PODOSTEMUM CERATOPHYLLUM AND PATTERNS OF FISH OCCURRENCE AND RICHNESS IN A SOUTHERN APPALACHIAN RIVER

by

JANE ELIZABETH ARGENTINA
B.S. Tufts University, 2000
(under the direction of Mary C. Freeman)

ABSTRACT

We estimated percent cover of *Podostemum ceratophyllum* Michx. and fish richness at twenty randomly selected shoals within a 42 kilometer reach of the Conasauga River to determine if changes in species richness or species presence are related to variable levels of *P. ceratophyllum* coverage. We also assessed fish behavioral shifts with changes in *P. ceratophyllum* coverage in an experimental study. *P. ceratophyllum* was correlated positively with increasing bed sediment sizes and decreasing canopy cover, and negatively associated with drainage area. Fish species richness peaked in the center portion of the study reach, declining further downstream, coincident with range contraction of six lotic fishes. Benthic insectivorous fishes showed significant preference for habitat with increased *P. ceratophyllum* coverage compared with low coverage. River-wide changes in *P. ceratophyllum* could influence fish productivity through lower insect biomass and abundances, although water quality may have a greater effect on fish abundance and persistence.

INDEX WORDS: Conasauga River, *Podostemum ceratophyllum*, fish richness, shoal, turbidity, drainage area, benthic insectivores, experimental manipulation, aquatic macrophytes

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TABLE OF CONTENTS

Page

Chapter One

Introduction

Species richness is shaped by the landscape and the riverscape at multiple scales in river systems. Benthic habitat structure and heterogeneity, in particular, can strongly influence local abundances of riverine biota. Aquatic plants are important habitat features for biota inhabiting low velocity areas such as sea grass beds (Heck and Wetstone 1977, Heck and Crowder 1991), lake edges (Werner and Hall 1988), and river margins (Newman 1991, Fritz et al. 2004). I have focused on a submerged aquatic plant, *Podostemum ceratophyllum* Michx., that is known to provide heterogeneous habitat to aquatic macroinvertebrates (Nelson and Scott 1962, Hutchens et al. 2004), and with which a number of benthic fishes have been reported to associate (Etnier and Starnes 1993, Marcinek 2003, Hagler 2006). *P. ceratophyllum* (Podostemaceae) is a completely submerged filamentous dicotyledon without true roots; it occurs in high gradient upland waters such as Appalachian streams and rivers (Hutchens et al. 2004, Hagler 2006), and shoal and riffle habitat of Piedmont rivers (Mullholland and Lenat 1992, Nelson and Scott 1962, Grubaugh and Wallace 1995, Marcinek 2003, Fritz et al. 2004). It characteristically occurs in fast-flowing waters where it attaches with hyphae to the surface of rocks. By increasing surface area, reducing scour, and increasing availability of organic matter and epiphytic periphyton, *P. ceratophyllum* enhances benthic habitat for aquatic invertebrates (Grubaugh and Wallace 1995, Hutchens et al. 2004), and may do the same for riverine fishes.

In an observational study (Chapter 2), I have examined longitudinal patterns of occurrence of *P. ceratophyllum* and of fishes in relation to natural features and land use in a biologically diverse southeastern Appalachian river. Specifically, I have evaluated relationships between *P. ceratophyllum* and habitat variables measured at three scales – local (i.e., habitat at the point of observation), shoal (channel slope, canopy cover) and landscape (drainage area, land use upstream and in the riparian buffer). I have also assessed relations between the longitudinal distribution of *P. ceratophyllum* in the Conasauga River and (1) fish species richness and (2) occurrence probabilities for lotic fish species, using survey methods that account for incomplete species detectabilities (Nichols et al. 1998, MacKenzie et al. 2002).

In an experimental study (Chapter 3), I tested the influence of *P. ceratophyllum* on benthic fish habitat use. Benthic habitats provide protection from predators and refugia from high velocities for a broad range of aquatic animals. Benthic aquatic macroinvertebrate biomass generally increases in areas with complex habitats, including heterogenous bed sediments, woody debris and aquatic plants (Rabeni and Minshall 1977, Anderson et al. 1978, Allan 1995, Meyer et al. 1997). I expected that benthic insectivorous fishes would show a behavioral response to increased coverage of *P. ceratophyllum* and that this response may be due to increases in food availability. To test for a behavioral response by benthic fishes, I manipulated *P. ceratophyllum* cover and quantified fish abundances, as well as insect density and biomass, two weeks after the manipulation, in two experimental patches measuring about 30 m^2.

Together these observational and experimental studies provide new data and insights on factors controlling abundance of *P. ceratophyllum* and fish responses to macrophyte coverage at shoal and local habitat scales.

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Chapter 2

Habitat and landscape effects on distribution of a benthic macrophyte, *Podostemum ceratophyllum* Michx, in a southern Appalachian river and associations with lotic fishes

Introduction

Rivers are influenced by the valley through which they flow (Hynes 1975, Allan 2004), and lotic plants and animals have evolved in the context of riverine and watershed influences. Factors commonly associated with patterns in occurrences of stream fish species include natural riverscape geomorphic features, such as stream size, gradient, velocity and sediment composition (Horwitz 1978, Aadland 1993, Burkhead and Jelks 2001, Walters et al. 2003, Goldstein and Meador 2004). Patterns are also associated with landscape influences from land use, including changes in water chemistry, light availability, sediment loads, and change in organic substrates including woody debris and aquatic plants (reviewed in Allan 2004). We examined longitudinal patterns of occurrences of an important benthic macrophyte, *Podostemum ceratophyllum,* and fish species richness in relation to natural features and land use in a biologically diverse southeastern Appalachian river.

The Conasauga River in northeast Georgia and eastern Tennessee has a diverse faunal assemblage of 92 fish species (Walters 1997) that includes three fishes (*Cyprinella caerulea*, *Percina antesella*, and *Percina jenkinsi*), as well as nine mussels (*Epioblasma metatriata*, *Epioblasma othcaloogensis*, *Hamiota altilis*, *Medionidus acutissimus*, *Medionidus parvulus*, *Pleurobema decisum*, *Pleurobema georgianum*, *Pleurobema perovatum*, *Ptychobranchus greeni*) that are federally protected, and additional state protected fishes. Many of these protected species occur exclusively or primarily in relatively larger streams, in this case the mainstem of the Conasauga River, and have been eliminated from portions of their range outside of the Conasauga system because of dams, flow alteration and poor water quality (Freeman et al. 2005). Thus, the Conasauga system is considered important to conservation of southeastern fauna. Protection of the Conasauga River fauna is dependent on an accurate understanding of the status of fish populations in the river and factors potentially leading to their decline.

A primary goal of this study has been to investigate a potentially important riverine habitat feature, the aquatic macrophyte *P. ceratophyllum* (Podostemaceae). *P. ceratophyllum* is a submerged aquatic macrophyte that uses hyphae to attach to stable bed sediments in flowing riverine environments. The range of *P. ceratophyllum* covers the U.S. eastern seaboard, but widespread population declines have been recorded across the species's range. Reasons for decline have not been studied explicitly, although some propose siltation, acidification, excess nutrients, and flow alteration as causative factors (Philbrick and Crow 1983, Meijer 1976, Munch 1993). *P. ceratophyllum* is extirpated in Rhode Island, and listed as endangered in Ohio, threatened in New York and a species of special concern in Massachusetts, Maine, Kentucky and Connecticut (USDA 2006).

P. ceratophyllum is primarily found in the clear, swift water of riffles and rapids of large rivers and streams, but can also be found in clear, moderately deep runs up to 1m deep. It is usually reported from high gradient upland areas such as Appalachian streams and rivers, but it is occasionally reported in Piedmont rivers (Mullholland and Lenat 1992, Fritz et al. 2004), where coverage can be extensive (Nelson and Scott 1962, Grubaugh and Wallace 1995, Marcinek 2003). Despite studies in which *P.*

ceratophyllum has been mentioned as important riverine habitat, features that describe causative factors of occurrence and abundance have not been studied. Therefore, we have assessed the longitudinal distribution of *P. ceratophyllum* in the Conasauga River mainstem and evaluated relationships between *P. ceratophyllum* and habitat variables measured at three scales – local (i.e., habitat at the point of observation), shoal (channel slope, canopy cover) and landscape (drainage area, land use upstream and in the riparian buffer).

A second goal in this study was to quantify longitudinal patterns of fish species distribution, and to investigate factors that may contribute to longitudinal variation in species richness and in the occurrence of species. In particular, although *P. ceratophyllum* provides habitat for macroinvertebrates (Grubaugh and Wallace 1995, Hutchens et al. 2004), little is known about the effects of diminished *P. ceratophyllum* cover on stream fish assemblages. For example, the riverweed darter, *Etheostoma podostmone*, was described as a close associate with *P. ceratophyllum*, but current studies show that even with loss of *P. ceratophyllum* cover, the riverweed darter persists in the Roanoke River in Virginia (Connelly et al. 1999). Therefore, we have also assessed relations between the longitudinal distribution of *P. ceratophyllum* in the Conasauga River and (1) fish species richness and (2) occurrence probabilities for lotic fish species. Finally, we tested for longitudinal changes in probabilities of species-specific occurrence generally, and determined whether longitudinal trends were related to land use patterns either in addition to or independent of changes in *P. ceratophyllum*. Together, these analyses quantify patterns of lotic species in relation to riverscape and landscape variation in a southeastern Appalachian river.

Methods

Study Sites and Focal Species

Study sites were located in the mainstem of the Conasauga River, a tributary to the Oostanaula River in the Coosa River basin. The Conasauga River begins in the Chattahoochee National Forest, flows west crossing the Tennessee and Georgia state line five times before turning south where it remains in Georgia until its confluence with the Oostanaula River. The upper portion of the Conasauga River is in Murray County, Georgia, and it eventually forms most of the boundary between Murray and Whitfield counties, Georgia. The Conasauga River is 148.5 river kilometers from headwaters to mouth. The study reach was a 5th - 6th order stream (1:24,000 topographic map) and included the mainstem from 36.6 river kilometers to 75.4 river kilometers upstream of the confluence (i.e. the confluence is river km 0). We focused on 30 lotic-dependent species from seven families, Cyprinidae, Catostomidae, Ictaluridae, Fundulidae, Cottidae, Centrarchidae, and Percidae (Appendix A), for modeling patterns of occurrence.

Habitat mapping and site selection

The focal fish species and *P. ceratophyllum* primarily inhabit shoals – relatively shallow areas of moderate to swift flow over coarse bed sediments. To estimate shoal distribution and coverage in the Conasauga River, a field crew canoed the length of the mainstem in the study area (Figure 1), a distance of 41.6 kilometers, during May and June 2006. During these trips, we used a handheld GPS to record the location of each shoal encountered. At each shoal we used a range finder to estimate total shoal length, and we visually assessed the presence or absence of *Podostemum ceratophyllum* and *Justicia* *americana*. We then divided the entire mainstem reach into four equidistant segments, and randomly chose five shoals within each of the four sections for further study.

Habitat and vegetation measurements

We measured habitat characteristics at each of the twenty randomly selected shoals, including percent coverage of *P. ceratophyllum* and factors we hypothesized would influence coverage including shoal slope and average bed sediment. I used a Topcon autolevel and stadia rod to measure local shoal slope and a hand-held tape-measure for shoal length. A spherical densiometer was used to estimate canopy cover. We used a one square foot (0.093 m^2) PVC frame subsampler subdivided into sixteen 3” x 3” (7.6 cm X 7.6 cm) squares to estimate bed sediment size and percent *P. ceratophyllum* coverage within each shoal at 50 randomly selected locations. At each sub-sampling location, we measured the medial axis of two sediment particles and counted the number of squares within each frame that contained *P. ceratophyllum* attached to the bed sediments. At three of the twenty shoals we collected the *P. ceratophyllum* at sixteen subsamples (four samples within each subclass of 1-4 squares, 4-8 squares, 9-12 squares and 13-16 squares occupied) to estimate the relationship between coverage at the shoal and plant biomass. We removed all *P. ceratophyllum* within these subsamples to plastic bags and returned the samples to a laboratory where the *P. ceratophyllum* was separated from remaining sediment and detritus, and then dried, ashed and weighed to estimate the ash free dry mass at variable levels of *P ceratophyllum* coverage.

Predictive models for P. ceratophyllum

We used an information-theoretic approach (Burnham and Anderson 2002) to determine the best-supported model from a suite of models to understand factors that predict *P. ceratophyllum* abundance at a given point. To evaluate effects of land use as well as physical factors that may influence *P. ceratophyllum* coverage, we used a hierarchical model (Proc **nlmixed**; SAS version 9.1 2002). The hierarchical model allowed us to incorporate all point observations of *P. ceratophyllum* cover in a regression model based on predictor variables measured at the scale of shoal and at the observation point (i.e., microhabitat), while including a random effect for shoal identity (i.e., to account for repeated observations within shoals; Singer 1998). We used logistic regression to model the probability of greater than 50% coverage ($\geq$ 8 squares in the sample frame) as a binomial variable. This percentage was chosen because samples with $\geq$ 8 squares containing *P. ceratophyllum* had a consistently higher level of aquatic insect biomass compared with samples having < 8 squares occupied (Chapter 3). We constructed models using five shoal- or watershed-level variables [drainage area (km^2), percent canopy cover, percent row crop land cover in the watershed, percent agriculture in a 100 m buffer 1 km upstream of each site, and slope (natural log transformed)] and two observation-point variables [average sediment size and position within the channel (classified as within the center 50% or on the outer 25% of channel width)].

Land cover above each sampling site was calculated using ArcView® 3.3 geographic information systems (GIS) software. We used digital U.S. Geological Survey (USGS) topographic maps to delineate watershed boundaries of each sampling site. Within each delineated watershed, we used a 1:24,000 hydrography network to further delineate 100m buffers that extended from each sample site along the mainstem to the headwaters from each site, and 100m buffers that extended along the mainstem1 km upstream of each site. We used the 2001 USGS National Land Cover Database zone 60 land cover to estimate percent urban, percent forested and percent agriculture for each sample watershed. To model *P. ceratophyllum* cover, we used a subset of the agriculture in the basin, percent row crop, in the watershed as a land cover variable. Similar to land use in catchments in the Etowah River basin (Roy et al. 2006), agricultural land cover in the watershed buffer was correlated with agricultural land cover within the watershed (r = 0.96). Therefore, we used the 1 kilometer buffer segments instead of buffer in the entire watershed to model *P. ceratophyllum* in relation to local buffer agricultural land use at each site.

The null model (i.e., no predictor variables) and models with all combinations of the shoal-level and observation-point variables were run, excluding models that included both buffer and watershed land use, for a total of 95 models. We selected all models that were within the 1/8th of the top model AIC weight as candidate models and calculated relative variable importance and model-averaged odds ratios for each parameter included in the candidate models (Burnham and Anderson 2002).

Fish species richness and species occurrence

A field crew performed visual snorkel or seine surveys to assess the species of fishes present at each of the 20 shoals. Before each fish survey we measured temperature, dissolved oxygen (mg/l), turbidity (NTU), and conductivity (uS/cm). Species lists were compiled during snorkel surveys by multiple (2-3) observers.

Snorkelers observed fishes for one hour, searching all available habitats and independently compiling lists of all species seen. If the turbidity was above 5 NTUs, we used a seine to sample fishes. During a seine sampling, we attempted to get as complete a fish list as possible by sampling all available habitat. We used hauls in low flow areas, or sets in flowing water in which two people held the seine perpendicular to the flow and others disturbed the substrate with their feet to corral fishes into the net, sampling approximately 3.35 m^2 with each set. Seine sampling was conducted for 45 minutes and covered the entire shoal. Sampling was repeated on a subsequent day to obtain a repeat sample.

Multiple snorkel observations and repeat shoal seine samples allowed us to estimate overall species richness while accounting for incomplete species detection (Nichols et al. 1998). Having repeated observations also allowed us to estimate the proportion of shoals occupied (i.e., patch occupancy) by individual species, again, while accounting for species-specific detectability that is less than one (MacKenzie et al. 2002).

We estimated species richness at each shoal using SPECRICH 2 (http://www.mbr-pwrc.usgs.gov/software/specrich2.html). The SPECRICH program estimates species richness based on species presence in replicate samples using a jackknife estimator for model M_h (White et al. 1978). Model M_h allows for heterogeneity (h) in detectabilities across species, while holding detectability for each species constant between samples. We estimated richness for all fish species and for species considered to be lotic-dependent. Fishes were considered “lotic” if they are not known to persist in lake or reservoir habitat (Etnier and Starnes 1993, Jenkins and Burkhead 1993). We used correlation analysis to examine relationships between total and lotic richness and *P. ceratophyllum* coverage, land cover, and river kilometer at the sampled shoals.

