

EFFECTS OF LONG-TERM PREY SUBSTITUTION ON FOOD PREFERENCE FOR AN ENDEMIC INSULAR PIT VIPER (*BOTHROPS ALCATRAZ*)

JOÃO MIGUEL ALVES-NUNES^{1,3}, ADRIANO FELLONE², AND OTAVIO A. V. MARQUES^{1,2}

¹Programa de Pós-Graduação em Biodiversidade, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista “Júlio de Mesquita Filho,” R. Cristóvão Colombo, 2265, São José do Rio Preto, São Paulo, Brazil

²Programa de Pós-Graduação em Ecologia e Evolução, Universidade Federal de São Paulo - UNIFESP, R. Prof. Artur Riedel, 275 Diadema, São Paulo, Brazil

³Corresponding author; e-mail: joao.miguel.jm16@gmail.com

Abstract.—Snakes use chemical and visual cues to recognize prey, with genetics, experience, nutritional value, and natural prey restrictions affecting dietary choices. Nevertheless, the effect of substituting natural prey with ancestral prey on dietary preferences remains unknown. To address this question, we carried out captive experiments using the snake, Alcatrazes Lancehead (*Bothrops alcatraz*), which has a unique diet of ectotherms resulting from insular evolution, compared to the primarily endothermic diets of other members of the *B. jararaca* group. We used wild caught and captive born individuals to conduct two experimental procedures to determine the impact of the long-term (10+ y) feeding of endothermic prey (mammals) to both naive and experienced individuals on prey preference. Despite somewhat conflicting results, our study indicates that, despite long-term prey restriction, *B. alcatraz* prefers natural ectothermic prey items (centipedes and lizards) over the ancestral endothermic prey. Although this study is limited by sample size, it suggests that the dietary preference of *B. alcatraz* is innate and conserved, with significant implications for the conservation of both this species and other island snakes.

Key Words.—ancestral prey; sensory ecology; conservation; animal behavior

INTRODUCTION

Dietary selection in snakes, which is driven by differentiation and selection of sensory cues (Cooper and Burghardt 1990), is strongly influenced by the consequences of plasticity, thus providing ideal model systems to examine the interaction between innate and learned behaviors on prey preference (Burghardt and Hess 1968; Burghardt 1993). Snakes employ both chemical and visual cues to recognize their prey during hunting (Chiszar et al. 1976; Drummond 1985; Burghardt 1993). Variations exist among species, however, as some rely more on visual and thermal cues (Chiszar et al. 1981), and others more on chemical cues (Saviola et al. 2012). Nonetheless, many studies that examine chemoreception in snakes often overlook visual cues as initiators of vomeronasal chemoreception and consider only chemical stimuli (Saviola et al. 2011). Therefore, it is necessary to carry out studies using diverse methods to test the appropriate sensory stimuli that elicit responses from each species.

Sensory studies in different snake species have revealed several variables influencing prey preference, encompassing a hereditary genetic basis (Arnold 1981; Waters and Burghardt 2005), ontogenetic

changes (Saviola et al. 2012), experience inducible by a single meal (Burghardt and Krause 1999; Waters and Burghardt 2013), and prey nutritional value (Burghardt et al. 2000). An additional variable that has been scarcely addressed is prey restriction, which may lead to diverse and differing outcomes on prey preference. Studies involving North American water snakes, for instance, demonstrated that snakes exhibited more pronounced responses to the scent of prey they had consumed over a year prior than to odors of foods they had never ingested (Gove and Burghardt 1975). Research on the pit vipers Pygmy Rattlesnake (*Sistrurus miliarius*) and Black-tailed Rattlesnake (*Crotalus ornatus*) have provided evidence for an innate preference of native prey scent cues over those of captive House Mice (*Mus musculus*) in naive individuals for feeding periods ranging from 1–5 y (Holding et al. 2016; Emerson and Johnson 2023). All of these studies are based on prey restriction that aligns with the normal diet of the studied species.

Dietary differences occur among the pit vipers in the *B. jararaca* group, making it an ideal model system for the study of evolutionary sensory ecology. Among these species, the Alcatrazes Lancehead (*Bothrops alcatraz*) stands out as having a distinct biology and

restricted diet resulting from approximately 11,000 y of insular isolation on Alcatrazes Island, Brazil, where the absence of small mammals has resulted in a reliance on ectothermic prey (Marques et al. 2002; Graziotin et al. 2006; Barbo et al. 2012, 2016). This vulnerable species is endemic to Alcatrazes Island and evolved from an ancestor similar to the common pit viper, Lancehead (*Bothrops jararaca*; Marques et al. 2002). Remarkably, the absence of small mammals on the island has forced *B. alcatraz* to rely upon ectothermic prey, primarily centipedes (representing 70% of their food items) and lizards (Marques et al. 2002).

