Applying general ecological models to positive interactions among lotic fishes: implications for population and community regulation at multiple spatial scales

Brandon K. Peoples

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Fisheries and Wildlife

Emmanuel A. Frimpong, chair
Lori Blanc
Donald Orth
C. Andrew Dolloff

24 February 2015
Blacksburg, Virginia, United States

Keywords: mutualism, symbiosis, biological markets, experiment, facilitation, stress gradient hypothesis, structural equation modeling, information-theoretic, co-occurrence, occupancy modeling, stream fish, nest association, lotic, habitat, *Nocomis*

Applying general ecological models to positive interactions among lotic fishes: implications for population and community regulation at multiple spatial scales

Brandon K. Peoples

Abstract

Positive biotic interactions such as mutualism, commensalism and facilitation are ubiquitous in nature, but historically have received considerably less research attention than negative interactions such as competition, predation and parasitism. The paucity of research on positive interactions is particularly evident in stream ecosystems and in vertebrate communities. Stream fishes clearly provide an ideal system for advancing research on positive interactions. Many minnows (Cyprinidae) of eastern North America engage in a potentially mutualistic reproductive interaction known as nest association, in which individuals of one species (nest associates) spawn in nests constructed by host species. In nest association, hosts provide unsilted gravel substrate for spawning nest associates, and increased parental care to associate broods. High associate: host egg ratios can create a dilution effect, reducing the probability that host eggs will be preyed upon by egg predators. Nest associative interactions are common, but are relatively understudied compared to other interactions among stream fishes.

The goals of this study were to apply general ecological models to this novel system to (a) gain new insight into the mechanisms structuring nest associative stream fish communities, and (b) to use inference from stream fish communities to potentially expand and improve the general ecological models. These goals required completion of three objectives, including (1) examining the influence of abiotic and biotic contexts on reproductive behavior and fitness outcomes between a cyprinid host and associate, using the biological markets model to generate predictions; (2) examining the utility of the nest web framework (previously only used for cavity nesting vertebrate communities) and the stress gradient hypothesis (previously applied almost exclusively to plant communities) for predicting which associate species spawn on nests built by various nest building species, and the consequences of these choices, respectively; and (3) using two-species occupancy modeling to determine the relative influence of biotic interactions and habitat covariates on the co-occurrence of a host and two nest associates.

To accomplish these goals, I conducted a large-scale experiment to manipulate presence of mutualists (*Nocomis leptocephalus*, host; *Chrosomus oreas*, associate), egg predators (biotic context) and habitat quality (abiotic context). I conducted behavioral nest observations and conducted repeated stream fish stream fish community surveys to collect demographic data. I constructed a nest web from observational data, and implemented structural equation modeling through an information-theoretic framework to identify nest web plausibility across a large spatial extent. I tested some predictions of the stress gradient hypothesis by regressing juveniles-per-nest and a metric of cyprinid community structure on a composite measure of physical stress (scaled gradients of catchment-scale agricultural land use and catchment area). I used two-species occupancy modeling to model co-occurrence of *N. leptocephalus* hosts and two associates, *C. oreas* and *Clinostomus funduloides*, and used an information-theoretic framework to compare hypotheses representing the importance of biotic interactions, habitat covariates or both at determining species co-occurrence.

Results corroborated some (but not all) model predictions, and identified room for improvement in each of the general models. Nest associative spawning by *C. oreas* was not context dependent; *C. oreas* did not spawn in the absence of a reproductively active male *N. leptocephalus* at any treatment level. However, the net fitness outcome of host and associate species was mutualistic, and the interaction outcome switched from commensalistic mutualistic with abiotic context. *N. leptocephalus* reproductive success was improved by *C. oreas* presence in less-silted habitats, but not in heavily-silted habitats. This is most likely because broods were subject to predation in both habitat types, but were also negatively affected by siltation in silted habitats. Accordingly, egg dilution by associates was not sufficient to support a mutualistic relationship in less favorable habitats. Results suggest that the biological markets model may be a useful tool for predicting fitness outcomes of nest associative mutualism, but may not be as useful for predicting the behavioral outcomes of obligate mutualisms. Future applications of the biological markets model should carefully consider species traits, specifically the degree to which trading behavior is obligate for participants. Future work with this model will yield more insight by considering highly facultative associates.

Nest webs constructed from nest observational data suggested an interaction topology in which strong (nearly-obligate) associates relied most frequently on *N. leptocephalus* nests, and less frequently on nests constructed by *Campostoma anomalum*. Weak (facultative) associates were seldom associated with nests constructed by either species, and probably spawned before hosts began nesting activity. Structural equation models corroborated this topology throughout the New River basin, although some less-supported model evidence specified some nest association by weak associates. Juveniles-per-nest of strong associates responded positively to physical stress, while this metric for other cyprinid reproductive groups showed no relationship. Proportional representation of *Nocomis* and strong associates also increased predictably with physical stress. This study suggests that the nest web framework can be informative to systems outside the ones for which it was developed; future studies may be able to use this framework to better understand the role of habitat-modifying species in communities other than cavity nesting terrestrial vertebrates and nest associative stream fishes. This work extended the nest web

framework by (a) modeling the outcomes of interactions instead of the interactions themselves, and (b) by using structural equation modeling to test nest web predictions with an information-theoretic framework. This study also suggests that the stress gradient hypothesis can be useful for understanding interaction dynamics in vertebrate communities; this represents the first direct evidence that this model can be used in vertebrate communities. Further, I demonstrate that the stress gradient hypothesis may be extended to predict community structure. However, more research in a diversity of systems will be needed to determine the extent to which this can be applied.

This study provides some of the first evidence of large-scale positive co-occurrence patterns in vertebrates. However, the precise roles of habitat covariates and biotic interactions were species-specific. Occupancy results suggest that co-occurrence between *N. leptocephalus* and nest associate *C. funduloides* is driven only by reproductive behavioral interactions. Alternatively, evidence suggests that co-occurrence between *N. leptocephalus* and *C. oreas* is driven by both nest association and habitat covariates. That two-species occupancy modeling can be a useful tool for comparing difficult-to-test hypotheses involving biotic interactions at large spatial scales. This study represents the first quantitative, multi-scale treatment of positive interactions in stream ecosystems.

This study demonstrates that applying general ecological models to stream fish communities can yield new insights about both the study system and the models themselves. While models of negative interactions, food webs and dispersal have been applied to stream fishes, we stand to gain much ground by also considering positive biotic interactions. In doing so, stream fish ecologists will also be able to contribute to the advancement of general ecology, and thus raise awareness for these understudied ecosystems and taxa.

Acknowledgements

I never intended to earn a PhD until just before I started working on my PhD. I had tricked myself into believing I wanted a slower-paced job at an agency, but Emmanuel Frimpong knew my career path before I did, and I thank him for all the opportunities he has given me. He entertains my outlandish ideas, answers my incessant questions, and points me in the right direction. He stays optimistic when times look rough, and is patient when I make (even big) mistakes. Emmanuel taught me to have confidence in my abilities and to seize opportunities such as projects, scholarships and even a postdoc position. He encouraged me to flesh out projects that compliment this dissertation and many that stand alone. He makes time for everyone, regardless of their status or how busy he is. Emmanuel has been an amazing role model, advisor and friend; I cannot thank him enough.

Jeremy Pritt and Richard Pendleton made conceptual contributions to early stages of some of the ideas presented in this dissertation. Our ideas first took shape in 2009 on a napkin in a van traveling east from Nashville. They are fleshed out in this dissertation, and I am eager to learn where they are going.

I owe much to my doctoral committee for their help throughout my time at Tech. They gave me feedback and advice on my research, wrote letters of recommendation for awards and postdoc positions, co-authored papers with me and provided invaluable advice for navigating the torrents of academia. Lori Blanc has been a true inspiration. She has been the strongest force pushing me outside of my taxonomy, and consequently has fostered much of the fundamental thinking behind the ideas presented in this dissertation. She even made me describe my research in a poem during my preliminary exams. Andy Dolloff is one of the busiest people I know, yet

still takes the time to kindly provide advice. Andy has taught me the value of practicality, and that I actually am more practical than I consider myself to be. Don Orth has practically been my step-advisor, having tolerated almost as many of my disruptions as Emmanuel. Working with Don and his students has been a great pleasure, and teaching his Ichthyology lab has been an amazing learning experience for which I am thankful. In one way or another, I could not have completed my PhD without the unique contributions of each of these three individuals.

The National Science Foundation Division of Environmental Biology awarded Emmanuel the grant that funded the experiment presented in Chapter 1, and subsequently brought me back to Virginia Tech. The United States Geologic Survey Aquatic Gap Program funded the stream fish community data presented in Chapters 2 and 3, during the collection of which I served as a happy technician.

I owe a mountain of thanks to my field crew—not just for help in the field, but for becoming some of the best friends I have ever had. Jason Emmel and Steve Watkins made significant contributions to the design of the experimental enclosures. The three of us put our heads together and built what many believed we couldn't—a channel-spanning enclosure system that could stand up to bankfull floods, over…and over…and over again. Stephen Floyd helped manage a 2013 field crew that swelled to nearly a dozen, all the while conducting his master's research on the side. These three individuals have been instrumental to the success of this project. Furthermore, I thank Christina Bolton, Joseph Buckwalter, Paige Crane, Kaylie Fitzgerald, Jian Huang, A. Dawn Mercer, Regan Mukai, Stanford Nettles, C. Thomas Olinger, Tom Russell, June Shrestha and Caitlin Worsham. These individuals worked dawn-to-dusk, for seven days a week on a project that often was, at best, a losing battle and, at worst, explicitly self-defeating. Thank you all for not giving up on me; you built this dissertation.

Several land owners and organizations were instrumental in the success of this project. I thank Palmer and Phyllis Price for access to their property on the North Fork Roanoke River in the pursuit of experimental data and turkey meat. I also thank Edward and Janet Yost for North Fork property access, stimulating conversation, delicious well water and for the view from their cabin porch. The Hiendel, Kohl, McPherson, Parsons and Sutphin families also provided us property access to hunt down enough white suckers for the experiment. I thank the town of Blacksburg, VA for allowing us to work on Toms Creek in Heritage Park. I specifically thank Dean Crane, director of Blacksburg Parks and Recreation, for facilitating our access, buffering our missteps and for introducing us to Paige. I thank the Brillhart families and the rest of the property owners in the Farmingdale community for their patience with the project. I also thank the Virginia Tech Catawba Sustainability Center, and Josh Nease, its director, for facilitating access to Catawba Creek.

I thank my fellow Frimpong lab mates—Yaw Ansah, Gifty Anane-Taabeah, Joe Buckwalter, Stephen Floyd, Iris Fynn, Jian Huang and Steve Watkins. It has been a pleasure to call you my teammates; good luck. I owe another big thanks to all my friends in the FiW department, who are too numerous to name. Thank you for your friendship, help and advice; you make grad school bearable.

Most importantly, I thank Alyson Peoples, my wife. She has gladly accompanied me wherever fishes have dragged me, and will have done so again by the time this document is published. She stuck with me when it felt like I was married to a flooded pile of T-posts and fencing. She stuck with me when I ran off to Honduras to shock fish in the jungle. Anyone else would have left. Anyone else would be no reason to keep going.

Attribution

Two co-authors contributed significantly to this dissertation, which is a compilation of three publications and manuscripts. This section specifies the contributions of each co-author to this work. Emmanuel Frimpong is my academic advisor and primary project supervisor. He wrote the grants to fund this study, and contributed heavily to the project design, analysis and writing of each manuscript. Lori Blanc serves on my advisory committee, and contributed much to the concepts, study design, and writing of Chapter 2. This dissertation is accordingly written in the first-person plural voice. Some information among chapters may appear redundant because each chapter is written as a stand-alone manuscript

Peoples, B.K. and E.A. Frimpong. Biological markets theory predicts context dependent outcomes in a mutualism between two freshwater fishes. *In review*. Emmanuel Frimpong co-authored Chapter 1. As of 23 April 2015, a version of this chapter is under review in *Ecology*.

Peoples, B.K., L. Blanc and E.A. Frimpong. Lotic cyprinid communities can be structured as nest webs and predicted by the stress-gradient hypothesis. Lori Blanc and Emmanuel Frimpong co-authored Chapter 2. As of 23 April 2015, this manuscript is under review in *Journal of Animal Ecology*.

Peoples, B.K. and E.A. Frimpong. Biotic interactions and habitat drive positive co-occurrence between facilitating and beneficiary stream fishes. Emmanuel Frimpong co-authored Chapter 3. AS of 23 April 2015, this manuscript is under review in *Ecological Applications*.

Table of Contents

List of tables

List of equations

List of figures

General introduction

Interspecific interactions act strongly on biotic processes, regulating patterns across levels of taxonomic organization (Boucher et al. 1982). Interactions can be characterized by the sign of generalized outcomes for each participant. For a single participant or a homogeneous group of participants, pairwise outcomes are often conceptualized as being positive (+), negative (-), or neutral (0). Although interactions are best described as a continuum of outcomes (Abrams 1987), they are often categorized as simple binary outcomes ranging from competitive (-,-) to mutualistic (+,+). Among the types of outcomes in Table 1, negative interactions (competition and predation) received more research attention during the twentieth century, at least by western ecologists (see Boucher 1982, 1988 and references therein). Until the early 1980s, the common understanding among ecologists was that negative interactions were the dominant force in nature, and that positive interactions (mutualism and commensalism) simply represented ecological idiosyncrasies (Boucher 1985, Keddy 1989, Cherif 1990). As a consequence, positive interactions were largely excluded from the historical ecological paradigm.

Table 1. Potential pairwise outcomes (upper), and associated characterizations (lower), of interspecific interactions. Adopted from Wootton (1998).

	Effect of species 1 on individual fitness of species 2		
Effect of species 2 on fitness of species 1	+	0	-
+	+,+ Mutualism	+,0 Commensalism*	-,+ Antagonism**
0	+,0 Commensalism	0,0 Neturalism	-,0 Amensalism***
-	-,+ Antagonism	-,0 Amensalism	-,- Competition

*Commensalistic interactions are considered “facilitations” when driven by dominant “foundation species” or “ecosystem engineers”

**Antagonistic interactions include predation and parasitism

***Amensalism is often referred to as asymmetric competition

The lack of classical attention given to positive interactions belies their importance in nature. Positive interactions are ubiquitous: they occur in every ecosystem, and among most taxa on earth (Boucher et al. 1982, Margulis and Fester 1991). Mutualisms are attributed to such large-scale processes as the evolution of the eukaryotic cell (Margulis 1981) and the massive radiation of the angiosperms (Midgley and Bond 1991). Many mutualisms (and their massive implications) are well-understood; countless studies have dissected mutualistic interactions between pollinators and plants (Kearns et al. 1998), mycorrhizae and tree roots (Gerdemann 1970), and coral and their endosymbiotic algae (Muscatine and Porter 1977). In recent decades, research on positive interactions (namely mutualism) has progressed beyond simple biological accounts to the point of full inclusion into mainstream ecology (reviewed by Bronstein 1994). Theoretical treatments of positive interactions in terms of evolution (Foster and Wensellers 2006, Ferrière et al. 2007), population regulation (Hoeksema and Schwartz 2001, Thompson et al. 2006), community regulation (Bertness and Callaway 1994, Hacker and Gaines 1997), species distributions (Linder et al. 2012), and conservation concerns (Bronstein et al. 2004, Byers et al. 2006) have been presented. However, many ecological subdisciplines have been slow to recognize the importance of positive interactions. Similarly, many concepts that are generally accepted by general ecologists remain untested in a diversity of taxa and ecosystems.

A contemporary goal of ecology involves characterizing contextual shifts in the outcomes and frequencies (or relative importance) of biotic interactions. Outcomes of interactions and their magnitudes are seldom discrete; contextual differences can dramatically influence effects on participants, or even the necessity for participation itself. Two general forms of context affect interactions: abiotic (e.g., habitat conditions or resource availability) and biotic (e.g., abundance of mutualists, hosts, predators, or competitors). Tertiary species' interactions with mutualists have been shown to interfere with mutualism dynamics (Bacher and Friedli 2002), causing some mutualistic species pairs to coevolve unique defense strategies (Althoff et al. 2005). Likewise, the abiotic contexts in which interactions occur affect outcomes. Changes in resource availability (e.g., light or nutrients) have been shown to shift pairwise interactions from one categorical outcome type to another (Kersch and Fonseca 2005, Miranda et al. 2011). These interaction dynamics are often characterized as biological "markets" where species must trade resources to ensure their persistence (Noë and Hammerstein 1994, Hoeksema and Schwartz 2001).

The frequency and relative importance of interactions for regulating community-level processes are also context dependent (Bertness and Callaway 1994). In general, competition has been shown to be most frequent (or important) in physically benign (i.e., stable and less stressful) habitats or when predation pressure is low, while positive interactions prevail in harsher habitats or when predation pressure is high (Bertness and Hacker 1994, Bertness and Yeh 1994, Callaway 1998). These observations led to the development of a well-supported community-level model: the stress-gradient hypothesis (SGH; Bertness and Callaway 1994, Bertness and Leonard 1997), which has been recently refined to consider species traits (Maestre et al. 2009), and stress thresholds (Malkinson and Tiebörger 2010).

Important interspecific interactions must be inferable at large spatial scales, and evident in species' geographic distributions (Van der Putten et al. 2010). Unfortunately, most studies identifying the influence of positive interactions on species distributions have done so only at relatively small spatial scales (Bertness and Grosholz 1985, Bertness 1989, Callaway 1992). Driven by "assembly rules" hypotheses (Diamond 1975), most studies at larger spatial scales have focused only on negative interactions (Araújo and Guisan 2006). Most of these studies suggest that large-scale patterns in species distributions are governed more by habitat attributes than biotic interactions (Olson et al. 2005, Bailey et al. 2009, Williamson and Clark 2011), although some suggest otherwise (Brown et al. 2000, Englund et al. 2009). The question of whether or not positive interactions can be reflect in species distributions remains unanswered, and should be investigated.

Generalized models describing biological processes should be applicable across ecosystems and taxa. One reason (among many) for disparity in application of ecological theory across taxa and systems is that many models have not been evaluated in ecosystems other than the ones in which they were developed. This is quite true for models of mutualism and facilitation (positive interactions driven by a dominant “foundation species”, typically commensalistic). Unfortunately, we still lack a concerted predictive framework for understanding context dependency (Agrawal et al. 2007), largely because of disproportionate research among ecosystems and taxa (He and Bertness 2014). For instance, context dependency in ant-plant and mycorrhizal mutualisms have been studied so thoroughly as to allow syntheses and meta-analyses (Chamberlain and Holland 2009, Hoeksema et al. 2010). Meanwhile, only a handful of studies have even recognized that both negative *and* positive interactions occur in freshwater ecosystem (Holomozuki et al. 2010); even fewer have investigated how those interactions may change with context (Chamberlain et al. 2014, Skelton et al. 2014). Further, very little is known about context dependency in mutualisms among vertebrates (Blanc and Walters 2008b, Gingins et al. 2013, Canestrari et al. 2014). Freshwater fishes clearly provide a novel system with which to test models predicting mutualism dynamics.

Although research on positive interactions in streams is increasing, such work has focused almost entirely on invertebrates (Holomuzki et al. 2010), and none have sought to evaluate interactions in terms of an encompassing theoretical model. Lotic fish ecologists have been slower to study the importance of positive interactions than their terrestrial and marine counterparts. Principles of competition and predation still dominate the current paradigm, as evidenced in well-cited predictive models of fish community structure (Schlosser 1987, Tonn 1990, Strange and Foin 1999). When describing various aspects of fish population or community structure, many contemporary studies still consider only negative biotic interactions (Peres-Neto 2004, Hoeinghaus et al. 2007, Crow et al. 2010, Gido and Jackson 2010). The focus on negative interactions in freshwater ecosystems is not unwarranted; their influences are pervasive. Additionally, positive interactions among stream fishes may not always be apparent, or may be brief (albeit important). However, before the regulatory processes of stream fish population and community structure can be fully understood, positive interactions must be considered.

Freshwater fishes participate in several general types of positive interactions; some are better studied than others. One of the most well-known is bioturbation, a process in which trophic or reproductive activities by a species disrupts and releases substrates and associated nutrients. Typically associated with large bodied fishes, bioturbation can be a critical component of nutrient dynamics in lotic systems (Flecker 1996, Moore et al. 2004). Like granivorous birds and mammals, fishes also engage in transport mutualisms with numerous terrestrial plants. By consuming and passing seeds on upstream migrations, fishes are critical agents of dispersal for many riparian plant species (Anderson et al. 2011, Horn et al. 2011). Fishes are often hosts to commensalic black fly larvae (Chironomidae), providing upstream dispersal protection from predators (Tokeshi 1993).

Nest association, a reproductive mode in which one fish species (the associate) uses nests build by another species (the host) for spawning (Johnston and Page 1992), is a potentially mutualistic interaction exhibited by stream fishes (Goff 1984, McKaye 1985, Johnston 1994b).

Because nest association is widespread across several continents and many families, its importance for stream fish population and community regulation may be great. Nest association interactions provide a unique system for testing the utility of population- and community-level models of positive interactions.

The goals of this study were to apply general ecological models to nest associative cyprinid communities of the eastern United States to (a) gain new insight into the mechanisms structuring these communities, and (b) to use inference from stream fish communities to potentially expand and improve the general ecological models. This research focused on both population and community level processes at multiple spatial scales. Three general objectives were necessary for achieving this goal: 1) investigating context dependency of a two-species nest association interaction; 2) identifying the abiotic contexts in which positive reproductive interactions play a significant role in organizing stream fish communities; and 3) assessing the large scale implications of biotic interactions by modeling species co-occurrence. Basing hypotheses on general ecological models of positive interactions enables the evaluation of the models' utility for making predictions in this "novel" ecosystem. Applying the models to nest associative stream fishes will either reinforce models in their current states, or will provide directions for model refinement.

