UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

TROPHIC SPATIAL ECOLOGY OF INVERTIVOROUS STREAM FISHES

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

DOCTOR OF PHILOSOPHY

By
DAVID PATRICK GILLETTE
Norman, Oklahoma
2007

Downloaded from SHAREOK. Persistent Handle URL: https://hdl.handle.net/11244/1292

UMI Number: 3291251



UMI Microform 3291251

Copyright 2008 by ProQuest Information and Learning Company.
All rights reserved. This microform edition is protected against
unauthorized copying under Title 17, United States Code.

ProQuest Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

TROPHIC SPATIAL ECOLOGY OF INVERTIVOROUS STREAM FISHES

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF ZOOLOGY

BY

William J. Matthews (Advisor)

Bruce W. Hoagland

Michael E. Kaspari

Jeffrey F. Kelly

Caryn C. Vaughn

© Copyright by DAVID PATRICK GILLETTE 2007
All Rights Reserved.

ACKNOWLEDGMENTS

My time at the University of Oklahoma has been extremely rewarding, and I have many people to thank for this experience. My respect and admiration for the students, faculty and staff associated with the University of Oklahoma, and particularly the Department of Zoology, has grown with every interaction I have had.

My major advisor, Bill Matthews, has been a consistent source of inspiration and guidance. His comments and suggestions have consistently guided my research in productive directions, and I hope in the future to affect young scientists in the manner he has affected me. My graduate committee (Bruce Hoagland, Mike Kaspari, Jeff Kelly and Caryn Vaughn) helped me develop my thoughts and ideas into the present dissertation. I am extremely grateful for the time they have spent helping me develop as a scientist. I thank the "fish lab" (Edie Marsh-Matthews, Ingo Schlupp, Rich Broughton, Melody Brooks, Rae Deaton, Chad Hargrave, Rachael Remington, Peter Unmack, Nate Franssen, Jeff Wesner, Courtney Franssen, Michi Tobler, Rudi Riesch, Laura Ewing, Sara Cartwright, Paulette Reneau and Rich Zamor) for field assistance, statistical advice and helpful discussion of this research.

I thank my parents, John and Linda Gillette, for their endless support and encouragement. I still remember the fishing trip at Hastings, NE, that sparked my interest in fishes, an interest that I have pursued to this day. My daughter, Ashiya and my son, Stephen, continue to inspire and encourage me with their curiosity and wonder at the world. Finally, I am grateful for the love, support and patience of my wife, Subala, who helped me in so many ways during the work for this dissertation, and without whose help and understanding I would not have been able to complete this work.

TABLE OF CONTENTS

ACKNOWLEDGMENTS ..... **iv**

TABLE OF CONTENTS ..... **v**

LIST OF TABLES ..... **vii**

LIST OF FIGURES ..... **x**

PREFACE ..... **xiii**

ABSTRACT ..... **xiv**

Chapter 1: Trophic subsidy effects on abundance and condition of a drift-feeding minnow: The importance of donor patch characteristics **1**
- **Abstract** **2**
- **Introduction** **3**
- **Methods** **4**
  - Study System **4**
  - Field Survey Methods **5**
  - Analyses **6**
- **Results** **9**
  - Model comparison **9**
  - Prey subsidy determinants **10**
- **Discussion** **11**
- **Acknowledgments** **14**
- **Literature Cited** **15**
- **Tables** **24**
- **Figures** **31**

Chapter 2: Variation among riffles affects prey use and feeding selectivity of the benthic insectovore *Etheostoma spectabile* **34**
- **Abstract** **35**
- **Introduction** **37**
- **Methods** **40**
  - Study System **40**
  - Field Survey & Lab Methods **40**
  - Evaluation of Sample Size for Diet Analysis **42**
  - Analyses **43**
- **Results** **46**
- **Discussion** **49**
- **Acknowledgments** **54**
- **Literature Cited** **54**
- **Tables** **66**
- **Figures** **69**

Chapter 3: Effects of trophic subsidy exlusion on consumer fitness: An esperimental test using stream fishes **76**
Abstract **77**
Introduction **78**
Methods **80**
Study System Description **80**
Experimental Design **81**
Diet Comparison **82**
Terrestrial Insect Access and Light Intensity **83**
Stable Isotope Analyses **83**
Body Condition **84**
Results **85**
Discussion **86**
Aschnowledgments **91**
Literature Cited **92**
Tables **102**
Figures **104**

LIST OF TABLES

Chapter 1

Table 1. Variables included and number of candidate models for eleven model categories predicting abundance (A), body condition (C) and foraging success (F) of *Notropis boops* in pools of Brier Creek, southern Oklahoma, USA.

Table 2. Model category, predictor variable(s), Akaike weight (w_i), and adjusted R^2 for best ten models predicting area-corrected abundance of *Notropis boops* in pools of Brier Creek. Models ranked in order of decreasing w_i. See Table 1 for predictor variable units.

Table 3. Model category, predictor variable(s), Akaike weight (w_i), and adjusted R^2 for best ten models predicting body condition of *Notropis boops* in pools of Brier Creek. Models ranked in order of decreasing w_i. See Table 1 for predictor variable units.

Table 4. Model category, predictor variable(s), Akaike weight (w_i), and adjusted R^2 for top ten models predicting foraging success of *Notropis boops* in pools of Brier Creek. Models ranked in order of decreasing w_i. See Table 1 for predictor variable units.

Table 5. Significance and effect direction of riffle variables predicting export of Ephemeroptera, Chironomidae (Diptera) and Amphipoda drift density (mg dry mass • m^{-3} water) from riffles into pools, in Brier Creek. Tests of global fit for all models presented are significant at $\alpha = 0.05$. (+ = positive effect, – = negative effect, NS = effect non-significant)

Chapter 2

Table 1. Results of testing for differences in abundance of common prey items in *Etheostoma spectabile* diet by body size (standard length) and by riffle. Body size effects tested using linear regression, and riffle effects using Kruskal-Wallis test. Results significant at $\alpha = 0.05$ denoted by an asterisk (*).

Table 2. Feeding selectivity of *Etheostoma spectabile* for common prey items. Combined results include all fishes and prey items from all riffles; items consumed in lower proportion than expected under H_0: random use of available prey denoted by a minus (-), and those consumed in greater proportion than expected by a plus (+). Tests significant at $\alpha = 0.05$ denoted by an asterisk (*). Large prey taxa denoted by a dagger (†). Results by riffle indicate the number of riffles in which a prey item was consumed in lower or greater proportion than expected under H_0. Note: Hydropsychid larva were not collected from riffle 1 or present in darter guts from this riffle, giving a total of only nine riffles tested for this prey item.

Table 3. Best-fit stepwise multiple regression models predicting differences between proportion consumed and proportion available for common prey items as a function of riffle habitat.

Chapter 3

Table 1. Mean and standard error density of benthic macroinvertebrates in experimental streams at the conclusion of the study in May 2006.

Table 2. Results of testing for effects of terrestrial exclusion on lipid mass, with body mass as a covariate. Tests significant at $\alpha = 0.05$ denoted by an asterisk (*).

LIST OF FIGURES

Chapter 1

Figure 1. Spatial autocorrelation (Moran's *I*) of water depth (A) and water flow velocity (B) as a function of channel lag distance in Brier Creek, southern Oklahoma, USA. Significant positive values over short lag distances, decreasing with distance before increasing once again, indicate a patchy spatial structure due to riffle and pool formation.

Figure 2. Akaike weights (w_i) of ten best models, grouped by model category, predicting area-corrected abundance (A), body condition (B) and foraging success (C) of Bigeye Shiner (*Notropis boops*) in pools of Brier Creek, southern Oklahoma, USA.

Chapter 2

Figure 1. Results of sensitivity analysis using gut contents data for Riffle 10, showing the positive decelerating relationships between number of fish in a sample and mean Percent Similarity Index (PSI) among all possible independent samples (Panel A), and number of fish in a sample and the PSI of all possible independent samples compared to the sample of ten fish (Panel B). Best-fit second order polynomial regression lines are shown; error bars indicate standard deviation.

Figure 2. Mean and standard error dry mass per individual for common prey taxa in riffles of Brier Creek, Oklahoma. Prey items sharing lowercase letters do not differ significantly. Items sharing a lowercase "a" were classified as "small" prey, and those sharing a lowercase "b" as "large" prey.

Figure 3. Mean and standard error abundance of invertebrate prey taxa consumed by *Etheostoma spectabile* (A) and available (B) in riffles of Brier Creek, Oklahoma. Abundance of available bivalves and ostracods were not quantified; these prey items are denoted by an asterisk (*) on panel B.

Figure 4. Relationship between prey head capsule width and *Etheostoma spectabile* standard length, showing best fit lines obtained using quantile regression. Slope of the 5^{th} (lower dashed line; P = 1.00) and 50^{th} (solid line; P = 0.44) quantiles did not differ significantly from zero. Slope of the 95^{th} quantile (upper dashed line) was marginally significant (P = 0.05).

Figure 5. Relationship between number of prey items consumed and darter standard length, showing best fit lines obtained using quantile regression. Slope of the 50^{th} (solid line; P = 0.14) and 90^{th} (upper dashed line; P = 0.27) quantiles did not differ significantly from zero. Slope of the 10^{th} quantile (lower dashed line) was marginally significant (P = 0.07).

Chapter 3

Figure 1. Terrestrial invertebrate input for control and exclusion streams, measured on three occasions over the course of the study. Means differing significantly (two-sample *t*-test) are denoted by an asterisk (*).

Figure 2. Mean and standard error diet composition for *Notropis boops*, *Etheostoma spectabile* and *Fundulus notatus* taken from experimental streams in May 2006.

Figure 3. Biplot showing mean and standard deviation stable isotope ratios of carbon and nitrogen in dorsal muscle tissue of *Notropis boops*, *Etheostoma spectabile* and *Fundulus notatus*, and whole chironomid larva taken from experimental streams in May 2006. Significant differences between control and exclusion means (two-sample *t*-test) are denoted by an asterisk (*).

Figure 4. Relationship between lipid and whole-body mass for *Fundulus notatus* from control and exclusion streams in May 2006. Vertical dotted line indicates value of body mass above which terrestrial invertebrate exclusion effect on lipid mass was significant, with body mass as a covariate, as determined by the Wilcox (1987) procedure.

PREFACE

The three chapters of this dissertation have been formatted for the journal *Ecology*.

ABSTRACT

In the past thirty years, ecologists have learned that the patterns they observe are the results of processes operating over many different spatial scales. Consequently, the decision of the spatial scale on which to conduct ecological research is an important one. To aid ecologists in this decision, the term "patch" was introduced to describe homogeneous, discrete habitat units within larger landscapes. A habitat patch thus defines an appropriate unit of ecological study, and ecologists have learned a great deal by examining the relationships between organisms within a habitat patch, and patch characteristics. Recently, investigators have begun to study the relationships between organisms in a given patch, and the characteristics of habitats adjoining the occupied patch, referred to as "patch context." Such information is necessary, because some species are reliant on resources derived in habitats adjacent to an occupied patch. In such a case, occupied patch characteristics do not sufficiently describe a species' ecology; connections to adjoining habitats, as well as the ability of adjoining habitats to produce necessary resources, must also be examined.

Streams are interesting ecosystems in which to study the relationship between species and habitat patches. Many streams are naturally divided into patches through the formation of riffles (shallow, swift-flowing habitats) and pools (deep, slow-flowing habitats). These habitats are repeated along a stream's length, such that pools are separated by riffles, and riffles by pools. Stream species are typically restricted to one of these habitat types, either living in riffles or pools. Finally, different species of stream fishes rely on different resources for food, and this provides a basis on which to predict the important of patch context to a species. For example, pool-dwelling species reliant

on invertebrates falling into the stream, or drifting into a pool from an upstream riffle, are more likely to be dependent on the context of an occupied patch than a species that feeds on resources derived within its occupied patch.

In my dissertation research, I investigated the manner in which trophic ecology links organisms to different habitats within the landscape. I studied three species: the Orangethroat Darter (*Etheostoma spectabile*), a fish that lives on the stream bottom and feeds on insect larva and other invertebrates; the Bigeye Shiner (*Notropis boops*), a minnow that swims in the middle of the water column and feeds on insect larva drifting downstream and terrestrial insects falling into the stream, and the Blackstripe Topminnow (*Fundulus notatus*), which swims just below the water's surface and feeds on insects falling into the stream from streamside vegetation. These different feeding habits suggest differences in dependence on habitats outside the patch occupied by a species. The Orangethroat Darter feeds on resources from its occupied patch, whereas both the Bigeye Shiner and Blackstripe Topminnow use resources produced either upstream or in the terrestrial environment adjacent to the stream. Thus, patch context is predicted to have a strong effect on the latter two species, whereas characteristics of the occupied patch are likely to be important for the Orangethroat Darter.

In Chapter 1, I studied the relationship between Bigeye Shiner populations and variables of habitat patches within the stream, and the land adjacent to the stream (the riparian zone). This work was done in Brier Creek, a small stream in southern Oklahoma. Results showed that the number of Shiners in a pool was best predicted by habitat variables of the riffle just upstream from a pool. These riffle variables also determined the amount of insect larva drifting from riffles into downstream pools at

night, which in turn predicted Shiner feeding success. Body condition of Shiners was best predicted by feeding success. These results illustrate the importance of upstream riffles to the pool-dwelling Bigeye Shiner, and provide an example of how feeding habits can lead to importance of patch context for a species. When animals consume resources originating in other habitats and "imported" into their occupied patch, connections among habitat patches are important to the species' survival.

In Chapter 2, I studied differences in feeding ecology among populations of Orangethroat Darters on riffles in Brier Creek. Results showed significant differences among riffles. The number of prey items consumed varied significantly among riffles, but was not affected by darter body size. Prey selection varied greatly among riffles, and for four of seven prey items was explained by habitat differences. Contrary to theoretical predictions, diet breadth of darters within riffles was not dependent on the abundance of energetically favorable prey, largely due to a lack of selection for these prey items. These results indicate that variation among riffles can have a strong effect on prey use by the Orangethroat Darter, and that this is an important spatial scale over which to study diet variation in this and similar species.

In Chapter 3, I asked if feeding habits of different fish species determined their dependence on insects entering the stream from the riparian zone (terrestrial insects). Using experimental streams, I excluded these insects from half of the experimental units for each fish species, and examined differences in fish diet and body fat. Under terrestrial insect exclusion, diet and body fat of the bottom-feeding Orangethroat Darter were unchanged. Bigeye Shiner switched their diet from terrestrial insects to aquatic resources, but body fat levels did not change. Blackstripe Topminnow also switched

their diet away from terrestrial insects, but, unlike Bigeye Shiner, body fat levels decreased when terrestrial insects were unavailable. These results indicate that reducing movement of trophic resources from one habitat to another affects different species in different ways, and that the feeding habits of species may help predict this response. This result is important in light of human landscape modification, which often alters the amount of insects moving into streams from the surrounding landscape.

My dissertation research has shed new light on the manner in which feeding ecology determines an organism's relationship to habitat over multiple spatial scales. Different species are dependent on different resources, some of which are not produced in their occupied habitat patch. For such species, the environment must be viewed on a spatial scale large enough to include those habitats that "export" resources to these consumers. Other species consume resources produced in an occupied patch, and move little between patches. Because of this, differences in habitat and resources among patches can result in different patterns of prey consumption for such species. Finally, interrupting movement of resources from one habitat to another does not affect all species in the same manner, with predictable differences based on feeding ecology of species. Collectively, these results provide new information on the relationships between fishes and resources produced in different areas of the stream, and contribute to the understanding of the spatial ecology of functionally diverse communities in ecosystems characterized by a high degree of resource transport among habitats.

CHAPTER 1: TROPHIC SUBSIDY EFFECTS ON ABUNDANCE AND CONDITION OF A DRIFT-FEEDING MINNOW: THE IMPORTANCE OF DONOR PATCH CHARACTERISTICS

DAVID P. GILLETTE

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

ABSTRACT

Although the importance of trophic subsidies is well documented for both aquatic and terrestrial systems, the extent to which donor patch variation affects consumers by regulating subsidy levels is not clear. I used a model comparison approach to examine variation in abundance, body condition and foraging success of the Bigeye Shiner (*Notropis boops*), a drift-feeding minnow in Brier Creek, southern Oklahoma, USA. Because the pool-dwelling *N. boops* typically forages on invertebrate input from upstream riffles and the riparian zone, I hypothesized that variation in *N. boops* abundance and body condition would be affected by upstream and riparian environmental variables that regulate levels of invertebrate subsidies exported into pools. After correcting for area effects, variation in *N. boops* abundance was best explained by models containing upstream environmental variables, and body condition by foraging success models. Foraging success was best explained by night invertebrate drift density, which was a function of upstream riffle characteristics. Riffles with higher substrate diversity, lower flow velocity, and higher densities of the benthic insectivore Orangethroat Darter (*Etheostoma spectabile*) exported higher night-time amounts of invertebrate drift to downstream pools. This indicates that adjacent habitats can influence local abundance and condition of species in tightly-connected systems such as streams, particularly if a species' trophic ecology makes it dependent on input of subsidies from donor patches.

INTRODUCTION

In tightly-connected ecosystems, trophic subsidies across ecotones can link consumers to adjacent habitats (Polis et al. 1997, Baxter et al. 2005). Organisms in such systems depend on allochthonous resources, showing strong numerical responses to inter-patch subsidies (Rose and Polis 1998, Bastow et al. 2002, Barrett et al. 2005). In some cases, consumers depend almost entirely on trophic subsidies to fulfill energetic requirements (Bastow et al. 2002). Although the importance of trophic subsidies is well documented, the extent to which donor patch variation affects consumers in recipient patches through variation in subsidy levels is not clear. In rivers and streams, evidence suggests that undisturbed riparian zones, particularly those forested and with dense canopy, contribute the most terrestrial invertebrates (Baxter et al. 2005). In other donor-recipient systems measures of connectivity such as perimeter to area ratio, patch permeability or distance between patches drive input levels (Polis et al. 1997). For consumers dependent on trophic subsidies, variables driving resource import from adjacent habitats can determine trophic resource levels (Palik et al. 2006).

I examined donor-recipient habitat linkages and their effects on the drift-feeding minnow Bigeye Shiner (*Notropis boops*) in Brier Creek, a midwestern (USA) stream. Brier Creek is a "riffle-pool" stream (*sensu* McMahon et al. 1996), comprised of alternating shallow, swift-flowing habitats and deep, slow-flowing habitats. *Notropis boops* is most common at the upstream ends of pools where it forages on invertebrates drifting off upstream riffles (Pflieger 1997), or falling from the riparian zone (Gillette, unpublished data). Because it consumes resources imported into its habitat, *N. boops* is

an appropriate organism to test for effects of adjacent patches on consumers via trophic subsidy level variation.

Using a model comparison approach (Burnham and Anderson 2002), I evaluated the relative strength of pool, upstream riffle, and riparian zone models in explaining variation in *N. boops* abundance, body condition and foraging success among pools. I then tested for effects of upstream and riparian environmental variables on levels of invertebrate subsidies drifting from riffles into pools, or falling into pools from the riparian zone. Specifically, this analysis tested the hypothesis that upstream and riparian habitat variables affected *N. boops* abundance, body condition and foraging success through variation in trophic subsidy levels.

METHODS

Study System

I studied a 1-km section of Brier Creek, a small prairie-margin stream in southern Oklahoma (Power and Matthews 1983, Power et al. 1985, Gelwick and Matthews 1992). Riffle fishes include Orangethroat Darter (*Etheostoma spectabile*), a benthic insectivore, and central stoneroller (*Campostoma anomalum*), an algivore. Pool assemblages include sunfishes (*Lepomis*) spp., largemouth bass (*Micropterus salmoides*) and spotted bass (*M. punctulatus*), catfishes, suckers and a topminnow (Matthews et al. 1994). *Notropis boops* is the only abundant drift-feeding minnow present in the system.

Riffle-pool structure creates a system of longitudinally repeated habitat units. A correlogram of points along the stream channel (Figure 1) indicates habitat patchiness (Legendre and Fortin 1989). This patchiness is a function of riffle-pool structure, as the

smallest riffles and pools are 10 m long, and this is the longest distance with significant positive values of Moran's *I* (Figure 1). This analysis indicates that conceptualizing riffles and pools as a system of interconnected patches is appropriate.

Field Survey Methods

From 30 July to 6 August 2004, I quantified in-stream and riparian environmental variables, aquatic and terrestrial invertebrates, and populations of *N. boops* in 10 study pools and *E. spectabile* in upstream riffles. These pools correspond to 10 of the 14 pools studied by Power and Matthews (1983) and Matthews et al. (1994). At 1-m points along cross-stream transects spaced every 5 m, I measured water depth and flow velocity (Marsh-McBirney flow meter), and estimated substrate type (Bain 1999). Percentage riparian canopy cover for each pool was quantified following Kelley and Krueger (2005). Percentage overhanging vegetation was calculated as the percentage of transect ends overhung by vegetation.

In each riffle and pool, vacuum benthos samples using 600 μm mesh (Brown et al. 1987) were taken at four randomly selected points. At the upstream end of each pool, invertebrate drift was sampled for one hour at midday (1400 hours) and at night (2200 hours) for two successive days, using 363 μm mesh drift nets. In each pool, invertebrate input from the riparian zone was sampled using four 0.065 m^2 pan traps filled with water and a small amount of surfactant. Traps were set twice for two consecutive 24 hours periods, then combined for each pool. All invertebrate samples were preserved in 70% alcohol, sorted to the lowest practical taxonomic level, counted, and weighed to determine biomass after drying at 60° C.

I used snorkel surveys to census pool fish populations (Harvey 1991, Matthews et al. 1994). I entered each pool from the downstream end, swimming slowly upstream and tallying numbers of *N. boops* and *Micropterus* spp. on dive slates. Clear water allowed bank to bank visibility. Only *Micropterus* spp. greater than 60 mm standard length (SL; "large bass") were considered potential predators of *N. boops*, because this gape-limited predator can consume prey half its size (Post 2003) and the smallest *N. boops* measured 30 mm SL during the survey. The following morning, ten *N. boops* from each pool were preserved in 10% formalin. In riffles, *E. spectabile* were sampled by kick-seining (Matthews 1990). Starting downstream, a 3 mm mesh seine was placed across each riffle every five meters, and the substrate disturbed from 5 m upstream down to the seine.