Based on the evolutionary history of *B. alcatraz*, our objective was to assess whether feeding only mammalian prey (similar to the ancestral condition) to captive individuals would influence prey preference of those individuals. To determine this, we used two experimental methods, one to isolate and determine variation in chemosensory response to scent cues and the other to test the interaction between visual, thermal, and olfactory stimuli from whole prey items. Because of phylogenetic conservatism and nutritional quality of the prey items, we expected that *B. alcatraz* would favor mammals over native prey, exhibiting plasticity in feeding preference.

MATERIALS AND METHODS

Animals.—We used eight snakes for experimental trials, five of which were wild caught (two males, three females) and three captive born from wild caught parents (one male, two females). We captured snakes on Alcatrazes Island in 2012, while captive born snakes were born in 2013. All snakes we used were adults based on snout-vent lengths > 44 cm (Marques et al. 2002). We individually housed snakes in plastic boxes (45 × 30 × 15 cm), furnished with a cardboard substrate and a water pot, and maintained at a temperature of approximately 25° C under natural light-dark photoperiod (approximately 12:12 h). Due to the difficulty of maintaining a natural feeding regime (centipedes and lizards), we fed snakes laboratory *Mus musculus* equivalent to 10% of their body weight on a monthly basis. Therefore, wild caught snakes had not been exposed to natural prey or their stimuli for more than 10 y, while captive bred individuals have never been exposed to natural prey items or their stimuli.

Experimental design.—For experiment 1, we investigated the olfactory feeding preference by

preparing and presenting aqueous extracts to snakes (Greene et al. 2002; Mullin et al. 2004; Pernetta et al. 2009; Weaver et al. 2012; Holding et al. 2016), then calculating the tongue-flick/attack score, TFAS(R), proposed by Cooper and Burghardt (1990) to quantify snake responses, which uses scents absorbed onto a cotton swab. Over a period of 300 s, we recorded the number of tongue movements directed at the swab. We prepared aqueous extracts of centipedes (*Cryptops* sp.), House Geckos (*Hemidactylus mabouia*), and *Mus musculus*. We collected centipedes and lizards (used in this and the following experiment) in an adjacent area of mainland Atlantic Forest near the Alcatraz Islands. We prepared all aqueous extracts by placing the prey item in a distilled water bath at a concentration of 0.25 g/ml for 4 h. We killed prey on the same day of the experiment. After preparation of aqueous extracts, we impregnated 15-cm sterilized swabs (Global Swab, Ltd. Harrow, England) with the appropriate extract, then passed 10 times on the dorsal and ventral surfaces of the body of the prey to increase the odor concentration. We only used the same swab for tests that were repeated on the same day.

For controls, we used a swab impregnated with distilled water as a negative control and a swab dipped in a cologne solution at a ratio of 1:10 as a pungency control. We presented scent cues in a randomized order by inserting a swab into a transparent acrylic block that was placed near the snake. We began experimental trials when the swab reached a distance of 5 cm from the head of the snake, eliciting an initial tongue-flick response. The performer of each experiment was blind to the conditions. We recorded all experimental trials for 30 s using a HDR-PJ200 camera (Sony Electronics, New York, New York, USA) and allowed a rest period of 480 s between each trial. We conducted all experiments in boxes measuring 26 × 18 × 15 cm. The acclimation period was approximately 1 d before the start of the experiment.

For experiment 2, we investigated whether the same patterns of feeding preference appear for living prey items following the methods established by Aubret et al (2006) and Li et al (2011). Living prey items provide visual, thermal, and olfactory cues. We placed snakes in a test arena measuring 58 × 40 × 34 cm, divided into two equal compartments by a sliding door on the other side of the arena (Fig. 1). We placed the snake on one side of the test arena, and living prey items (mice, centipedes or lizards) in one of two small containers covered with a 1 mm

