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Detectability of reptiles in standardised surveys: a test using grass snake *Natrix helvetica* models

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The ability to detect snakes in the field may be influenced by phenotypic and morphological variables attributable to the target species. These variables include body size, colouration, and body posture. To test what effect these variables had on detectability by surveyors, plasticine model grass snakes were distributed along a predetermined transect in reptile habitat. Detections of different types of snake models along the transect were compared between two groups of inexperienced students and those of a single experienced observer. The experienced surveyor detected 72 % of all the snake models, compared to 53 % and 58 % by the inexperienced groups. All groups detected more larger snakes than smaller snakes, and more uncoiled snakes than coiled snakes. The presence of a yellow/black collar did not influence the detection of the snakes. The results demonstrate the observer bias that may be inherent in surveys of snakes due to variation in size and posture of the target animals. Accounting for such biases in the design of reptile surveys and providing appropriate training and experience for volunteers may improve the validity and interpretation of data collected within citizen science programmes.

Keywords: population assessment, imperfect detection, citizen science, survey protocol

INTRODUCTION

A major issue associated with the surveying of cryptic species is the recording of false negatives whereby the species is present but goes undetected at the site (e.g. MacKenzie et al., 2002; Fitzpatrick et al., 2009; Guillera-Arroita et al., 2017). Failure to take imperfect detection into account can detrimentally impact the reliability of analysis in key areas such as population structure, abundance and species richness (Griffiths et al., 2015). With increasing engagement of volunteers in biodiversity surveying and monitoring programmes, it is important that any biases associated with variation between observers can be accounted for (Bird et al., 2014). Indeed, Schmeller et al. (2009) found that 86 % of participants in European biological monitoring schemes were volunteers, and the results from such surveys are often viewed critically (Lewandowski & Specht, 2014). Consequently, Fitzpatrick et al. (2009) caution against mixing participants with differing experience levels in the same survey as this can introduce sampling variation and increase the likelihood of both false negatives and false positives.

Cryptic reptile species can be difficult to observe in the field, especially in the case of smaller individuals and without the use of Artificial Cover Objects (ACOs) (Halliday & Blouin-Demers, 2015; Gregory & Tuttle, 2016). Detectability depends on the target species' behaviour, phenological traits, morphology, size and life stage as well as the sampling method and capture technique employed (Mazerolle et al., 2007; O'Donnell & Semlitsch, 2015; Willson, 2016). For example, a programme in Guam that used traps baited with mice to capture invasive brown treesnakes *Boiga irregularis* was effective for adult snakes but failed to trap immature snakes due to ontogenetic shifts in behaviour (Rodda et al., 2007). The cryptic nature of many immature reptiles also confounds detectability. Analysing data from five lizard species, Rodda et al. (2015) reported a capture disparity between juvenile and adult lizards with a consistent bias comprised of under-sampling of juveniles and a slight over-sampling of adults. Colour patterns may also affect detectability by observers and potential predators. Although a ring or collar around the neck has evolved in a range of lizards and snakes, such markings could serve either a disruptive or aposematic function (Jackson & Pounds, 1980; Madsen, 1987). Although frequently ignored, bias in sampling the sizes, stages and colour morphs of reptiles is therefore probably a widespread phenomenon and inherent in many survey programmes.

Although replica models have been previously used in ecological studies focusing on vulnerability to predation and aposematism (e.g. Madsen, 1987; Bittner, 2003; Mitrovich & Cotroneo, 2006; Posa et al., 2007; Saporito et al., 2007; Bateman et al., 2016; Rößler et al., 2018), the use of species-specific models to investigate detectability remains understudied. In Honduras,

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Albergoni et al. (2016) examined the effectiveness of volunteers visually surveying for model herpetofauna, including snakes, and found that detectability was improved by experience and working in larger groups. However, the models were both conspicuously coloured and generic in body form over any species-specific characteristics, thus enhancing the likelihood of being detected.

