CHANGES IN BENTHIC BIOTA AND NITROGEN SOURCES CONCURRENT WITH DECLINING FISH DIVERSITY IN THE CONASAUGA RIVER

by

CHRISTINA LYNN BAKER

(Under the Direction of Mary C. Freeman)

ABSTRACT

The Conasauga River in Georgia and Tennessee is a site of recent, rapid decline in biodiversity of fishes and mollusks associated with a downstream gradient of increasing agricultural land use. The purpose of this research was to quantify other changes in the Conasauga and to compare location of changes with that of declines. Elevations in $\delta^{15}N$ stable isotopes in primary consumer tissues indicated hotspots of anthropogenic nitrogen input located upstream of fish declines. Biomass, abundance, richness and composition of benthic macroinvertebrates shifted downstream and several individual taxa exhibited threshold declines upstream of fish declines and in conjunction with loss of an aquatic macrophyte. Algal accrual was more influenced by time period than location and was greatest during a period with long duration between rain events. If algal accrual is a mechanism by which eutrophication alters biotic communities in the Conasauga, then increased prevalence of drought might have dire consequences.

INDEX WORDS: Stable isotopes, algal accrual, macroinvertebrates, riverweed, *Podostemum,* Conasauga River, land use, thresholds, periphyton, nutrients, nitrogen, agriculture, declining biodiversity

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CHAPTER 1

INTRODUCTION

Anthropogenic disturbances often result in shifts toward less diverse benthic communities in lotic systems. The disturbed community becomes dominated by species that are adapted to survive under broader environmental conditions (Woodcock and Huryn 2007, Doledec et al. 2011). These taxa are typically more tolerant to pollution, produce more young or reproduce more often, and have less specific habitat requirements. Communities can be resistant to stressors at low levels but change abruptly when stressors reach some threshold (Groffman et al. 2006, Dodds et al. 2010). This threshold effect has been documented for several stressors to aquatic communities, such as impervious surfaces (King et al. 2011), nutrient concentrations (Evans-White et al. 2009), and fine sediment deposition (Wagenhoff et al. 2011). Some stressors, such as nutrients, can be a subsidy at low levels and a stressor at high levels (King and Richardson 2007, Wagenhoff et al. 2011). Nutrients can have an indirect stressor effect on benthic communities by promoting nuisance algal growth when other conditions are conducive to growth (Biggs 2000), or at very high concentrations, nutrients can be directly toxic to biota (Miltner and Rankin 1998).

Over recent decades researchers have documented declines in biodiversity along the Conasauga River, which is a highly diverse headwater refuge for many species that have disappeared in lower portions of the Coosa River Basin as a result of habitat loss (Freeman et al. 2005). The Conasauga River is located in northwest Georgia and southeast Tennessee and

originates in the Chattahoochee/Cherokee National Forest in the Blue Ridge Province and then flows out into the Valley and Ridge Province. Outside of the national forest, land use is predominately agriculture; suburban growth primarily occurs downstream of the reach where aquatic species declines begin. Previous research has documented declines in native mussels, snails, fishes, and the aquatic macrophyte, *Podostemum ceratophyllum* (Freeman et al. 2007, Sharpe and Nichols 2007, Wenger et al. 2009, Argentina et al. 2010a, Argentina et al. 2010b, Hagler et al. 2011). Fish declines are more apparent in downstream reaches and are relatively abrupt, indicating a change in habitat suitability for sensitive fishes downstream or an initial resistance to agricultural impacts upstream and a lack of resistance downstream (Freeman et al. 2006, Hagler et al. 2011). Declining detection of imperiled and sensitive fish species in the Conasauga River appears to be coincident in space and time with high nutrient concentrations. Algal blooms during periods of low flow provide additional evidence that nutrients are a potential stressor in the Conasauga (Freeman et al. 2006). However, researchers have not been able to establish the mechanism of decline and there are several working hypotheses including high nutrient levels, pesticide pollution, or a combination of stressors. To conserve biodiversity in the Conasauga, managers need to know the location of biotic declines along the river and which stressor inputs are concurrent with declining biodiversity. The purpose of this thesis is to investigate changes in other aspects of the biotic community (macroinvertebrates, *Podostemum* and algae) in the Conasauga, to evaluate whether these changes are discontinuous and concurrent with fish declines, and to assess evidence that anthropogenic sources of nutrients increase discontinuously downstream at specific hypothesized locations.

To detect locations of anthropogenic nitrogen loading, I measured nitrogen stable isotopes in primary consumer tissues. Several recent studies have assessed ^{15}N enrichment to

detect anthropogenic sources of nutrients. Manure and sewage are elevated in ^{15}N in comparison to natural and inorganic nitrogen sources, so high levels of water column or biotic ^{15}N can indicate a greater proportion of fecal nitrogen relative to atmospheric, soil, autotroph, or inorganic fertilizer sources. Researchers have measured ^{15}N along gradients of land use and increasing population (Cabana and Rasmussen 1996, Vander Zanden et al. 2005) to detect sewage and manure input. Primary consumers integrate fluctuations in nitrogen over time (Post 2002, Gustafson et al. 2007), and high signatures in tissues can indicate chronic anthropogenic nutrient enrichment (Vander Zanden et al. 2005). Therefore, I measured $\delta^{15}N$ in primary consumer tissues to evaluate whether tributaries or point sources contributed additional anthropogenic nitrogen that was unexplained by continuous change in percent agricultural land in the basin.

To examine changes in the biotic community along the Conasauga River, I measured functional and taxonomic shifts in the invertebrate community along with changes in biomass of *Podostemum ceratophyllum*. Macroinvertebrates vary in sensitivity to pollution, and stressors to rivers can have predictable effects on aquatic macroinvertebrate community composition (Statzner and Beche 2010). Richness of the sensitive invertebrate taxa Ephemeroptera, Plecoptera and Trichoptera is a widely used metric to evaluate anthropogenic impacts on a stream. However, this metric does not distinguish among the multitude of stressors associated with human impact (Pollard and Yuan 2010). Functional traits, such as feeding mode or food preference, and life history characteristics, like oviposition location (Doledec et al. 2006, Doledec et al. 2011), have been used in other studies to indicate mechanism of stressor effects on macroinvertebrates. For example, a longitudinal increase in scrapers or algivores might indicate that differing algal growth between upstream and downstream reaches is a mechanism of change

in macroinvertebrate composition. In this study, I compared multiple methods of measuring change in community composition to assess which types of taxa decline or increase downstream, and to evaluate evidence of a discontinuous shift in the macroinvertebrate community and in *Podostemum* biomass concurrent with a discontinuous shift in fishes and in a potential stressor (anthropogenic nitrogen loading).

To assess whether conditions promoting or restricting algal growth differed from upstream to downstream, I measured rate of accrual and net algal accrual on a standard artificial substrate without any experimental manipulation of factors that affect algal growth. I measured algal accrual on artificial substrates at sites upstream of, and within, the reach of fish declines. Nuisance algal growth is a possible mechanism by which high nutrient levels negatively impact Conasauga River biota. Nuisance algal growth can reduce light availability for aquatic macrophytes, reduce oxygen availability for macroinvertebrates and fishes, and smother habitat of macroinvertebrates and fishes that require clean substrate for feeding and reproduction (Harding et al. 1999). The relationship between nutrients and algal growth is complex, because many other factors such as grazing pressure, flow, light availability and temperature can limit or impact algal accrual. However, in temperate streams with open canopies, nutrients and stream flow are more often limiting factors of periphyton accrual (Biggs 2000).

This research is described in three chapters corresponding to investigation of upstream to downstream shifts in ^{15}N enrichment in primary consumers, benthic macroinvertebrate taxa abundance and biomass, and accrual of benthic algae. This work is intended to provide new information on changes specific to the Conasauga River, but also on methods for assessing biotic changes in relation to putative sources (such as tributary inputs or changes in land use) of

stressors to a river. Most broadly, my research contributes to an understanding of changing ecological conditions in river systems in response to anthropogenic drivers.

Literature Cited

Argentina, J. E., M. C. Freeman, and B. J. Freeman. 2010a. Predictors of occurrence of the aquatic macrophyte *Podostemum ceratophyllum* in a southern Appalachian river. Southeastern Naturalist **9**:465-476.

Argentina, J. E., M. C. Freeman, and B. J. Freeman. 2010b. The response of stream fish to local and reach-scale variation in the occurrence of a benthic aquatic macrophyte. Freshwater Biology **55**:643-653.

Biggs, B. J. F. 2000. Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. Journal of the North American Benthological Society **19**:17-31.

Cabana, G. and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences of the United States of America **93**:10844-10847.

Dodds, W. K., W. H. Clements, K. Gido, R. H. Hilderbrand, and R. S. King. 2010. Thresholds, breakpoints, and nonlinearity in freshwaters as related to management. Journal of the North American Benthological Society **29**:988-997.

Doledec, S., N. Phillips, M. Scarsbrook, R. H. Riley, and C. R. Townsend. 2006. Comparison of structural and functional approaches to determining landuse effects on grassland stream invertebrate communities. Journal of the North American Benthological Society **25**:44-60.

Doledec, S., N. Phillips, and C. Townsend. 2011. Invertebrate community responses to land use at a broad spatial scale: trait and taxonomic measures compared in New Zealand rivers. Freshwater Biology **56**:1670-1688.

Evans-White, M. A., W. K. Dodds, D. G. Huggins, and D. S. Baker. 2009. Thresholds in macroinvertebrate biodiversity and stoichiometry across water-quality gradients in Central Plains (USA) streams. Journal of the North American Benthological Society **28**:855-868.

Freeman, B. J., M. M. Hagler, S. J. Wenger, G. B. Anderson, and R. A. Katz. 2006. Identification and mapping of critical habitats in the Conasauga River corridor of Georgia and Tennessee 2006 Annual report to the Georgia Department of Natural Resources, Atlanta. Institute of Ecology, University of Georgia, Athens.

Freeman, B. J., M. M. Hagler, S. J. Wenger, G. B. Anderson, and R. A. Katz. 2007. Identification and mapping of critical habitats in the Conasauga River corridor of Georgia and Tennessee 2006 Annual report to the Georgia Department of Natural Resources, Atlanta. Institute of Ecology, University of Georgia, Athens.

Freeman, M. C., E. R. Irwin, N. M. Burkhead, B. J. Freeman, and H. M. Bart Jr. 2005. Status and conservation of the fish fauna of the Alabama River system. Pages 557-858 *in* J. N. Rinne, R. M. Hughes, and R. Calamusso, editors. Historical Changes in Large River Fish Assemblages of the Americas. American Fisheries Society Symposium 45, Bethesda, Maryland.

Groffman, P., J. Baron, T. Blett, A. Gold, I. Goodman, L. Gunderson, B. Levinson, M. Palmer, H. Paerl, G. Peterson, N. Poff, D. Rejeski, J. Reynolds, M. Turner, K. Weathers, and J. Wiens. 2006. Ecological thresholds: The key to successful environmental management or an important concept with no practical application? Ecosystems **9**:1-13.

Gustafson, L., W. Showers, T. Kwak, J. Levine, and M. Stoskopf. 2007. Temporal and spatial variability in stable isotope compositions of a freshwater mussel: implications for biomonitoring and ecological studies. Oecologia **152**:140-150.

Hagler, M. M., M. C. Freeman, S. J. Wenger, B. J. Freeman, P. L. Rakes, and J. R. Shute. 2011. Use of recent and historical records to estimate status and trends of a rare and imperiled stream fish, *Percina jenkinsi* (Percidae). Canadian Journal of Fisheries and Aquatic Sciences **68**:739-748.

Harding, J. S., R. G. Young, J. W. Hayes, K. A. Shearer, and J. D. Stark. 1999. Changes in agricultural intensity and river health along a river continuum. Freshwater Biology **42**:345-357.

King, R. S., M. E. Baker, P. F. Kazyak, and D. E. Weller. 2011. How novel is too novel? Stream community thresholds at exceptionally low levels of catchment urbanization. Ecological Applications **21**:1659-1678.

King, R. S. and C. J. Richardson. 2007. Subsidy-stress response of macroinvertebrate community biomass to a phosphorus gradient in an oligotrophic wetland ecosystem. Journal of the North American Benthological Society **26**:491-508.

Miltner, R. J. and E. T. Rankin. 1998. Primary nutrients and the biotic integrity of rivers and streams. Freshwater Biology **40**:145-158.

Pollard, A. I. and L. L. Yuan. 2010. Assessing the consistency of response metrics of the invertebrate benthos: a comparison of trait- and identity-based measures. Freshwater Biology **55**:1420-1429.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology **83**:703-718.

Sharpe, A. J. and E. G. Nichols. 2007. Use of stable nitrogen isotopes and permeable membrane devices to study what factors influence freshwater mollusk survival in the Conasauaga river. Environmental Monitoring and Assessment **132**:275-295.

Statzner, B. and L. A. Beche. 2010. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? Freshwater Biology **55**:80-119.

Vander Zanden, M. J., Y. Vadeboncoeur, M. W. Diebel, and E. Jeppesen. 2005. Primary consumer stable nitrogen isotopes as indicators of nutrient source. Environmental Science & Technology **39**:7509-7515.

Wagenhoff, A., C. R. Townsend, N. Phillips, and C. D. Matthaei. 2011. Subsidy-stress and multiple-stressor effects along gradients of deposited fine sediment and dissolved nutrients in a regional set of streams and rivers. Freshwater Biology **56**:1916-1936.

Wenger, S. J., M. M. Hagler, and B. J. Freeman. 2009. Prioritizing areas of the Conasauga River sub-basin in Georgia and Tennessee for preservation and restoration. Southeastern Fishes Council Proceedings **51**:31-38.

Woodcock, T. S. and A. D. Huryn. 2007. The response of macroinvertebrate production to a pollution gradient in a headwater stream. Freshwater Biology **52**:177-196.

CHAPTER 2

NITROGEN ISOTOPE COMPOSITION IN PRIMARY CONSUMERS AS AN INDICATOR OF ANTHROPOGENIC NITROGEN POLLUTION

Introduction

Increased nutrient availability (e.g., nitrogen and phosphorus) in aquatic systems can stimulate production across trophic levels. However, where nutrients are delivered in excess of a system's assimilative capacity, water quality is degraded, dissolved oxygen depleted (Rabalais 2002), and shifts in food resource availability (Davis et al. 2010) occur that may have negative effects across trophic levels. In excess, certain forms of nitrogen can be directly toxic to macroinvertebrates and stream biota, although such conditions may be localized or in the hyporheic zone where they are difficult to measure (Augspurger et al. 2003, Strayer et al. 2004). A more obvious effect of fertilization of aquatic systems is an increase in the production of algae, often resulting in algal blooms. Excess algal production alters stream habitats by coating bed sediments that may be important for benthic invertebrates and fishes. Algae or phytoplankton may reduce light availability for submerged macrophytes (Rabalais 2002, Hilton et al. 2006). As algal cells die and decompose, dissolved oxygen can be depleted. Further, algal respiration at night (i.e., in the absence of photosynthesis) can draw down dissolved oxygen levels, possibly to levels intolerable to fishes and other biota.

One of the tools available for identifying the nutrient sources that may be fueling primary production, is the analysis of stable isotopes of primary consumers. Stable isotopes are naturally occurring variations of an element that differ in mass by having a different number of neutrons.

Ratios of light to heavy nitrogen isotopes (e.g., $^{14}N/^{15}N$, respectively) vary predictably with chemical, physical and biological processes, and are therefore commonly used in ecological studies. For example, they can be used as a natural tracer of nutrient inputs (Cabana and Rasmussen 1996, Vander Zanden et al. 2005) and to elucidate food web structure (Peterson and Fry 1987). Because ^{15}N accumulates in tissues of animals higher on the food chain, an animal's ^{15}N signature can indicate trophic level. Each trophic transfer results in an increase in $\delta^{15}N$ of +3-5‰ on average (Peterson and Fry 1987). Isotopic compositions are reported as δ (delta) values that represent the difference from a standard in parts per thousand (ppt or permil); the standard for ^{15}N is atmospheric N at ~0‰. Since primary consumers feed at the base of the food chain, their ^{15}N signatures should be relatively low. This is especially true in forested systems where leaf litter is the main source of upland nutrients to the stream. As land use shifts from forested to agricultural, there are additional sources of nutrients (e.g., inorganic fertilizers and manure) that may contribute to primary production in streams, and these nutrient sources have distinct $\delta^{15}N$ values that are incorporated in the tissues of primary consumers (Anderson and Cabana 2005, Vander Zanden et al. 2005). Therefore, deviations from background $\delta^{15}N$ levels, and sudden shifts in $\delta^{15}N$, can indicate locations of increased nutrient input (e.g., hot-spots of sewage or septic runoff; Steffy and Kilham 2004).

Primary consumers are often used to develop a baseline for trophic studies because their isotopic signatures are more stable than that of their basal food resources. Algal cells turn over very quickly and respond rapidly to changes in resource availability, and therefore have high temporal and spatial variability in their $\delta^{15}N$ signatures that can make it difficult to observe underlying trends. In contrast, primary consumers live longer and are steadily "sampling" algae and particulates and integrating variability in nitrogen sources in their tissues (Cabana and

Rasmussen 1996, Post 2002, Gustafson et al. 2007). Therefore, $\delta^{15}N$ from tissues of primary consumers should provide a clearer picture of nutrient inputs over a longer time period than $\delta^{15}N$ from algae or water column nitrogen. Since primary consumers are constantly feeding and assimilating nutrients from all available resources, stable isotope analysis of those consumers allows for an integrated sample of nutrient sources over time and over a large spatial area.

Research along the Conasauga River in northwest Georgia and southeast Tennessee over the last two decades indicates changing biological conditions and species losses. The Conasauga River is valued for exceptional biodiversity, but scientists have noted a decline in the occurrence of several fish and mussel species of interest and a loss of the submerged aquatic macrophyte, riverweed (*Podostemum ceratophyllum*), known to provide important habitat for macroinvertebrates and fishes (Freeman et al. 2007, Sharpe and Nichols 2007, Wenger et al. 2009, Argentina et al. 2010a, Argentina et al. 2010b, Hagler et al. 2011). Increased levels of dissolved nutrients in stream water (especially nitrogen) and algal blooms have been observed over extensive reaches of the mainstem Conasauga River (Freeman et al. 2007). Eutrophication, or the oversupply of nutrients to aquatic systems, leads to a suite of changes that can result in a loss of biodiversity (Schindler 1990), and is one possible explanation of the changing conditions observed in the Conasauga River.

My approach to investigating sources of nitrogen loading in the river was to sample biota for isotope analysis along a gradient of forested to agricultural land use. In a study in the upper portion of the Conasauga River, Sharpe and Nichols (2007) documented a shift towards higher $\delta^{15}N$ in snails at sites in the Conasauga River outside of the national forest and in two tributaries. Building on their work (where sampling was conducted in 2004), I sampled over a greater spatial extent of the Conasauga River and at a finer spatial scale (i.e. more sites along the mainstem),

from the headwaters to downstream of Dalton, GA. The headwaters of the Conasauga River are protected as national forest land, but land use quickly shifts towards agriculture just outside of the national forest. The Conasauga River valley is a wide alluvial valley that has been farmed for centuries, and many floodplain farms (crop and pasture land) are located along the banks of the river. By including sites from across this reach, one can assess changing nitrogen sources in response to changing land use, and identify whether there are hot-spots of nitrogen enrichment.

I evaluated shifts in $\delta^{15}N$ in three primary consumer taxa, an algivorous minnow (*Campostoma oligolepis*), native snails (*Elimia sp.*), and the Asian clam (*Corbicula fluminea*), from 16 shoals along ~76 river-kilometers of the Conasauga mainstem. To evaluate the support of several competing hypotheses that might explain shifts in $\delta^{15}N$, I used a hierarchical modeling approach. The hypotheses ask the following: 1) can the proportion of agricultural land use explain shifts in $\delta^{15}N$ in biota?; 2) are shifts in $\delta^{15}N$ in biota spatially related to the confluence of large tributaries with the mainstem?; 3) is there evidence that a specific source in the floodplain of the river (e.g., confined animal production site) results in a shift in $\delta^{15}N$? The results of this analysis may inform nutrient management in the Conasauga River basin by identifying tributary watersheds or specific reaches of the mainstem where contributions of upland nutrients may be elevated and fueling primary production.

Methods

Site selection

I collected primary consumers from 16 sites located at shoals in the Conasauga River, along a reach that began in the national forest and ended at Airport Road (Table 2.1 and Figure 2.1). The site in the national forest (Site 1) was chosen because the watershed there is forested,

with little or no influence of agricultural land, and thus $\delta^{15}N$ was expected to be lowest at this site, representing baseline or background conditions. I sampled shoals upstream and downstream of the confluence of each major tributary of interest, including Mill Creek (Trib 1, Sites 3-4), Perry Creek (Trib 2, Sites 4-5), Sugar Creek (Trib 3, Sites 8-9), Sumac Creek (Trib 4, Sites 10-11) and Coahulla Creek (Trib 5, Sites 15-16). I also sampled a shoal upstream and downstream of a dairy along the Conasauga River (Dairy, Sites 6-7). Sites 2 and 12-14 were included to increase the spatial extent of the sampling, even though there were no major tributary confluences of interest associated with those sites.

Sampling Methods

Snails, clams, and the largescale stoneroller, *Campostoma oligolepis* (henceforth "fish") were collected from each site on a single occasion between June-August 2010. Due to the relative rarity of native mussels within the Conasauga River, I collected the invasive Asian clam. I searched the shoal for snails and clams until I found at least 10 individuals or for no more than 1 hour. No clams were found at the national forest site (site 1) and only one snail was collected at Airport Road (site 16). The fish were collected by seine until at least eight individuals were obtained. All samples were frozen on dry ice in the field and transported to a laboratory where they were kept at -20°C until they were processed for stable isotope analysis.

Lab Methods

The standard length of each fish, the aperture width of each snail shell, and the length of each clam shell were measured to the nearest 0.5 mm prior to tissue harvesting. Lateral muscle tissue was harvested from the caudal peduncle of five fish from each site. Muscle tissue was

chosen because it has a slow turnover rate and is therefore a better integrator of chronic nutrient pollution than whole body samples (Gustafson et al. 2007). Foot muscle tissue was harvested from three snails and three clams from each site (except at the national forest site and at Airport Road (see previous section)). Foot muscle was used in clams and snails because it is relatively easy to remove, it does not contain other tissues or gut material that might skew or increase variability of signatures and it is a large muscle with enough material that multiple subsamples can be taken. Tissue was analyzed from only a subset of the total number of individuals collected, with the remaining samples retained in case I found high variability among individuals within sites. A greater number of fish were analyzed than clams and snails to account for greater mobility in fish and the increased likelihood of a more varied diet. In total across the 16 sites, 171 samples were analyzed (80 fish, 46 snails, and 45 clams).

Individual tissue samples were rinsed with deionized water, placed in glass scintillation vials and freeze dried. After drying, a glass stir rod was used to pulverize and homogenize the sample in the scintillation vial with the aid of a vortexer. Homogenized samples weighing 0.25-2 mg were individually placed in 5 mm X 9 mm ultrapure tin capsules and weighed for analysis. Samples were analyzed for $\delta^{15}N$ by the Analytical Chemistry Lab in the Odum School of Ecology, University of Georgia. The samples were combusted in an elemental analyzer (Costech NA 1500 CHN Analyzer (Dumas method)). Combustion effluent was routed through a Conflo III interface (Thermo-Finnigan A) to an isotope ratio mass spectrometer (Thermo-Finnigan Delta V). Precision for ^{15}N analysis was <0.12‰ using NIST Bovine Liver (1577b) as the reference standard. Replicate subsamples were analyzed for 33 individuals to confirm adequate homogenization of samples (mean standard deviation was 0.21‰.).

Statistical Analysis

I used estimates derived from a Geographic Information System (GIS) for percent agricultural land use upstream of each site within the entire catchment and within the riparian corridor along the Conasauga River. Estimates were derived using ArcGIS 9.2 (ESRI 1999-2006) to summarize 2001 National Land Cover Data (USGS 2007), combining cultivated cropland with hay and pasture land to get total percent agricultural land. Percent agricultural land in the riparian area upstream of each study site was calculated by assessing land use within 100-meters to each side of the river. Percent agricultural land in the entire upstream catchment was similar to that in the riparian area alone (Table 2.1), but the former was the best predictor in models (see below), therefore percent agricultural land in the riparian area is not discussed further.

I used multi-level (or mixed effects) linear regression implemented in SAS 9.1 (SAS Institute Inc. 2002-2003) to predict $\delta^{15}N$ in study organisms based on explanatory variables. Multi-level regression allows one to explicitly account for hierarchically structured data; in this case, multiple individuals were collected per site. I included a site-level random intercept to allow for differences in the mean $\delta^{15}N$ among sites. The residual error in the model represents among-individual variation. I assumed there would be substantial differences in ^{15}N signatures that must be accounted for among organisms (i.e., because they consume somewhat different resources through different feeding modes), and between the national forest site and other sites (i.e., because the national forest site has no agricultural land upstream). Therefore, in the base-line or null model, I included two individual-level predictors to indicate (1) organism type (i.e., two binary parameters were used to distinguish among three different organisms), and (2) a site-level predictor indicating position downstream of the national forest (i.e., a single binary

parameter to distinguish Sites 2-16 from Site 1). All other models were compared to the base-line model.

In alternative explanatory models, a continuous predictor variable was included at the individual level to test whether body size influenced ^{15}N signature, because the food resource being consumed might differ across size classes of individuals. At the site level, I included both continuous and categorical explanatory variables to relate changing land use and watershed features to $\delta^{15}N$ in primary consumers. The continuous site-level predictors included the percent agricultural land upstream of each site and river-kilometer. A parameter indicating river-kilometer was included to account for gradients in $\delta^{15}N$ not explained by percent agriculture (i.e., a null hypothesis that would indicate a downstream trend unrelated to changes in land use). Finally, binary variables were included to examine the effect of being downstream (DS) of tributaries or point sources: DS Tribs 1-5 and DS Dairy. Candidate models were constructed using all possible combinations of the parameters listed above, given that they were uncorrelated based on Pearson correlation ($r^2 < 0.40$). Although there were 6 potential binary site-level variables, many were highly correlated with one another (especially those pairs indicating tributaries in close spatial proximity). Further, percent agricultural land was highly correlated with most binary site-level parameters. Once correlated combinations of parameters were removed, there remained 35 candidate models.

I used an information theoretic approach (Burnham and Anderson 2002) to evaluate support of models. Akaike's Information Criterion with correction for small sample size (AICc; Burnham and Anderson 2002) was used to rank candidate models from best-supported (lowest AICc) to least-supported. Models with Akaike weight within 10% of the weight of the top model were reported. The baseline model (i.e., including organism type and position

downstream of the national forest) was used to evaluate the proportion of additional variation explained by each model in the confidence set. Parameter estimates with 95% confidence intervals were used to interpret the effect size of each of the parameters in the best-supported model.

Results

Average $\delta^{15}N$ values for fish, snails and clams were 10.27‰, 8.68‰ and 7.24‰, respectively (Table 2.2). The trend that fish were the most enriched and clams were least enriched in $\delta^{15}N$ was consistent across all sites. Primary consumers had lower $\delta^{15}N$ values at the national forest site than at other downstream sites. The base-line model that accounted for organism type and site position outside of the national forest was, therefore, well-supported compared to the no-predictors model (Table 2.3). The parameters accounting for organism type and position downstream of the national forest explained 80% of the total variation in the data. Parameter estimates from the base-line model indicated that the $\delta^{15}N$ signatures of snails and clams were 1.60‰ and 3.42‰ lower, respectively, than the $\delta^{15}N$ signature of fish (Figure 2.2).

After accounting for the effect of organism type, 35% of the variation in $\delta^{15}N$ remaining in the model was among individuals (i.e., within sites). Despite differences in organism size (fish standard length 34-80 mm; snail aperture widths 8-10.5 mm; and clams length 12.5-23.5 mm), including size as an individual-level predictor did not substantially improve empirical support of candidate models, indicating there was little evidence that body size influenced $\delta^{15}N$ signatures among study individuals.

After accounting for the effect of being downstream of the national forest, 65% of the remaining variation in $\delta^{15}N$ was among sites. Of the variation among sites, 93% was explained

by the best-supported model, which included two binary predictor variables indicating site position downstream of Trib 1 and downstream of the dairy (Table 2.3). A model with Dairy alone explained 81% of the remaining among-site variation; however, DS Dairy was the second-best single predictor. The best single site-level predictor was percent agricultural land use in the catchment, explaining 87% of the remaining among-site variation (i.e., compared to the base-line model) (Figure 2.3); however, percent agricultural land did not appear as a parameter in the top four models. Percent agricultural land use was correlated with most other site-level predictor variables (except DS Trib 5), and therefore was not included in most models. The best-supported model that included percent agricultural land use in the catchment was 7 times less probable than the top model (Table 2.3).

