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POSITIVE INTERACTIONS IN FRESHWATER SYSTEMS

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Wildlife and Fisheries Biology

by
Samuel Clarence Silknetter
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Accepted by:
Dr. Brandon Peoples, Committee Chair
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Dr. Troy Farmer

ABSTRACT

The goals of this thesis are twofold, 1) to review the existing literature on positive interactions, and 2) to experimentally test the role of biotic context in a freshwater reproductive interaction. First, my co-authors and I have conducted a review of positive interactions in freshwaters to establish a direction for future research. By outlining case studies and causal mechanisms, we illustrate the diversity of positive interactions, and set the stage for a comprehensive look at the role of context in shaping interaction outcomes. Our research directive focuses on the value of both basic life history and experimental research, then using those findings to investigate these interactions under different contexts, in different levels of ecosystem structure, and at multiple geographic scales. Applications of positive interactions are discussed in regards to conservation, restoration, biological invasions, and in aquaculture production. Following this review, I experimentally investigate context dependency in the nest associative interaction between two stream fishes. By using three treatments of partner density while maintaining otherwise consistent conditions, shifts in interaction outcomes can be directly attributed to context dependency. Findings are incorporated into our understanding of this novel study system, and their contribution to broader ecological theory is discussed.

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GENERAL INTRODUCTION

Interactions between species have intrigued naturalists for centuries, and classical ecology rightly looked to these interactions to understand patterns of biodiversity. Historically, only antagonistic interactions were considered meaningful contributors to population dynamics, but new understanding of ecological processes has revealed, in part, the significance of positive interactions (Boucher et al. 1982). Positive biotic interactions, which include mutualism, commensalism, and facilitation are important drivers of population abundance and community structure (Boucher et al., 1982; Stachowicz, 2001; Bruno et al., 2003), yet remain understudied relative to negative interactions (competition, predation, and parasitism; Bronstein, 1994a, b, 2009). There is a dearth of knowledge related to positive interactions which relates directly to a lack of research, perhaps nowhere as pronounced as it is in freshwaters.

The history of positive interactions in ecological research started primarily with studies of mutualistic interactions involving plants (Way 1963, Janzen 1966, Galil and Eisikowitch 1968). Some plant systems have now been examined to the point where meta-analyses are appropriate (Rosumek et al. 2009, Hoeksema et al. 2010), yet positive interactions in freshwaters remain in a state of relative infancy in comparison. In recent years though, we have seen an increased focus on mutualism, commensalism, and facilitation and their roles in freshwaters. Owing to a few well-studied systems which will be discussed at length in this thesis, the study of positive interactions in freshwaters appears to have reached a point of critical mass. It now seems a worthwhile endeavor to review what has been accomplished so far and that which still needs our research attention. In recent years, positive interaction research in freshwaters have identified

significant roles of ontogeny, host control, partner density, and the presence of third-party taxa in shaping interaction outcomes, just to name a few. Through the incorporation of concepts borrowed from other disciplines and the synthesis of novel freshwater research, we can provide a research directive to further the study of positive interactions in freshwaters.

An important feature of positive interactions that has generated much research interest is that interaction outcomes can shift as a result of changing context (Bronstein, 1994b; Noë and Hammerstein, 1995). Positive interactions typically have reduced interaction strength when compared to antagonistic interactions that often have one-way transfers of energy (Sachs and Simms, 2006; Moore et al., 2006). Resource exchanges that characterize positive interactions require a balance of costs and benefits, and while these interactions appear quite stable evolutionarily (Frederickson, 2017), they are often quite complex and subject to specific environmental or biotic conditions (Chamberlain et al., 2014). There are many demonstrable environmental changes which the natural world is currently subjected to, and anthropogenic impacts are unlikely to cease. As the context in which species interactions occur shifts, it is vital that ecologists understand the responses of those taxa and that we are able to predict the impacts of future changes. Determining how costs and benefits of biotic interactions change as a result of context will improve general ecological models, and work in freshwater systems has already begun to contribute to a more comprehensive understanding of this phenomena. Future examinations of context dependency should address broad scales, from shaping responses of individuals and populations (Horn et al., 2011) to the processes of communities (Brown et al., 2002; Nakano et al., 2005) and ecosystems (Moore, 2006).

One study system especially well suited to examinations of context dependency is nest association, a common positive interaction among North American freshwater fishes. In this partnership, ‘associate’ species spawn in nests constructed by a host. In the eastern United States, the most prolific and widespread hosts are chubs of the genus *Nocomis*; their nests are used by more than associate species throughout their range (Johnston and Page 1992). Chubs provide associates with suitable substrate for reproduction and an element of parental care (Vives, 1990; Maurakis et al., 1992; Wallin, 1992). In exchange, associates provide a dilution effect (sensu McKaye and McKaye, 1977) to the host when predators are present; a decreased proportion of chub eggs on nests reduces their likelihood of predation (Johnston, 1994b). While large chub nests may attract hundreds of associates (McAuliffe and Bennett 1981, Meffe et al. 1988), others may attract few or none. Natural variability in associate density is high, especially across ecological gradients such as stream size and land use (Peoples et al., 2015). As the benefits of brood dilution are correlated with associate density, differences in partner abundance provide a form of biotic context that may determine interaction outcomes.

CHAPTER ONE

POSITIVE BIOTIC INTERACTIONS IN FRESHWATERS: A RESEARCH DIRECTIVE

Samuel Silknetter, Bryan Brown, Robert Creed, Emmanuel Frimpong,
James Skelton, and Brandon Peoples.

Abstract

Positive interspecific interactions (mutualism, commensalism, and facilitation) are ubiquitous in nature, but understudied in freshwater ecosystems. This review assesses the state-of-the-knowledge of positive interactions in freshwaters, and provides direction for future research. A few mutualistic relationships have received some research attention, namely seed-dispersing fishes, crayfishes and their branchiobdellidan symbionts, and communal-spawning stream fishes. Facilitative effects of a few habitat-modifying species have also been identified, as well as positive indirect trophic interactions. However, less is known about interactions in which participants directly exchange nutrients or protection. Most studies in freshwaters have been conducted using observations or experiments at small spatial scales. However, a cross-scale approach is necessary for a full understanding of how positive interactions operate. Likewise, research must seek to understand context dependency in positive interactions—how biotic (e.g. species or traits) and abiotic factors determine outcomes of positive interactions. Lastly, research on positive interactions must progress from a perspective of pairwise interactions, to a broader community context. A firm understanding of positive interactions will yield better predictions for managing freshwater ecosystems.

Introduction

Positive interspecific interactions (mutualism, commensalism, and facilitation) are ubiquitous in nature, and are important drivers of population abundance and community structure (Boucher et al. 1982, Bronstein 1994a, 1994b). Yet despite their ubiquity and importance, positive interactions remain understudied relative to negative interactions (competition, predation, and parasitism). Mutualisms have been observed and written about since the times of ancient Greece, but were not examined in an empirical scientific framework until the mid-1900s (Boucher et al. 1982). Because early experimental work was conducted mostly on pollination, seed dispersal, and protection mutualisms in plants, our current understanding of positive interactions is based largely on studies of plants in terrestrial ecosystems.

Research on positive interactions in freshwater ecosystems lags behind terrestrial systems (Figure 1). In fact, a review of biotic interactions in freshwater systems (Holomuzki et al. 2010) acknowledged that positive interactions are by far the least studied interaction type in this field. However, a few key interactions and taxa have been studied to allow for syntheses. For example, positive effects of a few habitat-modifying species were reviewed by Moore (2006), and seed-dispersal mutualisms between fishes and plants (ichthyochory) have had several reviews (Pollux 2011, Horn et al. 2011, Parolin et al. 2013). Although there remains a great deal that remains unstudied about positive interactions in freshwater systems, emerging insight from other systems is reaching a point to provide opportunity for a synthesis and identify key knowledge gaps and opportunities for new research.

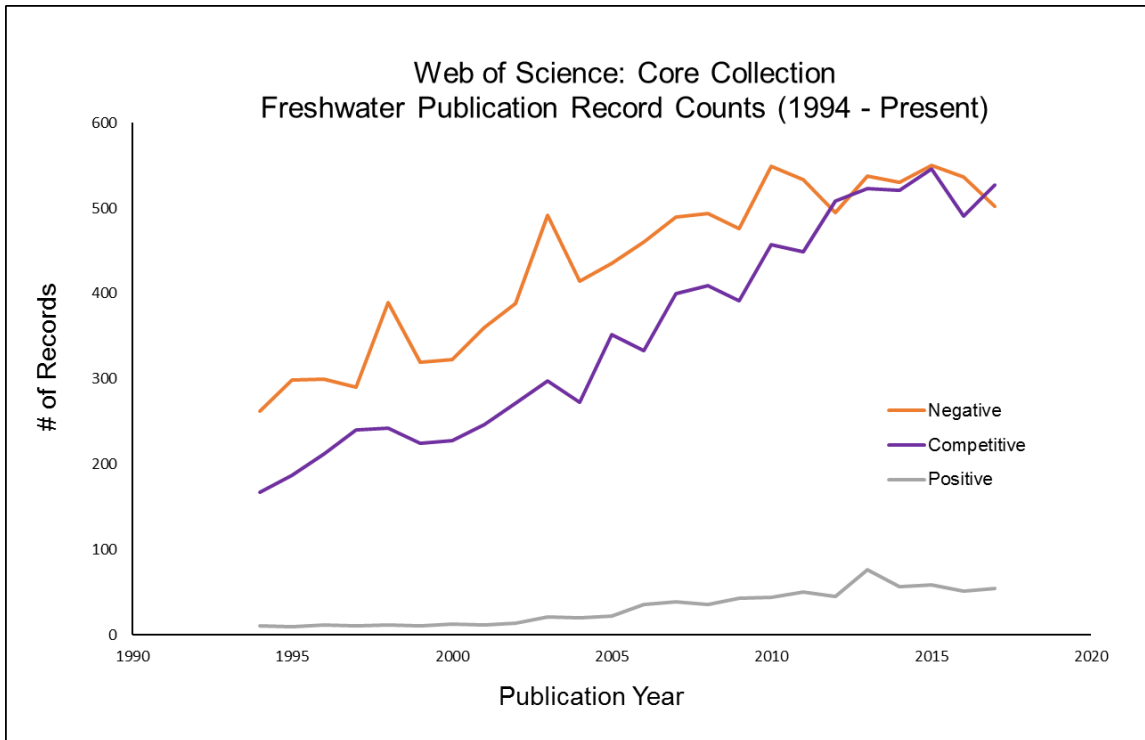


Figure 1.1: Web of Science core collection publications related to species interactions in freshwaters from 1994 - 2017. Freshwaters include wetlands, streams, rivers, lakes and their synonyms. Negative interactions include predation, parasitism, and herbivory; positive interactions include mutualism, commensalism, and facilitation.

In this review, we assess the state-of-the-knowledge of positive interactions in freshwaters, and provide direction for future research objectives. The scope of this review comprises direct, macro-organismal positive interactions in all global freshwaters (streams, rivers, lakes, wetlands). We include interactions ranging from obligate, endosymbiotic relationships to facultative and highly context-dependent benefits from organismal habitat modification. Interactions that occur in, but are not unique in form or function to freshwaters (such as gut microbe mutualisms or nitrogen-fixing bacteria), are excluded, except where differences can be drawn between freshwaters and other systems. Our goals were to synthesize current knowledge of positive interactions and how they relate to general ecological models, establish a directive to guide and streamline future

research, and illustrate the importance of understanding positive interactions for conservation and management of freshwater systems and biota. Accordingly, we present this review in five sections: i) collating and examining existing case studies and causal mechanisms, ii) addressing the stability of mutualisms and role of context-dependency in shaping interaction outcomes, iii) evaluating the implications of positive interactions at the community-level and how this can inform general ecological models, iv) proposing a research directive for advancing our understanding of positive biotic interactions in freshwater systems, and v) demonstrating the importance of positive interactions for conservation and management of freshwaters.

