

AQUATIC INVERTEBRATES

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INTRODUCTION

Aquatic invertebrates are important constituents of aquatic ecosystems, providing exploitable resources for fish and humans (e.g. crabs, shrimps and molluscs) and vital functions such as the decomposition of organic debris and release of plant nutrients. The term aquatic invertebrates includes the floating plankton, swimming nekton, organisms associated with plants (periphyton) and sediments (benthos) and the surface-dwelling neuston.

Aquatic organisms are exposed to the effects of agrochemicals in two ways: through the direct and deliberate application to water, such as the introduction of pesticides to control weeds or the vectors of disease-causing organisms (blackfly, snails, mosquitoes, etc.) and through indirect means, such as spray-drift deposition or run-off from riparian land. Laboratory and field studies show that aquatic invertebrates are at risk from virtually all groups of synthetic and natural insecticides. Their response to exposure *in vivo* is quite variable as it is related to the physical, chemical and biotic characteristics of their environment, but in general, aquatic invertebrates (including surface-dwelling species) are remarkably sensitive to insecticides. Being a high-risk group to low level pesticide exposure, they require a considerable degree of surveillance and monitoring; but their sensitivity can be used to provide a proxy measure of insecticide contamination of lotic (flowing) and lentic (still) waters, as bioindicators.

A selection of simple, low-cost and robust sampling techniques are given in this chapter that will enable biological monitoring of streams, rivers, swamps, lagoons, pools and lakes. The aim of the biological monitoring is to gather information about the relative abundance and composition of invertebrates over space and time from which decisions about potential agrochemical impacts can be deduced. This does not mean studying and collecting unmanageable amounts of data on all species – a frequent temptation – but rather focusing on key species or functions that are at risk. The techniques described below are a few of many but they have broad application and proved their worth in collecting qualitative and quantitative biological data from diverse aquatic habitats.

No single method will suffice to sample the diversity of species colonizing a body of water but it is rare that monitoring all the invertebrates within the biome is necessary for impact studies. Biological surveillance and monitoring should be accompanied by some basic aquatic physico-chemical monitoring, such as water pH, temperature, oxygen concentration and conductivity, as these parameters invariably help to interpret and discriminate change resulting from natural variation and agrochemical impact (see chapter 5).

STUDY DESIGN

The first step is to describe the observed or expected problem. For example, the regional plant protection service is intending to spray an area close to a wetland site to control a specific pest and this operation could lead to contamination of the river or lagoon by spray-drift of aerosol droplets. The planning strategy is to list the possible effects of such an action, form a testable hypothesis (null) and consider what variables might either

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hinder the testing of the hypothesis or complicate the interpretation of your results. Figure 1.1 in chapter 1 gives an example. Potential impact is determined from a desk study, i.e. from knowledge of a pesticide's formulation, dose, method and scale of application, persistence, ecotoxicology and physico-chemical properties. An hypothesis about the potential impact is made and a sampling strategy is then devised that will collect the organisms indicated to be at risk. Many field studies are invalidated by poor design and insufficient sampling but some of the pitfalls can be avoided by adhering to sound statistical principles and the guidelines provided below. The classic texts of Southwood (1996) and Elliot (1971) provide a range of ecological and statistical methods for hydrobiology.

Table 9.1 lists the aquatic invertebrate groups most susceptible to pesticides. However, as the dose and frequency of pesticide application vary with the type of pest control measure and also because pesticide bioavailability and toxicity to organisms is related to environmental factors, the list is only indicative. Many more groups and species are at risk from pesticides applied directly to water.

