INTEGRATIVE APPROACHES TO EXPLORING FUNCTIONAL ROLES OF CLANDESTINE SPECIES: A CASE STUDY OF AQUATIC SNAKES WITHIN ISOLATED WETLAND ECOSYSTEMS

by

JOHN D. WILLSON

(Under the Direction of J. Whitfield Gibbons)

ABSTRACT

Understanding the functional roles of species is critical to making informed conservation and management decisions. Although the roles of many common species have been thoroughly studied, species that are infrequently encountered are often assumed to be rare and of minor functional significance. Snakes are among the most secretive vertebrates and consequently, we know little about snake population ecology, predator-prey relationships, or the roles that snakes play within ecosystems. Prompted by this lack of knowledge, I used novel and integrative methods to investigate the roles of these inconspicuous and difficult-to-sample predators. Specifically, I (1) used mark-recapture, stable isotope analyses, and laboratory experiments to estimate the predatory role of aquatic snakes within an isolated fishless wetland, (2) investigated ways in which prey communities may mediate snake's roles, and (3) theoretically evaluated ways in which snakes, as ectotherms, may differ from comparable endothermic predators in their interactions with prey populations.

Using advanced mark-recapture analyses to develop a thorough understanding of factors that affect detectability in aquatic snakes, I estimated snake population density at Ellenton Bay,

an isolated, 6 ha freshwater wetland in South Carolina, USA. I found that snakes exist at extremely high densities (>170 snakes ha^{-1}) within this productive habitat. Using data on snake density, growth rate, diet, and mass conversion efficiency, I estimated that snakes consumed over 200 kg of amphibian prey annually at Ellenton Bay, translating into >150,000 kJ ha^{-1} of energy flow from secondary to tertiary consumers within the food web. Additionally, I demonstrated that aquatic salamanders, which are characteristic of fishless wetlands, represent a higher quality prey for watersnakes than sunfish, which are characteristic of permanent wetlands. Watersnakes consumed more and larger salamanders than sunfish with less impairment to post-prandial locomotor performance. Thus, although snakes can be extremely important predators within some aquatic ecosystems, snake's roles may be mediated by prey community composition. Finally, using a mathematical predator-prey model, I demonstrated that shifting predator numerical responses from those characteristic of endotherms to more linear curves characteristic of ectotherms was generally stabilizing to predator-prey dynamics, indicating that snakes may exhibit more stable predator-prey interactions than those of endothermic predators.

INDEX WORDS: black swamp snake, banded watersnake, *Seminatrix pygaea, Nerodia fasciata,* diet, stable isotopes, food webs, trophic niche, population dynamics, mark-recapture, program MARK, robust design, population estimation, detectability, physiological ecology, feeding ecology, prey handling, locomotor performance, digestive physiology, specific dynamic action (SDA), population modeling, predator-prey interactions, life-history, ectothermy, reptiles.

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A primary goal of my dissertation research was to explore new ecological fields and join my knowledge of snake ecology with innovative methods that have seldom been applied to reptiles. Thus, my dissertation relied heavily on collaboration and many co-authors were instrumental in my research. I would particularly like to thank Whit Gibbons, Bill Hopkins, Melissa Pilgrim, Pej Rohani, Chris Romanek, Brian Todd, and Chris Winne, all of whom were involved in data collection, analysis, or editing of my dissertation research.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

The turn of the 21st century has seen Earth's human population exceed six billion and has marked unprecedented levels of natural resource consumption, global transportation, and urban expansion. In light of the magnitude and diversity of effects that these activities are having on the environment, ecologists and conservation biologists are confronted with increasingly difficult decisions in their prioritization of conservation resources. The desire to maximize the success of limited conservation initiatives has prompted increased interest in ecosystem ecology, with the frequent goal of understanding the role of individual species or functional groups in maintaining overall ecosystem integrity (Grimm 1995; Lyons and Schwartz 2001; Vanni et al. 2002). Among the functional groups within ecosystems, particular attention has been paid to high-level predators, and the roles of predators have been demonstrated in a variety of terrestrial and aquatic systems (e.g., Paine 1966, 1969; Estes and Palmisano 1974; Soulé et al. 1988; McLaren and Peterson 1994). In many cases, predators play an important direct role in regulating prey populations (Erlinge et al. 1984; Hanski et al. 2001; Korpimaki et al. 2004; Sundell 2006), and predators can indirectly influence abundance of lower trophic levels through trophic cascades (Pace et al. 1999; Polis et al. 2000; Schmitz et al. 2000). So-called "keystone" predators can play roles disproportionate to their abundance by maintaining biodiversity through preferential predation on competitively-superior prey taxa (Paine 1966, 1969). Although major groups of predatory vertebrates include mammals, birds, fish, amphibians, and reptiles, the roles of

herpetofauna as predators remain largely unaddressed. Particularly, although all snakes are strictly carnivorous and many species are top predators within food webs (Greene 1997), our knowledge of the role of snakes within ecosystems is extremely limited.

Because of their secretive behavior, snakes are often encountered with a frequency that belies their true abundance and have historically been undervalued as ecosystem components. For example, in an early edition of the Golden Guide to Reptiles and Amphibians, Zim and Smith (1953) stated: "as a group they [reptiles] are neither 'good' nor 'bad,' but are interesting and unusual, although of minor importance. If they should all disappear, it would not make much difference one way or the other." However, due in part to their low energetic requirements, snakes often exist at population densities an order of magnitude or more greater than comparable endothermic predators (Pough 1980). Resting metabolic rates of reptiles are generally 10 – 20% of those of similarly-sized endotherms (Pough 1980). Snakes' low metabolic demands allow for efficient transfer of ingested energy across trophic levels. For example, adders (*Vipera berus*) and least weasels (*Mustela rixosa*), similarly-sized European predators that specialize on microtine rodents, have assimilation efficiencies of 49% and 2.3%, respectively (Pough 1980). Consequently, *V. berus* occur at population densities (4 -14 per hectare; Parker and Plummer 1987) 20 – 1400 times greater than those of *M. rixosa* (0.01 – 0.20 per hectare; Hanski et al. 2001). Small-bodied aquatic (*Regina alleni* and *Seminatrix pygaea*) and terrestrial (*Diadophis punctatus*) snakes have both been documented to reach densities of greater than 1000 per hectare in Florida (Godley 1980), and Kansas (Fitch 1975), respectively. Even large-bodied terrestrial snakes can reach high densities in suitable habitat. For example, one Chinese island hosts spectacular densities (274 per hectare) of a large endemic pit-viper, *Gloydius shedaoensis* (Sun et al. 2001). Because snakes often exist at high densities and are capable of efficient assimilation of

biomass and energy, they presumably comprise important functional components of many ecosystems. However, although numerous studies have examined the effects of prey availability on snake demography, growth, and survival (e.g., Forsman and Lindell 1991; 1997; Shine and Madsen 1997; Madsen and Shine 2000a, 2000b; Madsen et al. 2006; Sperry and Weatherhead 2008), and a few studies have examined the effects of snake predation on prey behavior (e.g., Getz et al. 1990; Kotler et al. 1993; Bouskila 1995), few studies have evaluated snakes' functional roles as predators or their impacts on prey populations.

Perhaps the most dramatic evidence of the potential impacts of snake predation arises from situations where non-native snakes have been introduced into naive ecosystems. For example, the accidental introduction of the brown treesnake (*Boiga irregularis*) into the island nation of Guam has had catastrophic effects on native birds, bats, and lizards, driving several species to extinction and leaving much of the island devoid of most native vertebrates (Fritts and Rodda 1998; Rodda and Savage 2007). Similarly, introduction of the viperine watersnake (*Natrix maura*) to the island of Mallorca has been proposed as a cause of the decline of an endemic midwife toad (*Alytes mulentensis*; Moore et al. 2004). Some cases where snake abundance or prey consumption has been quantified are difficult to interpret because the area occupied by foraging snakes is unknown. For example, Ineich et al. (2007) estimated that approximately 1,400 sea kraits (*Laticauda laticaudata* and *L. saintgironsi*) inhabiting a 6-ha islet in the South Pacific consumed over 36,000 eels (972 kg) from surrounding reefs annually. However, although their results clearly show that snakes are important predators in coral reef ecosystems, this predation pressure may be spread over as much as 130 ha of surrounding coral reef habitat (Ineich et al. 2007). Conversely, in his classic study of the foraging ecology of the striped crayfish snake (*Regina alleni*), Godley (1980) used systematic sampling to estimate snake

density and prey consumption over a quantified area within an unusually uniform habitat: mats of introduced water hyacinth (*Eichhorina crassipes*) in Florida. Godley (1980) employed a dredging method to systematically sample *R. alleni* and their primary prey, crayfish and odonate naiads. Godley (1980) calculated that the mean density of *R. alleni* was 1289 snakes per hectare and estimated that snakes consumed 9.6% and 90.7% of the standing biomass of crayfish and odonate naiads, respectively, during the fall, when snake feeding rates were highest. Despite a limited number of intensive studies such as Godley's, our overall knowledge of the effects of snake predation on prey populations is minimal.

Often, a necessary first step in evaluating functional roles of species within ecosystems is to determine the abundance of each species or functional group. Thus, we have gleaned much of our knowledge of the effects of community structure on ecosystem processes from studies of species groups that are relatively easy to survey, such as grassland plants (e.g., Tilman et al. 1997) or sessile invertebrates (e.g., Paine 1966, 1969; Pringle et al. 1999). Snakes are among the most secretive of all terrestrial vertebrates, leading some authors to label snakes as generally intractable (Parker and Plummer 1987) or question the feasibility of monitoring snake populations using conventional methods such as mark-recapture (Turner 1977; Shine 1987; Fitch 1987; Parker and Plummer 1987). Harvey (2005) demonstrated the cryptic nature of snakes by randomly centering 50 m by 50 m (0.25 ha) search plots on individual massasauga rattlesnakes (*Sistrurus catenatus*), located by radio-telemetry. In less that 5% of plot searches were observers able to locate the snake within a 20-minute search period. Due to snakes' cryptic behavior and low or sporadic activity patterns, many researchers have relied on counts or other indices of relative abundance as indicators of snake population density (Dorcas and Willson *in press*). However, using counts as indicators of density requires assuming a consistent (generally positive

and linear) relationship between population density and the index of abundance, an assumption that is likely seldom reasonable for snakes (Dorcas and Willson *in press*). For example, research on *B. irregularis* has found no correlation between visual counts and population density estimated by mark-recapture (Rodda et al. 2005) and has demonstrated that snake surveys may suffer from observer bias (Rodda 1993). Such evidence necessitates the incorporation of detection probability into population monitoring (Mazerolle et al. 2007).

Recent advances in mark-recapture analytical techniques allow researchers to estimate population density of species with low or variable recapture probability (Dorcas and Willson *in press*). Particularly powerful is the so-called "robust design" analysis (Pollock 1982) which uses a combination of demographically open and closed population models to estimate both population size and survivorship over long, demographically open intervals with minimal violation of model assumptions (Pollock et al. 1990; Bailey et al. 2004). Importantly, robust design also allows for incorporation of time and group (e.g., sex or age) varying capture and recapture probabilities, behavioral responses to traps (i.e., "trap-happy" or "trap-shyness") and temporary emigration (individuals becoming unavailable for capture during some sampling intervals; Kendall et al. 1997; Bailey et al. 2004). Although accounting for these factors can greatly improve accuracy and precision of population estimates, the implementation of robust design analysis requires careful design of sampling methodology. Consequently, these methods have only recently been applied to herpetofauna (Mazerolle et al. 2007) and have yet to be applied to snake populations.

Quantifying diet is also essential for understanding the functional role of species in regard to energy processing, transfer, and storage within ecosystems. Traditionally, gut or fecal contents have been examined to determine diet composition (e.g., Votier et al. 2003; Mushinsky

and Hebrard 1977; Mushinsky et al. 1982). However, such direct dietary analyses often incur bias due to difficulty in accurately measuring relative abundance of different prey items, lack of temporal integration of diet composition (i.e., samples are snapshots), and failure to account for variation in assimilation rates, potentially resulting in over or under-representation of certain items in diet (Bearhop et al. 1999; Votier et al. 2003; Bearhop et al. 2004). For snakes, these complications may be exacerbated by infrequent feeding intervals, changes in behavior related to meal size (e.g., extent of basking or reduced locomotor ability), near complete digestion of many food items, and difficulty of detecting small food items by manual palpation. For example, because snakes are able to consume spectacularly large whole prey (up to 150% of the snake's own body mass; Greene 1983), and because large prey take longer to digest (Andrade et al. 1997), impair snakes' abilities to crawl or swim (Garland and Arnold 1983; Ford and Shuttlesworth 1986; Shine and Shetty 2001; Mehta 2006), and are more obvious to the observer, it is likely that large diet items are overrepresented in many assessments of snake diet. Because stable isotope composition of consumer tissue reflects diet in predictable ways, examination of variance structure in stable isotope composition has been proposed as a robust tool for evaluating diet without incurring bias associated with gut content analyses (Beahop et al. 2004; Newsome et al. 2007). However, using stable isotopes as diet indicators requires careful consideration of spatial and temporal variation in prey availability and isotopic composition (Bearhop et al. 2004). Few studies to date have characterized suitable study systems or employed the sampling designs necessary to implement this powerful tool effectively.

Finally, thorough assessment of a predator's role requires not only knowledge of prey consumption, but also an understanding of the reciprocal interaction between predator and prey populations. The study of predator-prey interactions has shed considerable light on numerous

ecological processes. For example, investigations of the characteristically cyclic dynamics of high latitude microtine rodents (reviewed in Hanski *et al.* 2001; Korpimaki *et al.* 2004) have demonstrated that life-history differences between predator functional groups can result in profound differences in predator-prey interactions. The ability of generalist predators to switch prey types allows them to maintain high population densities and mount rapid functional responses to increases in prey abundance, stabilizing population dynamics (Andersson and Erlinge 1977; Hanski et al. 1991). Although snakes are strict carnivores and can exist at high densities, snake population biology and particularly how snakes interact with prey populations remain poorly understood (Gragg *et al.* 2007; Dorcas and Willson *in press*; Nowak et al. *in press*). For example, in a recent review Nowak et al. (*in press*) found that of 301 studies of predator-prey dynamics, none focused on terrestrial ectothermic predators. Moreover, ectotherms display a suite of physiological and life-history attributes that make them fundamentally different from mammalian and avian predators. However, the population dynamic consequences of these physiological and life-history differences on predator-prey interactions remain unexplored (Nowak et al. *in press*), and no studies have incorporated snake predation into theoretical predator-prey models.

To date, few studies have accurately determined snake population density, fewer have extended their results to assess the role that snakes play as predators, and none have incorporated snake predation into theoretical predator-prey models. My dissertation takes an integrative, multidisciplinary approach to generate a comprehensive understanding of functional roles that snakes, though infrequently observed, can play in ecosystems. My research draws on techniques and concepts from field population monitoring and mark-recapture statistics, stable isotope food-web ecology, laboratory physiological and performance experiments, and mathematical predator-

prey population modeling to investigate the roles that snakes play as predators. Although my dissertation focuses particularly on snakes, the approach is one that can be applied to a variety of inconspicuous or difficult-to-sample species.

In Chapters 2 – 4 of my dissertation I present a case study of aquatic snakes within Ellenton Bay, an isolated 6 ha fishless freshwater wetland in South Carolina, USA, to illustrate the role that snakes can play in some ecosystems. In Chapter 2, I use intensive field sampling and advanced robust design mark-recapture analytical techniques (Pollock 1982; Pollock et al. 1990) in program MARK (White and Burnham 1999) to evaluate factors influencing detectability of two species of secretive aquatic snakes. I construct several *a priori* mark-recapture models incorporating various combinations of time and group (sex)-varying capture and recapture probabilities, behavioral responses to traps (i.e., "trap-happiness" or "trap-shyness"), and temporary emigration and rank models for each species using Akaike's information criterion (AIC; Akaike 1973). I use patterns of model support and parameter estimates from those models to evaluate factors affecting the accuracy and precision of population estimates for secretive snake species.

In Chapter 3, I use stable isotope techniques to evaluate diets of aquatic snakes within an isolated wetland, Ellenton Bay, where amphibian breeding migrations cause dramatic seasonal shifts in prey abundance. Although stable isotopes have been proposed as an unbiased tool for quantifying diet (Bearhop et al. 2004; Newsome et al. 2007), their use requires careful consideration of spatial and temporal variation in prey availability and isotopic composition (Bearhop et al. 2004). I use stable isotopes (C and N) to examine trophic niche characteristics of two sympatric aquatic snakes (*N. fasciata* and *S. pygaea*) in response to seasonal migrations of amphibian prey. Specifically, I characterize snake and prey isotope compositions through time,

space, and ontogeny. I also assess seasonal availability of prey taxa and compare seasonal snake and prey isotope profiles to assess the effects of temporal resource pulses (breeding amphibian migrations) on trophic niche width and diet overlap between the two snake species.

In Chapter 4, I assess the functional role that aquatic snakes play as predators by estimating the biomass of amphibians consumed by snakes at Ellenton Bay over a one year period. To accomplish this goal, I synthesize snake population density estimates obtained using mark-recapture (Chapter 2), stable isotope and field data on snake diets (Chapter 3), snake growth rates measured in the field, and mass conversion efficiency data obtained in the laboratory. Specifically, I use these data to estimate snake density and standing biomass, biomass of prey consumed by snakes over a one year period, and distribution of consumption by snakes across amphibian prey taxa. My results shed light on snakes' roles as predators within fishless wetland ecosystems and their role in transfer of biomass and energy between the wetlands and surrounding terrestrial habitats.

Having used a case study to demonstrate the role that snakes can play within fishless wetland ecosystems, in Chapter 5, I evaluate how differing prey communities may cause variation in snakes' roles among aquatic ecosystems. Specifically, I evaluate ways in which prey characteristic of fishless wetlands may facilitate the important functional role that snakes play within these ecosystems. I present data on prey composition of isolated (fishless) and permanent (fish inhabited) wetlands to establish that aquatic salamanders (*Ambystoma*) are the primary prey available to snakes in isolated wetlands and centrarchid fish (sunfish; *Lepomis* spp.) are the dominant prey in permanent wetlands. I then conduct a series of three laboratory experiments that investigate ways in which *Ambystoma* differ from *Lepomis* as potential food items for juvenile *N. fasciata*. Specifically, I compare nutritional composition and morphology of the prey

taxa and use a series of repeated-measures experiments to examine handling (number of prey consumed, maximum prey size, and handling time), digestive metabolism (specific dynamic action, SDA), and post-prandial locomotor performance (reduction in maximum crawling speed) of snakes fed *Ambystoma* and *Lepomis*. My results document ways in which prey differ in their suitability as prey for snakes, potentially leading to variation in snakes' functional importance among aquatic ecosystems.

In Chapter 6, I broaden the scope of my findings to evaluate how snakes interact with prey populations over long time scales. Specifically, I develop a mathematical predator-prey model to theoretically explore how the physiological and life-history attributes of ectotherms make predator-prey relationships involving snakes different from those involving comparable endothermic predators (e.g., mammals). I argue that a fundamental difference between endothermic and ectothermic predators lies in their responses to variation in prey abundance. Due to high energetic demands, endothermic specialist predators expire rapidly when prey become scarce and have limited abilities to translate abundant prey into reproductive output. Functionally, this creates steep, approaching threshold, curves relating vital rates to prey abundance. Ectotherms, conversely, have superior capabilities for energy storage and higher reproductive potential, allowing vital rates to respond more linearly to increasing prey abundance. I construct a discrete-time predator-prey model that explores the population dynamic consequences of shifting vital rate curves from those characteristic of endotherms to more linear curves characteristic of ectotherms.

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CHAPTER 2

ECOLOGICAL AND METHODOLOGICAL FACTORS AFFECTING DETECTABILITY AND POPULATION ESTIMATION IN "UNCATCHABLE" SPECIES[1]

[1] Willson, J. D., B. D. Todd, and C. T. Winne. To be submitted to *Journal of Wildlife Management*.

INTRODUCTION

With anthropogenic impacts on global ecosystems expanding at an alarming rate, recognition of the critical need to monitor animal populations is growing rapidly. For some species or situations, population monitoring can be as simple as taking direct census counts. However, in most situations census counts provide an incomplete assessment of population status because detection probabilities are < 1 and may vary among individuals or over time (Pollock et al. 2002, Mazerolle et al. 2007). Mark-recapture models allow for incorporation of detectability into estimates of population size and vital rates (e.g., survivorship) and they have a long tradition as powerful tools in wildlife management (reviewed in Pollock et al. 1990). However, when capture, and especially recapture, rates are low, mark-recapture analyses quickly lose power, generating imprecise parameter estimates with large associated standard errors (Kendall et al. 1995), a factor that can hamper effective management of wildlife populations.

The application of mark-recapture techniques to particularly secretive species confronts the researcher with a unique set of challenges. For example, parameters related to detection probability (e.g., capture probability, recapture probability, temporary emigration) are often considered "nuisance parameters" when studying easily observed species (Murray and Patterson 2006). When detectability is low, however, understanding variation in capture probability is critical. Identifying and accounting for biological or methodological factors that contribute to low or variable detection probabilities can significantly improve the accuracy and precision of parameter estimates in secretive species (Bailey et al. 2004b, c). For example, application of advanced mark-recapture methods recently revealed that subterranean behavior was a major factor influencing capture probability of woodland salamanders (Bailey et al. 2004b). The appropriate modification of mark-recapture models increased capture and recapture probabilities

and dramatically improved the precision of population estimates (Bailey et al. 2004b, c). Recent advances in mark-recapture analytical methods have provided a variety of tools to researchers interested in monitoring populations of secretive species (e.g., Pollock 1982, Norris and Pollock 1996, Kendall et al. 1997, Pledger 2000, Bailey et al. 2004a, Yang and Chao 2005). However, few studies provide a comprehensive approach for implementing these techniques to optimize monitoring of species with low capture probability. Here we use aquatic snakes as a case study for applying advanced mark-recapture techniques to improve accuracy and precision of population parameter estimates for species that are poorly understood and notoriously difficult to sample.

Snakes are among the most secretive of all terrestrial vertebrates. Despite the fact that snakes often occur at high densities (e.g., Fitch 1975, Godley 1980, Sun et al. 2001), numerous reports exist where rates of snake capture per unit sampling effort are abysmally low and/or many snakes are marked, but few, if any, are ever recaptured (Dorcas and Willson 2009). Low recapture rates have led some authors to label snakes as generally intractable (Parker and Plummer 1987) or to question the feasibility of using mark-recapture methods to estimate snake population parameters (Fitch 1987, Parker and Plummer 1987, Shine 1987). Indeed, standard errors associated with snake population size and vital rate estimates are often large (reviewed in Parker and Plummer 1987) and in many cases, assumptions of mark-recapture models are obviously violated. However, intrinsic intractability is only one of several potential and not mutually exclusive phenomena that can contribute to low capture probability in snakes and other secretive wildlife. Specifically, several factors may deflate capture or recapture probability and result in biased or imprecise population and vital rate estimates. We discuss two of these factors in detail below.

Unequal catchability - Historically, the two most popular population estimators are the demographically closed Lincoln-Peterson index (Lincoln 1930; discussed in detail in Pollock et al. 1990) and the demographically open Cormack-Jolly-Seber model (Seber 1982; Lebreton et al. 1992). Classically, both of these models assume constant and equal capture probability across all individuals and sampling occasions (Pollock et al. 1990). Numerous authors have suggested that this assumption is seldom met, as capture probability is likely to vary between demographic groups (e.g., sexes, or sizes or reproductive classes), over time (e.g., across seasons, years, or with environmental conditions), or based on an animal's prior capture experience (e.g., animals may become trap-happy or trap-shy). The mistake of assuming constant capture probability in models when variation in the population actually exists can substantially bias parameter estimates (Pollock et al. 1990, Bailey et al. 2004b). Evidence suggests that the behavior of many species can lead to violation of the equal catchability assumption. For example, although few studies have estimated capture probability in snakes, reproductive female Aspic vipers (*Vipera aspis*) are much more catchable by visual encounter surveys than non-reproductive females (Bonnet and Naulleau 1996). Also, Gragg et al. (2007) demonstrated that prey abundance has a strong influence on capture probability of brown treesnakes (*Boiga irregularis*) in baited traps; application of rodenticide reduced prey abundance and increased snake capture probability by 22 – 65%.

Temporary emigration – Temporary emigration occurs when a portion of the population of interest is alive and present at the study site, but is unavailable for capture during a sampling interval (Kendall et al. 1997). Temporary emigration can result from a variety of factors including behavior (e.g., inactivity) or aspects of the sampling method or design. For example, if sampling is not homogenous across the population, temporary emigration can result from

animals moving between well-sampled and under-sampled areas. Additionally, it is possible to differentiate between two forms of temporary emigration in mark-recapture analyses: completely random temporary emigration, in which a random portion of the population is unavailable for capture during a given sampling occasion; and Markovian temporary emigration, in which an individual's likelihood of emigrating is dependent on whether it was available for capture during the previous sample (Kendall et al. 1997). If random temporary emigration exists, but is not accounted for, capture probability will be underestimated and the precision of parameter estimates will be reduced (Kendall et al. 1997, Bailey et al. 2004b). The consequences of Markovian temporary emigration on population size and survivorship estimates are complex and have not been thoroughly explored in the literature (Kendall et al. 1997).

Recent decades have seen rapid advances in the statistical analysis of mark-recapture data. Software packages (e.g., program MARK; White and Burnham 1999) are currently available that allow stratified analyses, wherein individuals are divided into demographic groups that may differ in capture probability (Mazerolle et al. 2007), and which allow the inclusion of individual covariates that may affect capture probability (Pollock 2002). Moreover, mark-recapture analyses have been developed that allow for incorporation of unequal catchability and temporary emigration (Pollock et al. 1990, Kendall et al. 1997). Particularly powerful is the so-called "robust design" analysis which uses a combination of demographically open and closed population models to estimate both population size and survivorship with minimal violation of model assumptions (Pollock et al. 1990, Bailey et al. 2004b). First introduced by Pollock (1982), robust design consists of a series of widely-spaced primary sampling intervals, across which survivorship is estimated using an open model approach. Each primary sampling interval consists of several short secondary samples (often successive days), across which population size

and capture probability parameters are estimated, assuming demographic closure. Because closed models are used within secondary sampling intervals, these models can incorporate time-varying capture probabilities and trap responses. Perhaps most importantly, robust design models are currently the only models that can account for the presence of temporary emigration (Kendall et al. 1997). Despite the obvious advantage of robust design analyses, their implementation requires careful study design. Consequently, these methods have only recently been applied to herpetofauna (Mazerolle et al. 2007) and have not yet been applied to snake populations (Dorcas and Willson 2009).

In this study, we evaluated factors influencing detectability of snakes using high-intensity robust-design sampling. We sampled two species of semi-aquatic snakes at an isolated wetland in South Carolina, USA over a one-year period, yielding > 2,500 captures of > 900 individuals. We constructed *a priori* models using various combinations of time and group (sex)-varying capture and recapture probabilities, behavioral responses to traps (i.e., "trap-happiness" or "trap-shyness"), and temporary emigration, and ranked models for each species using Akaike's information criterion (AIC; Akaike 1973). We use patterns of model support and parameter estimates from those models to interpret behavioral and methodological phenomena affecting detectability of these notoriously secretive species.

METHODS

Study site

Ellenton Bay is an isolated Carolina bay freshwater wetland located on the Department of Energy's Savannah River Site in the Upper Coastal Plain of South Carolina, USA. Ellenton Bay is characterized by relatively shallow water (generally < 1 m deep) and abundant emergent

vegetation, including grasses (predominantly *Panicum* sp.), water lilies (*Nymphaea odorata*), and water-shields (*Brasenia schreberi*). Although water levels are variable across years (Willson et al. 2006; Winne et al. 2006b), the bay typically holds water year-round and can achieve a surface area of approximately 10 ha in wet years. However, during the sampling period for this study (May 2005 – May 2006), water levels remained relatively constant with the wetland surface area covering approximately 5.4 ha. 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. Thus, Ellenton Bay is currently devoid of fish, crayfish, and large, permanently aquatic salamanders (*Siren sp.* and *Amphiuma means*). Importantly, Ellenton Bay is a discrete wetland isolated from other wetlands by an extensive matrix of forested uplands; the closest large wetland is 3.2 km away. Despite extensive sampling, only 2 of > 1,000 marked *N. fasciata* have been documented moving between Ellenton Bay and other wetlands and no inter-wetland movement was detected during the time period of this study. The closest known population of *S. pygaea* is 5.7 km from Ellenton Bay and no *S. pygaea* have ever been documented moving between Ellenton Bay and any other wetlands.

Study species

Although seven species of semiaquatic snakes are present at Ellenton Bay, two species dominate captures (94% of snake captures in 2005 and 2006): the black swamp snake (*Seminatrix pygaea*) and banded watersnake (*Nerodia fasciata*). *Seminatrix pygaea* is considered the smallest semi-aquatic snake in North America (maximum total length 555 mm; Gibbons and Dorcas 2004) and is endemic to the Coastal Plain of the Southeastern US from North Carolina to southern Alabama. Because it is highly aquatic, seldom leaving aquatic habitats or basking above water (Gibbons and Dorcas 2004, Winne et al. 2006b), its abundance and status across much of

its range are largely unknown and it is considered a species of concern in South Carolina, Georgia, and Alabama. *Nerodia fasciata* is larger (maximum total length 1588 mm; Gibbons and Dorcas 2004) and is ubiquitous in the Southeastern US, occurring in virtually all types of aquatic habitats. This species is occasionally observed foraging along wetland margins or basking on emergent vegetation. Both *S. pygaea* and *N. fasciata* feed primarily on amphibians at Ellenton Bay, with *S. pygaea* specializing on paedomorphic mole salamanders, *Ambystoma talpoideum* (Willson et al. *in review*). Both species are viviparous and give birth in July and August in our region. Moreover, when the wetland holds water, virtually all mature females of both species are reproductive (Winne et al. 2006b; J. D. Willson and C. T. Winne, Savannah River Ecology Laboratory, unpublished data), eliminating the need to separate adult females into reproductive and non-reproductive groups.

Capture methods

We captured snakes at Ellenton Bay from May 2005 to May 2006. Within this period, we designed a temporal sampling regime to fit Pollock's (1982) robust design. Robust design requires a demographically-open study period punctuated by primary sampling intervals that each consist of a series of secondary samples within which demographic closure is assumed. To meet this framework, we sampled snakes monthly (primary samples) from May–September 2005 for 10 consecutive days (secondary samples), and for 6 consecutive days in the additional primary periods of October 2005, March 2006, and April 2006.

We captured snakes using plastic minnow traps (model 700; N.A.S Incorporated, Marblehead, Ohio), the most effective method for capturing these species in heavily-vegetated aquatic habitats (Willson et al. 2005, Willson et al. 2008). During each primary sampling period, we set 465 traps spaced approximately 2 m apart in a continuous transect around the aquatic

periphery of the wetland. We placed traps in shallow water with the uppermost portion of the trap above water to prevent drowning of captured snakes. Although traps were not intentionally baited, incidental capture of amphibians resulted in 'natural baiting' of traps (Seigel *et al.* 1995, Winne 2005). We checked traps daily in the early morning and recorded each snake's capture location to within 10 m.

Following capture, we recorded sex (by inspection of tail morphology and/or cloacal probing), snout-vent length (SVL), and body mass for each snake captured. We then marked each snake with a unique code by branding ventral scales (Winne et al. 2006a) before releasing it at its capture location on the day of capture. Importantly, robust design studies assume that all individuals are available for capture on all sampling occasions. Because we always released snakes on their day of capture, all individuals were available for capture within the wetland on each day of sampling.

For simplicity, we restricted analyses to animals born before the study started. We identified *N. fasciata* born during the study (2005 young-of-year) by their body size and excluded them from analyses. *Seminatrix pygaea* are small enough at birth that they can escape through the mesh of minnow traps and are therefore uncatchable until they reach ~200 mm SVL (Willson et al. 2008), at greater than nine months of age (J. D. Willson and C. T. Winne, unpublished data). Thus, we included all captured *S. pygaea* in mark-recapture analyses. Our capture histories for both species included sex as a grouping factor to allow for testing of sex-specific effects on the parameters of interest in the model selection procedure described below.

Mark-recapture modeling

The primary goal of our mark-recapture analysis was to examine factors affecting detectability of two secretive aquatic snake species. We used an overall model selection

procedure that included the following steps: (1) we identified 8 *a priori* candidate models that offered contrasting biological representations of the role of capture probability, recapture probability, and temporary emigration in our study species, (2) we used our most highly parameterized candidate model to evaluate the role of sex in these factors, (3) where sex effects on parameters were supported, we modified our 8 candidate models to include sex effects for those parameters and then compared the fit of the 8 candidate models using AIC, and (4) we ran a subsequent *post hoc* analysis to investigate support for Markovian temporary emigration and its effect on detectability parameters.

We used Program MARK (White and Burnham 1999) to construct and evaluate mark-recapture models. We used the Huggins closed captures form of the robust design model which conditions population estimates separate from the model likelihood and generally performs better with sparse data resulting from small samples sizes or infrequent recaptures (Huggins 1989, 1991, L. Bailey, Patuxent Wildlife Research Center, personal communication). We assumed capture and recapture probabilities within primary periods were constant (i.e., no day-to-day variation among consecutive secondary samples). More general models are possible (Kendall et al. 1997), but are heavily parameterized, often failing to computationally converge when recapture rates are low or the number of primary periods is great. Thus, our most heavily parameterized model included capture (p) and recapture (c) probabilities that varied among primary periods and also allowed for a behavioral response to traps (i.e., $p \neq c$) and constant random temporary emigration (γ).

In our *a priori* selection of candidate models, we fixed survival rates to simplify model structure and increase precision of estimated parameters, following the methods of Bailey et al. (2004a). We used annual survival estimates from unpublished Cormack Jolly-Seber models of *N.*

fasciata and *S. pygaea* at Ellenton Bay from 2005–2008. Models found evidence for yearly variation in survivorship but no support for sex differences in survival during 2005-2006. Yearly survival estimates for *N. fasciata* and *S. pygaea* from 2005-2006 were 0.92 and 0.88, respectively. Repeating our model selection procedure (see below) using conservative yearly survival estimates of 0.50 and 0.70 for both species did not alter model rankings, demonstrating that our results are robust to survivorship assumptions. We used month as a common denominator between our primary periods and adjusted interval lengths in Program MARK accordingly. Therefore, we converted yearly survival estimates into monthly rates of 0.993 for *N. fasciata* and 0.989 for *S. pygaea*.

We first established eight candidate models (Table 2.1) to evaluate support for the presence of temporary emigration (γ) as well as behavioral responses to traps and temporal variation in capture and recapture probabilities among primary periods. These models were generated *a priori* to test all possible parameter combinations and were organized as follows:

Models 1 and 2 were heavily parameterized, allowing for behavioral responses to traps ($p \neq c$) and capture and recapture probabilities that varied across primary sampling periods. Model 1 included constant random temporary emigration whereas temporary emigration was fixed at zero in model 2.

Models 3 and 4 allowed for behavioral responses ($p \neq c$), but did not allow p and c to vary over primary periods. Model 3 included constant random temporary emigration whereas temporary emigration was fixed at zero in model 4.

Models 5 and 6 allowed p and c to vary over primary periods but did not include a behavioral response to traps ($p = c$). Model 5 included constant random temporary emigration whereas temporary emigration was fixed at zero in model 6.

Models 7 and 8 were the simplest models tested, including no behavioral response to traps and constant p and c among primary periods. Model 7 included constant random temporary emigration whereas temporary emigration was fixed at zero in model 8.

Possible sex differences greatly increased the number of alternative models required to test all parameter permutations. Rather than including all sex-varying model permutations in our primary model selection procedure, we first evaluated sex differences in detectability parameters (p, c, and γ) by modifying the most parameterized candidate model (Model 1; see Table 2.2). Our permutations of this model included: (1a) no sex differences in parameters; (1b) sex differences in γ; (1c) sex differences in p and c; and (1d) sex differences in all three parameters. If there was support for sex differences in any of these parameters, the affected parameter was allowed to vary between the sexes when subsequently testing the 8 candidate models to evaluable detectability (Table 2.1).

Markovian temporary emigration occurs when an animal's probability of being available for capture in the study area during a primary sampling period is dependent on whether it was available for capture during the previous primary period. It is accounted for computationally by allowing γ' – the probability that an animal stays away from the study area given that it was a temporary emigrant in the previous primary period, to differ from γ'' – the probability that an animal available for capture in the previous primary period becomes unavailable for capture in the current primary period. Because the causes and consequences of Markovian temporary emigration are poorly understood (Bailey et al. 2004b), we did not include Markovian temporary emigration in our primary model selection procedure. Instead, we performed a post hoc analysis comparing models with constant random temporary emigration, Markovian temporary emigration, and temporary emigration fixed at zero for each species. Apart from temporary

emigration parameters, these models were otherwise identical and included constant (to ease comparison of these parameters between model variants) but independent capture and recapture probabilities. As in the previous model selection procedure, we included sex effects where they were supported. This analysis allowed us to evaluate support for Markovian temporary emigration in each species as well as examine the effects of incorporating Markovian temporary emigration on estimates of other parameters.

