AMPHIBIANS AND REPTILES

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INTRODUCTION

Amphibians and reptiles are especially abundant in the world's tropical, sub-tropical and warm temperate regions. They are cold-blooded, or ectothermic, vertebrates, with an internal body temperature primarily dependent upon external warmth.

Amphibians are present in damp wetland habitats, occurring at the edges of ponds and streams, and have both aquatic and terrestrial life stages. Adults breed in water during seasonal rains, the eggs laid as spawn hatching into larvae (tadpoles), which are able to swim and feed initially on algae and later carrion. Some species (e.g. African clawed frog) depend on water throughout the year; others are inactive during dry periods. Invertebrates constitute amphibians' main prey. Amphibians are especially numerous in tropical rainforests.

Certain reptiles, such as crocodilians, freshwater chelonians (turtles and terrapins) and some snake and monitor lizard species, are also associated with water and damp habitats in the tropics and sub-tropics. All are predators, feeding primarily on fish, but also taking carrion. Freshwater chelonians additionally feed on invertebrate species such as crustacea, as well as fish.

Most species of reptile are terrestrial, and abundant in a wide range of habitats. Geckos and certain skink and agamid species inhabit rocks and trees, while others are ground-dwelling. Most lizard species prey on invertebrates, but monitors, for example, scavenge a wide range of prey and, like crocodilians, include carrion. Snakes depend mainly on lizards, anurans (frogs and toads) and other small vertebrates. Like their lizard prey, they are often found in dry, sandy regions, in savanna woodland, as well as in damp habitats and tropical rainforests. Surviving in arid conditions, lizards especially maintain activity during the dry seasons of the year. Tortoises are mostly herbivorous, seeking refuge in vegetation.

Amphibians and such insectivorous reptiles as lizards have an important function in linking invertebrates with more advanced vertebrates higher up the food chain. Not only do they constitute a food resource for such organisms, but they are a means by which chemical residues, especially residues of organochlorine pesticides taken in with contaminated prey, can enter food chains. Through bioconcentration, some of these chemicals find a way into the environment generally and, on occasion, into man. Lipid soluble pesticides tend to become sequestered in the body fat of reptiles. Their ectothermy additionally renders them dependent upon temperature for metabolizing pesticide residues, and a poor ability to do so results in accumulation in body tissues. Amphibians have a soft permeable skin and larval gill membranes, highly vascularized and allowing the entry of chemical contaminants. In contrast to birds and certain epigeal insects, both amphibians and reptiles also have a limited capacity for emigration and recolonization, or to adapt to rapid changes in habitat . With this range of characters they are, therefore, good indicators of the quality of terrestrial habitats, and residue loads are biomarkers of the level of contaminants entering food chains, and hence the environment generally.

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Amphibians and reptiles take up pesticides in a number of ways.

- Inhalation: near areas of contamination, pesticides may be taken up during breathing through the lungs, especially in reptiles.
- Contact: after treatment, pesticides may be taken up by amphibians, particularly through larval gill membranes and their permeable skin; reptiles have scaly skins and do not have aquatic larvae, and so pesticides are not taken up as readily by this means.
- Ingestion: both amphibians and insectivorous reptiles can take in pesticides through the ingestion of invertebrate prey which is contaminated, either with pesticide particles adhering to the cuticle or, in the case of species higher up the food chain, through prey with residues sequestered in body fat.

Where there are pesticide control campaigns, amphibians and reptiles can come into direct contact with pesticides as non-target organisms in treated habitats or in areas of spray drift. Amphibians in open water bodies may also be exposed to pesticides due to run-off from adjacent agricultural land on which chemicals are used to control crop pests.

The aim of this chapter is to describe techniques for monitoring amphibian and reptile populations, depending on species and habitat, which have use in pesticide impact assessment work.

OBJECTIVES

There are three main objectives for monitoring amphibian and reptile populations.

- To assess the direct effects of pesticide application and run-off on species and herpetofaunal diversity from the observation of living animals and collection of any specimens killed by pesticides (see chapter 6 on analysis for residues in the laboratory). Selected species are identified for use as bioindicators.
- To assess the indirect effects of pesticide treatment on a range of amphibian and reptile species through the effect on their mainly invertebrate prey, including ingestion of contaminated prey resulting in poisoning (collection of specimens for laboratory residue analysis), and on vegetation (in the case of herbicides) that provides refuge and also a harbour for prey.
- To collect and preserve specimens in the field – voucher specimens – for identification in the case of biodiversity studies, preserved material for gut content and residue analysis (organochlorines) in the laboratory, and living material for cholinesterase testing (mainly organophosphates) in the laboratory.

The method of monitoring the impact of pesticides on amphibian and reptile populations will depend on the pesticide type and formulation, the method of application, the receiving habitat and species involved, and the impact on herpetofaunal diversity (many species) or on bioindicator species (one or two). An estimate of population changes resulting from exposure is required: this may be an estimate of species diversity (richness and composition – percentage frequency), a population estimate (absolute numbers in an area), relative abundance (comparison of relative densities), or a measure of density (numbers per unit area).

