

# Strong response to anuran chemical cues by an extreme dietary specialist, the eastern hog-nosed snake (*Heterodon platirhinos*)

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**Abstract:** Squamate reptiles use the lingual–vomeronasal system to identify food using only chemical cues. In lizards, most of which are dietary generalists that consume a wide variety of arthropods and other small animals, correlated evolution has occurred between addition of plants to the diet and responsiveness to plant chemical cues. In snakes, many of which are dietary specialists, several studies have detected differences in response strength among populations that correspond to the importance of prey types in different geographic locations. In one species of *Thamnophis* Fitzinger, 1843, such variation in responsiveness has been demonstrated to have a genetic basis. We studied tongue-flicking and biting responses to chemical cues from a range of potential prey types by nine ingestively naive hatchlings of the eastern hog-nosed snake (*Heterodon platirhinos* Latreille in Sonnini and Latreille, 1801), which is an extreme toad specialist that less frequently eats other anurans. The snakes responded most strongly to chemical cues from the southern toad (*Bufo terrestris* (Bonnaterre, 1789)), as indicated by significantly greater tongue-flick rate. Only two individuals bit in response to chemicals cues, both to the toad cues. Elevated tongue-flick rates were also elicited by chemical cues from the green frog (*Rana clamitans* Latreille in Sonnini de Manoncourt and Latreille, 1801), but the mean rate for frog cues was less than for toad cues. Responses to other potential prey types did not differ from those of the control stimuli. Our findings are consistent with those of several other investigators in showing close correspondence between the inclusion and importance of dietary items and the intensity of chemosensory investigation in snakes. Studies of diverse dietary specialists are needed to establish the generality of this relationship in snakes and to demonstrate that diet and chemosensory responses to food cues co-evolve.

**Résumé :** Les reptiles squamates utilisent leur système lingual–voméronasal pour identifier leur nourriture à l'aide des seuls signaux chimiques. Chez les lézards, dont la plupart ont une régime alimentaire généraliste et qui consomment une variété d'arthropodes et d'autres petits animaux, il y a eu au cours de l'évolution une corrélation entre l'addition de plantes au régime et la capacité de réagir aux signaux chimiques des plantes. Chez les serpents, dont plusieurs ont un régime spécialisé, un grand nombre d'études ont décelé des différences de force de la réaction au sein des populations, correspondant à l'importance des divers types de proies dans les différentes régions géographiques. Chez une espèce de *Thamnophis* Fitzinger, 1843, il a été démontré que cette variation de réactivité a une base génétique. Nous avons étudié les réactions de claquement de la langue et de morsure en réaction à des signaux chimiques provenant d'une gamme de types potentiels de proies chez neuf couleuvres à nez plat (*Heterodon platirhinos* Latreille in Sonnini and Latreille, 1801) néonates, n'ayant jamais ingéré de proies; cette couleuvre est un prédateur fortement spécialisé des crapauds qui mange plus rarement d'autres anoures. Les couleuvres réagissent le plus fortement aux signaux chimiques du crapaud austral (*Bufo terrestris* (Bonnaterre, 1789)), comme l'indique le nombre significativement plus élevé de claquements de la langue. Seuls deux individus ont mordu en réaction aux signaux chimiques, tous les deux aux signaux de crapauds. Les signaux chimiques provenant de la grenouille verte (*Rana clamitans* Latreille in Sonnini de Manoncourt and Latreille, 1801) provoquent aussi des taux accrus de claquement de la langue, mais le taux de claquement en réaction aux signaux de grenouilles est inférieur à celui aux signaux de crapauds. Les réactions aux autres types potentiels de proies ne diffèrent pas de celles provoquées par les stimulus témoins. Nos résultats s'accordent avec ceux de plusieurs autres recherches en montrant une correspondance étroite entre l'inclusion et l'importance de certains éléments dans le régime et l'intensité de la recherche chimiosensorielle chez les serpents. Il serait nécessaire d'étudier une gamme étendue de serpents à régime alimentaire spécialisé afin d'établir la généralisation de cette relation chez les serpents et démontrer qu'il y a une co-évolution du régime alimentaire et des réactions chimiosensorielles aux signaux de la nourriture.

