UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

# EFFECTS OF FISH DENSITY, IDENTITY, AND SPECIES RICHNESS ON STREAM ECOSYSTEMS

A Dissertation

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

Doctor of Philosophy

By

CHAD WESLEY HARGRAVE
Norman, Oklahoma
2005

Downloaded from SHAREOK. Persistent Handle URL: https://hdl.handle.net/11244/849

UMI Number: 3163015



UMI Microform 3163015

Copyright 2005 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

EFFECTS OF FISH DENSITY, IDENTITY, AND SPECIES RICHNESS IN STREAM ECOSYSTEMS

A Dissertation APPROVED FOR THE
DEPARTMENT OF ZOOLOGY

BY

_____
William J. Matthews (Advisor)

_____
Elizabeth A. Bergey

_____
Michael E. Kaspari

_____
Robert W. Nairn

_____
Caryn C. Vaughn

© Copyright by Chad Wesley Hargrave 2005
All Rights Reserved.

## ACKNOWLEDGMENTS

The time I have spent at the University of Oklahoma has been extremely rewarding. I owe much of this experience to the tremendous faculty, staff, and students in the Department of Zoology and Biological Station. Especially, Dave Hambright, Larry Weider, Donna Cobb, Richard Page, Malon Ward, and Tammy Cluck, from the Biological Station, for giving me a home-away-from-home.

I owe a special thanks to Bill Matthews, my major advisor, for his excellent mentoring, critical evaluations of all my work, and his constant support. I have grown a lot through his guidance, and I only hope that one day I will be able to share with my students some of what he has taught me. My committee (Elizabeth Bergey, Michael Kaspari, Robert Nairn, and Caryn Vaughn) was extremely committed to helping me grow as a scientist. I thank them for their time and efforts that have helped shape my future. I thank the 'fish lab': Edie Marsh-Matthews, Melody Brooks, Rae Deaton, Katy Sutherland, Raul Ramirez, David Gillette, and Mike Eggleton for field assistance, statistical advice, critical discussion of this research, and for listening to an endless stream of practice talks.

Finally, I would like to acknowledge my family for moral support, my best friend for sticking by my side, and especially my grandfather who taught me the joys of being on the water. My love for water and its organisms is why I am an aquatic ecologist. This dissertation is dedicated to my daughter Savannah, who was born the summer I began this research. Her curiosity and love of learning has inspired me to never stop asking 'why'.

## TABLE OF CONTENTS

**ACKNOWLEDGMENTS** . . . . . iv

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

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

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

**PREFACE** . . . . . xii

**ABSTRACT** . . . . . xiii

**Chapter 1: Effects of fish density on stream ecosystem properties** . . . . . 1
- **Abstract** . . . . . 2
- **Introduction** . . . . . 3
- **Methods** . . . . . 5
  - Experimental design . . . . . 6
  - Fish . . . . . 6
  - Periphyton biomass . . . . . 7
  - Benthic invertebrates . . . . . 7
  - Benthic particulate organic matter . . . . . 8
  - Stomach contents . . . . . 8
  - Statistical analyses . . . . . 8
- **Results** . . . . . 9
  - Fish effects on periphyton biomass . . . . . 9
  - Fish effects on benthic invertebrates . . . . . 9
  - Fish effects on BPOM . . . . . 10
  - Stomach contents . . . . . 10
- **Discussion** . . . . . 11
  - The benthic grazer functional group . . . . . 11
  - The benthic invertivore functional group . . . . . 12
  - The surface insectivore functional group . . . . . 14
  - The benthic omnivore-disturber functional group . . . . . 15
  - The water column omnivore functional group . . . . . 15
  - Conclusions . . . . . 16
- **Acknowledgments** . . . . . 18
- **Literature Cited** . . . . . 19
- **Tables** . . . . . 23
- **Figures** . . . . . 30

**Chapter 2: Stream fishes affect benthic primary productivity through species-specific food web pathways** . . . . . 35
- **Abstract** . . . . . 36
- **Introduction** . . . . . 37
- **Methods** . . . . . 40

Experimental design . . . . . 41
Fish . . . . . 41
Stomach contents . . . . . 42
Terrestrial insect access . . . . . 43
Benthic primary productivity . . . . . 43
Benthic invertebrates . . . . . 43
Statistical analyses . . . . . 44
**Results** . . . . . 45
Terrestrial insect access . . . . . 45
Effects of Orangethroat Darter . . . . . 45
Effects of Western Mosquitofish . . . . . 46
Effects of Bullhead Minnow . . . . . 47
**Discussion** . . . . . 48
**Acknowledgments** . . . . . 54
**Literature Cited** . . . . . 55
**Tables** . . . . . 59
**Figures** . . . . . 64
**Chapter 3: Fish richness enhances stream ecosystem function: evidence for interspecific facilitation.** . . . . . 72
**Abstract** . . . . . 73
**Introduction** . . . . . 74
**Methods** . . . . . 76
Experimental design . . . . . 76
Effects on PPR and algae biomass. . . . . 78
Effects on benthic particulate organic matter . . . . . 78
Effects on benthic invertebrates . . . . . 79
Synergistic effects on algae biomass . . . . . 79
Statistical analyses . . . . . 81
**Results** . . . . . 81
Fish biomass and richness effects on PPR. . . . . 81
Synergistic response in algae biomass. . . . . 82
Algae composition overtime . . . . . 82
Assemblage composition related effects. . . . . 83
Effects on benthic invertebrates . . . . . 84
Effects on BPOM . . . . . 84
**Discussion** . . . . . 85
Effects of assemblage composition . . . . . 87
Potential mechanisms . . . . . 88
**Acknowledgments** . . . . . 91
**Literature Cited** . . . . . 92
**Tables** . . . . . 96
**Figures** . . . . . 103
**Chapter 4: Fishes affect primary productivity and periphyton biomass in natural stream ecosystems: an enclosure experiment** . . . . . 107
**Abstract** . . . . . 108
**Introduction** . . . . . 109

**Methods** . . . . . **110**
Fish enclosures . . . . . **110**
Fish treatments. . . . . **111**
PPR and periphyton biomass . . . . . **111**
Benthic invertebrates . . . . . **112**
Gut contents . . . . . **112**
Statistical analyses. . . . . **113**
**Results** . . . . . **113**
PPR and periphyton biomass . . . . . **113**
Benthic invertebrates . . . . . **114**
Gut contents . . . . . **114**
**Discussion** . . . . . **114**
Conclusions . . . . . **116**
**Acknowledgments** . . . . . **117**
**Literature Cited** . . . . . **118**
**Tables** . . . . . **120**
**Figures** . . . . . **121**

## LIST OF TABLES

*Chapter 1*

Table 1. Linear and quadratic regression models, *F* statistic, *P* value, and *R* squared for each species effect on chlorophyll-*a* biomass on days 15 and 30. Significant models are in bold. . . . . 23

Table 2. Linear and quadratic regression models, *F* statistic, *P* value, and *R* squared for each species effect on benthic invertebrate density on day 30. Significant models are in bold. . . . . 26

Table 3. Linear and quadratic regression models, *F* statistic, *P* value, and *R* squared for each species effect on benthic particulate organic matter on day 30. Significant models are in bold. . . . . 28

*Chapter 2*

Table 1. Predicted effects of Orangethroat Darter, Bullhead Minnow, and Western Mosquitofish on primary productivity and benthic grazing invertebrates in each stream half for all fish and terrestrial insect treatments. Horizontal line indicates no effect. Direction and number of arrows indicates direction and relative magnitude of effects. For example, Bullhead Minnow treatment with 25 fish per stream has greater PPR in half-A with 25 fish than half-B with no fish. . . . . 58

Table 2. Experimental design showing number of fish per stream mesocosm half within each treatment level of fish and terrestrial insect access. Each treatment has six replicates. . . . . 60

Table 3. Repeated-measure analysis of variance tables summarizing statistical results of fish and terrestrial insect treatment effects, stream half effect, and all interaction effects on benthic primary production (PPR) and benthic invertebrate density (Invertebrates) for each fish species . . . . . 61

*Chapter 3*

Table 1. Fish species and their trophic and functional designations based on published diet and behavioral traits. Species composition for replicate treatments based on random selection without replacement within each replicate. Each replicate is identified by letters a-e within each treatment. *Per capita* species effect ($x_i \times 10^{-4}$) on chlorophyll-*a*, generated from single-species experiments. . . . . 95

Table 2. Multiple regression table testing effects of fish richness and biomass on primary

productivity (PPR), benthic invertebrate density, benthic particulate organic matter (BPOM), and synergistic effect on algae biomass at each sample date. Regression for synergistic effects on algae on days 14 and 28 had df = 3, 30 and for Day 42 had df = 3, 28. All other regressions for days 14 and 28 have df = 3, 35, and for Day 42 has df = 3, 33. Values represent parameter estimates for each regression, and associated level of significance: NS, $P > 0.05$; *, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $P \leq 0.001$. . . . . 97

Table 3. Percent variance explained and vector loadings for each species on the first three principal component axes. . . . . 99

Table 4. Pearson's correlation coefficient and associated level of significance: NS, $P > 0.05$; *, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $P \leq 0.001$ for correlations between principal component scores defining each stream assemblage, and primary production (PPR), synergistic effects on chlorophyll-*a*, benthic invertebrate density, and benthic particulate organic matter (BPOM). . . . . 101

*Chapter 4*

Table 1. Repeated measures ANOVA table showing degrees of freedom (*df*), *F*- and *P*-values for main treatment effects, time effects, and treatment by time interaction for each response variable. . . . . 119

## LIST OF FIGURES

*Chapter 1*

Figure 1. Density effects of six fish species on periphyton biomass on days 15 and 30. The non-linear regression line was drawn in all cases where the non-linear model was significant. . . . . 31

Figure 2. Density effects of six fish species on benthic macroinvertebrate density on day 30. . . . . 32

Figure 3. Density effects of six fish species on accumulation of benthic particulate organic matter on day 30. The non-linear regression line was drawn in all cases where the non-linear model was significant. . . . . 33

Figure 4. Average percent occurrence of all food items consumed by each species when removed from stream mesocosms on day 30 of each experiment. Vertical bars represent 1 SD. . . . . 34

*Chapter 2*

Figure 1. Hypothesized consumer mediated pathways for (A) Orangethroat Darter (trophic cascade), (B) Western Mosquitofish (terrestrial nutrient translocation), and (C) Bullhead Minnow (bioturbation and trophic cascade). Large arrows connecting food web compartments indicate direction of energy flow. Small arrows adjacent to each food web compartment indicate hypothesized direct and indirect effects of fish on invertebrates, algae, and nutrients. . . . . 65

Figure 2. Depiction of stream mesocosms with fish barrier dividing each pool . . . 66

Figure 3. Effects of terrestrial insect barrier on terrestrial insect access collected in water surface traps. Probability values are from t-tests. Vertical bars represent 1 standard error (SE). . . . . 67

Figure 4. Average PPR measured from each mesocosm half (columns) for each treatment. Column shading indicates terrestrial insect treatment. Vertical bars represent 1 SE. . . . . 68

Figure 5. Average benthic invertebrate density measured from each mesocosm half (columns) for each treatment. Column shading indicates terrestrial input treatment. Vertical bars represent 1 SE. . . . . 69

Figure 6. Average percent occurrence of food items found in guts of 10 individuals of each species taken from mesocosms with and without terrestrial insect barriers. Vertical bars represent 1 SE. . . . . 70

*Chapter 3*

Figure 1. Mean primary production (mg $O_2$ $cm^{-2}$ $h^{-1}$ ± 1 SE) across richness treatments for days 14, 28. and 42. . . . . 103

Figure 2. Synergistic response in algae biomass (µg chlorophyll-*a* / $cm^{-2}$) on days 14, 28, and 42 for each species richness treatment. Synergistic effect was calculated as the difference between observed and predicted change in chlorophyll-*a* on each sample day. Open circles indicate differences, and filled circles are means of the differences within each treatment. . . . . 104

Figure 3. Relative dominance among algae taxa for each sample day, measured as the number of mesocosms in which each algae was the most abundant taxa. . . . . 105

*Chapter 4*

Figure 1. Mean response in primary productivity (PPR) on days 15 and 30. Results from contrasts indicated by letters above each bar. Contrasts were calculated on average PPR for both sample days because there was no significant time effect. Treatments with different letters had significantly different mean PPR on both sample days. Vertical bars are one standard error . . . . . 121

Figure 2. Mean response in periphyton biomass (estimated as chlorophyll-*a*) on days 15 and 30. Results from contrasts indicated by capital (Day 15) and lower case (Day 30) letters above each bar. Contrasts were calculated separately for both days because of a significant time effect. Treatments with different letters had significantly different chlorophyll-*a* on that sample day. Vertical bars are one standard error. . . . . 122

Figure 3. Mean response in benthic invertebrate density to treatments on days 15 and 30. Vertical bars are one standard error. . . . . 123

Figure 4. Average percent occurrence of food items found in guts of eight individuals ($n$ = 8) for each species recovered from enclosures on day 30. Vertical bars are one standard deviation . . . . . 124

## PREFACE

The four chapters of this dissertation have been formatted for submission to *Ecology*.

## ABSTRACT

Stream ecosystem properties and functions are important because they provide services such as water purification, oxygen production, and carbon fixation that help sustain life. Aquatic organisms can affect these properties and functions through different pathways linked to a species trophic and functional characteristics. It is necessary to understand how these ecosystem effects vary among taxa because as aquatic biota change as a result of random, natural, or anthropogenic influences, ecosystems properties and functions are likely to be affected.

Fishes are important consumers in stream ecosystems, having important regulatory roles for many ecosystem properties and functions. In this dissertation, I addressed the effects of fishes on stream ecosystems from three perspectives. I asked (1) if fish ecosystem effects were dependent on local fish density, (2) if fish effects were caused by different species-specific food web pathways, and (3) if co-occurring fish species had facilitative effects on ecosystems when in more specious assemblages.

In Chapter 1, I tested density effects of six fishes from different trophic and functional groups on benthic algae biomass (chlorophyll-*a*), benthic invertebrate density, and benthic particulate organic matter (BPOM). In general, Central Stoneroller, a benthic grazer, had no effect on algae biomass, a negative effect on benthic invertebrates, and a positive, non-linear effect on BPOM. Orangebelly Darter, a benthic invertivore, had a positive, linear effect on algae biomass, no effect on benthic invertebrates, and a positive, non-linear effect on BPOM. Brook Silverside, a surface insectivore, had a positive, non-linear effect on algae biomass, and no effects otherwise. Golden Redhorse, a benthic, disturbing omnivore, had a positive, linear effect on algae biomass, no effect on benthic

invertebrates, and a positive, non-linear effect on BPOM. Striped Shiner and Rocky Shiner, both water column omnivores, differed in effects. Striped Shiner had a positive, non-linear effect on algae biomass, but no effect on benthic invertebrates or BPOM. Rocky Shiner had no effect on any ecosystem property. This Chapter demonstrated that fish effects on benthic algae are highly dependent on density, increasing linearly in most cases with fish number. Fish effects on benthic invertebrates and BPOM also were density dependent but changed more non-linearly than effects on algae. Diet analyses and behavioral observations suggested that these fish effects were mediated through different species-specific food web pathways

In Chapter 2, I tested three hypothesized food web pathways (i.e., trophic cascade, terrestrial nutrient translocation, and nutrient translocation via bioturbation) for fish effects on primary productivity (PPR) of benthic algae. Orangethroat Darter, Western Mosquitofish, and Bullhead Minnow were used as models for each respective pathway. Orangethroat Darter, a benthic invertivore, increased PPR through an apparent trophic cascade, by localized reduction of benthic grazing invertebrate densities. Western Mosquitofish, a surface feeding insectivore, increased PPR by enhancing nutrients through terrestrial nutrient translocation. Bullhead Minnow, a benthic omnivore that disturbed sediments during foraging, increased PPR through nutrient enhancement via bioturbation. It also reduced benthic grazing invertebrates. Thus, this species may have affected PPR through a combination of bioturbation and trophic cascade mechanisms. This study indicated fish effects on PPR occurred through different pathways linked to species-specific trophic and functional characteristics. It is unknown if species-specific

ecosystem effects are additive on a *per capita* basis in more specious assemblages, or if fishes have facilitative interactions that might result in synergistic ecosystem effects.

In Chapter 3, I tested for synergistic effects of fish species richness on PPR and algae biomass, by randomly composing fish assemblages with richness ranging 1 to 6 species. Initial increase in PPR resulted from additive effects of individual species-specific effects. However, as the experiment progressed, PPR increased synergistically in treatments with two or more fish species, suggesting interspecific facilitation. These data support the biodiversity ecosystem function hypothesis in stream fishes, but the exact mechanisms of the positive, synergistic effects of fishes on benthic, stream algae is unknown. However, the data suggest that benthic and watercolumn fishes may have interacted, enhancing foraging efficiency of these co-occurring taxa and increasing rates of nutrient cycling and nutrient exchange between stream sediments and water.

This research suggests that the population size, the identity of species in the assemblage, as well as the number of species making up the assemblage can be important factors affecting stream ecosystem properties and functions. Chapters 1 – 3 were conducted in artificial stream mesocosms, and may have limited applicability to natural stream ecosystems where a variety of abiotic factors can influence ecosystems. In Chapter 4, I tested the ecosystem effects of three fish species from different functional groups on PPR, benthic algae biomass (as chlorophyll-*a*), and benthic invertebrate density in a natural stream ecosystem using field enclosures. Treatments were Blackstripe Topminnow (*Fundulus notatus*), a surface insectivore; Longear Sunfish (*Lepomis megalotis*), a watercolumn insectivore; Orangethroat Darter (*Etheostoma spectabile*), a benthic invertivore; and a fishless control. Primary productivity was

greater in all enclosures with fish than in enclosures without fish. Relative to control enclosures, benthic algae biomass was not different in Blackstripe Topminnow treatments, but was greater in Longear Sunfish treatments on both sample days, and was greater in Orangethroat Darter treatments on day 15. Benthic invertebrate abundance was not affected by any of the fish treatments. These data agree with results from previous mesocosm experiments. Thus, despite the limited realism of stream mesocosms, patterns observed in mesocosm experiments are likely to occur in natural stream ecosystems.

My dissertation research has supported the assumption that fishes are important for stream ecosystems, suggesting that fish assemblage properties such as population size, species identity in the assemblage, and species richness can be important factors regulating stream ecosystem function. Thus, factors impacting fish density, altering species composition, and reducing species richness could have negative effects on stream ecosystems through indirect food web pathways.

# CHAPTER 1: EFFECTS OF FISH DENSITY ON STREAM ECOSYSTEM PROPERTIES

CHAD W. HARGRAVE

*University of Oklahoma Biological Station and Department of Zoology, University of Oklahoma, Norman, Oklahoma 73019*

## ABSTRACT

The effects of most stream fishes on ecosystem properties remain unknown. I tested effects of six fish species (five functional groups) across a range of densities on periphyton biomass, benthic invertebrate density, and benthic particulate organic matter (BPOM), to determine if effects of species from different functional groups varied linearly or non-linearly with density. Central Stoneroller, a benthic grazer, had no effect on periphyton biomass, a negative effect on benthic invertebrates, and a positive, non-linear effect on BPOM. Orangebelly Darter, a benthic invertivore, had a positive, linear effect on periphyton, no effect on benthic invertebrates, and a positive, non-linear effect on BPOM. Brook Silverside, a surface insectivore, had a positive, non-linear effect on periphyton, and no effects otherwise. Golden Redhorse, a benthic, disturbing omnivore, had a positive, linear effect on periphyton, no effect on benthic invertebrates, and a positive, non-linear effect on periphyton. Striped Shiner and Rocky Shiner, both water column omnivores, differed in effects. Striped Shiner had a positive, non-linear effect on periphyton, but no effect on benthic invertebrates or BPOM. Rocky Shiner had no effect on any ecosystem property. In this study, fish effects on three ecosystem properties intensified with density. In most cases, fish effects on periphyton biomass were similar among species, but effects on benthic invertebrates and BPOM were different among species with unique trophic and function roles. This suggests that fish effects on PPR are likely to be mediated through different pathways, which depend on a species interactions with the ecosystem.

## INTRODUCTION

Organisms have a variety of effects on ecosystem functions and properties. Predators can affect primary and secondary production through both direct and indirect food web interactions (Slobodkin 1962, Fretwell 1987, Oksanen 1991, Pace 1999). Many organisms can affect nutrient cycling within ecosystems or enhance nutrient exchange between ecosystem compartments (McNaughton and Georgiadis 1986, Jonsson and Jonsson 2003). Some taxa can physically modify ecosystems (ecosystem engineers) through foraging or other physical activities, influencing abundance and availability of specific resources (Jones et al. 1994). At any given time within an ecosystem, there are many taxon-specific effects influencing ecosystem-level processes and functions. Understanding these effects is requisite for predicting consequences of species loss and importance of biodiversity.

Fishes are important consumers in aquatic ecosystems, having strong effects on a variety of ecosystem properties that vary with functional group, i.e., a species trophic and physical interactions with the ecosystem (Wootton and Power 1993, Matthews 1998). Generally, herbivorous fishes negatively affect algal biomass (Power and Matthews 1983, Matthews et al. 1987, Gelwick and Matthews 1992) and enhance nitrogen cycling (Grimm 1988). Predatory fishes that consume grazing invertebrates can increase primary production through trophic cascades (Power 1990, McIntosh and Townsend 1996). Insectivorous, water column and surface fishes can increase primary production through terrestrial nutrient translocation, e.g., by consumption of terrestrial insects and excretion of nutrients into the stream ecosystem (Dahl 1998, Gido and Matthews 2001, Fausch et al. 2002, Baxter et al. 2004). Omnivorous, benthic fishes can increase particulate organic

matter resuspension by disturbing stream sediments through foraging (Bioturbation; Flecker 1996). Bioturbation can increase availability of particulates and enhance ecosystem carbon budgets, and can result in nutrient translocation by releasing nutrient stores in the sediments (Schaus and Vanni 2000, Vanni 2002). Because fishes can regulate ecosystem properties through a variety of pathways, changes to natural fish assemblage composition may impact overall ecosystem functioning by influencing an array of potentially linked consumer mediated interactions.

Ecosystem effects have been tested in only a few fish species from the functionally diverse North American fish fauna (Matthews 1998), and the relative importance of fish density for ecosystem effects is not well understood. The goal of this study was to test fish effects across a range of densities on periphyton biomass, benthic invertebrate density, and benthic particulate organic matter (BPOM), and to determine if effects of species from different functional groups varied density and if such effects were linear or non-linear. I used six common fish species representing five widespread functional feeding groups: benthic grazer (Central Stoneroller; *Campostoma anomalum*), benthic, predatory invertivore (Orangebelly Darter; *Etheostoma radiosum*), surface feeding insectivore (Brook Silverside; *Labidesthes sicculus*), benthic, disturbing omnivore (Golden Redhorse; *Moxostoma erythrurum*), and water column omnivores (Striped and Rocky Shiner; *Luxilus chrysocephalus* and *Notropis suttkusi*). I expected ecosystem effects to intensify with density for all species. I predicted that all taxa would increase periphyton biomass, except the benthic grazer which was expected to decrease periphyton. The benthic herbivorous, invertivorous, and omnivorous fishes were

expected to decrease benthic invertebrates, and only the benthic disturbing omnivore was predicted to increase BPOM.

## METHODS

I conducted three 30-day experiments to test ecosystem effects of six fish species, with two species tested per experiment. All experiments were conducted in stream mesocosms at the University of Oklahoma Biological Station (UOBS), Marshall Co. OK, USA. Each mesocosm consisted of one pool (183 cm diameter and 80 cm deep) and one riffle (122 cm long and 5 –10 cm deep) as used by Gido and Matthews (2001). Mesocosms were lined with natural gravel-cobble substrate taken from a nearby stream. Prior to each experiment, mesocosms were drained and cleaned, and benthic sediments homogenized among individual units. Mesocosms remained dry for at least 5 days between experiments at which time they were refilled with city water and inoculated with a 500 ml of a natural periphyton slurry scraped from rocks in a nearby stream. Following inoculation, flow was continuously maintained by pumping water from a downstream collecting box to the head of each riffle with a 2500 L/h submersible pump. Pumps provided circulation of nutrients throughout each mesocosm, and aerated the water. Mesocosms remained fishless for at least 5 days after receiving the periphyton inoculation, which allowed establishment of a periphyton and snail assemblage dominated by *Oedogonium* and *Spirogyra*, and *Physella* from the slurry, respectively, and colonization of insect larvae, including dipterans, ephemeropterans, and odonates, by ovipositing winged adults.

### *Experimental Design*

I used a regression design to test effects of fish density on ecosystem properties. Density for each fish species was varied as 10, 20, 30, 40, and 50 individuals per stream (3.8, 7.6, 11.4, 15.2, and 19.0 fish $m^{-2}$), with two control treatments receiving no fish. Each species-density treatment was replicated twice.

### *Fish*

At least 350 individuals of each species were collected from streams in Oklahoma, transported to UOBS in insulated boxes, and randomly assigned to mesocosms. The day fish were assigned to mesocosms was day-1 for each experiment. Additional individuals remaining after experiments were started were held in a separate tank as replacements for any fish that died. Fish remained in mesocosms for 30 days, at which time all individuals were removed and preserved in 10% formalin. Later, fish from each mesocosm were dried at 60°C to a constant mass and weighed to determine fish biomass in each stream.

Central Stoneroller and Orangebelly Darter were collected from the Blue River, Johnston Co., OK., and put in mesocosms on 29 June 2001. Five Central Stonerollers and 27 Orangebelly Darters died during the experiment and were replaced. When the experiment ended, average dry mass of Central Stoneroller and Orangebelly Darter was 830 ± 242 mg and 218 ± 58 mg, respectively. Brook Silverside and Golden Redhorse (young-of-year) were collected from Blue River, and Pennington Creek, Johnston Co., OK, respectively, and put in mesocosms on 12 August 2001. Sixteen Brook Silversides and 10 Golden Redhorse died and were replaced. Brook Silverside and Golden Redhorse

averaged 147 ± 54 mg and 737 ± 397 mg dry mass at the end of the experiment, respectively. Striped Shiner and Rocky Shiner were collected from Blue River, and put in streams on 25 September 2001. Five Striped Shiners and nine Rocky Shiners died and were replaced. Average dry mass for Striped Shiner and Rocky Shiner at the end of the experiment was 821 ± 111 mg and 159 ± 19 mg, respectively.