Parameter estimates for the top model indicate that the largest shift in $\delta^{15}N$ was between the national forest site and other downstream sites (4.61‰ (95% CI = 3.94-5.28‰); Figure 2.4). Additional upward shifts in $\delta^{15}N$ occurred downstream of Trib 1 (Mill Creek; 0.98‰ (95% CI = 0.51-1.45‰)) and downstream of the dairy (Gregory Mill (1.12‰ (95% CI = 0.78-1.46‰)).

Discussion

Targeted sampling of $\delta^{15}N$ isotopes upstream and downstream of potential sources of anthropogenic nitrogen allowed detection of locations of elevated anthropogenic nitrogen input. A multiple model comparison approach indicated that change in $\delta^{15}N$ was better represented by parameters with specific locations of change rather than a parameter for continuous change with increasing percent agricultural land. Findings provided evidence of $\delta^{15}N$ enrichment indicative of manure or sewage sources in fish (largescale stoneroller), Asian clam, and snails (*Elimia* sp.) along the study reach, especially between the national forest site and other downstream sites.

Additional smaller enrichments in $\delta^{15}N$ occurred downstream of a tributary confluence and in close proximity to a dairy within the floodplain of the Conasauga River. This finding implies that nutrient management in the Conasauga basin can be focused on specific locations. My results indicated that nitrogen enrichment begins upstream of fish declines, so if nutrients are a threat to Conasauga biota then declines may occur further upstream in the future.

Consistent differences in the $\delta^{15}N$ signatures of the three study species likely reflected different feeding habits. In this study, the Asian clam had the lowest $\delta^{15}N$ of all study organisms, suggesting that the proportion of relatively enriched material that is assimilated is less than that of snails in the Conasauga River. Algae, biofilm, and small invertebrates are likely consumed by largescale stonerollers (fish), which were most elevated in $\delta^{15}N$, and also showed the greatest among-individual variation of the three study species. This variability was especially apparent at Sites 3 and 9 (Table 2.2). At Site 3, the fish collected for analysis were taken from a portion of the shoal that was upstream of, but close (within ~20m) to the mouth of Trib 1; fish may have been moving around within the site, potentially feeding in areas with higher $\delta^{15}N$ (i.e., downstream of Trib 1). Stable isotope studies using an algivorous fish as a primary consumer may need to account for increased mobility and more varied diet in fish by sampling a greater number of individuals or sampling over larger spatial areas.

Primary consumer $\delta^{15}N$ increased in the downstream direction and with increasing percent agricultural land use in the watershed. Percent agricultural land use was the best-supported single predictor of $\delta^{15}N$. However, a combination of binary variables, DS national forest, DS Trib 1, and DS Dairy, better reflected shifts in $\delta^{15}N$. The additive effect of these three variables in the best-supported model effectively divided the study sites into four reaches (Site 1, Sites 2-3, Sites 4-6, and Sites 7-16), thereby capturing shifts in $\delta^{15}N$ enrichment among sites

better than a single continuous variable (percent agricultural land use). For example, based on the estimate for increase with percent agriculture (0.13‰ per 1% agriculture land) I would expect an increase of 0.86‰ on average at sites downstream of the dairy, which is less than the observed 1.12‰ increase.

Inclusion of binary site-level parameters also captured a significant change nitrogen enrichment (+0.98‰) and percent agricultural land (+6%) at sites downstream of Trib 1 and upstream of the dairy (Sites 4-6). Sharpe and Nichols (2007) found that snails from within Trib 1 collected in 2004 had the highest mean $\delta^{15}N$ (11.82‰) found in all of their study sites. This was 1.11‰ $\delta^{15}N$ higher than the most-enriched individual snail sampled in my study (10.71‰ at Site 10, upstream of the mouth of Trib 4). A third point of ^{15}N elevation was at the dairy (+1.12‰). This increase was slightly higher than the Trib 1 increase and was associated with a much smaller increase in percent agricultural land (+2%). Sharpe and Nichols (2007) found the highest levels of TN and TP measured in their study (2.5 mg/L TN and 0.30 mg/L TP) at the dairy site while dairy manure was being applied to an adjacent agricultural field. The results of this study and Sharpe and Nichols (2007) suggest that the Trib 1 watershed and the dairy are significant contributors of nitrogen enrichment to the Conasauga River.

$\delta^{15}N$ signatures in primary consumers in the Conasauga River downstream of the national forest were similar to, though somewhat lower than, primary consumers in Danish lakes within agricultural areas (+13‰; Vander Zanden et al. 2005). Vander Zanden et al. found primary consumers in lakes in urban areas to be most enriched in $\delta^{15}N$ (+31‰) and in natural watersheds to be least enriched (+3‰). Snails collected from the Conasauga River within the national forest in 2010 (this study) and in 2004 (Sharpe and Nichols 2007) had similar $\delta^{15}N$ signatures, and these signatures were similar to those reported by Cabana and Rasmussen (1996) and Vander

Zanden et al. (2005) for primary consumers from pristine areas with low percent agricultural land (+3.3‰ and +3‰, respectively). $\delta^{15}N$ signatures for snails in the Conasauga River at four sites between the dairy and Lower Kings Bridge (Sites 7, 8, 9, and 12) were slightly less enriched on average in the summer of 2004 (mean +8.8‰, range +8.3 to +9.3‰; Sharpe and Nichols 2007) than in my study (mean +9.4‰, range +9.2 to +9.6‰).

Elevated primary consumer ^{15}N enrichment suggests anthropogenic sources of nitrogen, however it is difficult to determine contributions from various sources because natural processes of nitrogen transformation (e.g., denitrification and volatilization of ammonia) can cause once distinct ^{15}N signatures to overlap (Heaton 1986, Kendall 1998, Vander Zanden et al. 2005). Animal waste products (e.g., sewage and various types of manure) begin with signatures elevated in $\delta^{15}N$ (+10 to +20‰) and can become more enriched with nitrogen transformation. Inorganic fertilizers begin with a low $\delta^{15}N$ signature (-3 to +3‰), but enrichment increases as in-stream denitrification occurs; denitrification rates are generally higher with high nitrogen concentration in streams. Therefore both manure and inorganic fertilizer can contribute to elevation of $\delta^{15}N$. Both manure and inorganic fertilizer application have been increasing in Murray and Whitfield Counties (in number of acres fertilized), but manure application is increasing at a greater rate. Manure application doubled in Murray and Whitfield Counties between 2002 and 2007, while inorganic fertilizer application increased by 8% (NASS 2009).

Additional stable isotope analysis of primary consumers may be helpful to further identify areas contributing to $\delta^{15}N$ enrichment between the national forest site and Easley Ford (Sites 1-2). Other methods may be required, however, to differentiate nitrogen sources contributing to the enrichment. In this study, I was not able to identify whether primary consumers outside of the national forest are enriched because a large proportion of the nitrogen

fueling production is supplied by inorganic fertilizer that is enriched by denitrification (i.e., a quantity and quality difference), or because a small proportion of the nitrogen fueling production is from highly enriched manure or sewage sources (i.e., a quality difference), or because highly enriched sources are supplied in high quantities. Poor nutrient management in floodplain agricultural fields and/or the Trib 1 watershed is a likely cause of nitrogen enrichment, however, poorly maintained septic systems may also contribute to enrichment. Septic system failure or wastewater discharge can be distinguished from manure or animal waste by screening for optical brighteners, in conjunction with sampling for fecal bacteria. Optical brighteners are found in most household detergents and may indicate, for example, septic system failure, while bacteria sampling would help quantify the amount of waste reaching the stream (Hartel et al. 2007). The low density of residential areas along the upper Conasauga River makes it feasible to confirm or rule out leaking septic systems as a significant nitrogen source.

In either case, anthropogenic nitrogen sources appear to be fueling primary production in the mainstem Conasauga River. Although percent agricultural land was a strong predictor of ^{15}N in primary consumers, results of this work indicated that shifts in the upper Conasauga River (between Sites 1 and 2) were greater than would be expected from the increase in percent agricultural land use alone. Nitrogen enrichment in primary consumers, consistently high dissolved nitrate-nitrite concentrations (reported from base flow water samples taken within the Conasauga River), and anecdotal evidence documenting algal blooms along extensive reaches of the upper Conasauga River over the last decade all support the hypothesis that eutrophication may be a major driver of deteriorating conditions in the Conasauga River, possibly contributing to declines in fish and mussel species. Additionally, runoff of agricultural nutrients is likely

accompanied by runoff of other agricultural chemicals (e.g. pesticides). Locations identified in this study as points of nutrient input are probably also sites of loading of other toxins. Continued trends of increased agricultural runoff are likely incompatible with long-term persistence of the high biodiversity found in the Conasauga River.

Literature Cited

Anderson, C. and G. Cabana. 2005. Delta N-15 in riverine food webs: effects of N inputs from agricultural watersheds. Canadian Journal of Fisheries and Aquatic Sciences **62**:333-340.

Argentina, J. E., M. C. Freeman, and B. J. Freeman. 2010a. Predictors of occurrence of the aquatic macrophyte *Podostemum ceratophyllum* in a southern Appalachian river. Southeastern Naturalist **9**:465-476.

Argentina, J. E., M. C. Freeman, and B. J. Freeman. 2010b. The response of stream fish to local and reach-scale variation in the occurrence of a benthic aquatic macrophyte. Freshwater Biology **55**:643-653.

Augspurger, T., Keller, A.E., Black, M.C., Cope, W.G., and Dwyer, F.J. 2003. Water quality guidance for protection of freshwater mussels (Unionidae) from ammonia exposure. Environmental Toxicology and Chemistry **22**: 2569-2575.

Burnham, K.P., and Anderson, D.R. 2002. Model Selection and Multimodel Inference: a Practical Information-Theoretic Approach. Springer-Verlag, New York.

Cabana, G. and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences of the United States of America **93**:10844-10847.

Davis, J. M., A. D. Rosemond, S. L. Eggert, W. F. Cross, and J. B. Wallace. 2010. Long-term nutrient enrichment decouples predator and prey production. Proceedings of the National Academy of Sciences of the United States of America **107**:121-126.

ESRI. 1999-2006. ArcMap 9.2. Redlands, CA.

Freeman, B.J., Hagler, M.M., Wenger, S.J., Anderson, G.B., and Katz, R.A. 2007. Identification and mapping of critical habitats in the Conasauga River corridor of Georgia and Tennessee. 2006 Annual report to the Georgia Department of Natural Resources, Atlanta.

Gustafson, L., W. Showers, T. Kwak, J. Levine, and M. Stoskopf. 2007. Temporal and spatial variability in stable isotope compositions of a freshwater mussel: implications for biomonitoring and ecological studies. Oecologia **152**:140-150.

Hagler, M. M., M. C. Freeman, S. J. Wenger, B. J. Freeman, P. L. Rakes, and J. R. Shute. 2011. Use of recent and historical records to estimate status and trends of a rare and imperiled stream fish, Percina jenkinsi (Percidae). Canadian Journal of Fisheries and Aquatic Sciences **68**:739-748.

Hartel, P. G., C. Hagedorn, J. L. McDonald, J. A. Fisher, M. A. Saluta, J. W. Dickerson, L. C. Gentit, S. L. Smith, N. S. Mantriprayada, K. J. Ritter, and C. N. Belcher. 2007. Exposing water samples to ultraviolet light improves fluorometry for detecting human fecal contamination. Water Research **41**:3629-3642.

Heaton, T. H. E. 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere - a review. Chemical Geology **59**:87-102.

Hilton, J., M. O'Hare, M. J. Bowes, and J. I. Jones. 2006. How green is my river? A new paradigm of eutrophication in rivers. Science of the Total Environment **365**:66-83.

Kendall, C. 1998. Tracing nitrogen sources and cycling in catchments. Pages 519-576 *in* C. Kendall and J. J. McDonnell, editors. Isotope Tracers in Catchment Hydrology. Elsevier Science B.V., Amsterdam.

Peterson, B. J. and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics **18**:293-320.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology **83**:703-718.

Rabalais, N. N. 2002. Nitrogen in aquatic ecosystems. Ambio **31**:102-112.

SAS Institute Inc. 2002-2003. Statistical Analysis Software version 9.1. Cary, NC.

Sharpe, A. J. and E. G. Nichols. 2007. Use of stable nitrogen isotopes and permeable membrane devices to study what factors influence freshwater mollusk survival in the Conasauga River. Environmental Monitoring and Assessment **132**:275-295.

Schindler, D. W. 1990. Experimental perturbations of whole lakes as tests of hypotheses concerning ecosystem structure and function. Oikos **57**:25-41.

Steffy, L. Y. and S. S. Kilham. 2004. Elevated delta N-15 in stream biota in areas with septic tank systems in an urban watershed. Ecological Applications **14**:637-641.

Strayer, D.L., Downing, J.A., Haag, W.R., King, T.L., Layzer, J.B., Newton, T.J., and Nichols, S.J. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. Bioscience 54(5): 429-439.

National Agricultural Statistics Service (NASS). *2007 Census of Agriculture*. AC-07-A-2. Washington, DC: United States Department of Agriculture, 2009. http://www.agcensus.usda.gov/Publications/2007/Full_Report/usv1.pdf (accessed December 15, 2011).

USGS. 2007. NLCD 2001 Land Cover. U.S. Geological Survey, Sioux Fall, SD

Vander Zanden, M. J., Y. Vadeboncoeur, M. W. Diebel, and E. Jeppesen. 2005. Primary consumer stable nitrogen isotopes as indicators of nutrient source. Environmental Science & Technology **39**:7509-7515.

Wenger, S. J., M. M. Hagler, and B. J. Freeman. 2009. Prioritizing areas of the Conasauga River sub-basin in Georgia and Tennessee for preservation and restoration. Southeastern Fishes Council Proceedings **51**:31-38.

Table 2.1. Stable isotope site locations and land use percentages. US=upstream; DS=downstream. See also Figure 2.1.

Site No.	Site Name	Sample Date	River kilometer	Percent agricultural land in catchment	Percent agricultural land in riparian area
1	National Forest	7/21/2010	121.0	0%	0%
2	Easley Ford	8/10/2010	104.3	3%	3%
3	US Trib 1	6/9/2010	101.4	4%	3%
4	DS Trib 1	7/7/2010	101.1	10%	7%
5	DS Trib 2	7/7/2010	99.5	11%	8%
6	US Dairy - Carlton Petty Rd	7/7/2010	97.2	11%	9%
7	DS Dairy	7/8/2010	96.2	13%	10%
8	US Trib 3	7/8/2010	90.0	14%	11%
9	DS Trib 3 - Hwy 2	6/8/2010	87.9	16%	13%
10	US Trib 4	7/21/2010	80.0	16%	14%
11	DS Trib 4	7/21/2010	79.3	17%	15%
12	Lower Kings Bridge	6/16/2010	73.2	17%	15%
13	GA Hwy 286	7/6/2010	70.3	18%	16%
14	GA Hwy 76/52	7/6/2010	59.2	20%	18%
15	US Trib 5 - Tibbs Bridge	6/8/2010	51.2	20%	19%
16	DS Trib 5 - Airport Rd.	7/6/2010	44.6	22%	21%

Table 2.2. Summary of stable isotope data by site. Total number of samples collected (N) and mean $\delta^{15}N$ with standard deviation (SD) by site and organism in 2010 are shown.

	Fish			Snail			Clam		
Site	N	Mean(‰)	SD(‰)	N	Mean(‰)	SD(‰)	N	Mean(‰)	SD(‰)
1	5	4.11	0.66	3	3.43	0.69	0	NA	NA
2	5	8.60	0.70	3	7.10	0.19	3	5.14	0.21
3	5	9.73	1.34	3	7.55	0.42	3	6.08	0.04
4	5	10.49	0.16	3	8.34	0.33	3	6.24	0.43
5	5	10.02	0.56	3	8.26	0.07	3	6.22	0.53
6	5	10.34	0.69	3	8.40	0.56	3	6.55	0.70
7	5	10.76	0.53	3	9.32	0.21	3	7.52	0.10
8	5	10.80	0.46	3	9.17	0.57	3	7.49	0.37
9	5	11.12	1.27	3	9.36	0.06	3	7.55	0.58
10	5	11.63	0.48	3	9.89	0.71	3	7.65	0.62
11	5	12.03	0.40	3	9.81	0.42	3	7.56	0.57
12	5	10.67	0.22	3	9.58	0.22	3	7.84	0.44
13	5	10.77	0.18	3	9.39	0.28	3	8.27	0.39
14	5	11.40	0.35	3	10.02	0.56	3	8.20	0.14
15	5	10.72	0.32	3	9.40	0.27	3	8.41	0.20
16	5	11.21	0.78	1	9.90	NA	3	7.86	0.61

Table 2.3. Models relating tributaries, a point source and percent agricultural land use upstream to $\delta^{15}N$; number of parameters (K), ΔAIC_C, and model weights (w_i) are shown. No predictors and base-line models are included for comparison. The no-predictors model includes an intercept, a random site-level effect, and a residual error term. The base-line model includes those three parameters, plus two individual-level parameters to distinguish organism type and a site-level parameter to indicate position downstream of the national forest. All other models include those six parameters, plus other site-level predictors, as shown below. DS=Downstream.

Model	K	ΔAIC_C	w_i
DS Trib 1, DS Dairy	8	0	0.39
DS Trib 1, DS Dairy, DS Trib 4	9	0.6	0.29
DS Trib 1, DS Dairy, DS Trib 5	9	2.1	0.14
DS Trib 1, DS Dairy, DS Trib 4, DS Trib 5	10	2.8	0.10
% Ag in Catchment, DS Trib 5	8	3.9	0.05
% Ag in Catchment	7	4.4	0.04
Base-line model	6	30.6	3.93×10^{-8}
No-predictors model	3	362.8	NA

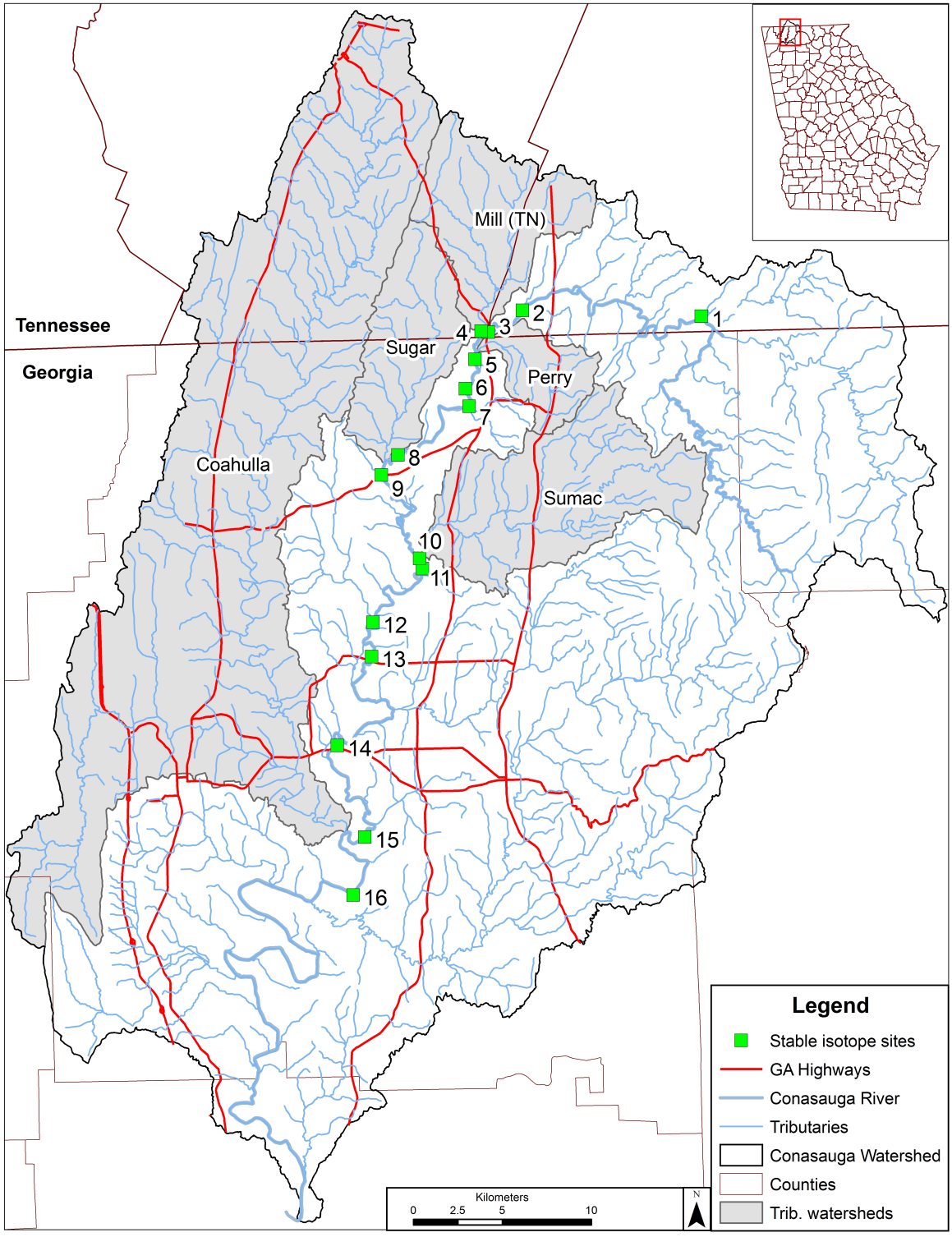

Figure 2.1. Map of stable isotopes sampling sites located along the Conasauga River. Sites were chosen in pairs, upstream and downstream of tributary watersheds of interest, and upstream and downstream of a waste lagoon (Sites 6-7). Additional sites were selected for greater geographic coverage; these were typically located at road crossings. See also Table 1.

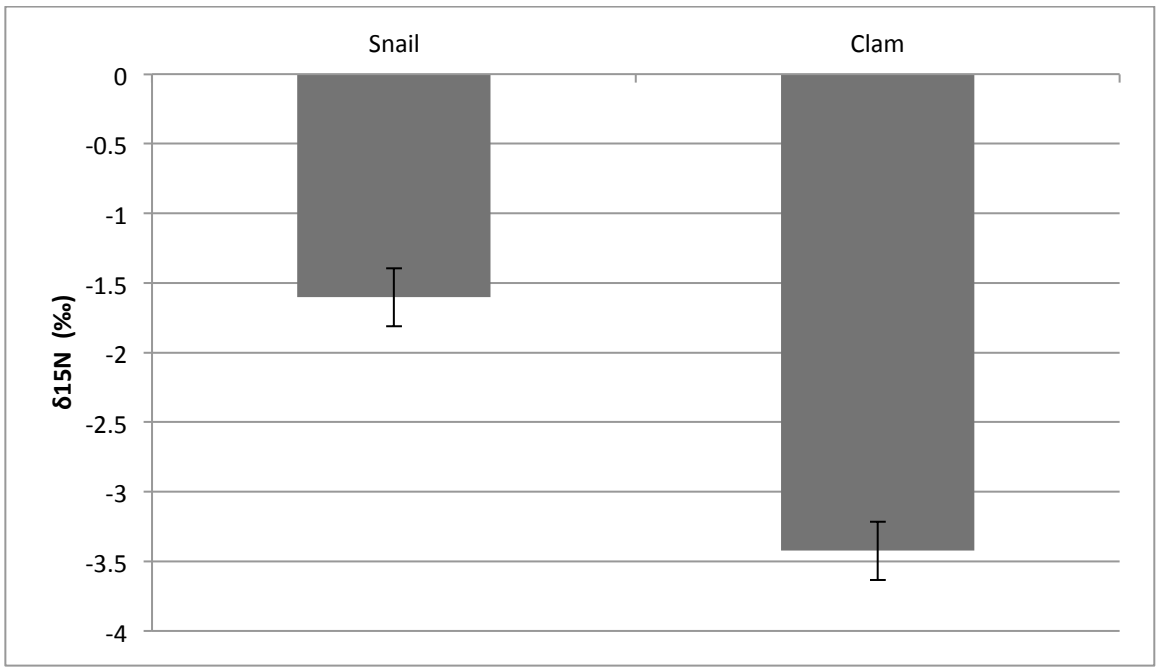

Figure 2.2. Parameter estimates (± 95% CI) from the best-supported model indicating the effect of organism type on $\delta^{15}N$ values relative to that of fish. Values indicate that fish are the most enriched in $\delta^{15}N$ and clams are the least enriched.

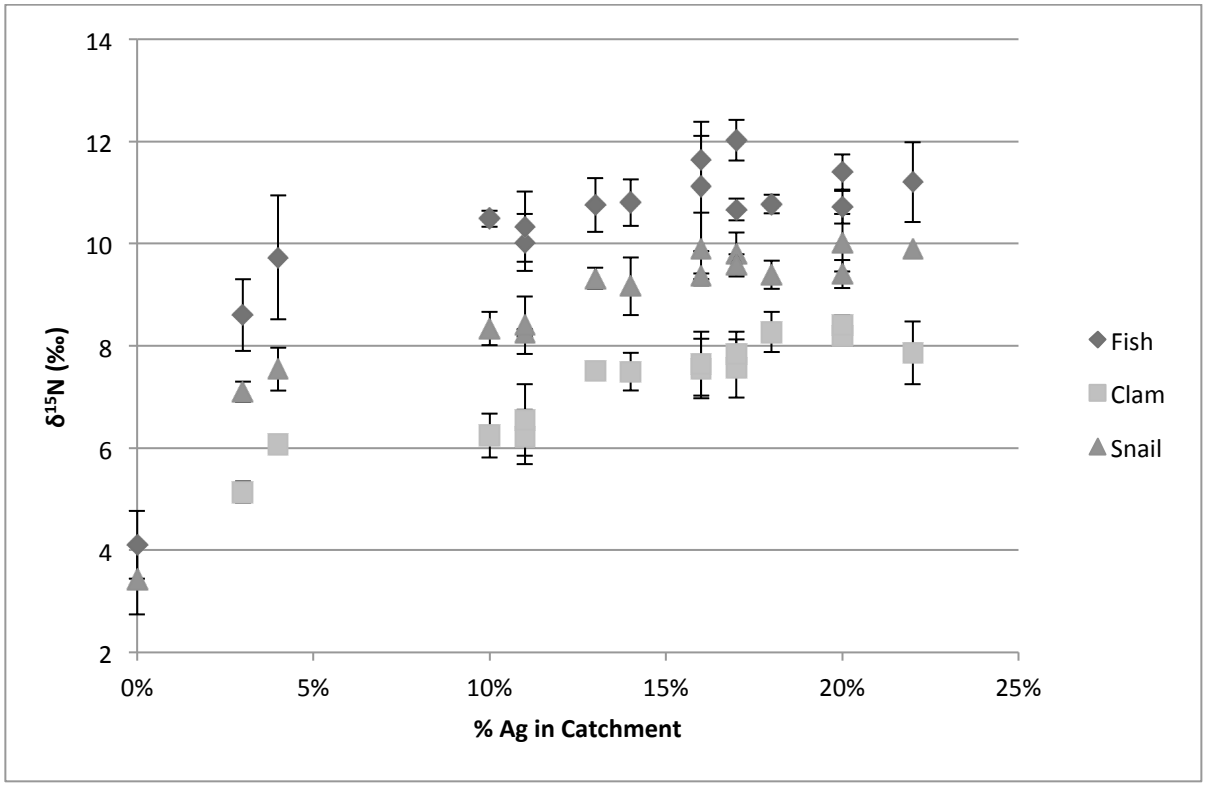

Figure 2.3. Mean $\delta^{15}N$ (± SD) of fish, snails and clams at each site, plotted against percent agricultural land use in the watershed upstream of each site.