RESULTS

Sixty-nine sampling days at Ellenton Bay yielded a total of 1,392 captures of 414 individual *N. fasciata* and 1,286 captures of 495 *S. pygaea*. Captures were highest in the summer for both species (Fig. 2.1). However, *N. fasciata* displayed a more uniform seasonal pattern of captures than did *S. pygaea*, with high capture rates from May – September and reduced capture rates in October and March. *Seminatrix pygaea* displayed a more strongly unimodal pattern of captures, with peak numbers of snakes captured in August and September and very low capture rates in October and March. Through time, captures of new, previously uncaptured individuals declined rapidly in both species, relative to total captures. However, despite intense sampling, novel individuals were captured in all months and the percentage of snakes that were recaptures plateaued at approximately 80% and 70% of total captures after the first three months of sampling for *N. fasciata* and *S. pygaea*, respectively (Fig. 2.1).

For *N. fasciata*, variations of Model 1 that we used to evaluate sex effects were ranked comparably and carried nearly equal weight with a ΔAICc of only 1.2 separating the four models. Consequently, because there was no unequivocal support for sex differences in p, c, or γ for *N. fasciata*, we did not allow parameters to vary between the sexes for this species in our

primary model selection procedure (Table 2.2). In contrast, for *S. pygaea*, model 1b (sex effects in γ) carried the greatest support of the four variants of model 1, differing from the next best model by a ΔAICc of 4.9. Thus, in subsequent analyses, we included a sex effect in γ for *S. pygaea* (Table 2.2).

We found strong support for time-varying capture and recapture probabilities across months for both species (Tables 3, 4). For *N. fasciata*, the 3 highest ranked models included time-varying p and c and all models with constant p and c had ΔAICc's of 100 or more greater than the best-ranked model. For *S. pygaea*, all models with constant p and c were ranked last, with AICc values at least 300 greater than the best-ranked model. Likewise, we found strong support for behavioral responses to traps ($p \neq c$) in both species, with the top two models for each species containing a behavioral response (Tables 3, 4). Initial capture probabilities (p) for *N. fasciata* were generally low (range: 0.02 – 0.05) and relatively consistent over time (Fig. 2). This species showed a strongly positive behavioral response to traps (i.e., "trap happiness") with recapture probabilities (c) several times greater than p in all seasons (range: 0.06 – 0.26). Moreover, recapture probabilities showed strong temporal variation, with high recapture probabilities (> 0.25) in July and August. Conversely, *S. pygaea* exhibited strong temporal variation in both capture and recapture probabilities, with high values of both parameters in the summer, and near-zero values in the spring (Fig. 2.2). Maximum initial capture probability estimates for *S. pygaea* were substantially greater than those for *N. fasciata*, approaching 0.10 in July and August. *S. pygaea* exhibited a trap-happy behavioral response similar to *N. fasciata*, with recapture probability always greater than initial capture probability. However, the strength of the behavioral response varied over time in *S. pygaea* with a strong trap-happy response (c >>

p) in June and July, and nearly equal capture and recapture probabilities in August and September.

The most strongly supported model for *N. fasciata* (Model 2) included time-varying capture probability with a behavioral response and no random temporary emigration (i.e., γ fixed at zero; Table 2.3). Thus, we conclude that there was little support for random temporary emigration in this *N. fasciata* population. Conversely, we found support for random temporary emigration in *S. pygaea*. The most strongly supported model for this species (Model 1) included time-varying capture probability with a behavioral response and γ that differed between the sexes (Table 2.4). This model was separated by a ΔAICc of at least 8.9 compared with less-supported models. Estimates of temporary emigration parameters (γ) from this model were 0.19 ± 0.09 and 0.36 ± 0.08 for male and female *S. pygaea*, respectively.

We evaluated support for Markovian temporary emigration subsequent to our primary model selection procedure by ranking models with completely random temporary emigration, Markovian temporary emigration, or no temporary emigration (γ fixed at 0) for each species. These analyses revealed strong support for Markovian temporary emigration in both species, with the Markovian model ranking better by a ΔAICc of at least 80 for both species (Table 2.5). For both species, γ'' estimates were between 0.2 and 0.4, but γ' estimates were very high (0.81 – 0.98). Incorporating Markovian temporary emigration nearly doubled initial capture probability (p) estimates in all cases, but had little effect on recapture probabilities.

DISCUSSION

We use snakes as a case study in applying advanced mark-recapture methods to improve accuracy and precision of population parameter estimates for notoriously difficult-to-sample wildlife species. In both aquatic snake species we found evidence that capture and recapture

probabilities were not equal, suggesting that the use of standard open population models would violate traditional modeling assumptions and yield biased estimates of population sizes and vital rates. Specifically, we found strong support for time-varying capture and recapture probabilities and strong trap-happy responses in both *N. fasciata* and *S. pygaea*. We also found evidence for temporary emigration in *S. pygaea* and that rates of temporary emigration varied between the sexes. Below we discuss the ecological relevance of these phenomena and the implications these results have for estimating population parameters and vital rates of intractable or otherwise difficult-to-study species.

Interpreting variation in detection probability parameters requires careful consideration of factors that influence capture rates using a given sampling method. Several capture methods are commonly used in snake population monitoring, including baited and unbaited traps, visual searches or road censuses, and turning of natural or artificial cover objects (i.e., coverboards; Fitch 1987, Dorcas and Willson 2009). Because each of these methods targets snakes involved in different activities, capture rates obtained using different methods may reflect different biological or behavioral attributes of snakes (Dorcas and Willson 2009). For example, road censuses only detect snakes that are actively moving through habitat, presumably engaged in activities such as foraging, mate-searching, or dispersal, whereas cover objects generally capture snakes that are inactive or thermoregulating. Thus, seasons or environmental conditions that are favorable for activity might produce high capture rates in road censuses but low numbers of snakes captured under coverboards. Although our traps were not intentionally baited, they readily accumulated aquatic prey, which were frequently consumed by snakes within traps (Seigel *et al.* 1995, Winne 2005). Thus, our capture rates are primarily indicators of foraging

activity. This idea is supported by an experimental study conducted at Ellenton Bay that demonstrated increased capture rates associated with presence of prey in traps (Winne 2005).

Time-varying capture probability

We found clear support for temporal variation in capture and recapture probability in both *N. fasciata* and *S. pygaea*. This result is not unexpected; the activity patterns of many ectotherms are strongly dependent on environmental conditions, resulting in highly seasonal patterns of activity in temperate regions (Gibbons and Semlitsch 1987). Both *N. fasciata* and *S. pygaea* displayed generally unimodal temporal patterns of capture and recapture probability with rates that peaked in the summer. This pattern likely reflects foraging activity, with the highest foraging rates occurring during months when warm air and water temperatures facilitate rapid digestion and allow for both diurnal and nocturnal activity. Many snakes exhibit reduced foraging during seasons that correspond with mating or gestation (Bonnet et al. 1998, Gregory et al. 1999). We found no strong support for variation between the sexes in capture or recapture probabilities and did not see a noticeable reduction in detectability parameters during seasons when female snakes were pregnant. Although unexpected, this may be due in part to the fact that females of both species feed during pregnancy (Winne et al. 2006b, J. D. Willson and C. T. Winne unpublished data) and because the homogeneity of the aquatic habitat does not result in spatial conflicts between foraging and thermoregulation (Winne et al. 2006b). Failure to account for the time-varying capture probabilities we observed would result in decreased precision of parameter estimates and apparent variation in population size among seasons.

Behavioral response to traps (trap-happiness)

Behavioral response to capture has long been appreciated as a factor that can bias population size and vital rate estimation (Nichols et al. 1984). Trap responses have been

documented in a variety of animal taxa, particularly birds and small mammals (Nichols et al. 1984), but have seldom been tested for in snakes or other herpetofauna (Dorcas and Willson 2009). We found compelling evidence of behavioral responses to traps in this study; in both species recapture probability was always greater than initial capture probability, indicating a strong trap-happy response. This positive trap response is likely due to bait accumulation in traps; high recapture rates presumably reflect an association of traps with feeding opportunities. Trap happy responses are frequently associated with baited traps in other taxa (Pollock et al. 1990) and it is likely that our results apply to most situations where snakes are sampled with baited traps. The only other study to explicitly examine trap responses in snakes noted a positive, but ephemeral, behavioral response in brown treesnakes (*Boiga irregularis*) sampled using baited arboreal funnel traps (Tyrrell et al. 2009). It is important to consider, however, that snakes sampled using other methods may exhibit different responses. For example, it is plausible that snakes sampled using coverboards might exhibit trap-shy responses associated with the lack of reward (food), stress of being captured, or perhaps degradation of the microclimate under coverboards after frequent turning. Indeed, negative trap responses have been noted in terrestrial salamanders sampled using cover objects (Bailey et al. 2004b). In our study, *N. fasciata* exhibited a stronger trap response than *S. pygaea*, with recapture probability up to five times greater than initial capture probability for *N. fasciata* in some seasons. This disparity might simply be due to the larger size and mobility of *N. fasciata* compared to *S. pygaea*, which could facilitate the ability of *N. fasciata* to forage more widely and locate traps. Finally, capture and recapture probabilities did not always vary synchronously in our system. For example, *N. fasciata* displayed a weak behavioral response in cooler months (e.g., October, when initial capture and recapture probabilities were comparable) but showed strong trap-happiness in the

summer (e.g., July and August, when recapture probability was more than five times greater than initial capture probability). This temporal variation in trap response strongly suggests seasonal differences in foraging rates. Because digestion is slower at lower temperatures, in cooler months snakes may exhibit longer refractory periods between feedings, reducing their likelihood of being captured multiple times within a sampling period.

Behavioral responses violate the equal catchability assumption of simplistic closed population models such as the Lincoln-Peterson estimator (Lincoln 1930), resulting in considerable bias in estimates of population size (Nichols et al. 1984, Pollock et al. 1990) or vital rates (Hines and Nichols 2002). Specifically, failure to account for a trap-happy response leads to underestimation of population size whereas a trap-shy response results in population size overestimation (Nichols et al. 1984, Pollock et al. 1990). For example, the most strongly supported model (Model 2, which included a behavioral response to traps) yielded a total *N. fasciata* population estimate of 306 individuals in August. The comparable model that did not incorporate a trap-happy response (Model 5) underestimated population size by a factor of nearly three (111 individuals). Most studies that have used mark-recapture to estimate population size in snakes either used open population models that cannot account for behavioral responses, or have focused on population estimation and not reported support for, or strength of, behavioral responses (e.g., Whiting et al. 1996, Lourdais et al. 2002). Our results demonstrate that pronounced trap responses can exist in snakes and that these responses, if unaccounted for, can result in strongly biased estimates of population size. We advise researchers to evaluate trap responses in any study attempting to estimate population size for snakes or other secretive wildlife species, especially for studies that use baited traps as a primary capture method. Moreover, standard open population models cannot incorporate trap responses (Nichols et al.

1984), necessitating the use of robust design (Pollock 1982) sampling and analyses for studies that aim to estimate population size over demographically open intervals in cases where trap responses exist (Nichols et al. 1984).

Temporary Emigration

Temporary emigration can result from a combination of an animal's physical location, its behavior, and the efficacy of the capture method across habitats and behaviors. Among animal groups, reptiles and amphibians might be particularly likely to exhibit inherent temporary emigration due to their low energetic requirements, which allow them to undergo prolonged periods of inactivity. Moreover, many reptile and amphibian species use habitats that are difficult to sample (e.g., subterranean or arboreal habitats), which can lead to a large portion of the population being unavailable for capture much of the time. Recently, several authors have examined temporary emigration in amphibians and their results have been compelling. For example, Bailey et al. (2004b) found high rates of temporary emigration in terrestrial salamanders, with an average of 87% being below ground and unavailable for capture during each primary sampling period. Temporary emigration models have also been used to evaluate populations of amphibians that are effectively sampled only at breeding sites (e.g., Bailey et al. 2004a, Fretey et al. 2004). These models allow for estimation of the non-breeding segment of the population that is unavailable for capture during surveys each year (Bailey et al. 2004a). Snakes, which have low energetic demands and frequently use difficult-to-sample habitats, may also exhibit inactivity due to digestion, pregnancy, or ecdysis. However, despite the theoretical likelihood of temporary emigration occurring in snake populations, studies have heretofore lacked the appropriate sampling designs (i.e., robust design) to examine this possibility.

We found evidence for moderate levels of temporary emigration in *S. pygaea*, with 19% of males and 36% of females being unavailable for capture during a given primary sampling period. In contrast, support for temporary emigration in *N. fasciata* was equivocal. This difference between the species leads us to believe that temporary emigration in *S. pygaea* likely reflects spatial distribution of the species within the wetland and reduced movement (i.e., smaller home range) of *S. pygaea* in relation to *N. fasciata*. It is likely that limited movement of *S. pygaea* due to its small body size precluded a segment of the population from encountering our trapping array over the course of a primary sampling interval. Conversely, *N. fasciata* may forage more widely, allowing all individuals to encounter traps. The difference in temporary emigration rates between the sexes in *S. pygaea* could be due to more extensive movement of males due to mate-searching behavior or to reduced movement by females during pregnancy, as is typical of many snake species (Gibbons and Semlitsch, 1897, Gregory et al., 1987). Regardless of its cause, more extensive movement by males would result in a smaller proportion of males failing to encounter a trap within sampling periods. Finally, it is unlikely that behavioral inactivity contributed strongly to temporary emigration in these species. Both *N. fasciata* and *S. pygaea* are consistently active within the wetland during the months we sampled and regularly enter traps and feed during ecdysis and pregnancy (Winne et al. 2006b; J. D. Willson and C. T. Winne unpublished data). Moreover, our use of long (10-day) primary sampling periods minimized the chance that snakes would remain inactive during the entire interval due to digestion, at least in warmer months.

Failure to account for temporary emigration, where it exists, does not bias estimates of overall population size in open population models (i.e., "superpopulation size" *sensu* Kendall et al. 1997, Bailey et al. 2004b), but does reduce precision of vital rate estimates (Kendall et al.

1997, Bailey et al. 2004b). However, because closed population models assume a static population, these models will underestimate population size when a portion of the population has temporarily emigrated, and thus is unavailable for capture within the sampling period. Thus, our analysis using robust design is necessary to generate precise and unbiased population size estimates in situations where temporary emigration exists (e.g., *S. pygaea*). Additionally, our post-hoc analyses revealed support for Markovian temporary emigration in both species. Markovian temporary emigration is poorly understood, and properties of Markovian temporary emigration models have not been thoroughly examined in the literature. Moreover, no published methods currently exist for estimating total population (superpopulation) size in the presence of Markovian temporary emigration. Thus, it is difficult to assess the biological phenomena that produce Markovian temporary emigration patterns in our system, or to generalize about its implications for population estimation. If Markovian temporary emigration proves to be a common attribute of snake populations, snakes may provide excellent subjects for investigation of this phenomenon. Moreover, because unaccounted Markovian temporary emigration can deflate capture probability, accounting for it might facilitate study of snake populations by increasing realized capture probability (Kendall et al. 1997, Bailey et al. 2004b).

Conclusion and implications

In many cases, accurately estimating population size or vital rates (e.g., survival) is a critical step in conservation or management of wildlife populations. For example, many systems that are used to prioritize conservation initiatives or assess species' status, such as the U.S. Endangered Species Act or World Conservation Union's (IUCN) Red List, base rankings wholly or partly on estimated population sizes and perceived population trends. Likewise, effective management frequently demands assessing effects of experimental manipulations (e.g., habitat

modification or predator reduction) on survival or recruitment rates. Finally, most methods for projecting population trends (e.g., life tables and population viability analyses) require vital rate estimates and are highly sensitive to the precision of these values. Historically, the secretive behavior of snakes has hindered many attempts to estimate or monitor populations, and in those cases where mark-recapture has been executed successfully, precision is often low (Pollock and Plummer 1987, Dorcas and Willson 2009). Thus, the status of most snake populations and species remains unknown (Todd et al. *in press*). In fact, according to the 2004 IUCN Global Species Assessment, less than 4% of the 7,833 squamate species have been evaluated, compared with 67% of turtles, 90% of mammals, 100% of birds, and 100% of amphibians (Baillie et al. 2004), despite several authors expressing concern that reptiles are declining world-wide (Gibbons et al. 2000, Todd et al. *in press*). Aquatic snakes represent a particularly imperiled group and many of North America's most imperiled snakes are those associated with wetland habitats (e.g., Lake Erie watersnake, *Nerodia sipedon insularum*; Concho watersnake, *Nerodia harteri paucimaculata*; copperbelly watersnake, *Nerodia erythrogaster neglecta*; Atlantic saltmash snake, *Nerodia clarkii taeniata*; giant gartersnake, *Thamnophis gigas*; San Francisco gartersnake, *Thamnophis sirtalis teatrataenia*; and eastern massassauga, *Sistrurus catenatus catenatus*). Thus, developing methods that allow for accurate and precise estimation of population size and vital rates for snakes in general, and aquatic snakes in particular, is critical.

To date, few studies have specifically addressed factors influencing capture probability of snakes (but see Gragg et al. 2007, Tyrrell et al. 2009). This, combined with the apparently low inherent capture probability of many species, has contributed to our general lack of knowledge about snake population ecology (Parker and Plummer 1987, Dorcas and Willson 2009). We used robust-design sampling to evaluate factors influencing detectability parameters of aquatic snakes

within an isolated population. In two snake species, we found strong evidence of several aspects of sampling methodology and snake behavior that, if not accounted for, can result in biased or imprecise population size or vital rate estimates. Thus, we recommend caution when analyzing snake mark-recapture data and strongly suggest evaluating factors that influence detectability and which may violate assumptions of some mark-recapture models. Robust design is the only sampling method that allows for estimation of population size and vital rates over demographically open time scales without violating assumptions about population closure or equal capture probability. Moreover, robust design is the only mark-recapture method that allows testing for temporary emigration. Thus, robust design sampling shows great promise for improving the efficacy of snake mark-recapture studies. Although some snakes may be inherently intractable, in many cases careful study design and incorporation of factors that can artificially deflate capture and recapture probabilities can make precise monitoring of snake populations feasible.

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Table 2.1 List of *a priori* candidate models designed to evaluate support for temporary emigration, behavioral responses to traps, and time-varying capture probability in aquatic snakes at Ellenton Bay, South Carolina, USA. Abbreviations: γ = temporary emigration, p = initial capture probability, c = recapture probability, (.) = parameter constant, (t) = parameter varies over primary (monthly) periods.

	Parameters					
	Temporary emigration		Capture Probability			
	None	Constant, Random	Constant time, No trap response	Time-specific, No trap response	Constant time, Trap response	Time-specific, Trap response
model	γ (.) = 0	γ (.)	p (.) = c (.)	p (t) = c (t)	p(.), c(.)	p (t), c (t)
1		X†				X
2	X					X
3		X†			X	
4	X				X	
5		X†		X		
6	X			X		
7		X†	X			
8	X		X			

† Models 1, 3, 5, and 7 allow γ to vary between the sexes in *S. pygaea*. See methods for explanation.

Table 2.2 Variations of the most parameterized candidate model (Model 1) evaluating support for sex effects in capture probability, recapture probability, or temporary emigration in aquatic snakes at Ellenton Bay, South Carolina, USA. Parameters abbreviations: γ = temporary emigration, p = initial capture probability, c = recapture probability, (sex) = parameter different between males and females, (.) = parameter constant, (t) = parameter varies over primary (monthly) periods.

	Parameters			
	Temporary emigration		Capture Probability	
	Constant, Random	Sex-specific	Time-specific, Trap response	Time-specific, Sex-specific, Trap response
model	γ (.)	γ (sex)	p (t), c (t)	p (t*sex), c (t*sex)
1a	X		X	
1b		X	X	
1c	X			X
1d		X		X

Table 2.3 Model rankings for *Nerodia fasciata* sampled over a one year period at Ellenton Bay, South Carolina, USA. Models are listed in decreasing order of support using Akaike's information criterion (AIC). Parameters abbreviations: γ = temporary emigration, p = initial capture probability, c = recapture probability, (.) = parameter constant, (t) = parameter varies over primary (monthly) periods.

	Description					
model	γ	p	c	# Parameters	Δ AICc	AICc Weights
2	0	(t)	(t)	16	0	0.68698
1	(.)	(t)	(t)	17	1.5721	0.31302
5	(.)	(t)	(t) = p	9	106.3237	0
4	0	(.)	(.)	2	115.4396	0
3	(.)	(.)	(.)	3	117.4484	0
7	(.)	(.)	(.) = p	2	265.0176	0
6	0	(t)	(t) = p	8	576.3685	0
8	0	(.)	(.) = p	1	782.9599	0

Table 2.4 Model rankings for *Seminatrix pygaea* sampled over a one year period at Ellenton Bay, South Carolina, USA. Models are listed in decreasing order of support using Akaike's information criterion (AIC). Parameters abbreviations: γ = temporary emigration, p = initial capture probability, c = recapture probability, (sex) = parameter different between males and females, (.) = parameter constant, (t) = parameter varies over primary (monthly) periods.

	Description					
model	γ	p	c	# Parameters	ΔAICc	AICc Weights
1	(sex)	(t)	(t)	18	0	0.98848
2	0	(t)	(t)	16	8.9282	0.01138
5	(sex)	(t)	(t) = p	10	17.7945	0.00014
6	0	(t)	(t) = p	8	126.8841	0
3	(sex)	(.)	(.)	4	310.7447	0
4	0	(.)	(.)	2	312.6714	0
7	(sex)	(.)	(.) = p	3	369.2051	0
8	0	(.)	(.) = p	1	640.604	0

Table 2.5 Temporary emigration parameters (γ'' and γ'), capture probably (p), and recapture probability (c) estimates for *Nerodia fasciata* and *Seminatrix pygaea* generated from models with no temporary emigration (TE), constant random temporary emigration, and Markovian temporary emigration. All models include constant and independent capture and recapture probabilities p(.), c(.). Sexes are allowed to differ in temporary emigration parameters for *S. pygaea*.

	no TE		random TE		Markovian TE	
N. fasciata	Estimate	SE	Estimate	SE	Estimate	SE
ΔAIC	100.66		100.66		0	
γ''	0		< 0.01	< 0.01	0.25	0.04
γ'					0.91	0.03
p	0.04	< 0.01	0.04	< 0.01	0.07	0.01
c	0.18	0.01	0.18	0.01	0.18	0.01
S. pygaea						
ΔAIC	82.4		80.48		0	
γ'' ♂	0		< 0.01	< 0.01	0.22	0.04
γ' ♂					0.98	0.04
γ'' ♀	0		0.21	0.08	0.38	0.06
γ' ♀					0.82	0.05
p	0.03	< 0.01	0.03	< 0.01	0.06	0.01
c	0.11	< 0.01	0.11	< 0.01	0.11	< 0.01

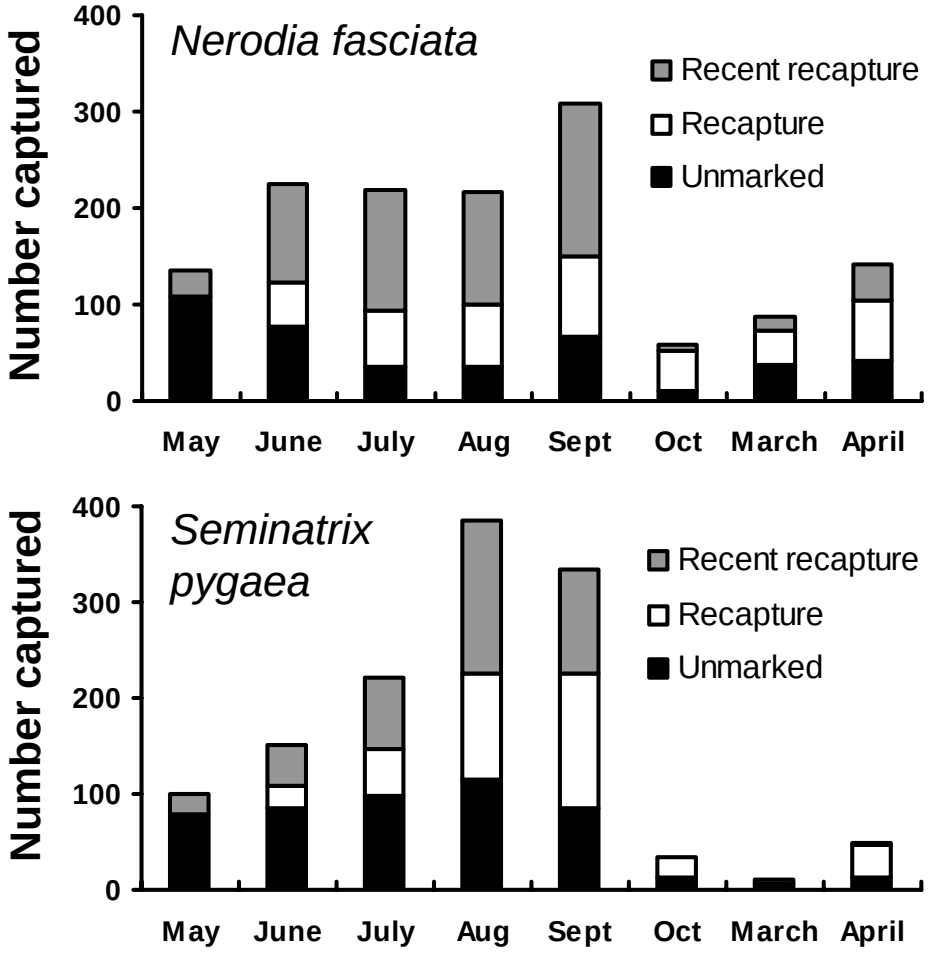

Figure 2.1 Capture summary for *N. fasciata* and *S. pygaea* at Ellenton Bay from May 2005 to May 2006. Bars represent total numbers of snakes captured in minnow traps, broken down into naive, unmarked individuals (black bars), snakes recaptured from previous primary sampling intervals (open bars), and recent recaptures within primary sampling intervals (gray bars).

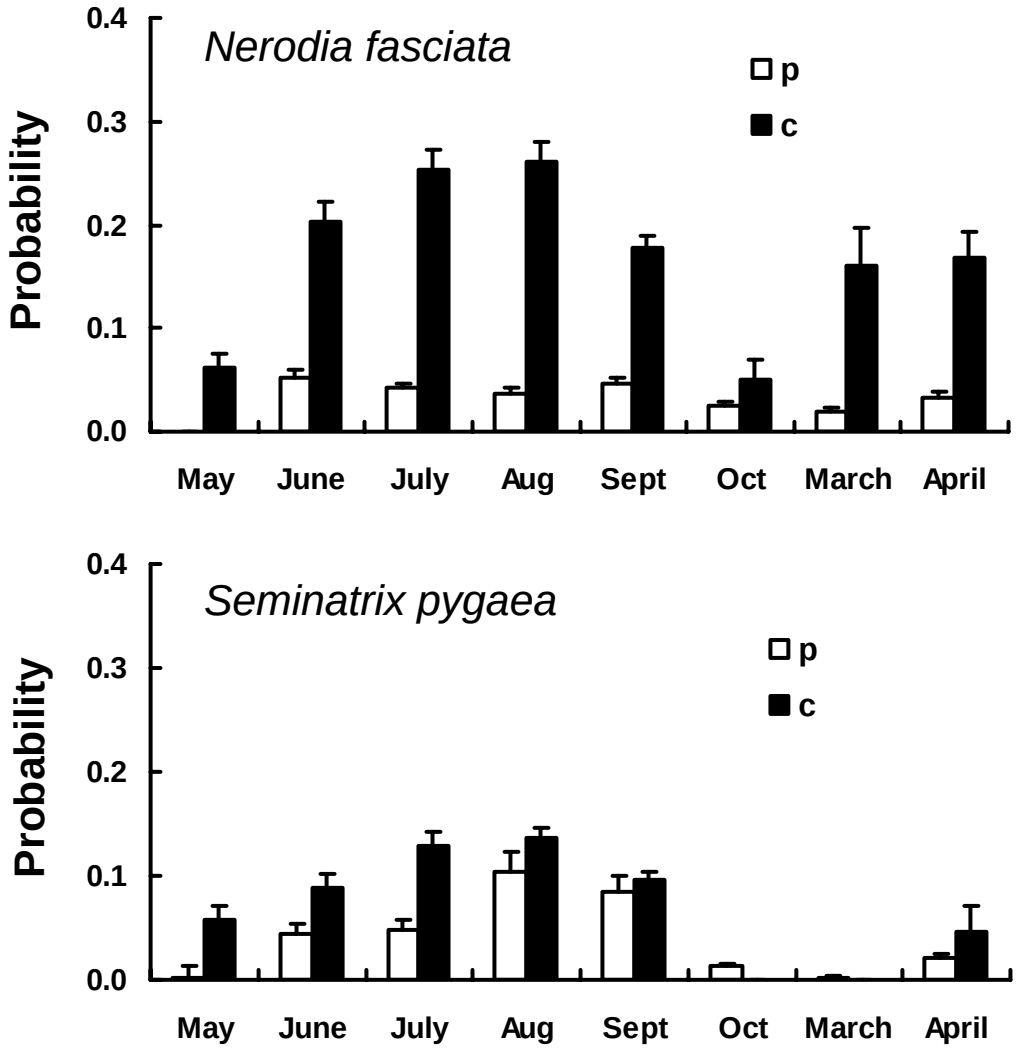

Figure 2.2 Seasonal variation in capture (p) and recapture (c) probabilities for *Nerodia fasciata* and *Seminatrix pygaea* at Ellenton Bay in 2005 and 2006. Parameter estimates for each species were generated from the favored model for each species, each of which included independent, time-varying, but not sex-varying capture and recapture probabilities.

CHAPTER 3

EFFECTS OF TERRESTRIAL RESOURCE PULSES ON TROPHIC NICHE WIDTH AND OVERLAP IN TWO SYMPATRIC AQUATIC SNAKE SPECIES: A STABLE ISOTOPE APPROACH [2]

[2] Willson, J. D., C. T. Winne, M. A. Pilgrim, C. S. Romanek, and J. W. Gibbons. Submitted to *Oikos*.

INTRODUCTION

Historically, ecological niche theory has been one of the most pervasive and controversial concepts in ecology. Despite its initial appeal, difficulties in defining, quantifying, and testing niche theory caused the concept to fall into disuse in the 1980's and 1990's (Chase and Leibold 2003). Recently, recognition of the magnitude and diversity of effects that human activities are having on the environment has prompted increased interest in ecosystem ecology, particularly in understanding the role that individual species play in ecosystems (Grimm 1995, Tilman et al. 1997, Vanni et al. 2002). Quantifying the role of species within a system involves assessing both the requirements of species from the system and the impacts of species on the system, concepts directly related to the classical Grinnellian (Grinnell 1917) and Eltonian (Elton 1927) views of the ecological niche, respectively (Chase and Leibold 2003). Indeed, the applicability of classical niche theory to modern population, community, and ecosystem ecology has been recognized, and efforts to consolidate niche theory and revise it into a theoretically sound, quantitatively useful, and applicable theory across multiple levels of biological organization are underway (Chase and Leibold 2003, Newsome et al. 2007).

Among the most important contributions of individual species to ecosystems is their role in energy use, storage, and transfer within the system (e.g., Heitchmidt et al. 1996, Lyons et al. 2005). Thus, assessing the functional role of a species depends in large part on quantifying its trophic niche width, which represents the richness and evenness of resources consumed (Bearhop et al. 2004). Interspecific niche overlap is often interpreted in light of potential competition and competitive exclusion or resource partitioning is often inferred from low incidence of niche overlap (Gause 1934, Pianka 1972). Traditionally, trophic niche characteristics have been described by either examining diet at one particular place and time or by pooling samples such

that niche dimensions represent an integration of dietary information over large temporal or spatial scales (Luiselli 2006a). Such lumping or splitting of dietary information precludes the evaluation of niche dynamics including spatial or temporal niche shifts. Although the dynamic nature of the trophic niche has been recognized in some taxa (e.g., Nieder 1997, Roy and Thomas 2003), the consequences of spatial and temporal variation in resource availability on niche characteristics of sympatric species remain poorly understood, particularly in herpetofauna (Luiselli 2006a).

Despite the apparent utility of the trophic niche as a tool for assessing community and food web structure, assessing diet composition for many species has proven difficult (Bearhop et al. 2004, Newsome et al. 2007). Direct dietary analyses, such as gut content or fecal analysis, often incur bias due to difficulty in accurately measuring relative abundance of prey taxa or failure to account for variation in digestibility and assimilation rates, potentially resulting in over or under-representation of certain food items (Bearhop et al. 1999, Votier et al. 2003, Bearhop et al. 2004). Recently, the examination of stable isotope composition (reported as $\delta^{13}C$ and $\delta^{15}N$ values) of consumer tissues has been touted as a robust tool for quantifying trophic niche width (Bearhop et al. 2004, Newsome et al. 2007). Indeed, Newsome et al. (2007) suggest that an organism's "isotopic niche," its range of stable isotope values expressed in δ-space, is comparable to Hutchinson's (1957) n-dimensional hypervolume representation of the niche. Because the isotopic composition of consumer tissues reflects assimilated diet in predictable ways, isotopic variation within a consumer population can be used to examine niche characteristics through space and time (Bearhop et al. 2004). For example, Inger et al. (2006) used stable isotopes to assess seasonal shifts in diet (from marine angiosperms to terrestrial grasses) and habitat use of wintering geese in Ireland. However, in order to use stable isotopes to

draw meaningful inferences about consumer trophic niche dimensions, it is necessary to quantify spatial and temporal variation in prey availability and isotopic composition (Bearhop et al. 2004). Specifically, stable isotope composition can only be used as a measure of a consumer's trophic niche if prey taxa are distinct in isotope composition and show little intraspecific variation through time and space (Bearhop et al. 2004). Consequently, despite the theoretical advantages of using stable isotopes to assess niche dimensions, few studies have characterized suitable study systems or employed the sampling designs necessary to implement this powerful tool effectively.

Typically, isolated freshwater wetlands dry periodically and lack fish, yielding simplified food webs often dominated by amphibians and reptiles (Gibbons et al. 2006). These systems provide an ideal opportunity to use stable isotopes to evaluate consumer trophic niches. Because amphibians receive source carbon from both terrestrial and aquatic plants, amphibian prey taxa are likely to vary widely in their carbon isotope composition. Moreover, amphibian-dominated systems exhibit dramatic shifts in resource availability as terrestrial adults of different species undertake seasonal breeding migrations to wetlands. Subsequently, their larvae grow within the wetland, and most species eventually metamorphose and emigrate into the surrounding uplands. In this study, we examine the diets of two sympatric aquatic predators inhabiting an isolated, fishless wetland in South Carolina; a generalist predator (the banded watersnake, *Nerodia fasciata*) and a species that specializes on aquatic salamanders (the black swamp snake, *Seminatrix pygaea*). We evaluate the effects of seasonal variation in amphibian prey availability on snake trophic niche dimensions and explore the ability of stable isotopes to reveal patterns of resource use. Specifically, we assess the isotope variance structure in our system as it relates to variation in snake and prey isotope profiles through time, space, and ontogeny. We subsequently

use trophic niche width and patterns of niche overlap to infer how fluctuating resource availability affects the potential for competitive interactions between the two species and make recommendations about using stable isotopes as trophic niche indicators.

METHODS

Study site

Ellenton Bay is an isolated Carolina bay freshwater wetland on the Department of Energy's Savannah River Site in the Upper Coastal Plain of South Carolina, USA. Although water levels are extremely variable (Willson et al. 2006, Winne et al. 2006b), the bay typically holds water year-round and covers approximately 10 ha when full. The water is generally shallow (< 1 m deep during 2003 - 2006) and is dominated by relatively uniform expanses of emergent grasses (predominantly *Panicum* sp.) with smaller patches of water lilies (*Nymphaea odorata*) and water-shields (*Brasenia schreberi*). Because Ellenton Bay dries periodically, it is currently devoid of fish, crayfish, and large aquatic salamanders (*Siren* sp. and *Amphiuma means*), making it a relatively uncomplicated aquatic ecosystem in which amphibians are the major vertebrate prey taxa and aquatic snakes are the major vertebrate predators (Willson et al. 2006, Winne et al. 2006b).

