ISSN 2634-1379

The Herpetological Journal

Volume 32, Number 4
October 2022

Published by the British Herpetological Society

British Herpetological Society

The Herpetological Journal is published quarterly by the British Herpetological Society and is issued free to members. Articles are listed in Current Awareness in Biological Sciences, Current Contents, Science Citation Index and Zoological Record. Applications to purchase copies and/or for details of membership should be made to the Hon. Secretary, British Herpetological Society, The Zoological Society of London, Regent's Park, London, NW1 4RY, UK. Instructions to authors are printed inside the back cover. All contributions should be addressed to the Scientific Editor.

Front cover: Puff adder *Bitis arietan*, see article on page 176. (© 4028mdk09 CC BY-SA 3.0)

Herpetological Journal

Volume 32, Number 4, 2022

Contents

Full papers

Volume 32, Number 3 was published on 1 July 2022

Herpetological Journal

FULL PAPER

https://doi.org/10.33256/32.4.176182

Published by the British Herpetological Society

Body size comparisons between wild and captive puff adders *Bitis arietans*

Daniel Kane & Christopher J. Michaels

Zoological Society of London, London Zoo, Regent's Park, London, UK, NW1 4RY

Captivity can provide a resource-rich environment for snakes which may lead to over-conditioning of individuals. We compared captive and wild populations of *Bitis arietans* to see if a difference in scaled mass index (SMI) existed between the samples. Male *B. arietans* had significantly higher SMI in captivity than in the wild; there was no difference between wild and captive females. A sex-related difference in SMI between wild male and female *B. arietans*, evident in the wild, was not found in captivity. These results suggest that the captive management of *B.arietans* may require review to prevent over-conditioning of male snakes, and that this may be informed by further comparative research on wild and captive populations of this species.

Keywords: Body condition, captivity, scaled mass index, Viperinae

INTRODUCTION

Captivity can provide a resource-rich environment. However, it is often combined with behavioural restrictions, generally in the form of a reduction or inability to perform such energetically costly behaviours such as intense physical activity or reproduction (Estevez & Christman, 2006). Therefore, an excess of adipose tissue is a common problem associated with captivity across many vertebrate taxa (Frye, 1984; Wadsworth et al., 1984; Bradley & Wright, 2000; Goodchild & Schwitzer, 2008; Vester et al., 2008; Rawski & Józefiak, 2014; Stoskopf, 2017). This condition is associated with negative pathological conditions (Salas & Manteca, 2016; Caravaggi et al., 2018), a reduced life expectancy (Clubb et al., 2008) and may reduce welfare in animals throughout their lives (D'Eath et al., 2009). Clearly this is a state which is best avoided. A commonly used method of assessing whether an animal is at an ideal body condition is through quantitative assessment and comparison with wild conspecifics, and a better understanding of body condition in captive animals may guide the way for improvements in welfare.

Body condition in snakes has been investigated, however published data seem only available for studies under natural conditions as opposed to in a captive environment (Naulleau & Bonnet, 1996; Madsen & Shine, 2000; Aubret et al., 2002; Waye & Mason, 2008). The most appropriate way of calculating body condition is by using a scaled mass index (SMI) which takes into account the allometric nature of morphological features of an animal - in other words, there is not a simple linear relationship between the increase of length and the increase in mass (Falk et al., 2017). Typically, there is a sigmoid (cubic) relationship between linear measurement and mass in animals; that is, larger animals are proportionately heavier per unit of length than are smaller individuals. Therefore, larger individuals show a corresponding greater increase in mass for a given increase in length. The methodology of Peig & Green (2009) is deemed a suitable method for this reason. The same process of calculating body condition has been applied to introduced *Boiga irregularis* on the island of Guam (Waye & Mason, 2008) and *Acrochordus arafurae* in Australia (Madsen & Shine, 2000) to assess temporal variation of body condition.

Venomous reptiles are frequently kept in zoological and private collections all over the world and large, heavy-bodied vipers are among the more commonly exhibited venomous snakes. Multiple factors, including their generally manageable size as well as the intricate body patterns and attractive colours of many species influence these animals' popularity. Species360, an organisation providing members with data collection and management software known as the Zoological Information Management System (ZIMS), lists 4676 individual snakes in the family Viperidae currently held in zoos and aquariums around the world (accessed 7/1/22), with 1288 individuals contained in the subfamily Viperinae and 336 in the genus *Bitis*. Puff adders, *Bitis arietans*, comprise 96 of those individuals, making this one of the most abundant species of viper among Species360 members. In addition to this, an unknown number of these snakes are also maintained in non-registered zoological institutions and private collections around the world.

Published literature suggest that in a typical captive setting some reptiles have the potential to grow to body conditions not often reached under wild circumstances (Beck, 2005; Mader, 2006). To further compound this, without recognised assessments for body condition in different reptilian taxa, there is often no clear-cut way to categorise physical states such as emaciation or obesity. Body condition scoring represents a standardised way for individuals using visual assessment to determine the body fat reserves of an animal (Vieira et al., 2015). To date only a small number of validated body condition scoring systems for reptiles exist (Deming et al., 2008; Thomson et al., 2009; Lamberski, 2013; Rawski & Józefiak, 2014; Falk et al., 2017). The many recognised diseases related to excess adipose tissue are likely to affect reptiles in the same way as demonstrated in other animals (German, 2006). Therefore, for individuals working directly with reptiles in ex-situ populations, having such information readily available is key to being able to provide the best welfare.

Correspondence: Daniel Kane (daniel.kane@zsl.org)

Herein, *B. arietans* are examined to explore the difference in SMI between wild and captive specimens. We view the most informative method of estimating the optimal body mass in relation to body length of captive *B. arietans* to be using data from wild populations of these animals, where a large enough dataset can be used to generate an 'ideal' value. This supposed ideal wild-population SMI can then be compared to ex-situ populations of *B. arietans*, with the null hypothesis that snakes in captivity will not differ in SMI from wild snakes. If this null hypothesis is rejected, and captive animals have a higher SMI, by lowering the SMI of the ex-situ population, welfare of these individuals may be increased. It is hoped that methods herein will allow an assessment of ideal SMI for this species to be quantified which may lead to a better understanding of fitness of the captive population as measured against the wild population.

METHODS

Figure 1. *Bitis arietans* from - **A)** Nigeria, **B)** Kenya, **C)** Tanzania and **D)** eSwatini. Each snake was photographed at the Liverpool School of Tropical Medicine by Edouard Crittenden. Black line illustrates measurements taken via ImageJ.

Total length and mass measurements of *Bitis arietans* were collected from published literature (Pitman, 1938; Rosi, 1988; Bonnet et al., 2001), institutional and personal correspondence (Liverpool School of Tropical Medicine [LTSM]; Ganger, E) field observations (Glaudas, X), and ZIMS (accessed 31/12/21). Wild snake length was typically measured directly while the snake was anesthetised (necessary for a separate telemetry study, for which ethical approval was granted), whereas in captivity snake length was calculated by photographing each snake next to a scale and digitally measuring total length via imageJ (Schindelin et al., 2012). A mean average of three lengths per captive snake was used to increase accuracy and precision (Fig. 1). Captive snakes were weighed using a digital balance (Adam Equipment CPWplus 15; Hendi 580233) and wild snakes with a portable electronic balance accurate to ± 1 g. All mass/length combinations were collected from individual snakes within one month of one another and without a feeding or defecation event in between to ensure that they were relevant to one another. Almost all measurements were taken simultaneously. Data for other *Bitis* spp. were unavailable in appropriate numbers for analysis. Snakes were allocated as male or female, captive or wild. Data from snakes of unknown sex were excluded. Snakes less than 540 mm in total length were regarded as juveniles and data from these animals were also excluded. This measure was achieved using the smallest size recorded for a male snake showing courtship or reproductive behaviour (Glaudas et al., 2020b), minus 10 % range buffer to account for potential variance.

Scaled Mass Indices (SMIs; M_i) were calculated according to the methodology of Peig & Green (2009), using the formula:

$$\hat{M}_i = M_i \left[\frac{L_0}{L_i}\right] b_{SMA}$$

where M_i and L_i are the body mass and the linear body measurement of individual irespectively; b_{SMA} is the scaling exponent estimated by the standardised major axis regression (SMA) of M on L; L_0 is an arbitrary value of L (in this case the arithmetic mean of the study population); and $\hat{M}_i$ is the predicted body mass for individual i when the linear body measurement is standardised to L_0. We calculated bSMA using the method of dividing the slope from the standard ordinary least squares regression of M on L (bOLS) by the Pearson's correlation coefficient *r*.

We used Shapiro-Wilk tests to check data for normality and found that this assumption was not met; non-parametric analyses were therefore used. Data were log-transformed to ensure homoskedastcity, confirmed via Bartlett's test. Data were analysed using RStudio (Version 1.4.1717) with an alpha of 0.05. We used the stats package (R Core Team, 2021) to perform Kruskall Wallis tests to investigate the effects of snake sex, source (wild or captive) and the interaction between these factors on SMI, and to use Mann-Whitney U tests to perform post-hoc comparisons, with Bonferroni corrections (adjusted alpha = 0.0083).

RESULTS

Masses and lengths of 84 wild (n male = 41, n female = 43) *Bitis arietans*, from Gauteng Province, South Africa, and 23 captive (n male = 17, n female = 12) *B. arietans*, from seven captive collections in Europe and the USA, were used for analysis (S1). Captive *B. arietans* with bloodlines originating from six countries were identified, and all but one individual were in captivity >12 months, with one female in captivity for nine months (S1). Data in the 'captive female' sample was comprised of 67 % snakes (n = 8) which did not reproduce in the same year in which weight was measured, 25 % (n = 3) snakes measured immediately prior to mixing male and female for breeding, following which each female produced a litter, and 8 % (n = 1) post-partum snakes. There was no information on reproductive status of wild snakes.

Median SMI was 597.4 g, 799.9 g, 784.5 g and 890.1 g for wild male, wild female, captive male and captive female snakes, respectively. There was a significant effect of source (captive or wild) (X^2_1 = 8.25, p = 0.004; captive higher than wild) and sex (X^2_1 = 23.06, p < 0.0001; females higher than males) of animals, and the interaction between the two variables (X^2_1 = 35.05, p < 0.0001) on SMI. Post hoc comparisons (Table 1) showed that SMI of wild and captive females, and of captive males, did not differ significantly, but all three were significantly higher than wild males (Fig. 2).

Table 1. P values derived from pairwise post hoc comparisons between scaled mass indices of wild and captive male and female *Bitis arietans*. An adjusted alpha of 0.0083 was used. Bold cells indicate a significant p value.

	Wild/ male	Wild/ female	Captive/ male	Captive/ female
Wild/male	x	x	x	x
Wild/female	**< 0.001**	x	x	x
Captive/male	**< 0.001**	0.9124	x	x
Captive/female	**< 0.001**	0.84148	0.77182	x

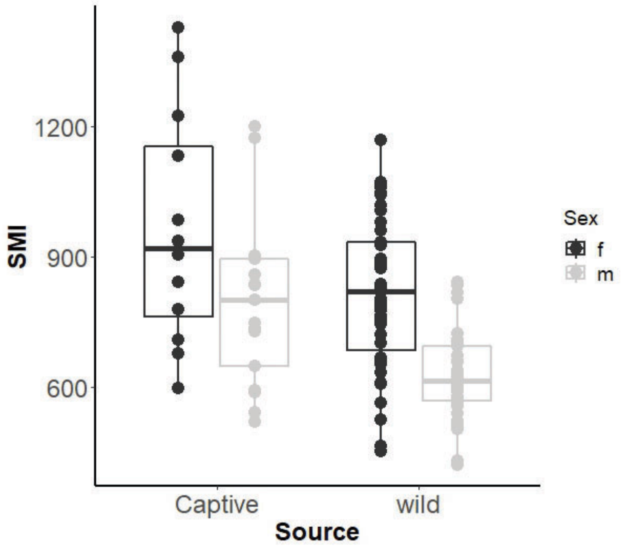

Figure 2. Median scaled mass index of 113 sampled *Bitis arietans* by source (captive or wild) and sex. Raw data are displayed for ease of interpretation; log-transformed data were analysed.

