

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

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## Summary

**1.** Little is known about how positive biotic interactions structure animal communities. Nest association is a common reproductive facilitation in which associate species spawn in nests constructed by host species. Nest-associative behaviour is nearly obligate for some species, but facultative for others; this can complicate interaction network topology.

**2.** Nest web diagrams can be used to depict interactions in nesting-structured communities and generate predictions about those interactions, but have thus far only been applied to cavity-nesting vertebrate communities. Likewise, the stress-gradient hypothesis (SGH) predicts that prevalent biotic interactions shift from competition to facilitation as abiotic and biotic stress increase; this model has been hardly applied to animal communities. Here, both of these models were applied to nest-associative fish communities and extended in novel ways to broaden their applicability.

**3.** A nest web was constructed using spawning observations over 3 years in several streams in south-western Virginia, USA. Structural equation modelling (SEM) was then implemented through an information-theoretic framework to identify the most plausible nest web topology in stream fish communities at 45 sites in the New River basin of the central Appalachian Mountains, USA. To test the SGH, the per-nest reproductive success of ‘strong’ (nearly obligate) nest associates was used to represent interaction importance. Eigenvectors were extracted from a principal coordinate analysis (PCoA) of proportional species abundances to represent community structure. Both of these metrics were regressed on physical stress, a combination of catchment-scale agricultural land use and stream size (representing spatiotemporal habitat variability).

**4.** Seventy-one per cent of SEM model evidence supported a parsimonious interaction topology in which strong associates rely on a single host (*Nocomis*), but not other species. PCoA identified a gradient of community structure dominated by *Nocomis* and associates, to communities dominated by other reproductive groups. Both metrics of interaction importance responded positively to physical stress.

**5.** This study demonstrates that nest webs can be useful in a variety of systems and that SEM can be a quantitative extension of this framework. Likewise, the SGH can be used to understand positive interactions in animal communities and can be extended to predict proportional representation of facilitating and beneficiary species in communities.

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

## Introduction

Positive biotic interactions such as facilitation and mutualism are common in nature. Yet despite their ubiquity, a

framework for integrating the roles of positive interactions with other important ecological processes is precluded by a lack of empirical studies on a broad suite of ecosystems and taxa (He & Bertness 2014). For example, it is common knowledge that facilitation has strong effects on plant community structure (Callaway *et al.* 2002), but

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very little research has considered the role of positive interactions in vertebrate communities (Barrio *et al.* 2013; Kamilar & Beaudrot 2013). The paucity of facilitation research is also particularly notable among freshwater animals (Holomuzki, Feminella & Power 2010; Fugère *et al.* 2012). Clearly, freshwater fishes provide an excellent system for understanding how positive interactions affect vertebrate communities, and for testing general ecological models in understudied systems.

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

Interaction topologies in fish communities structured largely by facilitator species can be complicated by certain aspects of their reproductive behaviour. First, many nest associates switch to using nests of other hosts (e.g. *Semotilus*, *Campostoma* or *Exoglossum*) when *Nocomis* are scarce, although these nests may not be as well guarded or silt-free as *Nocomis* nests (Pendleton *et al.* 2012). Secondly, other nest-building species may occasionally function as nest associates of *Nocomis* or as egg predators on *Nocomis* nests (Jenkins & Burkhead 1994; Sabaj, Maurakis & Woolcott 2000). Finally, plasticity in reproductive traits such as the dual spawning modes exhibited by many North American cyprinids (Johnston & Page 1992) can make it difficult to quantify proportions of associate nesting effort associated with host nests vs. simple broadcast spawning. Nest-associative behaviour for 'strong' associate species may be obligate (or nearly so; Wallin 1992; Mattingly & Black 2013). However, other 'weak' associates can opportunistically revert to the ancestral spawning mode of simple broadcast spawning.

One useful way to understand the complex interaction structure of nesting-based communities is through a nest

web diagram. Analogous to food webs, nest webs depict a hierarchy of direct and indirect interactions among species requiring or providing a common nesting resource (Martin & Eadie 1999). Nest webs are useful for tracing pathways of resource flow, identifying keystone relationships and making predictions about the consequences of altering key interactions. The nest web framework has provided insight for complex interactions among cavity-nesting bird species throughout the world (Martin, Aitken & Wiebe 2004; Blanc & Walters 2008a; Blanc & Martin 2012; Cockle, Martin & Robledo 2012; Orchan *et al.* 2013; Cockle & Martin 2015), but to date has not been used for any other taxa. Accordingly, the first goal of this study was to explore the potential of the nest web framework as a broadly applicable tool for understanding animal communities structured by facilitating species.

The second goal of this study was to identify the abiotic contexts in which nest-associative facilitation would be an important driver of stream fish community structure, using the stress-gradient hypothesis (SGH) to guide predictions (Bertness & Callaway 1994; Maestre *et al.* 2009). The SGH predicts that the frequency and/or importance of positive interactions should be greatest in highly stressful situations (high predation or physical stress) and that positive interactions should segue to competition as habitats become more benign. Since its development, a large body of research has demonstrated that the SGH can predict the relative roles of facilitative and competitive interactions in a variety of ecosystems (He, Bertness & Altieri 2013). However, this model has been tested mainly in vegetation communities with plant–plant interactions (Brooker *et al.* 2008), although the facilitative role of positive plant–animal interactions at determining vegetative community structure is becoming increasingly understood (Crain 2008; Smit, Rietkerk & Wassen 2009). However, the SGH has scarcely been considered in freshwater systems (Fugère *et al.* 2012) or animal–animal interactions in vertebrate communities (Barrio *et al.* 2013). Testing this model in vertebrate communities can bring new insight into the role of positive interactions among animals, as well as identify strengths and weaknesses of the model itself.

Applied to nest-associative lithophilic cyprinid communities, the SGH predicts that the frequency and/or importance of nest association should increase with physical stress, defined as any physical variable that may limit reproductive success of focal taxa (Maestre *et al.* 2009). In streams, anthropogenic land use (catchment-scale agricultural and residential development) is a ubiquitous stressor that elevates fine sediment loads, creating unfavourable reproductive and rearing conditions for lithophilic cyprinids. The negative relationship between this variable and reproductive success of lithophilic stream fishes is well-established (Berkman & Rabeni 1987; Wood & Armitage 1997; Kemp *et al.* 2011). Spatiotemporal habitat variability also represents physical stress; rapid and large changes in instream conditions (e.g. temperature, dissolved oxygen concentration, flow magnitude) can stress eggs and larvae

of lithophilic minnows. This variable can be approximated by catchment area (a measure of stream size) because headwater habitats are considerably less stable than downstream habitats (Schlosser 1987). Thus, the SGH would predict that the importance of positive interactions should increase with increasing agricultural land use and decreasing stream size.