This study examines the detectability of plasticine models of barred grass snakes *Natrix helvetica*, by volunteer surveyors of varying experience. The plasticine models for this study reflect the natural colouration of grass snakes making the challenges involved with observing them more realistic. This ensured that surveying effort would reflect a real-world scenario and consequently strengthen analyses when considering experience level. The study aims were therefore twofold. Firstly, we set out to determine the effects of size, body posture and colour markings (the yellow/black collar) on the detectability of grass snake models. Secondly, we compared the detectability of snake models between inexperienced and experienced observers. Collectively, the study aimed to shed light on potential survey limitations and improve the design of schemes utilising citizen scientists and interpretation of the data collected therein.

METHODS

Preparation of snake models

Snake models were made from non-toxic, pre-coloured modelling plasticine (Newplast®) using the colour 'ginger' for the heads and bodies, and 'yellow' for the distinctive collar and eyes. Eight different snake model types were created reflecting differences in size (large or small), posture (coiled or uncoiled) and colour pattern (with or without a yellow collar) (Fig. 1).

Large snake models were each made using 312.5 g of Newplast® and measured 96 cm while small snake models each comprised 125 g of Newplast® and measured 48 cm. Heating blocks of Newplast® in a preheated kitchen oven at 50° C for approximately 2 minutes made the material more pliable and easier to mould into shape.

Yellow collars and eyes were added after the main snake model structure had been made. Flank patterning and neck stripes around the yellow collars were replicated using a small paintbrush (Master Art "Premier" size 3) and black exterior masonry paint (B&Q Black Smooth Masonry Paint 50 ml tester pot). The dark colouring around the yellow iris of the eye and the circular pupil were drawn on using black, indelible pen (Sharpie Ultra Fine Tip permanent marker). The dorsal and ventral surfaces were left unmarked. When the paint had dried, the models were then packed in layers on greaseproof paper and put into boxes for transporting.

Experimental site

The study took place at an established reptile surveying site in Kent managed by the Forestry Commission. The site lies on a south-east facing chalk slope at the western edge of Kings Wood, an ancient mixed woodland system covering some 588 ha. The vegetation comprises rough calcareous grassland, bramble, bracken and scattered silver birch. Since 2014, the site has been surveyed several times a year using a standardised directed transect 350 m in length combined with 20–40 corrugated iron Artificial Cover Objects ('ACO tins': 50 x 50 cm). These surveys have revealed the presence of four reptile species *Anguis fragilis, Zootoca vivipara, Vipera berus* and *Natrix helvetica*.

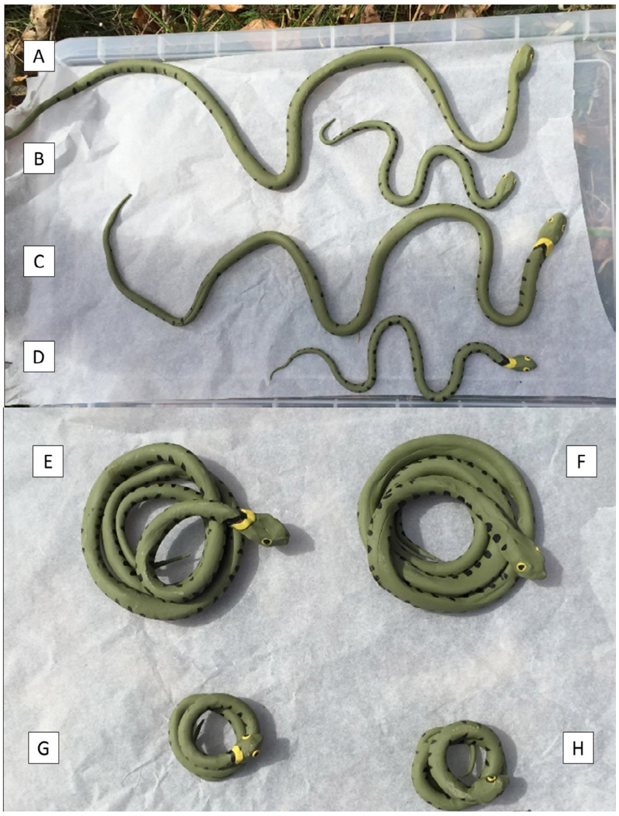

Figure 1. The eight different types of plasticine models used in the test. **A)** large uncoiled, no collar; **B)** small, uncoiled, no collar; **C)** large uncoiled, collared; **D)** small, uncoiled, collared; **E)** large, coiled, collared; **F)** large, coiled, no collar; **G)** small coiled, collared; **H)** small, coiled, no collar.

