

Stable Isotope Characterization of Community Trophic Structures in Assemblages of
Aquatic and Terrestrial Herpetofauna

By
Dale M. Jefferson

A Thesis Submitted to
Saint Mary's University, Halifax, Nova Scotia
in Partial Fulfillment of the Requirements for
the Degree of MSc. in Applied Science.

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Table of Contents

Acknowledgements	III
List of Tables	IV
List of Figures	V
Abstract	VI
General Introduction	1
Chapter 1: Identification of predictable patterns in the structure of natural aquatic amphibian assemblages using stable isotope analysis	
Abstract	10
Introduction	11
Materials and Methods	
Field Collections	15
Sample Preparation and Analysis	15
Data Analysis	16
Results	18
Discussion	20
Tables	28
Figures	30
Chapter 2: Application of stable isotope analysis to identify and quantify predictable patterns and in a herpetofaunal food web	
Abstract	35
Introduction	36
Materials and Methods	

Field Collections	39
Sample Preparation and Analysis	40
Data Analysis	41
Results	42
Discussion	45
Tables	53
Figures	55
Summary	60
Literature Cited	66

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“Human beings, who are almost unique in having the ability to learn from the experience of others, are also remarkable for their apparent disinclination to do so.”

-Douglas Adams, “Last Chance to See”

“The system of life on this planet is so astoundingly complex that it was a long time before man even realised that it was a system at all and that it wasn't something that was just there.”

-Douglas Adams, “Last Chance to See”

List of Tables

Chapter 1

Table 1.1: Surface areas of study ponds.

Table 1.2: Summary of aquatic specimen collections.

Table 1.3: Univariate F tests of larval amphibian species.

Table 1.4: Univariate F tests of collection sites.

Table 1.5: Univariate F tests for interaction between species and sites.

Chapter 2

Table 2.1: Summary of specimen collections.

Table 2.2: Morphometrics of snake specimens.

Table 2.3: Mean (± 1 SE) trophic position of herpetofauna and grasshoppers in Nova Scotia determined from stable isotope data.

List of Figures

Chapter 1

Figure 1.1: Map of wetland collection sites in Nova Scotia.

Figure 1.2: Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (± 1 SE) of coexisting amphibian species.

Figure 1.3: Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (± 1 SE) of larval spotted salamanders and wood frog tadpoles in ponds absent of spring peeper tadpoles.

Figure 1.4: Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (± 1 SE) of wood frog tadpoles and water tigers in natural ponds.

Figure 1.5: Comparison of mean stable isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) values (± 1 SE) of amphibian communities in nitrogen polluted and unpolluted ponds.

Chapter 2

Figure 2.1: Map of collection sites of snakes, redback salamanders, and grasshoppers in Nova Scotia.

Figure 2.2: Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (± 1 SE) of snakes and salamanders among locations.

Figure 2.3: Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (± 1 SE) of snakes, frogs, salamanders and grasshoppers.

Figure 2.4: Mean trophic positions (± 1 SE) of grasshoppers and herpetofauna.

Figure 2.5: Mean stable isotope values (± 1 SE) of snakes, frogs, alligators, and aquatic salamanders. Reproduced from Aresco and James (2005).

Stable Isotope Characterization of Community Trophic Structures in Assemblages of Aquatic and Terrestrial Herpetofauna

By Dale M. Jefferson

Abstract

Traditional methods of studying the trophic ecology of herpetofauna are problematic and are under increasing criticism because they may not accurately represent natural systems. The purpose of this study was to assess the utility of stable isotope analyses of carbon and nitrogen to quantify characteristics of trophic structures of natural aquatic and terrestrial assemblages of herpetofauna. Stable isotope analysis was performed on species in herpetofaunal communities in Nova Scotia, Canada. Recurrent patterns in the isotopic data provided quantified observations of potential predator-prey interactions among species, dietary overlap and partitioning, and was useful in estimating the relative trophic position of each species. The results of this study, in addition to providing quantified data of herpetofaunal trophic ecology, validated the application of stable isotope analysis in the study of natural communities. This study provides the basis for quantifying interactions and estimating trophic position of herpetofauna using stable isotope analysis.

November 6, 2008

General Introduction

The concept of biological communities originated from the observation of plants and animals coexisting in natural assemblages (Wilbur, 1972). Allee et al. (1949) defined communities as “a natural assemblage of organisms which, together with its habitat, has reached a survival level such that it is relatively independent of adjacent assemblages of equal rank; to this extent, given radiant energy, it is self-sustaining.” The goal of community ecology, as suggested by MacArthur (1972), is to describe general rules that govern the structure and organization of these assemblages. Conversely, Lawton (1999) and Simberloff (2004) both suggest the complex nature of communities make them resistant to the application of general rules. Nevertheless, underlying ecological processes have been shown to regulate the composition of communities by influencing the distribution and abundance of species (Aresco and James, 2005) producing predictable patterns (Paine, 1980). Identification of the mechanisms that determine community organization is a fundamental goal in ecology (Aresco and James, 2005; Dayton and Fitzgerald, 2001; Persson et al., 1992). Ecological studies have attempted to identify these mechanisms through general unifying theories (i.e., Diamond, 1975; Hubbell, 2001; etc.) and through vigorous assessment of interactions within specific communities (i.e., Skelly, 1995a; 1995b; 1996; 2002; Smith, 2006; Sredl and Collins, 1992; Syväranta and Jones, 2008; Wellborn et al., 1996; Wilbur 1972; 1987; Wilbur and Fauth, 1990; Wilbur et al., 1983; etc.).

Community structure arises through a number of mechanisms (Cortwright and Nelson, 1990). Interactions between the biological and physical environment can produce predictable patterns in community structure (Gibbons, 2003; Wellborn et al., 1996).

Biological composition, and interactions within communities are strongly influenced by physical characteristics of the habitat (Bridges, 1999; Connell, 1975; Eby et al., 2006; Griffiths, 1997; Wellborn et al., 1996). Species occupying temporary wetlands, for example, require specific adaptations, or life histories, that allow for their survival of drying periods; those that do not are naturally excluded (Griffiths, 1997; Wellborn et al., 1996). Interactions between organisms further organize and structure communities (Connell, 1975; Wilbur, 1972; Wilbur et al., 1983).

Trophic interactions, those involving the acquisition of dietary resources, are integral mechanisms governing the organization of natural communities (Chase, 2000; MacNally, 1983; Wilbur, 1987). Paine (1980) stated, "Strong trophic interactions can produce predictable, persistent patterns in the resource guilds". These interactions include competition, interactions within trophic levels, and predation, interactions between trophic levels (Connell, 1975). Predation strongly influences species composition, population densities, size structure, and competitive interactions (Morin, 1983a; 1983b; Wilbur et al., 1983; Cortwright and Nelson, 1990).

Dietary studies have traditionally focused on direct observations of feeding behaviour, analysis of gut and faecal material (Cree et al., 1999), predatory experiments (Skelly, 2002) and/or observations of feeding adaptations (Hillis, 1982; Seale, 1982; Wassersug, 2005). These methods, however, can produce ambiguous and subjective results (Hynes, 1950; Skelly, 2002). Cage effects can influence the outcome of experimental studies, while gut contents are subject to misidentification due to the effects of mastication and digestion of soft tissues (Hynes, 1950; Skelly, 2002). Gut content analyses only provide a snapshot of feeding practices and dietary preferences, and cannot discern consumed from

assimilated resources (McCarthy et al., 2004). Mesocosm studies have recently come under increasing criticism for their potential to overestimate ecological effects (Rubbo et al., 2006; Skelly, 2002).

Food chains and webs are the standard models, which represent observed trophic interactions (Cohen, 1977; Schmitz and Booth, 1997; Wilbur and Fauth, 1990). Elton (1927) coined the term “food chain” as a description of the chain of animal predation. Classic tri-level food chains depict the interaction between a top predator, an intermediate consumer and a primary producer (Aresco and James, 2005). Hansson et al. (1998) suggests food chain theory is one of the most thoroughly tested theories in aquatic ecology.

Food webs are formed from interlocking multiple food chains, and ideally represent the sum of community food chains (Aresco and James, 2005; Banasek-Richter, 2004; Polis, 1991). Paine (1980) described food webs as “idealized pictures of complex trophic patterns that change seasonally and geographically”. Food webs can be used as a framework to assess the importance and magnitude of trophic interactions (Link, 2002). Banasek-Richter (2004) stated that recognition of patterns and regularities in community food webs are required to understand their structure and function. These patterns can only be identified if the food webs among communities are comparable (Banasek-Richter, 2004).

Food chain models have traditionally been constructed from generalized empirical observations of pairwise interactions (Hastings and Powell, 1991; May, 1973; McCarthy et al., 1995; Pimm, 1982; Polis, 1991; Post, 2002a; Wilbur and Fauth, 1990). There is, however, great complexity in the organization and dynamics of natural food webs, which

complicates implementation of food chains (McCarthy et al., 1995; Schmitz and Booth, 1997). Hastings and Powell (1991) noted that models based on two interacting species represent caricatures of ecological systems that can only account for a limited number of naturally occurring phenomena. Polis (1991) stated, "... model systems decrease in stability with more species, more links (connectance), or greater linkage strength." Pimm (1982) noted that stable food web models are simple, consist of few trophic levels, and are compartmentalized with little omnivory or looping.

Food chains are oversimplifications that cannot cope with the inherent complexity of omnivory (Aresco and James, 2005) and looping (Polis, 1991) that commonly occur in natural food webs. Omnivory involves a species consuming prey from multiple trophic levels, and therefore does not conform to the traditional food chain model confounding the assignment of an omnivore to a specific trophic level (Aresco and James, 2005). Polis (1991) describes looping as "...a feeding interaction whereby species A eats species B and B eats C but either B (in mutual predation) or C (in a three species loop) eats A. Cannibalism is a "self-loop" (A eats A)." Complexity associated with natural interactions between more than three species within food webs traditionally results in intractable mathematics, and thus models are often based on simplified assumptions (Schmitz and Booth, 1997). Conversely, trophic position, estimated from isotopic data, is a continuous variable that incorporates the complexity of natural food webs and provides a relativistic hierarchy based on assimilated resources as opposed to feeding behaviour per se (Aresco and James, 2005).

Omnivory, feeding on more than one trophic level, is a common practice of both aquatic and terrestrial animals (Aresco and James, 2005). Omnivory is suggested to be an

influential factor in the regulation of food web dynamics and provides food web stability (McCann and Hastings, 1997; Polis, 1991). However, omnivory limits the application of food web models (Aresco and James, 2005). Additionally, animals often undergo dietary shifts associated with ontogeny (Jefferson and Russell, 2008) and/or environmental conditions (Aresco and James, 2005). Thus, traditional food web models are restricted in their ability to describe realized trophic positioning and trophic niches of animals. Ambiguity in the assessment of animal diets has confounded discrete trophic level assignment of species within food web models, and has resulted in the continued utilization of over simplified food web models (Aresco and James, 2005).

Analysis of stable isotope ratios of carbon and nitrogen is a technique that can be used alone or complementary to traditional methods in the study of trophic ecology (Haubert et al., 2005). Isotopic values indicate the ratio of heavy to light isotopes within a sample compared to that of a standard (Biasatti, 2004; Jardine et al., 2003). This is denoted by a delta symbol (δ) followed by the appropriate heavy isotope (i.e., $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$).

Enrichment is used as a relativistic term to denote differences in ratios of isotopic values among samples. A sample is considered enriched if the proportion of heavy isotopes is greater than that of another sample. Conversely, a sample is considered depleted if it has a lower proportion of heavy isotopes than a comparative sample. Preferential removal of lighter isotopes, through fractionation, results in enrichment because it increases the proportion of heavy isotopes within a sample (Berglund et al., 2001; Persic et al., 2004). Enriched samples exhibit relatively higher (or less negative) isotopic values, while depleted samples exhibit lower (or more negative) isotopic values.

$\delta^{13}\text{C}$ values vary to a greater extent among dietary sources, than among trophic levels

because trophic enrichment of ^{13}C is generally 1‰ or less (Das et al., 2003; Kohzu et al., 2005). $\delta^{13}\text{C}$ values are, therefore, useful as indicators of consumer diet (Das et al., 2003; Haubert et al., 2004; Kiriluk et al., 1999; Kohzu et al., 2005). These values relate to material consumed over a period of days, weeks, or months depending upon the tissues observed (Domi et al., 2005; Haubert et al., 2004; Kiriluk et al., 1999; Lepoint et al., 2004; Persic et al., 2004). $\delta^{13}\text{C}$ values are also useful in tracking carbon flow, and habitat occupation allowing for comparison of the aquatic versus terrestrial contribution to diet (Das et al., 2003; Persic et al., 2004; Sherwood and Rose, 2005). Values of $\delta^{13}\text{C}$ are often negative; this is because the standard used to calculate $\delta^{13}\text{C}$ values, Vienna Pee-Dee belemnite (VPDB), is typically enriched in ^{13}C relative to most sources of organic carbon (Jardine et al., 2003; Lepoint et al., 2004). $\delta^{15}\text{N}$ values undergo progressive trophic enrichment, and provide continuous variables of relative trophic positions of coexisting species (Persic et al., 2004). $\delta^{15}\text{N}$ values increase an average of 3 - 5‰ from one trophic level to the next (Minagawa and Wada, 1984; Peterson and Fry, 1987), however estimates of trophic ^{15}N enrichment are as low as 1.6‰ (Vanderklift and Ponsard, 2003). Values of $\delta^{15}\text{N}$ can then be used to track potential food chains by indicating trophic position of biota in relation to each other (Persic et al., 2004).

Utilization of multiple dietary sources creates a situation where consumers exhibit isotopic signatures that reflect the proportions of individual dietary resources assimilated (Gannes et al., 1997; Phillips and Gregg, 2003). Omnivory and/or consuming resources from different ecosystems (i.e., aquatic versus terrestrial) can produce $\delta^{15}\text{N}$ signatures in consumers that may not be intuitive based on the assumed trophic superiority of a species (Aresco and James, 2005; Ben-David and Schell, 2001; Phillips and Koch, 2002).

Similarly, consumer utilization of multiple dietary resources would reflect $\delta^{13}\text{C}$ values proportional to the importance of each source in the consumer's diet (Gannes et al., 1997). The relative proportions of consumer diets can be estimated using mixing models of stable isotope signatures given analysis of a consumer species and representative dietary resources (Phillips and Koch, 2002).

