

Heel sampling

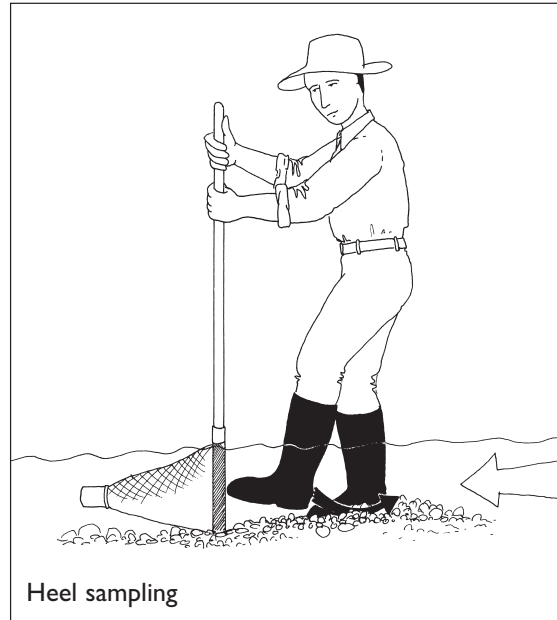
DON'T FORGET

EQUIPMENT: Hand net; plastic sample bottles; boots; permanent marker pen; 40% formalin (or 70% methanol).

Handle formalin with care (see chapter 3).

Method

- Label the collecting bottle with an identifying code, e.g. site and sample number using a thick permanent marker pen.
- Choose an area of stream to sample. Standardize substrate sampled at each site – stony riffles are preferable, but any substrate is possible provided there is a current.
- Face downstream holding a hand net in front of you so that the current enters the net.
- Grind or trample the substrate for 30 s with your heels while slowly walking backwards for a short distance (1–2 m) to dislodge organisms.
- Lift the net out of the water and splash stream water on to the outside of the net to wash invertebrates and debris stuck to the inside of net down into the collecting bottle.
- Unscrew the bottle from the net and cap, replacing the collecting bottle for the next sample. Alternatively, empty its contents into a separate, labelled container for transport.
- Add methanol or formalin unless processing is likely to be within a few hours of collection. Strain (through muslin to prevent loss of sample) off some water, about 25%, and replace with 40% formalin or 70% methanol.
- Repeat the operation at least twice more at each sample station to increase the number of species trapped but taking care not to sample from where you or others have already walked. If the sample station has two substrate types, stratify the sampling in proportion to the area: so if 60% pebble and 40% gravel with some sand, take three smaller samples (timing or area kicked) of the former and two of the latter.
- Processing: pour the contents of the bottle on to a white tray and separate the organisms from the sand, silt and debris by eye and using forceps or Pasteur pipettes for smaller organisms. Place them in bottles containing 70% alcohol: count and identify.



OTHER CONSIDERATIONS

Adjust the time of kicking according to the substrate to prevent clogging the net (= inefficient sampling) and sample sorting difficulties; 30 s samples are sufficient in riffles, 15 s in sediment. If you wish to increase the number of samples then you can reduce the time spent kicking or the distance sampled. This technique is useful for observing which organisms are alive/dead immediately after spraying provided no preservative is added after collection: place the contents of the bottle on a white tray before preserving.

Artificial substrates

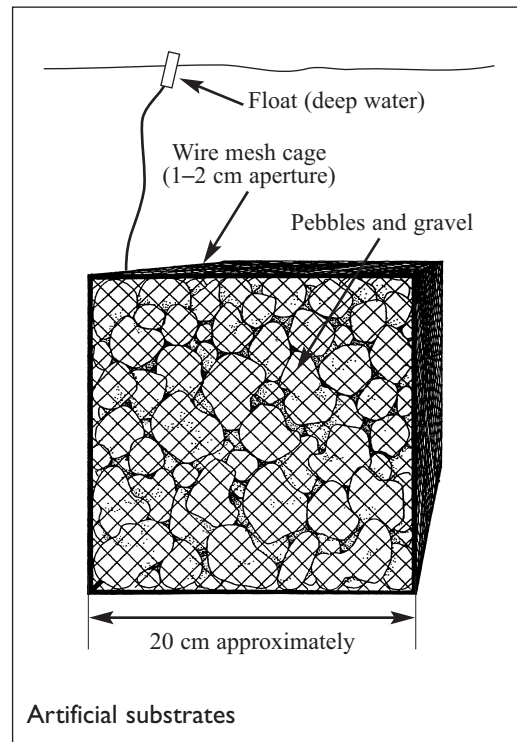
DON'T FORGET

EQUIPMENT: Samplers; wire; stakes; netting; floats (corks); paper and pencil; sample bottles; 2 inch paintbrush; plastic bags; bucket; permanent marker pen; 40% formalin.

