

RESOURCE PULSES AND SPATIAL SUBSIDIES IN OZARK KARST SPRINGS:
EFFECTS ON COMMUNITY STRUCTURE AND FOOD WEBS

BY

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Submitted to the graduate degree program in Ecology
and Evolutionary Biology and the Graduate Faculty
of the University of Kansas in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy.

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ABSTRACT

Spatial and temporal patterns of invertebrate community composition, biomass, functional diversity, foodweb dynamics, and foodweb complexity were examined in three Ozarks springs. Also examined was the effect of an experimental manipulation of algal production (function of light limitation) on foodweb pathways and complexity in one of the springs that has been intentionally deforested. Food source-consumer interactions were determined using carbon and nitrogen stable isotope and stoichiometric analyses. Isotope data were analyzed using the IsoSource, multi-food source, multi-isotope mixing model. Quantified food webs were constructed based on IsoSource predictions and biomass estimates of each consumer.

Spring invertebrate community analyses indicated that biocomplexity and functional diversity increased temporally and spatially along the spring source-springbrook gradient likely due to the corresponding variability in the composition and availability of food sources. Contrary to expectations, food web analyses indicated that the trophic base of the food web was autochthonous, shifting temporally towards a greater reliance on allochthonous resources. Spatial and temporal shifts in food source availability and utilization were associated with corresponding increases in food web complexity. Isotope ratios, based on manipulation of algal production, indicated a shift toward more allochthonous-based food web pathways and increases in omnivory and food web complexity in manipulated (shaded) sites compared to open sites in the spring. The contribution of studies in spring ecosystems to overall theories of benthic invertebrate community structure and food web dynamics is, unfortunately, still limited by the paucity of descriptive and experimental studies of these ecosystems. The study began with a synthesis and conceptual evaluation of springs around the world.

ACKNOWLEDGEMENTS

It has been a pleasure to study with Dr. James H. Thorp on this project. His counsel and support were instrumental in bringing my studies in Ecology and Evolutionary Biology at the University of Kansas to completion. I also wish to thank Dr. Chip Taylor, Dr. Don Huggins, Dr. Bryan Young, and Dr. Sharon Billings for serving on my committee. Their support and advice were greatly appreciated. I owe sincere thanks to Dr. Robert T. Pavlowsky and the people at the Ozarks Environmental and Water Resources Institute (OEWRI) at Missouri State University for their financial support. I could not have completed the project without it. Also, thanks goes to Marc Owen at OEWRI for creating maps of my spring sites. I would also like to thank the spring owners who graciously allowed me to work on their land, they are: Dr. Thomas Lynch; owner of Steury Spring, Mr. Russ Campbell; owner of Haseltine Spring, and Mr. Bob Lovitt; owner of Danforth Spring, and. In addition, I would like to give thanks to Mr. Loring Bullard from the Watershed Committee of the Ozarks for his assistance in locating spring sites, and his expertise on spring ecosystems. I also owe thanks to Jim Burks and Stephanie Gott at the Southwest Waste Water Treatment Plant of Springfield Missouri for their assistance with water quality assessment. I would also like to thank Lee Ann Bennett and Mary Anne Blackwood from KU for their assistance in invertebrate identification. In addition, thanks go to Dr. Craig Freeman, also from KU, for identification of my grass samples. I also thank the Ecology and Evolutionary Biology Department at KU for providing me travel funds to present my work at the North American Benthological Society annual conference. I would also like to thank Jennifer Shepard for her help in the field. I owe special thanks to Chad Copper for his assistance during the experimental portion of the project. I also owe a big thanks to the biology faculty at Drury University in Springfield, MO for their understanding and support. Finally, I would like to thank my husband and 3 daughters whose love got me through it all.

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INTRODUCTION

RESOURCE PULSES AND SPATIAL SUBSIDIES IN KARST SPRINGS: EFFECTS ON COMMUNITY STRUCTURE AND FOOD WEBS

Knowledge about the ecological importance of resource pulses in spatially subsidized ecosystems has been growing for nearly a decade (Polis *et al.* 1997). Organic pulses are defined as resources that occur episodically, enhance resource availability above the baseline level, and decay in availability through time (Ostfeld and Keesing 2000). Communities strongly affected by resource pulses tend to feature feeding generalist who can exploit these pulses but which also require alternative food sources to survive inter-pulse periods. The generalists themselves can also serve as a pulsed resource to their predators. The nature of the community will vary according to the degrees of consumer specialization on pulsed resources, consumer mobility, and population responses to pulsed resources (Ostfeld and Keesing 2000). Spatial subsidies are donor-controlled resources that originate in one habitat but move to recipients in other habitats, altering the dynamics of recipient populations and communities (Polis *et al.* 1997). Cross-habitat movement of nutrients, particulate organic matter (detritus), and organisms can have strong effects on community structure and food web dynamics (Anderson and Polis 1998) through top-down effects (subsidized consumers affect *in situ* resources and prey abundance) and/or bottom-up effects (increased productivity or detritus; Polis *et al.* 1997, Ostfeld and Keesing 2000). By tracing effects of resource pulses and spatial subsidies, we gain insights into spatial and temporal patterns and processes affecting food web structure and function which are central to the patch dynamics themes of fluvial landscape ecology.

In two respects, springs are arguably among the most important headwater ecosystems in the north temperate zone because their flow is more dependable than in the average surface-fed streams and their temperatures are more constant. These two characteristics provide refuges for aquatic species during droughts (and drinking water for many terrestrial species, including humans), during the heat of summer, and

in freezing conditions in winter when other stream sections face ice cover and scour. Their importance is likely to increase in those areas where global climate change is predicted to enhance variability of precipitation, as in parts of the Midwest (e.g., Dodds *et al.* 2004).

Before 1980 spring ecosystems were depicted as impoverished habitats with relatively few organisms of interest (Erman and Erman 1990). Several studies since then (e.g. Webb *et al.* 1995, Ferrington 1995) have shown that springs can be quite rich in species and are often habitats for a disproportionately large number of rare and endemic species, especially those requiring relatively constant temperatures (Erman and Erman 1995). Springs also promote greater benthic species richness in stream sections because they are more dependable sources of water (Mattson *et. al* 1995). Despite these factors, research on freshwater springs remains limited (Smith and Wood 2002).

Springs are by definition first order or headwater streams typically portrayed as heterotrophic systems dependent on allochthonous organic matter (e.g. Lindegaard 1995). Although the relative importance of autochthonous production has been noted in rivers (e.g., Thorp and DeLong 2002), the general consensus is that food webs in forested headwaters are primarily fueled by allochthonous production except possibly in areas where sufficient PAR reaches the stream bed (e.g., DeLong and Thorp 2006). The spatiotemporal importance of autochthonous and allochthonous organic matter to food webs in resource-pulsed, spatially-subsidized spring brooks has not been factored into our models of lotic ecosystems. Rather than springs being discrete self-contained microcosms as sometimes suggested (e.g. Gooch and Glazier 1991), I believe these seemingly autonomous systems instead, rely heavily on pulsed linkages between terrestrial and subterranean environments.

I suggest that the connectance between the subterranean resources and species characteristic of eucrenal (spring source) habitats and the epigeal resources and species of the rheocrenal (spring brook) habitats represent a dynamic interplay involving exchanges of water, organic matter, and nutrients. The resulting spatial

diversity of nutrient and organic subsidies within spring brook ecosystems can have significant impact on patterns of community structure and food web dynamics. For example, diversity of spring fauna typically increases with distance from the spring source (Williams and Danks 1991). Replacement of non-insect dominated spring fauna by a largely insect fauna downstream (Gooch and Glazier 1991) suggests that species interactions are important. These studies support the theory that responses by primary producers and generalist consumers to variation in spatial subsidies and pulsed inputs of nutrients into aquatic ecosystems will cause predictable changes in community dynamics (Ostfeld and Keesing 2000). Studies examining the effect of subsidies on species diversity in spring systems are, however, quite limited (Anderson and Wait 2001).

The descriptive and experimental studies described here represent a cohesive work that includes (a) a heuristic model for the diversity of origin and the delineation of spring types developed from a synthesis of literature that requires that springs be described as products of a “nested filtering” process; (b) an empirical study of seasonal community responses (diversity and biomass) along a downstream gradient in three spring brooks in response to pulses and subsidies; (c) an empirical, isotope-based study of how food web pathways and trophic complexity relative contributions of allochthonous and autochthonous organic matter alter with distance from the spring source and with temporal pulses and subsidies of organic matter; and (d) an experimental study of effects of resource manipulation on food web pathways along a longitudinal gradient from the spring source to the spring brook.

CHAPTER 1

PERSPECTIVES ON THE CLASSIFICATION OF SPRINGS BASED ON GEOLOGY, ORIGIN, AND RESIDENT BIOTA

Springs represent a vertical transition zone between surface and groundwater systems. Groundwater emerging from springs is stored in, and moves through permeable rocks called aquifers (Peale 1894). Geologically described as groundwater outcrops, springs represent the manifestation of subsurface flow or groundwater discharge that occurs either where groundwater is forced to the surface through fissures and other openings in the confining bed that overlies the aquifer, or as a result of gravity at the lowest ground elevation point within the aquifer (Freeze and Cherry 1979; van der Kamp 1995).

The earliest attempt at a complete classification scheme for springs was proposed by Keilhack (1912). Springs have been classified by the amount of water they discharge (Meinzer 1927), and by their temperature (Stearns *et al.* 1937; Waring 1965), origin (gravitational or non-gravitational; Bryan 1919), or mineral or chemical composition (Clarke 1924). Since development of these classification schemes, the scientific understanding of spring origin has improved considerably. For example, it is now known that geology and lithology (distribution of rock types in the subsurface, grain size, and grain packing) determine a spring's water source, surface texture and size of the components of the substrate, rate of flow, temperature, chemical composition, and its residence time (Egglshaw and Morgan 1964; Freeze and Cherry 1979; Danks and Williams 1991; van Everdingen 1991; Woodruff 1993).

The purpose of this chapter is to present a heuristic framework that illustrates the diversity of spring origin, the delineation of spring types, and the distinctive spring biota. The framework was developed from a synthesis of literature, and describes springs as products of a “nested filtering” process which includes: (i) geology and lithology; responsible for the structure and depth of spring origin; and (ii) the filtering effects of the thermostatic and chemostatic forces. These factors are collectively responsible for the composition of the residing fauna. As with any classification scheme, the model presented here is somewhat arbitrary as waters are

structurally and functionally dynamic; melding into each other often by imperceptible gradations, making it difficult to draw hard and fast lines of division between spring types. Hence, I emphasize and use the word heuristic here as a approach or method to aid in learning, discovery, or problem-solving, not as an exhaustive, definitive substitute for preexisting approaches to spring classification and faunal designations.

Mechanistic Agents in Predicting Spring Types and Characteristic Fauna

Geology / lithology

A fundamental aspect separating springs is the source of the water or the rock structure which brings it to the surface (Bryan 1919). All springs carry a history of the geology they touch, and can therefore be defined only in their individual geological setting (Danks and Williams 1991). Oscar E. Meinzer (1923) was one of the first to observe that the United States could be divided into discrete groundwater regions distinguished by consistent underlying geology. The Ground Water Atlas of the United States published by the U.S. Geological Survey (U.S.G.S.; Miller 2000) is a more recent designation of the principle aquifers of the United States based on the work of Meinzer and others (e.g. Thomas 1952; Heath 1984). These aquifers are defined in terms of six rock formations or geologic units designated by the U.S.G.S. that yield water to wells and springs, and also serve as the primary filter in the delineation of spring types illustrated in the model below.

Depth / residence time, thermalization, mineralization

Geology and lithology determine the length, depth, and intricacy of the underground conduit system, thereby establishing residence and surface return times, and regulating whether spring waters are affected by thermostatic and/or chemostatic forces. Waters that circulate through the geological aquifers illustrated above can roughly be considered as either deep-seated or shallow (Bryan 1919).

Deep circulating flow paths lead to increased residence times (often greater than 1000 years) and thermal, more mineralized spring waters (Bryan 1919; Freeze and Cherry 1979; Hynes 1983; Woodruff 1993; Bakalowicz 1994; Chapelle 1997).

Thermalization occurs when water molecules reach thermal equilibrium through contact with rock in areas of geologically recent volcanic activity or by deep subsurface circulation in areas where rocks have been faulted and intensely folded (Peale 1894; Stern *et al.* 1937; Waring 1965; van Everdingen 1991; Alfaro and Wallace 1994). The structural setting must be favorable for thermal patterns to occur. Underlying geology must provide enhanced vertical permeability zones (porous, fractured, and or folded) so that water moves deeply, gaining heat from the aquifer, and escapes, returning to the surface without thermal re-equilibration (Alfaro and Wallace 1994). Mineralization occurs when waters are infused with minerals leached from the rocks through which they pass. Although strongly related to aquifer geology, the extent of mineralization is often accelerated in heated waters (Peale 1894; Bakalowicz 1994; Alfaro and Wallace 1994; Hayford *et al.* 1995).

Cold, freshwater springs typically discharge water from relatively shallow, short flow systems (Freeze and Cherry 1979; Scanlon & Thrailkill 1987; van Everdingen 1991; Erman and Erman 1995; Chapelle 1997). Shallow waters have a relatively simple origin, derived largely from precipitation. Their movement is due to gravitational pressure through pore spaces and fractures of the rocks, both of which decrease with depth. However, a cold spring's groundwater flow path may penetrate to considerable depth (experiencing high temperatures), but the final ascent of the water to the surface is slow enough to allow complete loss of any acquired geothermal heat (van Everdingen 1991; van der Kamp 1995) through thermal conduction to the ground surface.

Regardless of the exact temperature or mineral present, the extent of thermalization or mineralization is influenced by not only the composition of the rock

types and depth from which the water issues, but also by the water's subsurface residence and surface return times (Felmlee and Cadigan 1982; van Everdingen 1991).

Ecological Importance of Springs

Springs can be a major structuring force that increases species richness in surface stream communities across North America (Mattson *et al.* 1995). Reasons for this could be increased primary productivity in response to relative constant physical and chemical conditions in spring ecosystems. The stable conditions in springs may also provide refugia for certain taxa during hydrologic spates. Indeed, the recolonization of flood-affected areas from spring tributaries is commonly reported (Armitage and Petts 1992).

Springs also support phylogenetically unique fauna, as many relict postglacial species immigrated to these ecosystems seeking refuge from increasing temperatures (Hynes 1970). Subterranean waters often contain endemic, primitive, or highly specialized turbellarians, hydrobiid snails, amphipods, isopods, decapods, and copepods (Pennak 2001); communities often distinct from those found in local surface waters. Of the 36 trichopteran species found by Erman and Erman (1995), ten are found only in small springs in Sierra Nevada, California. Other endemics have been found in desert springs with a natural range restricted to one or two isolated springs (Sigler and Sigler 1987). The predominance of pericaridan crustacean species, and lack of insect species has been reported for springs in eastern North America (Minckley 1963), while the opposite has been found in western North America (Ward and Dufford 1979). In addition, springs may represent habitats for speciation or evolution of endemics, as some spring species are often isolated from other water bodies due to non-emergent, totally aquatic life-styles (Erman and Erman 1990).

The Model: Paths of Spring Delineation and Faunal Composition

Groundwater flow paths described below, and illustrated in a graphic conceptual model (Fig. 1), represent a synthesis of ideas from the literature characterizing the diversity of spring origin, delineation of spring types, and description of the dominant biota (maximum of 3 most commonly cited species per genera). While the model appears rather dichotomous in nature, it is assumed that gradations of each filtering process occur. It is also assumed that components of groundwater systems can be arranged as: (a) a single unconfined aquifer; (b) two interconnected aquifers of equal hydrological importance; (c) a three-unit system consisting of an unconfined aquifer, a confined aquifer, and a confining bed; or (d) a complexly inter-bedded sequence of aquifers and confining beds (cf. Heath 1984). However, each groundwater flow path is described in terms of the dominating rock structure (geologic condition), or principal aquifer that brings significant amounts of water to the surface forming a particular spring type and defining its faunal composition.

Unconsolidated sand and gravel path

Overlying sand and gravel aquifers, while shallow, are responsible for some of the largest springs in the United States (Meinzer 1927; Fetter 1980). Springs in these aquifers issue from sand and gravel often underlain by volcanic (basalt) or carbonate (limestone) bedrock. Groundwater flow typically travels along short flow paths, resulting in short residence and surface return times; producing cold, freshwater springs (Fig. 1, path 1a). Average temperature and pH levels reported for these springs range from 6.7-23.0°C and 6.2-8.1 respectively. Dissolved oxygen concentrations are high (7.6-10.4 mg/L⁻¹); while total dissolved solids (TDS) typically range from 163-400 mg/L⁻¹.

Mineralization (NaCl due to marine origin) can occur at relatively shallow depths in the bedrock of these aquifers producing saline springs with TDS concentrations as high as 965 mg/L⁻¹ (USGS¹ 2008; Heath 1984; Miller 2000).

SPRING ORIGIN MODEL

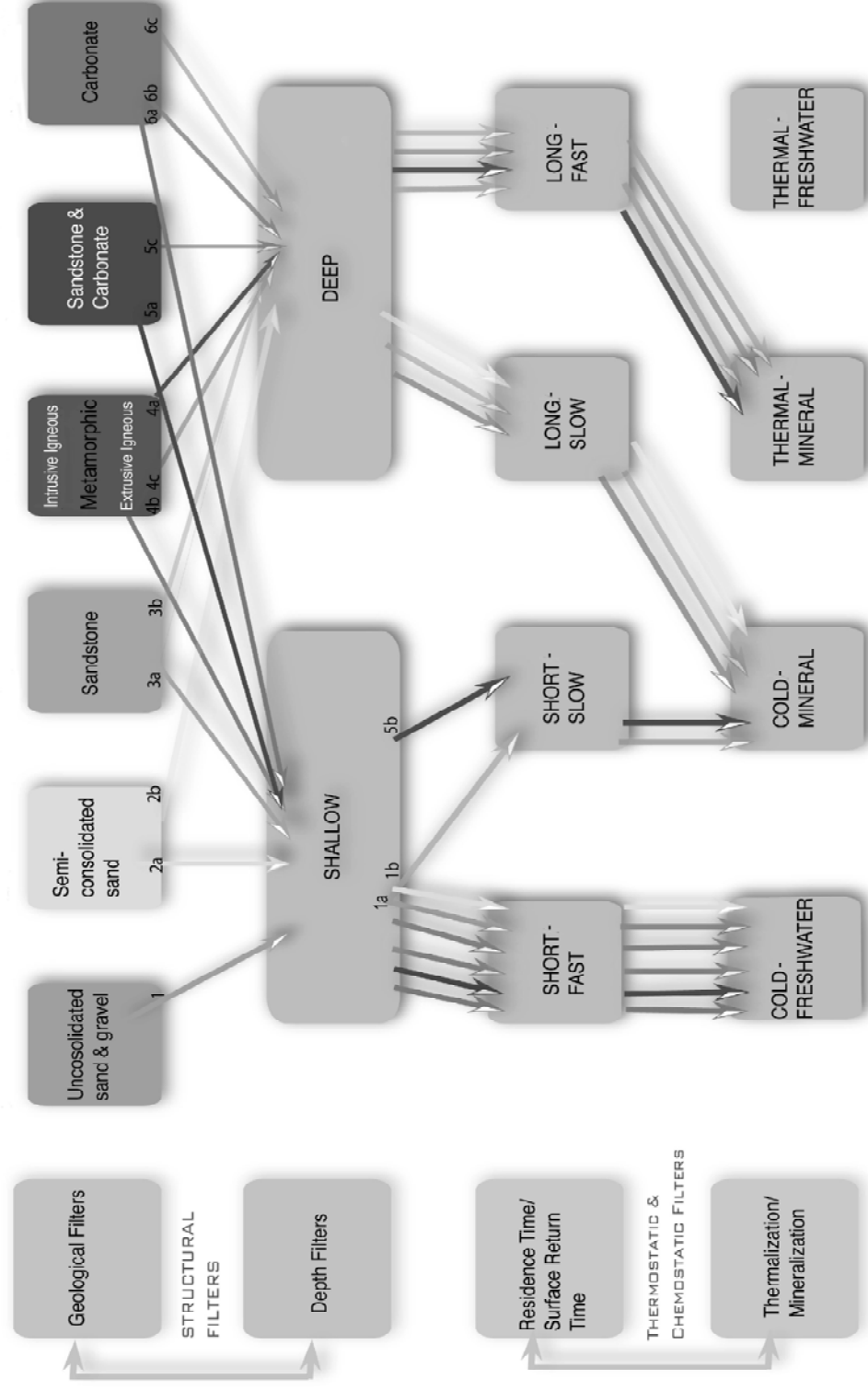


Figure 1 Model depicting spring origin and spring type based on the principal geological aquifer from which it issues.

Mineralization along such short, shallow flow paths is an indication of the complexity of the conduit system through which the water passes producing long residence and surface return times, and cold mineralized springs (Fig. 1, path 1b).

Vegetation in the springs is dominated by water cress (*Nasturtium officinale*), while the filamentous green algae *Spirogyra* sp. dominates the autotrophic community (Davidson and Wilding 1943; Resh 1983; Varza and Covich 1995; Gaskin and Bass 2000; Newell 2003). Springs in these aquifers have been characterized as both insect (Newell 2003; Resh 1983; Williams *et al.* 1997) and non-insect dominated ecosystems (Bass 2000; Varza and Covich 1995; Davidson and Wilding 1943). Non-insect taxa, characteristic of these aquifers include oligochaetes (especially *Limnodrilus* and *Lumbriculus*), turbellarians (*Dugesia*), amphipods (*Hyalella* and *Gammarus*), crayfish (Cambaridae), and snails (dominated by *Physa*). Predominant insect taxa include as many as 21 species of caddisflies (especially *Cheumatopsyche*, *Hydropsyche*, and *Ochrotrichia*), hydrophilid beetles (*Tropisternus*), damselflies (*Argia*), and numerous dipterans, especially chironomid midges (predominantly *Dicrotendipes*), biting midges (Ceratopogonidae: *Culicoides*), and various biting flies (Tabanidae: *Chrysops*). Several less abundant groups include dipteran families Simuliidae (*Simulium*), Dixidae, and Tipulidae (*Tipula*), as well as peaclams (*Pisidium castoraneum*), water mites (Acari), isopods (*Asellus* sp.), small beetles (*Helichus* and *Laccophilus*), and mayflies (especially *Paraleptophlebia*, *Baetis*, and *Stenonema*) (Davidson and Wilding 1943; Resh 1983; Varza and Covich 1995; Williams *et al.* 1997; Gaskin and Bass 2000; Bass 2000; Newell 2003).

Semi-consolidated sand path

Groundwater flow is local (short paths) in the shallow unconfined areas of these aquifers producing cold, freshwater springs (Fig. 1, path 2a). Water in the confined parts of the aquifer, however travels along deep, extremely long flow paths with TDS concentrations as high as 10,000 mg/L⁻¹ (path 2b). Flow is sluggish near the ends of the flow paths, commonly producing cold, often saline springs (Miller

2000; Schwartz and Zhang 2003). These soft beds produce many springs, but no truly thermal springs are known (Stearns *et al.* 1937).

Temperatures and TDS concentrations range from 13.98-21.21°C and 56.4-422 mg/L⁻¹, respectively. Dissolved oxygen levels are relatively low (1.28-6.25 mg/L), while pH is similar to that of the unconsolidated sand and gravel aquifers (6.1-8.0).

While vegetation in this type of spring consists primarily of water cress (*Nasturtium officinale*) and moss mats (USGS¹ 2008; Strayer 1988; Hakenkamp *et al.* 1994; Morse *et al.* 1997), little is known about the residing biota. By 1994, no quantitative studies had been published on the groundwater fauna of North American sandy aquifers (Hakenkamp *et al.* 1994). Currently, there remains a paucity of information on the biota residing in springs characterized by semi-consolidated sand geology. Meiofaunal studies tend to be the most common and show that nematodes, copepods, rotifers, oligochaetes, mites, and tardigrades are the primary constituents of these communities (Hakenkamp *et al.* 1994). Copepods are most diverse, represented by as many as 33 species (especially *Diacyclops*, *Bryocamptus*, and *Attheyella*), while crustaceans such as amphipods are few, represented primarily by *Stygobromus allegheniensis* (Strayer 1988). Sandy aquifers are supportive of diverse caddisfly (Trichoptera) populations (as many as 43 species cited) including representatives from the families Glossosomatidae (*Agapetus jocassee*), Hydropsychidae (dominated by *Hydropsyche alabama* and *Cheumatopsyche morsei*, which are endemic to springs of this aquifer type). Other caddisfly families found here are Hydroptilidae (*Hydroptila decia* and *H. setigera*), a diverse group of Lepidostomatidae (e.g., *Lepidostoma flinti*), Rhyacophilidae (*Rhyacophila accola*), and Uenoidae (*Neophylax auris*) (Morse and Barr 1990; Morse *et al.* 1997). Other than caddisflies, insects are represented relatively poorly in this type of spring and consist primarily of the orders Diptera (mostly Chironomidae), Ephemeroptera (Ephemerellidae, such as *Ephemerella* and Baetidae, such as *Baetis ochris*) and Plecoptera, with the last

dominated by the families Perlidae (*Beloneuria georgiana*) and Perlodidae (*Oconoperla innubila*) (Strayer 1988; Morse *et al.* 1997).

Sandstone path

Groundwater in sandstone aquifers typically moves through the shallow portions of the aquifer along short flow paths (Fig. 1, path 3a) progressing upward to discharge cool freshwater springs (Miller 2000). These are softwater springs (pH 5.4-7.0) with low concentrations of dissolved solids ($16.8\text{-}640\text{ mg/L}^{-1}$) and temperatures ranging from 5.5-20.0°C.

Groundwater flow in these aquifers will sometimes continue to percolate to deeper depths and move laterally over longer distances (path 3b) before discharging cold, often mineralized waters (Miller 2000). Mineralization is low ($<500\text{ mg/L}^{-1}$) in shallower parts of these aquifers (southeastern Minnesota, northeastern Iowa, southern Wisconsin, the upper peninsula of Michigan) but increases to $\sim 10,000\text{ mg/L}^{-1}$ in the deeply buried aquifer region of Missouri, producing typically saline springs (Heath 1984).

Dominant autotrophic organisms consist primarily of green algae (*Cladophora* and *Spirogyra*), and diatoms (especially *Achnanthes*) (Teal 1957; Ward and Dufford 1979; Glazier and Gooch 1987). Water cress (*Nasturtium officinale*) which is common in many spring types, is rarely cited as a vital component of sandstone spring flora (Wilhm 1970).

Insects dominate the fauna of most sandstone springs. Ephemeroptera (especially *Baetis*), Trichoptera (*Hydropsyche* and *Cheumatopsyche*), Plecoptera (*Leuctra*) and Odonata (*Argia* and *Ischnura*) reach their highest abundances in these softwater springs. Diptera is often the most diverse group, representing the majority of macroinvertebrate biomass and led by members of the true midge subfamily Chironominae (e.g., *Stictochironomus annulicrus* and *Chironomus*) as well as many representatives of Dytiscidae, Elmidae, and Tipulidae (*Tipula*). Common non-insect invertebrates include oligochaetes (*Limnodrilus* and *Lumbriculus*), and turbellarians

(*Dugesia* and *Phagocata gracilis*). Occasionally present are peaclams (*Pisidium*) physid snails, isopods (*Lirceus hoppelinae*) and amphipods (*Stygobromus* and *Hyaella*) (Meinzer 1927; Teal 1957; Wilhm 1970; Ward and Dufford 1979; Robinson 1981; Glazier and Gooch 1987; Gaskin and Bass 2000).

Igneous and metamorphic path

Igneous and metamorphic aquifers are composed of either crystalline-rock (intrusive igneous and metamorphic) or volcanic-rock (extrusive igneous; magma). Generally the permeability of crystalline rocks decreases with depth both because of fracture closing and because compressive forces cause fractures to occur more frequently at the surface. However, because the geological formations are similarly brittle at all depths, fracture permeability exists at great depths (Freeze and Cherry 1979). Commonly called fissure springs (Bryan 1919), these deep-seated waters typically have long residence times, high temperatures, and are highly mineralized (Fig. 1, path 4a). Faulting provides these springs with a quick return route to the surface, bringing with them the temperatures and minerals of the deeper crust (Bryan 1919; van Everdingen 1991). The sulfate-bearing minerals gypsum and anhydrite which are embedded in the crystalline rock produce sulfate or sulfurous springs.

Volcanic (extrusive igneous) rock can lie at or near the surface. Extrusive igneous, most of which is basalt, is often underlain by granite, metamorphic, and sedimentary rock, which are mostly impermeable. The cold temperatures (2.9-15.7°C) of these springs serve as an indication of shallow, quick flow paths through caverns and tunnels in the lava rather than through rock pores (Bryan 1919; USGS¹ 2008). These conditions result in short residence and return times back to the surface (path 4b) with little chance of mineralization (Scanlon and Thrailkill 1987; van Everdingen 1991; Erman and Erman 1995; Worthington and Ford 1995). Consequently, TDS concentrations range from 9.9-1590.0 mg/L⁻¹ (USGS¹ 2008). Springs issuing from extrusive igneous rock at moderate or considerable depths below the surface, originate from magmatic water, or water that has previously

contracted volcanic rock. These springs issue from deeper, longer flow paths (Fig. 1, path 4c) resulting in relatively longer residence times, and thereby experience greater thermalization and mineralization (Bryan 1919; Stearns *et al.* 1937). Large fissures, produced by faulting, ensure the water's quick ascension to the surface without thermal re-equilibration.

Non-thermal springs issuing from igneous and metamorphic formations are typically insect-dominated ecosystems characterized by moss mats, figwort (*Veronica americana*), and water cress, *Rorippa* sp. (= *Nasturtium officinale*) (Anderson and Anderson 1995; Erman and Erman 1995; Erman 1998). Dipterans are the most common insects with as many as 13 species of craneflies (Tipulidae: especially *Dicranota* and *Pedicia*), 15 species of biting midges (Ceratopogonidae; primarily *Culicoides* and *Dasyhelea*), 7 species of mothflies (Psychodidae: *Telmatoctopus*), 4 species of blackflies (*Simulium*), and members of the families Culicidae, Dixidae, Stratiomyidae, and Tabanidae. The chironomid midges (especially subfamilies Chironominae and Orthocladinae, with no dominant genus), however, are the most diverse group of dipterans. Caddisflies are typically the second most diverse insect taxa, represented by as many as 36 species (Erman and Erman 1995). The most frequently reported caddisfly taxa are *Rhyacophila*, *Hydroptila* rono, *Lepidostoma unicolor*, and *Micrasema*. The mayfly (Ephemeroptera; especially *Baetis*), and the stonefly (Plecoptera; especially *Amphinemura*), diversities are depauperate in comparison. Damselflies (Odonata: Zygoptera) are represented primarily by *Argia vivida* and *Ischnura perparva*, genera common in sandstone springs as well. *Argia vivida* is thermophilic and, therefore, also characteristic of thermal springs in these aquifers. Nematodes, oligochaetes, and mites are often quite abundant, while densities of groups such as Turbellaria, Mollusca (Bivalva and Gastropoda), and Amphipoda and are quite low.

Nearly all thermal springs are associated with this type of geology (Waring 1965). Temperatures in very warm thermal springs can range from 29-52°C, with much lower temperatures (24.3-34.6°C) in more moderate thermal settings. Dissolved

oxygen as well as TDS vary according to degree of thermalization, with values ranging from 0-5.4 mg/L⁻¹ and 22,000-50,000 mg/L⁻¹ respectively under high thermal conditions, and 5.8-7.1 mg/L⁻¹ and ~1,000 mg/L⁻¹ respectively, under moderate thermal conditions (Brock 1970; Prichard 1991; Hayford *et al.* 1995).

The autotrophic community in thermal springs consists of large moss or algal bacterial mats and the green algae *Oscillatoria* (Prichard 1991; Hayford *et al.* 1995), and invertebrate communities tend to be simple (Pritchard 1991). Water mites *Thermacarus nevadensis* (Hydrodromidae) and *Partnuniella thermalis* (Protsiidae) are typical biotic components of this ecosystem (Collins *et al.* 1976). It is the dipterans, however, that show the most interesting and diverse association to thermal springs. Chironomid midges (*Chrionomus*, *Paratendipes thermophilus*, and *Microspectra*) and brineflies (Ephydriidae; *Ephydra* and *Paracoenia trubida*) are dominant. Other common dipterans include soldier flies (Stratiomyidae; *Stratiomyia*) and biting midges (Ceratopogonidae: *Dasyhelea cincta*) (Brues 1927; Collins *et al.* 1976; Pritchard 1991; Hayford *et al.* 1995; Hayford and Hermann 1995). Other insect groups cited as being characteristic of thermal springs include small hydrophilid (*Paracymus*) and dytiscid beetles (*Bidessus*) and the thermophilic odonate *Argia vivida* (Odonata: Coenagrionidae). Insects in the orders Ephemeroptera, Megaloptera, and Plecoptera are rare (Collins 1976; Pritchard 1991; Hayford and Hermann 1995). Prevalant non-insect species include amphipods (*Hyalella azteca* and *Gammarus limnaeus*), and the isopod *Exosphaeroma thermophilum* (Brues 1927; Hayford and Hermann 1995).

Sandstone and carbonate path

Due to the mixed nature of the sandstone and carbonate rocks (limestone and dolomite) in these aquifers, large quantities of water move rapidly through the confined and unconfined carbonate underground solution cavities, while water moves slowly through the inter-granular sandstone pore spaces (Miller 2000). Limestone-dominated flow paths tend to be short and shallow producing cold, freshwater springs

(Fig. 1, path 5a) with temperatures ranging from 9.5-15.8°C and TDS concentrations of 92-275 mg/L⁻¹ (USGS¹ 2008). However, when aquifer regions are dominated by sandstone, residence time is long and surface return time is slow, producing cold, mineralized waters from shallow depths (path 5b) in the bedrock (Heath 1984; van Everdingen 1991). Dissolved oxygen levels are highly variable depending on proximity to the spring source (5.4-10.0 mg/L⁻¹), while pH levels are generally high (7.1-8.1) due to the influence of the carbonate geology. Less often, flow paths are long and deep with quick surface return times; producing mineralized thermal springs (path 5c).

Moss dominated by *Leptodictyum riparium*, *Barchytheceium*, and *Fontinalis*, jewel weed (*Impatiens capensis*), and water cress make up the majority of the macrophyte community. Filamentous algae are represented primarily by *Spirogyra*. (Tilly 1968; Stern and Stern 1969; Van Gundy 1973; Webb *et al.* 1995).

Biota in sandstone and carbonate aquifers is dominated by the amphipod *Gammarus minus* and the isopod *Asellus*, as well as the flatworm *Phagocata gracilis* (Turbellaria). Snails (Gastropoda: *Physa integra* and *Fontigens mickliniana*), and bivalve molluscs (*Pisidium casertanum* and *Spaherium*) are very abundant in some cases and completely absent in others. Oligochaetes are often strongly represented by species of *Lumbriculus* (Lumbriculidae) and *Limnodrilus* (Tubificidae), as are cambarid crayfish *Orcontectes* and *Cambarus*. Among the insects, mayflies (especially *Baetis*) and flies (Diptera) are the most abundant, especially the latter. Diptera include several chironomid midge genera from the subfamilies Orthocladinae and Chironominae, as well as crane flies (especially *Tipula* and *Antiocha*), the moth fly *Psychoda* (Psychodidae), the dancefly *Chelifera* (Empididae), the soldier fly *Stratiomys* (Dixidae), and the blackfly *Simulium*. Caddisflies are second in taxon richness behind dipterans and are represented primarily by *Rhyacophila parantra* and *Glossosoma intermedium*. Common beetles include *Stenelmis* (Elmidae) and *Ectopria* (Psephenidae), while megalopterans are represented by the alderfly *Sialis* and the fishfly *Chauliodes*. Stoneflies and odonates are weakly represented.

Carbonate path

Carbonate springs originate from carbonate rock (limestone and dolomite) and occur in many parts of the country as cold or thermal springs (van Everdingen 1991). Groundwater flow in carbonate aquifers is usually rapid, traveling through large solution openings, some of which may be several meters in diameter. As a result, large volumes of water move quickly in and out of these aquifers producing cold springs with temperatures ranging from 6.1-22.3°C (Fig. 1., path 6a) and low dissolved-solids concentrations (57-338 mg/L⁻¹). Dissolved oxygen levels are highly variable, ranging from 4.3 - 11.0 mg/L⁻¹. These spring systems are characterized as alkaline or carbonate, discharging clear waters with pH levels often as high as 9.1 and turbidity levels ranging from 1-4.2 NTU.

Exceptions to flow patterns stated above are the lower thick limits of the Floridian and Ozark Plateau aquifers where lateral and vertical movement of groundwater is retarded. This lengthens subsurface residence time and surface return time, thereby increasing mineralization of the water (Fig. 1, path 6b). Calcium is often the dominant cation in cold carbonate springs because surface outcrops of limestone and dolomite are commonly more than 95% calcium and magnesium carbonate (Worthington and Ford 1995). Also common in these aquifers are hardwater and saline springs containing sodium or calcium cations (or both), and chloride anions as their main constituents. Water in these carboniferous salt beds travels along typically shallow, short flow paths (van Everdingen 1991), producing cold saline springs such as those in Howard and Saline Counties of Missouri (Banner *et al.* 1989). High sulfate values are also frequently found in carbonate aquifers. Because the sulfate-bearing minerals (gypsum and anhydrite) are present in such small amounts, groundwater flow paths must be long and deep (path 6c) for mineralization to occur (Freeze and Cherry 1979). Springs with the highest sulfate concentrations then have the deepest, longest flow paths and are thermal, indicating quick surface return times due to fracture enlargements by dissolution of carbonate strata (Worthington and Ford 1995).