Study species

The banded watersnake (*Nerodia fasciata*) and black swamp snake (*Seminatrix pygaea*) are natricine watersnakes native to the southeastern United States and are the most abundant snakes at Ellenton Bay. The black swamp snake is considered the smallest semi-aquatic snake in North America (maximum total length 555 mm) and is highly aquatic, seldom leaving the aquatic habitat or basking above water (Gibbons and Dorcas 2004, Winne et al. 2006b). The

banded watersnake is larger (maximum total length 1588 mm) and can be observed along wetland margins or basking on emergent vegetation (Gibbons and Dorcas 2004). Both species are viviparous and give birth in July and August in our region. Although the banded watersnake is ubiquitous in the Southeast, occurring in virtually all aquatic habitats, the black swamp snake is generally uncommon and patchily distributed outside of peninsular Florida. Dense populations of black swamp snakes can occur, however, in isolated, heavily-vegetated wetlands (Gibbons and Dorcas 2004, Winne et al. 2005). Both banded watersnakes and black swamp snakes are known to consume a variety of aquatic prey, with a preference for fish and amphibians (Gibbons and Dorcas 2004). Although no published study has compared the diets of these two species where they occur in sympatry, gut content analysis suggests that both species prey predominantly on larval and paedomorphic *Ambystoma talpoideum* (mole salamanders) at Ellenton Bay (Fig. 3.1). The diet of black swamp snakes, particularly, is comprised nearly exclusively of salamanders. Banded watersnakes consume some other amphibians, including adult anurans and tadpoles, and consume these alternative amphibian prey types most frequently in the spring (Fig. 3.1).

Snake sampling

We sampled snakes at Ellenton Bay monthly from June – October 2005 and March – May 2006 and divided this study period into four biologically-relevant time periods (seasons) for all analyses: Spring (March-April), Early Summer (May- June), Late Summer (July – August), and Fall (September – October). We sampled snakes using plastic minnow traps (model 700; N.A.S Incorporated, Marblehead, Ohio), which is the most effective way to sample secretive semi-aquatic snakes in heavily-vegetated aquatic habitats in the Southeast (Willson et al. 2005, 2008, Winne 2005). For 5 – 10 days each month we set 465 traps approximately 2 m apart in a

continuous transect (~1 km long) around the periphery of the wetland. Although traps were not intentionally baited, incidental captures of aquatic prey resulted in "natural baiting" (Winne 2005). We checked traps daily for snakes and recorded each snake's capture location to within 20 m. At the laboratory, we recorded sex, snout-vent length (SVL, nearest mm), and body mass (nearest 0.01 g) of each snake captured. We identified recently-ingested prey items by forced regurgitation (Fitch, 1987) and adjusted diet proportions to account for differences in prey mass by multiplying the number of prey items (grouped by genus or life-stage: *Ambystoma talpoideum*, adult *Hyla sp.*, adult *Rana sp.*, *Acris sp.*, newly-metamorphosed *Rana sp.*, and tadpoles) recorded in snake's diets by the mean mass of prey within each group (generated from a database of 530 prey collected systematically at Ellenton Bay throughout the duration of the study). We then individually marked each snake by branding ventral scales (Winne et al. 2006a) before releasing it at its capture location on the day of capture.

Snakes used for isotope analyses were selected to represent all size classes, both sexes, and an even spatial distribution around the wetland. However, because of differences in catchability among size classes (Willson at al. 2008), size distributions for black swamp snakes were not uniform between sampling periods. During each trapping period (black swamp snake) or every other trapping period (banded watersnake) we collected ventral scale clips from approximately 10 (black swamp snake) - 20 (banded watersnake) individuals. Scale clips consisted of one to four ventral scales from the mid-body of the snake; however, tail clips were taken for some very small individuals. 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 clips and tail clips from the same individual do not differ substantially in isotopic composition (~ 0.20 ‰ difference; MAP, unpublished data).

Prey sampling

Although 24 amphibian species are known to occur at Ellenton Bay (Gibbons and Semlitsch 1991), most either exist at such low densities or enter the aquatic habitat so infrequently that they are likely only occasionally encountered by snakes. To conservatively identify all potentially important prey taxa, we examined a database of 2795 diet records for banded watersnakes and black swamp snakes at Ellenton Bay from 2003 – 2006 and recorded any prey species that had been documented as a prey item more than once. The resulting list of six prey species accounted for > 99% of the snake prey records: paedomorphic *Ambystoma talpoideum* (mole salamander), *Rana sphenocephala* (southern leopard frog), *R. clamitans* (bronze frog), *R. catesbeiana* (bullfrog), *Hyla gratiosa* (barking treefrog), and *Acris gryllus* (southern cricket frog). Based on seasonal availability and isotopic composition (see below), we grouped these prey taxa into six prey functional groups (aquatic salamanders, adult ranid frogs, adult cricket frogs, adult treefrogs, recently-metamorphosed anurans, and tadpoles) for subsequent analyses.

We assessed seasonal availability of aquatic amphibian species/life stages (aquatic salamanders and tadpoles) while sampling for snakes. During each sampling interval, we deployed 15 aquatic funnel traps, spaced equally around the perimeter of the wetland, as "prey traps." All prey captured in these traps were recorded daily and all trap contents were subsequently removed to avoid re-counting individuals the following day. Any traps containing snakes or large predaceous beetles (e.g., Belostomatidae or Dytiscidae) were excluded from the analysis. We classified aquatic prey groups as "available" during a given season if capture rates in aquatic funnel traps exceeded 0.5 animals per trap per night during that season.

We assessed seasonal availability of adult and newly-metamorphosed anurans (ranid frogs, cricket frogs, treefrogs, and anuran metamorphs) using captures in a terrestrial drift fence with pitfall and funnel traps that completely encircled Ellenton Bay from 1 Feb 2003 – 31 Jan 2004 (see Gibbons et al. 2006). To determine seasonal availability, we calculated the proportion of captures of each anuran prey group within each season and classified groups as available within a season if captures for a group during that season exceeded 10% of total captures for that group across the year. Although logistical difficulties prohibited us from operating the drift fence during years when snakes were sampled, these data represent general seasonal patterns of amphibian breeding activity, which are highly conserved across years (Blaustein et al. 2001).

To assess stable isotope composition of prey, we collected whole-body samples bi-monthly. In all seasons we collected prey samples one month prior to snake samples to allow for turnover time of snake scale tissue. For each bi-monthly sample we collected a minimum of ten salamanders and approximately five individuals from each other prey group (see results) available within the wetland during that season. When possible, prey items were collected from locations evenly spaced around the wetland. We fasted all prey items for 2-5 days to ensure that gut contents were voided prior to stable isotope analyses. In addition, we recorded body length (SVL) and mass of each captured prey item.

Stable isotope analyses

We dried snake scale clips in an oven (> 48 h at 40 - 50 ^{o}C) and prey samples in a freeze drier until they reached a stable dry mass (5-14 days). We homogenized dried prey items using a cryogrinder. We packaged ~1 mg of each sample (ground prey item or snake scale) into individual 3.5 x 5 mm tin capsules. We determined carbon and nitrogen isotope ratios of samples using a Finnigan DeltaPlus XL isotope ratio mass spectrometer at the Savannah River Ecology

Laboratory. We report stable carbon and nitrogen isotope compositions of samples in conventional delta notation (Ehleringer and Osmond 1989, Ehleringer and Rundel 1989), per mill (‰) units. We used laboratory (DORM-2, TURK) standards to calibrate samples to international standards (PDB and atmospheric air) for carbon and nitrogen, respectively.

Statistical analyses

Because young snakes are known to display residual maternal isotope signatures (Pilgrim 2007), we excluded the bottom 18% of the body size distribution of each snake species (black swamp snakes < 200 mm SVL, n = 7; banded watersnakes < 283 mm SVL; n = 12) from all analyses to avoid confounding diet-derived isotope signals with maternal signals. We tested for ontogenetic shifts in isotopic composition within each snake species and prey group using linear regressions of isotope values on SVL. For taxa (prey or snake) that did not exhibit significant ontogenetic shifts, we used Analyses of Variance (ANOVA) to compare isotope values among groups (i.e., spatial location, season). For taxa that displayed significant ontogenetic shifts in isotope ratios, we used Analysis of Covariance (ANCOVA), with SVL as the covariate. We tested for spatial variation in isotopic composition for the three prey groups (salamanders, ranid frogs, and cricket frogs) for which we had sufficient spatial distribution and sample size to subdivide samples within the wetland. We systematically divided the wetland into five equal sections and used ANOVA or ANCOVA to test for differences in isotope values among sections.

Isotopic mixing models (e.g., Phillips 2001, Harvey et al. 2002, Phillips et al. 2005, Carleton et al. 2008) have recently gained popularity as quantitative tools for explicitly translating consumer isotopic composition into diet. Although the mixing model approach is well-suited for investigations seeking to quantify the proportional contributions of multiple prey types to consumer diets, most mixing models assume that the consumers are in isotopic

equilibrium with their diets and model performance varies greatly depending on the trophic shift values use to parameterize the model. Because we suspect that snake tissues in this system are seldom in isotopic equilibrium with diet (see discussion) and data on trophic shift values for snake tissues are limited (but see Pilgrim 2005, Pilgrim 2007, Pilgrim et al., *in review*) we did not take a mixing models approach in this study. Alternatively, we adopt the approach of Bearhop et al. (2004) and use patterns of variation in stable isotope composition to gain insight on trophic niche characteristics for the two snake species.

We assessed overall trophic niche width of black swamp snakes and banded watersnakes by comparing variance of isotope values for each species, pooled across all seasons, using Levene's Test for Homogeneity of Variances, and assessed seasonal variation by comparing isotope values among seasons for each snake species using ANOVA or ANCOVA. We recognized statistical significance at $\alpha = 0.05$ and performed all statistical analyses using the STATISTICA software package (StatSoft, Inc. Tulsa, OK, USA 1998). We examined all data prior to each analysis and if statistical assumptions were not met we used appropriate transformations or non-parametric tests.

RESULTS

Prey availability and isotope composition

Availability of prey groups varied dramatically across seasons (Fig. 3.2). Some groups were abundant year-round (e.g., salamanders, Fig. 3.2a; ranid frogs, Fig. 3.2b), while other groups were available during some seasons, but were virtually absent during other seasons (e.g., tadpoles, Fig. 3.2a; treefrogs, Fig. 3.2b). Generally, prey richness was high in the spring and early summer (Fig. 3.2). This was primarily due to spring arrival of breeding anurans (e.g.,

treefrogs and cricket frogs) and the presence of their larvae (tadpoles) in the aquatic habitat. In contrast, prey richness was low in the summer and fall, when the primary available prey was salamanders (Fig. 3.2). Although available year-round, abundance of Ranid frogs peaked in the fall (Fig. 3.2).

To use isotope composition of consumer tissues as a meaningful measure of trophic niche width, prey must differ isotopically from each other and isotope signatures of prey must remain relatively stable over time (Bearhop et al. 2004). Plots of $\delta^{15}N$ and $\delta^{13}C$ values for all prey sampled (n=128) revealed that the six amphibian functional groups varied substantially in isotopic composition (Fig. 3.3). Tadpoles were most depleted in ^{13}C, whereas adult anurans (ranid frogs, cricket frogs, and treefrogs) were most enriched in ^{13}C. Adult anurans separated along the $\delta^{15}N$ axis, generally reflecting the degree of terrestrial habitat use; the most aquatic taxa (ranid frogs) were enriched in $\delta^{15}N$ relative to more terrestrial taxa (treefrogs).

The isotopic composition of prey functional groups showed little change across seasons (Fig. 3.4). For example, ranid frogs showed relatively high isotope values in all seasons, whereas tadpoles showed low isotope values whenever they were available. Thus, most temporal variation in the overall isotope profile of prey at Ellenton Bay was due to seasonal changes in prey availability rather than shifts in isotopic composition within prey taxa.

Salamanders exhibited a strong positive ontogenetic shift in nitrogen isotope composition (r^2 =0.501; $p < 0.001$). We did not detect any other significant ontogenetic shifts in prey isotope values (all $p > 0.05$). We found no spatial variation in isotopic composition of salamanders ($\delta^{13}C$, Kruskal-Wallace Test, $H_{4,55} = 5.43$, $p = 0.246$; $\delta^{15}N$, ANCOVA, $F_{4,49} = 1.42$, $p = 0.242$), adult ranid frogs ($\delta^{13}C$, ANOVA, $F_{4,21} = 0.27$, $p = 0.894$; $\delta^{15}N$, ANOVA, $F_{4,21} = 1.07$, $p = 0.394$), or adult cricket frogs ($\delta^{13}C$, ANCOVA, $F_{4,18} = 0.69$, $p = 0.611$, one outlier excluded; $\delta^{15}N$,

ANOVA, $F_{4,19} = 1.24$, $p = 0.329$). Thus, prey location within the wetland was not an important source of variation in prey isotopic composition at Ellenton Bay.

Variation in snake isotope composition

Overall, mean isotope values were similar for banded watersnakes (-26.0 ‰ $\delta^{13}C$ and 7.5 ‰ $\delta^{15}N$) and black swamp snakes (-26.2 ‰ $\delta^{13}C$ and 7.4 ‰ $\delta^{15}N$; Fig. 3.5). These values were slightly enriched in both carbon and nitrogen compared to mean isotope values of salamanders (Fig. 3.4). Although mean isotope values were not statistically different between banded watersnakes and black swamp snakes (Kruskal-Wallace Test: $\delta^{15}N$, $\chi^2 = 1.90$, $p = 0.17$; $\delta^{13}C$, $\chi^2 = 1.15$, $p < 0.28$), the species displayed strong differences in isotopic variance (Levene's Test for Homogeneity of Variances: $\delta^{15}N$, $F_{1,172} = 41.10$, $p < 0.01$; $\delta^{13}C$, $F_{1,172} = 21.53$, $p < 0.01$). Specifically, although the range of $\delta^{15}N$ values exhibited by the two snake species was similar (5 to 9 ‰), banded watersnakes displayed a much broader range of $\delta^{13}C$ values (-28.5 to -23.5 ‰) than black swamp snakes (-27.5 to -25 ‰). Thus, overall, stable isotope composition suggests that banded watersnakes have a broader trophic niche than black swamp snakes (Fig. 3.5).

Banded watersnakes exhibited no ontogenetic shift in carbon isotope composition (Fig. 3.6a; $r^2 = 0.01$; $p = 0.579$) but displayed a slight positive ontogenetic shift in nitrogen isotope values (Fig. 3.6b; $r^2 = 0.45$; $p = 0.034$). Black swamp snakes displayed highly significant ontogenetic shifts in both carbon (Fig. 3.6c; $r^2 = 0.15$; $p < 0.001$) and nitrogen (Fig. 3.6d; $r^2 = 0.78$; $p < 0.001$) isotope values, with larger individuals depleted in $\delta^{13}C$ and enriched in $\delta^{15}N$ relative to smaller individuals. R^2 values demonstrate that for black swamp snakes, 78% of the variation in nitrogen isotope composition was explained by body size (SVL), compared to only 45% for banded watersnakes. The ontogenetic shifts in black swamp snakes generally paralleled

those seen in salamanders (Fig. 3.6e, 3.6f), but were enriched slightly in both $\delta^{13}C$ and $\delta^{15}N$ (Fig. 3.6c-f).

Black swamp snakes exhibited no significant seasonal variation in carbon (Fig. 3.6c; ANCOVA, $F_{3,70} = 1.38$, $p = 0.256$) or nitrogen (Fig. 3.6d; Scheirer-Ray-Hare test, $F_{3,91} = 0.49$, $p = 0.693$) isotope values. Banded watersnakes exhibited significant seasonal variation in carbon values (Fig. 3.6a; ANOVA, $F_{3,94} = 15.41$, $p < 0.001$) but not in nitrogen isotope values (Fig. 3.6b; Scheirer-Ray-Hare test, $F_{3,94} = 0.47$, $p = 0.701$). The strongest seasonal shift in banded watersnake isotope values occurred between spring and early summer, when snakes showed strong enrichment in ^{13}C and a trend towards depletion in ^{15}N (Fig. 3.7). This shift occurred concomitant with availability of adult anurans, particularly treefrogs, which are enriched in ^{13}C and depleted in ^{15}N relative to other prey taxa (Fig. 3.4). Following early summer, banded watersnakes showed gradual depletion in ^{13}C through the late summer and fall (Fig. 3.7).

Seasonal shifts in prey consumption by banded watersnake resulted in shifts in overlap of isotope values between the two snake species (Fig. 3.4). In fall and spring the range of isotope values exhibited by banded watersnakes nearly completely encompassed those of black swamp snakes, whereas in summer the species exhibited reduced overlap in isotope values (Fig. 3.4).

DISCUSSION

Stable isotopes have been proposed as useful tools for examining consumer trophic niches (Bearhop et al. 2004, Newsome et al. 2007), but their use requires relatively simplified food webs with prey sources that differ in isotopic composition. Additionally, researchers must account for (or exclude) variation in isotopic composition within prey taxa across time and space. Recent studies have demonstrated that spatial variation in the isotopic composition of prey

taxa can occur on relatively small spatial scales (e.g., < 1 km; Pilgrim 2005), seriously complicating the use of stable isotopes as diet indicators. Because we did not detect spatial variation or strong temporal variation in prey isotopic composition within our small, well-defined study site, we were able to exclude the confounding effects of spatial and temporal heterogeneity in baseline isotope sources from our analyses. Thus, this system offered an ideal opportunity to explore the ability of stable isotopes to reveal patterns of resource use and trophic niche characteristics of sympatric predators in response to seasonally-fluctuating prey resources.

The black swamp snake (a dietary specialist)

Although black swamp snakes are known to feed on a variety of aquatic prey (Gibbons and Dorcas 2004), gut content analysis suggests they feed primarily on larval and paedomorphic mole salamanders at Ellenton Bay (Fig. 3.1). The low variance we observed in black swamp snake isotope values supports this conclusion, suggesting that this species exhibits a more specialized diet than the banded watersnake at Ellenton Bay. Black swamp snakes displayed strong positive ontogenetic shifts in $\delta^{15}N$ and $\delta^{13}C$ values with high r^2 values, indicating that much of the isotopic variation in black swamp snakes was attributable to ontogenetic shifts in isotope ratios. Ontogenetic shits in black swamp snakes paralleled salamander ontogenetic shifts, but were isotopically heavier, likely representing trophic enrichment (i.e., preferential incorporation of heavy diet isotopes into consumer tissues; Egger and Jones 2000). Thus, a logical explanation for the presence of an ontogenetic shift in black swamp snake isotope values is an ontogenetic shift in the size of salamanders consumed. Snakes are well-known to be gape limited predators and ontogenetic shifts in prey size have been demonstrated in many species (Mushinsky 1987, Arnold 1993), including some dietary specialists (e.g., Voris and Moffett 1981, Godley 1984). Due to the small body size of black swamp snakes, small individuals are

likely gape-limited in the size of salamanders that they can consume, with only the largest snakes able to ingest full-grown salamanders. A specialized diet of salamanders also is consistent with the lack of seasonal variation in black swamp snake isotope values, as salamanders are one of the few prey taxa that are abundant at Ellenton Bay year-round.

The banded watersnake (a dietary generalist)

Banded watersnakes are well-known to consume a variety of aquatic and semi-aquatic prey (Gibbons and Dorcas 2004) and displayed the most diverse diet of five sympatric natracine watersnake species in Louisiana (Mushinsky and Hebrard 1977). Gut content analyses suggested that banded watersnakes at Ellenton Bay feed primarily on aquatic salamanders but also consume tadpoles and adult anurans (Fig. 3.1). The results of our stable isotope analyses generally support this conclusion by demonstrating that banded watersnakes have a broader (more generalist) trophic niche than black swamp snakes with little ontogenetic variation in isotope values. Moreover, our isotope analyses revealed that unlike black swamp snakes, banded watersnakes showed strong seasonal shifts in isotopic composition. The most notable shifts in watersnakes occurred between spring and early summer. Between these seasons, banded watersnakes tissues displayed enrichment in ^{13}C and a trend towards depletion in ^{15}N. This pattern is consistent with banded watersnakes feeding on seasonally-available treefrogs and cricket frogs (which are enriched in ^{13}C and depleted in ^{15}N relative to other prey) in the spring. Although gut content analysis suggested that banded watersnakes also feed on tadpoles in the spring and early summer (Fig. 3.1), our stable isotope analyses do not support this conclusion. Tadpoles are depleted in both ^{13}C and depleted in ^{15}N relative to other prey. The only time period when watersnakes displayed simultaneous depletion in both ^{13}C and depleted in ^{15}N was between late summer and fall (Fig. 3.7), when tadpoles are rare within the wetland (Fig. 3.2). Thus, the patterns of stable

isotope composition suggest that our gut content analysis over-represented tadpoles and potentially under-represented treefrogs within snake diets. This likely reflects the fact that the gut content analysis was based on recently-ingested prey taken from snakes captured in aquatic traps. Snakes may have consumed larger numbers of aquatic tadpoles and smaller numbers of terrestrial/arboreal treefrogs within aquatic traps than they would at large within the wetland.

Interestingly, despite the fact that we sampled prey one month prior to snakes within seasons, isotopic shifts still appeared to be lagged relative to prey. After exhibiting the most enriched ^{13}C values in the early summer, banded watersnakes exhibited gradual depletion in ^{13}C through the late summer and fall. This lag corroborates evidence that although snake scale tissue can register diet isotopic composition on relatively short time scales (ca. 15 days, Pilgrim 2005, Pilgrim et al. *in review*), substantially longer amounts of time may be necessary for scale tissue to equilibrate with diet (e.g., 1.5 – 5.5 months, Pilgrim 2005, Pilgrim et al. *in review*). The lagged, short-term shifts we observed in snake isotope values suggest that isotopic mixing models (Phillips 2001, Phillips et al. 2005), which assume consumer tissue is in isotopic equilibrium with diet, should be used with caution, especially when attempting to use isotopes to assess diet over short time-scales.

Seasonal shifts in diet have been observed in other snake species and may allow generalist species to capitalize on seasonally abundant or particularly energy rich prey types during periods of high energetic demand. For example, during spring, female viperine snakes (*Natrix maura*) switch diets from fish to frogs, which are more abundant and are higher in energy content than other prey types (Santos et al. 2000). Because the timing of this dietary shift coincides with vitellogenesis, prey switching is presumably a strategy that offsets costs of reproduction in viperine snakes (Santos et al. 2000). Although our results suggest that the timing

of diet shifts in banded watersnakes largely reflected seasonal prey availability, the inclusion of large adult anurans during the spring months may be important for fueling reproduction as this corresponds with the timing of vitellogenesis for this species (JDW and CTW, unpublished data).

Effects of resource pulses on niche overlap

The role of interspecific competition in structuring snake communities remains unresolved (Reichenbach and Dalrymple 1980), but limited interspecific dietary overlap in many snake communities has prompted some researchers to conclude that snakes, particularly temperate aquatic species, partition prey resources (Mushinsky 1987, Luiselli 2006b). However, few studies have examined diet overlap between sympatric snake species within ecosystems with strong temporal variation in prey availability. Our results suggest that seasonal prey availability may be extremely important in shaping food webs. At Ellenton Bay, pulsed, seasonal movements of amphibian prey caused shifts in the trophic niche of the banded watersnake, leading to seasonal shifts in niche overlap that ranged from complete overlap between the species to only partial overlap. Thus, in situations where resources are limiting for much of the year, brief seasonal resource pulses could alter the potential for competitive interactions by reducing niche overlap between potential competitors. This, in turn, may promote community complexity by relaxing competitive exclusion during some seasons (Hutchinson 1959). Seasonal relaxation of competition pressure may be particularly advantageous for ectotherms, which can survive long periods when food is scarce (or competition is high) because of their exceptionally low metabolic costs, relative to endotherms (Pough 1980). For such species, short periods of relaxed competition due to seasonal prey abundance or seasonal availability of alternative prey taxa may allow individuals and/or species to persist within the community.

Implications for niche assessment

Evaluation of an organism's trophic niche can provide valuable insight into a species' role within its community and ecosystem (Chase and Leibold 2003). Unfortunately, because diet is generally examined either at one specific location and time or samples are pooled across wide geographic and temporal scales, niche dimensions often represent either instantaneous snapshots or averages over space and time, respectively (Luiselli 2006a). Stable isotopes have been proposed as useful niche indicators because isotopic composition represents an integration of diet over time and isotope analyses do not suffer from biases inherent in traditional gut or fecal content analyses (Bearhop et al. 2004). The characteristics of our study site and species allowed us to use a combination of traditional gut content analysis and stable isotope techniques to examine the trophic niches of banded watersnakes and black swamp snakes over differing spatial and temporal scales. In general, the two methods yielded similar conclusions (i.e., that both species feed largely on salamanders, but banded watersnakes consume some seasonally-available adult anurans in during the spring). However, our isotope analyses suggested that the gut content analysis may have over-represented tadpoles as diet items in branded watersnakes, likely due to consumption of tadpoles by snakes within aquatic traps. Thus, a combination of the two methods was necessary to produce a comprehensive understanding of diet in these snake species. Additionally, our conclusions about niche dimensions, position, and overlap between species were dependent on the scale at which data were assessed. When samples were pooled, banded watersnakes displayed broader niche width than black swamp snakes and the niches of the two species overlapped nearly completely. However, when samples were assessed within seasons, we found that niche overlap during some seasons was minimal. Thus, when assessing niche

dimensions, and especially in interspecific comparisons, careful delineation of the temporal and spatial scale across which the niche is being assessed can be critical.

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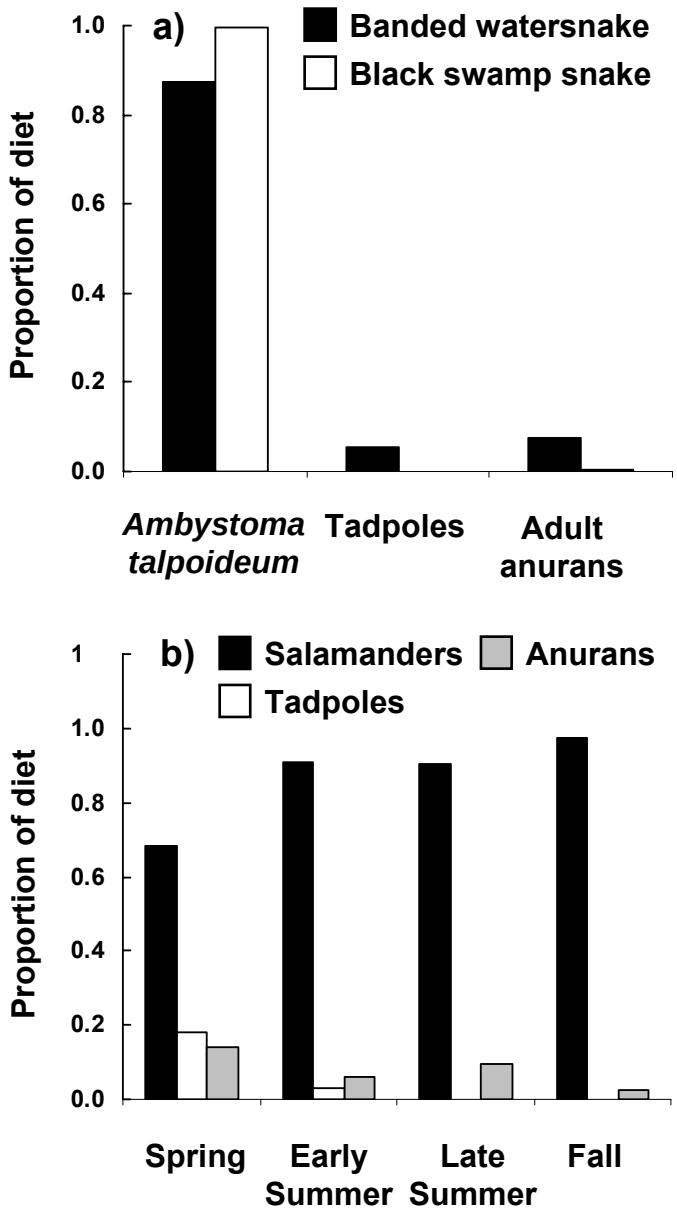

Figure 3.1 Diet composition of banded watersnakes and black swamp snakes at Ellenton Bay from May 2005 to April 2006 based on gut-content analysis. Overall diet composition for banded watersnakes and black swamp snakes (a) was derived from a total of 1,504 and 518 diet items, respectively, adjusted to account for differences in prey mass (see methods). Seasonal diet composition for banded watersnakes (b) demonstrates seasonal diet shifts in this species.

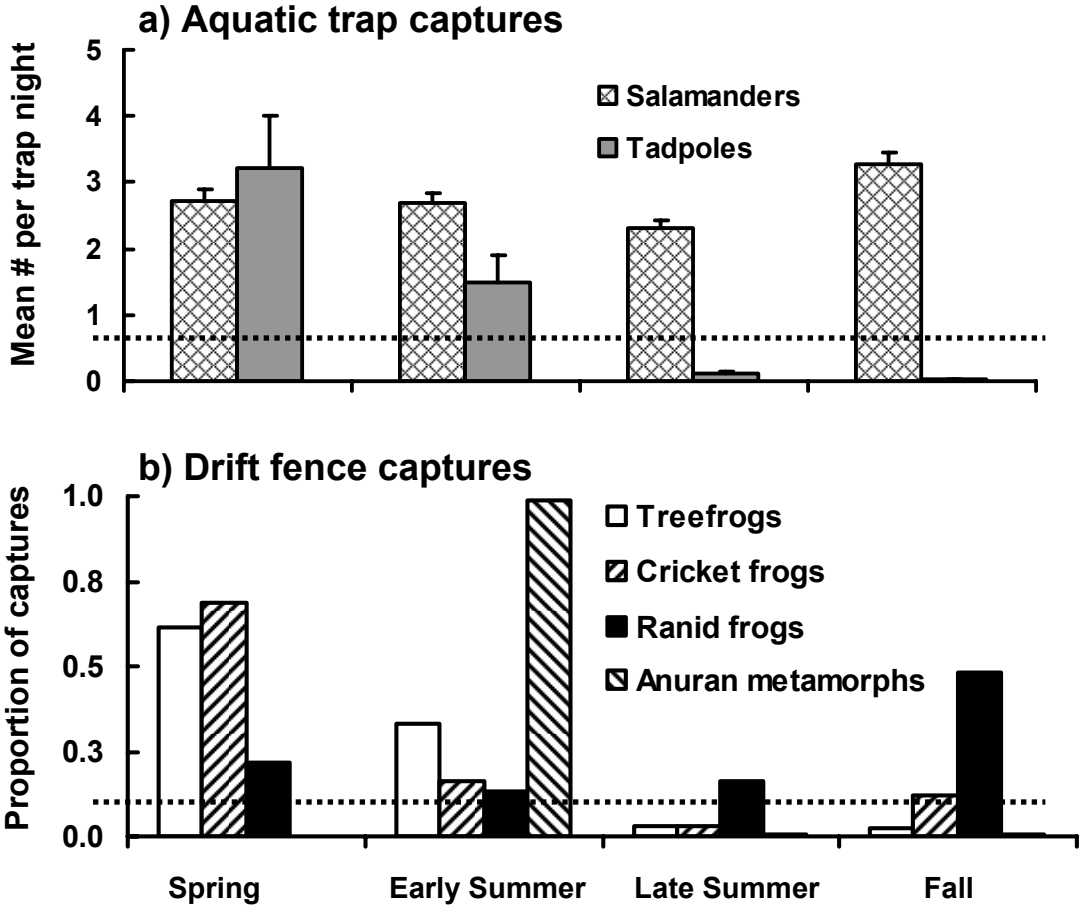

Figure 3.2 Seasonal availability of amphibian prey at Ellenton Bay including (a) fully aquatic groups (salamanders and tadpoles) and (b) migratory adult and metamorphic anurans. Bars in (b) represent the proportion of individuals within each prey taxon that were captured in a given season. Dashed lines represent thresholds above which prey were judged as available during a given season.

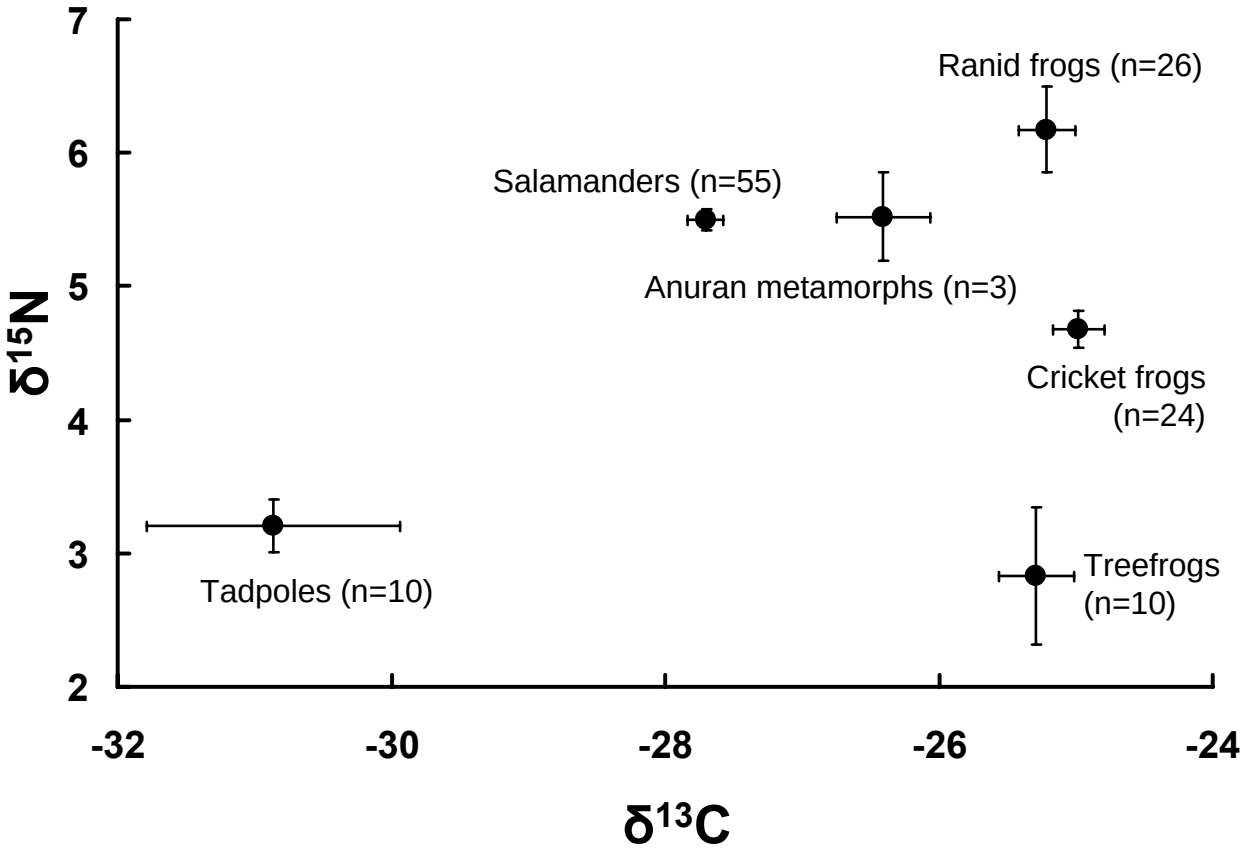

Figure 3.3 Average δ13C and δ15N values (± 1 SE) of amphibian prey captured at Ellenton Bay in 2005 and 2006. Prey were grouped into six functional groups based on similarity of isotopic composition and seasonal availability.

