

## Articles

# Intrinsic Prey Preference and Selection of the Giant Gartersnake: A Threatened Predator in a Nonnative Prey-Dominated Community

Julia S.M. Ersan,\* Brian J. Halstead, Erica L. Wildy, Michael L. Casazza, Glenn D. Wylie

J.S.M. Ersan, B.J. Halstead, M.L. Casazza, G.D. Wylie

U.S. Geological Survey, Western Ecological Research Center, 800 Business Park Drive, Suite D, Dixon, California 95620

E.L. Wildy

California State University, East Bay Department of Biological Sciences, 25800 Carlos Bee Blvd., Hayward, California 94542

## Abstract

The introduction of exotic species into an environment can introduce great change in the trophic dynamics of native species. This is of even greater concern if the native species is of conservation concern. The giant gartersnake, *Thamnophis gigas*, an endemic predator of the Central Valley of California and a species of conservation concern at the state and federal levels, has declined as a result of conversion of its once vast wetland habitat to agriculture. Another anthropogenic factor contributing to this snake's changing ecology is the introduction of nonnative prey into the species' habitats. These introductions have resulted in a prey community that is almost completely composed of exotic species and have potential for considerable effects. In order to assess prey preference and selection we performed three sets of behavioral trials on naïve neonates. We examined 1) neonate prey preference in response to olfactory cues of prepared prey extracts, 2) neonate consumption of different live prey items presented simultaneously; and 3) terrestrial feeding behavior and/or latency to successful attack. Results from the olfactory study suggest that native Sierran treefrogs, *Pseudacris sierra*, are preferred by neonates. Results from consumption trials suggest that neonates are more likely to select frog species than fish species. This is the first study that we are aware of that examines prey selection of this threatened species and serves to inform its conservation and management.

Keywords: diet; garter snake; introduced species; prey selection

Received: September 11, 2019; Accepted: February 26, 2020; Published Online Early: March 2020; Published: June 2020

Citation: Ersan JSM, Halstead BJ, Wildy EL, Casazza ML, Wylie GD. 2020. Intrinsic prey preference and selection of the giant gartersnake: a threatened predator in a nonnative prey-dominated community. *Journal of Fish and Wildlife Management* 11(1):164–173; e1944-687X. <https://doi.org/10.3996/062019-JFWM-051>

Copyright: All material appearing in the *Journal of Fish and Wildlife Management* is in the public domain and may be reproduced or copied without permission unless specifically noted with the copyright symbol ©. Citation of the source, as given above, is requested.

The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

\* Corresponding author: [jersan@usgs.gov](mailto:jersan@usgs.gov)

## Introduction

To fully understand the effects that the introduction of nonnative species has on an ecological system, an assessment of how species interact as well as how individual species are affected is critical (Sih et al. 2010). For example, foraging behavior of predatory species can change in systems into which new species have been

introduced. This not only directly affects the fitness of the individual consumers but can indirectly influence the long-term viability of the population to which those consumers belong (Shine 2010). Thus, studying interactions between native and exotic species is crucial for understanding the short-term and long-term consequences of the introductions on native species. This is

particularly important when the native species is of conservation concern.

The giant gartersnake, *Thamnophis gigas*, a state (California Department of Fish and Wildlife 2019) and federally threatened listed species pursuant to the U.S. Endangered Species Act (ESA 1973, as amended) and precinctive to the wetlands of the Central Valley of California (Fitch 1940), is a species that has experienced extensive human-mediated changes to its environment. Giant gartersnake population decline has largely been attributed to a mass conversion of its wetland habitat to various types of agriculture (U.S. Fish and Wildlife Service 1993; Zedler 1996). However, this snake has been able to persist in the northern part of its range, the Sacramento Valley, because of the presence of rice agriculture, which resembles the marsh-like conditions necessary for giant gartersnakes (Halstead et al. 2010).

In addition to habitat conversion, the giant gartersnake is also confronted with the introduction of many nonnative, hardy, generalist species into its habitat, many of which can serve as potential prey items. The diet of giant gartersnakes has not been extensively investigated; thus, the question of whether and how these introductions might affect the snake and potentially contribute to the snakes' population decline persists. Giant gartersnakes are generally thought to prey on anurans and fish (Rossman et al. 1996; Stebbins and McGinnis 2012). Though historically these snakes preyed upon native fishes and frogs such as Sacramento Blackfish *Orthodon microlepidotus* (Rossman et al. 1996), the now extinct Thicktail Chub *Gila crassicauda* (IUCN 2015), and California red-legged frogs *Rana draytonii*, the majority of prey now available to giant gartersnakes are introduced (Rossman et al. 1996). Of the two anurans known to co-occur with giant gartersnakes, one is the native Sierran treefrog *Pseudacris sierra* and the other is the invasive American bullfrog *Lithobates catesbeianus* (Stebbins and McGinnis 2012). Exotic fish species known to be consumed by these snakes include the Mississippi Silverside *Menidia beryllina*, Western Mosquitofish *Gambusia affinis*, Black Bullhead Catfish *Ameiurus melas* and several species in the minnow family (Cyprinidae; Ersan et al., in press). Many more introduced species, however, might comprise the diet of these snakes including many centrarchid fish species such as Green Sunfish *Lepomis cyanellus*, Bluegill *Lepomis macrochirus*, Black and White Crappie *Pomoxis* species, and Largemouth Bass *Micropoterus salmoides* (UC ANR 2015).

If giant gartersnakes are indeed consuming exotic species regularly, it is crucial that this activity is documented and the species identified so that any potential impact on giant gartersnake fitness can be examined. This study is an examination of diet preference and consumption in giant gartersnake neonates, born from females originating from habitats in which nonnative potential prey species occur. The use of captive-born neonates allows for measurement of a naïve response because these snakes have never been exposed to any prey and therefore have no learned experience. The objective of this study was to determine the relative strength of the predatory response of

neonate giant gartersnakes to native and introduced prey. We used two types of behavioral trials, one examining prey preferences of naïve neonate giant gartersnakes to chemical cues of different prey items, and a second quantifying the probability with which neonate giant gartersnakes consumed prey in a controlled laboratory setting.