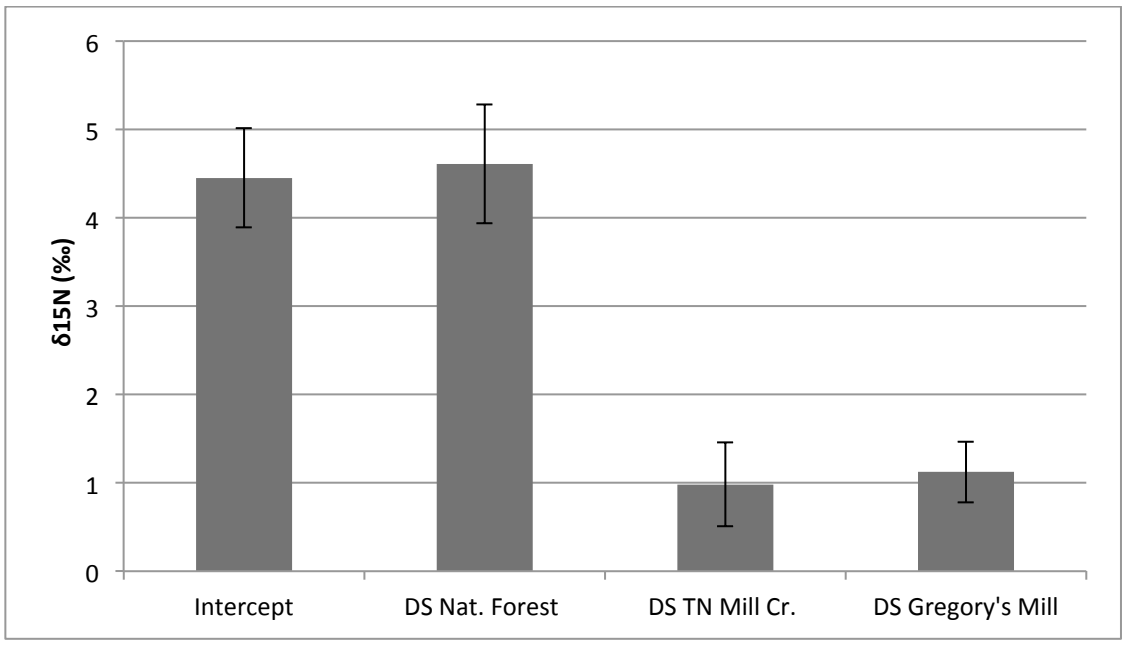

Figure 2.4. Site-level parameter estimates (±95% CI) for the top model. The intercept represents mean $\delta^{15}N$ in fish from the national forest site. "DS TN Mill Cr" is downstream from Trib 1; "DS Gregory's Mill" is downstream from the dairy.

CHAPTER 3

DETECTING DISCONTINUOUS CHANGE IN RIVERINE MACROINVERTEBRATE COMMUNITIES ALONG A LAND USE GRADIENT

Introduction

Anthropogenic disturbances, such as land use alteration and pollution, can alter taxonomic structure and functional composition of biotic assemblages. Often biotic communities change discontinuously along a disturbance gradient (Groffman et al. 2006). Aquatic invertebrate communities can be resistant to disturbances like increasing impervious surfaces (King et al. 2011) or nutrient loading (King and Richardson 2003, Evans-White et al. 2009) at low levels, but when disturbances reach some threshold the community changes rapidly. Identifying thresholds at which these discontinuous changes in communities occur is key for informing management. Traditionally, threshold detection methods are used to identify discontinuous responses of an ecosystem or community property along a gradient of an abiotic or biotic driver (Dodds et al. 2010). However, these methods can also be used to identify lags in community response to a management action (Clements et al. 2010) or disturbance (Gido et al. 2010). Presumably, threshold detection can be used to detect a suspected spatial discontinuity in the biotic community when the specific disturbance is unknown. In situations where the mechanism of community change is unknown, finding the location of change in space or time can provide useful information for determining the mechanism.

Aquatic invertebrates are commonly-used indicators of anthropogenic disturbance to freshwaters because taxa vary in their sensitivity to pollution as a result of differing life history strategies and physiological characteristics. Biotic indices use this variability in sensitivity to evaluate health of a stream. However, indices often fail to inform us of the stressor affecting a stream or the mechanism causing shifts in invertebrate community structure. In contrast, traits of invertebrate taxa that decline along a gradient can indicate which physiological or life history need is not being met, thus indicating the mechanism of community change in an impacted stream (Poff et al. 2006, Wagenhoff et al. 2011). For example, aquatic nymphs may be more vulnerable to excess algal growth if adult females lay eggs at or above the water surface rather than entering the water to select appropriate habitat (Doledec et al. 2006). If appropriately identified, traits of decreasing or increasing taxa can be used to evaluate competing hypotheses of the mechanisms behind changes in invertebrate community structure or declining biodiversity.

My research investigates trait-related shifts in macroinvertebrates along a spatial gradient where scientists suspect incipient decline in health of a river that is valued for conserving biological diversity. The Conasauga River in northwest Georgia and southeast Tennessee is a highly biodiverse headwater refuge for imperiled species that have lost habitat and been eliminated from lower portions of the Coosa River system (Freeman et al. 1996, Freeman et al. 2005). The Conasauga flows from the Chattahoochee National Forest in the Blue Ridge province, through the Valley and Ridge province, where agricultural land is prevalent, and then through an area of growing suburban development. Over recent decades researchers have documented declines in fishes, mussels and the aquatic macrophyte *Podostemum ceratophyllum* in the Conasauga River, and increasing incidence of extensive algal mat development during warm seasons (B. J. Freeman, personal communication; Freeman et al. 2007, Sharpe and Nichols

2007, Wenger et al. 2009, Argentina et al. 2010b, Hagler et al. 2011). Long-term monitoring of fish assemblages suggests that sensitive and imperiled species have primarily declined in reaches of the river downstream of the forested headwater but upstream of the direct influence of suburban development (Freeman et al. 2007, Hagler et al. 2011). Evidence suggests that the shift in the fish assemblage is discontinuous, but there is uncertainty in the location of the change. In order to mitigate loss of biodiversity in the Conasauga River, managers need to know where changes in the biotic community are occurring. Hypothesized causes of change in the Conasauga River fish and mussel assemblages include eutrophication, pesticide pollution, or a combination of stressors, but the mechanism is currently unknown. Shifts in occurrence, abundance or biomass of macroinvertebrate taxa concurrent with apparent declines in fishes have not been previously investigated. However, macroinvertebrates are important sentinels of ecosystem change, and could be useful indicators of the mechanism and location of biotic changes in the Conasauga River.

Ecologists employ a variety of analytical methods for understanding changes in community composition, including multivariate analyses (McCune and Grace 2002), examination of trends in multispecies metrics or measures of taxa diversity (Lenat 1993, Kerans and Karr 1994, Barbour et al. 1999), and analyses of taxon-specific shifts in species abundances or biomasses (Dufrene and Legendre 1997, Baker and King 2010). Here I use a combination of approaches to evaluate evidence of macroinvertebrate community change and to test *a priori* hypotheses concerning where along the study reach community shifts occur. Specifically, I compare analyses of macroinvertebrate sample data using linear regression and multi-model selection (Burnham and Anderson 2002), ordination analysis, and a recently developed community and taxon-specific threshold-analysis tool (Baker and King 2010). Additionally, I

use regression and multi-model selection to evaluate support for alternative a priori hypotheses concerning relations between species-specific traits and community change along the longitudinal gradient of the study reach. Together, these analyses evaluate the location of change in taxonomic and functional groups of macroinvertebrates, and potentially shed light on stressors that contribute to macroinvertebrate decline in the Conasauga River.

Methods

Study Location

The invertebrate study portion of the Conasauga River mainstem spanned 28.6 river kilometers (Figure 3.1), beginning at river kilometer 102 just upstream of the Georgia/Tennessee state line and extending to river kilometer 73.4 at Lower Kings Bridge Road near Dalton, GA. Water quality and fish occurrence and abundance data have been collected in this reach of river regularly over approximately 20 years. Over this period nutrient levels have increased and detection of sensitive, shoal-dependent fish species has declined. In addition to temporal declines, analysis of the fish community data indicate stronger evidence of decline in the reach downstream of Highway 2 (Freeman et al. 2007, Argentina et al. 2010b, Hagler et al. 2011), which I will refer to as the 'fish decline reach'. The invertebrate study portion encompassed the 'fish decline reach' and extended 15.3 kilometers upstream.

Macroinvertebrate sampling and processing

Invertebrates were collected from randomly selected shoals along the study portion of the Conasauga River in June and July 2009. Shoals were selected within five sections defined by confluences with major tributaries such that the number of shoals selected from each section was

weighted by the length of shoal habitat within the section. Shoal length was calculated from existing data of delineated shoals within the Conasauga (Argentina et al. 2010b) using ArcGIS (ESRI, Redlands, CA). If a shoal was selected more than once, I spaced the sample locations evenly along the length of the shoal; otherwise samples were collected from the mid-point of selected shoals. Using this strategy I collected 3 samples upstream of Mill Creek (TN), 2 samples between Mill Creek (TN) and Perry Creek, 16 samples between Perry Creek and Sugar Creek, 8 samples between Sugar Creek and Sumac Creek, and 4 samples downstream of Sumac Creek on each sampling date (Figure 3.1). A total of 66 samples were taken over two sampling dates in June and July 2009 (although one sample was lost, resulting in 65 used for analyses). A modified PVC T-sampler (Grubaugh and Wallace 1995) with a 11.5 cm diameter and 243 μm mesh net was used to collect samples from gravel and cobble in mid-channel where water velocity was moderate to swift. Gravel and cobbles within the T-sampler were scraped clean of the macrophyte *Podostemum ceratophyllum* and invertebrates, and the underlying sediment was disturbed to 5 cm depth. All material that flowed into the net was preserved in 5% formalin solution and dyed with Rose Bengal. Depth and water velocity (at 60% depth) were measured at each sample location using a wading rod and Marsh-McBirney flo-mate. I noted dominant substrate at the sample point as either coarse (medium cobble or larger) or fine (smaller than medium cobble).

Samples were returned to the laboratory and elutriated into a 250 μm mesh sieve to separate organic material from sediments. Remaining sediment was inspected with a magnifying lamp for shell or case-bearing macroinvertebrates that were not suspended during elutriation. Macroinvertebrates and *Podostemum* were separated from organic material under 12 X magnification and preserved in 70% ethanol solution and 5% formalin solution, respectively.

Insect taxa were identified to genus when possible with the exception of Chironomidae, which were classified as Tanypodinae or non-Tanypodinae. Non-insect taxa were identified to the lowest taxonomic level possible (e.g. Oligochaeta, Nematoda, Hydracarina). All invertebrates were measured to the nearest 0.1 mm using an ocular micrometer at a maximum of 50 X magnification. Body length or head width was converted to ash-free dry mass using published length-mass regressions and ash-free dry mass correction values (Benke et al. 1999). When genus level regressions were not available to convert length to dry mass, family or order level regressions were used. I used family level ash-free dry mass corrections for all taxa. *Podostemum* was dried, weighed, ashed and weighed again to obtain ash-free dry mass (AFDM) as a measure of standing-stock biomass within the sample.

Data analysis - Overview

I used three methods for evaluating changes in the invertebrate community in the Conasauga River and to test for existence of discontinuous points of change. First, I used regression analysis and an information theoretic approach to evaluate support for competing hypotheses of locations of discontinuous change versus an alternative hypothesis that change is continuous along the length of the study area, and a null hypothesis that there is no change in the invertebrate community. Hypothesized points of change were at the upstream end of the 'fish decline reach' (FDr, encompassing the lower 13.3 km of the study section) and at a point further upstream at the location of a dairy waste lagoon adjacent to the river, where previous research indicated a discontinuous increase in $\delta^{15}N$ isotope levels in biota. The reach downstream of this point was designated the 'high nitrogen reach' (HNr, see Chapter 2), and encompassed 23 km including the FDr. I used regression analyses to test specific hypotheses about the nature of

change in macroinvertebrate communities, incorporating the competing hypotheses about locations of change. Specifically, I tested the hypotheses that insects commonly consumed by fishes decline downstream, and that insect richness and EPT richness change downstream. To test whether taxon-specific changes in biomass were explained by functional or life-history traits, I used mixed-effects linear regression. In addition to regression analyses, I used nonmetric multidimensional scaling (NMDS) ordination of samples in species space to visualize community change in the river and to look for evidence of grouping of sites with similar community composition. Finally, I applied a new analysis tool developed by Baker and King (2010) to identify thresholds of change in communities and in individual taxa along a longitudinal gradient from upstream to downstream.

Invertebrate assemblages typically vary in taxa densities and composition in relation to variation in water velocity, water depth and substrate type (Gore 1978, Statzner et al. 1988, Gore et al. 2001). To account for habitat effects on among-sample differences in invertebrates, I tested for longitudinal trends in these habitat characteristics as measured at each sample location. *Podostemum ceratophyllum* (Riverweed) provides habitat for invertebrates and fishes (Hutchens et al. 2004, Argentina et al. 2010b), and was therefore another biotic component of interest in the Conasauga River. *Podostemum* was not present in every sample, so I used logistic regression to test for patterns of *Podostemum* occurrence using the entire dataset, followed by linear regression to test for trends in natural log transformed biomass of *Podostemum* for samples in which it occurred (54 of 65 samples; Gelman and Hill 2007). *Podostemum* is typically abundant in areas with coarse substrate (Argentina et al. 2010a) and flowing water (Nelson and Scott 1962). I regressed *Podostemum* occurrence and biomass against location variables, a variable for sample date, and sample-specific water velocity, substrate size, and water depth. I also regressed each of

the abiotic habitat characteristics against location variables to test for evidence of a longitudinal shift in habitat availability for *Podostemum* as well as invertebrates. Water depth and velocity were untransformed continuous variables modeled by linear regression. Substrate size was a binary variable describing substrate as coarse (1=medium cobble or larger) or fine (0=smaller than medium cobble) and was modeled by logistic regression.

Regression analysis of invertebrate quantity and community metrics

I used regression analysis to evaluate two broad hypotheses. First, I hypothesized that fish declines could reflect decreasing availability of invertebrate prey items, which were primarily the orders Ephemeroptera, Trichoptera, Plecoptera and Diptera for declining fish species (Etnier and Starnes 1993, Boschung and Mayden 2004). Therefore, I regressed total sample biomass and total sample abundance summed for these orders. Total sample biomasses were natural log transformed and regressed against alternative longitudinal variables and other covariates hypothesized to influence invertebrates: *Podostemum* biomass, water velocity and substrate size. I also included date as a binary predictor variable to account for variation between sample dates. Total sample abundances were similarly analyzed using negative binomial regression (to account for overdispersion in count data) with a log-link. Secondly, I hypothesized that if capacity of the river to support invertebrates declined along the study reach, then invertebrate total richness would decline if the entire community was affected, or EPT richness (a common indicator of aquatic ecosystem health) would decline if only sensitive invertebrates were affected. Richness was modeled with Poisson regression with a log-link and related to the same longitudinal and habitat variables as in the invertebrate prey analyses.

Regression parameter estimates were converted (by exponentiation for log-transformed response variables) to proportions of change in the response variable with every one-unit change in predictors. Continuous predictor variables (*Podostemum* biomass, water velocity and river kilometer) were standardized ((X_i-$\bar{X}$)/sd) prior to regression analyses. Effects of these predictors were interpreted as the proportion of change in the response variable with every standard deviation change in the predictor, and then rescaled to reflect biologically meaningful units of change. I used an information theoretic approach to evaluate relative model fit for competing hypotheses (specified in the previous section) about the location of longitudinal change in macroinvertebrate abundance, biomass and richness. Candidate models were constructed with all possible combinations of habitat and date variables without location parameters and with each of the three location parameters. Akaike's Information Criterion with correction for small sample size (AICc; Burnham and Anderson 2002) was used to rank candidate models from best-supported (lowest AICc) to least-supported, and those with a ΔAICc (candidate model AICc minus best-model AICc) of two or less were retained in the confidence set of models. Following Burnham and Anderson (2002), I eliminated models from the confidence set if they contained more parameters than the best-supported model and had similar log-likelihoods.

Mixed-effects regression of invertebrate functional traits

To evaluate mechanisms of invertebrate community functional shifts in the Conasauga River, I compiled a list of traits that could be used as indicators of changes in water quality and increased algal growth. I expected water quality to decline and algal accrual to increase downstream, so I developed hypotheses for responses of taxa with specific functional traits

accordingly (Table 3.1). I also included a trait predictor for predatory insects, which I expected to decline downstream in a pattern similar to insectivorous fishes.

Multivoltine and small-bodied invertebrates are better able to recover after a disturbance, so I used traits of voltinism and maximum body size as general indicators of disturbance. I used two binary predictors to indicate whether taxa were semivoltine (<1 generation per year), univoltine (1 generation per year; the baseline) or multivoltine (>1 generation per year). I also used two binary predictors to indicate whether the maximum size of each taxon was small (< 9 mm), medium (9-16 mm; the baseline), or large (>16 mm). The North Carolina Biotic Index (NCBI; Lenat 1993) was used to indicate changes in general water quality. The NCBI assigns tolerance values, ranging from 0 to 10 with 10 being most tolerant, to aquatic invertebrates of the southeast US. Taxon-specific NCBI value was included as a continuous predictor that was expected to increase with declining water quality.

The traits algivore, clinger and water-surface egg layer were expected to indicate changes in algal production or accrual. Algivores were expected to have a positive response to increased algal production. Conversely, excess algal accumulation on the surface of rocks and vegetation could lower habitat suitability for invertebrates that require clean, hard surfaces. Clingers require clean surfaces in areas with high water velocity and were expected to decline with increased algal accrual (Merritt et al. 2008). Water-surface egg laying insects were expected to respond negatively to algal accrual. Insects that lay eggs on or above the water surface do not come into contact with the substrate and may not be able to select clean substrate that is appropriate for egg deposition. Subsequently, development of nymphs may be hindered if eggs land in an area with excess algal growth (Doledec et al. 2006). Conversely, adult females of some taxa (e.g., many trichopterans) are adapted to swim or walk into the water and lay eggs

directly on the substrate and may be able to place their eggs in more appropriate habitats. These underwater egg-laying taxa were not expected to decline with increased algal accrual. Traits were compiled from Brigham et al. (1982), Lenat (1993), Wiggins (1996), Poff et al. (2006), Vieira et al. (2006), and Merritt et al. (2008). Only the 30 taxa for which all traits were known and which occurred in at least 5 samples were included in the analysis. Snails were analyzed separately, because their biomasses were much higher on average and overwhelmed trends in insect taxa. *Corbicula fluminea* were not included in regression analyses.

Taxon-specific occurrence (65 samples of 30 taxa = 1,950 occurrence estimates) and biomass (800 estimates of biomass, excluding 0 values) were related to invertebrate traits and river kilometer or reach parameters via mixed-effects linear regression using package lme4 (Bates et al. 2011) in R (version 2.14.0; R development Core Team 2011). Mixed-effects linear regression was used to account for the hierarchical structure in the data (Raudenbush and Bryk 2002). I initially modeled taxon-specific presence and biomasses with a random-intercept model to estimate the amount of variation among taxa and across samples. To account for variation in taxon-specific presence and biomass with sample-specific habitat, I added the habitat variables *Podostemum* biomass, water velocity, water depth and substrate size along with date to the random intercept model in all possible combinations. The best model with only habitat and date variables was then chosen as the baseline model to which location and trait variables were added. The effect of location was allowed to randomly vary among taxa (random slope effect). All trait models of biomass had the following form:

$$\text{Log}(Y_{ij}) = \beta_{0j} + \beta_{1j}*\textit{Podostemum}_i + \beta_{2j}*\text{water velocity}_i + \beta_{3j}*\text{date} + \beta_{4j}*\text{location}_i + \sigma^2_{ij}$$

Random intercept model: $\beta_{0j} = Y_{00} + Y_{01}*\text{trait}_j + \mu_{0j}$

Random slope model: $\beta_{4j} = Y_{10} + Y_{11}*\text{trait}_j + \mu_{1j.}$

Substituting the random effects into the model results in,

$$\text{Log}(Y_{ij}) = Y_{00} + Y_{01}*\text{trait} + \mu_{00} + \beta_{1j}*\textit{Podostemum}_i + \beta_{2j}*\text{water velocity}_i + \beta_{3j}*\text{date}_i +$$

$$Y_{10}*\text{location}_i + Y_{11}*\text{trait}*\text{location} + \mu_{11}*\text{location} + \sigma^2_{ij},$$

where Y_{ij} is the biomass for sample *i* of taxon *j,* Y_{00} is the grand mean intercept of biomass among taxa, Y_{01} is the effect of trait on the intercept, μ_{00} is the random intercept effect (normally distributed with a mean of zero) allowing intercepts to vary among taxa, Y_{10} is the mean slope for location among taxa, Y_{11} is the effect of trait on the slope for location, μ_{11} is the random slope effect (normally distributed with a mean of zero) allowing slopes for the location-effect to vary among taxa, and σ^2_{ij} is the residual variation among samples not accounted for by the model. All models also included a covariance parameter to account for correlation between slopes and intercepts. The model structure was similar for occurrence data, but modeled with a logit-link and not including a residual variation parameter.

One of three longitudinal parameters was included as a main effect in each model. These were the continuous predictor river kilometer, the binary predictor identifying the fish decline reach (FDr), and the binary predictor identifying the high nitrogen reach (HNr). Invertebrate traits (predator, scraper, clinger, surface egg layer, voltinism, size and NCBI) were included as main effects and in interactions with longitudinal parameters to evaluate whether taxa with specific traits shifted in biomass downstream. Biomass estimates were log transformed ($\log(Y_{ij})$)

for analysis, and all continuous predictors were standardized ($(X_i-\bar{X})/sd$). Parameter estimates of standardized continuous variables were exponentiated for interpretation and re-scaled to reflect biologically meaningful changes in predictor variables. AICc was used for model comparison to determine which location effect best predicted longitudinal patterns of macroinvertebrate biomass. Each random effect was counted as one additional parameter for the calculation of AICc. I reported effect sizes for all trait-by-location interactions that explained at least 30% of variation in slopes of the taxon-specific location effect.

Community shifts--Threshold Indicator Taxa Analysis (TITAN)

Program TITAN (Baker and King 2010) was used to identify thresholds of invertebrate community change and taxon-specific thresholds of increasing or decreasing biomass and abundance along a gradient of kilometers downstream from the top of the sample reach. Baker and King (2010) created TITAN to expand on change-point analysis (nCPA; King and Richardson 2003), which measures dissimilarity among and within sample groups along an environmental gradient to identify aggregate, community-level thresholds. TITAN combines nCPA with indicator species analysis to detect thresholds specific to each taxon. Indicator species analysis produces IndVal scores, which are a measure of association with groups of samples on either side of every candidate point of change (midpoints between two samples) based on taxon-specific occurrence and abundance. A high IndVal score indicates the taxon occurs consistently and in high abundance in one group of samples. In the case of TITAN, individual taxa are defined as increasing or declining in occurrence and abundance along the environmental gradient. A taxon-specific threshold is identified as the point along the environmental gradient where the IndVal is maximized. If the highest IndVal occurs to the left

of the candidate change point the taxon is defined as negatively responding and if to the right, as positively responding. TITAN re-scales taxon-specific IndVal scores to z-scores, which indicate degree of departure of the maximum IndVal score from the IndVal scores calculated from random permutations ((IndVal-mean of permutations)/sd of permutations). Standardizing IndVals as z-scores equalizes influence of extremely abundant or rare taxa on estimation of the community change point (Baker and King 2010). Separate community-level change points for negatively and positively responding taxa are determined by summing z-scores. The highest sum(z) score for each group defines the community threshold. Subsequently, TITAN produces two community level thresholds, positive response and negative response, and individual thresholds for each taxon.

Random permutations of the candidate change points allow estimation of the probability (p) of obtaining equal or greater IndVal scores by chance. To estimate uncertainty around change points and consistency of group assignment TITAN uses a bootstrap procedure. I followed the default procedure specified in Baker and King (2010) by using taxa with a minimum of 5 observations and running 250 random permutations and 500 bootstrap replicates. Biomass and abundance were log transformed prior to analysis ($\log(Y_{ij} + 1)$) to reduce influence of extreme observations. All 55 taxa present in 5 or more samples were evaluated for thresholds in abundance using TITAN. Biomass thresholds were evaluated for 53 taxa (2 taxa did not have published length-mass regressions). An additional 20 taxa were found in fewer than 5 samples, and therefore were not evaluated in any analysis. I also used TITAN to evaluate evidence of a threshold in *Podostemum ceratophyllum* biomass, but *Podostemum* was not included in estimation of the macroinvertebrate community change points. I ran TITAN analyses in R

(version 2.14.0; R development Core Team 2011) using code provided with the supplementary material available online with the Baker and King (2010) publication.

Community shifts—NMDS

I used nonmetric multidimensional scaling (NMDS; Field et al. 1982) to evaluate multivariate shifts in invertebrate community composition among samples from the 'high nitrogen reach' or the 'fish decline reach' compared to the upstream portion of the study area. Differences between samples in invertebrate biomass and abundance were represented with the Bray-Curtis dissimilarity metric. Bray-Curtis distances were then used to create an ordination of sites in species-space along a specified number of axes. Samples with similar composition and relative proportions of taxa were plotted in closer proximity to one another in the ordination. High stress (a measure of fit) affects interpretability of the ordination, so dimensionality of the ordination was selected such that final stress was reduced to an acceptable level (<20%; McCune and Grace 2002). I used the metaMDS function in package vegan (Oksanen et al. 2011) in R to create ordinations of 65 samples with abundance data of 55 taxa (those occurring in 5 or more samples) and biomass data of 53 taxa. Biomass and abundance were log-transformed ($\log(Y_{ij} + 1)$) for analysis. I used 20 random starting configurations. The environmental variables river kilometer, *Podostemum* biomass, water velocity and water depth were regressed against sample ordination scores using vector analysis and tested for significance using random permutation (function envfit in package vegan). Sites in the direction of the vector typically had high values of the environmental variable, and changes in taxonomic composition were considered to be associated with the environmental gradient.

Results

The 65 randomly selected samples used in the invertebrate analysis were collected from 35 of 60 shoals mapped within the study area. Of these samples, 14 were upstream of the 'high nitrogen reach' (HNr) and 51 were in the 'high nitrogen reach'; 41 samples were upstream of the fish decline reach (FDr) and 24 were in the 'fish decline reach'. The FDr was a subset of the HNr, therefore 24 of the 65 samples were collected from both reaches (Table 3.2). A 4.1 km section upstream of the HNr was inaccessible and was not sampled (Figure 3.1).

Habitat variables at sample locations differed among the reaches upstream and downstream of the hypothesized points of change (Table 3.2). *Podostemum ceratophyllum* biomass ranged from 0 to 279 grams AFDM per m^2 and only exceeded 100 g/m^2 in a middle portion of the study reach that crossed from the section upstream of the high nitrogen reach into the high nitrogen reach (between river kilometers 96.7 and 92.2). In contrast, nearly two-thirds of samples from the fish decline reach had no *Podostemum* or only trace amounts (<0.1 g/m^2). *Podostemum* was less likely to occur in the 'fish decline reach' and more likely to occur in deeper areas with coarse substrate (Table 3.3). Biomass of *Podostemum,* where it occurred*,* was reduced downstream as well, with an estimated reduction of 82% in the 'fish decline reach' or a 163% increase with each 10 km upstream (Table 3.3). *Podostemum* biomass was also influenced by water velocity, which varied from 0.22 to 1.13 m/s, but did not differ on average among sections (null model was best supported). Water depth was slightly greater on average in the 'fish decline reach'. Substrate also varied among reaches, with samples more likely to occur on coarse substrate upstream of the 'high nitrogen reach' (HNr; Table 3.4). Despite these trends, pairwise correlations of all habitat variables (*Podostemum*, water velocity, water depth and substrate size) and between habitat variables and date and location variables (Rkm, FDr, HNr)

were sufficiently low (r^2<40) to allow inclusion of these variables together in models to predict invertebrate community responses.

Longitudinal changes in invertebrate quantity and taxa richness

I estimated biomass of approximately 31,500 individual invertebrates and counted an additional 1,000 for which there were no published regressions. Raw data indicated a threshold decline in the number of samples with high total invertebrate biomass and abundance (excluding snails and *Corbicula fluminea*) in the fish decline reach (Figure 3.2). Biomass and abundance of "fish prey" (Ephemeroptera, Trichoptera, Plecoptera and Diptera) varied with habitat characteristics at sample points, but there was less evidence of a longitudinal shift in prey quantity. *Podostemum* biomass and water velocity parameters occurred in all models in the confidence set (delta AICc≤2) for prey biomass and abundance (Tables 3.5 and 3.6). Prey biomass increased by 77% with every 100 g/m^2 increase in *Podostemum* biomass and by 14% with every 0.1 m/s increase in water velocity (Table 3.5). Prey abundance increased by similar amounts with *Podostemum* and water velocity (Table 3.6). 'High nitrogen reach' and substrate size were also in the top model for prey biomass, but effects were imprecisely estimated with 95% confidence intervals that included zero (Table 3.5).

