ONTOGENETIC SHIFTS, HABITAT USE AND COMMUNITY STRUCTURE: HOW
FISHES USE AND INFLUENCE PROTECTED TALLGRASS PRAIRIE STREAMS

by

ERIKA C MARTIN

B.S., Ball State University, 2009

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Division of Biology
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2014

Abstract

This dissertation consists of three research-based chapters which focus on habitat association of prairie stream fishes and how these fish communities influence stream ecosystem properties. Chapter one introduces important concepts used throughout the chapters, and describes my study streams. In chapter two, I identify local habitat factors associated with the diversity and density of fishes in two protected prairie watersheds. Specifically, the relative importance of habitat factors associated with fish communities were evaluated along a stream-size gradient and across multiple seasons and years. I found that species richness was positively associated with pool area and discharge. Redundancy analyses showed common prairie fish species exhibit ontogenetic habitat associations, with adults in deep and juveniles in shallow pools. Chapter 3 addresses how fish species richness in small prairie streams affects whole-stream metabolism and biomass distribution of benthic organic matter, algal and macroinvertebrates. This study was conducted by stocking experimental stream mesocosms that included pool-riffle habitats with three different communities that represent a gradient of species richness of headwater prairie streams from one to three common prairie stream fish species. I illustrated how species influence ecosystems across multiple spatial scales and found that different communities altered the distribution of algal biomass from benthic surfaces to floating mats and from pools to riffles. The objective of the fourth chapter was to quantify how two size classes of herbivorous prairie stream fish species, central stoneroller *Campostoma anamolum* and southern redbelly dace *Chrosomus erythrogaster* differentially affect stream ecosystem properties. This study was also conducted in experimental stream mesocosms, where each unit consisted of one riffle and one pool. Using ANOVAs, I found large dace were associated with longer filaments ($F = 7.5$, $P = 0.002$, $df = 4$) and small fishes with less benthic organic matter (F

= 4.2, P = 0.02, df = 4). There was no evidence for ontogenetic shifts in diet and likely differences in energetic requirements and behavior drove the differences among treatments. My research finds that small-bodied prairie stream fishes have predictable habitat preferences and effects on stream properties are dependent on species identity, richness and size structure.

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was fitted for heterogeneous variances. Group indicates each partition within the model. Estimate is the estimated variance, followed by the standard error of each estimate. For example, algal filament lengths were over 300 times more variable in riffles than pools. . 101

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This dissertation would not have been possible without the guidance and support of numerous people. I thank Keith Gido for serving as my major advisor. Keith continually encouraged me and always had an open door for questions and problems, and to discuss research ideas. His unlimited enthusiasm for fishes and stream research is inspiring. I thank Walter Dodds for serving on my committee. Walter was a wonderful mentor, always willing to answer questions and discuss algae and metabolism, while keeping the best interest of students in mind and offering helpful and practical professional development advice. I thank Anthony Joern for serving on my committee. Tony provided insight as a great community ecologist, and offered a unique perspective as a terrestrial ecologist. Finally, I thank Nora Bello for serving on my committee. Nora shared her expertise in, and emphasized the value of, statistical analysis.

I am grateful to my many friends and colleagues for their continued support and the long discussions about science and future career paths. I would like to thank my labmates James Whitney, Matt Troia, Josh Perkin and Nate Cathcart for all the lively discussions, lab ice cream outings and Salsarita's Fridays that made graduate school wonderful. Thank you to all the members of the aquatic and ecology journal clubs for fun times, great discussions and camaraderie. Finally, I would like to thank Kaori Knights for all the animated conversations, venting sessions, and good times. His friendship during the past four years was invaluable.

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Dedication

This dissertation is dedicated to my family; Hobart (grandfather), Eileen (mother), Brent (father), Kirsten (sister) and Kenneth (nephew) Martin. Without my family, I never would have had the courage to begin my career, and without their unconditional support I would not have the humor and strength to have finished. I am forever grateful.

Preface

The contents of this dissertation represent concepts and approaches developed in collaboration with my major professor, members of my dissertation committee and fellow graduate students. Chapter 2 is published with Keith Gido, James Whitney, Kristin Hase as coauthors in the journal *The American Midland Naturalist*, volume 170, issue number 1, on pages 39-51. Chapter 3 is formatted for publication in the journal *Ecology* with Keith Gido, Nora Bello, Allison Veach and Walter Dodds as coauthors. Chapter 4 is formatted for publication in the journal *Ecology of Freshwater Fish*.

Chapter 1 – Overview of fish community and ecosystem dynamics in protected tallgrass prairie streams

North American tallgrass prairie is one of the most endangered ecosystems in the United States, with prairie losses estimated around 95% (Samson and Knopf 1994). Prairie streams are equally endangered because of fragmentation (Perkin and Gido 2011), channelization and alterations on channel morphology (Cross and Moss 1987), and agricultural runoff (Dodds et al. 2004). Further, numerous Great Plains fishes have been declining for decades because of changing landuse and stream flow patterns (Cross and Moss 1987, Taylor 2010, Gido et al. 2010). Maintaining biodiversity and natural ecosystem function in these systems is particularly critical because headwater streams are first in the hierarchical network which is important for downstream transport (Vannote et al. 1980) and make up roughly 70% of stream length (Lowe and Likens 2005).

Hydrologic variability is a hallmark of prairie streams, as flooding and drying are exaggerated in comparison to forested streams (Dodds et al. 2004). In smaller streams (i.e., 1st – 3rd order), the variability of runoff is mediated by climate and rainfall patterns (Dodds et al. 2004). Variable stream flow, particularly during drought, can cause temporary extirpation of fishes from part or all of a stream (Franssen et al. 2006), indicating abiotic factors might be important in regulating fish abundances in these systems (Schlosser 1985). Further, flow variability can be a much stronger influence on use of spatial resources by fishes than either interspecific competition or predation (Lobón Cerviá and Ricón 2004, Grossman et al. 1998). Variable flows influence substrate composition, instream cover, and habitat size, which can influence fish community structure in Great Plains streams (Fischer and Paukert 2008, Falke et

al. 2010), though the relationship between flow and fish community structure in prairie streams is still unclear (Franssen et al. 2006).

Prairie stream fishes can influence the recovery of stream ecosystem structure and function following disturbances (Wootton et al. 1996, Bertrand et al. 2009, Gido et al. 2010). Bertrand et al. (2009) found biomass and production of periphyton communities recover more quickly when grazing (southern redbelly dace; *Chrosomus erythrogaster*) and water-column (red shiners; *Cyprinella lutrensis*) minnows are present early after flooding. Murdock et al. (2011) found southern redbelly dace influenced structural environmental variables (algal biomass, benthic organic matter, benthic particle size) and biomass-specific gross primary productivity, early in recovery after flooding. My dissertation research complements this growing body of literature on context dependent fish effects by exploring community structure and ontogenetic shifts.

The effects of fish on stream ecosystem properties are dependent on functional traits of individual species as well as combined traits of entire communities. Power (1990), and later Capps and Flecker (2013), found that Armored catfish (*Loricariidae*) drastically alter sedimentation dynamics in tropical streams. Furthermore, small-bodied fishes can affect the algal community (Power 1984, Power et al. 1988, Evans-White et al. 2001, Evans-White et al. 2003, Bengtson et al. 2008), nutrient cycling (Vanni 2002, McIntyre et al. 2008, Schmitz 2008, Flecker et al. 2010), and stream metabolism (Taylor et al. 2006) in small headwater streams. In prairie streams, stonerollers compete with other taxa such as crayfish, and the presence of crayfish dampen the effects stonerollers have on stream properties (Evans-White et al. 2001). Southern redbelly dace also interact and compete with crayfish, but it is highly seasonal, where dace are more active during cooler seasons and crayfish during warm periods (Bengtson et al. 2008).

Bertrand et al. (2009) found that southern redbelly dace affect stream recovery post-flood differently than red shiners, where dace had greater effects on the stream properties than shiners; reducing algal filament lengths, particulate organic matter, and chironomid numbers. My work expands on this by including multiple species effects.

In streams, community structure is an important consideration, but organism size might also be critical, as size influences an organism's ability to use resources, energetic requirements and susceptibility to predation (Werner and Gilliam 1984). Ontogenetic shifts in diet and habitat use have been studied in a variety of species (sunfish; Osenberg et al. 1988, Osenberg et al. 1992, salmonids; Klemetsen et al. 2003, minnows, perch, sculpin, and stickleback; Nunn et al. 2007, gizzard shad; Pilati and Vanni 2007, cichlids; Burress et al. 2013, common minnow; Walker et al. 2013). For prairie stream herbivores, Bertrand and Gido (2007) found that small southern redbelly dace (*Chrosomus erythrogaster*) have a similar diet to larger individuals; consisting mostly of diatoms and filamentous algae. In contrast, Evans-White et al. (2001) used stable isotopes to suggest stonerollers got energy from invertebrates and found minor trends in gut contents becoming more algae filled with increasing length. My research builds on this by specifically comparing diet and ecosystem effects of different size groups of grazing minnows.

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Chapter 2 - Habitat associations of stream fishes in protected tallgrass prairie streams

Abstract

Describing fish habitat associations and their relevance to conservation remains a central challenge in stream fish ecology. Unfortunately, there are limited opportunities to investigate these associations in unaltered systems and identify critical habitats used by native fishes. Investigation of fish habitat associations in tallgrass prairie is especially vital, owing to their widespread destruction. Our study aim was to identify habitat factors associated with the distribution and density of fishes in two protected tallgrass prairie stream watersheds in eastern Kansas: Kings Creek on the Konza Prairie Biological Station (KPBS) and Fox Creek on the Tallgrass Prairie National Preserve (TPNP). We sampled fishes and measured eight habitat variables at three sites on KPBS (2006–2011) and four sites on TPNP (2008–2011). Multiple regression suggested that species richness was positively associated with pool area (partial r = 0.70) and discharge (partial r = 0.50) in Fox Creek (df = 15, Adj. R^2 = 0.60, P < 0.001). In Kings Creek, species richness was only associated with pool area (df = 17, R^2 = 0.44, P < 0.001). Redundancy analyses showed common prairie fish species exhibit ontogenetic habitat associations, partitioning adults in deep and juveniles in shallow pools. Strong species area relationships in these minimally altered systems indicates large volume habitats have greater species richness, suggesting water diversions or extractions that reduce habitat are likely to cause declines in native biodiversity.

Introduction

Both abiotic and biotic factors act concurrently over spatial and temporal scales to govern the distribution and abundance of species (Resh, 1988; Moyle and Light, 1996; Grossman et al., 1998; Magoulick, 2000; Jackson et al., 2001; Comita et al., 2009). For example biotic interactions such as algivory, competition, and predation can determine community structure and species persistence in streams (Jackson and Buss, 1975; Power and Matthews, 1983; Power et al., 1985; Power, 1992; Layman and Winemiller, 2004). Among potential abiotic influences on stream fish communities, stream flow is thought to be a master variable (e.g., Poff et al., 1997; Marchetti and Moyle, 2001; Lobo´n-Cervia´ and Rinco´n, 2004; Propst and Gido, 2004), but habitat structure (e.g., pool depth, large woody debris), which can be linked to flow, is also a strong determinant of community structure (e.g., Angermeier and Karr, 1984; Schlosser, 1987; Bond and Lake, 2003). The relative importance of biotic and abiotic factors likely varies across regions with different levels of intermittency, frequency, and predictability of discharge (Poff and Ward, 1989).

Prairie streams are characterized as having high flow variability and unpredictable disturbance events (Dodds et al., 2004), thus abiotic factors might be important in regulating fish abundances in these systems (Schlosser, 1985). Indeed, variable flows influence substrate composition, instream cover, and habitat size, which can influence fish community structure in Great Plains streams (Fischer and Paukert, 2008; Falke et al., 2010). For example variability in depth and flow parameters influence abundance, distribution, and persistence of Arkansas darter (Etheostoma cragini) in Colorado prairie streams (Labbe and Fausch, 2000). Similarly, Gelwick et al. (1997) found that variation in water depth affected habitat use in Oklahoma prairie streams. However, Statzner (1987) suggested biotic factors might govern prairie stream community

structure in pools but not riffles because hydrologic variability is greater in riffles. These studies illustrate that the relative importance of biotic and abiotic factors in structuring fish communities, even within prairie streams, can vary with community composition and the environmental template.

In prairie streams, the disturbance regime as well as habitat structure varies with stream size (Schlosser, 1987). Drying is typically more intense in shallow headwaters, but flooding is more intense and habitats are deeper downstream because of increasing catchment area (Leopold and Miller, 1956). This spatial variation in disturbances might decouple fish habitat associations via effect of flooding or drying on habitat availability and species persistence. Moreover, differences in habitat (e.g., depth) might favor species with different life history traits; small bodied species with rapid turnover occupy headwaters and larger species with slower turnover are more likely to occur downstream (Schlosser, 1987). Because extirpations in headwaters are common, the influence of regional species pool on local communities is dependent on system connectivity (Labbe and Fausch, 2000; Fausch et al., 2002; Hitt and Angermeier, 2008).

North American tallgrass prairie is one of the most endangered ecosystems in the United States, with prairie losses estimated around 95% (Samson and Knopf, 1994). Prairie streams are equally endangered because of fragmentation (Perkin and Gido, 2011), channelization and alterations on channel morphology (Cross and Moss, 1987), and agricultural runoff (Dodds et al., 2004). Further, numerous Great Plains fishes have been declining for decades because of changing landuse and stream flow patterns (Cross and Moss, 1987; Taylor, 2010; Gido et al., 2010). The objective of this study was to identify local habitat factors associated with the diversity and density of fishes in two protected prairie watersheds. Specifically, we evaluated the relative importance of habitat factors associated with fish communities along a stream size

gradient and across multiple seasons and years. Our temporal extent of sampling allowed us to identify the consistency of habitat associations across a variety of biotic and abiotic conditions both spatially and temporally and helped identify the relative importance of local habitat in structuring fish communities within stream networks. Matthews (1988) stated the need for a ''frame of reference'' for considering how these systems now differ from their pristine state. Many studies focus on systems that are currently under the influence of anthropogenic alteration. Our study focuses on two large portions of protected tallgrass prairie, and results might be used as a baseline for conservation and comparison for small bodied prairie stream fishes.

Methods

Study Area

Fishes were sampled at three locations on Kings Creek (17.5 km^2 watershed area above lowermost site), on the Konza Prairie Biological Station (KPBS), and from four locations on Fox Creek (88.4 km^2 watershed area above lowermost site) on the Tallgrass Prairie National Preserve (TPNP; Fig. 2.1). Kings Creek is a tributary in the Kansas River Basin and Fox Creek is located in the Cottonwood/Neosho River Basin. Our lowermost site on Kings Creek was roughly 5.9 rkm from the Kansas River (a 7th order river) and on Fox Creek was roughly 5.2 rkm from the Cottonwood River (a 5th order river). Both areas are owned by The Nature Conservancy and are managed by Kansas State University (KPBS) and the National Park Service (TPNP) with a combination of fire and grazing to maintain native tallgrass prairie. Bison are the primary grazers on portions of Kings Creek, whereas Fox Creek watersheds have both bison and cattle. In each watershed, one sample site was established on the mainstem stream and 2–3 sites were located in headwater tributaries (Fig. 2.1). Although riffles were sampled at mainstem sites when flowing, only pools were consistently sampled among sites (1–3 per site) and thus were the focus of our

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analysis. Moreover, because riffles were intermittent, species diversity was typically much lower and a subset of the species captured in pools.

Fish Collection

Fishes were sampled with single pass backpack electrofishing with two netters in May, Aug. and Nov. from 2006–2011 at Kings Creek and a combination of backpack electrofishing with two netters and a 4.6 x 1.8 m seine with 3.2 mm mesh from 2008–2011 at Fox Creek. Seining occurred at two tributary sites and one main stem site in the Fox Creek watershed that had habitats too deep (>1 m) to be efficiently sampled with backpack electrofishing. Fishes were identified to species, measured (total length, mm) and released into the pool from which they were collected. Pool area was measured and abundance was expressed as density (number of individuals per m^2).

To account for differences in species size structure, the most abundant species (>10% of total individuals captured) were subdivided by size class (juvenile or adult). Size classes were based on published literature accounts of size at maturity and notable breaks in size structure were verified by length frequency histograms from our monitoring (Table 1). Total density without size class separation was used for rare species (<10% of total individuals captured) to avoid excessive zero values in our data analyses.

Habitat Variable Measurements

In each pool, discharge, substrate, depth, velocity, width, and percent canopy cover were measured along three transects. Total length of each pool was measured and multiplied by mean pool width to calculate pool area. Depth (m), current velocity (m/s taken at 60% depth, using a Marsh-McBirney Model 2000 flowmeter) and substrate size class (based on modified Wentworth

scale; Cummins, 1962) were quantified at five points along each transect. Substrate size classes were numerically scored [1 (clay/bedrock), 2 (silt), 3 (sand), 4 (gravel), 5 (pebble), 6 (cobble) and 7 (boulder)] to give an average size for each habitat sampled. Discharge (m^3/s^2) was calculated by multiplying mean depth (m) and width (m) to get area, and then multiplying area by the average current velocity (m/s) for each habitat. Percent canopy cover for each pool was estimated by averaging the densiometer readings at the center of each of the three transects per pool. In stream cover throughout the pool was characterized as log complex, aquatic vegetation, undercut bank, log, brush pile, bank grass, or root wads. Area of cover was measured and divided by total pool area to give the percent of the pool containing cover.

Data Analysis

Regression analyses and ordination were used to evaluate the relative importance of habitat variables in structuring fish assemblages across multiple temporal scales in each watershed. Separate analyses were conducted for the two watersheds because they occurred in different drainages and had notably different species composition and abundance patterns. Habitat variables were tested for normality using normal probability plots (NPPs) and log transformed when necessary. To characterize associations among habitat variables and identify major gradients across watersheds we used principal components analysis (PCA). Principal components analysis was chosen after an analysis of bivariate relationships between habitat variables revealed linear relationships (Borcard et al., 2011). To avoid multicollinearity among habitat variables, we examined variance inflation factors (VIF) and removed redundant variables (VIF > 10; Borcard et al., 2011) prior to running the PCA. Multiple linear regressions for each watershed were used to evaluate which of the retained habitat variables from the above analysis were the best predictors of species richness. We tested the association between retained habitat

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variables and fish assemblage structure in each watershed using redundancy analysis (RDA).

