UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

TROPHIC CONNECTIONS BETWEEN STREAM AND TERRESTRIAL FOOD WEBS

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

DOCTOR OF PHILOSOPHY

By

JEFF SCOTT WESNER
Norman, Oklahoma
2010

TROPHIC CONNECTIONS BETWEEN STREAM AND TERRESTRIAL FOOD WEBS

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF ZOOLOGY

BY

Dr. William Matthews, Chair

Dr. Elizabeth Bergey

Dr. Bruce Hoagland

Dr. Caryn Vaughn

Dr. Gary Wellborn

ACKNOWLEDGEMENTS

This work would have been impossible without the support of my family, who have continually encouraged my pursuit of all things abstract, both emotionally and financially. I am very lucky to have their support, love, and humor to keep the right perspective.

I thank my dissertation committee for support through all phases of this project. Drs. Bill Matthews, Caryn Vaughn, Liz Bergey, Gary Wellborn, and Bruce Hoagland provided essential encouragement and criticism to greatly improve this dissertation. Special thanks go to Dr. Bill Matthews, my dissertation advisor. Dr. Matthews allowed me to stumble and trip my way towards a dissertation I could call my own. He enthusiastically supported the good ideas and gently steered me away from the (many) bad ones. I am indebted for his help and wisdom and can only hope to offer the same to my future students.

Finally, I thank my fiancée Chelsea Southerland, whose support is unwavering even if unwarranted. I could not have completed this work without her love and encouragement and could ask for no better partner. Anyone who will smile through twelve hour days of 100° heat, slippery rocks, sticky bugs, and smelly fish is most certainly a perfect match for me.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 1

Chapter 2

Chapter 3

LIST OF FIGURES

Chapter 1

Chapter 2

Figure 2. Fish effects on emerging insect assemblages and spider response. (a) emergence biomass of adult aquatic insects over three sample dates, (b) proportion of predator biomass in emergent insect assemblage over three sample dates, (c) counts of occupied spider webs above aquatic mesocosms. Data are means ± SE.

Figure 3. Potential roles of alternative adult aquatic communities in terrestrial food webs created by different predator regimes in aquatic habitats. Different sizes of pie charts reflect differences in total biomass of emergent insects seen in this study. Different arrow sizes reflect the magnitude of the impact of each community predicted from this study (top-down = consumption of terrestrial prey; bottom-up = energy source for terrestrial consumers)

Chapter 3

Figure 1. Effects of fish on (a) mean total insect emergence dry mass (mg/m^2/day), (b) mean trophic structure of the emerging insect assemblage (proportion of emerging predatory insects by biomass/m^2/day), (c) mean emergence of Chironomidae, and (d) mean density of tetragnathid spiders above mesocoms. Data are natural log transformed least squares means ± SE. Asterisks indicate significant planned contrasts between fish treatments and the control. * indicates marginal significance ($P = 0.015$ at $\alpha_{adj} = 0.0071$). ** indicates significance at $\alpha = 0.0071$. Fish abbreviations are Es (*Etheostoma spectabile*), Cl (*Cyprinella lutrensis*), and Ga (*Gambusia affinis*).

ABSTRACT

Most animals have complex life histories (CLH) in which an individual's niche shifts through ontogeny. These organisms often cross habitat or ecosystem boundaries as they develop from larvae to adults, coupling energy flow among food webs in separate ecosystems. As a result, ecological processes such as productivity and predation that govern the abundance of organisms during one stage of their life history can have effects that cascade beyond the boundaries of the focal ecosystem. However, empirical and theoretical studies often treat food webs as closed systems in which in situ ecological processes are the primary components regulating the structure and function of food webs. In my dissertation research, I examined how a group of CLH organisms, aquatic insects, couple stream and riparian food webs as they develop from aquatic larvae to terrestrial adults. I further examined how predation by fish on aquatic insects alters emergence of insects into terrestrial food webs.

Aquatic insects are ubiquitous in freshwater habitats where they spend the majority of their larval stages. During development in freshwater habitats aquatic insects occupy nearly every trophic level in aquatic food webs from herbivores (e.g. Trichoptera) to predators (e.g. Odonata). Most species of aquatic insects undergo metamorphosis during development in which they emerge from an aquatic pupal or nymphal stage to become winged adults in terrestrial habitats. When they emerge as winged adults from aquatic habitats, adult aquatic insects subsidize diets of terrestrial predators such as birds, spiders, lizards and bats. The importance of adult aquatic insects as subsidies in terrestrial food webs is ultimately determined by the abundance and biomass of the emerging insect assemblage, which in turn is driven by ecological interactions in aquatic

habitats. The life cycle of aquatic insects and the strong environmental boundary between aquatic and terrestrial habitats offer an ideal setting to study the consequences of spatial connectivity among food webs in physically distinct habitats

In Chapter 1, I measured the contribution of adult aquatic insects to terrestrial food webs along three streams in Oklahoma. I made monthly collections of all winged insects in the terrestrial habitats along each stream and sorted insects according to larval origin (aquatic or terrestrial). Overall, adult aquatic insects comprised more than one-third of all winged insects. This contribution peaked along a permanent spring stream, reaching as high as 94% of abundance and 86% of biomass in winter. The majority of adult aquatic insects were taxa that do not feed as adults (non-consumers), whereas most adult terrestrial insects fed (consumers). This resulted in a strong negative relationship between the relative biomass of adult aquatic insects and the relative biomass of consumers in the overall insect assemblage. Because winged terrestrial insects are important prey for terrestrial predators like birds, spiders, and lizards, this study demonstrated that insects emerging from streams substantially elevate prey availability in a terrestrial food web. Neither prey availability nor insect trophic structure in terrestrial habitats could be accurately predicted based on terrestrial productivity alone.

In Chapter 2, I tested the hypothesis that predation by fish on larval aquatic insects alters insect emergence from aquatic mesocosms to terrestrial habitats. I tested the effects of predation by two fish species with different foraging strategies (*Cyprinella lutrensis* – water-column feeder; and *Etheostoma spectabile* – benthic feeder). Both fish reduced emerging insect biomass by nearly 50% relative to fishless pools. Fish effects were strongest on emergence of dragonflies (*Pantala flavescens*), which are predators as adults in terrestrial food webs. Therefore, insect assemblages emerging from pools with fish had less overall biomass and fewer predators than assemblages emerging from pools with fish, regardless of fish foraging strategy. These results demonstrate that predation in streams can cascade to terrestrial habitats, altering biomass and trophic structure of adult aquatic insect subsidies in terrestrial food webs.

In Chapter 3, I tested the hypothesis that fish species richness in aquatic mesocosms alters insect emergence to terrestrial habitats. I also measured the distributional response of a terrestrial consumer (tetragnathid spiders) to shifts in insect emergence. Three fish species (with complementary habitat domains were the predators in a factorial design using all possible combinations of fish. Pools with high fish richness reduced insect emergence by more than 30% relative to control pools. Tetragnathid spiders responded to reductions in insect emergence by shifting their distribution away from pools with high fish richness. Fish effects in the high richness treatments (three fish species) were generally stronger than predicted based on individual fish species performance, suggesting that interactions among fish species in high richness treatments were synergistic. These results show that the effects of fish species loss in streams can cascade to adjacent terrestrial systems. Additionally, the strength of these effects are driven by the habitat domain of the fishes, supporting the idea that the effects of fish species loss can be predicted based on the foraging ecology of the fish.

My dissertation research demonstrates the importance of spatial context in food web studies. I found that the abundance, biomass and trophic structure of winged insect assemblages in terrestrial habitats is driven by the relative productivities of the both aquatic and terrestrial habitats. In turn, the contribution of adult aquatic insects to

terrestrial habitats is regulated by fish predation on larval insects in aquatic habitats. Fish reduce insect emergence, thereby reducing the amount of energy available to terrestrial predators, an effect that varies relative to fish species richness. These results show that ecological processes like predation have effects that cascade beyond the habitat of the predator, altering prey availability and the distribution of consumers in adjacent food webs.

PREFACE

Chapter 1 is formatted for submission to *Oikos*.

Chapters 2 and 3 are formatted for submission to *Ecology*.

CHAPTER 1: SEASONAL VARIATION IN THE TROPHIC STRUCTURE OF A SPATIAL PREY SUBSIDY LINKING AQUATIC AND TERRESTRIAL FOOD WEBS: ADULT AQUATIC INSECTS.

JEFF SCOTT WESNER

Department of Zoology, University of Oklahoma, Norman, OK 73019

Abstract

Research over the past decade has established spatial resource subsidies as important determinants of food web dynamics. However, most empirical studies have considered the role of subsidies only in terms of magnitude, ignoring an important property of subsidies that may affect their impact in recipient food webs: the trophic structure of the subsidy relative to *in situ* resources. This may be especially important when subsidies are composed of organisms, as opposed to nutrient subsidies, because the trophic position of subsidy organisms may differ from *in situ* prey. I explored the relative magnitude and trophic structure of a cross-habitat prey subsidy, adult aquatic insects, in terrestrial habitats along three streams in the south-central United States. Overall, adult aquatic insects contributed more than one-third of potential insect prey abundance and biomass to the terrestrial habitat. This contribution peaked along a permanent spring stream, reaching as high as 94% of abundance and 86% of biomass in winter. Trophic structure of adult aquatic and terrestrial insects differed. Nearly all adult aquatic insects were non-consumers as adults, whereas all but one taxon of terrestrial insects were consumers. Such a difference created a strong relationship between the relative contribution of the prey subsidy and the trophic structure of the prey assemblage: as the proportion of adult aquatic insects increased, the proportion of consumers in the prey assemblage declined. Specific effects varied seasonally and with distance from the stream as the taxonomic composition of the subsidy changed, but general patterns were consistent. These findings show that adult aquatic insect subsidies to riparian food webs not only elevate prey availability, but also alter the trophic structure of the entire winged insect prey assemblage.

Introduction

Spatial subsidies are important determinants of food web dynamics (Polis et al. 1997; Nakano and Murakami 2001; Holt 2004). Spatial subsidies are resources that originate in a donor habitat, and enter a food web in the recipient habitat as prey, nutrients, or detritus (Polis et al. 1997). Prey subsidies consist of mobile organisms that cross habitat boundaries through deliberate (e.g. life-history, migration) or accidental (e.g. wind) events, thereby coupling energy flow between physically separate habitats (Polis et al. 1997). Previous studies have demonstrated that the primary impact of prey subsidies in recipient food webs varies as a function of magnitude, whereby subsidies have the largest effect when they substantially elevate prey abundance above that produced in the recipient habitat alone (Nakano and Murakami 2001; Marczak et al. 2007). However, recent theoretical treatments of spatial subsidies suggest that in addition to magnitude, the functional traits of the prey subsidy relative to *in situ* prey can also impact food web dynamics (Leroux and Loreau 2008), but this has received little attention in empirical studies. Knowledge of both the magnitude and functional role of spatial prey subsidies is needed to more fully understand their impact in recipient food webs.

Spatial subsidies can affect food web dynamics in the recipient habitat by introducing resources to the recipient habitat during times of low *in situ* productivity, minimizing the effects of disturbance or inherent cyclical patterns of the *in situ* resource and stabilizing consumer abundance at the next highest trophic level in the recipient habitat (Huxel et al. 2002). For example, birds along a Horonai stream maintain higher densities in winter due to aquatic insect emergence than would be expected from terrestrial insect production alone, because terrestrial insect emergence is low in the winter when aquatic insect emergence is high (Nakano and Murakami 2001).

Subsidies may also differ functionally from *in situ* prey, potentially altering the structure and function of the total prey assemblage, though this has not been studied. For example, the adult mating stages of salmon (Hilderbrand et al. 1999) and aquatic insects form important resources for headwater streams and riparian food webs, but most of these species do not feed as adults, making them functionally distinct from otherwise similar *in situ* prey. As a result, their presence in recipient habitats elevates total prey abundance, but may also alter the trophic structure of the total prey assemblage.

In this study I use a common and relatively well-studied cross-habitat prey subsidy, adult aquatic insects entering terrestrial food webs, to analyze variation in both the magnitude and trophic structure of the subsidy, and their impact on the total flying insect prey assemblage along streams. I explore how this contribution varies in the recipient ecosystem across seasons and with increasing isolation from the source habitat (distance from the stream). Adult aquatic insects are insects that are aquatic as larvae, but terrestrial as adults. Winged insect prey assemblages along streams receive substantial inputs from adult aquatic insects, elevating terrestrial consumer abundance in these habitats (Gray 1993; Nakano and Murakami 2001; Sabo and Power 2002; Baxter et al. 2004; Marczak and Richardson 2007). For example, aquatic insect emergence from a Horonai stream subsidizes terrestrial spiders, increasing their abundance nearly seven fold compared to reaches where aquatic insect emergence is suppressed (Baxter et al. 2004), and similar patterns have been observed for terrestrial consumers such as birds (Nakano and Murakami 2001), lizards (Sabo and Power 2002), and bats (Fukui et al.

2006). However, the trophic level of emerging aquatic insects varies greatly among common taxa; some are consumers as adults (predatory dragonflies: Odonata) while others do not feed (mayflies: Ephemeroptera; caddisflies: Trichoptera). In contrast, nearly all insects with terrestrial larvae are consumers as adults (Daly et al. 1998). Knowledge of the trophic structure and relative abundance of adult aquatic insects is needed to better understand their role in terrestrial ecosystems.

Several studies have focused on the flux of adult aquatic insects across the aquatic-terrestrial boundary, emphasizing the importance of aquatic insect emergence to riparian prey abundance, and how this importance varies with distance from aquatic habitats (Jackson and Resh 1989; Collier and Smith 1998; Delettre and Morvan 2000; Petersen et al. 2004). With some notable exceptions (Petersen et al. 2004), aquatic insects decline rapidly with distance from streams. However, most of these studies have been limited to only a few taxa: Plecoptera (Briers et al. 2002; Petersen et al. 2004), Trichoptera (Collier and Smith 1998), Trichoptera and Ephemeroptera (Kovats et al. 1996; Petersen et al. 2004), and Chironomidae (Delettre and Morvan 2000). In contrast, only a few studies have attempted to quantify the distribution of all winged adult aquatic insects in a riparian habitat (Jackson and Fisher 1986; Jackson and Resh 1989; Nakano and Murakami 2001), and only two (both along tropical streams) have described the relative contribution of adult aquatic insects to terrestrial secondary production (Lynch et al. 2002; Chan et al. 2007). The abundance of subsidies relative to autochthonous production is likely a better predictor of importance to recipient food webs than total abundance alone (Marczak and Richardson 2007). Additionally, the trophic structure of adult aquatic insects has not been considered, though consumption by some adult aquatic insects can have strong effects in the terrestrial ecosystem (Knight et al. 2005). For example, predatory dragonflies were abundant around fishless ponds, causing a reduction in plant pollination through increased predation on pollinators (Knight et al. 2005).

This study examined the effect of adult aquatic insect movement across an aquatic-terrestrial boundary on the overall trophic structure and prey availability of winged riparian insects by addressing the following three questions: 1) What is the trophic structure of an aquatic-terrestrial insect prey subsidy in the riparian habitat along three streams? 2) Does the contribution of adult aquatic insects alter the trophic structure of the entire winged insect assemblage? 3) How does this contribution vary seasonally and with distance from the stream?

Methods

Study Sites

Insects were sampled along three streams that represent contrasting but common stream types in the south-central United States: Byrd's Mill Creek (medium-sized Arbuckle mountain spring), Spring Lake Creek (small headwater prairie spring), and Finn Creek (intermittent stream) (Table 1). Byrd's Mill Creek (34°36'53.00"N, 96°38'02.56"W) is a clear, spring fed tributary in the Clear Boggy drainage in the eastern Arbuckle Mountains in south-central Oklahoma, USA (Pontotoc County). The streambed is primarily bedrock and cobble, forming a repeated riffle-pool pattern. Collections occurred approximately 4 km downstream of the springhead. Spring Lake Creek (35°11'49.33"N, 98°57'28.38"W) is a spring fed tributary to the Washita River in western Oklahoma (Washita County). Collections occurred approximately 70 m downstream of the springhead, where the streambed is primarily mud, sand and small gravel. Finn Creek (34°58’42.14”N, 97°31’15.71”W) is a first-order intermittent tributary running through the University of Oklahoma’s Kessler Farm Field Lab in central Oklahoma (McClain County). At this location the stream is dammed upstream to create a small farm pond. Collections occurred approximately 0.8 km below the outflow of the pond. The entire reach dried completely for several weeks during a regional drought from July to September 2006, though data presented below represent collections that occurred when the stream was wetted during at least part of the collection period. Riparian vegetation at all sites generally consists of second-growth patches of trees, vines, and shrubs, which extend approximately 40 m on either side of the stream, at which point prairie grasses and scattered tree stands occur. A small number of cattle graze the land and each site. At Byrd’s Mill Creek and Spring Lake Creek, all insect traps were placed upstream of a major crossing for the cattle, thereby limiting the potential effects of increased sedimentation. Cattle did not enter the stream at Finn Creek.

Insect collection

At each site aerial insects were collected with sticky traps, which are commonly used for insect surveys (Sabo and Power 2002). Sticky traps have an advantage over several other passive collection devices, because they do not require a response from the insect (Daly et al. 1998). Like all collection techniques, sticky traps have biases, and have been shown less effective for large insects, which can avoid them, or are too large to stick (M. Power, pers. comm.). I assumed that this bias was the same for both aquatic and terrestrial insects, but it should be noted that the data presented here represent mostly small-bodied winged insects. Larger insects such as odonates (dragonflies and damselflies) and lepidopterans (butterflies and moths) were observed at the time of collection, but were relatively rare on sticky traps. Because odonates represent a potentially large proportion of emerging aquatic insect biomass, my estimates of the consumer potential of adult aquatic insects are likely conservative, especially in summer months, when most odonates were present.

Traps consisted of clear acetate sheets (567 cm^2) wrapped around clear plastic cylinders allowing continuous collection of insects from all directions. The acetate sheets were coated on one side with TangleTrap® aerosol insect coating (The Tanglefoot Company, Grand Rapids, MI, U.S.A.), an odorless, non-drying, adhesive. Each trap was suspended from a tree branch so that the trap height was approximately 1.5 m above the ground. An effort was made to position the traps at a sufficient distance from the trunks of trees to avoid overrepresentation of terrestrial insects emerging from these habitats.

