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TROPHIC BASIS FOR RESTORATION OF FISH FAUNA IN RESTORED URBAN STREAMS

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ABSTRACT

Urban streams are greatly impacted by runoff, construction, loss of riparian buffers, and input of various anthropogenic materials. Past studies have shown that terrestrial subsidies from riparian zones can be important to aquatic communities in pristine, non-urban systems. However, urban streams typically lack forested buffer zones. In this project, we evaluated the importance of terrestrial subsidies to fish populations in streams affected by urbanization including unrestored reaches, reaches with restored riparian zones and some channel restoration, and forested reaches that were below waste water treatment plants (WTTPs). We examined the importance of terrestrial subsidies using stable isotopes of N and C, and using gut analyses of fishes. Our previous work has shown that components of the food web below a WWTP are enriched in ^{15}N and ^{13}C relative to terrestrial leaf material, reflecting utilization of sewage-derived N and C in the food web. Our specific objectives were to (1) evaluate the effectiveness of stream restoration in restoring abundance and richness of stream fish communities compared to unrestored and wastewater influenced forested streams; (2) assess availability of aquatic food resources and terrestrial subsidies in each of the stream reaches; and (3) evaluate the importance of terrestrial subsidies from riparian zones in supporting fish diets and growth in restored versus unrestored and forested streams using traditional gut analyses studies and ^{15}N and ^{13}C stable isotope signatures.

Fish from restored sites showed significant $\delta^{13}C$ depletion and $\delta^{15}N$ enrichment compared to unrestored sites, which is consistent with increased utilization of terrestrial detritus as a basal resource. Restored sites also had significantly higher species richness than unrestored sites. Total abundances of fish showed no significant difference overall, although there was a strong trend toward more fish in the restored sites. Abundance and taxa richness of terrestrial and aquatic macroinvertebrate was not significantly different between sites. However, aquatic and terrestrial macroinvertebrates showed a trend for higher richness and abundance at restored sites. Aquatic macroinvertebrates had significantly higher $\delta^{15}N$ compared to terrestrial invertebrates. Seston and FBOM had significantly higher $\delta^{15}N$ in the forested compared to the restored and unrestored sites. Other basal resources, although not significant, showed a trend of greater ^{15}N enrichment at forested sites. Both isotope mixing models and gut content analysis supported the conclusion that most fish were relying primarily on aquatic resources.

The carbon isotope data suggest that these urban stream restorations efforts are beginning to result in a shift toward greater utilization of terrestrial detrital resources by the stream biota. Higher fish abundance and taxa richness in restored reaches show that riparian restoration has been effective in improving the aquatic habitat. Thus, riparian restoration has been beneficial to fish communities, and should be considered as a management option for urban streams.

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SUMMARY AND CONCLUSIONS

We evaluated the importance of terrestrial subsidies to fish populations in streams affected by urbanization including unrestored reaches, reaches with restored riparian zones and some channel restoration, and forested reaches that were below waste water treatment plants (WTTPs). We examined the importance of terrestrial subsidies using stable isotopes of N and C, and using gut analyses of fishes. Fish were significantly more depleted in ^{13}C at restored sites than at unrestored sites. This result is important because it illustrates the increased role of riparian subsidies to the stream food web. This isotopic shift in the fishes is most likely due to increased use of detrital resources by aquatic macroinvertebrates, which were the most important component of fish diets, although increased use of terrestrial insects would also contribute to a lower $\delta^{13}C$ value in fish . Despite these trends, aquatic carbon was still the dominant basal resource for most fishes. As riparian regrowth continues, the importance of the terrestrial subsidy should also increase.

At sites below sewage treatment plants, much of the carbon in fish tissue is derived from sewage. The $\delta^{13}C$ of treated sewage coming into the stream appears to be about -26‰ (value of seston at those sites). Fish at these sites are 2 trophic levels removed from seston, so are slightly enriched compared to that value, about -25.4‰ (comparison of Figures 1 and 6). Isotope data showed that all fish as well as aquatic invertebrates were enriched in ^{15}N downstream of the WWTPs. These results are important because all of the sites below WWTPs were in forested reaches of their watersheds, where we would expect terrestrial inputs to be important. However, the large quantity of treated sewage entering the stream, in the form of seston and dissolved organic carbon, apparently overwhelmed the signal of terrestrial detritus in the food web.

We expected to see an increase in abundance and richness of fish, as well as terrestrial and aquatic invertebrates, at restored sites compared to unrestored sites, due to the increased canopy cover and improved aquatic habitat at those sites. The patterns observed were all consistent with that hypothesis, although the trends were not significant for all metrics. The trend toward improved macroinvertebrate and fish abundance and richness at restored sites that we observed, in combination with the trend toward greater use of terrestrial carbon at restored sites, suggests that the improved habitat and water quality at these sites is beginning to show a long-term benefit to the aquatic biota.

Our study illustrates an approach that should be useful to managers for evaluating the degree to which stream restoration has been successful in restoring the ecological function in urban streams, particularly with respect to fish abundance and habitat distribution, and the trophic basis for their production. These results are directly applicable for planning and implementing future restoration projects, which are growing in number in many urban areas, and for anticipating the time course required for restoration of the stream biota.

RECOMMENDATIONS

In light of the high cost of restoration projects, consideration needs to be given to repairing biological functions in addition to physical improvements of the restored system. It is important to continue monitoring of restored sites. With increased riparian canopy development, terrestrial leaf litter should become an increasingly important subsidy to the stream. Because the $\delta^{13}C$ value of periphyton and leaf litter are distinct in these streams, analysis of stable isotopes of carbon provides and economical monitoring tool for detecting such a shift, and should be considered either instead of or in addition to more traditional monitoring efforts that involve a large investment in sample processing and a high level of taxonomic expertise.