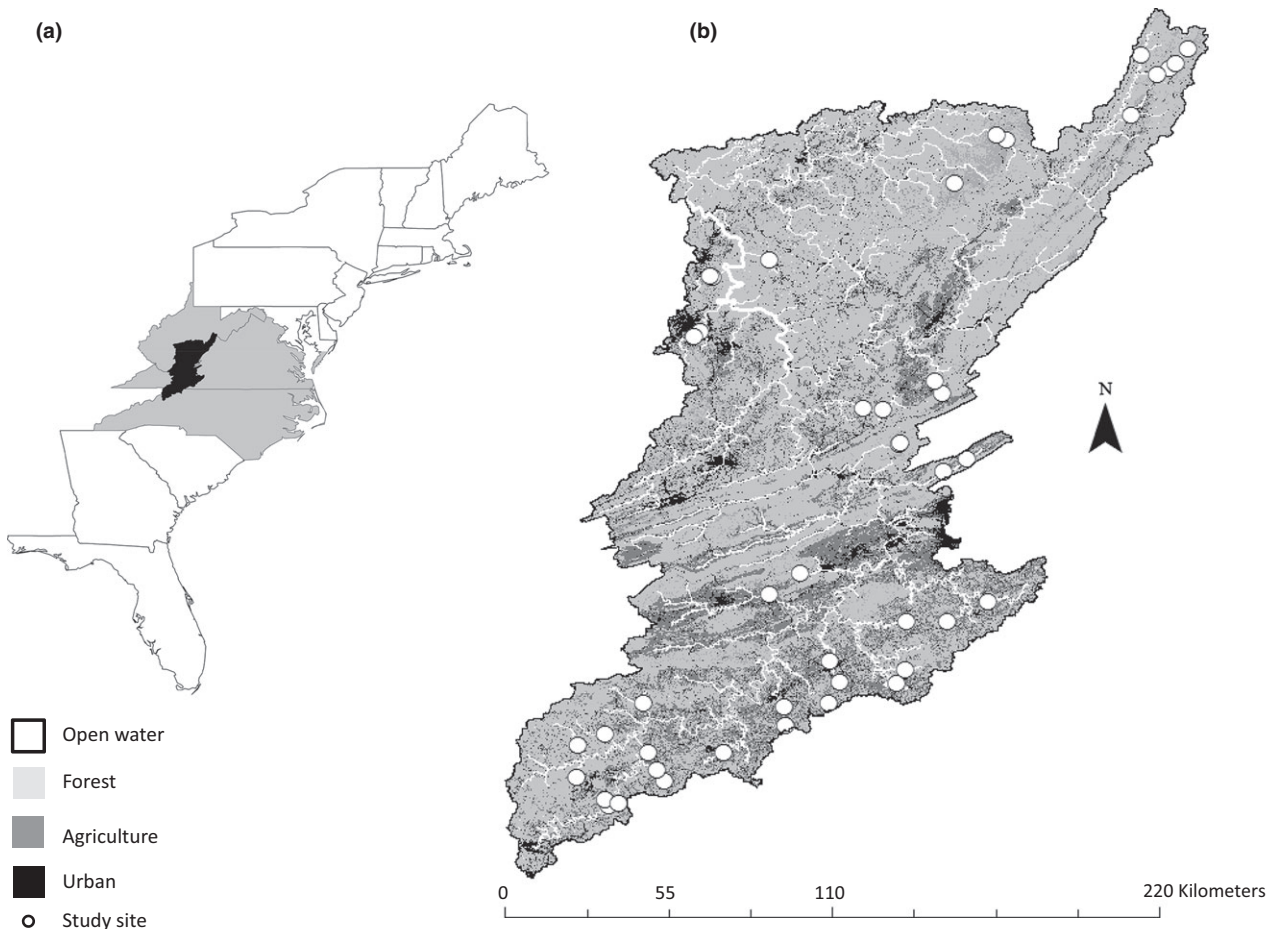
The first objective of this study was to use a limited sample of reproductive interaction data from a few streams to construct a nest web for lithophilic cyprinid communities in tributaries to the New River of the central Appalachian Mountains, USA. The second objective was to then apply a novel statistical approach [structural equation modelling (SEM), implemented through an information-theoretic framework] to test whether key interactions depicted in the nest web were generalizable across the basin. This new application of the nest web framework is intended to (1) generalize its use as a tool for understanding animal communities structured by facilitating species and (2) to move beyond some of its traditional limitations as a simple visual depiction (Blanc & Walters 2007) and thus extend the framework as a quantitative tool for comparing complex ecological hypotheses.

The third objective of this study was to determine whether or not the SGH could be a useful framework to predict interaction dynamics and community structure in vertebrate communities, using lotic Appalachian cyprinid communities as a model. This study also examined whether the SGH could be extended beyond predicting interaction frequency and importance to include predicting proportional representation of facilitating/beneficiary species in community structure. Specifically, it was hypothesized that (1) juveniles-per-nest of nest associates and (2) the proportional representation of *Nocomis* and associates would increase along a gradient of physical stress. Likewise, juveniles-per-nest of other reproductive groups should decrease or have no relationship with physical stress.

## Materials and methods

### STUDY AREA

This study was conducted in the New River basin of North Carolina, Virginia and West Virginia, USA (Fig. 1). Located in the central Appalachian Mountains, the New River drains approximately 18 000 km<sup>2</sup> of three major ecoregions: the Blue



**Fig. 1.** (a) The eastern United States of America; states in which the New River basin (black) is located are defined in grey. (b) Land use types and study sites within the New River basin.

Ridge, Valley and Ridge, and Central Appalachians (Omernick 1987). Elevations range from *c.* 200 to 1910 m. Underlying geology is largely granite in the Blue Ridge, limestone in the Valley and Ridge and a mix of sandstone and limestone in the Central Appalachians. Land use in the New River basin is primarily pasture for hay and cattle grazing, with lesser amounts of silviculture and row crop agriculture (mostly corn). Large tracts of eastern deciduous forest (Washington-Jefferson and Monongahela National Forests) exist in the basin, but a considerable amount of small private forests creates a patchwork of forest and agricultural land use throughout the basin (Jenkins & Burkhead 1994).