Traps were placed in transects perpendicular to the stream. Each transect consisted of three traps placed at the following distances from the stream edge: 0 m, 10 m, and 40 m. At two sites – Finn Creek and Byrd's Mill Creek – four transects were set; two on each side of pools approximately 50 m apart. Access at Spring Lake Creek was restricted to one side of the creek. Therefore, two transects were set at this site. Insects were identified to the taxonomic level needed to assign larval habitat (i.e., aquatic or terrestrial) (Borror and White 1970; Daly et al. 1998; Triplehorn and Johnson 2005), and measured for length (± 0.1 mm) for biomass estimation using published length-weight regression equations for each family or order (Sample et al. 1993). Insects were classified as consumers or non-consumers based on adult feeding descriptions given in

Triplehorn and Johnson (2005). Consumers are taxa that feed as adults, and non-consumers are taxa that do not feed or rarely feed (e.g. chironomids) as adults. Traps were deployed continuously for one year from April 2006 to April 2007, and acetate sheets were collected and replaced approximately monthly (trap deployment range: 27-62 days). However, due to logistic constraints, insects from only one month within each season (*summer*: June - August; *fall*: September – November; *winter*: December - February; *spring*: March – May) were identified and analyzed. Collections chosen for analysis were separated by at least one month from the previous collection, and were otherwise chosen based on the completeness of the collection (fewest number of missing traps; usually one or two). During collection, acetate sheets were removed from the cylinders and placed on a white paper background, and stored dry in the dark.

Subsampling

A large number of insects were collected on traps during pilot surveys in spring 2006. Therefore, I used the following subsampling protocol: upon collection, acetate sheets were placed on white paper, consisting of a grid of 2.54 cm x 2.54 cm squares. The paper was divided along the lines into four quadrants. Within each quadrant, three squares within each row were randomly sampled, resulting in a survey of 57 percent of the total coverage area of the sheet (323 cm^2).

Data Analysis

I used repeated-measures MANOVA (Proc GLM; SAS Institute, Cary, NC, USA) to analyze the effect of distance, season, and their interaction on the biomass and abundance of each response variable: total insects, proportion of insects that were aquatic, proportion of insects that were consumers, and proportion of adult aquatic insects that were consumers. ‘Distance from the stream’ was the between-subjects factor and ‘season’ was the within-subjects factor. Pillai’s Trace test statistic was used, which is typically more robust than other multivariate test statistics (Quinn and Keough 2002). If RM MANOVA was significant ($\alpha = 0.05$) for distance and/or season, but there was no interaction, I pooled distance or season data and tested for specific contrasts using Tukey’s multiple comparison test. If there was a significant interaction between season and distance I used Tukey’s multiple comparison test to compare distances within individual seasons. Data were log-transformed (total biomass and abundance) or arcsine-square root transformed (proportions) prior to analysis. Fallen traps on several dates resulted in missing values at Spring Lake Creek and Finn Creek. Missing values accounted for a small proportion of the monthly collections (< 8 %), but because of the low replication at each site (n=2), missing values would have prevented MANOVA. Therefore, I used the Expectation-Maximization algorithm in SAS (Proc MI) to impute missing values. The relationship between relative aquatic insect abundance and trophic structure of the total insect assemblage was compared using linear regression. Prior to regression, abundance data were natural log-transformed ($\ln(x + 1)$) to improve linearity. Non-linear regression is often used to describe the distribution of adult aquatic insects with distance from the stream. I used linear regression because it performed equally well compared to inverse-power or exponential regressions, and also allowed a comparison of adult aquatic and terrestrial insects, which were not expected to show non-linear patterns. Heterogeneity of variance was tested using Levene’s test. All data met assumptions of parametric statistics.

Results

Contribution of adult aquatic insects to overall insect prey availability

Adult aquatic insects averaged 41% of total insect abundance and 34% of total insect biomass, pooled for all sites, though patterns varied widely by site, season, and distance. On average, relative abundance and biomass of adult aquatic insects was highest in winter, when terrestrial insect production was low (Fig. 1 a,c,e**)**, but this trend was significant only at Byrd's Mill Creek (RM-MANOVA, Pillai's Trace: $F_{1,3} = 223.53$, $p = .0491$ for biomass; $F_{1,3} = 607.69$, $p = 0.0298$ for abundance).

Byrd's Mill Creek had the highest abundance of any site (18 952 total insects), and had the highest contribution of adult aquatic insects (67% of abundance and 63% of biomass). This contribution peaked in winter, when the total insect assemblage was comprised nearly entirely of adult aquatic insects (94% by abundance, 86% by biomass; Fig. 1 a). In all other seasons, adult aquatic insect production was never greater than 51% of total abundance or biomass (Fig. 1 a,c,e). Seasonal patterns were driven largely by variation in chironomid relative abundance, which was nearly two times greater in winter than any other season at Byrd's Mill Creek. In contrast, adult aquatic insects contributed 50% of abundance and 27% of biomass at Spring Lake Creek (8650 total insects) and 21% of abundance and 15% of biomass at Finn Creek (8508 total insects), the smallest creek in the study. However, total insect abundance and biomass varied significantly across seasons at Spring Lake Creek (RM-MANOVA, Pillai's Trace: $F_{1,3} = 6126.2$, $p = 0.0094$ for biomass; $F_{1,3} = 282.65$, $p = 0.0437$ for abundance) and Byrd's Mill Creek (RM-MANOVA, Pillai's Trace: $F_{1,3} = 7884.30$, $p = 0.0083$ for biomass; $F_{1,3} = 221.14$, p

= 0.0494 for abundance), suggesting that at Spring Lake Creek aquatic and terrestrial insects varied synchronously, but were out of phase at Byrd's Mill Creek.

Relative abundance and biomass of adult aquatic insects declined on average with distance from the stream at each site (Fig. 1 b,d,f), though this decline was significant only at Byrd's Mill Creek (RM-MANOVA between-subjects, $F_{4,6} = 163.02$, $p = 0.0068$ for biomass). At Byrd's Mill Creek the contribution of adult aquatic insects was higher at the stream edge than at 10 m, or 40 m (Tukey's comparisons with traps at the stream edge, $p \leq 0.05$, Fig. 1 b). At Finn Creek, relative abundance was nearly 2.5 times higher at the stream edge (35%) than at 10 m (12%) or 40 m (16%).

Four aquatic taxa showed significant decline with distance from the stream at Byrd's Mill Creek (Chironomidae: $r^2 = 0.127$, $p = 0.049$, Ceratopogonidae: $r^2 = 0.272$, $p = 0.005$, Trichoptera: $r^2 = 0.156$, $p = 0.032$, and Ephemeroptera: $r^2 = 0.426$, $p \leq 0\ 001$), but only Chironomidae declined significantly at all three sites (Spring Lake Creek: $r^2 = 0.147$, $p = 0.044$; Byrd's Mill Creek: $r^2 = 0.127$, $p = 0.049$; Finn Creek: $r^2 = 0.202$, $p = 0.018$). Overall adult aquatic insect abundance declined with distance from the stream only at Byrd's Mill Creek ($r^2 = 0.169$, $p = 0.026$). Terrestrial insects showed virtually no pattern with respect to distance from the stream, suggesting a uniform distribution.

Trophic structure of the prey subsidy

The trophic structure of the prey subsidy (adult aquatic insects) differed from *in situ* prey (terrestrial insects). Adult aquatic insects were dominated by non-consumers (Fig. 2). In contrast, nearly all terrestrial insects were consumers (> 90% by abundance and biomass). As a result, the trophic structure of the entire insect prey assemblage switched from consumer rich to consumer poor as relative abundance of adult aquatic insects increased (Fig. 3). An exception to this pattern occurred at Finn Creek, where predatory long-legged flies (Dolichopodidae) dominated adult aquatic insect emergence in the fall (> 65% of abundance and biomass); the only time when adult aquatic insects contained more consumers than non-consumers at any site (Fig. 2).

The trophic structure of adult aquatic insects varied seasonally at Byrd's Mill Creek (RM-MANOVA, Pillai's Trace, $F_{1,3} = 239.24$, $p = 0.0475$ for abundance) and Finn Creek (RM-MANOVA, Pillai's Trace, $F_{1,3} = 11575.4$, $p = 0.0068$ for biomass). At both sites, relative consumer abundance was lowest in winter, when consumers made up less than 4% of total aquatic abundance and biomass.

For the entire insect assemblage, trophic structure was stable across seasons at Byrd's Mill Creek (RM-MANOVA, Pillai's Trace, $F_{1,3} = 68.58$, $p = 0.0885$ for abundance; $F_{1,3} = 1.93$, $p = 0.4761$ for biomass) and Spring Lake Creek (RM-MANOVA, Pillai's Trace , $F_{1,3} = 35.22$, $p = 0.1231$ for abundance; $F_{1,3} = 0.20$ $p = 0.8908$ for biomass), but varied at Finn Creek (RM-MANOVA, Pillai's Trace, $F_{1,3} = 1654.00$, $p = 0.0181$ for abundance). At Finn Creek, the percent of consumers by abundance was lower in winter (55%) than in any other season (Tukey's post-hoc comparison with summer, fall, and spring, $p < 0.05$). The decrease in consumers at Finn Creek was due to an increase in relative abundance of adult aquatic insects (mostly non-feeding chironomids) in winter (29%) compared with summer (20%), fall (17%), and spring (15%).

The trophic structure of the total insect assemblage did not vary with distance from the source habitat at any site (RM-MANOVA between-subjects, $p \geq 0.05$), due to the relatively constant contribution of adult aquatic insects at each distance from the stream edge (RM-MANOVA between-subjects, $p \geq 0.05$ for abundance).

Discussion

Adult aquatic insects contributed a substantial amount of potential prey to the terrestrial ecosystem, but differed trophically from terrestrial insects (*in situ* prey). Most adult aquatic insects were non-consumer taxa, while most terrestrial insects were consumer taxa. Therefore, adult aquatic insects subsidized the prey assemblage along streams, but as the relative magnitude of the subsidy changed, so did the trophic structure of the overall prey assemblage.

Terrestrial insect production contributed less than 15 % of all winged insect biomass in the terrestrial habitat in some seasons, with the remainder subsidized from aquatic habitats. Such asymmetrical productivity, combined with trophic differences between terrestrial and aquatic insects, meant that the structure of the entire winged insect community was determined by the interaction of both the terrestrial and aquatic systems. Neither prey abundance nor insect trophic structure could be predicted without considering productivities of both source habitats. This was true even at distances of up to 40 m from the stream, where aquatic production contributed at least 25 % of all winged insects collected over one year at two sites. Recent research has demonstrated the importance of both local and regional processes in determining community structure (Beisner et al. 2006). Local processes such as predation and resource productivity can strongly affect community structure in the focal system (Liebold et al. 1997), but organism dispersal across habitat or ecosystem boundaries is also an important regional factor (Beisner et al. 2006). This study offers further support that local productivity alone may be an insufficient predictor of community structure, especially in heavily subsidized systems, such as riparian habitats.

Seasonal variation in the magnitude of subsidies can stabilize food webs by increasing resources at times of low *in situ* production (Nakano and Murakami 2001; Takimoto et al. 2002). Seasonal asymmetry may be especially likely for aquatic-terrestrial insect subsidies in temperate systems, because stream temperatures can be more stable than air temperatures, producing a constant source of insects in winter, when terrestrial habitats freeze (Nakano and Murakami 2001). This pattern was shown at Byrd's Mill Creek, when winter assemblages were dominated by adult aquatic insects. No seasonal changes in relative aquatic insect abundance occurred at Finn Creek or Spring Lake Creek, even though total prey abundance varied seasonally, suggesting that adult aquatic insects provided a constant proportion of prey availability at these sites.

Species composition also varied seasonally, resulting in changes to the trophic structure of the prey subsidy. At Finn Creek consumers dominated the adult aquatic insect assemblage in fall when predatory long-legged flies (Diptera: Dolichopodae) were abundant. At Spring Lake consumers were abundant in summer, when liquid-feeding ceratopogonids were at their peak abundance. Thus, the feeding potential of the prey subsidy varied seasonally due to species turnover, even when the magnitude of the subsidy remained stable. Such seasonal variation in the trophic structure of the prey subsidy is likely a common phenomenon in temperate streams, where emergence for many species occurs only during a small portion of the year, typically in warm summer months (Merritt and Cummins 1996). In this study the proportion of consumers in the adult aquatic insect assemblage generally increased in summer and fall, when air and stream temperatures were high and decreased in winter, when temperatures were low, though such changes were significant only at Finn Creek. Adult odonates (dragonflies and damselflies) were present at each site during summer, but were rare on sticky traps. Because these taxa are strong predators, my estimates of summer adult aquatic consumer biomass are likely conservative. Non-feeding chironomids were the only aquatic taxa consistently collected in each season, and were by far the most abundant winter adult aquatic insect, driving the pattern of consumer decline in winter.

Behavioral differences between consumer and non-consumer taxa may affect their distribution in terrestrial habitats. For example, emerging mayflies (Ephemeroptera) do not feed as adults and live only a few hours to days, typically forming large mating swarms near the stream and only rarely dispersing inland (Brittain 1982). Thus, mayfly swarms are available to terrestrial consumers in relatively short ‘bursts’, and are likely limited to consumers near the stream. In contrast, aquatic insects which require a blood meal, such as Ceratopogonidae and Tabanidae, may disperse several kilometers from the source habitat in search of food (Lillie et al. 1985; Lynch et al. 2002). Briers et al. (2005) found that adult aquatic insects contributed over 40% of riparian spider (Lycosidae) diets at the stream edge, but less than 1% at 20 m from the stream, due to the limited dispersal of adult stoneflies. In my study, adult aquatic insects were abundant up to 40 m from the stream edge, and linear regression showed a consistently significant decline at each site for only a single adult aquatic taxon (non-feeding chironomids), despite most aquatic taxa being non-consumers. Trends for most aquatic species showed a pattern of decline in abundance with distance from the stream, though the distances measured in this study may have been too short to document differences between consumer and non-consumer taxa.

The direct effect of adult aquatic insect subsidies in terrestrial systems is often measured as a response in the recipient consumer population, which is typically increased in the presence of subsidies (Baxter et al. 2004). Increases in the abundance of subsidized consumers may in turn result in stronger consumption of *in situ* prey populations, causing trophic cascades (Leroux and Loreau (2008). Leroux and Loreau (2008) hypothesized that highly subsidized systems have stronger trophic cascades than systems with low subsidy inputs, though strength varies depending on the feeding preferences of the recipient consumer. They showed that increasing subsidy inputs at the primary consumer level weakened the cascading effects of predators in the recipient ecosystem. In their model, they assumed that adult aquatic insects did not feed in the recipient food web. Data from my study generally support that assumption, but with important exceptions. Consumer taxa of adult aquatic insects were present in every collection, and comprised over 30% of adult aquatic insects in summer and fall at several sites. What, if any, effect the variation in trophic structure of adult aquatic insects has on the potential for trophic cascades is unknown, but is likely to vary according to the specific feeding preferences of the adult aquatic taxa, which ranged from predatory (Dolichopodidae) to sugar-feeding (Ceratopogonidae). For example, predatory dolichopodids, which were abundant in the fall at Finn Creek, may act as both predators and prey in terrestrial food webs, subsidizing both compartments simultaneously.

An important consequence of ecosystem openness is that alterations to the source habitat can impact the amount and type of energy entering the recipient ecosystem. For example, commercial ocean fishing reduces the return of salmon to headwater streams (Schindler et al. 2003), and fish predation in freshwater ecosystems reduces the biomass (Baxter et al. 2004) and consumer potential (Knight et al. 2005) of adult aquatic insects in the terrestrial ecosystem. Fish predation can also alter the trophic structure of emerging aquatic insect assemblages (Wesner, unpublished data). It seems likely that streams with different predator regimes could produce insect prey subsidies with different trophic structures than shown in this study, though this has not been well-studied. Freshwater systems are among the most highly modified in the world, and the unique and important subsidies they provide to terrestrial systems offer another way in which continued modification to freshwater systems, through species introductions and pollution, can have unintended consequences.

The recent focus in ecology on the importance of organism movement across habitat boundaries has shown that such movement can influence food web dynamics and community structure by subsidizing recipient systems (Polis et al. 1997; Schreiber and Rudolf 2008). To date nearly all studies assessing the impact of prey subsidies in food webs have focused on changes in magnitude of the subsidy (Nakano and Murakami 2001; Sabo and Power 2002; Baxter et al. 2004; Marczak and Richardson 2007). This study shows that insect prey subsidies emerging from streams can substantially alter not only prey availability in a recipient system, but also the trophic structure of the overall prey assemblage.

Acknowledgements

I thank my doctoral advisor, William Matthews, and my doctoral committee, Elizabeth Bergey, Bruce Hoagland, Caryn Vaughn, and Gary Wellborn, for advice through all phases of the study. Comments of three anonymous reviewers substantially improved the quality of the manuscript. Access to sites was graciously granted by the Kessler Farm Field Laboratory, Bruce Stewart, and Ronald Wesner. Funding was provided by Graduate Student Senate Research Grants (University of Oklahoma). This work was conducted in partial fulfullment of a Ph.D. degree from the University of Oklahoma, Department of Zoology.

Literature Cited

Baxter, C. V. et al. 2004. Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. – Ecology 85: 2656-2663.

Beisner, B.E. et al. 2006. The role of environmental and spatial processes in structuring lake communities from bacteria to fish. – Ecology 87: 2985-2991.

Borror, D. J. and White, R. E. 1970. A field guide to the insects of America north of Mexico. –Houghton Mifflin Company.

Briers, R. A. et al. 2002. Dispersal of adult stoneflies (Plecoptera) from upland streams draining catchments with contrasting land-use. – Arch. Hydrobiol. 155: 627-644.

Briers, R. A. et al. 2005. The lateral extent of the subsidy from an upland stream to riparian lycosid spiders. – Ecography 28: 165-170.

Brittain, J.E. 1982. Biology of mayflies. – Ann. Rev. Entomol. 27: 119-147.

Chan, E. K. W. et al. 2007. Contribution of adult aquatic insects to riparian prey availability along tropical forest streams. – Mar. Freshwat. Res. 58: 725-732.

Collier, K. J. and Smith, B. J. 1998. Dispersal of adult caddisflies (Trichoptera) into forests alongside three New Zealand streams. – Hydrobiologia 361: 53-65.

Daly, H. V. et al. 1998. Introduction to insect biology and diversity. Second edition. – Oxford Univ. Press.

Delettre, Y. R. and Morvan, N. 2000. Dispersal of adult aquatic Chironomidae (Diptera) in agricultural landscapes. – Freshwater Biol. 44: 399-411.

Fukui, D. et al. 2006. Effect of emergent aquatic insects on bat foraging in a riparian forest. – J. Anim. Ecol. 75: 1252-1258.

Gray, L. J. 1993. Response of insectivorous birds to emerging aquatic insects in riparian habitats of a tallgrass prairie stream. – Am. Midl. Nat. 129: 288-300.