### *Periphyton Biomass*

About 24 h after the addition of the natural periphyton slurry in each mesocosm, 8 unglazed clay tiles (225 $cm^2$ each) were placed in each mesocosm as a substrate for periphyton growth. On days 15 and 30, three randomly selected tiles were removed from each mesocosm. Periphyton was scraped into a common container for each mesocosm and stored on ice. Within 24 h, the scrapings were filtered under vacuum through a 0.45 μm filter. The filtrate was frozen for at least 24 h. Chlorophyll-*a* biomass was measured spectrophotometrically using the acetone extraction method with a correction for pheopigments (APHA 1995) .

### *Benthic Invertebrates*

On day 30, I took six sediment core samples (each 10 cm diameter by 7.5 cm deep) near the center of each mesocosm to estimate benthic macroinvertebrate density. Core samples were preserved in 10% formalin. Invertebrates were washed from sediments using a 250 μm sieve, identified and counted.

### *Benthic Particulate Organic Matter*

On day 1 of each experiment, I placed two plastic containers (12.7 × 12.7 × 4.76 cm deep), filled with cleaned 2 – 5 cm diameter gravel, flush with the substrate at opposite ends of each pool to estimate benthic particulate organic matter (BPOM) accumulation (Flecker 1996). I removed the containers from each pool on day 30 and preserved contents of each container separately with 5% formalin. In the laboratory, BPOM was vacuum filtered through a 41 μm mesh screen (Gelwick and Matthews 1992). The BPOM was dried at 60°C to a constant mass, weighed and combusted at 550°C for 1 h, and reweighed to estimate ash free dry mass of BPOM.

### *Stomach Contents*

At the end of each experiment (day 30) all fish were removed from the mesocosms and preserved in 10% formalin. Stomach contents were examined for 2 randomly selected individuals from each density treatment ($n$ = 10). I removed the anterior third of the alimentary tract (or the discrete stomach, if one existed), placed the contents on a gridded petri dish, and estimated percent occurrence for each major food category by counting number of grids occupied by each food type.

### *Statistical Analyses*

I used linear and non-linear regression to test for significant linear or quadratic effects of fish density on all ecosystem properties. I determined whether the linear or non-linear model was most appropriate by comparing model complexity to predictive power using model coefficients, $F$-value, $P$-values, and $R^2$.

## RESULTS

### *Fish Effects on Periphyton Biomass*

Periphyton biomass significantly increased with density of four fish species (Fig. 1). Central Stoneroller did not affect periphyton. Orangebelly Darter significantly increased periphyton on days 15 and 30. On day 15, there was at least a 4 fold increase in periphyton between treatments with 0 and 50 fish/mesocosm, with both linear and quadratic models significant (Table 1). On day 30, periphyton increased linearly by about 3 fold between treatments with 0 and 50 fish/mesocosm. Brook Silverside significantly increased periphyton on both sample dates. On day 15, average periphyton increased linearly by about 2.3 times between treatments with 0 and 50 fish/mesocosm. On day 30 periphyton increased non-linearly and was at least 6 times greater in the 50 fish/mesocosm treatment than 0 fish treatment (Table 1). Golden Redhorse significantly increased periphyton on both sample dates. On day 15 and 30, periphyton increased linearly by at least 4 fold between treatments with 0 and 50 fish/mesocosm on both days (Table 1). Striped Shiner had significant non-linear effects on periphyton biomass on days 15 and 30. On day 15 and 30, periphyton was about 3.5 and 12 times greater between the 0 and 50 fish/mesocosm treatments, respectively (Table 1). Rocky Shiner had no effect on periphyton biomass on days 15 or 30.

### *Fish Effects on Benthic Invertebrates*

Benthic invertebrate assemblages consisted of chironomids (52 ± 9% of total individuals), annelids (21 ± 2%), snails (22 ± 15%), ephemeropteran nymphs (3 ± 1%), odonate nymphs (1 ± 2%), coleopteran larvae (<1%) and ostracods (<1%) across all

experiments. Central Stoneroller was the only species to significantly affect benthic invertebrate density on day 30 (Fig. 2). Benthic invertebrates were about 2 fold less dense in treatments with 50 fish/mesocosm then 0 fish treatments. This effect was non-linear (Table 2).

### *Fish Effects on BPOM*

Central Stoneroller, Orangebelly Darter, and Golden Redhorse significantly increased total BPOM on day 30 (Fig. 3). BPOM was increased non-linearly and about 2.5, 2, and 3 times greater in Central Stoneroller, Orangebelly Darter, and Golden Redhorse treatments with 50 fish/mesocosms than 0 fish, respectively (Table 3).

### *Stomach Contents*

The species used in this study consumed an array of food items (Fig. 4) that corresponded with *a priori* functional group designations. Central Stoneroller was primarily algivorous, consuming mostly flocculent and filamentous algae. Orangebelly Darter was a benthic invertivore that consumed mostly benthic grazing invertebrates, including chironomids, snails, and ostracods. Brook Silverside was a surface feeder that foraged primarily on terrestrial insects, but some individuals consumed a small number of benthic invertebrates. Golden Redhorse was a benthic omnivore, consuming benthic invertebrates, terrestrial insects, and some algae. Striped Shiner was omnivorous, consuming food items, ranging from benthic invertebrates, to terrestrial insects, and algae. Rocky Shiner also was slightly omnivorous, but primarily ate terrestrial insects.

## DISCUSSION

I tested the effects of six fish species from five functional groups on several ecosystem properties. Periphyton biomass increased significantly with fish density for Orangebelly Darter, Brook Silverside, Golden Redhorse, and Striped Shiner. Benthic invertebrate abundance was significantly reduced with fish density for only one species (Central Stoneroller). This effect was non-linear. Density of three species (Central Stoneroller, Orangebelly Darter, and Golden Redhorse) significantly increased BPOM.

### *The Benthic Grazer Functional Group*

Central Stoneroller can decrease standing crops of periphyton through grazing (Power and Matthews 1983, Power et al. 1985, Gelwick and Matthews 1992). In my experiment, Central Stoneroller did not affect periphyton biomass as predicted. In fact, periphyton showed a slight but non-significant increase with Central Stoneroller density. Nutrient limitation within mesocosms could have caused these results. Periphyton biomass was very low in fishless, control streams, suggesting nutrients were unavailable for substantial periphyton growth (water came from a city source). As a result, it would have been difficult to detect a reduction in periphyton by grazing across density treatments. Furthermore, because nutrient availability was presumably low in this experiment, Central Stoneroller effects may have been linked more to nutrient regeneration than to grazing (Grimm 1988). This might have explained the slight increase in periphyton with fish density.

Central Stoneroller significantly reduced benthic invertebrates in this study. There appeared to be a threshold effect of fish density where reduction in benthic invertebrates was similar across all treatments with fish. I predicted this species would

reduce invertebrates by limiting periphyton food resources (Gelwick and Matthews 1992). However, Central Stoneroller did not reduce periphyton, so the negative effect of this species on benthic invertebrates may have resulted from predation or alterations to invertebrate habitat by mechanical disruption of the substrate through its grazing activity. Although typically a grazer, Central Stoneroller also consumes invertebrates (Evans-White, et al. 2001, Bergey and Weaver 2004). Grazing by this species affects benthic particulate size and availability in stream substrates (Gelwick and Matthews 1992, Gardner 1993). Increased particulates could potentially fill interstitial spaces, affecting invertebrate communities (Flecker 1996). Gut content data suggested limited consumption of benthic invertebrates by this species. Observations of feeding behavior of Central Stoneroller through Plexiglas viewing ports in each mesocosm indicated individuals often caused suspension of particulate matter into the water column. This apparently resulted in the significant increase in BPOM with fish density. The reciprocal effects of Central Stoneroller on BPOM and benthic invertebrate density indirectly supports my postulate that this species affected invertebrates by physically changing substrate architecture (Flecker 1996).

### *The Benthic Invertivore Functional Group*

The benthic invertivore, Orangebelly Darter, significantly increase periphyton biomass on days 15 and 30 as predicted. On day 15, effects on periphyton were non-linear reaching a maximum level at about 40 fish per mesocosm. On day 30, periphyton increased linearly with fish density.

I propose two mechanisms that could have been responsible for Orangebelly Darter effects on periphyton: (1) trophic cascade, and (2) nutrient enrichment through bioturbation. Gut content data and trends in invertebrate data suggest that this species affected periphyton through a trophic cascade. Orangebelly Darter primarily consumed chironomid larvae and small snails, and had a slight negative (although not significant) affect on benthic invertebrate density. Stream fishes that reduce grazing invertebrates densities have been linked to increased periphyton through a trophic cascade (Power 1990). It also is possible this species induced behavioral shifts in grazing invertebrates such as decreased foraging on exposed substrate surfaces, causing a trait-mediated trophic cascade (McIntosh and Townsend 1996, Schmitz et al. 1997). This would explain the weak effect of Orangebelly Darter on benthic invertebrate density. However, a nutrient enhancement mechanism also could have caused this species effect on periphyton. Species that disturb sediments during foraging can release unavailable nutrients from the sediments, promoting growth in primary producers (Vanni 2002). Orangebelly Darter was almost in constant contact with the mesocosm sediments, and apparently as a result of this behavior increased BPOM with fish density, peaking at a density of about 30 fish per mesocosm. Bioturbation could have released sedimentary nutrients (Schaus and Vanni 2000). To my knowledge, other studies have not addressed effect of benthic invertivorous fishes on BPOM or nutrient enrichment through bioturbation. The present study suggests that benthic invertivorous fishes could have potentially important roles in regulating BPOM and nutrient budgets as well as regulating periphyton biomass through predation.

*The Surface Insectivore Functional Group*

Brook Silverside increased periphyton biomass on days 15 and 30 as predicted. On day 15, periphyton increased linearly with fish density, but on day 30 periphyton increase was non-linear. There appeared to be a critical density at about 30 individuals per mesocosm, beyond which periphyton increased sharply with fish density. The strong dependence of periphyton on fish density suggested a nutrient mechanism responsible for this species' effect on periphyton.

Terrestrial insectivorous fishes might be important ecosystem components linking terrestrial nutrients to benthic stream compartments through nutrient translocation (Gido and Matthews 2001, Baxter et al. 2004). I suggest Brook Silverside increased periphyton by enhancing flux of nutrients into the mesocosms by consuming terrestrial insects and excreting nutrients into the water column (terrestrial nutrient translocation). Brook Silverside consumed mostly terrestrial insects, and rarely came into contact with the stream sediments (personal observation). Apparently as a result, this species had no effect on benthic invertebrate densities or BPOM. Thus, my data supported the nutrient translocation hypothesis, suggesting that fishes from functional groups that rarely physically interact with stream sediments can be linked to benthic ecosystem compartments through nutrient dynamics. The proposed terrestrial nutrient translocation effects were tightly coupled with fish density, such that there was a critical density (ca. 30 to 40 individuals) where fish effects on periphyton became prevalent.

*Benthic Omnivore-Disturber Functional Group*

As predicted, periphyton increased with Golden Redhorse density on days 15 and 30, which changed linearly with fish density on both days. Golden Redhorse was a benthic omnivore, consuming an array of food items and physically disturbing the mesocosm sediments. This species could have increased periphyton through mechanisms linked to foraging behavior. These included: (1) a trophic cascade, (2) terrestrial nutrient translocation, and (3) nutrient enrichment through bioturbation. Golden Redhorse consumed benthic invertebrates. Thus, it could have increased periphyton through a trophic cascade by reducing grazing invertebrate density (e.g., Power 1990). However, I detected no effect of this species on benthic invertebrate density. Rather, invertebrates showed a slight but non-significant increase, suggesting that nutrient enrichment could have caused this species effect. Golden Redhorse consumed terrestrial insects in addition to benthic invertebrates. Therefore, it could have positively affected periphyton through terrestrial nutrient translocation. The slight increase in benthic invertebrates supported this potential mechanism. However, Golden Redhorse was primarily a benthic species and disturbed the mesocosm substrate during foraging (personal observation), which caused a non-linear increase in BPOM with fish density that reached an asymptote at about 30 individuals per mesocosm. Thus, it is possible that this species increased periphyton by increasing nutrients from the mesocosm sediments through bioturbation.

*Water column Omnivore Functional Group*

Striped Shiner and Rocky Shiner, representing the water column omnivore functional group, had differing effects on the ecosystem properties measured in this

study. Rocky Shiner had no effect on periphyton, benthic invertebrate density, or BPOM. Striped Shiner significantly increased periphyton on both days 15 and 30 as predicted, but had no effect on benthic invertebrates, or BPOM. On day 15, effects on periphyton were non-linear. Striped Shiner effects appeared to plateau at about 30 individuals per mesocosm on day 15. Although the quadratic model was significant on day 30, it accounted for only an additional 1.2 % of the variation, and, thus, Striped Shiner effects on periphyton were mostly linear at this time. This species rarely disturbed the mesocosm sediments as indicated by no significant effect on BPOM and personal observations. However, Striped Shiner consumed benthic invertebrates as well as terrestrial insects. Thus, it is possible this species affected periphyton through a trophic cascade and terrestrial nutrient translocation. Because this species had no effect on benthic invertebrate density, a trophic cascade was likely not the primary mechanism for its ecosystem effects. I suggest terrestrial nutrient translocation is the most plausible explanation for Striped Shiner effect on periphyton biomass (Gido and Matthews 2001).

### *Conclusions*

This research showed that fish species from different functional groups are linked to several ecosystem properties. The exact mechanisms for their ecosystem effects remain unknown, illustrating the importance of further research for identifying specific mechanism causing different species effects. By the final day of the experiments, most fish effects on periphyton biomass were linear. However, terrestrial insectivores that potentially affect periphyton through terrestrial nutrient translocation might were an exception. This is probably because effects of this functional group are highly dependent

on nutrient flux into the ecosystem. Thus, at some critical fish density nutrient flux into the system becomes a dominant influence on periphyton growth. I do not suggest that the models calculated in this study for each species can be used in other systems to predict a species' effect at a given density, because magnitudes of these effects are likely to vary under different environmental contexts. However, I do suggest that linear models appear to be appropriate for predicting ecosystem effects of fish (and possibly other consumers). Thus, linear models can be used with some confidence to predict individual consumer effects as a baseline in biodiversity studies (Loreau and Hector 2001). The other ecosystem properties measured appeared to change in a more non-linear way. Thus, I advise caution in using linear models for predicting baselines for density effects on other ecosystem properties. In general, my data support the concept that fish from a variety of different functional groups can have important but different roles in regulating ecosystem functions and properties, and that fish density is an important predictor of a species' effect.

## ACKNOWLEDGEMENTS

I thank K. Hauger and R. Ramirez for help collecting fishes and starting experiments. I greatly appreciate the University of Oklahoma Biological Station faculty and staff including T. Cluck, D. Cobb, R. Page, M. Ward, and L. Weider for housing, equipment, and assistance maintaining experimental mesocosms. A review by R. Deaton improved early versions of this manuscript . I thank my doctoral committee (L. Bergy, M. Kaspari, W. Matthews, R. Nairn, and C. Vaughn) for critical discussion of this work during its planning phase and for critically reviewing this manuscript. Some funding for this project was provided by a National Science Foundation Dissertation Improvement Grant awarded to W. Matthews and C. Hargrave (NSF 0308729). This paper was submitted in partial fulfillment of the requirements for a Ph.D. degree at the University of Oklahoma, Department of Zoology

## LITERATURE CITED

American Public Health Association. 1995. Standard methods for the examination of water and wastewater, 19th ed. American Health Association, Washington, DC, USA.

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

Bergey, E. A. and J. E. Weaver. 2004. The influence of crevice size on the protection of epilithic algae from grazers. Freshwater Biology **49**:1014-1025.

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

Evans-White, M., W. K. Dodds, L. J. Gray, and K. M. Fritz. 2001. A comparison of the trophic ecology of the crayfishes *Orconectes nais* (Faxon) and *Orconectes neglectus* (Faxon) and the central stoneroller minnow *Campostoma anomalum* (Rafinesque): omnivory in a tallgrass prairie stream Hydrobiologia **462**:131-144.

Fausch, K. D., M. E. Power, and M. Murakami. 2002. Linkages between stream and forest food webs: Shigeru Nakano's legacy for ecology in Japan. Trends in Ecology and Evolution **17**: 429-434.

Flecker, A. S. 1996. Ecosystem engineering by a dominant detritivore in a diverse tropical stream. Ecology **77**:1845-1854.

Fretwell, S. D. 1987. Food chain dynamics: the central theory of ecology? Oikos **50**:291:301.

Gardner, T. J. 1993. Grazing and the distribution of sediment particle sizes in artificial

streams. Hydrobiologia **252**:127-132.

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.

Grimm, N. B. 1988. Feeding dynamics, nitrogen budgets, and ecosystem role of a desert stream omnivore, *Agosia chrysogaster* (Pisces: Cyprinidae). Environmental Biology of Fishes **21**:143-152.

Jones, C. G., J. H. Lawton, and M. Shachak. 1994. Organisms as ecosystem engineers. Oikos **69**:373-386.

Jonsson, B., and N. Jonsson. 2003. Migratory Atlantic salmon as vectors for the transfer of energy and nutrient between freshwater and marine environments. Freshwater Biology **48**:21-27.

Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature **412**:72-76.

Matthews, W. J. 1998. Patterns in freshwater fish ecology. Chapman and Hall, New York, New York, USA.

Matthews, W. J., A. J. Stewart, and M. E. Power. 1987. Grazing fishes as components of North American stream ecosystems: effects of *Campostoma anomalum*. Pages 128-135 *in*: W. J. Matthews and D. C. Heins, editors. Community and evolutionary ecology of North American stream fishes. University of Oklahoma Press, Norman. Oklahoma, USA.

Mcintosh, A. R., and C. R. Townsend. 1996. Interactions between fish, grazing

invertebrates and algae in a New Zealand stream: a trophic cascade mediated by fish-induced changes to grazer behavior? Oecologia **108**:174-181.

McNaughton, S. J., and N. J. Georgiadis. 1986. Ecology of African grazing and browsing mammals. Annual Review of Ecology and Systematics **17**:39-65.

Oksanen, L. 1991. Trophic levels and trophic dynamics: a consensus emerging. Trends in Ecology and Evolution **6**:58-60.

Pace, M. L., J. J. Cole, S. R. Carpenter, and J. F. Kitchell. 1999. Trophic cascades revealed in diverse ecosystems. Trends in Ecology and Evolution **14**:483-488.

Power, M. E. 1990. Effects of fish in river food webs. Science **250**:811-814.

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. E., W. J. Matthews, and A. J. Stewart. 1985. Grazing minnows, piscivorous bass, and stream algae: dynamics of a strong interaction. Ecology **66**:1448-1456.

Schaus, M. H., and M. J. Vanni. 2000. Effects of gizzard shad on phytoplankton and nutrient dynamics: role of sediment feeding and fish size. Ecology **81**:1701-1719.

Schmitz, O. J., A. P. Beckerman, and K. M. O'Brien. 1997. Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. Ecology **78**:1388-1399.

Slobodkin, L. B. 1962. Growth and regulation of animal populations. Holt, Rinehart, and Winston, New York, USA.

Vanni, M. J. 2002. Nutrient cycling by animals in freshwater ecosystems. Annual Review Ecology and Systematics **33**:341-370.

Wootton, J. T., and Power, M. E. 1993. Productivity, consumers, and the structure of a river food chain. Proceedings of the National Academy of Science **90**:1384-1387.

Table 1. Linear and quadratic regression models, *F* statistic, *P* value, and *R* squared for each species effect on chlorophyll-a biomass on days 15 and 30. Significant models are in bold.

| | Linear Models | Quadratic Models |
|---|---|---|
| **Species Effect Day 15** | | |
| Central Stoneroller | $y = 0.169 + 0.0020(x)$ | $y = 0.151 + 0.0048(x) - 0.0001(x^2)$ |
| *F* and *P* values | 1.829, 0.206 | 0.980, 0.412 |
| *R* squared | 0.155 | 0.179 |
| Orangebelly Darter | **$y = 0.250 + 0.0083(x)$** | **$y = 0.183 + 0.0184(x) - 0.0002(x^2)$** |
| *F* and *P* values | **9.570, 0.011** | **5.520, 0.027** |
| *R* squared | **0.489** | **0.551** |
| Brook Silverside | **$y = 0.263 + 0.0060(x)$** | $y = 0.296 + 0.0010(x) + 0.0001(x^2)$ |
| *F* and *P* values | **6.952, 0.025** | 0.3.449, 0.077 |
| *R* squared | **0.410** | 0.434 |
| Golden Redhorse | **$y = 0.215 + 0.0160(x)$** | **$y = 0.227 + 0.0141(x) + 0.0000(x^2)$** |
| *F* and *P* values | **18.879, 0.002** | **8.523, 0.008** |
| *R* squared | **0.654** | **0.654** |
| Striped Shiner | **$y = 0.059 + 0.0034(x)$** | **$y = 0.012 + 0.0103(x) - 0.0001(x^2)$** |
| *F* and *P* values | **7.607, 0.020** | **6.437, 0.018** |

| | | |
|---|---|---|
| *R* squared | **0.432** | **0.589** |
| Rocky Shiner | $y = 0.056 + 0.0002(x)$ | $y = 0.043 + 0.0022(x) - 0.0000(x^2)$ |
| *F* and *P* values | 0.172, 0.687 | 0.942, 0.425 |
| *R* squared | 0.017 | 0.173 |

**Species Effect Day 30**

| | | |
|---|---|---|
| Central Stoneroller | $y = 0.212 + 0.0044(x)$ | $y = 0.267 - 0.0039(x) + 0.0002(x^2)$ |
| *F* and *P* values | 3.129, 0.107 | 2.037, 0.186 |
| *R* squared | 0.238 | 0.312 |
| Orangebelly Darter | **$y = 0.245 + 0.0106(x)$** | $y = 0.195 + 0.0181(x) - 0.0001(x^2)$ |
| *F* and *P* values | **4.602, 0.051** | 2.201, 0.167 |
| *R* squared | **0.315** | 0.328 |
| Brook Silverside | **$y = 0.067 + 0.0351(x)$** | **$y = 0.377 - 0.0114(x) + 0.0009(x^2)$** |
| *F* and *P* values | **16.781, 0.002** | **11.599, 0.003** |
| *R* squared | **0.627** | **0.721** |
| Golden Redhorse | **$y = 0.314 + 0.0240(x)$** | $y = 0.251 + 0.0335(x) - 0.0002(x^2)$ |
| *F* and *P* values | **4.844, 0.052** | 2.223, 0.164 |
| *R* squared | **0.326** | 0.331 |
| Striped Shiner | **$y = 0.021 + 0.0118(x)$** | **$y = 0.059 + 0.0061(x) + 0.0001(x^2)$** |
| *F* and *P* values | **14.759, 0.003** | **6.983, 0.015** |
| *R* squared | **0.596** | **0.608** |

| | | |
|---|---|---|
| Rocky Shiner | $y = 0.064 + 0.0011(x)$ | $y = 0.039 + 0.0049(x) - 0.0001(x^2)$ |
| *F* and *P* values | 1.005, 0.339 | 1.004, 0.404 |
| *R* squared | 0.091 | 0.182 |

Table 2. Linear and quadratic regression models, *F* statistic, *P* value, and *R* squared for each species effect on benthic grazing invertebrate density on day 30. Significant models are in bold.

| **Species Effect Day 30** | Linear Models | Quadratic Models |
|---|---|---|
| Central Stoneroller | y = 708.02 – 6.084(x) | **y = 895.05 – 34.140(x) + 0.561($x^2$)** |
| *F* and *P* values | 2.696, 0.132 | **6.686, 0.016** |
| *R* squared | 0.212 | **0.598** |
| Orangebelly Darter | y = 730.36 – 4.246(x) | y = 946.47 – 36.662(x) + 0.648($x^2$) |
| *F* and *P* values | 0.791, 0.395 | 3.505, 0.075 |
| *R* squared | 0.073 | 0.438 |
| Brook Silverside | y = 557.07 – 1.335(x) | y = 533.06 + 2.267(x) – 0.072($x^2$) |
| *F* and *P* values | 0.070, 0.796 | 0.052, 0.950 |
| *R* squared | 0.007 | 0.011 |
| Golden Redhorse | y = 407.44 + 6.066(x) | y = 370.79 + 11.564(x) – 0.110($x^2$) |
| *F* and *P* values | 2.877, 0.121 | 1.414, 0.292 |
| *R* squared | 0.223 | 0.239 |
| Striped Shiner | y = 974.62 – 7.279(x) | y = 979.69– 8.037(x) + 0.015 ($x^2$) |
| *F* and *P* values | 0.718, 0.416 | 0.323, 0.731 |
| *R* squared | 0. 067 | 0.067 |

| | | |
|---|---|---|
| Rocky Shiner | $y = 1055.10 – 9.090(x)$ | $y = 1166.63 – 25.819(x) + 0.334(x^2)$ |
| *F* and *P* values | 1.883, 0.200 | 1.1553, 0.358 |
| *R* squared | 0.158 | 0.204 |

Table 3. Linear and quadratic regression models, *F* statistic, *P* value, and *R* squared for each species effect on benthic particulate organic matter on day 30. Significant models are in bold.

| **Species Effect Day 30** | Linear Models | Quadratic Models |
|---|---|---|
| Central Stoneroller | **$y = 0.931 + 0.0431(x)$** | **$y = 1.183 + 0.0053(x) + 0.0008(x^2)$** |
| *F* and *P* values | **7.981, 0.018** | **4.039, 0.056** |
| *R* squared | **0.444** | **0.473** |
| Orangebelly Darter | **$y = 1.509 + 0.0237(x)$** | **$y = 1.113 + 0.0831(x) – 0.0012(x^2)$** |
| *F* and *P* values | **5.634, 0.039** | **5.567, 0.027** |
| *R* squared | **0.360** | **0.553** |
| Brook Silverside | $y = 1.102 + 0.0374(x)$ | $y = 1.629 – 0.0416(x) + 0.0016(x^2)$ |
| *F* and *P* values | 2.517, 0.144 | 1.729, 0.231 |
| *R* squared | 0.201 | 0.278 |
| Golden Redhorse | **$y = 2.348 + 0.0726(x)$** | **$y = 1.767 + 0.1597(x) – 0.0017(x^2)$** |
| *F* and *P* values | **14.036, 0.004** | **8.571, 0.008** |
| *R* squared | **0.584** | **0.656** |
| Striped Shiner | $y = 1.598 + 0.0081(x)$ | $y = 1.411 + 0.0362(x) – 0.0006(x^2)$ |
| *F* and *P* values | 0.354, 0.565 | 0.339, 0.724 |
| *R* squared | 0. 034 | 0.069 |

| | | |
|---|---|---|
| Rocky Shiner | $y = 1.653 – 0.0038(x)$ | $y = 1.764 – 0.0205(x) + 0.0003(x^2)$ |
| *F* and *P* values | 0.210, 0.657 | 0.255, 0.780 |
| *R* squared | 0.021 | 0.054 |

## LIST OF FIGURES

Figure 1. Density effects of six fish species on periphyton biomass on days 15 and 30. The non-linear regression line was drawn in all cases where the non-linear model was significant.