Stable isotopes have been used to describe the structure of aquatic communities (Jepsen and Winemiller, 2002) and "isotopic niche" of organisms (Newsome et al., 2007). Stable isotope analysis integrates information over different temporal periods and is thus well suited to investigate the components of niche breadth (Newsome et al., 2007). Variance estimates of carbon and nitrogen isotope values can be viewed as measures of omnivory, where minimal variance estimates indicate a specialist feeding population (Sweeting et al., 2005). Variance measures of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values have been proposed as a direct measure of resource utilization or niche width (Bearhop et al., 2004). Therefore, this analytical method simultaneously incorporates trophic position and food web paradigms to illustrate carbon pathways within a community (Post, 2002b). Additionally, dietary variation within a population is exhibited in the variance of the isotopic data (Sv  ranta and Jones, 2008).

Stable isotope analysis is an effective technique in the description and quantification of food webs because it can depict the realized trophic positioning based on natural, and quantifiable data (Yi et al., 2006). Stable isotopes of carbon and nitrogen have been used to describe trophic relationships in a tropical amphibian community (Verburg et al., 2007), and shared resources between a declining anuran and introduced trout (Finlay and Vredenburg, 2007). Stable isotopes have been used to track seasonal movements and

diets in amphibians (Fenolio et al., 2005; 2006), as indicators of dietary resources of tuatara (Cree et al., 1999) and monitor lizards (Struck et al., 2002), and in numerous studies of diet and migration of marine turtles (i.e., Godley et al., 1998; Hatase et al., 2002; Reich et al., 2007; Wallace et al., 2006; etc.). Yi et al. (2006) and Aresco and James (2005) have both used this technique to describe complex food webs that have included herpetofaunal species.

Herpetofauna represent biologically and ecologically complex vertebrates that commonly occupy relatively low trophic levels, and which are integral to the health of many ecosystems (Gibbons, 2003; Griffiths, 1997; Willson and Dorcas, 2004). The unique biology and their life history of these animals make them vulnerable to environmental disturbances, and are currently experiencing global population declines as a result of anthropogenic activities (Araújo et al., 2006; Hecnar and M'Closky, 1997; Hamer et al., 2004; Mazerolle, 2003). Due to the relatively low trophic position of many herpetofauna, and relative lack of socioeconomic value (aside from the pet trade), their importance is often underestimated (Gibbons et al., 2000). However, disruptions at relatively low trophic levels can have consequential effects that resonate throughout food webs and alter community structure (Eby et al., 2006; Hecnar and M'Closky, 1997; Jonsson et al., 2006).

The objective of this project was to validate the usage of stable isotope analysis in the study of herpetofaunal ecology through identification of patterns in the isotopic data indicative of predictable species interactions. Henceforth this study focused on identifying and quantifying potential species interactions, overlap and partitioning of dietary resources among species, estimating trophic position, and indicating exposure to

disturbance specifically nitrogen pollution. This provides a method through which the various aspects of species interactions may be quantified and their importance can be later assessed. This will aid in the assessment of the position and influence of herpetofauna within ecosystems, and can be used to observe and assess direct and indirect effects of disturbances on herpetofaunal ecology.

In Chapter 1, I employed stable isotope analysis of carbon (C) and nitrogen (N) to identify and quantify predictable patterns in trophic interactions within temporary wetland communities. This chapter is primarily focused on predator-prey interactions important to the regulation of larval anuran population dynamics. Additionally, I discuss the relative dietary niche of aquatic amphibian and larval dytiscid species and indicate the effect of nitrogen pollution on stable isotopic signatures.

Stable isotope analysis is again employed in Chapter 2, however, its utility was expanded to depict and quantify the terrestrial herpetofaunal food web of Nova Scotia. In this chapter I discuss potential predator-prey interactions, trophic positioning and the partitioning/overlap in trophic niches among commonly coexisting herpetofaunal species.

Chapter 1

Identification of Predictable Patterns in the Structure of Natural Aquatic Amphibian Assemblages using Stable Isotope Analysis

By Dale M. Jefferson

Abstract

Temporary wetlands represent important habitats for complex assemblages of amphibians. Interspecific interactions and disturbances strongly influence the structure of these communities. Traditional methods used to characterize structural mechanisms are often awkward and potentially unrealistic in their assessment of natural communities. Stable isotope analysis of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) were used in an attempt to characterize and quantify trophic interactions and disturbances in natural communities of amphibians in ponds of Nova Scotia, Canada. Ponds from across Nova Scotia were sampled for larval spotted salamanders, spring peepers, wood frogs and dytiscids. Persistent patterns in the isotopic data indicated potential predator-prey interactions between spotted salamander larvae and spring peeper tadpoles, and between larval dytiscids and wood frog tadpoles. Additionally, the results suggest that co-existing tadpoles partition their diets, and therefore do not directly compete for these resources. Isotopic data also successfully illustrated larval amphibian exposure to nitrogen pollution in predictable areas. The application of stable isotope analysis was therefore successful in characterizing important trophic interactions and disturbances, which may influence the structure of these natural communities.

Introduction

Trophic interactions can produce predictable patterns within communities (Paine, 1980). Paine (1980) states, "One of the continuing triumphs of experimental ecology has been in identifying species native to marine benthic and small freshwater ecosystems that play major roles in community organization." Aquatic ecosystems provide a valuable model for the study of community ecology because they tend to be spatially contained and components can be manipulated (Denton and Beebee, 1997). Subsequently, community dynamics of small wetland ecosystems have been among the most widely studied, and have made significant contributions to the study of community ecology (Denton and Beebee, 1997; Halverson et al., 2003; Morin, 1986).

Amphibians commonly occupy temporary wetlands, and represent an ideal model for the study of community ecology (Babbitt et al., 2003; Denton and Beebee, 1997; Morin, 1983a; 1986; 1987; 1989; Morin et al., 1990). Complex life histories of amphibians occupying temporary wetlands involves rapid development from embryo to metamorphosed anuran or caudate to avoid desiccation or predation (Babbitt et al., 2003; Egan and Paton, 2004; Halverson et al., 2003; Loman, 2004; Wellborn et al., 1996). The transient nature and annually regenerative nature of these ecosystems provides a model of community development and succession (Dayton and Fitzgerald, 2001; Morin, 1987; Wilbur and Alford, 1985). Amphibian assemblages are strongly influenced by predatory and competitive interactions, as well as those with the physical environment (i.e., hydroperiod) (Egan and Paton, 2004; Halverson et al., 2003; Morin 1983a; 1983b; Wellborn et al., 1996; Wilbur, 1972; 1980; 1987).

Laboratory and mesocosm studies have been widely used to test the importance of

trophic interactions within amphibian communities (Skelly, 2002). However, these studies are currently experiencing increasing criticism and scrutiny (Rubbo et al., 2006; Skelly, 2002). Results of these studies potentially overestimate the effects of interactions and probably do not accurately reflect natural processes (Rubbo et al., 2006). Subsequently, the necessity for field experiments has greatly increased (Rubbo et al., 2006; Skelly, 2002). However, experimental specimens are often caged in natural wetlands in field experiments (Skelly, 2002) therefore the potential for cage effects persists. Dietary studies based on gut content analysis has been criticized because these studies only provide a snap shot of feeding practices and results can be subjective and biased (Hynes, 1950; McCarthy et al., 2004). Soft-bodied prey can be digested more rapidly than prey with exoskeletons or tough cell walls; therefore soft-bodied prey will inherently be identified less frequently (Cree et al., 1999; Hynes, 1950; McCarthy et al., 2004). Hynes (1950) identified problems quantifying prey species that have been masticated, such as plant material, where there is no means to distinguish the numbers of individuals consumed.

The criticism of traditional techniques is of particular importance to larval amphibians. Most studies have been overly vague and generalized in their description of larval amphibian diets, especially those of tadpoles (Skelly and Golon, 2003; Quammen and Durtsche, 2003). Due to the confusion in fundamental understanding of larval amphibian diets, it is difficult to assume artificial environments accurately depict natural ecosystem dynamics. It is, therefore, necessary to pursue techniques that reflect natural interactions and resource utilization. This method provides quantified data about the potential interactions among species chosen by the researcher, in natural systems. This largely

surmounts the subjectivity of empirical observations and potential for bias in identification of prey that inherent to analyses of gut contents. Additionally, the application of this method to specimens from natural communities negates the potential for cage effects.

Stable isotope analysis is an increasingly popular technique employed in the study of trophic ecology because it largely surmounts the problems of traditional techniques (Cree et al., 1999). Stable isotopes provide relatively long term dietary information for all individuals, including those with empty guts, does not show bias for or against soft or hard bodied prey, and is indicative of assimilated diet rather than solely upon the ingested resources observed (Cree et al., 1999). This last factor is important, because some species may not equally assimilate material from ingested resources (Skelly and Golon, 2003; Steinwacher and Travis, 1983). Stable isotope values of carbon ($\delta^{13}\text{C}$) typically undergo trophic enrichment of less than 1‰ and can, thus, be used as tracers of carbon pathways and indicators of animal diets (Syväranta and Jones, 2008). Nitrogen values ($\delta^{15}\text{N}$) undergo enrichment between 1.6-3.4‰ between trophic levels, and can be used as continuous variables to identify trophic levels (Berglund et al., 2001; Minawaga and Wada, 1984; Persic et al., 2004; Peterson and Fry, 1987; Vanderklift and Ponsard, 2003).

In this study, ratios of stable carbon and nitrogen isotopes were used to characterize amphibian community structures and dietary niche breadth in an assemblage of commonly co-occurring species in Nova Scotia, Canada. Larvae of the spotted salamander (*Ambystoma maculatum*) exhibit widespread distribution among temporary and fishless ponds of Nova Scotia (Gilhen, 1984). Tadpoles of spring peepers (*Pseudacris crucifer*), and wood frogs (*Lithobates sylvaticus*, formerly *Rana sylvatica*)

commonly coexist with larval spotted salamanders and predatory diving beetle larvae (Dytiscidae). Salamanders and predatory invertebrates represent apical predators within amphibian guilds in fishless wetlands (Sredl and Collins, 1992).

Materials and Methods

Field Collections

We sampled 7 small fishless wetlands across Nova Scotia; Cobequid (P1, 45°44.870'N, 63°47.786'W), Dale Avenue (P2, 45°34.831'N, 63°34.870'W), Harrietsfield (P3, 44°32.804'N, 63°38.467'W), Lewis Lake (P4, 44°41.426'N, 63°50.793'W), Middle Musquodoboit (P5, 45°03.186'N, 63°07.743'W), Point Pleasant Park (P6, 44°37.542'N, 63°34.347'W), and Spryfield (P7, 44°35.998'N, 63°36.427'W) between early May and mid-August 2005 (Fig. 1.1). Wetlands P1, P2, P5, and P6 were permanent ponds, while P3, P4 and P7 were ephemeral pools. All wetlands except P5 were surrounded by, or adjacent to woodland. P5 was an agricultural pond and was visibly eutrophic due to nutrient loading. Wetlands varied considerably in size from 52 m² (P2), to the largest, 2932 m² (P1) (Table 1.1).

Sample Preparation and Analysis

Larval anurans, ranging from Gosner (1960) stage 25-42, and larval salamanders, were collected by dipnet from occupied wetlands throughout the sampling period. Homogenized whole body samples were used to compensate for differences in fractionation between animal tissues (Biasatti, 2004). Frozen amphibian and insect tissues were coarsely homogenized, dried at 70°C for 48-72 hours, and then homogenized to a fine powder by mortar and pestle. Dry powder samples were weighed using a Sartorius microbalance to 0.200 mg (\pm 0.020). Weighed samples were packaged in Elemental Microanalysis Ltd. 5 x 3.5 mm tin capsules and loaded into 96 well culture trays and sent to the Stable Isotopes in Nature Laboratory (SINLab) for stable isotope analysis.

Stable isotope ratios in amphibian and insect samples were determined using a Finnigan

Mat Delta Plus isotope-ratio mass spectrometer (Thermo Finnigan, Bremen, Germany). Values of stable isotopes ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$) were found as the deviance (δ : delta) of the ratio of heavy to light isotopes (i.e., $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) within a sample, to that of an international standard, and expressed in parts per thousand (‰) (Biasatti, 2004; Schmidt et al., 2004). Stable isotope values were quantified and reported as described in Jardine et al. (2003).

Data Analysis

Assemblages of larval amphibians were tested in two groups, those that included wood frogs, spring peepers and spotted salamanders (P1, P3 and P7), and communities where spring peepers were absent (P2, P4 and P6), to test the potential effect of an absent prey source on the diet and trophic position of larval salamanders. Additionally, predator-prey interaction between water tigers and wood frog tadpoles were separately assessed in P3 and P6. Mann-Whitney tests were used to determine differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among Gosner (1960) stages of tadpoles at individual sites.

Normality was assessed using a Shapiro-Wilk test. Stable isotopic data of communities in P1, P3 and P7 violated parametric assumptions, and was subsequently rank transformed for parametric analysis. In the absence of appropriate non-parametric analytical methods, parametric analysis on rank transformed results is more robust to non-normality and heteroscedasticity than parametric tests on results that have not been rank transformed (Conover and Inman, 1981; Hesel and Hirsch, 2002). A two-way multivariate analysis of variance (MANOVA) was used to evaluate differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between larval salamanders, wood frog tadpoles and spring peeper tadpoles. Differences in stable isotopic values among species were assessed using Tukey

pairwise comparisons. A ranked multivariate analysis of variance was required in this instance because multiple independent variables existed and could not be pooled, and implementation of multiple non-parametric tests would have greatly increased the probability of incurring Type I errors. Multivariate analysis of variance was, therefore, used to control the experiment-wide error rate. Multivariate tests could not be used in subsequent analyses because of “empty cells” within the data set. Standard non-parametric tests were used for all further analyses.

Mann-Whitney tests were used to assess differences in stable isotopic signatures between wood frog tadpoles and larval salamanders, and between wood frog tadpoles and larval dytiscids. Differences in stable isotope values among communities of wood frog tadpoles and spotted salamander larvae, and wood frogs tadpoles and dytiscids were determined using Kruskal-Wallis and Mann-Whitney tests, respectively.

^{15}N enrichment of communities P1, P3 and P7 were compared to the P5 community to assess the potential for exposure of amphibians in P5 to nitrogenous pollution using Kruskal-Wallis and Mann-Whitney comparisons. Similarly, Kruskal-Wallis and Mann-Whitney tests were used to determine difference in ^{15}N enrichment of the community P6 compared to communities P2 and P4. These sites were compared due to the similarity in amphibian community composition among sites. A conservative testing procedure was adopted by adjusting significance levels using the Holm-Bonferroni method (Marcus et al., 1976), to reduce the risk of committing a type I error (Sokal and Rohlf, 1995). All analyses were performed on Systat (Wilkinson, 1998).