Chapter 1: Biological markets theory predicts context dependent outcomes in a mutualism between two freshwater fishes

By Brandon K. Peoples[1] and Emmanuel A. Frimpong[2]

Virginia Polytechnic Institute and State University, Department of Fish and Wildlife Conservation. 100 Cheatham Hall, Blacksburg, VA 24061. Phone: 540-231-6880, fax: 540-231-7580.

[1]bpeoples@vt.edu

[2]Corresponding author: frimp@vt.edu

Keywords: biological markets, mutualism, biotic interaction, context, fish, *Nocomis*, vertebrate, nest association, freshwater, stream

Abstract.- The development of encompassing general models of ecology is precluded by a narrow focus among taxa and systems. Models predicting context-dependent outcomes of biotic interactions have been tested using plants and bacteria, but their applicability at higher levels of taxonomic organization is largely unknown. We tested the biological markets model (BMM) in a context-dependent reproductive mutualism between two communal spawning stream fishes: mound nest building *Nocomis leptocephalus* and its associate, *Chrosomus oreas*. In an *in situ* experiment, we manipulated egg predator density and presence of both symbionts (biotic context), and replicated the experiment in habitats containing high- and low-quality spawning substrate (abiotic context). Deducing from the BMM, we hypothesized that increased predator density and decreased substrate availability would increase the propensity of *C. oreas* to associate with *N. leptocephalus* and decrease reproductive success of both species. We observed that *C. oreas* did not spawn without its host; this contradicts our first hypothesis but is supported by the BMM in a way we did not initially consider. The interaction outcome switched from commensalistic to mutualistic with changing abiotic and biotic contexts, although the net outcome was mutualistic. The BMM can be a useful tool for predicting outcomes of interactions among vertebrates.

Introduction

Outcomes of interspecific interactions are rarely static; they can shift from antagonistic to commensalistic to mutualistic, depending on abiotic and/or biotic context (Bronstein 1994a). Among types of pairwise interaction outcomes, mutualism is especially more likely to shift towards antagonism in changing contexts, largely because of asymmetric exploitation by participants (Chamberlain et al. 2014). Understanding context dependency in mutualisms is particularly important as anthropogenically mediated alterations in habitat such as climate change can affect interaction outcomes and significantly impact species distributions (Van der Putten et al. 2010). Unfortunately, we still lack a concerted predictive framework for understanding context dependency (Agrawal et al. 2007), largely because of disproportionate research among ecosystems and taxa (He and Bertness 2014). For instance, context dependency in ant-plant and mycorrhizal mutualisms have been studied so thoroughly as to allow syntheses and meta-analyses (Chamberlain and Holland 2009, Hoeksema et al. 2010). Meanwhile, only a handful of studies have even recognized that both negative *and* positive interactions occur in freshwater ecosystem (Holomozuki et al. 2010); even fewer have investigated how those interactions may change with context (Chamberlain et al. 2014, Skelton et al. 2014). Further, very little is known about context dependency in mutualisms among vertebrates (Blanc and Walters 2008b, Gingins et al. 2013, Canestrari et al. 2014). Freshwater fishes clearly provide a novel system with which to test models predicting mutualism dynamics.

Market theory (Noë and Hammerstein 1995) provides a useful framework for understanding context dependency in mutualism (Weyl et al. 2010). The biological markets model (BMM; Schwartz and Hoeksema 1998) applies the comparative advantage theory of economic specialization and trade to interspecific mutualism, and has been extended to include services (Hoeksema and Schwartz 2001) and differences in species resource requirements (Hoeksema and Schwartz 2003). This model has some distinct advantages of being based on individual decision (and selection), accounting explicitly for costs and benefits, the ability to predict when an interaction is mutually beneficial and specialization is adaptive. The BMM predicts that mutualism is adaptive when species perceive differences in costs of acquiring resources, relative to one another. When costs of acquiring resources in isolation is higher than the cost of trading, each species will specialize in acquiring one resource and will trade for another, thus engaging in mutualism. The BMM is particularly well-suited for predicting context dependency in mutualisms. Changing resource availability affects a species' ability to acquire resources on its own and, consequently, its perceived need/ability to trade. In general, the BMM predicts that decreased resource availability should promote trade, so long as at least one species is flexible in its ability to perceive changes in resource availability (e.g. facultative trading behavior can change contextually). Schwartz and Hoeksema (Schwartz and Hoeksema 1998) originally developed the BMM using plants and mycorrhizal fungi. This model has since been applied successfully to a wide variety of mutualisms among microbes (Werner et al. 2014), legumes and rhizobia (Simms et al. 2006), marine cleaner fishes and hosts (Bshary and Grutter 2002), and food-sharing bats (Carter and Wilkinson 2013). However, the BMM has not yet been extended into freshwater systems, and few studies have explicitly considered direct measures of fitness outcomes.

Our goal was to apply the biological markets model to investigate context dependency in a novel system: communal spawning stream fishes in eastern North America. *Nocomis* are minnows (Cyprinidae) that reproduce by carrying stones in their mouths to construct mounds in which their eggs are buried; only mature adult males construct nests. *Nocomis* facilitates the reproduction of over 35 other cyprinid species. Collectively termed “nest associates”, these species require host nests for spawning to various degrees. Nest association behavior may be nearly obligate for some “strong” associates, but many “weak” associates can either spawn with a host or revert to the ancestral behavior of open substrate broadcast spawning (Johnston and Page 1992, Pendleton et al. 2012). Nest associates are lithophils (requiring gravel substrate for spawning; *sensu* (Balon 1975) whose reproductive success depends on the presence of concentrated gravel and the relative absence of silt, which can smother eggs and larvae. *Nocomis* nesting ameliorates spawning habitat where gravel is scarce (McManamay et al. 2010) or heavily embedded with silt (Peoples et al. 2011), but the need for nest association may not be as dire in habitats where high-quality spawning substrate is abundant. Further, because associates do not guard their broods, egg burying by hosts can confer increased survival to associates that would be otherwise unattainable by spawning without a host (Johnston 1994a). Likewise, *Nocomis* can benefit from nest association through a dilution effect; high proportions of associate eggs on nests reduce the probability of host brood being eaten by egg predators. Therefore, participants in nest association trade two key resources: substrate and egg dilution. This interaction has the potential to be context-dependent; among various taxa and systems, pairwise outcomes of nest association have been documented as parasitic (Fletcher 1993, Yamana et al. 2013), commensalistic (Shao 1997a), and mutualistic (Johnston 1994b, Peoples and Frimpong 2013).

The basic model presented in 1998 is adopted here because it makes fewer assumptions. Consider a male *N. leptocephalus* and a male *C. oreas* in a forested stream with abundant reproductive substrate and minimal level of egg predators. The following conditions apply: (i) both species require two resources R1 and R2 for successful reproduction (i.e., egg survival in the form of dilution and gravel substrate). Eggs acquired in this case is measured in terms of dilution with the other species' eggs, which is adaptive for both species if predation does not increase in proportion to the increase in number of eggs (McKaye and KcKaye 1977) (ii) resource units are defined such that some quantity of substrate [R1] is equal in net reproductive value to a unit of eggs [R2]. Let units = the amount of each resource each individual can accrue in one breeding season (iii) a tradeoff in resource acquisition is required (e.g., when the males selects a spawning site, that determines the number of females attracted to spawn and the quantity of eggs fertilized. This tradeoff is initially represented by the Isolation Acquisition Isocline (IAI) and (iv) individuals are allowed to acquire resources at any point along their IAI (Figure 1.1).

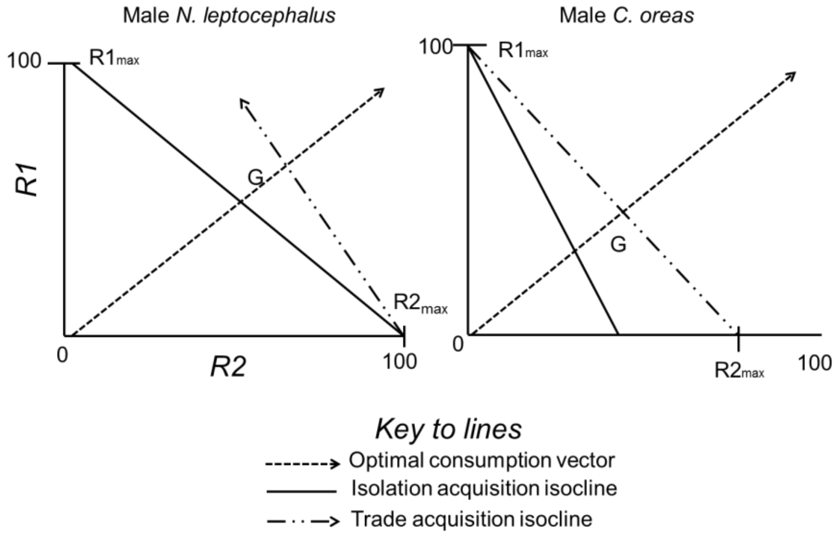

Figure 1.1. Depiction of comparative advantage and resource trade between *N. leptocephalus* (nest-builder) and *C. oreas* (nest associate). The two species trade hypothetical resources, R1 and R2. The potential gain in fitness, G, is the distance between the cost of acquiring resources in isolation and trading for resources.

R_{max} is the amount of the respective resource that the individual can acquire if it specialized completely on that resource. It is further assumed that each species is ideally adapted to this situation such that the cost of acquiring R1 and R2 is equal to the relative proportion that is required to maximize reproductive success, leading to the definition of the OCV. From the life-history of the two species, it is assumed that *N. leptocephalus* is equally good at acquiring both resources while *C. oreas* is better at acquiring egg dilution than substrate because of its relatively high fecundity and abundance. Isolation cost ratio, I, for each species is determined as $R1_{max}/R2_{max}$. Under the initial or baseline condition presented here $I_{N.\ leptocephalus} = 1$ and $I_{C.\ oreas} > 1$. Because one species (*C. oreas* in this case) perceives relative advantage in acquiring one resource (eggs) over the other (substrate) ≡ lower acquisition cost ratio of eggs to substrate (i.e., $R2_{max}/R1_{max}$) following the convention of the authors, and assuming that the two species have different consumption vectors (i.e., requirements), *C. oreas* will specialize in trading eggs for substrate, and the trade will benefit both species. Trade will result in a new isocline for each species (TAI). G, the distance between the two isoclines along the OCV, is the gain from trade in fitness units.

Species may have plastic responses to resource availability, for at least some of the resources, such that the maximum amount of a resource acquired depends on the amount in the environment. Schwartz and Hoeksema (1998) used a linear model to derive a new $R1_{max}$ and $R2_{max}$ in response to resource change. Specifically,

$$R_{max} = F_R \text{ x } R_{avail} + X_R, \tag{1}$$

where, F_R = flexibility of the species to adjust to changes in the resource, R_{avail} = availability of the resource, and X_R = the resource intercept or minimum amount of the resource required to sustain the species. This model was used symbolically and graphically to derive new values for $R1_{max}$ and $R2_{max}$ and new values of $I_{N.\ leptocephalus}$ and $I_{C.\ oreas}$ under decreased substrate and increased egg-predator conditions leading to prediction of changes in trade costs and gains

(Figure 1.2). Intermediate graphs leading to the final predictions are omitted for brevity. Under baseline conditions, *C. oreas* perceives a lower acquisition cost ratio of eggs to substrate. In all cases, as long as the two species trade at some trade cost ratio (T) between the isolation cost ratios for the two species, there is gain for both individuals. Under decreased substrate (R2) conditions such as is caused by urban or agricultural development, both species have decreased ability to acquire substrate but *N. leptocephalus* has more flexibility to move gravel and create substrate. Ability to acquire eggs ($R1_{max}$) is assumed to remain the same as the baseline for both species. The following changes in isolation costs result: $I_{N.\ leptocephalus} = R2_{max}/R1_{max} < 1$ and $I_{C.\ oreas} = R2_{max}/R1_{max} << 1$, implying isolation cost for both species shift to the left. Both species now perceive relative advantage in acquiring eggs. Furthermore, the perception of advantage is steeper for the *C. oreas* in the decreased substrate scenario and should encourage more associations with *N. leptocephalus*. Therefore *C. oreas* specializes in the acquisition of eggs and trade for substrate. Trade will be beneficial for both as long as they trade at a cost ratio, T, between their isolation cost ratios. There are possible absolute losses in fitness for both species because they both experience decreased $R1_{max}$, but both are better off with than without trade. Under the increased egg-predator condition, the availability of eggs ($R2_{max}$) will decrease from the baseline because spawning female numbers and their fecundity are assumed to stay constant in the short term (e.g., over one season). No flexibility is assumed for either species to acquire more eggs. As $R2_{max}$ stays constant, the following changes in isolation costs result: $I_{N.\ leptocephalus} = R2_{max}/R1_{max} > 1$ and $I_{C.\ oreas} = R2_{max}/R1_{max} \rightarrow 1$, implying isolation cost for both species shift to the right. Note that $I_{C.\ oreas} \rightarrow 1$ and NOT > 1. *N. leptocephalus* now perceives relative advantage in acquiring substrate so it will specialize in substrate and trade for eggs in the form of dilution.

The mutual resource trade between two species results in gains measured theoretically in fitness units (Schwartz and Hoeksema 1998). In ecological field studies fitness is difficult to measure directly for individuals or populations. In this study a framework that decomposes the relationships between the traits of an individual and its fitness (Arnold 1983, Violle et al. 2007) is adopted. This framework decomposes trait→fitness relationships into trait→performance and performance→fitness components, enabling the effect of traits on fitness in relative terms to be inferred by measuring one or more of the three components of performance (reproductive output, survival, and growth). Frimpong and Angermeier (2010) further linked this concept to population measurement made in observational field studies. In this framework the absolute abundance of individuals of a population can be used as a proxy for individual fitness when comparing different populations. For comparison of gains in fitness relative to baseline conditions, abundance of juveniles for the respective species will be the proxy for fitness. This kind of inference is routinely made (e.g., Damiani 2005; Afkhami and Rudgers 2009; Chamberlain and Holland 2009).

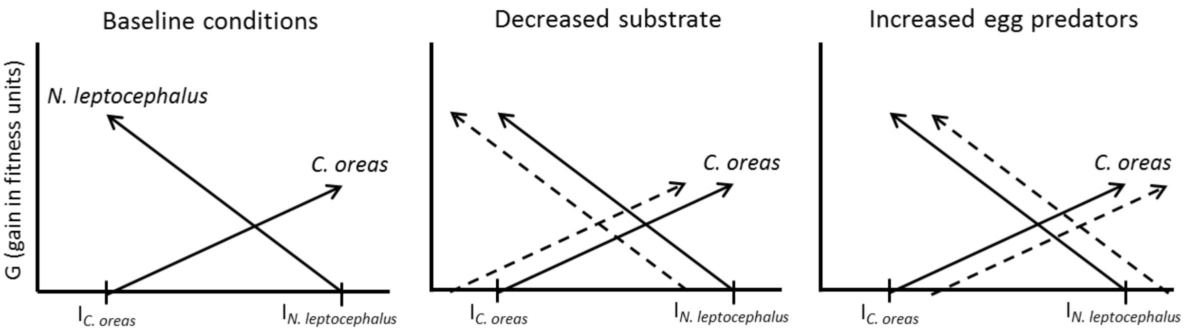

Figure 1.2. Depiction of trade cost and gains under three scenarios.

Using the biological markets model to guide predictions, we conducted a large-scale *in situ* experiment to investigate context dependency in the nest association between *Nocomis* *leptocephalus* and *Chrosomus oreas*, two common cyprinids in the central Appalachian Mountains, USA. *C. oreas* is a strong associate (Pendleton et al. 2012), but can also spawn by open-substrate broadcasting in the natural absence of a host (Jenkins and Burkhead 1994). Our objectives were to elucidate the effect of both abiotic (substrate availability) and biotic (predator density) context on the behavior and fitness outcomes (reproductive success) of the interaction. Substrate availability was represented by riparian land use types: forested (control) habitats with abundant high-quality gravel, and silted habitats in which all gravel was covered with a layer of silt. Biotic context was represented by densities of *Etheostoma flabellare* and *Catostomus commersoni*, two egg predators common throughout the Appalachians. These species do not use nest associative reproductive behavior, thus would not have to distinguish between their own eggs and cyprinid eggs. In a few experimental units, post-nuptial male *Campostoma anomalum* were used as a substitute for *C. commersoni* due to shortage of the latter. For comparison of gains in fitness relative to baseline conditions, we used juvenile (larval) abundance as the proxy for fitness (*sensu* Chamberlain and Holland 2009). Based on the predictions of the BMM, we hypothesized that increased predator density and decreased substrate availability would increase the propensity of *C. oreas* to associate with *N. leptocephalus* and decrease reproductive success of both species.

Methods

We conducted this study in spring of 2012 and 2013 in three 3rd to 4th order streams in the Valley and Ridge physiographic province of southwestern Virginia. Each stream represented a major drainage basin: Toms Creek (2012, Gulf of Mexico), North Fork Roanoke (2012, Atlantic) and Catawba Creek (2013, Chesapeake Bay). Control reaches were characterized by extensive riparian vegetation, stable banks and relatively little silt accumulation in riffles. Conversely, silted reaches were highly entrenched, had little to no riparian vegetation, and exhibited considerable sediment accumulation in riffles. Reaches on the same stream were separated by at least 1.5 fluvial km. Placing reaches in close proximity was logistically optimal, and allowed us to replicate the experiment in contrasting habitats without significant differences in stream size.

We conducted an *in situ* experiment of a balanced, split-plot 2^3 factorial design. Whole plots were replicated in control and silted habitats on each stream (n=3 whole plots per habitat type, 6 total). Each whole plot contained eight experimental units (EUs), randomly assigned a two-level (+,-) treatment of (a) predator density, (b) *N. leptocephalus* and (c) *C. oreas* (Figure 1.3a). This provided six replicates per treatment and 48 EUs. EUs were instream enclosures constructed of 6.4-mm mesh hardware cloth, supported by steel posts and backed by two-panel strips of ~5x10-cm welded fencing. To secure enclosures from fish movement among EUs, we partially excavated substrate directly upstream of fences and bent ~40 cm of the bottom portion of fences upstream to form an apron. We then buried fence aprons as much as possible, and secured the entire margin with 23-kg, form-fitting sandbags. We constructed fences in riffles to ensure each EU contained riffle habitat for potential non-associative spawning by *C. oreas*, pool tail habitat for potential *N. leptocephalus* nesting and pool habitat for feeding and resting. We constructed fences in a downstream-facing “V” shape to reduce water pressure on enclosures (Figure 1.3a). This design required daily maintenance, but withstood multiple large floods and required minimal post-flood repair.

We removed all fishes from EUs using triple-backpack electrofishing. We electrofished until no adult fishes (>40 mm) were captured; this required between four and ten electrofishing passes, depending on EU length and habitat complexity. During removal, we retained fish in holding tanks and monitored them for signs of handling stress. We then restocked fish at predetermined densities and released remaining individuals outside the experiment. Stocking densities are as follows: *N. leptocephalus* (-) = 0/unit and (+) = 16/unit; *C. oreas* (-) = 0/unit and (+) = 30/unit; predator (-) = 10/unit with only *C. oreas* or *N. leptocephalus* and (-) = 20/unit with both *C. oreas* and *N. leptocephalus*; predator (+) = 30/unit with only *C. oreas* or *N. leptocephalus* and 60/unit with both *N. leptocephalus* and *C. oreas*. There was no stocking in the (-, - , -) treatment, and he remaining treatment had 60 egg-predators. Adjustment to *C. oreas* and *N. leptocephalus* abundance is made to keep predation pressure constant across treatments.

We conducted spawning observations twice daily. Two workers wearing polarized sunglasses, one on each side of the stream, walked the length of the experiment and located fish to record whether or not they were spawning. Spawning of *N. leptocephalus* was evidenced by the presence of a conspicuous gravel mound in the experimental unit. Spawning of *C. oreas* was evidenced by multiple individuals in breeding color congregated in swift water and schooling vigorously. Because spawning by both species is conspicuous and can last for days, we are confident that no spawning events went undetected. If spawning was occurring, we video recorded (if water clarity permitted) or observed activities for at least half an hour each time to identify behavioral interactions.

At the onset of spawning, we attached 2-mm mesh vinyl screen to enclosures, leaving a 30-cm gap at the most downstream end of each fence (the point of the “V”, not shown in Figure 1.3b). At the gaps, we fastened 250-micron ichthyoplankton nets to capture drifting larval fishes from each unit (Figure 1.3b); these were collected twice daily for two weeks after the onset of spawning. We also collected larval fishes with cylindrical light traps designed specifically for sampling in shallow gravelly shoals of small streams. We set light traps at dusk and retrieved them each morning. All larval fish samples were preserved in 90% ethanol.

We identified larval fishes to species using discriminant function analysis of seven morphomeristic characters, corroborated by DNA barcoding using the mitochondrial COI locus. Details of this analysis are presented in Peoples et al. (*under review, Copeia*). We analyzed four response variables to test predictions of the BMM: (1 and 2) a binary variable representing whether or not each species spawned, and (3 and 4) the natural log-transformed counts of larval individuals of both species. We constructed mixed models to account for nested error structure by introducing a random factor of each whole plot nested within habitat type, and included second-order interaction terms (Potcner and Kowalski 2004). Variables in each model included predator density, habitat type, and symbiont presence (i.e. models predicting *C. oreas* responses included *N. leptocephalus* presence, and vice versa). We fit models using maximum likelihood estimation, and used contrasts to tease apart effects of specific factor levels and combinations within significant interaction effects. All analyses were conducted using SAS 9.3. Due to modest sample size, we drew inferences at α=0.10.

