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PRIMARY RESEARCH PAPER

Trophic ecology of a freshwater sponge (*Spongilla lacustris*) revealed by stable isotope analysis

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Abstract The vital roles that sponges play in marine habitats are well-known. However, sponges inhabiting freshwaters have been largely ignored despite having widespread distributions and often high local abundances. We used natural abundance stable isotope signatures of carbon and nitrogen (δ^{13}C and δ^{15}N) to infer the primary food source of the cosmopolitan freshwater sponge *Spongilla lacustris*. Our results suggest that *S. lacustris* feed largely on pelagic resources and may therefore link pelagic and benthic food webs. A facultative association between *S. lacustris* and endosymbiotic green algae caused *S. lacustris* to have significantly depleted carbon and nitrogen signatures that may reflect carbon and nitrogen exchange between sponges and their symbiotic algae. Isotopic data from specialist sponge consumers demonstrated that sponges hosting zoochlorellae were the major component of the diet of the spongillafly *Climacia areolaris* and the sponge-eating caddisfly *Ceraclea resurgens* suggesting that the symbiosis between freshwater sponges and algae is important to sponge predator trophic ecology. Our results help define the role of sponges in freshwater ecosystems and shed new light on the evolution and ecological consequences of a complex tri-trophic symbiosis involving freshwater sponges, zoochlorellae, and spongivorous insects.

Keywords Food webs · Energy flow · Symbiosis · Zoochlorellae · Sisyridae · *Ceraclea* · Sponge predators

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Introduction

Sponges (Porifera) are a diverse and inextricable component of the colorful and structurally complex aquascapes of coral reefs and play vital roles in energy flow, nutrient cycling, and community composition (Diaz & Rützler, 2001; Rützler, 2004; Becerro, 2008). Although most sponges are restricted to marine habitats, more than 200 species occur in freshwater (Reiswig et al., 2010). Sponges live in lakes, ponds, and streams world-wide (Reiswig et al., 2010) and they dominate some benthic communities (Jewell, 1936; Frost et al., 1982; Bailey et al., 1995). For example, sponges cover 44% of the benthos of the largest lake in the world (Lake Baikal, Russia; Pile et al., 1997) and contribute nearly half of the total

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benthic secondary production in parts of the River Thames, England (Mann et al., 1972). Despite their widespread distribution, often high local abundance, and potentially substantial contribution to ecosystem dynamics, sponges have been largely overlooked by ecologists studying freshwater systems.

In marine systems, sponges link benthic and pelagic food webs by filtering pelagically fixed carbon from the water column and converting it to biomass in the benthos (Lesser, 2006; Pile, 2006). Sponges in freshwater systems likely play a similar role, but this has not yet been evaluated. Although the types and sizes of particles that freshwater sponges are capable of ingesting are well-known (Frost, 1980, Reiswig et al., 1981, 2010), their diets in situ are virtually unknown (Reiswig et al., 2010). The many gaps that remain in our knowledge, at least in part, stem from the inherit difficulties of studying these organisms under natural conditions. In situ observations of trophic interactions are made impractical by the microscopic size of their food and an anatomical preclusion of traditional gut content analyses. Stable isotope analyses of carbon and nitrogen offer a powerful tool for overcoming these difficulties and gaining insight to sponge trophic interactions. In this study, we used carbon and nitrogen isotope signatures to assess the relative contributions of pelagic and benthic resources to the diet of the cosmopolitan freshwater sponge, *Spongilla lacustris* Linnaeus. We hypothesized that, like marine sponges, *S. lacustris* links pelagic and benthic communities by feeding extensively on pelagic phytoplankton. To assess our hypothesis, we compared the isotopic signatures of *S. lacustris* to pelagic and benthic baseline indicator species with well-known trophic ecology. We predicted that heterotrophic *S. lacustris* have isotopic signatures similar to primary consumers that feed on pelagic phytoplankton.