We estimated species-specific detectabilities and patch occupancy rates using the program Presence (MacKenzie et al. 2002). We held detectability for individual species constant across shoals, and evaluated models for each species either with constant occupancy across all shoals or with probability of occupancy allowed to vary as a function of percent *P. ceratophyllum* cover and biomass or river kilometer. We calculated P. ceratophyllum biomass at each shoal using a linear regression between AFDM (natural log transformed) and coverage. We compared AIC values for these alternative occupancy models to evaluate the support for (1) an effect of *Podostemum* cover or biomass or (2) an effect of longitudinal position in the mainstem, on species-specific probability of occurrence. We ran these models for a total of 22 lotic-dependent species, but not for lentic generalist species, or lotic species that either occurred at all shoals or that were extremely rare (occurring at fewer than 3 shoals). Where modeled species occurrence showed probability of occurrence at less than 0.65, we mapped occurrences during 2005 against historical collection sites to evaluate potential loss of range.

Results

We mapped 131 shoals in the 41.6 kilometer study reach of the Conasauga River (Figure 1). Total shoal habitat in that stretch of the river was 13.6 km out of the total 41.6 km (33 %). The twenty shoals randomly selected for sampling encompassed 1.5 km and 11 % of all available shoal habitat. The shoals were steeper and longer upstream and became less steep and shorter downstream. On average, shoals were separated upstream and downstream by 375 m from other shoals, and the distance between shoals increased downstream (Table 1).

Trends in P. ceratophyllum occurrence:

Percent *P. ceratophyllum* cover (quantified as the percent of squares within the sampling frame occupied) averaged from less than 1% to 55% across the 20 randomly selected shoals, with an average cover of 17%. At most sampled shoals, *P. ceratophyllum* biomass and surface area appeared sparse, even when measured cover (i.e., number of squares occupied) was high. Biomass increased with increasing cover of *P. ceratophyllum* ($p < 0.05$, $r = 0.59$; Figure 2).

Hierarchical modeling to relate physical variables to the probability of high *P. ceratophyllum* coverage (i.e., $\geq$ 8 squares occupied within a sampling frame) resulted in thirteen candidate models (i.e., with model weights within 1/8th of the top model; Table 2). The two observation-point variables, median particle size and location within the channel (within 25% of the edge or in the middle 50%), were present in every model, indicating habitat availability was a direct driver of the occurrence of *P. ceratophyllum*. For every 10 mm increase in particle size, *P. ceratophyllum* was 1.32 times more likely to be present in 50% or more of a sampling frame. *P. ceratophyllum* was 2.9 times more likely to be present in the center half of the channel rather than near the edges (Table 3). Each of the shoal-level variables, including percent canopy cover, drainage area, shoal slope, and percent of the watershed in row-crop agriculture, were present in at least one of the models. The best-supported model contained median particle size, location within the channel, drainage area and percent canopy cover. *P. ceratophyllum* cover decreased from upstream to downstream (Figure 3), with the probability of having high coverage decreasing 23% with every 50 km^2 increase in drainage area which ranged from about 255 to 800 km^2. The confidence interval crossed one, which would indicate uncertainty in the direction of the effect of watershed size, but the upper limit on the confidence interval only slightly greater than one. This indicates a high probability that the effect of drainage area is actually negative. From our estimated cover data, *P. ceratophyllum* cover was consistently low at drainage areas greater than about 500 km^2 (Figure 3). Canopy coverage showed a small negative effect on high *P. ceratophyllum* coverage, but the confidence intervals around the odds ratio included one (i.e., no effect: Table 3). The next best model was 64% as likely to be the best model and included, along with the previous four parameters, row crop land cover. Model-averaged effect of row crop land cover on *P. ceratophyllum* was positive; however, the confidence interval for the odds ratio included 1. For the two models that included agriculture in the buffer, the effect was positive in one model, and negative in the other. All the other models within the candidate set were less than 50% as likely to be the best model of those run. Overall, the strongest drivers of high *P. ceratophyllum* coverage were particle size, proximity to the center of the channel, drainage area and canopy cover.

Trends in species richness and species presence:

Estimated fish species richness was similar to observed species richness at most sampled locations, except at river kilometer 53.9, where estimated richness was much larger than observed richness because of large differences between the two observers.

Total richness and lotic fish species richness estimates were highest at shoals in the middle portion of the study reach (Figure 4; Table 4). Estimated total species richness was highest at river kilometer shoals 46.9 (31 species), 53.9 (28 species) and 60.7 (28 species). Estimated total species richness was lowest in the headwaters at the uppermost three shoals, RKm 75.4, 75.3, and 71.2. Species richness at the downstream-most shoal sampled, RKm 36.4, also was low, with an estimated richness of 18 species. Lotic fish richness followed a similar pattern with highest richness in the middle reaches. However, the proportion of estimated lotic specialists to total estimated richness declined in the downstream direction ($p < 0.05$, $r^2 = 0.38$, df = 19).

Each of the hypothesized drivers of species richness varied approximately linearly from upstream to downstream. On average, forested land cover in the watershed decreased downstream ($r = 0.94$) ranging from 98.7 percent forested for the upper most site to 67.8 percent forest near Dalton (Figure 5). This corresponded to an increase in developed land cover (low to high density developed), which ranged from 0.6% to 6% from upstream to downstream, and agricultural land covers (grassland, pasture and row crop), which ranged from 0.04% to 21.9% from upstream to downstream. Change in watershed land cover was also reflected in increasing turbidity levels (measured coincident with sampling) in the downstream direction, from < 2 NTU at the upstream-most five shoals to > 10 NTU at the downstream-most shoal ($r = 0.94$, $n= 20$; Figure 6). Thus, none of these variables were strongly correlated with species richness (which peaked in the center of the study reach). Percent *P. ceratophyllum* and shoal length also decreased downstream, and were weakly correlated with total and lotic richness (all r values ≤ 0.34).

Of the twenty lotic species for which patch occupancy models were appropriate, models for five species were most parsimonious with no covariates (which holds occupancy constant across all sites), models for fourteen species were improved with river kilometer as a covariate, and one model was improved with percent *P. ceratophyllum* coverage as a covariate (Table 5). None of the models using *P. ceratophyllum* biomass (AFDM) were better models than using coverage, and values are not reported. Models with no covariates were only slightly better (i.e., as indicated by similar model weights; Table 5) than models with river kilometer and *P. ceratophyllum* as covariates.

Eight species were more likely to occur further upstream than downstream. Of these eight, only one, *E. brevirostrum*, has a known historical range limited to the headwaters. Of the other seven, only one species (*E. coosae*) had an estimated probability of occurrence above 0.65. In contrast, of the six species more likely to be captured further downstream, probability of occurrence was greater than 0.8 except for *Luxilus chrysocephalus* (Psi = 0.68). In other words, even though the models indicated these six species are more likely to occur downstream, they occurred at shoals throughout the mainstem, whereas the six species that were more likely to occur further upstream primarily occurred upstream. For ten lotic species, observations were either too rare or the species occurred at all shoals, and we were unable to run the models.

Maps of the ten lotic species with probability of occurrences in 2005 less than 0.65 (based on best-supported models) were compared to historical observations (Figures 7-17). Four of these species occurred at few sites, but patterns in species occurrence did not indicate a loss from the upper portion of the mainstem. Six species, in contrast, showed evidence of loss in the downstream portion of the mainstem. Range restrictions to the upper portion of the watershed appear to have occurred with two minnows (*Cyprinella tricroistia*, and *Notropis xaenocephalus*), one bass (*Micropterus coosae*), and four darters *(E. trisella, P. antesella*, *P.* sp. *cf. P. macrocephala*, and *P. palmaris*), whose historical ranges included the downstream reach. Additional fish species historically recorded from this section of the river were not observed at all in 2005 or were observed only very rarely. This list includes *Noturus* sp. *cf. N. munitus, Ambloplites ariommus, Fundulus olivaceus, Moxostoma carinatum, Macrhybopsis* sp. *cf. M. aestivalis, E. ditrema*, *P. lenticula, P. jenkinsi* , and *P. shumardi*.

Discussion

Local features (sediment size, location in the channel) are better predictors of occurrence of the lotic aquatic macrophyte *P. ceratophyllum* than shoal slope or watershed land use. Furthermore, *P. ceratophyllum* cover declines from upstream to downstream along the mainstem of a southern Appalachian river. We have not found strong evidence that *P. ceratophyllum* cover has a large effect on either fish species richness, or on occurrence of most lotic fish species. However, several fish species display evidence of decline in the downstream portions of the study reach, coincident with higher turbidity, lower forest cover, and lower *P. ceratophyllum* cover.

P. ceratophyllum occurs in high velocity, wide, shallow shoal habitats, which generally correspond with coarse bed sediments and relatively steeper stream slope (Nelson and Scott 1962, Hutchens et al. 2004, Marcinek 2003, Hagler 2006). From our predictive models, we know *P. ceratophyllum* is more likely to occur in habitat that is in the center of the channel and on larger rocks, indicating a strong physical control on its presence. Because *P. ceratophyllum* does not have roots, the rocks to which its hyphae are attached are the only source of its stability. Hence, bedrock and bed sediments that only are moved with high stream velocity are likely to be better habitat than shifting gravel and sand. The importance of channel location suggests light also is a significant factor for the growth of *P. ceratophyllum*. Light could become limiting even in relatively shallow shoal habitats; e.g., when algae and diatoms covered much of the bottom of the channel, including *P. ceratophyllum*, during the drought of the late 1990s (B. J. Freeman, unpublished data). Increased turbidity could also limit light for benthic macrophytes (discussed below).

Landscape variables were less directly important than local variables in predicting the presence of *P. ceratophyllum*. Models presented here show a potentially positive impact of row crop agriculture on *P. ceratophyllum,* indicating a possible nutrient subsidy, but the confidence intervals around this estimate are large. However, agricultural land cover in the buffer in a one-kilometer reach above each site shows a negative effect on the probability of having high *P. ceratophyllum* coverage, but again with wide confidence intervals around this estimate. Thus, these data do not show clear, direct effects of replacing forest with agriculture on *P. ceratophyllum*.

Nonetheless, we found a general decline in *P. ceratophyllum* average cover in the downstream portion of the study reach, with consistently lower values at drainage areas greater than about 500 km^2. We do not expect a natural decline in *P. ceratophyllum* with increase in drainage area over this size range because we know it occurs in abundance in shoal habitats in other, larger Piedmont rivers with higher drainage areas (e.g., > 2000

km^2, Flint River, Marcinek 2003; > 400 to1500 km^2, Etowah River, Hagler 2006). In addition, unpublished data at river kilometer 54 (B. J. Freeman and J. E. Argentina) show an approximate 50% decline in *P. ceratophyllum* coverage from the late 1980s to 2005.

Two response patterns have been seen with fish populations within the Conasauga mainstem. One pattern we observed was an increase in richness from section one to section two of the sampled reach, where forest in the catchment and in the buffer decrease and nutrients increase (B. J Freeman, unpublished water quality data). Richness then declined downstream from section 2, as non-forest land covers further increased in the watershed. The pattern of increase in richness, followed by a decline in more impacted areas resembles a subsidy-stress response (Odum et al. 1979, Allan 2004). In this case, increasing nutrient inputs may drive increased productivity, supporting higher species richness in middle portions of the study reach. Further downstream, increasing anthropogenic nutrient inputs may become a stressor that reduces species occurrences, e.g., via toxicity, increased turbidity, eutrophication and consequent low dissolved oxygen. Whatever the mechanism, a number of studies have shown declining species richness in response to increasing agricultural and urban land uses (reviewed by Allan 2004).

The second pattern observed was a non-linear threshold response whereby species became rare, and possibly absent, at some longitudinal point along the mainstem. A non-linear response pattern may be seen if animals persist until a stress reaches a maximum level, beyond which populations decline dramatically (Allan 2004). A complete loss of a species may correspond with, e.g., threshold levels of turbidity, chemical toxicity, increased temperature, loss of food source or some unknown stresses. Stressor impact may be direct, through mortality from toxicity or gill tissue damage, or it may be indirect such as starvation or loss of spawning habitats. A non-linear response was seen as range contraction in six species (*Micropterus coosae*, *Cyprinella trichroistia, Notropis xaenocephalus*, *Percina palmaris*, *Percina antesella*, and *Percina* sp. *cf. P. macrocephala*, based on presence data, but population abundances may change more gradually.

These observations of declining *P. ceratophyllum* cover and loss of these six lotic fishes from downstream portions of their historic range strongly suggest declining ecological integrity in the lower Conasauga River mainstem. These biotic changes are coincident with increasing turbidity in the downstream direction, as has been observed in previous years in the Conasauga River (Freeman et al. 2003, 2004). By river kilometer 50.70, turbidity approached or surpassed 5 NTUs, so that snorkeling surveys were no longer feasible. High turbidity levels could limit light availability for benthic macrophytes. High turbidity levels, even at low flow levels, can affect fishes physically (Sutherland 2005) and by decreasing their ability to feed efficiently (Sweka and Hartman 2003). High turbidity can also interrupt visual cues used during spawning by stream fishes that develop nuptial coloration, such as *C. trichroistia* (Burkhead and Jelks 2001), a species that shows downstream decline in the Conasauga River. Elevated sedimentation can lead to changes in fish communities from those that need clean gravel or crevices for spawning to those tolerant of high sediment levels (Sutherland et al. 2003). In the Etowah River system, homogenization of fish communities (i.e., as a decline in endemic: cosmopolitan species ratios) occurs with increasing turbidity and fining of bed sediments (Walters et al. 2003a).

Other, unmeasured, changes may also impose stresses on river biota in the lower portion of the study reach. Stormflow sediment transport, and contaminant loading from agricultural lands and local urbanization, may increase in the lower portion of the study reach. Shoal habitat is also less numerous and further isolated from other shoal habitat in the lower Conasauga, which could limit fish dispersal and recolonization following disturbance, especially when compounded with human disturbances in the river. Local population stability of *P. ceratophyllum* may depend on vegetative growth, as seed production is low compared to other plants within the Podostemaceae family (mean of 12 per seed capsule, with only 39 capsules per plant) and dispersal ability is unknown but assumed to be low (Philbrick and Novelo 1997). It has been suggested that dispersal occurs via downstream movement with water and with birds carrying seeds upstream (Philbrick and Novelo 1997). Given limited dispersal ability, *P. ceratophyllum* may also be slow to recover following disturbances such as sediment scour or pulses of herbicides.

These results provide a baseline for further study in the Conasauga River aimed at species conservation. These results also suggest that, whereas benthic fishes may frequently occur with *P. ceratophyllum* (Etnier and Starnes 1993, Marcinek 2003, Hagler 2006), and may even reflect behavioral choices by the fish (Chapter 3), fish species persistence in a shoal is more strongly driven by other factors. In any case, declines in lotic species richness and *P. ceratophyllum* may respond similarly to anthropogenic stresses.

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Table 1. Physical characteristics of recorded shoals in each section of the Conasauga River, including number of shoals within each section, average distance between shoals (m), average length of shoals (m) and average slope (cm/m) of shoals. Average values of distance between shoals and length include all shoals in the sampled reach; average slope only includes 5 randomly selected shoals within each section. Error values are 95% confidence intervals. Sections are listed from most upstream reach to most downstream reach.

Section	**Number of shoals**	**Average distance between shoals (m)**	**Average length (m)**	**Average Slope (cm/m)**
1	48	199.7 (± 56.2)	134.7 (± 37.3)	0.855 (± 0.39)
2	33	292.3 (± 77.1)	117.6 (± 34.4)	0.257 (± 0.12)
3	34	454.8 (± 157.4)	64.2 (± 13.3)	0.155 (± 0.08)
4	15	949.3 (± 690.8)	62.8 (± 29.6)	0.153 (± 0.08)

Table 2. Logistic regression models of *P. ceratophyllum* cover $\geq$ 50% that were within one-eighth of the top model weight. Models included up to seven parameters: canopy, the average percent canopy cover over the entire shoal; med particle size, the average particle size (mm) within each sample; sq KM, drainage area (km^2) at the sampled shoal; location in stream, a binomial parameter with 0 equaling sites in the outer 25 % of the channel and 1 equaling sites in the center 50% of the channel; slope, gradient measured over the length of the shoal; row crop, the percent land cover in the watershed with row crop agriculture; and local buffer agriculture, the percent agricultural land cover within a 100 m buffer one kilometer upstream of the sample location.

