Prey Size and Feeding Rate Do Not Influence Trophic Morphology of Juvenile Water Snakes (*Nerodia sipedon*)

Meredith C. Swartwout[2], Philip Vogrinc, J. Alex Baecher[1], Chelsea Kross, and John D. Willson

Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701, USA

Abstract: Gape-limited predators, such as snakes, might rely on phenotypic plasticity to cope with variation in prey size, but experimental studies have found mixed evidence for plasticity in snake head morphology. Our study sought to determine whether variation in prey size and feeding rate induce head size plasticity in Northern Water Snakes (*Nerodia sipedon*). We avoided limitations of previous studies by growing all neonates to a consistent final size (400 mm snout–vent length [SVL]), and uncoupling feeding rate and prey size as potential factors influencing head size through four feeding treatments: big–fast (one large prey item every week), big–slow (one large prey item every other week), small–fast (two small prey items every week), and small–slow (one small prey item every week). Snakes in fast treatments grew faster than snakes in slow treatments, but there were no strong effects of feeding rate or prey size treatments on multiple head size metrics. Females grew faster than males, however, and had longer jaw lengths at 400 mm SVL than did males. Unlike previous studies, our results do not support the presence of phenotypic plasticity in head size in *N. sipedon*, indicating that head size plasticity in snakes can be species and context specific.

Key words: Allometry; Colubridae; Ecophysiology; Gape size; Growth; Jaw length; Plasticity

Phenotypic plasticity is the ability of individual genotypes to express alternate phenotypes in response to environmental conditions, allowing organisms to persist in variable or rapidly changing environments (Pigliucci et al. 2006; Charmantier et al. 2008; Hendry et al. 2008). Plasticity may be expressed through seasonal polyphenism, alternative behaviors, or polymorphism (West-Eberhard 1989). Some predators might use plasticity to quickly respond to shifts in prey type or size.

Exposure to variation in prey size might elicit plastic responses in predator morphology. We define trophic morphology as the size and shape of the feeding apparatus (i.e., head and jaw) necessary for capturing and consuming prey. Plasticity in trophic morphology in response to food size has been observed in crabs (Smith and Palmer 1994; Baldridge and Smith 2008), salamander and anuran larvae (Pfennig 1990, 1992; Walls et al. 1993; Whiteman et al. 2003; Schmidt et al. 2006), birds (Swennen et al. 1983; Gosler 1986), and mammals (Watt and Williams 1951; Lavelle 1983; Bouvier and Zimny 1987; Enomoto et al. 2010), among other organisms. Trophic morphology is particularly important for gape-limited predators, such as fishes and snakes, because these species typically consume their prey whole and can only swallow prey smaller than their gape size (but see Jayne et al. 2002). Diet-induced phenotypic plasticity of trophic morphology has been experimentally studied in a wide variety of fishes (e.g., Wimberger 1991; Day et al. 1994; Hegrenes 2001; Adams et al. 2003; Muschick et al. 2011), but only four studies have documented trophic plasticity in snakes through experimental manipulation of diet—in a water snake (Queral-Regil and King 1998), two vipers (Bonnet et al. 2001; Smith 2014), and an elapid (Aubret et al. 2004).

Trophic morphology in snakes can be influenced by shape and size of their primary prey. Large heads allow consumption of larger prey (Forsman and Lindell 1993; King 2002),

faster swallowing (Pough and Groves 1983; Aubret and Shine 2010), and shorter handling time (Vincent and Mori 2008), reducing the amount of time the snake is vulnerable to predation, and increasing foraging efficiency. Within a single snake species, there can be considerable variation in head size (e.g., European Adder [*Vipera berus*]; Forsman 1994), and this variation can result from evolutionary local adaptation of a population (Forsman and Shine 1997), a plastic response to prey size (Queral-Regil and King 1998), or a combination of the two. Tiger Snakes (*Notechis scutatus*) on Carnac Island, Australia feed on large Silver Gull (*Larus novaehollandiae*) chicks and have relatively larger heads than their mainland counterparts that eat smaller prey, with both population of origin (i.e., genetics) and diet-induced plasticity contributing to differences in head size among populations (Aubret et al. 2004; Fabien et al. 2004).

