

**Investigating Reproductive Phenology and Alloparental Care in Leuciscid Fishes  
using Niche Theory Approaches**

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

Master of Science  
In  
Fisheries and Wildlife Sciences

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(6th May, 2022)  
Blacksburg, VA

Keywords: (Hutchinsonian niche, Eltonian niche, reproductive niche, hypervolume, mutualism, habitat electivity, *Nocomis leptocephalus*, nest association, community ecology, parental care)

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## ABSTRACT

Mutualism is an understudied phenomenon across taxa, but is important to the persistence and structure of communities. The role of positive interactions in supporting threatened species is critically important given that freshwater fish are extremely vulnerable due to the combined effects of climate change and habitat degradation. To disentangle complex positive multi-species interactions, I applied both Eltonian and Hutchinsonian niche approaches to the reproductive mutualism of leuciscids known as nest association, a form of alloparental care characterized by an adult of one species guarding the offspring of another. I conducted this study with the leuciscid community of Toms Creek in which Bluehead Chub *Nocomis leptocephalus* is the primary nest builder and nest associate species include Rosyside Dace *Clinostomus funduloides*, Rosefin Shiner *Lythrurus ardens*, Mountain Redbelly Dace *Chrosomus oreas*, Crescent Shiner *Luxilus cerasinus*, White Shiner *Luxilus albeolus*, Central Stoneroller *Campostoma anomalum*, Creek Chub *Semotilus atromaculatus*, and Blacknose Dace *Rhinichthys atratulus*. I applied Hutchinsonian niche theory with respect to hydrology and water temperature to define the reproductive phenology of each species involved in the reproductive mutualism, then used Eltonian niche theory to define the role of each species in parental and alloparental care. My dual niche theory approach elucidates the reproductive requirements of each species and quantifies the contribution of nest associates to alloparental care, which historically have not been included in definitions of nest association. My results show that Blacknose Dace *Rhinichthys atratulus* and Creek Chub *Semotilus atromaculatus* are not true nest associates of Bluehead Chub *Nocomis leptocephalus* based on their reproductive phenology and observed nesting behaviors. In contrast to previous work on nest association, most associate species did contribute to parental care through nest defense and vigilance behaviors, and their specific behaviors were influenced by morphology. By clearly defining the reproductive niches of these species, this study lays the groundwork for future investigation of how various climate scenarios may impact multi-species reproductive mutualisms.

# Investigating Reproductive Phenology and Alloparental Care in Leuciscid Fishes using Niche Theory Approaches

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## GENERAL AUDIENCE ABSTRACT

Positive interactions are understudied throughout ecology, but are important to the persistence and structure of communities. The role of positive interactions in supporting threatened species is critically important given that freshwater fish are at high risk of extinction due to the combined effects of climate change and habitat degradation. To understand the complex positive interactions in freshwater fish, I applied two niche theory approaches to a mutualistic reproductive interaction known as nest association, where an adult of one species builds a nest on which it guards the offspring of other species (formally: a type of alloparental care). Niche theory is a way of describing how a species interacts with its environment. I conducted this study with the leuciscid (commonly known as minnow) community of Toms Creek in which Bluehead Chub *Nocomis leptcephalus* is the primary nest builder and nest associate species include Rosyside Dace *Clinostomus funduloides*, Rosefin Shiner *Lythrurus ardens*, Mountain Redbelly Dace *Chrosomus oreas*, Crescent Shiner *Luxilus cerasinus*, White Shiner *Luxilus albeolus*, Central Stoneroller *Camptostoma anomalum*, Creek Chub *Semotilus atromaculatus*, and Blacknose Dace *Rhinichthys atratulus*. For each species involved in the reproductive mutualism, I defined the seasonal timing of reproduction with respect to hydrology and water temperature, then defined their role in parental and alloparental care. My approach clarifies the reproductive requirements of each species and establishes the contributions of associate species to alloparental care, which historically have not been included in definitions of nest association. My results show that Blacknose Dace *Rhinichthys atratulus* and Creek Chub *Semotilus atromaculatus* are not true nest associates of Bluehead Chub *Nocomis leptcephalus* based on their reproductive requirements and observed nesting behaviors. In contrast to historic descriptions of nest association, most associate species did contribute to parental care through nest defense and vigilance behaviors, and their specific behaviors were influenced by their size and physical adaptations. By clearly defining the reproductive needs and roles of these species, this study lays the groundwork for future investigation of how various climate scenarios may impact positive relationships among species.

## ACKNOWLEDGEMENTS

I would like to thank my advisor Emmanuel A. Frimpong for his mentorship and support, and for introducing me to the magical world of Southeastern streams. I must also thank my committee members Holly Kindsvater, for her enthusiasm and patience, and inspiring me as a woman blazing paths in fisheries, and Eugene Maurakis, for his unique artistic approach to problem solving and attention to detail. Just as important are my lab mates Maddie Betts, Thomas Bustamante, Samantha Brooks, Houston Chandler, and Maddie Harris whose emotional support was critical to this endeavor. My technicians, Tal Tomlinson, Ty Stephenson, and Nathan Ferguson, brought boundless energy to our work and taught me about leadership and teamwork. I am fortunate to be supported by an amazing community of friends, mentors, and family who continue to inspire me daily to improve our planet. I also owe a debt of gratitude to the women who have preceded me in science and cleared a path for me in this field.

And lastly, I give my love to the minnows, without whom none of this would be possible.

## TABLE OF CONTENTS

|  |    |
|--|----|
| Acknowledgements.....  | iv |
| Chapter 1: Introduction.....   | 1  |
| Chapter 2: Hutchinsonian Niche Theory as a Framework for Multi-Species Mutualism...5   |    |
| Abstract.....  | 5  |
| Introduction.....  | 6  |
| Study system.....  | 8  |
| Objectives and Hypotheses.....   | 10 |
| Methods.....   | 10 |
| Field Methods.....   | 10 |
| Data Analysis.....   | 12 |
| Results.....   | 14 |
| Discussion.....  | 26 |
| Chapter 3: Applications of Eltonian Niche Theory to Alloparental Care in Fishes.....31 |    |
| Abstract.....  | 31 |
| Introduction.....  | 32 |
| Objectives and Hypotheses.....   | 34 |
| Methods.....   | 35 |
| Data Analysis.....   | 37 |
| Results.....   | 37 |
| Discussion.....  | 41 |
| Chapter 4: Conclusions.....  | 46 |
| References.....  | 48 |

## CHAPTER 1- INTRODUCTION

The field of ecology has long been divided on the interpretation and application of niche theory. The first formal niche description came from Grinnell in 1924 and focused only on the physical and climatic barriers to species distribution (Grinnell 1924). In 1927, Elton proposed instead that the niche describes the role of a species in its environment (Elton 1927). Expanding on Grinnell's work, Hutchinson later defined the ecological niche as an n-dimensional hypervolume with axes representing the environmental variables influencing population growth and persistence through reproduction and survival (Hutchinson 1957). The Hutchinsonian and Eltonian frameworks have been useful for answering questions of spatial distribution and community structure, respectively, but remain difficult to reconcile. Attempts to establish a unified niche theory have been proposed (Chase and Leibold 2004) but are not yet widely adopted.

The Hutchinsonian niche provides adequate structure to describe species' environmental needs, but fails to incorporate the reciprocal influence a species has on its environment or its contribution to interspecific interactions. Eltonian niche theory fills this gap by allowing us to categorize species based on their behaviors and ecological role (Rosado et al. 2016). The complexities of community interactions are therefore best viewed through multiple lenses, as the observed multi-species interactions are only interpretable in the full context of environmental conditions and unique behavior profile of participating species.

Traditional niche theory is centered on the impact of predation and competition through the competitive exclusion principle in Hutchinsonian theory, and resource exploitation patterns in Eltonian theory. Whereas competition and predation are widely understood to fundamentally shape species distributions, mutualism and other facilitative interactions are just as influential on community structure (Bruno et al. 2003). Niche theory is a useful tool for exploring mutualistic interactions (Batstone et al. 2018) given their context-dependency. Changes in species' behaviors or environmental conditions may result in a shift toward commensalism or even parasitism (Peoples and Frimpong 2016).

This context determining whether species' interactions are positive or negative becomes critically important when climate conditions shift within an ecosystem. Future climate models predict wider variability in flow regimes and temperatures around the globe (Trenberth 2011). Aquatic ecosystems are particularly vulnerable to these changes (Thackeray et al. 2016). The responses of fish, especially those with complex life history strategies, are understudied (Crozier et al. 2008). Fishes are among the most threatened taxa with almost 40% of North American fishes listed as imperiled (Jelks et al. 2008). Not captured by this statistic is the interconnected nature of species relationships. Mutualistic and facilitative relationships may support species in ways we do not yet understand, revealing the importance of full community conservation to slow biodiversity loss (Frimpong 2018).

Alloparental care, or the care of non-related young, in fishes can manifest as a mutualistic relationship. Alloparental care can occur with either embryos or free-

swimming young, and typically involves no care from the biological parents (Wisenden 1999). A commonly observed form is nest association, a behavior where a host species builds a nest on which associate species deposit embryos (Johnston 1994). Interestingly, in some systems, leuciscid associates have been observed guarding a mixed-brood nest in the absence of the leuciscid nest building host (Vives 1990). Because these systems are so understudied, there have been no detailed investigations of the pairwise interactions in these multi-species mutualisms or the potential contributions of associates to the care of their offspring. I aim to demonstrate that leuciscid associates do contribute to parental care through their defensive and vigilance behaviors around their hosts' nests.

In the context of changing climate and staggering biodiversity loss (Jelks 2008), scientists and managers need a holistic way to evaluate community relationships. By applying both Hutchinsonian and Eltonian niche theory, I can relate phenology and environmental sensitivity to species behaviors and roles in complex multi-species interactions. In this framework, it becomes critical to understand the overlap of partner species' niches in order to make predictions about how changing climates may impact the persistence of populations involved in mutualistic interactions.

I used the fish community of Toms Creek as a model system to examine the application of dual niche theory in a positive multi-species interaction involving alloparental care. Toms Creek is a third-order tributary to the New River located in Southwest Virginia and contains a diverse fish community including over a dozen species of leuciscids minnows. Several of these species have complex reproductive behaviors, including parental care of eggs deposited in benthic nests. The primary leuciscid nest

builder in this system is a male Bluehead Chub *Nocomis leptcephalus*, which constructs large gravel mounds during the spawning season. Two additional leuciscid nest building males occur in this system: Central Stoneroller *Campostoma anomalum* that create spawning pits, and Creek Chub *Semotilus atromaculatus* who builds a pit-ridge nest. A previous study reported eleven potential associates of *Nocomis* spp. in the New River basin (Pendleton et al. 2012), eight of which are found in Toms Creek.

In Chapter 2, I determine the Hutchinsonian reproductive niche of each species commonly observed on Bluehead Chub nests in Toms Creek with respect to hydrology and water temperature, and in Chapter 3, I measure the Eltonian reproductive niche of nest hosts and associates (*N. leptcephalus*, *C. anomalum*, *S. atromaculatus*, *Chrosomus oreas*, *Clinostomus funduloides*, *Luxilus albeolus*, *Luxilus cerasinus*, *Lythrurus ardens*, and *Rhynchys atratulus*) using defensive and vigilance behaviors to assess the contribution of each species to parental care. This dual niche theory approach elucidates the reproductive requirements of each species and quantifies previously unknown contributions of nest associates to this mutualism. By clearly defining the reproductive phenology of these species and their contributions to parental care, this study also lays the groundwork for future investigations of how various climate scenarios may impact multi-species reproductive mutualisms.