Permutational ANOVAs (Borcard et al., 2011) evaluated the significance of habitat variables used in the RDAs, and partial RDAs were used to evaluate the relative contribution of habitat variables while factoring out the effects of site, season, and year. Partial RDAs allowed us to evaluate the pure effects of pool habitat characteristics while controlling for spatial and temporal effects on community structure. Analyses were run in program R 2.9.2 using libraries vegan (Oksanen et al., 2009) and car (Fox, 2009).

Results

Habitat Associations

Habitat gradients and associations among variables summarized by PCA suggested major gradients associated with stream size (e.g., depth, area, discharge and velocity), instream cover, and seasonal changes in canopy cover. Stream width was removed from this analysis because it had a VIF >10 and was closely related to area. The two watersheds had similar habitat gradients; smaller streams generally had a greater proportion of instream cover and more canopy cover (Fig. 2.2). Canopy cover also changed seasonally with the greatest cover in May and August before leaf fall. Pools were deeper in mainstem sites (mean = 0.26 m, 0.83 m) than tributaries (mean = 0.11 m, 0.48 m) in both Kings and Fox creeks, respectively, but there was a high degree of overlap.

Fish Community Structure

Species richness was generally higher at sites in Fox Creek [mean = 11, standard deviation (SD) = 3.9] than in Kings Creek (mean = 7, SD = 2.2). In Fox Creek, *Lepomis cyanellus* (green sunfish), *Campostoma anomalum* (central stoneroller), *Luxilus cardinalis*

(cardinal shiner), *Lythrurus umbratilis* (redfin shiner), *Pimephales notatus* (bluntnose minnow), *Semotilus atromaculatus* (creek chub), and *Etheostoma spectabile* (orangethroat darter) all had <10% of the total number of individuals captured. The most abundant species in Kings Creek were *C. anomalum, Chrosomus erythrogaster* (southern redbelly dace), *S. atromaculatus*, and *E. spectabile*. Mainstem sites had higher richness than tributaries within watersheds. In Kings Creek, a mean of 8.0 (range = 4 to 13) species occurred in the mainstem, whereas mean richness in tributaries was 4 (range = 3 to 4). Mainstem Fox Creek has a mean species richness of 15.0 (range = 8 to 19) compared to 9.2 (range = 7 to 13) species in tributaries.

Fish Community Structure Habitat Associations

Multiple regression suggested that species richness was positively associated with area (partial r = 0.70) and discharge (partial r = 0.50) in Fox Creek (df = 15, Adj. R^2 = 0.60, P < 0.001; Fig. 2.3). In Kings Creek species richness was only associated with area (df = 17, R^2 = 0.44, P < 0.001; Fig. 2.3). Redundancy analysis followed by permutational ANOVA indicated that depth, area, percent canopy cover and substrate size were associated with fish community structure in both watersheds (Fig. 2.4). Partitioning variation among predictor variable categories (habitat, season, and year) with partial RDAs found that habitat variables explained 14% (Kings Creek) and 13% (Fox Creek) of the variance in fish community structure (Table 2). Spatial variability (i.e., Site) explained slightly less (<12%) variation than habitat in both watersheds. Although temporal variation in fish community structure was significant in Kings Creek [both year (9%) and season (6%) were significant], these variables were not significant in the Fox Creek model.

Individual Fish Species Habitat Associations

Prairie stream fishes exhibited ontogenetic shifts in habitat associations, where adults and juveniles of the same species responded differently to the same habitat variables (Fig. 2.4). Ontogenetic habitat shifts were more explicit in Kings Creek, where juveniles generally were associated with shallower habitats, as indicated by negative axis 1 and 2 scores in contrast to positive axis 1 or 2 scores for adults. Specifically, juvenile *C. anomalum*, *L. cardinalis and E. spectabile* were more abundant in shallow pools in Fox Creek and juvenile *P. erythrogaster*, *C. anomalum* and *E. spectabile* were more abundant in shallow pools in Kings Creek. In both watersheds, juveniles also were positively associated with increasing cover (proportional instream cover in Kings Creek, instream and canopy cover in Fox Creek). Adult fishes (and juvenile *S. atromaculatus*) were positively associated with depth and area in Kings Creek. In Fox Creek, adults were either positively associated with depth and area, or negatively associated with velocity (i.e., more abundant in slower pools).

Discussion

The objective of this study was to identify local habitat factors associated with fishes in two protected prairie watersheds, with a broader goal of laying a foundation for a ''frame of reference'' that might be used in considering how altered Great Plains systems now differ from their pristine state (see Matthews, 1988). We found that pool depth and surface area had the strongest relationship with assemblage composition and these variables likely reflect a gradient of stream size and permanence that is highly correlated with temperature and dissolved oxygen. Falke et al. (2010) found that local scale spawning habitat influenced both occupancy and relative abundance probabilities of plains fish larvae. Specifically, *Hybognathus hankinsoni* (brassy minnow) required large, deep, backwater habitats for successful spawning. They

proposed that while many stream fish species appear to be habitat generalists during spawning some taxa switch to habitat specialization. A diversity of habitats is likely important to plains stream fishes emphasizing the need to conserve all habitat types in prairie systems. We found juveniles use shallower waters likely as a refuge from piscivorous fishes (Gorman, 1988; Schlosser, 1988, Labbe and Fausch, 2000). Accordingly, positive associations of adult fishes and pool area and depth are explained partly because larger individuals are less effectively consumed by piscivorous fishes (*S. atromaculatus* and *L. cyanellus*). Deeper pools are also more likely to remain wet during dry periods and provide more food resources and refuge from terrestrial predators (Power, 1987; Lonzarich and Quinn, 1995). *Semotilus atromaculatus* is one of the largest of the common species in our samples, and terrestrial predator avoidance might explain why this species seems to prefer deep habitats. This result is concurrent with Grossman and Freeman (1987) who found that *S. atromaculatus* were significantly over represented in deep areas and Edwards (1997) who showed that *S. atromaculatus* avoids strong flows. The species depth relationships from this study provides empirical support for the conceptual model by Power (1987) that illustrated size specific shifts in depth preference, where small bodied fish use shallow waters to avoid aquatic predators and large bodied individuals used deeper areas to avoid terrestrial predators.

Fishes also showed associations with measures of pool cover. *Etheostoma spectabile* was positively associated with percent instream cover and percent canopy cover, which is consistent with studies that report associations with vegetation, brush, and rocks that provide protection (Kuehne and Barbour, 1983; Page, 1983). Similarly, our results indicated most juveniles were positively associated with percent instream cover or percent canopy cover (e.g., *E. spectabile, P. erythrogaster, C. anomalum, L. cardinalis*). This might further illustrate predator avoidance by

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these small individuals as mortality via predation can be 50% greater in habitats with less complex structures (Lonzarich and Quinn, 1995).

Seasonal changes explained little variation in assemblage structure suggesting pool assemblages were relatively stable over time. This finding is in contrast to the view that prairie streams are regulated by stochastic (not deterministic) forces but is consistent with the findings of Ross et al. (1985) that found a harsh drought summer had no lasting effect on stability of a prairie stream fish community. They concluded that fish communities in harsh prairie streams may be relatively stable and persistent, despite major changes in environmental quality, and suggest that resistance and adjustment stability are the main reasons driving community persistence. Schlosser (1987) had similar conclusions and suggested harsh headwaters to be dominated by small bodied, short lived ''colonizing'' species. Likewise, the majority of species present in these prairie streams are adapted to highly variable systems and can be classified as opportunistic life history strategists characterized by young age at maturation, low fecundity, and low juvenile survival (Winemiller and Rose, 1992). Although temporal variation in habitat availability should drive seasonal changes in assemblage structure (e.g., Grossman and Freeman, 1987), pool habitats in prairie streams can be stable if they are spring fed as was the case in our systems. Evidently, a combination of environmental filters and species adaptation is driving the assemblage composition in small prairie streams. Thus, maintenance of these permanent pools and habitat structure within them may be critical refugia for the persistence of species.

Other factors (i.e., functional groupings, biotic interactions) that were not the focus of this study might explain distributions or lack of habitat association for various fish species. Some common stream fishes in prairies, like *L. cyanellus*, can be classified as generalist species and might not be influenced by spatial or temporal variation in habitat availability. Further, habitat

associations of rare and potentially transient species (<10% total number of individuals captured) are difficult to detect because of small sample sizes. However, species that are rare based on abundance measurements are typically consistently sampled at our sites. Also, biotic interactions might be more influential than habitat in some circumstances where one species provides critical habitat for another species (Gorman, 1988). For example, *L. umbratilis* is a nest associate with *L. cyanellus* (Hunter and Wisby, 1961) which might make *L. cyanellus* presence more important that other habitat factors for *L. umbratilis* distribution. Similarly, *S. atromaculatus* are typically nest associates with *C. anomalum* (Becker, 1983).

Our data were collected from two streams in the Flint Hills, the only region with remaining tallgrass prairie watersheds, and might inform management and conservation of biodiversity of these systems. For example, ontogenetic shifts in habitat use by prairie stream fishes suggest the maintenance of connectivity among heterogeneous pools might be important for species persistence. This is especially true in Kings Creek where shallow pools (i.e., juvenile refugia) tend to occur in upstream headwaters. In contrast, Fox Creek sites had both deep and shallow areas within a pool or site. Further, natural spring flows that maintain stable pool volumes and habitat complexity in the form of cover are local habitat features that may be critical for the persistence of prairie stream fishes. Unfortunately, many of these spring fed streams are dammed,which has created refuges for lentic adapted species (e.g., *L. cyanellus*) and altered natural flow regimes (Kerns and Bonneau 2002). Indeed, the Fox Creek watershed has a number of small impoundments and species such as *L. cyanellus* are more dominant in this system than in Kings Creek which does not have any impoundments.

In conclusion whereas the dominant landcover of North America was historically prairie these ecosystems are one of the most endangered in the region (Samson and Knopf, 1994).

Maintenance of critical habitat features within this fragmented landscape is likely necessary to ensure the persistence of biodiversity in this highly endangered ecosystem.

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Table 2.1 Length at maturity and references for classification of abundant fish species (<10% of total individuals captured) as juvenile or adult.

Most abundant species			
Common name (*scientific name*)	Watershed	Length at maturity	Reference
Central stoneroller (*Campostoma anomalum*)	Kings/Fox	65	Lennon and Parker 1960, Becker 1983
Orangethroat darter (*Etheostoma spectabile*)	Kings/Fox	45	Small 1975
Creek chub (*Semotilus atromaculatus*)	Kings/Fox	75	Lewis and Elder 1953, Gunning and Lewis 1956
Southern redbelly dace (*Phoxinus erythrogaster*)	Kings	55	Stasiak 2007
Green sunfish (*Lepomis cyanellus*)	Fox	60	Carlander 1977, White 1971
Cardinal shiner (*Luxilus cardinalis*)	Fox	65	
Redfin shiner (*Lythrurus umbratilis*)	Fox	45	Matthews and Heins 1984
Bluntnose minnow (*Pimephales notatus*)	Fox	50	Becker 1983

Table 2.2. Proportion of variance explained by habitat factors, site, year, and month in RDA models for each watershed. The last column is the total constrained variation in fish assemblage structure explained by the full model.

Spatial scale	Habitat	Site	Year	Season	Total const. variation
Kings Creek	0.14	0.12	0.09	0.06	0.78
Fox Creek	0.13	0.11	NS	NS	0.88

NS = Not significant

Figure 2.1 Location of study sites (black dots) within Kings Creek and Fox Creek watersheds in eastern Kansas. Triangles indicate mainstem sites and circles indicate tributary sites.
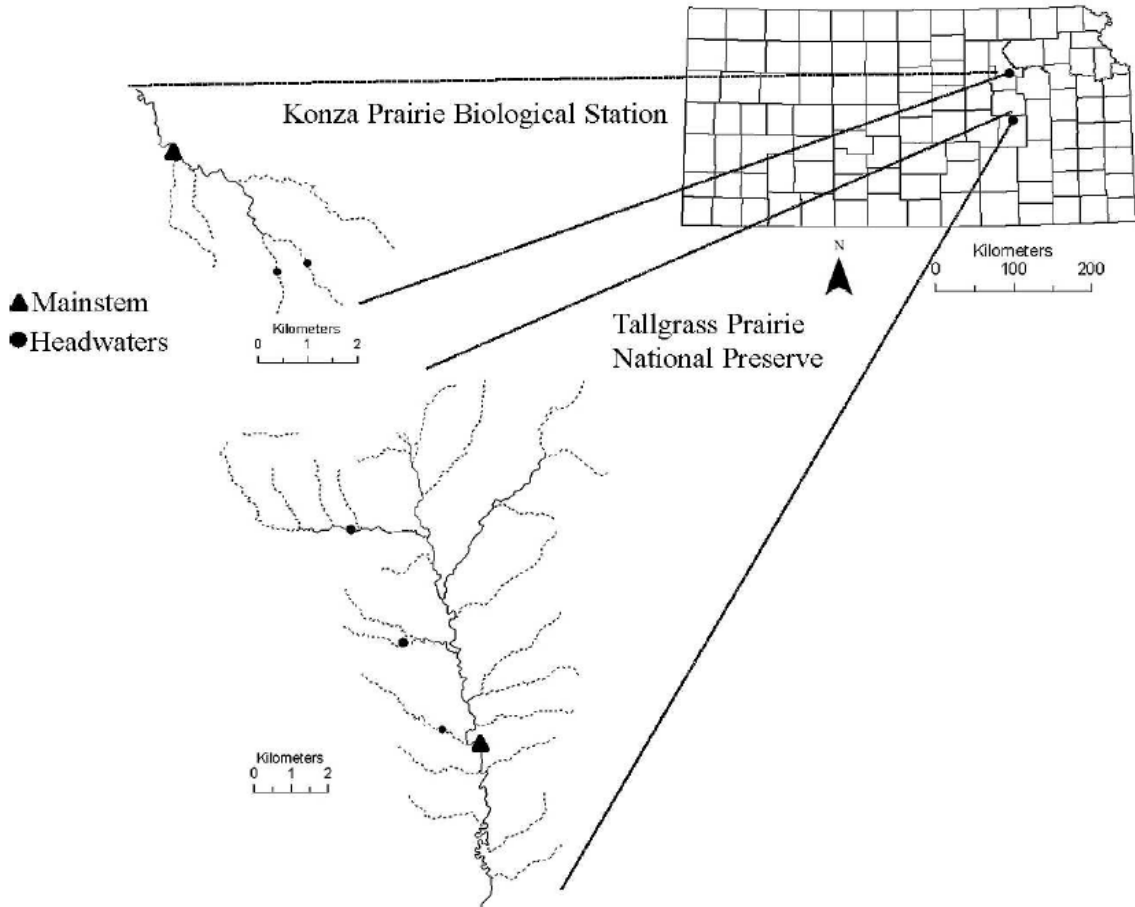
Figure 2.2 Principal components analyses (PCAs) including habitat and seasonal variables in Fox Creek (top) and Kings Creek (bottom). Each dot represents one sample site (e.g., pool 1, Nov. 2011) and symbols represent mainstem (circles) or tributary sample sites (triangles)