Total insect richness and EPT richness were best predicted by habitat variables. Both measures of richness increased by about 12% with every 100 g/m^2 increase in *Podostemum* biomass. The best-supported model for total insect richness also included predictors for substrate size and water velocity (Table 3.7). Substrate size was a predictor in every model in the confidence set for insect richness, and predicted a 20% increase in richness on coarse substrate according to the best-supported model. The effect of water velocity in the best-

supported model was positive but imprecisely estimated. All location parameters appeared in the confidence set for insect richness, but effects were imprecisely estimated. However, when habitat variables were left out of the models, location variables indicated a decline in richness in the downstream direction. River kilometer was the best of the three location parameters and the estimate from the location-only model suggested a 9.6% increase in richness with every 10 kilometers upstream. *Podostemum* was the only predictor in the top EPT richness model (Table 3.8). The second-best model indicated a positive effect of substrate size.

Longitudinal changes in invertebrate community composition

Occurrence and biomass of individual taxa varied in relation to sample-specific habitat, with less precise estimates of sample location effects. In models not including taxon-specific traits, higher *Podostemum* biomass and water velocity were associated with greater taxon-specific odds of occurrence and biomass (Table 3.9). River kilometer was the best-supported location variable for predicting taxa occurrence, with increasing odds upstream, although the effect was imprecisely estimated with *Podostemum* in the model. *Podostemum* declined downstream, so a downstream effect on taxon occurrence could have been overshadowed by the *Podostemum* effect. When *Podostemum* was removed from the model, the river kilometer effect was more precise and indicated an increase in occurrence upstream (Table 3.9). Given taxon occurrence, however, none of the location variables indicated a consistent increase or decline in biomass downstream (Table 3.9). There was stronger relative support for including the 'fish decline reach' parameter in a model of biomass, although the location effect was imprecisely estimated (Table 3.9). This imprecision largely resulted from high variation among taxa (shown

by large random slope effect; Table 3.9), indicating a longitudinal shift in community composition in the fish decline reach.

Two traits explained more than 30% of the variation among taxa when included in an interaction with location to predict either taxa presence or biomass. The trait semivoltine (< 1 generation per year) explained a large proportion of the variation in the 'high nitrogen reach' effect on taxa occurrence or biomass (Table 3.10; Figure 3.3). Odds of occurrence of semivoltine taxa declined by 65.5%, and biomass declined by a predicted 42% in the 'high nitrogen reach'. Conversely taxa with one or more generations per year increased downstream. The trait 'water-surface egg layer' explained large proportions of variation in effects on biomass of all three location variables (Table 3.10; Figure 3.4). Water-surface egg layers odds of occurrence increased by 119.9% and biomass increased by 120% in the 'high nitrogen reach', and biomass increased by 131% in the 'fish decline reach'. In contrast, taxa that lay eggs underwater declined downstream.

Snails (Pleuroceridae) were analyzed separately, because high snail biomass overwhelmed trends in insects. Untransformed data indicated a reduced number of samples with high snail biomass in the 'fish decline reach' as well as a linear decline in abundance (Figure 3.5). River kilometer was included as a predictor in the best-supported model for biomass and abundance analyses, but the effect on biomass was imprecisely estimated (Table 3.11). Abundance was estimated to be 142.1% greater with every 10 km upstream (Table 3.12). Snail abundance responded negatively to coarse substrate and to increased water velocity. Biomass was higher on the second sample date and lower on coarse substrate.

Points of change in invertebrate community and individual taxa-- TITAN

TITAN analysis suggested a point of invertebrate community decline within the 'high nitrogen reach' and upstream of the 'fish decline reach' at river kilometer 92.1 (9.85 kilometers downstream from the upstream end of the sample reach; Table 3.13, Figure 3.6). TITAN indicated a threshold decline in *Podostemum* at the same location as the negative community change point. The positive change point was less precise, and occurred further downstream but still upstream of the 'fish decline reach'. The nCPA estimate for the change point of the entire invertebrate community (Table 3.13) was similar to the negative change point in TITAN, but TITAN produced more precise estimates and separate values for negatively and positively responding taxa.

TITAN identified negative thresholds for twice the number of taxa for which it found positive thresholds, supporting the hypothesis that fewer macroinvertebrate taxa occurred in high abundance or biomass in the downstream direction. The biomass analysis identified thresholds for 26 negatively responding taxa and 11 positively responding taxa for which random permutations resulted in a p-value of ≤ 0.05. These groups of taxa were reduced to 9 negatively and 4 positively responding taxa based on a purity (proportion of bootstrap replicates that indicate a change-point response direction consistent with the observed response) restriction of ≥ 0.95 and reliability (proportion of bootstrap replicates with p-value ≤ 0.05) restriction of ≥ 0.90 (Figure 3.7a). Thresholds for most negatively responding taxa occurred within the 'high nitrogen reach' and upstream of the 'fish decline reach' (Figure 3.7a). Two of the taxon-specific biomass change points were located at the point of *Podostemum* decline. One negative taxon threshold was farther downstream in the fish decline reach. Positively responding taxon

thresholds were all within the 'high nitrogen reach', but most occurred downstream of the bulk of the negatively responding taxon thresholds.

The abundance analysis identified significant thresholds for 20 negatively responding taxa and 13 positively responding taxa. Of these, 10 negative and 5 positive threshold responses were pure and reliable (Figure 3.7b). The abundance analysis indicated negative thresholds for 4 taxa at the *Podostemum* point of change. Two negative abundance change points fell within the 'fish decline reach', one of which (Pleuroceridae; Figure 3.7b) was downstream of Sumac Creek (the downstream-most tributary confluence in the study area).

There was substantial overlap between the analyses based on biomass and abundance with regard to the identities of the taxa exhibiting clear thresholds. However, *Cheumatopsyche* (Trichoptera: Hydropsychidae) exhibited a negative threshold for biomass but not abundance, and *Chimarra* (Trichoptera: Philopotamidae) and Pleuroceridae (Gastropoda) had negative thresholds in abundance and not biomass. The abundance analysis indicated additional positive thresholds for *Hydroptila* (Trichoptera: Hydroptilidae) and an unidentified Leptoceridae (Trichoptera). There was not a pure abundance threshold for *Protoptila* (Trichoptera: Glossosomatidae), but there was a pure biomass threshold.

Shifts in invertebrate community composition--NMDS

Ordination of sites within species-space reflected a shift in community composition associated with river kilometer, *Podostemum* biomass and water velocity. Three-dimensional NMDS ordinations were used for both biomass and abundance data (final stress for each analysis <0.17). Axes one and three best illustrated the effects of longitudinal position on site scores and were chosen for interpretation for the biomass ordination (Figure 3.8 and 3.9). Axes two and

three were interpreted for the abundance ordination (Figure 3.10 and 3.11). River kilometer (r^2 = 0.14, p =0.027) and *Podostemum* biomass (r^2 =0.33; p=0.001) were significantly associated with site scores on the three axes based on biomass (Figure 3.8). Samples taken in the upstream portion of the study reach scored lower on axis 3, but samples from the separate reaches (upstream, HNr and FDr) were not clearly separated.

The ordination based on abundance was similar (Figure 3.10). Abundance-based ordination scores were significantly correlated with *Podostemum* biomass (r^2=0.45; p=0.001), water velocity (r^2=0.26; p=0.001) and river kilometer (r^2=0.40; p=0.001). Abundance ordination illustrated a clearer separation of sites in the FDr from upstream sites. Additionally, several sites from the upstream portion of the HNr, and typically with high *Podostemum* and low water velocity, clustered high on both axes and had a distinct community composition from the other sites.

Several taxa that TITAN identified as exhibiting a threshold decline from upstream to downstream were also strongly associated with high river kilometer (upstream sites), high *Podostemum* biomass or both. Of these taxa *Simulium* (Diptera: Simuliidae), *Plauditus* (Ephemeroptera: Baetidae), and *Nectopsyche* (Trichoptera: Leptoceridae) appeared more closely associated with *Podostemum* based on ordinations, whereas Dugesiidae, *Stenelmis* (Coleoptera: Elmidae), and *Neureclipsis* (Trichoptera: Polycentropodidae) were more closely associated with the river kilometer gradient (Figures 3.9 and 3.11). Perlidae (Plecoptera) and *Protoptila* (Trichoptera: Glossosomatidae) exhibited a threshold increase around mid-sample reach and downstream of the *Podostemum* decline. Ordination illustrated a negative relationship for these two taxa with *Podostemum,* and highest abundance and biomass were located mid-reach. Graphs of raw data for perlids and *Protoptila* seem to show a threshold increase followed by a threshold

decline, a pattern not picked up by TITAN. Other threshold increasing taxa appeared related to low *Podostemum* biomass and to occur in highest abundance and biomass at downstream sites.

Discussion

Use of multiple methods allowed for evaluation of hypotheses and generation of new hypotheses regarding the changing macroinvertebrate community in the Conasauga River. Findings provided additional evidence that *Podostemum* is a major driver of invertebrate communities. Changes in the macroinvertebrate community were spatially concurrent with previously documented declines in fishes and with decline of the aquatic macrophyte, *Podostemum ceratophyllum*. However, exploratory analysis methods indicated that *Podostemum* and some macroinvertebrate taxa exhibited threshold declines farther upstream and generated the hypothesis that biotic decline may have shifted upstream of previously documented declines.

Community change along downstream gradients

Taxonomic and functional composition of the invertebrate community changed from upstream to downstream, although not as predicted. The downstream decline in insect richness was consistent with other studies of macroinvertebrate response to pollution (e.g. Yuan 2010). However, EPT richness did not decline as expected. Invertebrates within broader taxonomic groups like family or order can vary considerably in ecological traits (e.g. habitat preference, feeding habits; Poff et al. 2006) or sensitivity to pollution (Lenat and Resh 2001). Responses of different EPT taxa may vary in relation to disturbance and land use gradients (Pollard and Yuan 2010). For this reason some EPT taxa may respond negatively while others respond positively, resulting in no net change in EPT richness. Trait-based approaches provide an alternative means

of grouping taxa to account for variation in function, physiology and life history among species or genera within broader taxonomic groups, and can provide a mechanistic explanation of the drivers of community change (Doledec et al. 2006, Poff et al. 2006, Pollard and Yuan 2010, Statzner and Beche 2010). However, my data showed the opposite of expected result for taxa categorized as 'water-surface egg layers'. Other studies found a decline in 'water-surface egg layers' with fine sediment deposition and nutrient inputs (Wagenhoff et al. 2011), and with pastoral land use (Doledec et al. 2011). Nutrients may impact surface egg layers indirectly by promoting nuisance algal accrual (Doledec et al. 2006). However, algal growth did not appear to be at levels that would smother habitat at the time of my study; this indirect effect between nutrients and 'water surface egg-layers' might be more pronounced during periods of algal blooms.

Analyses, in fact, reflected a consistent decline (i.e., two or more analyses reflected decline downstream) for several underwater egg laying taxa: baetid mayflies, hydropsychid caddisflies, and a few elmid genera. These taxa declined with agricultural and urban land use in other studies, with elmids showing particular sensitivity to sediment (Eyre et al. 2005, Braccia and Voshell 2007, Herbst et al. 2012). Soaps and other surfactants can also be harmful to elmids because they make it impossible for adults to maintain an air bubble required for breathing underwater (Brown 1987). Surfactants in herbicides have been hypothesized as possible toxins in the Conasauga River. Additional factors associated with downstream loss of *Podostemum* are likely indirectly impacting macroinvertebrates through habitat alteration. *Podostemum ceratophyllum* provides attachment surfaces and refuge for filter-feeding insects (Parker and Voshell 1983) and supports particularly high biomass and secondary production of filter-feeders as well as other invertebrates (Grubaugh and Wallace 1995, Grubaugh et al. 1997, Hutchens et

al. 2004, Argentina et al. 2010b). In addition to hydropsychids, other filter-feeding caddisflies (genera: *Chimarra, Neureclipsis, Psychomyia*) and the filter-feeding Dipteran *Simulium* declined downstream.

In addition to providing important habitat for invertebrates, *Podostemum ceratophyllum* also provides refuge and feeding habitat for fishes (Hagler 2006, Argentina et al. 2010b). Although this is the first study to quantify longitudinal changes in *Podostemum* biomass in the Conasauga, Argentina et al. (2010a) showed a downstream decline in *Podostemum* occurrence and anecdotal evidence suggests that *Podostemum* was historically much more prevalent in the 'fish decline reach' than it is today (B. J. Freeman, personal communication). Some species of fishes that have declined in the Conasauga, such as the Coosa chub (*Macrhybopsis* sp. cf *M. aestivalis*) and Coosa madtom (*Noturus* sp. cf *N. munitus*), are strongly associated with *Podostemum* elsewhere (Hagler 2006), and other fishes are more likely to be present in areas with high *Podostemum* coverage on the local shoal scale (Argentina et. al 2010b). Although declining fishes seem to be generalists in type of prey consumed, they may specialize in feeding in *Podostemum* mats. Thus, the downstream decline in *Podostemum* documented here may represent one of the causes of fish species decline observed in the Conasauga River in recent years (Freeman et al. 2007, Hagler et al. 2011).

Podostemum also was strongly associated with macroinvertebrate community composition, and loss of *Podostemum* downstream may, in fact, be a driver of biotic change in the Conasauga River. Biomass of *Podostemum* was considerably reduced in the 'fish decline reach', but the longitudinal threshold of *Podostemum* (identified by TITAN) indicated a decline further upstream in the 'high nitrogen reach' at the same point as the negative change point in the invertebrate community. Many declining invertebrate taxa exhibited a threshold response at the

same location as *Podostemum*. *Simulium* (Diptera: Simuliidae), *Plauditus* (Ephemeroptera: Baetidae), and *Nectopsyche* (Trichoptera: Leptoceridae) exhibited threshold declines in the range of the threshold for *Podostemum* and were more closely associated with *Podostemum* than river kilometer in ordinations. Studies from other parts of the world have found high densities of *Corydalus* and baetid mayflies in mats of Podostemaceae species (reviewed by Hutchens et al. 2004), and both of these taxa declined downstream in the Conasauga. Another study from Georgia found highest densities of pleurocerid snails in microhabitats with *Podostemum* mats (Krieger and Burbanck 1976). *Podostemum* was not supported as a predictor of snail biomass or abundance in Conasauga River data. However, TITAN indicated a threshold decline in pleurocerid snail abundance as a point downstream of that for *Podostemum* decline. Other taxa were not expected to decline with loss of *Podostemum*. Hutchens et al. (2004) expected scrapers to increase in proportion to other functional feeding groups after *Podostemum* removal, although those authors found no change. I also expected an increase in scrapers in downstream portions of the Conasauga, but instead saw a shift in scraper composition from less streamlined taxa (elmids and snails) to more streamlined or armored taxa (Caenidae and Glossosomatidae increased; Heptageniidae did not change). This may reflect a shift from scraper taxa that require the refuge from high water velocity that *Podostemum* provides, to those that are better able to maintain position in high flows. *Hydroptila* increased in abundance downstream in the Conasauga, and may find refuge in filamentous algal mats on which they feed (Wiggins 1996) rather than *Podostemum*.

Analytical methods of evaluating community change

It is difficult to identify mechanisms of change in ecosystems, because changes are often associated with multiple stressors with additive or interactive effects on biota. Indeed, many of the biotic indices used to evaluate stream ecosystem health were developed to evaluate changes in biota along gradients that are associated with multiple stressors (e.g., % urban land, % agricultural land; Karr and Chu 1997). These metrics generally do not identify the specific stressor(s) impacting streams (King and Richardson 2003). My study investigated biotic changes along a longitudinal gradient of river kilometer, which was associated with a gradient of increasing watershed agricultural land use. However, the specific stressor(s) causing decline in biota in the Conasauga River were unknown. Trait-based analyses of community change have an advantage over measures of diversity or biotic indices, because functional composition of invertebrate communities can vary predictably with stressors (Statzner and Beche 2010).

Evaluating change in composition of traits can help with identification of mechanisms of community change (Statzner and Beche 2010). However, in my study, trait composition did not vary in the ways predicted, and I was unable to infer support for *a priori* hypothesized stressors using this analysis. With TITAN, I was able to identify specific taxa that declined and increased, and more importantly, taxa that exhibited a discontinuous, threshold response along the river kilometer gradient. These TITAN results can be used to develop additional hypotheses about the stressor(s) affecting the macroinvertebrate community in the Conasauga. The TITAN program is uniquely able to identify threshold changes in individual taxa along disturbance gradients, however it can only assess changes with one gradient at a time. In my study, river kilometer was a well-supported predictor of *Podostemum ceratophyllum*, so with TITAN it would be difficult to assess whether taxa are more associated with river kilometer versus changes in *Podostemum*

downstream. NMDS ordination and vector analysis allowed for visualization of taxa association with multiple gradients, and specifically taxa that were more closely associated with river kilometer than *Podostemum*. Taxa that were associated more with *Podostemum* might have been indirectly affected by the same stressor(s) that directly affect invertebrates associated with river kilometer. On the other hand, these two groups of taxa might have declined as a result of different stressors.

Analytical methods of identifying discontinuous change along a gradient

Many methods exist for identifying thresholds of disturbances that result in discontinuous change in community composition or ecosystem function (see, e.g., Dodds et al. 2010). In this study, I used both hypothesis-based and exploratory approaches to determine location of discontinuous change in macroinvertebrates in the Conasauga River. Mixed-effects regression indicated that the *a priori* hypothesized point of change at the upstream end of the 'fish decline reach' was a point of greater change in community structure than the most upstream point of the 'high nitrogen reach'. Model selection results supported the hypothesis that change was discontinuous rather than continuous. Methods of threshold detection that are not hypothesis-based do not explicitly test alternative models for continuous versus discontinuous response. In fact, Change point analysis (nCPA) and TITAN can detect a threshold response even when community or taxon-specific change is continuous (Cuffney et al. 2011). With these methods the burden is placed on the user to determine if bootstrapped confidence limits are narrow enough to suggest a true threshold response (Qian et al. 2003, Baker and King 2010, King and Baker 2010).

Threshold responses can take various forms (i.e. step-function, broken-stick, dose-response; Cuffney et al. 2011). For this study, I was less interested in determining the shape of

the response than in determining if a discontinuous change was supported. Hence my regression analysis only tested for a difference in the means between taxon-specific biomass in reaches upstream and downstream of the point of change, and compared support for models with a point of change versus with continuous change. Other analyses, like piecewise regression, can be used to evaluate shape of threshold responses (Dodds et al. 2010). TITAN is useful for determining location of un-hypothesized change points along a gradient. However, Cuffney et al. (2011) demonstrate that TITAN is only suited for precisely estimating step-function thresholds. If a threshold response is broken-stick or dose-response shaped, TITAN will incorrectly identify the point of change (Cuffney et al. 2011) and confidence limits will indicate high uncertainty around the point (Baker and King 2010). For this reason, change points in TITAN need to be interpreted with care and uncertainty should be acknowledged. An additional problem with TITAN arises when there are multiple change points. Unless there is equal weight (equal IndVal) for two or more points of change, TITAN will choose the change point with more weight. In this study, two taxa (Perlidae and *Protoptila* (Family Glossosomatidae)) that were identified as discontinuously increasing with river kilometer actually show an increase followed by a decline within a short distance along the river.

Despite uncertainty in change points of individual taxa, TITAN was able to identify a point of negative change approximately midway between my *a priori* hypothesized points of change, and confidence limits were precise enough to suggest that the point of greatest negative invertebrate response was upstream of the 'fish decline reach'. The ability of TITAN to estimate points of change in negatively and positively responding taxa separately results in more precise estimates over other methods, like nCPA, that detect change points using the entire community. Also, from an evolutionary perspective, it makes sense to consider negatively and positively

responding taxa separately because organisms are expected to have differing adaptations and tolerances to novel environmental conditions (Baker and King 2010). The nCPA is also sensitive to distribution of samples along the environmental gradient (Daily et al. 2012), a problem avoided by TITAN by using IndVal scores that are independent of sample group size to estimate change points (Baker and King 2010).

Conclusions

My study lends additional support to the idea that *Podostemum ceratophyllum* plays an important role in structuring benthic communities. Loss of *Podostemum* in downstream reaches of the Conasauga likely explains some of the decline in invertebrate diversity and possibly fish losses. However, ordination analysis suggests that some changes in the invertebrate community in the Conasauga are a result of other stressors associated with the river kilometer gradient. The specific stressors altering communities in the Conasauga may include nutrient loading and contaminants. My study demonstrates the utility of threshold detection to identify potential sources of stressors, which in this case appear to occur farther upstream in the Conasauga than previously hypothesized. Additionally, my results suggest that analyses examining variation in responses among individual taxa (i.e. TITAN, trait-based regression analyses) are better able to detect patterns of community change than analyses based on aggregate measures of invertebrate biomass, abundance and richness.

Trait-based analysis can be particularly useful in identifying stressors to aquatic systems when all potential stressors are known, and when hypotheses about the relationship of potential stressors to shifts in trait composition can be developed *a priori*. However, in cases where potential stressors are overlooked or the hypothesized change in trait composition is

unsupported, analyses like TITAN or ordination can identify specific taxa that change, and this knowledge can then be used to develop testable hypotheses about the mechanism of change. Developing and testing such hypotheses, either experimentally or by monitoring changes in taxa predicted to decline, is a necessary next step in understanding and managing change in systems valued for species conservation.

Literature Cited

Argentina, J. E., M. C. Freeman, and B. J. Freeman. 2010a. Predictors of occurrence of the aquatic macrophyte *Podostemum ceratophyllum* in a southern Appalachian river. Southeastern Naturalist **9**:465-476.

Argentina, J. E., M. C. Freeman, and B. J. Freeman. 2010b. The response of stream fish to local and reach-scale variation in the occurrence of a benthic aquatic macrophyte. Freshwater Biology **55**:643-653.

Baker, M. E. and R. S. King. 2010. A new method for detecting and interpreting biodiversity and ecological community thresholds. Methods in Ecology and Evolution **1**:25-37.

Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish. EPA 841-B-99-002, U. S. Environmental Protection Agency; Office of Water, Washigton D. C.

Bates, D., M. Maechler, and B. Bolker. 2011. lme4: linear mixed-effects models using s4 classes. R package version 0.999375-42.

Benke, A. C., A. D. Huryn, L. A. Smock, and J. B. Wallace. 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. Journal of the North American Benthological Society **18**:308-343.

Boschung, H. T. and R. L. Mayden. 2004. Fishes of Alabama. Smithsonian Books, Washington.

Braccia, A. and J. R. Voshell. 2007. Benthic macroinvertebrate responses to increasing levels of cattle grazing in Blue Ridge Mountain streams, Virginia, USA. Environmental Monitoring and Assessment **131**:185-200.

Brigham, A. R., W. U. Brigham, and A. Gnilka. 1982. Aquatic Insects and Oligochaetes of North and South Carolina. Midwest Aquatic Enterprises, Mahomet.

Brown, H. P. 1987. Biology of riffle beetles. Annual Review of Entomology **32**:253-273.

Burnham, K. P. and D. R. Anderson. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. 2nd edition. Springer-Verlag, New York.

Clements, W. H., N. K. M. Vieira, and D. L. Sonderegger. 2010. Use of ecological thresholds to assess recovery in lotic ecosystems. Journal of the North American Benthological Society **29**:1017-1023.

Cuffney, T. F., S. S. Qian, R. A. Brightbill, J. T. May, and I. R. Waite. 2011. Response to King and Baker: limitations on threshold detection and characterization of community thresholds. Ecological Applications **21**:2840-2845.

Daily, J. P., N. P. Hitt, D. R. Smith, and C. D. Snyder. 2012. Experimental and environmental factors affect spurious detection of ecological thresholds. Ecology **93**:17-23.

Dodds, W. K., W. H. Clements, K. Gido, R. H. Hilderbrand, and R. S. King. 2010. Thresholds, breakpoints, and nonlinearity in freshwaters as related to management. Journal of the North American Benthological Society **29**:988-997.

Doledec, S., N. Phillips, M. Scarsbrook, R. H. Riley, and C. R. Townsend. 2006. Comparison of structural and functional approaches to determining landuse effects on grassland stream invertebrate communities. Journal of the North American Benthological Society **25**:44-60.

Doledec, S., N. Phillips, and C. Townsend. 2011. Invertebrate community responses to land use at a broad spatial scale: trait and taxonomic measures compared in New Zealand rivers. Freshwater Biology **56**:1670-1688.

Dufrene, M. and P. Legendre. 1997. Species assemblages and indicator species: The need for a flexible asymmetrical approach. Ecological Monographs **67**:345-366.

Etnier, D. A. and W. C. Starnes. 1993. The Fishes of Tennessee. University of Tennessee Press, Knoxville.

Evans-White, M. A., W. K. Dodds, D. G. Huggins, and D. S. Baker. 2009. Thresholds in macroinvertebrate biodiversity and stoichiometry across water-quality gradients in Central Plains (USA) streams. Journal of the North American Benthological Society **28**:855-868.

Eyre, M. D., G. N. Foster, and M. L. Luff. 2005. Exploring the relationship between land cover and the distribution of water beetle species (Coleoptera) at the regional scale. Hydrobiologia **533**:87-98.

Field, J. G., K. R. Clarke, and R. M. Warwick. 1982. A practical strategy for analyzing multispecies distribution patterns. Marine Ecology-Progress Series **8**:37-52.

Freeman, B. J., G. W. Benz, and D. E. Collins. 1996. A Stakeholder's Guide to the Conasauga River of Georgia and Tennessee, Conservation bulletin number 1. Southeast Aquatic Research Institute, Chattanooga, Tennessee.

Freeman, B. J., M. M. Hagler, S. J. Wenger, G. B. Anderson, and R. A. Katz. 2007. Identification and mapping of critical habitats in the Conasauga River corridor of Georgia and Tennessee 2006 Annual report to the Georgia Department of Natural Resources, Atlanta. Institute of Ecology, University of Georgia, Athens.

Freeman, M. C., E. R. Irwin, N. M. Burkhead, B. J. Freeman, and H. M. Bart Jr. 2005. Status and conservation of the fish fauna of the Alabama River system. Pages 557-858 *in* J. N. Rinne, R. M. Hughes, and R. Calamusso, editors. Historical Changes in Large River Fish Assemblages of the Americas. American Fisheries Society Symposium 45, Bethesda, Maryland.

Gelman, A. and J. Hill. 2007. Data Analysis Using Regression and Multilevel/Hierarchical Models. Cambridge University Press, Cambridge.

Gido, K. B., W. K. Dodds, and M. E. Eberle. 2010. Retrospective analysis of fish community change during a half-century of landuse and streamflow changes. Journal of the North American Benthological Society **29**:970-987.

Gore, J. A. 1978. Technique for predicting in-stream flow requirements of benthic macroinvertebrates. Freshwater Biology **8**:141-151.

Gore, J. A., J. B. Layzer, and J. Mead. 2001. Macroinvertebrate instream flow studies after 20 years: A role in stream management and restoration. Regulated Rivers-Research & Management **17**:527-542.

Groffman, P., J. Baron, T. Blett, A. Gold, I. Goodman, L. Gunderson, B. Levinson, M. Palmer, H. Paerl, G. Peterson, N. Poff, D. Rejeski, J. Reynolds, M. Turner, K. Weathers, and J. Wiens. 2006. Ecological thresholds: The key to successful environmental management or an important concept with no practical application? Ecosystems **9**:1-13.

Grubaugh, J. W. and J. B. Wallace. 1995. Functional structure and production of the benthic community in a Piedmont river - 1956-1957 and 1991-1992. Limnology and Oceanography **40**:490-501.

Grubaugh, J. W., J. B. Wallace, and E. S. Houston. 1997. Production of benthic macroinvertebrate communities along a southern Appalachian river continuum. Freshwater Biology **37**:581-596.

Hagler, M. M. 2006. Effects of natural flow variability over seven years on the occurrence of shoal-dependent fishes in the Etowah River. MS Thesis. University of Georgia, Athens, Georgia.

Hagler, M. M., M. C. Freeman, S. J. Wenger, B. J. Freeman, P. L. Rakes, and J. R. Shute. 2011. Use of recent and historical records to estimate status and trends of a rare and imperiled stream fish, *Percina jenkinsi* (Percidae). Canadian Journal of Fisheries and Aquatic Sciences **68**:739-748.

Herbst, D. B., M. T. Bogan, S. K. Roll, and H. D. Safford. 2012. Effects of livestock exclusion on in-stream habitat and benthic invertebrate assemblages in montane streams. Freshwater Biology **57**:204-217.