## CHAPTER 2- HUTCHINSONIAN NICHE THEORY AS A FRAMEWORK FOR MULTI-SPECIES MUTUALISM

### ABSTRACT

Global patterns of climate change are altering the temperature and hydrology of freshwater systems. The phenology of freshwater fish is closely linked to these factors, but species within a community respond differently to variation in climate. Reproductive synchrony is especially important in mutualisms where species depend on each other for survival and reproductive success. In this study I use hypervolume models of several environmental factors to capture multiple axes of reproductive phenology of the leuciscid community of Toms Creek. Many of these species participate in nest association, behavior where a host species builds a nest on which associate species deposit eggs. The hypervolume technique clarifies the niche separation between nest builders and verifies the relationship strength with each associate species. Using the Least Absolute Shrinkage and Selection Operator (LASSO) and Boosted Regression Trees (BRTs) I identified the environmental variables most influential to the phenology of each species and used them to construct Gaussian hypervolume models. I also quantified nesting habitat selection for nest building species as part of their realized Hutchinsonian niche. The phenology of each species was approximated with the water temperature associated with their peak presence on nests. Nest builders in Toms Creek selected for distinct habitat characteristics reflective of their nest structure and had different peak temperatures indicating some niche separation. Associates expressed strong total niche overlap with their primary host, Bluehead Chub *Nocomis leptocephalus*.

## INTRODUCTION

The phenology, or seasonal timing of life history events, of temperate freshwater fishes is dependent on predictable seasonal cues like temperature and hydrology (Krabbenhoft et al. 2014). Phenology can be expressed as a unimodal function of conditions, within which each species has a unique optimal range of preferred conditions. The current climate warming trend is causing changes to hydrologic systems and altering seasonal activities of many species (Pachauri and Meyer 2014). In many areas across North America, climate models predict precipitation patterns that may result in less frequent but more intense rainfall events (Trenberth 2011) which could drastically change the hydrologic cycles of small streams.

For many taxa, warming trends may lead to an extended breeding season, early arrival to spawning grounds, and advanced egg-laying dates (Post et al. 2008). Reproductive synchrony can shape communities through both intra- and interspecific interactions. Early breeding individuals produce offspring that benefit from their larger size in competition with individuals born later in the season, and these effects can carry through later life stages (Carter and Rudolf 2019).

The phenology of a species can be modeled as a response curve of reproductive activity along one axis or multiple axes of environmental variables, similar to the hypervolume concept described by Hutchinson (1957). Framing phenology with the Hutchinsonian niche theory provides a quantitative structure for considering the interactive effects of environmental conditions on species' behaviors. Across taxa, there

is currently no consensus on which environmental cues or intrinsic traits impact phenology (Woods et al. 2021). By identifying the most influential variables for each species, phenology can then be quantified as a hypervolume.

In this study, I propose that the niche of a species may be subdivided into conditions necessary for different life stages. For example, the conditions suitable for juvenile growth may not be identical to those needed to trigger spawning. Just as we consider specialization for feeding, so should we consider reproductive niche generalists and specialists for their environmental tolerances and use of spawning habitat (Colwell and Futuyma 1971). Much as Hutchinson (1957) described the realized niche as a spatial expression of the fundamental niche constrained by competition, I posit that the phenology of reproduction is the realized temporal expression of the fundamental reproductive niche requirements; therefore, the capacity of a species to alter its phenological response is constrained by its niche breadth.

Recent studies by Rudolph (2013), Post (2008), and Yang (2010) have investigated the impacts of reproductive phenology shifts on individual species' fitness or predator-prey relationships, but it is still unclear how complex multi-species interactions may be affected by climate induced phenology shifts. Evidence suggests that species within a community may respond differently to climatic variation (Both et al. 2009), potentially altering the periods when species may interact. As previously stated, a species may only shift its phenology within the constraints of its niche requirements; however, the niche includes not only abiotic variables but also biotic factors such as mutualist partners (Warren et al. 2014). Species involved in an obligate mutualism are therefore

constrained not only by their own environmental requirements but also by the requirements of their partners. Alternatively, the presence of a facilitating host may expand the niche expression of a species by making habitat or resources more readily available (Bruno et al. 2003, Batstone et al. 2018).

## STUDY SYSTEM

I used the fish community of Toms Creek as a model system to characterize the reproductive niche of eight leuciscid species through the lens of nest association. Toms Creek is a third-order tributary to the New River located in Southwest Virginia containing a diverse fish community. The leuciscid species found there frequently engage in nest association, a behavior where a host species builds a nest on which associate species deposit embryos (Johnston 1994). The primary nest builder in this system is the Bluehead Chub (*Nocomis leptcephalus*), which constructs large gravel mounds during the spawning season. A previous study in the New River basin reported eleven potential associates of *Nocomis* spp. (Pendleton et al. 2012), eight of which are found in Toms Creek. These include Rosyside Dace *Clinostomus funduloides*, Rosefin Shiner *Lythrurus ardens*, Mountain Redbelly Dace *Chrosomus oreas*, Crescent Shiner *Luxilus cerasinus*, White Shiner *Luxilus albeolus*, Central Stoneroller *Campostoma anomalum*, Creek Chub *Semotilus atromaculatus*, and Blacknose Dace *Rhynchys atratulus*. Through comparison of the reproductive behavioral traits, geographic range, spawning temperature, and substrate preferences of each associate, Pendleton et al. (2012) ranked species as either strong or weak associates. Variation in the strength of each species' association with

Bluehead Chub (Pendleton et al. 2012) provides a unique opportunity to explore the link between phenological synchrony and mutualism.

| <b>Species</b>                | <b>Nest association strength<br/>(Average rank)</b> |
|-------------------------------|---|
| <b>Rosyside Dace</b>          | 2.5   |
| <b>Rosefin Shiner</b>         | 2.5   |
| <b>Mountain Redbelly Dace</b> | 4.0   |
| <b>Crescent Shiner</b>        | 6.0   |
| <b>White Shiner</b>           | 6.0   |
| <b>Blacknose Dace</b>         | 7.0   |
| <b>Central Stoneroller</b>    | 9.0   |

Table 2.1- Summary of table from Pendleton et al. (2012) representing the relationship strength of associates present in Toms Creek with Bluehead Chubs. Ranks are based on the absolute value of scores derived from the ordination of a phylogenetically independent trait similarity matrix.

Pendleton et al. (2012) synthesized the current literature on spawning temperature ranges for *Nocomis* spp. and its associates across the New River basin, but there has been no fine-scale investigation of the mechanism driving the timing and synchrony of spawning among these species. I describe the ‘reproductive niche’ of a species by defining the temperature, hydrologic, and substrate requirements for reproduction. Since all species in Toms Creek are experiencing the same abiotic conditions, I can therefore consider each species’ unique spawning phenology as a proxy for its Hutchinsonian

reproductive niche. Nest builders must also select habitat for their reproduction; therefore the environmental conditions of substrate size, water depth and velocity, and the presence of instream cover or structure are also part of a host's niche (Lobb and Orth 1988).

## OBJECTIVE AND HYPOTHESES

The primary objectives of this study are to 1) define the Hutchinsonian reproductive niches of the Bluehead Chub and co-occurring leuciscids in Toms Creek and 2) quantify the strength of each species' association with the Bluehead Chub. In this chapter I present the following hypotheses: a) each associate species will have a distinct phenology that will be reflected in patterns of presence and relative abundance on Bluehead Chub nests throughout the season, b) the timing of spawning is a response to multiple dimensions of environmental conditions, c) the three nest building species require distinct microhabitats for nest construction, and d) the species identified as strong associates across their range in Pendleton et al. (2012) will have more overlap of their reproductive niche with the Bluehead Chub at the local scale than those identified as weak associates.

## METHODS

### FIELD METHODS

From 2016-2021, observers walked approximately 0.65 km of Toms Creek daily during the spawning season (April – July) each year, making notes of species' presence

and abundance on nests. Nikon V1 HD cameras were deployed underwater near active nests for at least 20 minutes to film fish activities on approximately 80 nests each year.

Instream and ambient air temperature (C) were recorded hourly to the nearest 0.1° C at Toms Creek with HOBO temperature loggers deployed from March to August 2016-2021. Some small data gaps were created by delay in deployment of loggers some years. To eliminate these data gaps, I predicted water temperature using two generalized linear models. The first related measured water temperature to measured air temperature, obtained from the Blacksburg Regional airport, and the second model linked records of Blacksburg air temperature to air temperature measured at the study site.

In 2016, 2017, 2020, and 2021, water level was measured in two locations on Toms Creek using a HOBO water level logger. I predicted the water level in unmeasured years by creating a multiple linear regression with hydrologic variables such as USGS stream gauge levels (USGS 2016) and rainfall data. Based on the results of a LASSO regression, the best predictors of water level in Toms Creek are prior rainfall (with a delay of 10 hours) and gauge height data from Catawba Creek. Catawba creek is of a similar size and gradient as Toms creek, and originates from the same mountain, so we can assume it experiences similar hydrologic conditions.

Available nesting habitat was measured at each of 277 permanent transects (~ 2-2.5m long) in Toms Creek in 2016, 2020, and 2021. Each transect was characterized by its wetted width, thalweg depth, thalweg velocity, thalweg substrate, secondary substrate,

degree of thalweg embeddedness, presence of gravel bars, and presence of instream structure or overhanging vegetation. Substrate was classified on the Wentworth scale using methods in Wentworth 1922. Embeddedness was categorized as either low, medium, or high based on a relative visual estimate of ambient siltation. Instream structures include large rocks, logs, or other vegetation that disrupt water flow. To track habitat usage trends across years, locations of all nests (mounds, pits, and pit-ridge) relative to each transect were identified when they were first observed, marked in situ, and noted in field notes for monitoring.

## DATA ANALYSIS

In order to quantify species presence and relative abundance on nests, I watched all nesting videos, pausing every two minutes to count all individuals of each species in the frame. Videos with poor clarity or low visibility were discarded. I averaged the count of each species per frame over the entirety of the video. If several videos were recorded on a single day, I averaged the totals from all videos to report one value per day.

To establish the environmental gradients present in Toms Creek, I converted hourly water temperature to reproductive degree days (RDD) using the procedure outlined in Chezik et al. (Chezik et al. 2014):

$$RDD_{daily} = \sum_{i:24} (\sum (T_{max\ hourly} - T_{min\ hourly}) + T_0/48) \quad (1)$$

where  $T_{\text{hourly}}$  is the water temperature measured once per hour,  $T_0$  is a threshold critical temperature, and the equation is summed over 24 hours. This method quantifies the accumulation of heat over time rather than using snapshot measurements. I identified significant changes in water level between days by performing a one-way ANOVA on 24 data points each day and using Tukey's honestly significant difference (Tukey 1991). This method efficiently evaluates pairwise differences in water level while keeping type I error low (Mchugh 2011). These results populated three nominal variables for stable, increasing, and decreasing water level compared to the previous day. Given the exploratory nature of this method, I retained raw water level as a variable as well. Using a density ridge function (Wilke 2021), I identified the average daily water temperature corresponding to the highest frequency of each species' presence based on their relative abundance on Bluehead Chub mounds (Fig. 2.2).

I modeled species' niches by first building boosted regression trees (BRT) using a suite of environmental covariates including various mutations of water temperature, periods of heat accumulation (represented as reproductive degree days), and water level, with the *gbm* package (Greenwell et al. 2020) in R. The boosted regression tree procedure identifies and ranks variables in order of importance. I used the top 3 variables (daily RDD, water level, and median temperature) to calculate Gaussian hypervolumes (Blonder 2022). Using the Jaccard similarity index, I then extracted the fractional overlap between each associate species and the Bluehead Chub.