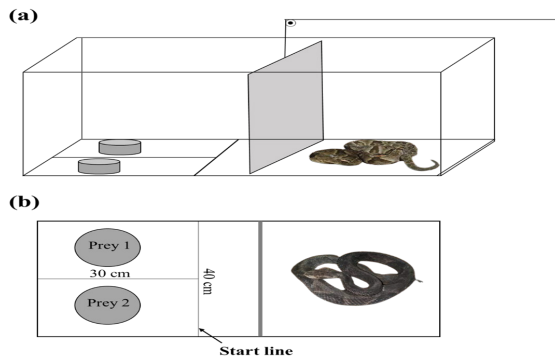


FIGURE 1. Diagram of the experiment 2 test arena used for investigating prey preferences in Alcatrazes Lancehead (*Bothrops alcatraz*), with a choice between two prey items that were placed in two small containers covered with a fine mesh screen and affixed to the substrate. Diagram a shows the general setup and b is the top view of the test arena.

mesh screen and fixed to the substrate on the other side of the arena, with the other container containing a different prey type. We marked the test arena substrate with a line separating the areas belonging to each prey box (Fig. 1).

After we introduced them to the empty compartment, we allowed the snakes to acclimate for 900 s. Then, we lifted the sliding door using a remote rope and pulley system. Experimental trials began as soon as the head of the snake crossed the starting line and continued for a period of 600 s. We recorded the time spent by the snake investigating around each prey container and counted tongue flicks directed at the prey box. Over a period of 600 s, we recorded the number of tongue movements directed at the prey box. We introduced the prey species in the trial room only during experimental trials and always kept them in a separate room to avoid introducing mixed odors into the experimental room or habituating the snakes to environmental odors (Burghardt and Layne 1995). We conducted three randomized trials: (1) mouse in one container/lizard in the other container; (2) mouse/centipede; and (3) centipede/lizard. As in experiment 1, we tested each animal three times.

Statistical analysis.—We used the scoring system for snakes that are active predators described by Cooper and Burghardt (1990). This method produces a tongue flick attack score (TFAS), where TFmax is the maximum number of tongue flicks elicited by the trial condition, TL is the length of the trial in seconds, and latency is the time in seconds to attack or to adopt the ambush posture toward the prey stimulus (Clark 2004). Here we adopt latency as attacks. Because we tested individual snake responses to multiple

prey cues, we used the TFAS(R) score for repeated measures on a single individual:

$$\text{TFAS(R)} = \text{TFmax} + (\text{TL} - \text{latency})$$

This scoring system assumes that an attack of a prey item scent cue is a stronger response than any number of tongue flicks, and latency to attack decreases with increasing response to the stimulus. Therefore, if an attack does not occur, TFAS(R) is equal to the number of tongue flicks that occurred during the presentation period.

For data analysis, we used Generalized Linear Mixed Models (GLMM) with Poisson distribution and a log link function. For experiment 1, TFAS(R) was the response variable, fixed factors were prey type and birthplace (captive born or wild caught), with individual and sex being input as random factors. For experiment 2, time of prey box inspection (measured in seconds) was the response variable, prey type was the fixed factor, with individual and sex being input as random factors. We transformed time using the $\log+1$ transformation to reduce the impact of extreme values and stabilize the variance. We used the same model to analyze these data as the one used in the previous experiment. The fixed variables included only the types of prey, while the random variables were the individuals and sex. We performed a Tukey's Test for *post hoc* pairwise comparisons. For all models, we evaluated data dispersion analysis, homoscedasticity, and delineate tests using model diagnostic values and plots, with the help of the package DHARMA (Hartig 2016) in R version 4.04 (R Core Team 2021).

RESULTS

In Experiment 1, the TFAS(R) was influenced by the interaction between prey type and birthplace. Mouse scent elicited a significantly higher number of tongue-flicks in both captive-born and wild-caught *B. alcatraz*, with a predatory attack by one snake from each collection group ($Z = 4.796$; $P < 0.001$). Additionally, there was a significantly higher TFAS(R) for centipedes over lizards among snakes born on the island ($Z = -2.708$; $P = 0.007$; Fig. 2). There was no apparent difference in TFAS(R) for prey among the groups with different birth origins ($Z = -0.655$; $P = 0.512$; Table 1).

In Experiment 2, the snakes reacted differently in relation to the tested prey. Among the snakes born in captivity, only one demonstrated interest in any prey during the experiment. This individual demonstrated