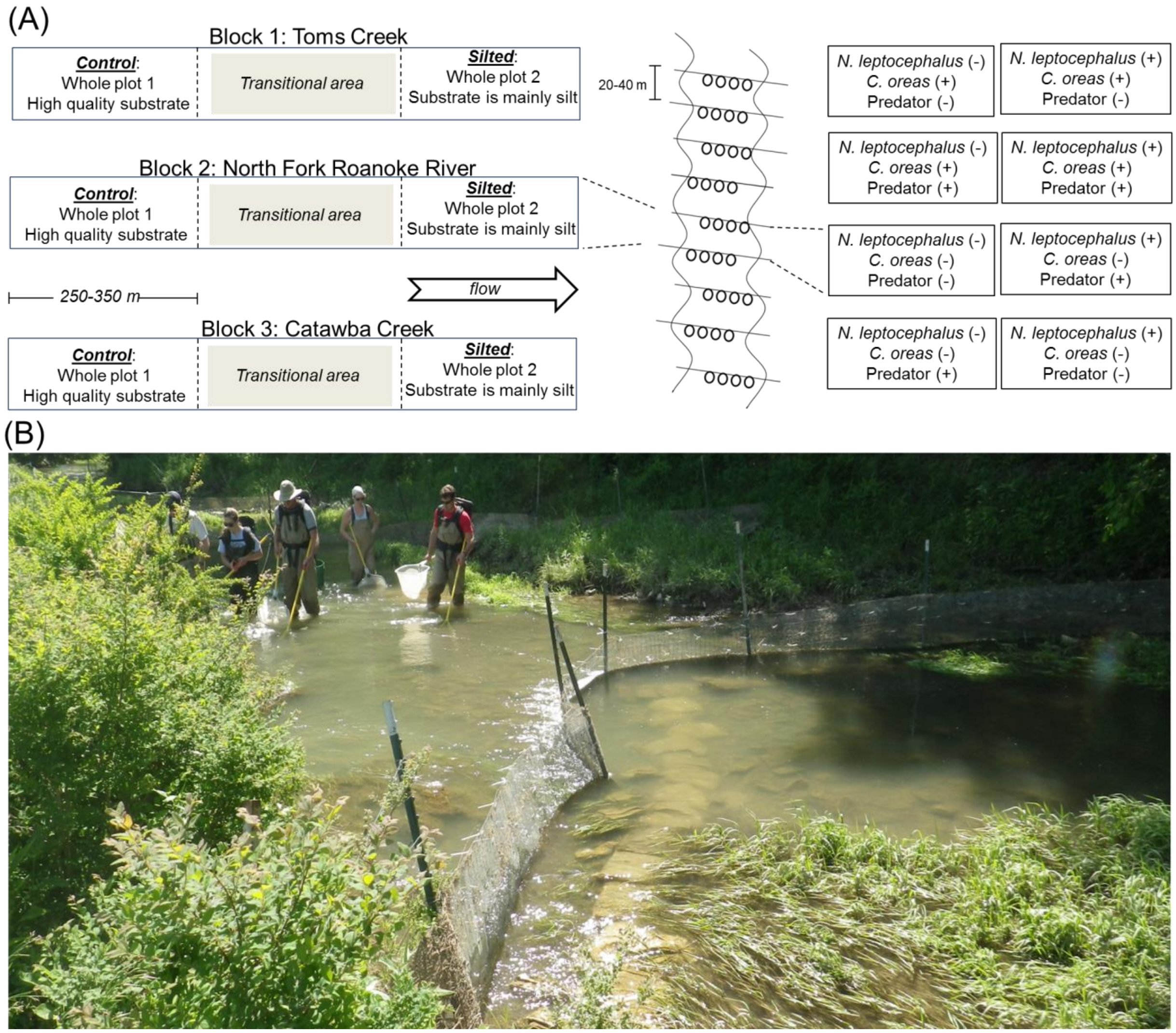

Figure 1.3. A) diagram of experimental design; B) depletion electrofishing to clear experimental units of all fishes before re-stocking.

Results

A large flood on Toms Creek near the end of reproductive activity breeched experimental units and washed away all nests. Behavioral analyses are therefore based on data from all three systems (*n*=48 EUs), but analyses of reproductive success were only possible for Catawba Creek and North Fork Roanoke (*n*=32 EUs). Results suggest that nest association behavior is obligate for *C. oreas,* which spawned only in 9 of 24 EUs in which it was present. *C. oreas* spawned only in the presence of breeding *N. leptocephalus*, and did not initiate reproduction until male *N. leptocephalus* began nest construction. *C. oreas* did not spawn in the three remaining experimental units with reproductively inactive *N. leptocephalus*. *C. oreas* in EUs without *N. leptocephalus* did not enter into intense breeding color, and were usually observed schooling sluggishly at medial depths in slow current; this is not spawning behavior. After experiments, female *C. oreas* from several EUs without *N. leptocephalus* were collected and sacrificed, and were found to be full of eggs; those in units with *N. leptocephalus* contained few to no eggs. Analyses revealed that the only factor predicting whether or not *C. oreas* spawned was the presence of *N. leptocephalus* ($F_{1,39}$=15.1, p=0.0004). Neither habitat type ($F_{1,39}$=0.56, p=0.4598) nor predator density ($F_{1,39}$=0.17, p=0.6785) predicted whether or not *C. oreas* would spawn. Conversely, *N. leptocephalus* constructed nests in 19 of 24 EUs, regardless of *C. oreas* presence. No factor predicted whether or not *N. leptocephalus* constructed nests (*p*>>0.10 for all).

The net outcome of nest association between *N. leptocephalus* and *C.* oreas was mutualistic; *N. leptocephalus* positively affected reproductive success of *C. oreas* ($F_{1,23}$=9.29, p=0.0057), and *C. oreas* moderately but also positively affected reproductive success of *N. leptocephalus* ($F_{1,23}$=2.91, p=0.1001). However, outcomes ranged from mutualistic to commensalistic, depending on abiotic and biotic context. Whereas habitat type did not directly affect reproductive success of either species in the main effects (p>0.10 for both), its effect on reproductive success of *N. leptocephalus* depended on the presence of *C. oreas* ($F_{1,39}$=4.18 p=0.0526). In the absence of *C. oreas*, *N. leptocephalus* reproductive success did not differ between habitat types ($F_{1,23}$=1.79, p=0.1923). In the presence of *C. oreas,* however, reproductive success of *N. leptocephalus* was significantly greater in forested than in silted habitats ($F_{1,23}$=4.12 p=0.0532)

(Figure 1.4). Thus, the outcome of the interaction switched from commensalistic in silted habitats to mutualistic in forested habitats. Predator density had no effect on reproductive success of either species (p>>0.10 for all direct effects and interactions).

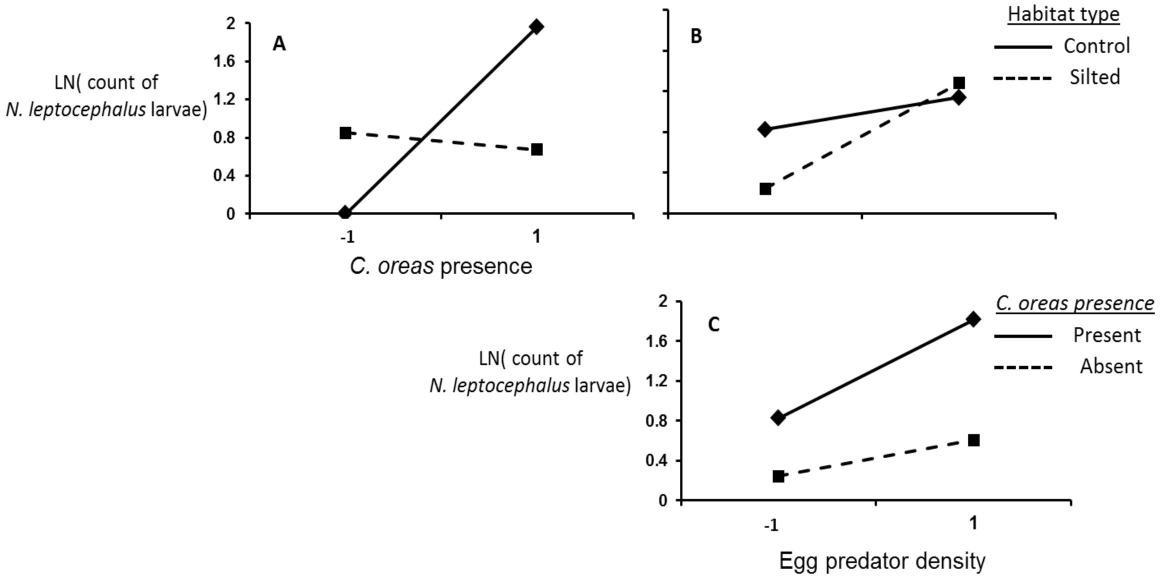

Figure 1.4. The presence of *C. oreas* interacted significantly with habitat type to predict reproductive success of *N. leptocephalus* (A). Interactions between predator density and habitat type (B), and predator density and presence of *C. oreas* (C.) were not statistically significant.

Discussion

The biological markets model predicted the behavioral dynamics and some of the fitness outcomes of nest association between *C. oreas* and *N. leptocephalus*, although not necessarily in the ways we expected. Using the BMM, we hypothesized that *C. oreas* would be more likely to spawn without *N. leptocephalus* in forested habitats, and in lower predator densities. We found, however, that nest association behavior is obligate for *C. oreas*. Schwartz and Hoeksema (1998) demonstrate mathematically that the ability of a species to maximize acquisition of a dynamically available resource (in this case, substrate availability) is determined by its flexibility to adapt to changing resource availability. If the species is inflexible, it cannot perceive changes in resource availability and must always associate. In other words, the BMM implies that the behavior of an inflexible obligate symbiont can only be contextual in whether or not its host is present *and* able/willing to trade. Additionally, trading behavior of the obligate symbiont cannot be dynamic *within* a particular state of host presence or absence. Therefore, as an obligate and inflexible associate, *C. oreas* was not capable of adjusting its reproductive behavior to meet changes in resource availability within a particular context of host presence or absence; *C. oreas* either spawned with *N. leptocephalus* or not at all. The possibility exists that *C. oreas* evolved alongside *N. leptocephalus* in a highly variable environment, thus making nest association an engrained, obligate behavior for *C. oreas.*

This study presents one of the first explicit tests of the biological markets model, and of context dependency in vertebrate interactions, to incorporate direct measures of participant fitness. As predicted, *N. leptocephalus* reproduced more successfully in forested than in silted habitats, but only in the presence of *C. oreas*. Analyses of behavioral and reproductive success data demonstrated that *C. oreas* benefitted from *N. leptocephalus*, regardless of context. The relationship was thus commensalistic in silted habitats where *C. oreas* did not improve reproductive success of *N. leptocephalus*, and only became mutualistic under improved habitat conditions. This experiment demonstrates that even at low densities (30 individuals per unit), the presence of associates can cause a mutualistic interaction, at least at the predator densities used in this study. In natural settings, *Nocomis* nests are typically swarmed with hundreds of associates representing up to six species, creating egg dilution by associates as much as 84% of all eggs on a nest (Cashner and Bart 2010). Accordingly, the low associate densities used in this study may have been unrealistic. It is likely that high associate densities can maintain a mutualistic relationship, even in heavily silted habitats. Spawning habitat amelioration via *Nocomis* nesting has been suggested as a mechanism facilitating colonization of associates in degraded stream habitats (Hitt and Roberts 2011). However, mutualism between nest association participants may also provide a positive feedback loop to perpetuate this process and expand distributions of mutualists (Afkhami et al. 2014). Future experimental work should seek to understand the effect of associate density on the outcomes of nest association.

Contrary to our hypothesis, we observed no effect of egg predator density on the reproductive success of either *N. leptocephalus* or *C. oreas*. This is surprising, given the strong effects of predation at determining the outcomes of nest associations in other systems (Baba et al. 1990, Fletcher 1993, Johnston 1994a). It is possible we chose relatively inefficient egg predators. This would explain the moderately significant effect of *C. oreas* presence on reproductive success of *N. leptocephalus* because the primary beneficial mechanism to hosts in nest association is egg dilution (Johnston 1994b). However, many egg predators of *Nocomis* nests are also associates that have already spawned or are actively spawning on another nest. This represents a case of cheating, which is ubiquitous among mutualisms (Bronstein 2009). Identifying cheaters and understanding the role they play at determining the net outcomes of nest association will be critical to future work in this system.

This study demonstrates that freshwater fishes can provide a tractable and informative system for testing general ecological models, and that ecologists can gain valuable new insight about their study systems through intertaxonomic thinking. We intend this study to stimulate discussion on the depth of applicability of general models, and to identify novel and creative applications of models to study systems much different than the ones for which they were originally intended. For instance, some results contradict our hypotheses, but corroborate the biological markets model in a way we did not initially consider. Our findings do not represent a misinterpretation of the BMM, but instead illustrate the lack of a general understanding about interspecific vertebrate symbioses. Very few studies have sought to empirically test the degree to which reproductive symbioses are facultative in fishes (Wallin 1992, Mattingly and Black 2013). A better understanding of the basic reproductive ecology of fishes is necessary for future theoretical research in this system of interactions; experiments with truly facultative associates (for example, *Rhinichthys atratulus*) will yield better insights into the ability of the BMM to predict behavioral context dependency in reproductive mutualisms among fishes. Pendleton *et al*. (2012) presented a gradient of obligation among potential associates in the study area. This gradient identifies species generally as "strong" (nearly obligate) and weak (mostly facultative) nest associates of *Nocomis*. This classification scheme could provide candidate alternate species for future studies improving on the design of the current experiment.

Another valuable insight from the study was that the simple presence of *N. leptocephalus* was not sufficient to induce spawning in *C. oreas*; a nest-building male was required. This demonstrates that the hosts exert partner control over associates by dictating timing of reproduction. Because nest associates of *Nocomis* are capable of utilizing multiple hosts (*Campostoma* and *Semotilus* spp.), it is possible that partner control/choice by all participants operates to stabilize the mutualism through time (Kaltenpoth et al. 2014). However, the actual cues that trigger spawning initiation are unknown. Authors have suggested that associates are attracted to nests based on the presence of adequate hosts (Shao 1997b), chemical cues (Hunter and Hasler 1965, Rakes et al. 1999), clean substrate (Cooper 1980, Vives 1990), the sound of nest construction (Steele 1978), or a combination of factors (Miller 1964, Wallin 1992). Outcomes of nest association can also vary as a result of the behavior of tertiary species. For example, Baba et al. (1990) found that nest association between *Coreoperca kawamebari* and its associate, *Puntungia herzi* became parasitic because a third species (*Zacco temminicki*) which superficially looked like *P. herzi* entered the nest unchallenged but preyed on eggs of both host and associate. Testing the BMM in this system from a community perspective is thus a logical next step that will yield better insight into how mutualisms structure communities at higher levels of taxonomic organization.

Chapter 2: Lotic cyprinid communities can be structured as nest webs and predicted by the stress-gradient hypothesis

by

Brandon K. Peoples[1], Lori A. Blanc[2], and Emmanuel A. Frimpong[1]*

[1]Virginia Polytechnic Institute and State University, Department of Fish and Wildlife Conservation. 100 Cheatham Hall, Blacksburg, VA, USA 24061

[2]Virginia Polytechnic Institute and State University, Department of Biological Sciences. 4082 Derring Hall, Blacksburg, VA, USA24061

*Corresponding author: frimp@vt.edu

Phone: 1-540-281-6880

Running title: *Facilitation in vertebrate communities*

Keywords*: ecosystem engineer, facilitation, fish, nest association, nest web, stream, Nocomis, stress-gradient hypothesis, structural equation modeling, vertebrate community*

Abstract.-Little is known about how positive biotic interactions structure animal communities. Nest association is a common reproductive facilitation in which associate species spawn in nests constructed by host species. Nest associative behavior is nearly obligate for some species, but facultative for others; this can complicate interaction network topology. Nest web diagrams can be used to depict interactions in nesting-structured communities and generate predictions about those interactions, but have thus far only been applied to cavity-nesting vertebrate communities. Likewise, the stress-gradient hypothesis (SGH) predicts that competition is an important interaction in benign habitats, but that facilitation increases in importance as habitats become more stressful; this model has been hardly applied to animal communities. The nest web framework and the SGH were applied to nest associative fish communities, and extended in novel ways to broaden their applicability. A nest web was constructed using spawning observations over three years in several streams in southwestern Virginia, USA. Structural equation modeling (SEM) was then implemented through an information-theoretic framework to identify the most plausible nest web topology in stream fish communities at 45 sites in the New River Basin of the central Appalachian Mountains, USA. To test the SGH, the per-nest reproductive success of "strong" (nearly obligate) nest associates was used to represent interaction importance. To quantify community structure, eigenvectors were extracted from a principal coordinate analysis (PCoA) of proportional abundances of members of the family Cyprinidae (the only species that participate in nest association in this system). Both of these metrics were regressed on physical stress, a combination of catchment-scale agricultural land use and stream size (representing spatiotemporal habitat variability). Seventy-one percent of SEM model evidence supported a parsimonious interaction topology in which strong associates rely on a single host (*Nocomis*), but not other species. PCoA identified a gradient of community

structure dominated by *Nocomis* and associates, to communities dominated by other reproductive groups. Both metrics of interaction importance responded positively to physical stress. This study demonstrates that nest webs can be useful in a variety of systems, and that SEM can be a quantitative extension of this framework. Likewise, the SGH can be used to understand positive interactions in animal communities, and can be extended to predict proportional representation of facilitating and beneficiary species in communities.

Introduction

Positive biotic interactions such as facilitation and mutualism are common in nature. Yet despite their ubiquity, a framework for integrating the roles of positive interactions with other important ecological processes is precluded by a lack of empirical studies on a broad suite of ecosystems and taxa (He and Bertness 2014). For example, it is common knowledge that facilitation has strong effects on plant community structure (Callaway et al. 2002), but very little research has considered the role of positive interactions in vertebrate communities (Barrio et al. 2013, Kamilar and Beaudrot 2013). The paucity of facilitation research is also particularly notable among freshwater animals (Holomuzki et al. 2010, Fugére et al. 2012). Clearly, freshwater fishes provide an excellent system for understanding how positive interactions affect vertebrate communities, and for testing general ecological models in understudied systems.

One conspicuous positive interaction among stream fishes is nest association, a breeding behavior in which individuals of one species (nest associates) spawn on nests built by host species (Johnston and Page 1992). Nest association occurs among many taxa in a diversity of aquatic habitats throughout the world (Johnston and Page 1992, Wong and Balshine 2011, Yamana et al. 2013). In North America, probably the most common nest association occurs between *Nocomis* (Cyprinidae) hosts and a number of cyprinid associates (over 30 species). Nesting male *Nocomis* select a very narrow range of gravel size and current velocity (Wisenden et al. 2009), creating hydrodynamic mounds of uniform, unsilted gravel. Nest associates, which are lithophilic spawners (requiring clean gravel substrate, Balon 1975), frequently rely on *Nocomis* nests for reproduction. In habitats where gravel is scarce or heavily embedded with silt, *Nocomis* nesting behavior facilitates nest associate reproduction by providing concentrated, unsilted gravel (Vives 1990) and parental care of broods (Johnston 1994a) that would have otherwise been unavailable to associates. High associate: host egg ratios often create a dilution effect for host broods, which reduces probability of predation on host eggs. The tradeoff between substrate provision/parental care by hosts and egg dilution by associates causes most cases of nest association to be mutualistic (Johnston 1994b).

Interaction topologies in fish communities structured largely by facilitator species can be complicated by certain aspects of their reproductive behavior. Firstly, many nest associates switch to using nests of other hosts (e.g. *Semotilus*, *Campostoma* or *Exoglossum*) when *Nocomis* are scarce, although these nests may not be as well guarded or silt-free as *Nocomis* nests (Pendleton et al. 2012). Secondly, other nest builders may occasionally function as nest associates of *Nocomis*, or as egg predators on *Nocomis* nests (Jenkins and Burkhead 1994). Finally, plasticity in reproductive traits such as the dual spawning modes exhibited by many North American cyprinids (Johnston and Page 1992) can make it difficult to quantify proportions of associate nesting effort associated with host nests versus simple broadcast spawning. Nest associative behavior for “strong” associate species may be obligate (or nearly so; Wallin 1992, Mattingly and Black 2013). However, other “weak” associates can opportunistically revert to the ancestral spawning mode of simple broadcast spawning.

One useful way to understand the complex interaction structure of nesting-based communities is through a nest web diagram. Analogous to food webs, nest webs depict a hierarchy of direct and indirect interactions among species requiring or providing a common nesting resource (Martin and Eadie 1999). Nest webs are useful for tracing pathways of resource flow, identifying keystone relationships, and making predictions about the consequences of altering key interactions. The nest web framework has provided insight for complex interactions among cavity nesting bird species throughout the world (Martin et al. 2004, Blanc and Walters

2008a, Blanc and Martin 2012, Cockle et al. 2012, Orchan et al. 2012, Cockle and Martin 2015), but to date has not been used for any other taxa. The first goal of this study was to explore the potential of the nest web framework as a broadly applicable tool for understanding animal communities structured by facilitating species. Accordingly, the first objective was to use a limited sample of reproductive interaction data from a few streams to construct a nest web for lithophilic cyprinid communities in tributaries to the New River of the central Appalachian Mountains, USA. The second objective was to then apply structural equation modeling, implemented through an information theoretic framework, to test whether key interactions depicted in the nest web were generalizable across the basin. This new application of the nest web framework is intended to (1) generalize its use as a tool for understanding animal communities structured by facilitating species, and (2) to move beyond some of its traditional limitations as a simple visual depiction (Blanc and Walters 2007) and thus extend the framework as a quantitative tool for comparing complex ecological hypotheses.

