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INTRODUCTION

Among mammals, species diversity and abundance world-wide are greatest for those animals collectively known as 'small mammals' (principally rats, mice, voles and shrews), amounting to over 1500 species within the orders Rodentia and Insectivora, and bats (order Chiroptera) of which there are almost 1000 species.

On account of their abundance and dependence upon plant or insect food, these two groups are themselves non-target casualties of pesticide spraying, and potential sources of secondary poisoning when eaten by predatory mammals or birds. Small mammals and bats that feed on insects are arguably more susceptible to poisoning from contaminated prey, and associated sub-lethal effects on body condition and breeding, as their higher metabolic rates require them to eat almost their own body weight of insects every day. In addition, these animals can suffer indirectly from pesticide-induced reductions in their prey populations.

Despite their obvious ecological importance, their mainly secretive or nocturnal lifestyles means that populations of small mammals and bats are not readily amenable to monitoring by observation but require trapping or specialized techniques for detection. However, most habitats will host species of both groups that can act as ecological indicators at the population level, and potentially also at a community level in diverse habitats of the tropics. Rodents and shrews normally spend their life cycles in high density populations within relatively small areas that can be effectively trapped and monitored, while insectivorous bats are efficient at integrating pesticides over more extensive areas that are subject to large-scale control operations against pests. Thus, wood mice were used as indicators of the ecological effects of different pesticide regimes used in cereal farming on the Boxworth Project (Johnson *et al.*, 1991a, b) and a community of tropical bats was used as the key indicator group to monitor the impact on small mammals of large-scale DDT spraying against tsetse fly in Zimbabwe (McWilliam, 1994).

Even assuming adequate resources, decisions made as to the size and scope of monitoring programmes depend upon factors such as pesticide toxicity, persistence, application/breakdown rate and resultant exposure of non-target fauna. Exposure and non-target response are in turn influenced by seasonal variation in climate and habitat, differences in susceptibility between species, sexes and age classes. For example, in contrast to adult males, reproductively active female bats were able to offload annually potentially lethal loads of highly persistent DDT metabolites through milk fed to their offspring. Individually marked adult wood mice, that had been fatally poisoned by methiocarb slug pellets within 2–4 days of application to fields in autumn, were rapidly replaced by juveniles immigrating from adjacent habitats.

In general, monitoring of small mammal populations should be carried out by specialists in view of the expertise required for identification and sampling. However, because these animals normally need to be individually handled, they can be marked and released to provide high quality data on the impact of pesticides over varying time periods and geographic scales. This chapter is thus intended as an introduction for managers to protocols

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and analyses of which they need to be aware when assessing the environmental impact of chemical treatments on such non-target groups.

PESTICIDE EFFECTS

The four major pesticide groups that small mammals are likely to be exposed to as non-target animals are: organochlorines, organophosphates, carbamates and pyrethroids. In general, any investigation of the impact of organochlorine insecticides requires a prolonged period of study as they (DDT and dieldrin) have a residual life of up to several years and being fat-soluble accumulate in the food chain. Thus, taxa at higher trophic levels, e.g. insectivorous or predatory small mammals and bats, are especially endangered. However, some organochlorines such as dieldrin and endosulfan are also acutely toxic when ingested (in food or by grooming) and sampling intervals need to be short enough to identify any post-application mortality. This would especially be the case, for example, with cover-spraying of a chemical such as endosulfan that is less persistent in the environment (half-lives recorded between 20 and 100 days) and is excreted from the body over a few days.

Although organophosphates and carbamates are not bio-accumulative, they are generally very toxic to vertebrates, both groups acting as neurotoxins by inhibiting the body's production of cholinesterase, an enzyme necessary for the transmission of nerve impulses. Pyrethroids have low persistence with half-lives of weeks and are also rapidly metabolized in mammals but are nevertheless acutely active, being neurotoxins that interfere with the sodium channel in nerve fibres. In general, studies looking at the effects of these three less persistent groups of pesticide need to concentrate more on detecting immediate post-application mortality over days and weeks rather than months and years for the persistent organochlorines. Although insectivorous animals are more likely to be affected through secondary poisoning from eating contaminated insects, it would also be appropriate to monitor herbivorous or seed-eating small mammals in situations where their food sources were sprayed (e.g. corn fields or grassland savannas).

