

Patterns of morphological variation following colonization of a novel prey environment

I. T. Clifton¹, J. D. Chamberlain² & M. E. Gifford¹

¹ Department of Biology, University of Central Arkansas, Conway, AR, USA

² Department of Biology, University of Arkansas at Little Rock, Little Rock, AR, USA

Keywords

colonization; morphology; snakes; adaptation; *Nerodia rhombifer*; prey size; phenotypic response; selective pressure.

Correspondence

Matthew E. Gifford, Department of Biology, University of Central Arkansas, 201 Donaghey Ave, Conway, AR 72035, USA.
Email: megifford@uca.edu

Editor: Mark-Oliver Rödel

Received 14 November 2016; revised 22 February 2017; accepted 27 February 2017

doi:10.1111/jzo.12459

Abstract

Colonization of new areas is accompanied by a variety of novel pressures, which can lead to rapid phenotypic change. We compared morphology of diamond-backed watersnakes (*Nerodia rhombifer*) among populations of recently colonized fish farms to examine responses to a potential selective pressure, prey size and evaluated intersexual differences in phenotypic responses. Our data suggest not only have these populations experienced morphological change but also that males and females might be responding differently to the shared selective pressure. We found that male snakes from two sites raising primarily small fish had smaller cranial elements than males from one of the sites raising large fish and did not differ from the other large-fish site. Similar to their male conspecifics, we found that females from both large-prey sites had longer quadrates than one of the small-prey sites. In addition, females from large-fish populations reached greater snout-vent lengths than females from small-fish sites. These findings are consistent with an adaptive response to prey size, and also a differential response between the sexes. Our study demonstrates the potential for rapid phenotypic response to a strong selective pressure following colonization.

Introduction

Natural selection is a constant process where individuals with certain traits survive and reproduce more frequently than other members of the population. The differential success in reproduction causes favored phenotypes, assuming they are heritable, to be more prevalent in future generations (Endler, 1986). The conservation and proliferation of favored phenotypes by natural selection results in locally adapted populations (Hoekstra, Krenz & Nachman, 2005; Hall & Willis, 2006).

In a constant environment, phenotypes within a population are generally at equilibrium with selection (i.e. stabilizing selection; Lande & Shannon, 1996) due to local adaptation; however, when a new area is colonized, the colonizing population is likely to be exposed to new selective pressures (Reznick & Ghalambor, 2001; Yeh, 2004). Under this novel environmental pressure one of two outcomes is possible: the pressure may be too intense for the population to handle resulting in extinction, or the population will adapt. Adaptation through natural selection takes place over time as more successful individuals contribute more genes to future generations. This causes the mean phenotype to shift away from the mean of the ancestral population (Losos *et al.*, 2001).

The morphology–performance–fitness paradigm (Arnold, 1983) provides a powerful means of investigating the link

between morphology and fitness. If a particular morphological trait hinders an organism's performance it can result in a decrease in fitness, but if the morphological trait increases performance the organism can experience increased fitness. Feeding morphology frequently is thought to be a target of natural selection (Boag & Grant, 1981; Carroll & Boyd, 1992; Brecko *et al.*, 2011) because even slight differences in form can substantially influence the feeding performance of an organism (Carroll *et al.*, 2004) and thus its overall fitness (Forsman & Lindell, 1993; Forsman, 1996) potentially leading to local adaptation (Boag & Grant, 1981; Carroll & Boyd, 1992). Feeding morphology may be influenced by prey size, prey shape, prey defenses or a variety of other prey characteristics.

Snakes provide an excellent model system to examine the influence of prey characteristics on feeding morphology because, as gape-limited predators, selection has several obvious targets on which to act. To consume larger prey, changes must occur in either body size or head size. This physical constraint has been overcome throughout the evolutionary history of snakes leading to repeated modification in cranial shape in response to prey size (Gans, 1961; Cundall & Greene, 2000). This impressive diversification of cranial morphology is exemplified in the macrostomatan snakes, which are the most derived of the snake lineages and characterized by large heads relative to body size as well as large gapes relative to head

size and highly kinetic skulls (Gans, 1961; Close & Cundall, 2014). This lineage encompasses a large number of snakes with a variety of specializations in feeding morphologies (Greene, 1997).

Increased relative length of head elements has been linked to better prey handling performance and larger gape in a range of snake species (Vincent *et al.*, 2006, 2009). This observed pattern is independent of body size. However, head size varies as an allometric function of body size and, as such, an increase in overall body size will also lead to a correspondingly larger head.

Diamond-backed watersnakes (*Nerodia rhombifer*) are piscivorous snakes (Mushinsky, Hebrard & Vodopich, 1982; Gibbons & Dorcas, 2004) that reproduce annually and reach sexual maturity in approximately 2 years (Gibbons & Dorcas, 2004). They are common in lentic systems such as ponds and bayous, where prey size can vary substantially, and artificial water bodies, such as fish farms, where prey sizes are generally far more constrained. Given that *N. rhombifer* primarily prey on fish and have a relatively short generation time, they are well suited to examining the effects of prey size on feeding morphology.

