SEMINATRIX PYGAEA, A MODEL OF ECOLOGICAL RESILIENCE TO DYNAMIC HABITATS

by

CHRISTOPHER T. WINNE

(Under the Direction of J. Whitfield Gibbons)

ABSTRACT

To capitalize on productive wetland habitats, organisms must cope with temporal variability in habitat suitability. Stochastic climatic variation causes variation in resource abundance, and extensive droughts can render isolated wetland habitats dry and devoid of aquatic prey for years. This dissertation documents strategies of *Seminatrix pygaea*, a freshwater aquatic snake, for long-term persistence in isolated wetlands.

Seminatrix pygaea survived drought by aestivating within a dried wetland, unlike many sympatric snake species, which leave wetlands during drought. The first wet season following drought, *S. pygaea* reproduced with similar frequency and fecundity compared to pre-drought years, suggesting reproduction was unaffected by prior aestivation. The ability to rebound rapidly from drought was due partly to *S. pygaea's* reproductive ecology, which was distinct from snakes exhibiting capital breeding and "adaptive anorexia." *Seminatrix pygaea* fed throughout pregnancy, rapidly translating high prey abundance into reproductive output through income breeding. Experiments using artificially enriched ^{15}N stable isotopes as biological tracers confirmed pregnant *S. pygaea* can incorporate income energy into maternal and offspring body tissues during pregnancy, and revealed substantial variation in reproductive allocation strategies among individual *S. pygaea*. Another experiment demonstrated that reproductive costs to locomotor performance differed between aquatic and terrestrial habitats, elucidating possible reasons why aquatic habitats may enable aquatic snakes to continue foraging during pregnancy.

The lack of aquatic prey during severe drought imposed significant survivorship pressures on *S. pygaea*, and the largest individuals, particularly females, were most adversely affected by resource limitation. Compared to pre-drought years, the largest *S. pygaea* were absent from the population immediately following drought, causing both maximum body size and sexual size dimorphism to be dramatically reduced. Conversely, strong positive correlations between maternal body size and indices of reproductive success in *S. pygaea* suggest females experience fecundity selection for large size during non-drought years. Following drought, *S. pygaea* quickly grow to pre-drought sizes or larger, reaching record body sizes and litter sizes within 2–4 years following wetland refilling. Overall, *S. pygaea* possess a distinctive suite of life-history traits enabling them to survive, reproduce, and thrive in isolated wetlands subject to periodic droughts and dramatic fluctuations in prey abundance.

INDEX WORDS: adaptive anorexia, aestivation, capital breeding, climatic variation, cost of reproduction, crawling, *Farancia abacura*, fecundity selection, income breeding, life history tradeoffs, litter size, locomotor performance, migration, *Nerodia fasciata*, *Nerodia floridana*, prey abundance, reproductive allocation, *Seminatrix pygaea*, swimming, relative clutch mass, reptile, reproduction, wetland conservation

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DEDICATION

To my wife Lisa, my son Austin, and my parents Mark and Diane Winne.

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A great number of people have helped shape or support my graduate program and dissertation research, and many have been directly involved as collaborators in some of my research projects. Whit Gibbons was an extraordinary advisor, always energetic and supportive, and he consistently displayed an enthusiasm for reptiles, amphibians, and the natural world that was (and is) truly infectious. The herpetology lab at the Savannah River Ecology Laboratory (SREL), past and present, has been a fun, engaging, and inspirational place to work. Numerous SREL members have aided me with data collection over the course of this dissertation research, including Tom Akre, Kimberly M. Andrews, Heather Brant, Kurt Buhlmann, Dean Croshaw, Mike Dorcas, Sarah DuRant, Andrew Durso, Evan Eskew, Luke Fedewa, Whit Gibbons, Xavier Glaudas, Judy Greene, Bill Hopkins, Justin Jones, Ben Lawrence, Tom Luhring, Brian Metts, Tony Mills, Dan Moen, John Nestor, Jason Norman, Mellissa Pilgrim, Sean Poppy, Robert Reed, David Scott, Richard Seigel, Brian Todd, Ria Tsaliagos, Tracey Tuberville, JD Willson, and Cameron Young. But, I must single out a few fellow graduate students and post-docs who have contributed the most, both in ideas and research efforts: JD Willson, Brian Todd, Mellissa Pilgrim, Xavier Glaudas, and Luke Fedewa. I also thank Dan Moen, Evan Eskew, Andrew Durso, and Ben Lawrence for allowing me to direct their NSF-sponsored REU (Research for Undergraduate Experience) or high school senior research projects at SREL – in each case it was a rewarding experience that taught me a great deal about mentoring students. I especially thank Bill Hopkins for numerous meaningful collaborations, excellent mentorship, and for serving as a co-major advisor during part of my dissertation studies, before he took a faculty position at

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Coping with climatic variation and associated fluctuations in resource levels is one of the greatest challenges to organisms in many ecosystems. Extreme drought, in particular, is among the most powerful selective forces and has been implicated in the evolution of numerous character traits and life-history attributes (e.g., Grant and Grant 1989, Grant and Grant 1996, Grant 1999). For aquatic organisms inhabiting isolated wetlands, droughts pose an obvious challenge to population stability and persistence. For example, severe droughts can result in osmotic stress, heat stress, increased predation risk, and decreased prey abundance (Bennett et al. 1970). In turn, these stressors can reduce survivorship and reproduction, and even cause local extinction (Seigel et al. 1995, Willson et al. 2006). Consequently animals have evolved numerous drought-survival strategies.

Two prevalent drought-survival strategies are migration and aestivation. Studies of drought effects on semi-aquatic snakes are limited, but suggest that many species migrate to other habitats to escape drought conditions. For example, banded watersnakes (*Nerodia fasciata*) may leave isolated wetlands when wetlands dry (Seigel et al. 1995) and return once wetlands refill (Willson et al. 2006). Similarly, in response to wet-dry cycles of tropical Australia, water pythons (*Liasis fuscus*) and Arafura filesnakes (*Acrochordus arafurae*) migrate between habitats to take advantage of rainfall-mediated changes in prey abundances (Shine and Lambeck 1985, Madsen and Shine 1996). In other taxa, smaller, more aquatic species are often ill-suited to overland travel and rely on aestivation, rather than migration, to abide drought (Chessman 1984). For example, in turtles, large-bodied emydids (e.g., *Trachemys scripta*, *Pseudemys floridana*) generally migrate to other water bodies during drought, while smaller or more aquatic species (e.g., *Deirochelys reticularia*, *Kinosternon subrubrum*, *Sternotherus odoratus*) generally remain at the wetland, either aestivating within the wetland, or burying themselves in adjacent uplands (Gibbons et al. 1983, Buhlmann and Gibbons 2001).

In addition to direct effects of drought on survival of wetland organisms, year to year variation in rainfall affects organisms indirectly through fluctuations in resource abundance. Indeed, prey resources within wetlands often vary widely between abundant and absent. Under such conditions, organisms are generally expected to exhibit large-scale variation in reproductive output among ‘good’ and ‘bad’ years (Seigel and Fitch 1985, Shine and Madsen 1997, Madsen and Shine 2000). For example, during severe droughts Florida snail kites (*Rostrhamus sociabilis*) suffer from reduced prey availability and experience decreased survivorship and reproductive output (Mooij et al. 2002). Similarly, in tropical Australia, Arafura filesnakes (*A. arafurae*) and water pythons (*L. fuscus*) show strong negative responses (e.g., decreased growth, reproductive output, and number of reproductive females) to decreases in prey abundance that are driven by rainfall patterns (Shine and Madsen 1997, Madsen and Shine 2000).

Animals have evolved alternative reproductive strategies to cope with temporal fluctuations in resource availability. Capital breeding is a strategy whereby animals accumulate energy (‘capital’) during periods of high productivity and allocate that energy towards reproduction once a threshold of stored energy has been met (e.g., Bonnet et al. 1998, Bonnet et al. 2002). Functionally, this permits reproductive output to be independent of resource availability at the time of reproduction. Aspic vipers (*Vipera aspis*), for example, can rely on stored energy to reproduce during years when mothers do not catch a single prey item (Lourdais et al. 2003). In contrast, income breeding is a strategy that relies on recently ingested energy (‘income’) to fuel reproductive output (Bonnet et al. 1998). In many vertebrate ectotherms, however, females do not eat during pregnancy, which can limit their ability to use income breeding and translate high resource abundance into viable offspring on a short time-scale (Bonnet et al. 1998).

Many snake species either do not feed or drastically reduce foraging during pregnancy (e.g., Gregory and Skebo 1998, Gregory et al. 1999, Brown and Shine 2004, Gregory and Isaac 2004), whereas other species continue to eat throughout pregnancy (e.g., Brown and Weatherhead 1997, Aldridge and Bufalino 2003, Shine et al. 2004). In many cases, optimal habitats and behaviors for gestation are incompatible with feeding (Gregory and Skebo 1998, Gregory et al. 1999). Females suffer from reduced locomotor speeds during pregnancy, which presumably reduces foraging efficiency and increases predation risk (Shine 1980, Seigel et al. 1987, Brown and Shine 2004, Webb 2004). Also, many females thermoregulate at higher temperatures and with greater precision during pregnancy (e.g., Charland and Gregory 1990), otherwise they risk longer gestation times and improperly developed offspring (Peterson et al. 1993, Arnold and Peterson 2002). Such precise thermoregulation may not be compatible with foraging behavior. Consequently, for some species, the inability or unwillingness to feed during pregnancy may be a form of ‘adaptive anorexia’ that reduces conflicts between feeding and thermoregulation during pregnancy (Mrosovsky and Sherry 1980, Gregory and Skebo 1998, Gregory et al. 1999).

This dissertation examines the ecological resilience of an aquatic snake, the Black Swamp Snake, *Seminatrix pygaea*, to periodic droughts. Because their small body size, reliance on aquatic prey, and high rates of evaporative water loss make them ill-suited to prolonged overland movement, I hypothesized that *S. pygaea* remain within dried wetlands and rely on aestivation to survive drought. Further, I predicted that if *S. pygaea* become anorexic during pregnancy and rely on a capital breeding strategy to fuel reproduction, then in the first season following drought the necessity to replenish depleted resources would preclude successful reproduction. In Chapter 2, I tested these hypotheses (i) using aquatic and terrestrial capture methods in the first wet year following a prolonged drought to assess the drought-survival strategy of *S. pygaea*, (ii) assessed reproduction during and after drought and compared these periods to pre-drought conditions using historical data, and (iii) examined the propensity of this species to feed during pregnancy.

In Chapter 3, I used *S. pygaea* as a study system to test the generality of the hypothesis that an antagonism exists between survivorship and reproductive selection pressures that act on body size and sexual size dimorphism (SSD). Specifically, I was able to examine temporal variation in body size structure, maximum body size, and SSD within a single *S. pygaea* population. I predicted that (i) the largest *S. pygaea* would be absent following prolonged, severe droughts, and that (ii) female-biased SSD would be more extreme in years following high food availability, compared to years following drought-induced aestivation and a shortage of aquatic prey. In addition, I examined the influence of maternal size on litter size and offspring characteristics in *S. pygaea* to demonstrate the potential for fecundity selection to counteract survivorship selection and, thus, maintain female-biased SSD within the population.

In Chapter 4, I used an experimental approach involving stable isotope techniques to investigate the timing of reproductive allocation in *S. pygaea*. Specifically, I manipulated the concentration of a naturally occurring stable isotope (^{15}N) in prey items and altered the time that we introduced labeled prey to reproductive females. By subsequently evaluating the isotopic composition of post-partum mothers and offspring I was able to experimentally test the hypothesis that *S. pygaea* transfer energy consumed during pregnancy to their offspring.

In Chapter 5, I examine the recovery of the Ellenton Bay aquatic snake community following a prolonged drought and examine species differences in drought-recovery strategies during years of high wetland productivity, revealing substantial differences in long-term trends of relative abundance and demography among species, which have important conservation and ecological implications.

In Chapter 6, I performed a simple experiment designed to address four basic questions about sexual differences in locomotor performance and reproductive costs to locomotion in non-marine semi-aquatic snakes: (i) are there sexual differences in locomotor performance between aquatic and terrestrial habitats? (ii) is the cost of reproductive locomotor impairment similar between aquatic and terrestrial habitats for females? (iii) is there a phenotypic tradeoff between reproductive investment and reproductive locomotor impairment costs? and (iv) if there is a phenotypic tradeoff observed in one habitat type, is the tradeoff equally apparent within another habitat?

In Chapter 7, I summarize and discuss the information presented in this dissertation.

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CHAPTER 2

INCOME BREEDING ALLOWS AN AQUATIC SNAKE (*SEMINATRIX PYGAEA*) TO REPRODUCE NORMALLY FOLLOWING PROLONGED DROUGHT-INDUCED AESTIVATION[1]

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[1] Winne, C.T., J.D. Willson, J.W. Gibbons. 2006. *Journal of Animal Ecology*. 75:1352-1360. Reprinted with permission of the publisher.

INTRODUCTION

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In this study, we examined the ecology of a small, aquatic snake, the black swamp snake *Seminatrix pygaea* (Cope), inhabiting an isolated freshwater wetland (Ellenton Bay) that is subject to periodic extreme droughts. Because their small body size, reliance on aquatic prey, and high rates of evaporative water loss make them ill-suited to prolonged overland movement, we hypothesized that *S. pygaea* remain within the dried wetland and rely on aestivation to survive drought. Further, if *S. pygaea* become anorexic during pregnancy and rely on a capital breeding strategy to fuel reproduction, we predicted that in the first season following drought the necessity to replenish depleted resources would preclude successful reproduction. To test these hypotheses, we (i) used aquatic and terrestrial capture methods in the first wet year following a prolonged drought to assess the drought-survival strategy of *S. pygaea*, (ii) assessed reproduction during and after drought and compared these periods to pre-drought conditions using historical data, and (iii) examined the propensity of this species to feed during pregnancy.

METHODS

Study species

Seminatrix is a monotypic genus of the cosmopolitan subfamily Natricinae and is endemic to a portion of the southeastern U.S. Coastal Plain. *Seminatrix pygaea,* the smallest aquatic snake in North America, is viviparous, and typically reproduces annually (Seigel et al. 1995b, Sever et al. 2000, Winne et al. 2005). Although capable of feeding on a wide variety of aquatic prey (Gibbons and Dorcas 2004), *S. pygaea* at our study site have fed nearly exclusively on aquatic larvae and paedomorphs of the salamander *Ambystoma talpoideum* (Holbrook) since the early 1990s (unpublished data). Adult *S. pygaea* have high rates of evaporative water loss compared to sympatric semi-aquatic snakes (Winne et al. 2001, Moen et al. 2005), rarely venture away from the water's edge (Gibbons and Dorcas 2004), and are abundant in some isolated wetlands, making them ideal for long-term investigations of population-level responses to drought.

Study site

Ellenton Bay, an isolated freshwater wetland in South Carolina, USA, has been the focus of numerous long-term herpetological studies (Gibbons 1990). The regional climate consists of hot, humid summers and mild, wet winters (mean annual precipitation c.a. 100 cm). Two multi-year droughts (1987-1990; 2000-2003) have occurred at Ellenton Bay during since 1975 (Fig. 2.1). We initiated the current study in February 2003 at the end of the second drought.

Ellenton Bay is currently fish-free but harbors a diverse assemblage of amphibians (24 species) and semi-aquatic reptiles (18 species) during most years (Gibbons and Semlitsch 1991, Gibbons et al. 2006). Ellenton Bay has the longest hydroperiod of non-permanent wetlands in the region. The only permanent wetland within 1.4 km of Ellenton Bay is a small, man-made pond c.a. 0.5 km from Ellenton Bay. No *S. pygaea* have ever been captured in the pond during extensive aquatic trapping over many years (unpublished data). The Ellenton Bay basin is approximately 10 ha when full, but water surface area and depth are extremely variable (Fig. 2.1), ranging from no water to a maximum depth of approximately 2 m. During droughts, a thick (up to 0.5 m) organic crust covers the entire basin but subsurface areas remain moist and up to 1 ha of viscous mud surrounds small open water areas during shorter dry spells. However, during the 2000 – 2003 drought, no standing water remained. Gibbons (1990) and Gibbons et al. (2006) provide further study site details.

Snake captures

From 1 February 2003 to 31 January 2004, Ellenton Bay was completely surrounded by a 1230-m long, 40-cm high, aluminum flashing drift fence, buried 6-10 cm into hard-packed soil (Gibbons and Semlitsch 1982). We installed 164 evenly-spaced traps (82 19-L buckets, 42 2.3-L buckets, 40 wooden box funnel traps) in pairs on opposite sides of the fence, allowing captures to be judged as entering or leaving the bay (Gibbons et al. 2006). Pitfall and funnel traps were checked a minimum of once daily. Captures of thousands of fossorial amphibians and reptiles, including salamanders, anurans, and snakes (Gibbons et al. 2006, Willson et al. 2006) strongly suggest that animals were seldom able to burrow under the drift fence and thus pass into the wetland undetected.

Aquatic trapping with minnow traps was conducted at Ellenton Bay from 1983 – 1987 (Seigel et al. 1995a, Seigel et al. 1995b) and May – June 1998 (Winne et al. 2005). Aquatic trapping was also conducted monthly from May – August 2003 (4,788 trap nights), immediately following the 2000 – 2003 drought. Traps were spaced approximately 2 m apart in transects along the margin of the bay, among emergent vegetation and checked at least once daily for snakes.

Sex, snout-vent length (SVL), tail length, body mass, and reproductive state were recorded for all captured snakes. Reproductive states of females were determined by palpating them for the presence of enlarged ova or developing embryos (Seigel et al. 1995b). Additionally, in some years (1983 - 1987), the presence or absence of prey items was identified by forced regurgitation (Fitch 1987). Each snake was marked with a unique code by clipping (1983 – 1998, Fitch 1987) or branding (2003, Winne et al. 2006) ventral scales before release at its capture location.

Feeding trials and maternal-litter relationships

To examine feeding rates of snakes in the laboratory, pregnant (n = 16) and non-pregnant (n = 7) females were collected between 21 May and 30 July 2003 and housed under laboratory conditions until parturition. Snakes were kept individually in plastic 5 L shoeboxes fitted with paper towels as a substrate and a large water dish (737 mL) that allowed snakes to fully submerge. Cages were placed within an environmental chamber at 25°C with a 14L:10D photoperiod. Water and towels were changed 2-3 times per week, and all snakes were offered live *A. talpoideum* larvae totaling 40 – 60% of the snake's mass every 7 – 10 days.

Approximately 12 h after feeding, all remaining prey were removed and weighed to determine the amount of prey consumed. During late July through August cages were examined once or twice daily for the presence of neonates. All pregnant females gave birth between 3 and 25 August 2003. Mass, SVL, and tail length of mothers and neonates were measured within 24 h of parturition.

Statistical analyses

A contingency table analysis was used to determine if frequencies of reproductive females were statistically different among years and to detect differences in frequencies of individuals containing food items among sexes and reproductive classes. Both Analysis of Variance (ANOVA) and Analysis of Covariance (ANCOVA; with maternal SVL as the covariate) were used to test whether litter size varied among years; data were natural log-transformed prior to analyses. The relationship between maternal SVL and litter size was determined using linear regression on natural log-transformed data. To compare feeding rates of pregnant and non-pregnant females, the Kruskal-Wallis test was used in lieu of a one-way ANOVA because the assumption of homogenous variances could not be met. All tests were performed by hand (asymmetric contingency table analysis) or using the STATISTICA for Windows software package (StatSoft, Inc. Tulsa, OK, USA 1998). Data were examined prior to each analysis and all statistical assumptions were met. Statistical significance was recognized at $\alpha = 0.05$. All means are presented as ± 1 standard error.

RESULTS

Drought survival strategy

Ellenton Bay was completely encircled with a terrestrial drift fence prior to the onset of rains in early February 2003 that refilled the wetland, which reached peak water level in July

(Fig. 2.1). This allowed us to detect any potential immigrants to the wetland prior to wetland refilling. Nonetheless, *S. pygaea* were not captured entering or exiting Ellenton Bay from 1 February 2003 through April 2003. During May three adults were captured leaving the bay at the drift fence (6, 18, and 21 May), and only one adult was captured entering (21 May). Despite the previous drought (Fig. 2.1) and the paucity of immigrants to Ellenton Bay (Fig. 2.2), aquatic trapping revealed that a substantial population of adult *S. pygaea* was resident within Ellenton Bay after it refilled (Fig. 2.2). However, in comparison to 1998, the population was highly skewed towards adults in 2003 ($\chi^2 = 19.24$; $P < 0.001$), with no juveniles captured in 2003 (Fig. 2.3). Thus, it appears that adult *S. pygaea* were able to survive within the dried wetland throughout the 2.5 yr drought but that no recruitment (and probably no reproduction) occurred.

Post-drought reproduction

Sixty nine individual females were captured in the aquatic habitat from May to August 2003. Of these, 49 (71%) were pregnant during one or more months, which is similar to the percentage (76%) pregnant in 1998 (Winne et al. 2005) and in the four years sampled during the 1980's (Seigel et al. 1995b; Fig. 2.4a). Despite notable changes in water level and the severe drought of 2000 – 2003 no significant differences in the frequency of reproductive females were found among these six years (contingency table; $\chi^2 = 6.2$; $P = 0.267$; Fig. 2.4a).

Litter size did not vary significantly among years (ANOVA, $F_{4,81} = 1.69$, $P = 0.160$; Fig. 2.4b), even after accounting for maternal length (ANCOVA, SVL as covariate, $F_{4,80} = 1.21$, $P = 0.314$). As expected, a significant positive relationship (slope = 3.43) existed between natural log-transformed maternal SVL and litter size in 2003 ($r^2 = 0.39$; $P = 0.01$). The slope of the regression was not significantly different from the slopes observed by Seigel et al. (1995b) in previous years (ANCOVA test for difference in slope, SVL as covariate, $F_{4,76} = 1.44$, $P = 0.229$; Fig. 2.4b).

Lack of anorexia during pregnancy

Most *S. pygaea* readily consumed large meals (20.8 ± 2.5% of their body mass per feeding) during pregnancy (Fig. 2.5a). The average percent body mass consumed by six of the pregnant females (range: 24 – 36%) was similar to consumption by non-pregnant females (range: 24 – 37%; Fig. 2.5a). Meal sizes were large for some pregnant females, with several consuming prey 40 – 57% of their prepartum body mass during single feeding events. Non-pregnant females consumed proportionally more prey, on average (30.7 ± 2.1% of their body mass), than pregnant females (Kruskal-Wallis; $H_{1,22} = 5.58$; $P = 0.018$; Fig. 2.5a), but field captures demonstrate that pregnant females readily feed during pregnancy in the wild. Records of adult females (84 non-pregnant, 70 pregnant) from June 1983 to August 1987 show that pregnant females were the most likely demographic to contain prey items: 67% of pregnant females contained one or more prey items, whereas only 40% of non-pregnant females contained prey items (Fig. 2.5b). Proportions of pregnant and non-pregnant females containing prey items ($\chi^2 = 2.230$; $P = 0.135$) did not vary significantly. Of 180 males, only 25% contained prey items, significantly less than the proportion of pregnant females with food ($\chi^2 = 10.6$; $P = 0.001$), but not statistically less than the proportion of non-pregnant females containing prey ($\chi^2 = 2.4$; $P = 0.122$). In accordance with our laboratory results, meal sizes of field-captured pregnant females were large. For example, one pregnant female captured in the field regurgitated two *A. talpoideum* larvae with a combined mass equaling 32% of the female's prepartum body mass.

DISCUSSION

Drought survival strategy

We documented the number of *S. pygaea* returning to Ellenton Bay as it refilled after being dry for over 2 years, as well as the relative abundance of snakes within the wetland after the water had returned to normal levels. None immigrated to the wetland prior to the refilling of the bay and only a few returned after the wetland refilled. Nonetheless, a substantial population of adult *S. pygaea* was resident within the wetland as soon as it refilled. Thus, despite their highly aquatic habits, *S. pygaea* appear well-adapted to survive multi-year droughts.

Although *S. pygaea* is the smallest aquatic snake species at Ellenton Bay (and thus least likely to trespass over the drift-fence), it accounted for only 0.8% of the snakes captured entering the wetland during spring 2003. Nonetheless, it was the most abundant snake within the aquatic habitat both before (1998; 69.7% of snake captures) and after (2003; 89.1% snake captures) the drought. Additionally, drift fence captures of adult and neonate *S. pygaea* during summer 2003 (see Fig. 6 in Winne et al. 2005)and in other drift fence studies, both at Ellenton Bay (Seigel et al. 1995a) and elsewhere (Dodd 1993), demonstrate that *S. pygaea* are readily captured in drift fences. Consequently, we conclude that *S. pygaea* captured within the aquatic habitat after Ellenton Bay refilled in 2003 must have been inside of the drift fence, within the dried basin of the bay, when the drought ended. Opportunistic searches and the use of artificial coverboards within the Ellenton Bay basin during the drought resulted in no *S. pygaea* captures (C. Winne, pers. obs.). Presumably, *S. pygaea* survived the multi-year drought by aestivating beneath the dried surface of the wetland, a phenomenon documented in a diversity of other taxa, including invertebrates (Dietz-Brantley et al. 2002), fish (Fishman et al. 1986, Sturla et al. 2002), amphibians (Loveridge and Withers 1981, Etheridge 1990, Withers 1993), and turtles (Grigg et al. 1986, Kennett and Christian 1994, Ligon and Peterson 2002), but not previously reported in snakes. Comparisons of relative abundance (snakes per trap night) within the aquatic habitat attest to the success of this strategy. Relative abundance of *S. pygaea* was similar between pre- (1998) and post-drought (2003) years, whereas sympatric semi-aquatic natricines (*Nerodia fasciata* and *N. floridana*) that did not aestivate experienced precipitous declines during the drought (Willson et al. 2006).

Despite unequivocal evidence from our study that a sizable portion of the *S. pygaea* population had aestivated within the wetland, data are not available to know what proportion of the population, if any, emigrated during the drought itself. Likewise, previous evidence for how *S. pygaea* survive droughts has been contradictory. Two studies suggested that they migrate between wetlands in response to drought (Dodd 1993, Seigel et al. 1995a). Seigel *et al.* (1995a) documented adult *S. pygaea* emigrating from Ellenton Bay in response to an earlier drought (1987 – 1990). Seigel *et al.* (1995a) did not monitor immigration or resident population levels immediately after the drought but noted that "despite the large number of individuals leaving the bay, many [others] did not emigrate," which suggests that some *S. pygaea* may have relied upon aestivation during that drought. Matthew J. Aresco (pers. comm.) found *S. pygaea* aestivating within a dried wetland in Florida during a severe drought. Similarly, Archie Carr (1940) noted that *S. pygaea* in Florida burrow deep (60 cm) into sphagnum and mud during winter. Thus, *S. pygaea* appears to be capable of adopting different drought-avoidance strategies in different situations, a phenomenon that has been noted for at least one species of amphibian (Lampert and Linsenmair 2002).