Figure 2. Density effects of six fish species on benthic macroinvertebrate density on day 30.

Figure 3. Density effects of six fish species on accumulation of benthic particulate organic matter on day 30. The non-linear regression line was drawn in all cases where the non-linear model was significant.

Figure 4. Average percent occurrence of all food items consumed by each species when removed from stream mesocosms on day 30 of each experiment. Vertical bars represent 1 SD.

Figure 1.

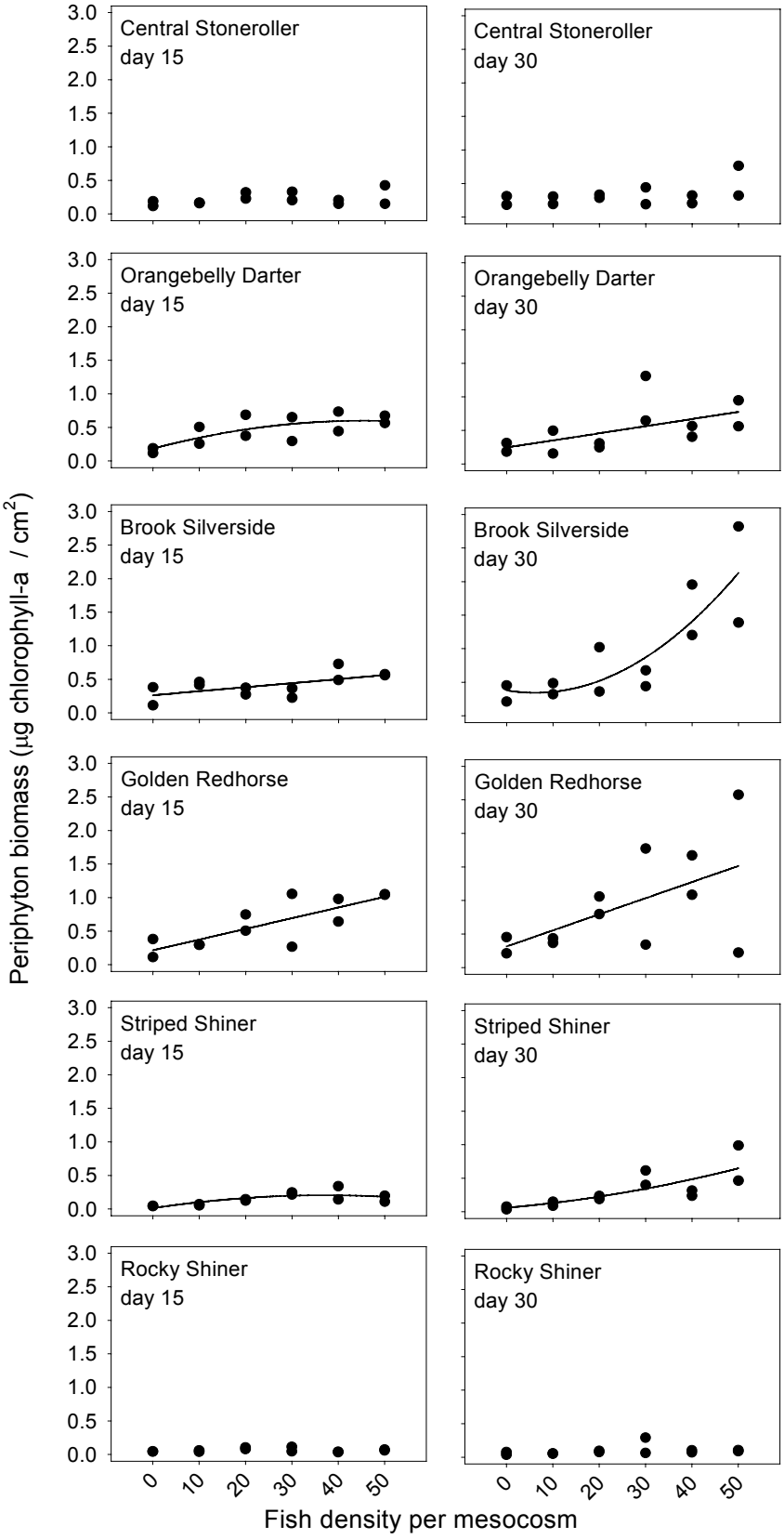

Central Stoneroller
day 15
Central Stoneroller
day 30
Orangebelly Darter
day 15
Orangebelly Darter
day 30
Brook Silverside
day 15
Brook Silverside
day 30
Golden Redhorse
day 15
Golden Redhorse
day 30
Striped Shiner
day 15
Striped Shiner
day 30
Rocky Shiner
day 15
Rocky Shiner
day 30
Periphyton biomass (µg chlorophyll-a / cm2)
Fish density per mesocosm
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
10
20
30
40
50

Figure 2.

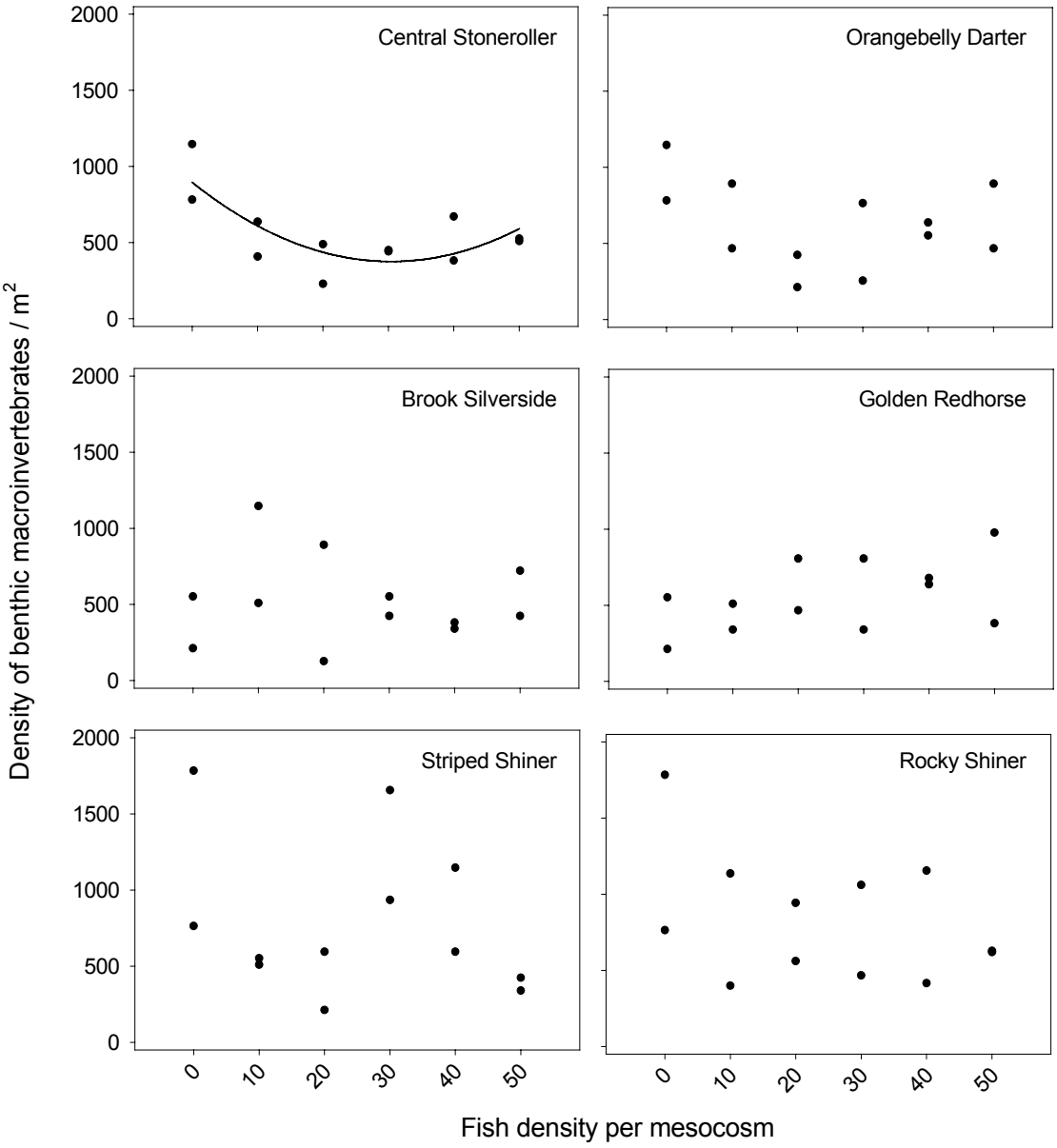

Central Stoneroller
Orangebelly Darter
Brook Silverside
Golden Redhorse
Striped Shiner
Rocky Shiner
Density of benthic macroinvertebrates / m2
Fish density per mesocosm
0
500
1000
1500
2000
0
10
20
30
40
50

Figure 3.

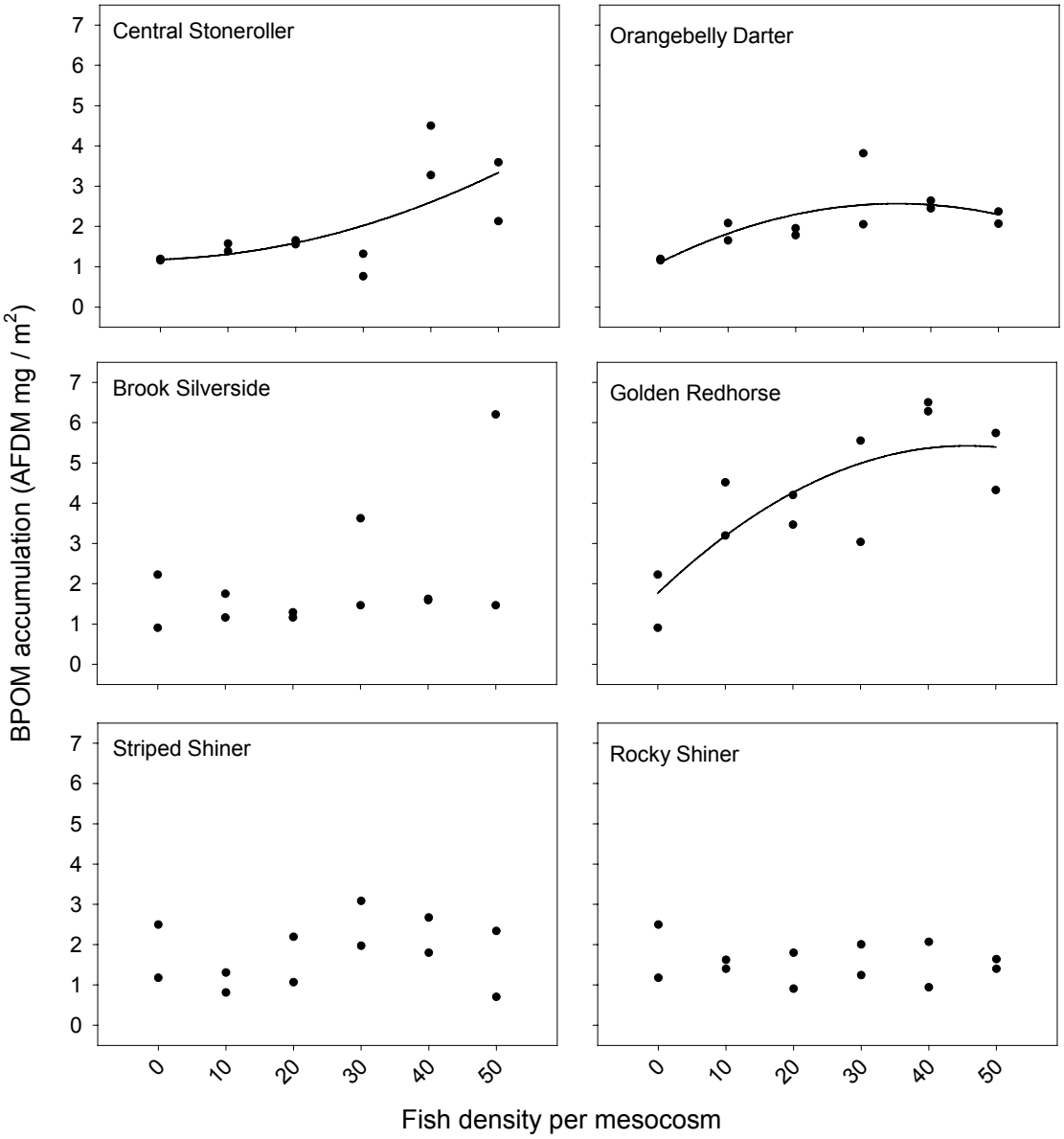
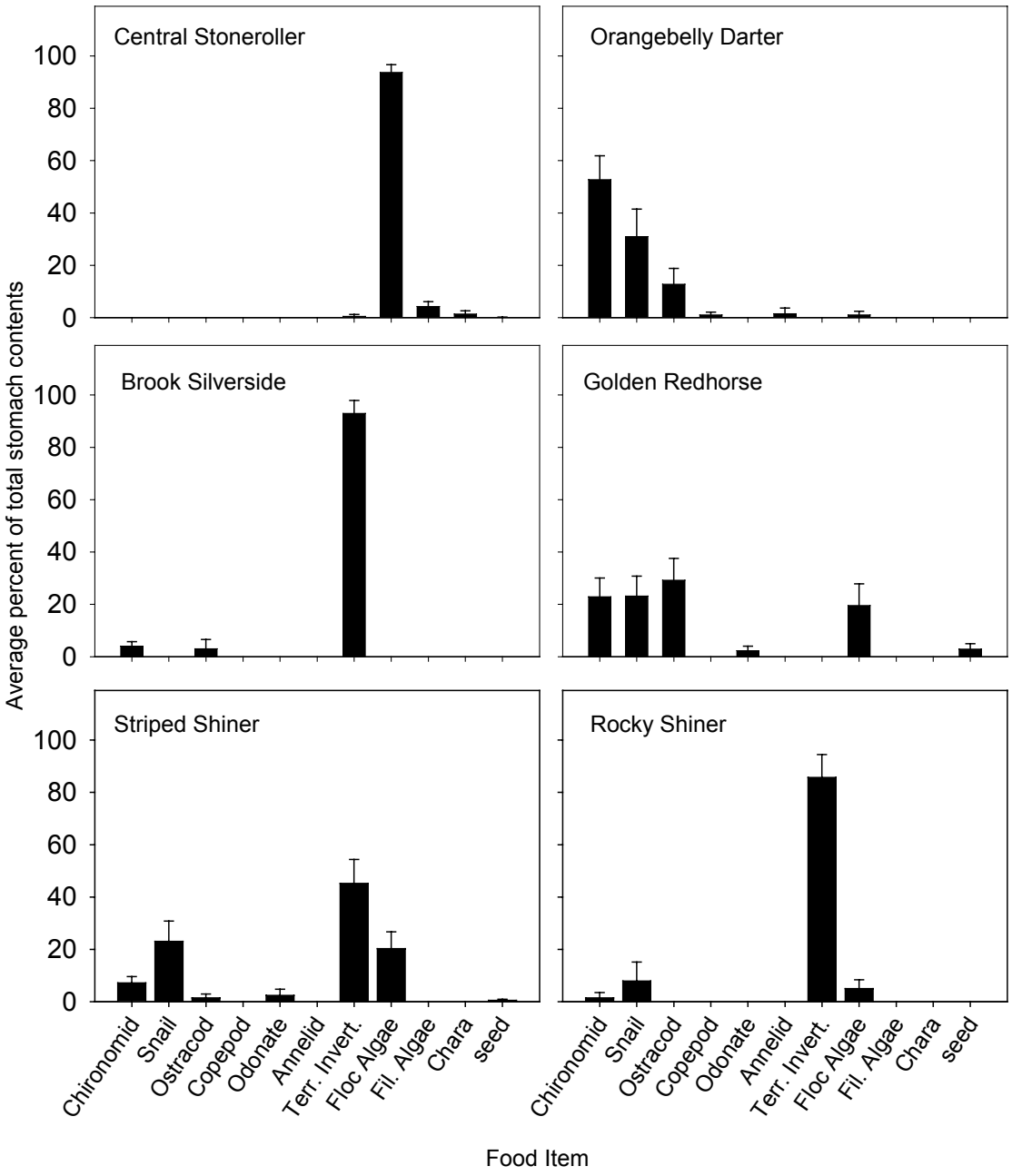

Central Stoneroller
Orangebelly Darter
Brook Silverside
Golden Redhorse
Striped Shiner
Rocky Shiner
BPOM accumulation (AFDM mg / m2)
Fish density per mesocosm
0
1
2
3
4
5
6
7
0
10
20
30
40
50

Figure 4.


Central Stoneroller
Orangebelly Darter
Brook Silverside
Golden Redhorse
Striped Shiner
Rocky Shiner
Average percent of total stomach contents
0
20
40
60
80
100
Chironomid
Snail
Ostracod
Copepod
Odonate
Annelid
Terr. Invert.
Floc Algae
Fil. Algae
Chara
seed
Food Item

# CHAPTER 2: STREAM FISHES AFFECT BENTHIC PRIMARY PRODUCTIVITY THROUGH SPECIES-SPECIFIC FOOD WEB PATHWAYS

**CHAD W. HARGRAVE**

*University of Oklahoma Biological Station and Department of Zoology*

*University of Oklahoma, Norman, Oklahoma 73019 USA*

## ABSTRACT

Consumers can affect ecosystem functions and properties through a variety of pathways. I tested three alternative hypotheses (i.e., trophic cascade, terrestrial nutrient translocation, and nutrient translocation via bioturbation) to examine the mechanistic roles of common, fishes in stream food webs. I used three fish species (Orangethroat Darter, Western Mosquitofish, and Bullhead Minnow) as model taxa to represent different functional groups with suspected different mechanistic effects on primary productivity (PPR). Stream mesocosms were fitted with fish and terrestrial insect barriers to address relative importance of localized fish predation versus access to terrestrial insects on effects of fish as consumers. Orangethroat Darter, a benthic invertivore, increased PPR through an apparent trophic cascade, by localized reduction of benthic grazing invertebrate densities. Western Mosquitofish, a surface feeding insectivore, increased PPR by enhancing nutrients through terrestrial nutrient translocation. Bullhead Minnow, a benthic omnivore that disturbed sediments during foraging, increased PPR through nutrient enhancement via bioturbation. It also reduced benthic grazing invertebrates. Thus, this species may have affected PPR through a combination of bioturbation and trophic cascade mechanisms. This study illustrates fishes affect PPR through pathways linked to species-specific trophic and functional characteristics.

## INTRODUCTION

Anthropogenic factors such as exotic introductions (Gido and Brown 1999), urbanization (Matthews and Gelwick 1990), and river impoundments (Rosenberg et al. 2000) negatively impact many aquatic ecosystems and their associated biota. Even small disturbances can affect a broad range of taxa and ecosystem processes because of the interconnected nature of food webs (Polis and Strong 1996). A mechanistic understanding of direct and indirect food web interactions is essential for predicting how communities and ecosystems might change in response to anthropogenic activities.

Because fishes are important consumers in many stream ecosystems, food web interactions involving fishes are potentially important regulatory processes of stream function. However, experimental studies testing mechanistic effects of fishes on stream functions are limited, and the consumer roles for most of the common, stream fish species in North America remain unknown (Matthews 1998). Using stream mesocosms, I tested three mechanistic hypotheses (Fig. 1) for consumer effects of fish on benthic primary productivity (PPR). I used three fish species from three functional groups common among the North American stream fish fauna. I expected each fish species to affect PPR through one of the three potential hypotheses. However, all three hypotheses were tested simultaneously for each species, identifying the most important consumer-mediated pathway for each taxon. I compared relative effects of localized predation and nutrient enrichment in these fishes by fitting stream mesocosms with barriers to exclude local fish presence from areas within mesocosms. To test the importance of terrestrial insect availability for fish effects, I placed barriers to terrestrial insects over stream mesocosms.

Localized predatory effects often result in positive effects on PPR (Hairston et al. 1960). In stream ecosystems, fish that consume or affect behavior of herbivores can indirectly increase PPR through a trophic cascade (e.g., Power and Matthews 1983, Power 1990, McIntosh and Townsend 1996). I hypothesized that Orangethroat Darter (*Etheostoma spectabile*), a benthic, invertivorous species, would increase PPR through a trophic cascade by locally reducing benthic grazing invertebrates (Fig. 1-A). Within mesocosms where fish were excluded from specific areas (mesocosm halves), I predicted Orangethroat Darter would increase PPR only in areas with fish locally present, and would have no effect on PPR in areas restricted from fish access (Table 1). I predicted the presence or absence of terrestrial insects would not influence this species effect on PPR, because its effect was not hypothesized to be linked to terrestrial insect availability (Fig. 1-A).

Organisms can affect the rate of nutrient exchange between ecosystem compartments (nutrient translocation), having positive or negative effects on ecosystem productivity (Vanni 2002). The translocation of nutrients between stream and terrestrial ecosystems has received much recent attention (Fausch et al. 2002, Baxter et al. 2005). Nutrient translocation out of streams into terrestrial ecosystems has been linked to production in vertebrates (e.g., Power 2001, Sabo and Power 2002). The opposite, i.e., translocation of terrestrial nutrients into stream ecosystems, can be an important energy source, influencing local fish abundance (Nakano and Murakami 2001).

Fish species that enhance the flux of terrestrial nutrients (terrestrial nutrient translocation) into streams should increase total soluble nutrients within the ecosystem, increasing PPR and benthic invertebrate densities (Gido and Matthews 2001). I tested the

terrestrial nutrient translocation hypothesis using Western Mosquitofish (*Gambusia affinis*), a small-bodied, surface feeding species. I hypothesized that Western Mosquitofish would consume terrestrial insects, excrete nutrients, and increase PPR and benthic grazing invertebrates (Fig. 1-B). I predicted that Western Mosquitofish would increase PPR only when terrestrial insects were accessible, and have no effect otherwise (Table 1). Change in PPR was predicted to increase with fish density because the overall nutrient flux into the mesocosm should be greater with more individuals. Enhancement of nutrients by fish was expected to permeate the fish barrier and occur throughout the mesocosm. Thus in mesocosms where Western Mosquitofish were excluded from specific areas, I predicted PPR would increase throughout the mesocosm regardless of local fish presence (Table 1).

In aquatic ecosystems nutrients accumulate in sediments, becoming unavailable to primary producers. Physical disturbance of sediments by organisms (bioturbation) can release these nutrients into the water column, enhancing PPR (Vanni 2002). This has been shown in invertebrates (Fukuhara and Sakamoto 1987) and fish (Schaus and Vanni 2000) in lake ecosystems. Bullhead Minnow (*Pimephales vigilax*), a benthic omnivore, disturbs the sediments through foraging. This results in suspension of benthic particulate matter and potentially releases sedimentary nutrients (pers. obs.). I hypothesized that Bullhead Minnow would increase PPR by enhancing nutrients through bioturbation (Fig. 1-C). I predicted Bullhead Minnow would increase PPR relative to mesocosms without fish. Increased PPR was expected to be linked to nutrient enhancement, which can permeate the fish barrier. Thus within mesocosms with Bullhead Minnow excluded from specific areas, I predicted PPR would increase throughout the mesocosm regardless of

local fish presence (Table 1). Terrestrial insect availability was not expected to influence Bullhead Minnow effects on PPR, because the nutrient enhancement by this species was predicted to come from sediments rather than terrestrial sources (Table 1). Bullhead Minnow consumes benthic invertebrates; therefore, I hypothesized it also would enhance PPR through a trophic cascade. Thus, within mesocosms with Bullhead Minnow excluded from specific areas, I predicted PPR to be greater in areas with local fish presence than in areas without (Table 1).