All *N. boops* were measured (SL) and the digestive tract removed from esophagus to anus (Heroux and Magnan 1996). Body and digestive tract were dried to constant mass (60° C) and weighed. Body condition was expressed as the residual of the $\log_{10}$ body mass against $\log_{10}$ SL linear regression (Schulte-Hostedde et al. 2005), and gut fullness as the residual of the $\log_{10}$ gut mass against $\log_{10}$ body mass linear regression (Sutton et al. 2000). Body condition was used to estimate stored energy (Booth and Keast 1986), a common fitness proxy for fishes (e.g., Seppa et al. 2001, Berumen et al. 2005). Gut fullness was used to quantify recent foraging success (Angermeier 1985).

Analyses

I used an information theoretic approach (Burnham and Anderson 2002) to evaluate explanatory models of *N. boops* abundance, body condition and foraging success in individual pools. For each model, I calculated Akaike's Information Criterion adjusted

for small sample size (AIC_c), Akaike weight (w_i), and adjusted R^2. Akaike weights were calculated using AIC_c, and represent the weight of evidence in favor of model *i* being the best among a set of possible models, summing to 1 across all candidate models (Burnham and Anderson 2002). I ranked models using w_i, with the best model having highest w_i. I also compared adjusted R^2 for each model because w_i only evaluates models relative to one another, not the fit of models to data (Burnham and Anderson 2002). For model comparison, I followed the rule of thumb (Burnham and Anderson 2002) that models with $\Delta AIC_c < 2.0$ from the best model have substantial support from data, and models with $\Delta AIC_c > 4.0$ considerably less support.

Model construction.- I grouped models into 11 categories (Table 1). Environmental variable models included abiotic parameters of pools, riffles and the riparian zone. Aquatic (see review in Matthews 1998) and riparian (see reviews by Pusey and Arthington 2003 and Baxter et al. 2005) environmental variables can affect stream fishes in multiple ways. Prey models included benthic density (mg dry mass • m^{-2}; for pool and riffle prey models), drift density (mg dry mass • m^{-3} water; for day and night drift prey models) and aerial input (mg dry mass • m^{-2} • day^{-1}; for riparian prey models) of known invertebrate prey items (Table 1). I classified ephemeropteran, trichopteran, and dipteran chironomid insect larva, amphipod crustaceans, and terrestrial and aerial invertebrates as *N. boops* prey based on gut contents surveys (D.P. Gillette, unpublished data). The pool prey category modeled the hypothesis that local prey density affects fishes within a patch, and the riffle prey category modeled the hypothesis that upstream prey density affects fishes in downstream patches. Some fishes also respond numerically to terrestrial invertebrate input (Kawaguchi et al. 2003). Invertebrate drift density can

affect growth of fishes (Harvey et al. 2006), and previous authors have noted the potential for drift density to limit stream fish growth (Schlosser 1998). I included drift density of prey items in both day prey drift and night prey drift categories to account for diel variation in availability of this resource.

Other models included density dependence, predation threat, and foraging success. Growth and survival of minnows can decrease as population density increases (Matthews et al. 2001), and small fishes often alter habitat use in response to presence of piscine predators (Power et al. 1985). Fish foraging success can depend on variables other than prey abundance (Angermeier 1985, Zhao et al. 2006). In such cases, foraging success may be a better predictor of consumer body condition than prey availability.

Within each category, models were constructed to include all possible variable combinations. I conducted separate model comparisons for abundance, body condition and foraging success of *N. boops*. The following sets of variables within a model category were collinear ($P[\rho=0] < 0.05$), so a single surrogate variable was chosen from the group. Maximum pool depth was selected to represent pool substrate diversity (positive correlate) and pool mean depth (positive). Pool chironomid larva density was selected to represent pool amphipod density (positive). All proportional data were arcsine square root transformed.

Determinants of prey subsidy levels.- I used stepwise multiple regression with backwards elimination to test predictors of prey export from riffles and the riparian zone into pools. Criterion for variable inclusion in each model was $\alpha < 0.05$, and criteria for variable removal was $\alpha > 0.10$. For riffles, independent variables were riffle environmental variables and prey density. I also included density of the benthic insectivore *E.*

spectabile, as invertebrate drift density can increase with predator density (Miyasaka and Nakano 2001). I conducted eight regressions, one for each prey group during the day and at night. For predictors of riparian export, I regressed input of all invertebrates and terrestrial invertebrates only against canopy cover and percent overhanging vegetation.

RESULTS

Model comparison

Abundance of *N. boops* was positively correlated with pool area ($R = 0.82$, $P = 0.004$). To correct for this, further analyses used residuals of the linear regression between patch area and abundance (area-corrected abundance). Area-corrected abundance was best explained by upstream riffle environmental variable (UREV) models (Figure 2A, Table 2); this was the only category with models substantially supported by the data. Pool prey was the only other category with a model in the best ten out of 97 candidate models, and relative likelihood of the best model from this category was only 14% that of the best UREV model. Substrate diversity and mean flow velocity were the strongest variables in UREV models. Variance in *N. boops* area-corrected abundance was well-explained, with adjusted R^2 ranging from 0.44 to 0.69 for the best ten models.

Body condition of *N. boops* was best explained by their foraging success (Figure 2B, Table 3); this was the only model receiving substantial support from the data. Pool habitat models were next best, with much lower relative likelihoods. The best four models (foraging success, two pool habitat models and one riffle model) out of 99 candidate models explained variance relatively well ($0.24 < R^2 < 0.56$).

Foraging success of *N. boops* was best explained by night prey drift models, followed by riparian prey and day prey drift models (Figure 2C, Table 4). Analyses did not reveal one model category to be much better than the rest; all four model categories produced at least one model with w_i >35% of the best model, and Pool Prey was the only category without a model that was substantially supported. However, only night prey drift models explained variance in foraging success well, with highest R^2 of 0.41.

Prey subsidy determinants

Drift density for all four prey groups was greater at night than midday (t-test, all $P < 0.001$). For three groups, drift export into pools was predicted by upstream riffle variables (Table 5). Only Trichoptera drift density had no significant predictors during the day or at night ($P [F = 0] > 0.05$ for all tests). Ephemeroptera drift density was a function of three habitat variables and benthos density during the day, and of five habitat variables and benthos density at night. Chironomid drift density was a function of two habitat variables and predator density during the day, and of four habitat variables, benthos density, and predator density at night. Amphipod drift density was not predicted by measured variables during the day, but was a function of four habitat variables, benthos density and predator density at night. Neither input of terrestrial invertebrates only (best fit model: $F_{1,9} = 1.93$, $P = 0.20$), nor that of all aerial invertebrates (best fit model: $F_{1,9} = 0.81$, $P = 0.40$) were predicted by riparian habitat variables.

DISCUSSION

Upstream riffle characteristics were important in explaining density and foraging success of the drift-feeding *N. boops*. UREV models best predicted area-corrected abundance, moreso than models using variables of the pools containing *N. boops*. Riffle substrate diversity was the strongest variable in these models; in addition to explaining *N. boops* abundance, it also positively influenced biomass of aquatic macroinvertebrate drift exported into downstream pools. Thus, riffles with high substrate diversity exported high levels of invertebrate trophic subsidies downstream, and those downstream pools were more densely populated by *N. boops*. Foraging success was best explained by night invertebrate drift, and body condition, in turn, was best explained by foraging success. Taken together, these results emphasize the importance of upstream riffles to pool-dwelling, drift-feeding minnows. This adds to the evidence that connections between habitats are crucial components of many organisms' ecologies (*e.g.*, Dethier et al. 2003, Franken and Hik 2004). For species reliant on allochthonous trophic resources, habitat trophic quality may be better predicted by donor habitat characteristics than by characteristics of the occupied patch.

Realization of the importance of habitat connectivity in lotic systems is not new. Hynes (1975) was among the first to emphasize it, and most conceptual models of lotic ecology incorporate movement of materials and organisms among habitats (*e.g.*, Vannote et al. 1980, Elwood et al. 1983, Pringle et al. 1988). However, increased awareness of the importance of spatial scale in ecology (Levin 1992, Schneider 2001) and specifically emergence of the field of landscape ecology (Wiens 1999) provide a contemporary

conceptual framework within which issues of habitat context and connectivity in these systems can be addressed (Schlosser 1991, Wiens 2002).

Landscape ecology emphasizes interactions between spatial pattern and ecological processes (Turner et al. 2001). An important component of spatial pattern is patch context, the landscape surrounding a habitat patch. In tightly-connected systems such as rivers and streams, patch context can be an important determinant of patch quality (Wiens 2002). The present study underscores the importance of patch context and habitat connectivity, by connecting attributes of adjacent habitats to trophic subsidy export levels.

Notropis boops body condition was best predicted by foraging success, suggesting that differences in pool-specific foraging success may remain consistent over time, allowing individuals in these patches to store more energy than individuals in patches with lower foraging success. Foraging success, in turn, was best predicted by night invertebrate drift densities of amphipods and Ephemeropteran larva. Although maceration by pharyngeal teeth makes summer gut contents unidentifiable, data from winter surveys showed these invertebrates to be important prey of *N. boops* (D.P. Gillette, unpublished data). In the same survey, foraging success was highest at 0300 hrs, suggesting that *N. boops* may forage nocturnally, a fact not previously reported.

Development of among-pool differences in body condition assumes a low rate of inter-pool movement. In general, stream fishes show leptokurtic movement distributions, with a few individuals dispersing long distances, while the majority of the population moves little (Skalski and Gilliam 2000, Fraser et al. 2001). For *N. boops* in Brier Creek this is likely the case; in a mark-resight study, 75% of marked fish remained in the same

pool 30 days after their release (Schaefer 1999). High-water events may relocate individuals among pools, but there were no such events in the month leading up to this study. In addition, recent work has shown that differences in drift input from riffles can remain consistent over long periods of time (Hansen and Closs 2007), suggesting that differences in resource availability among pools are temporally consistent, in the absence of "reset" events such as floods or droughts.

In the present study, macroinvertebrate prey drift from riffles into pools was predicted by riffle characteristics for three of four prey groups. Only drift of trichopteran larva was not significantly related to riffle variables at mid-day or during the night. Mean riffle flow velocity was most frequently significant (4 of 4 significant regression models), and always had a negative effect on drift. Substrate diversity, percentage gravel substrate, percentage bedrock substrate and riffle benthos density were each components 3 of 4 significant models. Of these, only substrate diversity had a consistent effect direction; riffles with higher substrate diversity exported higher invertebrate drift densities. Riffle density of the benthic insectivore *E. spectabile* increased density of amphipods and chironomid larva from the riffle. Although several studies have documented increased drift in the presence of benthic feeding fishes in laboratory systems (Culp et al. 1991) and field experiments (Dahl 1998, Miyasaka and Nakano 2001), this is among the first studies to show a positive effect of benthic foraging fish density on drift in natural systems.

Unlike aquatic macroinvertebrates, input of terrestrial invertebrates into pools was not a function of donor habitat variables. Riparian characteristics have been previously linked to invertebrate subsidy levels in streams (*e.g.*, Mason & MacDonald 1982, Piccolo

& Wipfli 2002), and evidence indicates that input levels may increase with canopy density (Edwards & Huryn 1995, Kawaguchi & Nakano 2001). The discrepancy between these results and the present study is likely due to spatial scale, as the authors above compared entire streams, or reaches much longer than pool size in Brier Creek. As Baxter et al. (2005) note, processes such as wind, dispersal and riparian patchiness can homogenize input at smaller spatial scales. Thus, riparian habitat may affect levels of terrestrial invertebrate input into Brier Creek, but is unlikely to effect individual pools via this pathway.

Knowledge of mechanisms linking habitat patches at multiple spatial scales is necessary to fully understand species-habitat relationships, and to identify appropriate scales of research and conservation. Results of the present study highlight the importance of trophic factors that can drive the spatial scale of species-habitat relationships. For taxa dependent on a supply of resources from adjacent patches, donor patch characteristics affecting resource export levels can have strong effects. Given the ubiquity of consumer dependence on trophic subsidies in both aquatic (Day and Branch 2002, Kaehler et al. 2006, Wernberg et al. 2006) and terrestrial (e.g., Schneider 2001b, Faeth et al. 2005, Hines et al. 2006) ecosystems, an understanding of donor patch dynamics and their role in regulating subsidy export may prove critical to understanding variation in consumer population size and individual fitness.

ACKNOWLEDGMENTS

I thank R. Cothran, S. Gillette, P. Jeyasingh, S. Jones and R. Remington for field assistance, and Mr. and Mrs. J. Martin for providing access to Brier Creek. D. Siegfried

and N. Franssen assisted with analyses. W. Porter helped modify the invertebrate sampler. Funding was provided by the United States Environmental Protection Agency Science to Achieve Results fellowship program, a Howard McCarley Research Grant from the Southwestern Association of Naturalists, and by the University of Oklahoma Graduate College. The University of Oklahoma Biological Station provided housing and logistical considerations. Research was conducted under University of Oklahoma Institutional Animal Care and Use Committee permit #R03-015 to David P. Gillette (DPG), and all fishes were collected under Oklahoma Department of Wildlife Conservation Scientific Collector's Permit # 3508 to DPG. This study was submitted in partial fulfillment of requirements for the Doctor of Philosophy degree in the Department of Zoology at the University of Oklahoma. I thank B. Hoagland, M. Kaspari, J. Kelly, C. Vaughn and W. Matthews for their service on my graduate committee.

LITERATURE CITED

Angermeier, P.L. 1985. Spatio-temporal patterns of foraging success for fishes in an Illinois stream. American Midland Naturalist 114: 342-359.

Bain, M.B. 1999. Substrate, p. 95–100. *In:* Aquatic Habitat Assessment: Common Methods. M. B. Bain and N. J. Stevenson (eds.). American Fisheries Society, Bethesda, Maryland.

Barrett, K, W.B. Anderson, D.A. Wait, L.L. Grismer, G.A Polis and M.D. Rose. 2005. Marine subsidies alter the diet and abundance of insular and coastal lizard populations. Oikos 109: 145-153.

Bastow, J.L., J.L. Sabo, J.C. Finlay and M.E. Power. 2002. A basal aquatic-terrestrial trophic link in rivers: Algal subsidies via shore-dwelling grasshoppers. Oecologia 131: 261-268.

Baxter, C.V., K.D. Fausch and W.C. Saunders. 2005. Tangled webs: Reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biology 50: 201-220.

Berumen, M.L., M.S. Pratchett and M.I. McCormick. 2005. Within-reef differences in diet and body condition of coral-feeding butterflyfishes (Chaetodontidae) Marine Ecology Progress Series 287: 217-227.

Booth, D.J. and J.A. Keast. 1986. Growth energy partitioning by juvenile Bluegill Sunfish, *Lepomis macrochirus* Rafinesque. Journal of Fish Biology 28: 37-45.

Borger L., N. Franconi, F. Ferretti, F. Meschi, G. De Michele, A. Gantz and T. Coulson. 2006. An integrated approach to identify spatiotemporal and individual-level determinants of animal home range size. American Naturalist 168: 471-485.

Brown, A. V., Schram, M. D. and P.P. Brussock. 1987. A vacuum benthos sampler suitable for diverse habitats. Hydrobiologia 153: 241-247.

Burnham, K.P. and D.R. Anderson. 2002. Model selection and multimodel inference: A practical information-theoretic approach. Second Edition. New York, NY: Springer.

Culp, J.M., N.E.Glozier and G.J. Scrimgeour. 1991. Reduction of predation risk under the cover of darkness: Avoidance responses of mayfly larvae to a benthic fish. Oecologia 86: 163-169.

Dahl, J. 1998. The impact of vertebrate and invertebrate predators on a stream benthic community. Oecologia 117: 217-226.

Day, E., and G.M. Branch. 2002. Effects of Sea Urchins (*Parechinus angulosus*) on recruits and juveniles of Abalone (*Haliotis midae*). Ecological Monographs 72: 133-149.

Dethier, M.N., K. McDonald and R.R. Strathmann. 2003. Colonization and connectivity of habitat patches for coastal marine species distant from source populations. Conservation Biology 17: 1024-1035.

Ebersole, J.L., W.J. Liss and C.A. Frissell. 2003. Thermal heterogeneity, stream channel morphology, and salmonid abundance in northeastern Oregon streams Canadian Journal of Fisheries and Aquatic Sciences 60: 1266-1280.

Edwards, E.D. and A.D. Huryn. 1996. Effect of riparian land use on contributions of terrestrial invertebrates to streams. Hydrobiologia 337: 151-159.

Elwood, J. W., J.D. Newbold, R.V. O´Neill and W. Van Winkle. 1983. Resource spiraling: An operational paradigm for analyzing lotic systems. In T. D. Fontaine & S. M. Bartell (Eds.), Dynamic of Lotic Ecosystems (pp. 3-27). Michigan: Ann Arbor Science.

Faeth, S.H., P.S. Warren, E. Shochat, and W.A. Marrusich. 2005. Trophic dynamics in urban communities. Bioscience 55: 399-407.

Franken, R.J. and D.S. Hik. 2004. Influence of habitat quality, patch size and connectivity on colonization and extinction dynamics of Collared Pikas *Ochotona collaris*. Journal of Animal Ecology 73: 889-896.

Fraser, D.F. and R.D. Cerri. 1982. Experimental evaluation of predator-prey relationships in a patchy environment: Consequences for habitat-use patterns in minnows. Ecology 63: 307-313.

Fraser, D.F., J.F. Gilliam, M.J. Daley, A.N. Le and G.T. Skalski. 2001. Explaining leptokurtic movement distributions: Intrapopulation variation in boldness and exploration. American Naturalist 158: 124-135.

Gelwick F.P. and W.J. Matthews. 1992. Effects of an algivorous minnow on temperature stream ecosystem properties. Ecology 73: 1630-1645.

Hansen, E.A. and G.P. Closs. 2007. Temporal consistency in the long-term spatial distribution of macroinvertebrate drift along a stream reach. Hydrobiologia 575: 361-371.

Harvey, B.C. 1991. Interactions among stream fishes: Predator-induced habitat shifts and larval survival. Oecologia 87: 29-36.

Harvey, B.C., R.J. Nakamoto and J.L. White. 2006. Reduced streamflow lowers dry-season growth of rainbow trout in a small stream. Transactions of the American Fisheries Society 13: 998-1005.

Heroux, D. and P. Magnan P. 1996. In situ determination of food daily ration in fish: Review and field evaluation. Environmental Biology of Fishes 46: 61-74.

Hines, J., J.P. Megonigal and R.F. Denno. 2006. Nutrient subsidies to belowground microbes impact aboveground food web interactions. Ecology 87: 1542-1555.

Hynes, H.B.N. 1975. The stream and its valley. Internationale Vereinigung für theoretische und angewandte Limnologie Verhandlungen 19:1-15.

Kaehler, S., E.A. Pakhomov, R.M. Kalin and S. Davis. 2006. Trophic importance of kelp-derived suspended particulate matter in a through-flow sub-Antarctic system. Marine Ecology – Progress Series 316: 17-22.

Kawaguchi, Y. and S. Nakano. 2001. Contribution of terrestrial invertebrates to the annual resource budget for salmonids in forest and grassland reaches of a headwater stream. Freshwater Biology 46: 303–316.

Kawaguchi, Y., Y. Taniguchi and S. Nakano. 2003. Terrestrial invertebrate inputs determine the local abundance of stream fishes in a forested stream. Ecology 84: 701-708.

Kelley, C. E. and W.C. Krueger. 2005. Canopy cover and shade determinations in Riparian zones. Journal of the American Water Resources Association 41: 37-46.

Legendre, P. and M.J. Fortin. 1989. Spatial Pattern and Ecological Analysis. Vegetatio 80: 107-138.

Levin, S.A. 1992. The problem of pattern and scale in ecology. Ecology 73:1943-1967.

Mason C.F. and MacDonald S.M. 1982. The input of terrestrial invertebrates from tree canopies to a stream. Freshwater Biology 12: 305–311.

Matthews, W.J. 1990. Spatial and temporal variation in fishes of riffle habitats: A comparison of analytical approaches for the Roanoke River. American Midland Naturalist 124:31-45.

Matthews, W.J. 1998. Patterns in Freshwater Fish Ecology. Chapman and Hall, New York. 756 pp.

Matthews, W.J., B. Harvey and M. Power. 1994. Spatial and temporal patterns in the fish assemblages of individual pools in a midwestern stream (U.S.A). Environmental Biology of Fishes 39: 381-397.

Matthews, W.J., K.B. Gido and E. Marsh-Matthews E. 2001. Density-dependent overwinter survival and growth of Red Shiners from a southwestern river. Transactions of the American Fisheries Society 130: 478-488.

McMahon, T.E., A.V. Zale and D.J. Orth. 1996. Aquatic habitat measurements. Pp. 83-120 in B.R. Murphy and D. W. Willis, eds. Fisheries Techniques. Second Edition. Bethesda, MD: American Fisheries Society.

Miyasaka, H. and S. Nakano. 2001. Drift dispersal of mayfly nymphs in the presence of chemical and visual cues from diurnal drift-and nocturnal benthic-foraging fishes. Freshwater Biology 46: 1229-1237.

Palik, B., D.P. Batzer and C. Kern. 2006. Upland forest linkages to seasonal wetlands: Litter flux, processing, and food quality. Ecosystems 9: 142-151.

Petty, J.T. and G.D. Grossman, 1996. Patch selection by Mottled Sculpin (Pisces: Cottidae) in a southern Appalachian stream. Freshwater Biology 35: 261-276.

Pflieger, W.L. 1997. The fishes of Missouri. Revised edition. Missouri Department of Conservation. Jefferson City, MO.

Piccolo J.J. and M.S. Wipfli. 2002. Does Red Alder (*Alnus rubra*) in upland riparian forests elevate macroinvertebrate and detritus export from headwater streams to downstream habitats in southeastern Alaska? Canadian Journal of Fisheries and Aquatic Sciences 59: 503–513.

Polis, G.A., W.B. Anderson and R.D. Holt. 1997. Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics 28: 289-316.

Post, D.M. 2003. Individual variation in the timing of ontogenetic niche shifts in largemouth bass. Ecology 84: 1298-1310.

Power, M.E. and W.J. Matthews. 1983. Algae-grazing minnows (*Campostoma anomalum*), piscivorous bass (*Micropterus* spp), and the distribution of attached algae in a small prairie-margin stream. Oecologia 60: 328-332.

Power, M., W. Matthews and A. Stewart. 1985. Grazing minnows, piscivorous bass, and stream algae: Dynamics of a strong interaction. Ecology 66: 1448-1456.

Pringle, C.M., R.J. Naiman, G. Bretschko, J.R. Karr, M.W. Oswood, J.R. Webster, R.L. Welcomme and M.J. Winterbourne. Patch dynamics in lotic systems: The stream as a mosaic. Journal of the North American Benthological Society 7: 503-524.

Pusey, B.J. and A.H. Arthington. 2003. Importance of the riparian zone to the conservation and management of freshwater fish: A review. Marine and Freshwater Research 54: 1-16.

