LIFE-HISTORY AND ENERGETICS OF THE DIAMOND-BACKED WATERSNAKE

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Jeremy David Chamberlain

B.S., Iowa State University, 2009
M.S., University of Texas at Tyler, 2011

This dissertation, "Life-history and energetics of the diamond-backed watersnake", is approved by:

Dissertation Advisor:

Robert S. Sikes, PhD
Professor of Biology

Dissertation Co-advisor:

Matthew. E. Gifford, PhD
Assistant Professor of Biology

Dissertation Committee:

William H. Baltosser, PhD
Professor of Biology

Darin E. Jones, PhD
Assistant Professor of Chemistry

John D. Willson, PhD
Assistant Professor of Biology

Program Coordinator:

Brian C. Berry, PhD
Assistant Professor of Chemistry

Interim Graduate Dean:

Abhijit Bhattacharyya, PhD
Professor of Systems Engineering

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LIFE-HISTORY AND ENERGETICS OF THE DIAMOND-BACKED WATERSNAKE, by Jeremy David Chamberlain, December 2016

ABSTRACT

Life-history research explores how environmental variation and physiological constraints shift the energy allocation decisions to competing functions of the body among individuals and populations. Variation in the energy available across the environmental landscape is of particular interest as it dictates the amount energy available for organisms to acquire and allocate to their life-history. Additionally, individuals vary in their ability to capture and assimilate energy from the environment.

This dissertation seeks to understand how environmental variation in energy availability and physiological constraints of attaining this energy shape allocation decisions among individuals and populations. I examined diamond-backed watersnakes (*Nerodia rhombifer*) from five populations that differed in their access to energy resources of prey. I compared measures of reproduction and growth to detect the presence of differential allocation decisions. I tested whether differences in allocation among individuals and populations arose as a constraint of their ability to assimilate energy resources in the environment, or as a function of the variation in prey characteristics across the landscape. Lastly, I measured how variation in reproductive investment may limit energy acquisition among individuals.

I dedicate this work to my parents, Marsha and Jim, for their constant love, support and encouragement to pursue my interest, no matter how wacky it may be

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PREFACE

Included within this dissertation is a chapter that has previously been accepted for publication. CHAPTER I was published in *Copeia*.

CHAPTER I

Physical and Physiological Costs of Reproduction in Watersnakes

This chapter was formatted in accordance with the guidelines set forth by Copeia (2016).

Abstract

Estimating the cost of reproduction is pivotal to understanding the trade-off between current and future reproductive success, a key prediction in life-history theory. Increases in the cost of each reproductive attempt theoretically reduce future reproductive ability. Further, costs may change as individuals grow thus changing the nature of this trade-off. Measuring changes in female locomotor ability during reproduction has been one effective method to measure the cost of reproduction for females. We measured female Diamond-backed Watersnake (*Nerodia rhombifer*) swimming speed during and after pregnancy to determine if there was a loss of locomotor ability. We then correlated these speeds with measures of reproductive burdening (as estimated by relative clutch mass) and body size to investigate if increased reproductive investment and body size changed locomotor ability and subsequent cost of reproduction. Female snakes swam slower during pregnancy than after. Larger relative clutch masses resulted in slower swimming speeds during pregnancy. Further, shorter individuals showed a greater decrease in swimming speed suggesting a greater cost of reproduction for smaller individuals. Lastly, we demonstrated that additional costs to locomotor ability may be incurred by the female due to weight loss during pregnancy from carrying the burden of reproductive material.

Introduction

A key tenet of life-history theory suggests that trade-offs between current reproduction and future reproductive success exist as a consequence of the costs of reproduction (Williams 1966; Stearns 1989). These costs can manifest themselves in animals in many forms including thermoregulatory constraints, increased predation, increased energetic demand, reduced prey consumption, and reduced locomotion (Shine 1980; Seigel et al. 1987; Stearn, 1989; Gregory et al. 1999; Angilleta and Sears 2000; Bonnet et al. 2002; Shine 2003; O'Donnell and Arnold 2005; Harshman and Zera 2007). However, these costs of reproduction have been difficult to demonstrate experimentally (Shine 1980).

While the costs to reproduction may take many forms among the various animal classes, loss of locomotor ability during pregnancy is often measured because of the relative ease of quantification in the laboratory. Squamate reptiles (i.e. snakes and lizards) are particularly good models to quantify this cost because of their body plans, foraging methods, and pivotal role in many food webs. The loss of mobility may represent a cost to female squamates by reducing their ability to evade predators or successfully forage (Seigel et al. 1987; Sinervo et al. 1991; Gregory et al. 1999; Miles et al. 2000; Olsson et al. 2000; Le Galliard et al. 2003; Shine 2003; Winne and Hopkins 2006; Cox and Calsbeek 2009; Brischoux et al. 2011). The resulting cost to a female may be more severe in organisms such as snakes that use lateral undulation requiring flexion of the lower body. In these cases, reproductive material may prevent this movement (Jayne 1985; Winne and Hopkins 2006). Live-bearing animals should also experience a greater cost than oviparous animals due to the extended time the reproductive material must be carried (Shine and Bull 1979; Jayne 1985; Shine 1988a).

The relative burden of reproductive material on a female can be calculated using relative clutch mass (RCM). RCM is calculated as:

$$RCM = \frac{LM}{Postparturient\ Mass} \quad (1),$$

where LM represents the total mass of reproductive material or litter mass (LM) and postparturient mass is the mass of the female after giving birth (Vitt and Price 1982). RCM allows females of different sizes to be compared. Thus, this measure can estimate how the relative burden of reproduction influences the mobility of different sized females (Seigel et al. 1986; Shine 1988a; Shine 1992; Winne and Hopkins 2006). Increasing RCM values represent more burdened females and presumably, more impeded locomotor ability, resulting in a greater cost of reproduction.

However, it is unclear whether the cost of reproduction in the form of impaired mobility is equivalent for different sized individuals within a species. If a relationship between body size and loss of mobility exists then there may be a selective pressure on growth rate and/or adult body size to reduce this cost and thereby increase fitness. Often in swimming vertebrates under non-reproductive conditions there is a relationship between swimming speed and body length. In some salamanders, shorter individuals swim faster than larger ones (Hammaker et al. 2003). However, in some snakes and fish there is a positive correlation between swimming speed and body length (Bainbridge 1957; Jayne 1985; Garland 1988; Weatherhead and Robertson 1992; Shine and Shetty 2001). Little attention has been given to whether this relationship is maintained when individuals are burdened by reproduction. By examining the relationship between body lengths and swimming speed during and after reproduction, one can evaluate how the cost of reproduction varies with body size.

A second possible explanation for the loss of mobility among females during reproduction may not be directly associated with the mass of the reproductive material but indirectly through physiological changes of the female during pregnancy or gravidity (Olsson et al. 2000). Often, there is a great energetic demand on females during pregnancy due to increased metabolic rates, differing thermal requirements, and reduction in feeding (Shine 1980; Gregory et al. 1999; Angilleta and Sears 2000; Lourdais et al. 2002; Ladyman et al. 2003; Brischoux et al. 2011). Thus, the loss of mobility in females during reproduction may not be simply due to reduced flexion in the body, but also due to loss of stored energy reserves to fuel these motions during pregnancy. Further, this loss of energetic reserves in the female's body can also represent another form of reproductive cost, particularly if her body condition prevents future reproduction.

In order to evaluate the cost of reproduction incurred from loss of mobility and the physiological changes during pregnancy, we studied the swimming speed of Diamond-backed Watersnakes (*Nerodia rhombifer*), a highly aquatic live-bearing snake, during and after pregnancy in the laboratory. We evaluate how the relative burden of reproductive material correlates with swimming speeds. We also measured the relationship of body length, using snout-vent lengths (SVL) and the loss of mobility due to reproduction. Lastly, we examined the possibility that the loss of mobility during reproduction is correlated with changes in female physiological condition during pregnancy.

MATERIALS AND METHODS

Study Animals

Thirty-three pregnant snakes were hand-captured 21June to 02 August, 2013 from sites in Lonoke and Mississippi Counties in Arkansas, USA. Pregnancy was determined by palpation of the lower abdomen to detect the presence of developing embryos in the uteri. Snakes were brought to the laboratory, where preparturient mass and snout-vent length (SVL) were measured. Females were individually housed in clear plastic totes (H15.2 x W41.3 x L58.4 cm) with aspen bedding and continual access to water. Snakes were offered 4-5 adult Goldfish (*Carassius auratus*) weekly. Frequency of feeding was recorded after all individuals were in the laboratory (2 August), but not the mass or number of fish consumed. Cages were checked daily for parturition.

After a female gave birth, neonates, stillborns, and yolks were individually weighed. Litter mass was calculated as the total mass of all the reproductive material (live neonates, stillborns and yolks). Relative clutch mass was calculated as LM divided by post-parturient mass for each litter to account for the large variation in body sizes among females.

Swimming Speed Trials

Each female had two swim trials, where the maximal swimming speed before giving birth (pre V_M) and after giving birth (post V_M) were recorded. The time between before and after measurements varied among females due to variation in the duration of pregnancy and time of capture for each female. All snakes were weighed and measured prior to swimming. Animals were individually placed in temporary plastic holding containers to await swimming speed trials, which were performed in a cement water trough (H 0.76 x W 0.72 x L 2.54 m), filled with 20 cm of water. Air and water temperature was measured before each day's runs, to account for potential variation in swimming speeds due to temperature. A one meter section was delineated on the bottom of the trough for the racetrack, with 0.25 m before and after the ends of the racetrack, denoted as the start and end blocks. Each snake was placed at the start block and the stopwatch was started as soon as the nose of the snake crossed the start line. The tail of each snake was gently tapped to encourage the snake to swim the entire length of the racetrack. The time was stopped as soon as the snake's nose crossed the end line. The time required for the snake to swim the one meter racetrack was recorded for each run and used to calculate maximal swimming speed. Each snake was run three times with at least thirty minutes between each run and the fastest speeds were used for pre and post V_M. Snakes that reversed direction or not did not swim in a straight line were brought back to the start block and re-run until a valid speed could be measured. If the snake failed to complete a full run within five minutes, no data was recorded for that run, the female was given at least 30 minutes before its next run, and maximal speed was calculated from the remaining two runs for that trial. Snakes were returned to their original cages immediately after trials.

Statistical Analyses

We calculated means and standard error of SVL, preparturient mass, postparturient mass, pre V_M, post V_M, ΔV_M, LM, RCM, and change in female mass (ΔM). Change in swimming speed was calculated as:

$$\Delta V_M = \text{pre } V_M - \text{post } V_M \quad (2).$$

Change in female mass was calculated as:

$$\Delta M = \text{postpartuient mass} - (\text{preparturient mass} - \text{LM}) \quad (3).$$

This value is used to examine the physiological cost of reproduction of the female, after removing the effect of weight loss due to reproductive material itself. We ran a paired t-test to detect a change in speed between pre V_M and post V_M. We ran a linear regression model using preparturient swimming speed as a response variable with RCM as an explanatory variable. Further, we correlated RCM with SVL to test if this relationship explained the effect of RCM on preparturient swimming speed. We computed a multiple linear regression to analyze the influence of SVL and LM on ΔV_M. We also correlated SVL to LM to test for collinearity between the explanatory variables, as SVL and LM are predicted to be highly correlated with each other in snakes. As a measure of female body condition changes during pregnancy we calculated female mass loss (ΔM). This measurement does not distinguish between any specific types of tissue loss and includes mass loss due to fat, muscle, and water loss. This value represents another potential cost of reproduction in females. Female mass change was then correlated with post V_M to estimate if mass change influences swimming speed as a potential cost of reproduction. Pearson product-moment correlations are reported for all significant relationships in regression models. Data were analyzed in the R platform. Interaction terms were removed from models when not significant. All analyses were run at a stated $\alpha = 0.05$.

RESULTS

Snakes were captured between 21 June and 02 August of 2015. All females gave birth between 24 August and 21 September. Swimming speed after giving birth was significantly greater than during pregnancy (t_{32} = 4.363, p = 0.0001, see Table 1). This translated into an average of a 13.09% decrease in speed during pregnancy compared to postparturient speed. Time in captivity did not correlate with mass or swimming speed change (ΔM: $F_{1,30}$ = 2.337, r = 0.2648, P = 0.1365; ΔV_M: $F_{1,30}$ = 5.143, r = -0.1277, P = 0.4787).

To test the hypothesis that swimming speed is reduced when the relative burden of reproductive material is increased, we correlated RCM and preparturient maximal swimming speed (pre V_M) and found a significant negative, albeit weak, relationship ($F_{1,31}$ = 5.008, r = - 0.3730, P = 0.0326; Figure 1). Thus, females that were relatively more burdened by the mass of their reproductive material swam slower during pregnancy than did less-burdened ones. Furthermore, this relationship is not simply an effect of body size as RCM and SVL were not correlated ($F_{1,31}$ = 0.1367, r = -0.0663, P = 0.714). Also, after giving birth, there was no correlation between RCM and post V_M ($F_{1,31}$ = 0.2152, r = -0.0831, P = 0.646). Animals with a higher RCM also fed less frequently in captivity ($F_{1,27}$ = 6.04, r = -0.4276, P = 0.0207).

The best predictor of change in swimming speed (ΔV_M) was female SVL. Litter mass was also significantly correlated with ΔV_M when SVL was included in the model (SVL: $F_{1,30}$ = 9.776, partial r = -0.5737, P = 0.0039; LM: $F_{1,30}$ = 5.827, partial r = 0.4033, P = 0.0221). Females with shorter SVL showed a greater change in their swimming speed than longer snakes after giving birth (Figure 2). However, females with heavier litter masses had greater changes in swimming speed after giving birth. There was a significant positive correlation between female SVL and LM, as expected ($F_{1,31}$ = 6.081, r = 0.3701, P = 0.0194; Figure 3).

To test the prediction of a physiological cost to females during pregnancy influencing swimming speed, we correlated female mass change (ΔM) and postparturient maximal swimming speed (post V_M). We found a strong positive relationship between these two variables ($F_{1,31}$ = 16.062, r = 0.5842, P = 0.0004). Females that lost more mass during their pregnancy swam slower after giving birth, while those individuals that loss less or gained weight during pregnancy swam faster (Figure 4). Additionally, longer individuals had greater weight change during their pregnancy, although this relationship is only marginally significant ($F_{1,31}$ = 3.8231, r = 0.3313, P = 0.0596). However, there was no relationship to number of feedings in captivity and the length of the animal ($F_{1,27}$ = 0.5566, r = -0.1421, P = 0.4621). But, frequency of feeding in captivity was negatively correlated with ΔM ($F_{1,27}$ = 17.61, r = -0.6283, P = 0.0003).

DISCUSSION

Through three separate measures, we have demonstrated a cost of reproduction in the form of loss of locomotor performance during pregnancy in Diamond-backed Watersnakes. This loss of locomotor ability appears to be a function of the increased physical burden of reproductive material a female must carry as well as a physiological cost through the loss of body mass during pregnancy. Furthermore, our results suggest that the cost of reproduction from a loss of locomotor ability is greater for smaller (shorter) individuals.

As the relative burden of reproductive material increases female watersnakes swim slower during pregnancy than less reproductively-burdened individuals, similar to Winne and Hopkins (2006) findings in the Black Swampsnake (*Seminatrix pygea*). Moreover, when the burden of reproductive material is removed after giving birth, there is no correlation between RCM and post V_M, suggesting that the reproductive burden was responsible for the slower speeds during pregnancy but not after. It is important to note that this relationship is independent of the size of the animal. The reproductive burden was estimated by relative clutch mass, a trait that we and others have found to be independent of body length within a given species of snake (Seigel and Fitch 1984; Seigel et al. 1986)

While others have documented reduced swimming speeds of pregnant snakes or lack thereof, from our examination this study is the first to demonstrate a higher cost to swimming speed on smaller snakes than larger ones (Seigel et al. 1987; Brown and Weatherhead 1997, Winne and Hopkins 2006). However, none of these studies explicitly tested for this relationship. Shorter snakes, carrying relatively higher than average litter masses suffer a greater loss in swimming speed compared to longer snakes also carrying higher than average litter masses. There was no relationship between SVL and pre V_M, but a strong negative relationship between SVL and post V_M. This relationship between swimming speed and body length may have profound effects on the overall life-history strategy of this species. Because of the potentially greater risk to reproducing at smaller sizes, selection may favor increased adult body size at first reproduction. This may result from increased offspring size, increased juvenile growth rates, or delaying the timing of first reproduction to achieve larger sizes. All of these functions influence fecundity in this species, as body-size is tightly correlated with reproductive output (Figure 3). Furthermore, these life-history consequences are likely if we assume that age (and not body size) at first reproduction is fixed, which it appears to be, at three years for females of this species (J.D. Chamberlain, unpublished data). Additionally, these traits may be exaggerated in populations where there is a higher risk to pregnant females due to predation or starvation.