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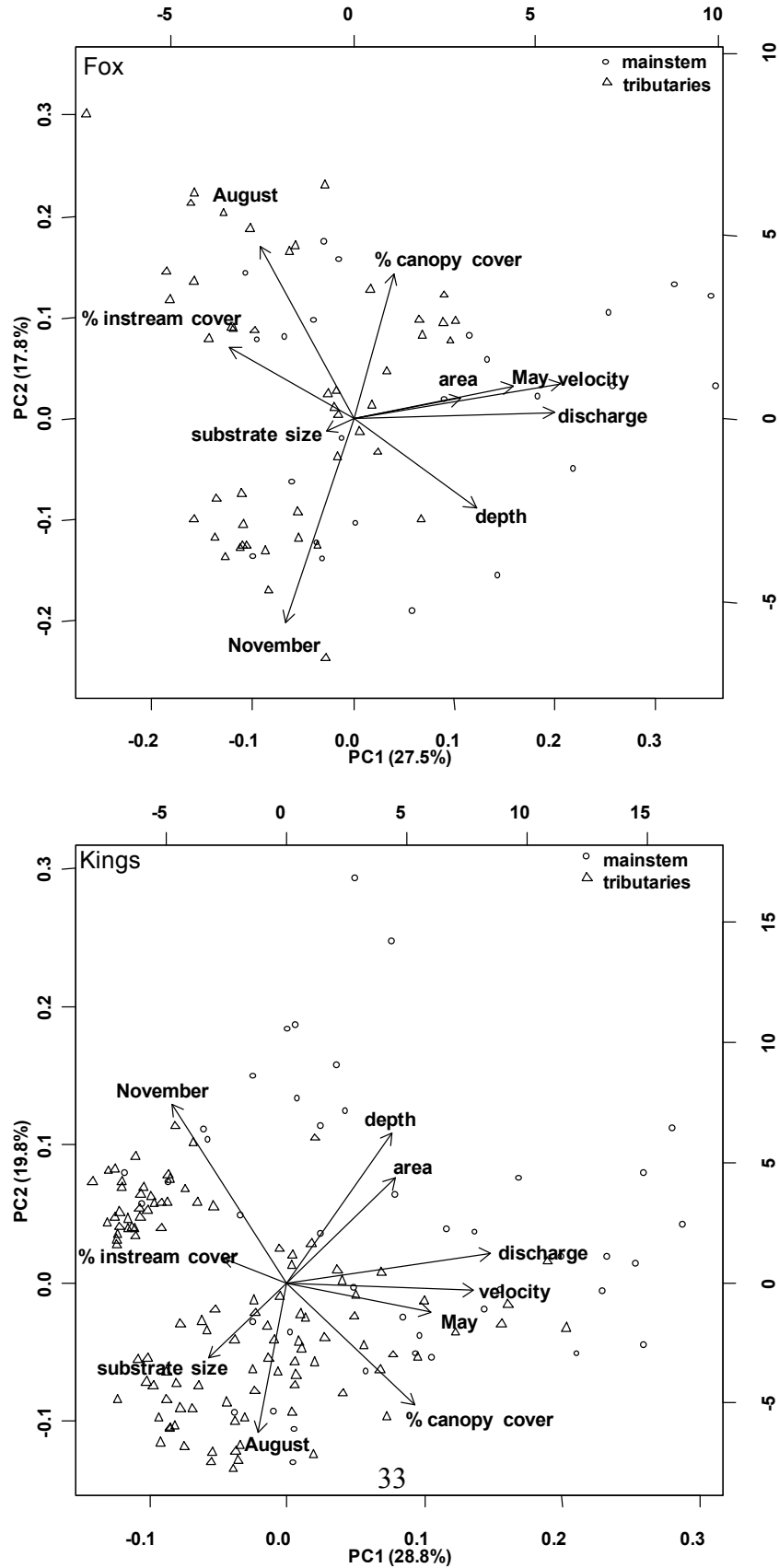
Figure 2.3 Multiple regression of pool area (independent variable) and species richness (dependent variable) for Fox Creek (closed circles; slope = 0.014, df = 15, r = 0.70, P < 0.001) and Kings Creek (open circles; slope = 0.018, df = 17, R^2 = 0.44, P < 0.001). In Fox Creek, species richness was positively associated with area (partial r = 0.70) and discharge (partial r = 0.50), this figure shows only area for both watersheds. An analysis of covariance (ANCOVA) found no significant difference between the slopes for Kings and Fox creeks.
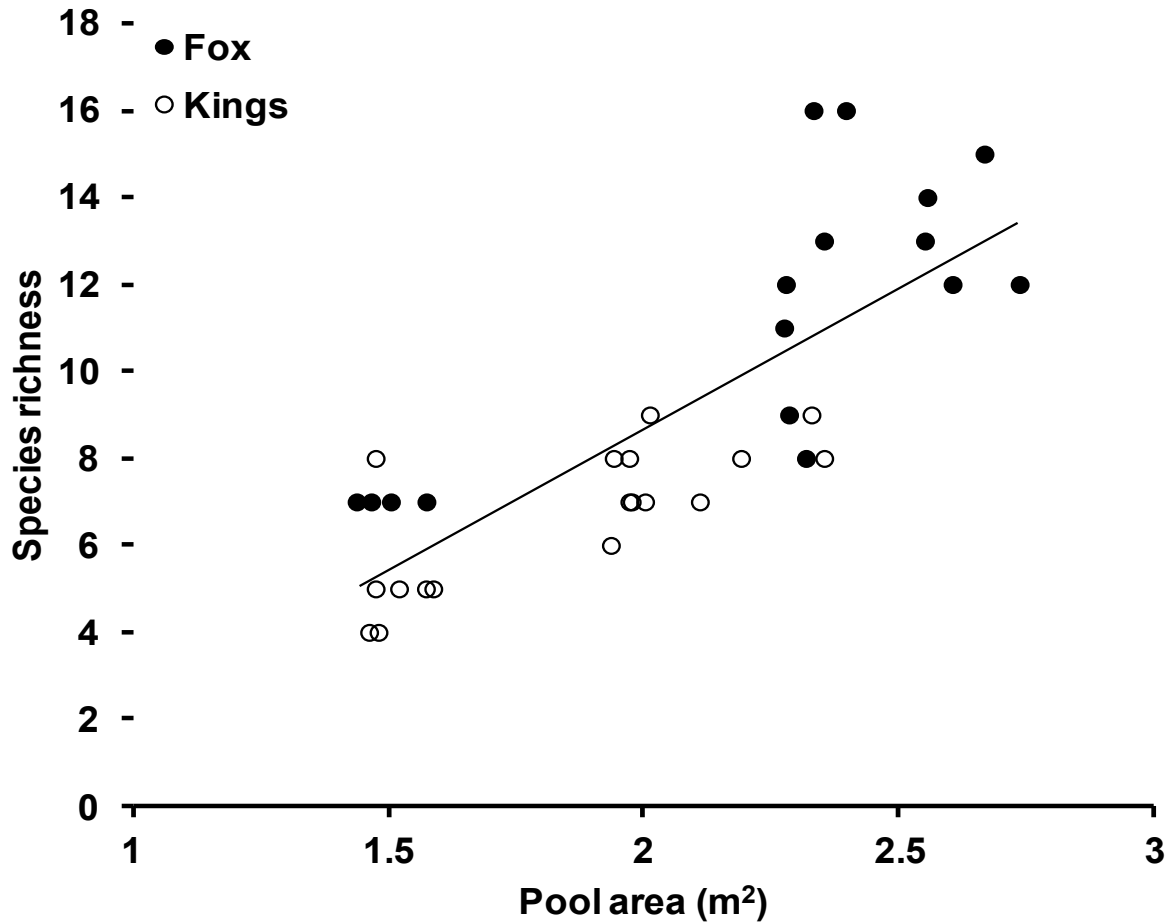
Figure 2.4 Redundancy analyses (RDAs) illustrating species-habitat associations of common species in two watersheds. Analyses included rare species, but were removed from the figure to alleviate crowding, and those species illustrated no associations. Axes 1 and 2 are significant (P < 0.05) for Kings and Fox creek RDAs. Upper (Fox Creek) and lower (Kings Creek) panels represent the habitat vectors and species loadings. Species codes are the first three letters of the genus and species, a 'J' or 'A' refers to the juvenile or adult size class (e.g., juvenile *C. anomalum* = Jcamano).
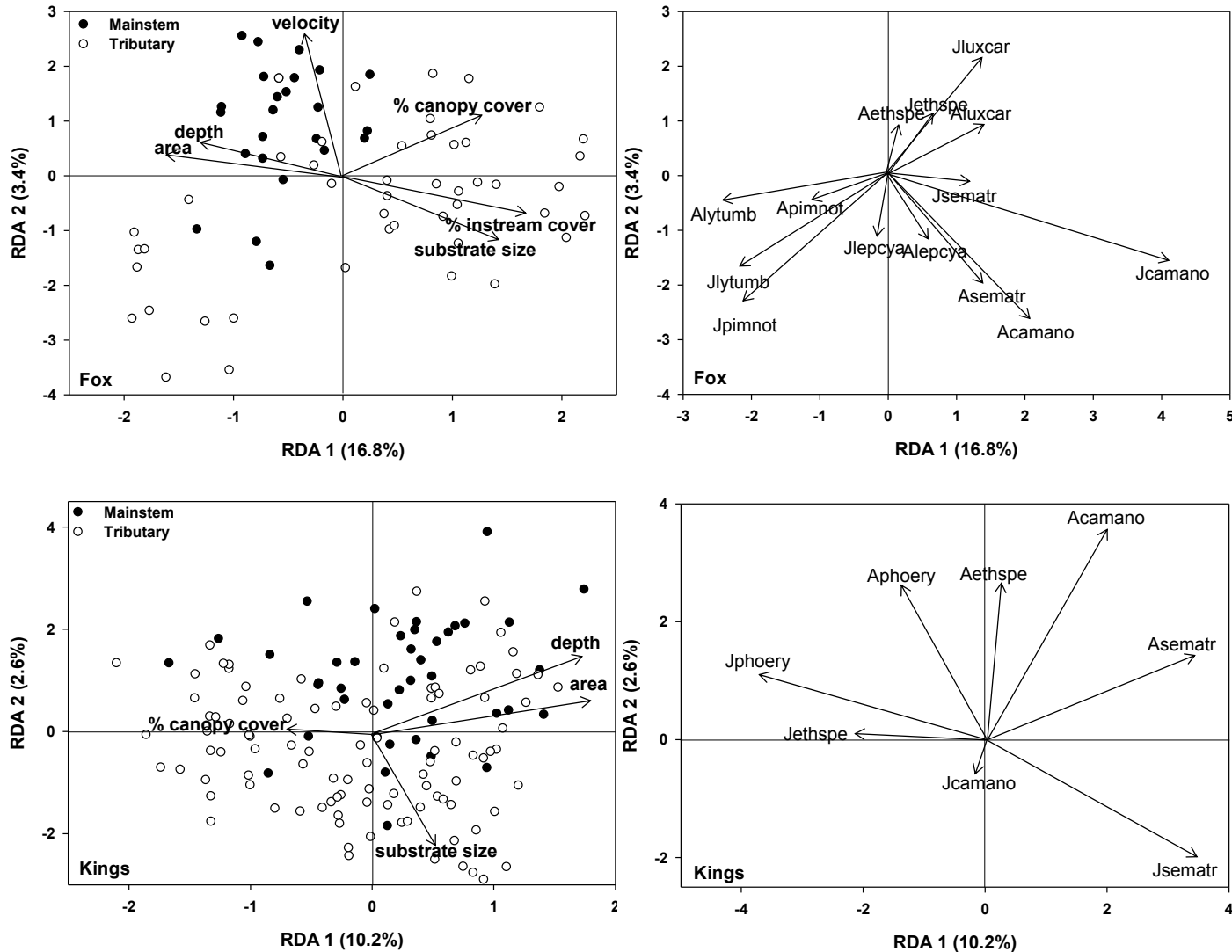
Chapter 3 – Influence of fish richness on ecosystem properties of headwater prairie streams

Abstract

Organisms can regulate their environment through a number of direct and indirect pathways, but identifying the specific circumstances under which populations or communities exert a strong influence on their environment is difficult. Taxonomic and functional richness of communities have been proposed to play a role in ecosystem processes, but evidence from observational studies has shown mixed results. In this study, we evaluated the effect of a natural gradient of taxonomic and functional fish richness on several key ecosystem properties in experimental streams by measuring responses in treatments with zero to three species and zero to two functional groups. We simulated headwater prairie streams using experimental stream mesocosms that consisted of an upstream-to-downstream gradient with pool-riffle structure at each section of the gradient. Ecosystem responses were analyzed individually using general linear mixed models. Increases in fish richness had complex effects on algal filament lengths, benthic and floating algae, benthic organic matter and macroinvertebrate biomass; often the effects of fish were dependent on habitat type and longitudinal stream location. Specifically, there were shifts in biomass of floating algal mats among treatments, with low mass of floating algae in two grazer species treatments to over 88% of the pool surface covered in the absence of fish. When two species of grazers (southern redbelly dace; *Chrosomus erythrogaster* and central stonerollers; *Campostoma anomalum*) were present, algal filaments were shortest and floating chlorophyll was at its lowest. In a monoculture treatment (stonerollers), no evidence of difference was found to that of two species, however, algal filament lengths had a large habitat

disparity. When an insectivorous species was introduced, response of some stream properties were intermediate between grazer no fish treatments. Our findings indicate that functional composition of fish communities alter the structural properties of prairie streams illustrating the potentially important role of fishes in mediating ecosystems in headwater prairie with species diversity.

Introduction

Effects of fishes on stream ecosystems have increasingly been recognized since the classic work by Power and colleagues identified the influence of grazing fishes on the structure of stream periphyton communities (Power and Matthews 1993, Power 1984, Power et al. 1988). Subsequent studies have illustrated the potential for stream macroconsumers (fish and crustaceans) to affect other aspects of ecosystems including changes in benthic invertebrate community structure (Gilinsky 1984) and nutrient cycling (Vanni 2002, McIntyre et al. 2008, Schmitz 2008, Flecker et al. 2010). Nevertheless, it is still difficult to predict the location and timing of fish effects on ecosystem properties (Vanni 2010) and there is need to identify the spatial and temporal patterns of fish effects.

Headwater streams make up over 70% of total stream length (Naiman 1983, Benda et al. 2005, Lowe and Likens 2005) and ecosystem processes in these systems affect larger downstream reaches through downstream transport of materials (Vannote et al. 1980), specifically water, nutrients, organic matter, invertebrates and larger debris (Wipfli and Gregovich, 2002, Compton et al. 2003, Gregory et al. 2003). Thus, headwater systems contribute to the ecological integrity of larger stream networks (Freeman et al. 2007). Fish affect numerous headwater stream properties including structure of periphyton and macroinvertebrate communities and stream function (most often measured as primary production and respiration).

Gelwick and Matthews (1992) measured how central stonerollers (*Campostoma anomalum*) influenced ecosystem attributes, including algal height, multiple measures of benthic organic matter, bacterial abundance, and several measures of primary productivity and nutrient concentrations / ratios. Specifically, they found grazers reduced organic matter biomass and primary production, and those effects lasted up to 55 days. These results agree with earlier studies that showed how small-bodied grazing fishes influence ecosystem properties (e.g., Power and Matthews 1993, Power 1984, Power et al. 1988). It may then be expected that differences in functional composition of headwater stream fish communities might influence the ecosystem response. For example, Flecker (1992) compared small-bodied (2 - 4cm) grazing fish (Loricoridae) to small-bodied insectivors (Crenuchidae) and found grazers reduced both the number of invertebrates and sediment dry mass more than insectivores. Understanding how changes in fish population or community structure in headwaters affect ecosystem properties is necessary to predict changes in structure and function at broader scales in the stream network.

Community composition has been proposed as a strong determinant of how fishes affect stream ecosystems through processes such as complementarily, resource use or trophic cascades (Hargrave 2009). Presence of some dominant species, such as grazing fishes (Gelwick and Matthews 1992, Bertrand and Gido 2007) and detritivores (Flecker 1996, Winemiller et al. 2006), has been associated with changes in algae and invertebrates, but those effects might be mediated by the presence of other species. For example, Power et al. (1985) showed grazing minnows reduced algal filament lengths in pools, but when predatory bass were added minnow grazing shifted to shallow pool margins and filament increased in deep areas. Other studies have shown how community composition influences stream ecosystems. Vanni (2002) found that nutrient (nitrogen and phosphorus) excretion rates varied approximately 10X across 26 species of

fishes in Venezuelan streams. Similarly, McIntyre et al. (2008) found that variation in fish community structure (e.g., body size, density, species composition) and biomass distribution were important drivers of nutrient loading in a tropical stream. Hargrave (2009) found that increasing fish species richness in stream mesocosms was associated with increased mesocosm primary production, and that relationship strengthened over the 42 day experiment. Combined, these studies suggest various mechanisms by which taxonomic and functional composition of fish communities may influence stream ecosystem properties.

Low taxonomic diversity of headwater streams (Schlosser 1982, Martin 2013) and relatively simple food web dynamics allows for straightforward manipulations of community structure to investigate the role of species and functional groups in properties of stream ecosystems. Because fish densities can be quite high (>15 individuals per m^2) in these habitats (Franssen et al. 2006), measuring the influence of variable functional and taxonomic diversity on ecosystem process in headwater streams might help understanding processes at the watershed scale. In this study, we evaluate how fish species richness in small prairie streams affects whole-stream metabolism and biomass distribution of benthic organic matter, algal and macroinvertebrate communities. We examined this question by stocking experimental stream mesocosms that included pool-riffle habitats across an upstream-to-downstream gradient with three fish communities reflecting the nested structure of headwater prairie streams. These communities included three common prairie stream fish species; central stoneroller, southern redbelly dace, and creek chub (*Semotilus atromaculatus*). Stonerollers are often the only species in small, ephemeral tributary stream reaches and comprised the single species community in our study; stonerollers and dace often occur concurrently in small, perennial stream reaches; and all three species exist together in larger, perennial stream reaches. It is unusual to find stream

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reaches where only dace or chub occur alone, and long-term monitoring of four sites of variable size and water permanence indicated no instances where these two species consistently comprise a community (Franssen et al. 2006, Martin et al. 2013).

The objective of this study was to quantify how changes in species richness and composition of small-bodied prairie stream fishes affect multiple stream ecosystem properties in experimental settings. We hypothesized that any effects of increased fish richness and corresponding compositional changes on measured ecosystem properties would depend on the relative importance of direct versus indirect effects (Fig. 3.1). The addition of either algivorous species (stoneroller or dace) is expected to decrease algal biomass via consumption and bioturbation, but the magnitude of this effect could be offset by nutrient remineralization. Grazing fishes may also homogenize resources within pools where they graze, but might increase heterogeneity among habitats by stimulating algal growth in riffles indirectly through excreted nutrients. The addition of the third species (creek chub, an insectivore) is anticipated to stimulate algal growth through both a trophic cascade (i.e. consuming grazing insects) and nutrient remineralization (i.e. relative to consumption/remineralization of the two grazing species), thus diminishing the influence of algivorous fishes on both abundance and distribution of resources.

Methods

Mesocosm Design and Treatments

For this study, we used experimental stream mesocosms (as described in Matthews *et al*. 2006) located on Konza Prairie Biological Station (KPBS). Reaches included a series of three 2.5m^2 pools connected by 0.8m^2 riffles (Fig. 3.2), for a total area of 10.1m^2 per reach. Flow was generated by pulling water through a 15.2 cm diameter plastic pipe from the downstream pool to

the upstream riffle with a trolling motor; allowing us to distinguish up-, middle-, and down-stream habitats. Prior to running the experiment mesocosms were power washed to remove the majority of organic matter (Bertrand et al. 2009). Reaches were randomly assigned to experimental treatments consisting of a control (i.e. no fish) and three fish communities, namely stonerollers alone, stonerollers with dace, and stoneroller, dace and creek chub) and a control with no fish. Single species treatment and combined species treatments were stocked at a constant density of 90 individuals per reach (26.6 fish/m^2; approximately 15g/m^2). The rationale for a constant stocking density across richness treatments was based on our long-term observations from Kings Creek (1995-2012) which found no evidence that small headwater stream richness is related to density of fishes (linear regression; $r^2 = 0.04$, $P > 0.102$). We also selected fish of similar sizes for all species (60-70mm total length, weight = 0.53 – 0.88g per individual; Table 3.1).

