Stream flow mediates biomass, associations, and nutrient cycling of dominant animal functional groups

by

Garrett William Hopper

B.S., Oklahoma State University, 2013
M.S., Kansas State University, 2015

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

2019

Abstract

Animals can be important mediators of resource heterogeneity and fluxes. While the properties of ecosystems generated by animals often result from interactions among multiple taxonomic and functional groups and environmental factors, most studies reduce these processes by examining processes performed by a single animal group under relatively static environmental conditions. Thus, our understanding of how animal-mediated ecosystem processes vary with abiotic and biotic context is limited. I propose a conceptual framework and present empirical evidence for animal-mediated nutrient cycling that considers potential effects of spatially overlapping animal groups within dynamic ecosystems to address this issue. First, I evaluate this framework by testing if biogeochemical hotspots generated by stable aggregations of mussels attract fishes. I quantified how different fish assemblage biomass was distributed between mussel bed reaches and reaches without mussel under different hydrologic conditions. I compared fish and mussel biomass at mussel beds to test whether differences in animal biomass mediate their contributions to nutrient cycling through nitrogen (N) and phosphorous (P) excretion. Hydrology influenced fish biomass distribution relative to stable mussel beds, with fish aggregating on mussel beds during low flows. Mussel biomass was consistently 10-fold higher than fish biomass, resulting in large differences among mussel and fish assemblage excretion rates, regardless of hydrologic conditions. Second, I evaluated the potential for spatial overlap of fish and mussels at fine spatial scales by conducting a 12-week field experiment to test if fish distribution was influenced by the presence of subsidies associated with live mussels or biogenic habitat of shells. I used underwater video footage to quantify fish occurrences at 50 0.25-m^2 enclosures stocked with either live mussels (two-species assemblages), sham mussels (shells filled with sand), or sediment only. Contrary to my predictions, I found that live mussels did not increase trophic subsidies to fish during the experiment, which may be attributed to unusually high stream flows homogenizing treatment effects. I also found that mussel shells (live and sham) influenced the distribution of fishes within the experimental reach and may provide habitat for fishes at fine spatial scales. Third, I investigated the potential for extreme low flow events to alter stream animals' nutrient contributions through shifts in species composition and biomass. I tested how biomass and nutrient cycling rates of an intermittent prairie stream community changed during a drought. I quantified the biomass and contributions to nutrient

cycling for assemblages comprising fishes, crayfish, and tadpoles in 12 isolated pools during the harshest drought on record for Kings Creek, KS. I found that assemblage biomass declined with decreasing pool size and assemblage composition shifted toward species with more drought resistant traits. Assemblage N excretion rates declined as pool biomass was reduced by mortality, emigration, or metamorphosis. P excretion rates were reduced initially but increased as species with high P excretion rates maintained similar proportional biomass and non-native fish biomass increased, consequently reducing assemblage excretion N:P. I conclude that taxonomically and functionally diverse animal groups coexisting in dynamic ecosystems generate the potential for periodic overlap of animal groups through facilitation or abiotic forcing. These studies demonstrate the context dependency of processes performed by animals, but also illustrate the general role of stoichiometric traits, biomass and density of organisms, and ecosystem size in governing animal processes.

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comprising fishes, crayfish, and tadpoles in 12 isolated pools during the harshest drought on record for Kings Creek, KS. I found that assemblage biomass declined with decreasing pool size and assemblage composition shifted toward species with more drought resistant traits. Assemblage N excretion rates declined as pool biomass was reduced by mortality, emigration, or metamorphosis. P excretion rates were reduced initially but increased as species with high P excretion rates maintained similar proportional biomass and non-native fish biomass increased, consequently reducing assemblage excretion N:P. I conclude that taxonomically and functionally diverse animal groups coexisting in dynamic ecosystems generate the potential for periodic overlap of animal groups through facilitation or abiotic forcing. These studies demonstrate the context dependency of processes performed by animals, but also illustrate the general role of stoichiometric traits, biomass and density of organisms, and ecosystem size in governing animal processes.

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Acknowledgements

I have been very fortunate to have worked alongside many talented individuals at Kansas State University. I owe many thanks to the Division of Biology graduate students, staff and faculty for making this an educational and enjoyable experience. I express my appreciation and gratitude to my advisor, Keith Gido, for the patient guidance and mentorship he has provided me with through the completion of this degree. I could not have asked for a better advisor. I would also thank my committee members, Walter Dodds, Michi Tobler, and Caryn Vaughn, for their guidance and suggestions that improved my dissertation. I'd also like to recognize Carla Atkinson, Kiza Gates, Thomas Parr and Traci DuBose for their contributions to my dissertation and graduate student experience. Members of the Gido lab are each friends of mine that enriched my experience at Kansas State University. Lab members also listened to and commented on my research and ideas. Many individuals contributed to field work including Cole Allen, Austin Earl, Tanna Fanshier, Jennie Grill, Andrew Hageman, Janell Hartwell, Croby Hedden, Sky Hedden, Ed Higgins, Johnathon Lopez, Katherine Murphy, Patrick Olson, Casey Pennock, Liz Renner, Noé Rodríguez, Emily Samuel, Brent Tweedy, Micah Waters, Robbie Weber. Rosemary Ramundo and Courtney Tobler provided technical assistance and performed nutrient analyses. Finally, I am forever grateful to my family members and Ella for the innumerable sacrifices they have made and overwhelming support they have given to me while I pursued this final degree.

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Dedication

I would like to dedicate this dissertation to my grandfather, Joe Bertoldo, who passed during my time in graduate school. Thank you for teaching me to appreciate the natural world.

Preface

The three chapters of this dissertation have been prepared in submission format and include co-authors. Chapter 2 is formatted for *Oecologia*, Chapter 3 is formatted for *Freshwater Science*, and Chapter 4 is formatted for *Freshwater Biology*. Chapter 2 has been adapted with permission from Spring Nature Centre, GmbH: **Springer**, *Oecologia*, Hopper et al., 2018 Chapter 2 is under review at *Freshwater Science*. Chapter 3 is submitted to *Freshwater Biology*.

Chapter 1 - Animal-mediated ecosystem effects

Identifying how species interactions influence ecosystems is a fundamental goal of ecology, especially in regard to environmental context (Polis, Power, & Huxel, 2004). Animals exert top-down effects through consumption (Menge, Lubchenco, Ashkenas, & Ramsey, 1986; McNaughton, 1988; Power, 1990) and bottom-up effects through excretion or egestion of nutrients (Vanni, 2002; Atkinson, Capps, Rugenski & Vanni, 2017, Sitters et al., 2017). While top-down control of ecosystems by animals has received much attention, few studies have established the importance of their bottom-up effects until recently (McNaughton 1984; Zaady et al. 1996, Meehan & Lindroth 2007; McIntyre et al., 2008, Atkinson &Vaughn, 2015). The relative importance of animal-mediated processes, such as nutrient cycling, varies spatially and temporally among taxonomic groups and ecosystems and is contingent upon the interaction of density, biomass, and organismal traits with environmental factors such as climate, ambient nutrient concentration, and ecosystem size (Benstead et al. 2010, Small et al., 2011, Atkinson et al. 2017). The influence of these interactions is often strongest when environmental conditions concentrate the biomass of one or more groups of animals. The properties of ecosystems mediated by animals result from interactions among multiple taxonomic and functional groups in combination with environmental factors; yet, most studies simplify these processes by considering a single animal group under relatively stable conditions (but see Evans-White & Lamberti 2005, 2006). Thus, estimation of animal-mediated processes in diverse and dynamic ecosystems has been limited by several key issues including: 1) identifying the abiotic and biotic mechanisms that lead to spatial or temporal overlap of taxonomically and functionally different groups of animals; 2) determining whether taxonomic and functional composition of assemblages alters the flux and stoichiometric contributions of animal assemblages to nutrient cycling, a key ecosystem function.

Spatial and temporal overlap of broadly different animal groups

Abiotic and biotic mechanisms regulate the overlap of multiple animal aggregations. For instance, diverse animal groups might aggregate during particular abiotic conditions, such as around a water source during drought conditions or at low elevation fields during winter (Western 1975; Ferrari & Garrott 2002; Redfern et al 2006). Aggregating animals might also overlap if the actions of one animal attracts the other, with the potential for positive feedbacks

resulting from ecosystem changes by those aggregations. For example, prairie dogs (*Cynomys ludovicianus*) occur as heterogeneously distributed colonies in prairie ecosystems that attract bison (*Bison bison*) grazing by causing compositional, structural, and nutritional changes in the vegetation through both top-down and bottom-up effects (Coppock et al. 1983). Grazing and urine and fecal deposits of bison then stimulate additional changes to the vegetation assemblage and increase nutrient cycling (Knapp et al. 1999), resulting in a positive feedback loop. For my dissertation, I predicted the potential for strong ecosystem effects should occur where abiotic and biotic mechanisms cause the spatial and temporal overlap of dominant animal functional groups. Understanding the potential for overlapping, aggregated animal groups to interact and influence nutrient and resource heterogeneity represents a fundamental knowledge gap. For the second Chapter of this dissertation, I developed a conceptual framework that considers how spatially overlapping aggregations of different animal groups might influence ecosystem properties. Because mussels and fish can maintain high biomass assemblages in streams, I was able to evaluate this framework by testing if nutrient hotspots generated by stable aggregations of mussels attract fishes. I compared how fish and mussel biomass varied with hydrology, their degree of spatial overlap, and in turn their effects on nutrient cycling. In this study I presented a generalizable conceptual framework and that provides a realistic test of the influence of animals on ecosystem function by evaluating animal-mediated nutrient cycling in the context of two co-occurring groups (mussels and fish) with the strongest documented ecosystem effects of aggregated stream biota.

In Chapter 3, I tested whether fish distribution within mussel beds is influenced by the presence of subsidies associated with live mussels or biogenic habitat of mussel shells. I quantified trophic resources to fish and used remote underwater video footage to quantify fish occurrences at experimental enclosures stocked with either live mussels (two- species assemblages), sham mussels (shells filled with sand). This study offers a mechanistic understanding of fine scale interactions between fish and mussels within mussel beds that I was unable to be detect in Chapter 2 and provides evidence that ecosystem effects of fish and mussels can overlap at fine spatial scales. Moreover, this study showcases the influence of environmental conditions over the ecosystem effects of animals when placed in the context of previous studies.

Biomass and stoichiometric traits within the context of drought

Extreme events may exacerbate ecosystem effects of animals because they control species' distributions (Boulton 2003; Lake 2003), trait expression and the direction and magnitude of how species' traits affect ecosystem function (Ackerly 2003). For the fourth chapter, I tested how drought (an extreme hydrologic event that concentrates animal biomass under harsh environmental conditions) might change animals' effects on ecosystems. I tracked how biomass and concomitant animal-mediated nutrient cycling of a prairie stream community changed during a severe drought by quantifying the biomass and contributions to nutrient cycling for assemblages comprising fishes, crayfish, and tadpoles in 12 isolated pools during a 3-month long field experiment during the harshest drought on record for Kings Creek, KS. This study highlights the potential for severe drought to influence the stability and function of ecosystem processes, such as nutrient cycling, by shifting species' roles based on their ability to tolerate harsh conditions. This study also illustrated that relative dominance of broad taxonomic groups with varying stoichiometric traits played a prominent role in stream nutrient dynamics and food web interactions, especially during periods of drought, which may result in additional losses in ecosystem function and changes in community structure.

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Chapter 2 - Biomass distribution of fishes and mussels mediates spatial and temporal heterogeneity in nutrient cycling in streams

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Background

Across all ecosystems, animals can have strong top-down effects through the consumption of resources (Power et al. 1988, Knapp et al. 1999) and bottom-up effects through excretion and egestion of nutrients (Small et al. 2011, Subalusky et al. 2015). The importance of animals in mediating and maintaining resource heterogeneity through indirect provisioning of nutrients is becoming widely accepted (Atkinson et al. 2017, Sitters et al. 2017). Animals ranging from ungulates to snails maintain resource heterogeneity and provide important nutrient subsidies to primary producers through urine, feces, and frass (McNaughton 1984, Zaady et al. 1996, Meehan and Lindroth 2007). The relative importance of animals in mediating nutrient heterogeneity varies temporally and spatially across species and ecosystems (Vanni 2002, Atkinson et al. 2017) and depends primarily on the interaction of density, biomass, and traits with environmental factors such as climate, ambient nutrient concentration, ecosystem size, and season (Benstead et al. 2010, Griffiths and Hill 2014). The effects of these interactions often become apparent at environmental extremes that redistribute the biomass of one or more groups of animals. For example, in stream ecosystems under hydrologic low flow conditions, a larger fraction of ecosystem nutrient demand may be supplied by animal excretion compared to catchment run-off (Grimm 1988, Atkinson et al. 2014, Childress et al. 2014). Animal biomass may be further redistributed if facilitation of one animal consumer group by another through the production of spatial subsidies concentrates animal biomass. Though properties of ecosystems produced by animals are often a product of interactions among multiple animal taxonomic and functional

groups and environmental factors, most studies have simplified these processes by investigating the role of a single animal group under relatively stable environmental conditions (Liess and Hillebrand 2004, but see Evans-White and Lamberti 2005, 2006).

Aggregating animals in particular, produce spatially-heterogeneous distributions of biomass which can generate biogeochemical hotspots – areas with disproportionately high rates of nutrient recycling and material flux (McIntyre et al. 2008). Such hotspots are dynamic and can be driven by environmental events such as hydrology and temperature (Atkinson and Vaughn 2015, Wetzel et al 2005). These patches of biogeochemical activity promote resource heterogeneity that maintains biodiversity (Bump et al. 2009) and can provide important nutrient subsidies in otherwise nutrient-limited systems (McIntyre et al. 2008, Atkinson et al. 2013). For example, nutrients and biological activity become locally concentrated and food web productivity increases in grazing ungulate systems (McNaughton 1984), bird roosting trees on the savanna (Dean et al. 1999), coral reefs (Allgeier et al. 2013), Everglade tree islands (Wetzel et al. 2005) and streams (Grimm 1988). While individual groups of animals such as these have been recognized for their ability to generate biogeochemical hotspots (McIntyre et al. 2008, Atkinson and Vaughn 2015), ecosystems comprise taxonomically and functionally diverse groups of animals that differ in their spatial overlap as well as their pathways and potentials for generating biogeochemical hotspots. Thus, understanding how overlapping, aggregated animal groups interact to influence nutrient and resource heterogeneity is a fundamental knowledge gap.

We propose a simple conceptual framework that considers how spatially-overlapping aggregations of different animal consumer groups might influence ecosystem properties (Fig. 1.1, Hopper et al., 2018). Spatial or temporal overlap by multiple groups of aggregated, animal consumers is common in many ecosystems and may be driven by either abiotic or biotic mechanisms, with potentially cumulative or synergistic (non-additive) ecosystem level effects (Fig. 1.1). Abiotic and biotic mechanisms might drive the overlap of multiple consumer aggregations. For example, consumer groups might aggregate during particular abiotic conditions, such as around a water source during drought conditions or at low elevation fields during winter (Western 1975, Ferrari, & Garrott 2002, Redfern et al. 2003).

Aggregating animal consumers might also overlap if the activities of one animal attracts the other, with the potential of resulting ecosystem changes by those aggregations to lead to a positive feedbacks (Fig. 1.1). For instance, prairie dogs (*Cynomys ludovicianus*) occur as

heterogeneously distributed colonies in prairie ecosystems that attract bison (*Bison bison*) grazing by triggering a broad array of compositional, structural, and nutritional changes in the vegetation through both direct and indirect effects (Coppock et al. 1983). Moreover, grazing and urine and fecal deposits of bison stimulate additional changes to the vegetation assemblage and increases nutrient cycling (Knapp et al. 1999). Thus, we predict the potential for strong ecosystem effects occurs where abiotic and biotic mechanisms cause the spatial and temporal overlap of dominant animal functional groups.

Stream ecosystems present an ideal opportunity to investigate the ecosystem consequences of overlapping consumer aggregations. Streams are spatially-heterogeneous, dynamic systems that expand and contract with hydrologic condition. Thus, the presence or absence of water fundamentally constrains the availability of habitat (Junk et al 1989, Grant et al. 2007). Stream animals have evolved several general adaptations to this constraint –high mobility, desiccation resistance, and/or high fecundity to compensate for the loss of adults through drying. Contrasting adaptations to stream drying are exemplified by mobile fish and sedentary unionid mussels (hereafter mussels), which can elicit some of the strongest documented ecosystem effects by stream animals (McIntyre et al. 2007, 2008, Atkinson and Vaughn 2015, Capps et al. 2015). While fish disperse as stream ecosystems expand, mussels populations are constrained to perennially wetted segments of the stream (Gough et al 2012). Mussels and fish commonly co-occur in streams of the southern United States as high biomass aggregations and both can form biogeochemical hotspots (McIntyre 2008, Atkinson and Vaughn 2015).

Mussels and fish have different life histories that influence how their distribution varies with hydrology, their degree of spatial overlap, and in turn their effects on ecosystem function. Mussels are long-lived (6 to >100 years), sedentary, filter feeders, that spend their adult life in dense, multi-species aggregations (up to 100 individuals m^{-2}) called mussel beds (Strayer 2008). Mussel beds are patchily distributed in streams because mussels are constrained to perennial stream reaches where sediments are stable with low shear stress (Allen and Vaughn 2010). Mussels have strong bottom-up effects through nitrogen excretion where they are abundant, which reduces nutrient limitation to primary producers leading to increased benthic algae (Vaughn et al. 2008), macroinvertebrates (Spooner and Vaughn 2006) and riparian spiders (Allen et al 2012). In contrast, stream fish are typically shorter-lived (2 to 5 years), mobile animals and their distribution and abundance are largely controlled by hydrology (Fausch et al. 2001,

Grossman 2010). Stream fishes can have strong top-down (Power et al 1985) and bottom-up effects (Gido and Matthews 2001), but those effects can be mediated by hydrology (Gido et al. 2010). Thus, the distribution of fish aggregations shifts seasonally and with stream discharge (Lobón-Cerviá 2009), while mussel beds remain stable (Strayer 2008). Therefore, mussels represent localized, stable hotspots that supply spatially predictable nutrient subsidies, while fishes are widespread, mobile hotspots that provide nutrient subsidies more dependent upon hydrological conditions. Consequently, there is great potential for co-occurring fish and mussel hotspots to overlap spatially or temporally, presenting an opportunity to investigate the potential for cumulative effects resulting from overlapping biogeochemical hotspots. Overlapping hotspots may also be generated independently of abiotic factors such as hydrology. Fish and mussel hotspots may overlap through positive feedback mechanisms where basal trophic resources stimulated by aggregations of mussels or habitat created by their shells facilitates habitat selection by fishes (Spooner and Vaughn 2006). Synergies may result when fishes, feeding on algal or insect prey, also excrete additional limiting nutrients thereby promoting more algal production (Gido and Matthews 2001). Thus, overlap of dominate animal functional groups may fundamentally alter ecosystem properties during periods of spatial overlap.

To understand the potential for spatial overlap to occur between fish and mussels, in the context of our conceptual model, we examined how aggregations of these two animal consumer groups were distributed relative to each other and estimated their potential contributions to nutrient cycling through excretion, especially with regards to hydrologic condition. We hypothesized that fish assemblage biomass would be greater in stream reaches with mussel aggregations compared to reaches with few mussels, because basal trophic resources stimulated by aggregations of mussels or habitat created by their shells may facilitate habitat selection by fishes (Spooner and Vaughn 2006). However, we expect aggregations of fish at mussel bed reaches to be greatest under low flow conditions because they will be more dispersed when habitat volume increases (Ross 1985, Schlosser 1991, Stanley et al. 1997). Finally, we hypothesized that spatial and temporal differences in the distribution of animal consumer group biomass would lead to different contributions of fish and mussel communities to nutrient cycling, a fundamental component of biogeochemical hotspots. We tested these hypotheses through field experiments conducted across two years. The objectives of these experiments were to 1) compare fish biomass at mussel bed reaches and non-mussel bed reaches, 2) test how mussel and fish

biomass differ when they co-occur at mussel beds and if differences in animal biomass and coarse taxonomic composition result in different flux and stoichiometric contributions to nutrient cycling through differential excretion of nitrogen (N) and phosphorus (P) and 3) evaluate spatial and temporal changes in flux and stoichiometric contributions to nutrient cycling of fish and mussel populations associated with assemblage composition and hydrology.

Materials and Methods

Study location

The Kiamichi River and Little River are adjacent tributaries to the Red River in the southcentral United States. The Kiamichi River (KR) drains approximately 4500 km^2 and is typically susceptible to extremely low water levels in the summer (Allen et al. 2013, Vaughn et al. 2015). The Little River (LR) drainage is 10,720 km^2 and is less hydrologically variable than the KR but experiences lower flows during the summer relative to the fall. The Glover River (GR) is an unimpounded tributary to the Little River, that drains approximately 828 km^2 and can experience almost complete desiccation to rapid flash flooding within a relatively short time period (Dauwalter and Fisher 2008). These well-studied rivers are recognized for their high fish (KR 86 species, LR 110 species, GR 33 species) and mussel (KR 31 species, LR 35 species, GR 22 species) diversity (Vaughn 2003, Matthews et al. 2005). In addition, animals are known to influence nutrient cycling in these rivers. For example, sites without mussels in the Kiamichi River and Little River, are N-limited while sites with high mussel biomass are co-limited by N and P (Atkinson et al. 2013, Vaughn et al. 2007), which should strengthen the role of animal aggregations in nutrient cycling. The locations and spatial extent of most mussel beds in these rivers have been mapped and their species compositions are well known (Spooner and Vaughn 2009, Atkinson et al. 2012, Atkinson and Vaughn 2015).

We selected paired reaches at seven locations within these rivers to understand the influence of mussel beds on fish biomass distribution and how mussel and fish aggregations influence nutrient cycling. Reaches were sampled for fish during the fall and summer to understand the influence of seasonal hydrological variation on fish biomass distribution and consumer driven nutrient cycling. Each location contained a ~ 100 m stream reach with a large mussel bed (mussel bed reach) and a ~100 m reach without mussels or with very low densities of

mussels (range 0 – 15.7 mussels m^{-2}, non-mussel bed reach). Mussel and non-mussel reaches were separated by an average distance of 346 m (range of 112-686 m). Non-mussel bed reaches served as references to test the effects of mussel beds on fish biomass distribution.

Overlapping fish and mussel biomass

To test our hypothesis that fish biomass would be higher in mussel bed reaches compared to non-mussel bed reaches, we sampled fish assemblages in each stream reach using a combination of backpack electrofishing and seining. Fish collection was accomplished through a two-pass closed population mark-recapture approach using two to six channel units per reach. Channel units were defined as relatively homogeneous areas of the channel that differ in depth, velocity, and substrate characteristics from adjacent areas (Bisson and Montgomery 2017). Individual fish collected during the first pass were identified to species, measured (total length, mm) and given a noticeable clip on the caudal fin prior to being returned to their respective channel unit. Individuals less than 40 mm were not marked to avoid high mortality related to handling stress (G. Hopper *personal observation*). Fish greater than 200 mm were also excluded because of their sparse distribution, high mobility and ability to avoid our sampling gear. Each reach was resampled 4-12 hours later using identical methods. Length-mass regressions from a subset of individuals collected on-site or previously collected individuals of the same species or genus were used to estimate wet mass (K. Gido *unpublished data*) of all captured individuals (Appendix Table A 1). The Chapman mark-recapture population estimator was used to calculate population sizes. Areal biomass was estimated for each channel unit separately as the product of the population estimate and the mean predicted mass of individuals collected from each channel unit, respectively (Seber 1982, Hayes et al. 2007). Within reach-level estimates used for comparisons were calculated from area-weighted averages of channel units. Reach estimates were calculated during August and October of 2015 and 2016; three paired reaches were not sampled during 2015 due to extreme flooding that prevented access to the stream. Finally, fish assemblage biomass was converted to dry mass, using measured wet-dry mass conversion ratios (dry mass = 22.9 % of wet mass, G. Hopper *unpublished data*). It was necessary to convert fish biomass to dry mass to compare with previously reported estimates of mussel dry soft tissue mass (shell excluded).

