Chapter 20

Sampling Amphibians and Reptiles

by

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Abstract

Amphibians and reptiles rank among the most charismatic vertebrates and have received a lot of taxonomic attention over time. Nevertheless, to improve the speed and quality of inventory and monitoring of these animals, we here provide an overview of the different sampling techniques used per taxon and detail the different ways of handling amphibians and reptiles. Also, we indicate the different types of data that are to be collected from specimens. Throughout the chapter tips and trick for safety are also provided.

Key words: amphibians, reptiles, sampling methods, handling methods, safety

1. Introduction

Amphibians and reptiles are important components of ecosystems. Recently, there is an increasing awareness that, like for many other taxa, herpetological diversity is threatened, and the growing list of declining populations suggests a worldwide crisis (Blaustein *et al.*, 1994). Efforts to collect baseline data about occurrence, distribution, and status of populations are relatively well advanced for amphibians, but much less so for reptiles for which recent data suggest that turtles and crocodilians are as threatened as anurans (Gibbons *et al.,* 2000).

In this chapter, we review field techniques for sampling reptiles and amphibians. However, as good body of literature that addresses in detail different topics of the sampling of reptiles and amphibians (see Heyer *et al*., 1994; Dodd, 2009; Graeter *et al*., 2008; Kok & Kalamandeen, 2008 and the references there contained) already exists, this work is only a general manual useful in ATBI initiatives. As such, it does not go into detail of all the aspects that need to be considered when inventorying and monitoring reptiles and amphibians.

Inventories may range from quick short-term surveys to extensive long-term monitoring programs. Inventories ideally involve the use of many collecting and observational techniques, in addition to the compilation of data from past surveys obtained from the literature and museum specimens. Monitoring programs ideally should use standardised techniques so that statistical analysis of data becomes possible.

2. Inventory and monitoring techniques for amphibians and reptiles

This is a condensed review of the most common techniques used in inventories and monitoring of amphibians and reptiles whereby we focus on the possibilities, limitations and materials needed.

2.1. Standardising the sampling effort

In monitoring, the importance of having standardised sampling procedures cannot be overemphasised. The methods mentioned hereunder are suitable for both terrestrial and aquatic habitats, and may be an efficient way of standardising monitoring surveys (*e.g.,* Brown 2001; Meik *et al.,* 2002). Next to recording biological data, the environmental conditions must also be scored and this again in a standardised way.

Standardisation of sampling can be achieved through: (i) time-constrained searches; (ii) area-constrained searches; (iii) quadrat sampling; (iv) transect surveys.

2.1.1. Time-constrained searches

The premise behind this technique is to actively search for animals in a given area for a pre-defined amount of time. If additional information will be collected from the animals found (*e.g.*, body measurements or marking individuals), then the time invested in these activities should not be considered as part of the search. Time-constrained searches are mostly applied during terrestrial surveys, although they can also be used in aquatic habitats, particularly for amphibians.

The main limitation of a time-constrained search is the long periods that the survey participants must commit to it. Furthermore, it must consider that the results of time-constrained searches are highly influenced by environmental factors such as time of the day, season, and weather (*e.g.,* it is well known that amphibian activity increases very much after rainfall). Another factor that will heavily influence time-constrained searches is the level of experience of the surveyors. Experts are likely to find more animals than inexperienced workers. It is vital to keep these factors in mind when designing a study.

For an inventory it is advisable to repeat the sampling to include several days with different weather conditions and to always follow the same previously planned search routine (*e.g.,* if the first search included turning stones, then that should also be included when repeating searches).

2.1.2. Area-constrained searches

With area-constrained searches the search is focused on a certain area and not on an amount of time. Area-constrained searches will give information in terms of absence or presence of species, and potentially some data on life history of the species such as time of reproduction, activity patterns, and habitat use. The size of the area to be searched might vary but it will depend either on the habitat type (*e.g.*, pond, creek, meadow, etc.) or on the focal species.

The main limitation of this technique rests with the effect of environmental conditions, the experience of the workers, and the planned search routine. As with time-constrained searches, the searches should be done during several days with different weather conditions or even different seasons to maximize the chance of encountering all species present in the area.

2.1.3. Quadrat sampling

In this technique, sampling arrays in a study area must be randomly distributed and the absence or presence of animals in these arrays verified. The sampling areas are usually squares (quadrats) that are thoroughly searched (Jaeger & Inger, 1994).

The main drawback of quadrat sampling is that the setup can be very time-consuming.

Within quadrat sampling, we can differentiate point sampling where small squares are used, and broad sampling where larger quadrats are used (Kok & Kalamandeen, 2008). Point sampling is preferred when studying single species in which the individuals are relatively small and densely distributed, while broad sampling is applied to species that are widely dispersed, large bodied or both, as well as for multispecies assemblages. In both cases, all quadrats need to be of the same size within each study area. A modification of this technique is called ‘patch sampling’, in which the sampling arrays are normally specific microhabitats (*e.g.,* logs, bushes, etc.). Patch sampling is applied when looking for specific target species, which we know or suspect that are confined to specific microhabitats within a larger habitat (Jaeger, 1994a). For both techniques some pre-requisites have to be met.

For quadrat sampling:

- Animals may not leave the quadrat before being observed.
- The quadrats are randomly distributed.

For patch sampling:

- Each patch must be defined precisely and in an operational way.
- All patches must be equally locatable by the observer without any bias.
- Animals may not leave the patch before being observed.

If these criteria are met, then quadrats and patches can be distributed randomly within the study area. Each of them then represents an independent sample, allowing statistical analysis of the obtained data if at least 25 to 30 quadrats were scored (Jaeger & Inger, 1994).