Model placement

The design aimed to compare the detectability of the eight different types of snake models by three groups of surveyors (two groups of inexperienced students and one expert surveyor). On the day before the first group of students were due to survey, snake models were placed non-randomly in likely reptile habitat identified by the authors based on their previous experience within 5 m of the transect route but >1.5 m from ACOs and at least 3 m from another model. Likely habitat was identified as an area on the edge of thick undergrowth and natural cover and avoided locations that would be too exposed or unusual such as the middle of a path or on a tree branch. A unique number from 1 to 104 was allocated to each snake model and written in indelible pen on the ventral surface to prevent repeat observations by the same group. The order in which the different types of snake models would be placed was randomised by inputting each model code 13 times into Excel and using the [=RAND()] function. Snake model locations were logged by GPS (eTrex30) to facilitate retrieval when the experiment had concluded.

Transect survey

The study utilised two inexperienced student groups (Group A (n=9) and Group B (n=10)) undertaking fieldwork over two days. No students in either group stated they had any previous experience of surveying for reptiles. An experienced observer with over three years of reptile survey experience also participated on day two, surveying the transect alone and recording observations of models independently from the inexperienced student groups. Group A participated on day one (8 April 2016) and Group B on day two (11 April 2016). Both inexperienced groups were accompanied by experienced reptile surveyors (Group A by three surveyors and Group B by two). The surveyors did not participate in the study but were present to help guide the student groups around the transect and to record the observations they made.

Prior to walking the transect, the groups were shown an example of a snake model and informed that they should try and detect as many as they could whilst on the walk. They were not told how many snake models were present at the site. To ensure snake models were not disturbed between trials, observers were asked to leave models *in-situ*. When an observation was made, one of the authors identified the snake model using its unique number.

A specific time limit to walk the transect was not allocated, but Group A and Group B took roughly an hour and a half to complete the transect while the experienced observer took two hours. Groups walked the same predetermined transect late morning / early afternoon in similar weather conditions (dry, hazy sunshine, no wind) and worked independently of each other.

Data analysis

Data analyses were performed in R version 4.1.0. A generalised linear mixed model (glmm function with a binomial family distribution) was used to explore the dependence of snake detectability (detected vs undetected) on fixed predictor variables: group (A, B and expert), snake size (large vs small), snake coiling (coiled vs uncoiled), and snake collar (with vs without yellow collar). As the locations of the snake models did not change over the course of the study and detectability may depend on location, this was included as a random factor in the models. Twelve models were constructed including different combinations of these predictors and their interactions in each (supplementary material), using the experienced observer as the baseline for 'group'. Model ranking was then carried out to determine the best-fitting models using AICc, and all models that fell within 2 ΔAICc units of the top-ranking model examined further (Burnham & Anderson, 2002).The influence of the random factor (i.e. location) was assessed by (1) comparing models with and without the random factor included using chi-squared (Field et al., 2014); and (2) calculating *marginal* R^2 that accounts for fixed effects only and *conditional* R^2 that accounts for both fixed and random effects (Nakagawa & Schielzeth, 2012).