It is well established in the literature that many squamates reduce or completely forego feeding during pregnancy because the burden of reproduction causes reduced rates of successful feeding, reduced ability to evade predators, and increased thermoregulatory needs (Shine and Bull 1979; Shine 1980; Seigel et al. 1987; Shine 1988b; Gregory et al. 1999; Lourdais et al. 2002; Brischoux et al. 2011). As a consequence of this reduction in feeding, female squamates may rely on stored energy reserves during pregnancy, resulting in weight loss during this period. Our data suggest that some snakes did forego feeding in captivity and consequently lost more weight during pregnancy, however the cause of this anorexia was not explored in this study. Further, we demonstrate that this weight loss associated with pregnancy inhibits a snake’s mobility after giving birth. This mass loss may be interpreted as a cost of reproduction as it may remove potential energy that could have been used in future reproductive attempts. Also, this mass loss may represent an added physiological stress that may have long-term negative effects on survival. The timing of locomotor loss after giving birth in autumn may prevent successful feeding during a critical foraging period for females. Meals acquired during this period may be used to regain lost mass and to store energy for the following reproductive year or hibernation.

To our knowledge, this is the first study to document that weight loss during pregnancy in snakes is correlated with the length of the animal. Longer individuals lost more weight during their pregnancy than shorter ones despite all females being offered size-appropriate meals weekly throughout their time in captivity. The mode of this weight loss cannot be directly explained from this study. However, three potential mechanisms of anorexia during pregnancy for snakes have been suggested: physical limitation through reduction of gut capacity, behavioral limitations such as reduced foraging ability or increased time thermoregulating, and physiological limitations such as suppression of appetite (Gregory et al. 1999; Lourdais et al. 2002; Brischoux et al. 2011). Lourdais et al. (2002) argue that feeding during pregnancy for snakes is only beneficial for future reproduction and does not contribute to the current reproductive attempt. Thus, older females receive a smaller benefit from potentially costly feeding during pregnancy and are more likely to forego this behavior. Since snakes show indeterminate growth and larger individuals are theoretically older, one way to interpret the pattern that longer snakes lost more weight during pregnancy is that they preferentially forewent feeding as the cost may outweigh the energy benefit for older animals. If this is the case, then anorexia during pregnancy in snakes may not simply be a function of physical constraints but also a function of behavioral or physiological choices made during pregnancy. Gregory et al. (1999) came to a similar conclusion after noting anorexia in late stage pregnant Western Terrestrial Gartersnakes (*Thamnophis elegans*) in the laboratory, despite being offered size-appropriate food. They argued that if the anorexia they documented was due solely to physical constraints, then snakes would simply feed on smaller meals more frequently, for which they found no support. In fact, when pregnant females did feed, meal sizes were the same as non-pregnant females after correcting for SVL. Combining the loss of reproductive advantage of older females with the potential of behavioral and physiological suppression of feeding during pregnancy may help to explain to relationship of female mass loss increasing with SVL in this species. However, more explicit studies are necessary to understand the mechanism for this relationship.

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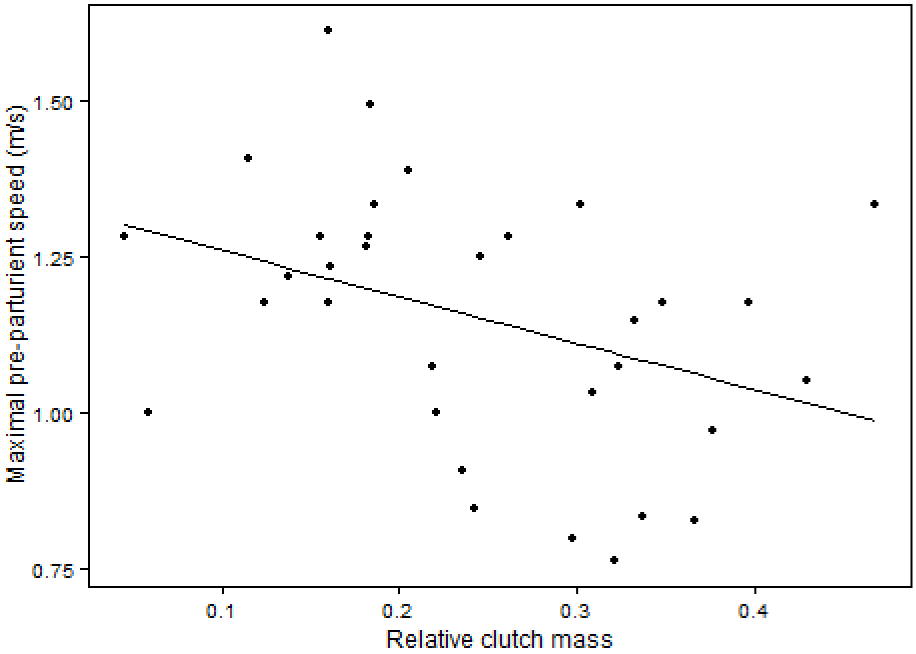

Fig. 1. Maximal swimming speed of each female before giving birth as a function of relative clutch mass (RCM). RCM is used as an estimate of a female's physical burden during reproduction ($F_{1,31}$ = 5.008, r = - 0.3730, P = 0.033) .

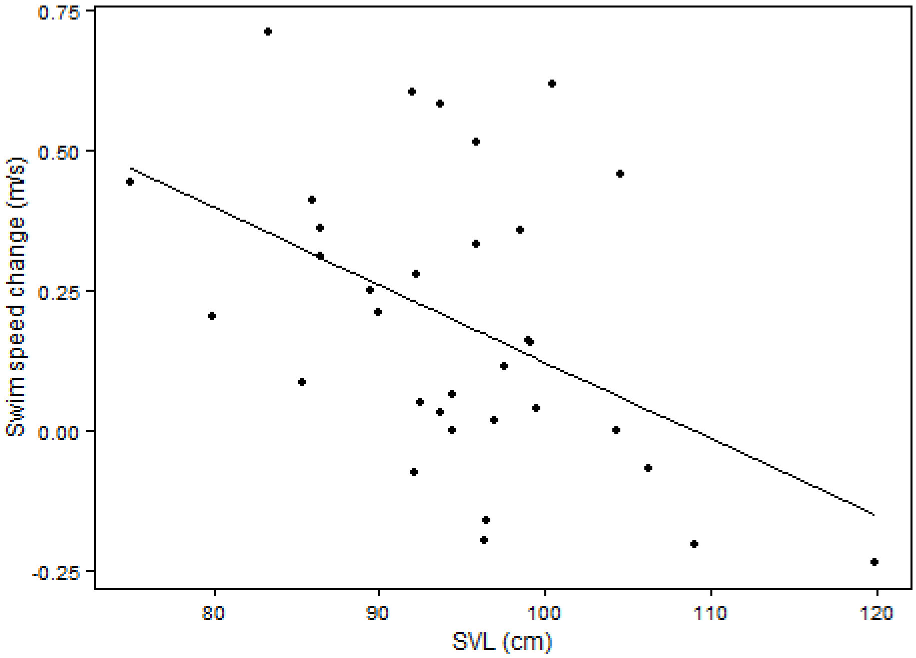

Fig. 2. Change in maximal swimming speed, speed after giving birth less the speed before giving birth, of each female as a function of snout-vent length ($F_{1,30}$ = 9.776, partial r = -0.5737, P = 0.0039).

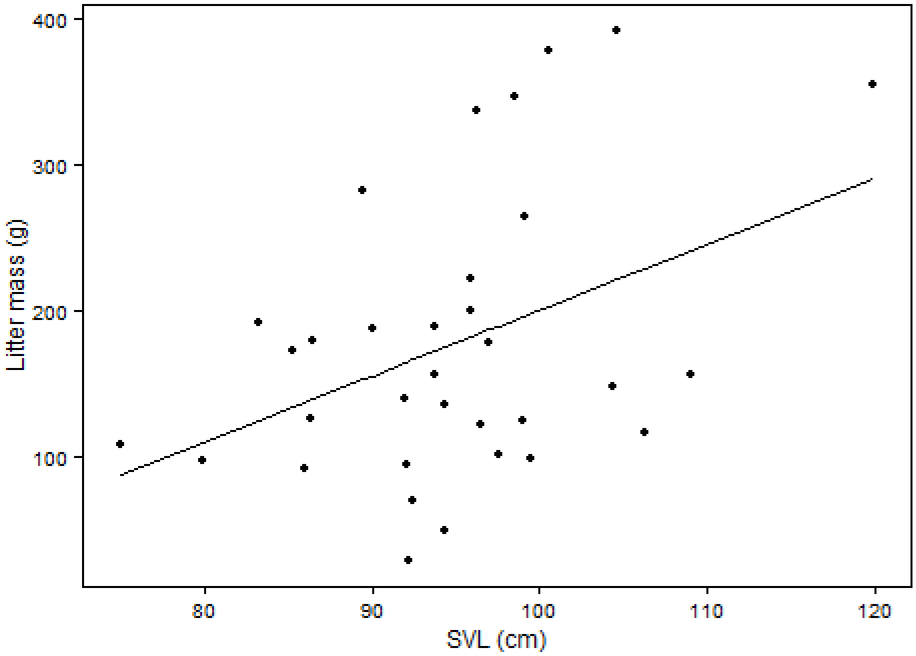

Fig. 3. The litter mass of each female as a function of snout-vent length ($F_{1,31}$ = 6.081, r = 0.3701, P = 0.019).

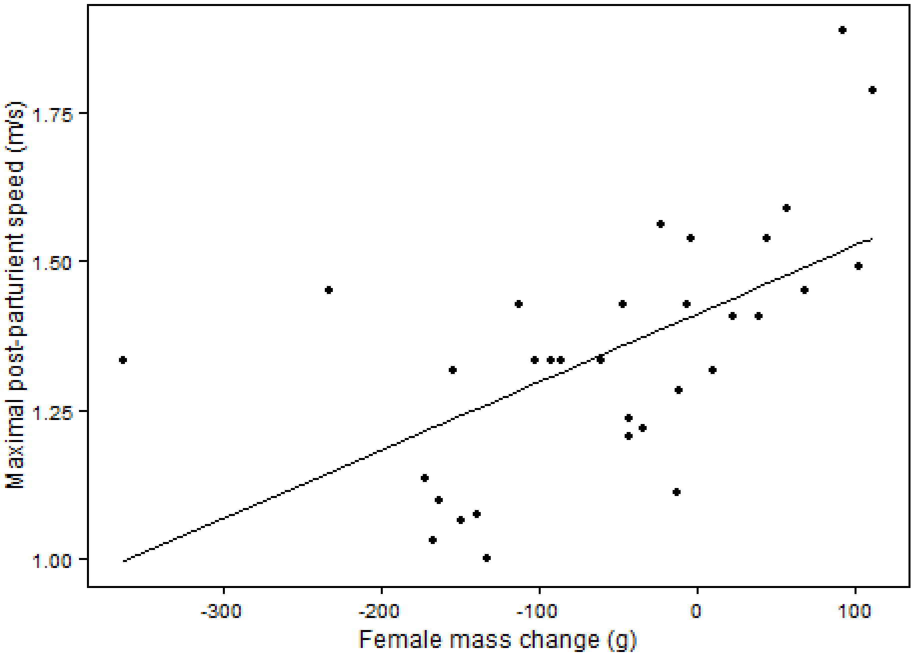

Fig. 4. Maximal swimming speed of each female after giving birth as a function of her mass loss during gestation ($F_{1,31}$ = 16.062, r = -0.5842, P = 0.0004). Female mass loss represents the mass lost due to the burden of carrying reproductive material for a female and may constitute an additional cost of reproduction for females.

Table 1. Means of reproductive characteristics and maximal swimming speed.

SVL (cm)	**Preparturient Mass (g)**	**Postparturient Mass (g)**	**Pre V_M (m/s)**	**Post V_M (m/s)**	**ΔV_M (m/s)**	**LM (g)**	**RCM**	**ΔM (g)**
94.8 ± 1.5	955.6 ± 51.7	722.4 ± 34.3	1.15 ± 0.04	1.35 ± 0.04	0.2 ± 0.04	177.2 ± 16.7	0.25 ± 0.02	-56.13 ± 18.09

Values are means ± s.e.m.

CHAPTER II

Influence of Prey Size on Reproduction Among Populations of Watersnakes

This chapter formatted in accordance with the guidelines set forth by the Journal of Zoology (2016).

Abstract

The average size of prey often varies across landscapes, resulting in populations of predators having differing access to energetic resources. Increasing the average size of prey within landscapes provides predators with increased access to energy resources. With additional energy potentially available for reproduction, females may maximize fitness by prioritizing allocation towards increasing the size of offspring, the number of offspring, or both to maximize fitness. Individuals with increased energy resources may also potentially allocate a higher relative proportion of their total energy to reproduction. To test this hypothesis, I evaluated the allocation patterns of diamond-backed watersnakes measuring the reproductive output of four populations that differed in average prey size; two dependent on small prey, two dependent on large prey. Snakes dependent on large prey produced longer, heavier babies compared to snakes from small prey sites. Statistical interactions among sites confounded my ability to compare differences in litter size, litter mass, and relative clutch mass. However, these interactions appeared to be driven by increased variation in these reproductive traits with female body length at a single site. Once this population was removed from analyses, patterns of litter size, litter mass, and relative clutch became interpretable. Snakes dependent on large prey produced similar numbers of offspring as snakes from one site dependent on small prey. Snakes from two sites dependent on large prey also allocated a similar proportion of their total energy to reproduction, as estimated by relative clutch mass, as one site dependent on small prey. I suggest that increasing energy availability from increased prey size results in additional energy being allocated to offspring size and not litter size, while maintaining the total proportion of energy allocation to reproduction.

Introduction

Early research on life-history began with examinations of offspring size and clutch/litter size (Lack 1947; Lack 1967; Smith and Fretwell 1974). These studies examined how organisms prioritize the allocation of energetic resources to reproduction. Understanding this complex relationship is still a focus of research. Central to this work are efforts to understand how variation in environmental factors shift priorities of allocation decisions (Cody 1966; Smith and Fretwell 1974; Nussbaum 1981; Seigel and Ford 1992; Iverson et al. 1993; Reznick and Yang 1993; Olsson and Shine 1997; Sun et al. 2006; Bownds, Wilson, and Marshall 2010; Ford and Seigel 2011). Increasing the average size of prey available in the environment provides predators increased energy intake (Arnold 1993; Chamberlain 2016, Chapter 4). When the total energy intake is increased, do the allocation decisions to reproduction shift?

There are four possible outcomes when individuals increase their energy intake (Hirschfield and Tinkle 1975): 1) additional energy can be allocated to increasing offspring size, litter size, or both; 2) the relative proportion of total energy allocated to reproduction can increase; 3) both the total energy allocated to reproduction (outcome 1) and the relative proportion of total energy to reproduction (outcome 2) can increase; 4) additional energy may not be shuttled to reproduction at all, and instead allocated to the remaining portion of the life-history.

If additional energy resources are allocated to reproduction (outcome 1), organisms may produce larger offspring or more offspring in order to maximize fitness. Theory suggests that if juvenile mortality is independent of the size of offspring, females should invest into producing as many offspring at the smallest viable size as possible.

However, if the probability of survival to reproduction increases with offspring body size, females should allocate to increasing offspring size to an optimum, beyond which offspring survival does not increase (Smith and Fretwell 1974; Stearns 1992). The question then becomes whether increasing energy availability by increasing prey size influences the survival of offspring in a size-dependent fashion.

The goal of this research, therefore, is to not only determine if increased environmental energy availability alters the total or proportional energy allocation pattern to reproduction, but also to explore whether increasing the average prey size available to predators results in increased offspring sizes, litter sizes or both.

Snakes provide a fitting model for examining the influence of environmental variation on reproduction because they exhibit variation in offspring and litter traits and they are gape-limited predators (Seigel and Ford 1992; Arnold 1993; Forsman 1996; Forsman and Shine 1997; Bronikowski and Arnold 1999; Bonnet et al. 2000; Shine 2005; Ford and Seigel 2011). Previous studies provide a basis for testing hypotheses concerning the reproductive responses to changing prey characteristics. Although environmental factors can select for shifts in offspring size and litter size, such as over-winter survival, starvation risk or size-specific predator avoidance, prey size should also influence allocation decisions during reproduction (Bonnet 1997; Shine and Downes 1999; Kissner and Weatherhead 2005; Sun et al. 2006).

As gape-limited predators, increasing body length, in an absolute-sense, drives a corresponding increase in gape size and the ability to handle larger prey (Forsman 1996). Therefore, increasing the initial body size of offspring should allow those neonates that are longer and have larger gapes to immediately begin foraging on larger, more calorically valuable prey (Chamberlain 2016, Chapter 4). This advantage should be strengthened when prey are smaller or rare. Similarly, if young snakes are able to grow quickly enough to obtain larger food, there should be an advantage to starting life at a larger body size by shortening the time necessary to access those resources. In contrast, if fitness does not differ with body size of neonates, mothers should favor larger litter sizes with smaller offspring because there is little to no additional risk to these small offspring (Smith and Fretwell 1974; Stearns 1992).