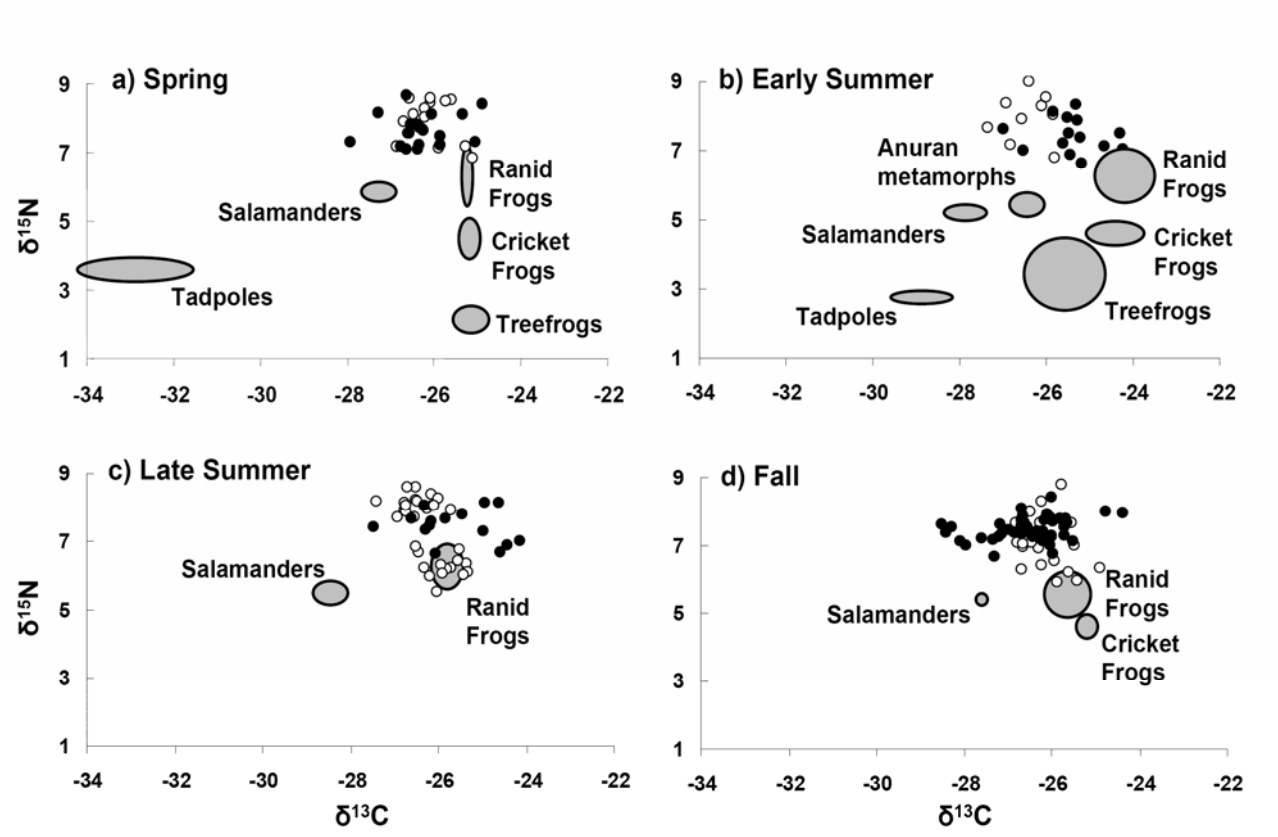

Figure 3.4 Seasonal shifts in isotopic composition of amphibian prey (shaded bubbles) and snakes (○ black swamp snakes, ● banded watersnakes) at Ellenton Bay. Shaded bubbles represent mean (± 1 SE) prey isotope values. Note that isotope values of prey groups are similar across seasons, thus shifts in overall seasonal isotope profiles are a result of shifts in availability, not shifts in isotope values within prey groups.

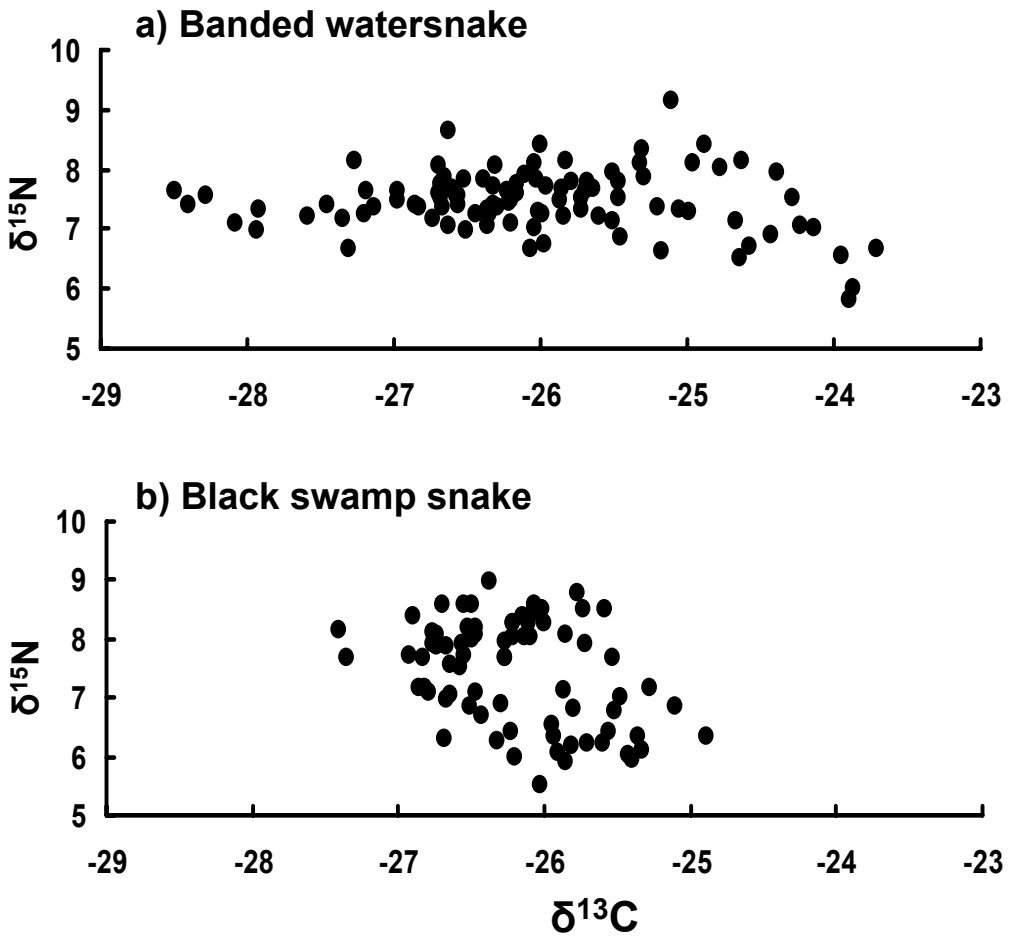

Figure 3.5 Overall trophic niche breadth of (a) banded watersnakes and (b) black swamp snakes at Ellenton Bay as indicated by carbon and nitrogen isotope composition.

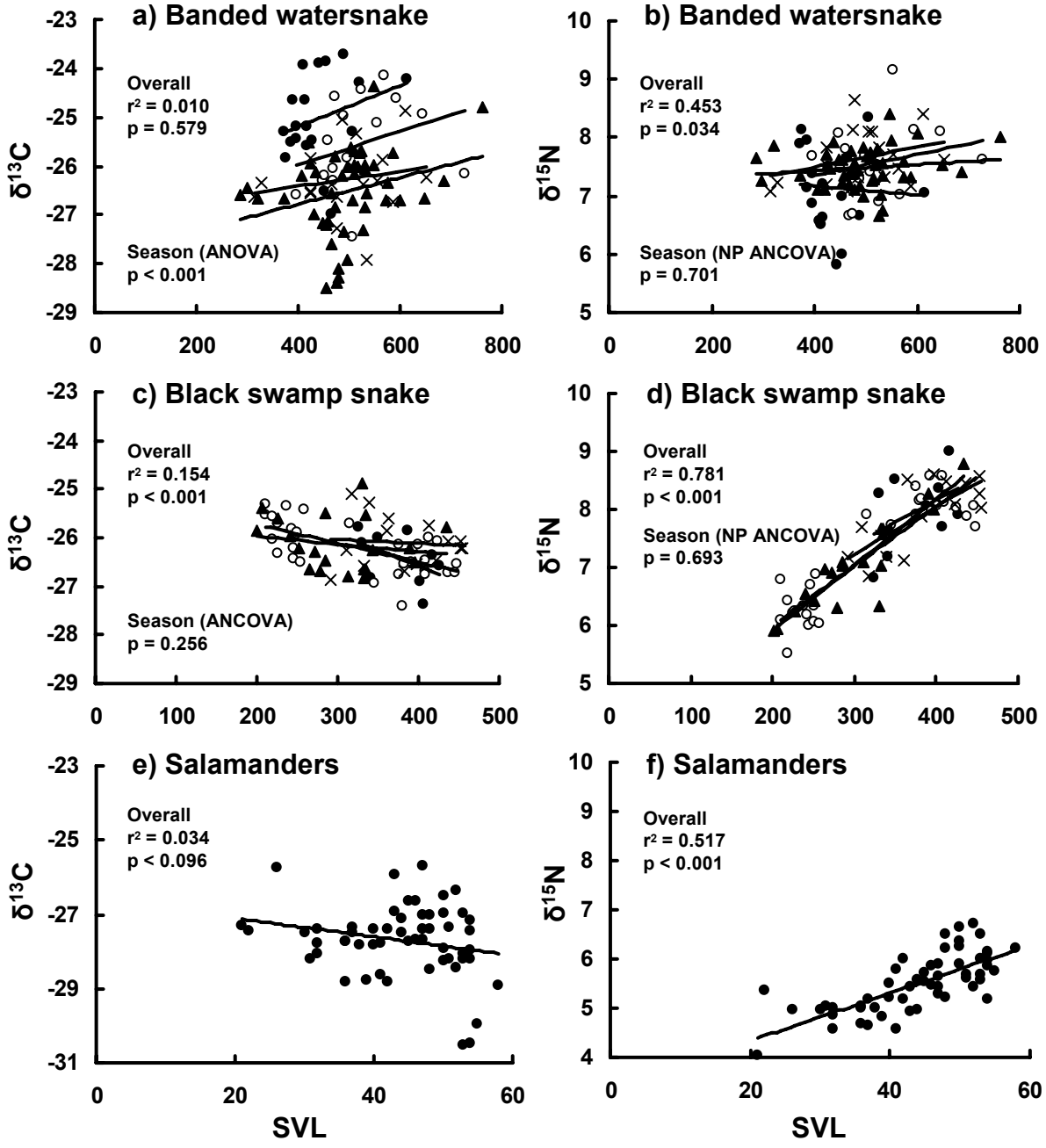

Figure 3.6 Ontogenetic and seasonal shifts in δ13C and δ15N values for banded watersnakes (a and b) and black swamp snakes (c and d). Symbols in a-d denote seasons: (X) spring, (●) early summer, (○) late summer, (▲) fall. Ontogenetic shifts in aquatic salamanders (e and f) are provided for comparison with black swamp snakes. Seasonal shifts in δ13C and δ15N values were evaluated using ANOVA, ANCOVA, or non-parametric ANCOVA (Scheirer-Ray-Hare test).

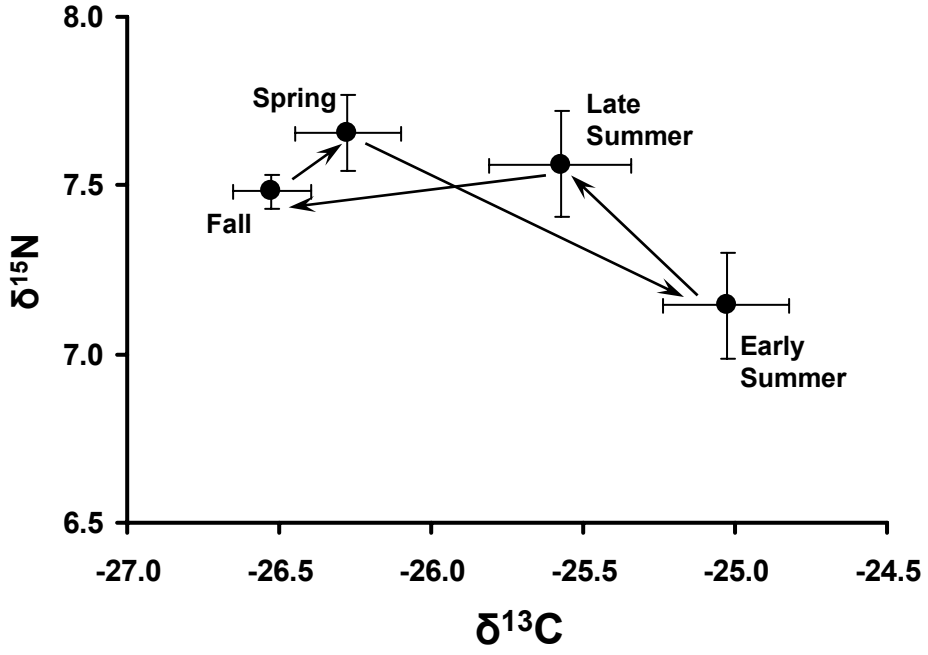

Figure 3.7 Seasonal shifts (arrows) in mean carbon and nitrogen isotope values (± 1 SE) for banded watersnakes at Ellenton Bay.

CHAPTER 4

THE PREDATORY ROLE OF AQUATIC SNAKES WITHIN AN ISOLATED WETLAND ECOSYSTEM: A CASE STUDY IN EVALUATING FUNCTIONAL ROLES OF CLANDESTINE SPECIES[3]

[3] Willson, J. D. and C. T. Winne. To be submitted to *Biological Conservation*.

INTRODUCTION

Recent recognition of the magnitude and diversity of effects that human activities are having on the environment has prompted increased interest in ecosystem ecology and particularly in understanding the role of species or functional groups within ecosystems (Grimm, 1995; Lyons and Schwartz, 2001; Vanni et al., 2002). Among functional groups of organisms, particular attention has been paid to predators, and their contributions to ecosystem function have been demonstrated in a variety of terrestrial and aquatic systems (e.g., Paine, 1966, 1969; Estes and Palmisano, 1974; Soulé et al., 1988; McLaren and Peterson, 1994). In many cases, predators have an important direct role in regulating prey populations (Erlinge et al., 1984; Hanski et al., 2001; Korpimaki et al., 2004; Sundell, 2006), and predators can indirectly influence abundance of lower trophic levels through trophic cascades (Pace et al., 1999; Polis et al., 2000; Schmitz et al., 2000). So-called "keystone" predators can play roles disproportionate to their abundance by maintaining biodiversity through preferential predation on competitively-superior prey taxa (Paine, 1966, 1969; Fauth, 1999). Although major groups of predatory vertebrates include mammals, birds, fish, amphibians, and reptiles, the roles of herpetofauna as predators remain largely unaddressed. Particularly, although all snakes are strictly carnivorous and many species are top predators within food webs (Greene, 1997), our knowledge of the roles of snakes within ecosystems is extremely limited.

Often, a necessary first step in evaluating the functional roles of species within ecosystems is to determine the abundance of each species or functional group. Thus, we have gleaned much of our knowledge of the effects of community structure on ecosystem processes from studies of species groups that are relatively easy to survey, such as grassland plants (e.g., Tilman et al., 1997) or sessile invertebrates (e.g., Paine, 1966, 1969; Pringle et al., 1999).

Conversely, the functional roles of secretive species remain largely unknown and in many cases infrequent observation prompts a perception of rarity and unimportance. Reptiles and amphibians are well-known for their cryptic habits (Gibbons et al., 2000; Mazerolle et al., 2007), leading some to refer to herpetofauna as "hidden biodiversity" (Gibbons, 1997). In recent years, however, field and analytical techniques have been developed that allow researchers to accurately estimate population density of species with low recapture probability (Dorcas and Willson, 2009; Willson et al., *in review-b*). For example, removal sampling has demonstrated that terrestrial reptiles and amphibians can occur at extraordinarily high densities (e.g., 52,800 lizards per ha, Rodda et al., 2001; 18,000 salamanders per ha, Petranka and Murray, 2001). Likewise, Kendall et al. (1997) developed statistical techniques for estimating rates of temporary emigration (individuals becoming unavailable for capture during some sampling intervals) based on data collected using Pollock's robust design (Pollock, 1982; Pollock et al., 1990). Bailey et al. (2004) used Kendall's methods to determine that during any given sampling interval 58 - 87% of terrestrial salamanders were unavailable for capture due to temporary use of subterranean habitats. Application of these techniques to amphibians has prompted a dramatic increase in the number of studies examining the role of amphibians as ecosystems components (e.g., Burton and Likens, 1975a; Davic and Welsh, 2004; Gibbons et al., 2006; Regester et al., 2006).

Among the terrestrial vertebrates, snakes are perhaps the most difficult large taxonomic group to study due to their cryptic behavior and low or sporadic activity patterns (Parker and Plummer, 1987, Dorcas and Willson, 2009). Although they are seldom conspicuous, snakes can exist at high densities compared to other terrestrial vertebrate predators (Pough, 1980). For example, aquatic snakes (e.g., striped crayfish snake, *Regina alleni,* and black swamp snake, *Seminatrix pygaea*) reach densities of > 1200 ha^{-1} in aquatic habitats in Florida (Godley, 1980),

and ringneck snakes (*Diadophis punctatus*) reach densities of > 1000 ha^{-1} in Kansas (Fitch, 1975). Even large-bodied terrestrial snakes can reach high densities in suitable habitat (e.g., 274 ha^{-1}; *Gloydius shedaoensis* – Shedao pit-viper; Sun et al., 2001). At such high densities, snakes may be among the most important predators in many ecosystems. However, our overall knowledge of the effects of snake predation on prey populations is minimal, at best (Nowak et al., 2008).

In this study we use aquatic snakes within an isolated, fishless wetland as a case study for examining the functional role of secretive predators within ecosystems. We use high-intensity sampling and advanced capture-recapture methodology to estimate population density of two species of aquatic snakes within Ellenton Bay, an isolated 5.4-ha wetland located in South Carolina, USA. We assess snake diets using direct examination of gut contents and stable isotope analyses. We then couple field growth rate measurements obtained from mark-recapture with data on mass conversion efficiency obtained in the laboratory to estimate prey consumption by snakes at Ellenton Bay over a one year period. Our results shed light on the important predatory role of snakes within this ecosystem and underscore the need to consider snakes in initiatives aimed at preserving overall ecosystem integrity.

METHODS

Study site

Ellenton Bay is an isolated Carolina bay freshwater wetland located on the Department of Energy's Savannah River Site (SRS) in the Upper Coastal Plain of South Carolina, USA. Ellenton Bay is characterized by shallow water (generally < 1 m deep) and relatively homogeneous expanses of emergent vegetation, including grasses (predominantly *Panicum* sp.),

water lilies (*Nymphaea odorata*), and water-shields (*Brasenia schreberi*). Although water levels are variable across years (Willson et al., 2006; Winne et al., 2006b), the bay typically holds water year-round and water levels remained relatively constant during the sampling period for this study (May 2005 – May 2006). The aquatic surface area of Ellenton Bay, measured in June 2006, was 5.37 ha. Due to periodic drying of the wetland during extreme droughts, Ellenton Bay is devoid of fish, crayfish, and large, permanently aquatic salamanders (*Siren* spp. and *Amphiuma means*), providing a relatively simple food web dominated by amphibians and reptiles. Importantly, Ellenton Bay is isolated from other wetlands by an extensive matrix of forested upland habitats, and represents a well-defined population of aquatic snakes (Willson et al., *in review-a*).

Study species

Although seven species of semiaquatic snakes are present at Ellenton Bay, two species dominate the community (94% of snake captures in 2005 and 2006): the banded watersnake (*Nerodia fasciata*) and black swamp snake (*Seminatrix pygaea*). *Nerodia fasciata* is larger (maximum total length 1588 mm; Gibbons and Dorcas, 2004) and is abundant and ubiquitous in the southeastern US, occurring in virtually all aquatic habitats. *Seminatrix pygaea* is considered the smallest semi-aquatic snake in North America (maximum total length 555 mm; Gibbons and Dorcas, 2004) and is endemic to the southeastern Coastal Plain from North Carolina to southern Alabama. Because *S. pygaea* is highly aquatic, seldom leaving aquatic habitats or basking above water (Gibbons and Dorcas, 2004; Winne et al., 2006b), it is seldom seen and its abundance and status across much of its range is largely unknown. Both *S. pygaea* and *N. fasciata* feed primarily on amphibians at Ellenton Bay, with *S. pygaea* specializing on paedomorphic mole salamanders, *Ambystoma talpoideum* (Willson et al., *in review-a*). Both species are viviparous and give birth

in July and August in the region. Moreover, when the wetland holds water, virtually all mature females of both species are reproductive (Winne et al., 2006b; unpublished data), negating the need to separate adult females into reproductive and non-reproductive groups.

Snake sampling

We captured snakes at Ellenton Bay over one year from May 2005 to May 2006. Within this period, we designed a temporal pattern of sampling to fit Pollock's (1982) robust design (Pollock et al., 1990). Robust design analysis requires widely-spaced primary sampling intervals, each consisting of a series of short secondary samples, across which population closure is assumed. To conform to this framework, we sampled snakes monthly (primary samples) from May–September 2005 for 10 consecutive days (secondary samples), and for 6 consecutive days in the additional primary periods of October 2005, March 2006, and April 2006.

We captured snakes using plastic aquatic funnel traps (i.e., minnow traps; model 700; N.A.S Incorporated, Marblehead, Ohio), which is the most effective method for capturing these species (Willson et al., 2005, 2008). During each sampling period, we set 465 traps, spaced approximately 2 m apart in a continuous transect around the aquatic periphery of the wetland. Although traps were not intentionally baited, incidental captures of aquatic prey resulted in 'natural baiting' of traps (Seigel et al., 1995; Winne, 2005). We checked traps daily in the early morning and recorded each snake's capture location to within 10 m.

Following capture, we recorded sex (by inspection of tail morphology and/or cloacal probing), snout-vent length (SVL to the nearest mm), tail length, and body mass (to the nearest 0.01 g using an electronic balance) for each snake captured. We identified recently-ingested prey items by forced regurgitation (Fitch, 1987) up to once per year per individual snake. We excluded from all analyses the mass for any snake with palpable prey that was not regurgitated

(i.e., snake body masses that were inflated due to prey). We then marked each snake with a unique code by branding ventral scales (Winne et al., 2006a) before releasing it at its capture location on the day of capture.

Estimating the predatory role of snakes

We estimated the functional role of snakes at Ellenton Bay in terms of prey consumption using data on snake density, diet, growth rate, and mass conversion efficiency. Because male and female snakes differed in body sizes, growth rates, and capture probability parameters, we divided snakes into three demographic groups per species for all analyses: males, females, and juveniles. *Nerodia fasciata* grow rapidly at this site, often reaching mature size in their second summer (Winne, 2008), therefore, the juvenile group for *N. fasciata* included only young born during the study (2005 young-of-year). *Seminatrix pygaea* are too small at birth to capture in aquatic traps (Willson et al., 2008) and are likely too small to contribute appreciably to overall vertebrate prey consumption. Thus, the juvenile group for *S. pygaea* included individuals from the size at which they become catchable in traps (ca. 200 mm SVL, 5 g; Willson et al., 2008) to the minimum size recorded for a reproductive female in this population (259 mm SVL). We assessed snake population sizes, diets, and growth rates on a seasonal basis, by subdividing the year into four biologically-relevant periods – late spring (May – June), summer (July – August), fall / winter (September – February), and early spring (March – April).

Snake density

We used the robust design format in Program MARK (White and Burnham, 1999) to construct and evaluate mark-recapture models. Robust design uses a combination of open and closed population models to estimate population size and other parameters with minimal violation of model assumptions (Pollock et al., 1990; Bailey et al., 2004). In a previous study, we

systematically evaluated factors influencing snake capture probability parameters using this dataset (Willson et al., *in review-b*). Here, we based our population estimation procedure on models that garnered the most support in that study. For both species, capture and recapture probabilities were independent (allowing for a behavioral response to traps) and were allowed to vary over time across primary samples. Based on the results of Willson et al. (*in review–b*), capture and recapture probabilities were set equal for male and female *N. fasciata* and for all demographic groups of *S. pygaea*. However, because unpublished models showed strong support for low capture and recapture probabilities in juvenile *N. fasciata*, we allowed these parameters to vary from adult parameters in this study. We held survivorship constant over time but allowed it to vary between demographic groups. As there was little support for random temporary emigration in *N. fasciata* (Willson et al., *in review-b*), we fixed the temporary emigration parameter at zero. For *S. pygaea*, however, we allowed constant random temporary emigration that differed between groups. To avoid nonsensical population estimates from months with few recaptures, we estimated population size for both species across each of the four seasons examined in this study (i.e., we held population size constant across months within seasons). Additionally, because neonate *N. fasciata* had particularly low recapture rates, we estimated their population size over only one interval, fall – spring. Because *S. pygaea* exhibited temporary emigration, we estimated the total population size (superpopulation size, *sensu* Kendall et al., 1997; Bailey et al., 2004) for each demographic group of *S. pygaea* at Ellenton Bay during each season ($N_{super,\ season}$) by correcting our population estimate (sampled population, $N_{sampled,season}$) for temporary emigration (TE_{season}) using the formula:

$$N_{super,season} = \frac{N_{sampled,season}}{\left(1 - TE_{season}\right)}$$

Snake diet

We evaluated diet composition of *N. fasciata* and *S. pygaea* at Ellenton Bay in 2005 – 2006 directly using gut contents manually regurgitated from snakes. We corrected diet proportions for prey mass by multiplying the number of prey items (grouped by genus or life-stage: *Ambystoma*, adult *Hyla*, adult *Rana*, *Acris*, newly-metamorphosed *Rana*, and tadpoles) recorded in snake's diets by the mean mass of prey within each group (generated from a database of 530 prey collected systematically at Ellenton Bay throughout the duration of the study). Subsequently, prey genera were lumped into functional groups (*A. talpoideum*, post-metamorphic anurans, and tadpoles) to ease visualization in figures. Our assessment of diet is supported by published studies that used stable isotope analyses to assess diet in this population (Willson et al., *in review-a*).

Snake growth and mass conversion efficiency

We calculated seasonal, group-specific growth rates for *N. fasciata* and *S. pygaea* at Ellenton Bay in 2005 – 2006 from individual mark-recapture data. We calculated growth (mass) for each individual snake for which we had an accurate mass (i.e., a mass without recently ingested prey) for at least two primary periods (months) within a season. We calculated growth rate by subtracting the latter mass from the earlier mass and dividing the result by the number of days elapsed between captures. Because captures of *S. pygaea* were very low in March we assessed growth in this species over only three intervals (late spring, summer, and fall – early spring). Due to females giving birth or snakes losing some mass over winter, snake demographic groups occasionally exhibited negative seasonal growth rates. In such cases, we set growth rate at zero for the season, rather than using a negative prey consumption value.

We determined mass conversion effeciency of *N. fasciata* feeding on amphibians in the laboratory. We used 21 captive-born *N. fasciata* (from mothers collected from uncontaminated sites on the SRS), representing a variety of ages (up to 2.5 yrs. old) and sizes (8.03 – 68.26 g) fed a diet of frozen *A. talpoideum* collected from Ellenton Bay. Snakes were maintained communally in 75.7 L aquaria, lined with paper towels, and provided with large water bowls, hide boxes, and basking lamps (12L:12D photoperiod) to provide thermal gradients within cages. Every 6 d snakes were offered 35% of their body mass in *A. talpoideum*. After 48 d, snakes were fasted to ensure post-absorptivity (6 d) and massed. Mass conversion effeciency was determined by dividing the mass gained over the course of the experiment by the cumulative wet mass of prey consumed. We tested for potential confounding effects of body size (starting mass) or feeding frequency (# of meals consumed and total mass consumed) on mass conversion efficiency using linear regressions and effects of sex on mass conversion efficiency using analysis of covariance (ANCOVA; independent variable = sex, dependent variable = mass gained, covariate = mass consumed, one individual excluded because sex was not recorded). Data were log transformed when statistical assumptions were not met and we recognized statistical significance at $P \leq 0.05$. We performed all statistical analyses using STATISTICA for Windows software package (StatSoft, Inc. Tulsa, OK, USA 1998). Because *S. pygaea* often refused to eat previously frozen prey, we did not determine mass conversion for this species. Instead, we assumed that mass conversion efficiencies were the same for both species. Likewise, as *A. talpoideum* comprised the vast majority of the diet of both species (see results) we used mass conversion effeciency values for *A. talpoideum* to assess consumption of other amphibian prey types (anurans and tadpoles). If invalid, these assumptions had minimal effect our results, given the dominance of *A.*

talpoideum in snakes' diets, the close taxonomic affinity of all prey types, and relatively minor contribution of *S. pygaea* to overall prey consumption.

Prey consumption by snakes

We calculated prey consumption for each snake demographic group within each season using the formula:

$$\text{Prey consumed (g group}^{-1}\text{ season}^{-1}) = \frac{G_{t,group} \times N_{t,group}}{MCE} \times \text{days in season}$$

where:

$N_{t,group}$ = population size during each season

$G_{t,group}$ = mean seasonal growth rate (g snake^{-1} day^{-1})

MCE = mass conversion efficiency (g gained g consumed^{-1})

We then summed prey consumption across seasons and demographic groups to calculate total mass of prey consumed by snakes at Ellenton Bay during the one-year study period. We converted prey mass into energetic equivalents using published values of energy content for *A. talpoideum* (4.06 kJ/g wet mass; Willson and Hopkins, *in review*). We subdivided prey consumption among prey taxa by multiplying the total prey mass consumed within each season by the proportion of diet (by mass) represented by each prey taxon within that season. We then divided the total mass of each prey taxon consumed by the mean mass of prey within that group to estimate the number of individual prey consumed.

RESULTS

Snake sampling

Sixty-eight days of sampling yielded 1,571 captures of 528 individual *N. fasciata* and 1,286 captures of 495 individual *S. pygaea* (Fig. 4.1). Both species exhibited seasonal shifts in

activity with the highest rate of captures in August and September and relatively few captures in October and March. Recapture rates increased rapidly in *N. fasciata* over the first three months of study, reaching 87% in July. However, recapture rates dropped again in the late summer, due to birth of neonates in August. Captures of *S. pygaea* were more strongly seasonal than those of *N. fasciata*, with very few captures in October, March and April. Although recapture rates also increased through 2005 in *S. pygaea*, reaching a maximum of 75% in September, a similar number of novel individuals were captured in each period from June to September. This pattern likely reflects a relatively constant influx of juvenile recruits as they grow large enough to become catchable in aquatic traps (Willson et al., 2008).

Snake density

Despite strong seasonal variation in numbers of snakes captured (Fig. 4.2), estimated population sizes for adult *N. fasciata* and *S. pygaea* were strikingly constant over time (Fig. 4.3). Population size estimates of adult *N. fasciata* ranged from 220 – 270 individuals across seasons, whereas those for adult *S. pygaea* ranged from 214 – 322 individuals. Total estimated population size of *N. fasciata* nearly doubled in the fall, when females gave birth and an estimated 222 neonates were added to the local population. Population size of juvenile *S. pygaea* was also more variable than that of adults, with a peak of 109 individuals in summer. Peak total population sizes of both species occurred in the fall, at which time an estimated 919 snakes inhabited Ellenton Bay, corresponding to a population density of 171 snakes ha^{-1} of wetland habitat. The mean standing biomass of snakes at Ellenton Bay in 2005 – 2006 was 7.77 kg ha^{-1}.

Snake diet

Our sampling yielded a total of 1,504 and 518 diet items for *N. fasciata* and *S. pygaea*, respectively. The diet of both species was dominated by larval and paedomorphic *A. talpoideum*

(Fig. 4.4a). *Seminatrix pygaea*, particularly, specialized on *A. talpoideum*, with only 7 diet records representing other taxa (*Acris* and newly-metamorphosed *Rana* and *Hyla*). *Nerodia fasciata*, conversely, included tadpoles and post-metamorphic anurans in their diet in somewhat higher numbers (91 and 47 records, respectively). The inclusion of these alternative prey taxa in the diet of *N. fasciata* was seasonal, with most anurans and tadpoles taken in the spring (Fig. 4.4b). Our assessment of snake diet is supported by comparisons of stable isotope composition of snakes and prey at Ellenton Bay (Willson et al., *in review-a*). Stable isotope profiles suggested that both *N. fasciata* and *S. pygaea* feed largely on *A. talpoideum* but that *N. fasciata* show seasonal diet shifts, feeding on seasonally-available adult anurans and tadpoles in the spring.

Snake growth and mass conversion efficiency

Nerodia fasciata showed strong seasonal variation in growth but adult females grew faster than adult males and juveniles in all seasons except fall/winter (Fig. 4.5a). Growth rates were highest (approaching 1 g mass gain per day in females) in late spring and were also high for females in early spring. Growth over the winter (Sept – March) was low in all groups. Due to their smaller body size, *S. pygaea* exhibited much lower absolute growth rates than *N. fasciata* (Fig. 4.5b). Female *S. pygaea* grew rapidly from fall through late spring, but lost mass in summer, due to parturition. Juvenile and adult male *S. pygaea* exhibited slow growth in spring and summer and negligible growth over the winter.

On average, *N. fasciata* gained 0.16 ± 0.01 g of body mass per g (wet mass) of *A. talpoideum* consumed in the laboratory. We detected no confounding effects of body size (Linear Regression; $R^2 = 0.03$, $P = 0.21$), sex (ANCOVA; $F_{1,17} = 1.02$, $P = 0.33$), number of meals consumed (Linear Regression; $R^2 = 0.02$, $P = 0.58$), or total mass consumed (Linear Regression;

$R^2 = 0.09$, $P = 0.13$) on mass conversion efficiency. Thus, we used 0.16 as our mass conversion efficiency value for all snakes in the study.

Prey consumption by snakes

We estimated that snakes consumed 213.35 kg of amphibian prey at Ellenton Bay from May 2005 – April 2006 (Table 4.1). Prey consumption was highest in the late spring, primarily as a result of high consumption rates by female *N. fasciata* during this season. Generally, *N. fasciata* contributed much more substantially to total prey consumption than *S. pygaea,* and females consumed more prey biomass than males in both species. When translated into energetic equivalents, we estimated that snakes consumed a total of 866,186 kJ of amphibian prey at Ellenton Bay over one year. This represents 161,301 kJ ha^{-1} of energy flow from amphibians into aquatic snakes within the Ellenton Bay food web.

When examined taxonomically, larval and paedomorphic *A. talpoideum* accounted for the majority (90%) of individual prey consumed, and we estimated that together *N. fasciata* and *S. pygaea* consumed a total of over 53,000 individual *A. talpoideum* over the course of the year (Table 4.2). Although they represented a relatively minor component of the diet of both snake species, tadpoles and adult anurans were also consumed in large numbers: 3,493 and 1,777 individuals, respectively.

DISCUSSION

Because snakes are often encountered with a frequency that belies their true abundance they have historically been undervalued as ecosystem components. For example, in the 1953 edition of the Golden Guide to Reptiles and Amphibians, Zim and Smith (1953) stated: “as a group they [reptiles] are neither ‘good’ nor ‘bad,’ but are interesting and unusual, although of

minor importance. If they should all disappear, it would not make much difference one way or the other." Nearly all snakes are incredibly secretive, which not only leads to the perception that they are rare, and perhaps of minor importance, but also impedes attempts to quantify snake density, a necessary first step in examining their functional roles. In many situations, snake captures are extremely low, sometimes prompting researches to resort to unstandardized or haphazard sampling methods (Dorcas and Willson, 2009). Even when sampling is standardized, counts, capture rates, or other indices of relative abundance are often poor indicators of snake density. For example, research on brown treesnakes (*Boiga irregularis*) has found no correlation between visual counts and population density estimated by mark-recapture (Rodda et al., 2005) and has demonstrated that snake surveys may suffer from observer bias (Rodda, 1993). Further, snakes have been shown to exhibit time and group (e.g., sex or age) varying capture and recapture probabilities, behavioral responses to traps (i.e., "trap-happiness" or "trap-shyness"), and temporary emigration (Tyrrell et al., *in press*; Willson et al., *in review-b*), all of which can bias estimates of population size. Due in part to the difficulties associated with accurately measuring snake density, the role that snakes play as predators in most ecosystems remains unknown.

Perhaps the most dramatic evidence of the potential impacts of snake predation arises from situations where non-native snakes have been introduced into naive ecosystems. For example, the accidental introduction of *B. irregularis* into the island nation of Guam has had catastrophic effects on native birds, bats, and lizards, driving several species to extinction and leaving large portions of the island devoid of native vertebrate fauna, save a few small lizard species (Fritts and Rodda, 1998; Rodda and Savage, 2007). Many cases where native snake abundance or prey consumption has been quantified are difficult to interpret because the area

occupied by foraging snakes is unknown. For example, although many snakes are easily captured as they enter and exit communal hibernacula (e.g., Gregory, 1984; Diller and Wallace, 2002; Weatherhead et al., 2002; Shine and Mason, 2004), it is difficult to translate den-based population estimates into snake densities across the surrounding landscape. In an analogous example, Ineich et al. (2007) estimated that approximately 1,400 sea kraits (*Laticauda laticaudata* and *L. saintgironsi*) which congregated at on 6-ha islet in the South Pacific consumed over 36,000 eels (972 kg) from surrounding reefs annually. Although their results clearly show that snakes are important predators in coral reef ecosystems, this predation pressure may have been spread over as much as 130 ha of surrounding coral reef habitat (Ineich et al., 2007). Conversely, in his classic study of the foraging ecology of the striped swamp snake (*Regina alleni*), Godley (1980) used systematic sampling to estimate snake density and prey consumption over a quantified area within an unusually uniform habitat: mats of exotic water hyacinth (*Eichhorina crassipes*) in Florida. Godley (1980) employed a dredging method to systematically sample *R. alleni* and their primary prey, crayfish and odonate naiads. Godley (1980) calculated that the mean standing crop of *R. alleni* was 30.79 kg ha^{-1} (1289 snakes ha^{-1}) and using data on diet, foraging rate, and prey density, estimated that snakes consumed 9.6% and 90.7% of the standing biomass of crayfish and odonate naiads, respectively, during the fall, when snake feeding rates were highest.

