FINAL REPORT
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***Project Title**:* Conservation planning for a geographically restricted headwater species, the Sandhills Chub

Project Summary: Although small in size, headwater streams account for the majority of stream length and land area drained in the U.S. and globally (Colvin et al. 2019) and contain fish species and assemblages that are not found in larger waters (Paller 1994). Thus, headwater streams play an important role in contributing to biodiversity (Meyer et al. 2007), and many small-bodied species that are considered imperiled due to geographically restricted ranges are only found in these habitats (Rohde and Arndt 1991; Meyer et al. 2007; Sterling and Warren 2017; Colvin et al. 2019). The Sandhills Chub (*Semotilus lumbee*) typifies imperiled headwater species; it is geographically restricted, is found in clear streams that are well oxygenated, and requires coarse substrate for reproduction. Because the global distribution of the Sandhills Chub is restricted to headwater streams of the Sandhills ecoregion in North and South Carolina, and it is a habitat specialist, it has been listed as imperiled by the American Fisheries Society (Jelks et al. 2008) and as a Species of Greatest Conservation Need by the South Carolina Department of Natural Resources (SCDNR) and North Carolina Wildlife Resources Commission (NCWRC).
Like many small stream, non-game fishes, little is known about population ecology of the Sandhills Chub, thus limiting our capability of providing data-driven guidance for conservation and restoration of the species. Therefore, the research conducted during our project has contributed important new findings. The data and knowledge from our research has provided education of graduate students via thesis completion and education of the public through outreach communication. These data also contribute to future conservation and management planning for the species. We quantified seasonal movement patterns of Sandhills Chub because understanding spatiotemporal movement is a key part of understanding a species' life history, and species' movement patterns influence the exchange of genetic material during spawning and therefore, population structure. We used archived tissue samples collected during 2020 to develop microsatellite genetic markers, which were then used to conduct a range-wide investigation of Sandhills Chub genetic population structure and characterize the genetic health of identified population(s). Because conservation of genetic diversity is a central tenet of conservation biology and partly a function of gene flow through movement of organisms, we investigated the influence of anthropogenic barriers on population structure, genetic diversity, inbreeding rates, effective population size, and gene flow. Finally, because the Sandhills Chub distribution partly overlaps with the distribution of the closely related and widely distributed Creek Chub (*Semotilus atromaculatus*), we investigated if hybridization is occurring between these two species. Our combined project results and recently completed study of Sandhills Chub habitat use were incorporated into a range-wide conservation strategy for the species, including prioritization of areas for conservation and reintroduction. With limited public

awareness of Sandhills Chub, we also conducted an outreach campaign through public presentations, social media, and production of brochures.

Justification and Need: Despite being considered Imperiled (Jelks et al. 2008) and a Species of Greatest Conservation Need, little research has focused on the Sandhills Chub, thus inhibiting data-based conservation decisions. For example, since the Sandhills Chub was formally described in 1978 (Snelson and Suttkus 1978), only two peer-reviewed papers on the species have been published, with the most recent paper being published 30 years ago (Rohde and Arndt 1991). Due to its restricted geographic distribution in small headwater streams that are susceptible to human disturbance, and habitat needs that often are not compatible with human development (e.g. cool, clear streams, with high levels of cover and dissolved oxygen, and access to pebble substrates), the SCDNR and NCWRC consider the Sandhills Chub a Highest Priority Species of Greatest Conservation Need in their State Wildlife Action Plans (SWAP). Additionally, the Sandhills Chub is classified as "Imperiled" (S2) in both North and South Carolina (NatureServe 2012).

Because of the Sandhills Chubs' conservation status, lack of previous research on the species, and increasing threats to its populations from anthropogenic land use and hydrologic alteration, collection of data necessary to develop a comprehensive conservation strategy for the species is needed. Working towards this goal, a CCU graduate student recently completed a study focused on identifying streams inhabited by Sandhills Chub in South Carolina and the development of a Sandhills Chub habitat use model (Herigan 2021). Locations where Sandhills Chub were collected were added to the database of Sandhills Chub occurrences and can be used to guide conservation initiatives.

Quantitatively and qualitatively describing movement patterns of a species provides insight on seasonal habitat and resource needs, reproductive behaviors, and dispersal. For example, movement patterns of small stream fish are influenced by a variety of factors including size, life stage, and season (Fraser and Sise 1980, Albanese et al. 2004). Because little work has focused on Sandhills Chub, we have no knowledge of their movement patterns. Thus, research focused on understanding movement of Sandhills Chub and factors influencing their movement will increase our understanding of their life history (as called for in NC and SC SWAPs; see below) and our ability to guide conservation and restoration of the species.

Understanding genetic diversity and gene flow are core tenets of conservation biology because genes are the base level of biological diversity (Trombulak et al. 2004). The evolution and application of genetic tools to conservation issues over the last thirty years has revolutionized the field of conservation biology. For example, use of microsatellite markers have allowed for identification of fine-scale genetic structuring of fish populations, investigation into legacy effects of stocking, and the provision of guidance for stocking and reintroduction of imperiled species (Kanno et al. 2011; Moyer and Darden 2013; Darden and Tarpey 2014; Rougemont et al. 2019). Aside from a recent study focused on phylogeography of the Creek Chub (*Semotilus atromaculatus;* Schönhuth et al. 2018) and its relation to other *Semotilus* species, there is no genetic information on Sandhills Chub. Thus, development of a comprehensive conservation plan for the Sandhills Chub is currently limited by lack of knowledge about its range-wide population genetic structure. Due to the recent sampling efforts of Herigan (2021) and accumulation of Sandhills Chub occurrence data from recent and historical sampling in NC and SC, we now have data on occurrence of Sandhills Chub throughout its distribution, and can efficiently and effectively sample them to complete a range-wide assessment of genetic population structure.

With habitat alteration and fragmentation potentially having major influences on population genetic structure, potential barriers to gene flow (e.g. dams) should be considered when studying the population genetics of fishes. For example, fine-scale genetic structuring can occur through natural processes, such as limited movement and dispersal by a species, but can also be a result of dispersal being unnaturally limited by anthropogenic barriers (Roberts et al. 2013). Understanding the mechanisms of genetic structuring also provides important management implications. In the case of natural, fine-scale genetic structuring, genetic conservation efforts would focus on conserving genetic diversity, but if genetic structuring is due to anthropogenic influences, actions that reduce barriers or promote gene flow would be more appropriate.

Small impoundments in the Sandhills are pervasive and thus represent potential barriers to Sandhills Chub movement and gene flow. Herigan (2021) documented an average of 46 impoundments within 12-digit hydrologic units (i.e. sub-watershed level) throughout the Sandhills Chub's distribution in SC. Given the extent of habitat fragmentation of streams in this region, it is imperative to understand how habitat fragmentation influences genetic diversity and gene flow of Sandhills Chub as we develop a conservation plan for the species. Therefore, we investigated the riverscape genetics of Sandhills Chub by quantifying gene flow, effective breeding population size (N_e), and diversity as a function of the intensity of habitat fragmentation.

Although habitat fragmentation resulting from impoundments is extensive throughout the Sandhills ecoregion and substantial human population growth is occurring in the regions around Fayetteville, NC at the northern edge of the Sandhills Chub distribution and Columbia, SC at the southern edge of its distribution, streams—although limited in number—with high quality habitats and stable Sandhills Chub populations still exist. Some of these populations occur on protected state and federal lands (e.g. Fort Bragg), where conservation initiatives can be more easily implemented compared to private lands. Because high quality habitats and stable Sandhills Chub populations still exist, and some of these populations occur on public lands, there is a high likelihood that conservation strategies for the species can be successful if data are available to guide development and implementation of those strategies. Therefore, development of a conservation strategy for the Sandhills Chub follows the directive for SWG funds to help "States focus on targeted species in a proactive fashion, to help identify and reverse species population declines before restoration becomes more difficult and costly."

Finally, public awareness of the Sandhills Chub is limited (like many small-bodied fishes). Therefore, providing opportunities for the public to learn about unique, but often unheralded species is an important strategy for conserving aquatic biodiversity. A diverse outreach strategy, including public presentations, and print and digital media is necessary for reaching audiences that consume information through a variety of ways, were utilized to bring attention to a species that the majority of people living in the Sandhills ecoregion do not know exists.

Recently, microfishing for small-bodied fishes that are not classified as game species has increased in popularity (Cooke et al. 2020) and may provide an additional opportunity for generating interest in species, such as the Sandhills Chub, that reaches beyond the scientific community. Endemic fishes bring a sense of uniqueness and pride in a place and demonstrate the conservation spirit of Teddy Roosevelt. Encouraging utilization and enjoyment of wild places and our nation's natural resources fosters a restoration of trust in resource agencies via the local communities that become co-stewards of the local species.

Purpose and Objectives - The intent of our project was to address the paucity of life history data for Sandhills Chub, define the genetic population structure for Sandhills Chub, quantify the influence of habitat fragmentation on population structure to guide and prioritize conservation of the species, and improve awareness of the species.

Objective 1**:** Quantify movement patterns of Sandhills Chub, because understanding spatiotemporal movement of a species is a key part of understanding their life history, and movement of organisms influences the exchange of genetic material.

Objective 2: Develop a microsatellite marker panel necessary for investigating genetic population structure.

Objective 3: Conduct a range-wide investigation of Sandhills Chub genetic population structure.

Objective 4: Quantify the influence of anthropogenic barriers on population structure, genetic diversity, inbreeding rates, effective population size, and gene flow.

Objective 5: Investigate if hybridization is occurring between Sandhills Chub and the closely related (but more widely distributed) Creek Chub.

Objective 6: Use the information from investigations 1, 3–5, and a recently completed study of habitat use to update (SC) and develop (NC) SWAP species accounts for Sandhills Chub, including prioritizing areas for conservation, reintroduction, and supplemental translocation.

Objective 7: Conduct outreach through public presentations, distribution of informational pamphlets, and social media to inform landowners and the public about Sandhills Chub.

Accomplishments -

Objective 1 – *Quantifying Movement Patterns*

SC-funded project personnel assisted with fieldwork throughout the duration of the study for this project objective, which was led by the NC-funded personnel. Field work occurred over ~16 full weeks of sampling during the 2.5-year study, and included sampling fish, collecting biological data on Sandhills Chub (length, maturity status, sex), genetic samples, and measuring water quality parameters. SC-funded project personnel are co-authors on the resulting manuscript from this study, which has been submitted for publication in a peer-reviewed journal (see attached; Ramsey et al.).

Objective 2 – *Microsatellite Panel Development*

We identified and screened prospective microsatellite loci for suitability in the Sandhills Chub genotyping panel. Genomic data were obtained from low-coverage, whole genome sequencing performed by NC State Genomics Sciences Laboratory. The program *msatcommander* identified 5,991 potential microsatellite loci from the Sandhills Chub genomic sequence data. We obtained M13-labeled primers and conducted initial screening on a subset of 191 of these loci that represent three-, four-, or five-base pair repeat motifs and whose primer melting temperatures were between 59-60 °C. Initial screening was performed with DNA from a set of 7 Sandhills Chub individuals (2 from the Wateree basin, 5 from Pee Dee basin) and was used to assess whether the primers successfully amplified DNA from a single genomic region (i.e. eliminate possibility of amplification of multiple genomic regions). Isolation of DNA from Sandhills Chub was performed using Qiagen DNeasy Blood and Tissue spin-column DNA isolation kits. Conditions for PCR amplification during primer screening were identical across primers and included: an initial denaturation at 94° C for 2 min, and 35 cycles of 94° C denaturation for 40 s, annealing for 20 s at 60° C, and extension at 72° C for 40 s, and ending with a final extension of 1 hour at 72° C.

Twenty-eight non-monomorphic loci that successfully amplified across our initial screening of seven individuals were further genotyped on a geographically more inclusive set of 30 Sandhills Chub individuals: 10 from the Cape Fear basin representing 6 collection localities, 11 from the Pee Dee basin representing 10 collection localities, and 9 from the Wateree basin obtained from two collection localities. From these, 23 loci successfully amplified and exhibited two or more alleles (mean = 6.6 alleles observed per locus). Based on the observed sizes of alleles at each locus (Table 1), we have organized these loci into multiplex groups and obtained labeled primers for use in generating genotype data.

Table 1. Microsatellite primers and multiplex group organization.

Panel	Locus	Repeat Motif	Sigma Dye	# Alleles Observed	Forward Primer Sequence	Reverse Primer Sequence
1	*Slu37*	ACGC	BDA-4 blue	3	GACGGTGGAGCTTTGAAGAG	GTCCGAATCTTTGTCCGACC
	Slu140	AAAAC	BDA-2 black	11	TGTCAATCAAACTCCTGGCG	ACACAACTGCCAGTAACGTC
	Slu15	AAT	BDA-4 blue	4	ATCATGGAGGATCAGGTGGG	TCTTGTCCTCTGCTCCATGG
	Slu40	AAAG	BDA-2 black	10	CGGCTACCTGTTTGTCTTCAC	TGGATGTTGCATTTGTTGACAC
	Slu157	ATC	BDA-3 green	4	TGAACTGTTGCTCATCGTCG	CACGAGACCTGCAGAACAAC
2	*Slu147*	ACT	BDA-2 black	2	ACACAAACCATCACGTCCATC	AGCGGCTGATGGTAGTAGTC
	Slu189	ACAGG	BDA-2 black	3	GCACAGCTAGCAGATATGGC	TTTCATGAGACTGCGTTGCC
	Slu4	AAG	BDA-4 blue	3	GCAGACCTCCTCGTACCAG	CTGTGGTCTGAATGGTTGCC
	Slu59	AAGAG	BDA-4 blue	10	CGTGGGAAAGACATGAGCAC	TGAGGCAAGAAGAGAATTGGTG
	Slu151	ATC	BDA-3 green	6	CTCCCAACACAGTTCAAGGC	GACGACCGAATCATGTGTCG
	Slu186	AAAGG	BDA-3 green	7	GACTCAACAAATGGAGGCCC	TAAACGAACCCTCTCCACCC
3	*Slu177*	ACTAT	BDA-2 black	3	TCACAGGAAACCAGAAACACAG	TCCCTTCAGTCTTTGCATGTG
	Slu124	ATCC	BDA-2 black	14	ACATACGGCAAGGAAATGGG	CCCTGATTGTAACGCTAGCC
	Slu54	AACAC	BDA-4 blue	3	TGGTCATCAGTCACACAACAG	GGCTTTACCCTGGTGTTGTG
	Slu164	ACGC	BDA-4 blue	5	GTTCAGCCCTCATCTCACAC	GCAGAACGAGAGCTGGAAC
	Slu30	ACGC	BDA-3 green	9	CTTGCGCCATCTAGAGTGTG	ATGCGTGTACAGTTCCACAG
	Slu66	AATAT	BDA-3 green	5	GTATGACAAGCAGCCCACAG	TCCCGATGACAGATACACCG
4	*Slu13*	AAG	BDA-2 black	3	TGTAAACGGTGCCTGAAACC	AACAGTTGGTGGCGGTAATG
	Slu50	AGAT	BDA-2 black	9	GGGTGACAGATGATAGCAGAC	GCTACGCAATACTCGTTCCC
	Slu51	AACAC	BDA-4 blue	5	GTGTTGGCTGGGAAGGATTG	AGTAGCGCATTCACCGTTTG
	Slu174	ACTC	BDA-4 blue	13	GGTGGCACTTTGGGCTTC	CCGGGAACTGTGCACTAAAC
	Seat412	TCTA	BDA-3 green	10	TGTTTGGGAACCGTTTGGT	GGTGTCACATCCACTGTAAGACA
	Slu7	AGG	BDA-3 green	5	AGCATCTGGTAGTGGCAGAG	CTTCAAAGGTCACATGGGCC

Objective 3 – *Evaluation of Genetic Population Structure*

During the course of our study, a total of 1,726 tissue samples (pelvic or caudal fin clips) were collected from Sandhills Chubs across their geographic range, and an additional 249 samples were collected as part of a previous Sandhills Chub study (Tables 2-4, Figure 1; total = 1,975). Sampling spanned 16, 10-digit HUCs, and Sandhills Chubs were collected from 13 of those HUCs (Tables 2-4). In total, we now have tissue samples from 58 locations (31 in NC and 28 in SC). Sandhills Chubs were not encountered at an additional 45 locations sampled as part of our project (Table 5), exceeding our goal of samples from 30 individuals at 30 locations. About 40% of samples are from the two streams where Sandhills Chub movement was quantified for Objective 1. These samples may allow for future, more in-depth genetic analyses (e.g. parentage, paternal and maternal effects). All genetic samples collected to date have been archived and accessioned into the SCDNR Population Genetics Tissue Collection archive.

Using the 23-locus microsatellite panel developed for Objective 2, we successfully genotyped 887 individual Sandhills Chub fin clips across 30 sampling locations (Table 3). We assessed population structure across the range of Sandhills Chub through the implementation of a Bayesian clustering analysis in *Structure* v. 2.3.4 (Prichard et al. 2000). Analysis of likelihood scores for assignment of individuals to varying numbers of genetic clusters in *Structure* allows the identification of the appropriate number of distinct populations (*K*). Analyses were performed in an iterative hierarchical manner, beginning with the range-wide dataset and followed by a series of analyses that assess finer spatial structure within each distinct population that was identified at the previous geographic level. *Structure* simulations were performed with and without consideration for sampling location (i.e., with or without location prior, in which collection location informs the analysis). All *Structure* analyses had a run length of 200,000 generations after an initial 50,000 generation burn-in period, and three replicate runs for each level of *K*. Likelihood scores were assessed using *StructureSelector* (Li & Lu 2018). Because analyses of population structure can be influenced by the presence of family groups within a dataset (Anderson and Dunham 2008; Rodriguez-Ramilo & Wang 2012), we used *Colony* 2.0.6.2 (Jones & Wang 2010) to identify related individuals (e.g. full-sibling or parent-offspring). For *Colony* analyses, we performed three short-length runs using a full- and pairwise-likelihood combined method, assuming a polygamous breeding system.

Colony analyses identified five collection localities with samples that were dominated by family groups (putative full siblings or parent-offspring relatives). These localities include: one in the Wateree system (population 1 in Figures 2, 3, and Table 6); three in the lower Pee Dee River system (populations 10, 11, 12 in Figures 2, 3, and Table 6); and one in the Cape Fear River system (population 30 in Figures 2, 3, and Table 6). Because of the potential for these family groups to drive clustering patterns in *Structure*'s algorithm, we performed *Structure* analyses with and without these locations/populations included. In the cases of the Wateree and three Lower Pee Dee River localities, it was necessary to also perform a series of *Structure* analyses that included these locations in spite of the predominance of family groups in order to study how the Wateree population behaves relative to the Pee Dee and Cape Fear River systems and observe potential population structure within the lower Pee Dee River, which includes the three relatively isolated populations of Sandhills Chub on the western side of the basin.

At the broadest spatial scale, the range-wide structure analyses support four major genetic partitions (Figure 2). These four populations do not directly follow boundaries between the three major river basins (Santee, Pee Dee, and Cape Fear). Instead, the four major sub-drainages of the Pee Dee River system (Lynches, Lower Pee Dee, Little Pee Dee, and Lumber Rivers) were each assigned to distinct genetic populations, including two instances where they belong to genetic clusters that include populations in either the Santee or Cape Fear. The population from the Lynches (Pee Dee River tributary) was assigned to a distinct genetic group with the Wateree population (Figures 2 and 3). Similarly, the seven localities sampled in the Lumber River (Pee Dee tributary) were assigned to a genetic grouping that also contained the five localities sampled in the Cape Fear River system (Deep River and Upper Cape Fear River). The Lower Pee Dee (7 localities) and Little Pee Dee (2 localities) were each assigned to their own distinct genetic population. Generally, ancestry assignments for population membership for each of the four major populations are uniformly high, with the exception of one headwater tributary of the Lower Pee Dee (population 15 in Figures 2 and 3), which has an average ancestry assignment of 9.3% to the population containing the Lumber and Cape Fear population. Also, some individuals in Lumber River collections have elevated ancestry assignments to the Little Pee Dee River population (e.g. populations 22 and 23 in Figures 2 and 3).

At more local spatial scales, *Structure* analyses indicated that collection localities shared highest proportional ancestry with those localities within the same HUC10 watershed unit, and subsequently with those localities most proximate by river-distance. For example, the eight sampling locations within the Lynches basin showed a higher-level clustering pattern with two groups each composed of four populations that are either in the south-flowing tributaries (populations 6, 7, 8, and 9 in Figures 2 and 3) or the north-flowing tributaries (populations 2, 3, 4, and 5 in Figures 2 and 3). Likewise in the Lower Pee Dee, three populations in the western tributaries formed a distinct cluster relative to the disjunct cluster of four populations on the far eastern side of the basin. Population structure in the Lumber, Cape Fear, and Deep River basins was more complicated. While collections from these three systems were assigned to a single genetic group at the broadest hierarchical analysis, subsequent hierarchical analyses showed that the Deep River (a Cape Fear tributary) has an allelic profile more similar to the Lumber River populations, with one location in the upper Lumber (locality 20 in Figures 2 and 3) having substantially more ancestry assignment to the Deep River genetic cluster than other Lumber River localities. Across the entire range, at the most local spatial scale, the iterative clustering analyses identified all collection localities as distinct from each other, with the exception of two localities (localities 6 and 8 in Figure 2) within the Lynches River system.

Our hierarchical analyses reveal that Sandhills Chub populations are highly structured which likely reflects processes driven by both the species' biology as well as the geological history of the Sandhills region. At the most local geographic scale, *Structure* analyses indicated that nearly all collection localities have unique allele frequencies, and that local populations form hierarchical genetic clusters with geographically proximate locations. This pattern may be driven by aspects of the species' life history, in which their limitation to small headwater stream habitats may result in relatively rapid genetic isolation and drift. In contrast, there are several instances where populations that are distributed on different sides of major watershed boundaries have similar allele-frequency profiles (e.g. the Wateree and Lynches River populations; the Lumber River and those populations in both the Deep River and Cape Fear River basins). These cross-watershed genetic groupings may reflect historic dispersal events driven by geologic (e.g. stream capture) or other events (e.g. flooding events across river basin divides).

Genetic health metrics associated with the individual populations identified through our *Structure* analyses suggest that Sandhills Chub populations tend to persist at relatively low genetic effective population sizes (N_e). Most populations have N_e values well below 100, and only seven populations had N_e estimates higher than this threshhold (Table 6). We used the Garza-Williamson (G-W) index to determine if populations exhibit genetic signatures of population bottlenecks. All populations were estimated to have low G-W values, suggesting all have experienced a historical bottleneck (Table 6). In spite of most populations exhibiting low effective population size and strong signature of historic population bottleneck, heterozygosity estimates (observed and expected heterozygosity, H_o and H_e, respectively) are relatively high (Table 6). The exception to the high heterozygosity scores are those populations that our *Colony* analyses determined were dominated by family groups (populations labeled 1, 10, 11, 12, and 30). We interpret these findings as evidence that Sandhills Chub persist in small, isolated populations in headwater streams and routinely experience population bottlenecks, but that heterozygosity may be maintained by aspects of their life history, such as a polygamous mating system and overlapping generations.

