The Mechanisms of a Successful Intraguild Predator

A Dissertation

Presented to the

Graduate Faculty of the

University of Louisiana at Lafayette

In Partial Fulfillment of the

Requirements for the Degree

Doctor of Philosophy

David A. Penning

Fall 2016

The Mechanisms of a Successful Intraguild Predator

David A. Penning

APPROVED:

Brad Moon, Chair Associate Professor of Biology	Paul Leberg Head and Professor of Biology
James Albert Professor of Biology	Daniel Povinelli Professor of Biology
Anthony Herrel Directeur de Recherche Muséum National d'Histoire Naturelle	Mary Farmer-Kaiser Dean of the Graduate School

ACKNOWLEDGEMENTS

In each chapter, I acknowledge the people who helped me with specific aspects of my dissertation as well as the funding sources that made this research possible. In addition to the help that I received with individual projects, I received immeasurable amounts of help, support, and inspiration throughout my education from many friends, colleagues, and mentors.

I want to first thank Brad Moon for providing invaluable resources, insights, and guidance throughout my dissertation. I would not have been able to accomplish what I have without his patience and encouragement. I also thank my committee members, Paul Leberg, James Albert, Daniel Povinelli, and Anthony Herrel. I also want to specifically thank Karen Smith for her help with histology techniques, sharing resources, and for always being willing to help. I also thank Suzanne Fredericq for allowing access to lab equipment.

There are many other people that I would like to thank: Missy Perkins for her help, unrelenting encouragement, and patience; Debbie and Sarah Penning for their constant support and willingness to help and listen; Ivan Moberly and Michael Fulbright for being excellent lab mates and friends. Landon Jones, Baxter Sawvel, and Tim Sullivan made my experience in the program more enjoyable and provided valuable advice. I also received help and support from project collaborators CJ Hillard and Schuyler Dartez.

For research funding for both my dissertation and side projects during my time at UL Lafayette, I thank the American Society of Ichthyologists and Herpetologists, Blink Films, BhB Reptiles, the Gans Collections and Charitable Fund, the Herpetologists’ League, the Kansas Herpetological Society, the Louisiana Board of Regents Doctoral Fellowship, Louisiana’s Rockefeller State Wildlife Fund, Miles of Exotics, the Society for the Study of Amphibians and Reptiles, the UL Lafayette Department of Biology, the UL Lafayette Ecology

Center, the UL Lafayette Graduate School, the UL Lafayette Graduate Student Organization, and the World Congress of Herpetology. I also thank the National Science Foundation and National Geographic for grants awarded to Brad Moon.

For access to animals and specimens, I thank Michael Fulbright, Nick Haertle, Katrina Hucks, Travis LaDuc, Travis Lyon, Brad Moon, Mark Miles, Landon and Ivan Moberly, Simon Pecnik, Debbie Penning, Missy Perkins, Marshall McCue, and the Kansas Herpetological Society.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I: Introduction

Predator–prey relationships are among the many kinds of interactions in nature, and involve complicated situations that come at a cost to both predators and prey (Gans, 1986; Lima, 2002). Body size plays a very important role in these interactions (Schmidt-Nielson, 1984; Cohen et al., 1993), and there is a general trend in predator–prey relationships in which the predator is larger than the prey (Arnold, 1993; Radloff and Du Toit, 2004). This size relationship is observed across ecosystems and trophic levels (Cohen et al., 1993). As many predators increase in size, they typically feed on larger prey (Vezina, 1985; Arnold, 1993). However, when prey begin to increase in size relative to their predators, they become less vulnerable to predation (Magalhaes et al., 2005). Only approximately 10% of predator–prey interactions have predators that are smaller than their prey (Cohen et al., 1993).

Looking through the lens of intraguild predation, the same trend in predator–prey size is apparent. Intraguild predation is the killing and eating of competitors that feed on many of the same organisms as the intraguild predator (Polis et al., 1989). Trophic specialization and relative body size are the two primary factors that influence intraguild predation, with the predator usually being larger than the prey (Polis et al., 1989; Lucas et al., 1998). The size difference between intraguild combatants has been well documented in both laboratory (Samu et al., 1999) and field studies (Donadio and Buskirk, 2006) across a diversity of intraguild competitors. For example, in 3,826 aquatic invertebrate interactions, the predator was always larger than the prey (Woodward and Hildrew, 2002). The same pattern has been observed in spiders (Wise, 2006), fish (Persson et al., 2004), salamanders (Gustafson, 1993), and in 97 pairwise interactions of mammalian carnivores (Palomares and Caro, 1999). Intraguild predation has been found to be more likely to succeed when body size differs between combatants by a factor of two (Palomares and Caro, 1999) to four (Buskirk, 1999).

Predator–prey interactions are typically investigated from an evolutionary, ecological, or behavioral perspective. Very little attention has been aimed at the functional morphology of predator–prey mechanisms during a predation event (Webb, 1986). Upon capture, the predator–prey bout is usually one-sided and over quickly, typically because the prey is small. Larger predators typically face fewer challenges with smaller prey (i.e., lizard vs. insect, raptor vs. rodent, etc.); the predators are limited morphologically (e.g., a large constricting snake could not physically place coils around a small animal) rather than energetically (Pough and Andrews, 1985). However, when the predator and prey are closely matched in size (and in offensive/defensive mechanisms), an attack can bring such a high risk to the predator that it may be avoided entirely even if the benefits of successful predation would be high (Donadio and Buskirk, 2006).

Few animals prey upon others that are of equal or greater size (Cohen et al., 1993), and even fewer do so with intraguild prey (Palomares and Caro, 1999). However, this is known to occur in several species of snakes (Greene, 1997; Ernst and Ernst, 2003; Jackson et al., 2004). Kingsnakes (genus *Lampropeltis*) are non-venomous, powerful constrictors who feed on a variety of different sizes and types of prey (Ernst and Ernst, 2003). Kingsnakes are resistant to the effects of viper venom (Weinstein et al., 1992), and their presence in habitats can affect the distribution and abundance of other snakes (Steen et al., 2014). In addition to feeding on vipers, kingsnakes feed on other snake-eating snakes and other constrictors (Ernst and Ernst, 2003), including constricting snakes that are larger than themselves (Jackson et al., 2004). In this scenario, the predator can be smaller than the prey and can still defeat the prey with the same mechanism that the prey use on their own prey.

The objective of my dissertation research was to better understand the link between muscle-level and whole-body anatomy, size, and performance as they relate to predator–prey interactions involving kingsnakes. I investigated predatory and defensive performance at the whole-body level and contractile performance at the individual muscle level. By studying key performance variables that are potentially important to predator–prey interactions, I hoped to identify and quantify the mechanisms of a seemingly paradoxical predator–prey interaction.

In Chapter 2, I quantified the effects of prey size and repeated feeding on constriction performance in thirty eastern kingsnakes (*Lampropeltis getula*) feeding on pre-killed rodents (*Mus musculus* and *Rattus norvegicus*). I measured the length of the constriction coil and peak constriction pressure during predation events. I found that prey size alone does not affect constriction performance, but when prey size is coupled with the effects of repeated feeding, performance can be significantly reduced in subsequent predation events.

In Chapter 3, I quantified peak constriction pressure as a measure of predation performance (n=182), pulling force as a measure of defensive (escape) performance (n=98), and the cross-sectional area of axial musculature (n=36) in six species of snakes (3 species of *Lampropeltis* and 3 species of *Pantherophis*). Axial musculature scaled similarly for all snakes, and there was no significant difference in defensive performance among species. However, all kingsnakes exerted significantly higher pressures on their prey (predation performance) than all ratsnakes (competitors and an intraguild prey). The similar defensive performance among species indicates that kingsnakes win in predatory encounters with ratsnakes because of their superior predation performance, not because ratsnakes have inferior defensive performance. The superior constriction performance by kingsnakes derives at least in part from their consistent and distinctive coil posture.

In Chapter 4, I described and quantified the muscle anatomy of two species of colubrid snakes, speckled kingsnakes (7 *Lampropeltis holbrooki*) and western ratsnakes (9 *Pantherophis obsoletus*). In addition to qualitative descriptions, I quantified the physiological cross-sectional area (PCSA) of nine muscles from each snake. I also compared the PCSA values to previous values of anatomical cross-sectional area (ACSA). There was no significant difference in PCSA of muscles between kingsnakes and ratsnakes. There was, however, a strong relationship between ACSA and PCSA measurements. I could not identify a significant difference in musculature between kingsnakes and ratsnakes that explains their different levels of constriction performance. Unmeasured components of muscle function, such as endurance and force production might account for differences in performance among two species with similar muscle structure.

Lastly, in Chapter 5, I quantified and compared the maximum isometric force *in vivo* and two measures of endurance in two of the largest epaxial muscle groups (semispinalis–spinalis complex and longissimus dorsi) between kingsnakes (8 *Lampropeltis holbrooki*) and ratsnakes (8 *Pantherophis obsoletus*). There was no significant difference in maximum isometric force or both measures of endurance between muscles or species. The results from all chapters together indicate that kingsnakes are able to produce significantly higher constriction pressures because of their consistent coil posture (behavior) and not because of differences in their muscle anatomy or physiology. Integrated studies of behavior and its underlying mechanisms, such as in these chapters, are critical to making strong inferences about relationships in predator–prey interactions and their outcomes.

Literature Cited

Arnold, S. J. (1993). Foraging theory and prey-size–predator-size relations in snakes. In *Snakes Ecology and Behavior* (ed. R. A. Seigel and J. T. Collins), pp. 87–115. New Jersey: The Blackburn Press.

Buskirk, S.W. (1999). Mesocarnivores of yellowstone. In *Carnivores in Ecosystems: The Yellowstone Experience* (ed. T. Clark, A. Curlee, S. Minta and P. Karieva), pp. 165–187. London: Yale University Press.

Cohen, J. E., Pimm, S. L., Yodzis, P. and Saldana, J. (1993). Body sizes of animal predators and animal prey in food webs. *J. Anim. Ecol.* **62**, 67–78.

Donadio, E. and Buskirk, S. W. (2006). Diet, morphology, and interspecific killing in Carnivora. *Am. Nat.* **167**, 524–536.

Ernst, C. H. and Ernst, E. M. (2003) *Snakes of the United States and Canada*. Washington DC: Smithsonian Institution.

Gans, C. (1986). Functional morphology of predator-prey relationships. In *Predator–Prey Relationships: Perspectives and Approaches from the Study of Lower Vertebrates* (ed. M. E. Feder and G. V. Lauder), pp. 6–23. Chicago: The University of Chicago Press.

Greene, H. W. (1997). *Snakes: The Evolution of Mystery in Nature*. Berkeley: University of California.

Gustafson, M. P. (1993). Intraguild predation among larval Plethodontid salamanders: A field experiment in artificial stream ponds. *Oecologia* **96**, 271–275.

Jackson, K., Kley, N. J. and Brainerd, E. L. (2004). How snakes eat snakes: the biomechanical challenges of ophiophagy for the California kingsnake, *Lampropeltis getula californiae* (Serpentes: Colubridae). *Zoology* **107**, 191–200.

Lima, S. L. (2002). Putting predators back into behavioral predator–prey interactions. *Trends Ecol. Evolut.* **17**, 70–75.

Lucas, E., Coderre, D. and Brodeur, J. (1998). Intraguild predation among Aphid predators: characterization and influence of extraguild predation. *Ecology* **79**, 1084–1092.

Magalhaes, S., Janssen, A., Montserrat, M. and Sabelis, M. W. (2005). Prey attack and predators defend: counterattacking prey trigger parental care in predators. *Proc. R. Soc. B.* **272**, 1929–1933.

Palomares, F., and Caro, T. M. (1999). Interspecific killing among mammalian carnivores. *Am. Nat.* **153**, 494–508.

Persson, L., Claessen, D., De Roos, A. M., Bystrom, P., Sjogren, S., Svanback, R., Wahlstrom, E. and Westman, E. (2004). Cannibalism in a size-structured population: Energy extraction and control. *Ecol. Monogr.* **74**, 135–157.

Polis, G. A., Myers, C. A. and Holt, R. D. (1989). The ecology and evolution of intraguild predation: potential competitors that eat each other. *Annu. Rev. Ecol. Evol. Syst.* **20**, 297–330.

Pough, F. H. and Andews, R. M. (1985). Energy costs of subduing and swallowing prey for a lizard. *Ecology* **66**, 1525–1533.

Radloff, F. G. and Du Toit, J. T. (2004). Large predators and their prey in a southern African savanna: a predator’s size determines its prey range. *J. Anim. Ecol.* **73**, 410–423.

Samu, F., Toft. S. and Kiss, B. (1999). Factors influencing cannibalism in the wolfspider *Pardosa agrestis* (Araneae, Lycosidae). *Behav. Ecol. Sociobiol.* **45**, 349–54.

Schmidt-Nielsen, K. (1984). *Scaling: Why is Animal Size So Important?* Cambridge: Cambridge University Press.

Steen, D. A., McClure, C. J. W., Sutton, W. B., Rudolph, D. C., Pierce, J. B., Lee, J. R., Smith, L. L., Gregory, B. B., Baxley, D. L., Stevenson, D. J. and Guyer, C. (2014). Copperheads are common where kingsnakes are not: relationships between the abundances of a predator and one of their prey. *Herpetologica* **70**, 69–76.

Vezina, A. F. (1985). Empirical relationships between predator and prey size among terrestrial vertebrate predators. *Oecologia* **67**, 555–565.

Webb, P. W. (1986). Locomotion and predator–prey relationships. In *Predator–Prey Relationships: Perspectives and Approaches from the Study of Lower Vertebrates* (ed. M. E. Feder and G. V. Lauder), pp. 24–41. Chicago: The University of Chicago Press.

Weinstein, S. A., DeWitt, C. F. and Smith, L. A. (1992). Variability of venom-neutralizing properties of serum from snakes of the colubrid genus *Lampropeltis*. *J. Herpetol.* **26**, 452–461.

Wise, D. H. (2006). Cannibalism, food limitation, intraspecific competition, and the regulation of spider populations. *Annu. Rev. Entomol.* **51**, 441–465.

Woodward, G. and Hildrew, A.G. (2002). Body-size determinants of niche overlap and intraguild predation within a complex food web. *J. Anim. Ecol.* **71**, 1063–1074.

CHAPTER II: The Gluttonous King: The Effects of Prey Size and Repeated Feeding on Predatory Performance in Kingsnakes

DAVID A. PENNING[1,2,3]

[1]Department of Biology, University of Louisiana at Lafayette, Lafayette, Louisiana, USA 70504- 43602

[2]Department of Biology and Environmental Health, Missouri Southern State University, Joplin, Missouri, USA 64801

[3] Corresponding author: David Penning, Department of Biology and Environmental Health, Missouri Southern State University, Joplin, Missouri, USA 64801, *davidapenning*@gmail.com

Abstract

Constriction is an evolutionarily and functionally important behavior that many snakes use to subdue a variety of prey. However, little work has examined the effects of prey size on constriction performance. Furthermore, many snakes are known to feed opportunistically even while previously consumed prey remain in the stomach. This temporary increase in mass may place constraints on subsequent predation performance. To test these effects, I investigated constriction performance in eastern kingsnakes (*Lampropeltis getula*) handling different sizes and quantities of rodent prey in two experiments by measuring coil length and peak constriction pressure. In the first experiment, constriction coil length and peak constriction pressure did not differ significantly between snakes feeding on either small (5% relative prey mass, rpm) or large (15% rpm) rodent prey. However, there was a significant interaction between prey size and repeated feeding. Snakes that had previously consumed small prey used similar coil lengths and peak constriction pressures on their second feeding compared to their first, but snakes that previously consumed large prey had significantly shorter coil lengths and lower peak constriction pressures when they fed for a second time (reductions of 60% and 51% respectively). In a second experiment, snakes were offered five sequential similarly sized prey (ca. 7% rpm). The snakes showed a regular decrease in coil length and peak constriction pressure across repeated feedings. During their final (fifth) feeding, snakes in the second experiment were using 45.7% shorter coils and exerting 50.1% lower peak constriction pressures compared to their first feeding. The results from both experiments showed that prey size alone did not affect constriction performance. However, predation performance was significantly affected by the prior consumption of prey ≥ 7% RPM in kingsnakes, and performance was further reduced during additional feedings.

Key Words: Constraint, Constriction, Feeding, *Lampropeltis*, Predation, Pressure, Snake

Introduction

Both predators and prey have morphological, physiological, and behavioral mechanisms for increasing their predation or escape success (Darwin, 1859; Dawkins & Krebs, 1979; Emerson, Greene, & Charnov, 1994). However, these mechanisms are subject to trade-offs (Zera & Harshman, 2001; Ings and Chittka, 2008) and constraints (Gould, 1980; Wainwright, 1988). For example, increased cranial kinesis in snakes simultaneously allowed for the ingestion of large prey (Gans, 1961), while likely reducing bite force (Frazzetta, 1970; Greene, 1983). Therefore, the ability to subdue large prey required additional behaviors and mechanisms such as constriction or venom (Greene, 1997; Cundall & Greene, 2000). Constriction behavior is a key innovation in the evolution and radiation of snakes (Greene & Burghardt, 1978; Greene, 1997), is integral to their feeding biology (Moon, 2000), and is therefore an evolutionary and functionally important predation mechanism (Greene & Burghardt, 1978; Penning & Dartez, 2016).

Many constricting snakes feed on a wide range of relative prey sizes (Greene, 1997; Arnold, 1993; King, 2002; Jackson et al., 2004) and are known to modify their prey-handling behaviors in response to different prey. For example, larger and more active prey are typically constricted whereas smaller prey may be seized and eaten alive (de Queiroz, 1984; Mehta, 2003, 2009). It is currently hypothesized that larger prey may take longer durations and greater strengths to subdue (Moon, 2000), requiring more of the body to be used during constriction (Hisaw & Gloyd, 1926). Moreover, larger prey may be less susceptible to constriction mechanisms (Hardy, 1994).

In addition to variations in prey size, snakes can encounter variations in prey quantity. Both terrestrial (Hisaw & Gloyd, 1926; Cunningham, 1959) and aquatic snakes (Lillywhite, 2014) are known to consume multiple prey in a single feeding. When a snake feeds, it quickly alters its total body mass (Cundall & Greene, 2000; Jackson, Kley, & Brainerd, 2004). Added body mass can leave a snake more vulnerable to predators by potentially reducing predator evasion (Shine, 1980; Mehta, 2006), sprint speed (Ford & Shuttlesworth, 1986; Mehta, 2006), endurance (Ford & Shuttlesworth, 1986; Herzog and Bailey, 1987), and can alter antipredator displays (Mehta, 2006; Herzog & Bailey, 1987). In addition to these consequences, the presence of extra mass in a snake's digestive tract is likely to affect subsequent predation performance. The previously ingested food may cause changes in mobility and possibly interfere with and limit axial bending. Limitations in axial bending would likely reduce constriction performance when a snake encounters subsequent prey. Despite the wide range of relative prey sizes taken by constricting snakes, and the importance of constriction in the evolution of snakes (Greene & Burghardt, 1978; Greene, 1983), investigations on the effects of prey size and repeated prey encounters on constriction performance are lacking. I conducted two experiments using eastern kingsnakes (*Lampropeltis getula*). Experiment 1 combined the effects of prey size, repeated feeding, and their interaction on constriction performance. Experiment 2 was an experimental test based on field observations of snakes that had fed on multiple prey items (Hisaw & Gloyd, 1926; Cunningham, 1959; Lillywhite, 2014), and quantified the effects of five sequential feedings of similarly sized prey on constriction performance.

Materials and Methods

For all experiments, each snake was housed individually and was provided water *ad libitum*, with light provided on a 12:12h cycle. Snakes were fasted for two weeks before trials but were otherwise fed pre-killed rodents weekly.

For Experiment 1 (see below), I used 20 kingsnakes (*Lampropeltis getula*; mass=102–810 g; snout-vent length [SVL]=50.4–120.4 cm). Experiment 1 tested the effects of prey size, repeated feeding, and their interaction on constriction performance. I randomly divided 20 snakes into two groups and assigned them to either the “small” prey category (5% relative mass) or the “large” prey category (15% relative mass). Body mass was not significantly different between the two groups of snakes (t_{18}=0.12, P>0.9). I offered each snake its first prey item (pre-killed *Mus musculus* or *Rattus norvegicus*) with an attached pressure sensor (0.5 or 2.0 ml, fluid-filled bulb) connected to a digital pressure transducer (Harvard Apparatus Pressure Transducer, Model 60-3002). I attached the sensor to the prey with wax-coated string. Upon presentation, all snakes readily struck at and constricted their prey. During constriction, I systematically shook the prey with forceps to elicit maximum constriction effort (Moon, 2000; Penning & Dartez, 2016), and I recorded the peak constriction pressure exerted on the prey. I did not record the durations of feeding bouts because the simulated prey movements affected the prey-handling durations. Once exertion began to decline (indicated by a gradual drop in pressure), I marked the beginning and end of the constriction coil with small pieces of tape. After marking the coil length, I clipped the string that held the pressure sensor to the prey and pulled the sensor out of the coil. When the snake loosened its coil and straightened its body, I took an overhead photo with a scale grid in view. I measured coil lengths using ImageJ. Immediately after the first feeding trial, I offered each snake a second prey item (of the same size as the first one) and repeated the procedure described above. Because I had to physically handle snakes to remove the sensor (and disturb their feeding), I did not measure ingestion durations.

For Experiment 2, I used 10 medium-sized kingsnakes (mass=105.3–171.4 g; SVL=57.1–73.9 cm). I used snakes and mice of specific sizes to measure changes in constriction performance during an ecologically relevant but dynamic predator–prey scenario. If a wild snake finds a mammal nest, it will likely feed on multiple prey; documented cases of 3–5 rodents within a snake's stomach are common (Hisaw & Gloyd, 1926; Cunningham, 1959). I presented each snake with five sequential mice of a size that would naturally be found in nesting groups (6.1–11.5 g; Schwartz & Schwartz, 1981). These mice represented a meal of ca. 7% relative snake mass (range=5.7–8.9%). Based on prey length (snout–tail base) and snake length (SVL), each prey spanned 6.6–11.2 % of each snake's SVL. I controlled prey mass to within 1 g for each snake, and later confirmed that there was no significant difference in prey mass across trials ($F_{4,6}$=1.4, P>0.26). For every sequential prey item, I recorded coil length and maximum constriction pressure following the methods described for Experiment 1.

Statistical Analyses

I log-transformed all data for all models. For Experiment 1, I used a multivariate analysis of variance for each dependent variable, with feeding event (1st or 2nd) as the within-subjects independent variable and prey size as the between-subjects independent variable. If an interaction was significant, I reported Tukey's post-hoc results for simple main effects (*P*-values based on the Studentized Range Statistic; Hammer et al., 2001). For Experiment 2, I used a one-way repeated-measures ANOVA for each dependent variable, with feeding event (1st through 5th) as the independent variable. If significant, I reported Tukey's post-hoc tests. I used Past 3.08 and JMP Pro (11.00.0) software for analyses and considered the results significant at P<0.05.

Results

In all experiments, kingsnakes readily accepted and constricted their prey by winding (Greene & Burghardt, 1978). For Experiment 1, all snakes constricted and consumed both prey, showing no signs of reduced interest during the second feeding. The kingsnakes quickly struck at and constricted their prey using 1–3 loops in their coils, resulting in coil lengths of 10.7–84.2 cm and peak constriction pressures of 6.5–32.7 kPa (kilopascals). There was a significant interaction effect between prey size (5% and 15% rpm) and repeated feedings (1^{st} and 2^{nd} prey) on coil length used ($F_{1,18}$=4.6, P<0.047; Fig. 1.1A). On the first feeding, there was no significant difference in coil length between the two prey-size groups (P>0.98). Additionally, for snakes feeding on small prey, there was no significant difference between coil lengths used during the first (45.7±7.2 cm) and second (32.9±3.9 cm) feedings (P>0.17). However, for snakes feeding on large prey, there was a significant difference between the coil lengths used during the first (46.6±7.3 cm) and second (18.6±2.8 cm) feedings (P<0.001; Fig. 1.1A, 1.2). The coil lengths used by snakes constricting a second large meal were significantly shorter than those used in all other trials (all P<0.001). In most cases, new constriction coils stopped where a prey bulge was visible (Fig. 1.2). During the second feeding on large prey, coil lengths were reduced by 60.1% of their original length. This large reduction in coil length is the cause of the significant interaction.

Peak constriction pressures followed the same pattern as coil lengths (Fig. 1.1) in Experiment 1. There was a significant interaction effect between prey size and repeated feedings on peak constriction pressure ($F_{1,18}$=18.1, P<0.001; Fig. 1.1B). On the first feeding, there was no significant difference in peak constriction pressure between the two prey-size groups (P>0.84) or between the first (23.7±1.8 kPa) and second (18.42±1.1 kPa) feedings by the small prey group (*P*>0.06). However, for snakes feeding on large prey, there was a significant difference between the peak constriction pressures during the first (22.5±2.3 kPa) and second (11.0±1.1 kPa) feedings (*P*<0.001). The peak constriction pressures used by snakes constricting there second large meal were significantly lower than those used in all other trials (all *P*<0.001). Similar to the results for coil length used, the significant reduction in peak constriction pressure by snakes feeding on large prey for the second time is the cause of the significant interaction.

For Experiment 2, all snakes constricted and consumed all five prey. Across the five repeated feedings, snakes showed signs of reduced interest in prey and no snake accepted a sixth prey item when offered. Coil length was significantly different across five sequential feedings ($F_{4,6}$=16.0, *P*<0.01; Fig. 1.3A) with more of the body being used in the first feeding (29.9±3.1 cm) compared to all others (all pair-wise comparisons *P*<0.05). Coil length was also significantly shorter in feedings four (17.4±1.6 cm) and five (14.7±1.2 cm) compared to the second feeding (22.3±1.8 cm; *P*<0.05). Coil lengths used in feedings three, four, and five were not significantly different from one another (all *P*>0.05). During feeding three, snakes were using 35% shorter coils compared to their first prey and during their fifth feeding, they used less than half their initial coil lengths (49.2%). Repeated feedings affected peak constriction pressure in the same manner as coil length. Peak constriction pressure was significantly different across five sequential feedings ($F_{4,6}$=19.8, *P*<0.01; Fig. 1.3B) with pressures being highest during the first feeding (24.7±2.3 kPa) compared to all others (all *P*<0.05). Peak constriction pressure was also significantly lower in feedings four (12.5±1.5 kPa) and five (13.4±1.5 kPa) compared to the second feeding (17.5±1.9 kPa). Peak constriction pressures in feedings three, four, and five were not significantly different from one another (all *P*>0.05).

By feeding three, snakes were exerting 36% lower peak constriction pressures compared to their first prey and during their fifth feeding, peak constriction pressure was reduced by 46% compared to their initial performance.