Using intensive, systematic sampling and advanced mark-recapture analyses, we estimated that, at peak density, 171 snakes ha^{-1} inhabited Ellenton Bay, corresponding to a mean standing biomass of 7.77 kg ha^{-1}. Further, we calculated that snakes at Ellenton Bay consumed over 200 kg of amphibians annually, translating into > 150,000 kJ ha^{-1} of energy flow from secondary to tertiary consumers within the food web. Ellenton Bay has been protected from

major anthropogenic disturbances for over five decades (Gibbons et al., 2006) and arguably ranks among the most pristine native wetlands where long-term herpetological research has been conducted. Thus, our data extend Godley's (1980) findings from highly-altered aquatic systems (exotic water hyacinth communities) by demonstrating that snakes also exist at high densities and are important predators in native aquatic ecosystems in the southeastern United States. An estimated 10,000 – 20,000 Carolina bay wetlands exist in the southeastern United States (Sharitz, 2003) and snakes likely serve similarly important roles in these and many other aquatic habitats throughout temperate and tropical regions of the world.

The high snakes densities we observed, and their important contributions to biomass and energy flow, may be due in part to characteristics of the amphibian prey community at Ellenton Bay. As ectotherms, amphibians are efficient at transfer of biomass and energy through food webs and occur at high densities in many ecosystems (Davic and Welsh, 2004). In an often cited example, Burton and Likens (1975a, 1975b) demonstrated that the average biomass of five species of salamanders was 1.77 kg ha^{-1} (2950 individuals ha^{-1}) in a New Hampshire forest. This biomass was more than twice that of birds during the breeding season and comparable to the biomass of small mammals. Fishless, often seasonal wetlands have received much attention as hotspots of amphibian biodiversity and productivity (Semlitch and Bodie, 1998; Gibbons et al., 2006). Further, aquatic salamanders may represent ideal prey for aquatic snakes because their elongate morphology allows snakes to consume large meals with minor impediments to post-prandial crawling speed (Willson & Hopkins, *in review*). Thus, it is likely that high abundances of amphibians, particularly *A. talpoideum*, are important in fueling high snake biomass within Ellenton Bay and other fishless wetlands.

Despite the fact that snakes at Ellenton Bay preyed primarily on *A. talpoideum*, we estimated that snakes also consumed nearly 1,800 anurans over the course of the year. Many of these anurans are primarily terrestrial as adults and are consumed by aquatic snakes only when they return to wetlands for short periods to breed (Todd and Winne, 2006; Willson et al., *in review-a*). Thus, we estimate that snakes are responsible for the transfer of over 15 kg of biomass, or 61,000 kJ of energy per year from surrounding uplands to Ellenton Bay. Because many amphibians are semi-aquatic and exhibit complex life histories they have been proposed to serve important roles in energy and nutrient flow between aquatic and terrestrial systems (Davic and Welsh, 2004; Gibbons et al., 2006; Regester et al., 2006). Our results demonstrate that snakes may also play a key role in linking terrestrial and aquatic habitats by sequestering biomass of migratory adult amphibians within wetlands.

The catastrophic global decline of amphibians has received much recent attention and studies have highlighted the important effects that loss of amphibians may inflict on ecosystems (Gibbons et al., 2006; Whiles et al., 2006). In fact, one hypothesized result of amphibian declines are subsequent declines of tertiary predators, including snakes (Jennings et al., 1992; Matthews et al., 2002; Whiles et al., 2006; Todd et al., *in press*). Although they have garnered less public attention, reptiles are also declining world-wide (Gibbons et al., 2000; Whitfield et al., 2007; Todd et al., *in press*). Our study demonstrates the important predatory role that snakes can play in some ecosystems and stresses the importance of questioning the assumption that infrequently encountered species are rare and of minor ecological importance.

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Table 4.1 Estimated consumption of amphibian prey by snakes at Ellenton Bay between May 2005 and May 2006. Prey consumption was calculated using mean seasonal growth rates for each snake demographic group, mass conversion efficiency, and population size estimates. Prey mass was converted into energetic equivalents using published values for energetic content of *Ambystoma talpoideum*.

	Prey consumption (kg)					
N. fasciata	**Late Spring**	**Summer**	**Fall / Winter**	**Early Spring**	**Total (kg)**	**Total (kJ)**
♂	29.89	13.72	9.69	8.19	61.49	249,655
♀	42.58	13.76	0.00	26.18	82.52	335,023
Juv	0.00	0.00	30.87	15.89	46.76	189,851
Total	72.47	27.48	40.56	50.26	190.77	774,529
S. pygaea						
♂	0.98	0.94	0.24	0.07	2.23	9,038
♀	4.91	0.00	9.22	3.35	17.48	70,966
Juv	1.10	1.77	0.00	0.00	2.87	11,652
Total	6.99	2.71	9.46	3.41	22.58	91,656
Grand Total	**79.46**	**30.19**	**50.02**	**53.68**	**213.35**	**866,186**

Table 4.2 Estimated distribution of prey consumption by snakes across prey taxonomic groups. Prey consumption (# of individuals) was calculated by multiplying total prey mass consumed within each season (see Table 4.1) by the proportion of diet (by mass) represented by each prey taxon within that season (see Fig. 4.4) and dividing the result by the mean mass of prey within each group.

	Prey consumption (individuals)		
N. fasciata	***Ambystoma***	**Tadpoles**	**Anurans**
Late Spring	18,979	668	487
Summer	7,171	0	284
Fall / Winter	11,383	24	107
Early Spring	9,859	2,801	766
Total	47,392	3,493	1,645
S. pygaea			
Late Spring	1,991	0	54
Summer	781	0	0
Fall / Winter	2,723	0	7
Early Spring	952	0	71
Total	6,446	0	132
Grand Total	**53,838**	**3,493**	**1,777**

Figure 4.1 Aquatic snakes are abundant at Ellenton Bay, though they are seldom seen during visual searches. Black Swamp Snakes (*Seminatrix pygaea*; pictured) are especially secretive and are virtually never seen by the casual observer. Photo by John D. Willson.

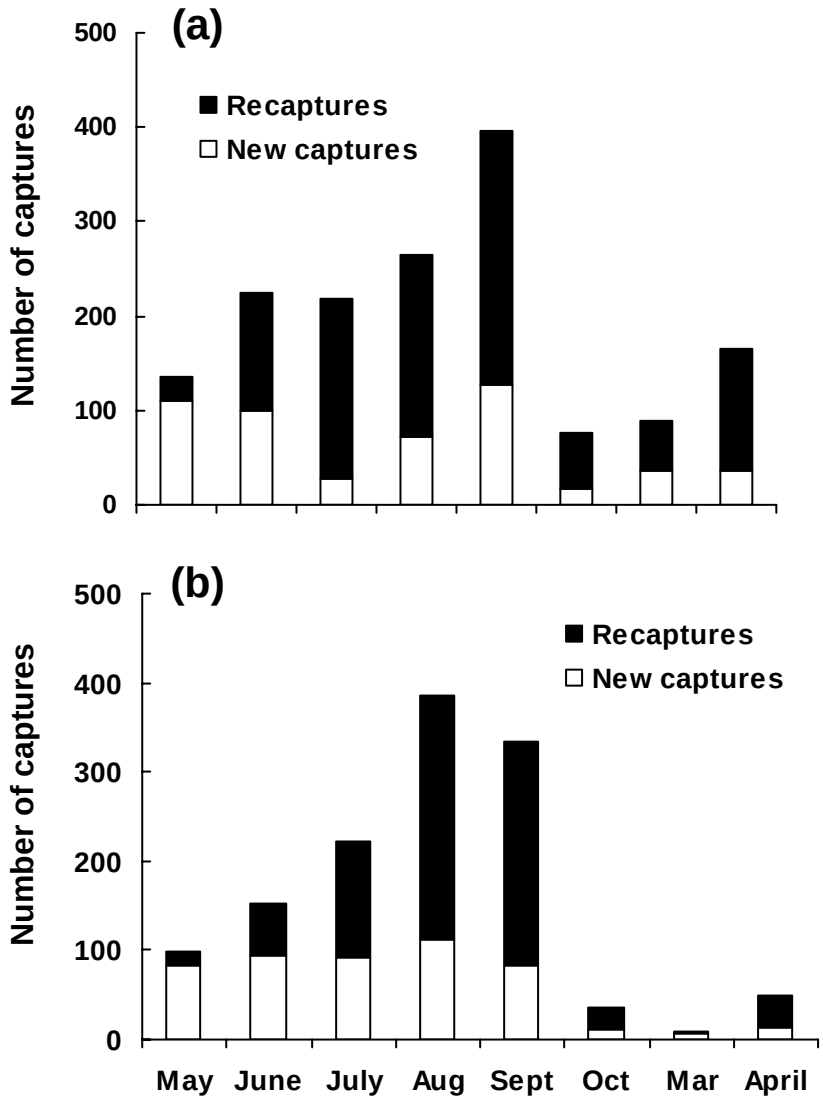

Figure 4.2 Seasonal distribution of captures of (a) *Nerodia fasciata* and (b) *Seminatrix pygaea* at Ellenton Bay from May 2005 to April 2006. During each season snakes were captured using 465 aquatic funnel traps (i.e., minnow traps) set in a continuous transect around the periphery of the wetland. Bars represent total captures over 10 consecutive days of trapping from May – September 2005 and 6 consecutive days of trapping in October 2005, April, and May 2006.

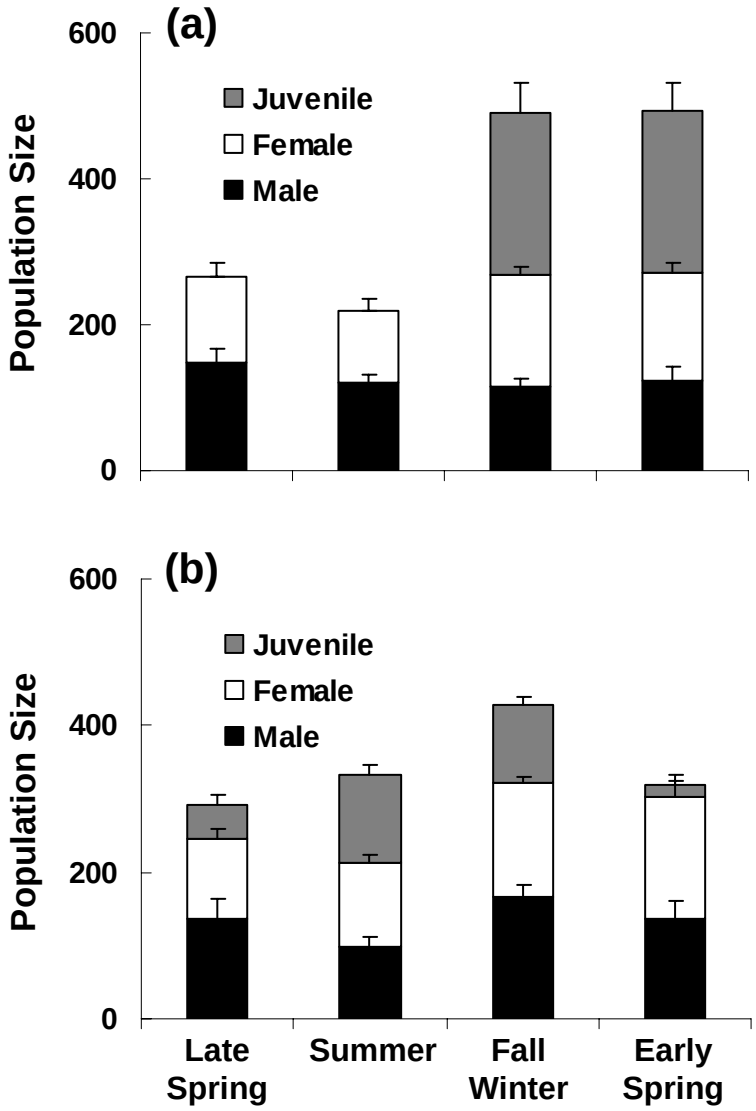

Figure 4.3 Population estimates (± 1 SE) of (a) *Nerodia fasciata* and (b) *Seminatrix pygaea* at Ellenton Bay from May 2005 to April 2006. Population sizes were estimated using robust design analysis in program MARK. Models included time-varying and independent (i.e., allowing for a behavioral response to traps) capture and recapture probabilities for both species and group-specific constant random temporary emigration for *S. pygaea*. Thus, population estimates for *S. pygaea* represent superpopulation size (i.e., population size corrected for temporary emigration).

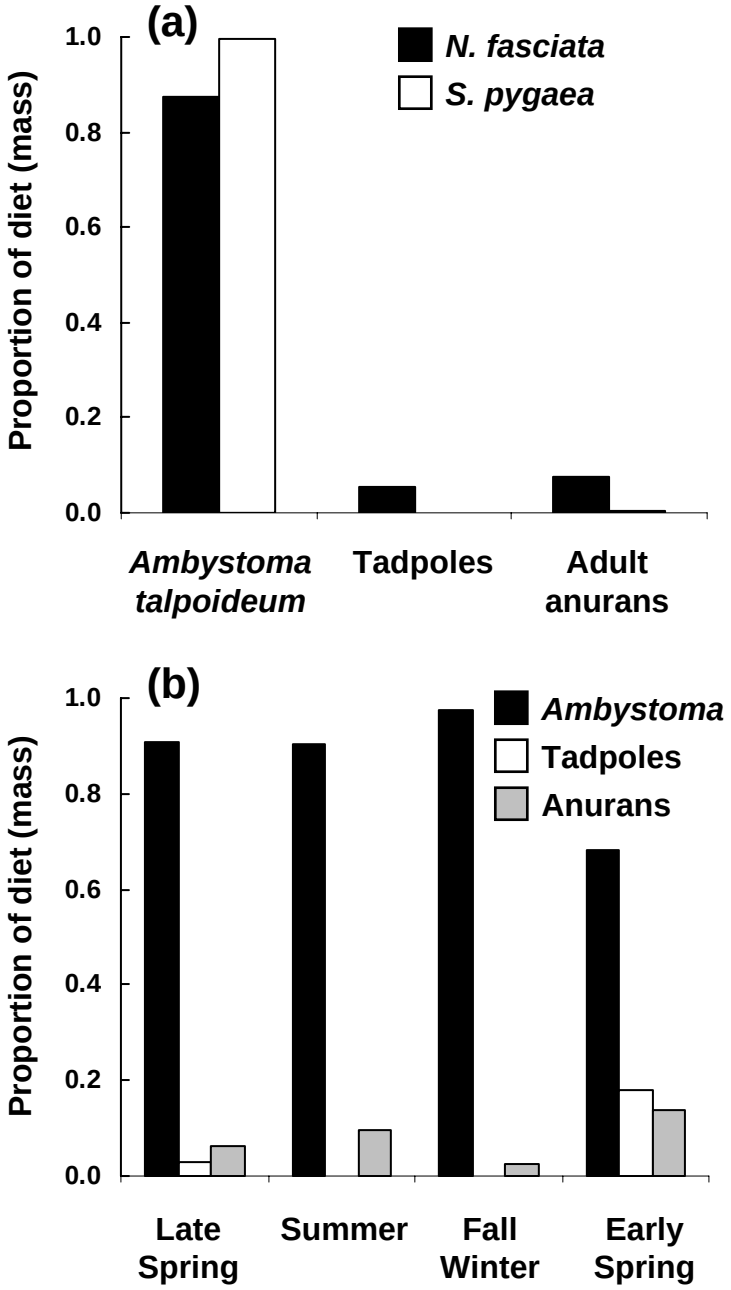

Figure 4.4 Diet composition of *Nerodia fasciata* and *Seminatrix pygaea* at Ellenton Bay from May 2005 to April 2006. Overall mass-corrected diet composition for *N. fasciata* and *S. pygaea* (a) was derived from a total of 1,504 and 518 diet items, respectively. Mass-corrected seasonal diet composition for *N. fasciata* (b) demonstrates seasonal diet shifts in this species.

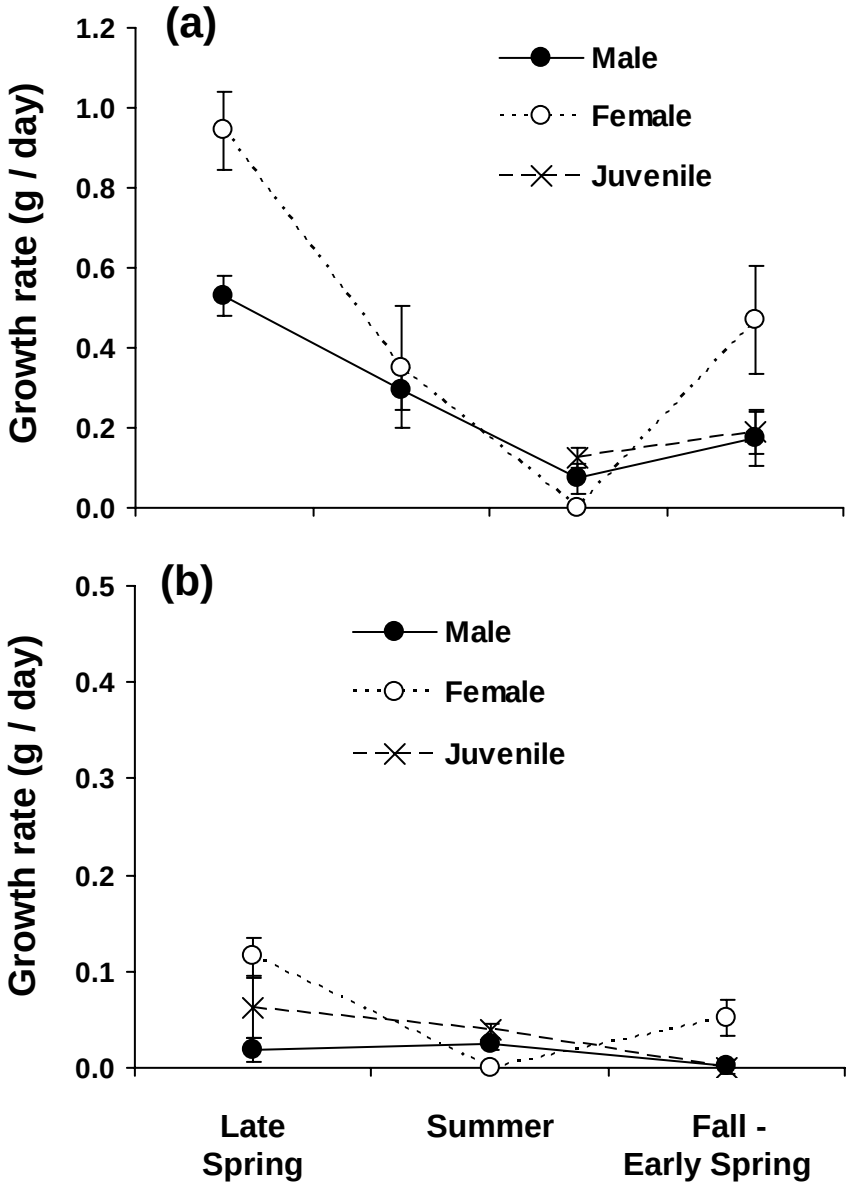

Figure 4.5 Mean (± 1 SE) seasonal growth rates of (a) *Nerodia fasciata* and (b) *Seminatrix pygaea* at Ellenton Bay from May 2005 to April 2006. In cases where snakes lost mass between capture intervals (due to females giving birth or snakes losing some mass over winter), growth rate was set at zero. Thus, if mass change was negative, we conservatively assumed no prey consumption rather than negative prey consumption.

CHAPTER 5

PREY MORPHOLOGY CONSTRAINS FEEDING ECOLOGY OF AN AQUATIC GENERALIST PREDATOR: IMPLICATIONS FOR WETLAND CONSERVATION [4]

[4] Willson, J. D. and W. A. Hopkins. To be submitted to *Ecological Monographs*.

INTRODUCTION

Classically, ecology seeks to understand factors that determine the distribution and abundance of organisms. Among the biotic and abiotic factors that can contribute to an organism's abundance, perhaps none is more obvious than the availability of suitable food resources (White 2008). In the extreme case of a dietary specialist, for example, a predator may be unable to persist outside of the geographic range of its specific prey. The simplest aspect of resource availability is prey abundance and many studies that use resource availability as an index of habitat quality focus exclusively on abundance (expressed numerically or in terms of biomass or volume) of potential prey taxa (e.g., Diaz and Carrascal 1991, Reaney and Whiting 2003, McCauley et al. 2006, Starr and Leung 2006). However, even for specialist species, a simple measurement of prey abundance can provide an inadequate assessment of true resource availability. For example, habitat structure or complexity can influence prey encounter or capture rates such that foraging success may vary among habitats with similar numerical prey abundances (Crowder and Cooper 1982, Mullin and Mushinsky 1997, Mullin and Cooper 2000). Assessing resource availability is further complicated for generalist species because prey taxa may differ in various aspects of quality, including nutritional composition and morphology. Nutritional composition of prey is easily measured and can have direct effects on a variety of fitness-related traits (Mayntz et al. 2003, Wilder and Rypstra 2008). For example, young Kittiwakes (*Rissa brevirostris*) fed low-lipid diets exhibit reduced growth and impaired learning abilities compared to those reared on high-lipid diets (Kitaysky et al. 2006). The consequences of variation in prey morphology, conversely, are generally poorly understood (Vincent et al. 2006).

Snakes provide an excellent model for studying the effects of prey morphology on feeding ecology. Historically, snakes have been underrepresented in the ecological literature, but

have recently gained appreciation, both as important ecosystem components (e.g., Godley 1980, Ineich et al. 2007) and as excellent subjects for investigating a broad range of ecological questions (Shine and Bonnet 2000, Dorcas and Willson 2009). All snakes are strict carnivores and are generally gape-limited in the size of prey that they can consume (Mushinsky 1987, Arnold 1993). Therefore, prey size and morphology ultimately determine both the maximum size of prey that can be consumed and the time and effort required for ingestion (Arnold 1993, Vincent et al. 2006). Moreover, snakes typically cannot adjust the size of potential prey by mastication and are forced to consume all components of each prey item, regardless of nutritional quality or digestibility. Because snakes lack limbs, they rely solely on their jaws and body musculature for both prey handling and locomotion (Cundall 1987). Therefore, the added burden of a large meal can hinder snake locomotor performance (Garland and Arnold 1983, Ford and Shuttlesworth 1986, Shine and Shetty 2001). For example, Mehta (2006) found that hatchling trinket snakes (*Elaphe helena*) fed large prey (> 50% of snake body mass) exhibited severely reduced maximum sprint speeds and exhausted quickly. Additionally, large meal sizes were associated with a switch from active to passive or cryptic defensive behaviors, presumably to compensate for locomotor impairment (Mehta 2006). Thus, snakes provide an excellent opportunity to evaluate the effects of prey morphology on various aspects of feeding ecology, physiology, and post-prandial locomotor performance.

The banded watersnake (*Nerodia fasciata* Linnaeus) is virtually ubiquitous in the southeastern United States, occurring in most aquatic habitats from temporary ditches to large lakes and rivers (Gibbons and Dorcas 2004). *Nerodia fasciata* is generally characterized as an opportunistic feeder and is known to consume a variety of aquatic vertebrate prey, with a particular preference for fish and amphibians (Mushinsky and Hebrard 1977, Gibbons and

Dorcas 2004). Paradoxically, in South Carolina *N. fasciata* are most abundant in wetlands that dry periodically, and consequently lack fish (JDW unpublished data), despite the fact that they experience precipitous declines in these habitats during periodic extreme droughts (Seigel, Gibbons and Lynch 1995, Willson et al. 2006). However, following drought-induced population declines, *N. fasciata* populations rebound rapidly, driven by high fecundity of surviving females and subsequent rapid growth to maturity of juveniles (Winne 2008). Presumably, this rapid recovery is fueled by characteristics of amphibian prey within fishless wetlands. The remarkable amphibian productivity of these habitats has been recognized, particularly following prolonged droughts (Gibbons et al. 2006). However, it is unknown whether other characteristics of amphibian prey, such as nutritional composition or morphology, may facilitate rapid growth of juvenile watersnakes and subsequent population recovery within ephemeral wetland habitats following drought.

In this study we investigated how morphological characteristics of dominant prey may constrain the feeding ecology of a generalist predator and therefore influence overall habitat quality for this species. We assessed prey available to juvenile *N. fasciata* in permanent and isolated, periodically-dry wetlands and compared quality of the dominant prey taxa from each wetland type, paedomorphic salamanders (*Ambystoma talpoideum*) and sunfish (*Lepomis marginatus*), respectively. Specifically, we compared nutritional composition and morphology of each prey taxon and used a series of repeated-measures experiments to examine prey handling (number of prey consumed, maximum prey size, and handling time), digestive metabolism (specific dynamic action, SDA), and post-prandial locomotor performance (maximum crawling speed) of snakes fed *Ambystoma* and *Lepomis*. We predicted that differences in prey morphology would constrain watersnakes' feeding and locomotor performance. Because *Ambystoma* are

more elongate and contain fewer impediments to handling and digestion (e.g., scales and spiny fin rays), we predicted that snakes would be able to consume larger *Ambystoma* than *Lepomis* and ingest *Ambystoma* more rapidly. Further, we expected that the large amounts of bony material present in *Lepomis* would result in a higher cost of digestion (SDA response) for *Lepomis* than *Ambystoma*. Finally, we expected that ingestion of *Lepomis* would induce greater body distension than ingestion of *Ambystoma*, resulting in increased post-prandial locomotor impairment (reduced maximum crawling speed) in snakes that consumed *Lepomis* compared to those that consumed *Ambystoma*.

METHODS

Experimental subjects

The banded watersnake (*Nerodia fasciata*) is a common, moderately large (maximum total length 1588 mm, Gibbons and Dorcas 2004) semi-aquatic snake that is found in a variety of aquatic habitats throughout its range in the southeastern United States (Gibbons and Dorcas 2004). Among snakes, this species is one of the most heavily–studied, including studies of diet (Mushinsky and Hebrard 1977, Kofron 1978, Mushinsky, Hebrard and Vodopich 1982), feeding morphology (Vincent et al. 2006, 2007), locomotor performance (Hopkins and Winne 2006), ecotoxicology (Hopkins et al. 1999, Hopkins et al. 2002, Hopkins and Winne 2006), and digestive physiology (Hopkins et al. 2004). To obtain experimental subjects, pregnant female *N. fasciata* were captured in aquatic funnel traps (i.e., minnow traps; Keck 1994, Willson et al. 2008) at various uncontaminated aquatic habitats on the U.S. Department of Energy's Savannah River Site (SRS), in South Carolina, USA in June and July 2005 and maintained in the laboratory until parturition (see Hopkins et al. 2005 and Hopkins and Winne 2006 for general housing

details). Following parturition, neonates were individually marked by ventral branding (Winne et al. 2006) and were maintained communally in 75.7 L aquaria between experiments. Cages were lined with paper towels and were equipped with large water bowls, hide boxes, and basking lamps (12L:12D photoperiod) to provide thermal gradients within cages. Snakes were offered *ad libitum* food [fish (*Gambusia holbrooki* and *Lepomis* spp.) or amphibian larvae (*Rana* spp. tadpoles and *Ambystoma talpoideum* larvae)] approximately once per week between experiments. To minimize the effects of prior feeding experience on our results we ensured that all snakes had previously consumed both prey types used in our experiments.

Prey availability and snake abundance

Between 30 May and 20 July, 2006, 16 wetlands on the SRS were sampled to characterize prey communities in permanent and isolated, periodically dry wetlands. Wetlands were selected to meet the following criteria: 1) were non-forested, 2) typically held water year-round, 3) had abundant emergent and sub-surface vegetation, and 4) were known to harbor populations of *N. fasciata*. Half (8) of the wetlands selected either had not dried within 20 yrs (based on long-term hydrologic surveys) or were occasionally connected to permanent water bodies. The remaining wetlands (n = 8) dried during severe droughts and were isolated from other water bodies. The functional consequence of these differences in hydrology was that permanent wetlands contained fish, whereas isolated, periodically dry wetlands contained only amphibians and invertebrates.

Each wetland was trapped for one night with 30 – 150 (depending on wetland area) cylindrical plastic minnow traps (model 700, N.A.S. Incorporated, Marblehead, Ohio) to assess prey availability. Traps were set in shallow water with the funnels submerged and were not baited. After 24 h, traps were checked and all fish and amphibians were identified to species and

counted. As our goal was to assess prey available to juvenile watersnakes, exceptionally large prey were excluded from analyses. For most prey types (fish and anurans) the small diameter of trap entrances (2.5 cm) excluded individuals too large to be considered potential prey. However, because the small maximum body diameter of giant, permanently aquatic salamanders (*Siren* and *Amphiuma*) allowed very large individuals to be captured (up to 236 g), we excluded these taxa from our comparisons. We grouped prey into functional groups including centrarchid fish (genera *Acantharchus*, *Centrarchus*, *Enneacanthus*, *Lepomis*, and *Micropterus*), *Gambusia* and *Fundulus*, "other fish" (genera *Ameiurus*, *Amia*, *Aphredoderus*, *Erimyzon*, *Esox*, and *Umbra*), larval and paedomorphic *Ambystoma talpoideum*, anuran tadpoles, and adult anurans (genera *Acris*, *Bufo*, *Hyla*, and *Rana*). We compared prey profiles between wetland types based on numerical abundance (mean number captured per trap night) and based on biomass. Prey biomass was calculated by multiplying the relative abundance of each prey functional group by the mean mass of prey within that group (derived from a database of 374 prey captured from a subset of the sampled wetlands).

Subsequent to prey sampling, traps were monitored for an additional 4 trap nights to assess differences in *N. fasciata* abundance among wetland types. Snakes were marked by clipping ventral scales to avoid recounting individual snakes and snake relative abundance at each wetland was calculated as the number of individual *N. fasciata* captured per 100 trap-nights of sampling.

Prey collection, morphology, and nutritional composition

Based on wetland prey community composition (see results) we selected representative taxa from the two most dominant (by biomass) prey groups from isolated and permanent wetlands: paedomorphic mole salamanders (*Ambystoma talpoideum*), and dollar sunfish

(*Lepomis marginatus*), respectively. Prey representing a variety of sizes were collected from uncontaminated aquatic habitats on the SRS using aquatic funnel traps. Prey were blotted dry, weighed to the nearest 0.01 g on an electronic balance, and individually frozen for use in subsequent experiments. Morphology of prey types was assessed by measuring maximum height and maximum width (to the nearest 0.01 mm using digital calipers) for 30 individuals of each prey type and testing for differences in maximum dimensions using ANCOVA, with prey mass as a covariate. Whole body prey samples (n = 4 per prey type, spanning the mass range used in the digestive metabolism experiment) were dried to constant mass in a freeze drier and analyzed for nutritional composition (protein, lipid, and energy content) at the University of Arkansas's Poultry Science Center. Lipid and protein content were determined using petroleum ether extraction and the Kjeldahl combustion technique, respectively, and energy content was determined using bomb calorimetry. Although dietary values are reported on a wet mass basis, differences in nutritional content between prey types were examined using analysis of covariance (ANCOVA) with absolute protein and lipid content, dry mass, and energy content (kJ) as dependent variables and prey wet mass as the covariate. In all experiments, prey rations were determined based upon the wet mass of the prey item.

To determine whether prey type influenced post-feeding morphology of snakes, we randomly assigned 16 similarly-sized snakes (mean mass = 18.9 ± 0.47 g) into the two prey-type treatments and fed each snake a prey item weighing 25.0 ± 0.4 % of its body mass. Maximum snake body width (occurring at the location of the prey item within the snake) was measured 1 h post-feeding and subsequently at 24 h intervals until body distension due to prey was no longer evident (120 h post-ingestion). Effect of prey type on body distension was evaluated over time using repeated-measures MANOVA (Profile Analysis). For all statistical analyses in this study,

if raw data failed to meet assumptions of parametric models, we used appropriate transformations. We recognized statistical significance at $P \leq 0.05$ and performed all statistical analyses using SAS (version 9; SAS Institute 2000) or the STATISTICA for Windows software package (StatSoft, Inc. Tulsa, OK, USA 1998).

Experiment I - prey handling

A set of two experiments was used to examine differences in prey handling (swallowing) between the two prey types. The first experiment used a repeated-measures design to determine if the number of prey consumed differed between prey types across a range of prey sizes (20 – 65% of snake body mass). Because this experiment demonstrated that snakes were able to swallow much larger salamanders than fish, a second experiment was designed, wherein snakes were offered only salamanders, to determine the maximum size of salamander that snakes could consume. Finally, data from all successful swallowing events were used to test for differences in handling time between the prey types and prey sizes.

In the first prey handling experiment juvenile *N. fasciata* (n=10/treatment; mass = 9.5 ± 0.61 g) were randomly assigned to a prey-size treatment (20, 35, 50, or 65% of the snake's body mass) and each snake was offered *A. talpoideum* and *L. marginatus* of the assigned size on separate occasions (prey type order determined randomly). Snakes were offered prey in excess (2 – 5 prey items per trial, depending on size treatment) and no snake consumed all prey offered in any trial. Number of prey consumed was compared between prey types using a Wilcoxon's matched pairs test separately for each prey size category. In the second experiment, juvenile *N. fasciata* (n=8-10/treatment; mass = 4.9 ± 0.26 g) were randomly assigned to a prey size treatment (75, 90, 105, or 120% of the snake's body mass) and each snake was offered two *A. talpoideum*

of the assigned size. Visual inspection of data allowed determination of the maximum prey size capable of being ingested by snakes.

All feeding trails were conducted between 0900 and 1800 h within a walk-in environmental chamber set at 30 °C. Snakes were fasted for 10 d prior to each trial. On the day of each feeding trial snakes were weighed and allowed to acclimate in feeding chambers within the environmental chamber for 1 h. Feeding chambers consisted of 739 ml clear plastic containers (GLAD sandwich boxes) to which we added c. 10 ml of room-temperature water to provide a suitable feeding environment for the watersnakes. Up to three snakes were run simultaneously during each trial in separate feeding chambers. Following acclimation, dead, defrosted prey were added to feeding chambers and a weighted plexiglass sheet was placed over the top of each feeding chamber, allowing detailed observation of feeding behavior. The observer then moved behind a blind and observed feeding snakes for the next 1 h, at which point the trial was terminated. During the trial, the observer recorded the time (nearest second) at which the snake positioned the prey into swallowing position (prey position aligned with the jaws and commencement of "jaw walking") and the time at first tongue flick after swallowing. Handling time was defined as the time elapsed between these two events (Krause and Burghardt 2001). If, at the terminus of the experiment, any snake was still in the process of swallowing a prey item, that individual was not disturbed and was allowed to continue until it either successfully swallowed the meal or regurgitated and discontinued contact with the prey item.

Data from successful ingestion events across both experiments were combined to compare prey handling times between prey types and sizes. Because differences existed between prey types in the maximum size of prey item that could be swallowed, handling times were not available for both prey types within all prey size categories. Thus, effects of prey type and prey

size on handling time were determined using a 2-factor analysis of variance (ANOVA) on log-transformed handling times within prey size treatments where both prey types were represented by more than one successful swallowing event (20% and 35% of snake body mass). In cases where individual snakes consumed more than one prey item of a given prey type within a size category, the mean handling time across all successful swallowing events was used to avoid pseudoreplication.

Experiment II –digestive metabolism

A repeated-measures experiment was used to compare metabolic responses of snakes fed salamanders and fish. For each of 11 juvenile *N. fasciata*, methods modified from Hopkins et al. (2004) were used to measure 1) standard metabolic rate (SMR; the metabolic rate of a resting, post-absorptive ectotherm at a specified temperature during the inactive phase of its circadian cycle; Bennett and Dawson 1976), 2) specific dynamic action (SDA; the increased energy expenditure associated with digestion, assimilation and biosynthesis; Kleiber 1975, Secor and Diamond 2000) after the snake had eaten a *L. marginatus* of 25% of the snake's body mass, and 3) SDA after it had eaten an *A. talpoideum* of 25% of the snake's body mass. Juvenile *N. fasciata* (n = 11) spanning a small range of body masses (15.92 – 22.71 g) were used in this experiment to minimize the confounding effects of allometric relationships between mass and metabolic rate on our results. Prior to respirometry trials, snakes were fasted for 10 days to ensure post-absorptivity. Snakes were then weighed and placed in individual chambers (1 L Erlenmeyer flask) within an environmental chamber (25 ^{o}C, constant dark), within which all metabolic measurements were taken. A 20 ml plastic sample bottle was affixed to the bottom of the flask with Velcro to provide water to snakes throughout the experiment. A single 25.4 x 9.52 cm paper

towel was folded lengthwise and placed loosely around the water dish to provide shelter and absorb excreta. The exterior of each chamber was wrapped with paper to reduce external stimuli.

