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Rapid assessments of reptile diversity

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18.1 Introduction

As of March 2015, a total of 10,178 reptile species have been described (Uetz and Hošek, 2015), at least 165 (>1.62%) of these in the preceding year (2014) alone. The conservation status of a majority of the newly described, or even of relatively familiar species, remains unknown. An earlier assessment of the conservation status of a large representative sample of the world's reptile fauna indicates that a significant proportion is threatened (Böhm et al., 2013). This makes a case for urgency in initiating studies on their conservation requirements, foremost among these being distributional and abundance data, as typically obtained during surveys constrained by time and other resources.

Reptiles play important ecological roles, *inter alia* forming significant animal biomass (Iverson, 1982), constituting important linkages in the ecosystem by providing dispersal mechanism for plants (Hnatiuk, 1978; Fialho, 1990; Olesen and Valido, 2010), contributing to environmental heterogeneity (Kaczor and Hartnett, 1990), having keystone functions in maintaining ecosystem structure (Ashton, 2010), and fostering important symbiotic associations with an array of organisms (Lago, 1991; Witz et al., 1991). Crocodilians are also known to maintain wet refugia during droughts, which are used by a variety of other organisms from macroinvertebrates and fish to turtles (Mazzotti et al., 2008). Many turtles and several crocodilians are scavengers, helping release nutrients locked up in dead tissue (Burroughs et al., 2014). Reptiles are regularly on the menu of predatory mammals, birds, fish, large invertebrates, including spiders, and even themselves (see Cook, 1987; Bauer, 1990; Martín and Lopez, 1990); they are also predators for a range of invertebrate and vertebrate species. Fossorial snakes may help aerate hard soils, allowing air to access rainforest tree roots (Rajendran, 1977) in a sense behaving as ecosystem engineers (sensu Jones et al., 1994) by significantly modifying and maintaining habitats.

Reptiles are important predators of insect (Bhanotar and Bhatnagar, 1976) and rodent (Lim, 1974; Whitaker and Advani, 1983) agricultural pests. Additionally, venom extracted from certain snakes is used for the production of life-saving drugs, including anti-venin serum for snake-bites (McCleary and Kini, 2013; Zouari-Kessentini et al., 2013). A number of large lizards and snakes, and nearly all of the world's turtles, are sought for food, medicine, or the pet trade (Valencia-Aguilar et al., 2013). Because of

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their lineage and life-history diversity and pan-global distribution, reptiles are considered model organisms for the study of vertebrate life (Pianka, 1986). Additionally, they feature in many indigenous cultural practices and belief systems, further studies of which may provide valuable insights into conservation strategies and management.

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A majority of the world's reptiles inhabit the tropics and subtropics, while temperate regions have far lower richness of reptiles. Nonetheless, many sites outside tropical areas remain to be surveyed, while a significant portion of the world's tropics have never been adequately inventoried for their reptile faunas.

18.2 What is an RA?

Rapid assessments (RAs) have been described as 'synoptic assessments that are often undertaken as a matter of urgency in the shortest timeframe possible to produce reliable and applicable results for its designed purpose' (Anonymous, 2006). RAs thus comprise short bouts of field data collection for estimation of species richness. Developed primarily in temperate regions, RAs collect data in a standardized (and therefore, comparable across time and space) and cost-effective manner, and are widely used for the preparation of environmental impact assessments (EIAs); they also may be useful for 'BioBlitz' surveys (or intensive surveys by competing groups to record biodiversity within a fixed period of time; Graham and Timpe, 2007; Robinson et al., 2013). RA techniques are favoured especially in cases of constraints on time, personnel, and other resources, and are themselves constrained by species' biology. The length of the sampling periods used to gather data during field work, purported to be 'rapid', appears not to be uniform in the herpetological literature, but generally is considered short enough to gather preliminary information, such as two weeks or less (see D'Cruze et al., 2006). The typical time frame for the Rapid Assessment Program (RAP) of Conservation International, a U.S. non-governmental environmental organization involved in international biodiversity conservation, is 4–6 weeks of field surveys, of which 5–7 days are allocated per specific site. In places showing seasonality, the decision of when to sample is therefore critical, and is likely best during the wet season when more species are actively foraging and breeding than during dry periods. However, this may not be possible in some cases for reasons of access to the study site (e.g. D'Cruze et al., 2006), delays in receiving permits, logistic arrangements, or other reasons. Given time limitations, it is important to target rare or threatened species in the survey area, as suggested by previously known distributional ranges and from information received through community questionnaire surveys (Section 18.4.1).