Hilderbrand, G.V. et al. 1999. Role of brown bears (Ursus arctos) in the flow of marine nitrogen into a terrestrial ecosystem. – Oecologia 121: 546-550.

Holt, R. D. 2004. Implications of system openness for local community structure and ecosystem function. – In: Polis, G.A. et al. (eds.), Food Webs at the Landscape Level. The Univ. of Chicago Press, pp. 96-114.

Huxel, G. R. et al. 2002. Effects of partitioning allochthonous and autochthonous resources on food web stability. – Ecol. Res. 17: 419-432.

Jackson, J. K. and Fisher, S. G. 1986. Secondary production, emergence, and export of aquatic insects of a Sonoran desert stream. – Ecology 67: 629-638.

Jackson, J. K. and Resh, V. H. 1989. Distribution and abundance of adult aquatic insects in the forest adjacent to a northern Californian stream. – Environ. Entomol. 18: 278-283.

Knight, T. M. et al. 2005. Trophic cascades across ecosystems. – Nature 437: 880-883.

Kovats, Z. E. et al. 1996. Inland dispersal of adult aquatic insects. – Freshwater Biol. 36: 265-276.

Leroux, S. J. and Loreau, M. 2008. Subsidy hypothesis and strength of trophic cascades across ecosystems. – Ecol. Lett. 11: 1147-1156.

Liebold, M.A. et al. 1997. Species turnover and the regulation of trophic structure. – Annu. Rev. Ecol. Syst. 28: 467-494.

Lillie, T.H. et al. 1985. The dispersal of Culicoides mississippiensis (Diptera: Ceratopogonidae) in a salt marsh near Yankeetown, Florida. – J. Am. Mosq. Control Assoc. 1: 463-467.

Lynch, R. J. et al. 2002. Adult aquatic insects: Potential contributors to riparian food webs in Australia's wet-dry tropics. – Aust. Ecol. 27: 515-526.

Marczak, L. B. and Richardson, J. S. 2007. Spiders and subsidies: results from the riparian zone of a coastal temperate rainforest. – J. Anim. Ecol. 76: 687-694.

Marczak, L. B. et al. 2007. Meta-analysis: Trophic level, habitat, and productivity shape the food web effects of resource subsidies. – Ecology 88: 140-148.

Merritt, R. W. and Cummins, K. W. 1996. An introduction to the aquatic insects of North America. Third edition. Kendall Hunt.

Nakano, S. and Murakami, M. 2001. Reciprocal subsidies: Dynamic interdependence between terrestrial and aquatic food webs. Proc. Natl. Acad. Sci. 98: 166-170.

Petersen, I. et al. 2004. Dispersal of adult aquatic insects in catchments of differing land use. – J. Appl. Ecol. 41: 934-950.

Polis, G. A. et al. 1997. Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. – Annu. Rev. Ecol. Syst. 28: 289-316.

Quinn, G.P. and Keough, M.J. 2002. Experimental design and data analysis for biologists. – Cambridge University Press, Cambridge, U.K.

Sabo, J. L. and Power, M. E. 2002. Numerical response of lizards to aquatic insects and short-term consequences for terrestrial prey. – Ecology 83: 3023-3036.

Sample, B. E. et al. 1993. Estimation of insect biomass by length and width. – Am. Midl. Nat. Naturalist 129: 234-240.

Schindler, D. E. et al. 2003. Pacific salmon and the ecology of coastal ecosystems. – Front. Ecol. Environ. 1: 31-37.

Schreiber, S. and Rudolf, V.H. 2008. Crossing habitat boundaries: coupling dynamics of ecosystems through complex life cycles. – Ecol. Lett. 11: 576-587.

Takimoto, G. et al. 2002. Seasonal subsidy stabilizes food web dynamics: Balance in a heterogeneous landscape. – Ecol. Res. 17: 433-439.

Triplehorn, C. A. and Johnson, N. F. 2005. Borror and DeLong's introduction to the study of insects. – Thomson Brooks/Cole.

Tables

Table 1. Physical characteristics of study sites. All data reflect measurements taken on the day of insect collection.

Characteristic	Byrd's Mill	Spring Lake	Finn
Max pool depth (cm)	82	52	26
Min pool depth (cm)	45	26	9
Max pool width (m)	6.6	7.1	2.3
Min pool width (m)	5	2.3	1
Max flow (m s^{-1})	0.8	0.56	0.13
Min flow (m s^{-1})	0.2	0.09	0.05
Max pool temp (ºC)	26	18	18
Min pool temp (ºC)	10	16	3
Max air temp (ºC)	29.4	22	20
Min air temp (ºC)	3.9	12	6

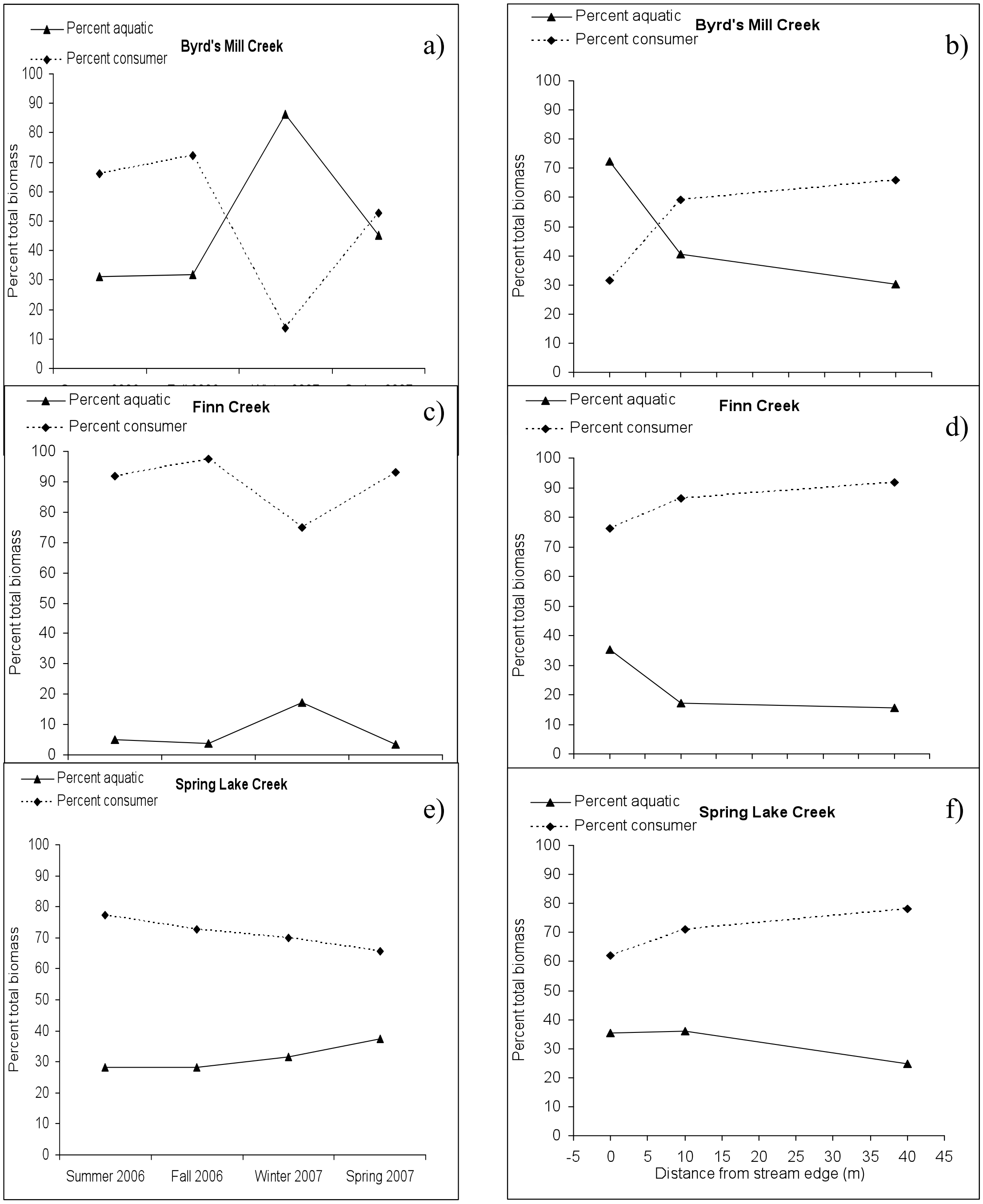
Figures

Figure 1. Relative biomass of adult aquatic insects and consumers in the total prey assemblage at each site by season (a,c,e) and distance from the stream (b,d,f). Data points for seasons represent means pooled for each distance, and data points for distance represent means pooled for each season. Error bars are excluded for clarity.

Figure 2. Relative biomass of consumers (gray) and non-consumers (black) in adult aquatic insect assemblages during each season for Byrd's Mill Creek, Spring Lake Creek, and Finn Creek. Different letters above bars indicate differences in percent abundance of consumers significant at alpha = 0.05 according to Tukey-Kramer multiple comparisons. Statistical results are for the comparison of percent of aquatic consumers across season using repeated measures MANOVA. Data were arcsine transformed prior to analysis, but are represented here as raw data.

Figure 3. Relationship between proportion of adult aquatic insect abundance and proportion of total consumers on sticky traps. Data points (n = 12) represent pooled data from all traps at each site (n = 3) for each season (n = 4).

Figure 1.



a)
Byrd's Mill Creek
Percent aquatic
Percent consumer
Percent total biomass
0
10
20
30
40
50
60
70
80
90
100
b)
Byrd's Mill Creek
c)
Finn Creek
d)
Finn Creek
e)
Spring Lake Creek
Summer 2006
Fall 2006
Winter 2007
Spring 2007
f)
Spring Lake Creek
-5
0
5
10
15
20
25
30
35
40
45
Distance from stream edge (m)

Figure 2.

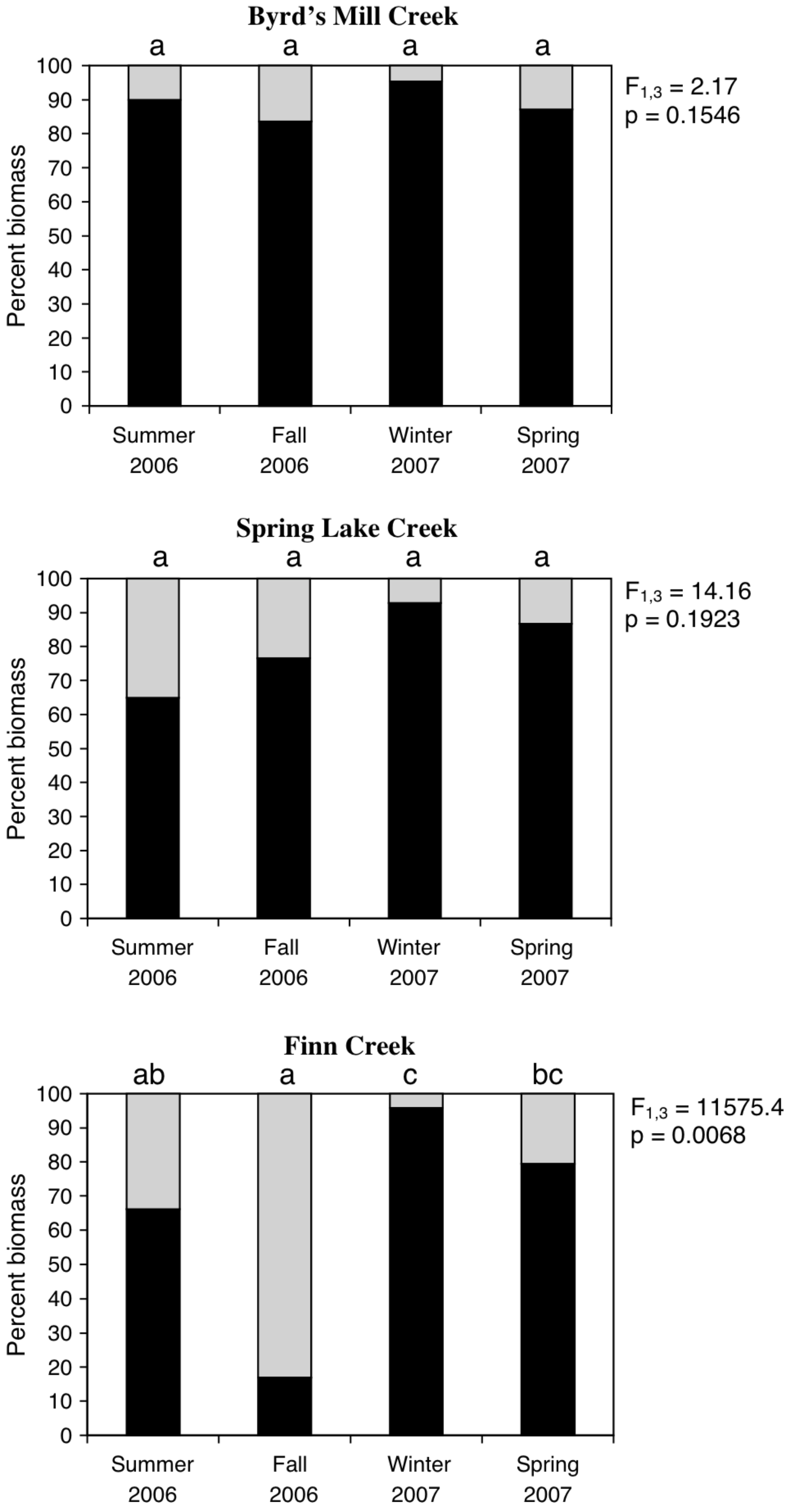
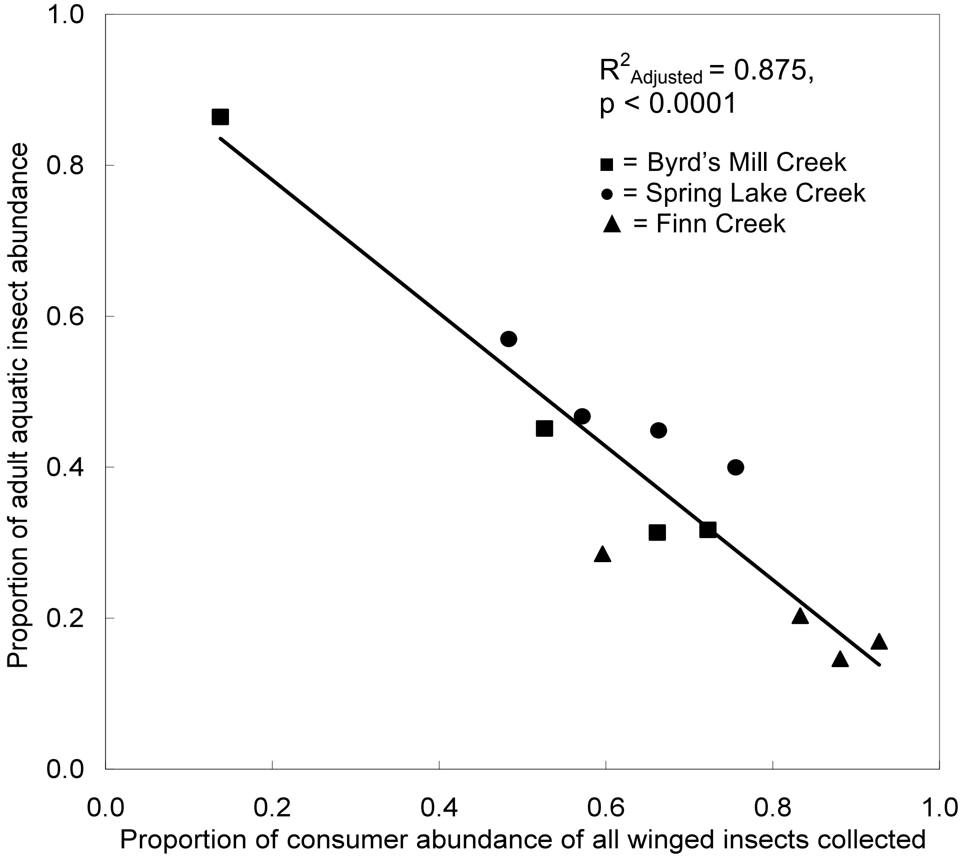

Byrd's Mill Creek
a
a
a
a
100
90
80
70
60
50
40
30
20
10
0
Percent biomass
Summer 2006
Fall 2006
Winter 2007
Spring 2007
$F_{1,3}$ = 2.17
p = 0.1546
Spring Lake Creek
a
a
a
a
100
90
80
70
60
50
40
30
20
10
0
Percent biomass
Summer 2006
Fall 2006
Winter 2007
Spring 2007
$F_{1,3}$ = 14.16
p = 0.1923
Finn Creek
ab
a
c
bc
100
90
80
70
60
50
40
30
20
10
0
Percent biomass
Summer 2006
Fall 2006
Winter 2007
Spring 2007
$F_{1,3}$ = 11575.4
p = 0.0068

Figure 3


$R^2_{Adjusted} = 0.875$,
$p < 0.0001$
■ = Byrd's Mill Creek
● = Spring Lake Creek
▲ = Finn Creek
Proportion of adult aquatic insect abundance
Proportion of consumer abundance of all winged insects collected
0.0
0.2
0.4
0.6
0.8
1.0
0.0
0.2
0.4
0.6
0.8
1.0

Appendix

Appendix 1. Mean abundance (individuals day^{-1} trap^{-1}) and biomass (mg day^{-1} trap^{-1}) of common insects (>1%) averaged across each sticky trap at each site. Trophic group is listed beside each family or order as C (consumer) or N (non-consumer). Data in parentheses are the percent abundance or biomass of each taxa in the entire collection for each site. L.O. =larval origin.