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## Introduction

The ability of snakes to detect, identify, and locate prey using chemical cues sampled lingually and sensed by the vomeronasal system has been the subject of intensive investigation (Burghardt 1970; Chiszar and Scudder 1980; Arnold 1981a). Tongue-flicking brings the ventral surfaces of the tongue's tips into contact with chemicals on substrates, and chemicals that adhere to the tongue are drawn into the mouth and pass through the vomeronasal ducts to the vomeronasal chemosensory epithelium. Therefore, tongue-flick rate provides a convenient index of the degree of chemosensory investigation elicited by various stimuli. Biting, which suggests predation attempts, indicates strong trophic response to a potential food stimulus.

Two types of evidence from studies using tongue-flicking rates and bites directed to sources of chemical stimuli suggest that diet may influence the strength of chemosensory responses to prey: coordinated intraspecific variation among populations in diet and response strength and strong, exclusive responses to chemical cues from their prey by extreme prey specialists. Geographic variation in response strength to potential prey taxa corresponds to geographic variation in diet in several species, including garter snake (*Thamnophis elegans* Baird and Girard, 1853; Arnold 1977, 1981a, 1981b), coachwhip (*Masticophis flagellum* (Shaw, 1802); Cooper et al. 1990), and racer (*Coluber constrictor* L., 1758; Cooper et al. 2000). One contrary observation is that the Japanese rat snake (*Elaphe quadrivirgata* (Boie, 1826)) responds strongly to frog chemical cues on an island where frogs are absent (Tanaka et al. 2001). However, because fewer island individuals responded to frog stimuli at all than did snakes from the mainland (Tanaka et al. 2001), natural selection may have begun to reduce response to frog stimuli in the absence of frogs.

Several studies suggest that dietary specialists respond much more strongly to chemical cues of their prey than to cues from other palatable taxa. For example, the crayfish specialists *Regina grahami* Baird and Girard, 1853 and *Regina septemvittata* (Say, 1825) exhibit strong responses to crayfish chemical cues, but not to chemical stimuli from a wide range of other taxa (Burghardt 1968; Waters and Burghardt 2005). Smooth greensnakes (*Ophedrys vernalis* (Harlan, 1827)), which specialize on arthropods, exhibited much stronger responses to cricket stimuli than to stimuli from any of 13 non-arthropod species representing several phyla (Burghardt 1970). The bandy-bandy (*Vermicella annulata* (Gray, 1841)), an ophiophage specializing on blind-snakes, follows scent trails of blindsnakes (Greenlees et al. 2005), and blindsnakes, which are ant specialists, in turn follow chemical trails of ants (Watkins et al. 1967; Gehlbach et al. 1971; Webb and Shine 1992). Hispaniolan vine snakes (*Uromacer frenatus* (Günther, 1865); Henderson et al. 1983) and the Australian elapid *Hoplocephalus bungaroides* Wagler, 1830 are lizard specialists that respond most strongly to lizard chemical cues (Downes 2002).

Geographic covariation of diet and chemosensory responses to various prey might be based on experience, genetic differences among populations, or both. Testing ingestively naive hatchlings, i.e., ones that have never eaten, has allowed investigators to eliminate experience as a factor

in many of the studies already cited. In the only study that has examined genetic bases of chemosensory responses to prey, three and possibly more groups of genes were found to influence responses by *T. elegans* to amphibians, slugs and leeches, and the particularly toxic salamanders of the genus *Taricha* Gray, 1850 (Arnold 1981a).

Although prey chemical discrimination has been demonstrated in a variety of taxa (e.g., Burghardt 1970; Cooper 1990; Lancaster and Wise 1996; Downes 2002; Cooper et al. 2000), the majority of studies focus on natricines (e.g., Burghardt 1968; Arnold 1981a; Waters and Burghardt 2005), many of which have rather broad diets. There are, however, numerous species of snakes in other taxa that are dietary specialists, some of them extreme specialists. This diversity offers an excellent opportunity to conduct studies designed specifically to test the generality of the hypothesis that each specialist responds most strongly to chemical cues from its own prey.

We report such a study for the eastern hog-nosed snake (*Heterodon platirhinos* Latreille in Sonnini and Latreille, 1801), which is a known dietary specialist that eats primarily toads of the genus *Bufo* Laurenti, 1768, but also consumes some frogs (Conant and Collins 1998). We studied tongue-flicking and biting responses to chemical cues from numerous potential prey including the southern toad (*Bufo terrestris* (Bonnaterre, 1789)), a toad sympatric with *H. platirhinos*. Using ingestively naive hatchlings as subjects allowed us to eliminate prior experience with food as a factor influencing responsiveness.