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INTRODUCTION

Healthy streams play a critical role in protecting downstream water quality through incorporation and retention of nutrients into algal and bacterial producers and transfer of those nutrients to macroinvertebrate and fish consumers. Stream restoration projects have been conducted or are underway in several NC cities and elsewhere. In the Pacific Northwest, much of the impetus for restoration efforts has been to restore salmon runs (Roni et al. 1996). In contrast, urban stream restoration projects have focused on bank stabilization, flood control, recreational value, and aesthetics (see Brady 1996, Kondolf 1996). A restoration effort in Strawberry Creek on the University of California-Berkeley Campus resulted in changes from a level of poor water quality to one of good water quality, including an improved NCBI biotic index, increased taxa richness, and improved ability to support native species (Charbonneau and Resh 1992). Changes in land use in the Yadkin basin have resulted in slow but detectable improvements in suspended sediment in the Yadkin River, NC (Richter et al. 1990). However, despite the interest in restoration, for most restoration projects little attention has been paid to effectiveness at restoring ecological services, including biotic integrity. The EPA (Brady 1996) and others have emphasized the need to focus on restoration of ecological processes in order for restoration efforts to be meaningful. To address this deficiency, we conducted a study to quantify the degree of recovery of the fish community and the trophic basis for fish production in restored and unrestored urban streams, and in forested streams that were impacted by sewage effluent.

The fish fauna of North and South Buffalo Creeks is dominated by species characterized as pollution tolerant, especially redbreast sunfish (*Lepomis auritus*), green sunfish (*Lepomis cyanellus*), mosquitofish (*Gambusia holbrooki*) and red shiner (*Cyprinella lutrensis*) (NC DENR 1999). Among 17 sites in the Cape Fear River Basin that were assessed by the NC DENR in 1998 (NC DENR 1999), 2 stations on South Buffalo Creek had the two lowest ratings, and the site on North Buffalo Creek was also rated as “Poor” on the NC Index of Biotic Integrity (NCIBI) for fish. These sites also received rating of Poor for macroinvertebrate NC Biotic Index (NCBI, see Lenat 1984, 1993) in the 1999 report. Substrate for macroinvertebrates is limited, densities are low, and dominant taxa are Chironomidae, which are less available and desirable to fish than many other macroinvertebrate taxa. Nonetheless, these restored areas appear to support high fish density. Given the impaired macroinvertebrate community, the trophic basis for supporting a high fish density in not clear. In this project, we evaluated the importance of terrestrial subsidies compared to aquatic resources to support their recovery. If important, terrestrial subsidies would provide further incentive for managing urban streams for forested buffer zones. We examined the importance of terrestrial subsidies using natural abundances of stable isotopes of nitrogen (^{14}N and ^{15}N) and carbon (^{12}C and ^{13}C). Our previous work has shown that components of the food webs of North Buffalo Creek are enriched in ^{15}N and ^{13}C relative to terrestrial leaf material, reflecting anthropogenic N and C sources to the stream (Ulseth and Hershey 2005). Thus, the $\delta^{15}N$ value of fish can be used to quantitatively measure the importance of assimilated terrestrial versus aquatic resources in supporting fish growth. Because most urban streams would be expected to be enriched in ^{15}N, this approach should be applicable to other systems. Our specific objectives were to (1) evaluate the effectiveness of stream restoration on restoring abundance and richness of stream fish communities compared to unrestored and wastewater influenced forested streams; (2) assess availability of aquatic food resources and terrestrial subsidies in each of the stream reaches; and (3) evaluate the importance of terrestrial subsidies from riparian zones in supporting fish diets and growth in restored versus unrestored and forested streams using traditional gut analyses studies and ^{15}N and ^{13}C stable isotope signatures.

METHODS

Study Sites

Study sites for this study were chosen based on previous studies of urban stream restoration in Greensboro, NC, (Ulseth and Hershey 2005, Lynam 2004) and advice from a water quality professional in Charlotte, NC (Peine, Charlotte-Mecklenburg Stormwater Services, personal communication). North and South Buffalo Creeks are mid-order streams, in the piedmont of North Carolina, with their headwaters originating in Greensboro, NC, a city of 223,891 people (US Census Bureau 2000). Both streams lie within the Haw River catchment of the Cape Fear River Basin. North and South Buffalo Creeks are directly impacted by the effects of urbanization. Wastewater treatment plants (WWTPs) on both creeks have resulted in poor water quality downstream of these plants (NCDENR 2004). Point source contamination from the WWTPs and non-point pollution from other urban sources have resulted in EPA 303(d) listings for both creeks with Total Maximum Daily Load (TMDL) plans under development (NCDENR 2004). In response to these problems, the city has made efforts over the past decade to restore certain sections of both streams in an effort to improve the biological health and recreational value of the streams.

Little Sugar Creek is a mid-order stream located in the Catawba River watershed, with headwaters in Charlotte, NC (population of 540,828, US Census Bureau 2000). Little Sugar Creek has a history of poor water quality and poor riparian buffers due to heavy urbanization and the WWTP effluent that it receives. Although the creek’s ratings have improved slightly for macroinvertebrates, water quality is still low (NCDENR 2003a). Some restoration efforts have been attempted on the stream in recent years, but most of the creek remains unrestored.

Three site types on each stream were assessed for this study: urban restored, urban unrestored, and non-urban forested. Urban restored sites were classified as those areas of the stream that have undergone riparian reconstruction or regrowth, and, in some cases, in-stream reconstruction in the last 11 years. Lake Daniel Park, located on North Buffalo Creek, has been allowed to regrow its riparian zone since 1994/1995 with additional riparian planting in recent years (Schneider, personal communication, City of Greensboro, Water Resources Dept.). Hillsdale Park, on South Buffalo Creek, was fully restored in 2003 with riparian replantings and instream structural restoration (Schneider, personal communication, City of Greensboro, Water Resources Dept.). Restoration of Little Sugar Creek at Freedom Park in Charlotte was completed in the summer of 2003 with the reconstruction of meanders in the stream, the landscaping and stabilization of riparian zones, and the structuring of in-stream pool-riffle sequences (Charlotte-Mecklenburg Government 2005).

Urban unrestored sites were classified as those parts of the stream within a highly urbanized section of the city with no previous restoration work done. The section of North Buffalo Creek in Latham Park is very impacted by extremely high stormwater runoff from Wendover Avenue and the surrounding neighborhood streets, leading to heavily incised banks and poor water quality. An unrestored site on South Buffalo Creek is also highly impacted as it runs parallel to Holden Road and under I-40. The Midtown portion of Little Sugar Creek is located in the middle of downtown Charlotte, underneath a parking deck, at the intersection of three major highways, and has frequent inputs of automotive parts and fluids from the auto shop adjacent to it.

Non-urban forested sections were located outside of the highly urbanized city centers with all three sites downstream from WWTPs. Near Greensboro, these forested sections of North and South Buffalo are located on private properties along Creekview and Harvest Roads, respectively. The forested section of Little Sugar Creek is located outside of Charlotte in a less populated area near the South Carolina border.