L.O	Order	Family – L.O.	Spring Lake Creek		Byrd's Mill Creek		Finn Creek	
			Abundance (%)	Biomass (%)	Abundance (%)	Biomass (%)	Abundance (%)	Biomass (%)
Aquatic	Diptera							
		Ceratopogonidae - C	0.18 (2.8)	0.003 (0.4)	0.23 (5.2)	0.003 (0.2)	0.03 (0.9)	0.001 (0.1)
		Chironomidae - N	2.64 (41.9)	0.180 (22.5)	2.34 (50.1)	0.549 (39.2)	0.52 (11.9)	0.026 (11.9)
		Dolichopodidae - C	0.10 (1.5)	0.018 (2.2)	0.05 (1.1)	0.015 (1.1)	0.22 (5.0)	0.033 (6.3)
		Psychodidae - C			0.12 (2.6)	0.005 (0.3)		
	Megaloptera - N				0.009 (0.2)	0.13 (9.4)		
	Trichoptera - N				0.16 (3.6)	0.093 (6.6)	0.02 (0.5)	0.006 (0.0)
	Ephemeroptera - N				0.10 (2.3)	0.080 (5.7)		
Terrestrial	Coleoptera - C		0.13 (2.2)	0.008 (1.0)	0.06 (3.0)	0.097 (6.9)	0.14 (3.2)	0.079 (14.1)
	Diptera							
		Cecidomyidae - N	0.11 (1.7)	0.002 (0.3)	0.05 (1.1)	0.002 (0.2)	0.16 (3.7)	0.006 (1.2)
		Chloropidae - C	0.06 (1.0)	0.016 (2.0)	0.05 (1.0)	0.030 (2.1)	0.06 (1.5)	0.022 (4.1)
		Muscidae - C	0.01 (0.2)	0.018 (2.3)				
		Ottidae - C	0.01 (0.2)	0.012 (1.5)				
		Phoridae - C	0.28 (4.5)	0.021 (2.7)	0.19 (4.2)	0.023 (1.6)	0.42 (9.7)	0.023 (4.3)
		Psychodidae - C	0.10 (1.7)	0.002 (0.2)			0.04 (0.9)	0.001 (0.1)
		Sciaridae - C	1.02 (16.2)	0.144 (18)	0.27 (5.9)	0.061 (4.3)	1.20 (27.9)	0.090 (27.9)
		Syrphidae - C	0.00 (0.0)	0.036 (4.5)				
		Tipulidae - C	0.01 (0.1)	0.093 (11.7)				
	Hemiptera							
		Aphididae - C	0.67 (10.7)	0.071 (8.9)	0.08 (1.9)	0.009 (0.6)	0.22 (5.0)	0.015 (2.9)
		Cicadellidae - C	0.11 (1.8)	0.015 (1.9)	0.14 (3.1)	0.050 (3.6)	0.45 (10.3)	0.064 (10.3)
	Hymenoptera - C		0.43 (6.8)	0.020 (2.5)	0.24 (6)	0.015 (1.1)	0.33 (7.6)	0.006 (1.1)
	Psocoptera - C		0.02 (0.4)	0.002 (0.2)	0.04 (1.0)	0.008 (0.5)	0.04 (1.0)	0.002 (0.3)
	Thysanoptera - C		0.04 (0.7)	0.000 (0.0)	0.05 (1.2)	0.001 (0.1)	0.07 (1.7)	0.001 (0.1)
N/A	Diptera							
		Unknown family	0.10 (1.6)	0.022 (2.7)			0.20 (5.0)	0.022 (0.0)
	Unknown		0.00 (0.1)	N/A	0.08 (1.8)	N/A	0.03 (0.7)	N/A
	All aquatic		3.07 (50)	0.21 (27)	3.02 (67)	0.88 (63)	0.84 (21)	0.08 (15)
	All terrestrial		3.10 (50)	0.56 (73)	1.53 (33)	0.47 (37)	3.32 (78)	0.43 (85)

CHAPTER 2: AQUATIC PREDATION ALTERS A TERRESTRIAL PREY SUBSIDY

JEFF SCOTT WESNER

Biological Station and Department of Zoology, University of Oklahoma, Norman, OK 73019.

Abstract

Organisms with complex life histories (CLH) often cross habitat or ecosystem boundaries as they develop from larvae to adults, coupling energy flow between ecosystems as both prey (bottom-up) and consumers (top-down). Predation effects on one stage of this life cycle can therefore cascade across ecosystems, magnifying the impact of local predation. The majority of predation studies have assessed effects only on a local level, within the habitat of the predator. I used large outdoor stream mesocosms to test the hypothesis that predation in an aquatic habitat alters the magnitude and trophic structure of a prey assemblage in a terrestrial habitat. I also tested how a consumer in the terrestrial habitat (web-weaving spiders) responded to these changes in prey export. Two fish species were the predators (red shiner, *Cyprinella lutrensis* and orangethroat darter, *Etheostoma spectabile*) in an experiment with three treatments: both fish species monocultures plus a fishless control. Fish predation reduced aquatic insect emergence biomass by 50% compared to the fishless control, and altered the trophic structure of the emergent community, reducing emerging insect predator biomass by 50%, but had no effect on other insect trophic groups. Spiders captured only insects that were unaffected by fish predation (mostly chironomids), and therefore did not respond numerically to overall changes in insect abundance or biomass. Patterns of insect emergence were largely driven by a strong negative relationship between fish and a predatory dragonfly (*Pantala flavescens*). The results of this experiment show that predation in one habitat can have strong effects on the biomass and trophic structure of subsidies entering adjacent habitats, resulting in contrasting predictions for the role of these subsidies in recipient food webs. In the absence of fish, aquatic habitats produced

terrestrial insect communities with higher biomass (bottom-up potential) and a higher proportion of predators (top-down potential) than when fish were present.

Introduction

Predation studies typically focus on direct or indirect effects of predation on local prey communities. However, a large percentage of organisms (~ 80%) exhibit ontogenetic niche shifts (Werner and Gilliam 1984), in which an organism's niche changes during development. In organisms with complex life-histories (CLH) such as amphibians and insects, niche shifts often occur between larval and adult stages, with each stage in separate habitats (Werner and Gilliam 1984). When prey communities are dominated by organisms that exhibit coupled ontogenetic and habitat shifts, predation effects in a local habitat can cascade across habitat or ecosystem boundaries, altering the abundance and biomass of the prey community in a second habitat or ecosystem (Baxter et al. 2004). These alterations can have strong effects on the structure and dynamics of food webs in the recipient habitat (Polis and Hurd 1996; Nakano and Murakami 2001; Baxter et al. 2004; Knight et al. 2005; Marczak and Richardson 2007).

Spatial subsidies involve the flow of nutrients, material and organisms across ecological boundaries, with their bottom-up effect in recipient food webs determined by the magnitude of the subsidy (Nakano and Murakami 2001; Sabo and Power 2002; Baxter et al. 2004). When subsidies consist of an assemblage of organisms, as opposed to nutrient subsidies, top-down effects may also be important, and will vary according to the trophic structure of the subsidy assemblage. Subsidy communities that are equal in magnitude, but different in species composition, may have different impacts on recipient food webs if the species occupy different trophic levels. For example, insects that emerge from aquatic to terrestrial habitats subsidize consumers in terrestrial food webs (Nakano and Murakami 2001; Baxter et al. 2004). Many aquatic insects do not feed as adults (e.g. chironomids, mayflies, caddisflies), while some are predators (e.g. damselflies and dragonflies), and can have strong consumptive effects in terrestrial ecosystems (Knight et al. 2005). Hence, the primary impact in terrestrial food webs of a subsidy assemblage of chironomids and mayflies (non-consumers) is likely as an energy source for terrestrial consumers. In contrast, a subsidy assemblage composed of damselflies and dragonflies (predators) may represent the same amount of biomass as the former subsidy, but could also exert strong top-down effects in a terrestrial food web.

Recent theory has emphasized how changes to the quality of larval habitat can influence the consumer potential of CLH communities in the adult habitat. Increased resources in the larval habitat can cause a counterintuitive decrease in larval abundance over time if adult reproduction is limited by low resource levels in the adult habitat, and vice versa. Such alternative stable states are thereby maintained by altering productivity in only one system (Schreiber and Rudolf 2008). Similarly, increased mortality of aquatic tadpoles decreases adult frog abundance around ponds. Because adult frogs are predatory, a decrease results in contrasting predator profiles around ponds ('predator shadows'). When applied to the landscape scale across a series of natural ponds, such reductions in adult predators alter the spatial patterning of terrestrial trophic cascades, based only on dynamics in aquatic habitats (McCoy et al. 2008).

In addition to resource availability, predation in the larval habitat may also regulate the consumer potential of prey subsidies in recipient food webs (*sensu* Knight et al. 2005), but this has not been studied. Additionally, to my knowledge only two studies have assessed the effect of predation in larval habitats on the magnitude of a subsidy entering a recipient habitat (Baxter et al. 2004; Finlay and Vredenburg 2007). In both cases predatory trout reduced the biomass of organisms emerging from aquatic to terrestrial habitats. Fish are often the top predators in permanent aquatic habitats. Fish species identity is typically related to feeding strategy, which can determine the strength of predation. Benthic feeding fishes may have stronger effects on in stream secondary productivity than surface-feeding fishes, because surface-feeding fishes are subsidized by terrestrial input, reducing pressure on benthic invertebrates (Dahl and Greenburg 1996). Knowledge of factors controlling both the magnitude and trophic structure of organism subsidies is needed to more fully understand how food web changes in one habitat can cascade into adjacent habitats.

I tested the hypothesis that top predators in aquatic habitats alter the magnitude and trophic structure of organisms entering adjacent terrestrial habitats. Using two fish species as predators, I predicted that fish predation would alter the trophic structure and reduce biomass of insects emerging from aquatic mesocosms into the surrounding terrestrial habitat, causing a reduction in abundance of terrestrial spiders that feed on emerging insects. I also predicted that the strength of the predation effect would correspond to fish-feeding strategy.

Methods

Predators

I manipulated the presence of two predatory fish species with different functional feeding strategies: benthic invertivore (*Etheostoma spectabile*) and water-column invertivore (*Cyprinella lutrensis*). Both species are common and widespread throughout the central United States and often co-occur. The orangethroat darter (*Etheostoma* *spectabile*) is an invertivore common to streams throughout eastern Oklahoma and feeds exclusively on the benthos, primarily on insects [chironomids, stoneflies, mayflies (Martin 1984; Miller and Robison 2004)]. Red shiner (*Cyprinella lutrensis*) is an abundant invertivorous local minnow in North American prairie streams (Matthews 1985). It typically lives in the water column and feeds at all levels of the water column, including benthic, mid-water, and surface habitats (Hale 1963). Insects are the primary prey of red shiner, but it can also include substantial amounts of algae and other invertebrates (Gido and Matthews 2001).

A predatory dragonfly species (*Pantala flavescens*) aerially colonized the mesocosms within minutes after filling with water, acting as an extra potential predator on aquatic insects in the mesocosms. I did not manipulate *P. flavescens* abundance. *P. flavescens* feeds on invertebrates (e.g. midges) and small vertebrates (tadpoles) (Sherratt and Harvey 1989).

Description of mesocosms and experimental design

This experiment was conducted using large outdoor aquatic mesocosms at the University of Oklahoma Biological Station (UOBS) near Lake Texoma, OK, USA (Matthews et al. 2006) over 46 days in June and July 2007. Each mesocosm consisted of an individual pool (183 cm diameter and 46 cm deep) with a riffle (122 cm long and 5-10 cm deep) both “upstream” and “downstream” (Figure 1). The side of each mesocosm has a Plexiglas viewing window (22 x 10 cm) just below water level. Partial shade was provided with shade cloths suspended approximately 150 cm above each mesocosm. The substrate was a mixture of cobble and gravel taken from Brier Creek (Marshall Co., OK, USA). Mesocosms were separated by marine plywood dividers placed in the riffles to prevent water from mixing between units. Plastic mesh screens (0.32 cm diameter) at the upper and lower attachment of each riffle restricted fish to pools. Flow was maintained by Little Giant submersible pumps (2500 L/h) (Little Giant Pump Co., Oklahoma City, OK). All units were filled with well water from a public supply (Marshall County Water Corporation) over a 24-hour period on May 24-25 and flow was initiated immediately after. On May 25, a slurry of algae obtained from nearby Brier Creek was added equally to each unit to introduce periphyton and filamentous algae. Small snails, cladocerans, and some larval insects were likely included in the algal inoculations. Units were maintained with flow but without fish for 17 days (May 25 – June 11) to allow establishment of algae and aerial colonization by invertebrates (mostly midges and dragonflies; Table 1). Mesocosms were not covered at any point during the experiment, allowing continuous oviposition. Temperature (31.3 ± 2.0 °C) and dissolved oxygen (9.3 ± 1.0 mg/l) were measured several times during the experiment. Conductivity was not measured in this experiment, but was measured between 385 and 490 μS in a previous experiment using the same mesocosms (Gido and Matthews 2001). Temperature, dissolved oxygen, and conductivity in the mesocosms are within the natural ranges of nearby streams (Miller and Golloday 1991).

The experiment included six replicates of each of three treatments: water column feeder (*C. lutrensis*), benthic feeder (*E. spectabile*), and fishless control. Treatments were assigned randomly among 18 individual mesocosm units. Approximately 150 individuals of similar size for each fish species were collected from nearby streams by seining on 11 June 2007. This marked the beginning of the experiment (Table 1). On the same day, 20 individuals of each species were assigned to each of 6 units, resulting in a density of 10.6 fish m^{-2}, commensurate with natural densities. All remaining fish were held in separate units, and were used to replace any dead fish (< 10 total, checked daily) on the day they were found.

Fish from a single randomly selected replicate from each fish treatment were observed three times over the course of the experiment (Table 1). Observations lasted 5 minutes during early afternoon following methods described in Hargrave (2009). Notes were taken at 30 second intervals on the number of fish visible and their position in the pool: benthic, water column, or surface. Feeding attempts and their location also were noted whenever they occurred during the 5 minute period. At the end of the experiment all fish were preserved in 10% formalin. Gut contents of 15 fish from each species (at least two per replicate) were identified to the lowest taxonomic level possible and classified as larval or winged. I compared fish diet overlap using the Morisita-Horn index (C_{MH}) (Gelwick and Matthews 2006):

$$C_{MH} = [2 \text{ x } \sum(an_i bn_i)]/[(da + db)aN \text{ x } Bn]$$

where aN is the number of individual prey in fish species A; bN is the number of individual prey in fish species B; an_i is the number of individual prey of the i^{th} species in fish species A; bn_i is the number of individual prey of the i^{th} species in fish species B; da $= \sum an_i^2/aN^2$, and db $= \sum bn_i^2/bN^2$. C_{MH} has values between 1 and 0, where 1 indicates that diets are identical with respect to proportional prey composition, and 0 indicates that fish diets are completely different.

Periphyton was sampled using ten porous silica discs (2.5 cm diameter), placed randomly on the substrate in each pool 13 days before the start of the experiment.

Periphyton was collected on days 15 and 42. On day 15, four discs were collected from each mesocosm and frozen overnight to lyse cells. The discs were then extracted overnight in 90% acetone. Chlorophyll *a* was estimated for each treatment spectrophotometrically with a correction for phaeophytin (American Public Health Association, 1998). Periphyton samples from day 42 were lost.

Floating emergence traps were deployed continuously for 2 to 4 days during three sampling periods (Table 1). Traps were made using a galvanized steel frame with a collection area of 0.16 m^2. Styrofoam was attached to the bottom of the traps for buoyancy. Nylon fabric surrounded the trap and was attached to a plastic collection bottle at the top, which was fitted with an inverted funnel. A small piece of an insecticidal strip was placed in each collecting jar (active ingredient = dichlorvos, Hot Shot No-Pest Strip, United Industries, St. Louis, MO). Upon collection, insects were transferred to individually labeled vials, and stored dry or in 95% ethanol. All insects were identified to family using Triplehorn and Johnson (2005), and measured for length to the nearest 0.1 mm for regression estimation of biomass (Sabo et al. 2002). Adult insects were classified as predators, omnivores, or non-consumers according to descriptions of family-level adult feeding roles in Triplehorn and Johnson (2005).

The emergence traps collected insects whose life-cycle contained a floating pupa stage, but underestimated insects which crawl out of the water to emerge (e.g. odonates). Odonate emergence was estimated by collecting exuviae from the upstream and downstream screens. Limiting collections to screens underestimated total dragonfly emergence for the pool. To correct for this and create a common area metric for analyses with emergence trap data, I multiplied the proportion of the pool circumference surveyed

(0.16) by the pool area (2.6 m^2). All dragonfly collections were divided by the resulting area (0.4176 m^2) to estimate emergence density. All mesocosms were checked for exuviae at least twice per week for the first four weeks, and then almost daily afterwards. All exuviae were preserved in 70% ethanol and stored for later identification and measurement. Biomass of dragonflies was estimated based on published regression equations using exuviae head width, measured to the nearest 0.1 mm (Sabo et al. 2002).

Benthic macroinvertebrates were sampled three times during the experiment using a steel cylinder (50 cm^2). The cylinder was driven approximately 5 centimeters into the substrate and the contents scooped into a sieve (500 μm), drained, and preserved in ethanol (70%). Three samples were taken from each pool and combined into a single sample. Macroinvertebrates were identified to order or family using Merritt and Cummins (1996).

Shifts in foraging behavior under the threat of fish predation are common (Sih 1980). To test for this potential effect in this experiment, I counted larval dragonflies that were perched on clay tiles five separate times (Table 1). I assumed the clay tiles represented risky habitats for dragonflies, because they contained no refuge and had light backgrounds. In addition, I observed dragonflies feeding from the tiles on several occasions through the viewing windows early in the experiment. On each observation date I approached each mesocosm slowly to avoid disturbing the dragonflies, and quickly counted the number of dragonflies on each tile. Four tiles were in each mesocosm, but algal growth sometimes obscured 1-2 tiles. I corrected for this by dividing the counts by the number of tiles observed.

Macrophytes can serve as refuge for benthic invertebrate prey (Gilinsky 1984), as can mats of filamentous algae. I measured this structural refuge by multiplying the percent coverage of benthic filamentous algae by the mean height of filaments to use volume of filamentous algae as an estimate of prey refuge. Two measurements were made towards the end of the experiment (Table 1). I measured height by placing a meter stick on the substrate in three random locations and recording the height (±1 cm) of each filament that touched the meter stick (usually 3-4).

Spiders naturally colonized the tops of mesocosms and served as terrestrial consumers. I measured their response to insect emergence by visual counts of occupied webs directly over each pool on four nights: two nights before fish introductions, and then on three nights after fish introductions (Table 1). Most spiders were tetragnathids, which commonly build webs on riparian vegetation and include a large percentage of emerging chironomid and mayfly insects in their diet (Williams et al. 1995). Webs were usually attached to the emergence trap cages, edges of the mesocosm, and/or the pvc pipe running above each pool.

Data analysis

I tested the null hypothesis of no differences between treatments using a separate repeated measures ANOVA (rmANOVA) for abundance of common taxa, abundance and biomass of trophic groups, proportion of each trophic group, overall biomass, and overall abundance. Fish treatment was the between-subjects factor, and time was the within-subjects factor. Relative abundance of macroinvertebrates in fish diets was analyzed with a one-way ANOVA using fish species as predictor variable. Linear regression was used to test for a relationship between overall insect emergence and spider abundance. If an ANOVA was significant for any variable, I used Tukey HSD Honestly Significant Differences (HSD) post-hoc test, which corrects for family-wise type 1 error (Quinn and Keough 2002). Abundance data were log transformed, counts of larval odonates were square-root transformed, and proportional data were arcsine square root transformed when needed to satisfy assumptions for ANOVA (Quinn and Keough 2002). For rmANOVA, sphericity was tested using Mauchly's W, and adjusted degrees of freedom were used when significant following the Greenhouse-Geisser adjustment. All statistical analyses were performed using SPSS 16.0 for Macintosh (SPSS, Chicago, Ill.).