## METHODS

I conducted three 25-day experiments were conducted in stream mesocosms at the University of Oklahoma Biological Station (UOBS), Marshall Co. OK, USA. Each mesocosm consisted of one pool (183 cm diameter and 46 cm deep) and one riffle (122 cm long and 5 –10 cm deep) (Gelwick and Matthews 1997, Gido and Matthews 2001). Mesocosms were lined with natural gravel-cobble substrate taken from a nearby stream. Prior to each experiment, mesocosms were drained and cleaned, and sediment homogenized among individual units. Mesocosms remained dry for 7 days between experiments at which time they were refilled with water and inoculated with a 1.0 L of a natural periphyton slurry scraped from rocks in a nearby stream. Following inoculation, flow was continuously maintained by pumping water from a downstream collecting box to the head of each riffle with a 2500 L/h submersible pump, maintaining circulation of nutrients throughout each mesocosm. Mesocosms remained fishless for at least 7 days after receiving the periphyton inoculation. This allowed establishment of a periphyton assemblage dominated by *Oeogonium* and *Spirogyra*, recovery of a *Physella* snail

assemblage which were in the slurry and previously present in the mesocosms, and colonization of insect larvae, including dipterans, ephemeropterans, and odonates, by ovipositing winged adults.

### *Experimental Design*

The experimental design included two main effects: fish (3 treatment-levels) and terrestrial input (2 treatment-levels). Each stream mesocosm was divided in half with a fish excluding screen (3.0 mm mesh; Fig. 2). Twelve randomly selected mesocosms received 25 fish in both mesocosm halves, 12 received 25 fish in one randomly-selected half, and 12 had no fish. Thus, total fish density per mesocosm was 0, 25, or 50 individuals (0, 13.7, or 27.3 fish/$m^2$). One of two terrestrial insect treatments (with or without access by flying adults) was randomly assigned among mesocosms within each fish treatment level (Table 2). Mesocosms without insect access had mesh screening (1.0 mm) covering the mesocosm. Treatments with insect access had screening suspended over the mesocosm providing about a 40 cm access gap for flying adults, while controlling for potential shading effects of the screen. All insect screening was in place on the first day of the experiment. All treatments were replicated 6 times, totaling 36 independent experimental units in each experiment.

### *Fish*

Fish were collected from nearby streams and randomly assigned to mesocosms. The day fish were assigned to mesocosms was day-1 for each experiment. Additional individuals remaining after experiments were started were held in a spare mesocosm as

replacements for any fish that died. Orangethroat Darters were collected from Buckhorn Ck, Murray Co., OK., and put in mesocosms on 16 June 2003. Fifteen individuals died during the experiment and were replaced. Average standard length (SL) and mass for Orangethroat Darter was 36.9 ± 0.7 mm and 100 ± 9 mg, respectively. Western Mosquitofish were collected from a pond on the University of Oklahoma campus, Cleveland Co., OK., on 9 September 2003. Seven individuals died during this experiment and were replaced. Western Mosquitofish averaged 30.3 ± 0.8 mm and 113 ± 26 mg in SL and mass, respectively. Bullhead Minnows were collected from Lake Texoma, Marshall Co., OK, and put in mesocosms on 28 July 2003. Twenty two individuals died during the experiment and were replaced. Average SL and mass for Bullhead Minnows was 40.6 ± 1.9 mm and 313 ± 176 mg, respectively.

### *Stomach Contents*

At the end of each experiment (day 25) all fish were removed from the mesocosms and preserved in 10% formalin. Gut contents were examined for 10 randomly selected individuals from each treatment. I removed the anterior third of the alimentary tract (or the discrete stomach, if one existed), placed the contents on a gridded petri dish, and estimated percent occurrence for each major food category (e.g., aquatic invertebrate, algae, terrestrial invertebrates) by counting number of grids occupied by each food type. Schoener's index was used to assess dietary overlap between individuals from mesocosms with and without insect access (Schoener 1971).

### *Terrestrial Insect Access*

To estimate insect access, I placed 16 pan traps with ca. a 2 cm layer of soapy water (each 26.5 cm long by 20.5 wide, 6.0 cm deep) on the water surface of eight mesocosms from both terrestrial insect treatments (Southwood 1978). Pan trapss were added on day 10, removed on day 12, and contents preserved in 10 % formalin. Preserved insects were filtered through a 41 μm screen, dried at 50°C, weighed, ashed at 550°C for 1 h, and re-weighed to estimate total input of insects into the mesocosms. T-tests were used to examine any differences between terrestrial insect treatment levels in each experiment.

### *Benthic primary productivity*

About 24 h after the addition of the natural periphyton slurry , 4 unglazed clay tiles (225 $cm^2$) were placed in each mesocosm half as a substrate for periphyton growth. I used the oxygen evolution method (Stewart 1987, Gelwick and Matthews 1992) to estimate PPR on one randomly selected tile from each half on day 25.

### *Benthic Invertebrates*

On day 25, I took two sediment core samples (5.8 cm diameter, 15 cm deep) from the center of each mesocosm half and preserved in 10% formalin. Invertebrates were washed from sediments using a 250 μm sieve, identified to family, and counted. Only numbers of benthic invertebrates that consumed some algae and, thus, could have negatively affected periphyton biomass were used in analyses.

*Statistical Analyses*

I used a two-way repeated measures ANOVA to test for effects of fish and terrestrial insect treatments on PPR and benthic invertebrate density. Samples from both mesocosm halves were modeled as the repeated measures because of the lack of independence between halves within each mesocosm unit. Independent contrast statements were used to test for differences among mesocosms with and without fish for each terrestrial insect treatment. Paired t-tests were used to test for differences between mesocosm halves within each fish and terrestrial insect treatment level. The following statistical interpretations were used as evidence supporting either of the three hypotheses:

(1) Trophic cascade. – A significant half by fish treatment interaction; a difference between mesocosm halves only for treatments with fish excluded from one half; and no significant terrestrial insect treatment effect or significant interactions with insect treatment was interpreted as support for the trophic cascade mechanism.

(2) Terrestrial nutrient translocation. – A significant interaction between fish and terrestrial insect treatment; significant fish treatment effect only in mesocosms with terrestrial insect access; and no significant mesocosm half effect or half by main treatment interactions was interpreted as support for the terrestrial nutrient translocation mechanism

(3) Nutrient Translocation through Bioturbation. – A significant fish treatment effect; no significant terrestrial insect treatment effect; no interaction between fish and terrestrial insect treatment; and no significant interaction between mesocosm half and either main treatment was interpreted as support for the nutrient translocation through bioturbation mechanism

SAS (2000) was used for repeated measures ANOVAs, independent contrasts, paired t-tests, and independent two-sample t-tests.

## RESULTS

### *Terrestrial Insect Access*

Terrestrial insect barriers significantly reduced mass of terrestrial insects captured in pan traps on stream mesocosm surfaces by 4.1, 7.4, and 11.1 fold during experiments with Orangethroat Darter, Western Mosquitofish, and Bullhead Minnow, respectively (Fig. 3).

### *Effects of Orangethroat Darter*

Orangethroat Darter effects on PPR supported a trophic cascade. Stream mesocosms with Orangethroat Darter had on average 1.89 times greater PPR than mesocosms without (Fig. 4). There was a significant mesocosm half effect and a significant half by fish treatment interaction (Table 3). Within mesocosms where Orangethroat Darter was excluded from one half, there was 2.3 times greater PPR in halves with fish than in halves without (paired t-test: with terrestrial insect access $t = 2.87$, $P = 0.035$; without terrestrial insect access $t = 3.24$, $P = 0.023$; Fig. 4). Terrestrial insect treatment effect was not significant. There was no significant interaction between fish and terrestrial insect treatments, or between mesocosm half and terrestrial insect treatment (Table 3).

Potential algivorous invertebrates in the Orangethroat Darter experiment included Chironomidae (relative abundance 44%), Physidae and Planorbidae (30%), and

Tricorythidae (11%). Orangethroat Darter effects on benthic invertebrate density supported a trophic cascade mechanism (Fig. 5). There was a significant mesocosm half by fish treatment interaction (Table 3). Within mesocosms with Orangethroat darter present in only one half, the halves without fish had about twice as many invertebrates as halves with fish (paired t-test: with terrestrial insect access $t = -2.18$, $P = 0.081$; without terrestrial insect access $t = 4.92$, $P = 0.004$; Fig. 5). The was no significant fish or terrestrial insect treatment effect on benthic invertebrate density, nor was their interaction significant (Table 3).

Orangethroat Darter consumed mostly grazing chironomid larvae. There was high diet overlap (63.0%) between Orangethroat Darter in streams with and without terrestrial insects. Although chironomid larvae were the most common food item consumed by Orangethroat Darter, there was a greater proportion of snails and fewer chironomids and ostracods in Orangethroat Darter guts from mesocosms without than from mesocosms with terrestrial insect access (Fig. 6).

### *Effects of Western Mosquitofish*

Western Mosquitofish effects on PPR supported the terrestrial nutrient translocation hypothesis (Fig. 4). Fish and terrestrial insect treatments effects were significant, as was their interaction (Table 3). Western Mosquitofish increased PPR by an average of 2.1 times in mesocosms with terrestrial insect access, but had no effect in mesocosms without terrestrial insects (Fig. 4). There was no significant mesocosm half effect or mesocosm half by main treatment interactions (Table 3).

Potential algivorous invertebrates in the Western Mosquito fish experiment included Chironomidae (41%) and Physidae and Planorbidae (38%), and Tricorythidae (3%). Invertebrate densities were highly variable among Western Mosquitofish treatments (Fig. 5). The half by main treatment interaction was the only significant source of variation accounting for invertebrate density (Table 3). This significant interaction occurred because the differences between mesocosm halves was greatest in mesocosms without terrestrial insect barriers than in mesocosms with barriers.

Diet overlap between Western Mosquitofish in mesocosms with and without terrestrial insect access was 52.5%, suggesting some diet switching in fish from the two terrestrial insect treatments. Western Mosquitofish consumed mostly terrestrial arthropods in mesocosms with terrestrial insect access, and consumed more benthic items and fewer terrestrial arthropods in mesocosms without terrestrial insects (Fig. 6).

### *Effects of Bullhead Minnow*

Bullhead Minnow effects on PPR were not fully consistent the bioturbation hypothesis (Fig. 4). Bullhead Minnow increased PPR by 1.70 times on average relative to mesocosm without fish. There was a significant fish treatment effect, but no significant terrestrial insect treatment effect. There was no fish by terrestrial insect treatment interaction (Table 3). Effect of mesocosm half, or half by main treatment interactions were not significant.

Potential algivorous invertebrates in this experiment included Chironomidae (33%), Physidae and Planoribidae (35%), and Tricorythidae (3%). Effects of Bullhead Minnow on benthic grazing invertebrates matched a trophic cascade (Fig. 5). There was

a significant mesocosm half by fish treatment interaction for invertebrates (Table 3). In mesocosms with Bullhead Minnow present in only one half, invertebrate density was 1.75 times greater on average in the half without Bullhead Minnow than in the half with fish (paired t-test: with terrestrial insect access $t = -8.88$, $P < 0.001$; without terrestrial insect access $t = 2.97$, $P = 0.031$; Fig. 5). Benthic invertebrate density was not affected by fish or terrestrial insect treatments, or their interaction (Table 3).

Bullhead Minnow consumed an array of food items ranging from benthic invertebrates to terrestrial insects and appeared to shift food habits between the two terrestrial insect treatments (Fig. 6). There was 34.7% overlap in diet between Bullhead Minnows from both terrestrial insect treatments. Filamentous algae, daphnia, and terrestrial arthropods comprised a greater proportion in Bullhead Minnow diet when in mesocosms with terrestrial insects; whereas, benthic invertebrates such as snails, chironomids, and ostracods comprised the greatest proportion of food items for Bullhead Minnow in mesocosms without terrestrial insects.

## DISCUSSION

Effects of Orangethroat Darter supported predictions for the trophic cascade hypothesis, which were Orangethroat Darter would decrease grazer densities, increasing PPR. In stream mesocosms with Orangethroat Darter present in only one half of the stream mesocosm (half), the fish effect on PPR was dependent on local fish presence, suggesting that localized predation pressure on invertebrates decreased potential algivory and, thus, increased periphyton standing crop. Most trophic cascade studies have found reciprocal predator-prey effects that result in increased PPR (Pace et al. 1999). Much like

the cascading predatory effects of fish shown in lake (Carpenter et al. 1985) and stream (Power 1990) ecosystems, Orangethroat Darter consumed mostly benthic, algivorous and detritivoroius invertebrates, reducing invertebrate densities and increasing PPR.

Trophic cascades occur in terrestrial (Moran and Hurd 1998), lake (McQueen et al. 1986), marine (Wooten 1995), and stream communities (Huryn 1998). My experiment with Orangethroat Darter was different than previous studies, however, because it simultaneously tested the predatory, nutrient translocation, and bioturbation effects of this fish on PPR. This allowed me to determine relative importance of these two pathways for regulation of PPR by benthic invertivores. Although Orangethroat Darter recycles nutrients through excretion, its effect on PPR likely was not driven by nutrient enrichment. I based this on the fact that PPR did not increase in mesocosm halves without fish present, and that terrestrial insect access had no influence on this species' effect. A positive response in PPR in these cases would have suggested nutrients were a significant part of the mechanistic pathway. Orangethroat Darter enhancing PPR via direct predation of algivorous and detritivorous invertebrates, and nutrient effects through bioturbation or consumption of terrestrial insects is inconsequential for this species.

Western Mosquitofish apparently affected PPR via terrestrial nutrient translocation. In mesocosms with Western Mosquitofish present in only one half, PPR increased on both sides of the barrier regardless of its local presence. This suggests that increased PPR resulted from factors able to cross the fish barrier, namely water soluble nutrients. Western Mosquitofish effects on PPR were absent in mesocosms without terrestrial insect access, suggesting the nutrient enhancement was linked to terrestrial

insect availability. This species consumed terrestrial insects when available, apparently enhanced soluble nutrients, and increased PPR. Gido and Matthews (2001) proposed a nutrient translocation hypothesis (converting surface insects to watercolumn nutrients) to explain increased PPR with increased density of Red Shiner (*Cyprinella lutrensis*), a watercolumn insectivore. Our data provide mechanistic support for the Gido and Matthews (2001) conclusions that fishes can have important roles linking terrestrial nutrient sources to benthic stream ecosystem compartments.

Fausch et al. (2002) also showed an indirect link between terrestrial inputs and stream algae, albeit through a mechanism different from the terrestrial nutrient translocation hypothesis. They found that in the absence of terrestrial inputs, Dolly Varden charr consumed benthic invertebrates, causing a trophic cascade. I observed this same interaction, but the strength was low and the diet shift in Western Mosquitofish to include more benthic invertebrates did not affect PPR through a trophic cascade. Diet preference of Western Mosquitofish in this experiment seemed somewhat limited to a specific number of food items. This possibly restricted its ability to switch to completely different food sources in the different terrestrial insect treatments. The dependence of Western Mosquitofish effects on PPR to terrestrial insect availability was likely exemplified by this limited prey switching ability. Because many fish species prefer terrestrial over benthic food items, the terrestrial nutrient translocation mechanism may be a common pathway in stream ecosystems. However, it is likely that this mechanism is dominant only during times of high terrestrial insect availability.

My data are partially consistent with the hypothesis that Bullhead Minnow affected PPR through nutrient translocation via bioturbation. In mesocosms where

Bullhead Minnow was present in only one half, PPR increased in the half without the fish. This suggests nutrients were enhanced in these mesocosms, permeating the fish barrier and increasing PPR in fishless halves. The nutrient enrichment in these mesocosms likely came from the sediments, because this effect was present in treatments without terrestrial insect access. In these mesocosm, however, PPR was not enhanced as predicted in the halves with local presence of Bullhead Minnow. I suggest that increased benthic foraging caused this observation. Gut content data indicated that Bullhead Minnow consumed a greater proportion of benthic food items in mesocosms without terrestrial insects, and consumed more terrestrial arthropods in mesocosms with terrestrial insect access. Increased benthic foraging activity by this species in mesocosms without terrestrial insects would have caused greater physical disturbance of the stream sediments. In turn, this would have reduced PPR in the mesocosm half with fish present, and increased PPR in the opposite half through nutrient enhancement.

Nutrient release from sediments by benthic feeding fishes has been shown to be a significant source of nutrient loading in lake ecosystems (Schaus and Vanni 2000, Vanni et al. 2005), but has been less studied in stream ecosystems (Grimm 1988). I suggest my data support the hypothesis that fish bioturbation in stream ecosystems can enhance PPR by releasing sedimentary nutrients in a manner similar to lake ecosystems.

My data suggest, however, that Bullhead Minnow also might have affected PPR through additional mechanistic pathways, which depended on physical fish presence or terrestrial insect availability. The effects of Bullhead Minnow on PPR did not match predictions for a trophic cascade, but the local effects of Bullhead Minnow on benthic invertebrate density did. Bullhead Minnow consumed a large proportion of benthic

grazing invertebrates. It also significantly reduced benthic invertebrates in mesocosm halves with fish present relative to halves without. It is possible that Bullhead Minnow locally affected PPR through a trophic cascade. Bullhead Minnow also consumed terrestrial insects when in mesocosms with insect access. Thus, the terrestrial nutrient translocation hypothesis could have caused the nutrient enrichment effect in treatments with terrestrial insect availability. The effects of Bullhead Minnow appeared to be highly context dependent. Local effects on PPR could have been linked to local predation. In the absence of terrestrial insects nutrient enhancement effects were likely linked to bioturbation, and in the presence of terrestrial insects nutrient enhancement effects could have been linked to terrestrial nutrient translocation. I suggest that this species' primary mechanistic pathway is likely to vary under different ecological scenarios.

These experiments simultaneously tested three alternative hypotheses for consumer regulation of PPR and benthic invertebrate grazers by three different fish species from different functional groups. I concluded that the Orangethroat Darter and Western Mosquitofish affected PPR through trophic cascade and terrestrial nutrient translocation, respectively. The Bullhead Minnow affected PPR through bioturbation, but also might have enhanced PPR through the other pathways was well. Thus, specific mechanisms might be highly context dependent for certain species particularly if an omnivore. Because the fish species used in this study represented common functional groups found in most small to moderate sized streams throughout the Mississippi River drainage in North America, I suggest that these different consumer mediated pathways are likely present in many stream ecosystems. It is likely these pathways have important regulatory effects on PPR in many natural stream food webs. Thus, it is logical to predict

that anthropogenic disturbances threatening functional composition in stream food webs would affect a variety of pathways linked to basic ecosystem functions such as PPR. Such impacts could have extensive consequences for many taxa, processes, and functions, linked by various direct and indirect interactions.

## ACKNOWLEDGEMENTS

I am grateful to R. Deaton, S. Hargrave, R. Remington, and P. Unmack for help collecting fishes and starting experiments. I thank T. Cluck, D. Cobb, R. Page, M. Ward, and L. Weider at University of Oklahoma Biological Station for use of facilities and housing. I thank my doctoral committee, E. Bergey, M. Kaspari, W. Matthews, R. Nairn, and C. Vaughn, for critical discussions and comments on this manuscript. The assistance of J. Mendoza (University of Oklahoma, Department of Psychology) was instrumental in statistical analyses and interpretations. Funding was provided by a National Science Foundation Dissertation Improvement Grant to W. Matthews and C. Hargrave (NSF 0308729), a Blanche Adams Memorial Scholarship, and a McCarley Graduate Research Grant from Southwestern Association of Naturalists. This work was submitted in partial fulfillment of Ph.D. degree at University of Oklahoma, Department of Zoology.

## LITERATURE CITED


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

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

Carpenter, S. R., J. F. Kitchell, and J. R. Hodgson. 1985. Cascading trophic interactions and lake productivity. Bioscience **35**:634-639.

Fausch, K. D., M. E. Power, and M. Murakami. 2002. Linkages between stream and forest food webs: Shigeru Nakano's legacy for ecology in Japan. Trends in Ecology and Evolution **17**:429-434.

Fukuhara, H., and M. Sakamoto. 1987. Enhancement of inorganic nitrogen and phosphate release from lake sediment by tubificid worms and chironomid larvae. Oikos **38**:312-320.

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

Gelwick, F. P., and W. J. Matthews. 1997. Effects of algivorous minnows (*Campostoma*) on spatial and temporal heterogeneity of stream periphyton. Oecologia **112**:386-392.

Gido, K. B., and J. H. Brown. 1999. Invasion of North American drainages by alien fish species. Freshwater Biology **42**:387-399.

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

Grimm, N. B. 1988. Feeding dynamics, nitrogen budgets, and ecosystem role of a desert stream omnivore, *Agosia chrysogaster* (Pisces: Cyprinidae). Environmental Biology of Fishes **21**:143-152.

Hairston, N. G., F. E. Smith, and L. B. Slobodkin. 1960. Community structure, population control, and competition. The American Naturalist **44**:421-425.

Huryn, A. D. 1998. Ecosystem-level evidence for top-down and bottom-up control of production in a grassland stream system. Oecologia **115**:173-183.

Matthews, W. J. 1998. Patterns in freshwater fish ecology. Chapman and Hall, New York, New York, USA.

Matthews, W. J., and F. P. Gelwick. 1990. Fishes of Crutcho Creek and the North Canadian River in central Oklahoma: effects of urbanization. Southwestern Naturalist **35**:403-410.

Mcintosh, A. R., and C. R. Townsend. 1996. Interactions between fish, grazing invertebrates and algae in a New Zealand stream: a trophic cascade mediated by fish-induced changes to grazer behavior? Oecologia **108**:174-181.

McQueen, D. J., J. R. Post, and E. L. Mills. 1986. Trophic relationships in freshwater pelagic ecosystems. Canadian Journal Fisheries Aquatic Sciences **43**:1571-1581.

Moran, M. D., and L. E. Hurd. 1998. A trophic cascade in a diverse arthropod community caused by a generalist arthropod predator. Oecologia **113**:126-132.

Nakano, S., and M. Murakami. 2001. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. Proceedings of the National Academy

of Science, USA **98**:166-170.

Pace, M. L., J. J. Cole, S. R. Carpenter, and J. F. Kitchell. 1999. Trophic cascades revealed in diverse ecosystems. Trends in Ecology and Evolution **14**:483-488.

Polis, G. A., and D. R. Strong. 1996. Food web complexity and community dynamics. The American Naturalist **147**:813-846.

Power, M. E. 1990. Effects of fish in river food webs. Science **250**:811-814.

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. E. 2001. Prey exchange between a stream and its forested watershed elevates predator densities in both habitats. Proceedings of the National Academy of Science, USA **98**:14-15.

Rosenberg, D. M., P. McCully, C. M. Pringle. 2000. Global-scale environmental effects of hydrological alterations: introduction. Bioscience **50**: 746-751.

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

SAS Institute. 2000. Version 8.01. Cary, North Carolina, USA.

Schaus, M. H., and M. J. Vanni. 2000. Effects of gizzard shad on phytoplankton and nutrient dynamics: role of sediment feeding and fish size. Ecology **81**:1701-1719.

Schoener, T. W. 1971. Theory of feeding strategies. Annual Review of Ecology and Systematics **2**:369-404.

Southwood, R. 1978. Ecological Methods: with particular reference to the study of insect populations. Chapman Hall, New York.

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

Vanni, M. J. 2002. Nutrient cycling by animals in freshwater ecosystems. Annual Review Ecology and Systematics **33**:341-370.

Vanni, M. J., K. K. Arend, M. T. Bremigan, D. B. Bunnel, J. E. Garvey, M. J. Gonzalez, W. H. Renwick, P. A. Soranno, and R. A. Stein. 2005. Linking landscapes and food webs: effects of omnivorous fish and watersheds on reservoir ecosystems. Bioscience **55**: 155-167

Wooten, J. T. 1995. Effects of birds on sea urchins and algae: a lower-intertidal trophic cascade. Ecoscience **2**:321-328.