Handle formalin with care (see chapter 3).

Method

- Choose an area of lake or river to sample (substrate normally silt, mud or solid bedrock). Standardize the placement at each site so that the samplers are, e.g. the same distance from vegetation and shoreline; or for rivers, in similar current strengths, and exposure, e.g. positions on bedrock, sand or sediment.
- Place 4–6 samplers at each site. In static water or slow flowing rivers, rest the sampler on the substrate and mark the position carefully – draw a map and use a cork float as a marker in deeper water. In faster flowing streams secure the samplers to the bedrock using wires tied to stakes jammed into crevices.
- Leave the samplers for at least 2 weeks before retrieving. Standardize the retrieval period for each site.
- To retrieve, gently but quickly ease the sampler into a mesh bag (1 mm aperture) before lifting the sampler free of the water. This will catch most organisms that are dislodged by the activity.
- Put the bag containing the sampler into a bucket of water, wash out the bag, shake the sampler, then remove the pebbles from the wire cage (if one was used) and brush the substrate with a paintbrush to remove the more tenacious organisms.
- Strain off water in the bucket as necessary to match the volume of the sample bottle. Pour the sample into a labelled sample bottle and preserve the contents of the bottle with 40% formalin (use 4 ml for every 100 ml of sample).
- Sort and identify invertebrates. Combine the results of replicate substrates at any one sampling station and enter the data into a statistical package for analyses. Use the mean and standard error for plotting.



OTHER CONSIDERATIONS

Sampling of the artificial substrates is (biologically) destructive, but samplers can be replaced if continued monitoring is required. However, they must be left submerged for a least a further 2 weeks to allow re-colonization before their retrieval.

Sweep net (aquatic)

DON'T FORGET

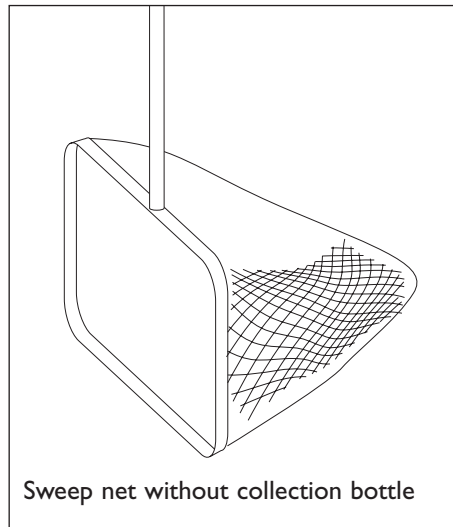
EQUIPMENT: Sweep net; sample bottles x 20; buckets x 3; permanent marker pen; plastic bags x 20; 40% formalin; muslin.

Handle formalin with care (see chapter 3).
Standardize the time taken to sweep vegetation at all sites.

ROOTED VEGETATION

Method

- Label the collecting bottle with an identifying code, e.g. site and sample number using a permanent marker pen.
- Choose an area of vegetation to sample. Standardize the substrate sampled at each site, e.g. papyrus and *Vossia*. If 80% papyrus and 20% *Vossia*, take four papyrus and one *Vossia* sweeps.
- Hold the net with two hands and scrape the submerged stems with the metal support of the net, then sweep the area between the stems and around the roots using a constant figure of eight-like motion that prevents organisms escaping from the net.
- Continue this routine for a fixed period of time such as 1 min. Lift the net out of the water and wash the organisms and debris stuck on the sides of the net down to the bottom of the net (splash water on the outside of net to achieve this).
- Either unscrew the collecting bottle (if fitted) and tip the contents into a labelled sample bottle or invert the net into a bucket of water and wash out the organisms, straining off water (through muslin) as necessary to match the volume of the sample bottle. Pour the sample into a labelled sample bottle. Preserve the contents of the sample bottle with 40% formalin (use 4 ml for every 100 ml of sample).
- Repeat the sampling 3–4 times at each site.



