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ORIGINAL ARTICLE

Food supplementation affects the foraging ecology of a low-energy, ambush-foraging snake

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Abstract
The effect of food availability on the spatial ecology of animals varies within and across study systems because a multitude of factors can affect the spatial activity of organisms. Low-energy specialists, such as ambush-foraging snakes, feed infrequently and can endure long periods without food. Because they have low-energy requirements, one possible tactic for feeding may be to simply ambush for longer periods when prey availability is low, thereby decreasing the potential costs associated with locating new ambush sites. We used radiotelemetry, supplemental feeding, and remote video cameras on free-ranging male puff adders (*Bitis arietans*) in South Africa to test the hypothesis that food intake affects the foraging ecology of extreme low-energy, ambush foragers and to quantify their natural feeding rates. Supplementally fed puff adders improved their body condition, spent less time foraging, and decreased distance traveled compared to control snakes. However, movement frequency and home range size did not differ between the two groups. These findings indicate that control snakes traveled farther within similar-sized home ranges compared to fed snakes and did so at no survival cost. Further, naturally foraging puff adders successfully caught a prey of small size once every 10 days on average. Hence, despite their "sit-and-wait" foraging strategy and their low-energy intake/requirements, underfed puff adders travel widely to presumably find appropriate ambush sites that maximize prey capture. Our research provides the first strong evidence that the spatial activity of a terrestrial vertebrate species with extremely low energetic demands is significantly affected by food intake.

Significance statement
Because animals travel their home range partly to forage, their space use can be affected by the amount of food available in nature. We show that, despite a low-energy lifestyle, the movement level of an ambush-foraging snake that feed infrequently is linked to the amount of food they eat. Underfed snakes traveled greater distances in search of prey, within similar-sized home ranges, compared to well-fed snakes, and they did so at no survival cost. Hence, our research adds to our understanding of the effect of food on the spatial ecology of animals, by providing conclusive evidence that the spatial response of an organism with extremely low-energy demands can be affected by food.

Keywords Feeding rate · Movement · Predator-prey relationship · Puff adder · Spatial ecology

Introduction

Because animals move through the landscape, in part, to forage, variation in food availability should be an important determinant of the spatial ecology of animals. All else being equal, individuals living in areas with high food availability are presumably able to meet their energetic requirements in smaller areas and therefore should decrease home range or territory size (Stenger 1958; Schoener 1968). Yet, studies have revealed that food availability does not necessarily affect the observed spatial behavior of animals; although most show a reduced home range when food is relatively abundant, some increase space use (Haapakoski and Ylönen 2010), whereas

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others fail to respond (see Boutin 1990; Adams 2001 for reviews). These contrasting patterns emphasize that factors other than the availability of food resources, such as predation risk (Desy et al. 1990; Lagos et al. 1995), competition (Myers et al. 1979; Fortier and Tamarin 1998), and mating system (Gaulin and FitzGerald 1988; Ims 1987, 1988), can also affect the spatial activity of animals.

Quantifying the significance of food availability on space use of animals has been facilitated by the use of food supplementation studies (Boutin 1990). Among terrestrial vertebrates, the vast majority of studies have focused on species with high-energy requirements and/or that feed frequently (e.g., endotherms and lizards; see Boutin 1990; Adams 2001 for reviews), biasing our understanding of the relationship between food and space use toward these organisms. Examining the spatial response of infrequently feeding, low-energy species to variation in food intake is, therefore, necessary to better understand the diversity of tactics used to cope with varying levels of food resources. For example, we may expect low-energy organisms, such as ambush-foraging predators, to be somewhat pre-adapted to not respond spatially to variation in food availability compared to species with higher-energy needs. That is, due to their low-energy requirements, their foraging strategy may be to simply lie in wait at ambush locations for longer periods of time—as opposed, for instance, to increasing foraging activity by moving between more ambush sites—when food is scarce (Beaupre 2002; Nowak et al. 2015), in which case food availability is unlikely to have an impact on space use.

Snakes, and specifically ambush-foraging species, have low metabolic rates and are extreme among vertebrates in their aptitude to endure prolonged starvation (Secor and Diamond 1998; McCue 2007; McCue et al. 2012). This ability provides the opportunity to examine the spatial response to variation in food levels in vertebrates with extremely low energy demands. Only three studies have examined the spatial response of snakes to supplemental feeding, and none provided strong evidence that food intake affected space use (Taylor et al. 2005; Tetzlaff 2015; but see Wasko and Sasa 2012 for partial evidence). This scarcity of snake studies is surprising given that snakes, and vipers in particular, have emerged as model organisms in ecological studies in part due to their amenability to experimental research (Shine and Bonnet 2000). Furthermore, because many ambush-foraging snakes can be easily approached with minimal disturbance in the field, subjects can be hand-fed individually in situ. This allows precise quantification of supplemental food intake and also provides an alternative method to feeding stations typically used in supplementation studies. The latter method can attract other members of the population and their predators in the study area, thereby increasing population and/or predator density (i.e., a numerical response), which may ultimately confound the true effect of food availability on space use (Taitt and Krebs 1981; Adams 2001).