Table 2. Site Totals for Sandhills Chub Genetic Samples 2020 through 2025
Sorted by HUC8 and HUC10 Watersheds
(Fin clip numbers: This study – 1726; Previously collected – 249; Total – 1975)

HUC8	**Pee Dee**				**Cape Fear**		**Santee**
30+ Fin Clips	25				5		1
HUC10	**Lumber**	**Little Pee Dee**	**Lower Pee Dee**	**Lynches**	**Deep**	**Upper Cape Fear**	**Wateree**
30+ Fin Clips	8	2	7	8	1	4	1

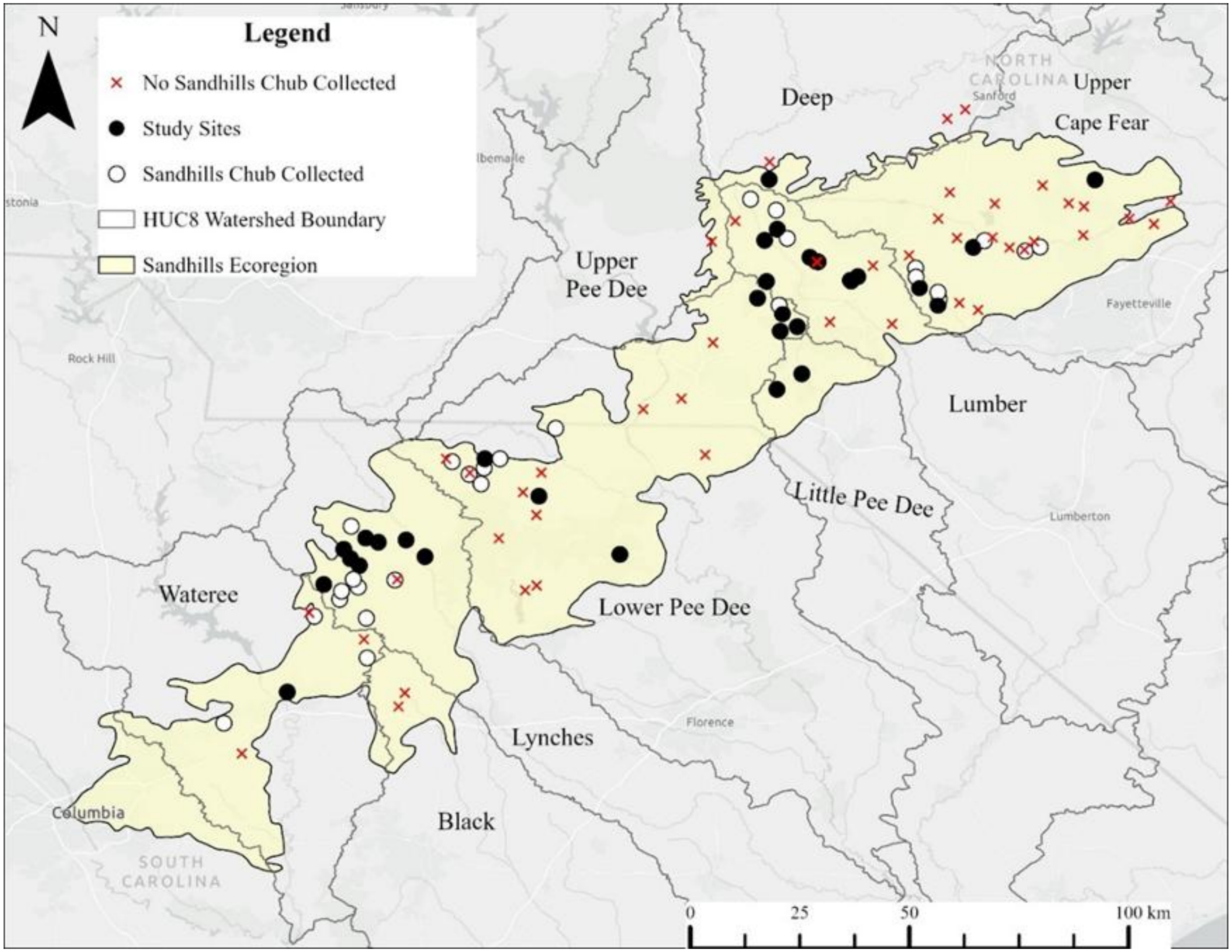

Figure 1. Locations where at least one Sandhills Chub was collected (hollow circle with black outline), or where no Sandhills Chubs were collected (red x's), during 2022−2024. Sites included in the genetic analyses (black points; 22−30 samples per site) were primarily sampled during 2022−2024, but archived samples from 2020 were used to increase sample sizes for some locations in South Carolina (10 sites). Basemap layer via Esri 2025, water boundary from USGS 2024, and ecoregion layer from USEPA 2012.

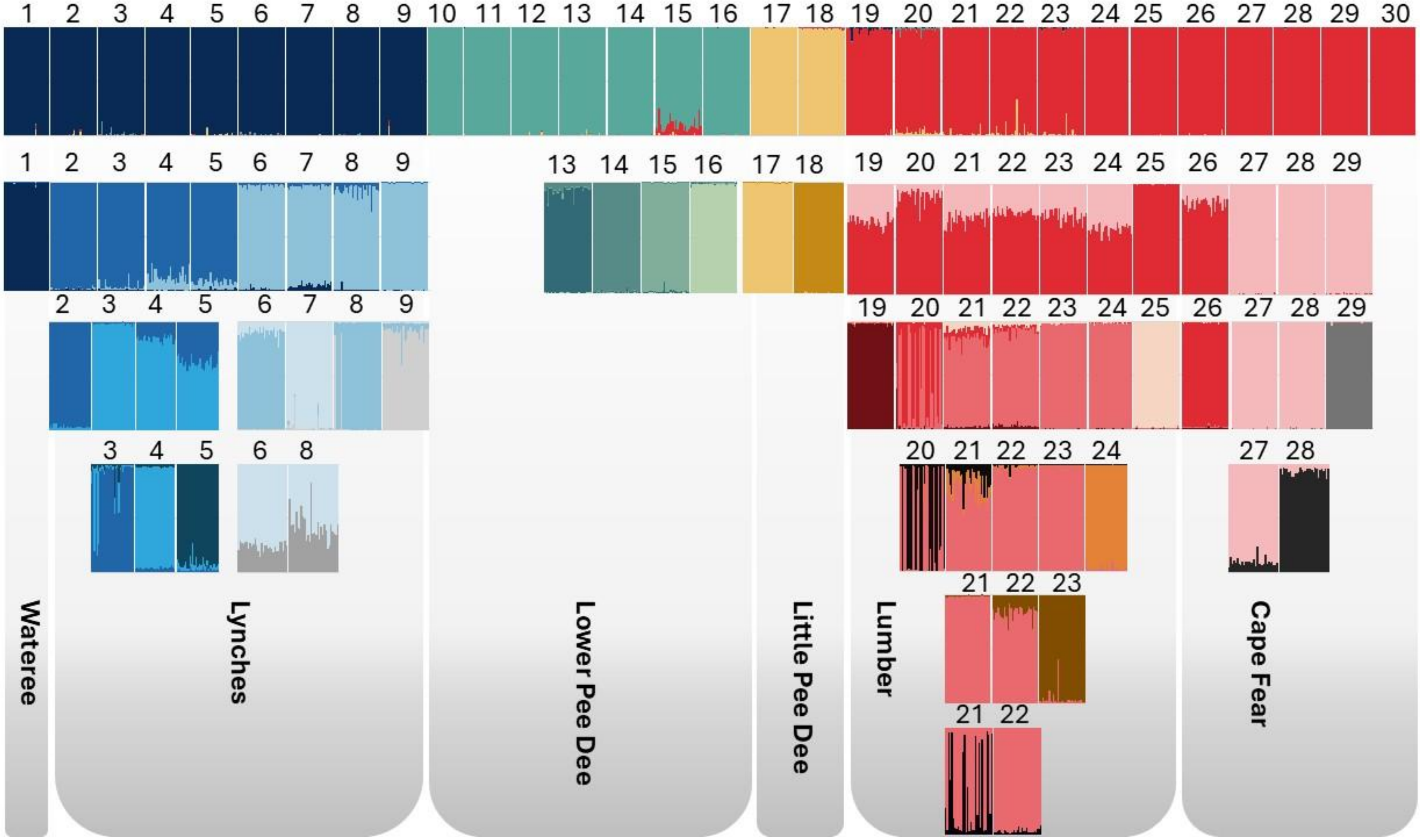

Figure 2. *Structure*-inferred ancestry assignment plots from hierarchical assessment of population genetic structure. Sampling localities are numbered (1-30) and correspond to locality labels mapped in Figure 3. Vertical lines within each rectangular block represent percent ancestry assignment for individual fish, with different colors indicating distinct genetic cluster membership at each hierarchical tier of analysis. White vertical lines separate localities. Sampling locations 10, 11, 12, and 30 were removed from *Structure* analyses after the first hierarchical tier due to predominance of full-sibling groups in collections.

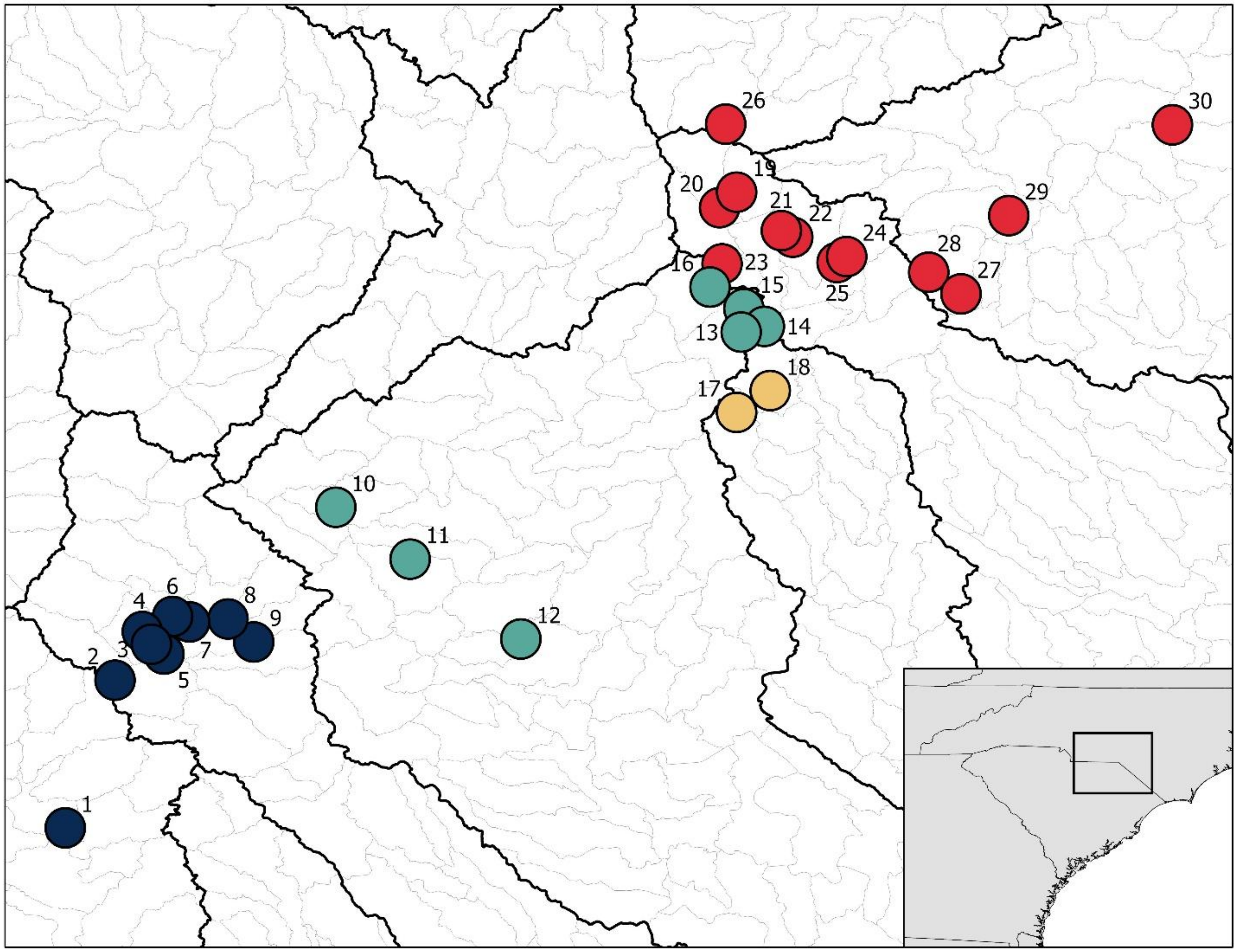

Figure 3. Map depicting collection localities (numbered 1 through 30, corresponding to population labels in Figure 2), with colors indicating the major population genetic grouping to which they belong as inferred from the range-wide population assignment inferred from *Structure* analyses.

Table 3. Sample locations in NC where Sandhills Chub were collected. RPFC indicates fin clips collected during this study, GHFC indicates fin clips archived from previous sampling, and total is the combined fin clip total. Streams with 30 or more fin clips are highlighted. Site with * is near another finished site.

Major Basin	HUC10 Subwatershed	Date	RPFC	GHFC	Total
Cape Fear	Headwaters Little River	6/16/2023	42	0	42
Cape Fear	Headwaters Little River	6/16/2023	4	0	4
Cape Fear	Middle Deep River	11/13/2023	31	0	31
Cape Fear	Outlet Little River	7/18/2022	2	0	2
Cape Fear	Outlet Little River	6/15/2023	4	0	4
Cape Fear	Outlet Little River	5/22/2023	32	0	32
Cape Fear	Rockfish Creek	7/21/2022	43	0	43
Cape Fear	Rockfish Creek	4/21/2023	4	0	4
Cape Fear	Rockfish Creek	01/14/2025	360	0	360
Cape Fear	Rockfish Creek	1/19/2023	5	0	5
Cape Fear	Rockfish Creek	4/20/2023	1	0	1
Cape Fear	Rockfish Creek	7/20/2022	8	0	8
Pee Dee	Hitchcock Creek	5/19/2023	31	0	31
Pee Dee	Hitchcock Creek	5/26/2023	1	0	1
Pee Dee	Hitchcock Creek	11/16/2023	31	0	31
Pee Dee	Hitchcock Creek	4/17/2024	38	0	38
Pee Dee	Hitchcock Creek	7/20/2023	35	0	35
Pee Dee	Lower Drowning Creek	01/20/2023	45	0	45
Pee Dee	Lower Drowning Creek	6/20/2022	4	0	4
Pee Dee	Lower Drowning Creek	6/19/2024	30	0	30
Pee Dee	Lower Drowning Creek	01/15/2025	425	0	425
Pee Dee	Lower Drowning Creek	10/9/2022	36	0	36
Pee Dee	Lower Drowning Creek	5/17/2024	30	0	30
Pee Dee	Upper Drowning Creek	5/26/2023	31	0	31
Pee Dee	Upper Drowning Creek	4/19/2023	1	0	1
Pee Dee	Upper Drowning Creek	5/18/2023	5	0	5
Pee Dee	Upper Drowning Creek	5/26/2023	32	0	32
Pee Dee	Upper Drowning Creek	7/21/2023	3	0	3
Pee Dee	Upper Drowning Creek	6/20/2024	30	0	30
Pee Dee	Upper Little Pee Dee	4/18/2024	35	0	35
Pee Dee	Upper Little Pee Dee	7/21/2023	38	0	38

Table 4. Sample locations in SC where Sandhills Chub were collected. RPFC indicates fin clips collected during this study, GHFC indicates fin clips archived from previous sampling, and total is the combined fin clip total. Streams with 30 or more fin clips are highlighted.

Major Basin	HUC10 Subwatershed	Date	RPFC	GHFC	TOTAL
Pee Dee	Little Lynches River	11/19/2020	0	8	8
Pee Dee	Little Lynches River	10/20/2020	0	1	1
Pee Dee	Little Lynches River	7/12/2023	24	23	47
Pee Dee	Little Lynches River	4/02/2024	17	17	34
Pee Dee	Little Lynches River	10/27/2020	0	7	7
Pee Dee	Little Lynches River	11/2/2020	0	2	2
Pee Dee	Little Lynches River	10/20/2020	0	1	1
Pee Dee	Little Lynches River	7/11/2023	12	19	31
Pee Dee	Little Lynches River	4/02/2024	40	0	40
Pee Dee	Reedys Branch-Great Pee Dee River	6/19/2023	29	3	32
Pee Dee	Thompson Creek	10/7/2020	0	3	3
Pee Dee	Thompson Creek	6/20/2023	21	11	32
Pee Dee	Thompson Creek	12/7/2023	1	0	1
Pee Dee	Thompson Creek	6/22/2023	30	6	36
Pee Dee	Thompson Creek	9/23/2020	0	1	1
Pee Dee	Upper Black Creek	10/19/2020	0	1	1
Pee Dee	Upper Black Creek	10/7/2020	0	6	6
Pee Dee	Upper Black Creek	11/10/2020	0	8	8
Pee Dee	Upper Lynches River	9/9/2020	0	5	5
Pee Dee	Upper Lynches River	10/4/2020	0	31	31
Pee Dee	Upper Lynches River	6/23/2023	47	9	56
Pee Dee	Upper Lynches River	7/11/2023	42	13	55
Pee Dee	Upper Lynches River	10/31/2020	0	59	59
Pee Dee	Upper Lynches River	10/2/2020	0	3	3
Santee	Middle Wateree River	7/13/2023	36	0	36
Santee	Middle Wateree River	10/14/2020	3	2	5
Santee	Upper Wateree River	7/14/2023	7	7	14
Santee	Upper Wateree River	10/18/2020	0	3	3

Table 5. Sample locations where Sandhills Chub were not collected during 2022 through 2024.

Major Basin	HUC10 Subwatershed	Stream	Date
Black	Scape Ore Swamp	Timber Creek on Timber Creek Rd	7/14/2023
Black	Scape Ore Swamp	Unnamed Tributary of Timber Creek	12/8/2023
Cape Fear	Headwaters Little River	Crane Creek	5/21/2023
Cape Fear	Headwaters Little River	James Creek off base	6/20/2022
Cape Fear	Headwaters Little River	Little River on Fort Bragg	6/21/2022
Cape Fear	Headwaters Little River	Reedy Branch	5/21/2023
Cape Fear	Headwaters Little River	Tributary of Little River	5/21/2023
Cape Fear	Headwaters Little River	Tuckahoe Creek at Bridge	6/22/2022
Cape Fear	Lower Deep River	Dry Fork	5/20/2023
Cape Fear	Lower Deep River	Raccoon Creek	5/20/2023
Cape Fear	Middle Deep River	Wet Creek on Bensalem Church Rd	7/20/2023
Cape Fear	Outlet Little River	Anderson Creek	5/19/2023
Cape Fear	Outlet Little River	Deep Creek	7/21/2022
Cape Fear	Outlet Little River	Hector Creek	6/15/2023
Cape Fear	Outlet Little River	Jumping Run Creek	5/19/2023
Cape Fear	Outlet Little River	Jumping Run Creek	6/15/2023
Cape Fear	Outlet Little River	Little River Tributary	5/21/2023
Cape Fear	Outlet Little River	Tributary of Little Bridge Branch	5/21/2023
Cape Fear	Outlet Little River	Tributary of Little River	5/19/2023
Cape Fear	Outlet Little River	Tributary of Little River	5/19/2023
Cape Fear	Rockfish Creek	Juniper Creek on Plank Rd	6/22/2022
Cape Fear	Rockfish Creek	Nicholson Creek	7/22/2022
Cape Fear	Upper Little River	Mire Branch	5/21/2023
Pee Dee	Hitchcock Creek	Hitchcock Creek on McNeil Rd	4/19/2024
Pee Dee	Lower Drowning Creek	Big Muddy Creek	4/18/2024
Pee Dee	Lower Drowning Creek	Deep Creek on Roseland Road	6/20/2022
Pee Dee	Lower Drowning Creek	Deep Creek on Roseland Road	7/21/2023
Pee Dee	Lower Drowning Creek	Mountain Creek off base	6/23/2022
Pee Dee	Lower Drowning Creek	Tributary of Aberdeen Creek in Park	4/19/2024
Pee Dee	Pee Dee-Blewett Falls Lake	Silver Creek	7/20/2023
Pee Dee	Reedys Branch-Great Pee Dee River	Phils Creek	7/13/2023
Pee Dee	Thompson Creek	Little Bear Creek	6/22/2023
Pee Dee	Thompson Creek	Tributary of Mount Prong Creek	6/22/2023
Pee Dee	Thompson Creek	Twitty Prong Creek	6/22/2023
Pee Dee	Upper Black Creek	Big Beaverdam Creek on Middendorf Rd	12/7/2023
Pee Dee	Upper Black Creek	Big Beaverdam Creek on Sander's Rd	12/7/2023
Pee Dee	Upper Black Creek	Little Black Creek	6/21/2023
Pee Dee	Upper Black Creek	Long Branch	6/21/2023
Pee Dee	Upper Black Creek	Unnamed Tributary of Little Black Creek	6/20/2023
Pee Dee	Upper Drowning Creek	Naked Creek	5/16/2024
Pee Dee	Upper Drowning Creek	Speeds Creek	6/20/2024
Pee Dee	Upper Drowning Creek	Unnamed Tributary of Pee Dee River	6/20/2024
Pee Dee	Upper Lynches River	Red Oak Camp Creek	6/23/2023
Santee	Lower Wateree River	Haig Creek	7/14/2023
Santee	Middle Wateree River	Big Pine Tree Creek	4/2/2024
Santee	Upper Wateree River	Grannies Quarter Creek	7/13/2023

Table 6. Genetic health metrics for populations of Sandhills Chub. Population numbers correspond to numbered localities on map in Figure 3. Linkage disequilibrium effective population size (LDN$_e$); Observed (H$_o$) and expected (H$_e$) heterozygosity; and Garza-Williamson index (G-W).

Major Basin	Population Number	LD N$_e$	H$_o$	H$_e$	G-W
Santee - Wateree	1	20.3 [12.9 - 35.7]	0.429	0.389	0.271
Pee Dee - Lynches	2	89.3 [55.1 - 205.3]	0.566	0.586	0.200
Pee Dee - Lynches	3	35.5 [28.0 - 47.0]	0.627	0.622	0.266
Pee Dee - Lynches	4	70.0 [46.2 - 132.1]	0.571	0.612	0.256
Pee Dee - Lynches	5	86.5 [55.9 - 173.7]	0.616	0.606	0.221
Pee Dee - Lynches	6, 8	42.4 [35.9 - 50.9]	0.556	0.586	0.220
Pee Dee - Lynches	7	106.9 [59.9 - 368.9]	0.546	0.585	0.229
Pee Dee - Lynches	9	29.3 [21.3 - 43.0]	0.499	0.474	0.286
Pee Dee – Lower	10	44.2 [12.7 - inf.]	0.241	0.245	0.295
Pee Dee – Lower	11	20.8 [12.7 - 39.5]	0.299	0.279	0.248
Pee Dee – Lower	12	10.8 [5.1 - 23.5]	0.198	0.216	0.254
Pee Dee – Lower	13	105.4 [46.7 - inf.]	0.450	0.438	0.223
Pee Dee – Lower	14	99.4 [46.2 - 8732.5]	0.482	0.459	0.245
Pee Dee – Lower	15	80.1 [46.1 - 230.4]	0.450	0.464	0.219
Pee Dee – Lower	16	-86.4 [-339.4 - inf.]	0.421	0.433	0.213
Pee Dee – Little	17	44.0 [25.1 - 114.0]	0.271	0.294	0.265
Pee Dee – Little	18	39.7 [25.8 - 72.9]	0.357	0.379	0.224
Pee Dee – Lumber	19	158.0 [60.1 - inf.]	0.455	0.451	0.240
Pee Dee – Lumber	20	13.8 [11.6 - 16.6]	0.508	0.503	0.256
Pee Dee – Lumber	21	23.8 [18.6 - 31.6]	0.509	0.488	0.238
Pee Dee – Lumber	22	266.8 [91.8 - inf.]	0.524	0.516	0.238
Pee Dee – Lumber	23	59.8 [39.5 - 110.9]	0.474	0.495	0.222
Pee Dee – Lumber	24	22.3 [16.6 - 31.3]	0.454	0.474	0.256
Pee Dee – Lumber	25	32.3 [20.5 - 60.9]	0.372	0.378	0.217
Cape Fear – Deep	26	47.7 [31.2 - 88.3]	0.461	0.459	0.270
Cape Fear - Upper	27	194.7 [60.5 - inf.]	0.370	0.393	0.212
Cape Fear - Upper	28	27.2 [19.0 - 42.8]	0.394	0.379	0.233
Cape Fear - Upper	29	37.0 [22.7 - 76.4]	0.364	0.373	0.207
Cape Fear - Upper	30	25.1 [13.3 - 66.8]	0.238	0.266	0.233

Objective 4 – *Evaluation of Genetic Relationships with Anthropogenic Barriers*

Although numerous studies have indicated negative effects of dams on riverine and migratory species, research on relationships between anthropogenic fragmentation of headwater streams and population genetics of fishes that live in these streams is limited. We used the newly-generated genetic dataset of 23 microsatellite loci from our study and Bayesian linear models to investigate relationships between anthropogenic dams and Sandhills Chub genetic differentiation, genetic diversity, and inbreeding. Genetic samples were collected from 887 Sandhills Chubs across 30 sites, spanning the entire geographic distribution of the species. Pairwise F_{ST} values ranged from 0.014 to 0.425 and were unrelated to the number of dams between sites. Instead, genetic differentiation was a function of whether sites were or were not within the same HUC-8 sub-watershed. Neither genetic diversity nor inbreeding coefficients were related to dams and site attributes (upstream drainage area and free-flowing stream reach). Although fragmentation can have deleterious genetic effects on populations through reduction of effective population sizes, gene flow, and genetic variation, barriers may have limited effects on genetics of sedentary species that evolved in isolated habitats, such as the Sandhills Chub. Full study details on methods, results, and conclusion are in the attached manuscript that has been submitted for publication in a peer-reviewed journal (Phelps et al.).

