Development of a habitat model for an endemic leuciscid, the Sandhills Chub (*Semotilus lumbee*)

Garrett Mitchell Herigan

*Coastal Carolina University*

Follow this and additional works at: https://digitalcommons.coastal.edu/etd

Part of the Biology Commons

**Recommended Citation**


https://digitalcommons.coastal.edu/etd/132

This Thesis is brought to you for free and open access by the College of Graduate and Continuing Studies at CCU Digital Commons. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of CCU Digital Commons. For more information, please contact commons@coastal.edu.
Development of a habitat model for an endemic leuciscid, the Sandhills Chub (*Semotilus lumbee*)

By

Garrett M. Herigan

Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Coastal Marine and Wetland Studies in the School of the Coastal Environment Coastal Carolina University 2021

Derek P. Crane, Ph.D, Major Advisor

John J. Hutchens, Ph.D, Committee Member

Christopher E. Hill, Ph.D, Committee Member

Mark C. Scott, Ph.D, Committee Member

Richard F. Viso, Ph.D, Program Coordinator

William G. Ambrose Jr., Ph.D, Vice Dean
Acknowledgements

I would like to extend a special thank you to Dr. Derek Crane for serving as my major advisor throughout my time at Coastal Carolina University and work on this project. His encouragement and teachings have pushed me to become the best scientist that I can be and will continue to affect the way I approach fisheries science as I begin my career. I appreciate all the hard work he has put in to get me to this point and I look forward to putting the things I’ve learned to good use as I continue to grow as a scientist and a professional.

Thank you to Dr. John Hutchens, Dr. Christopher Hill, and Dr. Mark Scott for serving on my committee, providing me with constructive criticism throughout this process, and editing this thesis. I also would like to thank all the volunteers and technicians that helped me to collect the data for this project out in the field including Craig Fleury, Justin McNabb, Garrett Elmo, Molly Takacs, Logan Masterson, and Nick Coleman. Thank you for all your help collecting fish and dragging the gear in and out of our machete-made paths through the Sandhills. Thank you to Fritz Rohde and Dustin Smith for your help with identifying sampling locations, suggesting the kick-seine technique, and stomping around some SC Sandhills streams with me. Thank you to the US Fish and Wildlife Service State Wildlife Grant Program, Coastal Carolina University, and the M. K. Pentecostal Ecology Fund for the funding and making this project possible.

Finally, thank you to my family, my friends, and my girlfriend Becca for all the love and support you have offered along the way, it meant more to me than you know, and I am extremely grateful.
Abstract

Headwater streams comprise the majority of stream length within a watershed and significantly contribute to drainage-wide species diversity by supporting rare and endemic species. Despite being common, headwater streams are often understudied compared to larger waters that support recreational and commercial fisheries. However, recent conservation efforts focused on native, non-game species have motivated research to better understand headwater stream fishes. The Sandhills Chub (*Semotilus lumbee*) is a leuciscid only found in the Sandhills ecoregion of North and South Carolina, and limited previous research indicates they prefer small, clear streams with sand or gravel substrate and little aquatic vegetation. Because of its limited geographic distribution, the Sandhills Chub is a species of conservation concern, and as a result, has been listed as imperiled by the American Fisheries Society and as a Species of Special Concern by the South Carolina Department of Natural Resources. It has been extirpated from several locations in South Carolina, therefore, there is a need for quantitative information to guide conservation and restoration efforts. To guide sampling of fishes in the low-conductivity streams of the Sandhills ecoregion, I first compared two fish sampling approaches: (1) a novel combination of gears (electrofishing coupled with kick-seining) and (2) standard three-pass electrofishing. At each site (n = 25), each method was used to sample separate reaches equal to 35 times the mean stream width. I compared fish density and species richness between the two methods and used logistic regression to estimate the probability of capturing a new species on the second and third passes when electrofishing. I calculated capture probabilities for the most common species encountered using the Carle-Strub depletion method with the three-pass electrofishing data. A total of 1479
fishes encompassing 30 species were collected, and three-pass electrofishing resulted in significantly greater density and species richness (0.21 vs. 0.13 fish/m² and 7.24 vs. 5.00 species, respectively) compared to the combination method. There was a 64% probability of capturing a new species on the second electrofishing pass and 27% probability of capturing a new species on the third pass when using three-pass electrofishing. Capture probabilities ranged from 0.50-0.87 for the 13 species examined. The use of kick seining after a single electrofishing pass provided no benefit compared to additional electrofishing passes. Therefore, I recommend making at least three passes while electrofishing when estimating relative abundance and species richness in low-conductivity wadeable streams, with the potential need for more passes depending on project objectives.

Results from this comparison of stream sampling techniques were then used to guide targeted sampling for Sandhills Chubs in low-conductivity headwater streams of the Carolina Sandhills. The goal of this study was to identify habitat and biological characteristics associated with presence of Sandhills Chub. A total of 431 Sandhills Chub were collected from 41 of 115 sites sampled during 2019 and 2020. Co-occurrence analysis and logistic regression were used to identify which habitat and biological features were associated with the presence or absence of Sandhills Chub. Cooccurrence analysis indicated positive relationships between Sandhills Chub and Dollar Sunfish (Lepomis marginatus), Creek Chubsucker (Erimyzon oblongus), and Margined Madtom (Notorus insignis) and negative relationships between Sandhills Chub and Largemouth Bass (Micropterus salmoides), Bluegill, Creek Chub (Semotilus atromaculatus), Eastern Mosquitofish (Gambusia holbrooki), and Lined Topminnow (Fundulus lineolatus).The
logistic regression models indicated that dissolved oxygen, instream cover, and the percent of substrates between 6 and 11 mm were positively associated with presence of Sandhills Chub. Results from this study will help guide management decisions for future conservation and restoration of Sandhills Chub.
# Table of Contents

Title Page ............................................................................................................................. i
Copyright ............................................................................................................................ ii
Acknowledgements............................................................................................................ iii
Abstract .............................................................................................................................. iv
List of Tables ................................................................................................................... viii
List of Figures .................................................................................................................... ix

Chapter 1: Comparison of two fish sampling techniques for low-conductivity, wadeable streams in the Carolina Sandhills........................................................................................ 1
  Abstract ........................................................................................................................... 1
  Introduction ..................................................................................................................... 3
  Methods ........................................................................................................................... 6
  Results ............................................................................................................................. 9
  Discussion ..................................................................................................................... 10
  References ..................................................................................................................... 14
  Tables and Figures ........................................................................................................ 17

Chapter 2: Habitat characteristics associated with the presence of the Sandhills Chub (Semotilus lumbee) ........................................................................................................... 21
  Abstract ......................................................................................................................... 21
  Introduction ................................................................................................................... 23
  Methods ........................................................................................................................... 26
  Results ........................................................................................................................... 32
  Discussion ..................................................................................................................... 34
  References ..................................................................................................................... 40
  Tables and Figures ........................................................................................................ 46
List of Tables

Table 1. List of species encountered while sampling in the Carolina Sandhills during summer and fall of 2019 (n = 25 sites sampled).

Table 2. List of most common species captured using three-pass electrofishing in the Carolina Sandhills during summer and fall 2019, including average estimated abundance and capture probabilities from all sites sampled (n = 25). Standard errors are provided in parentheses.

Table 3. Summary of comparison between the average capture probabilities for families of fish while electrofishing between my data, sampling in the Carolina Sandhills, and other published literature (Hense et al. 2010; Heimbuch et al. 1997; Wiley and Tsai 1983). Standard errors are provided in parentheses.

Table 4. Habitat characteristics for sites in the Carolina Sandhills where Sandhills Chub (Semotilus lumbee) were present or absent and overall means. Standard errors are provided in parentheses.

Table 5. Results of co-occurrence analysis between Sandhills Chub (Semotilus lumbee) and other species collected while sampling in the Carolina Sandhills in 2019 and 2020. Analysis was completed using the 'cooccur' package in R (Griffith et al. 2016). Species co-occurrence was calculated using a random sampling with replacement and hypergeometric distribution approach to the probabilistic model (Griffith et al. 2016; Veech 2013) which compares observed co-occurrence to the expected co-occurrence between pairs of species and searches for significant positive, negative, and random pairwise interactions (α = 0.05). P corresponds to the probability that the species co-occur more or less than what would be expected by random chance and can be interpreted as a P-value.

Table 6. Summary table of the top ten models fit to analyze habitat characteristics associated with presence of Sandhills Chub (Semotilus lumbee). DO = dissolved oxygen (mg/L), substrate = the percent of substrates in the 6-11 mm size class, cover = the percent of instream cover, impoundments = the number of stream impoundments within each 12-digit HUC, elevation = the elevation of the local catchment (m).

Table 7. Summary of the top four logistic regression mixed-models used to analyze habitat characteristics associated with presence of Sandhills Chub (Semotilus lumbee). Odds ratios and 5-fold cross validation values are reported with 95% confidence intervals in parentheses. Odds ratios for cover, substrate, impoundments, and elevation are according to a 10-unit increase and the odds ratio for DO is according to a 1-unit increase. DO = dissolved oxygen (mg/L), substrate = the percent of substrates in the 6-11 mm size class, cover = the percent of instream cover, impoundments = the number of stream impoundments within each 12-digit HUC, elevation = the elevation of the local catchment (m).
List of Figures

**Figure 1.** The number of species added by each sampling pass and the average total species richness for two stream sampling methods, three-pass electrofishing and combination method (one-pass electrofishing with one-pass kick seine) used in the Carolina Sandhills during summer and fall 2019. Error bars show +/- standard error of the mean.