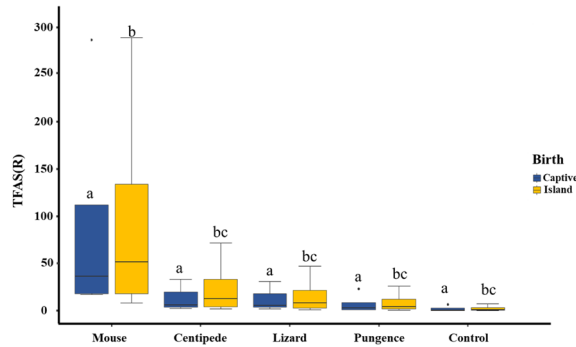


FIGURE 2. The tongue-flick/attack score (TFAS[R]) of Alcatrazes Lancehead (*Bothrops alcatraz*) from the experiment of odors from a swab impregnated with aqueous scent cues of different prey for snakes born in captivity (blue) and on the island (yellow). Horizontal lines are the medians, boxes are the second and third quartiles, vertical lines extending from the box indicate the dispersion of the data, excluding outlying values. The points beyond the whiskers represent outliers or extreme values in the data distribution. Different lowercase letters indicate a significant difference between groups ($P < 0.050$).

interest exclusively for centipedes, performing 35 tongue flicks and investigating the box with this prey 57.1% of the time. The snakes born on the island also showed a preference for centipedes over other prey. The tongue flick rate was significantly higher for centipedes compared to mice and lizards, with values 82% and 67% higher, respectively (centipede/mouse: $Z = -7.623$, $P < 0.001$; centipede/lizard: $Z = -6.224$, $P < 0.001$; Fig. 3). Additionally, snakes

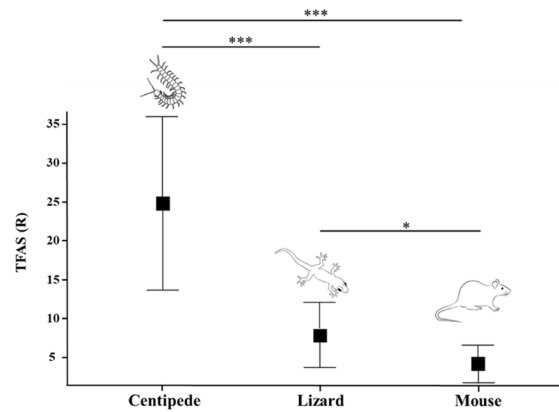


FIGURE 3. The mean (\pm standard deviation) of tongue-flick/attack score, TFAS(R), of Alcatrazes Lancehead (*Bothrops alcatraz*) born on the island for different prey (centipede, lizard, mouse) placed inside small containers covered with mesh screens. Asterisks and horizontal bars indicate significant differences in the frequency of behaviors in relation to the type of prey stimulus among each species (*** $P < 0.001$ and * $P < 0.050$).

significantly preferred living lizards over living mice prey, with a 47% higher TFAS(R) rate ($Z = -2.436$, $P = 0.015$; Table 1). The time that *B. alcatraz* spent investigating the prey box also varied depending on the type of food item. The snakes spent significantly more time inspecting the centipede box than those with mice ($Z = -1.980$, $P = 0.048$; Fig. 4, Table 2).

TABLE 1. Index model summary for Tongue Flick Attack Scores for multiple prey, TFAS(R), for Alcatrazes Lancehead (*Bothrops alcatraz*) with index estimates, standard errors (SE), Z test scores, and P values of significance for experiments 1 and 2. The intercept for experiment 1 was prey (mouse) and birth (captivity) and for experiment 2 was prey (lizard).

Fixed variables	Estimate	SE	Z value	P value
Experiment 1				
(Intercept)	384.71	0.802	4.796	< 0.001
Prey (Centipede)	-214.74	0.162	-13.193	< 0.001
Prey (Lizard)	-222.15	0.168	-13.201	< 0.001
Prey (Pungence)	-249.83	0.186	-13.398	< 0.001
Prey (Control)	-373.50	0.336	-11.096	< 0.001
Birth (Island)	-0.452	0.690	-0.655	0.513
Prey (Centipede): Birth (Island)	0.756	0.197	3.831	0.001
Prey (Lizard): Birth (Island)	0.399	0.214	1.860	0.063
Prey (Pungence): Birth (Island)	0.085	0.254	0.335	0.738
Prey (Control): Birth (Island)	0.041	0.464	0.089	0.929
Experiment 2				
(Intercept)	1.392	0.818	1.701	0.089
Prey (centipede)	1.091	0.175	6.224	< 0.001
Prey (mouse)	-0.626	0.256	-2.436	0.015