Post-drought reproductive ecology

Drought is energetically challenging for many animals, in part because food availability is often reduced or absent. To survive, non-migratory animals must rely on stored energy that might have otherwise been allocated to reproduction. Thus, many animals do not reproduce during drought and reproductive output is often reduced immediately following drought, until energy reserves are replenished. The conflicting requirements between reproduction and survival are evident in Galapagos marine iguanas (*Amblyrhynchus cristatus*) exposed to periodic food shortages caused by the El Nino-Southern Oscillation (ENSO) cycle. During ENSO events, *A. cristatus* allocate all of their stored energy to survival and do not reproduce (Laurie 1990, Wikelski and Thom 2000). Moreover, following an ENSO event, few females are able to gather enough resources to reproduce during the first season; thus, successful reproduction does not occur until two years following drought (Laurie 1990).

In contrast, *S. pygaea* reproduced in the same frequency and with the same fecundity in the first season following extreme drought as in pre-drought years. How did *S. pygaea* fuel reproduction following the drought? In 2003, we began aquatic trapping in May when most females already contained enlarged ova or embryos. We were therefore unable to directly estimate post-drought body condition, and, by extension, the amount of energy stores ('capital') available for reproduction (Bonnet et al. 2001, Gignac and Gregory 2005) for our population. Nonetheless, two lines of evidence indicate that *S. pygaea* began 2003 with little or no energy for capital reproduction. First, aestivation by *S. pygaea* during the drought and the absence of amphibian prey (Gibbons et al. 2006) suggest that feeding opportunities were limited or absent. Lack of successful reproduction during the drought, as evidenced by the absence of juveniles immediately following the drought, further supports the supposition that for *S. pygaea* to survive the drought, they would have been forced to rely upon energy stores primarily for maintenance metabolism. In addition, compared to pre-drought years, significantly fewer large individuals remained within the wetland immediately following the drought (unpublished data). For reptiles, the largest individuals within a population are generally the most vulnerable to energetic deficiencies: larger individuals have greater total metabolic demands and are more likely to be selected against during times of food shortage (Wikelski and Thom 2000, Beaupre 2002). The cumulative evidence suggests that *S. pygaea* were energetically constrained at the beginning of 2003 and relied primarily upon food consumed during vitellogenesis and/or pregnancy (i.e., income) to fuel reproduction. Such income breeding is considered rare for snakes (Bonnet et al. 1998, Gregory et al. 1999) but seems fitting for the ecology of *S. pygaea*.

Bonnet et al. (1998) stated that capital breeding is best suited to organisms for which simultaneous energy acquisition and expenditure is unlikely to be feasible. Thus, for many snakes capital breeding is an ideal strategy because it provides a disassociation between feeding and reproduction, a necessary requirement for snakes that become anorexic during pregnancy (Bonnet et al. 1998). Unlike many snakes, *S. pygaea* are not constrained to a capital breeding strategy because they do not exhibit 'adaptive anorexia,' but instead feed readily throughout pregnancy.

What allows *S. pygaea* to continue to feed during pregnancy? A possible explanation is that *S. pygaea* are not subject to the costs typically associated with feeding. For example, unlike most natricines, *S. pygaea* seldom bask out of the water and rarely leave the aquatic habitat (Gibbons and Dorcas 2004). Therefore, because pregnant *S. pygaea* are always in close proximity to prey, no obvious spatial conflicts arise between foraging and thermoregulation. Additionally, aquatic locomotion in *S. pygaea* is less impaired by pregnancy than terrestrial locomotion (Winne and Hopkins 2006), thus, selective pressures to reduce activity (e.g., Brodie III 1989) may be lower in aquatic habitats compared to terrestrial habitats (Brown and Weatherhead 1997). Finally, the nearly exclusive use of heavily-vegetated subsurface habitats, both during foraging and gestation, may reduce overall predation pressure on pregnant *S. pygaea* (compared to more exposed foragers) and therefore remove selective pressures to reduce foraging activity during pregnancy. These hypotheses certainly warrant further study but are supported by recent evidence that other aquatic snake species (e.g., *Emydocephalus annulatus*, *Nerodia sipedon*) also continue to feed during pregnancy (Brown and Weatherhead 1997, Aldridge and Bufalino 2003, Shine et al. 2004).

In addition to a lack of anorexia, aestivation may contribute to the ability of *S. pygaea* to reproduce in the first season following drought. By aestivating within the wetland, *S. pygaea* were able to emerge as soon as the wetland refilled and take immediate advantage of extraordinarily high abundances of explosively-breeding amphibian prey (Gibbons et al. 2006). Aestivation also allowed *S. pygaea* to exploit this abundant resource with virtually no competition because most other semi-aquatic snake and turtle species that inhabit Ellenton Bay either did not survive the drought or slowly immigrated to the wetland after it refilled (Gibbons et al. 1983, Willson et al. 2006). Consequently, prey availability was likely unlimited for *S. pygaea* immediately following the drought.

Conclusions

Our findings demonstrate that *S. pygaea* possess a distinctive suite of life history traits that permit them to survive and reproduce in isolated wetlands subject to periodic droughts and dramatic fluctuations in prey abundance. In contrast to snake species that leave aquatic habitats in response to drought, *S. pygaea* at Ellenton Bay apparently survived a multi-year drought by aestivating within the dried wetland. Furthermore, they reproduced at normal levels in the first season after the wetland refilled. The ability to rebound rapidly from the stresses of prolonged drought is due in part to *S. pygaea*'s reproductive ecology. As opposed to many ectotherms that exhibit capital breeding and 'adaptive anorexia,' *S. pygaea* readily feed throughout pregnancy, rapidly translating high prey abundance into reproductive output through income breeding. Collectively, these characteristics make *S. pygaea* well-adapted to isolated wetlands and an important model organism for future studies of reproductive ecology.

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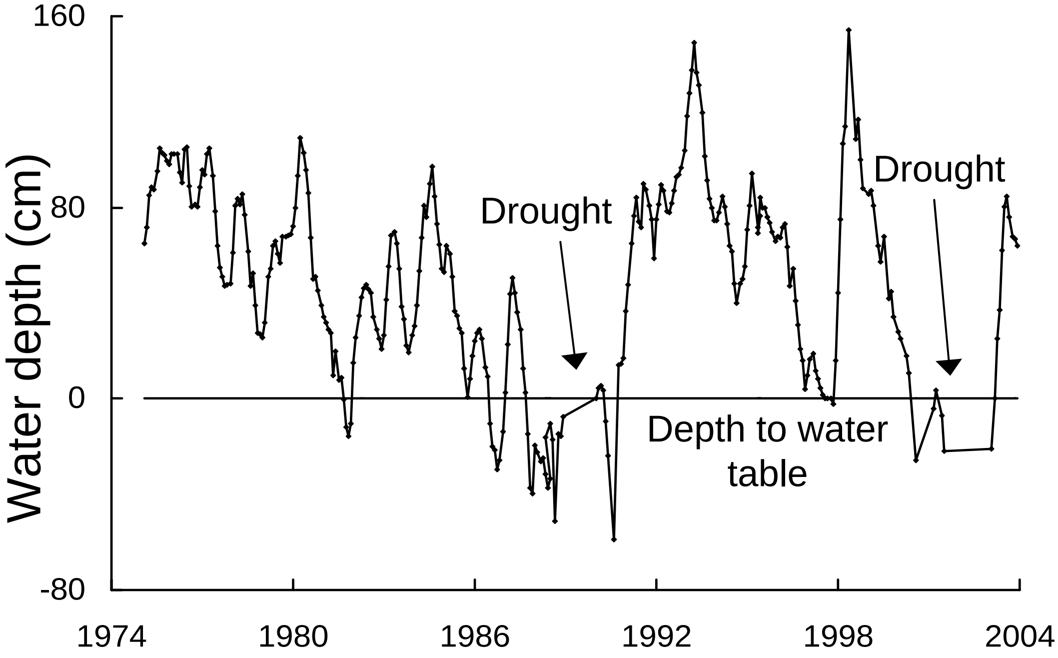

Figure 2.1. Mean monthly water depth at Ellenton Bay. During most droughts the depth to the top of the water table was determined by digging below the soil surface (represented by horizontal line); however, this depth was not determined between August 2001 and January 2003.

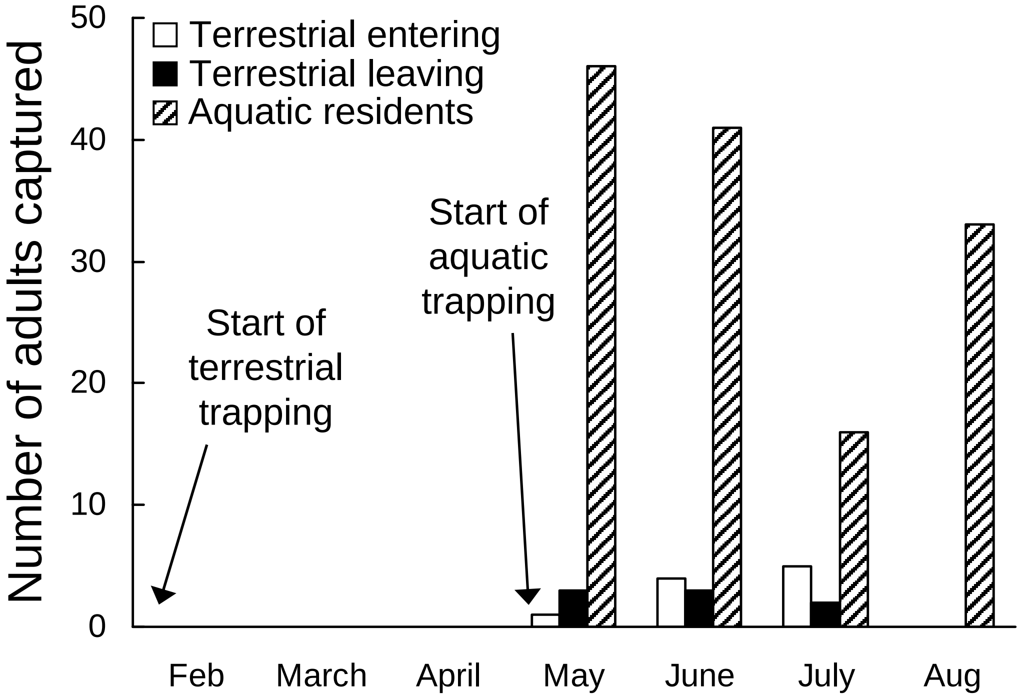

Figure 2.2. Terrestrial and aquatic captures of individual adult *Seminatrix pygaea* at Ellenton Bay in 2003. A drift fence served as a barrier to potential *S. pygaea* migrants and was operational in February, prior to the onset of rains that refilled the wetland. This allowed us to enumerate immigrant ('terrestrial entering') and emigrant ('terrestrial leaving') *S. pygaea* before and after the wetland refilled. Aquatic trapping began in May and revealed a large population of *S. pygaea* resident within the wetland ('aquatic residents') after heavy rains refilled the wetland.

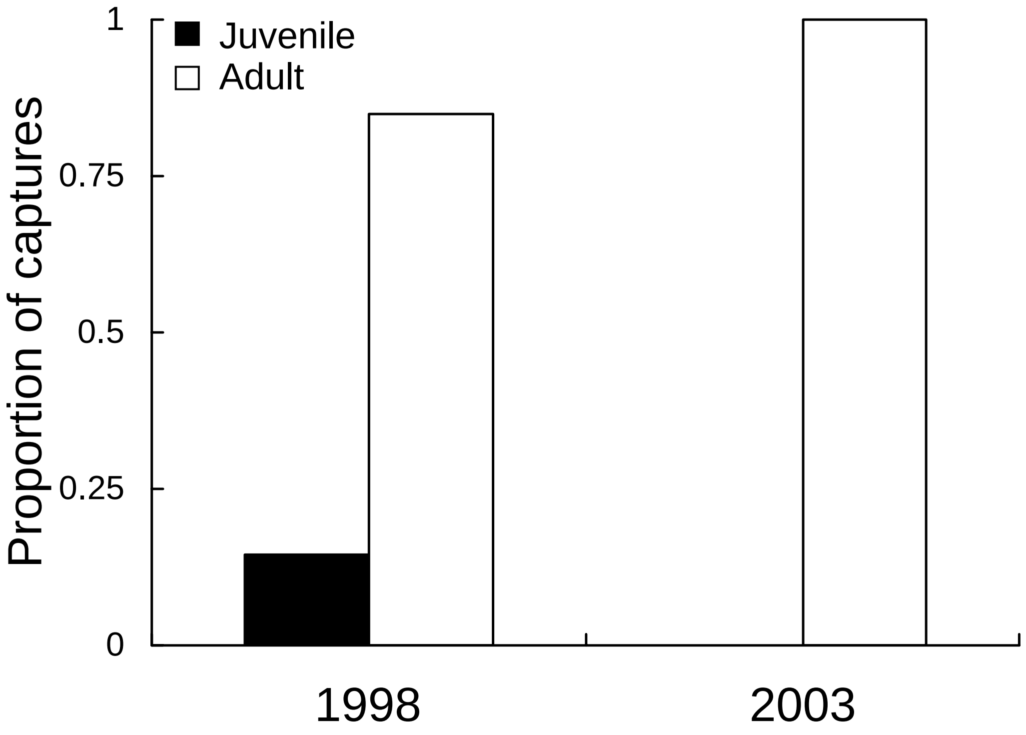

Figure 2.3. Proportion of juvenile *Seminatrix pygaea* captured at Ellenton Bay in 1998 and 2003. The lack of juveniles in 2003 suggests that no successful reproduction occurred during the 2000-2003 drought.

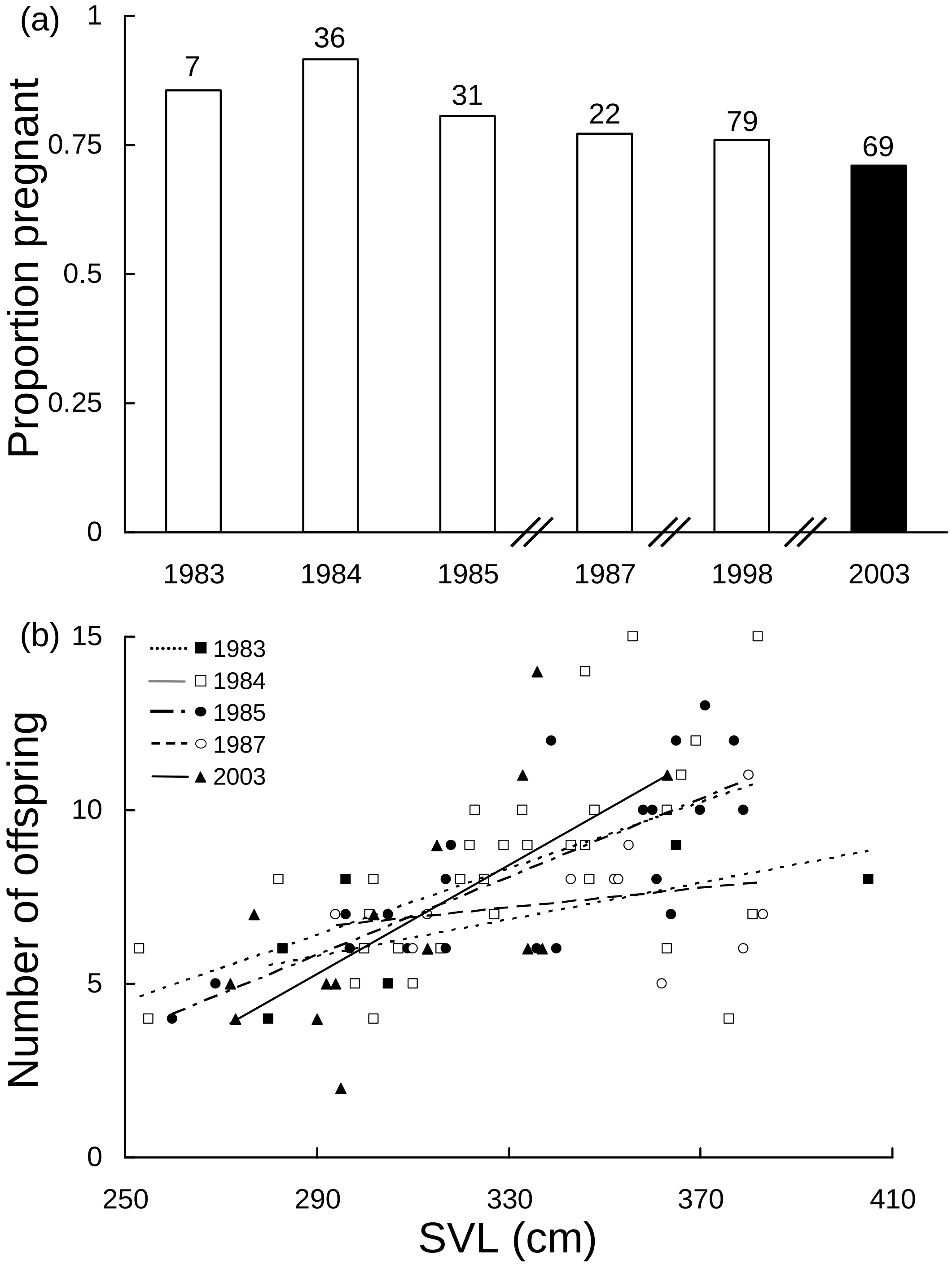

Figure 2.4. Reproductive ecology of *Seminatrix pygaea* at Ellenton Bay during pre- and post-drought years. a) Proportion of adult female *S. pygaea* that were pregnant in pre- (white) and post-drought (black) years. The number of adult females captured is given above each bar. b) Maternal-litter relationships among years.

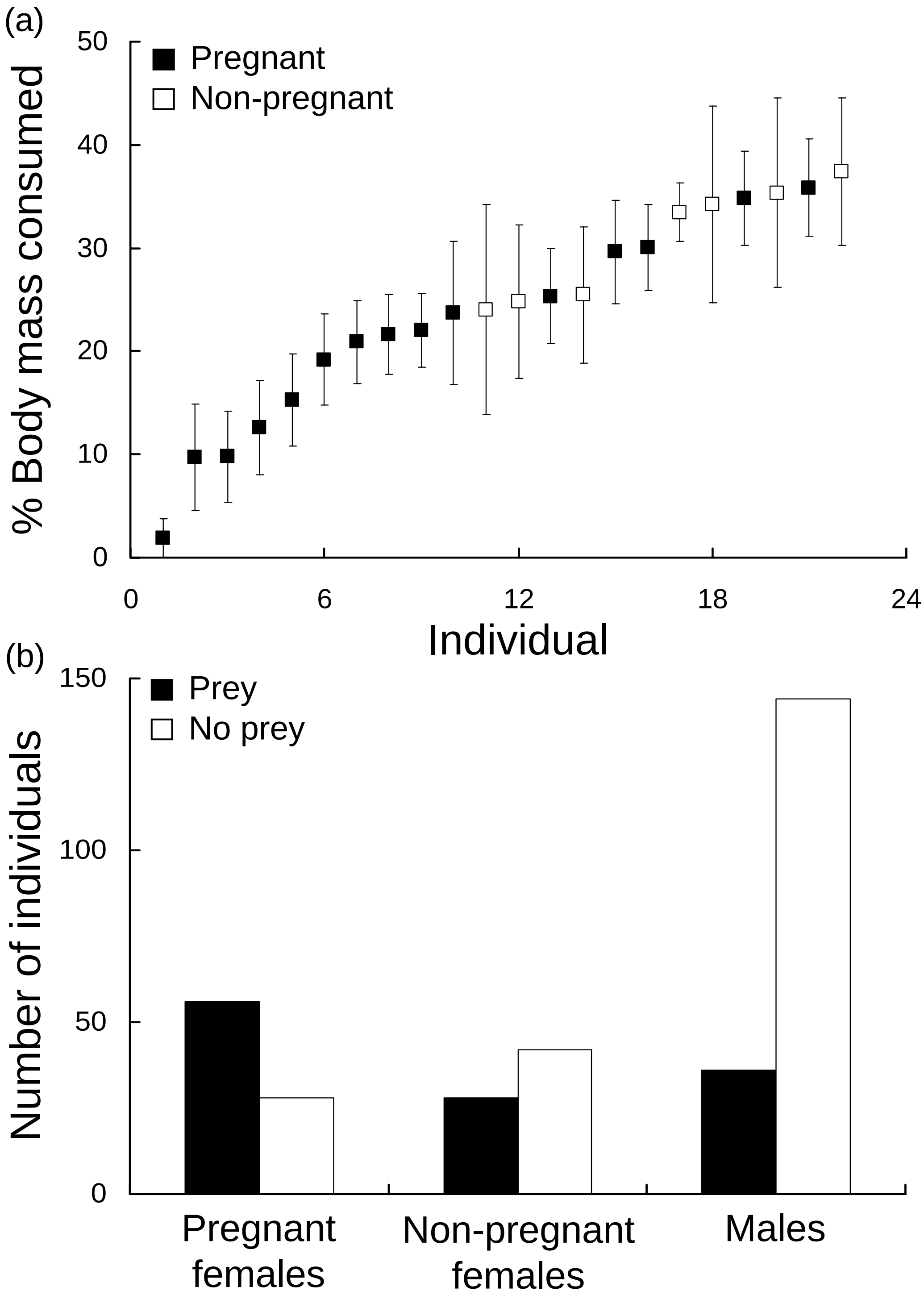

Figure 2.5. Effect of pregnancy on feeding in *Seminatrix pygaea* from Ellenton Bay. a) Mean (±1 SE) percent body mass consumed in the laboratory presented in order of increased prey consumption. b) Frequency of field-captured snakes containing food items.

CHAPTER 3

DROUGHT SURVIVAL AND REPRODUCTION IMPOSE CONTRASTING SELECTION PRESSURES ON MAXIMUM BODY SIZE AND SEXUAL SIZE DIMORPHISM IN A SNAKE, *SEMINATRIX PYGAEA*[1]

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[2] Winne, C. T., J. D. Willson, and J. W. Gibbons. To be submitted to *Oecologia*.

INTRODUCTION

Body size is one of the most obvious characteristics of any organism and plays an important ecological role by influencing nearly all physiological and life-history attributes, which in turn influence reproduction and survival (Peters 1983, Stearns 1992, Schmidt-Nielsen 1997). As a result, ecologists and evolutionary biologists have long studied the determinants and consequences of body size. A recurring theme is that the optimal body size for any given organism is context dependent and is often shaped by multiple, sometimes antagonistic forces (e.g., Darwin 1871, Case 1978, Wikelski 2005). In particular, two broad categories of selection act on body size: survival selection and selection for reproductive success, including sexual selection and fecundity selection (Preziosi and Fairbairn 1997, Wikelski and Trillmich 1997, Bonnet et al. 2000). Species that exhibit sexual size dimorphism (SSD) –sexual differences in body size– are of particular interest in this regard, because they provide the opportunity to explore the causes and consequences of different body sizes within a single species or population.

Ultimately, the direction and magnitude of SSD is determined by the ratio of different selection pressures on body size between each of the sexes (Arak 1988, Hedrick and Temeles 1989, Preziosi and Fairbairn 1997). Two dominant patterns of SSD are generally recognized, although more complex cases do exist (e.g., Madsen and Shine 1994). First, males are typically larger than females (male-biased SSD) in cases where the mating system is dominated by male-to-male combat (Darwin 1871, Clutton-Brock et al. 1977, Shine 1994). In this scenario, sexual selection for enhanced combat ability generally results in stronger selection for increased body size in males, compared to females. In contrast, females are usually larger than males (female-biased SSD) in populations with mating systems that do not involve male-to-male combat

(Darwin 1871, Shine 1994). Female-biased SSD is often explained by the fecundity advantage hypothesis, whereby there is strong selection for large females because they are able to carry more or larger offspring than small females (Darwin 1871, Semlitsch and Gibbons 1982, Seigel and Ford 1987, Shine 1994). Thus, in the absence of stronger selection for large size in males, fecundity selection will lead to female-biased SSD (Shine 1994).

Although selection pressures that generate sexual divergence in body size have been identified for many organisms, relatively few studies have examined selection pressures that moderate or exaggerate SSD within or among populations. For example, decreased food availability and/or prey size is known to negatively influence survivorship of larger individuals of reptiles (e.g., Wikelski and Trillmich 1997, Beaupre 2002, Wikelski 2005) and has been correlated with the evolution of island dwarfism in snakes and lizards (e.g., Case 1978, Boback 2003, Keogh et al. 2005, Jessop et al. 2006). The cause is generally straightforward: absolute metabolic energy requirements are positively correlated with body size (Bennett and Dawson 1976, Bennett 1982). In other words, although the metabolic rate per gram of tissue is lower for larger individuals (Bennett and Dawson 1976, Bennett 1982), absolutely more energy is required for larger individuals to support maintenance energy requirements, compared to smaller individuals, all else being equal (McNab 1971, Beaupre and Duvall 1998, McNab 1999, Bonnet et al. 2000, Beaupre 2002, Madsen and Shine 2002). Thus, unless larger individuals are relatively more efficient at foraging, smaller individuals will experience higher survivorship during periods of resource shortages (Forsman 1996, Beaupre 2002). In such cases, natural selection for ecological traits that increase survivorship during food shortages (e.g., smaller body size) may be in direct conflict with reproductive selection pressures that favor large body size (Forsman 1996, Beaupre and Duvall 1998).

On the Galapagos archipelago, Galapagos Marine Iguanas (*Amblyrhynchus cristatus*) provide an outstanding demonstration of the existence of this phenomenon (Wikelski and Trillmich 1997, Wikelski 2005). *Amblyrhynchus cristatus* exhibit male-male combat for females, resulting in sexual selection for larger males and male-biased SSD. However, during periods of food shortage caused by El Niño-Southern Oscillation (El Niño) events, the largest adults within populations experience the lowest survivorship as a result of their higher absolute energy requirements. As a result of fluctuating selection pressures, for large male body size in some years and small body size in other years, the degree of male-biased SSD fluctuates and is greatly reduced following El Niños. Moreover, mean adult body size and the degree of SSD differ among islands within the Galapagos archipelago; islands with greater food resources have larger lizards and a greater degree of male-biased SSD (Wikelski and Trillmich 1997, Wikelski 2005).

Recently, we have identified a system where a small aquatic snake species, the Black Swamp Snake (*Seminatrix pygaea*), is capable of surviving severe drought conditions by aestivating in dried wetlands (Winne et al. 2006b). Presumably, the lack of aquatic prey during extreme droughts (Gibbons et al. 2006) poses significant survivorship pressures on *S. pygaea*, analogous to those experienced by *A. cristatus* during El Niño events. In contrast to *A. cristatus*, however, *S. pygaea* exhibit female-biased SSD and no male-male combat (Gibbons and Dorcas 2004, Winne et al. 2005). Thus, our study system provides a unique opportunity to test the generality of the hypothesis that an antagonism exists between survivorship and reproductive selection pressures that act on body size and SSD. In particular, we are able to examine temporal variation in body size structure, maximum body size, and SSD within a single *S. pygaea* population. We predicted that (i) the largest *S. pygaea* would be largely absent following prolonged, severe droughts, and that (ii) female-biased SSD would be more extreme in years following high food availability, compared to years following drought-induced aestivation and a shortage of aquatic prey. In addition, we examine the influence of maternal size on litter size and offspring characteristics in *S. pygaea* to demonstrate the potential for fecundity selection to counteract survivorship selection and, thus, maintain female-biased SSD within the population.