Quadrat sampling has proved to be particularly useful in forests when searching for ground-dwelling amphibians and reptiles (Rodda & Dean-Braley, 2002). For best results in this methodology of quadrat (or patch) sampling, it will be important to apply the most appropriate searching technique within each of the quadrats (*e.g.*, using a rakes over leaf litter).

2.1.4. Transect surveys

A linear transect is established and the whole narrow strip (and nearby areas) is searched for animals. This is usually utilised for surveying herpetofauna across environmental gradients but can also be used within a single habitat (Jaeger, 1994a). However, for homogeneous study areas, quadrat sampling is recommended. If the design is properly randomized this method will provide a good representation of the occurring fauna over all habitat types. Depending on how the transects are set regarding the gradient, different information will be obtained. If transects are set in parallel to the gradient studied, then these surveys may be used to compare species across habitats. If on the other hands, transects are set perpendicularly to a gradient (*e.g.*, along a river), then one will be able to study changes in parameters of a given species along the gradient. The most common scale used in transect surveys is at the habitat level, but it is possible to work on a larger scale (ecosystem or landscape) by using, for example, aerial surveys across a large transect (Mourão *et al*., 2000).

Ideally, transect surveys have to meet the following assumptions:

- Specimens are randomly distributed throughout the transects.

- Transect lines are randomly chosen.
- All the specimens in the transect will be observed.
- Animals will not be counted twice within a transect and among transects.

When preparing transect surveys it is important to consider that some species will not meet all the method's assumptions. For example, cryptic species will not be observed or will flee from the observer without any notice, or many species do not have a random distribution, as they are associated to specific microhabitats.

2.2. Sampling Techniques

For selecting the most suitable sampling technique, it is necessary to evaluate :

- The objective of the study.
- The conspicuousness of the species of interest (their activity and habitat).
- The cost, time, and resources needed.

By and large, the methods that are more time- and resource-intensive will yield most information, and will allow more powerful statistical analyses. However, depending on the goal of the study, such intensive methods might provide data that are not needed (*e.g.,* obtain detailed ecological data when presence or absence of species would suffice). Furthermore, it is also more productive to use a combination of techniques instead of applying a single one, but again this will require more resources. Therefore, one must strike a balance between available resources for research and desired results before starting fieldwork.

The most common techniques used for sampling reptiles and amphibians can be divided into active and passive sampling, each with a number of specific techniques.

2.3. Active sampling

2.3.1. Visual encounter surveys (VES)

VES is by definition a time-constrained method in which observers sample for species richness and abundance along a survey path (Crump & Scott, 1994). The time spent in the field and the numbers of observers are taken into account. This technique is appropriate for both inventory and monitoring. VES might be particularly useful for detecting rare species that seldom fall into traps, and thus by using VES in combination with a passive sampling technique, it is potentially possible to obtain the complete species composition of the sampled area. Nevertheless, the efficiency of VES will vary much depending on the type of habitat (*e.g*. low vs. high vegetation) and the species biology (*e.g.* fossorial vs. arboreal). As a matter of fact, visual encounter surveys have a number of assumptions that in many occasions cannot be fully met :

- Each individual of every species must have the same probability of being encountered. This will not be met for example in species with a large sexual dimorphism were one of the sexes is much more visible than the other;

- Each individual is only recorded once during the survey. For this the use of individual marking may be the solution, but it implies a higher time investment;
- Each observer doing the survey must have similar experience and be able to potentially obtain the same results. The best approach to this problem is by training the workers in advance to ensure a similar level of experience.

Road cruising and aerial surveys could be cited as visual encounter surveys, although these are done at a different scale and have specific characteristics. In the case of road cruising, a road is used as a survey transect that is methodically driven through looking for both alive and roadkill specimens (Andrews, 2008). Aerial surveys are mostly used for estimating population size and distributions of large-bodied reptiles such as crocodilians or sea turtles (Glaudas, 2008).

2.3.2. Dipnetting and kick sampling

We refer to dipnetting when a dipnet is swept through an aquatic habitat to capture herpetofauna. When the dipnetting process is semi-standardised – the number of sweeps is recorded and compared among habitats – one may call it sweep sampling (Dodd, 2003).

Sweep sampling is used for sampling herpetofauna in small aquatic habitats (treeholes, springs, puddles, and ponds) where it is more efficient. However, sweep sampling may be used in larger aquatic habitats such as lakes as well with the aid of seines and nets. The main targets of this technique are amphibian larvae. It is important to consider that specific differences in animal positioning in the water column may result in differences in the ability to catch different species. Also not all species can be caught with the same net, so the type of net and its mesh size must be carefully selected depending on the ecology and size of the targeted species. Moreover, dipnetting should be scheduled in the season when the species are most likely to be found in the water. When these factors are taken into consideration sweep sampling may be a very effective sampling method that allows for comparisons among aquatic habitats that are somewhat homogeneous.

Kick sampling is a technique that is especially fit for aquatic habitats of small to intermediate size and with fast flowing current. It is predominantly used when looking for stream dwelling amphibians. It consists of lifting and removing all loose substrate from a stream bottom, kicking loose pebbles or even hand raking everything into a net. Typically, the most common nets used are those with a D-frame whose flat side may rest on the bottom of the stream. The most efficient way to kick-sample is with two-person teams, where one worker loosens the woody debris, rocks and other substrate, while the other holds the net in place. It is very important when sampling for herpetofauna to check the nets very often (every 5 minutes or less) to decrease animal stress and mortality. As before, mesh size has to be considered based on the target species size. The smaller the mesh size, the more species will be captured, but small mesh nets tend to clog up faster with debris, and thus require more frequent maintenance to maintain efficiency.