The New River is an ideal system for studying nest-associative interactions because of its relatively high cyprinid richness (29 species) and representation of cyprinid reproductive groups; these include six nest-building species (two of which were *Nocomis*), eight strong associates, 4 weak associates and 11 non-associates (Jenkins & Burkhead 1994). Data to populate nest webs (objective 1) were collected by conducting opportunistic nest searches on second- to third-order streams throughout Montgomery and Roanoke counties, Virginia, USA, during the breeding seasons (mid-April to early June) of 2012 through 2014. One of these streams was in the adjacent Roanoke River drainage, but being in the same ecoregion, it had the same fish species and indistinguishable instream habitat characteristics from the New River tributaries. Data for the second and third objectives were collected from 45 sites (second to fifth order, catchment areas ranging from 2 to 284 km<sup>2</sup>), representing gradients of catchment-scale agricultural land use (<1–46%), in July and August of 2012. Sites consisted of two spatially replicated reaches of 80–140 m in length (depending on stream size) within the same interconfluence stream segment, which was the actual sampling unit (Benda *et al.* 2004). Reaches were separated by at least one channel geomorphic sequence and were bounded by natural barriers (riffles or cascades) to prevent fish escapement during sampling. Data from both reaches were combined to represent a site.

## FIELD METHODS

Cyprinid nests were surveyed by two workers, walking on either side of the stream and wearing polarized sunglasses to reduce surface glare. When an active nest was located, workers watched each nest for at least 30 min and identified all species observed on the nest. Whereas some cyprinids are generally difficult to distinguish, those encountered in this study (Appendix S1, Supporting information) differ enough to allow visual identification without handling of specimens, especially when in breeding colours. The identity of nest-building species was evident by the structure of nests, although the resident male constructing the nest was often easily identifiable. Nests constructed by the two *Nocomis* species in the study area (*N. leptocephalus* and *N. platyrhynchus*) are large and dome-shaped (approaching a metre in diameter) and are constructed of fairly large gravels (up to 45 mm for *N. leptocephalus* and up to 75 mm for *N. platyrhynchus*; Bolton, Peoples & Frimpong 2015 and Maurakis 1998; respectively). Nests built by *Exoglossum* superficially resemble *Nocomis* nests, but are considerably smaller and are constructed of much smaller gravel. Alternatively, nests built by *Semotilus atromaculatus* resemble a long, low gravel ridge, while nests of *Campostoma anomalum* are simple gravel pits (Jenkins & Burkhead 1994). To minimize the chance a species went

overlooked, most nests were video-recorded and all were revisited multiple times. This method was more useful than snorkelling, which tended to disturb more skittish species.

Before fish sampling, instream habitat and *Nocomis* nest abundance were quantified along 10 transects per reach according to Barbour *et al.* (1999) and Peoples, Tainer & Frimpong (2011). Dominant substrate types were identified at ten equidistant points on each reach and classified according to a simplified Wentworth scale (e.g. bedrock, boulder, cobble, gravel, sand, silt and organic matter). Substrate embeddedness (%) was visually estimated at three equidistant points on each transect; one embeddedness estimate per transect was located in the thalweg. Fishes were sampled from 09 July to 14 August 2012 using single-pass, double-backpack electrofishing. Electrofishing effort was relatively intensive, averaging 3838 ± 209 s per site. All individuals were identified to species and measured to the nearest mm (total length).

## METRIC DEFINITIONS

Species were classified into reproductive groups (nest builders, strong associates, weak associates and non-associates) based on Jenkins & Burkhead (1994, and references therein), Pendleton *et al.* (2012) and Yamana, Watanabe & Nagata (2013). Species encountered in this study are listed in Appendix S1. An estimate of reproductive success of each reproductive group was necessary for comparing hypotheses specifying competing reproductive interaction topologies. To estimate reproductive success, individuals were classified as either juveniles (reproductively immature young-of-the-year) or adults by visually inspecting site-specific length-frequency histograms for modal breaks. This method is effective because length-frequency histograms of short-lived temperate fishes such as cyprinids show distinct modal breaks. This method typically agrees well with age estimation based on hard structures (Reid, Jones & Yunker 2008; Peoples, Tainer & Frimpong 2011). The purpose of the binary age classification was not to quantify actual age structure, but to partition demography into indices of reproductive success (juvenile abundance) and a major cause of reproductive output (adult abundance). A metric of nest-capable adult male *Nocomis* abundance was calculated by partitioning out individuals over 120 mm. This is not an exact estimate of the abundance of nest-capable males (females can reach lengths >120 mm), but all reasonable cut-off values for approximating this metric were correlated with nest abundance.

Observed interactions at nests may not translate directly to the importance of nest-associative spawning in this system; some may represent predation instead of spawning and others may spawn on nests but relatively infrequently compared to broadcast spawning, etc. Accordingly, juvenile abundance (catch-per-unit-effort, CPUE) of each reproductive group was estimated. This variable represents the *outcomes* of nest-associative and open broadcast spawning and can be used to infer community interactions when direct measurements of nest use at multiple locations are not possible across a large spatial extent. This approach represents a conceptual and analytical advancement of the nest web framework. Adult CPUE of each reproductive group was also calculated. For each site, a metric of unsilted gravel availability was calculated by multiplying the log-transformed proportion of gravel substrate by the average thalweg embeddedness. This metric was used in analyses of reproductive interaction topologies to represent the availability of quality (unsilted) spawning substrate



in habitat types (riffles) in which simple broadcast lithophils would spawn if they were not spawning on the nest of a host. *Nocomis* nests were not included in this calculation.

Catchment-scale percentages of agricultural land use and catchment area were used to represent physical stress in analyses testing the predictions of the SGH in this system. Catchment-scale percentage of agricultural land use is a useful proxy of instream siltation (Walser & Bart 2009), which is often difficult and time-consuming to measure accurately and simultaneously across a large spatial extent (Sutherland, Meyer & Gardiner 2002). The two metrics of physical stress were placed in comparable terms by scaling each variable to range from 1 to 10. Site-specific values for each variable were then placed into one of 10 equidistant bins. The two scaled variables were then summed to form a composite measure of stress, ranging from 2 to 20 in increments of 1. Based on this scheme, large streams with mostly forested catchments were defined as least stressful, and small agricultural streams were classified as most stressful. The purpose of defining physical stress in this way is demonstrative. By combining two measures of physical stress that are ubiquitous to streams, this approach allows for findings to be interpreted in terms of well-accepted conceptual frameworks (e.g. Schlosser 1987) and avoids making overly specific conclusions that are not generalizable beyond the present taxa and system.