FLOATING WEEDS

Method

- Sample whole floating weeds by sweeping them quickly up into the net – do not sweep slowly as the organisms will detect movement and detach themselves from the roots.
- Invert the net into a bucket of water containing a few drops of formalin and leave the vegetation for a few minutes to help release tenacious organisms.
- After shaking thoroughly, remove the vegetation to a labelled plastic bag. Transfer the main sample (the remaining water in the bucket) to a labelled sample bottle and preserve as above.
- Take five or six samples of floating vegetation to provide an estimate of the sample variation. With floating vegetation (as opposed to rooted, floating vegetation), it is useful to relate animal density as a function of the dry weight of the vegetation, or better still, root weight or volume, as this discounts bias from above surface biomass, which is variable throughout the year.
- Sort the vegetation to remove any fauna still adhering to it on return to the camp or laboratory and consolidate the sample organisms with those of the main sample.

OTHER CONSIDERATIONS

Sweep nets can be used to capture surface-dwelling insects in open water and around vegetation.

Cylinder or box sampling

DON'T FORGET

EQUIPMENT: Cylinder, box or Surber sampler; nets x 2 (one spare); sample bottles; permanent marker pen; formalin.

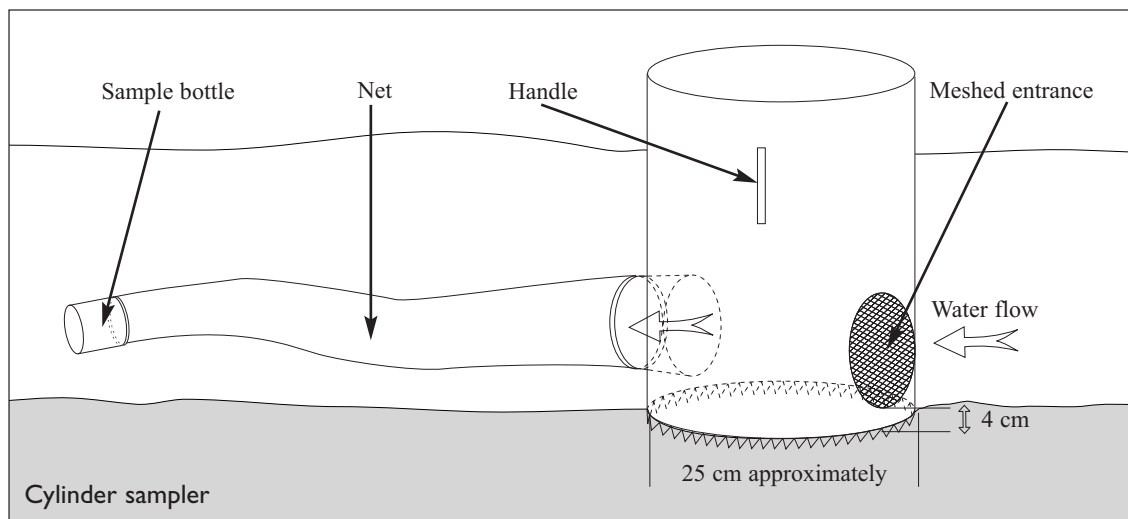
Handle formalin with care (see chapter 3).

Method

- Choose an area of stream to sample. Remember the key points: standardization of substrate sampled at each site – riffles are good; stratify the sampling if there is a strong demarcation of substrate type.
- Face upstream and drive the sampler about 5 cm into the substrate (using to-and-fro rotations) so that water enters the mouth of the cylinder or box and the net is downstream. Do not sample where you have trodden.
- Lift large stones within the cylinder and remove tenacious animals, e.g. molluscs, by hand. Then stir up substrate inside the sampler for 1–2 min to dislodge organisms, allowing the current to take them into the net.
- Lift the sampler out of the water so the net hangs down. Splash stream water on to the outside of the net to wash invertebrates and debris stuck to the inside of the net down into the collecting bottle.
- Unscrew the bottle from the net and put the cap on (or empty its contents into a separate, labelled container for transport).
- Label the collecting bottle cap with an identifying code e.g. site and sample number using a permanent marker pen.
- Add methanol or formalin.

TIP: Strain a small percentage of water from the sample and replace with preservative.

- Repeat the sampling procedure 4–8 times to achieve reasonable statistics. Sort samples on a white tray containing shallow water. Combine the results of replicate samples at any one sampling station and enter the data into a statistical package for analyses.



OTHER CONSIDERATIONS

Cylinder samplers can be used in sediment providing there is a water movement through the sampler that is sufficient to carry organisms into the net.