The second goal of this study was to identify the abiotic contexts in which nest-associative facilitation would be an important driver of stream fish communities, using the stress-gradient hypothesis to guide predictions (SGH; Bertness and Callaway 1994, Maestre et al. 2009). The SGH predicts that the frequency and/or importance of positive interactions should be greatest in highly stressful situations (high predation or physical stress), and that positive interactions should segue to competition as habitats become more benign. Since its development, a large body of research has demonstrated that the SGH can predict the relative roles of facilitative and competitive interactions in a variety of ecosystems. However, this model has been tested mainly in plant communities, and has scarcely been considered in freshwater systems (Fugére et al. 2012) or vertebrate communities (Barrio et al. 2013). Testing this model in vertebrate communities can bring new insight into the role of positive interactions among animals, as well as identify strengths and weaknesses of the model itself.

Applied to nest-associative lithophilic cyprinid communities, the SGH predicts that the frequency and/or importance of nest association should increase with physical stress, defined as any physical variable that may limit reproductive success of focal taxa (Maestre et al. 2009). In streams, anthropogenic land use (catchment-scale agricultural and residential development) is a ubiquitous stressor that elevates fine sediment loads, creating unfavorable reproductive and rearing conditions for lithophilic cyprinids (Berkman and Rabeni 1987). Spatiotemporal habitat variability also represents physical stress; rapid and large changes in instream conditions can stress eggs and larvae of lithophilic minnows. This variable can be approximated by catchment area (a measure of stream size) because headwater habitats are considerably less stable than downstream habitats (Schlosser 1987). This study used a stress gradient ranging from small, degraded streams as most stressful, to large forested streams as least stressful, employing a fitness-based, demographic metric of interaction importance (*sensu* Malkinson & Tiebörger 2010) that approximates reproductive success of beneficiary species (nest associates) in terms of the ameliorating activity of the facilitating species (“juveniles-per-nest”, see Methods). Finally, this study examined whether the SGH could be extended beyond predicting interaction frequency and importance to include predicting proportional representation of facilitating/beneficiary species in community structure. Specifically, it was hypothesized that (1) juveniles-per-nest of nest associates, and (2) the proportional representation of *Nocomis* and associates would increase along a gradient of physical stress. Likewise, juveniles-per-nest of other reproductive groups should decrease or have no relationship with physical stress.

Methods

To construct the nest web, opportunistic nest searches were conducted on several 2nd to 3rd-order streams throughout Montgomery and Roanoke counties, Virginia, USA during the breeding seasons (mid-April to early June) of 2012 through 2014. Cyprinid nests were surveyed by two workers, walking on either side of the stream and wearing polarized sunglasses to reduce surface glare. Species identities of nest building cyprinids are easily discernable: *Semotilus* nests resemble slightly-raised ridges, and *Campostoma* nests are simply excavated pits. *Nocomis* and *Exoglossum* nests are both raised gravel mounds, but *Nocomis* nests are constructed of larger gravel sizes, and in different habitat types (pool tails and runs vs. pool margins) than *Exoglossum* (Jenkins and Burkhead 1994). When an active nest was located, workers watched each nest for at least 30 minutes and noted all species observed on the nest. While some cyprinids are generally difficult to distinguish, those encountered in this study (Table 2.1) differ enough to allow visual identification without handling of specimens, especially when in breeding colors. To minimize the chance a species went overlooked, most nests were video recorded and all were revisited multiple times. This method was more useful than snorkeling, which tended to disturb more skittish species.

To quantify measures of interaction importance across a larger spatial extent, stream fish communities were surveyed in the New River basin of North Carolina, Virginia and West Virginia, USA. The New River is an ideal system for studying nest associative interactions because of its relatively high cyprinid richness (29 species) and representation of cyprinid reproductive groups; these include 6 nest-building species (2 of which were *Nocomis*), 8 strong associates, 4 weak associates and 11 non-associates (Jenkins and Burkhead 1994). The New River also contains the range of land use and associated instream conditions necessary for assessing a gradient of abiotic conditions. Forty-five sites (2^{nd} to 5^{th} order), representing gradients of catchment-scale agricultural land use (<1% to 46%), were sampled in July and August of 2012. Sites consisted of two spatially-replicated reaches of 80 to 140 m in length (depending on stream size) within the same interconfluence stream segment, which was the actual sampling unit (Benda et al. 2004). Reaches were separated by at least one channel geomorphic sequence, and were bounded by natural barriers (riffles or cascades) to prevent fish escapement during sampling. Data from both reaches were combined to represent a site.

Table 2.1. Reproductive groups of cyprinids encountered in the New River basin of NC, VA and WV, USA in summer 2012. Association strength was determined from Pendleton et al. 2012.

Species	Reproductive group
Nocomis platyrynchus	Mound nest builder
Nocomis leptocephalus	Mound nest builder
Cyprinella galactura	Non associate
Notropis photogenis	Non associate
Notropis scabriceps	Non associate
Notropis telescopus	Non associate
Phenacobius teretulus	Non associate
Pimephales notatus	Non associate
Campostoma anomalum	Secondary nest builder
Exoglossum laurae	Secondary nest builder
Exoglossum maxillingua	Secondary nest builder
Semotilus atromaculatus	Secondary nest builder
Chrosomus oreas	Strong nest associate
Clinostomus funduloides	Strong nest associate
Luxilus albeolus	Strong nest associate
Luxilus cerasinus	Strong nest associate
Luxilus coccogenis	Strong nest associate
Lythrurus ardens	Strong nest associate
Notropis rubricroceus	Strong nest associate
Notropis rubellus	Weak nest associate
Notropis volucellus	Weak nest associate
Rhinichthys atratulus	Weak nest associate
Rhinichthys cataractae	Weak nest associate

Instream habitat (substrate types and embeddedness) and *Nocomis* nest abundance were quantified along 10 transects per reach according to Barbour et al. (1999) and Peoples, Tainer &Frimpong (2011). Fishes were sampled from mid-July to mid-August 2012 using single-pass, double-backpack electrofishing. Electrofishing effort was relatively intensive, averaging 3838±209 seconds per site. All individuals were identified to species and measured to the nearest mm (total length). For each site, a metric of unsilted gravel availability was calculated by multiplying the log-transformed proportion of gravel substrate by the average thalweg embeddedness. This metric represents the availability of quality (unsilted) spawning substrate in habitat types (riffles) in which simple broadcast lithophils would spawn if they were not spawning on the nest of a host. *Nocomis* nests were not included in this calculation.

To estimate reproductive success, species were classified into reproductive groups (nest-builders, strong associates, weak associates and non-associates) based on Pendleton et al. (2012), Peoples and Frimpong (2013), and Jenkins and Burkhead (1994, and references therein). Species encountered in this study are listed in Table 2.1. Individuals were classified as either juveniles (reproductively immature young-of-the-year) or adults by visually inspecting site-specific length-frequency histograms for modal breaks. This method is effective because length-frequency histograms of short-lived temperate fishes such as cyprinids show distinct modal breaks. This method typically agrees well with age estimation based on hard structures (Reid et al. 2008, Peoples et al. 2011). The purpose of the binary age classification was not to quantify actual age structure, but to partition demography into indices of reproductive success (juvenile abundance) and a major cause of reproductive output (adult abundance). A metric of nest-capable adult male *Nocomis* abundance was calculated by partitioning out individuals over 120 mm. This is not an exact estimate of the abundance of nest-capable males (females can reach lengths >120 mm), but all reasonable cutoff values for approximating this metric were correlated with nest abundance.

A nest web was constructed by calculating the percentages of nests of each host on which associate species was observed (Martin and Eadie 1999). This nest web was then used as a guide for developing models representing competing hypotheses about interaction network topologies throughout the New River basin (Table 2.2). Observed interactions at nests may not translate directly to the importance of nest associative spawning in this system; some may represent predation instead of spawning and others may spawn on nests but relatively infrequently compared to broadcast spawning, etc. Accordingly, juvenile abundance (catch-per-unit-effort, CPUE) of each reproductive group was estimated. This variable represents the *outcomes* of nest-associative and open broadcast spawning and can be used to infer community interactions when direct measurements of nest use at multiple locations are not possible across a large spatial extent. This approach represents a conceptual and analytical advancement of the nest web framework.

All models contained a “base” structure that remained constant among models. The base model was constructed based upon the nest web results, prior evidence of relationships and logical intuition (e.g. the positive relationship between adult and juvenile abundance of a reproductive group). For example, models predicting nest abundance must contain large adult male abundance and gravel availability (Peoples et al. 2011). Because they reproduce almost exclusively on *Nocomis* nests, models predicting juvenile *Nocomis* and strong associate abundance must always contain nest abundance. To represent the dilution effect, models predicting juvenile *Nocomis* abundance must always contain strong associate juvenile abundance (Peoples and Frimpong 2013). Because they are mostly lithophilic spawners, models predicting abundances of juvenile weak- and non-associates and other (non-*Nocomis*) nest-builders must always contain gravel availability. Finally, models predicting abundances of juveniles of any group must contain adult abundances of that group. The base model represents a stand-alone hypothesis of interactions within a nest web in which *Nocomis* and strong associates participate in nest association and other groups do not.

Competing models were constructed by adding variables to the base model. Competing models contained combinations of four observed relationships that must happen often enough to drive cyprinid community structure and thus be evident in the interaction network topology: 1) strong associates spawn on non-*Nocomis* nests; 2) weak associates spawn on non-*Nocomis* nests; 3) weak associates spawn on *Nocomis* nests; and 4) other nest-builders spawn on *Nocomis* nests, as well as their own. For models in which a group is hypothesized to spawn on a nest, logical structure requires juvenile abundance of that group to be included in the model predicting the juvenile abundance of the host group, representing the dilution effect. Because non-*Nocomis* nests are inconspicuous and difficult to detect outside of the spawning season (especially nests of *C. anomalum*, by far the most abundant nest-builder in the study system other than *Nocomis*), abundance of adult non-*Nocomis* nest-builders were used as a proxy of their nesting activity. Varying the presence of each relationship in the nest web resulted in 16 parameterized models. Including a null (intercept-only) model, 17 models were compared (Table 2.2).

Models were fit using structural equation modeling (SEM). Before running SEMs, CPUE of juveniles and adults of each reproductive group was calculated by dividing counts of each group by electrofishing time (s). Each CPUE variable was then subjected to the arcsine square root transformation, and scaled and centered to mean=0 and variance=1. Nest abundance was log(x+1) transformed nest abundance. SEM models were estimated using diagonally weighted least squares regression. Relative support for competing models was evaluated based on Akaike's information criterion, adjusted for sample size (AICc); AICc was calculated from the global chi-square of each SEM. Model weights (w_i for each i^{th} model) were calculated to compare evidence ratios and weights-of-evidence for competing models. Model weights range from 0 to 1 and sum to 1 for all competing models. The best-supported model has the highest model weight, and models within two AICc units ($\Delta AICc \leq 2$) were considered equally plausible. One potential shortcoming of an information theoretic framework is that although a model may clearly out-compete others, it still may not approximate the data well, which is why competing models were also compared to an intercept-only (null) model, which was included in the competing model set. SEM models were fit using the *lavaan* package version 0.5-16 (Rosseel 2012) in R version 3.0.1.

To calculate a metric of physical stress for testing the SGH, catchment area and catchment-scale percentages of agriculture were placed in comparable terms by scaling each variable to range from 1 to 10. Site-specific values for each variable were then placed into one of ten equidistant bins. The two scaled variables were then summed to form a composite measure of stress, ranging from 2 to 20 in increments of 1. Based on this scheme, large streams with mostly-forested catchments were defined as least stressful, and small degraded streams were classified as most stressful. The purpose of defining physical stress in this way is demonstrative. By combining to measures of physical stress that are ubiquitous to streams, this approach allows for findings to be interpreted in terms of well-accepted conceptual frameworks (e.g. Schlosser 1987), and avoids making overly-specific conclusions that are not generalizable beyond the present taxa and system.

Juveniles-per-nest, a measure of nest associative interaction importance, was calculated by dividing juvenile abundance of reproductive groups by nest abundance (juveniles-per-nest). This metric represents the per-nest contribution of *Nocomis* nesting activity (habitat amelioration) to reproductive success of each reproductive group. The second SGH response variable, cyprinid community structure, is based on the assumption that if physical stress remains constant over several generations, differential reproductive success of facilitating and beneficiary species should proliferate temporally and be evident in the overall community structure (Bertness and Yeh 1994). This represents a potential extension of the SGH, which has been used to predict the contextual roles of interactions in communities, but not changes in community composition. Cyprinid community structure was represented as eigenvectors from a principal coordinates analysis (PCoA) of arcsine-square-root transformed proportions of cyprinid species based on a Bray-Curtis distance matrix using the *vegan* package (version 2.0-10) in R. The resulting Scree plot was inspected to determine appropriate dimensionality in the PCoA solution. Correlation coefficients between eigenvectors and species relative abundances were used to interpret eigenvectors. One axis representing the contrast between sites dominated by *Nocomis* and associates was used to represent interaction importance. To test the predictions of the SGH, juveniles-per-nest of strong associates and the community eigenvector were regressed on the physical stress metric.

Results

Nest web (both observational and SEM) results suggested an interaction network in which (a) strong associates spawn on *Nocomis* nests but may occasionally rely on other hosts, (b) weak associates infrequently rely on nests, and (c) non-associates never use nests for spawning (Figures 2.1 and 2.2, Table 2.2). Observations included 36 nests constructed by four nest-building species (*Nocomis leptocephalus*, *Campostoma anomalum*, *Semotilus atromaculatus* and *Exoglossum maxillingua*). Nest associates were observed on between one (*R. cataractae*) and 23 (*C. oreas*) individual nests. These nests were used by five strong associate species and two weak associate species. *C. anomalum*, a nest-building species that is known to occasionally use *Nocomis* nests for spawning, was observed at every *N. leptocephalus* nest. However, most *C. anomalum* behavior appeared to be predatory because most individuals detected were males that had spawned in prior weeks, as determined by the extent of tuberculation; few *C. anomalum* females were observed in the vicinity of *Nocomis* nests (hence the dashed line between *C. anomalum* and *N. leptocephalus* in Figure 2.1). For *S. atromaculatus* and *E. maxillingua*, only the nest builders and associated females were observed at nests (Figure 2.1). Every *Nocomis* nest was occupied by at least one strong associate species, while only a few *C. anomalum* nests attracted associates. Gravel use was unmeasured, and is implicit in Figure 2.1.

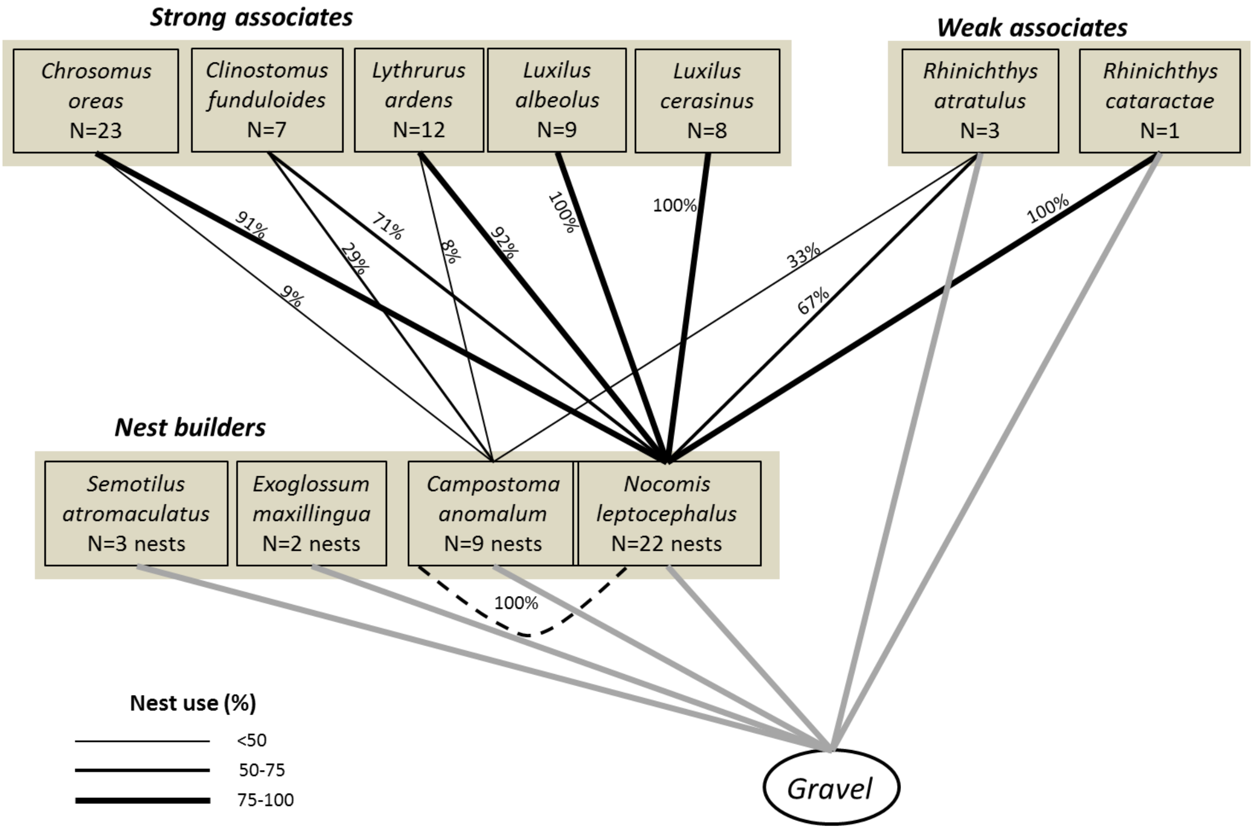

Figure 2.1. A nest web diagram depicting use of nests constructed by *Nocomis leptocephalus* and three other nest building fishes in southwestern Virginia, USA in the breeding seasons of 2012-2014. The dashed line between *Campostoma anomalum* and *N. leptocephalus* represents probable egg predation by *C. anomalum*.

SEM results determined that the most plausible interaction topology (as identified by the nest web) is parsimonious (Figure 2.2). Three models carried 86% of the weight-of-evidence; the remaining 14% of weight-of-evidence was spread diffusely over the remaining 14 models. The best-supported model (model 16, *w*=0.71) represented the hypotheses that strong and weak associates seldom rely on nest-builders other than *Nocomis* for reproduction, and that the reproductive success of weak associates and other nest-building species seldom depends on *Nocomis* nesting. The best model was 8 times better supported than the second-best model (model 12, *w*=0.09, ΔAICc=4.3), which differs from the best model only in specifying that weak associates spawn on *Nocomis* nests. The best model was 12 times more likely than the third best model (model 14, *w*=0.06, ΔAICc=4.9), which differed from the best model in specifying that weak associates are frequent users of nests built by species other than *Nocomis*. The best model was >490 million times more plausible than the intercept-only model (model 17, *w*<0.001, ΔAICc=40.0) (Table 2.2). Most parameter estimates in the best model were positive (Figure 2.2).

Table 2.2. Variables contained in (1= present, 0=absent) and fit statistics of seventeen competing structural equation models predicting interaction network topologies of gravel nesting stream fish communities in the New River basin, Virginia, USA.