In this field study conducted in South Africa, we used supplemental feeding and radiotelemetry to test the hypotheses that supplementally fed snakes would (1) improve body condition, (2) reduce time spent foraging, and (3) decrease movements compared to control snakes. Support for 1 and 2 would demonstrate that supplementally fed snakes experienced higher food intake and, as a result, decreased hunting behavior compared to unfed snakes. Furthermore, support for 3 would suggest that the costs of locating new ambush sites are offset by the foraging benefits acquired through increased spatial activity. For example, snake foraging success may be inversely related with residence time at ambush locations, if longer residence time increases snake detectability by prey and decreases the odds of capturing prey. Alternatively, rejection of 3 would indicate that the benefits of prospecting for new ambush sites are outweighed by the costs in these extreme low-energy, ambush specialists (Alexander and Marais 2007; McCue et al. 2012; this study). For example, searching for new ambush sites could expose snakes to predators and/or be energetically expensive, and hence, snakes may resort to decreasing these costs.

In addition to testing these hypotheses, we used fixed videography, which consisted of setting up continuously recording remote video cameras focused on ambushing animals, to record precise feeding data on ambush-hunting snakes (Clark 2006). Our extensive video dataset, which amounts to nearly 1500 h of recordings while snakes foraged, allows us to characterize in detail prey encounter and feeding rates of puff adders. The field experimental nature of our study combined with the use of radiotelemetry and remote video cameras allow us to provide one of the most detailed analyses of the link between food intake and foraging activity in an extreme low-energy terrestrial vertebrate.

Materials and methods

Study site and species

The study took place in the Dinokeng Game Reserve, a ca. 18,500 ha area in the Gauteng Province of South Africa (−25.38°S, 28.31°E; ca. 1100 m a.s.l.). The site, which is composed of a mosaic of savannas and open woodlands, falls within the savanna biome (Driver et al. 2005) and is seasonal with hot, wet summers and mild, dry winters (Shulze 1997).

The puff adder is a heavy-bodied medium-sized viperid snake (ca. 700–900-mm adult snout-to-vent length [SVL]), which occurs in savannas and open woodlands throughout most of sub-Saharan Africa and parts of the Arabian Peninsula. It is a primarily nocturnal ambush-foraging snake that—in our population—feeds largely on small mammals and amphibians and occasionally on birds and lizards (unpublished data; this study). In the area of study, puff adders are most active from the onset of the rainy season, typically in late October–November to the beginning of the dry season in June–July. Males decrease feeding and are primarily involved in mate-searching activities during the mating season from March to June. During the coldest months of the austral winter (July–August), puff adders do not hibernate but generally remain inactive on the surface, in burrows, under rocks, or in termitaria.

Radiotelemetry and supplemental feeding

As part of a larger study investigating the effect of food intake on male mate-searching activities and reproductive success, we conducted a food-supplementation experiment from January to March, just before the beginning of the mating season (March–June), for three consecutive field seasons (2013–2015). Because male snakes often forgo or drastically reduce feeding during the mating season (Prestt 1971; Aleksiuk and Gregory 1974; Slip and Shine 1988; but see Webber et al. 2012; Tetzlaff et al. 2015), we experimentally fed males prior to the mating season, but herein use the spatial data collected during the experimental feeding to examine the short-term response to feeding by adult males while they were still foraging.

Snakes were caught opportunistically and radiotransmitters (model SI-2, 13 g; Holohil Ltd., ON, Canada) were surgically implanted into the body cavity of 32 adult males in accordance with established procedures (Reinert and Cundall 1982; Reinert 1992; Alexander 2007). Because puff adders are secretive animals that are difficult to find, four of the snakes were used for two field seasons to increase the effective sample size (see results below). In total, we radiotracked six supplementally fed and five control snakes in 2013, six and seven in 2014, and six of each in 2015. Transmitter mass never exceeded 3% of the snake body mass. We released snakes at their capture locations typically 3–4 days following surgery and located snakes using a R1000 radio receiver (Communications Specialists Inc., CA, USA) ca. every 2–3 days (see results below) with a two- or three-element Yagi antenna (Africa Wildlife Tracking, Gauteng, South Africa).

Each year, we randomly allocated males to either a supplementally fed (experimental) or unfed (control) group, and it was not possible to record data blind because our study involved focal animals in the field. Puff adders are sedentary ambush predators that can easily be approached in nature with little disturbance. This allowed us to supplementally feed dead rodents to snakes using a pair of 1-m snake tongs once a week during the food supplementation period. (We minimized disturbance to the snakes by approaching them slowly, and although on a few occasions snakes fled, they typically did not react to our presence, which was also the case for unfed snakes.) Each male in the fed group was offered pre-killed striped mice (*Rhabdomys pumilio*), a natural prey species, or when in short supply, laboratory white rats (*Rattus norvegicus*), up to 15% of the snake's body mass (recorded at the start of the food supplementation period). Snakes sometimes refused food, usually during ecdysis, and therefore, we recorded the amount of supplemental food ingested by each individual. Each year, we terminated feeding within a week of the first evidence of mating activity, which varied slightly by year. We fed snakes from 8 January to 13 March in 2013, 15 January to 7 March in 2014, and 13 January to 5 March in 2015.