Objective 5 – *Determination of Hybridization*

Project personnel assisted with field work that included sampling at the northern and western edges of the Sandhills Chub distribution, where there is potential for sympatric Sandhills Chub and Creek Chub populations. Sandhills Chub and Creek Chub were collected together in three streams (Figure 4, Table 7). Tissue samples were collected in locations where the species occur together to determine if an intergrade zone occurs in this region.

Personnel screened known Creek Chub DNA samples with the newly developed 23 microsatellite panel to assess differences in patterns of amplification and allele ranges between Creek Chub and Sandhills Chub. Using PCR conditions that were optimized with Sandhills Chub, the Creek Chub samples did not amplify at seven loci (*Slu15, Slu30, Slu54, Slu74, Slu 124, Slu147,* and *Slu164*). Four loci (*Slu4*, *Slu40*, *Slu177*, and *Slu186*) amplified sufficiently for both species, and were found to have non-overlapping microsatellite allele distributions between Creek and Sandhills Chub specimens. We compared genotypes of Creek Chub individuals to those that were collected concurrently with Sandhills Chub (Table 7). Samples from each species produced genotypes that were consistent with amplification patterns and allele ranges observed for their respective species, suggesting a lack of hybridization. Therefore, genetic data do not show evidence of introgressive hybridization between Sandhills Chub and Creek Chub when they occur in sympatry.

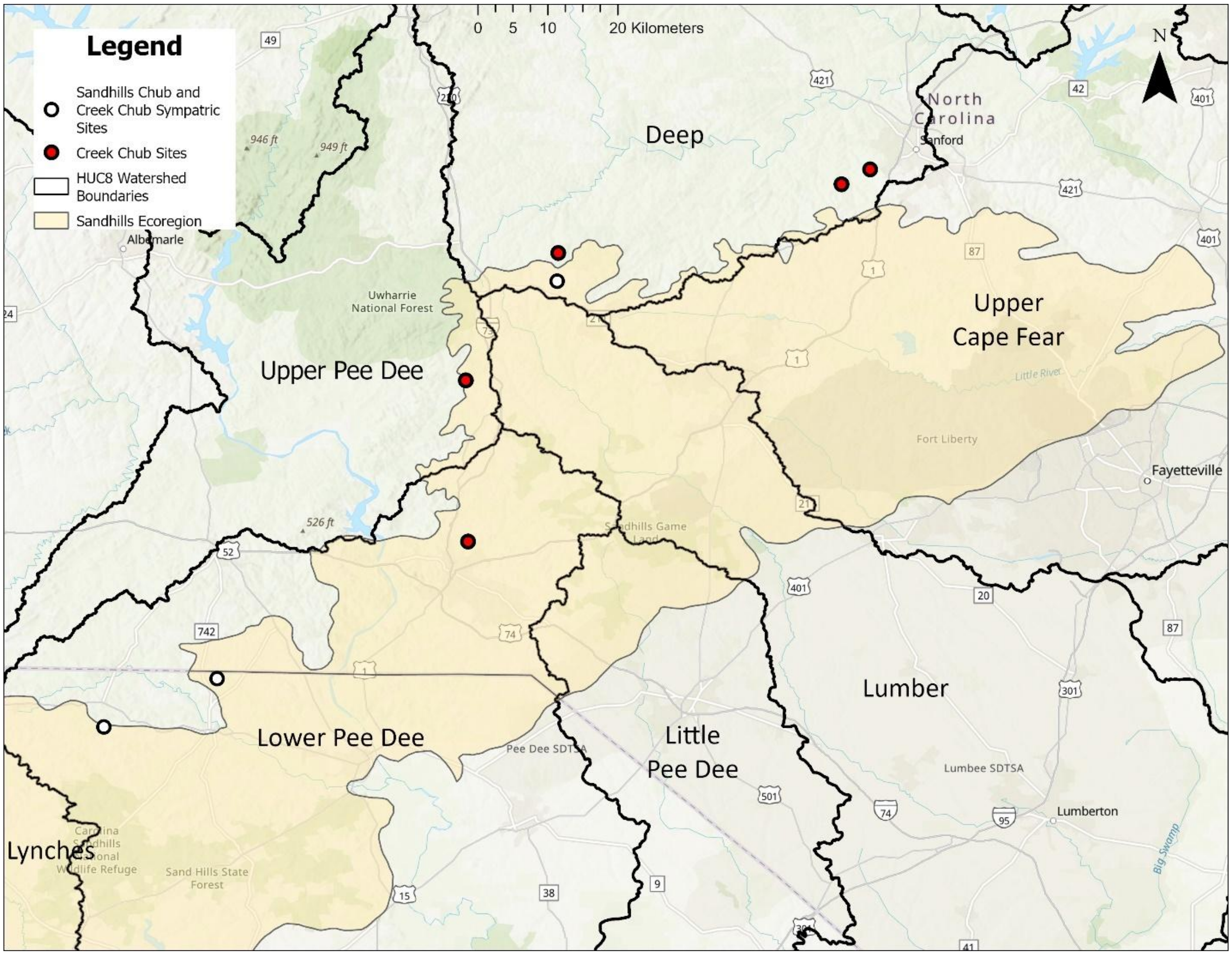

Figure 4. Locations where Creek Chubs were collected and Sandhills Chubs and Creek Chubs occur sympatrically.

Table 7. Locations where Creek Chubs and Sandhills Chubs were collected in sympatry during 2023-2024.

Major Basin	Stream	# Creek Chub	# Sandhills Chub
Pee Dee	Jennings Branch	7	21
Cape Fear	Wet Creek	7	31
Pee Dee	Jimmie's Creek	1	1

*Numerous Creek Chubs collected within 200 m downstream of where we began collecting Sandhills Chubs

Objective 6 – *Updating SC SWAP Species Description*

Following synthesis of the project results, project personnel drafted the updated 2025 SC SWAP species description (see attached) with new conservation and management recommendations. The SC Sandhills Chub distribution map was also updated and is available online.

Objective 7 – *Sandhills Chub Outreach*

We used social media, public presentations, mailing of educational material, and professional presentations to increase awareness of Sandhills Chub. Sixteen social media posts providing project updates and final results were posted to SCDNR (three Instagram, three Facebook, one Linkedin, and one *X*), NCWRC, (one Instagram, one Facebook, and one LinkedIn), NC Chapter of the American Fisheries Society (two Facebook), and North American Native Fishes Association (two Facebook) social media accounts. Graduate students Riley Phelps and Zach Ramsey organized a talk on Sandhills Chub, including tanks with local Sandhills species, at Cheraw State Park, SC in fall 2024. A second presentation was organized for Weymouth Woods Sandhills Nature Preserve, but had to be canceled due to state park closures associated with Hurricane Helene and state park staff being directed to recovery operations. Co-PI Crane gave presentations on the project at the Bimini Biological Field Station, Michigan State University, Sandhills Streamflow Stakeholders meeting, and for the NCWRC Science Communication Webinar Series (which is recorded and uploaded to YouTube). Graduate students Ramsey and Phelps presented their research at the annual meetings of the American Fisheries Society (two presentations), Southern Division of the American Fisheries Society (four presentations), SC Chapter of the American Fisheries Society (two presentations), and NC Chapter of the American Fisheries Society (two presentations). Phelps and Ramsey successfully defended their theses in May 2025 and manuscripts for publication in peer-reviewed journals have been submitted based on each of their theses.

Professional Presentations:
Phelps, R. W., D. P. Crane, T. Darden, R. Harrington, M. Scott, J. Hutchens, Z. Ramsey, B. Jones, and C. Bryan. 2025. Effects of anthropogenic stream barriers on Sandhills Chub (*Semotilus lumbee*) population genetics. 33rd annual meeting of the Southern Division of the American Fisheries Society. Asheville, NC.

Ramsey, Z. A., D. P. Crane, J. Hutchens, R. W. Phelps, B. Jones, T. Darden, M. Scott, and C. Bryan. 2025. Estimating population abundance and growth rates of Sandhills Chub *Semotilus lumbee* from two NC Sandhills headwater streams. 33rd annual meeting of the Southern Division of the American Fisheries Society. Asheville, NC.

Phelps, R. W., D. P. Crane, T. Darden, R. Harrington, M. Scott, J. Hutchens, Z. Ramsey, B. Jones, and C. Bryan. 2024 Effects of anthropogenic stream barriers on Sandhills Chub (*Semotilus lumbee*) population genetics: current progress. 154th Annual Meeting of the American Fisheries Society. Honolulu, HI.

Phelps, R. W., D. P. Crane, T. Darden, R. Harrington, M. Scott, J. Hutchens, Z. Ramsey, B. Jones, and C. Bryan. 2024. Effects of anthropogenic stream barriers on Sandhills Chub (*Semotilus lumbee*) population genetics: current progress. 32nd annual meeting of the Southern Division of the American Fisheries Society. Chattanooga, TN.

Phelps, R. W., D. P. Crane, T. Darden, R. Harrington, M. Scott, J. Hutchens, Z. Ramsey, B. Jones, and C. Bryan. 2024. Effects of anthropogenic stream barriers on Sandhills Chub (*Semotilus* *lumbee*) population genetics: current progress. Annual meeting of the South Carolina Chapter of the American Fisheries Society. Pickens, SC.

Ramsey, Z. A., D. P. Crane, J. Hutchens, R. W. Phelps, B. Jones, T. Darden, M. Scott, and C. Bryan. 2024. Movement of Endemic Sandhills Chub (*Semotilus lumbee*) in Headwater Streams of the NC Sandhills. 32nd annual meeting of the Southern Division of the American Fisheries Society. Chattanooga, TN.

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Ramsey, Z. A., D. P. Crane, J. Hutchens, R. W. Phelps, B. Jones, T. Darden, M. Scott, and C. Bryan. Movement of Endemic Sandhills Chub (*Semotilus lumbee*) in Headwater Streams of the NC Sandhills, 154th Annual Meeting of the American Fisheries Society, Honolulu, HI, United States. (2024).

R. W. Phelps, D. P. Crane, T. Darden, C. Bryan, B. Jones, M. Scott, and K. DeVilbiss. 2023. The effect of anthropogenic and natural stream barriers on Sandhills Chub population genetic structure. 34th annual meeting of the North Carolina Chapter of the American Fisheries Society. Durham, NC.

Z. A. Ramsey, D. P. Crane, J. Hutchens, R. W. Phelps, B. Jones, T. Darden, M. Scott, and C. Bryan. 2023. Movement characteristics of endemic Sandhills Chub (*Semotilus lumbee*). North Carolina annual American Fisheries Society meeting. Durham, NC.

Theses:
Phelps, R. 2025. Life history inhibits deleterious effects of dams on genetic health and structure of a headwater stream fish. Master's thesis. Coastal Carolina University, Conway, South Carolina.

Ramsey, Z. 2025. Investigating movement characteristics of stream fish in understudied headwater streams of the Sandhills ecoregion using endemic Sandhills Chub (*Semotilus lumbee*). Master's thesis. Coastal Carolina University, Conway, South Carolina.

Additional Information – *Pinewoods Darters*

Because the distribution of Sandhills Chub overlaps that of the more restricted distribution of Pinewoods Darter, we were able to collect data on Pinewoods Darter occurrences. Pinewoods Darters were collected at nine locations within the study area with collection and abundance data provided to NCWRC.

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Sandhills Chub
Semotilus lumbee

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DESCRIPTION

Taxonomy and Basic Description

The Sandhills Chub is a member of the minnow family (Leuciscidae) and the most recently described (Snelson and Suttkus 1978) of four species of the genus *Semotilus*, two of which occur in South Carolina (*S. lumbee* and *S. atromaculatus* Creek Chub) (Page et al. 2023). Minnows represent the most speciose family of freshwater fishes in North America (about 27% of the continental fauna), with at least 323 described taxa of predominantly small (<150 mm) and fusiform fishes (Warren and Burr 2014; Page et al. 2023).

Fish in the genus *Semotilus* are large minnows characterized by a robust body and large head. The Sandhills Chub typically ranges from 45−140 mm total length (Phelps 2025; Ramsey 2025) with a maximum reported total length of 240 mm (Rohde et al. 1994). The species appears to be reproductively mature by age-1, and males tend to be larger than mature females (Phelps 2025; Ramsey 2025), which may be a function of the need for males to have a large enough mouth gape to pick up gravel for building pit-ridge nests. There is a very small barbel in a groove above the upper lip near the corner of the mouth. The Sandhills Chub has relatively fine scales, a diffuse black lateral stripe, and a pinkish wash to the body in breeding season. This species can be distinguished from the similar Creek Chub (*S. atromaculatus*) by the absence of a dark spot in the dorsal fin near the anterior base in *S. lumbee* (present in *S. atromaculatus*) and a typical dorsal fin ray count of 9 (8 in *S. atromaculatus*).

Status

Globally, the Sandhills Chub is ranked as vulnerable to apparently secure (G3G4) (NatureServe 2024). It is considered imperiled (S2) in North Carolina and South Carolina, the only two states in which it occurs (NatureServe 2024). The Sandhills Chub was listed as vulnerable in fauna-wide assessments of the southeastern (Warren et al. 2000) and North American (Jelks et al. 2008) freshwater fishes and was selected as a Regional Species of Greatest Conservation Need in the Southeastern Association of Fish and Wildlife Agencies regional effort (Rice et al. 2019), receiving a designation of high concern.

POPULATION SIZE AND DISTRIBUTION

The Sandhills Chub occupies a narrow strip of the Sand Hills ecoregion (Griffith et al. 2002) of the inner coastal plain in south-central North Carolina and eastern South Carolina, from the Wateree (Santee) River basin eastward to the Cape Fear River basin (Snelson and Suttkus 1978; Rohde and Arndt 1991; Rohde et al. 2009; Tracy et al. 2020; Tracy et al. 2024). At the time of its description in 1978, the Sandhills Chub was only known from the Pee Dee (including Lynches) and Cape Fear River basins, and its presence in tributaries of the Wateree River (Santee basin) to the west was documented later (Rohde and Arndt 1991). In South Carolina, it is almost wholly restricted to the Sand Hills portions of the Wateree, Lynches, and Pee Dee River drainages. This species was observed in 1.4% of over 900 standardized statewide stream and river assessment samples across South Carolina between 2006 and 2023, but it can be relatively abundant in appropriate habitat within its narrow range (Rohde and Arndt 1991). Populations in the Lynches and Lumber (North Carolina) drainages are strongholds and exhibit higher genetic diversity than those in other drainages (Phelps 2025; Ramsey 2025). Abundance estimates in two representative populations were 218 per km in Aberdeen Creek (Pee Dee basin) and 137 per km in Gum Branch Creek (Cape Fear basin) (Ramsey 2025). Conversely, populations in the Wateree basin in the western portion of its range occur in comparatively low densities (SCDNR data; Rohde and Arndt 1991; Phelps 2025; Ramsey 2025).

HABITAT OR NATURAL COMMUNITY REQUIREMENTS

The Sandhills Chub is almost exclusively associated with small, flowing, often tannin-stained and acidic streams of the Sand Hills ecoregion (Snelson and Suttkus 1978; Rohde and Arndt 1991). Accordingly, in standardized sampling covering streams to small rivers of South Carolina, the Sandhills Chub exhibited its highest frequency of occurrence (14.3%) and average relative abundance (2.9%) in small streams (watershed area 0-15 km^2) of the Sand Hills. It is usually found in streams dominated by sand substrates with some gravel (Rohde and Arndt 1991; Rohde et al. 1994). This species is dependent on relatively cool, flowing, well-oxygenated headwaters with sufficient structure (i.e., wood); higher dissolved oxygen concentrations and greater amounts of cover in the stream section were both positively related to Sandhills Chub presence (Herigan et al. 2023). The Sandhills Chub shares its narrow Sand Hills-specific distribution with the Pinewoods Darter (*Etheostoma mariae*) and, in North Carolina where the latter is still known to occur, these species are often found in the same stream systems (Rohde and Arndt 1991).

Spawning primarily occurs in April and May, with the former month yielding the largest proportion of ripe individuals (Phelps 2025; Ramsey 2025). Males construct a pit-ridge nest characteristic of the genus (Woolcott and Maurakis 1988) using gravel in the 6 mm to 11 mm diameter range (Maurakis et al. 1990). Substrate composition is therefore important, and the amount of gravel in the 6-11 mm size range at the scale of the stream reach was a strong predictor of Sandhills Chub presence (Herigan et al. 2023).

Home range size in the Sandhills Chub is extremely small, with movement generally limited to relatively short stream reaches. In two North Carolina streams, 82% of almost 900 tagged individuals moved less than 200 m from their initial capture section, and only four individuals (<1%) moved distances greater than 1 km (Phelps 2025; Ramsey 2025). This limited movement has probably contributed to the high degree of genetic structuring observed across populations (Phelps 2025; Ramsey 2025).

CHALLENGES

A narrow range coupled with low mobility and specific habitat and life history requirements in headwater streams render the Sandhills Chub vulnerable to many anthropogenic threats. Its nearly exclusive association with small drainages places it at greater risk of extirpation from a given system due to acute or cumulative disturbances. The small headwater streams occupied by Sandhills Chub can be easily altered or fragmented, and this is compounded by the fact that headwater streams are afforded less regulatory protection than larger streams and rivers. Populations in small stream drainages flowing directly into considerably larger streams or rivers may be at heightened risk of extirpation (Sheldon 1987), since the larger downstream waterbodies could represent a physicochemical or behavioral barrier to emigration and subsequent recolonization, particularly given the low rates of movement exhibited by this species. The few known populations in tributaries of the Wateree (Santee) basin are inherently at higher risk of extirpation from this river basin due to their lower abundance and close proximity to areas of rapid human population growth such as the Columbia-Camden corridor.

Stable hydrology and associated physicochemical regimes are a defining feature of the Sand Hills ecoregion and serve as the template for the life history strategies of many regionally endemic species such as the Sandhills Chub. Even minor alterations to the natural hydrologic, thermal and sediment regimes, such as those typically associated with anthropogenic conversion of natural forested land cover to developed or impervious surfaces, have the potential to disrupt critical life history and ecological processes. Poor agricultural, silvicultural, and road construction practices often lead to stream siltation and non-point source water pollution (Waters 1995). For example, the reduction of natural forest and riparian cover in the watershed would be expected to increase water temperature (decrease dissolved oxygen), reduce wood loading for in-stream habitat and bank cover, and increase fine sediment inputs, all of which would decrease the probability of supporting Sandhills Chub (Marion 2008; Herigan et al. 2023). Unregulated use of motor vehicles in the stream bottoms also results in stream siltation and destruction of fish habitat.

Human-constructed impoundments cause additional impairment of Sandhills Chub habitat. Damming headwater streams to create ponds for golf courses eliminates important lotic habitats; the new lentic environments favor competing and often predatory species such as Largemouth Bass in unnaturally high densities. Impoundments also fragment habitat and can affect dispersal, prevent recolonization of streams after drought or other disturbances, and alter hydrology and sediment transport. Although impoundments are the most visible form of habitat fragmentation, culverts can also function as barriers if they are perched or if water velocity in a culvert prevents movement. The small streams occupied by Sandhills Chub are inherently more likely to be passed under roads by culvert rather than bridge, making full or partial barriers due to culverts a widespread factor affecting dispersal of this species.

CONSERVATION ACCOMPLISHMENTS

The Sandhills Chub has been the focus of two recent State Wildlife Grant investigations by Dr. Derek Crane and his graduate students at Coastal Carolina University since 2019 (Herigan et al. 2023; Phelps 2025; Ramsey 2025):

- 2019-2020: Identification of environmental and biological factors limiting occurrence of the Sandhills Chub (*Semotilus lumbee*) in South Carolina.

- 2022-2025: Conservation planning for a geographically restricted headwater species, the Sandhills Chub.

These investigations have produced important information on Sandhills Chub ecology and habitat requirements, allowing the development and refinement of conservation strategies for this species. Furthermore, this work yielded estimates of abundance, density and survival from two high-integrity populations that can serve as references for the evaluation of other populations.

South Carolina Stream Assessment data have facilitated the calculation of standardized abundance (density) estimates for this species at multiple spatial strata including statewide, river basin, level-IV ecoregion, and “ecobasin” (ecoregion x river basin). These estimates, for the first time, provide an objective measure of current population status that will serve as a baseline for following future population trends and gauging the effectiveness of conservation actions.

Following and building upon the dataset amassed from the South Carolina Stream Assessment study (2006-2011), additional standardized river and stream assessment efforts have enhanced the ability to assess the status of freshwater fishes across the majority of South Carolina’s freshwater habitats and understand the relationships between aquatic resource integrity and a rapidly changing landscape. The dataset now includes over 1,000 standardized samples spanning the past 20 years, providing the framework for modeling impacts to aquatic ecosystems and developing conservation tools aimed at preserving or restoring species status to former levels and mitigating future impacts on them. Aquatic resource conservation products and efforts completed or initiated since the previous SWAP include:

- The development and revision (C-SWG collaboration with North Carolina) of a web-based Aquatic Planning Tool (APT). This tool will allow practitioners to better manage SGCNs by providing predictive maps of the effects of proposed future development and restoration. The APT will have two primary functions, the first being an application to visualize current occurrence probabilities of SGCNs and their relationships with land use and climate across the Carolinas. The APT’s second function will be an interactive online tool allowing users to explore predicted impacts of future land management and climate scenarios on SGCNs at any stream segment in the Carolinas. This tool will be used by numerous and diverse conservation practitioners to guide future SGCN prioritization and surveys, restoration and reintroduction, and 'smart' city development to prevent the worst impacts of urbanization on freshwater fish SGCNs.
- Development of the first fish-based biotic index covering all freshwater stream regions in the state. The index provides a measure of stream biological (fish assemblage) integrity based on the relative abundance of region-specific and basin-specific indicator species. Current uses involve incorporating the index with fish vouchering and reporting into the

Office of Environmental Permitting's pipeline for wetland mitigation in an effort to assess success of mitigation and restoration projects.

- We conduct analysis of stream integrity based on the biotic index versus the watershed land use categories in an effort to determine thresholds of land-use-based disturbance and how fish communities respond.
- Provide collaborative input to the Office of Environmental Programs review of projects and permitting.
- Review fish collection permits for validity and ethics in regard to species and number of fish taken and the proposed purpose therein.
- We work closely with Aquatic Information Staff to promote awareness of SC aquatic resources and biodiversity.
- We collaborate with university researchers and graduate students to analyze relationships between aquatic species and environmental change.
- We produced eleven publications and manuscripts analyzing South Carolina stream and river resource response to environmental gradients and disturbances:

    - Marion, C.A., M.C. Scott, and K.M. Kubach. 2015. Multiscale environmental influences on fish assemblage structure of South Atlantic coastal plain streams. Transactions of the American Fisheries Society. 144(5):1040-1057.

    - Epstein, J.M., W.E. Pine III, C.M. Romagosa, M.C. Scott, C.T. Phillips, C.A. Marion, and B. Baiser. 2018. State- and Regional-Scale Patterns and Drivers of Freshwater Fish Functional Diversity in the Southeastern USA. Transactions of the American Fisheries Society. 147:1179-1198. https://doi.org/10.1002/tafs.10110

    - Peoples, B.K., E. Judson, T.L. Darden, D.J. Farrae, K. Kubach, J. Leitner, and M.C. Scott. 2021. Modeling distribution of endemic Bartram's Bass *Micropterus* sp. *cf. coosae*: Disturbance and proximity to invasion source increase hybridization with invasive Alabama Bass. North American Journal of Fisheries Management. 41:1309-1321. https://doi.org/10.1002/nafm.10637

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  - Mruzek, J.L., L.M. Bower, M.C. Scott, K. Kubach, and B.K. Peoples. *In review* 2024. Relative influence of flow regime, natural and anthropogenic environment on dimensions of local fish diversity. Hydrobiologia.

  - Cooper, C., K. Barrett, T. L. Darden, D. J. Farrae, K. Kubach, M. C. Scott, and B. K. Peoples. 2025. Competition or habitat: co-occurrence of endemic and cosmopolitan black bass species in a changing landscape. Ecology of Freshwater Fish 34(4):e70023. https://doi.org/10.1111/eff.70023

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  - Cooper, C., K. Barrett, L. M. Bower, T. L. Darden, D. J. Farrae, K. Kubach, M. C. Scott, and B. K. Peoples. 2025. Using multi-state occupancy models to quantify distribution and detection of endemic Bartram’s Bass and congeners. North American Journal of Fisheries Management 45(1):15-31 https://doi.org/10.1093/najfmt/vqae006

- Compilation of information and photographs for the development of nongame fish description web pages currently in development.