Freshwater sponges are inhabited by a wide variety of aquatic organisms including bacteria, algae, protists, hydrozoans, nematodes, rotifers, insects, and crustaceans (Roback, 1968; Reiswig, 1971; Matteson & Jacobi, 1980; Gaino et al., 2004; Parfenova et al., 2008). The diverse group of organisms commonly associated with freshwater sponges includes a relatively small number of highly specialized sponge predators including caddisflies of the genus *Ceraclea* (Trichoptera: Leptoceridae), all members of Sisyridae (Neuroptera), and several chironomid midge species (Diptera:Chironomidae). The rarity of spongivory among benthic invertebrates is presumably the result of highly effective chemical and physical sponge defenses (Sata et al., 2002; Reiswig et al., 2010). Consequently, spongivorous taxa display a range of morphological and behavioral adaptations for consuming freshwater sponges (Brown, 1952; Resh, 1976, Roque, 2004; b). For example, spongivorous caddisflies sequester the physical defenses of their prey by reinforcing their silken cases with sponge spicules (Fig. 1).

Like marine sponges, many freshwater sponges benefit from a mixotrophic association with endocellular algae and can thus act as both primary producers and consumers (Reiswig et al., 2010). In fact, for some freshwater sponges algae constitute more biomass than animal tissues (Frost & Elias, 1985). Although marine sponges host many types of photosymbionts, freshwater sponges typically host only green algae (zoochlorellae; but see Frost, 1997 for an exception). The presence of zoochlorellae gives freshwater sponges a distinctive green color (referred to hereafter as "green sponges"), whereas those without algal symbionts are typically white, off-white, or yellow (referred to hereafter as "white sponges"). Sponges that are green with zoochlorellae will quickly turn white when shaded (Frost & Williamson, 1980) as zoochlorellae are digested by their host (Williamson, 1979). Therefore, many populations of freshwater sponges contain both green and white individuals as a

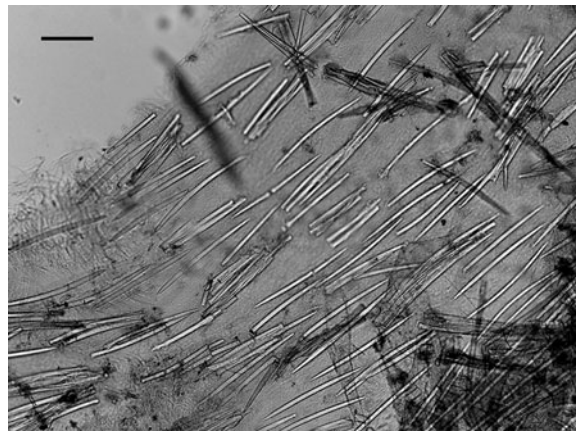

Fig. 1 Case of the sponge-eating caddisfly *Ceraclea resurgens* collected from Iron River near Big Bay, MI, viewed by light microscope. Case is composed of silk that has been reinforced with megasclere spicules sequestered from *Spongilla lacustris*. *Scale bar* 150 μm

result of spatial and/or temporal variation in light availability.

The presence of zoochlorellae may have major implications for specialist sponge predators. Results from marine systems have demonstrated that the endosymbiotic algae of sponges contribute largely to the nutrition of a sponge predator and strongly influence sponge predator distributions (Becerro, 2003). The contribution of zoochlorellae to the diets of freshwater sponge predators has not been previously reported, but histological examinations of sponge-eating caddisflies suggests that zoochlorellae may be an important component of their diet (Corallini & Gaino, 2001). In this study, we used natural stable isotope signatures to assess the importance of zoochlorellae to the diets of two freshwater sponge predators, a sponge-eating caddisfly (*Ceraclea resurgens* Walker) and a spongillafly (*Climacia areolaris* Hagen). To these ends, we assess the isotopic signatures of both sponge types and both sponge predators. Because zoochlorellae are exposed to the same boundary layer effects as other benthic primary producers and when present may constitute a considerable portion of the sponge's biomass (Frost & Elias, 1985) and overall production (Frost & Williamson, 1980), we predicted that sponges hosting zoochlorellae have carbon signatures similar to those of benthic primary producers. Based on previous observations that emphasize the importance of photosymbionts to the diets and food preferences of sponge predators (Corallini & Gaino, 2001; Becerro, 2003), we predicted that the isotopic signatures of sponge-eating caddisflies and spongillaflies would reflect diets comprised mainly of sponges hosting zoochlorellae.