Table 9.1 Aquatic invertebrate groups sensitive to pesticide contamination

Pesticide type	Indirect contamination	Direct contamination
Organochlorines	Crustacea, Ephemeroptera and Plecoptera	All zooplankton and benthos at risk
Organophosphates	Surface-dwelling Heteroptera, Coleoptera (particularly dytiscids), Ephemeroptera and Trichoptera	Plus Cladocera, Amphipoda and Diptera
Carbamates	Crustacea, Ephemeroptera, Trichoptera, Odonata and Zygoptera	All zooplankton and benthos at risk
Pyrethroids	Crustacea, Coleoptera, Heteroptera, Trichoptera, Ephemeroptera, Odonata and Zygoptera	Plus all benthos except Mollusca
Insect growth regulators	Macrocrustacea, zooplankton and other arthropods	All arthropods
Phenyl pyrazoles	Micro- and macrocrustacea, bivalve molluscs, filter feeders	All arthropods
Molluscicides	n/a	All benthos at some risk
Herbicides	Phytoplankton and invertebrate population shifts	Risk through oxygen deprivation (decaying plants)

The methods employed to sample these and other organisms are general collection techniques, i.e. they do not target specific groups and invertebrates, This versatility helps in observing wider population and behavioural change.

Flowing water

By way of example, a plant protection department will aerial spray 16 km² of grassland that borders a river. Having concluded from the desk study that the risk of spray-drift to the river is significant and that an impact on benthic invertebrates is very likely, the (null) hypothesis, that the organophosphate will not change the type or abundance of benthic invertebrates, must now be tested. Access permitting, choose the sampling sites so that substrate type, flow rate and rooted or emergent vegetation appear well matched. Look for riffle sections, i.e. shallow areas of turbulent flow over small rocks or gravel, when scouting the river as these are often the most productive areas, home to many 'sensitive' invertebrates (stoneflies, mayflies and crustaceans) and are easy to sample (see also 'Site Selection' in chapter 1, page 15).

Select at least two sites well upstream (e.g. 10 km) of the treated area to act as an unsprayed area (control), two or more in the target area and two well downstream of it, from which information on the extent of any effects may be gathered. There are no hard and fast rules about siting sampling stations and compromises from the ideal are the norm. Avoid sites likely to provide confounding data, such as just downstream of a village, clothes washing sites, abattoirs and industrial effluent, crops areas subject to localized pesticide application, etc. (Figure 9.1).