Results

Both fish species reduced emergent insect biomass by at least 55% compared to the fishless control (Figure 2a; Table 2), and significantly altered the trophic structure of the emergent community, reducing the proportion of predators when fish were present (Figure 2b). Predators averaged 55% of emergent biomass in the fishless treatment, but only 28 and 24% in *C. lutrensis* and *E. spectabile* treatments, respectively. This reduction was significant in the *E. spectabile* treatment and approached significance in the *C. lutrensis* treatment (Table 2). Overall predator proportion by biomass increased over time (Figure 2b; Table 2) with the onset of *P. flavescens* emergence (Table 2), but there was no interaction between time and treatment (rmANOVA within subjects $F_{2,12} = 0.532$,).

Fish had a strong negative effect on the emergence of a predatory dragonfly, *Pantala flavescens*, but not on any other taxon. Fish reduced mergence abundance of *P.*

flavescens from by at least 57% compared to the control (rmANOVA between subjects: $F_{2,14} = 10.314$, $P = 0.002$; Tukey HSD post-hoc comparisons between *C. lutrensis* vs. control: $P = 0.016$, *E. spectabile* vs. control: $P = 0.006$). Due to their relatively large size, reduction in *P. flavescens* emergence drove the large reductions in emergence of overall biomass and predator biomass. Overall predator biomass [*P. flavescens* and long-legged flies (Diptera: Dolichopodidae)] was reduced nearly five-fold in both fish treatments compared to the control (Table 2), and there was a significant treatment x time interaction (Table 2). Fish reduced emerging predator biomass on day 26 (one-way ANOVA: $F_{2,15} = 7.368$, $P = 0.006$), when *P. flavescens* emergence was at its peak, but had no effect on days 17 (one-way ANOVA: $F_{2,15} = 0.543$, $P = 0.592$) or 42 (one-way ANOVA: $F_{2,15} = 0.657$, $P = 0.532$).

Non-feeding insects (Chironomidae, Ephemeroptera, and Trichoptera) made up more than 90 percent of non-odonate individuals by abundance and were unaffected by fish presence. A total of 638 individuals representing nine insect taxa emerged from the mesocosms during the experiment. Fish had no effect on overall emergent insect abundance (Table 2).

Spiders were present above at least 83 percent of all mesocosms on each sampling date (n=4), but density was low (mean: 2.33 spiders/mesocosm, range: 0-8). I did not identify spiders below order (Araneae) during observations, but abundance appeared to be dominated by tetragnathids (Araneae: Tetragnathidae), horizontal orb weaving spiders that have been found to respond to fluctuations in aquatic insect abundance (Baxter et al. 2004; Marczak and Richardson 2007). Linear regression revealed no relationship between spider density and insect abundance ($R^2 = 0.0212$, $P = 0.5646$) or biomass ($R^2 =$

0.0624, $P = 0.3175$). Spiders showed no preference for fish treatments (Table 2) despite the reduction in emergent biomass when fish were present. On several dates I qualitatively examined spider webs to ensure that aquatic insects were being trapped. Food items in webs appeared to consist almost exclusively of chironomids, suggesting that spiders were subsidized by aquatic production. *Pantala flavescens* was not observed in any webs.

Benthic invertebrate abundance was dominated by small snails (64%; Gastropoda: Planorbidae) and chironomid larvae (21%). Among benthic insects, chironomid larvae made up approximately 90 percent of all insects by abundance. Fish had no impact on the abundance of any benthic invertebrate taxon (Table 2). *Pantala flavescens* was too rare in benthic samples to analyze, appearing in no more than 3 of the 18 pools on any sample date. This is in contrast to their relative abundance shown by exuviae counts, and suggests a sampling bias, which was likely due to the ability of *P. flavescens* larvae to evade capture during benthic samples due to their high mobility. In contrast to their rarity in benthic samples, visual benthic surveys of *P. flavescens* on clay tiles revealed a 5-6 fold higher density in fishless treatments (0.24/tile) relative to either *C. lutrensis* (0.03/tile) or *E. spectabile* (0.05/tile) treatments (rmANOVA between subjects, $F_{1,12} = 31.015$, $P = 0.011$, Tukey HSD post-hoc comparisons with control, $P \leq 0.019$).

I observed fish from a single pool for each species on three dates (Table 1). No darters were observed on day 30, because filamentous algal growth blocked them from my view. Nearly all of the 20 initial darters were collected at the end of the experiment and very little mortality occurred over the course of the experiment, meaning that darters were simply hidden on the benthos during observation periods. On days 7 and 14, I observed 2 and 3 darters, respectively. These individuals appeared to be actively feeding on the benthos. They moved in and out of rock crevices and under tiles and remained almost constantly in contact with the substrate. In contrast, nearly all red shiner individuals were observed on each day, and spent almost all of their time in the water column. On each occasion, only 2-3 shiners were seen feeding on the benthos at any time. The rest of the individuals remained in the water column and often broke the surface to feed. Diet analysis using the Morisita-Horn index (C_{MH}) revealed strong overlap in prey composition ($C_{MH} = 0.98$). On average, *C. lutrensis* fed on more terrestrial input (30%) than *E. spectabile* (<1%), but terrestrial input in *E. spectabile* was too rare ($n = 1$) to analyze statistically. Prey composition for both species was dominated by *Bosmina spp*. (Cladocera: Bosminidae), which made up 60% of food items. Cladoceran species typically occupy benthic and water-column habitats (Dodson and Frey 2001). The next most common food items, pooled for both species, were: chironomid larvae (12%), spiders (5%), *P. flavescens* (4%), unknown terrestrial insects (4%), terrestrial dipterans (3%), Collembola (3%), Planorbidae (3%), unidentifiable prey (2%), copepods (< 1%), chironomid pupae (< 1%), Plecoptera (< 1%), and Megaloptera (< 1%).

Chlorophyll *a* was similar across treatments on day 16 (mean: 6.85 (± 2.77) mg/m^2; Table 2). Prey refuge, estimated as volume of filamentous algae (mostly *Chara spp.* and *Oedegonium spp.*), was similar across treatments on day 29 ($F_{2,16} = 0.785$, $p = 0.475$) and day 41 ($F_{2,15} = 2.971$, $p = 0.087$).

Discussion

This study showed that predation by fish in an aquatic habitat strongly altered the trophic structure and biomass of an insect prey subsidy entering the terrestrial habitat. To my knowledge only two empirical studies have assessed the impact of predation in aquatic habitats on terrestrial food webs (Baxter et al. 2004; Knight et al. 2005). Trout predation reduced insect biomass export to the terrestrial system by nearly half, altering the abundance of terrestrial web-weaving spiders (Baxter et al. 2004), and predatory sunfish altered a terrestrial trophic cascade by reducing the abundance of predatory adult dragonflies in terrestrial habitats around ponds (Knight et al. 2005). My study complements the findings of Baxter et al. (2004) and Knight et al. (2005), showing that fish predation can simultaneously affect the magnitude and trophic structure of adult aquatic insect assemblages in terrestrial habitats. When predatory fish were present, insect emergence biomass was low and dominated by non-feeding insects. When predatory fish were absent, insect emergence biomass was high and dominated by predatory insects. The contrasting adult aquatic insect assemblages created by fish predation in this study are likely to have different effects in terrestrial food webs. In the presence of fish, the primary effect of adult aquatic insects in terrestrial food webs is likely bottom-up, as an energy source for terrestrial consumers (Figure 3). In the absence of fish, the likely effect of adult aquatic insects is bottom-up and top-down, as both prey and predators in terrestrial food webs (Figure 3).

Fish reduced emergence of adult insect predators by approximately 50%, nearly identical to the reduction found by Knight et al. (2005, as estimated from their Figure 2b). Modeling showed that a similar reduction in frog emergence from ponds reduced the predation profile around ponds (‘predator shadows’), and lead to an increase in herbivorous insect biomass, thereby reversing a trophic cascade (McCoy et al. 2008). Such effects of consumer movement across habitat and ecosystem boundaries are relatively unknown, but should be emphasized in future empirical studies to further understand the cascading effects of habitat alteration, especially when these habitats contain organisms with complex life histories.

Terrestrial web-weaving spiders did not respond to reductions in insect emergent biomass, in contrast to recent studies showing that this predatory guild is sensitive to such alterations. Baxter et al. (2004) showed that tetragnathid spiders were reduced along stream reaches with invasive rainbow trout (*Onchorhynchus mykiss*), due to a 35% reduction in emergent insect biomass. In their study, spiders were surveyed 2-4 m from the stream edge. In my study, spiders were surveyed directly above the water. Therefore, insects that crawl out of the water to emerge from the mesocosm walls (i.e., hemimetabolous insects) may have been able to avoid capture in spider webs, and the bulk of insects that spiders trapped were insects that emerge directly from the water surface (i.e., holometabolous insects). Such differences in emergence strategy could explain the lack of correlation between spider abundance and insect emergence, because chironomid emergence was similar across replicates, and chironomids were the most abundant prey taxon in spider webs. These data highlight the importance of prey and consumer functional traits in predicting the impact of subsidies (Marczak et al. 2007).

Fish in this experiment represented two different feeding strategies: benthic invertivore and water-column invertivore. Both fish species had similar overall predation effects, although *C. lutrensis* (water-column feeder) had somewhat more terrestrial insects in their diet than *E. spectabile* (benthic feeder) (30%, <1% of diet, respectively). When both fish species showed significant effects compared to the control, they were always stronger, based on p-values, in the *E. spectabile* treatment. Additionally, while both species reduced predatory insect emergence, only *E. spectabile* caused a reduction strong enough to significantly reduce the proportion of predator biomass in the emergent community.

Fish fed on a variety of prey items, but significant predation effects were limited to a single dragonfly species, *P. flavescens*. It is possible that fish reduced populations of prey taxa other than *P. flavescens*, but that these were masked by increased dragonfly predation on the same prey taxa in the control treatments (*sensu* Thorp and Bergey 1981). Several studies have found a strong predation effect of dragonflies on benthic aquatic community structure (Thorp and Cothran 1984) and abundance (Van Buskirk 1988). Cladocerans were the numerically dominant food item for both fish species, though were likely less important energetically than larger invertebrates given their small size. Cladocerans typically occur at all levels of the water column, though I only sampled invertebrates on the benthos. I do not know whether Cladocerans were present in the water column, or whether their prevalence in fish guts was due to both species feeding on the benthos or due to the presence of cladocerans at all levels of the water column.

Reduced insect emergence in the presence of fish could result from either direct or indirect predation. Direct predation occurs when predators consume prey. Indirect predation occurs when predators alter the behavior of prey by, for example, reducing oviposition in larval habitats with predators. It is likely that direct predation by fish on dragonfly larvae, and not behavioral avoidance by ovipositing dragonflies of mesocosms with fish, was the primary mechanism regulating insect emergence patterns in this study for the following reasons. The minimum development time from oviposition to emergence for *P. flavescens* is approximately 38 days (Suhling et al. 2004). Pools in my experiment were fishless for 17 days prior to fish introductions, meaning that initial oviposition occurred across pools that were equal in their predator threat. Peak dragonfly emergence occurred ~ 43 days after the mesocosms were filled with water, suggesting that the majority of *P. flavescens* emerging were the result of oviposition before fish introductions. While it is possible that dragonflies ovipositing after fish introductions avoided pools with fish (behavioral avoidance), this mechanism would not explain the strong differences seen in dragonfly emergence at the middle of the experiment. An alternative hypothesis is that *P. flavescens* larvae delayed emergence in fish treatments. Delayed emergence of a dragonfly species (*Lestes sponsa*) of up to 7 days was caused by the presence of a predatory fish (*Perca fluviatilis*) in a study by Brodin (2005). *P. flavescens* emergence from the *C. lutrensis* treatment increased in the final four days of the experiment (2.33 individuals/m^2/day vs. 0.12 individuals/m^2/day during the rest of the experiment), but this was due entirely to a single treatment, and emergence was not significantly different across treatments in the final collection period. Thus, direct predation by fish on larval *P. flavescens* was likely the primary mechanism driving emergence patterns, though it should be noted that indirect predation effects could create similar patterns in other systems.

Oviposition in the mesocosms began almost immediately upon filling with water. The source of insects is unknown, but is likely Lake Texoma, a large permanent reservoir located several hundred yards from the mesocosms. The exception is that some insects, snails, and cladocerans were likely introduced during algal inoculations at the beginning of the experiment, though it should be noted that the source of *P. flavescens* is almost certainly aerial, since it does not occur in the algal source habitat. Drift from the upstream riffles and flow rate in the mesocosms is low. The dynamics of colonization and predation therefore likely resemble small, isolated, slow flowing pool-riffle streams, all of which are common but understudied habitats in the central United States (Matthews 1988).

This study shows that predation in a donor habitat alters the magnitude and trophic structure of a prey subsidy entering an adjacent habitat. Subsidies alter food web dynamics in recipient systems from the bottom-up, as an energy subsidy to terrestrial consumers (Nakano and Murakami 2001; Baxter et al. 2004; Marczak et al. 2007) or from the top-down, as consumers themselves in recipient food webs (Knight et al. 2005). The type of impact is necessarily determined by both the magnitude and trophic structure of the subsidy, which in turn is determined by the quality of larval habitat (e.g., predation threat or resource abundance). Empirical studies have largely focused on the role of predation in regulating the biomass of prey subsidies. In this study, predation in the larval aquatic habitat altered both the biomass and trophic structure (proportion of predators) of a prey subsidy, reducing the amount of energy and predators entering the adult terrestrial habitat.

Acknowledgements

I thank my doctoral advisor, William Matthews, and my doctoral committee, Elizabeth Bergey, Bruce Hoagland, Caryn Vaughn, and Gary Wellborn, for advice through all phases of the study. This study would have been impossible without the help of Chelsea Southerland and the staff at the University of Oklahoma Biological Station: Larry Weider, Dave Hambright, Richard Page, Maylon Ward, and Donna Cobb. Funding was provided by a summer research fellowship from the University of Oklahoma Biological Station, M. Blanche Adams and M. Francis Adams Memorial Scholarship in Zoology, and Graduate Student Senate Research Grants. This research complied with current laws of the United States of America. This work was submitted in partial fulfillment of a Ph.D. degree at the University of Oklahoma, Department of Zoology.

Literature Cited

American Public Health Association. 1998. Standard methods for the Examination of Water and Wastewater. 20th edition. American Public Health Association, Washington, DC.

Baxter, C. V., K. D. Fausch, M. Murakami, and P. L. Chapman. 2004. Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. Ecology **85**:2656-2663.

Brodin, T. 2005. Predator effects on behaviour and life-history of prey. Doctoral Dissertation. Umea University, Umea, Sweden.

Dahl, J. and L. A. Greenberg. 1996. Impact on stream benthic prey by benthic vs. drift feeding predators: a meta-analysis. Oikos **77**:177-182.

Dodson, S.I. and D.G. Frey. 2001. Cladocera and other branchiopoda. In J. Thorp and A. Covich (Eds.), Ecology and classification of North American freshwater invertebrates. Second edition: 849-913, Academic Press, CA.

Finlay, J.C. and V.T. Vrendenburg. 2007. Introduced trout sever trophic connections in watersheds: consequences for a declining amphibian. Ecology **88**: 2187-2198.

Gelwick, F. P., W. J. Matthews. 2006. Trophic relations of stream fishes. In F. R. Hauer and G. A. Lamberti (Eds.), Methods in stream ecology. Second edition: 611-635, Academic Press, CA.

Gido, K. B. and W.J. Matthews. 2001. Ecosystem effects of water column minnows in experimental streams. Oecologia **126**:247-253.

Gilinsky, E. 1984. The role of fish predation and spatial heterogeneity in determining benthic community structure. Ecology **65**:455-468.

Hale, G. E. 1963. A comparative study of the food of the shiners *Notropis lutrensis* and *Notropis venustus.* Proceedings of the Oklahoma Academy of Science **43**:125-129.

Hargrave, C. W., R. Ramirez, M. Brooks, M. A. Eggleton, K. Sutherland, R. Deaton, and H. Galbraith. 2006. Indirect food web interactions increase growth of an algivorous stream fish. Freshwater Biology **51**:1901-1910.

Hargrave, C.W. 2009. Effects of fish species richness and assemblage composition on stream ecosystem function. Ecology of Freshwater Fish **18**:24-32.

Knight, T. M., M. W. McCoy, J. M. Chase, K. A. McCoy, and R. D. Holt. 2005. Trophic cascades across ecosystems. Nature **437**:880-883.

Marczak, L. B. and J. S. Richardson. 2007. Spiders and subsidies: results from the riparian zone of a coastal temperate rainforest. Journal of Animal Ecology **76**:687-694.

Marczak, L.B., R.M. Thompson, J.S. Richardson. 2007. Meta-analysis: trophic level, habitat, and productivity shape the food web effects of resource subsidies. Ecology **88**: 140-148.

Martin, F.D. 1984. Diets of four sympatric species of *Etheostoma* (Pisces: Percidae) from southern Indiana: intraspecific and intraspecific multiple comparisons. Environmental Biology of Fishes **11**:113-120.

Matthews, W. J. 1985. Distribution of Midwestern fishes on multivariate environmental gradients, with emphasis on *Notropis lutrensis*. American Midland Naturalist **113**:225-237.

Matthews, W.J. 1988. North American prairie streams as systems for ecological study. Journal of the North American Benthological Society **7**:387-409.

Matthews, W. J., K. B. Gido, G. P. Garrett, F. P. Gelwick, J. G. Stewart, and J. Schaefer. 2006. Modular experimental riffle-pool stream system. Transactions of the American Fisheries Society **135**:1559-1566.

McCoy, M. W., Barfield, M., and Holt, R.D. 2008. Predator shadows: complex life histories as generators of spatially patterned indirect interactions across ecosystems. Oikos **118**:87-100.

Merritt, R. W. and K. W. Cummins. 1996. An introduction to the aquatic insects of North America. Third edition. An introduction to the aquatic insects of North America. Kendall/Hunt, Dubuque, IA.

Miller, M. and S.W. Golladay. 1991. Effects of spates and drying on macroinvertebrate assemblages of an intermittent and a perennial prairie stream. Journal of the North American Benthological Society **15**:670-689.

Miller, R. J. and H. W. Robison. 2004. Fishes of Oklahoma. University of Oklahoma Press, Norman, OK.