## Methods

The experimental subjects were nine *H. platirhinos* that hatched between 11 and 14 August 2006 from a single clutch incubated on moist vermiculite at 30 °C. Although testing multiple clutches is preferable to eliminate the possibility of clutch-specific effects, it is not feasible for many species that are difficult to obtain. Fortunately, the probability that a single clutch of a prey specialist shows strong response only to cues from the special prey merely by chance must be extremely low because dietary generalists respond strongly to chemical cues from a wide taxonomic range of prey. The mother was collected 16 km northwest of Tuscaloosa, Alabama. The hatchlings were shipped from Tuscaloosa to Fort Wayne, arriving on 23 August. In Fort Wayne the snakes were kept in an animal care facility accredited by the American Association for Laboratory Animal Care. Light was provided by fluorescent bulbs on a 12 h light : 12 h dark cycle and the ambient temperature was fixed at 28 °C.

In the laboratory at Indiana University – Purdue University Fort Wayne, the snakes were housed individually in clean translucent plastic terraria (15 cm × 9 cm × 9 cm) having tight-fitting lids further secured by duct tape. Each box contained a plastic shelter and a water bowl. Water was available continuously, but the snakes were not fed. None of them had eaten between hatching and testing. Prior to being tested, the snakes lacked any experience with prey. The only opportunity for them to have experience with prey vomors, which are similar to odors but are sensed by the vomeronasal system rather than the olfactory system (Cooper

and Burghardt 1990a), would have been during transport, but toads and snakes were shipped in separate plastic containers within a larger box. Because vomodorants tend to be large molecules having high information content and low volatility (Cooper and Burghardt 1990a) and because such nonvolatile molecules are typically sampled by direct contact with the tongue, the hatchlings are very unlikely to have encountered detectable prey chemical cues before the experiments. Responses by hatchlings were conducted before any had shed their skins for the first time.

We measured lingual and biting responses by the ingestively naive snakes to cotton-tipped applicators bearing chemical stimuli from several potential prey species, most notably the southern toad (*B. terrestris*), which is a common prey of *H. platirhinos*. Additional chemical stimuli were cologne, which served as an indicator of response levels to a highly odorous nonfood stimulus, and deionized water, which is an odorless control for response levels in the experimental setting.

The experimenter began stimulus preparation by dipping the cotton tip of a 15 cm wooden applicator into deionized water, except in the case of the cologne stimuli. The water itself was the odorless control substance used to gauge responses in the absence of prey or predator cues. Cologne stimuli were prepared by dipping a dry applicator into Mennen Skin Bracer, Spice Scent<sup>®</sup> diluted by three parts deionized water to one part cologne. Undiluted cologne appears to be noxious to some squamate reptiles, but chemosensory response levels are not affected by cologne diluted 3:1 in lizards (Cooper et al. 2003). Experimental stimuli from animals were obtained by bringing cotton tips wetted with deionized water into contact with the skin of the potential prey and rolling it across dorsal and ventral surfaces on the head and body.

Prior to any trials for a particular experiment, cage lids were removed and the animals were not tested until at least 5 min later. This was done to minimize the effects of disturbance on locomotion because tongue-flick rates increase during locomotion (as in strike-induced chemosensory searching; Chiszar et al. 1983; Cooper et al. 1994). To begin a trial, the experimenter slowly approached a terrarium and moved the cotton tip to a position 1 cm anterior to the snake's snout. Starting with the first tongue-flick directed to the cotton, the number of tongue-flicks to the swab was recorded for a maximum of 60 s or for a shorter time if the snake moved away and would not resume tongue-flicking when the swab was repositioned in front of its head. If a snake bit a swab, the experimenter recorded the number of tongue-flicks and latency to the attack and terminated the trial. Snakes were not allowed to ingest the cotton. Each individual snake was tested in three experiments using a minimum intertrial interval of 30 min within each experiment and 1 day between experiments. If an individual crawled away before tongue-flicking the swab, the trial was discarded and repeated later.