STUDY DESIGN

It is difficult to be too prescriptive because study aims, environments and operational resources are so variable. However, investigations of the impact of pesticides on non-target fauna are normally based on comparing species abundance and population structure either between treated and control sites or before and after application at the same site.

Study plots need to be large enough to monitor mobile fauna – a minimum of 1 km² within a larger treatment area for small mammals and more in the order of 10 km² for insectivorous bats. In both cases, it is important to have replicates (randomized or stratified) to obtain a measure of the natural variation within treatments. Thus, it is statistically desirable to have at least three different sites when adopting the 'before and after' treatment approach or at least three different treated and control plots when making comparisons between different sites.

In the latter case, site selection is important as control and treated plots need to be carefully matched to reduce uncontrolled variation (i.e. compare like with like). To reduce variation caused by differences in sampling time, control and treated sites ideally should be sampled simultaneously. Therefore, they should be as close as possible to reduce travel but far enough apart to prevent any effect of treatment or exchange of populations. Naturally, if the pesticide treatment that requires monitoring is not homogeneous or evenly distributed throughout the environment, the sampling design needs to be adapted more to point surveys. For example, if termite mounds in savanna ecosystems or particular agricultural fields were targeted for control, it would be necessary to use these as the sampling replicates.

When carrying out before and after comparisons, it is also important to have an adequate pre-spray sampling period to assess natural variation in population abundance or composition. This can be problematic in assessing

the impact of emergency control operations but for most mammalian studies a minimum of 4 weeks is recommended and at least three sampling periods.

In practice, the timing and duration of study is largely determined by the nature of the chemical (degree of toxicity and persistence) and application chronology (regular and repeated treatment cycles or single control applications). However, when mammals are used as 'indicators', it is important to take into account the seasonal nature of their own population cycles as this can influence the interpretation of data. Many small mammals and bats have seasonal reproductive cycles as well as annual periods of relative inactivity (winter hibernation or dry season torpor), that critically determine relative abundance values derived from surveys. It is especially important to allow for these natural fluctuations in 'before and after' treatment comparisons, or indeed in any follow-up assessment. For example, population sizes of small mammals with large litters are greatly augmented as the young become mobile and this might mask any pesticide-induced mortality if age structure is ignored.

In general it requires weeks or months of survey to determine the severity of pesticide impact and/or recovery of mammal populations. The need for more 'precautionary' long-term monitoring is also influenced by:

- the scale of chemical application, which can be extensive in agriculture or pest outbreaks, thus reducing chances of local population recovery by gradual immigration
- the presence of protected species or habitats that need to be safeguarded.

Small mammal survey can be demanding of manpower and resources, particularly when large-scale emergency pest control operations (such as aerial control of locusts) require simultaneous monitoring of control and treated sites. As a rule of thumb, a minimum number of four people are required, even when sampling control and treated sites on alternate days. An efficient division of labour is achieved by having a team of one data recorder, one operator to empty traps, one animal handler/measurer and someone to re-bait and replace traps.

MONITORING METHODS

Although more sophisticated and intensive techniques involving radio-tagging are now available for studying small mammals, these are generally too expensive and labour-intensive for use in field assessments of non-target animals in the tropics, unless justified by a need to assess the impact of pesticides on rare or endangered species. A useful practical introduction to the subject has been presented by Kenward (1987).

The most common approach in assessing the impact of pesticides on small mammals involves their capture – mark – recapture (CMR) in a live-trapping programme using baited Longworth or Sherman traps. Grid-based layouts, although more labour-intensive, are preferable to line-trapping for long-term studies as they enable the survival, population density and home ranges of marked individuals on experimental plots to be compared before and after treatment with matched control areas.

Grids

The design of the trapping programme in respect of the length of trapping period, number and density of traps will be influenced by factors such as habitat type, density and abundance of small mammals, in addition to logistical considerations determined by the nature of the experimental or control situation. The following guidelines are derived from the literature on monitoring the response of small mammals to environmental impacts, such as pesticide applications (Douglass, 1989; Flowerdew, 1988; Greig-Smith and Westlake, 1988; Johnson *et al.*, 1991a, b; Tarrant *et al.*, 1990), will need to be modified to suit different field situations.