To capture the role of habitat on the niche of nest builders, I performed K- means clustering on the permanent transect characteristics of thalweg depth, thalweg velocity, wetted width, and substrate type. I used a bootstrap method to assess the stability of various number of clusters. Using the average Jaccard similarity of each cluster, I selected 0.8 as the cutoff for meaningful patterns. I identified the primary qualities of each of the resulting groupings to produce ecologically meaningful habitat descriptions of each transect, then compared the frequency of used and unused transects in the years 2016-2021 to find which habitat type each nest builder is selected using Ivlev's electivity index ( $I$ ):

$$I = (r-P)/(r+P) \quad (2)$$

where  $r$  is the proportion used by the species and  $P$  is the relative proportion of the resource in the environment (Ivlev 1975). Values close to 1 indicate strong selection for a resource and values near -1 indicate avoidance.

## RESULTS

All strong nest associates (*sensu* Pendleton et al. 2012) had mean temperature values aligned closely with the main nest builder, Bluehead Chub, at approximately 18° C. Central Stoneroller, Rosyside Dace, and Mountain Redbelly Dace were skewed toward lower temperatures. Crescent Shiner, Rosefin Shiner, and White Shiner skewed slightly toward higher temperatures. Blacknose Dace and Creek Chub distributions were skewed significantly toward lower temperatures. Of the three nest building species, Creek Chub

had the lowest mean spawning temperature. Bluehead Chub had the highest mean and median temperatures (table 2.2)

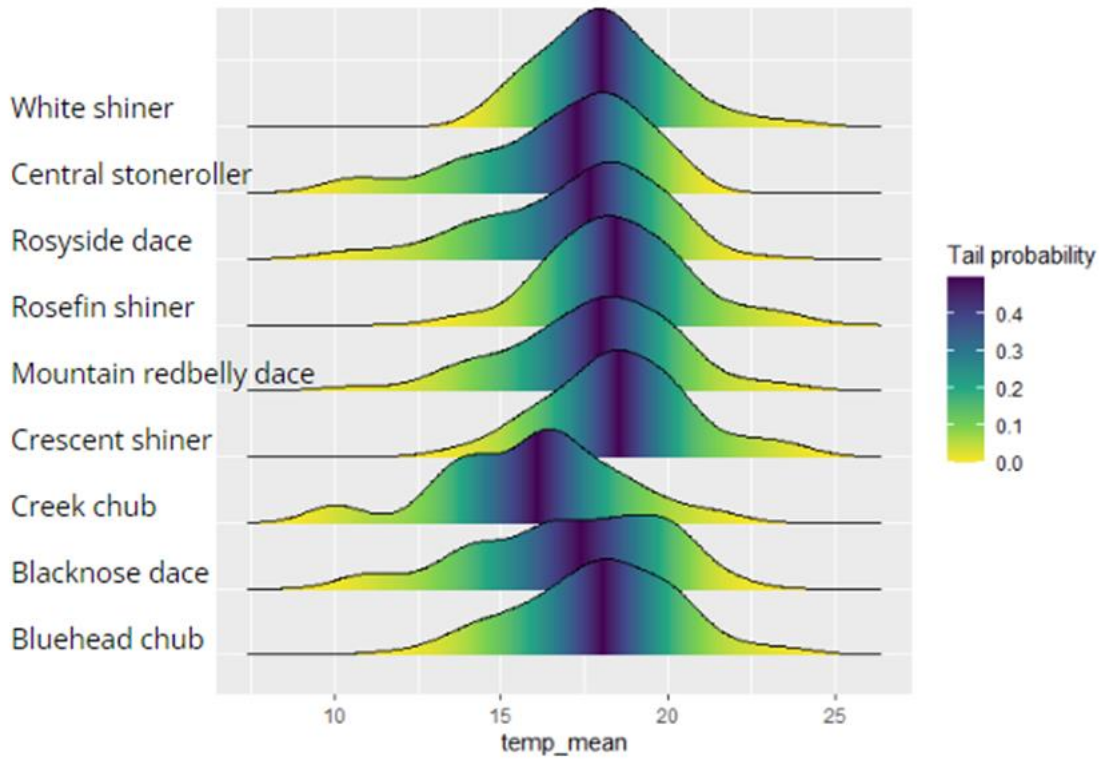


Figure 2.1- Occurrence of leuciscid species on Bluehead Chub nests in relation to average daily water temperature (C) in Tom's Creek, Montgomery Co., VA, 2016-2021.

|                         | <b>Mean Water Temp. (C)</b> | <b>Median Water Temp. (C)</b> | <b>Standard Error</b> | <b>Confidence Interval</b> |
|-------------------------|-----------------------------|-------------------------------|-----------------------|----------------------------|
| <i>S. atromaculatus</i> | 15.9                        | 16.1                          | 0.50                  | 14.92-16.88                |
| <i>C. anomalum</i>      | 16.8                        | 17.4                          | 0.26                  | 16.29-17.31                |
| <i>R. atratulus</i>     | 17.1                        | 17.4                          | 0.51                  | 16.1-18.1                  |
| <i>C. funduloides</i>   | 17.2                        | 17.6                          | 0.24                  | 16.73-17.67                |
| <i>C. oreas</i>         | 17.7                        | 18.1                          | 0.23                  | 17.25-18.15                |
| <i>N. leptcephalus</i>  | 17.9                        | 18.2                          | 0.24                  | 17.43-18.37                |
| <i>L. albeolus</i>      | 18.1                        | 18.1                          | 0.28                  | 17.55-18.65                |
| <i>L. ardens</i>        | 18.5                        | 18.4                          | 0.24                  | 18.03-18.97                |
| <i>L. cerasinus</i>     | 18.6                        | 18.4                          | 0.28                  | 18.05-19.15                |

Table 2.2- Daily mean and median daily water temperature (C) associated with the peak presence of each species on Bluehead Chub nests, at 95% confidence intervals on the mean are also reported.

The boosted regression trees using species abundance and a suite of environmental covariates including various mutations of water temperature, periods of heat accumulation (represented as reproductive degree days), and water level. The BRT results indicated that across all species, water level and daily mean and median temperatures were the most influential factors (Fig. 2.2). The most influential variables were those that explained the greatest variability in the data, and I used a natural-break method to choose how many variables should be retained.

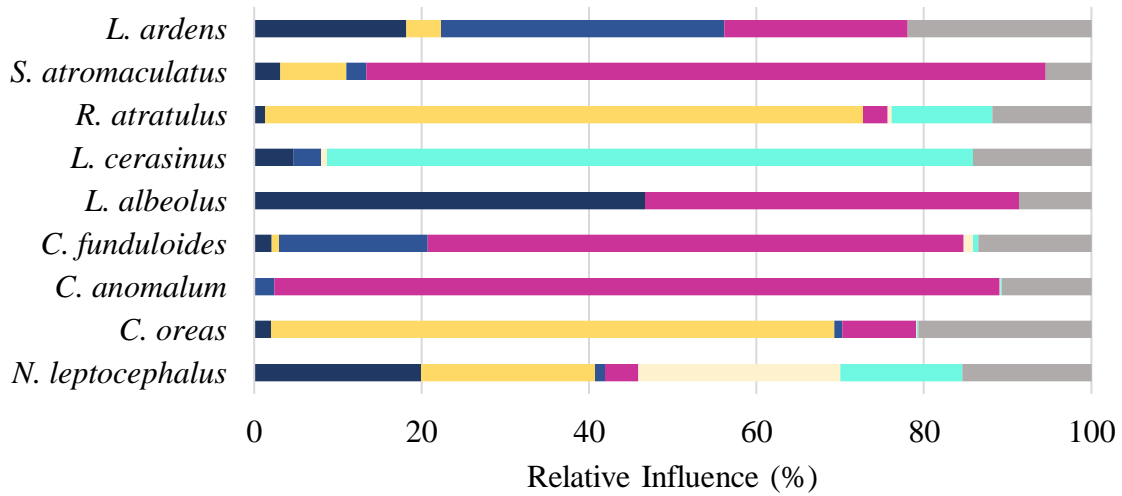
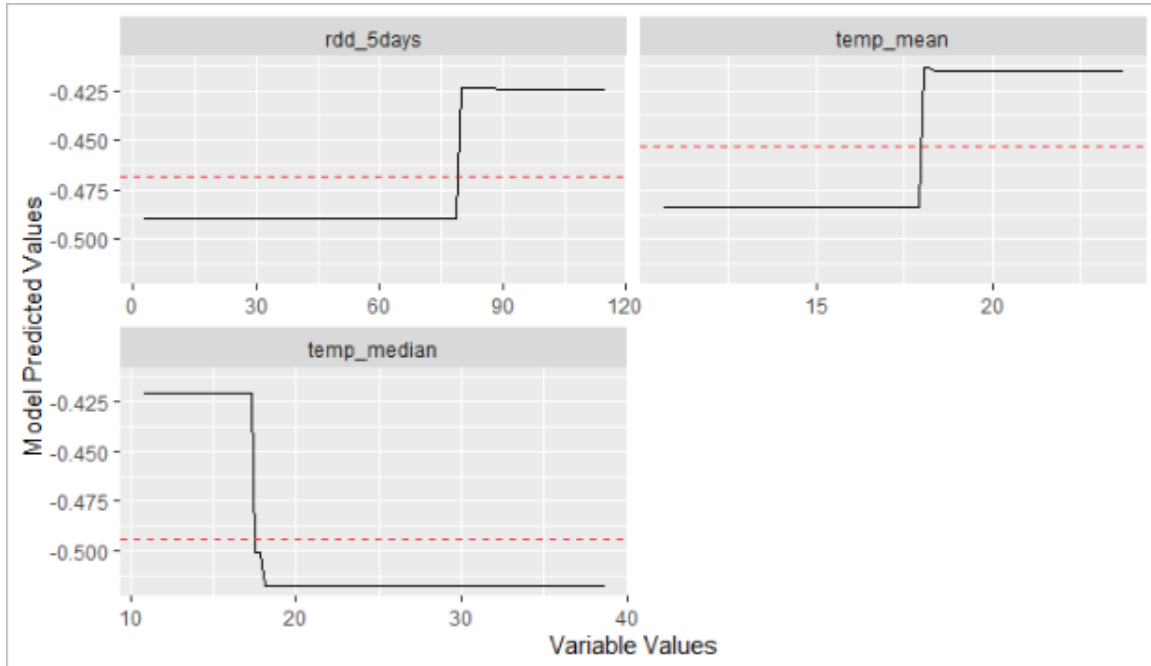


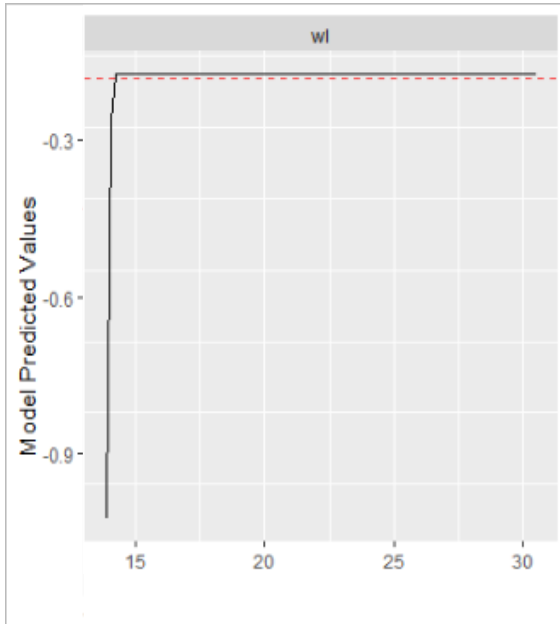
Figure 2.2- Relative influence of the boosted regression tree variables on the presence of each associate species on Bluehead Chub nests, as a percentage of total influence. The most common influential variables are colored while non-significant variables are grouped into “other”. Significant variables are daily mean temperature, daily median temperature, daily temperature range, water level, 5-day RDD (heat accumulation of water over a five-day period), and 6-day RDD (heat accumulation of water over a six-day period).

Partial dependence plots indicating threshold values for the most influential variables in each species BRT results are shown in figure 2.3.