Drift sampling

DON'T FORGET

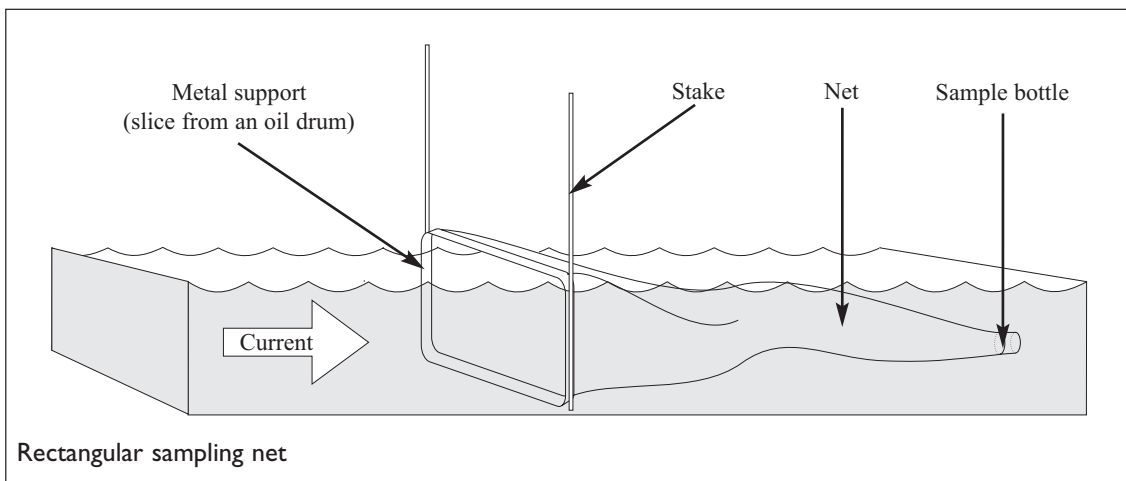
EQUIPMENT: Drift nets; hammer and stakes; tape measure; wire or string; screw-cap bottles and caps; an orange or cork; permanent marker pen; muslin; 40% formalin; flow meter.

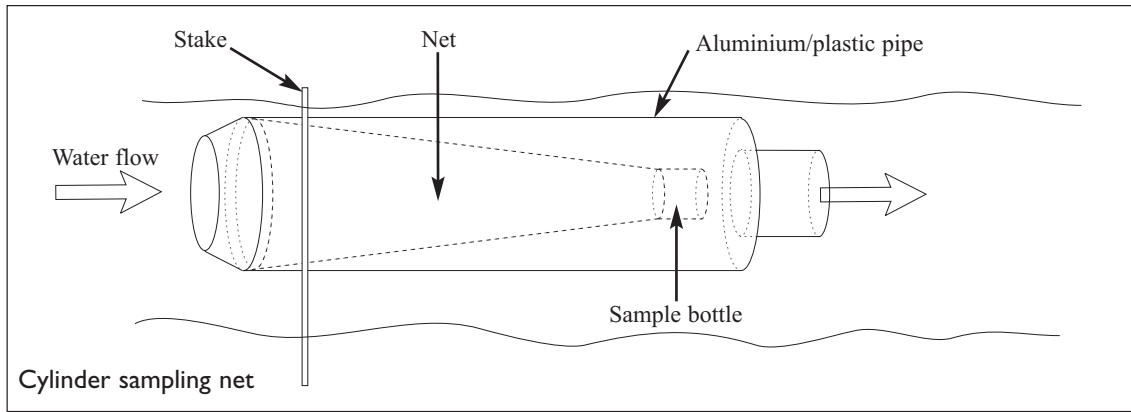
This is a two person job.
Handle formalin with care (see chapter 3).

Method

- Set the drift net in a part of the stream channel that can be waded: in a fast flowing stream this is a two person job. Hammer the stakes into the substrate and fix the frame of the net, with the opening submerged and facing upstream, to the stakes with wire. Concrete reinforcing rods make excellent stakes. Ideally, set 2–3 nets up and downstream of spraying.
- Screw on the sampling bottle and note the time. Set the nets at the same distance from the river bed and in similar currents at all sites. The current can be gauged using a flow meter held in the mouth of the net.
- Empty the net periodically, e.g. every 24 h. During heavy rain or insecticide spraying, the time interval is shortened, perhaps to 2–4 h. Tap the net on the outside to help wash invertebrates stuck to the inside of the net down into the collecting bottle before unscrewing.
- Note whether there are signs of the net being clogged, such as eddying or backflow from the mouth of the sampler. If so, note this in a notebook for the sample number, time, etc. Empty the net more regularly if these signs are evident.
- Label the collecting bottle with an identifying code, e.g. site, sample number and time collected using a permanent marker pen.
- As the bottle will be full of water, hold some muslin over the mouth of the bottle to prevent loss of specimens and pour out some of the water (about 25%). Top up the bottle with 40% formalin unless processing is likely to be within a few hours of collection.