Springs of this nature have diverse autotrophic communities, often with large moss mats (*Brachythecium selebrosum* and *Fissidens*) and thick beds of water cress (*Nasturtium officinale*). Benthic microalgal assemblages are diverse (*Cyanophyta*, *Chlorophyta*, and *Chrysophyta*), and *Spirogyra* and *Cladophora* are the dominant filamentous green algae (USGS¹ 2008; Sloan 1956; Odum 1957; Minckley 1963; Van Gundy 1973; Woodruff 1993; Miller 2000; Jackson 2001).

Invertebrate communities of alkaline or carbonate springs are often characterized as being peracaridan or non-insect dominated ecosystems (Van Gundy 1973; Glazier and Gooch 1987; Woodruff 1993). Dominant species include Amphipoda (*Gammarus minus*, *Hyaella azteca*, and *Crangonyx forbesi*), Isopoda (*Lirceus hoppinae* and *Asellus*), molluscs (*Physa*, *Elimia*, and *Pisidium*), flatworms (*Dugesia*, *Phagocata gracilis*, and *Polycelis*), oligochaetes (*Limnodrilus* and *Lumbriculus*), and the crayfish genera *Orconectes* and *Procambarus*. Insect groups are typically more diverse but low in abundance. Dipterans are the most abundant and diverse of the insect taxa led by chironomids including as many as 19 species of Tanypodinae (especially *Pentaneur* and *Procladius*), 25 or more species of Orthocladiinae (especially *Psectrocladius*), and over 46 species of Chironominae (especially *Stictochironomus*, *Endochironomus*, and *Cryptochironomus*). Other dipteran representatives include crane flies (*Tipula*), dance flies (*Herodromia*), black flies (*Simulium*), biting midges (*Culicoides*), and soldier flies (*Odontomyia*). Trichoptera, which are second in taxonomic richness to dipterans, are led in dominance by *Cheumatopsyche* and *Hydropsyche*, but also include *Glossoma*, (*Glossomatidae*), *Hydroptila* (*Hydroptilidae*), and both *Ochrotrichia* and *Helicopsyche* (*Helicopsychidae*). Other insect groups include Coleoptera (dominated by *Optioservus* [*Elmidae*], *Psephenus* [*Psephenidae*], and *Agabus* [*Dytiscidae*], Hemiptera (*Gerridae*: *Metrobates*), Megaloptera (*Sialidae*: *Sialis*), Odonata (*Coenagrionidae*: *Argia*), and Plecoptera (*Perlidae*: *Acroneuria* and *Perlesta*). Ephemeroptera are most strongly represented by *Baetis* (*Baetidae*) but also by

Stenonema (Heptageniidae), and *Ephemerella* (Ephemerellidae) (Van Gundy 1973; Glazier and Gooch 1987; Woodruff 1993).

Patterns in the Biota of Spring Ecosystems

The fauna of non-glaciated cold springs are characterized by amphipods, molluscs, turbellarians and oligochaetes, and the following insect groups (in order of dominance): chironomids, trichopterans, ephemeropterans, and plecopterans (Odum 1957; Tilly 1968; Gooch and Glazier 1991; Webb *et al.* 1995; Williams and Williams 1998). The non-glaciated, unconsolidated sand and gravel aquifers as well as the semi-consolidated sand aquifers are typically both insect and non-insect dominated. However, across all studies examined, Plecoptera and Ephemeroptera were weakly represented, and pericaridans, molluscs, turbellarians, and oligochaetes are very rare or absent from the cold springs of the non-glaciated igneous and metamorphic aquifers—both points of discrepancy with previously cited studies.

Likewise, glaciated cold spring fauna have generally been characterized as being dominated by chironomids, stoneflies, caddisflies, and amphipods, with turbellarians and molluscs weakly represented (Williams and Hogg 1988; Williams and Williams 1998). While this pattern seems consistent for insects in the largely glaciated sandstone, sandstone-carbonate, and glaciated portions of the carbonate aquifers, turbellarians and molluscs were abundant in these aquifers and amphipods were consistently rare in sandstone aquifers.

Specific faunal groups exhibited notable similarities across all spring types. Of the insect groups, Diptera (led by Chironomidae) followed by Trichoptera were consistently the most diverse and abundant faunal groups. Mayflies were also consistently reported. The dipteran pattern held true for thermal and mineral springs as well. *Cheumatopsyche* and *Hydropsyche* were the most common caddisfly genera, and *Baetis* was the most common mayfly genera. Of the non-insect groups, the gammarid amphipods were the most dominant. Several non-insect groups were consistently represented in the various types of springs by only two genera within the larger taxon. These were: the turbellarians *Dugesia* and *Phagocata gracilis*; the

molluscs *Physa* and *Pisidium*; the isopods *Asellus* and *Lirceus hoppinae*; and the oligochaetes *Limnodrilus* and *Lumbriculus*. Fauna were most diverse in coldwater springs than in thermal and mineral.

Patterns in floral diversity were also present among spring types. Water cress was the most abundant macrophyte among all types of springs. Algal communities were consistently dominated by filamentous *Spirogyra* and *Cladophora*. Moss mats were also very common in spring ecosystems. Their abundance may reflect the fact that mosses assimilate free carbon dioxide and are therefore found in habitats such as springs where free carbon dioxide is high (Stern and Stern 1969). The presence of moss is also an indication of spring constancy and persistence (Erman and Erman 1995).

The similarities noted above indicate that some spring species have considerable geographic ranges; indeed, at the order, and even genus levels, the biota of springs is remarkably similar world-wide (Ward and Dufford 1979). At the species level, however, the diversity is high within Coleoptera, Oligochaeta, Diptera (Chironomidae), Plecoptera, and Trichoptera. For example, Webb *et al.* (1995) found 17 oligochaete species in southern Illinois; Erman and Erman (1995) collected 36 trichopteran species in a cold water spring in California; and 66 species of chironomids were collected in Big Springs, Kansas (Ferrington *et al.* 1995). Some general patterns are described below for the major types of springs.

Unconsolidated sand and gravel and semi-consolidated sand aquifers: These springs are characterized as both insect and non-insect dominated. Non-insects tend to be more dominate however, with only a few common insect groups with low diversity including Trichoptera, Diptera (Chironomidae), and Coleoptera. All other insect taxa are poorly represented.

Sandstone and igneous/metamorphic aquifers: In springs issuing from these aquifers, peracaridan crustaceans and molluscs are typically absent and insects dominate, but again represented by only a few species within Trichoptera, Diptera (Chironomidae), Ephemeroptera, Plecoptera, and Coleoptera.

Sandstone-carbonate and carbonate aquifers: The primarily non-insect fauna of these springs is dominated by turbellarians and peracaridans (usually amphipods, but sometimes accompanied by isopods; Glazier 1991). *Gammarus minus* is the characteristic species of amphipods dominating hardwater spring communities. Limestone springs favor dense populations of gastropods as well. Both crustaceans and snails are absent in softwater springs (i.e. sandstone). Causal reasons for this could be the lack of calcium for shell formation (Hynes 1970), or the fact that both taxa show a strong affinity for watercress, a macrophyte missing from softwater springs. Thermal constancy of temperate cold springs favors non-emergent taxa, because thermal cues required for insect life-history patterns such as emergence and diapause are absent (Glazier 1991). These conditions should result in their dominance in all spring types. However, the low pH and alkalinity levels found in softwater springs are not favorable for crustaceans and molluscs. While insect groups are not dominant in sandstone-carbonate and carbonate aquifers, they are more diverse than the non-insect groups.

The synthesis of literature and patterns presented here illustrate that it is the geology of the aquifer that exerts a controlling effect on a spring's fauna, as it determines a spring's water source, surface texture and size of the components of the substrate, rate of flow, temperature, and chemical composition—all primary determinants of benthic species distribution.

CHAPTER 2

STRUCTURAL CHANGES IN A KARST SPRING COMMUNITY FROM CAVE SOURCE TO SPRINGBROOK

Introduction

Known as the aquatic ecologist's natural laboratory (Odum 1957), springs represent a groundwater/surface water ecotone where discharge, temperature, and chemical characteristics remain relatively constant (Sloan 1956; Teal 1957; Iverson 1988; Gooch and Glazier 1991; Danks and Williams 1991; van der Kamp 1995; Glazier 1998). Moreover, springs typically represent smaller, more isolated habitats with fewer large predators, reducing further the number of variables to be considered in field investigations (Stern and Stern 1969; Glazier 1991). In these respects, springs are arguably among the most unique headwater ecosystems in the North Temperate Zone and are ideal locations for population, community, and ecosystem investigation.

Springs harbor biological communities that are markedly different from streams in the same geographic region (McCabe 1998). Before 1980, however, spring ecosystems were depicted as impoverished habitats with relatively few species of interest (Erman and Erman 1990). Several studies since then (e.g. Ferrington 1995; Webb *et al.* 1995) have shown that springs can be quite rich in species and are often habitats for a disproportionately large number of rare, relict, and endemic species (Hynes, 1983; Erman and Erman 1995). Springs make an important contribution to benthic species richness in stream sections and have been shown to contribute as much as one third of regional freshwater biodiversity (Erman 1992; Mattson *et al.* 1995; Sabatino *et al.* 2003). The importance of spring habitats in sustaining high levels of biodiversity and their contribution to freshwater biodiversity at local and regional scales is likely to increase especially in areas where global climate change is predicted to enhance variability of precipitation, as in parts of the Midwest (e.g., Dodds *et al.* 2004). Despite their importance to freshwater ecology, research on these unique ecosystems remains limited and poorly understood (Ferrington 1995; Smith and Wood 2002; Wood *et al.* 2005).

Identification of factors that determine the abundance and distributional patterns of species in epigeal systems such as water chemistry, discharge, substrate, and the relative importance of allochthonous vs. autochthonous organic matter has been the subject of long-term research efforts (cf. Minshall 1967; Finn and Poff 2005). Water chemistry, for example, is an important determinant of faunistic organization in springs (cf. Erman & Erman 1995). High concentrations of dissolved ions such as calcium, chloride, and ammonium have been associated with spatial differences in macroinvertebrate community structure and increases in species richness (Williams *et al.* 1997; Erman 2002). Flow permanence has been shown to lead to greater species diversity in springs (Erman 1992), and contributes to the lack of aerial dispersal life-cycle patterns of non-insect groups (Smith *et al.* 2003); it also explains a significant fraction of the variability in species abundance (Williams 1991; Smith and Wood 2002). Thermal stability is also an important factor defining biotic spring assemblages allowing springs to harbor a unique group of species, of which many reproduce asynchronously all year (e.g. *Gammarus minus* Say) (Odum 1957; Teal 1957; Williams and Hogg 1988; Glazier 1991; Erman and Erman 1995; Smith *et al.* 2001).

Studies of small-scale patchiness of invertebrate density and diversity in a single habitat type are not new to aquatic research (Minshall 1984; Downes *et al.* 1993). Spring source/springbrook ecotones represent dynamic boundaries of patch connectedness that play important roles in determining the distributional patterns of resident spring biota. Conditions at the spring source are consistent with the stability of emergent groundwater (reduced thermal, discharge, and chemical fluctuations). Stable ground water influences diminish, however, with increasing distance from the source (eucrenal zone). Hence, insect genera at the spring source often include subterranean animals and typically vary from those in the lower reaches; moreover, faunal diversity commonly increases downstream as conditions of the springbrook become increasingly more influenced by dynamic ambient conditions of the surrounding watershed (Sloan 1956; Odum 1957; Teal 1957; Minckley 1963;

Minshall 1968; van Gundy 1973; Ward and Dufford 1979; Danks and Williams 1991; McCabe 1998; Smith *et al.* 2001). This creates a longitudinal mosaic of variable habitat patches and corresponding community dynamics over small spatial scales, thereby defining the spring source/springbrook ecotone effect.

Longitudinal position and seasonal effects have commonly been cited as influential determinants of taxonomic structure and function in streams (Vannote *et al.* 1980; Scarsbrook and Townsend 1993; Finn and Poff 2005). Longitudinal and seasonal variation in availability of a range of lotic and riparian food resources (allochthonous vs. autochthonous) is crucial in the regulation of spatially and temporally diverse faunal distributions (Thompson and Townsend 1999). Low species diversity at the spring source and the longitudinal fluctuations in community structure and composition found in springbrooks have been attributed to: (a) the lack of particular organic matter (POM) and subsequential transport of detritus from upper reaches (Ward and Dufford 1979; McCabe 1998); (b) the shift from subterranean dissolved organic carbon (DOC) to heavy reliance on allochthonous leaf detritus (Minshall 1967); and (c) community interactions such as energy transfers (Varza and Covich 1995; Glazier & Gooch 1987). These studies lend support to the idea that patterns of community structure in these fragile ecosystems can be attributed to the variation in spatial subsidies with distance from the spring source and the temporal (seasonal) pulses of organic inputs along the spring source/spring brook continuum.

Karst systems represent ideal systems for studying spatial and temporal variability in macroinvertebrate community structure. Although largely ignored in global biodiversity studies karst systems are at the roots of the conceptual foundation for groundwater ecosystems and represent an array of diverse habitats with substantial biodiversity (Gilbert *et al.* 1994). Knowledge of karst springs is significant from a biological perspective because the porous aquifers, typical of karst topography, are based on hydrological and geomorphologic features that are organized by karstification into flow pathways that contain pipe-like conduit systems. These wide, cavernous conduits are characterized by rapid flow, direct transmittance,

and low filtering capabilities which, in turn, yield interactive biological components by allowing the movement of particulate organic matter and larger subsurface organisms between subterranean and surface environments. Hence, the biodiversity of karst springbrooks is often subsidized by subterranean species from the spring source and underground limestone conduit systems, moving across habitat boundaries to locate better pupation sites and food resources (Hobbs 1992; Erman 2002).

Cold hardwater limestone (pH >7.0) springs and springbrooks in karstic regions are typically dominated by non-insect taxa such as gammarid amphipods, asellid isopods, triclads, and physid gastropods; along with limited insect species (Minshall 1967; Stern and Stern 1969; Glazier and Gooch 1987; Gooch and Glazier 1991). However, longitudinal changes in community composition and abundance have been observed, with diversity typically increasing with distance along the springbrook (Sloan 1956; Minckley 1963; Minshall 1968; Ward and Dufford 1979).

Research in spring habitats is limited in the Central Plains of the United States. With the exception of the work of Vineyard and Feder (1982), there is little substantial data for the springs of the state of Missouri (Ferrington 1995). Much of the work on springs has been performed by systematists in a single spring, often on a single taxon of animals (Erman 2002). The present study represents a temporal and spatial analysis of spring invertebrate communities, across seasons at multiple sites in multiple springs in the same geographic area.

This study compared springs within the Ozarks over time and examined the influence of habitat characteristics (water chemistry, discharge), reach (distance from the spring source), and season on macroinvertebrate community structure. The following principal questions were posed: (i) How does community structure (species composition, diversity, richness, evenness) vary spatially along a downstream gradient from the spring source (cave) in each of three karst springs; (ii) How does community structure in each of these 3 springs vary temporally (seasonally); and (iii) Do the three study springs have similar longitudinal and seasonal community patterns?

Based on pilot samples and literature sources, I hypothesized that: (i) species composition would shift from a community composed of non-insect taxa to one dominated by aquatic insects; (ii) community biocomplexity would vary spatially from more simplistic at the spring source to increasing complexity with distance along the spring brook largely as a function of differences in spatial availability of dominant carbon sources (allochthonous vs. autochthonous); (iii) community biocomplexity would vary temporally (seasonally) reflecting accessibility to, and availability of, dominant carbon sources (allochthonous vs. autochthonous) along this gradient; (iv) community functional feeding groups (FFG's) will vary temporally illustrating a shift in available carbon sources; and (v) each of the springs will exhibit similar longitudinal and seasonal biological patterns due to the fact that they occupy similar or adjoining watersheds in the same geographical area.

Methods

Study area and site descriptions

The study area encompassing all three springs is located in a karstic region of the Ozarks in southwestern Green County, Missouri. The Missouri Environmental Geology Atlas (MEGA) spring database currently lists more than 3,000 springs in the state. Greene County has hundreds of springs that flow all year, are the products of karst terrain, and represent the outflow points for karst drainage systems. The study area is located in the Springfield Plateau region (Fig. 2) of the Ozark uplift just north of the city of Springfield, and represents two adjoining watersheds: the Sac and the James River basins.

Greene County is underlain by two aquifers: the shallow unconfined Springfield Plateau Aquifer and the deeper confined Ozark Aquifer. Springs in this region were formed by Mississippian-aged carbonate rocks and older Ordovician dolomites. The Springfield Plateau is well known for extensive Burlington-Keokuk Limestone karst formations. The basin's floor consists of Jefferson City-Cotter dolomite (Fig. 3) followed in an upward direction by Compton limestone, North-view

shale, Pierson limestone, Elsey limestone, the Burlington-Keokuk limestone formation, and Warsaw limestone at the top (Thompson 1986). The Northview shale

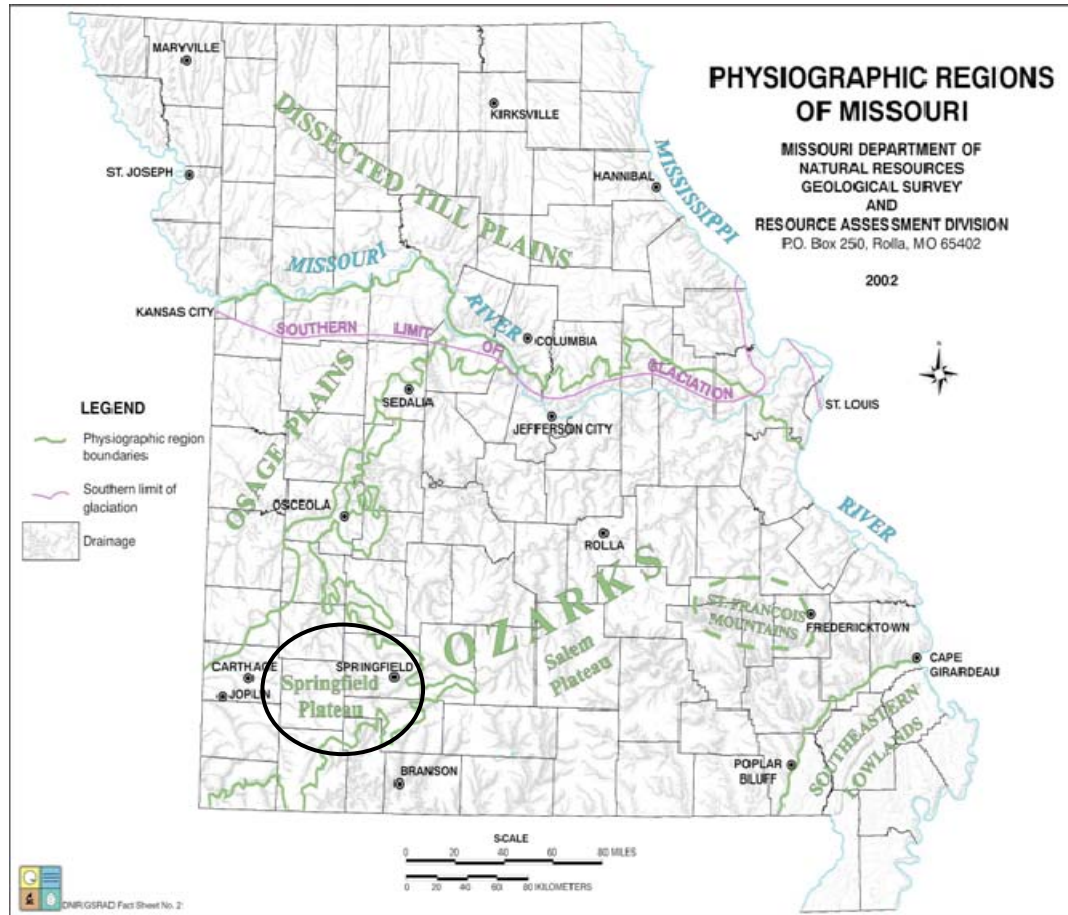


Figure 2 Map showing physiographic features of Missouri.

acts as an aquitard, allowing for the emergence of many springs and seeps in the basin. However, most of the springs and caves in this region are developed specifically in the Burlington-Keokuk limestone that has been dissolved along karst fractures (Vineyard and Feder 1982). This limestone is very fossiliferous, and parts of the formation are composed mostly of the remains of crinoids, ancient sea animals (Bullard *et al.* 2001). The study area has an average annual rainfall of 101.6 cm. The mean annual temperature is 15 ° Centigrade.

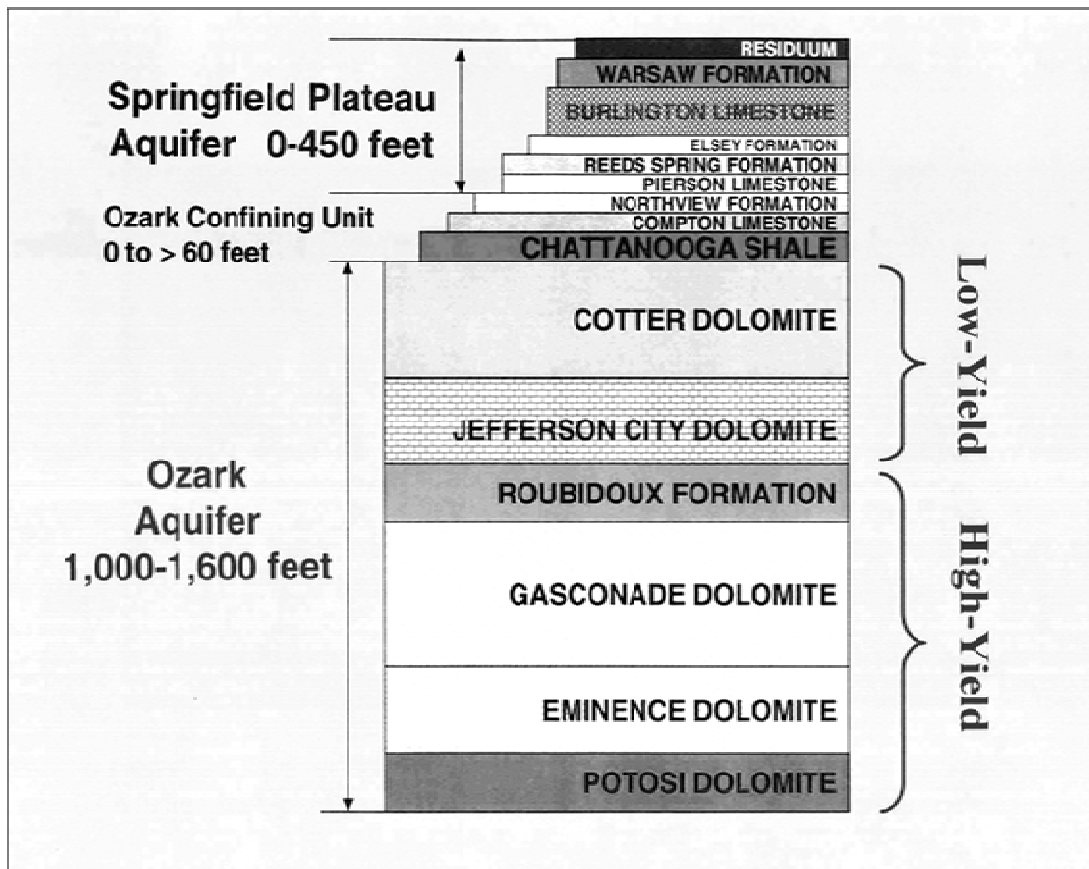


Figure 3 Composite stratigraphic section of the rock units in the Ozark and Springfield Aquifers of Greene County, Missouri. (Missouri Department of Natural Resources Geological Survey and Resources Assessment Division)

All 3 study springs can be defined as a fourth magnitude (Meinzer 1927) or medium volume ($0.01\text{-}0.5\text{ m}^3/\text{s}^{-1}$) cold, freshwater, permanent rheocrene (Danks and Williams 1991), with each originating from an open cave mouth (single point of issue) without any significant hydrostatic head (White 1988) and followed by a springbrook.

Haseltine Spring. This spring is just North West of the city of Springfield, Missouri R23W, T29N, Sec, 3 ($37^{\circ}25'$ N, $93^{\circ}43'$ W) and is located on the Campbell homestead in the Sac River Basin (Figs. 4 & 5). The Sac River basin is primarily rural. Land uses in this watershed are predominantly agricultural with livestock

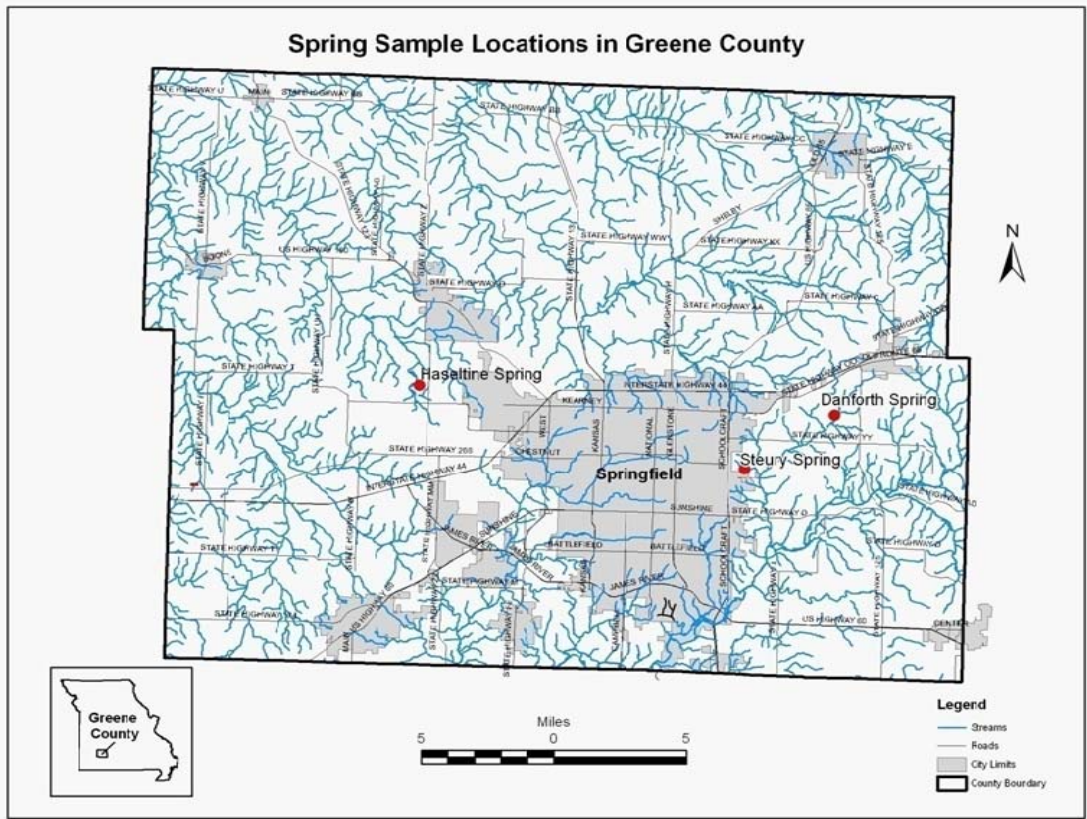


Figure 4 Spring locations in Greene County, near the city of Springfield, Missouri

with livestock production serving as the significant agricultural enterprise in the basin throughout its hiSteury (Missouri Department of Conservation [MDC₁]). Land cover in the Sac Basin is predominantly grassland and pasture (59%) with forest second in abundance (23%). The spring sits in an open valley with only one side of the channel having any riparian cover. Historic pictures of the site show that at one time, both sides of the channel were covered with deciduous trees. Water at the springhead emerges from Haseltine Cave ($\sim 38.98 \text{ m}^2$) and flows out of the cave, forming the springbrook.

Steury and Danforth Springs. These springs are near Missouri R21W, T29N, Sec. 22. (37°21' N, 93°21' W) and, Missouri R20W, T29N, Sec. 5. (37°24' N, 93°15' W), respectively, and they are located just east of Springfield in the James River

Basin (Figs.4 & 5). Fluvial systems in this basin are typically found in narrow, steep-sided valleys with high bluffs, and are characterized by high gradient and relief. Land use is primarily agricultural (mostly pasture and some row cropping), with much smaller proportions representing forest (30%) and urban (7%) land uses (MDC₂). *Steury* is located in a deciduous forest and emerges from a large cave (~63.50 m²) with a connecting natural bridge. The spring's cave was walled-up with rock in the 1930's for refrigeration. As the water passes out of the cave, a rapidly moving rheocrene forms whose flow is often hampered by massive watercress growth.

Danforth is located on the Lovett Pinetum with approximately 40 species and subspecies of North American pine *in situ*. Danforth is remembered locally for providing water to large numbers of people in the Springfield area during the drought of the 1930's (Bullard 2001). Subterranean waters at the Danforth springhead emerge from a smaller cave-like opening (~9.01 m²), at the base of a vertical limestone bluff, forming short rheocrene section (25m) that then cascades into a large limnocrene pool (~30 m²). From there, the pool spill-over forms a well-defined springbrook.

Haseltine Spring



Steury Spring



Danforth Spring

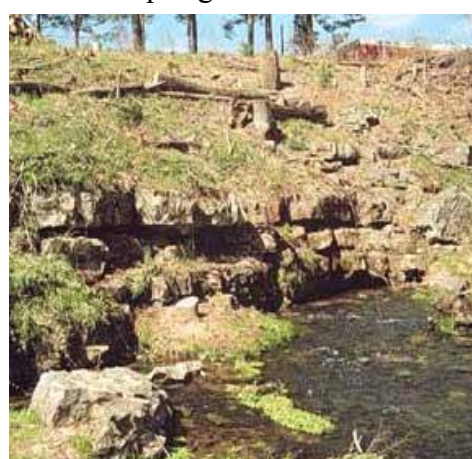


Figure 5 Photographs of the 3 springs at their points of origin.

Because spring ecosystems in the study area are situated within the spectrum between caves and streams, the biota share attributes and life forms with each of these habitats (Bullard *et al.* 2001). Spring fauna exhibit a large degree of endemism, with approximately 38 species from springs and subterranean habitats restricted to the Ozark region of Missouri (Vineyard and Feder 1982). The spring source fauna is subsidized by species otherwise known only from subterranean waters. These are such a persistent constituent of the springs that a clear line distinguishing the fauna of the springs from that of their subterranean sources is often unclear (Vineyard and Feder 1982).

Spring characterization

For the purpose of this study, spring source or spring issue refers to the area of spring origin at the cave and springbrook refers to downstream reaches below the source prior to the confluence with another tributary (Steury and Danforth) or termination into a deepwater lake (Haseltine). This designation was made because often there is not a single, well-defined spring source, but a series of sources that combine to produce a springbrook (Ward and Dufford 1979). The spring source for each spring was clearly defined because all issue from caves or cave-like openings in carbonate rock formations.

The inorganic substrate types in all three springs consisted primarily of cobble and gravel. The exceptions to this were Haseltine Cave (spring source) and the Danforth limnocrone (R2) area which are primarily sand, silt, clay, and gravel. The predominant substrate-current combination of each of the springbrooks is primarily a rubble-riffle complex.

Each of the springs is associated with distinctly different riparia (Table 1): a deciduous forest, a coniferous forest, and a combination of deciduous forest and open, mowed, grassland. Consequently, there were vast differences in amount of incident light that reached the wetted surface of each spring. Incident light values (% transmittance) were measured using an AccuPAR Linear PAR/LAI ceptometer

(Decagon Devices, Inc.). Mean light values were generated from an average of 12 measures taken in full leaf-out conditions at noon over the length of each spring (Table 1). As leaves fell in late fall, the amount of incident light increased until it corresponded with that of direct sunlight.

The distances from the spring source to the most downstream reach varied among springs from 101 to 145 m. These springbrook lengths were relatively long compared to the relatively short site lengths typical of most published spring studies (Stern and Stern 1969; Wilhm 1970; Resh 1983; Williams and Hogg 1988, Varza and Covich 1995; Erman and Erman 1995; Beierkuhnlein and Grasle 1998; Webb *et al.* 1998; Smith *et al.* 2001; Von Fumetti *et al.* 2006). Pilot studies confirmed that major changes in biocomplexity occurred over these distances.

Table 1 General Characteristics of Study Springs

Site	Flow regime	Elevation (m)	Mean width (m)	Length (m)	Riparian Vegetation	Cave Size (m ²)	Ground-water Residence Time	Avg. Incident Light (=‰t)
Steury Spring	Permanent	396.24	4.89	144.77	Heavy deciduous forest	63.5	48h 2.01 mi 0.02 m·s ⁻¹	0.35
Danforth Spring	Permanent	409.956	3.98	101.23	Heavy coniferous forest	9.01	90h 2.22 mi 0.01 m·s ⁻¹	0.41
Haseltine Spring	Permanent	350.52	3.49	103.1	Open lawn, with only 1 side of channel tree-lined for 34 m	38.98	192h 2.41 mi 0.01 m·s ⁻¹	0.72

Subsurface residence time (or ages) of spring waters was determined by examining dye tracing data (Missouri Environmental Geology Atlas; MEGA) and comparing averages of the amount of time from injection point to reception point over karst conduit distance traveled, and computing comparative measures of velocity

(Table 1). Traditional radioactive isotope methods for determining subsurface residence time could not be used because Haseltine, Danforth, and Steury Springs are non-inertial systems (Gibert *et al.* 1994) with small stores and short retention times (days or shorter). Previous work on these springs show average residence times ranging from 9 hours to 2 days (Loring Bullard; Ozarks spring expert, personal conversation, Tom Alley; Ozarks spring and dye tracing expert, personal conversation).

Physiochemical and biological collections

Hypotheses were tested with data from the spring source and three reaches of the spring brook (henceforth referred to as R1-R4), for each of the three spring ecosystems during two seasons: (i) in early summer when spring brook communities have access to the largest amount of autochthonous resources; and (ii) in early winter when access is high for allochthonous resources from the riparian zone. Each of the 4 sampling reaches were broken into 3 sampling transects: the head, middle, and base (length of each reach divided by 3). Because the springs in this study were small, substrate sampling represented a major disturbance factor in these habitats, and was therefore limited to two sampling periods during a given year to avoid unnecessary destruction to eucrenal or hypocrenal zones (cf. Erman 2002).

Macroinvertebrate samples, one from each of the three sampling transects in each of the four sampling reaches (R1-R4), in each spring were collected for both seasonal sampling periods. Each springbrook was divided into longitudinal reaches for sampling based on preliminary observations of distinct biotypes. Invertebrate sampling procedures were varied according to substrate characteristics (sand/silt vs. cobble). In shallow hypocrenal zones where the substrate is composed of primarily large and small cobble to coarse gravels, invertebrates were collected using a Surber sampler (500 μ m mesh) following disturbance of the substrate to a depth of \sim 15 cm for one minute. In deeper habitats (a shallow pool habitat) with silt, sand and clay substrates, invertebrates were collected with an Ellis-Rutter sampler (500 μ m mesh)

with substrate disturbance depth and timing similar to Surber collections. Because water velocity was typically low in this area, agitated water within the sample was hand-splashed into the net. Invertebrate density was standardized by sampler area. Samples were placed on ice for transport back to the laboratory where they were sorted into major taxonomic groups and placed in a refrigerator at 40 °C to clear the digestive tracts of the invertebrates. Samples were then frozen until later processing. Fauna were identified to species where possible, although some taxa were identified only to subfamily or family, (e.g. Chironomidae, and Oligochaeta respectively).

In association with each invertebrate sample, various habitat parameters and standard water quality variables were measured. Reach width (m) was measured along head, middle, and base transects. Three replicate velocity ($\text{m}\cdot\text{s}^{-1}$) measures were taken along each width transect (9 per reach) and reach depth (m) measurements were taken at ~30 cm intervals along each of the three width transects. Discharge was calculated from measures of current velocity (Marsh-McBirney, Inc. Flo-Mate™ Model 2000 portable flow meter). Total phosphorous (TP), orthophosphate, total organic and inorganic carbon (TIC, TOC), total nitrogen (TKN), nitrate (NO_3), nitrite (NO_2), ammonia (NH_3), calcium (Ca), and magnesium (Mg) levels of collected samples were analyzed by the Southwest Waste Water Treatment Plant of Springfield Missouri using standard methods (American Public Health Association 1998). Dissolved oxygen, pH, temperature, turbidity, and specific conductivity were measured *in situ* using a YSI 6920 Environmental Monitoring System Sonde and a YSI 650 Multi-parameter Display System (YSI Incorporated).

Statistical analyses

Macroinvertebrate community data were first examined graphically to discern longitudinal zonation patterns within each spring. To examine differences between invertebrate communities within and among springs, abundance, Margalef's species richness index, and Shannon's index of diversity (H') were calculated using $(\#/m^2)$, $D_{Mg} = (S-1) / \ln N$ (Clifford and Stephenson 1975), and $H' = -\sum p_i \ln p_i$, (α species

diversity software EstimateS; Colwell 2004), respectively. These indices were then used as dependent variables in a three-way analysis of variance (ANOVA; GLM, NCSS) to test for effects of reach, spring, and season. Statistical significance was determined at $\alpha = 0.05$. Tukey post-hoc tests were used to explore significant differences.