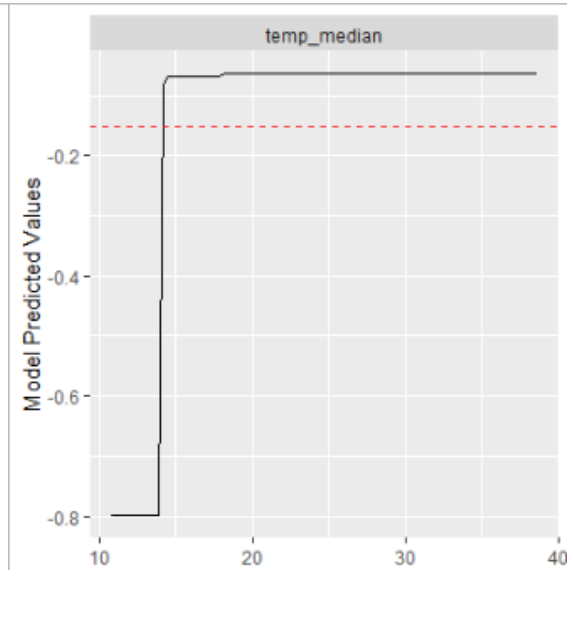
*N. leptcephalus*



*C. oreas*

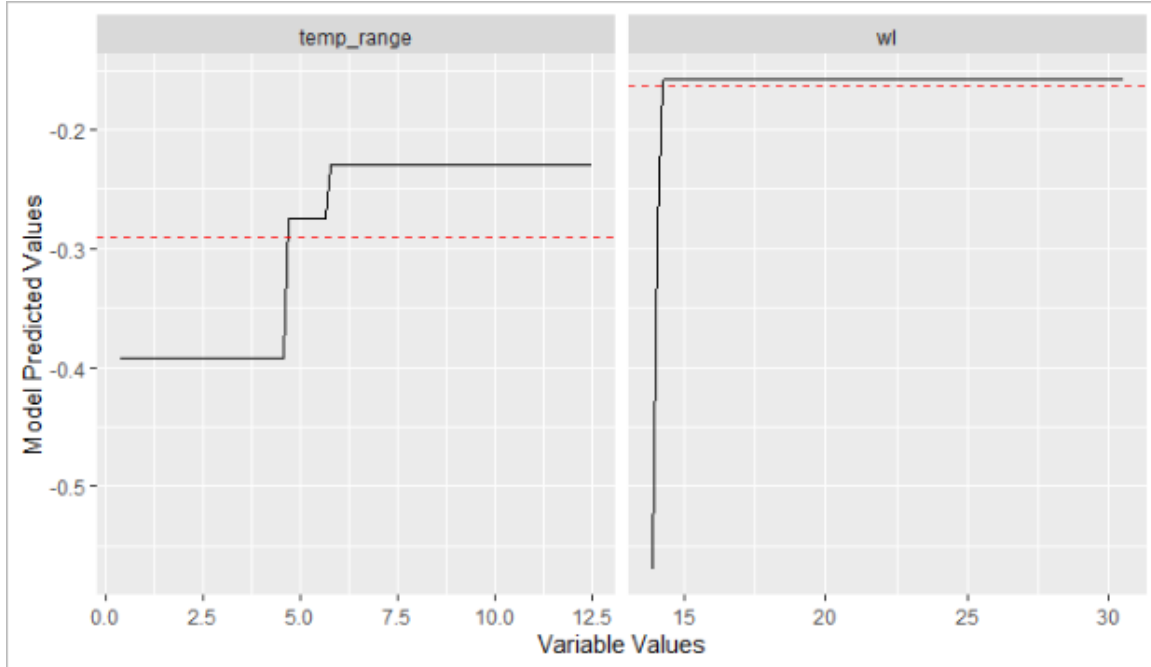


*C. anomalum*

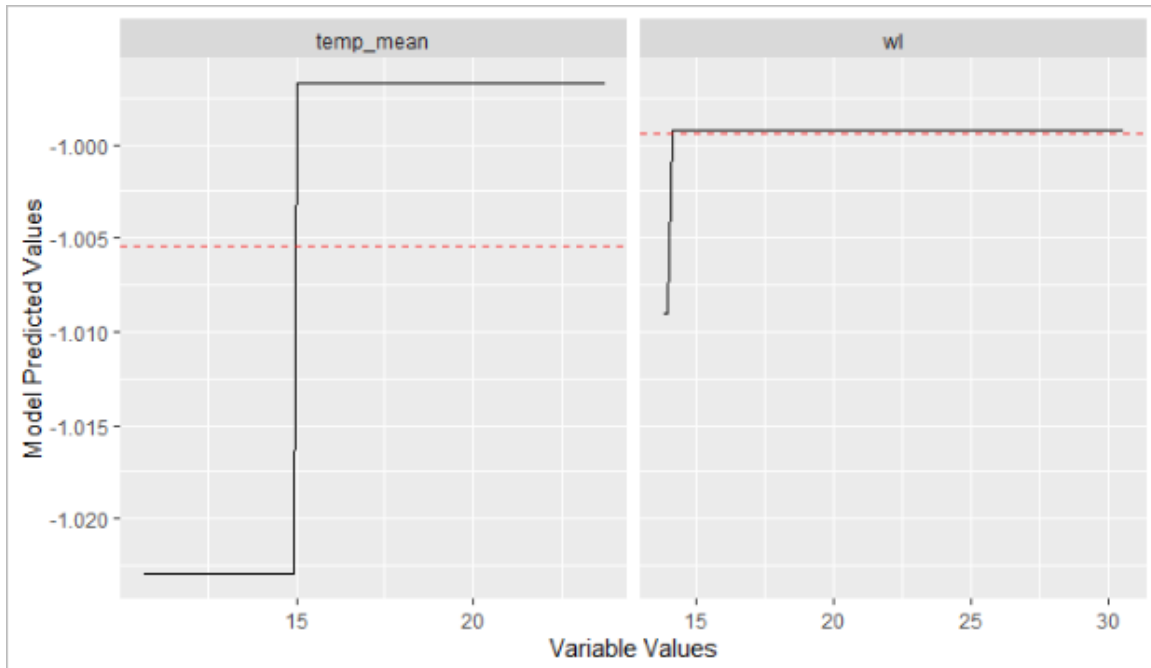


Variable Values

*C. funduloides*

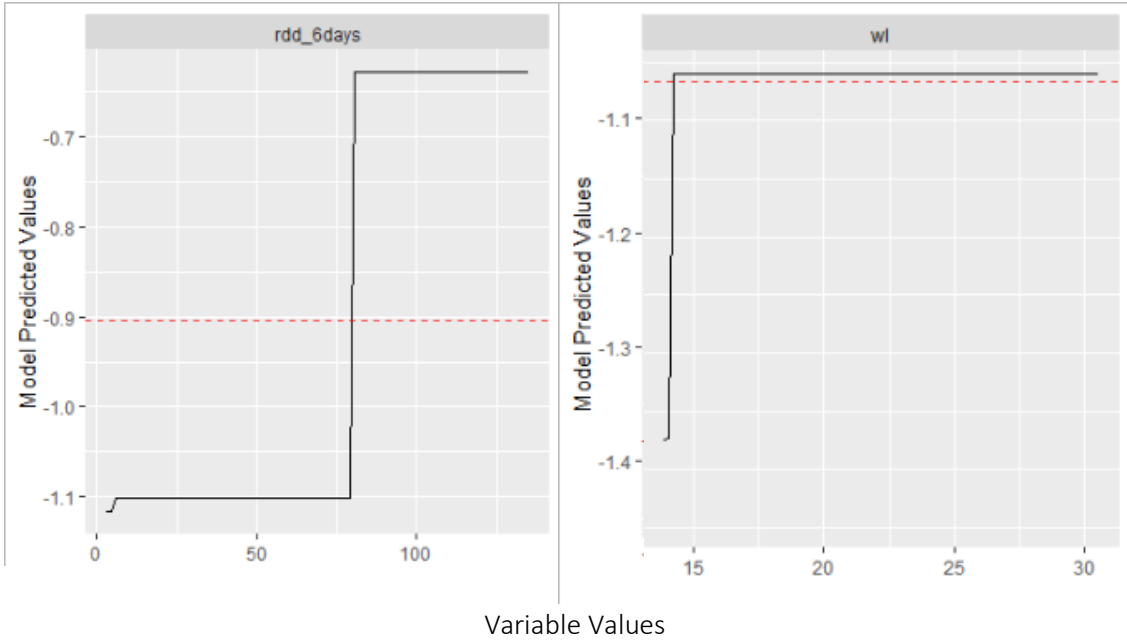


*L. albeolus*

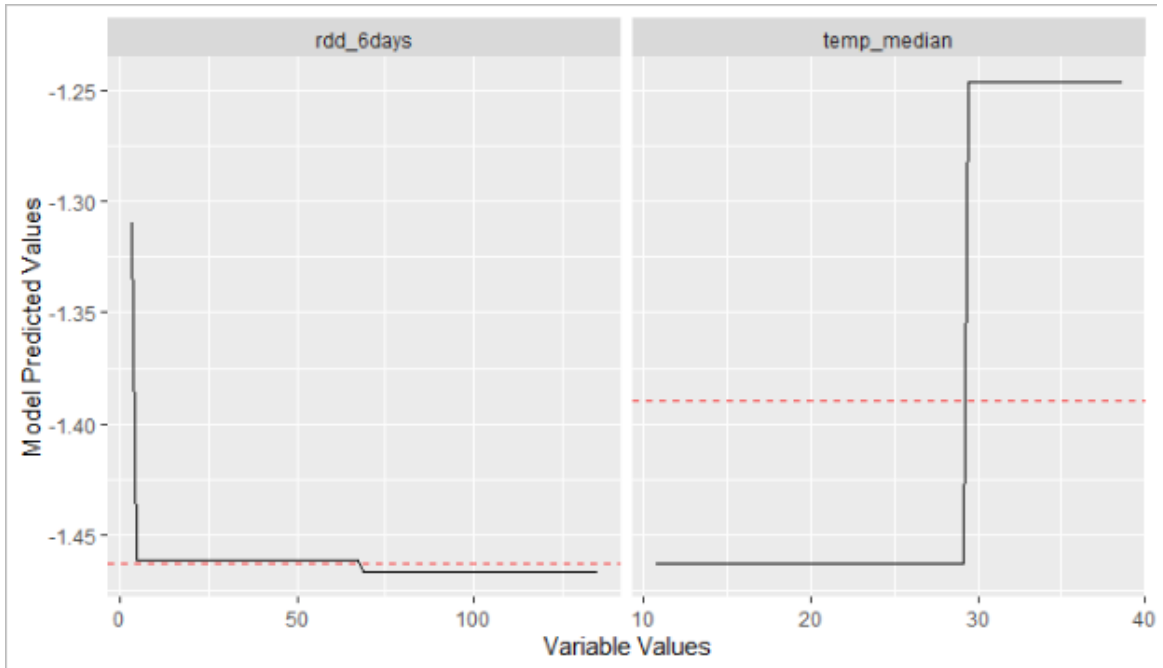


*L. cerasinus*

*S. atromaculatus*



*R. atratulus*



*L. ardens*

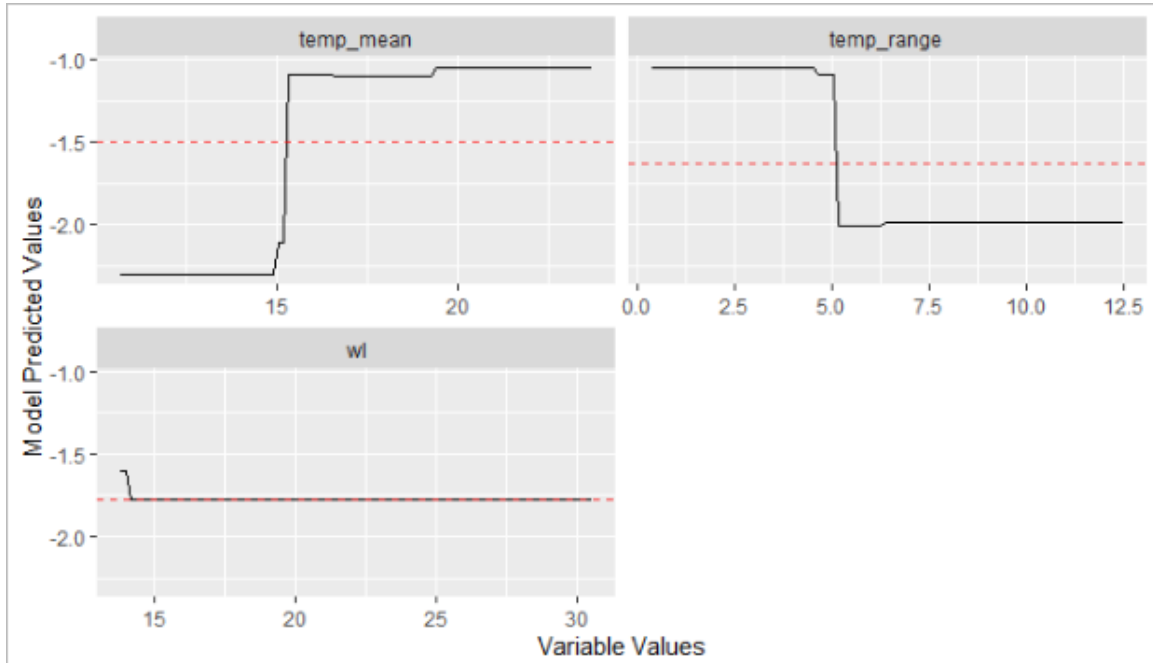


Figure 2.3- Partial dependence plots for most influential variables from the boosted regression trees. The top variables were selected with a natural break method.

Blacknose Dace had the least amount (46%) of niche overlap with Bluehead Chub whereas Creek chub had the greatest overlap (64%). There were no significant differences between fractional overlaps among other associate species (table 2.3).

| Species                 | Fractional Overlap |
|-------------------------|--------------------|
| <i>S. atromaculatus</i> | 0.64               |
| <i>C. funduloides</i>   | 0.58               |
| <i>L. albeolus</i>      | 0.57               |
| <i>C. oreas</i>         | 0.57               |
| <i>C. anomalum</i>      | 0.56               |
| <i>L. cerasinus</i>     | 0.54               |
| <i>L. ardens</i>        | 0.49               |
| <i>R. atratulus</i>     | 0.46               |

Table 2.3- Fractional niche overlap represented as the Jaccard index of similarity between hypervolume objects of associate species with Bluehead Chub. Hypervolume axes are water level, median daily water temperature, and daily reproductive degree day value.

The habitat in Toms Creek clustered into four groups (Fig. 2.5). The highest cluster stability was shown when the data was clustered in five or fewer groups (Fig. 2.4). Although the highest stability was in two or three clusters, I chose to use four to capture potentially biologically meaningful differences in habitat conditions.

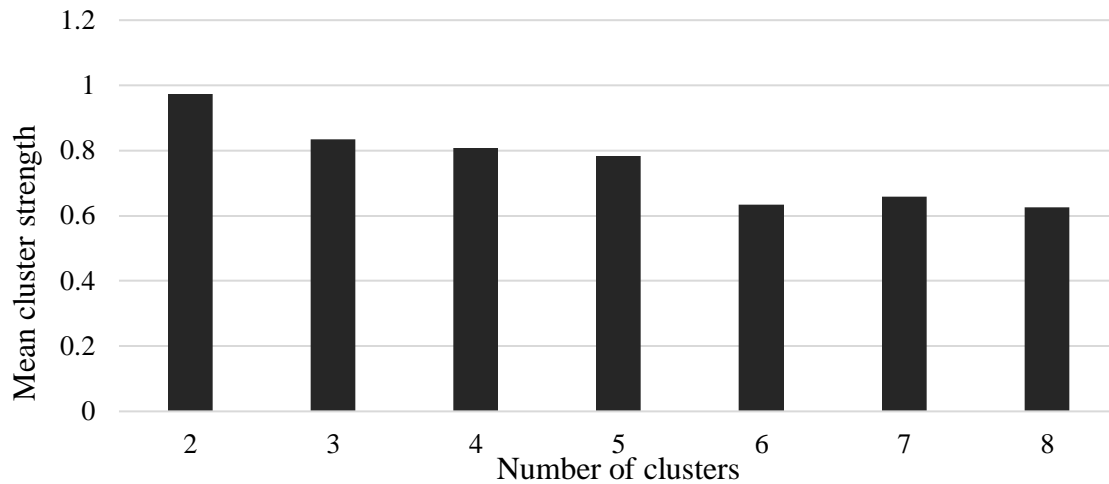


Figure 2.4- Mean cluster stability from bootstrapping of K-means on Toms Creek habitat characteristics.

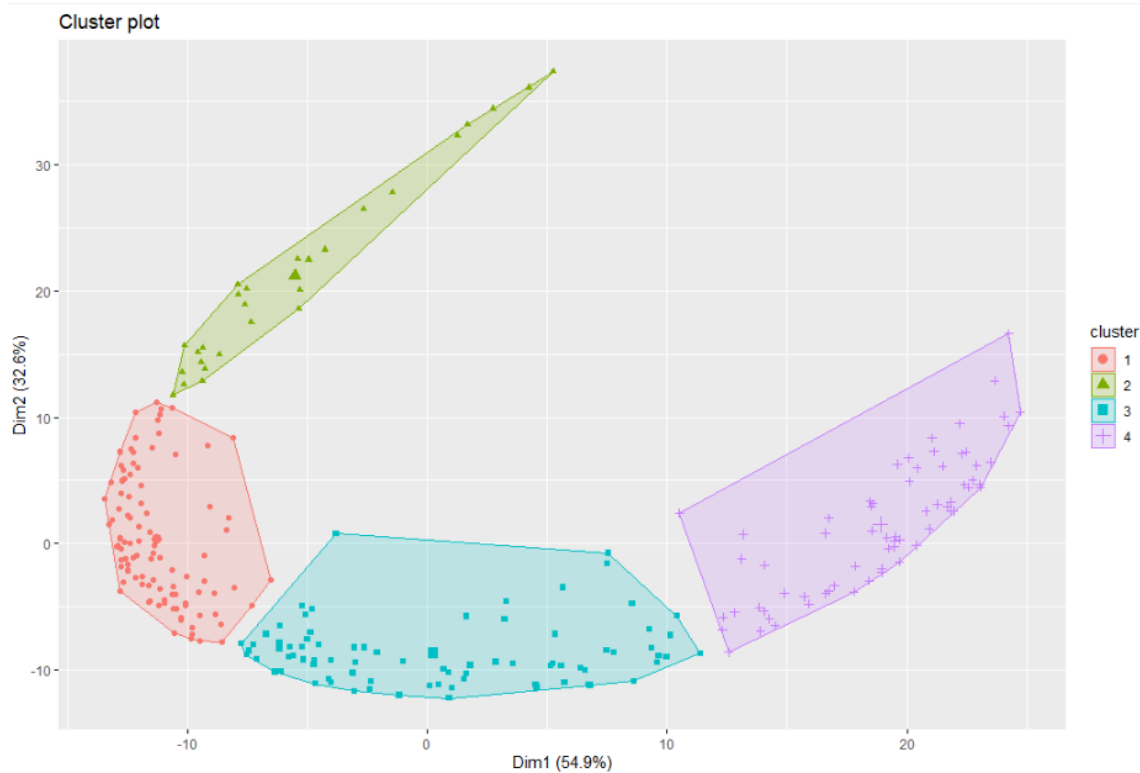


Figure 2.5- K-means clustering of Toms Creek transects. Each point represents a 2m segment of stream, with habitat variables including wetted width, depth, thalweg velocity, embeddedness, and primary substrate.

After examining the average characteristics of the transects in each cluster (Table 2.4), I derived descriptions of the primary habitat types and presented with a typical example of a transect from the study site (table 2.5).