Experimental evidence for diet-induced phenotypic plasticity of trophic morphology in snakes has been mixed. Several studies have found plastic responses in trophic morphology, but isolating the effects of prey size is often complicated by differences in growth rate and final body size between treatments, generally because treatments offering larger prey were also offering more total prey mass (e.g., Forsman 1996; Bonnet et al. 2001). Experiments that balanced total prey mass offered among feeding treatments found phenotypic plasticity of trophic morphology in Northern Water Snakes (*Nerodia sipedon*; Queral-Regil and King 1998), Australian Tiger Snakes (Aubret et al. 2004), and Prairie Rattlesnakes (*Crotalus viridis*; Smith 2014), but not *Boa constrictor* (Schuett et al. 2005). Even when total prey mass offered was balanced, however, growth trajectories sometimes differed between treatments (e.g., Queral-Regil and King 1998; Schuett et al. 2005).

Growth rate can influence head shape through either isometry (i.e., head size increases proportionally with body size) or ontogenetic shifts in the allometric relationship if head size growth diverges from whole-body growth rates (Blouin and Loeb 1991). Analysis of covariance (ANCOVA), using body size as a covariate, is a common analytical tool

[1] Present Address: School of Natural Resources and Environment, University of Florida, Gainesville, FL 32603, USA
[2] Correspondence: e-mail, mswartwo@uark.edu

used to compare relative head sizes among treatments that differed in growth rate and final body size. However, ANCOVAs cannot account for ontogenetic shifts in head–body allometry when body sizes of treatment groups are largely nonoverlapping. If there is large variation in body size measurements, error in measurement can become a source of error affecting ANCOVA interpretation (McCoy et al. 2006). Whether these limitations were addressed in studies that showed strong differences in growth among treatment groups is unclear.

We used a long-term factorial feeding experiment to examine the interactive effects of prey size and snake growth rate on trophic morphology of juvenile Northern Water Snakes. We achieved differences in growth by manipulating feeding rate. To avoid confounding effects of ontogenetic shifts in head–body allometry, we continued the experiment until all snakes reached the same final body size. Our study was partially motivated by anecdotal field observations of juvenile water snakes exhibiting fast individual growth rates that appeared to have relatively small heads compared with individuals experiencing slower growth rates. Thus, on the basis of our own observations and the literature, we hypothesized that (1) snakes fed larger prey would have larger heads than snakes fed smaller prey; (2) snakes fed at a higher rate (amount and frequency of prey) would have smaller heads than snakes fed at a lower rate; and (3) differences in snake head size would be greater for snakes fed large prey, as opposed to those fed small prey, regardless of feeding frequency. Available literature suggests that there is female-biased sexual size dimorphism in *Nerodia* head size and growth (i.e., King 1986; Weatherhead et al. 1995; Perkins and Eason 2019), so although it was not our main objective, we also hypothesized that female snakes would have larger heads and grow faster than males.

Materials and Methods
Study Species

Nerodia sipedon is an abundant semiaquatic natricine with a broad distribution in eastern and central North America (Ernst and Ernst 2003). They are ubiquitous in a wide array of aquatic habitats from large lakes and rivers to temporary wetlands and even brackish estuaries (Gibbons and Dorcas 2004). The diet of *N. sipedon* is also highly variable, made up primarily of fish and amphibians; over 80 fish taxa have been recorded as prey (Gibbons and Dorcas 2004). Like most North American water snakes, *N. sipedon* is fast growing and exhibits female-biased sexual size dimorphism. Male and female *N. sipedon* typically reach sexual maturity at 2 yr old, after reaching a snout–vent length (SVL) of 370–400 mm or 500–650 mm, respectively (Ernst and Ernst 2003), but growth rates are variable among studied populations. In Missouri, males can reach 430 mm within 1 yr (Bauman and Metter 1975). In Kansas, 1-yr-olds averaged 313 mm SVL. In a well-studied Lake Erie population, males mature in 2 yr at 430 mm SVL and females at 3 yr and 590 mm SVL (King 1986). Maximum (female) total length is reported as 1500 mm (Ernst and Ernst 2003).

Snake Collection and Characteristics

In Autumn 2013, we collected and maintained 37 neonate *Nerodia sipedon* in the laboratory (mean ± 1 SE SVL: 184 ± 1.9 mm, range = 164–218 mm; mass: 4.2 ± 0.1 g, range = 3.0–5.8 g). Between 18 September and 10 October 2013, we collected 19 neonates from two locations in northwest Arkansas, USA: Scull Creek (36°06′49.1″N, 94°09′40.7″W; in all cases datum = WGS84), and the Mulberry River (35°31′52.2″N, 94°02′28.0″W). A gravid female captured at the Scull Creek location gave birth to an additional 18 neonates in the lab on 18 September 2013. Four snakes died over the course of the experiment, leaving 33 neonates for the comparisons below.