Definitions and methods

For clarity, we first define all relevant terms and interaction types (Table 1). The scope of this paper is positive interactions, defined as direct relationships between two or more resource-exchanging species that benefit at least one partner while doing harm to neither (Stachowicz 2001). Using this broader approach expands the scope of positive interactions to include not only mutually beneficial pairwise symbioses, but also direct commensalism (one species benefits while the other receives no net cost or benefit), and facilitation (the presence of one species alters the environment in a way that directly enhances fitness of a second, neighboring species; Bronstein 2009). Positive interactions range from symbioses to facultative or incidental partnerships, and therefore require specific terminology. Here, we define symbiosis as an intimate interaction between different organisms, where at least one of the parties is obligatorily dependent on the association as a part of its life history (Leung and Poulin 2008). Using this definition, we discuss interactions herein as either symbiotic (between host and ‘symbionts’), or more

loosely as partnerships (between host and ‘partners’). Some system-specific terminology is used in the literature (i.e. cleaners and clients in cleaning interactions), but in all cases we will indicate or comment on whether the interaction is considered symbiotic. We follow Ferriere et al. (2002) and distinguish the host as the interacting species that provides commodities, and the symbiont or partner as the species that provide goods and/or services in return.

Table 1.1: Definitions of significant terms.

Mutualism	Interaction in which both species involved receive a measurable benefit
Commensalism	Interaction in which one species benefits while the other has no net cost or benefit
Facilitation	Interaction where presence of one species alters the environment (i.e. ecosystem engineering or habitat amelioration) in a way that directly enhances fitness of a neighboring species
Symbiosis	Intimate relationship between different organisms, where at least one organism is obligatorily dependent on the association for part of its life history
Partnership	Interspecific association with meaningful fitness consequences for at least one organism, but which is not biologically obligatory or lacks prolonged physical contact
Host	Interacting species that provides commodities
Symbiont/Partner	Interacting species that provides goods or services in exchange for commodity

To address the five sections outlined above and present the state-of-the-knowledge of positive interactions in freshwaters, a thorough review of existing literature was necessary. We conducted Web of Science core collection searches (Clarivate Analytics 2018) for interactions (mutualism, commensalism, facilitation and synonyms) and freshwater habitats (wetlands, streams, rivers, lakes and synonyms) covered by this review. Our search for analogous interactions in other ecosystem types (i.e. marine, terrestrial), conceptual models, general ecological principles etcetera were not exhaustive,

but come primarily from primary sources included in this review and the combined expertise of the authors.

Case studies and causal mechanisms

Positive interactions can be grouped in many different ways, reflecting their ubiquity and diversity. Boucher et al. (1982) were the first to group types of mutualisms, and successive studies have re-evaluated those groupings. We follow the schema of Bronstein (2009), which uses the concept of the ‘biological marketplace’ (Bull and Rice 1991, Noë and Hammerstein 1994) in which organisms trade easily produced resources/commodities in return for those they could not readily produce for themselves. Bronstein (2009) identified nutrition, transportation, and protection as the three resource or commodity types traded in mutualisms, but provided no freshwater examples to support these groupings. However, they can be used and expanded upon to address non-mutualistic positive interactions such as facilitation in freshwaters; for example, commodities provided by hosts may include habitat amelioration, predation release, or other valuable benefits. In the case studies discussed below, interaction types are grouped according to the resource type above which is provided to the host. We also discuss facilitation by habitat modifying species, which is somewhat analogous to hosts in that they provide a service for other species. This group includes ecosystem engineers (i.e. Moore 2006), but also species which make smaller, yet still biologically significant modifications. Still more examples of positive interactions exist, though they do not necessarily fit into the types mentioned above. These ‘other’ interactions nevertheless influence ecological structure, often through poorly understood mechanisms.

Nutrition

Nutritional interactions are those in which a host provides nutritional benefits to a symbiont in exchange for resources. While nutritional interactions are well-represented in ecological literature, most examples come from microbes (in animal guts, on plant roots, etc.). In freshwaters, nutritional symbioses appear to occur infrequently, at least from the perspective of interactions in which a singular host receives a nutritional benefit. One example of a nutrition-driven interaction is the complex association between algae, diatoms, cyanobacteria, heterotrophic microbes, and detritus that make up periphyton (Larned 2010). This matrix of organisms includes taxa supporting one another through resource exchanges and structural support, though parsing out costs and benefits to interacting species is difficult (Biggs et al. 1998). A pairwise nutritional example is the interaction between freshwater sponges (Spongillidae: *Spongilla* and *Ephydatia* spp.) and their endosymbiotic green algae (Chlorellaceae: *Zoochlorella* sp; Wilkinson 1980, Frost and Williamson 1980). Sponges do not require the algae to survive, but may benefit from algae-acquired glucose in exchange for providing a suitable, illuminated habitat for their symbiont. The algae undergo photosynthesis when the sponge is in a well-lit location, and provide extra nutrients to the host as a result. When in low-light conditions, algae cannot undergo photosynthesis and nutrient subsidies to the host cease. In this case, the host sponge will most often digest the algae, thus ending the interaction (Wilkinson 1980). More recent work has used stable isotope analyses to examine the trophic dynamics of *S. lacustris*, with results indicating that ‘green sponges’ (i.e. those inhabited by green algae) are exclusively selected by spongillafly predators (Skelton and Strand 2013).

Another nutritional interaction comes from a mutual facilitation between two species of oligochaete worms in European mesotrophic lakes (Milbrink 1993). In this case, laboratory and observational studies have shown that both *Potamothrix moldaviensis* and *Tubifex tubifex* achieve increased growth when they co-occur, compared to when they are isolated from one another. The apparent mechanism is that feeding on the fecal pellets of the opposite species provides a concentrated source of desirable bacteria colonies. While both species have highly similar diets, they only digest some of the bacteria taxa from their shared food sources, while other bacteria pass through the gut unmolested. Thus, bacteria are partitioned between these worms, and fecal pellets of the opposite species provide a readily accessible source of the desirable prey. Though facultative, this nutritional interaction is credited with the expansion of the *Potamothrix* genus throughout much of Europe like its already cosmopolitan cousin, *T. tubifex* (Milbrink 1993). This interaction provides an example in which there is no true host, only mutual ‘facilitator species’, illustrating both the complexity of interaction types and the difficulty in classifying them.

Transport

Transport interactions are those in which species, whether from symbioses or brief-exchange partnerships (sensu Leigh 2010), provide a means of dispersal for the host or host progeny. Transport interactions are best represented in freshwater systems by fish-borne seed dispersal, or ichthyochory, in which plants make their seeds accessible to fishes, by either dropping ripe fruits, or relying on flooding to inundate their low-hanging fruits (Horn et al. 2011). Pollux (2011) classifies the process into six stages: seed uptake, ingestion, retention, survival (intact passage as excreta), germination probability, and

germination rate after gut passage. Most ichthyochorous fish have evolved to be proficient herbivores (Drewe et al. 2004, Correa et al. 2007), and always benefit from their consumption of fruits and other plant materials. Plants benefit when seeds are successfully dispersed and germinate, and in general, fish are largely successful at doing so (Horn et al. 2011, Correa et al. 2015b).

Ichthyochory has been well documented in tropical South America, with key early studies focused on the Amazon River basin (Gottsberger 1978). Upland rivers in the Amazon basin are often characterized by poor instream productivity, but riparian zones provide pulses of allochthonous inputs during seasonal floods (Gottsberger 1978, Parolin et al. 2013, Correa et al. 2015a) which help to maintain high fish diversity and abundance (Horn et al. 2011, Correa and Winemiller 2014). Generally, plant diversity in the submerged riparian zones of Amazon basin is low (Gottsberger 1978), potentially the result of ichthyochory which broadly disperses seeds from a few highly dominant plant species. Pollux (2011) conducted a review of ichthyochory experiments; while it provides future directions for research in this field, it focuses mainly on the design and potential pitfalls of ichthyochory experiments. Major goals of ichthyochory studies have been to determine which fish are best at dispersing seeds and how best to conserve important mutualists, yet findings remain largely specific to commercially valuable fish species.

In addition to the diverse examples of ichthyochory in South and Central America, several studies have examined this interaction in Holarctic regions. Channel Catfish (Ictaluridae: *Ictalurus punctatus*) is a common ichthyochorous fish in temperate rivers of the United States. Channel Catfish have been shown to consume and successfully germinate seeds of the red mulberry (Moraceae: *Morus rubra*) and swamp privet

(Oleaceae: *Forestiera acuminata*) in the upper Mississippi River (Chick et al. 2003), and another case has documented ichthyochory by this species in the southeastern United States (Adams et al. 2007). Findings mirrored many of the studies from the Amazon, but with the notable differences that 1) flood pulses are irregular and anthropogenically altered in the study areas, and 2) that the temperate portions of the Mississippi River system examined are not considered nutrient-limited. Another recent study (VonBank et al. 2018) has illustrated the potential for an invasive species (Common Carp, Cyprinidae: *Cyprinus carpio*) to engage in ichthyochory, with implications for the disruption of native fish and hydrophyte populations.

Protection

Protection interactions occur when symbionts or partners protect their hosts from natural enemies or parasites (Hopkins et al. 2017). These interactions are perhaps best typified by ant-plant symbioses in which a defenseless host gains the protection of a symbiont in exchange for nutrition, shelter, or some other commodity. One freshwater example analogous to the ant-plant symbiosis is the partnership between the water strider (Gerridae: *Gerris nepalensis*) and its host, the water caltrop (Lythraceae: *Trapa natans*). An aquatic leaf beetle (Chrysomelidae: *Galerucella nipponensis*) consumes caltrop leaves and although the exact mechanisms remain unidentified, these injured caltrop leaves have been shown to increase the abundance of the predatory water striders (Harada et al. 2008, 2011). Striders release the caltrop from herbivory by feeding on the leaf beetles, thus benefitting from this food source but also by the provision of a suitable substrate (leaf surface) for laying their eggs. This interaction, though facultative, has significant impacts on the trophic dynamics of the systems where it occurs. Further

experimentation will seek to identify the attractant (chemical or other pathway) which draws water striders to depredated water caltrop, and potential costs to the host associated with high densities of water striders (Harada et al. 2011).

In addition to the characteristic protection interactions involving defenseless hosts are cleaning interactions in which hosts or ‘clients’ are groomed by ‘cleaners’ to remove parasitic or otherwise unwanted epibionts. Cleaning interactions among marine fishes are very well studied, but this behavior has evolved in freshwater fishes as well. In marine systems, reef fish often have ‘cleaning stations’, areas in which client fish solicit cleaners by displaying specific behaviors to make their desire to be cleaned known (Côté et al. 1998). Freshwater cleaner fishes have recently been found to exhibit the same cleaning behavior, in what some authors consider a temporary, proto-cooperative behavior (Severo-Neto and Froehlich 2016). Unlike other cleaning behaviors discussed below, this partnership is likely very brief and no physical attachment exists between cleaners and clients.

Another cleaning mutualism involves *Chaetogaster limnaei limnaei* (Naididae), a cosmopolitan symbiotic oligochaete that attaches to the body surface of its host. The aquatic pulmonate snail (Physidae: *Physa acuta*) is a primary host, where the oligochaete attaches to its mantle or pulmonary cavity and searches the passing water for microorganisms, such as protozoa, rotifers, diatoms, or other algae (Stoll et al. 2013). The oligochaete is often considered a commensal, as it benefits from the interaction without directly feeding on the snail or limiting its food sources (e.g. Zimmermann et al. 2011), but has also been shown to protect its host from trematode infections, suggesting the potential for mutualism (Ibrahim 2007). The interaction can be quite complex, with

serious direct and indirect effects for the oligochaete and host, but also for trematode populations and their predators (Zimmermann et al. 2011, Stoll et al. 2017). Protection from trematodes provided by oligochaetes may save the life of the host, but is also likely to reduce host fitness and reproductive success at high symbiont density (Stoll et al. 2017). Infection has even shown to shift the reproductive strategy of infested hosts (Stoll et al. 2013), and the snail lacks defensive adaptations to mitigate or minimize these costs.

Another cleaning symbiosis common in freshwaters is the interaction between crayfish and annelid worms of the order Branchiodbellida (hereafter, 'worms'). The worms, which were previously thought to be commensals, attach to the exterior of the host and feed on epibiotic parasites, primarily in the gills of the host (Brown et al. 2002). This increases respiration efficiency, and worm symbionts have been shown to significantly increase host growth while simultaneously reducing mortality (Brown et al. 2012). The worms benefit from a generally stable food source, and are only known to reproduce on the exterior of the crayfish; thus they appear to be obligate symbionts (Creed et al. 2015). This is a common interaction in global freshwaters, and the system can be readily manipulated in laboratory experiments.