Results

Physicochemical characteristics

The spring source and springbrook sites displayed little variability in the physiochemical variables examined (Table 2 & 3). Spring water was characterized as hard by its high Ca (range 71.9-109.2 mg/L) and CaCO_3 concentrations (range 199-287.2 mg l^{-1}). Variation in Ca and Mg ratios were representative of the variation in the depth of water from which the springs issue. Haseltine and Steury Springs originate from limestone bedrock with higher Ca to Mg ratios (Ca/Mg ratio ranges 88.3-94.3/2.2-2.3 mg l^{-1}), while Danforth issues from deeper dolomite bedrock (Ca/Mg with lower Ca to Mg ratios (63.9-77.2/5.5-6.6 mg l^{-1}). These parameters (indicative of the topography of the area), and related conductivity values that ranged from 407-553 $\mu\text{S}/\text{cm}$ (with intra spring variation ranging from only 1-38 $\mu\text{S}/\text{cm}$), represented the physiochemical measures that exhibited notable variability within and among springs. Temperature regimes of the three springs were comparable regardless of season. Intra- and inter- spring temperature variability was never greater than 1.1°C and 1.5°C respectively. Mean flow rates were comparable across all springs within a given season and indicative of fourth magnitude (0.01-0.5 m^3/s^{-1} ; Meinzer 1927) springs. Summer rates were 0.17, 0.15, and 0.09 m^3/s^{-1} for Haseltine, Danforth, and Steury respectively, while winter rates fell to 0.04, 0.03, and 0.02 m^3/s^{-1} . Intra-spring physiochemical variability was confined to a relatively narrow range for all other parameters. Dissolved oxygen levels varied by ≤ 1.7 mg l^{-1} , pH by ≤ 0.7 , and turbidity by ≤ 1 NTU. Likewise, intra- and inter-spring nutrient levels displayed little seasonal variability. There was no detectable ammonia and rarely any detectable

Table 2 Summary of the **summer sampling** mean temperature ($^{\circ}\text{C}$), discharge (m^3/s^{-1}), conductivity ($\text{C } \mu\text{S cm}^{-1}$), dissolved oxygen (DO mg l^{-1}), pH, turbidity (NTU), total inorganic carbon (TIC mg l^{-1}), total organic carbon (TOC mg l^{-1}), total hardness ($\text{CaCO}_3 \text{ mg l}^{-1}$), calcium (Ca mg l^{-1}), magnesium (Mg mg l^{-1}), ammonia ($\text{NH}_3 \text{ mg l}^{-1}$), nitrate ($\text{NO}_3 \text{ mg l}^{-1}$), total Kjeldahl Nitrogen (TKN mg l^{-1}), phosphate ($\text{PO}_4 \text{ P mg l}^{-1}$), and total phosphate TP (mg l^{-1}), recorded at source and 3 reaches in the springbrook within the three limestone springs. Mean and standard deviation (SD) values based on $n=9$ per reach. H= Haseltine Spring reaches, D = Danforth Spring reaches, S = Steury Spring reaches.

	site	H1	H2	H3	H4	D1	D2	D3	D4	S1	S2	S3	S4
Temp. ($^{\circ}\text{C}$)	mean	14.40	14.14	14.20	15.24	13.90	14.09	13.87	13.91	14.38	14.55	15.15	15.18
	SD	0.41	0.08	0.09	0.07	0.33	0.24	0.01	0.11	0.01	0.16	0.02	0.04
Discharge (m^3/s)	mean	0.09	0.27	0.13	0.17	0.09	0.22	0.13	0.14	0.13	0.10	0.08	0.05
	SD	0.06	0.06	0.03	0.05	0.01	0.11	0.01	0.01	0.07	0.05	0.02	0.01
Cond. ($\mu\text{S cm}^{-1}$)	mean	456.7	462.3	462.0	447.7	477.0	475.7	478.0	475.0	552.7	550.3	518.7	527.0
	SD	5.51	0.58	1.00	3.10	1.00	0.58	4.36	0.05	0.58	1.15	3.60	4.00
D.O. (mg l^{-1})	mean	6.20	6.84	7.91	7.98	9.37	9.55	9.75	10.10	7.74	7.68	7.41	7.13
	SD	0.22	0.71	0.20	0.04	0.17	0.13	0.10	0.02	0.05	0.06	0.08	0.17
pH	mean	7.03	7.15	7.26	7.62	7.27	7.47	7.52	7.60	7.45	7.68	7.80	8.15
	SD	0.02	0.03	0.08	0.16	0.05	0.17	0.11	0.53	0.03	0.09	0.03	0.42
Turbidity (NTU)	mean	1.63	1.67	2.10	2.00	1.23	1.00	0.93	1.60	1.47	0.97	1.13	1.43
	SD	0.32	0.38	0.17	0.10	0.15	0.10	0.21	0.36	0.25	0.15	0.68	0.15
TIC (mg l^{-1})	mean	13.40	12.60	11.20	13.30	14.80	13.70	13.40	13.30	14.00	14.10	13.20	14.00
	SD	0.45	0.50	0.37	0.53	0.85	0.80	0.70	0.30	1.00	0.85	1.05	0.66
TOC (mg l^{-1})	mean	4.23	2.71	2.87	3.36	2.19	2.81	2.69	2.29	2.98	3.20	2.81	2.86
	SD	0.13	0.24	0.23	0.26	0.13	0.24	0.23	0.26	0.17	0.30	0.17	0.09
CaCO_3 (mg l^{-1})	mean	226	232	233	237	214	206	220	220	271	269	282	267
	SD	8.50	2.52	7.02	9.50	8.50	7.00	2.00	4.00	7.41	2.66	4.66	1.76
Ca (mg l^{-1})	mean	87.10	87.70	88.70	90.90	75.40	72.30	77.20	77.20	104.30	103.40	108.80	103.10
	SD	1.05	0.90	0.70	0.40	0.85	0.38	1.00	0.94	1.00	0.46	0.37	0.12
Mg (mg l^{-1})	mean	2.10	2.20	2.30	2.40	6.40	6.10	6.60	6.60	2.40	2.50	2.60	2.40
	SD	0.17	0.07	0.15	0.17	0.40	0.27	0.43	0.20	0.25	0.08	0.12	0.07
NH_3 (mg l^{-1})	mean	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	SD	na	na	na	na	na	na	na	na	na	na	na	na
NO_3 (mg l^{-1})	mean	1.83	3.02	3.36	3.32	3.36	2.05	2.07	2.02	2.05	2.31	2.31	2.1
	SD	0.1	0.1	0.1	0.2	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1
TKN (mg l^{-1})	mean	0.03	NT	NT	0.24	0.32	0.32	0.01	0.23	0.21	0.12	0.34	0.3
	SD	0.1	na	na	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0
$\text{PO}_4 \text{ P}$ (mg l^{-1})	mean	NT	NT	NT	NT	0.03	0.02	0.02	0.02	NT	NT	NT	NT
	SD	na	na	na	na	0.1	0.1	0.1	0.1	na	na	na	na
TP (mg l^{-1})	mean	0.17	0.19	0.2	0.17	0.3	0.17	0.18	0.23	0.22	0.27	0.18	0.19
	SD	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.3	0.1	0.1	0.1	0.1

Table 3 Summary of the **winter sampling** mean temperature ($^{\circ}\text{C}$), discharge (m^3/s), conductivity ($\text{C } \mu\text{S cm}^{-1}$), dissolved oxygen (DO mg l^{-1}), pH, turbidity (NTU), total inorganic carbon (TIC mg l^{-1}), total organic carbon (TOC mg l^{-1}), total hardness ($\text{CaCO}_3 \text{ mg l}^{-1}$), calcium (Ca mg l^{-1}), magnesium (Mg mg l^{-1}), ammonia ($\text{NH}_3 \text{ mg l}^{-1}$), nitrate ($\text{NO}_3 \text{ mg l}^{-1}$), total Kjeldahl Nitrogen (TKN mg l^{-1}), phosphate ($\text{PO}_4 \text{ P mg l}^{-1}$), and total phosphate TP (mg l^{-1}), recorded at the source and 3 reaches in the springbrook within the three limestone springs. Mean and standard deviation (SD) values based on $n=9$ per reach. H= Haseltine Spring reaches, D = Danforth Spring reaches, S = Steury Spring reaches.

	site	H1	H2	H3	H4	D1	D2	D3	D4	S1	S2	S3	S4
Temp. ($^{\circ}\text{C}$)	mean	14.19	14.59	15.01	15.12	13.65	13.85	14.29	14.55	13.59	13.77	14.16	14.70
	SD	0.07	0.17	0.52	0.11	0.30	0.21	0.41	0.41	0.06	0.10	0.17	0.16
Discharge (m^3/s)	mean	0.02	0.05	0.03	0.06	0.28	0.03	0.06	0.01	0.03	0.02	0.02	0.02
	SD	0.01	0.01	0.01	0.02	0.03	0.06	0.02	0.01	0.02	0.01	0.01	0.01
Cond. ($\mu\text{S cm}^{-1}$)	mean	431.3	431.7	431.3	448.67	408.00	407.00	407.00	407.00	487.33	480.33	460.67	449.33
	SD	1.15	0.58	0.58	2.89	0.00	1.00	0.00	0.00	0.58	6.66	4.04	4.16
D.O. (mg l^{-1})	mean	6.24	6.43	6.93	7.84	8.62	8.98	9.24	9.76	9.03	9.26	9.17	9.46
	SD	0.15	0.18	0.14	0.06	0.13	0.20	0.01	0.28	0.03	0.16	0.06	0.09
pH	mean	7.31	7.45	7.56	7.64	7.79	7.85	7.87	8.15	8.16	8.20	8.34	8.31
	SD	0.11	0.13	0.10	0.05	0.30	0.10	0.10	0.10	0.05	0.10	0.11	0.10
Turbidity (NTU)	mean	2.30	1.83	2.83	2.37	1.70	1.97	1.97	1.77	1.67	1.73	2.23	1.70
	SD	0.5	0.5	0.3	0.5	0.6	1.0	0.8	0.3	0.8	0.2	0.5	0.2
TIC (mg l^{-1})	mean	39.37	41.13	40.27	38.37	32.73	32.53	32.83	31.80	44.00	31.90	39.67	43.00
	SD	0.8	1.0	0.7	0.6	0.9	0.6	0.7	0.8	1.0	0.7	0.6	0.3
TOC (mg l^{-1})	mean	36.07	38.50	39.20	35.83	32.17	32.27	32.30	29.87	40.37	31.01	37.94	40.93
	SD	1.0	0.7	1.0	0.8	0.3	1.0	0.6	0.3	1.0	0.4	0.7	1.0
CaCO_3 (mg l^{-1})	mean	229	228	231	233	184	186	182	188	245	242	241	241
	SD	1.7	1.7	1.6	3.4	4.8	1.7	0.4	2.1	1.6	1.2	0.4	2.1
Ca (mg l^{-1})	mean	88.30	87.70	88.80	89.40	64.80	65.30	63.90	65.90	94.30	93.10	93.00	93.10
	SD	0.5	0.7	0.2	0.8	0.6	1.0	0.9	0.2	0.4	0.4	1.0	0.7
Mg (mg l^{-1})	mean	2.20	2.20	2.20	2.30	5.50	5.50	5.50	5.70	2.20	2.20	2.20	2.20
	SD	0.1	0.1	0.3	0.3	0.4	0.1	0.2	0.3	0.5	0.3	0.2	0.2
NH_3 (mg l^{-1})	mean	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	SD	na	na	na	na	na	na	na	na	na	na	na	na
NO_3 (mg l^{-1})	mean	2.81	2.79	2.79	2.41	2.11	2.14	2.10	2.10	2.03	2.05	2.02	2.02
	SD	0.3	0.3	0.5	0.1	0.1	0.4	0.1	0.1	0.1	0.1	0.1	0.1
TKN (mg l^{-1})	mean	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	SD	na	na	na	na	na	na	na	na	na	na	na	na
$\text{PO}_4 \text{ P}$ (mg l^{-1})	mean	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	SD	na	na	na	na	na	na	na	na	na	na	na	na
TP (mg l^{-1})	mean	0.09	0.02	0.02	0.03	0.01	0.03	0.04	0.03	0.13	0.01	0.02	0.04
	SD	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.01	0.04	0.01	0.01	0.02

orthophosphate in the spring waters during the study. Intra-spring comparisons for total nitrogen and total phosphate showed that they generally varied by no more than 0.9 mg l^{-1} , while nitrate levels varied slightly ($\leq 1.5 \text{ mg l}^{-1}$).

Due to the low intra-spring variation, spring physiochemistry was not considered to be biologically significant in the determination of intra-spring variation in macroinvertebrate community composition, and was not included in further analyses. However, there were some noticeable seasonal differences. Discharge was 3-5 times greater across reaches within a given spring in the summer compared to winter due to greater amounts of water cress growth in the winter. Concentrations of TIC and TOC were ~ 3 and 10 times greater respectively in the winter; possibly reflective of the input and breakdown of allochthonous carbon to the systems. The effect of season on invertebrate community structure was examined through ANOVA techniques.

Flora and fauna

Organic substrate types consisted of a mixture of short vascular hydrophytes: the water cress, *Nasturtium officinale* (watercress), *Potamogeton amplifolius* (a large-leaved pond weed), and *Impatiens capensis* (jewel weed). *Impatiens* is a vigorously growing annual that thickly lined the banks of each spring with just a few small clumps in the active channel. *Nasturtium* grew in thick mats along the spring bank, as well as in the active channel and served as a major habitat for amphipods. During the winter months *Impatiens* and *Potamogeton* died back due to their primarily terrestrial origin. Because of the temporal stability of each springbrook and the plant's location within the spring channel, *Nasturtium* grew profusely year round. During summer months, however, *Nasturtium* was less dense in more open canopy springbrook reaches and died back to just small clumps along the spring bank in Haseltine Spring due to the total absence of any riparian canopy, and consequent vast amount of incident light over the spring channel. *Nasturtium* density was less dramatically reduced in the lower reaches of Steury and Danforth as deciduous and

coniferous riparian cover was present. Other instream primary producers included the three species of moss (Table 4) that grew on submerged rocks, served as a major invertebrate habitat, and grew in close association with the liverwort, *Marchantia polymorpha*. Algae, *Nitella* and *Chara*, present in springbrooks, were encrusted with lime, giving them a rough appearance. Spring sources at Haseltine and Steury had comparatively minimal amounts of these resources, unlike Danforth Spring that issued from a much smaller cave-like opening. *Lemna minor* was present only in the pooled area in Danforth Spring, and flowed down into springbrook reaches. *Lemna* is typically not a direct food source for herbivores but is incorporated into the detritus, which is a food source for many spring species (Tilly 1968). Grasses in the riparian and floodplain areas consisted of both C₄ and C₃ groups, including *Digitaria* sp., *Setaria* sp., *Festuca dactylis*, and *Poa annua*. Deciduous forests include a mixture of trees (hickory, oak, walnut, cedar) and shrubs.

A total of 31 macroinvertebrate taxa belonging to 26 families were quantified from samples taken from three springs across two seasons (Table 4). Of these, 68% were insect taxa, a proportion that would have been even greater had the chironomid been identified to genus like most other groups. The remaining 22% consisted of peracaridan crustaceans (10%) and non-hexapod invertebrates. Typically in limestone springs, there is only one numerically dominant peracaridan, or if two, one is an amphipod and the other an isopod (Glazier 1991) as was the case in these three karst springs. While aquatic insects were the most diverse group, they generally were lower in abundance than the peracaridan crustaceans.

Longitudinal zonation patterns

Macroinvertebrate communities exhibited distinct longitudinal zonation patterns along the spring source-springbrook continuum in all springs during both seasonal sampling periods. Spring sources supported a depauperate fauna, and were generally devoid of aquatic insects. In contrast, the springbrook supported a moderate diversity of aquatic insect and non-insect macroinvertebrates. Hence, species

composition shifted along this continuum from a community composed of non-insect taxa to one dominated by aquatic insects.

Table 4 The fauna and flora collected in three Ozark springs.

FAUNA			
TURBELLARIA		DIPTERA	
Planariidae	<i>Dugesia doratocephala</i>	Empididae	<i>Hemerodromia sp.</i>
OLIGOCHAETA		Tipulidae	<i>Tipula sp.</i>
Lumbriculidae		Simuliidae	<i>Simulium sp.</i>
HIRUDINEA		Stratyomyidae	<i>Stratimys sp.</i>
GASTROPODA		Chironomidae	<i>Chironominae</i>
Pleuroceridae	<i>Goniobasis potosiensis</i>		<i>Tanypodinae</i>
Physidae	<i>Physa remingtoni</i>	ODONATA	
Planorbidae	<i>Promenetus sp.</i>	Coenagrionidae	<i>Argia sedula</i>
DECAPODA		CAUDATA	
Cambaridae	<i>Orconectes sp.</i>	Plethodontidae	<i>Eurycea multiplicata griseogaster</i>
AMPHIPODA		SCORPAENIFORMES	
Crangonyctidae	<i>Crangonyx forbesi</i>	Cottidae	<i>Cottus bairdi</i>
Gammaridae	<i>Gammarus minus</i>	PERCIFORMES	
ISOPODA		Percidae	<i>Etheostoma punctulatum</i>
Asellidae	<i>Lirceus hoppinae</i>		
PLECOPTERA		FLORA	
Perlidae	<i>Acroneuria frisoni</i>	CAPPARALES	
EPHEMEROPTERA		Brassicaceae	<i>Nasturtium officinale</i>
Baetidae	<i>Baetis flavistriga</i>	ARALES	
Ephemerellidae	<i>Eurylophella bicolor</i>	Lemnaceae	<i>Lemna minor</i>
TRICHOPTERA		MARCHANTIALES	
Hydropsychidae	<i>Cheumatopsyche sp.</i>	Marchantiaceae	<i>Marchantia polymorpha</i>
	<i>Hydropsyche piatrix</i>	FISSIDENTALES	
Glossomatidae	<i>Agapetus sp.</i>	Fissidentaceae	<i>Fissidens debilis</i>
Heliocopsychidae	<i>Helicopsyche borealis</i>	HYPNALES	
Hydroptilidae	<i>Neotrichia sp.</i>	Campylaceae	<i>Drepanocladus aduncus</i>
	<i>Hydroptila sp.</i>	LEUCODONTALES	
	<i>Oxyethira sp.</i>	Fontinalaceae	<i>Fontinalis antipyretica</i>
	<i>Ochrotrichia contorta</i>	GERANIALES	
MEGALOPTERA		Balsaminaceae	<i>Impatiens capensis</i>
Sialidae	<i>Sialis sp.</i>	CHLOROKYBALES	
COLEOPTERA		Chlorokybaceae	<i>Nitella sp.</i>
Elmidae	<i>Optioservus sandersoni</i>	CHARALES	
Dytiscidae	<i>Oreodytes sp.</i>	Characeae	<i>Chara sp.</i>

Summer assemblages (Figs. 6a-c) in spring sources (R1) were dominated primarily by the peracaridan crustaceans *Lirceus hoppinae*, *Crangonyx forbesi*, and *Gammarus minus* considered endemic to springs and cave streams in this region of the Ozarks (Vineyard and Feder 1982). Gammarids were numerically dominant over crangonids, a characteristic of perennial springs when both are present (Minckley 1963). Because these crustaceans are shredding herbivores (Glazier and Gooch 1987), their food source was probably watercress (most dense at or near the source), benthic organic matter (from the groundwater and exterior of the cave), moss, and a small amount of terrestrial organic matter that had blown into the cave. Salamanders (*Eurycea multiplicata*) appeared only at R1 or at the head of R2, and they, along with the triclad *Dugesia doratocephala*, represented the predator guild. The crayfish *Orconectes* sp. and the oligochaete worm Lumbriculidae were present across all reaches, occupying the omnivore and collector guilds respectively.

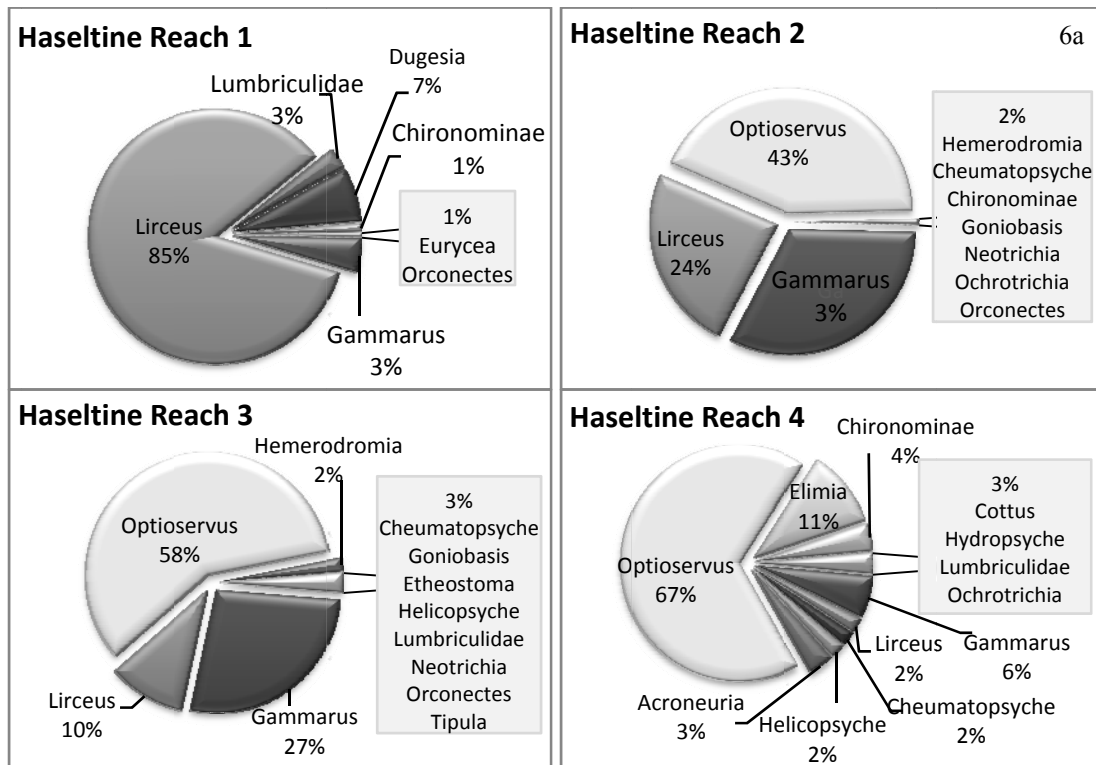


Figure 6 a-c Summer season pie charts showing longitudinal zonation patterns in community composition from the spring source (upper left) to the most downstream reach (lower right).

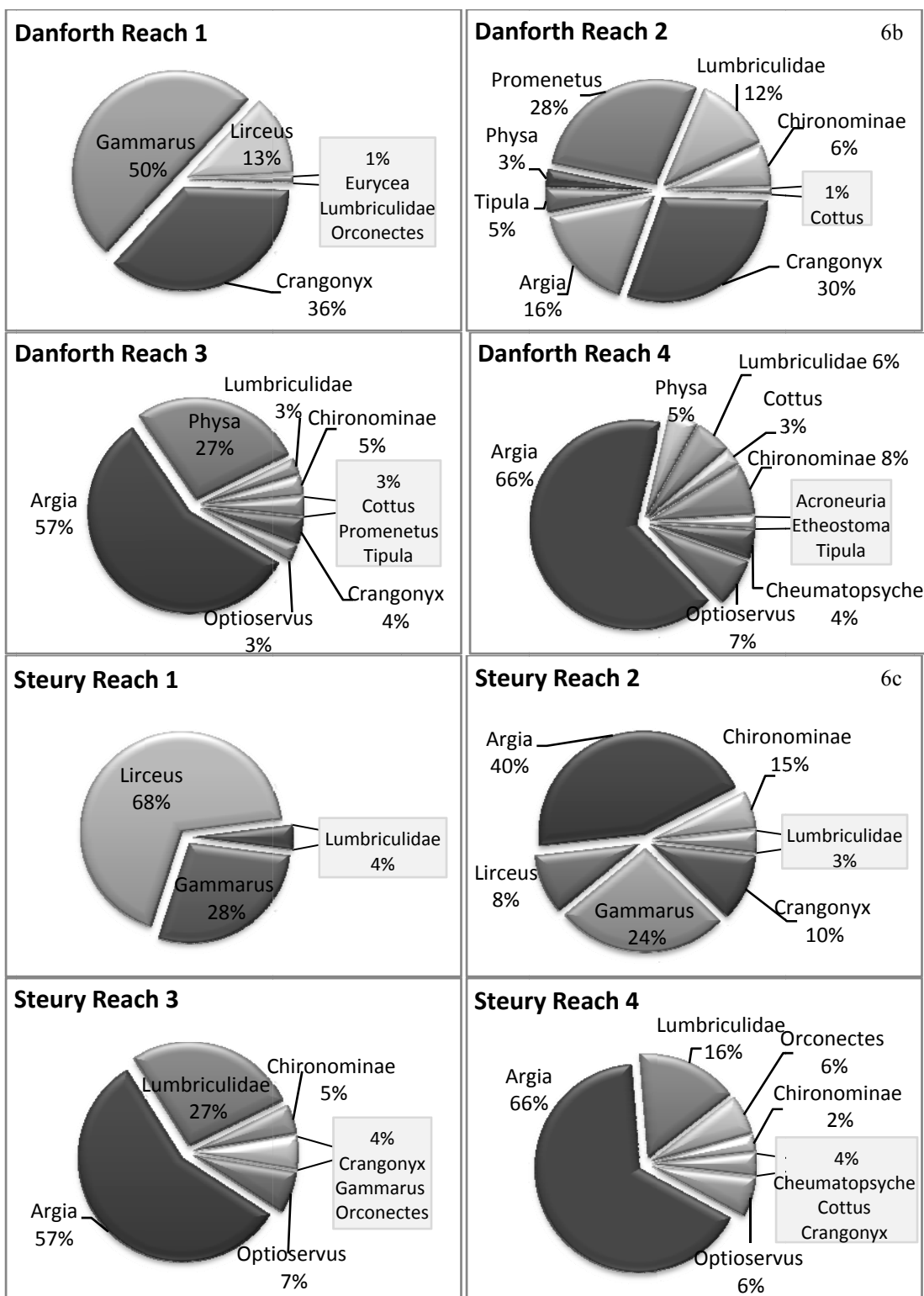


Figure 6a-c con't. Summer season pie charts showing longitudinal zonation patterns in community composition from the spring source (upper left) to the most downstream reach (lower right).

Dominance in summer springbrook communities shifted to the coleopteran *Optioservus sandersoni* in Haseltine (primarily open canopy) and to the damselfly *Argia sedula* in Danforth and Steury (coniferous and deciduous canopies, respectively). Caddisflies were the most diverse mid- and lower reach springbrook fauna, represented by as many as eight genera from four different families; they were never found at the spring issue. They, along with the snails *Goniobasis potosiensis*, *Promenetus* sp., and *Physa remingtonia*, dominated the collector/filterer and scraper guilds. Other downstream constituents included the crane fly *Tipula* sp., the stonefly *Acroneuria frisoni*, the sculpin *Cottus bairdi*, and the perch *Etheostoma punctulatum*. Stoneflies, known to be predators, were only found in reach 4, and sculpin and perch were never found above reach 3. Of note was the paucity of chironomids. Midges represented only 1%, 2%, and 3% of overall invertebrate abundance in Haseltine, Danforth and Steury respectively.

Estimates of the relative importance of different functional feeding groups to community organization were determined by examining dominance ratios (density of peracardians/insects to total invertebrate density). The proportion of peracaridan crustaceans to total invertebrates steadily decreased from 96-100% at reach 1 for the 3 springs to 0-8% by reach 4. Insect to total invertebrate dominance proportions correspondingly increased from 0% at reach 1 to 76-90% by reach 4. These patterns represented not only shifts in biodiversity but also shifts in functional diversity (FFG's), with functional composition shifting in dominance from predominantly shredders to collector/filterers and scrapers.

Few seasonal differences in zonation patterns or community constituents were noted at the spring source, but notable differences in springbrook constituents and functional feeding groups were present (Figs. 7a-c). Winter sampling showed that peracaridan remained dominant at the spring source, and the elm mid beetle *Optioservus sandersoni* in Haseltine, and the damselfly *Argia sedula* in Danforth and Steury remained dominant constituents of winter springbrook communities.

However, seasonal shifts in the relative abundance of certain FFG's occurred between reach 1 and 4 across all sites. The proportion of peracaridan-to-total-invertebrates decreased in the winter communities, shifting from 64-95% at reach 1 for the 3 springs, to 0-38% by reach 4. The proportion of insects-to-total-invertebrates correspondingly increased in upper reaches ranging 0-24% at reach 1 to 37-90% by reach 4. Lower reach (3-4) springbrook communities tended to have larger collector-gatherer abundances (especially in Danforth and Steury) as well as notably larger numbers of amphipods (especially in Haseltine) . This was likely due to the increases in allochthonous leaf litter and water cress density in lower springbrook reaches during the winter. Increases in members of the collector-gatherer guild included midges from the subfamily Chironominae, craneflies, danceflies (*Hemerodromia* sp.), and soldierflies (*Stratimys* sp.). Midges had been virtually absent from summer collections, but their numbers doubled in Haseltine and Danforth, and increased 10-

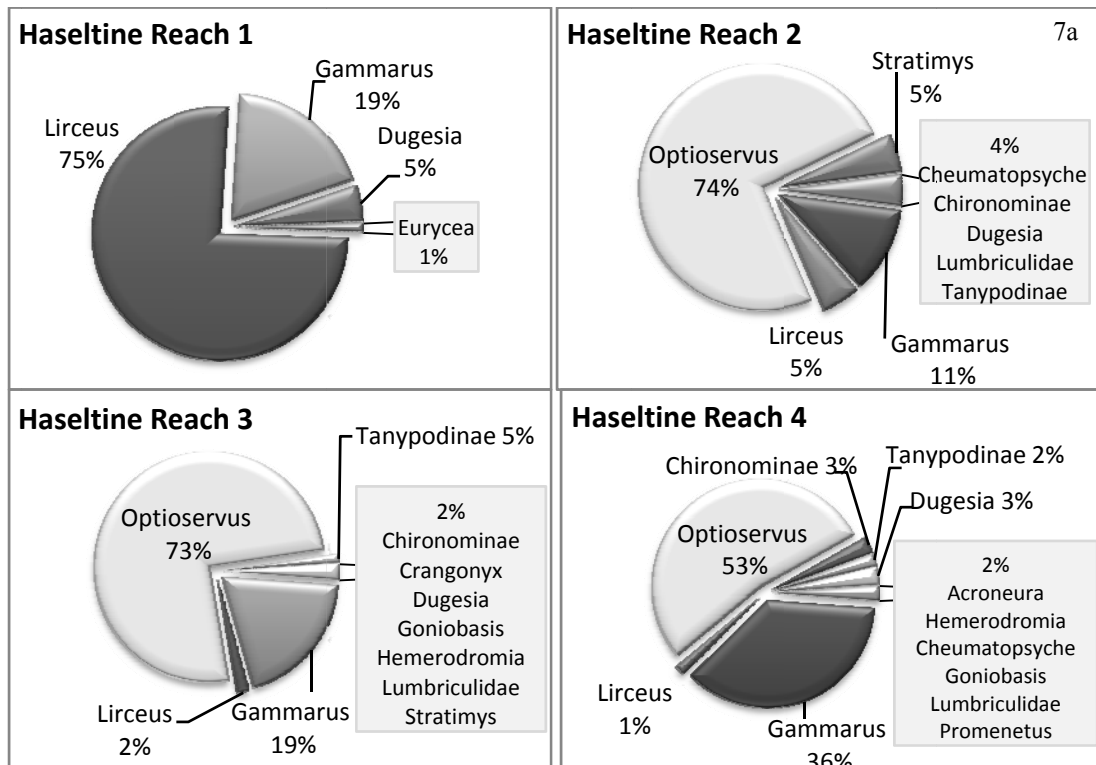


Figure 7 a-c Winter season pie charts showing longitudinal zonation patterns in community composition from the spring source (upper left) to the most downstream reach (lower right).

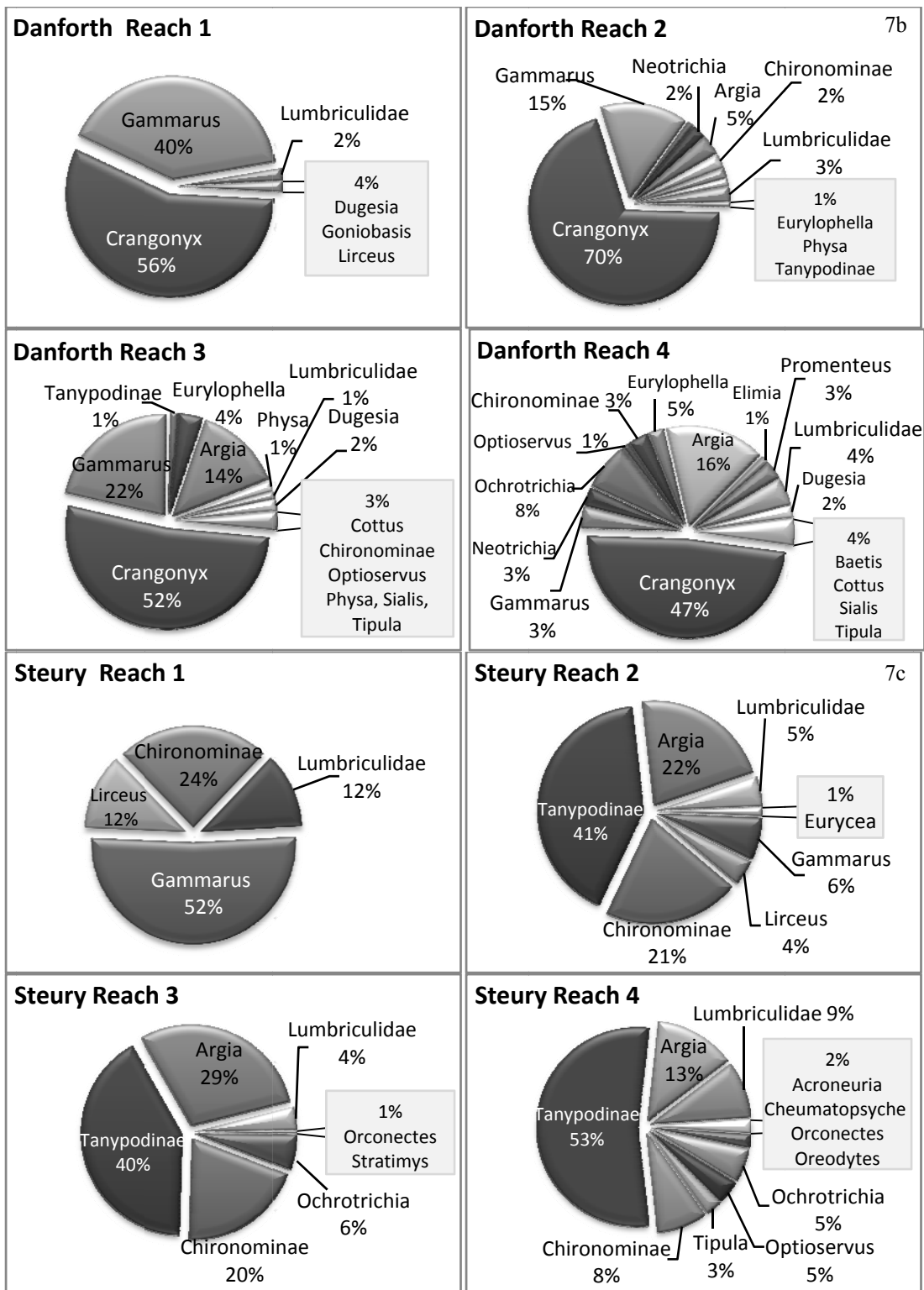


Figure 7 a-c con't. Winter season pie charts showing longitudinal zonation patterns in community composition from the spring source (upper left) to the most downstream reach (lower right).

fold in Steury, representing 60% of its winter community. Midge seasonal abundance patterns mimicked those described in a limnological study of a cold Tennessee springbrook (Stern and Stern 1969), where midges were most abundant in late autumn and winter, and few were present in the summer. Collectors such as worms, craneflies, and mayflies (*Baetis flavistrigas*, *Eurylophella bicolor*) increased by as much as 2 and 38 times their abundances found in summer. Amphipod abundance in reaches 3 and 4 increased 14 fold in Haseltine, and nearly 100 fold in Danforth over that found in summer. *Dugesia doratocephala*, known to feed on amphipods, was collected only at the spring issue during summer sampling: it was found across all reaches during winter sampling periods likely due to the downstream migration of amphipods during the winter.

Macroinvertebrate abundance, diversity, and taxon richness

Over the study period, the range in total macroinvertebrate abundance, was 3,720-41,462·m⁻² in Haseltine, 2,054-14,075·m⁻² in Danforth, and 355-4,979·m⁻² in Steury. The most abundant consumer species found at the issue (R1) in all three springs during both seasons were stygophilic amphipods and isopods. Gravid females were collected during both seasons, confirming year-round reproduction strategies. Damselflies were the most abundant insect species in the lower reaches of Danforth and Steury, while riffle beetles held that position in Haseltine. Significant variability in density was evident in response to differences in reach and spring type, (Table 5) but not in response to season. Across springs, invertebrate densities were significantly higher in Haseltine and lowest in Steury. Averaging across reaches, there was a significant increase from the issue to reach 2 as insect species began to join peracaridans, followed by a decrease in abundance by reach 4 with the near absence of amphipods and isopods. Because there were significant interactions among each of these factors, abundance did, in fact, vary temporally depending on the spring or reach in question. Averaging across springs, invertebrate abundance was significantly higher during the winter months (except in Haseltine) when spring invertebrate

populations are known to be at their maxima (Stern and Stern 1969). Averaging across reaches, abundance was also significantly higher in upper reaches during the summer and in reach 4 during the winter, likely due to the downstream migration of peracaridan species, as previously mentioned.