We quantified mussel densities in August 2015, 2016 and 2018 during low flow conditions when mussel abundance is most accurately estimated (Vaughn et al. 1997). Because they are sessile, it was not necessary to estimate abundance during higher flows. Mussels were sampled by excavating 15-20 (depending on the size of the mussel bed) haphazardly placed, 0.25 m^2 quadrats to a depth of approximately 15 cm at each mussel reach (Vaughn et al. 1997, 2015, Galbraith et al. 2010, Atkinson et al. 2014). Mussels were identified, counted, their longest axis measured and then returned to the stream alive. We used species-specific length-mass regressions to estimate individual mussel dry soft tissue masses (DW) (Atkinson and Vaughn 2015). When length-mass data were insufficient a global length-mass regression was generated using a bootstrapping procedure that subsampled (10,000 times) the existing data set so that no one taxon was represented by more than 10 individuals (Appendix Table A 2). Areal mussel biomass (g $DW \cdot m^{-2}$) was based on the sum of estimated dry soft tissue mass of all species within each quadrat. Reach-level estimates were calculated from averages of the quadrats.

Fish and mussel nutrient excretion rates

For fish assemblages, individual excretion rates were measured for four fish species that made up more than 80% of total biomass across reaches. Fish species included a grazing minnow (*Campostoma spadiceum*), benthic insectivore (*Etheostoma radiosum*), mesopredator (*Lepomis megalotis*) and water column insectivore (*Notropis boops*). Fish were collected from the Glover River using a seine and occasionally a backpack electrofishing unit to corral fish into the seine. Fish excretion rates were measured during 2016 in the spring (March) when temperatures ranged from 18.9-21.9 °C, summer (August) when temperatures ranged from 29.7-32.4 °C and fall (October) when temperature ranged from 20.0 - 22.9 °C. Individual excretion rates were measured for at least seven individuals of each species during each season, except for *N. boops*, which was not included in the October sample because we were unable to collect enough individuals > 40 mm. Captured fish were placed into a cooler of fresh stream water and allowed to recover for ~15 minutes. Individual fish were taken from the cooler and placed in a 1000 mL Nalgene bottle with a known volume of filtered stream water (GF/F; 0.7 µm pore size; Whatman Buckinghamshire, U. K.) and incubated for ~ 1 hour. Following the excretion experiment, total length and wet mass were recorded for individual fish and wet mass was converted to dry mass as described above.

Water samples were collected at the end of each trial, placed on ice and transported back to the laboratory for analysis. Nutrient analysis focused on NH_4^+ and soluble reactive phosphorus (SRP). Analyses were performed using the indo-phenol blue and ascorbic acid methods for NH_4^+ and SRP, respectively, using an O-I Analytical Flow Solution IV autoanalyzer (APHA 2005). Excretion calculations were based on the difference between nutrient concentrations of identical containers incubated simultaneously with and without fish. To compare mussel excretion measured as TP to fish excretion measured as SRP, we applied a conversion factor of 1.37 (SE ± 0.04, n = 7) to fish excretion values (TP = 1.37 • SRP). This conversion was based on a subsample of fish excretion samples where we measured both SRP and TP (G. Hopper *unpublished data*).

Size scaling of NH_4^+ and TP (hereafter N and P, respectively) excretion and molar N: P for all fish species was visualized using least-squares regression of $\log_{10}$-transformed excretion rates against $\log_{10}$-transformed dry mass. We removed measurements if they exceeded expected excretion rates of conspecifics by >10 fold to avoid the influence of outliers. For the N excretion data set a total of eight outliers were removed (4 % of the data set), and only a single individual was removed from the P data set (< 1% of the data set) using this criterion. When slopes for individual species were equal (overlapping confidence intervals), we used ANCOVA to test for interspecific differences of $\log_{10}$ transformed excretion rates and molar N: P ratios, using $\log_{10}$ transformed dry mass as a covariate. If no relationship was found between excretion rates and the covariate, we used ANOVA to test for interspecific differences in excretion. We found no differences in N or P excretion rates among fish species (see Results section and Table 1; P > 0.74) and were able to use a simple biomass model ($\log(E) = 0.84 + 0.67 \times \log(M)$) to predict fish N excretion rates and ($\log(E) = -0.11 + 0.49 \times \log(M)$) P excretion rates.

To derive areal excretion rates for mussel communities, we used previously published, field-measured excretion data collected in the summer at ~30 C by Atkinson et al. (2013) for four species of mussels that are common in mussel beds in these rivers: *Actinonaias ligamentina, Amblema plicata, Ptychobranchus occidentalis,* and *Cyclonaias pustulosa*, (Appendix Table A 2). Excretion rates were corrected for nutrient reuptake using a control with empty shells. Values were measured and calculated as μmol TN or TP g DW^{-1} hr^{-1} (Appendix A 2. Full methods in Atkinson et al. 2013). First, because excretion rates increase with increasing body size (Vanni and McIntyre 2016) we calculated the body-size dependent mass-specific excretion rate for each

individual of these four species ($excretion = b \times DW^a$). For species not measured, we used the overall scaling relationship derived from all observations in Atkinson et al. (2013). Second, we adjusted excretion rates for seasonal temperature differences. Mussel species have strong differences in thermal tolerances, which affect their excretion rates, particularly *A. ligamentina* and *A. plicata* which comprise the majority of mussel biomass in rivers in this region (Spooner and Vaughn 2008). To derive excretion rates for our mussel assemblages at 20°C (fall temperature), we used published laboratory data on the temperature dependence of excretion for six common mussel species: *A. ligamentina*, *A. plicata*, *Lampsilis cardium*, *Obliquaria reflexa*, *C. pustulosa*, *Truncilla truncata* (Spooner and Vaughn 2008). For these data, we fit 2nd order polynomials for each species and calculated the ratio of excretion at 20°C to excretion at 30°C. We then multiplied each species' field-measured excretion rates at 30°C by this ratio to estimate excretion rates at 20°C. It is important to note that our excretion estimates for fish and mussel assemblages are based on NH_4^+ and TN, respectively. This corresponds to a conservative estimate for fish N excretion while providing a maximum estimate for N excreted by mussels. Although this discrepancy exists, it is likely that fish excretion rates measured as TN would result in a similar pattern presented here since NH_4^+ is a majority of excretion measured as TN (Vanni 2002, Ramamonjisoa and Natuhara 2017).

Comparing mussel and fish contributions to nutrient cycling

We used spatially explicit mussel and fish species composition and biomass data to estimate the variation in aggregate nutrient excretion between mussel beds and associated fish assemblages. Mussel assemblage excretion estimates were calculated by multiplying species-specific excretion rates (μmol $P \cdot h^{-1} \cdot g\ DW^{-1}$, μmols $NH_4^+ \cdot h^{-1} \cdot g\ DW^{-1}$) by the total biomass estimate for a quadrat (g $DW \cdot m^{-2}$) or the mean excretion rates for all species if species specific rates were unavailable. We estimated assemblage excretion rates for fish by multiplying the measured excretion scaling equations by dry mass estimates for individuals in the assemblage data set. Species-level nutrient excretion was then calculated as the product of population estimates for fish and the per capita excretion rates. Assemblage excretion rates were estimated separately for each sampling unit (channel units for fish and quadrats for mussels), with reach-level estimates calculated from area-weighted averages. Averaging across sampling units within a reach yielded N and P areal excretion rates (μmol $m^{-2} \cdot h^{-1}$) for each assemblage. The estimated areal excretion rates of N and

P for each assemblage were used to calculate assemblage excretion N:P ratios. We used the variation among reaches in aggregate excretion rates and N:P to compare the contributions of fish and mussels to nutrient recycling in these reaches.

Data analysis

Paired t-tests were used to test for differences in fish assemblage biomass at mussel bed reaches and non-mussel bed reaches for each sampling period and log response ratios (lnR) were used to visualize proportional differences in areal biomass of fish assemblages at mussel bed reaches and non-mussel bed reaches. In addition to t-tests, we calculated 95% confidence intervals of lnR to determine if effects of mussel beds on fish biomass distribution were significant (not overlapping zero). We used linear models to compare fish and mussel biomass at reaches where they co-occur. "Consumer" (i.e., mussel or fish), "season", "reach", "year" and their interactions were included as factors. Finally, fish and mussel assemblage areal excretion rates for N and P were compared using linear models with "consumer, "season", "reach" and "year" and their interactions. Statistical analyses were performed in R version 3.4.4 (R Development Core Team 2016). We used the function *aov ()* to carry out linear models in the package car (Fox and Weisberg 2018). All biomass (g m^{-2}) and excretion (μmol m^{-2} h^{-1}) data were $\log_{10} + 1$ transformed prior to analyses to conform to assumptions of normality and homogeneity of variances.

Results

Fish assemblage biomass

Fish and mussel species richness and biomass were highly variable within and among reaches. Areal fish biomass estimates within reaches exhibited high spatial variation among channel units sampled, often varying an order of magnitude or more (Appendix Table A 3). Contrary to our prediction, there was no difference ($P > 0.05$) in fish assemblage biomass among mussel bed and non-mussel bed reaches during the summer and fall of 2015 (Fig. 1.2, 1.3). However, areal fish biomass was greater at mussel bed reaches during the summer of 2016 ($t_{0.05} = -3.41$, $df = 6$, $P = 0.007$, Fig. 1.3) compared to non-mussel bed reaches, but returned to the previous year's pattern during the fall of 2016. In support of our expectations, this result was driven by relatively higher

fish biomass at six mussel bed reaches during the summer 2016 sampling period, which followed a period of lower flow (Appendix Table A 3, Figs. A 4, 5, 6).

Fish and mussel excretion rates

Three fish species (*C. spadiceum*, *E. radiosum*, and *N. boops*) showed a significant positive relationship between body mass and measured N excretion rates ($P < 0.05$), while *L. megalotis* showed only a marginally significant relation between body mass and N excretion rates ($P = 0.07$). Similarly, *C. spadiceum*, *E. radiosum*, and *N. boops* P excretion rates were positively related to body mass ($P < 0.05$, Table 2). However, P excretion rates for *L. megalotis* were not significantly related to body mass. ANCOVA testing for interspecific differences among species with body mass as a covariate revealed no difference for rates of N excretion ($F_{3,114} = 0.42$, $P = 0.73$) or P excretion ($F_{3, 115} = 0.28$, $P = 0.8$). Estimated individual mussel N and P excretion rates (mean ± SD) used to estimate mussel assemblage areal excretion were much higher at 30°C (263.4 µmol N h^{-1} ± 135.2 and 42.9 ± 7.6 µmol P h^{-1}) compared to rates measured at 20°C (10.1 ± 5.33 µmol N h^{-1} and 0.7 ± 0.4 µmol P h^{-1}; Appendix Table A 2).

Fish and mussel contributions to nutrient cycling

Major differences in fish and mussel life history traits (i.e., mobility) resulted in an order of magnitude difference between mussel areal biomass and fish areal biomass during both fall and summer ($F_{6, 274} = 10.97$, $P < 0.05$; Fig 1.4a). This pattern generally increased with stream size (Fig. 1.5). We predicted biomass differences among mussel and fish assemblages would lead to considerable spatial and temporal differences among co-occurring fish and mussel areal excretion rates. Both mussel and fish areal excretion rates closely paralleled differences in animal biomass among reaches, with mussel areal N excretion rates being consistently an order of magnitude greater than fish areal excretion rates (Appendix Table A 3). Areal excretion rates for N differed among co-occurring mussel and fish assemblages (Fig. 1.4b) and showed substantial variation across sites and both seasons sampled ($F_{6,274} = 6.21$, $P < 0.05$). Mussel assemblage N areal excretion rates decreased from summer to fall as water temperature fell, while fish assemblage N areal excretion rates were similar although fish biomass distribution fluctuated with stream discharge across seasons ($F_{1,274} = 7.12$, $P < 0.05$, Fig. 1.4a and b). Similarly, mussel P areal excretion rates were an order of magnitude greater than fish

assemblage P excretion rates and both groups varied among reaches ($F_{6, 274} = 6.8$, $P < 0.05$, Fig 1.4c). In contrast to N areal excretion rates, fish or mussel P areal excretion rates did not differ significantly among seasons ($P > 0.05$).

The ratio of N: P excreted by mussel and fish assemblages varied considerably across seasons ($F_{1,274} = 15.04$, $P < 0.05$) as mussel assemblages responded to decreasing temperatures (Fig. 4d) by excreting at a lower N:P. Differences in mussel bed composition among reaches also led to distinct differences in mussel assemblage N:P compared to fish assemblage excretion N:P ($F_{6,274} = 10.76$, $P < 0.05$, Fig. 1.4d). In mussel beds with greater densities (mean = 1719.1 $g \cdot m^{-2}$, SE ± 106.5) of the thermally sensitive mussel species, *A. ligamentina*, assemblage excretion N:P (summer mean = 15.4, SE ± 0.4; fall mean = 9.9, SE ± 0.3) was consistently higher than fish excretion N:P (summer mean = 10.8, SE ± 0.9 fall mean = 7.2, SE± 1.0) but the magnitude of difference between co-occurring fish and mussel assemblages exhibited a strong decline at lower water temperatures during the fall (Vaughn et al. 2007, Atkinson et al. 2013). At three mussel beds where *A. ligamentina* was present at low densities (mean = 220.3 $g \cdot m^{-2}$, SE± 48.9), lower fall water temperatures reduced mussel assemblage excretion N:P (summer mean N:P = 11.1, SE ± 0.6; fall N:P mean = 6.7, SE ± 0.4) below the excretion stoichiometry of the fish assemblage (summer mean N:P = 9.8, SE ± 0.6; fall mean N:P = 8.8, SE ± 0.5). When *A. ligamentina* was absent, mussel assemblage excretion N:P (summer mean = 7.2, SE ± 1.1; fall mean = 5.0, SE ± 0.7) was lower compared to fish communities during summer (mean N:P = 11.0, SE ± 1.4) and fall (mean N:P = 8.7, SE ± 0.9).

Discussion

Aggregated animals can form biogeochemical hotspots that influence ecosystem function. The strongest effects should occur where abiotic and biotic mechanisms result in the highest spatial and temporal overlap of dominant animal groups (Fig. 1.1). We tested this prediction by examining the biomass overlap and ecosystem effects (nutrient recycling) of two dominant groups of stream animals, mussels and fish. We found that biomass of mussel aggregations was often an order of magnitude greater than fish biomass and was spatially concentrated and temporally stable. In contrast, fish biomass was temporally variable and was only aggregated in mussel beds during one relatively low flow period. Thus, using biomass as a metric to estimate the potential contributions of animals to nutrient cycling, we found strong ecosystem effects of

one mussel assemblages, but only weak effects from fish assemblages (Fig. 1.1, 1. 2). Although standing stock or biomass might reflect production, such as when production to biomass ratios are stable (Gido and Hargrave 2009), shifting the axes of the conceptual framework to biomass production or element specific production might offer a more accurate representation of consumer effects on nutrient dynamics, such as altering rates and supplies of key nutrients like N and P.

In our study, abiotic factors (i.e., hydrology) seemed to influence the distribution of fish aggregations relative to stable mussel beds, with fish aggregating on mussel beds during low flow conditions in summer 2016. However, mussel aggregations themselves did not generally appear to attract fish aggregations, as fish biomass was similar on and off mussel beds during all other sampling periods. We note that the conditions that we sampled were atypical of these rivers, which in most recent years have been prone to extremely low summer flows (Allen et al. 2013, Vaughn et al. 2015). Summer 2015 was a 100-year flood event for the Kiamichi River and we were unable to sample three sites there in 2015 because they were not accessible (Appendix Fig. A 5). While hydrologic conditions did not reach typical low flow extremes during the summer of 2016, we found that following periods of relatively low flows fish biomass can become concentrated on mussel beds, but that more extreme conditions may be required to aggregate fish and mussels, thus eliciting strong ecosystem level effects.

Mussel areal biomass was consistently and order of magnitude higher than fish areal biomass, although substantial spatial variation existed for both groups. The most apparent pattern was a longitudinal increase in biomass in more downstream reaches for mussels but not for fish (Fig, 1.4). In reaches where mussel densities were highest, the more than 100-fold difference between mussel and fish biomass resulted in a large difference in assemblage excretion rates during both summer and fall (Figs. 1.5b, c, Appendix Fig A 1). This longitudinal pattern in mussel biomass distribution means that mussel bed effects intensify as mussel density in beds increases downstream (Atkinson et al. 2012, Atkinson and Vaughn 2015).

Although our data suggest fish biomass was not spatially heterogeneous across a stream-size gradient, spatial heterogeneity was present across channel units within reaches (Fig. 1.5). For example, mussel bed Reach 1 comprises four unique channel units and fish areal dry mass within this reach ranged from 0.04 to 6.70 $g \cdot m^{-2}$ during fall and 0.01 to 12.73 $g \cdot m^{-2}$ during summer, suggesting that species-specific habitat preferences result in locally concentrated fish

biomass heterogeneously within reaches (Angermeier and Karr 1983). The mussel beds we sampled occurred in shallow, slow-moving runs, which were dominated by sunfish (Centrarchidae) comprising ~ 80% of fish biomass in our study reaches. In studies of tropical rivers, fish densities increased in riffle habitats (Taylor et al. 2006, McIntyre et al. 2008) that were rarely present at the reaches we sampled and associations between fishes and habitat type might offer a better explanation of fish biomass distribution at the scale we examined. Within the context of our conceptual framework, the combined excretion of mussels and fish at the scale of our stream reaches would likely fall within the lower right region (Fig. 1.1, 1.2). Large differences in biomass between co-occurring mussel and fish communities in mussel reaches suggest the mussels govern nutrient availability and overlapping fish communities perform a relatively minor role or their influence is concentrated at finer habitat scales. Although fish contributions to nutrient cycling were low compared to mussels within mussel bed reaches, the homogeneous distribution of fish likely means they contribute more broadly to nutrient dynamics compared to sedentary mussel hotspots.

Shifting distributions of fish assemblage biomass altered fish assemblage excretion rates among sampling periods (Appendix Fig. A 1) with fish assemblage excretion rates generally paralleling increases or decreases in fish biomass (Fig. 1.4a-c). However, the Reach 4 fish assemblage was an exception, and excretion rates increased from summer to fall although fish assemblage biomass declined (Appendix Fig. A 1).This increase in fish excretion rates was driven by a transition from many small bodied fishes with higher per capita excretion rates that were in high densities during fall sampling of 2015 and 2016 to larger fishes at other sampling periods, leading to a reduction in the assemblage excretion rate. Although our conceptual framework does not incorporate temperature or assemblage composition, it should still prove useful across systems given that biomass often determines the influence of animals on ecosystems (Atkinson et al. 2017, Hall et al. 2007).

We found that where fish and mussel communities overlap, the excretion stoichiometry of fish assemblages was more spatially and temporally stable relative to the excretion stoichiometry of mussels, which varied seasonally and with assemblage composition. In combination with earlier work, our data indicate that two co-existing, abundant species with opposing thermal optima (*A. ligamentina*, *A. plicata*) differentially dominated mussel assemblage biomass resulting in differences in excretion N:P. Previous work has demonstrated

how mussels mediated water column N:P that altered assemblage composition and dominance patterns among algal functional groups (Atkinson et al. 2013). Thus, variation in animal assemblage composition may cause differences in the competitive interactions among primary producers with varying tissue C:N and N:P (Atkinson et al., 2013). By feeding selectively on primary producer tissues with low C:N or high N:P, overlapping grazing fishes may exert top-down effects that help to maintain the balance among algal functional groups within mussel beds. In terrestrial ecosystems, herbivores increase the biomass and abundance of rapidly growing primary producers with low C:N ratios, because grazing stimulates nutritious regrowth of such plants, that increases localized N mineralization rates and N availability (Sitters and Venterink 2015). In summary, variation in animal community composition (i.e., mussels and fish) and associated physiological traits might mediate multiple aspects of consumer driven nutrient dynamics including excretion N:P, recycling rates, and total excretion volume (Atkinson et al. 2017).

Although mussels did not facilitate fish habitat selection at the scale of our study, we acknowledge that most of the fishes sampled in our study (i.e., sunfish) might not rely on the benthic resources stimulated by aggregations of filter feeding mussels. Our conceptual model, however, is applicable at finer spatial scales where biotic interaction are more likely to occur. For example, growth of juvenile Pacific lamprey aggregated in mussel beds is enhanced through the consumption of mussel derived spatial subsidies (Limm and Power 2011). Thus, it is possible that juvenile fishes that were excluded from our analyses, and benthic fishes may benefit by seeking cover or resources in aggregations of mussels that occur at the patch scale (Downing et al. 1993, Strayer and Ralley 1993). Indeed, fish biomass was spatially heterogeneous within mussel bed reaches and a more focused survey within mussel bed reaches may result in fine scale spatial overlap of mussels and fish species that are associated with the benthos such as the grazing minnow (*Campostoma spadiceum*) or benthic invertivores (darters).

The composition and structure of communities has been presented as one key factor influencing stream ecosystem structure and function (Flecker 1996, Vanni et al. 2002, McIntyre et al. 2007). Within this context, the effects of major functional groups on ecosystems have been largely investigated in isolation and under relatively static conditions. In nature, groups of animals with broadly different life histories often coexist in temporally dynamic ecosystems. Consequently, their effects on ecosystem structure and function operate simultaneously but can

shift both spatially and temporally, generating the potential for biogeochemical hotspots to overlap periodically. In rivers where mussels and fish coexist, it appears that the scales at which aggregations occur is variable between groups. While fish might be aggregated at micro- or meso-habitats, their biomass is widely distributed among stream reaches and might exceed that of mussels within the entire river system. Conversely, mussels are heterogeneously distributed among reaches, and within mussel bed reaches fish communities likely provide locally-concentrated, transient nutrient subsidies while aggregations of mussels provide stable, long-term nutrient subsidies that vary in importance with stream discharge and temperature (Vaughn et al. 2004, Atkinson and Vaughn 2015). By investigating two co-occurring groups of animal we were able to show discrepancies in the distribution of animal biomass and the potential for ecosystem-level effects by freshwater mussel and fish communities in two river systems in the southern United States.