| <b>Cluster</b> | <b>Velocity (m/sec)</b> | <b>Depth (m)</b> | <b>Primary Substrates</b> | <b>Undercut (%)</b> | <b>Overhang (%)</b> | <b>Instream (%)</b> | <b>Gravel bar (%)</b> |
|----------------|-------------------------|------------------|---------------------------|---------------------|---------------------|---------------------|-----------------------|
| <b>1</b>       | 0.2                     | 41.5             | Boulder/Bedrock           | 33.7                | 60.4                | 21.8                | 31.7                  |
| <b>2</b>       | 0.1                     | 61.1             | Boulder/Bedrock           | 33.3                | 40.74               | 51.9                | 25.9                  |
| <b>3</b>       | 0.2                     | 29.7             | Cobble/Boulder            | 25.6                | 31.71               | 18.3                | 40.2                  |
| <b>4</b>       | 0.3                     | 17.8             | Gravel/Cobble             | 23.9                | 17.91               | 11.9                | 37.3                  |

Table 2.4- Average velocity and depth of the thalweg, major substrate, and the percentage of each transect type with undercut banks, overhanging vegetation, instream structures, and gravel bars.





| Cluster | Name              | Description  | Example   |
|---------|-------------------|--|---|
| 1       | Moderate pool     | Moderately deep pool or run with boulder and bedrock substrate and abundant overhanging vegetation                       |    |
| 2       | Refuge pool       | Very deep boulder and bedrock pool with abundant instream or bank structure and some overhanging vegetation              |    |
| 3       | Coarse riffle/run | Fairly shallow, moderately fast water riffle, run or pool tail with boulder to cobble substrate and adjacent gravel bars |   |
| 4       | Shallow riffle    | Very shallow, fast water riffle or pool tail with cobble to gravel substrate and adjacent gravel bars                    |  |

Table 2.5- Primary habitat types with description of conditions and example locations within Toms Creek

Bluehead Chubs did not exhibit strong preference for most habitat types (Fig. 2.6), but selected strongly against shallow riffles. Central Stonerollers selected strongly for deep refuge pools and weakly for shallow riffles. Creek Chubs exhibited the strongest preferences, selecting against moderate pools and for shallower areas like runs and riffles.