	Hypotheses represented				Model attributes				
Model	Strong associates non-*Nocomis* nests	Weak associates use non-*Nocomis* nests	Weak associates use *Nocomis* nests	Other nesters use *Nocomis* nests	No. of parameters	AICc	ΔAICc	w_i	Evidence ratio
1	1	1	1	1	21	50.8	14.9	<0.01	1744
2	0	1	1	1	19	49.8	14.0	<0.01	1081
3	1	0	1	1	19	45.5	9.6	0.01	122
4	0	0	1	1	17	43.7	7.8	0.01	50
5	1	1	0	1	19	46.5	10.6	<0.01	204
6	0	1	0	1	17	44.7	8.8	0.01	83
7	1	0	0	1	17	46.5	10.6	<0.01	201
8	0	0	0	1	15	44.5	8.6	0.01	74
9	1	1	1	0	20	50.1	14.3	<0.01	1263
10	0	1	1	0	18	47.4	11.5	<0.01	319
11	1	0	1	0	17	41.7	5.9	0.04	19
12	0	0	1	0	15	40.1	4.3	0.09	8
13	1	1	0	0	17	42.2	6.3	0.03	24
14	0	1	0	0	15	40.7	4.9	0.06	12
15	1	0	0	0	15	42.8	7.0	0.02	33
16	0	0	0	0	13	35.8	0.0	0.71	1
17		intercept only			6	75.9	40.0	<0.01	490377046

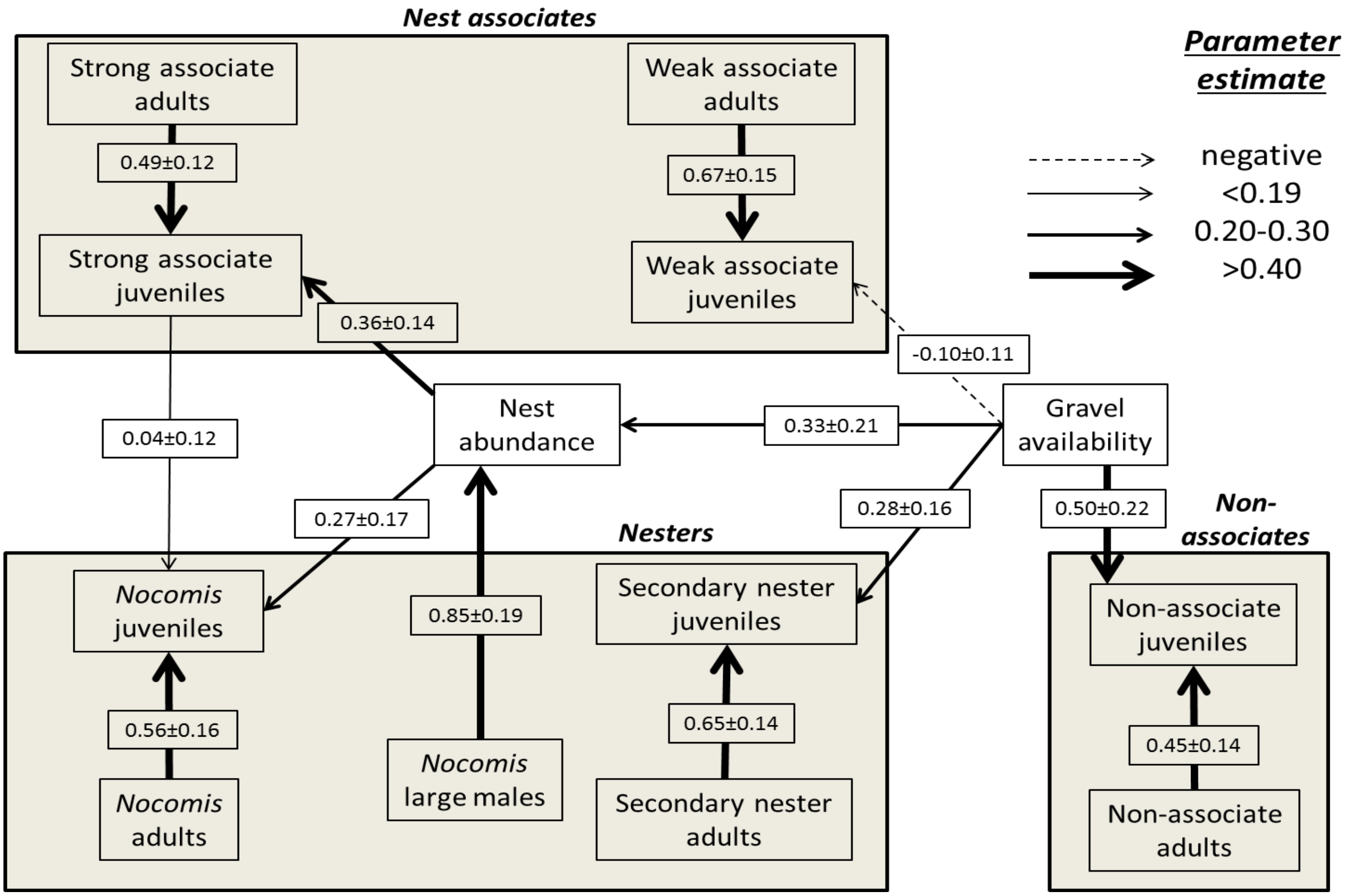

Figure 2.2. A nest web diagram depicting use of nests constructed by *Nocomis leptocephalus* and three other nest building fishes in southwestern Virginia, USA in the breeding seasons of 2012-2014. Each arrow represents a standardized regression coefficient ± standard error.

The PCoA results corroborate the nest web results, suggesting a gradient of cyprinid community structure ranging from sites dominated by *Nocomis* and a few strong associate species, to sites dominated by a suite of other nest-builders and weak- and non-associates (Figure 2.3). PCoA eigenvectors 1 and 2 described 32% and 29% (61% total), respectively, of the variation in cyprinid community structure. *N. leptocephalus* (*r*=-0.75) and the most common strong associates were negatively correlated with the first PCoA eigenvector (*Clinostomus funduloides*, *r*=-0.66; *Notropis rubricroceus*, *r*=-0.42 and *Chrosomus oreas*, *r*=-0.29). Less-abundant strong associates, which were captured diffusely throughout the study area, were weakly correlated (|*r*|<0.20) with the first PCoA eigenvector. Conversely, the most common and abundant members of other reproductive groups were positively correlated with the first PCoA eigenvector. These include a nest-builder (*C. anomalum, r*=0.64), a weak nest associate (*Rhinichthys atratulus, r*=0.41), and two non-associates (*Notropis telescopus, r*=0.32 and *Pimephales notatus*, *r*=0.28). The second PCoA eigenvector primarily was correlated with weak and non-associates associated with smaller (*R. atratulus*, *r*=-0.78 and *S. atromaculatus*, *r*=-0.47) versus larger (*Cyprinella galactura*, *r*=0.51; *N. telescopus*, *r*=0.40 and *P. notatus*, *r*=0.49) streams, although one patchily-distributed strong associate (*Luxilus albeolus*, *r*=0.53) was positively correlated with this eigenvector.

As predicted, juveniles-per-nest of strong associates increased exponentially with physical stress (*p*=0.0005, Figure 2.4a). Likewise, juveniles-per-nest of other groups showed no relationship with agriculture (*p*>0.05 for all, Figure 2.4b-d). Further, the PCoA eigenvector score decreased linearly with agricultural land use (*p*=0.0003, Figure 2.4e). Because the PCoA eigenvector was negatively correlated with *Nocomis* and several strong associates, its negative relationship with physical stress implies increasing proportional dominance of *Nocomis* and strong associates with increasing catchment-scale agricultural land use.

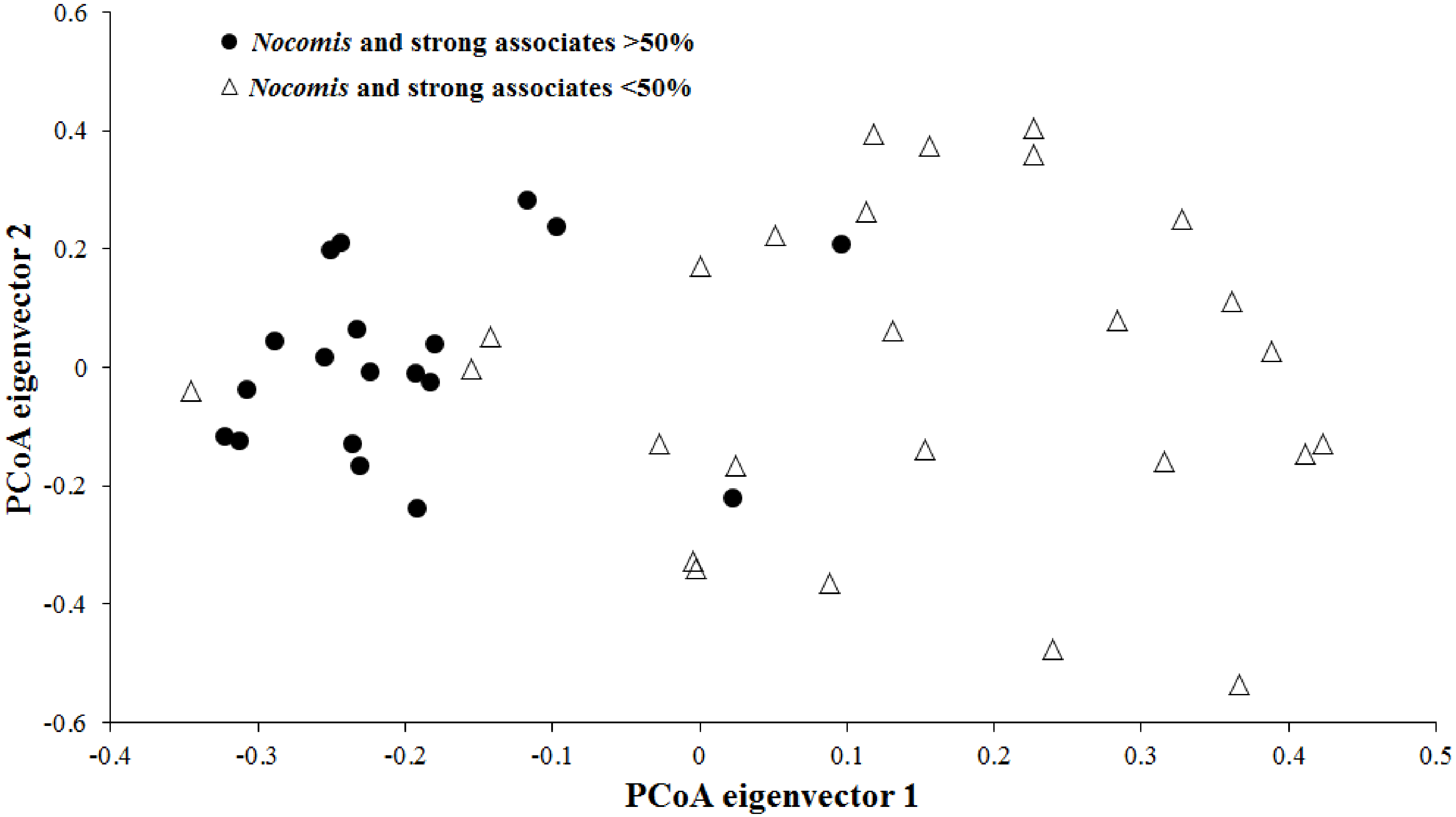

Figure 2.3. Two-dimensional solution of a principal coordinates analysis (PCoA) of Bray-Curtis similarities among cyprinid communities at 45 sites on tributaries to the New River, NC, VA and WV, USA. The PCoA results revealed a dichotomy between sites dominated by *Nocomis* and a few strong associates (black circles), to sites dominated by species of other reproductive groups (hollow triangles).

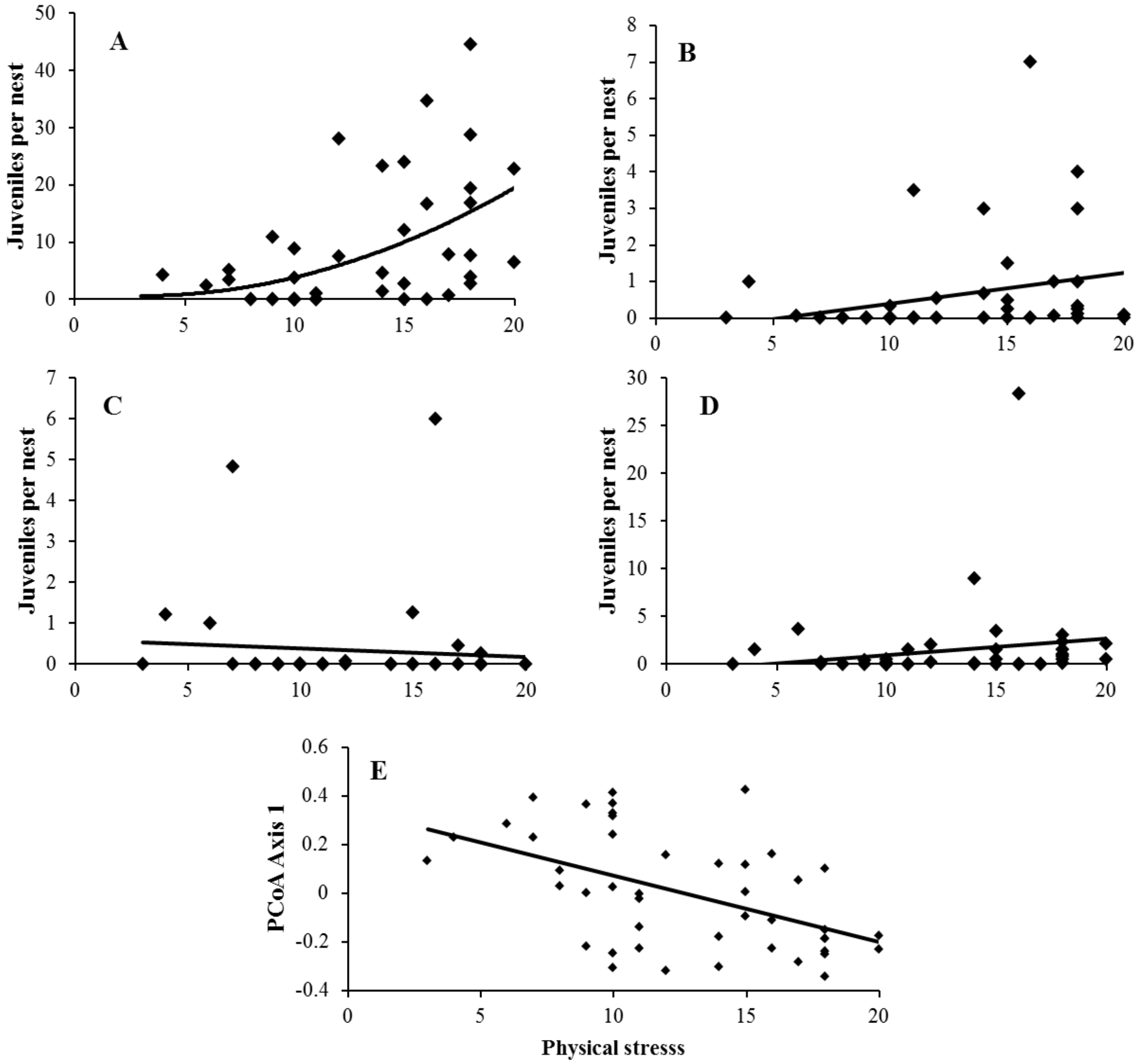

Figure 2.4. Number of juveniles-per-nest of *Nocomis* plus strong associates (A) responded predictably to physical stress, while weak associates (B), non-associates (C), and other nest-builders (D) did not. The first PCoA eigenvector responded negatively to increased physical stress (E). Decreasing eigenvector values represent increased proportional representation of *Nocomis* and strong associates in cyprinid community structure.

Discussion

The nest web framework provided valuable insight about the reproductive topology of lithophilic cyprinid communities across a large spatial extent. Evidence suggests cyprinid community structure in this system is driven largely by the nesting activity of *Nocomis*, and that the magnitude of the interaction importance depends on abiotic condition. Observational and model-based evidence suggest an interaction topology in which strong associates rely nearly exclusively on *Nocomis* nesting for reproduction, and other groups rely mostly on simple lithophily. Spawning mode plasticity of strong associates was poorly supported, but some model evidence suggested nest associative behavior and host preference is plastic for weak associates. Whereas weak associates may rely on nest association in certain habitats, their reproductive success is more dependent on ambient substrate conditions than the nesting activity of a host (Peoples and Frimpong 2013). Many beneficiaries of facilitator species are capable of utilizing multiple hosts, depending on abiotic context (Machiote et al. 2004). For instance, several studies found that habitat characteristics mediated the plasticity of host choices for secondary cavity-nesting birds, and that some species were more prone than others to using alternate hosts (Blanc and Walters 2008b, Robles and Martin 2013, Cockle and Martin 2015). Accordingly, the role of facilitating species at determining animal community structure may be affected in part by the degree to which associative behavior of beneficiary species is facultative. Abundance of associates that are more closely associated with facilitator species will be more likely to shift as habitats change to favor or inhibit facilitator species.

This study advances the nest web conceptual framework in several ways, demonstrating the potential of this framework for broader ecological application alongside more quantitative approaches (Blanc and Walters 2007). Firstly, this study demonstrates that the nest web framework can be applied to systems other than cavity nesting avian communities; we posit that this framework could be useful in any biotic community in which multiple species rely on one (or several) facilitating species. This model should be able to span taxa. Secondly, combining nest web diagrams with SEM (Blanc and Walters 2008b, the current study) to estimate standardized regression coefficients can provide a means to compare relationship strength of nest web pathways among systems, develop hypotheses about potential changes in nest webs across large geographic extents, and examine how interaction structure can change with context. Using network analysis to quantitatively model nest web diagrams (Blanc and Walters 2007, Cockle and Martin 2015) can uncover unexpected community dynamics, for example, how variation in environmental context (e.g., through perturbations) can impact the relative importance of perceived keystone species. Further, using indirect measures of interaction importance (i.e. as opposed to behavioral observations) by modeling the outcomes of nesting interactions can be a useful way of applying nest webs in systems where direct measures of interactions are difficult to measure across large spatial extents.

Nest webs can also help ecologists identify key interactions, resource states and processes that flow from unmodified nesting resources, through facilitating species, to beneficiary species to determine community structure. For example, Blanc and Martin (2012) re-designed the original nest web of a cavity nesting avian community in British Columbia by incorporating finer resolutions of resource states (i.e., decay states of trembling/quaking aspen *Populus tremuloides*) as opposed to simple species-level abundance at the nest web foundation, and found that resource availability was lower than previously believed. Similarly, quantifying gradients of gravel availability in the lithophilic cyprinid nest web could aid in predicting community-level shifts in reproductive behavior as resources become increasingly scarce, for example in urbanizing stream habitats (Peoples, Tainer & Frimpong 2011) or in sediment-starved rivers below dams (Peoples et al. 2014). Likewise, in the absence or reduced abundance of *Nocomis*, interpretation of the nest web would suggest strong associates would rely more heavily on secondary nesters. This may must occur in many localities, for example in small tributaries to the

Cumberland River, USA where *S. atromaculatus* functions as the primary fish host (Mattingly and Black 2013). Future observational studies will shed light onto alternate reproductive topologies of lotic cyprinid communities.

This study shows that at least some predictions of the SGH can be extended to animal communities structured by facilitative interactions. First, the importance of spawning habitat amelioration by *Nocomis* for the reproductive success of strong associates increased along the gradient of physical stress. Moreover, per-nest reproductive success of species that are less likely to rely on *Nocomis* showed no significant relationship with physical stress. This, coupled with the most plausible nest web, suggest that facilitation by *Nocomis* is a strong mechanism structuring these communities. Although the SGH predicts that positive interactions should be more prevalent under harsher environmental conditions, the ability of animals to participate in facilitation may be behaviorally constrained (in this case by nest association strength); that is, not all species can simply rely on facilitation as stress increases. Nest association may be an optimal spawning mode in physically stressful habitats, but using this behavior may be impossible for simple lithophilic non-associates that have not evolved the more complex spawning mode of nest association. Obligate symbionts of facilitators, in this case strong nest associates of *Nocomis*, may thus possess an evolutionary advantage at thriving in harsher habitats (Johnston 1999). This may suggest adaptation to pre-Anthropocene natural disturbance regimes (e.g. heavily siltation from landslides and glacial outflow). The system-specific role of facilitation at determining the presence and abundance of species may thus be constrained by larger-scale processes (e.g. species sorting and coevolution) establishing the biotic context in which species interact.

This study advances the SGH in two ways. Firstly, it demonstrates that the SGH can be a useful model for understanding the role of positive biotic interactions in animal communities.

Second, it shows that while the interactions themselves may be difficult to measure over a large spatial extent, the outcomes of those interactions (reproductive success of beneficiary species and overall adult community structure) can also be predicted by the SGH. This was particularly evident in the increasing proportional representation of *Nocomis* and strong associates with increasing physical stress. Similarly, Hitt and Roberts (2011) also showed that *N. leptocephalus* and its associates were much more likely to colonize streams whose catchments had undergone considerable land use change. Facilitation surely contributes to the colonization potential of these species. Future work on the SGH should seek to seek to determine whether proportional representation of facilitators and beneficiary species increase with physical stress in the same manner as interaction frequency. This could expand the utility as a more powerful predictive framework that can be applied across systems and taxa.

The utility of the SGH for understanding biological communities is becoming increasingly clear. Thus far, the SGH has been restricted to a few ecological subdisciplines because animal ecologists historically have been slower to investigate the hypothesis that intrinsic community-structuring mechanisms can include both positive and negative interactions. To date, only Barrio *et al* (2013) has examined the SGH in vertebrate communities; they found that competition was reduced in harsh environments. Like the present study, this contributes partial evidence supporting the SGH; evidence fully supporting the SGH would require demonstrating that the same interaction switches from competition to facilitative from low to high stress. However, these two studies provide preliminary steps toward a better understanding of the role of positive interactions in vertebrate communities. More work is necessary to determine if the relationship between *Nocomis* and associates becomes neutral or even negative, as may be evidenced in increasing cheating, parasitism, and competition (all of which are documented in nest association) under less stressful conditions. In this system, the period in which adult *Nocomis* and associates interact positively is fairly brief, although it defines the future recruitment success of all species involved; the spawning season lasts approximately three weeks, and pre- and post-spawn trophic interactions may be competitive. Any competitive trophic interactions could not have occurred in the absence of a prior reproductive facilitation (*sensu* Bertness and Yeh 1994). Additionally, success (or lack thereof) of positive nest associative interactions will set the context for potential density-dependent interactions in proceeding seasons.

Future work should seek to accommodate synergistic effects of different types of interactions (e.g. recruitment facilitation and associational defenses from egg predation), seasonal changes in interaction direction and magnitude (Biswas and Wagner 2014) and changes in interaction dynamics with ontogenetic shifts (Soliveres et al. 2010) . Finally, some studies have suggested that the importance of facilitation should decrease at very high stress thresholds. While we found no evidence of this pattern, it is possible that we did not survey the full gradient of physical stress. Future work should seek to evaluate the community-level effects of lotic ecosystem engineers across a larger gradient of physical stress, while avoiding nonsensical comparisons of extreme physical stress beyond the fundamental niche of the facilitator (He and Bertness 2014).