Rose, M.D. and G.A. Polis. 1998. The distribution and abundance of coyotes: The effects of allochthonous food subsidies from the sea. Ecology 79: 998-1007.

Schaefer, J. 1999. Unpublished Ph.D. Dissertation. University of Oklahoma.

Schlosser, I.J. 1991. Stream fish ecology: A landscape perspective. Bioscience 41: 704-712.

Schlosser, I.J. 1998. Fish recruitment, dispersal, and trophic interactions in a heterogeneous lotic environment. Oecologia 113: 260-268.

Schneider, D.C. 2001a. The rise of the concept of scale in ecology. Bioscience 51:545-553.

Schneider, M.F. 2001b. Habitat loss, fragmentation and predator impact: Spatial implications for prey conservation. Journal of Applied Ecology 38: 720-735.

Schulte-Hostedde, A.I., B.Zinnar, J.S.Millar, and G.J. Hickling. 2005. Restitution of mass-size residuals: Validating body condition indices. Ecology 86: 155-163.

Seppa, T., A. Laurila, N. Peuhkuri, J. Piironen and N. Lower. 2001. Early familiarity has fitness consequences for Arctic Char (*Salvelinus alpinus*) juveniles Canadian Journal of Fisheries and Aquatic Sciences 58: 1380-1385.

Skalski, G. and J. Gilliam. 2000. Modeling diffusive spread in a heterogeneous population: A movement study with stream fish. Ecology 81: 1685-1700

Sutton S.G., Bult T.P.and R.L. Haedrich. 2000. Relationships among fat weight, body weight, water weight, and condition factors in wild Atlantic salmon parr. Transactions of the American Fisheries Society 129: 527–538.

Thompson, A.R., J.T. Petty and G.D. Grossman. 2001. Multi-scale effects of resource patchiness on foraging behavior and habitat use by Longnose Dace, *Rhinichthys cataractae*. Freshwater Biology 46: 145-160.

Turner, M.G., R.H. Gardner and R.V. O'Neill. 2001. Landscape ecology in theory and practice: Pattern and process. Springer: New York, N.Y. 401 pages.

Vannote, R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell, and C.E. Cushing. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences. 37: 130-137.

Wernberg, T., M.A. Vanderklift, J. How, and P.S. Lavery. 2006. Export of detached macroalgae from reefs to adjacent seagrass beds. Oecologia 147: 692-701.

Wiens, J.A. 1999. The science and practice of landscape ecology. Pages 371-383 in Klopatek J.M. and Gardner, R.H. Eds. Landscape Ecological Analysis: Issues and Applications. New York: Springer.

Wiens, J.A. 2002. Riverine landscapes: Taking landscape ecology into the water. Freshwater Biology 47: 501-515.

Zhao, X.X., M.G. Fox and D.C. Lasenby. 2006. Effect of prey density, prey mobility and habitat structure on size selection and consumption of amphipods by a benthic feeding fish. Archiv fur Hydrobiologie 165: 269-288.

Table 1. Variables included and number of candidate models for eleven model categories predicting abundance (A), body condition (C) and foraging success (F) of *Notropis boops* in pools of Brier Creek, southern Oklahoma, USA. (UREV = Upstream Riffle Environmental Variable; PEV = Pool Environmental Variable)

Model Category	Analyses Used	Variables Included	Number of Models
PEV	A, C, F	Mean water flow velocity Maximum water depth Gravel substrate Bedrock substrate	15
UREV	A, C, F	Mean water flow velocity Mean water depth Substrate diversity Gravel substrate Bedrock substrate	31
Riparian Habitat	A, C, F	Canopy cover Overhanging vegetation	3
Pool Prey	A, C, F	Chironomid benthic density Trichoptera benthic density Ephemeroptera benthic	7

		density	
Riffle Prey	A, C, F	Chironomid benthic density	15
		Trichoptera benthic density	
		Ephemeroptera benthic density	
		Amphipoda benthic density	
Riparian Prey	A, C, F	Terrestrial invertebrate input	3
		Combined aerial invertebrate input	
Night Prey Drift	A, C, F	Chironomid drift density	15
		Trichoptera drift density	
		Ephemeroptera drift density	
		Amphipoda drift density	
Day Prey Drift	A, C, F	Chironomid drift density	7
		Trichoptera drift density	
		Ephemeroptera drift density	
Density	C, F	Number *N. boops* • m^{-2}	1

Dependence		pool area	
Predation Threat	A, C	Number large bass • $pool^{-1}$	1
Foraging Success	C	Gut fullness	1

.

Table 2. Model category, predictor variable(s), Akaike weight (w_i), and adjusted R^2 for best ten models predicting area-corrected abundance of *Notropis boops* in pools of Brier Creek. Models ranked in order of decreasing w_i. See Table 1 for model category abbreviations and predictor variable units.

Rank	*Category*	*Predictor Variable (s)*	ΔAICc	w_i	Adjusted R^2
1	UREV	Mean Flow Velocity (MFV), Substrate Diversity (SD)	0.000	0.251	0.69
2	UREV	SD	0.086	0.241	0.62
3	UREV	MFV, SD, Gravel Substrate	2.609	0.068	0.69
4	UREV	MFV, SD, Bedrock Substrate	2.923	0.058	0.68
5	UREV	SD, Bedrock Substrate	2.923	0.058	0.58
6	UREV	SD, Gravel Substrate	3.200	0.051	0.57
7	UREV	Mean Depth, SD	3.296	0.048	0.57
8	Pool Prey	Trichoptera Mass	3.992	0.034	0.44
9	UREV	Mean Depth, MFV, SD	4.078	0.048	0.57
10	Pool Prey	Trichoptera Mass, Ephemeroptera Mass	4.250	0.030	0.52

Table 3. Model category, predictor variable(s), Akaike weight (w_i), and adjusted R^2 for best ten models predicting body condition of *Notropis boops* in pools of Brier Creek. Models ranked in order of decreasing w_i. See Table 1 for model category abbreviations and predictor variable units.

Rank	*Category*	*Predictor Variable (s)*	ΔAICc	w_i	Adjusted R^2
1	Foraging Success	Gut Fullness	0.000	0.318	0.56
2	PEV	Gravel Substrate, Bedrock Substrate	2.445	0.094	0.41
3	PEV	Gravel Substrate	3.447	0.057	0.28
4	UREV	Bedrock Substrate	3.744	0.049	0.24
5	Pool Prey	Ephemeroptera Mass	4.592	0.032	0.15
6	UREV	Substrate Diversity	5.147	0.024	0.08
7	Density Dependence	Number fish • m^{-2}	5.185	0.024	0.07
8	Night Prey Drift	Amphipod Drift Density	5.804	0.017	-0.01
9	Night Prey Drift	Trichoptera Drift Density	5.852	0.017	-0.02
10	Riparian Habitat	% Overhanging Vegetation	6.003	0.016	-0.04

Table 4. Model category, predictor variable(s), Akaike weight (w_i), and adjusted R^2 for top ten models predicting foraging success of *Notropis boops* in pools of Brier Creek. Models ranked in order of decreasing w_i. See Table 1 for predictor variable units.

Rank	*Model Category*	*Predictor Variable (s)*	ΔAICc	w_i	Adjusted R^2
1	Night Drift Prey	Amphipoda drift density, Ephemeroptera drift density	0.000	0.145	0.41
2	Night Prey Drift	Ephemeroptera drift density	0.709	0.101	0.05
3	Riparian Prey	Aerial invertebrate input	0.968	0.089	0.01
4	Day Prey Drift	Trichoptera drift density	1.106	0.083	-0.01
5	Night Prey Drift	Amphipoda drift density	1.373	0.073	-0.05
6	Density Dependence	Number fish • m^{-2}	1.746	0.060	-0.10
7	Pool Prey	Trichoptera mass	2.028	0.052	-0.15
8	Pool Prey	Ephemeroptera mass	2.061	0.052	-0.15
9	Day Prey Drift	Chironomidae drift density	2.098	0.051	-0.16
10	Night Prey Drift	Chironomidae drift density	2.115	0.050	-0.16

Table 5. Significance and effect direction of riffle variables predicting export of Ephemeroptera, Chironomidae (Diptera) and Amphipoda drift density (mg dry mass • m^{-3} water) from riffles into pools, in Brier Creek. Tests of global fit for all models presented are significant at $\alpha = 0.05$. (+ = positive effect, – = negative effect, NS = effect non-significant)

	Ephemeroptera		Chironomidae		Amphipoda
	Mid-day	Night	Mid-day	Night	Night
Mean flow velocity ($m \cdot s^{-1}$)	–	–	–	–	–
Mean water depth (cm)	NS	–	NS	NS	NS
Substrate diversity (Shannon's *H*)	+	+	NS	+	+
% Gravel substrate	+	+	NS	–	–
% Bedrock substrate	NS	–	+	+	+
Benthos density (number • m^{-2})	–	–	NS	+	–
E. spectabile density (number • m^{-2})	NS	NS	+	+	+

LIST OF FIGURES

Figure 1. Spatial autocorrelation (Moran's *I*) of water depth (A) and water flow velocity (B) as a function of channel lag distance in Brier Creek, southern Oklahoma, USA. Significant positive values over short lag distances, decreasing with distance before increasing once again, indicate a patchy spatial structure due to riffle and pool formation.

Figure 2. Akaike weights (w_i) of ten best models, grouped by model category, predicting area-corrected abundance (A), body condition (B) and foraging success (C) of Bigeye Shiner (*Notropis boops*) in pools of Brier Creek, southern Oklahoma, USA. (PEV = Pool Environmental Variable; UREV = Upstream Riffle Environmental Variable)

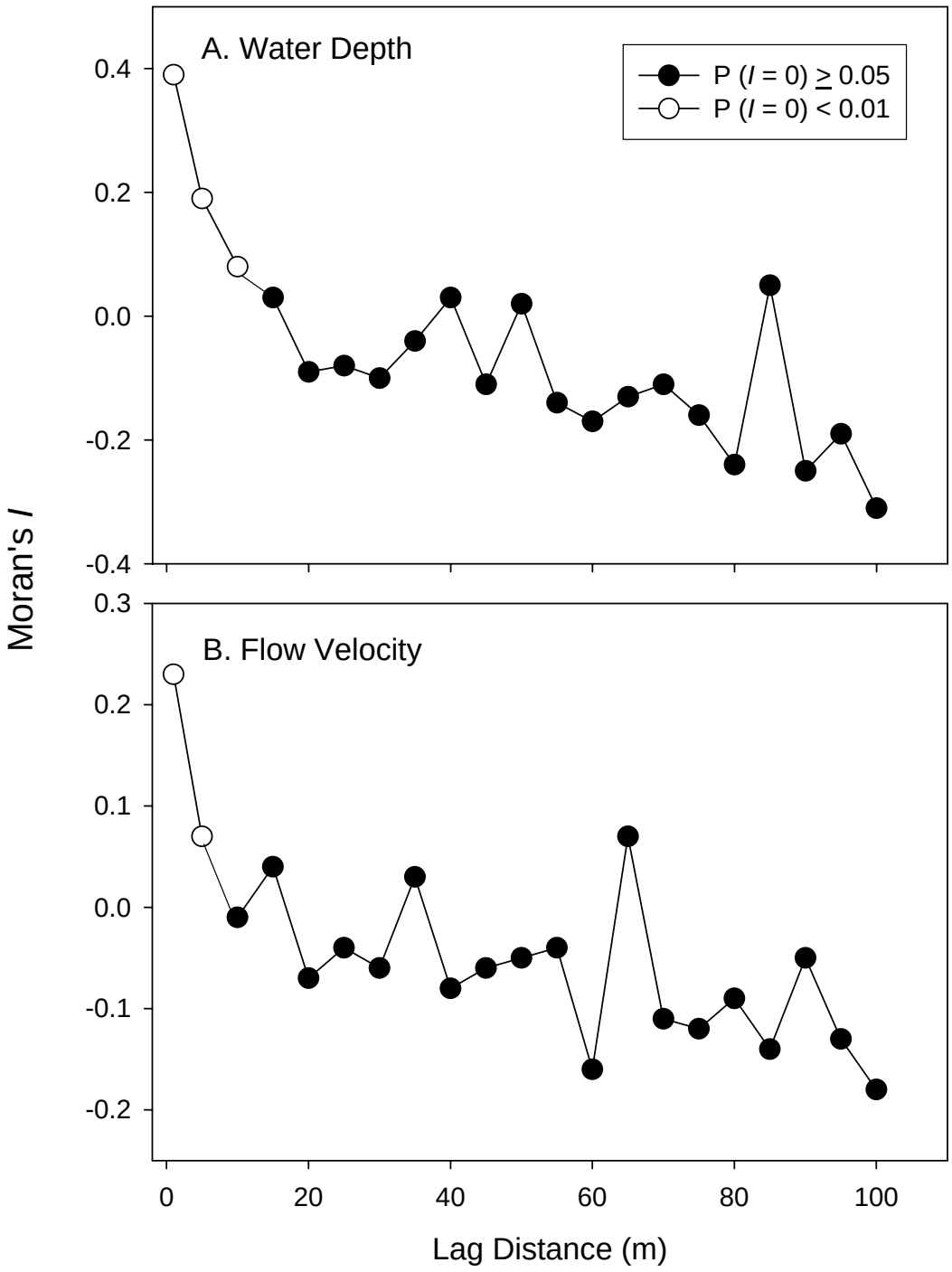

A. Water Depth
P (I = 0) ≥ 0.05
P (I = 0) < 0.01
B. Flow Velocity
Moran's I
Lag Distance (m)
0.4
0.2
0.0
-0.2
-0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
0
20
40
60
80
100

Figure 1.

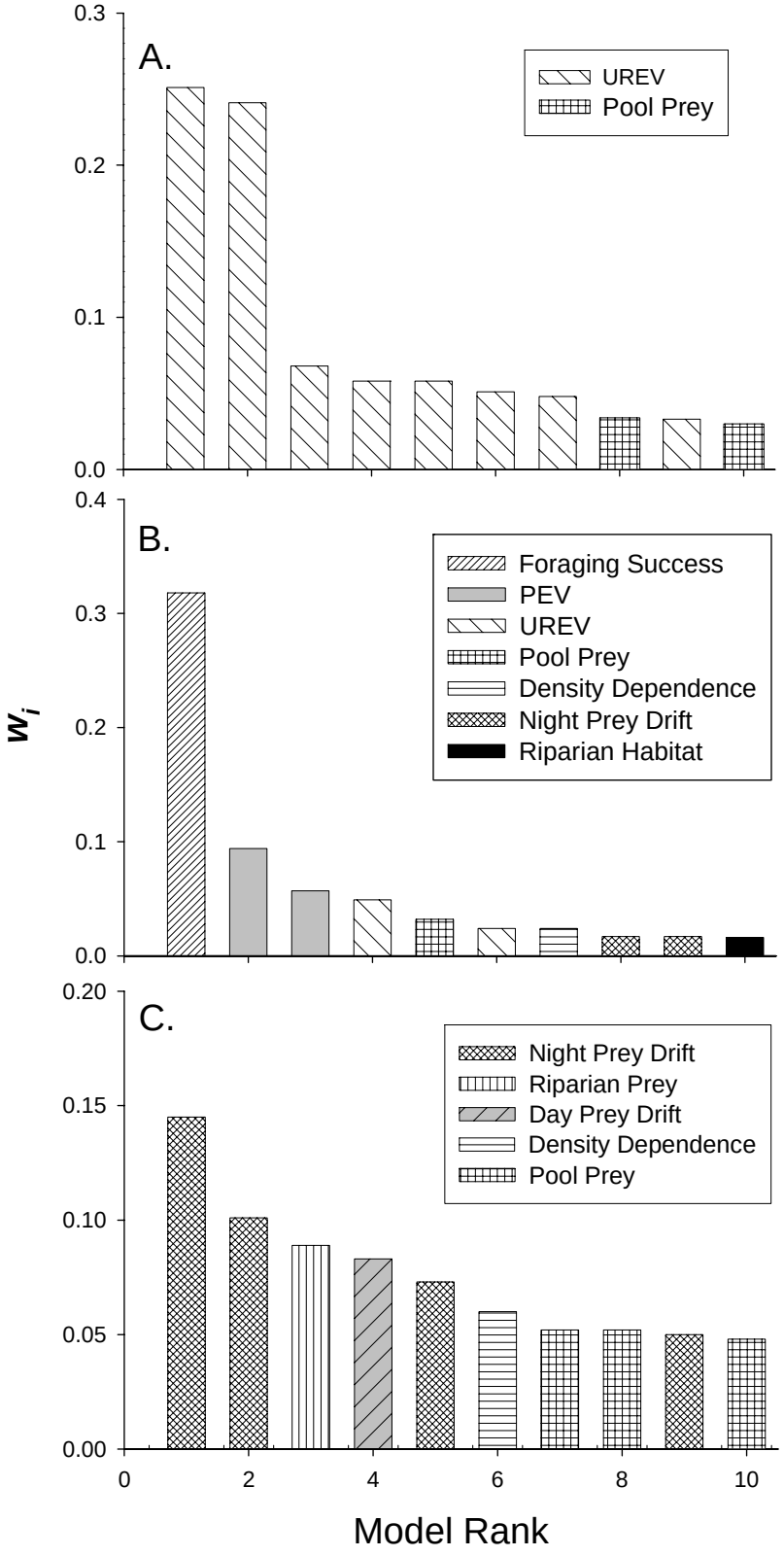

A.
UREV
Pool Prey
B.
Foraging Success
PEV
UREV
Pool Prey
Density Dependence
Night Prey Drift
Riparian Habitat
C.
Night Prey Drift
Riparian Prey
Day Prey Drift
Density Dependence
Pool Prey
w_i
Model Rank

Figure 2.

CHAPTER 2: VARIATION AMONG RIFFLES AFFECTS PREY USE AND FEEDING SELECTIVITY OF THE BENTHIC INSECTIVORE *ETHEOSTOMA SPECTABILE*

DAVID P. GILLETTE

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

ABSTRACT

Riffle-pool formation differentiates habitat in many lotic ecosystems. These habitat types possess distinctive fish and invertebrate assemblages, and may serve to sub-divide populations of organisms largely restricted to either riffles or pools. Such subdivision may lead to variation in ecological patterns driven by riffle- and pool-scale environmental variation. The present study examined prey use by the orangethroat darter (*Etheostoma spectabile*), a benthic insectivorous fish, in a midwestern (USA) riffle-pool stream. *Etheostoma spectabile* primarily occupies riffles, where it forages on benthic macroinvertebrates. I tested effects of body size and environmental variation among riffles on size and number of prey items consumed by *E. spectabile*, then examined selectivity for and against common prey items. I also compared variation in diet breadth among riffles to patterns predicted by foraging theory. Number of total prey items and of three common prey items consumed varied significantly among riffles. Overall number of prey items consumed was not affected by darter body size, and consumption of only one of seven common prey items increased with darter body size. Prey selection varied greatly among riffles, and for four of seven prey items was explained by habitat differences. Contrary to theory, diet breadth within riffles was not dependent on abundance of energetically favorable prey, largely due to a lack of selection for these prey items. These results indicate that variation among riffles can have a strong effect on prey use by *E. spectabile*, and that attempts to characterize foraging behavior over longer stream reaches may omit an important level of variation. For species restricted to small patches within larger habitats, ecological processes may be driven by local patch

characteristics more than by larger scale phenomenon, or intrinsic factors such as body size.

INTRODUCTION

Viewing habitats as collections of patches has helped ecologists realize the hierarchical structure of natural systems, and that different processes operate over different spatial scales (Wu and Loucks 1995). Although lotic ecosystems are patchy over many scales (Pringle et al. 1988, Townsend 1989), one of the strongest sources of habitat variability comes from riffle-pool formation (Richards 1976, Montgomery and Buffington 1997). In streams, invertebrate (Brown and Brussock 1991) and fish (Gelwick 1990, Taylor 1997, 2000) assemblages differ between these two habitat types. Because they alternate along a stream's length, pools are separated by riffles, and riffles by pools (Lonzarich et al. 2000). Thus, for organisms primarily limited to either of these habitat types, favorable habitat patches are separated by unfavorable patches. If this patchiness separates organisms into structured populations or sub-populations, even transiently, variation among such habitat types might create variation in ecological patterns and processes from riffle to riffle or pool to pool (Gelwick 1990, Matthews et al. 1994). Although several investigators have studied differences in fish foraging between riffles and pools (e.g., Fraser and Gilliam 1992, Bridcut and Giller 1995, Magoulick and Wilzbach 1998), the effects of variation within these habitat types (i.e., among individual riffles and pools) have not been addressed.

Optimal foraging theory (OFT; Pyke et al. 1977) states that evolution favors prey selection strategies that maximize net energy gain. One prediction of OFT is that prey types are added to a forager's diet in order of their profitability ranks, where profitability is quantified as the net energy gain acquired from consuming a prey type (Stephens and Krebs 1986). Consequently, diet breadth is predicted to increase as highly profitable

items become less and less available, and other, less profitable items are added to the diet (Werner and Hall 1974). Other variables being equal, larger prey items (within the limits of an organism's handling ability) are more energetically favorable, providing greater return for a given amount of energy spent acquiring prey (Kerr 1971, Wankowski and Thorp 1979). Accordingly, prey use varies with predator body size in many systems, as larger predators are able to overcome handling limitations and incorporate larger prey into their diets (Polis and McCormick 1986, Fisher and Dickman 1993, Wellborn 1994). Often, small predators consume small prey items, whereas large predators consume both small and large prey (Schoener and Gorman 1968, Gittleman 1985). In heterogeneous habitats, prey use can also vary among habitat patches. This variation can be the result of differences in prey abundance (Cowen 1986, Holmes and Schultz 1988, Beukers-Stewart and Jones 2004), or other factors such as habitat complexity (Gotceitas and Colgan 1989, Diehl 1992) that affect foraging. Under these conditions, prey use is constrained by local factors, resulting in spatial variation in predator diet.

For fishes, strength of the predator size-prey size relationship may vary with trophic ecology. A positive predator size-prey size relationship has repeatedly been shown for piscivores (Parsons 1971, Knight et al. 1984, Rudershausen et al. 2007), planktivores (reviewed by Zaret 1980) and drift-feeding insectivores (Bannon and Ringler 1986, Rincon and Lobon-Cervia 1999). For species with other trophic ecologies, such as benthic insectivory, the pattern is not as clear. Benthic insectivores are among the most abundant fishes in temperate streams (Matthews 1998), and can play important roles in lotic ecosystems (Dahl and Greenberg 1996, Williams et al. 2003, Hargrave et al. 2006). For these fishes, a positive body size-prey size relationship has been shown in

some studies (Matthews et al. 1982, Rakocinksi 1991, Baker 2002), but not others (Mathur 1973, Miller 1984). There are several reasons that these fishes may not follow the positive body size-prey size relationship. First, unlike water column feeders, benthic insectivores feed on the stream bottom, a structurally complex habitat that may preclude evaluation of all possible prey items. Second, many benthic fishes occupy small home ranges within a stream (Hill and Grossman 1987, Freeman 1995), so prey use may be influenced by spatial variation in prey availability and habitat.