Another protection interaction is nest association, which occurs most commonly among North American minnows (Leuciscidae; Tan and Armbruster 2018). In this interaction, 'associate' species spawn in nests constructed by a host. There are more than 35 known associate species (Johnston and Page 1992), which use nests constructed by at least three genera of hosts (*Campostoma*, *Nocomis*, *Semotilus*). Interactions range from obligatory associates who spawn nowhere but in hosts nests to opportunistic spawners that may use the nest for reproduction while simultaneously parasitizing its eggs (Pendleton et al.

2012). In nests of *Nocomis* spp., associates benefit as a result of suitable spawning substrate provided by the nest (Vives 1990, Maurakis et al. 1992, Peoples and Frimpong 2013) and an element of parental care (Wallin 1992, Johnston 1994a). Hosts benefit from a dilution effect (McKaye and McKaye 1977) in which high proportions of associate eggs on nests decrease the likelihood of predation on host eggs (Johnston 1994b, Silknetter et al. 2018). While direct effects are generally limited to the pairwise interaction between host and associate, the habitat amelioration provided has clear benefits for other members of these stream communities.

Habitat Modification

Habitat modification, also termed as ecosystem engineering (Jones et al. 1994), is a common behavior throughout many freshwater organisms. Previous work has examined habitat modification in streams, and has provided useful examples and case studies that have informed this review (Moore 2006). When species modify their habitats, they often ameliorate unsuitable conditions for themselves and other taxa. When interactions occur, they are often facilitative or incidentally beneficial in nature, as benefits to other species are generally not the ‘intent’ of the habitat modifier. Positive effects of habitat modifying species do not necessarily require reciprocal benefits from beneficiary species, although positive feedback loops sometimes occur. Whether a commensal interaction or a mutualism as a result of reciprocal benefits, understanding the nature of facilitation and identifying potential positive feedback loops should be a priority of freshwater ecologists. Beavers (*Castor* sp.) are a prime example of a habitat modifier and exemplifies ecosystem engineering with the massive dam complexes that they construct (Naiman et al. 1988). For beavers, damming is a behavior that is carried out for its own benefit; while

numerous other species benefit from it, this behavior does not represent a direct interaction between species. The beaver is also an example that illustrates the context component of facilitative interactions. While some species benefit from the habitat complexity of beaver dams, the thermal stress associated with dams is detrimental to cold-water taxa and can cause shifts in assemblage structure (Wright et al. 2004, Wright and Jones 2006). This facilitation nevertheless has direct benefits for a variety of taxa, and the scale of beaver impacts likely represent the greatest contribution of all habitat modifiers.

Though perhaps less obvious, there are numerous other cases of habitat modification in freshwaters which have been shown to structure ecological communities. For example, net-spinning caddisfly (Hydropsychidae) larvae construct retreats in high-flow stream systems to filter food from the water column. These retreats significantly reduce flows in the areas immediately downstream, creating low-flow microhabitats that facilitate other benthic invertebrates (Nakano et al. 2005) and increase sediment stability (Albertson et al. 2014). Removal of habitat modifying species like case-building caddisflies can also result in shifts in community composition (Nakano et al. 2007).

In addition to the protection mutualism described above, nest association in the leuciscid fishes provides an example of habitat modification in streams. Adult males of the nest-building genera, especially *Nocomis*, alter available substrate in streams as part of their spawning behavior. For example, adult male *Nocomis* may move thousands of pebbles (Reighard 1943, Lachner 1952), equating to a total pebble-carrying distance greater than 25 km to complete a single large nest (Wisenden et al. 2009). *Nocomis* nests are distinct features on the benthoscape that differ starkly from the surrounding substrate (Maurakis

et al. 1992, Bolton et al. 2015), and are often the only sources of concentrated, un-silted gravel in heavily embedded or sediment-starved reaches (McManamay et al. 2010, Peoples et al. 2014). Nest building by *Nocomis* thus provides critical microhabitat for lithophilic-spawning fishes, providing a mechanism for *Nocomis* and associates to reproduce and persist in reaches of poor substrate quality (Hitt and Roberts 2012, Peoples et al. 2015). *Nocomis* nests also facilitate a diversity of benthic macroinvertebrates that begin colonizing upon nest construction (Swartwout et al. 2016).

Context dependency in positive interactions

An important feature of positive interactions is that their outcomes are rarely static: they can switch from being positive to negative with changing context (Bronstein 1994b, Noë and Hammerstein 1995). Context dependency may arise from changing abiotic (i.e. the environment in which the interaction occurs, spatiotemporal factors) or biotic (i.e. identity, traits, or abundance of participants) factors. All direct antagonisms, though often discussed as distinct types of interactions, are characterized by unidirectional flows of energy. Positive interactions, however, involve more complex energy transfers and thus more frequently display context dependent outcomes (Chamberlain et al. 2014).

Mutualism and facilitation may also have reduced interaction strength relative to antagonism (Sachs and Simms 2006, Moore et al. 2017), though Frederickson (2017) provides excellent counterpoints to the notion of mutualism breakdown. Despite the balance of resource exchange necessary to maintain mutually beneficial interactions, there is scant evidence of positive interactions consistently changing to parasitism in either ecological or evolutionary time (Frederickson 2017). In a meta-analysis of plant and mycorrhizal fungi interaction studies, for example, mean outcomes were

overwhelmingly positive (Hoeksema et al. 2010). Negative interaction outcomes are likely to have been observed under extreme cases of environmental context, supporting the notion of stable positive interactions in natural settings (Frederickson 2017).

Understanding how context dependency in freshwaters may alter the costs and benefits of biotic interactions will improve general ecological models and provide better tools for predicting biological responses to environmental change.

Abiotic variables affect stream organisms through dynamic processes that operate across spatial scales and establish context for biotic interactions (Jackson et al. 2001, Heino 2013). Peoples and Frimpong (2016a) examined, in part, the role of riparian land cover (as a proxy of instream habitat quality) on nest associative behavior. Results indicated that associates provide a benefit to hosts only in forested stream reaches. In degraded reaches, the benefits of the interactions were not able to outweigh the costs of the poor abiotic conditions. Another experiment examined the role of environmental fouling on the interaction outcomes of the crayfish-worm symbiosis. Two levels of fouling conditions (low: stream water and sterilized cobbles, high: stream water, sand, silt, unscrubbed cobbles) and two levels of symbiont density (high and low density worms per crayfish) were established in a lab setting (Thomas et al. 2013). Results indicated that the level of environmental fouling affected the relative benefits provided by the worms, as well as the reproductive success of juvenile worms.

Biotic factors can also shift the outcomes of positive interactions. In pairwise or multi-species interactions, the condition, abundance, ontogeny, and other biotic factors related to participants drive the outcomes of interactions in which they take part. For example, in ichthyochorous *Pacu* spp. (Serrasalminidae), increased body size significantly increases the

benefit to the host plant: large *Pacu* pass seeds through their digestive tract intact, whereas smaller fish damage the seeds and significantly decrease germination rates (Galetti et al. 2008).

In the Branchiobdellidan worm – crayfish symbiosis, the worms may feed on crayfish tissue when epibiotic parasite loads are low, significantly impairing the host's ability to respire (Brown et al. 2012). However, crayfish colonized by worms have the ability to alter densities of their symbiont. Both *Cambarus chasmodactylus* and *Orconectes cristavarius* of the crayfish family Cambaridae can actively groom themselves, removing high densities of worms to prevent a mutualistic cleaning symbiosis from switching to parasitism (Farrell et al. 2014). Effects of ontogeny have also been examined in the crayfish-worm symbiosis, and host age (and therefore size) has been shown to have important ramifications for the outcomes of the interaction (Skelton et al. 2014, Thomas et al. 2016). Smaller crayfish are more efficient groomers than adults, and can remove large worms from their exoskeleton. The result is that these young crayfish are colonized primarily by smaller worm species. As crayfish become adults, however, they lose the ability to remove large worms, and these superior predators prey on the smaller worm taxa (Thomas et al. 2016). As crayfish age, their dominant symbionts shift; this brings up questions of symbiont transmission (i.e. Fisher et al. 2017), life-history tradeoffs by worms, and regional and temporal shifts in symbiont diversity. These examples illustrate both biotic context dependency and the interactive effects that ontogeny can have on interaction outcomes.

A major finding in many positive interactions, including terrestrial and marine systems, is that symbiont or partner abundance maximizes benefits to the host at intermediate

abundance (Morales 2000, Izzo and Vasconcelos 2002, Brown et al. 2012, Palmer and Brody 2013, Skelton et al. 2016). In these instances, the maximum benefit for at least one interacting species cannot be achieved without a fitness cost to the other; there exists a conflict of interest in terms of resource exchange. To manage these conflicting interests, some hosts have the ability to punish exploitation by partners (i.e. Frost and Elias 1985, Izzo and Vasconcelos 2002, Farrell et al. 2014, Creed and Brown 2018), whereas others appear to have no defenses for exploitation (Stoll et al. 2017) or exploitation is not predicted to occur (Peoples and Frimpong 2016b). A recent study examining context dependent outcomes in nest associative leuciscids found that parasitic outcomes only occurred at low partner abundance (Silknetter et al. 2018). Hosts benefited from a dilution effect (*sensu* McKaye and McKaye 1977) of reduced brood predation, but only if associate abundances were sufficiently high. In this case, both host and partner maximize reproductive success by spawning in high abundances, and therefore no conflict of interest exists. Better understanding the role of partner/symbiont density on interaction outcomes will likely provide further insight into the evolution and significance of conflicting interests as well as the mechanisms that may mitigate them.

A research directive for studying positive interactions in freshwater systems

We propose a four-step approach for advancing the study of positive interactions in freshwater ecosystems: i. examining life history to identify previously unknown positive interactions and their causal mechanisms, ii. evaluating the role of context dependency in interactions, iii. quantifying implications of interactions at population and community levels, and iv. examining interactions across spatial scales. As it is a prerequisite to each one of these four pathways, our first and foremost

responsibility should be to gain a mechanistic understanding of the factors driving these interactions. We have given examples of several study systems in which a foundational understanding of these mechanisms exist, and for that reason, those findings are beginning to be incorporated into broader ecological theory.

Identifying new positive interactions and causal mechanisms

Many ecologists contend that the study of basic species life history has gone by the wayside in favor macroecological studies, and seek to bring life history studies back in vogue (Frimpong 2018). The call for the ‘resurrection’ of these concepts (Able 2016) coincides with a realization that modeling, species inference, and big data are only as good as the foundational ecology behind them. It is incumbent upon researchers to synthesize their findings and to incorporate personal notes and observations into the conclusions of their work, especially as their career comes to a close (Matthews 2015). Many of the examples of positive interactions discussed here have stemmed from the observations of earlier naturalists seeking to simply describe and understand the phenomena they witnessed. There is no substitute for curious, thoughtful exploration of the natural world, and there must certainly be meaningful, yet currently unknown positive interactions yet to be described. As ecologists, we must also seek to take what we know from one location, taxon, or discipline, and apply it somewhere else.

We can use existing knowledge of positive interactions in other systems to predict where and when others may occur in freshwater ecosystems. Convergent evolution has resulted in similar interactions between highly diverse taxonomic groups. Cleaning symbioses, for example, have been known to occur in marine crustaceans and

fishes for decades (Trivers 1971), yet only relatively recently have cleaning behaviors been identified in comparable freshwater taxa (crayfish: Brown et al. 2002; fishes: Severo-Neto and Froehlich 2016). It is also unsurprising that freshwater corals, which diverged from a common marine ancestor, rely on mutualist zooxanthellae for nutrition (Frost and Williamson 1980), similarly to their marine cousins. Aside from future studies of life history, following clues from evolutionary biology may be the most obvious way to find new examples of positive interactions.

We can also use general ecological theories, often developed in other ecosystems and/or taxa, to guide predictions of where we expect to encounter positive interactions. One useful framework for understanding and predicting positive interactions is market theory. Ecological market theory predicts positive interactions will occur between species with differential surplus and deficit production of key fitness resources (i.e. nutrition, transportation, protection, etc.), therefore encouraging resource trade (Noë and Hammerstein 1994, 1995, Hammerstein and Noë 2016). Additionally, the inefficient use of resources may lead to ‘trade deficits’, which would provide an evolutionary benefit to positive interactions (Palmer et al. 2010). For example, when two species have a competitive advantage in the production of different resources, exchange of resources would confer a greater benefit to both species than competition. Excess production of a given resource also reduces or removes the cost of losing that resource to interacting species, thus, positive interactions may be predicted. If the interacting species exchange the excess resource for some good or service, the result is mutualism; if they exchange nothing, it is commensalism. If we assume that this excess of resource production is what

drives interactions, then market theory lets us hypothesize both when we will have positive interactions and when they will be replaced by antagonistic interactions.