Calculate drift density using the area of mouth (or partial area if the net was not completely submerged), the flow rate and numbers of animals caught in a known time. If, during the sampling period, a volume of 20.1 m^3 of water passed through the net, and the sample bottle contained 102 Nematocera, then express the result as number per m^3 , i.e. $102/20.1 = 5 \text{ Nematocera/m}^3$.





GAUGING FLOW

An approximation of the flow through the net can be made from the flow of the river – provided the net is not clogged and impeding flow. See the method sheet dealing with measurement of current using a floating object (chapter 5). If you can borrow a calibrated flow meter, that will be more accurate – especially if held in the exit pipe of a cylinder sampler. See method sheet for flow calculations.

OTHER CONSIDERATIONS

The dimensions of a drift net are not fixed. A common size for rivers is a 30 x 30 cm mouth; 30 cm x 15 cm (height) for streams.

In silt or mud the stakes must be long enough to secure the net. In rocky substrates, metal stakes can be hammered into the bed or the net tied to trees or stakes on the bank.

Note the phase of the moon, i.e. full, half and new, during sampling periods. Invertebrate drift is greatest just after sunset – moonlight will tend to change this pattern and affect catches.

Emergence traps

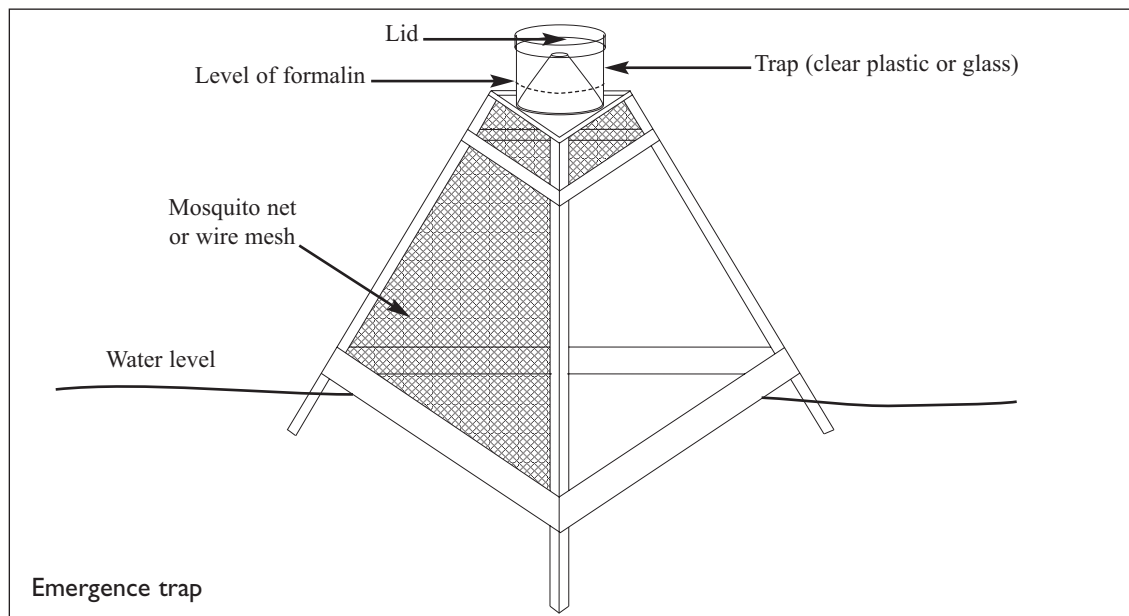
DON'T FORGET

EQUIPMENT: Emergence traps; collecting heads; string; rubber bands; knife; stones; sample bottles; permanent marker pen; formalin.

Handle formalin with care (see chapter 3).

Method

- Choose an area of stream or lagoon to sample that is readily accessible and not too deep: depth will be determined by the length of the trap's legs. Standardize the substrate sampled for each site, e.g. within weeds, over vegetation or sediment, etc. Emergence traps can be made with floats to sample deeper water.
- Without treading in the area over which you wish to site the trap, place the sampler in the water so that the base of the sides or cone is just beneath the water. Use rocks to level the trap or increase the height of the legs.