Table 1. Predicted effects of Orangethroat Darter, Western Mosquitofish, and Bullhead Minnow on primary productivity in each stream half for all fish and terrestrial insect treatments. Horizontal line indicates no effect. Direction and number of arrows indicates direction and relative magnitude of effects. For example, Bullhead Minnow treatment with 25 fish per stream has greater PPR in half-A with 25 fish than half-B with no fish.

| | Fish Treatment per Stream | | | | | |
|---|---|---|---|---|---|---|
| | 50 fish | | 25 fish | | 0 fish | |
| | 25 fish | 25 fish | 25 fish | 0 fish | 0 fish | 0 fish |
| Orangethroat Darter | half-A | half-B | half-A | half-B | half-A | half-B |
| Effects on PPR | | | | | | |
| With terrestrial insects | ↑ | ↑ | ↑ | – | – | – |
| Without terr. insect | ↑ | ↑ | ↑ | – | – | – |
| Western Mosquitofish | | | | | | |
| Effects on PPR | | | | | | |
| With terrestrial insects | ↑↑ | ↑↑ | ↑ | ↑ | – | – |
| Without terr. insects | – | – | – | – | – | – |

| Bullhead Minnow | | | | | | |
|---|---|---|---|---|---|---|
| Effects on PPR | | | | | | |
| With terrestrial insects | ↑↑ | ↑↑ | ↑↑ | ↑ | – | – |
| Without terr. insects | ↑↑ | ↑↑ | ↑↑ | ↑ | – | – |

Table 2. Experimental design showing number of fish per stream mesocosm half within each treatment level of fish and terrestrial insect access. Each treatment has six replicates.

| | Fish Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Total fish per Mesocosm | | | | | |
| | 50 | | 25 | | 0 | |
| Without Terrestrial Insect Barrier | half-A | half-B | half-A | half-B | half-A | half-B |
| | 25 | 25 | 25 | 0 | 0 | 0 |
| | $n = 6$ | | $n = 6$ | | $n = 6$ | |
| With Terrestrial Insect Barrier | half-A | half-B | half-A | half-B | half-A | half-B |
| | 25 | 25 | 25 | 0 | 0 | 0 |
| | $n = 6$ | | $n = 6$ | | $n = 6$ | |

Table 3. Repeated-measure analysis of variance tables summarizing statistical results of fish and terrestrial insect treatment effects, stream half effect, and all interaction effects on benthic primary production (PPR) and benthic grazing invertebrate density (Invertebrates) for each fish species.

| | | PPR | | Invertebrates | |
|---|---|---|---|---|---|
| Source of Variation | DF | *F* | *P* | *F* | *P* |
| Orangethroat Darter | | | | | |
| Fish (F) | 2,30 | **3.45** | **0.045** | 0.62 | 0.542 |
| Terr. Insect (T) | 1,30 | 0.23 | 0.638 | 0.01 | 0.905 |
| Stream Half (H) | 1,30 | **11.86** | **0.002** | 3.25 | 0.081 |
| Interaction terms | | | | | |
| F × T | 2,30 | 0.07 | 0.936 | 0.40 | 0.672 |
| H × F | 2,30 | **18.18** | **<0.001** | **4.26** | **0.024** |
| H × T | 1,30 | 0.08 | 0.780 | 0.42 | 0.522 |
| H × F × T | 2,30 | 0.73 | 0.488 | 1.39 | 0.264 |
| Western Mosquitofish | | | | | |
| Fish (F) | 2,30 | **12.03** | **<0.001** | 0.29 | 0.751 |
| Terr. Insect (T) | 1,30 | **26.83** | **<0.001** | 0.53 | 0.474 |
| Stream Half (H) | 1,30 | 0.85 | 0.365 | 1.39 | 0.248 |

| | | | | | |
|---|---|---|---|---|---|
| Interaction terms | | | | | |
| F × T | 2,30 | **4.84** | **0.015** | 0.96 | 0.394 |
| H × F | 2,30 | 0.59 | 0.560 | 1.32 | 0.283 |
| H × T | 1,30 | 0.56 | 0.457 | **7.41** | **0.012** |
| H × F × T | 2,30 | 0.93 | 0.407 | 0.12 | 0.885 |
| | | | | | |
| Bullhead Minnow | | | | | |
| Fish (F) | 2,30 | **4.38** | **0.021** | 1.08 | 0.352 |
| Terr. Insect (T) | 1,30 | 1.32 | 0.259 | 2.75 | 0.108 |
| Stream Half (H) | 1,30 | 1.94 | 0.173 | 3.33 | 0.078 |
| Interaction terms | | | | | |
| F × T | 2,30 | 0.54 | 0.589 | 0.60 | 0.556 |
| H × F | 2,30 | 1.65 | 0.209 | **12.15** | **<0.001** |
| H × T | 1,30 | 0.92 | 0.344 | 2.19 | 0.149 |
| H × F × T | 2,30 | 0.85 | 0.439 | 0.02 | 0.978 |

## LIST OF FIGURES

Figure 1. Hypothesized consumer mediated pathways for (A) Orangethroat Darter (trophic cascade), (B) Western Mosquitofish (terrestrial nutrient translocation), and (C) Bullhead Minnow (bioturbation and trophic cascade). Large arrows connecting food web compartments indicate direction of energy flow. Small arrows adjacent to each food web compartment indicate hypothesized direct and indirect effects of fish on invertebrates, algae, and nutrients.

Figure 2. Depiction of stream mesocosms with fish barrier dividing each pool.

Figure 3. Effects of terrestrial insect barrier on terrestrial insect access collected in water surface traps. Probability values are from t-tests. Vertical bars represent 1 standard error (SE).

Figure 4. Average PPR measured from each mesocosm half (columns) for each treatment. Column shading indicates terrestrial insect treatment. Vertical bars represent 1 SE.

Figure 5. Average benthic grazing invertebrate density measured from each mesocosm half (columns) for each treatment. Column shading indicates terrestrial input treatment. Vertical bars represent 1 SE.

Figure 6. Average percent occurrence of food items found in guts of 10 individuals of each species taken from mesocosms with and without terrestrial insect barriers. Vertical bars represent 1 SE.

Figure 1.

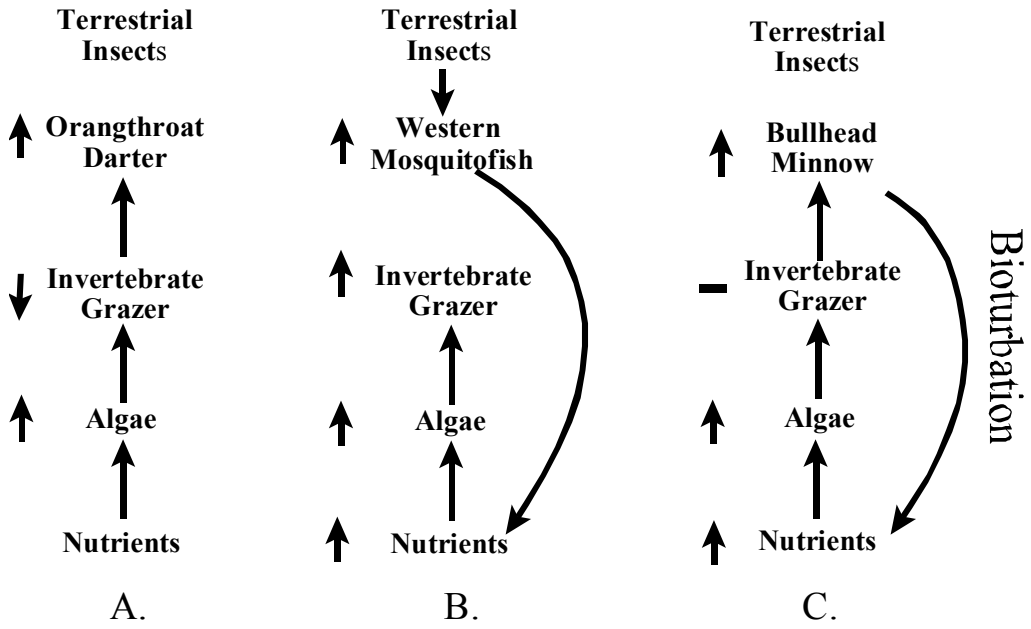

Terrestrial Insects
Orangthroat Darter
Invertebrate Grazer
Algae
Nutrients
A.
Terrestrial Insects
Western Mosquitofish
Invertebrate Grazer
Algae
Nutrients
B.
Terrestrial Insects
Bullhead Minnow
Invertebrate Grazer
Algae
Nutrients
Bioturbation
C.

Figure 2.

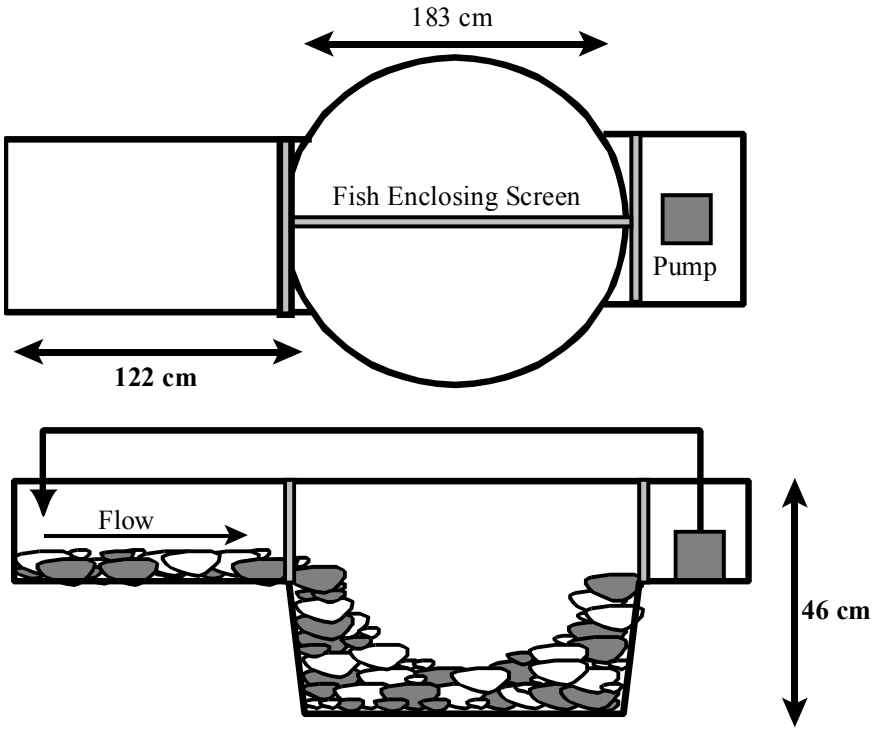


Figure 3.

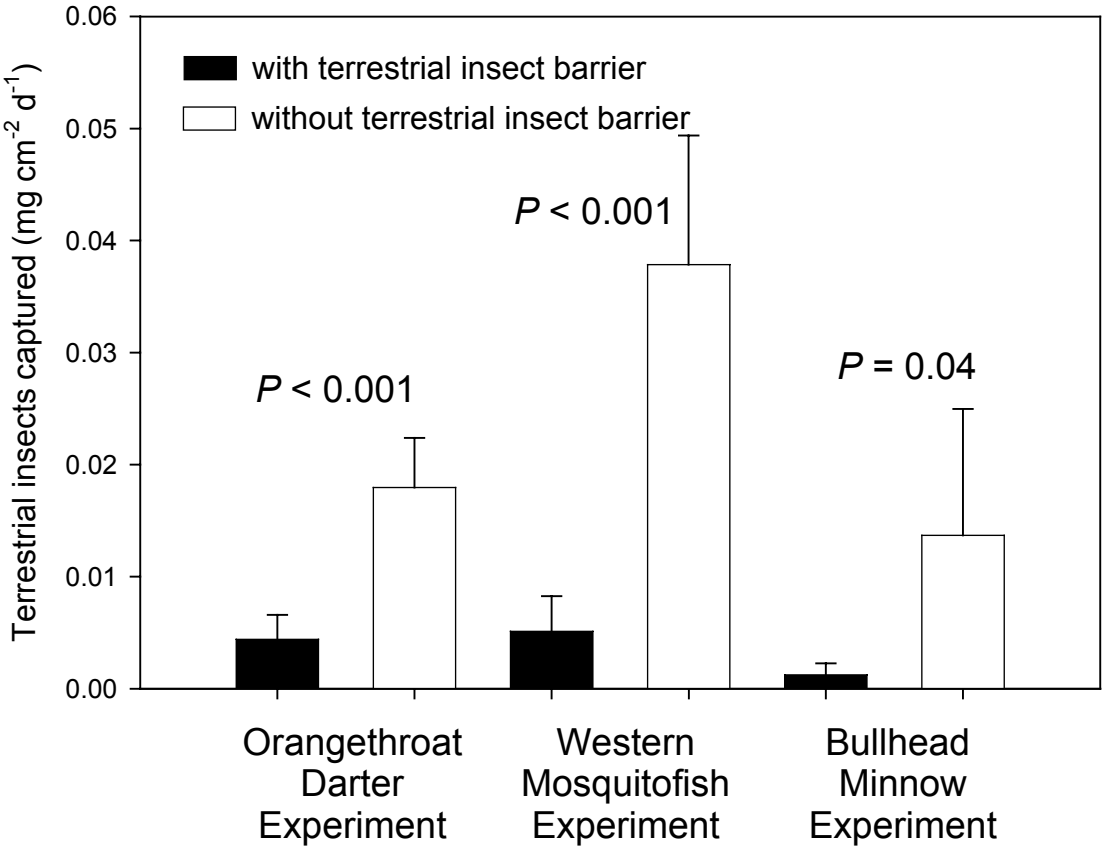

0.06
0.05
0.04
0.03
0.02
0.01
0.00
Terrestrial insects captured (mg cm-2 d-1)
with terrestrial insect barrier
without terrestrial insect barrier
P < 0.001
P < 0.001
P = 0.04
Orangethroat Darter Experiment
Western Mosquitofish Experiment
Bullhead Minnow Experiment

Figure 4.

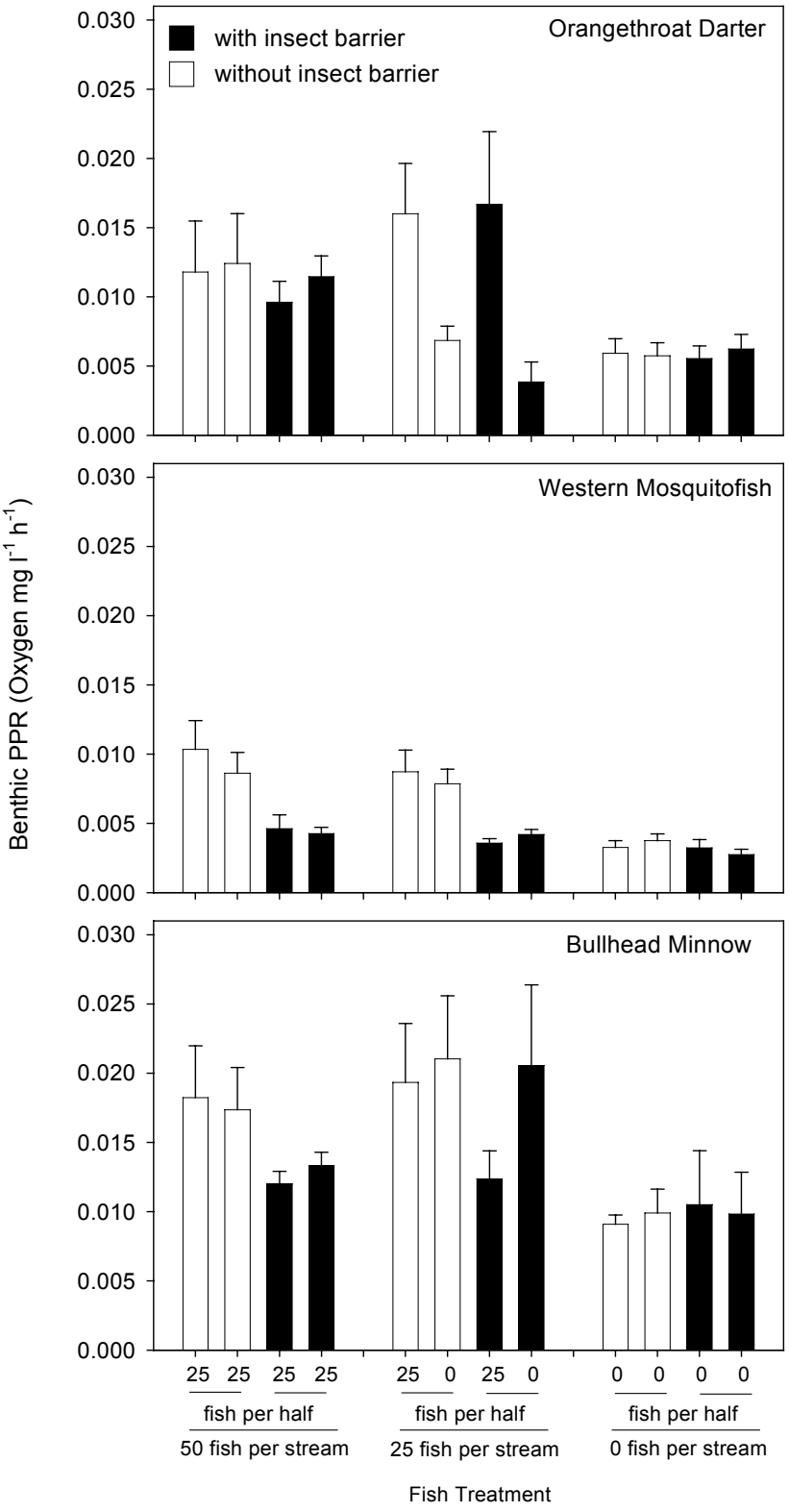

with insect barrier
without insect barrier
Orangethroat Darter
Western Mosquitofish
Bullhead Minnow
Benthic PPR (Oxygen mg l-1 h-1)
0.030
0.025
0.020
0.015
0.010
0.005
0.000
25 25 25 25 25 0 25 0 0 0 0 0
fish per half
50 fish per stream
25 fish per stream
0 fish per stream
Fish Treatment

Figure 5.

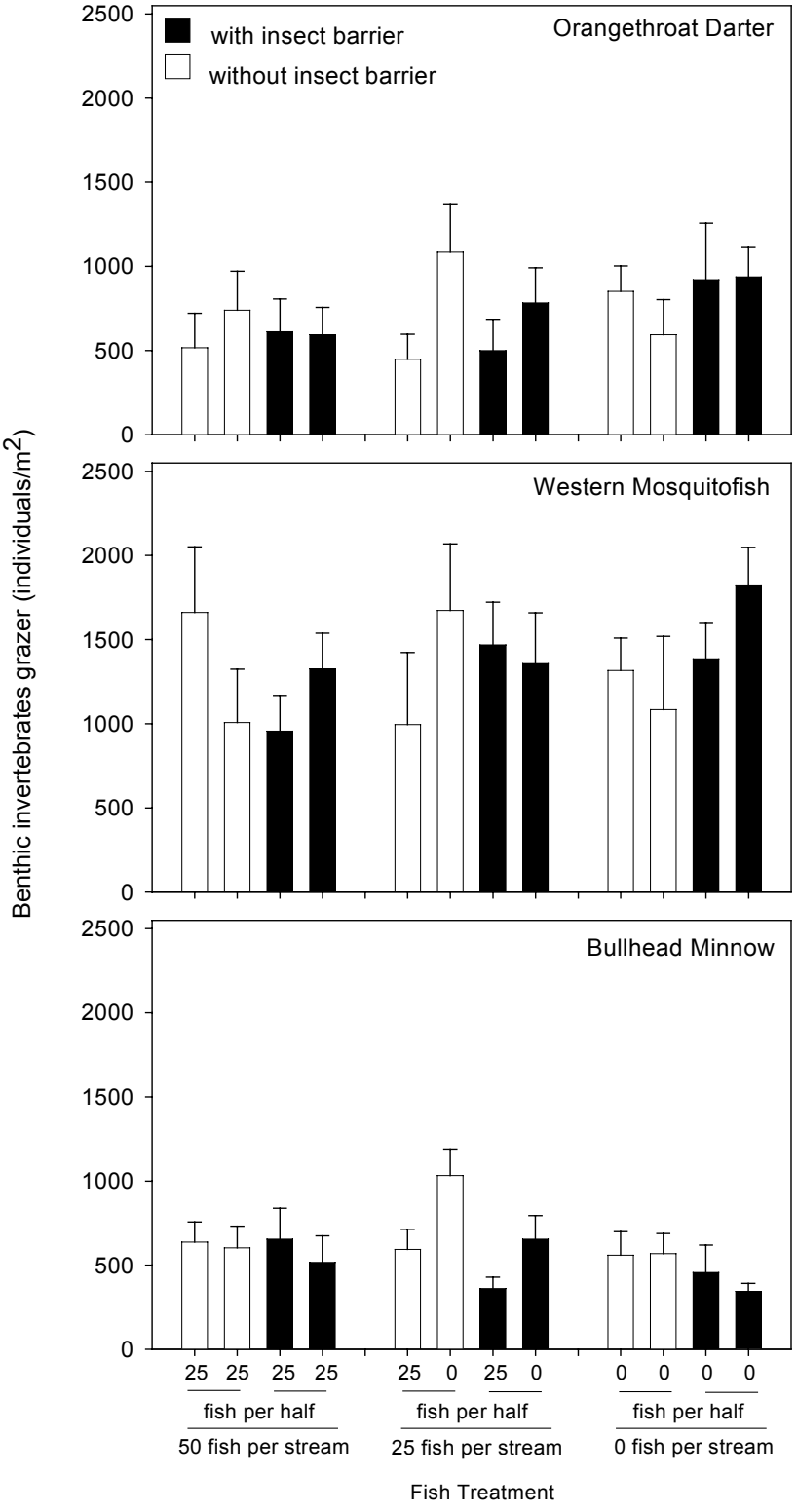
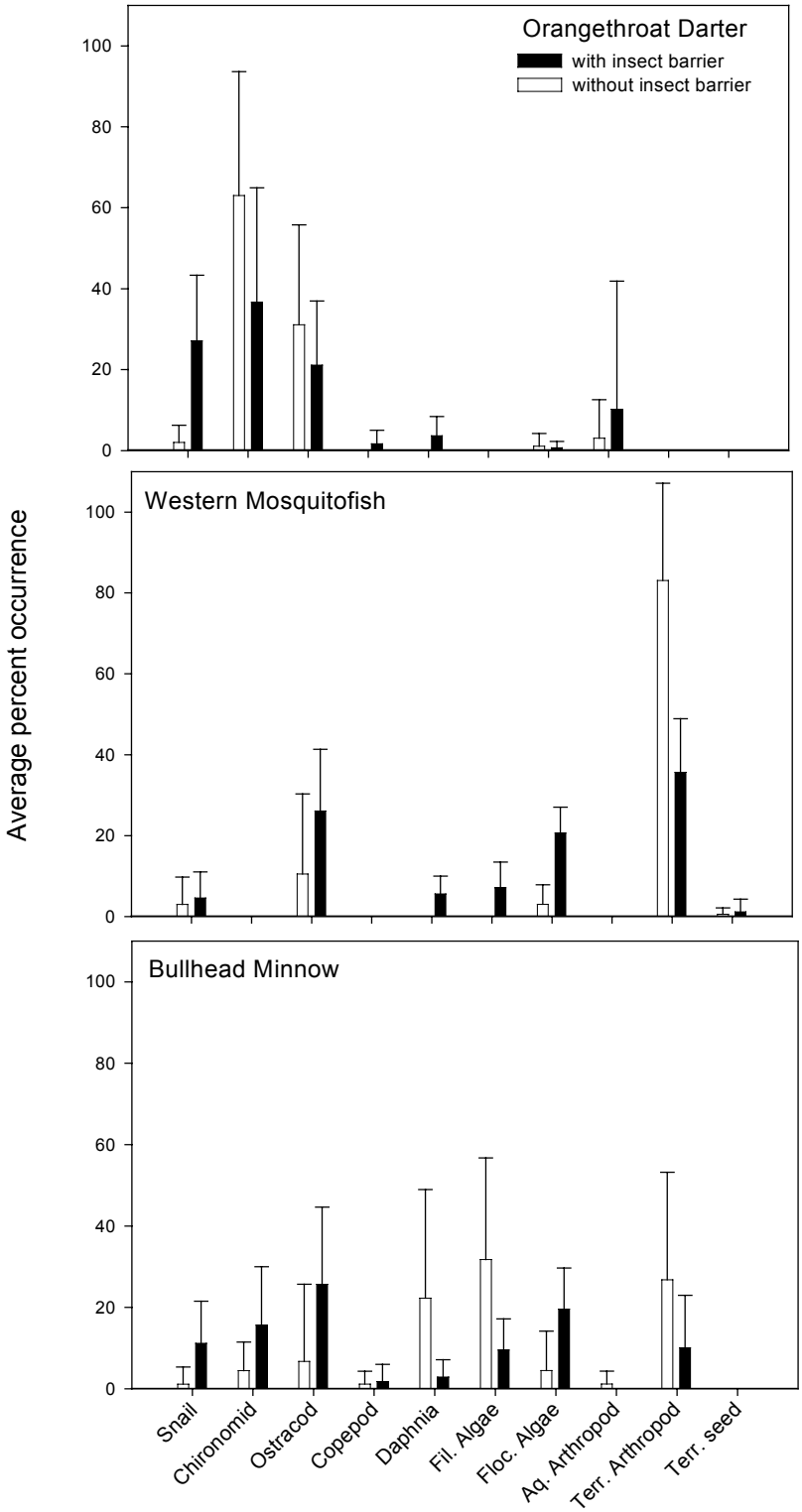

with insect barrier
without insect barrier
Orangethroat Darter
Western Mosquitofish
Bullhead Minnow
Benthic invertebrates grazer (individuals/m2)
2500
2000
1500
1000
500
0
25 25 25 25
25 0 25 0
0 0 0 0
fish per half
fish per half
fish per half
50 fish per stream
25 fish per stream
0 fish per stream
Fish Treatment

Figure 6.


Orangethroat Darter
with insect barrier
without insect barrier
Western Mosquitofish
Bullhead Minnow
Average percent occurrence
0
20
40
60
80
100
Snail
Chironomid
Ostracod
Copepod
Daphnia
Fil. Algae
Floc. Algae
Aq. Arthropod
Terr. Arthropod
Terr. seed

# CHAPTER 3: FISH RICHNESS ENHANCES STREAM ECOSYSTEM FUNCTION: EVIDENCE FOR INTERSPECIFIC FACILITATION

**CHAD W. HARGRAVE**

*University of Oklahoma Biological Station and Department of Zoology,*

*University of Oklahoma, Norman, Oklahoma 73019 USA*

## ABSTRACT

Fish species can have pervasive effects on ecosystems through different pathways that depend on a species' trophic and functional characteristics. It is unknown if co-occurring fishes can have facilitating interactions that could result in synergistic effects on ecosystem function in habitas with more taxa. In this study, I present evidence that number of fish species in an ecosystem can positively affect primary productivity (PPR) and algae biomass, apparently through interspecific facilitation resulting in synergistic enhancement of stream algae. To test for species richness effects of fishes, I randomly composed fish assemblages ranging in richness from 1 to 6 species in large outdoor-stream mesocosms. Benthic PPR was estimated about every 14 days for 42 days. On Day 14, PPR was not affected by fish species richness, but by days 28 and 42 PPR significantly increased with fish richness. The percentage of streams with synergistic effects on algae biomass (suggesting interspecific facilitation) was 48%, 92% and 88% on days 14, 28, and 42, respectively. The exact mechanisms of the positive, synergistic effects of fishes on stream algae is unknown. It is likely no one mechanism caused this response. Specific species combinations may have contributed to the observed pattern. My data suggest that benthic and watercolumn fishes may have interacted, enhancing foraging efficiency of co-occurring taxa and increasing rates of nutrient cycling and nutrient exchange between stream sediments and water. These data support the biodiversity ecosystem function hypothesis in stream fishes, suggesting that the number of fish species in a stream ecosystem as well as then species making up the assemblage can have positive, interactive effects on ecosystem function.