METHODS

Study organism

Seminatrix pygaea is a member of the cosmopolitan subfamily Natricinae and is endemic to various aquatic habitats throughout a portion of the southeastern US Coastal Plain. It is the smallest semi-aquatic snake in North America, reaching a maximum recorded snout-to-vent length (SVL) of 485 mm (Gibbons and Dorcas 2004), and published interspecific comparisons of SSD among snakes indicate that *S. pygaea* may be less sexually dimorphic than most or all other natricine species (e.g., see appendix one in Shine 1994). Like other new world natricines, *S. pygaea* is viviparous (Sever et al. 2000), and mothers typically give birth in late July or early August (Seigel et al. 1995b, Winne et al. 2005). *Seminatrix pygaea* is capable of feeding on a wide variety of aquatic prey (Gibbons and Dorcas 2004). However, both males and females have fed nearly exclusively on aquatic larvae and paedomorphs of the Mole Salamander (*Ambystoma talpoideum*) at our study site since the early 1990s (C.T. Winne and J.D. Willson, unpublished data). Adult *S. pygaea* have very high rates of evaporative water loss compared with sympatric semi-aquatic snakes (Winne et al. 2001, Moen et al. 2005) and, consequently, they rarely venture away from the water's edge (Gibbons and Dorcas 2004). Additionally, *S. pygaea* are abundant in some isolated wetlands, making them ideal for long-term investigations of population-level responses to drought (Winne et al. 2006b).

Study site

Ellenton Bay is an isolated freshwater wetland located on the U.S. Department of Energy's Savannah River Site (SRS) in the upper Coastal Plain of South Carolina, USA. Although water level is extremely variable, the bay generally holds water year-round, and when full, covers approximately 10 ha. During most years, Ellenton Bay is dominated by shallow water (< 1 m deep) and relatively uniform distributions of emergent grasses (predominantly *Panicum* spp.), water lilies (*Nymphaea odorata*), and water shields (*Brasenia schreberi*). However, severe droughts have rendered Ellenton Bay dry on at least three occasions in the past three decades, most recently during 1987–1990 and 2000–2003 (Seigel et al. 1995a, Willson et al. 2006, Winne et al. 2006b). When dry, a thick (up to 0.5 m) organic crust covers the entire basin but subsurface areas remain moist and up to 1 ha of viscous mud surrounds small open water areas during shorter dry spells. In drought years, Ellenton Bay is the last nonpermanent wetland to dry within the region (i.e., it has the longest hydroperiod). The only permanent wetland within 1.4 km of Ellenton Bay is a small, manmade pond *c.* 0.5 km from Ellenton Bay. However, no *S. pygaea* have been captured in the pond during aquatic trapping over many years (C.T. Winne, J.D. Willson, and J.W. Gibbons, unpublished data). In fact, the closest known *S. pygaea* occurrences to Ellenton Bay include populations at Risher Sloughs (SRS) and Castor's Bay (SRS), which are 5.7 and 8.7 km from Ellenton Bay, respectively (C.T. Winne, J.D. Willson, unpublished data). Consequently, the Ellenton Bay population is effectively isolated from all known populations of *S. pygaea* on the SRS. The habitat surrounding Ellenton Bay is a mosaic of old-fields in various stages of succession and second-growth mixed pine-hardwood forest. Ellenton Bay is currently fish-free but harbors a diverse assemblage of amphibians (24 species) and semi–aquatic reptiles (18 species) during most years (Gibbons and Semlitsch 1991, Gibbons et al. 2006).

Data collection

To assess post-drought changes in body size distributions of *S. pygaea* within Ellenton Bay, we conducted aquatic trapping from May to June of the following years: 1983–1987 (Seigel et al. 1995a, Seigel et al. 1995b), 1998 (Winne et al. 2005), and 2003 (Winne et al. 2006b). We used a combination of commercially available steel and plastic minnow traps (Willson et al. 2005) set approximately 2 m apart in transects along the margin of the bay amidst emergent vegetation. Although we did not purposefully bait the traps, they naturally accumulated amphibian larvae between daily trap checks and we left accumulated larvae in the traps (Seigel et al. 1995a, Winne 2005). All captured snakes were returned to the laboratory where we recorded SVL (nearest mm), body mass (nearest 0.1 g using an electronic balance), and sex (by visual inspection or probing). We marked each snake with a unique code by scale-clipping (1983–1987; 1998) or heat-branding (2003, Winne et al. 2006a). We released all snakes the following day (1983–1987; 2003) or at the end of each five-day trapping period (1998). Additionally, we used a terrestrial drift fence that completely encircled Ellenton Bay to document the body sizes of any *S. pygaea* that moved into or out of the wetland during 2003 (for details see Willson et al. 2006, Winne et al. 2006b).

To determine maternal-litter relationships for *S. pygaea* from Ellenton Bay, we collected 16 pregnant females from 21 May–30 July 2003 and housed them under laboratory conditions until parturition. We housed snakes individually in plastic 5-L shoeboxes, fitted with paper towels as a substrate and a large water dish that allowed snakes to fully submerge. We placed all cages in an environmental chamber at 25 ^{o}C with a 14L:10D photoperiod. We changed water and towels 2-3 times per week, and offered all snakes live salamander larvae (*A. talpoideum*) totaling 40–60% of the snake's mass every 7-10 days. During late July through August we examined cages once or twice daily for the presence of neonates. All pregnant females gave birth from 3–25 August 2003. We measured the SVL and mass of mothers and neonates within 24 h of parturition.

Statistical analyses

We compared size frequency distributions of *S. pygaea* among three time intervals to assess drought-associated changes in population size structure. Snake captures from 1983 to 1987 represent historical size frequency distributions, which occurred prior to a major drought that began in Autumn of 1987 and ended in 1990 (Seigel et al. 1995a). Captures from 1998 constitute the population size structure immediately prior to the recent drought (September 2000–February 2003) that is the focus of this paper. Snakes captured during 2003 comprise the drought survivors and, thus, yield estimates of post-drought size structure. By focusing exclusively on captures from May and June, we are able to document the population size structure that existed at Ellenton Bay prior to significant growth in body length that occurred during the summer and fall after the 2000–2003 drought. We have recently documented that aquatic minnow traps cannot reliably capture *S. pygaea* smaller than 200 mm SVL (Willson et al. 2008). Therefore, we excluded individuals smaller than 200 mm SVL from all figures and analyses.

We compared maximum body size among years using the largest 10% of individuals of each sex captured in a given year category (1983–1987, *n*=107; 1998, *n*=120; 2003, *n*=68) as our indicator of maximum body size. We used a two-way analysis of variance (ANOVA; independent factors: year and sex; dependent factor: SVL) to examine the effects of year, sex, and year-by-sex interactions. Subsequently, we used Tukey's honestly significant difference (HSD) test for post-hoc comparisons. We also calculated an index of SSD for each year, using the difference between the ratio of female to male SVL (based on largest 10% of individuals in each sex) and one (Shine 1994). Following the methods of Forsman (1991) and Wikelski and Trillmich (1997), we verified that using the largest 10% of individuals for each sex/year category was a robust estimate of maximum body size for our study. To do this, we calculated maximum body size of each sex (for each year category) using eight different metrics: the SVL of the single largest individual and the mean SVL of the two, three, four, five, or ten largest individuals, and also the mean SVL of the largest five percent of population and largest 10 percent of population. We found that indices of maximum body size were all highly correlated (Kendall's coefficient of concordance; males: W=0.89, p<0.001; females: W=1; p<0.001), indicating that annual comparisons of maximum body size in *S. pygaea* is insensitive to the number of individuals used in the calculation (Forsman 1991, Wikelski and Trillmich 1997).

We used linear regression (on natural log-transformed variables) to describe the relationships between maternal SVL and mean litter characteristics. To assess the effect of maternal length on relative post-partum body condition, we regressed ln(mass) against ln(SVL) and used the residuals from the analyses as our measure of relative body condition. We used the STATISTICA for Windows (1998) software package (StatSoft, Inc. Tulsa, OK, USA 1998) for all tests. All means are presented ± 1 standard error.

RESULTS

We observed dramatic differences in size-frequency distributions of *S. pygaea* captured before (1983–1987, 1998) and after (2003) prolonged drought (Fig. 3.1). In pre-drought years, a large proportion (25–32.7 %) of individuals was larger than 325 mm SVL (Fig. 3.1a, b).

However, following the 2000–2003 drought only one individual (1.5 % of the population), a female, was larger than 325 mm SVL (Fig. 3.1c). Hence, both males and females from these larger size classes (i.e., $\geq$ 325 mm SVL) were noticeably absent. Inspection of the size-frequency histograms indicates that females (typically the larger of the two sexes) experienced a greater shift toward smaller size than did males following the drought. After the drought, only nine *S. pygaea* entered Ellenton Bay (throughout all of 2003) and all of these animals were similar in size to those captured contemporaneously within the wetland (one female, 234 mm SVL; eight males SVL's 250–315 mm).

We found significant variation in maximum body size among years ($F_{2,24}=16.55$, $p<0.001$) and between the sexes ($F_{1,24}=38.88$; $p<0.001$). There was no significant interaction between year and sex ($F_{2,24}=0.90$; $p=0.418$). Temporal variation in maximum body size was due only to changes between pre- and post-drought comparisons ($p<0.001$), as there were no statistical differences in maximum body size between the two pre-drought samples ($p=0.542$). Comparisons of the SSD index reveal that SSD was greater in pre-drought years (1983–1987, SSD=0.147; 1998, SSD=0.161) than in 2003 (SSD=0.095), indicating that compared with males, females experienced a greater reduction in maximum body size. Further evidence of a post-drought decrease in SSD is provided by year-by-sex independent contrasts, which demonstrate that maximum body size was significantly reduced following drought for females ($p\leq0.001$), but not for males ($p\geq0.271$). As expected, independent contrasts showed no significant differences in maximum body size between pre-drought years for males ($p=0.998$) or females ($p=0.865$). Overall, maximum body size and SSD were greater in pre-drought years and were dramatically reduced after the 2000–2003 drought (Fig. 3.2).

As expected, there was a significant positive relationship between maternal SVL and litter size in 2003 (r^2=0.39; p=0.010) and in all years (1983–1987 and 2003; data not available for 1998) combined (n=86 litters; r^2=0.35; p<0.001; Fig. 3.3a). Also, longer mothers gave birth to longer (SVL: r^2=0.24; p=0.051; Fig. 3.3b) and heavier (r^2=0.24; p=0.054; Fig. 3.3c) offspring. Comparing maternal body length (SVL) to post-partum relative body mass yielded an inverse parabolic relationship in which mid-sized females had greater masses for their length (i.e., greater body condition) compared to small and large adult females (Fig. 3.3d.).

DISCUSSION

Drought-induced mortality

We predicted that extreme drought, such as the 2000–2003 drought that left Ellenton Bay effectively dry and devoid of amphibian prey for multiple years, would pose significant hardships to *S. pygaea.* In particular, we predicted that larger *S. pygaea* would suffer greater mortality during the drought, analogous to the survivorship patterns observed for *A. cristatus* during El Niño-induced resource shortages on the Galapagos archipelago (Wikelski and Trillmich 1997, Wikelski 2005). Aestivation ultimately allowed a substantial proportion of *S. pygaea* to survive the drought (Winne et al. 2006b), unlike many sympatric species of semi-aquatic snakes that do not aestivate and which experienced precipitous declines or local extirpations (Willson et al. 2006). Nevertheless, we observed substantial shifts in the demography of *S. pygaea* following the drought that were indicative of differential survival among individuals. Both average and maximum body size were significantly reduced after the drought, results that support our hypothesis and fit expected changes in body size of reptiles experiencing prolonged food scarcity. Simply put, in reptiles larger individuals have higher absolute metabolic rates and energy requirements (Bennett and Dawson 1976, Bennett 1982), making resource scarcity potentially more costly for larger individuals (e.g., Wikelski and Trillmich 1997, Beaupre 2002, Madsen and Shine 2002). For example, individual-based energetic models of rattlesnakes have demonstrated that, *ceteris paribus*, larger individuals are less likely to survive and reproduce than smaller individuals under resource limitation (Beaupre 2002). The disappearance of nearly all *S. pygaea* larger than 325 mm SVL supports the supposition that an upper body size threshold may exist for long-term survival of *S. pygaea* during prolonged droughts. Based on our results, it might be reasonable to predict that this threshold size decreases with increasing drought duration as fewer animals are able to meet their energy needs during periods of resource scarcity.

We observed a larger demographic shift in female than male *S. pygaea* following the drought. Typically, female *S. pygaea* attain larger maximum body size than males and few males grow larger than 325 mm SVL. Thus, one likely reason that females experienced greater post-drought decline in body size is that, being larger, more of them exhausted energy reserves before the drought ended. However, an additional reason is that costs associated with female reproduction may have left females with reduced energy reserves and contributed to lower survivorship following parturition (Madsen and Shine 1993a, Luiselli et al. 1996, Brown and Weatherhead 1997, Shine 2003). For example, female Water Pythons (*Liasis fuscus*) that allocate more energy to reproduction experience lower survival rates (Madsen and Shine 2000a). At Ellenton Bay the majority of *S. pygaea* give birth in late July or August (Seigel et al. 1995b, Winne et al. 2005). These months coincide with the beginning of the driest season in the region and in 2000 occurred approximately one month before Ellenton Bay dried completely from 2000 until early 2003. Females, therefore, would have had little, if any time to recover from the depletion of energy reserves allocated to reproduction before entering aestivation. Although all female *S. pygaea* typically invest a majority of their lipid reserves into reproduction (C.T. Winne, unpublished data), our comparison of post-partum body conditions revealed that mid-sized females (*c.* 295–315 mm SVL) had greater post-partum energy reserves than both smaller and larger reproductive females. This corresponds with our observation that the largest females had the lowest survivorship during the drought.

Three alternative interpretations of our results include: (1) selective emigration of large *S. pygaea* prior to the drought, (2) disproportionate shrinking of large individual *S. pygaea*, and (3) large *S. pygaea* of both sexes simply dying of old age. The available evidence does not support any of these hypotheses. First, we found that little overland migration occurred in *S. pygaea* following the 2000–2003 drought (Willson et al. 2006, Winne et al. 2006b). Only nine *S. pygaea* entered Ellenton Bay in 2003 and all were shorter than 325 mm SVL, indicating that our samples were based on the resident population and that no larger individuals survived. Furthermore, previous studies of *S. pygaea* have demonstrated that neonates and juveniles move overland far more frequently than adults (Dodd 1993, Winne et al. 2005). Also, because no *S. pygaea* have ever been captured in nearby wetlands (the closest *S. pygaea* occurrence is 5.6 km away; C.T. Winne and J.D. Willson, unpublished data), it is unlikely that the largest *S. pygaea* survived the drought by emigrating to nearby wetlands. Second, long-term studies spanning periods of severe food shortage suggest that significant shrinkage does not occur in individual snakes (Madsen and Shine 2001, Luiselli 2005). We observed no evidence of shrinking by any *S. pygaea* during our study, but we note that snakes were not permanently marked prior to the drought. Nonetheless, despite the ability of Galapagos Marine Iguanas to shrink up to 20% in body length during El Niños (Wikelski and Thom 2000), the largest iguanas still suffered disproportionate starvation-induced mortality. Third, in reptiles, survivorship generally increases with age or is independent of age after the first year (Turner 1977, Parker and Plummer 1987). Age and body size are often not highly correlated in adult reptiles because of substantial individual variation in growth trajectories (Madsen and Shine 2000b, Blouin-Demers et al. 2002). Thus, although we were not able to age adult *S. pygaea* in our population, it is unlikely that all of the largest individuals were also the oldest individuals. Additionally, evidence from another reptile demonstrates that when resources are limited, natural selection against large body size occurs independently of age (Wikelski and Trillmich 1997, Wikelski 2005).

Shifting sexual size dimorphism

Traditionally, sexual size dimorphisms have been treated as species-specific traits. More recently, a few studies have demonstrated that SSD can vary among populations (e.g., Forsman 1991, Madsen and Shine 1993c, Pearson et al. 2002) or among age classes (King et al. 1999) and have attempted to understand the ecological causes of this variation using comparative techniques. We predicted that, compared to pre-drought years, SSD would be reduced following periods of prolonged food shortage, such as the 2000–2003 drought. As predicted, we observed significant annual variation in SSD within a single population, with both maximum body size and SSD being reduced immediately following a severe drought. More broadly, the annual pattern of variation in SSD that we observed for *S. pygaea* supported our prediction that the larger sex of sexually dimorphic species should be most adversely affected by resource limitation, thus reducing maximum body size and the degree of SSD.

One obvious question that arises from our results is: if *S. pygaea* undergo periodic, drought-induced selection for reduced body size, then what are the potential mechanisms that maintain female-biased SSD in *S. pygaea*? That is, why do *S. pygaea* ever grow larger than a size capable of surviving prolonged droughts, and why do females grow even larger than males? The prevalence of female-biased SSD has been well-documented in natricine snakes (Shine 1994, King et al. 1999). There is strong theoretical and empirical evidence that fecundity selection generally favors large body sizes in female snakes (Semlitsch and Gibbons 1982, Shine 1994). For example, in *S. pygaea*, we found strong positive relationships between female body size and several measures of reproductive success, including litter size, offspring length, and offspring mass. The production of a greater number of offspring has obvious evolutionary advantages, but producing larger offspring is also beneficial because it can increase offspring survivorship in snakes (Saint Girons and Naulleau 1981, Kissner and Weatherhead 2005). Together these reproductive advantages to large mothers are suspected to be the evolutionary driving force behind female-biased SSD in natricines, including *S. pygaea* (e.g., Semlitsch and Gibbons 1982, Shine 1994).

Our data suggest that selection for increased reproductive output during non-drought years favors female *S. pygaea* that are too large to survive prolonged food shortages such as those occurring during prolonged droughts. There may be less selection pressure for large body sizes in male *S. pygaea*. For example, there is no evidence of male-male combat in aquatic natricine snakes, including *S. pygaea*, and therefore no reason to expect selection pressure to result in larger body sizes in males than females (Shine 1994). Additionally, genetic evidence demonstrates that adult male size does not influence reproductive success in wild populations of another aquatic natricine, *Nerodia sipedon* (Weatherhead et al. 2002). Nonetheless, larger male size can improve reproductive success in some circumstances for *N. sipedon* (Kissner et al. 2005) and other natricine species (Madsen and Shine 1993b, Shine et al. 2000). No data regarding the effect of body size on reproductive success of male *S. pygaea* are currently available, but we suspect that most male *S. pygaea* forgo becoming large under conditions of resource limitations and follow the low-energy, low-growth strategy employed by male *N. sipedon* (Weatherhead et al. 2002), at least partly as a means to remain small enough to persist through periodic droughts via aestivation.

Conclusions

By taking advantage of the temporally dynamic nature of our isolated wetland study site, we documented shifting selection pressures acting on body size in a population of *S. pygaea*. We found that *S. pygaea* experienced significant reductions in maximum body size and SSD following prolonged drought-induced food shortages and that the demographic shifts were greater in females, the larger sex. We attribute these patterns to differential mortality of snakes that were too large to support their basal maintenance requirements or that were depleted in energy stores (due to costs of reproduction) during the drought. Conversely, we found strong positive correlations between maternal size and several measures of reproductive success in *S. pygaea*, indicating that larger females are likely favored by fecundity selection during years of high food abundance (i.e., non-drought years). Our study emphasizes the dynamic interplay between selection pressures that act on body size in *S. pygaea* and is analogous to broader patterns predicted by theory (e.g., Beaupre 2002; Forsman 1996) and observed in other wild reptile populations (e.g., Forsman 1996, Wikelski and Trillmich 1997, Beaupre 2002). Ultimately, *S. pygaea* are better able to persist during droughts than sympatric natricine watersnakes that do not aestivate (Willson et al. 2006, Winne et al. 2006b), but our study suggests that the strategy of aestivation may come at the cost of reduced body size and SSD in *S. pygaea*. These results are particularly interesting given that *S. pygaea* is the smallest semi-aquatic snake in North America (Gibbons and Dorcas 2004) and one of the least sexually dimorphic natricine watersnake species (e.g., see appendix one in Shine 1994). Future comparative studies across populations of aquatic snakes inhabiting wetlands with differing hydroperiods and prey dynamics may be informative as to the effects of aestivation and prey availability on body size evolution in *S. pygaea* and other species.

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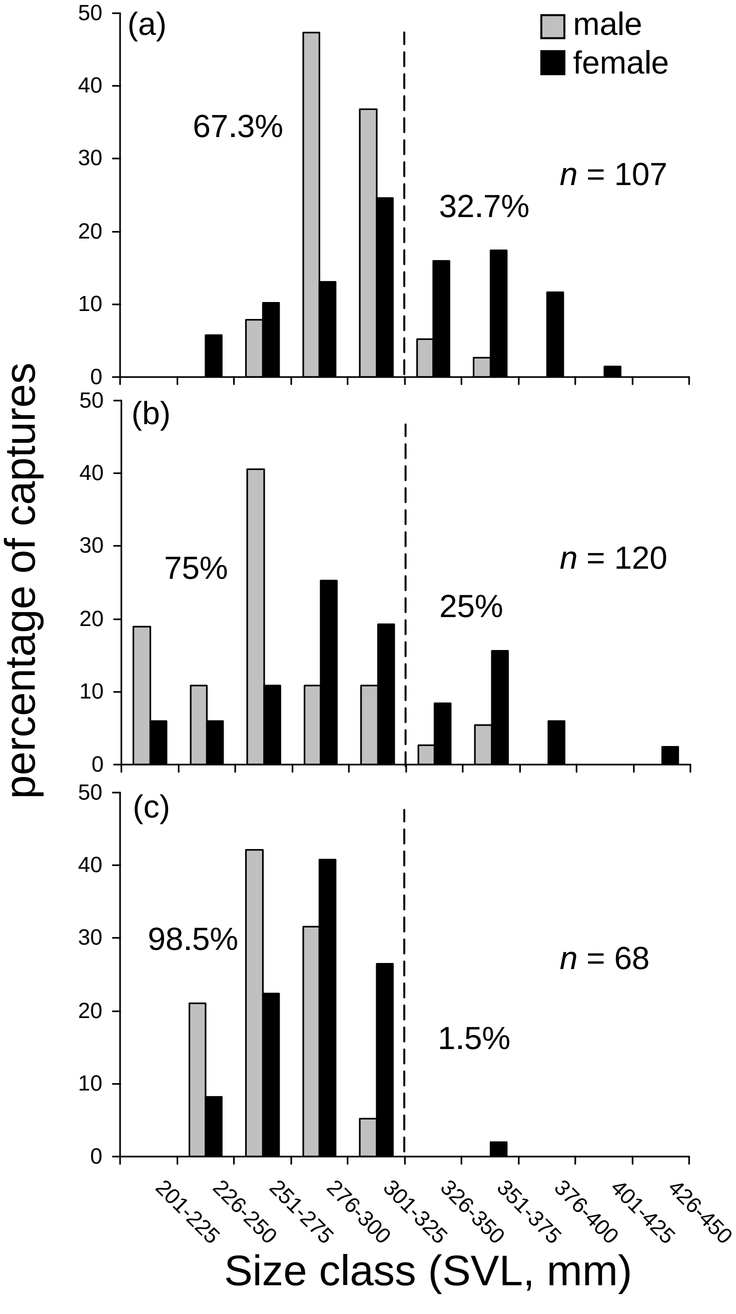

Figure 3.1. Percentage of *S. pygaea* captures at Ellenton Bay by size-class (SVL, snout-to-vent length) in 1983–1987 (a; pre-drought), 1998 (b; pre-drought), and 2003 (c; post-drought). Prior to the onset of the 2000–2003 drought (a, b), females were significantly larger than males and 25–32.7% of the population was larger than 325 mm SVL. In contrast, immediately following the drought (c) sexual size dimorphism was reduced, and only one snake (1.5 % of captures) was larger than 325 mm SVL. Individuals smaller than 200 mm SVL were excluded from figures (see methods). All snakes were captured in May or June.

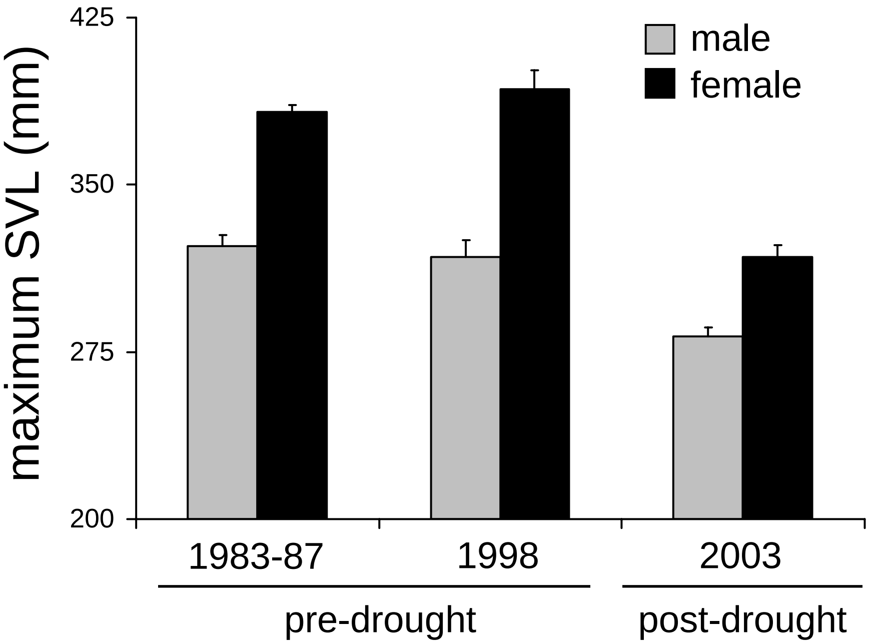

Figure 3.2. Maximum body size (SVL) of *S. pygaea* at Ellenton Bay. Maximum body size and sexual size dimorphism were greatest in pre-drought years and were significantly reduced in 2003, after the 2000–2003 drought (p<0.001). All snakes were captured in May or June.

