NETBIOME – ISLAND BIO-DIV -
SAMPLING REGIME – MICRO-INVERTEBRATES
Collembola

Extraction funnels

Soil collected in the field is subject to extraction in the lab using home made variants of tullgren funnels. Materials needed:

- 20cm diameter PVE piping (industrial piping, should be available from any large hardware supplier), cut into sections of 9cm depth.
- gauze. I gave both the La Reunion and Azorean teams an example of the gauze, but just in case here is a photo. I bought it in a hardware store. Needs to be durable.

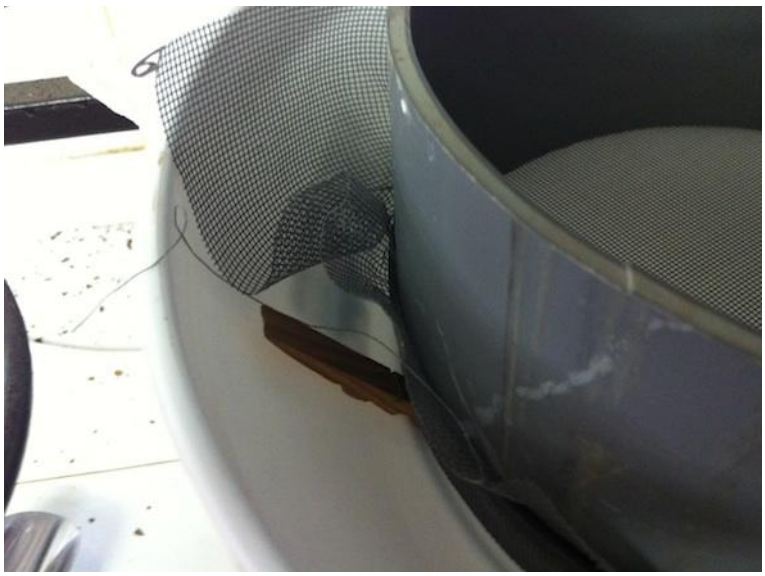
- large plastic zip ties.

Using the plastic zip ties, stretch the gauze over the plastic tubing and secure it

- large funnels capable (with a diameter greater than the PVC piping)
- Spacers to maintain an airflow between the sieve and the funnel
- 50 ml falcon tubes.
- A light source (40W bulb)

The sieves are filled to a dept of approx 5.5 cm with soil (see later) and placed in the funnels below a light source.

Four small spacers are used to elevate the sieve above the funnel to facilitate airflow. I use wooden clothes pegs (removing the metal spring and using the two bits of wood as two spacers:



A 50ml falcon tube (or similar), approximately 1/3 full with ethanol, is pushed onto the end of the funnel:

Sampling soil

Three extraction funnels are used per site. To fill each one to a volume of approximately 5.5 cm requires a total volume of 1.8 litres of soil to be collected per site. Thus, soil cores should be taken randomly within sites until such a volume has been sampled. I am not sure how deep the corers Christophe has suggested will sample to, but we should aim to sample to a standard depth that can be established when we have the corers (hopefully 10cm), but obviously less is soil is not sufficiently deep. Store the soil in plastic Ziploc bags for transport to the lab.

In the lab

Divide the collected soil among three sieves. Place the sieves in the funnels, ensuring the spacers allow for airflow. Finally, add the falcon tube with the ethanol. Do this last to avoid excess soil falling into the falcon tube. Soil will fall into the tube anyway, as the soil dries out, but the less the better, as it makes sorting of Collembola under the microscope more complicated. Leave the extraction for 7 days, checking at least once a day to be sure that bulbs have not blown. At the end of 7 days remove the falcon tubes (gently, to avoid more soil falling into it), and secure the lid onto it. Many of these tubes do not seal well, so make sure they do not lie on their side. Store falcon tubes in a fridge until the microscope stage.

Funnels and sieves should then be thoroughly cleaned before next use, using brushes and detergent.

Under the microscope

Under a binocular microscope, add the contents of each falcon tube to a petri dish (you will probably need more than one petri dish per falcon tube) and systematically sort through the material to remove Collembola, placing Collembola into a 2 ml screw cap tube with ethanol. Be sure that your screw cap tubes seal properly. I use a Gilson pipettes to remove Collembola (p200 and p1000), cutting the plastic tips to give a bore that facilitates sucking up Collembola from the petri dish.

After removal of Collembola, soil can be returned to the falcon tubes (okay to combine it into a single falcon tube per site if possible) and returned to the fridge (there will be a lot of Acari there that we may eventuall decide to use). The 2ml screw cap tubes of Collembola should be stored in the fridge as well.

IMPORTANT: cross contamination is a very real problem for the sequencing protocol we will use. Thus care is needed during the whole process. Additionally, label tubes and sieves appropriately to avoid any confusion. Also, it would be wise to have a practise run to ensure everything is working and that your set up extracts Collembola (and other interesting beasties!).