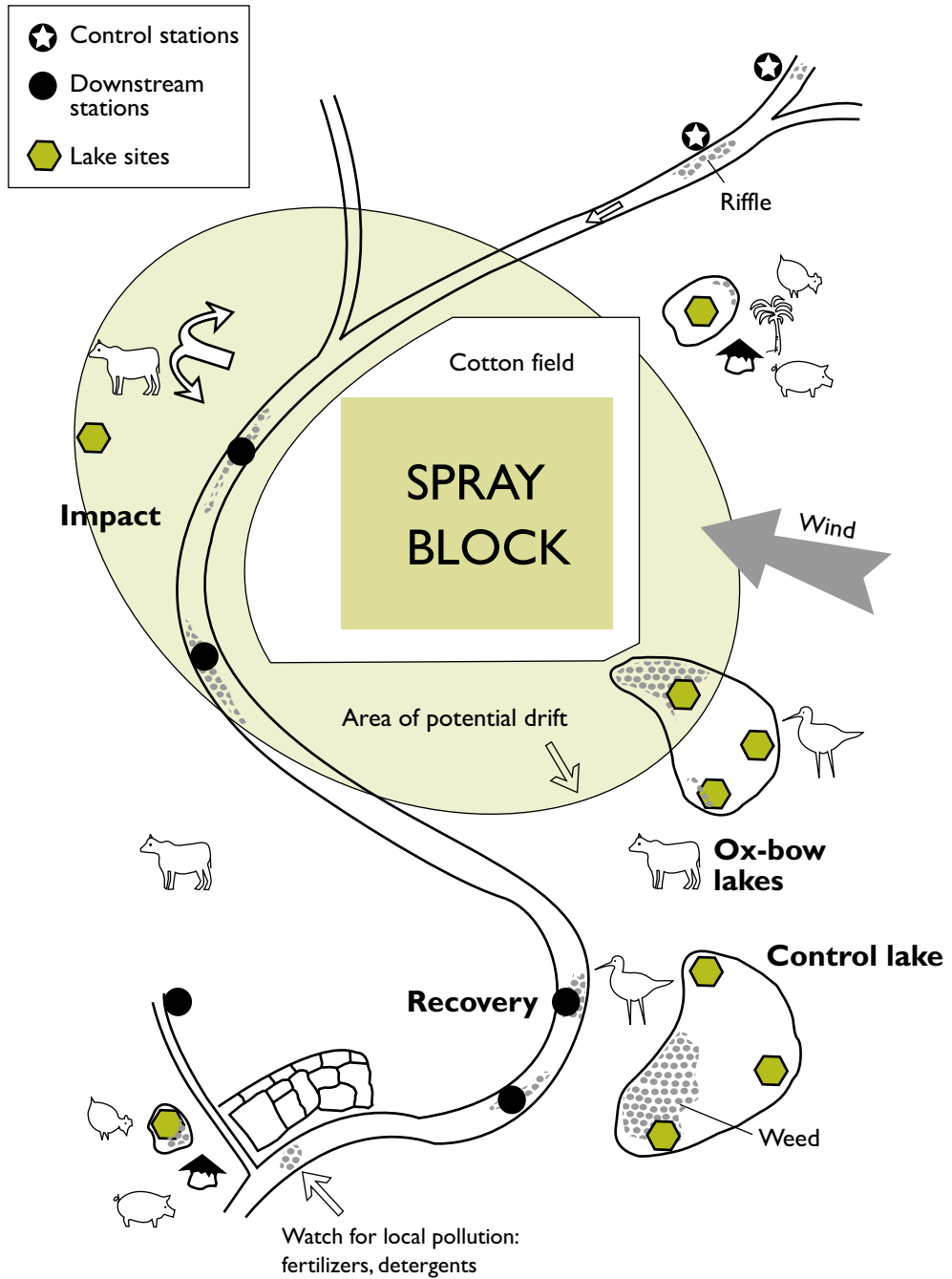


Figure 9.1: Potential lake and riverine sampling sites

Still water

Identification of population change as the means to assess the impact of pesticides on lentic biota is complicated by a usual lack of well defined treated and untreated areas of water. Change must be assessed from a before- and after-pesticide study and unless there are nearby water bodies of a comparable biological structure, distinguishing small, potential effects from natural variation is perplexing. Also, ponds, small lagoons and lakes are regarded, statistically, as one site, even though many replicate samples at more than one location across the lake may be taken (see 'Pseudo-replication' in chapter 2, page 58) and cause and effect reasoning may produce false positives.

Whether sampling flowing or static water, scout for possible sampling stations well in advance of monitoring to allow time to walk or boat to the banks and match site characteristics, access points and the uses to which the water is being put (effluent, irrigation, fish pools, etc.). Avoid choosing obvious access points such as fords or road bridges as sampling stations as they also provide easy access to local people for washing, playing, fishing, etc.

SAMPLING STRATEGY

A decision must be made about the level of data collection required, as this will affect the information value of the data and the reliability of any impact assessment. Qualitative methods provide species lists. They are useful for general collecting and assembly of baseline information on fauna. It is a mistake to think they are not time consuming, as 10 samples per site might be required to retrieve 80% of the species present and the taxonomic effort expended in sorting and identification is enormous. However, they are useful for post-spray recovery studies, where the objective is to determine survival of sensitive species affected or eliminated by pesticide use. Collecting data for comparing relative abundance or absolute numbers of species over space and time requires quantitative techniques and increased effort. Quantitative techniques are normally employed for pesticide impact studies, where identification of the degree of population change is frequently the objective. Obtaining reliable estimates of species abundance requires uniform, replicated sampling and some knowledge of species distribution and statistics. Table 9.2 shows a range of techniques that can be used to collect qualitative and quantitative information on aquatic biota. Their merits and limitations are discussed briefly in the 'Sampling Techniques' section and their use is described in the method sheets.

The following considerations apply to both lotic and lentic environments.