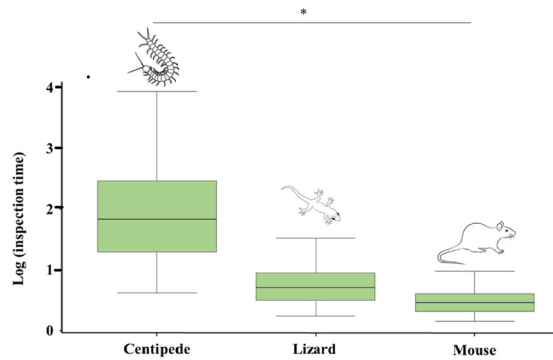


FIGURE 4. Inspection time of Alcatrazes Lancehead (*Bothrops alcatraz*) born on the island for different prey (centipede, lizard, mouse) placed inside small containers covered with mesh screens. Horizontal lines are the medians, boxes are the second and third quartiles, vertical lines extending from the box indicate the dispersion of the data, excluding outlying values. The points beyond the whiskers represent outliers or extreme values in the data distribution. The asterisk and horizontal bar indicate a difference in inspection time between centipedes and mice. The asterisk (*) and horizontal bar indicate significant differences in the frequency of behaviors in relation to the type of prey stimulus among each species ($P < 0.050$).

DISCUSSION

Our experiments yielded contradictory results, contrary to what we expected, and showed that feeding preference is innate in *B. alcatraz*. The results of experiment 1 suggested that both captive born and wild caught individuals prefer aqueous scent cues from mice but experiment 2 demonstrated that centipedes were preferred when presented with living prey items. Consistent with the findings of the present study, Burghardt and Abeshaheen (1971) discovered that newly hatched Fox Snakes (*Elaphe vulpina*) did not attack cotton swabs soaked in prey extract but responded with attacks to a wide range of visual stimuli. The movement of the prey, coupled with its odor, enhances the investigative and tongue-flicking response in some snakes (Burghardt and Denny 1983). Additionally, a strong response by the snakes to chemical cues from rodents may arise not from feeding preference, but from concentrated chemical cues from the numerous exocrine glands in mammals (Mateo and Johnston 2000), potentially explaining the high tongue flicking rates in response to the mouse scent cue during experiment 1 of our study. Furthermore, mammals are present in the diet of most *Bothrops* species, and were probably in the diet of the ancestors of the *B. jararaca* group (Martins et al., 2002). Thus, a high olfactory sensitivity to the smell of these animals may be conservative in this group. Other studies have shown that snake species

TABLE 2. Alcatrazes Lancehead (*Bothrops alcatraz*) time inspection model summary with index estimates, standard errors (SE), and Z and P test of significance for experiment 2. Intercept = Prey (centipede).

Fixed variables	Estimate	SE	Z	P
(Intercept)	0.5302	0.5624	0.943	0.3458
Prey (mouse)	-1.3958	0.7050	-1.980	0.0477
Prey (lizard)	-0.9491	0.5945	-1.596	0.1104

that specialize on mammalian prey respond more strongly to olfactory cues, while species that feed on different prey types tend to respond more strongly to visual cues, thus providing evidence for a correlation between the evolution of prey preference and the type of cue used for primary prey detection by snakes (Saviola et al 2012).

Given that the diet of *B. alcatraz* has been restricted to ectothermic prey (centipedes and lizards) for at least 11,000 y (Martins 2002), it is possible that this species has adapted to respond primarily to visual stimuli to elicit a predatory response. The second expectation that does not exclude the first is based on the phenomenon of food neophobia described by Barnett (1958). This phenomenon is defined as the fear of eating new foods and the hesitation to ingest them (Modlinska 2022). During experiment 1, based on olfactory ability, we observed a higher score for mice. During feeding management, mice are killed before being offered to the snakes, providing prey at ambient temperature and without movement. On Alcatrazes Island, where *B. alcatraz* lives, there are no endothermic prey, only potential aerial endothermic predators (Marques et al. 2002). This species possesses loreal pits sensitive to thermal stimuli (Goris and Terashima 1976). In the experiment with live prey, sensory stimuli (olfactory, visual, and thermal) were produced by the prey. Therefore, due to the olfactory characteristic used in experiment 1 and the naivety of the snakes towards endothermic prey, we propose that *B. alcatraz* avoided scents associated with mice due to food neophobia and maintained its innate preference for natural prey (centipedes and lizards). Future studies of food neophobia in this species could help interpret these findings.