Sampling Schedule

All sampling was completed during the summer months of 2004. Drift sampling, Rapid Bioassessment, and malaise trapping were conducted three times at each of the nine sites, and fish were sampled once at each site. All sites were sampled at approximately the same hour for each of the visits and in the same order. Stream sampling took place at base flow, or three days after a rain event.

Fish Sampling

Prior to sampling, a 100m transect was marked along the bank of each stream. Fish were collected by shocking one sweep upstream of the entrance site and returning downstream using back-mounted 12-volt battery-powered electrofishing units (BADGER™, Engineering Technical Services, UW-Madison, Madison, WI and Smith-Root, Inc., Vancouver, WA). Stunned fish were collected using dip nets and placed in buckets of stream water. At the end of the shocking runs, fish were identified using Menhenick (1991), measured for total length, and categorized by functional feeding groups (NCDENR 2001; Etnier and Starnes 1993; Rohde et al. 1994; Ross et al. 2003). For each species, 3-5 individuals were killed by cranial concussion and returned to the laboratory on ice. Remaining fish were returned to the stream. For isotope analysis, tail muscle was removed and dried. Guts were removed and placed in 70% ethanol for later gut content analysis.

Benthic Macroinvertebrate Collection

Stream macroinvertebrates were sampled using EPA guidelines for the Rapid Bioassessment Protocol established by Barbour et al. (1999). Samples collected by dip netting, kick netting, and rock surveys were stored in 20mL scintillation vials with 95% ethanol. In the laboratory, samples from each site were separated into their respective orders and identified to the lowest possible taxonomic level using the keys of Brigham et al. (1982), Epler (2001), and Merritt and Cummins (1996). Larval chironomids (Diptera) were mounted on microscope slides using CMC-10 Mounting Media (Masters Company, Inc., Woodale, IL) and kept on a slide warmer for at least 72 hours prior to identification in order to set the larvae in the media.

Additional samples of macroinvertebrates were collected from the streams for stable isotope analysis. Macroinvertebrates were stored in 250mL Nalgene™ bottles with stream water and placed on ice to slow metabolism for transportation to the laboratory. Samples were then passed through a 1mm sieve to remove the stream water and any associated particulate matter. The bottles were washed and filled with deionized water and the macroinvertebrates were held overnight for evacuation of gut contents. Macroinvertebrates were separated into taxonomic groups and dried for 48 hours in an oven at 60°C prior to grinding and processing for isotope analysis.

Malaise Trapping

Flying riparian insects were collected using malaise traps (Product No.: 2875AG, BioQuip, Inc., Rancho Dominguez, CA) mounted on each bank of each of the nine sites sampled in this study. Two traps were mounted per site and staked at randomly selected locations along the streams prior to dusk. Samples were collected in traps for approximately 15 hours before being taken down the following morning. Sample jars from the top of the malaise traps were removed and stored on ice for transport to the lab. Jars were frozen overnight in order to easily remove the trapped insects. Insects were transferred to 20mL scintillation vials and kept frozen until needed. Insects were then identified to order using Borror and White (1970) prior to being dried and processed for isotope analysis.

Particulate Organic Matter Sampling

Three types of organic matter were collected: coarse particulate organic matter (CPOM), fine benthic organic matter (FBOM), and suspended particulate organic matter (seston). CPOM are particles greater than 1mm in diameter, and included materials such as leaves and woody matter (Cummins 1974). A $0.093m^2$ Surber sampler was used to collect CPOM, in the form of leaf packs and woody debris. The Surber sampler was placed at a random spot on the stream bed with the open end facing upstream so that when the sample was removed from the stream, it would flow into the net. CPOM was taken to the laboratory on ice, where the samples were passed through a 1mm sieve to remove small particles. CPOM from each site was dried in a 60°C oven for 48 hours, and ashed in a muffle furnace at 550°C. Ash-free dry mass (AFDM) was calculated using methods in Minshall (1996) and biomass (g/m^2) was determined. Additional non-random samples of CPOM from each site were collected and dried in a 60°C oven for 48 hours and homogenized for stable isotope analysis.

Fine benthic organic matter (FBOM) was collected from the streambed at randomly selected sites along the reach using a 35mL turkey baster. Samples were placed in 250mL Nalgene™ bottles and placed on ice for transport to the laboratory, then passed through a 1mm sieve. The sieved sample was filtered onto pre-ashed 47mm Millipore™ glass fiber filters (Millipore Corp., Bedford, MA 01730) using vacuum filtration. Filtered samples were dried in a 60°C oven for 48 hours. Dried FPOM was acid washed using 1N HCl to remove any inorganic carbonate contamination that might influence $\delta^{13}C$ results.

Suspended particulate organic matter, or seston, was collected for isotope analysis. Although seston is not a major food source for fishes, it is important in the diets of many benthic macroinvertebrates (Merritt and Cummins 1996, Wallace and Cummins 1980), which fish do eat. Four-liter cubitainers were placed facing upstream at each site, allowing stream water to flow into them. Samples were placed on ice for transport to the laboratory. Seston was filtered onto pre-ashed 47mm Millipore™ glass fiber filters using vacuum filtration. Filters for both FBOM and seston were sent to Colorado Plateau Stable Isotope Laboratory for analysis.

Periphyton

Periphyton for stable isotope analyses was sampled from nine randomly selected rocks in each stream site. A wire brush was used to scrape the surface for periphyton, which was washed into a 250mL Nalgene™ bottle with DI water. Periphyton samples were then placed on ice for transport. Samples were filtered onto pre-ashed 47mm Millipore™ glass fiber filters using vacuum filtration and dried for 48 hours in a 60°C oven.

Additional samples of periphyton were taken from randomly selected rocks at each site. Using a plastic projector slide as a template, wire brushes were used to scrape periphyton from an $8.40cm^2$ area on the rocks. Samples were washed into foil-covered 250mL Nalgene™ bottles using DI water and filtered as above, then frozen until needed. The chlorophyll *a* in the samples was used to determine the biomass of periphyton in the stream (Steinman and Lamberti 1996, APHA, AWWA, and WEF 1992).