The narrow range of body sizes of our field-captured neonates (219–241 mm total length) that are well within the range of body sizes at birth documented for *N. sipedon* (218–301 mm total length; Ernst and Ernst 2003) indicates that these snakes were recently born and had limited or no feeding experience in the field before capture. Moreover, at the beginning of the experiment, field-captured (mean SVL = 179.4 ± 1.2 mm) and lab-born (mean SVL = 177.7 ± 3.7 mm) neonates were similar in body size (one-way analysis of variance [ANOVA]: $F_{1,31} = 2.27$, $P = 0.14$) and head measurements (one-way ANCOVAs: $F_{2,30} \leq 2.44$, $P \geq 0.10$). Nonetheless, we included collection group as a random effect in statistical models to account for nonindependence of siblings and those from the same locations. We also tested for differences in initial body sizes and head measurements by sex. There was no difference between sexes in initial SVL (one-way ANOVA: $F_{1,31} = 0.04$, $P = 0.84$), but female subjects had relatively greater mandible and rostral–occipital lengths than males at the start of the experiment (one-way ANCOVA: $F_{2,30} \geq 5.32$, $P < 0.01$).

Snake Maintenance

We maintained neonates communally (groups of two to five) in 75.7-L aquaria with a substrate of aspen wood shavings, a circular (22.5-cm diameter) hide box, and a large water bowl. We positioned a 100-W incandescent bulb above one end of each aquarium, providing a thermal gradient (ca. 20–46°C) within the cage during the day and allowing the cage to cool to ambient laboratory temperature at night. Individuals were randomly assigned to tanks, and groupings were rerandomized several times throughout the study to reduce any tank-related effects. Fresh water was added to snake water bowls ad libitum. We measured SVL (±1 mm on a ruler) three times and took an average and mass (±0.1 g on an electronic balance) of each snake weekly.

Prey Acquisition and Storage

We used sunfish (*Lepomis cyanellus* or *L. macrochirus*) acquired from local ponds and a fish hatchery in northwestern Arkansas as prey items for the experiment. We captured, preweighed, and sorted a large number of fish and stored them frozen, allowing us to select prey that closely matched the target mass for each snake in each feeding event. We offered thawed prey individually to each snake in the middle of each week, and if it was refused, offered food again 2 d later.

Experimental Design

Before the commencement of experimental feedings, we acclimated snakes to laboratory conditions for approximately 10 wk. During this time, snakes were offered frozen/thawed sunfish (*Lepomis* spp.) of relatively small size (<20% of

snake's body mass) approximately weekly to ensure that all snakes would accept sunfish as food during the experiment.

After the acclimation period, we randomly assigned snakes to one of four treatments in a fully factorial design including two levels of prey size (big and small) and two levels of feeding rate (fast and slow): big–fast (BF), big–slow (BS), small–fast (SF), or small–slow (SS). In designating our treatments, we used prey mass as our measure of prey size because it allowed us to offer snakes energetically similar meals and because the mass of *Lepomis* spp. is positively correlated with the maximum dimensions that present the primary challenge to ingestion (height, $R^2 = 0.88$; width, $R^2 = 0.78$; Willson and Hopkins 2011). Big prey items weighed approximately 30% of an individual snake's body mass, whereas small prey items weighed approximately 15%. We chose 30% as our size for big prey because it is at the upper limit of size of sunfish (*Lepomis* spp.) that can be consumed by *Nerodia*, thereby presenting a substantial challenge to gape during feeding (Willson and Hopkins 2011). Snakes in fast treatments were fed more frequently relative to slow treatments, but feeding was arranged so that the total mass of prey offered to snakes in big treatments was equivalent to that in small treatments. Therefore, snakes in BF treatments were offered one prey item equal to 29.87% ($\pm 0.97\%$ SD; $n = 594$) of the snake's mass per week, BS one prey item equal to 29.92% ($\pm 1.56\%$ SD; $n = 469$) of the snake's mass every other week, SF two fish individually equal to 15.20% ($\pm 1.60\%$ SD; $n = 795$) of the snake's mass every week, and SS one fish equal to 15.07% ($\pm 0.95\%$ SD; $n = 796$) of the snake's mass every week. Originally, we placed 9 snakes in each of three of the treatments and 10 in the BS treatment, with representation of both sexes, both collection locations, and lab-born and field-captured individuals in each treatment group; however, 4 snakes died during the experiment, resulting in 9 snakes in the BF treatment, 7 in BS, 8 in SF, and 9 in SS treatments (see Table S1).