The continued preference for natural prey, despite being fed nonnative prey for more than 10 y in captivity, provides evidence that prey preference in *B. alcatraz* is innate. Evidence for an innate origin of prey preference has been similarly documented in other pit vipers (Clark 2004). Dusky Pigmy Rattlesnakes (*Sistrurus miliarius barbouri*) primarily feed on lizards and anurans, but even when fed

mice from birth, displayed a preference for lizard scents (Holding et al. 2016). Naive neonate Eastern Black-tailed Rattlesnakes (*Crotalus ornatus*) that fed exclusively on non-native mammalian prey in captivity when exposed to native mammal and lizard stimuli exhibited their preference for these wild prey (Emerson and Johnson 2023). Similar patterns have also been shown in Western Terrestrial Garter Snakes (*Thamnophis elegans*), with one population that specializes in preying on banana slugs (*Ariolimax* spp.) producing offspring that maintain this dietary preference. On the other hand, this preference for slugs has not been verified in populations of garter snakes that do not interact with the slugs in nature (Arnold 1980). In addition to responses to prey stimuli, other pit viper species are known to possess prey-specific venoms that show more toxic effects to a narrow range of prey species that they frequently ingest (Gibbs and Mackessy 2009). *Bothrops alcatraz* venom has been shown to exhibit more toxic effects on arthropods than on mammalian prey items (Narvaes 2007), further indicating an evolutionary basis for dietary specialization.

Although our study has limitations on sample size and the sourcing of individuals used for our experiments, it indicates that prey preference of *B. alcatraz*, like that of a few other pit viper species, is likely innate in origin, as individuals used for this study had been fed nonnative prey items for more than 10 y and the captive-born individuals have never been exposed to native stimuli. Additionally, we show how use of varied methodologies can elucidate patterns about other sensory cues that do not become apparent when focusing on a single sense using a singular experimental methodology. Given that *B. alcatraz* is a vulnerable species and lives in insular inhabitant of an area with highly restricted access, this research and other such studies provide valuable information to guide and inform wise management practices into the future.

Acknowledgments.—We thank Selma M. Almeida-Santos for providing the animals for the study and being always available to help intellectually. We would like to thank Kelly Kish for the technical animal maintenance services and Rulon W. Clark for the encouragement and criticism of the work. We would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (grant number 311977/2023-5) and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP- 20/12658-4) for funding. An animal use

ethics committee (CEUA no 2825020921) cleared the animals used during this study.

LITERATURE CITED

- Arnold, S.J. 1980. The microevolution of feeding behavior. Pp. 409-453 In *Foraging Behavior: Ecological, Ethological and Psychological Approaches*. Kamil, A., and T. Sargent (Eds.). Garland Press, New York, New York, USA.
- Arnold, S.J. 1981. Behavioral variation in natural populations. I. Phenotypic, genetic and environmental correlations between chemoreceptive responses to prey in the garter snake, *Thamnophis elegans*. *Evolution* 35:489–509.
- Aubret, F., G.M. Burghardt, S. Maumelat, X. Bonnet, and D. Bradshaw. 2006. Feeding preferences in 2 disjunct populations of Tiger Snakes, *Notechis scutatus* (Elapidae). *Behavioral Ecology* 17:716–725.
- Barbo, F.E., J.L. Gasparini, A.P. Almeida, H. Zaher, F.G. Grazziotin, R.B. Gusmão, J.M.G. Ferrarini and R.J. Sawaya. 2016. Another new and threatened species of lancehead genus *Bothrops* (Serpentes, Viperidae) from Ilha dos Franceses, Southeastern Brazil. *Zootaxa* 4097:511–529.
- Barbo, F.E., F.G. Grazziotin, I. Sazima, M. Martins, and R.J. Sawaya. 2012. A new and threatened insular species of lancehead from southeastern Brazil. *Herpetologica* 68:418–429.
- Barnett, S. A. 1958. Experiments on ‘neophobia’ in wild and laboratory rats. *British Journal of Psychology* 49:195–201.
- Burghardt, G.M. 1969. Comparative prey-attack studies in newborn snakes of the genus *Thamnophis*. *Behaviour* 77–114.
- Burghardt, G.M. 1993. The comparative imperative: genetics and ontogeny of chemoreceptive prey responses in natricine snakes. *Brain, Behavior and Evolution* 41:138–146.
- Burghardt, G.M., and J.P. Abeshaheen. 1971. Responses to chemical stimuli of prey in newly hatched snakes of the genus *Elaphe*. *Animal Behaviour* 19:486–489.
- Burghardt, G.M., and D. Denny. 1983. Effects of prey movement and prey odor on feeding in garter snakes. *Zeitschrift für Tierpsychologie* 62:329–347.
- Burghardt, G.M., and E.H. Hess. 1968. Factors influencing the chemical release of prey attack in newborn snakes. *Journal of Comparative and*