18.3 Planning components of RAs

18.3.1 Assembling literature and other resources

Familiarity with the plant and animal literature (published, the so-called 'grey literature', reports, theses, and online material such as databases and electronic publications) concerned with the site where a RA will be conducted is essential for investigators. Moreover, to enable the activities that follow to be more useful, researchers need to be

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sensitive to topics of biology or human welfare that may be impacted by subsequent conclusions and recommendations.

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Tropical herpetofaunas are complex, and rarely are identification resources comprehensive or optimal for RA activities. Sources include monographs, traditional print editions of field guides, online guides, computer (delta) and traditional keys, and systematic and taxonomic papers to specific taxonomic groups or national faunas. Extensive library research and networking among co-specialists are essential in order to gather all relevant taxonomic and related literature for field and laboratory identification. Nonetheless, RAs also produce new species to science in poorly sampled parts of the world. Examination of critical museum specimens, especially type specimens and other comparative material and associated data, becomes essential, as is discussion and networking with colleagues in the same or related fields. Familiarity with the fauna is also helpful when investigators plan searches for threatened or other species of conservation importance that may be expected. Inclusion of local researchers is highly recommended. Not only do they tend to be familiar with the fauna and study site, they can also assist with logistical challenges onsite, besides helping in the permitting process (Chapter 2).

18.3.2 Permitting

Legislation surrounding resource access can be complex, and in all regions of the world permits from local and national agencies are required before survey teams can access the site. These range from villages and towns in the vicinity of the proposed sampling/ survey locality to local councils, district, county, state, and/or national governmental agencies that may be located many kilometres from the study sites. Exporting biological specimens, tissue samples and other biological material is similarly controlled by legislation, nationally or bilaterally enforced or under international regulations. The most important among these is the Convention in International Trade in Endangered Species of Wild Fauna and Flora (CITES), which lists species controlled under various Appendices that require export and import permits. Permitting regimes may be tedious (considering that rapid assessments are measurable in days), and may take many months to years to obtain from some agencies after submission of required documents. It is the responsibility of the field investigator to understand and be willing to comply with regulations before application for permits is made. After permits have been obtained, it is important that courtesy calls be made to local stakeholders, such as large landowners and village headmen, to brief them on research activities and possibly recruit assistants (including field researchers, porters, guides, and cooks) from among the local inhabitants. Researchers should consider providing as much economic assistance to these communities (in terms of homestays and purchase of provisions) as possible, in addition to on-the-job training as field biologists or parataxonomists.

18.3.3 Training of field personnel

Biodiversity surveys often are prone to observer bias, and when the time-frame for sampling becomes constrained, such bias can introduce significant problems in data reliability; in extreme cases, bias can result in fewer than expected sightings or collections (see

Zhang et al., 2014). Observer bias can result from: fatigue, poor weather conditions, visibility issues, heterogeneity in target species availability (i.e. observers tend to see animals that are more frequently available or are available in areas where they are more likely to be detected; Borchers and Samara, 2007), differences in observers' eyesight or capacity for walking transects, technical (especially in handling appropriate field instruments) and analytical skills (i.e. employing appropriate sampling methods as well as techniques for their analyses), field skills (such as the ability to interpret tracks and signs), and even soft skills, such as interpersonal skills and empathy with focus groups during interviews. Familiarity with field protocols will significantly decrease wastage of time, permitting investigators to 'hit the ground running'. Field investigators also need to be aware of dangers and annoyances in the field, such as venomous snakes and large crocodilians. Knowledge of first-aid and emergency medical plans are therefore essential, including location of the nearest primary health centre or hospital and whether they stock the appropriate anti-venin serum for locally occurring venomous snakes.