DISCUSSION

The results of this research show evidence for higher SMI in captive male *Bitis arietans* than in wild conspecifics. By using the SMI developed by Peig & Green (2009) we accounted for both the allometric relationship between mass and length data (Falk et al., 2017) and also for differences in size distributions between populations, by relating all data to the arbitrary length value T_0. The SMI has also been validated against some snakes (Peig & Green, 2009) and found to outperform other mass indices in terms of correlation to tissue composition. In *B. arietans*, the effects of source and sex on SMI were due to the interaction between variables; wild male snakes were less well conditioned than wild females and both sexes in captivity. The SMI of these three groups did not differ from one another.

In the field, *Bitis* ssp. growth and body condition has been demonstrated to be linked to food availability. Growth rates differ between populations of *B. caudalis* from arid and intermediate habitat zones, with snakes from areas with greater food abundance growing more quickly and appearing 'less thin' (Shine et al., 1998). Furthermore, supplementary feeding of wild *B. arietans* in South Africa showed changes in movement patterns when compared to control snakes, however home range remained similar (Glaudas & Alexander, 2017). Food intake was shown to directly affect allometric factors including growth rate, proportionate fang length and head width in captive juvenile *B. gabonica* (Bonnet et al., 2001). Data were not available for these features in our data set so they could not be compared. Food intake manipulation studies have revealed that growth rates can be substantially higher than those found under non-manipulated circumstances (Grubant et al., 1972), suggesting that growth in reptiles may be food-limited under certain natural conditions. Indeed, seasonal differences in food availability have been correlated with body condition of wild snakes (Madsen & Shine, 2000). This information should help inform husbandry aims and bring SMI of captive male *B. arietans* more in line with that of wild males.

In captivity, food provision may have a similar effect. Nutritional analysis of wild *B. arietans* prey is unknown, as for most snake species, however Dierenfeld et al. (2015) present a detailed nutritional analysis of the diet of wild *Drymarchon couperi*. Although this genus is phylogenetically distant from *Bitis*, the type of prey item consumed is broadly similar, both feeding on small mammals, amphibians and to a lesser extent birds and reptiles (Dierenfeld et al., 2015; Glaudas et al., 2017). Individual prey items in the diet of wild *D. couperi* contain 2-17 % crude fat. Crude fat percentage of lab-raised rats and mice, which are the staple food of many captive snakes including *Bitis* spp., average 20–30% (Dierenfeld et al., 2002). To potentially increase the effect of an excessively calorific diet in relation to a species' behaviour, an ambush predator such as *B. arietans* (Al-Sadoon, 1991) may be expected to require few calories to find prey. Captive diets for *B. arietans* are likely substantially higher in fat than wild diets. Glaudas & Alexander (2017) demonstrated that supplementary-fed wild *B. arietans* improved their body condition and decreased travel distance compared to control snakes, which may further intensify the increase in SMI for captive snakes fed a typical high-calorie diet of commercially raised mice and rats.

As well as higher fat content in captive diets, overall food quantity and feeding frequency may be higher. On average, *B. arietans* in South Africa feed once every 10 days through the active season. It is estimated that an adult male will feed on 12–15 occasions and consume 384–480 g, or 48–60 % of average body mass (Glaudas & Alexander, 2017). In captivity, adult *B. arietans* are often fed around once every two to three weeks (Rosi, 1988; pers. obs., D. Kane) which may resemble typical wild feed frequency. However, even being fed a modest sized commercially raised rodent of 80 g for nine months of the year (a typical 'active season' for a male *B. arietans*) would result in a food intake of 1440 g, or at least three times more than would be expected for a wild snake. This difference in prey mass intake can be explained by the fact that wild *B. arietans* will feed on relatively small prey, potentially of a size which may not be offered to captive snakes. Seasonality in feeding of wild *B. arietans* from more temperate latitudes is expected as this species is known to undergo bouts of torpor under unfavourable climatic conditions, and male snakes consume less food during the breeding season than at other times of year (Phelps, 2010). When viewed holistically, the factors outlined here lend support to the notion that the more food-rich the environment, the shorter distance a snake will move while foraging. The thermal environment, and likely also the space and complexity of the captive environment available to a typical squamate, dictates to a large extent the behaviour of the animal (Thomas et al., 2019). Therefore, when unnatural diet type is combined with artificially controlled environmental parameters the risk of serious obesity and other disease (Frye, 1984; Simpson, 2006) developing in captive snakes may be further increased. To counteract this effect in captivity it is recommended to provide food items most similar in size, frequency, and nutritional composition to those included in the diet of wild snakes.

The effect of increased dietary intake may be exacerbated by reduced opportunity to expend calories. A measure of space requirement can be inferred from daily movement patterns. Free-ranging, reproductively active, male *B. arietans* move 80 m day^{-1} during the breeding season, whereas non-breeding males and females generally move <10 m day^{-1} (Alexander & Maritz, 2015). Furthermore, the motivation, ability, or need, for a captive viper to exhibit movement patterns comparable to snakes in the wild may be reduced, again intensifying the effects of a diet excessively rich in calories.

Our findings point to a resource-rich environment in captivity generating male snakes with elevated body condition. Male snakes are likely less well conditioned than females in the wild due to seasonal resource expenditure in finding and courting females (Daltry et al., 1998; Glaudas & Alexander, 2017). Female snakes may also have an overall high threshold level of energy reserves to compensate for reproductive expense (Duvall et al., 1992). In captivity, however, this effect disappears such that sexes have similar body conditions. The trend for male *B. arietans* to have a higher SMI in captivity than in the wild is likely due to an increased intake of food in terms of quantity and calorific value, and less need to expend energy on active behaviours including foraging, mate-searching and other behaviours related to reproduction, when compared to wild males. This is a non-negligible difference, with captive male snakes having on average an SMI nearly 33 % larger than wild conspecifics.

Snakes allocate a significant proportion of stored reserves to meet metabolic costs associated with reproduction (Santos & Llorente, 2004). It is likely that any single breeding event may lower the female body condition below a reproductive threshold, with a corresponding decrease in SMI. Female *B. arietans* from wild and captive populations do not differ significantly in median SMI (799.9 g vs. 890.1 g). Wild female *B. arietans* are thought to reproduce infrequently on account of their large litter sizes and females are not equally fecund due to differing body conditions (Maritz & Alexander, 2013; Glaudas et al., 2020a). Our captive sample contains female snakes in a range of reproductive statuses, whereas the reproductive state of wild females is not known. Our sample size increases the validity of our results by reducing the likelihood of small sample size intensifying natural skew of snakes being allocated to either 'reproductive' or 'non-reproductive' body conditions and thus influencing results. Compared to male *B. arietans*, female *B. arietans* are relatively sedentary in the wild (Alexander & Maritz, 2015) so there may be less of an energy expenditure difference between wild and captive females compared to between wild and captive males. This may account for the difference in SMI between these four populations. It is unclear why wild and captive females do not significantly differ in SMI; the very high p value from the post-hoc comparisons suggests that the lack of difference is unlikely to be an artefact of sample size. It is possible that differences in behaviour (seasonally or otherwise) allow them to secure more prey items in the field, or create a smaller difference in energy expenditure between field and captive conditions. A factor we could not control for with our sample size was the origin of the snakes. Data from all wild snakes were from Gauteng province, South Africa, while none of the captive snakes were known to originate from this country. The possibility exists that cryptic lineages are hidden within '*B. arietans*' (Barlow et al., 2013) and future work may recognise multiple species. While wild and captive snakes originating from a broader geographic range would have provided the opportunity to test for locality or institution-specific differences in SMI, this was outside the scope of this work.

It is important to acknowledge limitations to BCIs based on morphometrics. However, as the differences in SMI between wild and captive populations of male *B. arietans* were significant, this is likely to have a real impact on the fitness of the captive population. Numerous pathologies associated with excess adipose tissue are reported from various taxa (Clubb et al., 2008; D'Eath et al., 2009; Salas & Manteca, 2016; Caravaggi et al., 2018). Clearly, unnatural levels of adipose tissue, likely linked to a high SMI, is a condition to be avoided should captive snake welfare be maximised. Snakes may be difficult to assess for body condition, however several studies have shown temporal variation in BCI (Madsen & Shine, 2000; Waye & Mason, 2008) albeit purely in wild populations. The reproductive state of snakes in this study was not considered as this information was not available for the majority of specimens; it is likely that female snakes in particular may show an elevated SMI prior to and during a reproductive season whereas male SMI decreases from the beginning to the end of the breeding season (Glaudas et al., 2020a). This highlights the need for long term data collection on body condition, preferably over several active seasons, from which reliable results can be inferred (as in Madsen & Shine, 2000). In the present work, we suggest that proportions of captive animals in different reproductive states in our data set do not differ significantly from those expected in a natural population; while this does not allow us to quantify the effects of reproductive state, it allows us to discount them in our comparisons.

Therefore, we suggest that a validated scoring system for body condition of *B. arietans*, based on visual appearance in conjunction with SMI calculations, may better account for the differing structural composition of an animal (Santos & Llorente, 2004; Jayson et al., 2018) and might be useful in informing the captive management of this species. Data on health impacts of high SMI in *B. arietans* are not available, therefore we suggest that future research should focus on health issues linked to body condition in this and other snake species.

In conclusion, the data presented in this study suggest that, on average, wild male *B. arietans* have a significantly lower SMI than do their captive counterparts whereas female snakes show no difference. Taking the median SMI of wild male snakes as ideal for this sex, captive male snakes appear to be over-conditioned. Applying the same comparison to female snakes suggests the body conditions of the two populations are the same. Given the negative pathologies associated with excess adipose tissue, linked to calorific imbalance and a higher SMI, the SMI of wild snakes is a condition which should be aimed for in a typical captive environment. The natural ambush-foraging behaviour of these snakes, and ability to ingest large meals, may predispose male snakes to maintaining an unnaturally high SMI, more so than other species with a higher metabolic rate (Al-Sadoon, 1991; Secor & Diamond, 1998). However, more detailed analysis is outside the scope of this work. The importance and benefits of maintaining captive vipers at an ideal wildtype SMI is, however, clear to be understood.

ACKNOWLEDGEMENTS

We are grateful to Xavier Glaudas who generously shared data on wild *B. arietans* with us. This wild data enabled the conclusions drawn from the statistical analyses to be far more robust than would otherwise be possible. Thanks are also due to Edouard Crittenden at the Liverpool School of Tropical Medicine and to Einzel Ganger for providing data on captive puff adders. We are thankful to Edmund Flach for sharing his many years of data on autopsied snakes, both wild and captive, at ZSL London Zoo. This provided valuable insights into snake physiology and the amount of variance between species and individuals. Finally, thanks are due to Benjamin Tapley for helpful comments on an earlier version of this manuscript and to the herpetology and veterinary team at ZSL London Zoo for ongoing husbandry of animals and assistance with data collection.

REFERENCES

Alexander, G.J. & Maritz, B. (2015). Sampling interval affects the estimation of movement parameters in four species of African snakes. *Journal of Zoology*, 297, 309–318.

Aubret, F., Bonnet, X., Shine, R. & Lourdais, O. (2002). Fat is sexy for females but not males: the influence of body reserves on reproduction in snakes (*Vipera aspis*). *Hormones and Behavior*, 42, 135–147.

Al-Sadoon, M.K. (1991). Metabolic rate-temperature curves of the horned viper, *Cerastes cerastes gasperetti*, the moila snake, *Malpolon moilensis*, and the adder, *Vipera berus*. *Comparative Biochemistry and Physiology Part A: Physiology*, 99, 119–122.

Barlow, A., Baker, K., Hendry, C.R., Peppin, L., Phelps, T., Tolley, K.A., ... & Wüster, W. (2013). Phylogeography of the widespread African puff adder (*Bitis arietans*) reveals multiple Pleistocene refugia in southern Africa. *Molecular Ecology*, 22, 1134–1157.