**Figure 2.** Map of the Sandhills ecoregion of South Carolina within the Catawba and Pee Dee river basins. Solid dots show sites where Sandhills Chub (*Semotilus lumbee*) were present and open circles with a plus show sites where they were absent.

**Figure 3.** Notched boxplots used to compare habitat characteristics at sites where Sandhills Chub (*Semotilus lumbee*) were present vs. absent. Notches represent 95% confidence interval of the median.

**Figure 4.** Plot of co-occurrence analysis showing the relationship between Sandhills Chub (*Semotilus lumbee*) and other fish species captured in the Carolina Sandhills. Species co-occurrence was calculated using a random sampling with replacement and hypergeometric distribution approach to the probabilistic model (Griffith et al. 2016; Veech 2013) which compares observed co-occurrence to the expected co-occurrence between pairs of species and searches for significant positive, negative, and random pairwise interactions ($\alpha = 0.05$).
Chapter 1: Comparison of two fish sampling techniques for low-conductivity, wadeable streams in the Carolina Sandhills

Abstract

Despite being common, low-conductivity, headwater streams are often understudied compared to larger waters that support recreational and commercial fisheries. However, recent conservation efforts focused on native, non-game species have created the need to develop and test sampling methods in these habitats. I compared a novel combination of gears (electrofishing coupled with kick-seining) to three-pass electrofishing for sampling fish assemblages in low-conductivity streams. At each site (n = 25), each method was used to sample separate reaches equal to 35 times the mean stream width. I compared density of fish captured and species richness between the two methods and used logistic regression to estimate the probability of capturing a new species on the second and third passes when electrofishing. I calculated capture probabilities for the most common species encountered using the Carle-Strub depletion method with the three-pass electrofishing data. Three-pass electrofishing, when compared to the combination method, resulted in significantly greater density and species richness (0.21 vs. 0.13 fish/m² and 7.24 vs. 5.00 species, respectively). There was a 64% probability of capturing a new species on the second pass and 27% probability of capturing a new species on the third pass when using three-pass electrofishing. Capture probabilities ranged from 0.50-0.87 for the 13 species examined. The use of kick seining after a single electrofishing pass provides no benefit compared to additional electrofishing passes. I recommend
making at least three passes while electrofishing when estimating relative abundance and species richness in low-conductivity wadeable streams, with the potential need for more passes depending on project objectives.
**Introduction**

Fisheries scientists are continuously investigating ways to increase sampling efficiency to best use time and resources, while still collecting high-quality data that are necessary to confidently address research objectives. Sampling effort and gear varies among projects based on project objectives, habitats being sampled, and target species because all gear types have biases associated with certain habitats (e.g., riffles or pools) or groups of fishes (e.g., species, size class, age class). Because many headwater streams do not support recreationally or commercially important fisheries, they have received less attention compared to large bodies of water with diverse fisheries, with the exception of streams that support salmonids. The U.S. Congress passed the State Wildlife Grant (SWG) Program in 2000 and funds from it are directed towards species of greatest conservation need to learn about and conserve native, particularly non-game, fishes, some of which occur in small, headwater streams. (US Fish and Wildlife Service 2019).

Similarly in South Carolina, the South Carolina Department of Natural Resources (SCDNR) completed a statewide wadeable streams assessment during 2006-2011, data from which has subsequently been used in conservation planning and decision-making.

Due to increased emphasis on sampling non-game fishes in a variety of previously understudied or infrequently sampled habitats, including headwater streams (first and second order), there is a need to evaluate and refine sampling techniques for these waters. Although many studies have investigated sampling fishes in wadeable streams (Simonson and Lyons 1995; Paller 1995; Bertrand et al. 2006), few have focused on low-conductivity headwater streams, and most of those studies focused on salmonids (Habera et al. 1999; Borgstrom and Skaala 1993). Low-conductivity (< 30 µS/cm) headwater
streams are common worldwide and are typically found in systems with geologies resistant to ionization when exposed to water (United States Environmental Protection Agency 2012). For example, an assessment of conductivity in wadeable streams (1st-4th order) throughout the conterminous USA, Griffith (2014) commonly observed values < 30 µS/cm.

Backpack electrofishing is one of the most common techniques used to sample fishes and estimate species richness and abundance in small streams (Bonar et al. 2009), and multi-pass electrofishing is commonly used to improve estimates of species richness and abundance as well as decrease the probability of failing to detect a species that is present (Vehanen et al. 2013). However, low conductivity (< 30 µS/cm) decreases effectiveness of electrofishing. When water conductivity is less than the conductivity of the fish being shocked, electrical current is directed toward and through the fish (Reynolds and Kolz 2012), which results in less than 100% power transfer from the water to the fish. Therefore, additional power must be applied to compensate for the difference in conductivity between the water and the fish targeted in order to elicit the desired response (Reynolds and Kolz 2012), and additional effort may be needed to confidently describe assemblages in low-conductivity waters. Other potential methods used to sample fish assemblages in wadable streams include electric seines (Bayley et al. 1989), piscicides such as rotenone (Gilowacki and Penczak 2005), and snorkeling surveys (Thurow et al. 2006). Because different sampling gears all have inherent biases, multiple gear types are often used to collect data at the fish assemblage level (Ruetz III et al. 2007; Onorato et al. 1998). Therefore, use of a combination of gears may be more effective than multi-pass electrofishing alone in low-conductivity habitats.
Seines are also commonly used to sample fishes in small streams. Pusey et al. (1998) suggested seining after electrofishing to avoid sampling bias and provide more accurate descriptions of fish assemblages. Kick seining can be used to flush fishes, especially benthic species, out of cover or drive fishes towards a net, where they are subsequently captured (Jordan and Jelks 2008). For example, in riffle habitats fast currents and reduced visibility of stunned fish associated with turbulent water surfaces can result in missed fish while sampling using traditional upstream backpack electrofishing techniques (Bozek and Rahel 1991). Therefore, researchers frequently kick seine while electrofishing downstream towards a net at the bottom of the riffle to increase capture of fishes. Given that the effectiveness of electrofishing is decreased in low-conductivity streams, kick-seining following electrofishing may improve capture probabilities and subsequently the accuracy of fish assemblage metrics for these habitats.

Small streams (>5m) in the Sandhills ecoregion of the Carolinas are characterized by naturally occurring low conductivity (Paller et al. 1996) and area low gradient with predominantly sand substrate. The goal of this study was to evaluate the effectiveness of using a single upstream electrofishing pass coupled with a single downstream kick seining pass (hereafter referred to as “combination method”) to sample low-conductivity streams in the Carolina Sandhills. I used kick seining rather than traditional pull seining because streams in the Sandhills contain large amounts of woody debris and undercut banks and these stream features may lower the capture efficiency by snagging the net and providing refuge for fishes. To evaluate the effectiveness of the combination method, I compared it with traditional three-pass electrofishing to determine which method resulted in greater density and species richness at paired sampling locations. Additionally, I
estimated the probability that a new species would be captured on additional passes with the backpack electrofishing unit or the combination method. Results from this study can be used to guide sampling of fishes in low conductivity, wadeable streams.

Methods

Study Site

Sampling was completed in the Sandhills ecoregion of South Carolina during June through October 2019. Twenty-five sites were randomly selected from the population of all wadeable, perennial streams in the Pee Dee and Wateree river drainages of South Carolina, using the SCDNR small stream database. On average streams were 2.4 m wide (SD = 0.90), 20.9 cm deep (SD = 8.7), and had a conductivity of 27.8 µS/cm (SD = 22.4).

Fish Sampling

At each site, one method was used to sample a length of 35 times the mean stream width (MSW) starting 50 m downstream of the selected location and the second method was used to sample a length of 35 times the MSW starting 50 meters upstream (Simonson and Lyons 1995). Methods were randomly assigned to either the upstream or downstream segments at each site, and block nets were placed at the upstream and downstream boundary of a segment while sampling using a given method. Time to complete each method was recorded and used to compare sampling effort between the two methods.

A single backpack electrofishing unit was used during sampling and at each site the three-pass and combination methods were completed using the same unit. The primary unit used was an ETS Electrofishing Systems model ABP-3 backpack unit (Madison, Wisconsin) with upgraded electrodes (anode: 3/8-inch diameter, cathode: 3 m
length with wired splayed) to improve effectiveness in low-conductivity conditions. Due to equipment failure, a second unit (Smith and Root model LR-24, Vancouver, Washington) was used at six sites. The same settings were used at all sites (AC, 600V, 25% duty cycle, and a frequency of 40 Hz) regardless of unit used.

For the three-pass method, I used a standard multi-pass electrofishing technique. Two people, both with dip nets and one with the backpack electrofisher, started at the downstream end of the sample site and sampled by zig-zagging in an upstream direction for three passes. Only two people were used to sample these streams because the average stream width was less than 3 m at most sites, limiting room for any additional individuals in the stream. After each pass, all fish were identified, counted, and then released below the downstream block net.