- Collaboration with SC Education Television in the filming and production of informative nature programs highlighting the diversity and status of South Carolina’s aquatic fauna

CONSERVATION RECOMMENDATIONS

- Use South Carolina Stream Assessment decision-support GIS modeling tools to identify levels and spatial distributions of critical habitat factors to sustain the species in geographic areas of interest.
- Use South Carolina Stream Assessment decision-support GIS modeling tools to identify priority regions and watersheds at greatest risk of decline in stream integrity.
- Describe additional life history and habitat requirements for the Sandhills Chub.
- Resample known locations to determine the population status of the Sandhills Chub and expand monitoring efforts within the Carolina Sandhills National Wildlife Refuge (NWR) in order to quantify the population within the refuge.
- Protect critical habitats for the Sandhills Chub from future development and further habitat degradation by following Best Management Practices(BMPs) and protecting and purchasing riparian areas.
- Promote land stewardship practices through educational programs both within critical habitats with healthy populations and in other areas that contain available habitat for the Sandhills Chub.
- Encourage responsible land use planning.
- Consider species needs when participating in the environmental permit review process.
- Continue to develop educational materials in order to raise public awareness of nongame fish species and their ecological importance to the natural history of South Carolina’s aquatic habitats.
- Educate off-road motor vehicle operators of the negative effects of crossing streams at multiple locations and using stream bottoms as trails.

MEASURES OF SUCCESS

Successful conservation of South Carolina's native aquatic species is best accomplished proactively by maintaining natural land cover, which supports aquatic habitat integrity and dependent species assemblages. For example, achieving the foremost goal of the Southeastern Aquatic Resource Partnership’s 2008 Southeast Aquatic Habitat Plan that 85% of lands within 30 m (100 ft.) of streams or rivers be maintained in natural vegetation would be a significant measure of success. Maintenance or recovery of natural land cover levels above the critical thresholds identified quantitatively using aquatic conservation planning tool modeling applications represents a foremost, highly-defensible measure of success in supporting species of greatest conservation need. At the species level, an indicator of effective conservation implementation could include a stable or increasing distribution and/or abundance relative to current levels as measured by statewide assessment data.

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How you can help:

- Protect and restore streamside native forests
- Pick up trash in streams
- Avoid installing culverts
- Don't move fish between streams
- Limit the use of pesticides or fertilizers near streams
- Every stream matters

Research Partners

SOUTH CAROLINA DEPARTMENT OF NATURAL RESOURCES

NORTH CAROLINA Wildlife RESOURCES COMMISSION

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Your land is near a stream that is inhabited by Sandhills Chub, a unique but often unknown fish!

Flip through to learn more!

What is a Sandhills Chub?

It is a species of minnow found **only** in small streams within the Sandhills ecoregion of North and South Carolina.

They are closely related to the widely distributed Creek Chub and may be easily misidentified. Creek Chub have a dark spot at the front of their dorsal fin (in the middle of their back), whereas Sandhills Chub don't have this spot.

Sandhills Chub vs Creek Chub

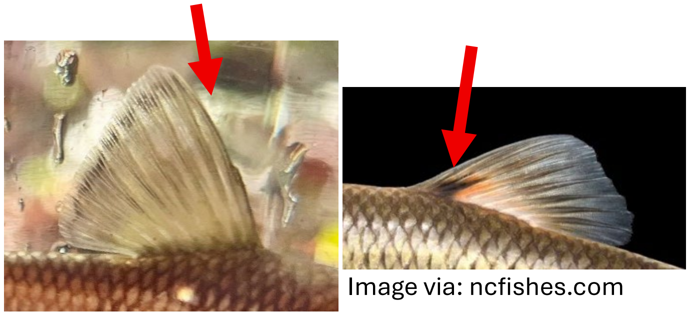

Image via: ncfishes.com

Ideal Habitat

Sandhills Chubs prefer small, clean, headwater streams with sand and gravel bottoms, and areas to hide such as woody debris or undercut banks.

Findings from our Research:

- Sandhills Chubs are sedentary, meaning they tend to stay within the same general area within a stream.
- Sandhills Chubs populations from different streams are genetically unique, even when the streams are close together.
- Several streams in North and South Carolina support healthy populations of Sandhills Chubs, but populations were likely lost or have declined in other streams.

Freshwater Biology

Original Article

Using movement ecology to guide conservation of a headwater stream specialist

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Additional Information

Research Topics

Data on vertebrate animals. Ethical review committee and relevant licence no.(s) in Acknowledgements

Files for peer review

All files submitted by the author for peer review are listed below. Files that could not be converted to PDF are indicated; reviewers are able to access them online.

Name	Type of File	Size	Page
ZacharyRamsey_Manuscript_09-09-25.docx	Main Document - MS Word	1000.0 KB	Page 4

Using movement ecology to guide conservation of a headwater stream specialist

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Keywords: diffusive spread, leptokurtosis, restricted movement paradigm, stream fish, Sandhills

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Ethics Statement

All work was done under collection permits through the NC Wildlife Resources Commission and procedures were approved by Coastal Carolina IACUC (protocol number 2021.03).

Conflicts of Interest

None.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abstract

1. Fish movement studies have traditionally focused on species that support commercial and recreational fisheries, but recently more focus has been given to non-game, stream fishes because of their recognized importance in maintaining biodiversity within these ecosystems. Stream fish movement is often “restricted” and follows a leptokurtic distribution via diffusive spread related to environmental and biological factors. Many studies of stream fish movement ecology in North America have occurred in high-gradient streams with coarse substrate in mountains or in streams of the interior plains. Streams in the Carolina Sandhills ecoregion of the southeastern USA are characterized as warm-water, low gradient, and dominated by sand with unique fish assemblages. Therefore, results from previous studies may not apply to Sandhills fishes. Our objectives were to use the Sandhills chub (*Semotilus lumbee*), a species of conservation concern that is endemic to the Carolina Sandhills ecoregion, to test hypotheses related to diffusive spread, the restricted movement paradigm, and environmental and biological drivers of movement in these understudied headwater streams.
2. From October 2022 through October 2024, Sandhills chub movements were measured using capture-recapture methods in 1,400-m reaches of two North Carolina, USA streams. We described net movement, total absolute movement, seasonal movement, and spawning movement by calculating kurtosis, skewness, median distance moved, and used a χ^2 to test for directional movement. We tested for diffusive spread using linear regressions between distance moved and time at large, and investigated if movement was related to environmental and biological factors using generalized linear mixed models.
3. Median distance moved was 0 m and 87% of fish moved ≤200 m. The limited movement of Sandhills chub suggested that they do not follow patterns of diffusive spread. Seasonal

and overall movement distributions were leptokurtic, but movement was not related to the environmental or biological factors we investigated. Movement distributions during the spawning season followed a similar pattern and were highly leptokurtic, with a median distance moved of 0 m. Additionally, there was no evidence of directional movement related to spawning or during spring, summer, or winter.

4. An ecological explanation of why small stream leucisids commonly display limited movement is not well developed, but several plausible hypotheses may explain our observations. First, like many small stream fishes in North America, the Sandhills chub co-evolved with beavers, which naturally fragmented headwater streams in this region, possibly restricting movement. Second, much of the habitat at our study locations was homogeneous and contained ample cover. Therefore, movement away from this habitat may increase the risk of predation. Finally, downstream movement may result in increased interspecific competition and risk of predation as additional and larger-bodied fishes become part of the assemblage in larger streams.
5. Our study provides insight into movement ecology of a headwater stream fish in an understudied habitat. As restricted movements are pervasive in headwater stream leucisids across a variety of stream types, future research to improve our mechanistic understanding of movement patterns will benefit conservation and restoration actions in these increasingly anthropogenically fragmented and degraded habitats.

1 | Introduction

Movement is a universal behavior of vertebrates to find food and mates, maintain individual- and population-level fitness, and escape adverse environmental conditions (Fryxell, Greever, & Sinclair, 1988; Liedvogel et al., 2013). Animal movement is often described as being migratory or nonmigratory. Migratory movement is the round-trip, seasonal movement of organisms from one location to another, which is uninterrupted by intervening resources, for the purposes of reproduction, feeding, or fulfilling specific life cycles (Dingle, 1996; Shaw & Couzin, 2013; Shaw, 2016). Nonmigratory movements occur in patterns that generally optimize an organism's abilities to forage, limit competition, and decrease predation (Heiler et al., 2008; Owen-Smith, Fryxell, & Merrill, 2010; Higdon et al. 2019). When resource distribution can be described by local patchiness, organisms commonly exhibit nonmigratory movement or residency (Shaw, 2016).

Fish movement and dispersal affect gene flow, community composition, and nutrient transfer within aquatic communities (Jackson, Peres-Neto, & Olden, 2001; Comte & Olden, 2018; Cooke et al., 2022). When changes occur within a fish's environment, one of their main responses is to move, especially when these changes have potentially negative effects (Baras & Lucas, 2001). Movement of fishes has mostly been categorized by function, habitat, temporal scale, and variation among individuals in a population (Brönmark et al., 2013). Movements for some species such as Pacific (*Oncorhynchus* spp.) and Atlantic (*Salmo salar*) salmons have been known for thousands of years because their predictable migrations allowed humans to use them as an important food source (Brönmark et al., 2013). However, it was not until 1956 when telemetry technologies, the use of tags and sensors to track the movement and behavior of organisms, were first used in fisheries research to track the passage of adult salmon at the Bonneville Dam on the Columbia River in Oregon (Hockersmith & Beeman, 2012).

Miniature radio transmitters, passive integrated transponder (PIT) tags, and visible implant elastomer tags, coupled with increased emphasis on conservation of non-game species, have allowed for investigating movement of small-bodied stream fishes (Cucherousset et al., 2005; Ficke & Myrick, 2009; Wells et al., 2017; McBaine, Hallerman, & Angermeier, 2022). For example, dispersal of banded sculpins (*Cottus carolinae*) was quantified in a small Tennessee stream using PIT tags in a capture-recapture study (Wells et al., 2017) and a portable PIT antenna was used to track movements of creek chub (*Semotilus atromaculatus*) and mottled sculpin (*Cottus bairdi*) in a small Piedmont stream in South Carolina, USA (Kelly et al., 2017). Early capture-recapture studies found that resident stream fishes within warmwater Indiana, USA, streams showed extremely restricted movements and often remained in the same pool or reach, resulting in the idea that stream fishes have a small "home range" for the duration of their adult lives (Gerking, 1953 & 1959).

Limited home ranges or the "restricted movement paradigm" (RMP) was largely accepted as the norm for stream fish movements, until Gowan et al. (1994) rejected the RMP for stream fishes because of bias associated with small study areas. Additionally, it was suggested that the small study areas may explain low recapture rates if fish were not recaptured (the majority) because they made longer movements outside of the sampling area (leading to underestimation of movement). Based on a review of resident stream salmonid movement and high immigration rates in their own study streams, Gowan et al. (1994) suggested movements for stream fishes are not restricted. However, differences in study reach length, duration of study, and time between recaptures (see Table 2 in Gowan et al. 1994) curtailed quantifying movement distributions across studies. Along with possible methodological issues, resident stream salmonids were found to be highly mobile (Gowan et al., 1994). Based on a meta-analysis of stream salmonid movements, Rodriguez (2002) concluded that the RMP is incomplete because it does not account for the small proportion of highly mobile individuals, which may be important for population processes. In freshwater fishes with few individuals moving among populations, gene flow may still be high enough to allow for the rapid spread of advantageous mutations but still too low to prevent differentiation at some loci through drift or local adaptations (Morjan & Rieseberg, 2004).

As knowledge of stream fish movements has increased, these movements can often be described by a leptokurtic distribution (Skalski & Gilliam, 2000; Petty & Grossman, 2004; Hicks & Servos, 2017). A leptokurtic distribution is defined as having a kurtosis value greater than three, with a higher, thinner peak centered around the mean and larger, heavier tails than a normal distribution (DeCarlo, 1997). Leptokurtosis is observed in stream fish movement distributions because the population consists of a large portion of sedentary individuals that result in a high, thin peak centered around zero horizontal distance movement, and a small number of highly mobile individuals that create heavier tails at the outer bounds of the distribution (Fraser et al., 2001; Radinger & Wolter, 2014). Leptokurtosis likely occurs because movement distributions are indicative of populations that are heterogeneous in their movement behavior (Skalski & Gilliam, 2000; Rodriguez, 2002; Radinger & Wolter, 2014) or because movement among fishes in small streams occurs via diffusive spread (Skalski & Gilliam, 2003). Heterogeneity in movements among individuals may be explained by intraspecific variability in phenotypic and personality traits (Cote et al., 2010; Fraser et al., 2001), differences in how individuals respond to changes in their environments such as temperature and flow conditions (Rasmussen & Belk, 2017), and biological factors such as competition, food availability, and reproductive state (Bonte et al., 2012; Bowler & Benton, 2005). When diffusive spread occurs, distances moved increase with time and the distribution of movements spreads out over a wider range of distances with time. This can lead to a leptokurtic distribution because as time increases, more mobile individuals that are at large for longer will have moved further, resulting in heavier tails and the high, thin peak in the distribution remains due to a large portion of the population remaining sedentary. Although leptokurtic distributions in movement are often attributed to diffusive spread, there has been limited investigation of its role in small stream fish populations (but see Skalski & Gilliam, 2000).

Most studies quantifying movements of small-stream fishes have focused on mountain streams (Young, 1996; Schmetterling & Adams, 2004; Hodges & Magoulick, 2011) and prairie or plains streams (Pennock et al., 2018; Ruppel et al., 2020). Additionally, there has been particular focus on widely distributed species such as salmonids (family Salmonidae), darters (subfamily Etheostominae), sunfishes (family Centrarchidae), creek chub (*Semotilus atromaculatus*), and bluehead chub (*Nocomis leptocephalus*). Our study focused on the Sandhills chub (*Semotilus lumbee*), an endemic headwater stream leuciscid that is only found within the Sandhills ecoregion of the Cape Fear, Pee Dee, and Santee River basins (Figure 1) of North and South Carolina (Rohde et al., 2009). They are a robust minnow and adults range in total length from 80 to 240 mm. Sandhills chub are one of two *Semotilus* species found in the region, the other being their sister species, the more common and widely distributed creek chub. Sandhills chub are habitat specialists that live in small streams (<3 m wide) with cool, clean, low pH water, and a substrate made of sand and fine gravel or pebble that males use to create pit ridge nests for reproduction (Rohde & Arndt, 1991; Rohde et al., 1994; Herigan et al. 2022). The Sandhills chub is listed as a species of special concern in both North and South Carolina (imperiled [S2] and ranked as vulnerable across its entire range [G3/G4]) because of its limited distribution and threats from habitat alteration (NatureServe, 2023). Although it is a species of conservation concern, it can be the dominant species in the headwater streams it is found in. Thus the combination of need for information on ecology of the species for conservation planning, and ability for it to be locally abundant make it a good candidate species for studying headwater stream movement ecology.

The Carolina Sandhills ecoregion is a narrow geographic area of the upper coastal plain ranging from Georgia to North Carolina, USA. Its streams differ from mountain, piedmont, and interior plains streams that have been the major focus of headwater stream fish movement in North America. Sandhills streams are generally warm, low gradient, and sandy-bottomed with low macroinvertebrate species richness (Feminella, 2000; Sefick et al. 2018) and instream cover provided by woody debris, aquatic vegetation, and undercut banks (Paller, Reichert, & Dean, 1996). Baseflow in headwater sections of Sandhills streams is typically high compared to other headwater stream systems because of groundwater flow through porous sediments in the surrounding landscape. Porous sediments and leaching of organic matter in Sandhills wetlands in this region result in streams with low conductivity (10–35 μS/cm) and pH (4.5–6.5). Low specific conductance and pH are negatively associated with productivity in aquatic ecosystems (Krueger & Waters, 1983), and intermediate (5.7–6.4) and low (4.0–4.4) pH streams have lower secondary productivity because of the absence of low-pH intolerant macroinvertebrate species, as well as the potential nonlethal effects that low pH can have on macroinvertebrate metabolism (Griffith, Perry, & Perry, 1994).

Given the characteristics of sandhill streams of the southeastern U.S. coastal plain, and the paucity of information on movement ecology of headwater species on the coastal plain, these streams are excellent systems to test the applicability of hypotheses related to stream fish movement ecology. We conducted a 2-year capture-recapture study of Sandhills chubs with the goal of increasing our understanding of stream fish movement and the ecology of an endemic species in an understudied ecoregion. Our objectives were to (i) test for diffusive spread in a small-bodied, small stream Sandhills species, (ii) quantify and describe Sandhills chub movements on a continuum of movement distributions and compare movement distributions to similar species, (iii) test if movement was related to biological and environmental factors such as fish length, season, stream, water temperature and flow, and (iv) determine if significant spawning period movements occurred. We hypothesized that: (i) distance moved would increase with time because of diffusive spread (Skalski & Gilliam, 2003), (ii) Sandhills chub movements would be highly leptokurtic, as has been observed for other small-bodied, headwater stream fishes (Skalski & Gilliam, 2000; Walker & Adams, 2016), but would not exhibit biases in movement direction, (iii) Sandhills chub movement would be related to water flow and total length of individual fish (Terui et al., 2021), with greater dispersal observed after high flows and for larger fish, and (iv) movement distributions would have an upstream bias during the spawning period to account for any downstream drift that may occur in early life stages (Steffensmeier et al., 2022).

2 | Methods

2.1 | Study Sites

Movement of Sandhills chub was tracked for two years from October 2022–October 2024 in Gum Branch Creek, North Carolina, USA, and an unnamed tributary of Aberdeen Creek, North Carolina, USA. These streams were selected because pilot surveys indicated Sandhills chub were abundant in both streams, both streams had relatively low levels of anthropogenic disturbance compared to many streams in the region, and the streams contained a long enough reach of accessible, continuous habitat to quantify movement. Therefore, movement in these streams should be reflective of movement observed in streams with high quality habitat and healthy Sandhills chub populations. Gum Branch Creek and Aberdeen Creek are first-order streams with sand substrates and small patches of fine gravel. These streams are ~1−3 m wide, ~0.5 m deep, and contain fallen trees, riparian vegetation roots, and undercut banks which provide cover for Sandhills chubs throughout the study reaches. The unnamed tributary of Aberdeen Creek is within the Lumber River basin in Pinebluff, Moore County, North Carolina, USA (Figure 2). Gum Branch Creek is a tributary of Rockfish Creek, within the Cape Fear River basin (Figure 2) and is in the southwestern portion of U.S. Army Base Fort Liberty in Hoke County, North Carolina, USA. For the first year of the study (October 2022–October 2023), we established a 900 m study reach to balance the feasibility of sampling and marking several hundred fish and ensuring the distance was long enough to adequately quantify movement based on previous studies of small stream leuciscids (e.g., Skalski and Gilliam 2000; Belica and Rahel 2007; Terui et al. 2021; Curtis et al. 2023). Each stream contained a barrier to movement upstream of our reaches; a large beaver (*Castor canadensis*) dam on Gum Branch Creek and a wetland with no defined streambed that was prone to intermittent drying in the unnamed tributary of Aberdeen Creek. Each 900-m reach was divided into thirty-six, 25-m sections. Each section was flagged and numbered in the downstream-to-upstream direction. Because a major critique of the RMP is that capture-recapture studies are not sensitive to fish movements outside of the study reach (Gowan et al., 1994), we expanded the reaches downstream by 500 m starting in October 2023 (new total reach length = 1,400 m, with 56, 25-m capture sections), 12 months after the initial sampling and tagging (see data analysis below for discussion of changing study reach lengths).

2.2 | Fish Sampling

Sampling occurred over three days at or near the midpoint of each meteorological season (January, April, July, and October) and monthly in May and June so environmental conditions were likely representative for that season. Therefore, movement between sampling periods should have been indicative of any seasonal patterns in movement. Sandhills chub were collected via single-pass backpack electrofishing (Edwards et al., 2003) using an ETS Electrofishing

Systems ABP-4 backpack electrofisher and dipnets. Electrofishing was conducted by a two-person team in the upstream direction, and at the end of each section any Sandhills chub that were captured were tagged (see below), measured (total length [mm]), and assigned a number corresponding to the section of capture. Block nets were deemed unnecessary after it was determined that deployment of such nets could cause potential biases by disturbing upstream reaches when putting the nets in place, and fish were commonly observed moving to cover within the sample reach rather than being pushed upstream during a pilot study.

Individual Sandhills chub ≥40 mm were implanted with 8 mm x 1.4 mm “skinny” PIT tags (Oregon RFID, Portland, OR) using similar methods to Cary et al. (2017). Incisions ~1 mm long were made lateral to the ventral line and anterior to the pectoral girdle using a #11 scalpel. Tags were inserted and massaged into the body cavity of the fish (Cary et al., 2017; Schumann et al., 2020). In a large study focused on safety and efficacy of PIT-tagging small-bodied stream fishes, including creek chubs, Cary et al. (2017) documented high retention and survival rates, and concluded that fish ≥40 mm TL could be effectively PIT-tagged. Blades were sterilized between each use with povidone-iodine solution. Incisions were not sutured and instead were left to heal on their own because longer handling time and potential injuries from sutures increases mortality associated with PIT tagging small-bodied stream fishes (Swarr, Myrick, & Fitzpatrick, 2022). Once the tag was inserted, Sandhills chub were left to rest in a bucket of aerated water from the stream until normal swimming activity resumed. Tagged fish were returned to the stream at the midpoint of the capture section. During recapture sampling, section number and total length were recorded for all previously tagged Sandhills chub and untagged fish were tagged as described above.

2.3 | Environmental Data

Water temperature (°C), conductivity (µS/cm), pH, and dissolved oxygen (mg/L) were measured and logged using a YSI multimeter each day of sampling. Water temperature and barometric pressure were recorded every hour by ONSET HOBO U20L water level loggers (Bourne, MA) that were placed in the nearest pool upstream and downstream of the sampling reach in October 2022. Only the downstream logger in Gum Branch Creek was able to be recovered because the upstream logger was covered by a beaver dam that was built during spring 2024. Barometric pressure was used to calculate water level using the Barometric Pressure Assistant in the HOBOware Pro® software system. In both study streams, water levels were not recorded during May 16−July 19, 2023, because the water level loggers reached storage capacity. The missing values were estimated using linear regression of the relationship between daily water level at each site and daily water level from a nearby United States Geological Survey water gauge in Rockfish Creek, Raeford, NC (USGS site #02104220; data retrieved from: https://waterdata.usgs.gov/monitoring-location/02104220/#dataTypeId=continuous-00065-0&period=P7D&showMedian=false).

2.4 | Movement Measurements

Movements were quantified in five ways: (1) movement between seasonal sampling periods, (2) absolute movement between seasonal sampling periods, (3) net distance moved during the entire study, (4) total absolute distance moved during the entire study, and (5) spawning period movements. We estimated the seasonal distance moved for each recaptured fish as the total distance moved by a fish between meteorological seasons. Movement was measured in meters, as the distance from the central point of recapture to the central point of initial capture. Negative values were assigned to downstream movements, and positive values were assigned to upstream movements (De Fries et al., 2022). Seasonal movement was only calculated between consecutive seasons. We calculated absolute distance moved between seasonal sampling periods because positive and negative distances can offset each other when modeling values based on the average. Net distance moved was also calculated for each fish that was recaptured at least once during seasonal sampling as the sum of all seasonal distances moved accounting for negative and positive movements for each recaptured Sandhills chub. For example, if a fish that was tagged in October was recaptured in January and moved -50 m, then recaptured again in April and moved 100 m, its net distance moved was 50 m. Total absolute distance moved was also calculated for all fish recaptured at least once during seasonal sampling, where movement direction was not considered, summing the total distance moved. Therefore, in our previous example, the fish would have an absolute distance moved of 150 m. Spawning period movements were calculated as the distance moved during the spawning period (April−May), including movement measurements from January to April, accounting for negative (downstream) and positive (upstream) movement.