We determined metabolic rate indirectly as O_2 consumption (adjusted for standard temperature and pressure) by connecting each chamber to a computer-controlled, closed-system respirometer (Micro Oxymax, Columbus Instruments, Columbus Ohio). Respirometry measurements commenced at 10:00–11:00 h and the first 48 h were used to calculate SMR. During this period, up to six snakes were run simultaneously and O_2 consumption was measured every 48 min. After every fourth sample, chambers were refreshed with ambient air equaling four times the headspace of each chamber. Thus, additional time (113 mins) was required following each fourth interval to allow for complete refreshment of air in the chambers. A total of 35 - 41 O_2 measurements was collected for each snake. Following SMR measurements, snakes were removed from the environmental chamber and fed one *A. talpoideum* or *L. marginatus* (order determined randomly) equal to approximately 25% (24.94 ± 0.14%) of the snake's body mass. Snakes were then returned to the environmental chamber and respirometry measurements were resumed for the following 6 d (144 h) to calculate SDA parameters. Up to four snakes (in individual chambers) were run simultaneously during each SDA trial and both prey types were represented within each trial. During SDA trials, O_2 consumption was measured every 36 min with longer intervals (120 min) every third sample to accommodate refreshing of chambers with ambient air. At the midpoint of the SDA trial (72 h post-feeding), snakes were removed, chambers cleaned, and instrumentation re-calibrated. Thus, we obtained a total of 130 O_2 measurements for each snake during each SDA trial. Following termination of each snake's first SDA trial, the snake was returned to its standard housing where it was fed again, fasted for 10 d, and then started on its second SMR and SDA trial, during which it was fed the second prey type.

Snake SMR was estimated by taking the mean of the lowest quartile of the O_2 measurements obtained for each snake during the 48 h SMR trial (Hopkins et al. 2004). Visual inspection of data plots confirmed that this technique removed all outliers from SMR measurements. To estimate SDA from post-prandial respiration measurements, a two-part smoothing technique was used to remove the effects of spontaneous activity from SDA curves. The smoothing technique used a moving central minimum of three values, followed by a moving central median of 11 measurements (Hopkins et al. 2004). Energy allocated to SMR during digestion was estimated by extrapolating SMR measured before feeding over the digestive period. The integrated $\dot{V}O_2$ allocated to SMR was then subtracted from the integrated area under each smoothed post-prandial curve to calculate the oxygen consumed to support SDA. Additionally, peak post-prandial O_2 consumption, digestive scope (peak O_2/SMR), time to peak O_2, time to 50% decrease from peak, and time to 75% decrease from peak were calculated for each snake. SDA was converted to energetic equivalents using a conversion factor of 19.8 J/mL O_2 (Secor and Diamond 2000) and the cost of digestion (SDA coefficient) was calculated as a percentage of total ingested energy allocated to the SDA response. Because individual snakes received both prey type treatments, individual was used as a blocking factor in a multivariate analysis of variance (MANOVA) to test for effects of prey type on SDA response, peak rate of O_2 consumption, digestive scope, time to peak, time to 50% decrease from peak, time to 75% decrease from peak, and SDA coefficient. SDA expressed as total kJ expended was excluded from the statistical analysis because this parameter is completely autocorrelated with SDA expressed as ml O_2 consumed. Although we controlled for mass by using a small range of snake sizes and blocking by individual, we initially tested for mass effects by including body mass as a

covariate. However, as no overall mass effect was detected ($F_{8,2} = 1.04$, $P = 0.576$), mass was subsequently removed from the statistical model.

Experiment III – post-feeding locomotor impairment

A repeated-measures design was used to compare post-feeding locomotor impairment of snakes fed salamanders and fish. Maximum crawling speed was selected as our measure of locomotor performance because watersnakes frequent terrestrial wetland edges while basking to aid digestion and are most abundant in heavily-vegetated habitats (Gibbons and Dorcas 2004, JDW unpublished data), where locomotion is more akin to crawling than swimming. Moreover, a previous study examining the relative effects of reproductive burden on swimming and crawling in a semi-aquatic snake found that burden affected crawling speed more strongly than it affected swimming speed (Winne and Hopkins 2006).

Sixteen juvenile *N. fasciata* (mean mass = 18.9 ± 0.47 g; mean SVL = 281 ± 3.7 mm) were randomly divided between fish and salamander treatments, and their maximum crawling speed was measured before feeding, immediately post-feeding, and subsequently at several time points during digestion using methods similar to those of Winne and Hopkins (2006). Throughout the experiment, snakes were housed at 28 ^{o}C within an environmental chamber but locomotor trials were conducted within a temperature-controlled laboratory (temperature range across all trials: 21.0 - 22.8 ^{o}C).

Maximum crawling velocity for each snake was determined using a 2.3 m linear sprint track lined with pairs of photocells projecting infrared beams at 10 cm intervals interfaced with a laptop computer (Columbus Instruments, Columbus, Ohio, USA; described in detail in Holem et al. 2006 and DuRant et al. 2007). The racetrack was lined with a strip of stiff plastic carpet ("astroturf") to maximize crawling performance (Winne and Hopkins 2006). Prior to the

experiment, snakes were fasted for 10 d and conditioned to the track by racing them twice in succession 48 h prior to the start of the experiment. Subsequently, snakes were raced twice in succession at six time intervals: 18 h before feeding and 1 h, 24 h, 48 h, 72 h, 96 h, and 120 h after consuming a *L. marginatus* or *A. talpoideum* equal to 25.0 ± 0.4% of the snake's body mass.

Prior to beginning each trial, individual snakes were placed in a box attached to the starting point of the track and left undisturbed for approximately one minute. A gate separating the start box from the sprint track was then lifted, and the snake was chased by hand down the track by lightly touching the snake's tail at approximately one second intervals to prompt a flight response (Huey et al. 1989, Winne and Hopkins 2006). Further motivation was provided by attaching a darkened shelter to the opposite end of the track (Bennett 1980). The time it took for each individual to crawl 60 cm was calculated for each 60 cm segment of the track and the single fastest velocity (expressed as cm/s; Shine et al. 2003) for each individual was used as an estimate of maximum locomotor performance in statistical comparisons. Effects of prey type on post-feeding maximum sprint velocity was evaluated using repeated-measures MANOVA (Profile Analysis). Although we attempted to minimize confounding effects of body size on crawling speed by using a small range of snake sizes, we include SVL as a covariate in our statistical model. However, as no effect of SVL was detected ($F_{1,13} = 0.19$, $P = 0.674$), SVL was subsequently removed from the model.

RESULTS

Prey availability and snake abundance

Permanent wetlands, or those with periodic connections to permanent water bodies, showed markedly different prey communities than wetlands that dried periodically and were isolated from other aquatic habitats (Fig. 5.1). Generally, prey communities within isolated wetlands were comprised exclusively of amphibians and were dominated by larval and paedomorphic *Ambystoma talpoideum*. Although permanent wetlands hosted small numbers of tadpoles and adult anurans, the prey communities of these wetlands were dominated by various fish species. Numerically, fish communities of permanent wetlands were dominated by *Gambusia holbrooki* and *Fundulus* sp. However, because these fish are small (0.65 ± 0.08 g), when prey mass was considered, centrarchid fish were the dominant prey type in permanent wetlands. Likewise, when prey mass was considered, other large prey taxa, such as adult anurans and "other fish," gained importance, whereas the importance of small but numerous prey taxa (e.g., tadpoles) declined. The patterns of abundance we documented agree well with other qualitative and quantitative assessments of wetland fish and amphibian assemblages in the region (e.g., Gibbons and Semlitsch 1999, Snodgrass et al. 1996). Continued trapping using aquatic funnel traps yielded a total of 132 individual *N. fasciata*. Isolated wetlands exhibited substantially greater *N. fasciata* abundance compared to permanent wetlands (Fig. 5.2).

Prey morphology and nutritional composition

Substantial differences in morphology were evident between salamanders and fish. Although *A. talpoideum* had a larger maximum body width than *L. marginatus* at a given mass (Fig. 5.3a; $F_{1,57} = 293.7$, $P < 0.001$), *L. marginatus* had substantially greater maximum height than *A. talpoideum* at all body sizes (Fig. 5.3b; $F_{1,57} = 1{,}224.9$, $P < 0.001$). In addition to

differences in maximum dimensions, other anatomical differences between the prey types were obvious. *Ambystoma talpoideum* possess well-developed legs and slimy skin, whereas *L. marginatus* have scales and fins, including dorsal and anal fins equipped with sharp spines (Fig. 5.3c). Following consumption of a large meal, snakes eating *L. marginatus* exhibited much greater body distension (maximum body width) than those consuming an equivalently-sized *A. talpoideum* (Fig. 5.3d). The difference in body distension between prey types declined over time (time-by-prey type interaction, $F_{5,70} = 8.35$, $P = 0.003$). By approximately 60 h post-ingestion, differences in maximum body width were no longer evident between snakes fed the two prey types (Fig. 5.3d).

Overall, nutritional composition was similar between the two prey types (Table 5.1). Among body composition variables, the greatest difference between the prey types was in water content, with *A. talpoideum* having 6% greater water content than *L. marginatus*. Likely due to differences in water content, *L. marginatus* had slightly higher protein content (expressed per unit wet mass) than *A. talpoideum*, but lipid and energy content were similar between the prey types (Table 5.1).

Prey handling

Snakes showed substantial differences in their ability to consume the two prey types. Although the maximum size of *L. marginatus* consumed by a juvenile *N. fasciata* was 50% of the snakes body mass (Fig. 5.4a), three snakes of 10 snakes were able to consume *A. talpoideum* equal to 105% of the snake's body mass (Fig. 5.4b). Within the smallest prey size category (20% of snake body mass), snakes consumed an average of 1.5 prey item, regardless of prey type (Wilcoxon's matched pairs test; $Z = 1.60$, $P = 0.109$). However, snakes consumed significantly more *A. talpoideum* than *L. marginatus* within all larger prey size categories tested: 35% of

snake body mass (Z = 2.20, P = 0.028), 50% of snake body mass (Z = 2.80, P = 0.005), 65% of snake body mass (Z = 2.67, P = 0.007).

Within prey size categories where both prey types could be consumed (20% and 35% of snake body mass), prey type had a significant effect on handling time ($F_{1,30}$ = 120.60, P < 0.001; Fig. 5.5). At 35% of snake body mass, mean ingestion time for *L. marginatus* (20.6 min) was nearly 15 times higher than that of *A. talpoideum* (1.5 min). Even handling time of *A. talpoideum* of 105% of snake body mass (9.0 min) was only half as long, on average, than handling time of *L. marginatus* of 35% of snake body mass (20.6 min). Prey size also had a significant effect on handling time ($F_{1,30}$ = 18.2, P < 0.001) with larger prey taking longer to ingest in both prey types (Fig. 5.5). However, no prey-type by prey-size interaction was detected ($F_{1,30}$ = 0.69, P = 0.411).

Digestive metabolism

Juvenile *N. fasciata* weighing 18.47 ± 0.29 g (treatments combined) had standard resting metabolic rates (SMR) of 0.66 ± 0.04 ml O_2/h and SMR did not differ between treatments (Table 5.2). After consuming meals of 25% of their body mass, snakes exhibited classic SDA response curves (Fig. 5.6) characterized by rapid increases in energy consumption for the first 24 h, a plateau at peak O_2 consumption from 24 – 48 h post-feeding, and a gradual decrease in energy consumption thereafter. Snakes returned to relatively steady levels of O_2 consumption by approximately 120 h post-feeding, but post-feeding minimum O_2 consumption was slightly higher than pre-feeding SMR, likely due to a slight increase in body mass.

Overall, SDA curves were remarkably similar between the two prey types, with only subtle differences in the shapes of the curves (Fig. 5.6). Indeed, we detected only a marginally-significant effect of treatment (prey type) on SDA response ($F_{8,3}$ = 7.49, P = 0.063). Individual comparisons revealed no effect of treatment on total $\dot{V}O_2$ consumed, peak O_2, digestive scope,

SDA coefficient (proportion of ingested energy allocated to SDA), and time to peak O_2 consumption (Table 5.2). However, treatment did significantly affect time to 50% and 75% decrease from peak O_2 consumption (Table 5.2), with snakes fed *A. talpoideum* declining more steeply towards baseline O_2 consumption rate than snakes fed *L. marginatus* (Fig. 5.6).

Post-feeding locomotor impairment

Prior to feeding, snakes in both treatment groups exhibited maximum crawling speeds of approximately 25 cm/s (Fig. 5.7). Following consumption of prey items equal to 25% of the snake's body mass, snakes in both treatment groups exhibited significant reductions in maximum sprint speed (time, $F_{6,84} = 16.71$, $P < 0.001$). This reduction, however, was much more pronounced in snakes fed *L. marginatus* than in snakes fed *A. talpoideum* (time-by-prey type interaction, $F_{6,84} = 2.67$, $P = 0.020$). When tested 1 h post-feeding, snakes fed *L. marginatus* displayed an average maximum sprint velocity of 13 cm/s, a reduction of nearly 45% from pre-feeding speed, while snakes fed *A. talpoideum* displayed a maximum sprint velocity of 21 cm/s, a reduction of only 23% from pre-feeding speed. Snakes gradually increased in mean sprint velocity during digestion. However, snakes that had consumed *L. marginatus* did not attain pre-prandial crawling velocities until approximately 100 h after feeding (Fig. 5.7).

DISCUSSION

Our results suggest that aquatic salamanders (e.g., *A. talpoideum*), which are characteristic of fishless wetlands in the South Carolina Coastal Plain, represent a higher quality prey for juvenile watersnakes than centrarchid fish (e.g., *L. marginatus*), which are characteristic of permanent wetlands. Juvenile *N. fasciat*a were able to consume larger *A. talpoideum* than *L. marginatus* and consumed more *A. talpoideum* at sizes where both prey types could be

consumed. *L. marginatus* induced greater post-prandial body distension in snakes than did *A. talpoideum*, resulting in severely-impaired locomotor performance. Cost of digestion (SDA response) was similar between the prey types, likely reflecting their similar nutritional composition. However, snakes fed *A. talpoideum* displayed significantly shorter times to 50% and 75% O_2 consumption rate, suggesting that digestion of *A. talpoideum* is slightly more rapid than *L. marginatus*. The differences in quality of dominant prey among fishless and permanent wetlands may contribute to the higher *N. fasciata* abundances we observed in fishless, compared to permanent wetland habitats.

Prey handling

Snakes are well-known for their ability to consume massive meals relative to their body size. In an extreme example, a juvenile lancehead (*Bothrops atrox*) consumed a lizard totaling 156% of the snake's body mass (Greene 1983). In many cases, however, evaluations of "prey size" consider only prey mass relative to snake body size (e.g., Mushinsky, Hebrard and Vodopich 1982, Shine 1991, Shine et al. 1998) and our understanding of how prey shape contributes to mechanics of feeding is limited (but see Kley and Brainerd 2002, Vincent et al. 2006). We found that prey morphology was extremely important in determining the maximum size of prey that could be ingested by snakes. Indeed, due to differences in prey morphology, the maximum size of *A. talpoideum* that snakes could consume (105 % of snake mass) was over twice the maximum size of *L. marginatus* (50% of snake mass). This result corroborates the findings of Vincent et al. (2006) who concluded that prey with great maximum dimensions were more difficult for snakes to consume than more elongate prey. Interestingly, the upper size of *A. talpoideum* that snakes were able to consume was apparently not dictated by gape limitation. In fact, two snakes attempting to swallow *A. talpoideum* of 105% of their body mass, and one

attempting 120%, all managed to get well past the widest point of the prey item (head) and swallow at least 2/3 of the prey's length before regurgitating. This observation suggests that snakes may be limited by the volume of their stomach, rather than their gape, when consuming *A. talpoideum*. Alternatively, these large prey may have filled the snake's body cavity so completely that cardiovascular or respiratory function would have been impaired had the snake not regurgitated. Other authors have noted that elongate, but massive prey represent the ideal prey morphology for snakes and it has been suggested that a switch to elongate prey was a precursor to the success, and subsequent diversification, of ancestral snakes (Greene 1983). Our results support this conclusion by demonstrating that, for snakes, feeding on large, elongate prey alleviates the phenomenon of gape limitation.

In addition to influencing the maximum ingested prey size, prey morphology also dictated the number of small prey ingested by snakes. Snakes consumed more *A. talpoideum* than *L. marginatus* when prey were small. Our small prey size treatments were well below the gape limitation threshold for both prey types. Thus, the fact that snakes consumed more *A. talpoideum* per feeding event presumably represented a choice by snakes, rather than morphological limitation. Previous studies have shown that given a choice, northern watersnakes (*Nerodia sipedon*) select smaller meal sizes than they are physically capable of ingesting (Andreadis and Burghardt 2005). Our results suggest that this decision may be influenced by prey type. It is plausible that the awkward shape of *L. marginatus* (narrow, but tall) make large meals of this prey type less desirable, perhaps due in part to the increased handling time and impaired locomotor performance incurred by snakes consuming large meals of this type.

In addition to differences in maximum meal size and number of prey consumed, we also observed striking differences in handling time between the two prey types. Handling time was

always longer for *L. marginatus*, and even *A. talpoideum* of over 100% of snake body mass took less than half as long to swallow as *L. marginatus* weighing 35% of snake's body mass. These results confirm the findings of Vincent et al. (2006) that prey morphology, independent of prey mass, can be an important determinant of snake feeding performance. They found that frogs, which have larger maximum dimensions, took *N. fasciata* longer and required more jaw protractions to swallow than fish (*Fundulus grandis*). Vincent et al. (2006) suggested that wide or bulky prey may interfere with a snake's ability to use anterior vertebral bending to transport prey past the jaws. Although we did not examine feeding mechanics in this study, it is likely that the narrow, tall morphology of *L. marginatus* contributed to the longer handling times we observed by limiting snake's abilities to use vertebral bending during ingestion. Although other studies have suggested that *Nerodia* rely primarily on jaw protractions to swallow fish (Kley and Brainerd 2002), it is likely that the elongate morphology of *A. talpoideum* facilitated the use of vertebral bending during ingestion (Kley and Brainerd 2002), resulting in rapid ingestion times, even for very large prey. Although the energetic cost of prey handling in snakes is likely trivial (Feder and Arnold 1982, Cruz-Neto et al. 2001), differences in handling time may be biologically relevant. For example, following optimal foraging theory, time spent consuming prey represents time lost from other important activities such as thermoregulation, reproduction, or pursuing subsequent prey (Arnold 1993). Perhaps more importantly, increased handling time may increase predation risk, especially in juvenile snakes (Arnold 1993, Vincent e*t al.* 2006). Thus, pursuing prey that require longer handling times not only decreases energy acquisition per unit time, but may also incur a fitness cost. Snakes have recently been documented to make foraging-versus-risk decisions (Aubret et al. 2007) and thus, may avoid prey that are risky to capture. Alternatively, snakes inhabiting habitats where only "risky" prey are available (e.g.,

permanent wetlands) may be subject to elevated levels of predation, consequently reducing population growth rates.

Cost of digestion

Previous studies suggest that nutritional composition, particularly protein content, is the primary driver of the SDA response in ectotherms (Andrade et al. 2005, McCue et al. 2005). This conclusion is based primarily on observations that SDA response correlates strongly with protein content of meals (Hailey 1998). Moreover, inhibition of protein synthesis has been shown to disable the SDA response in both fish (Brown and Cameron 1991a,1991b) and snakes (McCue et al. 2005). However, little is known about the effects of prey shape or structure on digestive physiology. In fact, most studies of SDA in ectotherms fed natural prey compare prey types that differ substantially in a variety of ways, including nutritional composition, taxonomy, structure, and amount of indigestible material (e.g., Secor and Phillips 1997, Hailey 1998, Secor and Faulkner 2002, Grayson et al. 2005, McCue et al. 2005, Secor and Boehm 2006). In such experiments, it is difficult to disentangle the effects of nutritional composition from other factors.

Few studies have compared SDA responses of ectotherms fed prey types that are similar in nutritional composition, but different in morphology. An intriguing result of one study was that Burmese pythons (*Python molurus*) fed pureed mice exhibited higher SDA responses than those fed intact mice (McCue et al. 2005). This counterintuitive result suggests that meal structure may make an important contribution to the magnitude and character of the SDA response in snakes. Studies of SDA in amphibians support the notion that there is more that contributes to the SDA response than simple nutritional composition. Grayson et al. (2005) found no differences in SDA responses of horned frogs (*Ceratophrys cranwelli*) fed prey types that differed in protein content by 46% (earthworms and newborn mice). In marine toads (*Bufo*

marinus), SDA also did not differ between earthworm and newborn mouse diets, but was elevated for prey types that possess chitinous exoskeletons (crickets and superworms; Secor and Faulkner 2002).

We compared SDA responses of snakes fed prey types that were similar in nutritional composition but substantially different in morphology. We hypothesized that *A. talpoideum* would be less costly to digest than *L. marginatus* because *A. talpoideum* lack scales, spiny fin rays, or other presumably difficult to digest materials. Surprisingly, we found that SDA profiles were similar for snakes digesting the two prey types. This result supports the general conclusion that nutritional composition of prey is the strongest determinant of SDA in snakes. However, we did detect slight differences between the prey types in the shapes of the SDA curves, (time to 50% and 75% decrease from peak O_2 consumption), suggesting that these prey morphologies may have subtle effects on digestive metabolism. Although the biological relevance of these differences is unknown, it is possible that the slightly faster processing speed of *A. talpoideum*, combined with the lack of post-feeding locomotor impairment, could allow for a greater turnover rate of prey items, and consequently faster growth of snakes.

Post-feeding locomotor performance

Because they lack limbs, trunk morphology is tied more intimately to locomotion in snakes than in other tetrapods. Indeed, snakes with slim, elongate morphology generally exhibit faster maximum crawling speeds than those with stouter (heavier relative to length) body forms (Ruben 1977, Shine and Shetty 2001, Shine et al. 2003). It follows that changes in snake body morphology have direct effects on locomotor performance. For example, female snakes suffer reduced locomotor performance during pregnancy, due in part to the added burden of offspring (Seigel et al. 1987, Winne and Hopkins 2006). Despite the fact that snakes are generally unable

to reduce prey size by mastication, the flexible head morphology of most snakes allows them to consume large meals relative to their body size. However, as with reproductive burden, the mass of a large meal comes at a cost to locomotor performance, both on land (Garland and Arnold 1983, Mehta 2006) and in the water (Shine and Shetty 2001). For example, Ford and Shuttlesworth (1986) found that checkered garter snakes (*Thamnophis marcianus*) fed large meals (10 - 50% of snake body mass) exhibited reduced maximum sprint speed, average crawling speed, and endurance duration. However, our study is the first to report differences in locomotor performance associated with differences in prey morphology, independent of mass. We found that although snakes fed both meal types experienced some post-prandial locomotor impairment, snakes fed *L. marginatus* exhibited much more severely reduced crawling speeds (45% reduction from pre-feeding) than those fed *A. talpoideum*. Moreover, those impediments persisted longer in *L. marginatus* than *A. talpoideum*, with snakes fed *L. marginatus* continuing to exhibit impaired crawling abilities for at least four days post-feeding. Our results are not unexpected given that *L. marginatus* induced a much greater distortion of normal body form than did *A. talpoideum*.

Presumably, reduction in sprint speed is associated with increased exposure to predators and subsequent reduction in survivorship (Shine 1980, Husak 2006a, 2006b). At least one study has reported a negative relationship between sprint speed and survivorship in juvenile garter snakes (*Thamnophis sirtalis*; Jayne and Bennett 1990). Thus, all else being equal, juvenile *N. fasciata* feeding on centrarchid fish could experience reduced survivorship compared to those feeding on aquatic salamanders. Additionally, impaired locomotor ability may reduce snake's abilities to procure future prey items, ultimately reducing individual growth rates, slowing time to maturity, and retarding population growth.

Implications for wetland conservation

Our results suggest that using resource availability as an index of habitat quality for generalist predators is complex, requiring consideration of more than simple prey abundance or even nutritional composition. For example, a cursory assessment of habitat quality, based solely on prey abundance, might conclude that both the permanent and the fishless (isolated, periodically dry) wetlands sampled in this study (Fig. 5.1a) represent suitable habitats for juvenile watersnakes, because both harbor high abundances of potential prey. However, we have shown that differences in prey morphology lead to substantial differences in overall quality of dominant prey between the two habitat types. Whereas isolated wetlands contain abundant high-quality prey (*A. talpoideum*), the most abundant (by biomass) prey type available in permanent wetlands (centrarchid fish) are of inferior quality. Consuming centrarchid fish imposes constraints on maximum prey size and feeding frequency and induces lengthened handling times and impaired crawling speeds that presumably increase a snake's risk of predation. Thus, in permanent habitats, juvenile snakes would be forced to either persist on a lower quality diet or resort to alternative prey types. Alternative prey could include those that are abundant but so small that they must be captured in large quantities to meet energetic demands (e.g., *Gambusia* and *Fundulus*) or those that are large but less abundant (e.g., adult anurans). Although they did not measure relative abundance of prey, one case provides insight into how *N. fasciata* may deal with this complex decision. In permanent cypress swamp habitats in Louisiana, juvenile *N. fasciata* fed predominantly on *Gambusia* and other small fish, while adults fed predominantly on frogs (Mushinsky et al. 1982), with neither demographic preying extensively on centrarchid fish, despite their availability (Mushinsky and Hebrard 1977, Kofron 1978).

The ecological value of fishless wetlands has received much recent attention (Whigham 1999, Semlitsch and Bodie 1998), particularly in light of recent weakening of federal protection for wetlands that are isolated from navigable waterways (Zedler et al. 2001, Downing et al. 2003). Isolated, often fishless, wetlands have received particular recognition as hotspots for amphibian biodiversity and productivity (Semlitsch and Bodie 1998, Gibbons et al. 2006). We demonstrate that aquatic salamanders (e.g., *A. talpoideum*), which are often the dominant amphibian in fishless wetlands in the southeastern United States, represent an optimal prey for juvenile aquatic snakes. The ability of snakes to consume very large salamanders with relatively minor impediments to locomotor performance, may facilitate the high abundances and rapid individual and population growth of *N. fasciata* we observed in fishless wetland habitats (Winne 2008), despite snake population declines during extreme droughts (Seigel, Gibbons and Lynch 1995, Willson et al. 2006). In fact, as *N. fasciata* disperse from these habitats during droughts (Seigel et al. 1995), and have subsequently been recorded moving to other wetlands at least 3 km away (J. D. Willson, Christopher Winne and Thomas Luhring, unpublished data), fishless wetlands may act as source populations for aquatic snakes across the landscape. Wetland loss has been cited as a major contributor to the imperilment of snake species (Todd et al. *in press*). For example, copperbelly watersnakes (*N. erythrogaster neglecta*) prefer ephemeral wetland habitats and move extensively between wetlands to capitalize on seasonally-abundant amphibian prey resources (Roe et al. 2004). Thus, loss or fragmentation of isolated, fishless wetlands has been proposed as the major threat to federally-threatened northern *N. e. neglecta* populations (Roe et al. 2003, 2004, 2006). Our results demonstrate that the morphological characteristics of amphibians make them ideal prey for aquatic snakes, underscoring the importance of fishless wetlands in snake conservation.

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Table 5.1 Nutritional composition of *Ambystoma talpoideum* and *Lepomis marginatus*. Values are reported as means ± 1 SE relative to the wet mass of the prey item. Significant differences between prey types (ANCOVA; dependent variables: dry mass (g), protein (g), lipid (g), kJ; covariate: wet mass) are indicated in bold.

	Ambystoma	***Lepomis***	$F_{1,5}$	**p**
% H_2O	81.33 ± 0.32	75.93 ± 0.42	59.59	**< 0.001**
% Protein	12.22 ± 0.35	14.62 ± 0.37	15.17	**0.011**
% Lipid	2.23 ± 0.18	2.37 ± 0.25	0.11	0.749
kJ / g	4.06 ± 0.12	4.63 ± 0.14	3.59	0.117

Table 5.2 Metabolic parameters (mean ± 1 SE) of *Nerodia fasciata* fed prey items (*Ambystoma talpoideum* and *Lepomis marginatus*) equal to 25% of snake body mass and held at 25 °C. Significant differences between prey types (MANOVA; individual as blocking factor) are indicated in bold. Differences between prey types in SDA expressed as total kJ expended were not tested statistically because this parameter is completely autocorrelated with SDA expressed as ml O_2 consumed.

Parameter measured (units)	***Ambystoma***	***Lepomis***	$F_{1,10}$	**P**
N	11	11		
Mass (g)	18.63 ± 0.49	18.31 ± 0.34		
SMR (ml O_2/h)	0.65 ± 0.06	0.67 ± 0.06	0.59	0.46
Peak O_2 rate (ml O_2/h)	4.00 ± 0.14	4.02 ± 0.09	0.00	0.98
Digestive scope (peak O_2/SMR)	6.53 ± 0.40	6.54 ± 0.65	3.23	0.10
Time to peak O_2 (h)	31.75 ± 2.91	24.91 ± 2.00	2.22	0.17
Time to 50% decrease from peak (h)	68.27 ± 1.53	74.33 ± 2.19	5.77	**0.04**
Time to 75% decrease from peak (h)	81.21 ± 1.72	95.94 ± 5.95	5.66	**0.04**
SDA				
Total ml O_2 consumed	229.54 ± 7.01	242.67 ± 8.88	0.49	0.50
Total kJ expended	4.54 ± 0.18	4.80 ± 0.18		
SDA coefficient (% ingested E utilized)	23.83 ± 0.47	22.98 ± 0.66	0.03	0.87

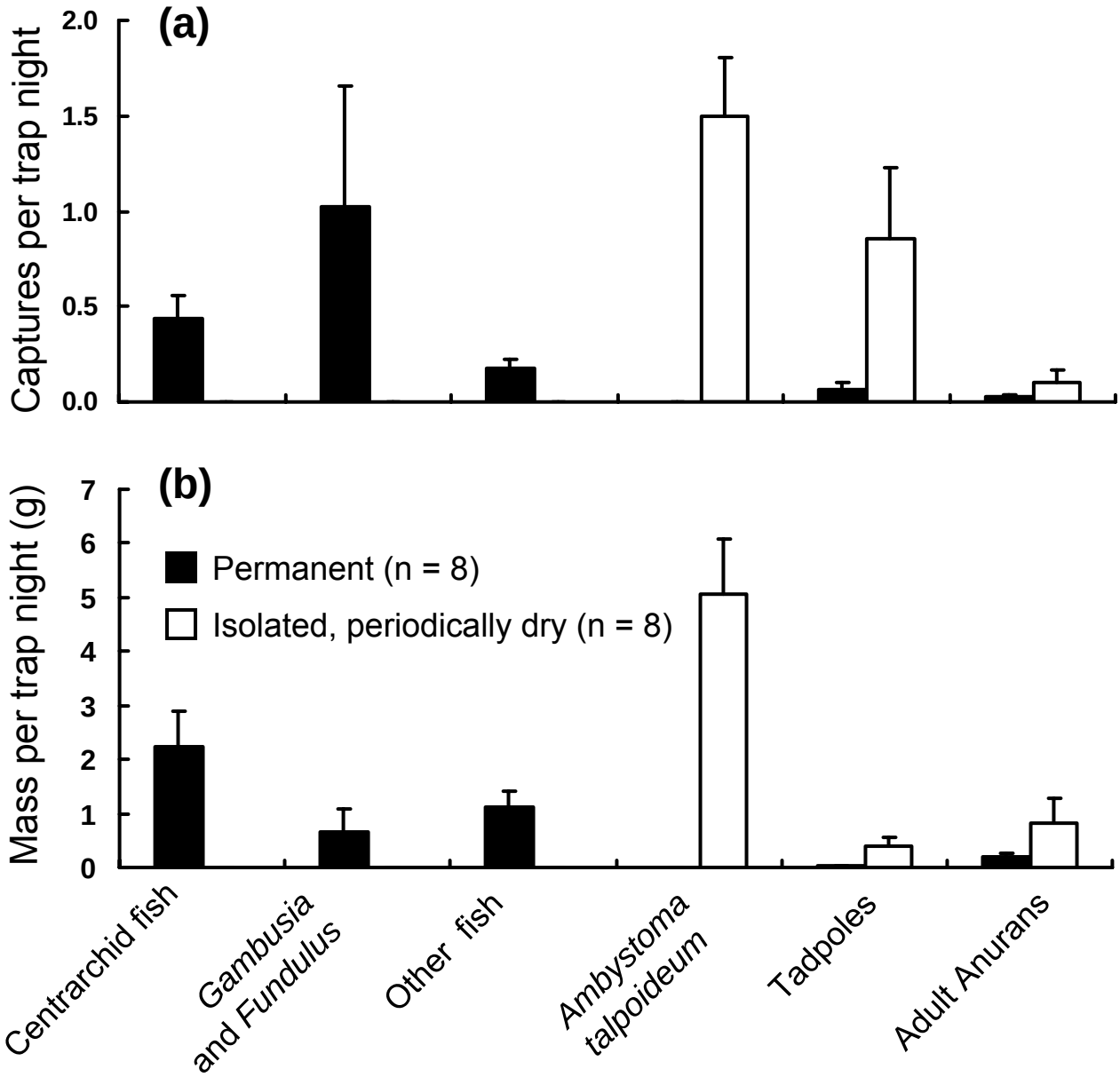

Figure 5.1 Prey profiles of permanent (n = 8) and isolated, periodically dry (n = 8) wetlands located on the Savannah River Site, South Carolina, USA, based on prey numerical abundance (a) and biomass (b). "Permanent" wetlands either had not dried within 20 yrs or were occasionally connected to permanent water bodies. "Isolated, periodically dry" wetlands dried during severe droughts and were isolated from other water bodies. Relative abundance of prey was measured as captures per trap-night in aquatic funnel traps (minnow traps). Prey are lumped into functional groups including centrarchid fish (genera *Acantharchus*, *Centrarchus*, *Enneacanthus*, *Lepomis*, and *Micropterus*), *Gambusia* and *Fundulus*, other fish (genera *Ameiurus*, *Amia*, *Aphredoderus*, *Erimyzon*, *Esox*, and *Umbra*), larval and paedomorphic *Ambystoma talpoideum*, anuran tadpoles, and adult anurans (genera *Acris*, *Bufo*, *Hyla*, and *Rana*).

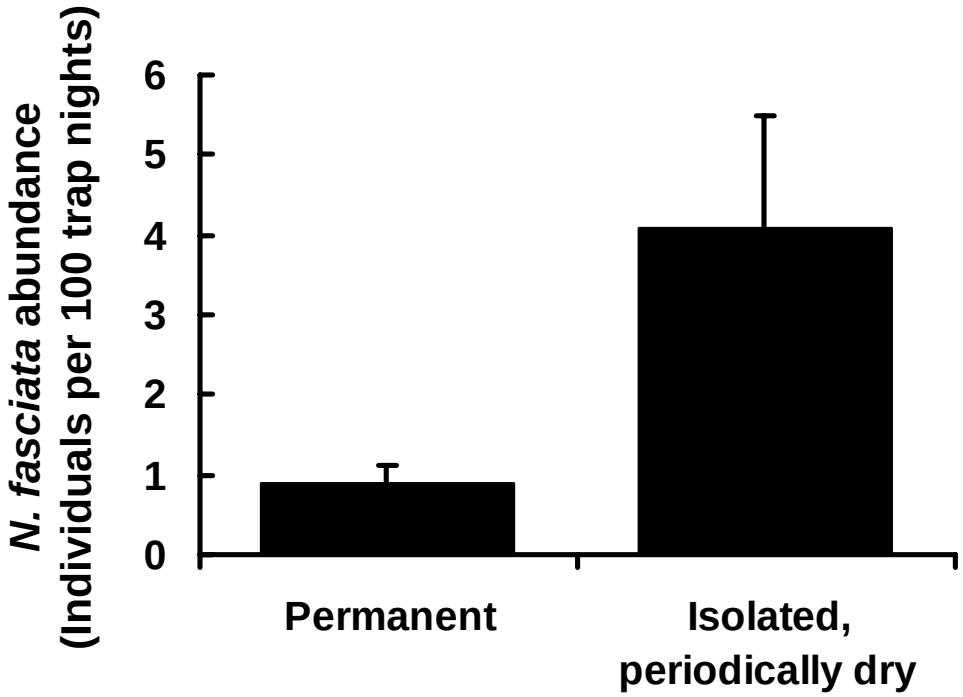

Figure 5.2 Mean (± 1 SE) abundance of *Nerodia fasciata* within permanent (n = 8) and isolated, periodically dry (n = 8) wetlands located on the Savannah River Site, South Carolina, USA. Snake relative abundance was assessed by calculating the number of individual snakes captured at each wetland per 100 trap nights of sampling with aquatic funnel traps (minnow traps). In total, 132 *N. fasciata* were captured in the 16 wetlands.