Two demographic, fitness-based metrics of interaction importance were used as response variables in SGH analyses. The first metric was calculated by dividing juvenile abundance of each reproductive group by nest abundance (juveniles-per-nest). The numbers of juveniles-per-nest represents the per-nest contribution of *Nocomis* nesting activity (habitat amelioration) to reproductive success of each reproductive group. The second SGH response variable, cyprinid community structure, is based on the assumption that if physical stress remains constant over several generations, differential reproductive success of facilitating and beneficiary species should proliferate temporally and be evident in the overall community structure (Bertness & Yeh 1994). This represents a potential extension of the SGH, which to date has been used to predict the contextual roles of interactions in communities, but not changes in community composition. This metric was calculated using principal coordinates analysis (PCoA) of proportional adult cyprinid abundance (see *Analyses* below).

## ANALYSES

A nest web was constructed by calculating the percentages of each nest type (which species constructed the nest) on which each associate species was observed (Martin & Eadie 1999). The nest web was then used as a guide for developing models representing competing hypotheses about interaction network topologies throughout the New River basin (Appendix S2).

Using the nest web as a guide, statistical models were constructed to represent competing hypotheses about reproductive interaction topologies. All competing models contained a 'base' structure that remained constant among models. The base structure was constructed based upon the nest web results, prior evidence of relationships and logical intuition (e.g. the positive relationship between adult and juvenile abundance of a reproductive group). For example, models predicting nest abundance must contain large adult male abundance and gravel availability (Peoples, Tainer & Frimpong 2011). Because they reproduce almost exclusively on *Nocomis* nests, models predicting juvenile *Nocomis*

and strong associate abundance must always contain nest abundance. To represent the dilution effect, models predicting juvenile *Nocomis* abundance must always contain strong associate juvenile abundance (Yamana, Watanabe & Nagata 2013). Because they are mostly lithophilic spawners, models predicting abundances of juvenile weak and non-associates and other (non-*Nocomis*) nest builders must always contain gravel availability. Finally, models predicting abundances of juveniles of any group must contain adult abundances of that group. The base structure represents a stand-alone hypothesis of interactions within a nest web in which *Nocomis* and strong associates participate in nest association and other groups do not.

Competing models were constructed by adding variables to the base structure. Competing models contained combinations of four observed relationships that must happen often enough to drive cyprinid community structure and thus be evident in the interaction network topology: (1) strong associates spawn on non-*Nocomis* nests; (2) weak associates spawn on non-*Nocomis* nests; (3) weak associates spawn on *Nocomis* nests; and (4) other nest builders spawn on *Nocomis* nests, as well as their own. For models in which a group is hypothesized to spawn on a nest, logical structure requires juvenile abundance of that group to be included in the model predicting the juvenile abundance of the host group, representing the dilution effect. Because non-*Nocomis* nests are inconspicuous and difficult to detect outside of the spawning season (especially nests of *C. anomalum*, by far the most abundant nest builder in the study system other than *Nocomis*), abundance of adult non-*Nocomis* nest builders was used as a proxy of their nesting activity. Varying the presence of each relationship in the nest web resulted in 16 parameterized models. Including a null (intercept-only) model, 17 models were compared (Appendix S2).

Models were fit using SEM. Each CPUE variable was first subjected to the arcsine-square-root-transformation, and scaled and centred to mean = 0 and variance = 1. Nest abundance was log ( $x + 1$ )-transformed. SEM models were estimated using diagonally weighted least squares regression. Relative support for competing models was evaluated based on Akaike's Information Criterion, adjusted for sample size (AICc); AICc was calculated from the global chi-square of each SEM. Model weights ( $w_i$  for each  $i$ th model) were calculated to compare evidence ratios and weights-of-evidence for competing models. Model weights range from 0 to 1 and sum to 1 for all competing models (Anderson & Burnham 2002). The best-supported model has the highest model weight, and models within two AICc units ( $\Delta\text{AICc} \leq 2$ ) are considered equally plausible. One potential shortcoming of an information-theoretic framework is that although a model may clearly out-compete others, it still may not approximate the data well, which is why competing models were also compared to an intercept-only (null) model. SEM models were fit using the LAVAAN package version 0.5-16 (Rosseel 2012) in R version 3.0.1.

For the second response variable in the SGH analysis, cyprinid community structure was represented as eigenvectors from a PCoA of cyprinid proportional abundance. Prior to analysis, the proportional abundance of each species at each site was arcsine-square-root-transformed. The PCoA was conducted on a Bray-Curtis distance matrix of the community data using the VEGAN package (version 2.0-10) in R. The resulting Scree plot was inspected to determine appropriate dimensionality in the PCoA solution. Correlation coefficients between eigenvectors and species relative abundances were used to interpret eigenvectors. One axis