Amphibian and reptile activity varies at different times of the year, and populations fluctuate seasonally. Some amphibians depend on seasonal rains for breeding, and most species are inactive during dry or cool periods of the year: certain reptile species may be active throughout the year, or less active during cooler or drier periods and breeding only at specific times of the year.

Effects of pesticide treatment may be compared *before* and *after* treatment, and in relation to time since treatment took place, in *treated* and *untreated areas*.

Sample collections are required for the following.

- Voucher specimens in biodiversity studies, preserved for later identification purposes.
- Analysis for organochlorine residues in the laboratory: specimens are preserved in formalin or deep frozen (see chapter 6). Residue levels are expressed as parts per million or mg kg^{-1} (µg g^{-1}) wet or dry body weight, or total lipid. Wet weight is the standard, and useful for estimating the level of residues entering food chains since predators usually ingest fresh prey; dry weight is used for comparing levels with those in other materials, e.g. soil and leaf litter, reflecting atmospheric and environmental levels generally. Lipid levels for determine effects on physiological processes of amphibians and reptiles themselves.
- Live samples of amphibians and reptiles especially lizards, are taken to the laboratory and maintained alive in cages for cholinesterase testing in the case of chemicals such as organophosphates.
- In the case of animals showing signs of acute poisoning, where the cause of morbidity is unknown, samples are killed immediately for biopsy and residue analysis.

STUDY DESIGN

The various techniques for the measurement of population differences in applied ecological studies still require standardization, and a useful work by Heyer *et al.* (1994) includes a description of the different methods for standardized monitoring of amphibian populations in particular, but also certain forms of reptile. Different sampling techniques may yield quite different results. Estimates of population size and density may be limited by differences in activity and behaviour of many species. Problems arise from the limitations of the survey techniques and replication of sites (see chapter 2).

Which pesticide?

Organochlorines

Organochlorines have both chronic and acute effects especially on amphibians, in particular dieldrin and its metabolites and BHC isomers. Residues accumulate and levels are measured from analysis in the laboratory of amphibian and reptile samples from sprayed and unsprayed areas. Organochlorines can have indirect effects on lizards through contamination and a reduction of invertebrate populations.

Organophosphates

Organophosphates, parathion in particular, can have acute effects on certain amphibians. Chlorpyrifos sprayed against locusts has caused death of lizards. Measurement of residue levels in amphibians and reptiles from sprayed and unsprayed areas may help to determine cause of death. Alternatively, the estimation of acetylcholinesterase levels can provide evidence of pesticide impact. Both are expensive and interpretation of resulting data is difficult. The latter procedure is specialized and also requires liquid nitrogen in the field and expensive test kits that are not readily available. Organophosphates may have indirect effects on lizards through contamination and a reduction of invertebrate prey populations.

Carbamates

Carbamates, e.g. bendiocarb, have been observed to affect lizards and probably also have indirect effects on lizards, through a reduction of the invertebrate population.

Pyrethroids

Pyrethroids are not very persistent in the environment but have acute effects on certain amphibians, especially the larvae. They may also have indirect effects on lizards through a reduction of invertebrate prey populations.

Insect growth regulators and biologicals

Insect growth regulators and biologicals have little or no direct effect on amphibians and reptiles; indirect effects through impact on prey have been indicated.

Herbicides

Herbicides have little known effects if any, but paraquat has been recorded to cause running eyes in tortoises. They can have an indirect effect on species through the removal of vegetation providing refuge and a harbour for invertebrate prey.

Where is it used?

- Agro-ecosystems: few amphibian and reptile species occur in agriculturally developed areas and so diversity is low; larval and adult frogs are found by irrigation channels and may be affected by run-off in paddy fields.
- Woodland/forest: amphibian and reptile faunal diversities are determined since they may be affected by pesticides; numbers of both arboreal and fossorial forms of both groups occurring in savanna woodland are determined, amphibians occur especially in tropical rainforests.
- Pasture/savanna: amphibian and reptile faunal diversities are determined, as in woodland/forest; numbers of surface-dwelling forms with burrow refuges are determined, especially lizards, snakes and tortoises, and certain toad species in adult terrestrial phase.

Application method

- Knapsack or tractor: such methods of application affect fauna on trees and shrubs, soil surface and soil.
- Ultra-low-volume: fauna on shrub vegetation and the ground surface are affected; if aerially applied, canopy and arboreal amphibians and arboreal reptiles are specifically affected.
- Fogging: canopy and arboreal amphibians are affected, together with arboreal reptiles.
- Aerial: fauna are affected as for ultra-low-volume and fogging treatments.
- Granules/seed dressing: such applications may affect fossorial amphibians and reptiles.