Beck, D.D. (2005). *Biology of Gila monsters and beaded lizards* (Vol. 9). Oakland: USA.

Bonnet, X., Shine, R., Naulleau, G. & Thiburce, C. (2001). Plastic vipers: influence of food intake on the size and shape of gaboon vipers (*Bitis gabonica*). *Journal of Zoology*, 255, 341–351.

Bradley, T.A. & Wright, K. (2000). Captive care and breeding of White's tree frog, *Pelodryas caerulea*. *Journal of Herpetological Medicine and Surgery*, 10, 21–25.

Caravaggi, A., Plowman, A., Wright, D.J. & Bishop, C.M. (2018). The composition of captive ruffed lemur (*Varecia* spp.) diets in UK zoological collections, with reference to the problems of obesity and iron storage disease. *Journal of Zoo and Aquarium Research*, 6, 41–49.

Clubb, R., Rowcliffe, M., Lee, P., Mar, K.U., Moss, C. & Mason, G.J. (2008). Compromised survivorship in zoo elephants. *Science*, 322, 1649.

Daltry, J.C., Wüster, W. & Thorpe, R.S. (1998). Intraspecific variation in the feeding ecology of the crotaline snake *Calloselasma rhodostoma* in Southeast Asia. *Journal of Herpetology*, 32, 198–205.

D'Eath, R.B., Tolkamp, B.J., Kyriazakis, I. & Lawrence, A.B. (2009). 'Freedom from hunger' and preventing obesity: the animal welfare implications of reducing food quantity or quality. *Animal Behaviour*, 77, 275–288.

Deming, C., Greiner, E. & Uhl, E.W. (2008). Prevalence of cryptosporidium infection and characteristics of oocyst shedding in a breeding colony of leopard geckos (*Eublepharis macularius*). *Journal of Zoo and Wildlife Medicine*, 39, 600–607.

Dierenfeld, E.S., Alcorn, H.L. & Jacobsen, K.L. (2002). *Nutrient composition of whole vertebrate prey (excluding fish) fed in zoos*. Beltsville (MD): US Department of Agriculture, Agricultural Research Service, National Agricultural Library, Animal Welfare Information Center.

Dierenfeld, E.S., Norton, T.M., Hyslop, N.L. & Stevenson, D.J. (2015). Nutrient composition of prey items consumed by free-ranging *Drymarchon couperi* (Eastern indigo snakes). *Southeastern Naturalist*, 14, 551–560.

Duvall, D., Arnold, S.J. & Schuett, G.W. (1992). Pitviper mating systems: ecological potential, sexual selection and microevolution. In: *Biology of the Pitvipers*, Campbell, J.A. & Brodie, E.D. (Eds.). Tyler: USA: 321–336.

Estevez, I. & Christman, M.C. (2006). Analysis of the movement and use of space of animals in confinement: The effect of sampling effort. *Applied Animal Behaviour Science*, 97, 221–240.

Falk, B.G., Snow, R.W. & Reed, R.N. (2017). A validation of 11 body-condition indices in a giant snake species that exhibits positive allometry. *PloSOne*, 12, e0180791.

Frye, F.L. (1984). Nutritional disorders in reptiles. In: *Diseases of Amphibians and Reptiles*, Hoff, G.L., Frye, F.L. & Jacobson, E.R. (Eds.). New York: USA. 794 pp.

German, A.J. (2006). The growing problem of obesity in dogs and cats. *The Journal of Nutrition*, 136, 1940–1946.

Glaudas, X. & Alexander, G.J. (2017). Food supplementation affects the foraging ecology of a low-energy, ambush-foraging snake. *Behavioural Ecology and Sociobiology*, 71, 5.

Glaudas, X., Kearney, T.C. & Alexander, G.J. (2017). Museum specimens bias measures of snake diet: a case study using the ambush-foraging puff adder (*Bitis arietans*). *Herpetologica*, 73, 121–128.

Glaudas, X., Rice, S.E., Clark, R.W. & Alexander, G.J. (2020a). Male energy reserves, mate-searching activities, and reproductive success: alternative resource use strategies in a presumed capital breeder. *Oecologia*, 194, 415–425.

Glaudas, X., Rice, S.E., Clark, R.W. & Alexander, G.J. (2020b). The intensity of sexual selection, body size and reproductive success in a mating system with male–male combat: is bigger better? *Oikos*, 129, 998–1011.

Goodchild, S. & Schwitzer, C. (2008). The problem of obesity in captive lemurs. *International Zoo News*, 55, 353–357.

Grubant, V.N., Rudaeva, A.V. & Vedmederya, V.I. (1972). Rearing young common adders in captivity. *The Soviet Journal of Ecology*, 3, 457–458.

Jayson, S., Harding, L., Michaels, C.J., Tapley, B., Hedley, J., Goetz, M., ... & Flach, E. (2018). Development of a body condition score for the mountain chicken frog (*Leptodactylus fallax*). *Zoo Biology*, 37, 196–205.

Lamberski, N. (2013). Body condition scores for desert tortoises. Retrieved from: http://www.avtcp.org/uploads/3/4/4/2/34426410/desert-tortoise-bcs-2013-lamberski-po.pdf.

Mader, R. (2006). *Reptile Medicine and Surgery, 2nd ed*. St. Louis: USA. 251–294.

Madsen, T. & Shine, R. (2000). Rain, fish and snakes: climatically driven population dynamics of Arafura filesnakes in tropical Australia. *Oecologia*, 124, 208–215.

Maritz, B. & Alexander, G.J. (2013). Namaqua dwarf adders must breed frequently: the problem with being small. *Journal of Zoology*, 289, 27–31.

Naulleau, G. & Bonnet, X. (1996). Body condition threshold for breeding in a viviparous snake. *Oecologia*, 107, 301–306.

Peig, J. & Green, A.J. (2009). New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos*, 118, 1883–1891.

Phelps, T. (2010). *Old World Vipers: a natural history of the azemiopinae and viperinae*. Frankfurt, Germany. 558.

Pitman, C.R.S. (1938). *A Guide to the Snakes of Uganda*. Kampala: Uganda. 362.

R Core Team (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Accessed from: URL https://www.R-project.org/

Rawski, M. & Józefiak, D. (2014). Body condition scoring and obesity in captive African side-neck turtles (*Pelomedusidae*). *Annals of Animal Science*, 14, 573–584.

Rosi, R. (1988): The breeding in captivity of the puff adder, *Bitis arietans* (Merrem, 1820). *Litteratura Serpentium*, 8, 123–133.

Salas, M. & Manteca, X. (2016). Assessing welfare in zoo animals: animal-based indicators. *Zoo Animal Welfare Education Centre*, 4, 2016.

Santos, X. & Llorente, G.A. (2004). Lipid dynamics in the viperine snake, *Natrix maura*, from the Ebro Delta (NE Spain). *Oikos*, 105, 132–140.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., ... & Tinevez, J.Y. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9, 676–682.

Secor, S.M. & Diamond, J. (1998). A vertebrate model of extreme physiological regulation. *Nature*, 395, 659–662.

Shine, R., Branch, W.R., Harlow, P.S. & Webb, J.K. (1998). Reproductive biology and food habits of horned adders, *Bitis caudalis* (Viperidae), from southern Africa. *Copeia*, 1998, 319–401.

Simpson, M. (2006). Hepatic lipidosis in a black-headed python (*Aspidites melanocephalus*). *Veterinary Clinics: Exotic Animal Practice*, 9, 589–598.

Species360 Zoological Information Management System (ZIMS) (2022). Accessed from: https://zims.species360.org/Login.aspx?ReturnUrl=%2fMain.aspx.

Stoskopf, M.K. (2017). Shark Health Management. In: *The Elasmobranch Husbandry Manual II. Recent Advances in the Care of Sharks, Rays and their Relatives*, Smith, M., Warmolts, D., Thoney, D., Hueter, R., Murray, M. & Ezcurra, J. (Eds.). Columbus: USA. 481.

Thomas, O., Kane, D. & Michaels, C.J. (2019). Effects of different heat sources on the behaviour of blue tree monitors (*Varanus macraei*) in captivity. *Herpetological Bulletin*, 149, 41–43.

Thomson, J.A., Burkholder, D., Heithaus, M.R. & Dill, L.M. (2009). Validation of a rapid visual-assessment technique for categorizing the body condition of green turtles (*Chelonia mydas*) in the field. *Copeia*, 2009, 251–255.

Vester, B.M., Burke, S.L., Dikeman, C.L., Simmons, L.G. & Swanson, K.S. (2008). Nutrient digestibility and fecal characteristics are different among captive exotic felids fed a beef-based raw diet. *Zoo Biology*, 27, 126–136.

Vieira, A., Brandão, S., Monteiro, A., Ajuda, I. & Stilwell, G. (2015). Development and validation of a visual body condition scoring system for dairy goats with picture-based training. *Journal of Dairy Science*, 98, 6597–6608.

Wadsworth, P.F., Jones, D.M. & Pugsley, S.L. (1984). Fatty liver in birds at the zoological society of London. *Avian Pathology*, 13, 231–239.

Waye, H.L. & Mason, R.T. (2008). A combination of body condition measurements is more informative than conventional condition indices: temporal variation in body condition and corticosterone in brown tree snakes (*Boiga irregularis*). *General and Comparative Endocrinology*, 155, 607–612.

Accepted: 20 August 2022

Please note that the Supplementary Material for this article is available online via the Herpetological Journal website: https://thebhs.org/publications/the-herpetological-journal/volume-32-number-4-october-2022

Herpetological Journal

FULL PAPER

https://doi.org/10.33256/32.4.183189

Published by the British Herpetological Society

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

Mikaella M.G. Lock & Richard A. Griffiths

Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, University of Kent, Marlowe Building, Canterbury, Kent, CT2 7NR, UK

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,

Correspondence: Richard Griffiths (r.a.griffiths@kent.ac.uk)

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*.

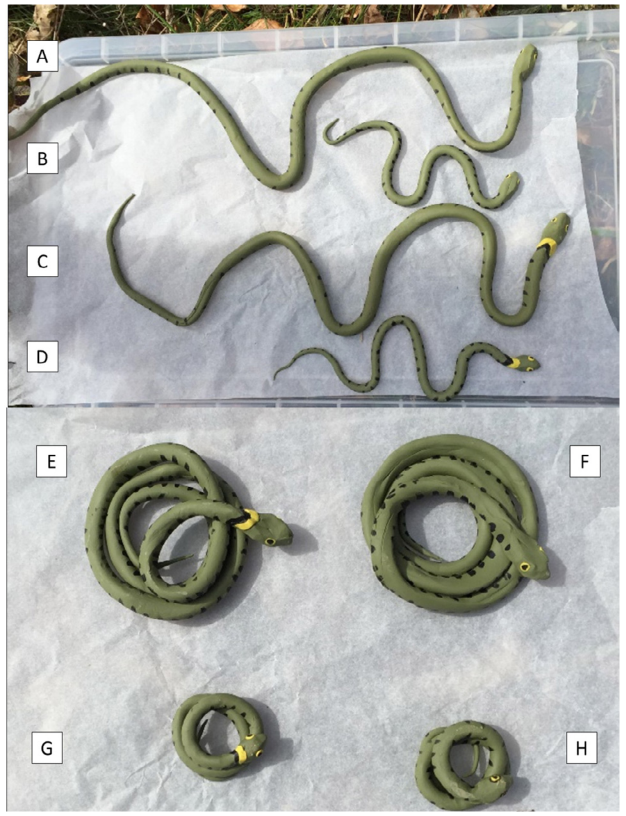

Figure 1. The eight different types of plasticine models used in the test. **A)** large uncoiled, no collar; **B)** small, uncoiled, no collar; **C)** large uncoiled, collared; **D)** small, uncoiled, collared; **E)** large, coiled, collared; **F)** large, coiled, no collar; **G)** small coiled, collared;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