Contrary to the observations of Willard (1977), most of the coils (in both experiments) involved the snake's venter facing towards the head (Fig. 1.2) instead of the dorsum. Regardless of the coil posture, many of the trials involved high peak constriction pressures. In Experiment 1, peak constriction pressures were similar to those shown to cause circulatory arrest in rodents (20.8 kPa; Boback et al., 2015) for snakes handling their first (23.7±1.8 kPa, 1-sample t_9=1.62, P>0.13) and second (18.4±1.1 kPa, t_9=2.0, P>0.06) small prey as well as snakes handling their first large prey (22.5±2.3 kPa, t_9=0.72, P>0.47). In Experiment 2, kingsnakes exerted similarly high pressures during the first two sequential prey encounters (24.0 ±2.2 kPa, t_9=1.5, P>0.17; 17.4 ±1.7 kPa, t_9=2.0, P>0.08 respectively) but constriction performance steadily dropped thereafter.

Discussion

Constriction is an important predation technique that may be affected by aspects of prey size and repeated prey encounters that have gone untested. The results of these experiments are some of the first quantitative tests of several assumptions of predator–prey dynamics involving constricting snakes, variations in prey size, and repeated encounters with prey.

Almost a century has passed since Hisaw & Gloyd (1926) stated that constricting snakes increase the size of the coil in proportion to prey size. Subsequent work has not addressed this observation, or has been unable to systematically control relative prey size (Moon and Mehta, 2007; Penning et al., 2015; Penning and Dartez, 2016). In contrast to the statement of Hisaw & Gloyd (1926), kingsnakes did not increase their coil lengths with increasing prey size. Kingsnakes used similar coil lengths on both small and large prey. Previous work has shown that the number of loops used in a coil (e.g., coil length) may (Moon and Mehta, 2007) or may not (Penning et al., 2015; Penning and Dartez, 2016) affect peak constriction performance for a variety of snakes (mainly pythons). Kingsnakes in both of my experiments showed the same patterns of variation in both coil length and peak constriction pressure. Constriction events involving significantly shorter coils had significantly lower peak constriction pressures (Fig. 1.1, 1.3). For kingsnakes, using more of the body in a constriction coil results in higher peak constriction pressure.

When snakes fed on a second large prey item (Experiment 1) or continued to encounter medium-sized prey (Experiment 2), they showed decreases in predation performance. At least three factors could cause a reduction in constriction performance during sequential encounters with prey. First, snakes may be experiencing fatigue during sequential feedings. However, all snakes experienced similar levels of prey struggling and similar constriction durations. If the snakes were fatigued, they should have all been experiencing similar levels of reduced performance because their constriction bouts were similar, regardless of prey size. Furthermore, when snakes were fed small prey (5% rpm, Experiment 1), performance with the second prey item was as high as the first, suggesting that fatigue is not a primary factor. Second, snakes may be learning what pressures are sufficient for sequential prey items and reducing their subsequent performance. Constriction is an energetically expensive behavior (Canjani et al., 2003; Penning and Dartez, 2016), and modulating constriction effort may reduce the energy necessary to kill prey (Penning et al., 2015). However, many constricting snakes will respond strongly to prey movement (Willard, 1977) by increasing constriction pressure and constricting for longer durations (Moon, 2000; Boback et al., 2012), and snakes feeding on small prey in Experiment 1 maintained similar coil lengths and high constriction pressures on subsequent prey. Third, the previously ingested prey may physically constrain axial flexion, coil length, and peak constriction pressure. Prey are somewhat compliant and malleable but their presence within a snake’s stomach probably makes movement more difficult. In most cases, the constriction coil stopped where a prey bulge was visible (Fig. 1.2) and sequentially adding new prey to the stomach increased the length of body that was not being used during subsequent constriction events. This constraint on coil length led to significant decreases in peak constriction pressure and followed similar patterns in both experiments. During the final prey encounters of Experiment 2, snakes contained four prey items within their stomachs, representing 27.9–42.0% of SVL (24.2–33.6% mass), coil length was reduced by 34.6–74.8% compared to their first prey encounter, and peak constriction pressure dropped by 28.4–68.8%.

Relative prey size is known to affect aspects of prey-handling (Mehta, 2003; 2009) and the way the coil is applied/formed in snakes (Greenwald, 1978; Moon, 2000). Moon and Mehta (2007) stated that constriction performance was also likely to vary with relative prey size. Furthermore, Hardy (1994) stated that relative prey size may play an important role in determining the proximate mechanism of death during constriction, with larger prey being killed by suffocation rather than circulatory arrest. However, on the first encounter with prey, kingsnakes exerted similar pressures on both small and large prey, regardless of relative prey size (Fig. 1.1B). These pressures were similar to the pressures shown to cause rapid incapacitation via circulatory arrest on prey of much larger absolute size (ca. 400g *Rattus rattus*; Boback et al. 2015).

When variation in prey size was coupled with the effects of repeated feeding, subsequent constriction performance was greatly reduced. The peak constriction pressures for several trials were significantly lower than those shown to cause rapid incapacitation via circulatory arrest (Boback et al. 2015). However, the lowest constriction pressures (mean range=11.0–15.36 kPa) were similar to the arterial blood pressure of rodents (10.0–16.7 kPa; Turney & Lockwood, 1986), and circulatory arrest can be caused by pressures high enough to interfere with venous blood flow (Moon and Mehta, 2007; Boback et al., 2015), which is much lower than arterial blood pressure (Hardy, 1994; Moon, 2000). Therefore, these reduced constriction pressures are likely still high enough to significantly impair circulatory function by stopping venous return (Moon, 2000; Moon & Mehta, 2007; Boback et al., 2015).

In this study, prey size alone did not affect constriction performance in kingsnakes. However, constriction performance was affected by the interaction between prey size and feeding encounter. When kingsnakes fed on additional prey (7–15% rpm) after their first encounter, they experienced significant reductions in predation performance. However, with only a few repeated feedings, these reductions in performance may not change the effectiveness of constriction or the mechanisms by which the prey are incapacitated.

Acknowledgements

I thank M. Fulbright, I. Moberly, B. Moon, M. Perkins, and L. Jones for their helpful discussion and support. I also thank J. Albert, A. Herrel, P. Leberg, B. Moon, and D. Povinelli for providing comments on earlier drafts that helped improve the quality of the manuscript. I thank BhB Reptiles, the Kansas Herpetological Society, the Louisiana Board of Regents (Doctoral Fellowship), and the National Geographic Society (grant 7933-05 to Brad Moon) for partial funding. This work was also made possible by the Herpetologists’ League Graduate Research Award.

Literature Cited

Arnold, S. J. (1993). Foraging theory and prey-size–predator-size relations in snakes. In *Snakes: Ecology and Behavior*: 87–116. Seigel, R.A., Collins, J.T. (Eds.). Caldwell, NJ: The Blackburn Press.

Boback, S. M., Hall, A. E., McCann, K. K., Hayes, A. W., Forrester, J. S., & Zwemer, C. F. (2012). Snake modulates constriction in response to prey's heartbeat. *Biol. Lett.* **11**:1–4.

Boback, S. M., McCann, K. J., Wood, K. A., McNeal, P. M., Blankenship, E. L., & Zwemer, C. F. (2015). Snake constriction rapidly induces circulatory arrest in rats. *J. Exp. Biol.* **218**: 2279–2288.

Canjani, C., Andrade, D. V., Cruz-Neto, A. P., & Abe, A. S. (2003). Aerobic metabolism during predation by a boid snake. *Comp. Biomech. Physiol. A.* **133**: 487–498.

Cundall, D., & Greene, H. W. (2000). Feeding in Snakes. In *Feeding: Form, Function, and Evolution in Tetrapod Vertebrates*: 293–327. Schwenk, K. (Ed.). San Diego, CA: Academic Press.

Cunningham, J. D. (1959). Reproduction and food of some California snakes. *Herpetologica* **15**:17–19.

Darwin, C. R. (1859) *On the Origins of Species by Means of Natural Selection*. London: John Murray.

Dawkins, R., & Krebs, J. R. (1979). Arms races between and within species. *Proc. R. Soc. B.* **205**: 489–511.

De Queiroz, A. (1984). Effects of prey type on the prey-handling behavior of the bullsnake, *Pituophis melanoleucus*. *J. Herpetol.* **18**: 333–336.

Emerson, S. B., Greene, H. W., & Charnov, E. L. (1994). Allometric aspects of predator-prey interactions. In *Ecological Morphology: Integrative Organismal Biology*: 123–139. Wainwright, P. C., & Reilly, S. M. (Eds.). Chicago, IL: University of Chicago Press.

Ford, N. B., and Shuttlesworth, G. (1986). Effects of variation in food intake on locomotory performance of juvenile garter snakes. *Copeia* **1986**: 999–1001.

Frazzetta, T. H. (1970). Studies on the fossil snake *Dinilysia patagonica* Woodward Part II. Jaw machinery in the earliest snakes. *Forma et Functio.* **3**: 205–221.

Gans, C. (1961). The feeding mechanism of snakes and its possible evolution. *Am. Zool.* **1**: 217–227.

Gould, S. J. (1980). The evolutionary biology of constraint. *Daedalus.* **109**(2): 39–52.

Greene, H. W. (1983). Dietary correlates of the origin and radiation of snakes. *Am. Zool.* **201**: 315–329.

Greene, H. W. (1997). *Snakes: The Evolution of Mystery in Nature.* Berkeley, CA: University of California Press.

Greene, H. W., and Burghardt, G. M. (1978). Behavior and phylogeny: constriction in ancient and modern snakes. *Science.* **200**: 74–77.

Greenwald, O. E. (1978). Kinematics and time relations of prey capture by gopher snakes. *Copeia* **1978**: 263–286.

Hammer, Ø., Harper, D.A.T., and Ryan, P. D. (2001). PAST-Palaeontological Statistics, ver. 1.89. *Palaeontol electron* **4**: 1–9.

Hardy D. L., Sr. (1994). A re-evaluation of suffocation as the cause of death during constriction by snakes. *Herpetol. Rev*. **229**:45–47.

Herzog Jr., H. A., & Bailey, B. D. (1987). Development of antipredator responses in snakes: II. Effects of recent feeding on defensive behaviors of juvenile garter snakes (*Thamnophis sirtalis*). *J. Comp. Psychol.* **101**: 387–389.

Hisaw, F. L., & Gloyd, H. K. (1926). The bull snake as a natural enemy of injurious rodents. *J. Mammal.* **7**: 200–205.

Ings, T. C., and Chittka, L. (2008). Speed-accuracy tradeoffs and false alarms in bee responses to cryptic predators. *Curr. Biol.* **18**: 1520–1524.

Jackson, K., Kley, N. J., & Brainerd, E. L. (2004). How snakes eat snakes: the biomechanical challenges of ophiophagy for the California kingsnake, *Lampropeltis getula californiae* (Serpentes: Colubridae). *Zoology* **107**: 191–200.

King, R. B. (2002). Predicted and observed maximum prey size–snake size allometry. *Funct. Ecol.* **16**: 766–772.

Lillywhite, H. B. (2014). *How Snakes Work: Structure, Function and Behavior or the World's Snakes*. New York: Oxford University Press.

Mehta, R. (2003). Prey-handling behavior of hatchling *Elaphe helena* (Colubridae). *Herpetologica* **59**: 469–479.

Mehta, R. (2006). Meal size affects antipredator behavior of hatchling trinket snakes, *Elaphe helena*. *Ethology* **112**: 649–656.

Mehta, R. (2009). Early experience shapes the development of behavioral repertoires of hatchling snakes. *J. Ethol.* **27**: 143–151.

Moon, B. R. (2000). The mechanics and muscular control of constriction in gopher snakes (*Pituophis melanoleucus*) and a king snake (*Lampropeltis getula*). *J. Zool. Lond.* **252**: 83–98.

Moon, B. R., & Mehta, R. S. (2007). Constriction strength in snakes. In *Biology of the Boas and Pythons*: 206–212. Henderson, R. W., & Powell, R. (Eds). Eagle Mountain, UT: Eagle Mountain Publishing.

Penning, D. A., Dartez, S. F., & Moon, B. R. (2015). The big squeeze: scaling of constriction pressure in two of the world's largest snakes, *Python reticulatus* and *Python molurus bivittatus*. *J. Exp. Biol.* **218**: 3364–3367.

Penning, D.A. and Dartez, S.F. (2016). Size, but not experience, affects the ontogeny of constriction performance in ball pythons (*Python regius*). *J. Exp. Zool. A.*in press

Schwartz, C. W., & Schwartz, E. R. (1981). *The Wild Mammals of Missouri Revised Edition*. Columbia: University of Missouri Press and Missouri Department of Conservation.

Shine, R. (1980). 'Costs' of reproduction in reptiles. *Oecologia.* **46**: 92–100.

Turney, T. H., & Lockwood, J. A. (1986). Systolic blood pressure in *Peromyscus* species: considerations for the murine hypertension model. *J. Zool*. **209:** 149–154.

Wainwright, P. C. (1988). Morphology and ecology: functional basis of feeding constraints in Caribbean labrid fishes. *Ecology*. **69**: 635–645.

Willard, D. E. (1977). Constricting methods of snakes. *Copeia.* **1977**: 379–382.

Zera, A. J., & Harshman, L. G. (2001). The physiology of life history trade-offs in animals. *Annu. Rev. Ecol. Syst.* **32**: 95–126.

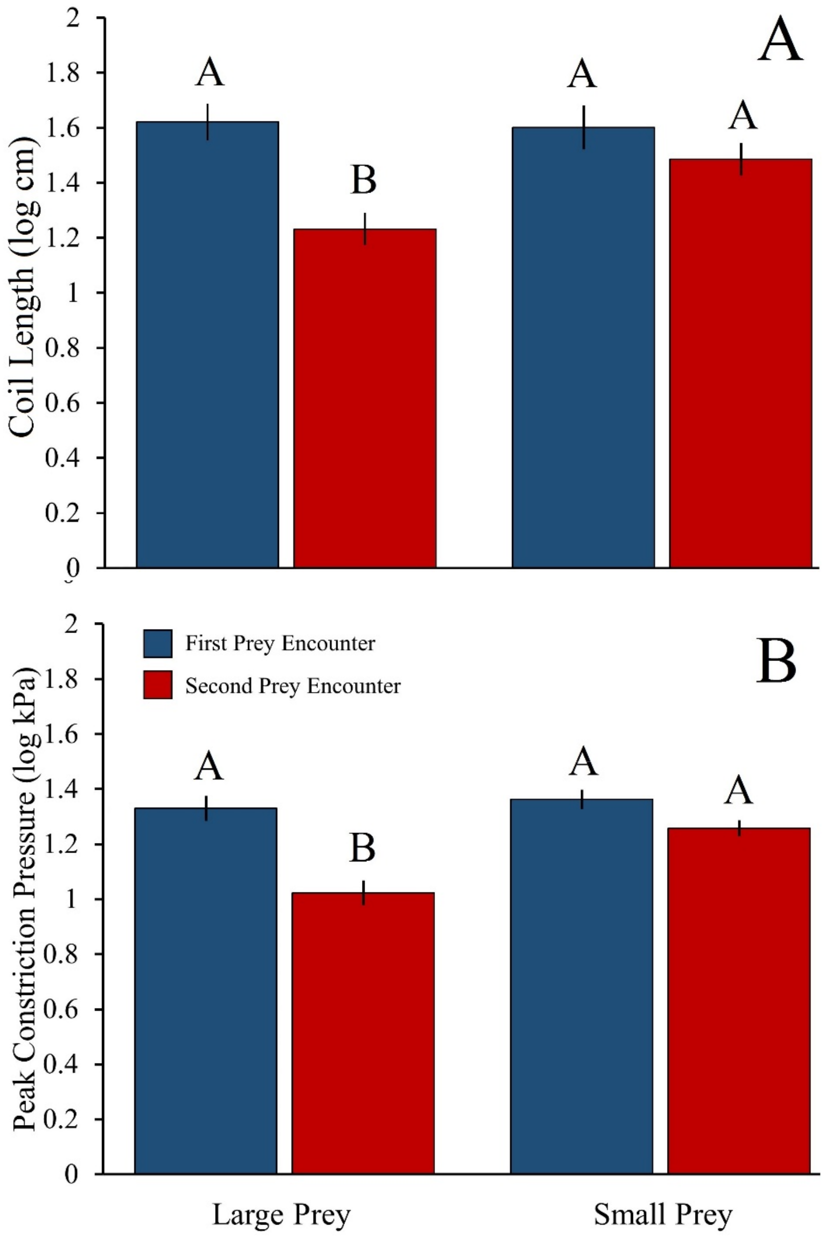

Figure 1.1 Coil length (A) and peak constriction pressures (B) for first and second feeding events on large and small prey by *Lampropeltis getula*. Bars and lines indicate means±standard errors. Different letters above bars denote pairwise significant differences based on Tukey's post-hoc tests.

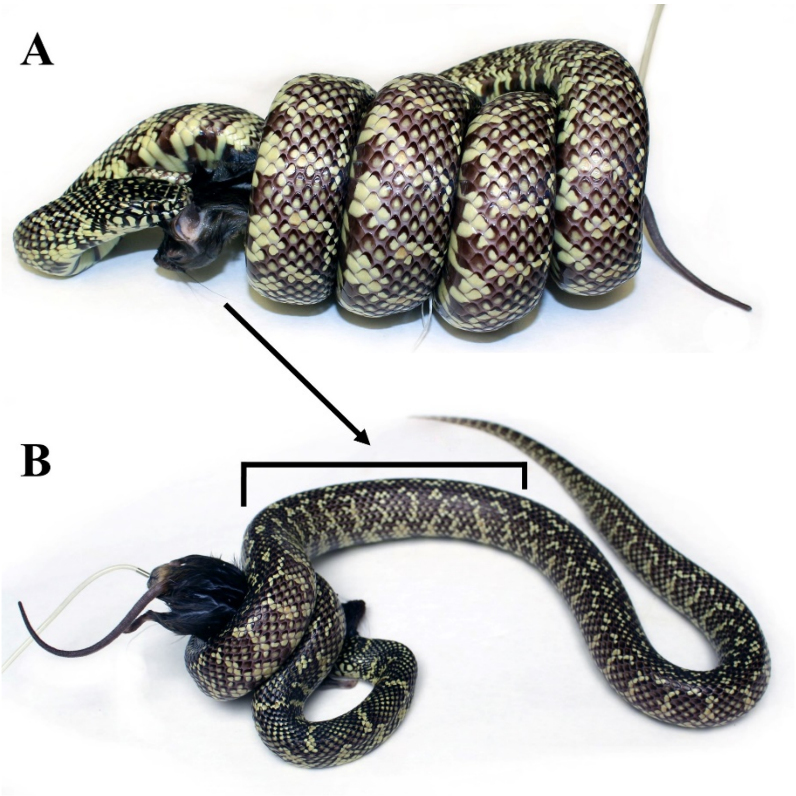

Figure 1.2. Kingsnake (*Lampropeltis getula*, 198 g) constricting its first (A) and second (B) mouse (15% relative mass each). Peak constriction pressure during the first encounter was 30.9 kPa with a coil length of 63.9 cm. During the second encounter, peak pressure was 13.3 kPa with a coil length of 23.5 cm; the coil stopped at the portion of the body where the previous prey was contained within the stomach.

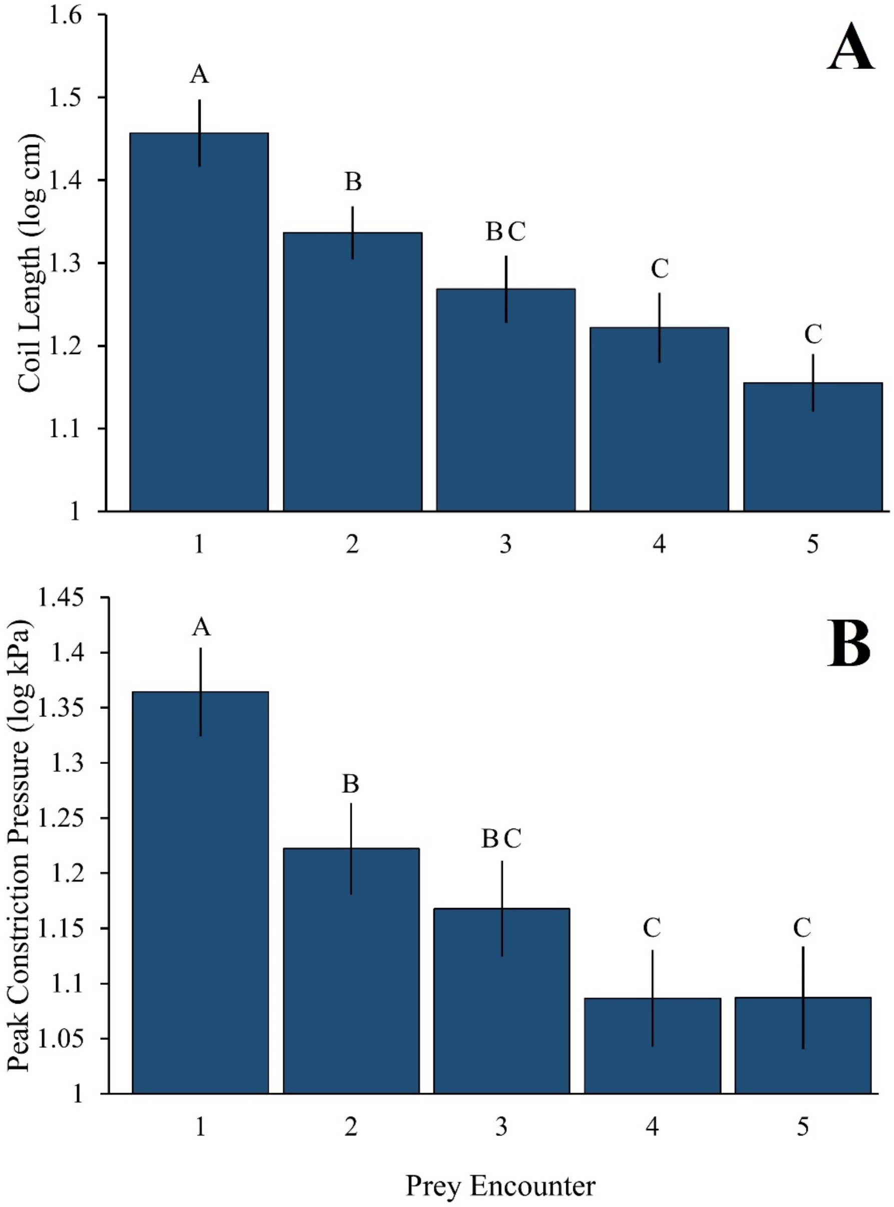

Figure 1.3. Coil length (A) and peak constriction pressure (B) for 10 *Lampropeltis getula* constricting mice during five sequential feedings (ca. 7% relative mass each). Bars and lines indicate means±standard errors. Letters above bars denote pairwise significant differences based on Tukey’s post-hoc tests.

CHAPTER III

The King of Snakes: Performance and Morphology of Intraguild Predators (*Lampropeltis*) and their Prey (*Pantherophis*)

Short Title: Kingsnake and Ratsnake Performance

DAVID A. PENNING[1,2,3], BRAD R. MOON[1]

[1]Department of Biology, University of Louisiana at Lafayette, Lafayette, Louisiana, USA 70504- 43602

[2]Department of Biology and Environmental Health, Missouri Southern State University, Joplin, Missouri, USA 64801

[3]Corresponding author E-mail: *davidapenning*@gmail.com

Abstract

Across ecosystems and trophic levels, predators are usually larger than their prey, and when trophic morphology converges, predators typically avoid predation on intraguild competitors unless the prey are notably smaller in size. However, a currently unexplained exception occurs in kingsnakes in the genus *Lampropeltis*. Kingsnakes are able to capture, constrict, and consume other snakes that are not only larger than themselves but that are also powerful constrictors (such as ratsnakes in the genus *Pantherophis*). Their mechanisms of success as intraguild predators on other constrictors remain unknown. To begin addressing these mechanisms, we studied the scaling of muscle cross-sectional area, pulling force, and constriction pressure across the ontogeny of six species of snakes (*L. californiae, L. getula, L. holbrooki, P. alleghaniensis, P. guttatus,* and *P. obsoletus*). Muscle cross-sectional area is an indicator of potential force production, pulling force is an indicator of escape performance, and constriction pressure is a measure of prey-handling performance. Muscle cross-sectional area scaled similarly for all snakes, and there was no significant difference in maximum pulling force among species. However, all kingsnakes exerted significantly higher pressures on their prey compared to all ratsnakes. The similar escape performance among species indicates that kingsnakes win in predatory encounters because of their superior constriction performance, not because ratsnakes have inferior escape performance. The superior constriction performance by kingsnakes derives from their consistent and distinctive coil posture and perhaps from additional aspects of muscle structure and function that need to be tested in future research.

Key Words: Constriction, Cross-Sectional Area, Force, Muscle, Pressure, Scaling

Summary Statement

Muscle cross-sectional area, pulling force, and constriction pressure increase with size in kingsnakes and ratsnakes. However, kingsnakes constrict with higher pressures than ratsnakes of the same size.

Introduction

The structure and function of an organism relate in part to predatory and anti-predator adaptations (Darwin, 1859; Wainwright, 1994). Predators are generally larger than their prey (Arnold, 1993; Radloff and Du Toit, 2004), and as prey increase in size relative to their predators, they become less vulnerable to predation (Magalhães et al., 2005). Furthermore, as trophic ranks (Holt et al., 1999) converge between predators (i.e., intraguild competitors; Polis et al., 1989), predators are less likely to attack prey greater than 25–50% of their own mass (Buskirk, 1999; Palomares and Caro, 1999; Wise, 2006). When the predator and prey are closely matched in size and have similar feeding morphology, an attack can bring such a high risk to the predator that it may be avoided entirely, even if the benefits of successful predation would be high (Donadio and Buskirk, 2006). However, some snakes consume intraguild prey that are well-matched in predatory abilities and in some cases are even larger than themselves (Jackson et al., 2004).

Feeding specializations have allowed many snakes to incorporate massive prey into their diets (Greene, 1997; Cundall and Greene, 2000), including snake-eating (ophiophagous) snakes (Traill, 1895; Smith, 1910; Jackson et al., 2004; Leong and Shunari, 2010). Many ophiophagous snakes are venomous (Greene, 1997), effectively offsetting the risks associated with trophic similarity between similarly sized combatants; often only a single bite is needed to subdue prey. Non-venomous ophiophagous snakes must use other prey-handling behaviors, such as constriction. Kingsnakes (genus *Lampropeltis* Fitzinger 1843) are non-venomous, constricting snakes that are well known for the ability to consume other snakes (Ernst and Ernst, 2003), including ones that are larger than themselves (Jackson et al., 2004). Surprisingly, kingsnakes are able to capture, constrict, and fully ingest other snakes (such as ratsnakes in the genus *Pantherophis* Fitzinger 1843) that seem well-matched in strength, in that they are effective constrictors on some of the same rodents that kingsnakes eat. Furthermore, the prey snake can be larger than the kingsnake (Ernst and Ernst, 2003; Jackson et al., 2004). There is currently no known mechanism that differentiates the abilities of these two constricting snakes—yet kingsnakes always seem to win (Jackson et al., 2004). How is this possible?