## Methods

In the spring of 2014, we collected three gravid female giant gartersnakes using modified minnow traps (Halstead et al. 2013) from each of three basins in California's Central Valley: Colusa Basin, Sutter Basin, and American Basin (Table 1). We brought the snakes back to the USGS Dixon Field Station (Dixon, CA) where they were maintained in the laboratory until parturition. We housed the snakes in 38-L glass aquaria lined with artificial turf that contained a metal water dish and an additional piece of rolled artificial turf that the snakes could use for shelter. We cleaned enclosures with  $\geq 3\%$  quaternary ammonium (Sparquat 256) after defecation. The laboratory had one window, which allowed for exposure to natural daylight; we also provided additional fluorescent lighting to subsidize the natural lighting present from 0900 to 1700 hours. The temperature was maintained between 23 and 28°C.

We offered adult snakes field-caught live prey (adult or tadpole bullfrogs) from their respective site of capture once a day and fresh water ad libitum. We maintained female snakes in captivity until they gave birth, after which we released them at their location of capture. We retained and housed neonate snakes by litter in the same manner described for the adults. We uniquely marked each neonate using a permanent marker as a means of identification and recorded their snout-vent length, tail length, and mass within 2 d of birth. Behavioral trials began 15 days after birth as has been convention in similar studies (Dix 1968; Cooper et al. 2000). We conducted two different behavioral experiments, one involving a set of olfactory trials and a second involving a set of consumption trials. We did not feed neonates until the second set of trials.

### Experiment 1: Neonate responses to olfactory chemical cues of potential prey

In July through October of 2014, between the times of 1030 and 1700 hours, we individually exposed 100 neonates, representing 9 litters, to a series of 11 chemical cues in random order presented on individual cotton swabs soaked with extracts of a potential prey animal or control cue. This trial was replicated 3 times for each neonate between the 15th and 20th day after birth. The extracts were of the following: 1) adult and 2) tadpole Sierran treefrog; 3) adult and 4) tadpole American bullfrog; 5) mosquitofish; 6) sunfish *Lepomis* spp.; 7) black bullhead catfish; 8) Louisiana red crayfish *Procambarus clarkii*; 9) water (visual control); 10) diluted mint extract *Mentha* spp. (olfactory control); and 11) California kingsnake *Lampropeltis californiae* (predatory control).



**Table 1.** Sums and percentages of prey types that neonate giant gartersnakes *Thamnophis gigas* within litters consumed July–October 2014. Left column lists litter identification and water basin where mother was collected. NA designates that the respective prey was not offered as a choice for that litter.

Litter identification and water basin	Treefrog tadpole	Bullfrog tadpole	Sunfish	Mosquitofish	Litter size
Four-choice group ( $n = 47$ )					
LN2224 (American)	4	1	0	0	5
Butler (Sutter)	4	15	1	0	20
LN2203 (American)	9	7	0	1	17
BKS 6 (American)	0	5	0	0	5
Three-choice group (no treefrog; $n = 33$ )					
CO4177 (Colusa)	NA	11	3	0	14
GI (Sutter)	NA	7	0	0	7
CO4176 (Colusa)	NA	12	0	0	12
Two-choice group (no frogs; $n = 7$ )					
Matt (Sutter)	NA	NA	6	1	13
CO4171 (Colusa)	NA	NA	0	0	4
BKS 6 (American)	NA	NA	0	0	2
Sum	17	58	10	2	—
Percentage ( $n = 87$ )	36.17	72.5	11.49	2.3	

We included a native predatory control for comparison with adult bullfrogs, which are known to prey on giant gartersnake neonates (Wylie et al. 2003). We created the extracts by soaking live prey items in deionized water at a ratio of 1:2 (grams of body mass:milliliters of water) for 30 min. We used these extracts either fresh or refrigerated at 8°C for up to 6 d. We allowed extracts to reach room temperature in the morning before use to allow for maximum odor volatilization when presented to the neonates. A third party labeled the extracts so the observer was blind to which chemical cue was presented in each trial.

Before each trial, the observer placed a neonate in an arena consisting of a clean plastic container measuring 8 × 25 × 15 cm with the container lid in place, and allowed it to acclimate for 5 min. Following this habituation period, the observer gradually moved the lid enough to expose the neonate and slowly introduced a cotton swab into the enclosure and brought it within 1 cm of the neonate's supralabials. The time record started when the neonate flicked its tongue in the direction of the swab for the first time. During the trials, if a snake moved toward the swab, the observer moved the swab away from the snake to maintain the 1-cm buffer. Alternatively, if a snake turned away from the swab, the observer kept the swab still.

The observer determined the degree of interest a neonate had in a particular extract using the number of tongue flicks it exhibited in response to the swab. Tongue-flick scoring techniques are well-established and broadly accepted as an indication of prey preference in reptiles (Cooper and Burghardt 1990). The method to gauge preference by measuring the response to chemical cues has been particularly well-developed in the genus *Thamnophis* (Burghardt 1969, 1970, 1975).

Throughout each trial, the observer counted tongue flicks exhibited by the neonate until the snake turned away and tongue-flicked >5 cm away from the swab or

after a maximum of 45 s had passed, whichever occurred first. The observer considered gapes (opens mouth while remaining still) and lunges (opens mouth and makes sudden movement toward the cotton swab) to be attacks and recorded them. Observers replicated each trial once per day, three times for each neonate between the ages of 15–20 d (Data S1, *Supplemental Material*).