We arbitrarily considered that a snake had moved between successive radiofixes if it traveled a distance ≥1 m from its previous position. We recorded the coordinates of each new location using a Garmin handheld GPS receiver (model GPSmap 78 s; datum WGS1984).

Fixed videography

Starting in September 2013, we used fixed videography to monitor puff adder feeding behavior. Each video camera unit was made of a closed-circuit television surveillance camera (model PC177IRHR-8, Supercircuit Inc., TX, USA), connected to a recording mini digital video recorder (model MDVR 14–4, Supercircuit Inc., TX, USA), and powered by a 12-V sealed lead-acid battery. The video cameras recorded in color during the day and automatically switched to nighttime vision using the built-in infrared LEDs at low light levels. We located radio-tagged snakes using radiotelemetry and set up the video camera units ca. 0.7 m in front of the snakes. The following day, we collected the memory cards and reviewed recordings to quantify time spent in ambush (and whenever possible time spent in ambush per day), prey encounter (any prey that came within 0.5 m of a snake) and capture (any successful strike at prey) rates, and prey identity to the lowest possible taxonomic level. We estimated prey length after the feeding event happened by placing a ruler at an angle that matched the prey trajectory in the camera's field of view and estimated size-specific prey mass by comparing prey caught on videos with intact congeners or conspecifics of similar size from the nearest locality available at the Ditsong Museum (formerly Transvaal Museum). We recorded observations on 16 adult males, including most of the control snakes, and on a few occasions, fed snakes, but always before we conducted the food supplementation experiment or long after snakes were supplementally fed. Therefore, these data allowed us to quantify the feeding rates of naturally foraging puff adders. We included all footage from November to late February, corresponding to the period of the active season when males forage.

Snake morphometrics

We recorded snake size (SVL; mm) using a measuring tape while snakes were anesthetized during surgery, the most precise technique to date to measure body length in snakes (Setser 2007; Cundall et al. 2016), and used these measures for the snake metric analyses when individuals were measured within a week of the start of the food supplementation period. In cases where snakes were measured more than a week before the start of the experiment, we first calculated the average growth rate (mm per day) of 16 adult males, for which we had at least 2 precise measurements, and then adjusted body size according to the calculated growth rate.

We weighed snakes at the start and at the end of the feeding experiment in the field using a portable scale (accuracy ±1 g) and calculated the body condition indices (BCI) of snakes at the start and end of the experiment using the residuals derived from a linear regression of ln-transformed mass on ln-transformed SVL (Weatherhead and Brown 1996; Waye and Mason 2008). Snakes with negative/positive BCI weigh less/more than average for their length and hence can be considered in a relatively bad/good body condition.

Snake behavior

We recorded snake behavior whenever possible. We defined snakes as resting when we found them in a coiled position with their head unraised, typically at the center of the body coil. We classified snakes as in ambush when the chin rested on the ground in a striking position with the body loosely coiled (i.e., the typical foraging position for puff adders) and traveling when the snake was found moving. Because postures were sometimes difficult to interpret, we included only unambiguous behaviors in the analysis. To avoid pseudoreplication, we calculated and used the proportion of times each individual exhibited a particular behavior in the analysis.

Spatial activity

We imported recorded geographic coordinates into the R statistical program, version 3.2.2 (R Core Team 2015), and used the package adehabitat for the R software (Calenge 2006) to calculate straight-line distance between successive radiolocations (m) and estimates of home range size (ha). The distance traveled between successive radiolocations was used to calculate the total distance traveled (TDT) during the food supplementation period, average distance per day (DTD), and average distance traveled per movement (DTM). We also calculated the movement frequency (MF) for each snake by dividing the number of observed movements by the total number of radiolocations, the total displacement of snakes (T DISP) by measuring the distance between the first and last radiolocations, and generated 95% and 50% minimum convex polygons (MCP). The 95% MCP provide an estimation of the total area used by snakes during the feeding period, while 50% MCP identify the size of the core areas within the home ranges. We used MCP as home range estimators instead of kernel density estimators, because the former are more appropriate for reptile and amphibian species (Row and Blouin-Demers 2006).

Statistical analyses

We analyzed most of our experimental data using general linear models. The factors included in the models zwere feeding treatment (fed, unfed) and year (2013, 2014, 2015). We initially included number of days tracked as a covariate and the feeding treatment × year interaction in all spatial analyses but removed these variables due to lack of significance. Feeding treatment and year were, respectively, modeled as fixed and random effects. For the spatial analysis examining the effect of feeding treatment and year on distance traveled per movement (DTM), *F* tests of all main effects were constructed using the mean square of individuals as the error term. Therefore, we included multiple movement data from each individual in this analysis, although the values from the same individual were averaged in the model to avoid pseudoreplication. Individual (nested within feeding treatment and year) was modeled as a random effect. Prior to data analyses, we inspected the data for extreme outliers (i.e., any values more than three times the interquartile range). A consistent outlier was a snake we tracked for only 15 days, the shortest tracking period of all snakes, and we therefore removed this individual from all spatial analyses. When necessary, the response variables were transformed to meet assumptions of normality and homoscedasticity, and we resorted to use non-parametric tests when transformed data still failed to meet assumptions for parametric testing. To quantify the rates at which snakes encountered and successfully caught prey, we calculated time spent in ambush and numbers of prey encountered and caught for each individual and averaged these values to derive the grand mean. We conducted all statistical analyses using STATISTICA, version 12.5 (StatSoft Inc. 2014), and SPSS, version 23 (IBM Corp. 2015). Values given are means ± SE unless otherwise mentioned, and all reported *p*-values are two tailed. Significance level for all tests was $\alpha = 0.05$.