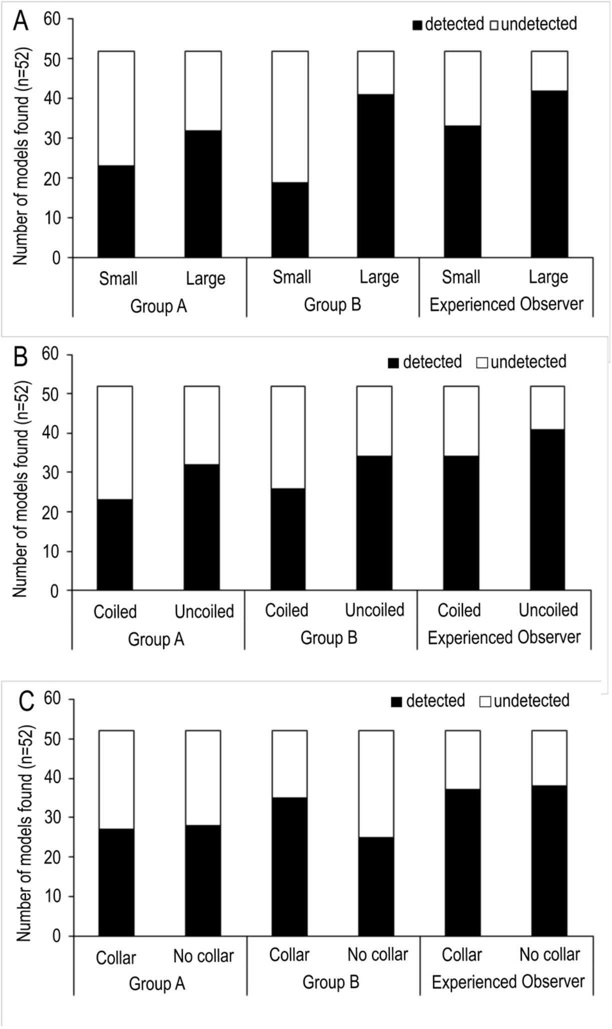

Figure 2. The relative numbers of different types of models detected/undetected by the three groups of observers. **A)** small versus large models; **B)** coiled versus uncoiled models; **C)** collared versus uncollared models.

RESULTS

All three groups detected more large snakes than small snakes (Fig. 2A). However, the single experienced observer detected more snakes (n=75; 72 % of the total present) than both Groups A (n=55; 53 % of the total) and B (n=60; 58 % of the total). Ten snakes - nine of which were small - were not detected by any group. The only large snake that remained undetected by any group was large, coiled and with a yellow collar. All three groups observed more uncoiled snakes than coiled snakes, but the presence of a yellow collar did not appear to influence detection.

Table 1. Summary of model ranking using AICc. The top four models all fell within <2 ΔAIC units of the best model, and all contained significant effects of group (a single experienced observer detected more snakes than both inexperienced groups) and snake size (more large snakes detected than small snakes). For 'Groups', the inexperienced groups were compared to the expert. Underlined variables are those that are significant within each model; R^2 values demonstrate the contributions of fixed factors only and fixed + random factor (i.e. including location) effects to the models. See supplementary material for full model outputs.

Model	Model no.	AICc	Δ AICc	weight	Log likelihood	df	*marginal* R^2 (fixed effects only)	*Conditional* R^2 (fixed + random effects)	Interpretation of significant variables
Group, Size, Group x Size	12	385.1	0	0.281	-185.351	7	0.156	0.398	expert>Group A, Group B
Group, Size, Coiling	5	385.7	0.66	0.202	-186.727	6	0.156	0.366	expert>Group A, Group B; large>small
Group, Size, Coiling, Collar, Group x Size	7	385.8	0.77	0.192	-183.620	9	0.175	0.396	expert>Group A, Group B
Group, Size	8	386.5	1.39	0.139	-188.130	5	0.141	0.366	expert>Group A, Group B; large>small
Group, Size, Coiling, Collar	6	387.1	2.07	0.100	-186.388	7	0.159	0.365	expert>Group A, Group B; large>small
Group, Size, Coiling, Size x Coiling	9	387.6	2.52	0.080	-186.609	7	0.156	0.366	expert>Group A, Group B; large>small
Size	1	394.1	9.00	0.003	-193.996	3	0.095	0.293	large>small
Size, Coiling, Size x Coiling	10	395.1	10.07	0.002	-192.474	5	0.110	0.293	large>small
Group	4	400.5	15.41	0.000	-196.175	4	0.044	0.367	expert>Group A, Group B
Group, Coiling, Group x Coiling	11	402.4	17.32	0.000	-194.008	7	0.070	0.381	none

Comparison of null (intercept only) model with random effects (location) model:
Null deviance = 417.58 df =311
Random effect deviance = 404.2 df = 310
Chi-squared = 13.38, df=1, P<0.001