In Arkansas, *N. rhombifer* are found in high densities on fish farms in the Mississippi River delta region. These farms, which specialize in raising either large- or small-bodied fish, provide an excellent opportunity to investigate the effects of prey size on the evolution of feeding morphology, specifically, head size. By comparing different populations of *N. rhombifer* from farms raising large-bodied fish and farms raising small-bodied fish, we can determine how prey size influences different head components. Each fish farm was established between 30 and 90 years ago (approximately 15–45 watersnake generations), potentially providing sufficient time for adaptive phenotypic change. Because of the low prey size diversity in these sites, we made two non-mutually exclusive predictions. (1) We predict snakes from populations with abundant large prey will have relatively larger heads than snakes from populations exposed to small prey. (2) Snakes exposed to large prey will reach greater maximum body size than snakes from small prey sites.

Materials and methods

We chose four fish farms in Lonoke County, Arkansas, containing high densities of *N. rhombifer*. These four locations can be characterized by the size of the fish they produce and therefore the prey available to snakes. The first two sites, Joe Hogan State Fish Hatchery (JOHO) and Keo Fish Farm (KEO), are characterized by the production of relatively large-bodied fish species. JOHO specializes in rearing channel catfish (*Ictalurus punctatus*), reaching a maximum length of 30 cm, and comprising nearly 80% of JOHO's total production. This farm also produces several other warm-water species including largemouth bass (*Micropterus salmoides*) and black crappie (*Pomoxis nigromaculatus*). KEO fish farm produces both hybrid striped bass (*Morone saxatilis/chrysops*) reaching a maximum length of 25 cm and triploid grass carp (*Ctenopharyngodon idella*) reaching a maximum length of 30 cm. The second two sites, Gentry and Canterbury Fish Farm (GNC) and Pool Fisheries (POOL) are both characterized by the production of relatively small-bodied goldfish (*Carassius auratus*) which reach a maximum size of 7.5 cm at both farms (Table 1). POOL and JOHO are the most centrally located of the four sites and are approximately 6 km apart (the minimum linear distance between any two sites) with POOL being approximately 6.5 km from GNC and 12 km from JOHO. KEO is the furthest from the other three sites at 20 km from JOHO, 25 km from POOL and 31 km from GNC. Further descriptions of each site and prey characteristics are described in Chamberlain (2016).

Standard length is used as a proxy for other morphometric traits as it is related to width, height and mass of each fish species. In JOHO channel catfish, a 1 cm increase in length corresponds to approximately a 1.7 mm increase in height, a 2 mm increase in width and a 1.2 g increase in mass. At KEO, a 1 cm increase in the length of a striped bass corresponds to a 2.6 mm increase in height, a 1.4 mm increase in width and a 5.3 g increase in mass; and a 1 cm increase in length for a grass carp corresponds to a 2.2 mm increase in height, a 1.6 mm increase in width and a 2.1 g increase in mass. For goldfish at GNC and POOL, a 1 cm increase in length is associated with a

Table 1 Summary of prey density of each fish species. Stocking and harvest values represent individuals stocked or harvested per hectare of pond. Stocking and harvest size denote the range in sizes of each fish at stocking or harvest. The term fry denotes fish that were spawned in the pond from eggs. Their initial size was not measured but is estimated as the size at hatching. Mean and maximum ingested prey sizes were measured from samples obtained through forced regurgitation

Site	Species	Stocking		Harvest Size (cm)		Mean Ingested Prey Mass (g)	Maximum Ingested Prey Mass (g)
		Size	Stocking	Harvest	Harvest		
GNC	Goldfish	Fry	250 000–500 000	2.5–5 and 5–7.5	100 000–500 000	19.13 (<i>N</i> = 39)	115
POOL	Goldfish	Fry	250 000–500 000	2.5–5 and 5–7.5	100 000–500 000	16.91 (<i>N</i> = 35)	94
KEO	Grass carp	Fry	50 000	10–25	50 000	43.76 (<i>N</i> = 137)	460
	Striped bass	Fry	20 000	2.5–25	20 000		
JOHO	Channel catfish (fingerling)	Fry	10 000	7.5	10 000	NA	NA
	Channel catfish (feeding)	7.5	4000–8000	15–30	4000–8000	NA	NA

GNC, Gentry and Canterbury Fish Farm; JOHO, Joe Hogan State Fish Hatchery; KEO, Keo Fish Farm; POOL, Pool Fisheries.

3.5 mm increase in height, a 1.5 mm increase in width and a 1.8 g increase in mass.