Morphology and physiology set the functional limitations of predator–prey dynamics (Webb, 1986), and behavior determines the ways that morphology and physiology are used (Hertz et al., 1982). For snakes that use constriction behavior, predation performance can be evaluated by measuring peak constriction pressure (Moon, 2000; Moon and Mehta, 2007; Boback et al., 2015; Penning et al., 2015; Penning and Dartez, 2016). Constriction pressure is a biologically important measure of performance (Moon and Mehta, 2007) because it can determine the time needed to subdue the prey and reduces the chances of prey escaping or causing injury to the snake. Potential ways of escaping from a constriction coil include pulling out of the coil, counter-constricting to make the aggressor release its coil, and clawing or biting to gain release. Therefore, pulling force is possibly an important performance measure in snakes because it indicates a snake’s ability to escape from the grip of a predator (Lourdais et al., 2005); it is ecologically relevant in this way and probably in some locomotor circumstances such as climbing, which our study species often do. Both predation (constriction pressure) and escape (pulling force) performance are affected by the cross-sectional area of muscle (CSA; Moon and Candy, 1997; Lourdais et al., 2005), and in principle can use up to about half of a snake's axial musculature (e.g., all the muscles on the concave parts of a constriction coil or on the concave parts of multiple axial bends used in pulling movements). Larger snakes have more muscle CSA (Moon and Mehta, 2007); therefore, changes in body size can be expected to have significant effects on these measures of performance in snakes (Moon and Mehta, 2007; Penning et al., 2015; Penning and Dartez, 2016). Although constriction pressure and pulling force are distinct variables that are typically related to different behaviors, they are appropriate indicators of predation and escape performance in snakes (Moon, 2000; Lourdais et al., 2005; Moon and Mehta, 2007; Boback et al., 2015; Penning et al., 2015; Penning and Dartez, 2016). Thus, they can be compared qualitatively to understand the factors that affect the outcome of this predator–prey interaction.

To understand how one constricting snake can capture, subdue, and consume another constricting snake with similar predatory and defensive mechanisms, we quantified and compared muscle CSA and measures of predation performance (constriction pressure) and escape performance (pulling force) across the ontogeny of two intraguild competitors: kingsnakes and ratsnakes. We used three species of kingsnakes (*Lampropeltis californiae* [Blainville 1835], *L. getula* [Linnaeus 1766], and *L. holbrooki* [Stejneger 1902]) and three species of ratsnakes (*Pantherophis alleghaniensis* [Holbrook 1836], *P. guttatus* [Linnaeus 1766], and *P. obsoletus* [Say 1823]; Pyron et al., 2013). We address several questions about how form and function change across ontogeny and differ between species. How does axial muscle CSA vary and change with size among kingsnakes and ratsnakes? What constriction pressures are exerted on prey and how do they change with size among species? What pulling forces can these snakes produce during escape attempts, and how do they change with size among species? We discuss several possible mechanisms that can determine the winner of predatory interactions between two constricting snakes.

Materials and Methods

We chose sample sizes based on available specimen and previously published work for both morphological (Jayne and Riley, 2007; Herrel et al., 2011) and performance investigations (Moon and Mehta, 2007; Penning et al., 2015; Penning and Dartez, 2016).

Morphology and Scaling

We measured the morphology of 36 preserved snakes (4 *L. holbrooki*, 9 *L. getula*, 8 *P. guttatus*, and 15 *P. obsoletus*) from a teaching collection (UL Lafayette) or the personal collection of DAP. We weighed each snake and measured its snout–vent length (SVL). We cut each specimen into sections at 20, 40, 60, 80, and 100% of its SVL, photographed each cross-section, and measured the anatomical CSA of major muscle groups (see below). For small cross-sections, we used a Canon (EOS Rebel T5i) digital camera attached to a Zeiss Stemi 2000-C stereoscopic microscope, with the cross sections immersed in 70% isopropyl alcohol; for larger cross-sections, we used an Olympus (Stylus Tough TG-630) digital camera. Each photograph included a scale; we confirmed that the images had square pixels, making a single scale appropriate. We measured the muscle CSA of five epaxial muscles (semispinalis–spinalis complex, multifidis, longissimus dorsi, and iliocostalis; Fig. 2.1) in each section of the body (Jayne and Riley, 2007; Herrel et al., 2011) using ImageJ (Herrel et al., 2011).

Predation Performance

All experimentation was approved by the University of Louisiana at Lafayette's Institutional Animal Care and Use Committee. We tested the constriction performance of 182 male and female snakes (21 *L. californiae,* 12 *L. holbrooki,* 56 *L. getula; 21 P. alleghaniensis, 22 P. guttatus*, and 50 *P. obsoletus*). Kingsnakes and ratsnakes eat a variety of prey, but all frequently incorporate small mammals into their diets (Ernst and Ernst, 2003). To compare constriction performance between snakes, we fed all snakes pre-killed rodents (mass ratio=15.2±0.6% snake mass) with a pressure sensor attached via dental floss as described below. For smaller snakes (ca.<0.5 m), we used a 0.5-ml water-filled latex balloon as the pressure sensor, and for larger snakes we used a 2-ml water-filled rubber pipette bulb as the pressure sensor. We attached pressure sensors to a Research Grade Blood Pressure Transducer (Model 60-3002, Harvard Apparatus, Holliston, Massachusetts) and offered prey to each snake with long forceps. We shook the prey to simulate movement and elicit maximum effort from each snake (following Moon and Mehta, 2007; Penning and Dartez, 2016). During the constriction event, we recorded peak constriction pressure from the digital readout on the transducer (which had a refresh rate of 2 Hz) and the number of loops used in the constriction coil. Once peak constriction pressure began to decline, we removed the pressure sensor from the prey and the snakes completed the feeding event. While snakes were swallowing their prey, we measured their maximum diameters with digital callipers.

Escape Performance

We tested maximum pulling forces of 98 male and females snakes (7 *L. californiae, 7 L. holbrooki, 32 L. getula; 8 P. alleghaniensis, 6 P. guttatus,* and *38 P. obsoletus).* To measure maximum pulling force, we anchored each snake to a large flat surface using gaffer’s tape placed just behind the head. We attached a Pesola® (Rebmattli 19, CH-6340 Baar, Switzerland) scale to the snake just anterior to the cloaca with gaffer’s tape. Pesola® scales provided similar and repeatable results when calibrated against an isometric force transducer

(MLT500/A, AD Instruments, Colorado Springs, Colorado). Once the scale was attached, we manually straightened the snake to its maximum length and anchored the spring scale to the flat surface. We released the snake and gently agitated it to elicit a pulling motion for a period of 5 min (Lourdais et al., 2005). We used a GoPro Hero 4 (Black) camera (GoPro, Inc., San Mateo, California) to record spring-scale displacements during the pulling movements (720p video at 60 frames per second). Using Tracker 4.87 software (Open Source Physics, http://www.opensourcephysics.org/index.cfm), we advanced frame by frame and recorded the maximum pulling force for each snake during its 5-min trial, and then converted the scale values from grams to Newtons (N).

Statistical Analyses

We used $\log_{10}$-transformed data for all models. To quantify the scaling of muscle CSA against body mass, we used reduced-major-axis (RMA) regression (Smith, 2009). To test for differences in slopes and elevations between kingsnakes and ratsnakes, we added snake species as a categorical variable to the RMA regressions; this is the RMA equivalent of ANCOVA (in the smatr 3 code package in RStudio; Warton et al., 2012) and allows for comparisons between slopes (factor A×factor B) and intercepts (factor A+factor B) in models with a categorical predictor. To evaluate constriction performance, we used ordinary least-squares (OLS) multiple regression with peak constriction pressure as the dependent variable, and snake species, maximum body diameter, and the number of loops used in a coil as independent variables. To evaluate pulling force, we used OLS multiple regression with pulling force as the dependent variable, snake species as a categorical variable, and snake body mass as the independent variable. Following previous methods (Herrel et al., 2011; Penning, 2016), and the general recommendations for regression analyses based on regression-line symmetry (Smith, 2009), we used RMA regression for comparisons between two morphological variables and OLS regression for comparisons between one morphological and one performance variable. We retained all data in all models because the results were the same with all data retained and with outliers removed. We performed analyses in JMP Pro 11.0.0 (SAS Institute Inc., Cary, NC, USA), RStudio (version 0.99.441; RStudio Team, 2015), and Past 3.08 (Hammer et al., 2001), and considered test results significant at $P<0.05$.

Results

Morphological Scaling

The five major epaxial muscles were easily delineated in most cross-sections (Fig. 2.1). For 11 of the cross-sections, we had to confirm muscle identities and boundaries with further probing and visual inspection. Muscle cross-sectional areas at the widest body diameter (60% SVL) were 0.030–0.93 cm^2 *for L. getula* (body mass=14–67 g), 0.024–0.10 cm^2 for *L. holbrooki* (11.4–628 g), 0.039–1.05 cm^2 for *P. guttatus* (8.6–572 g), and 0.044–1.94 cm^2 for *P. obsoletus* (13.9–1261 g). We did not have samples available for *L. californi*a and *P. alleghaniensis*. Muscle CSA increased with body mass in all cross-sections in all species (Table 2.1); in most sections and species, CSA also scaled with positive allometry (slope greater than 0.67). At each SVL cross-section, the slopes (mass×species interaction; Table 2.1) and intercepts (mass+species) for muscle CSA did not differ significantly between *Lampropeltis* and *Pantherophis* species (all $P>0.05$; Table 2.1). Across ontogeny, muscle CSA at each location increased similarly in all species (Table 2.1).

Predation Performance

All snakes readily struck at and constricted rodent prey vigorously using 1–3 loops of the body in the coil. Kingsnakes typically constricted using a single posture (Fig. 2.2A), with multiple loops forming a tight coil like that of a spring. Peak constriction pressures were 7.7–41.7 kPa (kilopascals) for *L. californiae*, 5.3–41.6 kPa for *L. getula*, and 9.9–26.8 kPa for *L. holbrooki*. Ratsnake constriction postures were much more variable than the typical kingsnake posture, with loops placed at different positions and angles on prey, loops that overlapped one another, and with the constrictor's head inside or outside the coil (Fig 2.2B). Peak constriction pressures were 3.2–17.3 kPa for *P. alleghaniensis*, 3.2–23.7 kPa for *P. guttatus*, and 3.2–19.7 kPa for *P. obsoletus.* Across all six species, there was no significant difference in the number of loops used in a coil (Kruskal–Wallis H=6.8, P>0.23).

Starting with a full-factorial model (pressure=diameter×number of loops×species), we sequentially removed non-significant factors to arrive at the final model (pressure=diameter+number of loops+species; $F_{7,174}$=61.3, P<0.001, adj. R^2=0.70). Diameter ($F_{1,174}$=148.1, P<0.0001), number of loops ($F_{1,174}$=9.88, P<0.003) and species ($F_{5,174}$=18.65, P<0.0001) were all significant factors in the final model. Within each genus, there were no significant pair-wise differences between covariate-adjusted means for peak constriction pressure (Tukey's HSD tests; Fig 2.3). However, all kingsnakes constricted with significantly higher pressures than all ratsnakes (Fig. 2.3).

To analyse the scaling of constriction performance across body size the same way as in previous work (Moon and Mehta, 2007; Penning et al., 2015; Penning and Dartez, 2016), we regressed peak constriction pressure against snake diameter. Diameter ($F_{1,175}$=300.1, P<0.0001) and species ($F_{5,175}$=18.4, P<0.0001) were significant factors. The interaction (diameter×species) was not significant ($F_{5,170}$=0.38, P>0.8), resulting in a similar scaling relationship between pressure and snake diameter for all six species (overall β=0.88). As with the full model, there were no significant differences between species in covariate-adjusted means within each genus (Tukey's HSD tests, all $P>0.05$). However, all kingsnake means were significantly higher than all ratsnake means (Tukey's HSD tests, all $P>0.05$; Fig. 2.4).

Escape Performance

In the tests of escape performance, snakes shortened their bodies into S-shaped curves and pulled against the Pesola® scale in attempts to free themselves. Restrained snakes typically exerted their maximum pulling forces <1.5 min into the 5-min trial. Maximum pulling forces were 2.5–19.0 N for *L. californiae*, 0.9–23.7 N for *L. getula*, 1.2–12.7 N for *L. holbrooki*, 1.5–14.3 N for *P. alleghaniensis*, 1.8–6.6 N for *P. guttatus*, and 0.8–24.5 N for *P. obsoletus*. In a full model (pulling force= mass×species), the interaction ($F_{5,86}=0.25$, $P>0.9$) and species factor ($F_{5,86}=2.1$, $P>0.068$) were not significant. Removing the interaction term did not result in a significant species factor, producing a final model that included only pulling force and mass. Larger snakes pulled with significantly higher forces than smaller individuals, regardless of species (pulling force=0.69×mass–0.68; $F_{1,96}=1967$, $R^2=0.95$, $P<0.0001$; Fig 2.5).

Discussion

Morphology and Scaling

In every section along the body, muscle CSA scaled positively with body mass in both kingsnakes and ratsnakes, and in most sections and species, muscle CSA scaled with positive allometry (Table 2.1). The lack of significant differences between species in muscle CSA and its scaling means that similarly sized kingsnakes and ratsnakes have the same amount of muscle that can be used in constriction or escape movements. However, there may be differences in muscle physiological cross-sectional area (the area of a muscle perpendicular to the muscle fibers), and hence maximum force production, that we have not yet detected.

Predation and Escape Performance

In constriction, if we assume that the muscles on the concave side of the coil contribute to force exertion, then up to half of the total musculature can be used and contribute to the constriction pressure. In a pulling-force test, if we assume that a snake bends into sinusoidal curves and the musculature on the concave part of each curve contributes to the pulling force, then up to half of the total musculature will be used and contribute to the pulling force. So although constriction pressure and pulling force are distinct variables used in different behaviors, they are appropriate indicators of predation and escape performance, and can be compared qualitatively to understand the factors that affect the outcome of the predator–prey interaction that we studied.

Across ontogeny, all species increased constriction performance in a similar manner (i.e., with similar slopes; Fig. 2.4). However, at every body size, kingsnakes produced significantly higher constriction pressures than identically sized ratsnakes. The similar escape performance among species indicates that kingsnakes win in predatory encounters because of their superior constriction performance, not because ratsnakes have inferior escape performance. With kingsnakes producing significantly higher constriction pressures while having similar musculature available for use, what are some possible mechanisms of their superior performance?

With all snakes using a similar number of loops, the orientation of loops in the coil may optimize force transmission by optimizing muscle fiber angles relative to the vectors of whole-body force exertion on the prey. Kingsnakes produced higher constriction pressures for a given number of loops in a coil (Fig 2.3), and qualitatively had a more uniform coil posture than ratsnakes (Fig 2.2). It is possible that the kingsnake coil posture may maximize the force applied (and therefore pressure) to the prey by reducing the need for coil adjustments and movement. Reducing the need for adjustments may enable both isometric and tetanic contractions that maximize force output, and may reduce periods of loosening that could allow prey to struggle more or escape. It is also possible that kingsnake muscle may be able to exert higher forces than ratsnake muscle by having muscles that can produce higher muscle stress by using different types of muscle contractions, or by having different muscle fibre angles and therefore higher physiological cross-sectional areas. Furthermore, in seeking to escape, a ratsnake must use shortening contractions, which exert lower forces than isometric and tetanic contractions (MacIntosh et al., 2006). Additionally, constriction bouts between kingsnakes and ratsnakes can last for hours (Jackson et al., 2004), suggesting that endurance may be important. Further work is needed in order to test these hypotheses. Experimental tests of muscle contractile force and endurance would help identify potential muscle-level differences between kingsnakes and ratsnakes.

The Effects of Constriction Pressure on Prey Animals

Constriction can disrupt breathing or circulation, cause structural damage to the spine, cause internal bleeding, and potentially disrupt brain function (McLees, 1928; Hardy, 1994; Moon, 2000; Moon and Mehta, 2007; Boback et al., 2015; Penning et al., 2015; Penning and Dartez, 2016). Although endotherms die quickly (within 10–78 s; McLees, 1928; Hardy, 1994) from constriction, ectothermic prey may be only fatigued by constriction and swallowed alive (Hardy, 1994; Boback et al., 2015). We have observed both escape by *Anolis* lizards after extended constriction, and their death from constriction in the same time taken to kill endothermic prey (ca. 1 min). Hence, the effects of constriction on ectotherms are variable and potentially complex; they may include harmful fluid movements, tissue distortions or damage, and neurological disruptions (Greene, 1983; Rivas, 2004; Moon, 2000; Moon and Mehta, 2007; Boback et al., 2015; Penning et al., 2015; Penning and Dartez, 2016). This variation and complexity indicate that we do not yet fully understand the effects of constriction, particularly on ectothermic prey. Constricting snakes probably experience internal pressures from the forces they exert on prey. However, blood pressures rise only slightly during constriction in boas, and less than during other behaviors such as hissing and swallowing (Wang et al., 2001). Changes in a snake's blood pressure during constriction are not yet well known and may affect circulation to parts of their musculature, but are clearly well tolerated by the constrictors. When prey are constricted, they experience circumferential pressure that may result in high internal pressures (Halperin et al., 1993; Boback et al., 2015; Penning, 2016) that have different effects on internal fluids than pressure applied at a specific point, and may be different from what the constricting snake itself experiences.

Kingsnakes often prey on other snakes, including other constrictors. When kingsnakes and their prey snakes are well matched in size, total muscular cross-sectional area, and escape performance, the kingsnakes succeed in predation because they have superior constriction performance. Their superior performance derives in part from their consistent and distinctive coil posture and potentially from additional aspects of muscle structure and function that need to be tested in future research. These abilities allow kingsnakes to succeed as intraguild predators on other snakes, including constrictors larger than themselves.

Acknowledgments

We thank T. Lyon, M. Miles, and L. Moberly for allowing access to their snakes and J. Albert, A. Herrel, P. Leberg, and D. Povinelli for providing comments on earlier drafts that helped improve the quality of the manuscript. DAP thanks M. Fulbright, L. Jones, I. Moberly,

M. Perkins, and B. Sawvel and for their helpful discussions and K. Smith and S. Fredericq for providing access to equipment and supplies. BRM thanks C. Gans, D. Hardy, N. Kley, and S. Secor for valuable insights.

Competing Interests

We declare no competing interests.

Author Contributions

DAP collected and analysed all data, drafted the manuscript, and provided funding. BRM helped design the project, edit the manuscript, provide several specimens, and provide funding. All approved the final manuscript.

Funding

Partial funding was provided by the Louisiana Board of Regents (grants RD-A-34 and ENH-TR-77 to BRM and Doctoral Fellowship to DAP), the National Geographic Society (grant 7933-05 to BRM), the National Science Foundation (IOS-0817647 to BRM), the Department of Biology and the Graduate Student Organization at the University of Louisiana at Lafayette (to DAP), the Louisiana Department of Wildlife and Fisheries Rockefeller State Wildlife Scholarship (to DAP), the Kansas Herpetological Society (to DAP), Miles of Exotics (to DAP), and BhB Reptiles (to DAP).

Literature Cited

Arnold, S. J. (1993) Foraging theory and prey-size—predator-size relations in snakes. In *Snakes: Ecology and Behavior* (eds. R. A. Seigel and J. T. Collins), pp. 87–115. New Jersey: Blackburn Press.

Boback, S. M., McCann, K. J., Wood, K. A., McNeal, P. M., Blankenship, E. L., and Zwemer, C. F. (2015) Snake constriction rapidly induces circulatory arrest in rats. *J. Exp. Biol.* **218,** 2279–2288.

Buskirk, S. W. (1999) Mesocarnivores of Yellowstone. In *Carnivores in Ecosystems: The Yellowstone Experience* (eds. T. W. Clark, A. P. Curlee, S. C. Minta and P. M. Karieva), pp. 165–187. Connecticut: Yale University Press.

Cundall, D., and Greene, H. W. (2000) Feeding in snakes. In *Feeding: Form, Function, and Evolution in Tetrapod Vertebrates* (ed. K. Schwenk), pp. 293–333. California: Academic Press.

Darwin, C. R. (1859) *On the Origins of Species by Means of Natural Selection*. London: John Murray.

Donadio, E., and Buskirk, S. W. (2006) Diet, morphology, and interspecific killing in Carnivora. *Amer. Nat.* **167**, 524–536.

Ernst, C. H., and Ernst, E. M. (2003) *Snakes of the United States and Canada*. Washington DC: Smithsonian Institution.

Greene, H. W. (1983) Dietary correlates of the origin and radiation of snakes. *Am. Zool.* **201**, 315–329.

Greene, H. W. (1997) *Snakes: The Evolution of Mystery in Nature.* California: University of California Press.

Halperin, H. R., Tsitlik, J. E., Gelfand, M., Weisfeldt, M. L., Gruben, K. G., Levin, H. R., Rayburn, B. K., Chandra, N. C., Scott, C. J., Kreps, B. J., et al. (1993). A preliminary study of cardiopulmonary resuscitation by circumferential compression of the chest with the use of a pneumatic vest. *N. Engl. J. Med.* **329**, 762–768.

Hammer, Ø., Harper, D.A.T., and Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeont. Electron.* **4**, 1–9.

Hardy, D. L. (1994) A re-evaluation of suffocation as the cause of death during constriction by snakes. *Herpetol. Rev.* **25**, 45–47.

Herrel. A., Huyghe, K., Oković, P., Lisičić, D., and Tadić, Z. (2011) Fast and furious: effects of body size on strike performance in an arboreal viper *Trimeresurus (Cryptelytrops) albolabris*. *J. Exp. Zool.* **315**, 22–29.

Hertz, P. E., Huey, R. B., and Nevo, E. (1982) Fight versus flight: body temperature influences defensive responses of lizards. *Anim. Behav.* **30**, 676–679.

Holt, R. D., Lawton, J. H., Polis, G. A., and Martinez, N. D. (1999) Trophic rank and the species–area relationship. *Ecology* **80**, 1495–1504.

Jackson. K., Kley, N. J., and Brainerd, E. L. (2004) How snakes eat snakes: the biomechanical challenges of ophiophagy for the California kingsnake, *Lampropeltis getula californiae* (Serpentes: Colubridae). *Zoology* **107**, 191–200.

Jayne, B. C., and Riley, M. A. (2007) Scaling of the axial morphology and gap-bridging ability of the brown tree snake, *Boiga irregularis*. *J. Exp. Biol.* **210**, 1148–1160.

Leong, T. M., and Shunari, M. (2010) Attempted predation on a dog-toothed cat snake, *Boiga cynodon* by a black spitting cobra, *Naja sumatrana* in Singapore (Reptilia: Squamata). *Nature in Singapore* **3**, 269–271.

Lourdais, O., Brischoux, F., and Barantin, L. (2005) How to assess musculature and performance in a constricting snake? A case study in the Colombian rainbow boa (*Epicrates cenchria maurus*). *J. Zool. Lond.* **265**, 43–51.

MacIntosh, B. R., P. F. Gardiner, and McComas, A. J. (2006) *Skeletal Muscle: Form and Function* 2nd edition. Illinois: Human Kinetics.

Magalhães, S., Janssen, A., Montserrat, M., and Sabelis, M. W. (2005) Prey attack and predators defend: counterattacking prey trigger parental care in predators. *Proc. R. Soc. Lond. B. Biol. Sci.* **272**, 1929–1933.

McLees, F. (1928) Killing by constriction. *Bull. Antivenin Inst. America* **1**, 105.

Moon, B. R. (2000) The mechanics and muscular control of constriction in gopher snakes (*Pituophis melanoleucus*) and a king snake (*Lampropeltis getula*). *J. Zool.* **252**, 83–98.

Moon, B. R., and Candy, R. (1997) Coelomic and muscular cross-sectional areas in three families of snakes. *J. Herpetol.* **31**, 37–44.

Moon, B. R., and Mehta, R. S. (2007) Constriction strength in snakes. In *Biology of the Boas and Pythons* (eds. R. W. Henderson and R. Powell), pp. 206–212. Utah: Eagle Mountain Publishing.

Palomares, F., and Caro, T. M. (1999) Interspecific killing among mammalian carnivores. *Amer. Nat.* **153**, 494–508.

Penning, D. A. (2016) The scaling of bite force and constriction pressure in kingsnakes (*Lampropeltis getula*): proximate determinants and correlated performance. *Integr. Zool. in press*

Penning, D. A., and Dartez, S. F. (2016) Size, but not experience, affects the ontogeny of constriction performance in ball pythons (*Python regius*). *J. Exp. Zool. A.***325**, 194–199.

Penning, D. A., Dartez, S. F., and Moon, B. R. (2015) The big squeeze: Scaling of constriction pressure in two of the world's largest snakes, *Python reticulatus* and *P. molurus bivittatus*. *J. Exp. Biol.* **218**, 3364–3367.

Polis, G. A., Myers, C. A., and Holt, R. D. (1989) The ecology and evolution of intraguild predation: potential competitors that eat each other. *Annu. Rev. Ecol. Evol. Syst.* **1989**, 297–330.

Pyron, R. A., Burbrink, F. T., and Wiens, J. J. (2013) A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evol. Biol.* **13**, 1–53.

Radloff, F. G., and Du Toit, J. T. (2004) Large predators and their prey in a southern African savanna: a predator's size determines its prey range. *J. Anim. Ecol.* **73**, 410–423.

Rivas, J. A. (2004) *Eunectes murinus* (green anaconda). Subduing behavior. *Herpetol. Rev.* **35**, 66–67.

RStudio Team (2015) RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

Smith, J. H. (1910) Krait and Landria (*D. trigonatus*). *J. Bombay Nat. Hist. Soc.* **20**, 863–864.

Smith, R. J. (2009) Use and misuse of the reduced major axis regression for line-fitting. *Am. J. Phys. Anthropol.* **140**, 476–486.

Traill, W. H. (1895) The food of the krait. *J. Bombay Nat. Hist. Soc.* **9**, 499.

Wang, T., Taylor, E. W., Andrade, D., and Abe, A. S. (2001) Autonomic control of heart rate during forced activity and digestion in the snake *Boa constrictor*. *J. Exp. Biol.* **204**, 3553–3560.

Warton, D. I., Duursma, R. A., Falster, D. S., and Taskinen, S. (2012) Smart 3—an R package for estimation and inference about allometric lines. *Methods Ecol. Evol.* **3**, 257–259.