Methods

Spongilla lacustris were sampled from Iron River and Morgan Pond in Marquette County, Michigan, USA. Iron River is a south shore tributary of Lake Superior. It is fed by a small spillway from Lake Independence, which is a shallow 752 ha mesotrophic lake fed primarily by Yellow Dog River. Land use around Lake Independence and along Iron River is limited to residential housing. The study site was a 7–10 m wide (wetted width) erosional reach immediately downstream of the spillway with a mostly open overhead canopy. The benthic substrate was composed mainly of granitic boulders and bedrock which were largely covered by green *S. lacustris* during peak sponge abundance (August–October). The underside of boulders and cobbles, and other shaded areas were often encased in yellowish-white, aposymbiotic sponges. Net-spinning caddisflies (*Ceratopsyche* sp.), perlid stoneflies (*Paragnetina media* Walker), flat-headed mayflies (*Stenonema tripunctatum* Banks), amphipods (*Hyalella azteca* De Saussure), rusty crayfish (*Orconectes rusticus* Girard), and sponge-eating caddisflies (*C. resurgens*) were abundant on and among the sponges.

Morgan Pond is a 2.6 ha, mesotrophic beaver pond fed by a first-order spring creek. Dominant aquatic macrophytes include *Potamogeton*, *Vallisneria,* and *Nuphar.* The pond edge is primarily colonized by speckled alder (*Alnus rugosa* Du Roi), sweet gale (*Myrica gale* Linnaeus) and cattail (*Typha* sp.) and is largely undeveloped. *Spongilla lacustris* occurred as distinctly individual and often large colonies composed of many long finger–like branches that either protruded from soft sediments or grew on woody emergent vegetation and debris.

We assessed the ratio of the heavy to light stable isotopes of carbon (^{13}C:^{12}C) and nitrogen (^{15}N:^{14}N) to infer the trophic status and primary energy source(s) of *S. lacustris* and sponge predators from Iron River and Morgan Pond. The methods, assumptions, and utility of this approach are discussed in Minigawa & Wada (1984), Peterson & Fry (1987), France (1995), Cabana & Rasmussen (1996), Vander Zanden & Rasmussen, (1999), Post (2002), and Fry (2006). We collected and individually analyzed 8 green and 8 white sponges from each site to assess the effects of zoochlorellae on sponge isotope signatures. Individuals of each type were selected arbitrarily from locations throughout the study sites. At Iron River, 8 late-instar (4th or 5th) sponge-eating caddisflies were collected from each sponge type and analyzed individually. All caddisflies were taken from different individual sponges. Individual spongillaflies collected from Morgan Pond were of insufficient mass to constitute a usable sample for isotope analysis. Therefore, each sample of spongillaflies was comprised of 3 penultimate instar larvae that were pulverized and homogenized. For all other taxa, we combined and homogenized many individuals within each taxon before isotope analysis (Table 1). Although pooling individuals of these taxa precluded any measurement of variance within each

population, our inclusion of many individuals per sample insured a robust estimate of the mean isotope signature of each population (Fry, 2006).