## INTRODUCTION

Fishes are the most species rich group of vertebrates (Nelson 1994). They can interact with their environment in a variety of ways, affecting ecosystem level properties and functions through species-specific trophic and functional interactions (e.g., Carpenter et al. 1985, Power et al. 1985, Wootton and Power 1993, Flecker 1996). For example, benthic invertivorous fishes can enhance primary productivity (PPR) through predatory control of invertebrate grazer density (Power 1990). Surface and watercolumn omnivorous fishes may enhance PPR by increasing the flux of terrestrial nutrients into aquatic ecosystems (Gido and Matthews 2001). Benthic omnivorous fishes that disturb sediments while foraging can increase PPR by releasing stored nutrients from the sediments into the watercolumn (Vanni 2002). The importance of fish species richness (number of species) for aquatic ecosystem function has not been tested. Thus, it is unknown whether fish species with different trophic and functional characteristics can have facilitating interactions on foraging effects of other co-occurring fish species, which might cascade through the food web and result in positive, synergistic effects on ecosystem properties or functions.

Taxa with interspecific, facilitating interactions that result in synergistic ecosystem effects include plants (e.g., Naeem et al. 1996, Tilman et al. 1996, Hector et al. 1999), protists (McGrady-Steed et al. 1997, Naeem and Li 1997), freshwater benthic insects (Jonsson and Malmqvist 2000, Cardinale et al. 2002), marine benthic invertebrates (Solan et al. 2004), pelagic cladocera (Norberg 2000), and terrestrial invertebrates (Heemsbergen et al. 2004). These examples, illustrating positive effects of biodiversity on ecosystem function, indicated that the biodiversity ecosystem-function

hypothesis (BEFH) applies across a broad range of taxa (Naeem 2002). These experiments, however, usually have focused on species richness within a single trophic level (Downing and Leibold 2002). Richness effects of consumer taxa that occupy a range of trophic levels are not well documented. Tests of the BEFH in fishes would likely extend the relevance of this hypothesis to a new suite of taxa, and underpin the potential importance of species richness in higher order consumers.

Because stream fishes are trophically and functionally diverse, they are an ideal model to test the BEFH in consumers from multiple trophic levels. For example, there are at least 17 different functional groups for freshwater stream fishes based on the food items they consume and their interactions with the environment (Matthews 1998). It is a plausible to predict that interspecific facilitation will occur among fishes in more specious assemblages, because some fishes species are likely to enhance the foraging success of other fishes in the assemblage, resulting in synergistic effects on ecosystem function. For example, in natural stream ecosystems benthic disturbing fishes can be observed suspending benthic materials (including food items) into the water column during foraging. In many cases, a school of water column and surface fishes follow these benthic species, consuming the suspended particulates (Matthews 1998). It is possible this interaction could increase the foraging effects of both species in these stream ecosystems (Matthews 1998). This could translate into synergistic effects on ecosystem function. In the present research, I tested the BEFH in stream fishes. I predicted fish species and functional group richness would have positive, synergistic effects on stream algae (measured as PPR and chlorophyll-*a*), resulting from interspecific facilitation among different fish species.

## METHODS

Experiments were conducted in 38 stream mesocosms located at the University of Oklahoma Biological Station (UOBS; Marshall Co., OK, USA). Each mesocosm consisted of one pool (183 cm diameter and 80 cm deep) and riffle (122 cm long and 5 – 10 cm deep; as used by Gido and Matthews 2001, Gelwick and Matthews 1997), and were lined with natural substrate from a nearby stream that was homogenized among mesocosm units. Mesocosms were filled with water on 27 May 2002 and inoculated with 1.0 L of a natural periphyton slurry. The periphyton slurry established a periphyton and snail assemblage dominated by *Oedogonium* and *Spirogyra*, and *Physella,* respectively. Flow was created by pumping water from a downstream collecting box to the head of each riffle with a 2500 L/h pump. From 27 May – 10 June mesocosms were allowed to be naturally colonized by aquatic insect larvae through oviposition by terrestrial adults resulting in an insect population of chironomids, ephemeropterans and odonates.

### *Experimental Design*

From 10–14 June, fishes were collected from nearby creeks, transported in insulated boxes to UOBS, and assigned to mesocosms based on the following experimental design. Each mesocosm was randomly assigned either a fishless control treatment or richness treatment ranging from 1 to 6 fish species. Treatment levels had five replicates except the 1-species treatment, which had eight replicates. I maintained fish density at 60 individuals per mesocosm (24 fish/$m^2$) throughout the experiment. If dead individuals were found they were replaced with fish from a holding tank.

Composition related effects were controlled by randomly selecting species for each replicate treatment from a pool of 12 potential species (Huston 1997, Allison 1999; Table 1). These species represented common fishes found in second-order streams in south-central Oklahoma,

Fish species were assigned to one of five functional groups based on food habits and foraging behaviors (Table 1). To document foraging behaviors for each species, On Day 28 (15 July) I conducted 5 minute focal observations of all species in each mesocosm through Plexiglas® viewing ports, and recorded each species' interaction with the ecosystem (e.g., time in contact with stream bottom, time in the water column, disruption of sediments, consumption of surface insects, etc.) on an ethogram. On Day 42 (26 July), all fishes were removed from mesocosms and preserved in 10% formalin. To document food habits, gut contents were identified for five preserved individuals of each species from each mesocosm. I removed the anterior one-third of the alimentary tract (or discrete stomach if one existed), opened the gut and placed contents on a gridded petri dish. The number of squares each food item occupied was counted to calculate a percent occurrence of each food item per species.

Wet mass of all fish recovered from each mesocosm was measured to estimate total fish biomass per mesocosm at the end of the experiment. Although juveniles of large species were used, size variation among different species could not be controlled completely. I statistically accounted for biomass variation among treatments by using total fish biomass recovered from each mesocosm as an independent variable in all multiple regression analyses.

*Effects on PPR and Algae Biomass*

On 14 June (Day 1), 10 unglazed clay tiles (225 $cm^2$ each) were placed in each stream as a substrate for periphyton growth. Benthic primary production (PPR), was estimated on days 14 (28 June), 28, and 42 by randomly selecting one tile from each stream on the sample date. I used the oxygen evolution method to estimate PPR, by placing each tile in a 3 L Ziploc® storage bag with stream water, and measuring dissolved oxygen when the tile was placed in the bag and after about a 2 h incubation period in sunlight (Gelwick and Matthews 1992).

I estimated algae biomass (as chlorophyll-*a*) by scraping periphyton from two randomly selected tiles into a common container from each mesocosm. Algae scrapings were stored on ice, filtered with a 0.45 µm glass fiber filter, and frozen at -4.0°C for at least 24 h. Chlorophyll-*a* was then extracted over a 24 h with 90% acetone and measured spectrophotometrically with a correction for pheopigments (APHA 1995).

Periphyton scrapings were preserved in 5% buffered formalin. Relative abundance of blue-green algae, diatoms, and filamentous algae from each mesocosm were ranked on a scale from 1 to 3 based from the most dominant to least dominant taxonomic group. These ranks were compared among sample days to determine if periphyton composition changed overtime.

*Effects on Benthic Particulate Organic Matter (BPOM)*

On Day 1, I placed two sediment traps (12.7×12.7, 4.76 cm deep), filled with cleaned gravel, flush with the substrate at opposite ends of each mesocosm pool to estimate BPOM sedimentation (Flecker 1996). On Day 28, particulates were recovered

from traps and preserved in 5% formalin. Particulate matter was filtered through 0.45 μm, dried at 60°C to a constant mass, weighed, combusted at 550°C for 1 h, and reweighed to estimate BPOM (Gardner 1993).

### *Effects on Benthic Invertebrates*

On days 14, 28, and 42, I took two sediment core samples (5.8 cm diameter, 15 cm deep) from the center of each mesocosm pool to estimate benthic invertebrate density. Samples were preserved in 10% formalin, washed through a 250 μm sieve, and invertebrates were identified and counted.

### *Synergistic Effects on Algae Biomass*

I calculated the predicted response in chlorophyll-*a* for each mesocosm based on the additive model

$$Y = \Sigma (x_i N_i d_i)$$

where Y was the predicted ecosystem response for each assemblage in a mesocosm, $x_i$ was the slope of the *per capita* effect of species i (Table 1), $N_i$ was the density of species i, and $d_i$ was the number of days species i was in the mesocosm. For example, mesocosm 10 had 3 fish species, Orangethroat Darter (*Etheostoma spectabile*), Striped Shiner (*Luxilus chrysocephalus*), and Brook Silverside (*Labidesthes sicculus*) with 20 individuals per species. On Day 42, the predicted effect for Orangethroat Darter, Striped Shiner, and Brook Silverside on chlorophyll-*a* was 0.162, 0.315, and 1.08 μg/cm$^2$, respectively. The sum of these individual effects was 1.55 μg/cm$^2$, which was used as the predicted effect on chlorophyll-*a*. Any synergistic response in chlorophyll-*a* resulting

from interspecific facilitation was estimated by subtracting the predicted chlorophyll-*a* from the observed chlorophyll-*a* actually measured in each mesocosm. Net values of zero suggested an additive response to individual species effects, values greater than zero suggested synergistic effects, and negative values suggested interspecific inhibition. In the above example, the observed effect on chlorophyll-*a* in mesocosm 10 was 2.21 $\mu g/cm^2$. Therefore, there was 0.65 $\mu g/cm^2$ greater chlorophyll-*a* than predicted by the additive model, presumably as a result of interspecific facilitation among taxa. The slope of each species *per capita* effect was based on a linear equation calculated with data from six previous single-species experiments (Table 1).

The single-species experiments were conducted from May 2001 – November 2002, and were designed to test individual fish effects on stream algae. The single-species experiments for 9 of the 12 species used a linear regression design, which examined effects of fish density on stream algae. From these results, I concluded that fish effects changed linearly with fish density (Chapter-1, Hargrave 2005). Therefore, the single-species experiments for 3 of the taxa used in the present experiment tested fish effects only for a single fish density. Because the single-species experiments and the present experiment took place at different times, I standardized *per capita* species-specific effects relative to background chlorophyll-*a* measured from fishless control streams in each experiment. Chlorophyll-*a* was used as the ecosystem property because this variable was consistently measured across all experiments and is correlated with PPR.

### *Statistical Analyses*

I used multiple regression to test the effects of total fish biomass per stream, and fish species and functional group richness on PPR, benthic invertebrate density, BPOM, and synergistic effects on algae. I used SAS (2000) for all multiple regression analyses.

I used principal components analysis (PCA) to quantitatively define assemblage composition in each mesocosm (Applied Biostatistics Inc. 2000). From a stream by species matrix, I classified each stream based on assemblage composition by calculating principal component scores for each stream on the first three PCA axes (PC-1, PC-2, PC-3). I examined effects of assemblage composition by correlating scores from PC-1, 2, and 3 with PPR, benthic invertebrate density, BPOM, and synergistic effects on algae (SAS 2000). Significant correlations suggested an assemblage composition effect. I identified species defining each assemblage using axis loading scores calculated for each species.

## RESULTS

### *Fish Biomass and Richness Effects on PPR*

Effects of fish biomass, and species and functional richness on benthic PPR varied across sample days. On day 14, only total fish biomass significantly accounted for variation in PPR among mesocosms (Table 2). Primary productivity was on average less than 1 times greater in treatments with 6 fish species than 1 species (Fig. 1), and fish richness did not significantly account for this increase in PPR across treatments (Table 2). On day 28 and 42, PPR increased on average about 2 fold greater between treatments

with 1 and 6 fish species per mesocosm (Fig. 1). This increase in PPR was significant with increased fish richness per mesocosm, but total fish biomass and functional richness had no significant effects on PPR at these sample days (Table 2).

### *Synergistic Response in Algae Biomass*

Number of mesocosms having a synergistic response in algae biomass increased over time (Fig. 2). On Day 14, only 48% of the mesocosms with two or more species had positive synergistic effects, and the average synergistic effect for most treatments was around zero. By days 28 and 42, the percentage of mesocosms with two or more species that had positive synergistic effects on algae increased to 92 and 88%, respectively (Fig. 2). The average synergistic response was near zero for treatments with one species, and greater than zero in treatments with more than one species. Neither total fish biomass, or species or functional group richness significantly accounted for the variation in synergistic response in algae biomass among mesocosm (Table 2).

### *Algae Composition Over Time*

The relative dominance of some algae taxa changed across sample days (Fig. 3). In general, the number of mesocosms with blue-green algae (mostly *Aphonothece* and *Chocoocus*) as the dominant taxa was similar across time. However, number of mesocosms with diatoms (mostly *Achnanthidium* and *Navicula*) as the dominant taxa decreased over time, while mesocosms with filamentous algae (mostly *Oegonium*, *Spirogyra*, and *Cladophora*) increased over time.

*Assemblage Composition Related Effects*

The first three principal component axes described about 50% of the variation in assemblage structure among mesocosms (Table 3). Mesocosms with positive PC-1 scores had assemblages with the benthic invertivores, Orangethroat Darter and Orangebelly Darter (*Etheostoma radiosum*), and mesocosms with negative PC-1 scores had assemblages with the water column omnivores, Red Shiner (*Cyprinella lutrensis*) and Rocky Shiner (*Notropis suttkusi*). Mesocosms with positive PC-2 scores had assemblages composed of the water column omnivores, Sand Shiner (*Notropis stramineus*), Red Shiner, and Green Sunfish (*Lepomis cyanellus*), the surface insectivore, Brook Silverside, and the benthic omnivore disturber, Common Carp (*Cyprinus carpio*). Mesocosm with negative PC-2 scores, had assemblages with the benthic grazer, Central Stoneroller (*Campostoma anomalum*). Mesocosms with positive PC-3 scores had assemblages with the water column omnivores, Striped and Rocky Shiners, and mesocosms with negative PC-3 scores had assemblages with the surface insectivore, Western Mosquitofish (*Gambusia affinis*), and the watercolumn omnivores Red Shiner and Rocky Shiner (Table 3).

There was evidence that fish assemblage composition influenced PPR measured from mesocosms, but did not influence synergistic effects on algae biomass. On days 28 and 42, PC-2 was significantly correlated with PPR (Table 4). Thus, Central Stoneroller was common in mesocosms with lowest PPR measurements, and Common Carp, Sand Shiner, and Red Shiner were common in streams with highest PPR measurements. There were no significant correlations between PPR and PC-1, or 3. The magnitude of the synergistic response in algae biomass was not correlated with PC-1, 2, or 3 (Table 4).

*Effects on Benthic invertebrates*

Benthic invertebrate assemblages were composed of chironomids, odonates, and ephemeropterans, as well as snails and annelids. Collector-gatherer Chironomidae was the dominate invertebrate taxa, comprising 65, 66, and 44% of the benthic invertebrate assemblage on days 14, 28, and 42, respectively. Annelids comprised 22, 22, and 40%, Physidae and Planorbidae comprised 7, 8, and 14%, and Tricorythidae comprised 5, 3, and 1% of the invertebrate assemblages on days 14, 28, and 42, respectively. Benthic invertebrate densities were highly variable among mesocosms, ranging 85 – 3142, 42 – 2760, and 85 – 2590 invertebrates/$m^2$ on days 14, 28, and 42, respectively, and decreased significantly with fish biomass on Day 42. There was no significant effect of any other independent variable on benthic invertebrate density (Table 2). Benthic invertebrate density was not correlated with PC-1, 2, or 3 (Table 3), indicating no assemblage composition effect on benthic invertebrate density.

*Effects on BPOM*

Benthic particulate organic matter ranged from 32 – 279 mg/$cm^2$ on day 28, but was not significantly affected by total fish biomass, or species or functional group richness (Table 2). There was evidence that fish assemblage composition affected BPOM abundance. Benthic particulate organic mater was positively correlated with PC-1 (Table 4). The benthic invertivores, Orangethroat Darter and Orangebelly Darter, benthic omnivore disturber, Common Carp, and benthic grazer, Central Stoneroller were common in mesocosms with highest BPOM measurements (Table 3).

## DISCUSSION

This study supports the robustness of the BEFH by demonstrating that it applies to stream fishes. However, species richness effects on stream primary productivity and algae biomass were temporally dependent. Initial fish effects (Day 14) on algae were not affected by species richness and did not increase synergistically in more specious treatments. Fish effects on Day 14 were additive, suggesting that at this time each species in the assemblage was independently affecting the ecosystem with little or no interactive effects. However, on days 28 and 42 fish richness had positive, synergistic effects on stream algae. Algae response in treatments with 2 or more species was greater than additive. This suggests that there was interspecific facilitation among taxa at these times, which resulted in synergistic effects on PPR in mesocosms with more than one fish species.

The dominant algae taxa found among mesocosms changed over sample days, which could have influenced the temporal development of richness effects. Specifically, filamentous algae became the dominant taxa in mesocosm by days 28 and 42. Filamentous algae are capable are accumulating large amounts of biomass by forming strands rather than being restricted to relatively flat mats. I measured some algal strands that were about 3 cm in length. Thus, fish effects that positively affected stream algae could have had stronger effects on filamentous forms that were able to continue to grow up off the stream bottom.

Most BEFH studies have not found a temporal component to richness effects on ecosystem function (e.g., Morin and McGrady-Steed 2004). My results are more consistent with the hypothesis that richness effects on productivity would be greater at

later stages of succession (Cardinale et al. 2004). My data suggest that richness effects of fishes could be influenced by environmental variability of the stream ecosystem, by affecting the temporal persistence of the occupying species. For example, when fish inhabitants are limited to a stream habitat for short time periods because of frequent droughts or unstable environmental conditions, their combined ecosystem effects are likely to result from additive, individual species-specific contributions. If fishes can occupy habitats for extended periods of time because of environmentally stability or permanent water, richness effects are likely to be important as interspecific facilitating interactions develop, resulting in synergistic regulation of ecosystem functions.

The fundamental thesis of the BEFH is that taxonomically rich assemblages have more interspecific, facilitative interactions resulting in a synergistic ecosystem effect or response (Loreau and Hector 2001). The synergistic response of algae in treatments with more than one species suggests interspecific facilitation occurred in my experiments. Further, the additive models appeared to be accurately predicting algae biomass in single species treatments because in these treatments the average difference between predicted and observed response was zero. I predicted however that the number of facilitative interactions would increase with increased species richness, which would have resulted in positive richness effects on degree of synergistic response in algae (Heemsbergen et al. 2004). This prediction was not supported. There was a saturation in synergistic response in algae biomass at the 2-species treatment. This observation could have occurred because (1) either the number of facilitating interactions did not increase with fish richness, or (2) the number of facilitating interactions increased while the relative strength of these interactions decreased on a *per capita* basis. The first explanation

would suggest that fish richness beyond 2 species in an assemblage does not contribute additionally to the ecosystem. The second explanation would occur if facilitating interactions are density-dependent. This would suggest that richness beyond the 2 species per assemblage could contribute additionally to the ecosystem if density of the species in the assemblage also increased with richness.

### *Effect of Assemblage Composition*

Richness effects often are caused by a single, dominant taxon or interactions between a few taxa. For example, richness effects on grassland primary productivity have been attributed to the combination of legumes and C4 grasses (Tilman et al. 1997). Similarly, I found evidence that fish assemblage composition in this study influenced PPR estimates among mesocosms. Specifically, mesocosms with the grazing minnow Central Stoneroller had lowest PPR estimates. Central Stoneroller consumed periphyton in this experiment and has been shown previously to reduce benthic algae in mesocosm as well as in natural streams (Gelwick and Matthews 1992, Gelwick and Matthews 1997). Thus, it is plausible to suggest this species reduced PPR in mesocosms where is was part of the fish assemblage. Mesocosms with the greatest PPR estimates had assemblages with the benthic, Common Carp, and the watercolumn omnivores Sand Shiner and Red Shiner. This composition effect could have resulted from species-specific effects of Common Carp, which had high per capita species effect (Table 1), or could have resulted from interspecific facilitative interactions among these taxa.

Specific fish assemblages were not replicated in this study. By limiting replication to only species richness, I focused on the fundamental question: does aquatic

ecosystem function increase on average with fish richness. Different species have varying effects on algae biomass (Table 1), which was not consistent within function group designations. This likely lead to variation among replicate treatments. In order to identify potentially dominant species or species combinations that may be resulting in greater synergistic effects on ecosystem function, it is necessary to conduct experiments that replicate fish assemblage structure within each richness level (e.g., Downing and Leibold 2002, Hector et al. 2002).

### *Potential Mechanisms*

Although I did not directly test mechanisms for fish richness effects, I propose two potential hypotheses and discuss the evidence for each. In short these hypotheses are that fishes affected PPR by (1) synergistic reduction of invertebrate algivores, and (2) increasing nutrient and particulate flux between sediments and the water column.

Because fishes can increase PPR by reducing benthic invertebrate grazer density (Power 1990), I suggest that positive effects of fish richness on PPR could have been mediated through synergistic reduction of algivorous invertebrates. The invertebrate taxa that consumed primarily algae comprised 75, 75, and 60% of the invertebrate assemblages in these mesocosms on days 14, 28, and 42, respectively. The dominant invertebrate taxa were chironomids which were mostly collector-gathers of flocculent algae particulates. Other taxa such as ephemeropterans and snails were less abundant but also consumed algae. There were no significant richness effects on benthic invertebrate density on any sample date. Only on Day 42 did total fish biomass per mesocosm significantly account for variation in invertebrate density, which could explain the shift

toward fewer invertebrate grazers on Day 42 than on previous sample days. Thus, I suggest the synergistic reduction of benthic invertebrates by more species rich fish assemblages as a potential mechanism was not the functioning mechanism. However, the variation in invertebrate density was high within each richness treatment, and statistical power could likely have been too low to detect significant treatment effects on benthic invertebrate densities.

Fishes can affect PPR by increasing soluble nutrients available for algae growth via excretion (Gido and Matthews 2001) or through physical disturbance of the sediments, releasing sedimentary organic nutrients (Vanni 2002). Synergistic enhancement of soluble nutrients in treatments with two or more species could have increased PPR. The benthic fishes Orangethroat Darter, Orangebelly Darter, Common Carp, and Central Stoneroller disturbed the sediments in this experiment, causing increased BPOM in mesocosms with these species. Access to autochthonous, benthic food sources (e.g., organic detritus, benthic invertebrates, etc.) by watercolumn foragers could have been enhanced by the physical disturbance caused by these benthic fishes. Therefore, in addition to contributing to the flux of allochthonous nutrients to the system by consuming terrestrial insects, watercolumn fishes, when co-occurring with these benthic taxa, could have contributed to autochthonous nutrient recycling by consumption of resuspended benthic foods. Enhanced rates of autochthonous nutrient recycling likely would increase PPR (Norberg 2000).

The two hypothesis presented above are based on functional properties of different taxa. However, functional group richness did not significantly increase PPR as I expected. This suggests that the functional group designations, alone, were not adequate

for predicting richness effects on PPR. I propose using species-specific characteristics is more appropriate than functional group characteristics for predicting richness effects on PPR. Recent diversity studies also have shown that species-specific differences among taxa within the same defined functional group can result in synergistic effects on ecosystem function (Jonsson and Malmqvist 2000, Norberg 2000, Cardinale 2002, Heemsbergen et al. 2004). Species-specific differences among consumer taxa, such as size, metabolic rates, nutrient ratios, feeding rates, feeding efficiency, feeding selectivity, activity, could all be important species-specific factors influencing consumer effects.

My data show that fish species richness positively affected primary production, lending support to the growing body of biodiversity ecosystem function studies that have shown a positive response in ecosystem function with increased taxonomic richness (Loreau et al. 2001). These data offer evidence that consumer taxa from multiple trophic levels can apparently have facilitative interactions, which can result in synergistic enhancement ecosystem function. Furthermore, I suggest that richness effects of consumers on ecosystem function may be temporally dependent, occurring only over longer periods of time. Thus, this work broadens the applicability of the BEFH to a new suite of taxa and suggests the importance of the temporal scale for biodiversity effects on ecosystem function.

## ACKNOWLEDGMENTS

I would like to thank T. Cluck, D. Cobb, R. Page, M. Ward, and L. Weider at University of Oklahoma Biological Station for logistical and operational assistance, and R. Deaton, K. Hauger, and M. Ratard for help collecting fishes and beginning experiments. I thank my doctoral committee (E. Bergey, M. Kaspari, W. Matthews, R. Nairn, and C. Vaughn) for thoughtful discussion, help designing these experiments, and for reviewing this manuscript. Partial funding for the work was provided by a Blanche Adams Memorial Summer Scholarship from the Department of Zoology, University of Oklahoma, and a National Science Foundation Doctoral Dissertation Improvement Grant awarded to W. Matthews and C. Hargrave (NSF 0308729). This work was submitted in partial fulfillment of Ph.D. degree at University of Oklahoma, Department of Zoology.

## LITERATURE CITED


Allison, G. W. 1999. The implications of experimental design for biodiversity manipulations. The American Naturalist **153**:26-45.

American Public Health Association. 1995. Standard methods for the examination of water and wastewater, 19th ed. American Health Association, Washington, DC, USA.

Applied Biostatistics Inc.. 2000. Numerical Taxonomic and Multivariate Analysis System Version 2.10, Setauket, New York, USA.

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

Cardinale, B. J., M. A. Palmer, and S. L. Collins. 2002. Species diversity enhances ecosystem functioning through interspecific facilitation. Nature **415**:426-429.

Carpenter, S. R., J. F. Kitchell, and J. R. Hodgson. 1985. Cascading trophic interactions and lake productivity. Bioscience **35**:634-639.

Downing, A. L., and M. W. Leibold. 2002. Ecosystem consequences of species richness and composition in pond food webs. Nature **416**:837-841.

Flecker, A. S. 1996. Ecosystem engineering by a dominant detritivore in a diverse tropical stream. Ecology **77**:1845-1854.

Gardner, T. J. 1993. Grazing and the distribution of sediment particle sizes in artificial stream ecosystems. Hydrobiologia **252**:127-132.

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

Gelwick, F. P., and W. J. Matthews. 1997. Effects of algivorous minnows (*Campostoma*) on spatial and temporal heterogeneity of stream periphyton. Oecologia **112**:386-392.