All capture-recapture movement studies have inherent biases because there are logistical and time constraints that limit the length of study reaches, which need to be balanced with capturing enough fish to test hypotheses. For example, because search areas are bound by barriers to movement or bounds are selected as part of the study design, the location where a fish is captured affects the maximum potential distance a fish may move within the study area. For example, in our study, a fish tagged in section 55 (near the upstream boundary) could move a maximum distance of 1,350 m downstream, whereas a fish tagged in section 28 could move a maximum of 700 m upstream. Some studies have overcome these challenges by using an initial mark site coupled with a recapture site where no fish are tagged that encompasses the initial mark site along with a specified distance upstream and downstream of the mark site (McBaine, Hallerman, & Angermeier, 2022). However, we could not use this method because Sandhills chub do not occur in high enough densities within small search areas, forcing us to use larger marking areas to attain a sufficient sample size. Additionally, having a centralized tagging location only alleviates this potential bias if tagging only occurs once and there is only one capture period. Once fish disperse from the central tagging area estimates of distances moved from multiple recaptures are subject to the same constraints as when fish are tagged throughout an entire study area. To test the assumption that the section in which a fish was captured did not influence its distance moved, we plotted and ran a linear regression between the maximum distance a fish could move (representing the capture section) and the actual number of sections a fish moved in its subsequent recapture (Figure 3). We found no relationship between where a fish was captured and distance moved ($R^2 = 0.0001$, $P = 0.765$). Although there was no relationship, the variance increased as the maximum potential movements increased. Because most Sandhills chub moved very little regardless of the section they were initially tagged in the increase in variance would not change our study conclusions.

2.5 | Data Analysis

All analyses were completed using capture-recapture data from the 1,400-m study reach in both study streams (Table 1). We chose to use data from the 1,400-m study reaches because adding movements from fish captured or recaptured in the 500-m reach added during year 2 did not appreciably change the distance of movements or study conclusions (Table 2). Additionally, it allowed us to detect longer movements that would have been missed if the data were censored to the initial 900-m study reach. We used all Sandhills chub movements, regardless of when they were tagged and recaptured, to test for diffusive spread. To quantify and describe the distribution of movements of Sandhills chub and test if movement was related to biological and environmental factors, we used movement data from Sandhills chub that were tagged and recaptured during seasonal sampling only (October, January, April, and July) because we quantified the movements in the scope of seasonal movement. We examined spawning period movement using only movements from Sandhills chub that were tagged and recaptured from January through May as spawning was documented to peak in April–May based on sampling in April through July, observations of nests, expression of gametes, and presence of breeding tubercles in our study.

We used a linear regression (‘lm’ function in the base R package) to examine the relationship between time at large (the number of days between a fish’s initial capture and its final recapture) and distance moved to test for diffusive spread in Sandhills chub movement. We also used linear regression to investigate the relationship between the maximum distance a recaptured fish moved and the amount of time it took for each fish to travel that distance after being tagged (i.e., time at large). If diffusive spread occurred, we expected the variance in distance moved to increase with time (Skalski & Gilliam, 2000), as well as a significant positive relationship between distance moved and time at large.

Histograms of net movement, total absolute movement, seasonal movement (positive and negative values), and absolute seasonal distance moved were created to test our hypothesis that Sandhills chub movements were highly leptokurtic but unbiased in movement direction. We pooled movement data across streams because movement did not statistically differ between our study steams (see Results). Kurtosis was calculated [‘kurtosis’ from the moments package in R (Komsta & Novomestky, 2022)] to determine the distribution (platykurtic, leptokurtic, or mesokurtic) of seasonal and net movements. Skewness of these distributions was calculated (‘skewness’ from the moments package in R) to determine if Sandhills chub exhibit biases in movement direction, with a positive skew indicating upstream movement and a negative skew indicating downstream movements. In addition to calculating skewness, a χ^2 test was also used to determine if fish exhibited biases in movement direction (Mitsuo et al., 2013).

We used generalized linear mixed models [GLMMs; ‘lmer’ function from the lme4 package of R (Bates et al., 2015)] to determine if Sandhills chub movement was related to season, study stream, fish total length, or water level. GLMMs were created for both seasonal movement and absolute seasonal movement. In this analysis, season, stream, total length (TL), and number of high-water days (HWdays) between sampling dates (water levels >75th percentile of daily water levels) were included as fixed effects and tag number (fishID) as the random effect. We examined all possible combinations of predictors including additive effects and two-way interactions, as well as a null model with only a random effect for fishID. We used Bayesian information criterion (BIC), delta BIC (ΔBIC), and Schwarz weights (BIC weights (w_i)) to select the most likely models among the suite of models examined (Aho, Derryberry, & Peterson, 2014), with Schwarz weights being the probability that a specific model is the most likely of the models being compared.

To test our hypothesis that spawning period movement distributions were different than other seasonal movement distributions and skewed towards upstream movement, we examined movement distributions of spawning movements using histograms and compared those to histograms of seasonal movement patterns. For this analysis, we did not pool movements across streams because of potential differences in the spatial distribution and amount of spawning habitat between them. Because winter to spring movements can be potential spawning movements and Sandhills chub spawning peaked in April–May, we created spawning period histograms of movement from January to May. We pooled movements from January to May across both years (2023 and 2024). After creating movement histograms, we calculated the kurtosis, skewness, and median of the distributions. As in our investigation of seasonal movements, we also ran a χ^2 test to determine if there were any differences in movement direction.

3 | Results

A total of 898 Sandhills chub were tagged between the two study streams (Table 1). Across all sampling periods, 364 Sandhills chub were recaptured at least once (41% recapture rate) and there was a total of 654 recapture events. Most fish were only recaptured once, but four Sandhills chub were recaptured seven times and one recaptured eight times. The tributary of Aberdeen Creek had more fish tagged (479), fish recaptured (235), and total recapture events (432) than Gum Branch Creek (419, 129, 222, respectively).

Sandhills chub displayed limited movement throughout the study. Median net distance moved was 0 m for all seasons. Similarly, median absolute distance moved was 25 m overall (Figure 5b) and across all seasons (Figure 6e–6h). Of the Sandhills chub recaptured during seasonal sampling (n = 192), 70 (36.5%) did not move, 142 (74%) moved ≤100 m, and 158 (82.3%) moved ≤200 m from their initial capture section. There were four Sandhills chub that moved >1,000 m from their initial capture location, with the longest movement being 1,225 m downstream. The largest range in movements occurred during autumn, with fish moving up to 1,225 m downstream and 700 m upstream.

Our hypothesis that distance moved would increase with time via diffusive spread was not supported. There was no relationship between time at large and distance moved (Figure 4a; R^2 = 0.0028, P = 0.3179) or maximum distance moved (Figure 4b; R^2 = 0.0002, P = 0.8104). Additionally, the variance of distance moved did not increase with time, providing further support that diffusive spread was not occurring in our streams over the timescale studied.

Sandhills chub movements were leptokurtic and only small biases in movement direction were observed. Net movement (kurtosis = 12.07) was highly leptokurtic (kurtosis > 3 is considered leptokurtic), and the distribution of net movement (Figure 5a; skewness = -0.98) and χ^2-test (χ^2 =

3.7059, df = 1, $P = 0.05422$) suggested there was some evidence of downstream movement bias. Seasonal movement distributions were also highly leptokurtic (Figure 6a–6d), and there was no evidence of movement direction bias during winter ($\chi^2 = 0$, df = 1, $P = 1$), spring ($\chi^2 = 0$, df = 1, $P = 1$), and summer ($\chi^2 = 0.0435$, df = 1, $P = 0.8348$). The distribution of fall movements (Figure 6d) had a downstream bias in movement direction (skewness = -1.94; $\chi^2 = 10.083$, df = 1, $P = 0.0015$).

There was no evidence that Sandhills chub movement was related to the biological and environmental factors we investigated; the null model best described movement (Table 3; BIC = 3493.68, ΔBIC = 0.00, $w_i = 0.66$). The most likely model of absolute movement (BIC = 3438.06, ΔBIC = 0.55, $w_i = 0.34$) had an indicator variable for stream (Table 3: BIC = 3437.51, ΔBIC = 0.00, $w_i = 0.45$), but the predicted difference in absolute movement (estimated difference = -71 m) between streams was not biologically meaningful. Movement distributions did not differ significantly between streams or season, even though there was slight bias in downstream movement (Figure 7a and 7b) and there were no significant relationships between movement and fish length or high-water days (Figure 7c and 7d), providing additional support for the null models.

Movement distributions associated with spawning were no different than other seasonal movement distributions, and there was no evidence of upstream bias. Spawning movement distributions were leptokurtic in the tributary of Aberdeen Creek (Figure 8a; kurtosis = 9.73) and Gum Branch Creek (Figure 8b; kurtosis = 8.78) and median distance moved was 0 m in both streams. Movements in Gum Branch Creek appeared to be upstream (skewness = 1.92), however, a χ^2-test indicated the evidence of upstream bias in movements was not statistically significant ($\chi^2 = 2.6667$, df = 1, $P = 0.1025$). In the tributary of Aberdeen Creek there was no evidence of upstream bias in movements (skewness = -0.01, $\chi^2 = 0.8621$, df = 1, $P = 0.3532$).

4 | Discussion

Sandhills chub displayed limited movement over 2 years in our study streams, with only 17.7% of recaptured fish moving more than 200 m from their initial capture section. Previous studies have asserted that resident, small-stream fishes have completely restricted movement (Gerking, 1953; Hill & Grossman,1987; Smithson & Johnston, 1999) or find that resident stream fishes are mobile and move freely in and out of their respective “home” pools or ranges (Riley, Fausch, & Gowan, 1992; Gowan et al., 1994). We examined movement and potential mechanisms of movement based on the concept that distance moved by stream fishes follows a continuum of movements. We found that Sandhills chub movements occur along this continuum, with a tendency of fish to remain near their initial capture location. As discussed in Gowan et al. (1994) and Rodriguez (2002), a challenge with testing the RMP is the difficulty in establishing quantitative criteria for accepting or rejecting it because distance moved is relative and occurs along a continuum. Therefore, no universal definition or distance exists that makes a fish’s movements “restricted” or “unrestricted.” We agree with these statements because classifying a movement as “restricted” is ultimately arbitrary and highly dependent upon the species and system of focus.

A major critique of using capture-recapture to investigate stream fish movements is that the method can negatively bias movement estimates because tagged fish that move outside of the study reach go undetected (Gowan et al., 1994; Albanese et al., 2003). We investigated this potential source of bias by adding an additional 500 m of search area in the second year of our study. Few individuals (34 out 282 [12%] fish recaptured in year 2 of the study) moved from the original study reach to the added reach. However, fish moving between the original 900-m reach to the added reach appeared to be highly mobile (median distance moved when a fish was initially captured in the original reach and recaptured in the new reach = 538 m). While it is possible that fish were moving beyond the additional 500 m downstream, there was no supporting evidence because of low number of fish that made long-distance movements within our search area. It is possible that fish could be dispersing during the first few months of life, before they can be tagged or detected during backpack electrofishing surveys. However, analysis of genetic data from 30 different Sandhills chub sites indicated that 28−29 of these sites were genetically distinct populations (Harrington, unpublished data), providing further evidence of limited movement regardless of life stage.

High turnover or presence of unmarked individuals, as well as low recapture rates have been proposed as evidence of movement for stream fishes (Cunjak & Randall, 1993; Gowan et al., 1994; Booth, Hairston Jr., & Fleck, 2013). However, it is unlikely that our recapture rate of 41% and continuous presence of untagged individuals each sampling season is due to Sandhills chub being a highly mobile species given the movement distributions we observed. Moderate recapture rates and high numbers of untagged individuals are more likely explained by low capture probabilities due to abundant cover, low conductivity (sometimes less than 11 μS/cm), and low annual survival (average annual survival = 0.21) within our streams (Ramsey, unpublished data). Therefore, high turnover rates within our streams are likely indicative of the sampling environment and lifespan of our study species, rather than high mobility of Sandhills chub.

The lack of movement displayed by Sandhills chub could be explained by the relatively homogenous habitat and stable flow conditions in our study streams. Fish movements generally occur in response to changes in their environmental and physical conditions, with fish usually moving to improve overall fitness (Railsback et al., 1999). Creek chub movements are strongly related to flow (Terui et al., 2021; Curtis et al., 2023), and habitat heterogeneity and complexity (Walker & Adams, 2016). For example, the probability and magnitude of creek chub movements increased during major and moderate flows within an intermittent urban stream in Ohio (Curtis et al., 2023). Compared to mountain and interior plains streams that are more runoff driven, water levels in Sandhills streams remain relatively constant because of the porous sand geology, substantial riparian buffer, and groundwater input. Water levels were relatively stable throughout our study, but we did experience two high flows during or before sampling our streams. There was 9.09 cm of rainfall over three days ~1 week before sampling in April 2023, which caused water levels to rise 0.11 m in the tributary of Aberdeen Creek and 0.21 m in Gum Branch Creek. In May 2024, 6.99 cm of rain fell over two days while we were sampling and water levels rose 0.12 m in the tributary of Aberdeen Creek and 0.41 m in Gum Branch Creek. However, we did not observe increased dispersal of fish associated with these increases in water levels and flow. Within Wyoming streams, Belica and Rahel (2008) found that creek chub moved a median distance of 49 m, with most movements being associated with movement between habitat patches. While we did not explicitly explore habitat patchiness, the habitat in our study reaches was relatively homogenous, with abundant cover available throughout. Therefore, it is likely that Sandhills chub do not need to move long distances for appropriate habitats.

Inter- and intraspecific competition can influence movement of stream fishes (Gerking, 1953; Hazelton & Grossman, 2009; Cooke et al., 2022). Because of the presumed low productivity in our streams, there is potential for high levels of competition within and among species. With increased intraspecific competition, non-dominant fish are likely to move more often and longer distances than the dominant fish in a pool to satisfy their needs (Hansen & Closs, 2009). However, assuming dominance is a function of size, we do not have evidence of dominant fish moving less because movement was unrelated to size. Risk of predation can affect the movement of organisms (Fraser, Gilliam, & Yip-Hoi, 1995; Fraser et al., 1999). Predator presence can potentially increase or decrease movement, as a fish might move (Gilliam & Fraser, 2001) or reduce movement and seek cover to avoid predation (Roberts & Angermeier, 2007). Based on their observed limited movements in the presence of predators such as eastern cottonmouth (*Agkistrodon piscivorus*) and redfin pickerel (*Esox americanus*), Sandhills chub might seek cover and remain in place to avoid being eaten. Similar behavior was observed in Roanoke darters (*Percina roanoka*) that did not attempt to move when predator density reached a certain threshold in adjacent pools (Roberts & Angermeier, 2007). A future study experimentally testing the effects of competition and predation would provide improved understanding of how Sandhills chub movement is related to these interactions.

Sandhills chub movements were highly leptokurtic, with movement distributions displaying extremely tall, thin peaks and larger, longer tails. Leptokurtosis of movement distributions is common among small-bodied stream fishes (Skalski & Gilliam, 2000; Belica & Rahel, 2008; Walker & Adams, 2016; Hicks & Servos, 2017; Pennock et al., 2018; Steffensmeier et al., 2022). Skalski & Gilliam (2003) explained leptokurtic movement using a classic advection-diffusion model and proposed that this movement distribution is explained by diffusive spread (increasing distance moved with time). If diffusive spread were occurring in this study, movement would have increased over time, or the variability of movement would have increased over time. The lack of a significant relationship between time and distance suggests that diffusive spread was not occurring among Sandhills chub and likely does not explain the observed leptokurtosis of movement distributions. Instead, heterogeneity in movement behavior among individuals is more likely to explain leptokurtic distributions of Sandhills chub (Skalski & Gilliam, 2000; Rodriguez, 2002; Radinger & Wolter, 2014). Heterogeneity in movements among individuals in a population could be the result of differences in sex, age, social status, and other phenotypic variations (Ketterson & Nolan Jr., 1985; Quinn & Brodeur, 1991; Armstrong, Braithwaite, & Huntingford, 1997). Variations in movement could potentially be explained by environmental heterogeneity (Gilliam and Fraser, 2001). However, we found no relationship between Sandhills chub movement and season, stream, water level, or total length. Another potential source of heterogeneity in movements could be variations in underlying behavioral traits responsible for movement (Gilliam & Fraser, 2001). Boldness, the propensity of an individual to explore unfamiliar space (Wilson et al., 1993), contributed to dispersal in Trinidad killifish (*Rivulus hartii*) and was a significant source of heterogeneity in movements (Fraser et al., 2001).

It was surprising that Sandhills chub movements did not increase in distance or direction during the spawning period because increased movements associated with spawning have been observed in a wide array of species, including stream fishes. In a southeastern Ohio stream, upstream movements of mature creek chub far exceeded downstream movements during spawning (Storck & Momot, 1981). Flathead chub (*Platygobio gracilis*) move long distances upstream to spawn, with some individuals in Fountain Creek, Colorado, USA, moving over 33 km during the spawning run (Walters et al., 2014). Habitat within our study streams and spawning behavior, particularly of males, was likely why we did not observe increased movements during the spawning period. Spawning habitat was relatively abundant and distributed throughout our study streams; therefore, males did not have to move far to build nests and females did not have to travel far to find nests to lay their eggs. Because Sandhills chub are found within headwater streams the amount of upstream habitat is limited, potentially limiting or prohibiting these fish from making long distance upstream movements during spawning. Because males provide parental care, it is unlikely that they move once they have established a "territory" to build and guard their nests. Future studies should consider weekly sampling during peak spawning as well as only tracking the movements of actively spawning fish, determined by the expression of gametes or visible breeding tubercles in males.

To our knowledge, our study is the first to explore movement of a small-stream Sandhills fish species. Understanding how small-stream fishes move and what influences their movements is important in implementing conservation measures for these species, especially as headwater streams are increasingly altered. Low movement rates among Sandhills chub, and their rareness, make them more vulnerable to extirpations, especially in chronically disturbed streams (Albanese, Angermeier, & Peterson, 2009) because of a low probability that declining populations will be rescued by colonists and their small population sizes (Brown & Kodric-Brown, 1977; Lande, 1993). Because Sandhills chub are mostly sedentary, colonization of new unoccupied reaches and population recovery would likely require a more active management approach, such as stocking or translocating fish to specific stream reaches, to facilitate successful colonization in habitats where Sandhills chub were extirpated but habitat quality has improved.

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Author Contributions

Conceptualisation: ZAR, DPC, BJ, TD, MS, JJH, Jr., CB. Developing methods: ZAR, DPC, BJ, TD, MS, JJH, Jr., CB, RWP. Data analysis: ZAR, DPC. Preparation of figures and tables: ZAR, DPC. Conducting the research: ZAR, RWP, DPC. Data interpretation and writing: ZAR, DPC, BJ, TD, MS, JJH, Jr., CB, RWP.

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Table 1 Summary of Sandhills chub (SHC) capture-recapture data from October 2022–October 2024 in study reaches from two first order streams in North Carolina, USA.

Table 2 Comparison of the percentage of Sandhills chub that moved ≤100 m and ≥500 m, the percentage of fish that moved a maximum distance ≤100 m and ≥500 m, and median distance and absolute distance in meters moved between the original 900-m study reach and the extended 1,400-m study reach. The original 900-m study reach was used to track Sandhills chub movements from October 2022–July 2023. In October 2023, we added an additional 500 m downstream to the study reach, giving us a 1,400-m study reach that we tracked movements in from October 2023–October 2024.

Table 3 Summary of top five generalized linear mixed models for Sandhills chub movement and absolute movement. Selection criteria included BIC values, delta BIC values, and BIC weights (w_i). BIC weights represent the probability that a model is the most likely model out of the suite of models examined. Predictor variables in the models were: total length in millimeters (TL), the study stream each Sandhills chub was sampled in (stream), the number of high-water days where water levels were greater than the 75th percentile of daily water level values (HWdays), and a random effect for each individual Sandhills chub (FishID).

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Figure 1 Map of the Sandhills chub (*Semotilus lumbee*) known distribution based on historical (within the last 50 years) and recent capture locations within the Sandhills ecoregion of NC and SC, USA. Black dots represent locations where at least one Sandhills chub has been captured. Watershed boundaries are at the hydrological unit code (HUC) 8 level, and are within the larger Cape Fear, Pee Dee, and Santee River basins. The grey underlay represents where the Sandhills ecoregion is found within these basins.

Figure 2 Map of Sandhills chub movement study sites from two North Carolina, USA, streams, an unnamed Tributary of Aberdeen Creek and Gum Branch Creek. Both sites are in south-central North Carolina within the Sandhills ecoregion. Rectangles represent where the study streams are with respect to the southeastern United States and where the 1,400-m study reach was within each study stream.

Figure 3 Relationship between the maximum potential distance that a Sandhills chub could move based on the section it was tagged in and the actual distance it was measured to have moved. Dots are weighted by the frequency of each observation; larger dots correspond to a higher frequency of observations.

Figure 4 Sandhills chub movement based on the number of days each recaptured fish was at large (a), with time at large being the amount of time between the initial capture and the last time each Sandhills chub was recaptured, and (b) the number of days it took each Sandhills chub to move its maximum distance from the section it was initially tagged in. All movement distances were measured in meters (m).

Figure 5 Distributions of (a) Sandhills chub net distance moved and (b) Sandhills chub total absolute distance moved. Net distance moved is the sum of all distances moved considering positive (upstream) and negative (downstream) movement from the initial capture section. Total absolute movement does not consider movement direction and is the sum of the absolute value of distance moved from the initial capture section. Plots include the median for each distribution, along with skewness and kurtosis in (a). The dashed line represents the median distance moved of Sandhills chub from October 2022–October 2024. Movement distance was measured in meters (m).

Figure 6 Distributions Sandhills chub distance moved, (a) – (d), and absolute distance moved distributions, (e) – (h), by season. The dashed line represents the median distance moved for each season. The kurtosis, skewness and median values of distributions were calculated for plots (a) through (d), and only the median values were calculated for plots (e) through (h) because absolute movement does not consider movement direction. Data were collected from October 2022–October 2024. All distances were measured in meters (m).

Figure 7 Sandhills chub movement by stream (a), season (b), total length (TL) (c), and number of high-water days in each season (d). Notches in boxplots represent the 95% CI centered around the median distance moved. Center lines show the medians, box limits indicate the 25th and 75th percentiles, the error bars extend 1.5 times the interquartile range from the 25th and 75th percentiles, and outliers are represented by dots. The total length of Sandhills chub was measured to the nearest millimeter. A high-water day was classified as a daily water level that was higher than the 75th percentile of daily water levels within our study streams. All data were collected from October 2022–October 2024 and all movements were measured in meters (m).

Figure 8 Sandhills chub movement distributions associated with the 2023 and 2024 spawning period from (a) the tributary of Aberdeen Creek and (b) Gum Branch Creek. Spawning movements are classified as movements that occur at the beginning or during the presumed spawning period. Only movements that occurred from January–April and January–May were analyzed because spawning peaked in April–May and winter to spring movements can correspond to spawning movements. The kurtosis, skewness, and median distance were calculated for movements from each stream and the median is also represented by the dashed vertical line. Distributions include movements that occurred during the 2023 and 2024 spawning periods.

Table 1 Summary of Sandhills chub (SHC) capture-recapture data from October 2022–October 2024 in 1,400-m study reaches from two first order streams in North Carolina, USA.

	Gum Branch Creek	Tributary of Aberdeen Creek	Total
Tagged SHC	419	479	898
Individual Recaptures	129	235	364
Recapture rate (%)	31	49	41
Recaptures	222	432	654
Recaptured 1 time	75	131	206
Recaptured 2 times	31	52	83
Recaptured 3 times	12	33	45
Recaptured 4 times	8	8	16
Recaptured 5 times	2	5	7
Recaptured 6 times	0	2	2
Recaptured 7 times	1	3	4
Recaptured 8 times	0	1	1
Never Recaptured	290	244	534

Table 2 Comparison of Sandhills chub movement based on the original 900-m study reach used from October 2022–July 2023 and the extended 1,400-m study reach used from October 2023–October 2024.

	900-m reach	1,400-m reach
Distance Moved ≤100 m (%)	82.3	79.2
Distance Moved ≥500 m (%)	3.7	5.8
Maximum Distance Moved ≤100 m (%)	78.8	75.3
Maximum Distance Moved ≥500 m (%)	4.4	6.8
Median Distance Moved (m)	0	0
Median Absolute Distance Moved (m)	25	25

Table 3 Summary of the top five generalized linear mixed models for Sandhills chub movement and absolute movement. Selection criteria included BIC values, delta BIC, and BIC weights (w_i). BIC weights represent the probability that a model is the most likely model out of the suite of models examined. Predictor variables were total length in millimeters (TL), study stream (stream), season, the number of high-water days (HWdays), and a random effect for each individual Sandhills chub (FishID).