Three experiments were conducted to assess responsiveness to chemical stimuli from several potential prey. In experiment 1, conducted on 24 August 2006 at 1025–1325 eastern daylight savings time (EDST), the stimulus sources were the southern toad (*B. terrestris*) collected in the same county as the mother of the snakes tested, green anole lizard

(*Anolis carolinensis* Voigt, 1832), domestic cricket (*Acheta domestica* L., 1758), cologne, and deionized water. Because experiment 1 established that response levels to the control stimuli cologne and deionized water were low and did not differ from responses to chemical cues from nonprey species, they were not included in the other experiments. In experiment 2, conducted on 25 August 2006 at 1030–1330 EDST, the stimulus sources were green frog (*Rana clamitans* Latreille in Sonnini de Manoncourt and Latreille, 1801) tadpole, yellow rat snake (*Pantherophis obsoleta quadrivittata* Holbrook, 1836 = *Elaphe alleghaniensis* (Holbrook, 1836)), fish (steelhead trout, *Oncorhynchus mykiss* (Walbaum, 1792)), mouse (*Mus musculus* L., 1758), and earthworm (*Lumbricus terrestris*). Tadpoles of *B. terrestris*, which might present chemical cues differing from those of adults, were unavailable. Tadpoles of *R. clamitans* were available and were included as stimulus sources to establish whether eastern hog-nosed snakes respond to chemical cues of anurans other than *Bufo* spp. and to cues from tadpoles, as well as adults. In experiment 3, performed on 26 August at 1400–1440 EDST, responsiveness to toad and tadpole stimuli were re-tested and compared. We have used the traditional names *Bufo* and *Rana*. Changes to the genera *Anaxyrus* Tschudi, 1845 and *Lithobates* Fitzinger, 1843, respectively, have been proposed for the species we used as stimulus sources (Frost et al. 2006), but too recently for consensus regarding their use.

Eastern hog-nosed snakes eat primarily toads, but occasionally will eat frogs, and some juveniles consume crickets or other insects (Conant and Collins 1998). They are sympatric with the lizard *A. carolinensis*, the snake *P. o. quadrivittata*, and numerous rodents, crickets, and earthworms. Although sympatric with tadpoles and fish of various species (not *O. mykiss*), the terrestrial *H. platirhinos* is unlikely to encounter them except when they are stranded on land after floods.

In each experiment each snake responded in all of the chemical stimulus conditions in a randomized block design. The stimulus sequence was selected by randomization in experiments 1 and 2, and was incompletely counterbalanced in experiment 3 to further reduce any possible bias owing to testing order. In experiment 3 the sequence was counterbalanced for eight individuals and the stimulus to be tested first was selected randomly for the remaining individual.

For experiments 1 and 2 the only variable analyzed was number of tongue-flicks per trial because no individuals bit. For experiment 3 we additionally analyzed tongue-flick attack scores for repeated-measure designs, TFAS(R) (Cooper and Burghardt 1990b), a widely used composite measure of overall response strength that is more appropriate for randomized block data than for the original tongue-flick attack score, TFAS (Burghardt 1967, 1970). TFAS(R) combines effects of tongue-flicks and biting, heavily weighting the latter because bites represent predatory attacks, whereas tongue-flicks show chemosensory investigation. TFAS(R) is the number of tongue-flicks in the trial if the predator does not bite. If the predator bites, TFAS(R) is the number of tongue-flicks prior to the bite plus 60 minus the latency to bite following the first tongue-flick.

The primary statistical tests used were analysis of variance for single-factor experiments having repeated-measure

(randomized block) designs. The assumption of homogeneity of variance was examined using Hartley's  $F_{MAX}$  tests followed by logarithmic transformation of data to reduce heterogeneity if necessary (Winer 1962). Normality was ensured by Kolmogorov–Smirnov tests (Sokal and Rohlf 1995). When significant main effects were detected in experiments 1 and 2, comparisons between pairs of condition means were conducted using Newman–Keuls tests (Zar 1996). Comparisons of responses to the same stimulus in different experiments were conducted using sign tests and a paired-sample  $t$  test (Zar 1996). Data are reported as means  $\pm$  1.0 SE. Significance tests were two-tailed with  $\alpha = 0.05$ .