In the present study, I examined prey use by a benthic insectivorous fish, the orangethroat darter (*Etheostoma spectabile*), in Brier Creek, southern Oklahoma. *Etheostoma spectabile* inhabits riffles, where it forages on macroinvertebrates gleaned from the substrate (Pflieger 1997, Vogt and Coon 1990). Because riffles in this system are separated by long pools containing piscivorous fishes (Power and Matthews 1983) and previous studies have shown low rates of darter movement in streams (Reed 1968, Scalet 1973, Freeman 1995, Labbe and Fausch 2000), inter-riffle movement is unlikely in the absence of high-discharge events. This creates an appropriate setting in which to examine effects of small scale habitat variation on foraging, by examining variation in prey use among darter populations on different riffles.

The objectives of this study were to test variation in *E. spectabile* diet among riffles, including patterns predicted by OFT, and to determine potential riffle attributes driving any variation. Specifically, I asked: 1. Do prey size, number of prey consumed and taxonomic prey composition vary with darter length or among riffles? 2. Is diet composition independent of variation in prey availability among riffles? 3. Do darters select for or against specific prey items? 4. Does selection for prey items vary among

riffles, and if so, is this variation explained by habitat variation? 5. Is diet breadth on individual riffles less when density of profitable prey items is higher?

METHODS

Study System

Brier Creek is a small, perennial tributary of Lake Texoma in the Red River basin of Marshall County, southern Oklahoma. A deciduous riparian zone separates the creek from ranchland, with a mix of grassland and sparse deciduous forest extending from the creek. The study reach consists of long pools separated by short, narrow riffles (Power and Matthews 1983). Substrate is primarily gravel and cobble, with exposed bedrock in some areas. Pools are occupied by *Lepomis* and *Micropterus* spp, some minnows (primarily *Notropis boops* and *Campostoma anomalum*), *Fundulus notatus*, and a few catostomids and ictalurids (Matthews et al. 1994, Matthews and Marsh-Matthews 2006). Riffles are inhabited primarily by *E. spectabile*. Detailed descriptions of Brier Creek habitat and ichthyofauna are given by Smith and Powell (1971), Ross et al. (1985), Mathews et al. (1994) and Matthews and Marsh-Matthews (2006).

Field Survey & Lab Methods

From July 30 to August 1, 2004, I took benthic invertebrate samples at four randomly selected points on each of ten riffles using a vacuum benthos sampler (Brown et al. 1987), with 600 μm mesh. Samples were preserved in the field in 70% ethanol. At mid-day on August 4, I sampled darters in each riffle by kick-seining (Matthews 1990).

Starting at the downstream end, a 3 mm mesh seine was placed across each riffle every five meters, and the substrate disturbed from 5 m upstream down to the seine. Fishes were counted, and ten individuals (or fewer when ten darters were not collected) preserved in 10% formalin. Ten individuals were collected from riffles 2 through 6 and 8 through 10; nine fishes were collected from riffles 1 and 3, and four from riffle 7.

In the laboratory, aquatic insect larvae from benthic samples were sorted to family, and other invertebrate taxa to class (Bivalvia and Gastropoda) or order (Amphipoda). To obtain mean biomass estimates for prey items, samples were grouped by taxa, dried to constant mass at 60° C, and weighed. All fishes were measured (Standard length; SL), and stomachs removed. Stomach contents were sorted to the same taxonomic level as benthic samples, and counted. Seed shrimp (Class Ostracoda) were present in fish guts, but were not counted in benthic samples, because their small size precluded efficient collection. Using an ocular micrometer, I measured head capsule width (HCW) to the nearest 30 μm for all prey taxa except sciomyzid (Diptera) larva and amphipods. Body width was measured for sciomyzids, and body length for amphipods.

I surveyed riffle habitat at 1-m points along cross-stream transects spaced every 5 m, measuring water depth and flow velocity (Marsh-McBirney flow meter), and estimating predominant substrate type on a modified Wentworth scale (Bain 1999). For water depth and flow velocity, points within each riffle were averaged to give means for each riffle. The number of points where a particular substrate type was dominant was divided by the total number of points surveyed to give percent substrate composition for each riffle.

Evaluation of Sample Size for Diet Analysis

Darter diet studies often examine hundreds of specimens (Scalet 1972, Matthews et al. 1982, Johnson and Hatch 1991, Strange 1993). Typically, diet is quantified over multiple seasons, over multiple stream reaches, and often for multiple species. Sample sizes for a single species at a site are much less, usually on the order of 10 to 50 individuals (e.g., Mathur 1973, Cordes and Page 1980, Matthews et al. 1982, Rakocinski 1991). To achieve the objective of this study, i.e., obtain a "snapshot" of *E. spectabile* diet on 10 different riffles at one point in time, 10 fish per riffle appeared to be an appropriate sample size based on previous studies. However, to test this assumption, I carried out a sensitivity analysis on one randomly selected riffle (Riffle 10). I first examined the relationship between Percent Similarity (Renkonen's) Index (PSI) of diet composition among all possible samples for a given sample size, and the number of fish in each sample (Figure 1A). I compared sample sizes from one to five, because independent sample pairs with $N > 5$ cannot be generated from a starting point of 10 fish. A positive, decelerating relationship indicated that increasing sample size above four fish did little to increase similarity among samples, suggesting that sampling more than ten fish from a riffle was unlikely to decrease variance among samples. A positive, decelerating relationship was also observed between the number of fish in a sample and the mean PSI of diet composition between samples and the complete sample of ten fish (Figure 1B). This relationship indicates that the ten fish sample provided a good estimate of diet composition of darters on the riffle, and that smaller sample sizes approach this estimate asymptotically.

Analyses

Unless otherwise noted, analyses used SAS v.9.1 or SPSS v.12.0. To examine effects of darter body size and riffle separately, independence of these two variables is necessary. I tested this by analysis of variance (ANOVA) with riffles as treatments, and body lengths of individual darters as replicates.

To test for effects of body size on prey size, I used quantile regression (Scharf et al. 1998). Quantile regression is useful for evaluating bivariate relationships in which the slope of the upper limit of a scatterplot differs from the slope of the lower limit (Scharf et al. 1998, Dunham et al. 2002, Rudershausen et al. 2005); this is often the case for predator size-prey size relationships (Scharf et al. 2002, Rudershausen et al. 2005). Based on published estimates of the sample size necessary to calculate each quantile (Scharf et al. 1998), I selected the 5^{th}, 50^{th} and 95^{th} quantiles for regression of prey HCW against darter SL. I used ANOVA to test for differences in size of prey consumed among riffles, with riffle as treatment and prey items as replicates. Similarly, I used regression of the 10^{th}, 50^{th} and 90^{th} quantiles to test for effects of darter SL on total number of prey consumed, and ANOVA to test for differences in total number of prey consumed among riffles.

To evaluate effects of body size on diet composition, I regressed numbers of common prey items consumed against darter SL. Common prey items were those consumed by at least 10% of all darters, and included seven taxa: the dipteran family Chironomidae, the mayfly families Baetidae, Tricorythidae and Leptophlebiidae, and the caddisfly families Philopotamidae, Hydroptilidae and Hydropsychidae. I also tested for

riffle effects on numbers of common prey items consumed. For each prey item, there were several fish that did not consume any. This resulted in negatively-skewed distributions which could not be normalized via transformation, so means were compared among riffles using the non-parametric Kruskal-Wallis test.

I used ANOVA with prey taxon as treatment and mean dry weight of taxon in each benthic sample as replicates to group prey items into size categories. This analysis indicated that prey taxa differed significantly in biomass per individual ($F_{6,223} = 12.91$, $P < 0.0001$), and that they fell into two groups: small prey (chironomid, hydroptilid, and baetid larvae), and large prey (philopotamid, hydropsychid, leptophlebiid and tricorythid larvae; Figure 2).

Diet breadth for each riffle was quantified using Levins' (1968) index: Diet Breadth (B) = $1 / \sum p_i^2$ where p_i is the fraction of total prey contributed by taxon i. An index of 1.0 indicates narrow diet breadth, with a single prey type comprising the entire diet; a maximum value of N occurs when each prey taxa is equally represented in the diet and N is the total number of prey taxa available (N = 13 for this study). I used multiple regression to test for effects of large prey density on diet breadth for each riffle, including darter density and total number of darters collected from each riffle as independent variables to control for their potential effects.

To test whether proportion of common prey items consumed was a function of their relative abundance in riffles, I constructed two triangular riffle-by-prey taxa matrices, one from benthic samples (available prey), and the other from gut contents (consumed prey). Matrices were relativized such that total abundance for each riffle summed to 1, so each cell gave the relative abundance of a prey item for a given riffle for

available or consumed prey. Data were arcsine square-root transformed, and significance of the correlation between matrices evaluated using Mantel's (1967) asymptotic approximation (PC-Ord v.4.17).

I used a randomization procedure (Manly 1991) to test the null hypothesis that prey items on each riffle were consumed randomly (i.e., in proportion to their availability; H_0). First, available prey and consumed prey were pooled for each riffle, and proportion of each prey item calculated. I then took 15,000 random samples of available prey from each riffle, with N equal to the number of prey items consumed (Resampling Stats v.5.0). For each sample, I calculated the proportion contributed by each prey item. Under H_0, the proportion available (P_a) and consumed (P_c) for each prey item are expected to be equal. For prey items with $P_c > P_a$, the probability of the observed difference occurring under H_0 is given by the proportion of random samples in which proportion of the prey item was greater than P_c. If less than 0.05, this test indicates selection for that prey item. Probability of a pattern of $P_c < P_a$ occurring under H_0 is given by the proportion of random samples in which the proportion of the prey item was less than P_c. In this case, a value less than 0.05 indicates selection against that prey item. If a prey item was collected on a riffle but was not present in guts, it was counted as less than expected under H_0; if a prey item was not collected from a riffle but was present in darter guts, it was counted as more than expected under H_0. On riffle 1, hydropsychid larvae were not collected in benthos samples and did not occur in darter guts, so no analysis was done. In addition to analyses of each common prey item separately, consumption of pooled large prey was also tested. After completing analyses for each riffle, I pooled data from all riffles and carried out the same analysis.

To evaluate effects of riffle habitat on prey selectivity, I used stepwise multiple regression with backwards elimination, with criteria for variable inclusion of alpha < 0.05, and criteria for variable removal of alpha > 0.10. First, I took the proportion each prey item contributed to consumed prey for each riffle, and subtracted the proportion it contributed to available prey. This difference represents the strength of selection for or against a prey item. I then regressed this value against mean water depth, mean flow velocity, and proportion substrate composition of gravel, pebble and cobble. All proportional data were arcsine-squareroot transformed. To test whether overall feeding selectivity on a riffle was a function of per capita prey levels, I summed the absolute value of the difference between proportions consumed and proportion available for all common prey items. This value ("diet shift") represents the extent to which use of common prey items differed from random, i.e., tended towards selectivity. Lower values indicate feeding patterns closer to random use of available prey. I used the ratio of common prey item density to *E. spectabile* density as a measure of per capita prey availability in each riffle, and regressed diet shift for each riffle against this value. A significant positive regression indicates higher feeding selectivity as per capita resource levels increase.

RESULTS

A total of 337 prey items were examined from 92 fish across the ten riffles. Ephemeropteran, trichopteran and dipteran larva comprised the majority of darter prey items, although a few other taxa, particularly ostracods, were also present (Figure 3).

Darter SL did not vary significantly among riffles ($F_{9,82}$ = 1.51, P = 0.16), allowing independent evaluation of riffle and body size effects.

Darter size did not affect the lower bound (P = 1.00) or median (P = 0.44) of prey size consumed. However, the upper bound of consumed prey size decreased as darter size increased (P = 0.05; Figure 4). Size of prey consumed varied slightly among riffles ($F_{9,326}$ = 1.75, P = 0.08). Darter size did not affect the median (P = 0.14) or upper bound (P = 0.27) of total number of prey consumed, but the lower bound decreased as darter size increased (P = 0.07; Figure 5). Slope of the best-fit line for the upper bound was steep; however, the large degree of variation within this quantile resulted in a non-significant test. Total number of prey consumed varied among riffles ($F_{9,82}$ = 2.41, P = 0.02).

Leptophlebiid mayfly larva was the only prey taxon for which its abundance in darter diet was affected by darter body size (Table 1); abundance increased with darter body size. Abundance of several prey taxa in darter diets varied significantly among riffles (Table 1).

Across all prey taxa, proportion consumed was dependent on the proportion available in each riffle (Mantel test t = 1.7, P = 0.086). Resampling results using pooled data from all riffles showed that darters selected for larva of the caddisfly families Hydroptilidae and Philopotamidae and the mayfly family Baetidae, and selected against larva of the dipteran family Chironomidae and mayfly families Tricorythidae and Leptophlebiidae (Table 2). For hydropsychid caddisfly larva, proportion of consumed and available prey did not differ significantly. Separate analyses of individual riffles produced 28 cases of prey consumed in lower proportion than their availability, 18 cases

of prey consumed in greater proportion than their availability, and 23 cases in which proportion consumed and available did not differ (Table 2). Each prey item was positively selected, negatively selected, and used in proportion to availability in at least one of the ten riffles surveyed.

For four of seven prey taxa, selectivity by darters varied with riffle habitat (Table 3). Selection for hydroptilid larva increased with water depth and percent substrate composition of gravel and cobble. Selection for both hydropsychid larva and tricorythid larva decreased as riffle depth increased. Selection for leptophlebiid larva increased as water flow, proportion gravel substrate and proportion cobble substrate decreased. Overall feeding selectivity on each riffle ("diet shift") was not a function of per capita resource levels ($F_{1,8} = 0.24$, P = 0.64).

Diet breadth varied widely across riffles, with values of Levins' B ranging from 2.7 to 7.0. However, this variation was not a function of large prey density, darter density, or number of darters collected from each riffle (best regression model $F_{1,8} = 1.05$, P = 0.34). When data were pooled for the entire study reach, large prey were consumed in lower proportion than expected under random use of available resources (P < 0.0001). On individual riffles, large prey were consumed in greater proportion than expected at only 1 out of 10 riffles, and in lesser proportion than expected in 5 riffles (Table 1). The differences between proportion of large prey available and consumed was negatively related to riffle mean water depth (Table 1), indicating stronger selection for large prey in shallower riffles.

DISCUSSION

In the present study, prey use by *E. spectabile* differed among riffles, as evidenced both by riffle effects on abundance of prey taxa consumed, and by inter-riffle variation in feeding selectivity. For most prey items, selection by darters was explained by riffle habitat variation. Effect of riffle on body size of consumed prey was marginally significant, as was a Mantel test of the relationship between prey availability and darter diet composition on each riffle. The positive body size-prey size relationship often shown for predatory fishes was not observed. Collectively, these results suggest that variation among individual riffles can strongly affect species such as *E. spectabile*, and that this is an important spatial scale on which to study foraging. In such systems, where organisms occupy isolated habitat patches, and where variation in habitat and prey availability among patches is strong, this variation may be a better predictor of foraging than factors such as body size.

This conclusion suggests that viewing rivers and streams as mosaics of smaller habitat patches can help to explain ecological processes in these systems (e.g., Pringle et al. 1988, Townsend 1989) that might otherwise go undetected if viewed only on large spatial scales. Although longitudinal gradients often dominate variation in lotic systems over large spatial scales (e.g., the River Continuum Concept; Vannote et al. 1980), riffle-pool structure can have strong effects on fishes over smaller scales (Gelwick 1990, Taylor 1997, 2000). Collectively, this suggests that hierarchical patch dynamics (Kotlier and Wiens 1990, Wu and Loucks 1995) provide a useful framework within which to view stream ecology, and results of the present study indicate that individual riffles can be an important spatial scale over which variation needs to be quantified.

Variation in darter diet among riffles did not follow predictions of foraging theory (Stephens and Krebs 1986). First, diet breadth was not a function of profitable (larger in this case) prey density, even when controlling for darter density and the number of darters collected from each riffle. Previous authors have shown predator diet breadth to be a function of the abundance of profitable prey, such that breadth is low when profitable prey are abundant, and high when low profitable prey levels necessitate incorporation of less profitable items into the diet (Charnov 1976, Goss-Custard 1977, Turner 1982, Agosto et al. 2003, Rodel et al. 2004). The mechanism behind such a pattern is positive selection for profitable prey items; prey items are not consumed in proportion to their availability. Lack of the predicted diet breadth-profitable prey abundance relationship in the present study can be traced to the lack of positive selection for profitable prey items. Of the four large prey taxa, only philopotamid caddisflies were selected for, whereas leptophlebiid and tricorythid mayflies were selected against. This suggests that darter prey selection is taxon-specific, and not based primarily on prey size. Additional work is needed to quantify the relative costs and benefits of consuming different benthic invertebrate prey taxa, many of which differ greatly in motility and other behavioral characteristics and morphology (Merritt and Cummins 1996). Such variation could affect encounter rate and prey handling time, variables assumed in this study to be equal for all prey taxa. In light of the result that selection for various prey types differed among riffles, and was associated with riffle habitat, it is possible that the relative profitability of prey items varies among riffles as a consequence of abiotic variation.

The assumption that larger prey are more energetically favorable than small prey is a simplification. In addition to energetic gain increasing with prey size, handling cost also often increases (Werner 1974). Gatz (1983) stated that bioenergetics of foraging is not well understood for benthic fishes, and the situation is still not well resolved. Rakocinski (1991), examining bioenergetics of foraging by three darter species, showed that even though handling cost increased with prey size, larger invertebrate prey items were still the most energetically favorable. Based on diet comparisons, Rakocinski (1991) concluded that small darters foraged more optimally than large darters.

Analyzing prey use separately for each riffle assumes a low rate of inter-riffle movement. Although *E. spectabile* movement has not been studied in Brier Creek, work in similar systems supports this notion, indicating that darters generally move little, although rare, long-distance movements can occur (Freeman 1995, Labbe and Fausch 2000). In addition, riffles in Brier Creek are separated by long pools containing piscivorous bass (*Micropterus* spp), likely precluding frequent inter-riffle movements by adult fish. Studying *E. spectabile*'s congener *E. radiosum* in a nearby riffle-pool system, Scalet (1973) found no inter-riffle movement of adults over time periods as long as three months. Larval drift can link riffle darter populations (Slack et al. 2004), and high stream discharge levels also likely move individuals (e.g., David and Closs 2002, Albanese et al. 2004). However, larvae were not examined in the present study, and stream discharge was near baseflow levels during and immediately before this study. Thus, it is likely that darters moved little before and during the survey period.

Diet of many fishes varies spatially with prey availability among sites (Cowen 1986, Delbeek and Williams 1988, Holbrook and Schmitt 1992, Beukers-Stewart and

Jones 2004). When they are unable to move among sites, fishes can grow less in habitats containing lower-quality prey (Henderson et al. 2004). Whether or not variation in prey use among riffles leads to variation in darter growth likely depends on the magnitude of differences in energetic benefits among riffles, and how consistent these differences remain over time, given the high levels of seasonal variation in stream invertebrate prey (Angermeier 1982, Magalhaes 1993).

Results of the present study also indicate an influence of riffle habitat on darter prey selection. Mean water depth, flow velocity and substrate composition predicted selection for mulitple prey items. Although the mechanisms for these effects were not studied here, previous studies have noted effects of habitat complexity on foraging (Gotceitas and Colgan 1989, Diehl 1992, Harrell and Dibble 2001). The stream bottom on which *E. spectabile* and other benthic-feeding fishes forage is structurally complex, consisting of particles ranging in size from silt to boulders, so it is not surprising that darter foraging varies with habitat characteristics. Although water depth and flow velocity are not direct measures of habitat structure, they have been shown to affect fish foraging (Flore et al. 2000, Asaeda et al. 2005). The potential for habitat variation to mediate effects of predation by benthic fishes on invertebrates bears further investigation, as these fishes can affect invertebrates through both direct (Dahl 1998, Williams et al. 2003) and indirect (Englund and Evander 1999, Miyasaka and Nakano 2001) pathways, leading to cascading ecosystem effects (Hargrave et al. 2006).

Several previous authors have shown a positive relationship between fish length and prey size. This relationship is well documented for water column planktivores (Zaret 1980), and drift feeding insectivorous stream fishes (Angermeier 1982, Lobon-Cervia and

Rincon 1994, Keeley and Grant 1997). In benthic-feeding stream fishes, results are more ambiguous. Miller (1984) showed that size of prey consumed by the frecklebelly madton *Noturus munitus* did not increase with body size. However, Baker's (2002) results indicated a positive prey size-predator body size relationship for the turquoise darter *Etheostoma inscriptum*. Phillips and Kilambi (1996) showed no difference in prey size consumed with body length for *E. spectabile*, logperch *Percina caprodes* and slender madtom *Noturus exilis*, whereas size of prey consumed increased with size for banded sculpin *Cottus carolinae*. Results of the present study suggest that inclusion of habitat heterogeneity data may help to describe patterns of prey use by these fishes, and that *in situ* investigations of foraging could be facilitated by controlling for habitat variation.

Because of their different feeding ecology, foraging by benthic insectivorous fishes may be better predicted by differences among habitat patches than by traditional energetics considerations. The stream bottom is structurally more complex than the water column, and many of these species show restricted movement patterns. Under such constraints, extrinsic environmental factors may have a stronger influence on prey use than intrinsic variables such as body size. Consequently, prey use by benthic insectivores may be better predicted by habitat structure and spatial variation among habitat patches, two extrinsic factors known to drive foraging behavior in many systems (Perry and Pianka 1997). Results of this study thus underscore the desirability of viewing streams at the scale of individual habitat patches, and of studying the effect of those patches on the behavior and ecology of organisms within them.

ACKNOWLEDGMENTS

I thank R. Cothran, S. Gillette, P. Jeyasingh, S. Jones and R. Remington for field assistance, and Mr. and Mrs. J. Martin for providing access to Brier Creek. W. Porter helped modify the invertebrate sampler. Funding was provided by the United States Environmental Protection Agency Science to Achieve Results fellowship program, a Howard McCarley Research Grant from the Southwestern Association of Naturalists, and by the University of Oklahoma Graduate College. The University of Oklahoma Biological Station provided housing and logistical considerations. Research was conducted under University of Oklahoma Institutional Animal Care and Use Committee permit #R03-015 to David P. Gillette (DPG), and all fishes were collected under Oklahoma Department of Wildlife Conservation Scientific Collector's Permit # 3508 to DPG. This study was submitted in partial fulfillment of requirements for the Doctor of Philosophy degree in the Department of Zoology at the University of Oklahoma. I thank B. Hoagland, M. Kaspari, J. Kelly, C. Vaughn and W. Matthews for their service on my graduate committee.