The main limitations of kick sampling are that it is very labour intensive and that it can cause habitat disruption. For the latter reason, it is very important to redeposit the habitat items (*e.g.* large stones, wood debris, etc.) that had been moved.

2.3.3. Stovepipe sampling

Stovepipe sampling is a quantitative method in which aquatic animals are trapped within an enclosure and later removed from it with a net (Shaffer *et al.*, 1994). The enclosures or samplers are typically pipe-like (one may use air conditioning ducts, culverts, stove pipes, and PVC pipes) (*e.g.,* Alford, 1986 or Skelly, 1996) or a rectangular box (*e.g.*, Harris *et al.*, 1988). These samplers are placed in the water, firmly set against the substrate, but with enough care as to not disturb the environment and cause the animals to flee. Once the sampler is in place, a net is swept within the enclosure to collect the animals. This technique is especially suitable for obtaining quantitative estimates of larval densities that can be used to estimate population size. Samplers should be placed randomly across the habitat, and their dimensions and the water depth recorded to obtain values of captured animals per volume. The best habitats to apply stovepipe sampling are shallow waters with sandy or mucky substrates, which allow to easily install the samplers. In habitats with water vegetation, pipe enclosures are easier to install than rectangular ones. This technique can be time intensive to use, so in case of large habitats or if we only want to determine the presence of a particular species, other methods such as dipnetting will be more useful.

2.3.4. Egg mass and nest counts

This is a method that can be used during breeding periods to monitor the reproductive activity in reptile and amphibian populations. In amphibians, egg masses are counted around a pond perimeter or within the pond and it is particularly useful for explosive breeders and those that reproduce in communal aggregations. For identification purposes it is recommended to photograph the egg masses or at least use detailed language to describe it. Mitchell (2000) recommends making the following observations:

- Is the mass globular or round?
- Are the eggs clumped, separated or on a string?
- What colour and shape are the embryos?
- Is jelly surrounding the eggs firm or loose?
- Is there a film on the surface of the mass?
- To what type of vegetation is the mass attached?

In the case of reptile egg nest counts, this technique is most useful for turtles and crocodilians. Normally a relatively large area must be checked and there is need of having some previous knowledge of nesting grounds, and sometimes the recognition of tracks can be very useful, as well as the leftover from predation over the nests or the remnants materials after the babies hatch (*e.g.*, broken egg shells). Egg mass and nests counts is a relatively simple and powerful method for determining the presence of species, and especially in the case of species that lay a single clutch per year it can be a reliable indicator of population size. This technique is nevertheless useless for amphibians that lay eggs in the land and for most squamata reptiles. Finally, it is important to consider that the lack of egg masses or nests cannot rule out the possibility of a species being present, but not reproducing.

2.3.5. Auditory surveys

Auditory surveys are very useful for estimating species richness of anurans. Male anurans in particular tend to be fairly conspicuous during breeding season when the use their mating calls for attracting females. These calls are species specific, so during the breeding season listening stations can be randomly selected along the breeding site to identify species presence and their relative abundance. This technique has the advantage of easily covering rather large areas while being hardly non-invasive.

Not all anurans are equally easy to detect, but with some training even non-expert workers can obtain good results. In inventory, regular auditory surveys are very helpful for determining species composition, but there are some limits when it comes to monitoring changes in a population because there is always a bias towards only observing declines in calling activity and it is difficult to evaluate if these are due to natural fluctuations. If the aim is monitoring, acoustical surveys should always be coupled with other sampling techniques. In the chapter on bioacoustics more information can be found.

Fig.1. Calling frog. (Photo by author).

2.3.6. Basking surveys and basking traps

Sampling techniques based on the animal's basking activity are applied in aquatic habitats, especially rivers were the observer can advance in parallel to the river bank while scanning basking sites with binoculars. The studied animals are normally turtles (Buhlmann & Vaughan, 1991; Lindeman, 1998), although it has also been applied on water snakes (Mills *et al.*, 1995).

Apart from species presence, basking surveys can also give information on sex ratios and juvenile recruitment, but when further information is needed, this technique is to be complemented with basking traps. These are wire traps that are attached to the underside of the basking log so when the animal instinctively jumps into the water, dives to the bottom of the trap giving the observer time to retrieve it. It is important to remember that basking traps must allow the animal to ultimately climb out of the trap if they fall in and the researcher is not present. The effectiveness of the basking surveys will depend on the amount of basking surface available, the time of the day or season when it is done and the animal's basking behaviour. The main limitation of this technique is that it depends on amount of basking surface available. If there are no basking sites, then no animals are observed, but it does not mean that the species is absent. For using basking traps it is absolutely necessary to identify first favourite basking sites, so a basking survey will always precede the setup of basking traps. In monitoring initiatives basking surveys and basking traps should always be made in conjunction with mark-recapture studies.

2.4. Passive sampling

2.4.1. Artificial cover

Many reptiles and amphibians use covers in the wild for hiding. Logs, rocks and even human debris provide refuge to many species, which implies that sampling these covers many times is an effective method. The problem with these "natural" covers is that quantifying their effectiveness is difficult.

By using artificial coverboards we can standardize the sampling effort maintaining the natural habitat and limit biases. The materials most commonly used for coverboards are solid wood boards, plywood boards, corrugated metal strips, tarpaper and horticultural plastic sheeting. These coverboards are set in array designs as linear transects, rectangular grids or webs, depending on the species and/or habitat sampled. Artificial coverboards have been used to sample many species of reptiles and amphibians (Parmelee & Fitch, 1995; Sutton *et al*., 1999; Houze & Chandler, 2002; Ryan *et al*., 2002; Smith *et al*., 2006). An additional benefit when using coverboards is that as they do not restrict movement, it does not require continuous surveillance as for example pitfall traps. Their maintenance is also easy and inexpensive when compared to pitfall traps. In studies where coverboards and pitfall trap arrays have been used, pitfall always captured more species and more individuals (Sutton *et al.*, 1999; Ryan *et al*., 2002), although coverboards detect species that are not found in pitfalls. In this sense this technique has proved to be particularly useful for small secretive snake species (Fitch, 1992).