Table 5 Summary of ANOVA examining effects of spring, reach, and season on the diversity, richness, and abundance of spring fauna. Significant *p* values in boldface.

Source	d.f.	SS	F	<i>p</i>
Diversity (Shannon H')				
Spring	2	1.991	154.37	<0.001
Reach	3	0.828	42.79	<0.001
Spring x Reach	6	0.06	1.56	0.179
Season	1	0.068	10.61	0.002
Spring x Season	2	0.341	26.47	<0.001
Reach x Season	3	0.118	6.09	0.001
Spring x Reach x Season	6	0.14	3.62	0.005
Richness (Margalef DM_g)				
Spring	2	2.449	12.82	<0.001
Reach	3	6.485	22.64	<0.001
Spring x Reach	6	1.869	3.26	0.009
Season	1	1.147	12.02	0.001
Spring x Season	2	1.272	6.66	0.002
Reach x Season	3	0.432	1.51	0.224
Spring x Reach x Season	6	2.117	3.7	0.004
Abundance (#/m²)				
Spring	2	3.572 x 10 ⁸	50.75	<0.001
Reach	3	4.668 x 10 ⁷	4.42	0.007
Spring x Reach	6	1.443 x 10 ⁸	6.83	<0.001
Season	1	2.320 x 10 ⁶	0.66	0.421
Spring x Season	2	1.055 x 10 ⁸	14.99	<0.001
Reach x Season	3	2.841 x 10 ⁷	2.69	0.056
Spring x Reach x Season	6	1.217 x 10 ⁸	5.76	<0.001

Regardless of which spring ecosystem examined, the community within the spring source was distinct from that in the springbrook. A longitudinal gradient of increasing species diversity and richness was evident in all three spring ecosystems

(Fig. 8). Species richness ranged from as low as 3 at the issue to as high as 15 at reach 4 during summer sampling, and from 4-20 at the issue and reach 4, respectively

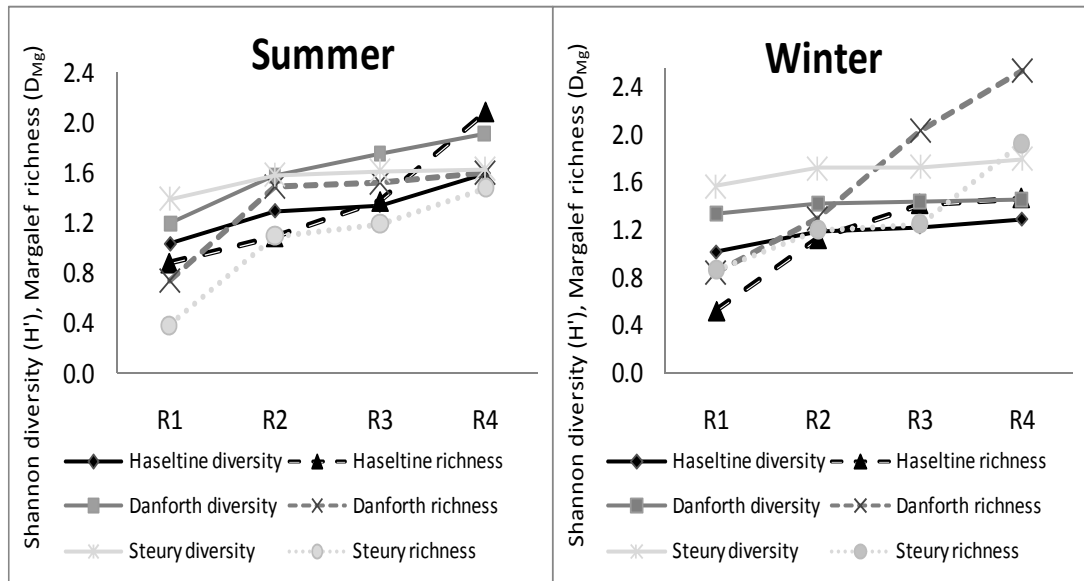


Figure 8 Changes in species richness and diversity along a downstream gradient from the spring source.

during winter sampling, Significant variability in richness and diversity were evident in response to spring, season, and reach treatments (Table 5). Richness significantly increased along the spring source-springbrook continuum, and was at its highest in reach 4 across all springs. Values were highest during the winter and in order among springs in Danforth, followed by Steury, and Haseltine across both seasons. Diversity was also significantly greater in reach 4, typically highest in the summer, with values greatest in Steury, followed by Danforth and Haseltine across both seasons.

Discussion

Macroinvertebrate community structure

Spring ecosystems have historically been considered challenging habitats whose invertebrate assemblages lack the diversity found in other lotic systems (Odum 1957; Teal 1957; Tilly 1968). More recently, studies have shown that many springs harbor a disproportionally large number of species, relative to their small size

(Williams and Hogg 1988; Glazier 1991; Erman 2002; Wood *et al.* 2005). While some spring species are confined to the spring source, others are distributed downstream, with species assemblages often changing rapidly along the spring source-springbrook gradient depending on the nature of the spring, increases in habitat diversity, characteristics of the riparian zone, and differences in functional requirements of resident species (Erman and Erman 1990; Erman 1992; Smith *et al.* 2003).

The results of this study indicate that faunal diversity, composition, and biocomplexity can vary temporally and spatially over very small spatial scales (cf. Bunn 1986; Downes *et al.* 1993). In support of my first hypothesis, the spatial gradient from reach 1 to reach 4, in each of these 3 karst springs, was accompanied by a predictable and consistent gradient in invertebrate community composition. In each spring, communities shifted from non-insect assemblages at the spring source to insect-dominated assemblages in the springbrook. The limitation of species at the spring issue concurs with similar findings reported elsewhere (Ide 1935; Sloan 1956; McKinley 1963; Danks and Williams 1991; Web *et al.* 1995; Erman 1998; Covich and Thorp 2001). The replacement of peracaridans by insect taxa is likely due to reduction in water cress density (known micro-habitat for peracaridans) and increases in aerial input of insect taxa along the spring source-springbrook gradient. Dominance by peracaridans, and the longitudinal patterns represented in this study, are consistent with the hypothetical framework proposed by Gooch and Glazier (1991) for carbonate springs. These patterns are also consistent with the notion that spring fauna are typically comprised of both more specialized groundwater species (e.g. stygophilic amphipods *G. minus* and *C. forbesi*, and asellid isopods *L. hoppinae*), as well as generalist species that inhabit a variety of lotic habitats (Williams and Danks 1991). More generally, this pattern reinforces previous studies that identified longitudinal location in lotic ecosystems as one of the most important predictors of benthic invertebrate distribution (Allan 1975; Minshall *et al.* 1985; Ward 1986; Williams and Hogg 1988). However, reach-scale variables have been shown to be

more important than longitudinal position in discerning differences in biotic distribution (Corkum 1990; Finn and Poff 2005), and there is evidence in this study to support that finding.

Spatial shifts in species composition were accompanied by spatial shifts in community biocomplexity (species richness and diversity) and functional diversity (functional feeding groups; FFG's). Springbrook species diversity and equitability were typically more than double that found in the spring source, as spring source fauna were replaced by more diverse insect faunal groups. This was predicted, as downstream increases in taxonomic richness and evenness in spring ecosystems have been widely noted (Ward and Dufford 1979; Milner and Petts 1994; Maiolini and Lencioni 2001; but see Resh 1983 for an exception). Functional diversity also shifted spatially from dominance by shredder species at the spring source, to collector/gathers and scrapers in the springbrook. Heino (2005) reported significant increases in functional biodiversity of macroinvertebrate assemblages along ecological gradients in boreal headwaters, and Ilg and Castella (2006) found increasing functional diversity with increasing distance from glacial stream origin in three glacial streams.

Biocomplexity and functional diversity in lower springbrook reaches also varied temporally. In each of the three springs, winter springbrook communities had a greater number of species across reaches, as larger numbers of collector-species joined community assemblages, possibly reflecting larger pools of detritus available at that time. Species richness increased in lower springbrook communities also due to the downstream migration of amphipods (shredders) and their predators (planarians), likely associated with increases in water cress density during the winter. Biocomplexity and functional diversity data are consistent with predictions made by the River Continuum Concept (RCC) for forested headwater systems, and suggests that spatial and temporal changes in the feeding guilds of macroinvertebrates reflected longitudinal and seasonal patterns in the contribution of autochthonous and

allochthonous inputs of organic matter along the reach 1 to reach 4 gradient (Vannote *et al.* 1980).

In contrast to my original expectation, the three springbrooks did not exhibit similar longitudinal patterns of biodiversity. While the springs lie in the same geographic region, in the same or adjacent watersheds, springbrook communities were quite individualistic in nature. Biological similarity differed significantly at similar points along the continuum, with spring identity accounting for 57% of the variation in macroinvertebrate community diversity (cf. Erman 2002). As these springs are distinguished primarily by their distinctly different riparia, this result suggests the importance of assessing the influence of riparian canopy, or complete lack thereof, on the subsequent nature of springbrook assemblages.

Mechanisms explaining distribution patterns

There are several possible mechanisms responsible for both the patterns of invertebrate community composition and the spatial and temporal variability in biocomplexity and functional diversity recorded in these three springs. These include: (i) the physicochemical nature of the springs; and (ii) the temporal and spatial (reach-scale) variability in the composition, abundance, and availability of different food sources.

The relatively high level of species richness in springbrook communities (given the size of these small ecosystems) is congruent with the first mechanism, as studies have shown that greater species-rich spring communities are associated with flow permanence and hard substrata such as limestone shoals rather than with soft-bottom substrates (Mattson *et al.* 1995; Wood *et al.* 2005). Also, in support of the first mechanism, Erman (2002) maintained that the chemical nature of springs with characteristically high concentrations of calcium, magnesium, alkalinity, and pH (such as Haseltine, Danforth, and Steury) are typically those with the greatest species richness and community complexity—all factors positively correlated with community stability. However, as these levels were high, they remained closely

comparable among springs along both temporal and spatial scales, as did the other physicochemical measures. This is not surprising, as carbonate springs are known to be thermally and chemically buffered ecosystems (Beckman and Hinchey 1944; Gooch and Glazier 1991). Beyond the physicochemical measures themselves, further evidence of their constancy was the dominance within invertebrate communities by non-emergent taxa (amphipods, isopods, gastropods), as both flow permanence and chemical and thermal constancy are known to favor non-dispersal life-cycle groups (i.e. those lacking aerial adults) in carbonate springs (Glazier 1991; Smith *et al.* 2003). In addition, Peracarida are indicators of long-term hydrogeological stability (Culver *et al.* 1994).

It may be important to note that my designation of spring source and springbrook differs substantially from the commonly accepted criteria proposed by Erman and Erman (1995). These authors and several others define the springbrook area as the point where water temperature differs by 2°C from the spring source (typically 5-10 m from the spring source), and they indicate that marked changes in biotic community structure and composition occur downstream of this point (Smith 2002; Smith *et al.* 2003; Wood *et al.* 2005; von Fumetti *et al.* 2006; Mori and Brancelj 2006; Barquin and Death 2009). In the karst springs, represented in this study, however, there was no such temperature shift along the 101-145 m between the head of the spring sources and the base of the springbrooks. Nonetheless, there were substantial changes in biodiversity and functional diversity along these lengths. This finding suggests that thermal variability is not always the mechanism responsible for defining longitudinal biodiversity patterns in springs and springbrooks. Therefore, while physiochemical constancy might explain (in part) the composition of the faunal communities, mechanisms other than water chemistry and temperature gradients must account for the gradient of biocomplexity found.

For at least four reasons, the biodiversity patterns appear to be more likely associated with the spatial (reach-scale) and temporal variability in the composition, abundance, and availability of different food sources. First, it is likely that low

species diversity at the spring sources was due to the absence of transported detritus from upper reaches which limited the diversity and quantity of available food (Ward and Dufford 1979). Each of these springs issue from cavernous openings in limestone bluffs, and there is little possibility for upstream detrital input. This explains why few collectors were found near the issue, since fine transported organic matter (FTOM) is mostly generated *in situ* (Glazier 1991).

Second, spatial and temporal variation among springbrook communities could be differentiated based on the variability in canopy cover (covered: Danforth and Steury; relatively little cover: Haseltine). The difference in canopy cover created vast differences in the amount of light available for primary production, the volume of organic leaf litter within the aquatic environment, and therefore differences in the amount and type of food available for consumption. These factors were then likely responsible for differences in community composition and biodiversity. For example, young damselfly larvae (*Argia sedula*), which are known to eat protozoans that colonize fine allochthonous detrital matter, were prominent in springbrooks with heavy riparian growth of coniferous and deciduous trees. In contrast, riffle beetles were extremely abundant in the Haseltine springbrook where the minimal canopy allowed high levels of incident solar radiation, favored periphyton growth, and promoted dominance of scrapers (riffle beetles).

In addition, temporal increases in springbrook biodiversity were associated with differences in the volume of leaf litter entering the systems. Biodiversity in springs with heavy riparian growth reflected higher abundances of collector/gatherer species; while increases in Haseltine biodiversity, where leaf litter input is minimal, were due primarily to the downstream migration of amphipods (as cited above) and, to a much lesser extent, by increases in collector/gatherer species. The seasonal increase in biodiversity is consistent with the notion that several species synchronize their life cycles to coincide with litter fall (Lindegaard *et al.* 1998; Williams and Williams 1998).

One might question how riparian growth could differ so dramatically in type (coniferous vs. deciduous) between the two covered springs, while springbrook communities were similar? Possible explanations could be that in spring systems where water is interconnected (Danforth and Steury are located in the same watershed) one might expect to find more similarity in spring invertebrate species (Erman 2002). Second, all types of forest canopies provide somewhat similar conditions including dense shade over the water in summer, leaf or pine needle input, and a low volume of soil erosion because of dense accumulations of litter on the forest floor. While these conditions result in reduced in-stream photosynthesis and algal growth, the allochthonous inputs often increase in-stream habitat heterogeneity. Moreover, while allochthonous detrital matter affords minimal direct nutrition for most species, it provides a microhabitat for nutritious microbial (algae, fungi, and bacteria) colonization, and pine needles could serve as a comparable substrate for at least periphyton growth. Hence, the patterns noted here lend additional support to the premise that the amount of riparian habitat along a springbrook is a dominate mechanism driving community composition within springs (Smith 2002; Smith *et al.* 2003), as well as in other lotic systems (Vannote *et al.* 1980; Cummins *et al.* 1989).

Third, springbrook community composition was associated with instream organic food sources. Piercer and scraper caddisflies, known to rely on moss and algae (Stern and Stern 1969), were more abundant and diverse than filtering caddisflies. Food for filtering caddisflies was comparatively in short supply as planktonic FTOM is rarely available in springs (Teal 1957; Minckley 1963) due to low, but constant discharge, and because it is mostly generated *in situ* (Anderson and Anderson 1995; McCabe 1998). Dense mats of water cress and moss, however, were in rich supply for piercer and scraper caddisflies. Evidence of this pattern was also seen in the positive relationship between increased water cress density and amphipod abundance in lower springbrook reaches in the winter, on the one hand, and the vast reduction in water cress density and upstream retreat of amphipods in the summer. This pattern was also noted by Gooch and Glazier (1991) after anthropogenic removal

of watercress in a Pennsylvania spring. This finding reinforces previous work suggesting that the amount of aquatic/emergent vegetation is a major determinant of macroinvertebrate community composition and distribution (Miller and Buikema 1977; Gooch and Glazier 1991; Williams and Williams 1999; Smith and Wood 2002).

Finally, total inorganic and organic carbon (TIC and TOC), along with discharge, represented the only physicochemical measures that exhibited substantial variability (seasonal); and these factors were also related to spatial and temporal patterns in the abundance and availability of different food sources. Seasonal shifts in the input and breakdown of allochthonous carbon to the systems and variability in subterranean respiratory (heterotrophic) and weathering process were likely responsible for winter increases in TIC and TOC. Likewise, seasonal increases in macrophyte growth patterns (watercress density) and input of terrestrial leaf litter were the reasons for winter reductions in flow velocity.

Conclusions/Implications

A number of classic works on springs provided comprehensive analyses of the structure and function of a single spring system (Odum 1957; Teal 1957; Minckley 1963; Tilly 1968). The results from this study are based on multiple spring systems that provide conclusions that can be applied more generally to the utility springs represent in their potential to contribute to a broader understanding of lotic community ecology and related theories of patch dynamics and riverine landscape ecology.

First, the physicochemical constancy of springs makes them ideal ecosystems for studying biotic community responses to environmental features, as the lack of variability more clearly reveals the mechanisms at work (e.g. life-history, species interactions) and reduces the number of possible confounding interactions. The physicochemical constancy of the springs in this study highlighted the role that organic food source mechanisms play in defining benthic community structure.

Spatial patchiness in the availability of resources, such as the lack of detritus from subterranean sources at the spring issue, or temporal pulses of allochthonous leaf litter, represent dynamic boundaries of patch connectedness along the longitudinal, lateral, vertical, and temporal dimensions of lotic systems, as defined by Ward (1989), and are central to patch dynamics themes of fluvial landscape community ecology (Pringle *et al.* 1988; Townsend 1989; Poole 2002).

Second, although spring communities are quite simple, they still demonstrate the majority of structural and functional properties seen in other lotic communities (Williams and Williams 1998). The lack of complexity makes them ideal locations to examine communities in their entirety, often impossible in more complex ecological systems. The small size of the communities in this study afforded a clearer view of their patch-within-patch design, how these composite patches changed over time and space, and made it possible to identify predictable changes in community dynamics (patches) based on the variability in spatial subsidies and pulsed inputs of organic matter to the systems. These findings: (i) reinforce tenet #1 of the Riverine Ecosystem Synthesis (RES) research framework (Thorp *et al.* 2006, 2008) which highlighted the patch-like, rather than continuum nature of species distributional patterns in riverine ecosystems; (ii) are central to the Hierarchical Patch Dynamics theory of ecology (Wu and Loucks 1995); and (ii) lend support to the currently developing theoretical base for the dynamics of spatially subsidized communities (Anderson *et al.* 2008).

Furthermore, the natural variation in community structure, evidenced in this study, along both temporal and spatial scales, suggests that ecosystem processes should exhibit corresponding changes. This makes springs excellent ecosystems in which to test questions regarding the spatial and temporal patterns of trophic dynamics whose variability is often too difficult to measure because of the spatial and temporal complexity of most lotic systems (Williams and Williams 1998). This is the focus of chapter 3.

Finally, the results of the present study provide a number of implications for the research and management of spring ecosystems and other fragile lotic

environments. The natural variability in structural and functional diversity in Danforth and Steury Spring communities was quite different from that seen in Haseltine Spring whose landscape had been anthropogenically modified (deforested and mowed lawn). Conserving these components of biodiversity in lotic systems requires an understanding that faunal composition and distribution patterns are responsive to ecological processes that reflect a mosaic of environmental gradients. Understanding these relationships also includes the use of research designs that assess structure and function along spatial scales small enough to discern such responses; responses that often disappear when considered at broader scales (Fukushima 2001; Wiens 2001). Understanding and conserving these components of biodiversity also requires assessing the ecological integrity of lotic systems from a riverine landscape perspective that discerns patterns along temporal, longitudinal, vertical, and lateral dimensions, as spatial patterns along each of these dimensions affect community structure and function.

CHAPTER 3

EFFECTS OF SPATIOTEMPORAL CHANGES IN ORGANIC INPUTS ON FOOD WEB PATHWAYS IN KARST CAVE SPRING ECOSYSTEMS

Introduction

Trophic dynamics of surface systems are often poorly understood, and research on them is usually limited in space and time (Sears *et al.* 2004), but information on the functional dynamics of spring ecosystems is even more restricted, with relatively few data available on spatial and seasonal effects (Danks and Williams 1991; Glazier 1998). Spring ecosystems are often defined as the ecotone between groundwaters and surface waters. Ecotones are characterized by the cross-habitat movement of nutrients, organic matter (detritus), and organisms across system boundaries that can have a significant impact on energy flow and trophic interactions (Anderson and Polis 1998). The ecological role of surface-subsurface exchanges is well rooted in the foundations of stream ecology, especially in floodplain and hyporheic zone studies (Dahm *et al.* 1998; Wroblicky *et al.* 1998; Tockner *et al.* 1999; Malard *et al.* 2002), but is lacking for spring ecosystems. A spring's filtration capacity, determined by its geologic composition and structure, is critical to inter-patch exchanges. The hydrogeomorphic organization of a karst spring produces surface-subsurface food webs that are tightly linked by energy fluxes across the interface between each habitat, making them model systems for studying the effects of temporal resource pulses and spatial subsidies on invertebrate community food web complexity (Glazier 1991).

Karst springs and springbrooks are characterized by hydrologically and seasonally driven, organic pulses which are spatially subsidized from the source to downstream by changes in both epigeal-hypogean flow and riparian-springbrook interactions. Subsidies of materials across hypogean-epigeal boundaries occur via vertical upwellings. Discharging groundwater carries dissolved matter and organic detritus to the surface from submerged caves and underground limestone conduit systems (Hobbs 1992). However, because the water that entered the underground

compartment of a karst spring came either from land, or a losing section of a surface stream, all or most of the organic matter at the spring source is likely to be directly or indirectly of terrestrial origin (allochthonous). In most cases, this organic matter will be recalcitrant carbon, especially if it is derived directly from land or has been underground more than a few days. Consequently, lotic species living in or immediately adjacent to the spring source must depend heavily on detrital, allochthonous organic matter as a source of energy. As the water moves downstream, more allochthonous carbon enters from overland surface flow. At the same time, however, the opportunities for instream primary production rise. Complicating this pattern are seasonally-charged resource pulses and spatial subsidies related to: (i) direct leaf fall into streams; (ii) overland flow of particulate (POM) and dissolved (DOM) organic matter derived from this leaf fall but entering later after heavy rains; (iii) subterranean resource pulses due to seasonal water table fluctuations; (iv) seasonal and spatial (canopy cover) variations in photosynthetically active radiation (PAR); (v) seasonal and spatial fluctuations in instream macrophyte production; and (vi) seasonal variability in macroinvertebrate distribution patterns. During low flow periods, the lack of overland surface flow greatly reduces terrestrial carbon pulses in both spring source and springbrook habitats, and invertebrate communities become even more dependent on underground resources.

Hence, rather than considering springs as discrete, self-contained microcosms, as has sometimes been suggested (e.g. Gooch and Glazier 1991), the present study starts from the premise that these seemingly autonomous systems rely heavily on spatial subsidies and pulsed linkages between terrestrial and subterranean environments. The cross-habitat exchanges of water, organic matter, nutrients, and organisms have the potential to alter macroinvertebrate food webs in both habitats. Although temporal pulses and spatial food web subsidies are closely bound due to the pulsed nature of many spatial subsidies, the theoretical basis behind studying these dynamic exchanges has only recently emerged (Anderson and Wait 2001).

This study focused on two major themes. First, because springs are characterized as first-order, headwater streams (Lindegaard 1995), current models would suggest that their food webs are supported primarily by allochthonous organic inputs (e.g., Webster *et al.* 1997; Cummins 1974; Vannote *et al.* 1980). However, the relative importance of autochthonous production has been noted in rivers (e.g., Thorp and DeLong 2002). Current models are founded primarily on forested, non-spring systems and thus may not apply to either karst spring systems or non-forested ecoregions such as the U.S. Great Plains (Dodds *et al.* 2004). The spatiotemporal importance of autochthonous and allochthonous organic matter to food webs in resource-pulsed, spatially-subsidized springbrooks has not been factored into current models of lotic ecosystems.

Second, traditional food web theory (May 1972; Cohen 1978; Pimm 1982; Briand 1983; Schoenly *et al.* 1991) would predict, especially given the small size of spring ecosystems that, food web complexity and connectance (proportion of actual links between food sources and consumers from the maximum number of possible links in the web) would be low and decrease with increasing web size (species richness), omnivory would be rare, species would interact directly (as predator or prey) with only 2-5 other species, the ratio of prey to predator species would be <1 , and linkage density (number of links per species) would remain constant (independent of web size/ species richness). However, these theories are being challenged by those that indicate that (i) connectivity is independent of web size, and in fact, can increase with species richness; (ii) linkage density increases with species richness; (iii) highly connected webs have a high proportion omnivores; (iv) omnivory actually increases complexity and connectivity; and (v) complexity promotes stability (Warren 1989; Martinez 1991; Polis 1991 and 1994; Hall and Raffaelli 1993; Polis and Strong 1996; Jaarsma 1998). Therefore, this study examined how the relative contributions of allochthonous and autochthonous organic matter to food web pathways, and food web complexity/connectivity varied temporally and spatially from the spring source to the downstream brook in three karst springs.

The springs examined (as discussed in chapter 2) were: Haseltine Spring which issues from a small cave, and whose springbrook is located in open, mowed grassland; Danforth Spring which arises from a small, cave-like opening at the base of a limestone bluff into a coniferous forest; and Steury Spring which issues from the largest cave into a deciduous forest. Based on the literature, I hypothesized that: (i) the dominant food source fueling spring food webs would be allochthonous; (ii) that dependence on autochthonous carbon sources would increase in lower reaches due to increased algal growth in springs with reduced canopy cover; and (iii) that the dominant food source would shift seasonally to a heavier reliance on allochthonous carbon due to increased allochthonous leaf-litter inputs. Additionally, I hypothesized that food web complexity would: (a) vary temporally, reflecting the availability of seasonally-pulsed carbon sources (autochthonous vs. allochthonous); (b) increase spatially along the gradient from the spring source to the springbrook; and (c) vary among the three springs due to relative differences in the nature of terrestrial and aquatic plant cover. The complexity of feeding relationships should increase in lower springbrook reaches because of changes in community composition (greater diversity of species; chapter 2) and availability of potential food sources.

Food source-consumer interactions and food web structure were determined using carbon and nitrogen stable isotope and stoichiometric analyses. These techniques have been broadly used because of their potential to reflect food assimilation, their help in resolving questions about the importance of specific food sources to invertebrate consumers, and their ability to elucidate trophic relationships (e.g. Rounick and Winterbourn 1986; Peterson and Fry 1987; Thorp *et al.* 1998; Delong and Thorp 2006).

Food web structure is considered to be important to the understanding of ecosystem function (Pimm 1982), but food webs are not static entities (Warren 1989). This study represents one of the few food web studies that examines both spatial and temporal patterns and replicates that examination in three similar ecosystems. By tracing the response of communities to temporal resource pulses and by examining

the effects of spatial subsidies, I analyzed how spatial and temporal patterns and processes affected food web structure and function and contributed to broader questions on the role of patch dynamics in riverine landscape ecology, and their application to newly developing theories on the interplay of temporal variation and spatial subsidies as major determinants of food web dynamics.

Methods

Sample collection and processing

To test hypotheses regarding temporal and spatial patterns in basal resource utilization (allochthonous vs. autochthonous), and the impact these patterns have on food web complexity, I collected 3 replicates of 15-20 biotic and abiotic units, representing either potential food sources or consumers, along 3 transects in each of the 4 reaches in all 3 springs during 2 seasons (summer and winter). Because isotope signatures can be heavily influenced by location, the same food source (e.g. epiphytic algae) can have different signatures within each system (Delong *et al.* 2001). For short distances, such as those in these three springbrooks, one might not expect these differences to be manifested. However, because the major source of spring water is from an underground karst system heavily influenced by calcium carbonate, the carbon signature of algae, for example, could vary substantially from upstream to downstream as the relative contributions of CO₂ from groundwater and atmospheric sources change. Consequently, replicate samples of similar food sources were taken along the spring source-springbrook gradient to fully assess spatial and temporal (seasonal) differences in diets of the zoobenthos community.

Terrestrial samples were placed in sterilized plastic bags, and aquatic samples were placed in acid-washed plastic vials. All were stored on ice for transport back to the laboratory where they were processed and dried for 48 h at 60°C. Because carbonate concentrations are high in karst systems, aquatic samples were acidified after drying and grinding to remove this source of isotopic carbon. Dried samples were ground to a fine powder with a Wig-L-Bug® and shipped to the Colorado

Plateau Stable Isotope Laboratory at Northern Arizona University, Flagstaff, Arizona for determination of carbon and nitrogen stable isotope signatures. Samples were analyzed with a DELTA V Advantage continuous flow mass spectrometer. The $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively) for samples were determined and reported as differences in parts per thousand ($‰$) between isotopic ratios of samples and standard compounds (PeeDee belemnite for carbon and atmospheric N for nitrogen).

Sources of organic matter

Terrestrial organic samples (C_3 and C_4 plants) collected included 5-6 terrestrial autotrophs in the riparian zone, including leaves from dominant tree species (deciduous in two springs, but coniferous in Danforth), grasses, and dominant brush. Aquatic sources from the springs included: (i) two particulate transported size classes: coarse (CTOM; $> 1\text{ mm}$ diameter) and fine (FTOM; $0.1\text{--}1\text{ mm}$ diameter); (ii) dissolved organic matter (DOM; $< 0.1\text{ mm}$ diameter); (iii) dissolved inorganic carbon (DIC) and dissolved inorganic nitrogen (DIN); (iv) benthic organic matter (BOM); (v) benthic algae (periphyton); and (vi) rooted macrophytes. Aquatic macrophytes were clipped above the roots and benthic algae were scraped from rock substrates. Water for particulate classes was collected at three random points along each of three transects at each sampling reach. Transect samples were then pooled into a single composite sample providing a clear representation of organic matter in the water column. Water samples were passed through coarse (1 mm) and fine (0.1 mm) sieves to collect CTOM and FTOM. DOM was obtained by filtering several liters of this pre-filtered water through an ultra-fine ($1\text{-}\mu\text{m}$ pore) glass-fiber filter. After adjusting the pH to 4.3 to remove inorganic forms of carbon, oxygen was bubbled through the DOM samples (cf. Thorp *et al.* 1998).

Standard procedures for collecting and analyzing isotopic signatures for DIC and DIN followed standard protocols. For DIN analysis, 250 mL water was collected in triplicate (to ensure a concentration of at least 0.05 mg N per L) in Nalgene bottles

from each sampling site in each of the 3 spring ecosystems during both seasons. DIN samples were filtered using Whatman 0.45 μm nylon membrane filters and a standard filtration device. Samples were frozen for preservation purposes within 6 hours of collection. The analytical procedure for analyzing $\delta^{15}\text{N}$ of dissolved inorganic nitrogen (DIN) involved converting the DIN to ammonia gas, driving the gas into the container headspace, and diffusing the ammonia through a Teflon membrane onto an acidified filter disk. The $\delta^{15}\text{N}$ ratios were determined using a Thermo Electron gas isotope-ratio mass spectrometer. For DIC analysis, water samples were collected and filtered into amber borosilicate vials with silicon Teflon lined septas using a 0.45- μm syringe filter assembly. A small volume of saturated mercuric chloride solution was added to each sample to impede microbial activity during storage. Samples were immediately stored on ice and prepared for shipment. Measurement of $\delta^{13}\text{C}$ isotopes for DIC was done using an O.I. analytical total organic carbon (TOC) analyzer. All DIN and DIC samples were shipped overnight delivery to the Colorado Plateau Stable Isotope Laboratory for analysis.

Consumers

Major invertebrate groups, representing major functional feeding groups were collected using a Surber sampler (500 μm mesh) and an Ellis-Rutter sampler (500 μm mesh) as described in chapter 2. Samples were placed on ice for transport back to the laboratory where they were sorted into major taxonomic groups and placed in a refrigerator at 40°C to clear their digestive tracts. Fauna were identified to species where possible, although chironomids and oligochaetes were identified only to subfamily and family, respectively. Functional feeding group designations were determined using Merritt and Cummins (2008). Fish and salamander samples collected were prepared for isotopic analysis by removing a small portion of muscle tissue in the laboratory. All samples were dried and prepared for isotope analysis as described above.

Biomass

Biomass was quantified as one measure the relative importance of different consumer components in each food web. The most commonly accepted technique for biomass estimation involves regression of dry mass on body length (Benke *et al.* 1999). Biomass estimates for invertebrate groups with densities >100 specimens per sample were made by establishing taxon-specific length-dry weight relationships (cf. Benke *et al.* 1999). Body lengths (exclusive of appendages) were measured to the nearest 0.05 mm using a dissecting microscope and an ocular micrometer. Specimens were dried at 60°C for 48 h and then weighed using a microbalance (sensitivity 0.002 mg) to determine dry mass. Dry-weight biomass measurements for taxonomic groups with <100 specimens per sample were determined directly. Biomass measures were then used as a measure of the potential energetic importance of different taxonomic groups in the food web.

Analyses

The isotope data were analyzed using the program IsoSource, a multi-food source, multi-isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) mixing model for determining the potential contribution of a broad suite of organic sources to the consumer diet using both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Phillips and Gregg 2003, Phillips *et al.* 2005). Stable isotopes of C and N provide quantitative information on trophic relationships and diet sources, and they can be used as surrogates for determining pathways of energy and nutrient flow between habitats and organisms (cf. Fry 2006). The IsoSource model (Phillips and Gregg (2003) creates all possible combinations of source proportions (each combination equals 100%), creates a set of predicted source mixtures that are compared against the observed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of the consumer, and quantifies the potential contribution of sources to the consumer diet. The results are presented as a 1-99th percentile range (minimum to maximum), or the range that a food source can feasibly contribute to a consumer's diet. Phillips and Gregg (2003) recommend using the 1-99th percentile range to represent the proportion (%) of the

diet accounted for by particular food sources, because of its sensitivity to small numbers of observations. Hence, a food's contribution at the first percentile (e.g. 40%) represents its minimum contribution to consumer diet, and its contribution at the 99th percentile represents its maximum. The IsoSource model also provides a mean percentage that a food source contributes. Hence, the 1-99th percentile range proportions are discussed in the text describing the food web models, where means are represented.

Corrections of food source ^{13}C and ^{15}N signatures were performed to account for expected levels of fractionation from one trophic level to the next (Phillips and Gregg 2003). Trophic enrichment levels for a single trophic level increase of 0.4 ‰ for $\delta^{13}\text{C}$ and 3.4 ‰ for $\delta^{15}\text{N}$ were used (cf. Delong and Thorp 2006). These same procedures were applied to secondary consumers if their food source was determined to be a primary consumer (Merritt and Cummins 2008). For omnivorous feeders or young damselflies (*Argia*) that eat protozoa (FTOM size) (Westfall and Tennesen 2008), however, the energy pathway in question requires examination of flow from basal resource to secondary consumer. In these cases, the trophic fractionation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of organic matter sources was increased using techniques similar to those employed by Delong and Thorp (2006). First, trophic position (TP) of each secondary consumer was calculated (Post 2002). Average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *Physa remingtoni* and *Goniobasis potosiensis* were used as the benthic feeding primary consumers to determine trophic position of the secondary consumers. Expected levels of fractionation for a single trophic level increase (as above) were then multiplied by $\text{TP} - 1$, and those values were added to the basal food sources for use in the IsoSource model.

Stable isotope analysis (SIA) focused on consumers that were either dominant in biomass or considered key players in the food web. For example, chironomids represented a maximum of only 1% of the overall biomass for any of the 3 springs in summer samples, and a maximum of only 2% of the overall biomass in Haseltine and Danforth in winter samples – not enough tissue to produce an isotope sample. Hence,

chironomids were used only in the SIA analysis for Steury Spring during the winter sampling season when they represented as much as 14% of the overall biomass in a given reach, and a total of 60% of the overall biomass for the entire spring.

Relationships between organic matter sources and consumers were first examined using standard comparative $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ bi-plots (Peterson and Fry 1987; Fry 1991). Foods choices selected for use with individual consumers in the IsoSource multi-mixing model were based on functional feeding group designations (Merritt and Cummins 2008; Thorp and Covich 2001) and examination of the bi-plots.

Food web models were developed from the predictions of the relative contribution of different food sources to consumer diets based on results from the IsoSource mixing model and biomass estimates of each consumer (Figs. 12-14). The importance of a food type (symbolized by arrow width) denotes the bulk of biomass (symbolized by circle size) supported by a particular food type. All links were quantified via direct trophic analysis by the IsoSource model and biomass estimates.

Food web complexity statistics were calculated and included both directed connectance and connectivity (linkage density and complexity) measures (Martinez 1991; Polis 1991; Closs and Lake 1994; Travers-Cromar and Williams 1996; Woodward *et al.* 2005). Directed connectance is defined as the proportion of documented directed links from the maximum number of possible directed links in the web, but also includes cannibalism and mutual predation interactions. It is calculated as $C_d = L/S^2$ where L is the number of directed links observed and S is the number of species in the web or web size (Martinez 1991; Woodward *et al.* 2005). The directed connectance metric, based on the constant connectance hypothesis (Martinez 1991), was selected over connectance metrics based on the link-species scaling law (Cohen and Newman 1985) because the expectation that each consumer's prey increases roughly in proportion to the number of species in the community (Martinez 1991) seemed more plausible than the expectation that species are linked to a constant number of species regardless of the number of species in the community (Cohen and Briand 1984; Pimm *et al.* 1991). Connectivity is defined as the number of

interactions (L) per component of the system (S) (Allen and Starr 1982). Linkage density was calculated as L/S (Warren 1989; Martinez 1991; Tavares-Cromar and Williams 1996; Woodward *et al.* 2005), and food web linkage complexity as $S \cdot C$ where $C = (L/(S[S-1]/2))$ (Polis 1991; Woodward *et al.* 2005). The number of directed trophic links was quantified by the 1-99th percentile range from the IsoSource distribution summary as described above. Connectance and connectivity metrics were used as dependent variables in an analysis of variance (ANOVA; NCSS) to test for effects of spring, reach, and season and their interaction on food web complexity. When treatment effects existed, the Tukey post-hoc test was used for comparing specific means. Statistical significance was determined at $\alpha = 0.05$.