LITERATURE CITED

Agosta, S.J., D. Morton and K.M. Kuhn. 2003. Feeding ecology of the bat *Eptesicus fuscus*: 'Preferred' prey abundance as one factor influencing prey selection and diet breadth. Journal of Zoology 260: 169-177.

Albanese, B., P.L. Angermeier and S. Dorai-Raj S. 2004. Ecological correlates of fish movement in a network of Virginia streams. Canadian Journal of Fisheries and Aquatic Sciences 61: 857-869.

Angermeier, P.L. 1982. Resource seasonality and fish diets in an Illinois stream. Environmental Biology of Fishes 7: 251-264.

Asaeda, T., T.K. Vu and J. Manatunge. 2005. Effects of flow velocity on feeding behavior and microhabitat selection of the stone Moroko *Pseudorasbora parva*: A trade-off between feeding and swimming costs. Transactions of the American Fisheries Society 134: 537-547.

Bain, M.B. 1999. Substrate, p. 95–100. *In:* Aquatic Habitat Assessment: Common Methods. M. B. Bain and N. J. Stevenson (eds.). American Fisheries Society, Bethesda, Maryland.

Baker, S. 2002. Food habits and feeding electivity of the turquoise darter, *Etheostoma inscriptum*, in a Georgia piedmont stream. Journal of Freshwater Ecology 17: 385-390.

Bannon, E. and N.H. Ringler. 1986. Optimal prey size for stream resident brown trout (*Salmo trutta*): A test of predictive models. Canadian Journal of Zoology 64: 704-713.

Beukers-Stewart, B. D. and G. P. Jones. 2004. The influence of prey abundance on the feeding ecology of two piscivorous species of coral reef fish. Journal of Experimental Marine Biology and Ecology 299: 155-184.

Bridcut, E.E. and P.S. Giller. 1995. Diet variability and foraging strategies in brown trout (*Salmo trutta*): An analysis from subpopulations to individuals. Canadian Journal of Fisheries and Aquatic Science 52: 2543-2552.

Brown, A. V., Schram, M. D. and P.P. Brussock. 1987. A vacuum benthos sampler suitable for diverse habitats. Hydrobiologia 153: 241-247.

Brown, A.V. and P.P. Brussock. 1991. Comparisons of benthic invertebrates between riffles and pools. Hydrobiologia 22:99-108.

Charnov, E.L. 1976. Optimal foraging: Attack strategy of a mantid. American Naturalist 110: 141-151.

Cordes, L.E. and L.M. Page. 1980. Feeding chronology and diet composition of two darters (Percidae) in the Iroquois River system, Illinois. American Midland Naturalist 104: 202-206.

Cowen, R. K. 1986. Site-specific differences in the feeding ecology of the California Sheephead, *Semicossyphus Pulcher* (Labridae). Environmental Biology of Fishes 16: 1-3.

Dahl, J. and L. Greenberg. 1996. Impact on stream benthic prey by benthic vs drift feeding predators: A meta-analysis. Oikos 77: 177-181.

Dahl, J. 1998. Effects of a benthivorous and a drift-feeding fish on a benthic stream assemblage. Oecologia 116: 426-432.

David, B.O. and G.P. Closs. 2002 Behavior of a stream-dwelling fish before, during, and after high-discharge events. Transactions of the American Fisheries Society 131: 762-771.

Davies, N.B. 1977. Prey selection and search strategy of spotted flycatcher (*Muscicapa striata*): Field study on optimal foraging. Animal Behaviour 25: 1016-1033.

Delbeek, J.C. and D.D. Williams. 1988. Feeding selectivity of four species of sympatric stickleback in brackish-water habitats in eastern Canada. Journal of Fish Biology 32: 41-62.

Diehl, S. 1992. Fish predation and benthic community structure: The role of omnivory and habitat complexity. Ecology 73:1646-1661.

Dunham, J. B., B.S. Cade and J.W. Terrell. 2002. Influences of spatial and temporal variation on fish-habitat relationships defined by regression quantiles. Transactions of the American Fisheries Society 131: 86–98.

Emlen, J.M. 1966. Role of time and energy in food preference. American Naturalist 100: 611-617.

Englund, G. and D. Evander. 1999. Interactions between sculpins, net-spinning caddis larvae and midge larvae. Oikos 85: 117-126.

Fisher, D.O. and C.R. Dickman. 1993. Body size-prey size relationships in insectivorous marsupials: Tests of three hypotheses. Ecology 74: 1871-1883.

Flore, L., W. Reckendorfer and H. Keckeis. 2000. Reaction field, capture field, and search volume of 0+ nase (*Chondrostoma nasus*): Effects of body size and water velocity. Canadian Journal of Fisheries and Aquatic Sciences 57: 342-350.

Fraser, D.F. and J.F. Gilliam. 1992. Nonlethal impacts of predator invasion: Facultative suppression of growth and reproduction. Ecology 73: 959-970.

Freeman, M.C. 1995. Movements by two small fishes in a large stream. Copeia 1995: 361-367.

Gatz, A.L. Jr. 1983. Do stream fishes forage optimally in nature? Pages 392- 402 *in* T.D. Fontaine III and S.M. Bartell, editors. Dynamics of lotic ecosystems. Ann Arbor Science, Ann Arbor, MI.

Gelwick, F.P. 1990. Longitudinal and temporal comparison of riffle and pool fish assemblages in a northeastern Oklahoma Ozark stream. Copeia 1990: 1072-1082.

Gittleman, J.L. 1985. Carnivore body size: Ecological and taxonomic correlates. Oecologia 67: 540-554.

Goss-Custard, J.D. 1977. Optimal foraging and size selection of worms by Redshank, *Tringa totanus*, in the field. Animal Behaviour 25: 10-29.

Gotceitas, V and P. Colgan. 1989. Predator foraging success and habitat complexity: Quantitative test of the threshold hypothesis. Oecologia 80: 158-166.

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.

Harrel, S.L. and E.D. Dibble. 2001. Foraging efficiency of juvenile bluegill, *Lepomis macrochirus*, among different vegetated habitats. Environmental Biology of Fishes 62: 441-453.

Henderson, B.A., G.E. Morgan and A. Vaillancourt. 2004. Growth, ingestion rates and metabolic activity of walleye in lakes with and without lake herring. Journal of Fish Biology 65: 1270-1282.

Hill, J. and G.D. Grossman. 1987. Home range estimates for three North American stream fishes. Copeia 1987: 376-380.

Holbrook, S.J and R.J. Schmitt. 1992. Causes and consequences of dietary specialization in surfperches: Patch choice and intraspecific competition. Ecology 73: 402-412.

Holmes, R.T. and J.C. Schultz. 1988. Food availability for forest birds: Effects of prey distribution and abundance on bird foraging. Canadian Journal of Zoology 66: 720-728.

Johnson, J.D. and J.T. Hatch. 1991. Life-history of the least darter *Etheostoma microperca* at the northwestern limits of its range. American Midland Naturalist 125: 87-103.

Keeley, E.R. and J.W.A. Grant. 1997. Allometry of diet selectivity in juvenile Atlantic Salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 54: 1894-1902.

Kerr, S.R. 1971. Prediction of growth efficiency in nature. Journal of the Fisheries Research Board of Canada 28**:** 809–814.

Knight, R. L., F. J. Margraf, and R. F. Carline. 1984. Piscivory by walleyes and yellow perch in western Lake Erie. Transactions of the American Fisheries Society 113: 677-693.

Kotliar, N. and J.A. Wiens. 1990. Multiple scales of patchiness and patch structure: A hierarchical framework for the study of heterogeneity. Oikos 59: 253-260.

Labbe, T.R. and K.D. Fausch. 2000. Dynamics of intermittent stream habitat regulate persistence of a threatened fish at multiple scales. Ecological Applications 10: 1774-1791.

Levins, R. 1968. Evolution in changing environments: Some theoretical explorations. Princeton Monographs in Population Biology no. 2. Princeton University Press, Princeton, N.J.

Lobon-Cervia, J. and P.A. Rincon. 1994. Trophics ecology of red roach (*Rutilus arcasii*) in a seasonal stream: An example of detritivory as a feeding tactic. Freshwater Biology 32: 123-132.

Lonzarich, D., M. Lonzarich and M. Warren. 2000. Effects of riffle length on the short-term movement of fishes among stream pools. Canadian Journal of Fisheries and Aquatic Sciences 57: 1508-1514.

Magalhaes, M. 1993. Feeding of an Iberian stream cyprinid assemblage: Seasonality of resource use in a highly variable environment. Oecologia 96: 253-260.

Magoulick, D.D. and M.A. Wilzbach, MA. 1998. Effect of temperature and macrohabitat on interspecific aggression, foraging success, and growth of brook trout and rainbow trout pairs in laboratory streams. Transactions of the American Fisheries Society 127: 708-717.

Manly, B.F.J. 1991. Randomization and Monte Carlo methods in biology, revised edn. London: Chapman and Hall.

Mantel, N. 1967. The detection of disease-clustering and generalized regression approach. Cancer Research 27: 209-220.

Mathur, D. 1973. Food habits and feeding chronology of Black-Banded Darter, *Percina nigrofasciata* (Agassiz) in Halawakee Creek, Alabama. Transactions of the American Fisheries Society 102: 48-55.

Matthews, W.J. 1990. Spatial and temporal variation in fishes of riffle habitats: A comparison of analytical approaches for the Roanoke River. American Midland Naturalist 124: 31-45.

Matthews, W.J., B.C. Harvey and M.E. Power. 1994. Spatial and temporal patterns in the fish assemblages of individual pools in a midwestern stream (USA). Environmental Biology of Fishes 39: 381-397.

Matthews, W.J. 1998. Patterns in Freshwater Fish Ecology. Chapman and Hall, New York. 756 pp.

Matthews, W.J., J.R. Bek and E. Surat. 1982. Comparative ecology of the darters *Etheostoma podomestone*, *Etheostoma flabellare* and *Percina roanoka* in the upper Roanoke River drainage, Virginia. Copeia 4: 805-814.

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

Miller, G.L. 1984. Trophic ecology of the Frecklebelly Madtom *Noturus munitus* in the Tombigbee River, Mississippi. American Midland Naturalist 111: 8-15.

Mittelbach, G.G. 1981. Foraging efficiency and body size: A study of optimal diet and habitat use by bluegills. Ecology 62: 1370-1386.

Miyasaka, H. and S. Nakano. 2001. Drift dispersal of mayfly nymphs in the presence of chemical and visual cues from diurnal drift-and nocturnal benthic-foraging fishes. Freshwater Biology 46: 1229-1237.

Montgomery, D.R. and J.M. Buffington. 1997. Channel-reach morphology in mountain drainage basins. Geological Society of America Bulletin 109: 596-611.

Parsons, J.W. 1971. Selective food preferences of walleyes of the 1959 year class in Lake Erie. Transactions of the American Fisheries Society 100: 474-485.

Perry, G. and E.R. Pianka. 1997. Animal foraging: Past, present and future. Trends in Ecology & Evolution 12: 360-364.

Pflieger, W.L. 1997. The fishes of Missouri. Revised edition. Missouri Department of Conservation. Jefferson City, MO.

Phillips, E.C. and R.V. Kilambi. 1996. Food habits of four benthic fish species (*Etheostoma spectabile*, *Percina caprodes*, *Noturus exilis*, *Cottus carolinae*) from northwest Arkansas streams. Southwestern Naturalist 41: 69-73.

Polis, G. and S.J. McCormick. 1986. Patterns of resource use and age structure among species of desert scorpion. Journal of Animal Ecology 55: 59-73.

Power, M.E. and W.J. Matthews. 1983. Algae-grazing minnow (*Campostoma anomalum*), piscivorous bass (*Micropterus* spp), and the distribution of attached algae in a small prairie-margin stream. Oecologia 60: 328-332.

Pringle, C., R. Naiman, G. Bretschko, J. Karr, M. Oswood, J. Webster, R. Welcomme and M. Winterbourne. 1988. Patch dynamics in lotic systems: The stream as a mosaic. Journal of the North American Benthological Society 7: 503-524.

Pyke, G.H., H.R. Pulliam and E.L. Charnov. 1977. Optimal foraging: Selective review of theory and test. Quarterly Review of Biology 52: 137-154.

Rakocinski, C. 1991. Prey size relationships and feeding tactics of primitive stream-dwelling darters. Canadian Journal of Fisheries and Aquatic Science 48: 681-693.

Reed, R.J. 1968. Mark and recapture studies of eight species of darters (Pisces: Percidae) in three streams of northwestern Pennsylvania. Copeia 1968: 173-175.

Richards, K.S. 1976. Morphology of riffle-pool sequences. Earth Surface Processes and Landforms 1: 71-88.

Rincon, P.A. and J. Lobon-Cervia. 1999. Prey-size selection by Brown Trout (*Salmo trutta* L.) in a stream in northern Spain. Canadian Journal of Zoology 77: 755-765.

Rodel, H.G., W. Volkl and H. Kilias. 2004. Winter browsing of brown hares: Evidence for diet breadth expansion. Mammalian Biology 69: 410-419.

Ross, S.T., W.J. Matthews and A.A. Echelle. 1985. Persistence of stream fish assemblages: Effects of environmental change. American Naturalist 126: 24-40.

Rudershausen, P.J., J.E. Tuomikoski, J.A. Buckel and J.E. Hightower. 2005. Prey selectivity and diet of striped bass in western Albemarle Sound, North Carolina Transactions of the American Fisheries Society 134: 1059-1074.

Scalet, C.G. 1973. Stream movements and population density of the Orangebelly Darter, *Etheostoma radiosum cyanorum* (Osteichthyes: Percidae). The Southwestern Naturalist 17: 381-387.

Scalet, C.G. 1972. Food habits of Orangebelly Darter, *Etheostoma radiosum cyanorum* (Osteichthyes: Percidae). American Midland Naturalist 87: 515-522.

Scharf, F.S., F. Juanes and M. Sutherland. 1998. Inferring ecological relationships from the edges of scatter diagrams: Comparison of regression techniques. Ecology 79: 448-460.

Scharf, F.S., J.A. Buckel and F. Juanes. 2002. Size-dependent vulnerability of juvenile bay anchovy *Anchoa mitchilli* to bluefish predation: Does large body size always provide a refuge? Marine Ecology-Progress Series 233: 241-252.

Schoener, T.W. and G.C. Gorman. 1968. Some niche differences in three lesser Antillean lizards of genus *Anolis*. Ecology 49: 819-830.

Slack, W.T., S.T. Ross and J.A. Ewing. 2004. Ecology and population structure of the bayou darter, *Etheostoma rubrum*: disjunct riffle habitats and downstream transport of larvae. Environmental Biology of Fishes 71: 151-164.

Smith, C. and C. Powell. 1971. The summer fish communities of Brier Creek, Marshall County, Oklahoma. American Museum Novitates 245: 1-30.

Stephens, D.W. and J.R. Krebs. 1986. Foraging Theory. Monographs in Behavior and Ecology, J.R Krebs and T. Clutton-Brock, eds. Princeton University Press, Princeton, N.J., USA.

Taylor, C. M. 1997. Fish species richness and incidence patterns in isolated and connected stream pools: Effects of pool volume and spatial position. Oecologia 110: 560-566.

Taylor, C.M. 2000. A large-scale comparative analysis of riffle and pool fish communities in an upland stream system. Environmental Biology of Fishes 58: 89-95.

Townsend, C. 1989. The patch-dynamics concept of stream fish ecology. Journal of the North American Benthological Society 8: 36-50.

Vannote, R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell and C.E. Cushing. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37: 130-137.

Vogt, G.F. and T.G. Coon. 1990. A comparison of the foraging behavior of two darter (*Etheostoma*) species. Copeia 1990: 41-49.

Wankowski, J.W.J., and J.E. Thorpe. 1979. The role of food particle size in the growth of juvenile Atlantic Salmon (*Salmo salar* L.). Journal of Fish Biology 14: 351–370.

Wellborn, G. A. 1994. Size-biased predation and prey life histories: A comparative study of freshwater amphipod populations. Ecology 75: 2104-2117.

Werner, E.E. 1974. The fish size, prey size, handling time relation in several sunfishes and some implications. Journal of the Fisheries Research Board of Canada. 31: 1531-1536.

Werner, E.E. and D.J. Hall. 1974. Optimal foraging and size selection of prey by Bluegill Sunfish (*Lepomis macrochirus*). Ecology 55: 1042-1052.

Williams, L.R., C.M. Taylor and M.L. Warren. 2003. Influence of fish predation on assemblage structure of macroinvertebrates in an intermittent stream. Transactions of the American Fisheries Society 132: 120-130.

Wu, J. and O. Loucks. 1995. From balance of nature to hierarchical patch dynamics: A paradigm shift in ecology. The Quarterly Review of Biology 70: 439-466.

Zaret, T.M. 1980. Predation and freshwater communities. New Haven, CT: Yale University Press. 187 pp.

Table 1. Results of testing for differences in abundance of common prey items in *Etheostoma spectabile* diet by body size (standard length) and by riffle. Body size effects tested using linear regression, and riffle effects using Kruskal-Wallis test. Results significant at $\alpha = 0.05$ denoted by an asterisk (*).

	Body Size Effects			Riffle Effects	
Prey Item	*F*	P (*F*=0)	*b*	χ^2	P (χ^2=0)
Chironomidae	0.038	0.85	--	20.29	0.026*
Hydroptilidae	0.124	0.73	--	8.79	0.46
Hydropsychidae	0.068	0.80	--	12.65	0.18
Philopotamidae	4.74	0.03*	0.22	14.21	0.12
Tricorythidae	1.12	0.29	--	25.58	0.002*
Baetidae	0.91	0.34	--	34.21	<0.001*
Leptophlebiidae	0.25	0.62	--	10.96	0.28

Table 2. Feeding selectivity of *Etheostoma spectabile* for common prey items. Combined results include all fishes and prey items from all riffles; items consumed in lower proportion than expected under H_0: random use of available prey denoted by a minus (-), and those consumed in greater proportion than expected by a plus (+). Tests significant at $\alpha = 0.05$ denoted by an asterisk (*). Large prey taxa denoted by a dagger (†). Results by riffle indicate the number of riffles in which a prey item was consumed in lower or greater proportion than expected under H_0. Note: Hydropsychid larva were not collected from riffle 1 or present in darter guts from this riffle, giving a total of only nine riffles tested for this prey item.

Prey Item		Combined		By Riffle		
Order	Family	Direction	*P*	Lower	NS	Greater
Diptera	Chironomidae	-	<0.001*	4	5	1
Trichoptera	Hydroptilidae	+	0.017*	2	5	3
Trichoptera	Hydropsychidae†	+	0.413	2	4	3
Trichoptera	Philopotamidae†	+	<0.001*	3	3	4
Ephemeroptera	Tricorythidae†	-	0.043*	7	1	2
Ephemeroptera	Baetidae	+	<0.001*	2	4	4
Ephemeroptera	Leptophlebiidae†	-	<0.001*	8	1	1
Large Prey		-	<0.001*	5	4	1

Table 3. Best-fit stepwise multiple regression models predicting differences between proportion consumed and proportion available for common prey items as a function of riffle habitat.

	Model Analysis of Variance			Independent Variable(s)	
Prey Item	*F*	P (*F*=0)	R^2	Habitat Variable	*b*
Chironomidae	$F_{1,8} = 2.77$	0.14	0.16	--	
Hydroptilidae	$F_{3,6} = 4.66$	0.05	0.55	Gravel substrate	0.78
				Cobble substrate	0.61
				Water depth	0.66
Hydropsychidae	$F_{1,8} = 4.49$	0.07	0.28	Water depth	-0.60
Philopotamidae	$F_{1,8} = 1.57$	0.25	0.06	--	
Tricorythidae	$F_{1,8} = 8.66$	0.02	0.46	Water depth	-0.72
Baetidae	$F_{1,8} = 1.90$	0.21	0.09	--	
Leptophlebiidae	$F_{3,6} = 11.47$	0.01	0.78	Water flow	-0.36
				Gravel substrate	-1.18
				Cobble substrate	-0.80

LIST OF FIGURES

Figure 1. Results of sensitivity analysis using gut contents data for Riffle 10, showing the positive decelerating relationships between number of fish in a sample and mean Percent Similarity Index (PSI) among all possible independent samples (Panel A), and number of fish in a sample and the PSI of all possible independent samples compared to the sample of ten fish (Panel B). Best-fit second order polynomial regression lines are shown; error bars indicate standard deviation.

Figure 2. Mean and standard error dry mass per individual for common prey taxa in riffles of Brier Creek, Oklahoma. Prey items sharing lowercase letters do not differ significantly. Items sharing a lowercase "a" were classified as "small" prey, and those sharing a lowercase "b" as "large" prey.

Figure 3. Mean and standard error abundance of invertebrate prey taxa consumed by *Etheostoma spectabile* (A) and available (B) in riffles of Brier Creek, Oklahoma. Abundance of available bivalves and ostracods were not quantified; these prey items are denoted by an asterisk (*) on panel B.

Figure 4. Relationship between prey head capsule width and *Etheostoma spectabile* standard length, showing best fit lines obtained using quantile regression. Slope of the 5th (lower dashed line; P = 1.00) and 50th (solid line; P = 0.44) quantiles did not differ significantly from zero. Slope of the 95th quantile (upper dashed line) was marginally significant (P = 0.05).

Figure 5. Relationship between number of prey items consumed and darter standard length, showing best fit lines obtained using quantile regression. Slope of the 50th (solid line; P = 0.14) and 90th (upper dashed line; P = 0.27) quantiles did not differ significantly from zero. Slope of the 10th quantile (lower dashed line) was marginally significant (P = 0.07).