When checking coverboards it is advisable to use tool such as snake hooks to avoid accidental bites. It is also advisable to flip the coverboards always towards the researcher to avoid the animals to escape. Finally, when sampling the coverboards it is also advisable to record environmental data such as the weather conditions, time of the day or the temperature. Sampling encompassing as many environmental conditions as possible will always yield better results.

2.4.2. Polyvinyl chloride (PVC) pipe surveys

PVC pipes are an easy and inexpensive technique for sampling hylid tree frogs. These PVC pipes can be placed in the ground or mounted on trees following a grid or transect setup. The ground-placed PVC pipes can be used nearby the breeding areas of the hylids or as a complement to pitfall traps and drift fences, which are normally easy to avoid for the tree frogs. The tree-mounted PVC pipes on the other hand are suitable for sampling the tree frogs even outside their breeding season (Dodd, 2003). For ground-placed pipes a good length is 1 m vertical pipe with around 60 cm sticking out of the surface, while tree-mounted pipes can be of around 60 cm with the bottom part set at a height of 2-4 m. These pipes should have the bottom sealed with a cap to retain some water, but holes should be made in the pipe at about 15 cm to allow draining the excess of water. A good average diameter for the pipes in both cases is about 2-5 cm. Nevertheless, tree frogs can be of many different sizes, so it might be necessary to try out pipes with different sizes and diameters until finding the most successful design for a given species. An important benefit of PVC pipes is that it causes no mortality on the sampled animals, so the frequency and timing of the checks can be very flexible. This allows accommodating this technique easily with other activities and also makes it suitable for using in remote field sites. The main limitation it has is that it is very specific (only for tree frogs) and that PVC pipes are rather conspicuous, so they can be subject of theft or unwanted manipulation. This technique is most useful for detecting presence/absence of species, and even for determining timing and dispersal from breeding grounds. On the other hand it is very tricky for comparing between sites because its results will depend very much on species assemblage and on the availability of other natural hiding sites. If the aim is monitoring through time in a same site, PVC pipes in conjunction with marking individuals can give much information.

2.4.3. Leaf-litterbag surveys

Leaf-litterbag surveys are specific for salamanders, which can be difficult to monitor due to their cryptic and fossorial nature. Litterbags have been commonly used for many years to estimate leaf litter breakdown in streams (*e.g.*, Peterson & Cummins, 1974), but it has been adapted for sampling stream-dwelling salamanders (Pauley & Little, 1998). This technique was successfully applied in the Great Smokey Mountains National Park to inventory various streams (Waldron *et al*., 2003). Their basic design consists of a square (50-90 cm per side, with 70 cm x 70 cm being the optimal size) piece of plastic netting with 1.9 cm mesh. Small rocks are placed on the netting in the field and covered with leaves before the corners are brought together and bound with cable ties to form the litterbag. Finished bags are placed in the stream at regular intervals and after an acclimation period of a couple of weeks, each bag is checked by placing a dip net underneath and lifting the bag into a bucket of water. Then, to extract the salamanders from the bag, dip the bag repeatedly in the bucket and then pour the water through the dip net. The salamanders are then processed and the bags are placed back into the stream.

Although this technique has proven to be successful for detecting the presence of salamander species, it is not capable of indexing populations sizes, so it cannot be applied on its own in monitoring programs.

2.4.4. Aquatic and terrestrial funnel trapping

Funnel trapping is a standard method for trapping many groups of animals including reptiles and amphibians. The principle behind these traps is pretty simple: animals are directed trhough a small opening in the trap via a funnel or ramp, and once inside, are unable to find their way out. This is a technique especially useful for capturing rare cryptic species and has the advantage of being suitable for standardizing. In addition as traps are used during a lapse of time, this technique is also less sensitive to biases resulting from temporal variations. On the other hand funnel trapping requires a substantial investment of time and equipment. The traps themselves can be expensive, and should be checked often to avoid mortality of the trapped animals. When applying funnel traps in inventories, the effort should focus on habitats and times when the target animals are more likely to be active, and the more different habitats sampled the more species we will likely detect. Nevertheless, funnel traps have generally a low capture rate, so for successful inventories, a high intensity sampling is recommended (several hundred trap-nights spread across the season).

Funnel traps are also very useful in long term monitoring programs as the trapping scheme can be easily replicated allowing comparisons. For this, traps can either be set in systematic or random arrays. Based on the capture rates detected we will be able to infer population status, but always with some reserves, as capture rates will depend not only in population size, but also in level of activity and the propensity of the species to enter and remain in the traps. The ideal situation is when traps are complemented by mark-recapture data.

Funnel traps can be used in aquatic and terrestrial habitats, and can be of different sizes, materials and shapes. The use of one or other will normally depend on the target species:

- **Small aquatic funnel traps**: These can be either cylindrical or rectangular and are normally used for trapping water snakes and aquatic amphibians. The traps that are commercially available are designed for capturing crawfish or eels, but these can also be used for amphibians. They are typically double ended and built of steel hardware cloth, plastic or nylon mesh. The plastic traps are normally the most suitable for trapping the smaller species. As an alternative, small and inexpensive traps can be made by inverting the top of a plastic soda bottle and anchoring it to the substrate with a stake (Willson & Dorcas, 2003).
- **Hoop-nets**: These are large funnel traps used primarily for trapping highly aquatic carnivorous turtles, although it is potentially useful for trapping any aquatic turtle. These traps are also commercially available in different sizes and made of twine or mesh. In their setting, the traps normally have a part above the surface allowing the captured turtles access to air. Normally hoop nets are baited to increase success and should be checked at least daily. In

occasions hoop nets can also capture large aquatic salamanders and large snakes.