Model	BIC	ΔBIC	w_i (BIC)
Movement			
Movement ~ FishID	3493.68	0.00	0.66
Movement ~ HWdays + FishID	3496.04	2.37	0.20
Movement ~ Stream + FishID	3499.16	5.48	0.04
Movement ~ TL + FishID	3499.19	5.51	0.04
Movement ~ TL + HWdays + FishID	3501.55	7.88	0.01
Absolute Movement			
ABSMovement ~ Stream + FishID	3437.51	0.00	0.45
ABSMovement ~ FishID	3438.06	0.55	0.34
ABSMovement ~ Stream + HWdays + FishID	3440.9	3.39	0.08
ABSMovement ~ TL + Stream + FishID	3442.37	4.87	0.04
ABSMovement ~ HWdays + FishID	3442.45	4.94	0.04

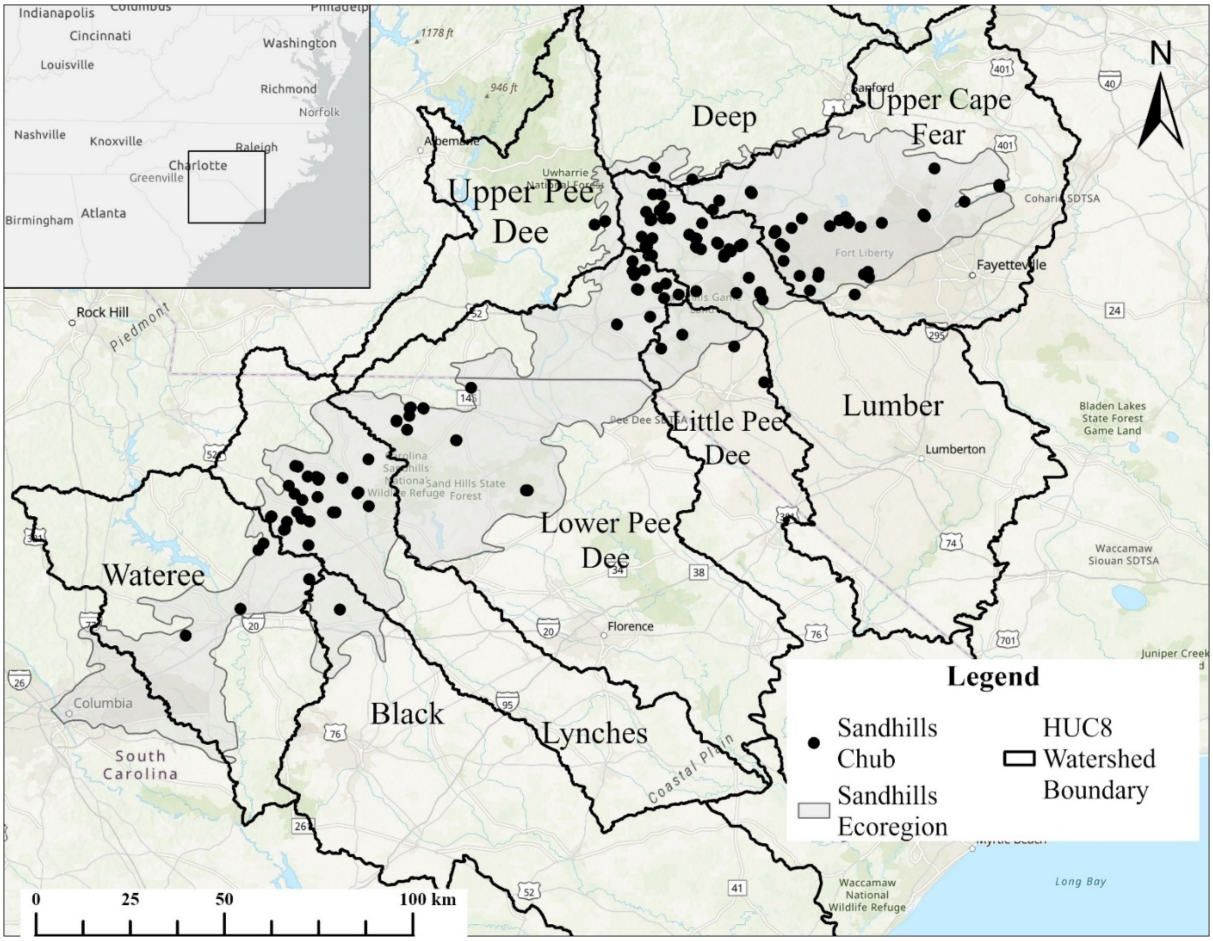


Figure 1 Map of Sandhills chub (*Semotilus lumbee*) known distribution based on historical (within the last 50 years) and recent capture locations within the Sandhills ecoregion of NC and SC, USA, including our two study streams. Black dots represent locations where at least one Sandhills chub has been captured. Watershed boundaries are at the hydrological unit code (HUC) 8 level, and are within the larger Cape Fear, Pee Dee, and Santee River basins. The grey underlay represents where the Sandhills ecoregion is found within these basins.

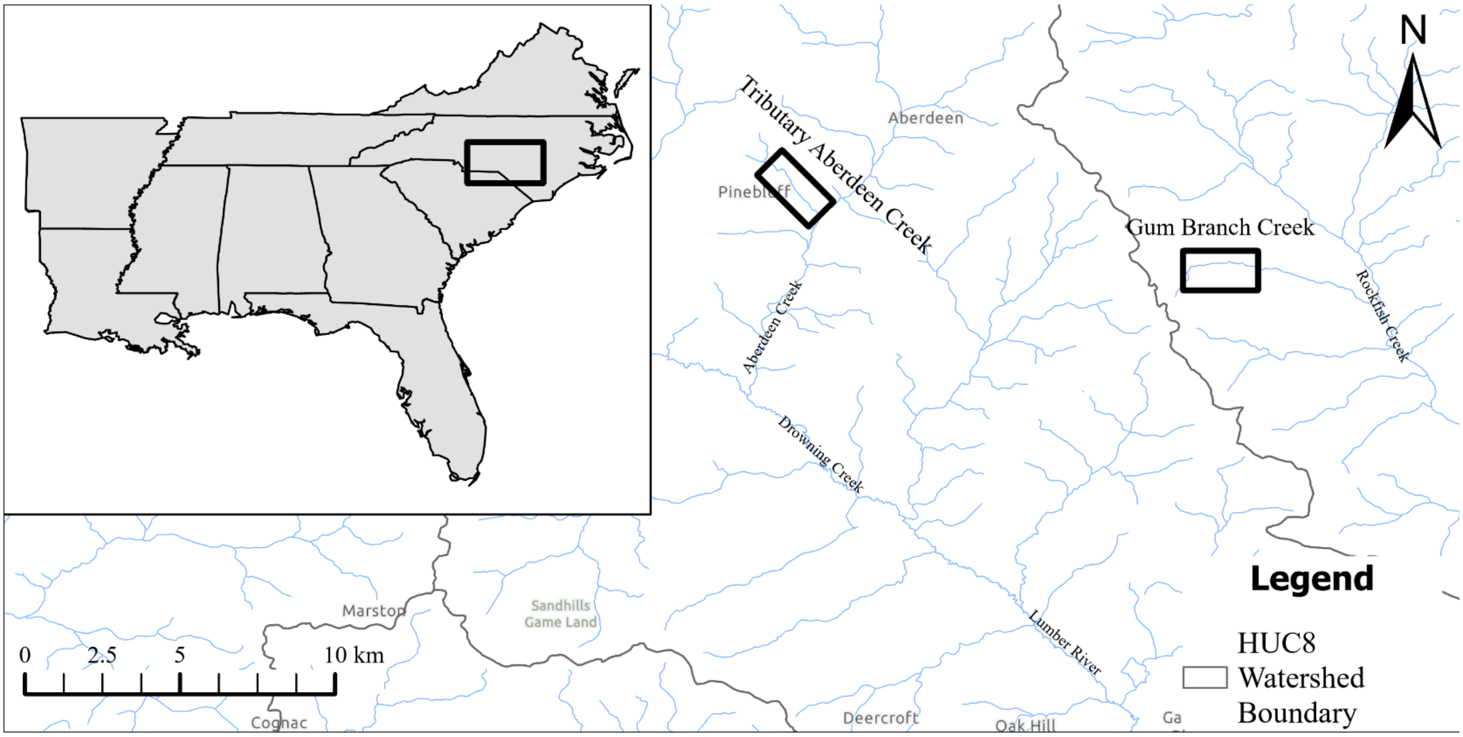


Figure 2 Map of Sandhills chub movement study sites from two North Carolina streams, an unnamed tributary of Aberdeen Creek and Gum Branch Creek. Both sites are in south-central North Carolina within the Sandhills ecoregion. Rectangles represent where the study streams are with respect to the southeastern United States and where the 1,400-m study reach is located within each study stream.

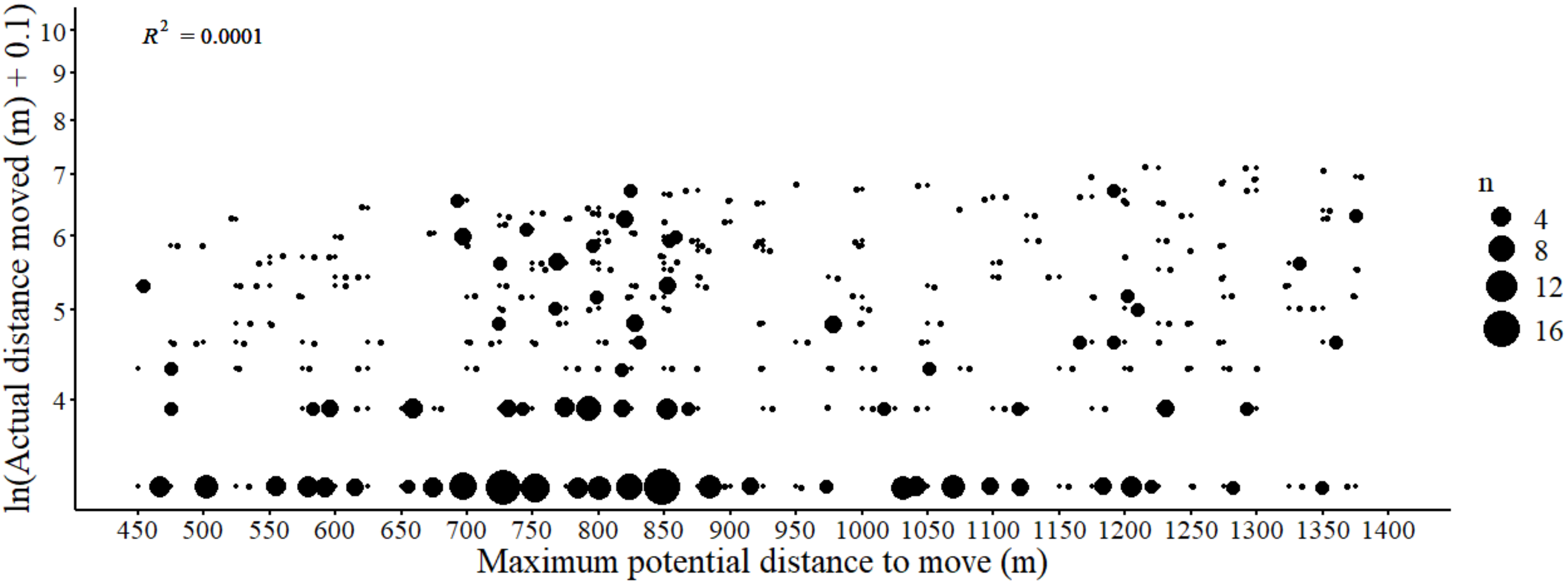


Figure 3 The relationship between the maximum potential distance that a Sandhills chub could move based on the section it was tagged in and the actual distance it was measured to have moved. Dots are weighted by the frequency of each observation; larger dots correspond to a higher frequency of observations.

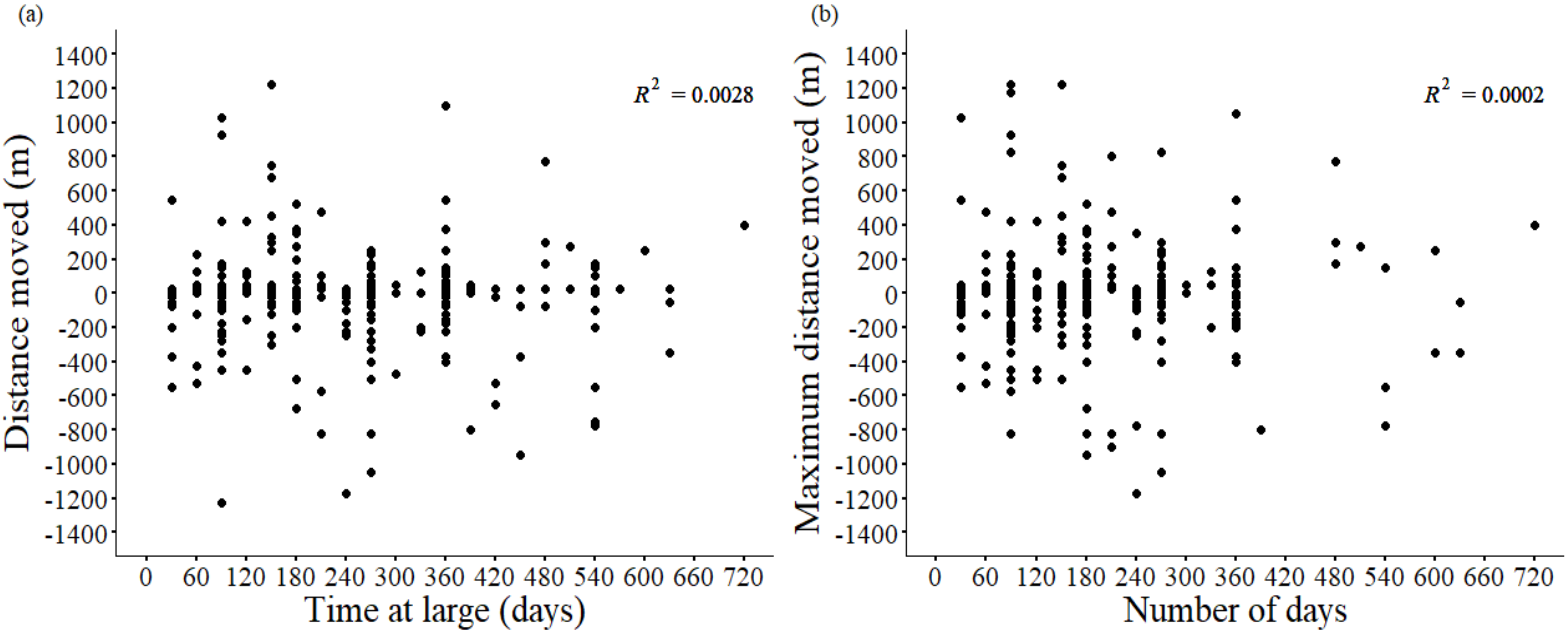


Figure 4 Sandhills chub movement based on the number of days each recaptured fish was at large (a), with time at large being the amount of time between the initial capture and the last time each Sandhills chub was recaptured, and (b) the number of days it took each Sandhills chub to move its maximum documented distance from the section it was initially tagged in. All movement distances were measured in meters (m).

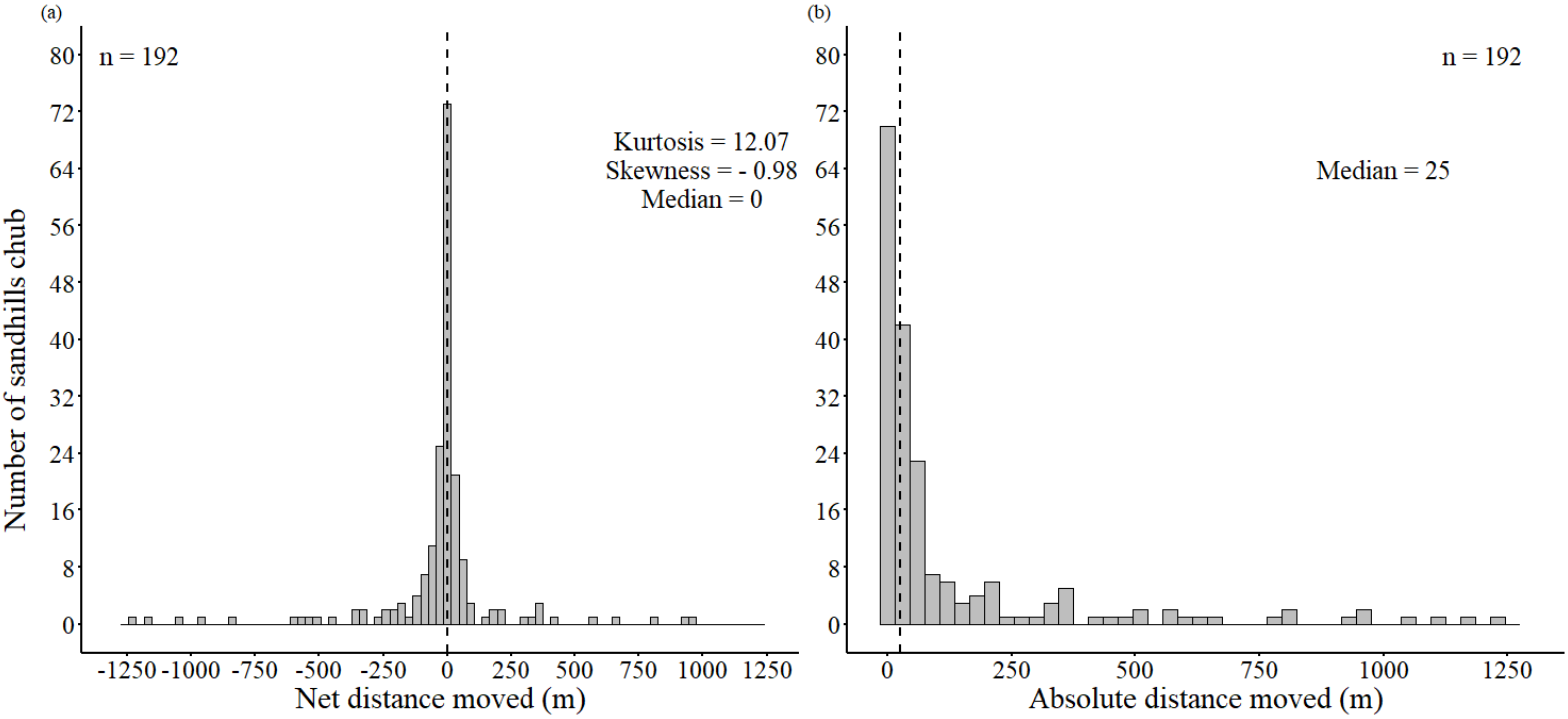


Figure 5 Distributions of (a) Sandhills chub net distance moved and (b) Sandhills chub total absolute distance moved. Net distance moved is the sum of all distances moved considering positive (upstream) and negative (downstream) movement from the initial capture section. Total absolute movement does not consider movement direction and is the sum of the absolute value of distance moved from the initial capture section. Plots include the median for each distribution, along with skewness and kurtosis in (a). The dashed line represents the median distance moved of Sandhills chub from October 2022–October 2024. Movement distance was measured in meters (m).

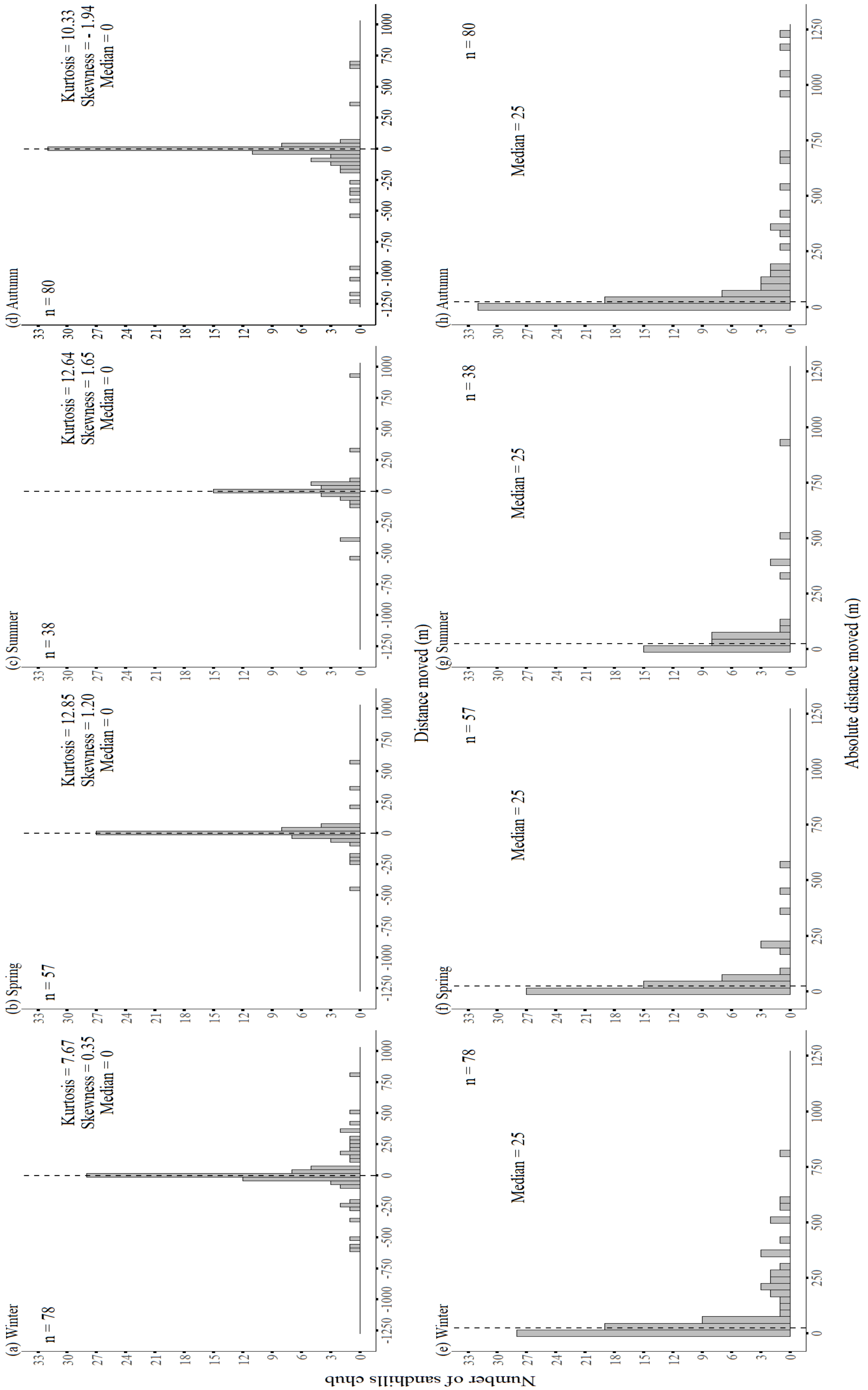
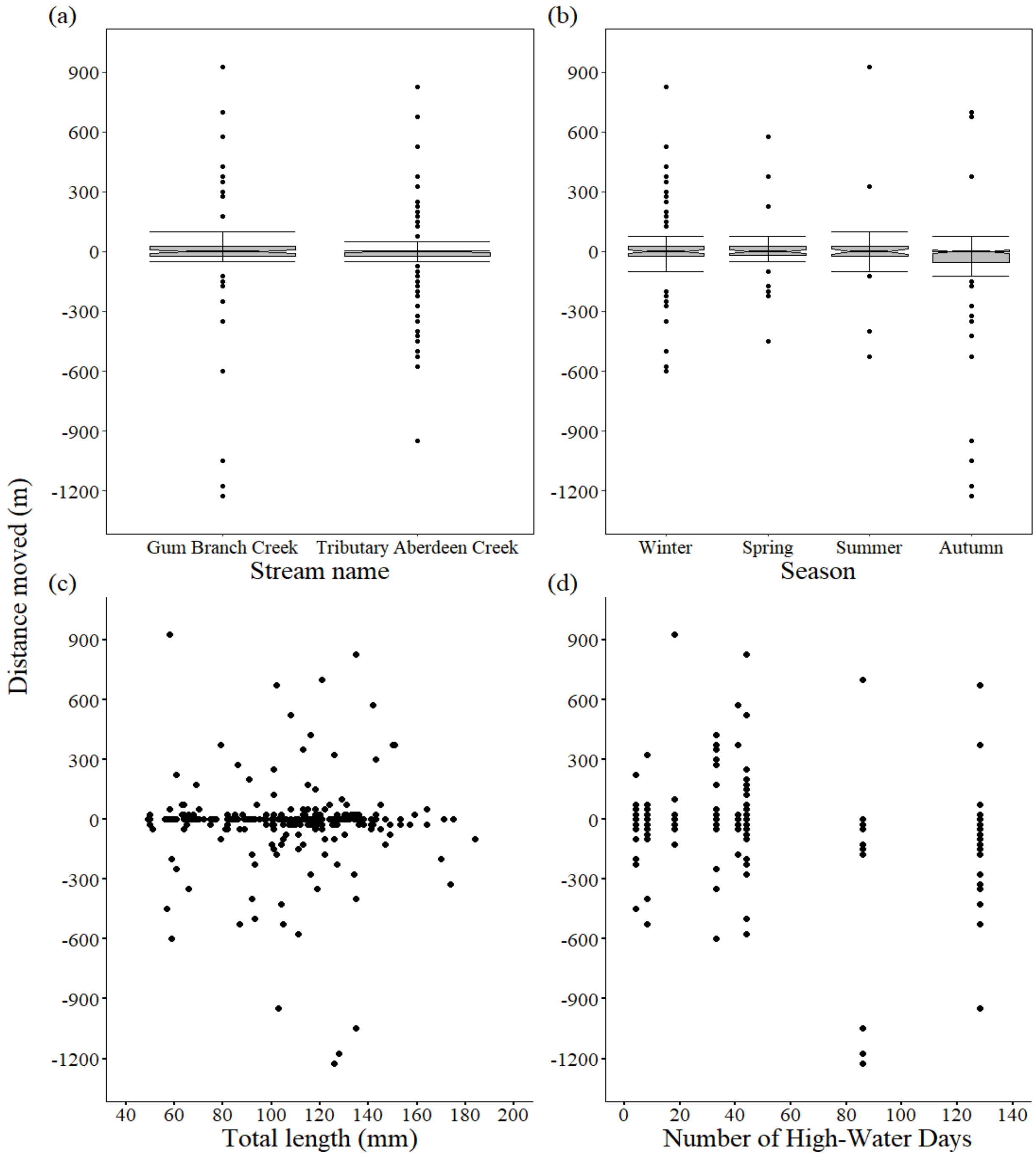

(a) Winter
n = 78
Kurtosis = 7.67
Skewness = 0.35
Median = 0
(b) Spring
n = 57
Kurtosis = 12.85
Skewness = 1.20
Median = 0
(c) Summer
n = 38
Kurtosis = 12.64
Skewness = 1.65
Median = 0
(d) Autumn
n = 80
Kurtosis = 10.33
Skewness = - 1.94
Median = 0
(e) Winter
n = 78
Median = 25
(f) Spring
n = 57
Median = 25
(g) Summer
n = 38
Median = 25
(h) Autumn
n = 80
Median = 25
Number of sandhills chub
0
3
6
9
12
15
18
21
24
27
30
33
Distance moved (m)
-1250
-1000
-750
-500
-250
0
250
500
750
1000
Absolute distance moved (m)
0
250
500
750
1000
1250

Figure 6 Distributions of Sandhills chub distance moved, (a)–(d), and absolute distance moved distributions, (e)–(h), by season from October 2022–October 2024. The dashed line represents the median distance moved for each season. The kurtosis, skewness and median values of distributions were calculated for plots (a) through (d), and only the median values were calculated for plots (e) through (h) because absolute movement does not consider movement direction. All distances were measured in meters (m).