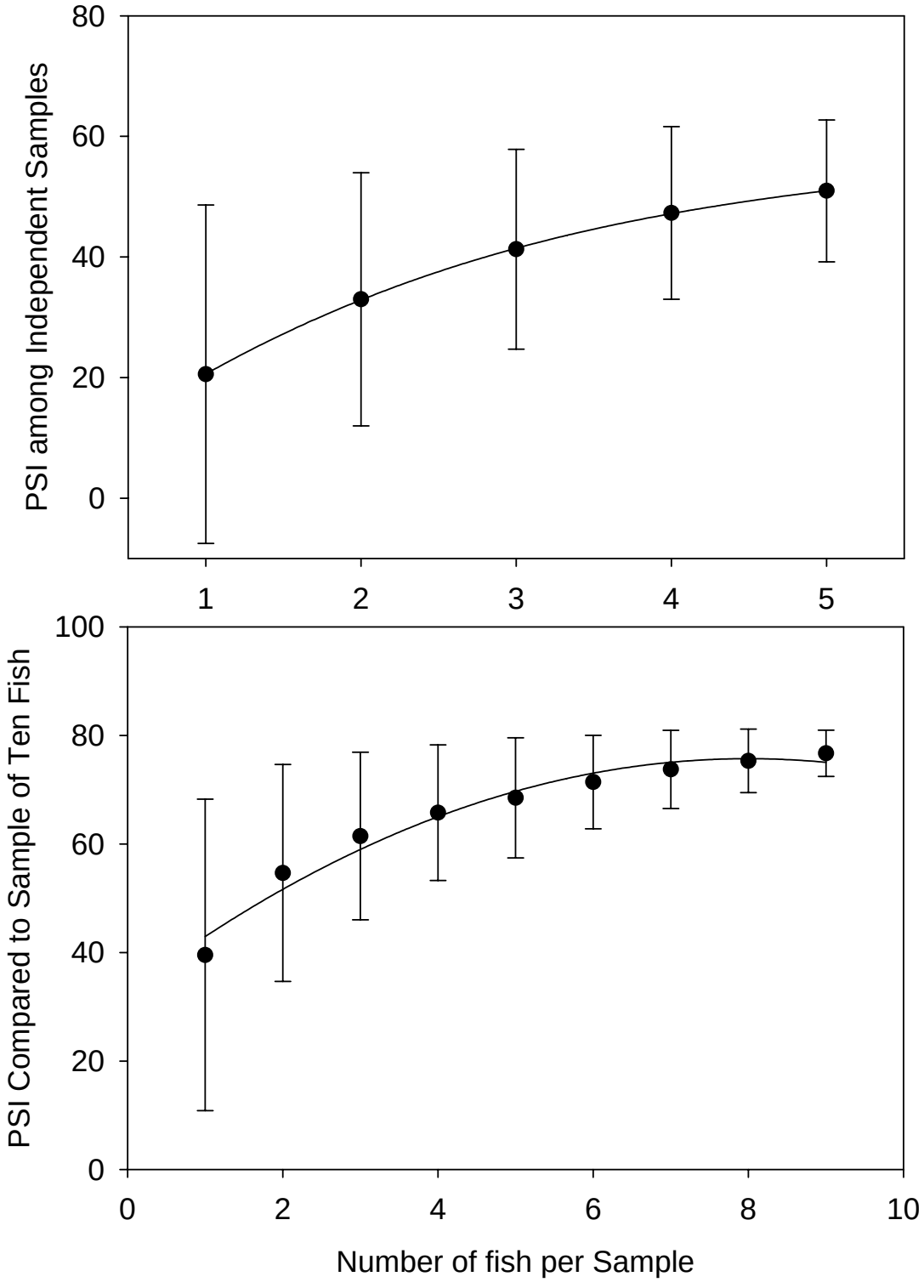

PSI among Independent Samples
80
60
40
20
0
1
2
3
4
5
PSI Compared to Sample of Ten Fish
100
80
60
40
20
0
0
2
4
6
8
10
Number of fish per Sample

Figure1.

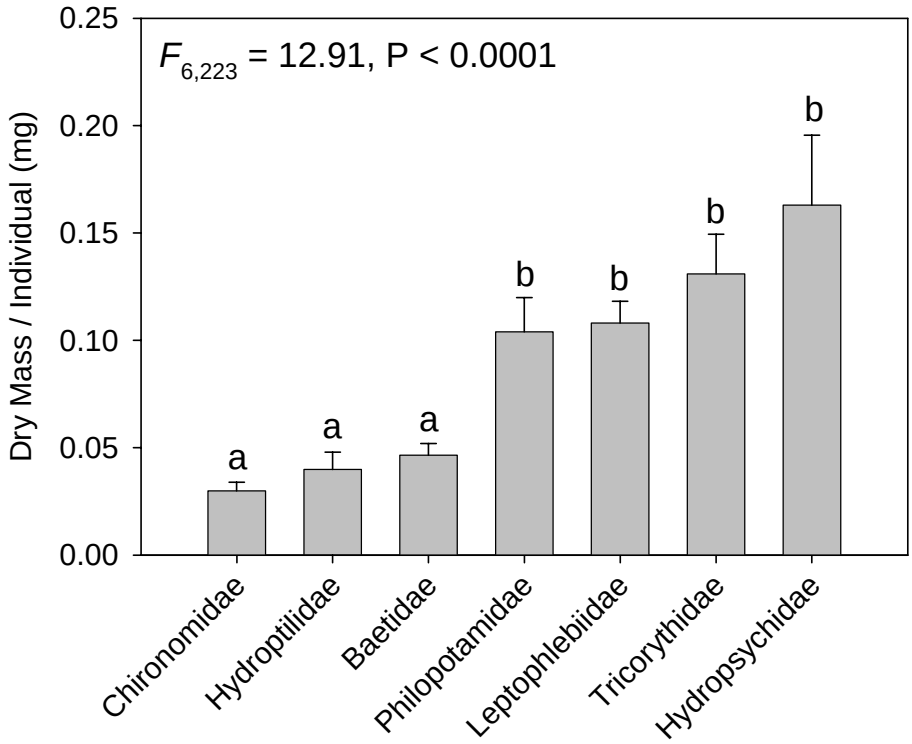

$F_{6,223}$ = 12.91, P < 0.0001
Dry Mass / Individual (mg)
0.25
0.20
0.15
0.10
0.05
0.00
a
a
a
b
b
b
b
Chironomidae
Hydroptilidae
Baetidae
Philopotamidae
Leptophlebiidae
Tricorythidae
Hydropsychidae

Figure 2.

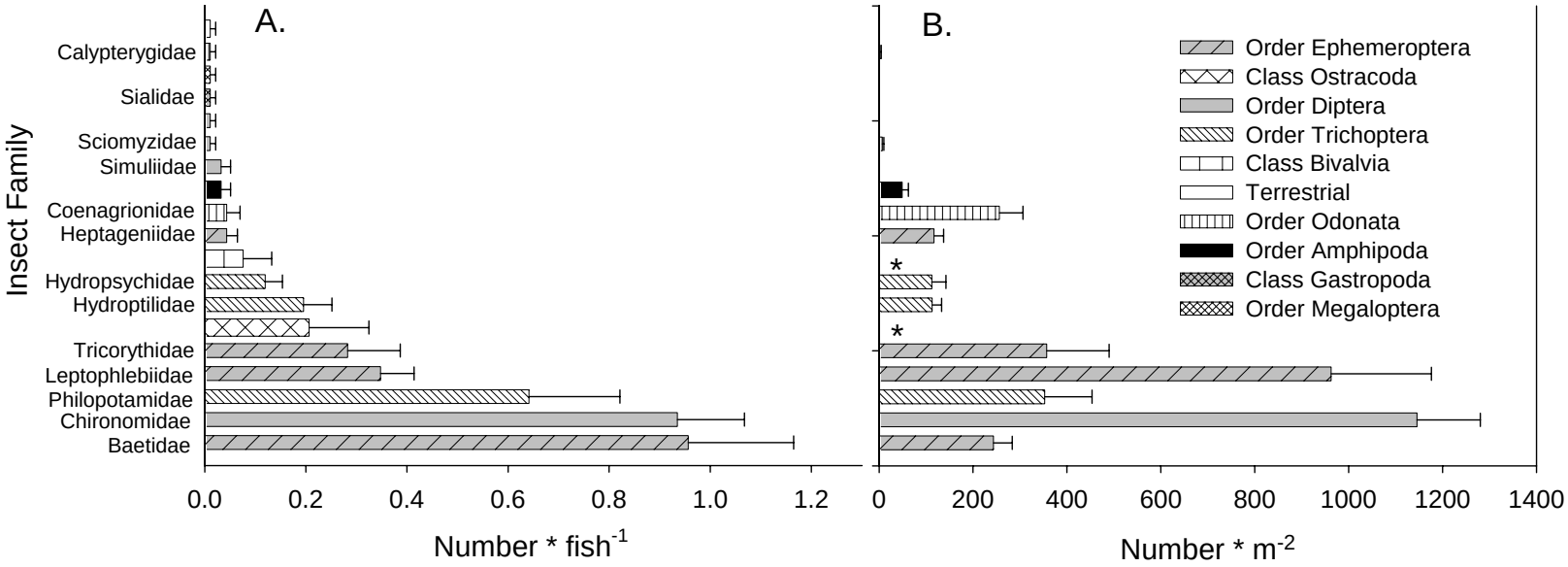

A.
B.
Insect Family
Calypterygidae
Sialidae
Sciomyzidae
Simuliidae
Coenagrionidae
Heptageniidae
Hydropsychidae
Hydroptilidae
Tricorythidae
Leptophlebiidae
Philopotamidae
Chironomidae
Baetidae
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Number * fish-1
0
200
400
600
800
1000
1200
1400
Number * m-2
Order Ephemeroptera
Class Ostracoda
Order Diptera
Order Trichoptera
Class Bivalvia
Terrestrial
Order Odonata
Order Amphipoda
Class Gastropoda
Order Megaloptera

Figure 3.

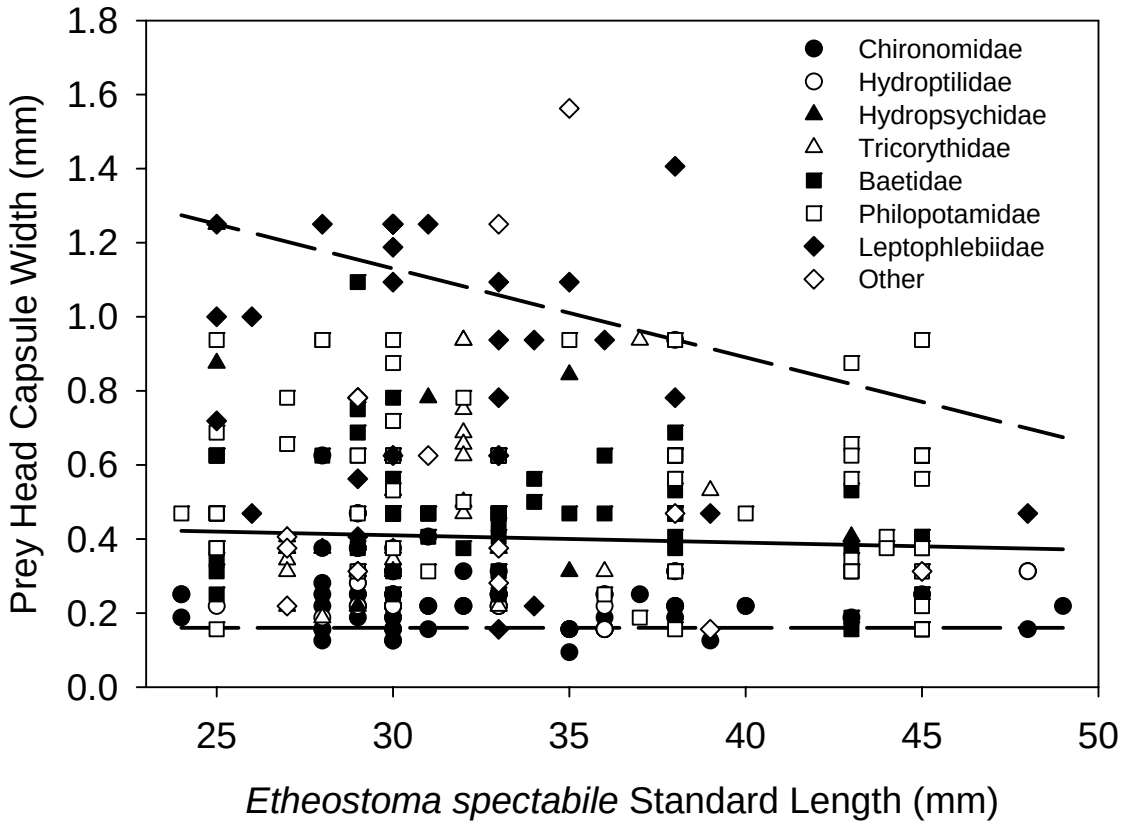

Prey Head Capsule Width (mm)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
25
30
35
40
45
50
Etheostoma spectabile Standard Length (mm)
Chironomidae
Hydroptilidae
Hydropsychidae
Tricorythidae
Baetidae
Philopotamidae
Leptophlebiidae
Other

Figure 4.

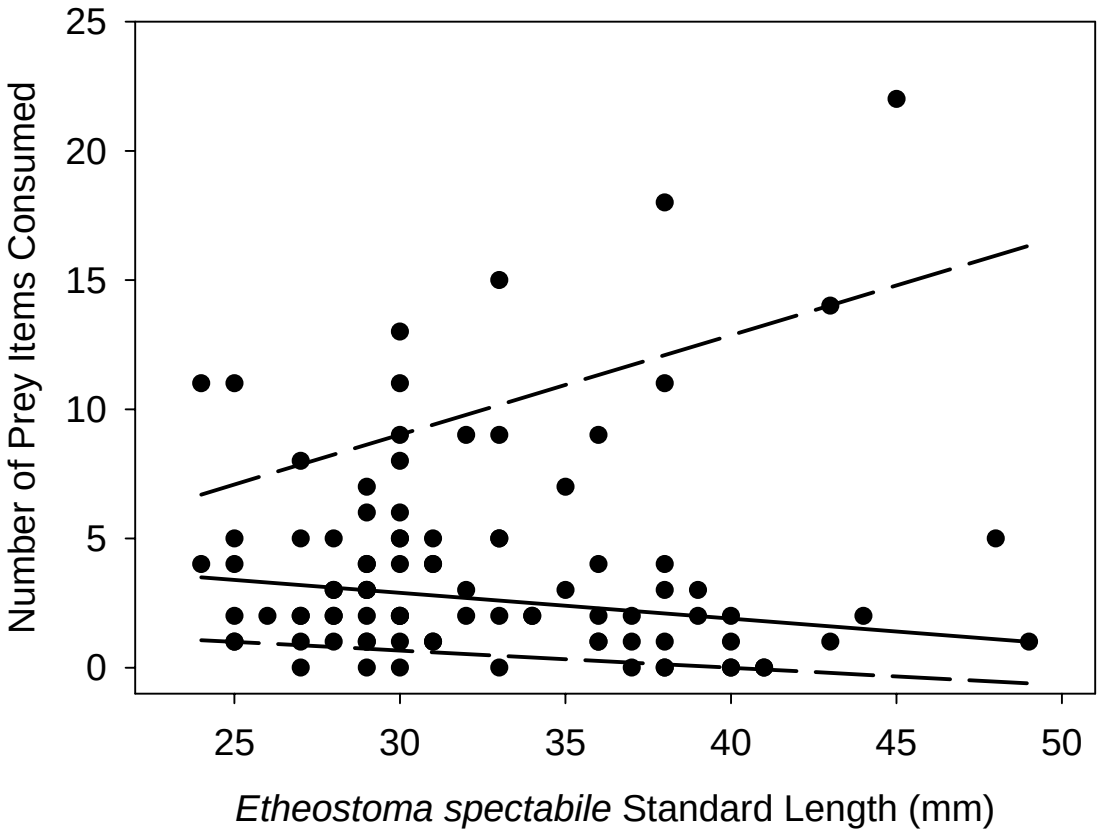

25
20
15
10
5
0
Number of Prey Items Consumed
25
30
35
40
45
50
Etheostoma spectabile Standard Length (mm)

Figure 5.

CHAPTER 3: EFFECTS OF TROPHIC SUBSIDY EXCLUSION ON CONSUMER FITNESS: AN EXPERIMENTAL TEST USING STREAM FISHES

DAVID P. GILLETTE

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

ABSTRACT

Trophic subsidies are important to consumers in many systems, and are an important means of connecting consumers in one habitat to resources derived from another. Despite the importance of trophic subsidies, there is little information on how these subsidies affect consumer fitness, and the extent to which species or functional groups in recipient habitats are affected to differing degrees. In the present study, I manipulated levels of terrestrial invertebrate input into experimental streams, and examined effects on three stream fishes differing in trophic ecology, using body condition as a fitness surrogate. Three species were studied, a terrestrial invertebrate specialist (Blackstripe Topminnow *Fundulus notatus*), an aquatic benthic invertebrate specialist (Orangethroat Darter *Etheostoma spectabile*), and a generalist invertivore (Bigeye Shiner *Notropis boops*). Although both *F. notatus* and *N. boops* shifted diets away from terrestrial invertebrates in the exclusion treatment, only *F. notatus* showed a decrease in body condition, likely because of its lesser ability to use aquatic resources. Analyses using stable isotopes showed that *Notropis boops* was the only species with a shifted $\delta^{13}C$ signature under terrestrial invertebrate exclusion, indicative of a trophic shift from a terrestrial to an aquatic carbon source. *Etheostoma spectabile* diet and body condition were unaffected by subsidy manipulation. These results indicate that species in diverse assemblages are not affected equally by alteration of trophic subsidy levels, but that effects may be predictable based on trophic ecology. Such information is useful in predicting patterns of assemblage change with spatial and temporal variation in trophic subsidy levels, which often change as humans alter landscapes.

INTRODUCTION

Trophic subsidies (input of resources from outside the boundaries of one system) are crucial to the functioning of many ecosystems, linking consumers to resources in adjacent habitats (Polis et al. 1997, Baxter et al. 2005). Such subsidies can drive local consumer abundance near ecotones, as demonstrated by numerical responses of drift-feeding fish (Kawaguchi et al. 2003), insectivorous birds (Davies 1976, Sweeney and Vannote 1982, Uesugi and Murakami 2007), bats (Power and Rainey 2000, Power et al. 2004), lizards (Sabo and Power 2002a) and spiders (Kato et al. 2003), and scavengers such as canines (Rose and Polis 1998, Roth 2003). Functional responses to changing subsidy levels have also been documented for many of these consumers (e.g., Nakano et al. 1999, Roth 2002, Waltham and Connolly 2006).

Despite their importance, little is known about how these subsidies affect consumer fitness (but see Sabo and Power 2002b), and the extent to which species or functional groups in recipient habitats are affected to differing degrees (but see Paetzold et al. 2007). Such information is important to understand food web dynamics (Polis et al. 1997), and to predict patterns of community change, as subsidy levels vary across habitats and over time. Stream fish assemblages are ideal systems in which to examine these questions. They assemblages, particularly in the central and southeastern Unites States, are functionally diverse, comprised of species feeding on different resources, or the same resources in different ways (Matthews 1998). The importance of trophic subsidies to consumers in these systems is also well-established. Consumption of terrestrial invertebrates by stream fishes has long been known (reviewed by Baxter et al.

2005), and recent experimental work has demonstrated numerical (Kawaguchi et al. 2003) and functional (Nakano et al. 1999, Baxter et al. 2004) responses of stream fishes to manipulation of terrestrial invertebrate flux into streams. Although direct quantification of fitness is difficult for any organism, body condition is well-established as a fitness proxy for fishes (Seppa et al. 2001, Berumen et al. 2005). Finally, terrestrial-aquatic subsidies flow over much smaller spatial scales than those of other donor-recipient systems (such as island-ocean ecotones), facilitating easier and more realistic experimental manipulation.

Ignoring indirect effects, there are three main scenarios under which species could be affected by subsidy reduction, depending on trophic ecology. If a species consumes subsidies when they are present, and is unable to switch resources in their absence (subsidy specialist), fitness may decrease. Alternatively, a species may avoid reduced fitness under subsidy reduction if: 1. It does not consume subsidies even when they are present (non-subsidy specialist) or, 2. It consumes subsidies when they are present, but is able to switch resources when they are absent (generalist). In the present study, I selected one fish species from each of these categories, based on published natural history accounts and personal observation. Blackstripe topminnow (*Fundulus notatus*) is a surface feeding insectivore, primarily consuming terrestrial invertebrates entering streams from the riparian zone (Thomerson and Wooldridge 1969, Atmar and Stewart 1972). With its supraterminal mouth and behavior of swimming just below the water's surface, it represents a subsidy specialist that may have difficulty switching resources. Orangethroat darter (*Etheostoma spectabile*) is a benthic insectivore, foraging on aquatic macroinvertebrates on the stream bottom (Vogt and Coon 1990, Strange 1992). With its

subterminal mouth and absent gas bladder, it represents a non-subsidy specialist, adapted for life on the stream bottom, rarely ingesting terrestrial invertebrates. Bigeye shiner (*Notropis boops*) is a water column minnow, feeding primarily on invertebrate drift composed of aquatic and terrestrial insects (Matthews et al. 2004). It represents a trophic generalist, consuming both terrestrial and aquatic invertebrates.

I hypothesized that effects of removing terrestrial invertebrate input would vary among these three species based on their trophic ecologies. Specifically, I made the three following predictions: 1. *Etheostoma spectabile* growth and body condition would not be affected, because this species does not normally feed on terrestrial subsidies; 2. *Notropis boops* growth and body condition would not be affected, because it is able to switch to aquatic resources in the absence of terrestrials; and 3. *Fundulus notatus* growth and body condition would decrease, because it feeds heavily on terrestrials, and may be unable to switch to aquatic resources when subsidies are eliminated.

METHODS

Study System Description

This experiment was conducted from November 5, 2005 to May 19, 2006, in outdoor experimental streams at the University of Oklahoma Biological Station (UOBS), Marshall County, Oklahoma (Gido and Matthews 2001, Matthews et al. 2001). See Matthews et al. (2006) for a detailed description of this experimental system. Streams were divided into 38 independent riffle-pool units containing sand, gravel and cobble substrates sculpted to create natural riffle-pool geomorphology. To equalize potential among-stream differences from previous experiments, a small amount of substrate from

each unit was mixed together on October 9, and this homogenate redistributed equally among units. Streams were filled with water on October 23, and inoculated with a slurry of algae and associated invertebrates from a local stream. Liquid fertilizer (5 ml; 12:8:8, N:P:K) was added to each unit to stimulate algal growth. Following fertilizer addition, units were left uncovered for two weeks to allow colonization by ovipositing winged adult insects.