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

Hector, A., B. Schmid, C. Beierkhnlein, M. C. Caldeira, M. Diemer, P. G. Dimitrakopoulos, J. A. Finn, H. Freitas, P. S. Giller, J. Good, R. Harris, P. Hogberg, K. Huss-Danell, J. Joshi, A. Jumpponen, C. Korner, P. W. Leadley, M. Loreau, A. Minns, C. P. H. Mulder, G. O'Donovan, S. J. Otway, J. S. Pereira, A. Prinz, D. J. Read, M. Scherer-Lorenzen, E. D. Schulze, A. S. D. Siamantziouras, E. M. Spehn, A. C. Terry, A. Y. Troumbis, F. I. Woodward, S. Yachi, and J. H. Lawton. 1999. Plant diversity and productivity experiments in European grasslands. Science **286**:1123-1127.

Hector, A., E. Bazeley-White, M. Loreau, S. Otway, and B. Schmid. 2002. Overyielding in grassland communities: testing the sampling effect hypothesis with replicated biodiversity experiments. Ecology Letters **5**:502-511.

Heemsbergen, D. A., M. P. Berg, M. Loreau, J. R. v. Hal, J. H. Faber, and H. A. Verhoef. 2004. Biodiversity effects on soil processes explained by interspecific functional dissimilarity. Science **306**:1019-1020.

Huston, M. A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. Oecologia **110**:449-460.

Jonsson, M., and B. Malmqvist. 2000. Ecosystem process rate increases with animal species richness: evidence from leaf-eating, aquatic insects. Oikos **89**:519-523.

Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature **412**:72-76.

Loreau M, S. Naeem, P. Inchausti, J. Bengtsson, J. P. Grime, A. Hector, D. U. Hooper, M. A. Huston, D. Raffaelli, B. Schmid, D. Tilman, D. A. Wardle. 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. Science **294**:804-808.

Matthews, W. J. 1998. Patterns in freshwater fish ecology. Chapman Hall, New York

McGrady-Steed, J., P. M. Harris, and P. J. Morin. 1997. Biodiversity regulates ecosystem predictability. Nature **390**:162-165.

Morin, P. J., and J. McGrady-Steed. 2004. Biodiversity and ecosystem functioning in aquatic microbial systems: a new analysis of temporal variation and species richness-predictability relationships. Oikos **104**:458-466.

Naeem, S. 2002. Ecosystem consequences of biodiversity loss: the evolution of a paradigm. Ecology **83**:1537-1552.

Naeem, S., and S. Li. 1997. Biodiversity enhances ecosystem reliability. Nature 390:507-509.

Naeem, S., K. Hakansson, J. H. Lawton, M. J. Crawley, and L. J. Thompson. 1996. Biodiversity and plant productivity in a model assemblage of plant species. Oikos **76**:259-264.

Nelson, J. S. 1994. Fishes of the world. J. Wiley, New York.

Norberg, J. 2000. Resource-niche complementarity and autotrophic compensation determines ecosystem-level responses to increased cladoceran species richness. Oecologia **122**:264-272.

Power, M. E. 1990. Effects of fish in river food webs. Science **250**:811-814.

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

SAS Institute. 2000. Version 8.01. Cary, North Carolina, USA.

Solan, M., B. J. Cardinale, A. L. Downing, K. A. M. Engelhardt, J. L. Ruesink, and D. S. Srivastava. 2004. Extinction and ecosystem function in the marine benthos. Science **306**:1177-1180.

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

Tilman, D., J. Knops, D. Wedin, P. Reich, M. Ritchie, and E. Siemann. 1997. The influence of functional diversity and composition on ecosystem processes. Science **277**:1300-1302.

Vanni, M. J. 2002. Nutrient cycling by animals in freshwater ecosystems. Annual Review Ecology and Systematics **33**:341-370.

Wootton, J. T, M. E. Power. 1993. Productivity, consumers and the structure of a river food-chain. Proceedings of the National Academy of Sciences **90**:1384-1387.

Table 1. Fish species and their trophic and functional designations based on published diet and behavioral traits. Species composition for replicate treatments based on random selection without replacement within each replicate. Each replicate is identified by letters a-e within each treatment. *Per capita* species effect ($x_i \times 10^{-4}$) on chlorphyll-*a*, generated from single-species experiments.

| Species and functional designations | species richness treatments | | | | | | $x_i$ |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | $\times 10^{-4}$ |
| **Water column omnivore** | | | | | | | |
| Striped Shiner, *Luxilus chrysocephalus* | c | - | b-d | c-e | c,e | b,c | 3.5 |
| Sand Shiner, *Notropis stramineus* | f | - | b | b,c,e | a,d,e | a,c-e | 3.0 |
| Green Sunfish, *Lepomis cyanellus* | g | c | e | b,e | b | a-c,e | 3.0 |
| **Watercolumn/surface insectivore** | | | | | | | |
| Brook Silverside, *Labidesthes sicculus* | - | - | a,c | c | c-e | c,d | 12.0 |
| Mosquitofish, *Gambusia affinis* | d | a,e | a,e | d | a,e | a,d,e | 2.5 |
| Red Shiner, *Cyprinella lutrensis* | - | a,c,e | a,b | e | b,d,e | a,c,d | 1.0 |
| Rock Shiner, *Notropis suttkusi* | e | d | d | b | - | c | 2.2 |
| **Benthic grazer** | | | | | | | |
| Stoneroller, *Campostoma anomalum* | - | e | e | a | a | b | 1.0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Benthic omnivore disturber** | | | | | | | |
| Bullhead Minnow, *Pimephales vigilax* | b | - | - | a | - | d | 4.0 |
| Common Carp, *Cyprinus carpio* | - | - | - | - | b,c,d | a,b,e | 8.0 |
| **Benthic invertivore** | | | | | | | |
| Orangebelly Darter, *Etheostoma radiosum* | a | b | c,d | a,c,d | a-c | a,b,e | 1.8 |
| Orangethroat Darter, *E. spectabile* | h | b | - | a,b,d | a-d | b,d,e | 4.0 |

Table 2. Multiple regression table testing effects of fish richness and biomass on primary production (PPR), benthic invertebrate density, benthic particulate organic matter (BPOM), and synergistic effect on algae biomass at each sample date. Regression for synergistic effects on algae on days 14 and 28 had df = 3, 30 and for Day 42 had df = 3, 28. All other regressions for days 14 and 28 have df = 3, 35, and for Day 42 has df = 3, 33. Values represent parameter estimates for each regression, and associated level of significance: NS, $P > 0.05$; *, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $P \leq 0.001$.

| | Regression Parameters | | | | | |
|---|---|---|---|---|---|---|
| Response Variable | Intercept | Mass | Species Richness | Functional Richness | $r^2$ | $F$ value |
| ***Day 14*** | | | | | | |
| PPR | 1.95*** | 0.021* | 0.33NS | –0.13NS | 0.34 | 5.24** |
| Invertebrates | 26.32*** | –0.22NS | 4.37NS | –3.20NS | 0.09 | 1.10NS |
| Synergism | 0.09NS | 0.0001NS | 0.009NS | –0.023NS | 0.03 | 0.20NS |
| ***Day 28*** | | | | | | |
| PPR | 3.50** | 0.02NS | 1.90** | –1.43NS | 0.37 | 6.05** |
| Invertebrates | 17.52*** | –0.06NS | 5.15NS | –5.30NS | 0.09 | 1.14NS |
| BPOM | 0.037NS | 0.001NS | –0.001NS | 0.002NS | 0.27 | 4.12* |
| Synergism | –0.07NS | 0.003NS | 0.143NS | –0.118NS | 0.16 | 1.85NS |

***Day 42***

| | | | | | | |
|---|---|---|---|---|---|---|
| PPR | 2.07** | 0.02NS | 1.12** | –0.75NS | 0.43 | 7.92*** |
| Invertebrates | 24.90*** | –0.21** | 2.63NS | –2.53NS | 0.19 | 2.65NS |
| Synergism | 0.24NS | 0.002NS | 0.17NS | 0.0002NS | 0.04 | 0.43NS |

Table 3. Percent variance explained and vector loadings for each species on the first three principal component axes.

| | Principal Component Axes | | |
|---|---|---|---|
| | PC-1 | PC-2 | PC-3 |
| Variance Explained | 18.5% | 15.8% | 13.7% |
| Loadings per species | | | |
| Striped Shiner | -0.0330 | 0.3405 | 0.4742 |
| Sand Shiner | -0.0640 | 0.6040 | -03678 |
| Green Sunfish | 0.2786 | 0.4877 | -0.1207 |
| Brook Silverside | -0.0722 | 0.4957 | 0.0440 |
| Western Mosquitofish | 0.1192 | -0.1809 | -0.7041 |
| Red Shiner | -0.4383 | -0.5504 | -0.4548 |
| Rocky Shiner | -0.3647 | 0.1506 | 0.4479 |
| Central Stoneroller | 0.4888 | -0.4650 | -0.1981 |
| Bullhead Minnow | -0.0862 | -0.2154 | -0.4512 |
| Common Carp | 0.6617 | 0.5394 | -0.0821 |
| Orangethroat Darter | 0.7600 | 0.0528 | 0.3293 |
| Orangebelly Darter | 0.7293 | 0.1087 | -0.0836 |

Table 4. Pearson's correlation coefficient and associated level of significance: NS, $P > 0.05$; *, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $P \leq 0.001$ for correlations between principal component scores defining each stream assemblage, and primary production (PPR), synergistic effects on chlorophyll-*a*, benthic invertebrate density, and benthic particulate organic matter (BPOM).

| | PRINCIPAL COMPONENT AXES | | |
|---|---|---|---|
| | PC-1 | PC-2 | PC-3 |
| Total PPR | | | |
| Week 2: *r* and *P*-value | 0.036 NS | 0.361 | 0.099 NS |
| Week 4: *r* and *P*-value | 0.230 NS | **0.566 **** | –0.105 NS |
| Week 6: *r* and *P*-value | –0.041 NS | **0.575 ***** | –0.245 NS |
| Synergistic Effect on Algae | | | |
| Week 2: *r* and *P*-value | 0.154 NS | 0.055 NS | 0.252 NS |
| Week 4: *r* and *P*-value | 0.189 NS | 0.274 NS | 0.205 NS |
| Week 6: *r* and *P*-value | 0.020 NS | 0.157 NS | 0.294 NS |
| Benthic Invertebrates | | | |
| Week 2: *r* and *P*-value | –0.023 NS | 0.070 NS | –0.108 NS |
| Week 4: *r* and *P*-value | –0.139 NS | –0.013 NS | –0.189 NS |
| Week 6: *r* and *P*-value | 0.044 NS | –0.001 NS | 0.008 NS |

| | | | |
|---|---|---|---|
| BPOM | | | |
| Week 4: *r* and *P*-value | **0.415 *** | 0.162 NS | –0.183 NS |

## LIST OF FIGURES

Figure 1. Mean primary production (mg $O_2$ $cm^{-2}$ $h^{-1}$ ± 1 SE) across richness treatments for days 14, 28. and 42.

Figure 2. Synergistic response in algae biomass (μg chlorophyll-*a* / $cm^{-2}$) on days 14, 28, and 42 for each species richness treatment. Synergistic effect was calculated as the difference between observed and predicted change in chlorophyll-*a* on each sample day. Open circles indicate differences, and filled circles are means of the differences within each treatment.

Figure 3. Relative dominance among algae taxa for each sample day, measured as the number of mesocosms in which each algae was the most abundant taxa.

Figure 1.

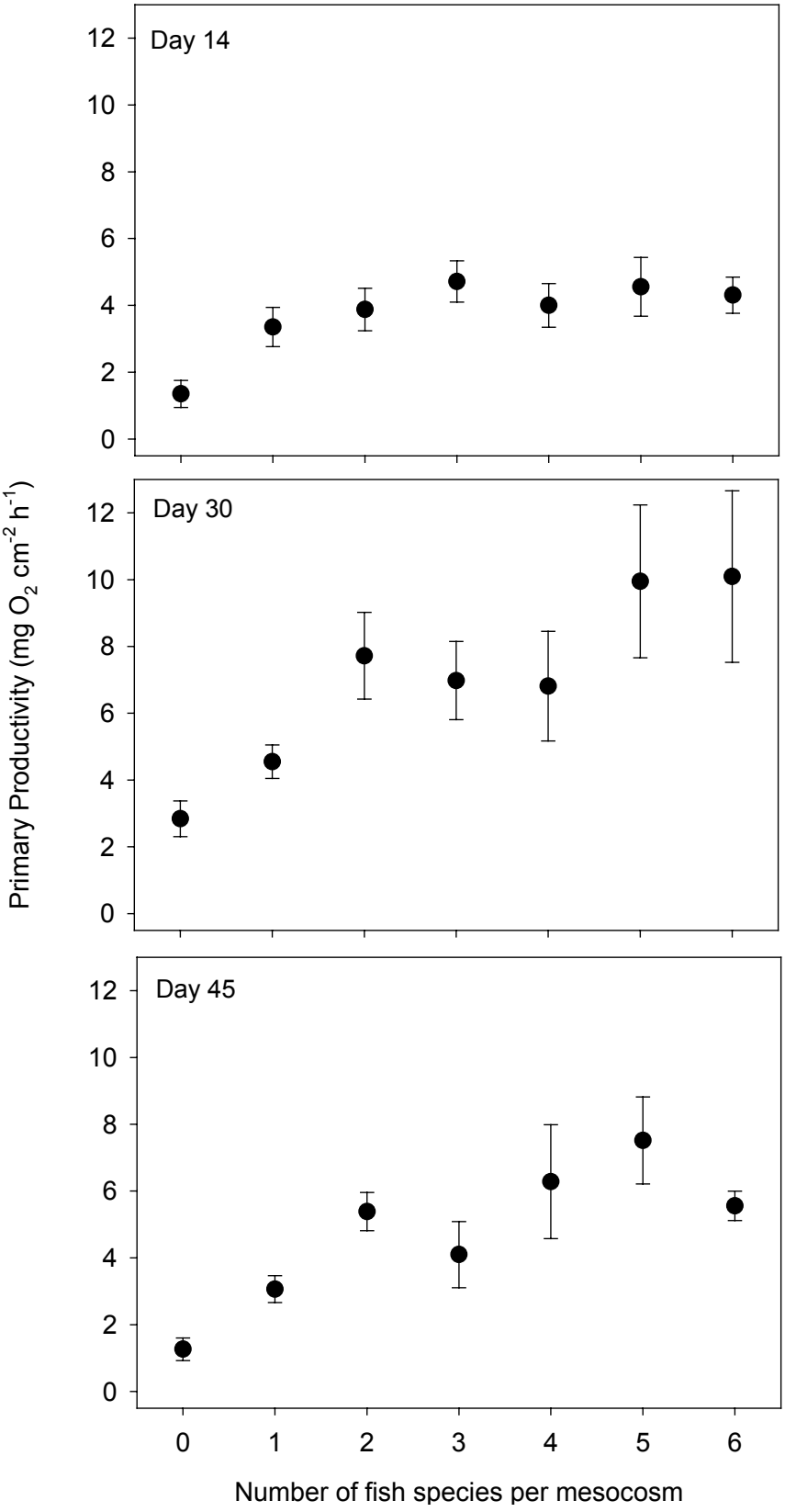

Day 14
Day 30
Day 45
Primary Productivity (mg $O_2$ $cm^{-2}$ $h^{-1}$)
Number of fish species per mesocosm
0
1
2
3
4
5
6
0
2
4
6
8
10
12

Figure 2.

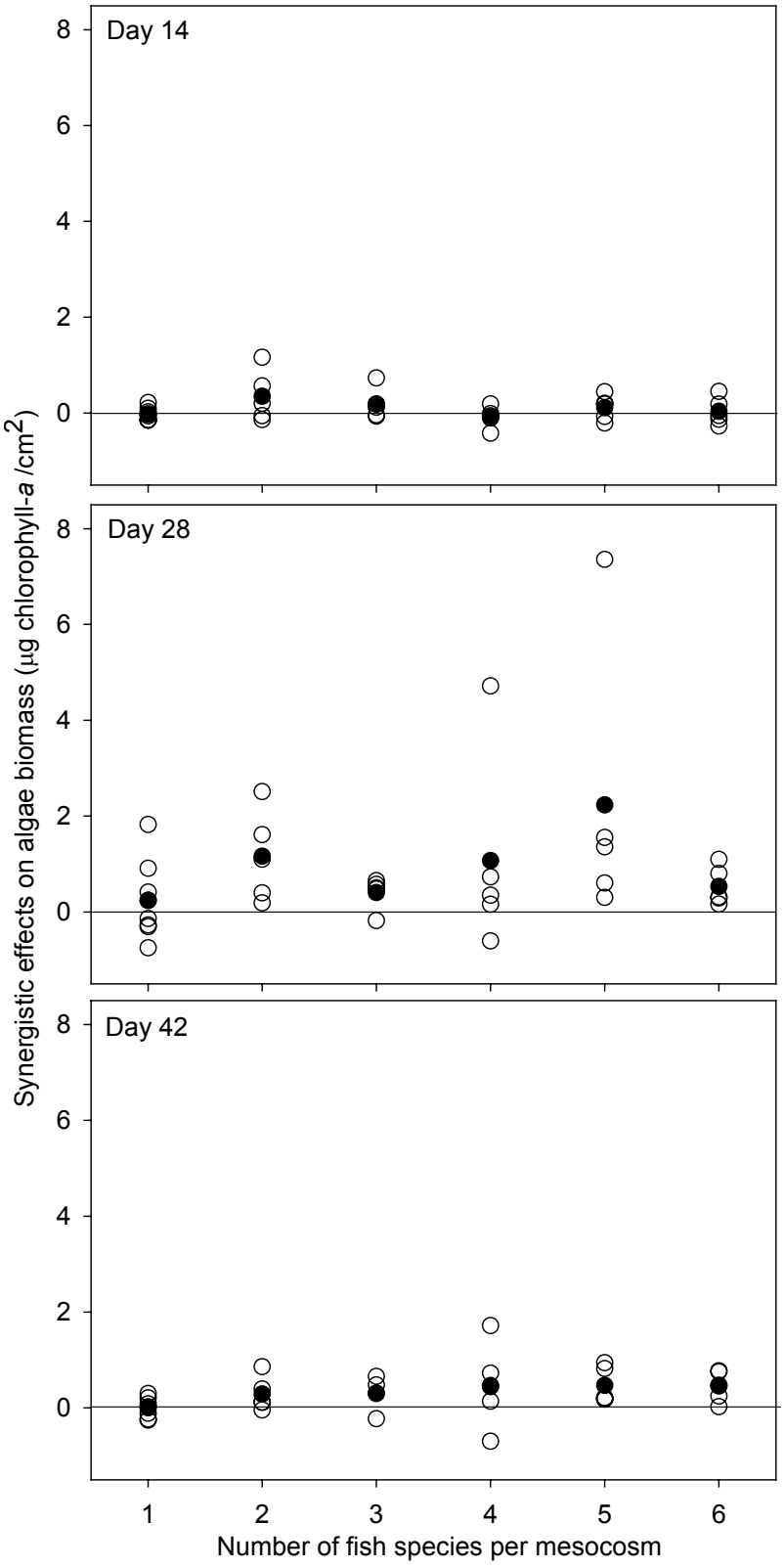
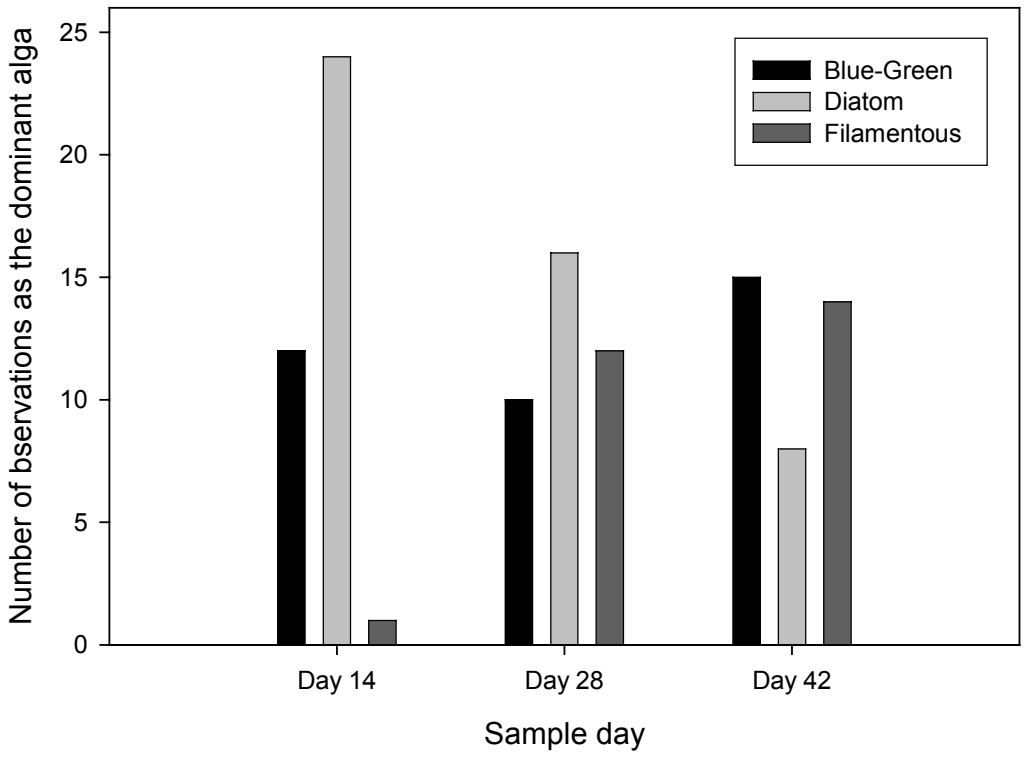

Day 14
Day 28
Day 42
Synergistic effects on algae biomass (µg chlorophyll-a /cm2)
Number of fish species per mesocosm
0
2
4
6
8
1
2
3
4
5
6

Figure 3.


Number of bservations as the dominant alga
25
20
15
10
5
0
Blue-Green
Diatom
Filamentous
Day 14
Day 28
Day 42
Sample day

# CHAPTER 4: FISHES AFFECT PRIMARY PRODUCTIVITY AND PERIPHYTON BIOMASS IN NATURAL STREAM ECOSYSTEMS: AN ENCLOSURE EXPERIMENT

CHAD W. HARGRAVE

*University of Oklahoma Biological Station and Department of Zoology*

*University of Oklahoma, Norman, Oklahoma 73019 USA*

**ABSTRACT**

In a 30 day experiment, I tested the ecosystem effects of three fish species from different functional groups using field enclosures in a natural stream ecosystem. Treatments were Blackstripe Topminnow (*Fundulus notatus*), a surface insectivore; Longear Sunfish (*Lepomis megalotis*), a watercolumn insectivore; Orangethroat Darter (*Etheostoma spectabile*), a benthic invertivore; and a fishless control. On days 15 and 30, I measured primary productivity (PPR), periphyton biomass (as chlorophyll-*a*), and benthic invertebrate density. On both sample dates, PPR was greater in all enclosures with fish than in enclosures without fish. Relative to control enclosures, chlorophyll-*a* was not different in Blackstripe Topminnow treatments, but was greater in Longear Sunfish treatments on both sample days, and was greater in Orangethroat Darter treatments on day 15. Benthic invertebrate abundance was not affected by any of the fish treatments. These data agree with results from previous experiments that tested fish effects in artificial stream mesocosms. Despite the limited realism of stream mesocosms, these data suggest that patterns observed in mesocosm experiments also are likely to occur in natural stream ecosystems.

## INTRODUCTION

Stream mesocosms are important for ecological research because they allow exploration of novel questions in controlled, replicated systems (McIntire 1993). They have been used to address general questions about hydrodynamics, algal-nutrient dynamics, macroinvertebrate growth, grazer-algal interactions, fish ecology, disturbance, toxicology, and longitudinal linkages in stream ecosystems (Lamberti and Steinman, 1993). Although using mesocosms is advantageous in many aspects, their simplistic nature can influence abiotic and biotic interactions, giving results that sometimes are not repeatable in natural streams (Gelwick and Matthews 1997, Fuller et al. 1998). Thus, applicability of data from stream mesocosm experiments to natural streams is limited and should be corroborated with field studies (McIntire 1993).

Using stream mesocosm experiments, I showed that fish density (Chapter-1; Hargrave 2005), species identity (Chapter-2; Hargrave 2005), and taxonomic richness (Chapter-3; Hargrave 2005) are potentially important factors affecting stream ecosystem properties and functions. These mesocosms were large outdoor units, filled with natural stream sediments, and had natural algae and invertebrate assemblages (illustrated in Gido and Matthews 2001). Thus, I assumed my experimental results from these artificial systems could apply to natural streams. There were, however, some basic differences between these mesocosms and natural stream ecosystems. Specifically, the water within each unit was recirculating, lacking variable, one-way flow, as well as ground water inputs or losses that are potentially important abiotic factors in many natural stream ecosystems (Craig 1993). Further, these mesocosms had no watershed thus lacked any potential effect due to runoff, which also could influence ecosystem processes and

functions. It is possible that abiotic factors such as these could limit the applicability of mesocosm data to real stream scenarios.

In the present experiment, I asked if fishes affect ecosystem properties and functions in a natural stream ecosystem. I tested ecosystem effects of three fish species representing different functional groups: Blackstripe Topminnow (*Fundulus notatus*), a surface feeding insectivore; Longear Sunfish (*Lepomis megalotis*), a water column omnivore; and Orangethroat Darter (*Etheostoma spectabile*), a benthic invertivore (Matthews 1998). Based on results from previous research with different fishes from the same functional groups, I predicted each species would increase primary productivity and periphyton biomass, and I predicted the benthic invertivore would reduce benthic invertebrate density.

## METHODS

### *Fish Enclosures*

I conducted a 30 day experiment in Brier Creek, Marshall County, Oklahoma (see Matthews 1998), using fish enclosures to test fish effects in this natural stream ecosystem. Sixteen fish enclosures were constructed using PVC as a support frame, and 0.31 mm polypropylene mesh screening to cover the bottom and sides of the frame (enclosure dimensions: 1 × 1 m base and 0.75 m sides). The top of the enclosures were not covered with screen.