We sampled GNC, KEO and JOHO a minimum of once per month beginning in late March and continuing into late October from 2013 to 2015 with POOL being added as a study site in 2014. During each sampling period, we walked the shoreline of individual farm ponds and captured snakes by hand. Sampling was conducted for approximately one hour per sampling period totaling to about six sampling periods per site per year.

For each snake captured, we measured the snout-vent length (cm; SVL), mass (g), head length (mm; measured from the tip of the rostrum to the caudal end of the skull where it articulates with C1), lower jaw length (mm; measured from the rostral tip of the mandible to the caudal tip of the mandible) and quadrate length (mm; measured from the dorsal tip where it articulates with the supratemporal bone to the ventral tip where it articulates with the mandible). We chose to measure these cranial elements because they are common metrics for the study of head size in snakes, together they contribute to gape size, and their importance has been demonstrated in previous studies (Vincent *et al.*, 2009; Hampton, 2011, 2014). Upon capture, we gently forced snakes with food in their guts to regurgitate prey and the prey item was identified and weighed (g) (Table 1). We generally did not cause snakes from JOHO to regurgitate prey due to the significant risk of injury caused by catfish spines; snakes were observed consuming large catfish on numerous occasions. We marked captured snakes with unique identification codes by clipping ventral scales to prevent repeated measurements. We released all snakes immediately after marking and measuring them.

We analyzed data from males and females separately due to clear sexual size dimorphism (Gibbons & Dorcas, 2004). We used data from two different datasets to test for differences in head shape and differences in adult body size (Table 2). The first dataset contained only animals with complete data for

cranial measurements and SVL and was used to analyze differences in cranial morphology. The second dataset was larger and was composed of the previous dataset along with additional animals for which only SVL measurements were available.

The first dataset consisted of 358 males ($n = 78$ from GNC, $n = 110$ from JOHO, $n = 111$ from KEO and $n = 59$ from POOL) and 511 females ($n = 113$ from GNC, $n = 169$ from JOHO, $n = 127$ from KEO and $n = 102$ from POOL) and was used for analysis of cranial morphology. To analyze differences in maximal adult body size, we used only the largest adult snakes from each population (largest 30% from each population sample) from the body size dataset. This measure provides a more direct assessment of maximal asymptotic body size by limiting the effects of past growth rate and age (Stamps & Andrews, 1992). This measurement provides an accurate estimate of asymptotic body size if the sample size is relatively large and is likely to include the largest individuals in a population. Before the dataset was narrowed to just the largest 30%, we had a total of 634 males ($n = 61$ from GNC, $n = 150$ from JOHO, $n = 368$ from KEO and $n = 55$ from POOL) and 816 females ($n = 129$ from GNC, $n = 172$ from JOHO, $n = 393$ from KEO and $n = 122$ from POOL). After identifying the largest 30% from each population, we were left with 190 males ($n = 18$ for GNC, $n = 45$ for JOHO, $n = 110$ for KEO and $n = 17$ for POOL) and 246 females ($n = 39$ for GNC, $n = 52$ for JOHO, $n = 118$ for KEO and $n = 37$ for POOL).

We analyzed data using analysis of covariance (ANCOVA) to test for differences in cranial elements among sites using body size (SVL) as a covariate. In instances where there were significant differences among sites that were confounded by differences in scaling relationships (i.e. differences in slope among populations), we used ordinary least squares (OLS) regression and reduced major axis (RMA) regression to characterize variation in scaling relationships among populations using the lmodel2 package in R (R Core Team 2016). Results of RMA regression were similar to OLS results and are reported in Tables S1 and S2. A TukeyHSD was used for post hoc analysis of differences when the main effect of “site” was statistically significant and scaling relationships did not differ among populations. We used analysis of variance (ANOVA) to evaluate differences in body size followed by TukeyHSD tests to determine population-level patterns of variation. All analyses tested for statistical significance at $\alpha < 0.05$; all data satisfied the assumptions of parametric testing.

Results

All measurements showed significant sexual dimorphism. As expected, all cranial elements were strongly related to SVL, although each cranial trait scaled with SVL differently in males and females (Table 3). In each case, cranial elements of female snakes exhibited steeper allometric slopes than for male snakes.

Males differed significantly among populations in head length, lower jaw length and quadrate length (Table 4); however, head length and lower jaw length each scaled differently

Table 2 Sample sizes of datasets used for analyses. Sample size for body size represents the largest 30% of each dataset (see text for details)

Site	Sex	Total	Body size (SVL)	Cranial morphology
GNC	M	61	18	78
	F	129	39	113
JOHO	M	150	45	110
	F	172	52	169
KEO	M	368	110	111
	F	393	118	127
POOL	M	55	17	59
	F	122	37	102
Total M		634	190	358
Total F		816	246	511
Total		1450	436	869

GNC, Gentry and Canterbury Fish Farm; JOHO, Joe Hogan State Fish Hatchery; KEO, Keo Fish Farm; POOL, Pool Fisheries; SVL, snout-vent length.

