

ORIGINAL ARTICLE



Role of phenotypic plasticity in morphological differentiation between watersnake populations

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Abstract

An individual's morphology is shaped by the environmental pressures it experiences, and the resulting morphological response is the culmination of both genetic factors and environmental (non-genetic) conditions experienced early in its life (i.e. phenotypic plasticity). The role that phenotypic plasticity plays in shaping phenotypes is important, but evidence for its influence is often mixed. We exposed female neonate diamond-backed watersnakes (*Nerodia rhombifer*) from populations experiencing different prey-size regimes to different feeding treatments to test the influence of phenotypic plasticity in shaping trophic morphology. We found that snakes in a large-prey treatment from a population frequently encountering large prey exhibited a higher growth rate in body size than individuals in a small-prey treatment from the same population. This pattern was not observed in snakes from a population that regularly encounters small prey. We also found that regardless of treatment, snakes from the small-prey population were smaller at birth than snakes from the large-prey population and remained so throughout the study. These results suggest that the ability to plastically respond to environmental pressures may be population-specific. These results also indicate a genetic predisposition towards larger body sizes in a population where large prey items are more common.

Key words: common garden, diamond-backed watersnake, local adaptation, phenotypic plasticity, trophic morphology

INTRODUCTION

Individuals must overcome a variety of environmental pressures to survive and reproduce. These pressures frequently shape the morphologies of individuals within and among populations. Phenotypes are the result of 2 processes: (i) expression of population-level genetic adapta-

tions over generations (e.g. Hoekstra *et al.* 2005; Hall & Willis 2006); and/or (ii) non-genetic phenotypic variation among individuals in response to environmental cues (i.e. phenotypic plasticity) (e.g. Queral-Regil & King 1998; Aubret *et al.* 2004).

Parsing the influence of these respective processes in shaping morphology is critical for our understanding of the long-term viability of populations in the face of environmental pressures. This is particularly true when organisms encounter new pressures such as those associated with colonization events (Reznick & Ghalambor 2001; Ghalambor *et al.* 2007). Because

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genetic adaptation takes many generations to occur and can be constrained by intrinsic factors (e.g. phenotypic and genetic correlations), it can be slow to produce phenotypes useful for coping with novel environmental pressures (Etterson & Shaw 2001; Futuyma 2010). Conversely, phenotypic plasticity occurs rapidly (i.e. within a single generation), but resultant phenotypes are not necessarily heritable to subsequent generations. Thus, phenotypic plasticity may constitute a temporary solution to a long-term environmental pressure, and in the absence of adaptive processes, the population will likely succumb to local extinction if the environmental pressure persists.

The morphology of feeding structures, or trophic morphology, is often a target of selective pressures (e.g. Mori & Vincent 2008; Segall *et al.* 2016) because even slight variation among individuals can result in substantial differences in performance, and, ultimately, fitness (Boag & Grant 1981; Arnold 1983). For example, Brecko *et al.* (2011) found evidence of differences in head size and head shape of dice snakes (*Natrix tessellate* Laurenti, 1768) with snakes that had consumed fish having more narrow heads, likely an advantage when capturing prey in water, than snakes that had fed on frogs. Environmental pressures on trophic morphology can be intense, especially in gape-limited predators (i.e. animals that only consume prey small enough to swallow whole) where morphological changes are relatively limited. The highly kinetic skulls of snakes have largely allowed them to overcome this limitation, enabling them to consume large prey relative to their size (Gans 1961; Cundall & Greene 2000; Vincent *et al.* 2006b).

The actual size of prey consumed by a snake, however, is constrained by both the size of its head relative to its body and the absolute size of the snake itself. A relatively large head leads to a relatively large gape, allowing the snake to ingest larger prey than what the body size alone might suggest (Vincent *et al.* 2006a, 2009). Alternatively, due to the relationship between head size and body size, larger snakes have absolutely larger heads than smaller snakes (e.g. Clifton *et al.* 2017), allowing them to consume larger prey than smaller conspecifics. Therefore, increases in either head size or in body size should increase the size of prey a snake can ingest. The ability to consume larger prey yields more energy per unit search effort, leading to a potential energetic advantage of having a larger trophic apparatus. The morphological constraint imposed by gape-limitation makes snakes an ideal model for testing plastic responses to environmental pressures. However, studies examining phenotypic plasticity's effect on trophic morphology in snakes has yielded mixed re-

sults (e.g. Queral-Regil & King 1998; Aubret *et al.* 2004; Schuett *et al.* 2005).