Isotopic analyses were supplemented by analysis of C:N ratios to quantify the relative importance of allochthonous and autochthonous food sources to consumers and to verify the allochthonous or autochthonous composition of organic food sources. While autochthonous organic matter typically has C:N ratios ranging from 8:1 (Thorp *et al.* 1998) to 12:1 (Wetzel 2001) or 15:1 (Kendall *et al.* 2001), allochthonous forms of organic matter typically range from 45:1 to 50:1 (Wetzel 1983) or are at least greater than 15:1 (Kendall *et al.* 2001). In this study, the C:N ratios of autochthonous food sources (water cress and moss) ranged between 7.01 and 12.5. The C:N ratios of allochthonous food sources for Haseltine and Steury (grasses and deciduous leaf litter) ranged from 16.59-28.23, while allochthonous material in Danforth (grasses and pine litter) ranged from 16.59-89.90. Due to the variability in C:N ratios for benthic algae, FTOM, DOM, and BOM, their allochthonous or autochthonous composition was determined by the classical approach where C:N values >15 were interpreted as indicating significant allochthonous contributions (Kendall *et al.* 2001).

Results

Stable isotope values for energy sources (boxes delineated by \pm SD about the means for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and consumers (circles with \pm SD bars) are shown for

Haseltine, Danforth, (Fig. 2), and Steury Springs (Figs. 9-11) for both summer and winter sampling seasons. There were a total of 907 stable isotope samples from the 3 spring sites: 462 were basal food sources and 445 were consumers (Table 6) identified mainly to species or genus (Chapter 2; Table 4). Filtration attempts failed to collect any CTOM fractions (>1mm diameter), which makes sense because turbidity levels were universally very low (<2.8 NTU). This also may explain why most springs lack true plankton (Teal 1957; Minkley 1963). Hence, transported organic matter sources used in food webs included only FTOM, and DOM fractions.

To improve the interpretive potential of the IsoSource multi-isotope mixing model output, mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of consumers with similar functional

Table 6 A summary of the samples of food sources and consumers and associated notations.

Food Source	Abbreviation	Consumer / Numeric Notation			
Dissolved Organic Matter	DOM	Amphipods	1	<i>Tipula sp.</i>	10
Fine Transported Matter	FTOM	<i>Lirceus hoppinae</i>	2	<i>Optioservus sandersoni</i>	11
Benthic Organic Matter	BOM	Lumbriculidae	3	Fish	12
Benthic Algae	Benthic Algae	<i>Orconectes sp.</i>	4	<i>Acroneturia sp.</i>	13
Moss	Moss	<i>Dugesia doratocephala</i>	5	<i>Argia sedula</i>	14
Water Cress	Water Cress	<i>Eurycea multiplicata</i>	6	<i>Eurylophella bicolor</i>	15
Liverwort	LW	Net-spinning caddisflies	7	<i>Stratimys sp.</i>	16
C ₃ Terrestrial Vegetation	C ₃	Case-building caddisflies	8	Chironominae	17
C ₄ Terrestrial Vegetation	C ₄	Gastropods	9	Tanypodinae.	18
Raccoon Scat	Raccoon Scat				

feeding strategies (Merritt and Cummins 2008; Thorp and Covich 2001) were calculated and included the following taxonomic combinations: amphipods (*Crangonyx forbesi* and *Gammarus minus*), gastropods (*Physa remingtoni*, *Promenetus sp.*, and *Goniobasis potosiensis*), case-building caddisflies (*Helicopsyche borealis*, *Neotrichia sp.*, and *Ochrotrichia contorta*), fishes (*Cottus bairdi* and *Etheostoma punctulatum*), and net-spinning caddisflies (*Cheumatopsyche sp.* and *Hydropsyche piatrix*).

An omnivore is often defined as an organism that eats at more than one trophic level (Pimm 1982). For the purpose of this study, omnivory is defined in broader terms to encapsulate omnivory that occurs when consumers (i) eat prey from many trophic levels, (ii) eat whatever resources are available or abundant; and (iii) change feeding preferences with ontogeny. All three types of omnivory affect food web complexity and connectivity (Polis *et al.* 1989; Polis 1991 and 1994). For example, young *Orconectes* crayfish can filter feed (FTOM), while both young and adults can feed at multiple trophic levels (Hobbs 2001). Young second instar *Argia* damselfly larvae can consume protozoa (FTOM size class) (Westfall and Tennesen 2008), and juvenile amphipods and isopods typically depend on algae and bacteria (FTOM) (Thorp and Covich 2001). For that reason, foods selected for use in the IsoSource model were selected to identify these forms of omnivory.

Isotopic signatures of food sources

Organic sources varied over space and time. Each organic matter food source, supporting metazoan production had distinct carbon and nitrogen stable isotope ratios at the spring source, but the degree of overlap often increased by reach 4 (Tables 7 and 8; Figs. 9-11). Allochthonous C₃ and C₄ food sources were generally absent in winter due to die back, but their contributions continued to be evident in winter transported and benthic organic matter isotope signatures and C:N ratios. Autochthonous food sources (watercress, moss, and algae) were prevalent throughout the year. Based on analysis of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ bi-plots, C₄ grasses were too enriched to be a reasonable food choice for consumers. Other foods such as benthic algae, BOM, and FTOM were also occasionally too C-enriched to be a carbon source for herbivores, as enrichment was associated with high C:N ratios. Autochthonous food sources, except for benthic algae, were generally more C-depleted (-45.33 to -29.12‰) than allochthonous C₃ leaf and grass litter (-31.40 to -27.77‰). Benthic algae were consistently more C-enriched (-21.73 to -7.80‰) than any other primary food source in Haseltine and Steury Springs, with signatures similar to that of C₄

Table 7 Summer stable carbon isotope ratios (‰) of sources and consumers in each of the 3 springs.

Sample Type	Haseltine Summer				Danforth Summer				Steury Summer			
	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
Primary Sources												
DOM	-25.2	-25.7	-28.2	-28.9	-28.9	-24.0	-27.1	-27.4	-28.5	-29.5	-28.8	-26.3
FTOM	-28.7	-25.1	-24.3	-24.0	-29.5	-33.1	-31.5	-32.9	-23.1	-25.2	-22.9	-23.4
BOM	-22.9	-22.2	-15.2	-11.3	-25.8	-26.0	-26.8	-27.9	—	-11.8	-11.9	-12.7
Benthic Algae	—	-13.4	-13.6	-20.3	-36.2	-36.4	-37.2	-38.0	-17.6	-16.7	-16.9	-17.0
Moss	-45.3	-45.2	-45.3	-38.6	-43.4	-43.3	-43.1	-41.3	—	-40.2	-40.8	-41.3
Water Cress	-29.1	-29.4	-29.4	-30.7	-33.2	-30.9	-31.5	-33.3	—	-30.9	-31.9	-33.3
Liverwort	—	—	—	—	—	—	—	—	—	-32.4	-32.1	-27.8
C ₃ Plants	—	-30.5	-30.6	-29.7	-31.4	-31.4	-30.4	-29.9	-27.4	-31.0	-30.7	-30.3
Raccoon Scat	—	—	—	—	—	—	—	—	—	—	—	—
Primary Consumers												
Amphipods	-32.4	-33.1	-36.8	-33.1	-37.4	-37.3	-36.9	—	-37.8	-35.6	-37.8	—
Lirceus	-32.0	-34.2	-34.8	-29.7	-32.7	—	—	—	-32.8	-32.5	—	—
Lumbriculidae	-31.6	—	-29.7	-30.9	-35.3	-32.2	-32.3	-33.6	-29.5	-28.7	-28.8	-28.8
Gastropods	—	—	-34.8	-29.3	—	-36.6	-37.7	-37.1	—	—	—	-33.3
Net Caddisfly	—	-36.4	-37.0	-38.3	—	—	—	-38.9	—	—	—	-39.1
Case Caddisfly	—	-37.6	-37.5	-37.3	—	—	—	—	—	—	—	—
Optioservus	—	-40.8	-41.3	-39.9	—	—	-42.8	-41.8	—	—	-42.8	-42.0
Tipula	—	—	-43.2	-42.3	—	-43.2	-43.2	-43.2	—	—	—	—
Secondary Consumers												
Argia	—	—	—	—	—	-40.2	-38.8	-29.9	—	-34.5	-30.9	-30.3
Acroneura	—	—	—	-35.2	—	—	—	—	—	—	—	—
Orconectes	-30.6	-33.5	-33.5	—	-32.4	—	—	—	—	—	-28.8	-28.8
Dugesia	-32.5	—	—	—	—	—	—	—	—	—	—	—
Eurycea	-36.1	—	—	—	-30.3	—	—	—	—	—	—	—
Fish	—	—	-34.7	-34.5	—	-32.8	-37.2	-37.1	—	—	—	-35.6

grass (mean -14.66‰). The exception to this was in Danforth Spring where algal signatures were often nearly as depleted as those of moss (Figs. 9-11). Stoichiometric analysis indicated that benthic algae and C₄ grasses had C:N ranges between 17.21 and 26.02 (Table 9).

Table 8 Winter stable carbon isotope ratios (‰) of sources and consumers in each of the 3 springs.

Sample Type	Haseltine Winter				Danforth Winter				Steury Winter			
	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
Primary Sources												
DOM	-22.7	-23.3	-30.9	-22.8	-30.1	-27.4	-29.7	-27.3	-27.3	-29.8	-28.6	-27.3
FTOM	-8.3	-9.7	-14.4	-14.5	-5.4	-16.2	-15.0	-29.5	-5.5	-20.6	-20.1	-20.8
BOM	-27.7	-27.7	-27.7	-27.8	-28.1	-28.0	-28.0	-27.8	—	-28.2	-28.2	-28.3
Benthic Algae	—	-5.3	-7.8	-13.0	-28.9	-29.4	-29.9	-30.0	-17.6	-18.4	19.3	-21.7
Moss	-41.0	-39.4	-37.6	-36.2	-42.3	-42.2	-42.6	-43.2	—	-26.3	-25.3	-24.9
Water Cress	-32.2	-32.6	-32.7	-31.7	-31.7	-31.5	-31.0	-30.6	—	-30.7	-30.5	-30.4
Liverwort	—	—	—	—	—	—	—	—	—	-27.1	—	—
C ₃ Plants	—	—	—	—	—	-28.0	-27.9	-27.8	—	—	—	—
Raccoon Scat	—	—	—	—	—	—	—	—	-27.4	—	—	—
Primary Consumers												
Amphipods	-33.0	-33.0	-30.7	-31.0	-31.9	-38.9	-36.8	-36.8	-31.5	-31.3	—	—
Lirceus	-27.0	-31.6	-27.4	-29.8	-29.0	—	—	—	-27.7	-30.0	—	—
Lumbriculidae	—	-30.5	-31.4	-30.0	-32.7	-36.2	-35.4	-35.3	-28.1	-28.2	-27.4	-28.1
Gastropods	—	—	-31.1	-34.5	-37.2	-39.2	-37.6	-37.7	—	—	—	—
Net Caddisfly	—	-31.7	—	-31.7	—	-40.8	-38.4	-37.8	—	—	—	-28.8
Case Caddisfly	—	—	—	—	—	-41.5	—	-40.0	—	—	-29.5	-29.9
Optioservus	—	-39.8	-38.6	-38.6	—	—	-39.8	-40.6	—	—	—	-34.3
Tipula	—	—	—	—	—	—	-43.5	-30.5	—	—	—	-43.6
Stratimys	—	-31.9	-30.3	—	—	—	—	—	—	—	—	—
Eurylophella	—	—	—	—	—	-41.0	-39.4	-42.0	—	—	—	—
Chironominae	—	—	—	—	—	—	—	—	—	-28.6	-29.0	-29.1
Secondary Consumers												
Argia	—	—	—	—	—	-35.8	-35.6	-36.2	—	-30.2	-31.4	-28.5
Acroneura	—	—	—	-35.2	—	—	—	—	—	—	—	—
Tanypodinae	—	—	—	—	—	—	—	—	—	-28.0	-28.3	-28.1
Orconectes	—	—	—	—	—	—	—	-37.5	—	—	-30.0	-29.3
Dugesia	-26.8	-27.3	-29.3	-29.4	-40.3	—	-34.1	-33.3	—	—	—	—
Eurycea	-29.1	—	—	—	—	—	—	—	—	-25.1	—	—
Fish	—	—	—	—	—	—	-39.1	-37.5	—	—	—	—

Summer samples showed considerable variability in FTOM, DOM, and BOM $\delta^{13}\text{C}$ signatures among spring sites. In Haseltine, FTOM generally resembled water cress; in Danforth, signature values were intermediate between C₃ plants and benthic algae; and in Steury, FTOM signatures were intermediate between C₄ and C₃ plant signatures. C:N ratios for FTOM were consistent with autochthonous values in

Haseltine, allochthonous carbon in Steury, and were intermediate between the two in Danforth (Table 4). FTOM was typically 3-6‰ more C-enriched than DOM in Haseltine and Steury, suggesting a potential contribution of enriched algal carbon in those sites. Moreover, FTOM in these two sites was enriched by as much as 10‰ over that of Danforth where algae were not enriched (Figs. 9-11; Table 7-8). In each spring, BOM was often as enriched as C₄ grasses, understandable as a source for BOM includes litter of terrestrial origin (Allan 1999). This was not the case in Danforth Spring as the coniferous forest, in which it resides, prevents much grass growth, and BOM was only slightly enriched over other food sources. Moss was consistently the most C-depleted food source.

Table 9 Mass C:N ratios for potential sources of organic matter from each of the 3 study springs. C:N values >15 were interpreted to indicate significant allochthonous contributions and are noted in bold. Sample values not available (NA) as R1 = cave.

		SUMMER				WINTER			
		DOM	FTOM	BOM	Algae	DOM	FTOM	BOM	ALGAE
Haseltine	<i>R1</i>	1.55	2.9	10.28	NA	2.04	23.22	20.42	NA
	<i>R2</i>	1.85	2.27	10.31	17.63	2	36.17	19.58	36.97
	<i>R3</i>	2.27	12.6	13.48	17.5	7.12	15.87	19.88	24.8
	<i>R4</i>	1.19	12.29	20.29	12.4	1.79	22.04	23.43	17.38
Danforth	<i>R1</i>	2.4	15.2	8.73	5.98	3.73	30.61	81.00	8.01
	<i>R2</i>	2.72	10.2	7.23	6.01	4.29	14.95	81.36	8.21
	<i>R3</i>	3.29	10.22	8.92	6.58	3.81	15.85	45.68	8.05
	<i>R4</i>	1.96	10.1	9.17	7.58	1.6	7.53	26.49	7.98
Steury	<i>R1</i>	1.2	22.72	NA	NA	2.17	56.42	NA	NA
	<i>R2</i>	2.98	20.76	15.06	24.49	5.15	33.04	33.04	19.08
	<i>R3</i>	3.86	16.87	14.98	23.98	7.14	16.6	33.61	15.51
	<i>R4</i>	3.13	14.87	14.87	22.4	8.69	16.03	34.34	12.41

Summer food sources showed less variation in $\delta^{15}\text{N}$ signatures. DOM was consistently the most N-enriched food source in each spring. Moss, considerably N-depleted in Haseltine, had $\delta^{15}\text{N}$ signatures similar to that of other food sources in the other two springs, where water cress was the most N-depleted food source (Figs. 9-

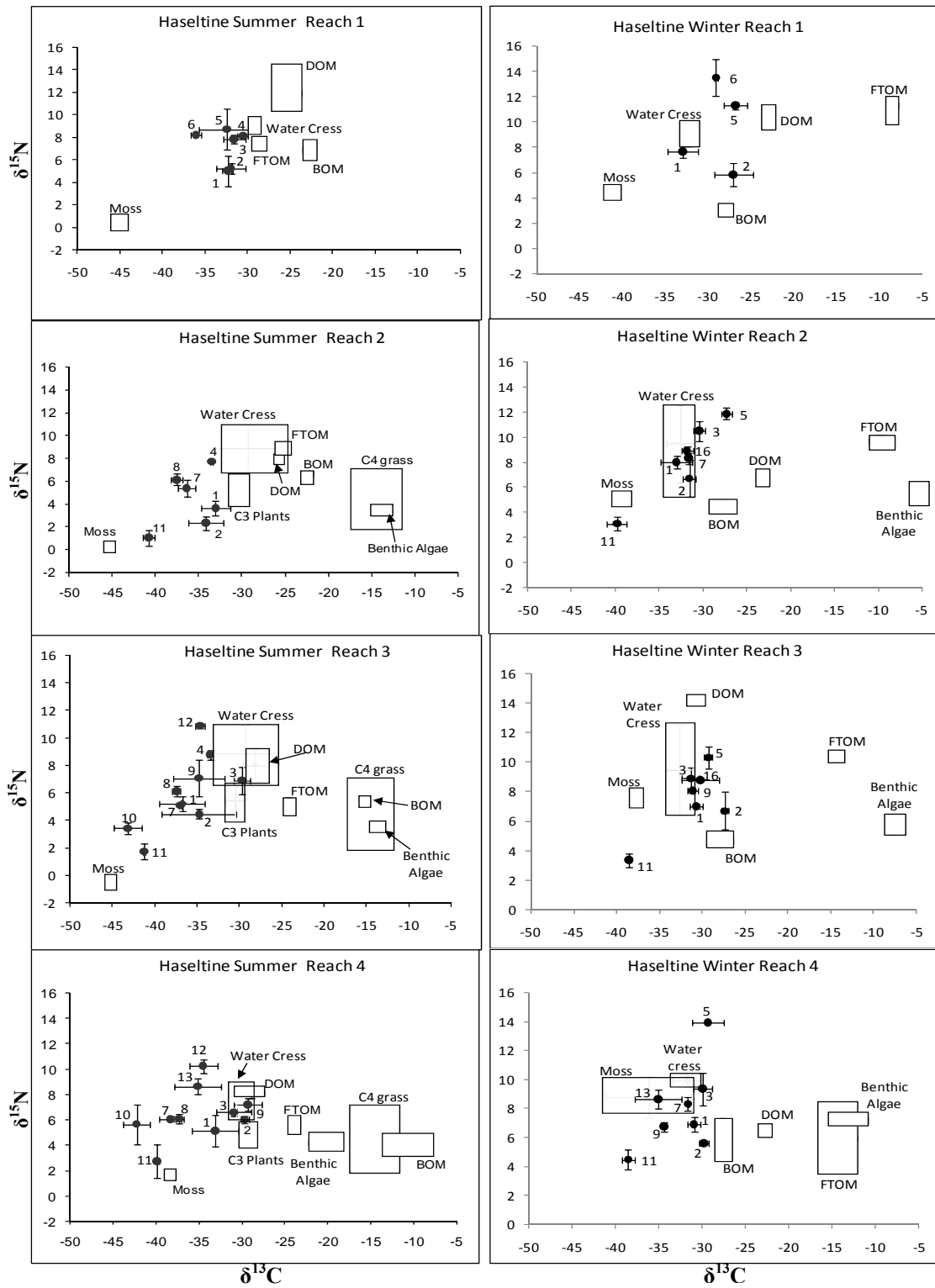


Figure 9 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (summer & winter) for Haseltine Spring. Potential Carbon/nitrogen sources shown as boxes delineated by 1 SD. Consumers indicated as circles (mean \pm 1 SD). See Table 1 for abbreviations and numeric notations.

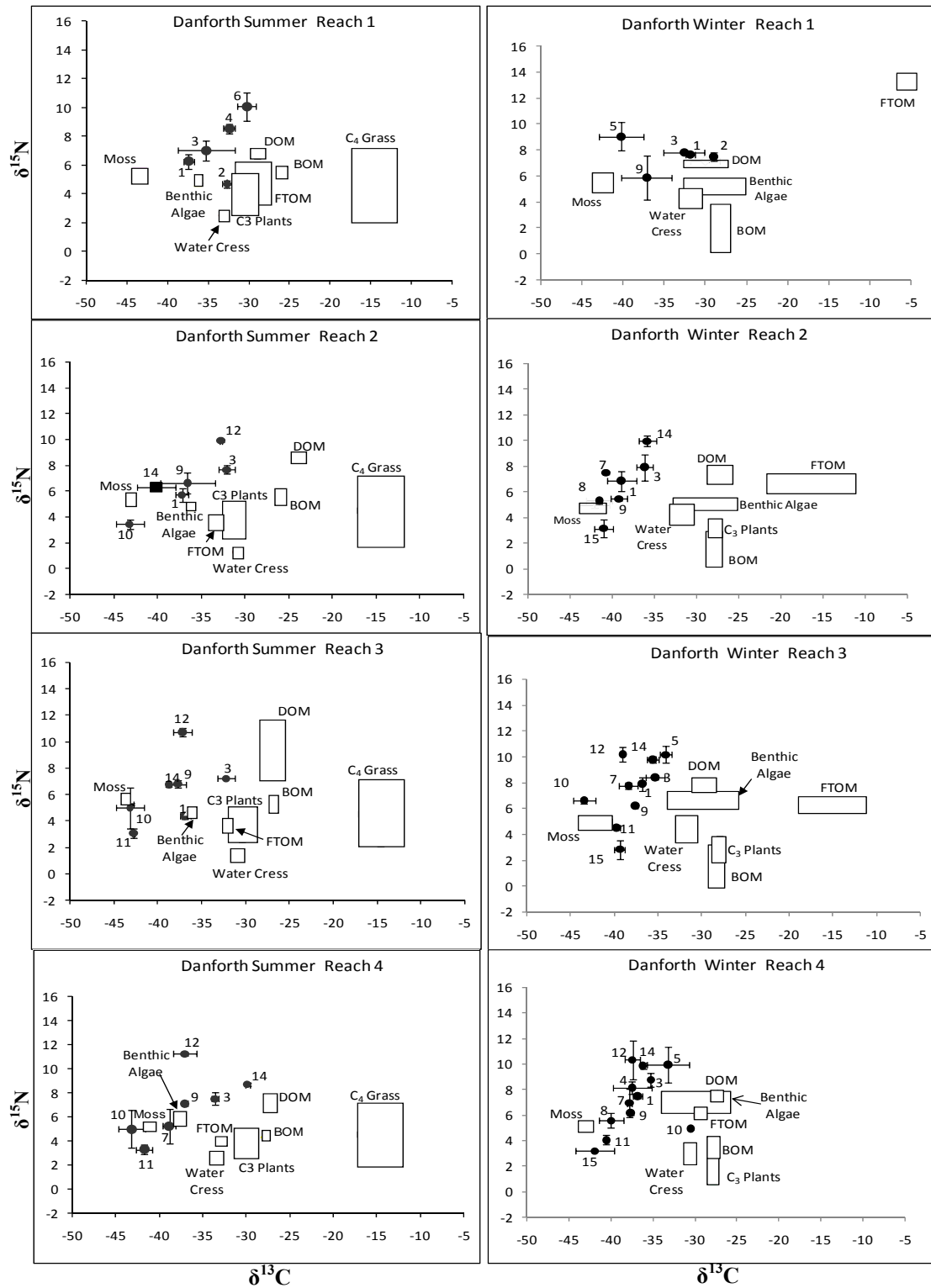


Figure 10 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (summer & winter) for Danforth Spring. Potential Carbon/nitrogen sources shown as boxes delineated by 1 SD. Consumers indicated as circles (mean \pm 1 SD). See Table 1 for abbreviations and numeric notations.

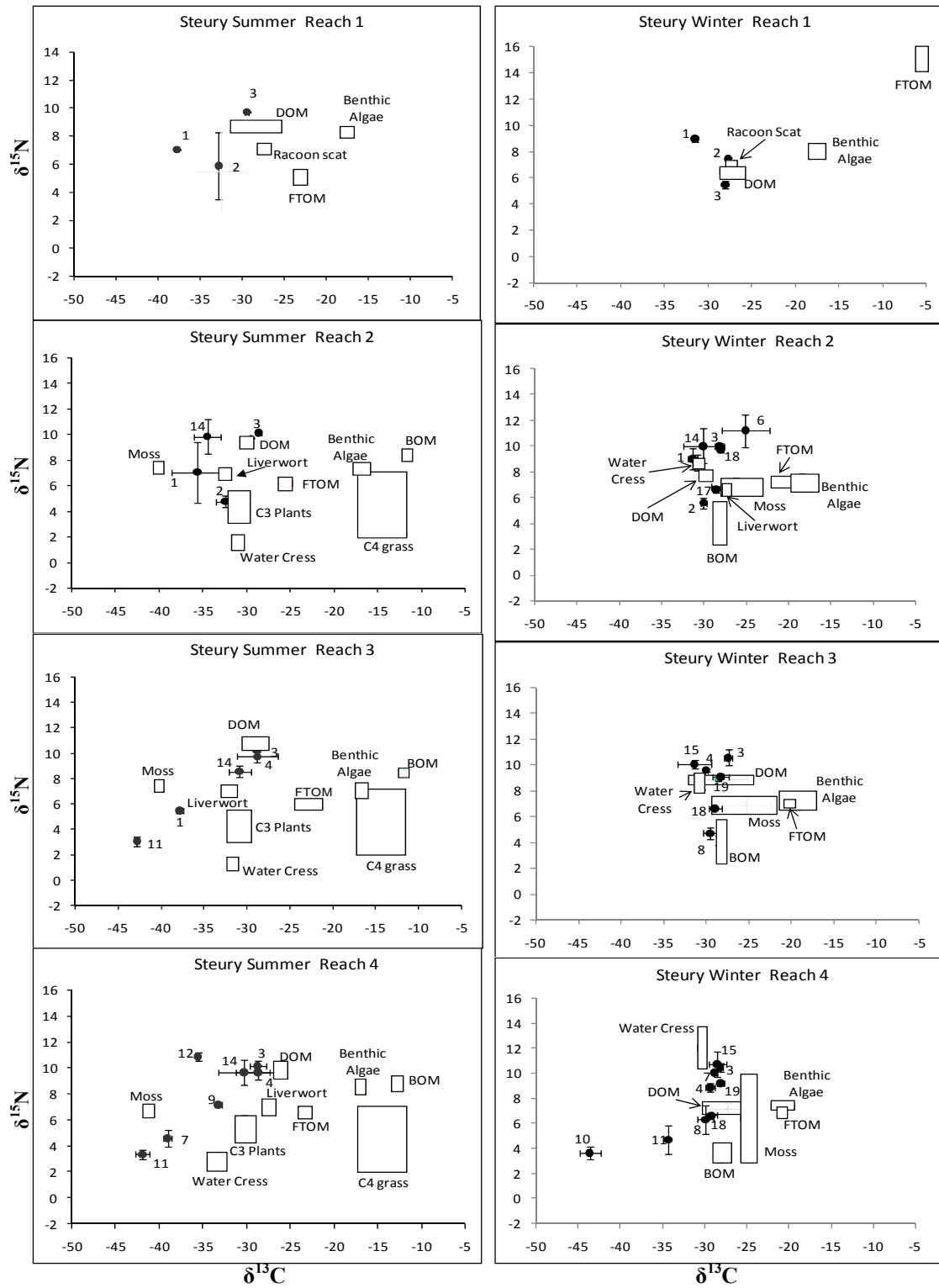


Figure 11 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (summer & winter) for Steury Spring. Potential carbon/nitrogen sources shown as boxes delineated by 1 SD. Consumers indicated as circles (mean \pm 1 SD). See Table 1 for abbreviations and numeric notations.

11). Overall, however, food sources were the most N-enriched in Haseltine, the most N-depleted in Danforth and intermediate between the two in Steury.

Stable isotope ratios of several energy sources varied substantially along spatial and temporal scales, illustrating that isotope signatures can be heavily influenced by spatial and temporal factors and emphasizing the importance of collecting like food sources across multiple sampling sites and seasons. Variation in food source $\delta^{13}\text{C}$ signatures along the spring source-springbrook gradient (R1 to R4) was common as some food sources were C-enriched, while others were consistently C-depleted along this gradient during both seasons. For example, in Haseltine summer samples, FTOM in reach 4, moss, and BOM $\delta^{13}\text{C}$ signatures were enriched by 5, 7, and 11‰ respectively, while DOM and benthic algae $\delta^{13}\text{C}$ signatures were depleted by 5‰ and 7‰, respectively from those in reach 1. Winter FTOM values in Danforth and Steury were depleted by 26 and 16‰ respectively in reach 4 compared to reach 1.

Spatial variation in $\delta^{15}\text{N}$ food source signatures was also noted, with values generally enriched in lower springbrook reaches although typically by a much lesser margin than that of carbon (1-2‰). The exception to this was in Haseltine, where both summer and winter DOM and FTOM $\delta^{15}\text{N}$ signatures in reach 4 were depleted by > 4‰ over those in reach 1, and in Danforth and Steury whose winter FTOM signatures were depleted by 7 and 6‰, respectively between reach 1 and 4.

Winter carbon and nitrogen signatures were generally enriched over summer values in all three springs (Figs. 9-11; Table 7-8). For example, Haseltine FTOM $\delta^{13}\text{C}$ values were enriched by 10-20‰, DOM by as much as 20‰, and moss and benthic algae by 5-8‰ compared to summer values. Danforth had comparable seasonal enrichment values for FTOM (16-26‰) and benthic algae (7-9‰). Steury FTOM was enriched by as much as 20‰ in reach 1, and moss $\delta^{13}\text{C}$ signatures, typically around -40‰ were enriched in winter samples by 14-17‰. BOM winter signatures were not enriched, but depleted by 5-17‰ from those of summer; a noted pattern associated with leaf decay (Schweizer *et al.* 1999). BOM signatures were

however, enriched over autochthonous carbon sources (water cress and moss) with values consistent with C₃ plants (pine trees) in Danforth (confirmed by C:N ratios of ~45.68-81.00; Table 9), and summer C₃ deciduous plant values (-28.27 to -28.24) in Haseltine and Steury.

Enrichment in $\delta^{15}\text{N}$ signatures, was less temporally variable (~1-4‰) except for FTOM whose values were enriched (especially in reach 1) over summer values by as much as 5, 9, and 11‰ in Haseltine, Danforth and Steury, respectively. In addition, winter moss samples (Haseltine) were substantially C-enriched over summer samples and were also more N-enriched (4-8‰) than summer samples. Stoichiometric analysis confirmed isotope signature shifts and the influence of allochthonous inputs because the primary shift in C:N ratios for FTOM and BOM between autochthonous and allochthonous composition occurred along temporal scales (Table 9).

Spring water DIC values (Table 10) indicated that spring water had passed through karst conduits of considerable heterotrophic activity as values were comparable to that of respired CO₂ (-27‰) from C₃ plants (Osmond *et al.* 1981).

Table 10 Dissolved inorganic carbon (DIC) and nitrogen (DIN) isotope ratios (‰) from each of the springs.

		DIC	DIN
Haseltine			
	Reach 1	-25.67	14.30
	Reach 3	-25.93	14.74
Danforth			
	Reach 1	-24.53	6.32
	Reach 3	-26.35	6.30
Steury			
	Reach 1	-25.59	7.96
	Reach 3	-25.57	7.46

Within-spring variability for DIN was low (Haseltine range 13.79-15.16; Danforth 5.65-6.83; Steury 7.57-8.05), but the $\delta^{15}\text{N}$ values for DIN in Haseltine were substantially enriched, indicating possible anthropogenic or agricultural influences.

In contrast, Danforth and Steury signatures were comparable and remained below the level indicative of animal or sewage waste (Kendall 2001). The $\delta^{15}\text{N}$ of DIN spring waters helps explain longitudinal within-spring similarities in food source $\delta^{15}\text{N}$ signatures, and differences among springbrooks (decreasing in N-enrichment from Haseltine to Steury and Danforth; Table 10).

Isotopic signatures of consumers

The range of $\delta^{13}\text{C}$ was -39.09 to $-26.84^{0/00}$ for all invertebrate consumers (excluding the crane fly *Tipula*, the mayfly *Eurylophella*, and the riffle beetle *Optioservus*), whereas $\delta^{15}\text{N}$ ranged from 1.50 to $11.23^{0/00}$. *Tipula*, *Eurylophella*, and *Optioservus* $\delta^{13}\text{C}$ values were much more depleted, ranging between -43.60 and $-39.41^{0/00}$. The signatures of these taxa were within ranges more similar to that of autochthonous food sources water cress and moss (-33.32 to -29.12‰ and 45.33 to -38.55‰ respectively) than to allochthonous C_3 plant sources (-31.40 to -27.77‰), and only similar to benthic algae in Danforth (-38.00 to -28.94‰). While less variable than food sources, consumer signatures varied along the gradient between reach 1 and 4.

For most invertebrates, the variability of isotope signatures (as ± 1 SD) was < 0.7 for $\text{‰}^{13}\text{C}$ and $< 1^{0/00}$ for $\delta^{15}\text{N}$. Some consumers however, had much broader diets (measured as ± 1 SD). Isopods and lumbriculid worms often exhibited a broad range in $\delta^{13}\text{C}$, while omnivorous amphipods (*Gammarus minus* and *Crangonyx forbesi*), snails, and crane flies exhibited broad ranges in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in Haseltine (Fig. 9-11). The $\delta^{15}\text{N}$ values of the crayfish *Orconectes* were as enriched as *Dugesia* and *Eurycea* illustrating its ability to feed at all trophic levels (Hobbs 2001).

Seasonal changes in $\delta^{13}\text{C}$ signatures of consumers corresponded with seasonal changes in food sources and were generally enriched over summer values. Additionally, a greater proportion of consumers had broader diets than in summer, exhibiting broad ranges (>0.7 for $\text{‰}^{13}\text{C}$ and $>1^{0/00}$ for $\delta^{15}\text{N}$) for carbon, nitrogen, or both across all three springs. The $\delta^{15}\text{N}$ values revealed that specifically worms and

net-spinning caddisflies were eating at one trophic level higher in the winter than in the summer. Winter collections of lumbriculid worms had $\delta^{15}\text{N}$ values similar to *Dugesia* and higher than those of the stonefly *Acroneuria* possibly due to dead animal matter suspended in the sediments.

Secondary consumers included planarians (*Dugesia doratocephala*), the polytrophic crayfish *Orconectes*, stoneflies (*Acroneuria*), midges (Tanypodinae), damselflies (*Argia*), salamanders (*Eurycea multiplicata*), and fishes (*Cottus bairdi* and *Etheostoma punctulatum*). Summer trophic positions for these consumers were calculated to be 2.5 for *Dugesia*, *Orconectes*, *Argia*, and *Acroneuria*, while *Eurycea* and the fishes were 2.9 and 3.1, respectively. The $\delta^{15}\text{N}$ values revealed that several were eating at one trophic level higher in the winter than in the summer, and trophic position calculations for all winter secondary consumers were slightly higher. *Dugesia*, *Eurycea*, and *Argia* were nearly 1 position higher in winter (3.3, 3.8, and 3.1 respectively) than in the summer. As expected, this group of invertebrates were highly enriched in $\delta^{15}\text{N}$, reflecting the known selective excretion of lighter isotopes with increasing trophic level (Peterson and Fry 1987).