Results

A total of 82 larval amphibians, and 9 water tigers were collected from 7 wetlands across Nova Scotia (Table 1.2). There were no differences in the isotopic ratios among tadpole developmental stages (Gosner, 1960) at individual collection sites (Mann-Whitney tests, all U not significant). Subsequently, isotope data for conspecific tadpoles at various developmental stages were pooled within collection sites for subsequent analysis.

Consistent patterns in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were observed at individual locations (Fig. 1.2 and 1.3). $\delta^{13}\text{C}$ signatures were similar in both spotted salamanders and spring peepers at individual ponds. Spotted salamanders consistently demonstrated greater $\delta^{15}\text{N}$ values than both anuran species, while wood frogs and spring peepers had similar $\delta^{15}\text{N}$ values. Water tigers were enriched in $\delta^{15}\text{N}$ relative to wood frog tadpoles, but exhibited similar $\delta^{13}\text{C}$ values (Fig. 1.4).

Overall significant differences in isotopic ratios were observed among amphibian species (Hotelling test; $F_{[4,80]} = 10.1$, $P < 0.001$) (Table 1.3) and for individual isotopes ($\delta^{13}\text{C}$, $F_{[2,42]} = 10.9$, $P < 0.001$; $\delta^{15}\text{N}$, $F_{[2,42]} = 10.7$, $P < 0.001$). There were significant differences in $\delta^{13}\text{C}$ values between spring peepers and wood frogs (Tukey pairwise comparisons; $P = 0.001$), and salamanders and wood frogs (pairwise comparisons, $P < 0.01$; Mann-Whitney test, $U = 308$, $P < 0.005$), and no differences detected between salamanders and spring peepers. Wood frogs were less enriched in ^{13}C (more negative) than both spring peepers and salamanders (Fig. 1.2 and 1.3). We observed significant differences in $\delta^{15}\text{N}$ between salamanders and spring peepers (pairwise comparisons, $P = 0.001$), and salamanders and wood frogs (pairwise comparisons, $P < 0.01$; Mann-Whitney test, $U = 250$, $P < 0.05$) and no differences between wood frogs and spring

peepers. Salamanders exhibited the greatest enrichment in ^{15}N (Fig. 1.2 and 1.3). Water tigers were significantly enriched relative to wood frog tadpoles in P3 ($U = 32$, $P = 0.02$) and P6 ($U = 21$, $P = 0.03$), but exhibited similar $\delta^{13}\text{C}$ values in both locations (Fig. 1.4).

There were significant differences in isotopic ratios among sites (Hotelling test, $F_{[4,80]} = 7.9$, $P < 0.001$) (Table 1.4), among sites for $\delta^{13}\text{C}$ ($F_{[2,42]} = 8.3$, $P < 0.001$) and $\delta^{15}\text{N}$ ($F_{[2,42]} = 9.4$, $P < 0.001$) of P1, P3 and P7, and among locations for $\delta^{15}\text{N}$ of P2, P4 and P6 (Kruskal-Wallis test, $H = 14.7$, $P < 0.01$). Additionally, differences in $\delta^{15}\text{N}$ values were observed between P5 and P1, P3 and P7 ($H = 18.9$, $P < 0.01$). *Post-hoc* testing revealed significant differences in $\delta^{13}\text{C}$ between P1 and both P3 and P7 (pairwise comparisons, both $P < 0.001$); no differences were observed between P3 and P7, or P2, P4 and P6. Significant differences in $\delta^{15}\text{N}$ values were observed between P6 and both P2 and P4 ($U = 4$, $P < 0.01$; $U = 18$, $P = 0.02$, respectively), and between P5 and P1 ($U = 0$, $P < 0.01$), P3 ($U = 0$, $P < 0.01$) and P7 ($U = 63$, $P = 0.005$). Differences in stable isotopic values in amphibians among ponds were probably due to differences in the isotopic signatures of basal nutrient sources unique to the individual ponds, and nitrogen pollution from the application of nitrogenous fertilizers to lands surrounding P5 and P6 (Fig. 1.5). Species X pond interactions were not significant in multivariate tests indicating relationships observed among tadpoles and salamanders were similar among ponds P1, P3 and P7 (Table 1.5).

Discussion

Patterns of isotopic values were consistent with the prediction of potential predation upon larval anurans from caudates and water tigers. Caudates commonly represent a predatory threat to coexisting larval amphibian larvae (Cortwright and Nelson, 1990; Brodie and Formanowicz, 1983, Formanowicz, 1986; Formanowicz and Brodie, 1982; Wilbur, 1972; 1987). Cortwright and Nelson (1990) found that predation from larval salamanders was the dominant factor in structuring experimental communities. Calef (1973) reported that 75% of natural mortality of red-legged frog tadpoles was due to salamander predation, and tadpole survival to metamorphosis was approximately 5%.

Consistent patterns in the stable isotopic data indicated potential predator-prey interactions between spring peeper tadpoles and larval salamanders in all cohabitated study ponds. Although spring peeper tadpoles are potential caudate prey, there are no observations of larval spotted salamander predation on spring peeper tadpoles in the literature. Larval spotted salamanders are known to consume red spotted newt larvae (Walters, 1975; Freda, 1983). Spotted salamander larvae also consume eggs and tadpoles of green frogs and grey tree frogs (Walters, 1975). Spotted salamander larvae become cannibalistic under crowded conditions (Degraaf and Yamasaki, 2001). Predation of spring peeper tadpoles has been described in other larger species of *Ambystoma*, as well as for adult red spotted newts (Morin, 1983b; 1986; 1995; Sredl and Collins, 1992).

Prey of larval spotted salamanders established in the literature includes the eggs and larvae of frogs, red spotted newt larvae, larval conspecifics, cladocerans, copepods, oligochaetes, aquatic insects, and other invertebrates (Branch and Altig, 1981; Degraaf and Yamasaki, 2001; Freda, 1983; Gilhen, 1984; Petranka, 1998; Pfingsten and Downs,

1989; Walters, 1975). In the absence of spring peeper tadpoles, larval spotted salamanders retained a superior trophic level relative to wood frogs. This indicates an omnivorous diet of larval spotted salamanders, commonly feeding upon invertebrates at lower trophic levels, as well as opportunistically consuming spring peepers and conspecifics (Degraaf and Yamasaki, 2001). Early pond occupation, oviposition and hatching, and rapid growth of wood frog tadpoles typically allow them to exceed the gape limitations of larval spotted salamanders. Wood frogs therefore represent, at best, infrequent prey of larval salamanders and appear to be at greater risk of predation from large predatory invertebrates (Fig. 1.4).

Wilbur (1972) observed that wood frog tadpoles represented an indirect competitor with larval salamanders by competing with the invertebrate prey of larval salamanders. This potential competition could reduce invertebrate abundance, and therefore negatively influence larval salamander growth and survival (Wilbur, 1972). Burley et al. (2006) found that wood frog tadpoles represent predators of larval salamander eggs. However, contradictory to the findings of Wilbur (1972), wood frog tadpoles did not appear to negatively effect larval salamander growth in a study by Burley et al. (2006). Burley et al. (2006) suggested predation on spotted salamander eggs by wood frog tadpoles releases larval salamanders from intraspecific competition, and offsets the effects of the indirect competition imposed by tadpoles. There was only one instance of overlap in trophic niche between wood frog tadpoles or larval salamanders observed in this study, in the spatially and temporally restrictive location P7 (Fig. 1.2). This suggests that any direct interaction between wood frog tadpoles and larval spotted salamanders is relatively infrequent, and possibly due to spatial restrictions of the habitat. Indirect interaction between these

species could not be confidently assessed because the proposed algal resource and competing invertebrates were not analyzed in this study. Therefore, similarity in diet of wood frog tadpoles and invertebrates, and therefore the potential for competition, could not be determined. Burley et al. (2006) noted that these interactions were density dependant. Therefore, experimental habitats used by Wilbur (1972) may have inflated the competitive effect of wood frog tadpoles on larval salamanders.

Patterns in stable isotopic values were indicative of water tiger predation on wood frog tadpoles in natural wetlands (Fig. 1.4). This supports the numerous studies that have previously observed significant predation on wood frog tadpoles from larval dytiscids (Formanowicz, 1982; Formanowicz and Bobka, 1989; Pearman, 1995; Rubbo et al., 2006). Larval dytiscids are voracious predators that appear indiscriminate to size preference in prey selection, and often engage prey exceeding their own size (Lundkvist et al. 2003). They have been observed consuming upwards of 20 tadpoles a day (Warfe and Barmuta, 2004). The voracity and indiscriminant feeding of water tigers have similarly been illustrated in reports of predation on fish (Lundkvist et al., 2003; Louarn and Cloarec, 1997). Drummond and Wolfe (1981) observed a larval *Dytiscus* (length 50 mm) that successfully attacked, and killed a young garter snake (*Thamnophis elegans*, length 190 mm, weight 2.8 g). Water tigers have also been observed consuming, smaller invertebrate prey (Stout, 1983). The tenacity observed in Dytiscidae larvae feeding behaviour has earned them the common name of water tigers (Boobar et al., 1998).

Water tigers use large piercing mandibles to inject paralytic and digestive enzymes into their prey (Boobar et al., 1998; Drummond and Wolfe, 1981). These enzymes function to paralyze and/or kill prey and subsequently facilitate preoral digestion of prey tissues

(Boobar et al., 1998; Drummond and Wolfe, 1981). This method of feeding frees water tigers from size limitation when selecting prey, and potentially allows them to avoid noxious substances produced within the skin of many tadpole species that make them unpalatable to most predators (Relyea, 2001). Relyea (2001) observed that chemically mediated unpalatability of tadpoles did not dissuade dytiscid predation, and that the skins of unpalatable tadpoles were not consumed. This method of feeding, however, complicates the identification of dytiscid prey through observations of gut content because the ingested material is digested and cannot be visually identified (Tate and Hershey, 2003). Identification of dytiscid diet through stable isotope analysis may also be complicated because dytiscids selectively consume and assimilate tissues of their prey. Differences in isotopic turnover rates among tissues often result in tissue specific isotopic signatures (Gannes et al., 1997; Gannes et al., 1998; Hobson and Clark, 1992a; Hobson and Clark, 1992b; Tieszen et al., 1983), therefore selective consumption and assimilation of prey tissues may influence the isotopic signature reflected by these predators. Most descriptions of water tiger diet have come from direct observations of dytiscid feeding (Tate and Hershey, 2003). However, Tate and Hershey (2003) successfully used molecular techniques (polyacrylamide gel electrophoresis) to identify a small number prey from dytiscid gut contents. Tate and Hershey (2003) also note the presence of multiple unidentified prey.

Water tigers typically use an ambush strategy to capture motile prey near the surface of the water (Formanowicz, 1982; Lundkvist et al., 2003). Wood frog tadpoles are relatively active and moderately sized. Spring peepers by comparison are small, benthic and relatively inactive (Skelly, 1995b). Therefore, wood frog tadpoles probably have a higher

encounter rate with water tigers than spring peeper tadpoles, and represent a more frequent prey species.

Both spring peeper and wood frog tadpoles are explosive breeders; they lay all of their eggs for one season within the span of a few weeks (Gilhen, 1984). This results in initially enormous tadpole populations, which precipitously decline due to high mortality rates in pre-metamorphic life stages (Berven, 1990; Calef, 1973; Herreid and Kinney, 1966). Previous studies have reported less than 10% survivorship of tadpoles to metamorphosis (Calef, 1973; Deevey, 1947; Herreid and Kinney, 1966; Turner, 1962), and suggest this is a typical survivorship rate (Calef, 1973). Amphibian survival, growth and development rates are often associated with drought, predation, pathogens and resource limitations (Brockelman, 1969; Cortwright and Nelson, 1990; Fraser, 1980; Licht, 1974; Morin, 1983a; 1983b; Travis, 1984; Turner, 1962; Wilbur, 1972; Wilbur et al., 1983). Predation can reduce (Wilbur, 1972) and even reverse the impact of competition between species (Morin, 1981). High tadpole densities can increase the risk of death from desiccation due to competition-induced reduction in pre-metamorphic growth rates (Morin, 1983b; Semlitsch, 1993; Wilbur, 1987; Wilbur and Fauth, 1990). Wilbur et al. (1983) suggest that predators positively influence growth in prey species by reducing prey densities before trophic demands of the population exceed available resources. Loman (2004) stated that relatively high population densities lead to relatively low survivorship in tadpoles.

Predictable and consistent patterns of community structure were observed using stable carbon and nitrogen isotopes to describe the “isotopic niche” of wood frogs, spring peepers, and spotted salamanders. Since $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures in the tissues of

consumers are primarily due to diet, differences among species can be attributed to differences in diet and/or differences in assimilation of ingested food (Steinwascher and Travis, 1983). Previous studies have shown detritus as the proportionally dominant material ingested by lithobatid (formerly ranid) tadpoles (Quammen and Durtsche, 2003). Much of this material is likely indigestible and therefore not assimilated into tadpole tissues. Similarities in $\delta^{15}\text{N}$ between wood frogs and spring peepers indicated that these amphibians feed at similar trophic levels, however significant differences in $\delta^{13}\text{C}$ between these species reflected differences in consumed and/or assimilated diet.

Morin and Johnson (1988), and Skelly (1995a, 2002) suggest that wood frog tadpoles compete with spring peeper tadpoles. However, the results of this study suggest tadpole species partition dietary resources. Skelly (2002) observed differences in the competitive effects of wood frog tadpoles on spring peeper tadpoles between mesocosm and natural pond experimental venues. This suggests competitive interactions among larval amphibians are potentially influenced by habitat characteristics and cannot be accurately described through mesocosm or laboratory experiments alone (Skelly, 1995b; 2002).

Trophic enrichment of ^{15}N measured between spring peeper tadpoles and salamander larvae is 1.6‰, and between dytiscids and wood frog tadpoles is 0.4‰. Nitrogen enrichment between predator and prey is typically between 1.6‰ and 3.4‰. The relatively low enrichment is indicative of omnivory of both tadpoles and their predators. Selective ingestion of prey tissues by water tigers may also influence the isotopic signatures they exhibit.