Frozen periphyton filters were placed in 20mL foil-covered falcon tubes with 10mL of 90% buffered acetone and frozen for 24 hours. After 24 hours, filters were removed and the extract was analyzed with a Thermo Spectronic Genesys 10 UV spectrophotometer or a TD 700 fluorometer (Turner Designs, Inc., Sunnyvale, CA). Results in mass per unit area ($\mu g/cm^2$) were obtained, in part, by following the methods of Steinman and Lamberti (1996).

Driftnetting

Drifting aquatic organisms were collected by mounting driftnets in each stream. Four driftnets were mounted, facing upstream, in the streambed using rebar. Nets were allowed to remain in the stream for 15 minutes to collect samples. Samples obtained from the nets were immediately frozen for analysis. Frozen samples were then dried in a 60°C oven for 48 hours and ground for stable isotope analysis.

Stable Isotope Processing

There were two major groups of isotope samples that were processed: filtered samples (periphyton, FBOM, seston) and dried organic samples (macroinvertebrates, fish, CPOM). Each sample was processed for natural abundance isotopes (^{13}C and ^{15}N). Dried sample filters for FBOM and seston were all placed in labeled 20mL scintillation vials for processing. All of the other samples were ground into a fine powder, placed in 4 x 6mm pressed tin capsules, sealed, and placed in 96-well plastic microtiter trays. All samples, both filtered and ground, were sent to the Colorado Plateau Stable Isotope Laboratory (CPSIL) of Northern Arizona University in Flagstaff, AZ for processing by gas isotope-ratio mass spectroscopy.

Mixing Models and Diet Composition

Seven species of fish were selected for the application of mixing models: an insectivore present at each site (*Lepomis auritus*), a top feeder (*Gambusia holbrooki*), a bottom feeder (*Erimyzon oblongus*), a catfish (*Ameiurus platycephalus*), and three representative shiners (Cyprinidae): (*Notemigonus crysoleucas, Notropis hudsonius, N.scepticus*). All three site types were respresented by these fish species, although some did not occur at each of the nine total sites.

Two-source mixing models (Fry and Sherr 1984; Post 2002; Vander Zanden and Vadeboncoeur 2002) were employed to discern contributions of terrestrial versus aquatic food resources in the diets of stream fish:

$$\% \text{ terrestrial} = [(\delta I_{consumer} - \delta I_{aquatic})/(\delta I_{terrestrial} - \delta I_{aquatic})] \times 100$$

where δI is the isotope concentration in ‰ relative to the standard (^{13}C or ^{15}N) from a given source (consumer, aquatic, or terrestrial). In addition to the above mixing model, the IsoSource model described by Phillips and Gregg (2003) was used. Calculations from IsoSource were based on isotopic ratios of both ^{13}C and ^{15}N for terrestrial insects, periphyton, and all aquatic macroinvertebrates available at that site. Fish gut contents were then compared to the results from the above mixing models in an attempt evaluate fish diets in these urban stream systems. All consumer (fish) isotopes were adjusted for one trophic position above their potential food sources before being placed in a mixing model; 2.2‰ for $\delta^{15}N$ and 1‰ for $\delta^{13}C$ (McCutchan et al. 2003).

Data Analysis

Normality of data was assessed using the Shapiro-Wilkins test. Only AFDM was found to be non-normal and was natural log transformed to resolve this issue. Two-way ANOVAs were performed on fish isotope, abundance, and richness data, using site type and functional feeding group (FFG) designations as factors. Another two-way ANOVA was used on macroinvertebrate isotope data using site type and source (aquatic vs. terrestrial) as factors. All other data were compared using one-way ANOVAs to assess site type differences. Tukey's HSD poc-hoc tests were applied to all significant ANOVA results to assess individual site type differences. All data was analyzed using SAS v.9.1.1 and JMP v.5.1.2 (SAS Institute, Inc., Cary, NC).

RESULTS

Fish

Highest species richness was found in the insectivore FFG compared to omnivores and piscivores ($p < 0.0001$). However, each of the fish FFGs had similar $\delta^{13}C$ ($p=0.3264$) and $\delta^{15}N$ ($p=0.3448$) mean values, suggesting less separation in diet sources than would be expected based on traditional taxonomic assignments to these groups. All fish from forested sites downstream of WWTPs showed significant $\delta^{13}C$ depletion ($p<0.0001$) and $\delta^{15}N$ enrichment ($p<0.0001$) compared to unrestored and restored sites (Figure 1). Restored sites were significantly depleted in ^{13}C compared to unrestored sites (Figure 1).

Restored sites had significantly higher species richness than unrestored sites, but did not differ significantly from forested sites (Figure 1). Forested and unrestored sites had similar species richness. Total abundances of fish showed no significant difference overall ($p=0.1397$), although there was a strong trend toward more fish in the restored sites (Figure 1).

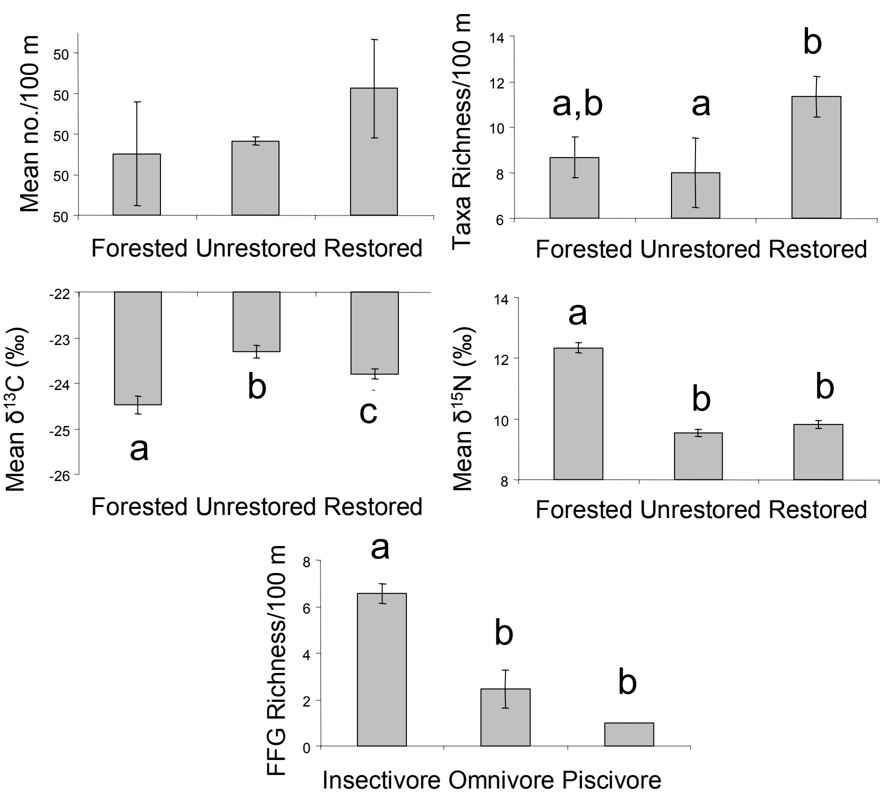

Figure 1. Differences in fish data for each site type, including (A) total abundance, (B) species richness, (C) mean $\delta^{13}C$ signatures, (D) mean $\delta^{15}N$ signatures, and (E) FFG designations. Error bars represent 1 SE of the mean. Bars with different letters were significantly different.