Wainwright, P. C. (1994) Functional morphology as a tool in ecological research. In *Ecological Morphology: Integrative Organismal Biology* (eds. P. C. Wainwright and S. M. Reilly), pp. 42–59. Illinois: University of Chicago Press.

Webb, P. W. (1986) Locomotion and predator-prey relationships. In *Predator-Prey Relationships: Perspectives and Approaches from the Study of Lower Vertebrates* (eds. M. E. Feder and G. V. Lauder), pp. 24–41. Illinois: University of Chicago Press.

Wise, D. H. (2006) Cannibalism, food limitation, intraspecific competition, and the regulation of spider populations. *Ann. Rev. Entomol.* **51**, 441–465.

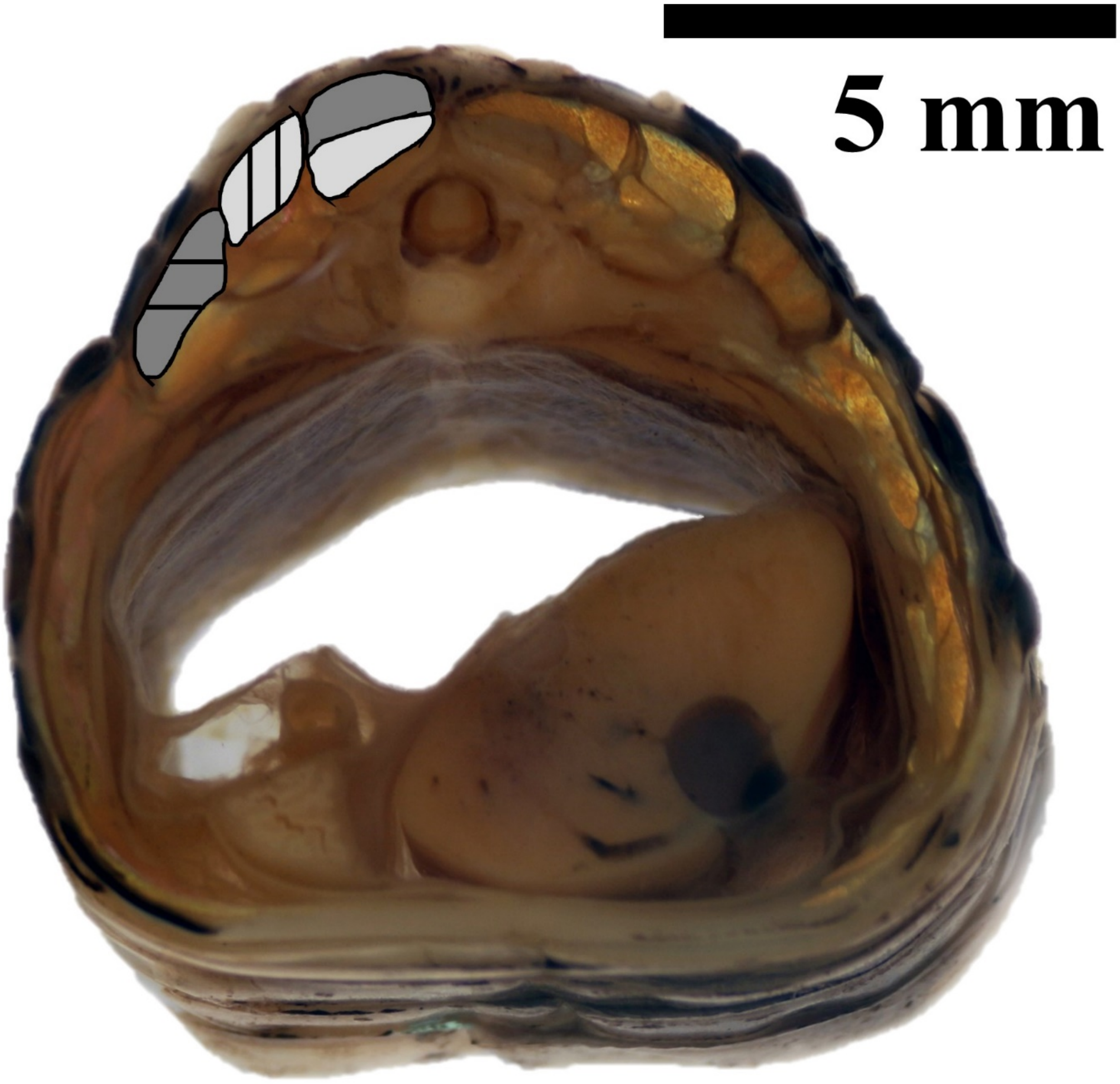

Figure 2.1. Photograph of anatomical cross-section taken at 40% of snout–vent length for a small (20.1g) kingsnake, *Lampropeltis getula*. Major epaxial muscles are delineated with plain dark gray (semispinalis–spinalis complex), plain light gray (multifidus), hatched light gray (longissimus dorsi), and hatched dark gray (iliocostalis).

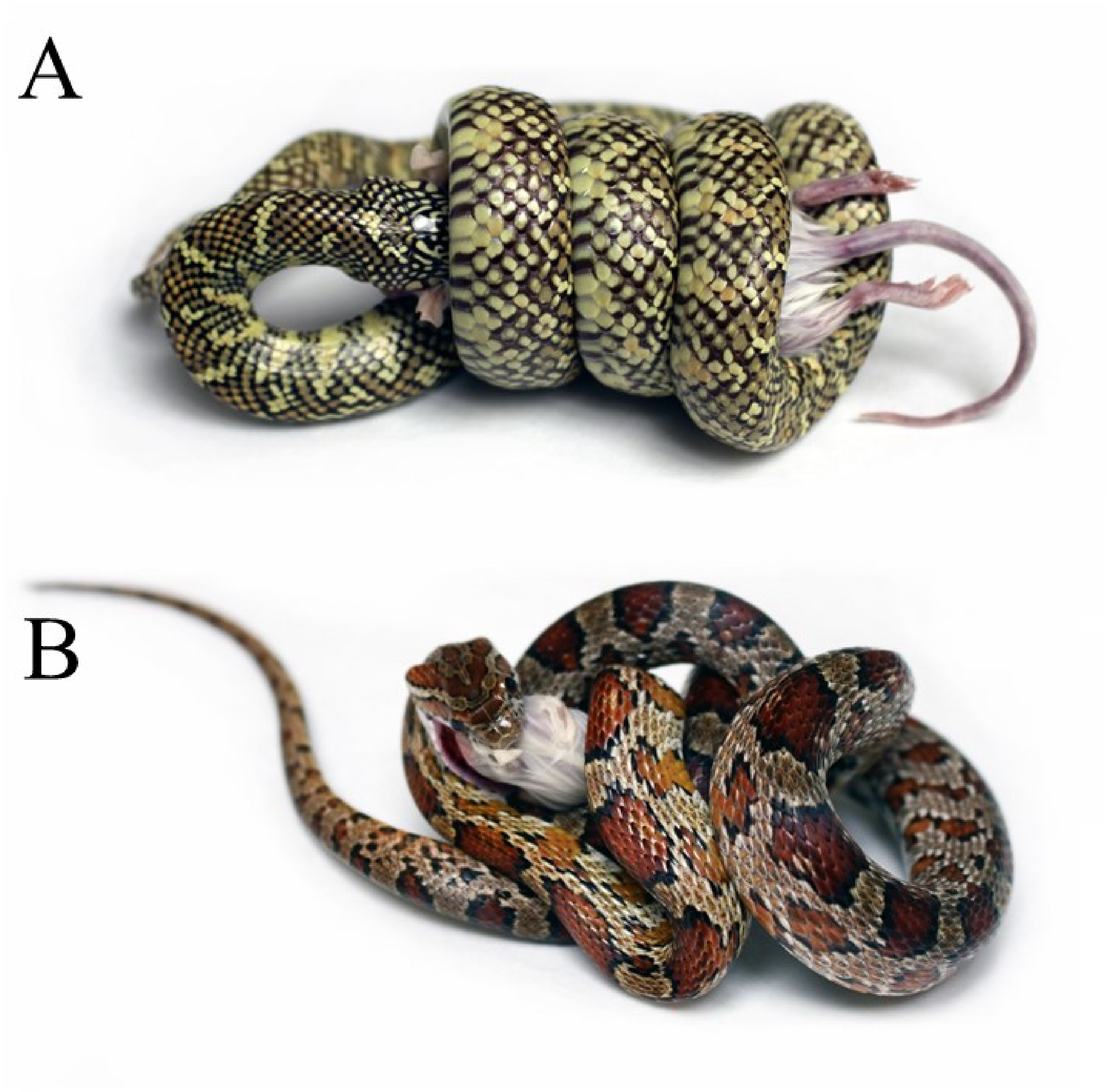

Figure 2.2. Constriction coil postures in a kingsnake, *Lampropeltis getula*, (92 g; A) and a ratsnake, *Pantherophis guttatus* (86 g; B). Both snakes were constricting similarly sized mice, *Mus musculus* (12 g).

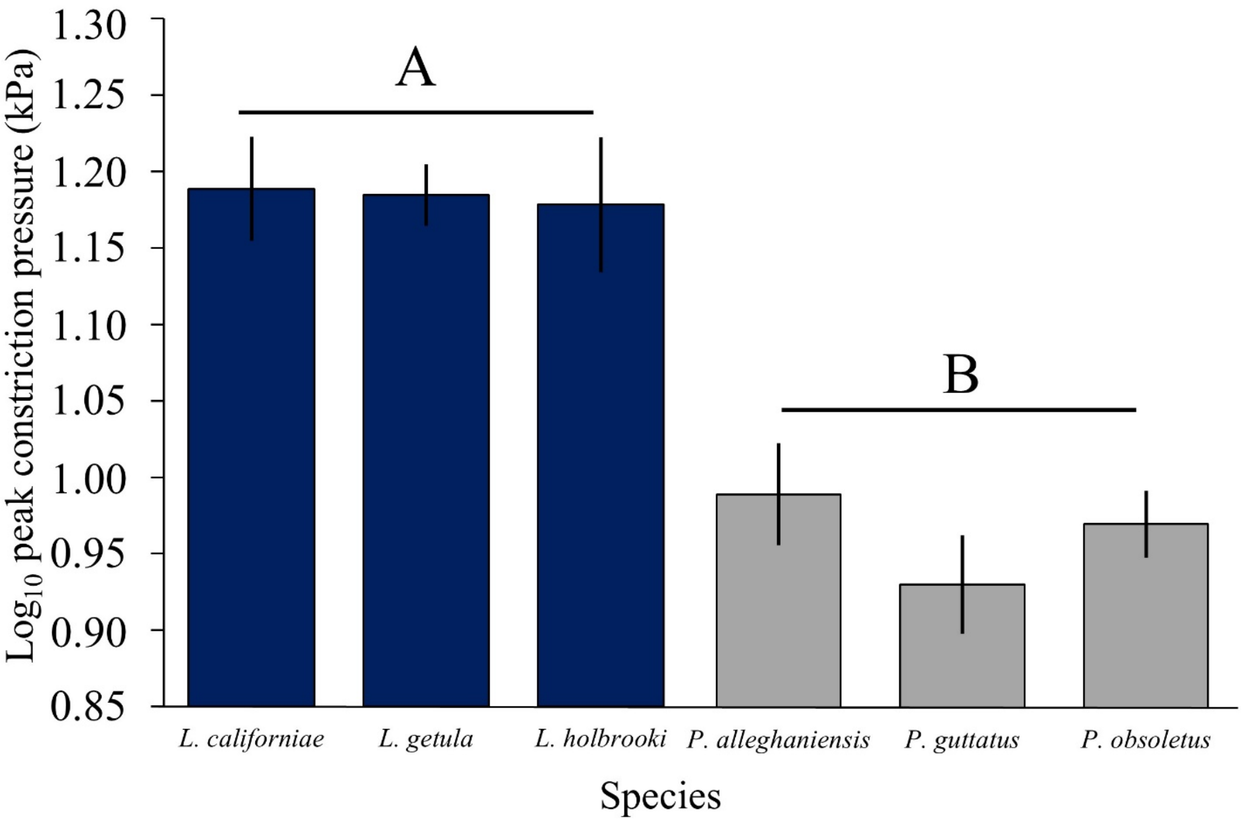

Figure 2.3. Peak constriction pressures (kPa) for six species of snakes (kingsnakes, *Lampropeltis spp*., are dark blue and ratsnakes, *Pantherophis spp*., are gray). Bars and lines indicate log-transformed covariate-adjusted means±s.e. for each species from a full model (pressure=diameter+species+number of loops). Significant differences (Tukey HSD, all P<0.05) are denoted with different letters (A,B). Sample sizes are as follows: *L. californiae*=21, *L. getula*=56, *L. holbrooki*=12, *P. alleghaniensis*=21, *P. guttatus*=22, and *P. obsoletus*=50.

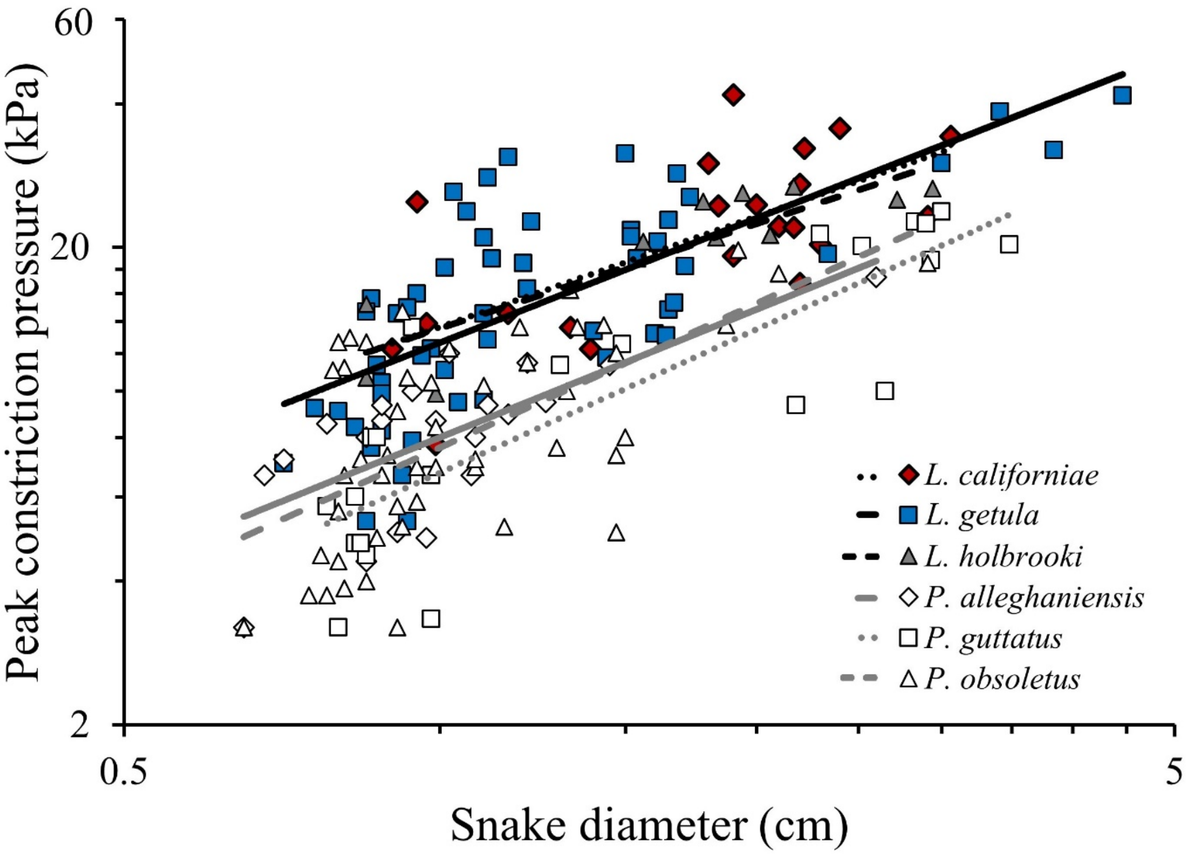

Figure 2.4. Peak constriction pressure (kPa) regressed against snake diameter (cm) for kingsnakes (*Lampropeltis californiae*=21, *L. getula*=56, and *L. holbrooki*=12) and ratsnakes (*Pantherophis alleghaniensis*=21, *P. guttatus*=22, and *P. obsoletus*=50). All slopes (denoted with species regression lines) are not significantly different from one another (see results). However, all kingsnake means are significantly higher than all ratsnake means (Tukey's HSD, P>0.05 for all comparisons).

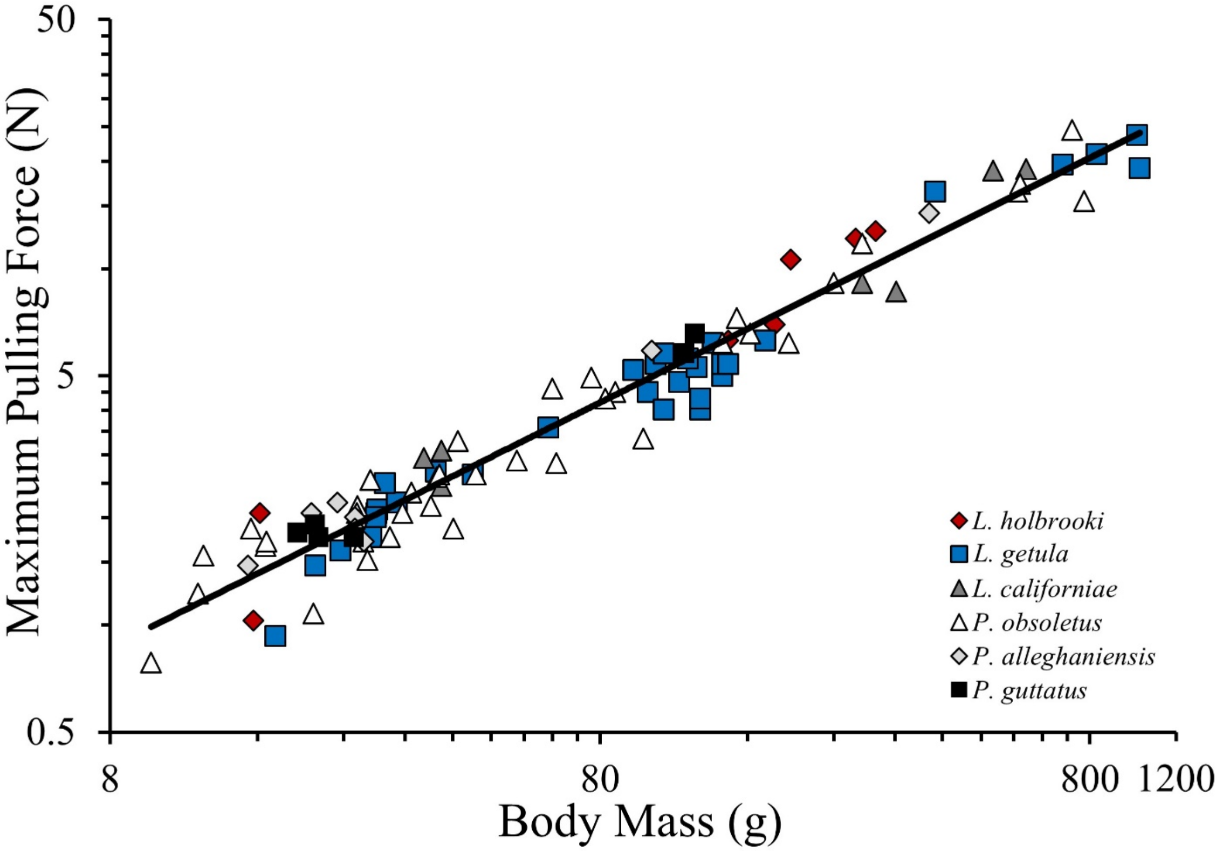

Figure 2.5. Maximum pulling force (N) regressed against snake mass (g) for kingsnakes (*Lampropeltis californiae*=7, *L. getula*=32, and *L. holbrooki*=7) and ratsnakes (*Pantherophis alleghaniensis*=8, *P. guttatus*=6, and *P. obsoletus*=38). There were no significant differences in slopes and intercepts between species, resulting in a single slope (black line) and intercept for all snakes (see Results).

Table 2.1. Species comparisons of the scaling relationships for muscle cross-sectional area (CSA) relative to body mass for kingsnake species (*Lampropeltis* spp.) and ratsnake species (*Pantherophis* spp.).

SVL Location	**Species**	**Slope**	**Intercept**	**R2**	**Genus × Mass**	**Genus + Mass**
20%	*L. getula*	1.25 (0.80, 1.94)	–3.04 (–3.86, –2.22)	0.98	*P* >0.23 (4.3)	*P* >0.53 (2.2)
	L. holbrooki	0.80 (0.74, 0.86)	–2.34 (–2.47, –2.21)	0.99	-	-
	P. guttatus	0.85 (0.69, 1.06)	–2.46 (–2.76, –2.16)	0.95	-	-
	P. obsoletus	0.79 (0.73, 0.85)	–2.34 (–2.49, –2.19)	0.98	-	-
40%	*L. getula*	0.95 (0.35, 2.53)	–2.60 (–4.17, –1.02)	0.86	*P* >0.91 (0.52)	*P* >0.63 (1.78)
	L. holbrooki	0.79 (0.72, 0.85)	–2.27 (–2.41, –2.13)	0.99	-	-
	P. guttatus	0.82 (0.64, 1.05)	–2.32 (–2.67, –1.98)	0.94	-	-
	P. obsoletus	0.80 (0.73, 0.89)	–2.31 (–2.51, –2.12)	0.97	-	-
60%	*L. getula*	0.83 (0.68, 0.98)	–2.48 (–3.96, –1.00)	0.84	*P* >0.95 (0.31)	*P* >0.18 (4.90)
	L. holbrooki	0.87 (0.73, 1.03)	–2.53 (–2.85, –2.20)	0.96	-	-
	P. guttatus	0.82 (0.68, 0.99)	–2.35 (–2.60, –2.10)	0.97	-	-
	P. obsoletus	0.83 (0.76, 0.91)	–2.36 (–2.54, –2.19)	0.98	-	-
80%	*L. getula*	0.63 (0.19, 2.07)	–2.33 (–3.68, –0.97)	0.76	*P* >0.48 (2.47)	*P* >0.39 (2.96)
	L. holbrooki	0.88 (0.72, 1.09)	–2.61 (–3.01, –2.22)	0.95	-	-
	P. guttatus	0.79 (0.62, 1.01)	–2.41 (–2.72, –2.10)	0.94	-	-
	P. obsoletus	0.92 (0.86, 0.98)	–2.63 (–2.77, –2.49)	0.99	-	-
100%	*L. getula*	0.55 (0.34, 0.88)	–2.41 (–2.80, –2.02)	0.97	*P* >0.16 (5.12)	*P* >0.39 (2.99)
	L. holbrooki	0.85 (0.68, 1.07)	–2.85 (–3.27, –2.43)	0.93	-	-
	P. guttatus	0.82 (0.68, 0.99)	–2.76 (–3.01, –2.51)	0.97	-	-
	P. obsoletus	0.91 (0.85, 0.97)	–2.88 (–3.28, –2.73)	0.99	-	-

Values are based on reduced-major-axis regressions on log$_{10}$ data with snake species as a categorical variable (muscle CSA=species×mass). Slope and intercept confidence limits (95%) are in parentheses. Comparisons between species for each section of the body are for differences between slopes (species×mass) and elevations (species+mass), and are given by *P* at each SVL site with test statistics in parentheses. Muscle CSA was measured in cm^2 and mass in g.

CHAPTER IV

Quantitative Axial Myology in two Constricting Snakes: *Lampropeltis holbrooki* and *Pantherophis obsoletus*

DAVID A. PENNING[1,2]

[1]Department of Biology, University of Louisiana at Lafayette, Lafayette, Louisiana, USA 70504- 43602

[2] Corresponding author: David Penning, Department of Biology and Environmental Health, Missouri Southern State University, Joplin, Missouri, USA 64801, davidapenning@gmail.com

Abstract

A snake's body represents an extreme degree of elongation with immense muscle complexity. Snakes have approximately twenty-five different muscles on each side of the body at each vertebra. These muscles serially repeat, overlap, interconnect, and rarely insert parallel to the vertebral column. The angled muscles mean that simple measurements of anatomical cross-sectional area (ACSA, perpendicular to the long-axis of the body) serve only as proxies for the physiological cross-sectional area (PCSA, area perpendicular to the muscle fibers), which determines muscle force. Here, I describe and quantify the musculature of two intraguild constrictors: speckled kingsnakes (*Lampropeltis holbrooki*) and western ratsnakes (*Pantherophis obsoletus*) whose predation performance varies considerably. Kingsnakes can produce significantly higher constriction pressures compared to ratsnakes of similar size. In both snakes, I carefully describe the anatomy and complexity of the muscles and tendons, identify a new lateral muscle, and provide some of the first quantitative measures of individual muscle and whole-body PCSA. Furthermore, I compare measurements of ACSA to measurements of PCSA. There was no significant difference in muscle PCSA between kingsnakes and ratsnakes. There is, however, a strong relationship between ACSA and PCSA measurements. I could not identify a significant difference in the musculature between kingsnakes and ratsnakes that explains their different levels of constriction performance.

Unmeasured components of muscle function, such as endurance and contractile force, might account for differences in performance between these two intraguild competitors. Alternatively, behavioral use of the musculature (i.e., constriction postures, proportions of the body used, etc.) might explain differences in constriction performance.

Introduction

For vertebrates, movement is brought about through the use of musculature that spans the vertebral and appendicular skeletal elements (MacIntosh et al., 2006; Schilling, 2011). The absence of limbs in snakes relegates all of their movements to bending and twisting movements of the head, vertebrae, ribs, and skin (Mosauer, 1935). While the general body plan of snakes may appear simple, it represents an extreme degree of elongation (Mosauer, 1935; Jayne and Riley, 2007) with immense diversity in form and function (Greene, 1997; Cundall and Greene, 2000; Lillywhite, 2014). The muscle anatomy of snakes is highly derived and extraordinarily complex (Mosauer, 1935), so much so that snakes have been excluded from comparative work on the evolution of vertebrate musculature (Schilling, 2011).

Investigations into snake musculature span centuries (Tyson, 1682–83; Nicodemo, 2012), but few accounts have evaluated the anatomy of multiple muscles within a single species (Gasc, 1981; Mosauer, 1935). Many muscles span only one joint, but several muscles span multiple joints with tendons of one to over thirty vertebrae in length that connect to skeletal elements, connective tissues, other muscles, and skin (Gasc, 1981; Jayne, 1982; Mosauer, 1935). This arrangement of segmental muscles and tendons with complex interconnections allows sophisticated control of movements that involve few to many joints along the body (Jayne, 1988; Moon, 2000a,b; Young, 2010; Young and Kardong, 2010). Historically, snake musculature was generally grouped by clade (boid, viperid, and colubrid type anatomy; Mosauer, 1935), but this categorization is oversimplified (Auffenberg, 1958, 1966; Gasc, 1981), and we lack anatomical descriptions for most snake species. Snakes are an extraordinarily diverse and important group of vertebrates (Greene, 1997; Lillywhite, 2014), yet we have a very limited understanding of their musculoskeletal anatomy and function. Considering the paucity of anatomical studies, the importance of anatomy to performance (Herrel et al., 2008), and the continued need for detailed morphological data (Pyron, 2015), investigations describing and especially quantifying the musculature of snakes are desperately needed in order to better understand evolution, diversity, function, and ecology.