Figures

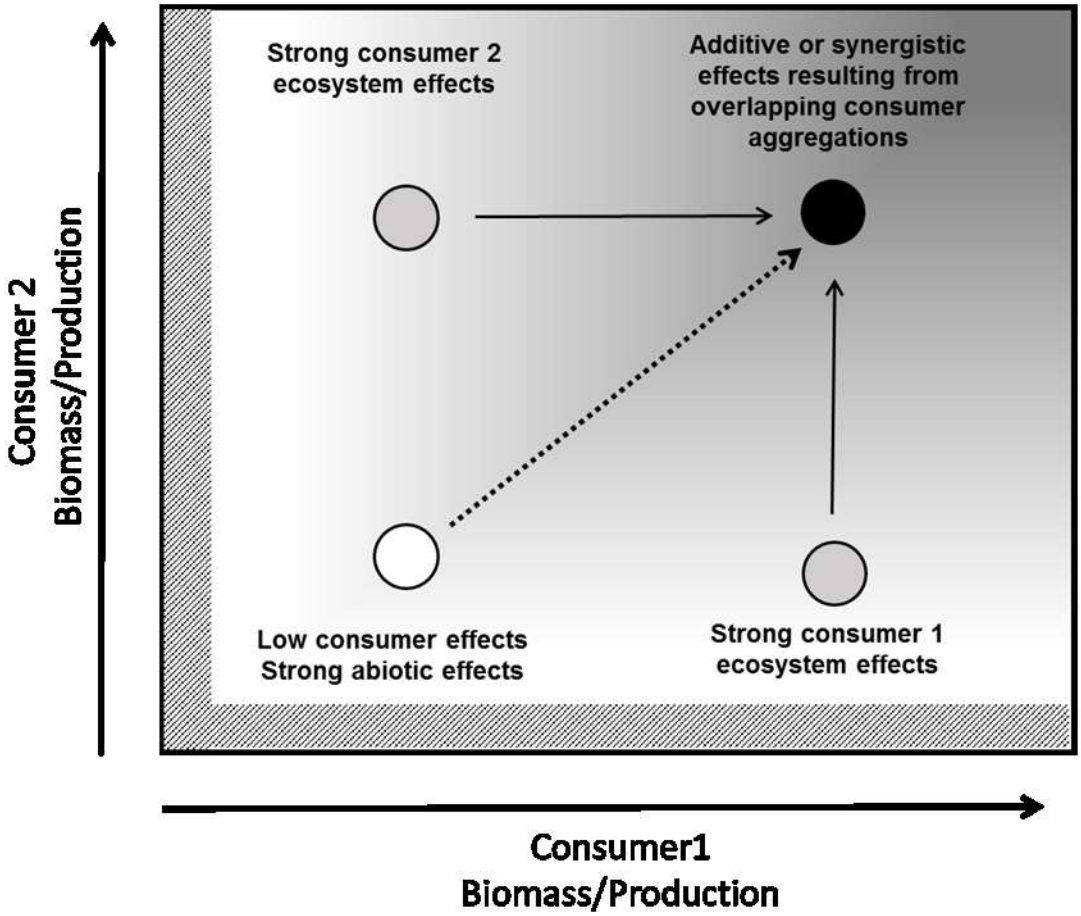

Figure 2.1. Conceptual diagram illustrating the importance of spatial overlap in regulating the ecosystem effects of animal consumer groups (hereafter consumers). Axes represent a gradient of either consumer biomass or production that should index their ecosystem effect. Darker shading indicates the strongest predicted effects by consumers. In the upper-left and lower-right regions of the figure a single consumer plays a dominant role in ecosystem function. Overlapping aggregations of consumer 1 and consumer 2 in the upper-right region create the highest potential for cumulative or synergist effects. The dashed arrow connecting the white and black circles represent the case of one consumer facilitating or attracting the other consumer through a resource subsidy, potentially generating a positive feedback on combined ecosystem effects. The solid arrows connecting white circles to the grey circles represent abiotic conditions (e.g., stream contraction) that force consumer aggregations to overlap. The hatched area along the X and Y axis represents the context in which most studies investigate the effects of consumers on ecosystem structure and function. For instance, increasing levels of consumer 1 or consumer 2 are only compared with very low levels of consumer 2 and consumer 1, respectively, or increasing levels of consumer 1 and consumer 2 are compared individually with zero presence of consumer 2 and consumer 1, respectively. Adapted with permission from Springer Nature Centre, GmbH: Springer, Oecologia, Hopper et al., 2018

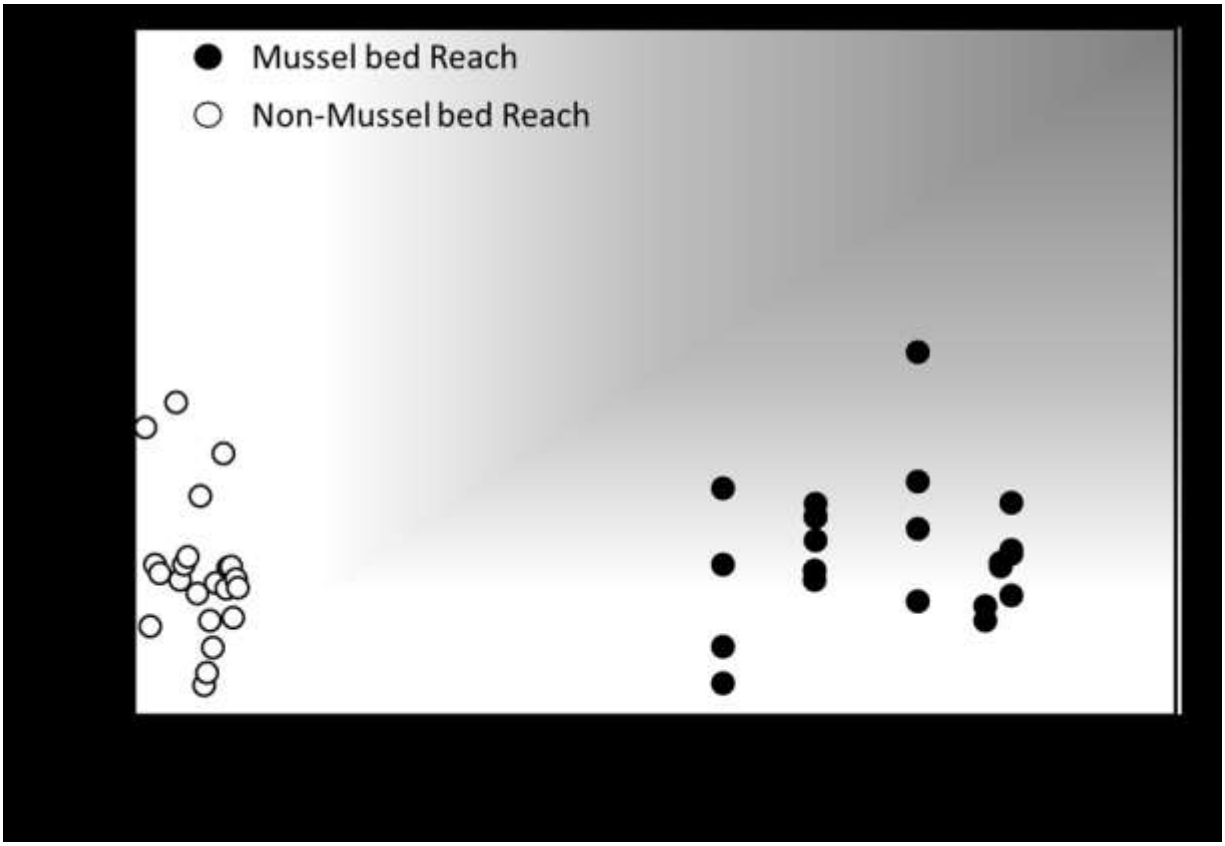

Figure 2.2. Empirical data of fish and mussel assemblage biomass estimated at seven paired mussel and non-mussel bed reaches across two years. Non-mussel fish biomass is staggered between 1 and 2.5 on the X axis to prevent overlap at zero. Figure shading corresponds to predicted ecosystem response of consumer biomass or production, where the darkest shading indicates the strongest predicted effects by consumers. Adapted with permission from Springer Nature Centre, GmbH: Springer, Oecologia, Hopper et al., 2018

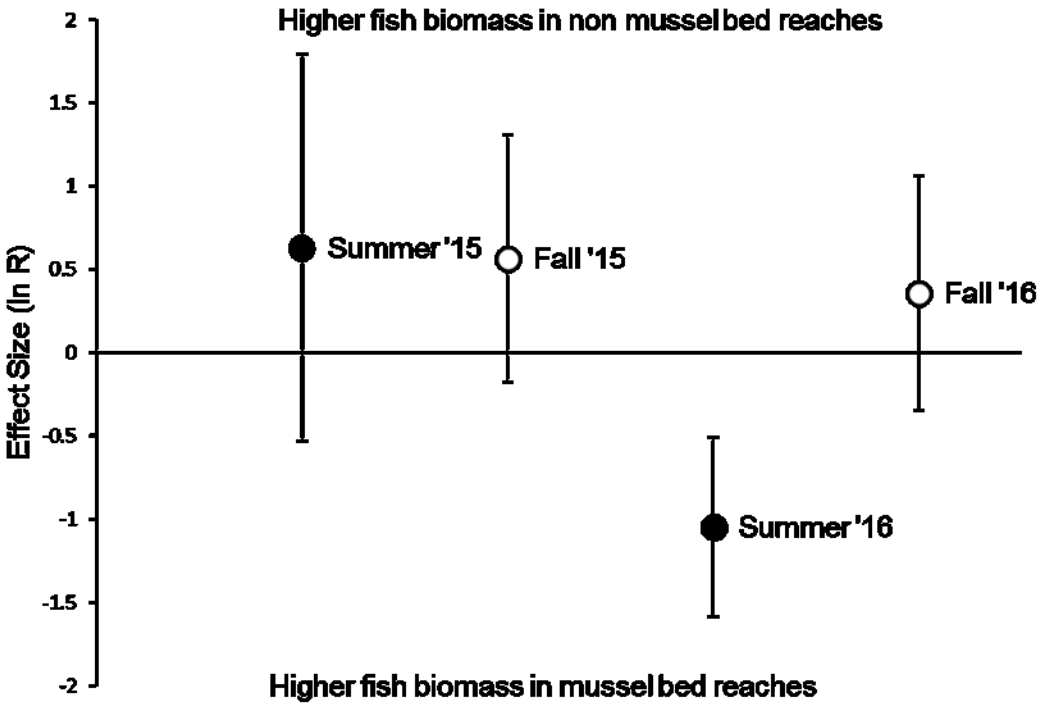

Figure 2.3.Effect sizes and 95% confidence intervals illustrating the proportional response of fish biomass to the presence of mussel beds during fall and summer over a two year study period. The season and year are listed to the right of their respective symbols. Adapted with permission from Springer Nature Centre, GmbH: Springer, Oecologia, Hopper et al., 2018

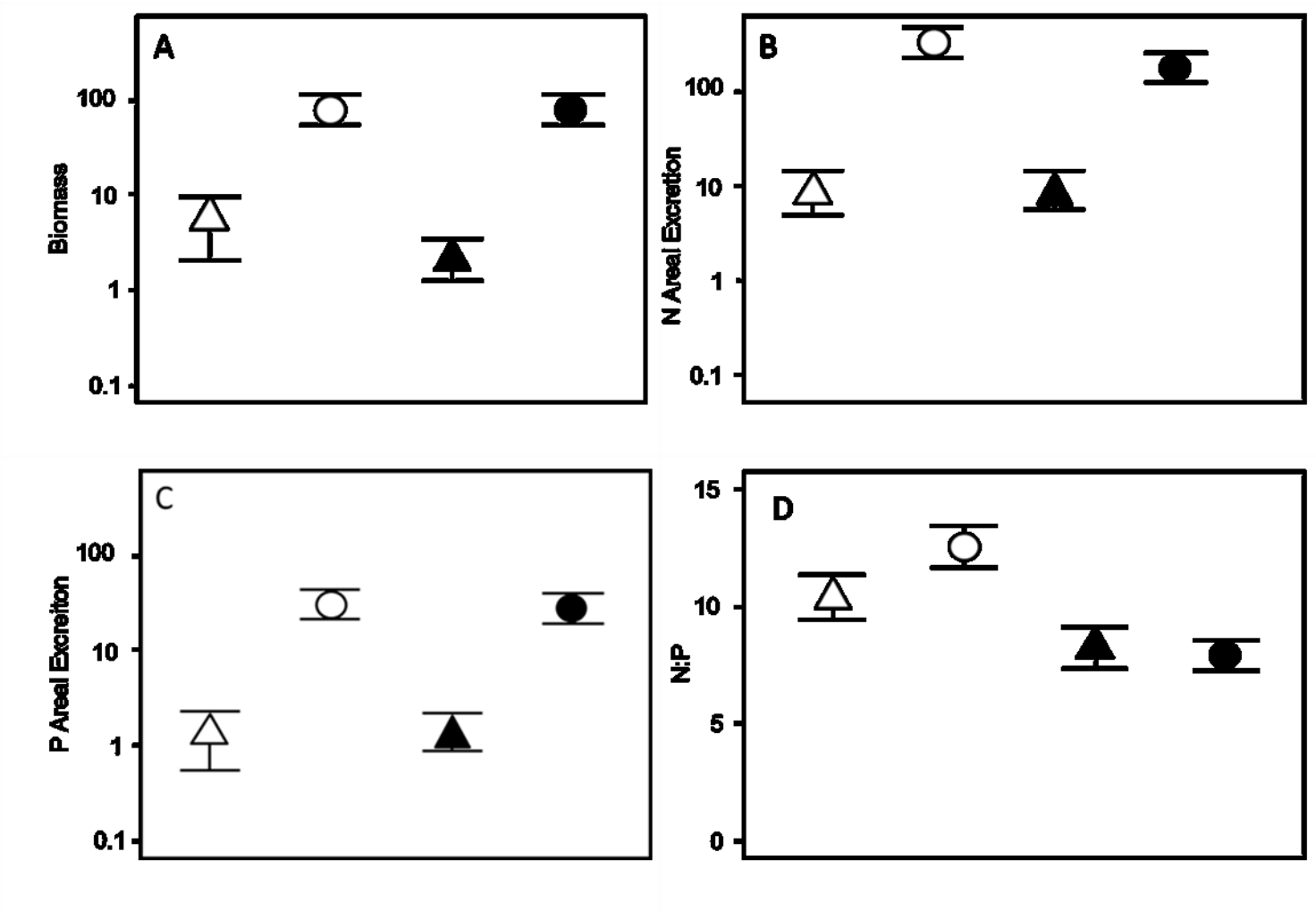

Figure 2.4. Summary of the seasonal comparison of fish and mussel (a) biomass grams DW·m-2), (b) areal nitrogen excretion rates (µmol $N \cdot m^{-2} \cdot h^{-1}$), (c) areal phosphorus excretion rates (µmol $P \cdot m^{-2} \cdot h^{-1}$), and (d) molar N: P of mussel and fish assemblage excretion averaged across seven mussel bed reaches (± 95% confidence intervals, N = 310). Mussels are represented as circles and fish are triangles. Summer sampling is represented by closed symbols and fall by open symbols. Adapted with permission from Springer Nature Centre, GmbH: Springer, Oecologia, Hopper et al., 2018

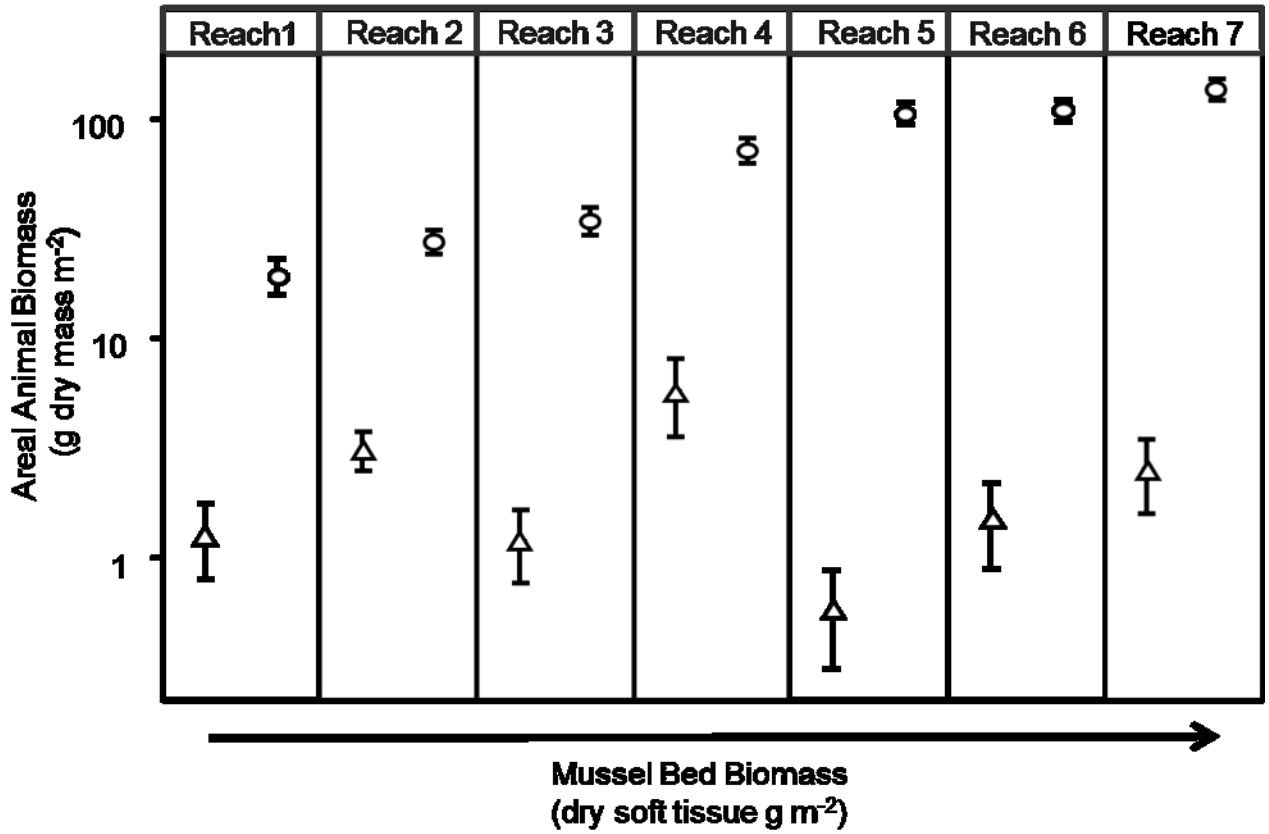

Figure 2.5. Comparison of fish (triangles) and mussel (circles) biomass (grams DW·m^{-2}) along the stream size gradient represented by each of the seven mussel bed reaches sampled (± 95% confidence intervals, N = 310). Reaches are arranged in order of increasing mussel bed biomass. Adapted with permission from Springer Nature Centre, GmbH: Springer, Oecologia, Hopper et al., 2018.

Tables

Table 2.1. Fish species for which ammonium and phosphorus excretion were directly measured. Linearized power functions were used to describe the scaling of excretion rates (E, µmol/h) relative to body dry mass (M, g): log (E) = a + b log (M). Bold font indicates statistically significant equations ($P < 0.05$). * The relationship between N excretion rate and body mass for L. megalotis was marginally significant (P=0.07).** indicates the equation used predict fish assemblage N and P areal excretion rates. Adapted with permission from Springer Nature Centre, GmbH: Springer, Oecologia, Hopper et al., 2018

			NH_4^+				TP		
Measured Taxa	N	Dry Mass (g)	a (SE)	b(SE)	R^2	n	a (SE)	b (SE)	R^2
Campostoma spadiceum	30	0.14- 0.76	**0.87 (0.07)**	**0.53 (0.15)**	**0.27**	33	**-0.01 (0.17)**	**0.78 (0.36)**	**0.13**
Etheostoma radiosum	36	0.11 - 0.48	**0.71 (0.07)**	**0.57 (0.10)**	**0.49**	33	**0.17(0.21)**	**1.03 (0.33)**	**0.24**
*Lepomis megalotis**	29	0.68 - 1.93	**0.83 (0.03)**	**0.50 (0.27)**	**0.50**	32	-0.37 (0.08)	0.55 (0.64)	0.02
Notropis boops	24	0.14 - 0.70	**0.86 (0.08)**	**0.74 (0.15)**	**0.51**	25	**1.07 (0.13)**	**1.07 (0.25)**	**0.44**
All species**	119	0.11 -1.93	**0.84 (0.02)**	**0.67 (0.05)**	**0.64**	123	**-0.11 (0.06)**	**0.49 (0.11)**	**0.13**

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Chapter 3 - Freshwater mussels alter fish distributions at fine spatial scales through habitat modifications.

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Background

Spatial subsidies are resources produced in one habitat that cross over into adjacent habitats (Polis et al. 1997, Nakano and Murakami 2001). Whereas spatial subsidies can occur across discrete habitat boundaries at broad spatial scales such as the reciprocal flux of insects among terrestrial and aquatic habitats (Baxter et al. 2005), resources also can cross fine scale boundaries within aquatic systems such as from pelagic to benthic habitats (Baustian et al. 2014, Jager and Diehl 2014) and may constitute spatial subsidies to some organisms. The effects of spatial subsidies are most pronounced when they substantially elevate resource abundance above that produced in the recipient habitat (Nakano and Murakami 2001, Marczak and Richardson 2007). Some organisms might also be considered ecosystem engineers and through physical modification, maintenance, or creation of habitat they can control the availability of spatial subsidies to other organisms (Jones et al. 1997). For instance, tree canopies create suitable habitat for other organisms by mediating understory and soil conditions (Holling 1992, Callaway and Walker 1997) and beaver dams create lentic habitat in otherwise flowing streams (Wright et al. 2002). When organisms are heterogeneously distributed, their physical engineering activities can amplify differences in resource production rates among patches and result in resource-rich and resource-poor patches (Wetzel et al. 2005, Chowdhury et al. 2016). Furthermore, the distribution of mobile animals may track the activities of temporally stable, and spatially heterogeneous, aggregations of animals that enhance or facilitate them. For example, prairie dogs (*Cynomys ludovicianus*) form spatially heterogeneously colonies that trigger numerous compositional, structural, and nutritional changes in the vegetation through both direct and indirect effects that attract bison (*Bison bison*, Coppock et al. 1983). Understanding the influence of animal interactions on the distribution of co-occurring groups is fundamental to ecology

because spatial and temporal overlap may enhance ecosystem functioning, such as nutrient cycling (Hopper et al. 2018).

Freshwater mussels (family Unionidae) are common in streams of eastern North America. They are patchily distributed in streams at multiple spatial scales. Mussels typically occur as dense, multi-species aggregations called mussel beds where densities are 10 to 100 times greater than areas outside of beds (Strayer 2008) and densities within mussel beds can vary along a gradient of stream size (Atkinson et al. 2012). Mussel beds exist in river channels that often experience significant sediment mobility, can persist in the same stream locations, and have similar abundance and community composition for decades (Sansom et al. 2018a). Heterogeneity also exists within mussel beds, with individual mussels aggregating in dense patches separated by areas with few or no mussels (Strayer and Ralley 1993, Vaughn and Spooner 2006). Through their filter feeding, mussels provide spatial subsidies that effectively link the pelagic and benthic food web compartments of the stream and stimulate primary and secondary production through nitrogen excretion and biodeposition of feces and pseudofeces (Atkinson and Vaughn 2015, Vaughn 2018). Since mussel beds elevate nutrients and resources that cross from pelagic to benthic habitats, mussel-derived resources might facilitate other organisms at both broad and fine spatial scales.

The distribution and abundance of mussels are closely linked to the distribution and abundance of fishes. Mussels are dependent on fish hosts for dispersal of their ectoparasitic larvae (Barnhart et al. 2008, Schwalb et al. 2011), thus mussels are only abundant and diverse where fish are also abundant and diverse (Vaughn and Taylor 2000, Modesto et al. 2018). In marine systems, some organisms exploit the shells of mussels to reduce physiological stress (Stephens and Bertness 1991) and injury or to avoid removal by currents or predators (Skilleter 1994). In streams, mussel shells create biogenic habitat for other organisms (Gutiérrez et al. 2003, Spooner and Vaughn 2006). In addition, nutrients excreted and biodeposited by mussels increase primary production and can shift the functional composition of algal communities (Atkinson et al. 2013). Densities of benthic macroinvertebrates are higher in sediment patches with mussels compared to patches without mussels and their community composition is different, likely due to mussels providing shell habitat, stabilizing sediments, and increasing algal food resources (Howard and Cuffey 2006, Vaughn and Spooner 2006, Spooner and Vaughn 2006, Vaughn et al. 2008). In the Eel River, CA, mussel biodeposits consumed by juvenile

Pacific lamprey significantly enhanced their growth (Limm and Power 2011). Thus, heterogeneously distributed mussels provide an opportunity to test whether fish abundance increases in response to spatial subsidies produced by freshwater mussels.

We hypothesized that patches of mussels would attract fishes through spatial subsidies, by concentrating their algal and invertebrate prey at fine spatial scales. Further, piscivorous fishes would be attracted to increased abundance of prey fishes feeding on increased algal or invertebrate biomass within mussel bed hotspots (Table 1). We hypothesized that spatial subsidies (i.e., trophic resources) generated within patches of live mussels would attract more fishes relative to shells or sediment. To test this hypothesis, we manipulated mussel occurrence within enclosures in a field experiment and used remote underwater video to quantify the abundance of fishes across treatments with live mussels, shells, and controls (sediment only).

Methods

Study system

We conducted our experiment in the Kiamichi River, a tributary (watershed area 4,560 km^2) of the Red River in the Ouachita Mountains of southeastern Oklahoma known for its high fish (86 species) and mussel (31 species) diversity (Matthews et al. 2005). There is considerable variation in seasonal discharge in this system (Vaughn et al. 2015; Appendix B Fig. B1), yet mussels are adapted to persist as temporally stable aggregations (Sansom et al. 2018a). To avoid confounding mussel legacy effects within our treatments, we installed the experiment in a river reach upstream of known mussel beds (Atkinson and Vaughn 2015) and transplanted mussels to the site (see below), similar to Atkinson et al. (2014). Our study site was a shallow, ~ 50 m reach with relatively homogenous depth and flow (Table 2) and the stream bottom was comprised mainly of sand, gravel and cobble. The lentic conditions of the experimental reach were representative of those in the Kiamichi River in late summer and fall, where mussel beds are contained in shallow, isolated reaches with long hydrologic residence times (Vaughn et al. 2004, Vaughn et al. 2015).