We maintained snakes in their treatments until they reached 400 mm SVL, at which point each snake was removed from the experiment. We chose 400 mm SVL as our cutoff because we wanted to allow snakes to grow for a substantial period under experimental conditions, but not so long that snake growth would slow on account of attaining sexual maturity. Five snakes in BS treatments grew so slowly that after 2.5 yr we switched them to feeding every week (BF feeding regime) until reaching 400 mm SVL (mean time in BF treatment after the switch = 7 wk; range = 1–14 wk). The experiment continued for 3 yr, until all snakes had reached 400 mm SVL.

Head morphology measurements.—We constructed a rectangular platform (91 × 42 cm) to take standardized photographs of the head of each snake from two fixed positions. We captured a dorsal image using a Nikon D60 DSLR camera and an AF-S Micro Nikkor 60-mm lens (Nikon Instruments) fixed to the platform 30.5 cm above a focal point where the head of each snake was placed. A Panasonic Lumix DMC-TS20 digital camera (Panasonic Corporation) was fixed to the platform 13.2 cm from the focal point to capture a lateral view. Starting at the initiation of feeding treatments on 21 January 2014, we photographed both dorsal and lateral views of each snake every 3 wk. To maintain consistency between photographs, we placed a tube

over the anterior portion of each snake's body, leaving the head exposed for photographs but limiting cervical movement and controlling position of the head (Fig. 1B). We gently manipulated snakes at the focal point to ensure their head was aligned with the body and the gular/chin region was flush with the surface of the platform. All efforts were made to avoid artificially changing the shape of the head via manipulation.

We measured interocular width (IOW), mandible length (ML), rostral–occipital length (ROL), and maxilla length (XL) in ImageJ (v1.x, National Institutes of Health, Bethesda, Maryland; available at http://rsb.info.nih.gov/ij/), using photographs taken at the beginning of the study and when snakes reached 400 mm SVL (Schneider et al. 2012; Fig.1). From dorsal photographs, we measured IOW as the distance between the outermost edges of the supraocular scales (Queral-Regil and King 1998), XL as the distance between the posteriormost upper labial scale and the leftmost posterior portion of the internasal scale where it meets with the prefrontal (Vincent et al. 2007), and ROL as the distance between the anterior edge of the rostral scale and the posterior union of parietal scales (Queral-Regil and King 1998; Schuett et al. 2005; Hampton 2014). From lateral photographs, we measured ML (or jaw length) as the distance from the anteriormost lower labial scale to the posterior of last lower labial scale (Forsman and Lindell 1993; Forsman 1996; Schuett et al. 2005). External measurements of ML (jaw length) and ROL (head length) are positively correlated with gape size in *Nerodia* (King 2002; Hampton 2014).

Data Analyses

We confirmed that there were no differences among treatments in initial SVL using a one-way ANOVA ($P > 0.1$; Sokal and Rohlf 1981). We used one-way ANCOVAs, with SVL as a covariate, to confirm that there were no significant differences in relative head morphology or relative body mass among treatments at the beginning of the experiment (all $P > 0.05$). To conform with the assumption of ANCOVA that the relationship between the response variable (ML or ROL) and covariate (SVL) is linear, we applied an inverse transformation to ML ($1/x$) and an inverse reflected transformation to ROL ($1/[-x + \max\{x\} + 1]$).