- Physiological Psychology 66:289–295.
- Burghardt, G.M., and M.A. Krause. 1999. Plasticity of foraging behavior in garter snakes (*Thamnophis sirtalis*) reared on different diets. *Journal of Comparative Psychology* 113:277–285.
- Burghardt, G.M., and D.G. Layne. 1995. Effects of ontogenetic processes and rearing conditions. Pp. 165–185 *In* Health and Welfare of Captive Reptiles. Warwick, C., F.L. Frye, and J.B. Murphy (Eds). Springer Netherlands, Dordrecht, Netherlands.
- Burghardt, G.M., D.G. Layne, and L. Konigsberg. 2000. The genetics of dietary experience in a restricted natural population. *Psychological Science* 11:69–72.
- Chiszar, D., K. Scudder, and L. Knight. 1976. Rate of tongue flicking by garter snakes (*Thamnophis radix haydeni*) and rattlesnakes (*Crotalus v. viridis*, *Sistrurus catenatus tergeminus*, and *Sistrurus catenatus edwardsi*) during prolonged exposure to food odors. *Behavioral Biology* 18:273–283.
- Chiszar, D., S.V. Taylor, C.W. Radcliffe, H.M. Smith, and B. O'Connell. 1981. Effects of chemical and visual stimuli upon chemosensory searching by garter snakes and rattlesnakes. *Journal of Herpetology* 15:415–423.
- Clark, R.W. 2004. Timber Rattlesnakes (*Crotalus horridus*) use chemical cues to select ambush sites. *Journal of Chemical Ecology* 30:607–617.
- Cooper, W.E., Jr. 1998. Prey chemical discrimination indicated by tongue-flicking in the eublepharid gecko *Coleonyx variegatus*. *Journal of Experimental Zoology* 281:21–25.
- Cooper, W.E., and G.M. Burghardt. 1990. A comparative analysis of scoring methods for chemical discrimination of prey by squamate reptiles. *Journal of Chemical Ecology* 16:45–65.
- Drummond, H. 1985. The role of vision in the predatory behaviour of natricine snakes. *Animal Behaviour* 33:206–215.
- Emerson, J.D., and J.D. Johnson. 2024. Evidence for an innate basis of prey preference in a desert ambush predator. *Evolutionary Ecology* 38:293–303.
- Falciglia, G.A., and P.A. Norton. 1994. Evidence for a genetic influence on preference for some foods. *Journal of the American Dietetic Association* 94:154–158.
- Gibbs, H.L., and S.P. Mackessy. 2009. Functional basis of a molecular adaptation: prey-specific toxic effects of venom from *Sistrurus* rattlesnakes. *Toxicon* 53:672–679.
- Goris, R.C., and S. Terashima. 1976. The structure and function of the infrared receptors of snakes. *Progress in Brain Research* 43:159–170.
- Gove, D., and G.M. Burghardt. 1975. Responses of ecologically dissimilar populations of the water snake *Natrix s. sipedon* to chemical cues from prey. *Journal of Chemical Ecology* 1:25–40.
- Grazziotin, F.G., M. Monzel, S. Echeverrigaray, and S.L. Bonatto. 2006. Phylogeography of the *Bothrops jararaca* complex (Serpentes: Viperidae): past fragmentation and island colonization in the Brazilian Atlantic Forest. *Molecular Ecology* 15:3969–3982.
- Greene, M.J., S.L. Stark, and R.T. Mason. 2002. Predatory response of Brown Tree Snakes to chemical stimuli from human skin. *Journal of Chemical Ecology* 28:2465–2473.
- Hartig, F. 2016. DHARMA: residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.1.2. <https://cran.r-project.org/web/packages/DHARMA/index.html>.
- Holding, M.L., E.H. Kern, R.D. Denton, and H.L. Gibbs. 2016. Fixed prey cue preferences among Dusky Pigmy Rattlesnakes (*Sistrurus miliarius barbouri*) raised on different long-term diets. *Evolutionary Ecology* 30:1–7. <https://doi.org/10.1007/s10682-015-9787-2>.
- Marques, O.A.V., and I. Sazima. 2009. Old habits die hard: mouse handling by a pitviper species on a rodent-free island. *Amphibia-Reptilia* 30:435–438.
- Marques, O.A.V., M. Martins, and I. Sazima. 2002. A new insular species of pitviper from Brazil, with comments on evolutionary biology and conservation of the *Bothrops jararaca* group (Serpentes, Viperidae). *Herpetologica* 58:303–312.
- Martins, M., and F.D.B. Molina. 2008. Panorama geral dos répteis ameaçados do Brasil. *Livro vermelho da fauna brasileira ameaçada de extinção*. Brasília: Ministério do Meio Ambiente 2:327–373.
- Martins, M., O.A.V. Marques, and I. Sazima. 2002. Ecological and phylogenetic correlates of feeding habits in Neotropical pitvipers of the genus *Bothrops*. Pp. 307–328 *In* Biology of the Vipers. Schuett, G.W., M. Höggren, M.E. Douglas, and H.W. Greene (Eds.). Eagle Mountain Publishing, Eagle Mountain, Utah, USA.
- Mateo, J.M., and R.E. Johnston. 2000. Kin recognition and the 'armpit effect': evidence of self-referent phenotype matching. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 267:695–700.