Source-consumer links

While omnivory was common in each spring, summer food webs showed that autochthonous foods (moss and water cress) constituted the major proportion of the diet supporting herbivore biomass (Figs. 12-14a). Across all reaches and springs, most herbivores had dietary ranges (1-99th percentile) indicating that moss contributed as much as 43-99% of their diet. In Danforth Spring for example, moss definitely contributed to the diet of snails (R2: 43-50%, R3: 35-64%, R4: 23-68%) and crane flies (R2: 59-86%, R3: 79-81%, R4: 80-96%). In Haseltine, moss contributed as much as 48-99% to the diet in 7 of 8 herbivores. Water cress also represented a major autochthonous food source. In Steury Spring, for example, it represented as much as 48-88% of the assimilated herbivore diet. Based on their C:N ratios (Table 9), DOM

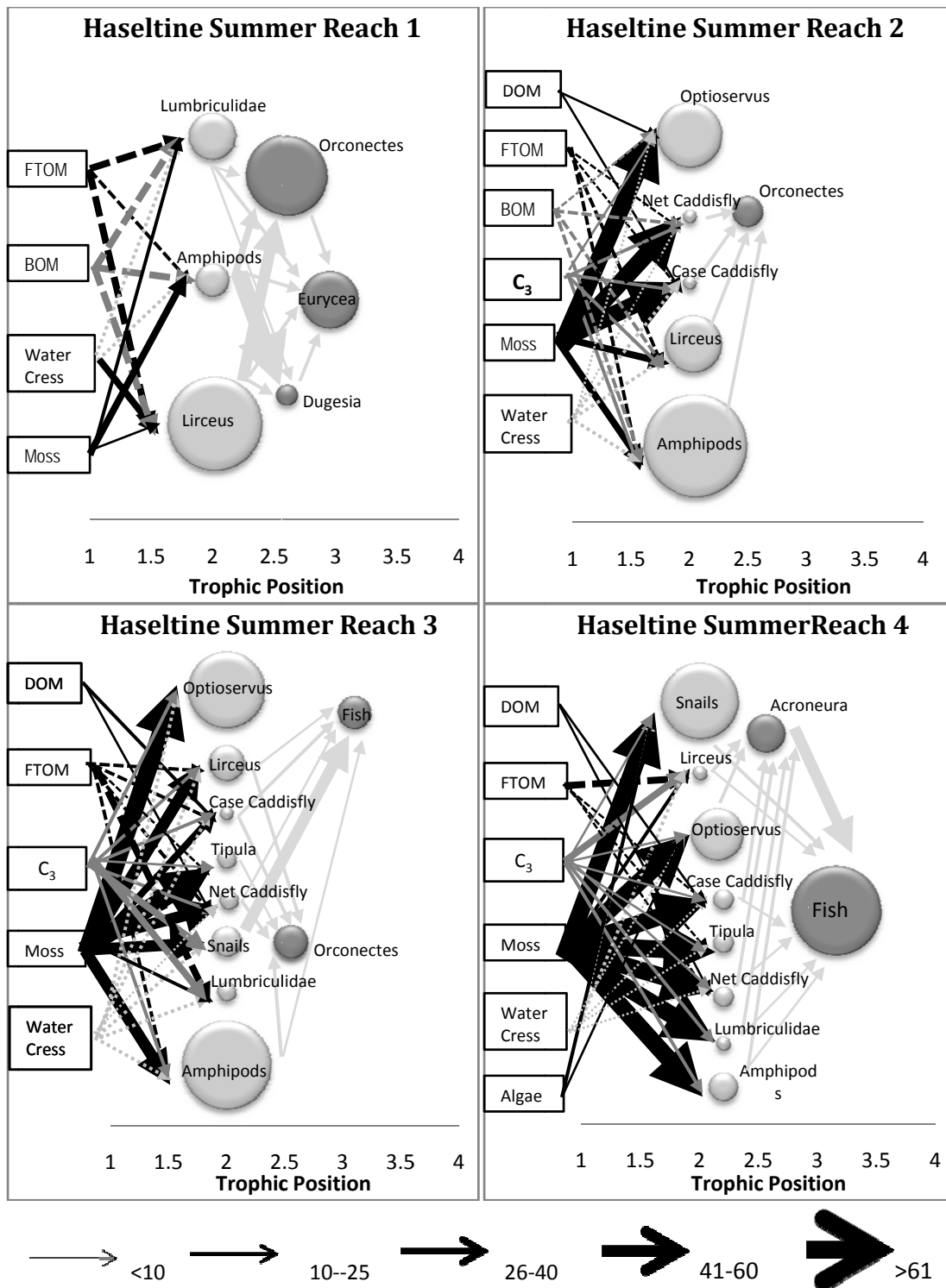


Figure 12a Quantified food webs for Haseltine Spring using mean dietary proportions that a lower trophic level contributes to the next higher level (arrow width). Bubble size represents the proportion of biomass contributed by each species. Species arranged by trophic position. See Table 1 for basal source abbreviations.

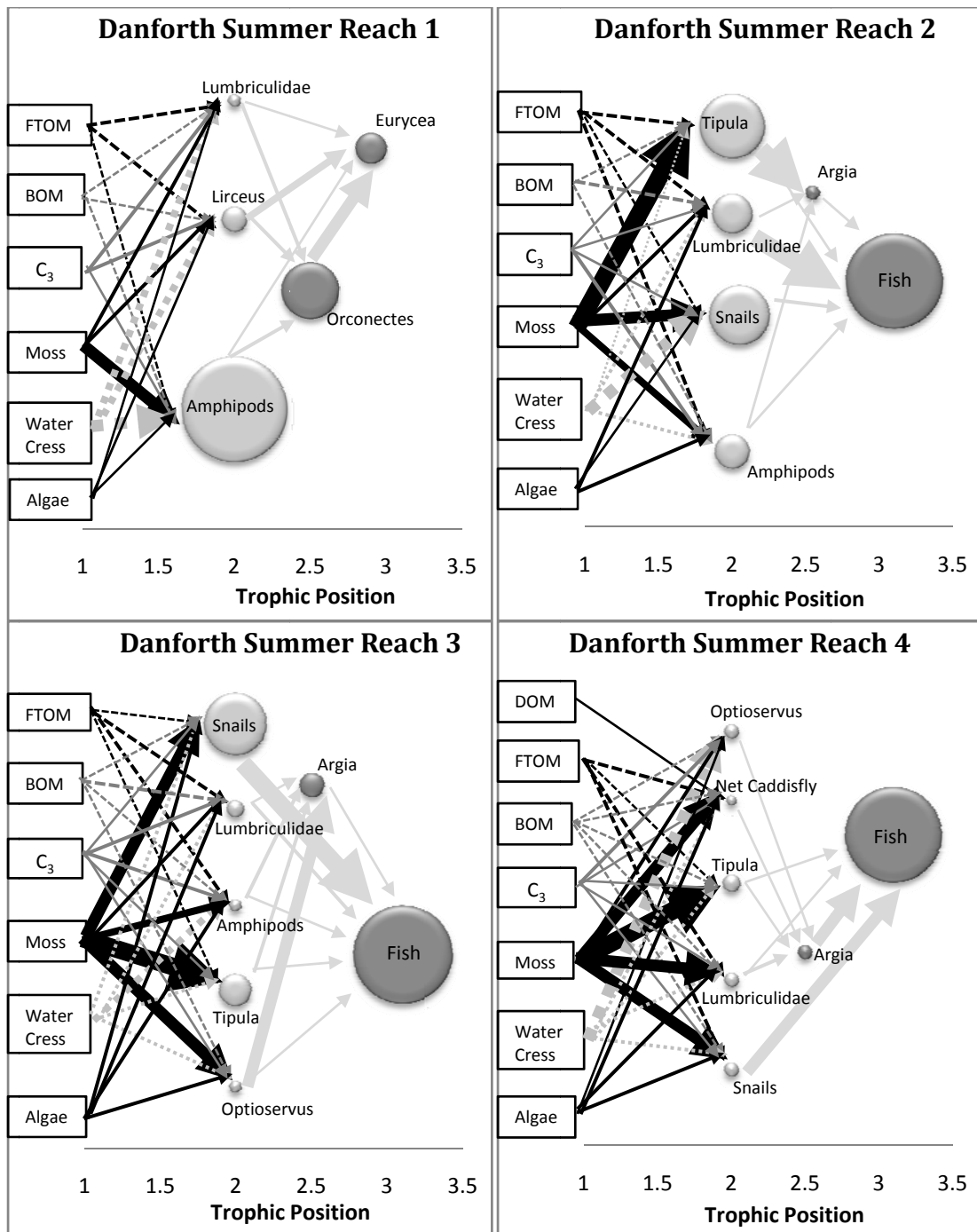


Figure 13a Quantified food webs for Danforth Spring using mean dietary proportions that a lower trophic level contributes to the next higher level (arrow width). Bubble size represents the proportion of biomass contributed by each species. Species arranged by trophic position. See Table 1 for basal source abbreviations.

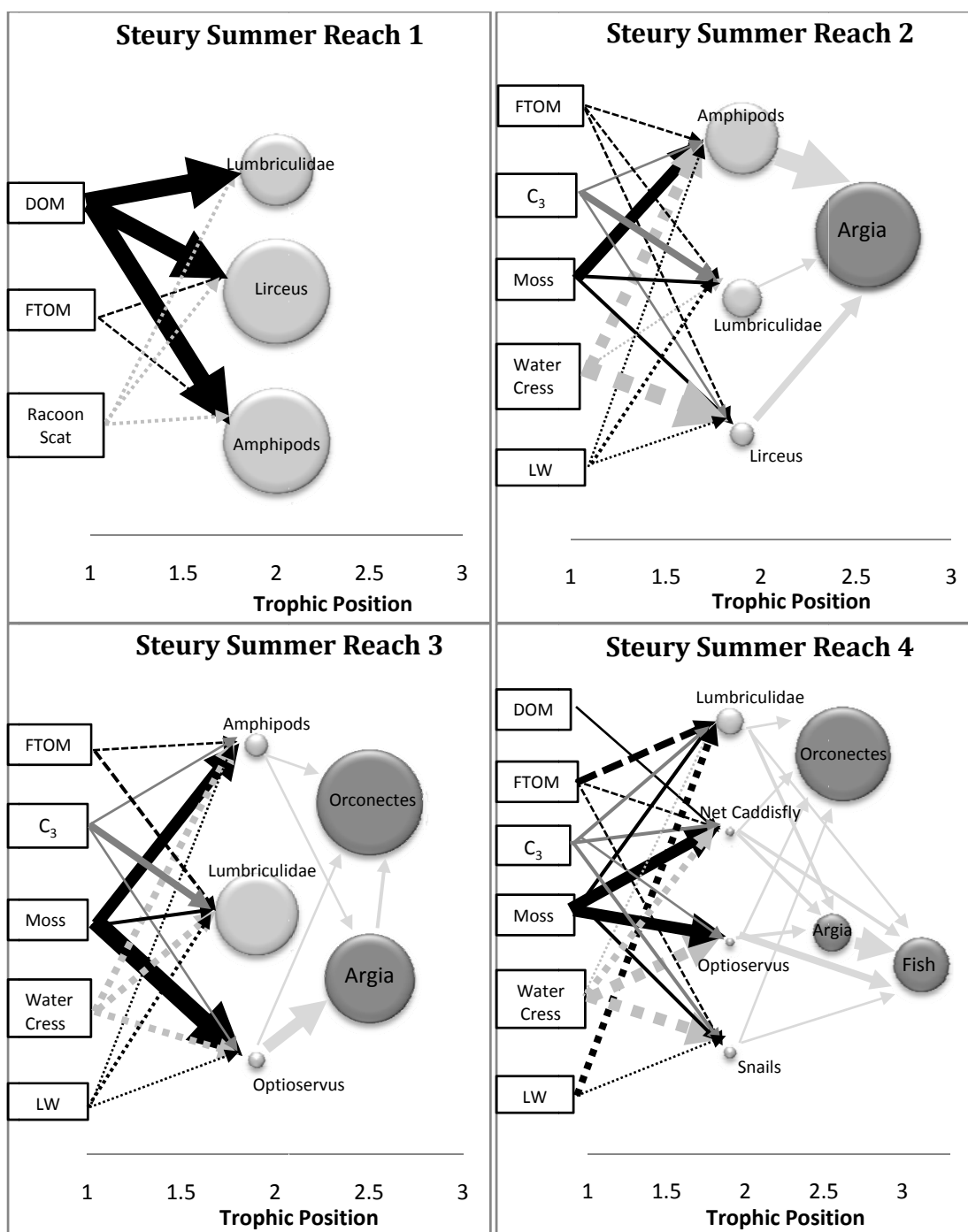


Figure 14a Quantified food webs for Steury Spring using mean dietary proportions that a lower trophic level contributes to the next higher level (arrow width). Bubble size represents the proportion of biomass contributed by each species. Species arranged by trophic position. See Table 1 for basal source abbreviations.

—→ <10 —→ 10–25 —→ 26–40 —→ 41–60 —→ >61

and BOM also represented autochthonous food sources. DOM, not an important food source in any other spring, constituted the major proportion of the diet (first percentile >78%) for herbivores in the Steury Spring cave. This was likely because food options were few, or because amphipods and isopods, representing the majority of herbivore biomass, were juveniles. BOM was too enriched in Steury and parts of Haseltine (reach 3 and 4) to be an important carbon source for consumers, but was substantially important to consumers in Haseltine reach 1 and 2, and moderately supported herbivore diets in Danforth. FTOM was of allochthonous origin (Table 9) in Steury and was only important in the diet of worms. FTOM in Haseltine and Danforth represented an autochthonous food source, based on C: N ratios (Table 9), and represented a moderately important component of the assimilated herbivore diets. Benthic algae was too enriched among all reaches in Steury, and in all but reach 4 in Haseltine to be considered a reasonable food source. Algae were not nearly as C-enriched in Danforth, and contributed substantial support to the diet of snails, worms, riffle beetles, and young amphipods. Allochthonous C₃ plant litter was generally as valuable to herbivores as FTOM or BOM, but all were second to water cress and moss in dietary importance.

Of particular interest was the observed spatial relationship between variability in the abundance and type of potential food resources, the number of herbivore species, and food web complexity. In each spring, the number and type of potential food resources increased along the spring source-springbrook gradient. Several food sources were not available at the cave source, or were often too enriched (BOM, algae) in other reaches for consumer use. But as food sources became available, consumer-food source links were established. Additionally, along this longitudinal gradient, the number of species increased. Consequently, the number of links (arrows) between food resources and consumers increased between reach 1 and 4, and food web complexity increased; as graphically displayed in the quantified food web models (Figs. 12-14).

Adult and ontogenetic omnivory was common in each spring. While higher level consumers such as planarians, salamanders, and crayfish fed on a variety of available prey (amphipods, isopods, and worms), their feeding patterns also responded to the spatial variability of basal food resources. Crayfish and damselflies relied on FTOM to a greater extent in lower springbrook reaches (Table 11) as FTOM is mostly generated *in situ*, and therefore increases along the spring source-springbrook gradient (Glazier 1991). Crayfish also relied on more basal resources in lower reaches as the number and type of potential food resources increased along this gradient. Fishes relied more on snails in Danforth, and in lower reaches of Haseltine where snail abundance was greatest; possibly associated with the nutritious nature of algae in these sites (C:N ratios; Table 9). Spatial shifts in higher level consumer feeding patterns also increased the number of consumer-resource feeding links.

Table 11 Extended diet of invertivores; mean proportion (%) of contribution of organic matter sources to the assimilated diet of secondary consumers in Haseltine Spring.

		SUMMER					WINTER		
		FTOM	BOM	WC	MOSS	C3	FTOM	BOM	MOSS
HASELTINE									
<i>Orconectes sp.</i>	<i>R1</i>	4							
	<i>R2</i>		3	4					
	<i>R3</i>	29	10	9	13	12			
	<i>R4</i>				8				
DANFORTH									
<i>Orconectes sp.</i>	<i>R1</i>	16		20		18			
	<i>R2</i>	1							
	<i>R3</i>	15							
	<i>R4</i>	78					11		
STEURY									
<i>Orconectes sp.</i>	<i>R3</i>	36		15		18	1	6	2
	<i>R4</i>	41		16	3	15	1	27	4
<i>Argia sedula</i>	<i>R3</i>	55					1		
	<i>R4</i>	60					19		
Tanypodinae	<i>R2</i>						12		
	<i>R3</i>						6		
	<i>R4</i>						10		

Winter and summer food webs were similar in that (i) autochthonous food sources (moss and water cress) still constituted a major proportion of the herbivore diet; (ii) algae were too enriched in Haseltine and Steury to be an important food

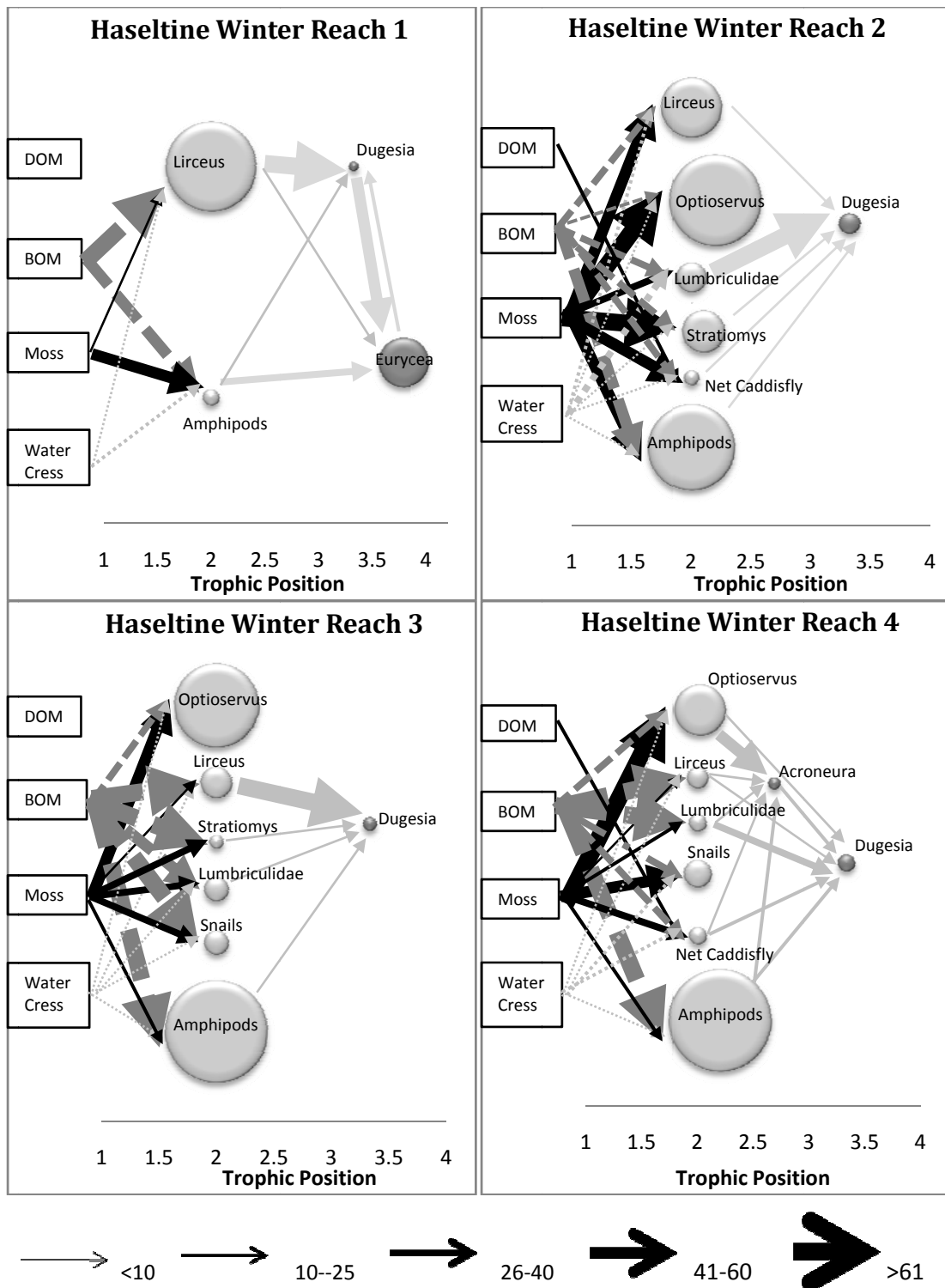


Figure 12b Quantified food webs for Haseltine Spring using mean dietary proportions that a lower trophic level contributes to the next higher level (arrow width). Bubble size represents the proportion of biomass contributed by each species. Species arranged by trophic position. See Table 1 for basal source abbreviations.

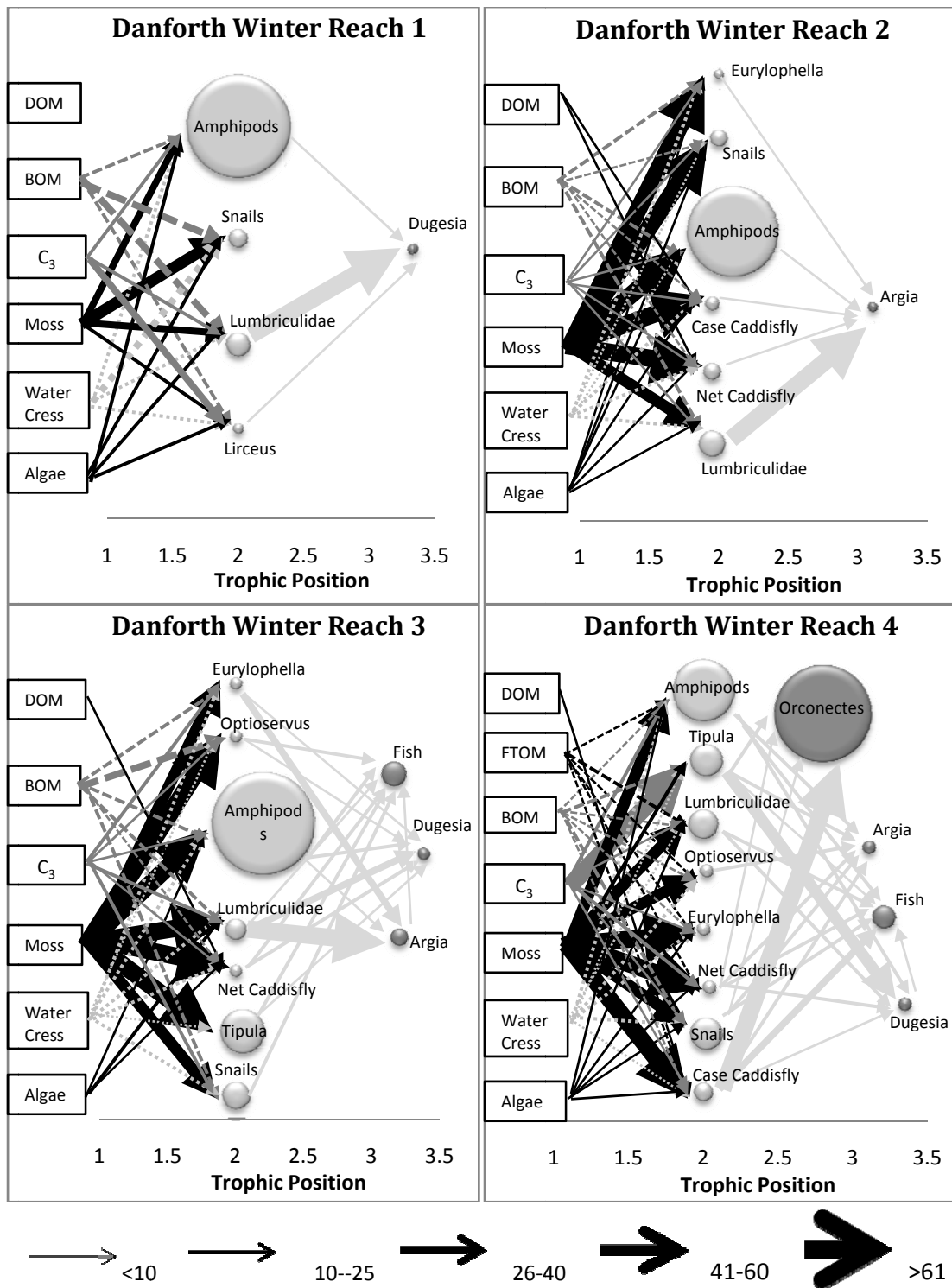


Figure 13b Quantified food webs for Danforth Spring using mean dietary proportions that a lower trophic level contributes to the next higher level (arrow width). Bubble size represents the proportion of biomass contributed by each species. Species arranged by trophic position. See Table 1 for basal source abbreviations.

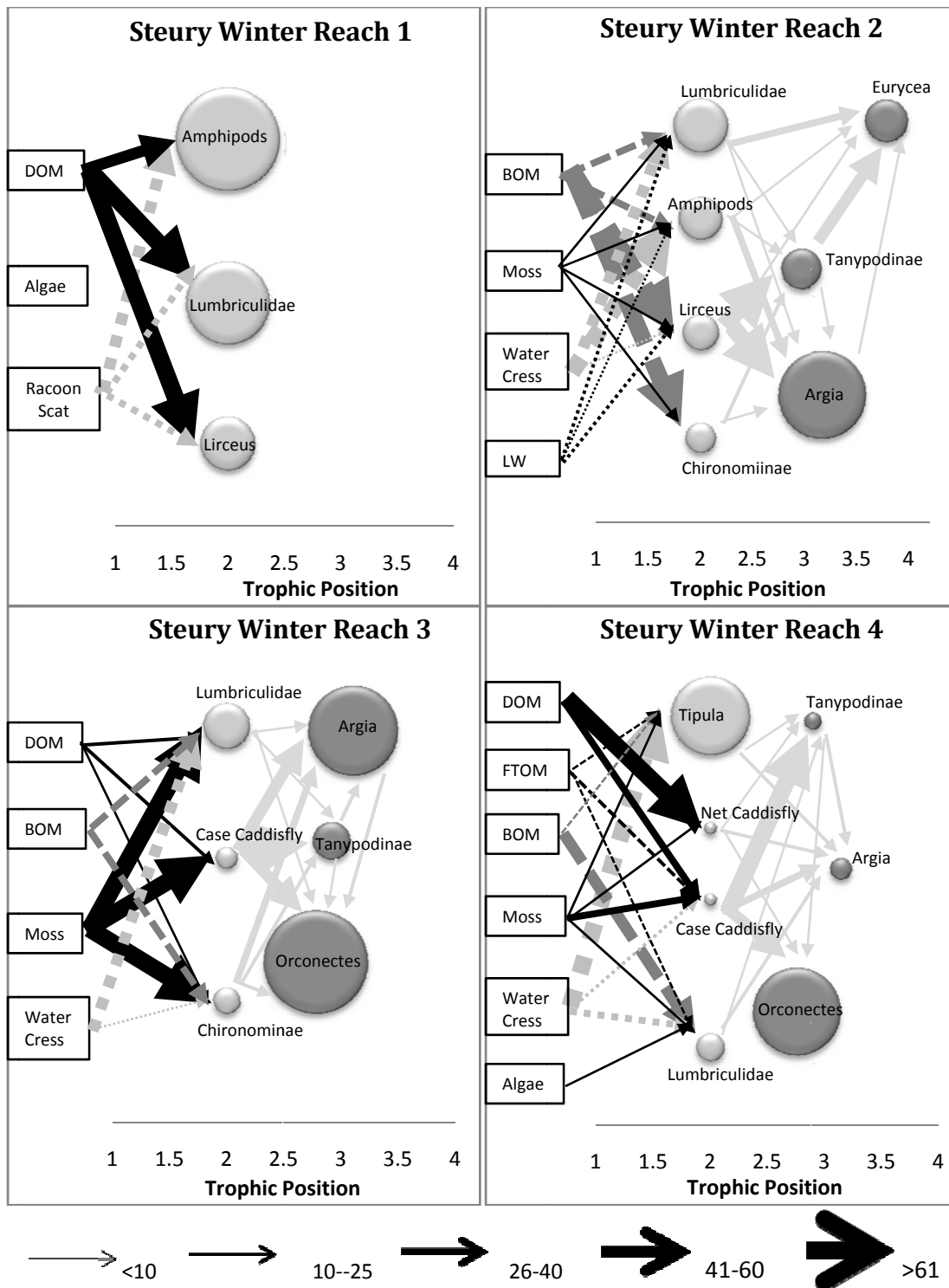


Figure 14b Quantified food webs for Steury Spring using mean dietary proportions that a lower trophic level contributes to the next higher level (arrow width). Bubble size represents the proportion of biomass contributed by each species. Species arranged by trophic position. See Table 1 for basal source abbreviations.

source, but were used by all herbivores in Danforth; and (iii) DOM was an important food source to all herbivores in the Steury cave, and to net and case-building caddisflies in each spring (Figs. 12-14b). However, temporal variability in resource use was evident in winter food webs, as the food base and diet of herbivores shifted to a noticeably greater dependence on allochthonous BOM. In Haseltine BOM was the dominant food source (first percentile >50%) for 7 of 7 herbivores. In Steury, BOM was definitively the dominant food source for chironomid midges (97-100%), isopods (61-93%), and case- and net-spinning caddisflies (38-70% and 53-90%, respectively).

Overall, there was a 13.5, 31.5, and 49% increase in the proportion of the diet supplied by allochthonous food resources in Danforth, Steury, and Haseltine, respectively, during the winter than that recorded for summer (Fig. 15). The temporal shift in the dominance of allochthonous food web pathways, associated with the addition of allochthonous food resources, was accompanied by an increase in the number of consumer-resource links and food web complexity, and is illustrated in seasonal comparisons of same-spring food web models (Figs. 12-14).

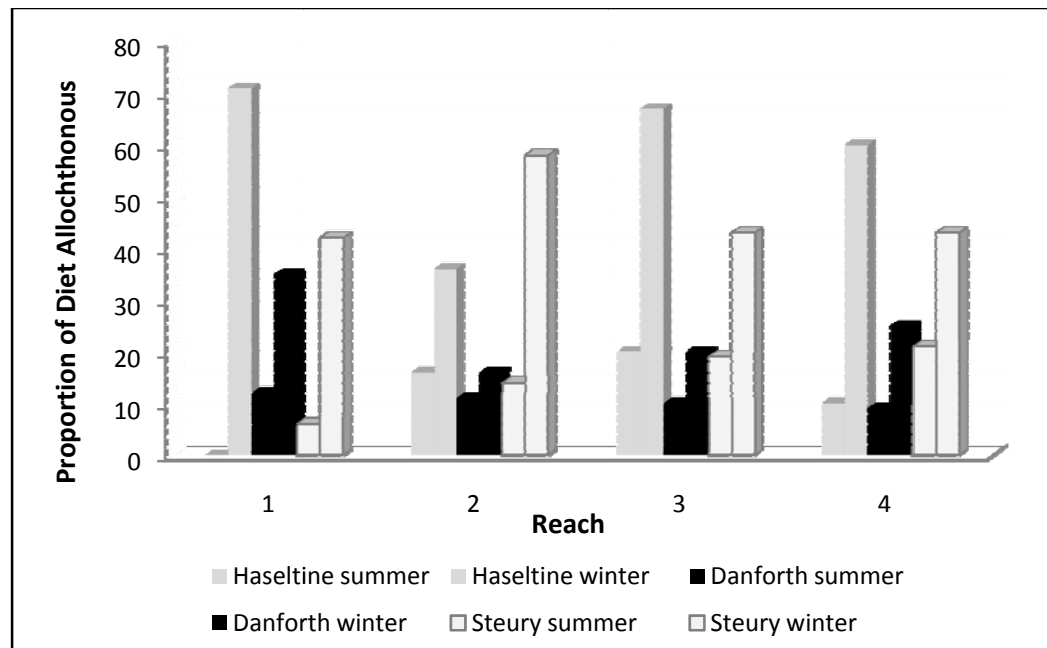


Figure 15 Comparative analysis of seasonal differences in the proportion of diet represented by allochthonous food sources in each of the three springs.

Winter invertivores also took advantage of the seasonal variability of available food resources. Haseltine planarian diets shifted from amphipods (summer) to more abundant isopods, except in reach 4 where the winter downstream migration of amphipods was associated with increases in water cress density, as cited in Chapter 2. Haseltine salamanders took advantage of winter increases in planarians. In Danforth crayfish took advantage of winter increases in species diversity and fed on a larger variety of herbivore prey and less on basal resources than during the summer. Danforth damselflies, consistently dependent on FTOM during the summer, shifted their dependence away from FTOM during the winter except in reach 4 as it was too enriched to be a possible food source in reach 1-3. Winter damselflies and crayfish in Steury made the same dietary shifts related to seasonal availability of FTOM. Tanypodinae, absent in Steury summer samples, represented an important winter food source for salamanders and damselflies. Temporal shifts in the feeding patterns of higher level consumers were also accompanied by an increase in the number of consumer-resource links.

Food web statistics

Food web metrics supported conclusions drawn from the quantified food web models. For these small spring communities, each food web had high connectance and connectivity values, and moderately long food chains. While web-size (S) increased spatially along the gradient from the spring source (reach 1) to reach 4 in each of the three springs (Table 12), maximum food chain length (4) remained consistent across springs and seasons. Connectance remained high among reaches, springs and seasons, but variability was low with values ranging from only 0.17 to 0.26. Connectance was noticeably higher (Table 12) than reported for other lotic food web studies for stream communities with comparatively more species (cf. Warren 1989; Travers-Cromar and Williams 1996; Woodward *et al.* 2005). Connectance values generally increased along the spring source-springbrook gradient as web size increased.

Significant variability in the number of feeding links (L) was recorded in response to differences in reach (F-ratio 6.58; $p < 0.01$) and spring (F-ratio 5.01; $p < 0.03$). The number of links increased by as much as 11-36 links, along the gradient between reach 1 and 4. Variation in the number of links among springs in descending order was Danforth, Haseltine, and Steury. Temporal variation in the number of links, while not found to be statistically significant, was consistently greater in Danforth and Steury during the winter (Table 12). Danforth and Steury had 63 and 21 more feeding links respectively in winter than in summer. Haseltine Spring was the exception to this pattern, consistent with its lack of canopy, and therefore potential for sizable leaf litter contributions.

Food web linkage complexity (SC) was affected by reach (F-ratio 6.88; $p < 0.01$) and spring (F-ratio 3.72; $p < 0.05$). Once again, complexity steadily increased along the gradient between reach 1 and 4 (Table 12) and was greatest in descending order in Danforth, Haseltine, and then Steury. Season did not have a significant

Table 12 Food web statistics as calculated for Haseltine, Danforth and Steury Spring food webs for both summer and winter seasons.

	SUMMER				WINTER			
	REACH 1	REACH 2	REACH 3	REACH 4	REACH 1	REACH 2	REACH 3	REACH 4
HASELTINE								
Number of links (L)	28	33	45	47	12	25	23	30
Web size (S)	11	12	15	16	8	11	11	12
Directed Connectance (C_d)	0.23	0.23	0.20	0.18	0.19	0.21	0.19	0.21
Linkage Complexity (SC)	5.60	6.00	6.43	6.27	3.43	5.00	4.60	5.45
Linkage Density (L/S)	2.55	2.75	3.00	2.94	1.50	2.27	2.09	2.50
DANFORTH								
Number of links (L)	28	32	39	39	22	37	53	74
Web size (S)	12	13	14	14	11	13	16	19
Directed Connectance (C_d)	0.19	0.19	0.20	0.20	0.18	0.22	0.21	0.20
Linkage Complexity (SC)	5.09	5.33	6.00	6.00	4.40	6.17	7.07	8.22
Linkage Density (L/S)	2.33	2.46	2.79	2.79	2.00	2.85	3.31	3.89
STEURY								
Number of links (L)	9	22	22	36	6	31	26	39
Web size (S)	7	10	11	14	6	12	10	13
Directed Connectance (C_d)	0.18	0.22	0.18	0.18	0.17	0.22	0.26	0.23
Linkage Complexity (SC)	3.00	4.89	4.40	5.54	2.40	5.64	5.78	6.50
Linkage Density (L/S)	1.29	2.20	2.00	2.57	1.00	2.58	2.60	3.00

effect on complexity. It was, however, slightly higher in winter (mean; Danforth 6.5, Steury 5.1) than in summer (mean; Danforth 5.5, Steury 4.4). Haseltine again was the exception to this pattern.

Linkage density (L/S) ranged from 1.50 to 3.00 in Haseltine, 2.00 to 3.89 in Danforth, and 1.00 to 3.00 in Steury. Significant variability in linkage density was recorded in response to reach (F ratio 7.25; $p < 0.01$), and spring (F ratio 4.14; $p < 0.04$), increasing again along the gradient between reach 1 and 4 and greater in Danforth, followed by Haseltine, and Steury. There was not a statistically significant effect of season on linkage density, but the number of links per species was, on average, greater during the winter than in summer. The exception again was in Haseltine likely due to its lack of leaf litter input.

Discussion

Karst springs are typically characterized as headwater systems, and as such, are considered strongly heterotrophic. Food webs in heterotrophic systems are either primarily supported by allochthonous organic inputs (e.g. Fisher and Likens 1973; Cummins 1974; Hynes 1983; Webster, *et al.* 1997; Vannote *et al.* 1980; Robinson *et al.* 2008; but see Zah 2001 for an exception), or require at least some allochthonous inputs. In contrast to the literature, and my original predictions, however, the results of this study revealed that these spring food webs are fueled primarily by autochthonous food sources, and therefore do not follow the conventional model for small headwater streams. Results also indicated that the spring food webs are dynamic in space and time despite the perceived constancy of environmental conditions in springs. As the relative importance of autochthonous feeding pathways and species richness (Chapter 2) increased spatially along the spring source-springbrook gradient, food webs became more complex. Additionally, the relative dependence on allochthonous food sources increased temporally, resulting in even greater food web complexity along this gradient during winter sampling periods.

Relative importance of basal food resources

For several reasons I had expected that springbrook consumers would rely more heavily on allochthonous materials throughout the year, especially in the two covered springs. First, the dominant functional feeding group, in terms of both abundance and biomass in each spring, was shredders (amphipods and isopods); these are known to rely primarily on allochthonous detritus for energy (Merritt and Cummins 2008). Second, the largest biomass of available food in forested headwater systems comes from allochthonous leaf litter (Vannote *et al.* 1980; Minshall 1983). Third, allochthonous matter often dominates the composition of FTOM (Rounick *et al.* 1982; Winterbourn *et al.* 1984; Lester *et al.* 1995). Despite these well-supported scientific observations, my results indicated that spring herbivores relied on autochthonous food resources, primarily moss and water cress.

The dependence by herbivores on these autochthonous food sources was surprising not only because these systems are considered heterotrophic, but because herbivory on these macrophytes is widely considered minimal due to their relatively high indigestible fiber (cellulose) and low nitrogen content (Gregory 1983; Mann 1988). Other studies, however, have reported macrophytes such as moss and liverwort to be important sources of carbon for aquatic consumers (Jones 1949; Minshall 1968; Stern and Stern 1969; Webster and Benfield 1986; Winterbourn *et al.* 1986; Mann 1988; Rosenfeld and Roff 1992; Robinson *et al.* 2008). In contrast, one would have expected periphyton, which was enriched in all springbrooks except Danforth, to be the more desirable and more nutritious autochthonous food choice because of its lower C:N ratios (Lau *et al.* 2008). However, when periphyton ratios are as high as those in Haseltine and Steury Springs (17.5-36.97), such algae may not represent the more nutritious food choice, and this may explain why herbivores were dependent on these unexpected macrophytic food sources. A second possible mechanism explaining the observed dependence on aquatic macrophytes is that peracaridans generally have higher cellulase activities, and therefore greater efficiencies in assimilating plants such as water cress and moss (Kesler 1983; Chamier 1991; Glazier 1991).