Sampling layout

The grid should be square for ease of marking and analysis, with a recommended 10 x 10 points trial-spaced at 5 m intervals in grassland, 10 or 15 m in woodland and 20 m in arable habitats. However, a less dense grid can be trapped over a longer period to achieve comparable catch-rates. At least two traps should be placed at each point to reduce the probability of an animal investigating an already occupied trap, although these can be of different sizes to suit particular species. In general, additional traps should be placed at each point if more than 50–60% of traps have caught animals at any one time.

Experimental design

In order to isolate treatment effects on study populations from environmental influences, it is necessary to operate simultaneously a minimum of two trapping grids before and after treatment, one in each of matched experimental and control sites. Habitat type, vegetation composition and structure need to correspond as closely as possible between the paired sites which should be separated by a distance at least great enough to prevent any spray drift into the control area. This allows each paired site to act as its own control, in addition to ensuring that any environmentally induced changes in populations on the control block can be differentiated from the effects of chemical application in the treated area. However, replication is desirable if resources are available, especially in the case of control operations that involve a variety of habitats or treatment regimes to validate and extrapolate findings between sites. With limited resources, it would be preferable to operate two replicate 7 x 7 grids instead of a single 10 x 10 array, both requiring the use of some 200 traps.

Although the length of pre- and post-treatment trial periods will be a compromise determined by balances between conflicting resource pressures and study aims, there are certain minimum requirements for a grid-trapping programme. In order to ensure that most of the grid population has been marked and enough recapture data gathered on individuals to establish their residency before treatment, a minimum of 8 days trapping data is necessary (1600 trap nights with two traps per point on a 10 x 10 array). Ideally, this should be carried out in two sessions of 4 nights, at least a week before application and over the 4 days immediately before treatment in order to distinguish resident from transient animals. Similarly, there should be at least two post-treatment monitoring sessions with trapping beginning 2 days after application to allow any immediate mortality to be detected and a follow-up survey beginning at least a week after treatment. However, time available for monitoring during pest control is often limited by operational considerations and valid differences in survivorship between control and treated grids can be obtained even when trapping is compressed into single 7-day sessions before and after treatment. If possible, it is best to plan the trapping programme to coincide with the dark phase of the moon, as catches are generally lower on clear moonlit nights. Naturally, four trapping sessions (one per week for a month) either side of application would provide better quality data.

Data analysis

The principal determinant of such trials is the proportion of resident marked individuals surviving the treatment compared with those alive after the same interval on the matched control grid. Such figures can be adequately analysed with simple non-parametric statistics like the chi-squared test.

An informative graphical method of portraying the impact of chemical applications is to plot both the cumulative number of captures and individuals against cumulative trapping effort (number of trap-nights). The position of any inflection points denoting a change in the slope of the curve can be related to treatment events. In addition, if the curve of numbers of individuals reaches a plateau, the asymptote denotes the population size at which it has been fully trapped. It is recommended that data are plotted daily to give an indication of the necessary sampling effort still required to sample most of the resident population.

However, to facilitate comparisons with other studies or between sites and sample periods some useful indices of population size and capture success are worth calculating. Although estimates of population size can be made from the proportions of marked and unmarked individuals in successive daily catches, the underlying assumptions to such models are often broken (Montgomery, 1987). Consequently, the minimum number of animals (MNA) known to be alive on the grid during the sample period is a more robust measure of its population size when most animals have been trapped (requiring high recapture rates). Another comparative index used to overcome slight differences in trapping effort between sample periods or sites is the number of captures per trap-night (divide the number of animals caught by the number of traps used and the number of nights spent trapping). Although there are various possible refinements (Gurnell and Gipps, 1989), population densities can be calculated and then compared by dividing the population size for each grid by its area.

Trap lines

Line-trapping can be used as a method to cover greater areas less intensively than a grid layout and consists of placing traps at equal intervals along line transects through a habitat.

Sampling layout and experimental design

Developed further for sampling large arable fields on the Boxworth Project, the technique consists of operating lines of 10 points at 20 m intervals with two traps per point. These are set out at a density of one trap-line for every 2 ha (i.e. each 200 m line is separated by some 100 m if equidistantly spaced) and run for 2 days only in a less labour-intensive approach. However, random sampling and replication can be ensured by running five trap-lines on a 10 ha study site (requiring 100 traps), the first line randomly assigned to say one of five 20 m intervals within the first 2 ha block and the remaining lines then spaced at equal intervals. To reduce the effects of weather, the trap-lines could be assigned randomly to different 2-day blocks over the sampling period. Again, a separate control area should be simultaneously monitored and a minimum of two pre- and two post-treatment trapping sessions, each spanning 3 days (2 full days and 2 full nights) can be carried out within 2 weeks if time is at a premium. However, it is recommended that the first post-treatment session should begin some 2 days after application, to give time for any impact from treatment, and the second trapping session to commence at least a few days after the first, say 7 days following application.