Much of the recent work on muscle anatomy in snakes has focused on the linkages between muscle cross-sectional area and measures of performance (Lourdais et al., 2005; Jayne and Riley, 2007; Herrel et al., 2011; Penning and Moon, 2016). Muscle cross-sectional area is used as an indicator of muscle force production and is often measured by bisecting specimens (or through imaging techniques on live animals) perpendicular to the vertebral column. However, snake muscles rarely insert parallel to the vertebral column (Mosauer, 1935; Gasc, 1981), making measurements based on simple whole-body cross-sections (i.e., anatomical cross-sectional area, ACSA) only approximations of the force-producing capacity of the musculature. Physiological cross-sectional area (PCSA, which is the area perpendicular to the muscle fibers) is the primary determinant of muscle force production (MacIntosh et al., 2006) and can be very different from ACSA. However, to my knowledge, there are no published measurements of PCSA of axial muscles for any snake.

Here, I describe and quantify the muscle anatomy of two colubrid snakes, speckled kingsnakes (*Lampropeltis holbrooki*) and western ratsnakes (*Pantherophis obsoletus*). A notable interaction occurs between these snakes: kingsnakes can capture, constrict, and fully ingest larger ratsnakes (Jackson et al., 2004). Kingsnakes and ratsnakes are both powerful constrictors, but kingsnakes can produce significantly higher constriction pressures than ratsnakes, despite having similar ACSAs (Penning and Moon, 2016). Whether or not any other muscular differences underlie these different levels of constriction performance is currently not known. To test for any such differences, I quantify and compare PCSAs for nine different muscles in these species. Lastly, I compare previously reported ACSA data (Penning and Moon, 2016) to the new PCSA data (this study).

Materials and Methods

The snakes I used for anatomical investigations (*L. holbrooki*=7, *P. obsoletus*=9) came from museum and personal collections (Table 3.1). Snakes are known to exhibit longitudinal variation in the anatomy of homologous muscles (Pregill, 1977; Nicodemo, 2012), so I analyzed the musculature at 50% snout–vent length (SVL; Jayne, 1982). Snakes were fixed in formalin and stored in a solution of 70% isopropyl alcohol (Simmons, 2015). Dehydration due to preservation may affect muscle mass but the relative water loss will be the same in all muscles (Herrel et al., 2014). Using neurosurgical tools, and a stereoscopic microscope (Meiji; 3.5–45X magnification) illuminated with a Fisher Scientific fiber optic ring lamp, I isolated, measured, and removed as many individual muscles from each specimen as possible (for a total of nine different muscles, identified below in the results). Prior to removal, I measured linear dimensions of each muscle with Mitutoyo digital calipers (0.01 cm). After removal, I dried each muscle by gentle blotting with paper towel and weighed it on a Mettler AE 50 digital scale (to 0.0001 g). I report average values whenever I was able to remove more than one segment of a muscle.

Measurements of muscle anatomy often include muscle mass, muscle length, and physiological cross-sectional area (Gans and Bock, 1965; Gans and De Vries, 1987; Lieber and Friden, 2000) calculated from the measurements described above (Alexander and Vernon, 1975; Sacks and Roy, 1987). Because all muscles had long parallel fibers, I calculated PCSA by dividing muscle mass by the product of muscle density (1.06 g/cm^3 from Mendez and Keys, 1960) and muscle length (Alexander and Vernon, 1975; Sacks and Roy, 1987). I used whole-muscle length instead of fiber length to calculate conservative measures of PCSA (Herrel et al., 2014). I also measure and report tendon lengths.

I follow the terminology of Gasc (1981) and Cundall (1982), and use the terms insertion, anterior, and posterior to avoid confusion when describing tendons (Moon, 2000a). In addition to linear measures, I report muscle and tendon lengths based on the number of vertebrae they spanned, including the vertebrae of insertion (Jayne, 1982). In addition to these quantitative measures, I give qualitative descriptions of muscle anatomy, insertion sites, and interconnections where the information adds to the previous literature. Lastly, I compare the ACSA data for the same species from Penning and Moon (2016) to my new measures of PCSA. Penning and Moon (2016) calculated ACSA at 20% SVL intervals. In order to estimate ACSA at 50% SVL, I averaged the ACSA values from 40 and 60% SVL.

Statistical Analysis

For quantitative comparisons, I log-transformed all data prior to analyses. Although I used adult individuals in all of my dissections, kingsnakes were significantly smaller than the ratsnakes (t_{14}=2.43, P<0.03). To account for size variation, I incorporated size (SVL) into models where body size would affect dependent variables. Because of the variation in the costocutaneous superior and inferior muscle insertions, and their miniscule size, I cannot be certain that I removed all of the muscle tissue. Therefore, I report their values (Table 3.2) but do not include them in quantitative analyses. To compare PCSA of all individually measured muscles between kingsnakes and ratsnakes while accounting for body size, I used a repeated-measures ANCOVA (multivariate approach; Vasey and Thayer, 1987). I treated each muscle as the repeated measure, species as a categorical independent variable, and SVL as a covariate. Because serially repeated muscle segments overlap along the body, multiple segments of each muscle contribute to the ACSA for each muscle measured from whole-body cross sections. Therefore, to compare the ACSA measurements from whole-body cross-sections to equivalent PCSA measurements, I needed to determine PCSA for the same number of overlapping muscle segments that contributed to the ACSA for each muscle in the whole-body cross sections. To do this, I multiplied individual muscle PCSA by the number of segments that overlap in a single whole-body cross section on one side of the body and then doubled the values to create a whole-body value. With these data, I performed the same analysis as above but with this calculated whole-body PCSA as the repeated-measures variable. To compare relative muscle length (muscle length divided by the sum of muscle and tendon length; Ruben, 1977), I performed a repeated-measures ANOVA with relative length of each muscle as the repeated measure and species as the categorical variable. Because some muscles lacked tendons (Table 3.2), I only included muscles in which tendons were present in every specimen.

To compare the scaling of PCSA between kingsnakes and ratsnakes, as done by Penning and Moon (2016), I summed the whole-body PCSAs determined above for each of the four largest, interconnected muscle groups (semispinalis–spinalis complex, longissimus dorsi, and iliocostalis) and used a reduced-major-axis (RMA) regression version of ANCOVA (Smith, 2009; Wharton et al., 2012) with SVL as the covariate and species as a categorical variable. I used RMA regression to measure scaling based on the methods used in similar studies (Herrel et al., 2011; Penning and Moon, 2016) and the general recommendations for regression analyses based on regression symmetry (Smith, 2009). Neither variable in this analysis is dependent upon the other (symmetrical relationship). The use of ordinary least squares regression will over/underestimate the relationship depending upon which variable is on the X and Y axis, making RMA regression the more appropriate analysis (Smith, 2009). Lastly, I quantified the relationship between whole-body ACSA (Penning and Moon, 2016) and whole-body PCSA for the four largest muscles by using another RMA ANCOVA with species as the categorical variable in order to quantify the relationship between these two measures. I performed analyses in JMP Pro (11.00.0) and RStudio (version 0.99.441) software and consider tests significant at $P<0.05$.

Results

For all 16 specimens, I was able to isolate and remove 250 individual muscle segments representing nine different muscles (1–4 segments of each muscle per snake). The remaining muscles were either absent or were so small and complex that I was not able to remove them intact. Previous work has shown the incredible complexity of snake axial musculature, and my dissections revealed even greater complexity involving new interlinkages, muscle slips, and a previously undescribed muscle. Below, I describe each muscle for both kingsnakes and ratsnakes. I quantify muscle and tendon lengths as noted above and in the text wherever additional details are necessary, and I provide descriptive statistics (Table 3.2). With the exception of the levator costa, each muscle consists of overlapping segments that make up longitudinal muscle columns.

Descriptions

Epaxial Muscles

Each multifidus (MF) is a single triangular-shaped muscle that inserts posteriorly on the neural spine via a short tendon and anteriorly onto vertebrae via two or three heads (Fig 3.1, Table 3.2). In kingsnakes, the MF has two anterior heads, a medial head that inserts onto a vertebra dorsal and posterior to the lateral head, which slopes downward to insert onto the next anterior vertebra. In ratsnakes, the anterior insertion of the MF is more complex, spanning up to three vertebrae and having three distinct heads that insert onto separate vertebrae, with the anterior-most head being the smallest.

The semispinalis–spinalis (SSP–SP) complex is a two-part muscle with multiple insertions and connections to other muscles (Fig 3.1, Table 3.2). The complex inserts anteriorly onto a vertebra by a long, ribbon-like tendon; the long anterior tendons of adjacent segments overlap and are intertwined within thick connective tissue (Moon, 2000a) that can seem stronger than the tendon itself. The muscle tissue spans 6–7 vertebrae in ratsnakes and 5–6 vertebrae in kingsnakes; these lengths reflect muscle tissue of both the SSP–SP overall and just the SSP part because it is longer than the SP part. Near the anterior end of the muscle tissue, the SSP–SP complex splits into two slips that become separate at their posterior ends. The SP slip inserts dorsally on the posterior tendon of the multifidus; the posterior tendon of the multifidis spans ca. two vertebrae overall. The SSP slip continues posteriorly onto a tendon that is continuous with the longissimus dorsi (Fig 3.1). Data in Table 3.2 for the posterior tendon of the SSP–SP complex refers only to the tendon of the SSP; there is no visible posterior tendon for the SP (Fig 3.1).

The longissimus dorsi (LD) is a sheet-like muscle that connects anteriorly via a forked tendon to both the SSP slip of the SSP–SP complex and the iliocostalis (IL), and posteriorly to a vertebra (Fig 3.1, Table 3.2). The posterior insertion of the LD has two heads that insert across two vertebrae onto the lateral side of each vertebra. The anterior tendon of the MF forms a sheet that forks anteriorly, connecting dorsally with the posterior tendon of the SSP slip of the SSP–SP complex and ventrally with the IL. The sheet-like tendon of the LD is an integral part of the connective tissue partitions among the three major epaxial muscle groups (SSP–SP complex, LD, and IL). The shared anterior tendon of the LD joins the SSP–SP complex to the IL, forming a long musculoskeletal chain (spanning on ca. 27 vertebrae in ratsnakes and 25 vertebrae in kingsnakes; Fig 3.1). Data in Table 3.2 for the anterior tendon of the LD refers to the length of the broad, sheet-like tissue before the tissue splits into two distinct tendons (Fig 3.1).

The iliocostalis (IL) is a long, slender muscle. Each segment of the IL muscle connects via tendons posteriorly to the LD and anteriorly to a rib (Fig 3.1, Table 3.2). The tendon connecting the LD to the IL passes through, and contributes to, a tough sheath of connective tissue that forms a distinct septum between the LD and IL columns in both species. From the anterior edge of LD muscle tissue to the posterior edge of IL muscle tissue, the ventral fork of the LD tendon spans up to 4.5 vertebrae in ratsnakes and kingsnakes. In ratsnakes, the anterior and posterior segments of the IL are similar in length and each span an average of seven vertebrae (Fig 3.1, 3.2). An intermediate tendon is less than one vertebra in length. As in some other colubrids, this intermediate tendon does not have direct connection to the ribs (Mosauer, 1935), but is encased in connective tissue that connects the serially repeating segments to one another. There is no intermediate tendon for the IL of kingsnakes (Fig 3.1). In ratsnakes, both segments of the IL have two small slips of fibers that angle dorsally to the next anterior segment and ventrally to the next posterior segment, braiding the adjacent segments together and making them difficult to separate in dissection (Fig 3.2). Kingsnakes show a similar but simpler pattern. Ratsnakes have four total slips that come off of the IL (two from each separate IL segment). Kingsnakes have only two slips that come off the single IL segment.

Hypaxial Muscles

The levator costa (LC) is a surprisingly large muscle (in mass) despite its short length (Table 3.2; Fig 3.3); it connects a vertebra to a rib and is presumed to elevate and protract the rib. Of the muscles that I describe, this is the only one that does not overlap along the body. This muscle can be accessed by clipping the anterior tendons of the IL muscle column and reflecting the entire IL muscle column laterally (Fig 3.3). The LC inserts anteriorly via a small tendon onto the prezygapophysial process of a vertebra and widens posteroventrally before inserting onto the surface of a rib approximately one-quarter of the distance from its base to rib tip.

The supracostalis lateralis superior (SLS) and supracostalis lateralis inferior (SLI) muscles connect nearby ribs; the muscle tissue runs posteroventrally and anterodorsally between insertions. In ratsnakes, both muscles often had short anterior and posterior tendons (Table 3.2). In kingsnakes, there were no obvious posterior tendons for either muscle, and there was no obvious anterior tendon on the SLI; the SLS had a short anterior tendon. Both muscles in both species often had muscle slips that interconnected adjacent segments. In both ratsnakes and kingsnakes, there was a previously undescribed muscle that separates the SLS and SLI (Fig 3.4). Considering its location between the two muscle groups, I describe it as the supracostalis lateralis centralis (SLC). The SLC fibers run the opposite direction (Fig 3.4) with fibers inclining anteroposteriorly. The SLC spans up to six vertebrae with small slips inserting onto each rib along the length of the muscle. As the SLC extends posteriorly, the segments of this muscle appear to run laterally then dorsally and then medially, giving a loosely twisted or rotated appearance. Without removal, the SLC is easily viewed after removing the costocuaneous muscles (Fig 3.4).

The costocutaneous superior (CCS) and inferior (CCI) are small muscles that connect the ribs to the skin, without obvious tendons. Segments of the CCS run downward posteriorly from ribs and attach diffusely to the skin (Fig 3.4). The CCI runs counter to the CCS; CCI segments run slightly upward anteriorly from ribs and attach diffusely to the skin.

Quantitative Analyses

For both kingsnakes and ratsnakes, the PCSA values for individual (single-segment) muscles ranged on average from 0.0038 to 0.039 cm^2 (Table 3.2). Of the individually measured muscles, the LC (kingsnakes=0.024 cm^2, ratsnakes=0.039 cm^2) had the highest PCSA; however, individual segments of the LC span only one vertebra and do not overlap along the body (Fig 3.3). For the single-segment PCSAs, there was no significant interaction between muscle type and species ($F_{6,7}=0.37$, $P>0.86$). While there was variation in PCSA across muscles (Table 3.2), there was no significant difference in PCSA between species ($F_{1,12}=0.05$, $P>0.82$; Fig 3.5A). In a whole-body muscle column, the total PCSA for all overlapping segments of a given muscle visible in the cross-section (on both sides of the vertebral column) was much higher in the longer epaxial muscles. The LD had the highest total column PCSA in kingsnakes (0.13 cm^2), while the IL had the highest total column PCSA in ratsnakes (0.24 cm^2). The result for total column PCSA for each muscle is similar to the results for single-segment PCSA. There was no significant interaction between species and muscle type ($F_{6,7}$=0.45, P>0.82). As with individual PCSAs, there was variation in total column PCSA (Fig 3.5B) but there was no significant difference in total column PCSA between species ($F_{1,12}$=0.21, P>0.65; Fig 3.5B).

The relative lengths of muscle tissue and corresponding tendons varied among muscles but not between species. There was no significant interaction between species and muscle identity ($F_{4,11}$=2.37, P>0.11) and relative muscle length was not significantly different between species ($F_{1,14}$=0.26, P>0.61). In general, short muscles tended to have short tendons, and long muscles tended to have long tendons, although there were some exceptions to these generalizations in both epaxial and hypaxial muscles (e.g., long posterior tendons of the MF and essentially undetectable posterior tendon of the LD). Of the major interconnected epaxial muscles, the SSP–SP complex had the shortest relative muscle length (ratsnakes=0.32±0.006, kingsnakes=0.31±0.02) while the IL had the longest (kingsnakes=0.80±0.01, ratsnakes=0.80±0.01).

To compare the scaling of PCSA between kingsnakes and ratsnakes (as in Penning and Moon, 2016), I summed the PCSAs of the four largest, interconnected muscle groups (SSP–SP complex, LD, and IL) and used a RMA regression version of ANCOVA (Smith, 2009; Wharton et al., 2012) with SVL as the covariate and species as a categorical variable. The summed PCSA of these four muscles was significantly related to SVL (PCSA=3.12×SVL–6.96, R^2=0.46, P<0.004; Fig 3.6). However, there was no differences between the slopes (P>0.48) or intercepts (P>0.89) of the kingsnake and ratsnake scaling patterns.

Eight snakes (four *P. obsoletus* and four *L. holbrooki*) from my dissections (and PCSA calculations) were used in the ACSA measurements of Penning and Moon (2016) and can therefore be compared quantitatively to the PCSAs in this study. The PCSA values were 55–

86% of the ACSA values, and no PCSA calculation was greater than the ACSA calculation, as would be expected for parallel-fibered muscles. The slopes ($P>0.25$) and intercepts ($P>0.86$) were not significantly different between kingsnakes and ratsnakes for these two measures. The overall relationship between ACSA and PCSA was positive (PCSA=0.95×ACSA–0.20, $R^2=0.80$, $P<0.002$) and isometric based on the 95% confidence intervals of the slope (slope CI=0.37–1.53, Fig 3.7).

Discussion

Descriptive Anatomy

In general, the epaxial muscle morphology of both ratsnakes and kingsnakes was similar to that previously described for other colubrid snakes (Mosauer, 1935; Gasc, 1981; Moon, 2000a; Jayne and Riley, 2007), but with some additional complexity. Longer muscles tended to have longer tendons and, except for the LC, all muscles overlapped along the axial system. Ratsnakes had an intermediate tendon to the IL whereas kingsnakes did not. Both kingsnakes and ratsnakes had more complex IL muscles than previously reported, with slips interconnecting adjacent segments and giving the IL a complex braided or mesh-like organization. To my knowledge, these slips have not been discussed as discrete muscle slips in the literature (Mosauer, 1935; Auffenberg, 1958; Pregill, 1977; Gasc, 1981; Moon, 2000a; Jayne and Riley, 2007); however, unnamed interconnecting fibers have been reported in several studies. Pregill (1977) noted that he could not separate individual IL segments from one another in *Coluber constrictor,* contrasting with the results of Mosauer (1935). Gasc (1981) did not discuss any interlinkages between adjacent IL segments, but they appear to be partially illustrated for *Xenodon merremi* (Fig 48 of Gasc, 1981) and perhaps *Hierophis viridiflavus* (Fig 38 of Gasc, 1981).

I describe a new hypaxial muscle in both species that to my knowledge has not been previously reported: the supracostalis lateralis centralis (Fig 3.4). Based on the location, size, and insertion of this muscle, it likely aids in stiffening or adduction and retraction of the ribs and in lateral flexion of the vertebral column. For example, it may work in concert with the SLS and SLI in locomotion (Gasc et al., 1989). The last muscle that warrants brief discussion is the LC. Very little is said of this muscle in the literature (Mosauer, 1935; Gasc, 1981), although it is hypothesized to be of major functional importance in “snake-like” forms (Gasc, 1981). This muscle has the highest PCSA of any individual muscle segment (Table 3.2); based on its size and location, it likely abducts and protracts the ribs, and hence plays an important role in locomotion, constriction, and other feeding movements. Given the large size and likely importance of this muscle, its function should be studied *in vivo* using electromyography, although instrumenting it might be challenging because of its position underneath the LD and IL.

Previous descriptions of snake musculature sometimes gave conflicting results (Mosauer, 1935; Auffenberg, 1958, 1966; Pregill, 1977, Ruben, 1977; Jayne, 1982). Considering the intraspecies variation observed in muscle and tendon lengths (Jayne, 1982; Table 3.2 this study), and the lack of ongoing anatomical work, it is currently impossible to separate the variation in anatomy caused by differences in dissection procedures from actual biological variation. In addition to differences in simple anatomical counts and qualitative descriptions, previous work proposes stark differences in the hypothesized functional consequences of anatomical complexity. Pregill (1977) stated that muscle variation was “unremarkable” with “minor” differences being additional tendons and interlacing muscles in colubrid snakes; Gasc (1981) viewed these differences much differently and hypothesized that differences in muscle organization would have functional (and taxonomic) significance. Furthermore, Herrel et al. (2008) showed that minor changes in muscle angle have significant consequences to performance in two interconnected muscles. Therefore, considering the incredible complexity and variation in snake musculature, it is likely that different interconnections and linkages will reveal differences in performance that are worth investigating and quantifying.

Quantitative Comparisons

Previous work has quantified snake musculature by using anatomical cross-sectional area as an indicator of the amount of muscle available for use (Lourdais et al., 2005; Jayne and Riley, 2007; Herrel et al., 2011; Penning and Moon, 2016). However, it is important to measure PCSA because it is a better indicator of the force-producing capacity of a muscle (Sacks and Roy, 1987; MacIntosh et al., 2006) and therefore offers a better foundation for understanding and predicting functions. To my knowledge, no prior quantitative data exist for PCSA in any snake, and therefore no comparisons of the actual force-producing capacities among snakes have been made. Penning and Moon (2016) showed that three species of kingsnakes are capable of producing significantly higher peak constriction pressures on rodent prey compared to three species of ratsnakes, despite all having similar ACSA of the major epaxial muscles. Penning and Moon (2016) noted that ACSA may not completely account for muscle-level differences among these species. Although there were differences in individual muscle masses, lengths, and PCSAs in this study (Table 3.2), there were no differences between *P. obsoletus* and *L. holbrooki* in the PCSA of different muscles when compared as individual muscles or whole cross-sections (Fig 3.5). With no significant differences in ACSA (Penning and Moon, 2016), PCSA, and relative muscle and tendon lengths, there is currently no identifiable anatomical mechanism that explains how kingsnakes exert significantly higher constriction pressures than ratsnakes. Similar muscle PCSA suggests that functional differences between these species, such as maximum force-producing abilities and feeding performance, should be minor. However, *in vivo* tests of muscle force and endurance could help explain the notable difference in whole-body constriction performance. Differences in muscle-level performance would indicate physiological differences (i.e., muscle stress, endurance, etc.) between these two intraguild competitors that ACSA and PCSA are not capable of identifying. Alternatively, similar muscle force and endurance in ratsnakes and kingsnakes would suggest that the ability of kingsnakes to produce higher peak constriction pressure is driven more by behavior (e.g., coil posture) than physiology (muscle-level performance).

ACSA and PCSA scale isometrically and there is a strong relationship between these two measurements (r^2=0.80; Fig 3.7). While there is a predictable relationship between these measures, it is important to note that the regression model does not pass through the origin (intercept ≠ 0). Therefore, ACSA overestimates PCSA in kingsnakes and ratsnakes. Nevertheless, these results suggest that simple ACSAs may be adequate proxies for predicting or qualitatively comparing whole-body performance capacity, particularly considering the difficulty of quantifying PCSA. However, simple whole-body ASCA is probably not a good proxy for quantitative predictions or comparisons of maximum forces or performance levels because ACSA overestimates the force-producing architecture of a muscle (PCSA).

Acknowledgments

I thank Brad Moon for his helpful insight and guidance on snake musculature and for providing access to several specimens. I also thank Karen Smith for allowing access to key pieces of equipment. Partial funding was provided by the Louisiana Board of Regents Doctoral Fellowship, the Department of Biology and Graduate Student Organization at the University of Louisiana at Lafayette, the Louisiana Department of Wildlife and Fisheries Rockefeller State Wildlife Scholarship, and the Kansas Herpetological Society.

Literature Cited

Alexander RM, Vernon A (1975) The dimensions of the knee and ankle muscles and the forces they exert. *J Hum Mov Stud* **1**, 115–123.

Auffenberg W (1958). The trunk musculature of *Sanzinia* and its bearing on certain aspects of the myological evolution in snakes. *Museum of Comparative Zoology* **82**, 1–12.

Auffenberg W (1966) The vertebral musculature of *Chersydrus* (Serpentes). *Quart J Florida Acad Sci* **29**, 155–162.

Cundall D (1982) Functional Morphology. In *Snakes: Ecology and Evolutionary Biology*. Seigel RA, Collins JT, Novak SS (eds). Pp. 106–140. New York, McGraw-Hill.

Cundall D, Greene HW (2000) Feeding in Snakes. In *Feeding: Form, Function, and Evolution in Tetrapod Vertebrates.* Schwenk K (ed), pp. 293–333. California, Academic Press.

Gans C, Bock WJ (1965) The functional significance of muscle architecture: a theoretical analysis. *Adv Anat Embryol Cell Biol* **38**, 115–142.

Gans C, De Vries F (1987). Functional bases of fiber length and angulation in muscle. *J Morphol* **192**, 63–85.

Gasc, J-P (1981) Axial Musculature. In *Biology of the Reptilia* Volume 11. Gans C, and Parsons TS (eds.), pp. 355–435. Academic Press, New York.

Gasc J-P, Cattaert D, Chasserat C, Clarac F (1989) Propulsive action of a snake pushing against a single site: its combined analysis. *J Morph*ol **201,** 315–329.

Greene HW (1997) *Snakes: The Evolution of Mystery in Nature*. Berkeley, University of California Press.

Herrel A, Schaerlaeken V, Ross C, Meyers J, Nishikawa K, Abdala V, Manzano A, Aerts P (2008) Electromyography and the evolution of motor control: limitations and insights. *Integr Comp Biol* **48**, 261–271.

Herrel A, Huyghe K, Oković P, Lisičić D, Tadić Z (2011) Fast and furious: effects of body size on strike performance in an arboreal viper *Trimeresurus (Cryptelytrops) albolabris*. *J Exp Zool* **315**, 22–29.

Herrel A, Redding CL, Meyers JJ, Nishikawa KC (2014) The scaling of tongue projection in the veiled chameleon *Chamaeleo calyptratus*. *Zoology* **117**, 227–236.

Jackson K, Kley NJ, Brainerd EL (2004) How snakes eat snakes: the biomechanical challenges of ophiophagy for the California kingsnake, *Lampropeltis getula californiae* (Serpentes: Colubridae). *Zoology* **107**, 191–200.

Jayne BC (1982) Comparative morphology of the semispinalis-spinalis muscle of snakes and correlations with locomotion and constriction. *J Morphol* **172**, 83–96.

Jayne BC (1988) Muscular mechanisms of snake locomotion: an electromyographic study of the sidewinding and concertina modes of *Crotalus cerastes, Nerodia fasciata,* and *Elaphe obsoleta*. *J Exp Biol* **140**, 1–33.