Diamond-backed watersnakes (*Nerodia rhombifer* Hallowell, 1852) are common in lentic systems of the southeastern United States. Unlike congeners that feed to varying degrees on both fish and amphibians depending on age and species, *N. rhombifer* are primarily piscivorous throughout their lives (Mushinsky *et al.* 1982). *N. rhombifer* have repeatedly colonized fish farms in central Arkansas, USA, where fish are raised in extremely high densities for commercial uses. These fish farms provide habitats where fish sizes are more constrained than would generally be found in nature. Furthermore, fish farms tend to specialize in the fish they produce, with some producing large-bodied fish and others producing small-bodied fish, effectively creating semi-natural mesocosms. Due to the restricted fish size and the limited overlap in prey size between fish farms (i.e. *N. rhombifer* populations), we have a unique opportunity to investigate the relative contributions of phenotypic plasticity and local adaptation to shaping trophic morphology between human-modified watersnake populations.

Using 2 populations of *N. rhombifer* that differ in prey size distributions, we attempted to elicit a plastic response in predator trophic morphology by exposing female neonate snakes to a diet of either large-bodied fish or small-bodied fish. Experimental studies that minimize extraneous environmental effects (e.g. common garden) allow us to identify the relative roles plasticity and adaptation play in shaping trophic morphology. If predator morphology is plastically influenced by prey size, we would expect neonate watersnakes only eating large-bodied fish to grow larger heads and/or larger bodies (SVL) than snakes eating only small-bodied fish, regardless of population; however, if patterns differ between populations irrespective of feeding treatment, it would suggest that local genetic differences (i.e. local adaptation) are influencing the morphological response to prey size.

MATERIALS AND METHODS

In August 2014, we hand-captured snakes from 2 study sites in Lonoke County, Arkansas, USA, approximately 30 km from one another. Each site specialized in raising different species that differed in size. At the large-prey site (LARGE), the fish farm specialized in raising large-bodied hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) and triploid grass carp [*Ctenopharyngodon idella* (Valenciennes, 1844)]. At the small-prey site (SMALL), the fish farm specialized in raising

small-bodied goldfish [*Carassius auratus* (Linnaeus, 1758)]. At the LARGE site, fish are stocked as fry and harvested at sizes between 2.5 and 25 cm. In contrast, fish at the SMALL site are stocked as fry and harvested at sizes between 2.5 and 7.5 cm. Thus, while smaller prey were available at the LARGE site, although in relatively low abundance, prey sizes were highly constrained at the SMALL site. More details on site traits, *N. rhombifer* natural history, and prey characteristics of these 2 sites have previously been described (Korfel *et al.* 2015; Chamberlain 2016; Clifton *et al.* 2017).

We collected 17 pregnant *N. rhombifer*, 9 from LARGE and 8 from SMALL. Females were maintained in the laboratory until parturition. We measured snout–vent length (SVL; cm), head length (mm; measured from the tip of the rostrum to the caudal end of the braincase), lower jaw length (mm; measured from rostral tip to 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) on all neonates. Each of the cranial measurements is related to the overall gape of the snake, and an increase in any of the 3 measurements would cause a corresponding increase in the animal's gape (Vincent *et al.* 2009; Hampton 2011, 2014). Two female offspring were randomly selected from each litter (18 from LARGE and 16 from SMALL). One was placed in a large-prey treatment ($n = 17$) and the other was placed in a small-prey treatment ($n = 17$). We used only females to remove the potential for sex-specific effects and because preliminary data from the wild populations suggested morphological differences were more pronounced in females than males (Clifton *et al.* 2017). Although several animals did not survive the entire study ($n = 14$ per treatment upon conclusion), all 34 ($n = 17$ large-prey; $n = 17$ small-prey) individuals were measured a minimum of 6 times and included in the analyses. After parturition mothers were released to their respective populations.