To test whether females from populations with access to differing sized prey allocate a different proportion of their total energy to reproduction (outcome 2), I propose the use of relative clutch mass (RCM = litter mass/ litter mass + post-parturient mass), a measure of the relative proportion of female's total mass that is comprised of reproductive material (Seigel, Fitch, and Ford 1986). Conceptually, this measure allows comparison of reproductive effort among individuals of the same species. If more total energy is allocated to reproduction (outcome 1), there should be an increase in average offspring size, litter size, or both among females from each site. To support outcome 3 females should increase both RCM and offspring size/litter size. Lastly, if additional energy is not being allocated to reproduction, then females should not differ in any of the measured reproductive traits.

While these predictions make theoretical sense, they are rarely tested in the field. In wild populations it can be difficult to determine how a particular environmental variable, specifically prey size, influences reproductive characteristics owing to the inability to control extraneous sources of variation. Therefore, replicate field sites that vary in prey size while simultaneously minimizing additional environmental variation would permit such comparisons. The aquaculture systems in Lonoke County, Arkansas provide a unique opportunity to study the effects of prey size on reproduction in wild populations of diamond-backed watersnakes (*Nerodia rhombifer*) in a replicated fashion. These farms raise different species of fish that vary in size while using similar culturing and pond techniques that minimize extraneous variation among the sites. My study examines the how variation in prey size found naturally among four fish farms in Arkansas shapes the allocation of energy to reproduction in diamond-backed watersnakes.

Methods

Site descriptions

Four fish farms in Lonoke County, Arkansas were selected for this study, two characterized by large prey and two characterized by small prey. The large prey populations (LARGE) are Joe Hogan State Fish Hatchery (JOHO) and Keo Fish Farm Inc. (KEO). JOHO is roughly 105 hectares and produces approximately 80% catfish species (*Ictalurus spp.*). The remainder of production on this farm consists of crappie and bass species (*Promoxis spp.* and *Micropterus spp.*). The maximum harvest size of catfish and crappie at JOHO is approximately 30 cm, and harvest size of bass is highly variable (Chamberlain 2016, Chapter 4). KEO is approximately 100 hectares but split between two properties, however only the smaller 40 hectare property was sampled for this study. This site specializes in the production of hybrid striped bass (*Morone saxatilis/chrysops*) and triploid grass carp (*Ctenopharyngodon idella*), with 50% of property dedicated to each fish species. The maximum harvest size for grass carp and hybrid striped bass at KEO is 25 cm (Chamberlain 2016, Chapter 4). The two small prey sites (SMALL) are

Gentry and Canterberry Fisheries LLC (GNC) and Pool Fisheries Inc. (POOL). Both sites specialize in the production of fathead minnows (*Pimephales promelas*) and goldfish (*Carassius auratus*). However, snakes at these sites only had access to the goldfish, which are harvested at a maximum size of 7.5 cm (Chamberlain 2016, Chapter 4). GNC is approximately 65 hectares and POOL is approximately 135 hectares in size.

I chose these sites because of their close proximity and environmental similarity. All sites consisted of series of rearing ponds ranging from 0.1-0.8 hectares with maximum depths of 3 m and exposed, grassy banks. All sites hosted avian and mammalian predators, such as great blue herons (*Ardea herodias*), great egrets (*Ardea alba*), American mink (*Neovison vison*), and striped skunks (*Mephitis mephitis*). Additionally, hybrid striped bass and catfish farmed at the LARGE populations were likely aquatic predators of neonate snakes. The maximum distance between any two sites was 15 km. However, due to lack of suitable habitat between sites, gene flow among sites was assumed to be limited.

Study Animals:

Pregnant snakes were hand-captured in late summers of 2012-2014 at all sites (total=124; 33, 33, 29, 29 from JOHO, KEO, GNC, and POOL respectively). Snakes were brought back to the laboratory and housed individually in size-appropriate plastic cages within a rack system. They were provided a thermal gradient of 23-32°C via thermal heating elements placed under the rear third of each cage (Vision Products, Canoga Park, CA). All snakes were provided aspen bedding, water, and fed once weekly *ad libitum* on size-appropriate goldfish. Dam snout-vent length (SVL) and body mass were measured at the time of capture, henceforth called pre-parturient mass. Upon parturition, dam body mass was re-measured, henceforth called post-parturient mass. A total of 2,149 offspring were measured (621, 609, 411, 508 from JOHO, KEO, GNC, and POOL respectively). Litter size (live only), litter mass (live only), and each offspring SVL and body mass were recorded. Using post-parturient mass and litter mass, I calculated relative clutch mass for each dam (litter mass/ litter mass + post-parturient mass) as a measure of individual reproductive effort. All dams and offspring were individually marked and released to their native population within a week of birth.

Statistical Analysis:

Data were analyzed in the R platform (R Core Team, 2014). All analyses on offspring mass and offspring SVL were performed using either a mean value for each litter. Data were tested for normality to meet the assumptions of parametric testing. Data were either log-transformed or square-root transformed to meet assumptions of normality where necessary. All data were analyzed using a nested design, with sites nested within prey type.

Because dam body-size is known to correlate with most reproductive characteristics in other snake species, analyses of covariance (ANCOVA) were used to measure population differences in reproductive traits with dam SVL as a covariate (Reviewed in Ford and Seigel 2011). I performed analysis of variance (ANOVA) and ordinary least squares regression if dam SVL did not correlate with a particular reproductive trait. ANCOVA models with significant interaction terms of dam SVL to factor terms were not interpreted, as they fail to meet the assumptions of common slopes. Non-significant interaction terms were removed from all models and only reported when their $p < 0.10$. When data were missing for a given trait, each litter with missing data was removed from the analysis. Statistical significance was determined at $\alpha < 0.05$. Significant ANCOVA, ANOVA, and linear models were further analyzed to detect population differences using post-hoc analyses with a Tukey correction.

Results

Offspring size:

Log-transformed offspring mass significantly correlated with female SVL and differed by prey type, with no interaction of SVL*PREY or PREY*SITE (SVL: $F_{1,116} = 32.152$, $P < 0.0001$; PREY: $F_{1,116} = 25.929$, $P < 0.0001$, Figure 1A). Snakes from LARGE sites produced heavier babies than snakes from SMALL sites ($t = -4.828$, $P < 0.0001$). Mean offspring mass was 23% larger at LARGE sites than at SMALL sites (LARGE = 10.5 ± 0.2 g; SMALL = 8.5 ± 0.2 g, mean ± s.e.).

Offspring SVL also significantly correlated with female SVL and differed by prey type, with no interaction of SVL*PREY or PREY*SITE (SVL: $F_{1,116} = 19.696$, $P < 0.0001$; PREY: $F_{1,116} = 14.444$, $P = 0.0002$, Figure 1B). Snakes from LARGE sites produced longer babies than snakes from SMALL sites ($t = -3.392$, $P = 0.0010$). Mean offspring length was 4.5% longer at LARGE sites than at SMALL sites (LARGE = 25.2 ± 0.2 cm; SMALL = 24.1 ± 0.1 cm, mean ± s.e.).

Litter size:

Litter size was square-root transformed to meet the assumption of normality. Litter size strongly correlated with female SVL ($F_{1,111} = 66.878$, $P < 0.0001$); however, two significant interactions within the full model made interpretations difficult (PREY*SITE: $F_{1,111} = 66.878$, $P = 0.0271$; SVL*PREY*SITE: $F_{1,111} = 2.894$, $P = 0.0385$). The SVL*PREY*SITE interaction suggested that litter size did not scale with SVL similarly among sites, nor did litter size scale with SVL similarly between sites within the same prey type. This can be more easily understood by visualizing the nature of these relationships by SITE instead of PREY (Figure 2A). It was clear from this analysis that the relationship of litter size to female SVL for snakes at GNC had a substantially shallower slope than the other SMALL site, POOL; whereas, both LARGE sites had similar relationships of SVL to litter size.

Litter mass:

Log-transformed litter mass was significantly correlated with female SVL; however, the significant interaction of SVL*PREY*SITE made differences between PREY types difficult to interpret (SVL: $F_{1,111} = 58.326$, $P < 0.0001$; SVL*PREY*SITE: $F_{2,111} = 3.261$, $P = 0.0428$). Similarly, visualizing this interaction in terms of SITE instead of PREY helped to decipher patterns (Figure 2B). Again, the relationship of litter mass to female SVL at GNC had a substantially shallower slope than the other SMALL site, as well as both LARGE sites.

Relative clutch mass:

SVL correlated with RCM, but it scaled differently between prey types and among populations as indicated by two significant interactions of SVL*PREY*SITE and PREY*SITE (SVL: $F_{1,108} = 5.512$, $P = 0.0207$; PREY*SITE: $F_{1,108} = 3.199$, $P = 0.0447$; SVL*PREY*SITE: $F_{1,108} = 3.001$, $P = 0.0337$). Again, visualizing by SITE rather PREY, as with litter size and litter mass, helped clarify this relationship (Figure 2C). SVL scaled negatively with relative clutch mass at GNCE, whereas it scaled positively at POOL.

A consistent pattern was evident in the relationships of litter size, litter mass, and relative clutch mass to maternal SVL; the regressions for GNC scaled with shallower slopes than those regressions from the corresponding SMALL site, POOL. Meanwhile, both LARGE sites behaved similarly when comparing their slopes of SVL to litter size, litter mass, and RCM. In an effort to explain the SMALL site pattern in the scaling of female SVL to each reproductive trait, I tested whether they differed in variability of these traits. I specifically tested whether shallower slopes at GNC were associated with increased variation in these traits for larger females as compared to POOL. I calculated the absolute value of the residuals of each reproductive trait regressed on female SVL for both populations and tested for a correlation between these residuals and female SVL for the three reproductive traits separately.

The relationship between variation in litter size and female SVL differed between POOL and GNC; GNC exhibited a positive slope compared to POOL, which had a slope that did not differ from zero (SVL: $F_{1,53}$= 9.196, P = 0.0037; SVL*SITE: $F_{1,53}$ = 7.666, P = 0.0077, Figure 3A). This suggested that at larger SVL's females from GNC produced litters that varied more in size than did females from POOL. Similar relationships occurred for litter mass and RCM; GNC snakes had larger residuals at longer SVL's compared to snakes from POOL (litter mass, SVL: $F_{1,53}$= 4.251, P = 0.0442; SVL*SITE: $F_{1,53}$ = 9.980, P = 0.0026; RCM, SVL: $F_{1,53}$= 3.981, P = 0.0512; SVL*SITE: $F_{1,53}$ = 9.789, P = 0.0029). These results suggest increased variation in litter mass and RCM at larger body sizes at GNC, which resulted in a steeper positive slope for these relationships compared to individuals from POOL (Figures 3A and B).

Interestingly, when GNC was removed from each analysis and the remaining three sites compared (using a non-nested model), litter size still strongly correlated with female SVL and there were significant differences among populations with no interaction of SVL*SITE (SVL: $F_{1,87}$= 64.658, $P < 0.0001$; SITE: $F_{1,87}$ = 3.674, P = 0.0294). Females from POOL had larger litters than females from KEO, but individuals at JOHO did not differ from either population (POOL:KEO, t = 2.654, P = 0.0253, POOL:JOHO, t = 1.042, P = 0.5521, KEO:JOHO, t = -1.779, P = 0.1824). Litter mass was significantly correlated with female SVL but there were no significant differences among the three populations of POOL, JOHO, and KEO (SVL: $F_{1,82}$= 61.704, P < 0.0001; SITE: $F_{1,82}$ = 1.233, P = 0.2969). Lastly, RCM significantly correlated with female SVL, but there wer no significant differences among sites (SVL: $F_{1,82}$= 6.880, P = 0.0104; SITE: $F_{1,82}$ = 1.969, P = 0.1460).

Discussion

Here I present evidence that prey size variation can have a profound influence on reproductive allocation patterns in diamond-backed watersnakes. Snakes feeding on larger prey had longer and heavier babies compared to similarly-sized snakes from sites that had access only to small prey. The influence of prey size on litter size, litter mass, and relative clutch mass is less clear because of significant interactions suggesting that, for these traits, factors of prey type and sites within prey type did not scale similarly with female SVL. Post-hoc examination suggests these interactions are likely due to a high degree of variation in reproductive traits in snakes with longer body sizes at GNC, which resulted in a similar shallow slope in the three regressions of litter size, litter mass, and RCM on female SVL. With the removal of GNC, litter size, litter mass, and relative clutch mass interaction terms were non-significant and allowed clearer inferences among sites. Litter size at POOL, a SMALL site, is significantly larger than KEO but not JOHO, the other LARGE site. I did not detect differences among POOL, KEO, and JOHO for litter mass or RCM. The lack of difference among sites for RCM suggests that snakes with access to larger prey did not increase the relative proportion of their total energy resources to reproduction, providing no support for outcome 2 or 3. The increase in offspring size at LARGE sites and lack of clear pattern in differences of litter size between one SMALL and both LARGE sites provide mixed support for an increase in total energy allocation to reproduction at LARGE sites, somewhat supporting outcome 1.

The strongest effect of increasing prey size was a dramatic increase in mass and length of offspring. While size-specific predation, starvation risk, and overwinter survival (Bonnet 1997; Shine and Downes 1999; Kissner and Weatherhead 2005; Sun et al. 2006) favor larger offspring size and could be influencing these sites, it is likely that snakes occupying all fish farms experience these environmental variables to a similar degree. I therefore conclude that the major selective force on offspring size in these populations is driven by differences in prey size.

Previous work has shown that average adult body size within a population is selectively driven by differences among populations in average prey size. Snakes colonizing islands with average prey sizes larger or smaller than the ancestral mainland population, for example, often result in gigantism or dwarfism (Forsman 1991; Boback 2003; Keogh, Scott, and Hayes 2005, Aubret and Shine 2007). Unfortunately, these studies typically examine prey size effects on adult body size and do not consider the effect of prey size on reproductive allocation or the response of offspring to increasing the average prey size. However, I am not the first to document that prey size can result in increasing mean offspring size in snakes. Sun et al. (2006) showed that average size of offspring in insular vipers was larger than mainland counterparts. These authors argued that the increase in offspring size was due to the need for neonatal vipers to quickly attain sizes that allow them to forage on proportional large birds that stopover during migration on their study island. Gape-limitation produces a strong selective pressure for increased SVL size of neonates. Mainland vipers, in contrast, feed on diverse prey with variable body sizes and produce offspring that are less than half the length of offspring from the insular population.

Thus, larger offspring may be an indirect effect of prey size. The ability to eat large prey is limited by gape elements of the skull in macrostomatan snakes (Cundall and Greene 2000). Longer gape elements increase the ability of macrostomatan snakes to swallow larger prey by aiding in the biomechanics of handling and swallowing prey (Cundall and Greene 2000, Vincent, Moon, and Shine 2006). If cranial gape elements are unable to respond to selection of larger prey independently, selection for an overall larger body size would achieve the same results as a function of positive allometries.

The selective force of prey size may operate at multiple levels in ontogeny to increase offspring size. If small prey are rare or unavailable at birth due to timing of life-histories of the prey or, in my case, because of anthropogenic interference associated with culturing fish-stock, selection should favor increasing neonate size to a point where neonates can begin to immediately forage. If prey is still too large at the time of birth, then increasing offspring mass could fuel post-natal growth from energetic reserves until neonates are large enough to begin foraging on available prey (Madsen and Shine 2002, Sun et al. 2006). Even if small prey are available to neonates, selection may still favor longer or heavier offspring if larger, more energetically-rich prey are available but un-attainable due to their size (Sun et al. 2006). Increasing both initial offspring mass and length should shorten the growth time necessary for neonates to access these more favorable prey.

Either or both of these explanations may be operating among my sites to drive differences in offspring size. While both LARGE sites stock small fish fry between April and July, small prey at the time of birth of neonate snakes in August to October are rare as only those fish stocked in June or July will not have grown beyond what neonate snakes can physically handle. Because of this time-delay of stocking, differential feeding of fish among ponds, and the retention of larger size classes of fish awaiting sale and shipment, larger and more calorically-valuable prey are always more abundant. Thus larger neonates at LARGE prey sites have access to more prey both immediately and through ontogeny. To further support this argument, observations of snake diet through ontogeny, including that of *N. rhombifer*, show that as snakes grow larger, individuals (particularly females) preferentially drop smaller prey items from their diet (reviewed in Arnold 1993, but see also Mushinsky, Hebrard, and Vodopich 1982, Plummer and Goy 1982).

The advantage of larger offspring to access larger prey is greatly diminished at SMALL sites because these sites have abundant small prey on which neonates can immediately begin foraging. But, there are still larger prey available, which might offer an energetic advantage, suggesting a benefit to starting life at a larger size. However, two factors may diminish this advantage compared to LARGE sites. First, the maximal prey size at SMALL sites is smaller, meaning that intermediate-sized snakes quickly lose a size advantage, and large snakes are likely to be constrained by needing to feed on relatively small prey. Slowing the rate at which larger body sizes are reached may be advantageous, which could be achieved by beginning as a smaller neonate. Secondly, the largest prey sizes at SMALL sites are rarer than smaller prey sizes due to commercial demand.