## Results

### Types of responses

All individuals tongue-flicked stimulus-bearing swabs in all conditions in all three experiments. In addition, some individuals performed species-typical defensive displays when the swab was being positioned. These included flattening the neck and expanding the width of the head in a manner reminiscent of cobras and a single lunge simulating a strike. Such behavior did not appear to affect relative responsiveness to chemical cues once tongue-flicking was initiated.

### Experiment 1

Number of tongue-flicks differed significantly among stimuli ( $F_{[4,32]} = 39.24$ ,  $P = 1.0 \times 10^{-6}$ ; Fig. 1). The variances in the numbers of tongue-flicks were homogeneous ( $F_{MAX[5,8]} = 5.68$ ,  $P > 0.10$ ). The distributions in the number of tongue-flicks did not deviate significantly from normality (Kolmogorov–Smirnov tests: toad,  $d = 0.12$ ; lizard,  $d = 0.18$ ; cricket,  $d = 0.19$ ; cologne,  $d = 0.22$ ; deionized water,  $d = 0.23$ ; each  $P$  value  $> 0.05$ ).

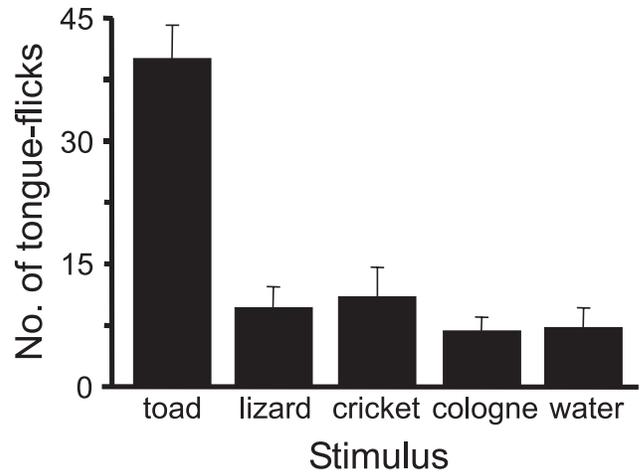
Numbers of tongue-flicks were much greater in response to toad surface chemicals than to all other stimuli (Fig. 1; Newman–Keuls tests for toad vs. lizard,  $P < 0.00013$ ; toad vs. cricket,  $P < 0.0014$ ; toad vs. cologne,  $P < 0.00014$ ; toad vs. deionized water,  $P < 0.00017$ ). No other differences between pairs of stimuli closely approached significance (each  $P$  value  $> 0.10$ ).

### Experiment 2

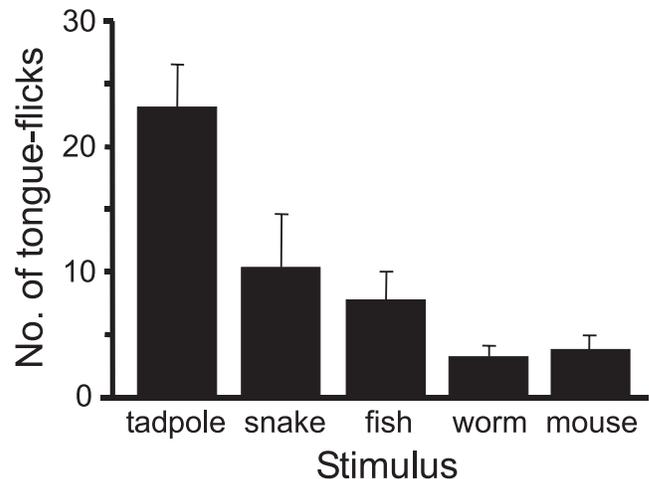
Although number of tongue-flicks differed significantly among stimuli using raw data ( $F_{[4,32]} = 9.81$ ,  $P = 2.7 \times 10^{-5}$ ; Fig. 2), variances in the numbers of tongue-flicks were significantly heterogeneous ( $F_{MAX[5,8]} = 51.51$ ;  $P \ll 0.01$ ). Although they remained heterogeneous for logarithmically transformed data ( $F_{MAX[5,8]} = 9.98$ ;  $P < 0.04$ ), we considered this minor heterogeneity to be acceptable because ANOVA is robust when heterogeneity of variance is not extreme. The distributions in the numbers of tongue-flicks did not deviate significantly from normality (Kolmogorov–Smirnov tests: tadpole,  $d = 0.11$ ; snake,  $d = 0.18$ ; fish,  $d = 0.22$ ; earthworm,  $d = 0.18$ ; mouse,  $d = 0.15$ ; each  $P$  value  $> 0.05$ ).