Carbon and nitrogen signatures of the primary producers that form the base of aquatic food webs vary substantially among systems (Cabana & Rasmussen, 1996; Vander Zanden & Rasmussen, 1999; Post, 2002). To determine baseline carbon and nitrogen signatures ($\delta^{13}C_{Base}$ and $\delta^{15}N_{Base}$) of Morgan Pond, we sampled the unionid mussel *Pyganodon* sp. and the snail *Amnicola limosa* Say. We sampled the mussel *Eliptio complanata* Lightfoot and snail *Campeloma decisum* Say to establish baselines for pelagic and benthic food webs for the Iron River site. Unionid mussels specialize on filtering phytoplankton and snails primarily scrape epilithic algae, therefore these taxa can be used to infer pelagic and benthic baseline carbon signatures (Post, 2002). We chose to sample primary consumers, rather than directly measuring baseline organisms (i.e., primary producers) because long-lived primary consumers can absorb much of the considerable short-term variation in baseline signatures and thus give a better estimate of the long-term average baseline signatures of a system (Post, 2002). Mussels and snails were not present immediately below the Lake Independence spillway on the Iron River and were consequently collected from Lake Independence, within 10 m above the spillway. Our primary interest was to assess the contribution of pelagic primary production within Lake Independence to the production in *S. lacustris* in the Iron River and mussels positioned immediately above the spillway were assumed to have access to the same pelagic resources as *S. lacustris* that were positioned immediately below the spillway. To assess potential differences in benthic carbon signatures above and below the spillway we also sampled *Hyalella azteca* from the river, a taxon that feeds extensively on periphyton (Hargrave, 1970).

Table 1 Sample type and size for all samples collected for isotopic analyses from Iron River and Morgan Pond

Sample type	No. of samples	Total no. of inds.
Iron River		
Spongilla lacustris		
Green	8	8
White	8	8
Ceraclea resurgens		
From green sponge	8	8
From white sponge	8	8
Baseline indicators		
Campeloma decisum	1	30
Eliptio complanata	4	12
Hyalella azteca	1	>50
Morgan Pond		
Spongilla lacustris		
Green	8	8
White	8	8
Climacia areolaris	4	12
Baseline indicators		
Amnicola limnosa	1	20
Pyganodon sp.	1	5

All samples were collected by hand or D-frame net during August of 2007. All invertebrates to be analyzed whole were kept at 5°C and allowed 24 h for gut clearance before being killed by freezing. We removed the shells and opercula of all snails and mussels because the isotopic composition of the shell and operculum is influenced by factors unrelated to diet, such as inorganic carbon sources (Post, 2002). All invertebrates were then cleaned in deionized water by cavitation using an ultrasonic wave cleaner for 30 s to remove any debris and then dried at 50°C for 24–48 h. Samples were pulverized to a fine homogeneous powder and fumed with concentrated HCl for 24 h to remove any inorganic carbon residues. All samples were then returned to 50°C for 24 h to drive off any remaining acid and were then weighed and loaded into 3.5 mm tin capsules and sent to The Alaska Stable Isotope Facility at the University of Alaska Fairbanks for analysis.

We used the carbon signatures of baseline indicator taxa to estimate the composite contributions of benthic and pelagic primary energy sources to *S. lacustris* using a two-end-member-mixing model: $\alpha = (\delta^{13}C_{consumer} - \delta^{13}C_{base2})/(\delta^{13}C_{base1} - \delta^{13}C_{base2})$, where α is the proportion of consumer carbon from the source base 1. For all sponge samples, base 1 was the pelagic baseline indicator species and base 2 was the benthic baseline indicator species. We did not correct for carbon fractionation during trophic transfer because this effect is usually small, variable, and often close to zero (Post, 2002). Specialist sponge predators are known to feed exclusively on freshwater sponges and we were interested in the relative contributions of each

sponge type (green vs. white) to their diet. Thus, to determine the contribution of each sponge type to the diet of sponge predators we used a mixing model in which green sponges were used as base 1 and white sponges as base 2.

Mean carbon and nitrogen values from sponges were compared using two-way analysis of variance (ANOVA) with site and sponge type as factors. Specific comparisons of mean isotope signatures were made using two-sample *t*-tests. Assumptions of equal variance and normality were verified using Fisher's *F* tests and Shapiro-Wilks tests, respectively.

Results

Carbon signatures of *S. lacustris* were significantly different among sites ($F_{1,28} = 2{,}529$, $P < 0.0001$), sponge types ($F_{1,28} = 3{,}306$, $P < 0.0001$), and there was a significant interaction between site and sponge type ($F_{1,28} = 1{,}402$, $P < 0.0001$). In general, the carbon signatures of sponges were more depleted in Morgan Pond than in Iron River and sponges hosting zoochlorellae had depleted carbon values compared to those without. The mean difference among carbon signatures between sponge types was considerably larger in Morgan Pond than Iron River (5.85‰ and 1.21‰, respectively). Nitrogen signatures of *S. lacustris* were also significantly different between sites ($F_{1,28} = 20.55$, $P < 0.001$), sponge types ($F_{1,28} = 144.07$, $P < 0.001$), and there was an interaction between site and type ($F_{1,28} = 63.44$, $P < 0.001$).