Another model we may ‘parasitize’ from another discipline is the stress-gradient hypothesis (SGH), which was developed to understand plant community dynamics (Bertness and Callaway 1994). The SGH predicts that species in more stressful environments are more likely to switch from competitive to mutually beneficial interactions (Callaway 2007, Brooker et al. 2008, He et al. 2013). Community benefits may be provided by facilitating species through habitat amelioration at high levels of physical stress, and benefits resulting from ‘neighborhood effects’ at high consumer pressure (Maestre et al. 2009, Malanson and Resler 2015). In freshwaters, this may include naturally stressful habitats like headwater streams, vernal pools and temporary wetlands, streambanks and shorelines, but also anthropogenically stressed habitats related to dams, reservoirs, or sites with impaired water quality. In disturbance ecology, the SGH provides a framework for research in which the inclusion of positive interactions studied at the local scale may inform broader spatiotemporal patterns and vice-versa (Nash et al. 2014).

While formulated for understanding plant community dynamics, the SGH has been applied to several stream-dwelling taxa as well. Fugère et al. (2012) found that in an assemblage of headwater stream detritivores, a decrease in resource quality (thus increased abiotic stress) shifted interactions from competitive to more neutral outcomes. Another study found that abiotic conditions influenced the factors structuring stream fish communities (Peoples et al. 2015). Specifically, sites with increased physical stress (i.e. land use impairments) were structured more by positive interactions than comparable

sites under more benign conditions (Peoples et al. 2015). While shoaling behavior and mixed-species aggregations have been shown to mitigate high levels of stress in marine fishes (Hoare et al. 2004), there is of yet no evidence of analogous interactions in freshwaters. This exemplifies how predictions in freshwaters can be informed by and extended from similar concepts in other systems. In environments which are highly stressful, facilitation may provide otherwise inaccessible niche space, and may increase biodiversity (McIntire and Fajardo 2014) or expand the ranges of participating species (He and Bertness 2014).

Lastly, known interactions should be evaluated to determine if outcomes might shift from negative or commensal to positive under some context. This may relate to cases of extreme environmental stressors, but may also reflect natural variation in outcomes. It is common for observational studies or experiments conducted *in situ* to exhibit different interaction outcomes. The meta-analysis by Hoeksema et al. (2010), for example, revealed that the mean interaction outcome of the plant-mycorrhizal fungi symbiosis was positive, but that negative interactions were also quite common. While meta-analysis is certainly not always possible, this finding illustrates why it is necessary for researchers to investigate interactions under a range of environmental and biotic conditions. A negative mean outcome does not discount the potential significance of positive interactions in shaping a given community and vice versa. Considering that mutualism is in fact reciprocal parasitism that results in net benefits to both individuals, a reexamination of presumably negative interactions (and especially parasitism) may reveal a range of conditions which includes positive outcomes.

Predicting context dependency in positive interactions

One way to understand context dependency in species interactions is through the use of modeling and game theory (Trivers 1971, Axelrod and Hamilton 1981, Bull and Rice 1991), which have given rise to the concept of the biological market (Noë and Hammerstein 1994). The biological markets model (BMM) seeks to explain exchange rates of resources using both raw supply and demand ratios, as well as opportunities for partner choice (Bshary and Bronstein 2004). Economic concepts are applied to ecology, and are used to evaluate when and why partners choose to cooperate (i.e. a mutually beneficial interaction). Using these ideas at ecological (as opposed to evolutionary) timescales, the BMM can incorporate context dependency to determine under what conditions a facultative symbiont will engage in an interaction. Most interspecific interactions in freshwater systems involve highly mobile organisms, and partner choice adds a great deal of complexity to this system when compared to terrestrial systems often dominated by sessile plants.

Nest association provides an illustrative example of how novel freshwater interactions challenge the predictions of the BMM. The traditional BMM framework predicts that for hosts to maximize their returns, they should i) have high initial investments to gain entry into the marketplace, and ii) that hosts should be selective when choosing trading partners. In nest association, the host has a high initial investment (loss of body condition) by building the nest, but hosts are not selective with associates, and associates are most beneficial to hosts at high abundances. The apparent lack of partner control allows for 'freeloading' by associates, challenging a classic assumptions of market economics and illustrating how BMM predictions may depends on the context of the system's specific biology. Including the unique features of some interactions in

freshwater systems may force a reevaluation of game-theoretic models, but should ultimately increase their applicability. The ability to make predictions at different spatial and temporal scales, from the individual to population level, make modelling approaches an invaluable asset for expanding our knowledge of positive interactions.

Positive interactions can be observed and tested at the individual level, but understanding the collective importance of positive interactions within a population or a community can be difficult. One way to address this difficulty is to propose multi-factor experiments to compare with and among experiments to address some biotic or abiotic gradients. The provision of a 'common currency' by using effect sizes (Agrawal et al. 2007) allows researchers to determine how sign and strength of interactions are context dependent along those gradients, and which are consistent across broad spatial or temporal ranges. Another consideration is how interaction outcomes are measured. While determining net outcomes are acceptable for most studies, these are often the product of bi-directional energy transfers, and positive or negative components of the interaction may be context dependent (Holzapfel and Mahall 1999). Thoughtful experimentation, combined with a strong understanding of related disciplines, will go a long way to further advise our predictions. Perhaps one benefit of lagging behind in respect to positive interactions in freshwaters is that we do not need to make the same mistakes or rehash arguments which have been resolved in other disciplines.

Recognizing implications of interactions at population and community levels

Theories seeking to explain the nature of populations and communities have historically lacked thoughtful considerations of positive interactions (Bronstein 1994a, Stachowicz 2001; but see Jones et al. 1994 for a classic example), and thus their inclusion may alter

many of those theories' predictions (Bruno et al. 2003, Agrawal et al. 2007).

Foundational concepts in ecology which may benefit from the inclusion of positive interactions are niche theory and species distributions, density dependence, and biodiversity – ecosystem functioning relationships. Because positive interactions in freshwaters have played such a small role in their revision, we expect that further exploration will yield insights into the peculiarities of freshwaters and help to better inform these concepts.

Population dynamics are largely the result of biotic context in the form density dependence, and it is well understood that increased density is a limiting factor to population growth. Increased population sizes in a fixed space results in increased competition and predation between and among species, and diseases and parasites often become more effective at infecting hosts. Negative density dependence causes populations to stabilize over time near their carrying capacity. One well-understood caveat to this relationship is the Allee effect, which describes positive density dependence resulting from intraspecific cooperation at low densities (i.e. well under carrying capacity). However, some work has shown that interspecific mutualism and facilitation, analogous to intraspecific cooperation, can result in positive dependence even at high density (Bertness et al. 1999). In freshwater systems where ontogenetic shifts (Skelton et al. 2014) or seasonal symbioses (Gottsberger 1978) occur, we may expect to find temporally discrete instances of positive density-dependence. Symbiont abundance is often directly proportional to community abundance and has been documented as a significant contributor to determining interaction outcomes (Johnston 1994b, Brown et al. 2012), thus illustrating the essential role of density dependence in positive interactions.

The Hutchinsonian niche concept is comprised of a ‘fundamental’ whole including all biotic and abiotic factors that the species may utilize, but also of a ‘realized’ reduced niche because of competitive interactions (Hutchinson 1957). However, positive interactions can counter some of these costs and thus significantly expand the realized niche; this has had major implications for breadth (Batstone et al. 2018), overlap (Bruno et al. 2003, Afkhami et al. 2014, Bulleri et al. 2016), and partitioning (Lee and Inouye 2010) of the niche. Freshwater communities subjected to habitat loss and other environmental change may contain species experiencing shrinking fundamental niches, resulting in lost connectivity, isolation, and potentially extirpation. However, if the community contains facilitator species that can ameliorate those stressful habitats, beneficiary species in the community may maintain niche breadth, experience greater niche overlap, or better to partition resources. The presence of facilitators and positive interactions may thus increase the resiliency of the community by mitigating losses to individual species fundamental niche. Though exhaustive work has documented the role of the niche in shaping freshwater communities (Jackson et al. 2001, Poff et al. 2006), positive interactions have been largely neglected. One notable exception includes the ability of mutualistic nest association between Bluehead Chub (*Nocomis leptcephalus*) and strong associate species to expand their ranges in the New River in North Carolina (Buckwalter et al. 2018). Future studies examining positive interactions in niche dynamics should seek to incorporate the distinctiveness of freshwaters in their predictions, particularly streams that are characterized by a continuum of dendritic and hierarchical networks.

We must seek to understand positive interactions in a community context. We have shown numerous examples of how positive interactions involve numerous taxa and can affect community structure. However, the majority of models and empirical studies reduce complex systems down to simpler pairwise interactions that are rarely ecologically realistic (Thrall et al. 2007, Palmer et al. 2015). This is understandable, as ecological systems are difficult to manipulate; conducting even simple pairwise experiments can often become a feat in and of themselves. However, many positive interactions occur in a community context, with different species' contributions resulting from specific traits (i.e. habitat modification) or interactive effects (i.e., third-party interactors) that result in variable interaction strengths and outcomes. Moving from a pairwise to a community-based framework of understanding positive interactions in freshwater systems will enable us to provide more realistic predictions of whole community response to environmental change in the context of positive interactions. Lastly, there is a need to incorporate positive interactions into our understanding of ecosystem function. A major finding in several recent reviews is that biodiversity plays a significant role in shaping a suite of ecosystem services (marine systems: Stachowicz et al. 2007; terrestrial plants: Hooper et al. 2012). Positive interactions can affect biodiversity in variable, but meaningful ways, exemplified by Engelhardt and Ritchie (2001), who looked at the benefits of diverse macrophyte assemblages on wetland ecosystem functioning. Greater richness of submerged wetland plants decreased competition due to a sampling effect, resulting in increased algal and total plant biomass (Engelhardt and Ritchie 2001). Positive interactions may have strong indirect benefits on diversity because of the sampling effect or complementarity (Cardinale et al. 2002, 2007,

Batstone et al. 2018). Furthermore, foundational species or habitat modifiers may have direct effects; symbiont or indirect benefactor diversity may increase, while non-associative species or weak, facultative symbionts may exhibit diversity declines (Hacker and Gaines 1997, Bulleri et al. 2016). Though freshwater lakes, reservoirs, and rivers make up less than 1% of all available waters globally (Carpenter et al. 2011), the biodiversity and ecosystem services they provide are vastly disproportionate to their volume. Quantifying the benefit of positive interactions to ecosystem function will better guide future predictions and allow for more effective management and conservation of biodiversity (Halpern et al. 2007).

Investigating positive interactions at multiple scales

Understanding the mechanisms that contribute to species coexistence is a fundamental goal of ecology. However, testing hypotheses about species interactions can be greatly affected by the spatial scale at which they are examined (Levin 1992, Fausch et al. 2002). At small (i.e. local, microhabitat) scales, predation studies often find patterns of avoidance, as prey species distributions are shaped largely by predator avoidance (Vuorinen et al. 1983, Power 1984). At the same scale, competition studies exhibit ‘checkerboard’ distributions as species confine themselves to optimal habitats devoid of their competitor (Pearson and Dawson 2003). At larger scales in the river network (reach, stream, watershed; Frissell et al. 1986) however, predation studies often find positive or null patterns as a result of similar habitat requirements, and because predator distributions are in part determined by the presence of suitable prey resources. Antagonistic interactions typically take a back seat to environmental conditions in shaping freshwater communities at large scales (Peres-Neto 2004), but there have been few examinations of

positive interaction patterns at different scales (but see Hopkins et al. 2017 for an investigation of defensive symbiont's role at multiple scales).