Using the logarithmically transformed data, the main effect of stimulus was highly significant ( $F_{[4,32]} = 9.33$ ,  $P = 4.1 \times 10^{-5}$ ). Numbers of tongue-flicks were consistently and substantially greater in response to chemical stimuli from tadpoles than from each of the other stimuli (Newman–

**Fig. 1.** Mean number of tongue-flicks by nine ingestively naive hatchlings of *Heterodon platirhinos* in response to chemical cues on cotton swabs in experiment 1. Stimulus sources were toad (*Bufo terrestris*), lizard (*Anolis carolinensis*), domestic cricket (*Acheta domesticus*), cologne, and deionized water. Error bars represent 1.0 SE.



**Fig. 2.** Mean number of tongue-flicks by nine ingestively naive hatchlings of *H. platirhinos* in response to chemical cues on cotton swabs in experiment 2. Stimulus sources were tadpole (*Rana clamitans*), snake (*Pantherophis obsoleta quadrivittata*), fish (*Oncorhynchus mykiss*), earthworm (*Lumbriculus terrestris*), and house mouse (*Mus musculus*). Error bars represent 1.0 SE.

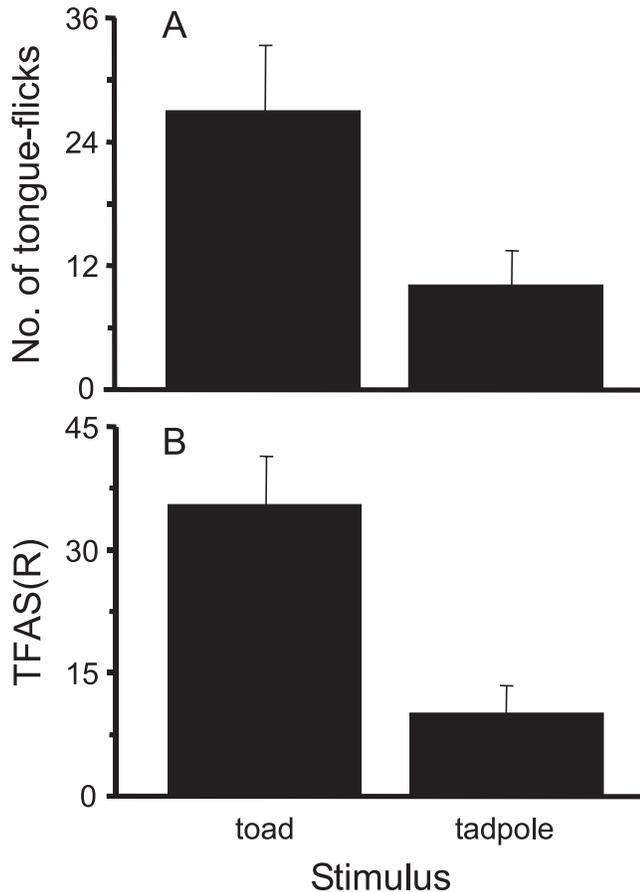


Keuls tests: snake,  $P < 0.0015$ ; fish,  $P < 0.0029$ ; earthworm,  $P < 0.00018$ ; mouse,  $P < 0.00024$ ). No other differences between pairs of stimuli were significant.

### Experiment 3

Toad stimuli elicited more tongue-flicks than did tadpole stimuli (Fig. 3a; toad range 2–52; tadpole range 1–23). Number of tongue-flicks was significantly greater in response to toad stimuli than to tadpole stimuli ( $F_{[1,8]} = 5.74$ ,  $P = 0.043$ ). Variances in the number of tongue-flicks were homogeneous ( $F_{MAX[2,8]} = 3.63$ ;  $P > 0.05$ ) and distributions did not deviate significantly from normality (Kolmogorov–Smirnov tests: toad,  $d = 0.14$ ; tadpole,  $d = 0.28$ ; each  $P$  value  $> 0.05$ ). Although no snakes bit swabs in experi-

**Fig. 3.** Responses by nine ingestively naive hatchlings of *H. platirhinos* to chemical cues from toads and *R. clamitans* tadpoles on cotton swabs in experiment 3. (A) Mean tongue-flicks. (B) Tongue-flick attack score (TFAS(R)). Error bars represent 1.0 SE.



ments 1 and 2, two bit swabs bearing toad chemical stimuli in experiment 3. None bit swabs bearing stimuli from tadpoles.