Iron River

The carbon signatures of pelagic and benthic baseline indicator species from Iron River were −32.51‰ and −22.43‰ (respectively) and carbon signatures of white *S. lacustris* from Iron River were similar to pelagic baseline indicators from Lake Independence (mean = −32.16; Fig. 2). Mixing model results indicated that the carbon from white *S. lacustris* in Iron River was almost exclusively of pelagic origin when either snails (mean ± SD = 0.98 ± 0.02, estimated proportion of pelagic carbon) or *H. azteca* (mean ± SD = 0.97 ± .02) were used as the benthic baseline for carbon signature. Green and white sponges had a small but significant difference in mean carbon signatures (mean $\delta^{13}C = -33.50$ and −32.16, respectively, $t = 16.63$, $df = 14$, $P < 0.0001$). We could not effectively use a mixing model to determine the relative contribution of each sponge type to the diets of sponge-eating caddisflies because their carbon signatures fell outside of the range bounded by green and white sponges. Sponge-eating caddisflies collected from green sponges had significantly depleted carbon signatures to those collected from white sponges ($t = -3.49$, $df = 14$, $P = 0.002$), and significantly depleted nitrogen signatures ($t = -1.82$, $df = 14$, $P = 0.045$).

Morgan Pond

In Morgan Pond, the carbon signatures of the baseline indicator species for littoral (34.49‰) and pelagic (35.45‰) carbon showed much less disparity than Iron River. During sample collection, *Cl. areolaris* were found only on green individuals of *S. lacustris.* Carbon signatures of *Cl. areolaris* were nearly identical to green *S. lacustris* (Fig. 2) and mixing model results indicated that the carbon of *Cl. areolaris* was exclusively from green sponges (mean ± SD = 0.98 ± .02, proportion of carbon from green sponges).

Discussion

Our results from Iron River suggest that *S. lacustris* may serve as an energetic link between pelagic primary production in an upstream lake and benthic production in a stream. Sponges that do not host zoochlorellae must obtain all of their carbon by heterotrophic processes and therefore their carbon signature is assumed to reflect the carbon signature of their diet. White *S. lacustris* from Iron River had carbon signatures that were nearly identical to the baseline indicator species for the pelagic primary producers (*E. complanata*), which was ∼10‰ depleted when compared to both benthic baseline indicators (*Ca. decisum* and *H. azteca*). The two benthic baseline indicators had similar carbon signatures (−22.45 and −23.55) and using either in our mixing model resulted in an estimated a 97–98% contribution of pelagically fixed carbon to the diet of *S. lacustris*. Previous work has emphasized the importance of phytoplankton in the diets of freshwater sponges (Frost, 1980; Frost, 1981; Pile et al., 1997). However, nitrogen signatures for white sponges in