- Modlinska, K. 2022. Food neophobia. Pp. 2778–2780 *In* Encyclopedia of Animal Cognition and Behavior. Vonk, J., and T.K. Shackelford (Eds.). Springer International Publishing, Cham, Switzerland.
- Mullin, S.J., H. Imbert, J.M. Fish, E.L. Ervin, and R.N. Fisher. 2004. Snake (Colubridae: *Thamnophis*) predatory responses to chemical cues from native and introduced prey species. *Southwestern Naturalist* 49:449–456.
- Narvaes, L.V.P. 2007. Isolamento e caracterização de toxinas do veneno de *Bothrops alcatraz* Marques, Martins e Sazima, 2002 e aspectos coevolutivos com a dieta. Ph.D. Dissertation, Universidade de São Paulo, São Paulo, Brazil. 30 p.
- Pernetta, A.P., C.J. Reading, and J.A. Allen. 2009. Chemoreception and kin discrimination by neonate Smooth Snakes, *Coronella austriaca*. *Animal Behaviour* 77:363–368.
- R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>.
- Saviola, A.J., W.E. Lamoreaux, R. Opferman, and D. Chiszar. 2011. Chemosensory response of the threatened Eastern Indigo Snake (*Drymarchon couperi*) to chemical and visual stimuli. *Herpetological Conservation and Biology* 6:449–454.
- Saviola, A., V. McKenzie, and D. Chiszar. 2012. Chemosensory responses to chemical and visual stimuli in five species of colubrid snakes. *Acta Herpetologica* 7:91–103.
- Waters, R.M., and G.M. Burghardt. 2005. The interaction of food motivation and experience in the ontogeny of chemoreception in crayfish snakes. *Animal Behaviour* 69:363–374.
- Waters, R.M., and G.M. Burghardt. 2013. Prey availability influences the ontogeny and timing of chemoreception-based prey shifting in the Striped Crayfish Snake, *Regina alleni*. *Journal of Comparative Psychology* 127:49–55.
- Weaver, R.E., W.H. Clark, and D.C. McEwen. 2012. Prey chemical discrimination by the Desert Nightsnake (*Hypsiglena chlorophaea*): a comparison of invertebrate and vertebrate prey. *Journal of Herpetology* 46:523–526.



João MIGUEL ALVES-NUNES is a Biologist who graduated from the University of Mogi das Cruzes (UMC), Cruzes, Brazil, and earned a Master's degree in Ecology and Animal Behavior from the São Paulo State University (UNESP), São Paulo, Brazil. He is interested in the complex interaction between variables and sensory factors in the predator-prey relationship, analyzing the perspective of an animal as both prey and predator. João works in the fields of sensory ecology and animal behavior of vertebrates and invertebrates, with an emphasis on venomous animals. (Photographed by Lais Caravelli H. Zago).



ADRIANO FELLONE earned a Bachelor's degree in Biological Sciences from Universidade Paulista, São Paulo, Brazil (2013). Since 2010, he has been working as a Scientific Research Support Agent at the Butantan Institute, São Paulo, Brazil, focusing on the management and conservation of endangered snakes in the Laboratory of Ecology and Evolution. Currently, Adriano is a Master's student in the Graduate Program in Ecology and Evolution (PPGEE) at the Federal University of São Paulo (UNIFESP), São Paulo, Brazil, where he develops projects on the behavior and feeding preferences of Neotropical snakes. (Photographed by João Miguel A. Nunes).



OTAVIO A.V. MARQUES has been a Scientific Researcher at the Instituto Butantan, São Paulo, Brazil, since 1993 and works in the Laboratório de Ecologia e Evolução. He conducts research on the natural history, ecology, and evolution of snakes, including studies on the insular pitvipers. Otavio is focused on studies that generate information on threatened species that support conservation actions. He is also actively involved in scientific dissemination of information aimed at the general public. (Photographed by Otavio A.V. Marques).