- Try to sample the same substrate type when taking replicate samples at a site. If the swamp bottom is 50% sand and 50% vegetation, stratify the samples and take half the samples from each habitat. The size of the sampling station should be large enough to allow enough replicate samples to be taken without trampling or otherwise disturbing the substrate to be sampled in the process.
- Think about the best sampling method for retrieving the information you require to meet your objectives, including the number of replicates required for statistical analysis: in practice, the latter is often a compromise between the optimal number (on the method sheets) and the time or resources available for processing. Too few replicate samples (<4) could render quantitative work on some species useless.
- Consider the way the pesticide is reaching the water and choose the most appropriate sampling method to measure the organisms most at risk, e.g. surface-dwelling organisms are at more risk from aerosol droplets than from surface run-off.
- Do not attempt to compare riffle sections with pools, weedbeds with clean gravel substrates, wet season with dry season data or sites just up and downstream of a confluence.
- Try to begin sampling at least 1–2 months before the chemical intervention. Sampling frequency is determined by logistical and environmental factors such as manpower, period of river flow, longevity of temporary pools, life cycle length, emergence periods, weather, etc. Sampling at 2-week intervals is a

reasonable goal for an intensive (short-term) study. Post-spray monitoring to determine whether recovery from identified impacts has occurred should continue, ideally, until full recovery is demonstrated; in practice this is rarely achieved, largely because of cost and natural variability of populations.

- With pre and post studies that lack a proper unsprayed control, try to find archive information that provides details of seasonal change in previous years.

Mesh sizes

Methods that employ nets to trap benthic invertebrates, such as sweep nets, cylinder and drift samplers, rely on the mesh size (aperture) to retain organisms of interest. Smaller organisms such as tubificid worms and first instar chironomid larvae may pass through nets with apertures >250 µm. Mesh of this size will clog up quickly when the substrate is disturbed (kick and cylinder sampling) or if samplers are left in place for long periods (drift sampling). A compromise is normally necessary: use larger mesh and anticipate losing some smaller organisms or reduce the mesh size and sample for shorter periods. A good aperture size for general collection of macro-invertebrates is 400 µm. Nylon mesh is much more durable than widely available muslin or mosquito netting, and whose apertures tend to be variable and closer to 1000 µm. Reduce the mesh size if you wish to make a special study of smaller species life stages.

Table 9.2 Aquatic sampling techniques: indicative

Method	Non-target biota					Habitat type			Pesticide
	Benthos	Surface-dwellers	Plankton	Algae	Epiphytic	Flowing	Static	Ephemeral	All
Qualitative									
Heel/kick	●					●		●	●
Drift	●	▲	○						●
Artificial substrate	●			●		●	●	●	●
Sweep net	●	●			●		●	●	●
Plant stems/roots					●	▲	●	●	●
Quantitative									
Cylinder/surber	●					●		●	●
Emergence	●					●	●	●	●
Plankton net			●	●			●	●	●
Grab	●						●	●	●
Artificial substrate	●			●	●	●	●	●	●
Drift	●	▲	○			●			●

● = best; ▲ = second best; ○ = possible if zooplankton retaining mesh size used.

SAMPLING TECHNIQUES

QUALITATIVE METHODS

Heel or kick sampling

This simple method for sampling benthic invertebrates in streams and rivers is excellent for general collecting and only requires a pond net. When replicated in rich habitats, the method can provide an impressive collection of fauna that may be ranked and subjected to non-parametric analysis – but at best it is only semi-quantitative. A pond or hand net (see method sheet) is held downstream of the operator, who grinds or tramples the substrate for a fixed period with boot heels to dislodge organisms which are swept into the net by the current. Heel sampling while walking backwards for a short distance will obtain larger samples. The method is suitable for general collecting of benthic invertebrates in sand, gravel and pebble substrates, but not large rocks or bedrock. There is rarely enough current over depositing substrates to use the method effectively and so tubificids (tube-dwelling worms) and other sediment dwellers (in-fauna) cannot be collected properly by this method. The frequency of sampling will be synchronized with spraying events and their severity: typically visit all sites every 10 days before spraying, then immediately after spraying and 3, 5, 10 and 20 days thereafter.

Limitations Qualitative; semi-quantitative at best. Cannot be used effectively in rivers deeper than the net height.

Processing Organisms are separated by eye from the debris using forceps and Pasteur pipettes and sorted into groups for counting. See section on page 193.

Resulting data Information on organisms are ranked at the family, genus or species level according to their relative abundance, e.g. 0–2 rare; 3–10 occasional; 11–50 frequent; 50–100 abundant. Set your own scale.