For the combination method, one pass with the electrofishing unit was made in the upstream direction. Once the upstream block net was reached, the area was sampled again by kick seining in the downstream direction. For the kick seining pass, one person aggressively kicked through the water, disturbing the substrate and flushing out cover to push fish toward the other researcher who was using a small seine (3.0 x 1.3 m, 5> mm mesh) stretched from bank to bank at neck-down areas to capture fish. This process was repeated approximately every five meters until the entire reach was sampled. Kick seining was completed after the electrofishing pass based on pilot work in the study area. Most of the streams had fine substrates and kick seining caused these substrates to erode and therefore reduced visibility for extended time after completion, which affected capture efficiency while electrofishing. All fish were identified, counted, and then
released below the downstream block net after the electrofishing pass and then again after kick seining.

Data Analysis

Density was calculated as the number of fish captured per m$^2$. I used density rather than the traditional method, number of fish per unit of time, to calculate CPUE because I wanted to remove the effect of time. Paired sample t-tests were used to compare density and species richness between the two methods. I then used logistic regression to estimate the probability of detecting a new species on the second and third electrofishing passes (one model for each pass). Based on examination of species richness data, logistic regression analysis could not be conducted for the combination method. Total richness, total abundance (based on three passes), and average width were used as my explanatory variables because these factors have been shown to significantly affect species richness estimates (Angermeier and Schlosser 1989; Ugland et al. 2003; Grenouillet et al. 2004). The probabilities that a new species would be captured on the second or third passes were calculated using average values for total richness, total fish, and stream width within the model.

Finally, using the three-pass electrofishing data, I estimated abundance and capture probability for the most common species (species that were captured from at least six sites) encountered using the removal() function of the FSA package in R (Table 1; Ogle et al. 2020). Again, I restricted this analysis to the three-pass method due to results from the above described analyses. The Carle-Strub depletion method for closed populations was used to estimate abundance and catchability because it can handle data where a greater number of individuals of a species were caught on the third pass than on
the first (Carle and Strub 1978). I then calculated average capture probabilities for
taxonomic families of fish by averaging the individual species averages. All analyses
were completed using R in RStudio (R Core Team 2020; RStudio Team 2020) with an
alpha level of 0.05.

Results

Three-pass electrofishing resulted in greater total catch, density, and species
richness compared to the combination method. However, on average the three-pass
method required almost twice as much time as the combination method (89.7 mins and
50.7 mins, respectively). A total of 1479 fish, representing 30 species were captured;
1003 fish with the three-pass method and 476 fish with the combination method. Nine
species were only captured using the three-pass method, whereas only two species were
exclusive to the combination method. However, the two species collected only using the
combination method were collected during the electrofishing pass. The three-pass
method resulted in significantly greater density (mean = 0.21 fish/m$^2$) than the
combination method (mean = 0.13 fish/m$^2$; $t = -4.48$, df = 24, $P < 0.001$). Species
richness was also significantly greater for the three-pass method (mean = 7.24) compared
to the combination method (mean = 5.00; $t = -4.04$, df = 24 $P < 0.001$; Fig. 1). For the
three-pass method, a new species was captured on the second pass at 15 of 25 sites while
the combination method only captured a new species at 2 of 25 sites. On the third pass
electrofishing, a new species was captured at 10 out of 25 sites.

All three electrofishing passes were needed to estimate species richness in low
conductivity streams. Pass two provided an average increase of 1.24 species and pass
three added an average of 0.64 species (Figure 1). I estimated that there was a 67%
probability that a new species would be collected during the second pass and 30% probability that a new species would be collected on the third pass. Logistic regression indicated that the odds of capturing a new species on the second pass increased by 55% (95% CI = 8-253%) with each additional species added to total richness at a site ($P = 0.038$). However, the number of species present at a site did not significantly affect the probability that an additional species was collected on the third pass ($P = 0.1022$). For the second and third passes, the odds of capturing a new species was not significantly related to abundance of fish ($P = 0.055$ and $P = 0.210$, respectively) and stream width ($P = 0.428$ and $P = 0.533$, respectively).

Species specific capture probabilities ranged from 0.50-0.87, with Tessellated Darter (*Etheostoma olmstedi*), Redfin Pickerel (*Esox americanus*), and Largemouth Bass (*Micropterus salmoides*) having the highest capture probabilities and Redbreast Sunfish (*Lepomis auritus*), Dusky Shiner (*Notropis cummingsae*), and Sandhills Chub (*Semotilus lumbee*) having the lowest capture probabilities (Table 2). When grouped by taxonomic family, capture probabilities of most families were 50-75%, with the exception of Percidae which was 87% (Table 3).

**Discussion**

Although a combination of gears is often beneficial when sampling fish assemblages, I found that in low-conductivity streams of the Carolina Sandhills, three-pass electrofishing resulted in greater density and species richness estimates than the combination method. Tiemann and Tiemann (2004) reported that in riffle habitats, kick seining produced higher average species richness (10.66 species), compared to electrofishing (8.28 species). However, their study only focused on riffle habitats and the
associated fish assemblages, whereas I was interested in sampling all habitats and species present. Riffle habitats were generally small in many of the streams I sampled. Therefore, although kick-seining may be beneficial for sampling streams with extensive riffle habitats, the benefits do not translate to lower gradient streams with fewer turbulent riffles. Additionally, the streams we sampled were small and shallow, which allowed the anode of the electrofishing unit to be relatively close to the fish being shocked (because I was able to move the anode through most of the available water). While the combination method required less than 60% of the time needed to complete three-pass electrofishing, two-pass electrofishing would be closer in average completion time to the combination method, while also producing an extra 1.2 species on the second pass compared to the kick seine. Therefore, even if time is a concern, electrofishing for only two passes will likely provide greater species richness estimates than one-pass with a kick seine in low-conductivity environments.

Although several studies exist comparing electrofishing and seining, to my knowledge there are no studies that compare multi-pass electrofishing to a combination of electrofishing and kick seining. However, several studies exist that compare electrofishing with other potential sampling techniques for low-conductivity streams such as rotenone poisoning and snorkeling surveys. Rotenone-based sampling is commonly used but is not desirable when working with species of conservation concern. Allard et al. (2014) evaluated the efficiency of electrofishing compared to rotenone poisoning in low-conductivity streams in New Guinea and found that electrofishing was a viable alternative if the streams were less than 25 cm deep and had conductivities of at least 46 µS/cm. More recently, Pottier et al. (2019) concluded that with the right equipment and
settings (1500 V) designed for electrofishing in low-conductivity environments, electrofishing can be effective at conductivities greater than or equal to 20 µS/cm in streams as deep as 55 cm. Therefore, when working with species of conservation concern, electrofishing should be implemented instead of rotenone due to the substantially decreased mortality of fish from near 100% for rotenone to <2% for electrofishing (Pottier et al. 2019). Chamberland et al. (2013) compared electrofishing to snorkeling surveys in Laurentian streams and found that snorkeling provided greater abundance estimates and greater or equal richness estimates at all sites. However, a potential problem with snorkel surveys is that they require good visibility (Jordan et al. 2008) and at sites similar to my study area, tannins reduce visibility and may therefore limit the effectiveness of visual surveys.

Based off my results along with several previous studies examining backpack electrofishing in wadable streams, more than three passes may be necessary if the objective of the study is to capture all species present, including rare species. However, if only the dominant species are of importance, standard three-pass electrofishing will suffice in low conductivity streams. Paller (1995) evaluated the amount of electrofishing effort needed when sampling in the Sandhills and found that the second and third passes each produced greater than a 10% increase in total species richness compared to the pass prior. However, passes four through seven all produced richness increases of less than 10%. My results are in line with those reported by Pusey et al. (1998) from Queensland, Australia. They concluded that in the Mary and Johnstone rivers, at least three electrofishing passes were necessary to obtain an accurate description of the fish assemblage. Conversely, Shank et al. (2016) reported that in wadeable streams of the
Mid-Atlantic, over 97% of species were captured after two passes and that one pass was sufficient in small (< 5m wide) streams. I attribute this difference to the fact that their study took place in streams with naturally higher conductivity than my study streams since the authors mentioned that all specific conductivity readings were well within the suggested range (30-500 µS/cm). As a result, the capture probabilities for their electrofishing unit were likely higher, leading to the recommendation of fewer passes. Bertrand et al. (2006) reported that when sampling fishes in prairie streams, all species were captured during the first pass at 14 of the 19 sites, however single-pass electrofishing often underestimated species richness as the total species richness of a reach increased.

My family-level capture probabilities are similar to other published backpack electrofishing capture probabilities (Hense et al. 2010; Heimbuch et al. 1997; Wiley and Tsai 1983; Table 3). The greatest observed differences between my study and others were for Percidae; my capture probabilities were more than two times that reported by Hense et al. (2010) and Wiley and Tsai (1983). I attribute this difference to that fact that in Hense et al. (2010), Wiley and Tsai (1983), and my study, capture probability was only presented for one percid species in each and therefore, variation between species was not accounted for through averaging.

For these reasons, I recommend multi-pass backpack electrofishing when sampling fishes in a low-conductivity environment with at least three passes to be confident that the majority of species present have been captured. If project objectives rely on the detection of rare and cryptic species, more than three passes may be necessary.
References


Gilowacki L. and T. Penczak. 2005. Species richness estimators applied to fish in a small tropical river sampled by conventional methods and rotenone. Aquatic Living Resources. 18:159-168.