Experimental Design

I used a factorial design with four fish species and two terrestrial invertebrate treatments. Treatments were randomly assigned to riffle-pool units. Fish treatments were *E. spectabile* (Mean Standard Length [SL] ± SD = 39.1 ± 6.0 mm), *N. boops* (57 ± 6.0 mm SL), *F. notatus* (44.8 ± 6.0 mm SL) and a fishless control. The terrestrial invertebrate treatment was either exclusion of terrestrial invertebrates, or a control. Units assigned to exclusion treatments were covered with insect screening (1.0 mm mesh); controls had the same covers suspended 40 cm above the water surface, to control for shading effects. Covers contained round holes (10 cm diameter) to allow for emergence of adult aquatic insects. Exclusion screens (3.0 mm mesh) within the water column restricted fishes to the pool portion of each unit. The upstream riffle portion was left uncovered to allow insect oviposition and drift of resulting larva into pools. Fishes were obtained from nearby streams and randomly assigned to riffle-pool units on November 5 at densities of 14 fish • m^{-2}. A subsample of each species was euthanized by an overdose of anesthetic (clove oil), and measured (Standard length, SL). At the end of the

experiment, I collected benthic invertebrate samples, subsampled fish for diet analyses, and took stable isotope samples.

Benthic macroinvertebrates were sampled with two benthic cores (78.5 cm^2 each) at randomly selected locations in each unit. Samples were pooled for each unit, preserved in 70% ethanol and sorted to family in the lab. Fish mean percent survival was calculated for each stream unit and compared for each species between treatments using a *t*-test. The same procedure was used to compare mean SL (mm) across treatments for each species. *Etheostoma spectabile* reproduced during the experiment, so survival and growth for this species were calculated after eliminating individuals from the most recent year class, by examining the length-frequency distribution, and using a 27 mm cutoff length.

Diet Comparison

At the end of the experiment, ten fish were randomly selected from each treatment for gut contents analysis. I dissected out the stomach (*E. spectabile*), or the anterior third of the digestive tract for species with no discrete stomach (*N. boops* and *F. notatus*). Gut contents were washed into a gridded Petri dish, and ingested items classified into one of seven categories: filamentous algae, detritus, dipteran chironomid larva, snails, microcrustaceans (including ostracods, copepods and cladocerans), other aquatic macroinvertebrates (non-chironomid), or terrestrial invertebrates. Diet composition was expressed as the number of grids covered by items from each prey category.

Terrestrial Insect Access and Light Intensity

I evaluated effectiveness of terrestrial invertebrate exclusion screens in early December 2005, late January 2006, and prior to ending the experiment in May 2006. Pan traps (0.065 m^2) were filled with water and surfactant and placed in ten randomly selected exclusion units and ten randomly selected control units. Sampling duration varied from four to eight days depending on sampling period, after which samples were preserved in 70% ethanol. Invertebrates were dried (60° C) and weighed, and a *t*-test used to compare mean terrestrial invertebrate input (mg dry mass • m^{-2} • day^{-1}) between exclusion and control treatments. I measured light intensity at the water surface on 6 December using a photometer (LI-Cor, Inc., Lincoln, NE). Ten units were randomly selected from both exclusion and control treatments, and mean light intensity (micromols • s^{-1} • m^{-2}) compared between treatments with a *t*-test.

Stable Isotope Analyses

At the conclusion of the experiment, I took samples of dorsal muscle tissue from each fish species, and whole chironomid larva for analysis of stable isotope ratios of carbon and nitrogen. After removal from algae-covered stones with forceps, chironomid larvae were held overnight to allow gut evacuation, then frozen. Five fish were randomly selected from each experimental treatment. Samples of fish tissue were taken from specimens previously fixed in formalin and isopropanol. This preservation process slightly alters isotopic ratios, making direct comparisons of preserved and non-preserved tissues problematic (Edwards et al. 2002). However, the magnitude of such alteration is typically small compared to levels of variation generated by natural fractionation

processes (Edwards et al. 2002). In addition, the primary comparison of interest in this study was between individuals of the same species in terrestrial exclusion and control treatments. Because all experimental fish were preserved in the same formalin, any minor alteration of isotope ratios should affect treatments equally, maintaining any differences between treatments.

Samples were dried for 48 hours at 50° C, homogenized by grinding in a mortar and pestle, transferred to tin capsules and weighed. Stable isotope ratios of carbon and nitrogen were determined using a Delta^{plus} XL mass spectrometer interfaced with a Costech Analytical ECS4010 elemental analyzer at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University. For each fish species, mean $\delta^{13}C$ and $\delta^{15}N$ values were compared between control and exclusion treatments using a *t*-test.

Body Condition

To assess body condition, soluble non-structural fats were extracted from dried, whole fish carcasses with five overnight extractions in petroleum ether (Meffe and Snelson 1993, Heulett et al. 1995). Fifteen fish were randomly selected from each treatment for analysis at the end of the study. I used Analysis of Covariance (ANCOVA) to test for effects of terrestrial exclusion on lipid mass of each species, with whole body dry mass as a covariate. In the case of a significant treatment effect and treatment-by-covariate interaction, I used Wilcox's modification of the Johnson-Neyman procedure (Wilcox 1987, Quinn and Keough 2002) to determine the range of the covariate over which the treatment effect was significant. This procedure compares groups in a pair-

wise manner along the range of the covariate, adjusting probability levels for the number of comparisons.

RESULTS

For all three sampling periods, mean biomass of terrestrial invertebrate input was greater for uncovered units (Figure 1). However, this difference was only significant in May (t_{18} = -2.45, P = 0.025) when input levels were high, and not in December (t_{18} = -0.76, P = 0.455) or January (t_{18} = -1.26, P = 0.223) when input levels were much lower. Light intensity did not differ between treatments (t_{18} = -0.24, P = 0.81).

Survival did not differ among treatments for any species (all P [t = 0] > 0.48). Standard length did not differ between treatments for *N. boops* (t_{219} = -0.86, P = 0.39) or *F. notatus* (t_{80} = -1.89, P = 0.063). Standard length of *E. spectabile* was significantly higher in the control treatment than in the exclusion treatment (t_{160} = 2.25, P = 0.025). However, means for both treatments were lower than mean SL for *E. spectabile* at the beginning of the experiment, indicating that the difference between treatments was likely due to size-specific mortality rather than growth rates, with more large individuals dying in the exclusion treatment.

The benthic macroinvertebrate assemblage in the experimental streams was dominated by chironomid larva (Table 1). Density of total macroinvertebrate and chironomid larva only did not differ between treatments for any species (all P [t = 0] > 0.20). *Notropis boops* and *F. notatus* shifted diets in the absence of terrestrial invertebrates, but *E. spectabile* did not (Figure 2). Both *N. boops* and *F. notatus* consumed more terrestrial invertebrates than any other prey item in control streams. In

exclusion streams, however, consumption of terrestrial invertebrates was greatly reduced. The terrestrial contribution to *N. boops* diet in the exclusion treatment was approximately 20% that of its contribution to control stream diets. No terrestrial prey item was found in guts of any *F. notatus* in exclusion streams. Detritus and algae contributed the most to gut contents of both these species in exclusion streams. *Etheostoma spectabile* fed primarily on chironomid larva and microcrustaceans in both exclusion and control streams.

All three species showed a general trend of ^{15}N depletion and ^{13}C enrichment under terrestrial invertebrate exclusion, shifting towards the signature of chironomid larva (Figure 3). However, there was no significant treatment effect on $\delta^{15}N$ values of any species (all P [$t = 0$] > 0.12). $\delta^{13}C$ values did not differ for *E. spectabile* ($t_8 = 1.28$, P = 0.24) or *F. notatus* ($t_8 = -1.22$, P = 0.26), but were significantly lower under terrestrial exclusion for *N. boops* ($t_7 = -4.72$, P = 0.002).

Fundulus notatus was the only species for which body condition differed between treatments (Table 2), showing decreased body condition with terrestrial invertebrate exclusion (Figure 4). Interaction of treatment-by-body mass was also significant ($F_{1,33} = 4.41$, P = 0.044). Analyses over the covariate range using the Wilcox procedure showed the treatment effect to be significant only for fishes with body mass greater than 0.26 g.

DISCUSSION

This study indicate that effects of trophic subsidy reduction vary with consumer trophic ecology, and that specialist taxa reliant on such subsidies may suffer fitness

consequences if they are unable to switch resources. Such information, coupled with knowledge of the variables that drive subsidy levels at the landscape level (e.g., Mason and McDonald 1982; Piccolo and Wipfli 2002; Orr et al. 2005), can help predict effects of landscape variation on fitness of consumers in recipient habitats. In the case of terrestrial invertebrate input into streams, variation can occur naturally due to variation in the riparian tree community (Mason and McDonald 1982, Piccolo and Wipfli 2002) or anthropogenically via landscape alteration such as mowing and cattle grazing (Edwards and Huryn 1996, Kawaguchi and Nakano 2001).

In light of the well-documented numerical and functional responses of many consumers to alteration in trophic subsidy levels (Polis et al. 1997, Baxter et al. 2005), it is not surprising that consumer fitness is affected when they are unable to vacate areas with low subsidy levels. Few studies, however have documented reduced consumer fitness under these conditions, either because organisms are not constrained to low-subsidy areas (Kawaguchi et al. 2003), or because species are able to switch resources (Baxter et al. 2004). One study demonstrating reduction in a fitness correlate was Sabo and Power (2002b), who showed reduced growth of riparian lizards held in streamside enclosures with reduced emergent aquatic insect input. In functionally diverse assemblages, consumers are likely to vary in their ability to respond both numerically and functionally to changing subsidy levels. For example, Paetzold et al. (2006) showed that riparian ground arthropods specializing on riverine subsidies showed stronger numerical responses to altered subsidy levels than ground arthropods consuming primarily terrestrial resources. The present study is among the first to demonstrate species-specific variation in the ability to respond functionally to altered subsidy levels. If different taxa

experience different fitness consequences, subsidy reduction has the potential to alter community structure of functionally diverse assemblages if such reduction remains consistent over time.

In the present study the fitness consequence of subsidy elimination was evident in reduced body condition, rather than in reduced growth or over-winter survival. Most fishes grow little during winter (Hurst and Conover 1998, Schultz et al. 1998, Garvey et al. 1998), so lack of a growth effect is not surprising. However, in many fishes, higher body condition increases overwinter survival (Newsome and Leduc 1975, Elliott 1976, Booth and Keast 1986). Stream fishes are not long-lived; the species studied here rarely survive past their third year (Pflieger 1997), and winter is typically the harshest season, with high mortality levels (Schlosser 1998). In the present study, however, reduced body condition did not lead to lower survival for *F. notatus*, likely for two reasons. First, the winter of 2005-06 was abnormally mild, with temperature well above average except for early December and mid February (http://www.srh.noaa.gov/oun/climate/graphs.php?city=OKC), leading to reduced stress and potentially lower mortality. Second, fishes were confined to experimental treatments in late fall, at the end of the summer when they were presumably already in good condition. Had fishes been in exclusion treatments leading up to the winter, they might have been in poor body condition as the winter began, potentially lowering overwinter survival. Thus, although survival was not affected by reduced subsidies in the present study, it would likely be if *F. notatus* is subjected to such conditions year-round.

Although body condition of both *E. spectabile* and *N. boops* was unaffected by terrestrial invertebrate reduction, the mechanisms by which they avoided its effect

differed. *Etheostoma spectabile* typically did not consume terrestrial invertebrates, and its diet was unchanged when subsidies were eliminated. *Notopis boops*, however, underwent a diet shift similar to that of *F. notatus*, consuming fewer terrestrial invertebrates in the exclusion treatment. Unlike *F. notatus*, the generalist *N. boops* was able to obtain adequate energy from aquatic resources, while *F. notatus*, the subsidy specialist, apparently was not. Indeed, comparison of muscle tissue $\delta^{13}C$ indicated that *N. boops* was the only species with a significant difference between control and exclusion treatments, shifting towards the more depleted signature of instream chironomid larva. Thus, the ability to obtain energy from an aquatic source when terrestrial invertebrates were excluded allowed the *N. boops* to avoid reduced body condition.

Although neither was affected in monoculture, the potential in natural streams for indirect effects between *N. boops* and *E. spectabile* to reduce available resources must be acknowledged. Prey-switching by generalist fishes has been shown to reduce benthic prey in other systems (Baxter et al. 2004), so prey switching by generalists such as *N. boops* in natural streams could reduce available resources for benthic specialists such as *E. spectabile*. In addition, *N. boops* could suffer a fitness consequence by having to compete with *E. spectabile* for benthic invertebrates, unlike in the present monoculture study.

Because stable isotope ratios of terrestrial invertebrates were not assayed in this experiment, it must be assumed that enrichment of *N. boops* muscle $\delta^{13}C$ under terrestrial exclusion is due to acquisition of carbon from aquatic sources, and not from some other allochthonous source. For example, terrestrial carbon signatures, particularly from plants using C4 and CAM photosynthetic pathways, can be as enriched, or more so, as the

observed carbon signature for chironomid larva (Raven 1987, Boutton 1991). Although CAM plants are rare in the vegetation surrounding the experimental stream site, C4 plants do occur, and could potentially be a source of carbon for terrestrial insects entering stream units. This scenario is unlikely, however, given that ^{13}C enrichment occurred in the absence (or near absence) of terrestrial input. If terrestrial and instream sources were equally depleted, no differences would be expected in isotopic signatures of fish tissue between treatments. In addition, the diet shift by *N. boops* is clear, indicating that fishes with enriched carbon signatures consumed fewer terrestrial invertebrates and a greater proportion of aquatic items. In light of these considerations, it is most likely that the shift in $\delta^{13}C$ observed in *N. boops* was the result of switching from a terrestrial to an aquatic diet, and assimilating aquatic carbon into muscle tissue.

In spite its significant isotopic shift, *N. boops* muscle tissue was still far from the isotopic equilibrium predicted based on a diet of primarily aquatic chironomid larva. $\delta^{13}C$ was more depleted, and $\delta^{15}N$ more enriched. This is likely due to cold winter water temperatures during the study. In fishes, turnover of muscle isotope signatures is affected both by growth rate (Hesslein et al. 1993, Trueman et al. 2005, Watanabe et al. 2005, Miller 2006, Zuanon et al. 2006) and metabolism (Logan et al. 2006). Indeed, Perga and Gerdeaux (2005) showed that whitefish muscle tissue only reflected food consumed in spring and summer, and not during winter. Conclusion of this experiment in May, coupled with the fact that the winter of 2005-06 was relatively warm, likely facilitated the incomplete shift in *N. boops* isotope ratios under terrestrial invertebrate exclusion.

As with many ecosystems worldwide, stream fish communities are becoming less distinctive, as endemic specialists are replaced by cosmopolitan generalist species

through the process of biotic homogenization (McKinney and Lockwood 1999, Rahel 2002). Results of the present study suggest yet another mechanism by which generalists may gain an advantage over specialists. Because the highest levels of terrestrial invertebrate subisidies are generally recorded in undisturbed forests with dense canopy cover (Cloe and Garman 1996, Nakano et al. 1999), continued alteration of riparian forests could potentially lead to reduced subsidy levels, with fitness consequences for trophic subsidy specialists.

Linkages between habitats have been emphasized in ecology in general (Saunders et al. 1991, Franklin 1993) and, recently, in stream ecology in particular (Ward 1998, Fausch et al. 2002). As knowledge of these linkages grows, so will the ability to understand the consequences of altering them. Results of the present study indicate that not all consumers will be affected equally if the flux of trophic subsidies is interrupted. Identification of functional traits (*sensu* McGill et al. 2006) useful in predicting species responses to altered subsidy levels will be important in allowing successful prediction of community level-responses to such a disturbance. Trophic ecology, particularly the degree to which species show resource specialization, is an important area likely candidate to become one such trait.

ACKNOWLEDGMENTS

I thank S. Gillette, R. Remington, P. Unmack and J. Wesner for field assistance. J. Kelly and N. Franssen assisted with stable isotope analyses. Thanks to N. Franssen, C. Hargrave, E. Marsh-Matthews, R. Remington and J. Wesner for valuable input and discussion. Funding was provided by the United States Environmental Protection

Agency Science to Achieve Results fellowship program, and a Howard McCarley Research Grant from the Southwestern Association of Naturalists. The University of Oklahoma Biological Station provided housing and research space, and T. Cluck, D. Cobb, R. Page and M. Ward provided logistical and other assistance. Research was conducted under University of Oklahoma Institutional Animal Care and Use Committee permit #R05-023 to David P. Gillette (DPG), and all fishes were collected under Oklahoma Department of Wildlife Conservation Scientific Collector's Permit # 3508 to DPG. This study was submitted in partial fulfillment of requirements for the Doctor of Philosophy degree in the Department of Zoology at the University of Oklahoma. I thank B. Hoagland, M. Kaspari, J. Kelly, C. Vaughn and W. Matthews for their service on my graduate committee.

LITERATURE CITED

Atmar, G.L. and K.W. Stewart. 1972. Food, feeding selectivity and ecological efficiencies of *Fundulus notatus* (Cyprinidontidae). American Midland Naturalist 88: 76-89.

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

Berumen, M.L., M.S. Pratchett and M.I. McCormick. 2005. Within-reef differences in diet and body condition of coral-feeding butterflyfishes (Chaetodontidae). Marine Ecology Progress Series 287: 217-227.

Booth, D.J. and J.A. Keast. 1986. Growth energy partitioning by juvenile Bluegill Sunfish, *Lepomis macrochirus* Rafinesque. Journal of Fish Biology 28: 37-45.

Boutton, T.W. 1991. Stable carbon isotope ratios of natural materials: II. Atmospheric, terrestrial, marine and freshwater environments. Pages 173-176 *in* E.A. Paul and J. M. Melillo, editors. Isotopic techniques in plant, soil and aquatic biology. Academic Press, New York, New York, U.S.A.

Brown, A.V., M.D. Schramm and P.P. Brussock. 1987. A vacuum benthos sampler suitable for diverse habitats. Hydrobiologia 153: 241-247.

Cloe W.W.I. and G.C. Garman. 1996. The energetic importance of terrestrial arthropod inputs to three warmwater streams. Freshwater Biology 36: 105–114.

Davies, N.B. 1976. Food, flocking and territorial behaviour of the Pied Wagtail (*Motacilla alba yarrellii* Gould) in winter. Journal of Animal Ecology 45: 235–254.

Edwards, E.D. and A.D. Huryn. 1996. Effect of riparian land use on contributions of terrestrial invertebrates to streams. Hydrobiologia 337: 151-159.

Edwards, M.S., T.F. Turner and Z.D. Sharp. 2002. Short- and long-term effects of fixation and preservation on stable isotope values ($\delta_{13}C$, $\delta_{15}N$, $\delta_{34}S$) of fluid-preserved museum specimens. Copeia 2002: 1106-1112.

Elliott, J.M. 1976. Energetics of feeding and metabolism and growth of Brown Trout (*Salmo trutta* L.) in relation to body weight, water temperature and ration size. Journal of Animal Ecology 45: 923-948.

Fausch, K.D., C.E. Torgerson, C.V. Baxter and H.W. Li. 2002. Landscapes to riverscapes: Bridging the gap between research and conservation of stream fishes. Bioscience 52: 483-498.

Franklin, J.F. 1993. Preserving biodiversity: species, ecosystems or landscapes. Ecological Applications 3: 202-205.

Garvey, J.E., R.A. Wright and R.A. Stein. 1998. Overwinter growth and survival of age-0 Largemouth Bass (*Micropterus salmoides*): Revisiting the role of body size. Canadian Journal of Fisheries and Aquatic Sciences 55: 2414-2424.

Gelwick, F. P. and W.J. Matthews. 1992. Effects of an algivorous minnow on temperate stream ecosystem properties. Ecology 73: 1630-1645.

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

Heulett S.T., S.C. Weeks and G.K. Meffe. 1995. Lipid dynamics and growth relative to resource level in juvenile Eastern Mosquitofish (*Gambusia holbrooki*: Poeciliidae). Copeia 1995: 97–104.

Hesslein, R.H., K.A. Hallard and P. Ramlal. 1993. Replacement of sulfur, carbon and nitrogen in tissue of growing Broad Whitefish (*Coregonus nasus*) in response to a chance in diet traced by delta-S-34, delta-C-13 and delta-N-15. Canadian Journal of Fisheries and Aquatic Sciences 50: 2071-2076.

Hurst, T.P. and D.O. Conover. 1998. Winter mortality of young-of-the-year Hudson River striped bass (*Morone saxatilis*): Size-dependent patterns and effects on recruitment. Canadian Journal of Fisheries and Aquatic Sciences 55: 1122-1130.

Kato, C., T. Iwata, S. Nakano and D. Kishi. 2003. Dynamics of aquatic insect flux affects distribution of riparian web-building spiders. Oikos 103: 113-120.

Kawaguchi, Y., S. Nakano and Y. Taniguchi. 2003. Terrestrial invertebrate inputs determine the local abundance of stream fishes in a forested stream. Ecology 84: 701–708.

Kawaguchi, Y. and S. Nakano. 2001. Contribution of terrestrial invertebrates to the annual resource budget for salmonids in forest and grassland reaches of a headwater stream. Freshwater Biology 46: 303–316.

Logan, J., H. Haas, L. Deegan and E. Gaines. 2006. Turnover rates of nitrogen stable isotopes in the salt marsh Mummichog, *Fundulus heteroclitus*, following a laboratory diet switch. Oecologia 147: 391-395.

MacNeil, M.A., K.G. Drouillard and A.T. Fisk. 2006. Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. Canadian Journal of Fisheries and Aquatic Science 63: 345-353.

Mason, C.F. and S.M. MacDonald. 1982 The input of terrestrial invertebrates from tree canopies to a stream. Freshwater Biology 12: 305–311.

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.

Matthews, W.J., A. Maria Miller-Lemke, M. L. Warren, Jr., D. Cobb, J. G. Stewart, B. Crump and F. P. Gelwick. 2004. Context-specific trophic and functional ecology of fishes of small stream ecosystems in the Ouachita National Forest. Pages 221-230 *in* Guldin, James M., tech. comp. 2004. Ouachita and Ozark Mountains

symposium: ecosystem management research. Gen. Tech. Rep. SRS–74. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station.

Matthews, W.J., K.B. Gido and E. Marsh-Matthews. 2001. Density-dependent overwinter survival and growth of red shiners from a southwestern river. Transactions of the American Fisheries Society 130: 478-488.

McGill, B.J., B.J. Enquist, E. Weiher and M. Westoby. 2006. Rebuilding community ecology from functional traits. Trends in Ecology & Evolution 21: 178-185.

McKinney, M.L. and J.L. Lockwood. 1999. Biotic homogenization: a few winners replacing many losers in the next mass extinction. Trends in Ecology & Evolution 14: 450-453.

Meffe, G.K. and F.F. Snelson, Jr. 1993. Lipid dynamics during reproduction in two livebearing fishes, *Gambusia holbrooki* and *Poecilia latipinna.* Canadian Journal of Fisheries and Aquatic Sciences 50: 2185–2191.