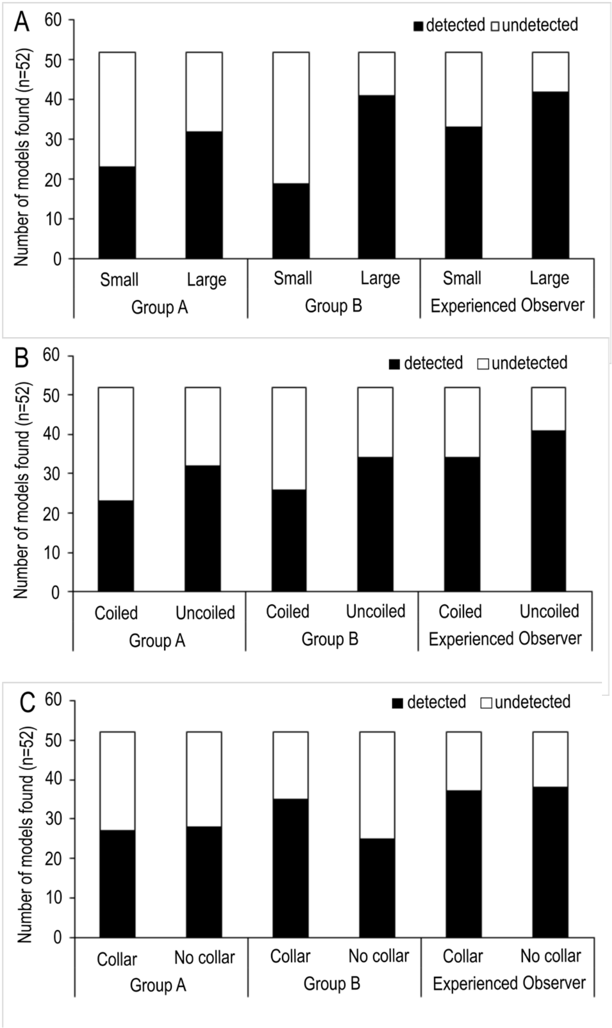

Figure 2. The relative numbers of different types of models detected/undetected by the three groups of observers. **A)** small versus large models; **B)** coiled versus uncoiled models; **C)** collared versus uncollared models.

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).

ACKNOWLEDGEMENTS

We are grateful to the BSc students who participated in this study; Rob Ward for acting as the experienced observer; and Forestry England for permission to work at the site. We thank David Gower, Dave Roberts, Rob Ward and Steve Allain for helpful discussions. The project was approved by the Research Ethics Committee, School of Anthropology and Conservation, University of Kent.

REFERENCES

Albergoni, A., Bride, I., Scialfa, Jocque, M. & Green, S. (2016). How useful are volunteers for visual biodiversity surveys? An evaluation of skill level and group size during a conservation expedition. *Biodiversity Conservation* 25, 133–149.

Bateman, P.W., Fleming, P.A. & Wolfe, A.K. (2016). A different kind of ecological modelling: the use of clay model organisms to explore predator-prey interactions in vertebrates. *Journal of Zoology* 301, 251–262.

Bird, T.J., Bates, A.E., Lefcheck, J.S., Hill, N.A., Thomson, R.J., Edgar, G.J., Stuart-Smith, R.D., Wotherspoon, S. et al. (2014). Statistical solutions for error and bias in global citizen science datasets. *Biological Conservation* 173, 144–154.

Bittner, T.D. (2003). Polymorphic clay models of *Thamnophis sirtalis* suggest patterns of avian predation. *Ohio Journal of Science* 103, 62–66.

Bonney, R., Shirk, J., Phillips, T., Wiggins, A., Ballard, H., Miller-Rushing, A. & Parrish, J. (2014). Next steps for citizen science. *Science* 343, 1436–1437.

Burnham, K.P. & Anderson, D.R. (2002). *Model Selection and Multimodel Inference: A Practical Information Theoretic Approach*. Second Edition, New York: Springer-Verlag.

Dickinson, J.L., Zuckerberg, B. & Bonter, D.N. (2010). Citizen science as an ecological research tool: challenges and benefits. *Annual Review of Ecology, Evolution, and Systematics* 41, 149–172.

Field, A., Miles, J. & Field, Z. (2014). *Discovering Statistics Using R*. London, Sage Publications.

Fitzpatrick, M.C., Preisser, E.L., Ellison, A.M. & Elkinton, J.S. (2009). Observer bias and the detection of low-density populations. *Ecological Applications* 19, 1673–1679.

Freilich, J.E. & LaRue Jr, E.L. (1998). Importance of observer experience in finding desert tortoises. *Journal of Wildlife Management* 62, 590–596.

Gregory, P.T. & Tuttle, K.N. (2016). Effects of body size and reproductive state on cover use of five species of temperate-zone natricine snakes. *Herpetologica* 72, 64–72.

Griffiths, R.A., Foster, J., Wilkinson, J.W. & Sewell, D. (2015). Science, statistics and surveys: a herpetological perspective. *Journal of Applied Ecology* 52, 1413–1417.

Guillera-Arroita, G., Lahoz-Monfort, J.J., van Rooyen, A.R., Weeks, A.R. & Tingley, R. (2017). Dealing with false positive and false negative errors about species occurrence at multiple levels. *Methods in Ecology and Evolution* 8, 1081–1091.

Halliday, W. & Blouin-Demers, G. (2015). Efficacy of coverboards for sampling small northern snakes. *Herpetology Notes* 8, 309–314.

Jackson, J.F. & Pounds, J.A. (1980). Of differential head-body pigmentation in snakes. *Journal of Herpetology* 14, 307–311.

Lewandowski, E. & Specht, H. (2014). Influence of volunteer and project characteristics on data quality of biological surveys. *Conservation Biology* 29, 713–723.

MacKenzie, D.I., Nichols, J.D., Lachman, G.B., Droege, S., Royle, J.A. & Langtimm, C.A. (2002) Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83, 2248–2255.

Madsen, T. (1987). Are juvenile grass snakes, *Natrix*, aposematically coloured? *Oikos* 48, 265–267.

Mazerolle, M.J., Bailey, L.L., Kendall, W.L., Royle, A J., Converse, S.J. & Nichols, J.D. (2007). Making great leaps forward: accounting for detectability in herpetological field studies. *Journal of Herpetology* 41, 672–689.

Mitrovich, M.J. & Cotroneo, R.A. (2006). Use of plasticine replica snakes to elicit antipredator behavior in the California ground squirrel (*Spermophilus beecheyi*). *The Southwestern Naturalist* 51, 263–267.

Nakagawa, S. & Schielzeth, H. (2012). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution* 4, 133–142.

Newman, C., Buesching, C.D. & Macdonald, D.W. (2003). Validating mammal monitoring methods and assessing the performance of volunteers in wildlife conservation – "Sed quis custodiet ipsos custodies?" *Biological Conservation* 113, 189–197.

O'Donnell, K.M. & Semlitsch, R.D. (2015). Advancing terrestrial salamander population ecology: the central role of imperfect detection. *Journal of Herpetology* 49, 533–540.

Oldekop, J.A., Bebbington, A.J., Berdel, F., Truelove, N.K., Wiersberg, T. & Preziosi, R.F. (2011). Testing the accuracy of non-experts in biodiversity monitoring exercises using fern species richness in the Ecuadorian Amazon. *Biodiversity Conservation* 20, 2615–2626.

Pierce, B.A. & Gutzwiller, K.J. (2004). Auditory sampling of frogs: detection efficiency in relation to survey duration. *Journal of Herpetology* 38, 495–500.

Posa, M.R.C., Sodhi, N.S. & Koh, L.P. (2007). Predation on artificial nests and caterpillar models across a disturbance gradient in Subie Bay, Philippines. *Journal of Tropical Ecology* 23, 27–33.

Reading, C.J. (1997). A proposed standard method for surveying reptiles in dry lowland heath. *Journal of Applied Ecology* 34, 1057–1069.

Rößler, D.C., Pröhl, H. & Lötters, S. (2018). The future of clay model studies. *BMC Zoology* 3. https://doi.org/10.1186/s40850-018-0033-6.

Rodda, G.H., Adams., A.A.Y., Campbell, E. & Fritts, T.H. (2015). General herpetological collecting is size-biased for five Pacific lizards. *Journal of Herpetology* 49, 507–512.

Rodda, G.H., Savidge, J.A., Tyrrell, C.L., Christy, M.T. & Ellingson, A.R. (2007). Size bias in visual searches and trapping of brown treesnakes on Guam. *Journal of Wildlife Management* 71, 656–661.

Rojas, B., Rautiala, P. & Mappes, J. (2014). Differential detectability of polymorphic warning signals under varying light environments. *Behavioural Processes* 109, 164–172.

Saporito, R.A., Zuercher, R., Roberts, M., Gerow, K.G. & Donnelly, M.A. (2007). Experimental evidence for aposematism in the dendrobatid poison frog *Oophaga pumilio*. *Copeia* 2007, 1006–1011.

Schmeller, D.S., Henry, P-Y., Julliard, R., Gruber, B., Clobert, J., Dziock, F., Lengyel, S., Nowicki, P. et al. (2009). Advantages of volunteer-based biodiversity monitoring in Europe. *Conservation Biology* 23, 307–316.

Sewell D., Guillera-Arroita, G., Griffiths, R.A. & Beebee T.J.C. (2012). When is a species declining? Optimizing survey effort to detect population changes in reptiles. *PLoS ONE* 7(8), e43387. doi:10.1371/journal.pone.0043387.

Snall, T., Kindvall, O., Nilsson, J. & Part, T. (2011). Evaluating citizen-based presence data for bird monitoring. *Biological Conservation* 144, 804–810.

Steger, C., Butt, B. & Hooten, M.B. (2017). Safari science: assess the reliability of citizen science data for wildlife surveys. *Journal of Applied Ecology* 54, 2053–2062.

Ward, R.J., Griffiths, R.A., Wilkinson, J.W. & Cornish, N. (2017). Optimising monitoring efforts for secretive snakes: a comparison of occupancy and N-mixture models for assessment of population status. *Scientific Reports* 7, 18074. doi:10.1038/s41598-017-18343-5.

Willson, J.D. (2016). In *Reptile Ecology and Conservation, A Handbook of Techniques*. 1st Ed. Edited by C. Kenneth Dodd, Jr. Oxford University Press: Oxford.

Wittmann, J., Girman, D. & Crocker, D. (2019). Using iNaturalist in a coverboard protocol to measure data quality: suggestions for project design. *Citizen Science: Theory and Practice* 4 21, 1–12.

Accepted: 8 September 2022

Please note that the Supplementary Material for this article is available online via the Herpetological Journal website: https://thebhs.org/publications/the-herpetological-journal/volume-32-number-4-october-2022

Herpetological Journal

FULL PAPER

https://doi.org/10.33256/32.4.190197

Published by the British Herpetological Society

Diet composition and tropic niche overlap of *Ameivula ocellifera* Spix 1825 (Squamata: Teiidae) and *Tropidurus cocorobensis* Rodrigues 1987 (Squamata: Tropiduridae), sympatric species with different foraging modes, in Caatinga

Patricia Marques do A. Oliveira[1,2], Carlos A. Navas[3] and Pedro M. Sales Nunes[1,2]

[1]Centro de Biociências, Departamento de Zoologia, Universidade Federal de Pernambuco, 50670-901 Recife, Pernambuco, Brazil
[2]Programa de Pós-Graduação em Biologia Animal, Centro de Biociências, Universidade Federal de Pernambuco, 50670-901, Recife, Pernambuco, Brazil
[3]Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, São Paulo/SP, Brazil

Lizard diets can be influenced by several factors, such as age, physiological aspects, food availability, behaviour and foraging mode. The latter can be an important predictor of the type of prey consumed. This study analysed *Ameivula ocellifera* and *Tropidurus cocorobensis* diets, both of which are psammophiles and coexist in an area of Caatinga in north-eastern Brazil, but use different foraging modes. Lizard stomachs were examined, and prey categories were quantified by frequency of occurrence, number, volume and relative importance index. We used PERMANOVA and SIMPER analyses to understand the dissimilarities among diets. Additionally, we estimated the degree of trophic niche overlap between species using the Pianka index. The most frequently consumed food item by *A. ocellifera* was Isoptera and Formicidae (Hymenoptera) for *T. cocorobensis*. The trophic niche overlap between the species was approximately 0.24 and, although there were many consumed prey categories in common, the proportion at which these prey categories were consumed was quite divergent. For example, the consumption of plant material, which was present in the diet of both species, was much more important for *T. cocorobensis* compared to the active forager *A. ocellifera*. Our results indicate that despite sharing the same space and consuming the same prey types, these species have significant differences in their diets. Furthermore, these divergences can be explained by several factors in the environment and even by the evolutionary history of each species, which are included in different families and are not evolutionarily close to each other.