## Tables and Figures

### Table 1. List of species encountered while sampling in the Carolina Sandhills during summer and fall of 2019 with X indicating capture using that method of sampling ($n = 25$ sites).

<table>
<thead>
<tr>
<th>Species</th>
<th>Sites present</th>
<th>Three-pass electrofishing</th>
<th>Combination method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Redfin Pickerel (<em>Esox americanus</em>)</td>
<td>18</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Pirate Perch (<em>Aphredoderus sayanus</em>)</td>
<td>16</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Margined Madtom (<em>Notorus insignis</em>)</td>
<td>15</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Yellow Bullhead (<em>Ameriurus natalis</em>)</td>
<td>15</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Dollar Sunfish (<em>Lepomis marginatus</em>)</td>
<td>14</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Dusky Shiner (<em>Notropis cummingsae</em>)</td>
<td>13</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Creek Chubsucker (<em>Erimyzon oblongus</em>)</td>
<td>11</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Bluegill (<em>Lepomis macrochirus</em>)</td>
<td>9</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Sandhills Chub (<em>Semotilus lumbee</em>)</td>
<td>9</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Largemouth Bass (<em>Micropterus salmoides</em>)</td>
<td>8</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Mud Sunfish (<em>Acantharchus pomotis</em>)</td>
<td>7</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Redbreast Sunfish (<em>Lepomis auritus</em>)</td>
<td>6</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Tessellated Darter (<em>Etheostoma olmstedii</em>)</td>
<td>6</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Warmouth (<em>Lepomis gulosus</em>)</td>
<td>4</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Bluehead Chub (<em>Nocomis leptocephalus</em>)</td>
<td>3</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Banded Sunfish (<em>Enneacanthus obesus</em>)</td>
<td>3</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Flier (<em>Centrarchus macropterus</em>)</td>
<td>2</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Blackbanded Sunfish (<em>Enneacanthus chaetodon</em>)</td>
<td>2</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Rosyside Dace (<em>Clinostomus funduloides</em>)</td>
<td>1</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Bluespotted Sunfish (<em>Enneacanthus gloriosus</em>)</td>
<td>1</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Golden Shiner (<em>Notemigonus crysoleucas</em>)</td>
<td>1</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Creek Chub (<em>Semotilus atromaculatus</em>)</td>
<td>1</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Redear Sunfish (<em>Lepomis microlophus</em>)</td>
<td>1</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Sawcheek Darter (<em>Etheostoma serrifer</em>)</td>
<td>1</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Pumpkinseed (<em>Lepomis gibbosus</em>)</td>
<td>1</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Lined Topminnow (<em>Fundulus lineolatus</em>)</td>
<td>1</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Chain Pickerel (<em>Esox niger</em>)</td>
<td>1</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Spotted Sunfish (<em>Lepomis punctatus</em>)</td>
<td>1</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Sea Green Darter (<em>Etheostoma thalassinum</em>)</td>
<td>1</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Eastern Mosquitofish (<em>Gambusia holbrooki</em>)</td>
<td>1</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Table 2. List of most common species captured using three-pass electrofishing in the Carolina Sandhills during summer and fall 2019, including average estimated abundance and capture probabilities from all sites sampled ($n = 25$). Standard errors are provided in parentheses.

<table>
<thead>
<tr>
<th>Species</th>
<th>Estimated abundance</th>
<th>Capture probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tessellated Darter (Etheostoma olmstedi)</td>
<td>2 (0.63)</td>
<td>0.87 (0.08)</td>
</tr>
<tr>
<td>Redfin Pickerel (Esox americanus)</td>
<td>4 (0.59)</td>
<td>0.73 (0.05)</td>
</tr>
<tr>
<td>Largemouth Bass (Micropterus salmoides)</td>
<td>1 (0.18)</td>
<td>0.68 (0.10)</td>
</tr>
<tr>
<td>Pirate Perch (Aphredoderus sayanus)</td>
<td>5 (1.29)</td>
<td>0.64 (0.06)</td>
</tr>
<tr>
<td>Dollar Sunfish (Lepomis marginatus)</td>
<td>9 (2.72)</td>
<td>0.62 (0.06)</td>
</tr>
<tr>
<td>Yellow Bullhead (Ameiurus natalis)</td>
<td>4 (1.05)</td>
<td>0.59 (0.05)</td>
</tr>
<tr>
<td>Bluegill (Lepomis macrochirus)</td>
<td>12 (8.62)</td>
<td>0.59 (0.07)</td>
</tr>
<tr>
<td>Margined Madtom (Notorus insignis)</td>
<td>5 (1.27)</td>
<td>0.59 (0.06)</td>
</tr>
<tr>
<td>Creek Chubsucker (Erimyzon oblongus)</td>
<td>4 (1.21)</td>
<td>0.56 (0.04)</td>
</tr>
<tr>
<td>Mud Sunfish (Acantharchus pomotis)</td>
<td>2 (0.42)</td>
<td>0.54 (0.09)</td>
</tr>
<tr>
<td>Redbreast Sunfish (Lepomis auritus)</td>
<td>7 (1.78)</td>
<td>0.54 (0.11)</td>
</tr>
<tr>
<td>Dusky Shiner (Notropis cummingsae)</td>
<td>13 (2.49)</td>
<td>0.53 (0.08)</td>
</tr>
<tr>
<td>Sandhills Chub (Semotilus lumbee)</td>
<td>13 (4.14)</td>
<td>0.50 (0.07)</td>
</tr>
</tbody>
</table>
Table 3. Summary of comparison between the average capture probabilities for families of fish while electrofishing between my data, sampling in the Carolina Sandhills, and other published literature (Hense et al. 2010; Heimbuch et al. 1997; Wiley and Tsai 1983). Standard errors are provided in parentheses.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Capture probability</td>
<td>Number of species</td>
<td>Capture probability</td>
<td>Number of species</td>
</tr>
<tr>
<td>Cyprinidae</td>
<td>0.51 (0.01) 2</td>
<td>0.57 (0.03) 5</td>
<td>0.71 (0.02) 9</td>
<td>0.68 (0.03) 9</td>
</tr>
<tr>
<td>Catostomidae</td>
<td>0.56 1</td>
<td>0.81 (0.17) 2</td>
<td>0.59 (0.02) 2</td>
<td>0.67 1</td>
</tr>
<tr>
<td>Ictaluridae</td>
<td>0.59 (0.00) 2</td>
<td>- 0</td>
<td>0.70 1</td>
<td>-</td>
</tr>
<tr>
<td>Aphredoderidae</td>
<td>0.64 1</td>
<td>- 0</td>
<td>- 0</td>
<td>-</td>
</tr>
<tr>
<td>Centrarchidae</td>
<td>0.59 (0.03) 5</td>
<td>0.54 (0.19) 2</td>
<td>0.35 (0.03) 3</td>
<td>0.69 (0.01) 2</td>
</tr>
<tr>
<td>Esocidae</td>
<td>0.73 1</td>
<td>- 0</td>
<td>0.56 1</td>
<td>-</td>
</tr>
<tr>
<td>Percidae</td>
<td>0.87 1</td>
<td>0.39 1</td>
<td>0.62 (0.03) 2</td>
<td>0.35 1</td>
</tr>
</tbody>
</table>
Fig 1. The number of species added by each sampling pass and the average total species richness for two stream sampling methods, three-pass electrofishing and combination method (one-pass electrofishing with one-pass kick seine) used in the Carolina Sandhills during summer and fall 2019. Error bars show +/- standard error of the mean.
Chapter 2: Habitat characteristics associated with the presence of the Sandhills Chub (Semotilus lumbee)

Abstract

Headwater streams comprise the majority of stream length within a watershed and significantly contribute to drainage wide species diversity by supporting many rare and endemic species. The Sandhills Chub (Semotilus lumbee) is a leuciscid only found in the Sandhills ecoregion of North and South Carolina, and generally occurs in headwater streams. Because of its limited geographic distribution, the Sandhills Chub is a species of conservation concern, and as a result, has been listed as imperiled by the American Fisheries Society and as a Species of Special Concern by the South Carolina Department of Natural Resources (SCDNR). It has been extirpated from several locations in South Carolina and quantitative information on habitat use and biological associations are needed to guide conservation and restoration efforts. The goal of this study was to identify habitat and biological characteristics associated with presence of Sandhills Chub in headwater streams. Co-occurrence analysis and logistic regression were used to identify which biological and habitat features were associated with the presence and absence of Sandhills Chub. Habitat and fish data were collected at 115 sites during 2019-2020 and 431 Sandhills Chub were collected at 41 out of 115 sites sampled.