Results

Four snakes were tracked for 2 years; one of these snakes was used as a control in two consecutive years (snake ID total distance traveled [year]; BIT14 307.1 m [2013], 1018.7 m [2014]), while the other three were first used as controls and then as experimental subjects (BIT5 1901.5 m [2013], 588.5 m [2014]; BIT8 711 m [2013], 751.4 m [2015]; BIT13 550.8 m [2013], 1096.2 m [2014]). Because these individuals' movement largely differed between years, we assumed measures for different years to be independent (including only the first year of data for each snake did not affect any of our spatial analyses).

In addition, snake-tracking effort varied by year (Kruskall-Wallis test $H_{(2)} = 32.4$, $n = 36$, $p < 0.0001$), but importantly not by feeding group (Mann-Whitney test $U = 171.5$, $n_{\text{fed}} = 18$, $n_{\text{unfed}} = 18$, $p = 0.77$); snakes were radiotracked on average every 1.7 days in 2013, 2.6 days in 2014, and 2.3 days in 2015. To control for the yearly differences in tracking effort, we adjusted the movement parameters using the regression equation derived from a previous study on the same puff adder population ($y = 8.74 \times x^{-0.025}$; Alexander and Maritz 2015), where y is the adjusted distance traveled and x is the recorded distance traveled.

Lastly, in 2013 and 2015, some snakes were initially captured after the start of the food supplementation study, and therefore, the tracking period varied somewhat between snakes. Periods of radiotracking ranged from 34 to 70 days per individual in 2013 (mean ± SD = 60 ± 14.2 days) and 15–50 days in 2015 (42.4 ± 13.8 days), but the number of days tracked did not differ between fed and unfed snakes during these years (Mann-Whitney test $U = 56$, $n_{\text{fed}} = 12$, $n_{\text{unfed}} = 11$, $p = 0.57$). In 2014, all snakes were tracked throughout the feeding experiment, i.e., 53 days. In total, we recorded 872 radiofixes and 523 movements.

Food intake and snake morphometrics

Snakes were fed 81 ± 12.6% ($n = 6$) of their initial body mass in 2013, 61.6 ± 7.9% ($n = 6$) in 2014, and 85.5 ± 8.7% ($n = 6$) in 2015. The amount of supplemental food ingested by fed snakes did not differ across years ($F_{2,15} = 1.63$, $p = 0.22$) and was positively associated with change in body condition indices (Δ BCI; linear regression $y = -0.08 + 0.0048x$, $r^2 = 0.81$, $n = 17$, $p < 0.0001$; Fig. 1). Fed and unfed snakes did not differ in initial SVL ($F_{1,30} = 0.34$, $p = 0.56$) or mass ($F_{1,30} = 0.22$, $p = 0.73$), and this was the case for each year (feeding treatment × year interaction; size $F_{2,30} = 0.7$, $p = 0.5$; mass $F_{2,30} = 0.25$, $p = 0.77$); fed males measured (mean ± SD) 790 ± 90 mm in SVL and weighed 757 ± 249 g, and unfed snakes measured 770 ± 90 mm in SVL and weighed 797 ± 311 g.

We investigated whether fed snakes increased BCI from the start to the end of the experiment compared to control snakes. In 2015, one of the fed snakes was eaten by a predator (we found the partially eaten carcass) before we could record its body mass at the end of the experiment, and we therefore did not include it in this analysis. The BCI of fed and unfed snakes at the start of the feeding experiment did not differ significantly ($F_{1,29} = 2.61$, $p = 0.11$). A repeated measure ANOVA revealed a significant effect for feeding treatment × BCI interaction ($F_{1,29} = 35.1$, $p < 0.0001$), with fed snakes improving their BCI while control snakes did not (Fig. 2). At the end of the experiment, fed snakes had better BCI than unfed snakes ($F_{1,29} = 10.8$, $p = 0.003$). The improvement in BCI of fed snakes was not explained by differences in movement (see below), as fed snakes still exhibited a significantly higher Δ BCI when TDT was included as a covariate (ANCOVA with ln-transformed TDT as the covariate; $F_{1,32} = 37.7$, $p < 0.0001$).