- Place the funnel trap assembly on the top and tie it down with string or rubber bands. Half fill the well with formalin and put on the lid. Locate another 3–4 traps in the vicinity remembering to standardize the substrate sampled within the site. Leave the traps in position for a period of at least 2 weeks and visit the trap every 2 days to empty the wells.
- To empty, suck out the contents of the well with a Pasteur pipette or use forceps to transfer the imagines to a sample bottle containing 4% formalin. Drop a label (pencil on paper) into the bottle and cap.
- Identify and count the imagines. Calculate the area sampled by each trap and report the density of imagines as number m^{-2} . Preserve the identified specimens in formalin, label and keep.

Tip: Keep a record of the meteorological conditions that prevail over the sampling period at all sites as rain, light and temperature can affect emergence.

OTHER CONSIDERATIONS

The example given is not the only design employed. See Mundie (1971) for other types of trap. People get curious about these traps. A notice about their purpose or a discussion with local people may reduce tampering or loss.

Plankton sampling

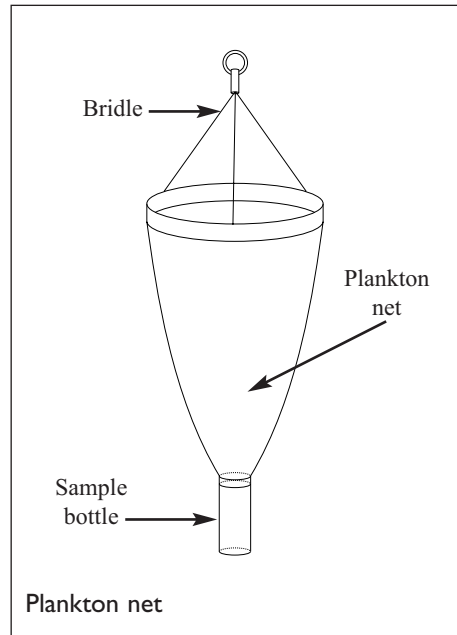
DON'T FORGET

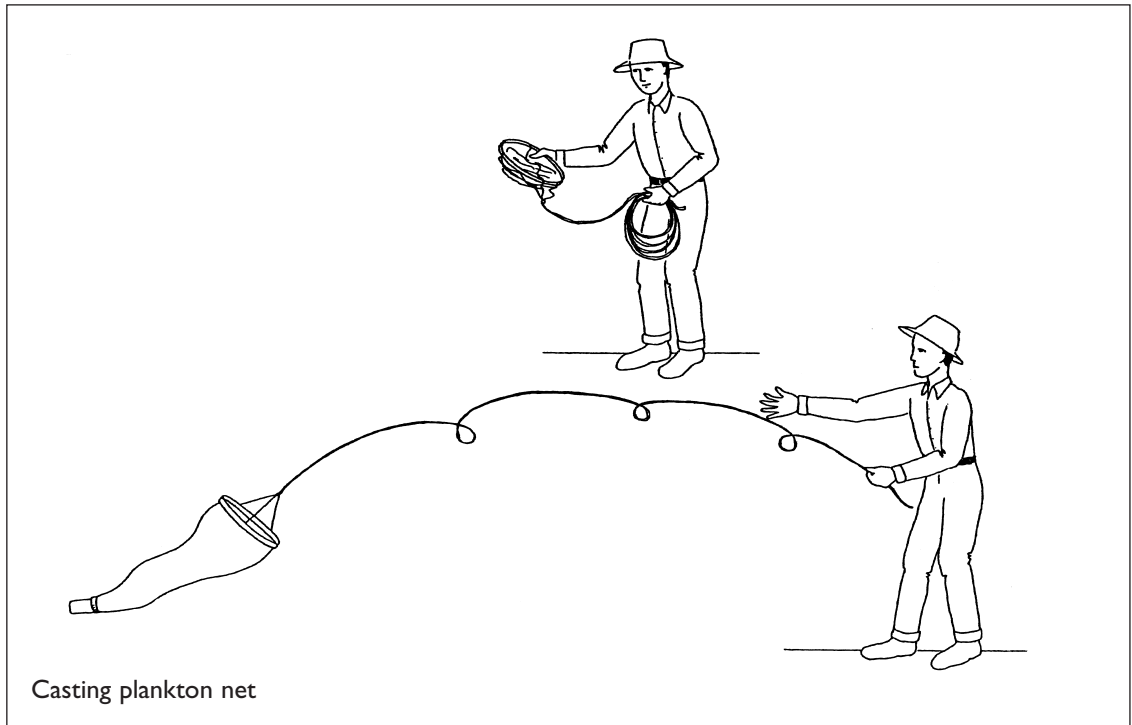
EQUIPMENT: Plankton net; 10 m tow-line; weighted glass bottle and stopper; screw-cap bottles and caps; thermometer; pencil and paper; 40% formalin.