Chapter 3: Biotic interactions and habitat drive positive co-occurrence between facilitating and beneficiary stream fishes

by

Brandon K. Peoples and Emmanuel A. Frimpong*

Virginia Polytechnic Institute and State University, Department of Fish and Wildlife Conservation. 100 Cheatham Hall, Blacksburg, VA 24061

*Corresponding author: frimp@vt.edu

Phone: 540-281-6880

Keywords: Biotic interactions, co-occurrence, occupancy model, facilitation, habitat, stream, fish, Nocomis, nest association, vertebrate community

Abstract:- Emerging evidence suggests that biotic interactions can drive species distributions in conjunction with abiotic factors. However, the prevailing knowledge suggests that biotic interactions among freshwater fishes are important only at fine scales, and to date positive interactions (mutualism and facilitation) have not been considered at large spatial scales. We used two-species occupancy modeling to examine patterns of co-occurrence between a reproductively facilitating fish (*Nocomis leptocephalus*) and two of its nearly-obligate associates, *Chrosomus oreas* and *Clinostomus funduloides*. Using an information theoretic approach, we compared models representing hypotheses specifying the importance of only habitat, only biotic interactions, or combinations of both. The best model for each species combination specified the importance of biotic interactions. For both associate species, probabilities that associates would occur in the absence of their host were considerably lower than probabilities of co-occurrence. Species interaction factors were greater than 1.0, indicating positive patterns of co-occurrence between hosts and associates. Models suggested that habitat variables mediated host-associate interactions for *C. oreas* but not for *C. funduloides.* This study provides the first evidence for large-scale patterns of positive co-occurrence among stream fishes, and demonstrates the importance of abiotic context for mediating biotic interactions. Two-species occupancy modeling may be a useful tool for parsing out the relative importance of biotic interactions and habitat variables for determining species distributions, although experiments and small-scale behavioral observations will also be necessary to confirm mechanisms.

Introduction

Understanding the factors that determine species distributions is essential for predicting organismal response to environmental change. It is well known that environmental factors drive species distributions, but ecologists increasingly acknowledge that interspecific biotic interactions can also be important (Wiens 2011, Wisz et al. 2013). While most research to date has focused on the effects of negative interactions (competition, predation and parasitism) on species distributions (Aragón and Sánchez-Fernández 2013, Ettinger and HilleRisLambers 2013), emerging studies suggest that positive interactions (facilitation and mutualism) can also play an important role (Heikkinen et al. 2007, Afkhami et al. 2014).

Despite the growing body of research among a diversity of taxa, relatively few studies have examined how biotic interactions influence distributions of freshwater fishes. Of those studies, most suggest that interactions can drive fine-scale distributional patterns (e.g. Werner *et al*. 1983; Power *et al*. 1985) but at larger scales, freshwater fish co-occurrence is driven only by habitat conditions (Peres-Neto 2004, Mouillot et al. 2007, Mouchet et al. 2013). Other evidence suggests that interactions can be important, but only in certain abiotic contexts (Hoeinghaus et al. 2007, Englund et al. 2009, Hein et al. 2014). Regardless, all research to date points to a diminished role of biotic interactions for explaining freshwater fish distribution at large spatial scales.

Thus far, large-scale studies of freshwater fish distributions have focused only on negative interactions; positive interactions have not been considered. One conspicuous positive interaction among stream fishes is nest association, a reproductive behavior in which individuals of one species (associates) deposit eggs in nests constructed by a host species (Johnston and Page 1992). Possibly the most common nest association in North America occurs between *Nocomis* (Cyprinidae) hosts and over thirty cyprinid associate species. *Nocomis* facilitate associate reproduction by building large gravel mound nests for spawning. Nest-building male *Nocomis* select a narrow range of substrate sizes and water velocities (Wisenden et al. 2009), creating what are often the only sources of appropriate spawning habitat for associates, which require unsilted gravel substrate to spawn. Tending male *Nocomis* also provide parental care through egg guarding, cleaning and burying that associates would not achieve by spawning without a host (Johnston 1994a).

In this study, we examined patterns of co-occurrence between *Nocomis leptocephalus* and two nest associates, *Chrosomus oreas* and *Clinostomus funduloides*, throughout the New River basin of the central Appalachian Mountains of North Carolina, Virginia and West Virginia, USA. Nest association behavior for these two associates is nearly obligate; their reproductive success is directly dependent on the nesting activities of their host (Pendleton et al. 2012, Peoples and Frimpong 2013). Specifically, we sought to evaluate the relative effects of habitat and facilitation on the co-occurrence of hosts and associates. We used two-species occupancy modeling to achieve this goal because it accounts for incomplete detection of focal species and allows for estimation of "species interaction factors" that indicate the pairwise outcome of the biotic interaction (Mackenzie et al. 2004, Richmond et al. 2010). This approach is also well suited for comparing hypotheses about the relative influence of biotic interactions and habitat covariates at determining species distributions (Haynes et al. 2014, Yackulic et al. 2014).

Methods

We sampled habitat and fishes at 61 sites in the New River basin during the summers of 2012 through 2014. We conducted three fish sampling visits to 47 sites, and two visits to the remaining 14 sites. A site comprised two spatially-replicated reaches of 80 to 140 m in length, depending on stream size. Reaches were located within the same interconfluence stream segment, which was the actual sampling unit (Benda et al. 2004). Reaches were separated by at least one channel geomorphic sequence, and were bounded by natural barriers (riffles or cascades) to prevent fish escapement during sampling. We combined data from both reaches to represent a site. We collected fishes using single-pass, double-backpack electrofishing. After sampling, all fishes were identified to species and released.

We measured instream habitat at 10 equidistant points along 20 transects per site (10 per reach). At each point, we measured depth (cm) and identified the substrate type. We also located and measured the deepest pool depth and shallowest adjacent downstream riffle depth. We subtracted the latter from the former to calculate residual pool depth (RPD), an informative metric of summertime habitat availability. We placed substrate types into six groups based on a modified Wentworth scale (bedrock, boulder, cobble, gravel, sand/silt and organic material; see Peoples *et al.* 2011 for details) and calculated the proportions of each substrate type at each site. For each sampled segment, we derived watershed area (km^2) and baseflow index (a measure of the proportion of streamflow that comes from groundwater) from extensions of the National Hydrography Dataset Plus version 2 (NHDPlus v2). We also calculated proportions of land use types within a 30-m riparian buffer (on each side of the stream) for each segment from the 2011 National Land Cover Database (Jin et al. 2013). We then summed proportions of land cover types representing human disturbance; these included suburban/urban, agricultural and pastoral land uses.

To reduce dimensionality in habitat data, we conducted a principal coordinate analysis (PCoA) of Bray-Curtis dissimilarities using the *cmdscale* function in R version 3.0.3. We then chose eigenvectors to represent habitat based on the resulting Scree plot, and used correlations with the original habitat data to interpret eigenvectors. We also “spatialized” habitat eigenvectors prior to analysis to account for spatial autocorrelation (Brind'Amour et al. 2005). To do so, we first calculated a matrix of pairwise network (fluvial) distances among sites using the NHDPlus v2 dataset. We then subjected this matrix to a principal coordinates of neighborhood matrices (PCNM) analysis using the *PCNM* function in the *PCNM* package of R. This procedure produces significance tests for Moran's *I* on each spatial eigenvector. Using all significant (α=0.05) spatial eigenvectors with positive eigenvalues from the PCNM analysis, we then used multiple linear regression with stepwise model selection (the *stepAIC function* in the *MASS* package of R) to identify the most important spatial eigenvectors predicting each habitat variable based on the lowest value of Akaike's information criterion (AIC). Next, we re-ran regressions using only the best predictors of each habitat variable. Finally, we extracted the predicted values of these regressions; the resulting values represented both site-specific habitat conditions and spatial gradients in each habitat variable. Before running occupancy models, we scaled and centered the spatialized habitat eigenvectors to mean=0 and variance=1.

We used two-species occupancy models to examine patterns of co-occurrence between *N. leptocephalus* and each associate species, separately. We used single-season models because we had no reason to believe that significant site-specific colonization and extinction of these species was occurring between visits. We specified models with survey-specific unconditional detection probabilities (detection probabilities that do not depend upon the presence of another species) that differed between the two species, but set conditional detection probabilities (detection probabilities that depend upon the presence of another species, Mackenzie et al. 2004) of each species equal to their survey-specific unconditional detection probabilities. That is, we had no reason to believe that the presence of one species affected the detectability of another species. Decreasing the number of estimated parameters in each model allowed for improved model stability.

We fit all models in program PRESENCE (Hines 2006), using the parameterization presented by Richmond et al. (2010). Using this parameterization, models estimate three occupancy parameters: occupancy of the "dominant" species (in this case, *N. leptocephalus*, Ψ^{A}), occupancy probability of the associate species, given the host is present (Ψ^{BA}), and occupancy probability of the associate species, given the host is absent (Ψ^{Ba}). We then derived the unconditional occupancy of nest associates (Ψ^{B}) using the equation:

$$\Psi^{B} = \Psi^{A}\Psi^{BA} + (1 - \Psi^{A})\Psi^{Ba} \tag{2}$$

We also derived the species interaction factor ("SIF", Φ) using the equation:

$$\Phi = \frac{\psi^{BA}}{\psi^{A}\psi^{B}} \tag{3}$$

SIF values are centered around 1.0: Φ <1.0 indicates negative co-occurrence, $\Phi \approx 1.0$ indicates independent co-occurrence, and Φ>1.0 indicates positive co-occurrence. We calculated standard errors for Φ using the delta method (Mackenzie et al. 2006).

We compared four types of models for each pair of species. The first and simplest specifies that biotic interactions do not affect the occurrence of either species (Ψ^{A} and Ψ^{B} are independent of each other; Φ =1.0). The second model specifies that only interactions between *N. leptocephalus* and associates affect occurrence of each species (Ψ^{BA} and Ψ^{Ba} are not necessarily equal). The third model is similar to the first one, but includes habitat covariate(s) of occupancy; this model implies no biotic interaction (Φ =1.0), but instead that species occurrence is driven only by habitat. The final model is similar to the second model, but specifies that both biotic interactions and habitat variables drive species occurrence. We compared models using multimodel inference based on AIC values and Akaike model weights (w_i). Models with the lowest AIC value are considered most plausible, and models within two AIC units (ΔAIC ≤ 2)

are considered equally plausible. Model weights range from 0 to 1.0, and sum to 1.0 for a given set of multiple hypotheses; the most plausible model has the highest model weight. We interpreted models in the 90% confidence set for each species (i.e. $\Sigma w_i \geq 0.90$).

Results

The first two PCoA eigenvectors explained a cumulative 90.8% of the variation in the habitat data. The first PCoA eigenvector (hereafter, *h1*) explained 68.6% of habitat variation; this eigenvector represented increases in watershed area (r=0.95), as well as decreases in baseflow index (r=-0.55) and proportions of fine substrates (silt and sand, r=-0.54). The second PCoA eigenvector (hereafter, *h2*) explained 22.2% of variation in habitat, and represented increases in residual pool depth (r=0.81). PCNM analysis identified nine significant spatial eigenvectors with positive eigenvalues. Accordingly, for competing occupancy models with covariates, we included models containing only *h1*, only *h2*, or both. Including a null (intercept-only) model and models without habitat covariates, we compared at total of nine two-species occupancy models for each host-associate pair (Table 3.1).

Table 3.1. Co-occurrence occupancy models for *N. leptocephalus* and either *Chrosomus oreas* or *Clinostomus funduloides*. Models are ranked by difference in Akaike's information criterion (ΔAIC) from the best model and model weight (w_i). Ψ represents occupancy parameters, and p represents detection probabilities. Letters A and B represent *N. leptocephalus* and an associate species; capitalization connotes assumed presence or absence. Parameter covariates are in parentheses; h1 and h2 represent eigenvectors from a principal coordinate analysis of ten habitat variables, s represents surveys, and (.) indicates a fixed parameter.

	Chrosomus oreas				
Model	No. parameters	-2Log likelihood	AIC	ΔAIC	w_i
$\Psi^{A}(h1)\ \Psi^{BA}(h1)\ \Psi^{Ba}(h1)\ p^{A}(s)\ p^{B}(s)$	10	291.5	311.5	0	0.65
$\Psi^{A}(h1)\ \Psi^{B}(h1)\ p^{A}(s)\ p^{B}(s)$	9	294.8	312.8	1.3	0.33
$\Psi^{A}(.)\ \Psi^{BA}(.)\ \Psi^{Ba}(.)\ p^{A}(s)\ p^{B}(s)$	9	301.2	319.9	7.7	0.01
$\Psi^{A}(.)\ \Psi^{B}(.)\ p^{A}(s)\ p^{B}(s)$	8	305.8	321.8	10.3	<0.01
$\Psi^{A}(h2)\ \Psi^{BA}(h2)\ \Psi^{Ba}(h2)\ p^{A}(s)\ p^{B}(s)$	10	304	324	12.5	<0.01
$\Psi^{A}(h1\ h2)\ \Psi^{B}(h1\ h2)\ p^{A}(s)\ p^{B}(s)$	10	304.5	324.5	13.05	<0.01
$\Psi^{A}(h1\ h2)\ \Psi^{BA}(h1\ h2)\ \Psi^{Ba}(h1\ h2)\ p^{A}(s)\ p^{B}(s)$	11	302.7	324.7	13.25	<0.01
$\Psi^{A}(h2)\ \Psi^{B}(h2)\ p^{A}(s)\ p^{B}(s)$	9	307.2	325.2	13.69	<0.01
Null	0	49735.8	49735.8	49424.4	<0.01
	Clinostomus funduloides				
$\Psi^{A}(.)\ \Psi^{BA}(.)\ \Psi^{Ba}(.)\ p^{A}(s)\ p^{B}(s)$	9	263.4	281.4	0	0.99
$\Psi^{A}(.)\ \Psi^{B}(.)\ p^{A}(s)\ p^{B}(s)$	8	274.9	290.9	9.55	<0.01
$\Psi^{A}(h1)\ \Psi^{BA}(h1)\ \Psi^{Ba}(h1)\ p^{A}(s)\ p^{B}(s)$	10	291.5	311.5	30.1	<0.01
$\Psi^{A}(h1)\ \Psi^{B}(h1)\ p^{A}(s)\ p^{B}(s)$	9	294.8	312.8	31.5	<0.01
$\Psi^{A}(h2)\ \Psi^{BA}(h2)\ \Psi^{Ba}(h2)\ p^{A}(s)\ p^{B}(s)$	10	303.9	323.9	42.6	<0.01
$\Psi^{A}(h1\ h2)\ \Psi^{B}(h1\ h2)\ p^{A}(s)\ p^{B}(s)$	10	304.5	324.5	43.2	<0.01
$\Psi^{A}(h1\ h2)\ \Psi^{BA}(h1\ h2)\ \Psi^{Ba}(h1\ h2)\ p^{A}(s)\ p^{B}(s)$	11	302.7	324.7	43.4	<0.01
$\Psi^{A}(h2)\ \Psi^{B}(h2)\ p^{A}(s)\ p^{B}(s)$	9	307.2	325.2	43.8	<0.01
Null	0	47893.8	47893.8	47612.4	<0.01

The most plausible models for both nest associate species specified that biotic interactions drive co-occurrence with *N. leptocephalus* (Table 3.1). Species interaction factors from the best models for both associate species were positive (*C. oreas*: Φ =1.12±0.04, *C. funduloides*: Φ=1.15±0.06), suggesting strong positive co-occurrence. Additionally, Ψ^{Ba} was considerably lower than Ψ^{BA} for both species (*C. oreas*: Ψ^{Ba}=0.22±0.02, Ψ^{BA}=0.53±0.04; *C. funduloides*: Ψ^{Ba}=0.25±0.13, Ψ^{BA}=0.78±0.06), indicating that associate occupancy probability was greatly reduced in the absence of *N. leptocephalus*.

The relative importance of habitat and biotic interactions differed between the two associate species. For *C. funduloides*, the best model (carrying 98% of model weight) did not include any habitat covariates; this model specifies that co-occurrence between *N. leptocephalus* and *C. funduloides* is driven only by biotic interactions. For *C. oreas*, however, the best model (w_i=0.65) contained parameters for both biotic interactions and *h1*, but the second-best model (w_i=0.33, ΔAIC=1.4) specified only the importance of habitat (*h1* only, Table 3.1). Occupancy probability of *C. oreas* decreased with increases in *h1*, despite a gradual increase in occupancy of *N. leptocephalus*. Accordingly, the probability of co-occurrence (Ψ^{BA}) and thus Φ decreased sharply with *h1*, sinking below 1.0 (indicating negative co-occurrence) as *C. oreas* became more scarce (Figure 3.1). We did not average parameters between the best two models for *C. oreas* because they estimated different occupancy parameters (Ψ^{B} vs. Ψ^{BA} and Ψ^{Ba}) for *C. oreas*, and we were not interested in the occupancy of *N. leptocephalus per se*.

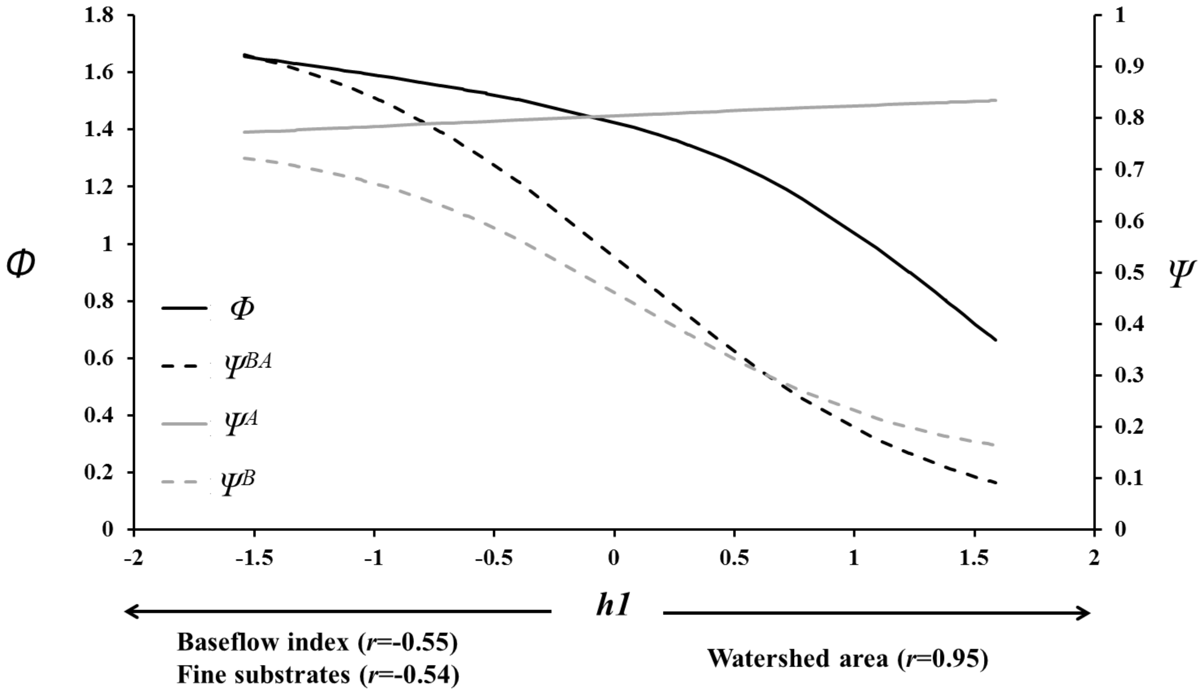

Figure 3.1. Habitat variables mediated the co-occurrence of *Chrosomus oreas* and its reproductive host, *Nocomis leptocephalus*. Although occupancy probability of *N. leptocephalus* (Ψ^{A}) increased slightly with *h1* (an eigenvector from a principal coordinate analysis of ten habitat variables that was highly positively correlated with watershed area), occupancy probability of *C. oreas* (Ψ^{B}) and thus the probability of co-occurrence (Ψ^{BA}) decreased. Accordingly, the species interaction factor (Φ) was positive at lower values of *h1*, but decreased sharply as *C. oreas* became less likely to occur.

Discussion

Few studies have inferred large-scale effects of positive biotic interactions on vertebrate distributions (Gutiérrez et al. 2005, Heikkinen et al. 2007). In this study, we demonstrate positive co-occurrence between a habitat-modifying fish and its associates, and show that the probability of associate occurrence is markedly reduced in the absence of the host. This study represents the first evidence of large-scale positive co-occurrence in freshwater fishes, although a few studies have provided some evidence of the importance of facilitation by *Nocomis* for determining distributions of nest associates. Firstly, Pendleton et al. (2012) demonstrated that geographic distributions of nearly obligate nest associates of *Nocomis* overlap considerably more with their host than do facultative associates, and that the distributions of many associates are nested entirely within that of *Nocomis*. At a finer scale, Hitt and Roberts (2011) found that streams whose watersheds had experienced punctuated agricultural disturbance in the past several between the 1960s and 2000s were much more likely to be colonized by *N. leptocephalus* and associates. Facilitative nest association has also been implicated in the expansion of an introduced nest associate throughout the Chattahoochee River basin in Georgia, USA (Walser et al. 2000). Johnston (1999) noted that cyprinids such as *Nocomis* whose reproductive mode includes nest building and/or parental care are disproportionately less imperiled than species with less complex reproductive behavior, and would thus be capable of expanding into vacant niches as other fishes become extirpated due to watershed disturbance. It is well known among numerous taxa and ecosystems that facilitator species ameliorate resources to facilitate the persistence and range expansion of associated species (Linder et al. 2012). Similarly, understanding the precise role played by *Nocomis* and other reproductive fish hosts (e.g.