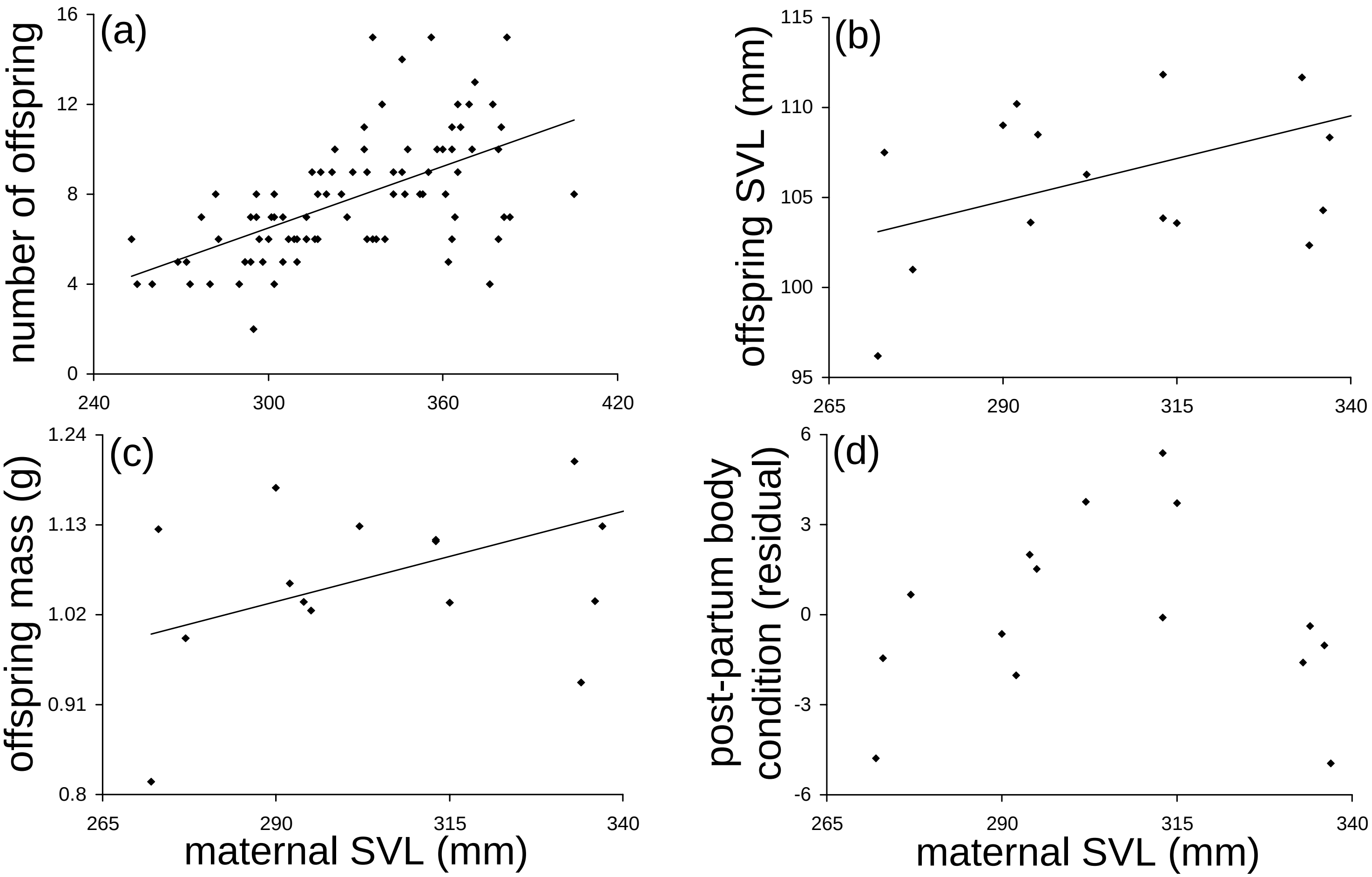

Figure 3.3. Relationships between maternal body size (SVL) and reproductive characteristics in *S. pygaea* at Ellenton Bay. There was a significant positive relationship between maternal SVL and a) litter size (p<0.001), b) mean offspring SVL (p=0.051), and c) mean offspring body mass (p=0.054). Comparing maternal SVL to post-partum relative body mass yielded an inverse parabolic relationship d), with mid-sized females having greater body mass for their length compared to small and large females.

CHAPTER 4

INCOME BREEDING IN A SNAKE, *SEMINATRIX PYGAEA*: EVIDENCE FROM MATERNAL TRANSFER OF STABLE ISOTOPES[1]

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[1] C. T. Winne, J. D. Willson, M. A. Pilgrim, and C. S. Romanek. To be submitted to *The Journal of Experimental Biology*.

INTRODUCTION

Animals acquire energy and partition it among the competing demands of maintenance, growth, and reproduction, with the evolutionary goal of translating energy into successful offspring. A number of reproductive allocation decisions must be made by animals, including “packaging decisions” such as the size, number, and type (e.g., egg vs. live) of offspring to produce (Stearns 1992, Roff 2002). However, the amount of energy available for ”packaging” offspring during a reproductive event is ultimately dependent upon resource availability, timing of resource allocation, and physiological mechanisms available to fuel reproduction, making these factors extremely important for understanding life history theory.

Many species inhabit dynamic habitats with fluctuating prey abundances, necessitating reproductive strategies for coping with temporal fluctuations in resource availability. In life history studies, a dichotomy is often drawn between “capital” and “income” reproductive allocation strategies (Drent and Daans 1980, Jönsson 1997, Bonnet et al. 1998, Houston et al. 2007). Capital breeding is a strategy used by animals to accumulate energy (“capital”) during periods of high resource productivity and store this energy in reserves such as lipids or muscle for long periods prior to reproduction. This allows reproductive output to be independent of resource availability at the time of reproduction (e.g., Lourdais et al. 2003) and can be especially advantageous for animals that require more than one season to accumulate sufficient energy for reproduction (Bonnet et al. 1998). In contrast, income breeding is a strategy whereby animals rely on recently ingested energy (“income”) to fuel reproductive output. Strict income breeders are able to take immediate advantage of increased resource abundance during the reproductive season but are unable to assemble large energy reserves for reproduction during other times due to costs of energy storage, such as metabolic costs or impediments to locomotion (Jönsson 1997, Houston et al. 2007).

Crucial factors that affect reproductive allocation strategies include costs of energy storage, availability of income, and costs of foraging during reproduction, among others (Bonnet et al. 1998, Houston et al. 2007). Taxonomic trends in reproductive allocation strategy are apparent: ectotherms such as reptiles are considered to be ideally suited to capital breeding because their relatively low metabolic rates and activity patterns reduce energy storage costs (Bonnet et al. 1998), whereas endotherms such as birds and mammals are more inclined towards income breeding (Drent and Daans 1980, Jönsson 1997, Houston et al. 2007). Within reptiles, most studied snake species are thought to rely principally on capital breeding, primarily because many species do not eat or dramatically reduce foraging during pregnancy (Gregory and Skebo 1998, Gregory et al. 1999). For such species, prey may be unavailable or limited in habitats used by snakes during gestation or pregnant snakes may simply cease foraging to decrease their susceptibility to predation (e.g., they are slower when pregnant, Seigel et al. 1987) or to allow them to more precisely thermoregulate at optimal temperatures for gestation (Charland and Gregory 1990, Peterson et al. 1993, Arnold and Peterson 2002). Not eating during pregnancy obviously reduces an organism's ability to finance offspring production and maternal maintenance with income energy but the unwillingness to eat during pregnancy, often referred to as "adaptive anorexia," is presumed to sometimes be an adaptive trait (Mrosovsky and Sherry 1980, Gregory and Skebo 1998, Gregory 2001). However, not all snakes exhibit pregnancy-induced anorexia (Aldridge and Bufalino 2003, Shine et al. 2004, Winne et al. 2006) and there is evidence that some snake species can modify litter or offspring sizes based on resources consumed early during the year (e.g., early spring) that they reproduce (Ford and Seigel 1989, Seigel and Ford 1991, Bonnet et al. 2001, Lourdais et al. 2003).

In reality, "capital" and "income" breeding represent extremes of a continuum and most species probably fall somewhere between these two alternative strategies (Bonnet et al. 1998, Houston et al. 2007). For example, Apsic Vipers (*Viper aspis*) are considered to epitomize the capital breeding strategy because they typically reproduce only every two to three years and they can reproduce during years in which they do not catch a single prey item (Bonnet et al. 1998, Lourdais et al. 2003). However, recent evidence has suggested that even *V. aspis* will incorporate supplemental income energy into its energy budget, when available, during offspring production (Bonnet et al. 2001, Lourdais et al. 2003). Indeed, there is growing evidence that some snakes rely on a mixed strategy and opportunistically use income energy to finance a second clutch (Brown and Shine 2002), grow during reproduction (Brown and Weatherhead 1997, Brown and Shine 2002), reproduce more frequently (Reading 2004), or increase litter or offspring sizes (Ford and Seigel 1989, Seigel and Ford 1991, Bonnet et al. 2001, Lourdais et al. 2003). In other cases, income energy may be required to reproduce following adverse years when capital cannot be accumulated (Winne et al. 2006). Still, the majority of evidence for income breeding (or mixed strategies) stems from correlative field observations of female body condition, prey abundance, and reproduction or from manipulative studies of prey availability beginning immediately post-hibernation (i.e., during early vitellogenesis) on reproductive output. Additionally, a few studies have examined changes in developing embryo composition throughout pregnancy (e.g., Clark et al. 1955, Stewart 1989) or used chemical tracers (Hoffman 1970) to investigate placental transfer of organic and inorganic materials from mothers to offspring in snakes (Stewart 1992), but the source (income vs. stored maternal resources) of transferred nutrients has not been examined. Consequently, we know little about the exact timing of resource allocation to offspring or the potential for viviparous snakes to transfer income energy directly to offspring during development. To fill this knowledge gap we need a method to directly investigate the timing of resource allocation by documenting the timing of nutrient transfer.

In recent years, stable isotopes have emerged as a powerful tool for tracing the flow of elements (e.g., carbon and nitrogen) among and within ecosystems. The use of stable isotopes as tracers within living systems relies on a conservative and predictable transfer of source isotopes into organism tissues. While most approaches focus on large-scale transfer of carbon and nitrogen (e.g., movement between aquatic and terrestrial environments, food web structure) stable isotope techniques can be used to investigate elemental cycling within organisms. For example, stable isotopes can be used to investigate reproductive allocation by providing the means to track the flow of maternal resources into offspring (Gannes et al. 1998). To date, the majority of studies using stable isotopes to investigate reproductive allocation have focused on taxa that rely upon distinctly different energy sources during reproductive and non-reproductive periods, for example insects with complex life histories (O’Brien et al. 2000, O'Brien et al. 2002, Min et al. 2006) and migratory birds (Gauthier et al. 2003, Hobson 2006). Nonetheless, recent studies have demonstrated that stable isotope ratios of prey items can be manipulated experimentally (e.g., MacNeil et al. 2006, Pilgrim 2007) and it may be possible to use these experimental techniques to identify sources of maternal nutrient allocation to offspring in taxa such as snakes that consume only a single general prey type.

We have been studying the ecology and physiology of Black Swamp Snakes (*Seminatrix pygaea*) inhabiting an isolated freshwater Carolina bay wetland in South Carolina, USA in response to variation in environmental conditions (drought) and prey availability. At this site, *S. pygaea* survives periodic, prolonged (multi-year) droughts by aestivating beneath the dried surface of the wetland, presumably without access to food (Winne et al. 2006, in review). Despite prolonged aestivation, high amphibian abundances occur at the study site immediately following drought, (Gibbons et al. 2006) and *S. pygaea* are able to reproduce in the same frequency and with the same fecundity immediately following emergence from aestivation as they did in pre-drought years (Winne et al. 2006). This result, in combination with the observation that pregnant *S. pygaea* readily consume prey during pregnancy (totaling up to 57% of pre-partum body mass in a single feeding event) has led us to conclude that *S. pygaea* relies on income breeding to fuel reproduction following prolonged drought (Winne et al. (2006). However, no direct evidence for maternal transfer of income energy to offspring was available for *S. pygaea*. Therefore, although *S. pygaea* used income energy consumed either during vitellogenesis or pregnancy to fuel offspring production, it is possible that resources consumed during pregnancy were allocated to maternal tissue rather than offspring. Here, we used an experimental approach involving stable isotope techniques to investigate the timing of reproductive allocation in *S. pygaea*. Specifically, we manipulated the concentration of a naturally occurring stable isotope (^{15}N) in prey items and altered the time that we introduced labeled prey to reproductive females. By subsequently evaluating the isotopic composition of post-partum mothers and offspring we were able to experimentally test our hypothesis that *S. pygaea* transfer energy consumed during pregnancy to their offspring.

METHODS

Study species

Seminatrix pygaea is a member of the cosmopolitan subfamily Natricinae and is endemic to the southeastern Coastal Plain of the United States. *Seminatrix pygaea* is the smallest aquatic snake in North America, with a maximum reported snout-vent length (SVL) of 485 mm (Gibbons and Dorcas 2004). Like other North American natricines, *S. pygaea* is viviparous and has a typical Type I or prenuptial reproductive cycle, with vitellogenesis occurring in spring (March to early June), ovulation in early June, and parturition in late July and early August (Dowling 1950, Seigel et al. 1995, Sever and Ryan 1999). However, the exact timing of ovulation and parturition is suspected to be somewhat flexible given the dynamic fluctuation of prey and environmental resources that *S. pygaea* rely upon at Ellenton Bay. *Seminatrix pygaea* is one of the most aquatic species of freshwater snakes within North America (Gibbons and Dorcas 2004). Although they are known to feed on a wide variety of prey, including small fishes, frogs, tadpoles, salamanders, leeches, and earthworms (Gibbons and Dorcas 2004), they have fed nearly exclusively on aquatic larval and paedomorphic Mole Salamanders (*Ambystoma talpoideum*) at our study site since the early 1990s (Willson et al. in review).

Animal collection and housing

We collected female *S. pygaea* (n = 18) from Ellenton Bay, a 10-ha Carolina Bay wetland located on the Savannah River Site in Aiken, SC, USA, from 3 – 8 May 2006. We recorded the initial SVL (nearest mm), tail length, and body mass (to the nearest 0.01 g using an electronic balance) of each snake captured. We then marked each snake with a unique code by branding ventral scales (Winne et al. 2006a). Females used in this experiment contained ova or early embryos at the time of capture (determined by gentle palpation) and gave birth from 25 July – 13

August, 2006 (mean parturition date: 2 August) to litters that ranged in size from 6 – 20 (mean ± SE: 13.4 ± 1.1) offspring.

Initially, we housed snakes communally (≤ 3 per cage, by treatments) in enclosures (48 x 24 x 72 cm) filled with well water (10 cm deep), to which we added vegetative structure in the form of sphagnum moss (*Sphagnum magelicum*) and live water hyacinths (*Eichhornia crassipes*). Enclosures provided snakes temperature gradients of ca. 26 – 36°C (via heat lamp) and UVA/UVB radiation (via fluorescent light) during the day (14L:10D photoperiod); at night, enclosure temperatures dropped to a steady 26°C. In mid-July, to facilitate our ability to monitor parturition dates without disturbing the pregnant females, we separated snakes and housed them individually in the enclosures. However, instead of filling the enclosures with water, we lined them with damp paper towels (changed regularly) and placed several large water bowls (filled with well water and vegetation) in each enclosure.

Isotopically Enriched Experimental Diet

To obtain an unambiguous isotopic signal that identified maternal transfer of nitrogen, we needed a diet artificially enriched in ^{15}N compared to naturally available diets. To achieve this goal, we enriched Canadian nightcrawlers (*Lumbricus terrestris*) in ^{15}N, following methods similar to MacNeil et al. (2006). We added 2.5 g of ammonium chloride ^{15}N concentrate (99.9% ^{15}N) to 750 ml of potato and water slurry. We allowed bacteria naturally present in the slurry mixture to absorb the ammonium chloride for eight days during incubation at 27°C. Following incubation, we thoroughly mixed the slurry into 21.5 litters of soil (equal parts organic topsoil and Canadian peat moss) and added 1100 – 1300 g of live Canadian Nightcrawlers. After 10 days at 17 – 20^{0}C, we haphazardly sampled 10 worms and analyzed them to be sure they had assimilated the isotope-enriched bacteria and attained ^{15}N levels greater than 300‰. We subsequently sampled 5 – 10 worms every 7 – 13 days to be sure they remained elevated above 300‰. We created a series of three replicate batches of enriched worms over the course of this experiment to ensure that enriched worms were available throughout pregnancy. The control diet consisted of identical worms, housed in an identical soil substrate, but without the addition of enriched ^{15}N slurry. We analyzed an equal number of control worms for ^{15}N during the enriched worm sampling periods. To compare enriched and control worm isotope values with those of natural prey items that are available to *S. pygaea* at our study site, we rely on natural prey data from Willson et al. (in review).

Experimental treatments and feeding protocol

We allocated female snakes to one of four experimental treatment groups: control A, control B, enriched early, and enriched late (Table 1). "Control A" snakes were never exposed to enriched worms or soil and, thus, served to provide baseline δ^{15}N values for maternal and offspring tissues. "Control B" snakes were exposed to enriched worms but did not consume them, allowing us to determine if simple exposure to the presence of enriched worms could contaminate the scale tissue of mothers or their offspring with enriched δ^{15}N values. "Enriched early" snakes began consuming enriched worms 77–83 days prior to parturition, providing a method for us to determine if ^{15}N consumed early in pregnancy is maternally transferred to offspring. "Enriched late" snakes began consuming enriched worms 23 days prior to parturition, allowing us to determine if ^{15}N consumed relatively late during pregnancy is transferred to offspring. We originally allocated the majority of snakes to the enriched treatments using stratified (with respect to SVL) random sampling. However, some snakes failed to consume enriched worms causing them to ultimately become part of the control B treatment. Also, one female assigned to the enriched early treatment consumed eight worms (six of which were enriched) but eventually underwent follicular atresia and was removed from the study.

We offered each snake 2 – 7 similarly sized live worms totaling 40% of the snake's body mass (determined the day of feeding) every 5 – 12 days (average: 8.5 days) throughout pregnancy, beginning on 12 May 2006. All snakes were fed on the same days and either received control or enriched worms, depending on their treatment. On two of the nine feeding occasions enriched worms were not available and, thus, we offered control worms to all snakes on those days. We fed snakes individually in plastic enclosures (23 x 9 x 55 cm) lined with paper towels and filled to a depth of 1 cm with well water. Snakes were allowed up to 12 hrs to consume the worms. After each feeding we counted the number of worms that were not consumed by each snake and returned snakes to their housing enclosures. Although we massed worms not consumed during feeding trials, there was wide variation in the amount of water absorbed by worms, making it difficult to directly estimate the amount of worm biomass consumed. Thus, for the purposes of this study we focus our analyses on the timing and number of worms consumed.

During all husbandry procedures, we exercised care to prevent contamination of the control treatment groups with enriched nitrogen. We always handled the control snakes and worms prior to working with the enriched treatment groups. We also thoroughly rinsed soil particles off of the worms prior to feedings and freezing for stable isotope analyses.

Sample Collection and Stable Isotope Analyses

We collected 2 – 5 mid-body ventral scale clips from mothers upon capture in May and immediately after parturition. Due to their smaller body size, we collected tail clips from all neonates within each litter immediately following birth. Scale clips have been shown to reflect isotopic composition of diet in snakes relatively soon (ca. 15 days) after a diet switch (Pilgrim

2005). Preliminary analyses have shown that scale and tail tissues from the same individual do not differ substantially in isotopic composition (~ 0.20 ‰ difference; M. A. Pilgrim, unpublished data). We collected whole-body samples of worms.

We dried all snake ventral scale and tail samples in an oven at 40 – 50°C for a minimum of 72 h. We dried the worm samples in a freeze drier, until they reached a stable dry mass. We homogenized each worm using a cryogenic grinder; separate cryogenic grinder vessels were used for control and enriched worms to reduce the opportunity for contamination. We packaged 1 mg of each sample (snake tissue or ground worm) into individual tin capsules and we determined their carbon and nitrogen isotope ratios using a Finnigan Delta+ isotope ratio mass spectrometer at the Savannah River Ecology Laboratory.

We report stable carbon and nitrogen isotope compositions of our samples as delta values. Delta values represent the ratio of heavy isotope to light isotope in a sample relative to the ratio of heavy to light isotope in a standard multiplied by 1000 (Ehleringer and Osmond 1989, Ehleringer and Rundel 1989). Thus, delta values are reported on a "per mil" (i.e., ‰) basis. Stable carbon isotope analysis determines the ratio of ^{13}C:^{12}C in a sample relative to a standard, while stable nitrogen isotope analysis determines the ratio of ^{15}N:^{14}N in a sample relative to a standard. PeeDee cretaceous belemnite (PDB) is the standard for carbon isotopes, while atmospheric air is the standard for nitrogen isotopes. When comparing two samples, the sample with a more positive delta value is enriched (contains more heavy isotope) relative to the other sample. When comparing two samples, the sample with a more negative delta value is depleted (contains more light isotope) relative to the other sample.

Statistics

To test for differences among treatments we used one-way analysis of variance (ANOVA), when assumptions of normality and homogeneity of variances could be met, and we used the non-parametric Kruskal-Wallis ANOVA when assumptions could not be met. We used litter means in our analysis of the effect of treatment on offspring $\delta^{15}N$ because siblings within a litter do not represent independent samples. We performed analyses using the STATISTICA for Windows software package (StatSoft, Inc. Tulsa, OK, USA 1998) and recognized statistical significance at $P \leq 0{\cdot}05$. We present all means as ± 1 SE.

RESULTS

We successfully created isotopically distinct diets of control and ^{15}N enriched worms. The three replicated batches of enriched worms were significantly elevated in $\delta^{15}N$ compared to control worms and natural amphibian prey (Kruskal-Wallis ANOVA: $H_{4,278} = 121.66$, $p < 0.001$; Fig. 4.1). Further, all enriched worms contained ^{15}N levels greater than 300‰, whereas all control worms and natural amphibian prey items contained ^{15}N levels under 6.3‰ and 10.2‰, respectively. Therefore, our diet design provided a strong, unambiguous isotopic signal capable of documenting the maternal transfer of recently ingested nitrogen.

At capture, treatment groups did not differ in ventral scale $\delta^{15}N$ (ANOVA: $F_{3,9} = 0.15$; $p = 0.925$; Fig. 4.2). However, by parturition, treatment groups differed significantly in maternal expression of ^{15}N within ventral scale tissue (ANOVA: $F_{3,9} = 4.41$; $p = 0.036$; Fig. 2). Females that consumed enriched worms either early or late in pregnancy exhibited dramatic (two- to seven-fold) or marked (two-fold) increases in $\delta^{15}N$ by parturition, respectfully (Fig. 4.2). Interestingly, there was considerable individual variation among enriched early snakes in $\delta^{15}N$ at parturition (Fig. 4.2). In contrast, females in the control A and control B groups did not exhibit any increase in $\delta^{15}N$ from the time of capture until parturition (Fig. 4.2). The lack of a detectable increase in $\delta^{15}N$ values of control snake maternal tissue indicates that the mere presence of enriched worms and our experimental protocols are not responsible for the elevated $\delta^{15}N$ observed in the enriched snake treatments. Instead, it is clear that our enriched diet provided mothers with a biologically useable form of ^{15}N and mothers were able to uptake ^{15}N and allocate it to their tissue.

We found significant differences among treatments in mean $\delta^{15}N$ of litters (Kruskal-Wallis ANOVA: $H_{3,17} = 10.34$, $p = 0.016$; Fig. 4.3). As predicted, most females that consumed enriched worms early (77 – 83 d prior to parturition) during pregnancy gave birth to offspring with elevated $\delta^{15}N$ values compared to control A, control B, and enriched late litters, providing direct evidence for maternal transfer of recently ingested ^{15}N to offspring (Fig. 4.3). Contrastingly, females that consumed enriched worms late (23 d prior to parturition) during pregnancy gave birth to offspring with $\delta^{15}N$ values similar to control A and control B litters, suggesting that ingested ^{15}N was not allocated to offspring. Collectively, these results indicate *S. pygaea* can transfer ^{15}N to offspring a minimum of 77 days prior to parturition, but perhaps not as late as 23 days prior to giving birth.

We observed individual variation in reproductive allocation strategies in *S. pygaea*. Among females that consumed enriched worms early during pregnancy, some females exhibited moderate enrichment of maternal tissue compared to the more substantial enrichment of offspring tissues, whereas other females showed the reverse pattern (Fig. 4.4). For example, one female consumed eight labeled worms throughout gestation and displayed elevated ^{15}N in maternal tissues, but failed to transfer substantial amounts of incoming ^{15}N to her offspring

(Table 1; Fig. 4.4). In contrast, another female consumed only one labeled worm but allocated considerably more ^{15}N to her offspring than to her own tissue (Table 1; Fig. 4.4).

DISCUSSION

Our results demonstrate that (i) stable isotopes can be artificially manipulated in prey and integrated into experiments to unambiguously document the timing and source of reproductive allocation in snakes, (ii) that *S. pygaea* is capable of allocating recently ingested income energy to both maternal and offspring tissues relatively late during pregnancy, and that (iii) individual variation may exist in reproductive allocation strategy of female *S. pygaea*.

In our experiment, we provided pregnant snakes with equal prey availability, relative to maternal mass, throughout gestation but manipulated the time at which we introduced ^{15}N enriched prey into their diets. All pregnant *S. pygaea* that consumed enriched worms exhibited increased $\delta^{15}N$ in their scale tissue. Thus, prey consumed by *S. pygaea* during pregnancy is used to build maternal tissue and this may be important for offsetting costs of reproduction, growth, or building energy stores. In other viviparous snake species high prey availability during pregnancy is associated with increased post-partum mass (Ford and Seigel 1989, Lourdais et al. 2002a, Gregory 2006), a trait that can significantly influence the chances of survival in female snakes (Luiselli et al. 1996, Bonnet et al. 1999, Madsen and Shine 2000a). Previously, however, most studies have been unable to discern whether increased post-partum mass was the result of income energy being used for maintenance (releasing them from the need to rely on energy stores during pregnancy) or tissue production (e.g., tissue turnover or growth). Our experiment confirms, at least for *S. pygaea*, that organic nutrient content from prey consumed during pregnancy is integrated into maternal tissue and, thus, is directly linked to a female’s post-partum mass.

Most females that consumed enriched prey early during pregnancy allocated enriched nitrogen to their offspring. This demonstrates unequivocally that *S. pygaea* can build offspring, at least partially, with income energy by transferring nitrogen products (e.g., amino acids or proteins) to offspring during pregnancy. A few other studies involving biological tracers have demonstrated placental transfer of radioactively-labeled nitrogen, amino acids, or proteins in other viviparous reptile species (e.g., Swain and Jones 1997), including snakes (e.g., Hoffman 1970). However, radioactive isotope studies have typically relied on intravenous injection of labeled molecules into mothers, rather than on consumption of labeled prey (see literature reviewed in Stewart 1992, Stewart and Thompson 2000). Thus, our study provides the first direct evidence that income energy consumed during pregnancy is allocated to offspring in viviparous snakes. Other studies of placental transfer in viviparous snakes, including detailed morphological studies (e.g., Blackburn and Lorenz 2003, Stewart and Brasch 2003) and chemical comparisons of excised uterine eggs or early embryos to live-born offspring in *Thamnophis ordinoides* (Stewart et al. 1990), *Virginia striatula* (Stewart 1989), and *Pseudechis porphyriacus* (Shine 1977), have also suggested that placental transfer of organic and inorganic nutrients may play an important role in the reproductive ecology of live-bearing species by providing supplemental or required income energy to offspring production. For example, although *V. striatula* are primarily lecithotrophic, females are able to augment yolk nourishment of young by increasing their nitrogen content during development, most likely through facultative placental transfer of amino acids or proteins (Stewart 1989, Sangha et al. 1996, Stewart and Brasch 2003). In a different study, Stewart et al. (1990) found no evidence of placental transfer of organic nutrients to offspring in the viviparous species *T. ordinoides*, though facultative placentotrophy did allow transfer of inorganic nutrients such as sodium and calcium. A limitation of these comparative studies, however, is that they have not focused on determining the original source (i.e., income vs. capital energy) of maternal nutrients involved in maternal-transfer.