### Experiment 2: Neonate selection and consumption of aquatic prey

At the age of 21 days, the day after the neonates completed the olfactory trials, we subjected 99 individuals to a prey choice experiment. Before each trial began, the observer placed neonates in a clean plastic container (8 × 30 × 20 cm) containing a metal pan (20 × 20 × 5 cm) filled with 600 mL of water and a number of live prey items. The observer weighed the prey items in a dry cup and then placed them into the dish pan in random order before the snake was introduced. The metal pan contained a choice of either four prey items, three prey items, or two prey items, depending upon the availability of prey. We grouped litters by the number of prey choices the individuals had: Group 1 (four litters,  $n = 47$ ): four prey choices (a treefrog tadpole, a bullfrog tadpole, a sunfish, and a mosquitofish), Group 2 (three litters,  $n = 33$ ): three prey choices (a bullfrog tadpole, a sunfish, and a mosquitofish), and Group 3 (two litters,  $n = 7$ ): two prey choices (a sunfish and a mosquitofish). Nine litters were represented in total (Table 1). Six individuals from litters CO4171 and BKS 6 that were offered a two-choice test did not select or consume a prey item after 45 min and we did not include them in the analyses. The observer placed neonates in the container on the rim of the dishpan with their head oriented toward the water. Often, neonates would move off the rim and explore the container space around the dishpan for several minutes before returning to the rim to begin to hunt. The time record would only begin when the snake returned to the



rim, oriented toward the prey items, and tongue-flicked (although sometimes a neonate would exhibit this behavior when first placed in the container, in which case the time record would start immediately). The observer placed a clear sheet of thermoplastic over the container and video-recorded the trial until the neonate caught one prey item or a maximum of 45 min passed, whichever occurred first. At this point, the observer removed the neonate. The observer replaced the selected prey item with a fresh individual of that prey type, but the remaining prey items and the water in the dishpan remained and were reused in consecutive trials (though this is not advised if this experiment were to be replicated because prey items are likely emitting stress and scent trails and tire after repeated trials). For this experiment, we tested each neonate just once. The observer watched the video in real time outside the room to monitor activities and then the same observer rewatched the video to record latency to attacks and the identity of the prey item involved for all attacks, both successful and unsuccessful (Data S2, *Supplemental Material*).

### Experiment 3: Neonate terrestrial feeding behavior

In a separate set of trials, we offered Sierran treefrog metamorphs to nine neonate giant gartersnakes that did not consume a prey item in the prior aquatic-prey-consumption trial to observe the feeding response of the snakes to the terrestrial life stage of this prey species and to determine whether there is a difference in the ease with which the neonates captured these prey animals (latency to successful attack). We conducted these trials separately from the trials with aquatic prey so that we could examine this issue without the additional complication of having multiple habitat regions within the testing arena.

To start, we placed one treefrog metamorph under a 125-mL cup (diameter 6.5 cm) in a clean plastic container (8 × 30 × 20 cm) with no metal pan and containing no water. Then, we placed the neonate in the container and removed the cup to reveal the metamorph. We recorded latency to a successful attack (Data S3, *Supplemental Material*). The day after feeding, we released the animals at the location their mother was captured.

### Data analysis

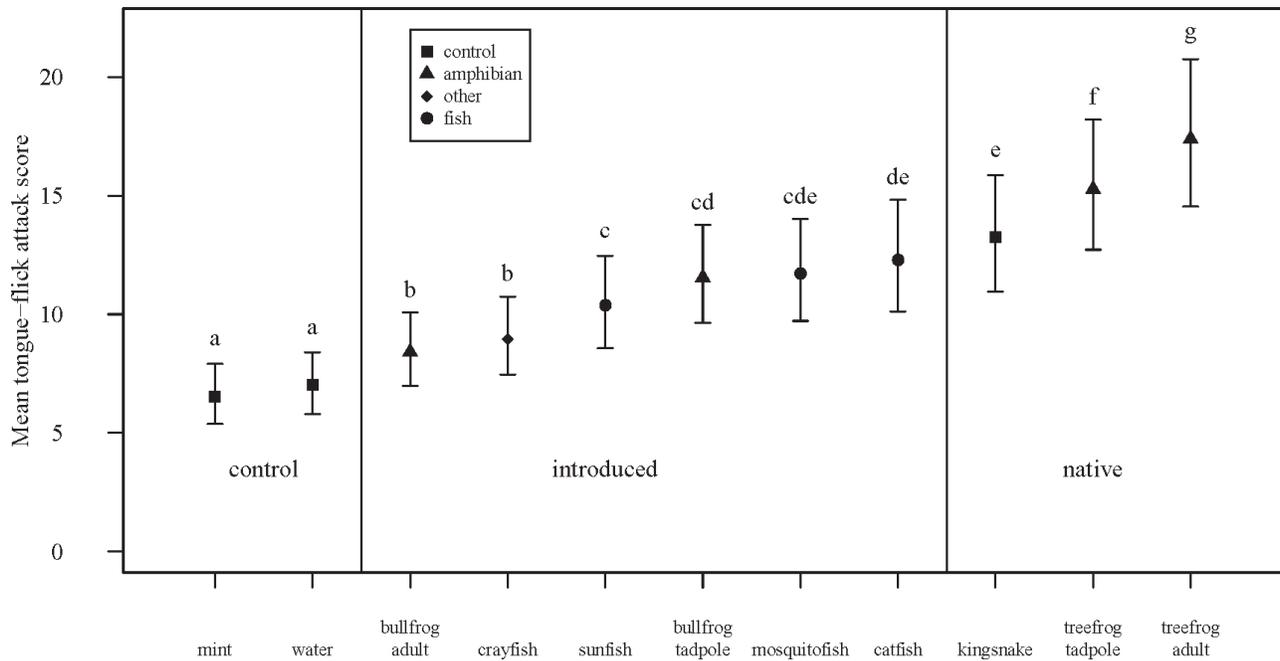
We analyzed the olfactory data using Bayesian hierarchical models with a Poisson distribution and a log-link function in Program JAGS 3.4.0 (Plummer 2013) called through R 3.1.0 (R Core Team 2014) using package 'rjags' (Plummer 2014). We calculated the tongue-flick attack score (TFAS) by subtracting the latency to attack (if any) from the length of the trial and adding the maximum number of tongue flicks for that individual from any trial (Cooper and Burghardt 1990). We estimated variation in TFAS across litters, individuals, and trials within individuals as log-normal random effects. We accounted for repeated measures and