TFAS(R) was significantly greater in the toad condition than in the tadpole condition ( $F_{[1,8]} = 32.28$ ,  $P = 0.00046$ ; Fig. 3b). Variances of TFAS(R) were homogeneous ( $F_{MAX[2,8]} = 3.04$ ,  $P > 0.10$ ) and distributions did not deviate significantly from normality (Kolmogorov–Smirnov tests: toad,  $d = 0.16$ ; tadpole,  $d = 0.28$ ; each  $P$  value  $> 0.05$ ).

#### Comparisons between experiments

Eight of nine snakes performed more tongue-flicks in the tadpole condition of experiment 2 than in experiment 3, indicating significantly stronger response during first exposure to the tadpole stimulus (sign test,  $P = 0.035$ ). The mean decrease in response to tadpole stimuli was large ( $12.67 \pm 3.30$ ;  $t_{[8]} = 3.83$ ,  $P < 0.0050$ ), and five individuals performed fewer than 5 tongue-flicks in response to tadpole stimuli in experiment 3 and an additional individual for which 19 total tongue-flicks were recorded moved away from the swab after 1 tongue-flick, whereas no snake exhibited less than 8 tongue-flicks in experiment 2.

In response to toad chemical stimuli, number of tongue-flicks was greater in experiment 1 than in experiment 3 for six of nine snakes (sign test,  $P > 0.10$ ). However, the two

individuals that bit in experiment 3 responded more strongly to toad stimuli than they did in experiment 1, yet had fewer tongue-flicks in experiment 3. For TFAS(R) four of nine snakes responded more strongly to toad stimuli in experiment 1 than in experiment 3 (sign test,  $P > 0.10$ ).

Only tadpole stimuli in experiment 2 elicited substantially more tongue-flicks than the control stimuli in experiment 1. All nine snakes performed more tongue-flicks in response to tadpole stimuli than to cologne (sign test,  $P = 0.0039$ ). Seven of nine snakes performed more tongue-flicks to tadpole stimuli than to deionized water, and the numbers of tongue-flicks were identical for the other two snakes (sign test,  $P < 0.016$ ). For these tests, five stimuli could possibly have been compared with each control stimulus. Thus, the difference between tadpole stimuli and cologne is significant after sequential Bonferroni adjustment, but the difference between tadpole stimuli and deionized water is marginal because 0.01 is needed for significance.

The more powerful ANOVAs showed that significantly more tongue-flicks occurred in trials with tadpole stimuli than with cologne ( $F_{[1,8]} = 26.68$ ,  $P < 0.00086$ ;  $F_{MAX[2,8]} = 4.09$ ,  $P > 0.05$ ) or deionized water ( $F_{[1,8]} = 14.21$ ,  $P < 0.0055$ ;  $F_{MAX[2,8]} = 2.07$ ,  $P > 0.05$ ).

#### Discussion

The primary result is a striking correspondence between diet and strength of response to chemical cues. Ingestively naive *H. platirhinos* responded much more strongly to surface chemical cues from toads, which constitute the large majority of their diet, than to surface chemical cues from a variety of other vertebrates and invertebrates. This is indicated by the significantly greater number of tongue-flicks elicited by toad stimuli than the other stimuli in experiment 1 and by the significantly greater number of tongue-flicks in response to toad stimuli than tadpole stimuli in experiment 3. Tests were not conducted to compare response rates between toad stimuli in experiment 1 and stimuli tested in experiment 2 because of the possibility that habituation to trial conditions might make the snakes less responsive in experiment 2. However, responses to tadpole stimuli in experiment 2 were significantly stronger than to the other stimuli in that experiment, whereas responsiveness to toad stimuli was significantly greater than to tadpole stimuli, strongly indicating that response strength was much greater for toad stimuli than for any of the other stimuli tested in experiment 2.