Macroinvertebrates

Abundance and taxa richness of terrestrial and aquatic macroinvertebrate was not significantly different between site typess. However, both groups of macroinvertebrates showed a trend for higher richness and abundance at restored sites (Fig. 2).

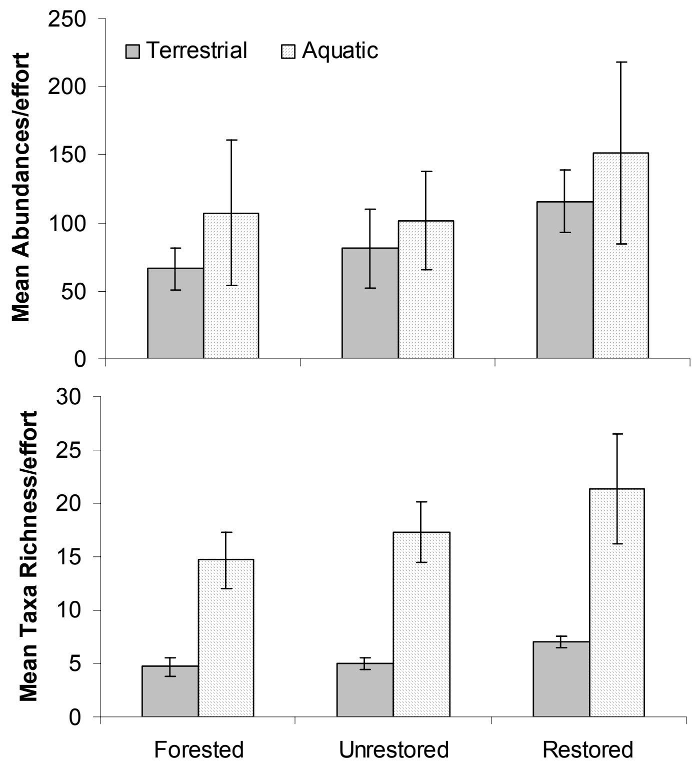

Figure 2. Differences in terrestrial and aquatic macroinvertebrate across sites for (A) abundance and (B) taxa richness. Error bars represent 1 SE of the mean.

Site types did not differ in total macroinvertebrate $\delta^{15}N$ (Figure 3), but there was a significant interaction between site type and invertebrate source (i.e., terrestrial versus aquatic). This interaction reflects that aquatic macroinvertebrates had significantly higher $\delta^{15}N$ overall, when compared to terrestrial invertebrates ($p=0.0012$, Figure 4). $\delta^{13}C$ did not differ significantly either among site types (Figure 3) or invertebrate source.

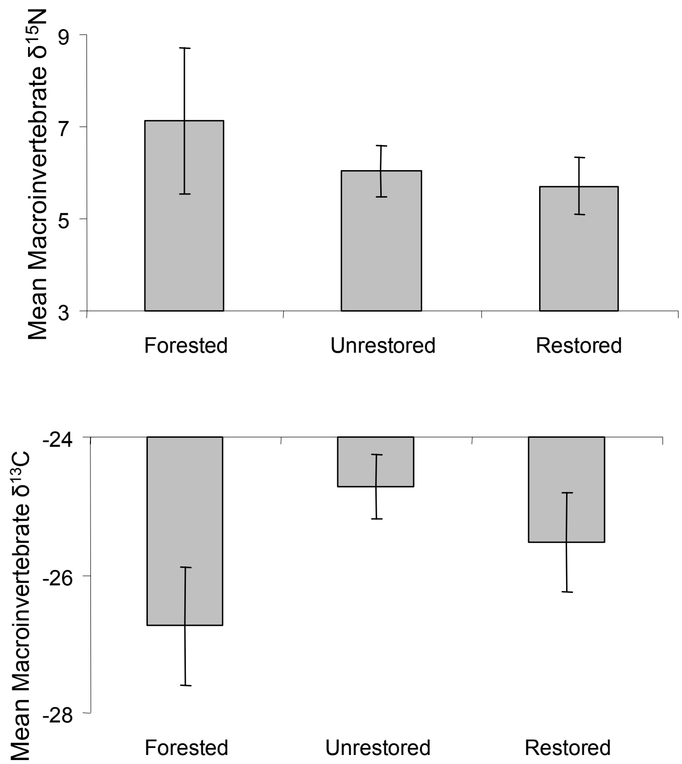

Figure 3. Differences in (A) 13C and (B) δ15N for terrestrial and aquatic macroinvertebrates. Error bars represent 1 SE of the mean.

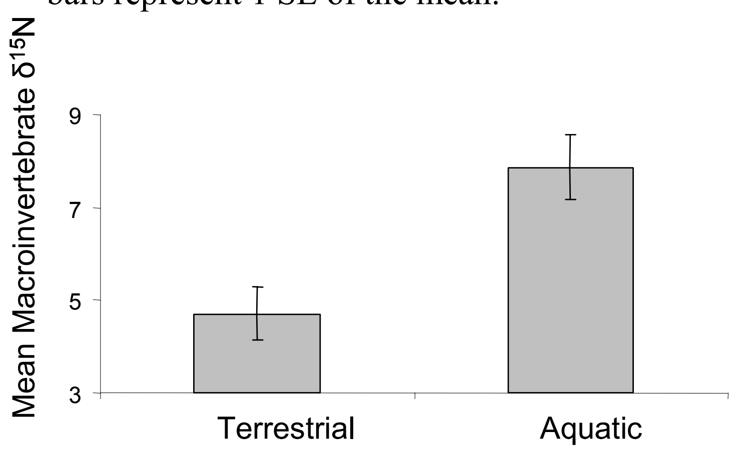

Figure 4. Differences in terrestrial and aquatic macroinvertebrate $\delta^{15}N$. Error bars represent 1 SE of the mean. Bars with different letters were significantly different.