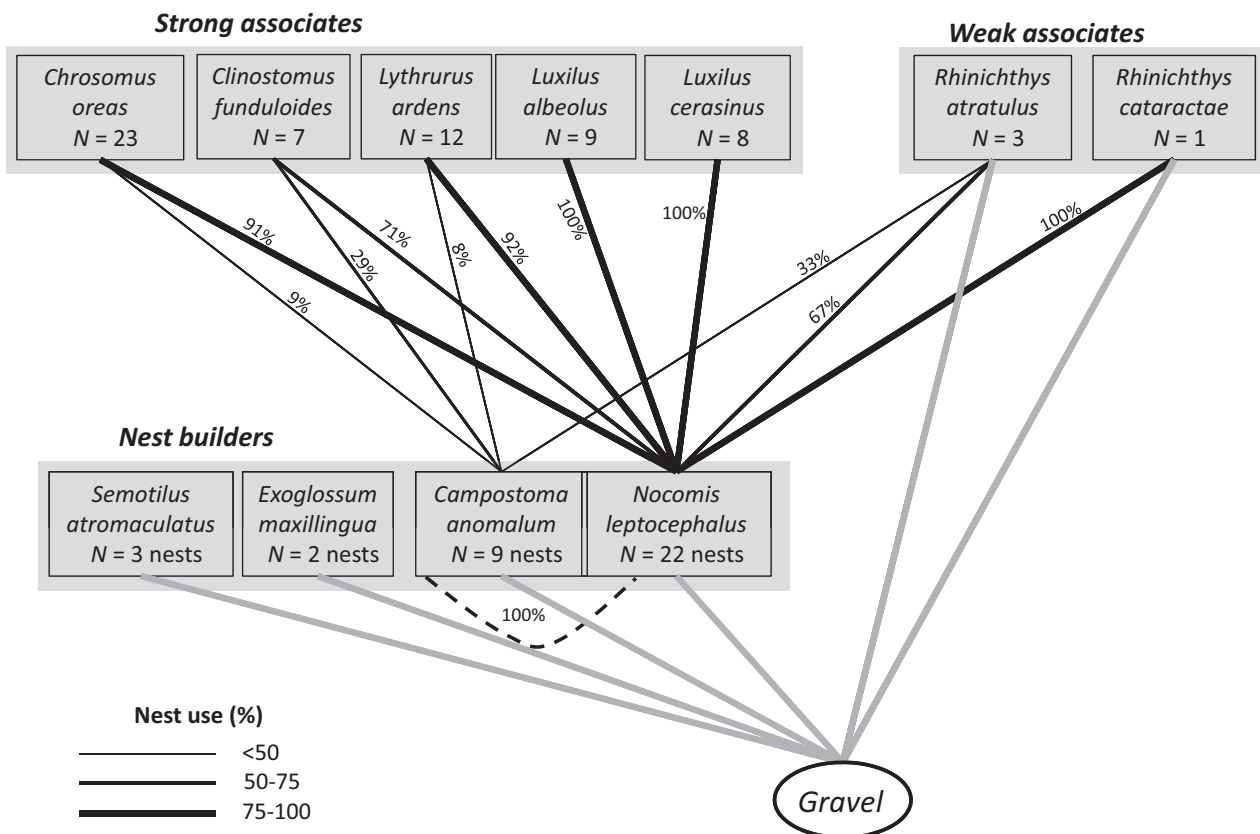
representing the contrast between sites dominated by *Nocomis* and associates was used to represent interaction importance. To test the predictions of the SGH, juveniles-per-nest of each reproductive group and the cyprinid community eigenvector were regressed on the physical stress metric.

## Results

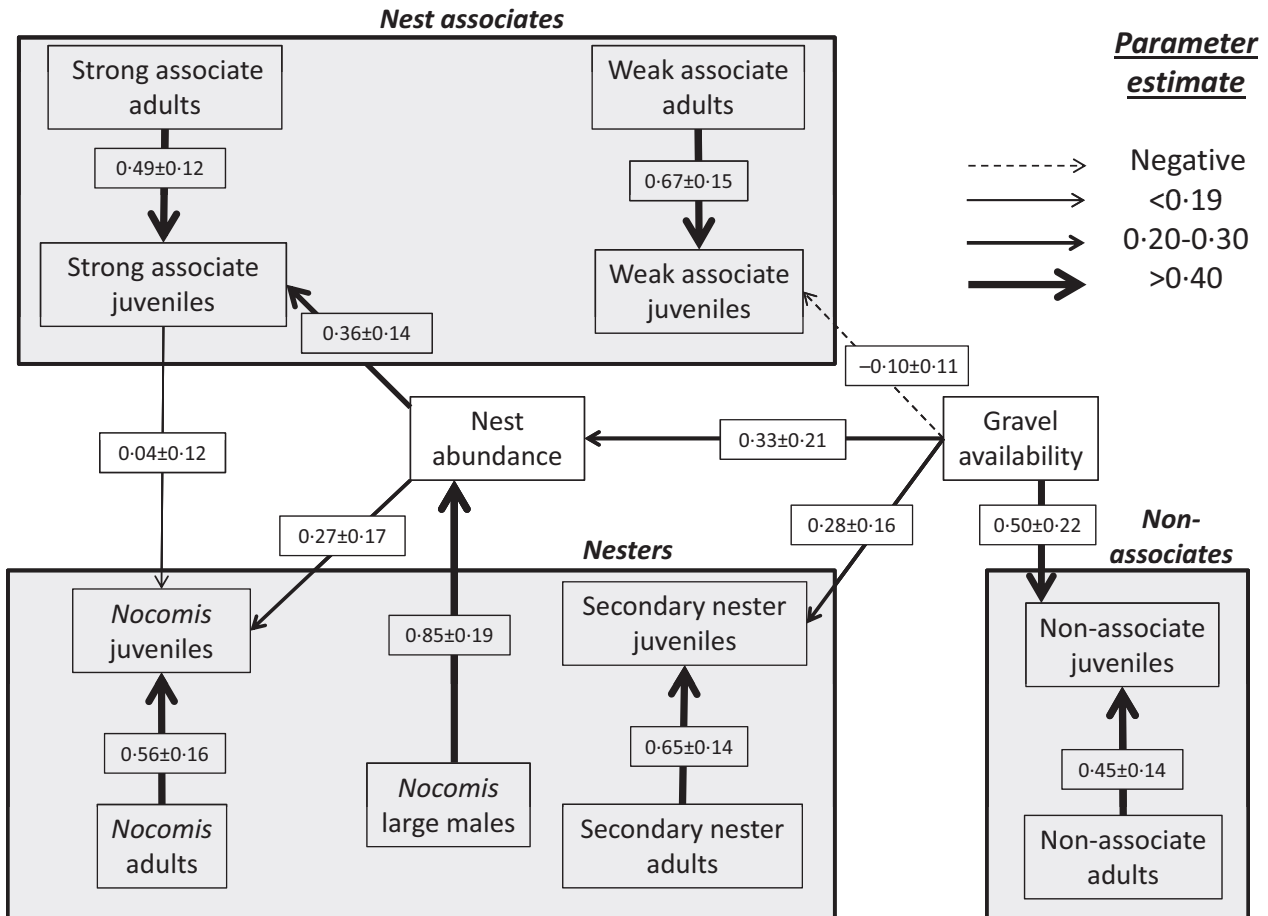
Nest web (both observational and SEM) results suggested an interaction network in which (1) strong associates spawn on *Nocomis* nests but may occasionally rely on other hosts, (2) weak associates infrequently rely on nests, and (3) non-associates never use nests for spawning (Figs 2 and 3, Appendix S2). Observations included 36 nests constructed by four nest-building species (*N. leptocephalus*, *C. anomalum*, *S. atromaculatus* and *Exoglossum maxillingua*). These nests were used by five strong associate species and two weak associate species. *C. anomalum*, a nest-building species that is known to occasionally use *Nocomis* nests for spawning, was observed at every

*N. leptocephalus* nest. However, most *C. anomalum* behaviour appeared to be predatory because most individuals detected were males that had spawned in prior weeks, as determined by the extent of tuberculation; few *C. anomalum* females were observed in the vicinity of *Nocomis* nests. For *S. atromaculatus* and *E. maxillingua*, only the nest builders and associated females were observed at nests (Fig. 2). Every *Nocomis* nest was occupied by at least one strong associate species, while only a few *C. anomalum* nests attracted associates of any species.