Data analysis

Treatments can also be compared by chi-squared tests using numbers caught per trap-night (or 100 trap-nights) and the proportion of animals recaptured after treatment. In addition, a density index can be calculated by summing captures of individuals over the 2-day trapping period for all trap-lines and dividing this by the sample area covered. In long-term studies where both grid and line-trapping are used concurrently, it is possible to calibrate the density index with the actual population densities found on the grids (Flowerdew, 1988).

PRACTICAL ISSUES

A good understanding of the practical live-trapping of small mammals (Gurnell and Flowerdew, 1994; Wilson *et al.*, 1996) is needed to expand on the points outlined below. The former reference has a comprehensive section on record keeping and trapping analysis and it is recommended that their formats for data sheets and summary tables be adopted.

Grid layout

Right-angles on the grid should be marked out with a prismatic compass and a 30 m tape used to measure trap intervals. Trap points can be marked out with canes, or 2 x 2 cm wooden staves cut so as to be just visible above the grass level (often over a metre high in tropical grasslands). Once an accurate baseline of canes has been

placed on one side of the grid, other points can be lined up by eye after measuring the correct interval. Both canes and associated traps should be given corresponding grid numbers with a permanent marker pen, so that animal positions can be recorded and mapped.

Traps

Longworth and Sherman traps are both suitable live-traps made from lightweight aluminium. However, where carriage space is limited, as during environmental monitoring of control operations in remote tropical areas, collapsible Sherman traps are recommended because they fold flat (see illustration on method sheet), come in a variety of sizes and strengths and can be transported in the plywood packing boxes in which they are exported by the suppliers (www.shermantraps.com).

Longworth traps are bulkier as they are made up of two sections: a tunnel with integral trip mechanism and separate nest box (see illustration on method sheet). Full details of their operation and sourcing can be found in Gurnell and Flowerdew (1994).

Traps should be placed within 1 m of the marker cane and trapping success can be improved by appropriate positioning, with the entrance flush to the ground and the trap aligned along runs in the grass or adjacent to grass tussocks. In wooded habitats or shrubby vegetation, they can be placed alongside fallen branches or logs and should always be sheltered beneath any available shade. Once the trap position has been selected on the first day, it should be kept there and care taken to keep the trip mechanism unobstructed by old bait or vegetation. The traps should be emptied of debris every morning and the bait replaced if necessary.

Baiting

Animals can be encouraged to enter traps by placing food inside them. A mixture found suitable for baiting in the tropics consists of: 1 part raisins, 2 parts peanut butter and enough rolled oats to make a mixture of a putty-like consistency. This can be rolled into balls and placed at the back of the trap, or just outside the trap if a pre-baiting period is found necessary. (Pre-baiting is the provision of bait for a limited period of 1 or 3 days with the door fixed open to familiarize animals with the traps. Although not generally recommended in time-limited field trials, it may be necessary in grassland during the first pre-spray session to discount initial avoidance of the traps, especially by voles, in any comparisons of catch rates with post-treatment periods.)

Maintenance

Traps need to be checked for animals at least twice per day – in the early morning, within 3 h of dawn, before the day warms up in order to minimize heat stress and replace bait. An additional visit in the late afternoon before dusk is required to ensure that all traps are baited and reset for the night's catch. Although the provision of bedding material in traps has not been found necessary in tropical conditions, if temperatures drop below about 10 °C, dried grass, hay, shredded paper or cotton waste should be introduced to the back of the traps.

In the tropics, experience has shown that fresh bait should be put down every second day as it tends to dry out in high temperatures and can also get depleted by insects. However, the oatmeal/raisin base of used bait can be 'refreshed' by the addition of more peanut butter and recycled. Plastic buckets with tight fitting lids are useful for preparing and carrying the bait.