Freshwaters, excluding isolated lakes, are hierarchical in nature (e.g., Poff 1997); scales range from hyper-local microhabitat to entire watersheds, and interaction types may scale even globally (Frissell et al. 1986, Domisch et al. 2015). Biotic interactions affect spatial patterns at local scales (including mutualism: Bascompte 2009), but beyond this fine-grained approach they have typically been dismissed as unimportant (Pearson and Dawson 2003). Species distribution modelling (SDM) is a suite of methods for predicting how species occur across landscapes (Guisan and Zimmermann 2000, Elith and Leathwick 2009), and has seen rapid growth as computational power has improved. With appropriate survey data and meaningful independent variables, range predictions can be highly accurate and are largely uninhibited by availability of appropriate models (Elith and Leathwick 2009). However, modelling for species with dynamic ranges (highly mobile) or using data that is limited in time or space can be more challenging and result in reduced predictive power. Biotic interactions are beginning increasingly incorporated into SDMs (Guisan and Thuiller 2005, Wisz et al. 2013, Godsoe et al. 2015, Joseph et al. 2016), but only recently have positive interactions been considered (Afkhami et al. 2014, Filazzola et al. 2017, Duffy and Johnson 2017). These models provide a framework to directly test for positive spatial correlations between species, even when those associations and their outcomes are context dependent (Tikhonov et al. 2017). (Peoples and Frimpong 2016a) examined species co-occurrence resulting from positive interactions, but there have been virtually no similar studies examining large scale patterns of positive co-occurrence in freshwaters. Referring back to the goal of

identifying new positive interactions, there are now models that infer species associations from large datasets (Morueta-Holme et al. 2016). This exemplifies the feedback between local observations and large-scale patterns, and perhaps a means for identifying previously unseen positive interactions. It is well known that species involved in positive interactions co-occur at small spatial scales, and many mutually beneficial symbioses are obligatory for one or both species. Understanding how positive interactions change across spatial scales, while accounting for catchment structure, continuous water quality conditions, and imperfect detections associated with freshwaters, should be a top priority.

Applying positive interactions into management

Understanding positive biotic interactions in freshwater systems is more than a pure ecological pursuit; it can have serious implications for conservation and management. One emerging concept involves conservation of positive interactions. One of the greatest problems with species conservation is the limited resources available to managers; getting the biggest ‘bang for the buck’ often results in funding for charismatic species or those with commercial value. As such, non-game species remain disproportionately imperiled, and freshwater biodiversity is and will likely remain under assault as the human population continues to climb (Jelks et al. 2008). Ichthyochory interactions, which are common throughout much of the Amazon basin, are exposed to increased human pressures including overexploitation of fisheries and the damming of large river sections (Correa et al. 2015a). If fish are harvested before they can reach maturity, or if natural flood pulses are removed, fish will either lose access to the fruits or prevent them from germinating. Examples like this show how the conservation of a few taxa may provide a means of benefitting multiple species, while only focusing efforts on one. Efforts focused

on host taxa which ameliorate habitats (dam, nest building), for instance, provide quantifiable benefits to multiple partners (Byers et al. 2006). The hosts are often generalists or locally abundant (beavers, minnows), yet support diverse taxa which may be particularly threatened by anthropogenic impacts. If necessity were truly the mother of invention, then as ecologists, we would do well to think creatively about how we can use positive interactions to conserve freshwater species.

One extension of conservation, and another potential avenue for the application of positive interactions, is in the restoration of freshwater systems (Byers et al. 2006, Halpern et al. 2007). Facilitative species are often necessary for colonization and succession to occur (Diamond 1986, Cardinale et al. 2001, Nakano et al. 2005), which is particularly important for high-gradient streams which are characterized by dynamic flows. In addition to successional species, it is often necessary to introduce foundational species in areas which are regularly exposed to stressful conditions. As shown in an arid terrestrial system by Filazzola et al. (2017), the management of dominant foundational species can have multiple community benefits through interaction networks. Managing foundational taxa in addition to making habitat improvements typical of restoration plans represents a novel way to leverage positive interactions for ecological benefit. Recent work has also examined the ability of positive interactions to cause alternative stable-states within certain ecosystems (Kéfi et al. 2016). Positive feedback loops are a necessary condition for alternative states to occur, and facilitation or other positive interactions appear to play an integral role. For example, environmental degradation (decreased resource availability, increased abiotic stress, etc.) may result in a shift from a highly productive stable state to one with decreased productivity. In this case, it may take

the restoration of facilitator or other positive-interacting species to restore the site to its previous state (Kéfi et al. 2016).

Positive interactions, by definition, must result in benefits to one or more of the species involved; however, this does not necessarily equate to what ecologists or society *writ large* may consider a ‘benefit’. For example, (Simberloff and Holle 1999) explained how positive interactions may result ‘invasion meltdowns’, in which non-native species may rapidly proliferate and cause changes to native community composition. Such proliferation may be the result of facilitation or mutualism between non-native or introduced species; chiefly through pollination, seed dispersal, and habitat modification (Simberloff and Holle 1999). Ichthyochory in the Illinois River Waterway (Mississippi River) provides at least one example of this phenomena, though the negative effects are difficult to quantify (VonBank et al. 2018). Here, the invasive Common Carp (Cyprinidae: *Cyprinus carpio*) feeds on and disperses seeds of both native and non-native vegetation. Though carp are already widely distributed throughout the Mississippi River, this vector may expand the dispersal of range-limited invasive plants; thus invasive species may facilitate the expansion of one another (VonBank et al. 2018). Though some evidence for such catastrophic ‘invasion meltdown’ does exist, such fears have been largely tempered after Simberloff’s (2006) follow-up examination of this phenomena. Another potentially negative outcome resulting from positive interactions is that non-native species may take advantage of or ‘hijack’ hosts. In the Chattahoochee River, the Rough Shiner (Leuciscidae: *Notropis baileyi*) has been introduced and appears to outcompete a native nest associate, resulting in altered community composition and reduced species evenness (Walser et al. 2000, Herrington and Popp 2004). While

disturbance often allows for the successful invasion of non-natives, many of the same traits that allow this colonization (early colonizers, habitat generalists) are shared by facilitative, foundational species. An interesting, though largely untested hypothesis, is that positive interactions may buffer those communities from disturbance. Clearly, context will be important in determining how positive interactions will affect the proliferation of invasive species and the structure of native communities (Bronstein 2009).

A final application of positive interactions in freshwater systems is in the service of aquaculture. A major challenge in aquaculture is to increase production while reducing environmental impacts (Whitmarsh et al. 2006). Raising positively interacting species in polyculture may provide a way to achieve these goals. In coastal marine systems where commercial aquaculture dominates, there is a growing body of work suggesting the benefits of polyculture and specifically, ‘integrated multi-trophic aquaculture’ (Nobre et al. 2010). Freshwater aquaculture is important and will continue to grow as an industry in the face of environmental change (Ficke et al. 2007), yet research into sustainable management practices lags far behind those in marine systems. Despite this fact, positive interactions can be applied to freshwater aquaculture even if they were developed for marine production. One example is the work by (Natrah et al. 2014), who have documented the significance of a mutualism between microalgae and bacteria in aquaculture. When used in flow-through systems and in feeding applications, polycultures of microalgae and bacteria can enhance the productivity and efficiency of aquaculture relative to monocultures of either. Another example of positive interactions in the service of aquaculture is the mutualism between euryhaline *Tilapia* sp. (Cichlidae)

and *Chlorella* sp. (Chlorellaceae). *Tilapia* provide carbon dioxide which would otherwise limit algae growth, and in exchange, *Chlorella* oxygenates and detoxifies water to increase growth of the fish (Gilles et al. 2008).

Conclusions

As illustrated by the extent of future directions discussed in this review, we as freshwater ecologists have a long way to go before we can fully appreciate and understand positive interactions. Though the unknown may seem to dwarf that which we do know, the studies discussed have shed light on a diverse array of systems and mechanisms that now form the foundation of our understanding. The interaction between freshwater cleaners fishes and their clients, for example, illustrates two important concepts which were evident throughout much of this review: i) freshwater interactions, even when analogous or sharing an evolutionary origin with interactions in other systems, are generally less studied, and ii) positive interactions need not be symbiotic, pairwise, long-term, or require significant investment to play a meaningful role in the shape of ecological communities. That freshwater cleaning stations have only been identified within the last several years emphasizes the point that the study of positive interactions is in an exploratory stage (Severo-Neto and Froehlich 2016). Examinations of basic life history will be necessary to identify examples and causal mechanisms driving positive interactions. Modern ecology has a strong focus on predicative ability and taxonomic inference, but there can be no substitute for direct observations of the phenomena themselves. Positive interactions may be evolutionarily stable (Frederickson 2017) but changing context in the span of ecological time has been demonstrated to affect interaction outcomes. Moreover, context and interactive effects at the community level or

higher may result in asymmetric outcomes or competition between partner species. The notion that fitness benefits are maximized at intermediate symbiont density may be a rule for species interactions, for example, but we have documented at least one exception. Identifying novel mechanisms and processes in freshwaters can help to inform general ecological principles, and in turn, we can use the predictions and theory from broader ecology to understand the interactions we seen in our streams, rivers, lakes, and wetlands. This give-and-take is the mark of a healthy research focus, and we are on our way to achieving such a balance.

CHAPTER TWO

MUTUALISM OR PARASITISM: PARTNER ABUNDANCE AFFECTS HOST FITNESS IN A FISH REPRODUCTIVE INTERACTION*

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Abstract

1. Mutualisms are ubiquitous in nature but are understudied in freshwater ecosystems. Mutualisms can be unstable, shifting to commensal or even negative outcomes with context. Quantifying context dependency in mutualisms is critical for understanding how biotic interactions will shift along disturbance gradients in freshwater systems.
2. A common reproductive interaction among stream fishes, *nest association* occurs when individuals of one species spawn in nests constructed by a host fish. Hosts benefit from a dilution effect: high proportions of associate eggs decrease the odds of host brood predation. Thus, partner abundance can be an important source of biotic context influencing the outcome of an association.
3. We conducted a large in situ experiment manipulating abundance of partner yellowfin shiner (Leuciscidae: *Notropis lutipinnis*) (absent, low, high) at constant abundance of host bluehead chub (Leuciscidae: *Nocomis leptocephalus*), and quantified chub reproductive success using genetic tools.
4. Evidence suggests that the nest association switched from mutualistic to parasitic outcomes as shiner abundance decreased. Chub reproductive success was highest at high

shiner abundances. However, chub reproductive success was actually higher in the complete absence of shiners than at low shiner densities.

5. This study shows that outcomes of biotic interactions in freshwater systems are context-dependent, and that partner abundance can be a key source of context-dependency in nest associations. We encourage future studies on freshwater mutualisms, which are thus far largely overlooked, relative to competition and predation.

Introduction

Positive biotic interactions such as mutualism and commensalism are important drivers of population abundance and community structure (Boucher, James, & Keeler, 1982; Bruno, Stachowicz, & Bertness, 2003), but are understudied relative to negative interactions (competition, predation, and parasitism; Bronstein, 1994a, 1994b, 2009). An important feature of positive interactions is that their outcomes are rarely static: they can switch from being positive to negative with changing context (Bronstein, 1994b; Noë & Hammerstein, 1995). Context-dependency may arise from reduced interaction strength, relative to antagonism (Moore, 2006; Sachs & Simms, 2006; but see Frederickson, 2017 for an alternative perspective), or complexity of resource transfers among participants (Chamberlain, Bronstein, & Rudgers, 2014). Context dependency may also arise from changing abiotic (i.e., the environment in which the interaction occurs; Lee, Kim, & Choe, 2009; Thomas, Creed, & Brown, 2013) or biotic (i.e., identity, traits or abundance of participants; Brown, Creed, Skelton, Rollins, & Farrell, 2012) factors. Understanding how context dependency alters the costs and

benefits of biotic interactions will improve general ecological models and provide better tools for predicting biological responses to environmental change.

Mutualisms and context dependency are understudied in freshwater systems; most of our knowledge on the subject comes from studies of terrestrial plants (He & Bertness, 2014). In fact, a review of biotic interactions in freshwater systems (Holomuzki, Feminella, & Power, 2010) included little information on mutualisms—not because of oversight by the authors, but because so few case studies exist outside of interactions with habitat modifying species (see Moore, 2006). Moreover, the review made no mention of the effect of context dependency on mutualisms. More mechanistic studies are required to quantify the roles of context-dependent mutualisms in shaping population- (Horn et al., 2011; Johnston, 1994a), community- (Brown, Creed, & Dobson, 2002; Johnston, 1994b; Nakano, Yamamoto, & Okino, 2005; Peoples, Blanc, & Frimpong, 2015; Skelton, Doak, Leonard, Creed, & Brown, 2016) and ecosystem-level (Moore, 2006; Skelton et al., 2016) processes in freshwater systems.