Model parameters	**Number of parameters**	**Δ AIC**	**relative likelihood**	**$w_{(i)}$**
canopy, med particle size, sq KM, location in stream	6	0	1.000	0.211
canopy, med particle size, sq KM, location in stream, row crop	7	0.9	0.638	0.135
med particle size, sq KM, location in stream	5	1.4	0.497	0.105
canopy, med particle size, location in stream	5	1.8	0.407	0.086
canopy, med particle size, sq KM, location in stream, slope	7	1.9	0.387	0.082
canopy, med. particle size, sq KM, location in stream, local buffer agriculture	7	2.4	0.301	0.054
canopy, med particle size, location in stream, row crop	6	2.7	0.259	0.055
canopy, med particle size, location in stream, row crop, slope, sq KM	8	2.9	0.235	0.050
canopy, med particle size, location in stream, slope	6	3.1	0.212	0.045
med particle size, sq KM, location in stream, slope	6	3.1	0.212	0.045
med particle size, sq KM, location in stream, row crop	6	3.1	0.212	0.045
med particle size, location in stream, slope, canopy	5	3.6	0.165	0.035
med particle size, sq KM, location in stream, local buffer agriculture	6	3.7	0.157	0.028

Table 3. Model-averaged parameter estimates and odds ratios with 95% confidence limits for logistic regression models of *P. ceratophyllum* cover $\geq$ 50%. Odds rations > 1 indicate a positive effect of the variable and < 1 indicate a negative effect of the variable.

Variable	Estimate	SE	Units for odds ratio	Odds ratio	95% Confidence Limits Lower	Upper
Point of observation						
Location in channel	1.079	0.233	Center	2.940	1.863	4.642
Sediment size	0.003	0.0004	10 cm	1.323	1.222	1.432
Shoal characteristics						
Canopy cover	-0.024	0.029	1%	0.976	0.922	1.033
Slope	0.121	0.362	1%	1.129	0.555	2.296
Landscape						
Drainage area	-0.004	0.002	50 km^2	0.809	0.642	1.019
Agriculture in buffer	-0.586	0.809	1%	0.557	0.114	2.716
Row crop land use	0.963	0.659	1%	2.619	0.720	9.528

Table 4. Observed species at each shoal sampled, with columns arranged from furthest upstream shoal to the furthest downstream shoal. Fishes are listed according to shoal occupancy with fishes caught only upstream in the top rows, fishes caught throughout the mainstem in the center rows and fishes only collected at the downstream shoals in the bottom rows. Richness is expressed as the total number of species observed and estimated species richness (standard error) at each shoal. * = lotic species.

	River kilometer																				
Species	75.4	75.3	71.2	67.3	66	63.2	62.7	61.6	60.7	58.7	56.2	55	53.9	50.7	46.9	44.75	44.7	42.8	41.1	40.7	36.4
*Etheostoma brevirostru	X	X	X	X																	
*Ambloplites arriomus		X																			
*Percina palmaris	X	X	X	X	X	X	X	X	X	X		X									
*Percina sp. cf. macrocephala		X	X	X	X			X	X	X	X	X	X								
Aplodinotus grunniens	X		X						X			X									
*Micropterus coosae	X		X	X	X	X	X	X	X	X		X			X						
*Notropis xaenocephalus	X	X	X	X	X	X	X	X		X	X	X	X					X			
*Cyprinella trichroistia	X	X	X	X	X	X	X	X	X	X		X	X								
*Campostoma oligolepis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
*Hypentelium etowanum	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
*Cyprinella callistia	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
*Cottus carolinae	X	X		X	X	X	X	X	X	X				X	X	X	X		X	X	X
*Cyprinella caerulea		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
*Etheostoma coosae	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
*Moxostoma duquesnei	X	X		X										X	X	X	X	X	X		X
*Etheostoma jordani	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X		X	X
*Percina nigrofasciata			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
*Phenacobius catostomus			X	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X
Micropterus salmoides	X		X	X	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X
*Percina kathae	X		X	X				X	X		X							X		X	X
Lepomis megalotis	X	X	X	X	X			X	X	X			X		X		X	X	X	X	X
Lepomis auritus	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X			
*Etheostoma rupestre			X			X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Micropterus punctulatus				X	X				X	X				X	X	X	X	X			
*Cyprinella venusta				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
*Notropis stilbius				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
*Etheostoma stigmaeum				X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lepomis macrochirus					X		X			X			X				X	X	X		
*Luxilus chrysocephalus					X		X	X	X	X	X	X		X	X	X	X	X	X	X	
*Fundulus stellifer					X				X						X	X					
*Percina antesella						X	X	X				X	X	X							
*Macrhybopsis aestivalis						X									X						
Moxostoma sp.								X	X			X									X
Pimephales vigilax									X	X	X	X	X	X	X	X	X	X			
Percina sp.										X											
*Etheostoma trisella											X		X	X	X	X					
Gambusia sp														X		X	X				
Pomoxis nigromaculatus															X						
Moxostoma poecilurum															X		X	X			X
Lepomis punctatus															X						
*Moxostoma carinatum																		X			
*Fundulus olivaceous																			X		
Ictalurus punctatus																			X		
Lepomis microlophus																			X		
Total number observed	17	16	20	24	23	20	21	25	26	26	19	23	22	21	27	21	23	24	18	16	17
Estimated species richness	17 (1.73)	17 (2.12)	20 (1.94)	24 (1.73)	26 (1.22)	22 (2.45)	22 (2.47)	27 (1.85)	28 (2.45)	27 (2.12)	20 (1.5)	23 (2.12)	28 (3.46)	21 (1.94)	31 (3)	22 (2.12)	24 (2.12)	24 (obs)	20 (2.29)	16 (obs)	18 (2.12)

Table 5. Probability of shoal occupancy (Psi) for lotic fish species modeled: as a constant across shoals, Psi(.), indicating a constant probability of occurrence across all shoals; as a function of river kilometer, Psi(RKm); or as a function of *P. ceratophyllum* coverage, Psi(Pod). Probability of detection (p) was modeled as a constant across all shoals in all models. Relative support (based on AIC values and model weights) was compared across the three models for each species; parameters in bold have the highest support.

	Psi(.)p(.)				Psi(RKm)p(.)					Psi(Pod)P(.)				
Species	Psi	p	² AIC	w_i	Psi	p	² AIC	w_i	direction of response	Psi	p	² AIC	w_i	direction of response
Etheostoma trisella	**0.412**	**0.551**	**0**	**0.553**	0.429	0.537	1.750	0.230		0.409	0.554	1.870	0.217	
Moxostoma duquesnei	**0.566**	**0.624**	**0**	**0.422**	0.554	0.644	0.015	0.419		0.567	0.624	1.952	0.159	
Fundulus stellifer	**0.319**	**0.387**	**0**	**0.531**	0.330	0.378	1.800	0.216		0.325	0.382	1.480	0.253	
Cottus carolinae	**0.867**	**0.691**	**0**	**0.568**	0.868	0.691	1.870	0.223		0.867	0.681	1.994	0.209	
Percina kathae	**0.571**	**0.500**	**0**	**0.484**	0.568	0.504	1.865	0.191		0.559	0.512	0.795	0.325	
Percina palmaris	0.530	0.948	22.267	0.000	**0.524**	**0.954**	**0**	**0.994**	**(+)**	0.526	0.952	10.230	0.006	
Percina antesella	0.297	0.649	1.017	0.305	**0.284**	**0.663**	**0**	**0.507**	**(+)**	0.302	0.642	1.978	0.188	
Notropis xaenocephalus	0.641	0.856	10.637	0.005	**0.628**	**0.868**	**0**	**0.988**	**(+)**	0.637	0.859	9.930	0.007	
Cyprinella tricroistia	0.646	0.718	18.750	0.000	**0.581**	**0.778**	**0**	**0.996**	**(+)**	0.600	0.761	10.937	0.004	
Micropterus coosae	0.507	0.811	5.230	0.055	**0.513**	**0.791**	**0**	**0.750**	**(+)**	0.507	0.806	2.691	0.195	
Percina sp. cf. macrocephala	0.530	0.735	10.720	0.005	**0.619**	**0.615**	**0**	**0.990**	**(+)**	0.565	0.685	10.346	0.006	
Etheostoma coosae	0.913	0.818	12.981	0.002	**0.857**	**0.857**	**0**	**0.998**	**(+)**	0.909	0.820	14.828	0.001	
Phenacobius catostomus	0.818	0.896	4.411	0.060	**0.814**	**0.901**	**0**	**0.544**	**(-)**	0.819	0.895	0.634	0.396	
Etheostoma stigmaeum	0.813	0.933	14.190	0.001	**0.810**	**0.937**	**0**	**0.976**	**(-)**	0.817	0.929	7.520	0.023	
Etheostoma jordani	0.912	0.909	1.330	0.278	**0.907**	**0.914**	**0**	**0.541**	**(-)**	0.911	0.910	2.200	0.180	
Etheostoma rupestre	0.781	0.844	3.370	0.126	**0.816**	**0.806**	**0**	**0.681**	**(-)**	0.780	0.845	2.530	0.192	
Luxilus chrysocephalus	0.787	0.787	3.616	0.141	**0.683**	**0.907**	**0**	**0.859**	**(-)**	-	-	-		
Percina nigrofasciata	0.906	0.971	11.180	0.003	**0.905**	**0.972**	**0**	**0.876**	**(-)**	0.905	0.972	3.960	0.121	
Cyprinella caerulea	0.873	0.866	4.032	0.076	0.906	0.832	0.990	0.350		**0.878**	**0.861**	**0**	**0.574**	**(-)**
Ambloplites ariommus[1]	-	-	-	-	-	-	-	-		-	-	-	-	
Macrhybopsis sp. cf. M. aestivalis[1]		-	-	-	-	-	-	-		-	-	-	-	
Moxostoma carinatum[1]	-	-	-	-	-	-	-	-		-	-	-	-	
Fundulus olivaceus[1]	-	-	-	-	-	-	-	-		-	-	-	-	
Cyprinella callistia[2]	-	-	-	-	-	-	-	-		-	-	-	-	
Campostoma oligolepis[2]	-	-	-	-	-	-	-	-		-	-	-	-	
*Cyprinella venusta**													-	

1= species that were seen too rarely to estimate detection or occupancy
2= species that were collected at all 21 shoals
+' = more likely to be upstream
-' = more likely to be downstream
*= models did not reach convergence

Figure 1. Location of all recorded shoals in the Conasauga River mainstem study reach. Locations sampled for *P. ceratophyllum* and fishes within each of the four sections are highlighted in red.

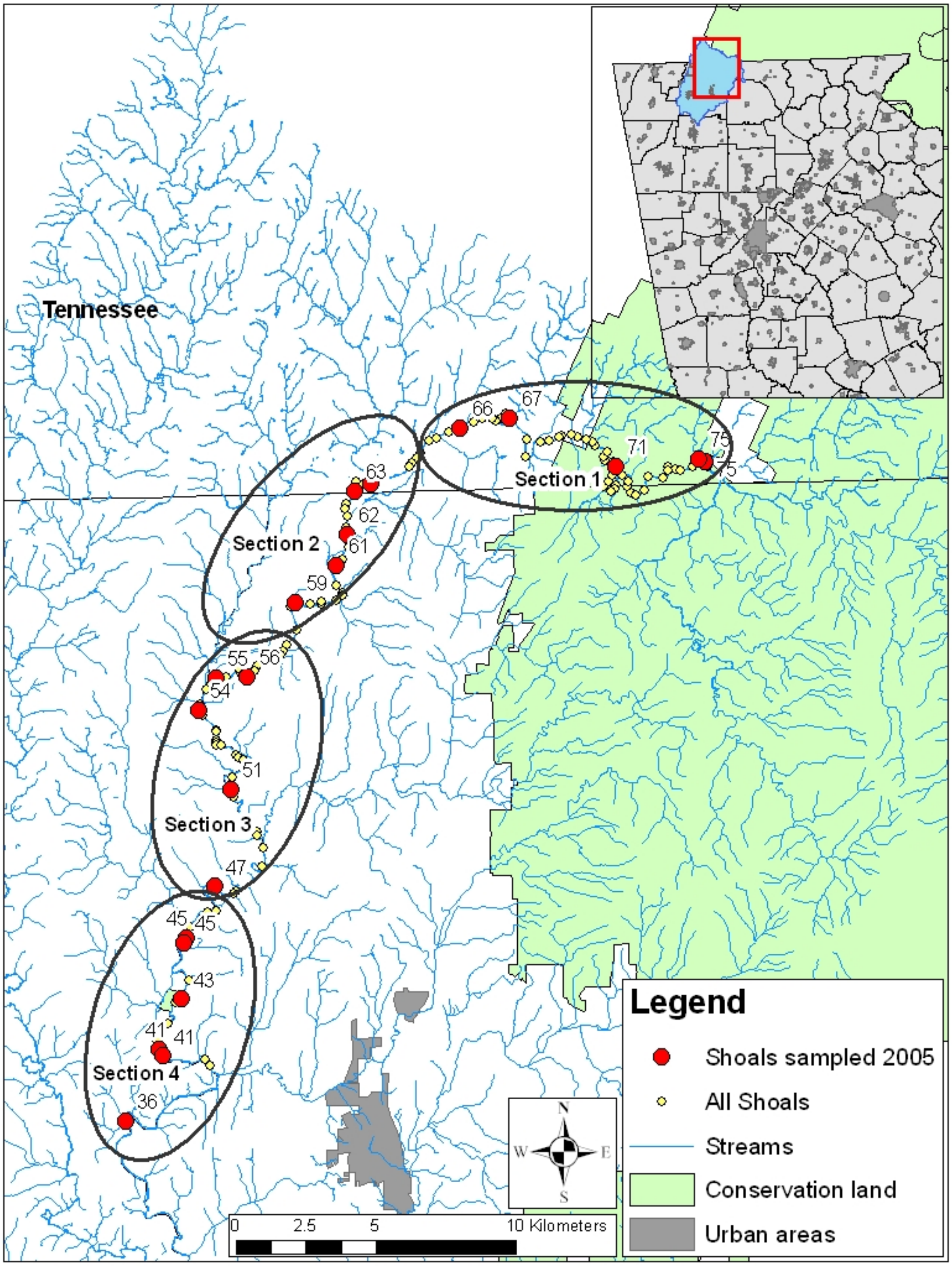
Tennessee
Section 1
Section 2
Section 3
Section 4
66
67
71
75
75
63
62
61
59
55
56
54
51
47
45
45
43
41
41
36
Legend
Shoals sampled 2005
All Shoals
Streams
Conservation land
Urban areas
N
S
W
E
0
2.5
5
10 Kilometers

Figure 2. Total ash-free dry mass of *P. ceratophyllum* (ln transform) in a 0.093 m^2 (1 ft^2) frame in relation to increasing plant coverage; data are for samples collected at three shoals.

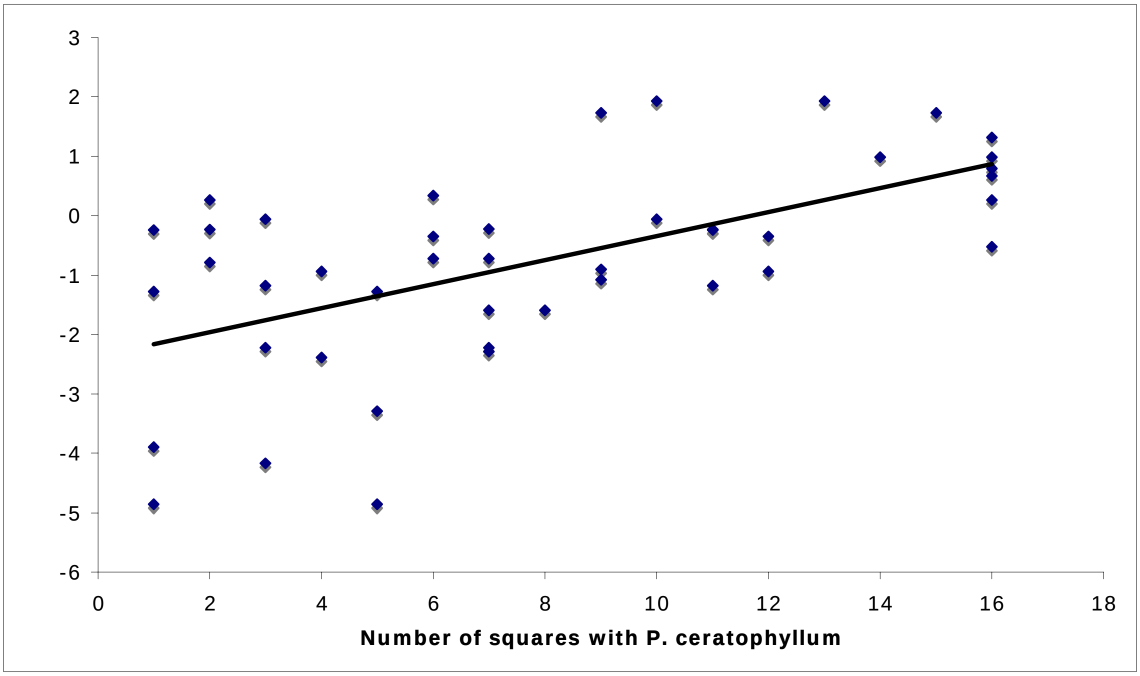
3
2
1
0
-1
-2
-3
-4
-5
-6
0
2
4
6
8
10
12
14
16
18
Number of squares with P. ceratophyllum

Figure 3. Change in average percent *P. ceratophyllum* coverage in the downstream direction. X-axis is arranged from the most upstream point to the most downstream point sampled in the Conasauga River. $r^2 = 0.46$.