Handle formalin with care (see chapter 3).

Method

- For zooplankton, use a 250–300 μm mesh net; for phytoplankton use 75 μm mesh net. Note the time of day, which should be standardized for the site being sampled.
- Tie the tow-line to the net bridle, screw on the collecting bottle and wet the net to make it heavier before casting. Find a suitable place on the river bank or pond edge from which to cast the net, i.e. free of overhanging trees, or rocks/weeds in the water. Coil the tow-line and hold it and the net by the hoop – see diagram. Hold the end of the rope and cast out the coil and net as far as possible. Note the distance: **Tip:** if the line has knots tied at 0.5 m intervals, the distance cast can be quickly measured.
- Haul in the net at a constant speed: slowly enough to prevent it surfacing and fast enough to prevent sinking. Lift out of the water by the bridle and wash down the net by splashing water on to the outside of the mesh. Unscrew the sample bottle, strain about 10% of the water and replace with 40% formalin and insert a paper label giving site, date and length of haul written in pencil. Cap bottle and invert to mix.
- Repeat the procedure six times, numbering replicates. (The net may also be towed for a known distance from a boat.)
- Alternatively, if the plankton is dense (greenish coloured water), fill a bottle with water, or in deeper water, lower a weighted bottle from a boat/bridge and unstopper with a string tied to the stopper when at the required depth. Replicate, preserve and label as above.
- Estimate the volume of water that passed through the net from the distance hauled (m) and the area of the mouth of the net.
If the diameter of net mouth = 30 cm, then the area of mouth = πr^2 or $3.142 \times 225 = 707 \text{ cm}^2$.
If the haul was 7 m then the volume of water sampled was area of mouth x length of haul = 4949 litre or 4.9 m^3 . (The volume of water sampled will be over-estimated because of back pressure caused by net resistance as it becomes clogged.)
- Process samples as soon as possible, as deterioration can occur (see treatment of plankton on page 191).





OTHER CONSIDERATIONS

In deep water and access to a boat, the plankton net can be hauled up vertically. Try and take plankton samples at the same time of day as they do change depth in response to light.

Grab sampling

DON'T FORGET

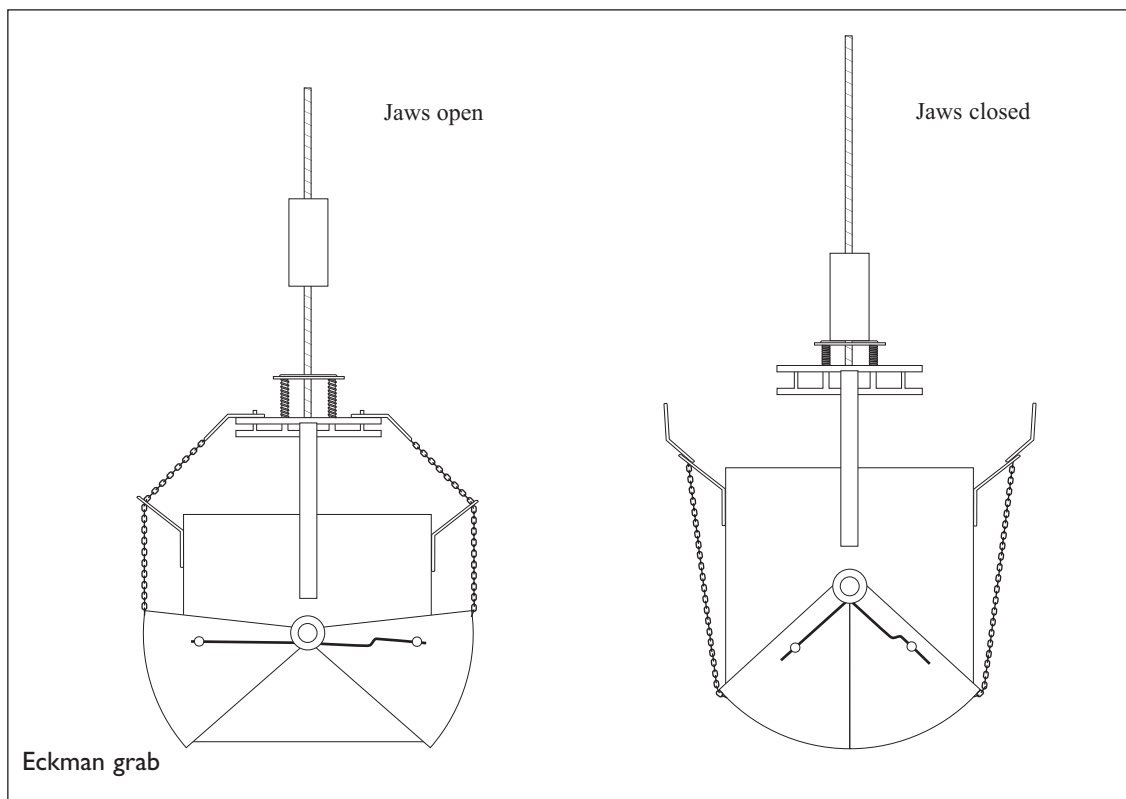
EQUIPMENT: Eckman or Petersen grab; pole; messenger or rope for grabs; strong plastic bags; wide mouth sample bottles (1 litre); buckets x 3; permanent marker pen; 40% formalin.