Additionally, herbivores could have been relying on detrital forms of these plants that had been processed by bacteria and fungi. These microbes are known to progressively reduce the cellulose content, while also increasing the nitrogen content of vascular macrophytes, making them more nutritious food options (Fenchel and Jorgensen 1977; Mann 1988).

The importance of autochthonous basal resources was not limited to primary consumers (herbivores). The strongest association between secondary consumers and basal resources was through autochthonous-based FTOM. This was not surprising for a number of reasons: (i) the FTOM fraction is the most strongly involved in energy flow through aquatic food webs (Hamilton and Lewis 1992); (ii) shredders (which were dominant in the three springs) have a vital role in making FTOM available to other consumers (Webster 1983; Lester *et al.* 1995) noted that a vital role for shredders (dominant in the three springs) is making FTOM available to consumers; and (iii) life history omnivory often includes the assimilation of FTOM (Hobbs 2001; Westfall and Tennessen 2008).

Dynamic food webs and dominant food web pathways

Mechanisms responsible for the dynamic nature of the food webs included: (i) the spatial and temporal variability in the composition, type, and/or availability of basal food resources; and (ii) the spatial and temporal patterns of community complexity, functional diversity, and biomass, as noted in Chapter 2. Autochthonous carbon sources were isotopically distinct (C-depleted) from allochthonous and were quite variable spatially due to spatial variability in dissolved inorganic carbon (DIC) pools from which the autotrophs obtain carbon. The primary carbon pools available to autotrophs in these spring waters are derived from dissolved atmospheric CO₂, dissolution of carbonate rocks (HCO₃⁻), and respired CO₂ from subterranean heterotrophic activity; with predicted $\delta^{13}\text{C}$ values of -7‰, +1‰, and -27‰, respectively (O'Leary 1981; Osmond *et al.* 1981; Finlay *et al.* 1999; Finlay 2001, 2004; Kendall 2001). Moss and water cress were likely C-depleted (-33.32 to -

29.12‰ and 45.33 to -38.55‰, respectively) as a result of use of the C-depleted biogenic (respiration) carbon source. Benthic algal signatures, however, were substantially enriched in Haseltine and Steury, a pattern noted in other studies (e.g. Hicks 1999; Bunn 2003). Because the major source of the water in these springs is from an underground karst system, heavily influenced by calcium carbonate, the carbon signature of the algae was likely influenced by the HCO_3^- pool. The fact that algae were most enriched in Haseltine is understandable as groundwater residence time is 2-4 times greater (see estimates based on dye tracing data; Chapter 2) in Haseltine than in Danforth or Steury. Additionally, the low flow velocity in these springs could have affected the carbon fixation of the algae, creating a thicker, stagnant boundary layer that typically results in more positive $\delta^{13}\text{C}$ values (France 1995; Finlay *et al.* 1999; Finlay 2004).

With distance from the spring source, groundwater influences on DIC pools decreased, and atmospheric and photosynthetic influences increased, resulting in spatial variability in not only food source signatures but also food source importance (as in the case of benthic algae). In addition, food source options were often limited at the spring source. The availability and type of food source increased between reach 1 and 4, as did species richness and functional diversity of the community (Chapter 2). Consequently, trophic interactions became more complex along this gradient, as evidenced by the increased intricacy of the quantified food web models. Examples of longitudinal changes in food web dynamics often include shifts in the dominant resource base or feeding guilds, and have been described in other freshwater systems (Hefti and Timka 1991; Voelz and Ward 1996; Webster and Meyer 1997; Rosi-Marshall and Wallace 2002).

Observed temporal variation in food source signatures was associated with the seasonal input of relatively enriched allochthonous leaf litter. Summer C:N values for FTOM and BOM fell within the range of autochthonous food sources indicating, for example, that instream autochthonous production (instream macrophytes and algal sloughing from benthic mineral materials and aquatic mosses) was the source of

FTOM for invertebrate consumers during the summer. Winter C:N values for these food sources were higher, similar to that of C₃ terrestrial plants, as sources of FTOM and BOM shifted to particulates from leaf litter input.

Seasonal change in basal resource composition (C-enrichment) was accompanied by corresponding changes in consumer C-signatures. These data suggest (as predicted) that the diet of herbivores in these three springs shifted temporally towards a greater reliance on allochthonous resources. Hence, the relative importance of the food base (autochthonous vs. allochthonous) shifts throughout the year based on the relative availability of carbon sources, and illustrates that these systems rely heavily on temporal pulses of nutrients and organic matter from the terrestrial environment. Accordingly, temporal shifts in basal resource utilization, along with increases in biocomplexity and functional diversity (Chapter 2), resulted in temporal increases in the trophic complexity of winter food webs. This finding is consistent with the work of several investigators who have found that allochthonous inputs often influence the trophic dynamics of the receiving system (Polis *et al.* 1997; Polis and Hurd 2001; Zhang *et al.* 2003)

The river continuum concept (RCC) is a useful conceptual model for predicting variability in food availability and biota along longitudinal gradients in lotic systems (Vannote *et al.* 1980). The results of this study, in part, are consistent with the RCC framework. First, community structure and function shifted in predictable patterns that correlated with environmental gradients along the spring source-springbrook gradient, as would be predicted by the RCC. Second, the relative importance of autochthonous resources (benthic algae) increased along the continuum. Third, temporal shifts in the relative importance of the food base (autochthonous to allochthonous) were related to variation in resource availability. In contrast to RCC predictions, however, the food webs in these first order systems are fueled primarily by autochthonous carbon sources, and therefore do not appear to be heterotrophic. In addition, the shift from a dominant consumption of allochthonous materials upstream to autochthonous food sources downstream did not occur. If shifts

in the composition of macroinvertebrate communities illustrate changes in resource use along the river continua, as stated by the RCC, then the food resources consumed should be reflective of the FFG composition. This pattern is not supported by my data, as autochthonous foods were consistently the dominant food source supporting metazoan biomass, along both temporal and spatial scales in each of the three springs, even though the dominant FFG was shredders. Rosi-Marshall and Wallace (2002) and Zah *et al.* (2001) noted similar findings when they determined that taxa classified as shredders were, in fact, consuming algae and autochthonous detritus rather than allochthonous leaf litter.

My data, instead, are more consistent with tenet #11 of the Riverine Ecosystem Synthesis model (RES; Thorp and Delong 2008) which highlights the importance of in-stream primary production (algae and other aquatic plants) and argues, as have others (Minshall 1978; Winterbourn *et al.* 1986; Zah *et al.* 2001; Bunn *et al.* 2003), that the importance of autotrophy in lotic ecosystems is often underestimated. Several investigators have reported that diverse and productive lotic communities are maintained through autochthonous food web pathways such as communities in arctic springs, arid-zone floodplain rivers, and glacier, desert, and alpine streams (Minshall, 1978; Ward, 1994; Jones *et al.* 1997; McKnight and Tate 1997; Jaarsma *et al.* 1998; Zah 2001; Bunn *et al.* 2003; Parker and Huryn 2006; Lau *et al.* 2009).

Food web complexity

Even in the relatively simple communities represented in this study, substantial variation in food web structure and complexity occurred. Species were highly interconnected. Connectance was noticeably higher (Table 5) than reported for other lotic food web studies of stream communities with considerably more species (cf. Warren 1989; Travers-Cromar and Williams 1996; Woodward *et al.* 2005). Connectance values were spatially quite stable along the spring source-springbrook gradient, but were generally higher in reach 4 as web size increased (number of food

sources and invertebrate species). The number of links per species (L), food web linkage complexity (SC), and linkage density (L/S) also increased along both temporal and spatial scales and were associated with increases in the number of species. Moreover, adult and ontogenetic omnivory was common (cf. Polis and Strong 1996).

According to early models, the patterns of connectance, complexity, linkage density, and omnivory observed in this study, would rarely be observed in natural systems and, if present, would reduce food web stability (Cohen 1978; Pimm 1982; Briand 1983; Cohen *et al.* 1986). However, more recent food web analyses do not conform to these patterns, and have, therefore, cast doubt on the validity of these models (Warren 1989; Martinez 1991; Polis 1991, 1994; Hall and Raffaelli 1993; Polis and Strong 1996; Jaarsma 1998; Winemiller 1998). Analyses based on more complete, data-rich food webs show that (i) most webs are highly interconnected and complex; (ii) connectivity and complexity increase with species richness; (iii) the ratio of prey to predator is >1 ; and (iv) omnivory is extremely common. Additionally, newer conceptual models suggest that food web dynamics are mainly controlled by resource availability (Polis and Strong 1996).

The results of this study are consistent with patterns presented in newer food web models. Spatial and temporal stability in spring food web connectance reflected corresponding increases in species richness and the amount, type, and availability of food resources along longitudinal and seasonal gradients in each spring. Increases in the number of links per species were especially associated with input of allochthonous leaf litter. High connectance, along with spatial and temporal increases in the number of links per species, indicates a community of more generalist consumers (Jaarsma *et al.* 1998). In contrast, low connectance values have been associated with specialist feeding patterns (Tavares-Cromar and Williams 1996). Huxel and McCann (1998) noted similar increases in the number of links per species with the input of allochthonous leaf litter, and found that this relationship served to stabilize food web dynamics when species preferentially feed on autochthonous food

sources. A number of investigators have indicated that the ability of organisms to switch diet in order to exploit changing resources both increases food web complexity and connectivity and promotes stability (Polis 1994; Polis and Strong 1996; Tavares-Cromar and Williams 1996).

Food web complexity, the number of feeding links, and linkage density were not independent of web size, nor were they independent of the relative contribution of autochthonous or allochthonous food sources. These observations suggest that experimental alteration in relative food availability (e.g., by altering autochthonous production through changes in incident PAR) would impact food web complexity. Such an experiment is discussed in Chapter 4.

Theoretical implications

A full comprehension of food web ecology must include an understanding of interactions among elements of the riverine landscape—that is, landscape ecology. Landscape ecology is concerned with spatial heterogeneity and functional interactions among a mosaic of habitat patches. Cross-habitat (patch) fluxes of nutrients and organic matter vary with both time and the level of connectance among the three spatial dimensions (vertical, longitudinal, and lateral) defined for lotic systems by Ward *et al.* (1989). As all food webs are influenced by the surrounding landscape, food web structure and function in one patch are often affected by adjacent patches (Polis and Hurd 2001). Hence, a focal community (patch) is embedded in a much larger landscape (patch), where the spatial heterogeneity of the landscape influences the community through allochthonous pulses or spatial subsidies of nutrients, organisms, and organic matter that can be major determinants of food web dynamics (Polis 1997; Polis *et al.* 2001; Woodward and Hildrew 2002; Sears *et al.* 2004; Vanni *et al.* 2004). Springs, for example, represent a focal community where functional interactions include those between groundwater, surface water, and terrestrial habitat patches.

As an example, isotopic signatures of aquatic plants such as benthic algae and water cress varied substantially along the spring source-springbrook gradient. Spatial variability in the signatures of these food sources likely reflect the patchy nature of DIC subsidies affected by subterranean, surface, and terrestrial landscape elements. Likewise, herbivore signatures were spatially variable even when they relied on the same food source, indicating their assimilation of these variable sources of carbon. However, further research is needed to discern the specific mechanisms responsible for such marked variation in food source composition. Temporal variation in food source signatures (enrichment) and consumers was recorded in association with temporal pulses of relatively C-enriched allochthonous leaf litter. In addition, food availability and type increased along spatial and temporal scales. Variability in the $\delta^{13}\text{C}$ value of autotrophs and their consumers in response to variability in inorganic carbon sources (DIC) or resource availability has been widely noted (Osmond *et al.* 1981; Rounick and Winterbourn 1986; Finlay 2001; Zah 2001; Bunn *et al.* 2003; but see Hicks 1997 for an exception).

Often patch-to-patch movement of organisms themselves serve as a spatial subsidy or pulsed resource, as seen in the downstream migration of stygophilic amphipods and their predators during the winter sampling period of my study. Several studies have noted that riparian predators such as spiders often serve as a spatially subsidized resource for aquatic insects (Paetzhold *et al.* 2005; Marczak and Richardson 2007; Robinson *et al.* 2008). Others have observed reciprocal energy subsidies across habitat patches (Nakano and Murakami 2001; Sabo and Power 2002; Baxter *et al.* 2005).

Evidence in my study for the interplay of temporal pulses and spatial subsidies of autochthonous and allochthonous food sources among elements of the riverine landscape come from the spatial and temporal variability seen in spring food web dynamics and food web complexity. First, the input of allochthonous material (leaf litter) produced temporal increases in food web complexity which emphasized the importance of exchanges across the lateral boundary between terrestrial and aquatic

systems; it also illustrated how the architecture of landscape boundaries (type of canopy, density of canopy) can limit the amount and type of litter entering the adjacent patch (Cadenaasso *et al.* 2004). Second, cross-habitat fluxes of organisms themselves served as a pulsed resource that increased the complexity of springbrook food webs. Third, the longitudinal shifts in food source composition, associated with spatial differences in subterranean to surface system exchanges and spatial availability of food sources, resulted in increased food web complexity. Longitudinal and vertical interactions, as well as terrestrialization of riverine food webs have been associated with shifts in food web dynamics in a variety of lotic settings (Lester *et al.* 1995; Hicks 1997; Ostfeld and Keesing 2000; Zah *et al.* 2001; Woodward *et al.* 2005; Lau *et al.* 2009). For example, Thompson and Townsend (1999) found that spatial and temporal variation in food web complexity in grassland stream food webs in New Zealand was positively correlated with variability in food supply.

These data suggest that springs show a high degree of functional complexity, as seen in other lotic systems and, therefore, provide relevant insight to the integration of themes related to riverine landscape ecology, food web ecology, and newly emerging theories on the interplay of temporal variation and spatial subsidies as major determinants of food web dynamics. The contribution of studies in spring ecosystems to the overall theory of food web dynamics is, unfortunately, still limited by the paucity of descriptive and experimental studies of these fascinating but fragile spring ecosystems.

CHAPTER 4

EFFECTS OF MANIPULATION OF BASAL RESOURCES ON FOOD WEB PATHWAYS IN A KARST CAVE SPRING ECOSYSTEM

Introduction

Studies of ecosystem function and food web dynamics in lotic systems have focused on understanding pathways of energy transfer and consumer-food source linkages. The relative importance of allochthonous and autochthonous organic matter in defining these linkages is still debated. Forested headwater streams are typically portrayed as systems fueled by allochthonous carbon in the form of leaf litter (Minckley 1963, Cummins *et al.* 1966; Minshall 1967; Tilly 1968; Hildrew *et al.* 1985; Warren 1989; Martinez 1991), with autochthonous carbon making only minor contributions (Vannote *et al.* 1980; Pimm 1982). However, more recent studies have identified autochthonous carbon as the key basal resource supporting metazoan production in some lotic food webs (Rosenfeld and Roff, 1992; Thorp *et al.* 1998; Bunn *et al.* 1999; Zah *et al.* 2001; Thorp and Delong 2002; Bunn *et al.* 2003; Delong and Thorp 2006).

While conclusions about the relative value of autochthonous and allochthonous carbon to epigeal stream communities are sometimes conflicting, information about their relative contribution in spring ecosystems is scarce. Springs have unusual attributes which may help us better understand the relative contributions of allochthonous and autochthonous food sources to lotic ecosystems. Springs are typically characterized as heterotrophic systems fueled by hypogean allochthonous materials (Lindegaard 1995); and while autochthonous food sources are available, they are considered inadequate to support spring consumers (Hynes 1983). Other stream ecologists, however, have found that small headwater systems like springs are dominated by fauna (e.g. pericardia crustaceans and oligochaetes) that feed primarily on autotrophic producers such as moss, algae, and water cress (Jones 1949; Minshall 1967; Stern and Stern 1969; Winterbourn *et al.* 1986; Webster and Benfield 1986; Mann 1988; Rosenfeld and Roff 1992). While herbivory on macrophytes such as

water cress and moss is considered minimal for streams in general by many scientists (e.g., Gregory 1983), this is not a universal sentiment (Webster and Benfield 1986; Mann 1988; Robinson *et al.* 2008). For example, Winterbourn *et al.* (1986) discovered small headwater tributaries in southeastern England where bryophytes were a prominent source of carbon incorporated by benthic invertebrates, and Jones (1949) found that moss was abundant in the gut contents of several invertebrate species.

Light availability is the major abiotic factor affecting autotrophic production, and thus ecosystem processes in aquatic systems (Odum 1957; Quinn 1997; Mosisch *et al.* 2001; Huggins *et al.* 2004; Mokany *et al.* 2008). Light reaching the benthic surface of a stream is reduced from surface radiation by dissolved, fine, and coarse transported organic matter (DOM, FTOM, CTOM), which at high concentrations, can drastically reduce the transmission of light (Wetzel 2001). Because of the clarity of spring water, however, there is minimal diffraction and reduction of impinging light (Odum 1957). Light can also be restricted by the riparian canopy, limiting instream primary production (Fisher and Likens 1973; Hill 1996; Bunn *et al.* 1999). The extent of riparian shading can affect the food base and diet of primary invertebrate consumers, altering their dependence on autochthonous and allochthonous foods, and thus changing their isotopic signatures (Winterbourn *et al.* 1986; Lester *et al.* 1995; Hicks 1997; March and Pringle 2003). While many studies have examined the effect of light limitation on lotic freshwater ecosystems (e.g. Quinn *et al.* 1997) few if any have done so in spring ecosystems.

Studies of ecosystem function and food web dynamics in lotic systems have typically involved the manipulation of a single trophic level rather than manipulation of basal resources (Woodward *et al.* 2005). In contrast, my study featured an experimental manipulation of microalgal biomass through light limitation in an Ozarks spring, and examined the resulting food web impact using stoichiometric analyses and stable isotopes of carbon and nitrogen. These assimilation-based techniques are commonly used in food web studies because of their ability to reflect

consumer-resource interactions and trophic relationships (Rounick and Winterbourn 1986; Peterson and Fry 1987; Fry 2006). I examined whether light manipulation (shaded/unshaded) and consequential reduction in basal resource production altered the relative importance of autochthonous and allochthonous organic resources to spring invertebrate communities and food web complexity. Hypotheses guiding the study were: (i) manipulation of algal production (as a function of light limitation) will alter the relative importance of the trophic food web base; and (ii) food web linkage complexity and linkage density will vary as a result of manipulation of the basal resource. In association with the first hypothesis, I predicted that there would be a shift toward a greater dependence on allochthonous-based food web pathways in shaded sections. For the second hypothesis, I predicted that there would be an increase in the importance of feeding generalists in shaded sections compared to open sections of the spring.

Methods

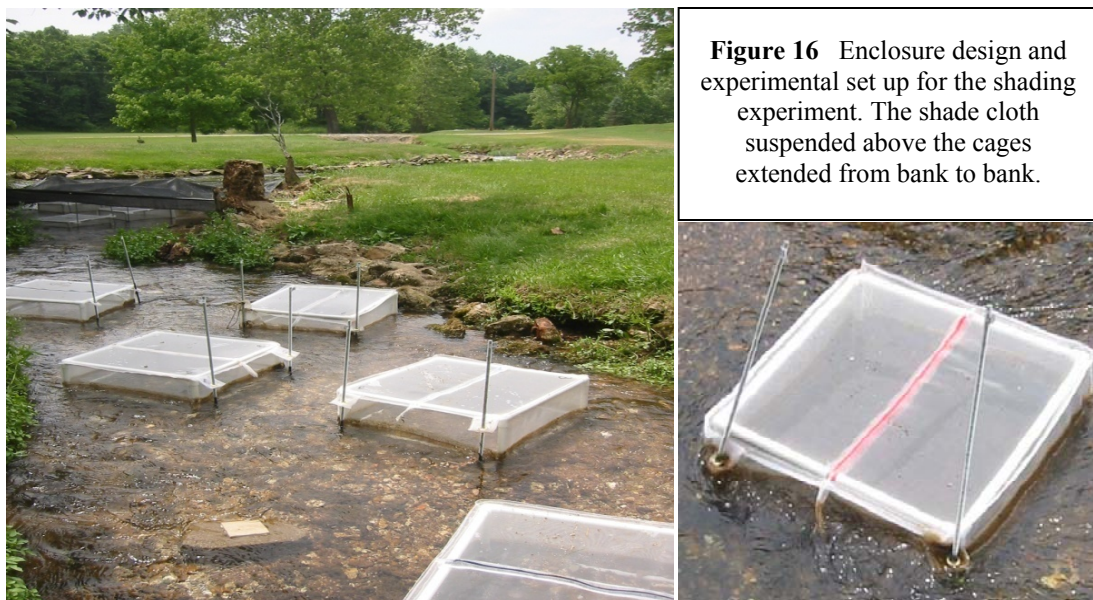
In situ food web experiment

Haseltine (as described in Chapter 2) is a karst spring that issues from a small cave. Most cave-based springs, like forested streams are highly shaded and autotrophic production is limited. However, this spring has experienced extensive anthropogenic deforestation, making it a useful site for these experiments. Riparian cover is so depleted that autochthonous production was expected to be the dominating basal resource. Experiments were conducted in three reaches, with reach 1 being closest to the cave mouth and reach 3 the farthest downstream. Because of land surface topography, and a small amount of riparian vegetation, reach one and two were naturally shaded during very early morning hours, and the lower-most portion of reach 1 was partially shaded for a short time during mid-day. Reach 3 was in the sun for the entire day. Ambient light conditions in each of the three study reaches have an average incident light transmittance (ILT) of 71%, 95%, and 100% in reach 1, 2, and 3, respectively (mid-day measurements).

Light and subsequent algal production was manipulated in a blocked, split-plot design in a 9-wk study during the summer of 2007. Availability of PAR (photosynthetically available radiation) was experimentally reduced by stretching a canopy of polyethylene, knitted shade cloth across three sites along the Haseltine springbrook (shading techniques that mimic the percentage of shading in two similar Ozarks springs; Danforth and Steury Springs, as discussed in Chapters 2-3). The percent of light penetration in shaded sections, as measured with a linear PAR/LAI ceptometer (AccuPAR: Model PAR-80; Decagon Devices, Inc.), equaled $\sim 20\%$. Each of these shaded sites was paired with an adjacent unshaded site, resulting in three pairs (reach blocks) of shaded and open sites. Invertebrate communities in each of these sites were secured in five cages (total = 30 cages). Enclosure cages and shade cloth were successfully tested for environmental durability for a period of 4 wk prior to the experiment. Open canopy cages were not placed in the lower portion of reach 1 because it was partially shaded.

Each of the 30 six-sided cages enclosed a bottom area of 0.186 m^2 , or roughly the area of two Surber samplers. Larger cages were not feasible because of both the small size of the springbrook and the number of benthic invertebrates which would need to be sacrificed in these fragile ecosystems. Cages were constructed of $\sim 1.3 \text{ cm}$ (0.5 inch) PVC pipe covered with Nitex nylon mesh (1000 μm pores) and secured to the bottom with steel stakes (Fig. 16). Cages in the same treatment area were off-set laterally by at least 0.5 m to avoid interference with water currents. Flow rates in these low-velocity streams differed by a mean of only $0.07 \text{ m}\cdot\text{sec}^{-1}$ between inside and outside of cages. The mesh cover of all cages reduced PAR inside by only 2% of the exterior levels, while the average difference in light between shaded and unshaded portions of the stream was 80%. Because only caged invertebrates were sampled in this study, the effect of cages was the same across treatments.

To begin the experiment, I added to each cage, benthic macroinvertebrates from two Surber (0.186 m^2) sampling events and 6-8 cm of benthic substrate from sections of the springbrook designated for cage placement. Any moss and vascular



macrophytes that occurred in those sections of the spring were also added to the cages to mimic natural conditions. No crayfish, salamanders, or fish from the springbrook were enclosed in cages because of the need of these consumers for larger foraging areas and because their densities were too low to sacrifice them at the end of the experiment for isotope analyses. After 9 wk, all macroinvertebrates were removed from cages and returned to the lab in a cooler. A subset of the invertebrates in each cage was frozen for stable isotope analysis (prepared for isotope analysis as described in Chapter 3), and the remainder were preserved for counting and identification.

Major sources of organic carbon (terrestrial and aquatic) were collected from each site and processed for stable isotope analysis, as described in Chapter 3, and included terrestrial C_3 and C_4 plants, fine transported organic matter (FTOM; 0.1-1 mm diameter), dissolved organic matter (DOM; < 0.1 mm diameter), benthic organic matter (BOM), benthic algae (periphyton), and rooted macrophytes. Epiphytic algae from water cress and moss were also collected for food web analysis because of extensive ^{13}C enrichment, high C:N ratios, and the lack of consumer dependence on epilithic algae seen in Chapter 3, and the possibility that organisms shown to be dependent on moss and water cress in Chapter 3 may have, in fact, actually been eating bacterial film, algae, or fine detritus associated with these plants.

Pigment analysis using Chlorophyll-*a* content of benthic biofilms was used to estimate the benthic algal biomass potentially available as food for enclosed consumers (Steinman and Lamberti 2007). Three clay tiles (25.8 cm²) were secured at the beginning of the experiment in the springbrook substrate at random locations in shaded and unshaded sections of each reach. At the conclusion of the 9-wk experiment, the organic covering of each tile was scrubbed loose and a slurry collected using deionized water. Chlorophyll-*a* was analyzed according to the methods of Steinman and Lamberti (2007). The slurry was filtered onto 0.45 µm Whatman GF/C glass fiber filters using a standard filtration device, and the photosynthetic pigments were extracted in a 90% buffered acetone solution in the dark for 24 h at 4°C. The Chl-*a* concentrations (in µg/cm²) of these solutions were measured with a spectrophotometer using an acidification step to account for phaeophytin content.

Analyses

Resource-consumer relationships were first assessed, as in Chapter 3, by examination of carbon and nitrogen bi-plot graphics (Peterson and Fry 1987; Fry 1991). Carbon and nitrogen stable isotope values were analyzed using the IsoSource multi-isotope mixing model (Phillips and Gregg 2003, Phillips *et al.* 2005), and used to determine the relative importance of allochthonous and autochthonous food sources to consumers. C:N ratios were used to determine the allochthonous or autochthonous composition of each food source (determinations as in Chapter 3; C/N >15 = allochthonous; < 15 = autochthonous). Quantified food web models were developed using IsoSource predictions and biomass estimates of each consumer. The IsoSource model (Phillips and Gregg (2003) examines all possible combinations of source proportions (each combination equals 100%), creates a set of predicted source mixtures that are compared against the observed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of the consumer, and quantifies the potential contribution of sources to the consumer diet. The results are presented as a 1-99th percentile range (minimum to maximum), or the

range that a food source can feasibly contribute to a consumer's diet. Phillips and Gregg (2003) recommended using the 1-99th percentile range to represent the proportion (%) of the diet accounted for by particular food sources, because of its sensitivity to small numbers of observations. Hence, a food's contribution at the first percentile represents its minimum contribution (e.g. 40%) to consumer diet, and its contribution at the 99th percentile represents its maximum. The IsoSource model also provides a mean percentage that a food source contributes. The 1-99th percentile range proportions are discussed in the text, and food web models were built based on the mean values.

Food web complexity statistics were calculated and included linkage density (L/S) and linkage complexity ($S \cdot C$) measures (Chapter 3). For linkage density, L = the number of feeding links in the web (as identified by the IsoSource model), and S = the number of species in the web. Linkage complexity is defined as the number of species in the system (S) times connectivity (C), calculated as $(L/(S[S-1]/2))$ (Warren 1989; Martinez 1991; Polis 1991; Tavares-Cromar and Williams 1996; Woodward *et al.* 2005). These food web metrics were chosen because of their applications to hypotheses on differences in generalist vs. specialist feeding patterns, and the complexity of those patterns. These metrics were then used as dependent variables in a two-factor analysis of variance (ANOVA; NCSS) to test for light and reach treatment effects. The Tukey post-hoc test was used for comparing specific means. Statistical significance was determined at $\alpha = 0.05$.

As part of the analysis, I pooled similar taxa from each of the five cages within a given reach and treatment. This step was necessary because the random selection of invertebrates added to each cage at the beginning of the experiment produced assemblages in cages where like taxa were not equally represented, as needed for treatment comparison. Because springs are small fragile systems (see Fig. 1), with generally low densities of the majority of the resident invertebrate fauna, natural collections were deemed a more appropriate approach, as multiple sampling efforts needed to produce replicate cage communities would have been too

destructive to the system. Moreover, characteristically short spring lengths made it impossible to collect further downstream to stock cages. This required pooling step produced an average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signature for each taxa represented in a given treatment and reach.

Results

Experimental communities

Food webs in experimental cages were simple from the perspective of number of trophic levels, just as the spring community itself is relatively limited in number of levels. Herbivores dominated cage communities (Table 13). Higher level consumers included midges from the subfamily Tanypodinae, dance flies (*Hemerodromia*), alderflies (*Sialis*), and planarians (*Dugesia*). Trophic position for these invertivores was calculated to be 2.23, 2.30, 2.38, and 2.74 for these midges, danceflies, alderflies, and planarians, respectively, with level 1.0 equal to a pure herbivore.

Cage communities in each reach were similar to the previous summer's collection discussed in Chapter 2. Peracaridans were dominant in reach 1, representing an average of 97.5% of the total fauna among treatments. Their proportions decreased to an average of 70% by reach 2. By reach 3, they represented only 5% of the community, as insects became dominant representing 85% of the total fauna on average.

Not all organisms collected in a given cage or treatment (sun/shade) were duplicated in the opposite treatment (Table 13), as stated in the rationale for the pooling step. For example, in reach 2 crane flies (*Tipula*), damselflies (*Argia*), and chironomid midges were only represented in cages in the sun, while Tanypodinae midges were only present in cages in the shade. Therefore, to make equivalent feeding comparisons among treatments, organisms selected for food web IsoSource analysis were only those represented in both treatments in a given reach. To improve the interpretive potential of the IsoSource multi-isotope mixing model, mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of consumers with similar functional feeding strategies (Merritt and

Table 13 A summary of the benthic community data set with the number of organisms standardized per m² (Surber Sampler area 0.093 m²).

	Reach 1-Cage 1		Reach 1-Cage 2		Reach 1-Cage 3		Reach 1-Cage 4		Reach 1-Cage 5	
	SUN	SHADE	SUN	SHADE	SUN	SHADE	SUN	SHADE	SUN	SHADE
Amphipods	46505.38	3408.60	13709.68	1989.25	13440.86	9946.24	42473.12	10483.87	1290.32	7795.70
<i>Lirceus hoppinae</i>	494.62	1279.57	2602.15	709.68	1752.69	860.22	1236.56	752.69	1290.32	741.94
Net Caddisfly	—	21.51	—	21.51	—	64.52	—	32.26	—	10.75
Case Caddisfly	—	—	—	—	118.28	—	—	—	—	—
<i>Tipula sp.</i>	—	—	43.01	32.26	—	—	—	—	10.75	—
Chironominae	—	139.78	—	43.01	—	107.53	—	21.51	—	—
<i>Optioservus sandersoni</i>	—	—	—	—	—	—	—	—	—	—
<i>Hemerodromia sp.</i>	—	—	—	—	—	—	—	—	—	—
Snails	—	21.51	—	—	—	21.51	—	21.51	—	21.51
Lumbriculidae	—	64.52	—	43.01	—	32.26	—	—	—	—
<i>Stratimys sp.</i>	—	—	—	10.75	—	53.76	—	—	—	10.75
<i>Argia sedula</i>	—	32.26	—	—	—	10.75	—	—	—	—
Tanyptodiinae	—	—	—	—	32.26	—	10.75	—	—	—
<i>Dugesia doratocephala</i>	161.29	1182.80	—	—	—	—	—	—	—	—
<i>Oreodytes sp.</i>	—	—	—	—	—	—	—	21.50	—	—

	Reach 2-Cage 1		Reach 2-Cage 2		Reach 2-Cage 3		Reach 2-Cage 4		Reach 2-Cage 5	
	SUN	SHADE	SUN	SHADE	SUN	SHADE	SUN	SHADE	SUN	SHADE
Amphipods	5483.87	4731.18	16666.67	11559.14	1322.58	1344.09	11021.51	3064.52	6989.25	13172.04
<i>Lirceus hoppinae</i>	526.88	150.54	—	268.82	161.29	204.30	537.63	193.55	1763.44	376.34
Net Caddisfly	43.01	—	43.01	—	—	32.26	10.75	—	10.75	43.01
Case Caddisfly	—	—	—	—	—	—	—	—	—	—
<i>Tipula sp.</i>	—	—	—	—	10.75	—	10.75	—	10.75	—
Chironominae	96.77	—	43.01	—	10.75	—	139.78	—	666.66	—
<i>Optioservus sandersoni</i>	1881.72	2075.27	2559.14	2827.96	1161.29	1881.72	1881.72	2935.48	2075.27	2096.77
<i>Hemerodromia sp.</i>	64.52	75.27	43.01	—	53.76	107.53	344.09	—	247.31	161.29
Snails	1053.76	849.46	279.57	1053.76	215.05	387.10	623.66	440.86	182.80	666.67
Lumbriculidae	—	268.82	—	—	21.51	53.76	32.26	10.75	10.75	32.26
<i>Sialis sp.</i>	—	—	—	—	—	—	—	—	—	10.75
<i>Argia sedula</i>	—	—	—	—	—	—	21.51	—	—	—
Tanyptodiinae	—	107.53	—	258.06	—	86.02	—	64.51	—	10.75
<i>Dugesia doratocephala</i>	1989.25	548.39	215.05	—	—	107.53	168.42	—	—	—

	Reach 3-Cage 1		Reach 3-Cage 2		Reach 3-Cage 3		Reach 3-Cage 4		Reach 3-Cage 5	
	SUN	SHADE	SUN	SHADE	SUN	SHADE	SUN	SHADE	SUN	SHADE
Amphipods	—	107.53	225.81	96.77	—	—	1204.30	53.76	—	—
<i>Lirceus hoppinae</i>	—	—	—	—	—	—	—	—	—	—
Net Caddisfly	—	—	—	—	—	—	—	—	—	—
Case Caddisfly	—	10.75	—	—	—	—	—	—	—	—
<i>Tipula sp.</i>	—	—	—	—	—	—	—	—	—	—
Chironominae	96.77	161.29	43.01	118.28	139.78	64.52	—	182.80	53.76	—
<i>Optioservus sandersoni</i>	537.63	1344.09	1236.56	2763.44	2064.52	2516.13	2634.41	1989.25	161.29	1483.87
<i>Hemerodromia sp.</i>	64.52	75.27	43.01	139.78	53.76	96.77	344.09	161.29	247.31	75.27
Snails	1053.76	376.34	279.57	—	21.50	236.56	623.66	430.11	182.80	118.28
Lumbriculidae	64.52	516.13	43.01	172.04	32.25	32.26	—	107.53	—	32.26
<i>Sialis sp.</i>	10.75	118.27	53.76	86.02	—	43.01	32.25	96.77	—	—
Tanyptodiinae	870.97	473.11	376.34	—	53.76	—	204.30	483.87	—	53.76
<i>Dugesia doratocephala</i>	—	32.25	—	—	0.00	—	—	—	—	—

Cummins 2008; Thorp and Covich 2001) were calculated and included the following combinations: amphipods (*Crangonyx forbesi* and *Gammarus minus*) and snails (*Physa remingtoni*, *Promenetus sp.*, and *Goniobasis potosiensis*).

Isotope signatures of sources and consumers

Autochthonous food source $\delta^{13}\text{C}$ values were typically more depleted than allochthonous. Their values ranged from -45.33 to -25.05‰ (mean; -31.35‰), -35.33 to -24.25‰ (mean; 28.12‰), and -40.32 to -26.33‰ (mean; -31.55‰) in reaches 1-3, respectively (Figs.17a-c). Allochthonous food sources ranged from -29.02 to -13.59‰ (mean; -21.60‰), -27.50 to -13.55‰ (mean; -18.77‰), and -29.65 to -16.24‰ (mean -24.63‰) in reaches 1- 3, respectively.

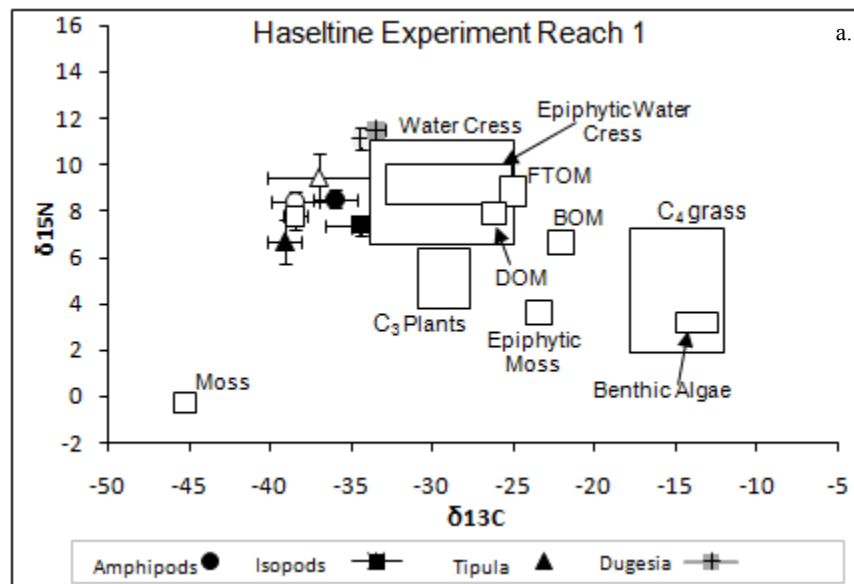


Figure 17a ^{13}C and $\delta^{15}\text{N}$ values for Haseltine Spring reach 1. Potential carbon/nitrogen sources shown as boxes delineated by 1 SD. Consumers symbols as per legend (mean \pm 1 SD) solid filled symbol closed for shaded, unfilled for open (sun) canopy samples.