Structural equation modelling results revealed that the most plausible interaction topology (as identified by the nest web) was parsimonious (Fig. 3). Three models carried 86% of the weight-of-evidence; the remaining 14% of weight-of-evidence was spread diffusely over the remaining 14 models. The best-supported model (model 16,  $w = 0.71$ ) represented the hypotheses that strong and weak associates seldom rely on nest builders other than *Nocomis* for reproduction and that the reproductive success of weak associates and other nest-building species



**Fig. 2.** A nest web diagram depicting use of nests constructed by *Nocomis leptocephalus* and three other nest-building fishes in south-western Virginia, USA, in the breeding seasons of 2012–2014.  $N$  = the number of spawning observations observed for that species. For nest builders, this represents individual nests; for nest associates, it represents the number of unique nests on which these species were observed spawning (multiple spawning events by the same species on the same nest were not counted). The dashed line between *Camptostoma anomalum* and *N. leptocephalus* represents probable egg predation by *C. anomalum*. Gravel is shown in grey because spawning observations only allowed us to assess breeding activity on nests. Data for the nest web are based only on (1) the presence of fish at a nest as opposed to the presence of eggs and (2) detection of fish at a host nest as opposed to a complete survey of all breeding effort within the area. Therefore, this nest web cannot illustrate the nature of interactions between species known to serve multiple roles (host, associate and/or predator), or convey the relative importance of nest use vs. broadcast spawning among weak associates. Non-associates were never observed at nests of any species.



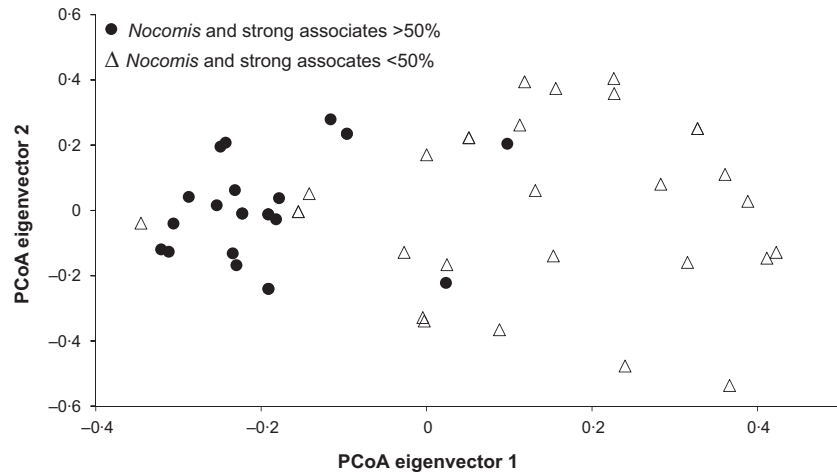
**Fig. 3.** Quantitative path diagram of the most plausible species interactions predicted by the nest web depicting cyprinid community structure in tributaries to the New River, NC, VA and WV, USA. Reproductive success of species that do not spawn via nest association was heavily dependent on the availability of unsilted gravel, while reproductive success of strong associates was dependent on gravel mound nests constructed by large male *Nocomis*. Weak associate reproductive success was not related strongly to nest abundance or gravel availability. Each arrow represents a standardized regression coefficient  $\pm$  standard error.

seldom depends on *Nocomis* nesting. The best model was 8 times better supported than the second best model (model 12,  $w = 0.09$ ,  $\Delta\text{AICc} = 4.3$ ), which differed from the best model only in specifying that weak associates spawn on *Nocomis* nests. The best model was 12 times more likely than the third best model (model 14,  $w = 0.06$ ,  $\Delta\text{AICc} = 4.9$ ), which differed from the best model in specifying that weak associates are frequent users of nests built by species other than *Nocomis*. The best model was far more plausible than the intercept-only model (model 17,  $w < 0.001$ ,  $\Delta\text{AICc} = 40.0$ ) (Appendix S2). Most parameter estimates in the best model were positive (Fig. 3).

The PCoA results corroborate the nest web results, suggesting a gradient of cyprinid community structure ranging from sites dominated by *Nocomis* and a few strong associate species, to sites dominated by a suite of other nest builders and weak and non-associates (Fig. 4). PCoA eigenvectors 1 and 2 described 32% and 29% (61% total), respectively, of the variation in cyprinid community structure. *N. leptocephalus* ( $r = -0.75$ ) and the most common

strong associates were negatively correlated with the first PCoA eigenvector (*Clinostomus funduloides*,  $r = -0.66$ ; *Notropis rubricroceus*,  $r = -0.42$  and *Chrosomus oreas*,  $r = -0.29$ ). Less-abundant strong associates, which were captured diffusely throughout the study area, were weakly correlated ( $|r| < 0.20$ ) with the first PCoA eigenvector. Conversely, the most common and abundant members of other reproductive groups were positively correlated with the first PCoA eigenvector. These included a nest builder (*C. anomalum*,  $r = 0.64$ ), a weak nest associate (*Rhinichthys atratulus*,  $r = 0.41$ ) and two non-associates (*Notropis telescopus*,  $r = 0.32$  and *Pimephales notatus*,  $r = 0.28$ ). The second PCoA eigenvector primarily was correlated with weak and non-associates associated with smaller (*R. atratulus*,  $r = -0.78$  and *S. atromaculatus*,  $r = -0.47$ ) vs. larger (*Cyprinella galactura*,  $r = 0.51$ ; *N. telescopus*,  $r = 0.40$  and *P. notatus*,  $r = 0.49$ ) streams, although one patchily distributed strong associate (*Luxilus albeolus*,  $r = 0.53$ ) was positively correlated with this eigenvector.

As predicted, juveniles-per-nest of strong associates increased exponentially with physical stress ( $P = 0.0005$ ,



**Fig. 4.** Two-dimensional solution of a principal coordinates analysis (PCoA) of Bray–Curtis similarities among cyprinid communities at 45 sites on tributaries to the New River, NC, VA and WV, USA. The PCoA results revealed a dichotomy between sites dominated by *Nocomis* and a few strong associates (black circles), and sites dominated by species of other reproductive groups (hollow triangles).