Jayne BC, Riley MA (2007) Scaling of the axial morphology and gap-bridging ability of the brown tree snake, *Boiga irregularis*. *J Exp Biol* **210**, 1148–1160.

Lieber RL, Friden J (2000). Functional and clinical significance of skeletal muscle architecture. *Muscle and Nerve* **23**, 1647–1666.

Lillywhite HB (2014). *How Snakes Work: Structure, Function and Behavior or the World's Snakes*. Oxford, Oxford University Press.

Lourdais O, Brischoux F, Barantin L (2005) How to assess musculature and performance in a constricting snake? A case study in the Columbian rainbow boa (*Epicrates cenchria maurus*). *J Zool* **265**, 43–51.

Mendez J, Keys A (1960) Density and composition of mammalian muscle. *Metab Clin Exp* **9**, 184–188.

MacIntosh BR, Gardiner PF, McComas AJ (2006) Skeletal muscle: form and function. 2nd edition. Illinois, Human Kinetics.

Moon BR (2000a). The mechanics and muscular control of constriction in gopher snakes (*Pituophis melanoleucus*) and a king snake (*Lampropeltis getula*). *J Zool* **252**, 83–98.

Moon BR (2000b) The mechanisms of swallowing and the muscular control of diverse behaviours in gopher snakes. *J Exp Biol* **203**, 2589–2601.

Mosauer W (1935) The myology of the trunk region of snakes and its significance for ophidian taxonomy and phylogeny. *Univ Cal Publ Biol Sci* **1**, 81–120.

Nicodemo PI (2012) Longitudinal Variation in the Axial Muscles of Snakes. Master's Thesis. Ohio, University of Cincinnati.

Penning DA, Moon BR (2016 *In Review*). The king of snakes: performance and morphology of intraguild predators (*Lampropeltis*) and their prey (*Pantherophis*). *J Exp Biol*

Pregill GK (1977) Axial myology of the racer *Coluber constrictor* with emphasis on the neck region. *San Diego Soc Nat Hist Trans* **18**, 185–206.

Pyron RA (2015) Post-molecular systematics and the future of phylogenetics. *Trends Ecol Evol* **30**, 384–389.

Ruben JA (1977) Morphological correlates of predatory modes in the coachwhip (*Masticophis flagellum*) and rosy boa (*Lichanura roseofusca*). *Herpetologica* **33**, 1–6.

Sacks RD, Roy RR (1982) Architecture of the hind limb muscles of cats: functional significance. *J Morphol* **173**, 185–195.

Schilling N (2011) Evolution of the axial system in craniates: Morphology and function of the perivertebral musculature. *Front Zool* **8**, 1–19.

Simmons JE (2015) *Herpetological collecting and collections management*. Herpetological Circular NO. 42. Utah, Society for the Studies of Amphibians and Reptiles.

Smith RJ (2009) Use and misuse of the reduced major axis regression for line-fitting. *Am J Phys Anthropol* **140**, 476–486.

Tyson E (1682–83) *Vipera caudi-Sona americana*, or the anatomy of the rattlesnake, dissected at the repository of the Royal Society in January 1682–83. *Phil Trans R Soc* **13**, 25–58.

Vasey MW, Thayer JF (1987). The continuing problem of false positives in repeated measures ANOVA in psychophysiology: a multivariate solution. *Psychophysiology* **24**, 479–486.

Warton DI, Duursma RA, Falster DS, Taskinen S (2012) Smart 3–an R package for estimation and inference about allometric lines. *Methods Ecol Evol* **3**, 257–259.

Young BA (2010) How a heavy-bodied snake strikes quickly: high-power axial musculature in the puff adder (*Bitis arietans*). *J Exp Zool*, **313**, 114–121.

Young BA, Kardong KV (2010) The functional morphology of hooding in cobras. *J Exp Biol* **213**, 1521–1528.

Table 3.1. Whole-body measurements for western ratsnakes, *Pantherophis obsoletus* (R), and speckled kingsnakes, *Lampropeltis holbrooki* (K). SVL, snout–vent length; TL, tail length; BV, body vertebrae; TV, tail vertebrae. Superscript ‘n’ denotes nipped tail; diameter is maximum body diameter.

snake ID	sex	mass (g)	diameter (cm)	SVL+TL (cm)	BV+TV
R1	M	352	2.2	102.5+20.9	237+81
R2	M	662	2.5	131.3+26.1	253+86
R3	F	1047	2.9	170.6+33.1	237+82
R4	F	633	2.7	133.5+29.2	240+84
R5	M	824	2.9	153.3+30.0	239+80
R6	F	1274	3.3	150.9+33.7	230+88
R7	M	850	2.7	143.3+30.6	232+91
R8	F	642	2.8	141.4+28.8	233+91
R9	M	678	2.6	145.2+27.5	244+81
K1	F	634	2.6	131.5+11.7^{n}	212+33^{n}
K2	M	534	2.3	131.3+16.4^{n}	217+45^{n}
K3	F	571	2.5	116.7+16.6	207+49
K4	F	492	2.7	113.0+11.3	214+35
K5	F	506	2.8	115.5+10.9	217+34
K6	F	612	2.6	129.5+14.2^{n}	210+31^{n}
K7	F	601	2.5	126.2+10.2^{n}	211+27^{n}

Table 3.2. Gross anatomical measurements for the musculature of *Pantherophis obsoletus* (n=9) and *Lampropeltis holbrooki* (n=7). Measurements are mean ± standard error. Note that the kingsnakes were smaller than the ratsnakes. “Post” indicates posterior, “Ant” indicates anterior, and values in brackets are ranges of vertebrae spanned (V).

Species	Muscle ID	Post Tendon (cm) [V]	Muscle Tissue (cm) [V]	Ant Tendon (cm) [V]	Muscle Mass (g)	PCSA (cm^2)
P. obsoletus	MF	0.8±0.3 [1.5–2]	1.1±0.4 [2–3]	absent	0.033±0.011	0.028±0.009
	SSP–SP	1.5±0.5 [2–3]	3.7±1.2 [6–7]	6.4±2.1 [11–13]	0.054±0.018	0.014±0.005
	LD	absent	3.0±1.0 [5–6.5]	0.8±0.3 [1]	0.051±0.017	0.016±0.005
	IL	1.5±0.5 [2–3.5]	6.0±2.0 [10–18]	1.7±0.6 [3–4.5]	0.060±0.020	0.010±0.003
	LC	absent	1.2±0.4 [1]	0.2±0.1 [0.25]	0.050±0.017	0.039±0.013
	SLS	0.4±0.3 [0–1]	3.7±1.2 [5.5–7]	0.9±0.3 [1–2.5]	0.027±0.009	0.007±0.002
	SLI	0.4±0.1 [0.25–1.25]	3.6±1.2 [5.5–7]	0.7±0.2 [1–1.5]	0.026±0.009	0.007±0.002
	CCS	absent	2.3±0.8 [3.5–4.5]	absent	0.017±0.006	0.007±0.002
	CCI	absent	2.3±0.8 [3–4]	absent	0.013±0.004	0.005±0.002
L. holbrooki	MF	1.1±0.4 [1.5–2]	1.2±0.4 [2]	absent	0.019±0.007	0.014±0.005
	SSP–SP	1.4±0.5 [3]	2.6±0.9 [5–6]	4.7±1.8 [9–11]	0.034±0.013	0.011±0.004
	LD	absent	2.1±0.8 [3.5–5.5]	1.3±0.5 [1]	0.042±0.016	0.013±0.005
	IL	1±0.4 [1–3.5]	5.5±2.1 [8–11]	1.0±0.4 [1.25–2]	0.037±0.014	0.005±0.002
	LC	absent	1.4±0.5 [1]	0.2±0.1 [0.25]	0.040±0.015	0.024±0.009
	SLS	absent	3.9±1.5 [6.5–7]	0.5±0.2 [0–1]	0.027±0.010	0.006±0.002
	SLI	absent	3.6±1.4 [6–7]	absent	0.019±0.007	0.005±0.002
	CCS	absent	1.8±0.7 [3–4]	absent	0.012±0.005	0.005±0.002
	CCI	absent	2.1±0.8 [3–4]	absent	0.009±0.003	0.004±0.001

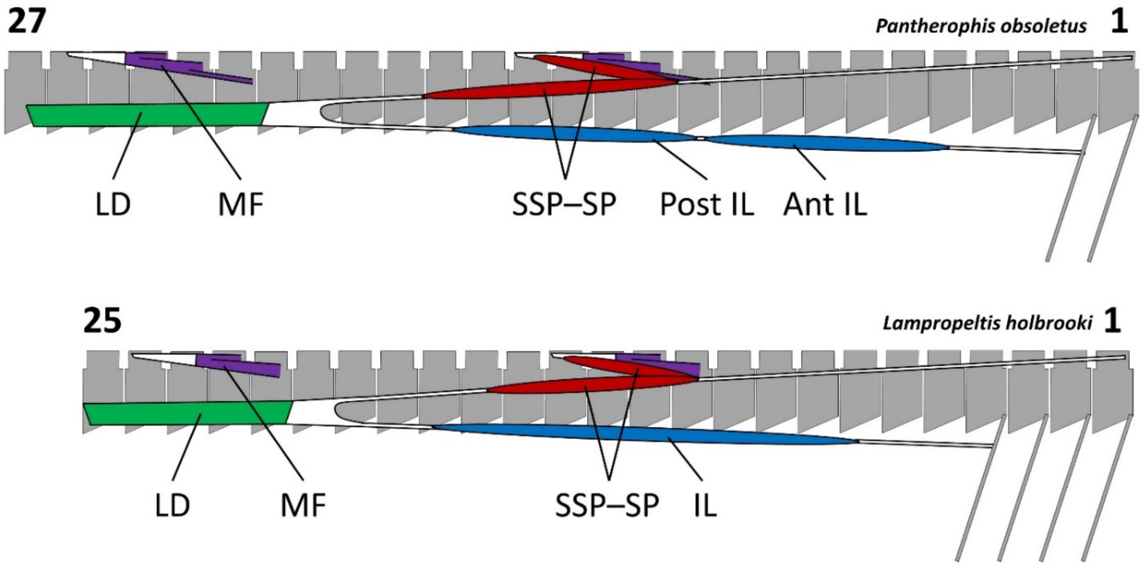

Figure 3.1. Simplified schematic right lateral view of several epaxial muscles in a western ratsnake, *Pantherophis obsoletus* (top) and speckled kingsnake, *Lampropeltis holbrooki* (bottom). Skeletal structure is gray with numbers representing the anterior (1) and posterior (25 and 27) attachment sites for the interlinked epaxial muscles. Colored areas represent contractile tissue and white areas represent tendons. Individual muscles are abbreviated from left to right: longissimus dorsi (LD), multifidus (MF), semispinalis–spinalis (SSP–SP), and iliocostalis (IL).

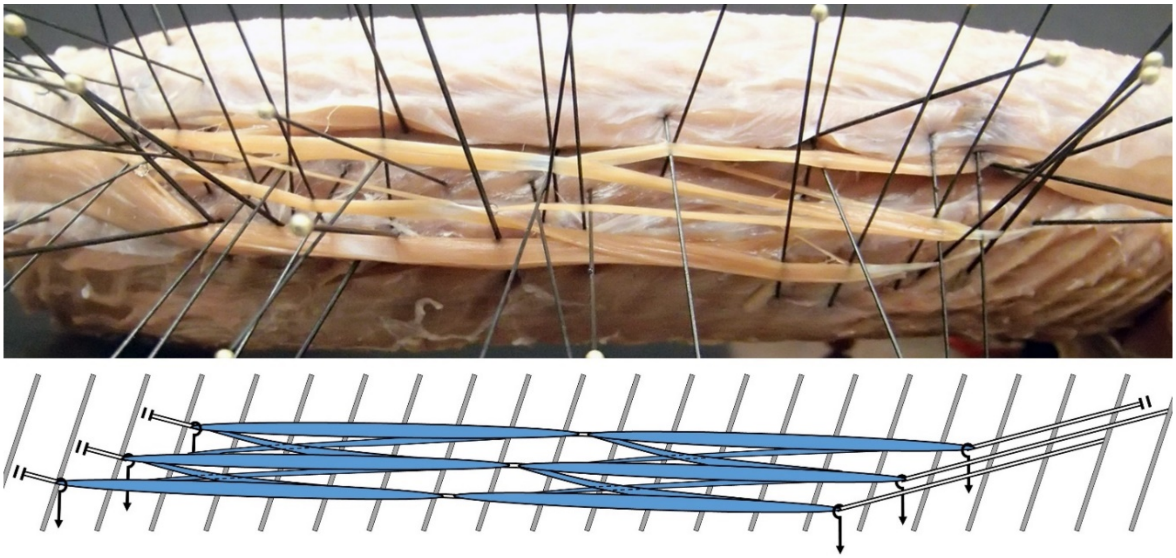

Figure 3.2. Right lateral view of three stretched-out iliocostalis muscle segments and their interconnecting slips in a western ratsnake *Pantherophis obsoletus* (top) and a schematic illustration of the same muscle linkages (bottom). Each muscle is pulled ventrally so that the smaller interlinkages can be seen.

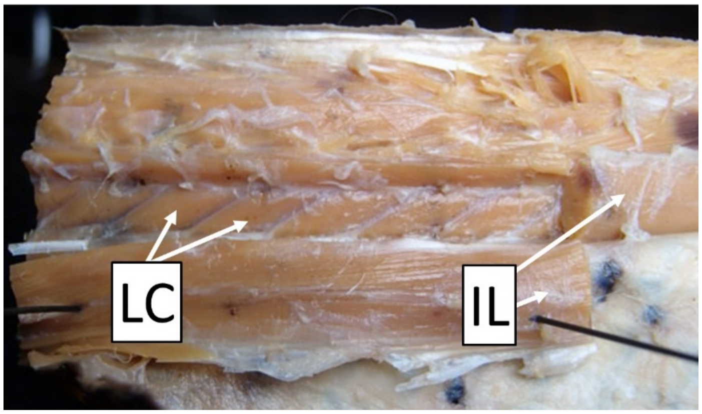

Figure 3.3. Right lateral view of the axial musculature in a western ratsnake *Pantherophis obsoletus*. The large superficial epaxial muscles (semispinalis–spinalis complex, longissimus dorsi, and iliocostalis [IL]) have largely been removed. Portions of the IL have been cut and reflected laterally to show the underlying individual levator costa (LC) muscles.

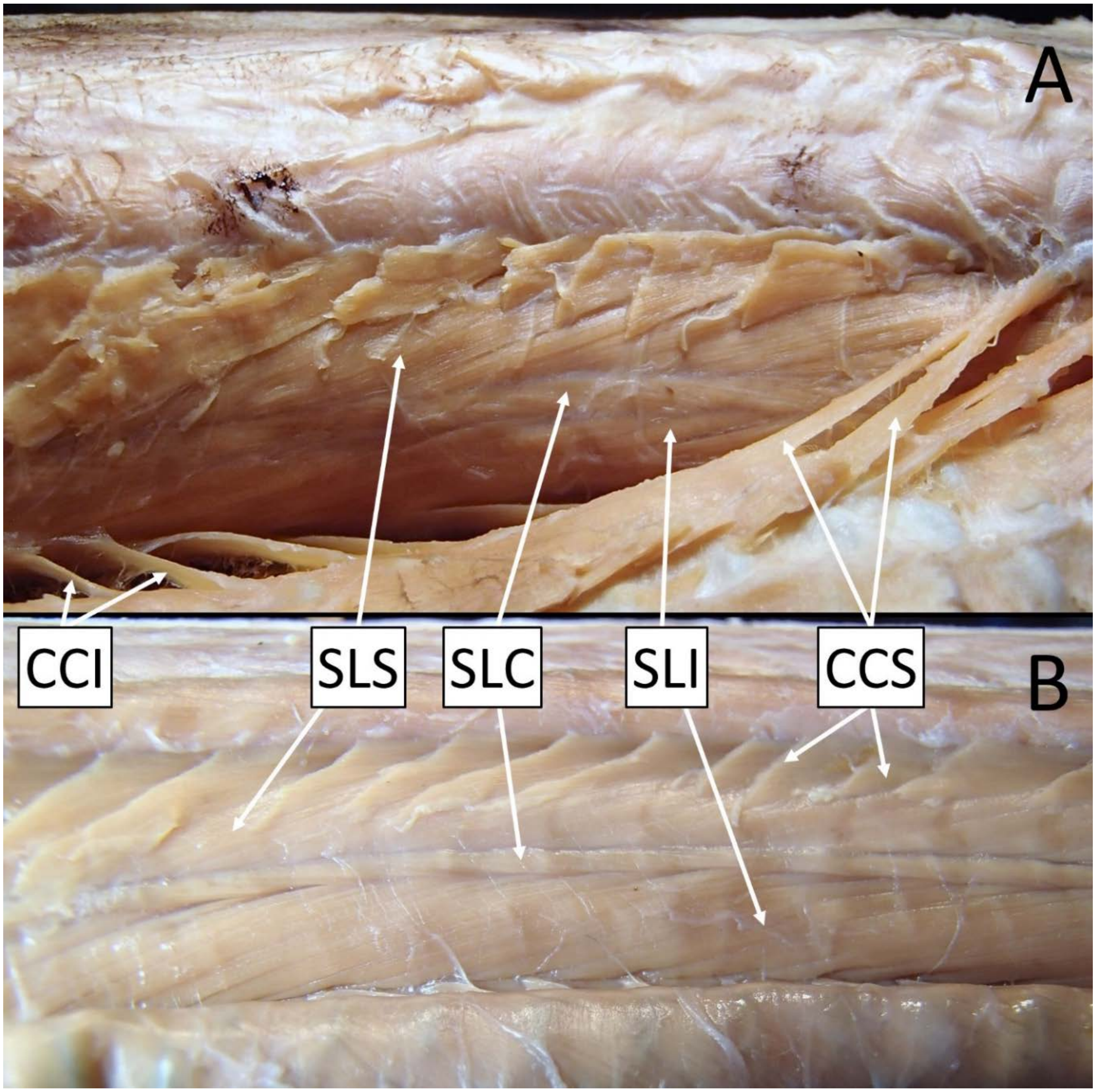

Figure 3.4. Right lateral view of the superficial hypaxial muscles in a western ratsnake, *Pantherophis obsoletus* (A), and a speckled kingsnake *Lampropeltis holbrooki* (B). This view is accomplished by making a mid-dorsal incision through the skin and reflecting it laterally, severing the costocutaneous muscle insertions on the skin and the fascia between the muscle and skin. Individual muscles are abbreviated from left to right: costocutaneous inferior (CCI), supracostalis lateralis superior (SLS), supracostalis lateralis centralis (SLC), supracostalis lateralis inferior (SLI), and costocutaneous superior (CCS).

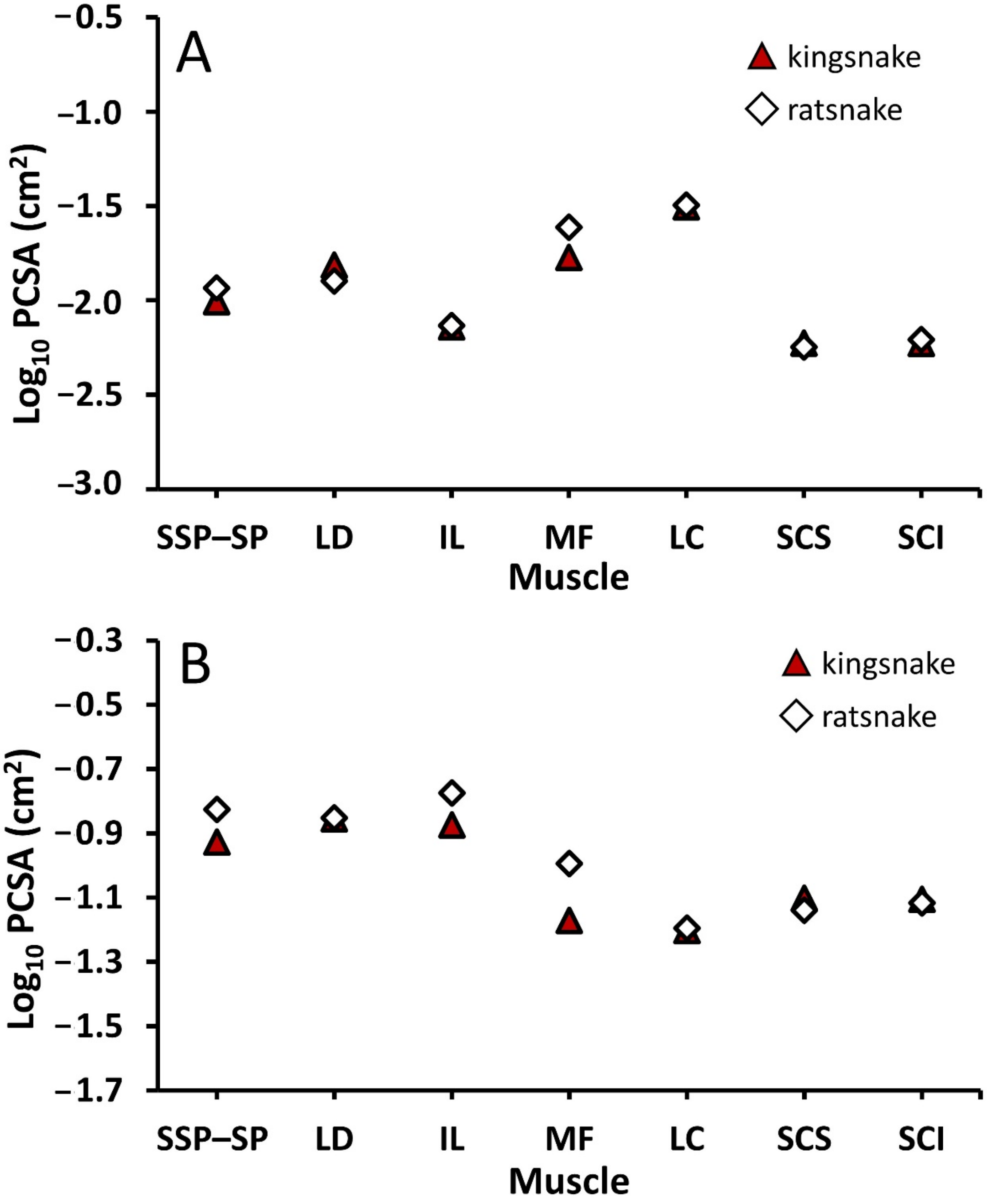

Figure 3.5. Covariate (SVL) adjusted means of $\log_{10}$ physiological cross-sectional area (PCSA) for individual muscles (A) and total column PCSA for each muscle (B) at ca. 50% snout–vent length. Red triangles denote speckled kingsnakes (*Lampropeltis holbrooki*), open diamonds denote western ratsnakes (*Pantherophis obsoletus*). Muscle names are abbreviated: semispinalis–spinalis complex (SSP–SP), longissimus dorsi (LD), iliocostalis (IL), multifidus (MF), levator costa (LC), supracostalis lateralis superior (SCS), and supracostalis lateralis inferior (SCI).

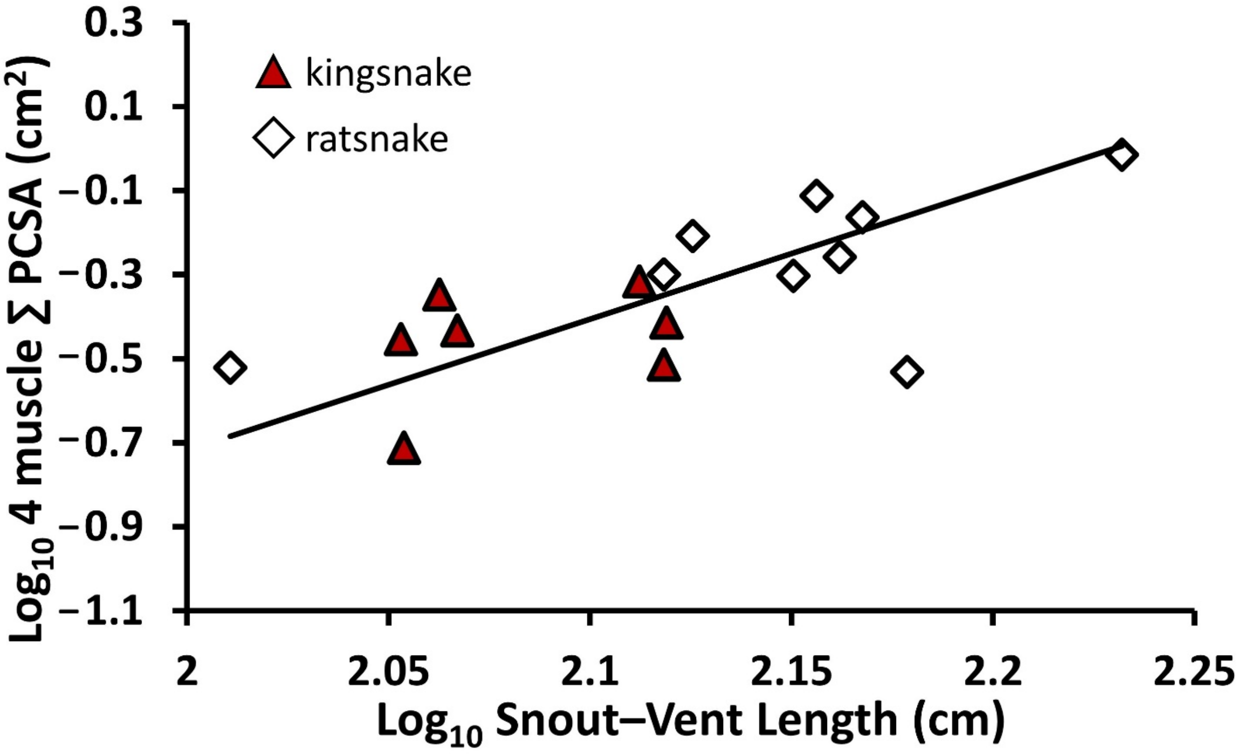

Figure 3.6. Sum of the $\log_{10}$ physiological cross-sectional areas (PCSA) for the four largest epaxial muscles (semispinalis–spinalis complex, longissimus dorsi, and iliocostalis) at ca. 50% snout–vent length (SVL) regressed against $\log_{10}$ total SVL. Red triangles denote speckled kingsnakes (*Lampropeltis holbrooki*), open diamonds denote western ratsnakes (*Pantherophis obsoletus*), and the black line represents the reduced major axis regression model (see results).