Stream ecosystem response variables

Response variables of interest to characterize the stream ecosystem included algal filament length [cm], benthic chlorophyll *a* [µg/cm^2], floating chlorophyll *a* [mg/cm^2], macroinvertebrate community structure and biomass [g/m^2], and benthic organic matter [mg/cm^2], whole-stream metabolism. These responses were measured four weeks after initiation of the study. Three mesh baskets (6 cm deep, 10x10 cm area) were placed in each habitat, location and treatment (N = 432). One basket was removed from each habitat and one pebble from the basket collected for chlorophyll analysis. Benthic chlorophyll *a* was extracted by submerging pebbles in a 95% ethanol solution that was heated for 5 minutes at 78°C and the extract analyzed using a spectrophotometer after 24 hours. Concentration of chlorophyll *a* was

corrected for cross-sectional area of pebbles (see Sartory and Grobbelaar 1984 and Bertrand and Gido 2007 for detailed methods).

Floating algal mats were quantified by first photographing the water surface of each stream mesocosm, and calculating the proportion of habitat covered with floating algae. Biomass (as chlorophyll *a*) was then measured for floating algal mats by taking a square core of the mat (10 by 10 cm) and following the above described procedure for extracting chlorophyll *a*. Only one sample was taken per pool and averaged across habitats from different stream units to calculate a grand mean density (g/m^2) of chlorophyll *a* in floating algal mats. We also calculated the benthic:floating chlorophyll ratio to illustrate the vertical distribution of algae.

To quantify structural properties of periphyton, algal filament length was sampled at three points along three different transects in each riffle (n = 9) and at six points along six different transects in each pool (n = 36) within each reach. Filament lengths was defined as the length of the longest filament attached to a pebble that occurred on each transect point.

Macroinvertebrates and benthic organic matter (BOM) samples were collected from three baskets in each habitat by emptying the pebbles into 8 L of water in a large bucket. The substrate was vigorously stirred and a 500 mL subsample of the slurry was collected for fine and course BOM. The remaining slurry was elutriated to separate inorganic substrate from organic matter and poured through a 250 μm mesh sieve to capture macroinvertebrates. Samples were preserved in 10% formalin and brought to the laboratory where invertebrates were counted and identified to order or family. Lengths of macroinvertebrates were measured for all individuals to calculate biomass for each habitat using standard length-mass relationships (Benke 1984). Chironomids were initially classified as *Tanypodinae* or non-*Tanypodinae*; however, because *Tanypodinae* chironomids constituted < 2.4% of sample biomass, all chironomids were

combined into a single group. The 500 mL benthic organic matter samples were preserved using 10% formalin and brought back to the laboratory where they were filtered, dried and ashed to obtain the amount of fine (GF/F 0.7μmmicrofiber filter) and coarse (1mm mesh filter) organic matter (Wallace et al. 2007). The ash-free dry mass (AFDM, g/m^2) was standardized by surface area of the basket.

Whole stream metabolism (respiration, gross primary production and net ecosystem production) was based on fluctuations in dissolved oxygen content of the water measured over a 24 hr period. These ecosystem rates were corrected for variation in temperature, dissolved oxygen, light, atmospheric pressure, stream morphology based on the modeling technique outlined in Riley and Dodds (2013). This method varies estimates of respiration, photosynthetic and aeration rates to minimize the sum square error between observed and predicted dissolved oxygen.

Fish distribution

Occurrences of individuals of each species were counted in each habitat to assess the association between fish distribution and properties of the stream ecosystem. Observations of fish positions were made through viewing ports on the sides of each pool (Fig. 3.2) and conducted every other day throughout the experiment at three time periods; early morning (8:00), afternoon (13:00) and evening (18:00).

Data Analysis

A general linear mixed model was fitted to each environmental response variable. The linear predictor included the fixed effects of treatment (i.e. 0, 1, 2, 3 species), habitat (pool vs riffle), and location (up-, mid- or down- stream) as well as all 2- and 3-way interactions. Random effects in the linear predictor included the reach nested within treatment to recognize the

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experimental unit for fish richness and composition. Also, reach was further crossed with habitat and with location to recognize the strip-plot design nested within each reach and corresponding experimental units for habitat and location, respectively. The overall experimental design may be described as a 3-way factorial in a strip plot arrangement nested within split-plot in a completely random design (Littell et al. 2006; Table 3.2, Fig. 3.3) Random effects were kept in the model even if their variance component converged to zero to recognize the full design structure of the experiment. As a consequence of variance components converging to zero, degrees of freedom were estimated using the default containment method.

Heterogeneous residual variances by habitat or treatment were deemed necessary to meet model assumptions and were further supported by enhanced model fit (Appendix A.1), as assessed with Bayesian Information Criterion. All variance components were estimates using residual maximum likelihood. Model assumptions (e.g., outliers, distribution, heterogeneity) were evaluated externally using studentized residuals and were considered to be appropriately met.

Statistical models were was fitted using the GLIMMIX procedure of SAS (Version 9.2, SAS Institute, Cary, NC) implemented using Newton-Raphson with ridging as the optimization technique. Estimated least square means and corresponding standard errors [SE] are presented. Relevant pairwise comparisons were conducted using either Tukey-Kramer or Bonferroni's adjustment, as appropriate in each case, to avoid inflation of Type I error rate due to multiple comparisons. We chose to explore marginal p-values, especially in the case of interactions, because interactions might include strong effects but are constrained by habitat or location

Responses on whole-stream metabolism (i.e. community respiration [R], gross primary production [GPP], and net ecosystem production [NEP]) were recorded using single

measurements obtained at the reach level and were evaluated using a standard ANOVA to assess the effect of fish richness and compositions. Externally studentized residuals were examined for heterogeneous variances and outliers. Analyses were conducted in the "stats" package in program R 2.15.3 (R Development Core Team (2008). Post-hoc comparisons were made using Tukey's Honestly Significant Difference (HSD).

To test if the number of individuals observed in pools (counts) varied among the three locations, visual counts were analyzed using repeated measures (rm) ANOVA with location as the main effects and time ($N = 11$ observation days) as the repeated factor. Separate rmANOVAs were conducted for each species and treatment combination (i.e., 6 tests) and pairwise comparisons were conducted using Tukey adjustment. Riffles were excluded from analyses because fish occurred in riffles <1% of occasions. Although fish distribution was measured three times each day, preliminary analyses found no differences in distribution within a day, therefore rmANOVA used only afternoon (13:00) observations.

Results

Algal filament length

Algal filament lengths exhibited heterogeneous variance between habitats (Appendix 1), with much greater variance in riffles than pools (Fig. 3.4). After accounting for heterogeneous variances, across all stream locations (up/mid/downstream), there was evidence for a 2-way interaction of treatment and habitat, where algal filament length was shortest in pools compared to riffles in control, single and three species treatments ($P_{adj.}$<0.05; Table 3.3). In turn, there was no evidence for habitat differences in the two species treatments ($P_{adj} = 0.40$), largely due to shorter filaments in riffles. There was also evidence for a treatment by location interaction effect

on filament lengths (P = 0.08) where downstream locations had shorter filaments in controls, but there was no evidence of location differences in fish treatments (P > 0.4).

Benthic and floating chlorophyll

Benthic chlorophyll exhibited heterogeneous variances among treatments (Appendix 1), with greater variance in treatments with fish compared to controls (Fig 3.5). After accounting for heterogeneous variances, there was marginal evidence for an interaction between treatment and habitat (treatment x habitat interaction P=0.07, Table 3.3) where single species riffles had higher benthic chlorophyll than control riffles (P_{adj}= 0.04), and single species riffles had higher benthic chlorophyll than single species pools (P_{adj}= 0.02). Other contrasts among treatments and habitat were not significant.

There was marginal evidence for differences among treatments in mean floating chlorophyll (P = 0.09; Table 3.3, Fig. 3.6). Mean floating chlorophyll was marginally higher in the control treatment than the two species treatment (P_{adj} = 0.09). The ratio of benthic to floating chlorophyll exhibited heterogeneous variances among treatments and habitats. After accounting for heterogeneous variances, we found no evidence for differences in the benthic to floating chlorophyll ratio among any main effects, or 2- or 3- way interactions (P>0.20).

Benthic organic matter

There was evidence for a 2-way interaction between location and treatment (P < 0.001) and marginal evidence for a 3-way interaction with treatment, habitat and location (P = 0.08, Table 3.3, Fig. 3.7) for BOM. The treatment by location interaction was characterized by higher BOM in downstream habitats than in middle and upstream habitats in the three species treatment (P_{adj} < 0.01), and BOM in upstream habitats was higher than middle or downstream habitats for controls (P ≤ 0.002). There was no evidence for significant differences in BOM among locations

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for single or two species treatments (P > 0.17). The 3-way interaction was attributed to increasing mean BOM from down- to up-stream in both riffles and pools in control treatments (P_{adj} < 0.01), but in three species treatments the downstream riffle was higher than other locations (P_{adj} < 0.007).

Whole-stream metabolism

There was no evidence for differences in respiration (g/m^2/d; LSmean= -2.34, SE= 0.39), gross primary productivity (g/m^2/d; LSmean = 2.91, SE= 0.30) or net ecosystem production (LSmean = 0.57, SD= 0.24) among treatments (P > 0.44).

Macroinvertebrate biomass

Macroinvertebrate biomass exhibited heterogeneous variances by treatment and habitat where variance was greater in riffles than pools (Appendix 1) and greater in fish treatments than in no fish controls (Fig. 3.8). There was evidence for marginal treatment effects on macroinvertebrate biomass (P=0.09; Table 3.3), characterized by a trend of higher macroinvertebrate biomass in treatments were fish were present (P>0.1).

Fish Distribution

Repeated measures ANOVA found evidence that fish distribution was dependent on fish treatment (Fig. 3.9). Tukey adjustment for multiple comparisons set significance to 0.008. In the single, two and three species treatments, no evidence was found that stoneroller numbers were different among locations (single species, $F_{2,86}$= 2.01, P = 0.14; two species, $F_{2,86}$= 2.86, P = 0.06; three species, $F_{2,86}$= 3.01, P = 0.05). In two and three species treatments, there was evidence that dace numbers differed among locations (two species, $F_{2,86}$= 20.93, P < 0.0001; three species, $F_{2,86}$= 5.36, P = 0.006), where dace were more common downstream than in

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middle or upstream pools ($P_{adj} < 0.02$). In three species treatments, no evidence was found that creek chub numbers varied among locations ($F_{2,86} = 1.41$, $P = 0.25$).

Discussion

Results of our study illustrate how changes in community (species richness) and functional (trophic guild) composition of fish communities can alter the distribution of primary producers and organic matter in small prairie streams. Responses of ecosystem properties to fish treatments were often dependent on habitat type and longitudinal stream location. A notable finding was a shift in biomass of floating algal mats among treatments, with minimal floating algae in two species treatments to over 88% of the pool surface covered in controls. Moreover, when two species of grazers (dace and stonerollers) were present, algal filaments were shortest and floating chlorophyll was at its lowest. Floating algal mats are formed by algal filaments reaching the surface, thus the ability of fish to crop filaments directly impedes the formation of mats. In single species treatments (i.e., only stonerollers), there also was a discrepancy between algal filament lengths and benthic algal biomass between riffles and pools. When an insectivorous species was introduced, effects were often somewhere between grazer treatments and no fish control values. The ability of fish to alter the distribution of algal biomass is likely the results of both direct and indirect effect (Fig. 3.1) and represents a mechanism in which abundance and composition of headwater fish communities can influence ecosystem properties.

A shift in the distribution of algal biomass from the benthic zone to floating mats can have important effects on ecosystem properties, such as shading benthic biota (Shigesada and Okubo 1981), serving as predation refugia for macroinvertebrates or small fishes (Power et al. 2008), an aid to dispersal and emergence of macroinvertebrates (Highsmith 1985, Power 1990), and reduction in edibility of algal resources (Chick et al. 2008). Power (1990) showed that

macroinvertebrate community structure was different in floating mats compared to benthic turf mats and that production and emergence was two to six times higher in floating mats, largely due to reduced fish predation in floating mats. Mature floating mats also might be less edible due to the calcareous matrix that deters fishes from foraging, as found by Chick et al. (2008) in the Florida Everglades. A lack of grazing on these mats could allow rapid succession of algal populations that leads to senescence and accumulation of detritus (Lamberti et al. 1987). Whereas, algal mats may provide additional habitat for macroinvertebrates, if anything, there was a slight trend for macroinvertebrates biomass to be lowest in control treatments where floating mats had the highest coverage. Because we did not sample the invertebrate community in algal mats it is possible we underestimated the total biomass of invertebrates in mesocosms, particularly in those with high amounts of floating algae.

The ability of fishes to influence abundance and structural properties of primary producer communities is likely dependent on abiotic conditions (Gido et al. 2010). In natural prairie streams, development of long algal filaments is impeded by flooding (Bertrand et al. 2009), and formation of floating mats are more likely during periods of low or no flow when fish might be congregated in high densities. Therefore we hypothesize that floating algae would most likely occur in natural stream locations during base flow 1) when no fish are present or 2) at sites with equal abundances of grazers and insectivorous fishes. It is also important to note that the presence of fishes might also complement effect of other consumers. For example, Murdock et al. (2010) found that fishes impeded the development of periphyton communities soon after disturbance, but after five weeks, herbivorous insects became the dominant grazers and maintained low biomass of periphyton in Kings Creek, Kansas.

While grazing fishes tended to decrease algal filament lengths, the benthic chlorophyll was higher in riffles of stoneroller only treatments and lowest in control riffles. This could be a factor of shading by floating mats, but selective grazing could also explain decreased filament lengths with increased benthic chlorophyll. For example, Hunter (1980) found that grazing snails decreased standing algal crop from over 30mg of dry weight when no grazers were present to under 7mg over a 45 day period, but while standing crop was greatly reduced there was over a three-fold increase in chlorophyll *a*. Habitat use and grazing behavior might explain the diminished effects of stonerollers on the distribution and structure of algae in riffles and pools when a second grazer (dace) was added. While both species preferred pools, stonerollers spend the majority of their time in contact with the benthic surface and dace occurred higher in the water column (Martin, personal observation). Moreover, Kohler et al. (2011) showed that diet of dace in experimental stream mesocosms reflects the riffle algal community composition more than the pool community composition. Thus, it is possible dace more readily feed on riffle algae either with short forays into riffles during the day or foraging in those habitats during night (Kohler et al. 2011). There also was evidence that replacing one third of the herbivorous fish biomass with invertivorous creek chub resulted in longer filaments and higher floating algal biomass relative to treatments with one or two grazers. We suspect this result is due to a decrease in biomass of herbivores, but nutrient remineralization by chub might also stimulate algal growth.

We found evidence that treatment, habitat and location interacted to affect the distribution of BOM. Similar to response of algal filament lengths, control treatments had a longitudinal pattern, where BOM was highest in up-stream locations and decreased down-stream; however, fish treatments had no evidence of longitudinal patterns. Flecker (1992) found

that very high biomass of grazing fishes can have strong negative effects on sediment in Venezuelan streams. Lack of BOM longitudinal patterns in fish treatments, like algal filaments, could be due to the mobility of fish causing a homogenization of BOM particles. Likely, the presence of fish results in a redistribution of organic matter throughout streams.

Analysis indicated that the heterogeneity of macroinvertebrate biomass and benthic chlorophyll was greatest in the presence of fishes. Alvarez and Peckarsky (2005) found that primary producer biomass heterogeneity was related to mobility of grazing invertebrates, where treatments with high grazer mobility had high producer biomass heterogeneity in artificial stream systems, and that the presence of fish (insectivorous trout) reduced the mobility of *Baetis* mayflies. Given that fish presence reduced the mobility of invertebrates, we might expect primary producer heterogeneity to be lowest in the presence of insectivorous fish, but no difference was found, suggesting a more complex relationship between fish, invertebrates and primary producers. Gelwick and Matthews (1997) measured spatial and temporal heterogeneity of algal filament height in experimental and natural streams and found that stonerollers reduced heterogeneity in artificial environments, but not in natural systems. We found no evidence that fish caused filaments to be more or less heterogeneous of filaments, but we found fish did increase benthic chlorophyll and macroinvertebrate heterogeneity. The Gelwick and Matthews (1997) measured algal filament heights at 1m transects in natural streams and 0.3m transects in artificial streams, whereas our artificial stream study measured algal filaments roughly every 0.07m in a grid, and across multiple habitats within each treatment reach, providing a broader scale with finer resolution and opposing results. It is reasonable that grazer density would affect heterogeneity. Our study stocked fish (60-70mm) at approximately 26.6 fish/m^2, and the Gelwick and Matthews (1997) study stocked grazing fish (45-7mm) at 9 fish/m^2. Differences in density,

scale (single habitats versus our multiple habitat reaches), and measurement resolution between studies could explain why we found fishes increase stream property heterogeneity but Gelwick and Matthews (1997) found stonerollers homogenize filament length. Furthermore, heterogeneous distribution of fishes (across habitats and locations) might be partly responsible for the increased heterogeneity pattern of macroinvertebrates and benthic chlorophyll in our study. Other research has shown that stream fish can affect spatial stream heterogeneity by creating "hotspots" of nutrients and nutrient recycling in natural Neotropical streams (McIntyre et al. 2008, Kapps and Flecker 2013).

Headwater streams (<3rd order) have relatively low taxonomic diversity (Schlosser 1982), but the combined influence of these streams affect the entire river network's nutrient cycling and organic matter processing via longitudinal transport (Freeman et al. 2007). Further, fish densities can be quite high (>15 individuals per m^2) in these habitats (Franssen et al. 2006), therefore measuring the influence of the fish that are present on variable functional and taxonomic diversity on ecosystem process in headwater streams might help understanding processes at the watershed scale. Since streams worldwide are experiencing catastrophic losses of biodiversity due to direct and indirect anthropogenic land- and river-scape alterations (Master et al. 1988, Allan and Flecker 1993, Dudgeon et al. 2006, Jelks et al. 2008), it is becoming increasingly urgent that we gather as much knowledge on protected systems as possible. Our study illustrates that functional composition of fish communities affect the spatial distribution of primary producers and potentially secondary consumers associated with those resources. Based on these results, we might expect that additions or losses of fish species from small prairie streams might alter the distribution of primary producer biomass from benthic to surface and from riffles to

pools. Such changes would likely influence the flux of materials (like insect emergence, see

Power et al. 2008) in and out of streams as well as nutrient cycling within streams.