This is a two person job.
Handle formalin with care (see chapter 3).

ECKMAN GRAB

Method

- Select an area of shallow water that is free of rooted vegetation and stones to sample. Standardize the placement of grabs at each site so that samples are the same distance from rooted vegetation or the shoreline; or for rivers, in similar current strengths or sized pools.
- Wade out slowly (to help maintain visibility) holding the pole of the sampler at arm's length in front of you. Firmly place the sampler on flat substrate and, depending on the action of the sampler, either twist the pole and push or send the messenger down to activate the jaws.
- Lift the grab vertically and check that no sticks or stones are preventing the jaws from closing. Hold the jaws over a bucket and release the jaws to deposit the sample. Wash the grab with a *little* water to flush the sediment stuck to the walls into the bucket.
- Pour the contents of the bucket into a heavy gauge plastic bag. Pour 50 ml of 40% formalin into the bag, place a pencil written label inside, close the bag and tie a label to the neck of the bag. Squeeze the bag gently for 1 min to disperse the formalin in the mud.
- Walk a few metres away from the previous sample and repeat the procedure, taking care not to sample where you have walked; 4–8 replicate samples are required for quantitative estimations. The grab can also be used from a boat in shallow water.



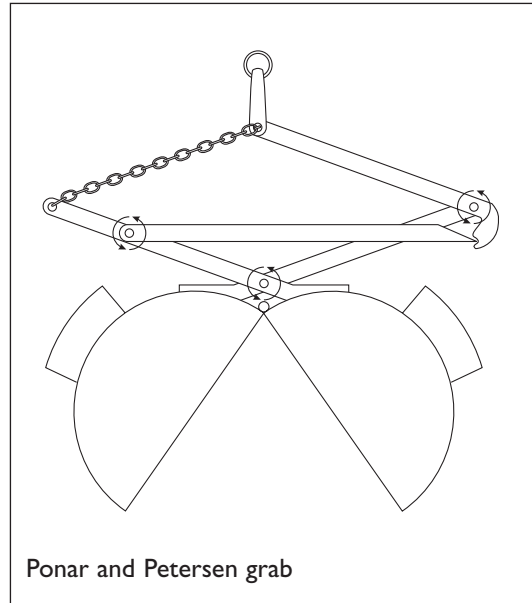
In deeper water use the Petersen grab from a boat.

PONAR AND PETERSEN GRAB

Method

- Check the depth with a stone tied to a line and then ensure you have enough rope tied to the grab. If the bottom is visible, avoid vegetation that might jam the jaws. Coil the rope, load the jaws, lift the grab over the rear of a boat/canoe and drop, allowing it to free-fall (do not burn your hands on the rope).
- Once the rope is slack, haul up the grab and check the jaws are closed properly before emptying and processing the sample as described for the Eckman grab above.

Tip: *Sieving the samples to remove mud and debris is easy to do in the lake, and the large quantities of water required to do it is frequently limiting at a camp.*



OTHER CONSIDERATIONS

Quantitation – the number of samples required will depend upon the abundance of species of interest. If the samples can be washed and sorted the same day, preservative is unnecessary – live organisms are easier to see on white trays.

Mud samples should be processed as soon as possible – within 1–2 days of return to the laboratory. Wash the mud through a series of sieves – 4 mm, 1 mm, 500 μm and 250 μm to remove stones and debris and separate organisms. Backwash the sieve contents on to a white tray and sort. Preserve the organisms in 4% formalin.