Miller, T.W. 2006.Tissue-specific response of delta N-15 in adult Pacific Herring (*Clupea pallasi*) following an isotopic shift in diet. Environmental Biology of Fishes 76: 177-189.

Nakano, S., H. Miyasaka and N. Kuhara. 1999. Terrestrial aquatic linkages: Riparian arthropod inputs alter trophic cascades in a stream food web. Ecology 80: 2435–2441.

Newsome, G.E. and G. Leduc. 1975. Seasonal changes in fat content in Yellow Perch (*Perca flavescens*) of two Laurentian lakes. Journal of the Fisheries Research Board of Canada 32: 2214-2221.

Orr, M., M. Zimmer, D.E. Jelinski and M. Mews. 2005. Wrack deposition on different beach types: Spatial and temporal variation in the pattern of subsidy. Ecology 86: 1496-1507.

Paetzold, A., J.F. Bernet and K. Tockner. 2006. Consumer-specific responses to riverine subsidy pulses in a riparian arthropod assemblage. Freshwater Biology 51: 1103-1115.

Perga, M.E. and D. Gerdeaux. 2005. 'Are fish what they eat' all year round? Oecologia 144: 598-606.

Pflieger, W.L. 1997. The fishes of Missouri. Revised edition. Missouri Department of Conservation. Jefferson City, Missouri, U.S.A.

Piccolo J.J. and M.S. Wipfli. 2002. Does Red Alder (*Alnus rubra*) in upland riparian forests elevate macroinvertebrate and detritus export from headwater streams to downstream habitats in southeastern Alaska? Canadian Journal of Fisheries and Aquatic Sciences 59: 503–513.

Polis G.A., W.B. Anderson and R.D. Holt. 1997. Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics 28: 289–316.

Power M.E. and W.E. Rainey. 2000. Food webs and resource sheds: Towards spatially delimiting trophic interactions. Pages 291-314 *in*: M.J. Hutchings, E.A. John and A.J.A. Stewart, editors. Ecological Consequences of Habitat Heterogeneity. Blackwell Scientific, Oxford, U.K.

Power, M.E., W.E. Rainey, M.S. Parker, J.L. Sabo, A. Smyth, S. Khandwala, J.C. Finlay, F.C. McNeely, K. Marsee and C. Anderson. 2004. River to watershed subsidies in

an old-growth conifer forest. Pages 217-240 *in*: G.A. Polis, M.E. Power and G.R. Huxel, editors. Food Webs at the Landscape Level. The University of Chicago Press, Chicago, Illinois, U.S.A.

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

Rahel, F.J.. 2002. Homogenization of freshwater faunas. Annual Review of Ecology and Systematics 33: 291-315.

Raven, J.A. 1987. The application of mass spectrometry to biochemical and physiological studies. Pages 127-180 *in*: D.D. Davies, editor, P.K. Stumpf and E.E. Conn, editors-in-chief. The biochemistry of plants: a comprehensive treatise. Volume 13: Methodology. Academic Press, New York, New York, U.S.A.

Rose, M.D. and G.A. Polis. 1998. The distribution and abundance of coyotes: The effects of allochthonous food subsidies from the sea. Ecology 79: 998-1007.

Roth, J.D. 2003. Variability in marine resources affects arctic fox population dynamics. Journal of Animal Ecology 72: 668-676.

Roth, J.D. 2002. Temporal variability in arctic fox diet as reflected in stable-carbon isotopes: The importance of sea ice. Oecologia 133: 70-77.

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

Sabo J.L. and M.E. Power. 2002b. River-watershed exchange: Effects of riverine subsidies on riparian lizards and their terrestrial prey. Ecology 83: 1860–1869.

Saunders, DA, R.J. Hobbs and C.R. Margules. 1991. Biological consequences of ecosystem fragmentation: A review. Conservation Biology 5: 18-32.

Schlosser, I.J. 1998. Fish recruitment, dispersal, and trophic interactions in a heterogeneous lotic environment. Oecologia 113: 260-268.

Schulz, E.T., D.O. Conover and A. Ehtisham. 1998. The dead of winter: Size-dependent variation and genetic differences in seasonal mortality among Atlantic Silversides (Atherinidae: *Menidia menidia*) from different latitudes. Canadian Journal of Fisheries and Aquatic Sciences 55: 1149-1157.

Seppa, T., A. Laurila, N. Peuhkuri, J. Piironen and N. Lower. 2001. Early familiarity has fitness consequences for Arctic Char (*Salvelinus alpinus*) juveniles. Canadian Journal of Fisheries and Aquatic Sciences 58: 1380-1385.

Stewart, A.J. 1987. Responses of stream algae to grazing minnows and nutrients: A field test for interactions. Oecologia 72: 1-7.

Strange, R.M. 1992. Winter food habits of the Orangethroat Darter, *Etheostoma spectabile*, from southwestern Indiana. Proceedings of the Indiana Academy of Sciences 101: 293-298.

Sweeney B.W. and R.L. Vannote.1982. Population synchrony in mayflies: A predator satiation hypothesis. Evolution 36: 810–821.

Thomerson, J.E. and D.P. Wooldridge. 1970. Food habits of allotropic and syntopic populations of topminnows *Fundulus olivaceous* and *Fundulus notatus*. American Midland Naturalist 84: 573-583.

Trueman, C.N., R.A.R. McGill and P.H. Guyard. 2005. The effect of growth rate on tissue-diet isotopic spacing in rapidly growing animals: An experimental study with Atlantic Salmon (*Salmo salar*). Rapid Communications in Mass Spectrometry 19: 3239-3247.

Uesugi, A. and M. Murakami. 2007**.** Do seasonally fluctuating aquatic subsidies influence the distribution pattern of birds between riparian and upland forests? Ecological Research 22: 274-281.

Vaughn, C.C., F.P. Gelwick and W.J. Matthews. 1993. Effects of algivorous minnows on production of grazing stream invertebrates. Oikos 66: 119-128.

Vogt, G.F. and T.G. Coon. 1990. A comparison of the foraging behavior of two darter (*Etheostoma*) species. Copeia 1: 41-49.

Waltham, N.J and R.M. Connolly. 2006. Trophic strategies of Garfish, *Arrhamphus sclerolepis*, in natural coastal wetlands and artificial urban waterways. Marine Biology 148: 1135-1141.

Ward, J.V. 1998. Riverine landscapes: Biodiversity patterns, disturbance regimes, and aquatic conservation. Biological Conservation 83: 269-278.

Watanabe, Y., T. Seikai and O. Tominaga. 2005. Estimation of growth and food consumption in juvenile Japanese Flounder *Paralichthys olivaceus* using carbon stable isotope ratio delta C-13 under laboratory conditions. Journal of Experimental Marine Biology and Ecology 326: 187-198.

Wilcox, R.R. 1987. Pairwise comparisons of J-independent regression lines over a finite interval, simultaneous pairwise comparison of their parameters, and the Johnson-Neyman procedure. British Journal of Mathematical & Statistical Psychology 40: 80-93 Part 1.

Zuanon, J.A.S., A.C. Pezzato, L.E. Pezzato, J.R.S. Passos, M.M. Barros and C. Ducatti. 2006. Muscle delta C-13 change in Nile Tilapia (*Oreochromis niloticus*): Effects of growth and carbon turnover. Comparative biochemistry B-Biochemistry &

Molecular Biology 145: 101-107.

Table 1. Mean and standard error density of benthic macroinvertebrates in experimental streams at the conclusion of the study in May 2006.

	Benthos Density (Number • m^{-2})	
	Mean	Std. Error
(Diptera) Chironomid larva	5677.7	885.7
(Diptera) Ceratopogonid larva	5.5	4.0
(Odonata) Libellulidae larva	3.7	2.5
Nematodes	122.5	61.1
Snails	64.0	32.6

Table 2. Results of testing for effects of terrestrial exclusion on lipid mass, with body mass as a covariate. Tests significant at $\alpha = 0.05$ denoted by an asterisk (*).

Species	Effect	*F*	*P*
Notropis boops	Overall Model	$F_{2,42} = 7.40$	0.002*
	Treatment	$F_{1,42} = 1.53$	0.223
	Body Mass	$F_{1,42} = 9.96$	0.003*
Etheostoma spectabile	Overall Model	$F_{2,37} = 3.81$	0.031*
	Treatment	$F_{1,37} = 1.11$	0.299
	Body Mass	$F_{1,37} = 5.00$	0.031*
Fundulus notatus	Overall Model	$F_{2,33} = 48.61$	<0.0001*
	Treatment	$F_{1,33} = 10.63$	0.003*
	Body Mass	$F_{1,33} = 97.20$	<0.0001*

LIST OF FIGURES

Figure 1. Terrestrial invertebrate input for control and exclusion streams, measured on three occasions over the course of the study. Means differing significantly (two-sample *t*-test) are denoted by an asterisk (*).

Figure 2. Mean and standard error diet composition for *Notropis boops*, *Etheostoma spectabile* and *Fundulus notatus* taken from experimental streams in May 2006.

Figure 3. Biplot showing mean and standard deviation stable isotope ratios of carbon and nitrogen in dorsal muscle tissue of *Notropis boops*, *Etheostoma spectabile* and *Fundulus notatus*, and whole chironomid larva taken from experimental streams in May 2006. Significant differences between control and exclusion means (two-sample *t*-test) are denoted by an asterisk (*).

Figure 4. Relationship between lipid and whole-body mass for *Fundulus notatus* from control and exclusion streams in May 2006. Vertical dotted line indicates value of body mass above which terrestrial invertebrate exclusion effect on lipid mass was significant, with body mass as a covariate, as determined by the Wilcox (1987) procedure.

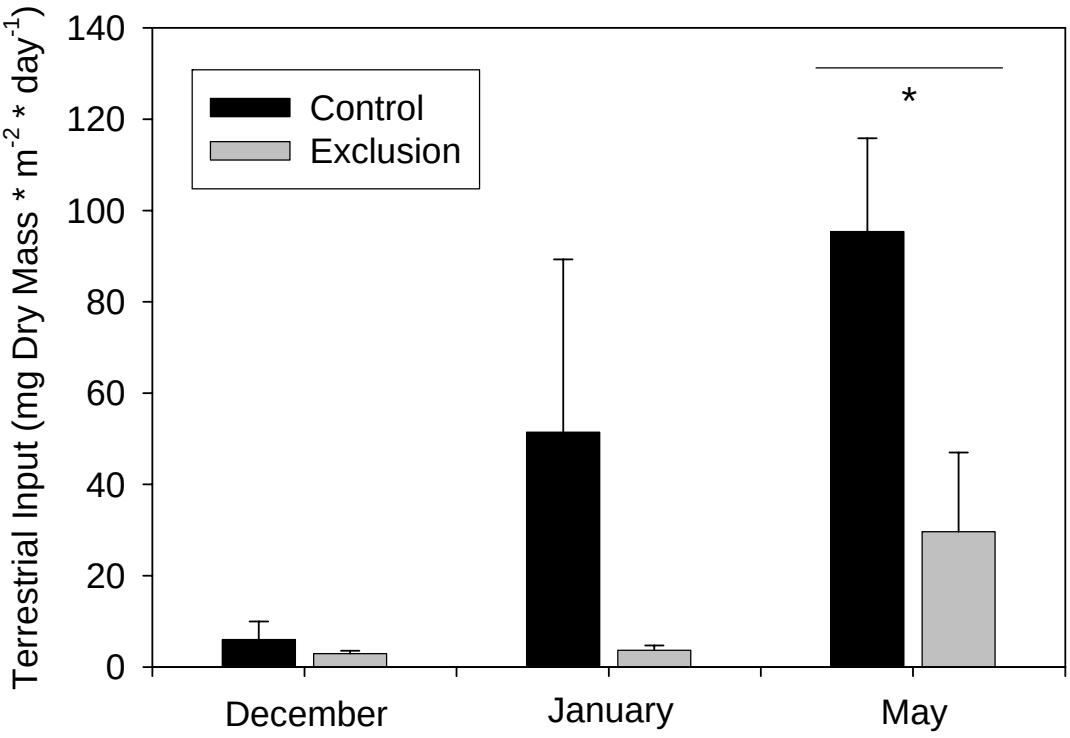

Terrestrial Input (mg Dry Mass * m-2 * day-1)
140
120
100
80
60
40
20
0
Control
Exclusion
*
December
January
May

Figure 1.

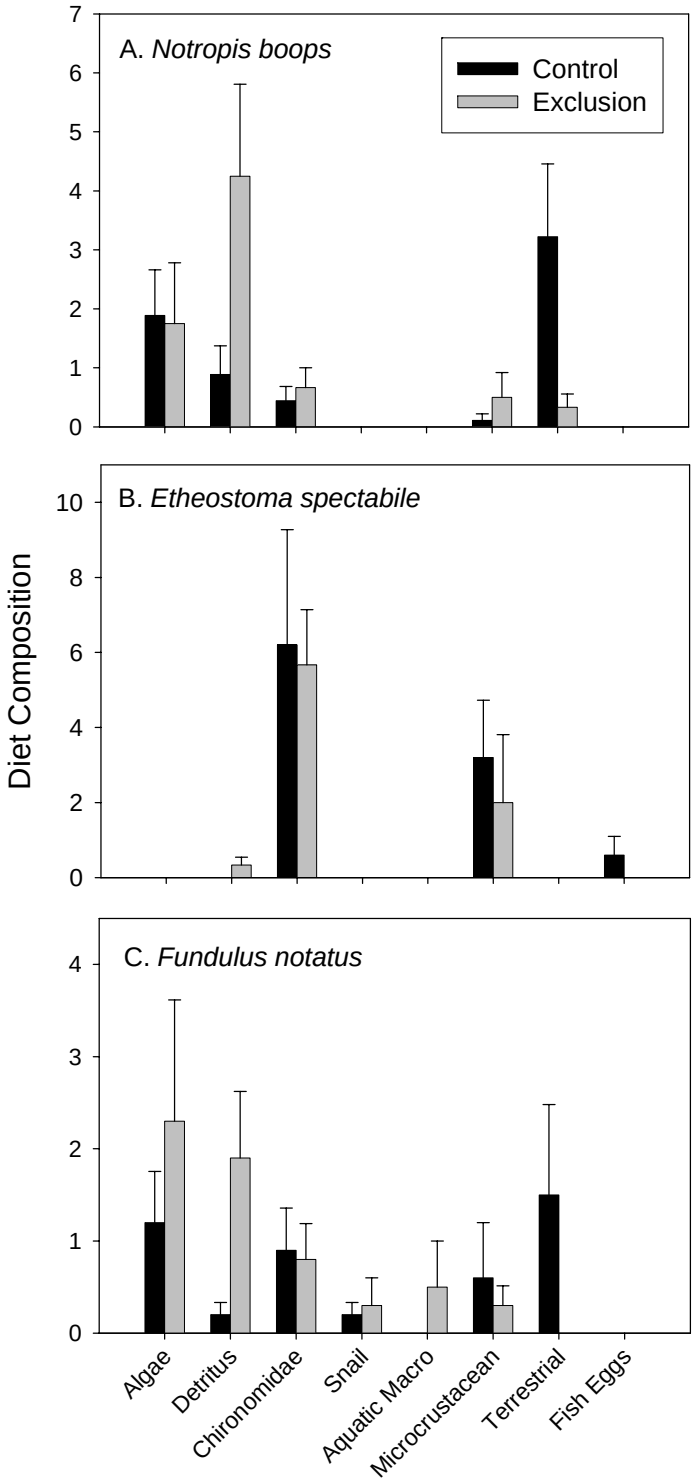

A. Notropis boops
Control
Exclusion
B. Etheostoma spectabile
C. Fundulus notatus
Diet Composition
Algae
Detritus
Chironomidae
Snail
Aquatic Macro
Microcrustacean
Terrestrial
Fish Eggs

Figure 2.

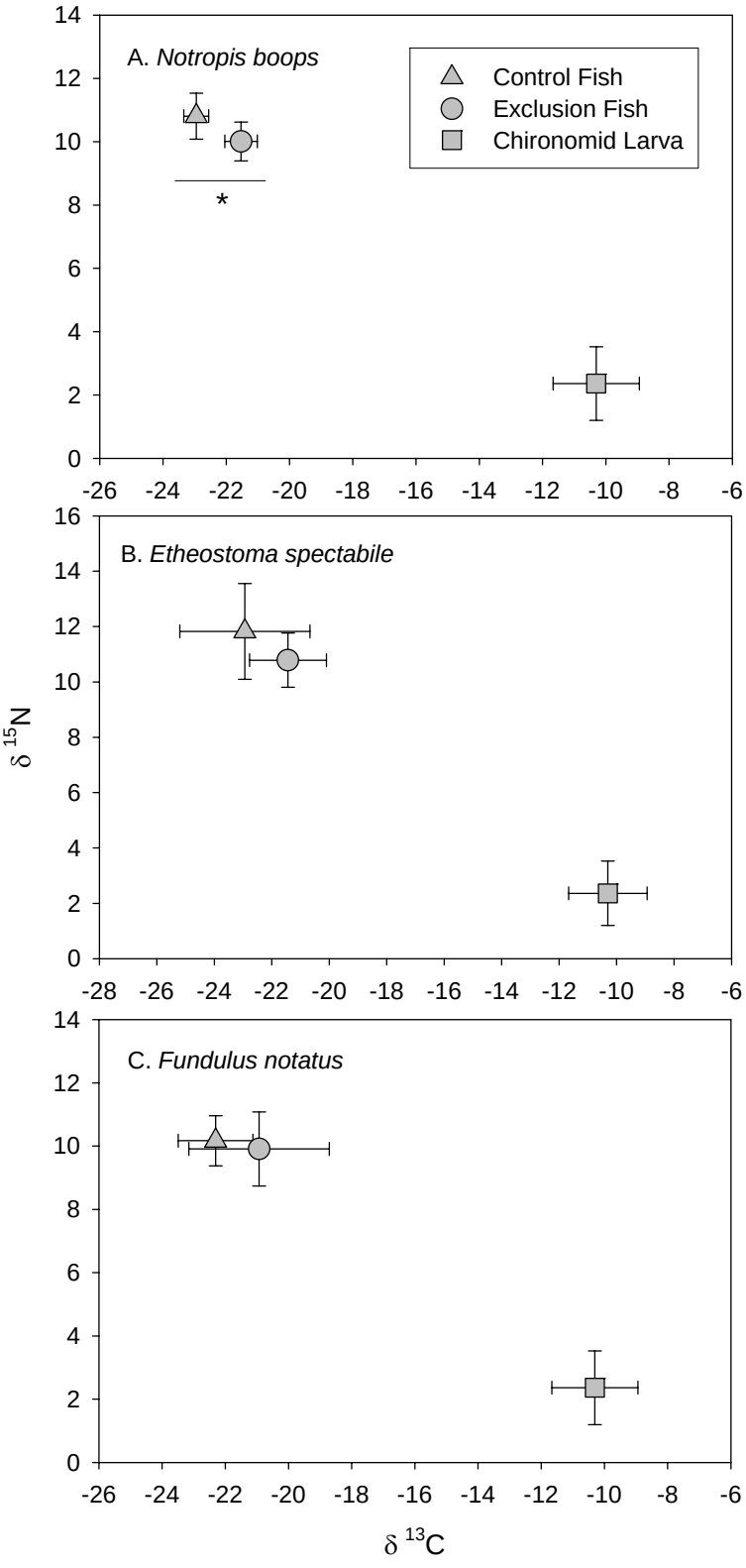

A. Notropis boops
Control Fish
Exclusion Fish
Chironomid Larva
*
B. Etheostoma spectabile
C. Fundulus notatus
δ 15N
δ 13C

Figure 3.

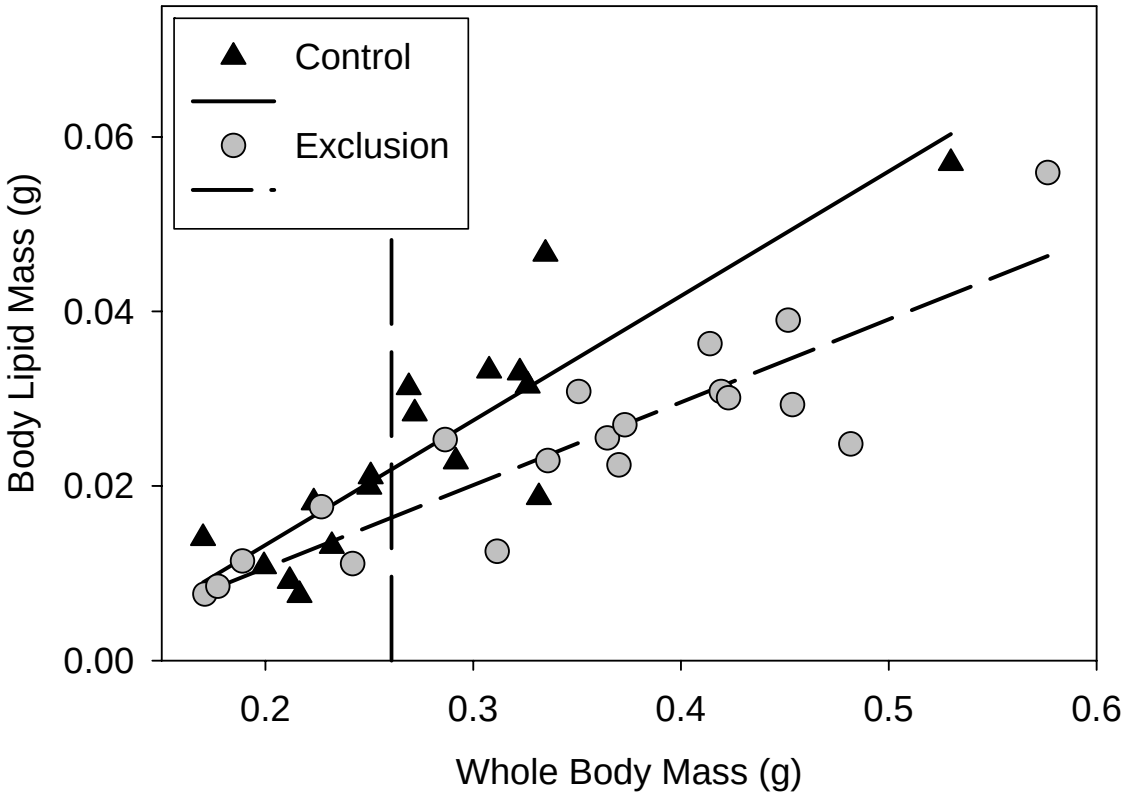

Control
Exclusion
Body Lipid Mass (g)
0.00
0.02
0.04
0.06
0.2
0.3
0.4
0.5
0.6
Whole Body Mass (g)

Figure 4.