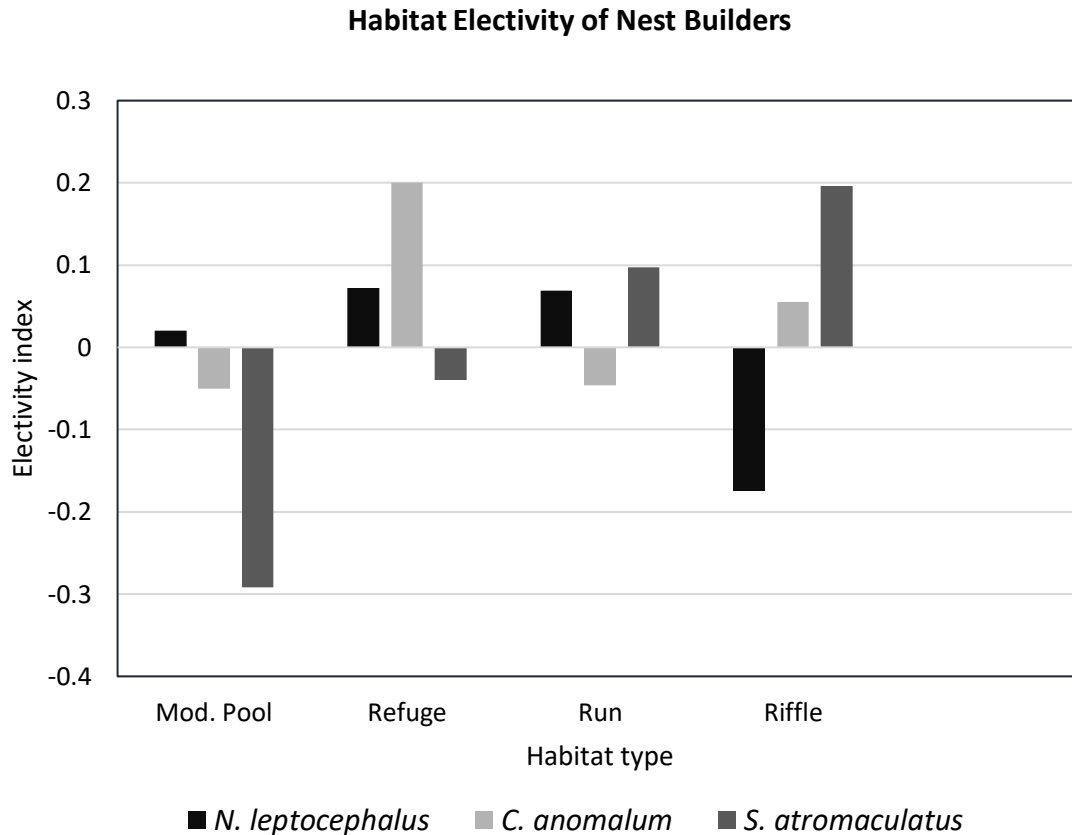


Figure 2.6 - Ivlev’s electivity index for habitat type by nest building species indicating the number of nests constructed in a transect classified a moderate pool (mod. pool), refuge pool (refuge), coarse riffle/run (run), or shallow riffle (riffle) over the years 2016-2021.

## DISCUSSION

My first objective in this study was to define the Hutchinsonian reproductive niche of the Bluehead Chub and co-occurring leuciscids in Toms Creek. I predicted that

each associate species has a distinct phenology reflected in its patterns of presence and relative abundance on Bluehead Chub nests. Comparison of presence against mean temperature gave an indication of each species' optimal reproductive temperature. These results support anecdotal observations of Creek Chubs initiating nest building in the spring, followed by Central Stonerollers, and finally Bluehead Chubs. The subtle shifts in optimal temperature explain why we observe variation in community composition over the season. A strong peak, like those exhibited by White Shiners, Crescent Shiners, and Rosefin Shiners, can be interpreted as a more specialized reproductive niche. In this system, a specialized niche presents as an obligate or exclusive association with Bluehead Chubs. Flatter curves indicate a weaker association with Bluehead Chubs. The species with flatter curves, including Mountain Redbelly Dace and Rosyside Dace, are often observed associating with Creek Chubs and Central Stonerollers, indicating a non-host specific relationship with the Bluehead Chub.

Habitat usage is an undeniably important axis in the niche of a nest building species. I predicted that the three nest builders require distinct microhabitats for nest construction. The habitat electivity demonstrated by each nest builder reflected the nest structure of the species. Mounds constructed by Bluehead Chubs require greater water depth than the pits of Central Stonerollers or Creek Chubs, which was evidenced by Bluehead Chub's avoidance of shallow riffle habitat. These findings are in agreement with those by Peoples et al. (2016) which found Central Stonerollers utilize habitat with finer substrate and shallower depth than do Bluehead Chubs. Bluehead Chubs habitat preferences align well with the available nesting locations in Toms Creek. Central

Stonerollers and Creek Chubs are likely more selective about their nest placement because they are dependent on the substrate present there, unlike the Bluehead Chub which can collect gravel from a larger radius around the selected nest location. Bluehead Chubs must also select locations that minimize the risks of as desiccation in late summer, predation from piscivores in deep water, and optimal current for embryos development (Bolton et al. 2015).

My second goal was to classify the strength of each species' association with the Bluehead Chub. To establish niche overlap, I modeled the timing of spawning as a response to multiple dimensions of environmental conditions. The boosted regression tree analysis revealed that for most species, presence on nests is predicted best by water level and daily heat accumulation (RDD). One factor included in the BRT was hydrograph direction, indicating whether the water level is increasing, decreasing, or stable from day to day. Although I expected this variable to be important for nest builders, it was not influential to any species. Instead, the raw water level measurements were much stronger. Interestingly, the Bluehead Chub was more sensitive to longer term trends in temperature, as the five-day RDD was significant for only that species to initiate nesting activities. This is likely because nest building or maintenance is energetically costly, so a host needs assurance of suitable weather conditions, in this case as a five-day increasing trend in water temperature. Additionally, associate species are dependent on the constructive actions of a nest builder so it is less important for them to be sensitive to weather conditions.

When I fed the variables identified in BRT analysis as commonly significant into the hypervolume model, most associates showed a great degree of overlap of their reproductive phenology niche with their host. My final hypothesis was that the species identified as strong associates across their range in Pendleton et al. (2012) would have more overlap of their reproductive niches with the Bluehead Chub in Toms Creek than those identified as weak associates. The hypervolume results among strong associates were fairly consistent with the other analysis and showed little variation between species (table 2.4). The low overlap of Blacknose Dace fits its classification by Pendleton et al. (2012) as a weak associate. This finding is also strengthened by work from Peoples et al. (2016) indicating that Blacknose Dace did not lay eggs on Bluehead Chub nests. Surprisingly, Creek Chub had the greatest overlap in reproductive niche, which was unexpected given that it is not considered an associate, selected for different habitat types (Fig. 2.6), and has never been observed spawning on Bluehead Chub nests (Peoples et al. 2016). This paradoxical result can be explained by expanding our view beyond the Hutchinsonian reproductive niche. Creek Chubs are frequent predators of both larvae and adult fish (Barber and McKinley 1971), therefore their presence on the nest of Bluehead Chubs is likely motivated by feeding rather than reproduction. My hypervolumes were based around the presence of species on Bluehead Chub nests, but if instead I considered the environmental covariates associates with Creek Chub nest construction, I predict a much greater distinction between the two species Hutchinsonian niches.

The persistence of nest association between the Bluehead Chub and other leuciscid species makes it unsurprising to observe highly similar reproductive niches for

all species involved. These models captured the broad scale temperature requirements of each associate, but to tease apart finer niche separations would require modeling each species with more axes of habitat and environmental variation. It would also be informative to model each species with only the variables identified as most influential to their unique phenology, but this comes with a loss of comparability between species. Precisely defining the reproductive niche of each species is not only informative for their individual conservation, but also a deeper understanding of the role of facilitation by other species in their continued survival.

## CHAPTER 3- APPLICATIONS OF ELTONIAN NICHE THEORY TO ALLOPARENTAL CARE IN FISHES

### ABSTRACT

Mutualism is chronically understudied, leading to underestimation of its influence on shaping community structure and species persistence. Eltonian niche theory provides a framework for examining a species' role in its environment and can be extended to complex phenomena such as alloparental care which may involve mutualistic interactions. In the nest association of leuciscids, there has been no evaluation of the contribution of nest associates to parental care. Historic definitions of nest association have focused on alloparental care within species and do not include any mention of distantly related associate species involving themselves in nest defense or care of mixed-brood offspring. Through the analysis of nesting behaviors of leuciscids in Toms Creek, I report that some associates do contribute to parental care through defensive and vigilance behaviors. This motivates investigation of positive and negative behaviors among species, to understand their fitness benefits to members of the associate species complex. Using Principal Coordinate Analysis (PCoA), I grouped species by their behaviors and examined the interspecific aggression patterns performed on Bluehead Chub *Nocomis leptocephalus* nests. Blacknose Dace *Rhinichthys atratulus* and Creek Chub *Semotilus atromaculatus* were distinct from the other associate species as they did not perform any spawning or defensive behaviors, but species with morphological adaptations for fighting did perform more defense. Smaller individuals, enjoying the protection of larger species, performed more spawning behaviors. Interspecific aggression patterns revealed a size structured social hierarchy within the association. Future studies should evaluate the potential influence of these aggressive behaviors on the reproductive success of associate species.

## INTRODUCTION

The species niche was defined by Elton (1927) as the unique way a species functions in its environment. While this definition is most commonly used to describe methods of feeding, species may have several different roles in their ecosystem. Additionally, the role of a species is not static over time. Throughout ontogeny and annual cycles, the behavior of a species changes as it shifts focus from growth and survival to reproduction. The functional role a species performs during the breeding season is therefore distinct from its ecosystem role during the rest of the year.

In several species of fish, males serve as ecosystem engineers during the summer, through the construction of gravel nests (Moore 2006). Their nests are a mecca for dozens of other fish species, known as nest associates, who utilize the clean substrate for their own reproduction, while others visit to prey on eggs or other individuals in the aggregation. Most nest associates are small bodied open substrate spawners and do not guard their young (Johnston 1999). When associates spawn on a nest, the host will bury their eggs along with its own. The nest building host provides additional alloparental care to the offspring of its associates by maintaining the cleanliness of the nest and defending against predators (Wisenden 1999). This transient relationship is crucial to the continued survival of many associate populations. The ability of a host to establish areas of clean substrate can mitigate the impact of anthropogenic sedimentation (Peoples et al. 2011) on

endangered species such as the Clinch Dace (*Chrosomus sp. cf. saylori*) (Hatcher et al. 2017). The interdependence of hosts and associates emphasizes the importance of reproductive behaviors in a species' ecosystem role.

In the Southeastern U.S., there are multiple nest building species that participate in such relationships. This study was conducted in Toms Creek, a third order stream in Southwest Virginia, where we find three species of nest builders. In this system, the nest building species (*Nocomis leptocephalus*, *Campostoma anomalum*, and *Semotilus atromaculatus*) serve as hosts, but the functional role of their associates has not been described. It is presumed that associates deposit eggs on the nest for a host to guard but that they provide no parental care themselves (Wisenden 1999).

The current hypothesis is that nest hosts tolerate nest associates because of the predator dilution effect and experience higher reproductive success in their presence (Johnston, 1994). Essentially, the presence of associate embryos increases the survival of host embryos by reducing the probability of their consumption. However, while observing the Toms Creek fish community, we have captured unexpected behaviors of nest associates on video. Some associate species have been observed guarding the spawning trough, chasing off nest predators, and performing vigilance behaviors. Based on these observations, I present the alternative hypothesis that associates contribute parental care to a nest in return for the privilege of spawning there as well as to protect their own offspring.

I define parental care in this system as vigilance behaviors on the nest, aggression toward other associates, aggression towards non-associate fish, and aggression toward non-fish predator species (ex. crayfish, snakes, snapping turtles). Nest associates vary in size and level of aggression, meaning they likely do not all perform the same, if any, defensive behaviors. Behavior may also be sexually dimorphic with one sex performing more defense than the other. An associate species' phenology might also drive changes in behavior within the breeding season. Individuals may cooperate more easily with nest hosts when ambient conditions align with their spawning requirements and become more disruptive when conditions are misaligned. Pendleton et al. (2012) categorized associates as either strong or weak based on reproductive behavioral traits, spawning temperature, substrate choices, and geographic range (Fig. 1.1).

## OBJECTIVES AND HYPOTHESES

My primary objective is to elucidate the behavioral mechanisms that distinguish each species involved in nest association. In this chapter I present the following hypotheses: a) nest associates contribute to parental care, b) associate behaviors will group according to strong, weak, and non-contributors to parental care, c) strong contributors to parental care are likely to be strong associates identified by Pendleton et al. 2012, and d) species' behaviors will be influenced by their morphology and life-history.