Nakano, S. and M. Murakami. 2001. Reciprocal subsidies: Dynamic interdependence between terrestrial and aquatic food webs. Proceedings of the National Academy of Sciences of the United States of America **98**:166-170.

Polis, G. A. and S. D. Hurd. 1996. Linking marine and terrestrial food webs: Allochthonous input from the ocean supports high secondary productivity on small islands and coastal land communities. American Naturalist **147**:396- 423.

Quinn, G. P. and M. J. Keough. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK.

Sabo, J. L., J. L. Bastow, and M. E. Power. 2002. Length-mass relationships for adult aquatic and terrestrial invertebrates in a California watershed. Journal of the North American Benthological Society **21**:336-343.

Sabo, J. L. and M. E. Power. 2002. Numerical response of lizards to aquatic insects and short-term consequences for terrestrial prey. Ecology **83**:3023-3036.

Schreiber, S. and V. H. W. Rudolf. 2008. Crossing habitat boundaries: coupling dynamics of ecosystems through complex life cycles. Ecology Letters **11**:576-587.

Sherratt, T.N. and I.F. Harvey. 1989. Predation by larvae of Pantala flavescens (Odonata) on tadpoles of Phyllomedusa trinitatis and Physalaemus pustulosus: the influence of absolute and relative density of prey on predator choice. Oikos **56**: 170-176.

Sih, A. 1980. Optimal behavior: can foragers balance two conflicting demands? Science **210**:1041-1043.

Suhling, F., K. Schenk, T. Padeffke, and A. Martens. 2004. A field study of larval development in a dragonfly assemblage in African desert ponds (Odonata). Hydrobiologia **528**:75-85.

Thorp, J. H. and E. A. Bergey. 1981. Field experiments on responses of a freshwater benthic macroinvertebrate community to vertebrate predators. Ecology **62**:365-375.

Thorp, J. H. and M. L. Cothran. 1984. Regulation of freshwater community structure at multiple intensities. Ecology **65**:1546-1555.

Triplehorn, C. A. and N.F. Johnson. 2005. Borror and DeLong's Introduction to the Study of Insects. 7th edition. Thomson Brooks/Cole, Belmont, CA.

Van Buskirk, J. 1988. Interactive effects of dragonfly predation in experimental pond communities. Ecology **69**:857-867.

Werner, E. E. and J. F. Gilliam. 1984. The ontogenetic niche and species interactions in size structured populations. Annual Review of Ecology and Systematics **15**:393-425.

Williams, D.D., L.G. Ambrose, and L.N. Browning. 1995. Trophic dynamics of two sympatric species of riparian spiders (Araneae: Tetragnathidae). Canadian Journal of Zoology **73**:1545-1553.

Tables

TABLE 1. Timeline showing dates of experimental setup and sampling of aquatic and terrestrial food web components from stream mesocosms at the University of Oklahoma Biological Station, near Lake Texoma, Oklahoma, USA.

Date in 2007	Day of experiment	Event
25 May		all mesocosms filled with water and inoculated with algae and small invertebrates; aerial colonization begins
29 May		periphyton substrates placed in each mesocosm
9 June		first spider counts
11 June	0	all fish collected and placed in mesocosms; beginning of experiment
18 June	7	first fish observations
19 June	8	first benthos sample
25 June	14	second fish observations
26 June	15	collected periphyton; first odonate counts; set emergence traps
27 June	16	measured temperature and dissolved oxygen
28 June	17	first emergence collection
2 July	21	second odonate counts; second spider counts
4 July	23	set emergence traps
7 July	26	second emergence collection; second benthos sample; third odonate counts
10 July	29	third fish observations; first macrophyte measurements
16 July	35	measured temperature and dissolved oxygen; fourth odonate counts; third spider counts
19 July	38	set emergence traps
22 July	41	fourth spider counts: second macrophyte measurements
23 July	42	third emergence collection; third benthos sample; fifth odonate counts; fish diet analysis; second periphyton collection (lost); end of experiment

Table 2. Repeated-measures ANOVA and Tukey's hsd post hoc comparisons of selected response variables.

Response	Source and post hoc	Hypothesis df	Error df	Effect size	*P*
Emergence					
Total abundance	treatment	2	15		0.549
Total biomass	treatment	2	15		0.018
	E. spectabile vs. control				
	C. lutrensis vs. control			0.91	0.040
Total predators, biomass	time	1.57	23.54†		0.025
	treatment	2	15		0.010
	treatment × time	1.57	23.54†		0.025
	E. spectabile vs. control			0.97	0.011
	C. lutrensis vs. control			0.69	0.041
Trophic structure, predator abundance (%)	treatment	2	13		0.222
Trophic structure, predator biomass (%)	time	2	22		0.004
	treatment	2	12		0.036
	E. spectabile vs. control			0.40	0.049
	C. lutrensis vs. control			0.59	0.064
Visual dragonfly counts	time	4	48		0.024
	treatment	2	12		0.003
	E. spectabile vs. control			1.70	0.006
	C. lutrensis vs. control			1.70	0.004
Chlorophyll *a*, day 16	treatment‡	2	17		0.360
Spiders					
Abundance (no./mesocosm)	time	2	30		0.001
	treatment	2	15		0.427

Notes: Units are abundance (no.$\cdot$m$^{-2}\cdot$d^{-1}), biomass (mg$\cdot$m$^{-2}\cdot$d^{-1}), trophic structure (proportion of predators by abundance biomass). Effect size is the difference between mean treatments/standard deviation. The predatory fish are *Etheostoma spectabile* and *Cyprinella lutrensis*. Time and treatment × time interactions are excluded unless significant.

† Degrees of freedom represent Greenhouse-Geisser adjustments.

‡ One-way ANOVA.

Figures

Figure 1. Schematic of a single mesocosm at the University of Oklahoma Biological Station. Shade cloth not shown.

Figure 2. Fish effects on emerging insect assemblages and spider response. (a) emergence biomass of adult aquatic insects over three sample dates, (b) proportion of predator biomass in emergent insect assemblage over three sample dates, (c) counts of occupied spider webs above aquatic mesocosms. Data are means ± SE.

Figure 3. Potential roles of alternative adult aquatic communities in terrestrial food webs created by different predator regimes in aquatic habitats. Different sizes of pie charts reflect differences in total biomass of emergent insects seen in this study. Different arrow sizes reflect the magnitude of the impact of each community predicted from this study [(top-down = consumption of terrestrial prey (bee); bottom-up = energy source for terrestrial consumers (spider)].

Figure 1

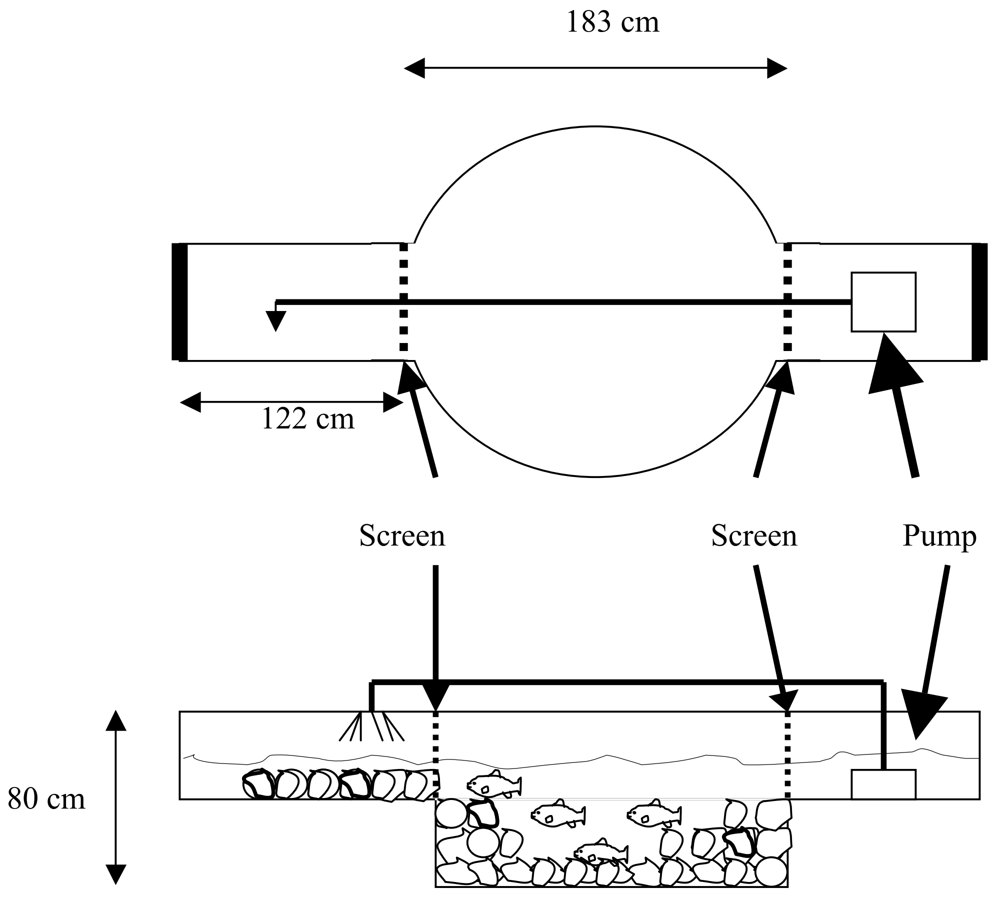


Figure 2

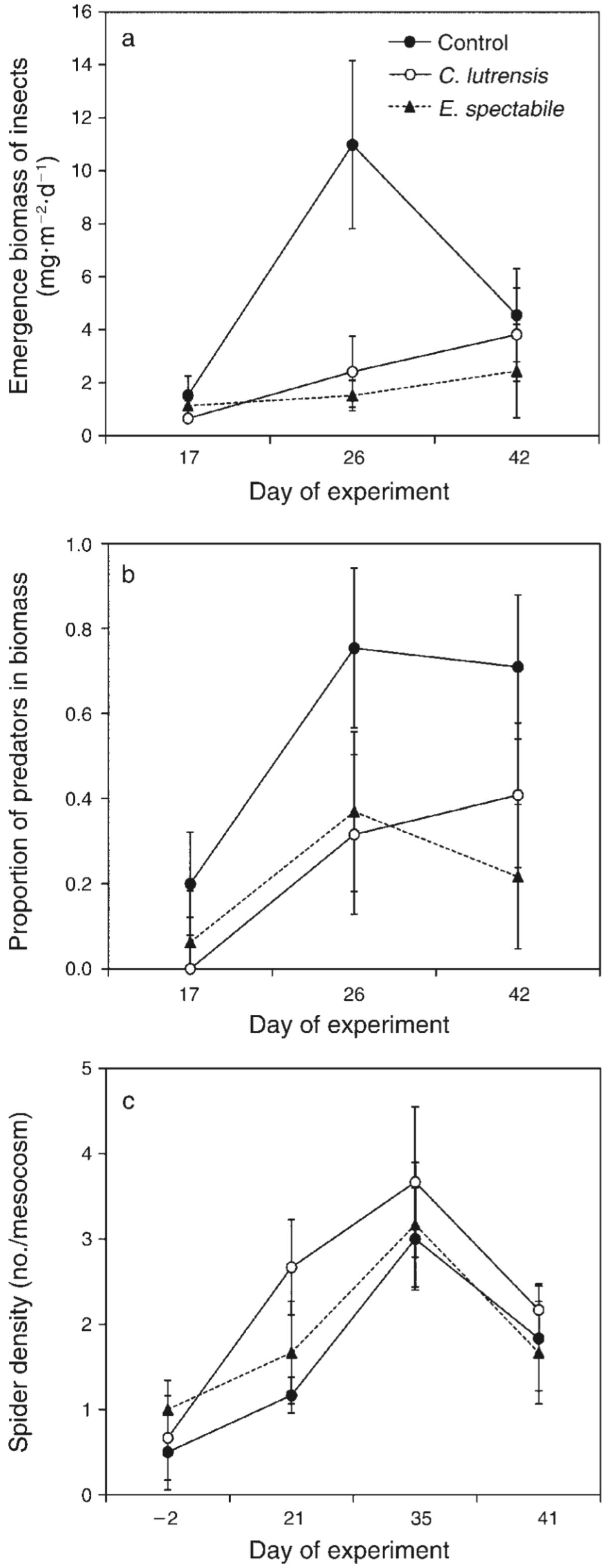
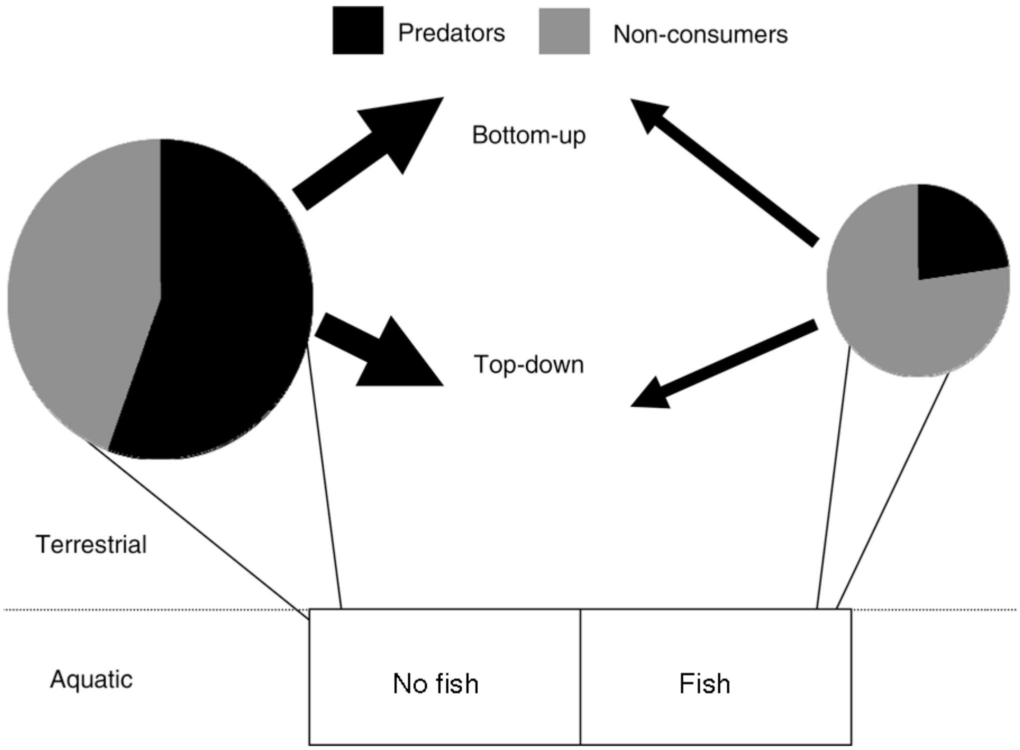

a
Control
C. lutrensis
E. spectabile
Emergence biomass of insects (mg·m⁻²·d⁻¹)
0
2
4
6
8
10
12
14
16
17
26
42
Day of experiment
b
Proportion of predators in biomass
0.0
0.2
0.4
0.6
0.8
1.0
17
26
42
Day of experiment
c
Spider density (no./mesocosm)
0
1
2
3
4
5
−2
21
35
41
Day of experiment

Figure 3


Predators
Non-consumers
Bottom-up
Top-down
Terrestrial
Aquatic
No fish
Fish

CHAPTER 3: BIODIVERSITY-ECOSYSTEM FLUX: AQUATIC PREDATOR RICHNESS ALTERS THE DISTRIBUTION OF A TERRESTRIAL CONSUMER

JEFF SCOTT WESNER

Biological Station and Department of Zoology, University of Oklahoma, Norman, OK, USA.

Abstract

Food webs in physically separate ecosystems are often connected through spatial resource subsidies. As a result the ecological effects of biodiversity loss in one ecosystem may cascade to adjacent ecosystems. I tested the hypothesis that predator diversity in aquatic food webs alters the flux of organisms into terrestrial food webs as measured by the emergence of insects from aquatic mesocosms to terrestrial habitats. I also measured the response of terrestrial insectivorous spiders to changes in insect emergence. Fish species with complementary habitat domains and a shared prey guild were the predators (*Etheostoma spectabile, Cyprinella lutrensis,* and *Gambusia affinis*) in a substitutable design using all possible combinations of fish. Total insect emergence biomass from pools with high fish richness was reduced by nearly 40%. Reduced emergence biomass caused a shift in the abundance of terrestrial spiders (Tetragnathidae), which were nearly four times less abundant above pools with high fish richness than pools without fish. For the overall prey assemblage (total emergence biomass and trophic structure), predation effects in polyculture were the average of fish performance in monoculture, suggesting redundancy. For common prey taxa and tetragnathid spiders, fish effects were generally synergistic in the high richness treatments, but redundant in the two species treatments. This study demonstrates that predator diversity effects are not limited to the habitat of the predator, but can propagate across habitat boundaries to adjacent systems.

Introduction

Two major goals of ecology are to understand how biodiversity affects ecosystem functioning (Reiss et al. 2009) and how physically separate ecosystems are coupled spatially by the movement of energy, material, and organisms (Polis and Hurd 1996; Holt and Loreau 2001). Species diversity enhances many ecosystem functions, such as biomass production, nutrient cycling, and decomposition (Reiss et al. 2009; Srivastava et al. 2009). These effects have been demonstrated in a variety of individual ecosystems: grassland (Schmitz and Sokol-Hessner 2002), freshwater (Nilsson et al. 2008; Hargrave 2009), and marine (Griffin et al. 2008). Although studies commonly focus on dynamics within a single ecosystem, ecosystems are rarely closed to external subsidies (Holt and Loreau 2001). The effects of biodiversity loss may therefore cascade across ecosystem boundaries through the emigration of mobile organisms that subsidize adjacent food webs. Knowledge of these effects in biodiversity studies has largely been neglected, but is critically important to understanding and mitigating the consequences of species loss.

Biodiversity studies historically have focused on primary producers, though recent studies have shown that predator diversity may have the strongest effects on ecosystem properties (Reiss et al. 2009). The effects of predator diversity on ecosystem functioning vary along a spectrum from negative to positive depending on the nature of interspecific predator interactions (Schmitz 2007). Interference interactions or intraguild predation can reduce predator consumption in polyculture, whereas facilitation or niche complementarity can enhance predation effects in polyculture relative to monoculture. Alternatively, predation effects in polyculture may simply be the average of individual species effects, resulting in redundancy (linearity). Predicting effects of multiple predators has been the focus of recent empirical and theoretical studies (Schmitz 2007, 2009). These studies demonstrate the usefulness of incorporating predator functional traits in *a priori* predictions of the direction of predator diversity effects (Schmitz 2007; Reiss et al. 2009). For example, Schmitz (2008) showed that the direction of cascading ecosystem effects caused by predatory spiders in a grassland ecosystem depended on their hunting mode: active versus sit-and-wait. Making accurate predictions of the consequences of predator species loss is especially important because species at higher trophic levels may be at greater risk of extinction than species at lower trophic levels (Pauly et al. 1998; Duffy 2003). For example, nearly 40% of freshwater fish species in North America are at risk of extinction, or are already extinct (Jelks et al. 2008). Many of these are predators that consume aquatic prey.