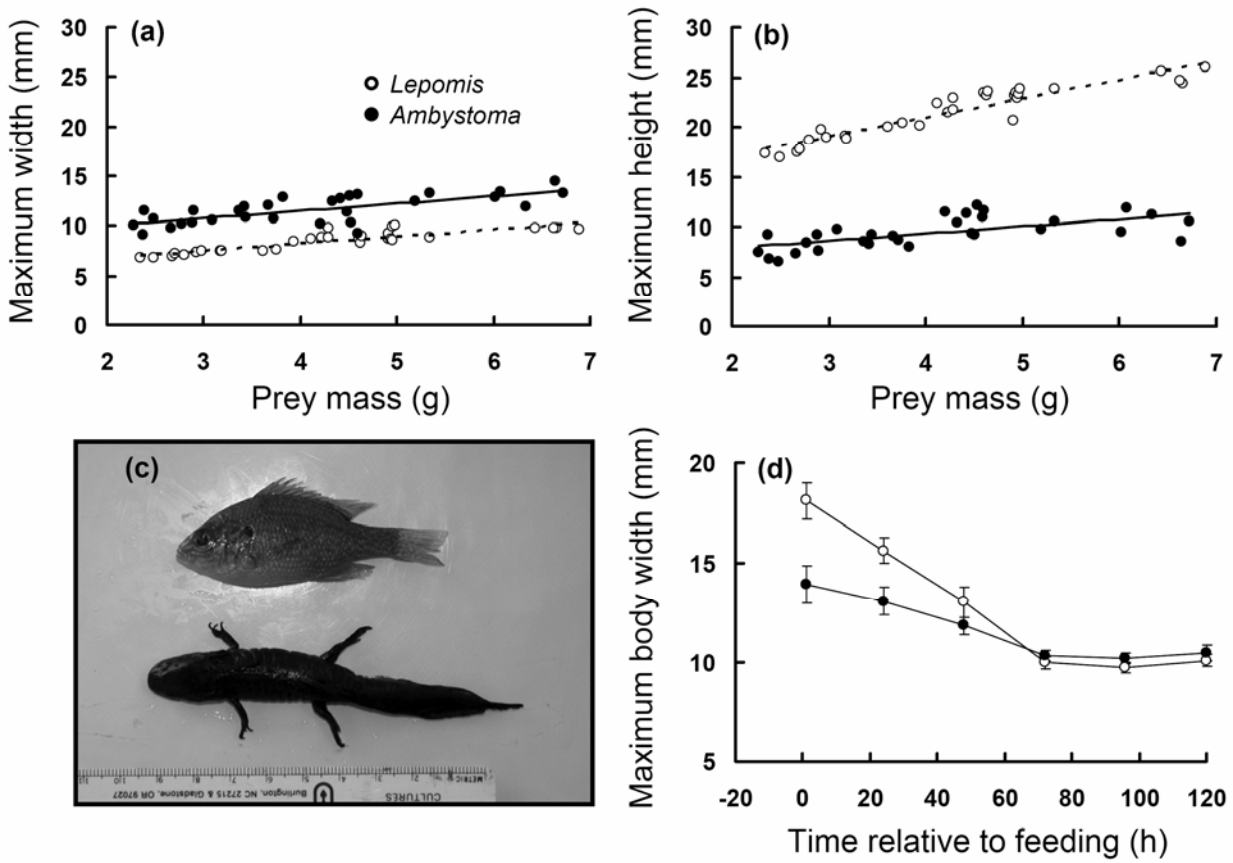

Figure 5.3 Morphological differences between paedomorphic *Ambystoma talpoideum* and *Lepomis marginatus*, including (a) maximum width and (b) maximum height of prey. Anatomical differences are evident in a photograph (c) of individuals of the two prey types of equal mass (6.6 g). Post-ingestion differences between the prey types are evident in a time-series of body distension (maximum body width) in snakes fed *L. marginatus* or *A. talpoideum* weighing 25% of snake body mass (d).

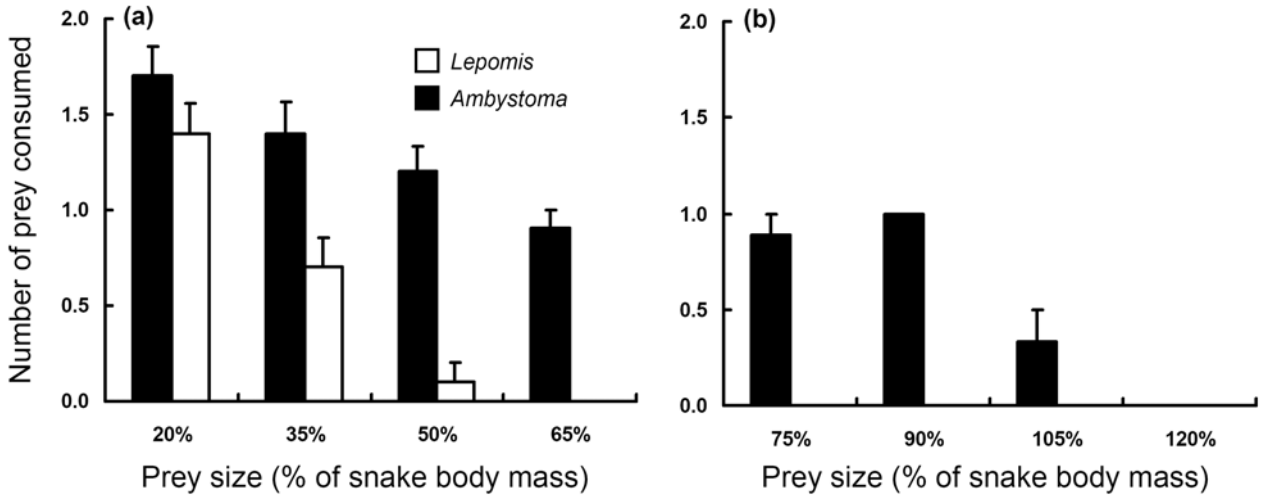

Figure 5.4 Number of prey consumed (mean ± 1 SE) by juvenile *Nerodia fasciata* fed *Ambystoma talpoideum* or *Lepomis marginatus* of different sizes (% of snake body mass). (a) Reports results of an experiment comparing number of prey consumed between prey types at relatively small meal sizes. In (a) snakes (n = 10 per size and prey type treatment) were offered *A. talpoideum* or *L. marginatus* of four size classes (20%, 35%, 50%, and 65% of snake body mass) in excess. (b) Reports results of an experiment determining the maximum size of *A. talpoideum* that can be consumed by juvenile *N. fasciata*. In (b) snakes (n = 8 – 10 per size treatment) were offered only *A. talpoideum* of four larger size classes in excess.

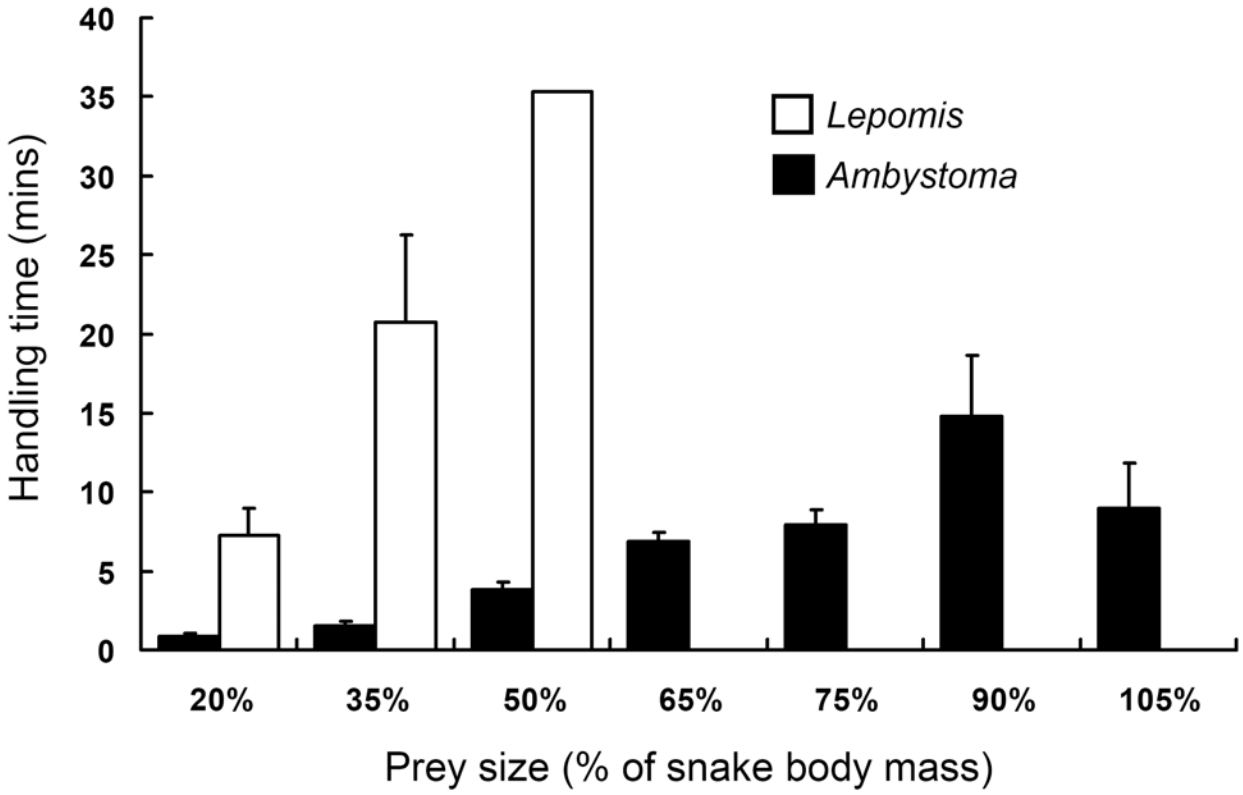

Figure 5.5 Handling times (mean ± 1 SE) of juvenile *Nerodia fasciata* fed *Ambystoma talpoideum* or *Lepomis marginatus* of different sizes (% of snake body mass). Handling time was defined as the time elapsed from the time the snake oriented the prey item into swallowing position until the first tongue flick following swallowing of the prey item.

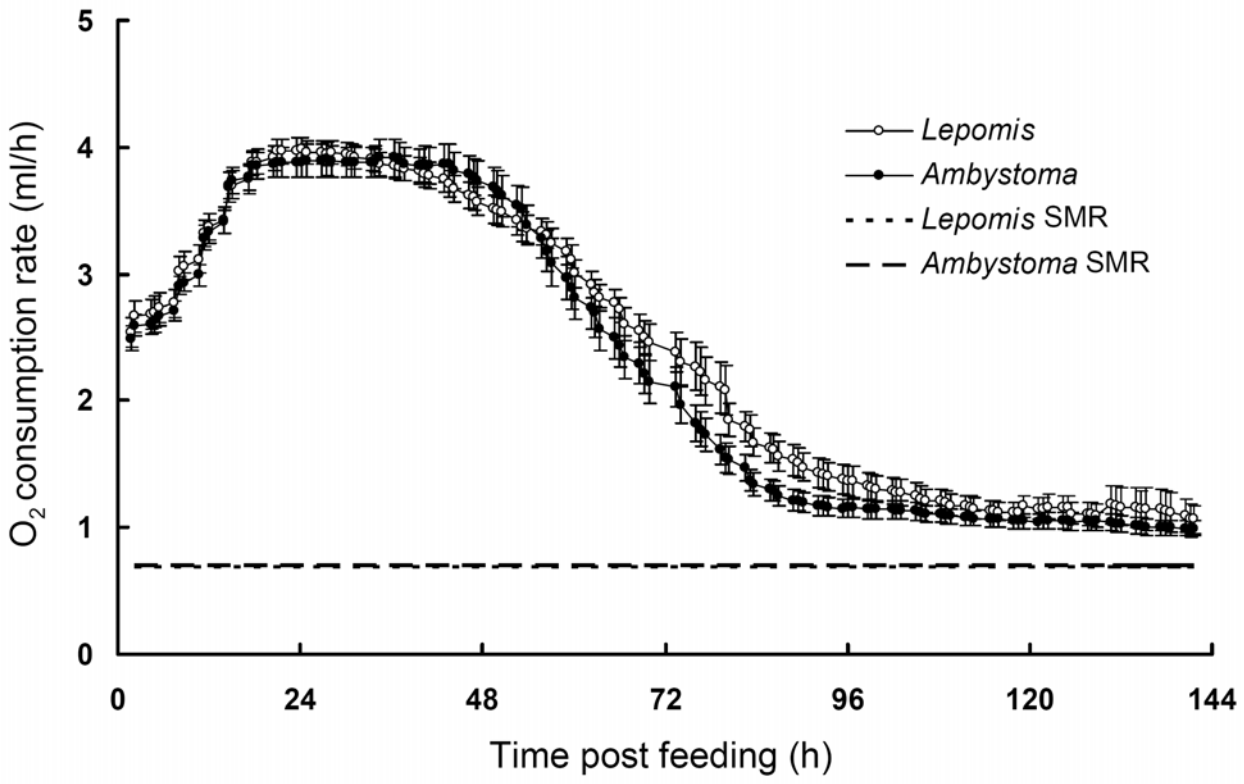

Figure 5.6 Mean (± 1 SE) oxygen consumption by juvenile *Nerodia fasciata* (n = 11 per treatment) fed an *Ambystoma talpoideum* (●) or *Lepomis marginatus* (○) equal to 25% of the snake's body mass at 25 °C. Dashed lines represent the mean SMR of snakes in each treatment group based on the lower quartile values of fasted snakes measured for 48 h prior to feeding.

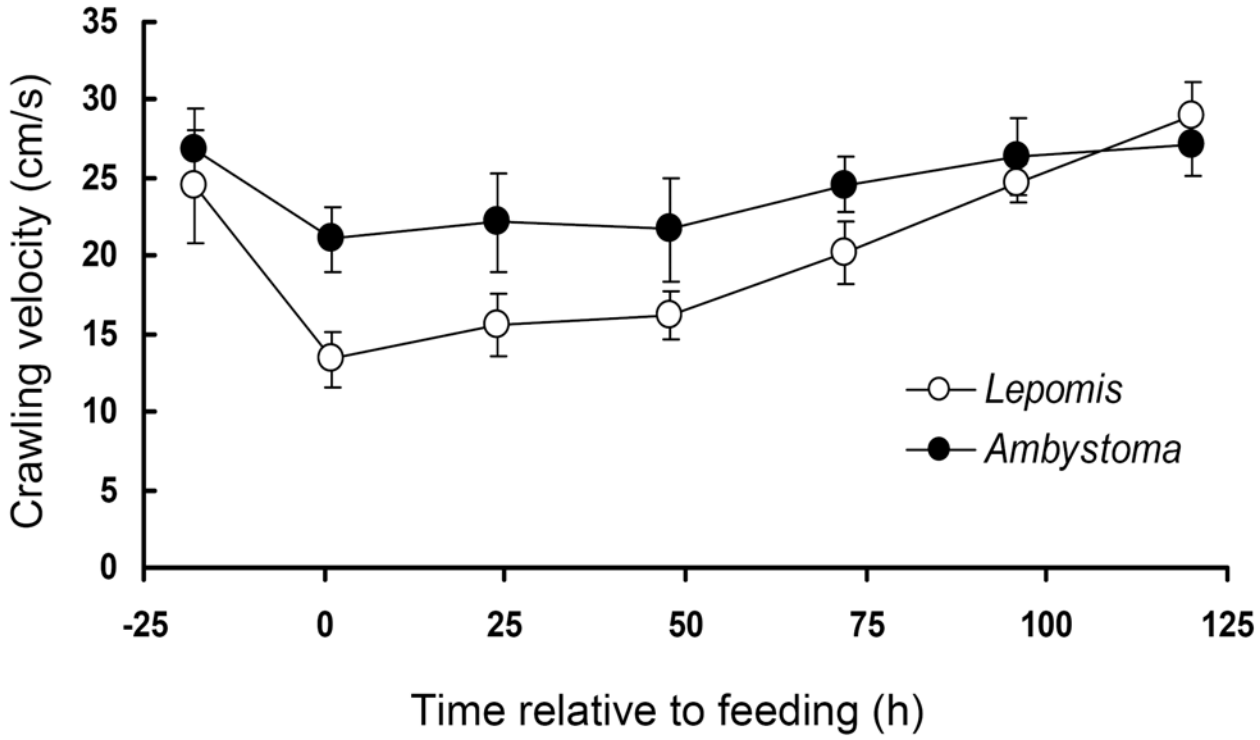

Figure 5.7 Post-feeding locomotor impairment of juvenile *Nerodia fasciata* (n = 8 per treatment) fed *Ambystoma talpoideum* (●) and *Lepomis marginatus* (○). Plots represent mean (± 1 SE) maximum crawling speeds over a 60 cm interval 18 h prior to feeding and 1 h, 24 h, 48 h, 72 h, 96 h, and 120 h after consuming a *L. marginatus* or *A. talpoideum* equal to 25% of the snake's body mass.

CHAPTER 6

THE ROLE OF ECTOTHERMY IN VERTEBRATE PREDATOR-PREY DYNAMICS: DOES INCREASING METABOLIC EFFICIENCY ALTER THE STABILITY OF PREDATOR-PREY INTERACTIONS?[5]

[5] Willson, J. D. and P. Rohani. To be submitted to *Proceedings of the Royal Society B*.

INTRODUCTION

Predator-prey interactions have long fascinated ecologists, prompting extensive theoretical and empirical investigation. Although the relative importance of bottom-up (resource-based), top-down (predation-based), and extrinsic (climate-based) forces in regulating animal populations remains hotly debated, the study of predator-prey interactions has shed considerable light on numerous ecological processes. Among the most fruitful in unraveling the impacts of predation on prey populations have been investigations of the characteristically cyclic dynamics of high latitude rodents, particularly microtine voles and lemmings (reviewed in Hanski *et al.* 2001; Korpimaki *et al.* 2004). A major conclusion of this vast body of literature has been that life-history differences between predator functional groups can result in profound differences in how predators and prey interact. For example, geographic differences in rodent-predator dynamics can result from differences in the relative abundance of specialist and generalist predators within ecosystems. The ability of generalist predators to switch prey types allows them to maintain high population densities and respond rapidly to increases in prey abundance, stabilizing population dynamics (Andersson & Erlinge 1977; Hanski, Hansson & Henttonen 1991).

Snakes have historically been underrepresented in the ecological literature (Shine & Bonnet 2000). Although snakes have recently gained popularity as study animals, snake population biology and particularly how snakes interact with prey populations remains poorly understood (Gragg *et al.* 2007; Dorcas & Willson 2009). For example, in a recent review Nowak, Theimer & Schuett (2008) found that of 301 studies of predator-prey dynamics, none focused on terrestrial ectothermic predators. Snakes are strictly carnivorous and many species prey partially or exclusively on small mammals (Mushinsky 1987). Thus, many snakes fall into the category of

specialist predators – species whose survival, growth, and reproduction are tied strongly to abundance of one prey species, or group of related species, though they may occasionally feed on other prey types – as the term is used in the predator-prey literature (Andresson & Erlinge 1977). Although secretive habits make snakes notoriously difficult to sample (Dorcas & Willson 2009), systematic studies have revealed that snakes can exist at incredibly high densities in some ecosystems (e.g. 1200 per ha, Godley 1980; 274 per ha, Sun *et al.* 2001). Moreover, snakes display a suite of physiological and life-history attributes that make them fundamentally different from mammalian and avian predators and snakes are thought to epitomize the 'low energy' lifestyle characteristic of ectothermic vertebrates (Bonnet, Bradshaw & Shine 1998). For example, snakes have extremely high assimilation efficiencies compared to endothermic predators [e.g., *Vipera berus* (adder) 49% vs. *Mustela nivalis* (least weasel) 2.3%; Pough 1980]. Requiring little energy to maintain their metabolism, snakes may allocate additional food ingested directly to storage, growth (juveniles), or reproduction (adults). However, the population dynamic consequences of these physiological and life-history differences on predator-prey interactions remains largely unexplored (but see Nowak, Theimer & Schuett 2008). Although at least two field studies have considered snakes as potential rodent predators, or have included snakes in predator censuses (Lin & Batzli 1995; Norrdahl & Korpimaki 2002), to our knowledge no studies have explicitly incorporated snake population dynamics into predator-prey models.

Predator numerical responses describe the relationship between the number of predators and prey abundance (Andersson & Erlinge 1977). Considerable attention has been paid to how predators differ in their numerical response curves. Migratory or mobile predators can show rapid numerical responses by congregating or migrating to areas of high prey density (Andersson

& Erlinge 1977; Hanski, Hansson & Henttonen 1991). Alternatively, among non-migratory (resident) specialist predators, the numerical response is primarily due to birth and death, resulting in a time-lagged response to changes in prey density (Andersson & Erlinge 1977; Hanski, Hansson & Henttonen 1991). Snakes are generally non-migratory and have small home ranges compared to comparable endothermic predators. Thus, specialist snake species undoubtedly display lagged numerical responses, perhaps even more strongly-lagged than those of endothermic specialists (Nowak, Theimer & Schuett 2008). However, the low energetic needs of ectotherms, combined with the ability to efficiently translate captured prey into reproduction or storage, make the shapes of vital rate curves (defined as the relationships between reproductive output, growth, or survival and prey abundance) profoundly different from those of endotherms (Nowak, Theimer & Schuett 2008). Although the dynamic consequences of lagged versus immediate numerical responses have been studied extensively, the sensitivity of vital rates to changes in prey density and the consequences of shifts in the slopes of vital rate curves remain largely unknown. Herein we compare numerical response and vital rate curves of endothermic and ectothermic vertebrate predators. We then develop a generalized predator-prey model to examine how shifting the shape (= slope) of vital rate curves within a resident specialist predator-prey systems affects predator-prey dynamics. We find that incorporating vital rate curves characteristic of ectothermic predators (specifically temperate snakes) is generally stabilizing to predator-dynamics, suggesting that these predators may exhibit relatively stable relationships with their prey in some cases.

Vital rates and the ectotherm numerical response

A conceptual representation of prey (= energy) allocation illustrates differences in allocation strategies between endothermic and ectothermic vertebrate predators (Fig. 6.1). Due to

endotherms' capabilities for high activity levels and rapid digestion, the upper limit of prey capture (μ_{max}, determined by handling/processing time) is much higher for endotherms than ectotherms (Nowak, Theimer & Schuett 2008). Likewise, a large proportion of the energy accrued by endotherms is allocated to maintenance or is lost as 'surplus killing' or 'waste' and is not translated into increased growth, reproduction, or survival. Thus, because only a relatively small portion of an endothermic predator's energy budget is allocated to reproduction, a direct relationship between overall prey capture rate and vital rates (reproductive output, growth, or survival) would not be expected. More likely, endotherms exhibit steeply-sloped correlations between vital rates and prey consumption above a threshold consumption rate necessary to meet metabolic demands (μ_{crit}). Indeed, authors have noted the inappropriateness of assuming linear relationships between vital rates (e.g., reproduction) and prey abundance in models of endothermic vertebrate predator-prey interactions (Hanski & Korpimaki 1995; Hanski *et al.* 2001).

Field data corroborate the presence of steep, approaching threshold, numerical responses in endothermic resident specialist predators. Both stoats (*Mustela erminea*) in Greenland and lynx (Lynx canadensis) the Yukon, showed steep lagged numerical responses to shifts in rodent density with apparent thresholds above which predator abundance increased exponentially in relation to prey abundance (Fig. 6.2a; O'Donogue *et al.* 1997; Gilg, Hanski & Sittler 2003; Gilg *et al.* 2006). Such responses are likely driven by extremely low reproductive output and/or low survivorship below threshold prey densities and rapid increase to maximum reproductive rate above thresholds. Few direct estimates of survivorship are available for specialist mammalian predators, but rapid rates of population decline following rodent population crashes (e.g., Hanski & Korpimaki 1995; King 1983) suggest that survivorship must be low in these cases. Likewise,

several authors have noted frequent cases of complete reproductive failure during years with low prey abundance across several mammalian specialist predator taxa including least weasels (Tapper 1979; Hanski & Korpimaki 1995) and lynx (O'Donogue *et al.* 1997).

Ectotherms, conversely, are characterized by low metabolic demands (Pough 1980) that may be altered by reducing activity or physiological state. Although increased handling and processing time reduces the maximum rate of energy acquisition in ectotherms (Nowak, Theimer & Schuett 2008), only a small portion of the energy budget must be allocated to maintenance (Fig. 6.1). Ectotherms have not been documented to engage in surplus killing (with the exception of occasional reports of snakes killing prey too large for them to swallow) and process captured prey very efficiently (Pough 1980), thus, the majority of acquired energy above μ_{crit} can be allocated directly into reproductive output, growth or storage. Moreover, low metabolic demands facilitate use of stored energy to buffer against resource shortages, which weakens the relationship between prey availability and survivorship. As a result, we expect vital rate curves of ectothermic predators to relate more linearly to prey abundance and have shallower slopes than those of endotherms.

Field data support these expected relationships. Whereas endotherms displayed steep, threshold-like numerical responses to rodent density (Fig. 6.2a), Australian water pythons (*Liasis fuscus*) displayed a much weaker and more linear lagged numerical response (Fig. 6.2b; Madsen *et al.* 2006). Indeed, variation in python numbers was only ca. two-fold, despite order-of-magnitude variations in prey density. In addition, snakes displayed relatively minor shifts in survivorship and reproduction in response to high variation in prey density (Fig. 6.2c, 2d). Even in years of minimal prey abundance, annual survivorship never dropped much below 50%. As opposed to endothermic predators that frequently experience complete reproductive failure

(Hanski & Korpimaki 1995; O'Donogue *et al.* 1997), some female pythons were reproductive in all years. Snakes' resilience to periods of low prey availability likely relates to their high capacity for energy storage and their ability to reduce metabolic demands through behavior. Indeed, female adders (*Vipera aspis*) can rely on stored energy and reproduce successfully even in years when they do not capture a single prey item (Lourdais *et al.* 2003).

It is clear that endothermic specialist predators are characterized by steep vital rate curves that do not relate linearly to overall prey consumption rate. Ecologists have accounted for this in predator-prey models by either 1) representing the relationship between vital rates and prey availability as a step function with a critical prey density, below which vital rates are zero and above which vital rates rapidly increase to their maximum (e.g., Hanski & Korpimaki 1995; Hanski *et al.* 2001; Korpimaki *et al.* 2002) or 2) representing the relationship as a smooth curve with steep slope (e.g., Hanski & Korpimaki 1995). Ectotherms, rather than displaying thresholds, are expected to show more linear relationships between vital rates and per capita prey consumption, allowing them to translate increases in prey consumption into increased vital rates up to the level where prey capture is limited by handling time. Below we formulate a mathematical predator-prey model to explore the dynamic consequences of altering the slope of vital rate curves and make predictions about the stability interactions between snakes and their prey.

MODEL

General predator-prey model

Here we describe a general model of a temperate terrestrial vertebrate predator-prey system, based largely on models of microtine rodents and their mammalian predators (e.g., May

1973; Hanski, Hansson & Henttonen 1991; Hanski & Korpimaki 1995; Turchin & Hanski 1997; Hanski *et al.* 2001; Korpimaki *et al.* 2002). Consider a seasonally-reproducing non-migratory specialist predator-prey system in which R_t and P_t are the total population sizes of the prey and predator, respectively. Predators are divided into two age classes, juveniles (J_t) and adults (A_t). The predators and prey interact in discrete time (1 yr time step) following a ratio-dependent predation process (Korpimaki *et al.* 2002). If μ_{max} is the maximum possible number of prey eaten per predator per time-step (year) and β is the prey density at which predators consume half their maximum number (half-satiation constant), the predator search area (h_t; Maynard Smith & Slatkin 1973) can be described by:

$$h_t = \frac{\mu_{max}}{(\beta + R_t)}$$

and the proportion of prey surviving predation by:

$$\omega_t(R_t, P_t) = \exp(-h_t P_t)$$

This function yields a type II functional response curve characteristic of resident specialist predators, whose upper asymptote and slope are determined by μ_{max} and β, respectively. Additionally, the discrete-time framework induces an implicit time-lag also characteristic of non-nomadic specialist predators.

We recognize that an ongoing debate exists regarding the choice between prey-dependent and ratio-dependent functional responses (Abrams & Ginzburg 2000). Despite the fact that snakes are non-territorial, we use a ratio-dependent predation process because: 1) Abrams & Ginzburg (2000) suggest that purely prey-dependent functional responses are rare and list several non-intuitive processes that may produce ratio-dependent functional responses and 2) ratio-

dependent responses in snakes are supported by field studies demonstrating that when adders (*Vipera berus*) occur at high densities, they exhibit reduced growth rates due to exploitative competition (Lindell & Forsman 1996).

In addition to predation, the prey population is mediated by its own intrinsic density-dependent rate of population change (ψ_t):

$$\psi_t(R_t, \omega_t) = e^{r\left(1-\frac{R_t\omega_t}{K}\right)}$$

Where *K* is the prey carrying capacity and *r* is the maximum rate of population change. We assume a specific sequence of demographic and predation events, with predation occurring prior to intrinsic density-dependence. Biologically, this sequence is equivalent to most predation occurring in the summer (a necessary caveat for temperate ectotherms) and prey density dependence occurring in winter. Altering the sequence of these events has little qualitative effect on our results. The iterative equation for the prey population can be written as:

$$R_{t+1}(R_t, P_t) = R_t\psi_t\omega_t$$

In the general model, the predator population is regulated solely through effects of prey density on predator survivorship. Given the proportion of the prey population surviving predation (ω_t), it follows that the proportion of prey consumed is ($1- \omega_t$) and the number of prey consumed per predator is $\mu_t = R_t (1- \omega_t) / P_t$. Thus, a function relating predator survivorship (θ_t) to per capita prey consumption (μ_t) can be written as follows:

$$\theta_t(R_t, P_t) = \frac{1}{1 + \exp(\mu_{crit} - \mu_t)} \qquad \text{(Equation 1a)}$$

where μ_{crit} is the per capita prey consumption at 50% survivorship. Equation 1a produces a sigmoid curve with a relatively steep slope, analogous to a threshold prey consumption rate (μ_{crit}), above which survivorship of predators is high and below which survivorship declines rapidly to zero. Predators are allowed to kill additional prey beyond μ_{crit}, up to μ_{max}, although this additional killing does not translate into increases in any predator vital rates (survivorship, birth rate, or recruitment rate). Thus, for endotherms, the distance between μ_{crit} and μ_{max} is analogous to the potential for surplus killing.

In the general model, all surviving juveniles at the end of a time step are recruited into the adult stage and all surviving adults reproduce at their maximum litter size (B_P). Likewise, because predators are non-migratory, additions to the adult population are due solely to recruitment of juveniles (i.e., no immigration or emigration occurs). Thus, the iterative equations for the predator-prey system can be expressed in matrix form as:

$$\begin{bmatrix} R \\ J \\ A \end{bmatrix}_{t+1} = \begin{bmatrix} \psi_t \omega_t & 0 & 0 \\ 0 & 0 & B_P \theta_t \\ 0 & \theta_t & \theta_t \end{bmatrix} \times \begin{bmatrix} R \\ J \\ A \end{bmatrix}_t \qquad \text{(Equation 2a)}$$

Modeling ectothermic predation

In Eq. 1a we define a survivorship vital rate function that is a sigmoid curve with a steep slope. As noted above, because ectotherms have much lower energetic demands, their vital rates are tied less tightly to current prey availability. We can simulate the effects of such resilience by

introducing a variable to Eq 1a that alters the slope of the survivorship curve (λ – the 'degree of ectothermy'). We can now define a general vital rate function (ϕ_t) that has a variable slope:

$$\phi_t(R_t, P_t) = \frac{1}{1 + \exp(\mu_{crit} - \mu_t)^{1-\lambda}} \qquad \text{(Equation 1b)}$$

Varying λ from 0 to 1 shifts the slope of the vital rate curve away from a steep, threshold-type curve towards a linear relationship between per capita prey consumption (μ_t) and vital rates (ϕ_t; Fig. 6.3).

Prey-dependent survivorship model

The general model includes a survivorship function (θ_t) that can be considered an extreme characterization of an endothermic predator with high and static metabolic demands. If these demands are not met ($\mu_t < \mu_{crit}$), survivorship declines rapidly to zero and surplus killing above μ_{crit} does not translate into higher vital rates. Because ectotherms have much lower energetic demands, their survivorship is tied less tightly to current prey availability and they are less prone to starvation during short-term prey shortages. We can simulate this difference by replacing θ_t (general predator survivorship) with ϕ_t (ectothermy-mediated survivorship). Now, high values of λ produce a relatively linear relationship between survivorship and prey consumption and 'surplus killing' (predation that does not translate into increased vital rates) does not occur.

Prey-dependent birth model

Thus far, litter size has been considered a static parameter and all surviving predators have been allowed to reproduce at their maximum litter size (B_P). Again, this could be

considered an extreme characterization of an endothermic predator that has a fairly small potential litter size and generally makes a 'decision' to reproduce at maximal rate or not reproduce at all based on current prey availability. Comparatively, most large ectothermic predators have relatively large maximum litter sizes and are able to adjust their litter size based on prey availability. Alternatively, many species alter reproductive output by adjusting frequency of reproduction, such that the birth function could also represent the proportion of females reproducing, rather than litter size per female. We can model the influence of ectothermy on the birth process by adjusting the maximum predator birth rate (B_P) by ϕ_t:

$$\begin{bmatrix} R \\ J \\ A \end{bmatrix}_{t+1} = \begin{bmatrix} \psi_t \omega_t & 0 & 0 \\ 0 & 0 & \phi_t B_P \theta_t \\ 0 & \theta_t & \theta_t \end{bmatrix} \times \begin{bmatrix} R \\ A \\ J \end{bmatrix}_t \quad \text{(Equation 2b)}$$

In Eq. 2b, when $\lambda = 0$, the model approximates a threshold response; below μ_{crit} predator reproduction declines rapidly to zero and above μ_{crit} predator reproduction rapidly rises to its maximum (B_P). As λ increases, the relationship between number of offspring produced and prey consumption becomes increasingly linear, such that instead to engaging in surplus killing, prey consumption above μ_{crit} is converted into additional offspring.

Prey-dependent recruitment model

Thus far, recruitment has been solely a function of time and survivorship. This is analogous to the recruitment process of most endotherms, which display fairly static juvenile periods, provided they survive to recruit. In most ectotherms, however, maturity is a function of accumulated growth, rather than time, and many ectotherms exhibit highly plastic juvenile periods that are tightly linked to resource availability. As in the previous models, we can

simulate the effects of ectothermy on growth and subsequent recruitment of juveniles by introducing ϕ_t to the recruitment function within the predator-prey matrix:

$$\begin{bmatrix} R \\ J \\ A \end{bmatrix}_{t+1} = \begin{bmatrix} \psi_t \omega_t & 0 & 0 \\ 0 & (1-\phi_t)\theta_t & B_P \theta_t \\ 0 & \phi_t \theta_t & \theta_t \end{bmatrix} \times \begin{bmatrix} R \\ J \\ A \end{bmatrix}_t \quad \text{(Equation 2c)}$$

In Eq. 2c, recruitment rate is modified by per capita prey consumption and increasing values of λ produce a more linear relationship between prey consumption and the proportion of juveniles recruited into the adult stage. The transition from juvenile to adult is now a product of recruitment (ϕ_t) and survivorship (θ_t), while the quantity $(1-\phi_t)\theta_t$ represents the number of surviving juveniles that remain in the juvenile stage.

PARAMETER ESTIMATION

Although our model represents a generalized predator-prey system, it is based on models of microtine rodents and mammalian predators (e.g., May 1973; Hanski, Hansson & Henttonen 1991; Hanski & Korpimaki 1995; Turchin & Hanski 1997; Hanski *et al.* 2001; Korpimaki *et al.* 2002). These studies have relied heavily on field data (their own and sources cited therein) to parameterize their models, providing substantial information on northern European microtine rodents and their predators. In northern Europe, the primary resident specialist predator of microtine rodents is the least weasel (*Mustela nivalis*). This species is particularly interesting from the perspective of this study because its basal metabolism is 2-3 times higher than would be expected for a mammal of its size, presumably due to its elongate body form (Erlinge 1974), and it readily engages in surplus killing (Oksanen, Oksanen & Fretwell 1985). Thus, the *M. nivalis*

represents and extreme characterization of an endothermic predator. Fortuitously, northern Europe is also home to a common snake, the adder (*Vipera berus*), that also specializes on microtine rodent prey (> 90% of snake diet, Lindell & Forsman 1996). *Vipera berus* is one of the most heavily-studied snake species, providing data on population biology, diet, physiology, and especially its responses to prey availability (Parker & Plummer 1987). Thus, the physiological and life history attributes of least weasels and adders, coupled with the availability of data make these species ideal model endothermic and ectothermic predators upon which to parameterize our model. Estimated parameter values for weasels and adders are provided in Table 6.1, along with parameter values used in model simulations. Of particular note, no previous studies have calculated μ_{crit} for *V. berus* directly. Thus, we estimated μ_{crit} for *V. berus* by calculating the number of voles necessary to meet the resting metabolic needs of an average-sized *V. berus* (125 g), using data provided in Lindell & Forsman (1996). We divided the mean resting metabolic rate of *V. berus* (358 kJ/yr) by *V. berus* assimilation efficiency (0.836) to ascertain that each adder must consume 428.23 kJ per year to survive. We then divided this value by the mean energy content of a vole (61.04 kJ) to obtain the number of voles that must be consumed per year. Finally, we divide this number by two since only female voles are considered in the model to arrive at a μ_{crit} value of 3.51 for *V. berus* (Table 6.1).