## **METHODS**

The study site was established at Deerfield park in 2008 by the Frimpong lab at Virginia Tech. In the years 2016-2021, observers walked approximately 0.65 km of Toms Creek daily from April-July, making note of which species were present on a nest and in what numbers. Approximately 80 nests are constructed each year within the study reach. I deployed cameras underwater near active nests for at least 20 minutes to film fish activity. To capture associate behaviors, I selected a subset of these videos for each year from 2016-2021 with the exception of 2018, as not enough videos were recorded throughout the season to produce a sufficient sample. Approximately 50% of videos from 2016 were reviewed, and an effort was made to choose an equal number of videos from early, mid, and late season. All suitable videos from 2017, 2019, 2020, and 2021 were selected. When multiple videos were taken on the same date, only one was selected for viewing based on video quality and uniqueness. Each daily observation of a nest was treated as independent.

Prior to reviewing the videos, I categorized potential behaviors as either defensive, breeding, or disruptive, and created an ethogram including common examples of each category (table 3.1). Nesting videos were viewed at half speed, rewinding often to observe different areas of the nest. Each video was examined by two trained observers for cross validation. Observers noted any instance of a behavior, taking special interest in interspecific aggression, mating, or defense against nest predators. Observations of each species were split by sex. Where observers were unable to determine the sex of a fish, we

defaulted to male. This ensured that behaviors were not missed due to inability to identify sex. Females performed fewer behaviors overall, so defaulting to male was more consistent with observed patterns.

| Category   | Behavior Description                                     |
|------------|--|
| Defensive  | Hovering over spawning trough                            |
|            | Swim at non-associate (non-fish)                         |
|            | Swim at non-associate (fish)                             |
|            | Circle perimeter of nest                                 |
| Spawning   | Spawning with conspecifics                               |
|            | Chase conspecific female                                 |
|            | Parallel or circle swim with conspecific (usually males) |
| Disruptive | Digging on the nest                                      |
|            | Disrupt spawning   |
|            | Swim at associate  |
|            | Attempted spawning with non- conspecific                 |
|            | Chase non-conspecific female                             |
|            | Parallel swim with other species                         |

Table 3.1- Ethogram of common fish behaviors in defensive, spawning, and disruptive categories.

To determine the relative abundance of each species, videos were paused every two minutes to count all individuals of each species in the frame. The count of each

species per frame was averaged over the entirety of the video. If several videos were recorded on a single day, the video totals were averaged.

## DATA ANALYSIS

The behavioral response matrix was summarized over the full season as the frequency of a behavior's presence over the frequency of a species' presence. I performed principal coordinate analysis (PCoA) on these proportions using a Minkowski distance matrix. With this method, species that cluster together exhibit more similar behaviors than those far apart.

Interspecific aggression patterns were extrapolated from the frequency of each species interaction including an attack. Observers identified the frequency of target and instigator species, and scaled the observations by the total number of the target species' presences recorded over the season (appendix for details).

## RESULTS

Mountain Redbelly Dace consistently dominated the nesting community, comprising up to 25% of individuals in peak season (Fig. 3.1). Rosyside dace and Central Stoneroller were the next most abundant, each averaging between 3-10% of the community. No other species reached a relative abundance over 3%.

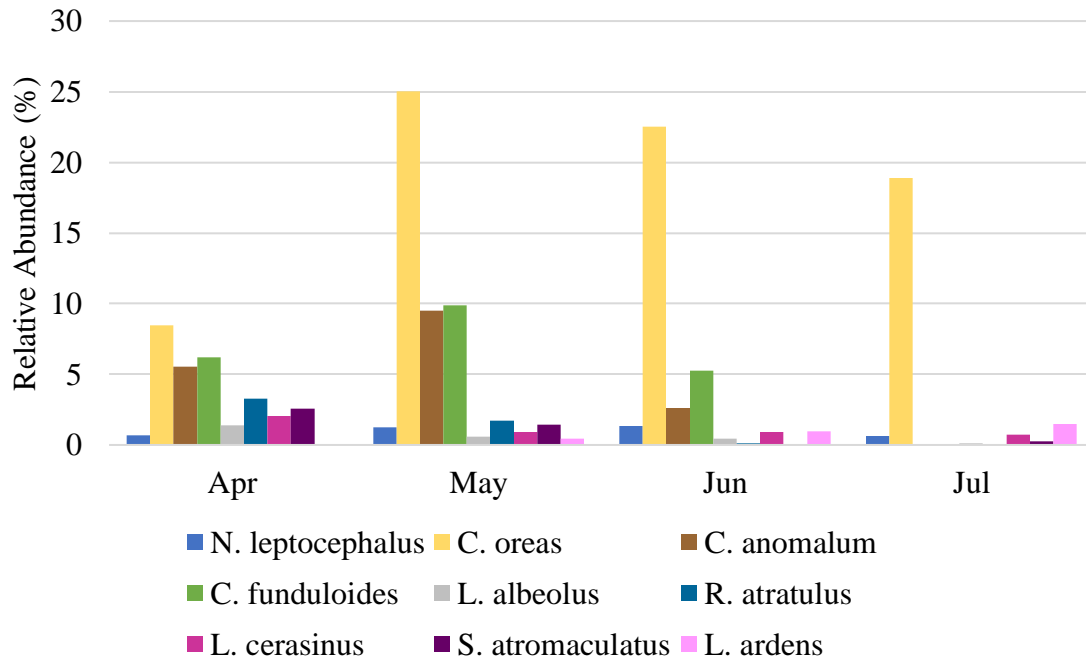


Figure 3.1- Relative abundance of species on Bluehead Chub nests, summarized by month, for the years 2016-2021. Abundance is represented as a percentage of the nest community on a given date.

In PCoA (Fig. 3.2) Bluehead Chub males fall in the center of a cluster of species, performing both defensive and aggressive behaviors including nest guarding as well as spawning. Among the male associates, Crescent Shiners, Rosefin Shiners, and Central Stonerollers exhibited more vigilance and defensive behaviors. Rosyside Dace and Mountain Redbelly Dace were more frequently observed spawning, but performed fewer defensive behaviors. White Shiners, Blacknose Dace, and Creek Chubs did not exhibit either spawning or defensive behaviors.

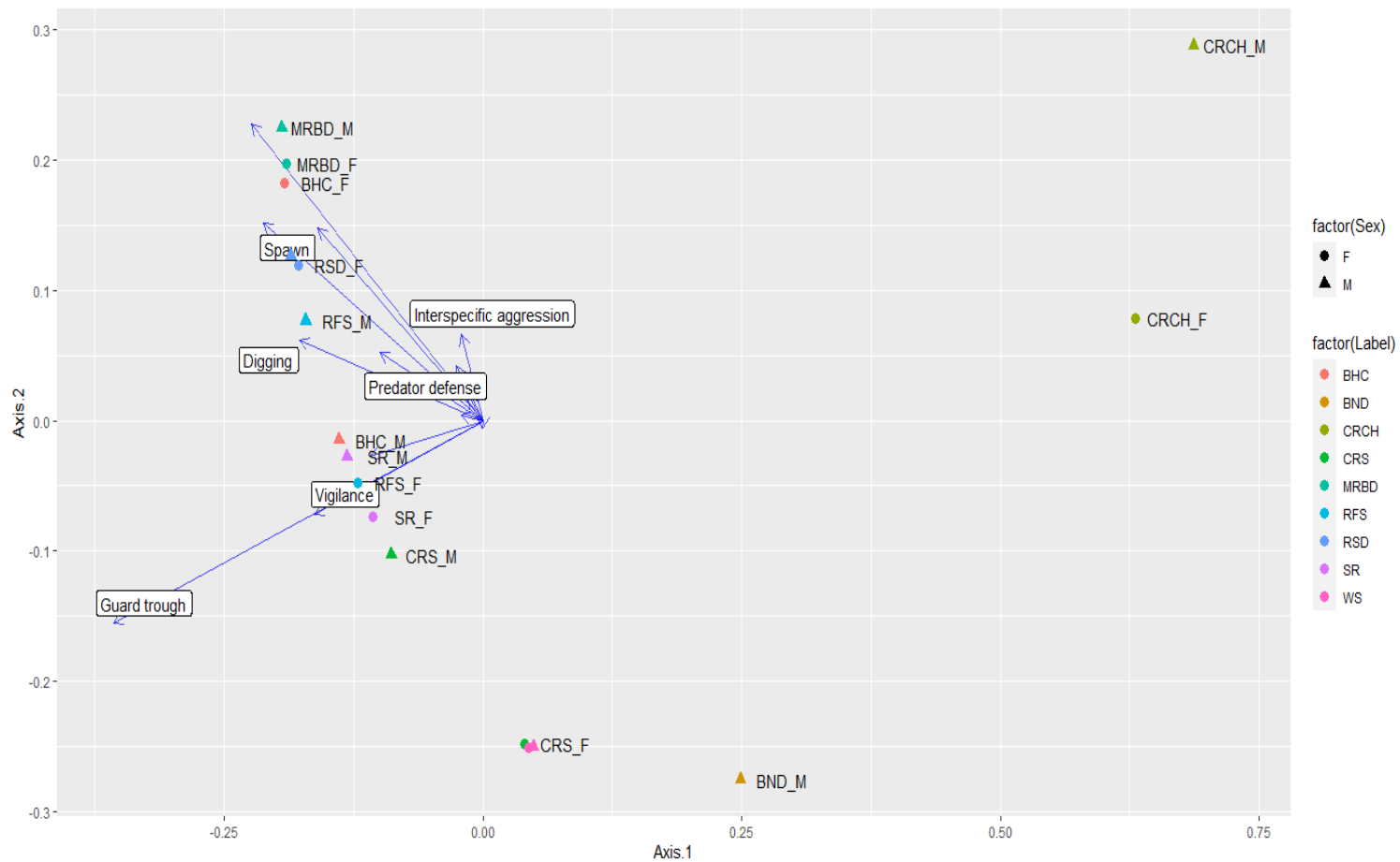


Figure 3.2- Principal coordinate analysis indicating behaviors that distinguish associate species. Influential behaviors are labelled along the blue arrows, refer to table 3.1 for details. Species abbreviations are BHC- Bluehead Chub *N. leptocephalus*, BND- Blacknose Dace *R. atratulus*, CRCH- Creek Chub *S. atomaculatus*, CRS- Crescent Shiner *L. cerasinus*, MRBD- Mountain Redbelly Dace *C. oreas*, RFS- Rosefin Shiner *L. ardens*, RSD- Rosyside Dace *C. funduloides*, SR- Central Stoneroller *C. anomalum*, and WS- White Shiner *L. albeolus*