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18.3.4 Timing

RAs essentially are snapshots of biodiversity over a short time span, and need to be scheduled with care, taking into account the phenology of the group being sampled and pre-existing knowledge of their activities. Behaviour and activity periods, both diel and seasonal, may be different, especially in seasonally dry-wet and/or hot-cold areas, and it becomes imperative to choose periods when the greatest number of species are active (usually, wet and warm periods) to obtain realistic estimates of species richness. A majority of reptiles are nocturnal or crepuscular. Many may be diurnal, however, such as most lizards of the families Agamidae, Cordylidae, Dactyloidae, Iguanidae, Lacertidae, Phrynosomatidae, Scincidae, Teiidae, Varanidae, and some members of the snake families Colubridae and Elapidae. Other members of these latter two snake families, and with few exceptions, the geckos (Gekkonidae), are nocturnal. Most sit-and-wait predatory reptiles, including many members of the snake families Boidae, Pythonidae, and Viperidae, crocodilians, and some turtles, may be encountered at any time of the day or night, whereas activity patterns of the fossorial snake families Dibamidae, Leptotyphlopidae, Typhlopidae, and Uropeltidae are largely unknown, although classified as nocturnal because they tend to be sighted after dark. In areas with strong seasonality, RAs may be conducted during different seasons (spring, summer, autumn, and even winter at some sites). Given the (often) vagaries of receiving permits and solving all logistic challenges, including access to sites in many tropical regions, pre-survey data on the life histories of the local reptiles are essential.

18.4 Field sampling

18.4.1 Community questionnaire surveys

For very short field visits or site reconnaissance prior to actual sampling, structured questionnaire surveys often prove useful for investigators, and arguably are the most cost-efficient techniques to gain new knowledge. Data sheets should be developed and

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tested for errors prior to being used at a field site on a particular focus group (local human inhabitants, who may be stakeholders of the survey site), and should include a series of questions addressing observed species in an area, seasons of observation, impressions of abundance, exploitation, habitat use, distinctive behaviour, folklore, and taboos. The list of questions should be relatively brief, inasmuch as the quality and accuracy of the responses decrease towards the end of a long questionnaire survey (Bogen, 1996). In some cases, anonymity may be desired by the interviewees.

The knowledge of local residents understandably varies according to age, aptitude, experience, and profession, information that should be recorded in the data sheets. Residents often reveal the existence of rare or cryptic species that may not be discovered during short inventory periods (such as aquatic turtles or snakes) when interviews are conducted with individuals most familiar with the environment, such as fishermen, hunters, and farmers. Other species that may be familiar to local human inhabitants include seasonally active species and those restricted to special habitats. Experience in conducting sociological studies and familiarity with the native language of the interviewee (including vernacular names of target organisms) is an asset for RA personnel collecting such data. Descriptions of morphology and distinctive behaviour of reptiles should be gathered from interviewees. In the absence of established vernacular names, images of species likely to occur in the area can be shown to respondents in order to avoid introducing bias into the reporting. RA personnel will do well to heed local taboos and restrictions, such as the capture of species that are culturally protected. Remuneration (money, other products, such as fishing gear, or even the prospect of future employment) may be appropriate in some cases to information providers. Semi-structured questionnaires, whereby interviewers collect additional (especially important anecdotal) data, are preferred. Attributes such as a genuine interest on the part of interviewees and general empathy enhance the accuracy of response. Data sheets should be sequentially numbered and cross-linked to georeferenced maps that include information on elevation, habitat/vegetation, wetland areas, and land cover. Community questionnaire surveys offer unparalleled opportunity for public education and outreach (Chapter 30), and skills in such areas are highly desirable.