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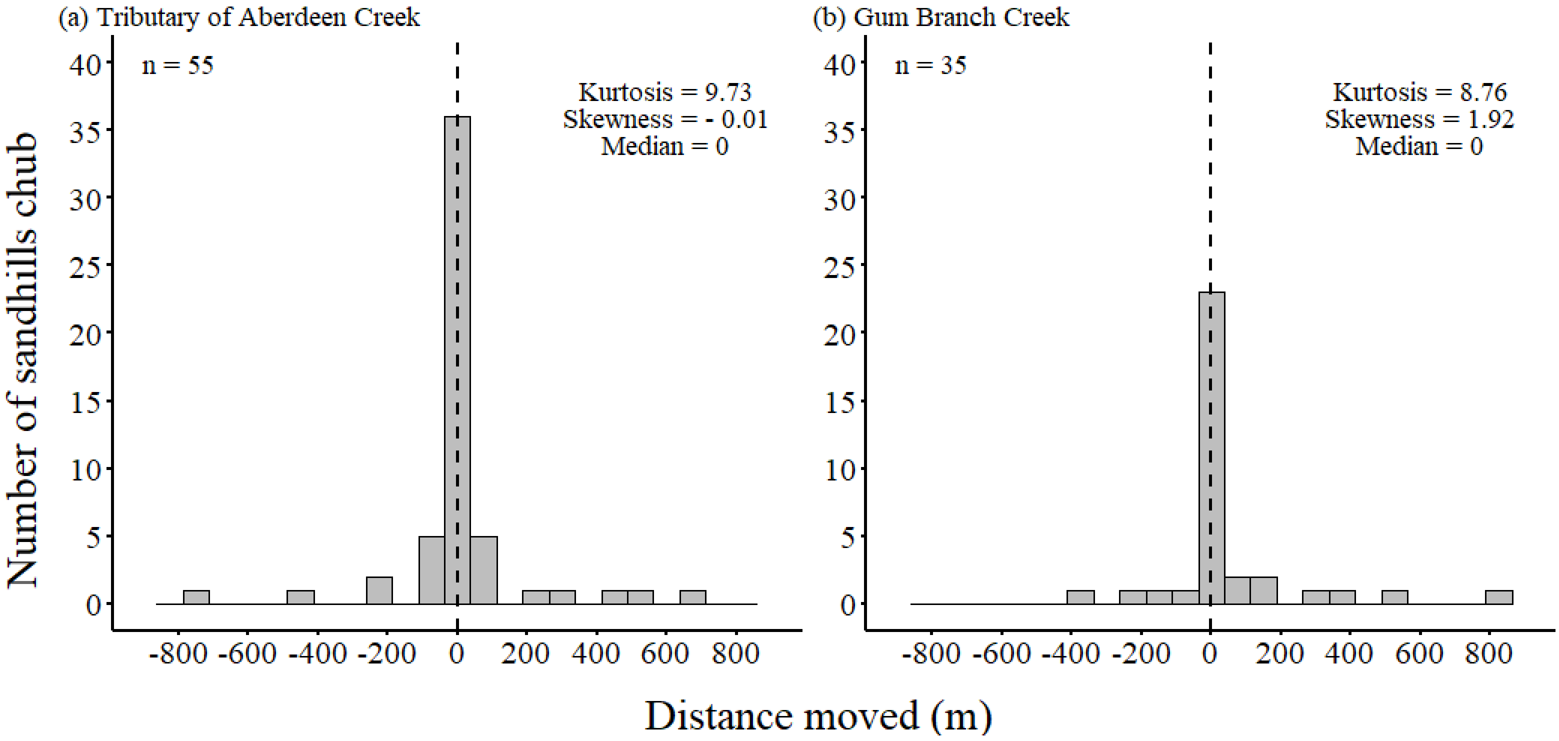
percentiles, and outliers are represented by dots. The total length of Sandhills chub was measured to the nearest mm. A high-water day was classified as a daily water level that was higher than the 75th percentile of daily water levels within our study streams. All data were collected from October 2022 – October 2024 and all movements were measured in meters.



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Life history inhibits deleterious effects of dams on genetic health and structure of a headwater stream fish

Running title: Genetics of a headwater stream fish

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Abstract

Habitat loss, fragmentation, and land use changes in river networks are common globally, which threatens biodiversity and can affect dispersal and subsequently gene flow and genetic health of species. Although numerous studies have indicated negative effects of dams on riverine and migratory species, research on relationships between anthropogenic fragmentation of headwater streams and genetics of fishes that live in these streams is limited. The sandhills chub (*Semotilus lumbee*) is an endemic headwater leuciscid that lives in highly fragmented streams of the Sandhills ecoregion in North and South Carolina. We used a newly-generated genetic dataset of 23 microsatellite loci and Bayesian linear models to investigate relationships between dam distributions and sandhills chub genetic differentiation, genetic diversity, and inbreeding rates. Genetic samples were collected from 887 sandhills chubs across 30 sites, spanning the entire geographic distribution of the species. Pairwise F_{ST} values ranged from 0.014 to 0.425 and were unrelated to the number of dams between sites. Instead, genetic differentiation was a function of whether sites were or were not within the same HUC-8 sub-watershed. Genetic diversity was moderate at most sites (mean $H_{\mathrm{E}} = 0.446$), inbreeding coefficients were small for all sites (mean $F_{\mathrm{IS}} = 0.020$), and both metrics were unrelated to dams and site attributes (upstream drainage area and free-flowing stream reach). Although fragmentation can have deleterious genetic effects on populations through reduction of effective population sizes, gene flow, and genetic variation, barriers may have limited effects on genetics of sedentary species that evolved in isolated habitats.

Keywords: sandhills, fragmentation, conservation genetics, headwater streams, population differentiation

Introduction

Habitat fragmentation is a focal point of conservation biology (Fazey et al. 2005) because it threatens biodiversity and increases extinction risk of species (Crooks et al. 2017; Kuipers et al. 2021). In addition to physically disconnecting habitats, fragmentation can limit movement of organisms and subsequently affect their gene flow, genetic health, and evolution (Franssen 2012; Junge et al. 2014; Wang et al. 2017; Valenzuela-Aguayo et al. 2019; Brauer and Beheregaray 2020). Stream networks have been extensively altered through anthropogenic fragmentation and the reduction in connectivity is known to be a major threat to aquatic biodiversity (Nislow et al. 2011; Perkin and Gido 2011; Lu et al. 2024). Only 37% of the Earth's rivers greater than 1,000 km are free-flowing and most are in remote regions of the world (Grill et al. 2019). Stream fragmentation can affect species' migrations (van Puijenbroek et al. 2019; Davies et al. 2021), result in loss of biotic and abiotic stream function (Freeman et al. 2007; Colvin et al. 2019), reduce genetic diversity, reduce effective population sizes (Machado et al. 2021), and can lead to one-way gene flow in fishes (Yamamoto et al. 2004). One-way gene flow is the result of fish often being able to move over or through a barrier in a downstream direction, but not upstream, thus, resulting in reduced genetic diversity in upstream reaches (Hernandez-Martich and Smith, 1997; Yamamoto et al. 2004). Anthropogenic fragmentation can also increase genetic differentiation between populations upstream and downstream of barriers (Raeymakers et al. 2008; Roberts et al. 2013). For example, genetic differentiation of Roanoke logperch (*Percina rex*) between sites separated by dams was similar to what would be expected across 1,200 km of undammed river (Roberts et al. 2013).

To understand relationships between habitat fragmentation and genetics, research has focused on anthropogenically fragmented habitats, such as the barriers to movement created by dams (Yamamoto et al. 2004; Skalski et al. 2008) or the division of habitats by roads (Vos and Chardon 1998; Shepard et al. 2008; Wang et al. 2017), yet many species inhabit naturally fragmented habitats. Natural fragmentation also has the potential to influence genetic structure and diversity of species (Deiner et al. 2007; Phillipsen and Lytle 2013). For example, Deiner et al. (2007) observed that rainbow trout (*Oncrhynchus mykiss*) had reduced genetic diversity upstream of waterfalls compared to downstream, likely due to isolation of upstream habitats, one-way gene flow due to waterfalls, and smaller population sizes (Deiner et al. 2007). Although natural and anthropogenic barriers to movement affect gene flow, there are numerous examples of species that thrive in a patchwork of isolated habitats and have demonstrated resistance to anthropogenic habitat fragmentation (Reid et al. 2008; Chiucchi and Gibbs 2010; Matesanz et al. 2018). Massasauga rattlesnakes (*Sistrurus catenatus*) naturally have low levels of gene flow between preferred habitat patches and genetic structure is unrelated to anthropogenic habitat fragmentation (Chiucchi and Gibbs 2010). A lack of relationship between anthropogenic fragmentation and genetic metrics has also been observed in aquatic environments. For example, genetic diversity of longear sunfish (*Lepomis megalotis*) did not differ between areas upstream and downstream of low-head dams (Smith et al. 2019). Thus, a reduction in genetic diversity or structuring of populations via anthropogenic fragmentation is not universal.

Headwater streams, typically considered small 1st- and 2nd- order streams, account for 79% of stream length in the U.S. and are important sources of biodiversity globally (Meyer et al. 2007; Finn et al. 2011; Creed et al. 2017; Colvin et al. 2019). Due to their small size and minimal protections, headwater streams are easily modified, fragmented, and removed from the landscape (Freeman et al. 2007). Habitat alteration and fragmentation have been shown to affect dispersal (Warren and Pardew 1998; Skalski et al. 2008; Lamphere and Blum 2012; Sterling et al. 2012), and genetic diversity of headwater species (Junker et al. 2012; Torterotot et al. 2014; Grubb et al. 2022). For example, low allelic richness and reduced effective population sizes were observed in the headwater pristine crayfish (*Cambarus pristinus*), which were likely due to anthropogenic habitat changes (Grubb et al. 2022). Furthermore, creek chub (*Semotilus atromaculatus*) genetic structure was associated with impoundments on rivers in Kansas but measures of genetic diversity remained high (Hudman and Gido 2013). However, some headwater fishes display limited dispersal and movement, and therefore genetic structure may be related to their natural ecology instead of fragmentation. Thus, it is important to account for population genetics, mobility, and life history when investigating the effects of habitat fragmentation on ecology and adaptive potential of headwater fishes.

The Sandhills ecoregion of North and South Carolina, USA is a narrow strip of land (~55 km at widest point) in the upper Coastal Plain of the eastern U.S. It is inhabited by unique biota such as pinewoods darter (*Etheostoma mariae*; Rohde and Arndt 1991), wiregrass (*Aristida stricta*; Burk 1959) and St. Francis satyr butterfly (*Neonympha mithcelli francisci*; Parshall and Kral 1989). The streams within the sandhills have experienced extensive habitat fragmentation over the last two centuries via impoundments for agriculture, stormwater retention, and recreation, with over 2,400 known dams documented on streams (Fig. 1). The sandhills chub is endemic to 1st- and 2nd-order headwater streams in the Sandhills ecoregion from northeast of Columbia, South Carolina to north of Fayetteville, North Carolina (Rohde and Arndt, 1991), and is a sister species to the widely distributed creek chub (Schönhuth et al. 2018). It is a robust, fusiform-shaped minnow that reaches a maximum total length of 240 mm (Snelson and Suttkus 1978). Compared to more intensively managed fishes that support recreational and commercial fisheries, there is limited information about sandhills chub, which was not officially described until 1978 (Snelson and Suttkus 1978). Sandhills chub are headwater stream specialists associated with well-oxygenated water with undercut banks, woody debris, root wads, and a mix of sand and gravel substrates, which they use to construct pit-ridge nests for reproduction (Rohde and Arndt 1991; Herigan et al. 2023). The South Carolina Department of Natural Resources (SCDNR) and North Carolina Wildlife Resources Commission (NCWRC) have listed the sandhills chub as a Species of Special Concern largely due to habitat loss in their already restricted distribution. Similarly, the American Fisheries Society lists the sandhills chub as vulnerable due to habitat loss (Jelks et al. 2008). Rohde and Arndt (1991) documented potential extirpation of sandhills chubs in several sites they were previously known to inhabit and hypothesized that these extirpations were the result of dams and habitat alteration. Due to the lack of genetic data on sandhills chub, status of sandhills chub as a species of conservation concern, and high-level anthropogenic stream fragmentation in headwater streams of the Sandhills ecoregion, we used a newly-generated genetic dataset of 23 microsatellite loci to investigate relationships between habitat fragmentation and genetic diversity, health, and structuring of sandhills chub across their geographic distribution. The objectives of this study were to (1) investigate if there was a relationship between population differentiation (pairwise F_{ST}) of sandhills chub, distance between sites, and the number of dams between sites, and (2) investigate if indices of genetic diversity and health (i.e., H_{E}, N_{A}, F_{IS}, and G-W) of sandhills chub were reduced in reaches upstream of dams compared to downstream reaches and unfragmented reaches. We hypothesized that genetic differentiation of sandhills chub would be related to the number of dams between sites because genetic differentiation of their sister species the creek chub was related to impoundments and dams between sites (Skalski et al. 2008; Hudman and Gido 2013). We also hypothesized that genetic diversity would be reduced in reaches upstream of dams due to one-way gene flow (Yamamoto et al. 2004), and observed decreases in genetic diversity upstream of reservoirs in studies of other headwater fishes (Schmidt and Schaefer 2018). Results from this study furthers our understanding of the interplay between life history, habitat, and population genetics of a headwater species and provides information to guide conservation of sandhills chub and other headwater fishes with similar life histories.

Methods

Study area and sample collection

Sampling of sandhills chubs was conducted in 1st- and 2nd-order headwater streams of the Cape Fear, Great Pee Dee, and Santee watersheds within the Sandhills ecoregion of North Carolina and South Carolina and encompassed the entire geographic distribution of sandhills chub. Historical locations of sandhills chub from Herigan et al. (2021, 2023), Rohde (1991), NCWRC, and a pilot study in 2022 were initially used to guide sampling efforts. We then used opportunistic sampling to collect fish in each HUC-8 (hydrologic unit) sub-watershed within the Sandhills ecoregion where there was habitat associated with presence of sandhills chub (Herigan et al. 2023). Tissue samples were collected from two main sources: archived samples collected during a previous investigation of sandhills chub-habitat associations during 2020 (Herigan et al. 2023), and samples collected for the current study during 2022−2024. The collection procedures were the same in both studies and all fish were sampled using an ETS ABP-4 backpack electrofisher (Electrofishing Systems, LLC, Madison, WI). Tissue samples were collected with a goal of collecting at least 30 samples from sandhills chubs >age-0 from at least one site in each HUC-12 unit where they occur. Each reach (referred to as a site) where 30+ genetic samples were collected was marked as a single GPS coordinate at roughly the midpoint of the sampled reach. Reach lengths ranged from 60−1400 m and had varying numbers of dams located upstream and downstream and varying distances of undammed stream connected to them, ensuring enough variation in the independent variables to conduct robust statistical analyses. We measured each sandhills chub (total length, mm) and clipped a ~5-mm^2 section of tissue from the pelvic or caudal fin. Samples were individually stored in tubes with sarcosyl-urea, 90% ethanol, or 100% ethanol. Between samples, scissors were sprayed with deionized water and wiped with a clean cloth to prevent cross-contamination. Fish were classified as >age-0 based on seasonal length-frequency histograms developed during a two-year mark-recapture study of sandhills chub (data from Ramsey 2025). Fish were classified as >age-0 if ≥ 70 mm (TL) in summer, ≥ 80 mm in autumn, and ≥ 90 mm in winter. All fish collected in spring were >age-0 because spring sampling occurred during or immediately after spawning, prior to age-0 fish recruiting to the electrofishing gear. We randomly selected samples for sites where there were more than 30 genetic samples for fish >age-0 using the 'ifelse' function (dplyr; Wickham et al. 2023) and 'month' function (lubridate; Grolemund and Wickham 2011) in RStudio version 2024.09.1+394 (Posit Team 2024). There were 16 sites that did not contain 30 samples from fish greater than the length thresholds, so remaining samples were randomly selected from fish less than those length thresholds for a given site using the dplyr package (Wickham et al. 2023) in RStudio to reach the desired number of samples when possible (n = 22, 28, 28, 29 for four sites).

Spatial analyses

Dam data for South Carolina was provided by Herigan et al. (2023). We used Landsat-8 and Copernicus Sentinel imagery in Google Earth Pro (7.3.4, Google, LLC, Mountain View, CA; European Space Agency 2022; United States Geological Survey 2022) and Esri's World Imagery Basemap (2022) in ArcGIS Pro 2.8 (Esri Inc. 2021) to plot dams intersecting flowlines in North Carolina. When available, dam ages were extracted from the Southeast Aquatic Resources

Partnership (SARP) aquatic barrier inventory (SARP 2024). Pairwise spatial measures used in the analysis of genetic differentiation (pairwise F_{ST}) were restricted to the Great Pee Dee River Basin because it only shares a contemporary connection with the Cape Fear and Santee river basins via the Atlantic Ocean. Additionally, since prehistoric connectivity of the headwaters in the Great Pee Dee Basin are unknown, we calculated both Euclidean and stream distances between site pairs as potential indices of connectivity. To do so, we made a network dataset using NHDPlus Version 2 stream flowline data (McKay et al. 2012) from the U.S. Environmental Protection Agency (USEPA) and the U.S. Geological Survey (USGS). Then, we used the Network Analyst Tool in ArcGIS Pro 3.0 (Esri Inc. 2022) to calculate pairwise stream distances between sites (km) and the number of dams between sites (PWB). Euclidean distances (EUD; km) were calculated between sites using the geosphere package in RStudio (Hijmans, 2024). We created a binary matrix for pairwise site comparisons if sites were or were not within the same HUC-8 sub-watershed (WS; the same sub-watershed = 0, across sub-watersheds = 1) because in some cases sites were in close geographic proximity but in different sub-watersheds.

Stream site attribute calculations

To better understand the relationships between anthropogenic dams and genetic diversity and health metrics, we calculated four stream site attributes in ArcGIS Pro 3.0 (Esri Inc. 2022): upstream drainage area of a site (UDA: km^2), cumulative length of free-flowing stream connected to a site (FFR; km), and the number of dams upstream and downstream from a site (UAI; DAI). We calculated the upstream drainage areas for each site using the Spatial Analyst Tool with one-meter resolution lidar-derived digital elevation models (DEM) from the 2015 NCFMP Lidar DEM (OCM Partners. 2024a) and 2020 USGS Lidar DEM (OCM Partners. 2024b). Genetic diversity commonly increases in the downstream direction for fishes (Hänfling and Weetman 2006; Dehais et al. 2010; Lamphere and Blum 2012), so upstream drainage area was used as an index of position within a headwater stream. We also calculated the cumulative length of free-flowing stream connected to a site using the Network Analyst Tool. The cumulative FFR was the total stream distance not intersected by an impoundment within the Sandhills ecoregion and reflected the total amount of Sandhills stream distance, including connected tributaries connected to a site. We included the cumulative length of free-flowing stream connected to a site because habitat size may be related to genetic diversity (Whiteley et al. 2013). To calculate the length of free-flowing stream reach connected to a site, we first created a network dataset using USGS NHDPlus Version 2 stream flowline data (McKay et al. 2012), from USEPA and USGS. Then we used the same dam point dataset that was used for the pairwise stream analyses to conduct a stream network trace in the network dataset that terminated when a flowline intersected a point layer of a dam, upstream start of an impoundment, or the Sandhills ecoregion border. After completing the stream network traces, we extracted the calculated length of free-flowing stream connected to each site. We chose to use the Sandhills ecoregion border as a barrier because movement outside of documented sandhills stream distribution was unlikely (based on extensive fish sampling of wadeable streams in South Carolina by South Carolina Department of Natural Resources [SCDNR]) and the trace would terminate at the Atlantic Ocean if there were no dams. We counted the number of dams upstream and downstream from a site using the dam and flowline layers in ArcGIS Pro 3.0.

Microsatellite genotyping and population genetic analyses

Molecular processing and genotyping of field samples were completed at the SCDNR's Marine Resources Research Institute Population Genetics Laboratory. A 23 microsatellite loci panel developed for sandhills chub was used to genotype samples and conduct standard population structuring and characterization analyses (Harrington, SCDNR, unpublished data). Pairwise F_{ST}

values (276 site pairs) and population (n=30) levels metrics of expected heterozygosity (H_E), observed heterozygosity (H_O), number of different alleles (N_A), and Garza-Williamson Index (*G-W*) were estimated using GenAlEx v6.5 software (Peakall and Smouse 2006; Peakall and Smouse 2012).