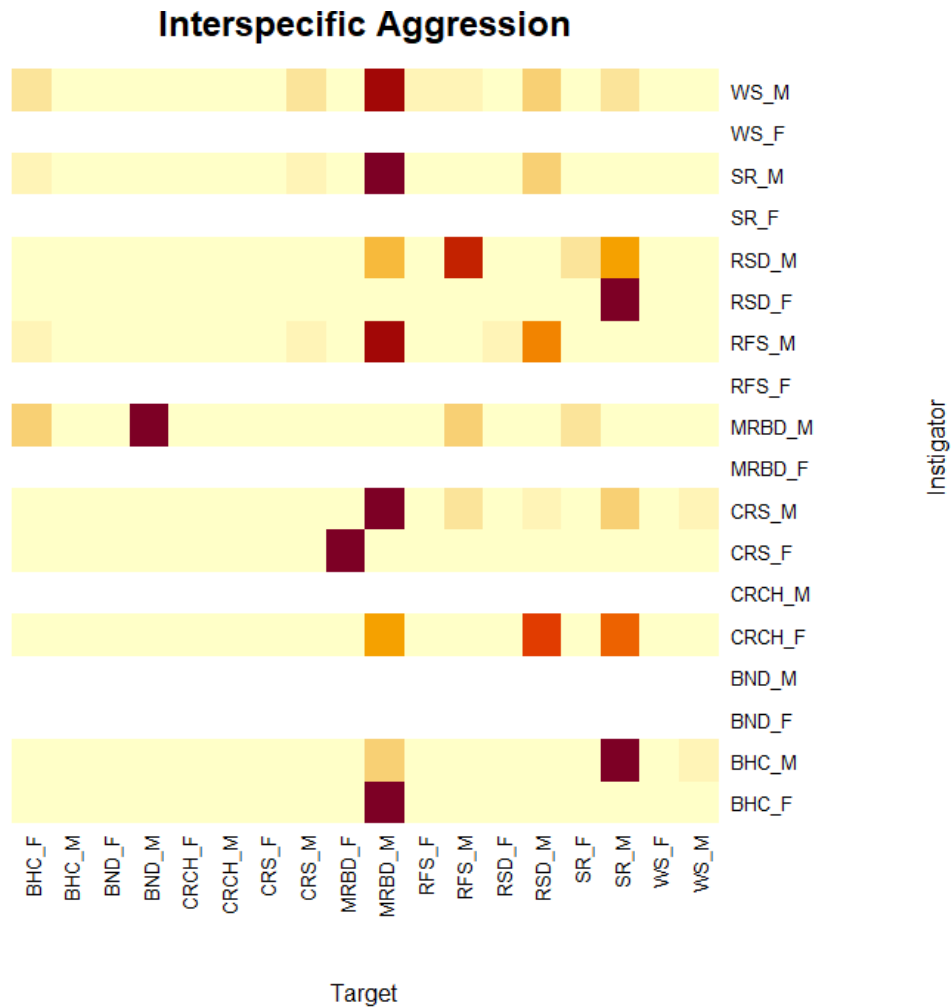


Figure 3.3- Heatmap of interaction frequency between species that instigate aggression and their targets. Darkest red indicates frequent interactions while light yellow indicates very little interaction. White indicates no interactions between those species were observed. Species are split by male and female, denoted with “M” or “F”. Species abbreviations are BHC- Bluehead Chub *N. leptocephalus*, BND- Blacknose Dace *R. atratulus*, CRCH- Creek Chub *S. atromaculatus*, CRS- Crescent Shiner *L. cerasinus*, MRBD- Mountain Redbelly Dace *C. oreas*, RFS- Rosefin Shiner *L. ardens*, RSD- Rosyside Dace *C. funduloides*, SR- Central Stoneroller *C. anomalum*, and WS- White Shiner *L. albeolus*

Mountain Redbelly Dace were the most frequent targets of aggression by all species, but exclusively instigated aggression toward Blacknose Dace. Bluehead Chub and Rosyside Dace targeted male Central Stonerollers. There were low levels of

aggression between Rosefin Shiners, Rosyside Dace, Crescent Shiners, and White Shiners (Fig. 3.3).

## **DISCUSSION**

Ordinating species by their nesting behaviors resulted in a spectrum of species behaviors ranging from mutualistic to parasitic to non-participatory (Fig. 3.5). Blacknose Dace, White Shiner, and Creek Chub were distinct from the other species in their lack of participation in both spawning and defense. White Shiners were not observed spawning on video, but their eggs have been genetically identified in historic sampling of Bluehead Chub mounds (Peoples et al. 2016). Mountain Redbelly Dace contribute to mutualism through the dilution effect by their sheer abundance, but not through defense. This could be viewed as less cooperative, but if we consider the combined dilution effect of both eggs and adults, their effect shifts toward mutualism. Central Stonerollers also contribute many eggs to the nest, but are also disruptive through their digging. Crescent Shiner and Rosefin Shiner contributed to both spawning and defense, but they spawn less frequently than Mountain Redbelly Dace or Central Stonerollers.

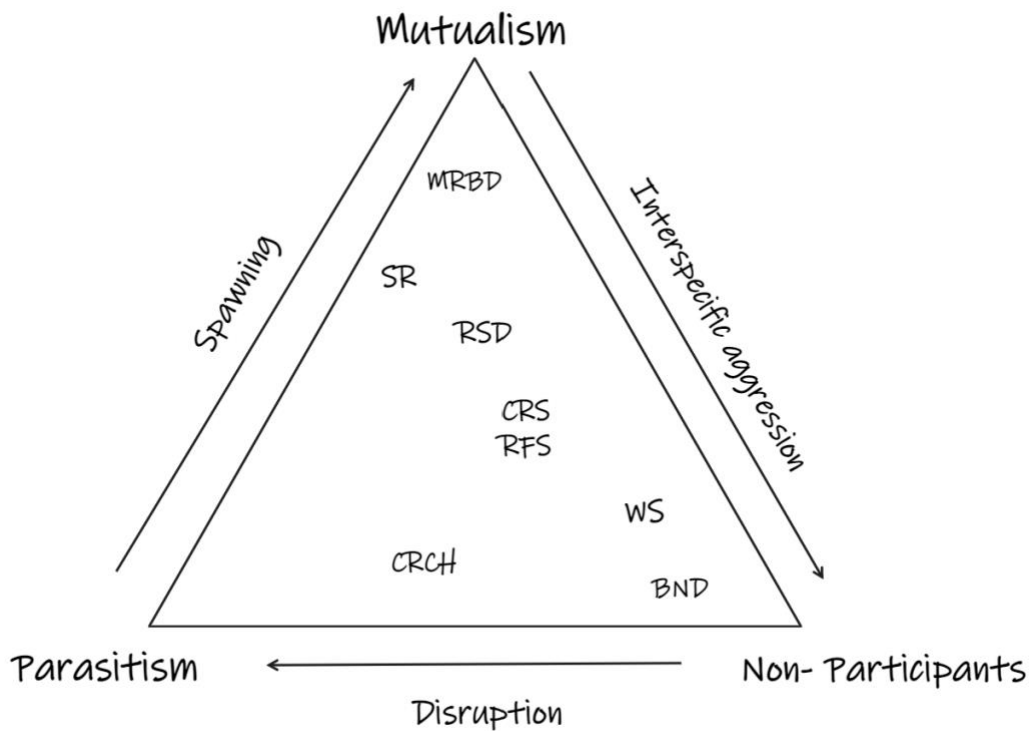


Figure 3.4- Theoretical diagram ordinating associate species based on the outcome of their interaction with Bluehead Chubs. Species abbreviations are BHC- Bluehead Chub *N. leptocephalus*, BND- Blacknose Dace *R. atratulus*, CRCH- Creek Chub *S. atromaculatus*, CRS- Crescent Shiner *L. cerasinus*, MRBD- Mountain Redbelly Dace *C. oreas*, RFS- Rosefin Shiner *L. ardens*, RSD- Rosyside Dace *C. funduloides*, SR- Central Stoneroller *C. anomalum*, and WS- White Shiner *L. albeolus*

I saw general agreement between the strength rankings of Pendleton et al. (2012) and the PCoA behavioral groupings (Fig. 3.2), with the exception of Central Stonerollers. Since I classified prolonged hovering over the spawning trough as a defensive behavior rather than an exploitative one (table 3.1) Central Stoneroller males grouped with strong associates who display the same behavior. A potential pitfall with my behavioral study is the challenge of attributing motivation to behaviors. Instead, we must objectively consider whether the outcome of the behavior or interaction is positive or negative for

each species involved. In this case, hovering Central Stonerollers may deter predators regardless of their intention to potentially consume eggs.

When examining the interspecific patterns of aggression (Fig. 3.3), I noted a potentially size structured aggression scheme. Larger fish with tubercles, a morphological traits adapted for fighting, are aggressive toward similar size and smaller fish. Small fish without spiked structures only fight with each other and non-associate (e.g. Fantail Darters *Etheostoma flabellare*) and non-fish nest predators (eg. Crayfish). Central Stoneroller males were targeted by Bluehead Chub males, likely because they spend a large portion of time digging on the nest. Bluehead Chubs may be threatened by disturbance the substrate and are aggressive in defense of their offspring. This also aligns more closely with Pendleton's et al. (2012) assignment of Central Stonerollers as weak associates.

Across all negative interactions, Mountain Redbelly Dace were the most frequent targets of aggression. In the behavioral observations, Mountain Redbelly Dace did not exhibit any threatening behaviors that would seem to provoke aggression, although they were observed digging to a lesser extent than Central Stonerollers. The simplest explanation is that they are present in such high abundance on the nest (Fig. 3.1) that they present a nuisance to other associates. When competing for limited space to lay eggs on the nest, larger associates push Mountain Redbelly Dace out of the way to make room for their own spawning endeavors.

The most interesting interaction revealed by my aggression frequency analysis was the targeting of Blacknose Dace almost exclusively by Mountain Redbelly Dace. One potential explanation is competition, given that these two species are the closest in size and share similar feeding strategies. Blacknose dace were not frequently observed disrupting nesting activities, but they were not afforded much opportunity given that Mountain Redbelly Dace chased them off the nest by within minutes of their arrival. Mountain Redbelly Dace may identify Blacknose Dace as a false associate and chase them away to deter cheating through egg consumption. This hypothesis is supported by both the weak temperature association (Fig. 2.1) of Blacknose Dace with Bluehead Chub as and the lack of spawning behaviors.

While this study was limited to Bluehead Chub nests, it would be interesting to observe the differences in species behavior on Central Stoneroller and Creek Chub nests. For example, associates may perform less defensive and more disruptive behaviors on the nest of a host with which they are less strongly associated. Observations of spawning on the nests of alternate hosts would also expand our understanding of the environmental limitations for species reproduction.

Despite the anecdotal observation of Vives (1990) that a Common Shiner (*Luxilus cornutus*) was guarding a mound in the absence of the Bluehead Chub, this study is the first formal evidence of associates contributing to parental care through vigilance and defensive behaviors. My investigation of both nesting behaviors and aggression patterns clarifies the reproductive role of each species and revealed fine-scale behavioral patterns

structuring the fish community in Toms Creek. This work lays the foundation to examine the strength of association of each species throughout its range and examine how their behaviors may change with community composition.

## CHAPTER 4- CONCLUSIONS

The primary objectives of this study were to define the Hutchinsonian and Eltonian reproductive niches of the Bluehead Chub and co-occurring leuciscids in Toms Creek, quantify the strength of each species' association with the Bluehead Chub, and elucidate the behavioral mechanisms that distinguish each associate species. The dual niche framework captured variability along all axes of the reproductive niche for these species. The Hutchinsonian niche provided phenological differences between species and was supplemented by the fine-scale patterns observed through the Eltonian niche; for example, while two species may have the same spawning temperature they do not necessarily share the same reproductive niche. Each species exhibited a unique reproductive niche profile defined by their combined behavior and phenology.

The variability in the association strength of each species with the Bluehead Chub, as shown by both their spawning temperature preferences and their nesting behaviors, aligns with the findings of Pendleton et al. (2012). While their study could not determine the mechanisms distinguishing associates of different strengths, my approach provides the added nuance of behaviors and interspecific interactions. By expanding on the dilution effect, my approach bolstered the explanation for persistence of a complex-multi species interaction. Although previously undescribed, associates do perform parental care and their contributions to nest association should not be overlooked. Parental care can take many forms, and in a nesting community the defensive behaviors of associate species do provide protection to their offspring.

A critical finding from this approach is that Blacknose Dace and Creek Chubs are not true associates of Bluehead Chubs. The temperature correlated with peak presence is significantly lower for Creek Chub (Fig. 2.4), and they were never observed spawning on bluehead chub nests. Blacknose Dace were also frequently targeted for aggression by Mountain Redbelly Dace. These differences indicate that Blacknose Dace should not be considered a true associate of the Bluehead Chub.

Future work should investigate differences between associate behaviors on the nests of different host species, or consider if presence of associates is correlated with nest size and location as a measure of success. It would also be interesting to consider how these relationships change across the range of the Bluehead Chub where it has a different pool of associates. These complex relationships could be mirrored in other fish communities. Understanding the subtleties of associate aggression and cooperation is important to our management of endangered species involved in similar interactions.

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