ANIMAL HANDLING

Small mammals **must** be handled with care and skill. Guidance and training from an expert is essential before undertaking any of the following activities. Occupied traps should be emptied into large, heavy duty, cloth bags, at least 20 x 30 cm, with a draw-string that can be tied around the top and looped on to a stick for returning

to a vehicle or local base for identification, categorizing, measuring and marking. It is normally sufficient to open one of the doors and shake the animal gently out. It can then be restrained in the bag opening by use of gloves or another bag to facilitate examination on a flat surface. Care must be taken not to suffocate these small animals by squeezing too hard in an effort to avoid being bitten. It is recommended that animals are held by the nape of the neck, where all the loose skin is grasped between finger and thumb and held firmly against the back of the skull, thus preventing the animal from being able to turn around and bite.

Identification

Species need to be identified with the aid of relevant field guides and taxonomic keys. However, if species identification is problematic, as in many tropical habitats, animals can be described and given a temporary ID before obtaining authority to take a specimen for later identification by an expert. Go no further than this without training from a mammalogist. If necessary, animals may be killed by soaking cotton wool in chloroform or ether and placing this with the cloth collecting bag in an air-tight plastic bag for some 10 to 15 min. This also allows collection of any ectoparasites which should be stored in 70% alcohol. The specimen's abdominal cavity should be cut open longitudinally and the diaphragm punctured through to the lungs to facilitate preservation, preferably in 70% alcohol or 10% formalin solution if residue analysis is contemplated.

As the teeth are an important taxonomic feature, it often helps to prop open the mouth with a small stick before preserving and the specimen needs to be labelled with details of location, date, captor, sex and any measurements taken. These would normally be: weight, total length (tip of nose to end of the last caudal vertebra), tail length (from the base of the tail to the end of the last caudal vertebra), hindfoot length (tip of the longest claw to the heel) and ear length (from the tip to the notch). Practical details on the preparation and preservation of taxonomic specimens have been summarized by Yates *et al.* (1996).

Measuring

A set of 'Pesola' spring balances (50 g, 100 g, 300 g, 1.5 kg) should be obtained for weighing (the BTO, www.bto.org). It is easy to determine the weight of animals by subtracting the weight of the cloth bag from the combined figure taken when weighing the animal inside the cloth collection bag. If the animals are small, they can be weighed inside a small polythene bag. Severe declines in body weight may indicate a change in body condition induced by pesticides, either acting directly or through a reduction in the food supply.

A 30 cm steel rule is probably adequate for most external measurements although a pair of callipers can be useful for greater accuracy (both are obtainable from the BTO, address above, which can additionally supply cloth bags – readily made to order by local tailors in the tropics). The standard measurements listed above as an aid to identification should be recorded on first capture as these are all characters used to distinguish between species.

Reproductive condition

Animals should at least be sexed, aged and weighed before marking and release. It is also useful to distinguish between adults and juveniles on the basis of size, pelage colour (usually very grey in juveniles) and reproductive condition. Adult males normally have scrotal testes and adult females can be recognized by pregnancy or the presence of suckled nipples. Juvenile females can be further distinguished by the presence of an imperforate vagina, which is still covered with a membrane. A guide to determining breeding condition among rodents is to be found in the recommended booklet by Gurnell and Flowerdew (1994).

Marking

For short-term field trials, that last for no more than a few weeks, fur clipping is least disturbing to animals and by trimming hair from different parts of the body various combinations can provide a series of individual identifications. For example, the combination of six patches on the left and right shoulders, flanks and haunches (e.g. denoted A to F) will give 41 possible marks for each sex of each species (see Gurnell and Flowerdew, 1994, Figure 3). If the fur is found growing back on recaptured animals it can always be cut again. For long-term studies, the use of ball-chain necklaces probably causes the least disturbance. These are made from linked stainless steel balls on to which are strung individually numbered or coloured metal split rings, the construction, application and sourcing details of which are described in Rudran (1996).

BIOCHEMICAL AND RESIDUE ANALYSIS

Exposure of animals to pesticides can of course be assessed directly by taking biological samples for laboratory studies. These can be divided broadly into biochemical or residue analyses. Both require special expertise and are expensive, although they are necessary for actual confirmation of exposure to chemical treatments. Prior to residue analysis, post-mortem examinations to determine pathological or histological effects can be carried out on any moribund animals or carcasses found after spraying (Tarrant, 1988). These should be double-wrapped in aluminium foil and immediately frozen in a portable freezer in the field for subsequent analysis (see chapter 6). Although post-mortem analyses will be restricted if specimens cannot be preserved by freezing, specific organs such as liver or brain, carcasses or even alimentary tracts stored in 10% formalin in aluminium canisters are amenable to residue analysis.