Previously, the best evidence for existence of income breeding in viviparous snakes has come from experimental manipulations of prey availability during vitellogenesis (Ford and Seigel 1989, Lourdais et al. 2003). Ford and Seigel (1989), for example, offered high and low rations of prey to Checkered Garter Snakes (*Thamnophis marcianus*) beginning immediately after females emerged from hibernation and continuing until parturition. They found that individuals offered high rations produced larger litters and were in better post-partum body condition compared to individuals that were offered low rations. In contrast, by manipulating prey availability during more specific time periods within pregnancy, Gignac and Gregory (2005) and Gregory (2006) observed that increased resource abundance during pregnancy did not affect litter or offspring size in *T. ordinoides* or *T. sirtalis*, respectively, although it did increase female postpartum mass in both species. Ultimately, experimental manipulations of prey availability during vitellogenesis are invaluable for understanding the net ecological effects of income energy on reproductive output (e.g., litter size, offspring size) and maternal characteristics (e.g., body size, post-partum body condition), but without the introduction of biological tracers they give little insight into the physiological pathways (e.g., placental transfer of organic nutrients to offspring) or exact timing of reproductive allocation decisions. For example, (a) does income energy used to build offspring originate from prey consumed during vitellogenesis, sometime during pregnancy, or both? (b) Is prey consumed during vitellogenesis/pregnancy allocated to maternal tissue and only maternally stored resources (capital) actually used to increase litter (or offspring) size? And (c) how late into pregnancy can income energy be allocated to offspring? Consequently, studies that use biological tracers, such as ours, provide an important direct link between consumed income energy and organic nutrient allocation to offspring in snakes.

The ability to transfer supplemental income energy to offspring, maternal tissue, or both during pregnancy may be particularly advantageous for species such as *S. pygaea* that inhabit dynamic habitats and experience dramatic, unpredictable fluctuations in prey abundance, because it allows considerable flexibility in reproductive allocation strategies. Indeed, we observed significant variation among individual *S. pygaea* in the use of income energy during pregnancy. Although the majority of females consumed worms throughout pregnancy, six of seventeen *S. pygaea* did not consume any prey (control or enriched) during our experiment. Despite the lack of income energy during pregnancy, these six females reproduced successfully, suggesting that the use of income energy during pregnancy is a facultative trait in *S. pygaea*. Additionally, among females that consumed enriched worms early during pregnancy, some females exhibited moderate enrichment of maternal tissue compared to the more substantial enrichment of offspring tissues, while other females showed the reverse pattern. The results provide evidence that some *S. pygaea* may use income energy primarily to provide energy supplements to offspring, whereas others may use it predominantly for their own nourishment. In the future, we hope to be able to determine the causative factors that underlie differences in reproductive allocation strategies among female *S. pygaea*. We suspect that they may include maternal characteristics such as age, body size, body condition, and growth rate, as well as environmental conditions (e.g., rain, water level, prey availability) during the current and previous year. All of these factors are known to exert a strong influence on life history decisions and demographic processes in animals in general (Stearns 1992, Roff 2002), and aquatic snakes in particular (e.g., Madsen and Shine 2000b, c, Lourdais et al. 2002b, Madsen et al. 2006).

Our experiment confirms that *S. pygaea* are capable of incorporating income energy into both maternal and offspring body tissues during pregnancy. In addition, our data reveal that substantial variation in reproductive allocation strategies may exist among individuals from a single population. Overall, our isotopically labeled diet served as an ideal experimental tool to document both the existence and timing of transfer of recently ingested nitrogen into maternal and offspring tissues, and we envision that future studies of snake reproduction can readily adopt similar methods. For example, many of the viviparous snake species that have served as models for snake reproduction studies, including *Thamnophis spp., Virginia spp.,* and *Storeia spp*., all readily consume worms as a natural part of their diet and in laboratory experiments (Ernst and Ernst 2003). Indeed, squamate reptiles offer considerable potential for understanding the evolution of viviparity and have become model organisms for morphological, physiological, and ecological studies on this topic (Blackburn 2006). Ultimately, by incorporating stable isotope labels or other biological tracers into manipulative experiments of prey abundance, we may be able to quantify and more fully understand how local resource availability influences reproductive allocation decisions, a central goal of many life history studies.

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Table 4.1. Snake experimental treatment group characteristics. Means are presented ± 1SE. Ranges are provided following means, in parentheses.

Treatment	Definition	n	Maternal SVL (mm)	Litter size	Enriched worms consumed	Control worms consumed
Control A	offered only control worms	2	429 ± 11 (418-440)	16 ± 4 (12-20)	0	5.5 ± 3.5 (2-9)
Control B	exposed to enriched worms but did not consume them	9	382 ± 18 (308-446)	13.9 ± 1.5 (8-20)	0	1.1 ± 0.7 (0-5)
Enriched early	began consuming enriched worms 77 - 83 d prior to parturition	4	396 ± 20 (343-450)	10.3 ± 1.6 (6-14)	5.8 ± 2.0 (1-11)	3.0 ± 1.4 (0-7)
Enriched late	began consuming enriched worms 23 d prior to parturition	2	391 ± 57 (334-448)	15 ± 5 (10-20)	1 ± 0 (1)	9 ± 0 (9)

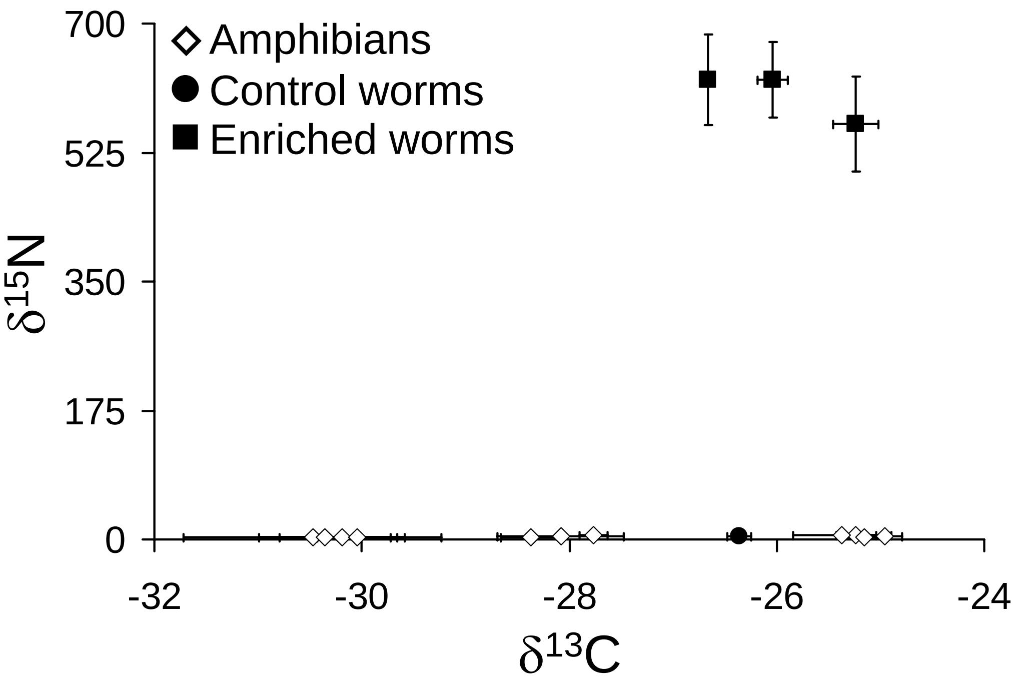

Figure 4.1. Stable isotope distribution of natural amphibian prey items (each point represents the mean for a different species or life stage) from Ellenton Bay and the experimentally introduced prey items, isotopically enriched and control worms. As expected, the three replicated batches of artificially enriched worms were significantly elevated in $\delta^{15}N$ compared to natural prey items and control worms ($p < 0.001$). Stable isotope values for natural amphibian prey reproduced from Willson et al., in review.

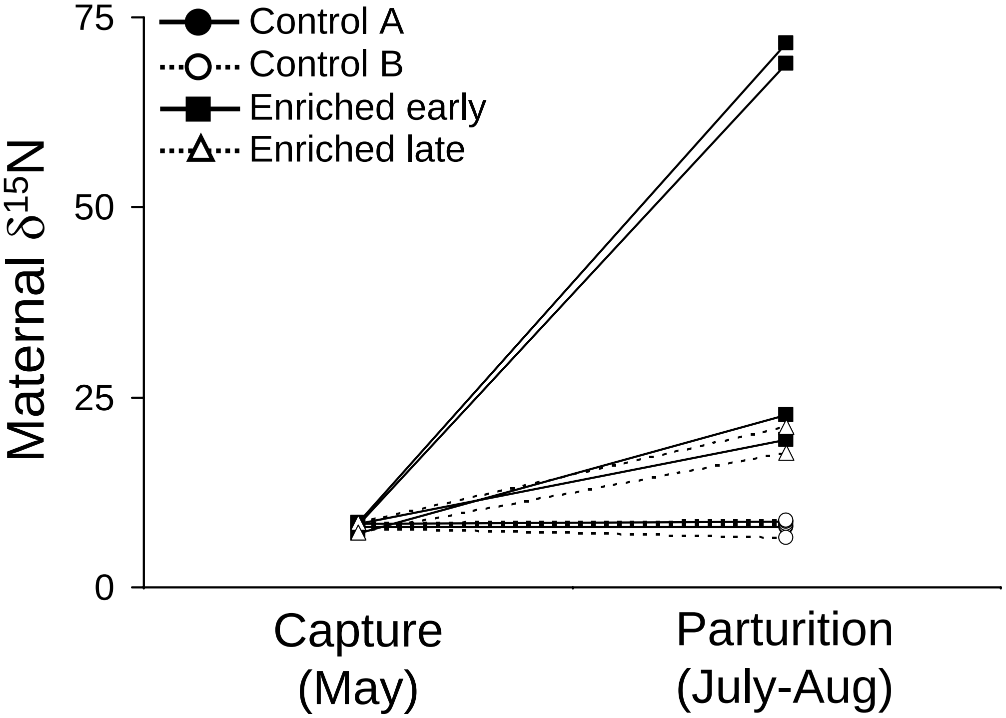

Figure 4.2. Maternal uptake of ^{15}N during pregnancy in *Seminatrix pygaea*. Females in the control A and control B groups did not exhibit any increase in $\delta^{15}N$ from the time of capture until parturition. In contrast, females that consumed enriched worms either early or late in pregnancy exhibited dramatic or marked increases in $\delta^{15}N$, respectfully.

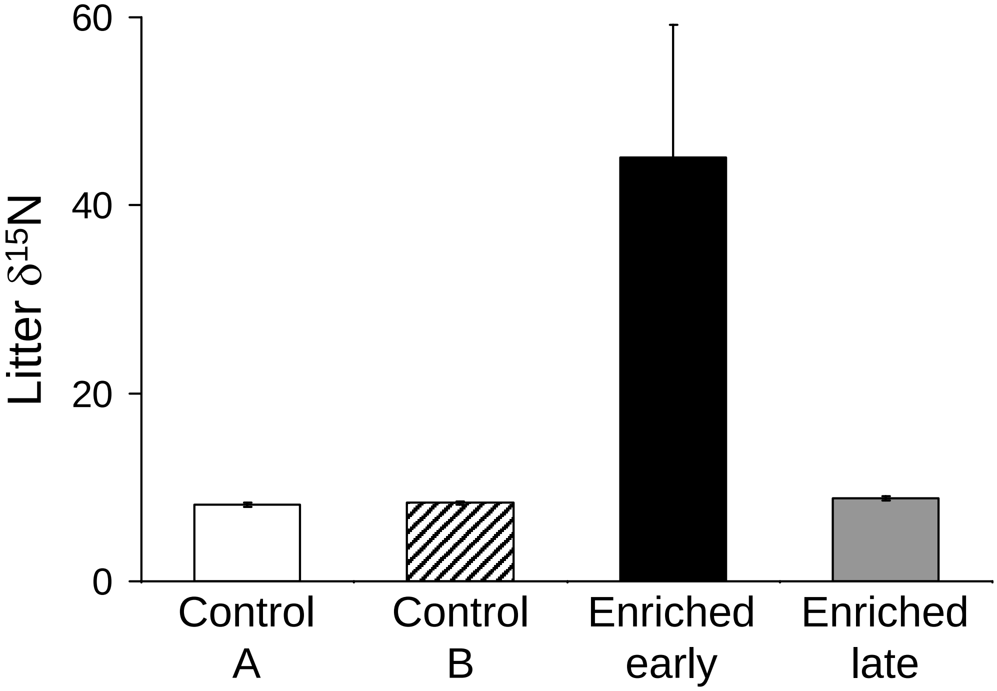

Figure 4.3. Maternal transfer of ^{15}N to offspring in *Seminatrix pygaea*. There was no maternal transfer of ^{15}N to offspring for control A, control B, or enriched late treatment groups. However, there was obvious maternal transfer of ^{15}N to offspring for the females that consumed enriched worms early during pregnancy, based on the elevated δ^{15}N values observed for enriched early litters. Treatment values are presented as grand means across litters.

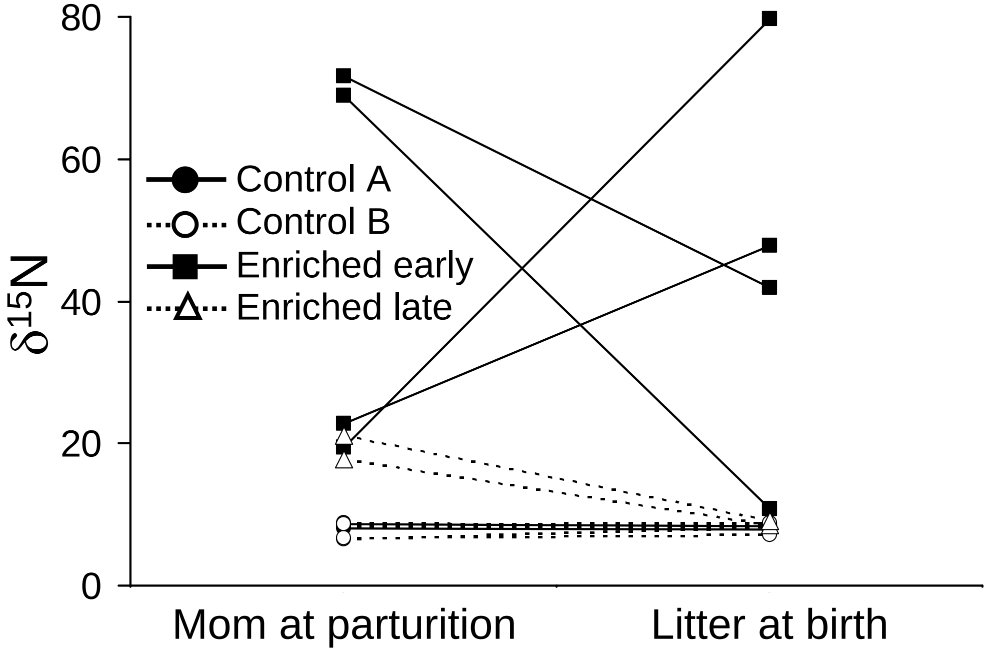

Figure 4.4. Individual variation in reproductive allocation strategies in *Seminatrix pygaea*. Among females that consumed enriched worms early during pregnancy, some females exhibited moderate enrichment of maternal tissue in ^{15}N compared to the more substantial enrichment of offspring tissues in ^{15}N, whereas other females showed the reverse pattern. Females that consumed enriched worms beginning late during pregnancy did not allocate observable levels of ^{15}N to their offspring. In control snakes, both maternal and offspring tissue contained only baseline $\delta^{15}N$ levels.

CHAPTER 5

POST-DROUGHT RECOVERY OF A WETLAND COMMUNITY: ECOLOGICAL RESILIENCE IN SEMI-AQUATIC SNAKES [1]

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[1] Winne, C. T. and J.D. Willson. To be submitted to *Journal of Zoology, London*.

INTRODUCTION

Isolated freshwater wetlands are hotspots of biological diversity and serve as critical habitat for many groups of organisms (e.g., Sharitz 2003). However, as temporally dynamic habitats they present a unique suite of challenges to organisms dependent upon them. Climatic variation leads to periods of high productivity (e.g., Gibbons et al. 2006), interspersed with episodes of predictable (i.e., periodic or seasonal) or unpredictable resource shortages (e.g., Polis et al. 1997, Madsen and Shine 2000, Madsen et al. 2006). Consequently, to capitalize on productive wetland habitats, organisms must be able to cope with temporal variability in habitat suitability. In particular, extensive droughts can leave isolated wetland habitats dry and devoid of aquatic prey for protracted periods, requiring species to stay and survive in the dried wetland, leave to seek refuge in other habitats until the wetland refills, or perish (e.g., Seigel et al. 1995a, Willson et al. 2006, Winne et al. 2006b, in review-a).

Although a central focus of ecology is to understand population dynamics and population-level responses of organisms to environmental perturbations, little is known about long-term population dynamics and demography in snakes (but see Webb et al. 2002, King et al. 2006, Madsen et al. 2006, Winne et al. 2007). Understanding the long-term dynamics of semi-aquatic snakes inhabiting freshwater wetlands is of particular interest, because they can occur at high abundances and play an important role as top predators within aquatic ecosystems (e.g., Godley 1980, Ineich et al. 2007). For example, Godley (1980) found that aquatic snakes (*Regina alleni* and *Seminatrix pygaea*) reach densities of >1200/ha in aquatic habitats in Florida and calculated that juvenile *R. alleni*, specialist predators upon aquatic odonate larvae, were capable of consuming *c*. 91% of the prey population annually. Moreover, in isolated wetlands semi-aquatic snakes are forced to contend with periodic, extreme droughts that can cause population declines and local extirpations of some species (e.g., Seigel et al. 1995a, Willson et al. 2006). Given the loss of wetland habitats and their susceptibility to drainage, pollution, and invasion by exotics (Dahl 1990), coupled with recognition that reptiles may be declining worldwide (Gibbons et al. 2000), it is increasingly important that we understand the population dynamics and conservation requirements of semi-aquatic snake species (e.g., Roe et al. 2003, 2004, King et al. 2006).

We studied the recovery of a wetland snake community on a large protected study site following a severe drought that lasted from 2000–2003. Our study site has hosted numerous long-term studies of effects of drought on reptiles and amphibians (Gibbons et al. 1983, Seigel et al. 1995a, Gibbons et al. 2006, Willson et al. 2006, Winne et al. 2006b, Glaudas et al. 2007, Winne et al. in review-a). In previous studies, we found clear differences in drought-survival strategies employed by semi-aquatic snakes, demonstrating that some can survive within isolated wetland habitats during drought, whereas other species experience precipitous declines or local extirpations (Willson et al. 2006, Winne et al. 2006b). Here, we detail the recovery of the aquatic snake community from 2003 to 2007, and we examine species differences in drought-recovery strategies during years of high wetland productivity. We reveal substantial differences in long-term trends of relative abundance and demography among species, which have important conservation and ecological implications.

METHODS

Study site

Ellenton Bay is an isolated freshwater wetland located on the Department of Energy’s 770 km^2 Savannah River Site (SRS) in the Upper Coastal Plain of South Carolina, USA, and is a typical Carolina bay (Sharitz 2003). Although water level is extremely variable (Fig. 5.1), the bay generally holds water year-round, and when full, covers approximately 10 ha. During most years, Ellenton Bay is dominated by shallow water (< 1 m deep) and relatively uniform distributions of emergent grasses (predominantly *Panicum* spp.), water lilies (*Nymphaea odorata*), and water shields (*Brasenia schreberi*). However, severe droughts have rendered Ellenton Bay dry on at least three occasions in the past three decades, most notably during 1987–1990 and 2000–2003 (Seigel et al. 1995a, Willson et al. 2006, Winne et al. 2006b). More recently, a series of shorter droughts have left the wetland dry from September to December 2006 and May to December 2007 (Fig. 5.1). When dry, a thick (up to 0.5 m) organic crust covers the entire basin but subsurface areas remain moist and up to 1 ha of viscous mud surrounds small open water areas during shorter dry spells. In drought years, Ellenton Bay is the last nonpermanent wetland to dry within the region (i.e., it has the longest hydroperiod). The only permanent wetland within 1.4 km of Ellenton Bay is a small, manmade pond *c.* 0.5 km from Ellenton Bay. Habitat surrounding Ellenton Bay is a mosaic of old-fields in various stages of succession and second-growth mixed pine-hardwood forest. Ellenton Bay is currently devoid of fish (*Gambusia holbrooki* were present until a severe drought in 1987; Seigel et al. 1995), but harbors a diverse assemblage of amphibians (24 species) and semi-aquatic reptiles (18 species), including seven snake species (Gibbons and Semlitsch 1991, Gibbons et al. 2006). In part because of its relative isolation from other wetlands and its importance –in terms of biodiversity and productivity– to surrounding habitats (e.g., Sharitz 2003, Gibbons et al. 2006), Ellenton Bay has been the host of a number of long-term studies of effects of drought on reptiles and amphibians (Gibbons et al. 1983, Seigel et al. 1995a, Gibbons et al. 2006, Willson et al. 2006, Winne et al. 2006b, Glaudas et al. 2007, Winne et al. in review-a). Seven semi-aquatic snake community occupy Ellenton Bay, including Cottonmouths (*Agkistrodon piscivorus*), Eastern

Mud Snakes (*Farancia abacura*), Rainbow Snakes (*F. erytrogramma*), Red-bellied Watersnakes (*Nerodia erythrogaster*), Banded Watersnakes (*Nerodia fasciata*), Florida Green Watersnakes (*N. floridana*), and Black Swamp Snakes (*Seminatrix pygaea*), making it an ideal study site for comparing interspecific differences in snake drought response.

Snake captures

We captured snakes at Ellenton Bay during 1998 and 2003–2007 using plastic minnow traps (model 700; N.A.S Incorporated, Marblehead, Ohio). This capture method is the most effective way to sample secretive semi-aquatic snakes in heavily-vegetated aquatic habitats in the Southeast (Willson et al. 2005, Winne 2005, Willson et al. 2008). In each year, we placed traps *c.* 2 m apart in transects along the margin of the bay amidst emergent vegetation. Although traps were not purposefully baited, incidental captures of amphibian larvae results in “natural baiting” of traps daily (Seigel et al. 1995a, Winne 2005). We checked traps once (2003–2007) or twice (1998) per day, at which time we removed all captured snakes.

At the laboratory, we recorded snout-to-vent length (SVL) to the nearest mm, body mass to the nearest 0.1 g (measured with an electronic balance), and sex (determined by visual inspection or probing). Snakes that contained prey items (determined by palpation) were gently forced to regurgitate before we measured snake body mass, but individual snakes were not forced to regurgitate more than twice per year. We recorded reproductive status of all female snakes (pregnant vs. non-pregnant, by palpation) and the number of enlarged ova or developing embryos for pregnant *S. pygaea* (by palpation, e.g., see Seigel et al. 1995b). We marked each snake with a unique code by scale-clipping (1998) or heat-branding (2003 – 2007, Winne et al. 2006a) and released snakes at their capture location.

To determine size at birth for the two most abundant snake species at Ellenton Bay, *N. fasciata* and *S. pygaea*, and litter size for *S. pygaea*, we held some pregnant females in laboratory enclosures until parturition (for housing details see Hopkins and Winne 2006, Winne and Hopkins 2006, Winne et al. in review-b) and recorded SVL and mass of all individual offspring within 24 hrs of birth.

Analyses

We compared aquatic snake captures in 1998 and 2003–2007 to assess patterns of drought-recovery in the aquatic snake community at Ellenton Bay, following the 2000–2003 drought (Fig. 5.1). For comparisons of relative abundance, community composition, and demography across years, recaptured snakes were not included in capture totals, and capture rates were standardized to sampling effort by dividing the number of snakes captured by the number of trap nights and multiplying by 100. Additionally, we restricted comparisons to include only similar, distinct periods within each year (April–June and/or August–September, hereafter referred to as “spring” and “autumn” for simplicity). We have recently documented that aquatic minnow traps cannot reliably capture *S. pygaea* smaller than 200 mm SVL (Willson et al. 2008). Therefore, we excluded individual *S. pygaea* smaller than 200 mm SVL from all figures and analyses.

To better understand how species rebound during the post-drought years, we examined annual changes in body size structure for our two most abundant species, *N. fasciata* and *S. pygaea*. We assigned groups of individuals to cohorts based on size at birth, visual inspection of size frequency distributions, and growth rates of marked known-age individuals. In addition, we compared reproductive output among years for one of these species, *S. pygaea*, using both analysis of variance (ANOVA) and analysis of covariance (ANCOVA). Year was the independent variable and litter size (natural log-transformed) was the dependent variable in both analyses; maternal SVL (natural log-transformed) was the covariate in the ANCOVA. Despite using data transformations, variances in litter size and maternal SVL were significantly heterogeneous among years for *S. pygaea* (Levene's test: litter size, $F_{7,220} = 2.39$; $p = 0.023$; maternal SVL, $F_{7,220} = 5.44$; $p < 0.001$). Consequently, in addition to the ANOVA and ANCOVA results, we also present the results of a non-parametric test, the Kruskal-Wallis ANOVA, on the effects of year on litter size. We used ova counts from palpations for pregnant snakes not kept in the laboratory until parturition because there is generally a strong positive correlation between palpation- and laboratory-observed litter sizes in *S. pygaea* (Seigel et al., 1995b; unpubl. data) and this allowed us to include four additional years in our analysis (1983–1985, 1987, data from Seigel et al. 1995b); it also allowed us to increase our sample sizes for 2003–2007. Comparable reproductive data are not available for *N. fasciata*.

RESULTS

Overall, aquatic snake capture frequencies in 2003 were reduced by about 29% compared to 1998, although August–September captures suggest that the post-drought reduction may have been as calamitous as *c.* 71% (Fig. 5.2). A similar but more dramatic reduction in aquatic snake capture rates was observed by Seigel et al. (1995a) following the extreme drought at Ellenton Bay that lasted from 1987 to 1990 (Fig. 5.2). Subsequent to 2003, autumn aquatic snake captures increased steadily and markedly each year, reaching pre-drought abundances by 2005 and 2006, respectively (Fig. 5.2). In contrast, annual spring capture frequencies of aquatic snakes were somewhat erratic, declining slightly each year from 2003 to 2005, remaining steady from 2005 to 2006, and then increasing rapidly to pre-drought abundances by 2007 (Fig. 5.2). In total, the four most commonly captured species–*S. pygaea*, *N. fasciata*, *F. abacura*, and *N. floridana*–

accounted for 98% of the individuals captured during spring and autumn from 1998–2007, and they will be the focus of the remainder of this paper.

We observed substantial interspecific variation among aquatic snakes in post-drought relative abundance and population recovery patterns (Figs. 5.3, 5.4). Despite *N. fasciata* accounting for nearly half of all aquatic snakes captured in 1998, no *N. fasciata* were captured in spring 2003 and only a few individuals (all adults) were captured in autumn 2003 (Figs. 5.3a, 5.4). Beginning in 2004, *N. fasciata* captures increased drastically each year, particularly in autumn (Fig. 5.3a). By 2005, *N. fasciata* again accounted for half of the aquatic snake captures in Ellenton Bay (Fig. 5.4). In contrast, *S. pygaea* were captured in relatively high abundances each year (Fig. 5.3a) and *S. pygaea* remained a central member of the aquatic snake community throughout the study (Fig. 5.4), though spring captures were generally more variable among years than were autumn captures (Figs. 5.3b, 5.4). *Nerodia floridana* were captured with moderate frequency in 1998 but was not captured again until 2005, at which point it was captured considerably less frequently each year compared to 1998 (Figs. 5.3b, 5.4). *Farancia abacura* were captured in 2003 and 2004 with moderate frequency and from 2005–2007 with lower frequency, despite not being captured in 1998 (Figs. 5.3d, 5.4).

