

Protocol C1:

Hard-bottomed Semi-quantitative



Requirements:

1. Waders or sturdy boots
2. D-net (0.5 mm mesh)
3. White tray or bucket
4. Sieve or sieve bucket (0.5 mm mesh)
5. Plastic screw-top sample containers (600-1000 ml volume)
6. Fine tweezers
7. Preservative
8. Labels and waterproof marker pen

Protocol:

1. Ensure that the sampling net and bucket/sieve are clean.
2. Select the appropriate habitat (e.g., riffle).
3. Sample beginning at the downstream end of the reach and proceed across and upstream.
4. Select an area of substrate (0.1 - 0.2 m²) to sample with a natural flow that will direct organisms into the net. Place the net on the streambed and step into the sampling area immediately upstream of the net, disturb the substrate under your feet by kicking to dislodge the upper layer of cobbles or gravel and to scrape the underlying bed. The area disturbed should extend no further than 0.5 metres upstream from the net. Remove the material from the net into the tray, bucket or sieve bucket if the net begins to get clogged.
5. Repeat Step 4 at several different locations within a 50 m stream reach and covering a variety of velocity regimes until a total area of 0.6 – 1.0 m² of riffle habitat has been sampled. Transfer this material to a white tray or bucket approximately half full of water, or to a sieve bucket. Wash or pick all animals off the net.
6. Rinse and remove any unwanted large debris items (e.g., stones, sticks, leaves) that may not fit into the sample container or will absorb and diminish the effectiveness of the preservative.
7. Transfer the sample to the sample container via a 0.5 mm sieve if a sieve bucket is not used. Inspect the sieve or sieve bucket and return any macroinvertebrates to the sample container. (Tweezers may be useful).
8. Add preservative. Aim for a preservative concentration in the sample container of 70 – 80% (i.e. allowing for the water already present). Be generous with preservative for samples containing plant material (leaves, sticks, macrophytes, or moss).
9. Place a sticky label on the side of the sample container and record the site code/name, date, and replicate number (if applicable) using a permanent marker. Write on the label when it is dry and do not rely on a label on the pottle lid! Place a waterproof label inside the container. Screw the lid on tightly. Make notes on the field data sheet describing the substrates sampled (cobble size, periphyton, embeddedness, etc.), the collector's name, sample type (e.g., D-net, 0.5 mm), and preservative used.

Protocol C2: Soft-bottomed, Semi-quantitative



Requirements:

1. Waders (chest)
2. D-net (0.5 mm mesh)
3. White tray or bucket
4. Sieve or sieve bucket (0.5 mm mesh)
5. Plastic screw-top sample containers (600-1000 ml volume)
6. Fine tweezers
7. Preservative
8. Labels and waterproof marker pen (or pencil)

Protocol:

1. Ensure that the sampling net and bucket are clean.
2. Sample a unit effort (0.3 m²) of woody debris, bank margins, or aquatic macrophytes using the following procedures. Avoid dredging the net along the bottom in mud or sand, and avoid leaves and algae if possible. Avoid hard (stony) substrates (or sample them separately using Protocol C1).
Woody Debris – Select submerged and partially decayed woody debris (50-250 mm diameter preferred). Place over the mouth of the bucket or sieve bucket. Pour water over the substrate while brushing the substrate gently by hand to remove organisms. Larger pieces may be sampled in situ by brushing the log while holding the net directly behind it. Each 1-metre section of woody debris has a sample area of about 0.3 m².
Bank Margins – Locate an area of bank with good structure and aggressively jab the net into the bank for a distance of 1-metre to dislodge organisms, followed by 2-3 cleaning sweeps to collect organisms in the water column. Each sample unit is about 0.3 m².
Macrophytes – Sweep the net through macrophyte beds for a distance of 1-metre to dislodge organisms, followed by 2-3 cleaning sweeps to collect organisms in the water column. Each sample unit is about 0.3 m².
3. Repeat Step 2 at 10 locations while moving progressively upstream. Remove sample material to a bucket or sieve bucket after each collection to avoid clogging the net. Select substrates to be sampled in proportion to their prevalence along a 50 - 100 m reach of stream. Record the reach length and the proportion of the sample taken from each substrate type (e.g., 50% wood, 25% banks, 25% macrophytes). After the 10th unit effort, wash or pick all animals off the net. The bucket or sieve bucket should now contain one entire sample comprising material dislodged from 3 m² of substrate.
4. Fill the bucket with water and rinse and remove any unwanted large debris items (e.g., sticks, leaves) that may not fit into the sample container or will absorb and diminish the effectiveness of the preservative.
5. Transfer the sample to the sample container via a 0.5 mm sieve if a sieve bucket is not used. Two containers may be needed; each container should be no more than 2/3 full with sample material. Inspect the sieve or sieve bucket and return any macroinvertebrates to the sample container. (Tweezers may be useful here).
6. Add preservative. Aim for a preservative concentration in the sample container of 70-80% (i.e., allowing for the water already present). Be generous with preservative for samples containing plant material (leaves, fine detritus, algae, moss, and macrophytes).
7. Place a sticky label on the side of the sample container and record the site code/name, date, and replicate number (if applicable) using a permanent marker. Write on the label when it is dry and do not rely on a label on the pottle lid! Place a waterproof label inside the container. Screw the lid on tightly.
8. Note the sample type (e.g., D-net), collector's name and preservative used on the field data sheet.
9. Record notes on the field data sheet describing the proportion of habitat units sampled (e.g., 4/5/1, woody debris/bank margins/macrophytes). Also describe on the field sheet the condition of the substrates sampled (woody debris diameter range, type of wood, %cover, periphyton, macrophytes species, bank structure, etc.).