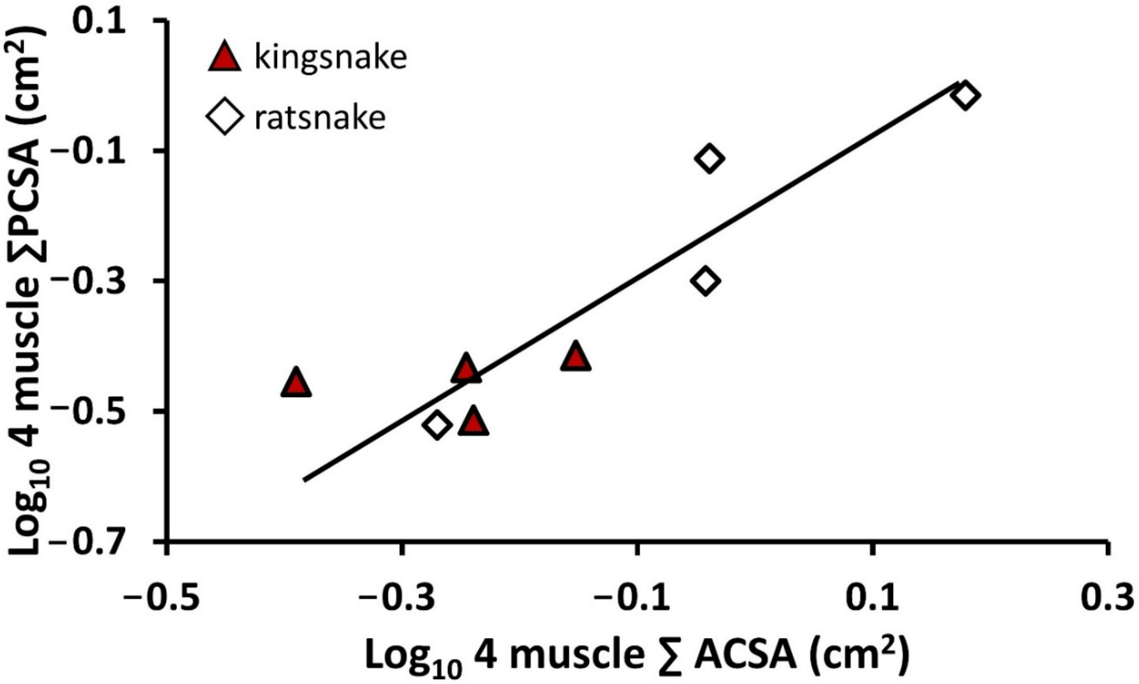

Figure 3.7. Sum of the $\log_{10}$ physiological cross-sectional areas (PCSA) from this study regressed against $\log_{10}$ anatomical cross-sectional areas (ACSA) for the four largest epaxial muscles (semispinalis–spinalis complex, longissimus dorsi, and iliocostalis) at ca. 50% SVL from Penning and Moon (2016). Red triangles denote speckled kingsnakes (*Lampropeltis holbrooki*), open diamonds denote western ratsnakes (*Pantherophis obsoletus*), and the black line represents the reduced major axis regression model (see results).

CHAPTER V

Identifying the Mechanisms of Predation: *In Vivo* Measurements of Force and Endurance in Epaxial Muscles of Kingsnakes (*Lampropeltis holbrooki*) and One of Their Prey Snakes (*Pantherophis obsoletus*)

Short Title: *In Vivo* Muscle Performance in Snakes

DAVID A. PENNING[1,2,3], BRAD R. MOON[1]

[1]Department of Biology, University of Louisiana at Lafayette, Lafayette, Louisiana, USA 70504- 43602

[2]Department of Biology and Environmental Health, Missouri Southern State University, Joplin, Missouri, USA 64801

[3] Corresponding author: David Penning, Department of Biology and Environmental Health, Missouri Southern State University, Joplin, Missouri, USA 64801, *davidapenning*@gmail.com

Abstract

Snakes are elongate vertebrates with hundreds of skeletal elements and thousands of muscles with complex interconnections. Many snakes use their musculature to constrict and kill by exerting forces around prey that the prey experience as pressures. In general, larger snakes produce higher constriction pressures than smaller ones, but previous work has shown that snake-eating snakes (kingsnakes; *Lampropeltis*) produce higher constriction pressures than similarly sized intraguild competitors (ratsnakes; *Pantherophis*). Although kingsnakes produce higher constriction pressures than their ratsnake prey, there are currently no identifiable differences in their muscle size and anatomy. Because of their similar anatomy, previous work has suggested that differences in constriction performance may be driven more by their posture (behavior) than by their muscle performance (physiology). However, quantitative analysis of muscle performance is still needed. Here, we investigated muscle physiology by quantifying and comparing controlled *in vivo* muscle force and endurance in two epaxial muscles of speckled kingsnakes (*L. holbrooki;* $N = 8$) and western ratsnakes (*P. obsoletus;* $N = 8$) in order to better understand how muscle performance relates to whole-body performance. The semispinalis–spinalis and longissimus dorsi muscles from larger snakes produced higher isometric forces (0.23–1.35 N) than those from smaller snakes, but there was no significant difference in maximum isometric force between muscles or species. In endurance tests, all muscles lost 25% of their maximum isometric force in an average of 58 s, and muscle force was reduced to an average of 38% of the maximum force over 4 min of stimulation. There was no significant relationship between body size and muscle endurance, and there was no difference in muscle endurance between muscles or snake species in our study sample. These and previous results suggest that kingsnakes are superior constrictors that can successfully prey upon other large constrictors not because of differences in their muscle physiology, but because of their more consistent use of an effective coil posture (behavior).

Key Words: Endurance, Isometric Force, Muscle, Physiology, Predation, Snake

Introduction

Predator–prey encounters are among the many interactions that are affected by an organism’s structure and function (Darwin, 1859; Webb, 1986; Wainwright, 1994). During predation attempts, the combatants have opposing goals of escape and consumption (Webb, 1986). These different goals require different behaviors, and therefore require the use of different muscles or muscle activation patterns. Snakes—the most diverse group of limbless terrestrial vertebrates—use their axial muscles for diverse movements, including escaping predators and, along with cranial muscles, capturing and handling prey. Snake musculature ranges from short, discrete muscles to multi-muscle complexes that span many vertebrae (Mosauer, 1935; Gasc, 1981; Penning, 2016c). The largest muscle group within a snake is formed by interconnections among the semispinalis–spinalis complex, longissimus dorsi, and iliocostalis (Mosauer, 1935; Ruben, 1977; Penning, 2016c). These muscles compose a large portion of the muscle mass within a snake’s body (Ruben, 1977), have the largest total physiological cross-sectional areas of muscles in snakes (Penning, 2016c), and together can span over twenty-five vertebrae (Moon, 2000; Jayne and Riley, 2007; Penning, 2016c). Snakes use this complex musculature for both predation and defense.

Snakes can use several methods to kill their prey including venom, biting, and constriction (Greene, 1997). Constricting snakes coil around their prey and exert forces that the prey experience as pressures. These pressures cause physiological disruptions in the prey, typically disabling it quickly. Much of the recent work on constriction performance has used mammalian prey (Moon, 2000; Penning et al., 2015; Boback et al., 2015; Penning and Dartez, 2016; Penning, 2016b). However, many snakes feed on a wide range of invertebrate and vertebrate prey (Ernst and Ernst, 2003). Kingsnakes (*Lampropeltis* spp.) are powerful constrictors known to feed on a wide variety of prey, including other snakes (Ernst and Ernst, 2003). Kingsnakes are resistant to the venom from vipers (Weinstein et al., 1992), and their presence in an ecosystem may change the distribution and abundance of other snakes (Steen et al., 2014). In addition to feeding on vipers, kingsnakes feed on and can kill other constricting snakes (*Pantherophis* spp.), including ones that are larger than themselves (Jackson et al., 2004), using the same mechanism of predation that the prey snakes use on other organisms. Previous work has shown that kingsnakes exert significantly higher constriction pressures on rodent prey than do ratsnakes, despite both kingsnakes and ratsnakes having similar muscle size and anatomy (Penning and Moon, 2016; Penning, 2016c).

How kingsnakes produce higher constriction pressures than ratsnakes remains unknown. Penning and Moon (2016) noted that kingsnakes use a coil posture that differs in both form and consistency from the more-variable coil postures of ratsnakes. Tests of muscle force and endurance could help elucidate the differences in whole-body constriction performance between kingsnakes and ratsnakes. Therefore, we quantified controlled *in vivo* maximum isometric force and endurance in two of the largest epaxial muscle groups; the semispinalis–spinalis complex and longissimus dorsi (Penning, 2016ac) in speckled kingsnakes (*L. getula*) and western ratsnakes (*P. obsoletus*). Differences in muscle performance could help explain how kingsnakes are able to produce higher peak constriction pressures and can overpower ratsnakes in predatory encounters. In particular, we tested the hypotheses that kingsnake muscles can produce higher forces and have greater endurance than similarly sized ratsnakes.

Materials and Methods

All experimentation was approved by the University of Louisiana at Lafayette's Institutional Animal Care and Use Committee. We used 16 snakes (8 *L. holbrooki* Stejneger 1902 and 8 *P. obsoletus* Say 1823) in tests of muscle performance. There was no significant difference in the body mass (*L. holbrooki* = 258.6 ± 24.0 g, *P. obsoletus*=250.8±52.1 g; t_{14}=0.69, P>0.50) or snout–vent length (SVL; *L. holbrooki* = 84.9±3.1 cm, *P. obsoletus*=88.0 ± 5.6 cm; t_{18}=0.34, P>0.74) of the kingsnakes and ratsnakes used. We maintained the snakes in captivity for at least 12 months prior to testing and fed them pre-killed rodents approximately weekly. We fasted snakes for two weeks prior to experimentation to aid pulmonary function under anesthesia (Bennett, 1991). We anesthetized each snake with volatile isoflurane via the open-drop method (Frye, 1991; Blouin-Demeres et al., 2000) and then transferred it to a surgical stage. The animals remained under anesthesia during all procedures and we periodically ventilated their lungs to ensure adequate oxygen and anesthesia delivery. We maintained the surgical stage within the voluntary temperature ranges of both species (27–29°C; Fitch, 1956; Brattstrom, 1965) with a heating pad, and we monitored snake body temperature with a cloacal thermocouple (Fisher Scientific; Model 15-077-11). Because snakes exhibit longitudinal variation in their axial musculature (Pregill, 1977; Nicodemo, 2012), we tested the muscles at 50% snout–vent length.

At 50% snout–vent length, we cut the skin with a mid-dorsal incision, reflected the skin laterally to reveal the large columns of epaxial muscles, and anchored the vertebral column in place with steel pins and tape straps. Once the skin was reflected and the snake was fixed in place, we isolated the anterior tendons of one of the two muscles to be measured. After completing the first muscle, we measured the second muscle. Because these measures were taken *in vivo*, and due the extreme complexity of snake musculature (Mosauer, 1935; Gasc, 1981, Penning, 2016c), we only measured performance for two of the larger epaxial muscle columns, the semispinalis–spinalis complex and the longissimus dorsi. The longissimus dorsi serves as the posterior anchor to two large epaxial muscles that connect to it anteriorly (Fig 4.1). The longissimus dorsi inserts posteriorly onto several vertebrae that can be anchored to the surgical stage and isometric force and endurance can be measured from its anterior tendon. The semispinalis–spinalis complex is composed of two fused muscles that have different posterior insertion sites. Measuring each segment of this muscle in isolation would be quite difficult and would likely result in muscle damage during removal. In pilot tests, we were unable to separate the muscle fibers of one segment from others in anesthetized specimens, but could easily isolate the anterior tendons. Here, we measured the maximum isometric force and endurance of the semispinalis–spinalis muscle via the anterior tendon of a single segment. The forces we measured may reflect some force sharing among adjacent muscle segments because the muscle segment being measured remained embedded in its connective tissues. However, the muscle anatomy and size are essentially identical in kingsnakes and ratsnakes (Penning and Moon, 2016; Penning, 2016c), making our data comparable between the species.

For both species, it was easy to access and isolate the anterior tendons of both muscles. However, it was more difficult to anchor the tendon of the longissimus dorsi to the transducer due to its anterior bifurcation (Fig 4.1). We tied both the dorsal and ventral forks of this tendon onto the line connecting to the transducer. For both muscles, when we cut the anterior tendon to secure it to the transducer, we could see the muscle shorten as soon as the tendon was severed. Once the anterior tendon was isolated, we secured it to an isometric force transducer

(AD Instruments, MLT500; 0.5 kg max load) oriented to measure force in line with the pull of the muscle (MacIntosh and Willis, 2000). The transducer was secured to movable manipulator stands (AD Instruments, MLA40) and connected to a ML856 Powerlab 26T system through a bridge pod amplifier (AD Instruments, ML301) to record the muscle force. To attach the muscle to the force transducer, we use a triple overhand knot that proved successful in pilot trials with braided line (Spiderwire ® Stealth®-Braid, 4.5 kg strain). This method showed no signs of slipping during trials used in analyses.

To begin testing, we moved the transducer away from the muscle until the line was taut with the muscle; any additional movement would result in muscle lengthening. We started measuring muscle force at this length in order to determine the optimal length for further testing. To record isometric force, we stimulated each muscle with 5V at 70 Hz and a pulse duration of 3 ms, for a total of 1.5 s. We chose these specifications based on preliminary recordings from pilot trials and previous research (Herrel et al., 2008); longer total stimulus durations or higher voltages did not increase maximum isometric force. We delivered the stimulations with a gold-plated brass electrode (AD Instruments, MLA0320) placed gently and directly on the surface of the exposed muscle tissue (Fig 4.2). After each stimulus, we stretched the muscle 2 mm by using an adjustable dial on the manipulator stand and then let the muscle rest for 5 minutes before delivering another stimulus (MacIntosh and Willis, 2000). We repeated this procedure, lengthening the muscle 2 mm each time, until we obtained maximum force. Once we obtained the optimal length for maximum contractile force, we let the snake rest under continuous anesthesia for 10 min before starting the endurance test.

In general, endurance is the ability to resist fatigue, which is the failure to maintain force (MacIntosh et al., 2006). However, endurance can be quantified in many ways depending on the muscle, species, and experiment (Garland et al., 1987; Ito et al., 1996; Sinervo et al., 2000; Clemente et al., 2009). For the endurance test, we delivered a continuous 4-min stimulation of 5 V at 70 Hz with a pulse duration of 3 ms. We quantified and report two measures of endurance. The first measure quantifies endurance time and is the duration from maximum force to a reduction in maximum force of 25%. The second measure is the endurance quotient and is the reduction in muscle force after 4 min of stimulation, quantified as the lowest force divided by the maximum force and multiplied by 100, which gives the percentage of maximum force at that time. After testing the first muscle, we moved 10 vertebrae anterior or posterior and tested the second muscle. After we obtained data from both muscles, we euthanized the still-anesthetized snakes with an overdose of Pentobarbital (Beuthanasia ®).

We digitized the force data at 1000 Hz using a low-pass digital filter with a cut-off frequency of 10 Hz. After filtering, we measured peak isometric force and the two endurance values (time at 25% reduction from peak force, and the endurance quotient after 4 min of stimulation), and $\log_{10}$-transformed the values for analysis. To compare maximum muscle force between kingsnakes and ratsnakes, we computed a 2-factor repeated-measures ANOVA using a multivariate approach (Vasey and Thayer, 1987). We used the single highest force from both muscles in analyses. We treated each muscle (semispinalis–spinalis complex and longissimus dorsi) as the repeated measure and snake species as a categorical independent variable. Although snakes in our study were similar in size, we included mass as a covariate in the initial analyses because size significantly affects performance in snakes (Moon and Mehta, 2007; Herrel et al., 2011; Penning and Moon, 2016); the results were similar when either mass or SVL were used as a covariate. We used similar analyses to compare both measures of endurance between muscles and species. We performed analyses in JMP Pro (11.00.0) and Past3.08 software and considered tests significant at $P<0.05$. Whenever applicable, we report mean ± standard error.

Results

The muscles of both species responded similarly to the experimental manipulations. After cutting each tendon, which allowed the muscle to shorten from its resting length in-vivo, and connecting it to the force transducer with no visible slackness, we typically had to stretch the muscle 6–18 mm to obtain maximum isometric force. There was no difference between kingsnakes and ratsnakes in the amount of muscle lengthening required to produce maximum force for the semispinalis–spinalis complex ($t_{14}=0.90$, $P>0.38$) and the longissimus dorsi ($t_{14}=0.89$, $P>0.39$) in our sample, suggesting that these muscles have similar resting lengths and tensions *in vivo*. Because a stimulus was delivered with every 2 mm stretch of each muscle, there was also no difference in the number of stimulations that each muscle received in both species.

Muscle length played an important role in maximum isometric force (Fig 4.3). After securing the tendon to the transducer, each muscle performed maximally at approximately 10 mm of stretch beyond the first stimulation with visible stretching (Fig 4.3). For the semispinalis–spinalis complex, maximum isometric force was 0.70±0.09 Newtons (N) in *Lampropeltis holbrooki* and 0.73±0.13 N in *Pantherophis obsoletus*. For the longissimus dorsi, maximum isometric force was 0.81±0.10N in *Lampropeltis holbrooki* and 0.76±0.18 N in *Pantherophis obsoletus*. There was no significant interaction between mass and species ($F_{1,12}=0.822$, $P>0.38$) in maximum isometric force. There was also no significant difference in maximum isometric forces between species ($F_{1,12}=0.004$, $P>0.94$) or muscles ($F_{1,12}=0.04$,

$P>0.85$). However, body mass significantly affected maximum isometric force ($F_{1,12}=10.2$, $P<0.008$) as expected, with larger snakes producing higher forces in both muscles (Fig 4.4).

For the semispinalis–spinalis complex, it took 60.9±12.8 seconds (s) for maximum isometric force to drop 25% below the peak value in *Lampropeltis holbrooki* and 80.3±27.7 s in *Pantherophis obsoletus*. For the longissimus dorsi, it took 56.3±7.6 s for maximum isometric force to drop by 25% in *Lampropeltis holbrooki* and 35.9±3.6 s in *Pantherophis obsoletus*. While there was variation in endurance duration across muscles and species, there was no significant interaction between mass and species ($F_{1,14}=0.56$, $P>0.47$), and mass did not significantly affect endurance time ($F_{1,14}=0.001$, $P>0.97$); there was also no significant difference between snake species ($F_{1,14}=1.21$, $P>0.29$) or muscle type ($F_{1,14}=0.86$, $P>0.37$). Both muscles from both species had similar endurance times, and there was no significant correlation between maximum isometric force and endurance time for either the semispinalis–spinalis complex (Pearson's $r=0.075$, $P>0.78$) or the longissimus dorsi (Pearson's $r=0.08$, $P>0.76$).

After a 4-min stimulation, all muscles experienced reductions in force output. In the semispinalis–spinalis complex, isometric force was reduced to 40.1±3.49% of the maximum in *Lampropeltis holbrooki* and 33.9±3.8% in *Pantherophis obsoletus*. In the longissimus dorsi, isometric force was reduced to 38.2±9.8% of the maximum in *Lampropeltis holbrooki* and 40.4±6.4 % in *Pantherophis obsoletus*. There was no significant interaction between mass and species ($F_{1,14}=0.51$, $P>0.49$) on loss of force after 4 min of stimulation. Mass did not significantly affect the endurance quotient ($F_{1,14}=0.07$, $P>0.79$) and there was no significant difference between snake species ($F_{1,14}=0.56$, $P>0.46$) or muscle type ($F_{1,14}=0.51$, $P>0.49$; Fig 4.5). There was no significant correlation between maximum isometric force and the endurance quotient for both the semispinalis–spinalis complex (Pearson's $r=-0.23$, $P>0.38$) and the longissimus dorsi (Pearson's $r=-0.18$, $P>0.49$).

Discussion

Morphology sets the functional limitations on performance, which in turn can affect behavior (Garland and Losos, 1984). However, behavior can also affect performance and fitness through modulation or through the use of different behaviors entirely (Irschick, 2000; Irschick et al., 2000). All of these interdependent components are profoundly affected by size (Schmidt-Nielsen, 1984). In general, as snakes increase in size, so does their axial musculature (Moon and Candy, 1997; Lourdais et al., 2005; Herrel et al., 2011; Penning and Moon, 2016; Penning, 2016c). Our results show that as snakes increase in size, so does the maximum isometric force of their muscles (Fig 4.4), which is probably a key part of the mechanisms underlying the strong relationship between snake size and whole-body performance (Lourdais et al., 2005; Moon and Mehta, 2007; Herrel et al., 2011; Penning et al., 2015; Penning and Dartez, 2016; Penning, 2016b). However, our muscle-level results do not explain how kingsnakes are able to exert significantly higher constriction pressures than ratsnakes. When quantifying the relationship between morphology and ecology it is important to consider both whole-animal performance and behavior (Garland and Losos, 1984). Here, we show the importance of considering whole-animal behavior when describing the relationship between muscle-level performance and whole-animal performance.

It is possible that we were not able to detect a significant difference in muscle force and endurance between kingsnakes and ratsnakes due to our limited sample sizes, and therefore limited statistical power (Button et al., 2013). However, our sample sizes are within the range of, or greater than, those from previous research on muscle-level performance, many of which detected significant relationships (Dial and Biewener, 1993; MacIntosh and Willis, 2000; Maganaris, 2001; Herrel et al., 2008). Furthermore, there are no quantifiable differences in the overall size, shape, or physiological cross-sectional area of the axial musculature of kingsnakes and ratsnakes across their ontogeny (Penning and Moon, 2016; Penning, 2016c). With both snakes having similar muscle morphology, there is no a priori reason to suspect a difference in their muscle-level performance that our sample could not detect.

While kingsnakes and ratsnakes have similar musculature, closely related species can differ in muscle properties as well as various aspects of performance (Bonine et al., 2001; 2005), and muscle-level properties may be part of a larger suite of traits that can interact in complex ways to determine whole-body performance. In previous research, we found that kingsnakes exert significantly higher constriction pressures than ratsnakes (Penning and Moon, 2016) even though both have similarly sized muscles (Penning and Moon, 2016; Penning, 2016c). We previously hypothesized that their coil posture (loop orientation and uniformity) allows kingsnakes to optimize pressure exerted on prey (Penning and Moon, 2016). However, it was also possible that kingsnakes have higher muscle force and endurance than ratsnakes, and that the difference in performance was more physiological than behavioral. Here we showed that there are no differences in maximum isometric force or endurance between muscles or species for two of the largest muscle groups in our sample. Therefore, kingsnakes appear to have superior constriction performance because of their behavior (coil posture) and not their physiology (muscle performance).

Larger snakes had muscles that produced significantly higher isometric forces, but size did not affect muscle endurance. Both large and small snakes maintained similar levels of force over time. In both muscles, kingsnakes and ratsnakes experienced a loss of 25% force output in approximately 58 s and experienced force reductions down to approximately 40% maximum force after 4 min of continuous contraction. However, whole-body endurance likely plays an important role in this predator–prey interaction and can differ from muscle-level endurance because muscles may not contract continuously *in vivo*. Ectotherms are generally expected to fatigue quickly with intense activity due to their extensive use of anaerobic respiration (Pough, 1983; Boback et al., 2015); however, predation events between kingsnakes and ratsnakes can take over 7 hr and involve continuous biting and constriction postures (Jackson et al., 2004). Either both animals are simultaneously experiencing similar levels of fatigue throughout the event or the snakes may be using their complex musculature differently, resulting in different levels of fatigue.

In addition, the opposing goals of escape and predation affect the animals differently. During this predator–prey interaction, the kingsnake is constricting the ratsnake while the ratsnake is attempting to escape the coil; the ratsnakes do not appear to counter-constrict. While it is likely that the kingsnake is experiencing some physiological effects from its own constriction behavior (Wang et al., 2001), the ratsnake is experiencing circumferential force around its body, which results in different pressure effects on the body (Halperin et al., 1993; Boback et al., 2015; Penning, 2016b). These constriction pressures are likely affecting blood flow in the prey ratsnake similar to what has been observed in mammal prey (Boback et al., 2015). Hence, the ratsnake’s muscles would be experiencing impaired blood flow that in turn affects their escape performance.

Kingsnakes are known for the ability to consume other snakes, including other constrictors such as ratsnakes (Ernst and Ernst, 2003; Jackson et al., 2004) that are seemingly well matched in both offensive abilities (biting and constriction) and defensive abilities (biting, possibly constriction, and locomotor forces). In seeking to elucidate the mechanisms of success when kingsnakes prey on other constrictors, we have provided descriptions of feeding behavior (Penning and Moon, 2016), qualitative and quantitative measures of muscle anatomy (Penning and Moon, 2016; Penning, 2016c), measures of whole body defensive and predation performance (Penning and Moon, 2016), and measures of *in vivo* muscle force and endurance (this study), for both kingsnakes and ratsnakes. Of all these measures, kingsnakes differ from ratsnakes only in using a more consistent coil posture and exerting higher constriction pressures. There are currently no detectable differences in their muscle anatomy, forces, or endurance. These results suggest that kingsnakes are superior constrictors that can successfully prey upon other large constrictors because of their more consistent use of an effective coil posture (Penning and Moon, 2016) that allows them to produce significantly higher peak constriction pressures than ratsnakes, making them the superior intraguild competitor and predator.

Acknowledgments

We thank J. Albert, A. Herrel, P. Leberg, and D. Povinelli for providing comments on earlier drafts that helped improve the quality of the manuscript.

Literature Cited

Bennett RA. 1991. A review of anesthesia and chemical restraints in reptiles. J Zoo Wildl Med 22:282–303.

Blouin-Demers G, Weatherhead PJ, Shilton CM, Parent CE, Brown GP. 2000. Use of inhalant anesthetics in three snake species. Contemporary Herpetology 4:1–7.

Boback SM, McCann KJ, Wood KA, McNeal PM, Blankenship EL, Zwemer CF. 2015. Snake constriction rapidly induces circulatory arrest in rats. J Exp Biol 218:2279–2288.

Bonine KE, Gleeson TT, Garland Jr T. 2001. Comparative analysis of fiber-type composition in the iliofibularis muscle of phrynosomatid lizards (Squamata). J Morpho 250:265–280.

Bonine KE, Gleeson TT, Garland Jr T. 2005. Muscle fiber-type variation in lizards (Squamata) and phylogenetic reconstruction of hypothesized ancestral states. J Exp Biol. 208:4529–4547.

Brattstrom BH. 1965. Body temperature of reptiles. Am Midl Nat 73:376–422.

Button, KS, Ioannidis, JPA, Mokrysz, C, Nosek BA, Flint, J, Robinson ESJ, Munafò MR. 2013. Power failure: why small sample size undermines the reliability of neuroscience. Nat Rev Neurosci 14:365–376.

Clemente CJ, Withers PC, Thompson GC. 2009. Metabolic rate and endurance capacity in Australian varanid lizards (Squamata; Varanidae; *Varanus*). Biol J Linnean Soc 97:664–676.

Darwin CR. 1859. On the Origins of Species by Means of Natural Selection. London: John Murray. 502 p.