One common positive interaction among North American freshwater fishes is nest association, in which “associate” species (partners) spawn in nests constructed by a host. Nest association can be considered a disjunctive symbiosis, as the species have an intimate short-term relationship, but lack the physical attachment or longevity typically associated with the more emblematic conjunctive symbioses. In North America, chubs (Leuciscidae: *Nocomis* spp.; Tan and Armbruster 2018) are widespread hosts; their nests are used by at least 35 associate species throughout their range (Johnston & Page, 1992). Adult male chubs build spawning nests in the spring and early summer by collecting gravel in their mouths and depositing the individual stones into a mound. Associates

benefit from nest association with chubs via two mechanisms. First, the concentrated gravel of the nest provides suitable spawning substrate and keeps eggs from smothering in silt (Maurakis, Woolcott, & Sabaj, 1992; Peoples & Frimpong, 2013; Vives, 1990). Second, male chubs provide an element of parental care by moving and adding stones even after their own spawning has ceased (Wallin, 1992), further protecting eggs from most predators (Johnston, 1994a). In return, hosts benefit from a dilution effect (*sensu* McKaye & McKaye, 1977) when predators are present; high proportions of associate eggs on nests decrease the likelihood of predation on chub eggs (Johnston, 1994b; Wallin, 1992). It is common for large chub nests to attract hundreds of individual associates, even when only one associate species is present (McAuliffe & Bennett, 1981; Meffe, Certain, & Sheldon, 1988). Brood dilution rates of up to 97% have been documented as a result of high adult associate abundance (Cashner & Bart, 2010; Wallin, 1992). However, associate abundance is naturally variable across ecological gradients such as stream size and land use (Peoples et al., 2015), resulting in some nests attracting low abundances of associates or even none (Y. Kanno, unpublished data). Because brood dilution is a key mechanism making the relationship beneficial for hosts, heterogeneity in associate abundance is a form of biotic context that may determine interaction outcomes.

In this study, we conducted an in situ experiment to examine associate abundance as biotic context in determining outcomes of a reproductive interaction between host bluehead chub *Nocomis leptocephalus* (hereafter, *chub*) and partner yellowfin shiner *Notropis lutipinnis* (hereafter, *shiner*), a common nest associate in the southeastern USA. In this system, shiners always benefit from the interaction (versus spawning in the

absence of chubs); thus, context dependency would be evident in differences in host reproductive success. Under a uniform treatment of predation, we hypothesised a commensalistic interaction at low associate abundances because the dilution effect on chub reproductive success should be negligible. We hypothesised the interaction would shift to being mutualistic at higher associate abundances due to the positive effects of brood dilution on chub reproductive success.

Methods

We conducted an in situ experiment with a randomised complete block design to test for effects of shiner abundance on the reproductive success of host chubs. We constructed 12 instream enclosures, removed non-focal species, and manipulated shiner abundance to three levels (absent, low and high) while holding constant abundance of chubs and piscine egg predators, which are necessary to provide a mechanism for brood dilution (i.e., brood dilution is not meaningful in the absence of predation). Three days after spawning, eggs were removed from nests and later identified to species using microsatellite genetic markers. Once all spawning had ceased, we re-ran the experiment with a new batch of individuals, resulting in four replicates of three treatment levels in each of two temporal blocks ($n = 24$). Using chub egg count as a proxy for host fitness, we compared treatment means to quantify effects of partner abundance on host reproductive success.

Study site and experimental methods

This study was conducted from April to June of 2017 in Six Mile Creek, a second-order tributary to the Savannah River of northwestern South Carolina, USA (34.822, -82.828). This stream is typical of the Piedmont ecoregion (Omernik, 1987),

with moderate gradient, regular pool/riffle sequences, and a narrow but intact buffer of riparian vegetation. The watershed is a mix of low-intensity agriculture (mainly livestock grazing) and deciduous forest, resulting in substrate dominated by sand in pools, and gravel and cobbles in riffles. Site selection was based on experimental feasibility—perennial flow, stream size, contiguous access from landowners (*c.* 500 m), and abundance of focal species.

We constructed 12 experimental units (EUs), consisting of instream enclosures constructed of 4.75 mm fabric block nets, supported by a frame of steel posts and backed by two-panel strips of 5 × 10 cm welded fencing (*sensu* Peoples & Frimpong, 2016; Wallin, 1992). Block net height was 122 cm, with >30 cm above the ordinary high-water mark (OHWM). Net width extended laterally beyond the OHWM as well, ranging from 20 to 50 cm per side. To prevent fish movement between EUs, a block net apron of ≥30 cm was anchored to levelled substrate using 23 kg form-fitting sandbags. Enclosures were constructed to provide each EU with the necessary spawning (Bolton et al. 2015; Wisenden et al. 2009) and feeding (Rohde et al. 2009) microhabitats for each species (typically one riffle-pool sequence).

We removed all fishes from EUs using double-backpack electrofishing. We electrofished until no fishes >40 mm were captured, then followed with a final pass using increased voltage; a minimum of seven electrofishing passes were conducted in each EU. Adult individuals of all focal species were retained in flow-through holding tanks and monitored for signs of handling stress; all non-focal species and focal species exhibiting stress (e.g., lethargy, laboured breathing, erratic swimming) were released outside of the experimental area. Focal species were then restocked at predetermined abundances

(Table 1). Each EU received two mature male chubs (with total length ≥ 115 mm total length and prominent nuptial tubercles; *sensu* Jenkins & Burkhead, 1994) and 15 female chubs (≥ 70 mm total length with visibly engorged abdomens; Jenkins & Burkhead, 1994) of approximately equal total length. Potential females not exhibiting obviously engorged abdomens were excluded to reduce the potential for mistakenly stocking immature males. Adult shiners (≥ 60 mm) were stocked at either high (80), low (15), or control (0) abundances, with each treatment randomly assigned to four EUs. Ambiguous secondary sexual characteristics prevented us from knowing exact sex ratios of shiners. However, we are confident that shiner stockings represented natural sex ratios because (a) all individuals came from within a close proximity of the experiment, and (b) individuals were randomly stocked. The control treatment lacking associates was necessary to determine a baseline level of reproductive success for chubs in the absence of a dilution effect. Shiner abundances in EUs were chosen to reflect abundances observed in nearby streams of similar characteristics, based on ongoing community sampling (Y. Kanno and B. Peoples, unpublished data), and are consistent with previous experimental studies of nest association (Peoples & Frimpong, 2016; Wallin, 1992). While as many as 500 shiners can be located on a nest at a given time in some streams (Meffe et al., 1988), our *high* treatment (80 individuals) is more realistic when considering the small stream size and limited number of host individuals per EU.

Table 2.2: Stocking abundances for each of the three experimental treatments. All fish were removed from each experimental unit via backpack electrofishing prior to stocking. Species codes are as follows: BHC (bluehead chub *Nocomis leptoccephalus*), YFS (yellowfin shiner *Notropis lutipinnis*), NHS (northern hogsucker *Hypentelium nigricans*), STJ (striped jumprock *Moxostoma rupiscartes*).

Treatment	BHC ♂	BHC ♀	YFS	NHS	STJ
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Control	2	15	0	1	1
Low	2	15	15	1	1
High	2	15	80	1	1

To standardize egg predation among EUs, we included one individual of each species of the egg predators striped jumprock (*Catostomidae: Moxostoma rupiscartes*) and northern hogsucker (*Catostomidae: Hypentelium nigricans*). These are large-bodied fishes that have been documented to prey on fish eggs (Frimpong & Angermeier, 2009), and this density reflects abundances observed in nearby streams of similar characteristics (Y. Kanno and B. Peoples, unpublished). Because a previous study of similar design (Peoples, Floyd, & Frimpong, 2016) found no effect of predator density (low versus high) on chub reproductive success, we did not vary predator density and instead focused only on the effects of partner density. Other co-occurring cyprinids may function as egg predators on chub nests, but also as nest associates, and were accordingly not used as egg predators in this experiment. Other potential egg predators include crayfishes (*Cambaridae: Cambarus* and *Procambarus* spp.; Dorn & Wojdak, 2004; Eversole, 2014), juvenile salamanders (*Plethodontidae: Desmognathus* and *Eurycea* spp.; Blaustein, Sadeh, & Blaustein, 2014; Parker, 1994), and various other predacious invertebrates that have been observed burrowing in chub nests in previous studies (Light, Fiumera, & Porter, 2005; Swartwout, Keating, & Frimpong, 2016) as well as the present one. Manipulating abundance of these egg predators was not feasible, and we assumed equal effects of these taxa across EUs.

Beginning the day after stocking, spawning observations were recorded at least twice daily using methods modified from Peoples et al. (2015) for the duration of the

experiment. Initial stocking of block 1 occurred on 07 May and observations continued until 20 May; block 2 was stocked on 27 May with observations continuing until 8 June. Wearing polarised sunglasses, one worker walked the length of the experimental area and located fish and nests to record whether they were spawning. All nests were measured daily for size (i.e., length, width and height) to indicate whether unobserved activity had occurred. Chub spawning was evidenced by the presence of a conspicuous gravel mound in the experimental unit. Several EUs had deep undercuts and/or pools, and in these areas underwater video observations were made periodically to ensure no nests went undetected. Due to the conspicuous spawning of both target species which can last for several days, and since the study stream was small (no more than 4 m wide and 1 m deep), we are confident that no nest construction went undetected. Spawning began in the EUs on 08 May 2017, and nest-building and spawning activity continued within the EUs until 26 June. Video and/or binocular observations were made when active spawning was identified. We harvested eggs from nests 3 days after initial nest observation to maximise the time available for spawning to occur without the risk of eggs hatching into larvae; in warm months, mobile larvae have been observed in as few as 3 days after initial spawning (Peoples & Frimpong, 2016). Consistent with Maurakis and Woolcott (1996), all nests were initiated at night, and thus all new nest observations were made in the early morning hours. To harvest eggs, we placed a 1 m², 500-µm drift net immediately downstream of the nest and anchored it to the substrate to prevent sample loss. Stones were removed from the nest by hand and agitated in the water column, allowing eggs, invertebrates and detritus to drift into the net. Once the nest had been completely

deconstructed, the contents of the drift net were transferred into 100% non-denatured ethanol.

Egg identification and statistical analysis

Eggs of confamilial species are very difficult to distinguish based on external characteristics. However, molecular tools are becoming increasingly useful for identifying eggs and larvae of lotic leuciscids (Cashner & Bart, 2010, 2018; Peoples, Cooper, Frimpong, & Hallerman, 2017). Eggs were identified to species using microsatellite genetic markers developed at the South Carolina Department of Natural Resources Populations Genetics Lab housed within the Hollings Marine Laboratory in Charleston, SC (details provided in Supplemental Materials). In brief, genomic DNA was isolated from eggs using a modified spin-column procedure. Isolated DNA was then amplified via polymerase chain reaction using a multiplexed group of three primer pairs, each with a unique allelic size range and dye colour, corresponding to one of three microsatellite loci (Ca5, Nme25C8.208 and RSD53) diagnostic for the two focal species. Fragment analysis was then conducted on the amplified DNA using capillary gel electrophoresis on a CEQ™ 8000 (Beckman Coulter, Inc; Fullerton, CA) automated sequencer. Finally, each chromatogram was scored for species identification using Beckman Coulter CEQ™ 8000 Fragment Analysis Software. Detailed methodology for selection of microsatellite loci and genetic analyses can be found in Supporting Information Appendix S1.

Due to variable egg abundances and the costs and logistics of genetic analysis, some EUs necessitated subsampling, while egg samples from other EUs could be analysed in total. When 35 or fewer eggs were collected from an EU, all eggs were

identified using molecular markers and direct abundance of chub eggs was determined. For EUs with >35 intact eggs, two separate subsamples were analysed, and the percentages of chub eggs were compared to ensure subsampling was representative of true proportions. In all cases, the proportion of chub eggs in the two subsamples were within 5% of one another, so the weighted average was calculated and that value was used for extrapolation. All activities were ethically reviewed and improved, and were conducted under the Clemson University Institutional Animal Care and Use Committee protocol number 2017-015.

We used a generalised linear model of a Poisson distribution (appropriate for count data), with trial number as a block, to quantify the effect of shiner abundance on chub egg abundance as a proxy for host fitness. The data were analysed with a blocked analysis of variance (ANOVA) fit to a Poisson distribution through the log-link function, to account for the count data of chub egg abundance. We then used a conservative post hoc Tukey's test to compare treatment means. All analyses were conducted in R version 3.4.3 (R Core Team, 2017).

Results

Chubs constructed nests in 20 of the 24 EUs, and chubs spawned in several EUs of each treatment. A total of 8,692 eggs were collected between the two blocks; genetic analysis identified 3,974 chub eggs and 4,718 shiner eggs. Although we never observed piscine egg predators disrupting nests, most nests we sampled contained high densities of juvenile salamanders and a diversity of predacious invertebrates.