Experimental design

Mussel treatments: Variation in spatial subsidies among mussel patches may be influenced by the traits of mussels occupying those patches (Howard and Cuffey 2006, Vaughn et al. 2007). To account for this, we used two mussel species in our experiment with traits that have been linked to food web dynamics and ecosystem function. *Actinonaias ligamentina* and *Amblema plicata* are both characteristic of the Interior Highlands mussel fauna (Haag 2010), and together comprise more than 70% of mussel biomass in the Kiamichi River (Vaughn and Pyron 1995). The two species differ in morphological, physiological and behavioral characteristics that influence their functional role in ecosystems (Vaughn 2010, Atkinson et al. 2018). *Actinonaias ligamentina* has a smooth shell and is more active than *A. plicata*, which has a ridged shell and tends to be sedentary (Vaughn et al. 2004, Allen and Vaughn 2009). Differences in algal and invertebrate communities occur on the shells of the two species (Spooner and Vaughn 2006, Vaughn et al. 2008, Atkinson et al. 2013). In addition, they have different temperature-dependent excretion rates, and in turn different tissue and excretion stoichiometry, which can mediate algal production and composition (Atkinson et al. 2018, Spooner and Vaughn 2012).

To represent natural variation in community composition and density in the Kiamichi River, we created mussel communities that were either dominated by live *A. ligamentina* (7 *A. ligamentina* and 3 *A. plicata*) or by live *A. plicata* (3 *A. ligamentina* and 7 *A. plicata*), resulting in a density of 40 individuals per m2. In addition, we had treatments that used sham mussels of both species in the same combinations and a sediment only control. Sham mussels were clean, empty shells filled with sand and glued together (Spooner and Vaughn 2006). Each treatment was replicated 10 times (n = 50). This design allowed us to separate effects of trophic resources (live mussels), structural features (sham mussels), and no mussels (sediment control). Mussels were collected from a downstream site, transplanted into enclosures, and returned to their collection site following the experiment.

Enclosures: We installed 50 enclosures (50 cm × 50 cm × 20 cm deep) on July 12, 2017. Enclosure frames were made from 3.3 cm schedule 40 PVC pipe and the sides and bottom were enclosed with 2.5 cm diameter poultry wire (Spooner and Vaughn 2006). Enclosures were placed in the stream reach >2 m from shore and approximately 2 m apart in a checkerboard pattern to minimize cage-effects on downstream enclosures. Sediment was removed from the stream bottom, homogenized, and then enclosures were buried 20 cm into the streambed and filled with

the homogenized sediment so the tops of the enclosures were level with the streambed and mussels could not escape. This allowed us to maintain constant mussel densities throughout the experiment while allowing invertebrates and fishes to move freely through both the sediment and water column.

Remote underwater video

We used camera-based methods to allow detailed observations of many experimental units simultaneously for extended periods. Remote underwater video (RUV) is commonly used in descriptive studies of marine environments but has become increasingly common in studies of freshwaters (Ebner et al. 2014, 2015, Wilson et al. 2015, Schmid et al. 2017). Although few of these studies have exploited the utility of RUV in an experimental setting, they have demonstrated the benefits of camera-based methods for illuminating ecological interactions among co-occurring organisms. Thus, camera-based methods provide powerful tools to test ecological questions using manipulative field experiments.

We used Activeon CX high definition cameras (www.activeon.com), with fixed focal length, continuous video, and a wide-angle field of view with a resolution of 1920 X 1080 pixels. These action cameras are a cost effective, yet reliable alternative to the handheld cameras commonly used in marine baited RUV studies (Struthers et al. 2015). The cameras were powered by lithium ion batteries (1200 mAh), allowing for a standard period of approximately 130 minutes of video recording, which was adopted for each deployment. The entire camera system was secured in a watertight case and attached to a flexible clamp mount (Captain FlexMount). Fish abundances at enclosures were observed by clamping a single camera to a 10 cm long PVC segment fitted into a “T” joint fixed to the top, upstream side of each enclosure. The 5.0 cm LCD screen on the back of each camera was used to position the camera in a downstream direction and ensure that the entire enclosure was within the field of view (Fig. 1).

We implemented underwater camera surveys exclusively during daylight between 0800 and 1700 hours to avoid light limitation at other times of day. Each enclosure was filmed for ~5 hours comprising two periods (~08:00-10:30, AM and ~15:00-17:30, PM) during a single day. All video samples were standardized to ~36 minutes of footage for analysis, beginning after the first ~36 minutes of filming when water clarity returned to normal following camera deployment. The footage used for the analysis was divided into 12, 30-second segments that were viewed in

real time, with five minutes separating each segment. The number of detected fishes for AM (mean 20.2 ± SD 6.7) and PM (mean 14.9 ± SD 5.7) sampling were similar and therefore were combined to increase the number of 30 second samples to 24 for each enclosure. Video quality for each enclosure was assessed by assigning a score ranging from 1 to 10 (zero visibility to highest clarity) to each 30 second sample. Following data collection, the lowest score at which fish were detected was determined and all samples below this threshold were removed (3% of 30-second samples). Percentage of usable video was determined for each enclosure for both week 9 and week 12 separately and compared across treatments (King et al. 2018; Appendix B Fig. B2). We use two different metrics to determine the response of fish to the treatments. The first, total fish detections, comprises the counts of maximum number of individuals within a particular 30 second segment (MaxN). We summed the MaxN for all 30 second samples for each enclosure to calculate total fish detections. The second metric, detection probability, was calculated as the proportion of 30 second segments in which at least one fish was detected. For instance, if at least one fish was detected in eight, 30 second samples out of a possible 24 at a single enclosure the detection probability was 0.33 for that enclosure. These metrics were quantified from the remaining "usable" video samples describe above. All fish observed were identified to the lowest taxonomic resolution possible and assigned to a size class by estimating their total length (mm) relative the known size of substrate baskets within the enclosures.

Quantifying spatial subsidies

To determine if live mussels and their shells increased spatial subsidies to fish, we quantified the biomass of benthic algae, organic matter and macroinvertebrates in each enclosure at 9 and 12 weeks following initiation of the experiment. Benthic algal biomass was measured by burying two ceramic tiles (width = 7.62 cm) with a glass fritted disc (diameter = 2.75 cm) mounted to the top flush with the sediment. Discs were removed at the 9 and 12-week sampling periods and frozen. Chlorophyll a was later cold-extracted with acetone and measured spectrophotometrically (American Public Health Association 2005). We placed six square mesh plastic baskets (6 cm deep, 100 cm2 surface area) filled with homogenized substrate from the experimental reach within each enclosure to measure the biomass of benthic organic matter and macroinvertebrates (Bertrand and Gido 2007). We removed three baskets from each enclosure at 9 and 12 weeks. We created a slurry by homogenizing the contents of the collected baskets in a bucket with a

known volume of stream water. A subsample of the slurry was filtered (GF/F; 0.7 μm pore size), frozen, and ashed to obtain ash free dry mass (AFDM) of benthic organic matter. Measurements of AFDM were then standardized to the volume of substrate sampled. The remaining slurry was processed for macroinvertebrates by elutriation followed by pouring it through a 0.175 mm mesh sieve and preservation in 70% ethanol. In the laboratory, macroinvertebrates were enumerated, identified to order or family (Merrit and Cummins 2008) and their length measured. We then used standard length-mass relationships (Benke et al. 1999, Giustini et al. 2008, Johnston and Cunjak 1999, Miserendino 2001, Stoffels et al. 2003, Miyasaka et al. 2008, Eckblad 1971, Obaza and Ruehl 2013) to estimate biomass. Length-mass relationships were calculated as $dry\ mass\ =\ a \cdot length\hat{}b$. Odonates longer than 10 mm were analyzed separately from the total invertebrate biomass, because their occurrence was rare in the dataset (< 1% of the data set) and, like fishes, large odonates could be attracted by treatment effects. Biomass was then standardized to the area of substrate sampled.

Statistical analyses

Variation in detection probability among treatments was assessed using ANOVA and total fish detections across treatments were compared using a generalized linear model with a Poisson distribution (Zuur et al. 2009). Fixed effects included treatment and sample date (week 9 or 12). We tested for assumptions of normality and heterogeneity of variances using Shapiro-Wilks tests and Levene's tests, for detection probability and total fish detections, respectively, before conducting statistical tests. Detection probabilities were arc-sin square-root transformed to meet assumptions of normality. We used the function *Anova*() in R to conduct likelihood ratio tests and obtain p-values for the generalized linear model as implemented in the package car.

We tested for differences in benthic invertebrates, large odonates, and algal and particulate organic matter biomass among treatments using ANOVA with treatment and sampling date as fixed effects. Before analyses were conducted it was necessary to $\log_{10}$ transform benthic invertebrate biomass and particulate organic matter data to conform to assumptions of normality and heterogeneity of variances. We used Tukey post hoc tests to conduct multiple comparisons if the null hypothesis of no difference among treatment means was rejected for each dependent variable. Statistical analyses were performed in R version 3.5.1 (R Development Core Team 2018).

Results

We detected 8 fish species from 580.5 hours of video samples. Juvenile *Lepomis* spp (~20-50 mm total length) made up nearly 90% of detections (Fig. 2A). In comparison to standardized fish surveys in a reach downstream (Hopper et al. 2018), abundances for Darters (Percidae) and Minnows (Cyprinidae) were much lower (Fig. 2B). Detection probability for fish did not vary significantly among treatments or sampling periods ($F_{4,90} = 1.36$, $P = 0.24$), although on average the probability of detection was lower at sediment only treatments (mean = 0.08, SE = 0.008) compared to detection probabilities for live (mean = 0.14, SE = 0.01) and sham treatments (mean = 0.14, SE= 0.03; Fig. 3A). However, the total number of fish detections varied significantly among treatments ($\chi^2 = 21.20$, $P < 0.001$). Multiple comparison tests indicated the total number of fish detected at either sham (mean = 3.6, SE = 0.80) or live mussel treatments (mean = 4.2, SE = 0.85) was nearly 2X higher than sediment only treatments (mean = 2, SE = 0.69); the number of fish detected did not differ significantly among live or sham mussel treatments and was not influence by mussel assemblage composition (Fig. 3B). The total number of fish detected was also influenced by time and increased from the week 9 (mean = 3, SE = 0.23) to week 12 (mean = 4.1, SE = 0.44; $\chi^2 = 8.65$, $P = 0.003$).

Benthic invertebrate biomass was highly variable across treatments, ranging from 0.03 - 2.93 g m^{-2} (mean = 0.87 g m^{-2}, SD ± 0.44) and did not differ among treatments ($P = 0.32$). Benthic invertebrate biomass increased significantly in all treatments from week 9 (mean =0.70 g m^{-2}, SD ± 0.33) to week 12 (mean = 1.05 g m^{-2}, SD ± 0.48; $F_{1,88} = 61.37$, $P < 0.001$). Odonate biomass did not differ among treatments or weeks (0.27 g m^{-2}, SD ± 0.24). Biomass of benthic algae was also highly variable among treatments (mean = 0.96 µg cm^{-2}, SD ± 0.88) and did not differ significantly among treatments ($P = 0.10$). Particulate organic matter also did not vary significantly among treatments ($P = 0.80$), but decreased significantly from week 9 to week 12 ($P < 0.001$).

Discussion

Mussel shells (live and sham) led to a heterogeneous distribution of fishes within our experimental reach and likely provide habitat for fishes at fine spatial scales. While the

probability of detection for fish did not vary significantly among treatments, the number of fish detections was greatest at either live mussel or sham treatments compared to sediment only. Our results are consistent with observations that both mussels and empty shells increase interstitial spaces in the substrate, which are important habitats for fish (Sechnick et al. 2011) and their prey (Cummins and Lauff 1969). Specifically, mussels and their spent shells might offer refuge from larger aquatic predators (Moy and Sparks 1991). For instance, during snorkel surveys in Brier Creek, Oklahoma (USA), small Green Sunfish (*Lepomis cyanellus*) wedge themselves horizontally beneath cobbles to avoid detection by observers that are confused as predators (W. J. Matthews, *personal communication*). Habitat complexity associated with mussel patches also might serve as critical flow refuge for the small fishes detected in our experiment. In comparison to a gravel bed, mussel patches significantly reduced near-bed flow velocity (Sansom et al. 2018b). Furthermore, in previous field studies, we have regularly observed active sunfish nests within mussel beds (C. Vaughn, *personal observation*), thus, stable stream sediments and the accumulation of dead shell material in and around patches of mussels may provide preferential spawning, nesting, or nursery habitat to certain fishes (Wisniewski et al 2013). Two species of cavity spawning madtoms, *Noturus gyrinus* and *N. eleuthurus* (Miller and Robison 2004) have been captured inside abandoned mussel shells in this river and others in the region (G. Hopper *personal observation*). Thus, habitat modifications by mussels at fine spatial scales appear to facilitate fish and can result in the heterogeneous distribution of fish within mussel beds.

Contrary to our hypothesis and previous studies in this river (Spooner and Vaughn 2006, Vaughn and Spooner 2006) mussel patches did not increase prey to fishes relative to sediment alone. Mussel biodeposits represent an abundant, high-quality food source for many stream invertebrates and even some fish (Limm and Power 2011, Atkinson et al. 2014). Increased nutrient cycling by mussels can modify benthic algal community composition and increase primary production that can increase invertebrate densities (Atkinson et al. 2013, Spooner and Vaughn 2006), and those effects can differ depending on the species. We used two mussel species with differing traits with documented effects on food web dynamics and ecosystem function (Spooner and Vaughn 2012, Atkinson et al. 2013), however, trophic resources to fish were not influenced by the physiological, behavioral or morphological traits of mussels occupying experimental patches. To avoid legacy effects from mussels, we located our experiment upstream of known mussel beds, near to the headwaters of the Kiamichi River. This

area is higher gradient than where previous experiments have been conducted and had relatively large substrate, which often has reduced macroinvertebrate densities compared to smaller substrates (Wise and Molles 1979). Spooner and Vaughn (2006) conducted an experiment like ours in the Kiamichi River with the same enclosure design, similar mussel species treatments (they had single species treatments rather than two-species assemblages), but conducted further downstream in a lower gradient area of the river. In their 12-month experiment they found that patches of mussels increased benthic invertebrate abundances, and that these effects were much greater during low flow summer periods than higher flows in autumn. While we planned for our experiment to encompass the typical summer low flow conditions in this river (Allen et al. 2013, Vaughn et al. 2015), mean discharge during the incubation time of the experiment (1.4 m^3 s^{-1}) was higher than in the previous 30 years (mean = 0.2 m^3 s^{-1}, SD = 0.4), and may have homogenized or prevented establishment of treatment effects (Appendix B Fig B1). Furthermore, continuously high flows through the reach may have reduced light availability to the benthos, reducing the importance of bottom up effects of nutrient recycling by mussels. While hydrologic conditions may have diminished bottom up effects by mussels, fishes still were concentrated near patches of mussels. We hypothesize that longer periods of low flow conditions may increase spatial subsidies derived in patches of live mussels (Spooner and Vaughn 2006). However, under the conditions of our experiment (higher flows) the attraction to trophic resources was less important than the structural features of mussel aggregations.

Throughout the experiment, we did not detect high densities or diversity of fishes. We hypothesized that mussels would increase prey densities and influence the distribution of benthic feeding fishes fish such as darters (Percidae) and grazing minnows (*Campostoma spadiceum*) that are prevalent in the system (Pyron et al. 1998, Figure 3.2B). The habitat in which we placed our enclosures, a low-velocity pool with cobble substrate was dominated by *Lepomis* spp. and few other species, such as darters which have higher abundances in riffles. The daytime deployment of our RUV cameras also greatly reduced the potential to detect nocturnal species, such as madtoms that may have occurred within enclosures. Indeed, two madtoms were captured in live and sham treatments during our basket sampling. Like most other sampling techniques, RUV has specific biases and limitations worth considering. For example, species identification may be limited if based solely on video footage (Pelletier et al. 2011, Cappo et al. 2004), especially for smaller individuals, fish with similar body shapes (i.e. *Lepomis* spp.) and fish that

can inhabit interstitial spaces (i.e., darters and madtoms). In addition, field and laboratory experiments revealed a nonlinear relationship between the abundance metric, MaxN used in our study, and true abundance, with underestimates increasing with abundance and mobility (Schobernd et al. 2014, Campbell et al. 2015). Given the high fish diversity previously documented in this system (Pyron et al. 1998, Matthews et al. 2005, Table 1), we can assume that the abundance of some small, schooling, and highly mobile species, such as cyprinids that inhabit the water column, has likely been underestimated in this experiment (Table 1, Figure 3.2B). Future studies might build on our results by conducting similar experiments along a gradient of stream conditions (e.g. depth or low flows), which might allow the responses of different suites of species to be quantified.

It appears that mussel patches at fine spatial scales (< 1 m^2) can facilitate fishes through direct modification of habitat in rivers where mussels and fish coexist. Despite low total fish detections in our experiment we found an effect of mussels on fish distributions at fine spatial scales, an effect that may become more apparent in downstream reaches with higher mussel densities (Atkinson et al. 2012). Understanding whether fish and mussels aggregate at fine spatial scales is important because they may interact to spatially concentrate the ecosystem effects of each group and we predict the strongest effects to occur where both mussel and fish densities are high (Hopper et al. 2018). Both habitat and trophic resources can act as spatial subsidies and aggregate species (Coppock et al. 1983, Vaughn and Spooner 2006). Although our experiment did not find a positive effect of mussel patches on the abundances of fish prey, it is likely that under different environmental contexts both habitat and nutrients supplied by mussels might be important. Investigators should seek out these types of interactions because overlapping aggregations of animals can form biogeochemical hotspots, and can have important consequences for ecosystem structure and function.

Figures

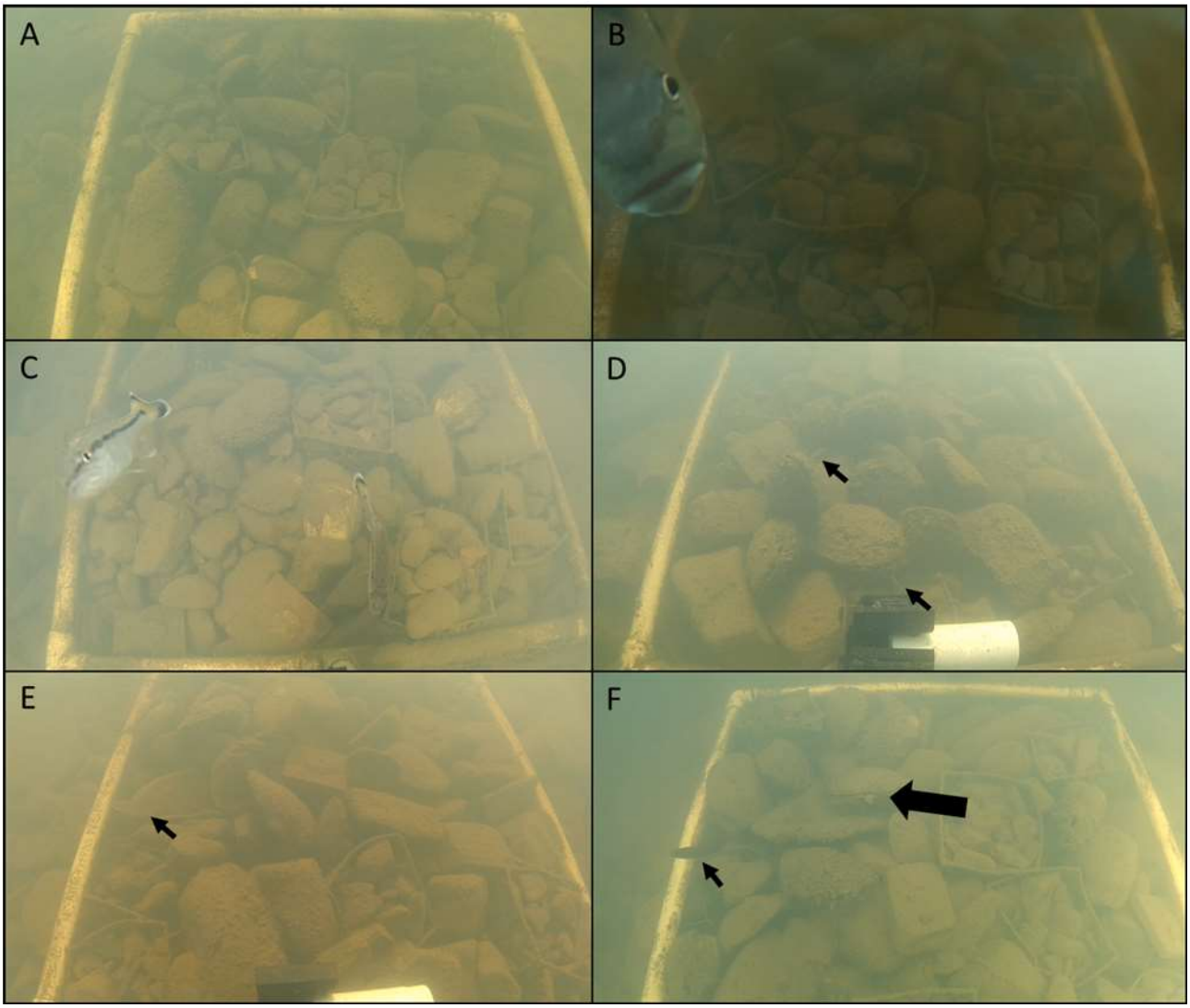

Figure 3.1 Example screen shots from remote underwater video (RUV) camera used to detected fish occurrence and diversity over experimental enclosures. View of live mussel enclosure and positioning of baskets used to quantify spatial subsidies (A); Adult Longear Sunfish (*Lepomis megalotis*) detected at a control enclosure (sediment only) (B); Two juvenile Spotted Bass (*Micropterus punctatus*) detected at a sham enclosure (C); Two juvenile sunfish *(Lepomis* spp) detected feeding on live mussels. Black arrows show the location of individuals (D); Longear Sunfish detected at a sham enclosure. Black arrow shows the location of the fish (E); Lepomis spp detected at a live mussel enclosure; small black arrow shows the location of the fish and a prominent mussel biodeposit is shown with a larger arrow (F).