All methods may have indirect consequences through contamination and disappearance of invertebrate prey.

Measurement of pesticide residues in amphibians and reptiles

Residue levels in amphibians and reptiles are generally expressed as mg kg^{-1} or µg g^{-1} (parts per million).

Wet weight

Residue levels are conventionally given as fresh (wet) weight. This measure is relevant to amphibians and reptiles for comparison with other organisms, and because they are prey of organisms higher up the food chain. Ingested intact or as large fleshy morsels, residue levels in such food material calculated as whole body wet weight provide information on pesticide levels entering the food chain. Amphibians and reptiles form a link in the food chain with invertebrate species, upon which they prey and which may be contaminated, and predators higher-up the food chain that prey on them in turn. Wet weight residue levels are also more useful for comparing amphibian levels with those in their aquatic environment.

Dry weight

Residue levels in materials like soil and leaf litter are given in dry weight. In order that levels in amphibian and lizard species can be compared directly, whole body dry weight is used. This allows the groups' levels to be placed in perspective by comparing them with baseline levels of materials in their habitats, with which they are closely associated, and which reflect environmental levels generally. Elevated levels in amphibians and reptiles are likely to indicate that the source of uptake is from contaminated prey.

Lipid

Residue levels in relation to body lipid provide information on the pesticide effects, on the physiology of the organisms themselves. Residues are sequestered in body fat, and levels are usually negatively correlated with percentage fat content, that is high residue levels (expressed as mg kg^{-1} lipid) correspond with low percentages of fat and vice versa. Fat is combusted during lean times of the year when the intake of food is at a low level. Residue levels then increase, and may cause chronic physiological or behavioural disorders, and even death when a lethal level is reached.

Population change

The treatment of areas with pesticides may reduce the population, or even result in the complete disappearance of amphibians and reptiles over a period of time. This is important for biodiversity studies. Alternatively, applications may give rise to a patchy distribution, the surveying of which presents its own problems (Swingland and Shorrocks, 1990). Populations are monitored for the extent of decline or rate of recovery. Halting spraying in an area may result in localization of populations at the start of recovery or re-immigration from adjacent untreated areas.

Distribution change

Reptiles may be repelled from areas with high levels of pesticide contamination. Species inhabiting tree canopies in forests, such as tree frogs, and certain snake and lizard species, for example, may be particularly susceptible to aerial spraying. Perch heights selected by amphibians and reptiles may be influenced by the technique used and target application of pesticide spraying in savanna woodland.

Percentage of habitat occupied

Amphibians and reptiles may depend upon certain habitat units such as trees or rocks. Recording the proportion of these units occupied provides a standardized technique of equal-effort monitoring that is independent of density.

Stage, maturity/age and sex

Pesticide contamination may affect growth and development in amphibians, resulting in deformed or otherwise abnormal animals, and can hinder or prevent larval stages from undergoing metamorphosis to become immature adults. Age and state of maturity, and if possible sex of adult amphibians (presence of nuptial pads in male anurans) are also recorded, especially in selected bioindicator species, to establish whether ratios change after treatment. Poisoning from ingested pesticide residues can also reduce longevity, and this may affect age structure in both amphibians and reptiles. Likewise, in lizards especially among reptiles which tend to go through three phases, i.e. hatchlings or juveniles during their first year, immature or sub-adult in the second, and adults from the third year on, poisoning may affect age structure. It is especially important in selected bioindicator species to determine the sex of adult lizards if possible (males are often differently and more brightly coloured than females).

INVENTORY, MONITORING AND SAMPLING TECHNIQUES

An estimate of species richness is required for an assessment of biodiversity. Amphibians and reptiles can be observed or collected during searches, and there are several well established methods for surveying and monitoring amphibian and reptile populations in the field. However, amphibians depend on water and damp habitats far more than reptiles, except for crocodiles, turtles and certain monitor and snake species. Most lizard species occupy arid or semi-arid habitats, which may lack amphibians, or in which amphibians are inactive except during periods of rain.

The techniques that follow are applicable either for both amphibians and reptiles, or for one group or another (Table 11.1). Techniques for amphibian surveying are treated separately from those for reptiles (lizards in particular) within the description of each methodology.

Table 11.1 Sampling techniques

Method	Non-target herpetofauna			Habitat							
				Terrestrial				Aquatic			
	Reptile	Amph larvae	Amph adult	Woodland	Forest	Savanna	Agro-eco	Woodland	Forest	Savanna	Agro-eco
Inventory	●	●	●	●	●	●	●	●	●	●	●
Visual survey	●		●	●	●	●	●	●	●	●	●
Block sampling	●		●	●	●	●	●	●	●	●	●
Patch sampling	●		●	●	●	●	●			●	●
Breeding survey		●	●		●		●		●	●	●
Quantitative	●		●					●	●	●	

Complete species inventorying

The aim in preparing a complete species inventory is to record all possible species in a habitat. It, therefore, constitutes a baseline study before pesticide treatment in an area, and provides information on species richness. It involves two parts: visual encounter surveying and microhabitat searching (see also under quadrats and transects). The making of inventories is extremely time consuming and not a normal objective in pesticide impact work.