We housed neonates individually in $45 \times 12.5 \times 9$ cm (L \times W \times H) plastic containers and provided them with a paper towel substrate, a hide, a water bowl and a thermal gradient ranging from 25 °C to 30 °C. We changed water regularly (2–3 times per week) and cleaned cages the day after feeding. Cages were randomly replaced on the rack after each feeding; thus, variation due to cage position was minimized. We measured the SVL, mass, head length, lower jaw length and quadrate length of each snake every 2 weeks for 4 months. SVL was measured by gently stretching the individual against a ruler fixed to the work surface and recorded to the nearest 0.1 cm. Each of the 3 cranial elements were measured using digital calipers and

measurements were recorded to the nearest 0.1 mm. Each measurement was taken only 1 time at each measuring period to minimize handling stress to the animal. Morphological measurements of snakes are subject to high degrees of observer error (Cundall *et al.* 2016). To minimize observer error, all measurements were taken by a single observer (I. Clifton) throughout the duration of the study as recommended by Cundall *et al.* (2016).

We offered snakes in both treatments equal masses of fish weekly for the duration of the study. We generally fed all snakes golden shiners (*Notemigonus crysoleucas* Mitchill, 1814); however, this was dependent on fish availability, particularly early in the experiment, and some snakes (primarily in the small-prey treatment) were fed a mixture of golden shiners and fathead minnows (*Pimephales promelas* Rafinesque, 1820) when small golden shiners were in short supply. We measured the mass (g) and the maximum height (measured at the rostral margin of caudal fin [mm]) of each fish that was offered as well as the number of fish each snake was offered. We removed any uneaten fish 24 h after feeding and recorded the number each snake consumed. The total mass offered was divided by the number of fish consumed to estimate the mass each snake consumed at each feeding period. Snakes in the large-prey treatment were fed larger fish (maximum height 7.05–12.15 mm, mean = 9.54 mm), with maximum height increasing over time, than their small-prey treatment conspecifics (maximum height 3.80–7.86 mm, mean = 6.67 mm). We increased the total mass of fish offered to both treatment groups (5.55–11.05 g, mean = 7.36 g) as the study progressed (Schuett *et al.* 2005).

We used 4 linear models in R (R Core Team 2016) and a linear mixed effects model in the lme4 package (Bates *et al.* 2015) in R to assess our feeding treatments. Linear Model 1 used the mean height of each fish offered for each feeding to confirm that the large-prey and small-prey treatments were fed different sized fish (model intercept) and the disparity in fish height increased with time (model slope). Linear Model 2 used the mean total mass of fish offered to each snake for each feeding to confirm that each treatment was offered the same amount of food (model intercept) and the mass remained equal over time (model slope). Linear Model 3 (LARGE population) and Linear Model 4 (SMALL population) used the mean mass of fish consumed to determine if mass of prey consumed was similar through the duration of the study (model slope). Linear Model 5, a linear mixed effects model with ID as a random effect, was used to confirm that snakes in the large-prey treatment were fed larger fish relative to their head size (mean prey height/head length) (model

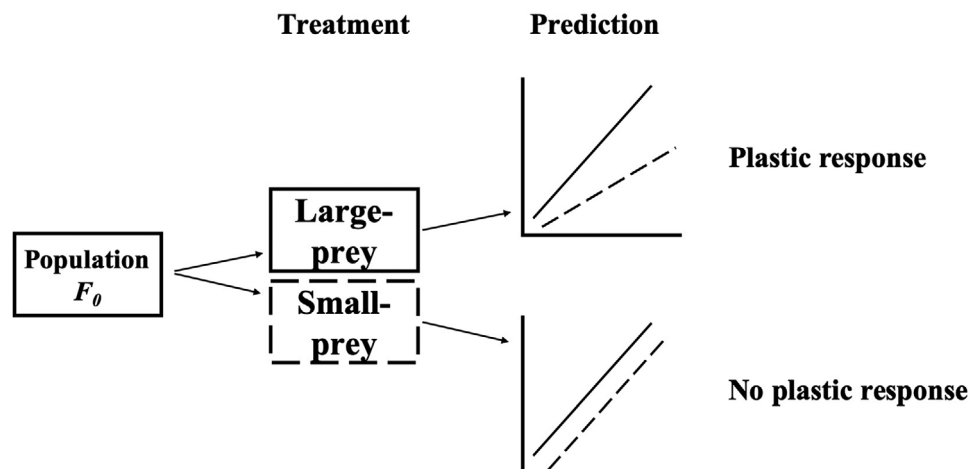


Figure 1 Study design schematic indicating neonate treatment allocation, and the predicted responses if prey size affects morphology.

intercept) and the disparity in relative prey size increased with time (model slope). Because snakes were fed weekly, but only measured every other week, we compared the head length of each individual to the mean prey height for the 2 consecutive feedings following each treatment.