I examined the effect of predator richness in aquatic habitats on the flux of prey to terrestrial habitats. Prey consisted largely of organisms with complex life histories, such as insects and amphibians, which cross habitat boundaries during development, coupling energy flow among aquatic and terrestrial ecosystems as both prey (Sabo and Power 2002; Baxter et al. 2004) and consumers (Knight et al. 2005). Predation effects on one stage of this life cycle can therefore cascade across ecosystems, altering the trophic structure (Wesner *in press*) and amount of energy (Baxter et al. 2004; Finlay and Vredenburg 2007) available to consumers in recipient systems. Here, I test the hypothesis that shifts in aquatic predator richness cascade to terrestrial food webs by altering the trophic structure and biomass of a terrestrial prey subsidy, which in turn alters the distribution of a terrestrial consumer that exploits adult aquatic insect prey (web-weaving spiders). I further used an *a priori* assigned functional trait of fish (habitat domain) to test the hypothesis that combinations of predators with non-overlapping habitat domains would show redundancy or synergism, but not antagonism, due to the reduced possibility of negative interspecific interactions.

Methods

Mesocosms

I manipulated fish species richness in a 57 day experiment using large outdoor stream mesocosms at the University of Oklahoma Biological Station (UOBS), Kingston, OK, USA. Mesocosms consisted of a riffle flowing into and out of a single pool (riffle unit: 122 cm length, 43 cm depth; pool unit: 183 cm diameter, 80 cm depth) and are described in detail in Matthews et al. (2006). Partial shade was provided with shade cloths suspended approximately 150 cm above each mesocosm. Units were never completely covered during the experiment, allowing continuous oviposition by frogs and aerial insects, which were abundant. Substrate in the mesocosms was a mixture of cobble and gravel taken from Brier Creek (Marshall Co., OK, USA), and was homogenized among pools prior to filling with water. Forty units were filled with well-water from a public supply (Marshall County Water Corporation) on 16 May 2008. On the same day each unit was inoculated with a slurry of algae (1L) scraped from rocks from Brier Creek. Filamentous algae (*Oedogonium* spp.) and macrophytes (*Chara* spp.) were abundant after several weeks and provided additional structural refuge, and their combined height and coverage area was equal across treatments at the end of the experiment (one-way ANOVA, $F_{10,38} = 0.255$, $P = 0.987$). Units were maintained without fish for 19 days to allow colonization by tadpoles and aerial invertebrates. The primary route of colonization is oviposition by insects and amphibians, though small snails and other invertebrates were likely also introduced with the algal inoculation. Plastic mesh screens (0.32 cm diameter openings) at the upper and lower attachment of each riffle restricted fish to pools. Flow was initiated on 18 May with submersible pumps (2500 L/h) (Little Giant Pump Co., Oklahoma City, OK). Temperature, dissolved oxygen, conductivity and flow in these mesocosms are commensurate with values of local streams (Matthews et al. 2006; Wesner *in press*).

Design

I manipulated richness (0, 1, 2 or 3 species) of three predatory fish species with complementary habitat domains: benthic (*Etheostoma spectabile*, orangethroat darter), water-column (*Cyprinella lutrensis*, red shiner), and surface (*Gambusia affinis*, western mosquitofish). Each is common and widespread throughout the central United States and they often co-occur. Species share a common prey guild (invertebrates), but differ in their habitat use. The darter (*E. spectabile*) feeds on the benthos, primarily on insects: chironomids, stoneflies, mayflies (Martin 1984; Miller and Robison 2004). The minnow (*C. lutrensis*) feeds in the water-column, but occasionally feeds in benthic and surface habitats (Hale 1963). The mosquitofish (*G. affinis*) feeds on the surface and includes both aquatic and terrestrial insects in their diet (Miller and Robison 2004). Fish of similar size were collected from nearby streams by seining and transported to holding tanks at UOBS no more than 48 hours before the start of the experiment.

Treatments consisted of each possible fish combination (one, two, or three fish species) plus a fishless control (eight total treatments). Five replicates of each treatment were distributed randomly among 40 pools on 4 June. I used a substitutable design, holding total fish density (n = 24) constant among each predator treatment. Therefore, multiple predator treatments contained 12 or 8 individuals of each species in the 2 or 3 species treatments, respectively. Single species treatments contained 24 individuals. Total fish density (9.13 fish/m^2) is commensurate with the natural density of each species. One replicate of the *C. lutrensis* treatment was lost due to a fish kill on day 33 and these data are excluded from analyses.

I tested for effects of predator richness on insect emergence (biomass and trophic structure), benthic prey biomass, terrestrial spider abundance, and aquatic primary production (chlorophyll *a*). Each response variable was measured several times during the experiment from each mesocosm (Appendix A). I measured insect emergence using a combination of emergence traps and exuviae counts. Floating emergence traps were deployed continuously for 2-4 days during each of four sampling periods (Appendix A). Traps with a galvanized steel frame and a collection area of 0.16 m^2 are described in detail in Wesner (in press). Upon collection, insects were transferred to individually labeled vials and stored in 70% ethanol. All insects were identified to family using Triplehorn and Johnson (2005) and measured for length to the nearest 0.1 mm for regression estimation of biomass (Sabo et al. 2002). Adult insects were classified as predators or non-consumers according to descriptions of family-level adult feeding roles in Triplehorn and Johnson (2005). Insects that crawl out of the water to emerge, such as odonates, can avoid emergence traps, but are easily estimated by counting discarded exuviae. I collected exuviae (all odonates) daily from the perimeter of each mesocosm.

Odonate adult biomass was estimated based on published regression equations using exuviae head width, measured to the nearest 0.1 mm (Sabo et al. 2002).

I sampled benthic prey (macroinvertebrates and tadpoles) twice during the experiment using a steel cylinder core (0.031 m^2; Appendix A). The cylinder was driven approximately 5 centimeters into the substrate and the contents scooped into a sieve (500 μm), drained, and preserved in 70% ethanol. On each sample date three random samples were taken from each pool and combined into a single sample (total area sampled = 0.093 m^2). Due to the large number of macroinvertebrates in samples, 30% of each sample by weight was sorted to order or family using Merritt and Cummins (1996). Tadpole abundance was visually estimated on several days by counting tadpoles on mesocosm walls.

I measured the response of spiders to insect emergence by counting occupied spider webs directly above each pool on several nights (Appendix A). Spiders were categorized as horizontal (Tetragnathidae) or vertical orb-weavers. Webs were usually attached to the emergence trap cages, edges of the mesocosm, and/or a pvc pipe above each pool.

I sampled periphyton using ten porous silica discs (2.5 cm diameter), placed randomly on the substrate in each pool 17 days before fish were introduced, to test for differences in primary production and evidence of trophic cascades. Three days before the end of the experiment I collected four discs from each mesocosm and froze them overnight to lyse cells. Discs were extracted in 90% acetone, and Chlorophyll *a* was estimated spectrophotometrically with a correction for phaeophytin (American Public Health Association, 1998).

To ensure fish occupied their *a priori* assigned habitat domains, I observed fish from a single randomly selected replicate from each fish treatment three times over the course of the experiment (Appendix A). Observations lasted 5 minutes during early afternoon following methods described in Hargrave (2009). Notes were taken at 30-second intervals on the number of fish visible and their position in the pool: benthic (lower 1/3 of water column), water column (middle 1/3 of water column), or surface (upper 1/3 of water column). Feeding attempts and their location also were noted. At the end of the experiment fish wet weight was converted to dry weight assuming 70% water content (Hoar and Randall 1969).

Data analysis

I tested the null hypothesis of no differences between treatments using a linear mixed model (PROC MIXED in SAS 9.1, Cary, NC), with treatment, time, and treatment x time as fixed effects. The response variables were benthic insect biomass, insect emergence biomass, proportion of predators in emerging insect assemblage, emergence biomass of common prey taxa, and spider abundance above mesocosms. Planned contrasts among fish treatments and the control used the least square means procedure and were adjusted for type I error using the sequential Bonferroni method (Rice 1989). Linear regression tested the relationship between fish species richness and mean insect emergence (biomass and trophic structure) and mean spider abundance. Chlorophyll *a* and mean counts of *Hyla* tadpoles on the meocosm walls were analyzed using a one-way ANOVA with fish treatment as a fixed factor in SPSS 16.0 for Macintosh (SPSS, Chicago, Ill). Multiple comparisons following ANOVA used the Tukey-Kramer post-hoc test (Quinn and Keough 2002). The Brown-Forsyth correction was applied when error variances of univariate ANOVA's were unequal according to Levene's test. All data were natural log transformed or arcsine square-root transformed (proportions) to improve normality (Quinn and Keough 2002). Shifts in habitat use by fish in single versus multiple species treatments was assessed by comparing the proportion of fish feeding in and occupying *a priori* assigned fish habitats (benthic, water-column, surface) using a Kruskal-Wallis test.

To test for linearity in multiple predator effects, I compared observed values of a response variable to values predicted from fish species performance in monoculture using a paired t-test (Vaughn et al. 2007). Predicted values were calculated by first obtaining a mean value per mg of dry weight of fish for each response variable in each of the three single species treatments (e.g., mean emergence biomass/m^2/day/mg dry weight of *E. spectabile*). This value was then multiplied by the dry mass of each species in the two and three species replicates and summed to obtain a predicted value for each mesocosm. A significant t-test indicates non-linearity where observed values are stronger (syngergism) or weaker (antagonism) than expected based on individual fish species performances. To test for species identity effects, the relationship between response variables and the relative dry mass of individual fish species was examined using linear regression. A significant positive relationship between a response variable and relative dry mass of one fish species, but not others, would indicate that a single fish species is driving differences among treatments, and not species richness per se.

Results

Insect emergence biomass varied significantly among treatments (Figure 1a; $F_{7,119} = 3.01$, *P* = 0.006), and was reduced nearly 65% in the high richness treatment compared to the control (Figure 1a; t = -3.35, *P* = 0.0011). Single and two species treatments reduced insect emergence nearly 25% on average relative to the control, but this difference was not significant (*P* > 0.05). Emergence patterns were largely driven by interactions between fish and *Pantala flavescens*, a dragonfly species that made up 31% of total insect emergence biomass. Fish reduced biomass of *P. flavescens* emergence from all treatments compared to the control ($F_{7,119} = 3.01$, *P* = 0.006, all post-hoc comparisons with control, *P* < 0.005). Chironomids were the most common emergent insect and were significantly reduced in the high fish richness treatment compared to the control (Figure 1c; t = -4.06, *P* = 0.0001). Fish had no significant effects on emergence of any other taxon (n = 18 total emerging insect taxa).

Trophic structure, defined as the relative biomass of predators in the emergent insect assemblage, increased over time following the onset of dragonfly emergence ($F_{7,109} = 11.15$, *P* < 0.0001), but did not vary significantly among treatments (treatment: $F_{7,109} = 1.54$, *P* = 0.1629; treatment x time: $F_{21,109} = 1.44$, *P* = 0.1169). However, planned contrasts revealed a significant reduction of the relative biomass of predators emerging from the *C. lutrensis* treatment relative to the control (Figure 1b; t = 3.06, P = 0.0028).

Abundance of horizontal orb-weaving spiders (Tetragnathidae) varied significantly among treatments (Figure 1d; $F_{7,96} = 2.28$, *P* = 0.0340), but not vertical web-weaving spiders ($F_{7,32} = 0.44$, *P* = 0.8676). Tetragnathid abundance above the three fish species treatment was only about one-fourth of that above the control, a difference that approached significance following the sequential Bonferroni adjustment (Figure 1d; t = -2.48, $P = 0.015$ at $\alpha_{adj} = 0.0071$). Tetragnathids appeared to capture primarily adult aquatic insects (mostly chironomids). Linear regression revealed a positive relationship between mean insect emergence biomass in July, when spiders were counted, and tetragnathid abundance ($r^2 = 0.179$, $P = 0.007$).

Tadpole abundance on the mesocosm walls was significantly reduced compared to the control in all fish treatments (one-way ANOVA: $F_{7,32} = 7.108$, $P < 0.0001$; Tukey-Kramer, $P \leq 0.014$ for all post-hoc comparisons between fish treatments and control). There was no variation among treatments for biomass of total benthic prey (invertebrates + tadpoles: $F_{7,64} = 0.96$, $P = 0.4655$), benthic insects ($F_{7,64} = 1.37$, $P = 0.2319$), or benthic dragonflies ($F_{7,64} = 0.51$, $P = 0.8212$). Chlorophyll *a* was similar among treatments (one-way ANOVA using Brown-Forsyth correction: $F_{\text{Brown-Forsyth}, 7,12.5} = 0.736$, $P = 0.646$). Linear regression between the natural log of chlorphyll *a* and the natural log of fish dry mass was not significant ($r^2_{adj} = 0.036$, $P = 0.529$), suggesting fish did not enhance primary production through nutrient recycling.

Fish observations confirmed their *a priori* assigned habitat domains. *Etheostoma spectabile* were rarely seen (n = 10 total observations) due to algal growth, but all observations of this species were on the substrate. *Cyprinella lutrensis* was observed in the water column 97% of the time, and *G. affinis* was observed near the surface 80% of the time. Fish also fed within habitats, with at least 100, 72, and 87% of observed feeding attempts occurring in the respective habitats for *E. spectabile*, *C. lutrensis*, and *G. affinis*, respectively. These data were also consistent across fish richness treatments according to Kruskal-Wallis test (percent observed: $\chi^2 = 1.249$, $P = 0.535$; percent feeding: $\chi^2 = 0.348$, $P = 0.840$), suggesting fish did not alter habitat use or feeding attempts in response to the presence of other fish species.

Linear regressions comparing response variables (total insect emergence biomass, trophic structure and tetragnathids spider abundance) across species richness treatments were significant only when the fishless control was included in the analysis (insect emergence biomass: $r^2 = 0.154$, $P = 0.012$; insect trophic structure: $r^2 = 0.106$, $P = 0.043$; tetragnathids abundance: $r^2 = 0.038$, $P = 0.230$). When the fishless control was excluded, linear relationships were not significant (insect emergence biomass: $r^2 = 0.079$, $P = 0.107$; insect trophic structure: $r^2 = 0.006$, $P = 0.651$; tetragnathid abundance: $r^2 = 0.030$, $P = 0.330$). This is perhaps not surprising, given that significant predation effects were largely limited to treatments with high fish species richness, but were minimal in treatments with low species richness. Therefore the relationship between species richness and response variables were largely curvilinear, rather than linear.

T-tests comparing observed total insect biomass and the proportion of predators emerging from polyculture pools with values predicted based on monoculture performance of individual fish species were not significant for any fish species combination (Table 1). For *P. flavescens* emergence biomass, tetragnathid abundance, and *Hyla sp.* abundance on mesocosm walls reductions in the high fish richness treatment was stronger than predicted from monoculture performance (Table 1). In other words, fish in treatments with high species richness had non-linear (synergistic) effects on these taxa, which could not be predicted from the performance of individual fish species. This result was true only for the three fish species treatments, with the exception of a synergistic effect on tadpoles in the *C. lutrensis* + *G. affinis* treatment (Table 1). In contrast to *P. flavescens* and *Hyla sp.*, multiple predator effects on chironomid emergence biomass were linear (Table 1), suggesting redundancy on this prey taxon. Linear regression comparing response variables and the relative biomass of a given fish species was never significant (*P* > 0.05), suggesting that enhanced effects in the high richness treatment were due to species richness and not species identity.

Discussion

In this study, aquatic predator richness altered the flux of organisms to terrestrial habitats, causing a shift in the distribution of a terrestrial consumer. Insect emergence from pools with high fish richness was reduced by 39% compared to pools without fish. In contrast, insect emergence biomass from pools with only one or two fish species was reduced by 19% compared to pools without fish. This effect cascaded to a terrestrial consumer, as tetragnathid spiders shifted their distribution away from pools with high fish richness, where emergence of aquatic insects was low. This study shows that the effects of predator diversity are not limited to the habitat of the predators, but cascade across habitat boundaries to affect consumers in adjacent systems.

One promising future avenue in biodiversity studies is the potential to use species traits to make *a priori* predictions of diversity outcomes (Schmitz 2008; Spooner and Vaughn 2009). This is important, because previous diversity experiments demonstrate a spectrum of effects ranging from antagonistic to redundant to synergistic, often in the same system, making it difficult for resource personnel to make management decisions based on primary literature (Bruno and Cardinale 2008). Recent theoretical (Schmitz 2007) and empirical (Schmitz 2008) evidence suggests that diverse outcomes of predator diversity studies may be linked to the hunting mode and habitat overlap of predators. In this study, predatory fish with identical hunting modes (active) and complementary habitat domains had synergistic effects only on individual prey species, but not on the overall prey community. At the community level (total insect emergence and trophic structure) multiple predator effects were linear, regardless of the number of fish species present, suggesting predation in multiple predator treatments was simply the average of individual species performances. When analyses were restricted to individual prey species, multiple predator effects were synergistic when predator richness was high for *P. flavescens* emergence and *Hyla* abundance. Emergence or abundance of these species in pools with high predator richness was lower than predicted based on average fish species performance in monoculture. In contrast, emergence of chironomid midges from pools with multiple fish species was simply the average of fish species performance in monoculture. Additionally, a synergistic multiple predator effect on tetragnathid spider density showed that indirect multiple predator effects in one habitat can cascade across habitat boundaries.

One possible explanation for these differences in prey susceptibility is that multiple predator effects are mediated by differences in prey escape behaviors. Larval dragonflies and tadpoles are active feeders during development and were visible throughout the water-column during the study. This mobility makes them vulnerable to predators, likely resulting in non-linear predation effects through facilitation. For example, when all three fish species were present, all microhabitats were occupied (benthic, water-column, surface). As a result, dragonflies and tadpoles attempting to evade *E. spectabile* by moving higher in the water column were then vulnerable to *C.*

lutrensis and *G. affinis*, and vice versa. This lack of safe refuge may have enhanced the effect of predation relative to treatments with only one or two fish species, in which at least one microhabitat was empty. Chironomids spend the majority of their larval life-cycle on the benthos, and often evade predation by burrowing in the benthos (Ball and Baker 1996), making them generally invulnerable to non-benthic predators until they enter the water column to emerge as adults. A further complication is that chironomids typically emerge at night, which reduces their vulnerability to visual fish predators. Thus, facilitation effects are unlikely for chironomids, and linear effects seen in this study are more likely.