Visual encounter surveying

Visual encounter surveys are the simplest kind of survey, and useful for determining herpetofaunal diversity in relation to number and frequency of pesticide applications in an area, and for comparing diversity before and after treatment or in treated and untreated areas. A measure of relative density is provided by the numbers sighted per unit of time, usually 1 h in relation to the number of observers (sightings frequency is expressed as numbers per man-hour), but several replicate surveys are required for statistical comparison. During visual encounter surveys, and depending on the ecology of species involved, the number and species of amphibian or reptile are recorded while one or more observers walk through an area or habitat for a measured period of time (timed searches). The path or paths taken by one or several observers can be a randomized zig-zag or straight line, or designed paths within a quadrat. These allow a faunal list to be compiled, with frequency of sightings in an assemblage (for species composition), and an estimate of relative abundance (numbers per man-hour) that is an expression of relative density. Actual density cannot be determined since only a proportion of individuals of a species is seen, and some species are fossorial and, therefore, under cover (these species are monitored using microhabitat searches).

For most amphibian surveys of this kind, searching is done at night using a wide-beamed spotlight, as the eyes of most forms reflect light, e.g. tree frogs at heights on forest trees in terrestrial habitats, or on banks and in water for rivers and other large water bodies. Weather conditions (air temperature, cloud cover, rain, etc.) should be recorded before and after surveys. For most diurnal species of lizard, such as lacertids, skinks and agamids, in terrestrial habitats, surveys are made during the day. For nocturnal lizards such as geckos (and especially forms whose eyes reflect light, e.g. turtles and crocodiles in large water bodies), surveys are made at night using a wide-beamed spotlight. Weather conditions (air temperature, cloud cover, etc.) should be recorded before and after surveys.

Limitations Only a proportion of the individuals of species seen on open ground, or those resting exposed and visually unimpeded by vegetation; activity varies with time of day, temperature and other seasonal weather conditions. Replicate surveys are required for purposes of comparison.

Resulting data Relative abundance (percentage species composition); sightings frequency (numbers per man-hour) reflecting relative density. Species richness and diversity within an assemblage.

Fauna sampled Active adults of most anuran (and lizard) species.

Sampling period Day and/or night, depends on species, or range of species or animal group's activity and behaviour.

Equipment Watches or stop-watches, digitometers, wide-beam spotlight (at night), thermometer or whirling hygrometer (see chapter 5) must be purchased. Plastic bags can be bought locally. Metal screw-lid aluminium (purchased) or glass containers (jam jars bought locally) are required for the preservative solution (45% formalin for dilution to 8–10% can be bought at a local hospital).

Staff required 2 or more observers; searching time (man-hours) increased, and hence numbers of animals sighted, in a specific time period (1 man-hour for fewer than 10 species, say; 5 man-hours for more than 10 and several observers).

Quadrat block sampling

This is a useful method to determine species present (richness and composition), and both their relative abundances and area density, where there has been pesticide spraying at ground level. The method applies especially for wooded areas with deep ground litter and a carpet layer of vegetation obscuring species, and rendering visual encounter surveys for certain species of amphibians and fossorial form of reptiles difficult or impossible. The method enables the determination of species present and composition, and area density (e.g. numbers per hectare). It involves thorough searching for amphibians and/or reptiles inside a series of square blocks that have been randomly selected within a matched habitat. One side of the habitat can be marked off, and the location of squares chosen (distance from one end) on the basis of random numbers given in a table. With approximate density of animals estimated beforehand, the size of squares chosen depends on vegetation quality and quantity. Depending on habitat type, microhabitat searches in woodland may involve turning stones, raking through leaf litter, probing holes and crevices with sticks, splitting or dismantling old and rotten logs, removal of epiphytes, etc., and recording time spent searching (area covered depends on number of animals recorded in relation to vegetation cover, quality and quantity). Searching need not continue when no further species are recorded; alternatively, a time limit of one or more man-hours is made, depending on number of species and individuals found, number of searchers and habitat type (1 man-hour with few individuals of species; 5 man-hours with 10 or more species and several observers involved).