The 2 populations were analyzed separately due to differences in body size at birth that were maintained throughout the study (Aubret *et al.* 2004). Consequently, the absolute head size of the snakes also differed between the 2 populations (Suppl. Table S1). We tested for a plastic response in the growth rate of cranial elements (head length, lower jaw length and quadrate length) relative to body size (SVL), and a plastic response in the growth rate of body size itself for both populations using linear mixed effects models (Fig. 1). The SVL*Treatment interaction term was used to test for differences in the rate of change of cranial elements with size. When testing for differences in SVL growth rate, an Age*Treatment interaction term was used for change in size with time. In all models, individual ID was included as a random effect to account for repeated measures.

RESULTS

Linear Model 1 confirmed that snakes in the large-prey treatment were offered significantly larger prey ($F_{1,60} = 855.3$, $P < 0.001$) and that prey size increased in the large-prey treatment ($F_{1,60} = 50.7$, $P < 0.001$). Linear Model 2 confirmed that snakes in the 2 feeding treatments did not differ in the mass of food offered ($F_{1,60} = 0.8$, $P = 0.373$) and that mass was kept equal

between the 2 treatments during the study ($F_{1,60} = 0.2$, $P = 0.673$). Linear Model 3 confirmed that snakes in both treatments from the LARGE population consumed similar prey masses during the study ($F_{1,30} = 2.4$, $P = 0.135$) (Fig. 2a). Linear Model 4 indicated that snakes from the SMALL population in the large-prey treatment consumed greater prey masses as the study progressed than snakes in the small-prey treatment ($F_{1,30} = 4.8$, $P = 0.036$) (Fig. 2b). Linear Model 5 confirmed that snakes in the large-prey treatment were fed significantly larger prey relative to their head size ($F_{1,478.8} = 397.2$, $P < 0.001$) and that prey size increased relative to head size in the large-prey treatment ($F_{1,478.8} = 124.6$, $P < 0.001$) (Fig. 3).

Linear mixed effects models for the SMALL population indicated no significant differences in the growth rate of cranial elements (head length, lower jaw length and quadrate length) between the large-prey and small-prey treatments ($F_{1,125.7} = 1.1$, $P = 0.293$; $F_{1,129.7} = 0.2$, $P = 0.665$; $F_{1,85.7} = 0.9$, $P = 0.347$, respectively). We also found no evidence of a difference in the body size growth rate between treatments for snakes from the SMALL population ($F_{1,116.2} = 1.32$, $P = 0.255$; Fig. 4).

The linear mixed effects models for the LARGE population did not provide evidence of significant differences between treatments in the growth rate of head length, lower jaw length or the quadrate length relative to SVL ($F_{1,145.1} = 0.3$, $P = 0.617$; $F_{1,149.9} = 0.004$, $P = 0.949$; $F_{1,149.4} = 3.7$, $P = 0.057$, respectively). However, our model indicated that snakes in the large-prey treatment grew more rapidly than those in the small-prey treatment ($F_{1,134.3} = 8.7$, $P = 0.004$; Fig. 4).