Sampling period 2 min.

Equipment Hand net and screw-cap bottles, glass jars or plastic containers.

Staff required 1.

Sampling surface-dwellers

Surface-living invertebrates such as the beetle families Gyrinidae, Veliidae, Hydrometridae and Gerridae are hard to sample. Counting the number of whirligig beetles in part of a river has little meaning as their sudden absence or appearance could be interpreted in many ways. A surface-breaking drift net is a fairly effective tool for trapping surface-dwellers affected by insecticides, whether from aerosol spray-drift or deliberate introductions to streams. The net traps disoriented or dead organisms and gives a wide picture of how a stretch of river is being affected by a toxin. With some types of pesticide application, terrestrial invertebrates in overhanging trees fall into the river too, and this increases the burden of processing. Other groups that are hard to sample such as backswimmers and water boatmen are also trapped. The drift net technique will provide no comparable data at control sites.

Drift nets (see below and method sheet) are staked to sample the top few centimetres of river instead of the main channel flow but otherwise the siting, periodicity of sampling and processing are no different from the sampling of invertebrate drift.

Limitations With only one net in each of the treated or untreated stretches of river, the method has no quantitative application.

Processing and resulting data As for heel samples.

Sampling period Between 1 h and 4 h after a spray event, depending upon how clogged the net becomes.

Equipment Drift net, flow meter and stakes to secure net.

Staff required 1 or 2.

Artificial substrates

Bedrock, sand, lake mud and weed beds can be difficult to sample with nets, especially in static water. Artificial substrates provide surfaces upon which organisms can alight and eventually colonize. Provided that sufficient colonization time is available, stones, tiles, bricks, plastic balls and tubes are all suitable materials to place in water,

either in a mesh bag or box on the bottom or suspended in water. After 2 weeks or more in water they may be removed, examined, washed in a bucket and replaced for another period. The interval spent submerged should be uniform at and between sites. About 4–8 artificial substrate samplers per site should provide enough information for statistical purposes. If the substrate sits on a fine mesh net that can be pulled over the sampler on retrieval, a quantitative result, relating numbers to surface area of substrate, can be achieved.

Limitations If the substrate presented is of uniform area, a semi-quantitative result is obtained that is useful for between site comparisons, *but the fauna sampled may not reflect the structure of the normal community of the underlying substrate.*

Processing The bucket washings are sieved and the contents sorted into groups using a white tray. Preserve for identification and counting. See section on page 193.

Resulting data Number of organisms per unit area.

Sampling period Minimum of 2 weeks.

Equipment Wire mesh and stones or other suitable substrate.

Staff required 2 is most efficient.

Sweep net sampling

Sweep nets can be used to sample qualitatively fauna associated with plant stems and roots of submerged and emergent vegetation (e.g. papyrus and *Vossia* stands), the roots of floating vegetation (e.g. *Eichhornia crassipes*, water hyacinth) or whole floating plants (*Salvinia*, *Piscia*).

Limitations The data collected are normally hard to rank and analyse statistically, but despite these limitations, the method provides information on species richness and can detect changes in relative abundance, e.g. the sudden absence of a shrimp or mayfly nymph that may be biologically significant. When used to sweep up whole plants such as *Salvinia*, the fauna may be related to the wet or dry weight of vegetation. A triangular pond net is useful for timed foraging in amongst weed beds and the rhizomes of papyrus and other grasses.

Processing Sweep net samples are processed in the same way as for heel samples.

Resulting data Data are normally expressed in catch per unit effort, such as the number of shrimp caught in a 3-min sweep of plant roots.

Sampling period 2–5 min.

Equipment Sweep net – these are easily fabricated locally – and collecting bottles.

Staff required 1.

Aquatic weeds and roots

Rooted vegetation provides a relatively stable substrate for invertebrate colonization. Trichoptera, Ephemeroptera, Chironomidae, Ostracoda, Isopoda and simuliids may be qualitatively sampled by cutting weed mats but comparing densities of organisms between sites is not straightforward, given the sampling difficulties and variation. It is probably as useful and faster to employ timed sweep netting of rooted vegetation or the roots of floating weeds rather than attempt semi-quantitation using dry weight or weed area.