Biochemical analysis

Exposure to some chemicals can be detected by biochemical changes in the blood. For example, death by organophosphate or carbamate poisoning is presumed to result from asphyxiation, through tetanus of the diaphragm, brought on by excessive stimulation of the central nervous system. This arises from an accumulation of acetylcholine as a result of the inhibition of cholinesterase enzymes by these compounds.

Sub-lethal exposure to relatively low levels of such pesticides can now be assessed for non-target bird and mammal populations (as well as people involved in application) by measuring the degree of esterase inhibition in blood serum harmlessly taken from individuals (Thompson and Walker, 1994). Although this has been successfully carried out for small mammals on the Boxworth Project in Britain, the technical resources required are considerable. In addition, the necessity to obtain good baseline control data from a large pre-spray sample of the indicator species limits the usefulness of the technique in habitats where non-target species are difficult to catch. Also, the degree of inhibition is greatly influenced by the time since exposure and varies both between species and individuals. In conclusion, the logistical constraints of fieldwork in the tropics and practical limitations of the methodology precludes this approach to detecting exposure, unless part of an intensive and long-term research project. Rather, any non-target casualties or captured animals whose behaviour indicates poisoning should be preserved for confirmatory residue analysis.

SURVEY METHODS FOR BATS

Insectivorous bats are widespread throughout most tropical areas, although their abundance is largely determined by the availability of insects, making them an excellent indicator group for monitoring the 'health' of habitats after chemical application. Their presence while flying at night can be determined by specialized live-capture techniques (mist-net, a mesh of fine nylon supported on poles; harp-trap, a rectangular frame strung vertically with fishing line), or by electronic 'bat detectors' that convert their ultrasonic echolocation calls into sounds which are audible to people.

To date there has been only one study that has used these techniques to monitor the impact of large-scale pesticide application on a community of bats, coupled with residue analysis to assess exposure (McWilliam, 1994). This involved a detailed and long-term investigation of the persistent organochlorine, DDT, with resources not generally available in most wildlife trials. As the capture and handling of bats at night is time consuming and requires comprehensive training (and in some countries formal licencing) to avoid injury to animals, the use of bat detectors is recommended for monitoring the relative abundance of bats in ecotoxicological research.

Bat detectors

Even with additional sound processing and recording equipment enabling echolocation calls to be assigned to individual species (Fenton and Bell, 1981; Vaughan *et al.*, 1997), bat detectors only provide an index of relative abundance as the bat detector cannot discriminate between individual bats. In effect, several detection events at a site, or 'bat passes', could as well be made repeatedly by the same bat passing through the detection space as by several bats flying across it in succession. In addition, the technique is biased towards those species with more intense calls that register at longer distances from the instrument. Nevertheless, the number of bat passes per unit time or transect length are measures of bat activity that can be compared between habitats or, in the context of pesticide impact, between pre-spray or control and post-spray treatments. This applies equally whether the entire ultrasonic spectrum is being covered by a 'broad-band' unit to monitor the whole fauna, or a more sensitive 'narrow-band' detector is used to sample a frequency common to the echolocation calls of a majority of species. In this respect, most surveys of general bat activity have used a frequency of 40 or 45 kHz (McWilliam, 1994; Walsh *et al.*, 1995).

A good introduction to the principles and practicalities of bat detector use is available from the Bat Conservation Trust, which can also be contacted for information on the current availability of hand-held models suitable for monitoring bat activity (www.bats.org.uk). Relatively inexpensive models used in Britain at the time of publication include the Batbox III from Stag Electronics (www.batbox.com), the Mini III from Ultra Sound Advice (www.ultrasoundadvice.co.uk), and the Skye SBR 1200 from Skye Instruments Ltd (www.skyeinstruments.com). These have been compared by Waters and Walsh (1994), who found that the Batbox III was the most sensitive of the models, although this was at the cost of less accuracy at higher frequencies. These suppliers do produce more sophisticated models which are worth considering if budgets allow. Highly regarded models of varying specification are also produced by Petersson Elektronik AB (www.batsound.com).

It is worthwhile consulting general reviews and compilations on bat detection to gain access to the literature (see 'Further Reading', page 254).