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Table 3.1 Number of individuals of different species stocked in different treatments used to test the influence of species richness on ecosystem properties of experimental streams. All individuals were approximately the same length and mass, so that biomass across treatments was kept constant ($15g/m^2$).

Treatment	*Species*	*Number*
Single species	Stoneroller	90
Two species	Stoneroller	45
	Dace	45
Three species	Stoneroller	30
	Dace	30
	Chub	30

Table 3.2 Strip-plot design nested within a split-plot in completely random design ANOVA table for linear mixed model. Factor abbreviations are: treatment (Trt; no fish, stonerollers, stonerollers+dace, stonerollers+dace+chub), habitat (Hab; pool, riffle), and location (Loc; up-, middle-, down-stream).

Factor	Degrees of Freedom	
Trt	(4-1) = 3	
Reach(Trt)	4(3-1) = 8	whole plot
Hab	2-1 = 1	split plot
Trt*Hab	(4-1)*(2-1) = 3	strip 1
Reach(Trt)*Hab	8(2-1) = 8	
Loc	3-1 = 2	
Trt*Loc	(4-1)*(3-1) = 6	strip 2
Reach(Trt)*Loc	8(3-1) = 16	
Loc*Hab	(3-1)*(2-1) = 2	
Trt*Loc*Hab	(4-1)*(3-1)*(2-1) = 6	
Reach(Trt)*Loc*Hab	8(3-1)*(2-1) = 16	
Total	72-1 = 71	

Table 3.3 Type III ANOVA results from general linear mixed models. Models for benthic chlorophyll, floating chlorophyll, and algal filament length accounted for heterogeneous variances across habitats and models macroinvertebrate biomass accounted for heterogeneous variances across treatments and habitat. Bold indicate effects with P-values < 0.10.

Response variable	Effect	Df_{num}	Df_{den}	F	P
Algal filament length	Treatment	3	8	3.6	**0.064**
	Habitat	1	8	59.3	**<.0001**
	Treatment*Habitat	3	8	3.6	**0.065**
	Location	2	16	3.5	**0.054**
	Treatment*Location	6	16	1.4	0.280
	Habitat*Location	2	15	3.0	**0.083**
	Treatment*Habitat*Location	6	15	1.1	0.396
Benthic chlorophyll	Treatment	3	8	3.8	**0.045**
	Habitat	1	8	70.9	0.989
	Treatment*Habitat	3	8	6.7	**0.067**
	Location	2	16	3.1	0.259
	Treatment*Location	6	16	0.5	0.739
	Habitat*Location	2	15	0.7	0.674

	Treatment*Habitat*Location	6	15	0.5	0.350
Floating chlorophyll	Treatment	3	8	3.1	**0.092**
	Habitat	1	8	3.9	0.237
	Treatment*Habitat	3	8	2.3	0.270
	Location	2	16	6.7	0.575
	Treatment*Location	6	16	1.7	0.137
	Habitat*Location	2	16	0.9	**0.001**
	Treatment*Habitat*Location	6	16	1.1	0.576
Benthic organic matter	Treatment	3	8	3.5	**0.071**
	Habitat	1	8	41.6	**<0.001**
	Treatment*Habitat	3	8	0.7	0.582
	Location	2	16	2.4	0.121
	Treatment*Location	6	16	10.2	**<0.001**
	Habitat*Location	2	15	0.5	0.635
	Treatment*Habitat*Location	6	15	2.4	**0.077**
Macroinvertebrate biomass	Treatment	3	8	3.1	**0.087**
	Habitat	1	8	1.5	0.259
	Treatment*Habitat	3	8	2.5	0.130

Location	2	16	0.8	0.463
Treatment*Location	6	16	1.5	0.253
Habitat*Location	2	15	2.9	**0.084**
Treatment*Habitat*Location	6	15	2.1	0.110

Figure 3.1 Conceptual diagram illustrating potential direct and indirect effects of headwater prairie stream fishes. Solid lines represent direct negative effects (e.g., consumption, bioturbation) and dashed lines indicate indirect effects (e.g., excretion, remineralization). Line thickness indicated strength of relationships. Panel A illustrates the potential effects of grazers, where the addition of either algivorous species (stoneroller or dace) is expected to decrease algal growth via consumption and bioturbation. The addition of the third species (B; creek chub, an insectivore) should stimulate algal growth through both a trophic cascade (consuming grazing insects) and nutrient remineralization (relative to consumption/remineralization of the two grazing species), thus diminishing the influence of algivorous fishes on both abundance and distribution of resources. Drawings and photos by E.C. Martin.
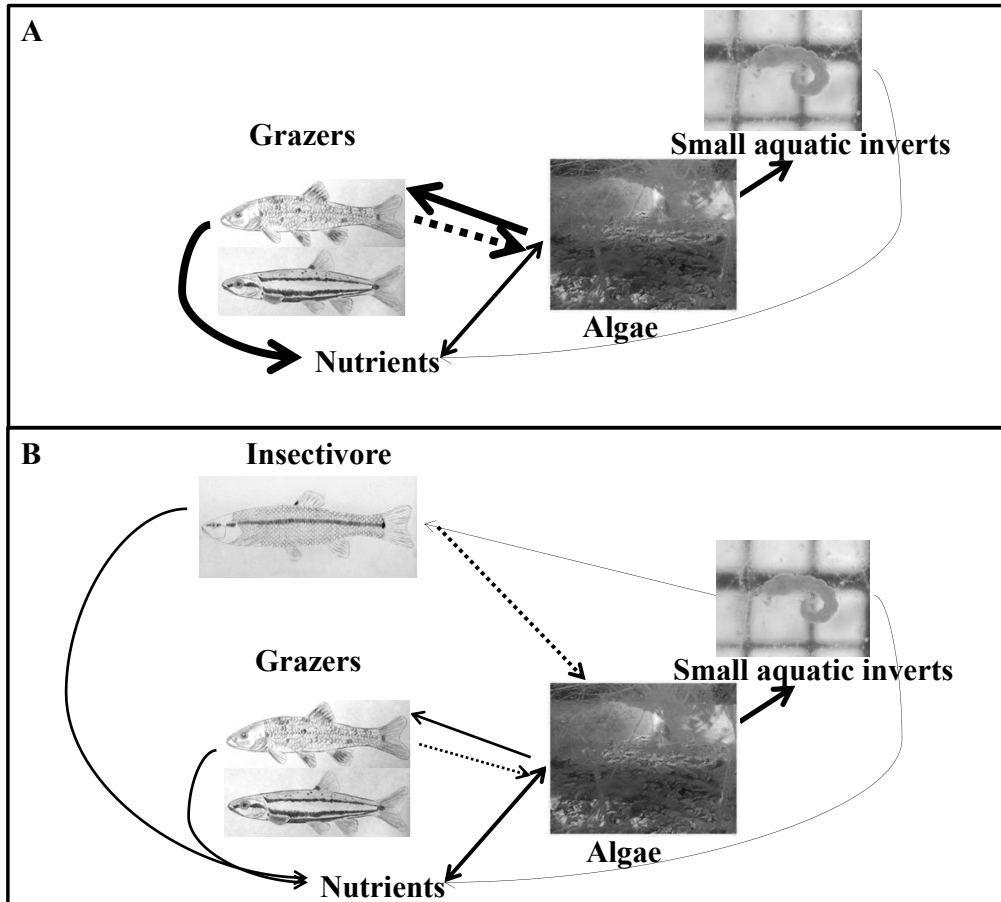
Figure 3.2 Diagram (top) and photograph (bottom) of experimental stream mesocosms on the Konza Prairie Biological Station. Mesocosms are located outdoors under a canopy that provides 60% shading. Flow is generated from a trolling motor in the upstream riffle that pulls water from the downstream pool through a 15 cm diameter plastic pipe buried under the substrate. Fresh spring water continuously flows into the stream from a pipe running across the top of all streams (see photo) and water overflows through holes in each riffle. Observations windows are located on each pool.
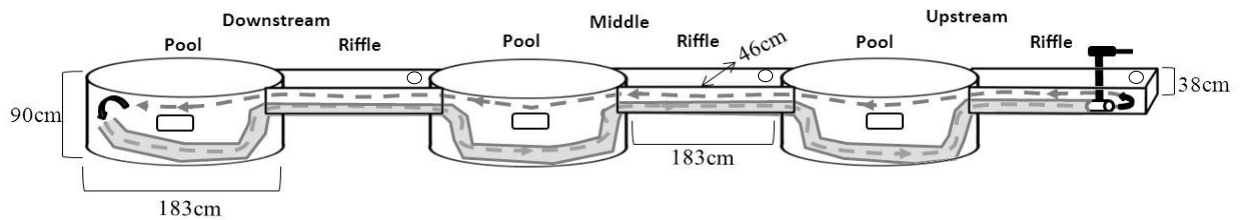
Figure 3.3 Diagram of an experimental reach. Dashed and dotted lines indicates sizes of experimental units for the treatments factors (i.e. habitat and location) in a strip-plot design nested within each reach. Reaches were randomly assigned to treatments. Each fish richness and composition factor (N=4) was replicated three times. Fixed and random effects corresponding to this treatment and design structure, as listed in the legend, were included in the statistical model used for analyses.
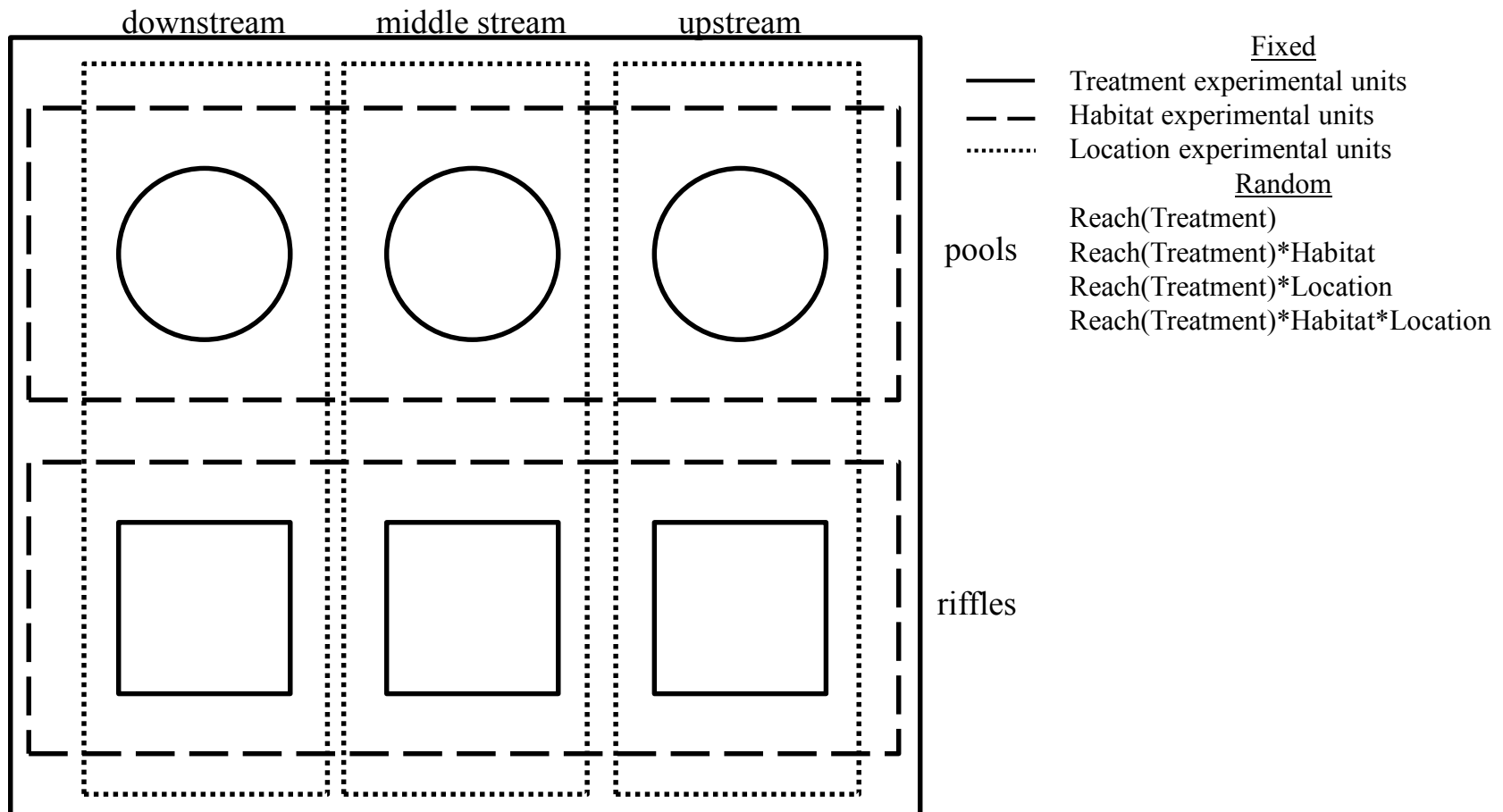
Figure 3.4 Least square mean estimates and standard error (SE) for algal filament lengths across treatments and habitats. Letters indicate significant differences from post-hoc tests with Bonferroni adjustments.
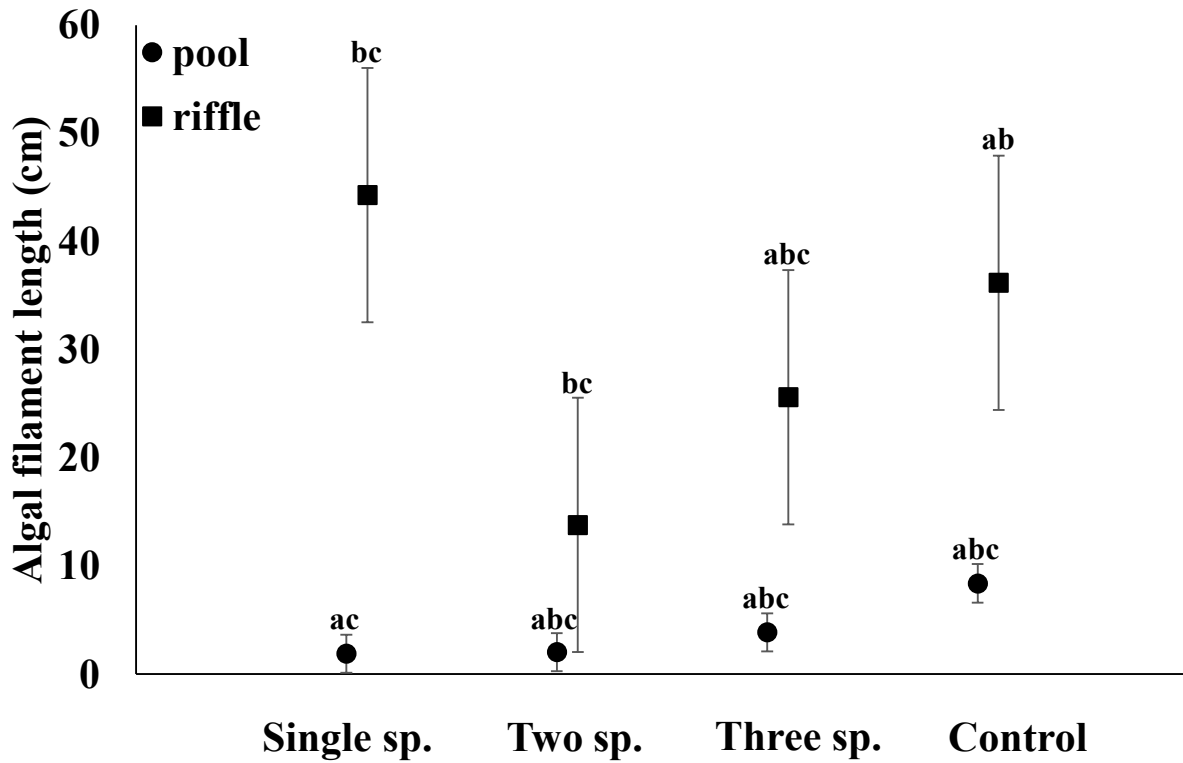
Figure 3.5 Least square mean estimates and standard error (SE) for benthic chlorophyll across

treatments and habitat. Letters indicate significant differences from post-hoc tests with