On average, forested sites appeared to score slightly higher on the North Carolina Biotic Index than restored and unrestored sites, although the differences were not significant. On a site by site basis, Archdale, a forested site, scored best, with a score of 3 (Good-Fair) and Holden, an unrestored site, scored the worst, with a 1 (Poor).

Basal Resources

Periphyton chlorophyll *a* was not significantly different at each site type (p=0.9596), and there was no trend in chlorophyll *a* among site types (Figure 5). Periphyton AFDM also did not differ significantly among sites, although there was a trend toward greater AFDM in unrestored sites (Figure 5).

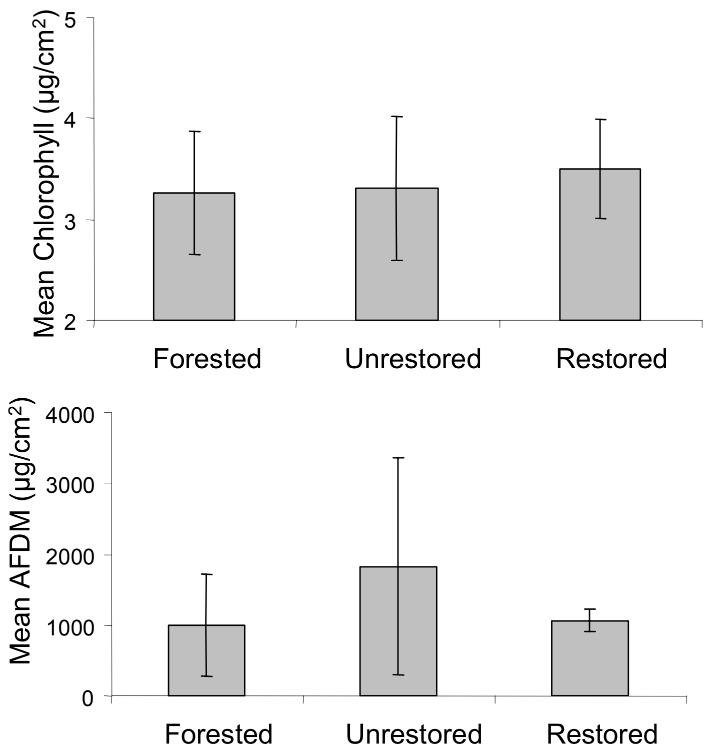

Figure 5. Mean biomass (µg/cm2) of (A) Chlorophyll *a* and (B) Ash-free Dry Mass (AFDM) across site types. Error bars represent 1 SE of the mean.

Basal Source Isotopes

Seston and FBOM showed a non-significant trend toward lower $\delta^{13}C$ in the restored sites (Figures 6). Periphyton signatures were, on average, slightly more depleted in ^{13}C in the forested sites (Figure 6). Seston and FBOM had significantly (p=0.0324) higher $\delta^{15}N$ in the forested compared to the restored and unrestored sites (Figure 7). The other basal resources, although not significant, showed a trend of greater ^{15}N enrichment at forested sites (Figure 7).

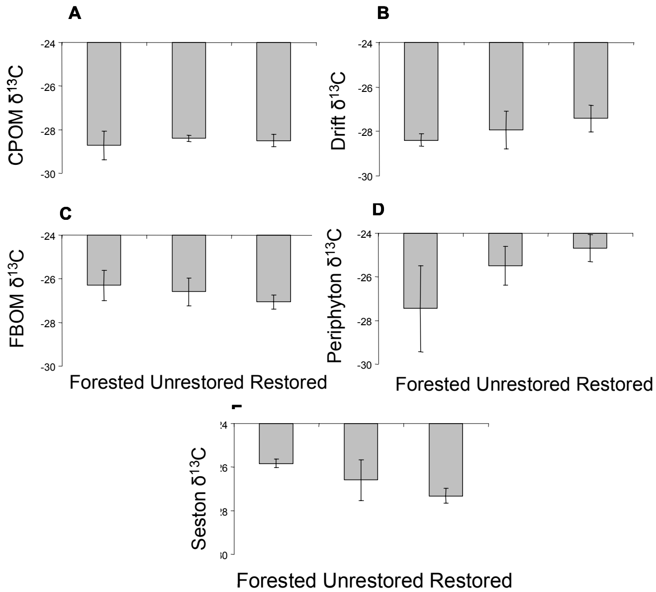

Figure 6. Mean δ13C signatures for (A) CPOM, (B) Drift, (C) FBOM, (D) Periphyton, and (E) Seston across site types. Error bars represent 1 SE of the mean.

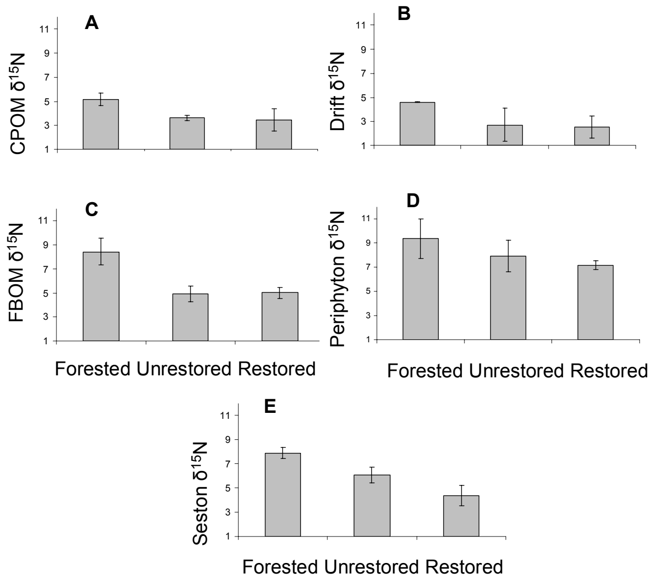

Figure 7. Mean $\delta^{15}N$ signatures for (A) CPOM, (B) Drift, (C) FBOM, (D) Periphyton, and (E) Seston across site types. Error bars represent 1 SE of the mean. Bars with different letters were significantly different.