Differences in basal level nutrient sources are probably responsible for fundamental differences in isotopic signatures of conspecifics among locations. Biogenic methane,

produced in anaerobic sediments, is relatively depleted in ^{13}C and can influence carbon signatures of biota within the local food web (Hornibrook et al., 2000; Kelly et al., 2004; Kiyashko et al., 2004; Orphan et al., 2001; Woltemate et al., 1984). Nitrogenous fertilizers can potentially enrich $\delta^{15}\text{N}$ values of soil nitrate, which subsequently enter wetlands in runoff and groundwater (Karr et al. 2001; McCutchan et al., 2003; Rubenstein and Hobson, 2004). Enrichment in ^{15}N of communities in P5 and P6 is probably due to exposure to anthropogenic nitrogen originating from fertilizers applied to surrounding agricultural and recreational lands, respectively (Fig. 1.5) (Jefferson and Russell, 2008). Exposure of amphibians to elevated concentrations of nitrogenous compounds can have lethal and chronic sublethal effects, and has been implicated in their population declines (Baker and Waights, 1994; Berger, 1989; Hamer et al., 2004; Johansson et al., 2001; Xu and Oldham, 1997). While these results represent a potential problem in comparing aquatic communities using stable isotope analysis, they also reflect the potential application of $\delta^{15}\text{N}$ values as indicators of aquatic biota exposure to nitrogen pollution (Jefferson and Russell, 2008).

Stable isotope analyses of carbon and nitrogen present a useful technique to evaluate community structure, predator-prey interactions and resource partitioning among species in palustrine communities. This study demonstrated predictable patterns of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures among amphibians and predatory invertebrates, and thus quantitative estimates of “isotopic niche”. However, putative interspecific interactions derived from stable isotope relationships among species require validation through direct observation. Additionally, differences in isotopic enrichment among wetlands confound comparison of communities unless isotopic values are appropriately adjusted (i.e., Aresco and James,

2005).

The persistent patterns in isotopic data identifying interspecific interactions validated the utility of stable isotope analysis in the study of natural communities. Traditional methods, although useful in identifying and predicting interactions, are relatively inadequate to stable isotope analysis in quantifying these interactions in natural communities. Stable isotope analysis provides the means by which to quantify the importance of species interactions within natural communities, and subsequently produce models reflective of natural systems.

Tables

Table 1.1: Surface areas of study ponds.

Location	Surface Area (m ²)
P1	2932
P2	52
P3	139
P4	72
P5	1466
P6	548
P7	78

Table 1.2: Summary of aquatic specimen collections.

Wetland	Species	Stage	n
P1	Spotted salamander	Larvae	3
	Spring peeper	25	3
	Wood frog	39	3
	Wood frog	44	3
P2	Spotted salamander	Larvae	6
	Wood frog	42	6
P3	Spotted salamander	Larvae	4
	Spring peeper	25	8
	Wood frog	42	6
	Dytiscid	Larvae	6
P4	Spotted salamander	Larvae	6
	Wood frog	41	6
P5	Spotted salamander	Larvae	4
	Spring peeper	38	3
P6	Spotted salamander	Larvae	3
	Wood frog	28	4
	Wood frog	31	3
P7	Spotted salamander	Larvae	3
	Spring peeper	25	2
	Spring peeper	42	3
	Wood frog	25	3
	Dytiscid	Larvae	3

Table 1.3: Univariate F tests of larval amphibian species; wood frogs, spring peepers, and spotted salamanders.

Source	SS	df	MS	F	P
¹³ C	374.6	2	187.3	8.3	0.001
Error	951.8	42	22.7		
¹⁵ N	436.2	2	218.1	9.4	< 0.001
Error	974.4	42	23.2		

Table 1.4: Univariate F tests of collection sites; P1, P3, and P7.

Source	SS	df	MS	F	P
¹³ C	497.8	2	248.9	10.9	< 0.001
Error	951.8	42	22.7		
¹⁵ N	498.6	2	249.3	10.7	< 0.001
Error	974.4	42	23.2		

Table 1.5: Univariate F tests of interactions between species and collection sites; species X site.

Source	SS	df	MS	F	P
¹³ C	25.3	4	6.3	0.3	0.889
Error	951.8	42	22.7		
¹⁵ N	43.8	4	10.9	0.5	0.756
Error	974.4	42	23.2		

Figures

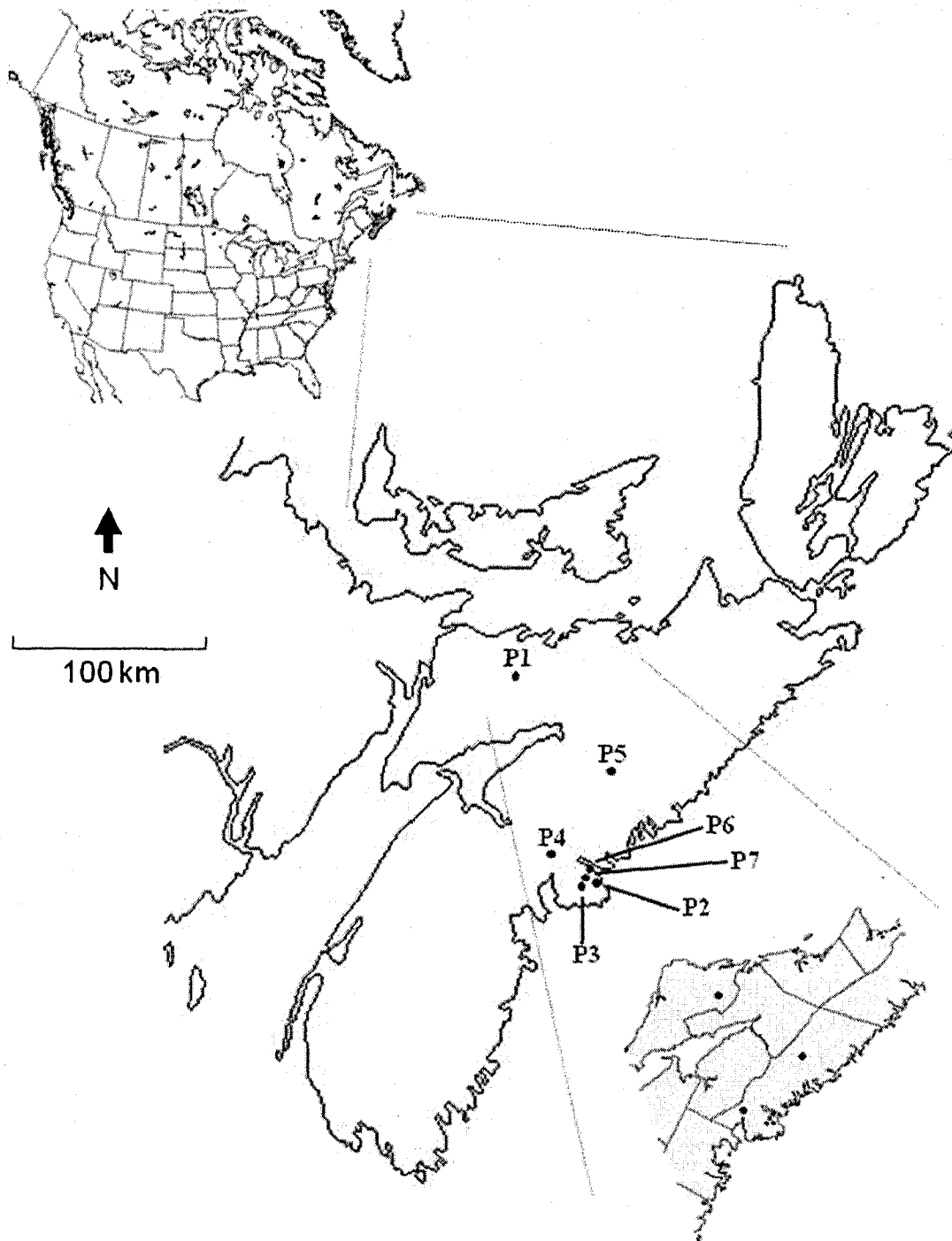


Figure 1.1: Map of wetland collection sites in Nova Scotia.

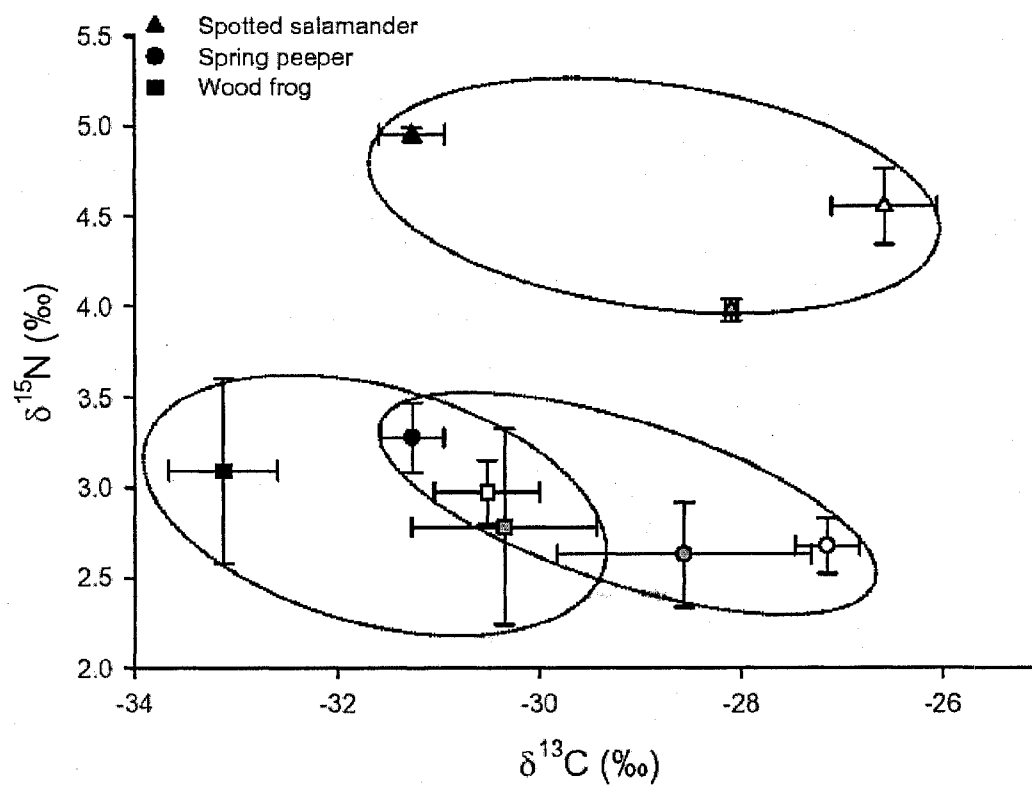


Figure 1.2: Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (± 1 SE) of coexisting amphibian species. Black symbols represent P1, white symbols P3, and grey symbols P7.

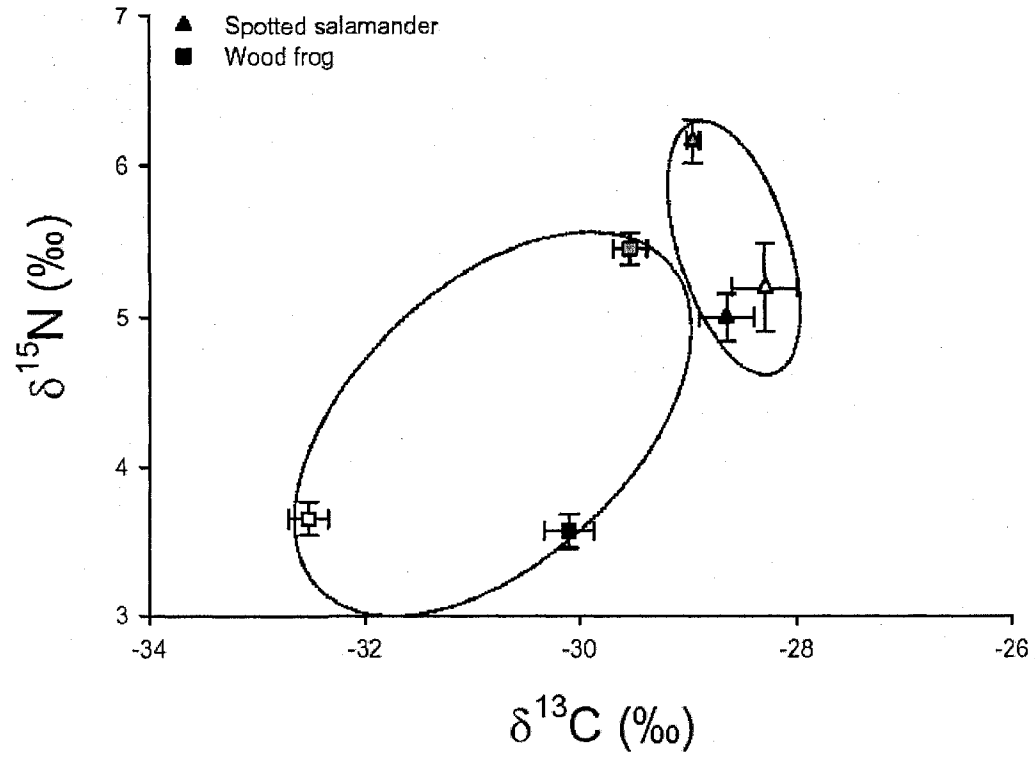


Figure 1.3: Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (± 1 SE) of larval spotted salamanders, and wood frog tadpoles in ponds absent of spring peeper tadpoles. Black symbols represent P2, white symbols represent P4 and grey symbols represent P6.