Hutchens, J. J., J. B. Wallace, and E. D. Romaniszyn. 2004. Role of *Podostemum ceratophyllum* Michx. in structuring benthic macroinvertebrate assemblages in a southern Appalachian river. Journal of the North American Benthological Society **23**:713-727.

Karr, J. R. and E. W. Chu. 1997. Biological Monitoring and Assessment: Using Multimetric Indexes Effectively. EPA 235-R97-001. University of Washington, Seattle.

Kerans, B. L. and J. R. Karr. 1994. A benthic index of biotic integrity (B-IBI) for rivers of the Tennessee Valley. Ecological Applications **4**:768-785.

King, R. S. and M. E. Baker. 2010. Considerations for analyzing ecological community thresholds in response to anthropogenic environmental gradients. Journal of the North American Benthological Society **29**:998-1008.

King, R. S., M. E. Baker, P. F. Kazyak, and D. E. Weller. 2011. How novel is too novel? Stream community thresholds at exceptionally low levels of catchment urbanization. Ecological Applications **21**:1659-1678.

King, R. S. and C. J. Richardson. 2003. Integrating bioassessment and ecological risk assessment: An approach to developing numerical water-quality criteria. Environmental Management **31**:795-809.

Krieger, K. A. and W. D. Burbanck. 1976. Distribution and dispersal mechanisms of *Oxytrema* (=*Goniobasis*) *suturalis* Haldeman (Gastropoda-Pleuroceridae) in Yellow River, Georgia, USA. American Midland Naturalist **95**:49-63.

Lenat, D. R. 1993. A biotic index for the southeastern United States – derivation and list of tolerance values, with criteria for assigning water quality ratings. Journal of the North American Benthological Society **12**:279-290.

Lenat, D. R. and V. H. Resh. 2001. Taxonomy and stream ecology - The benefits of genus- and species-level identifications. Journal of the North American Benthological Society **20**:287-298.

McCune, B. and J. B. Grace. 2002. Analysis of Ecological Communities. MJM Software Design, Glenedon Beach, OR, US.

Merritt, R. W., K. W. Cummins, and M. B. Berg, editors. 2008. An Introduction to the Aquatic Insects of North America. 4th edition. Kendall/Hunt Publishing Company, Dubuque.

Nelson, D. J. and D. C. Scott. 1962. Role of detritus in the productivity of a rock-outcrop community in a Piedmont stream. Limnology and Oceanography **7**:396-413.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. Stevens, and H. Wagner. 2011. vegan: community ecology package. R package version 2.0-2.

Parker, C. R. and J. R. Voshell. 1983. Production of filter-feeding Trichoptera in an impounded and a free-flowing river. Canadian Journal of Zoology-Revue Canadienne De Zoologie **61**:70-87.

Poff, N. L., J. D. Olden, N. K. M. Vieira, D. S. Finn, M. P. Simmons, and B. C. Kondratieff. 2006. Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. Journal of the North American Benthological Society **25**:730-755.

Pollard, A. I. and L. L. Yuan. 2010. Assessing the consistency of response metrics of the invertebrate benthos: a comparison of trait- and identity-based measures. Freshwater Biology **55**:1420-1429.

Qian, S. S., R. S. King, and C. J. Richardson. 2003. Two statistical methods for the detection of environmental thresholds. Ecological Modelling **166**:87-97.

Raudenbush, S. W. and A. S. Bryk. 2002. Hierarchical Linear Models : Applications and Data Analysis Methods. 2nd ed. edition. Thousand Oaks, Calif.Sage Publications, Thousand Oaks, Calif.

Sharpe, A. J. and E. G. Nichols. 2007. Use of stable nitrogen isotopes and permeable membrane devices to study what factors influence freshwater mollusk survival in the Conasauaga river. Environmental Monitoring and Assessment **132**:275-295.

Statzner, B. and L. A. Beche. 2010. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? Freshwater Biology **55**:80-119.

Statzner, B., J. A. Gore, and V. H. Resh. 1988. Hydraulic stream ecology – observed patterns and potential applications. Journal of the North American Benthological Society **7**:307-360.

Vieira, N. K. M., N. L. Poff, D. M. Carlisle, S. R. Moulton, M. L. Koski, and B. C. Kondratieff. 2006. A database of lotic invertebrate traits for North America. U. S. Geological Survey

Data Series 187. US Geological Survey, US Department of Interior, Reston, Virginia. (Available from: http://pubs.usgs.gov/ds/ds187/).

Wagenhoff, A., C. R. Townsend, N. Phillips, and C. D. Matthaei. 2011. Subsidy-stress and multiple-stressor effects along gradients of deposited fine sediment and dissolved nutrients in a regional set of streams and rivers. Freshwater Biology **56**:1916-1936.

Wenger, S. J., M. M. Hagler, and B. J. Freeman. 2009. Prioritizing areas of the Conasauga River sub-basin in Georgia and Tennessee for preservation and restoration. Southeastern Fishes Council Proceedings **51**:31-38.

Wiggins, G. B. 1996. Larvae of the North American Caddisfly Genera (Trichoptera), 2nd edition. University of Toronto Press, Toronto.

Yuan, L. L. 2010. Estimating the effects of excess nutrients on stream invertebrates from observational data. Ecological Applications **20**:110-125.

Table 3.1. Hypotheses for taxon-specific biomass response with trait-by-location interactions. In addition to the specified interaction effect, all regression models included an intercept, model parameters for effects of sample-specific habitat characteristics, main effects for the trait and location parameters, random intercept and slope effects, and residual variation. Each of three alternative location variables was assessed for each trait, resulting in 27 candidate models for testing hypotheses about longitudinal changes associated with taxa traits.

Hypothesis	**Model interaction effect**	**# of taxa with trait**
Predators decline downstream	Predator X location	10
Multivoltine taxa increase downstream	Multivoltine X location	9
Semivoltine taxa decline downstream	Semivoltine X location	8
Small taxa increase downstream	Small X location	15
Large taxa decline downstream	Large X location	2
Taxa with higher NCBI increase downstream	NCBI X location	all (continuous variable)
Scrapers increase downstream	Scraper X location	14
Clingers decline downstream	Clinger X location	25
Surface egg layers decline downstream	Surface egg layer X location	12

Table 3.2. Habitat characteristics within reaches defined by alternative hypothesized points of change in invertebrate metrics. The fish decline reach (FDr) is downstream and a subset of the high nutrient reach (HNr) (Fig. 1). Mean and standard error (SE) of habitat measures at samples (n) taken within in each section are reported. Proportion coarse substrate within reaches is calculated as the proportion of samples taken from areas dominated by medium cobble or larger substrate.

		Podostemum Biomass (g/m^2 AFDM)		Water Velocity (m/s)		Water Depth (m)		Proportion coarse substrate
Reach	n	mean	SE	mean	SE	mean	SE	
Upstream of HNr	14	26.50	16.99	0.67	0.06	0.39	0.03	0.71
Within HNr	51	27.29	9.42	0.67	0.03	0.35	0.02	0.47
Upstream FDr	41	42.18	12.43	0.64	0.04	0.33	0.02	0.59
Within FDr	24	1.38	0.71	0.71	0.04	0.41	0.02	0.42

Table 3.3. Location and habitat effects on *Podostemum* presence and standing-stock biomass. Model variables and parameter estimates (β, standard error in parentheses) are listed for models included in the confidence set (ΔAICc < 2). Effects on *Podostemum* odds of presence or on biomass are estimated for biologically relevant changes in predictors. Location variables include the binary predictor for within, versus upstream of, the fish decline reach (FDr) or the continuous variable river kilometer (Rkm). Models with only habitat variables are identified with NA in the location variable column. The null, or no-predictors, model is included for comparison. K is the number of parameters in the model; ΔAICc is the difference between the AICc value of each candidate model and the best-supported model. Akaike weights (w_i) are scaled to sum to 1 within each confidence set.

Model Variables		Intercept		Location		Depth or Velocity		Substrate size			
Location	Habitat	β1	β2	Effect	β3	Effect	β4	Effect	K	ΔAICc	w_i
Occurrence Analysis				**% change in odds of presence**		**% change in odds per 0.1 m depth**		**% change in odds on coarse substrate**			
FDr	Depth, Substrate	3.07 (0.93)	-3.33 (1.17)	- 96% in FDr	1.46 (0.55)	+ 224%	1.62 (0.96)	+ 405%	4	0.00	0.63
FDr	Depth	3.59 (0.85)	-3.26 (1.06)	- 96% in FDr	1.45 (0.50)	+ 222%	-	-	3	1.03	0.37
Null Model		1.59 (0.33)	-	-	-	-	-	-	1	15.5	-
Biomass (log AFDM)				**% change in biomass**		**% change in biomass per 0.1 m/s velocity**					
Rkm	Water Velocity	-4.48 (0.41)	0.77 (0.39)	+ 163% per 10 km upstream	-1.03 (0.42)	- 38%	-	-	4	0.00	0.41
FDr	Water Velocity	-3.88 (0.49)	-1.73 (0.91)	- 82% in FDr	-0.97 (0.43)	- 36%	-	-	4	0.13	0.39
NA	Water Velocity	-4.39 (0.42)	-	-	-1.13 (0.43)	- 41%	-	-	3	1.52	0.20
Null Model		-4.45 (0.44)	-	-	-	-	-	-	2	5.97	-

Table 3.4. Location effects on dominance of coarse substrate in samples. Location variables, parameter estimates and standard errors (SE) are shown for models in the confidence set (ΔAICc < 2; K is the number of parameters in the model; ΔAICc is the difference between the AICc value of each candidate model and the best-supported model. Akaike weights (w_i) are scaled to sum to 1 within each confidence set.

Model	β0 Intercept		β1 Location			K	ΔAICc	*w*i
Location Variable	Estimate	SE	Estimate	SE	Effect			
HNr	0.916	0.592	-1.034	0.654	64% lower odds of coarse substrate in HNr	2	0.0	0.37
Rkm	0.094	0.253	0.410	0.262	67% greater odds of coarse substrate with every 10 km upstream	2	0.1	0.35
Null Model	0.092	0.248	-	-	No effect of location	1	0.5	0.29

Table 3.5. Location and habitat effects on invertebrate prey biomass (log(AFDM)). Model variables and parameter estimates (β, standard error in parentheses) are listed for models included in the confidence set (ΔAICc < 2). Effects of continuous variables on prey biomass are estimated for biologically relevant changes in predictors. Models with only habitat variables are identified with NA in the location variable column. K is the number of parameters in the model; ΔAICc is the difference between the AICc value of each candidate model and the best-supported model. Akaike weights (w_i) are scaled to sum to 1 within the confidence set.

Model	β1 Intercept	β2 Location	β3 *Podostemum* Biomass		β4 Water Velocity		β5 Substrate Size	β6 Date	K	ΔAIC c	wi
Location Variable	Estimate (SE)	Estimate (SE)	Estimate (SE)	% change per 100 g/m^2	Estimate (SE)	% change per 0.1 m/s	Estimate (SE)	Estimate (SE)			
HNr	0.783 (0.250)	0.370 (0.237)	0.378 (0.100)	+77.2	0.287 (0.099)	+14.2	0.378 (0.198)	-	6	0.0	0.18
NA	1.106 (0.131)	-	0.384 (0.101)	+78.9	0.287 (0.100)	+14.2	0.314 (0.196)	-	5	0.1	0.17
NA	1.212 (0.158)	-	0.362 (0.102)	+72.9	0.285 (0.099)	+14.1	0.379 (0.200)	-0.284 (0.198)	6	0.4	0.15
NA	1.127 (0.098)	-	0.411 (0.101)	+86.3	0.288 (0.101)	+14.3	-	-	4	0.5	0.14
FDr	1.011 (0.168	0.223 (0.212)	0.412 (0.105)	+86.6	0.277 (0.100)	+13.7	0.340 (0.198)	-	6	1.4	0.09
HNr	1.050 (0.210)	0.278 (0.237)	0.410 (0.101)	+86.0	0.288 (0.101)	+14.3	-	-	5	1.4	0.09
Rkm	1.090 (0.142)	-0.100 (0.102)	0.407 (0.104)	+85.2	0.284 (0.100)	+14.1	0.346 (0.199)	-	6	1.5	0.09
NA	1.369 (0.138)	-	0.399 (0.102)-	+82.9	0.287 (0.101)	+14.2	-	-0.200 (0.197)	5	1.7	0.08
Null Model	1.271 (0.111)	-	-	-	-	-	-	-	2	14.5	-

Table 3.6. Habitat effects on invertebrate prey abundance. Model variables and parameter estimates (β, standard error in parentheses) are listed for the one model included in the confidence set (ΔAICc < 2). Effects of continuous variables on prey abundance are estimated for biologically relevant changes in predictors. K is the number of parameters in the model; ΔAICc is the difference between the AICc value of each candidate model and the best-supported model.

Model	β1 Intercept		β2 *Podostemum* Biomass			β3 Water Velocity			K	ΔAICc	w_i
	Estimate	SE	Estimate	SE	% change per 100 g/m^2	Estimate	SE	%change per 0.1 m/s			
Podostemum Biomass Water Velocity	5.577	0.078	0.384	0.08	+78.9	0.246	0.081	+12.1	4	0.0	1.0
Null Model	5.663	0.092	-	-	-	-	-	-	2	19.6	-

Table 3.7. Location and habitat effects on total insect richness. Model variables and parameter estimates (β, standard error in parentheses) are listed for models included in the confidence set (ΔAICc < 2). Effects of continuous variables on insect richness are estimated for biologically relevant changes in predictors. Models with only habitat variables are identified with NA in the location variable column. K is the number of parameters in the model; ΔAICc is the difference between the AICc value of each candidate model and the best-supported model. Akaike weights (w_i) are scaled to sum to 1 within each confidence set.

Model	β1 Intercept	β2 Location	β3 *Podostemum* Biomass		β4 Substrate Size		β5 Water Velocity	K	ΔAICc	w_i
Location Variable	Estimate (SE)	Estimate (SE)	Estimate (SE)	% change per 100g/m^2	Estimate (SE)	% change on coarse substrate	Estimate (SE)			
NA	2.647 (0.048)	-	0.076 (0.03)	+12.2	0.182 (0.064)	+20.0	0.057 (0.032)	4	0.0	0.34
NA	2.649 (0.048)	-	0.063 (0.029)	+10.0	0.182 (0.064)	+20.0	-	3	0.8	0.23
HNr	2.729 (0.08)	-0.093 (0.075)	0.066 (0.029)	+10.5	0.166 (0.065)	+18.1	-	4	1.6	0.14
Rkm	2.655 (0.048)	0.04 (0.033)	0.055 (0.03)	+8.7	0.169 (0.065)	+18.4	-	4	1.7	0.14
FDr	2.683 (0.056)	-0.081 (0.07)	0.053 (0.03)	+8.4	0.173 (0.065	+18.9	-	4	1.8	0.14
Null Model	2.751 (0.031)	-	-	-	-	-	-	1	11.5	-

Table 3.8. Habitat effects on EPT richness. Model variables and parameter estimates (β, standard error in parentheses) are listed for models included in the confidence set (ΔAICc < 2). Effects of continuous variables on EPT richness are estimated for biologically relevant changes in predictors. K is the number of parameters in the model; ΔAICc is the difference between the AICc value of each candidate model and the best-supported model. Akaike weights (w_i) are scaled to sum to 1 within each confidence set.

Model	β1 Intercept		β2 Podostemum Biomass			β2 Substrate Size		K	ΔAICc	w_i
	Estimate	SE	Estimate	SE	% Change per 100 g/m^2	Estimate	SE			
Podostemum Biomass	2.206	0.041	0.078	0.037	+12.5	-	-	2	0	0.44
Substrate Coarseness	2.131	0.062	-	-	-	0.145	0.083	2	1.4	0.39
Null Model	2.209	0.041	-	-	-	-	-	1	1.9	0.17

Table 3.9. Location and habitat effects on insect taxon-specific presence and standing-stock biomass. Model variables and parameter estimates (β, standard error in parentheses) are listed for models the top location and habitat model and for the location and habitat model without the effect of *Podostemum*. Estimates in bold indicate effects with confidence limits that do not overlap zero. Effects on average taxon-specific odds of occurrence or biomass are estimated for biologically relevant changes in predictors. The effect of date was included in the model to account for variation and is not interpreted in this table. K is the number of parameters in the model; ΔAICc is the difference between the AICc value of each model and the best-supported model. Akaike weights (w_i) are scaled to sum to 1 for each analysis.

Model Variable	Y_{00} Int.	β_{1j} Podostemum Biomass		β_{2j} Water Velocity		β_{3j} Date	Y_{10} Location		μ_{0j} Ran. Int.	μ_{1j} Ran. Slope	σ^2_{ij} Resid. Var.	K	Δ AICc	*w*i
Location	Est. (SE)	Est. (SE)	% change per 100 g/m^2	Est. (SE)	% change per 0.1 m/s	Est. (SE)	Est. (SE)	% change per 10 km upstream	Est.	Est.	Est.			
Occurrence Analysis														
Rkm	-0.72 (0.36)	**0.23 (0.06)**	+41.1	**0.21 (0.06)**	+10.1	0.51 (0.12)	0.20 (0.12)	-	3.57	0.26	-	8	0.0	1.0
Rkm	-0.55 (0.35)	-	-	**0.15 (0.06)**	+7.3	0.46 (0.12)	**0.25 (0.11)**	+22.2	3.50	0.25	-	7	11.8	0.0
Biomass Analysis														
FDr	-2.60 (0.23)	**0.30 (0.06)**	+59.0	**0.23 (0.06)**	+11.2	0.23 (0.11)	0.05 (0.24)	-	1.25	0.93	2.32	9	0.0	1.0
FDr	-2.45 (0.23)	-	-	**0.16 (0.06)**	+7.8	0.16 (0.11)	-0.13 (0.24)	-	1.29	0.99	2.40	8	26.9	0.0

Table 3.10. Trait-by-location interactions that explain 30% or more of the variation in the location effect. Average % change in biomass was calculated for taxa with and without traits. SEL= water-surface egg layer taxa; FDr= fish decline reach; HNr= high nitrogen reach; Rkm= river kilometer.

Trait X Location	% slope variance explained	Y_{01} Trait Estimate (SE)	Y_{10} Location Estimate (SE)	Y_{11} Trait X Location Estimate (SE)	% Change In Taxa With Trait	% Change In Taxa Without Trait
Occurrence Analysis						
Semivoltine X HNr	32%	0.489 (1.011)	0.162 (0.241)	-1.227 (0.465)	-65.5% odds of presence in HNr	+17.6% odds of presence in HNr
SEL X HNr	34%	-0.567 (0.888)	-0.450 (0.249)	0.908 (0.441)	+58.1% odds of presence in HNr	-36.2% odds of presence in HNr
Biomass Analysis						
SEL X FDr	41%	-0.267 (0.456)	-0.437 (0.255)	1.273 (0.403)	+130.7% in FDr	-35.4% in FDr
SEL X Rkm	40%	0.169 (0.408)	0.155 (0.109)	-0.503 (0.173)	-35.7% with every 10 km us	+21.8% with every 10 km us
SEL X HNr	62%	-0.655 (0.537)	-0.185 (0.178)	0.973 (0.299)	+119.9% in HNr	-16.9% in HNr
Semivoltine X HNr	98%	0.991 (0.521)	0.350 (0.158)	-0.897 (0.302)	-42.1% in HNr	+41.9% in HNr

Table 3.11. Location and habitat effects on snail biomass. Model variables and parameter estimates (β, standard error in parentheses) are listed for models included in the confidence set (ΔAICc < 2). Effects of continuous variables on snail biomass are estimated for biologically relevant changes in predictors. K is the number of parameters in the model; ΔAICc is the difference between the AICc value of each candidate model and the best-supported model. Akaike weights (w_i) are scaled to sum to 1 within the confidence set.

Model	β1 Intercept	β2 Location	β3 Date		β4 Substrate		β5 Water Depth	K	ΔAICc	w_i
Location Variable	Estimate (SE)	Estimate (SE)	Estimate (SE)	% change on 2nd date	Estimate (SE)	% change on coarse substrate	Estimate (SE)			
Rkm	-1.854 (0.472)	0.557 (0.295)	1.874 (0.585)	+551.6	-1.980 (0.597)	-86.2	-	5	0.0	0.33
FDr	-1.443 (0.539)	-1.110 (0.601)	1.813 (0.584)	+512.8	-1.924 (0.592)	-85.4	-	5	0.2	0.30
-	-1.941 (0.474)	-	1.576 (0.598)	+383.5	-1.533 (0.601)	-78.4	-0.488 (0.300)	5	0.9	0.20
-	-1.925 (0.480)	-	1.763 (0.594)	+483.0	-1.739 (0.595)	-82.4	-	4	1.4	0.17
Null Model	-1.967 (0.318)	-	-	-	-	-	-	2	10.5	-

Table 3.12. Location and habitat effects on snail abundance. Model variables and parameter estimates (β, standard error in parentheses) are listed for models included in the confidence set (ΔAICc < 2). Effects of continuous variables on snail abundance are estimated for biologically relevant changes in predictors. K is the number of parameters in the model; ΔAICc is the difference between the AICc value of each candidate model and the best-supported model. Akaike weights (w_i) are scaled to sum to 1 within the confidence set.

Model	β1 Intercept	β2 Location		β4 Substrate		β5 Water Velocity		K	ΔAICc	w_i
Location Variable	Estimate (SE)	Estimate (SE)	% change per 10 km Upstream	Estimate (SE)	% change on coarse substrate	Estimate (SE)	% change per 0.1 m/s			
Rkm	3.354 (0.116)	-0.702 (0.092)	+142.1	-1.089 (0.173)	-66.3	-0.470 (0.084)	-19.5	5	0.0	1.00
Null Model	3.117 (0.122)	-	-	-	-	-	-	2	57.2	-

Table 3.13. Community change points with 95% confidence limits from TITAN and nCPA analysis. Change points are expressed in units of kilometers downstream from the upstream boundary of the study portion of the mainstem. For reference, the upstream ends of HNr and FDr are 5.6 and 15 river kilometers downstream, respectively. The sumz(-) indicates the threshold point of synchronous decline of negatively responding taxa. sumz(+) is the threshold for positively responding taxa.

	Biomass			**Abundance**		
	Change Point	5% CI	95% CI	Change Point	5% CI	95% CI
TITAN sumz(-)	9.85	5.31	13.78	9.85	5.30	13.78
TITAN sumz(+)	12.98	6.86	27.09	12.98	8.00	27.09
nCPA (Bray-Curtis)	9.85	1.10	22.03	9.77	7.10	19.90

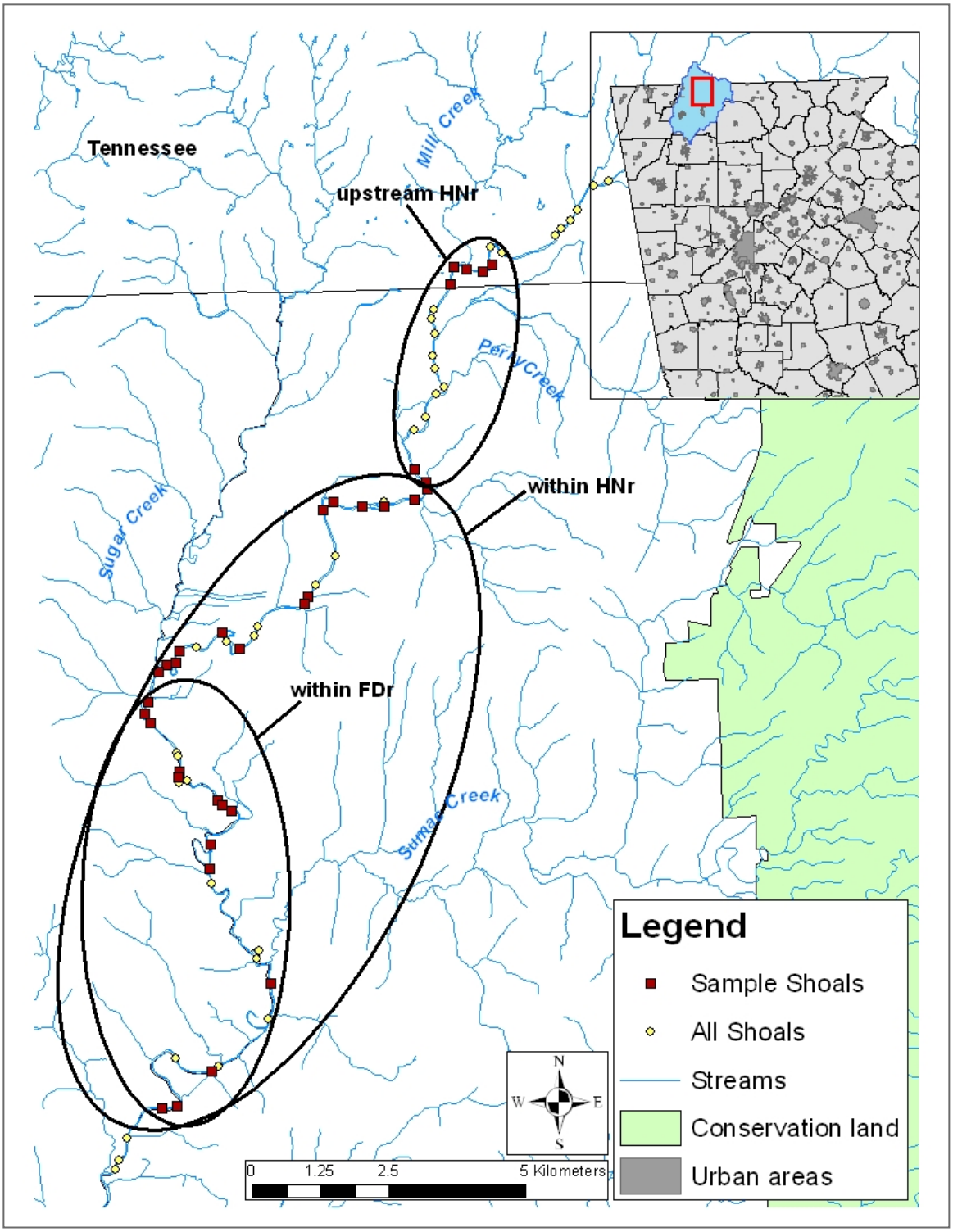

Figure 3.1. Map of invertebrate sample shoals with circles indicating sample reaches. Multiple samples were collected from some shoals.

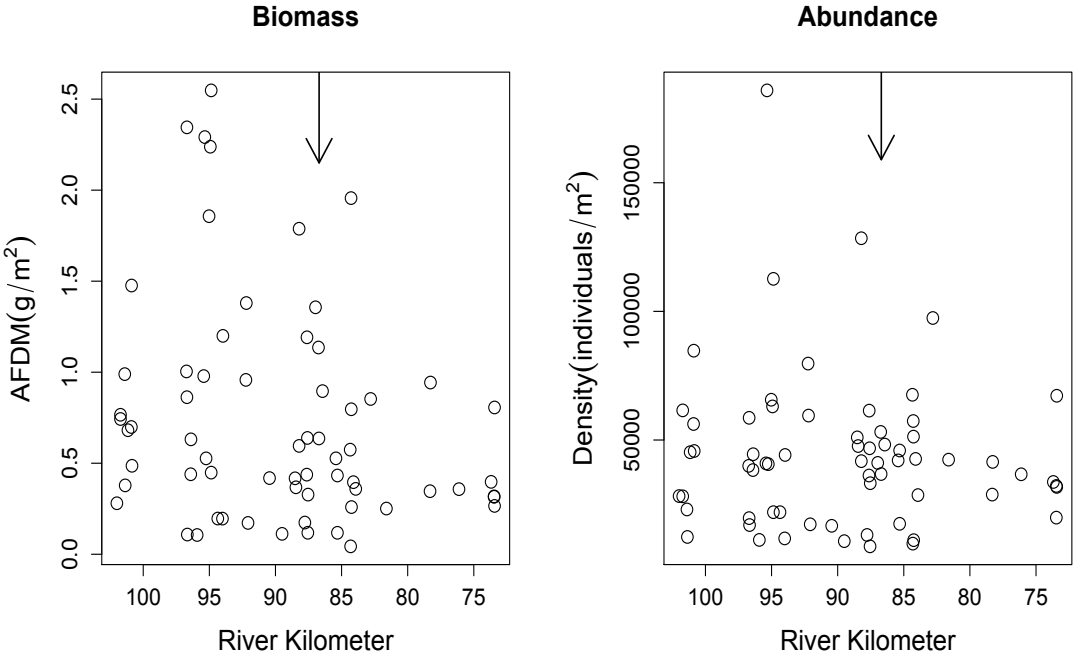

Figure 3.2. Raw biomass and abundance scaled up to 1m^2 area for all invertebrates collected. Arrows indicate the upstream end of the 'fish decline reach'.