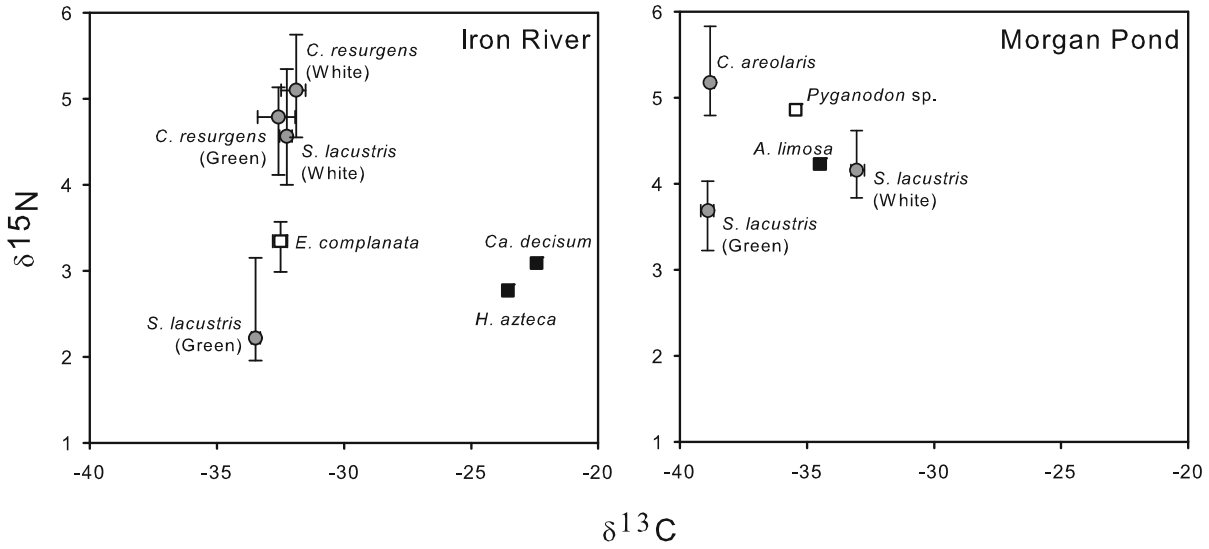

Fig. 2 Bi-plots of carbon and nitrogen signatures (δ^{13}C and δ^{15}N) for samples from Iron River (*left*) and Morgan Pond (*right*). *Symbols* indicate the mean carbon and nitrogen signatures for each taxon (or type). *Open* and *closed squares* indicate pelagic and benthic baseline indicators, respectively. Whiskers show total observed ranges. *S. lacustris* (*green*) indicates sponges hosting zoochlorellae. *S. lacustris* (*white*) indicates sponges not hosting zoochlorellae. *C. resurgens* (*green*) indicates individuals collected from *greens sponges*. *C. resurgens* (*white*) indicates individuals collected from *white sponges*

Iron River were considerably higher than our baseline indicators species (Fig. 2), which are known primary consumers. This suggests that other nitrogen sources may be important to the sponge diet. Other possible nitrogen sources include colloidal organic matter, heterotrophic protists, and bacteria (Reiswig, 1971). Because freshwater sponges can comprise a considerable portion of the benthic biomass and secondary production in lakes and streams (Jewell, 1936; Frost et al., 1982; Bailey et al., 1995; Pile et al., 1997), our results suggest that freshwater sponges may serve as an important energetic link between pelagic and benthic foodwebs in systems where sponges are dominant members of the benthic community.

The spatial distribution of freshwater sponges may reflect the importance of lake derived resources. In Iron River, sponges are abundant within ~300 m of the spillway, and scarce or absent further downstream (J. Skelton personal obs.). A similar pattern was noted by Jewell (1936) in a survey of northern Wisconsin lakes that identified lake outlets as the preferred habitat of several sponge species. It seems likely that this distributional pattern is the result of high suspended resource availability immediately downstream from lake outlets.

Benthic and pelagic indicator species from Morgan Pond had similar carbon signatures and were therefore not useful for separating pelagic and benthic carbon sources. Morgan Pond is small (~2 ha) and shallow (max depth <3 m). It is possible that its small size may limit the effects of the physical processes responsible for creating different littoral and pelagic baseline signatures (France, 1995). Second, the benthic baseline indicator species (*A. limnosa*) is much smaller and shorter-lived than the pelagic baseline indicator (*Pyganodon* sp.). As a result, there is a potential for temporal discontinuity in our estimates of pelagic and littoral baseline signatures because the tissues of the mussels would have integrated baseline variation over a greater timeframe than those of the snails (Post, 2002). Therefore, the carbon signatures of these two taxa may represent the mean baseline signature over two different time scales.