I was unable to interpret of the effect of prey size on litter size, litter mass, and RCM initially due to the increased variation in these traits at GNC. Why did variation in litter size, litter mass, and RCM increase with body length at this site and not the other SMALL site, POOL? I have identified two potential explanations that may address this discrepancy. 1) While these sites raise the same fish species to approximately the same sizes, they might not raise them at the same frequency. If GNC does not use all of its rearing capacity, either by not stocking as many ponds at one time or by not stocking at the same level throughout the year, prey may become more patchily distributed and harder to acquire for pregnant females. 2) Even if sites have the same size fish, raised at the same frequencies, these sizes may not be available in the same proportions. GNC might raise the largest-sized fish at a lower proportion of its total. This would require large females to either begin foraging on smaller prey more often, or move more frequently to find ponds with large prey. In either explanation, larger females that need more fuel to supply greater reproductive demands (since reproductive output is correlated with body size) will suffer a greater cost than smaller females when large prey become scarce. Both mechanisms would result in some large females successfully foraging while others would fail to locate appropriate food resources. Consequently, some large females will be able to fuel reproduction while others will not, leading to increased variation in reproductive output as female body size increases (Van Noordwijk and De Jong 1986).

The differences in litter size between POOL and KEO females suggest that there may be a trade-off between offspring and litter size, where POOL females produce larger litters of smaller offspring, while KEO females produces smaller litters of larger offspring. However, these differences were not consistent with a prey size effect, as JOHO did not differ from POOL or KEO in litter size, but differed from POOL in offspring size. The lack of difference between POOL and JOHO provides evidence to support outcome 1 that increased energy availability results in increased allocation to reproduction in terms of absolute energy.

Relative clutch mass, however, appears to not vary significantly among populations (except GNC), regardless of prey type. In other words, similar-sized females from POOL, KEO, and JOHO all contribute nearly the same proportion of reproductive effort from their own reserves in a given year, providing evidence to reject outcome 2. This suggests that there is little variation in the physiological controls of relative clutch mass among populations. Seigel et al. (1986) also found little variation in RCM within species of snakes. They argue the major driving force of differences in RCM among species results from major differences in ecological characteristics (i.e. reproductive mode, foraging strategies, etc.), which are unlikely to differ among populations.

Finally, while tangential to the major aims of this study, my results suggest a fecundity advantage to female body size that is driven by access to larger prey. Analysis of field data from these populations shows that maximal female body size differs substantially (Chamberlain 2016, Chapter 3). Female snakes from LARGE prey sites reach substantially longer (and heavier) sizes than females from SMALL sites. While many factors may explain this difference, the result on female fecundity should not go unnoticed. Females from POOL may produce larger litter sizes after correcting for body length. But, on an absolute scale and increase in length yields a corresponding increase in litter sizes. Thus, females from LARGE sites, which may be constrained to produce large offspring at the cost of litter size, may be able to compensate for a comparatively lower litter size by simply growing longer.

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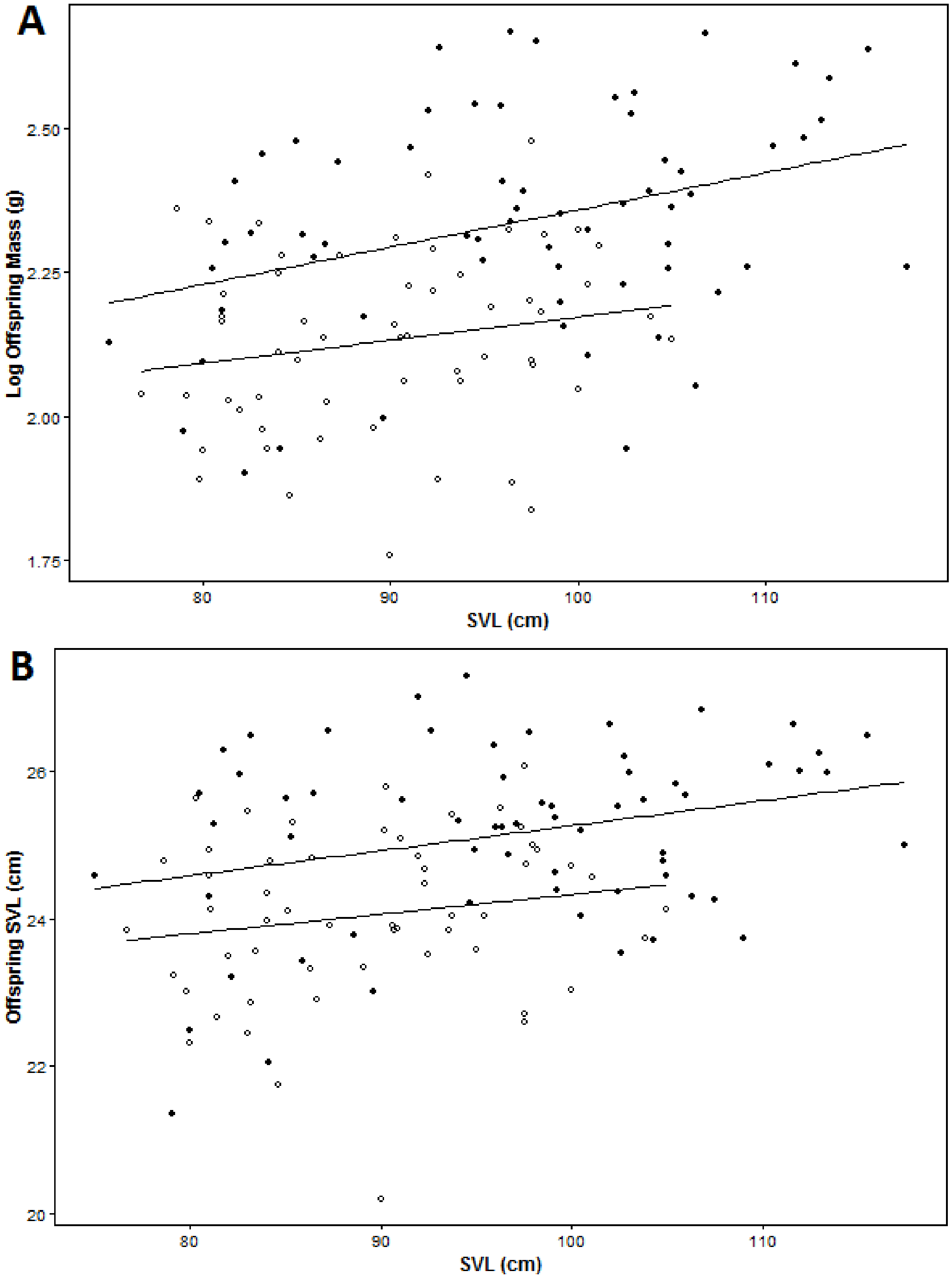

Figure 1 A and B. LARGE and SMALL sites significantly differ in both mass (Panel A) and length (Panel B) of offspring correcting for the effect of maternal body-size. Female watersnakes at LARGE sites produce offspring that are both heavier and longer than offspring from SMALL sites. There is no significant interaction between female SVL and either offspring mass or SVL. LARGE prey sites are represented by (●) and SMALL prey sites by (○).

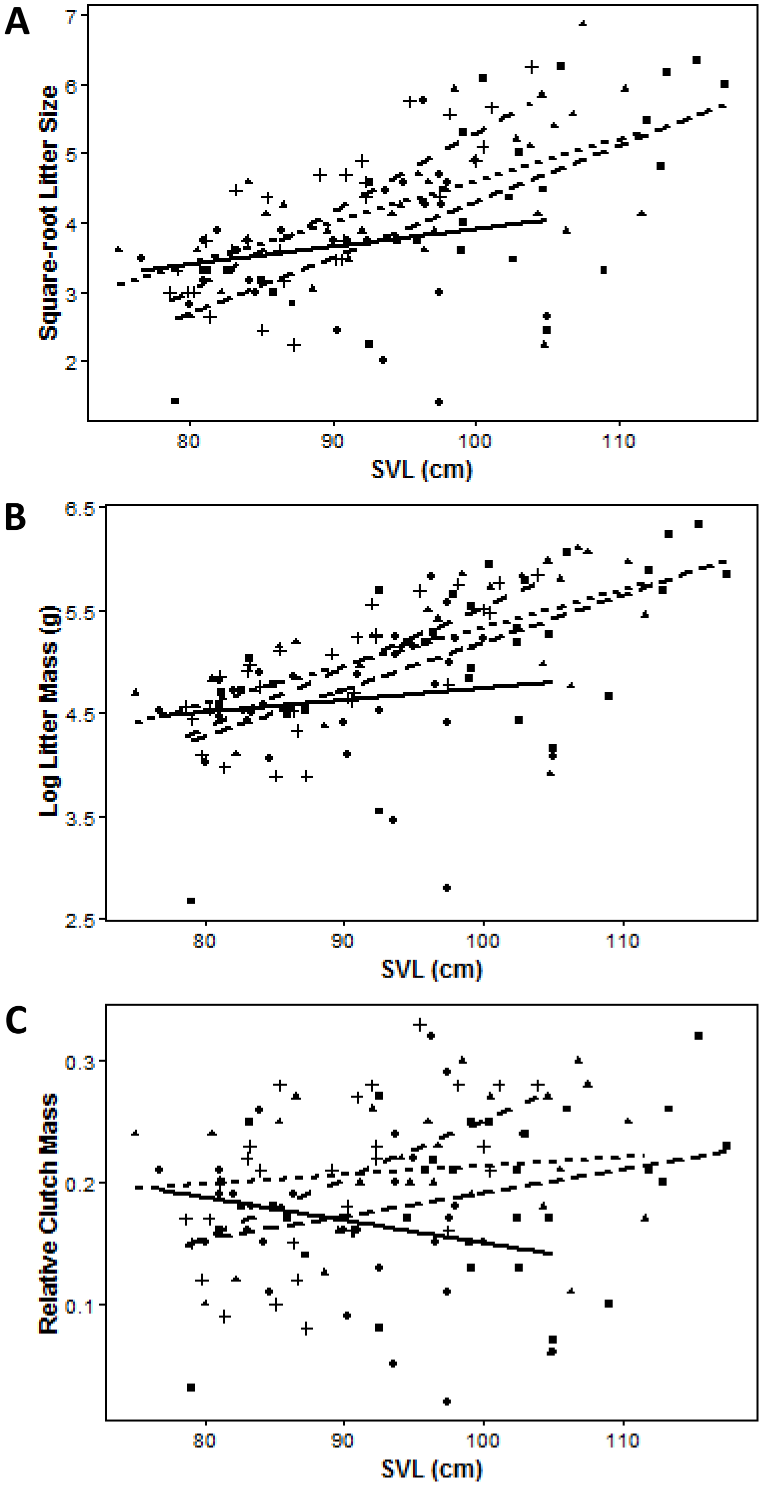

Figure 2 A-C. Strong interactions of female body length and site exist litter size (Panel A), litter mass (Panel B), and relative clutch mass (Panel C). In all three panels, GNC consistently behaves differently than its corresponding SMALL site POOL, while both LARGE sites behave similarly for all three traits. This suggests that the significance of these interactions may be driven by GNC. In all panels, GNC is represented by a (-●-), POOL by a (-+-), KEO (-■-), and JOHO (-▲-).

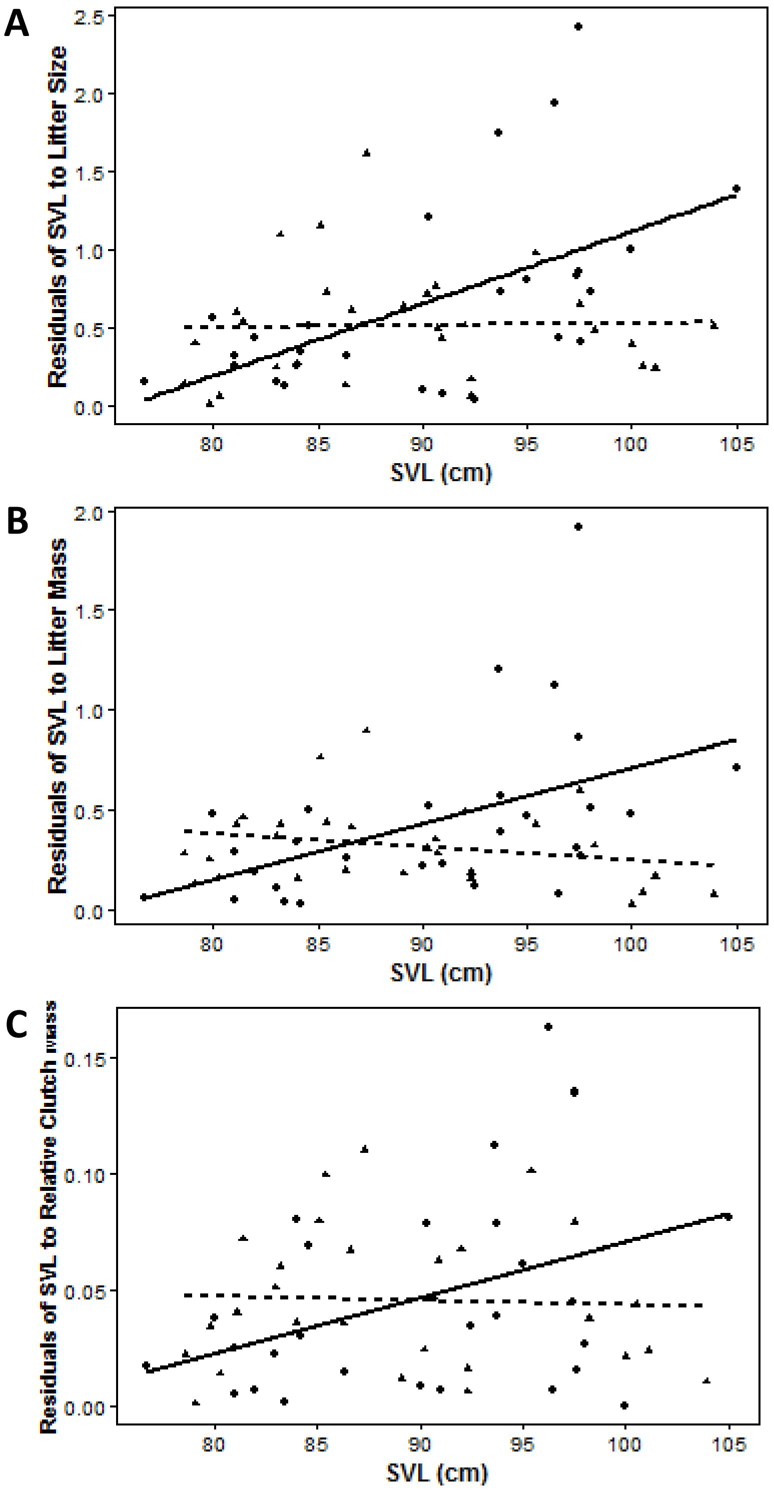

Figure 3 A-C. Variation in the residuals of litter size (Panel A), litter mass (Panel B), and relative clutch mass (Panel C) increase with female body length at GNC, represented by a (-●-), resulting in a positive correlation. The other SMALL site POOL, represented by a (-▲-), residuals do not correlate with female body length, resulting in near flat slopes.

CHAPTER III

Effects of Prey Size and Density on Growth Rate Among Populations of Watersnakes: A function of plasticity or local adaptation?

Abstract

Differences in growth rates among populations can have major fitness consequences, resulting in potential shifts in the entire life-history strategy. Determining the source of these growth rate differences, whether through local adaptation or phenotypic plasticity, is key to understanding how populations respond to selective pressures. Variation in prey characteristics is predicted to alter growth rates among populations. Therefore, I designed this study to characterize how the juvenile growth rates of individual watersnakes would respond to populational differences in prey size and density. I also tested whether growth rate of individuals in these populations was adaptive or phenotypically plastic. Lastly, I considered whether differences in growth rates of juveniles resulted in differences in the age/size at maturity or maximal adult body size, two important components of the life-history strategy. Through use of a common-garden growth study and data from a multi-year mark-recapture study of four populations of watersnakes that differed in their access to prey, I found that neither laboratory nor field growth rates of juveniles differed. The lack of differences in laboratory growth suggest that there is likely not an adaptive response of juvenile growth to prey characteristics. But, the lack differences in field growth rate suggest that prey size and density may not be selective pressures that act on juvenile growth in general. However, sample sizes of field growth rates were low, and interpretation of these results should be limited. While I show that the age and size at maturity did not differ among populations, I found strong evidence that maximal adult body sizes differ among populations. This suggests that populations differ in age structure or that adult growth rates differ, possibly driven by differences in prey characteristics.