Because all individuals were grown to 400 mm SVL, our study design allowed us to compare final head sizes without the introduction of potential bias from differences in head–body allometry. We used linear mixed-effect models (LMMs) to test for differences in final head measurements and days required to reach 400 mm SVL by feeding treatment: prey size treatment, feeding rate treatment, and their interaction. To account for potential influence of subject origin, we included collection group (Scull Creek Field, Lab-born, or Mulberry River) in the models as a random effect, and calculated intraclass correlations 1 and 2 (ICC1 and ICC2) to estimate the amount of variance in the model outcome accounted for by the random factor and how reliably levels within the random factor can be differentiated by the response variable (Zuur et al. 2009). Because of an experimenter error, one final ML measurement from a BF individual was lost, so we used the value from that individual's last measurement, 28 d earlier (SVL = 395 mm). However, excluding that data point from analyses did
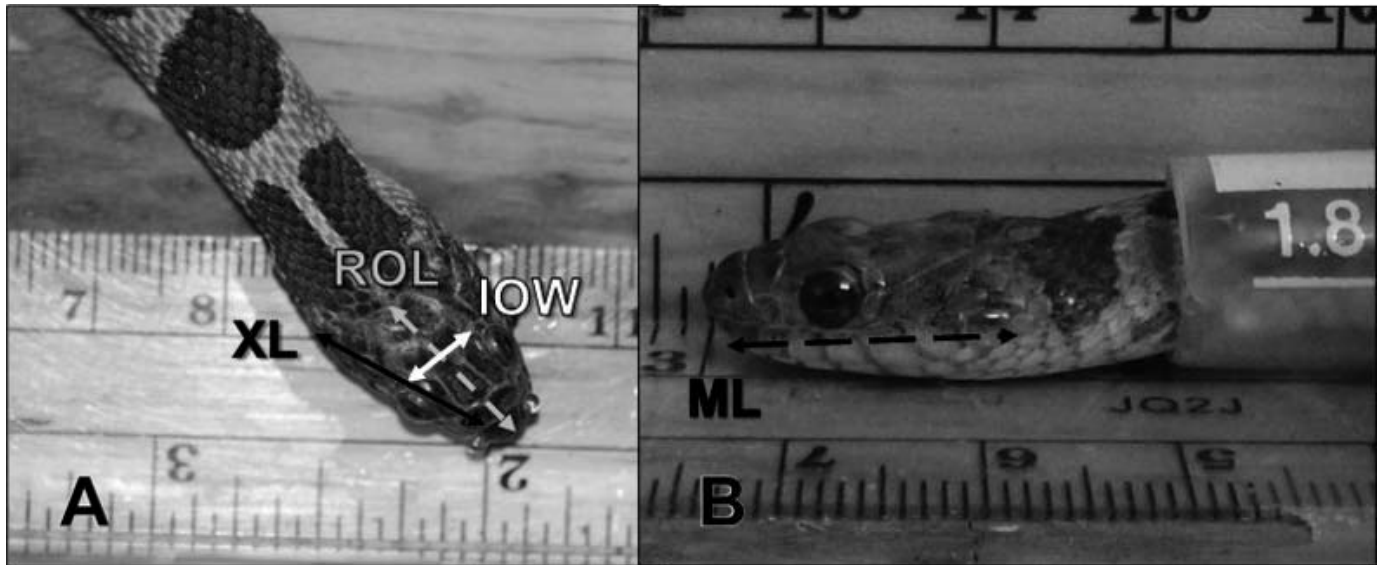
Fig. 1.—Head measurements taken from representative photographs of a juvenile Northern Water Snake (*Nerodia sipedon*). (A) Dorsal view: solid white line = interocular width, dashed gray line = rostral–occipital length, solid black line = maxilla length. (B) Lateral view: dashed black line = mandible length.

not change the results. To conform to the normality assumptions of linear models, we reflected IOW measurements so that $N = -x + \max(x) + 1$, where $x = $ IOW, and then used an inverse square-root transformation such that the dependent variable was equal to $1/\sqrt{(N)}$.

We used LMMs to test for an effect of sex on time until snakes reached 400 mm SVL and final head measurements, with collection group as a random effect. We conducted exploratory analyses that included sex as a main effect in our analyses of treatment effects. These models yielded some complex interactions that were difficult to interpret, given our relatively small sample sizes, but did not contradict results of the simpler models that did not include sex. Thus, to increase our power to detect treatment effects, and because sex effects were of secondary interest, we chose to analyze sex separately.

We used a Wald chi-square test to assess significance of main effects on growth and head measurements. For all head measurement analyses, we used a Bonferroni-adjusted level of significance of $\alpha = 0.01$ to account for the number of dependent variables run in independent ANOVAs (five: maxilla, mandible, IO, ROL, and days to 400 mm SVL). The Bonferroni method was appropriate for our experiment because we used no more than five tests with the same independent variable (Bender and Lange 2001). We conducted all data analyses using the car (Fox and Weisberg 2011), lme4 (Bates et al. 2015), and multilevel (Bliese 2016) packages in R v3.2.2 (R Core Team 2015). Unless otherwise indicated, response metrics are reported as mean ± 1 SE.

Results

Mean time required to reach 400 mm SVL differed among treatments (Fig. 2), with BS snakes growing the slowest (826 ± 37.6 d) and SF snakes growing fastest (342 ± 22.6 d). Time to 400 mm SVL differed significantly between feeding rate treatments (LMM, $\chi^2 = 31.40$, $P < 0.01$) and subjects in the big treatments took relatively longer to grow

than those in small treatments. When applying the Bonferroni adjustment, however, there was no significant effect of prey size ($\chi^2 = 5.37$, $P = 0.02$) or the interaction of prey size and feeding frequency ($\chi^2 = 0.76$, $P = 0.38$) on body growth. The random factor (collection group) did not explain much of the variation in the model (ICC1 = −0.03, ICC2 = −0.43).