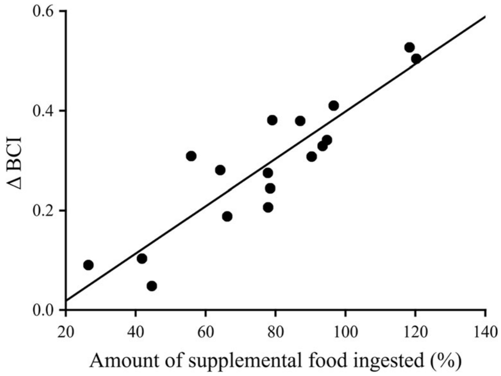

Fig. 1 The relationship between the amount of supplemental food ingested (expressed as percent of snake body mass at the start of the experiment) and change in body condition indices (Δ BCI) in fed male puff adders

Snake behavior

Control snakes were found in ambush significantly more often than fed snakes (square-root transformed; $F_{1,30} = 4.8$, $p = 0.04$), and fed snakes were found resting more often ($F_{1,30} = 4.3$, $p = 0.05$). Furthermore, the proportion of times snakes were found in ambush and resting positions varied by year (ambush $F_{2,30} = 5.2$, $p = 0.01$; resting $F_{2,30} = 5.1$, $p = 0.01$). Snakes were found in ambush more often in 2015 (0.65 ± 0.03) compared to 2014 (0.49 ± 0.05; Tukey HSD post hoc test, $p = 0.02$) and rested more often in 2014 (0.64 ± 0.06)

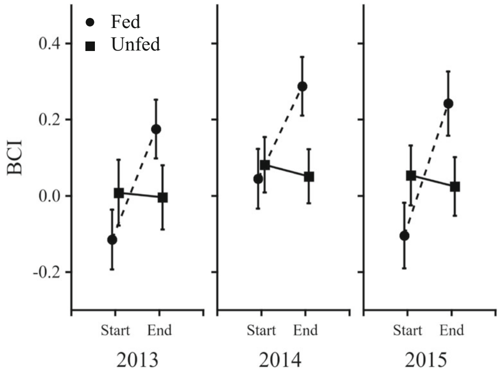

Fig. 2 The average body condition indices (BCI ± SE) of fed and unfed male puff adders at the start and end of the food supplementation period by year

compared to 2015 (0.4 ± 0.08; Tukey HSD post hoc test, $p = 0.01$). The proportion of times snakes were found moving did not differ by feeding treatment (Mann-Whitney test $U = 182$, $n_{\mathrm{fed}} = 18$, $n_{\mathrm{unfed}} = 18$, $p = 0.54$).

Spatial activity

Fed snakes had shorter TDT ($p = 0.04$; Fig. 3a) and DTD ($p = 0.05$; Fig. 3b) during the food supplementation period compared to unfed snakes. Fed snakes also exhibited significantly shorter DTM compared to unfed snakes ($p = 0.02$; Fig. 3c). Feeding treatment did not affect snake MF ($p = 0.73$), but year did ($p = 0.003$). Snakes moved more often in 2015 (0.7 ± 0.03) compared to 2013 (0.5 ± 0.06; Tukey HSD post hoc test, $p < 0.005$). Further, T DISP was similar between fed and unfed snakes ($p = 0.16$).

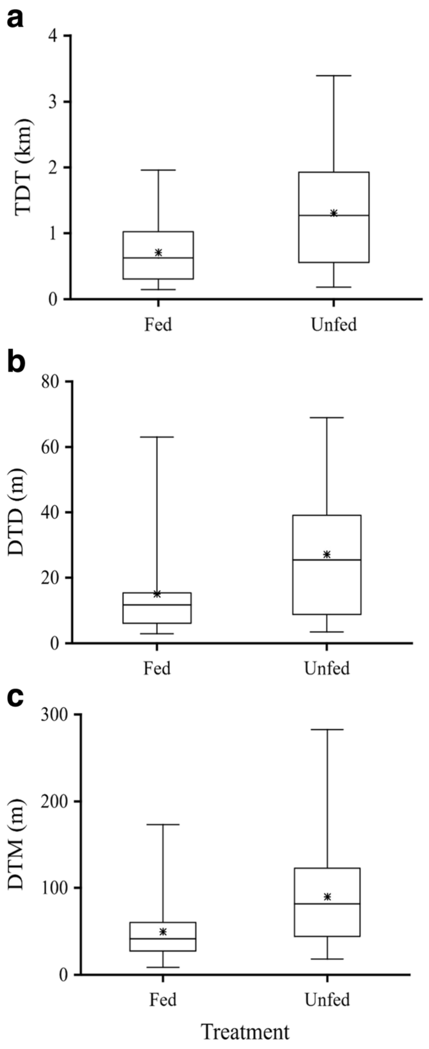

Fig. 3 The effect of feeding treatment on untransformed **a** total distance traveled (TDT; km), **b** distance traveled per day (DTD; m), and **c** distance traveled per movement (DTM; m) in male puff adders. The *lines within the boxes* are the medians, the *boxes* are the interquartile range, the *whiskers* are the minimum and maximum values, and the *asterisks* are the mean values. Note the different scales on the *Y* axes. All response variables are significantly different between feeding treatments at $p \leq 0.05$

The 95% MCP analysis did not meet the assumption of homoscedasticity when we included both the year and feeding treatment variables in the model. Therefore, we resorted to using separate one-way ANOVAs for the analysis; the 95% MCP did not differ by feeding treatment (ln-transformed variable; $F_{1,33} = 1.73$, $p = 0.2$; Fig. 4) or across years ($F_{2,32} = 0.7$, $p = 0.5$). Further, core area size was similar between feeding treatments (50% MCP; $F_{1,31} = 0.003$, $p = 0.96$; Fig. 4) and among years ($F_{2,31} = 0.35$, $p = 0.71$).