Introduction

In a seminal review of organismal growth rates, Arendt (1997) argued that growth rates are often adapted to local environmental conditions. Historical ideas of individual growth rates suggested this trait should be maximized, under the assumption that larger individuals should have greater survival, reproductive success, and increased foraging capability. However, much of the empirical evidence supported the idea that individuals grow well below their maximum in wild populations. Arendt suggested four mechanisms to explain why individual growth rates are often optimal and not maximal and thus may vary among populations within a species: 1) slower growth rates may be adaptive in nutrient stressed populations; 2) trade-offs with development may favor slower than maximal growth; 3) rapid growth should be favored when a minimum size must be reach quickly; and 4) rapid growth may evolve to compensate for slowed growth triggered from environmental conditions.

Different juvenile growth rates among populations can also arise as a phenotypically plastic response to environmental conditions. As Stearns and Koella (1986) demonstrated in models of growth rate reaction norms, a single genotype can produce substantial variation in growth rates simply due to environmental stressors altering juvenile or adult mortality rates, such as from nutrient stress or competition. Thus, if local environmental conditions differ between populations, an optimal growth rate for individuals can differ, even if no genetic variation is available on which selection can act or if little time has elapsed since population divergence.

Whether growth rates of individuals among populations differ due to natural selection or arise from phenotypic plasticity, a shift in the age or size at sexual maturity is predicted. Stearns and Koella (1986) suggested five potential consequences of slowing growth among individuals: Slower growing individuals 1) may mature later and at a smaller size; 2) mature later at the same size; 3) mature later at a larger size; 4) mature earlier at a smaller size; or 5) mature at the same age at a smaller size. Shifts in these critical life-history characteristics can have profound fitness consequences among individuals, particularly when body-size is correlated with survival or reproductive success, and therefore merit greater effort toward understanding these patterns (Peters 1983, Stearns 1992). In this study I examine how variation in prey size and quantity shape growth rate, age and size at maturity, and maximal adult body-size among populations of a predator.

Prey size and quantity shape life-histories by influencing the growth of individuals, potentially triggering variation in the age or size at maturity, survival, and maximal adult body size (Forsman 1991, Lourdais et al. 2002, Madsen and Shine 2000, Madsen and Shine 2002, Reznick and Yang 1993, Reznick et al. 2002, Salamolard et al. 2000, Siems and Sikes 1998). Slower growth rates may be favored where prey density is low or stochastic, potentially as a mechanism to deal with increasing frequency of starvation events (Arendt and Wilson 1997, Arendt and Reznick 2005, Miller at al. 2011, Siems and Sikes 1998). Conversely, faster growth rates should be favored in environments with dense, stable food sources (Bronikowski 2000, Bronikowski and Arnold 1999, Byars et al. 2010, Madsen and Shine 2000). If larger predators have access to large, dense, and calorically valuable prey then reaching larger body-sizes faster can result in an energetic advantage (Shine 1988, Chamberlain 2016, Chapter 4). Thus, faster growth rates may be favored in environments where average prey size is increased (Forsman 1991).

I measured juvenile growth rate, size and age at sexual maturity, and maximal adult body-size of diamond-backed watersnakes (*Nerodia rhombifer*) from four wild populations that differed in average prey sizes and prey density (Chamberlain 2016, Chapter 4). I tested for population differences in juvenile growth, and explored whether differences were driven by plasticity, adaptation, or both using common-garden growth experiment and field mark-recapture studies. I then tested whether any observed differences in growth rate could generate population-level differences in the age and size at maturity. Lastly, using the measurements of age and size at maturity, I tested whether differences in prey size and density among populations resulted from differences in maximal adult body-size.

Methods

Study species:

The diamond-backed watersnake is an ideal species for examinations of life-history differences as it is an abundant, often conspicuous predator that exhibits substantial variation in life-history across its range (Keck 2004). As a gape-limited predator that actively forages for fish, variation in prey size and density are predicted to have strong effects on the morphology and energetics of this species (Forsman 1996, Forsman and Shine 1997, Vincent et al. 2006). Further, this species adjusts well to laboratory conditions, making it a good candidate for common garden laboratory experiments and reproductive studies. Snakes in these analyses were part of a larger study examining the influence of prey characteristics on life-history strategies among populations. The populations used in this study have shown marked effects of prey size on offspring size (Chamberalain 2016, Chapter 2).

From the literature, it is estimated that males reach sexual maturity in the spring of their second year, whereas females do not reach maturity until the spring of their third year (Trauth et al. 2004, Keck 2004). Mating in this species occurs soon after emergence from hibernation and continues into May (J.D Chamberlain, pers. obs.). Females begin vitellogenesis in mid-May and continue until mid-June at which point embryos can be detected in the oviducts (J.D. Chamberlain, pers. obs.). Males begin spermatogenesis at the end of July, as evidenced by the presence of enlarged testes (J.D. Chamberlain, pers. obs.). Females begin parturition in late August, extending to the second week of October (J.D. Chamberlain, pers. obs.).

Study sites:

Four study sites in Lonoke and Mississippi counties, AR, offered variation in prey size, prey density, or both. Three sites were aquaculture farms characterized by a highly altered but stable environment, providing resident snakes with a constant, dense source of food throughout the active season. However, these farms differed substantially in the average size of fish raised. Two farms were characterized by large average fish sizes. Keo Fish Farm LLC (KEO) specializes in the production of triploid grass carp (*Ctenopharyngodon idealla*) and hybrid striped bass (*Morone saxitilis/chrysops*). Joe Hogan State Fish Hatchery (JOHO) produces several warm-water fish species including largemouth bass (*Micropterus salmoides*), black crappie (*Pomoxis nigromaculatus*), and channel catfish (*Ictalurus punctatus*), but 80% of their production is catfish. The average prey size on these two sites was significantly larger than other study sites (Chamberlain

2016, Chapter 4). Gentry and Canterberry Fisheries LLC. (GNC) specialized in production of goldfish (*Carassius auratus*) for the pet trade and fishing industry. Representative of natural prey density (nearly a hundredth of the densities at aquaculture sites) and size, the Big Lake Wildlife Management Area and Mallard Lake (MAL), run by the Arkansas Game and Fish Commission, comprised a 4986 hectare network of lakes, sloughs, canals, and ditches. This site is likely characteristic of historic conditions for diamond-backed watersnakes, and provides prey densities and sizes similar to un-disturbed conditions. It represents the presumed ancestral prey conditions under which these snakes evolved. Fish farms sites, therefore, represent divergence from this ancestral condition.

While extraneous sources of environmental variation can influence the factors of interest, comparisons of fish farm populations minimize this variation by using similar aquaculture techniques. The thermal, climatic, and predator environments at these sites are assumed to be similar because of proximity and similar management techniques.

Field growth rates:

All sites were part of a four year (2012-2015) mark-recapture study. The sites were sampled using visual surveys and hand captures monthly during the active season from 1 March to 31 October. All individuals captured were measured to the nearest 0.1 cm for snout-vent length (SVL), weighed to the nearest 0.1 g, individually marked with a unique scale-clip, and released back to their native population. If I recaptured individuals at subsequent sampling events, they were re-measured and the date recorded. To increase the probability of recapturing juveniles, neonates born in the laboratory from females collected from each population were also uniquely scale-clipped and released to their native populations.

I calculated field growth rate as the difference in SVL divided by the number days between measurements in recaptured specimens less the days spent in brumation, as it is assumed that minimal growth occurs during this period. Growth days, therefore, were defined as the number of days between capture less the days spent in brumation. I assume brumation occurred between 1 November and 28 February for all populations following Trauth et al’s (2004) estimation for *N. rhombifer* activity in Arkansas. Thus, I subtracted 120 days from “days between capture” for those individuals that were recaptured in two consecutive years. Similarly, I subtracted 240 days for those individuals recaptured after three consecutive years. As I was interested only in juvenile growth rate up to sexual maturity, recaptures of individuals marked as adults were not included in analyses.

Common-garden laboratory growth rates:

I collected pregnant females from each site in July of 2013 (n = 39 total, 11, 8, 10, and 10 for JOHO, KEO, GNC, and MAL, respectively) and allowed them to give birth in the laboratory as part of a larger reproduction study (Chamberlain 2016, Chapter 2). Parturition dates ranged from 23 August to 1 October. Six neonates, 3 male and 3 female, from each litter were held in captivity for the growth study. I marked and released all remaining neonates from each litter to their native populations as a part of the field growth study. I only used individuals that survived the duration of the study in subsequent analyses. With 11 individuals removed due to mortality, the total number of individuals used in analyses was 223 (n = 66, 45, 57, 55 from JOHO, KEO, GNC, and MAL, respectively).

Each neonate was individually housed within a rack system (Vision Products, Canoga Park, CA) and provided aspen bedding and continual access to water. The rack system enclosures (46 cm x 13 cm x 9 cm) provided a thermal gradient ranging from 25 to 31°C by including a heating element under the rear third of each tub. I estimated total weekly consumption of each snake as a covariate in growth rate analyses. I fed snakes live fathead minnows (*Pimephales promelas*) weekly. Fish were placed in the waterbowl of each snake in the evening and uneaten fish were removed and counted the following morning. The mass of fish consumed was estimated as the average mass of one fish times the number of fish eaten. The number of fish provided to each snake was counted at each feeding. Average weight of each fish was estimated as the average mass of a subsample of 10 fish from each weekly feeding. The number of fish provided each week was the maximum number of fish eaten the previous week by any individual snake plus one. Snakes initially fed 1-2 weeks after birth, often before the beginning of the common-garden growth experiment. Thus, I only calculated the total mass of fish consumed during the actual growth experiment for use as a covariate.

Body mass, SVL, and date were recorded at the time of birth and again at the beginning of the experiment. The growth experiment began on 2 October, 2013 and finished 31 January, 2014. During the study, I weighed and measured snakes every two weeks. Biweekly growth rate for length was calculated as the change in SVL divided by the number of days between measurements, as individual SVL growth is a more reliable measure than body mass. The growth rate used in analyses was the average of all biweekly growth rates. This value was selected to minimize individual variation in growth rate over the course of the study.

I compared growth rates using mixed effects models with site and sex as fixed effects, and maternal ID as a random effect. I used body size at the beginning of the common-garden trial and total mass of consumed fish as covariates, as these are predicted to have a positive correlation with growth rate and will compensate for potential effects of differences in age. I determined significance of fixed effects within the mixed model by using a Satterthwaite approximation for denominator degrees of freedom and the lmerTest package in R (Bates et al. 2014, Schaalje et al. 2002). Significance of the random effect of maternal ID was determined by a likelihood-ratio test, comparing two models, one including all fixed and random effects and one with all fixed effects, but where the random effect of maternal ID was removed. Non-significant interactions were removed from all models.

Size at sexual maturity:

I estimated the size at sexual maturity by combining two separate methods for females. First, I compiled the SVL's of all females that gave birth in the laboratory from 2012-2015 (n = 182 total, 89, 41, 33, and 19 for JOHO, KEO, GNC, and MAL respectively). Second, to this list I added the SVLs of dissected females that were a part of a seasonal fat-cycling study (J. D. Chamberlain, unpub. data) that were pregnant (i.e. developing embryos in the oviduct; n = 21 total, 11, 5, 4, 1 for JOHO, KEO, GNC, and MAL, respectively). From this dataset I identified the smallest pregnant female from each population sample, the average length of the smallest 10% of females, and the smallest 20% of females from each population. These values, though likely conservative, should encompass individuals reproducing for the first time and thus should reflect the average size at sexual maturity for females from each population. The smallest individual from each population sample might reflect a minimum critical size required for reproduction. Thus, if an individual with slower growth within a population does not reach this critical size, it may reach maturity a year later than the rest of its cohort. To compare populations, I examined the range of means among each site, but no statistical test was used, owing to the small sample size for some populations.

Size at maturity for males from each population was determined from the SVL of individuals with motile sperm in seminal ducts of dissected males from the seasonal fat-cycling study (n = 81 total, 25, 23, 24, 9 from JOHO, KEO, GNC, and MAL, respectively) (J. D. Chamberlain, unpub. data). Again, I identified the smallest mature male from each population, the average length of the smallest 10%, and the smallest 20% of males from each population and compared the range of these means among populations.

Using my estimate of juvenile growth rates of females in the field and the average size at sexual maturity for each population, I calculated the average number of days required for individuals born in each population to reach maturity. These values were compared for each population to test for differences in the year-age at sexual maturity in the field.

Lastly, I compared the range of means for the largest female measured in the field from each population to determine differences in maximal adult size. I also compared the average of the largest 10% and 20% of female lengths from each population using an ANOVA to determine if differences in the average size of the largest size class of females differed. Population differences were determined using a post-hoc t-test with a Tukey correction. Since the number of recaptured males was too small to calculate a field growth rate, I did not compare the predicted time to reach sexual maturity for each population. However, ample field data were available for all populations for males, so I estimated maximal adult male size and compared the average size of the largest 10% and 20% of male lengths for each population.

Results

Common-garden laboratory growth rates:

SVL growth rates among populations did not differ after accounting for the effects of maternal ID and total consumption, with sex having no effect on growth rates and no interactions of total consumption by site (Site: $F_{3, 35.005} = 30.767$, $p = 0.8675$, Total consumption: $F_{1, 183.776} = 376.190$, $p < 0.0001$, Sex: $F_{1,183.155} = 178.250$, $p = 0.3619$, Maternal ID: $\chi^2 = 5.083$, $p = 0.0242$). Total consumption and initial SVL were positively correlated ($r = 0.422$, $p < 0.0001$) and thus collinear with growth rate, therefore only total consumption was used as a covariate in the model. Further, absolute growth rates, uncorrected by any covariate but still accounting for non-independence of maternal ID, did not differ among populations (Site: $F_{3, 35.059} = 1.390$, $p = 0.2603$, Maternal ID: $\chi^2 = 24.724$, $p < 0.0001$), as seen in Figure 1.

Field growth rates:

Recaptures of marked juveniles that were still juveniles at the time of recapture were low among populations, with only 17 recaptures, 11 of which were female. No recaptures were measured from the natural population (MAL), most likely due to the large area for juveniles to disperse. I excluded recaptures of juveniles that were above adult size at the time recapture. These individuals would underestimate juvenile growth rate because growth slows after sexual maturity in most indeterminate growers, including snakes. Since sample sizes of females were roughly even, large enough for comparison between GNC (n = 5) and large prey sites (KEO and JOHO collectively, n = 6), I only examined juvenile female growth rates from field-sampled animals. I also pooled growth rates from KEO and JOHO on the grounds of they are both characterized as large prey fish farms and in order to balance the data for comparison. The average female growth rate in the field was 0.1434 cm*day^{-1} for GNC and 0.1334 cm*day^{-1} for the pooled Large populations. However, there was no statistical support for a difference between GNC and the Large prey sites (Student-t = 0.332, p = 0.7491), even after running non-parametric Mann-Whitney U-test, to account for small sample sizes (W = 24, p = 0.7308), likely due to the lower power of this analysis (d = 0.02, power = 0.06).

Size at maturity:

There appears to be a slight difference in the minimum size at sexual maturity for females among populations, with GNC (75.9 cm) and JOHO (75.0 cm) having lower, but similar, sizes at maturity compared to KEO (79.0 cm) and MAL (79.0 cm), which have identical minimum sizes (total range among all sites = 4.0 cm). However, when examining the average SVL of the smallest 10% and 20% of reproductive females, these differences are diminished (range at 10% = 3.5 cm, range at 20% = 2.8 cm), order changes, and there is considerable overlap among populations. Average size at sexual maturity among all populations is thus similar (Table 1).

There appears to be little difference among populations for minimum SVL at maturity of males (range = 0.7 cm) or the average SVL of the smallest 10% or 20% of males with motile sperm (range at 10% = 0.9 cm, range at 20% = 2.0 cm). Again, there is considerable overlap in the variation around the means and order changes, suggesting similar average sizes at maturity among populations for males (Table 1).

Maximal adult female body sizes differ substantially among populations, with large prey fish farm sites (KEO and JOHO) and natural population (MAL) having larger maximal adult sizes than the small prey fish farm site (GNC). GNC had a significantly shorter average SVL of the largest 10% and 20% size classes of females as compared to the other three populations (20%: $F_{3.317} = 8.811$, $p < 0.0001$, 10%: $F_{3,157} = 18.261$, $p < 0.0001$, Figure 2 C and D). At both 10% and 20%, GNC was significantly shorter than all other sites, while there were no differences among KEO, JOHO, and MAL (Table 2). This pattern was maintained when examining the largest female from each population. The two large prey fish farm sites, KEO and JOHO had maximal female sizes of 125.0 cm and 117.3, respectively. MAL, the natural site, had a comparable maximal female size of 116.4 cm. All three of these populations differed significantly from GNC, the small prey fish farm site, which had a maximal adult female size of 106.5 cm

Differences in maximal adult male body size among sites was substantially less extreme compared to differences in females (total range: 5 cm). KEO had the largest maximal body size among males (95.5 cm) whereas JOHO, the other large prey fish farm site, had the smallest (90.5 cm). The average SVL of the largest 10% and 20% also reflect a pattern of KEO and MAL males being larger and JOHO and GNC males were smaller. There were significant differences among populations in both the average of largest 10% and 20% of males (20%: $F_{3,266} = 13.713$, $p < 0.0001$, 10%: $F_{3,131} = 5.645$, $p = 0.0012$, See Figure 2 A and B). At 10% and 20%, males from KEO were significantly larger than males from GNC and JOHO, but sizes of males from GNC, JOHO, and MAL did not differ from each other, nor did MAL males differ from KEO males (Table 2).