Rates of prey refusal were higher for snakes in our big treatments (17% ± 3.5% of prey refused on average) and fast treatments (20% ± 2.9%) than snakes in the small treatments (8.0% ± 2.2% of prey refused) and slow treatments (3.8% ± 1.2%). However, we still achieved relative growth rates that varied by treatment as expected,
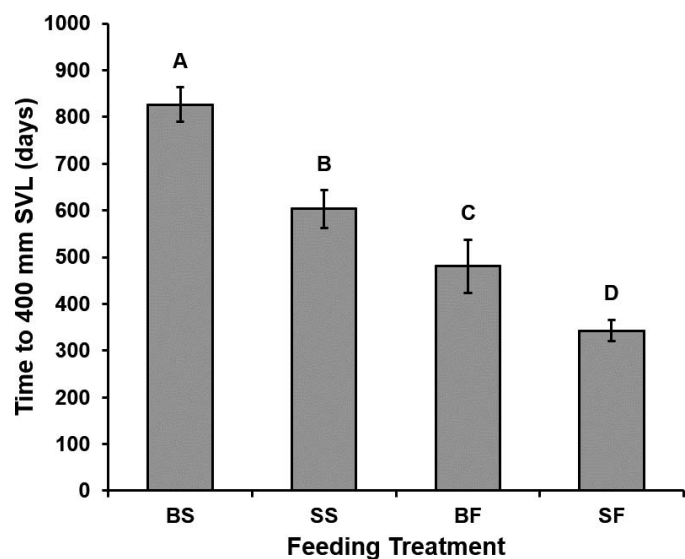

Fig. 2.—Mean time (days) for neonate *Nerodia sipedon* to reach 400 mm snout–vent length (SVL) in experimental feeding treatments. BS = big–slow, SS = small–slow, BF = big–fast, SF = small–fast. Error bars represent 95% confidence intervals; different superscripts indicate statistically significant differences in the response variable.
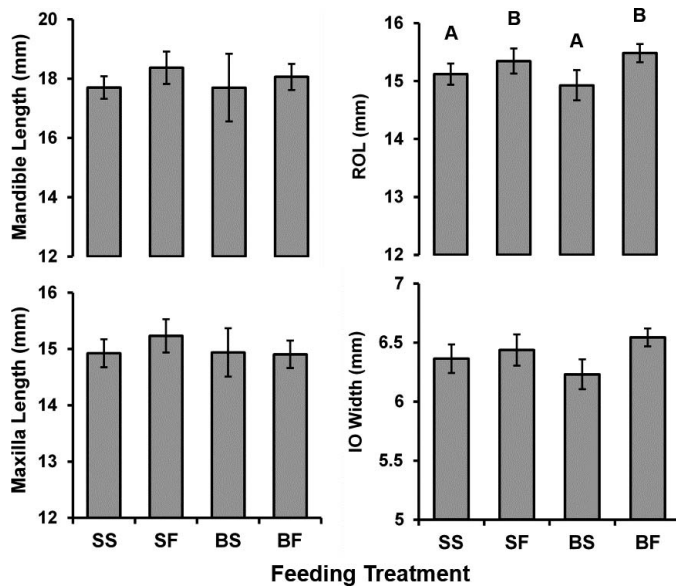
FIG. 3.—Mean values for mandible length, rostral-occipital length (ROL), maxilla length, and interocular width (IO Width) of *Nerodia sipedon* at the time that subjects attained 400 mm snout–vent length. BS = big–slow, SS = small–slow, BF = big–fast, SF = small–fast. Error bars represent 95% confidence intervals; different superscripts indicate statistically significant differences in the response variable.
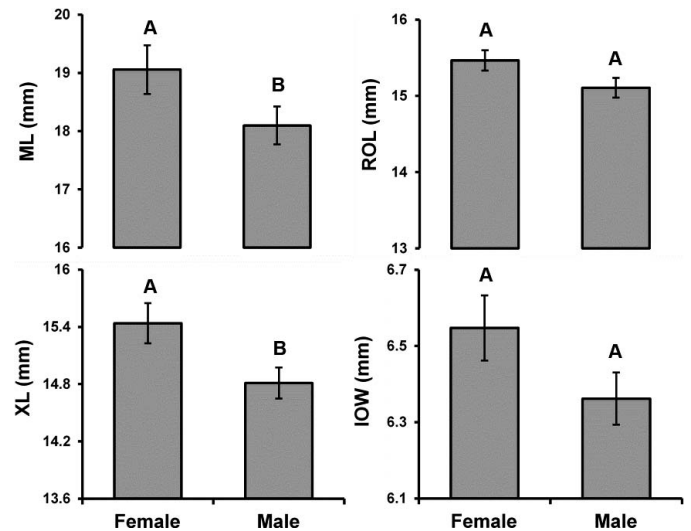