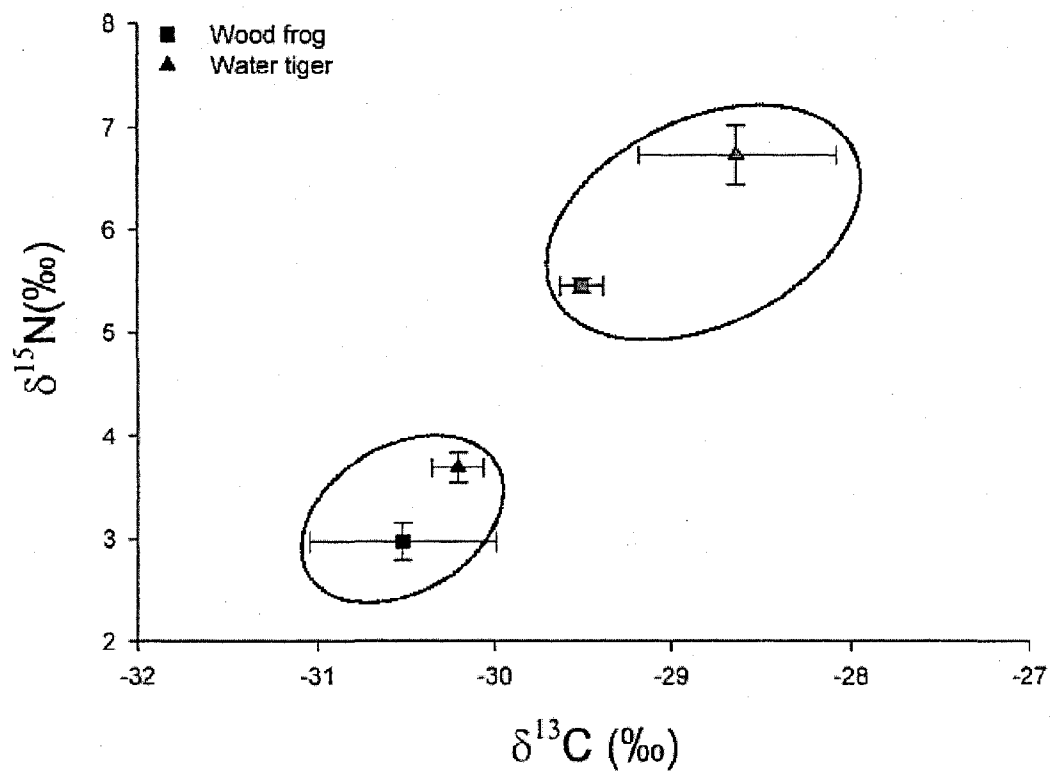


Figure 1.4: Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (± 1 SE) of wood frog tadpoles and water tigers in natural ponds P3 (black symbols) and P6 (grey symbols).

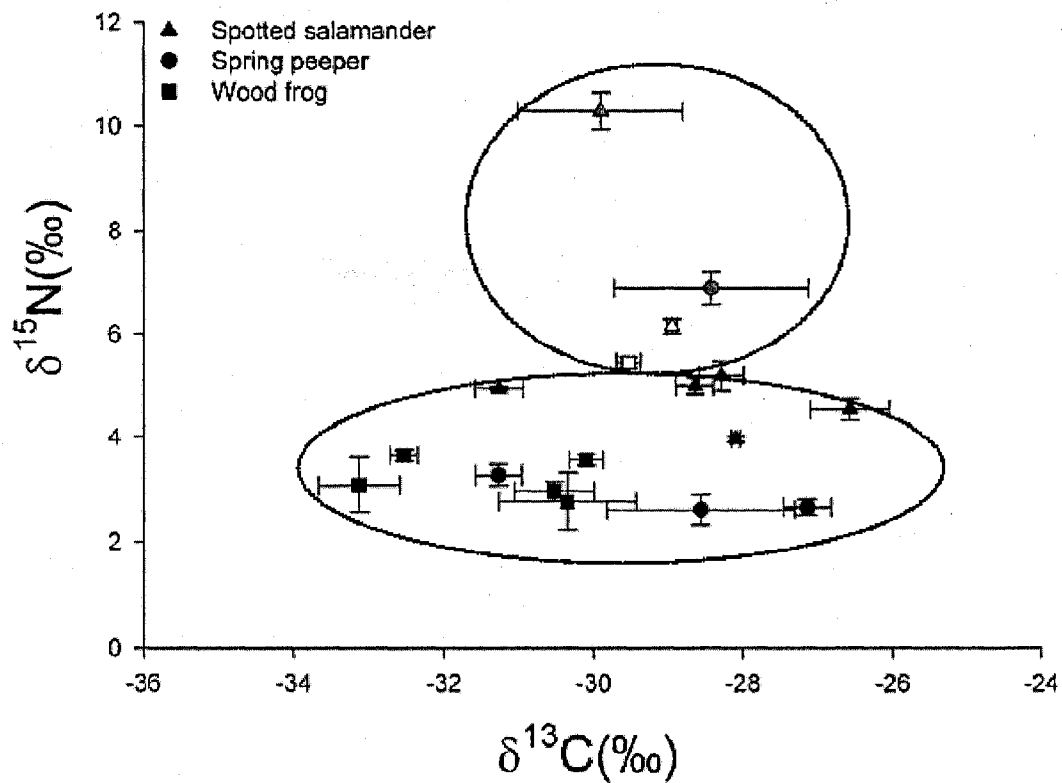


Figure 1.5: Comparison of mean stable isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) values (± 1 SE) of amphibian communities in nitrogen polluted, grey and white symbols (ponds P5 and P6, respectively), and unpolluted ponds, black symbols (ponds P1-P4 and P7). Lines encircle the two distinct groups (polluted and unpolluted) of ponds.

Chapter 2

Application of stable isotope analysis to identify and quantify predictable patterns in a herpetofaunal food web

By Dale M. Jefferson

Abstract

Complex interactions among species of herpetofauna are important in the transfer of nutrients across ecosystem boundaries and between disjunct trophic levels. These interactions have been described in studies using traditional methods, however they have not been well quantified using these methods. Natural abundances of stable isotopes of carbon (C) and nitrogen (N) were used to characterize a food web of primarily terrestrial herpetofauna through identification and quantification of interspecific interactions and relative trophic positioning of species. Prevalent species of snakes, salamanders and grasshoppers were actively collected from three locations in central mainland Nova Scotia, and anurans were collected from numerous locations across mainland Nova Scotia. Results indicated homogeneity in the diets of conspecifics, for each study species, despite spatial and/or temporal separation. Persistent patterns in the isotopic data were indicative of predictable interspecific interactions identified in the existing literature. This validated the application of stable isotope analysis in the characterization of trophic interactions among species within a natural herpetofaunal food web. However, identification of improbable species interactions reinforced the necessity for validation of observations using isotopic data through direct methods. Additionally, although stable isotopic data is more inclusive in terms of dietary information, trophic positioning of species based on ^{15}N signatures may be non-intuitive due to the effects of complex trophic interactions and feeding habitats among species.

Introduction

O'Neill et al. (1986) stated one can "...define ecosystems as the smallest units that can sustain life in isolation from all but atmospheric surroundings. However, one is left with the problem of specifying the area that should be included" (Post et al., 2007). Biota that traverse ecosystem boundaries represent important facilitators of nutrient transfer between these ecosystems (Helfield and Naiman, 2001; Naiman et al., 2002; Pryor, 2003). Studies of ecosystem coupling have primarily focused on the transfer of nutrients between marine/estuarine/freshwater/terrestrial systems and involving fishes (i.e., Doucette et al., 1996; Helfield and Naiman, 2001; Naiman et al., 2002). Ecosystem coupling may be vital to maintaining food webs (Nakano and Murakami, 2001) and to groups of animals including many species of herpetofauna (Gibbons, 2003; Griffiths, 1997).

Herpetofauna include two classes of animals (Amphibia and Reptilia) that form integral parts of ecosystems and are indicators of environmental quality (Gibbons et al., 2000; Willson and Dorcas, 2004). In some regions herpetofauna comprise a large portion of the vertebrate biomass and assume important roles of predators and prey (Aresco and James, 2005; Willson and Dorcas, 2004). However, public opinions of herpetofauna, traditionally, have not been reflective of their importance. This is reflected in Zim and Smith (1953) who stated, "As a group [reptiles] are neither 'good' nor 'bad', but are interesting and unusual, although of minor importance. If they should disappear, it would not make much difference one way or the other". Although our current awareness of herpetofaunal importance is greater today than it has been historically, populations of both reptiles and amphibians are now in precipitous global decline, largely due to

anthropogenic disturbances (Gibbons et al., 2000; Mazerolle, 2003; Rouse et al., 1999; Xu and Oldham, 1997).

Understanding the trophic dynamics of biota is fundamental to comprehension of community and ecosystem ecology (Paine, 1980). Food webs are often constructed from over simplifications of observational studies (Hastings and Powell, 1991), and our understanding of herpetofaunal trophic ecology has been reflective of our misconceptions of these animals (Aresco and James, 2005; Gibbons et al., 2000). This is detrimental to the understanding of herpetofaunal ecology, and subsequently conservation and protection efforts.

An important aspect of herpetofaunal ecology is the transfer of nutrients between disjunct trophic levels and across ecosystem boundaries (i.e., between aquatic and terrestrial habitats) (Waringer-Löschenkohl and Schagerl, 2001). Additionally, herpetofauna are often involved in complex ecological interactions that include omnivory and looping (Aresco and James, 2005; Polis and Myers, 1985). The complexity of these natural systems is often ignored or disregarded as insignificant in the production of food web models, because such complexity creates intractable mathematics (Aresco and James, 2005; Hastings and Powell, 1991; Schmitz and Booth, 1997).

Stable isotope analysis provides an analytical method of characterizing and quantifying natural food webs. The relatively long-term integration of dietary information provided by using this technique reduces the potential for subjectivity in trophic positioning of biota (Cree et al., 1999). Similarly, stable isotope analysis reflects the mixed signatures of all assimilated dietary resources (Aresco and James, 2005). Therefore, omnivory and looping are incorporated into the results of the analysis, and the traditional problems

involving trophic positioning, arising from these complex interactions, are resolved (Aresco and James, 2005). Stable isotope values of carbon are useful in tracing the carbon pathways through food webs, while those of nitrogen are useful as continuous variables identifying relative trophic position.

In this study, I used ratios of stable carbon and nitrogen isotopes to quantify characteristics of a terrestrial herpetofaunal food web in Nova Scotia, Canada. Maritime garter snakes (*Thamnophis sirtalis*), eastern smooth green snakes (*Liochlorophis vernalis*), northern ringneck snakes (*Diadophis punctatus*) and northern redbelly snakes (*Storeria occipitomaculata*) are common native snake species occupying field and forest habitats across Nova Scotia. Redback salamanders (*Plethodon cinereus*), American toads (*Anaxyrus* (formerly *Bufo*) *americanus*), green frogs (*Lithobates* (formerly *Rana*) *clamitans*), wood frogs (*L. sylvaticus* (formerly *Rana sylvatica*)), pickerel frogs (*L.* (formerly *Rana*) *palustris*) and spring peepers (*Pseudacris crucifer*) exhibit nearly ubiquitous dispersal across Nova Scotia, and represent a potentially important prey of many snake species (Gilhen, 1984). Similarly, red-legged grasshoppers (*Melanoplus femurrubrum*) represent a common terrestrial herbivore and potential prey of both amphibians and snakes. Trophic level distribution of species is expected to follow predictable patterns based on observations of diets from Gilhen (1984). The objective of this study was to assess the utility of stable isotope analysis to characterize the trophic structure of a herpetofaunal food web, and to quantify species' trophic position, trophic interactions, and relative omnivory.

Materials and Methods

Field Collections

We sampled 3 terrestrial sites within Halifax County, Nova Scotia; Clayton Park (S1, 44°39.560'N, 63°39.700'W), Uniacke Estates (S2, 44°54.167'N, 63°50.515'W) and Pockwock water shed (S3, 44°50.267'N, 63°47.924'W) (Fig. 2.1). These sites were chosen due to their accessibility, and the presence of all four snake species, redback salamanders and red-legged grasshoppers. All three sites contained open field and wood land habitats, however S2 and S3 include large riverine and lacustrine systems, while S1 contains mainly vernal pools and a few ephemeral palustrine systems. Additionally, S1 is part of an expanding municipal commons surrounded by an urban neighborhood. Woodland and open field habitats of S1 are highly fragmented by roads, athletic fields and complexes and are subsequently subject to a high degree of human disturbance relative to S2 and S3.

Sites were repeatedly sampled between August and September 2007. Inclement weather in the summer of 2007 delayed and truncated the sampling period for both snakes and salamanders as both species were often not found for a period of days following cold wet weather (snakes) or prolonged periods of hot, dry weather (salamanders). These sites were thoroughly traversed over an average period of two hours during each sampling expedition. Various habitats were searched within each collection site. Snakes were often found basking in the open, or hiding under flat stones, logs, boards and/or anthropogenic debris. Redback salamanders were found exclusively under logs, rocks and debris in dark moist environments. Grasshoppers were collected in fields and along dirt roads and paths. Anurans were collected from numerous collection sites across Nova Scotia in years prior to the initiation of this research. All specimens were collected by hand.

Collections of snakes focused on the four most common species in Nova Scotia; maritime garter snakes, eastern smooth green snakes, northern ringneck snakes and northern redbelly snakes. Redback salamanders and large adult grasshoppers were collected as potential snake prey. Redback salamanders are the most common salamander species in eastern Canada and the United States, and are commonly identified as snake prey. Similarly, grasshoppers are frequently listed as snake prey, and red-legged grasshoppers were found in all collection sites. Grasshoppers represent a primary consumer and potential prey of both reptiles and adult frogs.

Sample Preparation and Analysis

Total body lengths and gape sizes were measured and recorded for all snake specimens. Snake, caudate and insect tissues were coarsely homogenized, dried at 70°C for 48-72 hours, and then homogenized to a fine powder by mortar and pestle. Homogenized whole body samples were used to compensate for differences in fractionation between animal tissues (Biasatti, 2004). Dry powder samples were weighed using a Sartorius microbalance to 0.200 mg (± 0.020). Weighed samples were packaged in Elemental Microanalysis Ltd. 5 x 3.5 mm tin capsules and loaded into 96 well culture trays and sent to the Stable Isotopes in Nature Laboratory (SINLab), with associated loading forms, for stable isotope analysis.

Stable isotope ratios in tissue samples were determined using a Finnigan Mat Delta Plus isotope-ratio mass spectrometer (Thermo Finnigan, Bremen, Germany). Values of stable isotopes ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$) were found as the deviance (δ : delta) of the ratio of heavy to light isotopes (i.e., $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) within a sample, to that of an international standard,

and expressed in parts per thousand (‰) (Biasatti, 2004; Schmidt et al., 2004). Stable isotope values were quantified and reported as described in Jardine et al. (2003).