As expected given the intense sexual size dimorphism in this species, maximal body sizes of adult males were much shorter than females for all populations. The degree of difference between the maximal size for females and the maximal size for males within a population differed among populations (29.5 cm, 26.8 cm, 24.4 cm, and 14.9 cm for KEO, JOHO, MAL, and GNC, respectively) with both large prey fish farms having the greatest difference and the small prey farm having the smallest difference.

Estimated age at maturity:

If I use a common juvenile female growth rate in the field for all fish farm populations, based on a lack of statistical difference, then with some basic assumptions about the age and size at birth and time spent in hibernation, I can estimate the relative age at sexual maturity for each fish farm population. The average growth rate was 0.1380 cm*day^{-1}. From study of reproductive traits of these populations, I was able to derive the least-squares mean predicted SVL of offspring born from KEO, JOHO, and GNC (Chamberlain 2016, Chapter 2). These values reflect the average size of offspring from a population after correcting for the effect of maternal SVL on offspring size from an ANCOVA analysis. GNC had an average offspring SVL of 24.1 ± 0.2 cm, JOHO offspring averaged 24.9 ± 0.2 cm, and KEO offspring averaged 25.2 ± 0.2 cm. These differences were statistically significant, with neonates from GNC being smaller than both JOHO and KEO, but JOHO and KEO neonates did not differ from each other (Chamberlain 2016, Chapter 2). Additionally, the modal date on which the greatest number of females gave birth was 2 September, 5 September, and 17 September for KEO, JOHO, and GNC, respectively.

Assuming an average growth rate of 0.1380 cm*day^{-1}, an average birth date of 17 September, and an average SVL at birth of 24.1 cm, it would require GNC neonates 375 growth days and 615 total days to reach the minimum size at sexual maturity, which was 75.9 cm. Similarly, it would 420 growth days or 660 total days to reach the average SVL of the smallest 20% of females from GNC, which was 82.1 cm. Further, using the total number of days from the modal date of birth, females should reach the minimum size by 25 May two years after birth and should reach the average SVL of the smallest 20% of females by 9 July two years after birth.

Using an average growth rate of 0.1380 cm*day^{-1}, an average birth date of 2 September at KEO, and mean offspring size of 25.2 cm, 390 growth days or 630 total days are needed for neonates at KEO to reach the minimum size at maturity of 79.0 cm. It would require 420 growth days or 660 total days to reach the average SVL of the smallest 20% of reproductive females, which was 83.1 cm. Females at KEO therefore should reach the minimum size at sexual maturity by 25 May two years after birth and the average SVL of smallest 20% of females by 24 June two years after birth. With similar average offspring size and only a slightly later average date of birth for JOHO compared to KEO, patterns between these two populations are similar. I predict a 24.9 cm neonate born on 5 September at JOHO needs 363 growth days or 603 total days to reach the minimum size at maturity of 75.0 cm for this population, and 408 growth days or 648 total days to reach 81.2 cm, the average SVL of the smallest 20% of reproductive females for this population. Therefore, a JOHO neonate should reach the minimum size at maturity by 1 May two years after its birth, and reach the average SVL of the smallest 20% of females for JOHO by 15 June after two years.

Juveniles from GNC, KEO, and JOHO all appear to reach their respective minimum sizes at maturity by May two years after their birth. Snakes from GNC, the small prey site, putatively reach their minimum size at maturity on the same date as KEO, one of the large prey sites, 25 May. The time required to reach these minimum sizes differs by 27 days, with KEO juveniles taking longest and JOHO juveniles the least. But, dates for minimum size at maturity only differ by 24 days.

Discussion

Prey size and density do not appear to influence juvenile growth rate. In a common garden environment, juvenile snakes from populations characterized by dense large prey, dense small prey, or naturally varying prey sizes and densities exhibited similar growth rates over the course of a four month period. Mean field growth rates for juvenile females also did not differ among populations. Substantial variation within populations and small sample size, due to low recapture rates, resulted in a great deal of overlap and provided weak statistical power. Unsurprisingly, therefore, I did not see a statistically significant difference in the age or size at maturity, even when initial offspring SVL differed among populations. Interestingly, however, despite similarity in age and size at sexual maturity, maximal adult body sizes, particularly in females, differed substantially among populations. Maternal ID was a significant factor in my models, which suggests a genetic or maternal influence on juvenile growth rates.

The lack of differences in growth rates in both the field and laboratory are surprising given the strong differences in reproductive characteristics among these populations associated with prey size (Chamberlain 2016, Chapter 2). Large prey fish farms clearly produced longer and heavier babies than the small prey farm, GNC. Larger body sizes at birth should allow snakes to begin feeding on larger, more calorically valuable prey faster, thus increasing their growth rate. Further, despite the nearly 1 cm difference in offspring length at birth of large prey farms compared to the small prey fish farm, size and age at maturity remained similar among these three sites. These results suggest that some difference in growth rate must be present. My measure of growth rate in the field may be too variable or not sufficiently precise to detect subtle differences in growth rates among populations, particularly given my small sample sizes.

Assuming the lack of difference in average field growth reflects actual biological effects and not a statistical consequence of small sample sizes, discrepancies between differences in offspring size at birth and lack difference at age and size of maturity can still be explained. My measurement of field growth rate is an average across the entire time period measured. Use of this measure assumes growth rate is constant over the entire period for all populations. However, if juveniles at large prey fish farms have a variable growth rate, such that early in life growth is slow due to lack of appropriate sized food but increases as their larger body size allows them to access larger, higher quality prey, then the overall average growth rate could result in a growth rate similar to the small prey farm, which might have a nearly constant growth rate.

Juvenile growth may be constrained if prey sizes appropriate for neonate snakes at large prey fish farms are rare. However, as these neonates grow, foraging success should increase as appropriate sized prey becomes more common, thereby allowing for faster growth. Thus, the increase in body size of offspring at KEO and JOHO may be a strategy to increase survival of neonates by avoiding starvation until larger body sizes can be reached. This phenomenon is also in seen in an insular population of pit-vipers that need to begin quickly foraging on much larger prey items compared to their mainland counterparts (Sun et al. 2006).

My estimates for the age and size at maturity may also explain why smaller females gave birth later in the season than larger individuals. Data suggest that juveniles reach the minimum size of reproduction during the time period measured for the onset of vitellogenesis mid-May to mid-June, as observed from dissection data across all populations (J.D. Chamberlain, unpub. data). Therefore, the smallest 20% of individuals may not begin vitellogenesis and ovulation until the end of this window, which ultimately delays the fertilization and development of ova and thus the date of parturition. While the estimate for the timing of vitellogenesis and ovulation is only a rough approximation and may vary among populations, it suggests smaller individuals may need additional feeding and growth post-hibernation to increase energy stores before they are able to begin reproduction. In comparison, females that were already of adult body size and condition the previous season may be able to initiate vitellogenesis earlier, fueling their reproduction from stored fat reserves from the previous season.

Population differences in maximal adult body size in response to prey size seems to be a common response in snake species. Forsman (1991), in a study of insular and mainland adders, found that as prey size of voles increased among islands, so did the maximal body size of both male and female adders. He argued, as do I, that the increased maximal body size of females is driven by a fecundity advantage of larger individuals. Larger females produce either larger or more offspring compared to smaller individuals.

Therefore larger body sizes may be favored if the prey environment can fuel additional reproductive effort by larger individuals. I also found a similar pattern in sexual size dimorphism. There is likely less reproductive advantage of large adult body sizes in male *N. rhombifer*, as they exhibit scramble competition for mates such that multiple paternity of litters is probable (Uller and Olsson 2008). Thus, prey size appears to drive only one sex to reach larger maximal sizes, causing the greater disparity between males and females at large prey sites compared to MAL or GNC (Houston and Shine 1993; Shine 1986; Shine 1990). Lastly, I cannot show whether this maximal adult body size difference is a result of faster adult growth rates or longer life-span as this requires longer term mark-recapture and much higher detection of previously marked individuals.

Size at sexual maturity does not appear to be a good predictor of maximal adult size. Snakes from all fish farm populations had similar sizes at maturity, but differed significantly in maximal adult body size of both males and females. This pattern does not match the one observed for insular adder populations that had access to larger prey than mainland vipers (Forsman 1991). In Forsman's study islands, larger average prey sizes resulted in snakes exhibiting both faster juvenile growth and larger maximal adult body sizes.

Unfortunately, because of poor recapture rates and difficulty of sampling the natural population of MAL, I am unable to confidently infer whether the patterns of juvenile growth at fish farm populations differ from patterns of juvenile growth in natural environments as predicted. Thus, I cannot determine if either or both small prey or large prey fish farms have deviated from the putative ancestral condition. Additionally, size at first reproduction appears to vary little among populations, suggesting that this minimal size may be constrained physiologically or morphologically in diamond-backed watersnakes. However, a closer investigation of body condition at maturity is merited, and may show variation among populations. Finally, maximal adult female body sizes at MAL are similar to large prey fish farms, suggesting that prey size is in fact driving maximal size. MAL hosts prey items that are substantially larger than the maximal ingestible prey size of any watersnake occurring there, a pattern shared with large prey farms (Arkansas Game and Fish Commission Reports, 1987-2006). Thus, they may also benefit from a larger-female fecundity advantage.

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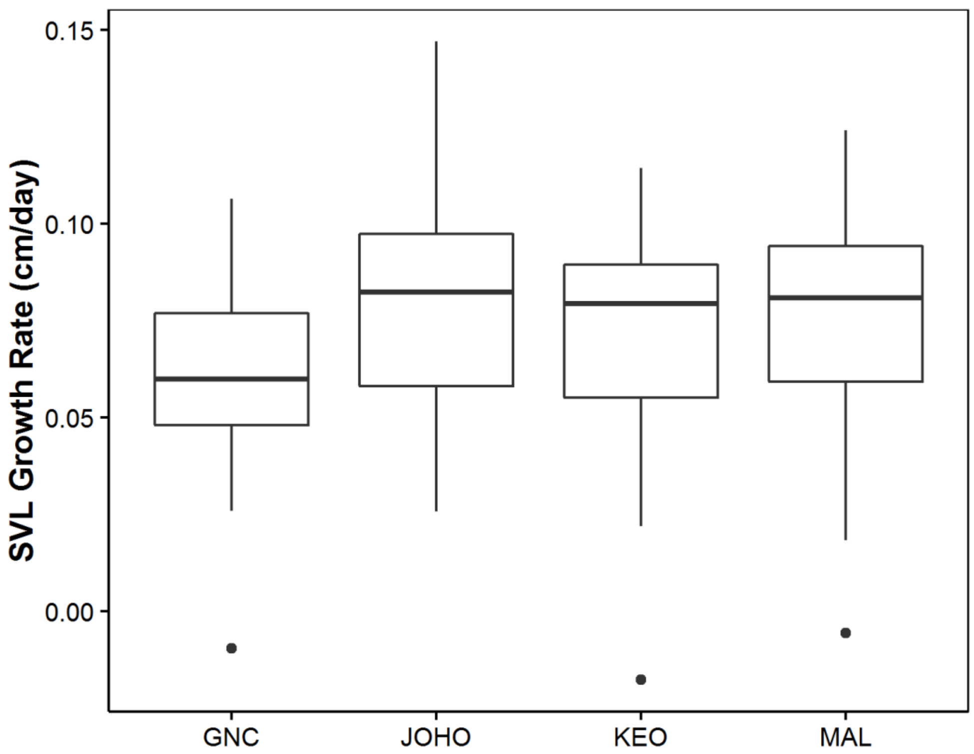


Figure 1. Laboratory common-garden growth rates of SVL did not differ among populations. These values represent un-corrected litter means of growth rate.

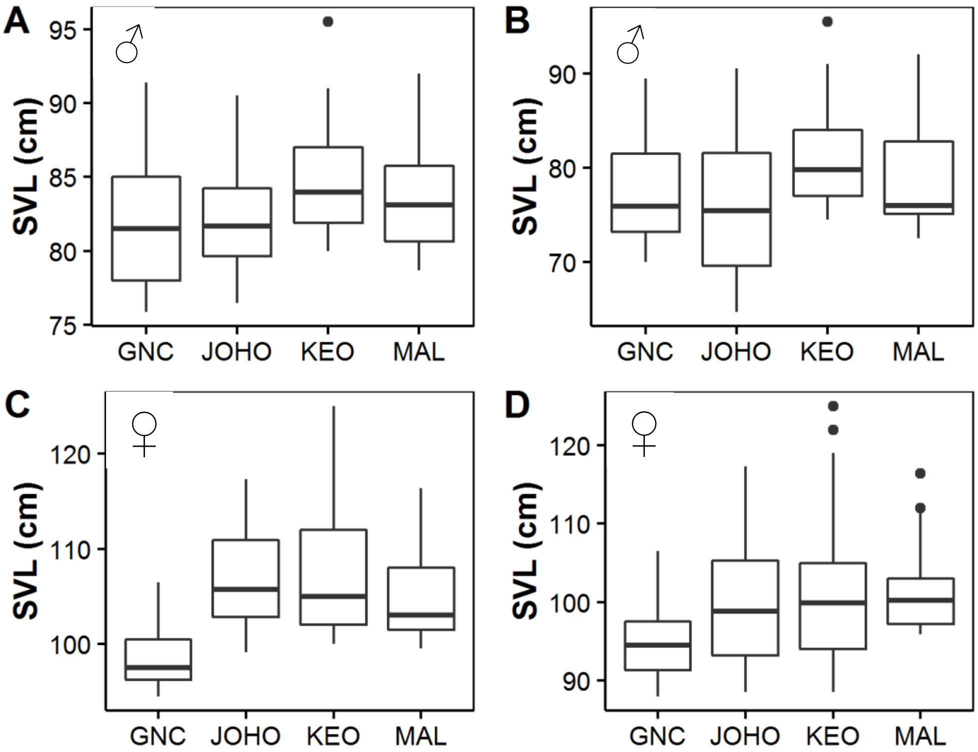
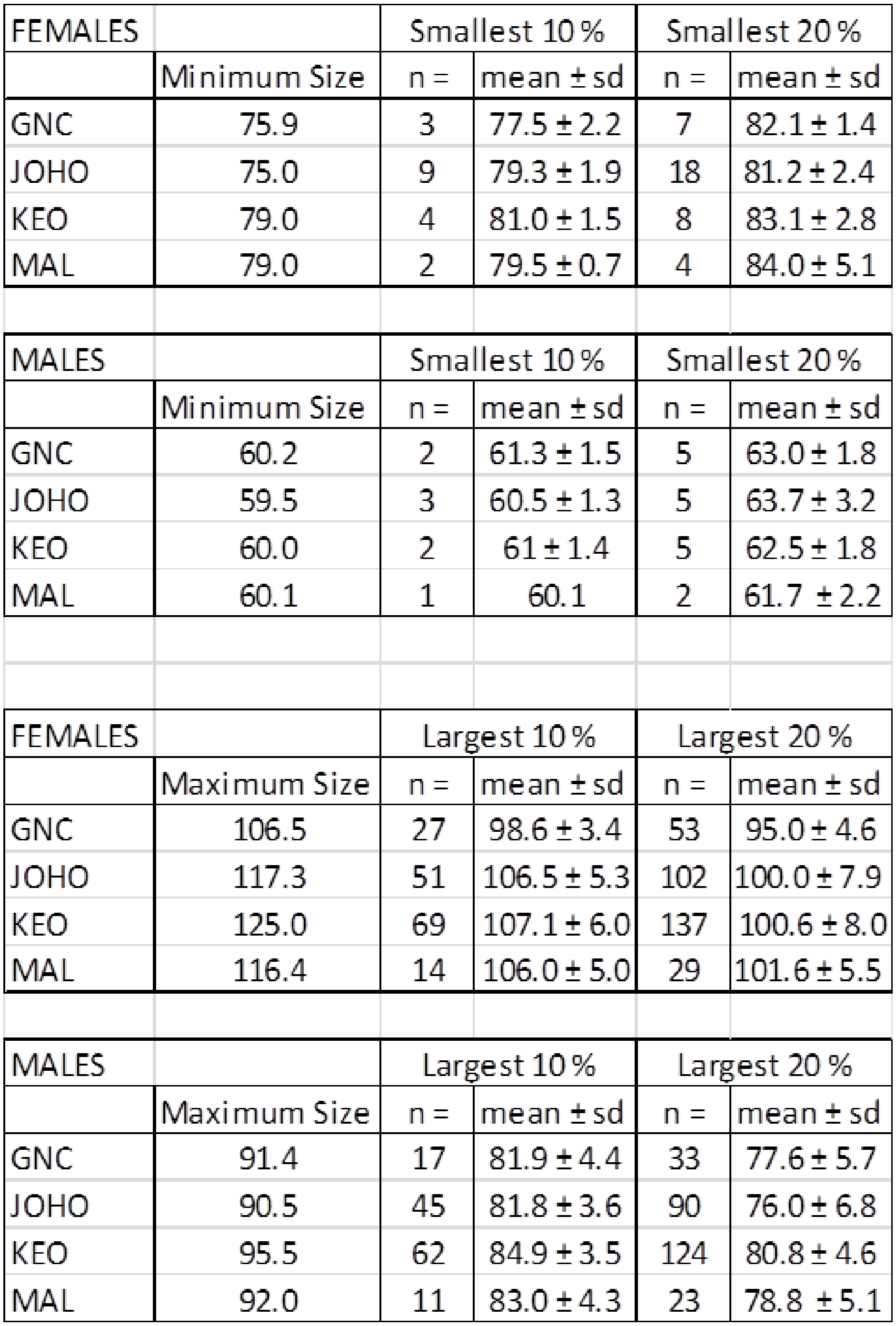


Figure 2 A-D. Panels A and B show the largest 10% and 20% of male SVL from each population, while panels C and D represent the largest 10% and 20% of female SVL from each population. Maximal male size does not differ among populations. However, maximal female size differs among populations, with GNC having a significantly smaller maximal size than JOHO, KEO, and MAL at both 10% and 20%.