Cooccurrence analysis indicated positive relationships between Sandhills Chub and Dollar Sunfish (Lepomis marginatus), Creek Chubsucker (Erimyzon oblongus), and Margined Madtom (Notorus insignis) and negative relationships between Sandhills Chub
and Largemouth Bass (*Micropterus salmoides*), Bluegill (*Lepomis macrochirus*), Creek Chub (*Semotilus atromaculatus*), Eastern Mosquitofish (*Gambusia holbrooki*), and Lined Topminnow (*Fundulus lineolatus*). I observed that Sandhills Chub presence was positively associated with dissolved oxygen levels, instream cover, and the percent of substrates between 6 and 11 mm. In addition, when an extreme outlier HUC was removed from the analysis, a negative relationship was seen between Sandhill Chub presence and the number of impoundments within each 12-digit HUC, however, further investigation is required to determine the validity of this relationship. Results from this study will help guide management decisions for the future conservation and restoration of Sandhills Chub.
Introduction

Headwater streams are an important source of biodiversity worldwide because of the unique habitats created by the variable nature of these systems. These streams are common and first and second order streams make up 79% of the total stream length in the U.S. (Colvin et al. 2019). Low-order streams contribute to regional fish diversity and provide full time inhabitants, as well as migratory species, with food resources, refuge from predators or competitors, thermal relief, and spawning and nursery habitats (Meyer et al. 2007). While being abundant, headwater streams are often overlooked, because most do not support important commercial or recreational fisheries with the exception of salmonids. Moreover, these streams are highly susceptible to disturbance due to their small drainage areas. While headwater streams do not contain large numbers of sport fish species, they are home to many rare and endemic non-game species.

The Sandhills Chub (Semotilus lumbee) is a leuciscid only found in the Sandhills ecoregion of North and South Carolina, and limited previous research on the species suggests it prefers small, clear, headwater streams with sand or gravel substrate and little aquatic vegetation (Rohde and Arndt 1991). Although the Sandhills Chub can be abundant within streams it occupies, it is classified as “Vulnerable” (NatureServe Global Conservation Status of G3; NatureServe 2021) because of its limited geographic distribution, and human development in the region. The Sandhills Chub has also been listed as imperiled by the American Fisheries Society (Jelks et al. 2008) and as a Species of Special Concern by the South Carolina Department of Natural Resources (SCDNR) and North Carolina Wildlife Resources Commission. It has been extirpated from several locations in North and South Carolina and is at risk to disappear from others (Rohde and
Arndt 1991), with habitat alteration as a result of impoundment, development, and agriculture as the main threats for local extirpation of the Sandhills Chub.

Small impoundments in the Sandhills are pervasive and have been created for recreation, golf courses, and irrigation. Additionally, the successful reintroduction of American Beaver (*Castor canadensis*) into the area (Rohde and Arndt 1991) has resulted in numerous beaver dams on headwater streams. Impoundment of streams can alter the substrate composition, temperature, and flow regime. Waters directly upstream from an impoundment experience decreased water velocity leading to warmer temperatures and increased siltation (Mammoliti 2002). This newly created habitat favors lentic species such as centrarchids and non-native species which may compete with or prey on Sandhills Chub. Impoundments also restrict movement of fishes, which can prevent recolonization of upstream reaches after periods of drought or low flow. Kashiwagi and Miranda (2009) found that Blackside Darter (*Percina maculata*) were collected throughout their study area except in upstream reaches of impounded streams. The darter was thought to have been eliminated from areas upstream of impoundments during droughts and was unable to return to these areas due to discontinuity of habitat caused by impoundments.

Urban, exurban, and agricultural development are other forms of anthropogenic disturbance potentially affecting Sandhills Chub habitat especially in the northern and southern edges of their distribution. The southern extent of the Sandhills Chub distribution is within the Columbia, SC metropolitan statistical area, which has experienced a population growth of 26.8% since 2000 and is projected to increase by an additional 9.7% by 2030 (South Carolina Department of Employment and Workforce...
The northern edge of their distribution is located near Fayetteville, NC, which has grown in population by 15.3% since 2000 and is projected to increase by 6.4% by 2030 (Nash et al. 2006). Although the core portion of Sandhills Chub distribution is sparsely populated, development of roads and clearing of land for silviculture and agriculture, as well as mining, represent threats to headwater streams in this region. Additionally, small streams in this region have been altered to an unknown degree due to historical land use such as timbering of Longleaf Pine (*Pinus palustris*) forests. Poor construction practices associated with development and road construction, agriculture, and removal of riparian buffers commonly result in increased siltation of stream substrates, which can have negative effects on stream ecosystems. Reed (1977) observed a decrease in the species richness of benthic macroinvertebrates and fishes (23% and 40% respectively) downstream of road construction as a result of increased siltation. Sandhills Chub are pit-ridge nest builders, which means that during spawning season the males construct a pit-ridge nest on the stream bed (Rohde et al. 2009). First, the male excavates a pit and deposits the substrates upstream to create a ridge. Then as the female lays her eggs in the upstream end of the nest, the male places larger substrates like gravel and pebbles on the eggs to protect them (Woolcott and Maurakis 1988). Pit-ridge building fishes are represented in higher proportion within the list of imperiled minnows than among minnows overall (Johnston 1999). Increased siltation may cause a decrease in the availability of larger substrate for ridge construction and therefore lead to decreased reproduction of Sandhills Chub. Additionally, stream siltation may indirectly affect Sandhills Chub by decreasing the abundance of macroinvertebrates, which they consume.
Even though the Sandhills Chub is a species of conservation concern, research on the species is limited. The most recent publication focusing on the Sandhills Chub was Rohde and Arndt (1991) and only two other publications focus primarily on the species (Woolcott and Maurakis 1988; Snelson and Suttkus 1978). The objective of this study was to create a predictive model of Sandhills Chub presence or absence using biotic factors, microhabitat features, and watershed characteristics. To accomplish this objective, I collected fish and habitat data from headwater streams in the Sandhills ecoregion of South Carolina during summer through fall, 2019-2020. Results from this study can be used by fisheries managers to develop informed conservation strategies for the Sandhills Chub.

Methods

Study Site

Sampling was completed in the Sandhills ecoregion portions of the Pee Dee \( n = 98 \) and Wateree \( n = 17 \) watersheds of South Carolina during June through October 2019 and August through November 2020 (Figure 2). Sites were selected using a stratified random sampling design from the population of all wadeable, perennial streams with a drainage area between 2-50 km\(^2\). This drainage area limitation was used because Sandhills Chub are known to be restricted to low-order headwater streams. The two strata used in the sampling design were streams that Sandhills Chub were previously collected in and streams where presence of Sandhills Chub was unknown. I used these two strata to force in sites where Sandhills Chub were present to ensure a sufficient number of events (Sandhills Chub presence) to allow for logistic regression analysis (i.e., fully random
sampling may have resulted in too few samples for statistical analysis). This sampling design resulted in a total of 24 sites from streams where Sandhills Chub were previously collected and 91 sites where their status was unknown.

**Fish Sampling**

At each site, three-pass backpack electrofishing was used to sample a length of 35 times the mean stream width (MSW; Simonson and Lyons 1995). The primary unit used was an ETS Electrofishing Systems model ABP-4 backpack unit (Madison, Wisconsin) with upgraded electrodes (anode: 3/8-inch diameter, cathode: 3 m length) to improve effectiveness in low-conductivity conditions. Due to equipment failure, a second unit (Smith and Root model LR-24, Vancouver, Washington) was used at six sites. The same settings were used at all sites (AC, 600V, 25% duty cycle, and a frequency of 40 Hz) regardless of unit used. Despite low conductivity, pilot work and results from this study demonstrated that three-pass electrofishing is effective at detecting Sandhills Chub; Sandhills Chub were detected on the first pass at 36 of the 41 sites where they were collected, on the second pass at five sites and never collected for the first time on the third pass. Therefore, the probability of a type-II detection error using three-pass electrofishing to collect Sandhills Chub is low. I used a standard multi-pass electrofishing technique with block nets at the upstream and downstream end of the reach. Two individuals, both with dip nets and one with the backpack electrofisher, started at the downstream end of each site and sampled by zig-zagging in an upstream direction for three passes. Only two people were used to sample these streams because the average stream width was less than 3 m at most sites, limiting room for any additional individuals in the stream. A single backpack electrofishing unit was used at a time when sampling
sites <3m wide. At eight sites, where MSW was >3m, two electrofishing units were used simultaneously with three people sampling. After each pass, all fish were identified, counted, and then released below the downstream block net.

**Habitat Sampling**

Habitat sampling was completed after fish sampling at each location. Habitat characteristics were collected at the site level using the transect method. Since most of the streams in this study were small (MSW <3 m), 13 evenly-spaced transects were used at each site (Simonson et al. 1994). Width was recorded at each transect and depth was recorded at three points along each transect; one point at the deepest spot in the stream (thalweg) and the other two equally distanced (Simonson 1993). Stream cover was also quantified at each site as present or absent for each transect if any form of instream cover (i.e. undercut bank, log jam, rock, aquatic vegetation, root ball) capable of concealing a fish greater than 10 cm was intersected by the transect. Water quality data (conductivity, dissolved oxygen, pH, and temperature) were collected at each site using a Hach (Hqd) portable multiprobe (Loveland, Colorado). Measurements were taken from the middle of the water column in an area of moderate flow. Substrate data were recorded using the Wolman pebble count method (Wolman 1954). One person zig-zagged from bank to bank stopping 100 times to pick up the piece of substrate nearest the big toe on their right foot and then measured the piece of substrate using a gravelometer along the intermediate-axis. This information was used to calculate d50 as well as the percentage of substrate falling within the 6-11 mm range. I calculated percentage of substrate within the 6-11 mm size class at each site because this is the primary size substrate used by Sandhills Chub to construct their nests (Maurakis et al. 1990).
Impoundments and Watershed Characteristics

To investigate the relationship between stream impoundments and presence of Sandhills Chub, I used Google Earth Pro (7.3.3.7786, Google LLC, Mountain View, CA) to determine the total number of impoundments present in each 12-digit hydrologic unit code (HUC) sampled. First, I imported shape files into Google Earth from the National Hydrography Dataset (USGS 2018a; USGS 2018b) and Watershed Boundary Dataset (USGS et al. 2020) for 12-digit HUC boundaries, NHDwaterbodies, and NHDflowlines. Then, I quantified the number of impoundments visually, using satellite imagery. To prevent potential effects of including ponds not created through impoundment of streams, I only counted impoundments that directly intersected NHDflowlines.