The smallest pregnant *N. fasciata* and *S. pygaea* captured at Ellenton Bay after the 2000–2003 drought were 469 and 259 mm SVL, respectively; thus, we considered these body lengths to represent minimum size at maturity for these populations. For each species, we assume that males mature at a similar or smaller size than females, as is true for all other closely related species that have been studied (Gibbons and Dorcas 2004). *Nerodia fasciata* displayed strong demographic shifts in body size structure throughout the drought-recovery years, as relatively distinct cohorts grew to maturity and large adult size (Fig. 5.5). Distinct cohorts were born in the late summer of each year and, as confirmed with marked known-age individuals (unpubl. data), rapidly grew to maturity (Fig. 5.5). For example, in 2004 we captured relatively few *N. fasciata* and they did not form readily identifiable cohorts, although a few individuals (i.e., the 2003 cohort) were positively identified as being born after the 2000–2003 drought (Fig. 5.5a). Yet, by the following year, 2005, the majority of *N. fasciata* captures consisted of offspring born following the 2000–2003 drought (Fig. 5.5b). Moreover, most mature *N. fasciata* captured at Ellenton Bay in 2006 and 2007 were born post-drought (Fig. 5.5a, b). *Seminatrix pygaea* displayed similar, but more dramatic demographic shifts in body size structure (Fig. 5.6). Body size distributions of *S. pygaea* captured in 2003 were severely truncated compared to pre-drought data from 1998, but drought-survivors grew considerably each subsequent year (Fig. 5.6). The first wave of *S. pygaea* cohorts, born in late summer 2003, began reaching maturity by 2005 (Fig. 5.6d), but the entire 2003 cohort did not reach maturity until 2006. Thus, most female *N. fasciata* begin reproducing by their second year of life (Fig. 5.5) and most *S. pygaea* begin reproducing by their second or third year of life (Fig. 5.6).

Seminatrix pygaea exhibited large and significant differences in litter size among years (ANOVA: $F_{7,220} = 17.38$, $p < 0.001$; Kruskal-Wallis: $H_{7,228} = 84.36$, $p < 0.001$). On average, *S. pygaea* had larger litters in the second, third, and fourth years of drought recovery (2004-2006) than during the first wet year following drought (2003) or any of pre-drought years (1983-1987; Table 5.1, Fig. 5.7a). The record litter size of *S. pygaea* at Ellenton Bay consisted of 15 offspring in years prior to 2004 (Fig. 5.7b). In contrast, from 2004 to 2006 *S. pygaea* routinely gave birth to litters with more than 15 offspring and the maximum litter size consisted of 24 offspring (Fig. 5.7b). Litter sizes varied significantly among years, independent of maternal body size (ANCOVA: $F_{7,219} = 2.36$, $p = 0.024$; Fig. 5.7b). Generally, females gave birth to the largest litters in 2004, 2005, and 2006, after accounting for maternal body length (Table 5.2; Fig. 5.7b). Additionally, slopes of regressions of ln-litter size on ln-maternal SVL were not significantly different among years (ANCOVA, test of parallelism: $F_{7,212} = 0.85$, $p = 0.546$; Fig. 5.7b). In 2007, *S. pygaea* did not reproduce at Ellenton Bay because the wetland dried.

DISCUSSION

To capitalize on productive wetland habitats, organisms must be able to cope with temporal variability in habitat suitability. Stochastic variation in climate leads to variation in resource abundance, and during extensive droughts isolated wetland habitats can be dry and devoid of aquatic prey for prolonged periods. We have previously discussed drought-survival strategies employed by semi-aquatic snakes, demonstrating that some species persist within isolated wetland habitats during drought, whereas other species experience precipitous declines or local extirpations (Willson et al. 2006, Winne et al. 2006b). Here, we detail the recovery of the aquatic snake community following a prolonged drought and examine species differences in drought-recovery strategies during years of high wetland productivity, and we reveal substantial differences in long-term trends of relative abundance and demography among species, which have important conservation and ecological implications.

Post-drought population recovery

We found that *N. fasciata* and *N. floridana* experienced dramatic declines in relative abundance as a result of the 2000–2003 drought. Compared to pre-drought levels, both species were noticeably absent from the wetland community during spring of 2003, the first wet year following more than two years of extensive drought conditions (Willson et al. 2006). Although both species suffered traumatic population declines, we found a considerable difference in population recovery rates between the species. *Nerodia fasciata* recovered from the population crash rapidly, with steady increases in capture rates each season and each year, beginning in autumn 2003. In contrast, we did not capture *N. floridana* again until 2005, and capture rates of *N. floridana* throughout our study never approached pre-drought levels. Seigel et al. (1995a) observed similar population crashes in these two species during an earlier drought at Ellenton Bay (1987–1990) and noted that *N. fasciata* were captured within one year following drought, but that *N. floridana* were not captured again until five years post-drought. Collectively, our studies suggest that *N. fasciata* are more resilient to prolonged drought than *N. floridana*, at least in relatively isolated wetlands such as Ellenton Bay.

In contrast to the two *Nerodia* species, we found that *S. pygaea* persisted at Ellenton Bay throughout the study and always comprised a substantial portion of the snake community, despite the drought of 2000–2003. The ability of *S. pygaea* to survive at Ellenton Bay during droughts is undoubtedly dependent upon their ability to aestivate beneath the dried wetland surface during unfavorable conditions (Winne et al. 2006b), though in some years they may also benefit from migration (Seigel et al. 1995a). Annual shifts in relative abundance of *S. pygaea* were erratic in spring, but were largely stable among years during autumn. These patterns likely indicate that relative abundance is particularly sensitive to temporal shifts in behavior (activity) in *S. pygaea*, rather than there truly being erratic fluctuations in population size. Prolonged, severe droughts likely reduce *S. pygaea* population size (particularly for large females, Winne et al. in review-a), but overall the population was not as negatively affected by drought as were *N. fasciata* and *N. floridana*. Similarly, although *F. abacura* were not captured at Ellenton Bay during 1998, they occupied Ellenton Bay in earlier years (1980’s and 1990’s, Seigel et al. 1995a; in 1999, CTW, pers. obs.), and we captured them at Ellenton Bay in every post-drought year of our study. The ability of *F. abacura* to aestivate, like *S. pygaea*, is a major factor that enables them to persist at Ellenton Bay throughout the drought (Willson et al. 2006).

Wetland repopulation

To understand the ecology and conservation needs of semi-aquatic snakes that use isolated wetlands, we must not only determine how snake populations fair as a result of drought, but we also must establish how populations that periodically suffer dramatic declines or local extirpations rebound following drought. For *N. fasciata*, the ability to recover rapidly following drought appears to be the result of immigration, high reproductive capacity, and fast growth to maturity. Willson et al. (2006) captured immigrant *N. fasciata* in a drift fence that encircled Ellenton Bay in 2003, and in later years we captured *N. fasciata* in surrounding terrestrial habitats that subsequently immigrated to Ellenton Bay (unpubl. data), suggesting that repopulation of Ellenton Bay by *N. fasciata* is contingent upon immigration from other aquatic habitats or adjacent terrestrial habitats. However, our evaluation of shifts in relative abundance, coupled with body size distributions and growth rates of known-age individuals suggests the rapid post-drought recovery of *N. fasciata* at Ellenton Bay may also be driven largely by population recruitment of offspring from resident female reproduction, once immigrant adults become established.

In 2003, we did not capture any *N. fasciata* in aquatic traps until August, despite high sampling effort. We only captured three individuals in the aquatic habitat in 2003, but all were adults, and one was a reproductive female carrying numerous offspring. The following spring (2004) we captured a few individuals that were born the previous year (based on size), but overwhelmingly size distributions of *N. fasciata* were widely scattered, indicating that a majority of individuals were probably immigrants from other habitats rather than a discrete cohort born at

Ellenton Bay in the previous year. Throughout the remaining years, however, large proportions of the captures were cohorts of offspring clearly born after the drought. Moreover, each year we captured considerably more *N. fasciata* in autumn (i.e., after parturition) than in spring, presumably because of the increase in population size due to recruitment of a large number of neonates from reproduction. Indeed, female *N. fasciata* are known to be highly fecund, with individual litters of up to 83 offspring, though an average of about 21 is more common (Ernst and Ernst 2003, Gibbons and Dorcas 2004). And, our finding that *N. fasciata* grow quickly and mature within two years demonstrates that individuals born at Ellenton Bay after the 2000–2003 drought began producing offspring of their own as early as 2005, with more post-drought individuals capable of reproduction added to the population each year thereafter.

In contrast to *N. fasciata*, substantially fewer *S. pygaea* were observed immigrating to Ellenton Bay following the drought of 2000–2003 (Willson et al. 2006), and the substantial population of *S. pygaea* that survived the drought by aestivating was in place to begin repopulating the wetland via reproduction during 2003 (Winne et al. 2006b). The offspring of drought-survivors formed tractable cohorts throughout the study, and some offspring born after the 2000–2003 drought become reproductive as early as 2005, though most females probably began reproducing in 2006. The reliance of *S. pygaea* on aestivation and subsequent reproduction of resident females for long-term population survival in Ellenton Bay is likely related to traits that are ill-suited for overland travel and rapid founding of a new population. For example, adult *S. pygaea* are smaller in body size, have higher rates of evaporate water loss, and much smaller litter sizes than *N. fasciata* (Winne et al. 2001, Ernst and Ernst 2003, Gibbons and Dorcas 2004). Additionally, *N. fasciata* are ubiquitous in the southeastern United States and on the Savannah River Site, and are found in almost any aquatic habitat, including a nearby permanent pond only *c.* 0.5 km from Ellenton Bay (Ernst and Ernst 2003, Gibbons and Dorcas 2004, unpubl. data). Contrastingly, *S. pygaea* have more restricted distributions on the Savannah River Site and, despite aquatic trapping in many locations over many years, the closest known *S. pygaea* occurrences to Ellenton Bay include populations at two isolated wetlands 5.7 and 8.7 km from Ellenton Bay, respectively (unpubl. data). Consequently, the Ellenton Bay population of *S. pygaea* is effectively isolated from all known populations on the Savannah River Site, but the *N. fasciata* population is not.

Post-drought wetland productivity

We observed surprisingly extreme shifts in body size distributions of *S. pygaea*, with both average and maximum body size being significantly larger each year from 2003 to 2006. During these post-drought years, we also observed drastic shifts in reproductive output for female *S. pygaea*, with litter sizes surpassing all previous records of reproductive potential for this species, at Ellenton Bay or elsewhere (Dowling 1950, Seigel et al. 1995b, Ernst and Ernst 2003, Gibbons and Dorcas 2004). The unprecedented shifts in reproductive output was predominantly due to larger maternal body sizes, which is correlated with litter size in other snake species (Seigel and Ford 1987, Shine 1994). However, we also found that females gave birth to a larger number of offspring in these later years independent of their body size, suggesting that greater food availability during the post-drought years may have been an important contributor to increased offspring production. Food resource availability has been linked to reproductive output in a number of snake species, both experimentally (e.g., Ford and Seigel 1989, Lourdais et al. 2003) and through field studies (e.g., Madsen and Shine 1999, 2000, Madsen et al. 2006), and we have documented the extraordinary productivity and abundances of amphibians that occurs at Ellenton Bay following drought (Gibbons et al. 2006). Although previously undocumented in snakes, “supranormal” reproductive events have been routinely observed in long-legged wetland wading birds following extensive droughts and are suggested to result from exceptional productivity and/or availability of aquatic prey (e.g., Frederick and Ogden 2001). We expect that high productivity of wetlands following drought is a general characteristic of isolated wetland systems and an important factor facilitating the rapid recovery of populations that we have observed in our study system.

Implications for conservation of wetland snakes

While it is recognized that maintaining large core terrestrial habitats surrounding wetlands is necessary for many semi-aquatic reptiles and amphibians (Gibbons 2003, Semlitsch and Bodie 2003), Roe and Georges (2007) recently argued that wetland conservation practices should also include managing landscapes to preserve the natural heterogeneity of wetland complexes and provide permeable travel corridors among wetlands. Our study suggests that such conservation efforts may be vital for long-term persistence of species such as *N. fasciata* and *N. floridana* in isolated wetlands, because they are highly susceptible to periodic extreme droughts, and they appear to rely on metapopulation (Levins 1969) or source-sink (Pulliam 1988) dynamics to recolonize Ellenton Bay following drought. A number of other semi-aquatic snake and turtle species use multiple wetlands throughout life and population persistence may be dependent upon factors such as terrestrial buffer size, the presence of corridors, and proximity to other wetlands (reviewed by Roe and Georges 2007). However, for highly aquatic species such as *S. pygaea*, that are not adapted for overland dispersal and rely upon aestivation to survive droughts, conservation measures that protect the occupied wetland itself, even if dry, is necessary. Worldwide, isolated wetlands are hotspots of biodiversity and are vital habitats for numerous taxa, including many amphibians and semi-aquatic reptiles. Ultimately, landscape management approaches that facilitate the conservation of multiple wetland types that vary in size, hydroperiod, and proximity to other wetlands is critical for maintaining biodiversity, productivity, and landscape function, particularly for wetland-dependent amphibians and semi-aquatic reptiles (Semlitsch and Bodie 1998, Semlitsch and Bodie 2003, Attum et al. 2007, Roe and Georges 2007).

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Table 5.1. Probability values from post-hoc comparisons (Tukey's HSD) of *Seminatrix pygaea* litter sizes among years at Ellenton Bay.

	1984	1985	1987	2003	2004	2005	2006
1983	0.985	0.979	0.999	1.000	0.332	**0.008**	**0.066**
1984	x	1.000	1.000	0.919	**0.083**	**0.000**	**0.000**
1985	x	x	1.000	0.942	0.260	**0.000**	**0.006**
1987	x	x	x	1.000	0.351	**0.001**	**0.035**
2003	x	x	x	x	**0.001**	**0.000**	**0.000**
2004	x	x	x	x	x	**0.063**	0.802
2005	x	x	x	x	x	x	0.575
2006	x	x	x	x	x	x	x

Table 5.2. Probability values from post-hoc comparisons (Tukey's HSD) of *Seminatrix pygaea* litter sizes among years at Ellenton Bay.

	1984	1985	1987	2003	2004	2005	2006
1983	0.947	0.927	0.995	1.000	0.095	**0.000**	**0.006**
1984	x	1.000	1.000	0.776	**0.008**	**0.000**	**0.000**
1985	x	x	0.999	0.829	**0.061**	**0.000**	**0.000**
1987	x	x	x	1.000	0.105	**0.000**	**0.002**
2003	x	x	x	x	**0.000**	**0.000**	**0.000**
2004	x	x	x	x	x	**0.005**	0.555
2005	x	x	x	x	x	x	0.272
2006	x	x	x	x	x	x	x

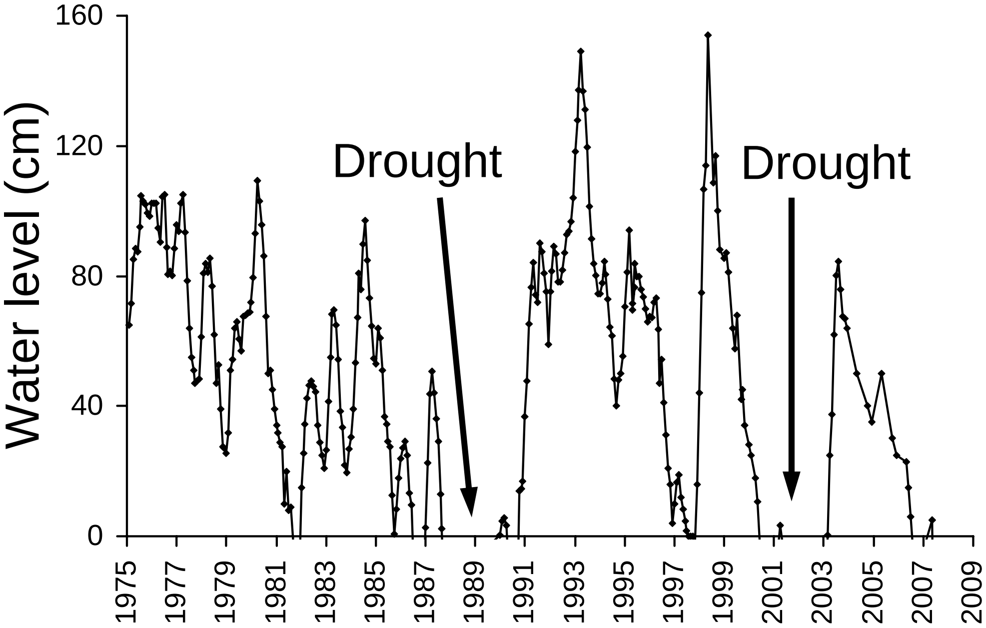

Figure 5.1. Water level at Ellenton Bay in the years 1974 – 2007. Note severe droughts in 1987–1990 and 2000–2003. Water depth was measured at a point in the interior of the wetland that represented general maximum basin depth, although a few isolated deeper holes existed in the muddiest central sections of the wetland. The focal years of drought recovery in this study are 2003–2007, although data on snakes from other years is also included herein.

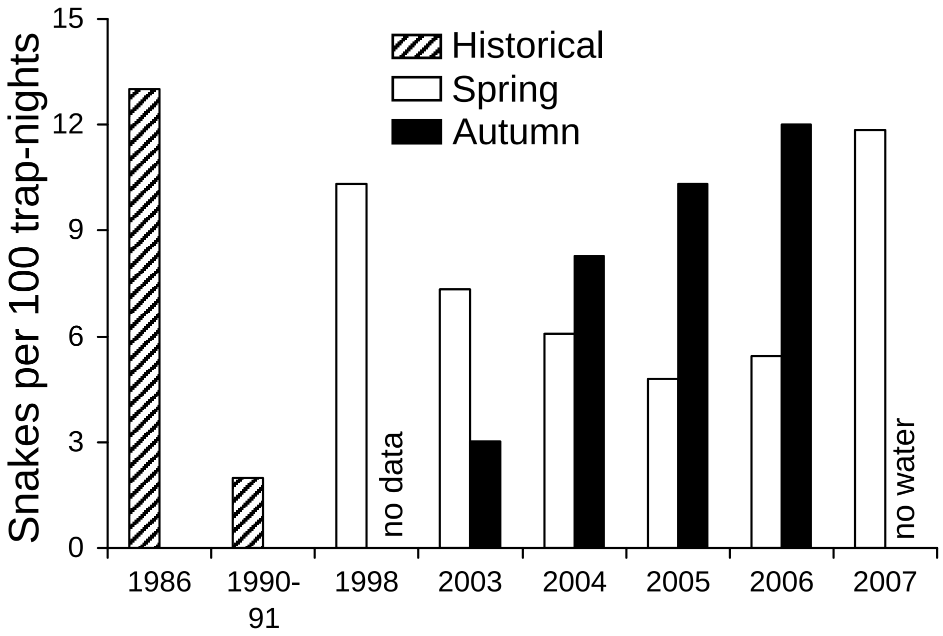

Figure 5.2. Relative abundance of snakes within Ellenton Bay as measured by aquatic trapping success in 1986 and 1998 (pre-drought years) and 1990–1991 and 2003–2007 (post-drought years). Data from 1998 and 2003–2007 represent individual snakes of all aquatic species captured per 100 trap nights. Historical data (Seigel et al. 1995a) represents overall number of snakes captured per 100 trap nights and does not specify species composition or season of capture. Capture success was not determined in autumn of 1998 and Ellenton Bay did not hold water during autumn 2007.

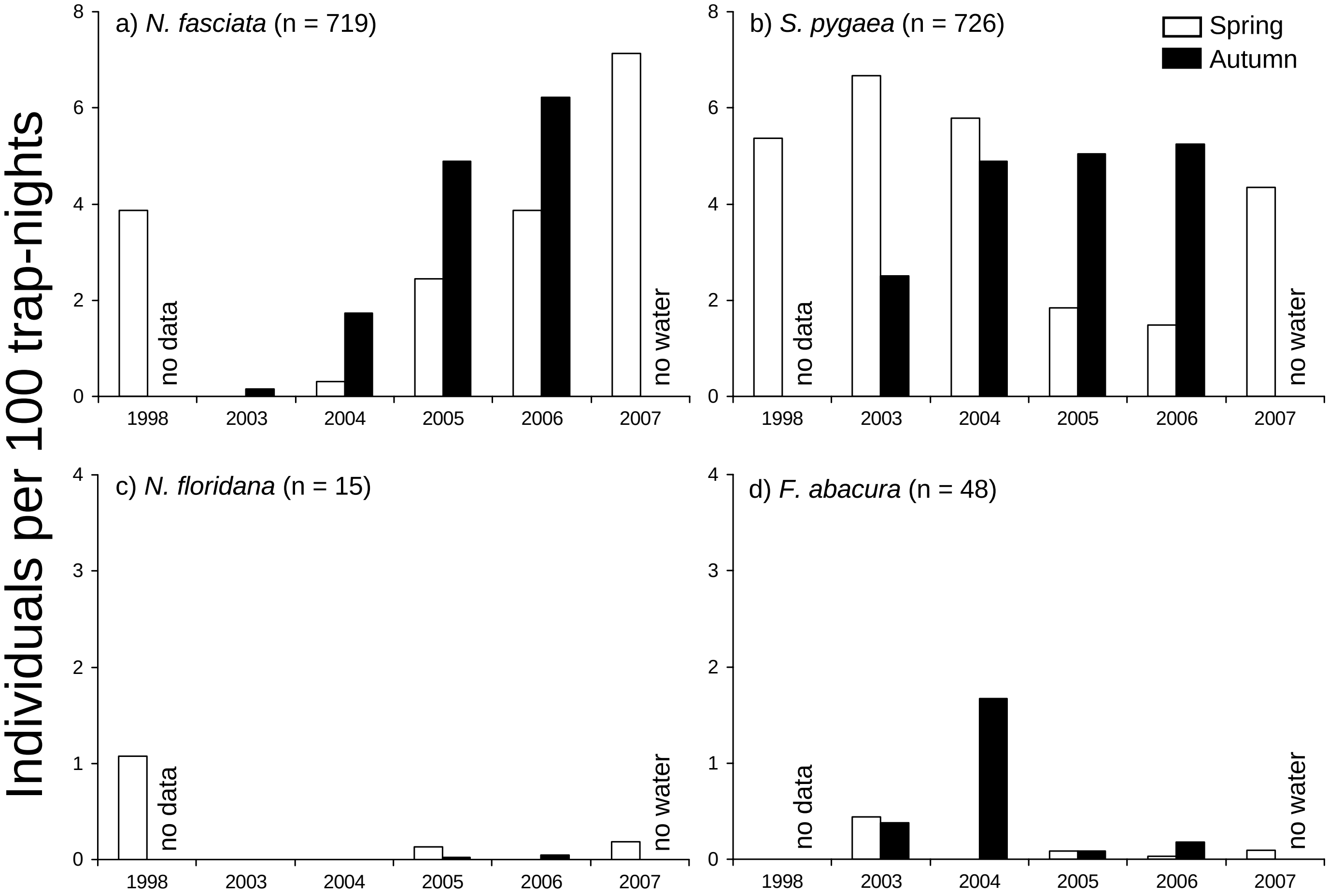

Figure 5.3. Relative abundance of a) *Nerodia fasciata*, b) *Seminatrix pygaea*, b) *Nerodia floridana*, and d) *Farancia abacura* within Ellenton Bay. Bars represent the number of individual snakes captured per 100 trap nights, using aquatic funnel traps, in pre- (1998) and post-drought (2003–2007) years. Capture success was not determined in autumn of 1998 and Ellenton Bay did not hold water during autumn 2007.

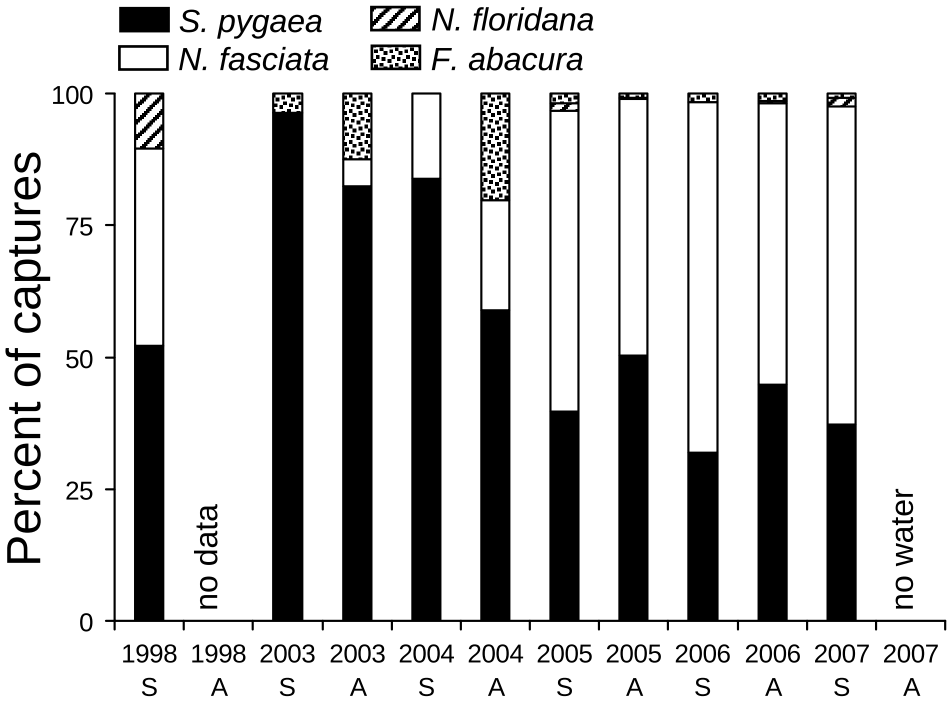

Figure 5.4. Community composition of semi-aquatic snakes within Ellenton Bay, as measured by the percent of captured individuals of each of the four most commonly captured species. All captures were made using aquatic funnel traps in 1998 (pre-drought year) and 2003–2007 (post-drought years). Spring (S) and autumn (A) captures are denoted below years of capture. Community composition was not determined in autumn of 1998 and Ellenton Bay did not hold water during autumn 2007.