Fig. 5a), and juveniles-per-nest of other groups showed no relationship with physical stress ( $P > 0.05$  for all, Fig. 5b–d). Further, the PCoA eigenvector score decreased linearly with physical stress ( $P = 0.0003$ , Fig. 5e). Because the PCoA eigenvector was negatively correlated with *Nocomis* and several strong associates, its negative relationship with physical stress implies increasing proportional dominance of *Nocomis* and strong associates with increasing catchment-scale agricultural land use.

## Discussion

The nest web framework provided valuable insight about the reproductive topology of lithophilic cyprinid communities throughout the New River basin. Evidence suggests that by facilitating the reproductive success of species with certain reproductive traits (strong nest-associative behaviour), *Nocomis* drive cyprinid fish community structure in this system via their habitat-modifying nesting activity. Further, the magnitude of the importance of this interaction depends on abiotic context.

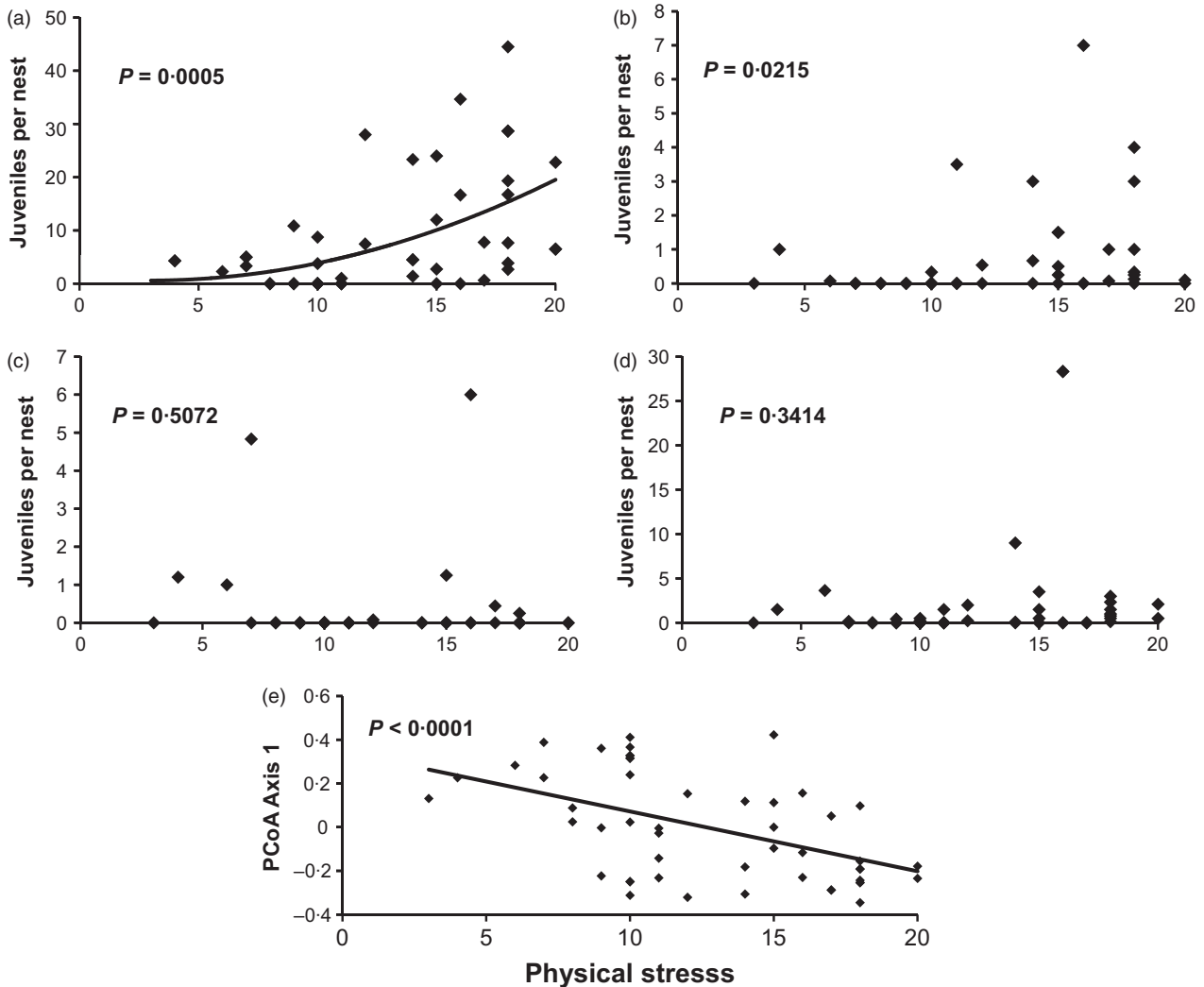
Both observational and model-based evidence suggest an interaction topology in which strong associates rely nearly exclusively on *Nocomis* nesting for reproduction, and other groups rely mostly on simple lithophily. It is possible that strong associates spawned primarily with *Nocomis* simply because *Nocomis* and their nests were more abundant. However, a paired t-test of *Nocomis* adult CPUE and secondary adult CPUE revealed that, across sites, *Nocomis* adults were significantly less abundant than adults of other nest-building species ( $t_{44} = 3.09$ ,  $P = 0.0034$ ). This reinforces the fact that associates were choosing specifically to spawn on *Nocomis* nests. Spawning mode plasticity of strong associates was poorly supported, but some model evidence suggested nest-associative behaviour and host preference is plastic for weak associates. Whereas weak associates may rely on nest association in certain habitats, their reproductive success is more dependent on ambient substrate conditions than the nesting activity of a host (Yamana, Watanabe &

Nagata 2013). Many beneficiaries of facilitator species are capable of utilizing multiple hosts, depending on abiotic context (Machiote, Branch & Villareal 2004). For instance, several studies found that habitat characteristics mediated the plasticity of host choices for secondary cavity-nesting birds and that some species were more prone than others to using alternate hosts (Blanc & Walters 2008b; Robles & Martin 2013; Cockle & Martin 2015). Accordingly, the role of facilitating species at determining animal community structure may be affected in part by the degree to which associative behaviour of beneficiary species is facultative.