Watershed characteristics were analyzed using the National Aquatic Resource Surveys’ StreamCat dataset (Hill et al. 2016). Sample locations were linked to the corresponding COMID number and data for percent forested land, percent forested land within 100 m stream buffers, percent of land developed, percent of land developed within 100 m buffers, canal density, road density, road crossing density, percent of land used for crops and hay, and percent of land used for crops and hay within 100m stream buffers were extracted for each watershed. Elevation data was also extracted for the local catchment at each site.

Data Analysis

All analyses were completed using R in RStudio (R Core Team 2021; RStudio Team 2020). First, I used the ‘cooccur’ package (Griffith et al. 2016) to analyze any potential biological relationships between Sandhills Chub and other species encountered while sampling. Species co-occurrence was calculated using a random sampling with
replacement and hypergeometric distribution approach to the probabilistic model (Griffith et al. 2016; Veech 2013) which compares observed co-occurrence to the expected co-occurrence between pairs of species and searches for significant positive, negative, and random pairwise interactions ($\alpha = 0.05$). This analysis was completed to see if co-occurrence data suggests ecological interactions between Sandhills Chub and other species that may facilitate or prevent their occupation of a site and should therefore be included in the model.

Second, I used a hierarchical generalized linear mixed-model (binary response: present or absent) to investigate the relationships between environmental variables and the probability of presence for Sandhills Chub. Samples were taken at the site level and were nested within 42 different 12-digit HUCs, which were nested within 10, 10-digit HUCs. Therefore, I used 12-digit HUCs nested within 10-digit HUCs as random effect grouping factors. Prior to conducting the analysis, I tested for multicollinearity among variables using the vif() function in the ‘car’ package (Fox and Weisburg 2019). Because including too many predictor variables relative to the number of events (e.g., locations where Sandhills Chub were present) in a logistic regression model can result in poor model fit and incorrect conclusions (Peduzzi et al. 1996; Vittinghoff and McCullough 2006; Ranganathan et al. 2017), I investigated if the large number of a priori predictor variables could be reduced to a statistically justifiable number, given the number of sites that had Sandhills chub. Peduzzi et al. (1996) reported that for models with less than 10 events per variable, regression coefficients were biased; however, Vittinghoff and McCullough (2006) reported that this rule of thumb can be relaxed as long as results are interpreted with caution and more complex models are compared to models containing
fewer predictors. Therefore, because my dataset only included 41 sites where Sandhills Chub were present, I needed to reduce the dimensionality of the data and limit the analysis to the most biologically plausible set of predictor variables.

To select the initial suite of variables included in the analysis, I examined co-occurrence plots and notched boxplots of relationships between environmental variables and the presence or absence of Sandhills Chub, and considered this information in the context of what is known about Sandhills Chub ecology and ecology of species it may have negative or positive associations with (Figure 3; Figure 4). In notched box plots the notches represent the 95% confidence interval of the median and when notches between two plots do not overlap that is good evidence that the medians differ (Chambers 1983). Variables where the notches clearly overlapped were removed from consideration and variables with notches that were close to overlapping were investigated further by considering the range and scale of the units. For example, box plots for depth, velocity, and width were very close to overlapping but when the scale of the units was taken into consideration the median differences were at a very small scale (i.e., approx. 5 cm for depth, 0.03 m/s for velocity, and 0.3 m for width). The same logic was applied to the density of roads within the watersheds where notches did not overlap however, the difference in medians was only about 0.5 km/km² and road density throughout the sample area was low. In addition, roads were accounted for in the development metric as impervious surfaces and no significant difference were highlighted by examination of the boxplots for watershed wide development or within the 100 m stream buffers. The box plots for conductivity also did not overlap in notches however, this variable was excluded from the model because there is no evidence of conductivity acting as a limiting factor.
for fishes; conductivity was generally low throughout the sample area. Next, I fit models for all possible combinations of the suite of biologically relevant variables (including the nested random effects in all models) and ranked them based on AICc and calculated ΔAICc and Akaike weights. Model fit was examined using the ‘DHARMa’ package (Hartig 2020) to check for overdispersion, underdispersion, and issues with the distribution of residuals, and no significant issues with model fit were identified. Finally, I completed 5-fold cross-validation on the most likely models to calculate the receiver operating characteristic (ROC) curve and estimate the area under the curve (AUC) using the ‘pROC’ package (Robin et al. 2011). The ROC curve is a plot that shows specificity versus sensitivity for varying levels of a classification threshold in logistic regression. Area under the ROC is a metric used to measure performance of classification models where AUC indicates the probability that the model will rank a true positive higher than a true negative value (i.e., values closer to one indicate more accurate classification).

Results

A total of 7788 fish, representing 52 species were captured at the 115 sites sampled. The most common species encountered included Dusky Shiner (Notropis cummingsae), Bluehead Chub (Nocomis leptocephalus), Yellow Bullhead (Ameriurus natalis), and Bluegill, with 431 Sandhills Chubs collected across 41 sites. Sampled streams were narrow (mean = 2.58 m wide, SD = 1.04; Table 4), shallow (mean = 21.4 cm deep, SD = 9.1), and had low conductivity (mean = 32.1 µS/cm, SD = 21.7). Overall land-use in the study area was primarily forested with low levels of development and
riparian buffers were generally intact; however, the prevalence of impoundments was high (Table 4).

Cooccurrence analysis indicated significant positive relationships between Sandhills Chub and Dollar Sunfish (*Lepomis marginatus*), Creek Chubsucker (*Erimyzon oblongus*), and Margined Madtom (*Notorus insignis*; Table 5). Negative relationships were found between Sandhills Chub and Largemouth Bass (*Micropterus salmoides*), Bluegill, Creek Chub (*Semotilus atromaculatus*), Eastern Mosquitofish (*Gambusia holbrooki*), and Lined Topminnow (*Fundulus lineolatus*; Figure 4). Because examination of these associations did not suggest interactions that facilitated or inhibited presence of Sandhills Chub (see Discussion), fish associations were not included in the logistic regression analysis.

Based on examination of boxplots and consideration of Sandhills Chub biology, variables for instream cover, percent of substrate between 6 and 11 mm, dissolved oxygen, the number of impoundments in the 12-digit HUC, and elevation of the catchment were included in the logistic regression analysis. The top four models based on AICc were within 2ΔAICc, so I interpreted and reported results from all four (Table 6). The four most likely mixed-models indicated that instream cover, dissolved oxygen, and the amount of substrate between 6 and 11 mm were important predictors of Sandhills Chub presence within their range (Table 7). A one unit (mg/L) increase in dissolved oxygen content resulted in an increase in the odds of presence by 74-79%. As instream cover increased by 10%, the odds of presence increased by 34-38%. Finally, a 10% increase in the amount of substrate between 6 and 11 mm resulted in an increase in the odds of Sandhills Chub presence by 101-126%. Although the most likely model included
the terms for the number of impoundments within each HUC12 and the elevation of the local catchment, the 95% confidence interval for these odds ratios (Table 7) overlapped zero and therefore the number of impoundments and elevation were not reliable predictors of Sandhills Chub presence.

**Discussion**

Endemic headwater stream specialists like the Sandhills Chub are at high risk of disturbance from environmental change due to their limited distributions compared to species with wide ranging distributions. I determined that high dissolved oxygen levels, presence of instream cover, and availability of the appropriate size substrate for nest construction are important habitat characteristics associated with presence of the Sandhills Chub in headwater streams of South Carolina.

Dissolved oxygen content in streams is important in shaping fish assemblages because different species have different metabolic requirements and oxygen demands (Ostrand and Wilde 2001). The lowest dissolved oxygen level at a site where Sandhills Chub was present was 5.40 mg/L and no chubs were found at any of the 13 sites sampled with dissolved oxygen less than this, suggesting that Sandhills Chub require oxygen rich streams.