The top four GLM models that were fitted all lay within <2 ΔAIC units of the best fitting model and had a cumulative weighting of 0.814 (Table 1). Indeed, the top eight models all included 'group' and 'size of the snake' as explanatory variables for detecting snakes. There was also some support for 'coiling' as an explanatory variable (Fig. 2B, Table 1), but generally little support for the presence of a 'collar' influencing detection (Fig. 2C, Table 1). Examination of the z-tests confirmed strong support for the single experienced observer detecting more snakes than the inexperienced groups, and for larger snakes being more detectable than smaller snakes. However, neither coiling nor the presence of a collar were statistically significant, and interaction terms were generally unimportant (Table 1; supplementary material). In all cases, models that included location as a random factor showed improved fits over models without the factor (all chi-squared tests P<0.05), and this was also reflected in higher *marginal* R^2 values for models including location as a random factor (Table 1). The location of the snake models within the study site therefore had a strong influence on detectability.

DISCUSSION

Sampling method and size and posture bias

Larger snakes were clearly easier to detect than smaller snakes by both the experienced observer and the inexperienced groups. Such a size bias has implications for population monitoring and sampling surveys for snakes in the field. This is especially true for smaller, cryptic species and for snakes of earlier life stages (Halliday & Blouin-Demers, 2015; Gregory & Tuttle, 2016; Willson, 2016). Previous research on grass snakes indicates that adults are more likely to be found in the open and immature snakes under refugia (Reading, 1997; Gregory & Tuttle, 2016). This underpins the importance of selecting a sampling method that (1) accounts for the behaviour of the study species, and (2) uses techniques that minimise size bias as far as possible. Confining the survey protocol to a simple visual encounter survey (VES) for a species such as the grass snake, for example, would likely incur a size bias that could potentially confound any analyses of population size or structure.

Albergoni et al. (2016) also found that volunteers conducting a visual survey for herpetofauna in Honduras observed more large models than small models. Our findings build on this by demonstrating that, in combination with other predictors, coiling had a limited effect on detectability. A limitation of the study is that uncoiled snakes are typically mobile rather than stationary, and coiled grass snakes will usually uncoil and seek cover if disturbed. Nevertheless, coiling may assist crypsis in the field, and in grass snakes the black bars along the flanks provide disruptive colouration that reduce detectability by visually guided surveyors or predators.

There was no evidence that the presence of a yellow collar bordered by black markings influenced detectability. The yellow collar is particularly intense in younger snakes, and Madsen (1987) believed that neonate grass snakes were particularly conspicuous during his surveys because of the colour of the collar. Indeed, he found that neonate plasticine models received more predatory bird pecks than melanistic models without a collar and hypothesised that the yellow and black marking may be aposematic colouration mimicking the unpalatable insects that birds avoid. This advantage may decline with age, and larger snakes often have less conspicuous collars (Madsen, 1987). Grass snakes sometimes coil up with the head and collar hidden (pers. obs.) and the collar may be most visible when the snake is moving. A study on ground squirrel attacks on rattlesnakes found attacks focused more around the head in smaller snakes than larger ones (Motrovich & Cotroneo, 2006). It is plausible that the yellow collar in grass snakes - particularly the intense coloration exhibited in juveniles - not only serves to distract predators by mimicking unpalatability (Madsen, 1987), but also serves to break up body outline as the snake flees, diverting an attack to a less vulnerable part of the body (Jackson & Pounds, 1980).