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

Nest webs can also help ecologists identify key interactions, resource states and processes that flow from





**Fig. 5.** Juveniles-per-nest of strong associates (a), weak associates (b), non-associates (c) and other nest builders (d) as a function of physical stress. In (e), the first PCoA eigenvector of lotic cyprinid community structure is also regressed on physical stress. Decreasing eigenvector values represent increased proportional representation of *Nocomis* and strong associates in cyprinid community structure. Physical stress represents a linear combination of scaled (1–10) catchment-scale percentages of agricultural land use and catchment area ( $\text{km}^2$ ). Regression results ( $P$ -values) are presented for each response variable, and fitted lines are presented for statistically significant ( $P < 0.05$ ) regression.

unmodified nesting resources, through facilitating species, to beneficiary species to determine community structure. For example, Blanc & Martin (2012) redesigned the original nest web of a cavity-nesting avian community in British Columbia by incorporating finer resolutions of resource states (i.e. decay states of trembling/quaking aspen *Populus tremuloides*) as opposed to simple species-level abundance at the nest web foundation and found that resource availability was lower than previously believed. Similarly, quantifying gradients of gravel availability in the lithophilic cyprinid nest web could aid in predicting community-level shifts in reproductive behaviour as resources become increasingly scarce, for example in urbanizing stream habitats (Peoples, Tainer & Frimpong 2011) or in sediment-starved rivers below dams (Peoples *et al.* 2014). Likewise, in the absence or reduced

abundance of *Nocomis*, interpretation of the nest web would suggest strong associates would rely more heavily on secondary nesters. This must occur in many localities, for example in small tributaries to the Cumberland River, USA, where *S. atromaculatus* functions as the primary fish host (Mattingly & Black 2013). Future observational studies will shed light onto alternate reproductive topologies of lotic cyprinid communities.

This study shows that at least some predictions of the SGH can be extended to animal communities structured by facilitative interactions. First, the importance of spawning habitat amelioration by *Nocomis* for the reproductive success of strong associates increased along the gradient of physical stress. Moreover, per-nest reproductive success of species that are less likely to rely on *Nocomis* showed no significant relationship with physical

stress. This, coupled with the most plausible nest web, suggest that facilitation by *Nocomis* is a strong mechanism structuring these communities. Although the SGH predicts that positive interactions should be more prevalent under harsher environmental conditions, the ability of animals to participate in facilitation may be behaviourally constrained (in this case by nest association strength); that is, not all species can simply rely on facilitation as stress increases. Nest association may be an optimal spawning mode in physically stressful habitats, but using this, behaviour may be impossible for simple lithophilic non-associates that have not evolved the more complex spawning mode of nest association. Obligate symbionts of facilitators, in this case strong nest associates of *Nocomis*, may thus possess an evolutionary advantage at thriving in harsher habitats (Johnston 1999). The system-specific role of facilitation at determining the presence and abundance of species may thus be constrained by larger-scale processes (e.g. species sorting and co-evolution) establishing the biotic context in which species interact.

This study advances the SGH in two ways. First, it demonstrates that the SGH can be a useful model for understanding the role of positive biotic interactions in animal communities. Secondly, it shows that while the interactions themselves may be difficult to measure over a large spatial extent, the outcomes of those interactions (reproductive success of beneficiary species and overall adult community structure) may also be predicted by the SGH. This was particularly evident in the increasing proportional representation of *Nocomis* and strong associates with increasing physical stress. Similarly, Hitt & Roberts (2011) also showed that *N. leptocephalus* and its associates were much more likely to colonize streams whose catchments had undergone considerable land use change. Future work on the SGH should seek to determine whether proportional representation of facilitators and beneficiary species increases with physical stress in the same manner as interaction frequency. This could expand the utility as a more powerful predictive framework that can be applied across systems and taxa.

The utility of the SGH for understanding biological communities is becoming increasingly clear. Thus far, the SGH has been restricted to a few ecological subdisciplines because animal ecologists historically have been slower to investigate the hypothesis that intrinsic community-structuring mechanisms can include both positive and negative interactions. To date, the only studies examining the SGH in vertebrate communities (Barrio *et al.* 2013; and the present study) have contributed partial evidence supporting the SGH; evidence fully supporting the SGH would require demonstrating that the same interaction switches from competition to facilitative from low to high stress. More work is necessary to determine whether the relationship between *Nocomis* and associates becomes neutral or even negative, as may be evidenced in increasing cheating (Baba, Nagata & Yamagishi 1990), parasitism (Fletcher 1993) and competition for space (Sabaj, Maurakis &

Woolcott 2000) under less stressful conditions. In this system, the period in which adult *Nocomis* and associates interact positively is fairly brief, although it defines the future recruitment success of all species involved; the spawning season lasts approximately three weeks, and pre- and post-spawn trophic interactions may be competitive. Any competitive trophic interactions could not have occurred in the absence of a prior reproductive facilitation (*sensu* Bertness & Yeh 1994).

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

This study does not imply that facilitation or even biotic interactions are the only factor structuring stream fish communities. Numerous studies have identified the importance of habitat complexity (Gorman & Karr 1978), dispersal (Muneepeerakul *et al.* 2008), disturbance (Ross, Matthews & Echelle 1985) and water quality/habitat degradation (Karr 1981) (just to name a few mechanisms) for determining stream fish community structure. Instead, this study demonstrates that evidence for strong effects of positive biotic interactions exists in the lotic cyprinid communities of the New River basin and that the importance of the interaction is mediated by habitat conditions. Little research has sought to parse out the relative importance of biotic interactions and habitat conditions; this is particularly true for stream fish communities (Peres-Neto 2004; Hoeinghaus, Winemiller & Birnbaum 2007; Mouchet *et al.* 2013). Further, positive interactions have not been included in well-accepted conceptual frameworks predicting stream fish community structure (Schlosser 1987; Strange, Moyle & Foin 1993; Fausch *et al.* 2002). The SGH can be a useful framework for doing so because (1) it includes the three primary biotic interactions (competition, predation and mutualism/facilitation) and (2) because it includes a role for abiotic factors. Future studies of stream fish communities should consider whether or not positive biotic interactions can operate in their systems. More research is needed to determine the scale and magnitude at which positive interactions affect stream fish communities. This will contribute to the inclusion of all types of biotic interactions (not just negative interactions) into mainstream thinking about stream fish communities and will improve community-level predictions.

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## Data accessibility

All data used in this manuscript are available from the Dryad Digital Repository at <http://dx.doi.org/10.5061/dryad.2ds6h> (Peoples, Blanc & Frimpong 2015).

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## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Appendix S1.** Reproductive groups of cyprinids encountered in the New River basin of NC, VA and WV, USA, in summer 2012.

**Appendix S2.** Variables contained in (1 = present, 0 = absent) and fit statistics of seventeen competing structural equation models predicting interaction network topologies of gravel nesting stream fish communities in the New River basin, Virginia, USA.