With experience and acoustic analysis it is possible for experts to distinguish between species on the basis of the character of their echolocation calls (frequency range, whether constant frequency [CF], frequency modulated [FM] in different pulse lengths and delivery combinations). However, in initial ecotoxicological surveys by novices, fewer assumptions are made if all detected sequences of at least two echolocation pulses are treated as one bat pass, without any attempted differentiation between species. In some situations it may be worthwhile separately noting bat passes that, by their increasing pulse repetition rates (a 'feeding buzz'), identify individuals that are actually foraging rather than being merely in transit. However, these categories can be combined during analysis.

If resources are available, it is possible to set up automatic monitoring stations for recording bat activity, say at equidistant points along a transect route, which are based on the recording of bat passes through the linkage of bat detectors to voice-activated tape recorders. However, although significantly reducing time spent in the field, this does require a substantial input into setting up the equipment and analysing the recordings and expert assistance is essential.

Transects

Bat detectors are conveniently used on transects, whether operated continuously while walking or driving at a standard speed through habitats or for timed periods at points selected randomly or regularly located along the route. Although some species will rarely be recorded due to the low intensity of their calls, the presence of bats generally can be detected within 10 to 50 m of most models. If chemical application is uniform over large-scale areas, such as achieved by aerial spraying, some form of stratified sampling is recommended as bats will generally forage near water, especially in the dry season, and in richer more complex habitats such as riverine forest or woodland where insect abundance is likely to be greater. Thus, after monitoring bat activity on transects through woodland in assessing the impact of ground-spraying DDT against tsetse fly in Zimbabwe, marking and recapture of bats was concentrated at matched sites in control and treated areas around seasonal pools in woodland, rivers and adjacent vegetation and around permanent water at dams (McWilliam, 1994).

In relatively safe locations, continuous sampling can be carried out on foot, perhaps in combination with timed point counts. However, in many less secure tropical habitats where wildlife or people can be encountered at night, it is advisable to carry out timed counts at predetermined points along a transect, from the roof of a 4-WD vehicle if necessary. This has the advantage of making it easier to record meteorological data at each point (cloud cover, degree of moonlight, temperature and wind speed) and make notes. Thus, even in the tropics, temperature is a determinant of the level of bat activity, mediated through its influence on insect activity.

Sampling and experimental design

Where more localized chemical application has been carried out, e.g. along linear habitats such as roadside verges, field margins, woodland edges, river or lake sides, randomly positioned or regularly spaced transects can be monitored. It is recommended that replicate transects at least 1 km in length are surveyed in both control and treated areas, with a minimum of 100 m between points if timed counts are carried out. When the area treated is less linear and more extensive, 10 km² or greater, some 15–20 points spaced 200–250 m apart can be monitored to ensure good coverage. If the treated area is extensive enough to allow parallel transects, these should be no closer than 250 m to each other. In locations where transects can be walked, a uniform speed should be adopted of between 2 and 3 km h⁻¹ and data noted or recorded on tape for later transcription, of the time taken and the number of bat passes per transect segment.

On timed point counts, the length of time required for monitoring at each spot depends upon the level of bat activity. However, from experience, a 5 min sampling period followed by an interval of 5 min to allow travel to the next spot is a reasonable compromise, allowing a 16 point transect to be covered in about 2.5 h. Monitoring should start at some fixed time between 15 and 30 min after sunset to cover the early evening peak in foraging activity. It is recommended that each replicate transect in control and treated areas is monitored over at least two sessions spaced about a week apart of 4 nights each, both before and after treatment. Two days or so should be allowed for the chemical application to be mediated by the insect population before commencing the first post-treatment session. If possible, all monitoring should be carried out during the dark phase of the moon as bat activity is often reduced on moonlit nights.

RESIDUE ANALYSIS FOR BATS

Monitoring may indicate that an acute effect is taking place and that mortality from poisoning is implicated, rather than the emigration of bats to richer food patches. In this case, specimens can be taken by a specialist (with permission from the relevant wildlife authorities), using mist nets or harp traps and preserved for residue analysis (McWilliam, 1994). If possible, specimens should be double-wrapped in aluminium foil and frozen. Alternatively, they can be stored in 10% formalin solution after opening their abdominal cavities and puncturing the diaphragm to ensure effective preservation (see chapter 6).

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