Chub egg abundance differed among the three treatments ($F_{2,23} = 30.1, p < 0.0001$), indicating that associate abundance affected host reproductive success (Figure 1). Nest association was mutualistic at high shiner abundance; in this treatment, chub egg abundance ($\bar{x} = 400.0$, standard error [SE] = 297.7) was significantly greater than control ($Z_{1,24} = 32.0, p < 0.0001$) and low abundance treatments ($Z_{1,24} = -10.5, p < 0.0001$). The Tukey's test also revealed that host egg abundance was significantly reduced at low associate abundance ($\bar{x} = 0.6, SE = 0.5$) when compared with the control ($\bar{x} = 146.4, SE = 81.4$), suggesting a parasitic interaction ($Z_{1,24} = -8.5, p < 0.0001$). Of more than 7,000 eggs deposited in high abundance treatments, more than 60% were identified as shiner ($\bar{x} = 671.9, SE = 400.5$). In contrast, only a total of 15 shiner eggs were identified in all low abundance treatments ($\bar{x} = 3.0, SE = 2.3$). See Figure S1 in Appendix S2 for a plot of all chub egg abundance data for each nest per treatment.

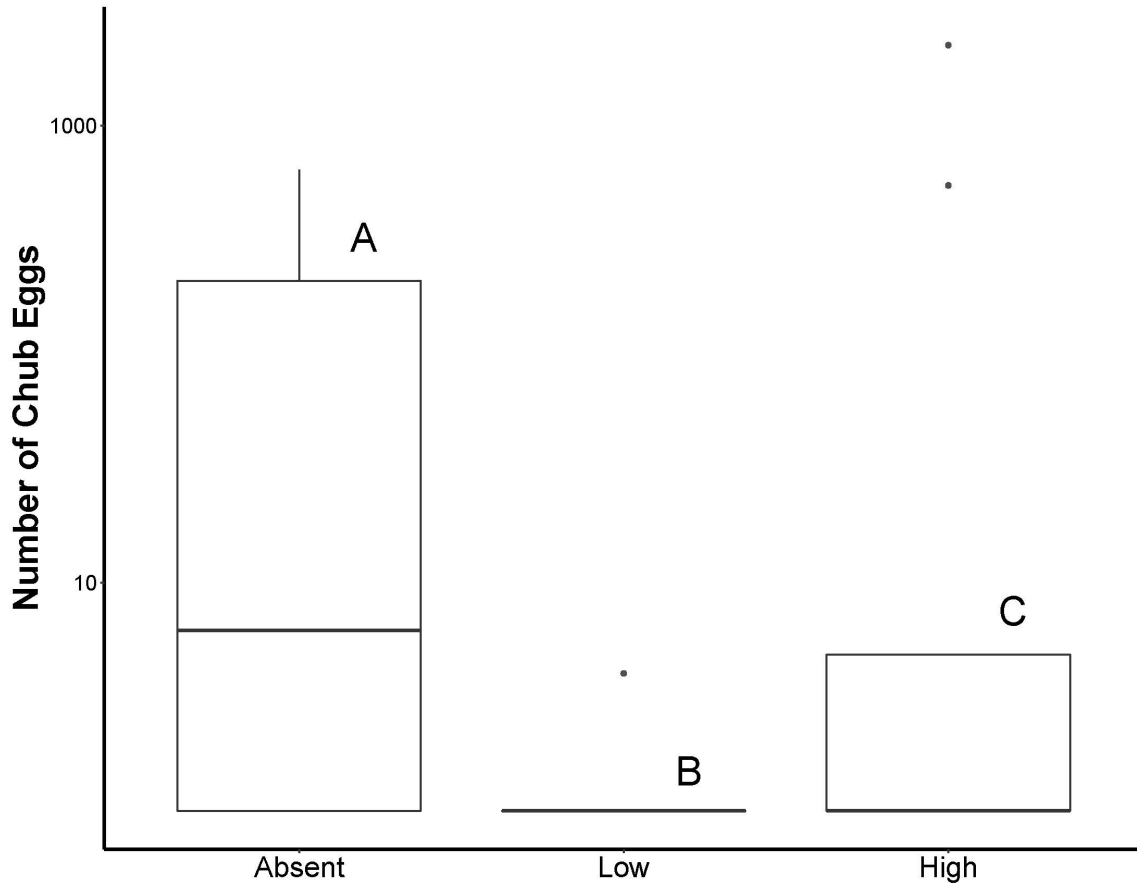


Figure 2.1: Bluehead chub (host) egg abundance for each treatment of yellowfin shiner (associate) abundance. The solid black midline represents the treatment mean, and the surrounding box depicts standard error. Significant differences among treatment means were determined using a post hoc Tukey's test and are signified by unique letter labels. Our results indicate significant differences between each of our three treatments. Number of nests sampled for each treatment was: absent = 8, low = 5, high = 7

Discussion

This work represents one of the first studies to document interaction outcome shifts from mutualism to parasitism that result solely from changes in partner abundance. Shiners should always benefit from spawning with a host (Johnston, 1994a) based on the simple fact that they must have a host to spawn (Wallin, 1992); they will not spawn in the absence of a nest-building host. Even though the outcome remained positive, the per-capita benefit for shiner varied drastically; we observed a 200-fold increase in per-capita egg abundance from low to high density experimental units. Reproductive

success of host chub, however, varied with shiner abundance and caused a shift in the interaction outcome from mutualism at high partner abundance to parasitism at low partner abundance. These findings support our hypothesis of mutualism at high shiner abundance, but do not support our hypothesis of commensalism at low shiner abundance. Thus, our results provide several key insights into context dependency in our study system. First, the outcomes of nest association depend on biotic context. Second, nest association appears to be mutualistic only when associate abundance is high enough for the benefits of brood dilution to outweigh the costs of egg predation. Finally, our results suggest chubs will benefit from higher reproductive success when disengaging entirely from associative spawning than spawning together with a low number of shiners.

In this system, brood dilution is the most likely mechanism that makes partner abundance function as a source of biotic context. Mutualisms incur both costs and benefits, and an interaction is only mutualistic if all participants receive a net benefit. As large piles of concentrated gravel, chub nests are conspicuous features on the streambed, advertising food availability for egg predators and representing a baseline cost for chub reproduction. However, chub spawning bouts are generally brief and inconspicuous (Sabaj, Maurakis, & Woolcott, 2000), drawing little attention to the nest. Conversely, spawning groups of shiners are highly conspicuous and can last for days on a nest. Congregated shiners on chub nests represent an additional fitness cost to host chubs because they make the nest even more conspicuous to egg predators. With high associate abundances typical of productive streams, associate eggs comprise the majority of eggs on an active nest (Cashner & Bart, 2010; Wallin, 1992). While predation was not directly observed in the experiment, the presence of predatory invertebrate taxa in the nests

suggests that egg predation did occur. Alternatively, the presence of egg predators may have altered the behaviour of chubs through perceived rather than actual predation. Thus, it is possible that female chubs did not reciprocate the male's invitation to spawn.

Regardless, brood dilution by associates still affords a mechanism for host benefits. As the strength and mode of predatory behaviour may provide additional sources of biotic context, examining these conditions represents a logical next step. Future work should include control treatments without piscine or other egg predators to untangle host responses to perceived versus realised egg predation.

Because it is so important to host fitness, many hosts have evolved unique strategies to manipulate symbiont or partner abundance to their own advantage (Cunning et al., 2015; Parkinson, Gobin, & Hughes, 2016; Parkinson et al., 2017). For example, crayfish hosts (Cambaridae: *Cambarus chasmodactylus* and *Orconectes cristavarius*) actively reduce density of branchiobdellidan worms to prevent a mutualistic cleaning symbiosis from switching to parasitism (Farrell, Creed, & Brown, 2014). Host chubs may also engage in a form of partner control by withholding spawning activity until associate abundance is high enough to benefit the host. Indeed, results of daily surveys concomitant with our experiment (Y. Kanno, unpublished data) in nearby streams suggest that nearly a third of chub nests are immediately abandoned upon being constructed. While not all nests constructed are utilised for spawning by associates, no abandoned nests were observed to attract any associates. Further research on the factors determining nest abandonment will yield important insight into the role of partner control in nest associative interactions.

Like many other studies, we simplified our system to quantify pairwise interaction outcomes between two participants. However, it is widely recognised that mutualism must be understood in a whole-community context (Palmer, Pringle, Stier, & Holt, 2015; Thrall, Hochberg, Burdon, & Bever, 2007). To the best of our knowledge, *Nocomis* occurs nowhere without at least one associate species (Pendleton, Pritt, Peoples, & Frimpong, 2012), and spawns with up to six associates simultaneously in parts of its range (Peoples et al., 2015). Moreover, associates usually have the opportunity to spawn among several nest-building host species, each with slightly different nesting habits (Peoples et al., 2016). Quantifying interactions between partner diversity and abundance is necessary for understanding context dependency in this system.

Partner abundance is a key source of context dependency in symbioses (Chomicki & Renner, 2017; Cunning & Baker, 2014; Kiers, Palmer, Ives, Bruno, & Bronstein, 2010). Maximum host fitness occurs at intermediate partner density in many symbioses (Brown et al., 2012; Izzo & Vasconcelos, 2002; Morales, 2000; Palmer & Brody, 2013). For example, a common finding is that interaction outcomes switch from commensalistic or mutualistic at lower to intermediate partner abundances, to parasitic at high abundances (e.g., Brown et al., 2012; Thomas et al., 2013). However, the fitness outcomes we observed along our continuum of partner abundance differed from these other studies. We found the opposite pattern, with parasitism at low partner abundances and mutualism at high abundances; this is probably due to the novelty of the resources being traded, spawning substrate/parental care and brood dilution, between hosts and associates. Unlike cleaning symbioses where high symbiont densities can be detrimental

to hosts (i.e., switching from mutualism to parasitism with increasing symbiont density), we can think of no mechanism that would cause increased brood dilution by associates to decrease host fitness. Our findings illustrate that, although partner abundance is a key source of biotic context, predictions on abundance-related fitness outcomes will require detailed system-specific information.

Although ecologists broadly recognise that mutualism is ubiquitous in nature (Bronstein, 1994a; Herre, Knowlton, Mueller, & Rehner, 1999; Sachs, Mueller, Wilcox, & Bull, 2004; Sachs & Simms, 2006; Stachowicz, 2001), it has until recently been largely overlooked in freshwater systems (Holomuzki et al., 2010). In addition to a few other interaction types (worm/crayfish cleaning symbiosis; Brown et al., 2002, 2012; Lee et al., 2009; Skelton et al., 2013; Thomas, Creed, Skelton, & Brown, 2016; frugivorous fish seed dispersal—Correa et al., 2015; Horn et al., 2011), nest associative spawning fishes provide an excellent model system for understanding mutualisms and context dependency in freshwater ecosystems. Mutually beneficial nest associations have been documented previously in systems of other nest building taxa (Goff, 1984; Johnston, 1994b; Wisenden & Keenleyside, 1992), and more recently with *Nocomis* hosts (Peoples & Frimpong, 2013). Moreover, studies have found variable outcomes of nest association with varying biotic context; for example body size of host sunfishes (Shao, 1997a, 1997b), or host brood parasitism by spawning partners (Baba, Nagata, & Yamagishi, 1990; Fletcher, 1993; Yamane, Watanabe, & Nagata, 2013). Continued research into nest associative spawning will help shed light on the role of mutualisms in freshwater ecosystems.

Mutualisms form the foundation for many fundamental ecological processes (Bronstein, 2009) and conserving mutualism will be a key component of conserving biodiversity under global change (Bronstein, Dieckmann, & Ferrière, 2004; Correa et al., 2015). Understanding context dependency is critical to predicting how interaction outcomes, and their consequent effects on population and community processes, will shift under changing scenarios. In freshwater systems, which are home to some of the richest, and yet most imperilled faunas on earth (Jelks et al., 2008), this is particularly evident. Identifying mutualisms and their context dependency will be important for understanding dynamics of freshwater ecosystems.