Keywords: diet, niche overlap, foraging mode, plant consumption, competition

INTRODUCTION

The diet composition of lizard species is an important source of information on trophic interactions between these reptiles, other animals, plants, and the environment in general (Duffield & Bull, 1998). Additionally, dietary composition is influenced by the ecological characteristics of species, such as foraging mode, which can affect energy expenditure, morphology, life history traits, and prey selection (Huey & Pianka, 1981; Verwaijen & Van Damme, 2007). Huey & Pianka (1981) observed that small to medium-sized foraging lizards ingest abundant prey that have high rates of movement, e.g. ants, since lizards that exhibit this type of foraging strategy encounter this type of prey more frequently. In contrast, small to medium-sized active foraging lizards ingest proportionally more sedentary, conglomerate prey, such as insect larvae and termites, as well as large and often inaccessible prey for sit-and-wait lizards (e.g. scorpions).

Environmental factors may also influence the dietary patterns of lizards (Griffiths & Christian, 1996; Whitfield & Donnelly, 2006; Perez-Cembranos et al., 2016), especially in regions with defined climate seasonality. This is the case of the Brazilian Caatinga, an ecoregion with high annual average temperatures (25–30 °C), particularly during the long dry season, and a rainfall regime which is restricted to three months of the year (annual average 773 mm) (De Andrade et al., 2018). This type of seasonality affects the trophic ecology of lizards, specifically, by reducing the diversity of ingested prey categories during the dry season, thereby generating physiological challenges which must be overcome (Vasconcellos et al., 2010; Sannolo & Carretero, 2019). Consequently, the trophic niches of lizard species in this environment are dynamic, both in relation to intraspecific niche breadth and niche overlap between species (Ribeiro & Freire, 2011; Ferreira et al., 2017).

The sharing of dietary resources among sympatric species is an important element when considering trophic niche (Huey & Pianka, 1977; Sutherland, 2011; Sousa et al., 2017), particularly when competition is involved. Hypothetically, niche overlap between two or more sympatric species should be limited in coexisting taxa (Pianka, 1974). For example, in desert regions, congeneric fossorial lizards tend to eat different sized prey when in sympatry, compared to when in allopatry, thus they appear to segregate trophic niches and consequently reduce competition (Pianka, 1973). According to this niche theory, sympatric species must differ in at least one of the niche dimensions (spatial, temporal or trophic) for their coexistence to be viable (Pianka, 1974).

In this study we explore the diet composition and trophic overlap between two heliophilous lizard species, *Ameivula ocellifera* Spix 1825, and *Tropidurus cocorobensis* Rodrigues 1987, that are often sympatric in the Brazilian Caatinga (Rodrigues, 1996; Menezes et al., 2011; Pedrosa et al., 2014), considering their distinct foraging strategies. *Ameivula ocellifera* is a small to medium-sized (males up to ~ 100 mm SVL and females up to ~ 80 mm SVL), active foraging teiid lizard (Menezes et al., 2006; Zanchi-Silva et al., 2014; Sales & Freire, 2015), whose diet is based on small arthropods, and commonly predates upon termites and larvae (Sales & Freire, 2015; Ferreira et al., 2017). *Tropidurus cocorobensis* is a small-sized iguanian lizard (males up to ~ 73 mm SVL and females up to ~ 62 mm SVL), which uses a sit-and-wait foraging strategy, and is restricted to sandy environments (Rodrigues, 1987; 2003). There is currently no published data on its diet.

Correspondence: Patricia Marques (patricia.marques@ufpe.br)

METHODS

Study Area

This study was carried out in the Catimbau National Park (hereafter PARNA Catimbau), a conservation unit inserted in the Caatinga ecoregion, between the geographical coordinates 08°24'' S and 08°36' S and 37°09' W and 37°14' W, in the state of Pernambuco, Brazil. The PARNA Catimbau comprises approximately 62,000 ha, with an altitude varying between 700 m and 1000 m a.s.l (PROJETO RADAMBRASIL, 1983), and located in a transition zone between the Brazilian mesoregions known as Agreste and Sertão. The climate in the region is considered hot semi-arid (BSh), according to the Köppen classification, with an average precipitation of 600 mm and an average annual temperature of 26 °C (Gomes et al., 2006). The PARNA Catimbau area is covered by outcrops of sand conglomerates and sandy soil (PROJETO RADAMBRASIL, 1983) and the vegetation is typical of the Caatinga, i.e. predominantly xeromorphic, where families such as Cactaceae, Euphorbiaceae, Mimosaceae and Fabaceae are the most common (Gomes et al., 2006). In general, the landscapes of the PARNA Catimbau are similar to other areas in the Caatinga. During the dry season (usually from March to May) the climate is warm and dry and it is possible to observe dry plants without their leaves, however during the rainy season (usually from September to January) the plants bloom fully, their leaves sprout and some flooded areas can be formed due to rainwater accumulation.

Data Collection

The *A. ocellifera* and *T. cocorobensis* specimens were collected during two expeditions to the PARNA Catimbau in November 2020 and January 2021, during the dry season (Fig. 1). Lizards were collected using pitfall traps (Cechin & Martins, 2000; Foster, 2012) and by lasso (“noosing” in Fitzgerald, 2012). There is no evidence of significant differences between the methods used for stomach content analyses of captured lizards (Costa et al., 2008). Soon after collection the subjects were euthanised using an overdose of liquid lidocaine, based on experimental procedures which were approved by the Committee of Ethics on Animal Use (CEUA-UFPE process 0004/2020). All collections were authorised by the Brazilian government environmental entities (permit SISBIO #73617). All collected specimens were deposited in the Herpetological Collection of the Federal University of Pernambuco (CHUFPE).

In the laboratory the stomach and intestines of each specimen were removed and the contents analysed under a stereomicroscope. The ingested prey categories were identified to the Order level and, specifically for Hymenoptera (Formicidae), at the Family level. Prey category measurements (maximum length and width) were taken using a digital caliper (precision of 0.1 mm).

Data Analysis

For each prey item category, we calculated the frequency of occurrence, and the number and volume (in mm^3) of prey per stomach. The volume was estimated using the ellipsoid formula, using the length (l) and width (w) of each prey item, according to Dunham (1983):

$$V = \frac{4}{3}\pi\left(\frac{w}{2}\right)^2\left(\frac{l}{2}\right)$$

Additionally, we calculated the relative importance index (R.I) (Howard et al., 1999) for each type of prey that was ingested. Wheren X represented the average number of times that item X was repeated within the stomachs; fX: the number of stomachs in which item X was found and vX: the average volume of food item X in mm³. N, F and V correspond, respectively, to the general sum of the number, frequency and volume of all grouped prey categories:

$$Ix = \frac{\left[\left(\frac{nX}{N}\right)+\left(\frac{vX}{V}\right)+\left(\frac{fX}{F}\right)\right]}{3}$$

We also calculated the food niche overlap (Ojk) between species according to Pianka’s (1974) index, considering the volumetric proportion of each prey category in the lizards’ diets. In the formula, "pij" corresponds to the proportion of prey categories in the diet of *A. ocellifera* and "pik", to the proportion of prey categories in the diet of *T. cocorobensis*:

$$Ojk = \frac{\sum pij * pik}{\sqrt{\sum pij^2 * pik^2}}$$

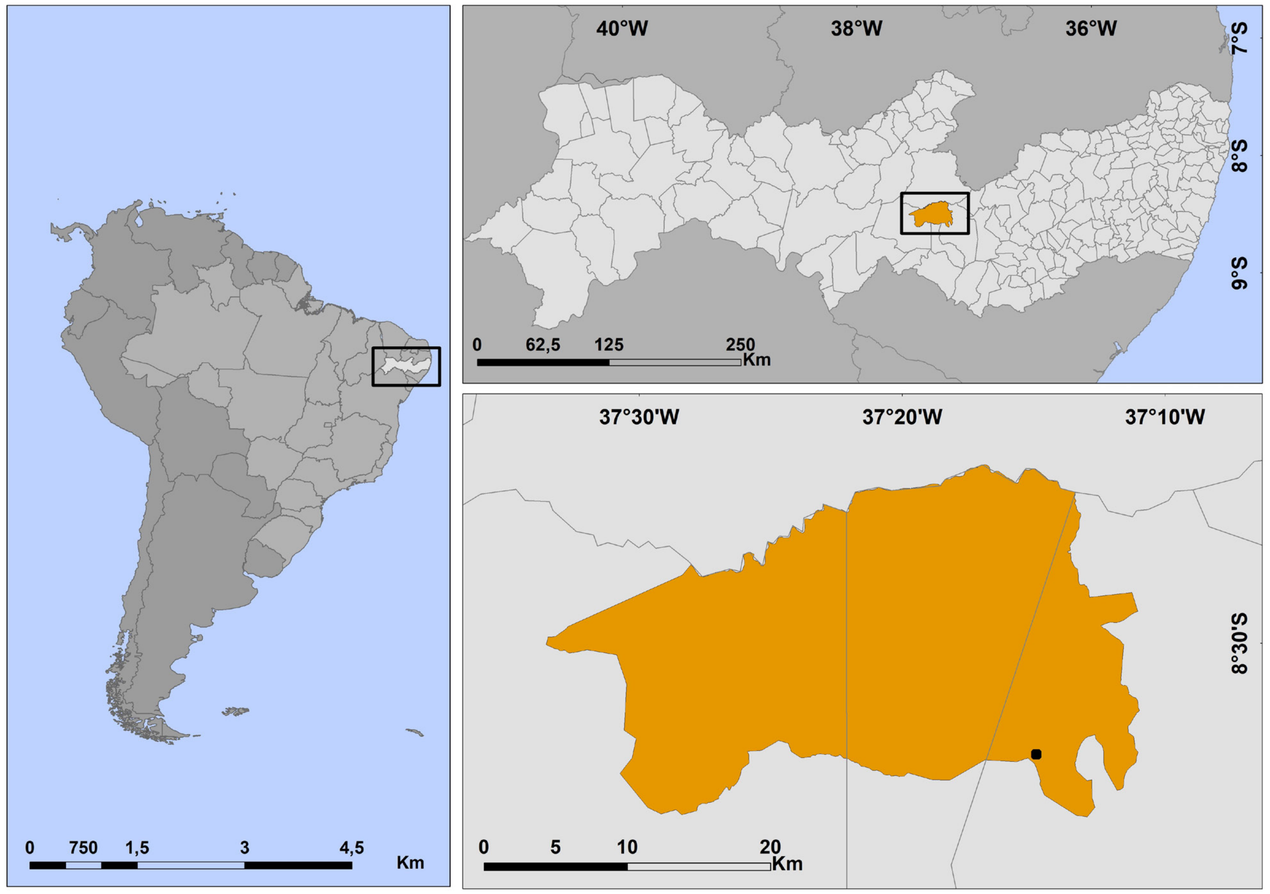

Figure 1. Map with the location of Catimbau National Park. The black dot represents the collection area.

To compare the diet compositions of the two different species, we performed a Permutational Multivariate Analysis of Variance (PERMANOVA), based on a similarity matrix of the number of individuals of each item per stomach, using the Bray-Curtis index (Anderson, 2014), using the 'vegan' package (Oksanen et al., 2019) in R 3.5.1 (R Core Team, 2022). In order to identify the cumulative contributions of the most influential prey types to the dissimilarity between the diets of each species, we used the Analysis of Similarity Percentages (SIMPER) also using the 'vegan' package (Oksanen et al., 2019) in R 3.5.1 (R Core Team, 2022).