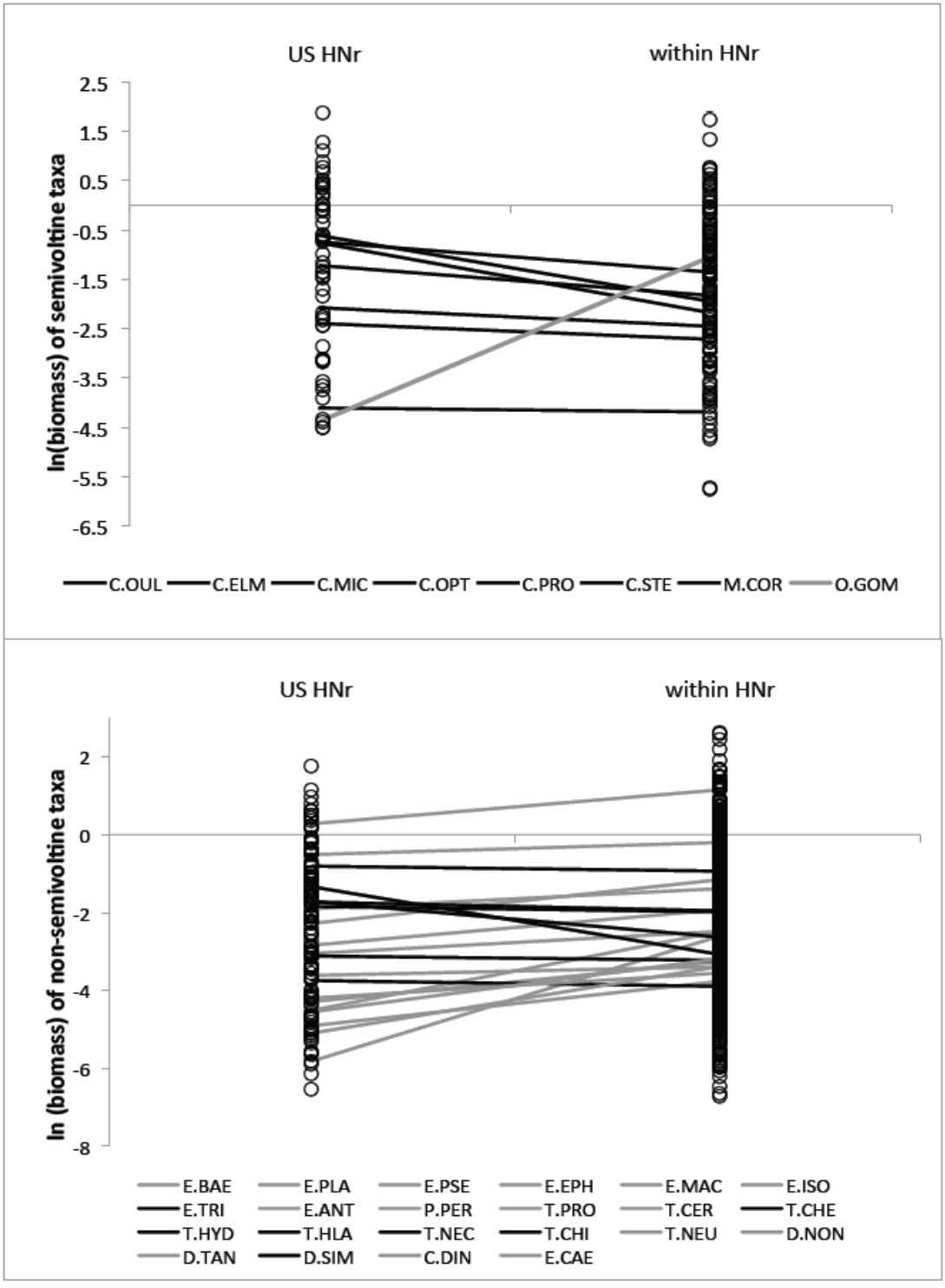

Figure 3.3. Change in biomass (ln(ash-free dry mass)) of (a) semivoltine and (b) non-semivoltine taxa in the 'high nitrogen reach' (HNr) versus the reach upstream of the 'high nitrogen reach' (US HNr). Grey lines indicate increasing taxa; black lines indicate declining taxa. Taxa codes are listed in Appendix A.

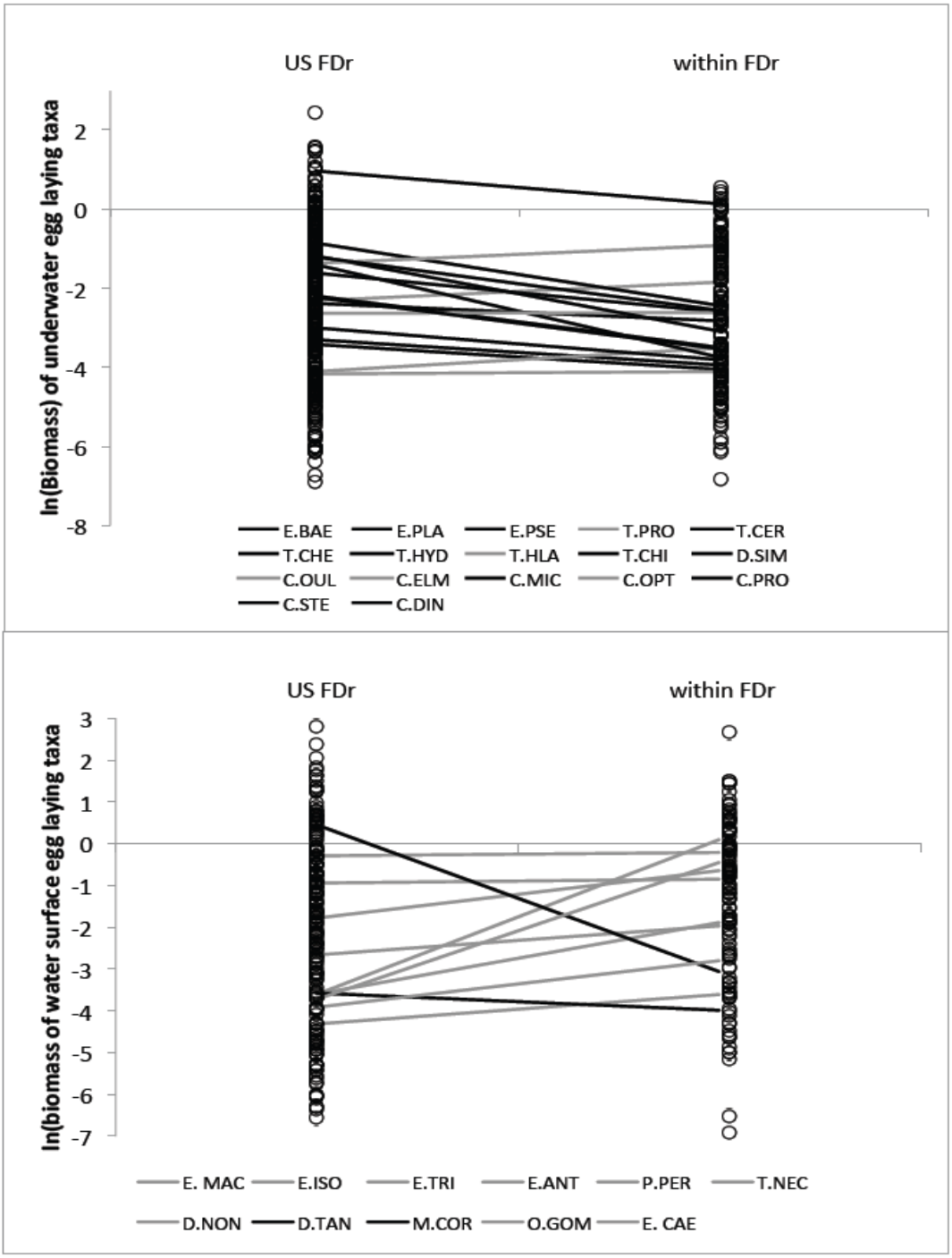

Figure 3.4. Change in biomass (ln(ash-free dry mass)) of (a) underwater egg laying taxa and (b) water surface egg laying taxa in the 'fish decline reach' (HNr) versus the reach upstream of the 'fish decline reach' (US HNr). Grey lines indicate increasing taxa; black lines indicate declining taxa. Taxa codes are listed in Appendix A.

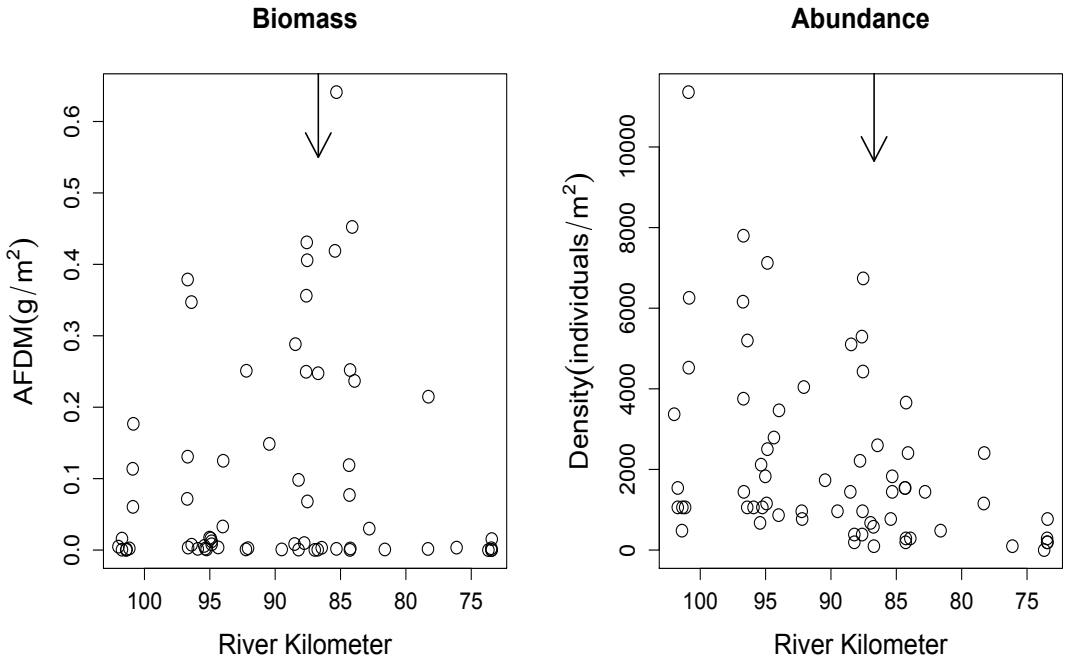

Figure 3.5. Raw biomass and abundance scaled up to $1m^2$ area for pleurocerid snails. Arrows indicate the upstream end of the 'fish decline reach'

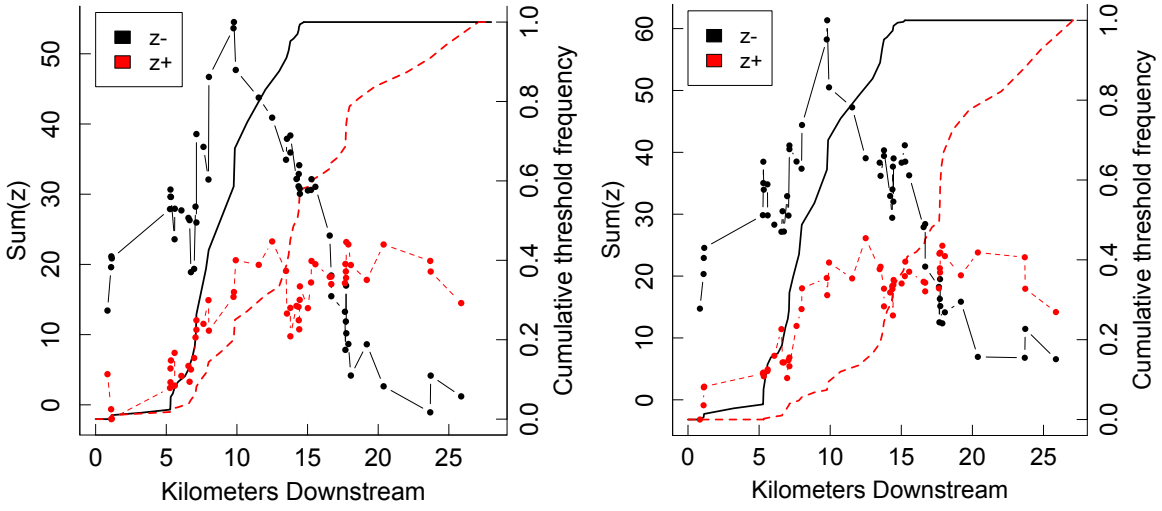

Figure 3.6. TITAN sum z-scores for (a) biomass and (b) abundance plotted at each candidate change-point for negatively (black points) and positively (red points) responding taxa. Smooth lines represent cumulative frequency curves of change points for negatively (solid-black line) and positively (dotted-red line) responding taxa.

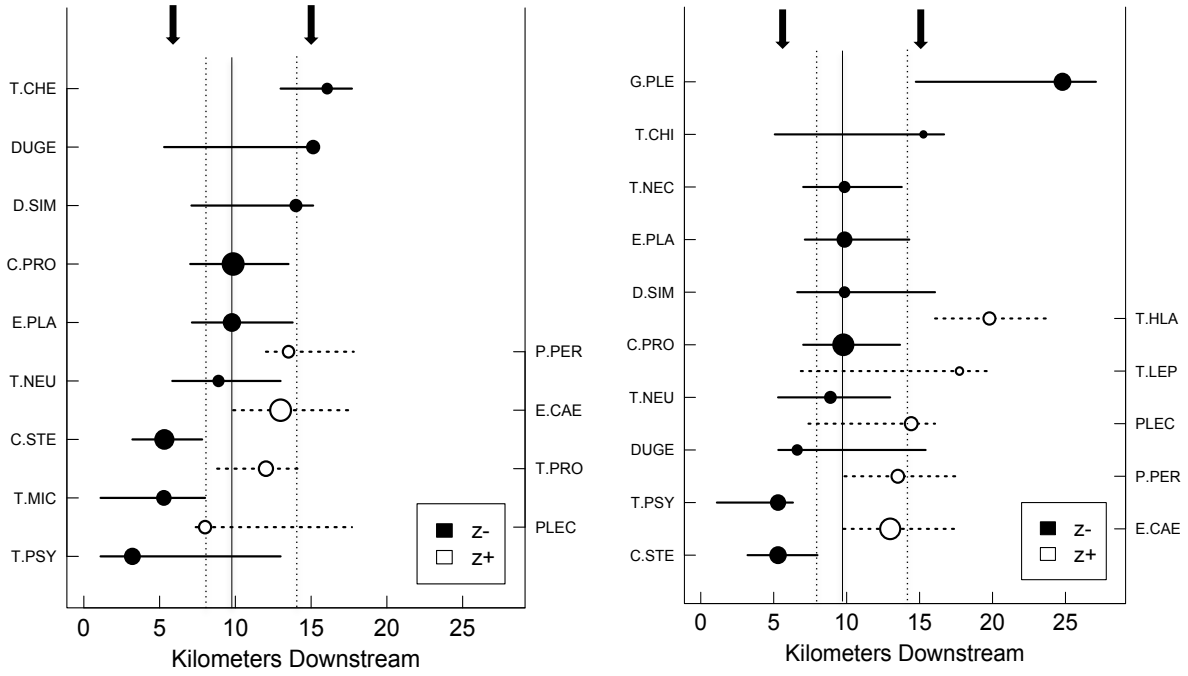

Figure 3.7. Individual taxon change points from (a) biomass and (b) abundance TITAN analyses for pure (≥ 0.95) and reliable (≥0.90) taxa. Black dots represent negative thresholds and white dots represent positive thresholds. Horizontal lines represent 90% confidence intervals from 500 bootstrap replicates. Vertical black lines indicate the negative change point for *Podostemum* and dotted lines show confidence intervals. Arrows signify hypothesized points of change at the upstream end of HNr and FDr. Taxa codes are listed in Appendix A.

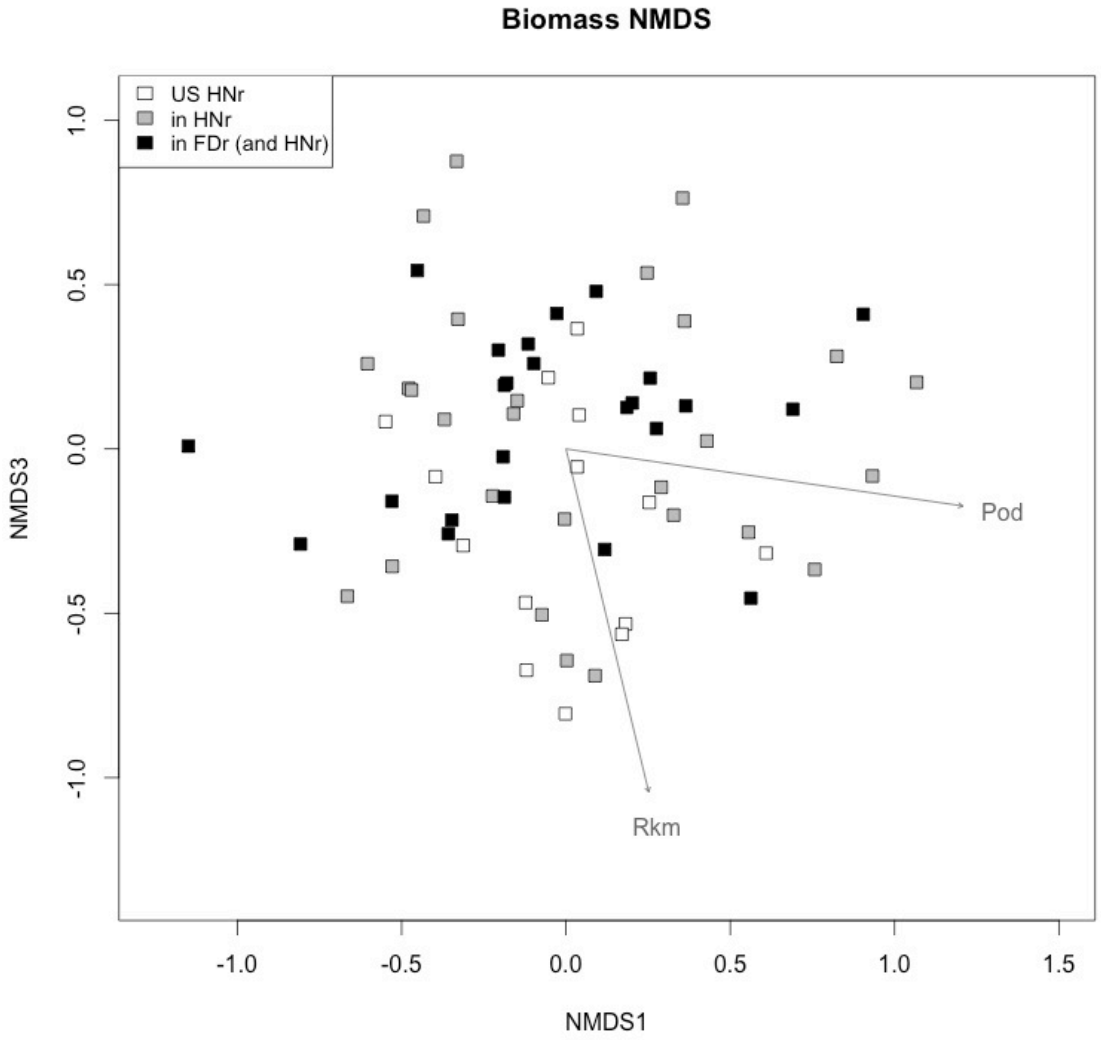

Figure 3.8. NMDS ordination of invertebrate taxon-specific biomass data displaying sites in species space. Symbol color represents reach: upstream (US HNr), 'high nitrogen reach' (HNr), and 'fish decline reach' (FDr). The 'fish decline reach' is a subset of the 'high nitrogen reach'. Environmental vectors (Pod=*Podostemum ceratophyllum* biomass, Rkm=river kilometer) are drawn from regression coefficients with site ordination scores on each axis. Grey vector lines indicate significant correlations.

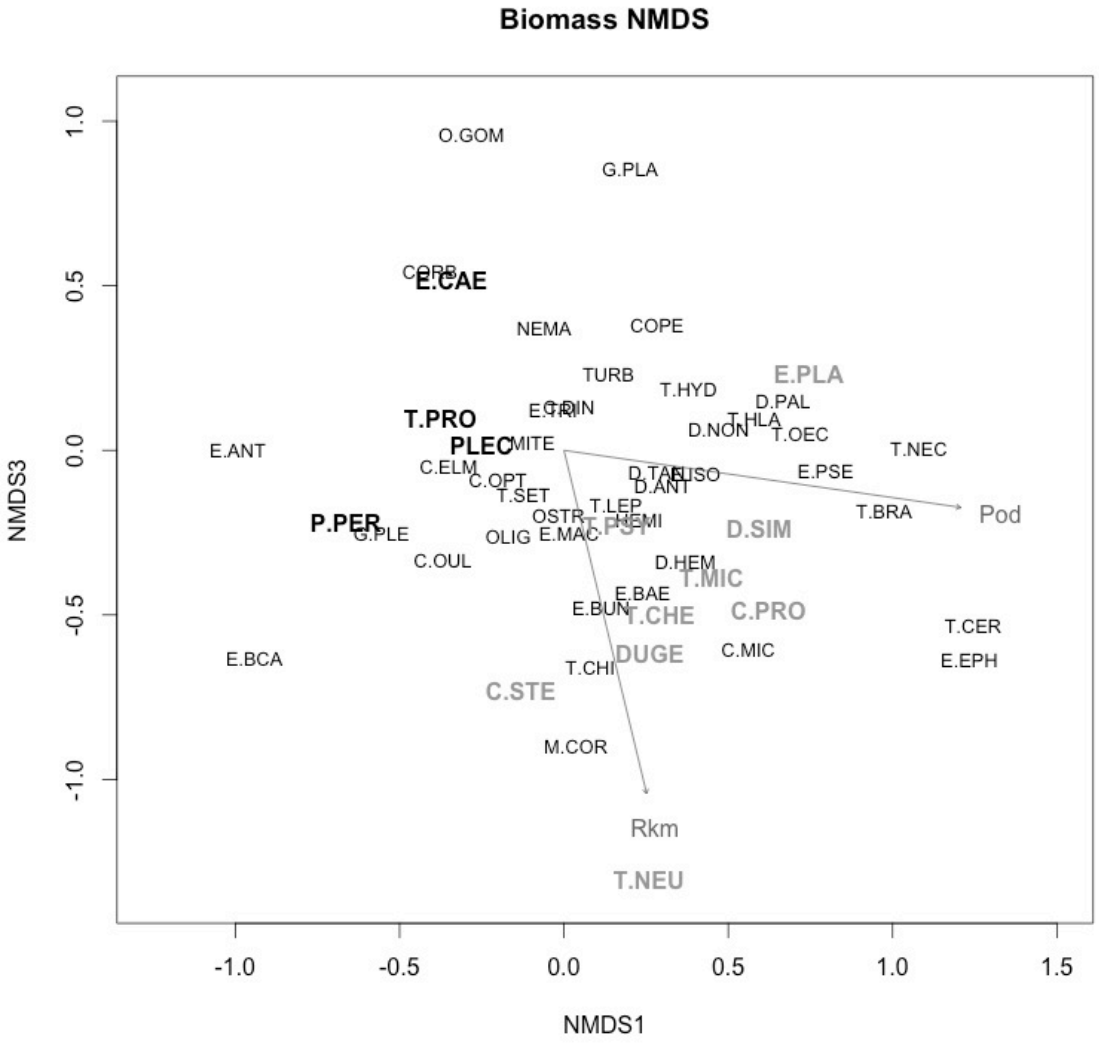

Figure 3.9. NMDS ordination of invertebrate taxon-specific biomass data displaying taxa abbreviations. Taxa exhibiting thresholds in TITAN analysis are in bold text. Bold black text signifies a positively responding taxon and bold grey text signifies a negatively responding taxon with respect to kilometers downstream. Environmental vectors (Pod=*Podostemum ceratophyllum* biomass, Rkm=river kilometer) are drawn from regression coefficients with site ordination scores on each axis. Grey vector lines indicate significant correlations.

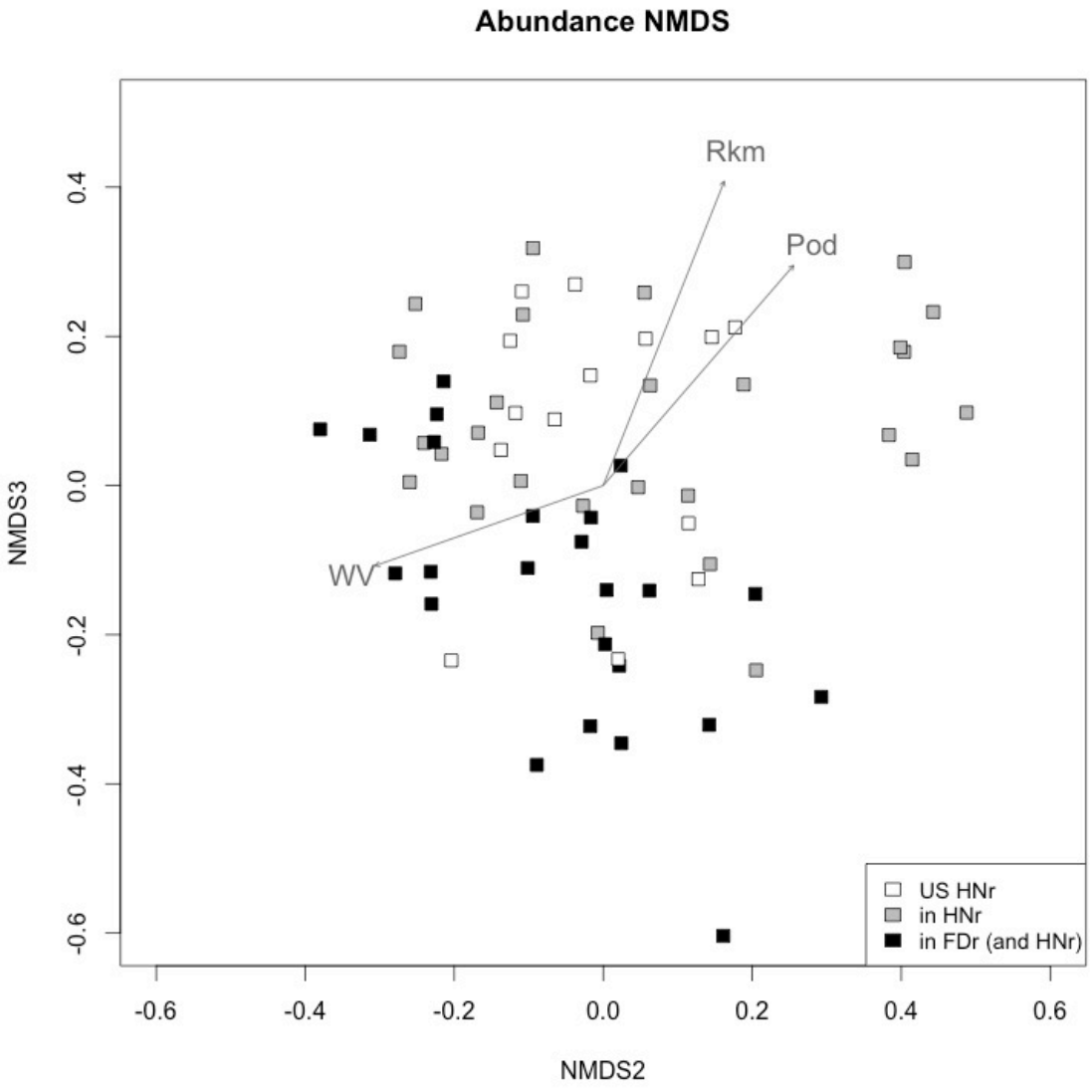

Figure 3.10. NMDS ordination of invertebrate taxon-specific abundance data displaying sites in species space. Symbol color represents reach: upstream (US HNr), 'high nitrogen reach' (HNr), and 'fish decline reach' (FDr). The 'fish decline reach' is a subset of the 'high nitrogen reach'. Environmental vectors (WV=water velocity, Pod=*Podostemum ceratophyllum* biomass, Rkm=river kilometer) are drawn from regression coefficients with site ordination scores on each axis. Grey vector lines indicate significant correlations.

