted, if you were to process the rest of the sample would you be able to count at least 20 total individuals for any of the species? Estimate the number of individuals you would see by multiplying the number individuals you have counted with how much more liquid is left to process (ex: I have counted 10 *dentifera* in a 100 mL subset of a 400 mL sample, I multiply the 10 *dentifera* by 4 to estimate that there is 40 *dentifera* are in the entire sample).

i. If yes, go back to step 3 and make a new subset of the sample or process the whole sample.

ii. If no, you are done processing

13. Once you are done processing the sample, record your counted totals into the field book. For the Duffy Lab, we record the numbers of each subset individually and not as a sample total.