Protocol C3: Hard-bottomed, Quantitative



Requirements:

1. Waders or sturdy boots
2. Surber sampler (area 0.1 m², 0.5 mm mesh)
3. Brush
4. White tray
5. Sieve or sieve bucket (0.5 mm mesh)
6. Plastic screw-top sample containers (600 ml volume)
7. Preservative
8. Labels and waterproof marker pen, or pencil

Protocol:

1. Ensure that the sampling net is clean.
2. Select a suitable sample reach and habitat (e.g., riffle). Sample beginning at the downstream end of the reach and proceeding across and upstream.
3. Place the sampler on the streambed ensuring a good fit around the perimeter. The sampler should be positioned so that the water current washes dislodged material into the net.
4. Brush material from the upper surface of all cobbles contained within the sample quadrat. Pick up each cobble and, holding it immediately in front of the net mouth, brush all sides of the cobble clean. Repeat for all of the larger substrate elements within the sampler quadrat. Place clean cobbles outside of the sampler quadrat. Disturb the finer substrate remaining within the quadrat to a depth of 5 – 10 cm. Beware of broken glass and other sharp objects.
5. Remove the sampler from the water, rinse the net several times to concentrate the sample in the bottom of the net (take care not to lose material during this process), and return to the stream bank. Remove and discard large substrate elements that may have entered the net, taking care to remove adhering invertebrates before disposal. Remove sample from collection net either by inverting net into a suitable container, or by removing container attached to end of collection net. Elutriation may also be required (i.e., repeated rinsing of sample to separate organic and inorganic fractions).
6. Let the sample settle for a few minutes and decant off excess water via the sieve. Return any macroinvertebrates that are washed out with the water to the sample container. (Tweezers may be useful here).
7. Add preservative. Aim for a preservative concentration in the sample container of 70 - 80% (i.e., allowing for the water already present). Be generous with preservative for samples containing plant material (leaves, sticks, macrophytes, moss or periphyton).
8. Place a sticky label on the side of the sample container and record the site code/name, date, and replicate number (if applicable) using a permanent marker. Write on the label when it is dry and do not rely on a label on the pottle lid! Place a waterproof label inside the container. Screw the lid on tightly.
9. Note the sample type (e.g., Surber 0.1 m²), collector's name and preservative used on the field data sheet.