CHAPTER 2 SUPPLEMENTAL INFORMATION

S1: Microsatellite genetic marker analyses

DNA Isolation

DNA was isolated from egg samples preserved in 100 percent non-denatured ethanol (EtOH) using a spin column isolation protocol. The egg sample was transferred to a clean microfuge tube and dried to evaporate all remaining EtOH. DNA was isolated from egg samples according to a modified Wizard SV Genomic DNA Purification System protocol (Promega Corporation, Fitchburg, Wisconsin). Each egg was placed in 200 μ L of digestion solution comprised of nuclei lysis solution (72.8%), 0.5 M EDTA (18.2%), 20 mg/mL proteinase K (7.2%), and RNase (1.8%). After incubation for 3 hours at 55 °C, 180 μ L of lysis buffer were pipetted into the sample and vortexed, allowing the lysis buffer to mix with the digestion solution. The entire sample lysate was then transferred to a spin column assembly, where a vacuum manifold pulled non-DNA components of the sample through the column while retaining DNA on the filter. Eight hundred microliters of Wizard SV Wash Solution, containing EtOH, were added to each spin column and allowed to pass through four separate times. Spin columns and their collection tubes were then spun at 13,000 rpm for 3 minutes to dry the columns; columns were transferred to clean, labeled 1.5 mL microcentrifuge tubes. Genomic DNA was then eluted into 30 μ L of 55 °C nuclease-free water and incubated at room temperature for 2 minutes. Tubes were spun at 13,000 rpm for 1 minute, followed by a final elution with 50 μ L of 55 °C nuclease-free water. Samples were transferred to a -20 °C freezer for long-term storage.

Table S1.1: Multiplexed microsatellite markers used for identification of Bluehead Chub and Yellowfin Shiner. The allelic size range for Bluehead Chub (BHC) and Yellowfin Shiner (YFS) are based solely on samples used in the experiment, collected from watersheds in or adjacent to the Six Mile Creek drainage. Forward primers in each primer pair were labeled with a fluorescent WellRED dye (D2 = black, D3 = green, D4 = blue).

Locus	BHC Size Range (bp)	YFS Size Range (bp)	CEQ Dye	Primer Concentration (μ M)	Repeat Motif	Reference
Ca5	235-347	No Amp.	D3	0.29	(TAGA) ₁₅	(Dimoski et al. 2000)
Nme25C8.208	210-236	210-224	D4	0.03	(TG) ₉	(Burridge and Gold 2003)
RSD53	156-178	No Amp.	D2	0.09	(AC) ₁₃ (AT) ₄	(Pitcher et al. 2009)

Microsatellite Genotyping

For all samples, a multiplexed group of three microsatellite loci was amplified using polymerase chain reaction (PCR). No microsatellite primers have been developed specifically for Bluehead Chub and Yellowfin Shiner, so 40 microsatellite primers from closely related cyprinids were tested on 15 samples from each species. Three microsatellite primers (Table S1), originally developed for Central Stoneroller (Leuciscidae: *Campostoma anomalum*) (Ca5), Redside Dace (Leuciscidae: *Clinostomus elongates*) (RSD53), and Cape Fear Shiner (Leuciscidae: *Notropis mekistocholas*) (Nme25C8.208), were chosen based on amplification success and diagnostic capability. All three primers amplify in Bluehead Chub, but only Nme25C8.208 amplifies in Yellowfin Shiner. Nme25C8.208 thus acts as a positive control, confirming the identity of the Yellowfin Shiner egg and simultaneously distinguishing it from Bluehead Chub eggs. The forward primers in each pair were labeled with a WellRED fluorescent dye (Table S1). All amplifications occurred in 11- μ L reaction volumes containing 1 \times HotMaster Buffer, 0.2 mM dNTPs, 1.5 mM MgCl₂, 0.45 μ M forward and reverse primers, 0.03 U HotMaster Taq DNA polymerase, and ~5 ng genomic DNA. All samples

were amplified with two negative controls to detect any contamination. PCR was performed using I-Cycler thermocyclers (Bio-Rad Laboratories, Hercules, California) with the following reaction profile: initial denaturation at 94 °C for 3 min; 20 cycles of denaturation at 94 °C for 30 sec, primer annealing at 65 °C (decreased by 1°C every 2 cycles) for 30 sec, and extension at 72 °C for 40 sec; 20 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec, and extension at 72 °C for 40 sec; and a final extension at 72 °C for 1 hour.

Following DNA amplification, PCR products were separated via capillary gel electrophoresis on a Beckman CEQ™ 8000 (Beckman Coulter, Inc., Fullerton, California). The DNA was denatured with formamide and supplemented with a size standard (400 bp; Beckman Coulter) for accurate fragment length analysis. Chromatograms were analyzed using the frag3/PA version 1 analysis algorithm to determine the size of the alleles at each locus. Two readers independently scored the chromatograms using CEQ™ 8000 Fragment Analysis Software (Beckman Coulter); their scores were compared using Compare Spreadsheets software (Office Assistance LLC) and differences were resolved by conference or repeating amplification and analysis.

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Pitcher, T.E., Beausoleil, J.J., Abbott, J.A. and Vandereerden, J.L., 2009. Sperm design and function in the redbreasted sunfish *Clinostomus elongatus*. *Journal of fish biology*, 75(4), pp.924-931.

S2: Supplemental abundance plot

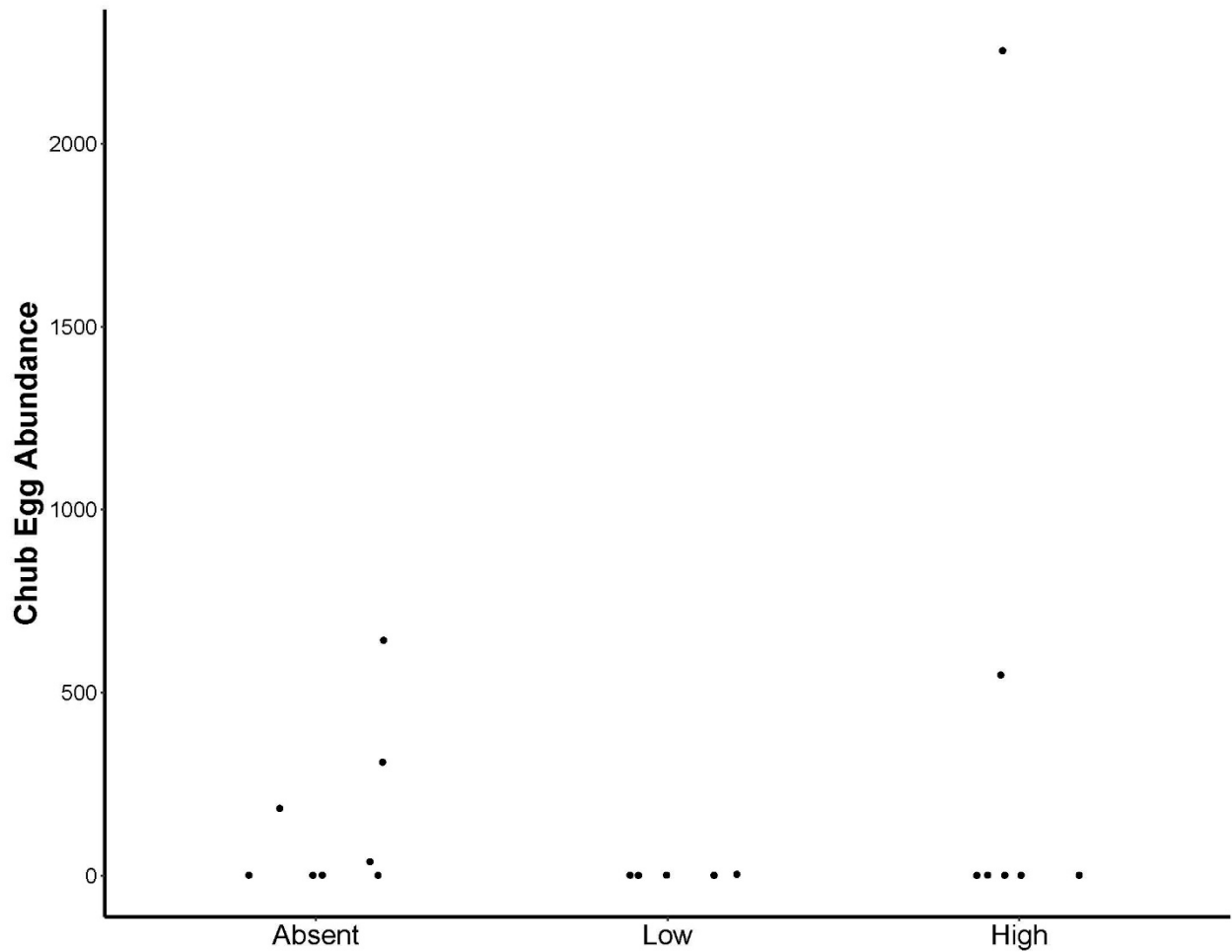


Figure S2.1: Bluehead Chub (host) egg abundance for each nest per treatment of Yellowfin Shiner (associate) abundance. Each point represents a single nest. Points in each treatment bin were plotted using a jitter function to display all points without overlap; horizontal placement within each bin is random and inconsequential. Number of nests sampled for each treatment was: Absent = 8, Low = 5, High = 7.

GENERAL CONCLUSIONS

The study of positive interactions in freshwaters has benefitted from concepts forged in other disciplines, but those concepts will need modified to suit the needs of freshwaters. The basic patterns of the stress-gradient hypothesis do not necessarily hold up for freshwater invertebrates (Fugère et al. 2012), but using that framework leads to biologically meaningful results and interpretation. Positive interactions in terrestrial animal-plant systems are often shown to display high rates asymmetry and nestedness (P. Vázquez et al. 2007, Bascompte 2009), a tendency which is expected to hold true for other mutualisms. In a coral reef cleaning symbiosis, nestedness was found to be even greater than in the terrestrial systems (Guimaraes et al. 2007). Cleaning interactions in crayfish-worm symbiosis will differ from these terrestrial and marine interactions, but along with research into asymmetric competition (i.e. Hudina et al. 2011), these studies provide evidence that allows for new, testable hypotheses in freshwaters. A common theme that holds true for many freshwater interactions, even when analogous or sharing an evolutionary origin with interactions in marine systems is that they are generally less studied. Furthermore, positive interactions like cleaning stations in freshwaters (Severo-Neto and Froehlich 2016) need not be symbiotic, long-term, or require significant investment to play a meaningful role in the shape of ecological communities. Streams, rivers, lakes and wetlands provide an abundance of novel species and interactions, and by borrowing from other fields and disciplines, we can better understand freshwaters without having to reinvent the wheel.

A major directive of our review was that future research must include both explicit tests and general life history studies. To that end, an experiment was conducted

which blends these two themes to further our knowledge of positive interactions in freshwaters. Much of the recent literature on *Nocomis* nest association originates in the New River and surrounding drainages in the mid-Atlantic region of the eastern United States (Peoples et al. 2011), a region with a diverse assemblage of associate species. However, the experiment conducted as part of this thesis was conducted in the upper Savannah River basin in upstate South Carolina, in a watershed with only two nest associates. Previous fieldwork and ongoing observational studies at Clemson University have led to a comprehensive understanding of the life histories of the species involved in the interaction (Y Kanno and B. Peoples, personal communication). This knowledge allowed us to test for the most meaningful form of biotic context observed in the system. Partner density is highly variable even within the same stream system, whereas the identity of potential partners and predators would have provided a much narrower range of realistic biotic context. The findings of this study provide new knowledge in our understanding of nest association, and it does so by merging life history and explicit experimentation to ask and answer the driving questions.

Global climate change predicts increases in water temperatures, anthropogenic impacts have resulted in increased pollution, and habitat alteration and deforestation have left many of our global freshwaters sedimented and in a general state of disrepair. Under increased temperature regimes, for example, crayfish will burrow to reduce thermal stress. When this occurs, the majority of symbiotic branchiobdellidan worms leave their host and crayfish growth ceases (Ames et al. 2015). Similarly, light conditions in freshwater sponges affect their symbiosis with green algae. In high light conditions, sponges turn green as the result of dense aggregations of the algae within them – if

conditions change and the sponges are left in the dark, photosynthesis ceases and the sponge will ingest their symbiont and the interaction ceases (Frost and Williamson 1980).

Though extreme cases, these examples show how plausible changes in conditions can cause interactions to cease, not just change in strength or outcome. In the face of anthropogenic impacts, it is vital to recognize how positive biotic interactions may be negatively affected, or how they might help to maintain ecosystem function. Positive interaction research in freshwaters has been fruitful thus far, and this thesis should provide motivation for that trend to continue.

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