overdispersion with a log-normal observation-level random effect. We also included the effect of age on TFAS in the model. We tested goodness-of-fit with a Bayesian *P*-value using Pearson residuals and data simulated from the model. Uninformative priors included a Uniform (minimum = 0, maximum = 10) distribution for standard deviations of random effects and a Normal (mean = 0, SD = 10) distribution for the intercept and coefficients. We ran the model using 5 independent chains, a 100,000 iteration burn-in, and 100,000 sampling iterations after the burn-in. We thinned the output by a factor of 50. We examined history plots and the Gelman–Rubin diagnostic (Gelman and Rubin 1992) to assess convergence, and found no evidence for lack of convergence (Gelman–Rubin diagnostic <1.1 for all monitored parameters).

We analyzed the consumption data using Bayesian hierarchical models with a multinomial ( $\geq 3$  prey types offered) or binomial (2 prey types offered) distribution for each set of consumption trials in Program OpenBUGS 3.2.3 (Thomas 2004; Thomas et al. 2006) called through R 3.1.0 using package 'R2OpenBUGS' (Sturtz et al. 2005). We analyzed the litters independently (with weighted averages based on litter size) and then analyzed as a pooled group for comparison. Weighted averages and pooled results were very similar, so we calculated odds ratios for pooled groups. We interpreted litters in which all individuals consumed the same prey as 100% likely to consume that particular prey item (no analyses were performed). As an uninformative prior, the probabilities for selection of each type were equally likely and between 0 and 1 (e.g., for 2 prey types, each prey type had a prior probability of 0.5; for 3 prey types, each prey type had a prior probability of 0.33, etc.). We used a binomial distribution for the group with two prey choices and a multinomial distribution for groups with three or four prey choices. We ran the model using 5 independent chains, a 20,000 iteration burn-in, and 20,000 sampling iterations after the burn-in. We thinned the output by a factor of 10. History plots depicted no evidence for lack of convergence.

We analyzed latency to successful capture data using a fixed-effects analysis of variance in Program JAGS 3.4.0 called through R 3.1.0 using packages 'runjags' (Denwood 2016) and 'modeest' (Poncet 2012). We log-transformed latencies to meet normality assumptions. Uninformative priors included a Normal (0, 100) distribution for mean latency to attack for each prey type, and a Uniform (0, 100) distribution for the standard deviation of the residual error. We tested goodness-of-fit with a Bayesian *P*-value using Pearson residuals and data simulated from the model. The model ran using 5 independent chains, a 9,000 iteration burn-in, plus 20,000 iterations after the burn-in. We assessed history plots and the Gelman–Rubin diagnostics (Gelman and Rubin 1992) for convergence, and found no evidence for lack of convergence (Gelman–Rubin diagnostic <1.1 for all monitored parameters).





**Figure 1.** Mean tongue-flick attack scores of neonate giant gartersnakes *Thamnophis gigas* in response to chemical cues of extracts in prey preference trials, July–October 2014. Different letters represent significant differences (95% credible intervals of differences in mean tongue-flick attack score did not include zero). Points represent posterior medians; error bars represent 95% credible intervals.

**Results**

**Prey preference trials**

The three native species odors elicited the strongest responses from the neonates. The strongest tongue-flick responses were exhibited toward the native prey species, *P. sierra* adult and tadpoles, followed by the native predator, the California kingsnake. The weakest responses were exhibited toward the visual and olfactory controls. All other responses to introduced species were intermediate in strength with respect to controls and natives (Figure 1). Responses were more consistent among litters (posterior median of SD [log scale] = 0.202, 95% credible interval [CRI] = 0.099–0.435) and individuals (median = 0.281, 95% CRI = 0.234–0.338) than within individuals (median = 0.646, CRI = 0.623–0.669). Variation among individuals in their response to cues was least for the native treefrog metamorph extract and greatest for the introduced catfish extract and controls (Table 2). As neonates increased in age by one day, TFAS decreased 1.11 (CRI = 1.09–1.13) times. Attacks were elicited in 25 of 100 neonates and occurred in 45 of 3086 (0.015 %) trials.

**Prey selection trials**

Little difference in estimation existed between the pooling method versus calculating probabilities independently for each litter, then calculating the weighted mean; therefore, we present pooled results. In the group that had four prey choices, neonates did not consume either tadpole species more than the other (odds of consuming treefrog over bullfrog: 0.363 [0.116–1.150]) or

either fish type more than the other (odds of consuming sunfish over mosquitofish: 1.08 [0.02–41.00]; Figure 2). There was, however, significant selection of both tadpole species over both fish species in general (odds of consuming either tadpole over either fish 746.0 [57.0–32,600.0]). In the group with three prey choices (treefrog tadpole not offered), the bullfrog tadpole was heavily selected over both fish types (odds of consuming bullfrog over sunfish: 15.9 [1.4–331.0], odds of consuming bullfrog over mosquitofish: 5.12E + 31 [1,150–∞], odds of consuming bullfrog over either fish: 166.0 [16.2–1,890.0]; Figure 2) and there also was a significantly higher probability of consuming sunfish over mosquitofish (odds of consuming sunfish over mosquitofish: 2.39E + 30 [81.0–∞]). However, in the group with only sunfish and mosquitofish offered, there was no evidence for selection of either fish species over the other (odds of consuming sunfish over mosquitofish: 3.99 [0.89–31.20]). In this group, 12 individuals in total, and all individuals in one litter, did not catch a prey item after 45 min and we did not include them in this analysis.