Protocol C4:

Soft-bottomed, Quantitative - Macrophytes



Requirements:

1. Waders (chest)
2. D-net (0.5 mm mesh)
3. Buckets with lids
4. Field balance able to weigh to at least 5 g
5. 0.5 mm sieve or sieve bucket
6. 500 ml wash bottle
7. Plastic screw-top sample containers (250 ml) and zip-lock plastic bags
8. Preservative
9. Labels and waterproof marker pen/pencil

Protocol:

1. Ensure that the sampling net is clean.
2. Approach sample site by moving upstream through the waterway. Determine plant species to be sampled. Consistency in plant species is important for comparisons between sites, although not always possible. Standardise the depth/velocity conditions of sampling points, where possible.
3. Collect replicate samples ($n \geq 4$) of submerged macrophyte tips (approx. 100 g wet weight of top 20-30 cm of plant, which is equivalent to 1.5 – 2L of weed) by moving net upstream into macrophyte bed and breaking off required portion of plant material. Place each replicate sample in a separate bucket. Rinse net thoroughly between replicates.
4. Add approx. 1L of clean water to each bucket and firmly attach lid. Shake bucket vigorously (20x) to detach invertebrates from macrophyte material.
5. Pour dislodged macroinvertebrates and detritus through a 0.5 mm sieve. Rinse each sample twice more in a similar manner.
6. With the aid of a wash bottle, transfer material retained on the sieve to a plastic container.
7. Add preservative. Aim for a preservative concentration in the sample container of 70-80% (i.e., allowing for the water already present). Be generous.
8. Place a sticky label on the side of the sample container and record the site code/name, date, and replicate number (if applicable) using a permanent marker. Write on the label when it is dry and do not rely on a label on the pottle lid! Place a waterproof label inside the container. Screw the lid on tightly.
9. Note the sample type, collector's name and preservative used on the field data sheet.
10. Drain the plant material of excess water (leave to stand in sieve for two minutes) and then weigh to the nearest 5 g using a spring balance. If greater precision is required place plant samples in labelled plastic bags and return to laboratory for drying (70°C for at least 24 hrs) and weighing.
11. Record wet weight of macrophyte material associated with each replicate sample. Also record the species and condition (i.e., senescent, flowering, covered in epiphytes) of the macrophyte bed from which the sample was taken.

Protocol P1:

Coded – Abundance

Requirements:

1. Running water – tap with hose attached recommended
2. Endecott® sieves (e.g., 0.5 & 4.0 mm)
3. Several white trays
4. Petri dishes
5. 2 pairs of fine forceps (#4 or #5)
6. Binocular microscope
7. Identification keys & taxonomic references
8. Preservative
9. Glass vials
10. Labels and sharp pencil

Protocol:

1. All samples received should be recorded in a “laboratory log”. A unique job number, the date received, number and type/s of samples, analyses required, results-required-by date, job manager, and sample processor’s name should also be recorded. The date completed should be entered once sample processing has finished. The fate of samples can then be verified in conjunction with a Chain-of-Custody form.
2. Ensure that the Endecott® sieves are clean and stack them, in the bottom of a sink, with the 4mm sieve on top of the 0.5 mm sieve. Intermediate sieve sizes (e.g., 1 mm & 2 mm) can also be useful.
3. Tip the sample into the top (4 mm mesh) sieve using water to wash all material from the sample container. The objective is to separate the sample into several fractions by size of debris and animals to make it easier to find macroinvertebrates amongst the debris.
4. Wash material through the stack of sieves using water from the hose. Do not use too much water pressure as animals could be damaged.
5. When most of the smaller material has passed through the 4 mm sieve, place the sieve in a white tray. Use the hose to spray water onto the sieve until the tray contains about 30 mm depth of water. Gently move the sieve up and down until no more debris and animals pass through. Pour the water and material from this tray back into the sieves remaining in the stack.
6. Invert the contents of the 4 mm sieve into another white tray and wash all material off the sieve into the tray.
7. Repeat steps 5 and 6 until the contents of all sieves are contained in white trays.
8. Try not to have too much material in each tray. If necessary, use additional trays for the finer fractions so that animals can be seen clearly amongst the debris. (In each tray no more than 70-80% of the tray bottom should be covered by the sample).
9. If there is a lot of heavy material (gravel and stones), lighter material can be decanted off into another tray. Check the stones for heavy animals such as snails and stony-cased caddisflies.
10. Starting with the largest size fraction, work systematically across each tray recording the taxa present and keeping an overall tally of the numbers present so that each taxon can be assigned to one of five coded abundance categories:- R = Rare = 1-4; C = Common = 5-19; A = Abundant = 20-99; VA = Very Abundant = 100-499; VVA or XA = Very, Very Abundant or eXtra Abundant = 500+ animals per sample. Microscopic examination should not be necessary at this stage.
11. Place up to 20 (but at least 1-5) representatives of each taxon encountered into a Petri dish to confirm identifications by microscopic examination (if necessary) and to be placed into a vial containing 70% ethanol for storage and QC.
12. The minimum level of identification required is that specified in Appendix B. Do not include aerial adult insects, terrestrial invertebrates, empty snail shells, insect pupae, caddisfly cases, or exuviae. Examination of late pupae can, however, assist greatly with larval identifications.
13. Place a label in the vial noting the site code/name, date, sample type, and collector’s name. This vial must contain all animals removed from the sample including at least one representative of every taxon found.
14. On completion of sample processing, there should be (1) a vial containing representatives of all taxa encountered in the sample; and (2) the preserved sample residue in its original plastic pottle with original label.