Fish effects were strongest on tadpoles and dragonflies, both of which couple food web dynamics between aquatic and terrestrial ecosystems as they develop (Knight et al. 2005; McCoy et al. 2008). Fish reduced dragonfly emergence biomass, which drove a reduction in total insect emergence biomass. I did not estimate frog emergence, so it is unknown whether reductions in visual tadpole counts were due to fish consumption, thereby reducing frog emergence, or whether this simply represented a behavioral shift of tadpoles from mesocosm walls to benthic habitats where they were less visible. It is likely that both mechanisms operated simultaneously, as I observed *C. lutrensis* swarm-feeding on tadpoles in a non-experimental pool. As a result, the actual reduction in total emergence biomass (amphibians plus insects) from aquatic to terrestrial habitats is likely greater than that reflected from insects alone, given the large size of frogs relative to most adult insects. These results complement previous findings that changes in frog emergence from ponds create a patchwork mosaic of trophic cascade potentials, because frog abundance, and therefore consumption, is greater around ponds with increased primary production (McCoy et al. 2008). Here, I show that shifts in aquatic predator richness potentially alter frog emergence; a result that should be further tested to determine the potential for trophic cascades in terrestrial food webs across a gradient of aquatic predator diversity.

The link between emerging aquatic insects and terrestrial consumers has been demonstrated in a variety of natural settings from temperate (Nakano and Murakami 2001; Sabo and Power 2002; Baxter et al. 2004) to tropical (Marczak and Richardson 2007). Fish are often the top predators in many aquatic habitats, and can reduce adult aquatic insect biomass substantially in natural streams (Baxter et al. 2004; Wesner *unpublished data*) and ponds (Knight et al. 2005). Therefore, because the effects shown here using mesocosms are reflected in part in studies in natural habitats, they are unlikely to simply be an artifact of mesocosms, but instead highlight the potential for losses in aquatic biodiversity to affect food webs beyond the boundary of the aquatic habitat. This result is especially important given the current biodiversity crisis for freshwater fishes (Jelks et al. 2008; Magurran 2009).

The rich literature of biodiversity and ecosystem function demonstrates that changes in plant (Tilman et al. 1996; Hooper and Vitousek 1997) and consumer diversity (Bruno and Cardinale 2008; Schmitz et al. 2007; Nillson et al. 2008) can substantially alter ecosystem functioning. This study explicitly demonstrates that diversity effects in one system can cascade across habitat boundaries to adjacent systems. Specifically, aquatic habitats with high fish richness altered the flux of organisms across habitat boundaries, causing a shift in the distribution of a terrestrial consumer that is subsidized by this flux. Freshwater habitats are among the most altered in the world (Richter et al.

1997). Approximately 40% of all freshwater fish in North America, many of which are predators, are at risk of extinction or are already extinct (Jelks et al. 2008). This study shows that human alterations to fish diversity may have consequences that cascade beyond the boundaries of the aquatic ecosystem.

Acknowledgements

I am grateful to the University of Oklahoma Biological Station (UOBS) for support of this research: L. Weider, D. Cobb, and R. Page. C. Southerland, R. Zamor, and J. White provided field and lab assistance. W. Matthews, L. Bergey, C. Vaughn, G. Wellborn, and B. Hoagland provided valuable advice during the entire experiment. This work was funded by a UOBS Summer Research Fellowship, and by an Adams Scholarship from the Department of Zoology, University of Oklahoma. This work was submitted in partial fulfillment of a Ph.D. degree from the University of Oklahoma.

Literature Cited

American Public Health Association. 1998. Standard methods for the Examination of Water and Wastewater. 20th edition. American Public Health Association, Washington, DC.

Ball, S. L. and R. L. Baker. 1996. Predator-induced life history changes: Antipredator behavior costs or facultative life history shifts? Ecology **77**:1116-1124.

Baxter, C. V., K. D. Fausch, M. Murakami, and P. L. Chapman. 2004. Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. Ecology **85**:2656-2663.

Bruno, J. F. and B. J. Cardinale. 2008. Cascading effects of predator richness. Frontiers in Ecology and the Environment **6**:539-546.

Burdon, F. J. and J. S. Harding. 2008. The linkage between riparian predators and aquatic insects across a stream-resource spectrum. Freshwater Biology **53**:330-346.

Cardinale, B. J., A. R. Ives, and P. Inchausti. 2004. Effects of species diversity on the primary productivity of ecosystems: extending our spatial and temporal scales of inference. Oikos **104**:437-450.

Duffy, J. E. 2003. Biodiversity loss, trophic skew and ecosystem functioning. Ecology Letters **6**:680-687.

Hoar, W.S. and D.J. Randall. 1969. Fish physiology: excretion, ionic regulation, and metabolism. First edition. Academic Press, Inc., New York, NY.

Finlay, J.C., and V.T. Vrendenburg. 2007. Introduced trout sever trophic connections in watersheds: consequences for a declining amphibian. Ecology **88**: 2187-2198.

Gelwick, F. P., and W. J. Matthews. 2006. Trophic relations of stream fishes. In F. R. Hauer and G. A. Lamberti (Eds.), Methods in stream ecology. Second edition: 611-635, Academic Press, CA.

Griffin, J. N., K. L. De la Haye, S. J. Hawkins, R. C. Thompson, and S. R. Jenkins. 2008. Predator diversity and ecosystem functioning: Density modifies the effect of resource partitioning. Ecology **89**:298-305.

Hale, G. E. 1963. A comparative study of the food of the shiners *Notropis lutrensis* and *Notropis venustus.* Proceedings of the Oklahoma Academy of Science **43**:125-129.

Hargrave, C.W. 2009. Effects of fish species richness and assemblage composition on stream ecosystem function. Ecology of Freshwater Fish **18**:24-32.

Holt, R. D. and M. Loreau. 2001. Biodiversity and ecosystem functioning: The role of trophic interactions and the importance of system openness. The functional consequences of biodiversity: empirical progress and theoretical extensions Princeton University Press, Princeton, NJ, USA, pp 246-262.

Hooper, D. U. and P. M. Vitousek. 1997. The effects of plant composition and diversity on ecosystem processes. Science **277**:1302-1305.

Jelks, H.L., S.J. Walsh, N.M. Burkhead, S. Contreras-Balderas, E. Díaz-Pardo, D.A. Hendrickson, J. Lyons, N.E. Mandrak, F. McCormick, J.S. Nelson, S.P. Platania, B.A. Porter, C.B. Renaud, J.J. Schmitter-Soto, E.B. Taylor, and M.L. Warren, Jr. 2008. Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries **33**:372-389.

Knight, T. M., M. W. McCoy, J. M. Chase, K. A. McCoy, and R. D. Holt. 2005. Trophic cascades across ecosystems. Nature **437**:880-883.

Magurran, A. E. 2009. Threats to freshwater fish. Science **325**:1215-1216.

Marczak, L. B. and J. S. Richardson. 2007. Spiders and subsidies: results from the riparian zone of a coastal temperate rainforest. Journal of Animal Ecology **76**:687-694.

Martin, F.D. 1984. Diets of four sympatric species of *Etheostoma* (Pisces: Percidae) from southern Indiana: intraspecific and intraspecific multiple comparisons. Environmental Biology of Fishes **11**:113-120.

Matthews, W. J., K. B. Gido, G. P. Garrett, F. P. Gelwick, J. G. Stewart, and J. Schaefer. 2006. Modular experimental riffle-pool stream system. Transactions of the American Fisheries Society **135**:1559-1566.

McCoy, M. W., M. Barfield, and R.D. Holt. 2008. Predator shadows: complex life histories as generators of spatially patterned indirect interactions across ecosystems. Oikos **118**:87-100.

Merritt, R. W. and K. W. Cummins. 1996. An introduction to the aquatic insects of North America. Third edition. Kendall/Hunt, Dubuque, IA.

Miller, R. J. and H. W. Robison. 2004. Fishes of Oklahoma. Second edition. University of Oklahoma Press, Norman, OK.

Nakano, S. and M. Murakami. 2001. Reciprocal subsidies: Dynamic interdependence between terrestrial and aquatic food webs. Proceedings of the National Academy of Sciences of the United States of America **98**:166-170.

Nilsson, E., K. Olsson, A. Persson, P. Nystrom, G. Svensson, and U. Nilsson. 2008. Effects of stream predator richness on the prey community and ecosystem attributes. Oecologia **157**:641-651.

Pauly, D., V. Christensen, J. Dalsgaard, R. Froese, and F. Torres. 1998. Fishing down marine food webs. Science **279**:860-863.

Polis, G. A. and S. D. Hurd. 1996. Linking marine and terrestrial food webs: Allochthonous input from the ocean supports high secondary productivity on small islands and coastal land communities. American Naturalist **147**:396-423.

Quinn, G. P. and M. J. Keough. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK.

Reiss, J., J. R. Bridle, J. M. Montoya, and G. Woodward. 2009. Emerging horizons in biodiversity and ecosystem functioning research. Trends in Ecology & Evolution **24**:505-514.

Richter, B. D., D. P. Braun, M. A. Mendelson, and L. L. Master. 1997. Threats to imperiled freshwater fauna. Conservation Biology **11**:1081-1093.

Rice, W.R. 1989. Analyzing tables of statistical test. Evolution **43**:223-225.

Sabo, J. L., J. L. Bastow, and M. E. Power. 2002. Length-mass relationships for adult aquatic and terrestrial invertebrates in a California watershed. Journal of the North American Benthological Society **21**:336-343.

Sabo, J. L. and M. E. Power. 2002. Numerical response of lizards to aquatic insects and short-term consequences for terrestrial prey. Ecology **83**:3023-3036.

Schmitz, O.J. 2007. Predator diversity and trophic interactions. *Ecology* 88: 2415-2426.

Schmitz, O. J. 2008. Effects of predator hunting mode on grassland ecosystem function. Science **319**:952-954.

Schmitz, O.J. 2009. Effects of predator functional diversity on grassland ecosystem function. Ecology **90**:2339-2345.

Schmitz, O. J. and L. Sokol-Hessner. 2002. Linearity in the aggregate effects of multiple predators in a food web. Ecology Letters **5**:168-172.

Spooner, D.E., and C.C. Vaughn. 2009. Species richness and temperature influence mussel biomass: a partitioning approach applied to natural communities. Ecology **90**:781-790.

Srivastava, D. S., B. J. Cardinale, A. L. Downing, J. E. Duffy, C. Jouseau, M. Sankaran, and J. P. Wright. 2009. Diversity has stronger top-down than bottom-up effects on decomposition. Ecology **90**:1073-1083.

Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature **379**:718-720.

Triplehorn, C. A. and N.F. Johnson. 2005. Borror and DeLong's Introduction to the Study of Insects. 7th edition. Thomson Brooks/Cole, Belmont, CA.

Vaughn, C. C., D. E. Spooner, and H. S. Galbraith. 2007. Context-dependent species identity effects within a functional group of filter-feeding bivalves. Ecology **88**:1654-1662.

Wesner, J.S. Aquatic predation alters a terrestrial prey subsidy. Ecology, in press.

Tables

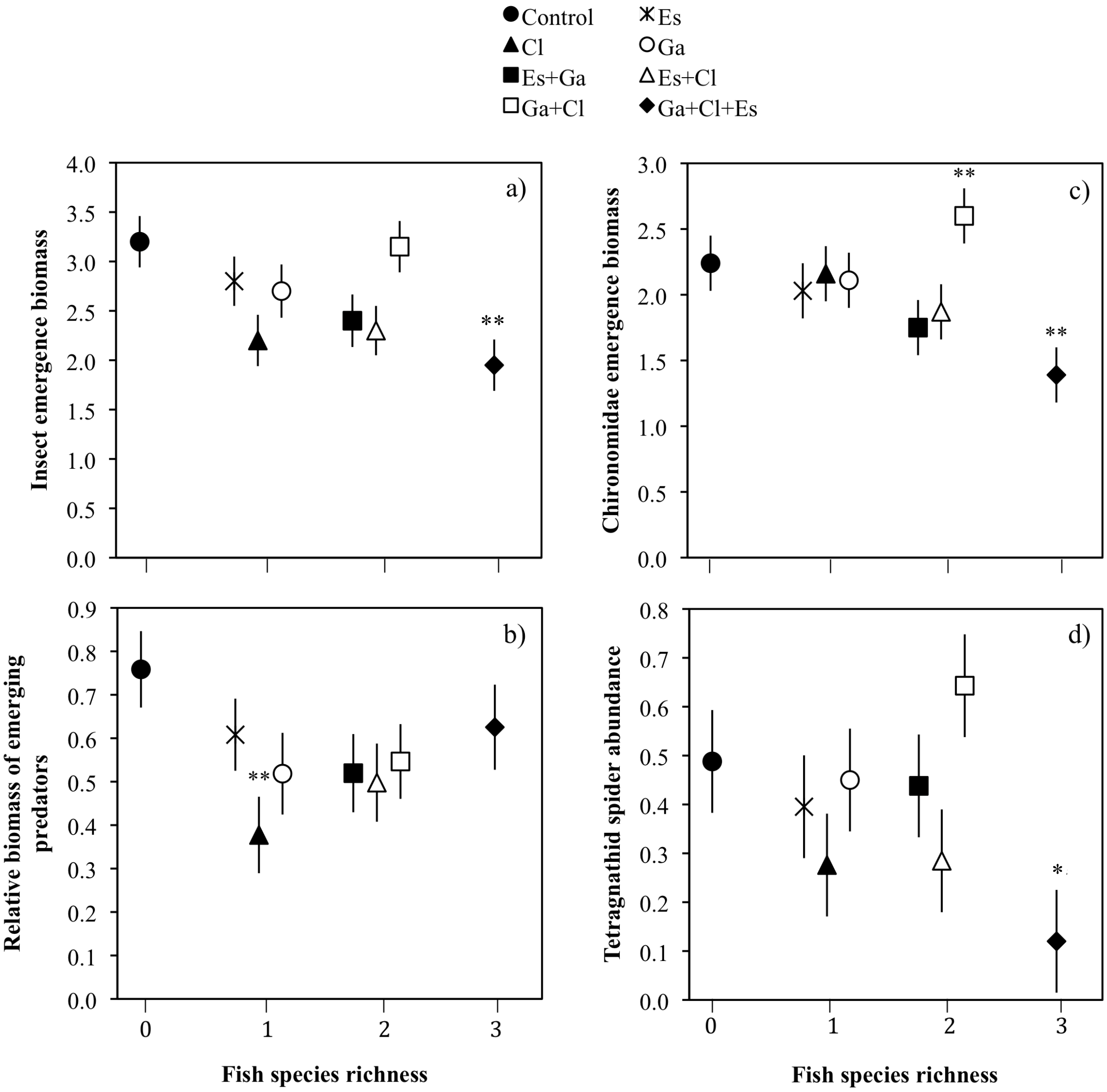
Table 1. Results of a paired t-test comparing values of response variables observed in fish polyculture with values predicted from fish species performance in monoculture. Trophic structure is defined as the proportion of emerging predatory insects by biomass/m^2/day. Degrees of freedom = 4 for all comparisons. *Es* = *Etheosoma spectabile*, *Cl* = *Cyprinella lutrensis, Ga* = *Gambusia affinis.* Values in bold indicate significance at alpha = 0.05.

	Insect emergence biomass		Emerging insect trophic structure		*P. flavescens* emergence biomass		*Hyla* spp. abundance on mesocosm walls		Chironomidae emergence biomass		Tetragnathid spider abundance	
Fish treatment	t	*P*	t	*P*	t	*P*	t	*P*	t	*P*	t	*P*
Es + *Ga*	-2.237	0.089	-0.105	0.354	-1.865	0.136	-1.848	0.138	-2.350	0.078	-0.332	0.756
Es + *Cl*	-1.879	0.133	0.243	0.820	-0.092	0.931	1.209	0.293	-1.889	0.132	-0.469	0.663
Es + *Ga*	-0.448	0.677	0.712	0.546	-1.977	0.119	-7.325	**0.002**	-0.360	0.737	-0.332	0.266
Es + *Ga* + *Cl*	-1.681	0.168	-0.507	0.639	-3.838	**0.018**	-3.368	**0.028**	-1.511	0.205	-3.068	**0.037**

Figures

Figure 1. Effects of fish on (a) mean total insect emergence dry mass (mg/m^2/day), (b) transformed mean trophic structure of the emerging insect assemblage (relative emergence of predatory insects; biomass/m^2/day), (c) mean emergence of Chironomidae, and (d) mean density of tetragnathid spiders above mesocoms. Data are natural log transformed or arcsine squareroot transformed (proportions, b) least squares means ± SE. Asterisks indicate significant planned contrasts between fish treatments and the control. * indicates marginal significance ($P = 0.015$ at $\alpha_{adj} = 0.0071$). ** indicates significance at $\alpha = 0.0071$. Fish abbreviations are Es (*Etheostoma spectabile*), Cl (*Cyprinella lutrensis*), and Ga (*Gambusia affinis*).

Figure 1



●Control
✶Es
▲Cl
○Ga
■Es+Ga
△Es+Cl
□Ga+Cl
◆Ga+Cl+Es
a)
Insect emergence biomass
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
c)
Chironomidae emergence biomass
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
**
b)
Relative biomass of emerging predators
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
**
d)
Tetragnathid spider abundance
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
*
0
1
2
3
Fish species richness
Fish species richness

Appendix

Appendix A. Timeline of aquatic and terrestrial food web samples from mesocosms.

Date	Day of experiment	Event
5/16/08		All mesocosms filled with water and inoculated with algae and small invertebrates; aerial colonization begins;
6/4/08	0	Distributed fish to treatments; experiment begins
6/5/08	1	first benthic sample
6/6/08	2	first tadpole counts
6/10/08	6	set emergence traps; first fish observation; second tadpole counts;
6/11/08	7	first fish observations; first spider counts
6/12/09	8	first emergence collection
6/16/08	11	second fish observation; third tadpole counts; second spider counts
6/22/08	18	third spider counts
6/25/08	21	third fish observations
6/26/08	22	set emergence traps; fourth tadpole counts
6/28/08	24	second emergence collection
7/2/08	28	fifth tadpole counts
7/9/08	35	fourth spider counts; fourth fish observations
7/10/08	36	set emergence traps; sixth tadpole counts
7/13/08	39	third emergence collection
7/21/08	47	fifth fish observation
7/23/08	49	sixth tadpole and odonate counts; fifth spider counts
7/27/08	53	set emergence traps
7/28/08	54	collected periphyton
7/29/08	55	fourth emergence collection
7/30/08	56	second benthic sample
7/31/08	57	collected all fish; end of experiment