The neonates’ average latency to successful attack differed significantly among the prey types (Figure 3). The mean latency to attack response for treefrog metamorphs (median = 29.3 s, CRI = 12.0–70.5 s) was significantly shorter than all other prey types. The mean latency to successful attack did not differ among treefrog tadpoles, bullfrog tadpoles, and mosquitofish; but latency to successful attack was shorter for treefrog tadpoles (median = 104 s, CRI = 52–212 s) than sunfish (median = 426 s, CRI = 192–949 s).

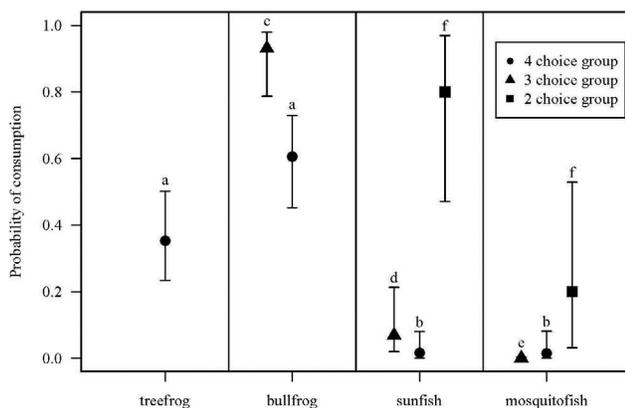
**Table 2.** Posterior quantiles of individual variation of tongue-flick attack scores of neonate giant gartersnakes *Thamnophis gigas* in response to chemical cues in prey preference trials, July–October 2014.

Cue	Standard deviation (log scale)		
	2.50%	50.00%	97.50%
Mint	0.061	0.266	0.416
Water	0.234	0.281	0.338
Bullfrog adult	0.005	0.121	0.287
Crayfish	0.005	0.080	0.234
Sunfish	0.010	0.163	0.327
Bullfrog tadpole	0.004	0.082	0.233
Mosquitofish	0.012	0.148	0.308
Catfish	0.104	0.287	0.423
Kingsnake	0.006	0.157	0.314
Treefrog tadpole	0.004	0.071	0.216
Treefrog metamorph	0.002	0.059	0.182

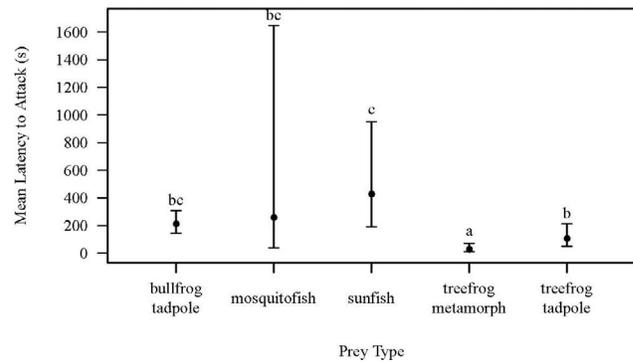
### Discussion

Giant gartersnake neonates innately prefer native prey and can distinguish a native predator. The focal result suggests that giant gartersnakes innately prefer native treefrogs and their larvae. The preference for native prey species also exists in other snakes, including *Thamnophis hammondi* (Hale 2010), *T. elegans* (Arnold 1977), *Coluber constrictor* (Cooper et al. 2000), and *Nerodia sipedon* (Gove and Burghardt 1975). It is not particularly surprising that a predator would innately prefer native prey; however, preference in this context might simply mean that neonates innately recognize treefrogs as prey and might not recognize the other species as prey. Although the mechanisms leading to preference of native prey are unknown and potentially complex, neonate giant gartersnakes clearly exhibit the strongest response to cues from native prey.

Neonates exhibited the third highest response toward the native predator, the California kingsnake. A response



**Figure 2.** Consumption probabilities of neonate giant gartersnakes *Thamnophis gigas* presented with prey choices in consumption trials, July–October 2014. Four-choice group:  $n = 47$ ; three-choice group:  $n = 33$ ; two-choice group:  $n = 7$ . Different letters represent significant differences within groups. Points represent posterior medians; error bars represent 95% credible intervals.



**Figure 3.** Mean latency to attack (in seconds) and successful capture of prey by neonate giant gartersnakes *Thamnophis gigas* in consumption trials, July–October 2014. Different letters represent significant differences. Points represent posterior medians; error bars represent highest posterior density intervals.

of this nature is not unprecedented. High TFASs toward ophiophagous snakes were also observed in studies on other snakes in the genus *Thamnophis* (Weldon 1982) and *Coluber constrictor* (Cooper et al. 2000) and were interpreted as recognition of a predator. Adult bullfrogs are also predators of giant gartersnake neonates (Wylie et al. 2003), but because the response of neonates to the adult bullfrog was substantially less than that exhibited toward the California kingsnake, the data do not support innate recognition of adult bullfrogs as predators. The occurrence of intraguild predation between giant gartersnakes and bullfrogs might cause selection pressures to counter each other and thus result in a neutral response. Alternatively, interpretation of a high TFAS toward the California kingsnake could be attributed to the condition of it being native and therefore innately recognized.

In the consumption trials, we did not find treefrog tadpoles to be selected significantly more often than bullfrog tadpoles. This seemingly contradicts the results of the olfactory trials where neonates exhibited a preference for native treefrog tadpoles over bullfrog tadpoles. However, what these data probably suggest is that neonates use multiple sensory cues to identify and select for prey and there are many variables that are contributing to that selection. For instance, although bullfrog tadpoles are less preferred, they might be easier to catch. We made no locomotory measurements in this study, but different tadpole species differ in swimming kinematics (Wassersug 1989) and antipredator behavior (Lawler 1989). Furthermore, though we made attempts to reduce size variation among prey items, bullfrog tadpoles tended to be larger (as much as 0.5 g) than other prey items. This could have influenced both locomotory performance among tadpole individuals as well as selection by neonates for larger prey items (Arnold 1993). Additionally, different species of frog larvae have shown variation in their refuge-seeking response when exposed to chemical cues of native and introduced predators (Pearl et al. 2003), thus supporting the hypothesis of additional behavioral variability between tadpole species. Perhaps treefrogs, which have evolved with giant gartersnakes, have a more developed

antipredatory response to giant gartersnakes than do bullfrogs, which did not evolve with giant gartersnakes (but did evolve with other natricines).