RESULTS

In total, 31 *A. ocellifera* specimens (14 during the first and 17 during the second expedition) and 53 *T. cocorobensis* specimens (30 during the first and 23 during the second expedition) were collected, all of which were adults. The average size (SVL) was 66.43 mm for *A. ocellifera* and 71.32 mm for *T. cocorobensis*. All the lizards, of both species, had food in their stomachs. Interspecific comparisons demonstrated that the diets of *A. cellifera* and *T. cocorobensis* differed significantly, when taking into account the number (N) of cover type of prey, as indicated by the results of the PERMANOVA analysis (Factor Species~ Number (N): $p<0.001$). And, according to the SIMPER analysis, the prey types responsible for the main differences between the species' diets are Hymenoptera Formicidae (0.720) and Isoptera (0.408).

The *A. ocellifera* and *T. cocorobensis* specimens sampled in the PARNA Catimbau most frequently ingested insects (Table 1). No digestive tracts were found empty. In general, the number of prey categories ingested by the two species was very similar, where 17 different categories were ingested by *A. ocellifera* and 18 by *T. cocorobensis*. The most frequently identified prey in the diet of *A. ocellifera* was Isoptera (present in 24.66 % of stomachs) (Table 1), and Formicidae (Hymenoptera) (28.75 %) in the diet of *T. cocorobensis* (Table 1). Considering the volumetric proportion of food, the main item for *A. ocellifera* was Blattodea (6049.95 mm³) and for *T. cocorobensis* the main item was plant material (in total, 37033.5 mm³) (Table 1). Among the types of plant materials found, flowers occupy a greater volume in the diet composition of *T. cocorobensis* (23154.22 mm³) and in the stomach contents of *A. ocellifera*, leaves occupy a greater volume (2014.58 mm³).

Regarding the Relative Importance index, Isoptera was the most important item for *A. ocellifera*, where R.I=0.47 (Table 1, Fig. 2). The other prey categories ingested by the species were quite distant from this value, where the second and third most important prey categories presented values of 0.14 (Blattodea) and 0.07 (Hymenoptera) (Table 1, Fig. 2). In the *T. cocorobensis* diet, plant material had the highest R.I (in total, 0.38), followed by Formicidae (Hymenoptera) and Isoptera (0.28 and 0.16) respectively (Table 1, Fig. 2). The trophic niche overlap between both species was 0.2465 (24.65 %). In addition, here we observed the second record of saurophagy for the species *T. cocorobensis* (first: Oliveira & Nunes, 2020), in the present study the prey was a juvenile individual of *A. ocellifera*.

Table 1. Diet composition of *Ameivula ocellifera* and *Tropidurus cocorobensis* in Catimbau National Park, Pernambuco, Brazil. F represents the frequency of items in the diet, N represents the number, V the volume in mm³ and R.I the value of the relative importance.

	Ameivula ocellifera (N=31)				*Tropidurus cocorobensis* (N=53)			
Prey Types	F (%)	N (%)	V (mm³)	R.I	F (%)	N (%)	V (mm³)	R.I
Hexapoda								
Blattodea	2 (2.74)	4 (0.37)	6049.95	0.14	1 (0.65)	1 (0.06)	121.88	0.00
Coleoptera	3 (4.11)	5 (0.47)	294.73	0.02	4 (2.61)	9 (0.54)	523.34	0.01
Coleoptera (Larvae)	5 (6.85)	5 (0.47)	247.55	0.03	2 (1.31)	13 (0.79)	86.61	0.01
Diptera	-	-	-	-	3 (1.96)	3 (0.18)	41.03	0.01
Hemiptera	-	-	-	-	2 (1.31)	3 (0.18)	34.61	0.01
Homoptera	1 (1.37)	1 (0.09)	11.24	0.01	3 (1.96)	4 (0.24)	21.78	0.01
Hymenoptera								
Formicidae	11 (15.06)	33 (3.08)	301.75	0.07	44 (28.75)	826 (49.97)	2658.63	0.28
Formicidae (Larvae)	-	-	-	-	3 (1.96)	3 (0.18)	3.18	0.01
Non Formicidae	10 (13.69)	14 (1.14)	928.63	0.07	28 (18.30)	44 (2.66)	1905.32	0.08
Isoptera	18 (24.66)	957 (89.36)	4125.17	0.47	15 (9.80)	483 (29.22)	3870.43	0.16
Mantodea	-	-	-	-	2 (1.31)	2 (0.12)	324.86	0.01
Odonata	1 (1.37)	1 (0.09)	194.98	0.01	-	-	-	-
Orthoptera	3 (4.11)	5 (0.47)	1534.46	0.05	3 (1.96)	3 (0.18)	530.91	0.01
Phasmatodea	1 (1.37)	1 (0.09)	6.70	0.01	1 (0.65)	2 (0.12)	15.16	0.00
Unidentified Insect	3 (4.11)	3 (0.28)	775.38	0.03	-	-	-	-
Arachnida								
Araneae	4 (5.48)	4 (0.37)	533.41	0.03	3 (1.96)	3 (0.18)	2064.75	0.02
Pseudoscorpiones	2 (2.74)	2 (0.19)	2.16	0.01	1 (0.65)	1 (0.06)	1.32	0.00
Scorpiones	1 (1.37)	1 (0.09)	75.30	0.01	-	-	-	-
Chilopoda								
Scolopendromorpha	1 (1.37)	1 (0.09)	183.99	0.01	-	-	-	-
Mollusca								
Gastropoda	4 (5.48)	4 (0.37)	53.75	0.02	1 (0.65)	1 (0.06)	8.68	0.00
Vertebrate								
Squamata	-	-	-	-	1 (0.65)	1 (0.06)	363.92	0.00
Matter Plant								
Flowers	2 (2.74)	4 (0.37)	659.39	0.05	20 (13.07)	144 (8.71)	23154.22	0.23
Leaves	1 (1.37)	26 (2.43)	2014.58	0.02	16 (10.46)	121 (6.47)	13879.28	0.15
Total		**1071**	**17993.12**			**1653**	**49609.90**	

DISCUSSION

Ameivula ocellifera and *Tropidurus cocorobensis* ingest a wide variety of different insects, and a few other animal groups such as arachnids and gastropods, however, plant material appears to be the main item in the diet of *T. cocorobensis* (Table 1). Although these lizards are sympatric in the study area and their diets are composed of many of the same prey categories, the proportions of these prey categories are notably different, thus their diets are significantly distinct (Table 1). Isoptera was the most frequently ingested item by *A. ocellifera* specimens (24.66 %), followed distantly by Formicidae (15 %), whereas Formicidae (28.75 %) and plant material (23.53 %) were the most frequently consumed items in the *T. cocorobensis* diet, with similar frequency values. Such

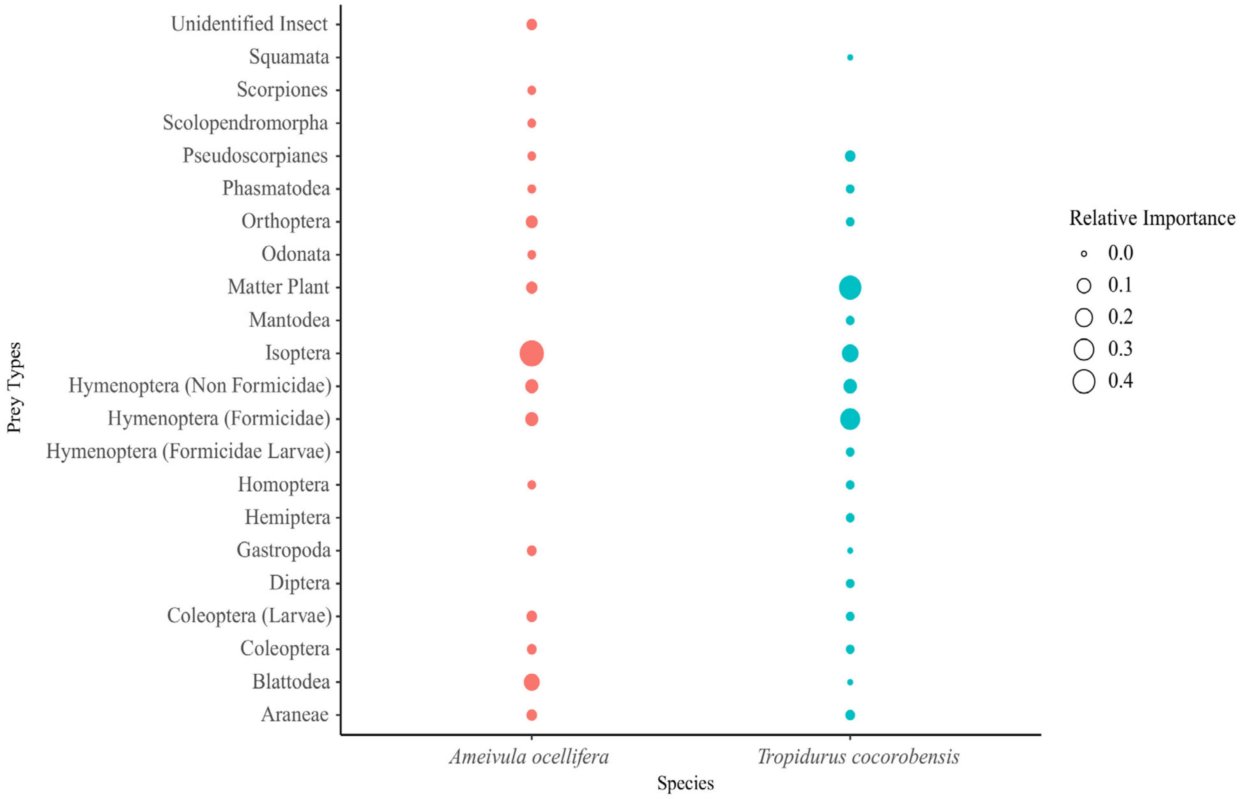

Figure 2. Graph representing the relative importance (R.I) of each item ingested by the species *Ameivula ocellifera* (first column) and *Tropidurus cocorobensis* (second column) collected at Catimbau National Park. The larger the symbol, the greater relative importance of prey.

differences in the most frequently consumed prey items of these lizards are in accordance with the observations of Huey & Pianka (1981).

If niche segregation is achieved, then competition should decrease, thus favoring the coexistence of different species (Pianka, 1973; 1974). In this study, *A. ocellifera* and *T. cocorobensis* seemed to share similar spatial (Pedrosa et al., 2014) and temporal niches, since both species are diurnal and heliophilous lizards (Rodrigues, 1987; Menezes et al., 2011). Differences regarding niche segregation are theoretically possible, but we lack information on important variables such as the time of day for peak of activity and the time of day at which foraging starts, among others, which are only known for *A. ocellifera* in other areas of north-eastern Brazil (Menezes et al., 2011; Albuquerque et al., 2018).

Considering that both species share many ecological similarities, including the same microhabitat (both psamophiles) and temporal niches, niche differentiation may be mainly trophic, as our results suggest for the populations in the PARNA Catimbau. Furthermore, foraging strategy appears to be an important factor when considering diet. The sit-and-wait forager, *T. cocorobensis*, consumes a higher frequency of active prey (i.e. ants), whereas the more active forager, *A. ocellifera*, ingests fewer active prey items (i.e. termites), and these prey items represent exactly the greatest dissimilarity between the diets of these lizards. These results corroborate the previously cited proposals by Huey & Pianka (1981).

However, historical influences should also be considered as an important factor influencing the differences in the proportions of consumed prey categories by both species (i.e. by number, frequency, and volume), which are nested in very distant clades within Squamata (Teiioidea and Iguania; Pyron et al., 2013; Simões & Pyron, 2021). The consumption of plant material is vastly documented, for Iguania and Tropiduridae in particular (e.g. Fialho et al., 2000; Van Sluys et al., 2004; Kolodiuk et al., 2010; Garda et al., 2012; Siqueira et al., 2013; Verrastro & Ely, 2015; García-Rosales et al., 2020; Tan et al., 2020), where Tropiduridae is included as one of the families whose diet comprises the highest percentage of omnivorous species and the highest average percentage of plant material in their diet (Cooper & Vitt, 2002).