Limitations Mainly for use in woodland or similar habitat containing inactive amphibians and/or fossorial reptiles.
Resulting data Relative abundance (percentage species composition); sightings frequency (numbers per man-hour) reflecting relative density when timed microhabitat searches are made. Species richness and diversity within an assemblage.
Fauna sampled Primarily yields both active and inactive species, fossorial species, or individuals of species seeking the refuge of vegetation.
Sampling period Day or night, provided the exact time of the start of the search, air temperature and other weather conditions are recorded, but easier during daylight hours with clear visibility.
Equipment Watch or stop-watch, compass, digitometer, thermometer or whirling hygrometer and altimeter must be purchased. Machetes, vegetable rakes and plastic bags can be bought locally. Metal screw-lid aluminium (purchased) or glass containers (jam jars can be bought locally) are required for the preservative solution (45% formalin for dilution to 8–10% can be bought at a local hospital).
Staff required 2 or more observers; searching time (man-hours) increased, and hence numbers of animals sighted in a specific time period (1 man-hour for fewer than 10 species, say; 5 man-hours for more than 10 and several observers).

Transect block sampling

Transects, as an alternative to quadrats, utilize a similar microhabitat searching procedure, and depending on habitat type, may involve turning stones, raking through leaf litter, probing holes and crevices with sticks, splitting or dismantling of old and rotten logs, removal of epiphytes, etc., recording time spent searching (area covered depends on number of animals recorded, in relation to vegetation cover, quality and quantity). As for quadrats searching need not continue when no further species are recorded; alternatively, a time limit of one or more man-hours is made, depending on number of species and individuals found, number of searchers and habitat type. Transects can be laid out as strips to determine amphibian or reptile population clines over a distance of continuously changing habitat, or in relation to increasing or declining levels of pesticide usage. Blocks at a distance from one end of sections along the transect are selected for intensive searching by random numbers in a table. The method enables determination of species present and composition, and area density (e.g. numbers per hectare) along the transect. Density from visual transects is still only relative density (e.g. numbers per hectare), for a proportion of animals will be in their refuges and not active and not, therefore, counted. If walked, or otherwise travelled, transects are also timed, then relative abundance as sighting frequency (numbers per man-hour) will also be obtained, and can be used as a check from statistical correlation with area density.
Limitations Mainly for use in woodland or similar habitat containing inactive amphibians and/or fossorial reptiles.
Resulting data Relative abundance (percentage species composition); sightings frequency (numbers per man-hour) reflecting relative density if timed microhabitat searches are made. Species richness and diversity within an assemblage.
Fauna sampled Primarily yields both active and inactive species, fossorial species, or individuals of species seeking the refuge of vegetation.
Sampling period Day or night, provided the exact time of the start of the search, air temperature and other weather conditions are recorded, but easier during daylight hours with clear visibility.
Equipment Watch or stop-watch, compass, digitometer, thermometer or whirling hygrometer and altimeter must be purchased. Machetes, vegetable rakes and plastic bags can be bought locally. Linen bags can be made locally. Metal screw-lid aluminium (purchased) or glass jars are required for the preservative solution (45% formalin for dilution to 8–10% can be bought at a local hospital).
Staff required 2 or more observers; searching time (man-hours) increased, and hence number of animals sighted in a specific time period (1 man-hour for fewer than 10 species, say; 5 man-hours for more than 10 and several observers).

Patch sampling

High densities of amphibians and certain reptile species are often associated with specific microhabitats or patches in an area. Patches are selected randomly within an area of uniform widespread pesticide application for comparison with those in similar untreated, or less treated, habitats. One side of the area is measured along a straight line, and patches are selected at distances perpendicular from points on a straight line at a set distance apart using random numbers from a table. Material making up the patch is removed or broken up, e.g. turn over rocks, separate out logs or cut down bush; record the numbers of each species sampled, ensuring that all of the animals associated with each patch are included. The method is used to determine the number, relative abundance and densities of species in the overall area.

Limitations Mainly for use in woodland or similar habitat containing inactive amphibians and/or fossorial reptiles.

Resulting data Relative abundance (percentage species composition); sightings frequency (numbers per man-hour) reflecting relative density if timed microhabitat searches are made. Species richness and diversity within an assemblage.

Fauna sampled Primarily yields fossorial species, or individuals of species seeking the refuge of specific vegetation or ground cover type.

Sampling period Day or night, provided the exact time of the start of the search, air temperature and other weather conditions are recorded, but easier during daylight hours with clear visibility.

Equipment Watch or stop-watch, compass, digitometer, thermometer or whirling hygrometer and altimeter must be purchased. Machetes, vegetable rakes and plastic bags can be bought locally. Metal screw-lid aluminium (purchased) or glass containers (jam jars can be bought locally) are required for the preservative solution (45% formalin for dilution to 8–10% can be bought at a local hospital).

Staff required 2 or more observers; searching time (man-hours) increased, and hence number of animals sighted in a specific time period (1 man-hour for fewer than 10 species, say; 5 man-hours for more than 10 and several observers).