QUANTITATIVE METHODS

Cylinder or box sampling

Quantitative information on benthic fauna inhabiting stream and river beds can be obtained using a cylinder or box which encloses a known area of stream bed (0.05 m² is a practical size but smaller is workable where the substrate is gravel or pebble). Compared with a box sampler, which is not easily rotated in stony substrates, the cylinder is more versatile and can be used in soft and stony substrates. Both are of limited use on bedrock, although a foam rubber skirt fitted on the bottom of the sampler can seal off the area of smooth rock to be sampled. The cylinder is driven into the substrate to a depth of about 5 cm. Water passing through the meshed entrance, which faces upstream, washes the animals displaced within the enclosed area into a net tied to the downstream exit (see method sheet). The ideal number of samples taken at any one site is 4–8.

Limitations Stream depth cannot be greater than the height of the samplers (30–40 cm) and they cannot sample large rocks. A Surber sampler can be used in the same way but it has disadvantages: the quadrat only rests on the substrate, sampling is a two person job, and it is effective at a lesser water depth (10 cm).

Processing Organisms are sorted on a white tray into groups and stored in alcohol while awaiting identification. See section on page 193.

Resulting data To maximize the information returned from quantitative samples, organisms are normally identified, where possible, to species. The mean, standard deviation or confidence limits of the mean number of a group or species are presented graphically against, for example, time or site number.

Sampling period A typical sampling frequency would be every 2–3 days immediately after spraying, and weekly or biweekly later on. The frequency will depend upon the severity of the response – the more severe then the greater the frequency. In small streams the number and frequency of samples may be determined also by the surface area of substrate available – the exact same area should not be sampled more than once every 2 weeks, allowing time for recolonization.

Equipment A cylinder, metal box and nets can be fabricated locally. Catering size coffee cans or plastic pipes can also be utilized.

Staff required The ideal number of staff required for all quantitative sampling techniques is 2.

Invertebrate drift sampling

Invertebrates in streams and rivers periodically drift downstream. The largest number of animals normally drift at night, just after dark, but during periods of heavy rain or drought, drift densities soar. Insecticides may cause sensitive species to drift in huge numbers and for many hours after they contact water. The response may be orders of magnitude greater than normal drift densities and can be used as a biological indicator of contamination of water, even at very low concentrations of chemical.

Ideally, drift nets are located above and below the site of pesticide application. Chemical aerosols are often carried considerable distances by prevailing winds and remain airborne for some days. Upstream ‘control’ drift sites should, therefore, be 10 km or more from the nearest site of chemical application. Two or three well-spaced, downstream, drift net sites are preferable to one but access or stream length/depth often determines the number. Methods of measuring current for the estimation of drift density are provided in chapter 5.

In fast flowing streams and during the wet season, nets quickly clog up with debris. Larger mesh sizes will reduce clogging but decrease the catch of smaller organisms. The simplest solution to clogging is to empty nets frequently.

Limitations Although drift can be quantified easily, drift density cannot be reliably related to production or standing populations of benthic invertebrates. This is a consideration if time or manpower is a constraint: it may be worth considering using quantitative population estimates and fewer drift nets. Some invertebrates tend to drift more than others and so the technique is selective.

Processing The samples are washed in a sieve (same aperture size as net mesh) and placed on a white tray. Invertebrates are sorted into groups, preserved in alcohol (70%) or formalin (4%) and later identified and counted. See section on page 193.

Resulting data Drift density is calculated 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. Graphic presentations are very effective at communicating the results.

Sampling period Following insecticide contamination of water, invertebrates may clog downstream nets within 30–60 min. Nets should be observed to ensure water is not ‘backing-up’ at the mouth of the sampler otherwise drifting organisms will be deflected away from the mouth of the net. Under normal conditions, 24 h is a convenient sampling interval as it covers the natural photoperiod to which many invertebrates respond. During or after insecticide use, the frequency of sampling is determined by the amount of material collecting in the net. Samples are taken until the downstream drift density once more approximates that upstream of the contamination.