Table 1. Summary of the minimum size at maturity, the smallest 10% and 20% of sexually mature males and females, the maximal size, and the largest 10% and 20% of males and females for each population.



FEMALES		Smallest 10 %		Smallest 20 %	
	Minimum Size	n =	mean ± sd	n =	mean ± sd
GNC	75.9	3	77.5 ± 2.2	7	82.1 ± 1.4
JOHO	75.0	9	79.3 ± 1.9	18	81.2 ± 2.4
KEO	79.0	4	81.0 ± 1.5	8	83.1 ± 2.8
MAL	79.0	2	79.5 ± 0.7	4	84.0 ± 5.1

MALES		Smallest 10 %		Smallest 20 %	
	Minimum Size	n =	mean ± sd	n =	mean ± sd
GNC	60.2	2	61.3 ± 1.5	5	63.0 ± 1.8
JOHO	59.5	3	60.5 ± 1.3	5	63.7 ± 3.2
KEO	60.0	2	61 ± 1.4	5	62.5 ± 1.8
MAL	60.1	1	60.1	2	61.7 ± 2.2

FEMALES		Largest 10 %		Largest 20 %	
	Maximum Size	n =	mean ± sd	n =	mean ± sd
GNC	106.5	27	98.6 ± 3.4	53	95.0 ± 4.6
JOHO	117.3	51	106.5 ± 5.3	102	100.0 ± 7.9
KEO	125.0	69	107.1 ± 6.0	137	100.6 ± 8.0
MAL	116.4	14	106.0 ± 5.0	29	101.6 ± 5.5

MALES		Largest 10 %		Largest 20 %	
	Maximum Size	n =	mean ± sd	n =	mean ± sd
GNC	91.4	17	81.9 ± 4.4	33	77.6 ± 5.7
JOHO	90.5	45	81.8 ± 3.6	90	76.0 ± 6.8
KEO	95.5	62	84.9 ± 3.5	124	80.8 ± 4.6
MAL	92.0	11	83.0 ± 4.3	23	78.8 ± 5.1

Table 2. Summary of the post-hoc tests for differences in SVL of the largest 20% and 10% of males and females from each population

		LARGEST 20%			**LARGEST 10%**		
		Mean Difference	**95% Confidence Interval**	**P-value**	**Mean Difference**	**95% Confidence Interval**	**P-value**
FEMALE	JOHO-GNC	4.990	1.799-8.180	0.0004	8.057	4.772-11.341	<0.0001
	KEO-GNC	5.638	2.590-8.686	<0.0001	8.553	5.424-11.681	<0.0001
	MAL-GNC	6.654	2.302-11.006	0.0006	6.630	2.291-10.969	0.0006
	KEO-JOHO	0.648	-1.816-3.112	0.9049	0.496	-2.066-3.058	0.9583
	MAL-JOHO	1.664	-2.301-5.630	0.6995	-1.427	-5.377-2.524	0.7846
	MAL-KEO	1.016	-2.835-4.868	0.9041	-1.923	-5.744-1.899	0.5601
		Mean Difference	**95% Confidence Interval**	**P-value**	**Mean Difference**	**95% Confidence Interval**	**P-value**
MALE	JOHO-GNC	-1.604	-4.546-1.338	0.4946	-0.012	-2.738-2.715	>0.9995
	KEO-GNC	3.279	0.447-6.111	0.0159	2.721	0.102-5.340	0.0384
	MAL-GNC	1.196	-2.731-5.123	0.8602	1.619	-2.187-5.424	0.6857
	KEO-JOHO	4.883	2.881-6.885	<0.0001	2.733	0.844-4.621	0.0014
	MAL-JOHO	2.800	0.578-6.178	0.1424	1.630	-1.715-4.976	0.5844
	MAL-KEO	-2.083	-5.365-1.199	0.3577	-1.102	-4.360-2.155	0.8148

CHAPTER IV

A Comparison of Prey Quality, Quantity, and Assimilation Efficiency among Populations of Watersnakes

Abstract

I sought to characterize the energetic or physiological correlates to the observed life-history divergences among populations of diamond-backed watersnakes occupying fish farms in Lonoke County, AR. These farms share similar structure, climate, and predator regimes, but differ substantially in the species and size of fish they culture. Snakes occupying these farms, consequently, have access to prey species that differ in sizes and densities. It is unknown whether these snakes have become specialized to consuming fish on specific farms by adjusting their digestive physiology. This study was designed to examine the factors driving the observed life-history differences among populations of these snakes. I characterized the species, size distributions, energetic quality and densities for each fish cultured among four fish farms to address the role of these mechanisms in driving these life-history divergences among these populations of snakes. I quantified water content, calcium content, and the energetic-density as measures of prey quality for each species. I then compared energy assimilation efficiency of female snakes from each population to test for dietary specialization. While densities, quality, and species of fish differed among populations, none of these traits were consistent with patterns of life-history divergence. I found little support of digestive adaptation within any fish farm population and therefore argue that differences among these populations are driven from the energetic consequence of eating differing-sized fish.

Introduction

Differences in life-history strategies among populations are a common theme among most taxa. These alternative energy allocation patterns may arise from phylogenetic history, natural selection, or plastic responses to environmental conditions (Angilletta et al 2004; Lande 1982; Shine 2005; Stearns 1992; Zera and Harshmann 2001). Regardless of their cause, the fundamental assumption behind life-history strategies is that organisms prioritize allocation of assimilated energy among the competing functions of growth, reproduction, storage, and self-maintenance (Gadgil and Bossert 1970). Although researchers seldom consider whether energy allocation patterns among populations may diverge due to fundamental differences in the amount of energy available to individuals and their ability to assimilate it (Angilletta 2001; Sun et al. 2006; Zandoña et al 2011). Systemic differences in the quality and abundance of energy resources, as well as population-level differences in individuals' ability to capture, digest, and assimilate those energy resources could profoundly influence life-histories (Angilletta 2001; Ballinger and Congdon 1980; Madsen and Shine 1999; Walsh and Reznick 2008).

Here I evaluate the energetic environment and underlying digestive physiology of a predator shown to have divergent life-histories correlated with differences in prey characteristics. To this end, I estimated prey size, quality, and density of four populations of diamond-backed watersnakes (*Nerodia rhombifer*) occurring at fish farms rearing different species and sizes of fish. I also measured energy assimilation efficiency of adult females to test for a physiological adaptation to local prey.

Snakes were sampled for this study from fish farms that can be characterized as either “large-prey”, with a relatively large average prey size or “small-prey”, with a substantially smaller average prey-size. I have demonstrated that watersnakes occupying large-prey farms reach larger adult body sizes because of greater post-maturational growth, higher relative fat storage, and greater annual reproductive effort with larger relative offspring sizes compared to small-prey populations (Chamberlain 2016, Chapters 2 and 3, Chamberlain unpubl. obs.). But, all populations have similar juvenile field growth rates and ages and sizes at maturity (Chamberlain 2016, Chapter 3). Ostensibly, these life-history divergences are driven by differences in the average size of prey available to each population. Theoretically, however, these differences may also arise from differences in the abundance and quality of prey or differences in assimilation efficiency among populations. In this study I evaluate prey size, prey quality, prey density, and assimilation efficiency as explanatory variables for the patterns of life-history divergence among watersnakes occupying large and small prey fish farms.

Methods

Study sites:

Four fish farms in Lonoke County, AR were part of an on-going study to examine life-history differences in diamond-backed watersnakes. All sites were similar in size, structure, and aquaculture technique but differed in the fish species cultured. Two populations, Gentry and Canterberry Fisheries LLC (GNC) and Pool Fisheries (POOL), were characterized by production of relatively small-bodied goldfish (*Carassius auratus*). Conversely, Keo Fish Farm (KEO) and Joe Hogan State Fish Hatchery (JOHO) specialized in production of relatively large-bodied fish. KEO produced triploid grass carp (*Ctenopharygodon idealla*) and hybrid striped bass (*Morone saxitilis/chrysops*), and JOHO produced several warm-water species including channel catfish (*Ictalurus punctatus*) and black crappie (*Pomoxis nigromaculatus*), with 80% of their production being catfish.

Prey quality and density:

I obtained fish from each fish farm, euthanized them in the laboratory by immersion in MS-222 and froze them for later analysis. Where possible, I analyzed fish species in size classes representative of the average sizes harvested at each site. Each fish farm determined these size classes independently as they are the sizes classes they sell. Because fish were donated from each farm and large fish were substantially more economically valuable, sample sizes among size classes and fish species were not equal. I used a minimum of five and a maximum of ten individuals per species of each size class from each site. From KEO, two size classes of grass carp (small, n =10, and large, n = 6) and three sizes of hybrid striped bass (small, n = 10, medium, n = 10, and large, n = 7) were measured. At JOHO, two size classes of channel catfish (small, n = 10, and large, n = 10) were sampled, whereas black crappie were distributed along a size gradient and therefore not split into any size class (n = 7). Three size classes of goldfish were available from both POOL and GNC and the sample sizes were the same (small, n = 10, medium, n = 10, and large n = 10, total, n = 60). Some overlap in size classes of fish occurred, as growth rate in the ponds was variable. To account for this variation, I measured each fish independently, and used its length as a covariate in my analyses.

Fish were thawed and total wet mass (to the nearest 0.1 g) and total body lengths (to the nearest 0.1 cm) were recorded. I then dried each fish at 60°C to a constant mass, re-weighed, and homogenized them using mortar and pestle or blender, depending on the size of the fish. Homogenized samples were then stored under vacuum to await calorimetry analysis.

I used data each farm provided on fish stocking and harvest per hectare for each species analyzed as a measure of prey density. Each farm also provided data on stocking regimes, average stocking sizes, average harvest sizes, and fish feed used. I quantified prey quality using energetic-density of dried fish, percent water content of fish, and percent calcium content. Energetic-density was estimated from bomb calorimetry using a Parr 1281 bomb calorimeter (Parr Instrument Company, Moline, IL). To obtain energetic-density of each fish, I sub-sampled dried tissue weighing between 0.1 g and 1.0 g and combusted it using a mineral oil spike and a cotton thread fuse. Kilojoules per gram of tissue were then calculated, after correcting for energy release from the spike and fuse. Samples that weighed more than 1.0 g, were sub-sampled and run in duplicate to assure proper homogenization. If duplicate sub-samples differed by more than 2%, they were further homogenized and re-analyzed until they fell within this margin. For samples weighing less than 1.0 g, the entire sample was combusted. The calorimeter was standardized using benzoic acid with a known energetic-density of 26.435 kJ/g. I only ran samples after the calorimeter was calibrated to a 99.999% accuracy, representing less than a 0.07 kJ divergence from the benzoic acid standard. I calculated percent water content as the difference between the total wet mass and the total dry mass, divided by the total wet mass. Since calcium in sub-samples did not combust, I was able estimate percent calcium for each sample burned. I calculated percent calcium by weighing the residual calcium and divided this value by the mass of the dried whole tissue sub-sample. Assuming complete homogenization of the fish sample, this value should reflect the percent calcium in the entire dried fish. I did not collect percent calcium on the small size class of catfish from JOHO, therefore analyses of percent calcium reflect only the large size class for catfish.

Using fish body length as a covariate, I analyzed energy density, percent water content, and percent calcium of each fish species independently by analyses of covariance (ANCOVAs). The presence of significant interaction terms between fish species and body length were used to determine differences in traits of interest. As the focus of this study was to determine whether differences in prey species were associated with life-history differences between large-prey and small-prey farms, goldfish samples from POOL and GNC were pooled for analyses.

Energy Assimilation:

I calculated energy assimilation efficiency as the difference between total energy of fish consumed and total energy present in the excreta and egesta divided by ingested energy (Woods 1982):

$$Assimilation\ Efficiency = \frac{I - (E_g + E_x)}{I}$$

Where I is the total ingested energy and $E_g + E_x$ represent the energy content in the total fecal material that contained both egesta (fecal waste) and excreta (nitrogenous metabolic waste). I used assimilation efficiency, which is a less direct measure of energy extraction from food compared to digestive efficiency, because it is difficult to separate the excreta from the egesta in *N. rhombifer* waste. Therefore, I refer to the combination of egesta and excreta as fecal material. Assimilation efficiency is widely accepted as a valid method of estimating energy extraction from food as excreta represents ingested energy that was not utilized (Woods 1982).

I fed all individuals a fixed ratio of energy to mass of snake to prevent confounding snake body size with prey size. This ratio was determined by dividing the energy in the smallest individual of the largest fish species used in the trial (hybrid striped bass) by the mass of the smallest snake from KEO, which was the only population offered hybrid striped bass. However, due to a shortage of live striped bass, these samples were not used in analyses. This value (0.5 kJ of fish per gram of snake wet mass) represented the lower limit of whole fish that could be offered to the smallest snake. The total energy value offered to each snake was determined by multiplying the wet mass of each female snake by this ratio. To determine the actual mass of whole fish to be offered to each female based on the total energy as estimated above, I first calculated a least-squares linear regression that related the energy per gram of dry weight of fish to grams of fish wet mass separately for each species. I attempted to provide each population of snakes with fish that were raised from their respective farms to account for possible digestive adaptions of snakes to specific prey items. However, because it was not possible to use live catfish, crappie, or striped bass for this analysis, I substituted goldfish (8-10 cm), which had similar energetic densities as small catfish (catfish < 10 cm), as demonstrated in my calorimetry analyses. Site-specific goldfish (8-10 cm) were fed to snakes from POOL and GNC, whereas site-specific grass carp (10 cm) were fed to snakes from KEO.

Forty non-reproductive sub-adult (60 cm<SVL < 75 cm) and adult female snakes (75 cm < SVL< 100 cm) were collected from each site (N = 10 per site) in September of 2015 and housed individually in the laboratory. Only 31 individuals actually fed over the course of the trial (n = 6, 9, 7, and 9 for GNC, JOHO, KEO, and POOL, respectively). I provided females with constant access to water, aspen bedding, and a thermal gradient ranging from 23-32 °C. Snakes were offered size-appropriate goldfish weekly until the beginning of the assimilation trial. The longest any individual was held in captivity before the trials began was approximately 1 month.

Two weeks prior to the start of the trial, I withheld food to clear the digestive tract, thereby ensuring that fecal material collected was produced from the current meal. At the start of the trial, snakes were weighed, measured, and all bedding was removed from cages to facilitate collection of fecal material though water was available throughout the trial. Live fish were weighed, counted, and provided to the snakes in their water bowls. I left fish in cages overnight, then counted and weighed uneaten fish the following morning to estimate the actual mass and energy content of fish consumed by each snake. Because of the necessity of feeding live fish and variation in the size of fish available from each farm, the target mass of fish was often surpassed, but never exceeded more than 125% of the estimated value.

I checked cages twice daily for fecal material, which was collected by scraping the inside of the cage with a razorblade. If I found fecal material in the water bowl, I allowed it settle and decanted off the water until I could collect the pellet. All fecal material was dried to a constant weight at 60°C. The dry weight of the fecal material was measured, homogenized, and stored until calorimetry analysis was performed. I conducted calorimetry of fecal material using the same methods as described above for calorimetry of fish. Sub-samples were run in duplicate and weighed between 0.1 and 1.0 g. I estimated total energetic content of the fecal material by multiplying the dried weight of the fecal material by its energy density.