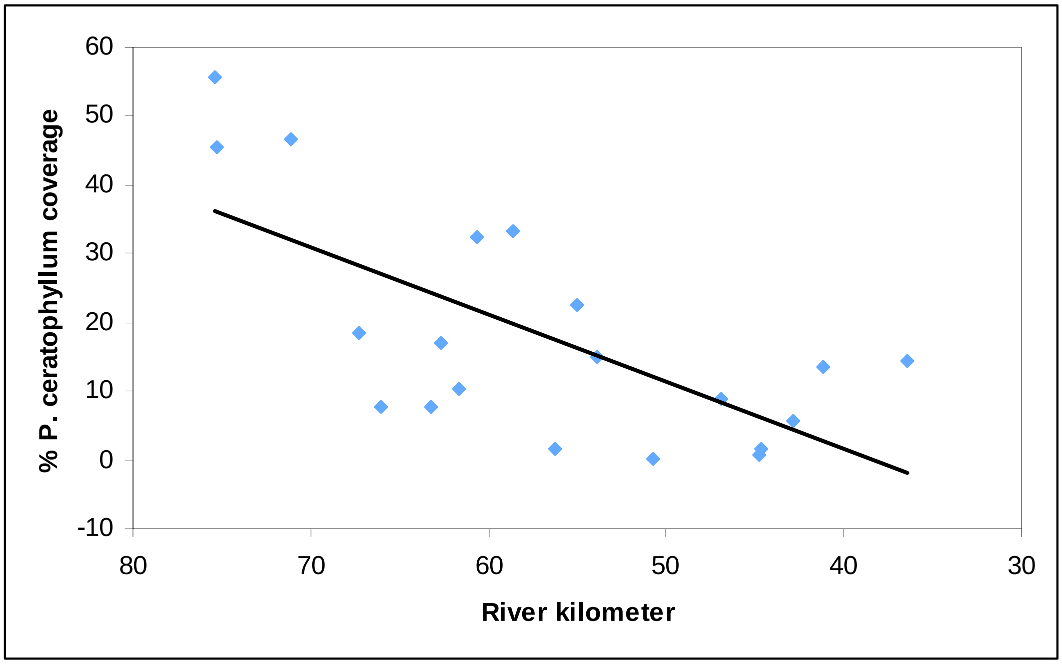
60
50
40
30
20
10
0
-10
% P. ceratophyllum coverage
80
70
60
50
40
30
River kilometer

Figure 4. Estimated species richness for lotic fishes (white bars) and all fish species (grey bars) at shoals along the mainstem of the Conasauga River. X axis is river kilometers arranged from the most upstream point (75.4 km from confluence) to the most downstream site (36.4 km from confluence). Total species regression: $y = -0.020x^2 + 2.23x - 35.8$; $r^2 = 0.47$; lotic species regression: $y = -0.015x^2 + 1.72x - 29.4$, $r^2 = 0.50$.

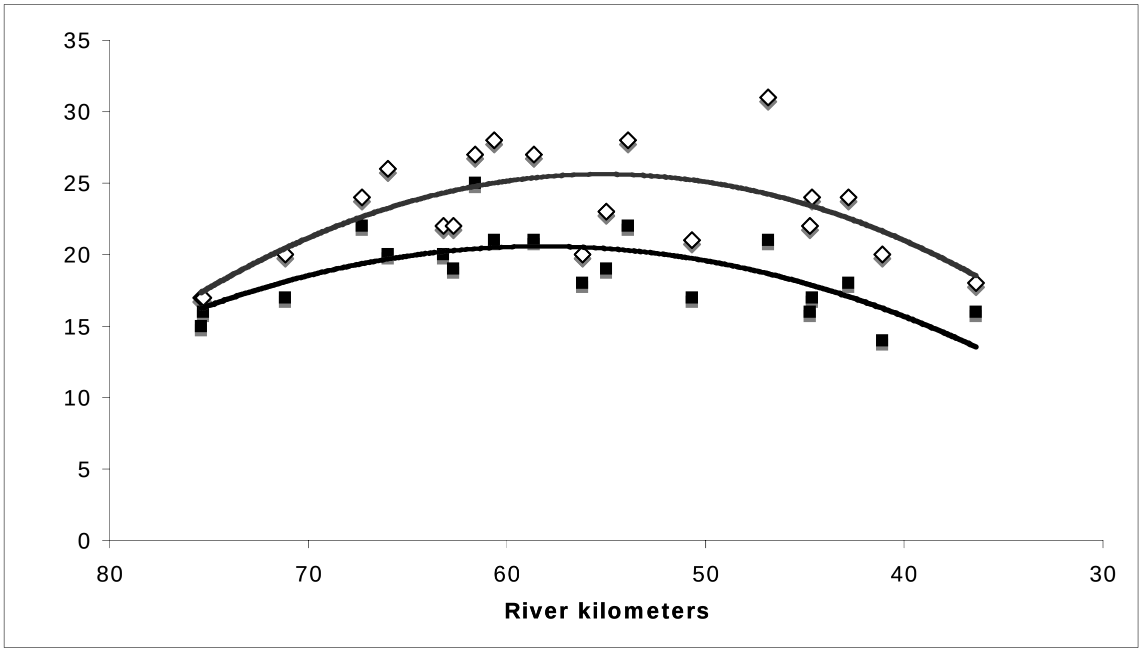
35
30
25
20
15
10
5
0
80
70
60
50
40
30
River kilometers

Figure 5. Change in percent forest in the watershed in the downstream direction along the mainstem of the Conasauga River. X axis is river kilometers arranged from the most upstream point (75.4 km from confluence) to the most downstream site (36.4 km from confluence); % forest = 0.8811(rkm) + 32.531, $r^2 = 0.89$.

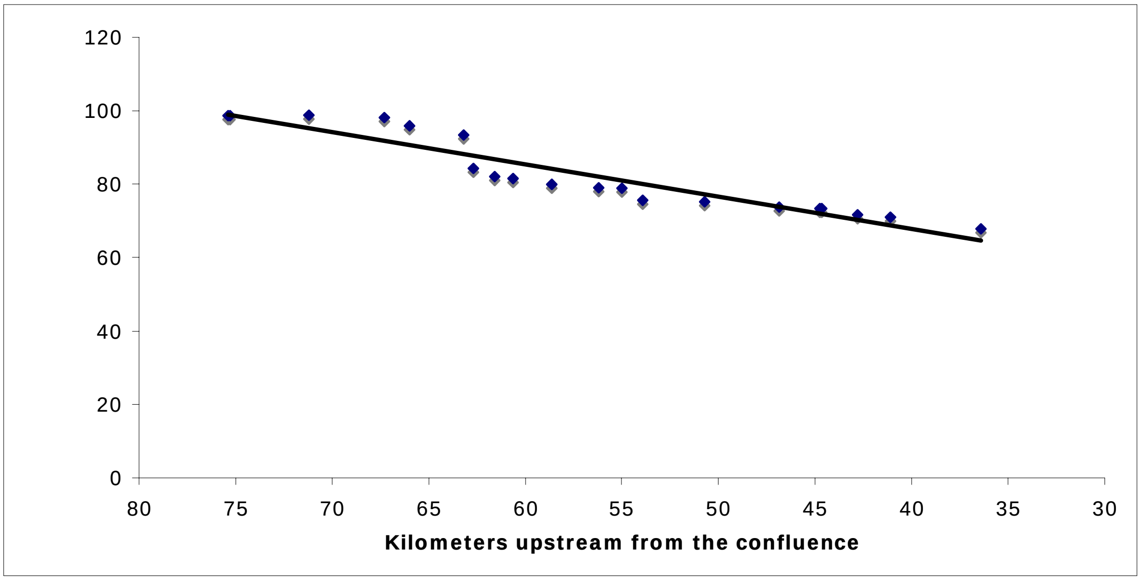
120
100
80
60
40
20
0
80
75
70
65
60
55
50
45
40
35
30
Kilometers upstream from the confluence

Figure 6. Change in baseflow turbidity (NTU) from upstream to downstream along the mainstem of the Conasauga River, 2005. X axis is river kilometers in distance from the mouth of the Conasuaga River; (Turbidity = 0.2804 (distance) + 20.059, $r^2 = 0.88$).

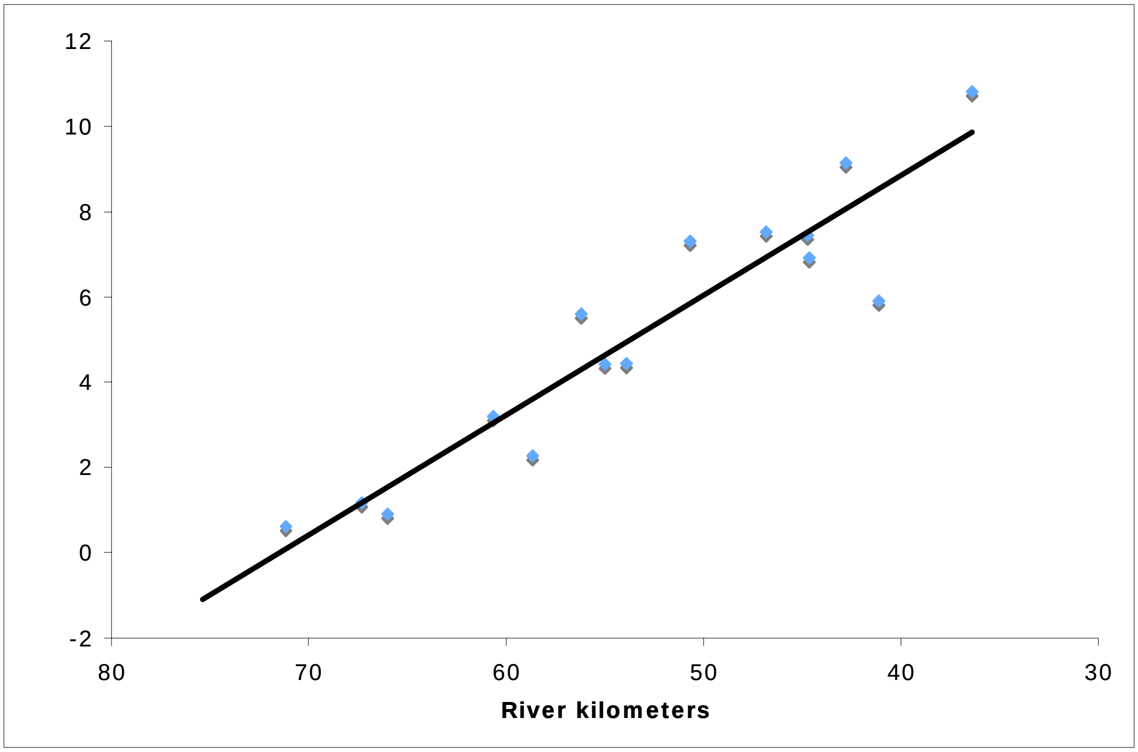
12
10
8
6
4
2
0
-2
80
70
60
50
40
30
River kilometers

Figure 7. Historical and 2005 range of *Cyprinella trichroistia*, tricolor shiner.

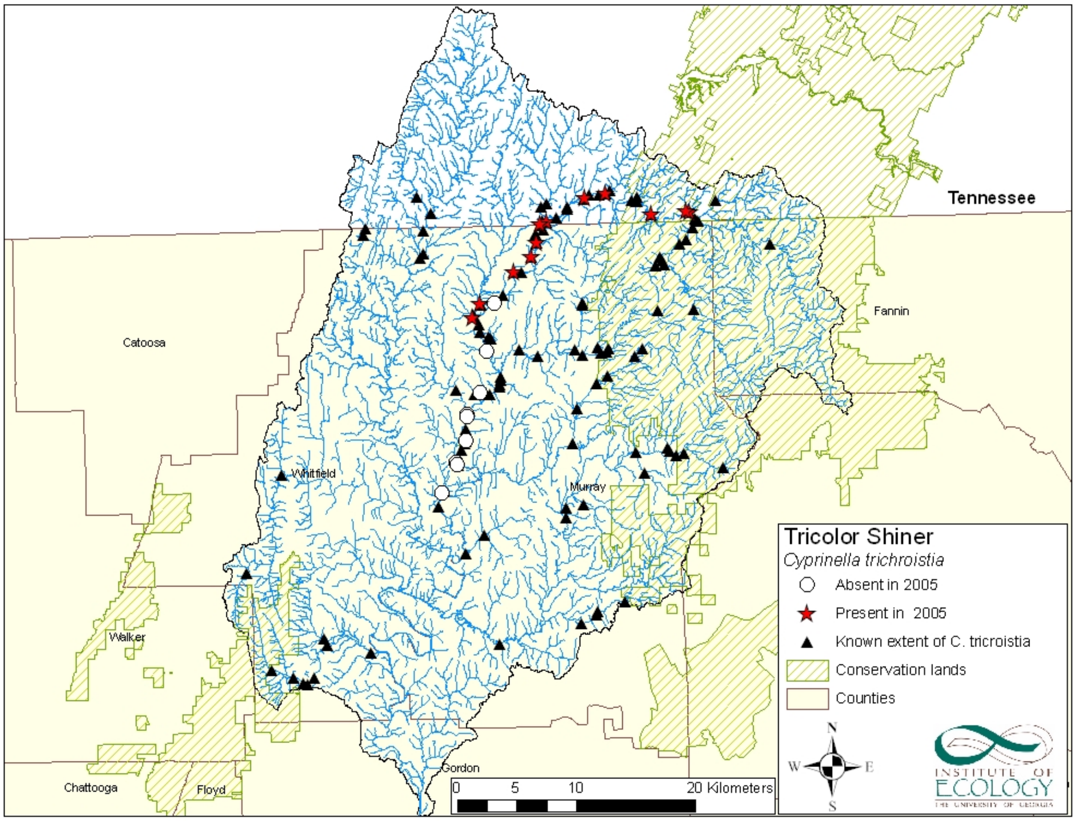
Tennessee
Fannin
Catoosa
Whitfield
Murray
Walker
Chattooga
Floyd
Gordon
0
5
10
20 Kilometers
Tricolor Shiner
Cyprinella trichroistia
○ Absent in 2005
★ Present in 2005
▲ Known extent of C. tricroistia
▨ Conservation lands
☐ Counties
N
W
E
S
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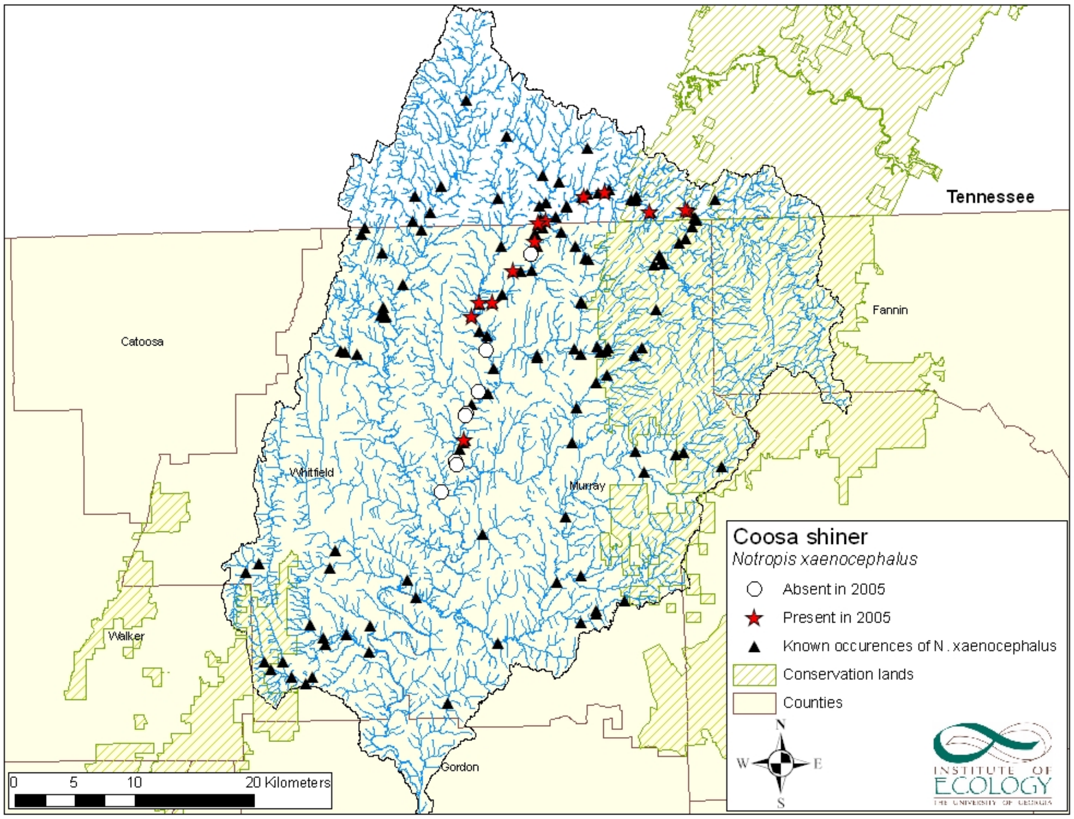
Figure 8. Historical and 2005 range of *Notropis xaenocephalus,* Coosa shiner.