Table 3 Summary statistics of ordinary least square regression of cranial elements against snout-vent length examining differences in scaling among sexes. Slopes and intercepts are followed by their 95% confidence intervals in parentheses

	Slope	Intercept	Allometry
Male			
Head	0.643 (0.619, 0.667)	0.191 (0.148, 0.233)	Negative
Lower jaw	0.783 (0.761, 0.805)	0.035 (−0.004, 0.074)	Negative
Quadrate	0.969 (0.939, 0.999)	−0.688 (−0.741, −0.635)	Isometric
Female			
Head	0.750 (0.732, 0.768)	0.037 (0.004, 0.070)	Negative
Lower jaw	0.882 (0.865, 0.899)	−0.099 (−0.131, −0.068)	Negative
Quadrate	1.093 (1.074, 1.111)	−0.854 (−0.889, −0.819)	Positive

Table 4 ANCOVA results testing for differences among populations in male and female head length, lower jaw length and quadrate length using snout-vent length (SVL) as the covariate

Cranial element	Factor	Degrees of freedom	F-value	P-value
Male				
Head length	SVL	1,350	2954.28	<0.001
	Location	3,350	4.1	0.007
	SVL × location	3,350	2.72	0.0447
Lower jaw length	SVL	1,350	5252.62	<0.001
	Location	3,350	5.11	0.002
	SVL × location	3,350	3.00	0.031
Quadrate length	SVL	1,350	4221.16	<0.001
	Location	3,350	4.78	0.003
	SVL × location	3,350	1.37	0.251
Female				
Head Length	SVL	1,503	7647.62	<0.001
	Location	3,503	13.54	<0.001
	SVL × location	3,503	11.25	<0.001
Lower jaw length	SVL	1,503	12 377.99	<0.001
	Location	3,503	18.79	<0.001
	SVL × location	3,503	13.63	<0.001
Quadrate length	SVL	1,503	13 572.42	<0.001
	Location	3,503	5.58	<0.001
	SVL × location	3,503	1.30	0.273

with SVL among populations. In both cases, GNC had the steepest slope followed by KEO, then JOHO, with POOL having the shallowest slope (Table 5; Fig. 1). Males from KEO had significantly longer quadrates than males from GNC and POOL regardless of SVL ($P = 0.014$ and $P = 0.028$, respectively), whereas males from JOHO were not significantly different than males from any of the three other populations. Despite these differences in slope (as indicated by the ANCOVA), the confidence intervals for the slope estimates from OLS and RMA regressions generally overlap among populations. After removing the interaction term (SVL × Location), there was still significant variation among populations in head and lower jaw ($F_{3,353} = 22.79$, $P = 0.008$ and $F_{3,353} = 22.79$, $P = 0.002$, respectively). Males from KEO had

Table 5 Summary statistics of ordinary least squares regression of cranial elements against SVL for differences in scaling among populations in male and female snakes. Slopes and intercepts are followed by their 95% confidence intervals in parentheses

	Slope	Intercept	Allometry
Male			
Head			
KEO	0.679 (0.637, 0.721)	0.131 (0.057, 0.758)	Negative
JOHO	0.627 (0.591, 0.663)	0.222 (0.158, 0.591)	Negative
GNC	0.704 (0.639, 0.770)	0.074 (−0.046, 0.193)	Negative
POOL	0.602 (0.533, 0.672)	0.0258 (0.133, 0.384)	Negative
Lower jaw			
KEO	0.816 (0.777, 0.854)	−0.016 (−0.084, 0.052)	Negative
JOHO	0.763 (0.728, 0.798)	0.072 (0.010, 0.134)	Negative
GNC	0.849 (0.792, 0.905)	−0.088 (−0.190, 0.015)	Negative
POOL	0.757 (0.696, 0.818)	0.076 (−0.034, 0.186)	Negative
Quadrate			
KEO	0.972 (0.917, 1.027)	−0.685 (−0.783, −0.588)	Isometric
JOHO	0.966 (0.923, 1.010)	−0.758 (−0.758, −0.603)	Isometric
GNC	1.062 (0.981, 1.143)	−0.864 (−1.011, −0.717)	Isometric
POOL	0.961 (0.872, 1.049)	−0.679 (−0.839, −0.519)	Isometric
Female			
Head			
KEO	0.779 (0.744, 0.814)	−0.008 (−0.072, 0.055)	Negative
JOHO	0.698 (0.670, 0.726)	0.140 (0.088, 0.192)	Negative
GNC	0.849 (0.806, 0.893)	−0.154 (−0.234, −0.073)	Negative
POOL	0.731 (0.692, 0.770)	0.059 (−0.013, 0.130)	Negative
Lower jaw			
KEO	0.924 (0.890, 0.958)	−0.161 (−0.223, −0.099)	Negative
JOHO	0.831 (0.805, 0.857)	−0.003 (−0.052, 0.045)	Negative
GNC	0.976 (0.936, 1.015)	−0.278 (−0.351, −0.205)	Isometric
POOL	0.879 (0.848, 0.911)	−0.106 (−0.164, −0.048)	Negative
Quadrate			
KEO	1.092 (1.055, 1.129)	−0.846 (−0.913, −0.778)	Positive
JOHO	1.081 (1.1052, 1.110)	−0.830 (−0.885, −0.775)	Positive
GNC	1.143 (1.094, 1.192)	−0.948 (−1.039, −0.858)	Positive
POOL	1.092 (1.043, 1.140)	−0.862 (−0.950, −0.773)	Positive