Detectability and the use of volunteers

The reliability of data generated by volunteer citizen science schemes varies widely and depends on species, species rarity, available technology, and the study area (Dickinsen et al., 2010; Bonney et al., 2014; Steger et al., 2017). The ability of volunteers to adhere to sampling protocols, complete different tasks, and collect and record high quality data can determine the success or the failure of a conservation project (Albergoni et al., 2016). As the recruitment of volunteers into biodiversity monitoring schemes continues to increase so do issues concerning the reliability of volunteer-derived data (Lewandowski & Specht, 2014). For example, occupancy modelling seeks to account for imperfect detection while estimating the probability that a target species is present (or absent) from a sample of study areas (e.g. MacKenzie et al., 2002; Sewell et al., 2012; O'Donnell & Semlitsch, 2015; Ward et al., 2017). However, this type of modelling requires repeated surveys recording presence / absence data at each study site. Different observers have different identification skills and differing approaches to search effort (Freilich & LaRue Jr., 1998; Lewandowski & Specht, 2014; Albergoni et al., 2016; Wittman et al., 2019) but inter-observer variation - in particular variation between experienced and inexperienced observers - remains relatively understudied (Fitzpatrick et al., 2009). In some cases, volunteer bias can be beneficial. Snall et al. (2011) suggest that volunteer-led opportunistic survey schemes focused on rare species yield comparatively more data than systematic schemes with strict protocols. Moreover, developing methods that enable researchers to better engage with volunteers will produce better quality data.

Volunteer characteristics can influence accurate data collection remarkably. Physical fitness, education background, visual acuity and hearing, previous biological surveying experience, and commitment and willingness to undertake tasks are all elements that can bias data collection (Newman et al., 2003; Mazerolle et al., 2007). Moreover, volunteer group size should be tailored to the survey work required, as detectability may either decrease or increase depending on the size of the group. This is most likely due to participants becoming distracted or suffering from survey fatigue (Albergoni et al., 2016). Visual encounter surveys of reptiles require concentration and appropriate fieldcraft. Our work with student groups that have been provided with the relevant search images of target species but which otherwise lack experience has shown that levels of concentration can rapidly decrease as the survey progresses, or after the target species has been observed a few times (pers. obs). Likewise, although Pierce and Gutzwiller (2004) found that a 15-minute survey of anuran calls yielded more detections than surveys conducted over five or ten minutes, longer survey times showed a pattern of decreasing detection efficiency. In the case of volunteers, excessive survey duration may decrease volunteer willingness to visit other sites during the same survey period. It may also detrimentally impact volunteer retention and result in increases in variation between surveys conducted by different volunteers in different years (Pierce & Gutzwiller, 2004).

Dim light or very bright light can affect visual acuity (Rojas et al., 2014), and inclement weather may not only adversely affect visibility but also participant motivation to complete the study (Albergoni et al., 2016; Mazerolle et al., 2007). Moreover, the height above ground at which observers are focusing on during surveys can influence detectability of the target species. For example, the study conducted by Albergoni et al. (2016) showed that volunteers recorded more model sightings at middle-level (43 %) with little difference between ground level models (29 %) and top-level models (28 %). Our data also showed that the location of the snake models - which was the same for all three groups - had a clear effect on detectability: ten snake models were not found by any of the groups. Variation in both the topography of the site and the microhabitats used by the target species are inherent factors that will influence detectability of both model snakes and live snakes, and the design of directed transect surveys need to take into account these factors.

In real-world surveys, the goal may be to assess presence-absence, abundance or population size, and statistical tools are available to account for imperfect detection using all of these approaches (Griffiths et al., 2015). Moreover, such tools can also be used to incorporate covariates of detectability, such as surveyor expertise, weather conditions and habitat. However, robust survey design is needed to ensure that the quantity of data is sufficient to allow reliable estimation of such parameters: the more information to be extracted from a survey the more data that will be needed to build the appropriate model.

Conclusion

Phenotypically accurate models such as the plasticine snakes used in this study are a useful tool for researchers to gain a better understanding of detectability biases, volunteer skills, and the accuracy of data reported by observers. This is important for two reasons. Firstly, the dependency on volunteer data drawn from biological surveys has increased dramatically in recent years. This could be due to online engagement through 'citizen science' monitoring programmes and the ease by which data can be uploaded to monitoring platforms (Schmeller et al., 2009). Secondly, volunteer data are often excluded from final analyses due to the concern that it is fundamentally flawed (Lewandowski & Specht, 2014). Depending on the sampling methodology employed, researchers can use models to test for detectability bias in advance. This can help inform survey design, training needs and the composition of survey teams, and ensure detectability biases are considered. By targeting sampling methods to the skill-sets of participants, researchers can obtain sound results without significant variation between skill levels (Freilich & LaRue Jr., 1998; Newman et al., 2003; Oldekop et al., 2011).

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