Tennessee
Catoosa
Fannin
Whitfield
Murray
Walker
Gordon
Coosa shiner
Notropis xaenocephalus
○ Absent in 2005
★ Present in 2005
▲ Known occurences of N. xaenocephalus
▨ Conservation lands
☐ Counties
N
W
E
S
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0
5
10
20 Kilometers

Figure 9. Historical and 2005 range of *Micropterus coosae*, redeye bass.

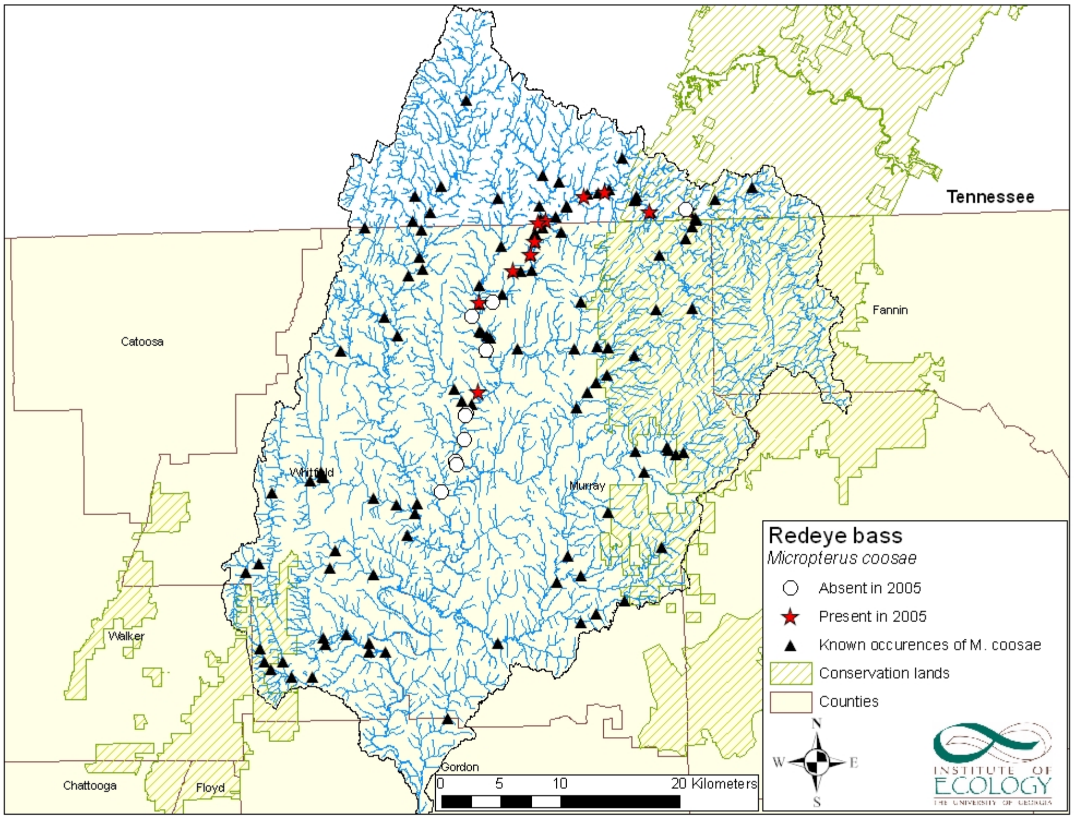
Tennessee
Catoosa
Fannin
Whitfield
Murray
Walker
Gordon
Chattooga
Floyd
Redeye bass
Micropterus coosae
Absent in 2005
Present in 2005
Known occurences of M. coosae
Conservation lands
Counties
0 5 10 20 Kilometers
N
W
E
S
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Figure 10. Historical and 2005 range of *Etheostoma trisella*, trispot darter.

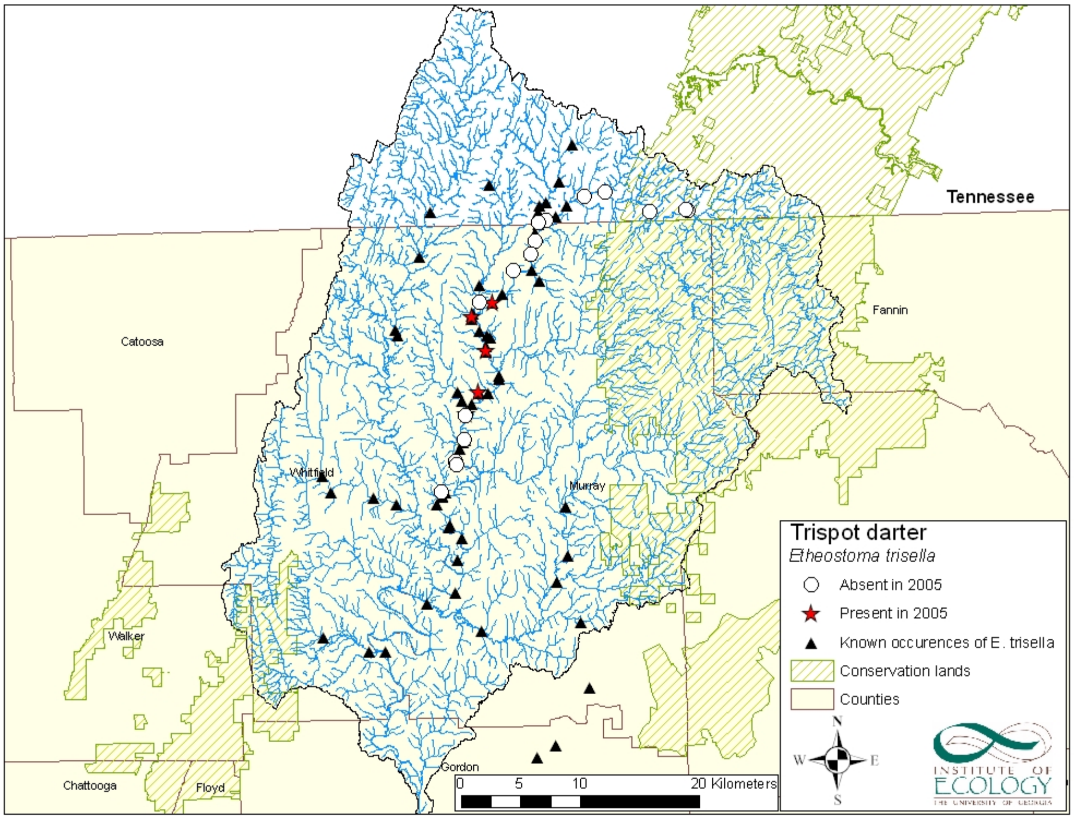
Tennessee
Fannin
Catoosa
Whitfield
Murray
Walker
Chattooga
Floyd
Gordon
Trispot darter
Etheostoma trisella
○ Absent in 2005
★ Present in 2005
▲ Known occurences of E. trisella
▨ Conservation lands
☐ Counties
N
W
E
S
0 5 10 20 Kilometers
INSTITUTE OF ECOLOGY
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Figure 11. Historical and 2005 range of *Percina antesella,* amber darter.

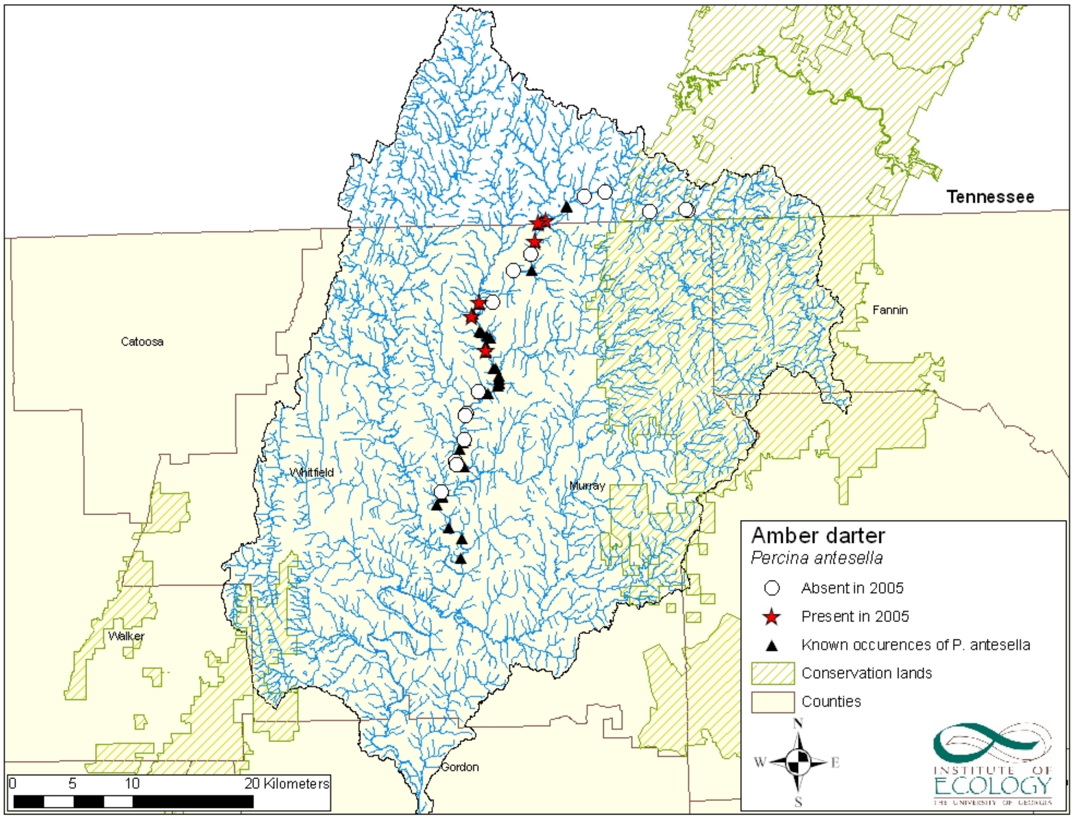
Tennessee
Fannin
Catoosa
Whitfield
Murray
Walker
Gordon
0 5 10 20 Kilometers
Amber darter
Percina antesella
○ Absent in 2005
★ Present in 2005
▲ Known occurences of P. antesella
▨ Conservation lands
☐ Counties
N
W
E
S
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Figure 12. Historical and 2005 range of *Percina* sp. *cf. P. macrocephala*, upland bridled darter.

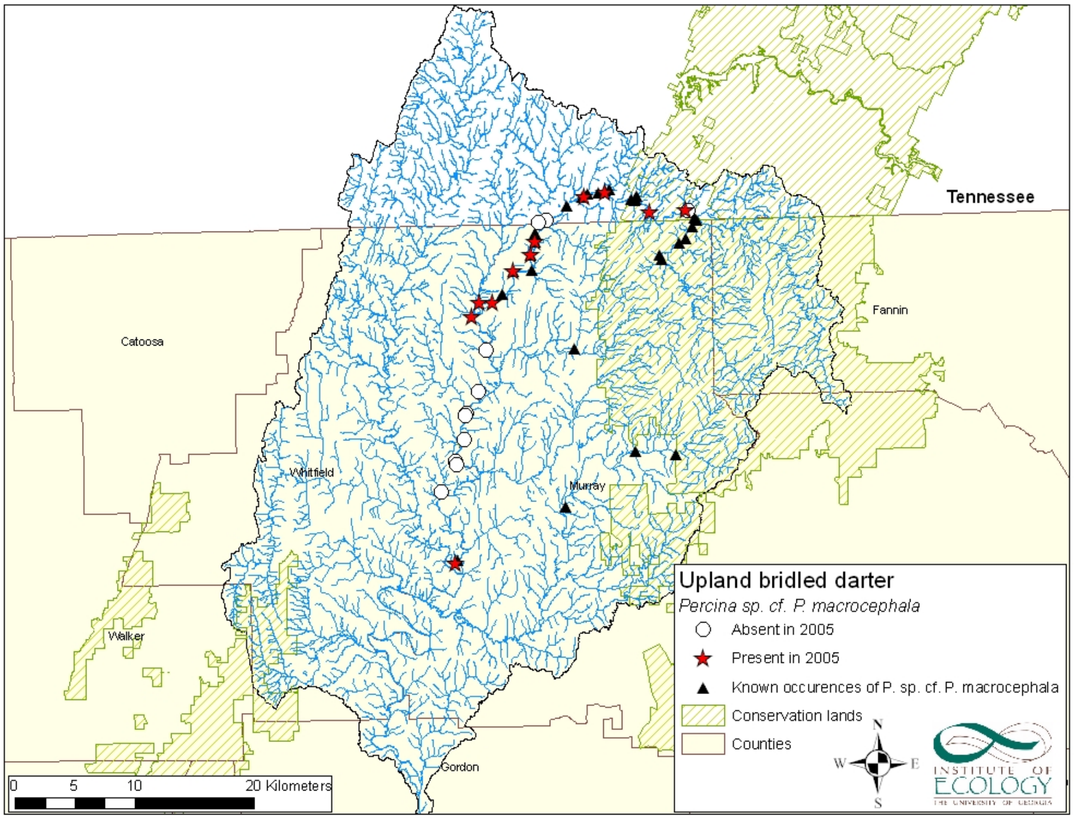
Tennessee
Fannin
Catoosa
Whitfield
Murray
Walker
Gordon
Upland bridled darter
Percina sp. cf. P. macrocephala
Absent in 2005
Present in 2005
Known occurences of P. sp. cf. P. macrocephala
Conservation lands
Counties
0
5
10
20 Kilometers
N
W
E
S
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Figure 13. Historical and 2005 range of *Percina palmaris,* bronze darter.

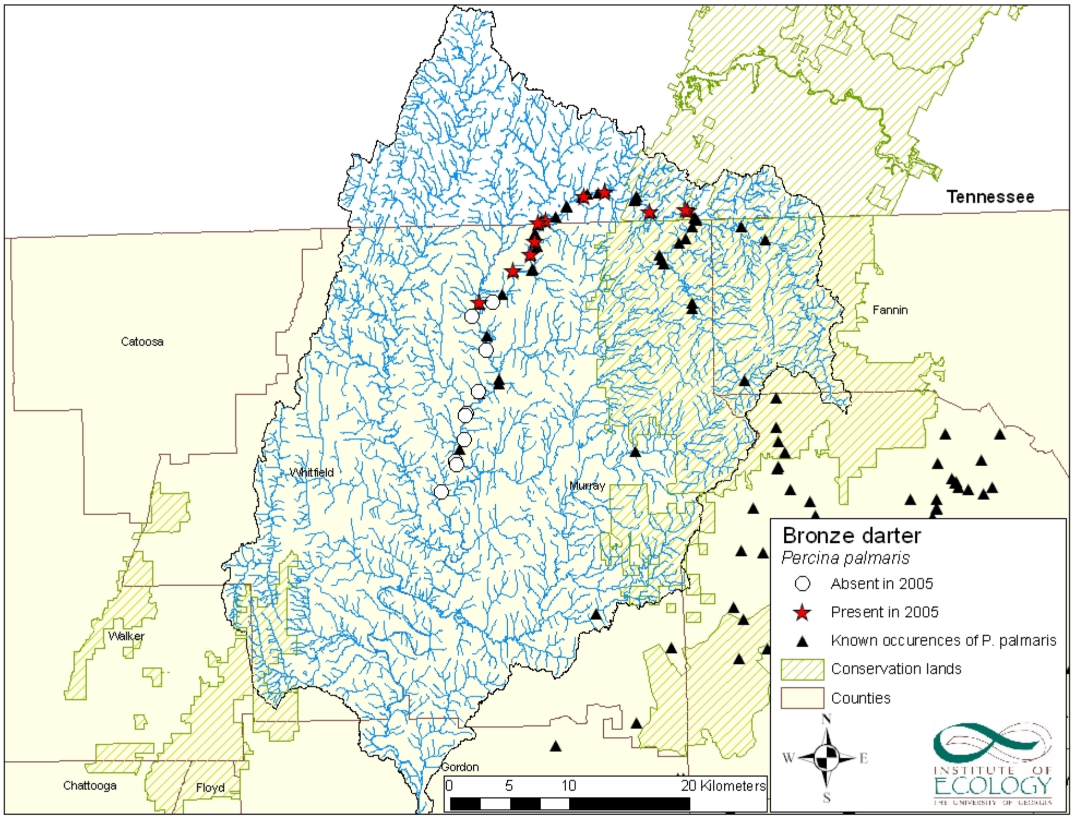
Tennessee
Catoosa
Fannin
Whitfield
Murray
Walker
Gordon
Chattooga
Floyd
0 5 10 20 Kilometers
Bronze darter
Percina palmaris
○ Absent in 2005
★ Present in 2005
▲ Known occurences of P. palmaris
▨ Conservation lands
☐ Counties
N
W
E
S
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Figure 14. Historical and 2005 range of *Moxostoma duquesnei*, black redhorse.