Equipment Drift nets, preferably with collection bottle attached, stakes, current meter and sample bottles.
Staff required 2 is ideal.

Plankton sampling

Phytoplankton and zooplankton densities may change markedly in response to the application of insecticides, herbicides and fertilizers near lakes, ponds, swamps and rivers. In rivers and oligotrophic standing water, plankton normally needs to be collected with a net that is hauled vertically (deep water) or horizontally to concentrate their numbers sufficiently for counting. As nutrient levels increase, as in river pools, ox-bow lakes and eutrophic waters, plankton becomes more abundant and may, in the absence of a net, be sampled with a wide-mouth bottle.

Sampling strategies for rivers will be similar to those for benthic invertebrates, i.e. sites upstream and downstream of the perturbed area would be sampled as 'unsprayed' control and treated areas. Where whole ponds and lakes are affected by some form of agrochemical intervention, an unaffected water body might act as a control site, or if the intervention is deliberate (e.g. mosquito, snail or weed control) or the timing known (e.g. aerial tsetse control), then pre-treatment data can be gathered. Difficulties in interpretation of post-spray data in standing water are eased if the natural variation of plankton abundance is known from a matched control site, but in practice, these are hard to find.

Limitations Oscillations in plankton densities occur regularly and in response to changing water temperature and light. Direct and indirect effects of agrochemicals on crustaceans, rotifers, diatoms, and green and blue-green algae are not easy to determine, especially when the timing of the intervention is unknown, e.g. run-off of chemical occurring over an extended period or when the deposition of chemical on a water body is low (as with spray-drift). Sorting plankton to main taxonomic groupings is easily achieved. Where species *x* of a group is clearly affected by a chemical, seek specialist help with identification.

Processing If the water sampled was green or the collecting bottle was teeming with zooplankton before preservation, it is likely that sub-sampling or dilution is necessary to reduce the number of organisms prior to counting. Alternatively, a haemocytometer can be used to count phytoplankton in a small volume of water without the need for dilution. Other specialized counting chambers are available for counting low density populations (e.g. Sedgewick-Rafter chamber), but a small Petri dish standing on graph paper will normally suffice, provided wind and heat do not circulate the contents. Zooplankton can be removed from a phytoplankton sample by sieving through fine nylon mesh or muslin. Zooplankton and rotifers are easily counted in a Petri dish sitting on graph paper.

Resulting data Calculate serial dilutions before expressing abundance as numbers (or biomass) per unit volume of water.

Sampling period In ponds and lagoons, the length or depth of the haul determines the sampling period. A 10 m haul is sufficient where plankton are abundant.

Equipment Microscope, counting chamber, Petri dishes, plankton net and bottles.

Staff required 1.

Emergence traps

Insect emergence from water can be assessed with emergence traps. Emergence is not a proxy measure of population density but it is indicative of insecticide impact on a crucial phase in an insect's life cycle. The dynamics of emergence are a function of life history, temperature, light and wind and as these are so variable a large number of traps (5–10) must be deployed to reduce sampling errors. Emergence samplers are useful for quantifying the effects of insect growth regulators and microbial insecticides on insect metamorphosis or nymphal development, where cylinder or other sampling techniques cannot. The traps are set just below the water surface to prevent non-emerging insects from flying inside. In theory, they sample a known area of substrate but riverine traps may not because of the current. Insects that emerge by crawling up emergent plants (e.g. Odonata – dragonflies and damselflies) are generally not sampled, but the method is more efficient for

chironomids and other Nematocera. Traps can be used in shallow littoral regions of still waters or they can be floated on a lake surface. Mundie (1971) reviewed trap construction and use.

Limitations The numbers of emerging insects can be very low at certain times of year and consideration should be given to the value of using the method when low trap catches can seriously reduce the power of the statistical comparison. Traps must be anchored in the wet season.

Processing Traps are emptied periodically and the contents sorted on a white tray. See section on page 193.

Resulting data The density of insects is expressed as numbers of species or groups per unit area (m^2).

Sampling period Two weeks before and after treatments are administered (minimum) but the traps should be emptied every 2–3 days and the formalin recharged.