- **Interruption traps and fake nets**: These traps are suitable for complementing the hoop nets. In this case the trap is unbaited, but uses nets or natural channels to draw the turtle towards the funnel. Essentially they work like drift fences but on the water. The design of the trap can include unbaited hoop-nets, swing door traps or pressure plate traps at the end of the channels of nets. As with the hoop-nets, although these traps are mainly for turtles, they can capture other species such as large amphibians or large snakes (Vogt, 1980).
- **Terrestrial funnel trapping**: Terrestrial funnel traps are typically used in conjunction with pitfall traps along drift fences. The design of the trap can be very variable, although the most common variation consists of a wire hardware cloth cylinder with inverted hardware cloth funnels pinned into each side (Fitch, 1987). It is advisable to set the traps in the shade or cover them with a board to make them more attractive and to protect the captured animals from the rain and the heat. In the case of amphibians it is also advisable to use some kind method to moisture the inside of the trap (*e.g.*, a moist sponge). Terrestrial funnel traps can also be constructed of wood boxes, which makes their building more complex and time-consuming, but in different studies have proved to capture almost any snake, reptile or amphibian possible (*e.g.*, Burgdorf *et al*., 2005; Enge, 2001; Greenberg *et al*., 1994).

2.4.5. Terrestrial drift fences and pitfall traps

Drift fences have proven to be effective for sampling most amphibians and squamata reptiles (Nelson & Gibbons, 1972; Semlitsch *et al*., 1981; Hanlin *et al*., 2000; Enge, 2001; Russell *et al*., 2002; Ryan *et al*., 2002, Todd *et al*., 2008). The basic design of a drift fence is a straight fence buried slightly below ground, and standing up to 50 cm high. Pitfall traps are then buried at floor level and placed at a certain interval alongside the fence. The spatial arrangement of the fence can vary, and we can separate drift fence arrays into:

- **Straight-line drift fences**: These can be set up in X or Y-shaped arrays and are normally used for sampling upland habitat (Corn, 1994).
- **Continuous or partial drift fences**: This setting is commonly used to circle partially or completely wetlands (Dodd & Scott, 1994).

The capture rates and effectiveness of this technique may differ very much between sites, but it is clear that this technique is particularily useful for determining species richness and relative abundance (see Ryan *et al*., 2002 for comparisons with coverboards and time-constrained visual surveys). The main limitations are as follows:

- Expensive and hard to set up. After installing, the traps should be visited at least once in a day.
- Capture biases. Some species may show trap avoidance or even attraction towards the pitfall traps.

- Many species such as large snakes or tree frogs can escape from the pitfall traps. This can be somewhat avoided with putting plastic collars on top of the pitfall traps or using double-pit systems.
- Species associated to certain microhabitats might not be sampled.

The best way to improve the success of drift fence arrays is to combine pitfall traps with funnel traps. This technique is normally used on long-term monitoring programmes due to the relatively high amount of time and funding needed to install them.

3. Capturing and handling animals

When sampling animals they should be handled in a way that allows further study (vouchering, photographing, marking, etc.). Handling is generally done by hand, but several tools and utensils can ease the task and increase the safety of both the sampler and the specimen.

3.1. Snakes

Prior to identification, all snakes should be considered potentially venomous. When identified as venomous, ONLY EXPERIENCED AND TRAINED PROFESSIONALS SHOULD EVER ATTEMPT TO CAPTURE AND HANDLE THEM.

The most common tools used for capturing snakes are hooks and tongs that are used to immobilize the snake and keep it at a safe distance from the researcher. The usual procedure for manipulating a snake is using the hook or tong for lifting up the animal gently from the mid-front body while keeping hold of the snake's tail to avoid it from turning around.

For hand-catching snakes, we should set it in an open area and press its head gently, but firmly against the floor, using for example the bottom of the hook, so we can safely manipulate it. We can secure the head between the thumb and fingers of one hand, and use the other hand to sustain the rest of the body weight to make sure the snake does not suffer spinal injuries. Although giant snakes (boas and pythons) are not poisonous we should never underrate their strength. They should never be handled by only one person and special care must always be paid to their heads. These animals need to bite in order to strangle and their bites can easily infect due to the bacteria in their mouth.

Fig. 2. Handling a small snake. (Photo by author).

For smaller harmless and fast moving colubrids, hooks and tongs might not be appropriate and collecting directly by hand with thick protection gloves is recommended.

Hooks can easily be handcrafted, but tongs are more difficult to manufacture and are normally purchased from supply companies. Currently both hooks and tongs from different brands are readily available through the Internet. They should be made of a light but resistant materials such as anodized aluminium or titanium. The size of the hook and tongs will depend on the size of snake we target. The handles of both tongs and hooks should be made of a material that will not slip during the manipulations, such as rubber. Finally the material that will be in contact with the snake should minimize the chance of injuring the snake while manipulating it (*e.g*., rubber coated).

3.2. Lizards

Lizards on average can be quite difficult to capture by hand due to their size and fast movements, so to assist on their capture we can use a small noose. The noose can be built with a long, slender pole such as a bamboo stick or a telescopic fishing pole where a thread of dental floss or fishing line can be attached. It is common lizard behaviour to flee upon sensing something approaching and then freeze shortly, and it is then when the noose can be placed over the head to trap the animal from a certain distance (see Marcellini & Jenssen, 1991). In the case of large lizards, caution must be taken when handling as they can cause injuries with their claws, and deliver powerful bites that can easily become infected. It is recommended to manipulate these animals wearing heavy-duty gloves to prevent any possible wounds. It is very important to avoid capturing lizards by the tail as it will break off in many occasions.