Bonferroni adjustments.
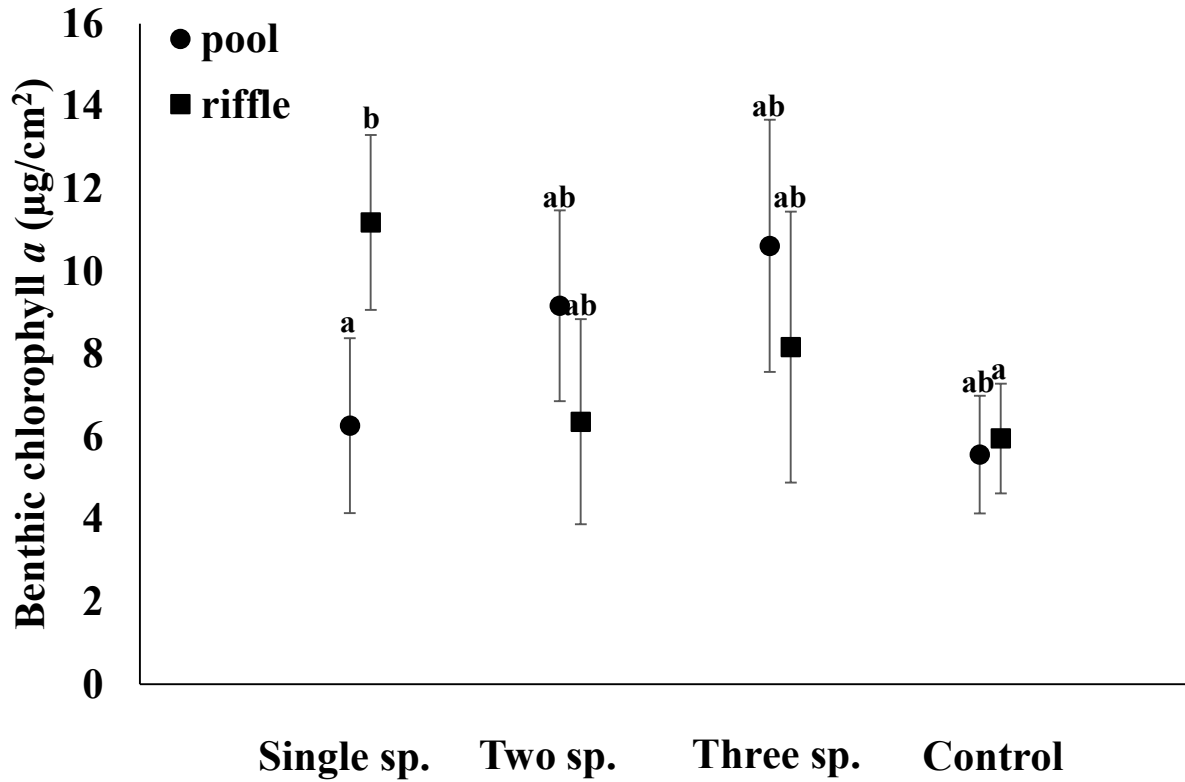
Figure 3.6 Least square mean estimates and standard error (SE) for floating chlorophyll across treatments. Letters indicate significant differences from post-hoc tests using Tukey adjustments.
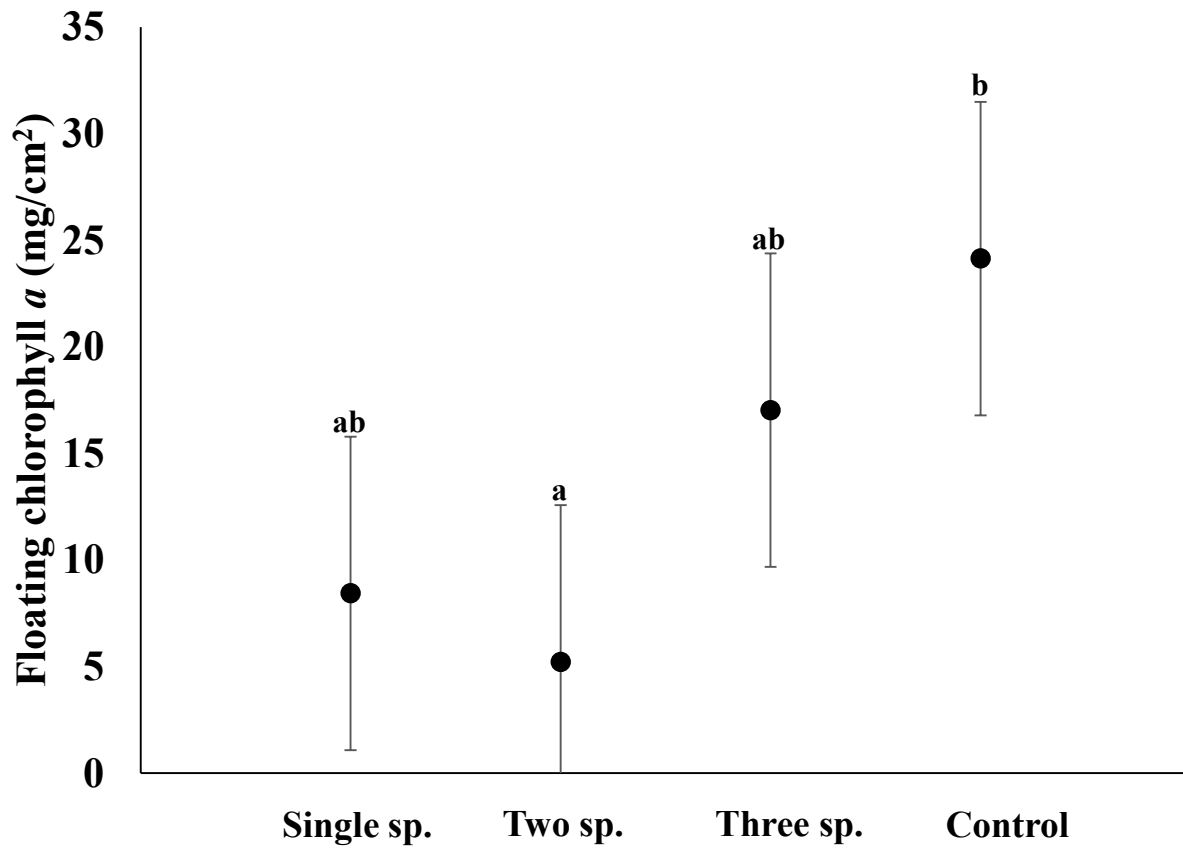
Figure 3.7 Least square mean estimates and standard error (SE) for benthic organic matter across treatments, habitats and locations for pools (top) and riffles (bottom). Letters indicate significant differences from post-hoc tests with Bonferroni adjustments.
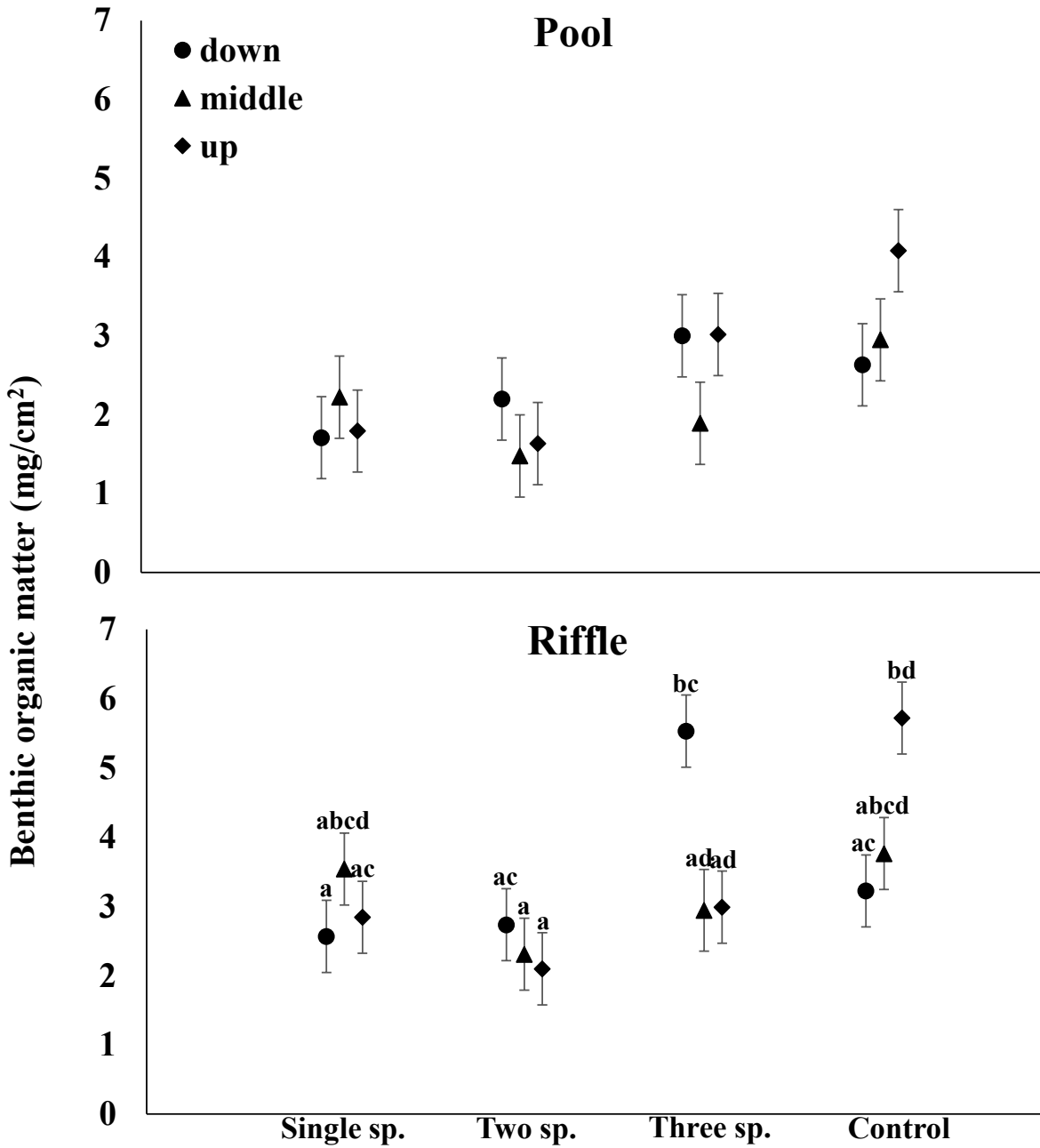
Figure 3.8 Least square mean estimates and standard error (SE) for macroinvertebrate biomass across treatments.
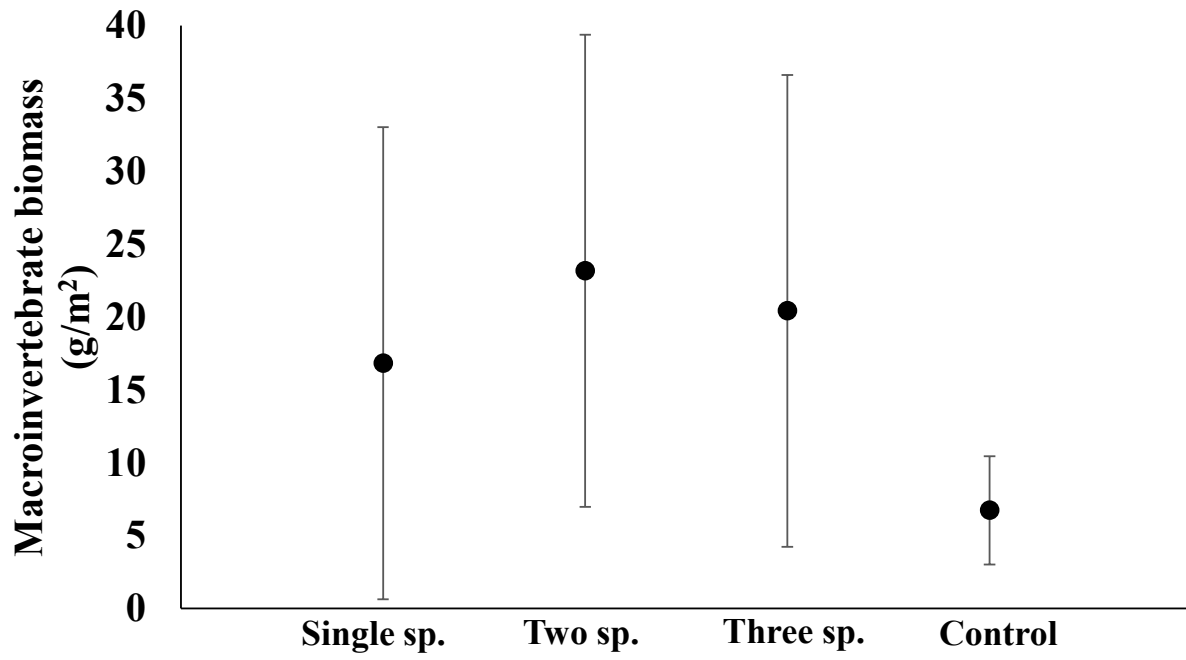
Figure 3.9 Mean proportion of fish among longitudinal pool locations for each species in each treatment. Letters indicate significant differences from post-hoc tests using Tukey adjustments.
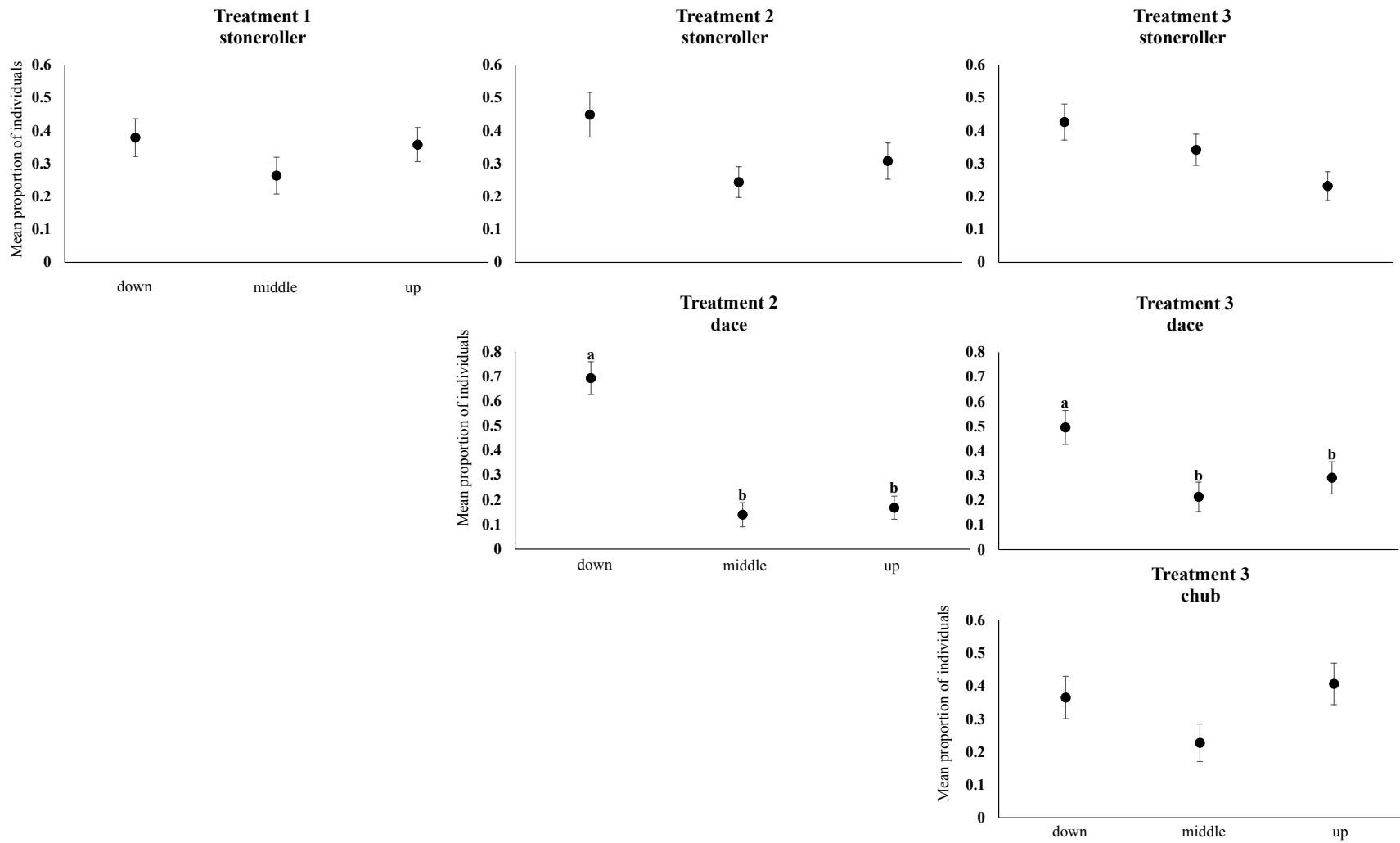
Chapter 4 – Influence of size-structure of herbivorous fishes on prairie stream ecosystem properties

Erika C Martin

Abstract

Per capita foraging rates of smaller individuals should be higher than larger individuals due to metabolic scaling. For herbivorous stream fishes, I hypothesized that when controlling for biomass, juvenile fishes would have a larger per capita impact on benthic organisms than adult fishes. I used mesocosm experiments to test the response of benthic algal biomass, algal filament lengths, organic matter size fractions and macroinvertebrates to the presence of juveniles and adults of two herbivorous minnow species; central stoneroller (*Campostoma anomalum*) and southern redbelly dace (*Chrosomus erythrogaster*). Position in the water column and gut contents of these species and size classes were quantified to assess the role of inter- and intraspecific differences in habitat use and diet. Results indicated large dace treatments had longer filaments than other treatments ($F = 7.5$, $P < 0.01$, $df = 4$). Small individuals of both species were associated with decreases in coarse benthic organic matter ($F = 4.2$, $P = 0.02$, $df = 4$), but only small stonerollers were associated with decreased fine benthic organic matter ($F = 4.1$, $P = 0.02$, $df = 4$). Regardless of size, dace occupied habitats in the middle of the water column and near the bottom, whereas stonerollers occurred near the bottom or had contact with the substrate. Diet of both species and size classes was almost exclusively algae; therefore differences in energetic requirements, rather than diet shifts, are likely driving this response. This study illustrates the importance of considering ontogenetic changes in fishes' energetics and behavior when assessing the influence of herbivorous fishes on stream ecosystem properties.

Introduction

Ecosystems worldwide are experiencing catastrophic losses of diversity due to direct and indirect anthropogenic alterations (Cardinale et al. 2012). In streams, declines in fish diversity and abundance have been extensively documented, both globally (Dudgeon et al. 2006) and regionally (e.g., Xenopoulos et al. 2005, Gido et al. 2010a). Because fishes are the dominant vertebrates in many streams, knowledge of species composition and species' environmental influence are critical to forecast the consequences of their losses to the physical and functional changes in stream ecosystems.

Small-bodied grazing fishes influence ecosystem properties (e.g., Power and Matthews 1993, Power 1984, Power et al. 1988) and can have an unusually large impact on stream ecosystems (Power et al. 1996, Bertrand and Gido 2007, Capps and Flecker 2013). These fishes can affect algal communities directly through consumption (Power 1984, Power et al. 1988, Evans-White et al. 2001, Evans-White et al. 2003, Bengtson et al. 2008) and nutrient cycling (Flecker et al. 2010). In one of the most comprehensive studies, Gelwick and Matthews (1992) found grazers reduced organic matter biomass and primary production, and those effects lasted up to 55 days. Although the above cited studies illustrate the large potential herbivorous fishes have to influence stream ecosystems, effects of these fishes are highly context dependent (sensu Power et al. 1996) and are likely a function of species biomass, ambient nutrient conditions and physiology (Gido et al. 2010b).