Data Analysis

A Shapiro-Wilk test was used to assess the normality of the data. Kruskal-Wallis comparisons were used to assess differences in body lengths and gape size in each snake species among sampling locations, with the exception of green snakes where a Mann-Whitney test was used. Kruskal-Wallis tests were also used to assess differences in stable isotopic signatures between conspecifics of snakes, amphibians and grasshoppers among collection sites. A conservative testing procedure was adopted where the experiment-wise error was Bonferroni corrected to $\alpha = 0.003$ ($0.05/15$). Parametric testing procedures assume a normal distribution in data, where this assumption was violated, original data was log transformed. A one-way multivariate analysis of variance (MANOVA) was performed on the log transformed stable isotope data to assess differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among all species. Tukey pairwise comparisons of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among species and communities were used to evaluate ecological interactions.

Trophic positions were estimated using the equation $\text{TP} = (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{base}} / \Delta_n) + \lambda$, from Estrada et al. (2003). Where TP is trophic position, $\delta^{15}\text{N}_{\text{consumer}}$ is the nitrogen signature of target species, $\delta^{15}\text{N}_{\text{base}}$ is the nitrogen signature of the base species (red-legged grasshoppers), $\Delta_n = 2.3\text{‰}$ which is the mean enrichment of $\delta^{15}\text{N}$ suggested by Aresco and James (2005) and McCutchan et al. (2003), and $\lambda = 2$ as the trophic position assigned to the base species. Red-legged grasshoppers are considered a primary consumer and were assigned a trophic position of 2 as suggested by McCutchan et al. (2003). All analyses were performed on Systat (Wilkinson, 1998).

Results

We collected 44 snakes, 16 salamanders and 15 grasshoppers from three sites in Halifax and Hants counties, and 64 anurans were collected from locations across Nova Scotia (Table 2.1). The total lengths and gape sizes were similar between individuals of each snake species across Nova Scotia (all H were not significant, and U were not significant for green snakes). Total lengths of snake specimens were consistent with those of average adult snakes described by Gilhen (1984) (Table 2.2).

No significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of conspecifics were observed among locations (Fig. 2.2), therefore species data was pooled from across collection locations for further analysis (Fig. 2.3). There were overall significant differences in stable isotopic ratios between species (Hotelling test, $F_{[20,246]} = 18.7$, $P < 0.001$) and for individual isotopes ($\delta^{13}\text{C}$, $F_{[10,125]} = 17.9$, $P < 0.001$; $\delta^{15}\text{N}$, $F_{[10,125]} = 20.1$, $P < 0.001$).

Mean stable isotopic signatures of terrestrial species ranged from -28.0‰ (grasshoppers) to -24.2‰ (redback salamanders) for $\delta^{13}\text{C}$, and from 1.0‰ (grasshoppers) to 6.9‰ (ringneck snakes) for $\delta^{15}\text{N}$ (Fig. 2.3). Grasshoppers exhibited $\delta^{15}\text{N}$ values significantly lower than all other species in this study (Tukey pairwise comparisons; all $P < 0.001$) (Fig. 2.3). Correspondingly, estimates of trophic position placed red-legged grasshopper at the basal trophic position of 2.0 as a primary consumer. Ringneck snakes were enriched in ^{15}N relative to all species and occupied the highest trophic position of all species, 4.6. Spring peepers occupied the lowest trophic position of all herpetofaunal species, 2.8. Redbelly snakes were observed at a trophic position of 3.1, and green snakes and redback salamanders both occupied a trophic position of 3.2. Garter snakes occupied a trophic position of 3.9, pickerel frogs and wood frogs were both observed at a trophic

position of 4.0, green frogs occupied a slightly superior trophic position of 4.3 and American toads occupied an inferior trophic position of 3.6 (Table 2.3 and Fig. 2.4).

Similarity in isotopic values of both carbon and nitrogen between species was used as an indicator of potential dietary resource overlap between species using Tukey pairwise comparisons. Pickerel frogs exhibited the greatest overlap sharing similar stable isotopic values with all species except grasshoppers. Garter snakes also exhibited a high degree of overlap and had isotopic values similar to those of redbelly snakes, ringneck snakes, toads, wood frogs and green frogs. Overlap was observed in the realized diets among all Lithobatid frogs studied (green frogs, wood frogs and pickerel frogs) (no significant differences among $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values in Tukey comparisons).

Predator-prey interactions between species were observed through Tukey pairwise comparisons where both species shared similar $\delta^{13}\text{C}$ values and the potential predator exhibited a significantly enriched $\delta^{15}\text{N}$ value relative to the prey (Fig. 2.3). Garter and ringneck snakes exhibited similarity in carbon signatures and significantly enriched $\delta^{15}\text{N}$ values relative to spring peepers ($P = 0.001$ and $P < 0.001$ respectively). Similarly, green snakes and pickerel frogs both exhibited similar $\delta^{13}\text{C}$ values as grasshoppers, and were both significantly enriched in ^{15}N (both $P < 0.001$). Ringneck snakes had significantly greater $\delta^{15}\text{N}$ values than redback salamanders ($P < 0.01$) and these species did not significantly differ in carbon isotope values. Green frogs shared similar $\delta^{13}\text{C}$ values, and were significantly enriched in ^{15}N relative to green snakes ($P < 0.001$) and redbelly snakes ($P = 0.006$). Wood frogs were significantly enriched in ^{15}N relative to spring peepers ($P = 0.003$), and both species exhibited similar $\delta^{13}\text{C}$ values. Redbelly snakes

exhibited similar $\delta^{13}\text{C}$ values as ringneck snakes, and were significantly depleted in ^{15}N relative to ringneck snakes ($P = 0.001$).

Discussion

Stable isotopic values depicted persistent and predictable patterns in resource utilization and trophic position of species. Individual snakes, within each species, were comparable based on similarity in total lengths and gape size; the trophic ecology discussed is considered to be that of adult snakes. Isotopic data suggests similarity in the general diets of conspecifics of all study species among collection sites, however snakes generally partitioned dietary resources among snake species. Conversely, carbon resource overlap between amphibians and between snakes and among amphibians was relatively common. Dietary studies based on traditional methods corroborate the trophic interactions and relatively similarities in diet among species.

Stable isotope signatures of ringneck snakes and redback salamanders suggest a predator-prey interaction between the two species (Fig. 2.3), which is supported by existing literature (Gilhen, 1984; Blanchard et al., 1979). Additional prey of ringneck snakes noted in the literature include other small species of salamanders (i.e., *Plethodon glutinosus*, *Eurycea bislineata*, *Desmognathus fuscus*), ground skinks (*Lygosoma laterale*), five lined skinks (*Eumeces fasciatus*), smooth green snakes and redbelly snakes, various species of anurans, insects and earthworms (Barbour, 1950; Blanchard et al., 1979; Bush, 1959; Hamilton and Polack, 1956; Jaeger, 1971; Wright and Wright, 1957). Similarly, isotopic data suggests that spring peepers and redbelly snakes also represent potential prey of ringneck snakes (Fig. 2.3). The superior trophic position of ringneck snakes in this study suggests they primarily consume prey from a higher trophic position compared to prey of heterospecifics (Fig. 2.4). Additionally, ringneck snakes exhibited trophic enrichment of ^{15}N of approximately 3.1‰ relative to redback

salamanders. Most studies have traditionally used ^{15}N enrichment of 3.4‰ as the standard trophic enrichment between a consumer and a single dietary source (Minawaga and Wada, 1984; Peterson and Fry, 1987). The relatively high trophic enrichment of ringneck snakes relative to their established prey (redback salamanders) (Gilhen, 1984), and their relative trophic position (Fig. 2.4) suggests their prey all occupy a similar trophic position.

Garter snakes represent the largest and potentially most omnivorous snake species in Nova Scotia (Gilhen, 1984; Brown, 1979). Garter snakes commonly consume a wide variety of prey from multiple trophic levels and occasionally among ecosystems (Gilhen, 1984; Brown, 1979). The prey of garter snakes observed in the literature includes numerous species of insects, earthworms, mammals, birds, fish, amphibians and snakes (Brown, 1979; Gilhen, 1984; Heinen and Hammond, 1997). Similarity in the trophic position of garter snakes and Lithobatid frogs is probably the result of omnivorous diets of both snakes and frogs. Lithobatid frogs also represent potential prey of garter snakes (Brown, 1979; Carpenter, 1952; Gilhen, 1984; Heinen and Hammond, 1997). Brown (1979) noted that a large proportion of garter snake diet consists of amphibians and earthworms.

Large species of anurans, including green frogs and bullfrogs, are aggressive predators that can consume snakes, and amphibians in addition to invertebrate prey (Frost, 1935; Gilhen, 1984; Korschgen and Baskett, 1963; Korschgen and Moyle, 1955; McKamie and Heidt, 1974; Minton, 1949; Smith, 1977), and may aggressively engage vertebrates far larger than themselves, including humans (Smith, 1977; *pers. obs.*). Similarly, Aresco and James (2005) observed similarity in the $\delta^{15}\text{N}$ values of coexisting American

alligators and adult amphibians (Fig. 2.5). This was counterintuitive to the expectations of Aresco and James (2005) because alligators are typically considered apical predators, capable of consuming all of the species that exhibited similar or enriched $\delta^{15}\text{N}$ values. Aresco and James (2005) suggest the relatively low $\delta^{15}\text{N}$ values of alligators were probably due to omnivory, assimilation of inadvertently consumed detrital material, and consumption of terrestrial prey, which typically have relatively lower nitrogen signatures than aquatic prey. A survey of stomach contents of herpetofauna by Bush (1959) identified a significant proportion of sediment, detritus, and sandy/rock material was consumed by all anurans surveyed. Therefore, similarity in $\delta^{15}\text{N}$ of garter snakes and anurans may similarly be due to their omnivorous diets, assimilation of non-target resources (i.e., detritus, sediment, etc.), looping that occurs between these species, and potential consumption of aquatic prey by amphibians. Aquatic prey can exhibit enriched nitrogen signatures relative to terrestrial prey species (Aresco and James, 2005).

Redback salamanders and American toads are both considered potential prey of garter snakes (Cochran, 1911; Madison et al., 1999; Gilhen, 1984), however stable isotope signatures of these species did not support this interaction. Gilhen (1984) suggested juvenile garter snakes are reliant upon redback salamanders as prey, however adult garter snakes generally consume larger prey. The absence any evidence of a predator-prey interaction, in this study, supports the idea that redback salamanders do not significantly contribute to the diet of adult garter snakes. American toads were not common in the particular locations in Nova Scotia where snakes were collected, and therefore did not represent significant prey. Conversely, spring peepers were relatively abundant in all areas sampled and observed as potential prey of garter snakes through stable isotope

analysis. Spring peepers are rarely identified as prey of garter snakes in the current literature, however Carpenter (1952) and Heinen and Hammond (1997) suggests garter snakes may consume spring peepers and/or closely related species. It is suspected that spring peepers do represent potential prey of garter snakes, however, the current literature identify Lithobatid frogs as prey more frequently (Gilhen, 1984; Heinen and Hammond, 1997). The superior trophic position of *Lithobates*, relative to garter snakes, is probably due to their predation on conspecifics (Fox, 1975; Petranka and Thomas, 1995; *pers. obs.*), other herpetofaunal species, and on aquatic prey (Gilhen, 1984). Species from aquatic habitats are often enriched in ^{15}N relative to terrestrial species (Aresco and James, 2005). Omnivory and highly generalist feeding behaviours of Lithobatids and garter snakes probably explains the dietary overlap among these and other species (Gilhen, 1984). Additionally, direct observation of gut contents of both species indicate similarity in consumed prey among most species of snakes and amphibians in Nova Scotia (Gilhen, 1984).

Green snakes, redback salamanders, redbelly snakes, and spring peepers all occupy a similar and relatively low trophic position (Fig. 2.4). Significant differences in $\delta^{13}\text{C}$ values suggests partitioning of dietary resources among these species, with the exception of redbelly snakes and spring peepers whose carbon signatures were not significantly different which suggests dietary overlap among these species. This suggests the respective prey of each species occupy a similar trophic position and/or a similar degree of omnivory among these species. Gilhen (1984) observed that the diet of all three species are primarily composed of herbivorous and/or detritivorous invertebrate prey. Redbelly snakes have been suggested to feed almost entirely upon slugs, suggesting a

relatively low degree of omnivory in their diet (Gilhen, 1984). Although green snakes, redback salamanders and spring peepers have been reported to consume a diverse range of invertebrate species, they are probably no more omnivorous than redbelly snakes.

Grasshoppers occupy the lowest consumer position observed in this study, which is indicative of their herbivorous diet (Fig. 2.4). Halaj et al. (2005) observed similar isotopic values in grasshoppers in an analysis of a forest food web as observed in this study (Fig. 2.3). Additionally, grasshoppers are potential prey of green snakes and green frogs (Fig. 2.3), which conforms to traditional observations of the diets of both species (Brown, 1979; Gilhen, 1984; Wilson and Friddle, 1950).

Herpetofaunal communities represent an important nexus through which nutrients are distributed between disjunct trophic levels and across ecosystem boundaries (Pryor, 2003; Waringer-Löschenkohl and Schagerl, 2001). In addition to omnivory, these systems are subject to looping. Looping is a situation of mutualistic predation among two or more species and also includes cannibalism (Polis, 1991). Many food web theorists have traditionally dismissed loops as “unreasonable structures” (Polis, 1991). Pimm (1982) states, “I know of no cases, in the real world, with loops”, but later revised this view to concede the occurrence of loops in age structured aquatic systems, but maintained loops were rare in terrestrial systems (Pimm and Rice, 1987). Conversely, Polis (1991) states that cannibalism, a self loop, has been reported in more than 1300 species, and is vital to population dynamics of many communities. Cannibalism is relatively common among reptiles and amphibians (Griffiths, 1997; Polis, 1991; Polis and Meyers, 1985). A number of studies have reported cannibalism in larval and adult frogs (Crump, 1986; Lehtinen, 2004), salamanders (Wildy et al., 1998), crocodiles (Polis

and Myers, 1985), turtles (Polis and Myers, 1985), snakes (Polis and Myers, 1985), chameleons (Keren-Rotem et al., 2006), and anolis (Gerber and Echternacht, 2000). Additionally, herpetofauna are commonly involved in interspecific loops. Amphibians often exhibit predator-prey role reversals resulting from ontogenetic changes, community size structure and priority effects (Gilhen, 1984). Drummond and Wolfe (1981) observed a larval dytiscid (an aquatic diving beetle) successfully attack and kill a young garter snake. Stable isotope analysis is beneficial in that looping inherently influences the isotopic signatures of consumers, and therefore provides a more realistic estimate of trophic position (Aresco and James, 2005). However, this is also a weakness in the technique in that stable isotope analysis alone cannot be used to observe loop structures as both species may exhibit similar isotopic values.