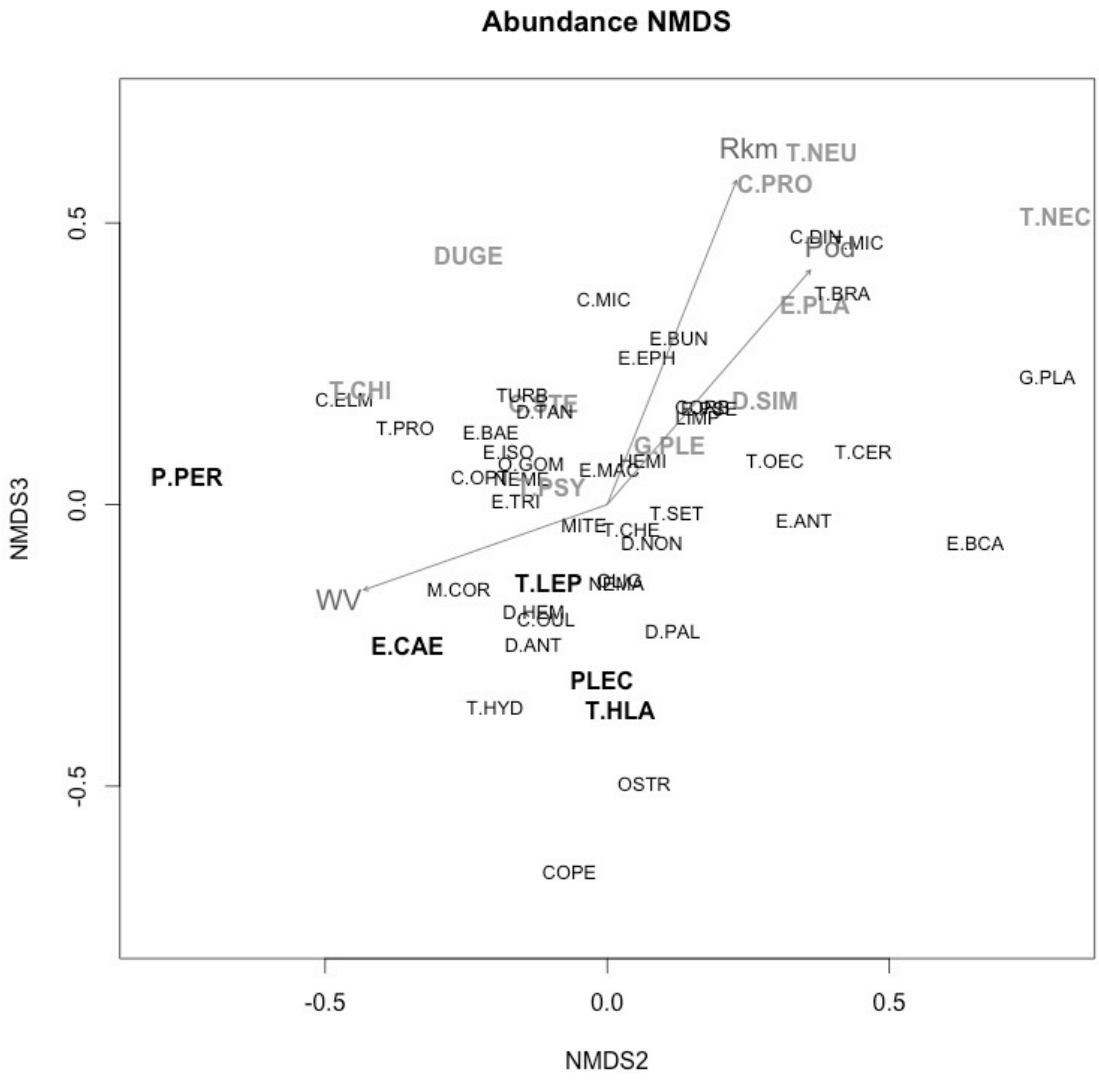

Figure 3.11. NMDS ordination of invertebrate taxon-specific biomass data displaying taxa abbreviations. Taxa exhibiting thresholds in TITAN analysis are in bold text. Bold black text signifies a positively responding taxon and bold grey text signifies a negatively responding taxon with respect to kilometers downstream. Environmental vectors (WV=water velocity, Pod=*Podostemum ceratophyllum* biomass, Rkm=river kilometer) are drawn from regression coefficients with site ordination scores on each axis. Grey vector lines indicate significant correlations.

CHAPTER 4

ALGAL ACCRUAL AS A MECHNISM OF NUTRIENT-INDUCED CHANGES IN BIOTA IN THE CONASAUGA RIVER

Introduction

Periphyton (attached algae) is a primary food resource for biota in low-order rivers with open canopies and exists in a matrix with heterotrophic microbes and fine organic particles. In general, accrual of periphyton is influenced by temperature, grazing pressure, nutrient and light availability, and stream flow (Rosemond 1993, Biggs 1996). In open-canopy streams with high nutrient concentrations, stream flow is often the limiting factor of periphyton accrual (Biggs 2000). High flow events that suspend sediment can cause scouring of benthic algae (Francoeur and Biggs 2006). Conversely, low flow periods reduce scour and concentrate nutrients, creating conditions conducive to algal blooms (Biggs 1996, 2000, Suren et al. 2003b, Hilton et al. 2006). Although algae are an important basal resource for stream ecosystems, algal blooms can smother habitats for other benthic organisms (Harding et al. 1999, Suren et al. 2003a), alter periphyton composition (Ferragut and Bicudo 2012), and reduce light availability for macrophytes (Hilton et al. 2006). Additionally, decay of blooms can reduce dissolved oxygen availability for macroinvertebrates and fishes (Rabalais 2002).

Research along the Conasauga River in northwest Georgia and southeast Tennessee over the last two decades indicates changing biological conditions and species losses. The Conasauga River is valued for exceptional biodiversity, but scientists have noted a decline in the occurrence

of several fish and mussel species of interest and a loss of the submerged aquatic macrophyte, riverweed (*Podostemum ceratophyllum*), known to provide important habitat for macroinvertebrates and fishes (Freeman et al. 2007, Sharpe and Nichols 2007, Wenger et al. 2009, Argentina et al. 2010a, Argentina et al. 2010b, Hagler et al. 2011). Eutrophication, or the oversupply of nutrients to aquatic systems, leads to a suite of changes that can result in a loss of biodiversity (Schindler 1990), and is one possible explanation of the changing conditions observed in the Conasauga River. Middle portions of the Conasauga River flow through a region of primarily agricultural land use, where nutrient concentrations (i.e. total nitrogen and total phosphorus, Sharpe and Nichols 2007; dissolved inorganic nitrogen and soluble reactive phosphorus Freeman et al. 2007, Table 4.1) in the river often exceed levels of eutrophic potential as defined by Dodds et al. (1998). Two widespread algal blooms have been documented in middle portions of the Conasauga in 1999 and 2007, both drought years, suggesting low flows in combination with high nutrient levels increase algal growth (Freeman et al. 2006). Nuisance algal accrual is a hypothesized stressor to biota in the Conasauga and may be a mechanism linking increased nutrients to biota losses. Coincidence of higher algal accrual with downstream declines in biota would support this hypothesis.

The goal of this study was to measure rates of periphyton accrual at sites upstream of versus within reaches where algal blooms have been documented in the past. I expected that algal accrual rates would be higher at downstream sites even under normal flow conditions. Anticipating, however, that nutrient availability may not limit algal growth in the agriculturally-influenced study reach, I also predicted that differences in factors such as light availability (canopy cover and turbidity), water velocity and depth would be correlated with periphyton accrual and these variables would differ among sites.

Methods

Field Methods

I measured algal accrual using a standard substrate of unglazed red clay quarry tiles at four shoals in the Conasauga River in June, July and August 2010. One shoal ("US") was located at the Tennessee/Georgia state line (RKM 101), upstream of the reach in which algal blooms have been observed. The other three shoals were selected within the bloom reach and at sites that were easily accessible but not in areas heavily utilized for recreation. The most upstream bloom-reach site was at the Hwy 2 road crossing (RKM 88 "DS1") which has been identified as the upstream end of a reach in which fish declines have been documented (Freeman et al. 2007). The other two sites were downstream of the Lower Kings Bridge crossing (RKM 73; "DS2") and at the Tibbs Bridge Road crossing (RKM 50; "DS3"). Tiles were deployed over a time period during which there were fluctuations in flow conditions due to storm events.

Because of unforeseen losses of tiles, I conducted two trials using different sampling strategies. Tiles were initially installed in early June (Trial 1). I installed 4 groups of 5 tiles within each of the 4 shoals, and the objective was to remove one randomly selected tile weekly from each group at all sites (for a total of 16 tiles on each sample date). However, all tiles were vandalized at two of the sites prior to the first sample date. I continued collecting a minimum of 2 tiles a week from the remaining two sites (DS1 and DS3), over a 5-week period.

Trial 2 was conducted over a 7-week period, beginning in early July. I installed 2 groups of 4 tiles at each of the 4 shoals. In Trial 2, I again collected two tiles from each site weekly, but I replaced tiles that were collected or lost at each site with a new, clean tile. Tiles were thus in place in the shoal for varying durations from 1 to 4 weeks, which produced a larger sample size of 1- to 2-week tiles having different installation dates.

Tiles were installed against the riverbed using 2 large binder clips and 2, 10-inch galvanized steel spikes that were hammered into the substrate. To account for variability in effects of habitat variables on algal accrual at each tile, I measured depth and water velocity using a Marsh-McBirney flo-mate on each sampling occasion prior to tile removal. At each tile group, I measured canopy cover at the time of installation using a spherical densiometer. Turbidity was measured at each site on each sample date. When tiles were removed, I cleaned excess material off the bottom and sides of the tile and placed the tile in a bag with stream water. Tiles were transported back to the lab on ice and processed for analysis the same day.

Lab methods

I subsampled periphyton from each tile and measured ash-free dry mass and chlorophyll *a* in order to estimate algal accrual rates. I scraped periphyton from a measured area on the surface of the tile into a known volume of stream water. The algal slurry was homogenized using a stir plate and a known volume was vacuum-filtered onto a glass fiber filter with 0.7μm pore size. One filter was stored in a -80°C freezer until processing for chlorophyll *a* analysis. Before analysis, filters were cut into small pieces, placed in a tube with 10 mL of buffered 90% acetone, shaken to disturb the algae, and extracted for 22 hours in a freezer. Extracts were analyzed in a spectrophotometer to estimate mass of algae per unit area using chlorophyll *a* corrected for pheophytin pigments (Steinman et al. 2007). An additional pre-combusted and weighed filter was used for ash-free dry mass (AFDM). The filter for AFDM was stored in a 45°C drying oven for a minimum of 72 hours after filtration and then combusted in a 500°C oven.

Statistical Methods

Prior to testing for site effects on periphyton accrual, I regressed AFDM and chlorophyll *a* against habitat variables measured at tile locations: canopy cover, water depth, water velocity at the streambed and at 60% depth, and turbidity (one measure per site per sampling event). Depth, water velocity, and turbidity were measured on multiple dates during the deployment period for most tiles, and I averaged values for regression analyses such that each tile had a separate estimate of average conditions over the duration of deployment. In addition to environmental variables measured at sites, I also evaluated support for effects of storm events on algal accrual using USGS real-time precipitation data. USGS discharge data were incomplete during my study period, so I was not able to evaluate effects of stream flow directly. Biggs (2000) regressed chlorophyll *a* against the predictor "days of accrual between flood events >3 times median flow"; 0.6 inches of rain corresponded with similar flow increases in the Conasauga during the study period. I used a second precipitation parameter for days since a rain event ≥ 0.25 inches to capture effects of rain events at a greater frequency. Precipitation data were obtained from the USGS data for Conasauga River at Eton (02384500). I counted days of accrual after rain events for each tile, because tiles were deployed for different time periods. If no rain event occurred during tile deployment, I counted days since deployment. Each environmental variable was evaluated separately to estimate effects on periphyton biomass.

I used tiles from Trial 2 to test for among-site differences in periphyton accrual, by regressing ln-transformed values for each periphyton measure (AFDM and chlorophyll a) as a function of site and number of days the tile was deployed (duration). I evaluated relative support for models with either a linear term for the effect of duration or with an additional quadratic term to model a rapid increase in accrual followed by a plateau. Testing for among-site differences in

rate of periphyton accrual required adding interactions between variables coding for site and duration. To avoid over-fitting the data, I combined data for sites in sequence to test for faster accrual downstream compared to upstream. Specifically, I evaluated support for three models in which site was coded using a single variable to contrast rate of accrual in: DS3 versus US, DS2 and DS1 combined; DS3 and DS2 versus DS1 and US; and all three downstream sites (DS1, DS2, and DS3) versus US. I compared relative support among models with no predictors (null model), duration only, site and duration, and a model with site and duration interactions. The most complex model included 7 parameters (intercept, residual, and effects of: duration, duration^2, site combination, site combination X duration, and site combination X duration^2) and was fit using data for 68 tiles. For Trial 1, I evaluated support for an effect of duration and for site differences for DS1 and DS3 (28 tiles). Additionally, I tested for differences in accrual and in environmental variables between trials for sites DS1 and DS3 combined (59 tiles) using a binary predictor for trial. I evaluated support for effects of environmental variables on chlorophyll *a* and AFDM using tiles collected at these two sites over the course of both trials.

Duration was standardized prior to analysis and estimates were rescaled to represent change per day for interpretation. In models with interactions, the effect of site was estimated at mean duration. I used an information theoretic approach (Burnham and Anderson 2002) to evaluate support of models with and without quadratic effects, with site effects, and with or without interactions. Akaike's Information Criterion with correction for small sample size (AICc; Burnham and Anderson 2002) was used to rank candidate models from best-supported (lowest AICc) to least-supported, and those with a ΔAICc (candidate model AICc minus best-model AICc) of two or less were retained in the confidence set of models. Following Burnham and Anderson (2002), I eliminated models from the confidence set if they contained more

parameters than the best-supported model and had similar log-likelihoods. I also used AICc to evaluate whether environmental variables were supported as predictors of periphyton biomass over models with no predictors.

Results

Periphyton accrual was measured in two trials after I had 100% loss of tiles at two of the four sites in Trial 1. Trial 2 was analyzed separately and was successfully completed with 12 to 22 tiles collected from each site. Biomass (AFDM, chlorophyll *a*) of periphyton increased with duration of tile deployment and accrual was best predicted by a quadratic function that allowed rate of accrual to decline over time (Figure 4.1). Mean chlorophyll *a* and AFDM were 20.4 mg/m^2 and 4.9 g/m^2, respectively, across all sites and both trials.

Total algal accrual, but not rate, differed among sites in Trial 2. However, only the most downstream (DS3) site had higher algal accrual than sites upstream (US, DS1, and DS2). Alternative models had essentially no support (for models without interactions, ΔAICc values ranged from 8.7 to 10.5 for chlorophyll *a* and 13.5 and 15.8 for AFDM; null model ΔAICc = 56.9 and 199.8). Biomass of chlorophyll *a* and AFDM were 73.2% and 66.9%, respectively, higher on average at site DS3 than at the other three sites (Table 4.2). The rate of increase of algal biomass did not differ between sites (ΔAICc values for models with interactions between days and sites = 0.5 to 14.9 for chlorophyll *a* and 2.7 to 19.1 for AFDM). However, no tiles were collected for any site before 6 days of deployment and by the sixth day much of the algal biomass at DS3 had already accrued (Figures 4.2 and 4.3). Therefore, the analysis of rate did not include the initial period of exponential growth for some sites.

The best-supported environmental predictor of chlorophyll *a* and AFDM was days of accrual following a 0.6 inch rain event (Table 4.3; Figure 4.4). Five precipitation events exceeded 0.6" during Trial 2, with net accrual increasing >20% for each day between an event and tile collection. Water velocity at 60% depth was substantially more supported than the null model for AFDM but not for chlorophyll *a*. All other variables had similar log-likelihoods to the null models. Models using days since a 0.6" event were much more supported than those using days since a 0.25" event (ΔAICc scores = 18.4 to 19.5). Variables describing days since a rain event did not differ between DS3 and upstream sites, because each site had tiles sampled during a similar distribution of time periods. Other environmental variables did differ among sites, but not on average between DS3 and all upstream sites combined. Canopy cover and turbidity were lower at the most upstream site (US; Table 4.4), possibly confounding upstream to downstream site effects on periphyton accrual.

Tiles collected from Trial 1 showed higher periphyton accrual than during Trial 2 (Table 4.5), with no evidence of differences between the two sites with data. The highest chlorophyll *a* (0.0211 mg/cm^2) and AFDM (5.11 mg/cm^2) values were measured at site DS1 in Trial 1 (Figure 4.5). Although Trial 2 indicated a lower level of algal accrual at DS1 relative to DS3, Trial 1 indicated a similar level of accrual at the two sites. Contrary to the results of Trial 2, there was no trend of increasing algal accrual over time at DS1 and DS3 in Trial 1 (Figure 4.5). Data collection in Trial 1 began after a two-week conditioning period, and by the time of first sample the accrual of periphyton had plateaued. Additionally, all environmental variables were different between trials with the exception of water velocity at 60% depth. However, the best-supported predictor of periphyton and algal biomass was days of accrual following a 0.6 inch rain event (Table 4.6). All other models had ΔAICc values much greater than 2.

Discussion

This study provided additional evidence that scouring flow events constrain algal accrual and might be more important than other environmental conditions or nutrients in regulating algal accumulation in high nutrient rivers. I found that periphyton growth in the Conasauga River differed more between time periods than between sites with differing light and nutrient availability. The best environmental predictor of periphyton biomass across sites and trials was a measure of days of accrual between flood events. The highest algal biomasses were measured after 23 days between storm events, which was the longest duration without storms.

Periphyton accrual plateaued within a short period of time during this study, indicating that algal growth was constrained. Nutrient concentrations measured over multiple years in the Conasauga have been high, especially in downstream reaches (Table 4.1), such that periphyton growth was likely limited by other factors, possibly flow. Other studies have suggested that low flow periods result in higher coverage and biomass of periphyton and successional change from diatoms to cyanobacteria to filamentous green in nutrient enriched rivers (Suren et al. 2003b, Stevenson et al. 2006). Biggs (2000) found that greater than 50 days of accrual time between flood events resulted in increased frequency of algal blooms and that accrual time, as opposed to nutrient concentrations, was more related to biomass. The maximum chlorophyll *a* measure from my study (0.0211 mg/cm^2 or 211 mg/m^2) occurred within 23 days and was above levels indicating eutrophic conditions (200 mg/m^2; Dodds 1998). The mean chlorophyll *a* value across both trials was in the range for mesotrophic condition. In algal bloom years, however, chlorophyll *a* values have likely far exceeded thresholds indicating eutrophic conditions (B. J. Freeman, personal communication).

Hilton et al. (2006) suggest in a conceptual model of eutrophication in rivers, that eutrophic rivers with short-residence times will switch from dominance by macrophytes, to dominance of *Cladophora*, and eventually to thick benthic mats. In the Conasauga, primary producers seem to have shifted from *Podostemum ceratophyllum* to benthic algae in downstream portions of the river where biotic declines occur. Based on anecdotal observations, it seems that periphyton might restrict light availability for *Podostemum*, similarly to other studies of epiphyton accrual on macrophytes during low flow periods and with high nutrient conditions (Ham et al. 1981, Spink et al. 1993).

My study was designed to measure rate of periphyton accrual, which is often used as a proxy for primary productivity (Greenwood and Rosemond 2005). Rates of algal accrual were not different between upstream and downstream sites. However, at some sites accrual had already begun to plateau at the time of first sample (6 days of accrual). Many studies allow for a colonization period, and time to first sample can range from 3 days to 12 days or more (Greenwood and Rosemond 2005, Kominoski et al. 2007). This study indicates that in high nutrient conditions, it is important to begin sampling early in order to capture the rapid initial rate of increase. Other studies have measured rates by dividing accrued biomass over duration of substrate deployment, but this assumes a linear change in biomass over time, which may not be realistic (Kevern et al. 1966). The shape of the accrual of biomass over time can indicate whether increasing biomass is constrained at some level or if it continues to increase freely over time. Sampling early and modeling different hypothesized response shapes can allow better understanding of factors affecting periphyton accrual. Additionally, the strategy of replacing tiles after removal was intended to compensate for tile disturbance, and may also have resulted in

more representative samples of algal accrual over time because multiple date ranges of deployment were represented for each duration.

Nutrient levels in the Conasauga have been on an increasing trajectory over time (B. J. Freeman and M. M. Hagler, unpublished data). Additionally, low flow periods during drought likely exacerbate nutrient effects by reducing flow limitation and concentrating nutrients and other agricultural pollutants. The southeast U.S. is projected to have longer periods between rain events, and rain events are projected to be flashier as a result of climate change (Wang et al. 2010). This has ecological implications for the Conasauga, because flashy rain events increase pollutant loading and low flows contribute to algal blooms. To slow biodiversity loss in the Conasauga, managers need to focus on reducing pollutant runoff from agricultural land. My study has provided evidence that a large portion of the Conasauga River, possibly extending to the upstream-most site assessed, is susceptible to algal blooms and associated effects on other biota. Monitoring algal dynamics, especially during low-flow periods, can provide information on changes in ecological condition of the river and on the efficacy of management efforts to reduce nutrient inputs.

Literature Cited

Argentina, J. E., M. C. Freeman, and B. J. Freeman. 2010a. Predictors of occurrence of the aquatic macrophyte *Podostemum ceratophyllum* in a Southern Appalachian River. Southeastern Naturalist **9**:465-476.

Argentina, J. E., M. C. Freeman, and B. J. Freeman. 2010b. The response of stream fish to local and reach-scale variation in the occurrence of a benthic aquatic macrophyte. Freshwater Biology **55**:643-653.

Biggs, B. J. F. 1996. Hydraulic habitat of plants in streams. Regulated Rivers-Research & Management **12**:131-144.

Biggs, B. J. F. 2000. Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. Journal of the North American Benthological Society **19**:17-31.

Burnham, K. P. and D. R. Anderson. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. 2nd edition. Springer-Verlag, New York.

Dodds, W. K., J. R. Jones, and E. B. Welch. 1998. Suggested classification of stream trophic state: Distributions of temperate stream types by chlorophyll, total nitrogen, and phosphorus. Water Research **32**:1455-1462.

Ferragut, C. and D. D. Bicudo. 2012. Effect of N and P enrichment on periphytic algal community succession in a tropical oligotrophic reservoir. Limnology **13**:131-141.

Francoeur, S. N. and B. J. F. Biggs. 2006. Short-term effects of elevated velocity and sediment abrasion on benthic algal communities. Hydrobiologia **561**:59-69.

Freeman, B. J., G. W. Benz, and D. E. Collins. 1996. A Stakeholder's Guide to the Conasauga River of Georgia and Tennessee, Conservation bulletin number 1. Southeast Aquatic Research Institute, Chattanooga, Tennessee.

Freeman, B. J., M. M. Hagler, S. J. Wenger, G. B. Anderson, and R. A. Katz. 2006. Identification and mapping of critical habitats in the Conasauga River corridor of Georgia and Tennessee 2006 Annual report to the Georgia Department of Natural Resources, Atlanta. Institute of Ecology, University of Georgia, Athens.

Freeman, B. J., M. M. Hagler, S. J. Wenger, G. B. Anderson, and R. A. Katz. 2007. Identification and mapping of critical habitats in the Conasauga River corridor of Georgia and Tennessee 2006 Annual report to the Georgia Department of Natural Resources, Atlanta. Institute of Ecology, University of Georgia, Athens.

Freeman, M. C., E. R. Irwin, N. M. Burkhead, B. J. Freeman, and H. M. Bart Jr. 2005. Status and conservation of the fish fauna of the Alabama River system. Pages 557-858 *in* J. N. Rinne, R. M. Hughes, and R. Calamusso, editors. Historical Changes in Large River Fish Assemblages of the Americas. American Fisheries Society Symposium 45, Bethesda, Maryland.

Greenwood, J. L. and A. D. Rosemond. 2005. Periphyton response to long-term nutrient enrichment in a shaded headwater stream. Canadian Journal of Fisheries and Aquatic Sciences **62**:2033-2045.

Hagler, M. M., M. C. Freeman, S. J. Wenger, B. J. Freeman, P. L. Rakes, and J. R. Shute. 2011. Use of recent and historical records to estimate status and trends of a rare and imperiled stream fish, *Percina jenkinsi* (Percidae). Canadian Journal of Fisheries and Aquatic Sciences **68**:739-748.

Ham, S. F., J. F. Wright, and A. D. Berrie. 1981. Growth and recession of aquatic macrophytes on an unshaded section of the River Lambourn, England, from 1971 to 1976. Freshwater Biology **11**:381-390.

Harding, J. S., R. G. Young, J. W. Hayes, K. A. Shearer, and J. D. Stark. 1999. Changes in agricultural intensity and river health along a river continuum. Freshwater Biology **42**:345-357.

Hilton, J., M. O'Hare, M. J. Bowes, and J. I. Jones. 2006. How green is my river? A new paradigm of eutrophication in rivers. Science of the Total Environment **365**:66-83.

Kevern, N. R., J. L. Wilhm, and G. M. v. Dyne. 1966. Use of artificial substrata to estimate the productivity of periphyton. Limnology and Oceanography **11**:499-502.

Kominoski, J. S., P. A. Moore, R. G. Wetzel, and N. C. Tuchman. 2007. Elevated CO2 alters leaf-litter-derived dissolved organic carbon: effects on stream periphyton and crayfish feeding preference. Journal of the North American Benthological Society **26**:663-672.

Rabalais, N. N. 2002. Nitrogen in aquatic ecosystems. Ambio **31**:102-112.

Rosemond, A. D. 1993. Interactions among irradiance, nutrients, and herbivores constrain a stream algal community. Oecologia **94**:585-594.

Sharpe, A. J. and E. G. Nichols. 2007. Use of stable nitrogen isotopes and permeable membrane devices to study what factors influence freshwater mollusk survival in the Conasauaga river. Environmental Monitoring and Assessment **132**:275-295.

Spink, A. J., K. J. Murphy, S. M. Smith, and D. F. Wetlake. 1993. Effects of eutrophication on *Ranunculus a*nd *Potamogeton*. Journal of Aquatic Plant Management **31**:113-117.

Steinman, A. D., G. A. Lamberti, and P. R. Leavitt. 2007. Chapter 17 - Biomass and pigments of benthic algae. Pages 357-379 Methods in Stream Ecology (Second Edition). Academic Press, San Diego.

Stevenson, R., S. Rier, C. Riseng, R. Schultz, and M. Wiley. 2006. Comparing effects of nutrients on algal biomass in streams in two regions with different disturbance regimes and with applications for developing nutrient criteria. Hydrobiologia **561**:149-165.

Suren, A. M., B. J. F. Biggs, M. J. Duncan, L. Bergey, and P. Lambert. 2003a. Benthic community dynamics during summer low-flows in two rivers of contrasting enrichment 2. Invertebrates. New Zealand Journal of Marine and Freshwater Research **37**:71-83.

Suren, A. M., B. J. F. Biggs, C. Kilroy, and L. Bergey. 2003b. Benthic community dynamics during summer low-flows in two rivers of contrasting enrichment 1. Periphyton. New Zealand Journal of Marine and Freshwater Research **37**:53-70.

U. S. Fish and Wildlife Service (USFWS) 2000. Mobile River Basin Aquatic Ecosystem Recovery Plan. Atlanta, GA. 128 pp.

Wang, H., R. Fu, A. Kumar, and W. H. Li. 2010. Intensification of summer rainfall variability in the southeastern United States during recent decades. Journal of Hydrometeorology **11**:1007-1018.

Wenger, S. J., M. M. Hagler, and B. J. Freeman. 2009. Prioritizing areas of the Conasauga River sub-basin in Georgia and Tennessee for preservation and restoration. Southeastern Fishes Council Proceedings **51**:31-38.

Table 4.1. Summary of nutrient data from algal accrual study sites collected in summer months (June, July, August) from 1997 to 2010. Percent elevation ("% elev") refers to frequency of samples having values of soluble reactive phosphorus (SRP) or combined nitrate and nitrite higher than indicated thresholds. Data are from B. J. Freeman and M. M. Hagler, unpublished.