This high intake of plant material seems to be a result of historical heritage. Although the primitive condition of Iguania seems to be carnivory, and the primitive condition of Tropiduridae is uncertain, plant consumption is widespread throughout the crown groups of the family and appears to be the condition at the root of the *torquatus* group, in which *T. cocorobensis* is nested (Frost et al., 2001; Cooper & Vitt, 2002). On the other hand, carnivory appears to be the primitive condition for all Scincoidea species, for the family Teiidae, and for the genus *Ameivula* (Cooper & Vitt, 2002). Furthermore, there are records of plant material consumption by some teiid lizards in Brazil, but at much lower levels compared to tropidurids (Menezes et al., 2006; 2011; Sales et al., 2011; Sales & Freire, 2015). However, plant consumption in the family Teiidae is greatly reduced when compared with the observed in Tropiduridae (Cooper & Vitt, 2002).

In the present study, all data were collected during the dry season, but it is important to consider the different responses of each species to environmental variations when comparing the use of food resources, especially in environments with marked seasonality, such as the Caatinga. In this environment the ingestion rates of some species of *Tropidurus*, such as *T. hispidus* and *T. semitaeniatus*, may vary depending on the different seasons, consuming mainly ants and termites in both seasons, and becoming opportunistic predators of arthropod larvae during the rainy season, this prey being poorly available in the warmer season (Ribeiro & Freire, 2011). In some species of the Teiidae family, seasonal changes in trophic ecology are not present, for example *Glaucomastix littoralis* in coastal sandbank environments, which experience reduced seasonality and constantly high air humidity and rainfall (Teixeira-Filho et al., 2003). On the other hand, in a population of *A. ocellifera* in the Caatinga, seasonal changes in diet have been recorded for the three most important prey categories during each season (insect larvae, termites and orthopterans during the rainy season, contrasting with spiders, hemipterans and insect larvae during the dry season) (Sales & Freire, 2015), which seems to reinforce the influence of seasonality on diet.

Despite inhabiting the same microhabitat, there is a low trophic niche overlap between *A. ocellifera* and *T. cocorobensis* and their diets differ significantly. The predation of termites by *A. ocellifera* in the Caatinga and Cerrado ecoregions is well documented (Menezes et al., 2011; Sales & Freire, 2015), but populations of this species in Amazonian savannahs mainly consume orthopterans (Mesquita & Colli, 2003). Specimens of *T. cocorobensis* consume several of the same prey categories as their congeners and ants are the most frequently ingested prey category, similar to *T. hispidus* and *T. semitaeniatus* (Ribeiro & Freire, 2011), which are also sympatric with *T. cocorobensis* and *A. ocellifera* in the Catimbau National Park (Pedrosa et al., 2014).

Our results show that although the study species share microhabitats and the same food resources, the trophic niche overlap between these species is low and their diets differ significantly. This may be related to competition strategies, historical constraints, and/or responses to environmental variations. We suggest that future studies consider the possible temporal niche overlap of these species, in order to provide more information on the activity period of *T. cocorobensis*. Our study contributes to the knowledge of resource sharing among sympatric species of lizards from different families in the Caatinga.

ACKNOWLEDGEMENTS

The authors thank the Instituto Chico Mendes de Conservação da Biodiversidade and to the Comitê de Ética em Uso Animal of the Universidade Federal de Pernambuco for animal collection and handling permits permissions (ICMBio permits nº 73617-1; CEUA-UFPE nº 0004/2020). The authors also thank the Projeto Ecológico de Longa Duração-PELD/CNPq Catimbau and its coordinator M. Tabarelli for lodging and equipment used during field work. PMAO is thankful to the Pro-reitoria para Assuntos de Pesquisa e Pós-Graduação da UFPE and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (IBPG- 1566-2.04/19) for financial support. PMSN is grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq Fellowship #313622/2018-3 and 309253/2021-7).

REFERENCES

Albuquerque, R.L., dos Santos Protázio, A., Barbosa de Queiroga Cavalcanti, L., Lopez, L.C.S. & Mesquita, D.O. (2018). Geographical ecology of *Tropidurus hispidus* (Squamata: Tropiduridae) and *Cnemidophorus ocellifer* (Squamata: Teiidae) in a Neotropical region: a comparison among Atlantic Forest, Caatinga, and coastal populations. *Journal of Herpetology*, 52, 145–155.

Anderson, M.J. (2014). Permutational multivariate analysis of variance (PERMANOVA). *Wiley stats ref: statistics reference online*, 1–15. doi: https://doi.org/10.1002/9781118445112.stat07841.

Cechin, S.Z. & Martins, M. (2000). Eficiência de armadilhas de queda (pitfall traps) em amostragens de anfíbios e répteis no Brasil. *Revistabrasileira de zoologia*, 17, 729–740.

Cooper Jr, W.E. & Vitt, L.J. (2002). Distribution, extent, and evolution of plant consumption by lizards. *Journal of Zoology*, 25, 487–517.

Costa G.C., Mesquita, D.O. & Colli, G.R. (2008). The effect of pitfall trapping on lizard diets. *The Herpetological Journal*, 18, 45–48.

De Andrade, E.M., do Nascimento Aquino, D., Chaves, L.C.G. & Lopes, F.B. (2018). Water as capital and its uses in the Caatinga. In: *Caatinga: the largest tropical dry forest region in South America*. Springer, Miami, EUA.

Duffield, G.A. & Bull, C.M. (1998). Seasonal and ontogenetic changes in the diet of the Australian skink *Egernia stokesii*. *Herpetologica*, 414–419.

Dunham, A.E. (1983). Realized niche overlap, resource abundance, and intensity of interspecific competition. *Lizard ecology* 261–280. doi: https://doi.org/10.4159/harvard.9780674183384.c15.

Ferreira, A.S., de Oliveira Silva, A., da Conceição, B.M. & Faria, R.G. (2017). The diet of six species of lizards in an area of Caatinga, Brazil. *The Herpetological Journal*, 26, 151–160.

Fialho, R.F., Rocha, C.F.D. & Vrcibradic, D. (2000). Feeding ecology of *Tropidurus torquatus*: ontogenetic shift in plant consumption and seasonal trends in diet. *Journal of Herpetology*, 2000, 325–330.

Fitzgerald L.A. (2012). Finding and Capture reptiles. pp.77–87 In: *Reptile biodiversity - standard methods for inventory and monitoring*. University of California Press, Berkeley. McDiarmid R.W., Foster M.S, Guyer C., Gibbons J.W. & Chernoff, N. (Eds.).

Foster, M.S. (2012). Preparing reptiles as voucher specimens.

Reptile Biodiversity: Standard Methods for Inventory and Monitoring. University of California Press, Berkeley.

Frost, D.R., Rodrigues, M.T., Grant, T. & Titus, T.A. (2001). Phylogenetics of the lizard genus *Tropidurus* (Squamata: Tropiduridae: Tropidurinae): direct optimization, descriptive efficiency, and sensitivity analysis of congruence between molecular data and morphology. *Molecular phylogenetics and evolution,* 21, 352–371. doi: https://doi.org/10.1006/mpev.2001.1015.

García-Rosales, A., Arriaga-Noguez, A. & Ramírez-Bautista, A. (2020). Natural History of the Black Iguana *Ctenosaura similis* (Squamata: Iguanidae) in Isla Contoy, Quintana Roo, Mexico. *Acta Biológica Colombiana*, 25(3), 394–402.

Garda, A.A., Costa, G.C., França, F.G., Giugliano, L.G., Leite, G.S., Mesquita, D.O., ... & Colli, G.R. (2012). Reproduction, body size, and diet of *Polychrus acutirostris* (Squamata: Polychrotidae) in two contrasting environments in Brazil. *Journal of Herpetology*, 46(1), 2–8.

Gomes, A.P.S., Rodal, M.J.N. & Melo, A.L. (2006). Florística e fitogeografia da vegetação arbustiva subcaducifólia da Chapada de São José, Buíque, PE, Brasil. *Acta Botanica Brasílica,* 20, 37–48. doi: https://doi.org/10.1590/S0102-33062006000100005.

Griffiths, A.D. & Christian, K.A. (1996). Diet and habitat use of frillneck lizards in a seasonal tropical environment. *Oecologia*, 106(1), 39–48.

Howard, A.K., Forester, J.D., Ruder, J.M., Parmerlee, J.S. & Powell, R. (1999). Natural history of a terrestrial Hispaniolan anole: *Anolis barbouri*. *Journal of Herpetology,* 33, 702–706. doi: https://doi.org/10.2307/1565590.

Huey, R.B. & Pianka, E.R. (1977). Patterns of niche overlap among broadly sympatric versus narrowly sympatric Kalahari lizards (Scincidae: Mabuya). *Ecology*, 58(1), 119–128.

Huey, R.B. & Pianka, E.R. (1981). Ecological consequences of foraging mode. *Ecology*, 62(4), 991–999.

Kolodiuk, M.F., Ribeiro, L.B. & Freire, E.M. (2010). Diet and foraging behavior of two species of *Tropidurus* (Squamata, Tropiduridae) in the Caatinga of northeastern Brazil. *South American Journal of Herpetology*, 5, 35–44.

Menezes, V.A., Amaral, V.C., Van Sluys, M. & Rocha, C.F.D. (2006). Diet and foraging of the endemic lizard *Cnemidophorus littoralis* (Squamata, Teiidae) in the restinga de Jurubatiba, Macaé, RJ. *Brazilian Journal of Biology*, 66(3), 803–807.

Menezes, V.A., Van Sluys, M., Fontes, A.F. & Rocha, C.F.D. (2011). Living in a caatinga-rocky field transitional habitat: ecological aspects of the whiptail lizard *Cnemidophorus ocellifer* (Teiidae) in northeastern Brazil. *Zoologia*, 28, 8–16.

Mesquita, D.O. & Colli, G.R. (2003). Geographical variation in the ecology of populations of some Brazilian species of *Cnemidophorus* (Squamata, Teiidae). *Copeia*, 2003(2), 285–298.

Oksanen, J., Blanchet, G.F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., ... & Wagner H. (2019). vegan: Community EcologyPackage. R package version 2.5–6. Available from: https://CRAN.R-project.org/package=vegan.

Oliveira, P.M. & Nunes, P.M.S. (2020). An endemic lizard of Catimbau National Park, Pernambuco, Brazil, found in stomach contents of *Tropidurus cocorobensis* (Squamata, Tropiduridae). *Herpetology Notes*, 13, 305–307.

Pedrosa, I.M.M.D.C., Costa, T.B., Faria, R.G., França, F.G.R., Laranjeiras, D.O., Oliveira, T.C.S.P.D., ... & Garda, A.A. (2014). Herpetofauna of protected areas in the Caatinga III: The Catimbau National Park, Pernambuco, Brazil. *Biota Neotropica*, 14(4), e20140046.

Perez-Cembranos, A., Leon, A. & Perez-Mellado, V. (2016). Omnivory of an insular lizard: sources of variation in the diet of *Podarcis lilfordi* (Squamata, Lacertidae). *PloSOne*, 11(2), e0148947.

Pianka, E.R. (1973). The structure of lizard communities. *Annual Review of Ecology and Systematics*, 4(1), 53–74.

Pianka, E.R. (1974). Niche overlap and diffuse competition. *Proceedings of the National Academy of Sciences*, 71(5), 2141–2145.

PROJETO-RADAMBRASIL. (1983). Folhas SF 23/24 Rio de Janeiro/Vitória, geologia, geomorfologia, pedologia, vegetação e uso potencial da terra, 32. CPRM, Brazil.

Pyron, R.A., Burbrink, F.T. & Wiens, J.J. (2013). A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC evolutionary biology*, 13(1), 1–54.