Quantitative sampling of amphibian larvae (and aquatic reptiles)

Sampling methods, mainly for counting amphibian larvae, in pools and lakes, and slow-moving streams include seining, dipnetting and trapping, and enclosure sampling in known volumes of water. The relative merits of funnel-ended cylinder trapping vis-à-vis sweep netting and torch-surveying have been discussed by Griffiths (1985). The number of larval and/or adult amphibians caught are recorded in relation to the size of the pond (in the case of seining), number of net-dips or trap, or volumes of water sampled. A net is used to sample all microhabitats in a pond, which may include open water, under weed, edge of bank or in soft surface mud at the bottom of the water body.

The methods are used to obtain amphibian larval species richness, density and population size of amphibians, in relation to pesticide run-off from surrounding land.

Limitations Applicable in mainly open still water (pools, lakes or slow-moving streams); yields mainly tadpoles.

Resulting data Relative abundance (percentage species composition); tadpole density in relation to the size of the pond or volume of water; frequency (numbers per trap over a set time period, e.g. 24 h) reflecting relative density. Species richness and diversity within an assemblage.

Fauna sampled Tadpoles of water-associated anuran species.

Sampling period Day or night, provided the exact time of the start of the search, air temperature and other weather conditions are recorded, but easier during daylight hours with clear visibility. The activity of tadpoles may vary between day and night.

Equipment Thermometers, waders or hip boots, and long-handled dipnets and headlamps need to be purchased. Many of the net types, etc., could probably be made locally. Plastic bags and spare batteries can be bought locally, as can certain other items like notebooks, etc. Metal screw-lid aluminium (purchased) or glass containers (jam

jars can be bought locally) are required for the preservative solution (45% formalin for dilution to 8–10% can be bought at a local hospital).
Staff required 1 observer with 1–2 assistants.

Breeding site surveying for amphibians

Amphibians congregate, often during season rains, at sites adjacent to water to breed. Adults are counted along visual or aural transects. Larvae are present in water for longer periods than the adults. The surveys are mainly conducted in relation to long-term monitoring of populations of amphibians and reptiles in areas or regions where pesticides have been applied, or where water in breeding sites is known to be contaminated from surface run-off.
Limitations Applicable along edges of open pools, and shorelines of lakes or streams; yields adult anurans.
Resulting data Relative abundance (percentage species composition); sightings frequency (numbers per man-hour) reflecting relative density. Species richness and diversity within an assemblage.
Fauna sampled Adult anurans of water-associated species.
Sampling period Day or night, provided the exact time of the start of the search, air temperature and other weather conditions are recorded, but easier during daylight hours with clear visibility. Some anurans are only active at night.
Equipment Watch or stop-watch, thermometer or whirling hygrometer must be purchased. Waders or hip boots and wet suits (if needed), and long-handled dipnets and headlamps also need to be purchased, but the last could be made locally. Plastic bags and spare batteries can be bought locally, as can certain other items like notebooks, etc. Metal screw-lid aluminium (purchased) or glass containers (e.g. jam jars) are required for the preservative solution (45% formalin for dilution to 8–10% can be bought at a local hospital).
Staff required 2 or more observers; searching time (man-hours) increased, and hence number of animals sighted, in a specific time period (1 man-hour for fewer than 10 species, say; 5 man-hours for more than 10 and several observers.

Additional methods for amphibians

Specific methods of sampling amphibians are described by Heyer *et al.* (1994), which are more suitable for specialist application. These include:

- straight-line drift fences and pitfall traps as surface barriers, under the supervision of a herpetologist, directing ground-dwelling species enter pit-fall or funnel traps (used primarily for inventorying and long-term monitoring of populations of adult amphibians over a period of, for example, several months or seasons in areas or regions where pesticides have been applied)
- drift fences encircling amphibian breeding ponds act as surface barriers, under the supervision of a herpetologist, like the previous technique, but used to monitor amphibians as they enter and leave sites, and conducted in relation to long-term changes from pesticide application in an area
- audio strip transects for many frog species that have characteristic calls; calls are recorded with a tape recorder, after recognition of the species making them, to estimate the relative abundance of calling males, and thus of all adults (after determining sex ratios from ground studies), species composition, and breeding site use and phenology
- artificial ponds, which are placed in an area long enough for amphibians to find them (useful for frog diversity assessment and larval abundance)
- artificial cover, in which flat planks of wood or sheets of corrugated iron are placed on the ground for species to seek refuge under (useful for estimating populations of certain amphibian species)

- light trapping, after darkness is useful for long-term population monitoring of species that seek insect prey attracted to light, e.g. toads during the terrestrial phase in relation to widespread application of pesticides over a number of years; animals are recorded at, say, 30 or 60 min intervals, depending on numbers, for 2–4 h after sunset)
- automatic acoustic monitoring of frog calls (useful for determining male populations, and thus adult numbers)
- radio tracking with transmitters and receivers (used to investigate habitat use by amphibians outside the breeding season)
- radioactive tag tracking used to locate tagged individuals in relation to movement
- geographical information system (GIS) and remote sensing techniques used for determining habitat associated with species at known densities.