Correlates of snake morphometrics and spatial activity

The three movement variables (TDT, DTD, DTM) that were affected by feeding treatment were highly correlated (ln-transformed variables; $r^2 \geq 0.85$, $p < 0.0001$ in all cases), and therefore, we performed analyses using the TDT variable only, which we believe provide the best estimate of snake spatial activity overall. Snake SVL was not related to ln-transformed TDT (linear regression $y = 4.9 + 0.02x$; $r^2 = 0.06$, $n = 35$, $p = 0.15$). However, TDT was negatively associated with Δ BCI overall ($y = 6.9–2.2x$; $r^2 = 0.26$, $n = 34$, $p = 0.002$), and the relationship between TDT and Δ BCI differed by feeding treatment. When assessed separately, the regression was statistically significant for unfed snakes only (unfed snakes $y = 6.88–5.4x$; $r^2 = 0.34$, $n = 17$, $p = 0.01$; fed snakes $y = 6.64–1.18x$; $r^2 = 0.06$, $n = 17$, $p = 0.35$; Fig. 5).

Natural prey encounter and feeding rates

We recorded information on natural feeding ecology for 16 adult males from 124 video recordings, totalling 1480 h of videos while snakes foraged. Recorded time spent in ambush per individual varied from 1.7 to 292.1 h and averaged (mean ± SD) 92.5 ± 104.5 h, and snakes used 3.9 ± 3.7 foraging sites (range 1–14). In total, puff adders encountered 53 prey; snakes struck at 17 prey (32% of encountered prey), of which 13 were successfully captured (a 76.5% striking success; Appendix). Average prey mass was 32 ± 35 g (3–124 g), and prey encounter and capture rates per hour spent in ambush averaged 0.04 ± 0.04 (0–0.12) and 0.01 ± 0.017 (0–0.06), respectively. Year did not affect prey encounter (one-way ANOVAs; $F_{2,19} = 0.7$, $p = 0.93$) nor capture rates (square-root transformed; $F_{2,19} = 0.33$, $p = 0.72$). Because some snakes commuted—sometimes for several days in a row—between the same resting and foraging sites, we were able to accurately quantify time spent in ambush per day; 11 individuals spent 10 ± 1.6 h (7.6–13.6; $n = 56$) foraging per day, typically at night. Therefore, on average, snakes encountered a prey every 2.5 days and fed every 10 days. Assuming that males forage for ca. 4–5 months per active season, we estimate that adult male puff

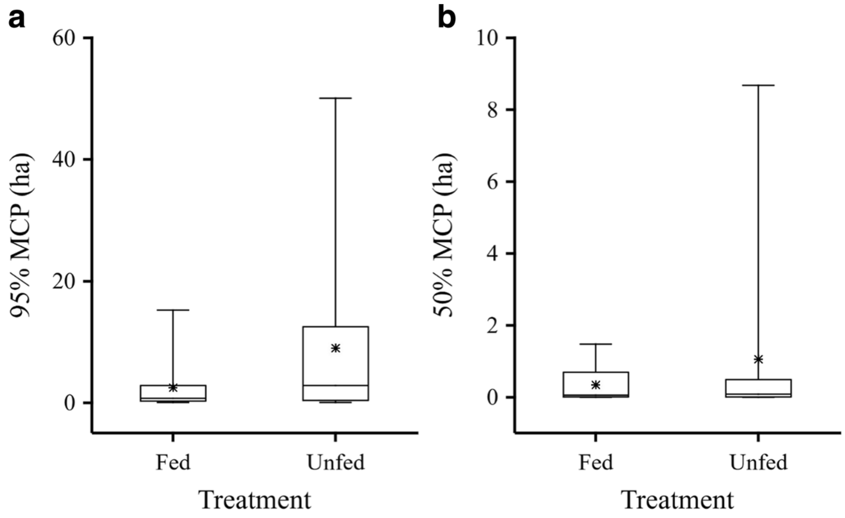

Fig. 4 The effect of feeding treatment on untransformed **a** 95% MCP (minimum *convex polygons*) home range size and **b** 50% MCP home range size in male puff adders. The *lines within the boxes* are the medians, the *boxes* are the interquartile range, the *whiskers* are the minimum and maximum values, and the *asterisks* are the mean values. Note the different scales on the *Y* axes. The sizes of home range did not differ between feeding treatments

adders catch 12–15 prey items totalling 384–480 g of prey each foraging season (48–60% of average male body mass).

Discussion

Increased food intake due to supplemental feeding caused male puff adders to move shorter distances (TDT, DTD, and DTM). However, supplemental feeding did not affect movement frequency nor home range size. This is in spite of the fact that supplemental feeding significantly improved body condition and resulted in reduced time spent in ambush and that naturally foraging puff adders fed infrequently (although not as infrequently as commonly recognized for some ambush-foraging snakes; Secor and Nagy 1994; Secor and Diamond 1998). We interpret these trends as conclusive evidence that puff adders move farther in search of ambush sites in response to low food intake but remain within defined home ranges.