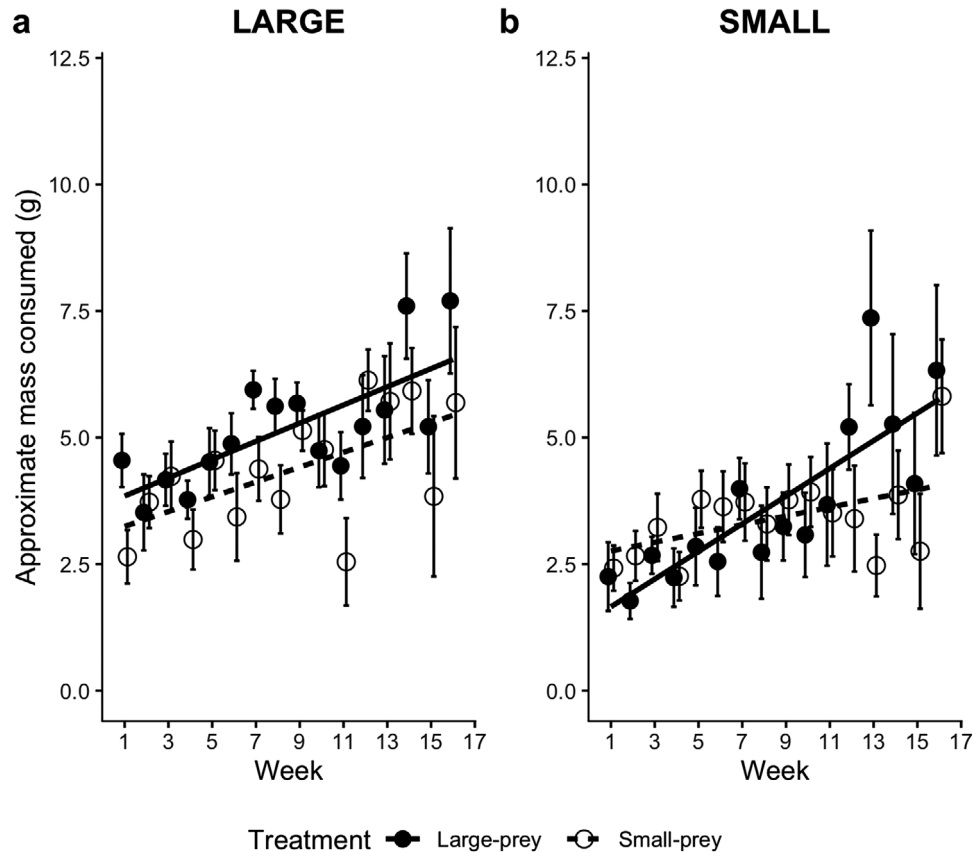


Figure 2 Approximate mass of fish consumed by snakes from the LARGE population during the study (a). Approximate mass of fish consumed by snakes from the SMALL population during the study (b).

DISCUSSION

Our results suggest a population-level difference in the plastic response of snake morphology in response to variation in prey size. Chiefly, snakes in the LARGE population exhibited a greater body size growth rate in response to larger prey items that was not seen in SMALL snakes (Fig. 4). We found no evidence of treatment-related changes in the growth of the 3 cranial elements we examined relative to body size, suggesting that prey size does not affect the allometric relationship between body size and the 3 cranial elements that we measured in these animals. These results indicate a capacity for phenotypic plasticity in some morphological traits, specifically body size, in response to the prey size encountered during ontogeny. However, these results also suggest that this capacity for plastic responses may be population-specific.

Our results here suggest a potential mechanism explaining patterns previously observed in field studies of these same populations: specifically, females from pop-

ulations where large prey were frequently encountered reached greater SVLs than females from populations where small prey were more common (Clifton *et al.* 2017). Here we find that female snakes from the LARGE site are consistently larger in SVL and grow more rapidly when they frequently encounter large prey.

The evidence for phenotypic plasticity in cranial elements of snakes has been mixed in the literature. For example, Schuett *et al.* (2005) found that prey size did not influence the cranial elements in neonate *Boa constrictor* when neonates in a small-prey treatment were fed 8–21-g mice throughout the study versus neonates in a large-prey treatment that were fed 32–260-g rats throughout the study. In contrast, Aubret *et al.* (2004) found that tiger snake neonates (*Notechis scutatus* Peters, 1861) fed larger prey than conspecifics (1.7–1.8 g and 2.3–2.5 mm circumference versus 1.8–5.0 g and 2.5–4.7 mm in circumference) grew longer mandibles. Similarly, Queral-Regil and King (1998) found evidence of a plastic response in both SVL and mandible length, with snakes