Instream cover has been shown to be an important habitat characteristic for numerous species of fishes (Blair et al. 2021; Angermeier and Karr 1984). Cover such as woody debris provides concealment from predators and influences the depth, velocity, and substrates of a stream (Angermeier and Karr 1984). The sister species of the Sandhills chub, the Creek Chub, has been shown to be highly associated with instream
cover such as woody debris and aquatic vegetation (Belica and Rahel 2008). The addition of woody debris as instream cover is common in stream restoration and conservation efforts focused on improving habitat for salmonid populations (Lehane et al. 2002; Sweka and Hartman 2006). In addition, Gatz (2007) reported that the addition of woody debris into a stream in central Ohio significantly increased the total number of Creek Chub individuals found within the experimental reach. Instream cover for fishes can come in a variety of forms. Similar to woody debris, undercut banks provide refuge from aquatic and terrestrial predators. Blair et al. (2021) reported that undercut bank volume was positively related to Brook Trout (Salvelinus fontinalis) abundance, particularly for adults, in headwater streams of Ontario. The largest Sandhills Chub captured and measured in my study was 19.5 cm. Therefore, being a relatively small-bodied fish, almost all Sandhills Chubs are susceptible to predation from a variety of terrestrial and aquatic predators in locations lacking instream cover. Finally, Woolcott and Maurakis (1988) also noted that nests of Semotilus species were typically located near instream cover. Sandhills Chub may be exposed while constructing nests, therefore quick access to cover is likely important for survival when they are otherwise vulnerable during nest building.

Substrate composition is important to a species like the Sandhills Chub that relies on the availability of specific sizes of substrates to construct a nest and successfully spawn. Sandhills Chub move substrates with their mouth, so substrate size for nest building is limited to a narrow range based on mouth gape. Maurakis et al. (1990) reported that 82% of substrates in Sandhills Chub pit-ridge nests were in the 6-11 mm range. Substrates within the size 6-11 mm size class play an important role in decreasing
water velocity in the pit downstream of the ridge which facilitates the deposition of eggs into the nest (Maurakis et al. 1990). These observations, along with the strong effect of this size class of substrates in my models, suggests that streams lacking in substrates of this size may not be suitable for Sandhills Chub reproduction. Increases in siltation due to agriculture, development, and stream impoundment may lead to a loss of spawning habitat and reduced reproduction in Sandhills Chub populations. Substrate augmentation has been used as a method for increasing spawning habitat for a variety of species (Taylor et al. 2019) and consideration of species-specific substrate requirements are needed when completing these projects. For example, Crane and Farrell (2013) reported that the addition of coarse gravel retained Walleye (Sander vitreus) eggs better than larger substrates and should be used when creating Walleye spawning habitat. Similarly, Zeug et al. (2013) reported that substrate augmentation increased spawning in Steelhead Trout (Oncorhynchus mykiss) and Chinook Salmon (Oncorhynchus tshawytscha) in the Lower American River, CA; however, substrate size selection led to differing effects between the two species. Where appropriate (e.g., areas where spawning habitat has been lost or degraded), additions of substrates in the 6-11 mm size class coupled with addressing any causes of stream siltation, may increase reproduction by Sandhills Chub.

Although the confidence interval for the impoundment parameter estimate overlapped zero, thus indicating a limited effect of impoundments on presence of Sandhills Chub, overall effects of impoundments on Sandhills Chub are not well understood. For example, post-hoc analyses indicated that the impoundment parameter estimate was substantially influenced by one 12-digit HUC with an extreme number of impoundments (139) but presence of Sandhills Chub at five out of the eight sites sampled
within this HUC. When this outlier HUC was removed from the dataset and the model was fit with the remaining data, the number of impoundments in each 12-digit HUC had a strong negative relationship on the odds of presence of Sandhills Chub. Impoundments likely serve as a barrier preventing the recolonization of Sandhills Chub in upstream reaches after disturbances such as extreme high and low flows as well as a barrier preventing gene flow. Hudman and Gido (2013) reported that impoundments can act as a barrier to gene flow for Creek Chub populations upstream of impoundments and this effect could be amplified for habitat specialists or if the isolated habitat is relatively small. Therefore, since Sandhills Chub are headwater specialists and impoundment of streams in the Sandhills is frequent, there may be a pronounced effect on gene flow in the species, which should be investigated further.

Co-occurrence analysis indicated a limited number of positive and negative relationships between Sandhills Chub and other fishes, but I do not suspect that these relationships are inhibiting or facilitating the presence of Sandhills Chub based on biological interactions. Largemouth Bass, Bluegill, Eastern Mosquitofish, Lined Topminnow, and Creek Chub had negative cooccurrence relationships with Sandhills Chub. Largemouth Bass and Bluegill are lentic species, so they would not be expected to occur with Sandhills Chub and therefore, are likely not competing for resources. It is possible that when streams are impounded, Sandhills Chub occurring in the impounded area may be preyed upon by Largemouth Bass. Additionally, Largemouth Bass that wash into downstream areas may prey upon Sandhills Chub; however, long-term survival of individual Largemouth Bass in all but the deepest pools in these small headwater streams is unlikely. Eastern Mosquitofish and Lined Topminnow are small-bodied species and
therefore, are also likely not competing with Sandhills Chub for resources. Creek Chub and Sandhills Chub are sister species (Schönhuth et al. 2018) and the negative relationship in cooccurrence between the two species is likely due to limited overlap in their distributions (Snelson and Suttkus 1978; Rohde et al. 2009). This lack of overlap may be caused by the negative relationship between presence of Creek Chub and the percent of sand in the watershed (Maloney et al. 2013). During my sampling, I collected Sandhills Chub and Creek Chub at only one of 41 sites where Sandhills Chub were present and overall Creek Chub were only collected at 13 sites on the western edge of the Sandhills.

Although this study represents extensive sampling across the Sandhills Chub’s entire distribution in SC, the number of sites sampled limited the extent of our statistical analyses. For example, we could not investigate potential interactions between predictor variables. Future research should encompass the entire range of this species throughout the North and South Carolina Sandhills. Additional samples may also help to clarify the effects of habitat fragmentation through the construction of impoundments leading to habitat discontinuity for the species, which will be particularly important as sprawl from population centers at the northern and southern edges of Sandhills Chub distribution continue.

Conclusion

The Sandhills Chub prefers streams that have high dissolved oxygen content, presence of instream cover, and the substrates necessary to construct their pit-ridge nests. My study identified several new locations where Sandhills Chub occur as well as locations with sizable populations. This information can be used to prioritize
conservations efforts in the Sandhills by (1) protecting locations with larger populations
(2) restoring habitat at sites with low numbers of Sandhills Chub and (3) potential
reintroduction of Sandhills Chub to locations containing suitable habitat but where
Sandhills Chub have been extirpated. Protection of riparian zones from development in
the future and restoration of degraded sites should assist in maintaining cooler water
temperatures, which will in turn allow the stream to hold more oxygen, increase
recruitment of woody debris for instream cover, and also limit sedimentation of the
stream bed. The addition of instream cover such as woody debris for refuge and
substrates between the size of 6 and 11 mm for nest construction may help to improve
habitat for Sandhills Chub in locations where these habitat features are missing. My
model also allowed me to identify sites with high quality habitat where Sandhills Chub
are not present, for example, sites that the models estimated high probability of Sandhills
Chub presence but did not contain Sandhills Chub. These locations should be
investigated further and if longer reaches within the stream do not document Sandhills
Chub, reintroduction into these sites is an option.
References