Organism size influences its ability to use resources, energetic requirements and susceptibility to predation (Brooks and Dodson 1965, Werner and Gilliam 1984, Klemetsen et al. 2003, Burress et al. 2013, Walker et al. 2013). Differences in juvenile and adult diet are typically an adaptation to maximize growth and survival during the most vulnerable stages of the life

cycle (Pilati and Vanni 2007). Ontogenetic diet shifts are an evolutionary adaptation to alleviate intraspecific competition. Juveniles might prefer smaller, easier to digest prey while adults can forage on larger prey (Nunn et al. 2007, Burress et al. 2013). For example, Pumpkinseed (*Lepomis gibbosus*) exhibit a strong ontogenetic diet shift: small juveniles feed primarily on soft-bodied invertebrates while large adults feed on snails (Osenberg et al. 1992). Gizzard shad (*Dorosoma cepedianum*) in western Lake Erie also have an ontogenetic diet shift, where juveniles feed on zooplankton with high energy content, switching to phytoplankton and detritus as they grow (Price 1963, Mundahl 1988). Evans-White et al. (2001) used stable isotopes to suggest central stonerollers (*Campostoma anomalum*) obtained about 1/5th of their energy from invertebrates and found minor trends in gut contents becoming more algae filled as fish length increased. In contrast, small (30-50mm) southern redbelly dace (*Chrosomus erythrogaster*) had similar diet contents as larger (>60mm total length) individuals; consisting mostly of diatoms and filamentous algae (Bertrand and Gido 2007). In this study, I used two herbivorous species, one that exhibits ontogenetic diet shifts (central stoneroller, hereafter "stoneroller") and one that does not (southern redbelly dace, hereafter "dace"), to test if an ontogenetic shift can alter the impact of a species effect on stream ecosystem properties.

Energetic requirements of fishes is likely to influence the breadth of impact individuals and communities can have on stream properties. In general, the energetic requirements per individual is a function of respiration, waste and growth (Brett and Groves 1979), where an increase in organism mass corresponds to declines in specific metabolic rates (all other variables equal; Peters 1986, Clarke and Johnston 1999). If we use the conventional metabolic scaling equation of Brett (1962), metabolism for a single fish weighing 20 grams is less than 50% of 20 fish weighing one gram each. This illustrates the potential for small fish to have a

disproportionally large impact on stream properties when compared to similar biomass of larger fish. Using an experimental manipulation for this study allowed me to keep external variables constant while manipulating organism size and species.

Both dace and stonerollers impact structural and functional properties of streams (e.g., Power and Matthews 1983, Gelwick and Matthews 1992, Vaughn et al. 1993, Bertrand and Gido 2007, Bengtson et al. 2008); however few studies have directly compared juveniles and adults. Comparison of ontogenetic variation of these two herbivorous species that often dominate abundance in small streams could provide useful because the proportion of small and large fish often varies in stream spatially and seasonally. Long-term monitoring of fishes in Kings Creek, Kansas (see Franssen et al. 2006) shows that proportion of juvenile stonerollers and dace varied annually (Fig. 4.1). At this site, the proportion of juvenile stonerollers varied from 0 to 90% annually, and proportion of juvenile dace from 30 to 89%. I hypothesized that, given equal biomass, smaller fish would have a greater per capita impact on stream properties, based on energetic model predictions, and that the ontogenetic shift would be stronger in stonerollers.

Methods

Mesocosm Experimental Design

I used 20 experimental stream mesocosms (as described in Matthews et al. 2006) located on Konza Prairie Biological Station (KPBS), KS, USA, to test the influence of ontogenetic variation of two grazing prairie fish on stream ecosystem properties. Experimental units (streams) included two macrohabitats; one riffle ($0.8m^2$) upstream of one pool ($2.5m^2$). Flow was generated by a trolling motor pulling water through a 15.2 cm diameter plastic pipe connecting the pool to the riffle. I used five experimental treatments based on observations of the two most

common herbivorous species (stoneroller and dace) in Kings Creek, the stream located on the KPBS. I selected these five variables based on extensive work in natural and experimental streams (Evans-White et al. 2001, Bertrand and Gido 2007, Murdock et al. 2011). All fishes were collected from nearby prairie streams and stocked on the same day. Each species was represented with two size classes creating four fish treatments: juvenile stonerollers (mean length 56mm, [3.9mm SD]; mean weight 2.0g [0.4g SD]), adult stoneroller (81mm [2.7mm SD]; 5.9g [0.5g SD]), juvenile dace (31mm [1.6mm SD]; 0.45g [0.2g SD]), and adult dace (53mm [1.1mm SD]; 1.5g [0.1g SD]). Biomass of fish was held constant between 15 and 20 g/m^2. The fifth treatment was a control, where no fish were added to the streams. Each treatment was replicated four times. To assure flow and depth were homogeneous across treatments, these attributes were measured at three points along three transects in each riffle (n = 9) and at five or seven (five for shorter transects, seven for longer transects near the center of pool) points along four transects in each pool (n = 19) within each reach for roughly 10 measurements per m^2 for each habitat. Based on ANOVA, there were no differences in flow or depth among treatments (P = 0.42 and 0.29, respectively).

Ecosystem Response Variables

I measured ecosystem properties in pools after 30 days (October 1 to 31, 2012). To quantify structural properties of the periphyton, algal filament lengths were measured at three points along three transects in each riffle (n = 9) and at five or seven (five for shorter transects, seven for longer transects near the center of pool) points along four transects in each pool (n = 19) within each reach for roughly 10 measurements per m^2 for each habitat. Filament lengths were the length of the longest filament attached to a pebble that occurred on the transect point. I also placed three 10 cm^2 mesh baskets in each experimental pool to collect benthic chlorophyll *a*,

macroinvertebrates, and organic matter. One basket was removed for chlorophyll *a* analysis:

three pebbles were collected, and chlorophyll *a* was extracted. Concentration of chlorophyll *a*

was corrected for cross-sectional area of pebbles (see Sartory and Grobbelaar 1984 and Bertrand

and Gido 2007 for detailed methods).

A second basket was removed for benthic organic matter analysis. The basket was placed

into 8L of water and the substrate vigorously stirred before 500 mL subsample of the slurry was

collected. The 500 mL benthic organic matter sample was preserved using 10% formalin,

brought back to the laboratory and filtered through two mesh sizes (coarse [1-mm mesh filter]

and fine [GF/F 47 mm microfiber filter]), dried at 60°C, weighed, ashed at 450°C , and re-

weighed to determine ash-free dry mass (AFDM) (Wallace et al. 2007). AFDM was

standardized by surface area of the basket. Macroinvertebrates were sampled by a similar

procedure to organic matter, where a third basket was placed in 8L of water, but the resulting

slurry was elutriated to separate inorganic substrate from organic matter and poured through a

sieve (250 μm mesh) to capture macroinvertebrates. Samples were preserved in 10% formalin

and brought to the laboratory where invertebrates were counted and identified to order or family

(Thorp 2001, Merritt 2008). Chironomids were initially classified as *Tanypodinae* or non-

Tanypodinae; however, because *Tanypodinae* chironomids constituted < 1% of sample biomass,

all chironomids were combined into a single group. Lengths of macroinvertebrates were taken on

all individuals to calculate biomass for each macrohabitat using standard length-mass

relationships (Benke 1984). Macroinvertebrate density (g/m^2) was calculated by dividing the

total biomass of the sample by the surface area of the basket. To test for effects of treatments on

ecosystem function, I monitored dissolved oxygen concentration as an approximation for whole

stream metabolism for the last day of the experiment, beginning at 05:00, taking measurements

every hour, and concluding at 17:00. Gross primary productivity (GPP; g O_2/m^2/d) was based on diurnal changes in dissolved oxygen measurements from a handheld oxygen probe (YSI 550 A). Whole stream metabolism (respiration, gross primary production and net ecosystem production) was estimated from dissolved oxygen and temperature measured every hour for 12 hours at the end of the experiment. The modeling fitting approach using the "solver" function in Excel as described by Riley and Dodds (2013) was used to estimate respiration (R), initial slope of photosynthesis-irradiance curve (α) and maximum rate of photosynthesis (P_{max}) at the measured temperature. I used the reaeration coefficient (k=0.432/d), which was derived from previous research conducted in this system (Murdock et al. 2010). Water was recirculated at the same velocity and the bed-form was similar in all experimental units so turbulence-induced aeration was similar across experimental stream channels.

At the conclusion of the experiment, I collected five stonerollers and five dace from both size classes to characterize diet. Diet items in the foregut were obtained and analyzed under a dissecting microscope. Food items sorted into three generalized classes; detritus, algae or macroinvertebrate animal matter. I calculated total area of gut contents (mm^2) and proportion of each food item (Franssen and Gido 2006).

Data Analysis

I tested for differences in each response variable by performing single-factor ANOVA to test treatment effects ($\alpha = 0.05$). Variables were tested for normality, heterogeneous variance and outliers using studentized residual plots. If the ANOVA was significant, a Tukey's Honestly Significant Difference (HSD) post-hoc test was performed. All statistical analyses were run using R 2.15.3 (R development team 2012) and library MASS (Venables and Ripley 2002).

Because diet was homogenous across species and size classes, no statistical analyses or diet overlap indices were used (see Results).

Results

Environmental response variables

Mean algal filament length ranged from zero to 24 cm, depending on treatment. Large dace treatments had some of the longest filaments, and small dace the shortest. ANOVA results indicated that small dace, small stonerollers and large stonerollers were associated with shorter filaments than large dace (F = 7.5, P = 0.002, df = 4; Fig. 4.2). Small dace and small stoneroller treatments had shorter filaments than controls, and controls had intermediate algal filament lengths between large dace and large stonerollers. Mean chlorophyll *a* concentration ranged from 0.62 to 1.1 μg/cm^2, and there was no evidence that chlorophyll differed among treatments (F = 0.3, P = 0.86). Mean gross primary productivity ranged from 6.4 to 6.8 g O$_2$/m^2/d with no evidence of differences among treatments (F = 0.84, P = 0.52).

Mean coarse benthic organic matter ranged from 0.16 to 0.86 mg/cm^2 per treatment. Results from ANOVA indicated that small dace and small stonerollers were associated with lower concentrations of coarse benthic organic matter than other large fish treatments and controls (F = 4.2, P = 0.02, df = 4; Fig. 4.3). Mean fine benthic organic matter ranged from 0.95 to 1.65 mg/cm^2. Small stonerollers had approximately 1.5 times less fine benthic organic matter concentrations than large dace treatments (F = 4.1, P = 0.02, df = 4). Large stoneroller, small dace and controls had intermediate values. Mean macroinvertebrate biomass ranged from 4.23 to 27.49 mg/cm^2, depending on treatment. I found no evidence of differences among treatments for macroinvertebrate biomass (F = 0.61, P = 0.66).

Fish behavior and diet

There were significant differences in behavior between stonerollers and dace, where dace spent the majority of time in the middle or bottom portion of the water column ($F = 4.1$, $P < 0.001$, df = 3) and stonerollers in the bottom or had contact with the substrate ($F = 4.1$, $P = 0.05$, df = 3; Fig. 4.4). Volume of diet for all 20 individuals consisted of almost exclusively filamentous algae (>95%) for both species and size classes, thus no statistical analyses were conducted.

Discussion

This study suggests that size distribution of a species might alter their impact on the structural stream environment. Results partially supported my prediction that smaller fish can have a more intense impact on stream properties than larger fish of the same species. Whereas both small and large stonerollers were associated with shorter algal filament length, small dace decreased filaments and large dace did not. Reductions in algal filaments by grazing fish is consistent with previous research on stonerollers (Power and Matthews 1983, Power et al. 1985, Power et al. 1988) and dace (Bertrand and Gido 2007). However, results from this study are in contrast to Bertrand and Gido (2007), who found both small and large dace decreased algal filament lengths. Moreover, Bertrand and Gido (2007) found that only large dace decreased chlorophyll *a*.

Previous studies on ontogenetic diet shifts of herbivorous fishes are mixed. Bertrand and Gido (2007), found small (30-50mm) southern redbelly dace (*Chrosomus erythrogaster*) had similar diet contents as larger (>60mm total length) individuals; consisting mostly of diatoms and filamentous algae. A study on stonerollers found minor trends in gut contents becoming more algae filled as fish length increased Evans-White et al. (2001). Similar to Bertrand and

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Gido (2007), I did not see a diet shift in either species, thus differences in diet within and among species is not likely driving differential effect on algal filament lengths.

Small stonerollers and small dace had decreased fine and coarse benthic organic matter and were different from treatments with larger individuals. Similarly, Murdock et al. (2011) found dace decreased total benthic organic matter (1-500µm) in experimental mesocosms early after a flood in spring, but these effects were diminished over time. Bertrand and Gido (2007) found that, under more stable conditions in summer, dace increased fine particulate organic matter (98 - 1 µm size range). Disparity in fish effects on organic matter might be due, in part, to the seasonality of organic matter concentration which, in natural streams, can vary greatly by season (Kemp and Dodds 2001), where fine benthic organic matter is typically highest in spring and lowest in autumn, and coarse benthic organic matter is highest in autumn and lowest in spring. My experimental mesocosm study occurred in autumn, when coarse benthic organic matter can be highest. However, the effects of fishes on benthic organic matter are likely a combination of size and the relationship between organic matter and algae. Indeed, I found a positive relationship among algal filament length and fine (F = 5.8, R^2 = 0.24, P = 0.02) and coarse (F = 17.3, R^2 = 0.46, P <0.001) benthic organic matter. If the relationship between algal filaments and BOM was the only factor, we would expect large stonerollers to have similar values as small stonerollers and dace, but large stonerollers are not different than large dace. This suggests that there is a body size effect fish have on BOM, where the intensity of bioturbation likely increases with individual size and abundance. Previous studies have shown stonerollers influence benthic organisms through bioturbation (Adámek and Maršálek 2013) by scraping algae off substrates (Fowler and Tabor 1985, McNeely 1987).

Interestingly, while I found differences among treatments in structural measures (AFL, BOM), functional measurements in streams (GPP) were not different among treatments. This might be explained by the scale of measurements; AFL and BOM measurements were taken in 10 x 10 cm baskets from pools, whereas GPP integrates processes across pools and riffles. If fish are spending the majority of their time in one habitat, productivity in the other habitat might increase, especially given the recirculating flow (i.e., fish graze in pools, reducing filaments, but high nutrient waste water is pulled from pools and recirculated over riffles, exaggerating their impact on whole stream GPP).

The effects on stream properties discovered in this study can be attributed to a combination of both behavior and energetics. Differences in time spent foraging in different habitats might explain the similarities among small individuals and large stonerollers in their effects on algal filament length. Previous studies in the experimental streams found that large stonerollers and large dace primarily use pool habitats and are rarely found in riffles in experimental streams (Chapter 3). Although I did not compare habitat use of large and small individuals, they might be different, as smaller fish prefer shallower stream habitats in natural systems (Power 1987, Martin et al. 2013). In an experimental study, adult dace gut contents were found to be more similar to riffle algal communities than pools, suggesting they drift feed on riffle algae (Kohler et al. 2011). Thus, large dace may forage less on pool algal filaments than stonerollers and juvenile dace. This study found that stonerollers spent more time in contact with the bottom than dace, which might explain why large stonerollers have a similar impact on algae as small stonerollers and small dace. Along with differences in fish behavior, energetics also likely play a role in explaining the effects different sized fish have on the stream environment. Using the metabolic scaling equation from Brett (1962), I calculated the estimated metabolism

per individual in the experiment using mean weight (X) of fishes and extrapolated the values to account for the differences in total number of individuals per treatment (Table 4.1). I found that, assuming a is constant among these small bodied species (which was a conservative estimate, as values of a are typically higher in larger individuals), adult and juvenile dace treatments had 1.6 to 6 times higher metabolic O_2 consumption rates than stonerollers adult and juvenile treatments, respectively. Small and large stoneroller treatments had similar metabolic rates, but combined small dace metabolism was nearly four times higher than large dace. Based metabolism, it is apparent to expect stonerollers of both size classes to have similar effects on stream properties, but dace to have opposing effects between size classes, which is precisely what this paper has shown. Differences in ecosystem effects between the two species (stonerollers and dace), then, might be attributed to aforementioned behavior variation.

To extrapolate this information to natural systems, my study has shown that, along with abundance and distribution measurements, size structure could provide additional information on variation in population effects. Previous research has shown that juvenile fish use different stream habitats than adult fish, where juveniles prefer shallower areas and cover and are more abundant in small stream (<3rd order) headwaters than the mainstem (Martin et al. 2013). Therefore, streams could experience a reduction in structural properties like algal filament lengths and organic matter concentration where juvenile fishes congregate. Furthermore, fish population dynamics are often cyclical, especially in regards to reproductive or colonization/migration timing. Post-spawning events, when eggs have hatched and fry and juveniles are abundant might have a particularly large impact on stream properties due to sudden consumption of resources. Likewise, fish effects on stream properties might be exacerbated when

small headwater stream reaches are re-wetted and colonized by large individuals when algal and macroinvertebrate biomass might already be low (Murdock et al. 2011).

This experiment used a density of fishes between 15 and 20 g of fish/m^2, which is within the range of densities observed in Kings Creek, but density is highly variable and these densities are only present during some sites and time of year (Franssen et al. 2006). Thus, differences in stream properties between size classes could be exaggerated during certain times of the year, at particular stream locations. Regardless, while biomass is likely the main driver of fish affects in stream ecosystems, this study suggests that size structure is likely an important consideration. The ontogenetic shifts of grazing fishes in these experimental mesocosms is the first step in understanding the complex spatial and temporal effects these species might have in natural stream systems.