18.4.2 Visual encounter survey

Perhaps the easiest RA technique for reptiles is the visual encounter survey (VES; Crump and Scott, 1994; Guyer and Donnelly, 2012; see Chapter 10). A VES comprises time-constrained searches along pre-established transects for visually or acoustically locating animals from the target group. Techniques include using rakes or sticks to turn over leaf litter and logs, looking inside tree holes and rock crevices, and netting streams and other water bodies (Chapters 10, 11 and 17). Transect location and position are important in the planning phase. If the objective of the RA is to record as many species as possible (approaching a comprehensive species inventory), representative habitats should be covered. For quick comparisons of species richness among sites or habitats, the effort expended in different habitats needs to be equivalent to permit standardization. Every individual is accurately identified to species and georeferenced to the point of observation; each data sheet should contain a record of individuals, description of the

habitat, time of survey, and number of field personnel employed in order to estimate survey effort. Sites for assessment need to be selected carefully based on available maps and transects established during daylight hours. As transects are typically established along existing forest trails, investigators need to be aware of potential bias. In such edge habitats, visibility may be significantly greater with different species assemblages than within forested habitats. If multiple transects are used, these should be located sufficiently distant from one another to avoid recounting the same individuals and to exploit the within-habitat diversity. Where faunal turnover with distance is high, such as within tropical sites, the transect technique is quantitatively more effective and easier to use than pitfall trapping or cover boards (Sung et al., 2011).

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18.4.3 Species list technique

Originally a technique for rapidly estimating bird species within a small geographical area when constrained by time, the species list technique (SLT) of MacKinnon and Phillipps (1993) has been used for herpetofaunas (Muir and Muir, 2011) and consists of preparing faunal lists of three, five, or 10 species, and once completed, a second list started, and the process repeated during the length of the survey period. The strength of the technique is that multiple observers can pool their data into one large database. The cumulative species richness then is related to the number of observations, rather than to space and time, thus permitting moderate differences in techniques employed and inter-observer skill.

18.4.4 Trapping

A variety of trapping techniques is utilized for sampling reptiles, and while a majority are suitable for long-term studies, a few may be employed during sampling periods that last a fortnight or less (suiting the requirements of a RA). Traps typically sample species that are not encountered during VES, and result in data on presence (but not absence) in addition to valuable life history information and perhaps relative abundance. One trapping technique is pitfall trapping (Chapter 10), which comprises burying an array of buckets flush with the ground surface. Buckets need to be as large as possible (as the depth of the same will determine the size of animal to be trapped) and with smooth sides. In association with a drift fence that directs animals to the mouth of the bucket, captures can be made of a variety of surface-dwelling or subfossorial species of squamates and small turtles (in addition to small mammals, amphibians, and arthropods). Initial capture rates may be high in pitfall trapping. These traps often capture species not otherwise encountered, making the technique appropriate for reptile RAs.

18.4.5 Taxon-specific techniques

Sea turtles

Beach surveys are a useful RA technique for gathering information on sea turtles. When time is limited, sea turtle presence may be indicated through egg shells, crawl tracks, and nesting pits that are species-specific (see Pritchard and Mortimer, 1999, for details of tracks and nests). These can be examined carefully and classified at least as fresh (e.g.

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made within the last 24 hours or a few days, and indicative of ongoing nesting) or aged (tracks of some species, such as *Dermochelys coriacea* may persist for weeks or even months under fair weather conditions). Survey platforms include ground and aerial surveys, both having their strengths and weaknesses, and a combination of these two techniques is naturally the most useful (see Chapter 15).

Non-marine turtles

The frequent low densities of tortoises and freshwater turtles make them difficult candidates when applying RA techniques for reptiles. Successful methods are frequently restricted to a few species, including visually locating terrestrial turtles on horseback, using trained dogs, detecting well-used trails and burrows, using blunt-tipped metal rods to probe holes underground ('sounding'), using a rake in vegetation-choked waterbodies, hooks, and electroshocking (Plummer, 1979; Vogt, 2012; Chapters 13 and 14).