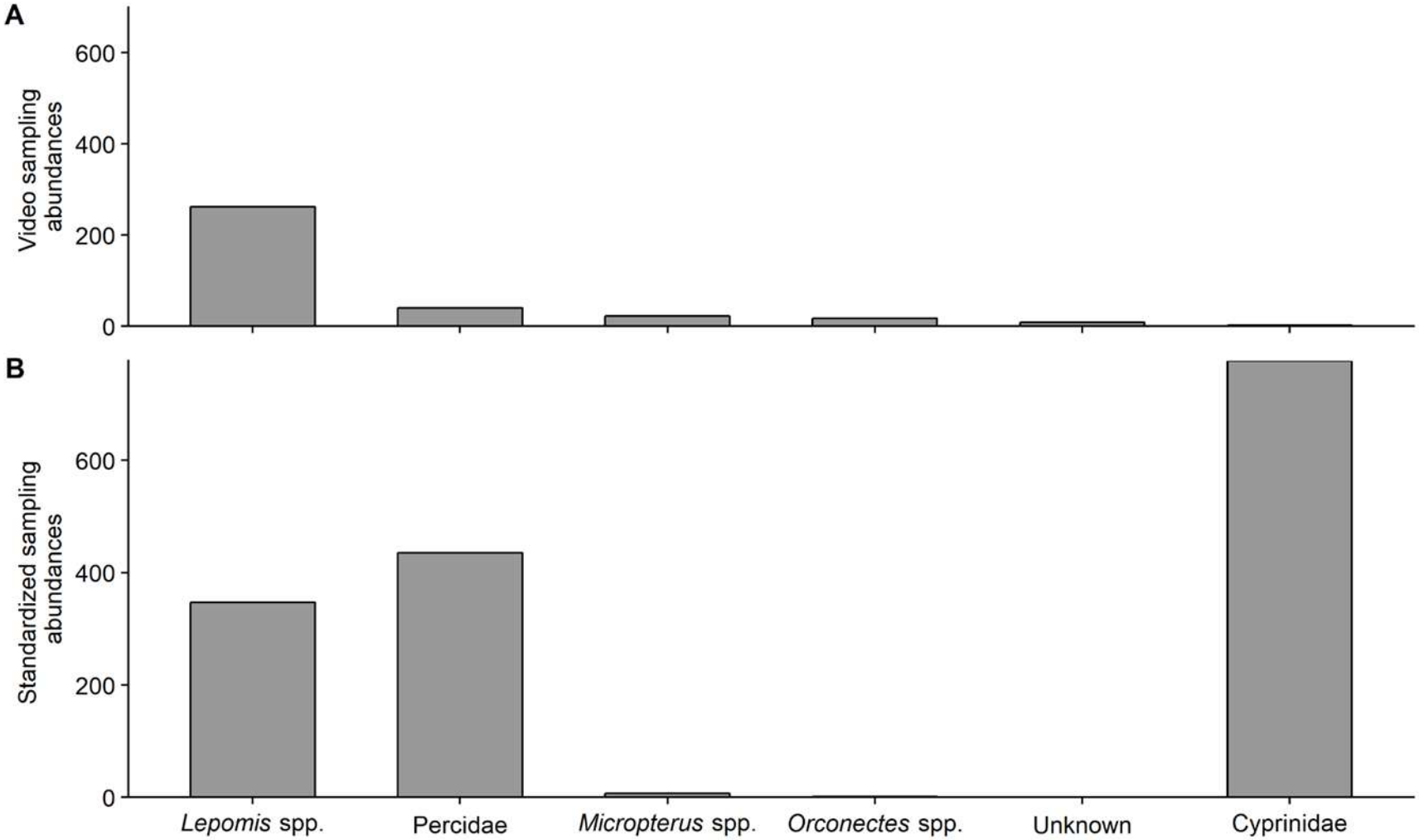

Figure 3.2 Rank-abundance for fishes and crayfish detected using the remote underwater video at enclosures placed in the Kiamichi River, OK (A). Organisms are categorized to the lowest taxonomic level possible based on visibility. Abundance of fish and crayfish at a site downstream of the experimental reach (B) and organized for comparison to abundances measured by underwater video.

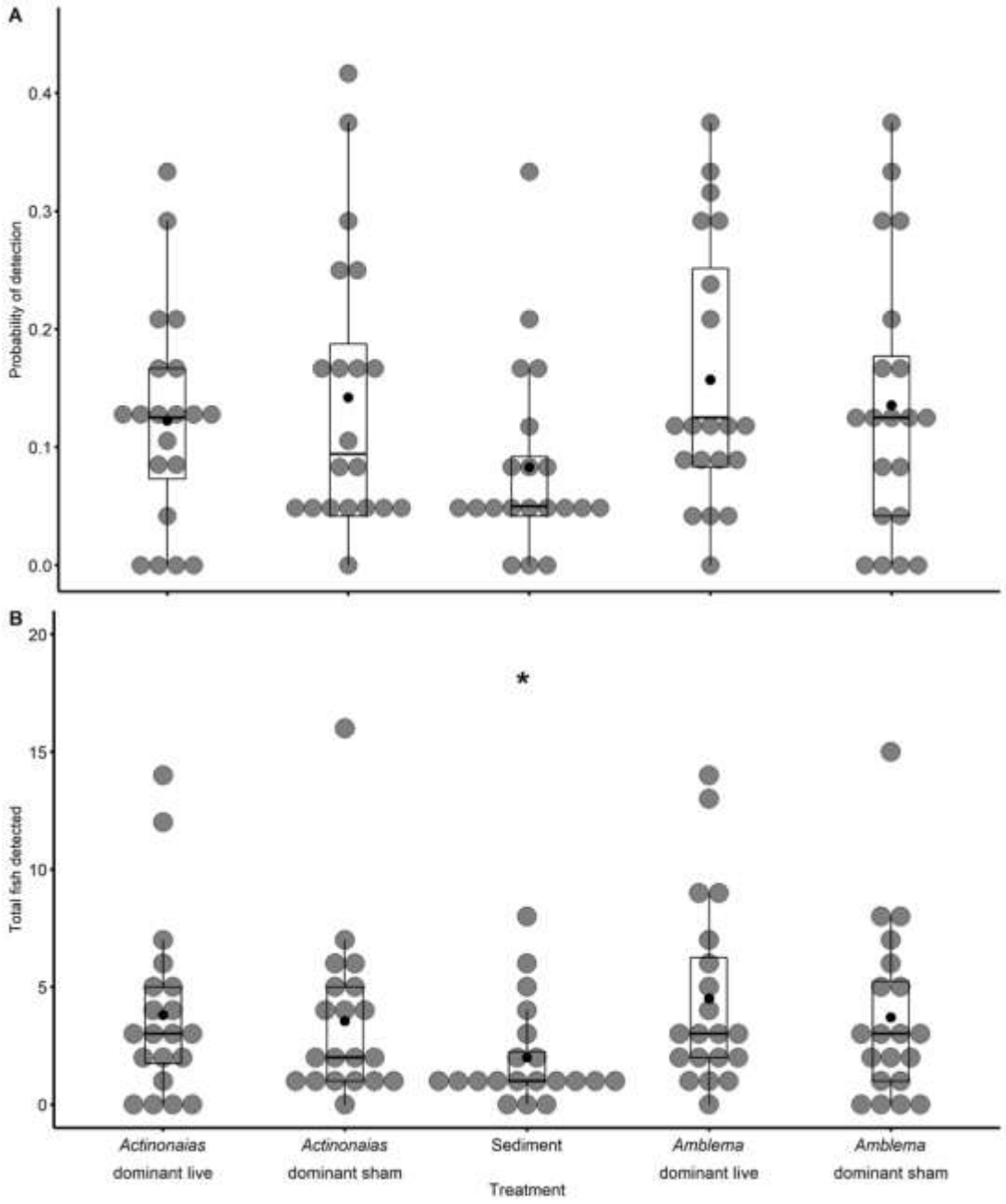

Figure 3.3 Detection probability for fish at enclosures treatments containing live mussels, sham mussels or sediment (A). Total number of fish detected in 24, 30 second samples across enclosure treatments (B). The * indicates a significant difference among the treatments and control (sediment only). Boxes cover the first through third quartile of the data; horizontal black lines indicate the median and black circles indicate the mean.

Tables

Table 3.1 Composition of fish communities in the Kiamichi River, Oklahoma, downstream of the experimental reach based on fish collected using standardized seining and electrofishing (Hopper *unpublished*). The predicted numerical response (direction and cause) is based on the trophic guilds and vertical stream position for each species. The direction of predicted response is described as either an increase (++) or no change (NA). Trophic guilds are coarsely split into: A=Algivore; D=Detritivore; I= Invertivore; and P=Piscivore. Position in stream is either the Surface, stream bottom (Benthic), or water column (WC).

Species	Predicted Numerical Response to Mussels/Shams	Trophic Guild	Position in Stream	Predicted cause of Numerical Response
Etheostoma radiosum[a]	++	I	Benthic	Habitat & invertebrates
Lepomis megalotis[a]	NA or ++	I	WC	Habitat & invertebrates
Lepomis cyanellus[a]	NA or ++	I	WC	Habitat & invertebrates
Lepomis macrochirus[a]	NA or ++	I	WC	Habitat & invertebrates
Etheostoma nigrum[a]	++	I	Benthic	Habitat & invertebrates
Micropterus punctulatus[a]	NA or ++	P/I	WC	Invertebrate & fish
Orconectes palmeri[a]	++	D	Benthic	Habitat & detrital
Percina copelandi[a]	++	I	Benthic	Habitat & invertebrates
Campostoma spadiceum	++	A	Benthic	Habitat & Algae
Lythrurus umbratilis	NA	I	WC	
Cyprinella whipplei	NA	I	WC	
Notropis boops	NA	I	WC	
Percina sciera	++	I	Benthic	Habitat & invertebrates
Labidesthes sicculus	NA	I	Surface	
Fundulus notatus	NA	I	Surface	
Gambusia affinis	NA	I	Surface	
Pimephales notatus	++	D/I	Benthic	Habitat & detrital
Etheostoma gracilis	++	I	Benthic	Habitat & invertebrates
Lepomis humilis	NA or ++	I	WC	Habitat & invertebrates
Lepomis gulosus	NA or ++	I	WC	Habitat & invertebrates
Pimephales vigilax	++	I	Benthic	Habitat & detrital
Ameiuris natalis	++	P/I	Benthic	Habitat & invertebrates
Micropterus salmoides[a]	NA or ++	P/I	WC	Invertebrate & fish
Lepomis microlophus	NA or ++	I	WC	Habitat & invertebrates
Moxostoma erythrurum	++	I	Benthic	Invertebrates
Noturus nocturnus	++	I	Benthic	Habitat & invertebrates
Pylodictis olivaris	NA or ++	P	Benthic	Fish

Note: [a] Species detected at experimental enclosures at the Kiamichi River, OK using remote underwater video (RUV).

Table 3.2 Abiotic and biotic characteristics measured across experimental treatments during week 9 and week 12. Odonate biomass is abbreviated "Od. Biomass". Values in parentheses are the standard deviation of the mean for each treatment. AFDM decreased significantly from week 9 to week 12 and Invertebrate biomass increased significantly from week 9 to week 12.

	Week 9						Week 12					
Treatment	Depth (m)	Current Velocity (m^3 sec^{-1})	Chl a (µg cm^{-2})	BOM (g m^{-2})	Invert. Biomass (g m^{-2})	Od. Biomass (g m^{-2})	Depth (m)	Current Velocity (m^3 sec^{-1})	Chl a (µg cm^{-2})	BOM (g m^{-2})	Invert. Biomass (g m^{-2})	Od. Biomass (g m^{-2})
Actinonaias Dominant Live	0.56 ± 0.09	0.02 ± 0.02	0.79 ± 0.43	1.50 ± 1.2	0.54 ± 0.24	0.01 ± 0.01	0.55 ± 0.08	0.02 ± 0.01	0.87 ± 0.52	0.3 ± 0.2	1.00 ± 0.30	0.02 ± 0.10
Actinonaias Dominant Sham	0.56 ± 0.06	0.01 ± 0.02	1.58 ± 0.84	1.70 ± 1.3	0.66 ±0.27	0.02 ± 0.01	0.59 ± 0.06	0.02 ± 0.01	1.33 ± 1.20	0.2 ± 0.1	1.06 ± 0.34	0.03 ± 0.03
Amblema Dominant Live	0.54 ± 0.07	0.01 ± 0.01	0.79 ± 0.35	1.10 ± 0.4	0.88 ± 0.43	0.03 ± 0.03	0.56 ± 0.06	0.01 ± 0.01	1.20 ± 1.05	0.4 ± 0.1	1.21 ± 0.71	0.05 ± 0.04
Amblema Dominant Sham	0.52 ± 0.09	0.02 ± 0.02	1.10 ± 0.72	1.30 ± 0.6	0.74 ± 0.29	0.03 ± 0.03	0.55 ± 0.07	0.02 ± 0.02	0.35 ± 1.63	0.6 ± 0.16	1.08 ± 0.63	0.02 ± 0.01
Sediment	0.58 ± 0.09	0.02 ± 0.01	1.08 ± 0.08	1.30 ± 3.0	0.69 ± 0.37	0.02 ± 0.01	0.59 ± 0.08	0.01 ± 0.02	0.80 ± 0.34	0.4 ± 0.3	0.88 ± 0.32	0.02 ± 0.01

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Chapter 4 - Biomass loss and species turnover during severe drought shift stream community excretion stoichiometry

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Background

The significance of animals to nutrient cycling (animal-mediated nutrient cycling) is now widely accepted across ecosystems (Atkinson *et al.* 2017; Sitters *et al.* 2017). Animal-mediated nutrient cycling is the product of the stoichiometric requirements (Capps & Flecker 2013), biomass and density of organisms (McIntyre *et al.* 2008; Atkinson & Vaughn 2015), background nutrient conditions (Wilson & Xenopoulos 2011), and ecosystem size (Benstead *et al.* 2010). The results of these interactions are often most apparent when environmental conditions shift the biomass distribution of dominant animal groups. For example, in stream ecosystems, excretion from aggregated animals under low flow conditions can supply a larger fraction of ecosystem nutrient demand relative to catchment run-off (Grimm 1988; Childress, Allan & McIntyre 2014; Atkinson & Vaughn 2015). However, extreme events may exacerbate ecosystem effects of animals, because they control abundances and occurrences of species (Boulton 2003; Lake 2003), species' trait expression and the direction and magnitude of how species' traits affect ecosystem function (Ackerly 2003).

In streams, drought represents one extreme of the hydrological continuum and is characterized by unpredictable periods of low flow (Lake 2003; Lennox 2019). Stream organisms experiencing regular drought evolved adaptations to water scarcity, although these adaptations may not entirely protect them from the suite of stresses associated with intense drought events (Boulton 2003). Moreover, drought is an extreme ramp disturbance, in which the perturbation intensifies over time and may prove more challenging for organisms due to a lack of

predictability in timing or duration (Lake 2003; Lynch & Magoulick 2016). For example, the formation of isolated pools during extreme drought prevents the normal transport of nutrients, biota and organic matter downstream and can produce distinct lentic conditions among pools (Lake 2003). In some pools, especially those with open canopies, algal blooms may develop (Dahm *et al.* 2003), conductivity and temperatures may rise (Matthews, Surat & Hill 1982), and stratification may occur (Wood, Fisher & Grimm 1992), all of which can severely stress or kill stream biota. It is during these times that ecosystem processes shift from being hydrologically to biologically controlled, with internal processes (e.g., sedimentation of autochthonous material, nutrient cycling and consumption) shaping conditions in pool habitats (Tockner *et al.* 1999).

Biogeochemical processes underlying ecosystem function can be affected during and following extended periods of drying (Baldwin & Mitchell 2000; Dahm *et al.* 2003). Drought decreases inputs of dissolved organic carbon, phosphorus (P) and nitrogen (N) to intermittent streams, leading to a shift from predominantly heterotrophic (microbial) to autotrophic organisms (Dahm *et al.* 2003). Subsequent low dissolved oxygen from overnight respiration also may kill some animals (Matthews & Maness 1979; Ostrand & Marks 2000). Either stratification and/or stagnant conditions can lead to nutrient accumulation, increasing the risk of toxic algal blooms (Ha *et al.* 1999; Colley 2004), which can cause further mortality of stream biota. Mortality of fishes and invertebrate populations will, in turn, affect animal-mediated ecosystem functions such as nutrient cycling. Of the few studies that have tested the impacts of droughts on stream biogeochemistry (Williams & Melack 1997; Wall, Phillips & Riva-Murray 1998; Morecroft *et al.* 2000), most focus on microbial-mediated processes and responses that follow drought (Foster & Walling 1978; Bayley *et al.* 1992).

Few studies have tested the influence of drought on the ecosystem effects of aquatic animals (Matthews & Marsh-Matthews 2003; Atkinson, Julian & Vaughn 2014). Indeed, animal contributions to stream food web dynamics and nutrient cycles can differ during drought years or during periods of low flow (Wootton, Parker & Power 1996; Vaughn, Gido & Spooner 2004; Power, Parker & Dietrich 2008). The influence of drought on the effects of aquatic animals in ecosystems may be related to the flux of individuals or materials. For instance, consumption of resources is intensified in local patches during low-flow conditions when densities are high (Canton, Short & Ward 1984; Matthews & Marsh-Matthews 2003) and feces, nutrients and fragmented particulate organic matter tend to persist in isolated pools (Conallin *et al.* 2011;

King, Tonkin & Lieshcke 2012). Moreover, positive feedbacks between physiological or stoichiometric traits of animals and ambient conditions may occur until habitat conditions deteriorate beyond physiological thresholds and mortality leads to loss of animal biomass (Boulton 2003; Atkinson *et al.* 2014). Specifically, nutrient cycling rates by aquatic animals should increase with temperature as animal metabolic rates increase, causing an increase in nutrient availability, which if converted to algal biomass, would result in oxygen depletion. Given the potential consequences of these feedbacks, understanding how animal-mediated nutrient cycling is influenced during drought represents a fundament knowledge gap (Dahm *et al.* 2003; Matthews & Marsh-Matthews 2003).

We examined whether observed shifts in animal assemblage composition and biomass influenced animal-mediated nutrient cycling in Kings Creek (Kansas, USA), an intermittent prairie stream, during the worst drought on record for this system. We compared the biomass of fishes, crayfishes and tadpoles along a gradient of drying pools and estimated their contributions to nutrient cycling through excretion of N and P. Our objectives were to: 1) test how much the biomass of aquatic animal assemblages differ along a gradient of isolated, drying pools, 2) quantify abiotic conditions among the pools and associate changes in abiotic factors with changes in the taxonomic composition of pool assemblages and 3) determine if temporal changes in assemblage biomass and taxonomic composition among pools result in different flux and stoichiometric contributions to nutrient cycling through differential excretion of N and P. We hypothesized assemblage biomass would decrease with pool size as habitat became increasingly limited and abiotic conditions (i.e., water temperatures, dissolved oxygen, and ammonium concentrations) became increasingly harsh. Furthermore, we hypothesized that temporal differences in assemblage biomass and taxonomic composition would lead to different contributions of pool assemblages to nutrient cycling and stoichiometry.

Materials and Methods

Study location and the onset of drought

Kings Creek is located on the Konza Prairie Biological Station (KPBS) in the Flint Hills region of Kansas (USA) and drains 3487 ha of native tallgrass prairie. Tallgrass prairie is the dominant land cover type, but trees occur in riparian areas in the lower portions of the catchment. Grazing

by bison (*Bison bison*) occurs in the headwaters with some row-crop agriculture also present in lower reaches of the catchment where the study took place. Discharge in Kings Creek is highly variable, but tends to peak during April, May and June (Dodds *et al.* 2004). During mid- to late-summer (July–September) a lack of precipitation typically leads to drying of middle reaches in Kings Creek while downstream reaches continue to flow. Severe drought during 2018 resulted in near-complete stream drying throughout the catchment. The middle reaches were first to dry, followed by headwater springs, and finally in downstream reaches that historically remained flowing year-round. This main-stem perennial reach ceased to flow by early June, leading to the development of isolated pools (Figure 4.1).

To test our hypothesis that animal assemblage biomass would be highest in larger pools, we selected 12 isolated pools (no surface water connectivity) within the main stem of Kings Creek that varied in size (surface area and depth) and sampled the animal assemblages and abiotic factors within each pool monthly during June, July and August (Table 1). Additionally, we logged hourly temperature in each pool throughout the study with HOBO UA-002-64 Pendant Data Loggers (Onset Computer Corporation, Bourne, MA) placed in the middle of the pool at approximately 0.5 m depth. Pool dimensions were characterized during animal assemblage sampling by measuring the length of each pool and width at three transects (m) to calculate surface area (m^2). Depth (cm) was taken at 5 equidistant points along each transect. We calculated pool volume (m^3) as the product of cross-sectional area and mean depths of each transects. We also tracked monthly palmer drought severity index (PDSI) values for the study area using data from the North American Drought Monitoring program (www.ncdc.noaa.gov). Variation in nutrient concentrations among pools was quantified by collecting a 250 mL unfiltered water sample during each sampling period. Water samples were placed on ice within 30 min of collection and transported back to the lab where they were kept frozen until analysis. Because ammonium (NH_4^+) can be toxic to aquatic animals in high concentrations, water sample nutrient analysis focused on NH_4^+ (Mayes *et al.* 1986). Analyses were conducted with the indophenol blue method using an O-I Analytical Flow Solution IV autoanalyzer (APHA, 2005).

Assemblage composition and biomass variation among pools

We used one or more seine hauls (4.6 × 1.8 m, 3.2-mm mesh) to sample the entire length of each pool to estimate animal biomass across a pool-size gradient. Given the severity of the drought,

we chose seining rather than electrofishing to minimize stress to animals occupying the potentially harsh conditions. Similar to previous studies (Allen et al., 1992), we found seining to be very effective when pools were narrow and isolated, limiting the ability of animals to escape around the seine. All fish, crayfish and tadpoles were identified to species, enumerated and their total lengths measured (mm). At a later date, we determined capture efficiencies (q) using a two-pass closed population mark-recapture approach at seven of the pools used in the study. Individuals collected during the first pass were identified to species, measured and given a noticeable clip on the caudal fin or uropod (crayfishes) prior to being returned to the pool. Each pool was resampled several hours later using identical methods. Because recaptures for some species were insufficient in some pools (Table S1), we averaged q across species and used linear regression to test whether the relationship between q and pool volume was significantly different than zero. Raw values for q were arc-sine square-root-transformed to meet assumptions of normality and heterogeneity. Because regression indicated that q significantly declined in pools with greater volume ($P = 0.04$, $R^2 = 0.52$), we estimated capture efficiencies for each of the 12 pools during the 3 sampling periods using the linear model ($\sin^{-1}\sqrt{(q)} = -0.00338 \times volume + 0.85$). Back-transformed, modeled capture efficiencies for pools in Kings Creek were similar to those of Allen et al., (1992) and ranged from 0.19 – 0.55. Modeled capture efficiencies were applied to the relative abundance of species captured during standardized sampling to estimate absolute abundances for all species. Length-mass regressions from a subset of individuals collected on-site or previously collected individuals of the same species, or a species with similar body shape were used to estimate wet mass (K. Gido *unpublished data*) of all captured individuals (Table S2). Assemblage biomass (g m^{-2}) was estimated for each pool separately as the product of the absolute abundance estimate and the mean predicted mass of individuals collected divided by the area of their respective pool. Pool estimates were calculated during June, July and August of 2018.

Estimating animal-mediated nutrient cycling among pools

We hypothesized spatial and temporal differences in the distribution of biomass and taxonomic composition would lead to different contributions of animal assemblages to nutrient cycling. To test this hypothesis, individual excretion rates were measured for 5 fish, 2 tadpole, and 1 crayfish species that comprised more than 80% of total biomass across pools. Individual excretion rates were measured for at least 8 individuals of each species (Table 2). Animals were collected from

pools using a seine and placed in a cooler with water from the pools and allowed to recover for 15 min. Individuals were then placed in a plastic container with 100-200 mL of filtered stream water depending on the size of the animal (GF/F; 0.7 μm pore size; Whatman Buckinghamshire, U. K.) and incubated for 1 hour. Following the excretion experiment, total length and wet mass were recorded for all individuals. Water samples were collected at the end of each trial, placed on ice and transported back to the lab where they were kept frozen until analyses. Nutrient analyses focused on NH_4^+ and soluble reactive phosphorus (SRP). Analyses were performed using the indophenol blue and ascorbic acid methods for NH_4^+ and SRP, respectively, using an O-I Analytical Flow Solution IV autoanalyzer (APHA, 2005). Excretion calculations were based on the difference between nutrient concentrations of identical containers incubated simultaneously with and without animals.

Spatially explicit species composition and biomass data were used to compare variation in assemblage nutrient excretion rates among 12 isolated pools at our three discrete sampling periods during the drought. We applied our excretion estimates to areal biomass estimates to derive areal excretion rates (μmol m^{-2} h^{-1}) for each assemblage during each sampling period. The estimated excretion rates of N and P for each animal assemblage were used to calculate assemblage excretion N:P molar ratios. We then compared assemblage areal excretion rates and N:P across the gradient of assemblage biomass to detect changes in the contributions of animals to nutrient cycling in isolated pools.

Analyses

Assemblage composition and biomass variation among pools

We hypothesized assemblage biomass would decrease with pool size as habitat became limited and abiotic conditions became increasingly harsher. We tested for temporal differences in animal biomass among drying pools using analysis of covariance (ANCOVA) with "biomass" as a response variable, "sample period" as a fixed effect and "surface area" as a covariate. We tested if assumptions of normality and heterogeneity of variances were met using Shapiro-Wilks tests and Levene's tests, respectively, before conducting statistical tests. It was necessary to log_{10}-transform "biomass" data to conform to assumptions of normality and heterogeneity of variances.