RESULTS

We simulated the general model across varying levels of 'surplus killing' (SK) and predator birth rate (B_P; Fig. 6.4). As the general model includes steep vital rate curves, broadly representing an endothermic predator, the potential for SK in the general model is analogous to the distance between μ_{crit} and μ_{max}. Thus, we simulated increasing levels of SK by holding μ_{crit}

constant and varying μ_{max}. Our results demonstrate that in the general model, increasing SK is profoundly destabilizing to predator-prey dynamics (Fig. 6.4). In fact, except at very low B_P, only levels of SK below 25% produce stable annual cycles. Increasing SK above 25%, predator-prey dynamics pass through small regions of stable multi-annual cycles but quickly move into chaotic dynamics. Although higher levels of SK produce stable dynamics at very low B_P (< 1), only chaos or predator extinction occur when SK is increased above 120%. Increasing B_P also destabilizes predator-prey dynamics, with the most stable dynamics seen at very low rates of predator reproduction ($B_P < 1$). Due to the destabilizing effects of increasing SK and B_P we varied these parameters in further simulations to determine whether increasing the 'degree of ectothermy' by varying the slopes of vital rate curves influences the stability of predator-prey dynamics.

When the three model variants (survivorship, birth, and recruitment) were simulated across varying levels of λ ('degree of ectothermy'), SK, and B_P, it was clear that increasing λ had a generally stabilizing effect on predator-prey dynamics (Fig. 6.5). This trend was particularly pronounced for the survivorship model (Fig. 6.5a, 6.5d), suggesting that an approximately linear relationship between prey consumption and survivorship has a particularly strong stabilizing effect on predator-prey interactions. At low values of λ, only very low values of SK and B_P produced stable dynamics. However, increasing λ resulted in much larger regions of stability. In fact, increasing λ above 0.5 resulted in stable dynamics across large ranges of both SK and B_P. At values of λ between 0.5 and 0.9, stable dynamics were produced at levels of SK up to 150% (Fig. 6.5a). Likewise, at levels of λ between 0.6 and 0.9, stability became insensitive to maximum reproductive rate, with stable dynamics produced even at $B_P > 30$ (Fig. 6.5d). In the survivorship model, stable multiannual dynamics were only prevalent at relatively low values of

λ ($\lambda < 0.6$; Fig. 6.5a, 6.5d), suggesting that multiannual dynamics are more likely to occur in endothermic predator-prey interactions.

The prey-dependent birth model also showed increasing regions of stability with increasing λ (Fig. 6.5b, 6.5e). However, the effect was much less pronounced than that observed in the survivorship model; with levels of SK above 5% or $B_P > 4$ resulting in chaotic dynamics, even at high levels of λ. Modifying the model to make recruitment proportional to prey consumption had a slight negative effect on the relationship between SK and stability of predator-prey interactions (Fig. 6.5c). However, varying λ in the recruitment model did influence the stability of increases in B_P (Fig. 6.5f). Increasing λ above 0.2 in this model produced large regions of stable multiannual cycles.

DISCUSSION

Our results demonstrate that increased potential for surplus killing (predation that does not translate into increased vital rates) by specialist endothermic predators is destabilizing to predator-prey dynamics. This result is not unexpected; endothermic predators have high energetic requirements but can only make limited use of surplus food killed and the cost of handling prey soon outweighs the energetic gain of killing another food item (Oksanen, Oksanen & Fretwell 1985). The result is a lagged response to prey abundance, leading to overcompensation and instability of predator-prey dynamics. Likewise, increasing predator reproductive rate destabilizes dynamics by amplifying the predators' potential to 'overshoot' prey populations, leading to complex dynamics and chaos (May 1974; Hassell, Lawton & May 1976).

For an ectothermic predator, however, little food is necessary for maintenance and surplus prey can be allocated directly to reproductive output, growth (analogous to recruitment in ectotherms), and storage (Andrews 1982; Pough 1980). Concordantly, adjusting the slopes of vital rate curves, allowing a more linear relationship between prey capture rate and vital rates, generally stabilized increases in both μ_{max} relative to μ_{crit} (surplus killing) and maximum predator reproductive rate. Although specialist snake species respond with a lagged numerical response, the ability to compensate for prey density through adjusted survival (during prey declines), birth rate, and recruitment (during increases in prey density) may minimize overshooting that leads to unstable dynamics in lagged systems. Yodzis & Innes (1992) support this conclusion in their analysis of the impacts of body size and metabolic category on consumer-resource dynamics. Using simple models they demonstrate that high maximal ingestion to metabolic respiration rate ratios are stabilizing and conclude that ectotherms should exhibit more stable dynamics and persist on lower resource levels than endotherms. Our results further suggest that of the three vital rates, survivorship may be most influential in stabilizing predator-prey dynamics. This result is not unexpected, as the survivorship function affects all predators, whereas birth and recruitment functions act only on single life stages. Allowing more direct relationships between prey abundance and reproductive and recruitment rates generally stabilized increases in maximum birth rate but did little to improve stability of increases in SK. Finally, a partial mechanism underlying our findings may be our selection of a ratio-dependent functional response (which is translated into the numerical response through μ). The ratio-dependent relationship between prey density, predator density, and vital rates allows some degree of density dependence in snake vital rates which is generally stabilizing to population dynamics (Neubert & Caswell 2000).

Although our results suggest that ectothermic specialist predators are more likely than endotherms to exhibit stable interactions with their prey, the question of how important snakes are as predators within ecosystems remains unresolved (Nowak, Theimer & Schuett 2008). At least two studies have demonstrated that snakes can be important predators in some systems. Godley (1980) estimated that during some seasons juvenile striped swamp snakes (*Regina alleni*), specialist predators upon aquatic odonate larvae, consumed 90.7% of odonates within a Florida water hyacinth ecosystem. Likewise, Ineich *et al.* (2007) estimated that sea snakes (*Laticauda* sp.) consume 36,000 eels annually from the waters surrounding a small (6 ha) islet in the South Pacific Ocean. Is it possible that adders play a role in the dynamics of northern European microtine rodents, despite the fact that they are infrequently encountered during predator surveys (Norrdahl & Korpimaki 2002)? Snakes are notoriously secretive and studies have shown that visual counts are poor indicators of snake abundance (Rodda *et al.* 2005; Dorcas & Willson 2009). Adder densities have been estimated using mark-recapture at 4 – 14 individuals per ha (Table 6.1; Parker & Plummer 1987). This corresponds to 20 – 70 times the maximum densities reported for least weasels during high phases of rodent cycles (Hanski, Hansson & Henttonen 1991; Hanski *et al.* 2001). At such densities, it is probable that adders exert significant predation pressure on vole populations, as predicted for vipers in general by Nowak, Theimer & Schuett (2008). Indeed, Lindell & Forsman (1996) estimated that adders consumed up to 9.4 – 66.1 voles per ha per year on six Swedish islands and noted that islands with more adders hosted lower vole densities. Moreover, experimentally increasing adder density resulted in vole declines, although these trends were not statistically significant (Lindell & Forsman 1996).

We have used vipers and microtine rodents as a model for investigating how one aspect of ectothermic predation (the slopes of vital rate curves) differs from that of endotherms. However, numerous other differences exist between ectothermic and endothermic vertebrate predators that may influence predator-prey interactions. For example, although no snake functional responses have been derived from field data, Nowak, Theimer & Schuett (2008) recently hypothesized that vipers will exhibit lower functional responses than similarly-sized endothermic predators. They also predicted that snakes, especially species that typically rely on capital breeding (e.g., temperate vipers) will display strongly-lagged numerical responses due to the need to accumulate sufficient energetic stores (capital) to fuel reproduction (Nowak, Theimer & Schuett (2008). Investigations of the population dynamic consequences of these and other differences in the mechanics of predation between ectotherms and endotherms, in conjunction with field and laboratory experiments of snakes and their prey, will continue to shed light on the importance of these often-overlooked predators.

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Table 6.1 Model parameter estimates for stereotypical specialist endothermic and ectothermic predators, the least weasel (*Mustela nivalis*) and the adder (*Vipera berus*), and parameter values used in simulations. All values are listed per time step (year). Densities are given per km^2 and all values except population density consider only the female segment of the population. Parameters that are varied in simulations are noted (var). Population density is not a parameter used in simulations but is provided for comparison. Sources: (1) Hanski & Korpimaki 1995, (2) Korpimaki *et al.* 2002, (3) Lindell & Forsman 1996, (4) Andren & Nilson 1983, (5) Hanski *et al.* 2001, (6) Parker & Plummer 1987.

Parameter		***Mustela nivalis***	***Vipera berus***	**used**	**Source**
r	Prey intrinsic rate of increase	-	-	1.5†	
K	Prey carrying capacity	-	-	10,000	1
β	Predator half-satiation constant (prey density)	250 – 1,000	unknown	500	1
μ_{max}	Maximum predator kill rate	420-600	unknown	var	1, 2
μ_{crit}	Consumption at 50% vital rate (= survival threshold)	360	3.51	30	2, 3††
B_p	Maximum predator litter size	3	7	var	2,4
λ	'Degree of ectothermy' (= slope of vital rate curve)	low	high	var	
	Population density (males and females)	1-20	400-1,400	-	5, 6

† Field data demonstrate that microtine rodents can exhibit very high maximum rates of increase ($r > 5$; Hanski & Korpimaki 1995). As high growth rates generally destabilize predator-prey interactions, we selected a substantially lower value of r when parameterizing our model.

†† μ_{crit} for *V. berus* was estimated by calculating the number of voles that must necessarily be consumed for an average-sized *V. berus* (125 g) to meet its resting metabolic needs, using data provided in Lindell & Forsman (1996). See text for details.

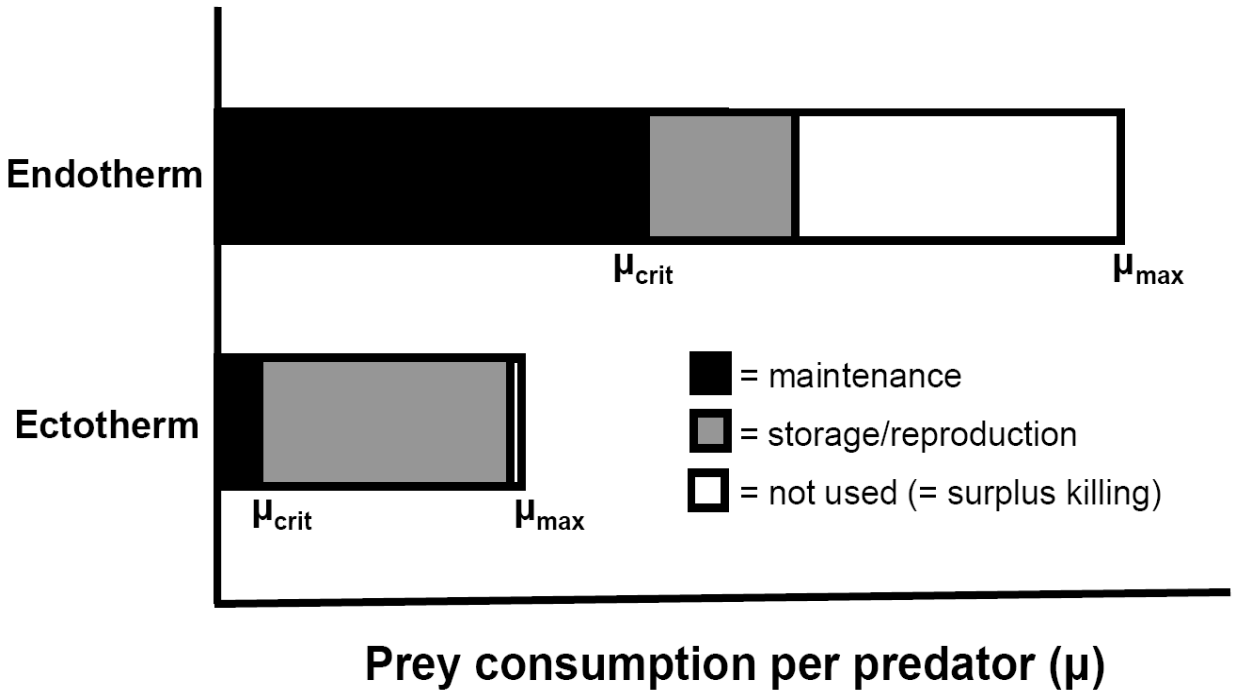

Figure 6.1 Conceptualized representation of prey (= energy) allocation in endothermic and ectothermic vertebrate predators. Predators are limited in the maximum number of prey they can capture (μ_{max}) by handling time, with μ_{max} much higher for endotherms than ectotherms. Predators allocate energy (= captured prey) to maintenance up to the minimum energy required to meet metabolic demands (μ_{crit}). Above μ_{crit}, energy may be allocated to reproduction or storage, with ectotherms having relatively higher capabilities for such conversions than endotherms. At high levels of prey availability, endothermic predators are allowed substantial harvest of prey that does not translate into increased reproduction or storage, 'surplus killing.'

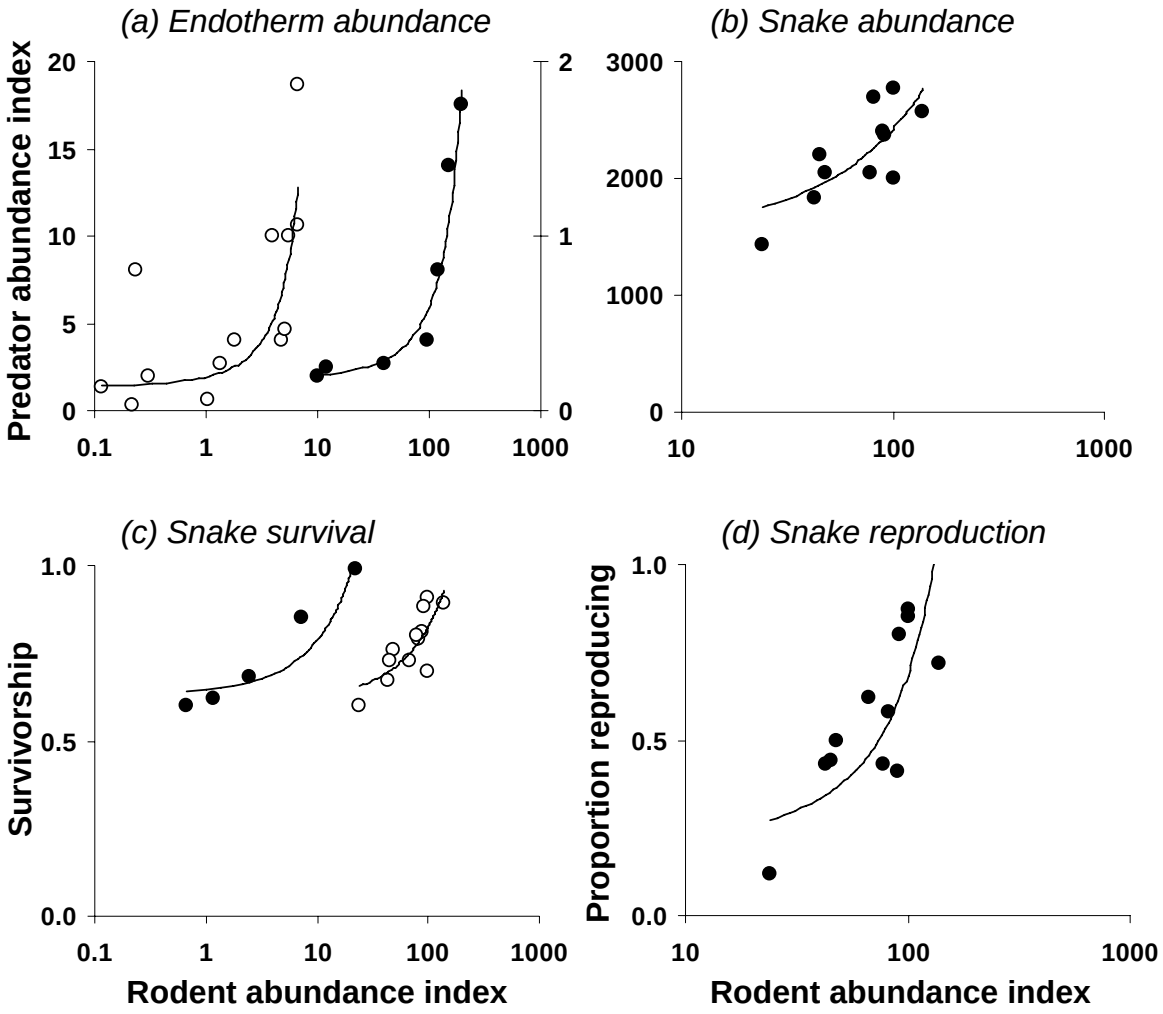

Figure 6.2 Numerical response and vital rate curves of rodent-eating snakes and endothermic specialist predators. (A) Abundance of lynx (*Lynx canadensis*) in the southwest Yukon (●) and stoats (*Mustela erminea*) in Greenland (○) in relation to rodent abundance. (B) Abundance of Australian water pythons (*Liasis fuscus*), and (C) survivorship of snakes, adders (*Vipera berus*) in Sweden (●) and water pythons (○), in relation to prey (rodent) abundance. (D) Reproduction (proportion reproductive) of water pythons at varying prey densities. Predator numerical responses (A and B) are expressed as lagged relationships (i.e., predator abundance is correlated with prey abundance in previous seasons). Note that curves for endotherms (A) are steep, approaching threshold responses, whereas those of ectotherms have much shallower slopes and are more linear. Data are adapted from Gilg *et al.* 2006 (A), O'Donoghue *et al.* 1997 (A), Madsen *et al.* 2006 (B, C, D), and Forsman & Lindell 1997 (C).

ϕ_t

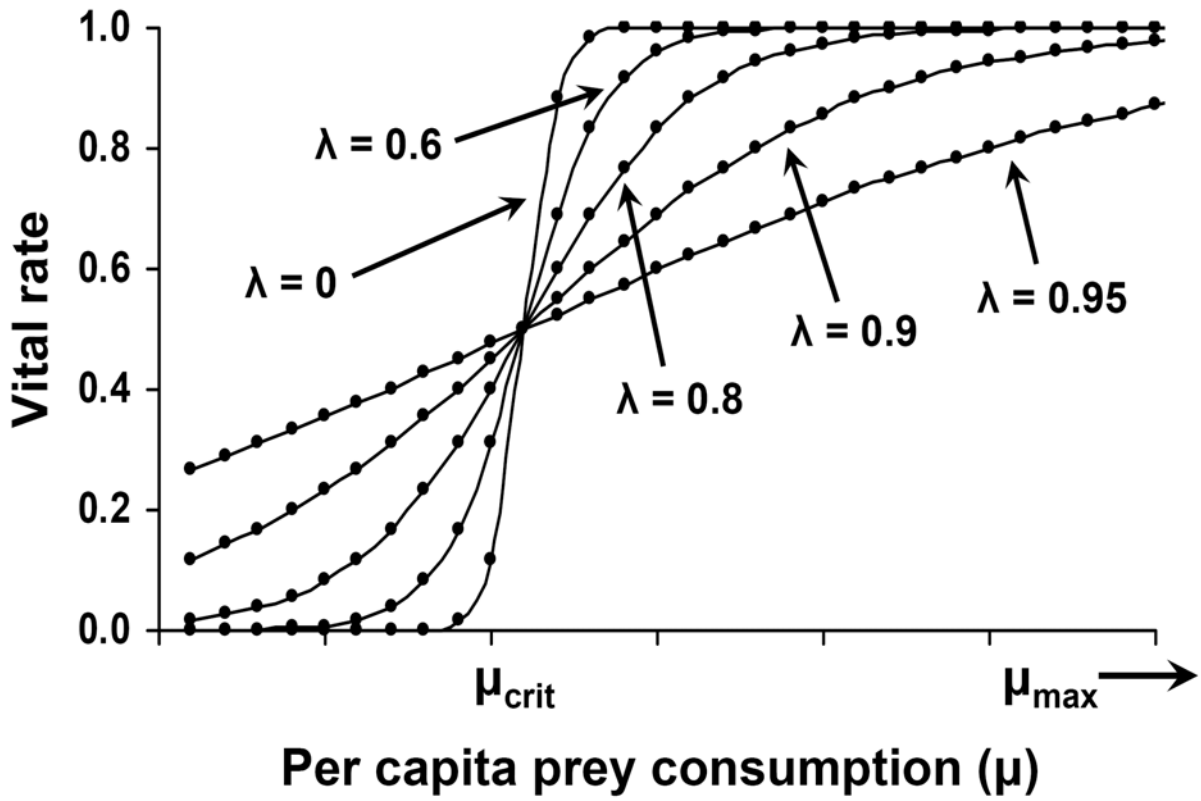

Figure 6.3 Vital rate (survival, reproductive rate, or recruitment rate) curves obtained from the generalized vital rate function (φ_t, Eq. 1b) and varying values of λ ('degree of ectothermy'). Low values of λ produce steep curves, representing vital rate thresholds in endotherms, whereas high values of λ produce progressively linear curves characteristic of ectotherms.

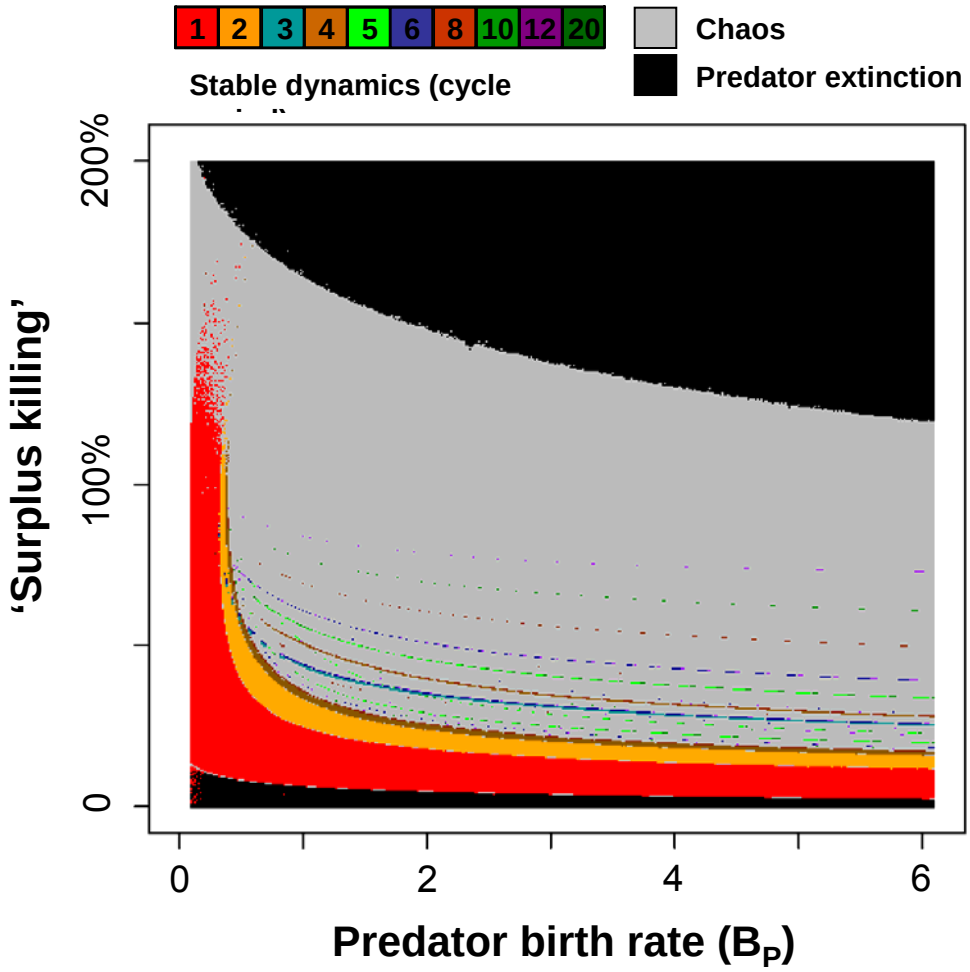

Figure 6.4 Operating diagram of the general predator-prey model, illustrating the destabilizing effects of surplus killing and increased predator birth rate on predator-prey dynamics. 'Surplus killing' is the potential for killing above the minimum needed to meet metabolic requirements (μ_{crit}), expressed relative to μ_{crit}: 'surplus killing' = 100% x (μ_{max} - μ_{crit}) / μ_{crit}. Parameter combinations that produce stable predator-prey dynamics are indicated by colors (annual cycles in red, multiannual cycles in other colors) whereas those that produce unstable dynamics are indicated by gray (chaos) or black (predator extinction).

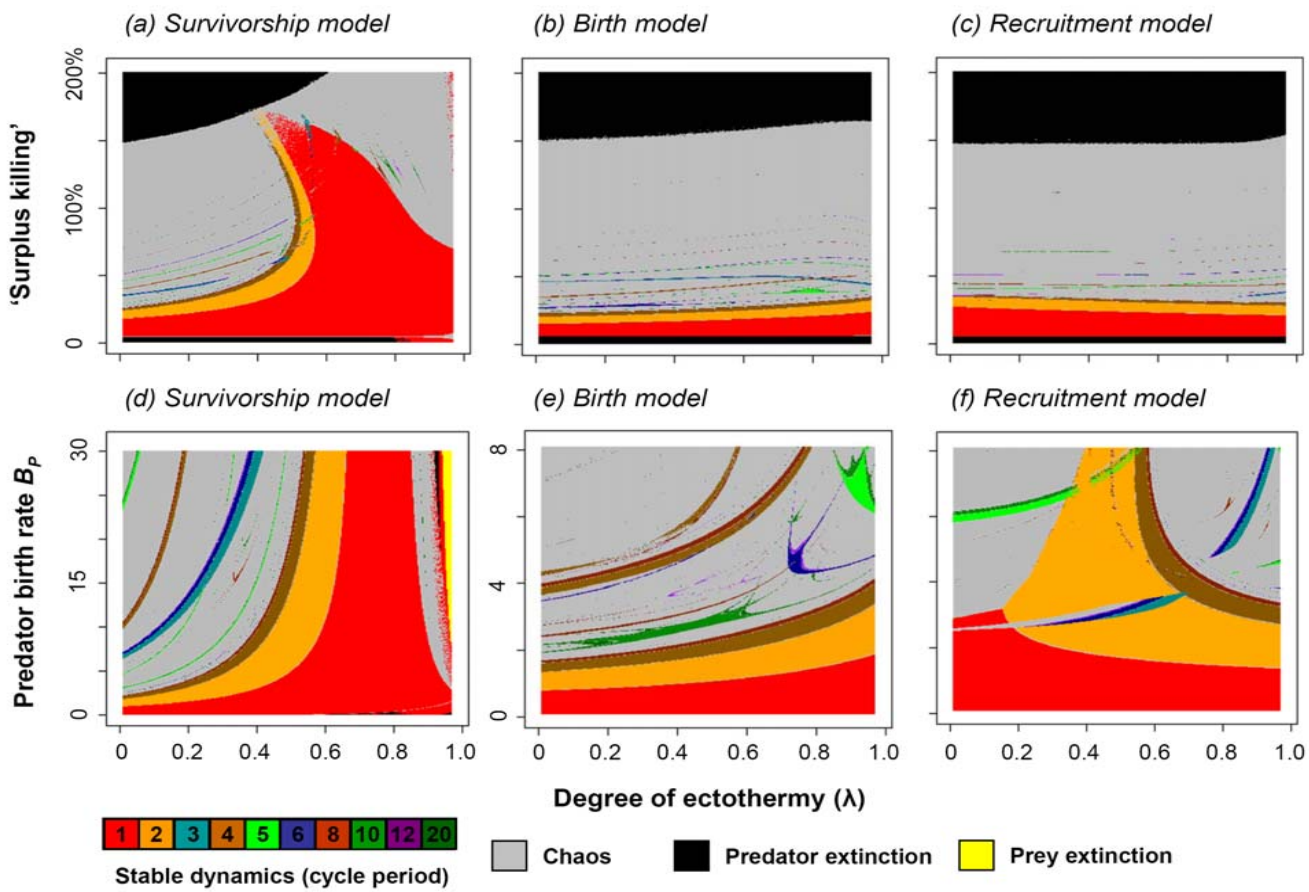

Figure 6.5 Operating diagrams illustrating the stability of predator-prey interactions in which slopes of vital rate curves (survivorship, predator birth rate, recruitment rate) are altered to represent increasing 'degree of ectothermy' (λ) at increasing levels of 'surplus killing' (A-C) and maximum predator reproductive rate (D-F). Low values of λ represent steeper curves characteristic of endotherms. 'Surplus killing' is the potential for killing above the minimum needed to meet metabolic requirements (μ_{crit}), expressed relative to μ_{crit}: 'surplus killing' = 100% x (μ_{max} - μ_{crit}) / μ_{crit}. In (A-C) predator birth rate is held constant at B_P = 2. In (D- F) 'surplus killing' is held constant at 25%. Parameter combinations that produce stable predator-prey dynamics are indicated by colors (annual cycles in red, multiannual cycles in other colors) whereas those that produce unstable dynamics are indicated by gray (chaos), black (predator extinction), or yellow (prey extinction).

CHAPTER 7

CONCLUSION

Understanding the functional roles of species and their contributions to ecosystem function is critical to making informed decisions about the prioritization of limited conservation resources. Unfortunately, most of our knowledge of species' roles is derived from study of common and widespread species, whereas those that are infrequently encountered are often assumed to be rare and of minor functional significance. Snakes are among the most secretive vertebrate taxa and consequently, we know little about snake population ecology, predator-prey relationships, or the roles that snakes play in communities or ecosystems. Prompted by this lack of knowledge, my dissertation sought to use novel and integrative methods to investigate the roles of this inconspicuous and notoriously difficult-to-sample animal group. Specifically, I aimed to 1) use advanced mark-recapture methods, stable isotope analyses, and laboratory experiments to estimate the predatory role of aquatic snakes within an isolated fishless wetland, 2) investigate ways in which prey communities may mediate the roles of aquatic snakes across wetland types, and 3) theoretically evaluate ways in which snakes, as ectotherms, may differ from comparable endothermic predators (e.g., mammals) in their interactions with prey populations.

In Chapter 2, I used aquatic snakes as a case study in applying advanced capture-recapture methods to improve accuracy and precision of population parameter estimates for secretive species. For both *Nerodia fasciata* and *Seminatrix pygaea*, I found evidence that

capture and recapture probabilities were not equal, suggesting that use of standard open population models would violate traditional modeling assumptions and yield biased population size and vital rate estimates. Specifically, I found strong support for time-varying capture and recapture probabilities and strong trap-happy responses in both snake species. I also found evidence for temporary emigration in *S. pygaea* and that rates of temporary emigration varied between the sexes in this species. These results set the stage for generating accurate and precise population estimates for aquatic snakes at Ellenton Bay. Moreover, my study is among the first comprehensive assessments of factors affecting detectability in snakes, providing a framework for studies aimed at monitoring populations of these secretive and often underappreciated species.

In Chapter 3, I used stable isotope techniques to evaluate diets of aquatic snakes at Ellenton Bay, where amphibian breeding migrations cause dramatic seasonal shifts in prey abundance. Although stable isotopes have been proposed as an unbiased tool for quantifying diet, their use requires careful consideration of spatial and temporal variation in prey availability and isotopic composition. I determined that amphibian prey at Ellenton Bay clustered into functional groups based on similarity of isotopic composition and seasonal availability, and that these functional groups varied little in isotopic composition over time. Thus, Ellenton Bay meets provides an ideal opportunity to use stable isotopes as diet indicators. Isotope variance structure indicated that the trophic niche width of *N. fasciata* was broader (more generalist) than that of *S. pygaea*. Moreover, *N. fasciata* exhibited seasonal variation in isotope composition, suggesting seasonal inclusion of adult anurans within snake diets in the spring. Conversely, *S. pygaea* exhibited no seasonal variation but displayed strong ontogenetic shifts in carbon and nitrogen isotope composition that closely paralleled ontogenetic shifts in their primary prey,

paedomorphic mole salamanders (*Ambystoma talpoideum*). These results support my direct assessments of snake diet obtained by conventional gut content analysis. Moreover, although niche dimensions are often treated as static, my results demonstrate that seasonal shifts in niche dimensions can lead to changes in niche overlap between sympatric species. Such short-term fluctuations in niche overlap have implications for our understanding of competitive interactions and consequently the structuring of communities and ecosystems.

In Chapter 4, I assessed the functional role that aquatic snakes play as predators by estimating the biomass of amphibians consumed by snakes at Ellenton Bay over a one year period. Specifically, I synthesized data from Chapters 2 and 3 to estimate snake density and standing biomass at Ellenton Bay, biomass of prey consumed by snakes over the year, and distribution of predation across amphibian prey taxa. Using intensive, systematic sampling and advanced mark-recapture analyses, I estimated that, at peak density, 171 snakes ha^{-1} inhabited Ellenton Bay, corresponding to a mean standing biomass of 7.77 kg ha^{-1}. Further, I calculated that snakes at Ellenton Bay consumed over 200 kg of amphibians annually, translating into > 150,000 kJ ha^{-1} of energy flow from secondary to tertiary consumers within the food web. This total represented nearly 50,000 individual amphibians, the majority of which were *A. talpoideum*. Thus, my data demonstrate that snakes can exist at high densities and play important predatory roles in native aquatic ecosystems in the southeastern United States. Moreover, snakes may play a key role in linking terrestrial and aquatic habitats by sequestering biomass of migratory adult amphibians within wetlands. My results highlight the important predatory role that snakes can play in some ecosystems and stress the importance of questioning the assumption that infrequently encountered species are rare and of minor ecological importance.

Having determined that snakes can be important predators within fishless wetlands, in Chapter 5 I evaluated ways in prey communities may mediate snakes' roles among wetland types. My results suggested that aquatic salamanders (*Ambystoma*), which are characteristic of fishless wetlands, represent a higher quality prey for juvenile watersnakes than sunfish (*Lepomis*), which are characteristic of permanent wetlands. Juvenile *N. fasciat*a were able to consume larger *Ambystoma* than *Lepomis* and consumed more *Ambystoma* at sizes where both prey types could be consumed. *Lepomis* induced greater post-prandial body distension in snakes than did *Ambystoma*, resulting in severely-impaired locomotor performance. Cost of digestion (SDA response) was similar between the prey types, likely reflecting their similar nutritional composition. However, snakes fed *Ambystoma* displayed significantly shorter times to 50% and 75% O_2 consumption rate, suggesting that digestion of *Ambystoma* is slightly more rapid than *Lepomis*. My results document ways in which prey morphology can constrain feeding ecology in gape limited predators, potentially leading to variation in snakes' functional importance among aquatic ecosystems. Specifically, my results suggest that amphibian prey may be particularly important in fueling high densities and rapid individual and population growth of aquatic snakes. Thus, preservation of fishless wetlands may be critical for conservation of aquatic snake species, many of which are imperiled.

In Chapter 6, I broadened the scope of my findings to evaluate how snakes interact with prey populations over long time scales and how snakes may differ in their predatory interactions with prey from comparable endothermic predators (e.g., mammals). I argue that due to high energetic demands, endothermic specialist predators expire rapidly when prey become scarce and have limited abilities to translate abundant prey into reproductive output. Functionally, this creates steep, approaching threshold, curves relating vital rates to prey abundance. Ectotherms,

conversely, have superior capabilities for energy storage and higher reproductive potential, allowing vital rates (birth rate, survival, and recruitment) to respond more linearly to increasing prey abundance. Using a predator-prey model, I explored the population dynamic consequences of these differences and found that shifting vital rate curves from those characteristic of endotherms to more linear curves characteristic of ectotherms was generally stabilizing to predator-prey dynamics. This result suggests that ectothermic vertebrate predators may exhibit relatively stable relationships with their prey and provides an important step in understanding fundamental differences between endothermic and ectothermic predator-prey interactions.