GNC, Gentry and Canterbury Fish Farm; JOHO, Joe Hogan State Fish Hatchery; KEO, Keo Fish Farm; POOL, Pool Fisheries; SVL, snout-vent length.

significantly longer heads than males from GNC ($P = 0.03$), whereas males from JOHO and POOL were not statistically different from either KEO or GNC. Males from KEO had significantly longer lower jaws than males from both GNC and POOL ($P = 0.012$ and $P = 0.004$, respectively). Lower jaws of males from JOHO did not vary significantly from males of any of the other three populations.

The scaling relationship did not differ significantly among populations for quadrate length in females, but mean quadrate length did (Table 4). Specifically, females from KEO and JOHO had significantly longer quadrates than females from POOL ($P < 0.001$ and $P = 0.016$, respectively). Conversely, head length and lower jaw length differed in allometry among populations (Table 5; Fig. 1), making it difficult to identify a specific pattern of variation among populations. Furthermore, variation in scaling relationships among populations did not follow a predictable pattern associated with prey size among

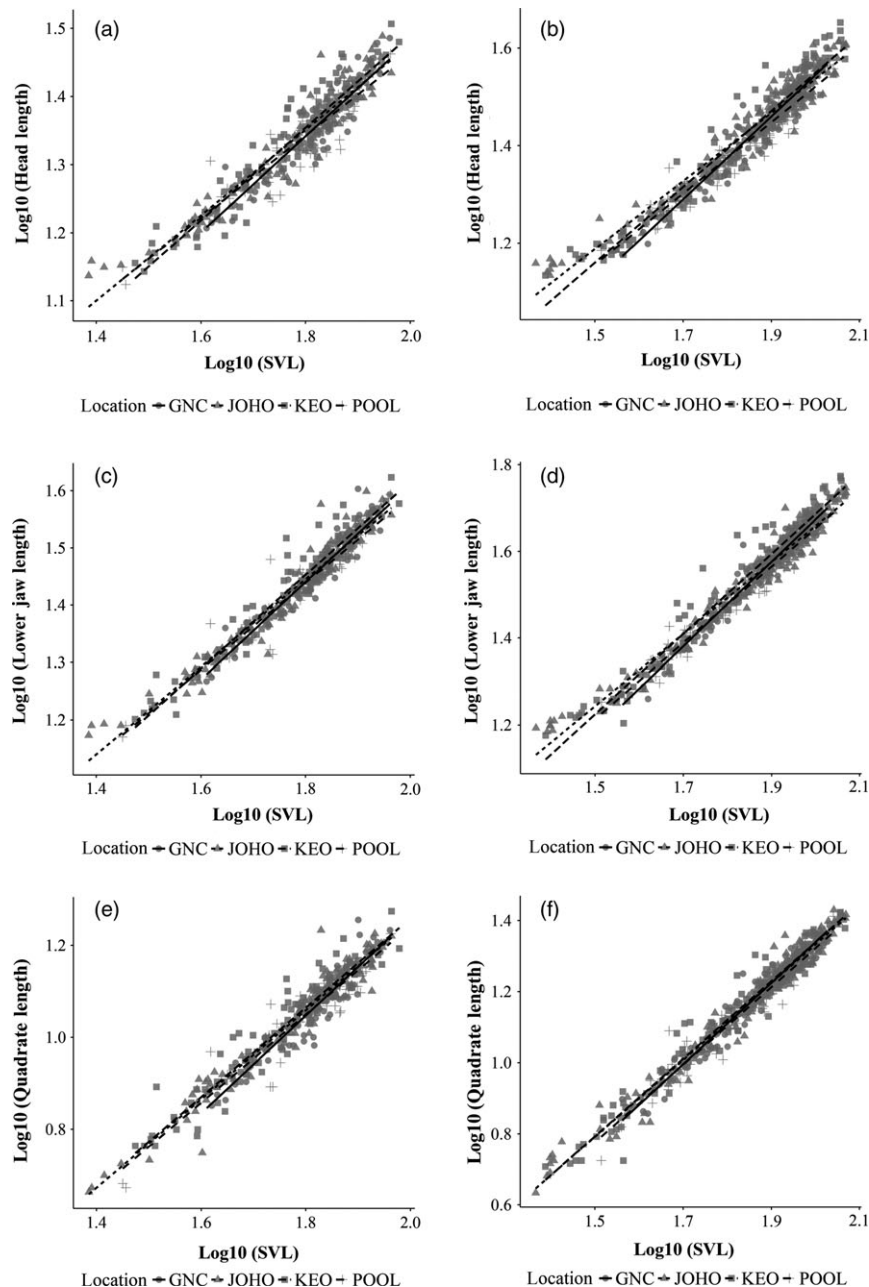


Figure 1 Variation in cranial elements as a function of snout-vent length (SVL) for males and females among each population after \log_{10} transformation. Male head length (a) next to female head length (b), male lower jaw length (c) next to female lower jaw length (d), and male quadrate length (e) next to female quadrate length (f).

populations. For both head length and lower jaw length we found that female snakes from GNC had the steepest slopes, whereas JOHO had the shallowest slopes. The slopes for KEO and POOL were located between the other two sites (Table 5; Fig. 1).

Mean asymptotic body size (SVL) differed significantly among populations for females ($F_{3,241} = 22.79$, $P < 0.001$; Fig. 2b), but not for males ($F_{3,186} = 1.63$, $P = 0.184$; Fig. 2a). Specifically, females from KEO and JOHO were significantly

larger than females from GNC ($P < 0.001$ for both comparisons) and POOL ($P < 0.001$ for both comparisons). Mean asymptotic body size of females from KEO and JOHO was, on average, 8% larger than females from GNC and POOL.

Discussion

We found strong support for the second hypothesis that snakes exposed to large prey will reach larger body sizes – but only

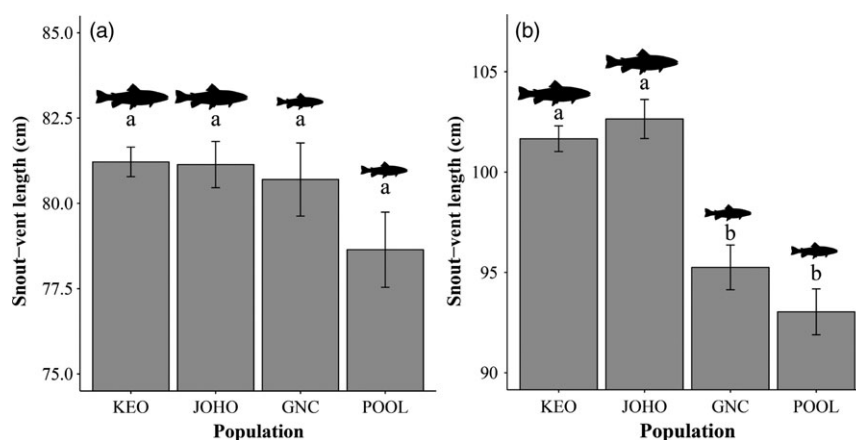


Figure 2 Variation in maximal (asymptotic) adult body size snout-vent length (SVL) among populations for males (a) and females (b) (mean \pm SE). Site type is indicated by either a large fish (large-prey site) or a small fish (small-prey site) above each bar.