We used canonical correspondence analysis (CCA, ter Braak, 1987) in the package 'vegan' (Oksanen *et al.* 2019) to summarize spatial and temporal variability in pool assemblage structure and to evaluate the relationship between abiotic environmental variables (NH_4+, pool volume, temperature) and variation in pool assemblage structure, using species-specific biomass estimates for each sampling period. CCA is a multivariate ordination technique that selects a linear combination of environmental variables to maximize the dispersion of species scores, while preserving Chi-square distances among samples, thus reflects differences in proportional abundance of species across samples (Gauch 1982). Axes gradient lengths provide a measure of faunal turnover, and sample scores separated by four standard deviations should have few species in common (Gauch 1982; ter Braak 1987). This analysis produces a diagram with vector arrows that represent the relative importance of environmental factors in describing variation among pool assemblages. Rare species (<5 occurrence across samples) were excluded from these analyses because their occurrence in samples is more likely random and does not represent true differences in assemblage biomass structure across space or time. We combined biomass of northern cricket frog (*Acris crepitans*) and northern leopard frog (*Lithobates pipiens*) because *A. crepitans* only occurred in one pool but was the dominant animal biomass and would have been removed otherwise. This resulted in a core community of 10 species. Monte Carlo simulations (999 iterations) were used to test whether eigenvalues from the CCA were significantly ($P < 0.05$) greater than those generated from a randomized matrix. Prior to CCA, variance inflation factors were used to check variables for multicollinearity. All values were <10 so we concluded that multicollinearity was minimal. All analyses were performed in R version 3.5.1 (R Core Team 2018).

Animal-mediated nutrient cycling among pools

We tested for interspecific differences in NH_4^+ and SRP (hereafter N and P) excretion rates for all species. Size scaling of N and P was visualized using least-squares regression of $\log_{10}$-transformed excretion rates against $\log_{10}$-transformed wet mass. When slopes for individual species were equal (overlapping confidence intervals), we used ANCOVA to test for interspecific differences of $\log_{10}$-transformed excretion rates, using $\log_{10}$-transformed wet mass as a covariate. If no relationship was found between excretion rates and the covariate, we used ANOVA to test for interspecific differences in excretion rates. Differences among species

excretion N:P were assessed using ANOVA. Tukey pairwise test for multiple comparisons were used if the null hypothesis of no difference among species excretion N:P was rejected.

We tested how changes in areal biomass influence nutrient cycling rates of pool assemblages using ANCOVA with areal N excretion, areal P excretion as response variables, sample period as a fixed effect and biomass as a covariate. Areal N and P were $\log_{10}$ transformed prior to analyses to meet assumptions of normality and heterogeneity of variances. Assemblage excretion N:P was $\log_{10}$-transformed to improve normality and meet assumptions of heterogeneity and differences among pool assemblage excretion N:P during drought was assessed using ANOVA (Schminder *et al.* 2010).

Results

Drought induced changes in assemblage composition

A total of 16,426 individuals representing 19 species was captured across the 12 pools throughout the study. Fishes comprised the majority of biomass in 10 of 12 pools during June, but only 5 of the remaining pools in August (Table 4.3; Figure C1). In June, *C. erythrogaster* was the most abundant fish (69% of individuals captured), the dominant biomass in 8 of the 12 pools and the only fish species to occur in all pools (Table 4.3; Figure C1). However, as the drought continued, the biomass of *C. erythrogaster* decreased, was completely lost from Pool 6 and Pool 8 by July, and was the dominant biomass of only 3 pools in August. One species, water nymph crayfish (*Orconectes nais*), was ubiquitous in June (Table 4.3; Figure C1). Pool 2 was dominated by *A. crepitans* during June and July, but their abundance was reduced during August when western mosquitofish (*Gambusia affinis*) numerically dominated and made up > 40% of assemblage biomass. Due to either mortality, emigration (for crayfish) or metamorphosis (for tadpoles), biomass of nearly all species declined from June to August (Table 4.3, Figure C1). One exception was that *G. affinis* biomass increased to more than 20% of assemblage biomass in 4 pools where the species occurred in low abundances at the outset of the study. None of the remaining species comprised more than 11% of sampled biomass.

Severe drought conditions (PSDI between -3 and -4) persisted throughout the study. Consequently, Pool 5 dried completely before July sampling (Figure 4.1), and Pool 6 and 10 dried by August. CCA characterized the association between assemblage structure and abiotic

conditions across the 12 pools and three sample periods (Figure 4.2). The first and second axis cumulatively explained 91.96% of the constrained variation among pools and sample periods. Pool surface area was the most important explanatory variable in the CCA ($F_{1, 28} = 4.28$; $P = 0.02$), while temperature ($F_{1, 28} = 1.66$; $P = 0.13$) and NH_4^+ concentrations ($F_{1, 28} = 0.75$; $P = 0.52$) did not appear to influence assemblage composition. Higher Axis I scores reflected increased abundance of *C. erythrogaster* in larger pools with lower water temperatures. The remaining species had lower Axis I scores and were typical of smaller pools with warmer temperatures characteristic of conditions in July and August. *Gambusia affinis* was the most abundant fish species in August and had the lowest Axis I score. These differences in assemblage structure resulted in clear separation of the assemblages sampled during June (larger pools) having generally higher Axis I scores and July and August (smaller pools), which had lower Axis I scores.

Animal mediated nutrient cycling

Size-scaling of N excretion rates was significant for all species ($P < 0.05$, Table S3). ANCOVA revealed the relationship between N excretion and mass for the non-native *G. affinis* was significantly different than other species and increased more than proportionately with wet mass (Table C3). However, the per capita N excretion rate of *G. affinis* was less than half that of all native fishes, crayfish and tadpoles (Table 4.2). Only four fish species, *C. erythrogaster*, *G affinis*, common shiner (*Luxilus cornutus*) and creek chub (*Semotilus atromaculatus*) showed significant size-scaling for P excretion rates ($P < 0.05$, Table C2). ANCOVA testing for interspecific differences in P excretion rates showed that *C. erythrogaster* and *S. atromaculatus* increased proportionately with wet mass (Table C3). *Orconectes nais* per capita excretion rate was nearly 10-fold lower (0.01 μmols g^{-1} h^{-1}) than other species (Table 4.2). No species showed size-scaling between excretion N:P and wet mass. However, excretion N:P differed significantly among species ($F_{7, 96} = 25.70$, $P < 0.001$). Post hoc tests, indicated *O. nais* excreted at higher N:P relative to other species. *Gambusia affinis*, had the lowest N:P and was significantly lower compared to others species (Table 4.2).

Drought induced changes in biomass and animal-mediated nutrient cycling

Assemblage biomass was generally greater in pools with larger surface area (slope = 0.85$F_{1, 26}$ = 12.14, P = 0.002) and this pattern was consistent across months (Figure 4.3A). Temporally distinct assemblage biomass mediated differences in assemblage areal N excretion rates among pools ($F_{2, 26}$ = 9.61, P = 0.001), with greater N excretion rates occurring in June when assemblage biomass was highest (Figure 4.3B). In contrast, P areal excretion rates increased during August ($F_{2, 26}$ = 8.02, P = 0.002; Figure 3C). Accordingly, molar N:P of assemblage excretion decreased significantly throughout the drought ($F_{2, 26}$ = 4.44, P = 0.02; Figure 4.4) and post hoc comparisons indicated molar N:P of assemblage excretion was significantly lower in August compared to June (P = 0.03) and July (P = 0.04).

Discussion

Our study quantified shifts in the ecosystem effects of aquatic animals resulting from biomass loss and structural changes in isolated pool assemblages during a severe drought. Deteriorating stream conditions caused large reductions in the biomass of pool assemblages and subsequent changes to animal-mediated nutrient cycling. For example, we saw a large decline in N (average of 91% among pools) and P excretion (average of 45% among pools) by pool assemblages following drought related reductions in biomass and alterations to assemblage composition. Variable abiotic conditions among isolated pools also led to changes in their assemblage structure. Pool volume had the greatest influence on the structure of pool assemblages. These changes were driven by the complete loss or massive decline of *C. erythrogaster* and the recruitment of *G. affinis* during drought. In contrast to the other species, *G. affinis* is a non-native fish in this catchment and appeared to proliferate under conditions imposed by drought (Casterlin & Reynolds 1977; Hubbs 2000), becoming abundant in four pools by August. Increasing biomass of *G. affinis*, in combination with the high per capita P excretion of this species, appear to have contributed to the rise of assemblage P excretion in August (Figure 4.3C and C1). Thus, our study highlights the potential for severe drought to influence the stability and function of ecosystem processes, such as nutrient cycling, by shifting species' roles associated with their ability to tolerate harsh conditions.

Pool assemblage biomass primarily mediated fluxes of N with a reduction in assemblage excretion rates coinciding with biomass declines. However, assemblage P excretion was only

initially reduced with biomass loss and tended to increase in small pools remaining in August. Animal assemblages at the end of the drought had higher proportional biomass of species that excreted P at higher rates, whereas most pools early in the drought were dominated by *C. erythrogaster* (Figure C1), which had a relatively low P excretion rate (Table 4.2). Excretion of P by late drought assemblages might contribute to toxic cyanobacterial blooms by contributing excess P, especially where solar irradiance and temperatures are high (Donnelly, Grace & Hart 1997; Ha *et al.* 1999). Thus, we hypothesize that nutrients excreted by animals in isolated pools is likely important to algal and cyanobacteria dynamics during drought and may contribute to further reductions in animal biomass and associated ecosystem functions through local extinction of sensitive taxa.

Loss of biomass and shifts in assemblage structure over the course of the drought led to a decline in assemblage excretion N:P. Animal excretion was measured near the beginning of this study (late June) and may not reflect physiological consequences of thermal stress and starvation such as when organisms catabolize their tissues and excrete at higher N:P (Spooner & Vaughn 2008). Furthermore, stoichiometric models predict reductions in consumption rates can increase excretion N:P (Moody *et al.* 2018) and certain animals may be limited by resource availability during drought, which may explain the high excretion N:P of the herbivorous minnow, *C. erythrogaster* in our study (mean = 25.4; Table 4.2) compared to other studies (mean = 9.8, SD = 2.7, n = 10; Macmanamay *et al.* 2010). Reduced foraging is expected to enhance nutrient use efficiency and decrease excretion rates as an adaptive response to the extrinsic mortality threat posed by predators (Dalton & Flecker 2014). The relatively low excretion rates measured for some taxa during drought compared to other studies (Vanni & McIntyre, 2016) may signal an adaptive response to mortality imposed by harsh conditions of intense drought similar to that posed by predation threat, but further tests of this hypothesis are needed.

Distinctive lentic conditions developed among pools and resulted in unique assemblages forming among the 12 pools with potentially important consequences to ecosystem processes. A single pool, Pool 2, was dominated by the tadpole, *A. crepitans* during June and July comprising 94% and 98% of assemblage biomass, respectively. Nevertheless, this species was nearly absent from Pool 2 in August when *G. affinis* numerically dominated (40% of assemblage biomass, Figure C1). In contrast to fish, tadpole biomass in Pool 2 was probably reduced by an environmentally-cued developmental switch to adult frogs (Crump 1989; Gerland *et al*., 2005)

that emigrated rather than died. Fish and amphibians can both represent nutrient sinks by retaining P to construct bones, however, differences in fish and amphibian life histories probably affect their role in nutrient cycling during severe drought. For example, Capps et al., (2015), modeled the influence of metamorphosing wood frogs (*Lythobates sylvaticus*) on P export from an aquatic habitat into surrounding terrestrial habitats and found wood frogs were net exporters of nutrients from a vernal pool over 21 years. Whereas drought induced fish mortality may contribute to remineralization of P through decomposition (Vanni, Boros & McIntyre 2013; Boros, Takacs & Vanni 2015), life stage shifts by amphibians during extreme drought likely lead to rapid transfer of nutrients out of isolated pools and into adjacent ecosystems (Regester & Whiles 2006; Regester, Whiles & Lips 2008). This result highlights the potentially important role of biphasic amphibians on energy and nutrient transfer across terrestrial and aquatic margins during drought, and warrants further investigation of the variable contributions of co-occurring taxonomic and functional groups on ecosystem function.

The supply ratios of nutrients that limit primary producers and bacteria can depend on the stoichiometric traits of the dominant animal group (Elser *et al.* 1988, 1995). While fish species had reduced biomass, crayfish maintained relatively high biomass and likely serve as important nutrient cyclers in isolated pools during severe drought. In combination with previous work investigating somatic stoichiometry of crayfish (Evans-White & Lamberti 2005), the high N:P of crayfish excretion we observed indicates crayfish might act as P sinks in isolated pools. Although our study was conducted during the most severe drought on record for this stream, we suggest nutrients controlled by crayfish may be important to ecosystem functions, including microbial respiration and leaf decomposition in isolated pools dominated by this group (Rosemond *et al.* 2002). Overall, our study contributes evidence that the relative dominance of broad taxonomic groups (e.g. crustaceans or fishes) may have a prominent influence on stream nutrient dynamics and food web interactions, especially during periods of drought.

With expectations of a drier future for this region, we should anticipate significant changes to stream food webs and nutrient dynamics as events like the one documented here appear more frequently and with greater intensity (Mishra & Singh 2011; Dai 2013; Langerwisch *et al.* 2013; Diffenbaugh, Swain & Touma 2015). Physiological tolerance and stoichiometric traits could be combined with population vital rates to assess and predict the immediate and lasting consequences of such disturbances to stream nutrient dynamics (Atkinson *et al.* 2014).

Organismal traits including thermal tolerance, feeding, and life history have previously been used to evaluate risks to both drought and climate change (Wenger *et al.* 2011; Villnäs *et al.* 2012; Chessman 2013), and thermal tolerance may drive changes in community composition as climate change takes hold and anthropogenic modifications to hydrologic regimes continue (Spooner & Vaughn 2008; Perkin *et al.* 2017). Although the full implications of shifts in the composition of freshwater communities to ecosystems are not known, our study fills a fundamental knowledge gap by illustrating that biomass loss and turnover of species with varying stoichiometric traits alter the availability of nutrients in a prairie stream during severe drought, which may result in additional losses in ecosystem function and changes in community structure.

Figures

Figure 4.1 Time series of Pool 5 during an extreme drought that impacted Kings Creek, USA in summer 2018. Top photo was taken June 18th, middle photo was taken July 3rd and bottom photo was taken July 10th

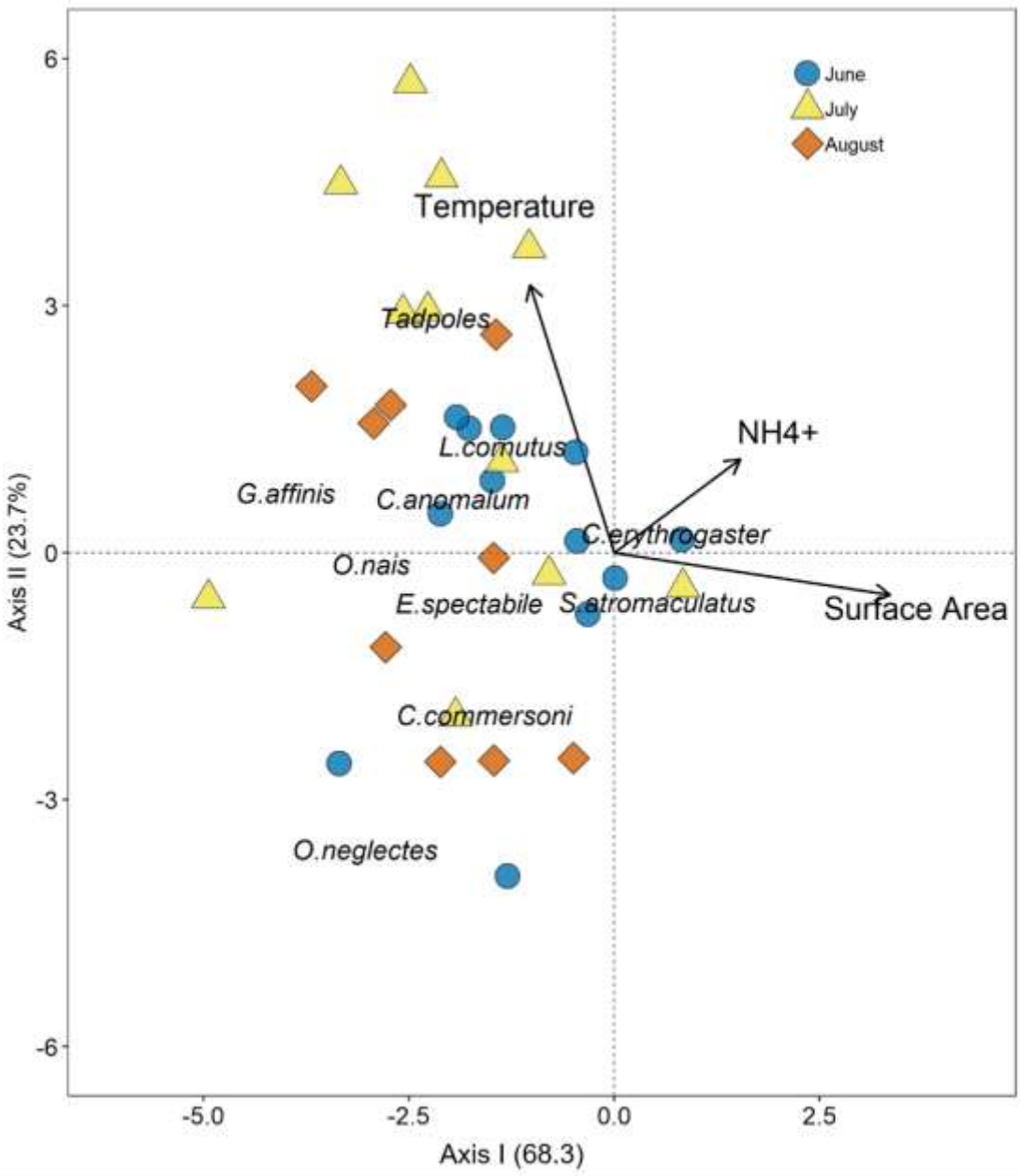

Figure 4.2 Canonical correspondence analysis of the aquatic animal community (i.e., fish, crayfish and tadpoles) across 12 isolated pools in Kings Creek, USA sampled during June, July and August of 2018. The first and second axes had eigenvalues of 0.185 and 0.064, respectively. The species score for Tadpoles combines biomass of the two species *Acris crepitans* and *Lithobates pipiens*. Labels for environmental vectors: Temperature is the maximum temperature (°C) measured for each pool during each month; NH_4^+ is µmol L^{-1} of ammonium measured from water samples during each sample period; and Surface Area was measured at each sampling period (m^3). One pool dried completely before July sampling and two pools dried before August sampling (n = 32).

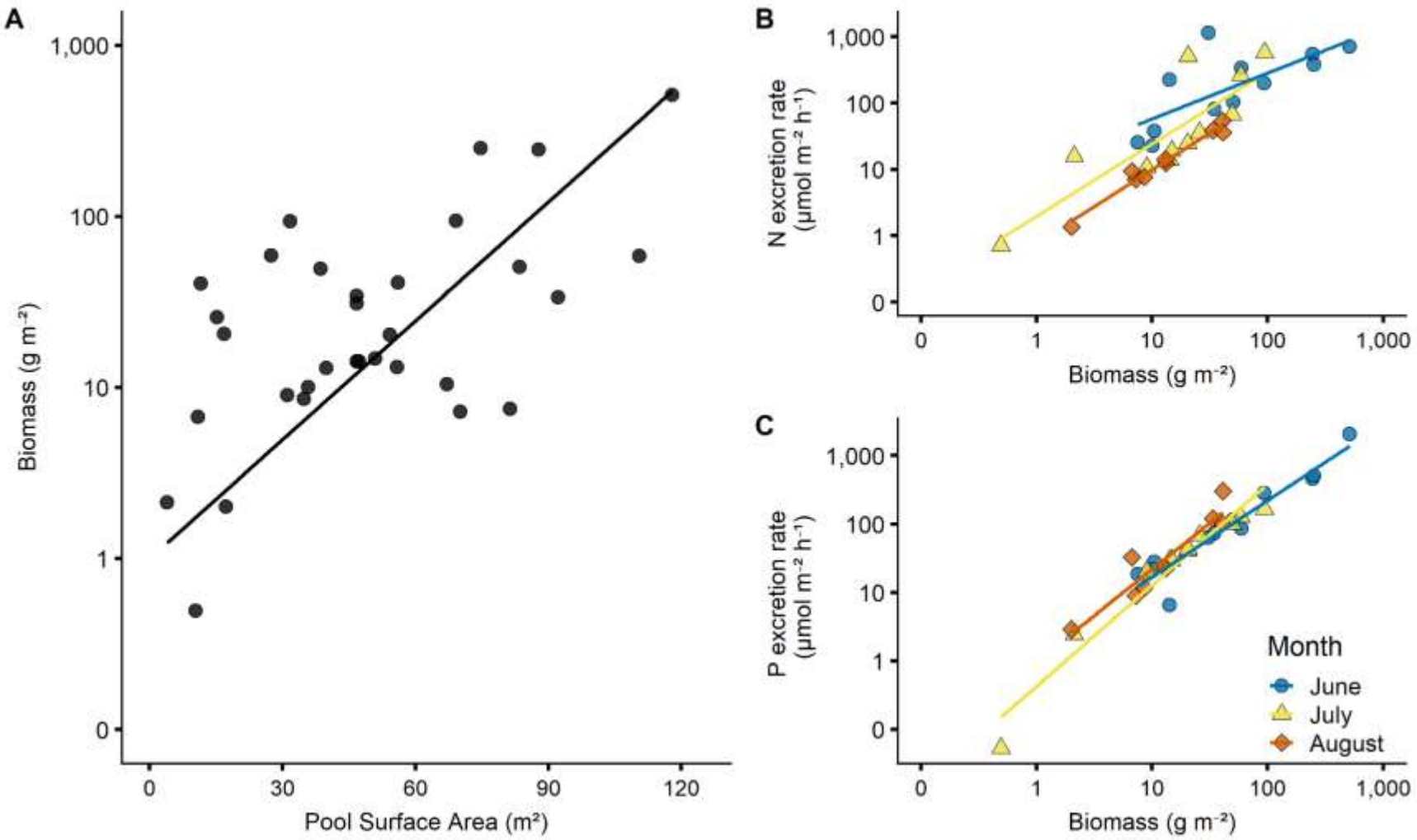

Figure 4.3 Relationship between pool assemblage biomass and surface area (A) and assemblage N excretion (B) and P excretion (C) rates and pool assemblage biomass from 12 isolated pools in Kings Creek, USA sampled during June, July and August of 2018. One pool dried completely before July sampling and two pools dried before August sampling (n = 32).