Results from consumption trials indicated that although neonates will consume fish, they are consumed with lower probability than anuran prey. A variable that could have contributed to the modest, albeit present selection of fish by neonates, could be the design of the experimental setup itself. In another highly aquatic snake species, *Nerodia clarkii*, individuals varied in fish capture success with differences in mangrove density (Mullin and Mushinsky 1995). It is possible that the limited size and structural simplicity of the dishpan affected the ability of giant gartersnake neonates to capture fish they were offered relative to what might be observed in their more structurally complex natural habitats. It is also important to note that we used no native fish in trials because few native fish occurred in sampled areas. Additionally, nonnative fish that evolved in the presence of exclusively piscivorous snakes could arguably have better antipredatory defenses than native fish inhabiting the Central Valley, which did not evolve in such circumstances.

We found additional evidence for the predilection of treefrogs in the consumption trials where we offered neonates treefrog metamorphs. The data collected during the different series of consumption trials cannot be directly compared because they were independently run and because of the differences in aquatic and nonaquatic conditions of the trial designs. That notwithstanding, latencies to attack on metamorphs were substantially shorter than in trials where neonates were offered the aquatic prey in dish pans. Moreover, with aquatic prey, several failed attempts typically occurred before capture (if capture even occurred), whereas in the terrestrial trials, every neonate was able to capture the metamorph during its first attempt.

Preference for native prey might not be constant throughout an individual's life. For example, innate preference for native prey by *T. hammondi* was shown to be modified such that they learned to prefer nonnative prey (Hale 2010). This confirmed the finding that wild-caught *T. hammondi* preferred nonnatives including centrarchids and an anuran, *Xenopus laevis* (Mullin et al. 2004). Bullfrogs were introduced to California in 1896 (Heard 1904), which might have provided giant gartersnakes enough time to evolve to innately recognize them as alternative prey, especially because giant gartersnakes might have historically consumed a related species, the California red-legged frog *Rana draytonii* (Rossman et al. 1996).

There are many negative effects that these nonnatives can have on the giant gartersnake, such as serving as vectors of both parasites and disease. For example, bullfrogs, *Gambusia* spp. (Coyner et al. 2003), and *Lepomis* spp. (Cone and Anderson 1977) can serve as intermediate hosts of nematodes (i.e., *Eustrongylides* spp.) and can transmit these parasites to their ophidian predators (Jiménez-Ruiz et al. 2002). Many giant gartersnakes exhibit cysts of larval nematodes that form subcutaneously (U.S. Fish and Wildlife Service 1999) and though direct effects of nematodes on giant

gartersnake fitness are unknown, nematode infestations have been correlated with mortality in *Thamnophis* spp. (Lichtenfels and Lavies 1976). Catfish and Centrarchids can also cause harm to giant gartersnakes by predation (Young et al. 2013) or through injury to snakes after they have been consumed. The spines in the fins of these fish can penetrate snakes' body cavities, causing deleterious effects or even death (Emmons et al. 2016; Stellati et al. 2019). Even more mild effects, such as reduction of crawling speed of the snake due to consumption of Centrarchids, could lead to compromised exposure to predators (Willson and Hopkins 2011).

Nonnative fishes and bullfrogs may potentially harm giant gartersnake populations through indirect means. For example, there is evidence that bullfrog tadpoles (Kupferberg 1997) and nonnative fish (Adams 2000; Gilliland 2010; Pearl et al. 2005; Preston et al. 2012) have significant detrimental effects on treefrog tadpoles. Extensive stocking of mosquitofish in sampling locations and the overwhelming abundance of other nonnative fish and bullfrogs likely have adverse and perhaps synergistic effects on treefrog tadpole success in the community of the giant gartersnake as well. Based on the findings in the current study, management to increase the abundance of treefrogs would likely benefit giant gartersnake populations.

The introductions of bullfrogs and nonnative fish into the Central Valley of California have multifaceted community effects. An understanding of the relationship between these introduced species and the existing native species is required to effectively manage species of conservation concern. Though in their current environment, giant gartersnakes have exhibited both habitat and dietary plasticity by persisting in rice agriculture and consuming these available nonnative prey types, it remains important to understand the relationship between a threatened native predator and its prey in this complex modified system. The results of this study indicate giant gartersnakes have an innate prey preference for a native treefrog, which contributes to the understanding of this predator-prey interaction (Sih and Moore 1990) and provides support for better conservation of both species.

## Supplemental Material

Please note: The *Journal of Fish and Wildlife Management* is not responsible for the content or functionality of any supplemental material. Queries should be directed to the corresponding author for the article.

**Data S1.** Refer to Figure. 1 Mean tongue-flick attack scores of neonate giant gartersnakes *Thamnophis gigas*. Columns refer to individual neonates' basin of capture of mother, identification of mother, neonate individual identification, type of prey extracts, matrix formatting of extracts for analysis, duration of each of three trials for each extract, age of neonate for each of three trials for each extract, tongue-flick total for each of three trials for each extract, tongue-flick attack score (TFAS) for each of three trials for each extract. TFAS is calculated by

subtracting the latency to attack (if any) from the length of the trial and adding the maximum number of tongue flicks for that individual from any trial.

Found at DOI: <https://doi.org/10.3996/062019-JFWM-051.S1> (116 KB XLSX).

**Data S2.** Refer to Figure. 2 Consumption probabilities of neonate giant gartersnakes *Thamnophis gigas* presented with prey choices in consumption trials. Columns refer to litter identification and number of neonates that consumed prey type.

Found at DOI: <https://doi.org/10.3996/062019-JFWM-051.S2> (10 KB XLSX).

**Data S3.** Refer to Figure. 3 Mean latency to attack and successful capture of prey by neonate giant gartersnakes *Thamnophis gigas* in consumption trials. Columns refer to mothers' location of capture, litter identification, individual identification (by number, then code), latency to successful attack of prey item, choice of prey item.