Additional methods for reptiles

Specific methods of sampling reptiles (e.g. O'Shea, 1992), which are more suitable for specialist application, include:

- artificial cover in which flat planks of wood or sheets of corrugated iron are placed on the ground for species to seek refuge under (useful for estimating populations of certain lacertid, gecko, skink and snake species)
- tracking threads in which a reel of cotton is attached to the animal and paid out as the animal moves through its habitat (has been used successfully for recording tortoise movements under supervision of an herpetologist)
- straight-line drift fences and pitfall traps, as surface barriers, under the supervision of an herpetologist, directing ground-dwelling species into pit-fall or funnel traps (used primarily for inventorying and long-term monitoring of reptile populations over a period of, for example, several months or seasons, in areas or regions where pesticides have been applied)
- quantitative sampling of aquatic reptiles using seine nets for turtles (numbers caught are recorded in relation to the size of pond, and thus density from indirect effects due to pesticide run-off from surrounding land)
- light trapping, after darkness (useful for monitoring such species as geckos, seen on adjacent even, pale-coloured surfaces seeking insect food attracted to light). Animals are recorded at, say, 30 or 60 min intervals (depends on numbers) for 2-4 h after sunset in relation to long-term changes in population numbers with widespread application of pesticides in an area over a number of years
- radio tracking with the use of transmitters and receivers (used to investigate habitat use by snakes)
- radioactive tag tracking used to locate tagged individuals in relation to movement
- GIS and remote sensing techniques used for determining habitat associated with species at known densities.

TAXONOMY

To determine species richness (composition and frequency), some basic taxonomy will be involved. In the absence of a specialist herpetologist with local knowledge of the fauna, a field guide with identification key is useful, although some skill is still required to work through the key. Field guides for amphibians and reptiles do not exist throughout the world, so a specific example cannot be cited that covers all tropical, sub-tropical and warm temperate areas. Specimens of uncertain species can be collected, labelled, preserved and taken to a museum specialist to confirm identification.

DIVERSITY ASSESSMENT

Herpetofaunal diversity can be reduced by blanket aerial spraying of habitat with pesticides, especially forests. Quantitative sampling will provide information on diversity (the number of species present in a sample of certain size). The formula most commonly adopted is the Index of Diversity using the Shannon-Weiner function (H'). The formula is given by:

$$H' = \sum_{\gamma=1}^{s} p_i \log_e p_i$$

in which p_i is the proportion of individuals for the ith species out of the total number of individuals (i.e. the number of individuals of a species divided by total number of individuals recorded in sample), while $\log_n p_i$ is usually the natural logarithm ($\log_e$) of p_i.

The following case studies are based on actual observations.

Amphibians

Table 11.2 Diversity of rainforest species compared in primary and adjacent man-managed (including pesticide exposure) secondary forest (Peninsular Malaysia, March 1995)

Species counted	Primary rainforest			Secondary rainforest		
	No.	p_i	$p_i{}^{l}n p_i$	No.	p_i	$p_i{}^{l}n p_i$
1	13	0.200	– 0.322	25	0.338	– 0.367
2	9	0.138	– 0.274	13	0.176	– 0.306
3	9	0.138	– 0.274	11	0.149	– 0.283
4	7	0.108	– 0.240	8	0.108	– 0.240
5	4	0.062	– 0.172	5	0.068	– 0.182
6	3	0.046	– 0.142	5	0.068	– 0.182
7	3	0.046	– 0.142	2	0.027	– 0.096
8	2	0.031	– 0.107	2	0.027	– 0.096
9	2	0.031	– 0.107	2	0.027	– 0.096
10	2	0.031	– 0.107	2	0.027	– 0.096
11	2	0.031	– 0.107			
12	2	0.031	– 0.107			
13	2	0.031	– 0.107			
14	1	0.015	– 0.064			
15	1	0.015	– 0.064			
16	1	0.015	– 0.064			
17	1	0.015	– 0.064			
18	1	0.015	– 0.064			
Totals	65	(1.000)	– 2.528	74	(1.000)	– 1.906

Worked example

p_i is the proportion of the number of a species (i) of amphibian out of the total, i.e there are 13 (= I) of the first species in primary forest out of a total of 65 amphibians recorded. Then p_i = 13/65 = 0.200, and $p_i \times \log_e p_i$ = –0.322; $\sum p_i \log_e p_i$ (the sum of $p_i \log_e p_i$ for all 18 species) = -2.528, and thus H' = 2.528. Note that the total of p_i equals 1, and provides a check that there are no calculation errors. Index of Diversity using the Shannon-Wiener function (H') is usually between 1 and 3 (below 1 is low diversity; above 2 is high).

Thus, 65 individuals contained 18 species in primary rainforest (Shannon-Wiener function H' is 2.528), while 74 contained 10 species (H' = 1.906) in adjacent secondary forest. Amphibian diversity was, therefore, greater in primary forest, and this can be confirmed statistically (t = 4.33, 139 d.f., P<0.001) using a test described by Magurran (1988).