The enclosures were placed in Brier Creek on 20 September 2003. Four, 5-gallon buckets of dry gravel from the stream bank were placed inside each enclosure to anchor the enclosures and provide a natural substrate. Enclosures were placed near the center of

the stream channel, in areas with similar depth (ca. 0.4 m), flow (ca. 0.01 m/s), and canopy cover. Enclosure positions were arranged in a staggered fashion and were at least 3 m apart to avoid any potential upstream effects from adjacent enclosures.

### *Fish Treatments*

On 28 September 2003, I collected 60 individuals of each species from Brier Creek by seine, and randomly assigned fish treatments among enclosures, providing four replicates per treatment. Density was constant at 20 individuals per enclosure. The fish remained in the enclosures from 28 September through 29 October 2003, at which time they were removed with nets and preserved in 10% formalin. Number of individuals and wet mass of fishes recovered from each enclosure was determined in the laboratory. All 60 Blackstripe Topminnow were recovered from enclosures, and individual mass averaged 0.8 ± 0.3 g. Recovery rates of Longear Sunfish averaged 87% and ranged from 16 – 18 individuals, and mass per individual averaged 11.5 ± 4.5 g. Recovery rates for Orangethroat Darter averaged 80% and ranged from15 – 17 individuals, and average mass per individual was 1.1 ± 0.5 g.

### *PPR and Periphyton Biomass*

Four unglazed clay tiles were placed on the substrate in each enclosure on 28 September 2003. On days 15 and 30, I removed one randomly selected tile to estimate benthic primary productivity (PPR) and periphyton biomass. I used the oxygen evolution method to estimate PPR (Gelwick and Matthews 1992); placing each tile in a Ziploc® storage bag with stream water. Dissolved oxygen of the water was measured when the

tile was placed in the bag and after about a 2 h incubation period in sunlight. Following the oxygen evolution method, the periphyton from each tile was scraped into separate plastic containers and stored on ice. In the laboratory the periphyton slurry in each container was filtered through a 0.45 um filter. The filter with filtrate were placed in a vial and frozen for at least 24 h at 4°C. Following freezing, chlorophyll-*a* was extracted from the filtrate for 24 h using a 90% acetone solution. The extract was measured spectrophotometrically to determine chlorophyll-*a* biomass with a correction for pheopigments (APHA 1995).

### *Benthic Invertebrates*

I sampled macroinvertebrates on days 15 and 30 by taking one core sample (100 $cm^2$ and 5 cm deep) of stream sediments near the center of each enclosure. The samples were preserved in 5% formalin and returned to the lab for identification and enumeration.

### *Gut Contents*

Two individuals from each enclosure ($n$ = 8) were examined for gut contents. I dissected out the anterior third of the alimentary tract from each fish (or the discrete stomach, if one existed), placed the contents on a gridded petri dish, and estimated percent occurrence for each major food category by counting number of grids occupied by each food type.

### *Statistical Analyses*

I used an One-way Repeated Measures ANOVA to test for significant treatment effects, time effects between sample dates, and treatment by time interaction. Independent contrasts were performed to detect pair-wise differences among means. If there was a significant time or time by treatment interaction, comparisons among means were made separately for each sample day, but if there was no time effect or time by treatment interaction, I compared the combined average of both sample days among treatments. I used SAS (2000) for all analyses.

## RESULTS

### *Primary Productivity and Periphyton Biomass*

Primary productivity differed significantly among treatments, but did not change significantly between sample dates (Table 1). Enclosures with Blackstripe Topminnow, Longear Sunfish, and Orangethroat Darter had on average about 2, 4, and 2.5 times greater PPR, respectively, than enclosures without fish (Fig. 1).

Treatment and time effects on chlorophyll-*a* were slightly different than on PPR. Chlorophyll-*a* was significantly different among fish treatments and different between sample dates (Table 1). There was no treatment by time interaction, indicating that chlorophyll-a decreased from days 15 to 30 similarly across all treatments. On average chlorophyll-*a* was about 5, 13, and 5.5 times greater in enclosures with Blackstripe Topminnow, Longear Sunfish and Orangethroat Darter, respectively, than in fishless enclosures (Fig. 2). However, enclosures with Blackstripe Topminnow or Orangethroat

Darter did not have significantly greater chlorophyll-*a* than enclosures without fish on both sample days or on day 30, respectively (Fig. 2).

### *Benthic Invertebrates*

Benthic invertebrate assemblages were composed primarily of chironomids (55%) and ephemeropteran nymphs (25%). Less common invertebrate taxa included trichopteran (2%), odonate larvae (3%), and coleopteran larvae (1%), as well as snails (3%) and annelids (10%). Benthic invertebrate densities varied from about 630 to 980 individuals/$m^2$ (Fig. 3), but there were no significant treatment or time effects on invertebrate density (Table. 1).

### *Gut Contents*

Gut contents confirmed my hypothesized functional roles for each species (Fig. 4). Blackstripe Topminnow was primarily a surface feeder, with terrestrial insect making up the largest proportion of its gut contents. Longear Sunfish was omnivorous, consuming an array of food items ranging from terrestrial and benthic invertebrates to algae. Orangethroat Darter was a benthic invertivore, consuming primarily benthic invertebrates.

## DISCUSSION

This experiment showed that fishes can affect ecosystem properties and functions in natural stream ecosystems, suggesting that, although mesocosms are structurally and functionally simplistic relative to natural streams, results from such systems might apply

to natural stream ecosystems. Other studies have corroborated mesocosm results with experiments in natural systems. For example, Resetarits (1991) found similar predatory effects of fish on salamanders in mesocosms and natural stream ecosystems; and Schafer (1999) showed that factors affecting fish movement across riffle barriers were similar in mesocosms and natural streams.

I observed that the general pattern of fish ecosystem effects was repeatable from mesocosms to natural streams, but the effect size was different. In the field enclosure experiment, I found that primary productivity (PPR) and periphyton biomass was greater in enclosures with fish versus those without. In previous mesocosm experiments, fish from the same functional groups also increased periphyton biomass relative to fishless controls. However, fish effects in mesocosms were greater in magnitude than fish effects in field enclosures. On average, mesocosms with 20 fish/$m^2$ had about a two-fold greater effect on periphyton than did fish in field enclosures. This could indicate that fish effects in stream mesocosms were intensified relative to their effects in real streams, possibly as a result of continued stable flow or nutrient build-up (Gelwick and Matthews 1993)

I found no fish effects on benthic invertebrate density in this enclosure experiment. However, there was a slight but non significant decrease in invertebrates in the Orangebelly Darter treatment as would be expected from its food habits. This pattern is consistent with results from mesocosm experiments, in which I rarely have detected fish effects on benthic invertebrates. Invertebrate densities were highly variable among treatments in this field study (as well as in previous mesocosm experiments), thus,

statistical power was likely too low to detect significant fish effects on this ecosystem property.

The concordance in results between mesocosms and natural streams is likely to depend on the similarity in conditions between the two systems at that point in time. When my experiments were conducted in Brier Creek, flow was very low (ca. 0.01 m/s). This natural flow was similar to that measured in previous stream mesocosm experiments. Thus, abiotic effects linked to flow may have been minimal in Brier Creek when I conducted my experiments. Further, there was little rain in the Brier Ck. drainage area during my the field experiment, limiting watershed effects such as nutrient or sediment inputs that could have influenced fish ecosystem effects. I suggest that fish effects in natural systems likely are context specific, such that results from mesocosms are likely to apply to natural streams under a limited set of environmental conditions.

### *Conclusions*

Based on my dissertation research using artificial stream mesocosms and a natural field experiment, I suggest that under certain environmental conditions (e.g., reduced flow) fish can have pervasive effects on stream ecosystem properties and functions. These effects are likely tightly coupled with fish density, and likely vary among species from different functional groups. It also is probable that the number of fish species in a stream ecosystem is linked to rates of ecosystem functioning, such that loss of fish species from stream ecosystems could possibly have negative ecosystem consequences.

## ACKNOWLEDGEMENTS

I am grateful to D. Hambright and P. Jeyasingh for helping collect fish and starting this field enclosure experiment. This research would not have been possible without the gracious permission of James Martin of Kingston, Oklahoma to conduct these experiments in Brier Creek. I thank the faculty and staff, T. Cluck, D. Cobb, R. Page, M. Ward, and L. Weider, at the University of Oklahoma Biological Station for facilities and equipment. Finally, I thank my doctoral committee, E. Bergey, M. Kaspari, W. Matthews, R. Nairn, and C. Vaughn, for reviewing this manuscript and for critical discussion. This work was supported by a McCarley Research Grant awarded to C. Hargrave by the Southwestern Association of Naturalists, and partially supported by a National Science Foundation Doctoral Dissertation Improvement Grant awarded to W. Matthews and C. Hargrave (NSF 0308729). This work was submitted in partial fulfillment of a Ph.D. degree at University of Oklahoma, Department of Zoology.

## LITERATURE CITED

American Public Health Association. 1995. Standard methods for the examination of water and wastewater, 19th ed. American Health Association, Washington, DC, USA.

Craig, D. A. 1993. Hydrodynamic consideration in artificial stream research. Journal of the North American Benthological Society **12**:324-327.

Fuller, R. L., C. Ribble, A. Kelley, E. Gaenzle. 1998. Impact of stream grazers on periphyton communities: a laboratory and field manipulation. Journal of Freshwater Ecology **13**:105-114.

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

Gelwick, F. P., and W. J. Matthews. 1993. Artificial streams for studies of fish ecology. Journal of the North American Benthological Society **12**:343-347.

Gelwick, F. P., and W. J. Matthews. 1997. Effects of algivorous minnows (*Campostoma*) on spatial and temporal heterogeneity of stream periphyton. Oecologia **112**:386-392.

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

Lamberti, G. A., and A. D. Steinman. 1993. Research in artificial streams: applications, uses, and abuses. Journal of the North American Benthological Society **12**:314-317.

Matthews, W. J. 1998. Patterns in freshwater fish ecology. Chapman and Hall, New York, New York, USA.

McIntire, C. D. 1993. Historical and other perspectives of laboratory stream research. Journal of the North American Benthological Society **12**:318-323

Resetarits, W. J. 1991. Ecological interactions among predators in experimental stream communities. Ecology **72**:1782-1793.

SAS Institute. 2000. version 8.01. Cary, North Carolina, USA.

Schafer, J. F. 1999. Movement by three stream dwelling cyprinids (*Notropis boops*, *Campostoma anomalum*, and *Cyprinella venusta*). Unpublished Dissertation, University of Oklahoma.

Table 1. Repeated measures ANOVA table showing degrees of freedom (*df*), *F*- and *P*-values for main treatment effects, time effects, and treatment by time interaction for each response variable.

| Source of variation | *df* | F | P |
|---|---|---|---|
| PPR | | | |
| Treatment Effect | 3,12 | 26.40 | <0.0001 |
| Time Effect | 1,12 | 3.16 | 0.1008 |
| Interaction | 2,12 | 1.26 | 0.3313 |
| Chlorophyll-*a* | | | |
| Treatment Effect | 3,12 | 11.84 | 0.0007 |
| Time Effect | 1,12 | 18.08 | 0.0011 |
| Interaction | 2,12 | 2.23 | 0.1378 |
| Benthic Invertebrates | | | |
| Treatment Effect | 3,12 | 0.47 | 0.7064 |
| Time Effect | 1,12 | 0.04 | 0.8399 |
| Interaction | 2,12 | 0.09 | 0.9020 |

## LIST OF FIGURES

Figure 1. Mean response in primary productivity (PPR) on days 15 and 30. Results from contrasts indicated by letters above each bar. Contrasts were calculated on average PPR for both sample days because there was no significant time effect. Treatments with different letters had significantly different mean PPR on both sample days. Vertical bars are standard errors.

Figure 2. Mean response in periphyton biomass (estimated as chlorophyll-*a*) on days 15 and 30. Results from contrasts indicated by capital (Day 15) and lower case (Day 30) letters above each bar. Contrasts were calculated separately for both days because of a significant time effect. Treatments with different letters had significantly different chlorophyll-*a* on that sample day. Vertical bars are standard errors.

Figure 3. Mean response in benthic invertebrate density to treatments on days 15 and 30. Vertical bars are standard errors.

Figure 4. Average percent occurrence of food items found in guts of eight individuals (*n* = 8) for each species recovered from enclosures on day 30. Vertical bars are standard deviations.

Figure 1.

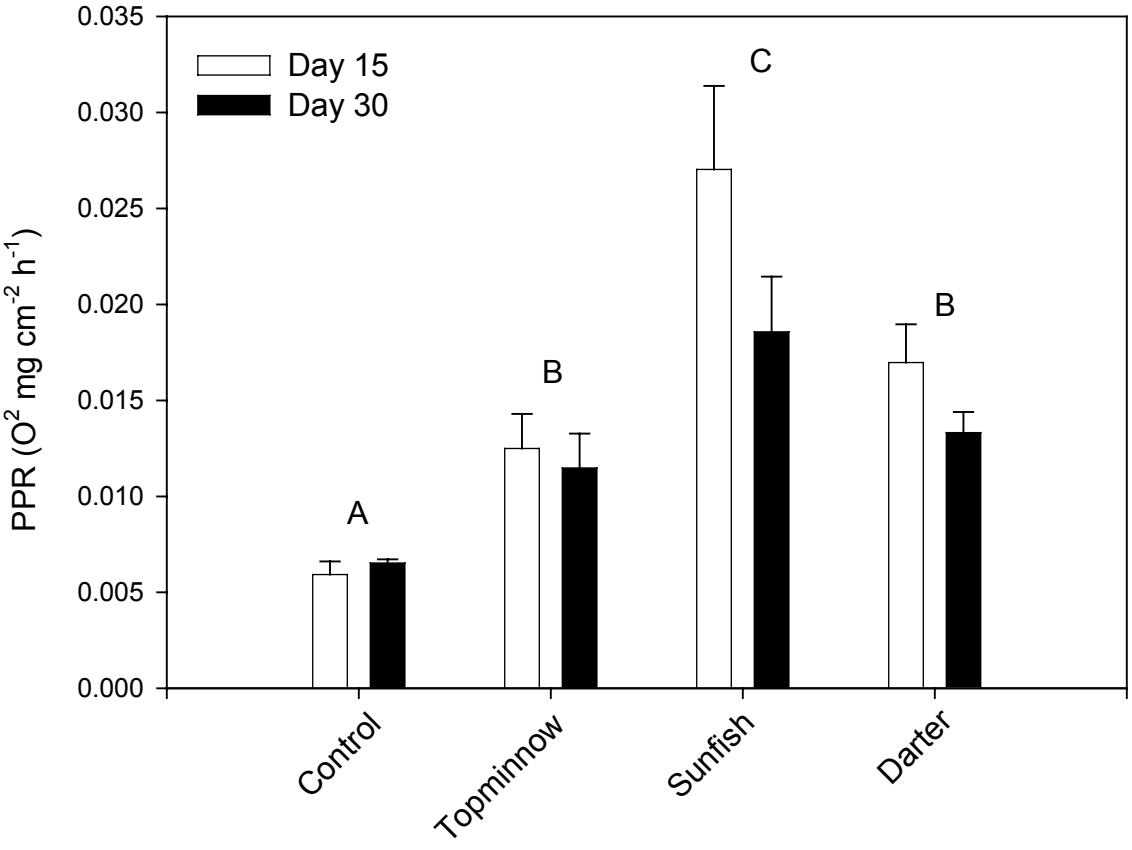

0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
PPR ($O^2$ mg $cm^{-2}$ $h^{-1}$)
Day 15
Day 30
A
B
C
B
Control
Topminnow
Sunfish
Darter

Figure 2.

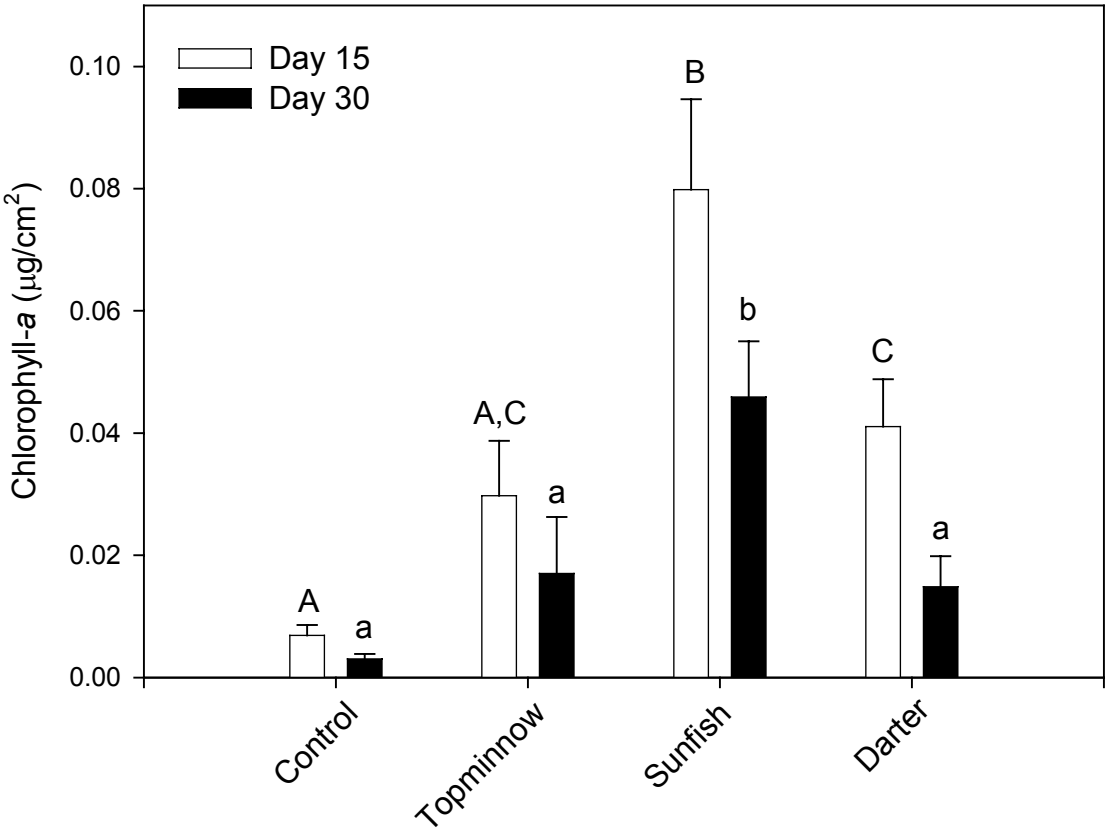

Day 15
Day 30
Chlorophyll-a (μg/cm²)
0.10
0.08
0.06
0.04
0.02
0.00
A
a
A,C
a
B
b
C
a
Control
Topminnow
Sunfish
Darter

Figure 3.

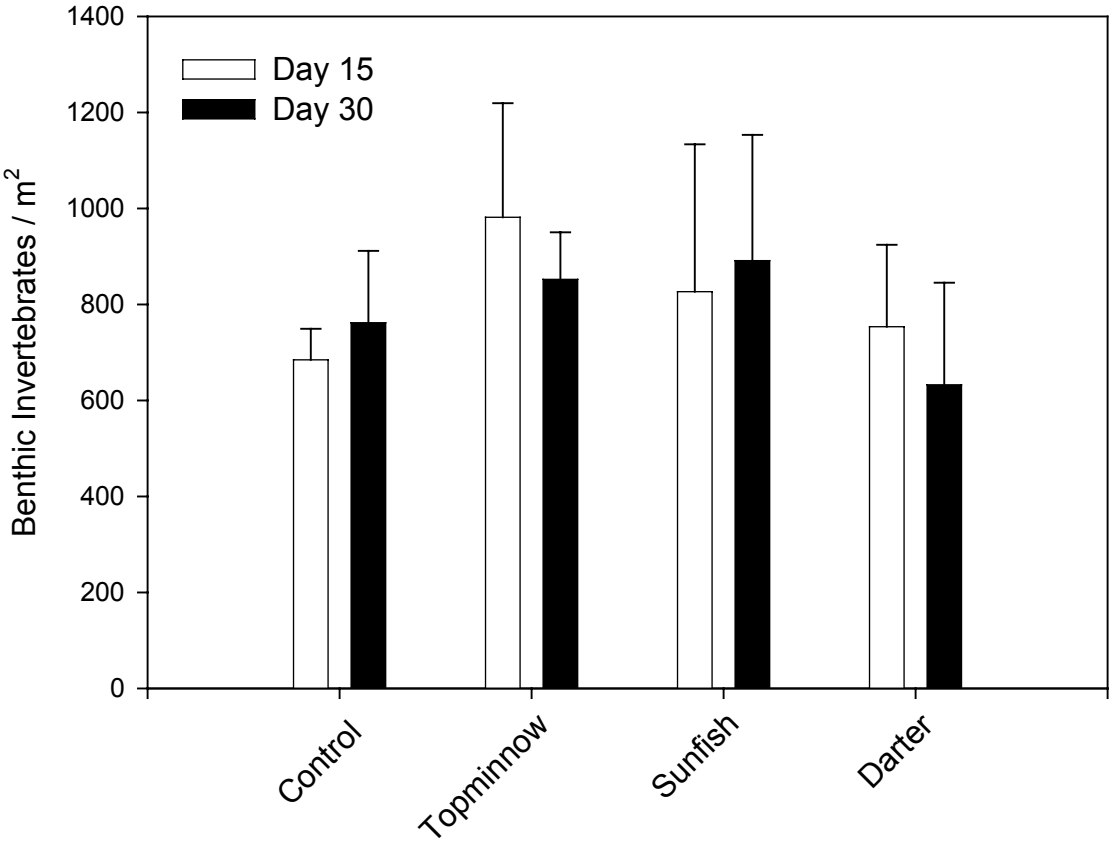
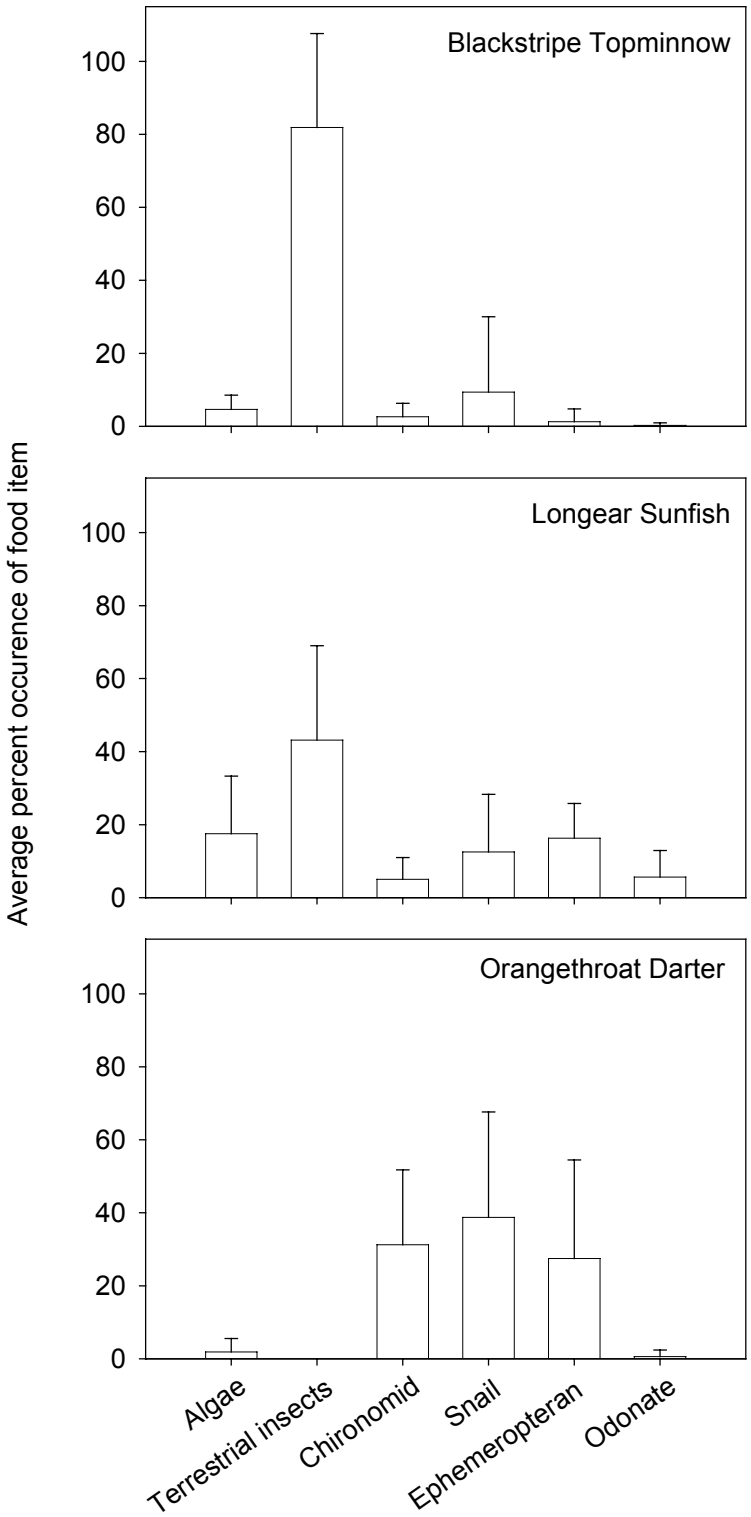

Day 15
Day 30
Benthic Invertebrates / m2
1400
1200
1000
800
600
400
200
0
Control
Topminnow
Sunfish
Darter

Figure 4.


Blackstripe Topminnow
Longear Sunfish
Orangethroat Darter
Average percent occurence of food item
0
20
40
60
80
100
Algae
Terrestrial insects
Chironomid
Snail
Ephemeropteran
Odonate