Isotope Mixing Models

In many cases, neither two-source mixing models nor IsoSource were able to effectively predict fish diet compositions (Tables 1-2). Two-source mixing models showed that redbreast sunfish

(*Lepomis auritus*) were using mostly aquatic sources for both nitrogen (99.2%) and carbon (50.4%) at Holden, an unrestored site (Table 1). Isosource also indicated that redbreast sunfish were using mostly aquatic sources at Holden (Table 2). Redbreast gut contents (Table 3) indicate a mixture of food sources, but most were of aquatic origin.

Mosquitofish (*Gambusia holbrooki*) demonstrated similar diet composition to Redbreast sunfish. At unrestored sites mosquitofish obtained about 92.5% of their nitrogen and 63% of their carbon from aquatic sources, according to two-source mixing models (Table 1). IsoSource modeling (Table 2) and gut contents (Table 3) also indicated major usage of aquatically derived food sources. Creek chubsuckers (*Erimyzon oblongus*), did not give consistent enough results to make any conclusions about their diet composition (Tables 1-3).

Flat bullhead catfish (*Ameirus platycephalus*) appeared to have more diverse diets. For example, at Archdale, a forested site, terrestrial nitrogen (58.7%) and aquatic carbon (80.9%) appeared to be more important (Table 1). But at a restored site, Freedom, an opposite trend was seen, with aquatic nitrogen (71.2%) and terrestrial carbon (81.2%) being more important (Table 1). Both IsoSource and gut data appeared to corroborate these mixing model results by showing a mixture of terrestrial and aquatic sources in the forested site, but mainly aquatic sources were important in the restored site (Tables 2-3).

Finally, for the shiner species examined (*Notemigonus crysoleucus, Notropis hudsonius,* and *Notropis scepticus*), the only consistent diet data came from unrestored sites (Tables 1-3). Two-source mixing model results indicate primarily aquatic contributions of both nitrogen (93.4%) and carbon (83.8%) (Table 1). As with other fish species, both IsoSource and gut content data agree with the two-source model by indicating only aquatically-derived food items for these fish diets (Tables 2-3).

Table 1. Two-source mixing model for selected fishes. Numbers indicate percentage of the source used in the fish diets. **§** = no fish of this species found, *****= model did not converge to give a statistically reliable result. $\mathbf{d^{15}N_{terr}}$ = contribution of terrestrial ^{15}N to the diet, $\mathbf{d^{15}N_{aq}}$= contribution of aquatic ^{15}N to the diet, $\mathbf{d^{13}C_{terr}}$ = contribution of terrestrial ^{13}C to the diet, $\mathbf{d^{13}C_{aq}}$= contribution of aquatic ^{13}C to the diet.

		Forested			**Restored**			**Unrestored**		
Species	Isotope	Creekview	Harvest	Archdale	Lake Daniel	Hillsdale	Freedom	Latham	Holden	Midtown
Redbreast Sunfish	$d^{15}N_{terr}$	*	*	*	0.171	*	*	0.447	0.008	*
(*Lepomis auritus*)	$d^{15}N_{aq}$	*	*	*	0.829	*	*	0.553	0.992	*
Insectivore	$d^{13}C_{terr}$	*	*	*	*	0.455	*	*	0.496	*
	$d^{13}C_{aq}$	*	*	*	*	0.545	*	*	0.504	*
Mosquitofish	$d^{15}N_{terr}$	§	*	*	§	§	*	0.075	*	*
(*Gambusia holbrooki*)	$d^{15}N_{aq}$		*	*			*	0.925	*	*
Insectivore	$d^{13}C_{terr}$		*	*			*	*	0.37	*
	$d^{13}C_{aq}$		*	*			*	*	0.63	*
Creek Chubsucker	$d^{15}N_{terr}$	§	§	*	*	*	*	*	*	*
(*Erimyzon oblongus*)	$d^{15}N_{aq}$				*	*	*	*	*	*
Omnivore	$d^{13}C_{terr}$				*	0.43	*	*	0.449	*
	$d^{13}C_{aq}$				*	0.57	*	*	0.551	*
Flat Bullhead	$d^{15}N_{terr}$	0.123	0.116	0.587	0.225	*	0.288	§	§	§
(*Ameirus platycephalus*)	$d^{15}N_{aq}$	0.877	0.884	0.413	0.775	*	0.712			
Insectivore	$d^{13}C_{terr}$	*	*	0.191	*	0.411	0.812			
	$d^{13}C_{aq}$	*	*	0.809	*	0.589	0.188			
Shiners	$d^{15}N_{terr}$	§	*	*	*	*	*	0.066	*	§
(*Notemigonus crysoleucus*)	$d^{15}N_{aq}$		*	*	*	*	*	0.934	*	
(*Notropis hudsonius*)	$d^{13}C_{terr}$		0.983	*	0.976	*	*	*	0.162	
(*N. scepticus*)	$d^{13}C_{aq}$		0.017	*	0.024	*	*	*	0.838	

Table 2. Isosource results for several fish species at each site. All figurein () are the possible contribution of each source from the 1st – 99th percentile at a tolerance of 0.01%. °= tolerance of 0.05%, § = no fish of this species found, *= model did not converge to give a statistically reliable result.

	Forested			**Restored**			**Unrestored**		
Species	Creekview	Harvest	Archdale	Lake Daniel	Hillsdale	Freedom	Latham	Holden	Midtown
Redbreast Sunfish (*Lepomis auritus*) Insectivore	Crayfish (87-88%) Zygoptera (6%) Terr. Insects (6%)			*	*	Zygoptera (58-72%) Trichoptera (16-27%)	Crayfish (28-48%) Trichoptera (12-51%)	Terre. Insects (44-48%) Periphyton (32-37%) Zygoptera (5-18%) Trichoptera (2-8%)	Zygoptera (54-67%) Crayfish (12-35%)
Mosquitofish (*Gambusia holbrooki)* Insectivore	§	Zygoptera (67-68%) Anisoptera (17-18%) Periphyton (7-11 %) Trichoptera (3-8%)	Trichoptera (10-53%) Zygoptera (5-56%) Anisoptera (12-31%) Periphyton (7-16%) Terr, Insects (5-10%)	§	§	*	*	*	Periphyton (61-74%) Trichoptera (8-15%) Zygoptera (1-9%) Crayfish (1-14%)
Creek Chubsucker (*Erimyzon oblongus)* Omnivore	§	§	*	*	*	*	*	*	*
Flat Bullhead (*Ameirus platycephalus*) Insectivore	*	Trichoptera (26-38%)	Anisoptera (44-65%) Terr.Insects (9-15%) Small Fish (1-14%)	Small Fish ° (37-49%) Terr. Insects ° (2-35%)	*	Trichoptera (1-47%)	§	§	§
Shiners (*Notemigonus crysoleucus)* (*Notropis hudsonius)* (*Notropis scepticus)*	§	*	*	*	*	*	*	Periphyton (13-37%)	§