Protocol QC1:

Quality Control for Coded – Abundance

Protocol:

1. All samples received, processed and identified should be recorded in a “laboratory log”. The fate of samples can then be verified in conjunction with a Chain-of- Custody form.
2. Ten percent of the sorted samples to be re-examined by another sorter. The second sorter must be familiar with sorting procedures and the full range of macroinvertebrate taxa from running waters in New Zealand and will be provided with the results from the first sorter.
3. **Taxonomic accuracy.** On average, the number of taxa that are identified as different taxa between the two taxonomists must be < 10% of the total taxa recorded from the sample. For example, a sample with 31 taxa passes QC when no more than 3 taxa are identified differently between the two taxonomists. If the correct taxonomic identification of an organism is disputed, then an agreed expert should check a specimen.
4. **Abundance coding 1 (missed taxa).** New taxa must not be in the A-abundant, VA-very abundant, or VVA-very very abundant categories. A new taxon is one that is not listed on the original data sheet as recorded by the original sorter/taxonomist.
5. **Abundance coding 2 (accuracy).** On average, the total number of taxa re-allocated to an abundance code differing by greater than one abundance code category must be < 10% of total number of taxa allocated an abundance code during the first sort. For example; a sample with 31 taxa passes QC when no more than 3 taxa have abundance codes that are off by more than one category (e.g., VA-very abundant should have been C-common). If average > 10% more organisms are found then a further 10% of samples are to be re-checked. If the criterion is still not met then **all** samples must be re-processed.
6. Trainee sorters should have at least 50% of samples re-checked for QC, and can be considered competent sorters when < 10% of checked samples are returning < 10% new taxa, or < 10% re-codes compared with the first sort.
7. After a sample has been completely sorted all sieves, trays and equipment should be cleaned thoroughly and picked free of organisms and debris before the next sample is processed.

Protocol P2:

200 Fixed Count + Scan for Rare Taxa

Requirements:

1. Running water – tap with hose recommended
2. 0.5 mm sieve
3. Clean, flat-bottomed, white tray marked in 6 cm x 6 cm grids
4. 6 cm x 6 cm cookie cutters
5. Fine forceps
6. 70% ethanol preservative
7. Specimen vials with stoppers
8. Bench lamp
9. Labels and sharp pencil
10. Counter
11. 500 ml wash bottle
12. Identification keys & taxonomic references
13. Binocular dissecting microscope and light source for species identification

Protocol:

1. All samples received should be recorded in a “laboratory log”. A unique job number, the date received, number and type/s of samples, analyses required, results-required-by date, job manager, and sample processor’s name should be recorded. The date completed should be entered once sample processing has finished. The fate of samples can be verified in conjunction with a Chain-of-Custody form.
2. Thoroughly rinse sample in a clean 0.5 mm sieve to remove preservative and fine sediment. Large organic material (whole leaves, twigs, algal or macrophyte mats, etc.) not removed in the field should be rinsed, visually inspected for organisms, and discarded. Gently mix the sample by hand while rinsing, and continue until wash water runs clear and the sample is thoroughly homogenised (i.e., break down lumps of algae etc). A coarse sieve (e.g., 4 mm) can be helpful for removing larger pieces of unwanted organic material so long as all macroinvertebrates are picked out and placed into the 0.5 mm sieve.
3. After washing, transfer contents of sieve to a white sorting tray marked with grids approximately 6 cm x 6 cm (use black indelible marker). Visually check sieve before washing in preparation for next sample. Using the wash bottle spread the sample evenly across the tray. There should be enough water to just cover all material. If the samples have been preserved in alcohol some organisms (particularly ostracods and early instar insects) may float on the surface. If this is occurs add a drop of washing detergent and stir gently.
4. Use a random numbers table to select a starting grid square within the tray. A cookie cutter (6 x 6 cm) is recommended to delineate the chosen grid square. Moving systematically across the square remove all organisms visible to the naked eye. Place captured organisms in a separate labelled vial (add preservative), counting each individual with a counter. When complete, do a final check of the square’s contents to ensure no animals have been missed.
5. Any organism that is lying over a line separating two grids is considered to be in the square containing its head. In those instances where it may not be possible to determine the location of the head (worms for instance), the organism is considered to be in the square containing most of its body.
6. After all visible organisms have been removed use forceps and/or a suction device to transfer remaining detritus to a container labelled as “sorted residue”. Include location and date information (as per original sample label). Add preservative. This provides material for sorting QA/QC procedures.
7. If a total of at least 200 organisms have been obtained sample sorting ceases. However, if less than 200 organisms have been enumerated, place another cookie cutter on a second randomly chosen square. Continue this process until at least 200 animals have been captured.
8. Once a square has been started it should be finished, even if the 200 individual total is exceeded. The total number of grid squares covered should be noted, along with the total individual count.
9. Save the remaining unsorted sample debris residue in a separate container labelled "sample residue"; this container should include the original sample label. Add preservative.
10. The “sample residue” and vial containing the 200 individuals must be sorted by an experience taxonomist. (Note: In situations where the sorter is an experienced taxonomist, invertebrate identification and counting can be carried out during the sorting process to save time). Pour the 200 individual sample into a Petri-dish or Bogorov tray and observe under a binocular microscope. Compile a taxa list and count the numbers of each taxon. Return the 200 individuals to a labelled vial and add preservative. This sample will be used for taxonomic QA/QC (see below).

continued overleaf

Protocol P2 continued

11. The minimum level of identification required is that specified in Appendix B. Do not include aerial adult insects, pupae, terrestrial invertebrates, empty snail shells, caddisfly cases or exuviae. Examination of late pupae can, however, assist greatly with larval identifications.
12. Complete the taxa list by scanning the "sample residue" for rare taxa. This is carried out with the sample spread in white sorting trays. Any rare taxa obtained should be placed in a labelled vial with preservative. This is also an opportunity to remove larger (e.g., late instar) or better-conditioned individuals of taxa already encountered to assist in identification.
13. The vial containing the 200 individuals, and the vial containing rare taxa should be taped together. Record the taxa found in the scan for rare taxa separately from the 200 fixed count data.
14. Return the "sample residue" to its container with the original labels.
15. On completion of sample processing there should be: (1) A labelled container holding the sample residue (already scanned for rare taxa); (2) A labelled container holding the sorted residue (required for QC procedures to assess sorting efficiency); (3) A labelled vial containing the 200+ individuals; and (4) A labelled vial containing the rare taxa (not included in the 200+ sample) removed from the sample residue.

Protocol QC2: Quality Control for Fixed Count

1. Protocol:
2. All samples received, processed and identified should be recorded in a "laboratory log". The fate of samples can then be verified in conjunction with a Chain-of- Custody form.
3. Ten percent of the sorted samples to be re-examined by another sorter. The second sorter must be familiar with sorting procedures and the full range of macroinvertebrate taxa from running waters in New Zealand and will be provided with the results from the first sorter.
4. The fixed count protocol requires examination of the sample residue (were all rare taxa removed by the first sorter?) and the sorted residue (were any animals missed during the collection of the 200+ individual sub-sample?). A check on the taxonomic efficiency of both the 200+ sub-sample and the vial of rare taxa are also required.
5. **Taxonomic accuracy.** On average, the number of taxa that are identified as different taxa, in either the full 200+ individual vial, or the rare taxa vial, between the two taxonomists must be < 10% of the total taxa recorded from the sample. For example, a sample with 31 taxa passes QC when no more than 3 taxa are identified differently between the two taxonomists. If the correct taxonomic identification of an organism is disputed, then a specimen should be checked by an agreed expert.
6. **Sorting accuracy 1 (missed taxa).** If average > 10% new species are found in the sample residue then the scan for rare taxa is deemed to have failed and a further 10% of samples are to be re-checked. If the criterion is still not met then all samples must be re-processed.
7. **Sorting accuracy 2 (missed individuals).** If average > 10% more organisms are found in the sorted residue then a further 10% of samples are to be re-checked. If the criterion is still not met then all samples must be re-processed.
8. Trainee sorters should have at least 50% of samples re-checked for QC, and can be considered competent sorters when < 10% of checked samples are returning < 10% new taxa, or < 10% re-codes than first sort.
9. After a sample has been completely sorted all sieves, trays and equipment should be thoroughly cleaned and picked free of organisms and debris before the next sample is begun.