3.3. Aquatic turtles and tortoises

Aquatic turtles can sometime be captured by hand and with the aid of a dip net, although the usual way of capturing turtles is using traps (see survey methods). In the case of turtles or tortoises they should always be handled with care as they can deliver powerful bites, but this is easily avoided by keeping your hands away from their head. Normally turtles can easily be held at mid- or back-body, although additional attention should also be paid for some species' claws that can be elongated and inflict deep wounds. As with large lizards the use of thick gloves to manipulate the animals is also recommended.

Fig. 3. Handling turtles. (Photo by author).

3.4. Crocodilians

Due to their size and dangerous bites, crocodilians should exclusively be handled by experts. Normally their capture is done by several people and with the aid of a noose. While small and young animals can be grabbed from behind the head with one hand, using the other hand to support their weight (as you would do with a large lizard), larger animals have to be handled by several people. It is important to make sure that the jaws are closed, for example by wrapping duct tape around them, before doing any measuring, and extreme caution must be paid to the tail which can deliver powerful strokes. It is highly advisable to cover the animals' eyes to reduce their stress.

Fig. 4. Handling large animals. A. Small crocodilian; B. Large lizard. (Photos by author).

3.5. Amphibians (frogs, toads, newts, salamanders and caecilians)

Aquatic amphibians can be captured by hand and with the aid of a dip net before they jump into the water or while floating in shallow waters. Most amphibians are nocturnal, so a flashlight can also be used to temporarily blind them and get close enough to them. In the case of terrestrial amphibians the challenge is locating them, as on average capturing them by hand is not difficult. Nevertheless we should have in mind that all amphibians have some degree of toxicity in their skins. Cutaneous glands are a shared character of all adult amphibians and they are normally the main source of biological active compounds found in the amphibians skin. The level of toxicity depends on the exact components of these substances and can range from noxious to highly toxic depending on the animals. The highest toxicity is due to the presence of alkaloids that in most cases derive from the arthropods the animals eat in the wild. Alkaloids have been found in some salamanders, but especially in Dendrobatidae and Mantellidae (Daly, 1998). The secretion of these compounds will be increased when the animals are stressed due to handling so the use of latex gloves or an inside out Ziploc bag is recommended to avoid direct contact with the skin. If none of these are available, and we must necessarily have direct contact with the animal, hands should always be thoroughly washed after manipulating them, making sure we avoid contact with our eyes or mouth. In the same way, any surface that has been in contact with the animals should be thoroughly rinsed and cleaned with water.

Fig. 5. Stressed frog. (Photo by author).

For safely handling frogs and toads, they should be held between the fingers and thumbs around the waist of the animal. For some specific measurements or for photographing the frogs should be grabbed from one of the front legs between the thumb and index finger while sitting on top of the hand. The grab should be firm enough to avoid the animal from escaping using their strong back legs, but with much care to avoid any damage to the front limbs. In the case of salamanders and newts, we should hold them in the entire hand gently restraining the animal between the thumbs and fingers just behind the head, in a similar way as it is done with medium and small-sized lizards.

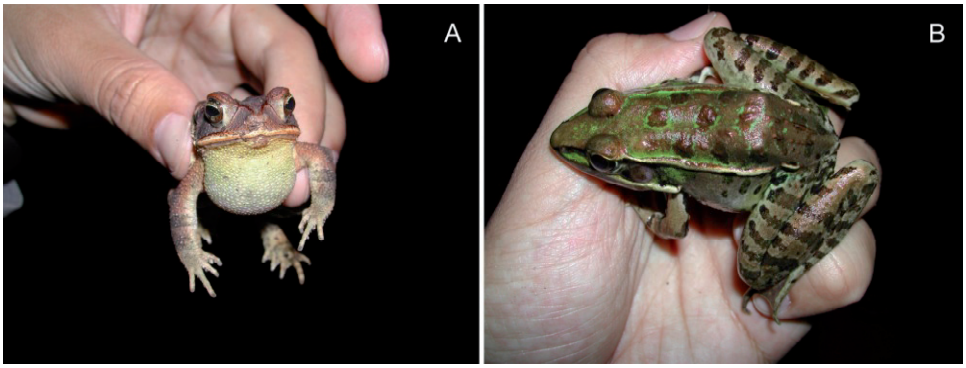

Fig. 6. Handling frogs. A. Holding the animal safely; B. Handling position for measurements. (Photos by author).

Finally, it is important to consider that when handling different amphibian specimens in the field, a researcher can involuntary become a vector for transmitting pathogens such as chytrid fungi. The chytrid fungus *Batracochytridium dendrobatidis* is behind the disappearance of entire populations of amphibians around the world, so if your are going to handle amphibians in the wild, there are a number of rules you should strictly respect to avoid the transmission of chytrid fungi between populations or sites:

- Never move individuals of adult amphibians, larvae or egg between distinct places even if they are very close since this could contribute to the dispersion of the pathogens;
- Never introduce animals, plants or any other organism in the environment, because, besides interfering with native species, they may carry pathogens. We know that fish can transmit viruses that affect amphibians, and in many countries the native amphibians are infected by introduced amphibian species that carry the chytrid fungus. If you detect introduced (allochtonous) organisms in your area, get in contact with an expert;
- Avoid accidentally transporting the pathogens yourself. The chytrid fungus does not have a stage that is resistant to desiccation but it can survive in whatever type of organic material that maintains humidity. As such, after a trip to the field wash well at the site all the objects that have been in touch with the environment (*e.g.*, boots and sample nets). After submerging them in bleach (a bath of 30 seconds is sufficient if you use domestic bleach with at least 4% sodium hypochlorite) or in other suitable disinfectants put them out in the sun for as long as possible;
- If you do not want to use bleach to clean your field material, you can use commercial products specifically sold in veterinary stores. Some suitable commercial products are: Halamid® (www.alpharmaanimalhealth.co.uk) and Virkon® (www.antechh.com);
- If you hold amphibians use disposable gloves or if it is necessary to keep them for some time use disposable containers or ones that have been previously sterilised. Do not put them in touch with specimens from other areas if you are going to return them to the natural environment. Remember that you must sterilise all equipment before using it;
- Inform when possible about the problem of emerging diseases in amphibians and how it is possible to avoid contributing to its spread.