Biting in response to chemical cues from toads by two snakes in experiment 3 is a further indication that the snakes responded to the chemical cues from toads as prey cues. The snakes had not undergone the first ecdysis, but most had opaque eyes during testing, and were thus relatively unlikely to eat (Bellairs 1970). This might have resulted in lower tongue-flick rates and reduced tendency to bite. The eyes of the two snakes that bit had cleared prior to ecdysis. It is likely that a higher proportion of snakes would have bitten in trials with toad stimuli if they had been tested after shedding or just prior to shedding when the eyes were clear.

The hatchling snakes also exhibited elevated response to chemical stimuli from the tadpole stage of the frog *R. clamitans*, as shown by the higher tongue-flick rate in experiment

2 to frog stimuli than to any of the other stimuli. This further indicates that responsiveness is fine-tuned to diet because *H. platirhinos* is a toad specialist known to also eat frogs, and some juveniles eat insects (Conant and Collins 1998). Although we did not detect any significant increase in tongue-flicking rate in response to cricket stimuli relative to control stimuli, the mean tongue-flick rate for cricket stimuli was higher than for all stimuli in experiment 1 other than toad stimuli, but was only about a fourth of that for toads.

Low response rates were elicited by cologne and fish stimuli despite their being highly odorous stimuli. This finding further supports the hypothesis that the observed high response strength to southern toad and green frog stimuli is a reflection of the specific chemical characteristics of these anuran species, and not merely a consequence of strong scent.

Eastern hog-nosed snakes may eat insects occasionally, but tongue-flick rates to cricket stimuli for the single litter tested did not differ from those to control stimuli or stimuli from animals not eaten. Nevertheless, the mean response rate was slightly greater in response to cricket chemical stimuli than to other non-amphibian stimuli and was highly variable (2–34 tongue-flicks). However, none of the snakes fixated on a swab bearing cricket cues, whereas all did so for at least a substantial portion of trials with toad stimuli by directly facing the swab and examining it with short tongue-flicks that contacted the cotton. Thus, responses to cricket stimuli do not seem to indicate any interest in crickets as food.

None of the other animal species used as stimulus sources are known prey for hog-nosed snakes and none elicited large numbers of tongue-flicks. Like responses to cricket cues, responses to snake stimuli were quite variable (range 1–33), with five snakes tongue-flicking 3 times or less and two snakes tongue-flicking more than 30 times. The source of this variability and its interpretation are unclear, but might simply indicate differences in disturbance among snakes. Hog-nosed snakes did not exhibit defensive reactions to snake chemicals.

Responses by inexperienced *H. platirhinos* to chemical cues are exactly as predicted by the hypothesis that natural selection has favored correspondence between the dietary specialization and the responsiveness to chemicals associated with the specialized prey. Whether correlated evolution has occurred between diet and chemosensory behavior cannot be determined based on a single species. Testing for such correlated evolution poses two problems in snake studies. First is the controversial state of snake phylogeny, but recent advances have greatly lessened this problem. If taxa having uncertain relationships are studied, the problem might be avoided by testing chemosensory responses in pairs of closely related populations and species that differ in diet. A second problem is availability of extreme dietary specialists such as egg, slug, and centipede eaters. It is difficult to obtain sufficient numbers of adults of many taxa to conduct experiments, as well as to locate clutches of hatchlings that have never been exposed to prey.

Similar problems apply to studies of other lizards, snakes being a subgroup of lizards, but it has been established that transitions from exclusively animal diets to omnivory and herbivory are accompanied by de novo evolution of respon-

siveness to plant food chemicals (Cooper 2002, 2003). Because so many snakes in diverse lineages have very narrow diet breadth, thorough assessment of the hypothesis that strong chemosensory response evolves in tandem with dietary specialization is a long-term undertaking that will require contributions by many investigators. Nevertheless, the striking correspondence between specialized diet and strong response to chemical cues from the limited food eaten by the crayfish specialists *R. grahamii* and *R. septemvittata* (Burghardt 1968; Waters and Burghardt 2005), the slug-eating populations of *T. elegans* (Arnold 1981a), the lizard specialists *U. frenatus* (Henderson et al. 1983) and *H. bungaroides* (Downes 2002), and the toad specialist *H. platirhinos* strongly suggests that the hypothesis is correct in at least some taxa and that comparative investigation of this relationship will be fruitful.

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