Found at DOI: <https://doi.org/10.3996/062019-JFWM-051.S3> (13 KB XLSX).

### Acknowledgments

We thank J. Demianew, N. Dotson, A. Essert, K. Fouts, A. Jordan, D. Knapp, B. Larsen, D. Mackell, S. McNally, K. Ober, and S. Skalos for assistance in data collection and caring for the snakes. Carlton Rochester, the Associate Editor, and two anonymous reviewers provided valuable comments that improved this manuscript. All experimental procedures were reviewed and approved by the U.S. Fish and Wildlife Service (Recovery Permit TE-157216-2) and California State University, East Bay Institute for Animal Care and Use (Protocol # JE-0617). Animals were collected under California Scientific Collecting Permit # 10779. This work was supported by California Department of Water Resources and The Natomas Basin Conservancy.

Any use of trade, product, website, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

### References

- Adams MJ. 2000. Pond permanence and the effects of exotic vertebrates on anurans. *Ecological Applications* 10:559–568.
- Arnold SJ. 1977. Polymorphism and geographic variation in the feeding behavior of the garter snake *Thamnophis elegans*. *Science* 197:676–678.
- Arnold SJ. 1993. Foraging theory and prey size–predator size relation in snakes. Pages 87–112 in Seigel RA, Collins JT, editors. *Snakes: ecology and behavior*. Caldwell, New Jersey: The Blackburn Press.
- Burghardt GM. 1969. Comparative prey-attack studies in newborn snakes of the genus *Thamnophis*. *Behaviour* 1:77–114.
- Burghardt GM. 1970. Interspecific geographical variation in chemical food cue preferences of newborn garter snakes (*Thamnophis sirtalis*). *Behaviour* 36:246–257.
- Burghardt GM. 1975. Chemical prey preference polymorphism in newborn garter snakes *Thamnophis sirtalis*. *Behaviour* 52:202–224.
- California Department of Fish and Wildlife. 2019. California Natural Diversity Database Endangered and Threatened Animals List. Available: <https://wildlife.ca.gov/Data/CNDDDB/Plants-and-Animals> (March 2020).
- Cone DK, Anderson RC. 1977. Parasites of pumpkinseed (*Lepomis gibbosus* L.) from Ryan Lake, Algonquin, Ontario. *Canadian Journal of Zoology* 55:1410–1423.
- Cooper WE, Burghardt GM. 1990. A comparative analysis of scoring methods for chemical discrimination of prey by Squamate reptiles. *Journal of Chemical Ecology* 16:45–65.
- Cooper WE, Burghardt GM, Brown WS. 2000. Behavioural responses by hatchling racers (*Coluber constrictor*) from two geographically distinct populations to chemical stimuli from potential prey and predators. *Amphibia–Reptilia* 21:103–115.
- Coyner DF, Spalding MG, Forrester DJ. 2003. Epizootiology of *Eustrongylides idnotus* in Florida: transmission and development in intermediate hosts. *Journal of Parasitology* 89:290–298.
- Denwood MJ. 2016. runjags: an R package providing interface utilities, parallel computing methods and additional distributions for MCMC models in JAGS. *Journal of Statistical Software* 71:1–25. <https://doi.org/10.18637/jss.v071.i09>
- Dix MW. 1968. Snake food preference: innate intraspecific geographic variation. *Science* 159:1478–1479.
- Emmons ID, Nowak EM, Lauger KK. 2016. Prey availability and foraging events of the Northern Mexican Garter-snake (*Thamnophis eques megalops*) in northern central Arizona. *Herpetological Review* 47:555–561.
- Fitch HS. 1940. The biogeographical study of the ordinoides Artenkreis of garter snakes (Genus *Thamnophis*). University of California Publications in Zoology 44:69–73.
- Ersan JSM, Halstead BJ, Wildy EL, Casazza ML, Wylie GD (in press). Giant Gartersnakes (*Thamnophis gigas*) exploit abundant non-native prey while maintaining their appetite for native anurans. *Herpetologica*
- Gelman A, Rubin DB. 1992. Inference from iterative simulation using multiple sequences. *Statistical Science* 7:457–472.
- Gilliland KE. 2010. The presence of *Micropterus salmoides* (Largemouth Bass) influences the populations of *Rana draytonii* (California Red-legged frog) and *Pseudacris regilla* (Pacific treefrog) in two ponds in Santa Barbara County, California. Master's thesis. San Luis Obispo: California State Polytechnic University.
- Gove D, Burghardt GM. 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.
- Hale KB. 2010. Prey preferences as a function of feeding experience and prey type in neonate gartersnakes (Colubridae: *Thamnophis*). Master's thesis. Charleston: Eastern Illinois University.
- Halstead BJ, Wylie GD, Casazza ML. 2010. Habitat suitability and conservation of the giant gartersnake (*Thamnophis gigas*) in the Sacramento Valley of California. *Copeia* 2010:591–599.
- Halstead BJ, Wylie GD, Casazza ML. 2013. Efficacy of trap modifications for increasing capture rates of aquatic snakes in floating aquatic funnel traps. *Herpetological Conservation and Biology* 8:65–74.
- Heard M. 1904. A California frog ranch. *Out West* 21:20–27.
- [IUCN] International Union for Conservation of Nature. 2015. The IUCN red list of threatened species. “*Gila crassicauda*.” <http://doi.org/10.2305/IUCN.UK.2013-1.RLTS.T9183A18229941.en>
- Jiménez-Ruiz FA, García-Prieto L, Pérez-Ponce de León G. 2002. Helminth infracommunity structure of the sympatric garter snakes *Thamnophis eques* and *Thamnophis melanogaster* from the mesa central of Mexico. *Journal of Parasitology* 88:454–460.
- Kupferberg SJ. 1997. Bullfrog (*Rana catesbeiana*) invasion of a California river: role of larval competition. *Ecology* 78:1736–1751.
- Lawler SP. 1989. Behavioral responses to predators and predation risk in four species of larval anurans. *Animal Behaviour* 38:1039–1047.
- Lichtenfels JR, Lavies B. 1976. Mortality in red-sided garter snakes, *Thamnophis sirtalis parietalis*, due to larval nematode, *Eustrongylides* sp. *Laboratory Animal Science* 26:465–467.
- Mullin SJ, Imbert H, Fish JM, Ervin EL, Fisher RN. 2004. Snake (Colubridae: *Thamnophis*) predatory responses to chemical cues from native and introduced prey species. *The Southwestern Naturalist* 49:449–456.
- Mullin SJ, Mushinsky HR. 1995. Foraging ecology of the mangrove salt marsh snake, *Nerodia clarkii compressicauda*: effects of vegetational density. *Amphibia-Reptilia* 16:167–175.
- Pearl CA, Adams MJ, Schuytema GS, Nebeker AV. 2003. Behavioral responses of anuran larvae to chemical cues of native and introduced predators in the Pacific Northwestern United States. *Journal of Herpetology* 37:572–576.
- Pearl CA, Adams MJ, Leuthold N, Bury RB. 2005. Amphibian occurrence and aquatic invaders in a changing landscape: implications for wetland mitigation in the Willamette Valley, Oregon, USA. *Wetlands* 25:76–88.
- Plummer M. 2013. JAGS: a program for analysis of Bayesian graphical models using Gibbs sampling. Pages 1–10 in Hornik K, Leisch F, Zeileis A, editors. *Proceedings of the 3rd international workshop on distributed statistical computing (DSC 2003)*, 20–22 March, Technische Universität Wien, Vienna, Austria. ISSN 1609–395X. Available: <https://www.r-project.org/conferences/DSC-2003/Proceedings/Plummer.pdf> (March 2020).
- Plummer M. 2014. rjags: Bayesian graphical models using MCMC. R package version 3.14. Available: <http://CRAN.R-project.org/package=rjags> (February 2020).
- Poncet P. 2012. modeest: mode estimation. Program R package version 2.1. Available: <http://CRAN.R-project.org/package=modeest> (February 2020).
- Preston DL, Henderson JS, Johnson PT. 2012. Community ecology of invasions: direct and indirect effects of multiple invasive species on aquatic communities. *Ecology* 93:1254–1261.
- R Core Team 2014. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available: <http://www.R-project.org> (February 2020).
- Rossman DA, Ford NB, Seigel RA. 1996. The garter snakes: evolution and ecology. Volume 2. Norman: University of Oklahoma Press.
- Shine R. 2010. The ecological impact of the invasive cane toads (*Bufo marinus*) in Australia. *The Quarterly Review of Biology* 85:253–291.
- Sih A, Moore RD. 1990. Interacting effects of predator and prey behavior in determining diets. Pages 771–796 in RN Hughs, editor. *Behavioural mechanisms of food selection*. Germany: Springer.
- Sih A, Stamps J, Yang LH, McElreath R, Ramenofsky M. 2010. Behavior as a key component of integrative biology in a human-altered world. *Integrative and Comparative Biology* 50:934–944.
- Stebbins RC, McGinnis SM. 2012. *Field guide to amphibians and reptiles of California*. Berkeley: University of California Press.
- Stellati, L., Borgianni N, Bissattini AM, Buono V, Haubrock PJ, Balzani P, Tricarico E, Inghilesi AF, Tancioni L, Martinoli M, Luiselli L, Vignoli L. 2019. Living with aliens: suboptimal ecological condition in semiaquatic snakes inhabiting a hot spot of al biodiversity. *Acta Oecologica* 100:103466.
- Sturtz S, Ligges U, Gelman A. 2005. R2WinBUGS: a package for running WinBUGS from R. *Journal of Statistical Software* 12:1–16.
- Thomas A. 2004. OpenBUGS. Available: <http://www.openbugs.net/> (February 2020).
- Thomas A, O'Hara B, Ligges U, Sturtz S. 2006. Making BUGS open. *R News* 6:12–17. Available: <https://www.r-project.org/doc/Rnews/bib/Rnewsbib.html> (February 2020).
- [UC ANR] University of California, Division of Agriculture and Natural Resources. 2015. California fish website. Available: <http://calfish.ucdavis.edu/species> (February 2020).
- [ESA] U.S. Endangered Species Act of 1973, as amended, Pub. L. No. 93-205, 87 Stat. 884 (Dec. 28, 1973). Available: <http://www.fws.gov/endangered/esa-library/pdf/ESAall.pdf> (March 2020).