Campostoma and *Semotilus*) will be useful for predicting how distributions of reproductive symbionts respond to changing environments.

Biotic interactions are often mediated by abiotic context. Emerging evidence suggests that habitat-mediated predator occurrence can affect the distribution of prey fishes at large scales. Englund et al. (2009) found that by varying with lake depth, dissolved oxygen concentration affected colonization of lakes by predators, which in turn caused extirpation of prey species. Similarly, Hein et al. (2014) showed that predation by invasive pike *Esox lucius* leads to extirpation of native brown trout *Salmo trutta* in Swedish lakes, but only in warm lakes in which *E. lucius* is more efficient at predation. However both of these studies were conducted in lentic systems. In streams, the prevailing knowledge suggests that fish co-occurrence is entirely habitat-mediated; no study has found large-scale importance of biotic interactions in these systems (Peres-Neto 2004, Mouillot et al. 2007, Mouchet et al. 2013).

Alternatively, we found that habitat conditions mediated the basin-wide co-occurrence of *N. leptocephalus* with *C. oreas*, but not with *C. funduloides*. Given the strong positive correlation of *h1* with watershed area, we interpret the relationship between *h1* and occupancy parameters to represent primarily a biotic response to a gradient of stream size and associated variables. This is intuitive, given that both species (and most *Chrosomus* species) occur commonly in smaller streams, but only *C. funduloides* is found consistently in larger streams (>4th Strahler-order, Jenkins and Burkhead 1994). Model results for *C. funduloides* do not imply that habitat variables do not influence the distribution of this species; it is well known that *C. funduloides* is a microhabitat specialist (Facey and Grossman 1992) that responds predictably to a variety of environmental stressors (Hazelton and Grossman 2009, Peoples and Frimpong 2012). Further, it is very likely that a species distribution model with only habitat covariates would identify significant predictors of *C. funduloides* distribution. Instead, results of this study demonstrate that, compared to the ten habitat variables and nine significant spatial gradients we identified, the relative importance of nest association is far greater for predicting occurrence of this species than the habitat covariates.

While the effects of negative interactions on stream fish co-occurrence remain unclear at large spatial scales, it is possible that nest association is more apparent because it is a reproductive interaction. Because obligate (or nearly so) associates require a host for reproduction, they cannot persist for multiple generations in the absence of host without dispersal from spawning habitats into habitats where the host is absent. Contrastingly, differences in habitat may mediate co-occurrence of predators and prey or competitors, giving rise to fine-scale checkerboard patterns of co-occurrence that dissolve when examined at a coarser grain and larger spatial extents (Hoeinghaus et al. 2007). More work is needed to determine how habitat mediates the interaction between reproductive hosts and facultative nest associates (Pendleton et al. 2012), or other positive interactions among stream fishes such as mixed-species schooling (see Matthews 1998, Chapter 9).

At fine spatial scales, it is well known that outcomes of biotic interactions often depend on abiotic context (Bronstein 1994a). However, the implications of these fine-scale outcomes for determining co-occurrence at large scales have been more difficult to infer. Recent applications of two-species occupancy modeling have proven this tool to be useful in that regard. For instance, Haynes et al. (2014) found that two loon species (*Gavia* spp.) competitively exclude one another on small lakes with simple shorelines, but that lake size and shoreline complexity facilitate co-occurrence. Using temporally dynamic two-species occupancy models, Yackulic et al. (2014) demonstrated that competition between two owl species (*Strix* spp.) led to elevated extinction probability among patches, which impacted the relationship between patch occupancy and habitat suitability. In this study, we show that two-species occupancy models can also be used to assess hypotheses about positive co-occurrence patterns. Inference can be made on the mechanism driving these patterns of co-occurrence because of previous observational (Peoples and Frimpong 2013) and experimental (Wallin 1992, Johnston 1994b) studies. Accordingly, we caution interpretation of species interaction factors from these models without corroborating experiments and/or behavioral observations at finer spatial scales to confidently identify the mechanism(s) that drive them.

Conclusions

Two main themes link the chapters of this dissertation: 1) using general ecological models to draw new inference for stream fish ecology, and 2) using stream fishes as a novel system to test and improve general models of ecology. These themes proceed from smaller to larger scales of taxonomic and spatial organization—from population-level inferences drawn at micro- and mesohabitat scales (Chapter 1), to community-level inference among stream segments (Chapter 2) and at the watershed scale (Chapter 3). This section provides a brief summary of those inferences.

How general ecological theory can improve inference for stream fish ecology

This dissertation demonstrates that biotic interactions can be important drivers of stream fish population and community processes, and can operate across spatial scales. This contradicts the prevailing thought, which suggests that biotic interactions are important at small scales (Power et al. 1985), but play a relatively diminished role at determining community structure (Hoeinghaus et al. 2007) and are typically not evident at larger spatial scales (Peres-Neto 2004, Mouchet et al. 2013). Firstly, I found that nest association has contextual outcomes for the population-level fitness of symbionts (Chapter 1). Secondly, I show that differences in symbiont reproductive traits can set the context for how biotic interactions affect adult community structure (Chapter 2). I also show that nearly-obligate symbiotic behavior has strong effects on large-scale patterns of co-occurrence between hosts and associates, and that these patterns are not always habitat-mediated (Chapter 3). A major conclusion of this dissertation is that accounting for biotic interactions can potentially improve inference for studies of stream fishes at several levels of biological and spatial organization.

This dissertation also demonstrates that positive interactions can be important drivers of biotic processes in streams. The ubiquity and importance of positive interactions have been well accepted among several ecological sub-disciplines, but have gained little traction among stream fish ecologists, or even vertebrate ecologists. Competition and predation currently are moderately integrated into the major conceptual frameworks of stream fish community ecology, but positive interactions remain overlooked. Accordingly, this dissertation joins the ranks of only a very few studies of stream fishes that have explicitly examined positive interactions (Wallin 1992, Johnston 1994a, b, Moore 2006, Hitt and Roberts 2011) interactions. It is my hope that this dissertation will catalyze interest in other positive interactions among freshwater fishes. In addition to examining nest association, future studies must seek to understand the roles of other ubiquitous and potentially positive biotic interactions among stream fishes, such as mixed-species schooling (*see* Matthews 1998, Ch. 9), transport mutualisms (Horn et al. 2011) and positive density dependence (Jungwirth et al. 2015).

Lastly, this dissertation demonstrates that interactions among stream fishes rarely are static; they can vary with abiotic and biotic context, and their outcomes and consequences can be predicted by general ecological models. In Chapter 1, I used biological market theory (Noë and Hammerstein 1995, Schwartz and Hoeksema 1998, Hoeksema and Schwartz 2003) to show that both abiotic and biotic contexts affected the fitness outcomes of a reproductive mutualism between *Nocomis leptocephalus* and *Chrosomus oreas*. *C. oreas* was not able to reproduce without a reproductively active male *N. leptocephalus*; the implications of obligate reproductive symbiosis for this and other strong nest associates (Wallin 1992) are clear. Likewise, *C. oreas* improved the reproductive success of *N. leptocephalus*, but only in less-silted abiotic conditions. Thus, the net interactions between the two species switched from commensalistic to mutualistic with changing abiotic context. Accordingly, Chapter 1 demonstrates that the BMM can be used to predict context dependent fitness outcomes of potentially positive interactions among stream fishes, although it may not be the most useful model for understanding behavior of obligate interactions (at least not at the spatial scale at which this study was conducted). Similarly, in Chapter 2 I used the nest web conceptual framework (previously only used for cavity-nesting avian communities, Martin and Eadie 1999) to elucidate reproductive interaction network topology among cyprinid communities in the New River. This approach showed that differences in species reproductive traits have community-level consequences for reproductive success. I also showed in Chapter 2 that the stress-gradient hypothesis (previously used nearly exclusively for plants, Bertness and Callaway 1994) can be used for predicting the abiotic contexts in which facilitation will be an important driver of stream fish community structure. Overall, the components of this theme demonstrate that creative application of general theories and models developed for disparate ecosystems and taxa can provide novel insight across systems if scientists are willing to think beyond their focal taxa.

How inferences from stream fishes can improve general models

In the first two chapters, I applied general ecological models to stream fish communities. While these models provided new insight into stream fish communities, applying them to this novel system resulted in new insights about the models themselves. For instance, in Chapter 1, the lack of spawning by *C. oreas* in units without reproductively active *N. leptocephalus* demonstrates that the BMM may not be a very useful model for understanding interaction dynamics of obligate mutualisms. Fishes also presented a unique challenge for observing reproductive interactions across a large spatial extent. We were able to overcome this issue by instead modeling fitness-based, demographic metrics that approximate interaction outcomes; this represents a functional extension to tools that can be used to generate and test general ecological hypotheses. In doing so, I was able to apply the nest web framework to a new system (Chapter 2) and demonstrate its applicability outside the sub-discipline of avian ecology. Based on these findings, I now posit that the nest web framework can be a useful tool for understanding any animal community structured by facilitating species. I also showed in Chapter 2 that the SGH can be applied to stream systems and vertebrate communities. Not only do I provide some of the first quantitative evidence of the utility of this model outside of plant ecology (Fugére et al. 2012, Barrio et al. 2013), but I also offer a potential extension of the SGH by demonstrating how it can be used to provide general predictions about community composition. Future work in other systems should examine whether the findings presented in this dissertation represent tools that can be generalizable across ecosystem and taxonomic boundaries, or are system-specific novelties confined only to stream fish communities.

Two unique statistical applications are presented in this dissertation. Firstly, I know of no other study that has applied an information-theoretic (IT), multimodel inferential approach to structural equation modeling as in Chapter 2. A common approach to SEM model selection involves removing factors from a global model until every sub-model and the trimmed SEM is significant at a predefined alpha level. However, this approach can be subjective and forces researchers to assume that there is only one plausible hypothesis describing the data (Hobbs and Hilborn 2006, Barrett 2007). By applying an IT approach to SEM, researchers can benefit from both the correlative complexity of SEM and the unbiased, relativistic inference provided by an IT framework. Secondly, in Chapter 3, I used two-species occupancy modeling to examine patterns of species co-occurrence. Compared to traditional methods for examining co-occurrence (e.g. null model comparisons, *see* Gotelli 2000), this method is ideal because it (a) can incorporate habitat covariates, which can mask or create spurious patterns of species co-occurrence and (b) because it allows for statistical comparison of complex hypotheses describing patterns of co-occurrence. Specifically, two-species occupancy modeling allows researchers to evaluate the relative and combined importance of habitat variables and biotic interactions at determining patterns of species occurrence. Such information can be very useful for evaluating combined effects of habitat degradation and species introduction or loss. Furthermore, Chapter 3 of this dissertation represents the first application of two-species occupancy models for examining positive patterns of species co-occurrence.

Lastly, this dissertation represents a call for integrating models describing ecological processes that typically are studied alone. For instance, potential effects of multiple biotic interactions are seldom examined in the same study; this dissertation is no exception. While the SGH makes predictions about the simultaneous roles of all three major biotic interactions (facilitation/mutualism, competition and predation), studies designed to fully test these predictions are necessary. More broadly, ecology still lacks a firm conceptual framework describing the spatial scales at which biotic interactions operate. While this dissertation suggests that reproductive facilitation can function (or at least be evident) across scales, these findings do not refute the large body of literature suggesting biotic interactions only affect relatively small-scale processes. The great diversity of biotic interactions in nature complicate such a process, but including spatial hierarchy into our conceptual understanding of the ecological role of biotic interactions will be a major step toward a fully integrated ecological model. Broader still, an ultimate goal of ecology should be a model integrating our understanding of the relative roles of major ecological processes such as dispersal and metapopulation/metacommunity dynamics, density dependence, biotic interactions, phylogeny, spatial dependence, genetic issues and macroecology (to name a few). Such a model would be spatiotemporally hierarchical, and would link process across levels of biological organization—from individuals to biomes. Clearly, a long road lies ahead.

References

Abrams, P. A. 1987. On classifying interactions between populations. Oecologia **73**:272-281.

Afkhami, M. E., P. J. McIntyre, and S. Y. Strauss. 2014. Mutualist-mediated effects on species' range limits across large geographic scales. Ecology Letters **17**:1265-1273.

Afkhami, M. E. and J. A. Rudgers. 2009. Endophyte-mediated resistance to herbivores depends on herbivore identity in the wild grass *Festuca subverticillata*. Environmental Entomology **38**:1086-1095.

Agrawal, A. A., D. D. Ackerly, F. Adler, A. E. Arnold, C. Cáceres, D. F. Doak, E. Post, P. J. Hudson, J. Maron, K. A. Mooney, M. Power, D. Schemske, J. Stachowicz, S. Strauss, M. G. Turner, and E. Werner. 2007. Filling key gaps in population and community ecology. Frontiers in Ecology and the Environment **5**:145-152.

Althoff, D. M., K. A. Segraves, and O. Pellmyr. 2005. Community context of an obligate mutualism: pollinator and florivore effects on *Yucca filamentosa*. Ecology **86**:905-913.

Anderson, J. T., T. Nuttle, J. S. S. Rojas, T. H. Pendergast, and A. S. Flecker. 2011. Extremely long-distance seed dispersal by an overfished Amazonian frugivore. Proceedings of the Royal Society of London B **278**:3329-3335.

Aragón, P. and D. Sánchez-Fernández. 2013. Can we disentangle predator-prey interactions from species distributions at a macro-scale? A case study with raptor species. Oikos **122**:64-72.

Araújo, M. B. and A. Guisan. 2006. Five (or so) challenges for species distribution modelling. Journal of Biogeography **33**:1677-1688.

Arnold, S. J. 1983. Morphology, performance and fitness. American Zoologist **23**:347-361.

Baba, R., Y. Nagata, and S. Yamagishi. 1990. Brood parasitism and egg robbing among three freshwater fish. Animal Behavior **40**:776-778.

Bacher, S. and J. Friedli. 2002. Dynamics of a mutualism in a multi-species context. Proceedings of the Royal Society of London B **269**:1517-1522.

Bailey, L. L., J. A. Reid, E. D. Forsman, and J. D. Nichols. 2009. Modeling co-ocurrence of northern spotted and barred owls: Accounting for detection probability differences. Biological Conservation **142**:2983-2989.

Balon, E. K. 1975. Reproductive guilds of fishes - proposal and definition. Journal of the Fisheries Research Board of Canada **32**:821-864.

Barbour, M. T., J. Gerristen, B. D. Snyder, and J. B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, 2nd Edition. O. o. W. United States Environmental Protection Agency. Washington, DC.

Barrett, P. 2007. Structural equation modelling: adjudging model fit. Personality and Individual Differences **42**:815-824.

Barrio, I. C., D. S. Hik, C. G. Bueno, and J. J. Cahill. 2013. Extending the stress-gradient hypothesis - is competition among animals less common in harsh environments? Oikos **122**:516-523.

Benda, L., L. Poff, D. Miller, T. Dunne, G. Reeves, G. Pess, and M. Pollock. 2004. The network dynamics hypothesis: how channel networkds structure riverine habitats. Bioscience **54**:413-427.

Berkman, H. E. and C. F. Rabeni. 1987. Effect of siltation on stream fish communities. Environmental Biology of Fishes **18**:285-294.

Bertness, M. D. 1989. Intraspecific competetion and facilitation ina northern acorn barnacle population. Ecology **70**:257-268.

Bertness, M. D. and R. Callaway. 1994. Positive interactions in communities. Trends in Ecology and Evolution **9**:191-193.

Bertness, M. D. and E. Grosholz. 1985. Population dynamics of the ribbed mussel, *Geukensia demissa*: The costs and benefits of an aggregated distribution. Oecologia **67**:192-204.

Bertness, M. D. and S. D. Hacker. 1994. Physical stress and positive associations among marsh plants. American Naturalist **142**:718-724.

Bertness, M. D. and G. H. Leonard. 1997. The role of positive interactions in communities: lessons from intertidal habitats. Ecology **78**:1976-1989.

Bertness, M. D. and S. M. Yeh. 1994. Cooperative and competitive interactions in the recruitment of marsh elders. Ecology **75**:2416-2429.

Biswas, S. R. and H. H. Wagner. 2014. A temporal dimension to the stress gradient hypothesis for intraspecific interactions. Oikos ***123***:1323-1330.

Blanc, L. A. and K. Martin. 2012. Identifying sutiable woodpecker nest trees using decay selection profiles in trembling aspen (*Populus tremuloides*). Forest Ecology and Management **286**:192-202.

Blanc, L. A. and J. R. Walters. 2007. Cavity-nesting community webs as predictive tools: where do we go from here? Journal of Ornithology **148**:417-423.

Blanc, L. A. and J. R. Walters. 2008a. Cavity-nest webs in a longleaf pine ecosystem. The Condor **110**:80-92.

Blanc, L. A. and J. W. Walters. 2008b. Cavity excavation and enlargement as mechanisms for indirect interactions in an avian community. Ecology **89**:506-514.

Boucher, D. H. 1985. The idea of mutualism, past and future. Pages 1-28 *in* D. H. Boucher, editor. The Biology of Mutualism. Croom Helm, London, UK.

Boucher, D. H. 1988. The Biology of Mutualism: Ecology and Evolution. Oxford University Press, Oxford.

Boucher, D. H., S. James, and K. H. Keeler. 1982. The ecology of mutualism. Annual Review of Ecology and Systematics **13**:315-347.

Brind'Amour, A., D. Boisclair, P. Legendre, and D. Borcard. 2005. Erratum: multiscale spatial distribution of a littoral fish community in relation to environmental variables. Limnology and Oceanography **50**:465-479.

Bronstein, J. L. 1994a. Conditional outcomes in mutualistic interactions. Trends in Ecology and Evolution **9**:214-217.

Bronstein, J. L. 1994b. Our current understanding of mutualism. The Quarterly Review of Biology **69**:31-51.

Bronstein, J. L. 2009. Mutualism and symbiosis. Pages 233-238 *in* S. A. Levin, editor. Princeton guide to ecology. Princeton University Press, Princeton.

Bronstein, J. L., U. Dieckmann, and R. Ferière. 2004. Coevolutionary dynamics and the conservation of mutualism. Pages 305-326 *in* R. Ferrière, U. Dieckmann, and D. Couvet, editors. Evolutionary and conservation biology. Cambridge University Press, Cambridge.

Brown, J. H., B. J. Fox, and D. A. Kelt. 2000. Assembly rules: desert rodent communities are structured at scales from local to continental. The American Naturalist **156**:314-321.

Bshary, R. and A. S. Grutter. 2002. Experimental evidence that partner choice is a driving force in the payoff distribution among cooperators or mutualists: the cleaner fish case. Ecology Letters **5**:130-136.

Byers, J. E., K. Cuddington, C. G. Jones, T. S. Talley, A. Hastings, J. G. Lambrinos, J. A. Crooks, and W. G. Wilson. 2006. Using ecosystem engineers to restore ecological systems. Trends in Ecology and Evolution **21**:493-500.

Callaway, R., R. W. Brooker, P. Choler, Z. Kikvidze, C. J. Lortie, R. Michalet, L. Paolini, F. I. Pugnaire, B. Newingham, E. T. Aschehoug, C. Armas, D. Kikodze, and B. J. Cook. 2002. Positive interactions among alpine plants increase with stress. Nature **417**.

Callaway, R. M. 1992. Effect of shrubs in recruitment of *Quercus douglasii* and *Quercus lobata* in California. Ecology **73**:2118-2128.

Callaway, R. M. 1998. Competition and facilitation: a synthetic approach to interactions in plant communities. Ecology **78**:1958-1965.

Canestrari, D., D. Bolopo, T. C. J. Turlings, G. Röder, J. M. Marcos, and V. Baglione. 2014. From parasitism to mutualism: unexpected interactions between a cuckoo and its host. Science **343**:1350-1352.

Carter, G. G. and G. S. Wilkinson. 2013. Food sharing in vampire bats: reciprocal help predicts donations more than relatedness or harassment. Proceedings of the Royal Society B **280**.

Cashner, M. F. and H. L. Bart. 2010. Reproductive ecology of nest associates: use of RFLPs to identify cyprinid eggs. Copeia **2010**:554-557.

Chamberlain, S. A., J. L. Bronstein, and J. A. Rudgers. 2014. How context dependent are species interactions? Ecology Letters.

Chamberlain, S. A. and J. N. Holland. 2009. Quantitative synthesis of context dependency in ant-plant protection mutualisms. Ecology **90**:2384-2392.

Cherif, A. 1990. Mutualism: the forgotten concept in teaching science. The American Biology Teacher **52**:206-208.

Cockle, K. L. and K. Martin. 2015. Temporal dynamics of a commensal network of cavity-nesting vertebrates: increased diversity during an insect outbreak. Ecology ***In press***.

Cockle, K. L., K. Martin, and G. Robledo. 2012. Linking fungi, trees, and hole-using birds in a Neotropical tree-cavity network: Pathways of cavity production and implications for conservation. Forest Ecology and Management **264**:210-219.

Cooper, J. E. 1980. Egg, larval and juvenile development of longnose dace, *Rhinichthys cataractae*, and river chub *Nocomis micropogon*, with notes on their hybridization. Copeia **1983**:469-478.

Crow, S. K., G. P. Closs, J. M. Waters, D. J. Booker, and G. P. Wallis. 2010. Niche partitioning and the effect of interspecific competetion on microhabitat use by two sympatric galaxiid stream fishes. Freshwater Biology **55**:967-982.