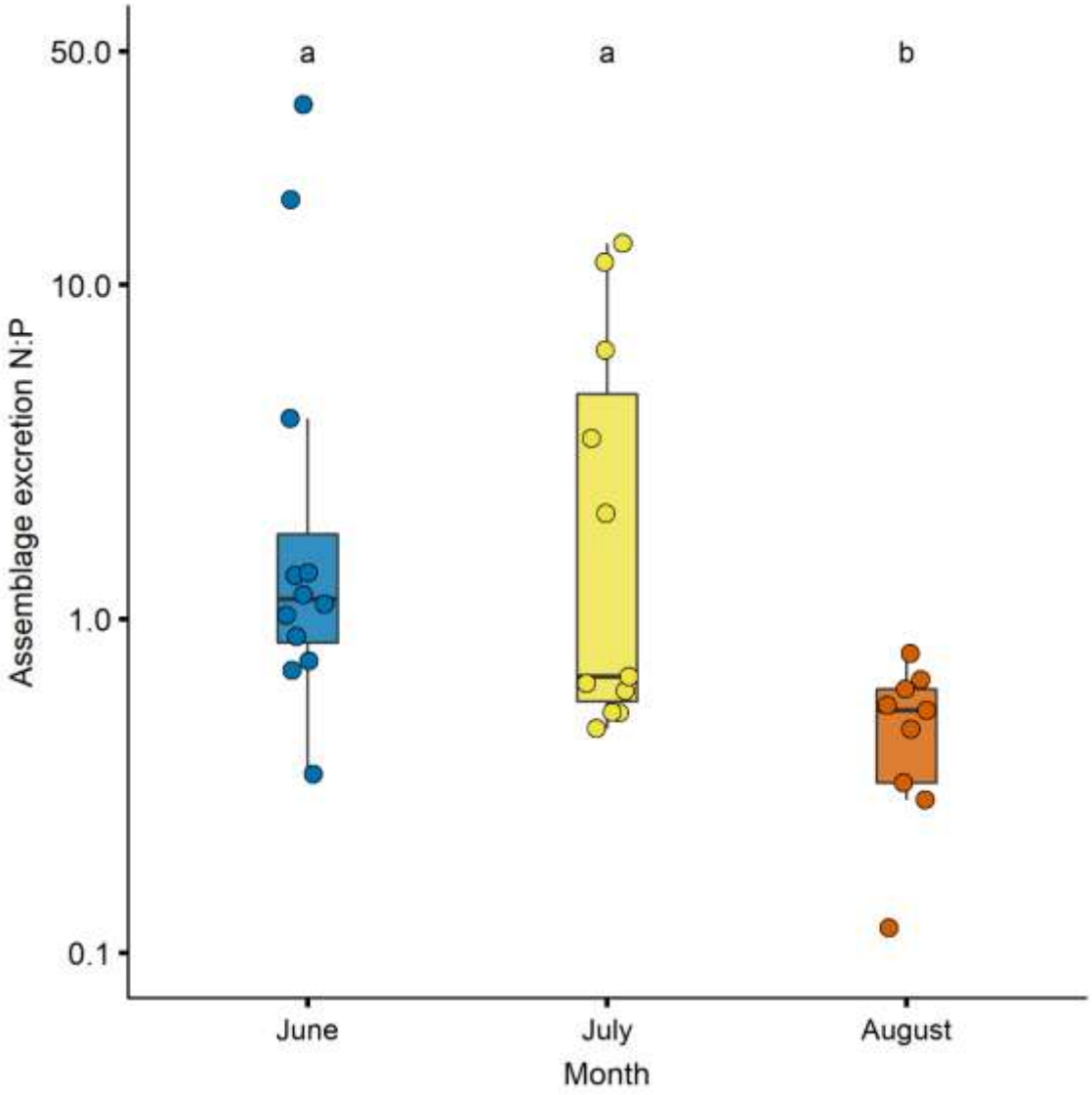

Figure 4.4 Assemblage excretion N:P (molar) for 12 isolated pools in Kings Creek, USA sampled during June, July and August of 2018. Boxes cover the first through third quartile of the data; horizontal black lines indicate the median of each month. Whiskers

represent the 5th and 95th quartile. Lowercase letters represent pairwise comparisons from the Tukey post hoc test. One pool dried completely before July sampling and two pools dried before August sampling (n=32).

Tables

Table 4.1 Abiotic characteristics monitored in 12 isolated pools in Kings Creek. Abbreviations represent dry pools (D) or periods when data loggers were not deployed (ND).

Abiotic factor	Month	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6	Pool 7	Pool 8	Pool 9	Pool 10	Pool 11	Pool 12
Surface area (m^2)	June	87.8	46.8	117.9	83.5	46.8	27.5	74.8	37.8	46.8	35.8	67.1	81.4
	July	69.1	16.8	84.8	50.9	D	4	38.6	15.2	31	D	47.3	54.3
	August	56	17.3	92.2	11.5	D	D	34.9	10.9	39.9	D	55.8	70.1
Temperature range (°C)	June	20.6-29.7	22.7-37.5	17.9-27.9	19.5-27.9	19.0-35.4	19.9-27.9	19.25-33.7	18.8-37.9	19.5-40	23.0-40.8	21.5-32.5	16.1-21.8
	July	24.3-28.7	18.4-39.2	17.8-28.7	16.2-33.9	D	17.8-28.7	18.9-39.4	19.3-39.5	21.5-41.8	D	19.7-32.6	16.4-24.5
	August	ND	18.6-22.8	17.9-22.3	16.3-21.5	D	D	19.4-22.9	19.4-28.5	ND	D	19.8-22.7	16.7-19.2
NH_4^+ ($\mu mol\ L^{-1}$)	June	37.2	54.8	615.7	50.8	524.1	89.2	48.6	48.4	110.1	89.2	35.5	30.7
	July	30.9	1457.5	116	1723.5	D	1106.5	59.9	41.6	1574.7	D	30.6	24
	August	117.3	238.6	105.1	939.4	D	D	235.7	115.4	200.6	D	161	92.4

Table 4.2 Per capita nutrient excretion rates (mean ± SD) for 9 aquatic animals found in 12 isolated pools of Kings Creek, USA during 2018. Tadpoles combines excretion rate of the two species *Acris crepitans* and *Lithobates pipiens*.

Species	n	N excretion rate ($\mu mol\ g^{-1}\ h^{-1}$)	P excretion rate ($\mu mol\ g^{-1}\ h^{-1}$)	Excretion N:P (Molar)
Campostoma anomalum	10	1.86 (0.61)	0.21 (0.15)	18.70 (16.52)
Chrosomus erythrogaster	24	1.37 (0.31)	0.07 (0.04)	25.40 (11.82)
Etheostoma spectabile	10	1.97 (0.40)	0.20 (0.06)	10.86 (3.16)
Gambusia affinis	15	0.45 (0.17)	0.20 (0.12)	4.37 (4.12)
Luxilus cornutus	8	1.40 (0.43)	0.11 (0.03)	13.37 (4.64)
Semotilus atromaculatus	9	1.47 (0.50)	0.12 (0.06)	19.18 (16.00)
Orconectes nais	11	0.70 (0.19)	0.01 (0.00)	140.80 (77.04)
Tadpoles	17	1.00 (0.50)	0.10 (0.11)	21.77 (20.40)

Table 4.3 Total biomas (g wet mass), proportional biomass, and the number of pools occupied for fish, crayfish and tadpole species captured from 12 isolated pools in Kings Creek for June, July and August of 2018. Rare taxa with < 5 occurrences and low biomass across samples are not included in the table, except for tadpoles which dominated the biomass in one pool throughout June and July. Tadpoles combines biomass of the two species *Acris crepitans* and *Lithobates pipiens*.

	June			July			August		
Taxa	Total Biomass	Proportional Biomass	Pools occupied	Total Biomass	Proportional Biomass	Pools occupied	Total Biomass	Proportional Biomass	Pools occupied
Campostoma anomalum	5726	0.05	10	1447.3	0.07	8	194	0.02	8
Catostomus commersoni	881.8	0.01	7	932	0.05	2	522.2	0.06	2
Chrosomous erythrogaster	93748.7	0.82	12	9244.6	0.48	8	2765.6	0.34	8
Etheostoma spectabile	968.5	0.01	10	1418.9	0.07	8	679.9	0.08	8
Gambusia affinis	166.7	0.00	8	272.7	0.01	8	367.9	0.05	8
Luxilus cornutus	914.2	0.01	5	1096.1	0.06	3	21.6	0.00	3
Semotilus atromaculatus	6791.8	0.06	10	853.6	0.04	6	888.4	0.11	6
Orconectes nais	2959.3	0.03	12	3073.2	0.16	10	1964.5	0.24	11
Orconectes neglectes	541.5	0.00	6	625.6	0.03	6	583.1	0.07	6
Tadpoles	1366.4	0.01	2	484.2	0.02	4	47.6	0.01	2

Note: Species with < 5 occurrences: *Amerius natalis* (yellow bullhead), *Cyprinella lutrensis* (red shiner), *Etheostoma nigrum* (johnny darter), *Lepomis cyanellus* (green sunfish), *Moxostoma erythrurum* (golden redhorse), *Noturus exilis* (slender madtom), *Phenacobius mirabilis* (suckermouth minnow), *Pimephales notatus* (bluntnose minnow)

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Chapter 5 - Conclusions

Understanding how interactions among taxonomically and functionally diverse animal groups influence resource distribution and fluxes is a fundamental goal of ecology. Estimates of animal-mediated processes have been limited by several key issues that I addressed in Chapters 2, 3, and 4 of this dissertation using experimental and observational methods.

Spatial and temporal overlap of broadly different animal groups

Species with different functional traits may overlap either through abiotic or biotic mechanisms, with potentially additive or synergistic (non-additive) effects on ecosystem functions. In Chapter 2, I developed and tested a conceptual framework, using a broad-scale field experiment to assess whether fish assemblages were attracted to biogeochemical hotspots produced by stable aggregations of mussels. Major differences in life history traits (i.e., mobility) of dominant animal groups (fish and mussels) influenced how their biomass varied with hydrology, their degree of spatial overlap, and their effects on ecosystem properties. Although biomass was the main driver of different nutrient cycling rates among fish and mussel assemblages, mussel assemblage composition was also important in determining mussel nutrient contributions. Dominant mussel species in these rivers have different temperature-dependent excretion rates and vary in tissue and excretion stoichiometry, which can regulate algal production and composition (Spooner and Vaughn, 2008; Atkinson et al. 2013). In contrast, nutrient contributions of fish were not affected by species identity or temperature. The fish species tested in Chapter 2 are fundamentally different in many aspects of their ecology but appear to be redundant in regard to their nutrient cycling effects under the conditions of this study. Thus, ecosystem level nutrient cycling by mussels may be fundamentally altered if shifts in mussel assemblage composition occur, such that functional replacement of species with unique stoichiometric traits may not be possible.

In Chapter 3, I used experimental enclosures with live mussels, shams, or sediment only and remote underwater video recordings to test whether fish distributions at fine spatial scales are influenced by biotic effects of mussels or biogenic habitat created from shells. Contrary to my predictions, live mussels did not increase subsidies to fishes. I found no difference between two experimental mussel assemblages comprised of two species of mussels with different, morphological, physiological, and behavioral traits (Spooner and Vaughn 2008). These mussels

and their traits are well studied and are linked to their effects on food web compartments (Spooner and Vaughn, 2006, 2008, Atkinson 2013) and stream nutrient cycling (Vaughn et al 2004; Atkinson and Vaughn 2015, Chapter 2). However, these results are supported by previous studies demonstrating the reduced ability of freshwater mussels to influence ecosystem processes with increases in stream flow and water volume (Vaughn et al., 2004). Although the traits of living mussels did not appear to influence fish occurrences, habitat generated by mussel shells (live and dead) appeared to attract co-occurring fishes. Within the context of this study, structural habitat maintained by the ecosystem engineering effects of mussels likely concentrates the effects of fish at fine spatial scales within mussel bed hotspots. At reduced flows, I would predict the biological activities of mussels to have a greater influence over fish distribution at fine spatial scales. Together, Chapter 2 and 3 illustrate that scales at which aggregations of mussels and fishes occur is variable among groups. While fish biomass is homogenous among stream reaches and may exceed that of mussels within the entire river system, fish tend to be aggregated at fine spatial scales around particular meso-habitats (Chapter 2), such as clumps of mussels (Chapter 3). Conversely, mussel biomass is heterogeneously distributed among reaches (Chapter 2). Within mussel bed reaches fish communities likely provide locally-concentrated, but transient nutrient subsidies while aggregations of mussels maintain stable, long-term nutrient subsidies that vary in importance with stream discharge and temperature

Biomass and stoichiometric traits within the context of drought

Few studies have tested the influence of drought on animal-mediated processes. However, animal contributions to stream food web dynamics and nutrient cycles can differ during drought years or periods of low flow. In Chapter 4, I tested how a severe drought influenced animal biomass, assemblage structure and subsequent nutrient cycling. I found that animal-mediated nutrient cycling was altered through biomass loss and the stoichiometric traits of taxa that differed in their occurrences and ability to tolerate abiotic conditions produced under drought conditions. In contrast to Chapter 2, excretion rates varied among fishes, suggesting that species experience harsh environmental conditions differently and their physiological responses shift their effects on nutrient cycling (Spooner and Vaughn 2008; Vaughn 2010). This result emphasizes how disrupting the balance between animal traits and their environment can alter their role of ecosystem function and underscores the potential for the stability and function of

ecosystems to be altered when species shift their roles in respect to their abundances. Moreover, differences among species' ability to tolerate drought conditions resulted in distinct pool assemblages with biomass represented by fishes, tadpoles, or crayfish. Life history, physiological, and stoichiometric traits of remaining taxa contributed to differences in animal-mediated nutrient cycling during drought. It would be informative to combine these traits with population vital rates to assess and predict the proximate and lasting impact of drought disturbances on stream ecosystems structure and function.

In combination, these studies showcase the context dependency of animal-mediated processes governed by interactions among diverse taxonomic and functional groups along abiotic and biotic gradients. Spatial scales at which animal groups aggregated was related to major differences in life history (e.g., mobility, reproduction, life stage) and abiotic factors, such as hydrologic condition. Such differences in animal distribution led to differences when and where their ecosystem effects overlapped. The conceptual framework presented in Chapter 2 allows for predictions about when and where overlapping biogeochemial hotspots will have the strongest effects and is widely generalizable because spatial or temporal overlap by aggregating animals is common across all ecosystems. Moreover, numerous generalizations can be made among each chapter and previous studies of stream ecology. For example, habitat size and associated abiotic gradients were important factors driving distributions (Chapters 2, 3, and 4) and relative abundances of species within assemblages (Chapters 3 and 4). Biomass and stoichiometric traits of taxa are also important, and can explain animal-mediated nutrient cycling across ecosystems (Chapters 2 and 4). These studies contribute to the conservation of aquatic biodiversity and associated ecosystem functions by identifying the spatial and temporal contexts under which diverse animals groups influence the stability and function of ecosystem processes.

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Appendix A - Chapter 2 supplemental tables and figures

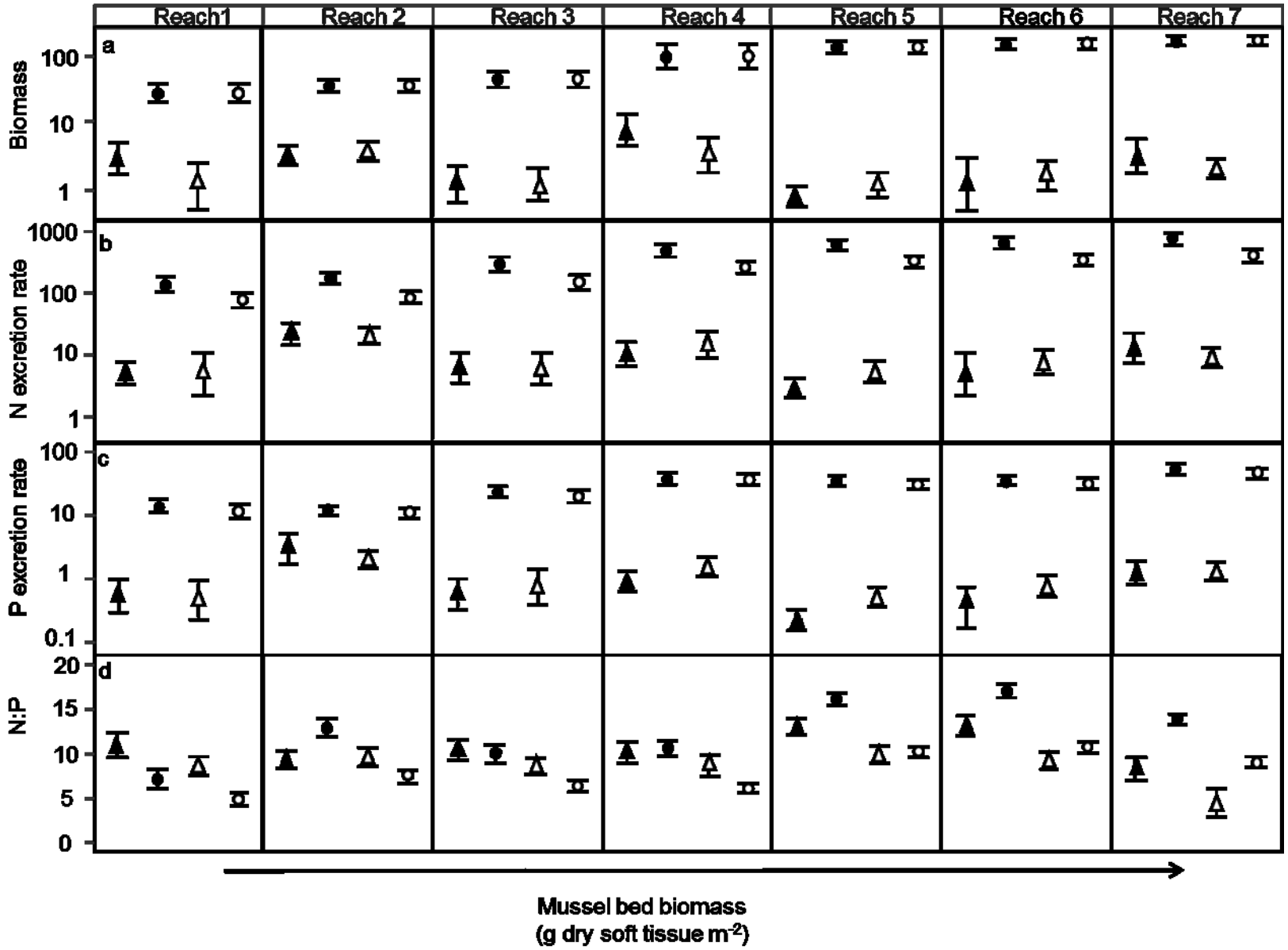
Reach1
Reach 2
Reach 3
Reach 4
Reach 5
Reach 6
Reach 7
a
b
c
d
Biomass
N excretion rate
P excretion rate
N:P
100
10
1
1000
0.1
20
15
5
0
Mussel bed biomass
(g dry soft tissue m-2)

Figure A 1 Comparison of fish (triangles) and mussel (circles) (a) biomass (grams DW·m^{-2}), (b) areal nitrogen excretion (umol N·m^{-2}·h^{-1}), (c) areal phosphorus excretion (umol P·m^{-2}·h^{-1}), and (d) molar N: P of mussel and fish community excretion at seven mussel bed reaches. Site abbreviations are listed at the top and are arranged left to right in order of increasing mussel community biomass. Summer sampling is represented by closed symbols and fall by open symbols. Adapted with permission from Springer Nature Centre, GmbH: Springer, Oecologia, Hopper et al., 2018

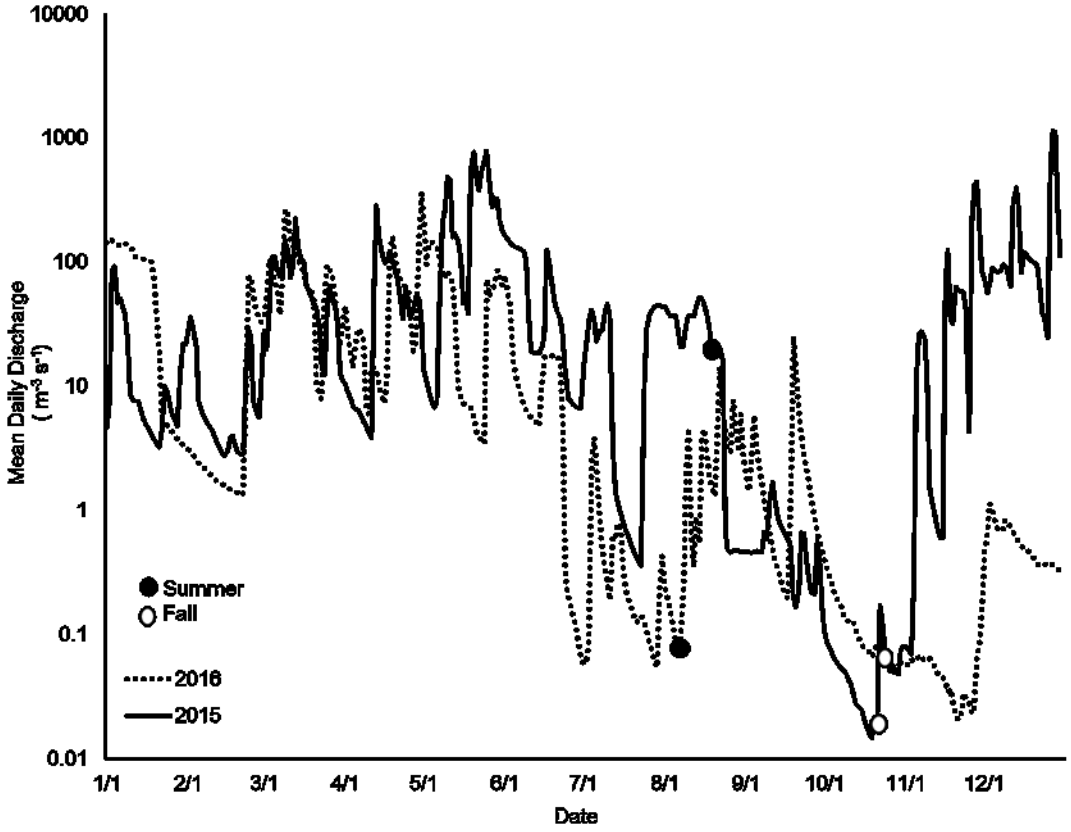

Figure A 2 USGS gage number 07335790 located at Clayton, OK on the Kiamichi River. Summer and fall dates when the fish community was sampled are indicated by circles on the hydrograph. Adapted with permission from Springer Nature Centre, GmbH: Springer, Oecologia, Hopper et al., 2018

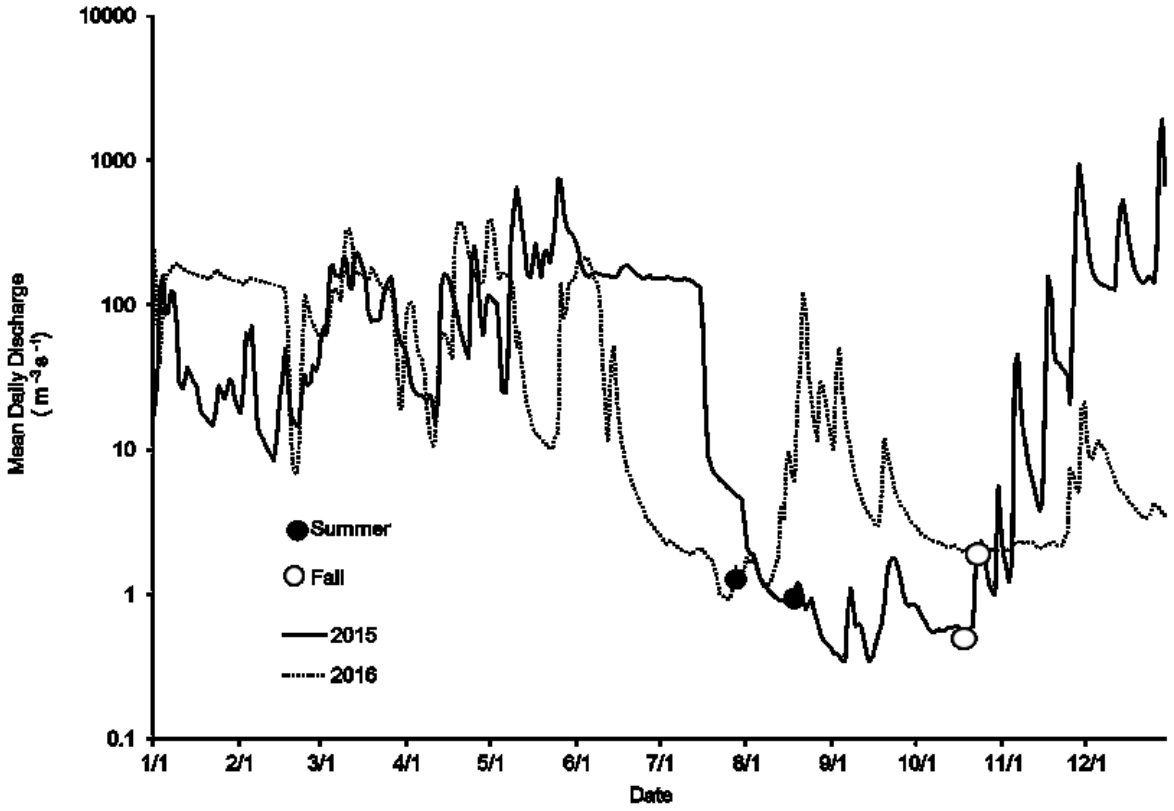

Figure A 3 USGS gage number 07338500 located at the confluence of Lukfata Creek on the Little River. Summer and fall dates for when the fish community was sampled are indicated by circles on the hydrograph. Adapted with permission from Springer Nature Centre, GmbH: Springer, Oecologia, Hopper et al., 2018

Figure A 4 USGS gage number 07337900 located at state highway 3 on the Glover River. Summer and fall dates for when the fish community was sampled are indicated by circles on the hydrograph

Table A1 Fish and mussel species for which lengths and weights were directly measured. Linearized power functions were used to describe the scaling of body mass (M, g) relative to total length (TL, mm): $\log(M) = a + b \log(TL)$. Total length for fish was measured from the tip of the snout to the end of the caudal fins. Total length for mussels was measured as the longest axis across a valve. All models were statistically significant at $P < 0.05$. Adapted with permission from Spring Nature Centre, GmbH: Springer, Oecologia, Hopper et al., 2018.