Site	% elev SRP	% elev nitrate + nitrite	SRP (mg/L; summer)			nitrate + nitrite (mg/L; summer)		
	(>100 μg/L)	(>1500 μg/L)	Mean	SE	N	Mean	SE	N
US	7%	7%	0.004	0.002	14	0.485	0.252	15
DS1	13%	10%	0.035	0.002	12	0.267	0.083	11
DS2	0%	9%	0.006	0.002	11	0.759	0.556	12
DS3	33%	33%	0.034	0.034	3	1.328	0.669	6

Table 4.2. Biofilm and algal accrual as a function of days of tile deployment and site. Accrual of biofilm components was modeled as a quadratic function of duration (days) of tile deployment. The parameter estimate for Site DS3 is the difference in algal accrual between the most downstream site and all other sites.

Site Variable	Intercept Estimate (SE)	Days Estimate (SE)	$Days^2$ Estimate (SE)	Site Est. (SE)	Site % change
AFDM					
DS3	-1.403 (0.098)	1.006 (0.066)	-0.385 (0.071)	0.512 (0.125)	+66.9
Chlorophyll a					
DS3	-6.972 (0.128)	0.751 (0.086)	-0.240 (0.093)	0.549 (0.163)	+73.2

Table 4.3. Models of environmental effects on chlorophyll *a* and AFDM for Trial 2 tiles. The ΔAICc is difference from AICc of best-supported model. Periphyton biomass measures were both ln-transformed.

	Intercept		Environmental variable			
	Estimate	SE	Estimate	SE	% change	Δ AICc
Chlorophyll *a*						
Day since 0.6" rain	-8.427	0.296	0.202	0.041	+22.4 per day	0
Null	-7.0573	0.110				18.4
AFDM						
Day since 0.6" rain	-3.191	0.321	0.229	0.045	+25.7 per day	0
Water velocity at 60% depth	0.063	0.598	-3.150	1.084	-27.0 per 0.1 m/s	14.4
Null	-1.6421	0.121				20.4

Table 4.4. Environmental variables measured at each algal accrual site during Trial 2. Water velocity, depth and turbidity were measured multiple times over the course of the study.

Site	% Canopy Cover			Water Depth (m)			Water Velocity at Bed (m/s)			Water Velocity at 60% (m/s)			Turbidity (NTU)		
	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N
US	16.46	10.94	2	0.28	0.014	55	0.26	0.014	55	0.49	0.027	55	5.77	0.28	5
DS1	27.58	8.57	2	0.25	0.015	45	0.26	0.016	45	0.53	0.028	45	11.79	0.76	5
DS2	49.04	2.90	2	0.26	0.011	54	0.35	0.016	54	0.59	0.020	54	11.82	0.70	5
DS3	31.70	0.00	2	0.24	0.014	51	0.26	0.010	51	0.49	0.025	51	14.99	0.98	5

Table 4.5. Difference in chlorophyll *a* and AFDM for Trial 2 compared to Trial 1. The AICc score for the null model indicating no difference between trials is included for comparison. Periphyton biomass measures were both ln-transformed.

	Intercept		Trial 2				
	Estimate	SE	Estimate	SE	% change	AICc	Null AICc
Chlorophyll *a*	-5.798	0.140	-1.062	0.192	-65.4	136.0	159.0
AFDM	-0.206	0.145	-1.249	0.200	-71.3	140.4	169.0

Table 4.6. Best-supported model of environmental effects on chlorophyll *a* and AFDM for sites DS1 and DS3, data combined for Trials 1 and 2. The AICc score of the null model is included for comparison. Periphyton biomass measures were both ln-transformed.

	Intercept		Days since 0.6 inches of rain				
	Estimate	SE	Estimate	SE	% change per day	AICc	Null AICc
Chlorophyll *a*	-7.005	0.200	0.069	0.018	+7.1	147.8	159.0
AFDM	-1.806	0.195	0.100	0.017	+10.5	144.4	167.0

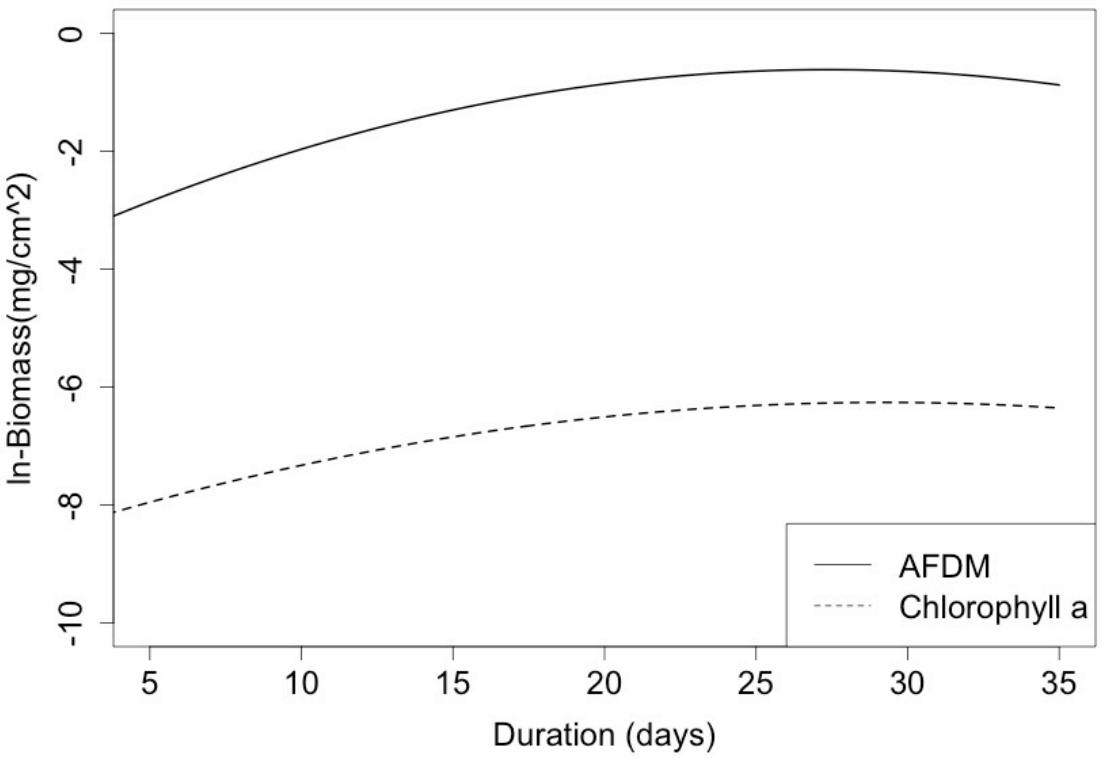

Figure 4.1. Predicted accrual of periphyton and algal biomass over time across four sites on the Conasauga mainstem. Curves are based on best-supported regression models relating accrual to days tiles were deployed, using data from Trial 2. Regressions are ln(AFDM) = -1.243 + 1.006*duration – 0.405*duration^2 and ln(Chlorophyll *a*) = -6.80 + 0.751*duration -0.261*duration^2. Duration is standardized for the regressions (i.e., the intercept is accrual at mean duration of 15.5 days); plotted predicted values are for actual days deployed.

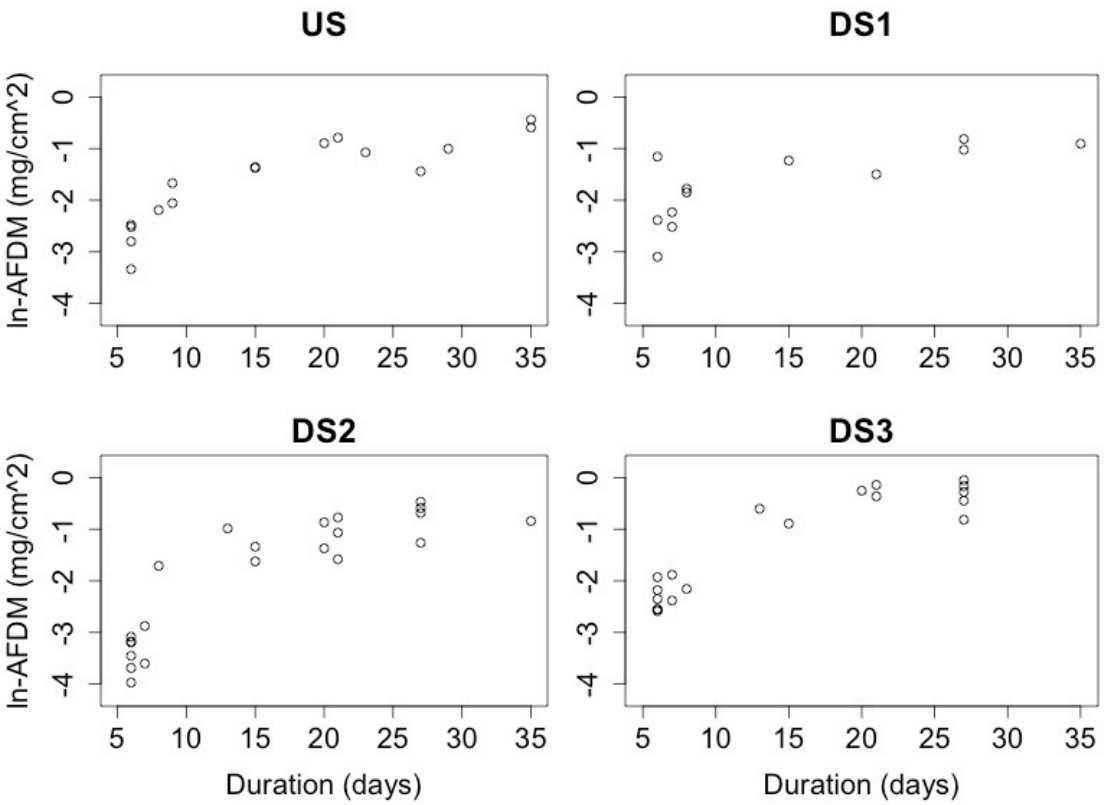

Figure 4.2. Periphyton biomass (measured by AFDM) at each site relative to the number of days of tile deployment. Multiple date ranges are represented for each duration. Only data for Trial 2 are included in this figure.

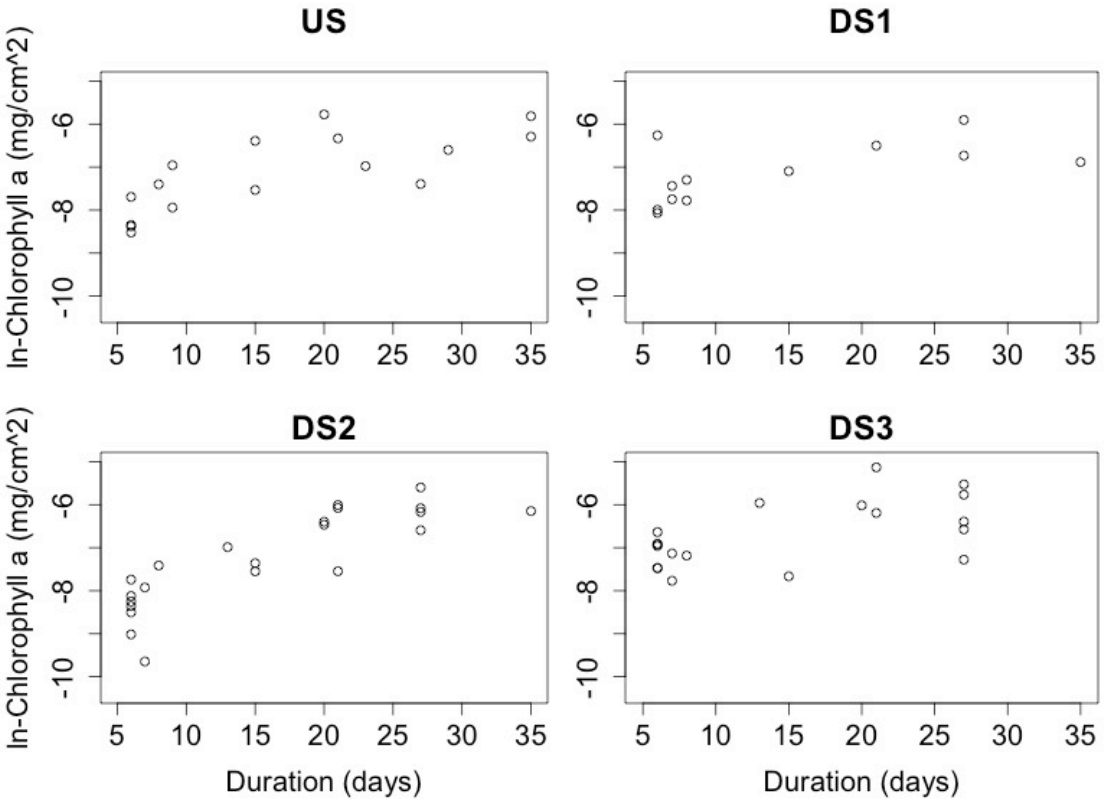

Figure 4.3. Algal biomass (measured by chlorophyll *a*) at each site relative to the number of days of tile deployment. Multiple date ranges are represented for each duration. Only data for Trial 2 are included in this figure.

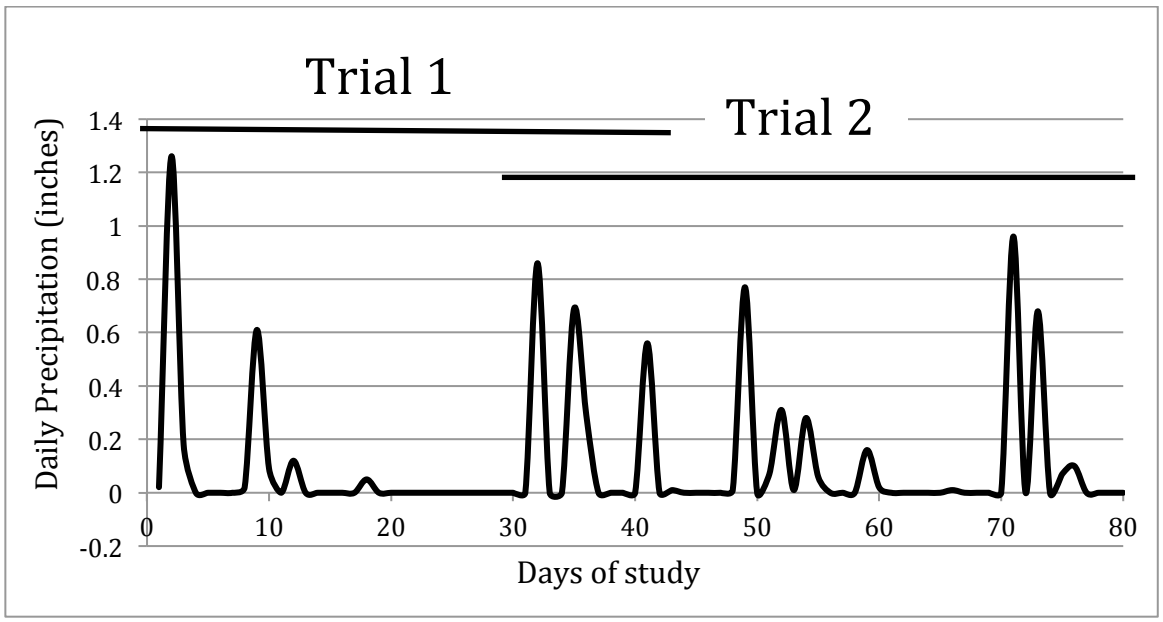

Figure 4.4. USGS daily precipitation data during study period. Time frame of each trial is represented by horizontal black bars.

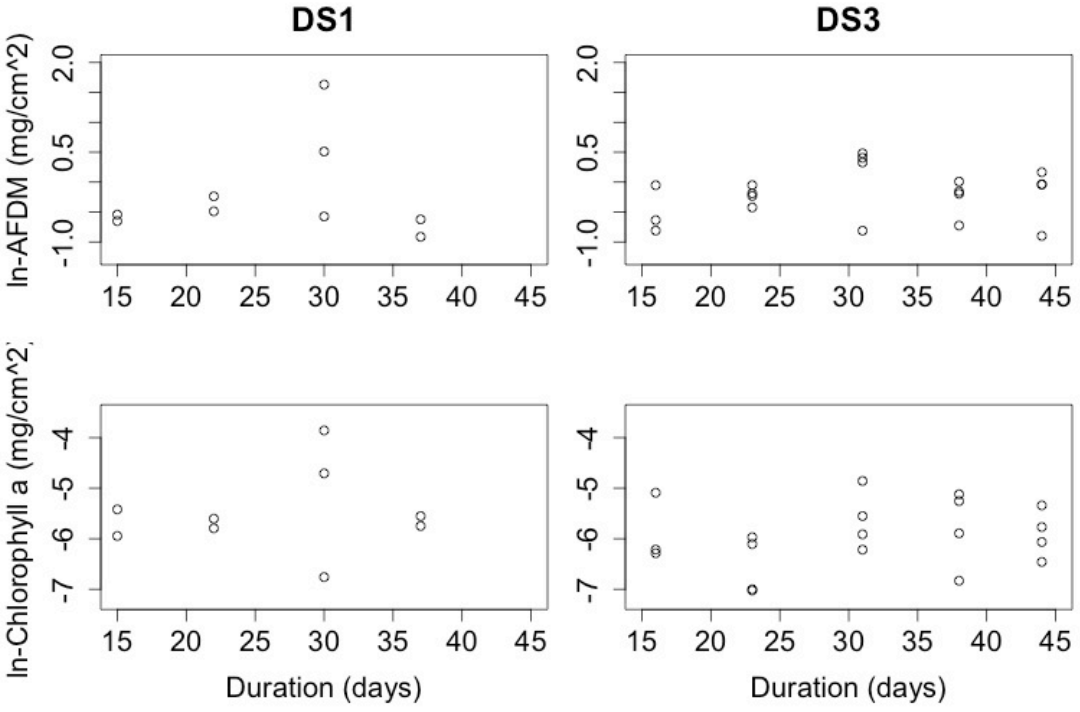

Figure 4.5. Periphyton measures from the two sites at which tiles were collected in Trial 1.

CHAPTER 5

CONCLUSIONS

This thesis provides additional evidence that diversity of Conasauga River biota declines from upstream to downstream along a reach that has been valued for biodiversity conservation, and that these declines span multiple trophic levels from aquatic primary producers and consumers, to invertebrate predators and insectivorous fishes. Additionally, some invertebrate taxa are resistant to stressors associated with land use and either maintain populations in downstream reaches or increase in abundance. Fishes show a similar pattern, as cosmopolitan species are found in similar abundances upstream and downstream (Freeman et al. 2007). I also find evidence that algal accrual is higher at the most downstream site studied, indicating that some primary producers may increase downstream even as a dominant macrophyte is declining. Mechanisms of biotic community change are currently unknown. However this study provides additional evidence that the contribution of nitrogen from manure sources increases discontinuously from upstream to downstream in the Conasauga River.

^{15}N in primary consumers as an indicator of anthropogenic nitrogen pollution

Anthropogenic nutrient loading has been hypothesized as a potential stressor to Conasauga biota. Consumer tissue ^{15}N indicated an increase in contribution of anthropogenic sources downstream of the national forest concurrent with an increase in percentage of agricultural land in the basin. However, I detected spikes in ^{15}N that were unexplained by the

continuous change in agricultural land, indicating increases in relative contribution of anthropogenic nitrogen. The greatest increase was at the first site downstream of the national forest, and subsequent increases were located at a major tributary confluence and in close proximity downstream of a dairy. Increased proportion of agricultural nitrogen at these points could also indicate that there are increased inputs of other agricultural stressors such as phosphorus and pesticides. Results of this analysis can be used to prioritize management efforts in subwatersheds and at point sources where a signal of elevated manure nitrogen was detected.

This study also supports the use of primary consumer ^{15}N as an integrator of long-term nutrient enrichment. Primary consumer tissues are often used as a baseline measure of nutrient conditions, because water column and algal ^{15}N turns over much more quickly. Conversely, consumers integrate fluctuations in nitrogen sources over time (Post 2002, Gustafson et al. 2007). My results show consistent ^{15}N enrichment across three differing consumer taxa (an herbivorous fish, snail and mussel) showing that a range of primary consumer taxa may be appropriate for using ^{15}N as an indicator of manure or sewage inputs (Vander Zanden et al. 2005).

Discontinuous downstream change in biotic communities

Previous evidence suggested a discontinuous change in composition of the fish community along the Conasauga River and my study contributed additional evidence that biotic changes are discontinuous along a river kilometer gradient. *Podostemum* was substantially reduced in the fish decline reach. Macroinvertebrate data exhibited an apparently abrupt reduction in the number of samples with high biomass coincident with the fish decline reach. Both results supported the hypothesis that benthic communities changed in the area where fish

taxa appear reduced. However, taxa-specific analyses using TITAN suggested a threshold decline in *Podostemum* at a point upstream of the fish decline reach, and that most threshold declines in invertebrate taxa were concurrent with declines in *Podostemum* at a point between the two *a priori* hypothesized points of change.

A combination of hypothesis-based and non-parametric methods was useful for determining that discontinuous change was statistically supported and that changes in some aspects of the biotic community occurred upstream of fish declines. Results from TITAN and ordination can be used to develop additional testable hypotheses about biotic changes in the Conasauga.

Algal accrual as a mechanism of nutrient induced changes in biota

Algal blooms in the Conasauga are a potential mechanism linking nutrient inputs to biotic decline. Algal accrual is sometimes substantial but appears episodic. My algal accrual study provides little evidence that algal biomass or growth rates are consistently higher in the fish decline reach. However, variability in conditions between the two trials, and occurrence of highest accrual during the trial lacking an upstream site for comparison limit conclusions. The variability in growth between trials might indicate that flow conditions rather than nutrients limit algal growth. Many other studies indicate that algal growth and nutrients are poorly correlated and that other factors might be more important for limiting algal growth (Biggs 2000). In periods of low flow with long durations between storms, differences in algal accrual between upstream and downstream reaches might be more evident and more tied to nutrient concentration. Phosphorus concentrations in the Conasauga typically exceed levels that support nuisance algal growth when storm frequency is low (Biggs 2000, Freeman et al. 2006). My

study also indicates that artificial substrates should be sampled soon after deployment to capture the exponential growth phase of algal accrual in rivers with high nutrient concentrations.

Synthesis

Conditions in the fish decline reach are altered relative to upstream reaches, but points of elevated input of anthropogenic nitrogen and shifts in some components of the benthic community occur farther upstream. This thesis did not support the hypothesis that algal growth is a link between nutrients and downstream changes in Conasauga River biota. However, this hypothesis might be supported if similar studies are conducted during periods of algal blooms. Given the close associations between *Podostemum* and macroinvertebrates and fishes, experimental studies to determine which stressors in the Conasauga are likely influencing *Podostemum* could provide key insights to mechanisms of change. If algal accumulation inhibits growth of *Podostemum* in the Conasauga, increased frequency of drought — a projected outcome of climate change for the Southeast U. S. — will have dire consequences to biota due to the apparent link between low flow and algal blooms. In addition to focusing on algal and *Podostemum* dynamics, future efforts to quantify changes in sensitive fishes and macroinvertebrates in relation to variation in flow, nutrients, and possibly contaminants may be key to understanding and managing changes in rivers valued for biodiversity, such as the Conasauga River.

Literature Cited

Biggs, B. J. F. 2000. Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. Journal of the North American Benthological Society **19**:17-31.

Freeman, B. J., M. M. Hagler, S. J. Wenger, G. B. Anderson, and R. A. Katz. 2006. Identification and mapping of critical habitats in the Conasauga River corridor of Georgia and Tennessee 2006 Annual report to the Georgia Department of Natural Resources, Atlanta. Institute of Ecology, University of Georgia, Athens.

Freeman, B. J., M. M. Hagler, S. J. Wenger, G. B. Anderson, and R. A. Katz. 2007. Identification and mapping of critical habitats in the Conasauga River corridor of Georgia and Tennessee 2006 Annual report to the Georgia Department of Natural Resources, Atlanta. Institute of Ecology, University of Georgia, Athens.

Gustafson, L., W. Showers, T. Kwak, J. Levine, and M. Stoskopf. 2007. Temporal and spatial variability in stable isotope compositions of a freshwater mussel: implications for biomonitoring and ecological studies. Oecologia **152**:140-150.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology **83**:703-718.

Vander Zanden, M. J., Y. Vadeboncoeur, M. W. Diebel, and E. Jeppesen. 2005. Primary consumer stable nitrogen isotopes as indicators of nutrient source. Environmental Science & Technology **39**:7509-7515.

APPENDICES

APPENDIX A: SUPPLEMENTARY MATERIAL FOR CHAPTER 3

Table 1. List of insect and non-insect taxa with taxon-specific codes used in invertebrate analysis figures. Rare taxa (occurring in fewer than five samples) are not listed as they were not used in analyses.

Insect Taxa

Taxon Code	*Order*	*Family*	*Genus*
E.BAE	Ephemeroptera	Baetidae	*Baetis*
E.PLA	Ephemeroptera	Baetidae	*Plauditus*
E.PSE	Ephemeroptera	Baetidae	*Pseudocloeon*
E.BUN	Ephemeroptera	Baetidae	UNKNOWN
E.BCA	Ephemeroptera	Baetiscidae	*Baetisca*
E.CAE	Ephemeroptera	Caenidae	*Caenis*
E.EPH	Ephemeroptera	Ephemerellidae	UNKNOWN
E.MAC	Ephemeroptera	Heptageniidae	*Maccaffertium* or *Stenonema*
E.ISO	Ephemeroptera	Isonychiidae	*Isonychia*
E.TRI	Ephemeroptera	Leptohyphidae	*Tricorythodes*
E.ANT	Ephemeroptera	Potamanthidae	*Anthopotamus*
P.PER	Plecoptera	Perlidae	UNKNOWN
PLEC	Plecoptera	UNKNOWN	
O.GOM	Odonata	Gomphidae	UNKNOWN
HEMI	Hemiptera		
C.MIC	Coleoptera	Elmidae	*Microcylloepus*
C.OPT	Coleoptera	Elmidae	*Optioservus*
C.OUL	Coleoptera	Elmidae	*Oulimnius*
C.PRO	Coleoptera	Elmidae	*Promoresia*
C.STE	Coleoptera	Elmidae	*Stenelmis*
C.ELM	Coleoptera	Elmidae	UNKNOWN
C.DIN	Coleoptera	Gyrinidae	*Dineutus*
T.BRA	Trichoptera	Brachycentridae	*Brachycentrus*
T.MIC	Trichoptera	Brachycentridae	*Micrasema*
T.PRO	Trichoptera	Glossosomatidae	*Protoptila*
T.CER	Trichoptera	Hydropsychidae	*Ceratopsyche*
T.CHE	Trichoptera	Hydropsychidae	*Cheumatopsyche*

T.HYD	Trichoptera	Hydropsychidae	*Hydropsyche*
T.HLA	Trichoptera	Hydroptilidae	*Hydroptila*
T.NEC	Trichoptera	Leptoceridae	*Nectopsyche*
T.OEC	Trichoptera	Leptoceridae	*Oecetis*
T.SET	Trichoptera	Leptoceridae	*Setodes*
T.LEP	Trichoptera	Leptoceridae	UNKNOWN
T.CHI	Trichoptera	Philopotamidae	*Chimarra*
T.NEU	Trichoptera	Polycentropodidae	*Neureclipsis*
T.PSY	Trichoptera	Psychomyiidae	*Psychomyia*
M.COR	Megaloptera	Corydalidae	*Corydalus*
D.PAL	Diptera	Ceratopogonidae	*Palpomyia*
D.NON	Diptera	Chironomidae	Non-Tanypodinae
D.TAN	Diptera	Chironomidae	Tanypodinae
D.HEM	Diptera	Empididae	*Hemerodromia*
D.SIM	Diptera	Simuliidae	*Simulium*
D.ANT	Diptera	Tipulidae	*Antocha*

Non-Insect Taxa

Taxon Code	*Higher Classification*	*Family*	*Genus*
TURB	Turbellaria		
DUGE	Turbellaria	Dugesiidae	
NEMA	Nematoda		
NEME	Nemertea		
LIMP	Gastropoda (Limpets)		
G.PLA	Gastropoda	Planorbidae	
G.PLE	Gastropoda	Pleuroceridae	
CORB	Bivalvia	Corbiculidae	*Corbicula*
OLIG	Oligochaeta		
MITE	Hydracarina		
OSTR	Ostracoda		
COPE	Copepoda		