Benthic algae were consistently more enriched than any other primary food source. There was however considerable variability in algal $\delta^{13}\text{C}$ signatures. In reach 1 and 2 algal ^{13}C signatures were approximately -13.59‰, consistent with the notion that boundary layer diffusion (in slow flowing waters such as Haseltine; $\delta^{13}\text{C}$

typically -10 to -15‰) and HCO_3^- uptake ($\delta^{13}\text{C}$ typically -5‰) in carbonate waters may determine the $\delta^{13}\text{C}$ value of submerged aquatic plants (Osmond *et al.* 1981). By reach 3, algal values were more C-depleted and resembled other autochthonous food sources. Stoichiometric analysis indicated that algal C:N ratios shifted along this gradient, with algal ratios consistent with allochthonous values in reach 1 and 2, but autochthonous by reach 3 (Table 14).

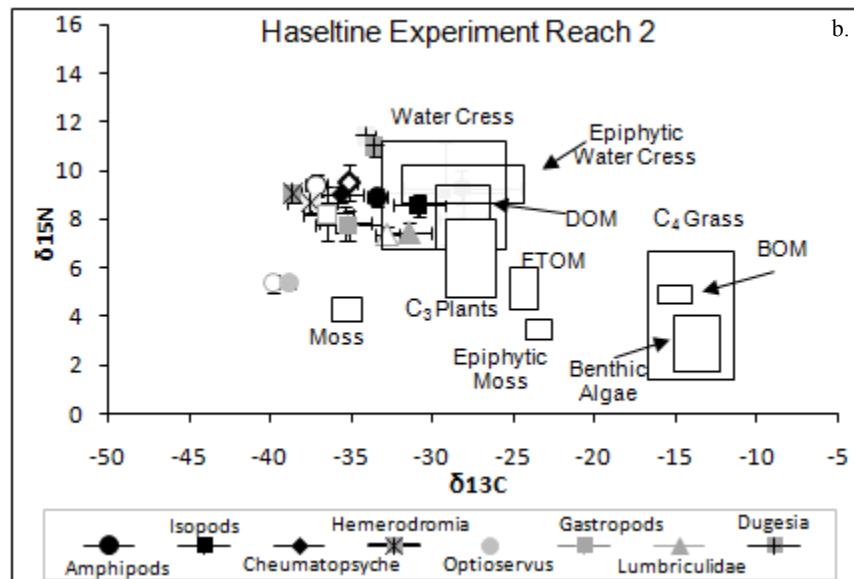


Figure 17b $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for Haseltine Spring reach 2. Potential carbon/nitrogen sources shown as boxes delineated by 1 SD. Consumers symbols as per legend (mean \pm 1 SD) solid filled symbol closed for shaded, unfilled for open (sun) canopy samples.

Algae were not the only food source too enriched in carbon for food web use (Figs 17a-c). Analysis of the C:N bi-plots revealed that BOM in reach 2, FTOM in reach 3, and C_4 plants were also too C-enriched to represent feasible food options. In addition, the $\delta^{15}\text{N}$ signatures of several foods in reach 3 (DOM, water cress, moss, and epilithic algae) were more enriched than that of most consumers, and therefore not possible food sources for food web analysis (Fig. 17c).

Benthic organic matter signatures (BOM) were consistent with terrestrial food source values. In reach 1, BOM values fell between C_4 terrestrial grasses and autochthonous food sources; but by reach 2, BOM resembled C_4 grasses as lawn mowing in the riparian zone on one side of the spring threw these grasses into the

spring, especially at this site. As stated above, BOM was too enriched to be a likely C source for metazoan consumers in this reach. By reach 3, however, BOM resembled C₃ plants. Observed C:N ratios of BOM indicated an allochthonous origin across reaches.

Dissolved organic matter (DOM) $\delta^{13}\text{C}$ values were similar to water cress in each of the three experimental reaches, which is consistent with the conclusion that DOM originates as biological products from plant organic matter (Allan 1999). Carbon and nitrogen ratios confirmed that DOM was indeed autochthonous in origin along the reach 1 to 3 gradient.

Table 14 Mean C:N ratios for potential sources of organic matter from each of the 3 study springs. C:N values >15 indicate significant allochthonous contributions and are noted in bold.

	Haseltine Experiment C:N			
	DOM	FTOM	BOM	ALGAE
R1	2.27	1.67	15.51	17.64
R2	1.85	12.61	17.28	17.52
R3	4.08	15.34	18.10	10.26

Observed FTOM isotope signatures resembled water cress in reach 1 and epiphytic algae from moss in reach 2, as the source of FTOM for invertebrate consumers is typically from sloughing of algae and aquatic instream macrophytes (Allan 1999). By reach 3, however, FTOM was as enriched as C₄ grasses consistent with the fact that FTOM can also include particulates from the forest floor (Allan 1999). The close correlation between isotopic signatures and C:N ratios confirmed that FTOM was indeed of autochthonous origin in reach 1 and 2, but shifted to allochthonous origin by reach 3 (Table 14).

Food source nitrogen signatures were variable and overall increased substantially by reach 3. DOM had increased by only 1‰ by reach 2, but increased by 6‰ more by reach 3. FTOM and BOM lacked this variability, but moss and algae showed the most dramatic enrichment, increasing by as much as 14 and 12‰, respectively, between reach 1 and 3. These lower reach increases in nitrogen were likely due to greater watershed influence with distance from the cave.

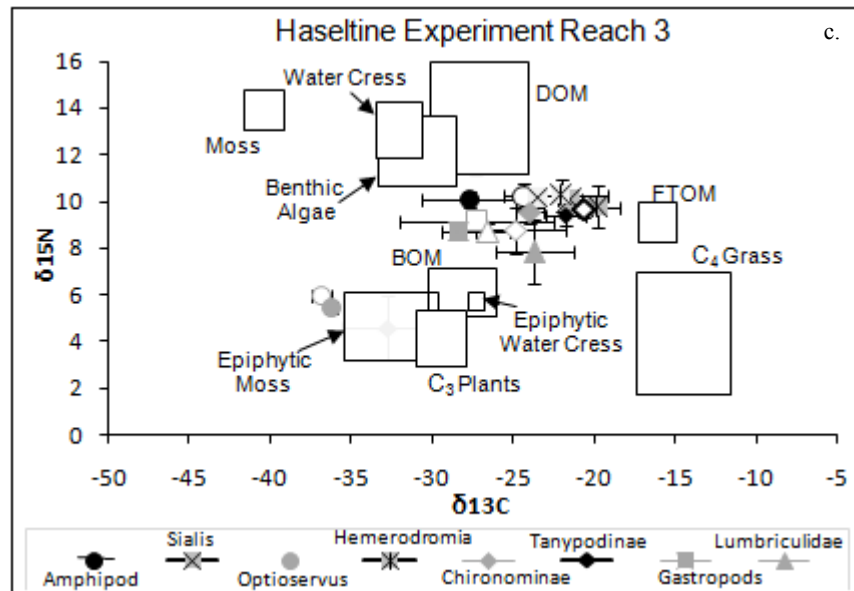


Figure 17c $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for Haseltine Spring reach 3. Potential carbon/nitrogen sources shown as boxes delineated by 1 SD. Consumers symbols as per legend (mean \pm 1 SD) solid filled symbol closed for shaded, unfilled for open (sun) canopy samples.

Invertebrate communities responded to light manipulations. Invertebrates from shaded blocks had $\delta^{13}\text{C}$ values that were typically more enriched than those in open plots, indicating that food resources used by invertebrates in the shade were primarily of terrestrial origin (Figs. 17a-c). In open blocks in each reach, invertebrates exhibited generally more negative (C-depleted) values indicating a greater dependence on autochthonous materials. Observed consumer $\delta^{15}\text{N}$ signatures did not appear to be affected by light treatment.

Algal biomass

Data on benthic algal biomass (estimated as Chl-*a*) represented the net algal accrual over the period of the 9-wk experiment. Microbial accrual in experimental sites (shaded) was strongly influenced by light regime (Table 15). Chlorophyll-*a* concentrations ranged from 0.24 $\mu\text{g}/\text{cm}^2$ to greater than 26 $\mu\text{g}/\text{cm}^2$, with a mean of 5.57 $\mu\text{g}/\text{cm}^2$ across all treatments (sun/shade). The mean concentration on artificial substrates in sun plots was between 5 and 7 times greater than in shaded plots. By the

end of the experiment, periphyton (Chl-*a*) was significantly greater on tiles in the open sites (ANOVA; $F = 7.66$, $p < 0.01$).

Table 15 Mean values for Chlorophyll-*a* in each light treatment, for Haseltine Spring

	SUN	SHADE
	$\mu\text{g}/\text{cm}^2$	$\mu\text{g}/\text{cm}^2$
Reach 1	18.56	2.66
Reach 2	5.99	1.06
Reach 3	4.26	0.89

Relationships between consumers and primary carbon sources

Estimates resulting from the IsoSource mixing model confirmed C:N bi-plot analysis, indicating that autochthonous food sources were more important to consumers in open rather than shaded sites in each of the three reaches (Fig. 18 a-c). This was the case even though algae were too enriched in ^{13}C to be a significant food source for any of the consumers, as enrichment was associated with high C:N ratios (cf. Hicks 1997; Bunn 2003).

In reach 1 (Fig. 18a), instream moss constituted the major proportion of the diet (First percentile $> 42\%$; 1-99th % tile values) for consumers representing the greatest biomass (peracaridans) in open cages. Epiphytic algae were second to moss in their importance as an autotrophic food source, and represented a larger average contribution to herbivore diets in open cages (32%) than in shaded (20%). Other autochthonous food sources (FTOM and water cress) were similar in their contributions, typically representing as much as 23-40% of herbivore diets in open treatments. Allochthonous BOM (Table 14) was not a significant carbon source for any of the open canopy consumers, and C_3 plants represented only a moderate proportion of their diets. Herbivores in reach 1 shaded cages, however, relied less heavily on moss and water cress, and more heavily on allochthonous BOM and C_3 plants with ranges (1-99th percentile) indicating these sources could contribute up to 30 and 48% to their diet respectively. The only source connecting basal resources to invertivores was FTOM, contributing $\sim 20\%$ to the diet of planarians, the only predator collected in reach 1.

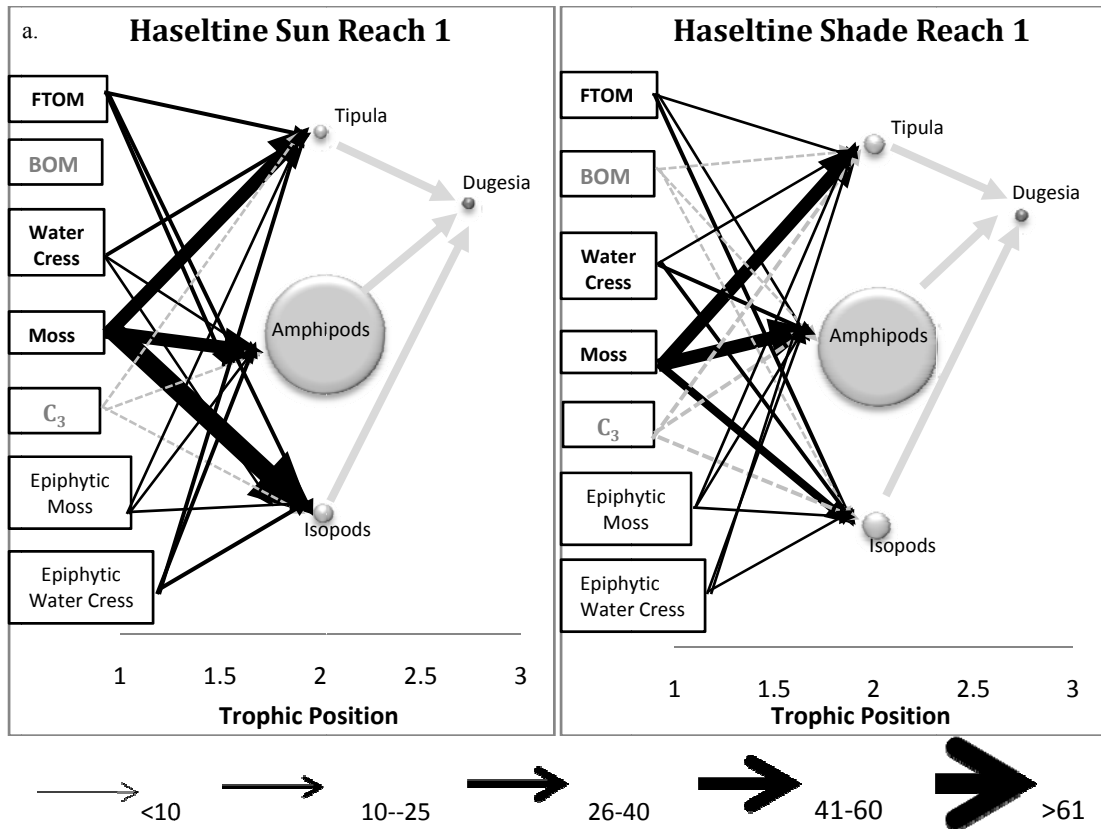


Figure 18a. Quantified food webs for Haseltine Spring using mean dietary proportions that a lower trophic level contributes to the next higher level (arrow width). Circle size represents the proportion of biomass contributed by each species. Species arranged by trophic position. Black arrows = autochthonous, Grey dotted = allochthonous food source (FTOM, DOM, BOM origin determined by C:N stoichiometric analysis).

By reach 2 (Fig. 18b), larger variability in open versus shaded site consumer feeding patterns was observed. Moss remained the dominant food source in both open (99th percentile: 60-65%) and shaded cages, but much less so in shaded (99th percentile: 30-45%). Water cress represented as much as 35-58% of herbivore diet across both treatments. Epiphytic algal sources, on average, were used to a greater extent in open cages than in shaded cages (open 55%; shaded 42%). Epiphytic algal sources, for example, were much more important to snails and riffle beetles in open cages (first percentile: 58 and 100%, respectively) than in shaded cages (first percentile: 1 and 3%, respectively). The value of transported FTOM to herbivore diets was similar across treatments, and DOM was an important source only to net-

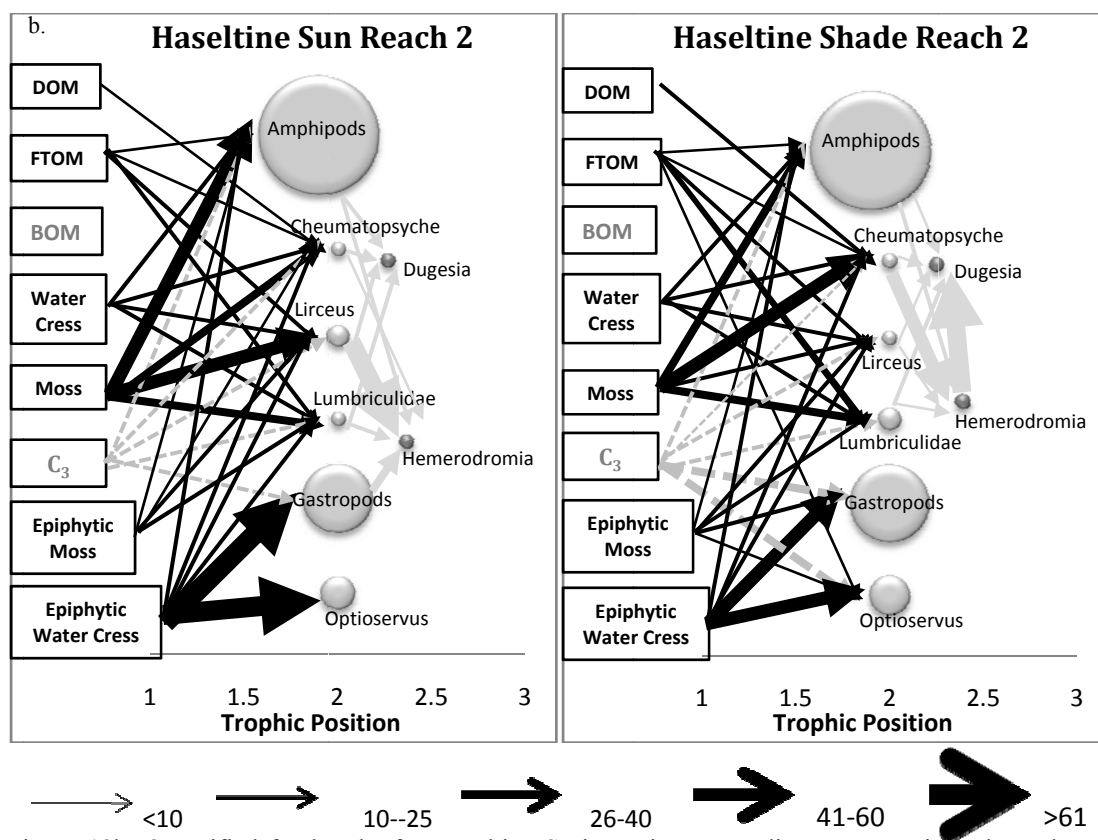


Figure 18b. Quantified food webs for Haseltine Spring using mean dietary proportions that a lower trophic level contributes to the next higher level (arrow width). Circle size represents the proportion of biomass contributed by each species. Species arranged by trophic position. Black arrows = autochthonous, Grey dotted = allochthonous food source (FTOM, DOM, BOM origin determined by C:N stoichiometric analysis).

spinning caddisflies. Allochthonous C_3 plants represented a more important food source to herbivores in shaded cages (1- 99th % percentile: 48-92%) than in open cages (99th % percentile: 41-55%). Planarians relied on autochthonous FTOM (Table 14) to a greater extent in open cages, while dependence on C_3 plants by danceflies was greater in shaded cages.

By reach 3, DOM, water cress, moss, benthic algae, and FTOM were either too N or C-enriched to be used as a possible food sources. Epiphytic algal sources represented a major fraction of the herbivore diet. Again, the dependence on these epiphytic algal sources was greater in open sites (64%) than in shaded (50%). Epiphytic algae from moss were especially important to riffle beetles and snails in open cages (66-96 and 0-58%, respectively) and slightly less for those in shaded

cages (55-95 and 0-38%, respectively). Epiphytic algae from water cress represented as much as 92 and 83% of amphipod and snail diets, respectively in open cages, and 65 and 61% of their diet in shaded cages. Allochthonous BOM was actually used to a greater extent by amphipods and chironomid midges in open cages, but on average was used more by the remaining herbivores in shaded cages. Terrestrial C_3 plants were a moderately important part of the diet in all 5 herbivore taxa in shaded plots and 3 of 5 in unshaded areas. These differences represent an overall greater dependence on autochthonous food sources by consumers in open plots and a greater dependence on allochthonous foods in shaded plots.

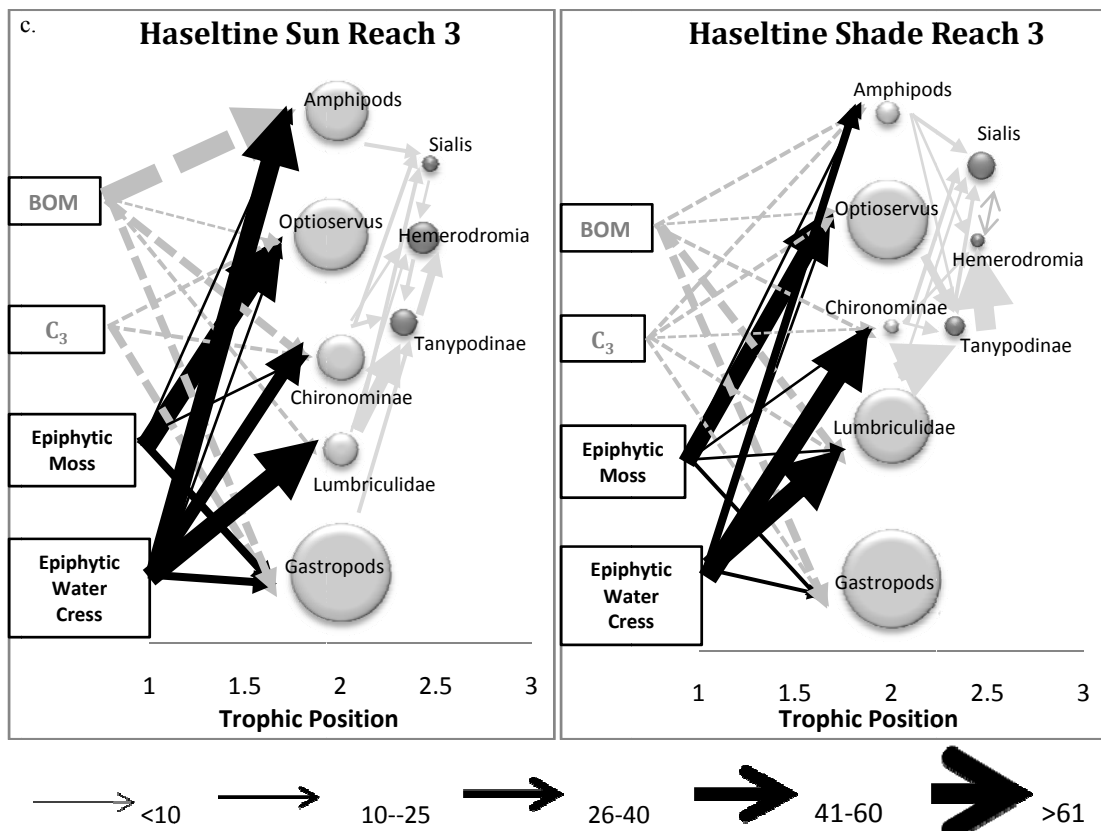


Figure 18c. Quantified food webs for Haseltine Spring using mean dietary proportions that a lower trophic level contributes to the next higher level (arrow width). Circle size represents the proportion of biomass contributed by each species. Species arranged by trophic position. Black arrows = autochthonous, Grey dotted = allochthonous food source (FTOM, DOM, BOM origin determined by C:N stoichiometric analysis).

Figure 19 shares the overall differences in the relative importance of autochthonous and allochthonous food resources for the two treatments in each reach of Haseltine Spring. Histogram values represent an average of the proportion of the diet attributed to autochthonous food sources for each consumer in a given reach for both treatments, and provide a more general picture of the effect of light treatment on feeding pathways for the entire experimental community. In each reach, autochthonous foods significantly (ANOVA; $F = 6.80$, $p < 0.01$) represented a greater proportion of herbivore diets in open versus shaded treatments.

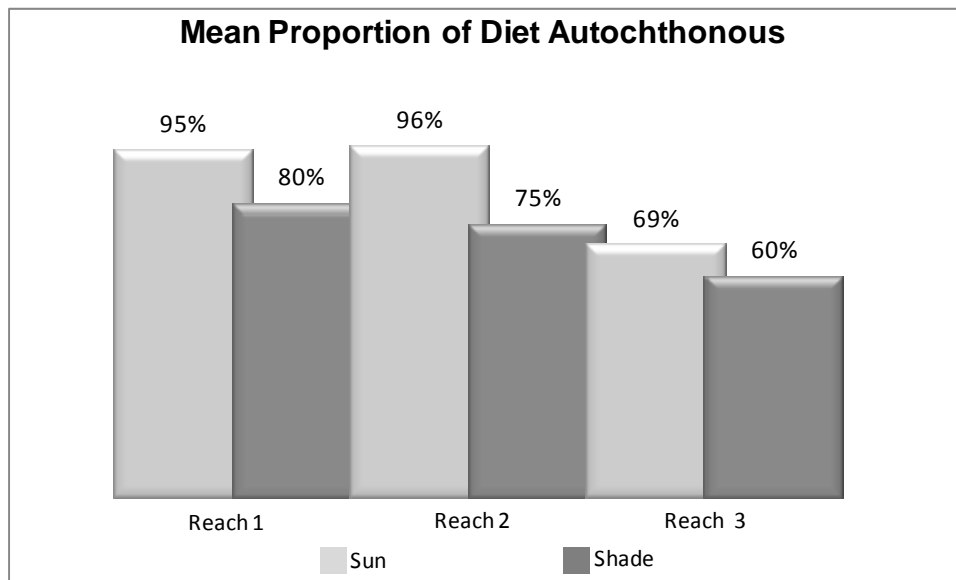


Figure 19 Mean proportion of diet (averaged across all consumers) attributed to autochthonous food sources for each treatment (light/shade) averaged across each reach.

Web structure: Linkage complexity and density

In each of the study reaches, linkage density (L/S) and linkage complexity (SC) measures provided insights to whether food selectivity of consumers was altered by light treatments. In each of the study reaches significant differences in linkage density were recorded (Table 16) in response to differences in light treatment (open/shaded) ($F = 44.66$, $p < 0.02$) and reach ($F = 61.68$, $p < 0.01$). The number of links per species was higher in shaded treatment plots, and increased in order from

reach 1 to 3. This indicates that invertebrates were using a greater diversity of carbon resources in shaded spring plots, or had more generalized feeding patterns. Food web linkage complexity was also significantly higher in response to light ($F = 47.69$, $p < 0.02$) and reach ($F = 59.11$, $p < 0.01$) treatments (Table 16). Linkage complexity was again higher in shaded plots and increased progressively along the gradient from reach 1 to 3.

Table 16 Food web statistics for Haseltine Spring for both open (sun) and shaded light treatments.

Light Treatment	Reach	Web Size	Number of Links	Linkage Complexity	Linkage Density
		S	L	SC	L/S
SUN	R1	11	22	4.40	2.00
SHADE	R1	11	25	5.00	2.27
SUN	R2	15	37	5.29	2.47
SHADE	R2	15	44	6.29	2.93
SUN	R3	12	32	5.82	2.67
SHADE	R3	12	37	6.73	3.08

Discussion

Headwater streams, which includes springs (Lindegaard 1995) are commonly characterized as heavily shaded, heterotrophic systems where instream primary production should contribute very little to the total annual energy flux, often less than 1-2% (Fisher and Likens 1973; Cummins 1974; Lewis *et al.* 2001; Thorp and Delong 2002). Haseltine Spring, however, has been intentionally deforested. This lack of riparian cover in Haseltine enabled me to quantify the flows of organic matter, consumer biomass, and strength of consumer-resource links in response to an experimental reduction in algal biomass (as a function of light reduction with shade cloth). Working in a tree-less spring was necessary and useful for this study because it allowed me to duplicate aspects of springs with characteristically heavy canopies. The only alternative would be to chop down trees surrounding an already fragile

spring ecosystem. This tactic was not an environmentally sound option, nor possible because of private ownership of the land within which the forested springs reside.

The lack of riparian canopy has been shown to alter the structural and functional integrity of lotic ecosystems (cf. Fisher and Likens 1973; Rounick *et al.* 1982; Lester *et al.* 1995). Evidence of this relationship was seen in Chapters 2 and 3, as the composition of the Haseltine springbrook fauna and their diets were more consistent with a reliance on autotrophic, rather than heterotrophic food sources. Manipulative approaches to aquatic food web research provide estimates of the relative importance of allochthonous and autochthonous food web pathways to lotic consumers. Hence, I expected that the experimental manipulation of algal biomass would alter the food base supporting metazoan production and change food web complexity.

Trophic base of the food webs

The results of this study indicated that not only was the food base and diet of aquatic consumers in this spring fueled by autochthonous production but also that the nature of the food base varied with the relative abundance of epiphytic and epilithic algal food sources. Treatment differences in the $\delta^{13}\text{C}$ signature of invertebrate consumers corresponded with differences in the $\delta^{13}\text{C}$ signature of nearby allochthonous and autochthonous food sources. Consumers from shaded plots had $\delta^{13}\text{C}$ values indicating that they responded to the decrease in algal food resources with a greater reliance on food resources of terrestrial origin. Open site consumers, in contrast, exhibited generally more negative $\delta^{13}\text{C}$ values, indicating greater use of autochthonous materials. Likewise allochthonous food source-consumer links, represented in the quantified food web models, were greater in number and strength, and supported a greater proportion of herbivore biomass in treatment plots than in open plots. Hence, species groups in shaded sites became relatively more dependent on allochthonous based food web pathways than identical species groups in open canopy sites, where variability in the relative abundance of algae had not been

altered. In addition, dietary shifts by consumers in treatment plots resulted in increases in generalist feeding patterns (omnivory) and consequent increases in food web complexity. These results indicate that algal availability for consumption is the mechanism defining (i) the trophic base of the food web; (ii) the degree of food specialization by consumers; and (iii) the level of food web complexity.

Importance of autotrophy in lotic systems

The relative amount of algal production was key in defining the structure and function of treatment food webs in shaded and open plots in Haseltine Spring. Predictions regarding the relative importance of autochthonous and allochthonous food sources are typically based on stream-order position in a riverine network (headwaters to large river) and/or on the related presence or absence of riparian canopy, as proposed by traditional models such as the river continuum concept (RCC) (Vannote *et al.* 1980). This model proposed that consumers rely on: (i) allochthonous organic matter (leaf litter) in forested, headwater streams; (ii) autochthonous material in wider, mid-sized streams; and (iii) terrestrial carbon in large, non-floodplain rivers in the form of fine particulate organic matter (FPOM) leached from upstream processing (Cummins *et al.* 1973; Fisher and Likens 1973; Vannote *et al.* 1980). It follows, therefore, that one would expect that subterranean or particulate detritus from the surface would strongly influence the structure and function of the food web in springs issuing from caves (Huxel and McCann 1998; Simon *et al.* 2003). There is a growing body of research, however, that indicates that autotrophy is underestimated in a broad range of lotic systems.

Despite its often low standing biomass (due to riparian shading), algal production can support substantial secondary production (McIntire 1973; Minshall 1978; Rounick *et al.* 1982; Hamilton *et al.* 1992; Lewis *et al.* 2001; McCutchan and Lewis 2002). Several investigators have reported that autochthonous production (particularly algae) is the major driver of aquatic food webs in systems typically characterized as allochthonous-based (Thorp *et al.* 1998; Bunn *et al.* 2003; Thorp and

Delong 2002). Mayer and Likens (1987) found that the small, second order, forested Bear Brook system in New Hampshire was supported by autotrophic production in the form of diatoms. Rosemond (1994) found that the heavily forested brook known as Walker Branch (Tennessee) supported a vast community of algal grazing snails (1000/m²). Zah *et al.* (2001) studied an alpine stream in the Swiss Alps and predicted that autochthonous production would be the dominant food source in the portions of the stream above the tree line. They also predicted marked shifts in the energy base from autochthonous to allochthonous in subalpine forested areas (heavy allochthonous input). In contrast, they found that regardless of the canopy cover or season, autochthonous organic matter constituted the main energy base. Bunn *et al.* (2003), studying a large arid zone floodplain river in Australia, predicted on the basis of the RCC) that aquatic primary production would be limited and food webs would depend on terrestrial carbon sources. They found, however, that benthic algae was the most important food source despite its limited biomass. Recent studies in the tropics indicated that autochthonous foods are important to consumers even in streams with large inputs of allochthonous leaf litter (March and Pringle 2003; Yam and Dudgeon 2005; Lau *et al.* 2008; Lau *et al.* 2009).

These studies, which are clearly not consistent with predictions of the RCC, support alternative views of riverine ecosystem function such as those proposed by the Riverine Productivity Model (Thorp and Delong 1994, 2002) and the Riverine Ecosystem Synthesis (Thorp *et al.* 2006, 2008). These models stress the importance of autochthonous primary production in many portions of riverine ecosystems and argue that the role of autotrophs, such as microalgae, has been underestimated in systems typically characterized as heterotrophic. Data from the manipulative experiments in this study provide evidence that algae can be major drivers of food web structure and function in spring ecosystems as well. Spring ecosystems, then provide yet another viable avenue for sorting out feeding pathways between herbivores and the heterogeneous mix of basal food sources that has been a long-term

focus of ecological research (Woodward *et al.* 2005). In addition, the findings of this study suggest the importance of assimilated-based analyses (isotope, C:N ratios) and experimental manipulation in assessing mechanisms responsible for ecosystem functioning, rather than relying on predictive models whose principles may or may not apply.

The role of omnivory in food web complexity

Resource limitation has been shown to alter food web dynamics, often having a greater impact than the impact of predator limitation (Osenberg and Mittelbach 2001). Reduction in basal resources often promotes omnivorous feeding patterns when shifts in the trophic base of the food web causes consumers to switch to another resource (Oksanen *et al.* 1981; Wallace *et al.* 1999; Hall 2000). In this study, reduction in algal food resources was significantly associated with increases in the importance of omnivory (i.e., consumers eating whatever resources are plentiful or obtainable at the time; Polis 1994). This was evidenced by increases in linkage density (the number of links per species; L/S) by consumers in shaded plots. This indicates that identical species groups varied in the degree of food specialization between treatments (cf. Warren 1990; Tavares-Cromar and Williams 1996). Moreover, increases in omnivory were tightly linked with increases in food web complexity. Evidence of this relationship comes from comparison of the relative intricacy of the quantified food web models and food web complexity (SC) metrics of consumers in shaded vs. open control sites.

Not only do the results from this study reinforce previous work on the impact of resource limitation, they also highlight the role that omnivory plays in food web complexity. In contrast to early food web literature (e.g. May 1972; Cohen 1978; Pimm 1982), more recent, quantitative food web studies reveal that omnivory is common and that it increases food web complexity (Warren 1989; Winemiller 1990; Polis 1991; Martinez 1991; Tavares-Cromar and Williams 1996). Moreover, relationships between increases in omnivory and associated increases in food web

complexity are associated, in current food web studies, with food web stability (Martinez 1991; Hall and Raffaelli 1993; Polis 1994; Polis and Strong 1996; Jaarsma 1998; Woodward *et al.* 2005). Fundamental principles in food web ecology have shifted away from the well established view that simplicity generates stability (May 1972; Pimm 1982). More recent advances in food web modeling and experimentation now suggest that that complexity (i.e. high linkage density and linkage complexity), enhances food web stability (Polis 1991; Polis 1994; Williams and Martinez 2000). But, are populations of omnivores (generalist consumers) more stable because of their ability to switch to a different type of resource? Polis and Strong (1996) have provided strong support for the role omnivory plays in food web complexity and subsequent stability of complex webs. They argue that generalist consumers can not only switch to and from pulsed resources as they are made available, but also can feed on various resources which prevents particular resources from being driven to extinction; both actions tend to create more biologically stable food webs. Other investigators have suggested that shifts in the nature of the resource base from autotrophy to heterotrophy, often due to shifts in resource availability (such as seen in this study), may lead to greater donor-control, which could also increase web stability (De Angelis 1975; Woodward and Hildrew 2002). Thus, both resource abundance, and the degree of preferential feeding influence food web stability (Huxel and McCann 1998). While I lack experimental evidence directly pertaining to issues of stability in my spring, the increases in omnivory and complexity found in shaded plots could suggest an increase in food web stability in these treatment sites. This in turn could indicate that anthropogenic deforestation of headwater ecosystems, such as Haseltine, could destabilize the food web dynamics of residing invertebrate communities. Much more research is needed to determine the link between the relative efficiencies of resource use by omnivores and complexity and stability in food web dynamics.

Implications

Stream ecosystem health monitoring has only recently begun to incorporate measures of ecosystem processes in the determination of the health of stream and river ecosystems (Bunn *et al.* 1999; Fellows *et al.* 2006). For example, biomanipulation of food web structure is being used in lake and river ecosystems for the development of better water quality and nutrient loading management strategies (Gulati *et al.* 1990). It is also employed to determine how over-exploitation of fishes from the marine environment alters marine food webs (Sherman 1990). This study indicates that measures of ecosystem process would be effective indicators of ecosystem health in situations where disturbance (deforestation) influences those processes. Not only did the lack of forest canopy alter the structure of the benthic communities, as seen in Chapter 2, it also altered the functional integrity of the ecosystem. The importance of the forest in maintaining the functional and structural integrity of stream ecosystems has been widely emphasized where large-scale watershed practices, such as clear-cutting, have altered the food base of invertebrate consumers (Fisher and Likens 1973; Rounick *et al.* 1982; Bunn *et al.* 1999). Additionally, clearance of riparian vegetation, often tied with agricultural land use, can diminish the amount of suitable habitat for both aquatic and terrestrial invertebrates. This can remove a major energy source from both habitats' food webs as reciprocal fluxes of terrestrial invertebrates to streams from the riparian canopy and emergences of adult insects from the stream often provide large amounts of carbon to the food webs of the receiving system (Nakano and Murakami 2001; Sabo and Power 2002; Baxter *et al.* 2005). Thus, resource limitation would alter the food webs in both habitats. Conservation and management of lotic systems must include management of the terrestrial landscape as well.

Springs are being lost at an alarming and accelerating rate (Erman and Erman 1995). Agricultural intensification and groundwater contamination are constant threats to spring ecosystems. Additionally, springs are threatened through their use as sources of drinking water, commercial bottled water operations, water for livestock,

irrigation, and fish hatcheries (van Everdingen 1991). Some springs also serve as fishing and tourist attractions. Spring-associated plants and biota depend on the year-round constancy of spring microhabitats which would be lost with the impact of urban development. Application of food web research to detect ecosystem stress (Ulanowicz and Kemp 2001) or the effect of chemical contamination (Reagan and Fordham 1992) is essential to the preservation of lotic waterways such as springs. Thus, the impact that management strategies leave on the energetic transfers among trophic elements of a lotic ecosystem must be incorporated into federal, state, and local management practices.

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