for female snakes. Females from KEO and JOHO, both large-prey sites, reached significantly larger asymptotic sizes than snakes from the two small-prey sites. These data suggest that female snakes from large-prey sites have responded to frequent encounters with large prey by adjusting maximal body size. Such a change would result in a larger absolute head size in females from large-prey populations relative to those from small-prey sites.

Variation in prey size constrains the ability of predators to ingest some prey items, and in many cases has been overcome by changes in size of cranial characters (e.g. Carroll *et al.*, 2004; Vincent *et al.*, 2006). We found mixed support for this hypothesis in our study. Male *Nerodia rhombifer* from KEO, one of the large-prey sites, had significantly longer cranial elements than snakes from at least one, if not both, small-prey sites. In general, male snakes from JOHO did not differ statistically from males from the other large-prey site, KEO, in the relative length of cranial elements, or did males from JOHO differ from the males from the two small-prey sites. Like males, females differed significantly in quadrate length, but with a slightly different pattern. Females from both large-prey sites had longer quadrates than females from POOL, a small-prey site, and females from GNC did not differ significantly from any of the other three populations. We found that each cranial trait scaled differently with body size (SVL) between sexes. The growth trajectory of each trait in females, regardless of population, was steeper than those of males. With only one exception (quadrate), each trait exhibited a negative allometry; a finding consistent with other studies of cranial morphology in snakes (e.g. Vincent *et al.*, 2007; Hampton, 2014). Both males and females were found to vary in scaling relationships among populations, at least for head length and lower jaw length. Snakes from GNC, both males and females, consistently had the steepest slopes followed by snakes from KEO. The absence of a clear pattern in cranial allometry suggests that populations have not diverged predictably in allometric relationships of head length and lower jaw length; however, the consistently shallow slope observed in JOHO may suggest disproportionate selection within that large-prey population in

favor of larger relative heads as juveniles. Carrier (1996) hypothesized that juveniles have exaggerated features that increase performance thus compensating for their smaller size. However, more recent investigators have found little evidence to support this hypothesis in feeding structure (Herrel & Gibb, 2006; Hampton, 2014). The patterns revealed in our study are consistent with these latter studies.