I tested for differences in assimilation efficiency among populations using analysis of variance (ANOVA) followed by a Tukey's post-hoc comparison to examine pairwise differences among populations. Data were analyzed two ways, first examining populations independently using a one-way ANOVA, and then by nesting populations within their corresponding prey size group (large or small) using a nested ANOVA design. I also tested whether assimilation efficiency scaled with body length differently for each species using an analysis of covariance (ANCOVA) with site as a factor and SVL as a covariate.

Results

Prey quality and density:

Fish densities and average sizes differed based on data provided by each farm for each species in these analyses (Table 1). These values represented estimated average sizes at harvest for each species, rather than an actual sample average, as size varied within a given pond.

Energetic-density scaled differently with body length among fish species (Length*Species: $F_{4,110} = 43.994$, $p < 0.0001$, Figure 1). Energetic-density did not significantly correlate with body length in goldfish ($r = 0.135$, $p = 0.335$) or crappie ($r = -0.230$, $p = 0.6202$) over the size range measured. Energetic-density had a weak negative correlation with body length in striped bass ($r =- 0.410$, $p = 0.0524$) and was strongly negatively correlated with body length in grass carp ($r =- 0.928$, $p < 0.0001$) over the size range measured. Catfish alone showed a strong positive correlation between energetic-density and fish body length ($r = 0.9101$, $p < 0.0001$).

Percent water content also scaled differently with body length among fish species (Length*Species: $F_{4,112} = 14.589$, $p < 0.0001$). Percent water content was not significantly correlated with size in striped bass over the size range I measured ($r =- 0.337$, $p = 0.1158$). However, it was negatively correlated with body length for both goldfish ($r =- 0.271$, $p = 0.0453$) and crappie ($r =- 0.864$, $p = 0.0122$). There was also a strong negative correlation between percent body water and body length for catfish ($r =- 0.825$, $p < 0.0001$). Grass carp showed a weak positive correlation of percent body water to body length ($r = 0.461$, $p = 0.0726$).

Percent calcium content scaled differently with body length among fish species (Length*Species: $F_{4,75} = 6.3639$, $p = 0.0002$). Goldfish and crappie both showed little correlation between percent calcium content and body length (Goldfish: $r =- 0.952$, $p = 0.610$; Crappie: $r = 0.590$, $p = 0.1637$). Percent body calcium positively correlated with body length in both striped ($r = 0.544$, $p = 0.0131$) and grass carp ($r = 0.913$, $p < 0.0001$). Only catfish showed a negative correlation between percent body calcium and body length ($r =- 0.703$, $p = 0.0159$), but this analysis did not include catfish in the smallest size class.

Energy Assimilation:

Assimilation efficiency was transformed by raising it to the fourth power to meet the assumption of normality. I found no difference in assimilation efficiency among sites ($F_{3,27} = 0.2295$, $p = 0.875$), nor by prey type, with site nested within prey type (Prey Type: $F_{1,27} = 0.6443$, $p = 0.84292$; Prey Type*Site: $F_{2,27} = 0.0221$, $p = 0.9781$). However, after running a power analysis on Prey Type, with a sample size of 15 and 16 at an $\alpha = 0.05$, I found this model had very low power at 0.12. I thus ran these same data using a non-parametric Kruskal-Wallis test to adjust for small sample sizes and still did not detect a difference among prey types ($H = 1.6$, $p = 0.2059$). I also tested whether assimilation efficiency scaled with snake body length and found that this relationship did not differ from 0 at any site (SVL: $F_{1,23} = 0.8455$, $p = 0.3674$; SVL*SITE: $F_{3,23} = 0.9653$, $p = 0.4260$), however, this analysis also suffered from low power at 0.15.

Discussion

If prey density drives life-history differences between large-prey and small-prey sites, one might predict that large-prey populations should experience higher prey densities, allowing for their increased adult growth, higher annual reproductive output, and increased fat storage, as these individuals may be able to feed more frequently and fuel increased energy output. While there is a clear difference in the density of fish between small prey sites raising goldfish and large prey sites raising either carp/bass or catfish/crappie, these differences did not explain patterns of life-history. The data suggest that large-prey populations had lower prey densities but higher annual reproductive output and adult growth (Chamberlain 2016, Chapter 2 and 3).

While higher prey densities at small-prey sites results in snakes experiencing prey at higher rates while foraging, any advantage of higher prey density at small prey sites may be offset by the size restriction of the prey. Larger snakes require more energy to maintain the increased demand of a greater tissue mass (Peters 1983). If the maximum energy available from a single prey item is fixed within a population but the body sizes of snakes continues to increase, this requires larger bodied snakes to consume greater numbers of prey items to maintain the same energetic intake as a smaller bodied snake within the same population (Arnold 1993; Schoener 1971). Thus, snakes at small prey sites may be exposed to more goldfish, but larger snakes within these populations must also consume more of them.

Interestingly, while the total number of fish per hectare was an order of magnitude greater at small prey sites, the mass of fish per hectare was similar among sites. I first approximated the average wet mass of the average size class of fish at the time harvest (based on second order polynomial regressions of fish length to wet mass: $R^2 = 0.985$, 0.956, 0.997, 0.989, and 0.998 for catfish, goldfish, grass carp, striped bass, and crappie, respectively, all with $p < 0.001$). I then multiplied this value by the maximum harvest of fish per hectare as estimated by each farm. The resulting values suggest that all farms were on the scale of $1 * 10^6$ grams/hectare. Further, when examining densities of fish per hectare and wet mass of fish per hectare, all fish farms had 1-2 orders of magnitude greater densities of fish per hectare and 1 order of magnitude greater wet mass of fish per hectare than a similar natural body of water in Arkansas (based on 19 years of sampling on Mallard Lake, Arkansas Game and Fish Commission Reports, 1987-2006). Thus, snakes occupying fish farms experience far greater prey densities than the conditions from which they likely originated. At some theoretical prey density, saturation of prey must occur, such that increasing the prey density would not change the foraging success of the predator (Sih 1984). Given the extreme prey densities found on all fish farm sites, this value may be surpassed. It is possible, then, that differences in prey density among fish farms may not result in differences in prey capture, and subsequent energy assimilation, thus negating it as a possible mechanism for life-history differences between large and small prey fish farms.

Patterns of energetic-density against body length of fish vary widely among species, resulting in inter-population differences in prey quality for snakes among fish farm sites. For example, a 10 cm catfish or goldfish had between 16-21 kJ/g dry weight, whereas striped bass and grass carp contained 21-25 kJ/g dry weight. Large prey fish species did not show a consistent pattern of energetic density, where catfish were of poorer quality than striped bass or grass carp. Due to these conflicting patterns, prey quality is unlikely to be the sole explanation for the differences in life-history patterns between large prey and small prey fish farms.

Energetic-density of goldfish did not vary with body length, suggesting that if all feeding costs were equal, a snake eating one 10 g goldfish would receive the same energetic content as eating ten 1 g goldfish. However, catfish at JOHO, a large-prey fish farm exhibited a positive correlation between energetic-density and body size, suggesting that a snake eating one 10 g catfish would receive proportionally more energy than eating ten 1 g catfish. But, at the other large-prey site, KEO, both carp and striped bass exhibited a negative relationship between energy-density and fish body length, suggesting a potential cost to feeding on large prey. Yet, KEO and JOHO exhibited statistically similar patterns of life histories (Chamberlain 2016, Chapter 2 and 3).

In many snake species, including *N. rhombifer*, it is well documented that small prey items are often dropped from the diet as body size increases, particularly in piscivorous species (Arnold 1993; Mushinsky et al. 1982; Plummer and Goy 1984). Arnold (1993), in a review of this phenomenon, suggested that a possible explanation may lie in the energetic cost of finding, handling and digesting prey. He suggested that the total energetic cost to a snake in capturing, handling, and digesting multiple small prey items versus a single large prey item of the same energetic value may result in a lower net energy gain for a snake feeding on multiple smaller fish. Consequently, even snakes at small prey sites should forage on the largest prey items they can handle. Total energetic content of a fish species scales positively with the mass of the prey and thus, even for fish species where the energetic-density decreases with length, it is likely to have only marginal effects on total energetic content. This is a consequence of mass increasing at a cubic rate with body length, whereas energy density scales in a linear fashion with body length. Larger fish within a population, therefore, will provide more total energy, particularly in species that are able to reach larger body sizes or increase in energetic-density with size. This point is clearly demonstrated in Figure 2. At small body sizes all fish have total energetic values between 4-40 kilojoules. However, as large-bodied fish continue to grow, total energetic content jumps 1-3 orders of magnitude at the largest sizes measured, even in striped bass and grass carp where energetic-density decreased with body size.

My research also provides an explanation for the differences in energy-density scaling with body size among fish. By calculating the residuals of energetic-density to body length and regressing these values against the residuals of percent calcium to body length, one can examine the influence of percent calcium on energetic-density for species where energetic-density changes with body length. Calcium provides no energetic value to prey items, so if the percentage of total mass composed of calcium increases as a prey item grows, the energetic-density should decrease. In both grass carp and striped bass, where I had a complete data set for percent calcium in all size classes, there was a strong negative correlation between the residuals of percent calcium content and residuals of energetic-density (Striped bass: $r = -0.569$, $p = 0.0088$; Grass carp: $r = -0.877$, $p < 0.0001$). I predict that had I measured percent calcium in all catfish samples, there would likely be no correlation or a positive correlation between these residual values, as it is likely that percent calcium increases more slowly with body size in catfish because of the absence of calcium-rich scales.

Although my data sets suffer from the constraints of small sample size, they suggest no differences in energy assimilation among populations. Therefore, if I interpret my results as actual measures of patterns in the field, differences in energy assimilation cannot explain differences in life-history strategy among sites. However, small sample sizes are a typical constraint of energy assimilation trials of squamates (Michel and Bonnet 2010; Cox and Secor 2007; Slade et al. 1994). The high degree of variation in assimilation efficiency within and among each population supports that the lack of differences among populations being biologically relevant and not a statistical artifact. If this is the case, then assimilation efficiency may be highly plastic in diamond-backed watersnakes.

I assert that while differences in prey density and quality exist among populations of diamond-backed watersnakes with access to differing sized prey, they are unlikely to fully explain life-history differences observed in the field among these populations. Differences in prey density among populations run counter to the directions necessary to explain life-history differences between large and small prey populations. Prey quality likely accounts for some portion of the differences in life-history between small and large prey populations. However, quality alone is unable to account for all patterns of life-history. Large-prey populations of JOHO and KEO exhibit similar patterns of life-history variation, but the fish species occurring at each these farms differed in the direction of correlation between energetic-density and body length. Further, life-history differences among these sites were not correlated with mean population differences in energy assimilation efficiency. Snakes from all populations acquired a similar percentage of energy from their meals, despite the possibility that the total energetic content differs among these sites. After accounting for differences due to prey density and quality, my data suggest that life-history differences among populations of watersnakes occurring at fish farms arise from differences in maximal prey-size available.

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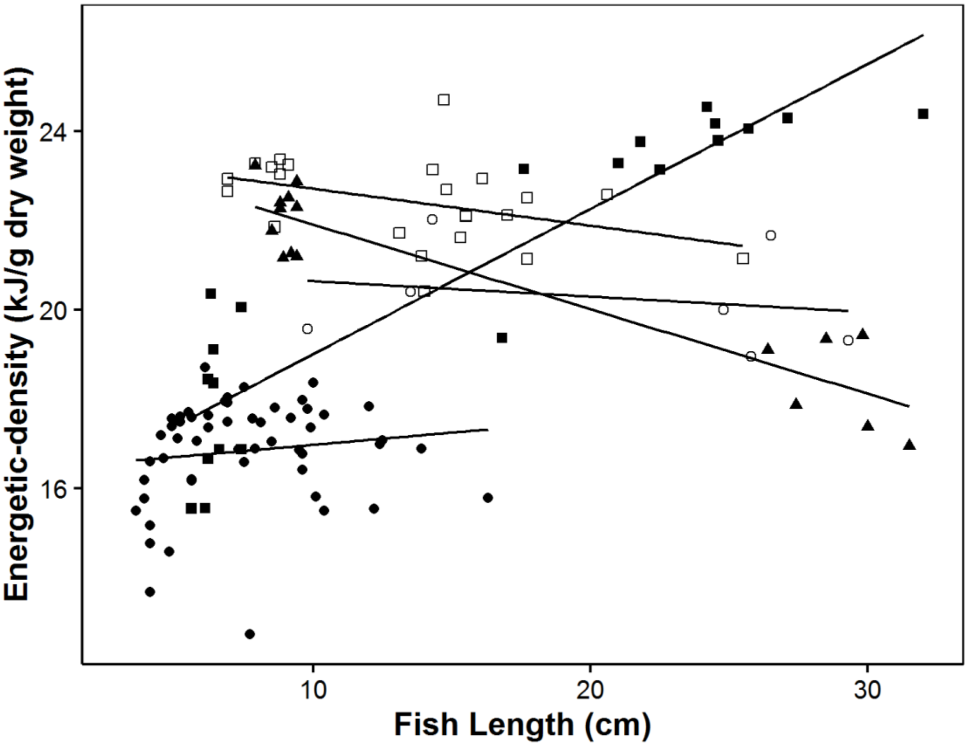

Figure 1. The energetic density of each fish species as it scales with fish body length. Catfish are represented by (■), goldfish by (●), grass carp (▲), striped bass (□), and black crappie (○). Body size does not correlate with energetic density in goldfish or black crappie over the body sizes measured as depicted from their nearly horizontal slopes. Both grass carp and striped bass exhibit a negative correlation between body length and energetic density, both having substantial negative slopes. Catfish alone demonstrate a strong positive relationship between length and energy density with a positive slope.

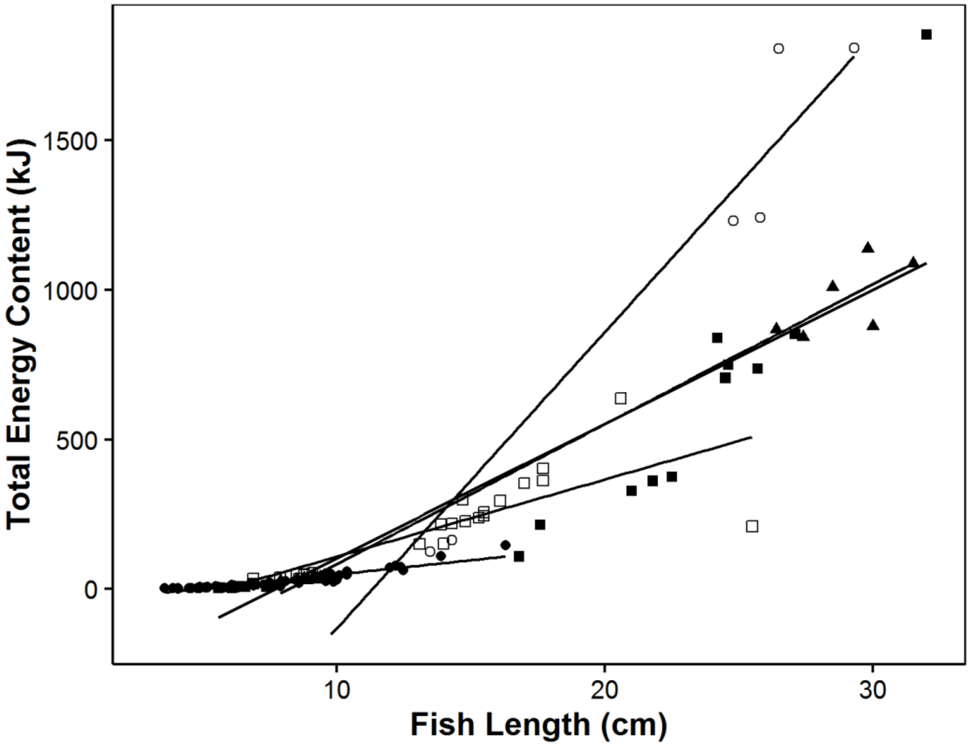

Figure 2. Total energy content of each fish species as it scales with fish body length. Catfish are represented by (■), goldfish by (●), grass carp (▲), striped bass (□), and black crappie (○). Snakes in all populations derive an energetic advantage of eating larger fish, even in fish species where energetic density decreases with body length. Thus, populations with access to larger fish should have a greater energetic input to fuel their life-history.

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 are measured in cm and 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.

Species	**Stocking Size**	**Stocking**	**Harvest Size**	**Harvest**
Goldfish	Fry	250,000-500,000	2.5-5 and 5-7.5	100,000-500,000
Grass Carp	Fry	50,000	10-25	50,000
Striped Bass	Fry	20,000	2.5-25	20,000
Channel Catfish (fingerling)	Fry	10,000	7.5	10,000
Channel Catfish (feeding)	7.5	4000-8000	15-30	4,000-8,000
Black Crappie	Fry	Natural	15-30	Variable