Dial, KP, Biewener AA. 1993. Pectoralis muscle force and power output during different modes of flight in pigeons (*Columba livia*). J Exp Biol 176: 31–54.

Ernst, CH, Ernst EM. 2003. Snakes of the United States and Canada. Washington DC: Smithsonian Institution. 680 p.

Fitch HS. 1956. Temperature responses in free-living amphibians and reptiles in northeastern Kansas. Univ Kans publ, Mus Nat Hist 8:417–476.

Frye FL. 1991. Biomedical and Surgical Aspects of Captive Reptile Husbandry Volume II. Florida: Krieger Publishing Company. 712 p.

Garland Jr T, Else PL, Hulbert AJ, Tap P. 1987. Effects of endurance training and captivity on activity metabolism of lizards. Am J Physiol Regul Integr Comp Physiol 252:R450–R456.

Garland Jr T, Losos, JB. 1984. Ecological morphology of locomotor performance in Squamate reptiles. In: Wainwright PC, Reilly SM, editors. Ecological Morphology: Integrative Organismal Biology. Illinois: University of Chicago Press. pp 240–302.

Gasc J-P. 1981. Axial Musculature. In: Gans C, Parsons TS, editors. Biology of the Reptilia. Volume 11. New York: Academic Press. pp 355–435.

Greene HW. 1997. Snakes: The Evolution of Mystery in Nature. Berkeley: University of California Press. 365 p.

Halperin HR, Tsitlik JE, Gelfand M, Weisfeldt ML, Gruben KG, Levin HR, Rayburn BK, Chandra NC, Scott CJ, Kreps BJ, Siu CO, Guerci AD. 1993. A preliminary study of cardiopulmonary resuscitation by circumferential compression of the chest with use of a pneumatic vest. N Engl J Med 329:762–768.

Herrel A, Schaerlaeken V, Ross C, Meyers J, Nishikawa K, Abdala V, Manzano A, Aerts P. 2008. Electromyography and the evolution of motor control: limitations and insights. Integr Comp Biol 48:261–271.

Herrel A, Huyghe K, Oković P, Lisičić D, Tadić Z. 2011. Fast and furious: effects of body size on strike performance in an arboreal viper *Trimeresurus (Cryptelytrops) albolabris*. J Exp Zool 315:22–29.

Irschick, DJ. 2000. Effects of behaviour and ontogeny on the locomotor performance of a West Indian lizard, *Anolis lineatopus*. Funct Ecol 14:438–444.

Irschick, DJ, Macrini, TE, Koruba, S, Forman, J. 2000. Ontogenetic differences in morphology, habitat use, behavior, and sprinting capacity in two West Indian *Anolis* lizards. J. Herpetol. 34:444–451.

Ito T, Shirado O, Suzuki H, Takahashi M, Kaneda K, Strax TE. 1996. Lumbar trunk muscle endurance testing: an inexpensive alternative to a machine for evaluation. Arch Phys Med Rehabil 77:75–79.

Jackson K, Kley NJ, Brainerd EL. 2004. How snakes eat snakes: the biomechanical challenges of ophiophagy for the California kingsnake, *Lampropeltis getula californiae* (Serpentes: Colubridae). Zoology 107:191–200.

Jayne BC, Riley MA. 2007. Scaling of the axial morphology and gap-bridging ability of the brown tree snake, *Boiga irregularis*. J Exp Biol 210:1148–1160.

Lourdais O, Brischoux F, Barantin L. 2005. How to assess musculature and performance in a constricting snake? A case study in the Columbian rainbow boa (*Epicrates cenchria maurus*). J Zool 265:43–51.

MacIntosh BR, Willis JC. 2000. Force-frequency relationship and potentiation in mammalian skeletal muscle. J Appl Physiol 88:2088–2096.

MacIntosh BR, Gardiner PF, McComas AJ. 2006. Skeletal Muscle: Form and Function. 2nd edition. Illinois: Human Kinetics. 432 p.

Maganaris CN. 2001. Force–length characteristics of in vivo human skeletal muscle. Acta Physiol Scand. 172:279–285.

Moon BR. 2000. The mechanics and muscular control of constriction in gopher snakes (*Pituophis melanoleucus*) and a king snake (*Lampropeltis getula*). J Zool 252:83–98.

Moon BR, Candy T. 1997. Coelomic and muscular cross-sectional areas in three families of snakes. J Herpetol 31:37–44.

Moon BR, Mehta RS. 2007. Constriction strength in snakes. In: Henderson RW, Powell R, editors. Biology of the Boas and Pythons. Utah: Eagle Mountain Publishing. pp 206–212.

Mosauer W. 1935. The myology of the trunk region of snakes and its significance for ophidian taxonomy and phylogeny. Univ Cal Publ Biol Sci 1:81–120.

Nicodemo PI. 2012. Longitudinal Variation in the Axial Muscles of Snakes [Master's Thesis]. Cincinatti (OH): University of Cincinnati. 39 p. Available from: https://etd.ohiolink.edu/pg_10?0::NO:10:P10_ACCESSION_NUM:ucin1346171520

Penning DA, Dartez SF, Moon BR. 2015. The big squeeze: Scaling of constriction pressure in two of the world's largest snakes, *Python reticulatus* and *P. molurus bivittatus*. J Exp Biol 218:3364–3367.

Penning DA, Dartez SF. 2016. Size, but not experience, affects the ontogeny of constriction performance in ball pythons (*Python regius*). J Exp Zool A 325:194–199.

Penning DA. 2016a. The gluttonous king: The effects of meal size and repeated feeding on constriction performance in kingsnakes (*Lampropeltis getula*). J Zool

Penning DA. 2016b. The scaling of bite force and constriction pressure in kingsnakes (*Lampropeltis getula*): proximate determinants and correlated performance. Integr Zool

Penning DA. 2016c. Quantitative Axial Myology in Two Constricting Snakes: *Lampropeltis holbrooki* and *Pantherophis obsoletus*. J Anat

Penning DA, Moon BR. 2016. The king of snakes: morphology and performance of intraguild predators (*Lampropeltis*) and their prey (*Pantherophis*). J Exp Biol

Pough FH. 1983. Amphibians and reptiles as low-energy systems. In: Aspey WP, Lustick SI, editors. Behavioral energetics: the cost of survival in vertebrates. Columbus: Ohio State University Press. pp 141–188.

Pregill GK. 1977. Axial myology of the racer *Coluber constrictor* with emphasis on the neck region. San Diego Soc Nat Hist Trans 18:185–206.

Ruben JA. 1977. Morphological correlates of predatory modes in the coachwhip (*Masticophis flagellum*) and rosy boa (*Lichanura roseofusca*). Herpetologica 33:1–6.

Schmidt-Nielsen, K. 1984. Scaling: Why is Animal Size so Important? Cambridge: Cambridge University Press. 241 p.

Sinervo B, Miles DB, Frankino A, Klukowski M, DeNardo DF. 2000. Testosterone, endurance, and Darwinian fitness: natural and sexual selection on the physiological bases of alternative male behaviors in side-blotched lizards. Horm Behav 38:222–233.

Steen DA, McClure CJW, Sutton WB, Rudolph DC, Pierce JB, Lee JR, Smith LL, Gregory BB, Baxley DL, Stevenson DJ, Guyer C. 2014. Copperheads are common where kingsnakes are not: relationships between the abundances of a predator and one of their prey. Herpetologica 70:69–76.

Vasey MW, Thayer JF. 1987. The continuing problem of false positives in repeated measures ANOVA in psychophysiology: a multivariate solution. Psychophysiology 24:479–486.

Wang T, Taylor EW, Andrade D, Abe AS. 2001. Autonomic control of heart rate during forced activity and digestion in the snake *Boa constrictor*. J Exp Biol 204:3553–3560.

Wainwright PC. 1994. Functional morphology as a tool in ecological research. In: Wainwright PC, Reilly SM, editors. Ecological Morphology: Integrative Organismal Biology. Illinois: University of Chicago Press. pp 42–59.

Webb PW. 1986. Locomotion and predator-prey relationships. In: Feder ME, Lauder GV, editors. Predator-Prey Relationships: Perspectives and Approaches from the Study of Lower Vertebrates. Illinois: University of Chicago Press. pp 24–41.

Weinstein SA, DeWitt CF, Smith LA. 1992. Variability of venom-neutralizing properties of serum from snakes of the colubrid genus *Lampropeltis*. J Herpetol 26:452–461.

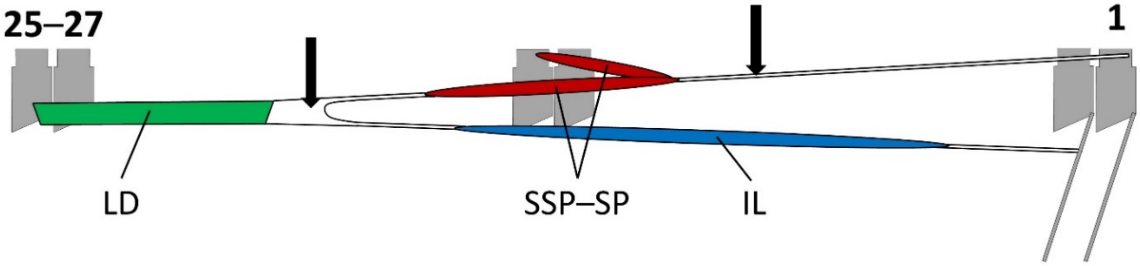

Figure 4.1. Schematic illustration of the right lateral view of the four major epaxial muscles in the speckled kingsnakes (*Lampropeltis holbrooki*) and western ratsnakes (*Pantherophis obsoleta*) used in this study; longissimus dorsi (LD), semispinalis–spinalis complex (SSP–SP), and iliocostalis (IL). Numbers indicate vertebrae with 1 being the most anterior insertion and 25–27 representing the range of most posterior vertebrae spanned by the interlinked muscles in both species (Penning, 2016a). Arrows indicate where we severed the tendons to connect them to the force transducer.

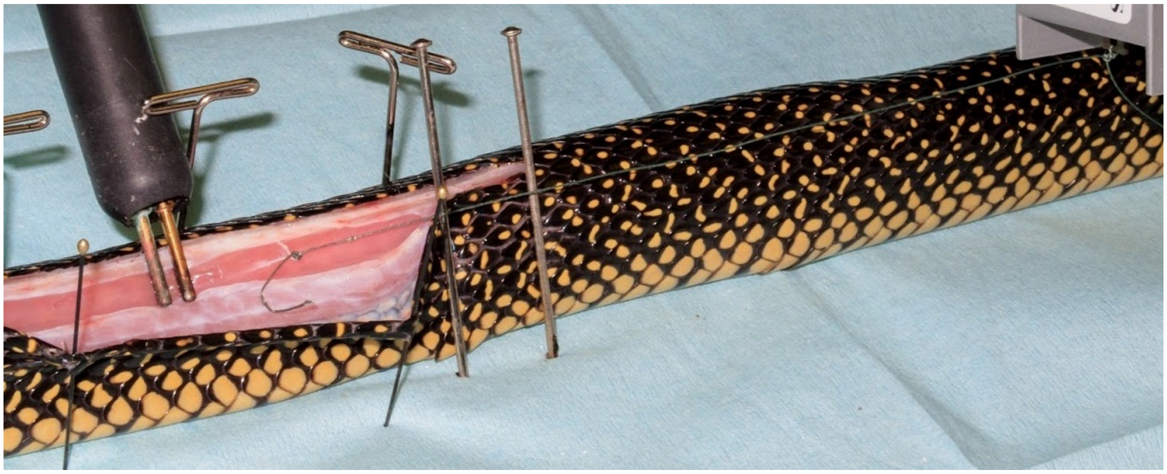

Figure 4.2. Oblique view of the right side of a speckled kingsnake, *Lampropeltis hiolbrooki* under anesthesia with the force transducer attached (top right). The stimulating electrode is resting on the semispinalis–spinalis muscle complex (left); the tendon connected to the force transducer is visible just to the right of the stimulating electrode.

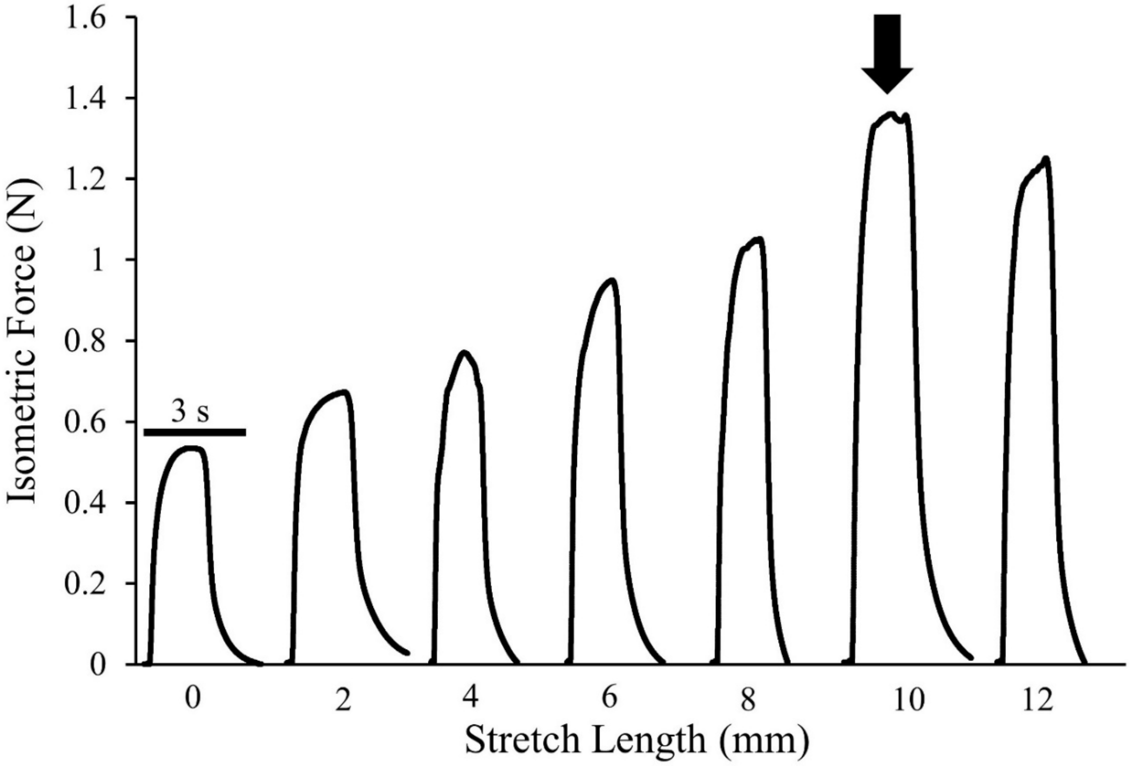

Figure 4.3. Individual force responses from stimulations of the longissimus dorsi muscle of a large western ratsnake, *Pantherophis obsoletus* (534 g, 109 cm SVL) used to find the muscle length that produced the maximum isometric force (black arrow). Each curve represents an individual stimulation at a given muscle length (X-axis). There was a five-minute rest period between each lengthening and stimulation.

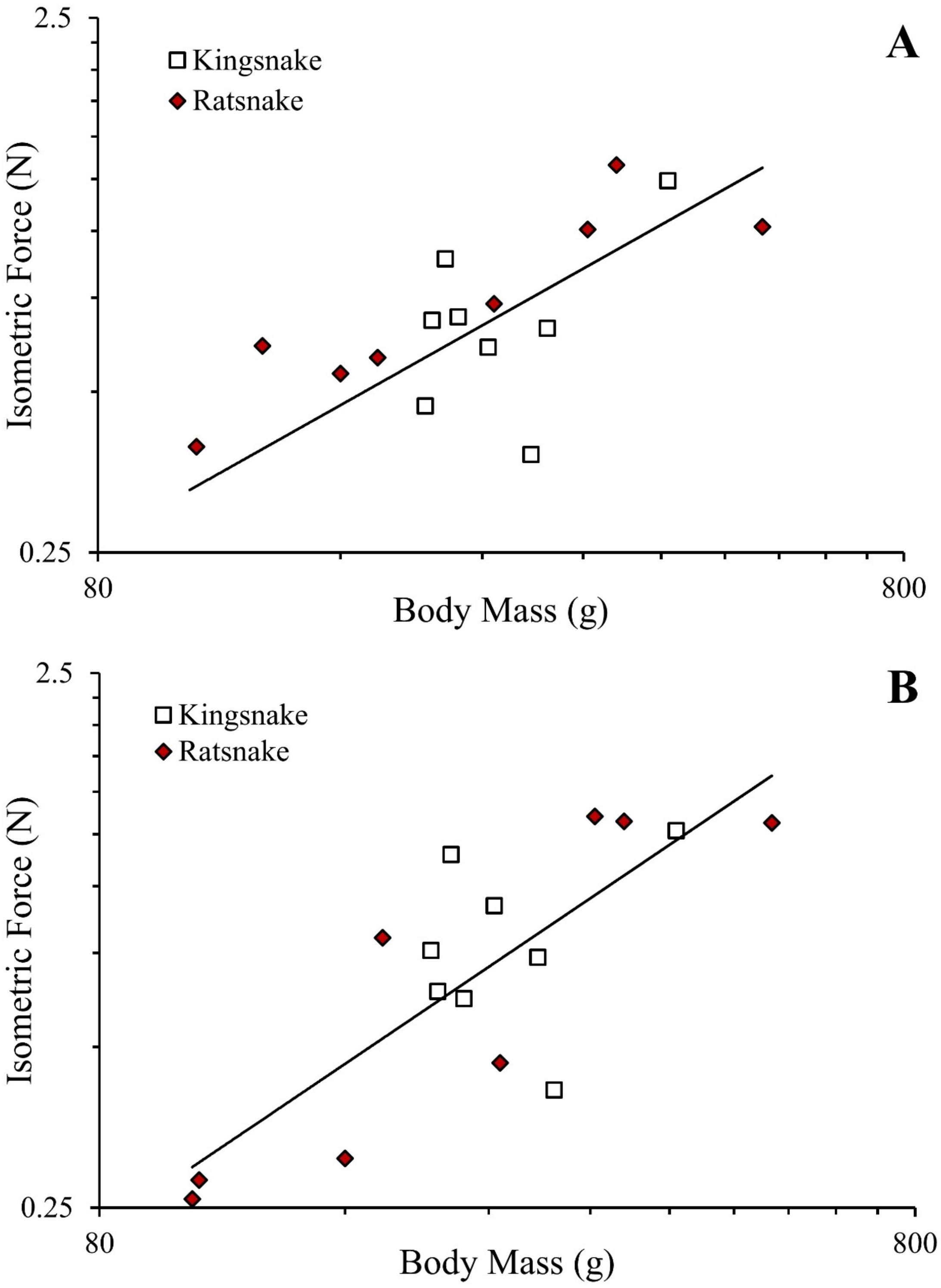

Figure 4.4. Scatterplot of maximum isometric force (Newtons) for the semispinalis–spinalis complex (A) and longissimus dorsi muscle (B) regressed against body mass for western ratsnakes (*Pantherophis obsoletus*) and speckled kingsnakes (*Lampropeltis holbrooki*).

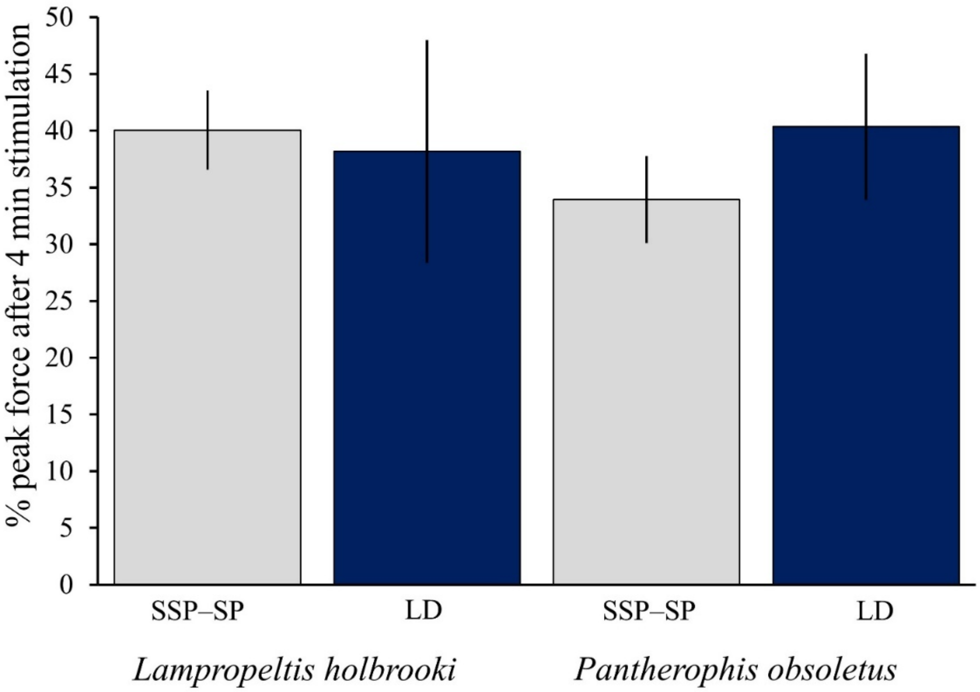

Figure 4.5. Muscle endurance in the semispinalis–spinalis complex and longissimus dorsi muscle of speckled kingsnakes (*Lampropeltis holbrooki*) and western ratsnakes (*Pantherophis obsoletus*). Endurance was measured as the percentage of maximum isometric force (mean ± SE) after 4 min of continuous stimulation.

APPENDIX

David A. Penning
Department of Biology
University of Louisiana at Lafayette
410 E. Saint Mary Blvd, Rm 108
Lafayette, LA 70503

8/1/2016

Brad Moon
Department of Biology
University of Louisiana at Lafayette
410 E. Saint Mary Blvd, Rm 108
Lafayette, LA 70503

Dear Brad Moon:

This letter is a request for your permission to include in my dissertation material that you have co-authored. I am completing a doctoral dissertation at the University of Louisiana at Lafayette, and the title of my dissertation is “The Mechanisms of a Successful Intraguild Predator.” I would like your written permission to include in my dissertation excerpts from or replications of the material described below. Your signing of this letter will confirm that you co-own the copyright to the material described below and that you are authorized to grant this permission. As a co-author, if you no longer own the rights to the material requested in this letter, will you kindly direct me to the person and/or entity now owning these rights?

The source of the material requested is our work together on chapters three and five of this dissertation.

The requested permission extends to any future revisions and editions of my dissertation, including non-exclusive rights in all languages, and to the prospective publication of my dissertation. This authorization is extended to ProQuest/UMI Dissertations Publishing, Ann Arbor, Michigan, for the purpose of reproducing and distributing copies of this dissertation. These rights will in no way restrict [republication or publication] of the material by you or by others authorized by you.

If these arrangements meet with your approval, please sign this letter where indicated below and return it to me in the enclosed stamped return envelope. Thank you very much.

Sincerely,

David A. Penning

As a/the copyright owner of the material described herein, I am granting permission for the use of the same requested material:

Name: ____ (Please print)

Signature: ____

Professional Title: ______________________________________

Date Signed: ____

Acknowledgment of Responsibility for Copyright Compliance

(Signed form to be attached to "Application for Admission to Candidacy.")

As a graduate student writing a thesis, dissertation, or synthesis project at the University of Louisiana at Lafayette, I declare that I understand the contents of the following statements and that I intend to uphold the agreements therein.

1. I understand and agree that if my dissertation or thesis contains copyrighted materials, either implied or indicated, I am solely responsible for obtaining written permission, where necessary, for same prior to the completion of any work as submitted for final review prior to its approval. It is my responsibility to maintain these written permissions.

2. I agree to indemnify and hold harmless the University of Louisiana at Lafayette, its employees, agents and representatives, in any claim or lawsuit that may be made or filed, which arises from my fulfillment of the requirements for my graduate degree, either thesis, dissertation, or synthesis project, or similar work.

Name: ____
(Please print)

Signature: ____

SS#/CLID: ____

Date: ____

Penning, David A. Bachelor of Science, University of Central Missouri, Fall 2010; Master of Science, University of Central Missouri, Spring 2012; Doctor of Philosophy, University of Louisiana at Lafayette, Fall 2016
Major: Environmental and Evolutionary Biology
Title of Dissertation: The Mechanisms of a Successful Intraguild Predator
Dissertation Director: Dr. Brad Moon
Pages in Dissertation: 126; Words in Abstract: 349

ABSTRACT

The objective of this research was to quantify and better understand the mechanisms of performance in constricting snakes. Many non-venomous snakes use constriction to subdue and kill different types, sizes, and quantities of prey. Using eastern kingsnakes (*Lampropeltis getula*), I measured the effects of prey size and repeated feeding on constriction performance. I found that prey size alone did not affect constriction performance, but when kingsnakes encountered additional prey of medium and large sizes, they experienced significant reductions in the length of the body used and peak constriction pressure. In addition to feeding on a variety of different mammalian prey, kingsnakes (*Lampropeltis spp.*) are known to feed on other snakes, including other constrictors (*Pantherophis ssp.*). To begin addressing how this is possible, I studied the scaling of muscle cross-sectional area, pulling force as an indicator of escape performance, and constriction pressure as a measure of predation performance across the ontogeny of six species of snakes (three kingsnake and three ratsnake species). Muscle cross-sectional area and pulling force scaled similarly for all snakes, but all kingsnakes were able to exert significantly higher peak constriction pressures on their prey than ratsnakes. The ability to exert higher pressures suggests that kingsnakes may have differences in muscle anatomy and physiology that have gone untested. In another experiment, I described and quantified nine different muscles in speckled kingsnakes (*L. holbrooki*) and western ratsnakes (*P. obsoletus*) in order to better compare their anatomy. There were no significant differences

in quantitative measures of musculature between these two species. Finally, I compared individual muscle performance between kingsnakes and ratsnakes by testing *in vivo* muscle force production and endurance. There was no difference between muscle force and endurance in our sample of kingsnakes and ratsnakes. The results from all chapters together indicate that kingsnakes are able to produce significantly higher constriction pressures because of their consistent coil posture (behavior) and not because of differences in their muscle anatomy or physiology. Integrated studies of behavior and its underlying mechanisms, such as in these chapters, are critical to making strong inferences about relationships in predator–prey interactions and their outcomes.

BIOGRAPHICAL SKETCH

David Penning received his Bachelor of Science in Biology from the University of Central Missouri in 2010. David then went on to receive a Master of Science in Biology at the University of Central Missouri. At the University of Central Missouri, David's thesis, advised by Dr. Stefan Cairns, tested the effects of prey size on predation behavior in naïve snakes. During that time, David developed an interest in understanding predation mechanisms in snakes. His interests in predation and behavior lead him to the University of Louisiana at Lafayette, where he earned his doctorate in the Fall of 2016 under the advisement of Dr. Brad Moon.