FIG. 4.—Mean values for mandible length (ML), rostral-occipital length (ROL), maxilla length (XL), and interocular width (IOW) of *Nerodia sipedon* at the time that subjects of each sex attained 400 mm snout–vent length. Error bars represent 95% confidence intervals; different superscripts indicate statistically significant differences in the response variable.

and the treatments with the highest average prey refusal (SF and BF) took the shortest amount of time to reach 400 mm SVL (Fig. 2).

None of the final head measurements differed between prey size treatments (LMM: XL, $\chi^2 = 0.42$, $P = 0.52$; IOW, $\chi^2 = 0.38$, $P = 0.54$; ROL, $\chi^2 = 2.35$, $P = 0.13$; ML, $\chi^2 = 0.16$, $P = 0.69$) or by feeding rate treatments for XL ($\chi^2 = 0.68$, $P = 0.41$), IOW ($\chi^2 = 1.13$, $P = 0.29$), or ML ($\chi^2 = 0.14$, $P = 0.71$; Fig. 3). ROL was significantly longer in subjects that were fed with greater frequency ($\chi^2 = 7.06$, $P < 0.01$), but ICC values were high (ICC1 = 0.12, ICC2 = 0.73), where ICC2 values >0.7 indicate that the random factor, collection group, can be reliably differentiated by ROL, the response variable (Kensler et al. 2009). There were no interactive treatment effects on any head measurements ($\chi^2 \leq 3.09$, all $P > 0.05$).

Female snakes grew significantly faster than males ($\chi^2 = 8.05$, $P < 0.01$), taking an average of 425 ± 42.5 d to reach 400 mm SVL, whereas males took 643 ± 44.1 d. Female snakes had significantly longer XL ($\chi^2 = 14.90$, $P < 0.01$) and ML ($\chi^2 = 6.84$, $P < 0.01$) measurements relative to males. ROL ($\chi^2 = 5.90$, $P = 0.02$) and IOW ($\chi^2 = 1.03$, $P = 0.31$) also tended to be longer for females than for males (Fig. 4) but did not differ significantly between sexes after applying the Bonferroni adjustment.

DISCUSSION

Previous feeding experiments have yielded mixed support for the existence of diet-induced trophic morphology plasticity in snakes. Experimental design has varied widely across these studies, and none has experimentally uncoupled the influences of prey size and feeding rate on head size plasticity. In this experiment, we grew all experimental snakes to the same body size and successfully achieved

differences in growth rate among feeding rate treatments, allowing us to examine effects of growth rate and prey size on trophic morphology, without confounding treatment effects with ontogenetic changes in head–body allometry. Although we did not observe strong treatment effects on final head measurements, there were differences among sexes and a trend for snakes in the fast treatments to have longer heads. The absolute difference in head lengths between feeding rate treatments was 0.351 mm; it is unclear if this difference is biologically significant. These results do not support any of our hypothesized plastic responses of trophic morphology to prey size or feeding rate, and we conclude that there is limited plasticity in *Nerodia sipedon* head morphology in the populations we studied.

The observed lack of treatment effects on head size in this study contrasts most directly with Queral-Regil and King (1998), who found that juvenile *N. sipedon* fed larger prey had relatively longer jaws than those fed smaller prey. This difference might be partially explained by water snakes in our study feeding less frequently, and growing more slowly, than snakes in other studies (large prey average = 1.02 mm/d, and small prey average = 0.84 mm/d; Queral-Regil and King 1998). Growth rates in our study (0.33 ± 0.01 mm/d) were similar to Lake Erie island water snakes (0.20 ± 0.07 mm/d; King 1986), but slower than the growth rates other studies have documented for *N. sipedon* in the field (median = 0.62 mm/d in Michigan, Feaver 1977; mean = 0.47 ± 0.12 mm/d in Ontario, Brown and Weatherhead 1999). It is also possible that we could not detect the anticipated effects because differences in head size are not apparent until snakes are more than 400 mm SVL. However, Queral-Regil and King (1998) saw differences in *N. sipedon* head size in subjects matching the body size (mean = 392 mm final SVL) of those used in the present study. Given that it took 3 yr for some of our subjects to reach 400 mm SVL, a longer experiment with more frequent feeding would be logistically challenging. It is possible that subtle differences in head shape existed