Equipment Emergence traps, formalin and sample bottles.

Staff required 1.

Grab sampling

In general, sediment-dwelling invertebrates are better protected from incidental pesticide deposition into water bodies than plankton and nekton, as adsorption of chemicals on to the sediment reduces their immediate biological availability and toxicity. Impact assessment and biological monitoring of tubicolous worms (e.g. Tubificidae) and molluscs is not, therefore, commonplace. Where in-fauna are exposed to high concentrations of insecticide, however, such as during the control of the vectors of onchocerciasis, schistosomiasis and malaria, there is good reason to monitor populations of target (e.g. snails) and non-target organisms by sampling the sediment. Assessment of the indirect impacts of dense floating weed (reduced light penetration), weed cutting and herbicide use (deoxygenation/algal growth) on the range and abundance of in-fauna also requires quantitative methodology.

Sediment-dwelling organisms are easily sampled in lakes and rivers by use of grabs, which gouge out a small area of mud using sprung or weighted jaws. (In very shallow swamps, *dambos* and rice fields, a piece of plastic drainpipe may be quicker to use as a coring device.) The Eckman grab is ideal for use in rivers and inshore areas of lakes, where the water is a few feet deep and can be waded. At depths greater than that, a small Petersen grab operated from a boat or canoe is recommended.

Limitations and processing Separation of in-fauna from organic debris and mud is tiresome, and flushing sediment through a sieve series (e.g. 5 mm, 2 mm, 750 μm and 400 μm) is a necessary preliminary step to sorting and counting organisms on white trays. (See 'Sample Processing' on page 193.) Some substrates are not suitable for grab sampling, e.g. sand, rock and stony aggregates.

Resulting data The results are expressed as numbers of species or groups per unit area (m^2).

Sampling period Take samples at least every 3 days after spraying for up to a month after last spray.

Equipment For shallow (wading height) water use an Eckman grab: in deeper water, a boat and a Ponar or Petersen grab are required. These are all relatively expensive.

Staff required 2.

Physico-chemical methods

Measurements of basic parameters such as the water temperature, oxygen concentration, pH, conductivity, turbidity and flow rate (current) are very useful in aiding the interpretation of biological data. For example, dissolved oxygen levels may differ markedly between pools or up and downstream of a non-pesticide source of pollution and would, if undetected, influence the assessment of the pesticide impact on the fauna. Physico-chemical measurements should be made at the time of biological sampling and recorded in a notebook. Descriptions and method sheets for these techniques are given in chapter 5.

SAMPLE PROCESSING

Sorting invertebrate samples is a time consuming and tedious process. After rinsing samples collected in the field through sieves to remove mud, sand and preservative, there is no real substitute for hand sorting invertebrates on white trays in natural light. Backwash the sieve contents on to a tray that is divided into roughly equal segments (c. 6 cm x 6 cm) with a black permanent marker pen and sort like organisms into vials containing preservative using forceps and Pasteur pipettes. When sorting samples in water on a white background, most biologists find it more efficient to focus on groups of organisms, picking up, for example, shrimps first, then mayflies, then worms, etc., rather than sorting all individuals in one pass. Occasional lifting of a corner of the tray to create water movement helps to reveal specimens against a background of sand or sediment. Place a label (pencil on paper) showing the date, sample site and other pertinent information inside the vial. If samples can be sorted fresh in the field, separation is aided by invertebrate movement. Preserve the collections with 70% alcohol and prepare a reference collection of specimens that can be used to identify all others, whether by taxonomic name or, at least initially as morphospecies, by a distinguishing code (sp A, B, etc.). Ensure vial cap seals are not perished as loss of alcohol by evaporation will quickly ruin the collection. Taxonomic knowledge speeds up sample processing but a team of unskilled 'sorters' can be separating like species within a few days given basic, initial guidance. A low-power dissecting microscope, taxonomic keys and the help of specialists will be required to identify the reference specimens and enumerate the samples collected. All enumerations should be recorded in pencil in a notebook. Assistance with the identity of specimens is normally available from national museums, university departments and agricultural colleges, and there are a number of international specialists in aquatic groups who may be willing to assist (contact curators of national museums).

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