Damiani, C. C. 2005. Integrating direct effects and trait-mediated indirect effects using a projection matrix model. Ecology **86**:2068-2074.

Diamond, J. M. 1975. Assembly of species communities. Pages 342-444 *in* M. L. Cody and J. M. Diamond, editors. Ecology and evolution of communities. Harvard University Press, Cambridge, MA.

Englund, G., F. Johansson, P. Olofsson, J. Salonsaari, and J. Öhman. 2009. Predation leads to assembly rules in fragmented fish communities. Ecology Letters **12**:663-671.

Ettinger, A. K. and J. HilleRisLambers. 2013. Climate isn't everything: competitive interactions and variation by life stage will also affect range shifts in a warming world. American Journal of Botany **100**:1344-1355.

Facey, D. E. and G. D. Grossman. 1992. The relationsihp between water velocity, energetic costs, and microhabitat use in four North American stream fishes. Hydrobiologia **239**:1-6.

Ferrière, R., M. Gauduchon, and J. L. Bronstein. 2007. Evolution and persistence of obligate mutualists and exploiters: competetion for partners and evolutionary immunization. Ecology Letters **10**:115-126.

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

Fletcher, D. E. 1993. Nest association of dusky shiners (*Notropis cummingsae*) and redbreast sunfish (*Lepomis auritus*), a potentially parasitic relationship. Copeia **1993**:159-167.

Foster, K. R. and T. Wensellers. 2006. A general model for the evolution of mutualisms. Journal of Evolutionary Biology **19**:1283-1293.

Frimpong, E. A. and P. L. Angermeier. 2010. Trait-based approaches in the analysis of stream fish communities. Pages 109-136 *in* K. Gido and D. R. Jackson, editors. Community ecology of stream fishes: concepts, approaches, and techniques. American Fisheries Society Symposium 73. American Fisheries Society, Bethesda, MD.

Fugére, V., P. Andino, R. Espinosa, F. Anthelme, D. Jacobsen, and O. Dangles. 2012. Testing the stress-gradient hypothesis with aquatic detritivorous invertebrates: insights for biodiversity-ecosystem functioning research. Journal of Animal Ecology **81**:1259-1267.

Gerdemann, J. W. 1970. The significance of vesicular-arbuscular mycorrhizae in plant nutrition. Pages 125-129 *in* T. A. Tousson, R. V. Bega, and P. E. Nelson, editors. Root diseases and soil-borne plant pathogens. University of California Press, Berkeley.

Gido, K. and D. A. Jackson. 2010. Community ecology of stream fishes: concepts, approaches, and techniques. American Fisheries Society Symposium 73. American Fisheries Society, Bethesda, MD.

Gingins, S., J. Werminghausen, R. A. Johnstone, A. S. Grutter, and R. Bshary. 2013. Power and temptation cause shifts between exploitation and cooperation in a cleaner wrasse mutualism. Proceedings of the Royal Society B **280**:20130553.

Goff, G. P. 1984. Brood care of longnose gar (*Lepisosteus osseus*) by smallmouth bass (*Micropterus dolomieui*). Copeia **1984**:149-152.

Gotelli, N. J. 2000. Null model analysis of species co-occurrence patterns. Ecology **81**:2606-2621.

Gutiérrez, D., P. Fernández, A. Seymour, and D. Jordano. 2005. Habitat distribution models: are mutualist distributions good predictors of their associates? Ecological Applications **15**:3-18.

Hacker, S. D. and S. G. Gaines. 1997. Some implications of direct positive interactions for community species diversity. Ecology **78**:1990-2003.

Haynes, T. B., J. A. Schmutz, M. S. Lindbert, K. G. Wright, B. D. Uher-Koch, and A. E. Rosenberger. 2014. Occupancy of yellow-billed and Pacific loons: evidence for interspecific competition and habitat mediated co-occurrence. Journal of Avian Biology **45**:296-304.

Hazelton, P. D. and G. D. Grossman. 2009. The effects of turbidity and an invasive species on foraging success of rosyside dace (*Clinostomus funduloides*). Freshwater Biology **54**:1977-1989.

He, Q. and M. D. Bertness. 2014. Extreme stress, niches, and positive species interactions along stress gradients. Ecology **95**:1437-1443.

Heikkinen, R. K., M. Luoto, R. Virkkala, R. G. Pearson, and J. Körber. 2007. Biotic interactions improve prediction of boreal bird distributions at macro-scales. Global Ecology and Biogeography **16**:754-763.

Hein, C. L., G. Öhlund, and G. Englund. 2014. Fish introductions reveal the temperature dependence of species interactions. Proceedings of the Royal Society B **281**:20132641.

Hines, J. E. 2006. PRESENCE-Software to estimate patch occupancy and related parameters. USGS-PWRC.

Hitt, N. P. and J. H. Roberts. 2011. Hierarchical spatial structure of stream fish colonization and extinction. Oikos **121**:127-137.

Hobbs, N. T. and R. Hilborn. 2006. Alternatives to statistical hypothesis testing in ecology: a guide to self teaching. Ecological Applications **16**:5-19.

Hoeinghaus, D. J., K. O. Winemiller, and J. S. Birnbaum. 2007. Local and regional determinants of stream fish assemblage structure: inferences based on taxonomic vs. functional groups. Journal of Biogeography **34**:324-338.

Hoeksema, J. D., V. B. Chaudhary, C. A. Gehring, N. C. Johnson, J. Karst, R. T. Koide, A. Pringle, C. Zabinski, J. D. Bever, J. C. Moore, G. W. T. Wilson, J. N. Klironomos, and J. Umbanhowar. 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecology Letters **13**:1-14.

Hoeksema, J. D. and M. W. Schwartz. 2001. Modelling interspecific mutualisms as biological markets. Pages 173-183 *in* R. Noë, J. v. Hooff, and P. Hammerstein, editors. Economics in nature. Cambridge University Press, Cambridge, UK.

Hoeksema, J. D. and M. W. Schwartz. 2003. Expanding comparative-advantage biological market models: contingency of mutualism on partner's resource requirements and acquisition trade-offs. Proceedings of the Royal Society B **270**:913-919.

Holomuzki, J. R., J. W. Feminella, and M. E. Power. 2010. Biotic interactions in freshwater benthic habitats. Journal of the North American Benthological Society **29**:220-244.

Horn, M. H., S. B. Correa, P. Parolin, B. J. A. Pollux, J. T. Anderson, C. Lucas, P. Widmann, A. Tiju, M. Galetti, and M. Goulding. 2011. Seed dispersal by fishes in tropical and temperate fresh waters: the growing evidence. Acta Oecologia **37**:561-577.

Hunter, J. and A. Hasler. 1965. Spawning association of the redfin shiner, *Notropos umbratilis*, and the green sunfish *Lepomis cyanellus*. Copeia **1965**.

Jenkins, R. E. and N. M. Burkhead. 1994. Freshwater fishes of Virginia. American Fisheries Society, Bethesda, MD.

Jin, S., L. Yang, P. Danielson, C. Homer, J. Fry, and G. Xian. 2013. A comprehensive detection method for updating the National Land Cover Database to circa 2011. Remote Sensing of Environment **132**:159-175.

Johnston, C. E. 1994a. The benefit to some minnows of spawning in the nests of other species. Environmental Biology of Fishes **40**:213-218.

Johnston, C. E. 1994b. Nest association in fishes: evidence for mutualism. Behavioral and Ecological Sociobiology **35**:379-383.

Johnston, C. E. 1999. The relationship of spawning mode to conservation of North American minnows (Cyprinidae). Environmental Biology of Fishes **55**:21-30.

Johnston, C. E. and L. M. Page. 1992. The evolution of complex reproductive strategies in North American minnows (Cyprinidae). Pages 600-621 *in* R. L. Mayden, editor. Systematics, historical ecology, and North American freshwater fishes. Stanford University Press, Stanford, CA.

Jungwirth, A., D.Josi, J. Walker, and M. Taborsky. 2015. Benefits of coloniality: communal defence saves anti-predator effort in cooperative breeders. Functional Ecology ***In press.***

Kaltenpoth, M., K. Roeser-Mueller, S. Koehler, A. Peterson, T. Y. Nechitaylo, J. W. Stubblefield, G. Herzner, J. Seger, and E. Strohm. 2014. Partner choice and fidelity stabilize coevolution in a Cretaceous-age defensive symbiosis. Proceeding of the National Academy of Sciences of the USA **111**:6359-6364.

Kamilar, J. M. and L. Beaudrot. 2013. Understanding primate communities: recent developments and future directions. Evolutionary Anthropology:174-185.

Kearns, C. A., D. W. Inouye, and N. M. Waser. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. Annual Review of Ecology and Systematics **29**:83-112.

Keddy, P. A. 1989. Competition. Chapman and Hall, London, UK.

Kersch, M. F. and C. R. Fonseca. 2005. Abiotic factors and the conditional outcome of an ant-plant mutualism. Ecology **86**:2117-2126.

Linder, H. P., O. Bykova, J. Dyke, R. S. Etienne, T. Hickler, I. Kühn, G. Marion, R. Ohlemüller, S. J. Schymanski, and A. Singer. 2012. Biotic modifiers, environmental modulation and species distribution models. Journal of Biogeography: doi:10.1111/j.1365-2699.2012.02705.x.

Machiote, M., L. C. Branch, and D. Villareal. 2004. Burrowing owls and burrowing mammals: are ecosystem engineers interchangeable as facilitators? Oikos **106**:527-535.

Mackenzie, D. I., L. L. Bailey, and J. D. Nichols. 2004. Investigating species co-occurrence patterns when species are detected imperfectly. Journal of Animal Ecology **73**:546-555.

Mackenzie, D. I., J. D. Nichols, J. A. Royle, K. H. Pollock, L. L. Bailey, and J. E. Hines. 2006. Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Academic Press, Boston.

Maestre, F. T., R. M. Callaway, F. Valladares, and C. J. Lortie. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. Journal of Ecology **97**:199-205.

Malkinson, D. and K. Tiebörger. 2010. What does the stress-gradient hypothesis predict? Resolving the discrepancies. Oikos **119**:1546-1552.

Margulis, L. 1981. Symbiosis in cell evolution. Freeman, San Francisco.

Margulis, L. and R. Fester. 1991. Symbiosis as a source of evolutionary innovation. MIT Press, Cambridge, MA.

Martin, K., K. E. Aitken, and K. K. Wiebe. 2004. Nest sites and nest webs for cavity-nesting commnities in interior British Columbia, Canada: nest characteristics and niche partitioning. The Condor **106**:5-19.

Martin, K. and J. M. Eadie. 1999. Nest webs: a community-wide approach to the management and conservation of cavity-nesting forest birds. Forest Ecology and Management **115**:243-257.

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

Mattingly, H. T. and T. R. Black. 2013. Nest association and reproductive microhabitat of the threatened blackside dace, *Chrosomus cumberlandensis*. Southeastern Naturalist **12(sp4)**:49-63.

McKaye, K. R. 1985. Cichlid-catfish mutualistic defense of young in lake Malawi, Africa. Oecologia **66**:358-363.

McKaye, K. R. and N. M. KcKaye. 1977. Communal care and kidnapping of young by parental cichlids. Evolution **31**:674-681.

McManamay, R. A., D. J. Orth, C. A. Dolloff, and M. A. Cantrell. 2010. Gravel addition as a habitat restoration technique for tailwaters. North American Journal of Fisheries Management **30**:1238-1257.

Midgley, J. J. and W. J. Bond. 1991. How important are biotic pollination and dispersal to the success of the angiosperms? Philosophical Transactions of the Royal Society of London B **333**:209-215.

Miller, R. J. 1964. Behavior and ecology of some North American cyprinid fishes. American Midland Naturalist **72**:313-357.

Miranda, M. I., M. Omacini, and E. J. Chaneton. 2011. Environmental context of endophyte symbioses: interacting effects of water stress and insect herbivory. International Journal of Plant Science **172**:499-508.

Moore, J. C. 2006. Animal ecosystem engineers in streams. Bioscience **56**:237-246.

Moore, J. W., D. E. Schindler, and M. D. Scheurell. 2004. Disturbance of freshwater habitats by anadromous salmon in Alaska. Oecologia **139**:298-308.

Mouchet, M. A., M. D. M. Burns, A. M. Garcia, J. P. Vieira, and D. Mouillot. 2013. Invariant scaling relationship between functional dissimilarity and co-occurrence in fish assemblage of the Patos Lagoon estuary (Brazil): environmnetal filtering considtently overshadows competitive exclusion. Oikos **122**:247-257.

Mouillot, D., O. Dumay, and J. A. Tomasini. 2007. Limiting similarity, niche filtering and functional diversity in coastal lagoon fish communities. Estuarine, Coastal and Shelf Science **71**:443-456.

Muscatine, L. and J. W. Porter. 1977. Reef corals: mutualistic symbioses adapted to nutrient poor environments. Bioscience **27**:454-460.

Noë, R. and P. Hammerstein. 1994. Biological markets: supply and demand deterimine the effet of the partner. Behavioral and Ecological Sociobiology **35**:1-11.

Noë, R. and P. Hammerstein. 1995. Biological markets. Trends in Ecology and Evolution **10**:336-339.

Olson, G. S., R. G. Anthony, E. D. Forsman, S. H. Ackers, P. J. Loschl, J. A. Reid, K. M. Dugger, E. M. Glenn, and W. J. Ripple. 2005. Modeling of site occupancy dynamics for northern spotted owls, with emphasis on the effects of barred owls. The Journal of Wildlife Management **69**:918-932.

Orchan, Y., F. Chiron, A. Schwartz, and S. Kark. 2012. The complex interaction network among multiple invasive bird species in a cavity-nesting community. Biological Invasions:DOI: 10.1007/s10530-10012-10298-10536.

Pendleton, R. M., J. J. Pritt, B. K. Peoples, and E. A. Frimpong. 2012. The strength of *Nocomis* nest association contributes to patterns of rarity and commonness among New River, Virginia cyprinids. American Midland Naturalist **168**:202-212.

Peoples, B. K. and E. A. Frimpong. 2012. A snapshot analysis of age distributions of fishes in urban and forestted Virginia streams. Urban Ecosystems **15**.

Peoples, B. K. and E. A. Frimpong. 2013. Evidence of mutual benefits of nest association among freshwater cyprinids and implications for conservation. Aquatic Conservation: Marine and Freshwater Ecosystems **23**:911-923.

Peoples, B. K., R. A. McManamay, D. J. Orth, and E. A. Frimpong. 2014. Nesting habitat use by river chubs in a hydrologically variable Appalachian tailwater. Ecology of Freshwater Fish **23**:283-293.

Peoples, B. K., M. B. Tainer, and E. A. Frimpong. 2011. Bluehead chub nesting activity: a potential mechanism of population persistence in degraded stream habitats. Environmental Biology of Fishes **90**:379-391.

Peres-Neto, P. R. 2004. Patterns in the co-occurrence of fish species in streams: the role of site suitability, morphology, and phylogeny versus species interactions. Oecologia **140**:352-360.

Potcner, K. K. and S. M. Kowalski. 2004. How to analyze a split-plot experiment. Quality Progress **37**:67-74.

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.

Rakes, P. L., J. R. Shute, and P. W. Shute. 1999. Reproductive behavior, captive breeding, and restoration ecology of endangered fishes. Environmental Biology of Fishes **55**:31-42.

Reid, S. M., N. E. Jones, and G. Yunker. 2008. Evaluation of single-pass electrofishing and rapid habitat assessment for monitoring redside dace. North American Journal of Fisheries Management **28**:50-56.

Richmond, O. M. W., J. E. Hines, and S. R. Bessinger. 2010. Two-species occupancy models: a new parameterization applied to co-occurrence of secretive rails. Ecological Applications **20**:2036-2046.

Robles, H. and K. Martin. 2013. Habitat-mediated variation in the importance of ecosystem engineers for secondary cavity nesters in a nest web. PLoS ONE **9**:e90071.

Rosseel, Y. 2012. lavaan: an R package for structural equation modeling. Journal of Statistical Software **48**:1-36.

Schlosser, I. J. 1987. A conceptual framework for fish communities in small warmwater streams.*in* W. J. Matthews and D. C. Heins, editors. Community and Evolutionary Ecology of North American Stream Fishes. University of Oklahoma Press, Norman.

Schwartz, H. W. and J. D. Hoeksema. 1998. Specialization and resource trade: biological markets as a model of mutualisms. Ecology **79**:1029-1038.

Shao, B. 1997a. Effects of golden shiner (*Notemigonus crysoleucas*) nest association on host pumpkinseeds (*Lepomis gibbosus*): evidence for a non-parasitic relationship. Behavioral and Ecological Sociobiology **41**:399-406.

Shao, B. 1997b. Nest association of pumpkinseed, *Lepomis gibbosus*, and golden shiner, *Nogemigonus crysoleucas*. Environmental Biology of Fishes **50**:41-48.

Simms, E. L., D. L. Taylor, J. Povich, R. P. Shefferson, J. L. Sachs, M. Urbina, and Y. Tausczik. 2006. An empirical test of partner choice mechanisms in a wild legume-rhizobium interaction. Proceedings of the Royal Society B **273**:77-81.

Skelton, J., R. P. Creed, and B. L. Brown. 2014. Ontogenetic shift in host tolerance controls initiation of a cleaning symbiosis. Oikos **123**:677-686.

Soliveres, S., L. DeSoto, F. T. Maestre, and J. M. Olano. 2010. Spatio-temporal heterogeneity in abiotic factors modulate multiple ontogenetic shifts between competition and facilitation. Perspectives in Plant Ecology, Evolution and Systematics **12**:227-234.

Steele, B. D. 1978. The reproductive strategy and energetics of rosefin shiners, *Notropis ardens,* in Barebone Creek, Kentucky. University of Louisville, Louisville, KY.

Strange, E. M. and T. C. Foin. 1999. Interaction of physical and biolgoical processes in the assembly of stream fish communities. Pages 311-337 *in* E. Weiher and P. keddy, editors. Ecological assembly rules: perspectives, advances, retreats. Cambridge University Press, Cambridge, UK.

Thompson, A. R., R. M. Nisbet, and R. J. Schmitt. 2006. Dynamics of mutualist populations that are demographically open. Journal of Animal Ecology **75**:1239-1251.

Tokeshi, M. 1993. On the evolution of commensalism in the Chironomidae. Freshwater Biology **29**:481-489.

Tonn, W. M. 1990. Climate change and fish communities: a conceptual framework. Transactions of the American Fisheries Society **119**:337-352.

Van der Putten, W. H., M. Macel, and M. E. Visser. 2010. Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels. Proceedings of the Royal Society B **365**:2025-2034.

Violle, C., M. L. Navas, D. Vile, E. Kazakou, c. Fortunel, I. Hummel, and E. Garnier. 2007. Let the concept of the trait be functional! Oikos **116**:882-892.

Vives, S. P. 1990. Nesting ecology and behavior of hornyhead chub *Nocomis biguttatus*, a keystone species in Allequash Creek, Wisconsin. American Midland Naturalist **124**:46-56.

Wallin, J. E. 1992. The symbiotic nest association of yellowfin shiners, *Notropis lutipinnis*, and bluehead chubs, *Nocomis leptocephalus*. Environmental Biology of Fishes **33**:287-292.

Walser, C. A., B. Falterman, and H. L. Bart. 2000. Impact of introduced rough shiner (*Notropis baileyi*) on the native fish community in the Chattahoochee River system. American Midland Naturalist **144**:393-405.

Werner, G. D. A., J. E. Strassman, A. B. F. Ivens, D. J. P. Engelmoer, E. Verbuggen, D. C. Queller, R. Noë, N. C. Johnson, P. Hammerstein, and E. T. Kiers. 2014. Evolution of microbial markets. Proceeding of the National Academy of Sciences of the USA:1237-1244.

Weyl, E. G., M. E. Frederickson, D. W. Yu, and N. E. Pierce. 2010. Economic contract theory tests models of mutualism. Proceeding of the National Academy of Sciences of the USA **107**:15712-15716.

Wiens, J. J. 2011. The niche, biogeography and species interactions. Philosophical Transactions of the Royal Society of London B **366**:2336-2350.

Williamson, R. and J. D. Clark. 2011. Evaluating interactions between river otters and muskrats at bridge crossings in Kentucky. Journal of Mammalogy **92**:1314-1320.

Wisenden, B. D., A. Unruh, A. Morantes, S. Bury, B. Curry, R. Driscoll, M. Hussein, and S. Markegard. 2009. Functional constraints on nest characteristics of pebble mounds of breeding male hornyhead chubs *Nocomis biguttatus*. Journal of Fish Biology **75**:1577-1585.

Wisz, M. S., J. Pottier, and e. a. W.D. Kissling. 2013. The role of biotic interactions in shaping distributions and realized assemblages of species: implications for species distribution modeling. Biological Reviews of the Cambridge Philosophical Society **88**:15-30.

Wong, M. and S. Balshine. 2011. The evolution of cooperative breeding in the African cichlid fish, *Neolamprologus pulcher*. Biological Reviews **86**:511-530.

Wootton, R. J., editor. 1998. Ecology of Teleost Fishes, Second Edition. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Yackulic, C. B., J. Reid, J. D. Nichols, J. E. Hines, R. Davis, and E. Forsman. 2014. The roles of competititon and habitat in the dynamics of populations and species distributions. Ecology **95**:265-279.

Yamana, H., K. Watanabe, and Y. Nagata. 2013. Diversity of interspecific interactions between a nest-associating species, *Puntungia herzi*, and a multiple host species. Environmental Biology of Fishes **96**:573-584.