among our treatments that were not captured by our measurements or analyses. Landmark-based morphometric analysis could reveal complex changes in head shape (Vincent et al. 2004; Smith 2014). Likewise, examining the underlying bone structure with X-rays (as in Schuett et al. 2005), or preserved skulls might have revealed more subtle differences in morphology. Finally, Queral-Regil and King (1998) used a repeated-measures analytical approach that explicitly accounted for initial interindividual variation in head dimensions in their assessment of plasticity in head growth. With relatively small sample sizes and more experimental treatments, a comparable analysis of our data was not possible, leaving open the possibility that we did not detect differences in growth by not accounting for initial variability in head morphology among individual snakes.

In our study, snakes in our big prey feeding treatments refused their prey more frequently than snakes in the small feeding treatments; thus, differences in growth between those treatments might have been related to consumption rate. The high rate of refusals might have occurred because large *Lepomis* were too wide bodied for snakes to process. Although we selected *Lepomis* for our study because their morphology poses a challenge to gape, the diet of wild *N. sipedon* is often dominated by cyprinid fishes, with centrarchids (including *Lepomis*) comprising less than 10% of the diet in two well-studied populations (King 1993; Himes 2003). Furthermore, *N. sipedon* reared on a narrow-bodied fish food source (Cyprinidae: *Pimephales promelas*) grew faster and had relatively larger heads in feeding treatments involving larger prey sizes (Queral-Regil and King 1998).

Female snakes in our experiment grew faster and had longer jaws than male snakes, corroborating previous study findings on *N. sipedon* growth and sexual dimorphism (King 1986, 1989; Weatherhead et al. 1995; Queral-Regil and King 1998; Brown and Weatherhead 1999; Perkins and Eason 2019). Although they did not study *N. sipedon*, Mushinsky et al. (1982) found evidence of divergence in size of preferred prey among the sexes in two piscivorous congeners (*N. rhombifer* and *N. cyclopion*), with large females consuming larger fish than males at a given body size. Females might have a faster growth trajectory to optimize fitness because larger females can produce more offspring, whereas male mating success in *N. sipedon* is less dependent on adult body size (Weatherhead et al. 1995, 2008). Hormones could also be important regulators of male growth, as in garter snakes (*Thamnophis sirtalis*), where early androgen production inhibits body and relative jaw length growth in males (Shine and Crews 1988).

Despite differences in methodology, the disparity in results between our study and that of Queral-Regil and King (1988) suggests that capacity for phenotypic plasticity in snake trophic morphology might be species or context specific. Some insular populations of Australian Tiger Snakes that feed on large prey display phenotypic plasticity of trophic morphology, whereas mainland populations do not (Aubret and Shine 2010). Among *Nerodia* species, the capacity for phenotypic plasticity of trophic morphology might be more important for wetland-dwelling species such as *N. fasciata* and *N. erythrogaster* that use larger heads to consume wide-bodied anuran prey (Mushinsky et al. 1982;

Vincent et al. 2007), whereas *N. sipedon* typically consumes fish throughout ontogeny (Himes 2003). Intraspecific differences in diet were correlated with differences in head shape for Grass Snakes (*Natrix tesselata*) as well, with fish-eating snakes having narrower heads than frog-eating individuals (Brecko et al. 2011). Head shape in aquatic snakes is likely constrained by foraging method and prey behavior in addition to prey size (Segall et al. 2016). For example, piscivorous Diamond-Backed Water Snakes (*Nerodia rhombifer*) use open-mouth foraging and have a relatively larger head than syntopic *N. sipedon* in Kentucky (Perkins and Eason 2019). Given the importance of environmental and evolutionary context for the induction of phenotypic plasticity, future studies should thoroughly control for origin of snakes in experimental studies. Capture location was a potentially confounding factor in our study that we attempted to address, but this factor might still have obscured small treatment effects. A comprehensive survey of head size variation across multiple populations of *N. sipedon*, paired with long-term feeding experiments, would be necessary to determine the extent of geographic variation in the capacity for plasticity of trophic morphology in this species.

SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at https://doi.org/10.1655/Herpetologica-D-18-00007.S1

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