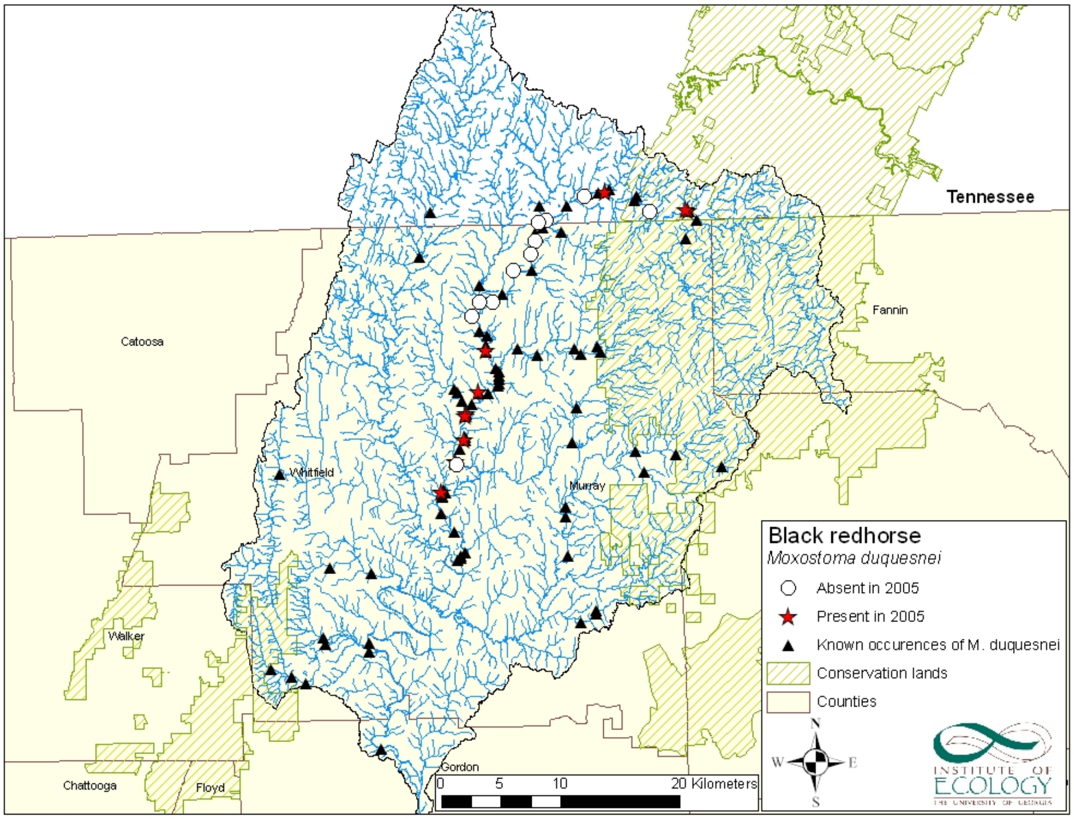
Tennessee
Fannin
Catoosa
Whitfield
Murray
Walker
Chattooga
Floyd
Gordon
Black redhorse
Moxostoma duquesnei
○ Absent in 2005
★ Present in 2005
▲ Known occurences of M. duquesnei
▨ Conservation lands
☐ Counties
N
W
E
S
0 5 10 20 Kilometers
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Figure 15. Historical and 2005 range of *Fundulus stellifer*, southern studfish.

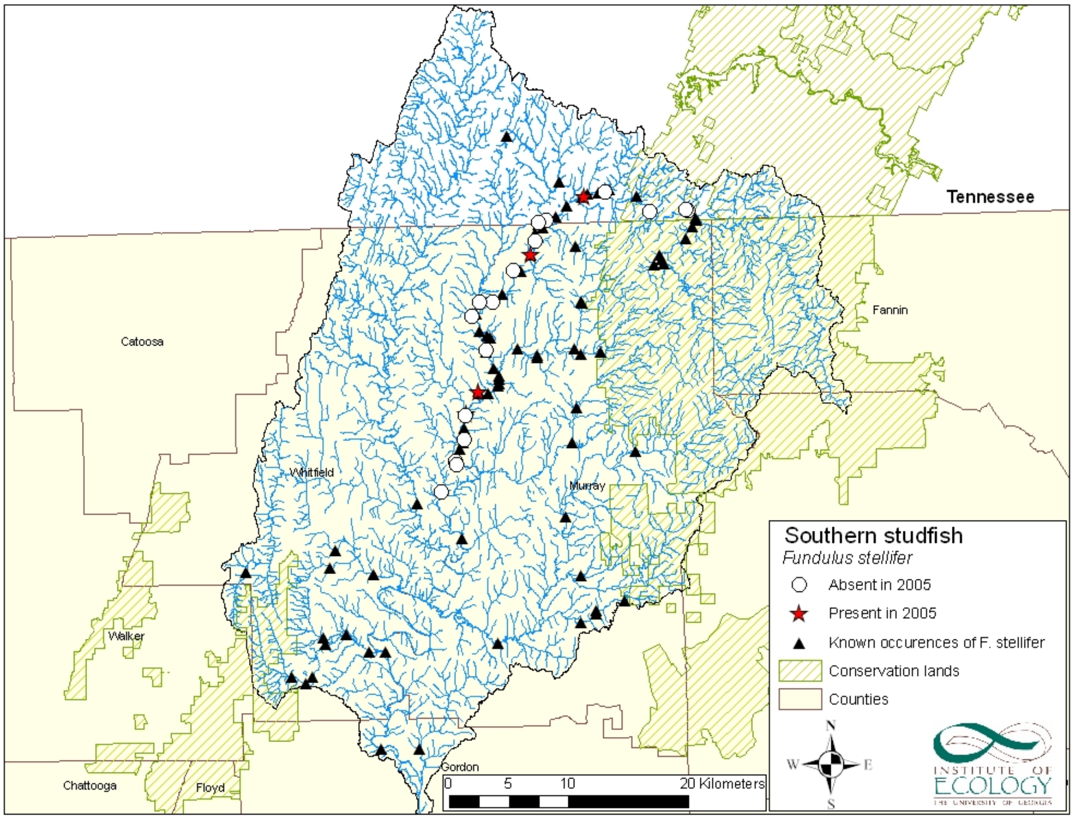
Tennessee
Fannin
Catoosa
Whitfield
Murray
Walker
Chattooga
Floyd
Gordon
0
5
10
20 Kilometers
Southern studfish
Fundulus stellifer
○ Absent in 2005
★ Present in 2005
▲ Known occurences of F. stellifer
▨ Conservation lands
☐ Counties
N
W
E
S
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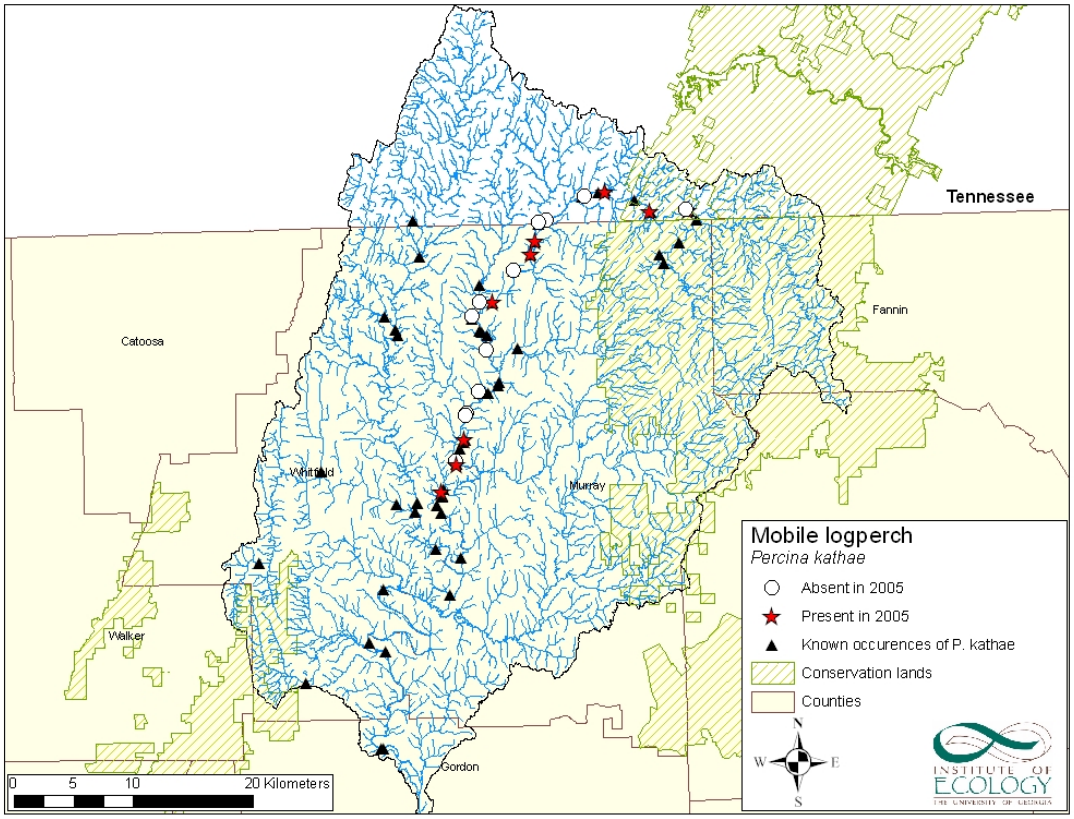
Figure 13. Historical and 2005 range of *Percina kathae*, Mobile logperch.


Tennessee
Fannin
Catoosa
Whitfield
Murray
Walker
Gordon
Mobile logperch
Percina kathae
○ Absent in 2005
★ Present in 2005
▲ Known occurences of P. kathae
▨ Conservation lands
☐ Counties
N
W
E
S
0 5 10 20 Kilometers
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Appendix 1. Lotic species of the Conasauga River and their conservation status according to Warren et al. 2000. CS = currently stable, E = endangered, T = threatened, V = vulnerable, and an asterisk denotes federally protected status.

Species	Common name	Status
Cyprinidae		
Campostoma oligolepis	largescale stoneroller	CS
Cyprinella caerulea	blue shiner	T*
Cyprinella callistia	Alabama shiner	CS
Cyprinella trichroistia	tricolor shiner	CS
Cyprinella venusta	blacktail shiner	CS
Luxilus chrysocephalus	striped shiner	CS
Macrhybopsis sp. cf. *M. aestivalis*	“Coosa chub”	V
Notropis stilbius	silverstripe shiner	CS
Notropis xaenocephalus	Coosa shiner	CS
Phenocobius catostomus	riffle minnow	CS
Catostomidae		
Hypentellium etowanum	Alabama hog sucker	CS
Moxostoma carinatum	river redhorse	CS
Moxostoma duquesnei	black redhorse	CS
Ictaluridae		
Noturus sp. cf. *N. munitus*	“Coosa madtom”	T
Fundulidae		
Fundulus olivaceous	blackspotted topminnow	CS
Fundulus stellifer	southern studfish	CS
Cottidae		
Cottus carolinae zopherus	Coosa banded sculpin	CS
Centrarchidae		
Ambloplites arriomus	shadow bass	CS
Micropterus coosae	redeye bass	CS
Percidae		
Etheostoma sp. cf. *E. brevirostrum*	“Conasauga snubnose darter”	T
Etheostoma coosae	Coosa darter	CS
Etheostoma ditrema	coldwater darter	T

Etheostoma rupestre	rock darter	CS
Etheostoma stigmaeum	speckled darter	CS
Etheostoma trisella	trispot darter	E
Percina antesella	amber darter	E*
Percina jenkinsi	Conasauga logperch	E*
Percina kathae	Mobile logperch	CS
Percina lenticula	freckled darter	T
Percina sp. cf. *P. macrocephala*	“Coosa bridled darter”	V
Percina nigrofasciata	blackbanded darter	CS
Percina palmaris	bronze darter	CS
Percina shumardi	river darter	CS

Chapter 3

Benthic fish response to experimentally manipulated density of an aquatic macrophyte, *Podostemum ceratophyllum*, Michx.

Introduction

Benthic aquatic habitat structure and heterogeneity strongly influence abundances of a broad range of animals in aquatic environments. While some animals specialize on particular size or types of substrate, a diversity of substrate types, particle sizes and organic substrates have been shown to increase the abundance and diversity of invertebrates (Rabeni and Minshall 1977, Allan 1995). Substrates such as woody debris have been shown to enhance habitat quality for insects (Anderson et al. 1978, Meyer et al. 1997), salmon (Roni and Quinn 2001), and other fishes (Quist and Guy 2001) in rivers. Aquatic plants are important habitat features for biota inhabiting low velocity areas such as sea grass beds (Heck and Wetstone 1977, Heck and Crowder 1991), lake edges (Werner and Hall 1988), and river margins (Newman 1991, Fritz et al. 2004). Fewer studies have focused on the effects of aquatic plants in flowing systems. We have focused on one aquatic plant in particular, *Podostemum ceratophyllum*, to examine its importance at a local scale as habitat structure in fast-flowing waters for riverine fishes.

P. ceratophyllum (Podostemaceae) is a completely submerged filamentous dicotyledon without true roots. It characteristically occurs in fast flowing waters near the center of the channel in riffles and shoals where it attaches to the surface of rocks with hyphae. It occurs in the United States primarily along the east coast, from Louisiana through Maine, and as far west as North Dakota and Oklahoma (Philbrick and Crow

1983). Populations also exist in Honduras, Dominican Republic and Nova Scotia, New Brunswick and Québec in Canada (USDA 2006).

Aquatic macrophytes can have large effects on the physical features of streams, including stream flow and sedimentation rates. Within stream macrophyte beds, flow can be altered depending on the physical structure of the plant and channel stream velocity (Madsen et al. 2001, Sand-Jensen 1998). *P. ceratophyllum* similarly alters the physical structure of shoals and riffles by changing flow regimes (Grubaugh and Wallace 1995), which can change sedimentation rates, organic deposition rates, and nutrient concentrations in the sediments. Current velocities within *P. ceratophyllum* beds can be decreased by more than 50% compared to flow above the plant beds in a Piedmont stream (Grubaugh and Wallace 1995). Sediment transport downstream can be slowed by *P. ceratophyllum* by two mechanisms: through increased sedimentation rates and/or by stabilizing the river bed substrate with hyphae, slowing the rate of downstream bed migration. Water velocity is slowed in macrophyte beds causing suspended sediment to be deposited, which in turn decreases turbidity downstream (Madsen et al. 2001). These deposited particles can be high in organic matter and total phosphorus in low velocity streams (Sand-Jensen 1998). Sediments can also become trapped in the stem and leaves of *P. ceratophyllum*, which can decrease the movement of fine particles downstream (Nelson and Scott 1962). The root structures of the plant may also act to stabilize the bed sediments as hyphae attach to sand, gravel and cobble, decreasing the likelihood that the sediments will move downstream during periods of high flow. Detritus settling out of the water column in *P. ceratophyllum* beds may increase food availability to macroinvertebrate gatherers. Epiphytes on aquatic plants are an important food source for aquatic macroinvertebrate grazers. Epiphytes can contribute up to 50% of total production in beds of some aquatic macrophytes (Cattaneo and Kalff 1980). The surface area of a benthic bedrock habitat is increased by 3-4X in tributaries to the Little Tennessee River (Hutchens et al. 2004), which can substantially increase area for epiphytic growth, and for the attachment of filter feeders such as Hydropsychidae.

P. ceratophyllum creates heterogeneous habitat in rivers, fundamentally changing the benthic environment of rivers. Increased surface area, reduced scour and increased availability of organic matter and epiphytic periphyton are expected to lead to a response from benthic biota. *P. ceratophyllum* has been shown to increase invertebrate productivity of Piedmont streams (Grubaugh and Wallace 1995); invertebrate biomass and species richness similarly increased in areas with *P. ceratophyllum* compared to open bedrock in Blue Ridge streams (Hutchens et al. 2004). Filter feeders such as Chironomidae and Hydropsychidae are especially dependent on attachment sites created by *P. ceratophyllum*. Benthic insects are a primary food source in streams and rivers for fish. The majority of fishes in eastern (Goldstein and Meador 2004) and central U.S. rivers (Horitz 1978). In this overview of central U.S. stream fish (183 species), one third of the fish species were classified as benthic insectivores, another 22 percent fed on insects in the water column, while few fish species fed on detritus and plant material (less than 7%) (Horwitz 1978).