We manipulated food intake in our population by hand-feeding individual snakes in the field. As we hypothesized, food-supplemented snakes improved in body condition and spent less time in ambush compared to control snakes, demonstrating that fed snakes consumed more food than control snakes and that supplemental food altered foraging behavior. Our results also show that puff adders in our population were food limited during the experimental feeding; in contrast to fed snakes, unfed snakes did not improve their body condition for the 3 years of our study. On average, naturally foraging puff adders successfully caught a prey item every 10 days, but these prey were generally small, averaging 32 g, indicating that puff adders indeed fed infrequently and had low consumption rates. Other snake studies, which all used viperid snakes, reported similar effects with fed snakes experiencing an increase in mass or body condition compared to unfed snakes (Taylor et al. 2005; Wasko and Sasa 2012; Tetzlaff 2015), suggesting that food limitation may be common in these typical “sit-and-wait” predators. Although available data are scarce, the combination of low prey encounter rate and moderate strike success (Clark 2006; Clark et al. 2012; Putnam et al. 2016) and active avoidance by prey (Jones et al. 2001; Punzo 2005; Glaudas and Rodríguez-Robles 2011a) could convincingly explain the food limitation experienced by some ambush-hunting snakes.

In addition, we tested the hypothesis that food intake would affect the spatial activity of puff adders. We controlled for two variables that are known to typically affect the spatial behavior of snakes. First, we focused our study on one sex. Sexual differences in movement are well known, with males often traveling farther than females (Gibbons and Semlitsch 1987). Second, male snakes actively locate females during the mating season, and male activity drastically increases while they search for mates (Jellen et al. 2007; Smith et al. 2009; Glaudas and Rodríguez-Robles 2011b). To control for this seasonal effect on spatial activity, we conducted our food-supplementation study before the mating season when males

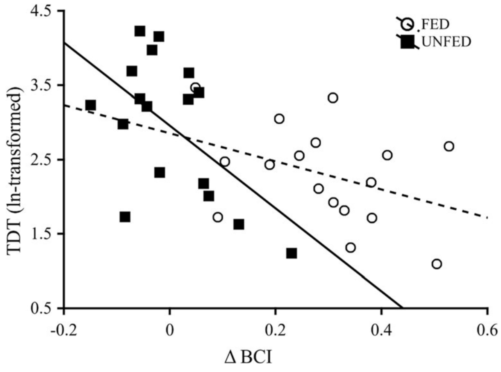

Fig. 5 The relationships between change in body condition indices (Δ BCI) and total distance traveled (TDT) by feeding treatment in male puff adders. The relationship is statistically significant for unfed snakes ($p = 0.01$) but not for fed snakes ($p = 0.35$)

were still engaged in foraging activities. Our findings demonstrate that food intake significantly affects the spatial ecology in this extreme ambush forager. Unfed puff adders increased TDT, DTD, and DTM compared to fed snakes, yet total home ranges and core areas were similar in size between feeding groups. Taken together, these results indicate that unfed snakes traveled farther within similar-sized home ranges compared to fed snakes, suggesting that they invested greater effort in foraging. It is worth mentioning, however, that despite the lack of statistical significance, total home range and core area size for unfed snakes were on average more than three times larger compared to fed snakes (see Fig. 4), indicating high variance within feeding treatments.

We also used change in body condition indices (Δ BCI) by snakes as proxies for foraging success (Madsen and Shine 2000; Lindell 2011) and regressed this variable against TDT. Overall, TDT was negatively affected by Δ BCI. Interestingly, the difference in TDT between fed and unfed snakes was the highest in 2015 (see Fig. 3a), the year with the most consistent decrease in Δ BCI among unfed snakes, longest time spent foraging, and highest movement frequency by snakes. Further, investigating the relationship between TDT and Δ BCI by feeding treatments revealed different patterns; the relationship was significant for unfed snakes but not for fed snakes. Our regression analysis, therefore, supports our experimental findings that successful foragers travel less compared to relatively unsuccessful snakes and also suggests that snakes may use a threshold in Δ BCI as a proximate cue to adjust foraging activity.

The spatial response of snakes to variation in food availability and/or intake has been studied both descriptively and experimentally. Correlation analyses of snake-prey populations demonstrated that water pythons (*Liasis fuscus*) seasonally track the predictable distribution of their modal prey, dusky rats (*Rattus colletti*; Madsen and Shine 1996), but the same population failed to respond spatially to stochastic climatic events that depleted the rat population (Ujvari et al. 2011). Recently emerged prairie rattlesnakes (*Crotalus viridis*) migrated long distances away from their den sites, until they reached suitable foraging grounds where prey number was artificially augmented (Duvall et al. 1990). However, western diamond-backed rattlesnakes (*C. atrox*) showed a weak spatial response to food and water provisioning of potential prey, possibly because the experimental manipulation did not attract rodents, a major food source for many adult rattlesnakes (Nowak et al. 2015).