Crocodiles

Inventories for crocodilians are facilitated by the fact that the group contains the fewest number of extant species relative to other groups of reptiles, and rarely do more than two species occur in syntopy. Additionally, community questionnaire surveys (Section 18.4.1) can be useful for investigators in a RA for learning much about which species occur at a site and other biological data, including habitat use and even estimates of abundance. Techniques that can be employed for rapid collection of data relative to RA requirements include boat surveys when investigators use spotlights to detect reflective eyeshine of crocodilians at night, especially under conditions of low tide (Bayliss, 1987). Bright lights from headlamps or hand-held flashlights are employed to detect eyeshine during these surveys, although too bright a light (outside the 50,000 and 20,000 candlepower range) may fail to detect crocodilians in the vicinity of the investigators (Mazzotti, 2012). Another RA technique includes the use of either fix-winged aircraft or helicopters to count crocodilians during the day, where a constant height and speed is recommended, although aerial surveys have only been successfully carried out in tidal forests and other relatively open landscapes (Mazzotti, 2012). Other RA techniques for crocodilians are in Chapter 16.

Squamates

Snakes and lizards comprise the bulk of reptiles encountered at most field sites. Fitch (1987) described several methods used by investigators in locating snakes, including the behaviour of prey species (e.g. amphibian distress calls, bird mobbing activities), road-cruising (where sampling is conducted over the same stretch of road 'transect' by car and live and dead snakes and lizards are recorded), and trapping. The latter may involve several techniques, most of which require a relatively large effort and time. One applicable technique for a RA is the use of cover boards (strategic placement of sheet metal or boards that are quick heating), under which thermophilous squamates may shelter, especially in temperate regions. Species' activities, natural history observations, and perhaps relative abundance data can be collected through the use of cover boards. Cover boards have not been used very successfully in tropical areas.

Pitfall trapping is a useful technique for sampling terrestrial and fossorial reptiles (Chapters 10 and 11). Because of the time expended in locating appropriate sites, establishing arrays of pitfalls and associated drift fences, and removing them at the end of the sampling period, it may be an unnecessarily tedious and time-consuming technique for many RAs targeting reptiles, especially relative to VES. Nonetheless, the technique is recommended when sufficient resources exist, as it can rapidly aid in the acquisition of additional reptile taxa that are not encountered using VES, such as fossorial or subsurface active lizards and snakes.

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18.4.6 Environmental DNA

Development of techniques in environmental DNA promises a new approach to monitoring reptiles in aquatic environments (Pilliod et al., 2013; Goldberg et al., 2015; see Chapter 25). The concept is based on the fact that aquatic animals leave DNA—either nuclear or mitochondrial DNA, in cellular or extracellular (dissolved DNA) form, through excretion, or shed skin (environmental DNA or eDNA). The presence of target species in biodiversity surveys can thus be potentially detected by analysing water samples for eDNA without the necessity of observing them. In water, eDNA is diluted and transported by currents and other hydrological processes, and may last 7–21 days, depending on environmental conditions (Dejean et al., 2011).

Studies on eDNA have targeted invertebrates, fish, frogs, and small mammals in North America and Europe (Ficetola et al., 2008; Kelly et al., 2014). More recently, studies are being initiated using eDNA for rare or secretive freshwater turtles in North America and China. The costs of detecting species via eDNA has been argued to be lower than field surveys, with a better chance of detection, especially of ecologically cryptic species. The procedure includes collection of eDNA by sampling the water body. The usually miniscule quantity of DNA is amplified via a polymerase chain reaction (PCR). By using species-specific primers that bind to the DNA of a target species, the presence of the species is confirmed via bar-coding (that helps identify species using short DNA sequences from a standard position in the genome). Apart from presence data for focal species in a particular habitat, other potential uses include estimating species abundance, noting the presence of invasive species, and generating species inventories from eDNA samples (Chapter 25).