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ribeiro, L.B. & Freire, E.M. (2011). Trophic ecology and foraging behavior of *Tropidurus hispidus* and *Tropidurus semitaeniatus* (Squamata, Tropiduridae) in a caatinga area of northeastern Brazil. Iheringia. *Série Zoologia*, 101, 225–232.

Rodrigues, M.T. (1987). Sistemática, ecologia e zoogeografia dos *Tropidurus* do grupo torquatus ao sul do Rio Amazonas (Sauria, Iguanidae). *Arquivos de Zoologia*, 31, 105–230.

Rodrigues, M.T. (1996). Lizards, snakes, and amphisbaenians from the Quaternary sand dunes of the middle Rio São Francisco, Bahia, Brazil. *Journal of Herpetology*, 513–523.

Rodrigues, M.T. (2003). Herpetofauna da caatinga. In: E*cologia e conservação da Caatinga*. Editora Universitária UFPE, Recife, Brazil.

Sales, R.F., Ribeiro, L.B. & Freire, E.M. (2011). Feeding ecology of *Ameiva ameiva* in a caatinga area of northeastern Brazil. *The Herpetological Journal*, 21(3), 199–207.

Sales, R.F. & Freire, E.M. (2015). Diet and foraging behavior of *Ameivula ocellifera* (Squamata: Teiidae) in the Brazilian semiarid Caatinga. *Journal of Herpetology*, 49(4), 579–585.

Sannolo, M. & Carretero, M.A. (2019). Dehydration constrains thermoregulation and space use in lizards. *PloSOne*, 14(7), e0220384.

Simões, T.R. & Pyron, R.A. (2021). The squamate tree of life. *Bulletin of the Museum of Comparative Zoology*, 163(2), 47–95.

Siqueira, C.C., Kiefer, M.C., Sluys, M.V. & Rocha, C.F.D. (2013). Variation in the diet of the lizard *Tropidurus torquatus* along its coastal range in Brazil. *Biota Neotropica*, 13(3), 93–101.

Sousa, J.G., Teixeira, A.A., Teles, D.A., Araújo-Filho, J.A. & Ávila, R.W. (2017). Feeding ecology of two sympatric geckos in an urban area of Northeastern Brazil. *Acta Herpetologica*, 12(1), 49–54.

Sutherland, D.R. (2011). Dietary niche overlap and size partitioning in sympatric varanid lizards. *Herpetologica*, 67(2), 146–153.

Tan, W.C., Herrel, A. & Measey, J. (2020). Dietary observations of four southern African agamid lizards (Agamidae). *Herpetological Conservation and Biology*, 15(1), 69–78.

Teixeira-Filho, P.F., Rocha, C.F.D. & Ribas, S.C. (2003). Relative feeding specialization may depress ontogenetic, seasonal, and sexual variations in diet: the endemic lizard *Cnemidophorus littoralis* (Teiidae). *Brazilian Journal of Biology*, 63, 321–328.

Van Sluys, M., Rocha, C.F.D., Vrcibradic, D., Galdino, C.A.B. & Fontes, A.F. (2004). Diet, activity, and microhabitat use of two syntopic *Tropidurus* species (Lacertilia: Tropiduridae) in Minas Gerais, Brazil. *Journal of Herpetology*, 38(4), 606–611.

Vasconcellos, A., Andreazze, R., Almeida, A.M., Araujo, H.F., Oliveira, E.S. & Oliveira, U. (2010). Seasonality of insects in the semi-arid Caatinga of northeastern Brazil. *Revista Brasileira de Entomologia*, 54, 471–476.

Verrastro, L. & Ely, I. (2015). Diet of the lizard *Liolaemus occipitalis* in the coastal sand dunes of southern Brazil (Squamata-Liolaemidae). *Brazilian Journal of Biology*, 75(2), 289–299.

Verwaijen, D. & Van Damme, R. (2007). Does foraging mode mould morphology in lacertid lizards? *Journal of Evolutionary Biology*, 20(5), 1950–1961.

Whitfield, S.M. & Donnelly, M.A. (2006). Ontogenetic and seasonal variation in the diets of a Costa Rican leaf-litter herpetofauna. *Journal of Tropical Ecology*, 22(4), 409–417.

Zanchi-Silva, D., Borges-Nojosa, D.M. & Galdino, C.A.B. (2014). Reproduction of a whiptail lizard (*Ameivula ocellifera*, Teiidae) from a coastal area in northeastern Brazil. *Anais da Academia Brasileira de Ciências*, 86(3), 1263–1272.

Accepted: 19 September 2022

THE HERPETOLOGICAL JOURNAL
SUBMISSION PROCESS

The Herpetological Journal is an international peer-reviewed publication of the British Herpetological Society, with open-access publication options. *The Journal* has a broad focus relating to behaviour, ecology, evolution, systematics, taxonomy, physiology, anatomy, functional morphology, pathology, natural history, method development and conservation of reptiles and amphibians. All articles should appeal to a general herpetological audience and have a solid grounding in natural history. We are committed to open science and avoiding unconscious biases so moving forward we will operate a double-blind peer review process.

Manuscripts that describe natural history observations, range extensions or checklists are not appropriate submissions (unless they address a bigger question) and would be better suited to our sister publication, *The Herpetological Bulletin*.

The Herpetological Journal welcomes contributions in the following categories:

- Full length research articles
- Short communications
- Reviews
- Perspectives/Opinion pieces

Files to be uploaded to the OJS system: Cover Page, Main Text, Tables, Figures, Supplementary Files.

Cover Page:
This will not be seen by peer reviewers in order to comply with a double-blind peer review process. The cover page should include Title, Authors, Author Affiliations, Ethical Statement and Author Contributions (if editors do not deem that authors contributed substantially to the research then the article will be rejected).

Main Text:
Nb. author names or affiliations should not be included in this file.

Full length research articles should be between 2,500 and 6,000 words and include the following sections: Title, Abstract (maximum 300 words), Keywords (five words that are not used in the title), Introduction, Methods, Results, Discussion, Acknowledgements, Data Accessibility, References, Figure captions, Table captions. The word limit excludes Data Accessibility and References. There are no limits to the number of figures and tables.

Short communications should be less than 2,500 words and include the following sections: Title, Abstract (maximum 250 words), Keywords (five words not used in the title), Main Text (NOT separated into Introduction, Methods, Results and Discussion), Acknowledgements, Data Accessibility, References, Figure captions, Table captions. The word limit excludes Data Accessibility, References, and Figure and Table captions. Short communications can have a maximum of ONE figure and ONE table.

Reviews are either solicited by editors or a short email enquiry should be sent to the Editor-in-Chief (bhsherpetologicaljournal@gmail.com) to enquire about the suitability of a proposed review. Reviews should be between 2,500 and 6,000 words. Section headings can be specified at the authors discretion.

Perspectives/Opinion pieces will be considered if they address a new or controversial topic/idea, or if they are comments about newly published articles in *The Herpetological Journal*. Perspectives/Opinion pieces should be a maximum of 1,500 words (excluding references) and can include ONE figure and ONE table.

Tables:
Tables should be provided in a separate Word file. Tables should be numbered in Arabic numerals, e.g. Table 1. Tables should be as simple as possible and typed double-spaced on separate sheets with a title/short explanatory paragraph above the table. Horizontal and vertical lines should be avoided, as should tables that split over more than one page or that need to be set in landscape format.

Figures:
Figures should initially be submitted in a single Word or PDF file. Graphs, line drawings and photographs should be numbered in sequence in Arabic numerals, e.g. Figure 1. If a figure has more than one part, each should be identified as (a), (b), etc. Figure captions should be included at the end of the main text. After acceptance figures should be submitted as separate image or pdf files with a minimum resolution of 300dpi and a maximum file size of 5MB.

Supplementary Data/Files:
To conform with an open science process, it will be necessary for datasets, code, supplementary figures etc. to be deposited in an online repository (e.g. https://osf.io/) and made available after publication. At the initial submission stage, at a minimum, any code and supplementary figures should be uploaded in the submission portal for review. Any new taxonomic changes should be recorded on ZooBank.

It is a fundamental condition that submitted manuscripts have not been published and will not be simultaneously submitted or published in another journal. However, as a journal we do support the submission of articles on preprint servers (e.g., bioRxiv) as long as the preprints are linked to final published articles.

By submitting a manuscript, the authors agree that the copyright for their article is transferred to the publisher if and when the article is accepted for publication. The copyright covers the exclusive rights to reproduce and distribute the article, including reprints and photographic reproductions. Permission to use images after publication will almost always be granted but must be sought in advance from the Editors.

Papers should be written in British English (including figure labels) and spelling should be that of the Oxford English Dictionary.

Times and dates should conform to the following formats: for time of day use 0900, 1000 etc; for dates use 7 July 2017 etc. Please avoid using bold text, all caps or small caps for emphasis. If emphasis is required, use italics. Common names should be in lower case unless a proper noun is used.

All submissions must adhere to the British Herpetological Society's Ethical Policy and Guidelines, which can be found here – https://www.thebhs.org/images/stories/BHS-ethicspolicy.pdf.

Open Access Policy: *The Herpetological Journal* supports "green" open access, as outlined by the Research Councils UK, to facilitate deposition of articles e.g. at institutional repositories. *The Herpetological Journal* also offers the option of "gold" open access for individual articles (free of charge for members of the British Herpetological Society, and at an article processing charge of £97 for non-members).

REFERENCE STYLE
CITATION IN TEXT:

- Chronological then alphabetical
- Use “et al.” (not italicised) for more than two authors
- Last name (s) and year separated by comma
- Names separate by “&”
- References separated by semicolon

Ex. 1: (Heyer et al., 1988; Weygoldt et al., 1989; Eterovick et al., 2005)
Ex. 2: (Smith et al., 2004; Jones & Smith, 2008)
Ex. 3: (Smith et al., 2015)
Ex. 4: “Although Smith et al. (2008) did not include -”
Ex. 5: "- as observed by Smith & Jones (2017)"

REFERENCES

- Authors
- Last name separated from initials by comma
- Initials capitalised and separated by period (no space)
- Names separated by commas
- Last name separated by “&”

Ex.: Smith, A.H., Jones, R.D. & Lloyd, K.A.
Ex.: Smith, A.H. & Jones, R.D.

Year:
- In parentheses, followed by a full stop.

Title:
- Only first letter capitalised except book titles (in this case, All First Letters Capitalised).

Journal:
- Journal name should be written in full, italicised, followed by a comma
- Volume and pages separated by comma and ending with full stop (not italicised).

Journal article:
- Authors. (Year). Title. *Journal*, Volume (Issue), xx–xx.

Book:
- Authors. (Year). Book Title. City: Country. Xxx p.

Book chapter:
- Authors. (Year). Chapter title. In: *Book Title*, Book editor (s). (Ed./Eds.) City: Country. Xxx p.

Ex. 1:
Lebboroni, M. & Corti, C. (2006). Road-killing of lizards and traffic density in central Italy. In: *Herpetologia Bonnensis II: Proceedings of the 13th Ordinary General Meeting of Societas Europaea Herpetologica*, 81–82. Vences, M., Köhler, J., Ziegler, T. & Böhme, W. (eds). Bonn: Societas Europaea Herpetologica.

Ex. 2:
Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989). Preparation and Analysis of Eukaryotic Genomic DNA. In: *Molecular Cloning: A Laboratory Manual*, 2nd Eds. Cold spring Harbor Laboratory Press, New York, USA.

Websites:
Lang, J., Chowfin, S. & Ross, J.P. (2019). *Gavialis gangeticus*. The IUCN Red List of Threatened Species 2019: e.T8966A149227430. Downloaded on 3 October 2019. http://dx.doi.org/10.2305/IUCN.UK.2019-1.RLTS.T8966A149227430.en.

All contributions should be addressed to the Scientific Editor:

Marcio R. Pie, Edge Hill University, UK.
E-mail: bhsherpetologicaljournal@gmail.com

Advertisements:
The Herpetological Journal accepts advertisements subject to approval of contents by the Managing Editor, to whom enquiries should be addressed.

Herpetological Journal website: https://www.thebhs.org/publications/the-herpetological-journal