While our results are consistent with an adaptive response to accommodate ingesting large prey, we are unable to rule out effects of developmental plasticity as a possible explanation for observed patterns as others have noted (e.g. Bonnet *et al.*, 2001; Krause, Burghardt & Gillingham, 2003; Schuett *et al.*, 2005). In populations of Australian tiger snakes (*Notechis scutatus*), Aubret, Shine & Bonnet (2004) demonstrated a plastic response in jaw length in response to prey size. In another feeding experiment, Queral-Regil & King (1998) found evidence for plasticity in body size, but only limited evidence in cranial traits (lower jaw) in *N. sipedon*. However, preliminary results from feeding trials in neonate *N. rhombifer* from two populations used in this study (KEO and GNC) did not indicate a strong plastic response in cranial morphology through the first 3 months of a neonate's life (Clifton and Gifford, unpubl. data). The prevalence of plasticity in cranial traits in other snake systems means the patterns observed here may be due to a plastic response or a combination of plastic and adaptive responses that cannot be parsed out with the available data.

Prey size can play an important role in shaping predator phenotypes, especially in the case of feeding morphology in gape-limited predators. In our system, it appears that *N. rhombifer* have changed morphologically to consume large prey at sites where larger prey are more frequently encountered. However, the specific morphological changes in response to larger prey apparently differed between males and females. Where females have experienced a multi-level change (relative quadrate length and absolute head size), males appear to have changed only in relative length of some cranial elements. Ultimately, changing the absolute size of the head and the relative size of cranial elements should have a similar consequence, allowing individuals to consume larger prey. The two large-

prey populations appear to have experienced rapid phenotypic change (occurring within or among generations or a combination of both) in response to colonization of habitat exposing them to a high frequency of large prey.

Acknowledgments

We want to thank Keo Fish Farms Inc., Gentry and Canterbury Fish Farm LLC., Pool Fisheries Inc. and Joe Hogan State Fish Hatchery for allowing us to use their properties. We owe thanks to T. Clay, A. Anderson, B. Perlinger, D. Filipek, M. Polett, R. Rowland, N. Ledbetter, C. Robinson, L. Monday and A. White for their assistance in the field. We are also thankful to C. Robinson for comments on previous drafts of this manuscript. Comments from two anonymous reviewers greatly improved this manuscript. This research was partly funded by the Theodore Roosevelt Grant from the American Museum of Natural History and the UCA College of Natural Science and Mathematics Student Research Grant. All methods were approved by the University of Central Arkansas Institutional Animal Care and Use Committee approval #14-006.