18.5 Data analyses and limitations

The limitations of time and, typically, collectors' bias have the potential to seriously underestimate biodiversity and, consequently, undermine the scientific value of RAs. Developing a solid theoretical basis for understanding the relationship between (collecting) effort and the number of species obtained, therefore, becomes important. Species identification errors need to be kept minimal, and if collection is not possible, digital images of vouchers (Chapter 5) and non-lethal DNA sampling (such as buccal swabs) may be appropriate (Chapter 25).

Since estimating species diversity and richness is challenging, especially at tropical sites, statistical extrapolation of data gathered may be helpful in many cases. Derived

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from this is the species accumulation curve (Chapter 21), a widely used predictive tool that has been argued to be without bias of collectors' attention given to uncollected species (Colwell and Coddington, 1994). Species accumulation curves are produced by plotting the cumulative number of new species encountered after each period of sampling against sampling effort (either transect length or hours of observations), other factors being constant (including weather and number of observers). The asymptote of the curve indicates the likely species richness for that habitat. For all models employed, a well-defined asymptote is required for reliable estimates of species richness at a particular geographical area. The general trend is for a rapid accumulation of species encountered (representing the more abundant representatives), reaching a higher 'shoulder', and plateauing off (including at this stage, the relatively more rare species), to show that all species have been sampled at the site. At sites with high reptile species richness, curves rise to a point of clear upper inflection or 'crest', indicative of a need for greater sampling that may be beyond the scope of a RA. Thus, in most tropical sites, curves are crested, and finding a model that can accurately predict species richness may be challenging. Species accumulation curves are influenced by ecological characteristics of the sites (Thompson et al., 2003), and the comparative ecologically cryptic nature of the study organisms, including their rarity, non-trapability, and their transient presence.

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The SLT familiar to ornithologists has been recently employed with herpetofaunal groups. A bias observed in the use of the technique for avian groups is towards solitary and terrestrial species (as opposed to monospecific flocking species). Consequently, the SLT may not reflect community structure, as quantified using other techniques (O'Dea et al., 2004). When such information is desirable, a combination of techniques needs to be employed in order to gather additional species names in lists of sites where the RA is employed.

18.6 Summary

A successful RA programme depends much on having realistic objectives, project planning, addressing resources available for the task, and understanding the limitations of the work itself (see also Chapter 2). It is important to recognize that absolute estimates of species diversity and richness are elusive figures even for long-term studies in most parts of the world, and complete species inventories may take up a person's lifetime (Myers and Rand, 1969; Das, 1996). Limitations on assessing richness and diversity increase with the size and vegetation complexity of the geographical region sampled (e.g. tropical rainforests, deserts) or when shy, ecologically cryptic species (such as fossorial squamates or aquatic turtles) are concerned. Current inventory techniques are biased towards reptile faunas of terrestrial and aquatic environments; those that are fossorial and arboreal tend to be poorly sampled because of limited sampling methods (Das, 2012; Chapter 11). Nonetheless, RAs have the potential to reveal the existence of unexpected and undescribed species (see Hawkins et al., 1990) and the identification of areas of high species richness (Graham et al., 2010). RAs are superior to another rapid source of acquiring similar information, that is, compilations based on publications, databases and museum materials that tend to be biased and/or of low temporal or spatial resolution (Elith and Leathwick, 2007).

Indeed, many RAs targeting reptiles are conducted in poorly-known tropical or other species-rich sites and remain the only source of information for years. Thus, rapid surveys of reptile biodiversity need careful planning and execution, thereby eliminating the mismatch between expectations and results. Investigators would do well to incorporate emerging technologies, perhaps in related disciplines, into RAs for reptiles. These include the use of environmental DNA and bar-coding, camera trapping for larger species (sea turtles, squamates, and crocodilians), and new methods of accessing tall trees in rainforest canopies. Finally, every field technique has its strengths and weaknesses, and a combination of field methods may be appropriate for reptile assessments that are of short duration.

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