Fish	n	a (SE)	b (SE)	R^2	applied to
Ameiurus natalis	20	-4.94 (0.24)	3.02 (0.13)	0.97	*Ameiurus natalis*
Campostoma spadiceum	31	-5.31 (0.25)	3.13 (0.14)	0.94	*Campostoma spadiceum*
Cyprinella whipplei	13	-5.73 (0.55)	3.27 (0.30)	0.91	*Cyprinella spp*
Etheostoma radiosum	28	-5.13 (0.60)	3.01 (0.35)	0.73	*Etheostoma radiosum, E. nigrum, E. gracile*
Etheostoma spectabile	237	-4.56 (0.16)	2.77 (0.10)	0.78	*Etheostoma spectabile*
Fundulus notatus	3	-6.41 (0.12)	3.76 (0.07)	0.99	*Fundulus notatus*
Ictalurus punctatus	18	-5.32 (0.18)	3.11 (0.09)	0.99	*Ictalurus punctatus*
Labidesthes sicculus	14	-5.29 (0.24)	2.98 (0.15)	0.97	*Labidesthes sicculus*
Lepomis cyanellas	10	-4.88 (0.25)	3.07 (0.12)	0.99	*Lepomis cyanellas*
Lepomis machrochirus	5	-5.40 (0.24)	3.33 (0.13)	0.99	*Lepomis machrochirus*
Lepomis megalotis	32	-5.17 (0.21)	3.19 (0.12)	0.97	*Lepomis megalotis, L. gulosus, L. miniatus, L. humilis*
Luxilus cardinalis	20	-6.21 (0.27)	3.60 (0.14)	0.97	*Luxilus chrysocephalus*
Micropteris dolomieu	37	-5.36 (0.14)	3.27 (0.07)	0.98	*Micropteris spp.*
Notropis boops	15	-5.12 (0.30)	2.99 (0.16)	0.95	*Notropis boops, N. volucellus, Lythrurus spp.*
Notropis stramineus	1047	-5.28 (0.50)	3.16 (0.03)	0.92	*Notropis stramineus*
Noturus exilis	10	-4.66 (0.36)	2.76 (0.23)	0.94	*Noturus spp.*
Percina sciera	13	-5.11 (0.41)	2.99 (0.23)	0.93	*Percina spp.*
Pimephales notatus	26	-5.71 (0.32)	3.41 (0.19)	0.93	*Pimephales notatus*
Pimephales vigilax	253	-5.57 (0.08)	3.36 (0.05)	0.96	*Pimephales vigilax*
Pylodictis olivaris	14	-5.25 (0.14)	3.13 (0.06)	0.99	*Pylodictis olivaris*
Mussels					
Cross-species bootstrap	80	-4.14 (0.20)	2.37 (0.11)	0.86	All mussel species not listed
Actinonaias ligamentina	46	-6.03 (0.42)	3.36 (0.20)	0.86	*Actinonaias ligamentina*
Amblema plicata	86	-4.82 (0.11)	2.72 (0.06)	0.96	*Amblema plicata*
Fusconaia flava	17	-2.89 (0.43)	1.62 (0.25)	0.72	*Fusconaia flava*
Lampsilis teres	3	-4.90 (0.16)	2.73 (0.08)	0.99	*Lampsilis teres*
Obliquaria reflexa	11	-5.26 (0.66)	3.03 (0.39)	0.86	*Obliquaria reflexa*
Ptychobranchus occidentalis	10	-4.73 (1.14)	2.63 (0.59)	0.67	*Ptychobranchus occidentalis*

Table A 2 Mussel species for which ammonium and phosphorus excretion were directly measured (Atkinson et al. 2013). Power functions were used to describe the scaling of excretion rates (E, µg g dry tissue^{-1} h^{-1}) relative to body dry mass (M, g): $E = a * M^b$. Adapted with permission from Spring Nature Centre, GmbH: Springer, Oecologia, Hopper et al., 2018

	TN		TP	
Measured taxa	a	b	a	b
Actinonaias ligamentina	192.34	-0.60	32.37	-0.81
Amblema plicata	194.13	-0.80	33.64	-0.74
Ptychobranchus occidentalis	148.14	-0.61	45.27	-0.95
Cyclonaias pustulosa	93.41	-0.70	37.76	-0.96
All species	158.64	-0.59	39.42	-0.90

Table A 3 Summary of hydrologic conditions for the four sampling periods. Mean daily discharge (Q) from USGS gaging stations was collected for the day sampling occurred, seven and 14 days prior to sampling. The sampling period during which fish biomass was higher at mussel bed reaches compared to non-musselbed reaches is highlighted in bold. Adapted with permission from Spring Nature Centre, GmbH: Springer, Oecologia, Hopper et al., 2018

River (USGS gage)	Sample Period	Mean Q 1 day	Mean Q 7 days	Mean Q 14 days
Glover River (7337900)	Summer 2015	0.04	0.03	0.05
	Fall 2015	0.05	0.03	0.03
	Summer 2016	**1.31**	**0.11**	**0.075**
	Fall 2016	0.08	0.08	0.13
Little River (7338500)	Summer 2015	0.94	0.93	1.15
	Fall 2015	0.58	0.58	0.57
	Summer 2016	**1.57**	**1.18**	**1.33**
	Fall 2016	2.03	2.05	2.06
Kiamichi River (7335790)	Summer 2015	28.14	43.79	38.6
	Fall 2015	0.01	0.02	0.03
	Summer 2016	**0.06**	**0.11**	**0.24**
	Fall 2016	0.05	0.07	0.08

Appendix B - Chapter 3 supplemental tables and figures

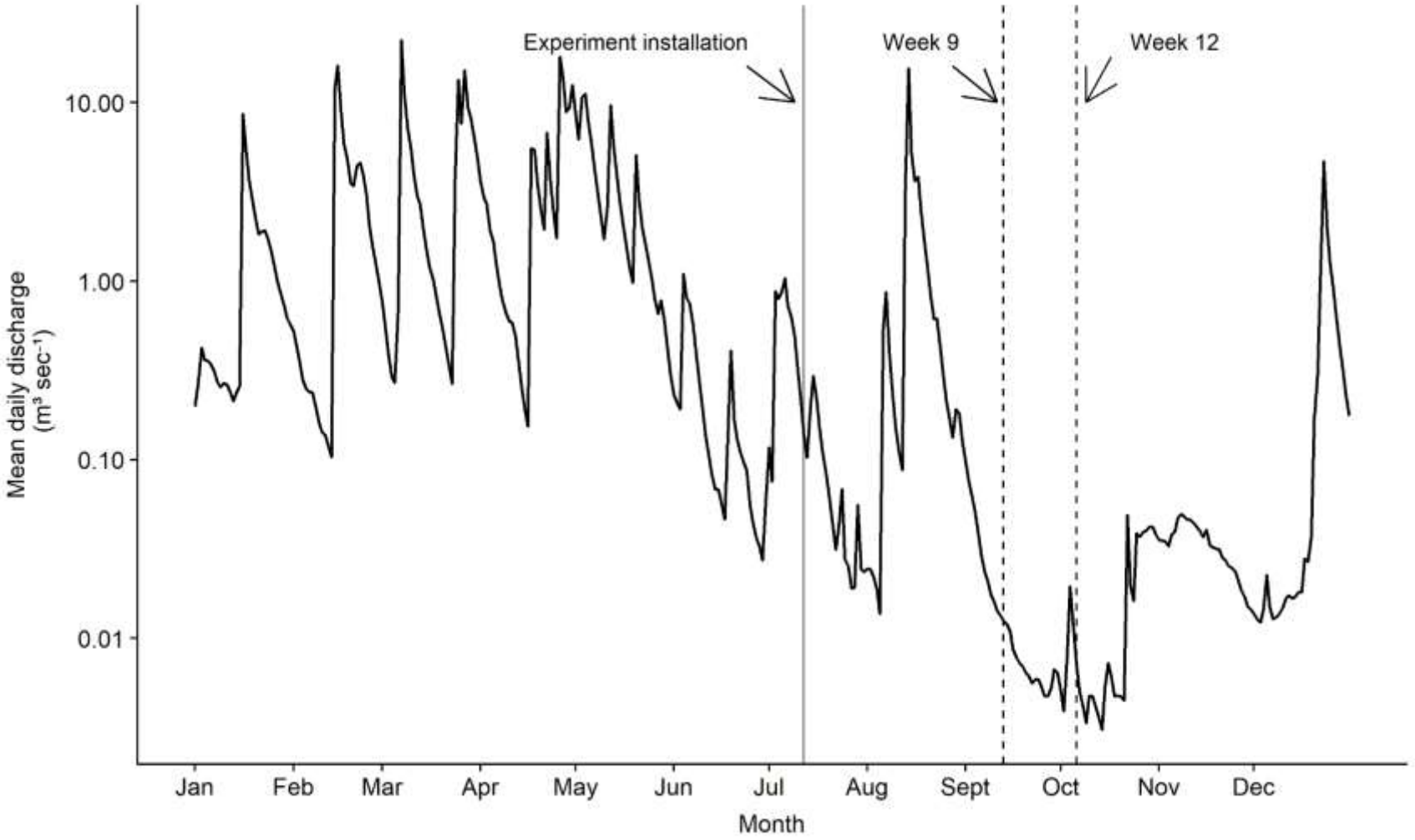

Figure B 1 Mean daily discharge in 2017 for the Kiamichi River, OK 20 km upstream of the experimental reach (USGS gage 0735700). The experiment began July 12 and ended October 6, 2017.

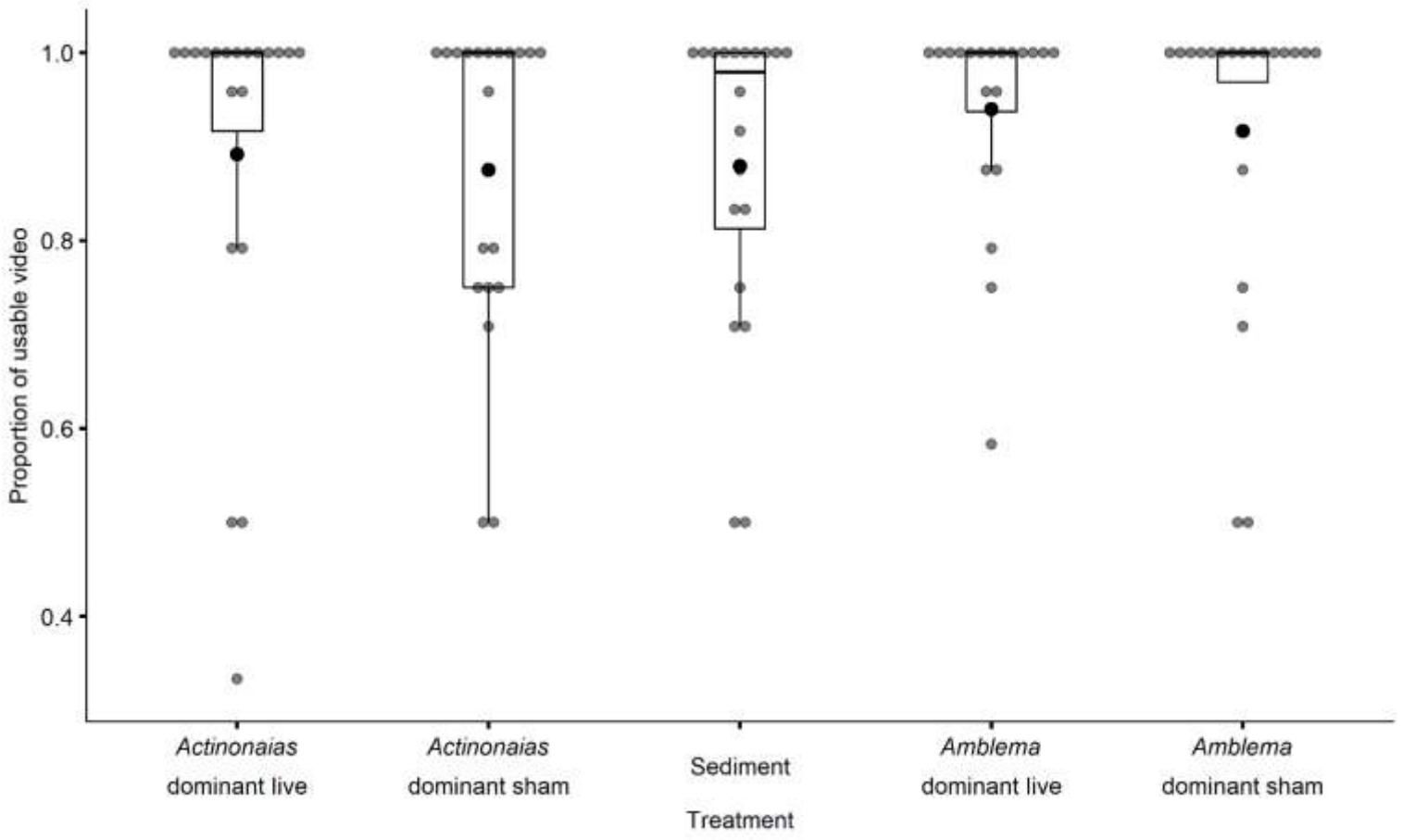

Figure B 2 Boxplots of the proportion of usable video among enclosure treatments from the Kiamichi River, OK that contain two species assemblages of live mussels, two species assemblages of shams (shells filled with sand) or sediment controls. Boxes cover the first through third quartile of the data; horizontal black lines indicate the median and black circles indicate the mean.

Appendix C - Chapter 4 supplemental tables and figures

Table C 1 Capture probabilities (*q*) for aquatic animals across a gradient of isolated pools during a drought in Kings Creek, USA.

Pool	Pool 1	Pool 2	Pool 3	Pool 7	Pool 8	Pool 9	Pool 10
volume (m^3)	94.98	27.50	109.52	33.65	16.53	23.21	12.79
Campostoma anomalum	0.29	0.00	0.27	0.21	0.00	0.09	0.33
Catostomus commersoni	0.33	0.00	0.28	1.00	0.00	0.00	0.00
Chrosomus erythrogaster	0.24	0.20	0.06	0.63	0.67	0.50	0.22
Etheostoma spectabile	0.09	0.08	0.09	0.46	0.00	0.00	0.00
Gambusia affinis	0.34	0.33	0.13	1.00	0.67	0.54	0.58
Luxilus cornutus	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Notorus exilis	0.00	0.00	0.00	0.00	0.00	1.00	0.00
Orconectes spp.	0.00	1.00	0.00	0.20	0.00	0.06	0.00
Semotilus atromaculatus	0.00	0.00	0.38	0.00	0.00	0.00	0.00
Tadpoles	0.00	0.00	0.20	0.00	0.00	0.00	0.00
Average *q*	0.26	0.40	0.20	0.58	0.67	0.44	0.38

Table C 2 Species for which length and mass were directly measured. Linearized power functions were used to describe the scaling of mass (g) relative total length (M, g): log (M) = a + b log (TL).

Source Taxa	n	a (SE)	b (SE)	R^2
Campostoma anomalum	391	-4.79 (0.018)	2.87 (0.01)	0.94
Chrosomus erythrogaster	59	-4.73 (0.24)	2.79 (0.14)	0.87
Etheostoma spectabile	237	-4.56 (0.16)	2.77 (0.10)	0.78
Gambusia affinis	15	-5.76 (0.21)	3.56 (0.14)	0.97
Luxilus cornutus	28	-4.01 (0.66)	2.40 (0.36)	0.61
Orconectes virilis†	90	-3.48 (0.42)	2.26 (0.24)	0.49
Semotilus atromaculatus	8	-4.58 (1.06)	2.85 (0.59)	0.730
Acris crepitans	9	-2.32 (0.70)	1.32 (0.50)	0.50
Lithobates pipiens.	8	-1.09 (0.41))	0.88 (0.23)	0.72

†Length-mass relationship was used to estimate biomass for *O. nais*, and *O. neglectes*.

Table C 3 Species for which ammonium (NH_4^+) and phosphorus excretion rates were directly measured. Linearized power functions were used to describe the scaling of excretion rates (E, μmol h^{-1}) relative to body wet mass (M, g): log (E) = a + b log (M).

NH_4^+ Excretion

Source Taxa	**n**	**a (SE)**	**b (SE)**	**R^2**	**P**
Campostoma anomalum	10	0.25 (0.04)	0.67 (0.12)	0.77	<0.001
Chrosomus erythrogaster	25	0.12 (0.02)	0.95(0.08)	0.85	<0.001
Etheostoma spectabile	10	0.27 (0.07)	0.96 (0.20)	0.71	<0.001
Gambusia affinis§	15	-0.23 (0.07)	1.34 (0.12)	0.90	<0.001
Luxilus cornutus	7	0.12 (0.08)	1.06 (0.30)	0.61	0.01
Lythobates spp.	17	-0.05 (0.03)	0.66 (0.06)	0.89	<0.001
Orconectes nais	14	0.02(0.08)	0.66 (0.13)	0.75	<0.001
Semotilus atromaculatus†	9	0.25 (0.10)	0.60 (0.31)	0.26	0.09
Global	117	0.17 (0.02)	0.64 (0.05)	0.59	<0.001

SRP Excretion

Source Taxa	**n**	**a (SE)**	**b (SE)**	**R^2**	**P**
Campostoma anomalum	10	0.68 (0.07)	-0.19 (0.22)	-0.03	0.43
Chrosomus erythrogaster§	25	0.27 (0.05)	0.94 (0.15)	0.63	<0.001
Etheostoma spectabile	10	0.61 (0.11)	0.54 (0.31)	0.19	0.12
Gambusia affinis	15	-1.02 (0.14)	0.53 (0.24)	0.22	0.05
Luxilus cornutus	7	0.55 (0.10)	0.87 (0.33)	0.46	0.03
Lythobates spp.	17	0.26 (0.05)	0.10 (0.10)	-0.001	0.34
Orconectes nais	14	-0.43 (0.22)	0.35 (0.35)	-0.001	0.34
Semotilus atromaculatus§	9	0.26 (0.20)	1.83 (0.65)	0.46	0.03
Global††	117	-0.99 (0.05)	-0.19(0.11)	0.03	0.08

†Mass-scaling of N excretion for *S. atromaculatus* was marginally significant; ††Mass-scaling of P for the global model applied to species for which excretion rates were not directly measured was marginally significant. § Indicate the relationship between wet mass and excretion rates differ significantly from the other species.

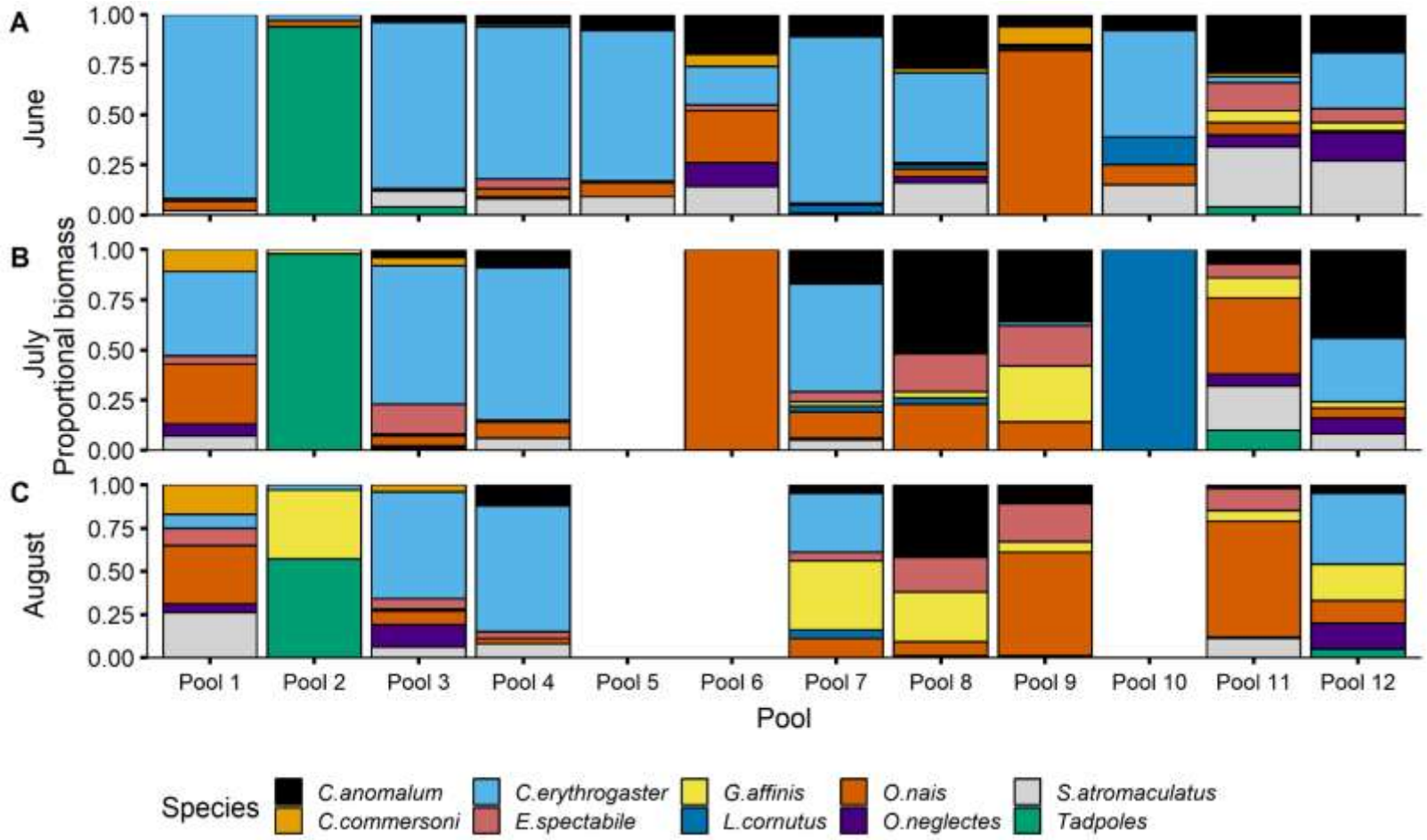

Figure C 1 Proportional biomass of core species captured from 12 isolated pools in Kings Creek, USA sampled during June (A), July (B) and August (C) of 2018. Pools are arranged from upstream to downstream. Tadpoles combines biomass of the two species *Acris crepitans* and *Lithobates pipiens*. Biomass of rare species (< 5 occurrences) are not included. Species with < 5 occurrences: *Amerius natalis* (yellow bullhead), *Cyprinella lutrensis* (red shiner), *Etheostoma nigrum* (johnny darter), *Lepomis cyanellus* (green sunfish), *Moxostoma erythrurum* (golden redhorse), *Noturus exilis* (slender madtom), *Phenacobius mirabilis* (suckermouth minnow), *Pimephales notatus* (bluntnose minnow)