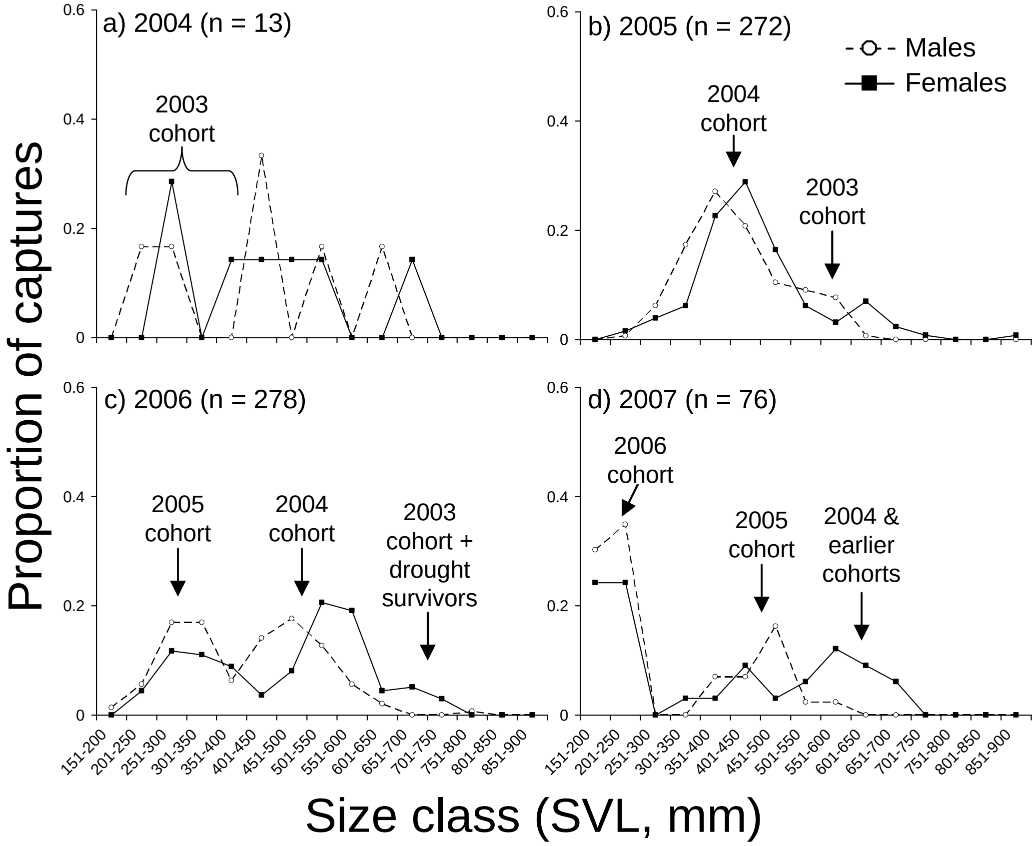

Figure 5.5. Body size distributions of male and female *Nerodia fasciata* captured within Ellenton Bay in a) 2004, b) 2005, c) 2006, and d) 2007. All captures were made in April–June (i.e., “spring”) using aquatic funnel traps and within-year recaptures were excluded from the dataset. Cohort assignments refer to year of birth for a group of similarly-sized individuals and minimum size at maturity for females is 469 mm SVL. Body sizes were not measured in 1998 and no *N. fasciata* were captured in spring 2003.

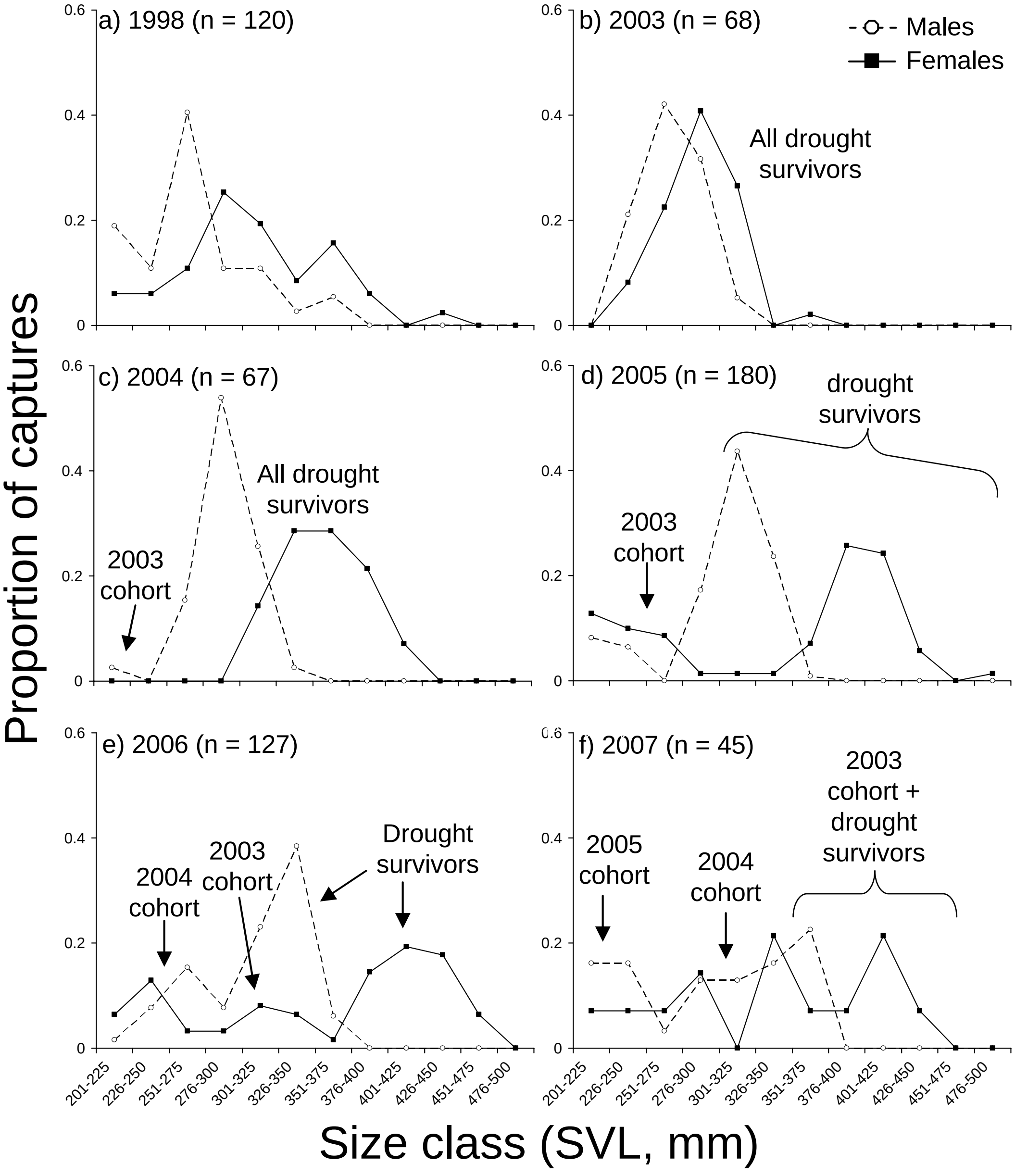

Figure 5.6. Body size distributions of male and female *Seminatrix pygaea* captured within Ellenton Bay in a) 1998 (a pre-drought year), b) 2003, c) 2004, d) 2005, e) 2006, and f) 2007. All captures were made in April–June (i.e., "spring") using aquatic funnel traps. Within-year recaptures and snakes smaller than 200 mm SVL were excluded from the dataset. Cohort assignments refer to year of birth for a group of similarly-sized individuals and minimum size at maturity for females is 259 mm SVL.

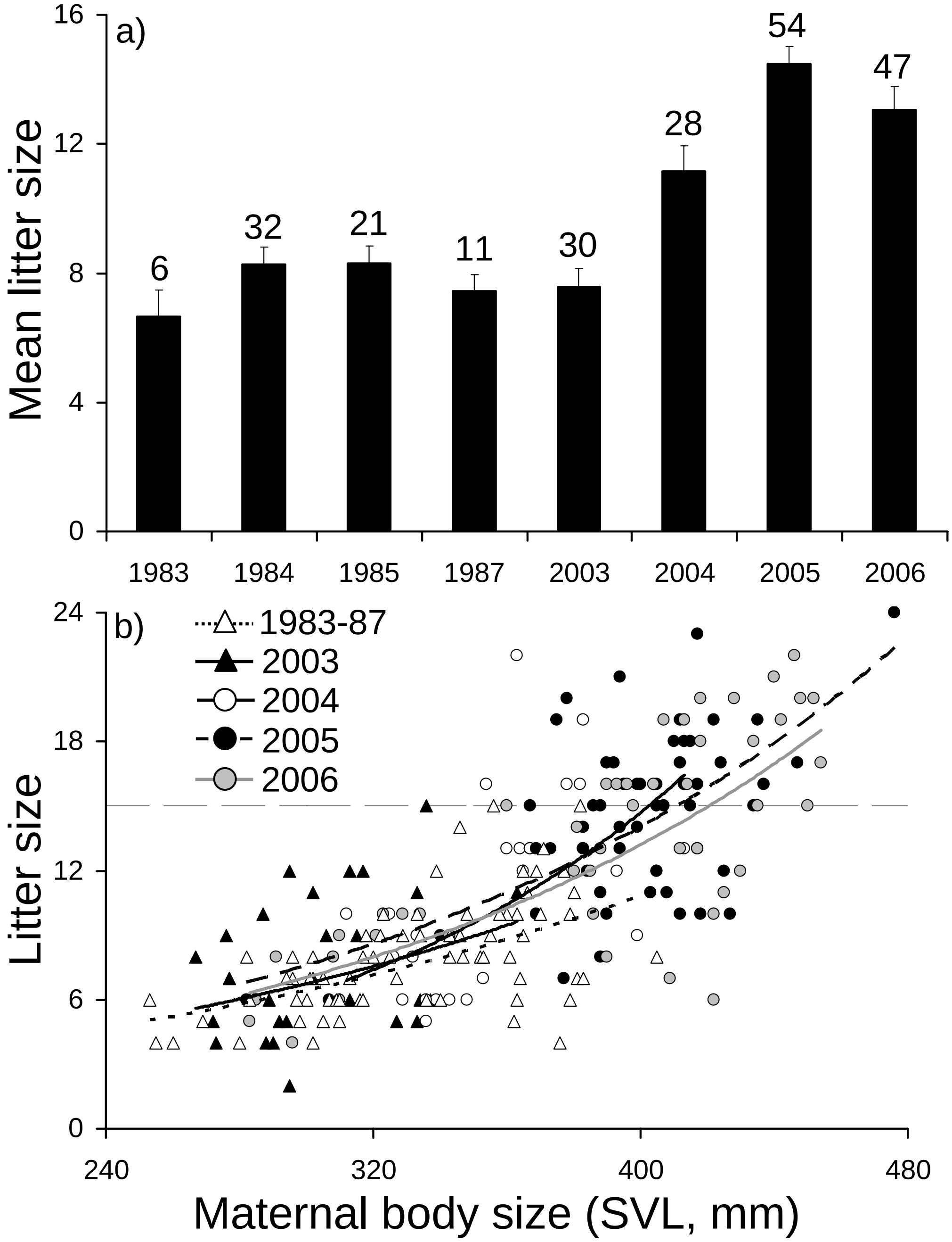

Figure 5.7. Litter sizes of *Seminatrix pygaea* captured within Ellenton Bay. a) Mean litter size among years, with sample sizes indicated above bars. Litter size varied significantly among years (p<0.001), with the females having the largest litters in 2004–2007. b) Relationship between maternal body size and litter size for each year. Dashed, horizontal line represents the maximum known litter size for *S. pygaea* prior to 2004. Litter sizes varied significantly among years, independent of maternal body size (p=0.024). In 2007, *S. pygaea* did not reproduce at Ellenton Bay because the wetland dried. Data from 1983–1987 was provided by Seigel et al. (1995b).

CHAPTER 6

INFLUENCE OF SEX AND REPRODUCTIVE CONDITION ON TERRESTRIAL AND AQUATIC LOCOMOTOR PERFORMANCE IN THE SEMI-AQUATIC SNAKE *SEMINATRIX PYGAEA*[1]

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INTRODUCTION

Cost of reproduction is defined as a tradeoff between current and future reproduction and has become a central focus in life history evolution (Roff 1992, Stearns 1992). Life history evolution models have generally recognized two categories of reproductive costs (Shine 1980). First, current reproductive investment can ultimately reduce future fecundity by diverting energy away from somatic growth and storage (Congdon et al. 1982). Second, reproduction can decrease current and future survival probability and therefore limit the probability of subsequent reproductive events. This second cost of reproduction – the survival cost – has been noted for males and females of a wide variety of taxa (Magnhagen 1991) and has emerged as a primary topic of investigation among ecologists interested in life history theory.

The survival costs of reproduction are fundamentally different between the sexes of most organisms. In squamate reptiles, male survival costs are generally associated with increased movements during the mating season and increased visibility during mating aggregations or displays (Andrén 1985, Madsen 1987). As a consequence males of some species are faster than females, perhaps as a mechanism to compensate for survival costs or mating needs (Shine and Shetty 2001, Shine et al. 2003). In contrast, females suffer reproductive survival costs attributable to reduced locomotor performance and increased basking behavior during pregnancy (Shine 1980, Madsen 1987, Seigel et al. 1987, Miles et al. 2000). For example, pregnant (or gravid) reptiles often bask in open, sunny habitat more frequently than non-reproductive individuals to increase developmental rates and quality of their offspring (Peterson et al. 1993, Arnold and Peterson 2002); thus, they are more visible to predators (Shine 1980, Andrén 1985, Madsen 1987). Concomitantly, because sprint speed and endurance are significantly impaired by pregnancy, pregnant females generally have a higher probability of being predated than non-reproductive individuals (Shine 1980, Seigel et al. 1987, Miles et al. 2000).

Most life history models predict a direct tradeoff between reproductive investment and reproductive costs (but see Reznick et al. 2000). In terms of reproductive costs to locomotor performance, such models predict that for every increase in reproductive investment there should be a concurrent decrease in sprint velocity or endurance and a decrease in survivorship. The most commonly used measure of reproductive investment in reptiles is relative clutch mass (RCM, Shine 1980, Seigel and Fitch 1984). Indeed, correlations between RCM and foraging and predator escape modes exist for reptiles and suggest that locomotor impairment can have implications for life history evolution on a broad scale (Vitt and Congdon 1978, Huey and Pianka 1981, Vitt and Price 1982). For example, Shine (1988) compared RCM among aquatic and terrestrial species of snakes and concluded that reproductive investment was constrained to a greater extent in morphologically-specialized aquatic snakes because of the plausible negative consequences of carrying eggs or offspring while swimming. Further, there is strong selection for fast-start swimming and increased reproductive investment in Trinidadian guppies (*Poecilia reticulata*) in the presence of piscivorus fish, but populations that evolve higher reproductive effort in the presence of predators suffer greater locomotor costs during pregnancy compared to populations that have lower reproductive effort (Ghalambor et al. 2004). One common approach to quantifying the potential effect of reproductive investment on survivorship is to test for negative phenotypic correlations between RCM and locomotor impairment (e.g., Seigel et al. 1987). Nonetheless, evidence supporting the expected negative phenotypic correlations between reproductive investment and locomotor impairment within species is mixed; in some cases a direct tradeoff exists (e.g., Shine 1980, Seigel et al. 1987, Miles et al. 2000, Wapstra and

O'Reilly 2001), whereas in other cases tradeoffs have not been observed (e.g., Brodie 1989, Olsson, Shine and Bak-Olsson 2000, Webb 2004). Although numerous studies have demonstrated reproductive costs in terms of reduced terrestrial locomotor performance, our understanding of pregnancy-induced locomotor impairment in other environments is limited (Webb 2004). This knowledge gap is important because (i) many organisms rely on multiple habitat types during pregnancy and (ii) locomotor performance in non-reproductive animals can be severely affected by substrate (Scribner and Weatherhead 1995, Finkler and Claussen 1999, Shine and Shetty 2001, Shine et al. 2003, Bonnet et al. 2005).

In this study, we performed a simple experiment designed to address four basic questions about sexual differences in locomotor performance and reproductive costs to locomotion in non-marine semi-aquatic snakes: (i) are there sexual differences in locomotor performance between aquatic and terrestrial habitats? (ii) is the cost of reproductive locomotor impairment similar between aquatic and terrestrial habitats for females? (iii) is there a phenotypic tradeoff between reproductive investment and reproductive locomotor impairment costs? and (iv) if there is a phenotypic tradeoff observed in one habitat type, is the tradeoff equally apparent within another habitat? First, because males of most snake species move more frequently and cover greater distances than females, we predicted that males would be faster than females in both habitat types. Second, we predicted that both forms of locomotion would be impaired by pregnancy, but that swimming would be less impacted compared to terrestrial locomotion. We based this supposition on three lines of evidence: swimming is generally a more efficient mode of locomotion (Lillywhite 1987), non-pregnant snakes nearly always swim faster than they crawl (Scribner and Weatherhead 1995, Finkler and Claussen 1999, Shine and Shetty 2001, Shine et al. 2003), and the increased mass and surface area of females during pregnancy is expected to greatly increase friction during crawling, but have less of an effect in an aquatic medium (Jayne 1985, Lillywhite 1987, Scribner and Weatherhead 1995). Lastly, we predicted that increased reproductive investment would increase locomotor impairment in both habitats.

METHODS

Study species

The black swamp snake (*Seminatrix pygaea*, Cope) has a unique combination of ecological traits that make it well-suited for this investigation. *Seminatrix pygaea* belongs to a monotypic genus of the cosmopolitan subfamily Natricinae and is endemic to a portion of the southeastern Coastal Plain of the United States (Gibbons and Dorcas 2004). It is the smallest aquatic snake in North America and, like other North American natricines, is viviparous and typically reproduces annually (Seigel et al. 1995, Winne et al. 2005). Unlike most reptiles, *S. pygaea* seldom bask out of the water or in direct sunlight (Gibbons and Dorcas 2004), and therefore predator-induced survival costs associated with reproduction should be primarily restricted to locomotor impairment (rather than basking) in this species. Second, *S. pygaea* are among the most aquatic of the North American semi-aquatic snakes and routinely forage for aquatic prey during pregnancy (Winne 2005, Winne et al. 2006). On the other hand, anecdotal reports suggest that females give birth on land, along the terrestrial margins of wetlands (Gibbons and Dorcas 2004). Thus, swimming and crawling performance are both presumably relevant to the ecology of *S. pygaea* during pregnancy. Third, *S. pygaea* do not possess specific gross morphological adaptations to swimming (e.g., dorso-ventrally flattened, paddle-like tails). Therefore, the interactive effects of habitat type and reproductive state on locomotion can be experimentally isolated, without having to consider how morphological adaptations for either terrestrial or aquatic locomotion affect habit-specific impairment (e.g., Shine 1988). Finally, pregnant females can be extraordinarily stout and exhibit a wide range of litter sizes and reproductive burdens, which increases our ability to detect tradeoffs between reproductive burdens and pregnancy-induced decrements to locomotor velocity.

Experimental subjects

Adult female (n = 15, snout-vent length $[\mathrm{SVL}]_{\mathrm{range}}$ = 325 – 383 mm, pregnant body $\mathrm{mass}_{\mathrm{range}}$ = 34.4 – 70.8 g, post-partum body $\mathrm{mass}_{\mathrm{range}}$ = 22.3 – 35.4 g) and adult male (n = 8, $\mathrm{SVL}_{\mathrm{range}}$ = 273 – 326 mm, body $\mathrm{mass}_{\mathrm{range}}$ = 12.9 – 21.5 g) *S. pygaea* were collected May – June 2004, from Ellenton Bay, a large isolated wetland located on the U.S. Department of Energy's Savannah River Site, in South Carolina, USA. The snakes were housed individually in 5 L plastic shoeboxes, fitted with paper towels as a substrate and a large water dish (737 mL). The snakes were then placed inside an environmental chamber (27°C, 14L:10D photoperiod), except during locomotor trials, and offered mole salamander larvae (*Ambystoma talpoideum*) totaling 30 – 50% of their body mass every 7 – 10 days. During July all of the cages were examined twice daily, and the mass, SVL, and tail length of the mother and her neonates were measured within 24hrs of parturition.

Locomotor performance

Because *S. pygaea* typically reproduce every year (Seigel et al. 1995, Winne et al. 2005), adult non-pregnant snakes were not readily available at the time of our study. Therefore, a repeated measures experimental design was used to determine the effect of pregnancy on maximum swimming and crawling velocity; all females used in this study were first tested during pregnancy, and then again, at least 3 weeks after parturition. Prior to parturition snakes were raced in water and on land (mean [$\pm$ 1 SE] days prior to parturition = 14.4 $\pm$ 1.7), and then returned to their plastic houses until after parturition. After giving birth, snakes were raced again in the two environmental media. All post-partum trials occurred 2.5 – 3 wks after parturition to allow females to recover. Male trials were run concurrently with post-partum females. The swimming and crawling trials were performed on separate but consecutive days for each snake, with half the animals tested in water first and half tested on land first. All trials were performed at 30 ± 0.5°C, by conducting the trials inside a walk-in environmental chamber, and the snakes were allowed to acclimate to this temperature for 3 – 6 h prior to trials. Each snake was conditioned to the racetrack and procedures (see below) two days prior to the start of the experiment and was post-absorptive at the time of the trials. All trials occurred between 1000 and 1600 h.

Maximum swimming and crawling velocities for each snake were determined using a 3-m race track similar to that previously described in Hopkins et al. (2005) and Hopkins and Winne (2006). The racetrack was 8-cm wide and placed inside a wooden track with sides 18 cm high to reduce escape attempts during trials. The racetrack was filled to a depth of 3 – 4 cm for swimming trials. For crawling trials the racetrack was drained and lined with a strip of stiff plastic carpet to maximize crawling performance.

Snakes were prodded as frequently as necessary for them to swim/crawl the full distance of the track. In a previous experiment (Hopkins et al. 2005), snakes were forced to swim three consecutive laps of the track. In that study, snakes achieved their fastest velocity 76% of the time during the first lap and 97% of the time within the first two laps. Therefore, snakes were only raced two consecutive lengths of the track in the current study (Hopkins and Winne 2006). Swimming and crawling occurred over a background marked at 1.0 cm increments and was recorded using a digital video camera (Canon GL1 Mini DV Camcorder). Maximum velocity for each lap at each sample interval was later calculated using a frame-by-frame advance on a VCR with accuracy to 0.03 sec (Hopkins et al. 2005, Hopkins and Winne 2006). To remove bias from the review process, the identity of snakes was concealed from the video tape reviewer. The time it took for each individual to swim 30 cm was calculated for each 30 cm segment of the track (after subtracting the initial portion of the track where the snake was placed). The single fastest swim velocity (expressed as cm/sec, Shine et al. 2003) for each individual was used as an estimate of maximum swimming/crawling performance in statistical comparisons.

Data analyses

Female *S. pygaea* are larger than males, both in our study and in natural populations (Winne et al. 2005). Therefore, following Shine et al. (2003), the effects of sex were tested with two different analyses, one that accounted for body size differences and one that did not. First, a two-factor repeated measures analysis of variance (ANOVA) was used to test for the effects of sex (males vs non-pregnant females), substrate (repeated factor), and sex-by-substrate interaction. Second, to account for body size, maximum velocity was expressed relative to body size (i.e., SVL / s), following the methods of Van Damme and Van Dooren (1999) and Shine et al. (2003). This method was chosen because preliminary analyses indicated that there were no significant relationships between body size and maximum velocity for either sex (all $P \geq 0.078$), or when males and non-pregnant females were pooled (all $P \geq 0.334$), and because there was no overlap in size between the sexes. Therefore, size-based covariates would have been inappropriate for our ANOVA model (Sokal and Rohlf 1995). Similarly, a two-way repeated measures ANOVA was used to test for the effects of pregnancy, substrate, and pregnancy-by-substrate interaction (independent variables) on maximum velocity (response variable). Body size corrections were not used for female-only analysis because the repeated measures design tested individuals against themselves and individuals did not change in body length between the tests. One-tailed p-values are reported for the ANOVA results when our tests were based on unidirectional *a priori* predictions. All data were normally distributed and exhibited homogeneous variances, and thus met all of the assumptions required for ANOVA.

Non-parametric correlations (Kendall's Tau) were used to (1) examine the correlation between reproductive burden and locomotor velocity and (2) to determine if females with larger reproductive burdens showed more dramatic increases in velocity after parturition, because residuals of parametric regressions did not meet assumptions of equal variance, despite transformation attempts (Sokal and Rohlf 1995). Relative litter mass (total litter mass / post-partum maternal mass) was used as our measure of reproductive burden. The litter mass from one female was not measured; therefore, the sample size was 14 for analyses that required this variable. STATISTICA for Windows software package (StatSoft, Inc. Tulsa, OK, USA. 1998) was used for all tests and statistical significance was recognized at $P \leq 0.05$. All means are presented as ± 1SE.

RESULTS

Sex

Both males and females always swam faster than they crawled (substrate: $F_{1,21} = 91.11$, $P < 0.001$, sex-by-substrate interaction: $F_{1,21} = 0.030$, $P = 0.865$, Fig. 6.6.1). Females (SVL = 354.5 ± 4.8 mm) were significantly larger than the males (SVL = 301.1 ± 6.2 mm, one-factor ANOVA, $F_{1,21} = 44.33$, $P < 0.001$). There was no significant difference between the sexes in maximum velocity when body size was not accounted for in the model ($F_{1,21} = 0.042$, $P = 0.420$, Fig. 6.1a). However, after controlling for size, there was still a significant effect of substrate ($F_{1,21} = 102.91$, $P < 0.001$, Fig. 6.1b) and no sex by substrate interaction ($F_{1,21} = 1.08$, $P = 0.311$), but the differences in relative velocity among the sexes became apparent ($F_{1,21} = 4.65$, $P = 0.021$). As expected, males were significantly faster for their size than were non-pregnant females (Fig. 6.1b).

Reproductive state

All tests of pregnant locomotor velocities began on the same day. Consequently, females were tested at different stages of pregnancy: females were tested 4 – 29 days prior to parturition (mean = 14.4 ± 1.7). To determine whether this affected our results we tested for relationships between stage of pregnancy (dependent variable = number of days prior to parturition) and post-partum increases in velocity. There were no significant relationships between these variables for either swimming (Kendall's Tau, $\tau = -0.254$, $Z = -1.318$, $P = 0.187$) or crawling ($\tau = -0.176$, $Z = -0.913$, $P = 0.361$) and therefore this factor was eliminated from further analyses.

Substrate and pregnancy

There were highly significant effects of substrate on locomotor velocity, with snakes always swimming faster than they crawled ($F_{1,28} = 43.37$, $P < 0.001$, Figs 1 and 2). As predicted, pregnancy significantly reduced both swimming and crawling velocity ($F_{1,28} = 51.48$, $P < 0.001$, Fig. 6.2). However, pregnancy impaired crawling velocity significantly more than swimming velocity (pregnancy-by-substrate interaction, $F_{1,28} = 2.92$, $P = 0.049$). The mean of the individual percent increases in crawling and swimming velocity after parturition was 72.8 ± 21.6 and 59.4 ± 12.8 %, respectively (note: the mean percent increases based on the grand means reported in Fig. 6.2 are 53.9% and 49.0%, respectively).

Tradeoffs

There was a large range of reproductive burdens, which increased our ability to detect tradeoffs between reproductive investments and locomotor costs. In our sample, relative litter mass ranged from 0.32 – 0.84 (mean = 0.54 ± 0.04) and litter size ranged from 6 – 22 (mean = 12 ± 1.2). Females with higher reproductive investment swam and crawled slower than females that invested less in reproduction (Fig. 6.3a), although the results were only significant for crawling velocity (swimming: $\tau = -0.206$, $Z = -1.026$, $P = 0.300$; crawling: $\tau = -0.582$, $Z = -2.901$, $P = 0.004$). To more accurately measure the effects of reproductive burden on locomotor impairment in each habitat, individual variability in pre- and post-parturition velocity must be accounted for. Thus, we measured the correlations between pregnancy-induced locomotor impairment (i.e., post-partum velocity increase) and reproductive burdens. Relative litter mass was significantly related to the degree of locomotor impairment in water ($\tau = 0.341$, $Z = 1.697$, $P = 0.033$, Fig. 6.3b). In contrast, there was no significant relationship between relative litter mass and locomotor impairment on land ($\tau = 0.429$, $Z = 2.135$, $P = 0.090$), although snakes with larger relative litter masses tended to be more impaired (Fig. 6.3b).