Table 3. Selected fish species, their NCDENR (2001) FFG designations, and their gut contents for each site type. §=no fish found in these sites

Species	Forested Sites	Restored Sites	Unrestored Sites
Redbreast Sunfish (*Lepomis auritus*) Insectivore	Coleoptera larvae adult Hymenoptera Anisoptera larvae mantidfly adult Diptera Trichoptera larvae adult Zygoptera Chironomid larvae Collembola	adult Diptera Zygoptera larvae Tipulid larvae Coleoptera larvae Diptera pupae plant matter Ephemeroptera larvae	Diptera pupae Ephemeroptera larvae Chironomid larvae adult Diptera Orthoptera adult plant matter adult Coleoptera adult Hymenoptera Zygoptera larvae Trichoptera larvae
Mosquitofish (*Gambusia holbrooki)* Insectivore	Chironomid larvae Diptera pupae adult Arachnid Trichoptera larvae adult Hymenoptera Diptera adult	Diptera adult	plant matter Diptera pupae Diptera adults
Creek Chubsucker (*Erimyzon oblongus)* Omnivore	periphyton Chironomid larvae	periphyton Chironomid larvae	Chironomid larvae
Flat Bullhead (*Ameirus platycephalus*) Insectivore	*Lepomis macrochirus* Crayfish plant matter *Gambusia holbrooki* Chironomid larvae Diptera pupae Physid snail adult Hymenoptera Zygoptera larvae	Chironomid larvae Ephemeroptera larvae filamentous algae	§
Shiners Golden Shiner (*Notemigonus crysoleucus)* Omnivore Spottail Shiner (*Notropis hudsonius)* Omnivore Sandbar Shiner (*Notropis scepticus)* Insectivore	plant matter Ephemeroptera larvae Chironomid larvae Trichoptera larvae filamentous algae	Ephemeroptera larvae plant matter adult Hymenoptera Chironomid larvae	plant matter filamentous algae Ephemeroptera larvae

DISCUSSION

Potential basal sources of carbon for stream fishes include leaf litter from terrestrial plants ($\delta^{13}C$ approximately -28‰), stream algae ($\delta^{13}C$ approximately -24.5‰ at restored sites and -25.5‰ at unrestored sites), and miscellaneous anthropogenic sources such as sewage leaks (not measured). Fish $\delta^{13}C$ at restored sites was slightly but significantly more depleted in ^{13}C compared to unrestored sites. This pattern is opposite what would be expected of a diet with periphyton as the sole basal resource (i.e., fish at the restored sites should be slightly enriched in ^{13}C), but consistent with the hypothesis that leaf litter is becoming a more important source of carbon to the food web as riparian development increases at the restored sites. Similarly, macroinvertebrates $\delta^{13}C$ values show the same trend as do fish, also suggesting that riparian inputs are becoming more important. Despite these trends, aquatic carbon was still the dominant basal resource for most fishes, a conclusion that is supported by the mixing model results.

of periphyton (about 7‰) was distinct from terrestrial detritus (CPOM, about 3‰), but there was little difference in either of these basal resources between restored and unrestored sites. We would expect fish to be approximately two trophic levels above their basal resource, thus showing a trophic $\delta^{15}N$ shift of about 6‰ above their basal resource. Combined terrestrial and aquatic invertebrate $\delta^{15}N$ was about 6‰ at both restored and unrestored sites, consistent with an N source of primarily leaf material (CPOM) rather than periphyton. However, aquatic invertebrates were more enriched in ^{15}N than terrestrial invertebrates (Figure 4) suggesting that they were utilizing primarily periphyton whereas the terrestrial invertebrates were utilizing unconditioned leaves (which are ^{15}N depleted compared to CPOM, see Ulseth and Hershey 2005). Fish had $\delta^{15}N$ of about 9.5 to 10‰, consistent with a diet of aquatic insects, and consistent with the mixing model results that they were using primarily aquatic resources.

At sites below sewage treatment plants, much of the carbon in fish tissue is derived from sewage. The $\delta^{13}C$ of treated sewage coming into the stream appears to be about -26‰ (value of seston at those sites). Fish at these sites are 2 trophic levels removed from seston, so are slightly enriched compared to that value, about -25.4‰ (comparison of Figures 1 and 6).

Isotope data showed that all fish as well as aquatic invertebrates were enriched in ^{15}N downstream of the WWTPs. Ulseth and Hershey (2005) previously showed that sewage effluent and most aquatic invertebrates were also significantly enriched in ^{15}N compared to other resources in North Buffalo Creek. Thus, our present results are consistent with incorporation of sewage-derived N into the fish from the aquatic environment via feeding on aquatic insects.

We expected to see an increase in abundance and richness of fish, as well as terrestrial and aquatic invertebrates, at restored sites compared to unrestored sites, due to the increased canopy cover and improved aquatic habitat at those sites. The patterns observed were all consistent with that hypothesis, although the trends were significant only for fish richness (Figures 1 and 2). In an earlier study of the effects of restoration on macroinvertebrate and water quality at several of these same sites, Lynam (2004) found little evidence of improved macroinvertebrate parameters, although she did see significant improvements in water quality (dissolved oxygen and nitrate concentration). The trend toward improved macroinvertebrate and fish abundance and richness at restored sites that we observed, in combination with the trend toward greater use of terrestrial carbon at restored sites, suggests that the improved habitat and water quality at these sites is beginning to show a long-term benefit to the aquatic biota.

It is important to continue monitoring of restored sites. With increased riparian canopy development, terrestrial leaf litter should become an increasingly important subsidy to the stream. Because the $\delta^{13}C$ value of periphyton and leaf litter are distinct in this stream, increased recovery should result in a greater shift in utilization of this detrital resource by the invertebrate and fish communities of the stream. Stable isotope analysis provides and economical monitoring tool for detecting such a shift.

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