4. Transporting and housing captured animals

If the captured animals must be transported to the lab and housed for some time it is necessary to use appropriate containers.

In the case of amphibians it is most important to keep them in moist substrate in containers or sealed plastic bags. It is a good practice to include some leaves or leafy branches to prevent squashing and maintain humidity. A moist paper towel or standing water in the container usually is effective depending on the needs of the species in question. For tadpoles, plastic containers filled in with water from the capture site can be used, and these containers should be transported in lightly chilled coolers to keep the tadpoles with a relatively low metabolic rate.

Small containers with ventilation are useful for holding small snakes, small turtles, and most lizards. Cloth bags of all sizes, including pillow-cases, are useful for temporarily holding even the largest lizards, turtles, snakes, and small crocodilians. One must be careful not to allow the animals to suffocate or drown while transporting them, and avoid placing them in direct sunlight where any container can rapidly overheat and the animals inside die.

Once in the lab, the setting prepared for short term housing the animals can be very simple. One must make sure that the temperature is suitable for the animals, that natural photoperiods are respected and that the containers are clean and have sufficient water and food.

5. Collecting information from captured animals

5.1. Measurements

All amphibians, squamata reptiles (lizards and snakes) and crocodilians the standardized measure used is the snout-vent length (SVL) that is defined as the distance between the tip of the head and the end of the cloaca. In addition, the tail length can also be recorded to have the total length of the animal, but salamanders and squamata reptiles have the ability to loose their tails as a defensive mechanism upon being attacked by a predator.

Together with the measurements of the body length, the typical measurement is weight. Most herpetofauna can be weighted with either a spring scale or an electronic scale, but for larger species (giant snakes, crocodilians, large turtles) a truck scale will be necessary.

Due to the ectotherm nature of reptiles and amphibians, in many occasions, it will also be of interest to obtain the cloacal temperature of the animals. Ambient temperature can be used as an approximation if it is not possible to measure body temperature, but it must be remembered that there can be significant differences between both measurements due to fluctuations that the animals metabolism can produce in their body temperature. The body temperature of the animals will affect their activity, so this information can be relevant for comparing between sampling periods in a monitoring activity. For measuring the body temperature we can use cloacal thermometers or digital thermometers with a probe. Take into consideration that, especially for smaller specimens, contact with our hands will affect their body temperature, so the measuring of temperature should be done immediately upon capturing the animal.

After collecting the animals it can sometimes be necessary to preserve them as vouchers. The preservation of specimens is a key element for taxonomic identification and when accompanied by properly compiled field notes, it becomes an excellent resource for scientific research in many branches of biology. For example, historical data from museum specimens can allow researchers to detect and assess changes in biodiversity in an area over time. For the preparation of vouchers it will be necessary to kill the animals, although in some cases it is possible to use animals that are already dead due to traps or road mortality. We should collect the minimum number of specimens possible depending on the aims of our study. Although it can depend on how common the animal in question is, it would be advisable to preserve around 20-30 animals for scientific studies and a minimum of 4 for voucher specimens (Graeter *et al.*, 2008). It is mandatory to follow any institutional guidelines that may apply or to request the necessary permits. The procedure to euthanize the sampled animals should be humane and should preserve the condition of the animal. The most preferred techniques for killing reptiles and amphibians are by injecting or submerging the animals in lethal doses of one of the following:

- Sodium pentobarbital
- Hydrous chlorobutanol
- Tricaine methenesulfonate
- Cloretone
- Ethanol
- Other anesthetics

In the case of amphibians, due to their permeable skin, immersion in anaesthetic solutions is the most frequent way of humanely killing them. The most common products used are chlorobuthanol and tricaine methanesulfonate, also called MS-222 (Andreone *et al*., 2008). The minimum concentration should be 250 mg/l (concentrations >500 mg/l must be buffered with an equal weight of sodium bicarbonate as it is an acidic product).

In the case of reptiles, sodium pentobarbital has traditionally been used injected intravenously, intra-abdominally or intrapleuropitoneally (Cooper *et al*., 1989), but recently the use of MS-222 has also been recommended through intracoleomic injections of 250 to 500 mg/kg at 1% solution (Conroy *et al.,* 2009).

The fixation of the specimens should only begin once we are sure that the animals are dead. As chemical fixation affects the proteins in the tissue of the animals, we should attempt to fix them in positions that preserve their morphology and that allows for the observation of key identification characters. The fixation in 10% formalin (obtained by diluting 40% formol) allows a better preservation of morphology so it is ideal for the animals that will be used for formal taxonomic description or for exhibit. Formalin is carcinogenic, flammable and dangerous if fumes are inhaled, so the appropriate cautions must be taken when working with it. In addition it will not allow to use the specimens for posterior DNA analysis so it is advisable to collect tissue samples before fixing the entire specimen, and fix these in pure ethanol. In case formalin is not available, 70% ethyl alcohol can be used, but other alcohols are not recommended (McDiarmid, 1994).