Patterns observed in stable isotope data also described redbelly snakes and green snakes as potential prey of green frogs. This is an unlikely interaction as both snake species are typically found occupying fields or rocky outcroppings, while adult green frogs typically remain relatively close to the perimeter of ponds. Stable isotopic data also suggested predation of spring peepers by wood frogs, however there is no support for either of these interactions could be found in the existing literature and they are, at best, considered to be infrequent events. These observations are probably the result of similarity in stable isotopic signatures of the perceived prey species to those of more common prey of green frogs and wood frogs. Overlap in stable isotopic values would make improbable prey indistinguishable from common prey using stable isotope analysis alone.

Trophic position, which is based on the realized diet of biota, is incommensurable through traditional dietary observations. Complex interactions highlight the subjectivity

of assigning organisms to trophic levels, which tend to be based on a behavioural hierarchy as opposed to realized diet (Aresco and James, 2005). The dismissal of such complexity within natural systems does not advance our understanding of these systems, but rather excuses the continued and persistent use of traditional models in spite of their limitations.

Analyses of stable isotopes of carbon and nitrogen present a useful technique to quantify community structure, predator-prey relationships, resource partitioning or overlap and to estimate trophic position of terrestrial herpetofauna. Consistent, predictable patterns in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures among species observed in this study demonstrate the utility of this technique in producing quantified estimates of “isotopic niche”. Relative trophic position and ^{15}N enrichment between predator and prey species also provided a method to indicate the relative degree of omnivory of individual species. Observations of improbable interactions highlight the necessity for interspecific interactions to be validated through direct observation. Similarly, mixing of isotopic signatures from multiple dietary sources, most notably arising from omnivory and consuming prey from disparate ecosystems, created a situation where potential prey (*Lithobates* frogs) of garter snakes exhibited relatively enriched $\delta^{15}\text{N}$ values and therefore did not conform to the expected results. Prior identification of such interactions and the application of appropriate mixing models would be required to quantify and assess such interactions.

This study indicates that stable isotope techniques are useful in their ability to quantify interspecific trophic interactions and to estimate relative trophic position of species within natural communities. However, since this data indiscriminately incorporates all of

the dietary resources assimilated by an organism, including those that involve complex interactions and feeding from multiple habitats, estimates of trophic position based on ^{15}N signatures can be non-intuitive. Additionally, similarity in carbon signatures among species may not necessarily be indicative of dietary overlap or predation but similarity in the isotopic signatures among distinct diets of the species of interest. Although this study offers harsh criticisms of traditional methods, I acknowledge their necessity in providing the basis for identifying species interactions and the theoretical importance of these interactions to community organization. Traditional methods are important to the validation of observations made through stable isotope analysis. However, the utility of traditional methods exists in physical identification of species interactions and predicting their importance and influence in natural systems, while that of stable isotope analysis is in its ability to quantify these interactions and test these predictions in natural communities.

Tables

Table 2.1: Summary of specimen collections.

Location	Species	n
S1	Grasshopper	6
	Redback salamander	6
	Garter snake	6
	Green snake	6
	Redbelly snake	1
	Ringneck snake	4
S2	Grasshopper	4
	Redback salamander	6
	Garter snake	2
	Redbelly snake	3
S3	Ringneck snake	3
	Grasshopper	5
	Redback salamander	4
	Garter snake	3
	Green snake	5
	Redbelly snake	6
Across NS	Ringneck snake	5
	Green frog	21
	Wood frog	7
	Pickerel frog	3
	Spring peeper	29
	American toad	4

Table 2.2: Morphometrics of snake specimens. All measurements are in mm.

Location	Species	Mean Total Length (± 1 SE)	Mean Gape (± 1 SE)
S1	Garter snake	363.7 (38.7)	16.7 (1.7)
	Green snake	340.3 (25.5)	14.8 (1.4)
	Redbelly snake	191.0 (N/A)	6.0 (N/A)
	Ringneck snake	351.0 (23.7)	11.0 (0.9)
S2	Garter snake	353.0 (82.0)	15.0 (5.0)
	Redbelly snake	240.3 (30.2)	7.0 (0.6)
	Ringneck snake	314.8 (47.2)	11.0 (1.9)
S3	Garter snake	380.6 (40.6)	13.0 (1.4)
	Green snake	330.0 (11.5)	10.8 (0.2)
	Redbelly snake	232.4 (9.9)	7.5 (0.3)
	Ringneck snake	280.2 (35.0)	7.8 (0.9)

Table 2.3: Mean (± 1 SE) trophic position of herpetofauna and grasshoppers in Nova Scotia determined from stable isotope data.

Species	Trophic Position (± 1 SE)
Garter snake	3.9 (0.08)
Green snake	3.2 (0.11)
Redbelly snake	3.1 (0.13)
Ringneck snake	4.6 (0.06)
American toad	3.6 (0.25)
Green frog	4.3 (0.20)
Pickerel frog	4.0 (0.12)
Spring peeper	2.8 (0.08)
Redback salamander	3.2 (0.10)
Grasshopper	2.0 (0.23)

Figures

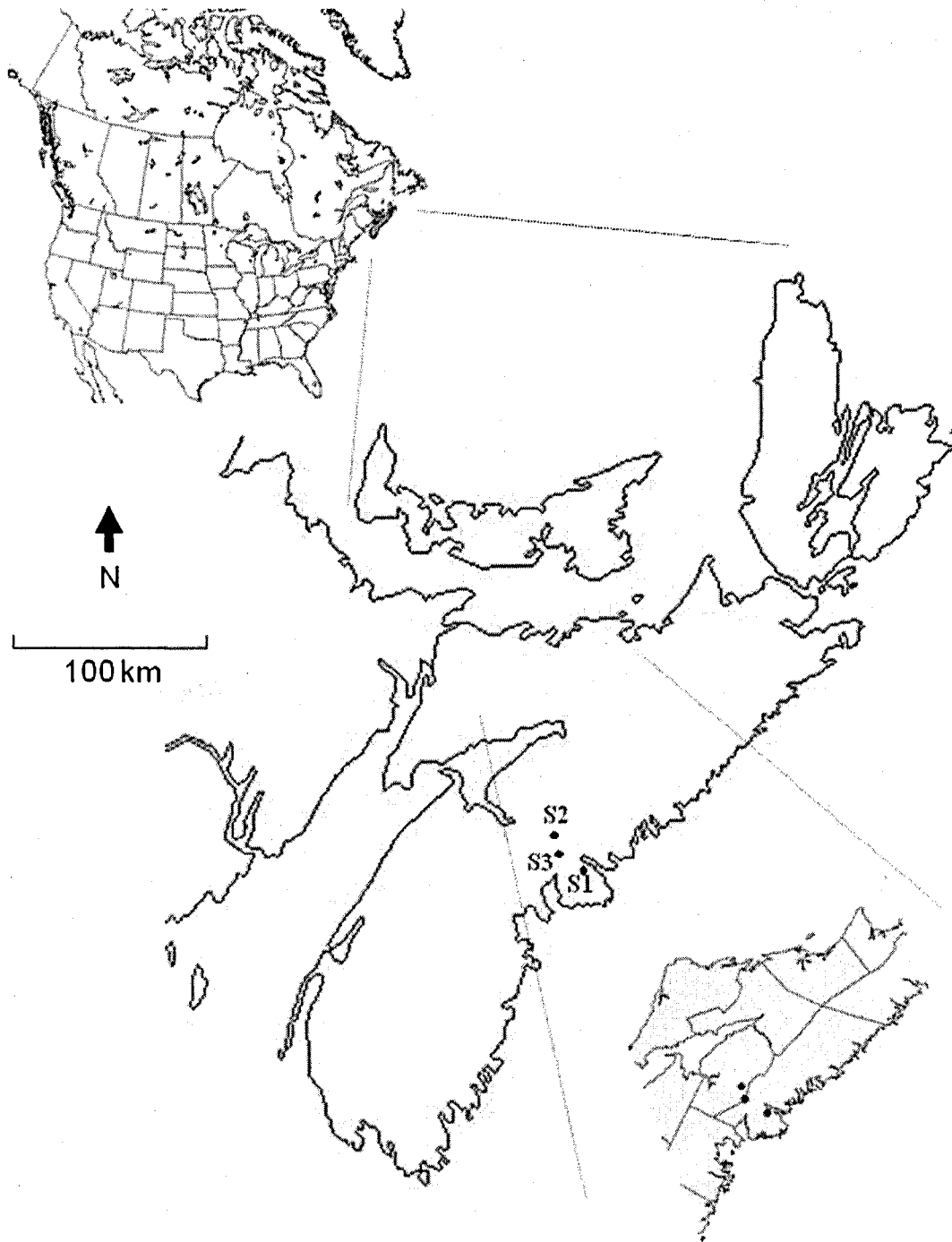


Figure 2.1: Map of collection sites of snakes, redback salamanders and grasshoppers in Nova Scotia, Canada.

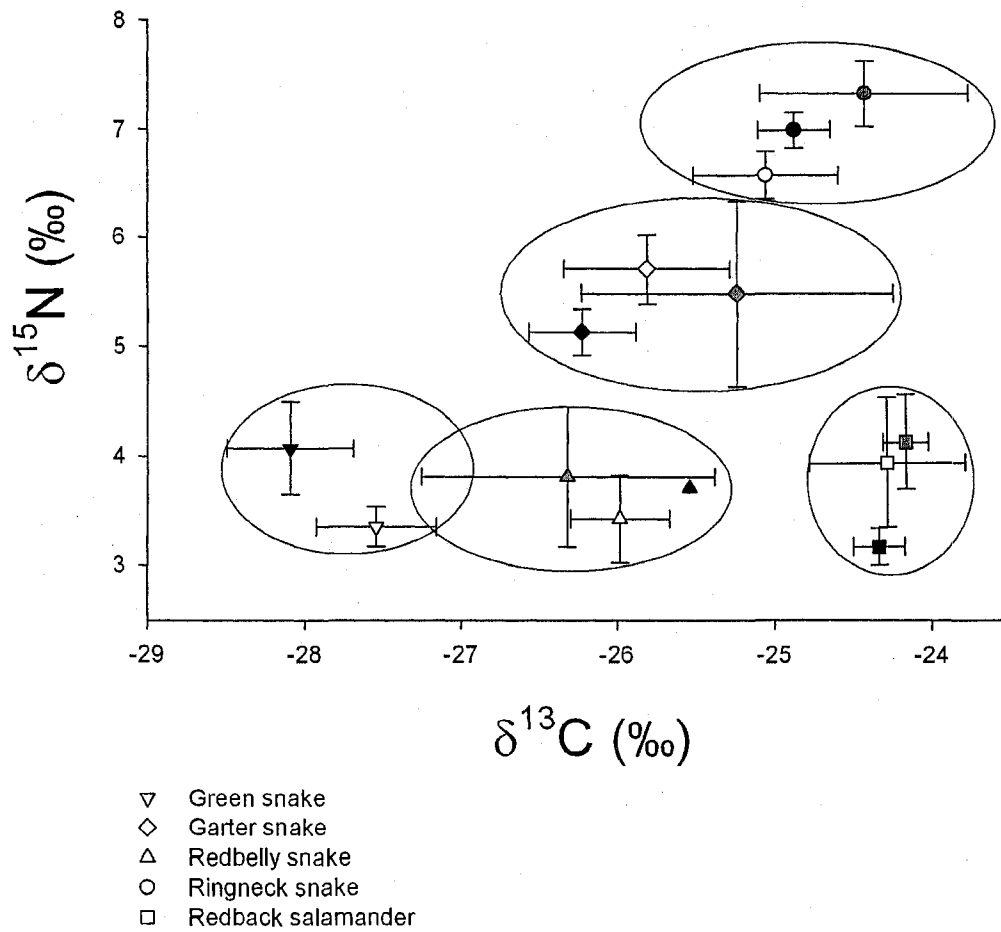


Figure 2.2: Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (± 1 SE) of snakes and salamanders among locations. Black symbols represent S1, grey symbols represent S2 and white symbols represent S3.

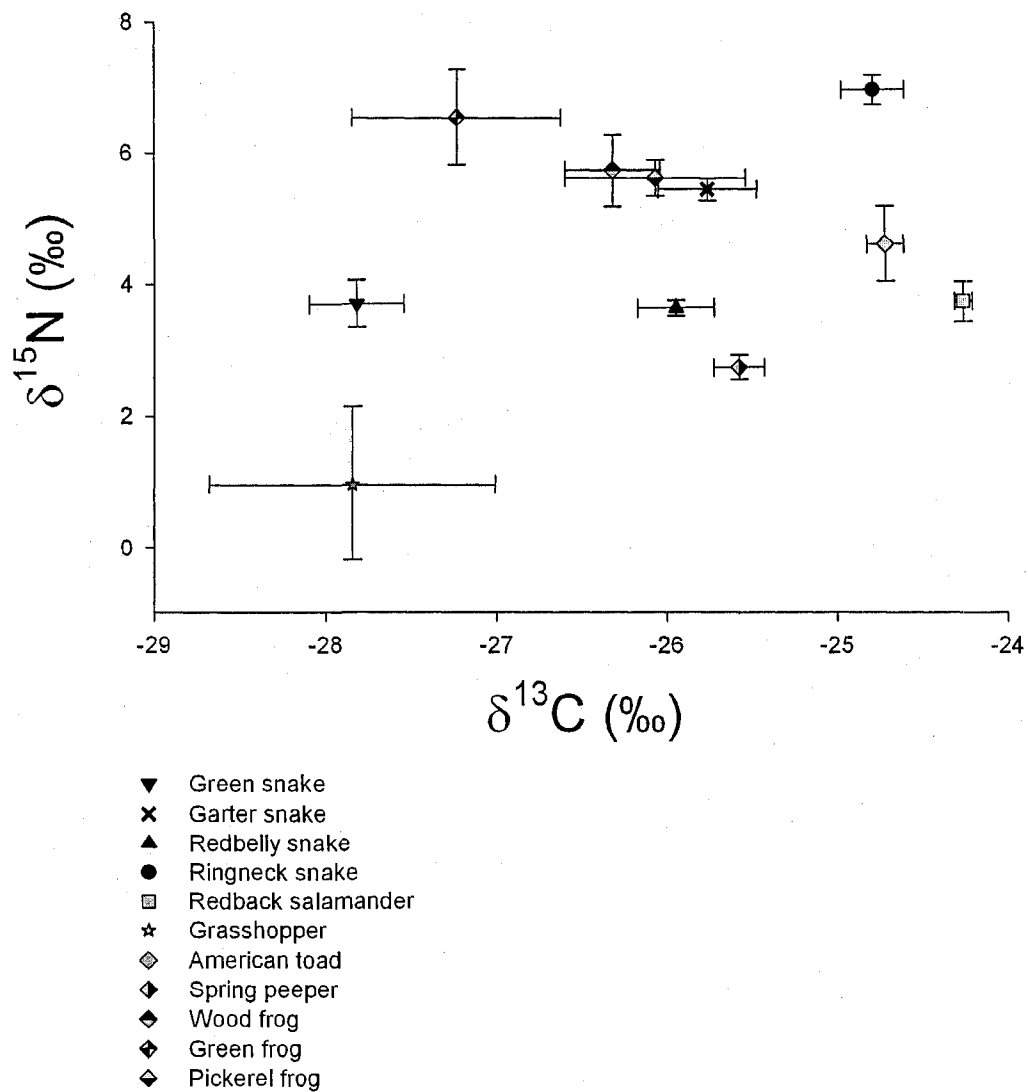


Figure 2.3: Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (± 1 SE) of snakes, frogs, salamanders and grasshoppers.