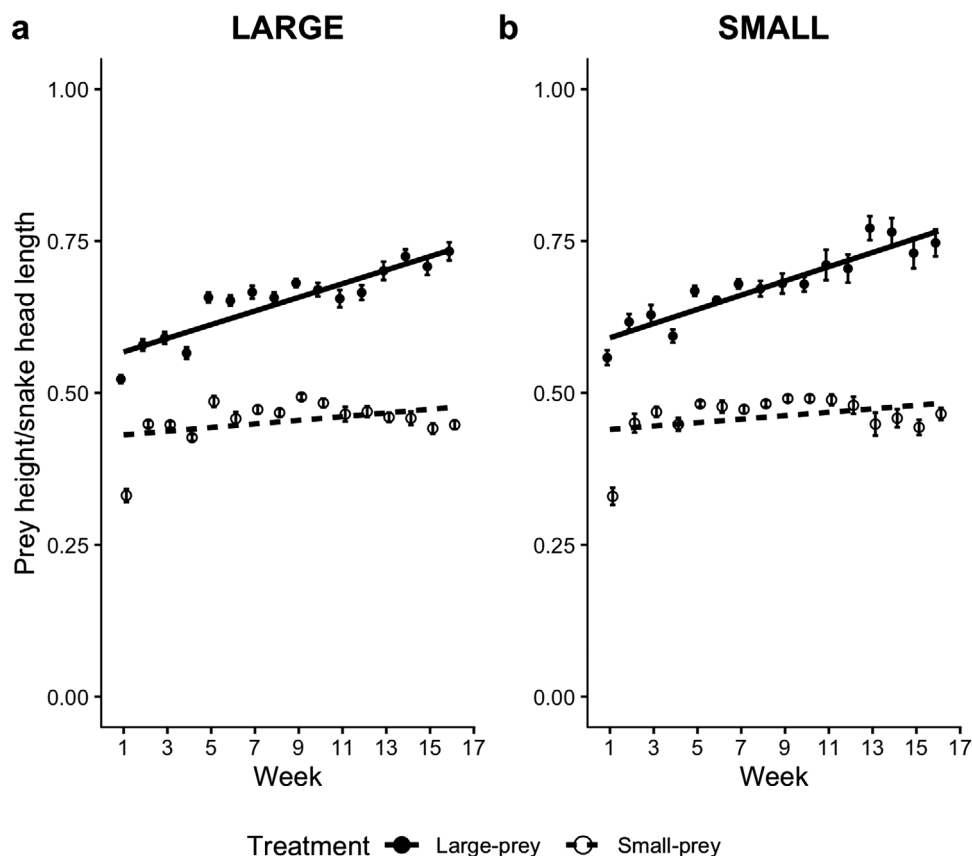


Figure 3 Relative prey height offered to the LARGE population (a) and the SMALL population (b) during the study.

being offered larger fish (1.6–3.0 g) growing longer and growing longer mandibles than snakes that were offered smaller fish (0.5–1.2 g). Our findings are more consistent with those of Queral-Regil and King (1998), at least in the LARGE population, where we found that SVL exhibited a plastic response to variation in prey size. The fact that prey size did not affect cranial elements but did have an effect on SVL may suggest that being larger is more important than the relative size of a given cranial element.

It is possible that differences in the prey masses consumed between the large-prey and small-prey treatment of snakes from the SMALL population (Fig. 2b) influenced the observed growth rates. However, the substantial increase in mass of prey consumed by individuals from this population in the large-prey treatment seems to be driven by low prey consumption early in the study, followed by a relatively sharp increase. This may indicate that snakes from the SMALL population had a more difficult time consuming large prey than LARGE population conspecifics until they reached a critical size. In fact, the point where both treatments from the SMALL population

are consuming approximately the same mass does not occur until the 7th week of the study (Fig. 2b) when snakes from the SMALL population are approaching similar SVLs to those of snakes from the LARGE population at birth (Fig. 4). This may lend credence to the idea that being bigger is more important than relative head size, particularly early in life when prey options are more constrained.