Many fishes are associated with *P. ceratophyllum* at a local scale, but the extent to which these associations are obligatory is unknown. For example, the spotfin chub, *Erimonax monachus,* has been seen to spawn in crevices in bedrock and boulders covered by *P. ceratophyllum* in the Little Tennessee River (Sutherland 2005). The riverweed darter, *Etheostoma podostemone*, was described as a close associate with *P. ceratophyllum* in the Roanoke River in Virginia (Connelly et al. 1999). In the Etowah River, Georgia, *Noturus munitus* is known to nest under cobbles covered with *P. ceratophyllum* (Etnier and Starnes 1993). At least six benthic fish species show positive associations with *P. ceratophyllum* in the Etowah River, GA (Hagler 2006). One hypothesis for the association between benthic fishes and *P. ceratophyllum* is fish preference for sites with increased food availability. However, we know of no experimental evidence for stream fish affinities for *P. ceratophyllum*.

Understanding the importance of *P. ceratophyllum* to stream fishes is of interest in part because *P. ceratophyllum* has shown widespread population declines across its range. The species is listed as historical in Rhode Island, endangered in Ohio, threatened in New York and as a species of special concern in Massachusetts, Maine, Kentucky and Connecticut. One study in Pennsylvania has shown population numbers of less than 30 distinct patches, with only a few large populations (Munch 1993). Widespread *P. ceratophyllum* loss can have a long-term effect on the survival of *P. ceratophyllum*, because most growth is vegetative, and seed and seedling production is low (Philbrick and Novelo 1997). Population losses have been attributed to habitat loss, decreased water quality, including from strip mining and acidification, and flow alteration (Philbrick and Crow 1983, Meijer 1976, Munch, 1993).

Bottom-dwelling fishes may use *P. ceratophyllum* as a refuge from predators, a refuge from swift currents or as a source of prey. Reduced flow could decrease energy expenditure by insects and fishes foraging in or near *P. ceratophyllum*, possibly decreasing the food requirements per day. There is also the possibility, however, that fishes in shoals with *P. ceratophyllum* would prefer areas lacking the plant, given the choice. For example, *P. ceratophyllum* could actually increase refuge for invertebrate prey and reduce foraging efficiency by benthic fishes. The purpose of this study has been to investigate the behavioral response of fishes to manipulation of *P. ceratophyllum* in a southeastern U.S. river to address this question: Do observed species' affinities for *P. ceratophyllum* reflect preference? Specifically, we have tested two hypotheses, against the null hypothesis that fishes will have no preference for habitat in which *P. ceratophyllum* has been added or removed:

1. An increase in *P. ceratophyllum* will lead to increased abundance and biomass of aquatic insects, while removal will lead to a similar decline.
2. Benthic insectivorous fishes will increase in areas where *P. ceratophyllum* cover is increased, and decrease where it is removed.

Results provide insight into potential mechanisms underlying use of macrophytes by benthic stream fishes.

Study site

The study was conducted at the first shoal upstream of Gregory Mill Bridge (River kilometer 59.90) in the Conasauga River, a 6th order tributary of the Oostanaula River, in Murray County, Georgia. The stream bed slope is moderate, and gravel and cobble dominate the bed sediments. The shoal was approximately 40 long and 10-20 m wide. The approximately 30 m wide riparian zone on either side of the shoal was forested, although the surrounding area is dominated by row crop agriculture. Both experimental areas were at this shoal, with experiment one approximately 15 m upstream of experiment two. There is a small, wooded island in the middle, which divides the flow into two channels. Experiment one took place in the river left channel alongside the island, and experiment two took place just downstream of the island.

Methods

To determine if there is a behavioral response due to preference for habitat with or without *P. ceratophyllum*, we manipulated the density of *P. ceratophyllum* in two areas measuring approximately 30 m^2. Both experiments took place in October 2005. The initial sampling/manipulation and resample occurred two weeks apart at each site, with the same protocol performed each time, for a total of four fish and aquatic insect sampling occasions.

To ensure habitat manipulation only changed *P. ceratophyllum* density and not bed sediment size or mean depth and velocity, we measured these parameters before manipulating the study area and after two weeks, before the second fish sampling occasion, generally after the nets were placed in the experimental area so no fishes could not escape the sample area. Depth was measured with a wading-rod and velocity was measured at 60% water column depth using a Marsh-McBirney Flo-mate 2000 at 5 regularly spaced locations within the center of each half of the experimental area. We used a 0.09 m^2 (1 ft^2) PVC frame subsampler subdivided into sixteen 7.62 cm x 7.62 cm (3” x 3”) squares to estimate *P. ceratophyllum* cover and bed sediment size. At nine evenly spaced locations in each half of the experimental area, we measured the medial axis of two sediment particles, and we counted the number of squares within each frame that contained *P. ceratophyllum* attached to the bed sediments. Sediment size, depth and velocity were compared between sides, pre- and post manipulation using Student’s t-test assuming unequal variances.

To enclose the fish sampling area for a closed population mark-recapture design, we set up five 6 x 2.5 m block nets. A net blocked each side of the enclosed area, and the fifth created a barrier down the middle of the enclosed area, creating two adjacent blocked-off rectangles. The center block net was sewn to the center of the downstream block net to aid in rapid installation. All nets were set with the lead-lines attached to lengths of rebar that were driven into the bed sediments to ensure the bottom of each net did not shift during the experiments. Large cobbles were used to flatten the bottom of the nets to the river bed, making a barrier to fish movement in and out of the experimental area. We also pulled the top of the nets up and secured them using a tag-line attached to trees on either bank.

We sampled fish on each side of the enclosed area by kick-setting with a 6’ x 8’ seine until thoroughly sampled (10-15 minutes). We then identified, measured standard length and marked captured fish using small fin clips. We marked the caudal fins in the first experiment and pectoral fins in the second experiment to eliminate confusion about when each fish had been previously captured. We clipped opposite fins on fishes captured on each side of the partitioned area so that we could detect escapes through the center block net. We then replaced the fish in the blocked off area from which they were captured and let them redistribute themselves. After a 30 minute acclimation period we resampled for the same amount of time as used initially, again catching as many fish as possible. We identified and measured standard length of each individual, and noted each marked individual. We also checked the block nets at the end of the second sample for dead or trapped fish. Block nets were removed following sampling.

After the initial fish sampling, we randomly selected which side would have *P. ceratophyllum* removed and which would have it added. On the removal side, we collected every rock with *P. ceratophyllum* attached and moved it to the addition side. We replaced the removed rock with a rock of similar size from the addition side to maintain average bed sediment size. We collected additional *P. ceratophyllum* from surrounding shoal habitat to increase coverage in the addition area and also added similarly size cobbles lacking *P. ceratophyllum* to the removal side.

We sampled macroinvertebrates at ten locations, five on the removal and five on the addition area spaced at 1-m intervals, in two experimental areas for a total of twenty pre-manipulation samples and twenty post-manipulation samples. Benthic macroinvertebrates were sampled using a modified PVC T-sampler (English 1987) with a 12.7 cm (five inch) diameter and a 243 micron mesh bag net. Insects were sampled in the top 2.5 cm of sediment and all large rocks were rubbed to dislodge attached macroinvertebrates. All *P. ceratophyllum* in the macroinvertebrate sample area was taken for later examination and to calculate ash-free dry mass. All insects were preserved in 10% formalin, with phloxine B added to aid in sorting and identification. Insects were separated from organic matter in the lab and sorted at 10x magnification. All insects were identified to family, counted and measured for length to the nearest μm. Insect length was converted to ash-free dry mass (AFDM) using published family level length-mass regression equations (Benke et al. 1999). Abundances and biomass were transformed {ln (x)} to normalize distributions and compared using Student’s t-test assuming equal variance.

We used a closed-capture model run in program MARK (White and Burnham 1999) to estimate benthic, insectivorous fish abundances before and after the experimental treatment. Because of unexpected pressure from water flow during the pre-manipulation sampling of the second, the nets were not secured well enough and became dislodged after the first fish sample. Therefore, we were unable to resample the experimental area to get a detection estimate. We used an average detection estimate from the second experiment post-manipulation samples to estimate pre-manipulation fish abundances (i.e., as number caught divided by estimated detection); we did not estimate confidence intervals for these abundance estimates. For the other three occasions, we used profile likelihood confidence intervals for abundances, computed in MARK.

Results

Changes in habitat

We successfully manipulated the density of *P. ceratophyllum*, increasing it to almost complete coverage on the addition side, and almost completely removing all plants on the removal side. Post-manipulation *P. ceratophyllum* coverage on the addition side was similarly higher in both experiments (84% in experiment one and 86% in experiment two) than on the experimental removal sides (7% in both experiments; coverage on addition vs. removal sides $p < 0.01$). *P. ceratophyllum* density prior to manipulation was lower (38%) in experiment one than experiment two (48%).

Discharge decreased throughout the experiment, from 73.8 cubic feet/second (cfs) on the first sampling date (10/08/05), to 55.26 cfs (10/14/05), 46.11 cfs (10/22/05) and 45.77 cfs (10/29/05) on subsequent dates (discharge recorded at USGS gage 2384500, located 17 km downstream from the study site). Despite falling discharge, habitat remained relatively constant between sides and between dates in each experimental area (Table 1). Velocity decreased significantly in experiment one and increased significantly in experiment two (despite decreasing discharge), and depth tended to increase (with decreasing discharge) between dates, but actual changes were small and conditions were similar between addition and removal sides (Table 1). In experimental area two, sediment sizes did not change after we manipulated the *P. ceratophyllum*. Sediment sizes were not measured before *P. ceratophyllum* manipulation in experiment one, but sediment sizes were not significantly different between the sides ($p = 0.38$).

Changes in fishes and aquatic insects

Variation among aquatic insect samples within sampling dates and between experimental areas was high (Table 2). Initial insect mean biomasses and abundances in experimental site two were higher than final mean abundances and biomass in experimental site one. However, the change in abundance and biomass of aquatic insects followed predicted results. In experiment one, aquatic insect biomass and abundance increased in *P. ceratophyllum* addition and decreased with removal (Table 2). Post-manipulation insect biomass was approximately 3X higher with macrophyte addition. In experiment two, aquatic insect mean biomass decreased in both removal and addition sides, although after manipulation the addition side again had significantly higher mean biomass as well as abundance than the removal side (Table 2).

Eight species of benthic, insectivorous fishes were captured across the two experiments (Appendix B). To allow better abundance estimates, I combined counts for all species of darters, madtom and sculpin. Estimated combined benthic fish abundance increased with *P. ceratophyllum* addition and decreased with *P. ceratophyllum* removal in both experiments. Benthic fish abundances were significantly higher in the addition side than in the removal side on the second date (i.e., post-manipulation) in both experiments (Table 3).

Discussion

Previous studies have shown an increase in macroinvertebrate productivity in areas with high *P. ceratophyllum* coverage (Nelson and Scott 1962, Hutchens et al. 2004). In this study, benthic fish abundances increased in both experiments in areas with increased *P. ceratophyllum* coverage and decreased in experiment one with reduced *P. ceratophyllum* coverage (there was no change in experiment two). After habitat manipulation, fish abundances were significantly higher in areas with high coverage than in areas with low coverage in both experiments. Aquatic insects also responded to changes in *P. ceratophyllum* coverage, although differences were less statistically significant because of high sample variability. Macroinvertebrate biomass and abundance were significantly higher in addition areas than in removal areas. Although some of the habitat variables were significantly different before and after the manipulation, all depth and velocity measurements were within the range of natural variability in which many shoal dependent species live (Aadland 1993).

This study was designed to determine if there is a behavioral response of benthic fishes to increased availability of large (2.5 m x 6 m), high-density patches of *P. ceratophyllum*. Because *P. ceratophyllum* appears to be declining in parts of its range, we wanted to understand potential consequences to fishes of reduced *P. ceratophyllum*. As is characteristic of temperate streams, most of the shoal fish species in the Conasauga River are insectivorous; if food is a limiting resource, population sizes could depend on the abundance and productivity of their prey. Benthic fishes using habitat in *P. ceratophyllum* are likely feeding in that habitat as well, because most benthic fishes are ambush foragers, feeding on the substrate over relatively small areas (Freeman and Freeman 1994, Inoue et al. 2005). Therefore, we also wanted to quantify aquatic insect use of *P. ceratophyllum* to verify that macrophyte manipulation also affected prey. We know from previous studies that insect biomass can be higher with high densities of *P. ceratophyllum* (Grubaugh and Wallace 1995, Hutchens et al. 2004), but the response of fishes to increased *P. ceratophyllum* coverage has not been investigated.

In other studies, benthic insectivorous fishes have been shown to respond to heterogeneity in prey availability by increasing use of habitats with high prey densities (Thompson et al. 2001). *Cottus bairdi*, a related species to the *Cottus carolinae* found in this study site, has similarly been shown to select habitat patches with high prey abundance over physically similar habitat with low prey abundance (Petty and Grossman 1996). In this study, we observed higher benthic fish densities in areas with higher biomass of *P. ceratophyllum* and aquatic insects, while physical variables remained relatively constant. Higher densities and biomass of aquatic insects may increase individual fitness of fishes if foraging ability increases with insect abundances, in which case higher fish abundances could be attributed to the increase in prey availability. Although unmeasured in this study, water velocities in *P. ceratophyllum* has been shown to be up to 50% slower than in surrounding habitat. Slower flows may decrease the energy expended to remain in a specific location, decreasing energy needs of fishes.

Although macrophyte density and abundance is generally shown to increase invertebrate abundances, increases in productivity are not always observed in higher trophic levels. Dense macrophytes can actually impede feeding by invertebrate predators because of decreased feeding efficiency in complex habitat structure (Warfe and Barmuta 2004). Macrophytes growing at high densities can also create a refuge for aquatic insects, keeping prey populations high and apparently increasing the overall food resource (reviewed in Orth et al. 1984). This study did not test whether foraging by benthic fishes was enhanced in areas with higher coverage of *P. ceratophyllum* because of increased biomass and density of aquatic insects.

A second, untested hypothesis is that benthic fishes may be using *P. ceratophyllum* as cover from predators. Riffle and shoal habitat may be effective refugia from large piscivorous predators such as large mouth bass (*Micropterus salmoides*), which generally are found in deeper pool habitats (Schlosser 1987). Smaller piscivorous predators such as redeye bass (*Micropterus coosae*) are often observed in shoals (personal observation) and may be important predators of benthic fishes. *P. ceratophyllum* may provide cover within shoals from aquatic predators such as redeye bass and also from avian predators. Stream fishes have been shown to seek shelter when they detect a threat from from avian predators (Allouche and Gaudin 2001). Avian predation risk has not been quantified for shoal dependent species, but many avian predators, including kingfishers (*Ceryle alcyon*) and herons (Ardeidae) are often seen along the Conasauga River (personal observation). Although many fishes caught in this experiment forage during the day, the freckled madtom, *Noturus leptacanthus*, is a noctural feeder and was caught only in the addition side in experiment two. These fish may be using *P. ceratophyllum* as refugia from predation during the day and foraging more broadly in the shoal at night.

Few studies have attempted an experimental manipulation of habitat of this size in a riverine environment (but see Fritz et al. 2004). Because *P. ceratophyllum* was scarce in the Conasauga River in 2005 (although more abundant in previous years; B. J. Freeman, personal communication), finding a site with enough plant coverage was challenging. Additionally, setting up the block nets within the main channel to perform this capture-recapture experiment was a challenge for two reasons. First, high discharge created strong downstream pull on the nets. Second, minimizing disturbance in the sampled habitat was important, so we had to work quickly and without entering the experimental area as much as possible until all nets were in place. Although this study was logistically difficult, a laboratory study of an environment this large would be unreasonable. Field experiments also allowed us to evaluate fish response given natural variability in patchiness of aquatic insects and environmental stochasticity. However, a laboratory study that tested fish foraging efficiencies in *P. ceratophyllum* at different prey densities would enhance understanding of the extent to which enhanced prey biomass translates into higher foraging rates.

In summary, this study has provided evidence that a suite of benthic riverine fishes will preferentially occupy, and likely benefit from, shoal habitats with enhanced coverage of *P. ceratophyllum*, compared to areas with lowered coverage. Thus, this supports the reasoning that fish population may respond to changes in rivers with changing levels of *P. ceratophyllum* coverage.

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Table 1. Physical habitat variables taken on addition and removal sides of experimental areas, before macrophyte manipulation and at the time of the second sampling, (i.e. 2 weeks later).

	Addition			*Removal*		
	before	*after*	*p*	*before*	*after*	*p*
Experiment 1						
Depth (m)	0.23	0.22	0.39	**0.18**	**0.23**	**< 0.05**
Velocity (m/s)	**0.47**	**0.21**	**< 0.05**	**0.37**	**0.20**	**< 0.05**
Sediment (mm)	.	53.50	No test	.	45.00	No test
Experiment 2						
Depth (m)	**0.22**	**0.25**	**< 0.05**	0.23	0.24	0.76
Velocity (m/s)	**0.21**	**0.31**	**< 0.05**	**0.20**	**0.26**	**< 0.05**
Sediment (mm)	45.00	44.67	0.97	33.67	32.11	0.81

Table 2. Insect density and biomass (g AFDM) in samples[1] taken on *P. ceratophyllum* addition and removal sides of experimental areas, before and after macrophyte manipulation. Comparisons between addition and removal means are based on ln-transformed data.