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Table 4.1 Table using the energetics equation from Brett (1962) $Y = aX^b$ to calculate the metabolism per individual (Y) based on rate of O_2 consumption per unit weight ($a = 0.3$), mean weight of fishes used in the experiment (X) and the constant b (0.8). The number of individuals (N) was multiplied by the individual estimate (O_2/g; Y) to calculate the total metabolism per treatment.

	Stoneroller		Dace	
	Juvenile	Adult	Juvenile	Adult
Estimated Y	0.61	1.45	0.18	0.48
Mean length (cm)	5.8	7.5	3.5	5.8
X	2.1	4.7	0.5	2.4
N	25	9	500	50
Y*N	15.3	13.1	90.0	24.0

Figure 4.1 Proportion of juvenile dace (black) and stoneroller (grey) in Kings Creek in mainstem stream sites in autumn from 2001 to 2011.
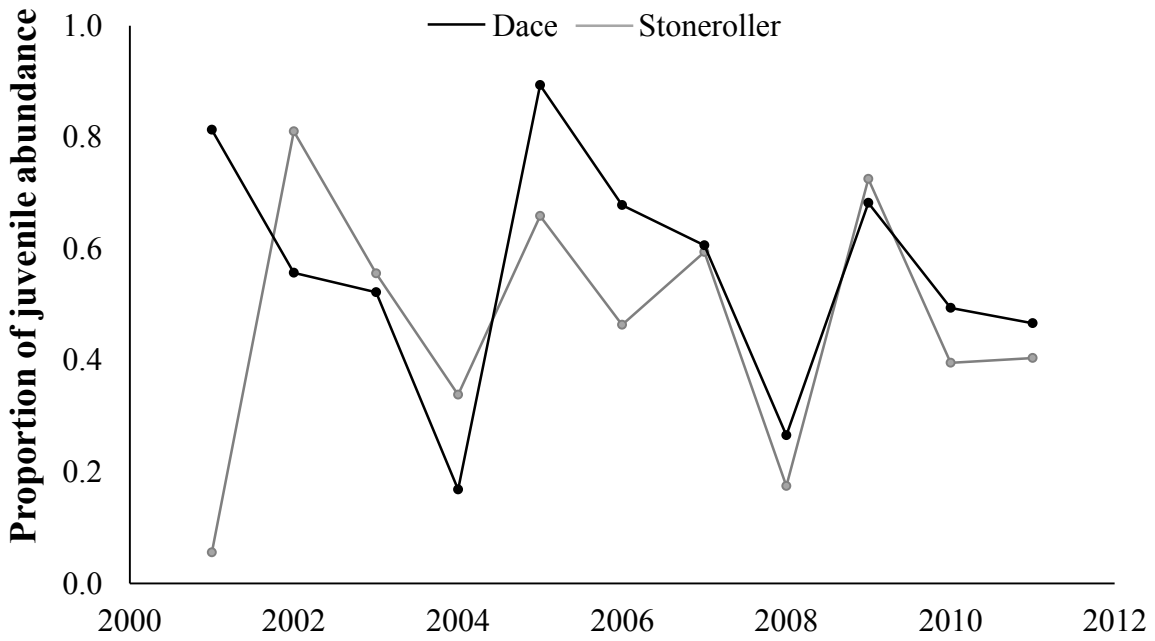
Figure 4.2 Means for environmental response variable algal filament length by treatment. Bars represent standard error. Letters indicate significant (P < 0.5) differences based on Tukey Honestly Significant Difference (HSD).
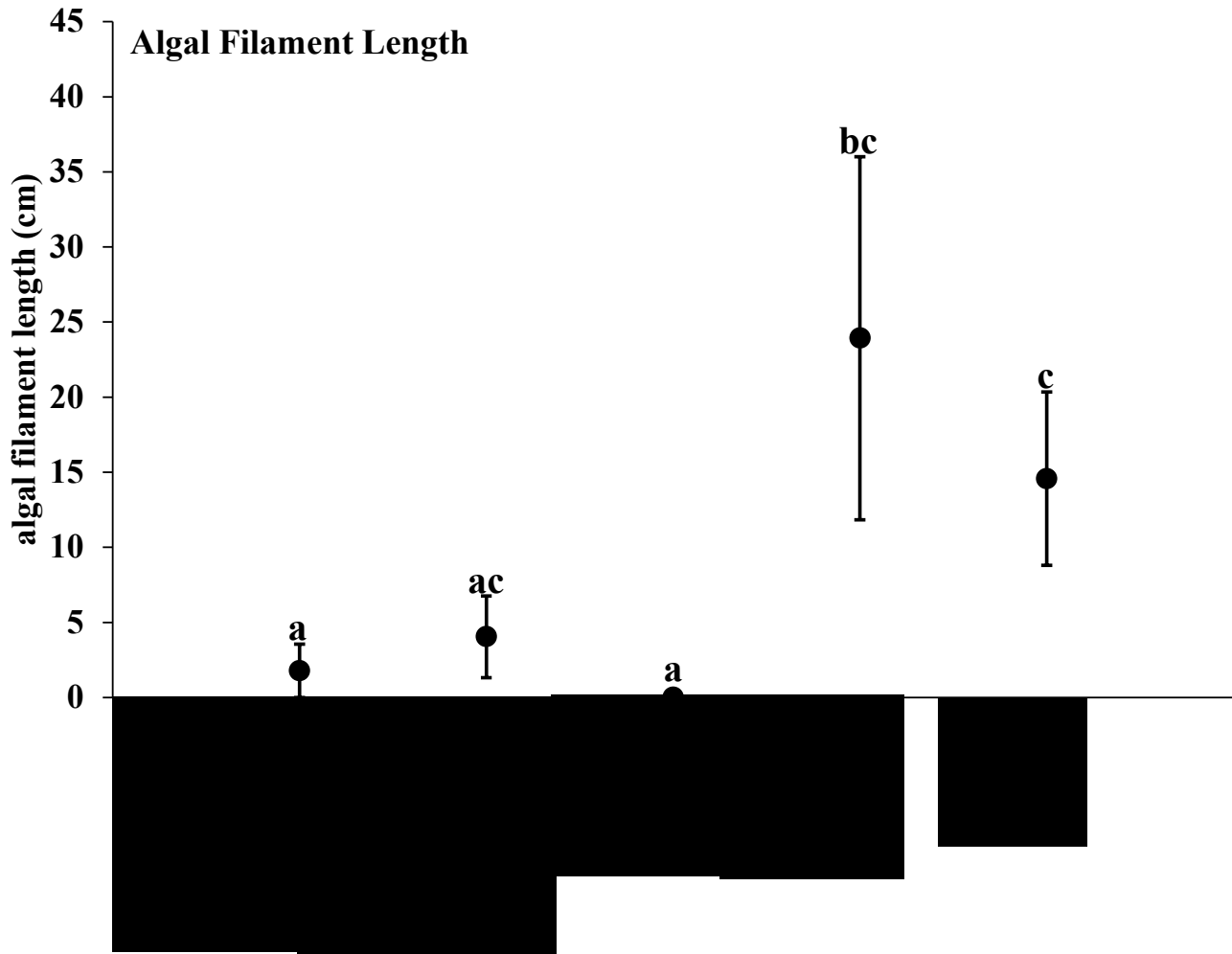
Figure 4.3 Means for environmental response variables fine (top) and coarse (bottom) benthic organic matter by treatment. Bars represent standard error. Letters indicate significant (P < 0.5) differences based on Tukey Honestly Significant Difference (HSD).
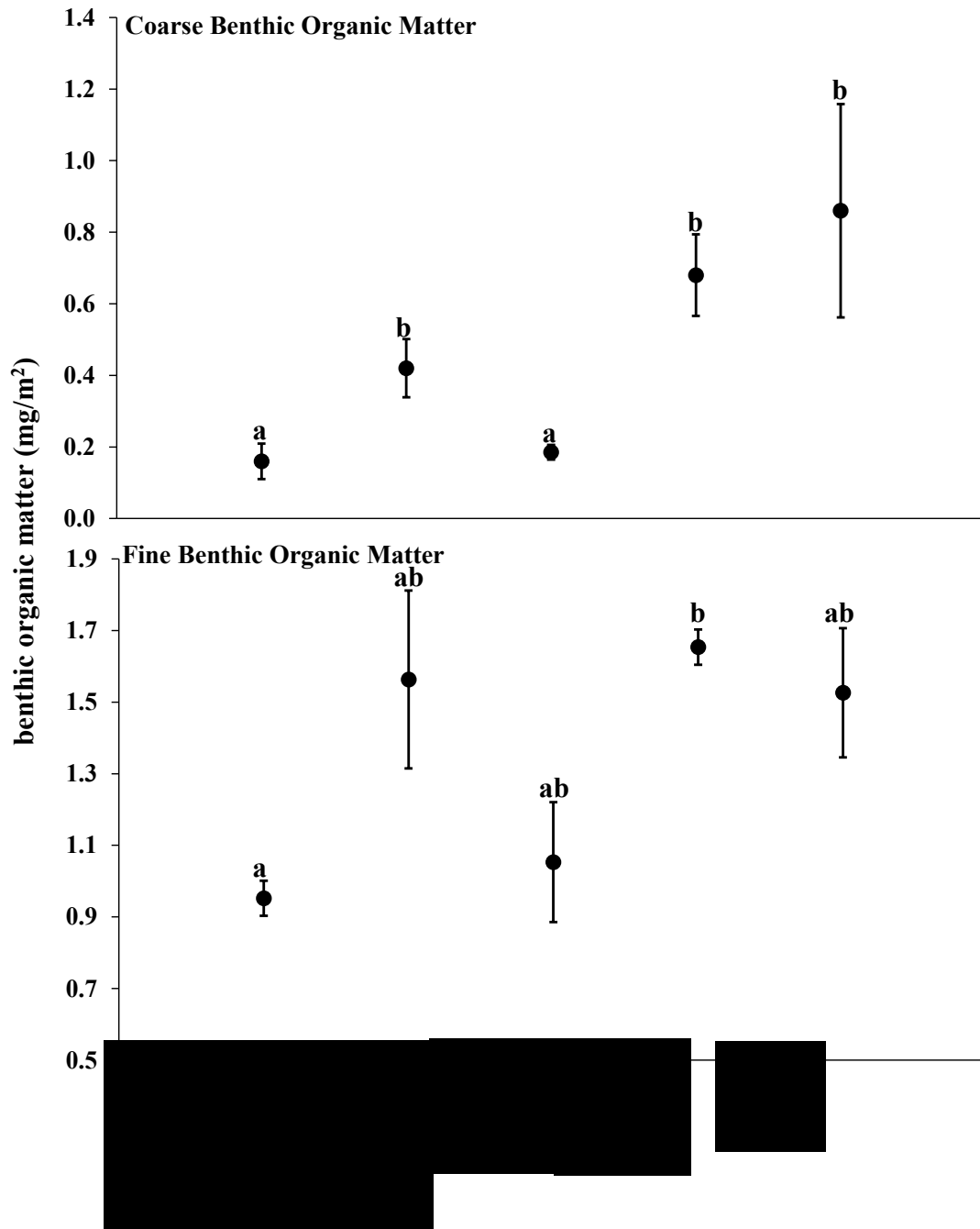
Figure 4.4 Mean number of stonerollers and dace at different water column depths. Bars represent standard deviation.
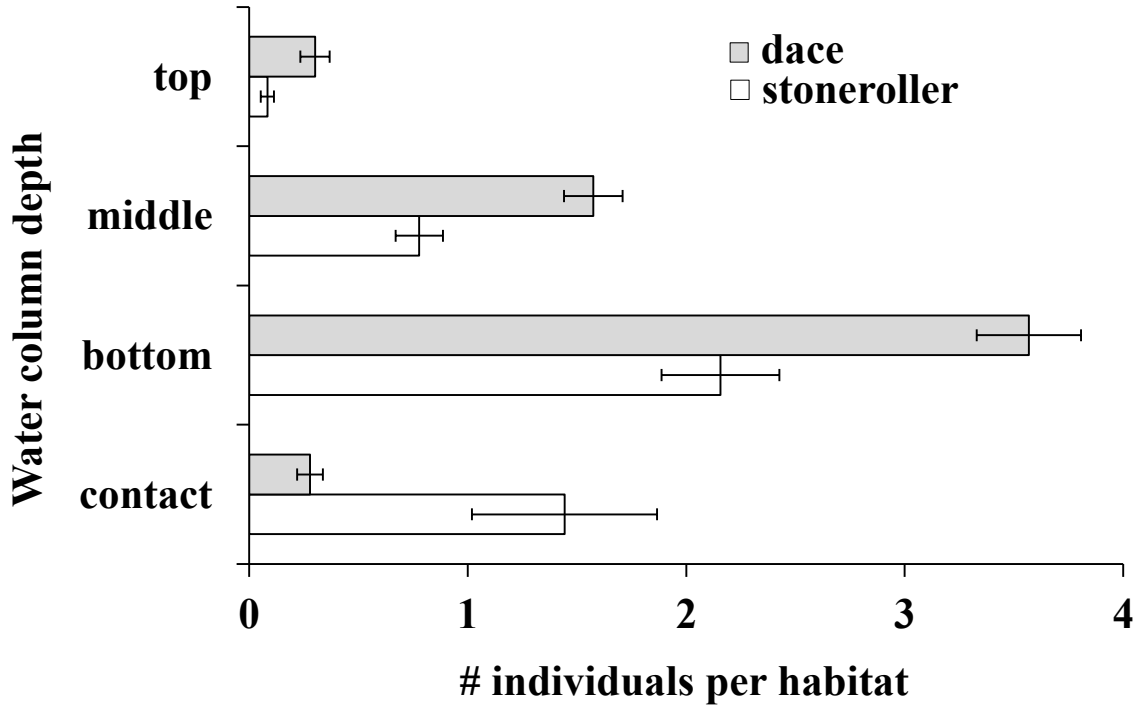
Chapter 5 - Conclusions

Maintaining natural biodiversity and ecosystem function in small headwater streams is particularly critical because headwater streams are first in the hierarchical network of habitats that transport materials downstream (Vannote et al. 1980). Small streams also make up roughly 70% of stream length (Lowe and Likens 2005). Habitat availability, community structure, and organism size are important considerations when quantifying or predicting the effects fishes might have on stream properties.

In Chapter 2, I identified local habitat factors associated with the diversity and density of fishes in two protected prairie watersheds, in Chapter 3, I addressed how fish species richness in small prairie streams affects whole-stream metabolism and biomass distribution of benthic organic matter, algal and macroinvertebrate communities, and finally, in Chapter 4, I quantified how two prairie stream fish species, the central stoneroller (*Campostoma anomalum*) and southern redbelly dace (*Chrosomus erythrogaster*) differentially affected stream ecosystem properties based on their size classes. Pool depth and surface area had the strongest relationship with assemblage composition, where juvenile fishes use shallower waters likely as a refuge from piscivorous fishes and positive associations of adult fishes and pool area and depth are explained partly because larger individuals are less effectively consumed by piscivorous fishes. These small-bodied fishes (central stonerollers, southern redbelly dace, and creek chub) are dependent on richness, habitat type, and directional flow. In pools, increases in fish richness increased benthic chlorophyll, algal filament lengths and benthic organic matter. This pattern was reversed in riffles for benthic chlorophyll, where increased richness is associated with decreased benthic chlorophyll. Often a longitudinal trend of flow was evident, though the up- to down-stream pattern was moderate and dependent on treatments and habitats. Further, the magnitude of effects

of fishes in stream properties is dependent on size. Small grazers (stonerollers and dace) are associated with reduced fine and course benthic organic matter than larger individuals of the same species. However, small dace, small stonerollers and large stonerollers were all associated with shorter filaments than large dace. Ontogenetic shifts are complex, and depend on the variables of interest and species identity, not necessarily functional group.

The complexity of how and when fish affect stream properties is becoming better understood as more studies are conducted; however, there is still a substantial knowledge gap. For example, other factors (i.e., functional groupings, biotic interactions) that were not the focus of the studies presented above might explain distributions or lack of ecosystem effects for various fish species. Also, biotic interactions might be more influential than habitat in some circumstances, but those were not quantified here. No evidence was found that species richness and the subsequent shift from benthic to floating algae influenced macroinvertebrate community structure. However, failure to sample the floating invertebrate community likely underestimated the biomass of invertebrates in mesocosms that had high amounts of floating algae. My research illustrates the importance to include, as much as possible, appropriate habitat and community structure variables and to perform experiments that characterize the interaction of species effects and confounding factors.

Understanding where fish are more likely to be found in a stream can indicate where fish ecosystem effects are more likely to occur. I found adult fishes prefer deeper pool habitats and juveniles shallower pool habitats. I also found that juvenile grazing fishes are associated with shorter filament lengths and less benthic organic matter. Based on these results, we might expect longer filaments and more benthic organic matter where larger fishes occur. Research that

furthers our understanding of how species impact stream ecosystems in headwater systems is critical to interpreting and predicting broader scale consequences.

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Appendix A

Variance components in the general linear mixed model

Table A.1 Variance components for each environmental response variable fitted with the general linear mixed model approach. Parameter indicates which group (treatment, habitat, location) was fitted for heterogeneous variances. Group indicates each partition within the model. Estimate is the estimated variance, followed by the standard error of each estimate. For example, algal filament lengths were over 300 times more variable in riffles than pools.

Response variable	Parameter	Group	Estimate	Standard Error
Algal filament length	Habitat	Pool	1.31	0.48
		Riffle	406.83	117.69
Benthic chlorophyll	Treatment	Single species	13.47	5.50
		Two species	16.00	6.82
		Three species	27.99	11.94
		Control	5.29	2.25
Floating chlorophyll	NA	-	-	-
Benthic organic matter	NA	-	-	-

Macroinvertebrate biomass	Treatment*Habitat	Single species Pool	892.22	515.12
		Single species Riffle	329.40	190.18
		Two species Pool	2.41	1.39
		Two species Riffle	1964.85	1134.40
		Three species Pool	368.73	212.89
		Three species Riffle	125.00	79.06
		Control Pool	19.89	11.48
		Control Riffle	70.69	40.81