In the LARGE population, snakes in the large-prey treatment often consumed more prey mass (Fig. 2a), although not significantly more, than small-prey treatment conspecifics. Because this consistent difference in mass of prey consumed is maintained throughout the study, it seems that the most likely explanation is the feeding regime itself. Specifically, snakes in the large-prey treatment were able to consume more mass from fewer individuals. Furthermore, this pattern may appear only in the LARGE population, because unlike the SMALL population, snakes from the LARGE population were more capable of consuming larger prey from birth due to their larger starting sizes. This same phenomenon may also

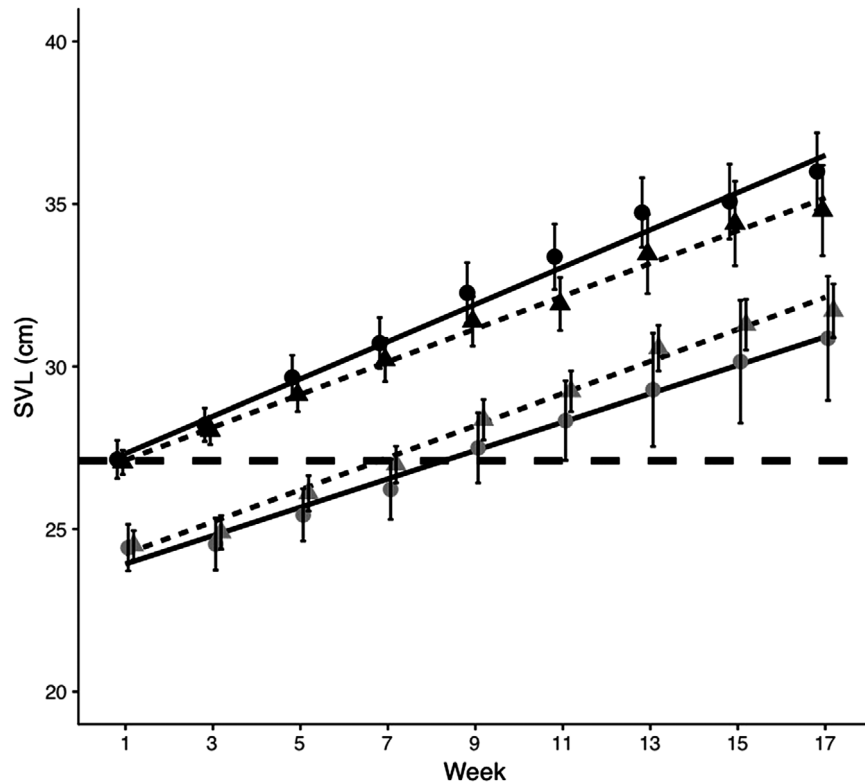


Figure 4 Snout-vent length (SVL) of snakes from the SMALL population (gray) and the LARGE population (black) in large-prey treatment (circles) and small-prey treatment (triangles). The black dashed line indicates the mean birth SVL of the LARGE population and the approximate size when snakes from large-prey treatment of the SMALL population were consuming an equal mass as the small-prey treatment.

explain why snakes from the SMALL population in the small-prey treatment had a higher, but nonsignificant, growth rate than their large-prey conspecifics (i.e. large-prey treatment individuals from the SMALL population had a limited capacity to consume the prey we offered until reaching a critical size). This would mean that snakes in the small-prey treatment from the SMALL population actually had more energy to allocate towards growth than their large-prey treatment counterparts.

Our results may elucidate some of the underlying processes that have led to the previously described patterns of differentiation in trophic morphology found in these watersnake populations (Clifton *et al.* 2017). Specifically, it appears that females that frequently encounter large prey have responded to this pressure through changes in body size, and associated changes in absolute head size, both genetically and plastically (i.e. large sizes at birth and increased growth rate when given large prey to consume).

Our study provides evidence of phenotypic plasticity in the growth rate, resulting in differences in body

size (SVL), but only in the population where large prey are frequently encountered. The ability to exhibit a plastic response can be an adaptive trait (reviewed in Wund 2012), and, along with being born larger and maintaining a greater SVL regardless of treatment throughout the study, likely indicates that snakes from the LARGE population have adapted to deal with the large prey they frequently encounter at their home site. These population-specific patterns support the idea that both local adaptation and phenotypic plasticity are important mechanisms mediating morphological responses to prey size.

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SUPPLEMENTARY MATERIALS

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Results of linear mixed effects models of differences in SVL, absolute head length, absolute lower jaw length, and absolute quadrate length between LARGE and SMALL. Significant results are indicated in bold and demonstrate that snakes from LARGE were bigger at birth and the differences were maintained throughout the course of the study.

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