Other food supplementation studies, which are similar in design to ours, all used viperid snakes, and none provided robust evidence for an effect of food on spatial activity. Although the focus of their study was to investigate whether female western diamond-backed rattlesnakes increased reproductive frequency when supplementally fed, Taylor et al. (2005) reported no effect of food on home range size or surface activity. Further, male eastern massasaugas (*Sistrurus catenatus*) did not increase movement patterns in response to supplemental feeding (Tetzlaff 2015), but the males were fed and tracked largely during the breeding season when they were primarily involved in mate-searching activities. The only other snake study that reported an effect of food on spatial activity found that food-supplemented terciopelos (*Bothrops asper*) moved less frequently and made shorter movements than control snakes (Wasko and Sasa 2012). However, the results of the latter study are difficult to interpret because of the small sample of snakes and the treatment of multiple observations on individual snakes as independent.

Because of the low-energy requirements of ectotherms (Pough 1980; Spotila and Standora 1985; Nowak et al. 2008) and the ability of many snakes (including *Bitis* spp.) to endure long periods without food (i.e., a unique characteristic among the major vertebrate groups; McCue 2007; McCue et al. 2012), it has been hypothesized that a strategy for ambush-foraging snakes may be to simply decrease costly activities, such as “widely” prospecting for ambush sites when prey availability is low, and wait for better times (Beaupre 2002; Nowak et al. 2015). For example, ambush-hunting snakes have the ability to down-regulate their gastrointestinal tract during fasting to lower their metabolic rate (Secor and Diamond 2000). It is possible that this explanation accounts, at least partially, for the lack of spatial response exhibited by most snake species to date. However, our findings show that the spatial activity of puff adders is affected by food intake and therefore provides the first conclusive evidence that the spatial ecology of an ambush-foraging snake with extremely low energy requirements is affected by food intake.

We propose three factors that could explain why underfed puff adders increased movement. First, although puff adders in our population experience a high mortality rate at certain times of the year (XG and GJA unpublished data), only a single supplementally fed snake was eaten by a predator during the experiment. Therefore, the increased spatial activity of underfed puff adders during our study came at no survival cost. Second, the cost of rectilinear locomotion in a heavy-bodied snake such as puff adders may be less than expected. If the energetic cost of foraging forays is low, it may be advantageous for puff adders to invest in locating optimal ambush sites to catch prey. Evidence suggests that three forms of snake locomotion, namely, sidewinding, lateral undulation, and concertina locomotion, are energetically expensive (Walton et al. 1990; Secor et al. 1992). Yet, the cost of rectilinear locomotion, the typical mode used by viperid snakes, has never been quantified, and we encourage future studies to fill in this knowledge gap. Third, we experimentally fed the snakes just prior to the beginning of the mating season. Males travel surprisingly long distances to find females during the March to mid-June mating season, and although they sometimes forage, movement is essentially devoted to locating females (unpublished data). Consequently, males in relatively poor body condition may increase foraging effort to increase the "capital" that might be necessary to fuel upcoming mate-searching activities. The capital breeding strategy has almost exclusively been applied to female snakes (Gregory 2009, 2011), but future studies—including our ongoing work—may reveal where male snakes fit in the continuum of strategies found between the two extremes of capital and income breeding tactics.

Conclusions

Our study demonstrates that food intake affects the spatial ecology of a low-energy, ambush-foraging species. Despite their "sit-and-wait" foraging strategy and their low-energy intake/requirements, underfed puff adders traveled widely—at no survival cost—presumably to find appropriate ambush sites that maximize prey capture. Hence, the term "mobile ambusher" best describes this predator and other ambush-hunting snakes (Greene 1992, 1997). Although our data do not allow us to thoroughly examine the relationships between foraging success, distance from previous foraging locations, or residence time at ambush locations, puff adders may increase prey capture rate by traveling longer distances between consecutive ambush locations and/or decreasing residence time at individual ambush sites. Future studies should consider addressing these questions to test the functional link between foraging success and increased spatial activity in ambush predators. Finally, we believe that ambush-foraging snakes are ideally suited for food supplementation studies. The combination of radiotelemetry and remote video cameras, techniques for which these organisms are well suited, allows the collection of high-quality data that could ultimately elucidate the complex relationship between space use and foraging success in ambush predators.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (University of the Witwatersrand, animal protocol #2012–42-04). Specimens were collected under scientific research permits CPF6–0167 and CPF6–0024, issued by the Gauteng Department of Agriculture and Rural Development.

Informed consent Informed consent was obtained from all individual participants included in the study.

Appendix

Prey captured by naturally foraging male puff adders: amphibians (n = 7; 3 *Schismaderma carens*, 1 *Amietophrynus gutturalis*, 1 *Amietophrynus* sp., 1 bufonid, 1 unknown amphibian), aves (n = 2; 1 *Passer diffusus*, 1 *Phylloscopus trochilus*), mammals (n = 2; 1 *Rattus* sp., 1 soricid), and reptiles (n = 2; 1 *Acanthocercus atricollis*, 1 *Gerrhosaurus flavigularis*).

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