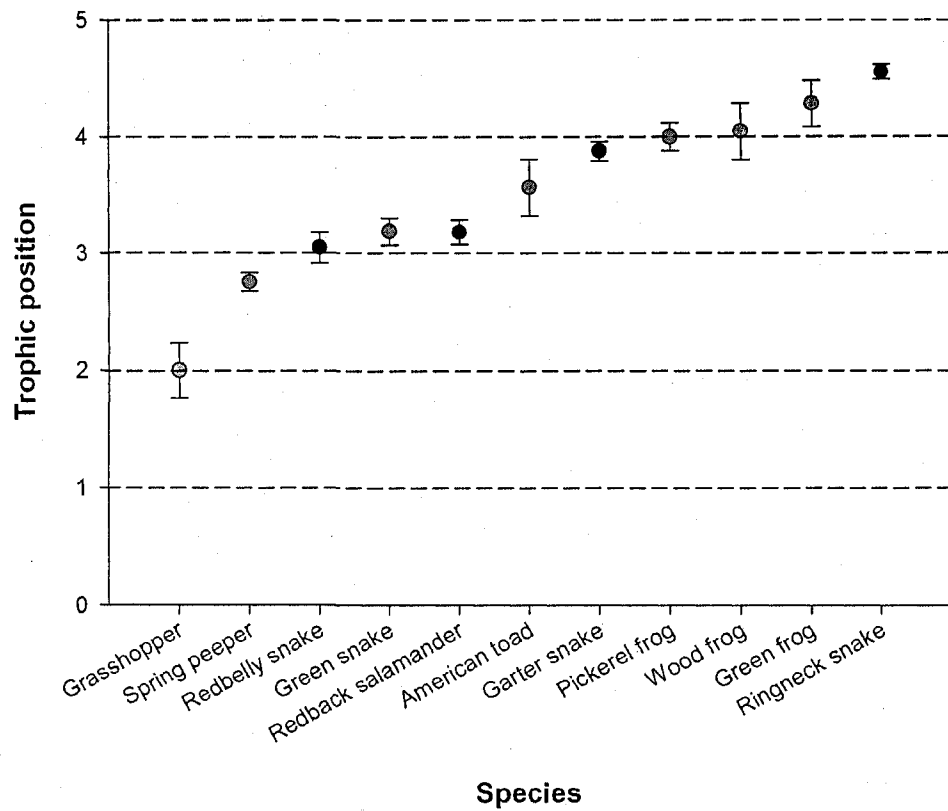


Figure 2.4: Mean trophic position (± 1 SE) of grasshoppers and herpetofauna. Grasshopper samples are represented by the light grey symbol; amphibians are represented by dark grey symbols; and snakes are represented by black symbols.

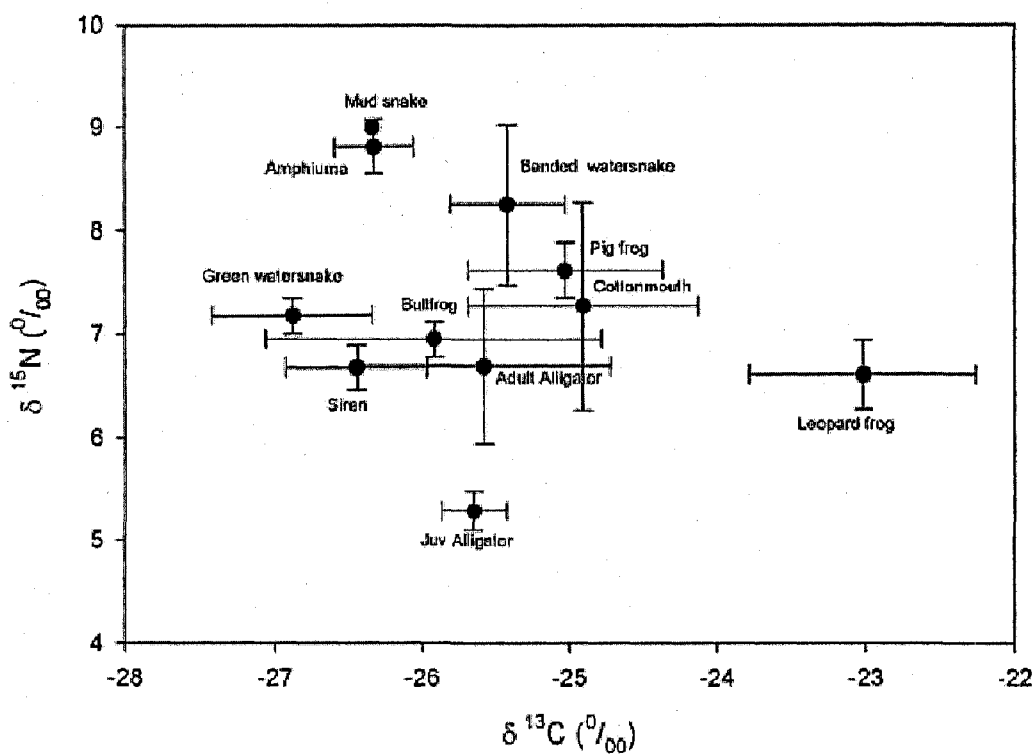


Figure 2.5: Mean stable isotope values (± 1 SE) of snakes, frogs, alligators, and aquatic salamanders. Samples were collected 2001–2004 at Lake Jackson, Leon County, Florida, from muscle of road-killed individuals and claw clips of live individuals. Reproduced from Aresco and James (2005).

Summary

Stable isotope analysis of herpetofaunal assemblages was successful in describing and quantifying predictable interspecific interactions and trophic positions of individual species. Models based on stable isotope analyses depict progression of assimilated nutrients from all assimilated dietary resources (the realized diet) as opposed to traditional food web models, which depict a hierarchy of feeding behaviour, often supported by population dynamics (Aresco and James, 2005; Phillips and Koch, 2002; Post, 2002a). The utility of this technique is that it can be used to quantify interactions within natural communities. This can then be used to assess the importance of specific interactions in community organization. This provides the means to produce realistic models of natural communities that will improve our understanding of how they function, how they are structured, and potentially predict and/or assess the impacts of direct and indirect disturbances.

However, Gannes et al. (1997) notes that the ease of use, and assumed simplicity in interpretation of isotopic results make them subject to error in interpretation. Spurious interactions identified using stable isotopic data are possible, and very probable due to the potential for overlap in isotopic signatures among species. Fundamental understanding of the technique, as well as the biological and ecological information of study species must be well understood to avoid misinterpretations. It is important to realize the necessity of traditional studies as correlative references, in the form of existing literature and concurrent experimentation, to validate observations made through stable isotope analysis. Layman et al. (2007) concluded, "Any single methodology, including stable isotope ratios, cannot provide a complete description of food web structure and function.

Application of a variety of research methodologies, as well as a thorough understanding of natural history of organisms and of species interactions, will lead to the most thorough understanding of food webs.”

Justification for the interactions described in this study was provided using an extensive review of the existing literature. In the absence of any direct observation of an interaction in the case of spotted salamander and spring peeper larvae, I have presented data that supports the potential for this observed interaction to be a highly probable and influential predator-prey interaction in ponds of Nova Scotia. Conversely, interactions observed to be improbable (i.e., wood frog predation upon spring peepers) lacked support in the literature, and based on personal observations of the species appear to reflect interactions that are, at best, infrequent.

Isotopic signatures are sensitive to differences in the signatures of basal level resources among distinct ecosystems (Aresco and James, 2005; Cabana and Rasmussen, 1996; Gannes et al., 1997). Differences in dispersal of nutrients between terrestrial and palustrine ecosystems are highlighted by the distinct contrast in similarity of isotopic signatures of conspecifics among distinct locations. Ephemeral wetlands accumulate water and nutrients from terrestrial runoff making them nutrient rich and productive seasonal ecosystems (Griffiths, 1997). Additionally, aquatic biota is often restricted to occupation of delineated aquatic habitats with distinct isotopic signatures. Conversely, similarity of stable isotope signatures of conspecifics in terrestrial ecosystems is probably due to a more homogenous dispersal of nutrients among terrestrial habitats. This difference is highlighted by comparison of the similarity in stable isotope signatures among conspecific tadpoles, and among adult frogs.

Wilbur (1997) notes that food webs are means of predicting ecological events (i.e., the effect of removing a prey species), but censures the implication that the food webs these models represent are static structures. Stable isotopes integrate trophic information over a relatively long period of time and therefore reflect mean trophic position and interactions. Alterations, or differences in food webs can be studied by comparing temporally or spatially distinct data sets. Layman et al. (2007) suggests that the extensive development of community-level isotope databases by ecologists will provide “insights into food web structure, particularly as a means to assess variation of trophic diversity and/or trophic redundancy in and among food webs.”

$\delta^{15}\text{N}$ values provide an effective indicator of realized trophic positions, however they may not produce results that correspond to traditional trophic level models (Aresco and James, 2005). Trophic levels depict a hierarchy of species based on their ability to influence the populations of other species through predation. Trophic position is estimated based on the flow and mixing of nutrients, which may not provide entirely intuitive results. This was observed in the relative enriched $\delta^{15}\text{N}$ values of *Lithobates*, and subsequently their higher trophic position, relative to garter snakes (Ch. 2, Fig. 2.3 and Fig. 2.4 respectively); a common predator of these frogs. Aresco and James (2005) highlighted this effect by observing inferior positioning of American alligators relative to bullfrogs (Ch. 2, Fig. 2.5). Trophic positions based on $\delta^{15}\text{N}$ values do not necessarily reflect traditionally models of trophic hierarchy. As a result, not all interspecific interactions may be identifiable using this technique, unless the importance of this interaction has been directly observed and appropriate mixing models can be implemented. This also has ramifications to studies dependant upon traditional

predictions of trophic levels including studies of bioaccumulation of pollutants (i.e., mercury) in food chains. Therefore, an understanding of the fundamental biology and ecology of studied biota must be understood before isotopic results are interpreted as they may lead to misleading or erroneous conclusions.

Lehtinen (2004) stated, "The classic view of anuran larvae as herbivores (Wassersug, 1975) is slowly being replaced by a more complex and dynamic description of diet invoking opportunistic use of available resources that may include ingestion of con- and heterospecifics." If this ideology were extended to food chains and webs it should indicate that our current understanding of trophic levels is restrictive, biased, and inaccurate. Stable isotope analyses can provide a realistic estimate of biota trophic position based on the trophic ecology of biota as opposed to behavioural or population dynamic data. Currently, experimental studies have mainly been used to describe rates of isotopic turnover among animal tissues, to assess isotopic enrichment between consumers and their diet, and to assess isotopic mixing (Robbins et al., 2002; Tieszen et al., 1983). Stable isotope analysis could alternatively be used to compare food web characteristics of natural and experimental communities to assess the realism of experimental studies.

The application of stable isotopic and laboratory experiments to refine mixing models in future research will be useful in identifying the importance of multiple resources in a consumer's diet. This could then be applied to provide estimates of interspecific interaction strengths, and if combined with studies of population dynamics this could, potentially, provide more accurate descriptions of community ecology. Additionally, comparison of basal level nutrient sources (i.e., sediment or representative primary

producer) can be used to adjust isotopic signatures of aquatic biota to compare aquatic communities (Aresco and James, 2005). Traditional observations, laboratory studies and mesocosm studies may then be used in conjunction with stable isotope analysis providing greater accuracy and realism to studies of trophic ecology and community ecology.

Implementation and improvement of studies utilizing stable isotope analyses could greatly improve our understanding of the natural trophic ecology and community dynamics of complex herpetofaunal assemblages. The impact to natural food webs by anthropogenic disturbances, introduced species and loss of biodiversity can be assessed using this technique. The success of food web restoration could also be assessed.

Additionally, this technique can reduce mortality of herpetofaunal specimens needed for research as analyses can be conducted on frozen tissues, specimens of opportunity (i.e., road kill), tissue samples acquired through non-lethal methods and on faecal samples (Gloutney and Hobson, 1998; Struck et al., 2002). Utilization of pre-existing specimens, specimens of opportunity and non-lethal sampling techniques are of particular importance when studying rare or protected species (Struck et al., 2002). This is becoming an increasingly important factor due to the continuing decline in reptile and amphibian populations.

There is a necessity for more comprehensive studies into the dietary composition of reptiles and amphibians using both gut content analysis and stable isotope analysis. Gut content analysis can be used to identify potential dietary resources of individual species. Stable isotope analysis of these identified resources and consumer species can be used to identify the relative importance of each resource using mixing models. Niche widths could also be compared among individuals (Araújo et al., 2007), among populations or

temporal periods (Syväranta and Jones, 2008), and potentially among species. Difficulties still remain, however, in identifying diets of tadpoles using gut content analysis, and the size of many tadpole species may make it difficult if not impossible to determine niche widths of individuals using stable isotope analysis. Current studies describing the use of stable isotopes to quantify niche width focus on fish species, and therefore are not subject to the problems of studying diminutive species of tadpoles (i.e., Araújo et al., 2007).

This study validates the application of stable isotope analysis in the characterization of interactions that structure natural herpetofaunal communities. In addition, this provides an assessment of the practical benefits and limitations of the technique. Additionally, this study provides the foundation for future studies to further quantify interactions and indicate their importance to the organization of natural communities.

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