Once the specimen is fixed, it is extremely important to attach each specimen with data such as the field number and any information recorded from the field (GPS coordinates, time, habitat, initial identification, collector, sampling method or weather conditions). It is advisable to use acronyms in the field number referring to the collector, followed by a progressive number and keep the same structure within sampling efforts. This should be printed in hard paper resistant to ethanol and formalin; either hand-written or printed with water resistant ink as there is a risk of loosing the information during transport or long-term storage.

Dependent of the aim of the study it could only be necessary to take a tissue sample or biopsy from the captured animals instead of preserving the whole specimen.

Blood samples are the most common procedures as when it is correctly done it may be less invasive than taking other tissues. In the case of DNA analysis rather small amounts of blood will be necessary, although the amount will be larger for physiological studies. Turtle blood can be obtained from a femoral or jugular vein, a carotid artery, the retrorbital space or the paired cervical sinuses (Dessauer, 1970). In medium and large sized lizards blood is typically collected from orbital sinuses (*e.g.*, Haenel *et al*., 2003), and in crocodilians blood is normally taken from internal jugular or caudal veins. In the case of amphibians, only the larger species can endure blood sampling and this can be done through the midline abdominal vein. Finally for most relatively large reptiles and amphibians heart puncture can also be a viable way to extract blood although this can cause mortality if done by inexperienced workers. In the case of smaller animals, heart puncture will be the only way to take blood samples and will necessarily be fatal. The blood samples can be collected through heparinized capillary tubes.

Alternative tissue samples that can be collected in reptiles and amphibians are tail clips from salamanders, lizards, turtles or snakes. Toe clips may be used as well in salamanders, frogs and lizards, while clipping scutes of the tail of crocodilians and ventral scales from snakes are also common practice. These sampling techniques have the additional benefit of potentially being very useful to researchers who need to mark animals for individual identification.

Finally for DNA studies there is the possibility of using other non traditional sources of tissue which are not aggressive but can later prove difficult to analyze due to the low molecular weight and concentration of DNA in the samples. The most relevant of these sources in amphibians and reptiles are feces, although orifice swabs and shell or scale remnants can also be useful (Poschadel & Moller, 2004). For methods to better preserve tissues for future DNA analyses, we refer to the chapter of Gemeinholzer *et al*. (this volume) on organizing specimen and tissue preservation techniques in the field for subsequent molecular analyses

5.2. Photo-vouchering

Photo-vouchering entails using photographs to document the occurrence of encountered wildlife. This is particularly useful in herpetology as it is very possible to make photographs of the animals accenting the key features that allow for a doubtless identification. These photo-vouchers, if correctly complemented with additional information will provide long-term evidence that those species exist or existed in a given geographical location. A literature record complemented with a photograph will make the report reliable without the shadow of a doubt. In addition photo-vouchers can be the alternative to traditional vouchers in the case of rare, threatened and endangered species or the alternative to the records of animals difficult to capture, such as basking water turtles. In the cases when the preparation of vouchers specimens is absolutely unavoidable, photographs of the living animal will also be of much help as after fixation specimens tend to lose their colours and even some patterns. The ideal situation of documenting the occurrence of a certain species is having the voucher specimen for detailed analysis complemented with photographs of the specimen before fixating.

Currently the use of digital cameras has made photographing cheaper. It is possible to quickly review the photographs taken and make as many pictures as necessary, although we should remember that digital files can also become corrupted and the information lost. Some recommendations for preparing photo-vouchers are:

- If the photographs are going to eventually be deposited in a natural history museum or other repositories we should obtain information on the format, size and resolution needed;
- Include some kind of scale in the photograph to have information on the size of the animal photographed;
- Make the photographs of the animals as soon as possible after capturing, as especially some amphibians tend to change colours and patterns after being captured;
- If the animals are very active, it can be useful to lightly chill them in a refrigerator, but never in the freezer. The amount of time should never be over a few minutes depending on the size, and if the animals are later going to be released back to the wild, first make sure that it has returned to normal temperature before doing so.

6. Field notes and data collection

Most serious shortfalls in gathering and managing descriptive data on amphibians and reptiles can be avoided through planning and preparation prior to collecting data. The list below compiled by Greene (2008) includes common issues and problems that need to be addressed when implementing an inventory or monitoring program:

- Research and study goals and the specific data to be gathered must be clear to all parties involved (*e.g.*, funding agency representatives, researchers, and technicians);
- Data must be gathered in an organized, consistent manner. Design a datasheet that is objective and simple to use, and which includes all relevant information in sufficient detail. If funds and expertise allow it, invest in personal digital assistants (PDAs) or electronic laboratory notebooks which can be programmed with customized forms for direct data entry in the field (this can help minimize data entry and data transfer errors);
- All personnel involved must be trained to gather data in the same manner. Attention to detail and consistency are paramount. Handwriting must be legible;
- Store data routinely in one place until the data can be entered into a database. Keep electronic backups or photocopies of the originals in a different secure location. More than one person should be familiar with the procedure and storage locations;

- Consider how the data will be used and then enter the data into an appropriately designed database. A spreadsheet such as Microsoft-Excel is adequate for many straightforward datasets. Microsoft-Access may be a better option if the data are a subset of a bigger relational database. Copy the data on a weekly basis at minimum to a portable storage medium and keep the files in a separate location;
- Review the data and the data management system early in the process and then periodically on a regular basis. This will allow early detection of errors and inconsistencies, which can be identified and corrected before valuable information is lost;
- One competent, detail-oriented person should oversee the entire process from data collection to data entry to data storage.

For some examples of datasheets that can be used during inventories and monitoring, I refer to Graeter (2008).

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