References

- Arnold, S.J. (1983). Morphology, performance and fitness. *Am. Nat.* **23**, 347–361.
- Aubret, F., Shine, R. & Bonnet, X. (2004). Adaptive developmental plasticity in snakes. *Nature* **431**, 261–262.
- Boag, P.T. & Grant, P.R. (1981). Intense natural selection in a population of Darwin's finches (Geospizinae) in the Galápagos. *Science* **214**, 82–85.
- Bonnet, X., Shine, R., Naulleau, G. & Thiburce, C. (2001). Plastic vipers: influence of food intake on the size and shape of Gaboon vipers (*Biris gabonica*). *J. Zool.* **255**, 341–351.
- Brecko, J., Vervust, B., Herrel, A. & Van Damme, R. (2011). Head morphology and diet in the dice snake (*Natrix tessellata*). *Mertensiella* **18**, 20–29.
- Carrier, D.R. (1996). Ontogenetic limits on locomotor performance. *Physiol. Zool.* **69**, 467–488.
- Carroll, S.P. & Boyd, C. (1992). Host race radiation in the soapberry bug: natural history with the history. *Evolution* **46**, 1052–1069.
- Carroll, A.M., Wainwright, P.C., Huskey, S.H., Collar, D.C. & Turingan, R.G. (2004). Morphology predicts suction feeding performance in centrarchid fishes. *J. Exp. Biol.* **207**, 3873–3881.
- Chamberlain, J. D. (2016). Chapter 4: A comparison of prey quality, quantity, and assimilation efficiency among populations of watersnakes. PhD Dissertation, Life-history and energetics of the diamond-backed watersnake. 83–106.
- Close, M. & Cundall, D. (2014). Snake lower jaw skin: extension and recovery of a hyperextensible keratinized integument. *J. Exp. Zool.* **321A**, 78–97.
- Cundall, D. & Greene, H.W. (2000). Feeding in snakes. In *Feeding: form, function and evolution in tetrapod vertebrates*: 293–327. Schwenk, K. (Ed.). San Diego: Academic Press.
- Endler, J.A. (1986). *Natural selection in the wild*. Princeton: Princeton University Press.
- Forsman, A. (1996). Body size and net energy gain in gape-limited predators: a model. *J. Herpetol.* **30**, 307–319.
- Forsman, A. & Lindell, L.E. (1993). The advantage of a big head: swallowing performance in adders, *Vipera berus*. *Funct. Ecol.* **7**, 183–189.
- Gans, C. (1961). The feeding mechanism of snakes and its possible evolution. *Am. Zool.* **1**, 217–227.
- Gibbons, J.W. & Dorcas, M.E. (2004). *North American watersnakes: a natural history*. Norman: University of Oklahoma Press.
- Greene, H.W. (1997). *Snakes: the evolution of nature in mystery*. Berkeley, CA: University of California Press.
- Hall, M.C. & Willis, J.H. (2006). Divergent selection on flowering time contributes to local adaptation in *Mimulus guttatus* populations. *Evolution* **60**, 2466–2477.
- Hampton, P.M. (2011). Feeding performance in the western ribbon snake (*Thamnophis proximus*): ontogeny and the effects of prey type and size. *Can. J. Ecol.* **89**, 945–950.
- Hampton, P.M. (2014). Allometry of skull morphology, gape size and ingestion performance in the banded watersnake (*Nerodia fasciata*) feeding on two types of prey. *J. Exp. Biol.* **217**, 472–478.
- Hoekstra, H.E., Krenz, J.G. & Nachman, M.W. (2005). Local adaptation in the rock pocket mouse (*Chaetodipus intermedius*): natural selection and phylogenetic history of populations. *Heredity* **94**, 217–228.
- Herrel, H. & Gibb, A.C. (2006). Ontogeny of performance in vertebrates. *Physiol. Biochem. Zool.* **79**, 1–6.
- Krause, M.A., Burghardt, G.M. & Gillingham, J.C. (2003). Body size plasticity and local variation of relative head and body size sexual dimorphism in garter snakes (*Thamnophis sirtalis*). *J. Zool.* **261**, 399–407.
- Lande, R. & Shannon, S. (1996). The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution* **50**, 434–437.
- Losos, J.B., Schoener, T.W., Warheit, K.I. & Creer, D. (2001). Experimental studies of adaptive differentiation in Bahamian *Anolis* lizards. *Genetica* **112–113**, 399–415.
- Mushinsky, H.R., Hebrard, J.J. & Vodopich, D.S. (1982). Ontogeny of water snake foraging ecology. *Ecology* **63**, 1624–1629.
- Queral-Regil, A. & King, R.B. (1998). Evidence for phenotypic plasticity in body size and relative head dimensions in response to amount and size of prey. *Copeia* **423–429**.
- R Core Team. (2016). *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.
- Reznick, D.N. & Ghalambor, C.K. (2001). The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica* **112–113**, 183–198.
- Schuett, G.W., Hardy, D.L. Jr, Earley, R.L. & Greene, H.W. (2005). Does prey size induce head skeleton phenotypic

- plasticity in early ontogeny in the snake *Boa constrictor*. *J. Zool.* **267**, 363–369.
- Stamps, J.A. & Andrews, R.M. (1992). Estimating asymptotic size using the largest individuals per sample. *Oecologia* **92**, 503–512.
- Vincent, S.E., Dang, P.D., Herrel, A. & Kley, N.J. (2006). Morphological integration and adaptation in the snake feeding system: a comparative phylogenetic study. *Evol. Biol.* **19**, 1545–1554.
- Vincent, S.E., Moon, B.R., Herrel, A. & Kley, N.J. (2007). Are ontogenetic shifts in diet linked to shifts in feeding mechanics? Scaling of the feeding apparatus in the banded watersnake *Nerodia fasciata*. *J. Exp. Biol.* **210**, 2057–2069.
- Vincent, S.E., Brandley, M.C., Herrel, A. & Alfaro, M.E. (2009). Convergence in trophic morphology and feeding performance among piscivorous natricine snakes. *J. Exp. Biol.* **22**, 1203–1211.

- Yeh, P.J. (2004). Rapid evolution of a sexually selected trait following population establishment in a novel habitat. *Evolution* **58**, 166–174.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Summary statistics of reduced major axis regression of cranial elements against SVL examining differences in scaling among sexes. Slopes and intercepts are followed by their 95 % confidence intervals in parentheses.

Table S2. Summary statistics of reduced major axis regression of cranial elements against SVL for differences in scaling among populations in male and female snakes. Slopes and intercepts are followed by their 95% confidence intervals in parentheses.