DISCUSSION

Male *S. pygaea* were faster than females in both habitats after differences in body size were accounted for. This sex difference in relative locomotor ability supports a general pattern that has emerged from other studies of amphibious (Scribner and Weatherhead 1995, Shine and Shetty 2001, Shine et al. 2003, but see Aubret 2004) and terrestrial snakes (Kelly et al. 1997). For laticaudid sea snakes, sexual differences in locomotor performance are exaggerated on land, perhaps because of greater reliance of males on terrestrial locomotion for finding mates (Shine and Shetty 2001, Shine et al. 2003). Similar interactions between sex-specific locomotor velocities and habitat type have been found in some North American natricines (Scribner and Weatherhead 1995). In contrast, we found that sexual differences in locomotor performance were not influenced by habitat type in *S. pygaea*, a result consistent with the notion that males and females of this species are similarly adapted to aquatic and terrestrial habitats. The morphological traits and selective pressures that have contributed to sexual differences in velocity within *S. pygaea* are unknown, but may reflect a trend for intrinsic physiological differences in locomotor ability and greater muscle mass in male snakes compared to females (Bonnet et al. 1998). An alternative explanation may be that females were not able to fully recover following parturition. However, we attempted to ameliorate this possibility by providing females weekly meals for 3 weeks prior to post-partum locomotor trials. To fully eliminate the effect of recovery, future studies could focus specifically on sexual differences in speed during early spring, prior to pregnancy.

Most studies of reproductive costs in females have focused on locomotor impairment in terrestrial environments. Terrestrial velocity is negatively affected by pregnancy in a wide variety of squamates (e.g., Shine 1980, Seigel et al. 1987, Miles et al. 2000, Wapstra and O'Reilly 2001), including semi-aquatic snakes (Brown and Shine 2004). Consequently, we predicted that pregnancy would also significantly impair locomotor performance in *S. pygaea*, both on land and in the water. Our predictions were supported: pregnancy significantly reduced both crawling and swimming speed in *S. pygaea*. Given the large number of published studies on reproductive ecology and life history evolution in semi-aquatic species (e.g., semi-aquatic snakes, turtles, and pond-breeding amphibians), surprisingly few have examined the effects of reproduction on swimming velocity. Previous studies are primarily limited to fish and a single salamander species, *Ambystoma maculatum*, which have demonstrated significant reproductive costs to swimming performance (Plaut 2002, Finkler et al. 2003, Ghalambor et al. 2004). Among snakes, only two species have been previously examined. Pregnant northern water snakes (*Nerodia sipedon*), tested early in gestation did not exhibit reductions in swimming velocities

(Brown and Weatherhead 1997). However, swimming velocity in an Elapid snake species, the death adder (*Acanthophis praelongus*), was significantly impaired by pregnancy (Webb 2004).

Habitat type and mode of locomotion are known to exert strong influences on locomotor velocity in a diverse array of snakes (e.g., Scribner and Weatherhead 1995, Finkler and Claussen 1999, Shine and Shetty 2001, Shine et al. 2003, Bonnet et al. 2005). Comparisons of aquatic and terrestrial locomotion in particular have demonstrated that swimming is generally a faster and more efficient means of moving than crawling (Lillywhite 1987, Scribner and Weatherhead 1995, Finkler and Claussen 1999, Shine and Shetty 2001, Shine et al. 2003). Therefore, we predicted that aquatic locomotor performance would be less affected by pregnancy than would performance on land. The results supported our hypothesis: (i) all pregnant *S. pygaea* swam faster than they crawled, and (ii) the post-partum increase in velocity was higher in the terrestrial habitat, indicating that locomotor impairment was more severe on land than in water. These results suggest that the costs of reproduction, in terms of locomotor performance, may be reduced for semi-aquatic snakes that rely heavily on swimming rather than crawling (Brown and Weatherhead 1997). Further, the relatively lower loss in aquatic velocity may partially explain why some species of aquatic snakes, including *S. pygaea*, continue to forage during pregnancy (Brown and Weatherhead 1997, Aldridge and Bufalino 2003, Shine et al. 2004, Winne et al. 2006), whereas many terrestrial snakes reduce activity and become anorexic (Brodie 1989, Bonnet 1998, Gregory and Skebo 1998, Gregory et al. 1999).

Shine (1988) predicted that carrying eggs or offspring in the posterior part of the body would more seriously impair locomotor performance in water compared to on land. Although he did not actually measure reproductive locomotor impairment among aquatic and terrestrial environments, he did demonstrate that, relative to terrestrial species, RCM was constrained in specialized aquatic snake species (i.e., marine snakes and sea kraits, Shine 1988). Our experiment provided a more direct test of the relative impacts of pregnancy on swimming and crawling velocities because we measured performance in a single species, *S. pygaea*, which does not possess any gross morphological adaptations to swimming (i.e., dorso-ventrally flattened, paddle-like tails). In contrast to Shine's (1988) hypothesis, our results demonstrate that overall swimming velocity is less affected by pregnancy than crawling velocity.

There are few other studies with which to compare our results and none have directly compared locomotor performance of pregnant and non-pregnant snakes in multiple habitats. A study of the effects of gravidity on locomotor performance in spotted salamanders, *Ambystoma maculatum*, found that burst swimming speed was negatively affected by gravidity, but that burst crawling speed was not (Finkler et al. 2003). Thus, compared to *S. pygaea* the degree of locomotor impairment appears to be reversed in salamanders and may be related to differences in limbed locomotion. Nonetheless, like snakes, *A. maculatum* swam faster than they crawled (Finkler et al. 2003). Although they did not measure effects of pregnancy, Shine and Shetty (2001) found that yellow-lipped sea kraits, *Laticauda colubrina*, containing prey items were significantly slower than unfed snakes, but that reductions in locomotor velocities were similar on land and in water. Clearly, more studies are needed on the effects of habitat type on pregnancy-induced locomotor impairment.

Models of life history evolution generally assume that a tradeoff exists between reproductive investment and locomotor impairment. Following this assumption, we expect the degree of locomotor impairment to scale proportionally with the level of reproductive effort (i.e., RCM). Such a phenotypic tradeoff has been demonstrated in some organisms (e.g., Shine 1980, Seigel et al. 1987, Miles et al. 2000), but not others (e.g., Brodie 1989, Olsson et al. 2000, Webb

2004). In *S. pygaea*, there was a significant tradeoff between locomotor performance and RCM for swimming, but not for crawling. While it is possible that increased sample size would result in a significant tradeoff for crawling velocity, our experiment suggests that increased fecundity-dependent tradeoffs may be associated with aquatic locomotion. That the tradeoff is more pronounced in one habitat than the other may be related to differences in the biomechanics of locomotion in aquatic and terrestrial habitats (Jayne 1985, Cundall 1987), and requires further investigation. Regardless of the cause, evidence for a stronger tradeoff in swimming performance, compared to crawling performance lends support to Shine's (1988) hypothesis that clutch size would affect swimming velocity more than terrestrial velocity during pregnancy.

Although we demonstrated that pregnancy incurs a significant cost in terms of reduced locomotor performance, the question remains: does this locomotor cost translate into a "true" cost of reproduction, in terms of reduced survivorship? Studies of terrestrial locomotor performance have demonstrated a negative effect of pregnancy on survival of reptiles (e.g., Miles et al. 2000). Little is known regarding the influence of pregnancy on survivorship of snakes in aquatic habitats, but some aspects of aquatic habitats seem likely to reduce the effects of locomotor impairment on survivorship (Brown and Weatherhead 1997). Heavily vegetated underwater habitats, such as those used by *S. pygaea* during foraging, should decrease a snake's vulnerability to visually-oriented predators. Also, submerged aquatic vegetation, rocks, or other structures and murky water offer many aquatic snakes nearly instantaneous escape from many predators (e.g., Brown and Weatherhead 1997).

In summary, male *S. pygaea* were faster than non-pregnant females in aquatic and terrestrial habitats, mirroring a trend that is common in other amphibious and terrestrial snakes. In female *S. pygaea*, pregnancy significantly decreased both aquatic and terrestrial locomotor velocities, but reproductive costs to locomotion were significantly higher in the terrestrial environment. Consequently, swimming may be more effective than crawling for escaping predators during pregnancy because swimming results in faster velocities and is less impaired by pregnancy. There was a direct tradeoff between reproductive investment and aquatic locomotor impairment: snakes that invested more in offspring experienced larger decreases in swimming velocity. However, evidence for such a tradeoff in the terrestrial habitat was weaker. In combination, our results provide mixed support for Shine's (1988) hypothesis and demonstrate that pregnancy-induced locomotor costs may be higher overall on land, but more sensitive to increases in reproductive burden in aquatic habitats. Undoubtedly, if among-habitat differences in reproductive costs to locomotion are pervasive, quantifying such differences will be important for understanding the evolution of reproductive life history traits and habitat use.

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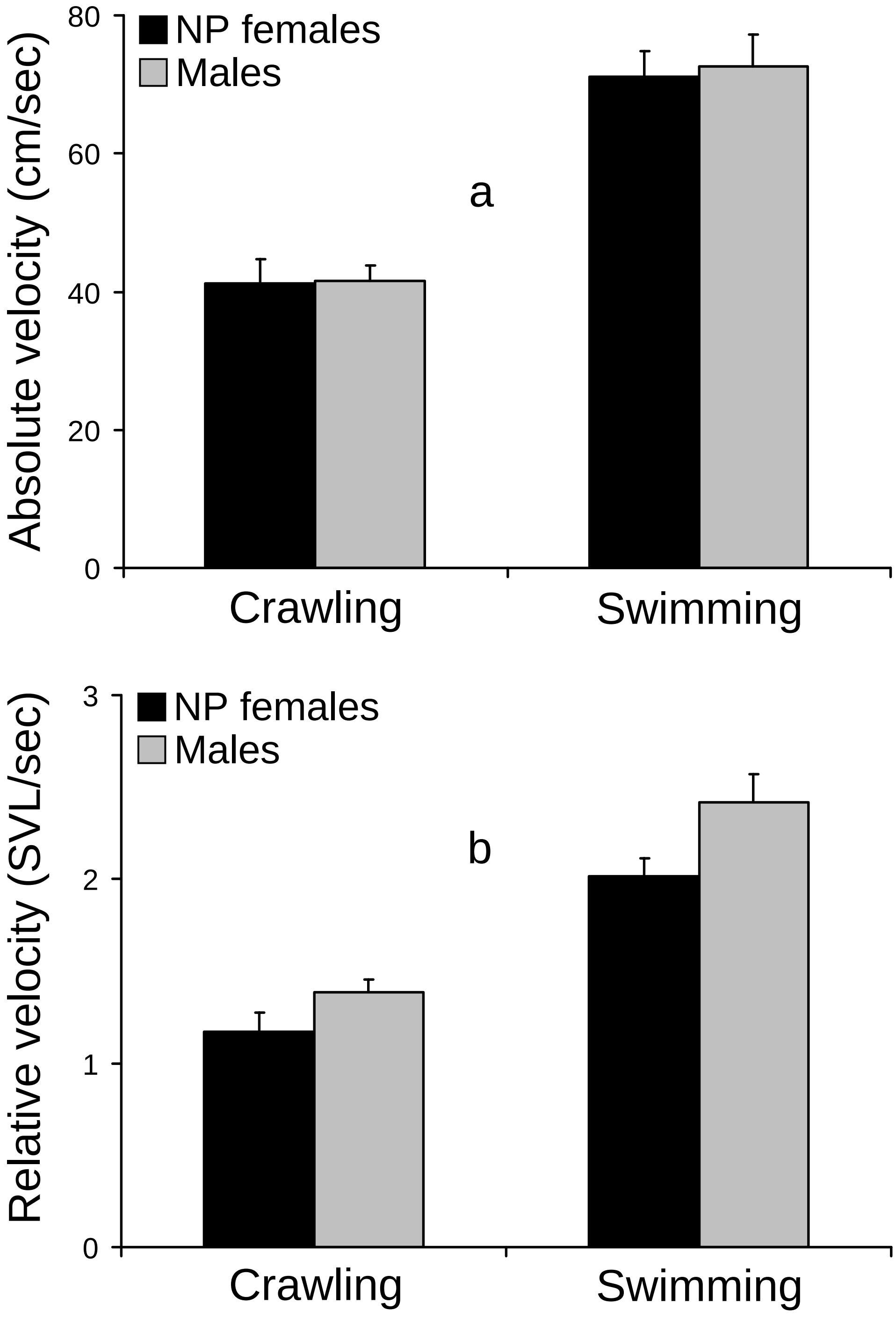

Figure 6.1. Effects of substrate and sex on maximum velocity in *Seminatrix pygaea*. a) Absolute sprint velocity. b) Sprint velocity relative to body length.

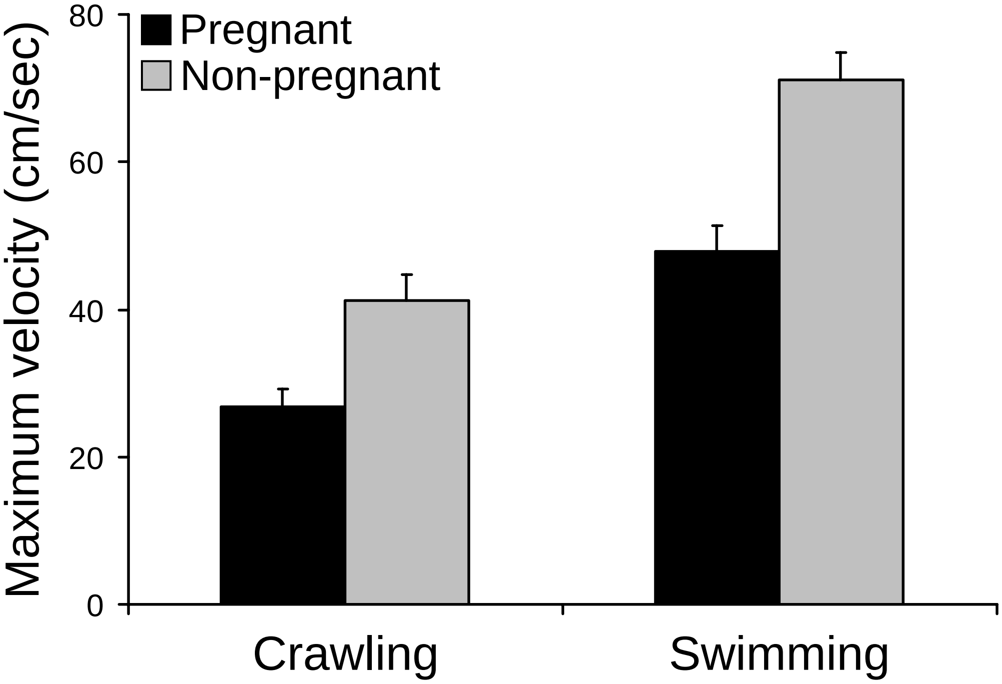

Figure 6.2. Effects of substrate and pregnancy on maximum velocity in female *Seminatrix pygaea*.

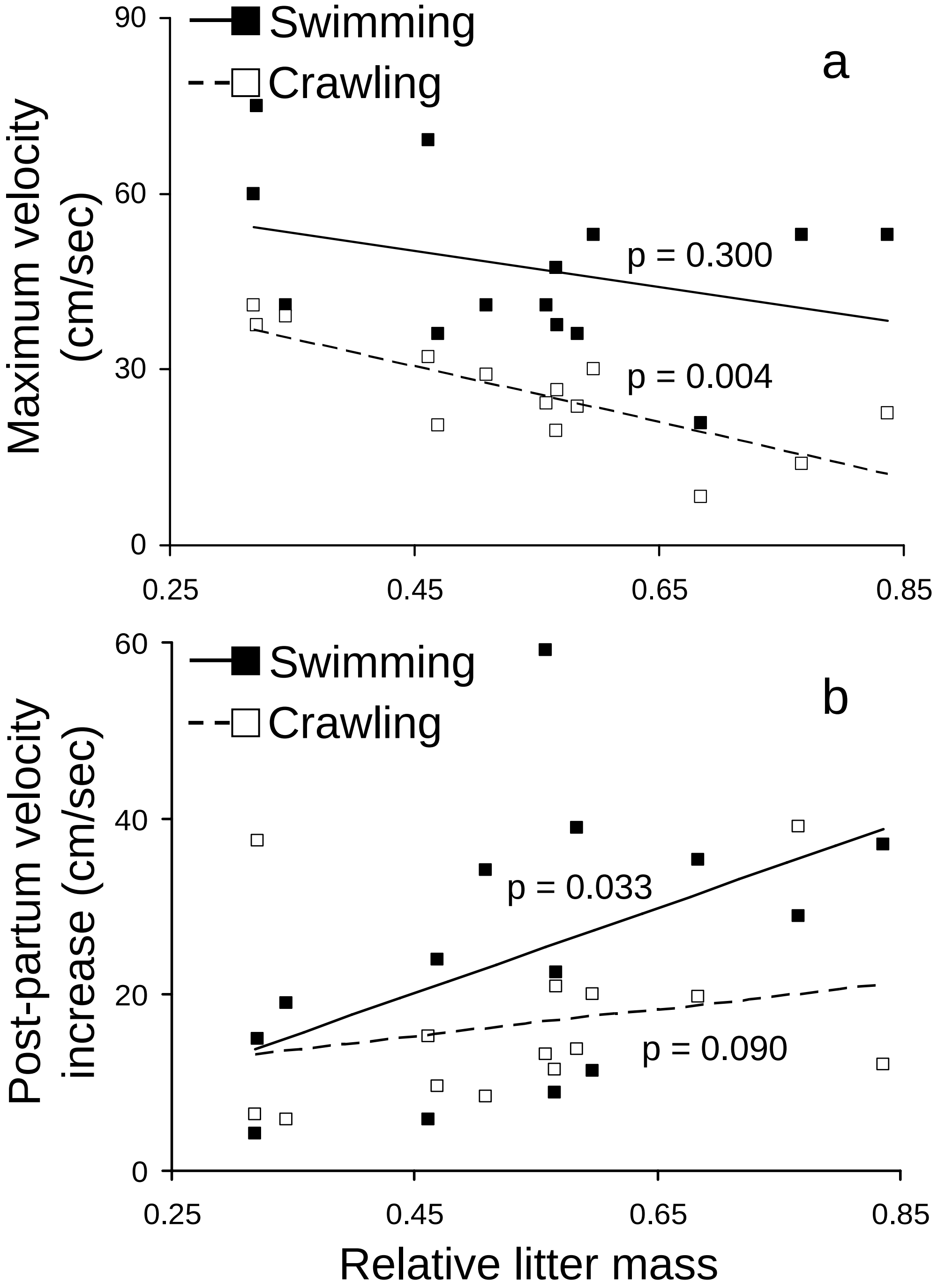

Figure 6.3. Effects of reproductive burden on locomotor impairment during pregnancy in *Seminatrix pygaea.* a) Relationship between relative litter mass and maximum velocity. b) Relationship between relative litter mass and post-partum increase in maximum velocity.

CHAPTER 7

CONCLUSION

The goal of this dissertation was to document the ecological resilience of a freshwater aquatic snake, *Seminatrix pygaea*, inhabiting an isolated wetland to prolonged drought. Specifically, my goals were to determine (1) drought survival strategy and reproductive ecology, (2) selection pressures operating on body size within a dynamic habitat, (3) reproductive allocation strategy ("income" vs. "capital" breeding), (4) long-term population dynamics and temporal shifts in demography in *S. pygaea* compared to sympatric semi-aquatic snake species, and (5) the influence of habitat (aquatic vs. terrestrial) and pregnancy on locomotor velocity.

Chapter 2 examines drought survival strategy and reproductive ecology of *S. pygaea* in an isolated wetland. *Seminatrix pygaea* are atypical from many sympatric snake species in that (i) their small body size, reliance on aquatic prey, and high rates of evaporative water loss make them ill-suited to overland movement, and (ii) they may not be subject to costs typically associated with feeding during pregnancy in snakes. Chapter 2 tests the hypothesis that *S. pygaea* survive periodic multiyear droughts by aestivating within the dried wetland, a survival strategy heretofore undocumented in snakes. Further, Chapter 2 tests the hypothesis that if *S. pygaea* rely on reproductive strategies involving "adaptive anorexia" and capital breeding, reproductive output would be reduced in the first wet year following drought. By encircling a 10-ha wetland with a continuous drift fence before it refilled, I demonstrated that *S. pygaea* were present within the dried wetland prior to the onset of spring rains that refilled the wetland in 2003. The results indicate that *S. pygaea* are capable of surviving multiyear droughts by aestivating within the dried wetland. Despite having presumably depleted energy reserves during the drought, *S. pygaea* reproduced with the same frequency and fecundity during the first season following refilling of the wetland as in pre-drought years. The ability of *S. pygaea* to rebound rapidly from stresses associated with prolonged drought was due in part to their reproductive ecology. *Seminatrix pygaea* readily fed throughout pregnancy and presumably were able to rapidly translate high post-drought prey abundances into reproductive output through income breeding.

Chapter 3 tests the hypotheses that a lack of aquatic prey during severe droughts imposes significant survivorship pressures on *S. pygaea*, and that the largest individuals, particularly females, would be most adversely affected by resource limitation. The findings suggest that both sexes experienced selection against large body size during severe drought, when prey resources were limited, as nearly all *S. pygaea* were absent from the largest size classes and maximum body size and sexual size dimorphism (SSD) were dramatically reduced following drought. Conversely, strong positive correlations between maternal body size and reproductive success in *S. pygaea* suggest that females experience fecundity selection for large size during non-drought years. Collectively, Chapter 3 emphasizes the dynamic interplay between selection pressures that act on body size and supports theoretical predictions about the relationship between body size and survivorship in ectotherms under conditions of resource limitation. The results are particularly interesting given that *S. pygaea* is the smallest semi-aquatic snake in North America and one of the least sexually dimorphic natricine watersnake species.

Chapter 2 documented that *S. pygaea* feed readily during pregnancy, leading to the hypothesis that they used income breeding to fuel reproduction following prolonged drought-induced aestivation. Still, direct evidence of nutrient transfer from recently-ingested prey to offspring is largely nonexistent in snakes. Therefore, Chapter 4 uses artificially enriched concentrations of a naturally occurring stable isotope (^{15}N) in prey items (nightcrawlers, *Lumbricus terrestris*) to test the hypothesis that *S. pygaea* transfer energy consumed during pregnancy to their offspring. The $\delta^{15}N$ of labeled prey (603.9 ± 38.3 ‰) was significantly elevated above natural levels for *S. pygaea* (5.5 – 9.0 ‰) and their natural amphibian prey (2.8 – 6.5 ‰), providing an ideal tool to document income breeding. Pregnant *S. pygaea* were offered prey items every 7-12 days from capture until parturition, but the timing of labeled prey item introduction was altered among treatment groups: control A (unlabeled prey), early (labeled prey early in gestation), and late (labeled prey late in gestation). Additionally, a fourth treatment included snakes that did not consume labeled prey but were exposed to them. By subsequently measuring the $\delta^{15}N$ of mothers and their offspring, I was able to determine if ^{15}N was transferred to either maternal or offspring tissue. The experiment confirmed that *S. pygaea* are capable of incorporating income energy into both maternal and offspring body tissues during pregnancy, providing direct evidence that *S. pygaea* can transfer ^{15}N to offspring a minimum of 77 days prior to parturition, but probably not as late as 23 days prior to giving birth. In addition, data revealed that substantial variation in reproductive allocation strategies may exist among individuals from a single population. For example, one female consumed labeled prey throughout gestation and displayed elevated ^{15}N in maternal tissues, but failed to transfer incoming ^{15}N to her offspring. Other females did not consume any prey during pregnancy but still reproduced, suggesting that they may have relied on a capital breeding strategy or income collected earlier during the same reproductive year.

Chapter 5 details the recovery of a wetland snake community on a large protected study site from 2003 to 2007, following a severe drought that lasted from 2000–2003, and examines species differences in drought-recovery strategies during years of high wetland productivity. *Nerodia fasciata* and *N. floridana* both experienced precipitous declines or local extirpations as a result of the 2000–2003 drought, whereas *S. pygaea* survived within the isolated wetland habitat by aestivating in the dried wetland. *Nerodia fasciata* recovered from the population crash rapidly, with steady increases population size each season and each year beginning the first year of wetland refilling. In contrast, *N. floridana* were not captured until the third year the wetland held water and capture rates of *N. floridana* throughout the study never approached pre-drought levels. Captures of *N. fasciata* immigrating into the wetland following drought, coupled with temporal shifts in demography indicated that *N. fasciata* may rely on metapopulation or source sink dynamics to persist long-term within isolated freshwater wetlands that occasionally experience prolonged droughts. *Seminatrix pygaea*, on the other hand, persisted throughout the study and always comprised a substantial portion of the snake community. Although the largest *S. pygaea*, particularly females, experienced drought-induced mortality in the isolated wetland, temporal shifts in demography indicate that drought survivors rapidly grew to pre-drought sizes or larger, reaching large body sizes, displaying increased female-biased sexual size dimorphism, and experiencing supernormal reproduction events during the 2nd to 4th wet years following drought. Collectively, the poor ability of adult *S. pygaea* to migrate overland long distances, their ability to aestivate during severe droughts, and their ability to thrive during productive post-drought years make *S. pygaea* uniquely suited for persisting in isolated wetland habitats, and suggests that protection of such wetlands may be vital for the conservation of *S. pygaea*.

Most life-history models assume a trade-off between reproductive investment and parental survival. Several studies have documented reproductive costs in terms of reduced locomotor performance in terrestrial habitats. However, few studies have determined the reproductive costs of pregnancy in aquatic environments, or compared pregnancy-induced locomotor costs among habitats. This knowledge gap is important because many organisms rely on multiple habitat types during pregnancy. Consequently, Chapter 6 examines sexual differences in maximum locomotor velocity and the relative impacts of pregnancy on locomotor performance in aquatic and terrestrial environments for a semi-aquatic snake (*S. pygaea*). In addition, because most life-history models predict a direct trade-off between reproductive investment and reproductive costs, Chapter 6 quantifies the relationship between reproductive investment and postpartum increase in velocity for both habitat types. Both males and non-pregnant females always swam faster than they crawled, but males were significantly faster for their size than were non-pregnant females. These results mirror sexual differences known to exist in other snakes, but differ in that the degree of sexual divergence in velocity did not vary with habitat for *S. pygaea.* Pregnancy significantly reduced both crawling and swimming velocity. Moreover, pregnancy impaired crawling velocity significantly more than swimming velocity. Also, there was a direct trade-off between reproductive investment and aquatic locomotor impairment: snakes that invested more in offspring experienced larger decreases in swimming velocity. However, evidence for such a trade-off in the terrestrial habitat was weaker. Overall, the results demonstrated that the cost of reproduction for semi-aquatic organisms may differ between aquatic and terrestrial habitats in complex ways. Swimming may be more effective than crawling for escaping predators during pregnancy, because swimming results in faster velocities and is less impaired by pregnancy. However, the assumption of a direct trade-off between reproductive investment and locomotor impairment may be stronger for swimming performance compared with crawling performance.