Protocol P3:

Full Count with Subsampling Option

Requirements:

1. Running water tap and sink
2. Endecott® sieves (0.5, 1.0, 2.0, & 4.0 mm)
3. Grided white trays
4. Petri dishes
5. 2 pairs of fine forceps (#4 or #5)
6. Binocular microscope
7. Identification keys & taxonomic references
8. 70% ethanol preservative
9. Glass vials and/or pottles
10. Labels and sharp pencil

Protocol:

1. Sieve and place the sample in grided sorting trays following Protocol P1.
2. Starting with the largest size fraction, work systematically across each tray removing all of the organisms in the sample. Normal eyesight should be precise enough to detect organisms > 1mm in total length. Do not use magnification.
3. Place the organisms of each taxon encountered into separate Petri dish to confirm identifications by microscopic examination (if necessary). Place sorted animals into vials or pottles containing 70% alcohol for storage and QC.
4. The minimum level of identification required is that specified in Appendix B. Do not include aerial adult insects, pupae, terrestrial invertebrates, empty snail shells, caddisfly cases or exuviae. . Examination of late pupae can, however, assist greatly with larval identifications.
5. Place a label in the vial or pottle noting the site code/name, date, sample type, and collector's name. Label multiple containers (e.g., "1 of 2, 2 of 2).
6. On completion of sample processing, there should be (1) labelled vials or pottles containing sorted organisms, and (2) the preserved sample residue in its original plastic pottle with the original label.

Subsampling Option:

(Note: Only very abundant taxa should be subsampled. Full counts should be made for all other taxa).

1. Subsampling of very abundant taxa (> 500 individuals) can save considerable time.
2. Count the number individuals of each very abundant taxon from a fixed fraction (between 10% and 50% recommended) of the sample grids for each sorting tray. Estimate the total abundance for that taxon by multiplying the number counted by between 10 (for 10% fraction) and 2 (for 50% fraction) according to the fraction of the sample that was counted.
3. Record the count estimate on the bench data sheet and note that the value is a subsampling estimate (e.g., 25% fraction).
4. Remove 10-20 representatives of each taxon subsampled and store in a separate vial or pottle from that containing the other sorted organisms.

Protocol QC3:

Quality Control for Full Count with Subsampling Option

Protocol:

1. All samples received, processed and identified should be recorded in a “laboratory log”. The fate of samples can then be verified in conjunction with a Chain-of- Custody form.
2. Ten percent of the sorted samples to be re-examined by another sorter. The second sorter must be familiar with sorting procedures and the full range of macroinvertebrate taxa from running waters in New Zealand and will be provided with the results from the first sorter.
3. **Taxonomic accuracy.** On average, the number of taxa that are identified as different taxa between the two taxonomists must be < 10% of the total taxa recorded from the sample. For example, a sample with 31 taxa passes QC when no more than 3 taxa are identified differently between the two taxonomists.
4. **Sorting accuracy.** On average, the total number of each taxon found in the remnant sample must be < 10% of total for each taxon counted during the first sort. If the QC sorter finds less than an average 10% more organisms than recorded in first sort then the sample passes QC requirements. If average > 10% more organisms are found then a further 10% of samples to be re-checked. If the criterion is still not met than all samples must be re-processed and resorted. If the correct taxonomic identification of an organism is disputed, then a specimen should be checked by an agreed expert.
5. Trainee sorters should have at least 50% of samples re-checked for QC, and can be considered competent sorters when < 10% of checked samples are returning < 10% more organisms and < 10% new taxa than first sort.
6. After a sample has been completely sorted all sieves, trays and equipment should be thoroughly cleaned and picked free of organisms and debris before the next sample is introduced.