	Addition		*Removal*		
	Mean	(*SE*)	*Mean*	(*SE*)	*p*
Experiment 1					
Density					
Before	168.6	(27.13)	182.6	(40.24)	0.89
After	240.8	(103.46)	121.4	(30.81)	0.24
Biomass					
Before	18.71	(3.06)	18.47	(5.00)	0.81
After	30.78	(11.97)	8.23	(1.87)	**0.06**
Experiment 2					
Density					
Before	372.0	(125.26)	267.4	(21.04)	0.77
After	281.5	(56.76)	93.8	(13.5)	**<0.01**
Biomass					
Before	49.57	(15.29)	28.90	(5.61)	0.21
After	33.60	(7.76)	8.93	(2.24)	**0.01**

1 Area sampled = 0.126 m^2.

Table 3. Estimated abundances of benthic insectivorous fishes on *P. ceratophyllum* addition and removal sides of experimental areas, before and after macrophyte manipulation. Profile likelihood confidence intervals (CI) are shown except for before-manipulation in experiment 2, when abundances were estimated using an average capture probability for the after-manipulation sample (see text).

	Addition		*Removal*		
	Abundance	*CI*	*Abundance*	*CI*	*p*
Experiment 1					
Before	40	39-49	51	43-75	N.S.
After	53	48-68	33	32-40	**< 0.05**
Experiment 2					
Before	27	-	19	-	No test
After	47	46-55	17	17-17	**< 0.05**

Chapter 4

Conclusions

Local and watershed variables appeared to influence occurrence of the aquatic macrophyte *Podostemum ceratophyllum* and fishes. *P. ceratophyllum* presence was positively influenced by local variables (increasing bed sediment sizes and location in the center of channel), and also showed a negative effect of watershed size, corresponding to a landscape variable. Lotic fish richness also showed a decline with watershed area, driven by reduction of sensitive species in the lower portion of the study reach where forested land cover decreased and agricultural and developed land cover increased. However, because the change in lotic species richness was not linear, but rather peaked in the center of the study reach, neither decline in *P. ceratophyllum* cover or changes in land cover correlated with richness. Additionally, modeled occurrences of most fish species were best predicted by shoal location along the mainstem, with about half the species more likely to occur in the upper portion of the sampled reach. Average cover of *P. ceratophyllum* was a good predictor of the presence at a shoal of only one species of fish, *Cyprinella caerulea* (which actually was less likely to occur with high macrophyte cover). Turbidity levels increased in the downstream direction, which could impact both *P. ceratophyllum* populations as well as the persistence of sensitive species and is indicative of anthropogenic disturbances in the watershed having a negative impact on stream integrity.

Whereas the observational study did not provide strong evidence of associations between lotic fishes and *P. ceratophyllum*, the experimental study did show a response by benthic insectivorous fishes at a local scale to higher *P. ceratophyllum* density. Higher cover of *P. ceratophyllum* corresponded with higher densities and biomass of aquatic macroinvertebrates. In two experiments, benthic insectivorous fishes and aquatic macroinvertebrates were significantly higher after two weeks in plots with increased *P. ceratophyllum* cover than in plots where the plant was removed. Behavioral response can be attributed to increased food resources, but could also be an attraction to habitat with increased protection from predators and high stream velocities.

Differences between results of the observational and experimental studies may reflect differences in scale, measurement units (presence vs. abundance) and uncertain relationships between coverage and biomass of *P. ceratophyllum*. The experimental study reflects short-term behavioral responses, whereas the observational study reflects persistence at a shoal level. Fish abundance, rather than presence, could provide more information as to how populations are actually changing at different locations in the mainstem, and could provide a more accurate understanding of how populations may be responding to differing levels of *P. ceratophyllum* coverage. Additionally, better understanding of how plant biomass changes with respect to coverage at a shoal could lead to a stronger correlation between changes in *P. ceratophyllum* coverage and fish species presence. Biomass may be a better predictor of fish species presence because the quality and quantity of habitat changes with plants that have been grazed or are otherwise damaged as opposed to dense plants with long leaves.

These results provide a baseline for further study in the Conasauga River aimed at species conservation. These results also provide insight into the mechanisms underlying patterns of benthic fish occurrence in association with *P. ceratophyllum* at a local scale, observed in other studies. Specifically, local associations may reflect choices by benthic fishes to utilize *P. ceratophyllum*, whereas species persistence is more strongly driven by other factors. In any case, lotic species richness and *P. ceratophyllum* may decline similarly in response to anthropogenic stresses.

Appendix A. Species detections in replicated observations at 21 Conasauga River shoals sampled in 2005. Shoals are identified by river kilometer, measured from the mouth of the river. Only one sample was taken at river km 42.8 and 40.65; at river km 55, a third observer sited *P. antesella*, but did not record other species.

Species	Field number and river kilometer														
	MCF 05-31		MCF 05-32		MCF 05-45		MCF 05-46		MCF 05-33		MCF 05-28		MCF 05-29		
	75.4		75.3		71.2		67.3		66		63.2		62.7		
Campostoma oligolepis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Notropis stilbius	0	0	0	0	0	0	1	1	1	1	0	1	0	1	1
Notropis xaenocephalus	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Phenacobius catostomus	0	0	0	0	1	1	1	1	1	1	0	0	0	1	0
Cyprinella callistia	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Cyprinella caerulea	0	0	0	1	1	0	1	1	1	1	0	1	1	0	1
Cyprinella venusta	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
Cyprinella trichroistia	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
Luxilus chrysocephalus	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0
Macrhybopsis aestivalis	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Onchorhyncus mykiss	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypentelium etowanum	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Moxostoma sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Moxostoma carinatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Moxostoma duquesnei	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0
Fundulus stellifer	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Fundulus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fundulus olivaceous	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cottus carolinae	0	1	0	1	0	0	1	1	1	1	0	1	1	0	0
Ambloplites arriomus	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Lepomis megalotis	1	0	1	1	1	1	1	1	1	1	0	0	0	0	0
Micropterus coosae	1	1	0	0	1	1	1	1	1	1	0	1	0	0	1
Percina sp. cf. P. macrocephala	0	0	1	1	1	1	1	0	1	0	0	0	0	0	0
Percina nigrofasciata	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1
Percina kathae	0	1	0	0	1	1	1	1	0	0	0	0	0	0	0
Percina palmaris	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Percina sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Percina antesella	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
Etheostoma brevirostrum	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0
Etheostoma coosae	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1
Etheostoma stigmaeum	0	0	0	0	0	0	1	1	0	0	1	1	1	1	1
Etheostoma jordani	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
Etheostoma trisella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Etheostoma rupestre	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0

Species	Field number and river kilometer													
	MCF 05-30			JER 05-13		MCF 05-34		MCF 05-35		MCF 05-36			JER 05-14	
	61.6			60.7		58.65		56.2		55			53.9	
Campostoma oligolepis	1	1	1	1	1	1	1	1	1	1	1	.	1	1
Notropis stilbius	1	1	1	1	1	1	1	1	1	1	1	.	1	1
Notropis xaenocephalus	1	1	1	0	0	1	0	1	0	1	1	.	1	0
Phenacobius catostomus	1	1	1	0	0	1	1	1	1	0	1	.	1	0
Cyprinella callistia	1	1	1	1	1	1	1	1	1	1	1	.	1	1
Cyprinella caerulea	1	1	1	1	1	1	1	1	1	1	1	.	1	0
Cyprinella venusta	0	1	1	1	1	1	1	1	1	1	1	.	1	1
Cyprinella trichroistia	1	0	1	1	1	1	0	0	0	0	1	.	1	0
Luxilus chrysocephalus	1	1	1	1	1	1	1	1	1	1	1	.	0	0
Macrhybopsis aestivalis	0	0	0	0	0	0	0	0	0	0	0	.	0	0
Onchorhyncus mykiss	0	0	0	0	0	0	0	0	0	0	0	.	0	0
Hypentelium etowanum	1	1	1	1	1	1	1	1	1	1	1	.	1	1
Moxostoma sp.	0	0	1	1	1	0	0	0	0	0	1	.	0	0
Moxostoma carinatum	0	0	0	0	0	0	0	0	0	0	0		0	0
Moxostoma duquesnei	0	0	0	0	0	0	0	0	0	0	0	.	0	0
Fundulus stellifer	0	0	0	1	1	0	0	0	0	0	0	.	0	0
Fundulus sp.	0	0	0	0	0	0	0	0	0	0	0	.	0	0
Fundulus olivaceous	0	0	0	0	0	0	0	0	0	0	0	.	0	0
Cottus carolinae	1	1	0	0	1	1	0	0	0	0	0	.	0	0
Ambloplites arriomus	0	0	0	0	0	0	0	0	0	0	0	.	0	0
Lepomis megalotis	1	0	1	1	0	1	1	0	0	0	0	.	1	0
Micropterus coosae	1	1	0	0	1	1	1	0	0	1	1	.	0	0
Percina sp. cf. P. macrocephala	1	1	1	1	0	1	1	1	1	1	1	.	1	0
Percina nigrofasciata	1	1	1	1	1	1	1	1	1	1	1	.	1	1
Percina kathae	0	1	0	1	0	0	0	1	0	0	0	.	0	0
Percina palmaris	1	1	1	1	1	1	1	0	0	0	1	.	0	0
Percina sp.	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Percina antesella	0	0	1	0	0	0	0	0	0	0	0	1	1	0
Etheostoma brevirostrum	0	0	0	0	0	0	0	0	0	0	0	.	0	0
Etheostoma coosae	1	1	1	1	1	1	1	1	1	1	1	.	1	0
Etheostoma stigmaeum	1	1	1	1	1	1	1	1	1	1	1	.	0	1
Etheostoma jordani	1	1	1	1	1	1	1	1	1	1	1	.	1	0
Etheostoma trisella	0	0	0	0	0	0	0	1	1	0	0	.	1	0
Etheostoma rupestre	1	1	1	1	1	1	1	1	1	1	1	.	1	0

Species	Field number and river kilometer															
	MCF 05-37/49		MCF 05-38/50		MCF 05-39/47		MCF 05-40/48		MCF 05-41		JER 05-16/18		JER 05-17		JER 05-15/19	
	50.7		46.85		44.75		44.65		42.8		41.1		40.65		36.4	
Campostoma oligolepis	1	1	1	1	1	1	1	1	1	.	1	0	1	.	0	1
Notropis stilbius	1	1	1	1	1	1	1	1	1	.	1	1	1	.	1	1
Notropis xaenocephalus	0	0	0	0	0	0	0	0	1	.	0	0	0	.	0	0
Phenacobius catostomus	1	1	1	1	1	1	1	1	1	.	1	1	1	.	1	1
Cyprinella callistia	1	1	1	1	1	1	1	1	1	.	1	1	1	.	1	1
Cyprinella caerulea	1	1	1	1	1	1	1	1	1	.	1	1	1	.	0	0
Cyprinella venusta	1	1	1	1	1	1	1	1	1	.	1	1	1	.	1	1
Cyprinella trichroistia	0	0	0	0	0	0	0	0	0	.	0	0	0	.	0	0
Luxilus chrysocephalus	1	1	1	1	1	1	1	1	1	.	1	1	1	.	0	0
Macrhybopsis aestivalis	0	0	0	1	0	0	0	0	0	.	0	0	0	.	0	0
Onchorhyncus mykiss	0	0	0	0	0	0	0	0	0	.	0	0	0	.	0	0
Hypentelium etowanum	1	1	1	1	1	1	1	1	1	.	1	1	1	.	1	1
Moxostoma sp.	0	0	0	0	0	0	0	0	0	.	0	0	0	.	0	1
Moxostoma carinatum	0	0	0	0	0	0	0	0	1		0	0	0		0	0
Moxostoma duquesnei	1	1	1	1	1	0	1	0	1	.	0	1	0	.	1	0
Fundulus stellifer	0	0	0	1	1	0	0	0	0	.	0	0	0	.	0	0
Fundulus sp.	0	0	0	0	0	0	0	0	0	.	0	0	0	.	0	0
Fundulus olivaceous	0	0	0	0	0	0	0	0	0	.	0	1	0	.	0	0
Cottus carolinae	1	1	1	1	1	1	1	1	0	.	1	0	1	.	1	1
Ambloplites arriomus	0	0	0	0	0	0	0	0	0	.	0	0	0	.	0	0
Lepomis megalotis	0	0	1	1	0	0	1	1	1	.	1	1	1	.	0	1
Micropterus coosae	0	0	0	1	0	0	0	0	0	.	0	0	0	.	0	0
Percina sp. cf. P. macrocephala	0	0	0	0	0	0	0	0	0	.	0	0	0	.	0	0
Percina nigrofasciata	1	1	1	1	1	1	1	1	1	.	1	1	1	.	1	1
Percina kathae	0	0	0	0	0	0	0	0	1	.	0	0	1	.	1	0
Percina palmaris	0	0	0	0	0	0	0	0	0	.	0	0	0	.	0	0
Percina sp.	0	0	0	0	0	0	0	0	0	.	0	0	0	.	0	0
Percina antesella	1	0	0	0	0	0	0	0	0	.	0	0	0	.	0	0
Etheostoma brevirostrum	0	0	0	0	0	0	0	0	0	.	0	0	0	.	0	0
Etheostoma coosae	1	1	0	1	1	1	0	1	1	.	0	0	0	.	0	0
Etheostoma stigmaeum	1	1	1	1	0	1	1	1	1	.	1	1	1	.	1	1
Etheostoma jordani	1	1	0	1	0	0	1	1	1	.	0	0	1	.	1	1
Etheostoma trisella	1	1	0	1	0	1	0	0	0	.	0	0	0	.	0	0
Etheostoma rupestre	1	1	1	1	1	1	1	1	1	.	0	0	1	.	1	1

Appendix B. Capture data for two *Podostemum* manipulation experiments.

Week 0 Addition			
Species	1st pass	2nd pass	marked
Cottus carolinae	9	12	6
Percina nigrofasciata	1	2	1
Cyprinella callistia	3	5	2
Phenacobius catostomus	2	1	1
Etheostoma jordani	12	17	13
Percina palmaris	2	3	1
Campostoma oligolepis	7	1	1
Etheostoma coosae	1	1	1

Week 0 Removal		1st pass	
Species	1st pass	2nd pass	marked
Campostoma oligolepis	10	2	1
Cottus carolinae	3	15	2
Etheostoma jordani	7	7	2
Etheostoma rupestre	3	5	4
Etheostoma coosae	4	3	3
Phenacobius catosomus	1	1	1
Percina palmaris	1	2	1
Percina nigrofasciata	1	0	0
Cyprinella callistia	0	1	0

Week 2 Addition			
Species	1st pass	2nd pass	marked
Cottus carolinae	12	14	10
Percina nigrofasciata	2	2	1
Cyprinella callistia	1	0	0
Phenacobius catostomus	0	1	0
Etheostoma jordani	16	15	9
Percina palmaris	2	1	0
Campostoma oligolepis	2	0	0
Etheostoma coosae	0	2	0
Hypentelium etowanum	3	1	1

Week 2 Removal			
Species	1st pass	2nd pass	marked
Campostoma oligolepis	4	3	1
Cottus carolinae	8	12	8
Etheostoma jordani	10	9	8
Etheostoma rupestre	2	2	2
Etheostoma coosae	1	2	1
Phenacobius catosomus	0	0	0
Percina palmaris	2	2	2
Percina nigrofasciata	0	1	0
Cyprinella callistia	0	0	0
Hypentelium etowanum	1	1	1

Week 0
Addition

Species	1st pass	2nd pass	marked
Cyprinella callistia	1	-	-
Etheostoma rupestre	4	-	-
Noturus leptacanthus	2	-	-
Percina palmaris	1	-	-
Cottus carolinae	7	-	-
Etheostoma jordani	11	-	-

Week 0
Removal

Species	1st pass	2nd pass	marked
Etheostoma rupestre	3	-	-
Percina palmaris	5	-	-
Cottus carolinae	4	-	-
Etheostoma jordani	3	-	-
Notropis stilbius	1	-	-
Percina nigrofasciata	2	-	-
Campostoma oligolepis	1	-	-

Week 2
Addition

Species	1st pass	2nd pass	marked
Etheostoma rupestre	1	3	1
Etheostoma coosae	1	0	0
Percina palmaris	1	0	0
Cottus carolinae	20	24	19
Etheostoma jordani	9	7	6
Noturus leptacanthus	5	4	4
Hypentelium etowanum	1	0	0
Etheostoma stigmaeum	0	1	0

Week 2
Removal

Species	1st pass	2nd pass	marked
Cyprinella callistia	0	2	0
Etheostoma rupestre	2	2	2
Cottus carolinae	11	11	10
Etheostoma jordani	1	1	0
Percina nigrofasciata	0	1	0