- U.S. Fish and Wildlife Service. 1993. Endangered and threatened wildlife and plants: determination of threatened status for the Giant Garter Snake. Federal Register 58:54053–54066.
- U.S. Fish and Wildlife Service. 1999. Draft recovery plan for the giant garter snake (*Thamnophis gigas*). Portland, Oregon: U.S. Fish and Wildlife Service.
- Wassersug RJ. 1989. Locomotion in amphibian larvae (or “Why aren’t tadpoles built like fishes?”). Integrative and Comparative Biology 29:65–84.
- Weldon PJ. 1982. Responses to ophiophagous snakes by snakes of the genus *Thamnophis*. Copeia 1982:788–794.
- Willson JD, Hopkins, WA. 2011. Prey morphology constrains the feeding ecology of an aquatic generalist predator. Ecology 92:744–754.
- Wylie GD, Casazza ML, Carpenter M. 2003. Diet of bullfrogs in relation to predation of giant garter snakes at Colusa National Wildlife Refuge. California Fish and Game 89:139–145.
- Young ME, Boyarski VL. 2013. *Thamnophis eques megaloops* (northern Mexican gartersnake). Predation. Herpetological Review 44:158–159.
- Zedler JB. 1996. Ecological Issues in wetland mitigation: an introduction to the forum. Ecological Applications 6:33–37.