Contrary to our predictions, the carbon signatures of green sponges were significantly *depleted* at both sites. One possible explanation for this pattern is that zoochlorellae utilize the isotopically depleted metabolic CO_2 respired by the sponge. A similar association is known for zooxanthelle and corals (Muscatine et al., 1989). Nitrogen signatures were also significantly

depleted in green sponges. Similar to carbon, nutrient recycling of zoochlorellae may explain the depleted nitrogen signature of green sponges. Nitrogen signatures of consumers increase with each trophic step because consumers tend to excrete the lighter isotope at a higher rate than the heavier isotope (Minigawa & Wada, 1984). Therefore, the nitrogenous wastes of a consumer have a nitrogen signature that is more depleted than the consumer's tissues. Nitrogen transfer from sponges to their algal symbionts has been previously demonstrated in marine systems. Pile et al. (2003) showed that the total nitrogen demand of the rhodophyte *Ceratodictyon* can be met by the heterotrophic processes of its symbiotic sponge partner *Haliclona.* Thus, the depleted nitrogen signature of green *S. lacustris* may be explained by zoochlorellae assimilating isotopically light nitrogenous waste of *S. lacustris*, preventing its excretion from the sponge.

We observed a significant interaction between site and sponge type in their combined effects on sponge carbon signatures that might be explained by physical characteristics of the two sites sampled. The mean difference in carbon signatures between green and white sponges was much greater in Morgan Pond (5.85‰) than in Iron River (1.21‰). Based on observations of coral and zooxanthelle isotope signatures at varying depths, Muscatine et al. (1989) hypothesized that in shallow water there is less discrimination of ^{13}C during fixation by zooxanthelle because the rate of photosynthesis greatly exceeds the rate of coral respiration. Therefore, zooxanthelle have a carbon signature that approaches the value of the internal pool which is supplied mainly by heterotrophic respiration and is thus similar to the animal tissues. Photosynthesis is surpassed by respiration at greater depths. Consequently, ^{13}C discrimination during fixation is greater in deeper water and there is a larger discrepancy in carbon signatures between the host and its photobionts. The green sponges of Morgan Pond were typically found at a depth of $\sim$1 m, whereas those in Iron River were typically found in $<$0.5 m of water. Thus some of the inter-site variation that we observed may be attributable to the effects of colony depth and resultant differences in insolation that in turn affect the rate of zoochlorellae photosynthesis.

Symbioses between invertebrates and photoautotrophs can have implications for the ecology and evolution of other consumers. For example, the opistobranch *Tylodina perversa* prefers individuals of the sponge *Aplysina aerophoba* that host high concentrations of symbiotic cyanobacteria (Becerro, 2003). Sponges hosting high concentrations of symbiotic cyanobacteria and *T. perversa* are limited to shallow water, suggesting that the prevalence of photobionts in shallow waters has shaped the ecological distribution of *T. perversa* (Becerro, 2003). Our results provide evidence of a similar spongivore preference in response to the presence of zoochlorellae. Spongillaflies collected from Morgan Pond were found only on green sponges and their carbon signatures indicated that they fed exclusively on green sponges. Although we could not use a carbon source mixing model to estimate the relative contributions of each sponge type to the diet of sponge-eating caddisflies, their nitrogen signatures suggest that green sponges are their predominant food source. Consumers typically have nitrogen signatures that are $\sim$3.4‰ enriched from their food (Minigawa & Wada, 1984; Vander Zanden & Rasmussen, 2001; Post, 2002). The nitrogen signatures of sponge-eating caddisflies were $\sim$3‰ enriched over green sponges, and $<$1‰ enriched over white sponges. However, it is clear that sponge-eating caddisflies feed on both sponge types to some degree. In addition to finding sponge-eating caddisflies on both sponge types, we also observed furrows left in sponge tissue as a result of caddisfly feeding activity that transected adjacent green and white sponges, indicating that individual sponge-eating caddisflies may switch from one type to the other. Sponge-eating caddisflies collected from white sponges had slightly but significantly enriched carbon and nitrogen signatures compared to those collected from green sponges, suggesting that they did not frequently switch from one type to the other. Together, our observations suggest that the sponge-eating caddisfly *C. resurgens* makes use of both sponge types, but that green sponges comprise the larger fraction of their diet.

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