Reptiles

Table 11.3 Diversity of woodland savanna species compared in an unpopulated tributary valley (Tug Gabibta) exposed to pesticide run-off and the main river valley (Tug Marodijeh) with human habitation (Hargeisa, Somaliland, March 1993)

Species counted	Tributary valley			Main inhabited valley		
	No.	p_i	$p_i \ln p_i$	No.	p_i	$p_i \ln p_i$
1	31	0.323	– 0.365	58	0.574	– 0.319
2	26	0.271	– 0.354	11	0.109	– 0.241
3	13	0.135	– 0.271	8	0.079	– 0.201
4	6	0.063	– 0.173	7	0.069	– 0.185
5	2	0.021	– 0.081	4	0.040	– 0.128
6	2	0.021	– 0.081	3	0.030	– 0.104
7	2	0.021	– 0.081	2	0.020	– 0.078
8	2	0.021	– 0.081	2	0.020	– 0.078
9	2	0.021	– 0.081	2	0.020	– 0.078
10	1	0.010	– 0.048	1	0.010	– 0.046
11	1	0.010	– 0.048	1	0.010	– 0.046
12	1	0.010	– 0.048	1	0.010	– 0.046
13	1	0.010	– 0.048	1	0.010	– 0.046
14	1	0.010	– 0.048			
15	1	0.010	– 0.048			
16	1	0.010	– 0.048			
17	1	0.010	– 0.048			
18	1	0.010	– 0.048			
19	1	0.010	– 0.048			
Totals	96	(1.000)	– 2.048	101	(1.000)	– 1.596

In the same way as for rainforest amphibians, 96 individuals contained 19 species in the tributary valley (Shannon-Wiener function H' is 2.048), while 101 contained 13 (H' = 1.596) in the town main valley. Diversity was, therefore, greater in the unpopulated tributary valley, and was confirmed statistically (t = 2.52, 195 d.f., P<0.01).

LABELLING

Specimens of uncertain species should be collected (Simmons, 1987) and preserved for purposes of identification, with information on distribution. The specimens must be carefully labelled giving at least:

- date of capture
- exact locality (preferably with coordinates using GPS)
- name of collector
- basic habitat information if possible (e.g. rocks, on tree, in water, by human dwellings).

The label should be of parchment, cloth or white plastic, which can be written on, and attached securely by cotton thread to the hind limb of the animal, or neck region of snakes and legless lizards. Information on the label should be written in pencil or permanent marker pen.

BIOINDICATORS

Amphibians, and especially lizards among reptiles, possess qualities that render them useful as bioindicator organisms.

Amphibians

Frogs, toads and other amphibians have both aquatic and terrestrial life stages that expose them to pollutants in either or both habitats. Chemicals are rapidly absorbed through the gelatinous outer layer of their eggs, larval gill membranes and larval and adult skin. Skeletal growth defects caused by contaminants are soon detected in the rapidly developing larvae (tadpoles). Both amphibians and reptiles are primitive vertebrates, with simple enzyme systems, and they are unable to detoxify chemical residues that as invertebrate predators they take in with contaminated prey. Cold-bloodedness or ectothermy results in a poor ability to metabolize chemical residues, and results in the accumulation of pesticides such as organochlorines, especially in fat, and also in the liver and other body tissues (including the brain) at readily detectable levels. Residue levels rise, until eventually a lethal level is reached, especially when fat is combusted during lean periods of the year.

High residue levels in turn present a hazard to predators higher up the food chain that ingest them (many amphibian species constitute a food resource for raptorial birds and other vertebrate predators in aquatic and terrestrial ecosystems). With increasing residue burdens, chronic effects become evident and amphibians, like many lizards also, show changes in behaviour and physiology and may not be able to respond and avoid exposure to toxic conditions during aestivation or hibernation. Being active, and visually and vocally conspicuous (especially at breeding ponds), population monitoring is facilitated in the field. Amphibians are also experimentally versatile in the laboratory and field.

Lizards and other reptiles

Lizards particularly among reptiles share many characteristics as pollution bioindicators with amphibians, but unlike most amphibian species, especially in the tropics, they occur in arid habitats and are generally active during both dry and wet seasons of the year. Lizards are relatively immobile with poor powers of emigration, and, therefore, numbers respond to habitat changes, including chemical contamination. Diurnal species especially are also active and visually conspicuous, enabling population monitoring in the field. Like amphibians, lizards are insectivorous and take in pesticides with contaminated prey and, as a food resource, form an important link in food chains between invertebrates and raptorial birds and other vertebrate predators in terrestrial ecosystems. With intra- and inter-specific competition for food and refuges (many lizards in particular are also territorial), their behaviour and physiology are likely to reflect differences between species due to pollutants. Lizards are also versatile experimental animals in the laboratory and field.

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