### Table 4. Habitat characteristics for sites in the Carolina Sandhills where Sandhills Chub (Semotilus lumbee) were present or absent and overall means. Standard errors provided in parentheses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Present</th>
<th>Absent</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fish</td>
<td>60.5 (2.0)</td>
<td>71.5 (10.1)</td>
<td>67.7 (7.3)</td>
</tr>
<tr>
<td>Richness</td>
<td>8.2 (0.5)</td>
<td>8.4 (0.6)</td>
<td>8.3 (0.4)</td>
</tr>
<tr>
<td>Width (m)</td>
<td>2.67 (0.17)</td>
<td>2.53 (0.12)</td>
<td>2.58 (0.10)</td>
</tr>
<tr>
<td>Depth (cm)</td>
<td>22.8 (1.4)</td>
<td>20.6 (1.1)</td>
<td>21.4 (0.85)</td>
</tr>
<tr>
<td>Velocity (m/s)</td>
<td>0.11 (0.01)</td>
<td>0.09 (0.01)</td>
<td>0.09 (0.01)</td>
</tr>
<tr>
<td>d50</td>
<td>5.48 (0.80)</td>
<td>7.67 (1.30)</td>
<td>6.89 (0.89)</td>
</tr>
<tr>
<td>Substrate between 6-11mm (%)</td>
<td>20.2 (2.3)</td>
<td>11.2 (1.3)</td>
<td>14.4 (1.2)</td>
</tr>
<tr>
<td>Instream cover (%)</td>
<td>63.4 (2.89)</td>
<td>47.3 (2.90)</td>
<td>53.1 (2.25)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>20.6 (0.6)</td>
<td>21.4 (0.5)</td>
<td>21.1 (0.4)</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>8.04 (0.18)</td>
<td>7.12 (0.22)</td>
<td>7.45 (0.16)</td>
</tr>
<tr>
<td>Conductivity (µS/cm)</td>
<td>27.53 (3.89)</td>
<td>34.62 (2.26)</td>
<td>32.09 (2.03)</td>
</tr>
<tr>
<td>pH</td>
<td>4.79 (0.13)</td>
<td>5.15 (0.10)</td>
<td>5.02 (0.08)</td>
</tr>
<tr>
<td>Number of impoundments</td>
<td>45.0 (6.0)</td>
<td>58.5 (4.5)</td>
<td>53.7 (3.6)</td>
</tr>
<tr>
<td>Development - watershed (%)</td>
<td>8.32 (1.31)</td>
<td>9.05 (1.02)</td>
<td>8.79 (0.80)</td>
</tr>
<tr>
<td>Development - watershed within 100 m buffer (%)</td>
<td>4.54 (0.98)</td>
<td>4.41 (0.63)</td>
<td>4.45 (0.54)</td>
</tr>
<tr>
<td>Forested land - watershed (%)</td>
<td>58.08 (1.86)</td>
<td>59.88 (1.57)</td>
<td>59.22 (1.21)</td>
</tr>
<tr>
<td>Forested land - watershed within 100 m buffer (%)</td>
<td>79.98 (1.50)</td>
<td>81.50 (1.01)</td>
<td>80.96 (0.84)</td>
</tr>
<tr>
<td>Agricultural land - watershed (%)</td>
<td>13.21 (1.55)</td>
<td>15.83 (1.28)</td>
<td>14.90 (1.00)</td>
</tr>
<tr>
<td>Agricultural land - watershed within 100 m buffer (%)</td>
<td>3.53 (0.67)</td>
<td>5.30 (0.63)</td>
<td>4.67 (0.47)</td>
</tr>
<tr>
<td>Road density - watershed (km/km²)</td>
<td>1.81 (0.12)</td>
<td>2.13 (0.11)</td>
<td>2.01 (0.09)</td>
</tr>
<tr>
<td>Road density - watershed within 100 m buffer (km/km²)</td>
<td>1.62 (0.13)</td>
<td>1.82 (0.12)</td>
<td>1.74 (0.09)</td>
</tr>
<tr>
<td>Road steam crossings - watershed (crossings/km²)</td>
<td>0.42 (0.05)</td>
<td>0.43 (0.04)</td>
<td>0.43 (0.03)</td>
</tr>
<tr>
<td>Canal density - watershed (km/km²)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td>Elevation - catchment (m)</td>
<td>113.7 (3.3)</td>
<td>101.7 (3.9)</td>
<td>105.9 (2.8)</td>
</tr>
</tbody>
</table>
Table 5. Results of co-occurrence analysis between Sandhills Chub and other species collected while sampling in the Carolina Sandhills in 2019 and 2020. Analysis was completed using the 'cooccur' package in R (Griffith et al. 2016). Species co-occurrence was calculated using a random sampling with replacement and hypergeometric distribution approach to the probabilistic model (Griffith et al. 2016; Veech 2013) which compares observed co-occurrence to the expected co-occurrence between pairs of species and searches for significant positive, negative, and random pairwise interactions (α = 0.05). P corresponds to the probability that the species co-occur more or less than what would be expected by random chance and can be interpreted as a P-value.

<table>
<thead>
<tr>
<th>Species</th>
<th>Observed cooccur</th>
<th>Expected cooccur</th>
<th>Relationship</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dollar Sunfish (<em>Lepomis marginatus</em>)</td>
<td>29</td>
<td>23.4</td>
<td>(+)</td>
<td>0.023</td>
</tr>
<tr>
<td>Creek Chubsucker (<em>Erimyzon oblongus</em>)</td>
<td>22</td>
<td>16.7</td>
<td>(+)</td>
<td>0.029</td>
</tr>
<tr>
<td>Margined Madtom (<em>Notorus insignis</em>)</td>
<td>22</td>
<td>16.4</td>
<td>(+)</td>
<td>0.020</td>
</tr>
<tr>
<td>Bluegill (<em>Lepomis macrochirus</em>)</td>
<td>10</td>
<td>18.2</td>
<td>(-)</td>
<td>0.001</td>
</tr>
<tr>
<td>Largemouth Bass (<em>Micropterus salmoides</em>)</td>
<td>9</td>
<td>15.6</td>
<td>(-)</td>
<td>0.006</td>
</tr>
<tr>
<td>Eastern Mosquitofish (<em>Gambusia holbrooki</em>)</td>
<td>7</td>
<td>16</td>
<td>(-)</td>
<td>0.000</td>
</tr>
<tr>
<td>Creek Chub (<em>Semotilus atromaculatus</em>)</td>
<td>1</td>
<td>4.8</td>
<td>(-)</td>
<td>0.016</td>
</tr>
<tr>
<td>Lined Topminnow (<em>Fundulus lineolatus</em>)</td>
<td>0</td>
<td>3.3</td>
<td>(-)</td>
<td>0.012</td>
</tr>
</tbody>
</table>
Table 6. Summary table of the top ten models fit to analyze habitat characteristics associated with presence of Sandhills Chub (*Semotilus lumbee*). DO = dissolved oxygen (mg/L), substrate = the percent of substrates in the 6-11 mm size class, cover = the percent of instream cover, impoundments = the number of stream impoundments within each 12-digit HUC, elevation = the elevation of the local catchment (m).

<table>
<thead>
<tr>
<th>Predictors</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>Akaike weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>cover + DO + substrate + impoundments + elevation + (1</td>
<td>HUC10:HUC12)</td>
<td>119.95</td>
<td>0</td>
</tr>
<tr>
<td>cover + DO + substrate + impoundments + (1</td>
<td>HUC10:HUC12)</td>
<td>120.62</td>
<td>0.67</td>
</tr>
<tr>
<td>cover + DO + substrate + (1</td>
<td>HUC10:HUC12)</td>
<td>120.65</td>
<td>0.7</td>
</tr>
<tr>
<td>cover + DO + substrate + elevation + (1</td>
<td>HUC10:HUC12)</td>
<td>120.82</td>
<td>0.87</td>
</tr>
<tr>
<td>DO + substrate + impoundments + elevation + (1</td>
<td>HUC10:HUC12)</td>
<td>122.66</td>
<td>2.71</td>
</tr>
<tr>
<td>DO + substrate + impoundments + (1</td>
<td>HUC10:HUC12)</td>
<td>123.15</td>
<td>3.2</td>
</tr>
<tr>
<td>DO + substrate + elevation + (1</td>
<td>HUC10:HUC12)</td>
<td>123.72</td>
<td>3.77</td>
</tr>
<tr>
<td>DO + substrate + (1</td>
<td>HUC10:HUC12)</td>
<td>123.76</td>
<td>3.81</td>
</tr>
<tr>
<td>cover + DO + elevation + (1</td>
<td>HUC10:HUC12)</td>
<td>125.31</td>
<td>5.36</td>
</tr>
<tr>
<td>cover + DO + impoundments + elevation + (1</td>
<td>HUC10:HUC12)</td>
<td>125.43</td>
<td>5.48</td>
</tr>
</tbody>
</table>
Table 7. Summary of the top four logistic regression mixed-models used to analyze habitat characteristics associated with presence of Sandhills Chub (*Semotilus lumbee*). Odds ratios and 5-fold cross validation values are reported with 95% confidence intervals in parentheses. Odds ratios for cover, substrate, impoundments, and elevation are according to a 10-unit increase and the odds ratio for DO is according to a 1-unit increase. DO = dissolved oxygen (mg/L), substrate = the percent of substrates in the 6-11 mm size class, cover = the percent of instream cover, impoundments = the number of stream impoundments within each 12-digit HUC, elevation = the elevation of the local catchment (m).

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover</td>
<td>1.34 (1.02-1.79)</td>
<td>1.36 (1.02-1.81)</td>
<td>1.38 (1.04-1.84)</td>
<td>1.36 (1.02-1.82)</td>
</tr>
<tr>
<td>DO</td>
<td>1.75 (1.10-2.78)</td>
<td>1.79 (1.12-2.88)</td>
<td>1.76 (1.09-2.83)</td>
<td>1.74 (1.09-2.77)</td>
</tr>
<tr>
<td>Substrate</td>
<td>2.08 (1.16-3.73)</td>
<td>2.26 (1.25-4.09)</td>
<td>2.20 (1.20-4.03)</td>
<td>2.01 (1.10-3.65)</td>
</tr>
<tr>
<td>Impoundments</td>
<td>0.81 (0.64-1.04)</td>
<td>0.84 (0.67-1.06)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Elevation</td>
<td>1.21 (0.90-1.63)</td>
<td>-</td>
<td>-</td>
<td>1.14 (0.85-1.53)</td>
</tr>
<tr>
<td>5-fold AUC</td>
<td>0.79 (0.60-0.97)</td>
<td>0.79 (0.61-0.96)</td>
<td>0.81 (0.62-0.98)</td>
<td>0.81 (0.62-0.98)</td>
</tr>
</tbody>
</table>
Figure 2. Map of the Sandhills ecoregion of South Carolina within the Catawba and Pee Dee river basins. Solid dots show sites where Sandhills Chub (*Semotilus lumbee*) were present and open circles with a plus show sites where they were absent.
Figure 3. Notched boxplots showing differences between habitat characteristics at sites where Sandhills Chub (*Semotilus lumbee*) were present vs. absent. Notches represent 95% confidence interval of the median.
Figure 4. Plot of co-occurrence analysis showing the relationship between Sandhills Chub (Semotilus lumbee) and other fish species captured in the Carolina Sandhills during sampling in 2019 and 2020. Species co-occurrence was calculated using a random sampling with replacement and hypergeometric distribution approach to the probabilistic model (Griffith et al. 2016; Veech 2013) which compares observed co-occurrence to the expected co-occurrence between pairs of species and searches for significant positive, negative, and random pairwise interactions (\(\alpha = 0.05\)).