Dams and population differentiation

We used Bayesian linear models (BLMs; Roberts et al. 2013) to investigate the relationship of pairwise F_{ST} with distance and the number of dams between sites within the Great Pee Dee River watershed (n = 276 site pairs based on 24 sites). Models were fitted in R statistical software version 4.4.2 (R Core Team, 2024) via RStudio version 2024.09.1+394 (Posit team, 2024) with Just Another Gibbs Sampler (JAGS; Plummer 2003) and the R2jags package (Su and Yajima 2024). The presence of prehistoric connections across sub-watersheds and their importance to gene flow compared to contemporary connectivity and hydrology of these systems is unknown. Therefore, we tested Euclidean and stream distance to investigate isolation by distance (IBD) within models. We fitted 14 models for pairwise F_{ST}, including an intercept only null model, a single predictor for each variable (Euclidean distance, stream distance, number of dams between sites, and if a site pair was within the same HUC-8 sub-watershed), additive models for all combinations of variables (only one distance variable used per model), and two full models that included interactions between distance and the number of dams (Euclidean distance x dams or stream distance x dams). Stream distances between sites were correlated with the variable indicating if a site pair was within the same HUC-8 sub-watershed (VIF = 11.04; car package; Fox and Weisberg 2019), so the indicator variable was not included in models with stream distance. Due to differences in scale for stream distance, Euclidean distance and the number of dams between sites, we z-score transformed all three to center values to a mean of zero. We assumed pairwise F_{ST} to be normally distributed and chose weakly informative priors (μ= 0, T = 10; JAGS uses precision represented by tau) for three chains, 10,000 iterations, a burn-in period of 5000 iterations, and a thinning interval of 10, resulting in 1500 iterations (500 per chain) for posterior parameter estimation. We checked Markov Chain Monte Carlo (MCMC) trace plots (Fig. A.1) and that $\hat{R}$ values were near 1.0 and less than 1.1 for each parameter to confirm model convergence (Gelman et al. 2013). We performed leave one out model selection using the LOO package in R, where smaller expected log predictive density values (ΔELPD) show greater model support (Vehtari et al. 2024). We checked posterior coefficient values for the top models and calculated 95% credible intervals for each parameter. To assess model fit, we created posterior prediction plots using the 'ppc_dens_overlay' function in the bayesplot package with the ggplot2 package, which overlays the posterior distribution over the observed distribution for the response variable (bayesplot: Gabry and Mahr 2024; ggplot2: Wickham 2016).

Based on initial model results, we used Bayesian linear models to investigate the bimodal distribution of pairwise F_{ST}. We initially used a Bayesian mixture model, assuming two normal distributions with an indicator variable for if a site pair was within the same HUC-8 sub-watershed, but models did not converge or $\hat{R}>1.1$. So, we split the data based on whether or not site pairs were within the same HUC-8 sub-watershed and conducted separate analyses for each. Because the variability in number of dams between site pairs was greatly reduced for sites within the same sub-watershed, we used a binary indicator for presence or absence of dams between sites (for both datasets). For site pairs within the same sub-watershed, we initially fit 16 models assuming a normal distribution for pairwise F_{ST}. We also fit models assuming a beta distribution, but they had poor model fit, and will not be discussed. The series of models included: (1) an intercept only model, (2) models with a single predictor for each variable (log-transformed Euclidean distance, log-transformed stream distance, and dam presence), (3) additive models for all combinations of variables (only one distance variable per model), (4) two full models that included interactions between the distance variables and dam presence, and (5) models listed in 1−4 with the addition of a categorical variable for the sub-watershed site pairs were within, and an interaction between dam presence and the sub-watershed variable. We log-transformed pairwise F_{ST} and the distance variables to meet assumptions of normality and linearity. Because sample sizes were reduced after splitting the data, models were fit using the 'kfold' function in the jagshelper package (jagshelper; Tyers 2024). We used non-informative priors ($\mu = 0$, $T = 0.001$) for three chains, 20,000 iterations, a burn-in period of 10,000 iterations and a thinning interval of 10 for 3000 posterior iterations. After fitting models, we checked Markov Chain Monte Carlo (MCMC) trace plots and that $\hat{R}$ values were near 1.0 and <1.1 for each parameter to confirm model convergence (Gelman et al. 2013). We then compared root mean square error (RMSE), from the 'kfold' function to assess model performance (jagshelper; Tyers 2024). Models with smallest RMSE and the null model were then refit with all the data using the 'jags' function in the R2jags package (R2jags; Su and Yajima 2024) to get posterior parameter estimates and assess model fit. We checked posterior coefficient values for these models and calculated 95% credible intervals for each parameter. To assess model fit, we created posterior prediction plots using the 'ppc_dens_overlay' function in the bayesplot package with the ggplot2 package (bayesplot: Gabry and Mahr 2024; ggplot2: Wickham 2016).

We fit the same models for site pairs not within the same sub-watershed as those listed above except (1) we did not include a categorical variable for the sub-watershed sites were in (because of being in different HUC-8 sub-watersheds), and (2) we fit a model adding a quadratic term for Euclidean distance based on initial scatterplots of Euclidean distance and pairwise F_{ST}. Euclidean distance was z-scored to center values to a mean of zero due to initial difficulty sampling the probability distribution. All models were fit using the ‘kfold’ function in the jagshelper package (jagshelper; Tyers 2024), using the same priors, packages, iterations, model checks, and posterior prediction checks as previously described for the models of site pairs within the same sub-watershed.

Dams and population genetic health

To investigate relationships between indices of genetic diversity and dams, we modeled four response variables (H_E, N_A, $G\text{-}W$, and F_{IS}) using Bayesian Linear Mixed Models (BLMMs). The response variables were modeled independently as functions of three fixed effects: upstream drainage area from a site, free-flowing stream length, number of dams downstream from a site, and a hierarchical effect for the HUC-8 sub-watershed the site was within. Because these analyses did not rely on pairwise values, data for all 30 sites were used in the models. Due to differences in scale for upstream drainage area, number of dams downstream of a site, and free-flowing stream reach, we z-score transformed all variables. We assumed H_E, N_A, $G\text{-}W$, and F_{IS} to be normally distributed and used weakly informative priors (H_E, F_{IS}, $G\text{-}W$: $\mu = 0$, T = 10; N_A: $\mu = 0$, T = 0.01). We ran three chains, with 20,000 iterations, a burn-in period of 10,000, and a thinning interval of 10. Cross-validation was performed as described above using ‘kfold’ function in the jagshelper package (jagshelper; Tyers 2024). We compared RMSE for each model, where smaller RMSE reflects better model performance to predict observed values (Tarekegn et al. 2020). We fit eight models for each response variable including a null model, a single predictor for each variable, and additive models for all combinations of variables. Levels of upstream drainage area, number of dams downstream from a site, and free-flowing stream length were not dependent on the levels of each other, so no interaction terms were included. We did not include the number of upstream dams in the models due to correlation with drainage area (VIF = 2.1; car package; Fox and Weisberg 2019), difficulty with model convergence when upstream drainage area and the number of dams upstream of a site were both included, and many of the upstream dams occurring at the start of the headwater streams, where they were not functioning as barriers to movement. The models that showed support based on smaller RMSE were re-fit in RStudio with JAGS (Plummer 2003) via the R2jags package (Su and Yajima 2024) using the full dataset to get posterior means and credible intervals. Due to the incorporation of the HUC-8 hierarchical effect and low sample sizes within sub-watersheds, 3000 iterations were ran for the posterior parameter estimates. We checked MCMC trace plots (Fig. A.2, A.3, A.4, A.5) and verified that $\hat{R}$ values were near 1.0 and <1.1 for each parameter to confirm model convergence (Gelman et al. 2013). After convergence was verified, we extracted posterior coefficient values for supported models and calculated 95% credible intervals for each parameter. To assess model fit, we created posterior prediction plots using the 'ppc_dens_overlay' function in the bayesplot package (Gabry and Mahr 2024) with the ggplot2 package (Wickham 2016).

Results

Dams and population differentiation

Genetic data were available for 887 sandhills chubs, across 30 sites (Table 3; Fig. 2). Differentiation was high among the 24 sites in the Great Pee Dee River basin ($n = 276$ pairs; mean pairwise F_{ST} (±SD) = 0.198 ± 0.093). Pairwise F_{ST} for sites from different HUC-8-level sub-watersheds were variable (range: 0.046 – 0.319), but tended to be greater than pairwise F_{ST} values for sites within a given HUC-8 sub-watershed (Table 1). Pairwise F_{ST} for sites within the Lower Pee Dee sub-watershed were an exception, with values similar to those observed for pairs of sites from different sub-watersheds (Table 1). Euclidean distances between sites ranged from 1.6 to 122.3 km and averaged 55.7 km, stream distances between sites ranged from 2.8 to 617.8 km and averaged 406.9 km, and the number of dams between sites ranged from 0 to 6 (mean = 1.86; Table A.2). Age data were only available for a small number of dams (n = 7 dams) between sites and ages ranged from 36 to 165 years old (mean = 109 years; SARP 2024). The number of dams between sites were similar if they were within the same sub-watershed, but on average, Euclidean and stream distances were greater for site pairs from different sub-watersheds (Table A.2).

The most likely model for pairwise F_{ST} included the number of dams between sites and if site pairs were within the same sub-watershed (Table 2). Although there was support for five of the models examined, the variable indicating if site pairs were within the same sub-watershed was the only variable included in all five (Table 2). For the most likely model, the mean posterior estimate of pairwise F_{ST} only increased by 0.011 (95% CI: 0.002 – 0.019) with a one SD increase in the number of dams between sites (SD = 1.68 dams between pairs). Pairwise F_{ST} was 0.132 (95% CI: 0.112 – 0.153) greater for sites not within the same sub-watershed compared to site pairs within the same sub-watershed (with number of dams between sites held constant). Regardless, the posterior prediction plot indicated poor model fit for the best supported model, because of the bimodal distribution of pairwise observed values (Fig. 3). The two models which contained only stream or Euclidean distance did not have good support.

For site pairs within the same sub-watershed, the model of log-transformed pairwise F_{ST} with the lowest RMSE included terms for Euclidean distance between sites, the indicator variable for presence or absence of a dam, the watershed site pairs were within, and an interaction between Euclidean distance and dam presence or absence (Table 3). However, the 95% credible interval for the interaction term overlapped zero. Based on the principle of parsimony in model selection and comparison of PPC plots for the models with the lowest RMSE values, we concluded the model with the second lowest RMSE, which did not include an interaction term, had the most support (Table 3; Fig. 4). The back-transformed mean posterior estimate of pairwise F_{ST} was 0.063 (95% CI: 0.027 – 0.141) for the Little Pee Dee sub-watershed in the absence of a dam and at the average Euclidean distance (mean = 19.24 km) between sites. Compared to the Little Pee Dee sub-watershed, F_{ST} was 1.609 times greater (95% CI: 0.728 – 3.633; model predicted $F_{ST} = 0.101$) in the Lower Pee Dee, 0.668 times less in the Lumber (95% CI: 0.303 – 1.519; model predicted $F_{ST} = 0.042$), and 0.824 times less (95% CI: 0.380 – 1.818; model predicted $F_{ST} = 0.052$) in the Lynches. Mean posterior estimates of pairwise F_{ST} (back-transformed) increased by 8.67% (95% CI: 5.86% – 11.45%) for a 25% increase in Euclidean distance between sites. Presence of a dam was associated with a 56.67% (95% CI: 24.73% – 96.99%) increase in pairwise F_{ST} (back-transformed) for site pairs within the same sub-watershed. However, the scatterplot of the relationship between pairwise F_{ST} for sites within the same sub-watershed and Euclidean distance, as a function of a dam being present or absent between sites, suggests this relationship is not universal (we tested for the interaction between presence or absence of dams and sub-watershed, but lack of site pairs without a dam between them in the Lower Pee Dee and Little Pee Dee sub-watersheds resulted in poor model performance). Site pairs with a dam between them in the Lumber sub-watershed had greater log-pairwise F_{ST} compared to site pairs with no dam between them, but genetic differentiation in the Lynches basin appears unrelated to presence of a dam (Fig. 7).

Site pairs not within the same HUC-8 sub-watershed had similar RMSE values for all models (Table 4). Posterior prediction plots for the model including a quadratic term for Euclidean distance indicated poor model fit. The most likely model included parameters for stream distance, dams, and an interaction between the two predictors (Table 4), but had similar model fit to the null model (Fig. 5), and 95% credible intervals of these parameters overlapped zero. The mean posterior estimate of log-transformed pairwise F_{ST} for site pairs in different HUC-8 sub-watersheds for the null model was -1.491 (95% CI: -1.528 – -1.455). Based on the null model, the back-transformed, mean posterior estimate of pairwise F_{ST} for site pairs in different HUC-8 sub-watersheds was 0.225 (95% CI: 0.217 – 0.233).

Dams and population genetic health

Expected heterozygosity was variable among the 30 sites in the Great Pee Dee River, Cape Fear, and Santee River basins (range: 0.216-0.622; mean $H_E \pm SD = 0.446 \pm 0.112$; Table 5). When pooled at the HUC-8 sub-watershed level, the Lynches sub-watershed had the greatest mean expected heterozygosity ($n = 8$; mean $H_E \pm SD = 0.576 \pm 0.048$), and the smallest was in the Little Pee Dee ($n = 2$; mean $H_E \pm SD = 0.336 \pm 0.060$). Inbreeding coefficients were close to zero for all sites, indicating no evidence of inbreeding (range: -0.060 – 0.114; mean $F_{IS} \pm SD = 0.020 \pm 0.052$). The number of different alleles was variable among the 30 sites, ranging from 1.826 to 5.957 (mean $N_A \pm SD = 4.127 \pm 1.236$). The Lynches sub-watershed had the greatest mean number of different alleles (mean $N_A \pm SD = 5.413 \pm 0.673$), and the Wateree sub-watershed had the lowest number of different alleles ($N_A = 2.696$). Garza-Williamson index values were similar among all 30 sites and suggested historical distribution-wide population bottlenecks (range: 0.1996 – 0.2955; mean $G\text{-}W \pm SD = 0.241 \pm 0.025$).

Null models had similar levels of support compared to the suite of models containing potential predictors of H_E, F_{IS}, N_A, and *G-W,* and RMSEs varied little across models (Table 6). The best supported model for H_E included the number of downstream dams of a site (Table 6), but the mean posterior estimate suggested H_E increased by 0.049 (95% CI: 0.015 – 0.080) with a one standard deviation increase in number of downstream dams (SD = 1.368 dams downstream). The second best supported model was the null (Table 6), where the mean posterior estimate of H_E was 0.413 (95% CI: 0.303 – 0.500) and in a posterior prediction plot had comparable fit to the most likely model (Fig. 6). The most likely models included upstream drainage area of a site for F_{IS} and N_A (Table 6), but posterior prediction plots had similar fit for the null models (Fig. 6). Similar to H_E, the most likely model of *G-W* included the number of downstream dams (Table 6), but model fit was comparable to the null (Fig. 6) Mean posterior estimates in the null models were 0.020 (95% CI: -0.007 – 0.046) for F_{IS}, 3.835 (95% CI: 2.738 – 4.868) for N_A was, and 0.241 (95% CI: 0.228 – 0.256) for *G-W* (Table 7).

Discussion

Sandhills chub displayed high levels of genetic differentiation between site pairs, but there was limited evidence that habitat fragmentation through construction of dams is related to population structuring across their entire distribution, and any relationship between dams and population structuring occurs at smaller spatial scales (i.e., sub-watershed), and is variable across sub-watersheds. A companion study of population genetic structure using the same samples indicates almost all 30 sites used in this study are distinct populations, and only two sites that were 3.7 river km apart group as a single population (Richard Harrington, SCDNR, unpublished data). Although habitat fragmentation, particularly dams, have been shown to be related to genetic differentiation of aquatic organisms, some populations have evolved in naturally fragmented habitats, exhibit minimal dispersal, and subsequently are genetically structured at fine spatial scales (Phillipsen and Lytle 2012; Baker et al. 2018; Smith et al. 2019). Headwater streams within the Carolina Sandhills ecoregion contain natural barriers such as plunge pools, log-jams, and abundant North American beaver (*Castor canadensis*) dams that may contribute to naturally low gene flow among sandhills chub populations. Sandhills chub display limited movement, and in a mark-recapture study in two of our study streams, Ramsey (2025) found that 79.2% of recaptured sandhills chubs moved less than 100 m from their initial capture location after being at large for 1-21 months. Limited dispersal may be characteristic of resident headwater fishes (Mitsuo et al. 2013; Curtis et al. 2023; Ramsey 2025). For example, Mitsuo et al. (2013) investigated movement of a headwater specialist species (*Lefua echigonia*) in Japan, and on average, 75% of recaptured fish moved less than 100 m from initial capture after being at large for 12 months. Furthermore, Creek Chub, the sister species to sandhills chub had a mean (±SD) absolute movement of 65 ± 99 m during an 11-month period (Curtis et al. 2023). Numerous studies have reported increased genetic differentiation of fishes related to dams but these studies often focused on more mobile species, in larger river systems (Dehais et al. 2010; Roberts et al. 2013; Smith et al. 2019; Eschenroeder and Roberts 2020; Escobar et al. 2024). There are fewer studies investigating the relationship of dams on gene flow in less mobile, headwater stream fishes, and their observations have been mixed (Hänfling and Weetman 2006; Hudman and Gido 2013; Schmidt and Schaefer 2018). Given the limited movement of sandhills chubs and other headwater stream specialists, naturally high levels of genetic differentiation at small spatial scales may be an inherent component of the landscape ecology of fishes in these waters regardless of anthropogenic stream fragmentation.

The most supported model of pairwise F_{ST} did not fully capture the observed variation, due to a bimodal distribution of F_{ST} values. Surprisingly, inclusion of an indicator variable for whether or not a pair of sites was within the same sub-watershed could not capture this variability. Further investigation of this bimodal relationship in post-hoc analyses found differing relationships depending on if site pairs were or were not within the same HUC-8 sub-watershed. Site pairs that were not within the same HUC-8 sub-watershed are likely separated by distances too large (interquartile ranges: stream distance = 594.7 – 479.1 km; Euclidean distance = 99.7 – 30.1 km) to be relevant given the limited dispersal of sandhills chub. In contrast, site pairs within the same HUC-8 sub-watershed, where distance between sites was comparably smaller (interquartile ranges: stream distance = 88.4 – 21.7 km; Euclidean distance = 18.5 – 6.4 km), we observed some support for isolation-by-distance and presence of a dam. This is not surprising given that other studies of headwater stream fish that observed an isolation-by-distance relationship had sites separated by distances similar to the distances our site pairs were separated by when our sites were within the same sub-watershed (Junker et al. 2012; Watson et al. 2024). However, we only observed small increases in pairwise F_{ST} as a function of increasing Euclidean distance and when a dam was present between sites for the dataset of site pairs within the same sub-watershed, although the relationship between pairwise F_{ST} and Euclidean distance appears fairly strong (see Fig. 7). Scatterplots of genetic differentiation as a function of dams, distance, and sub-watershed suggest variation in relationships among these variables across sub-watersheds. There may also be characteristics within stream networks that could explain genetic differentiation of sandhills chub. Stream and Euclidean distances may contribute differently to observed relationships in pairwise F_{ST} within sub-watersheds. If headwaters were prehistorically connected, observed genetic differentiation may be best explained by Euclidean distance, but site pairs without prehistoric connections may be best explained by stream distance. The Sandhills ecoregion is a more recent geologic formation (sediments dating to 6,000−75,000 years before present; Swezey et al. 2016) and isolation of once connected headwaters may have occurred, which can lead to genetic differentiation and eventual speciation (MacGuigan et al. 2023). Information on historical connections of streams and rivers of the Sandhills ecoregion were not available for this study, but in a companion study on genetic population structure we observed genetic grouping of sites that were geographically close (Euclidean distance) but in different sub-watersheds (not close based on stream distance; Richard Harrington, SCDNR, unpublished data). This suggests previous connections (potentially during geologic or hydrologic events) between some sites.

Although we were able to build a robust dataset of dams to investigate relationships between genetic metrics and habitat fragmentation, there are other potential anthropogenic barriers we were unable to investigate due to data limitations. Culverts are common throughout the U.S. and are a substantial cause of headwater stream fragmentation (Warren and Pardew 1998; Fuller et al. 2015; Zarri et al. 2022). Unfortunately, there is not a database for culverts in the current study region and developing one would be challenging because there are numerous types of culverts, and the degree to which they can restrict movement of fishes is variable (Warren and Pardew 1998; Macpherson et al. 2012; Briggs and Galarowicz 2013). Additionally, although many culverts occur at road crossings that can be surveyed, there are numerous but unknown numbers of culverts on private lands with more restricted access. Many studies choose to investigate movement or genetics of fishes in association with perched culverts, which can prevent upstream movement (Torterotot et al. 2014; Gido et al. 2023; Watson et al. 2024). However, streams within the Sandhills ecoregion are gently sloped in comparison to higher gradient streams (Blocksom et al. 2025), and although perched culverts are present in the Sandhills ecoregion, there are likely fewer there than within the piedmont and mountain regions of the southeastern U.S. Regardless, we acknowledge that the presence of culverts could potentially limit movement of sandhills chub and therefore patterns of genetic differentiation − better documentation is needed to investigate this relationship at localized scales where individual culverts can be more easily assessed.

Despite populations of sandhills chub being isolated with low gene flow occurring among populations, genetic diversity metrics (H_E, H_O, and N_A) tended to be relatively moderate. The Lynches, Lumber, and Deep River sub-watershed sites exhibited higher genetic diversity in comparison to the Lower Pee Dee, Little Pee Dee, Wateree, and Upper Cape Fear sub-watersheds. However, sandhills chub heterozygosity was similar to values observed in studies of their widely distributed sister species, the creek chub (H_E: 0.417 – 0.572; Skalski et al. 2008; mean $H_E = 0.58$; Schmidt and Schaefer 2018). The Lumber and Lynches sub-watersheds contained the greatest number of sites per sub-watershed (n = 7 and 8 respectively) and appear to be strongholds for sandhills chub populations, so it is unsurprising they contained greater genetic variation. Our analyses of genetic diversity and health metrics as a function of upstream drainage area from a site, free-flowing stream length, number of dams downstream from a site suggested relationships that were likely spurious (e.g., a small increase in H_E with increasing numbers of dams downstream), inconclusive (e.g., small effect sizes of upstream drainage area with 95% credible intervals overlapping for models of F_{IS} and N_A), or subtle (a -0.013 [95% CI: -0.024 − -0.002) decrease in $G\text{-}W$ based on a 1 SD increase in the number of dams). Previous studies investigating relationships between downstream dams and genetic diversity have been mixed (Yamamoto et al. 2004; Deiner et al. 2007; Reid et al. 2008; Van Leeuwen et al. 2018). Greater numbers of alleles were observed in white-spotted charr in reaches downstream of dams compared to sites upstream of dams (Yamamoto et al. 2004). In contrast, genetic diversity of rainbow trout in reaches downstream of dams was similar to upstream reaches (Deiner et al. 2007). Co-evolution with North American beavers may also have played a role in the life history of sandhills chub resulting in the observed landscape ecology that appears independent of anthropogenic dams. North American beaver dams can act as barriers and limit movement of fishes, especially during low flows

(Schlosser 1995; Cutting et al. 2018), although beaver dams have successional stages and are not permanent (Naiman et al. 1988). North American beaver dams are prevalent within the Sandhills ecoregion, which may have led to adaptations in sandhills chub that allow for maintaining genetic diversity in naturally fragmented streams. Although genetic diversity is often related to population size because larger populations can minimize effects of genetic drift (Allendorf et al. 2022), we observed relatively high genetic diversity despite small N_{E} estimates, thus providing evidence that sandhills chubs have adaptations for maintaining.

Despite populations from most of the study sites exhibiting moderate genetic diversity, we observed several sites with lower genetic diversity relative to others ($N_{\mathrm{A}} \leq 3.043$, H_{E} and $H_{\mathrm{O}} < 0.3$). These sites tended to be more isolated from other healthy sandhills chub populations or at the edge of the sandhills ecoregion, compared to sites in the Lumber and Lynches basins which are less isolated and located more interiorly. Lower genetic diversity of populations that are isolated or at the edge of their distribution are not uncommon (Fluker et al. 2010; Pregler et al. 2018). However, since there is no prior data for genetic diversity of sandhills chub, we are unable to detect if genetic variation has been changed from prehistoric levels.

Even though populations were isolated, there was no evidence of inbreeding depression. Absence of inbreeding was surprising because only a few pit-ridge nests were observed in two streams with healthy sandhills chub populations that were intensively sampled during the spawning period (Ramsey 2025) and contemporary effective populations sizes in 1400 m reaches of these same streams were small ($N_{\mathrm{E}} = 25.2$ [95% CI: 18.7 – 35.9] and $N_{\mathrm{E}} = 26.4$ [95% CI: 19 – 39.3]; Richard Harrington, SCDNR, unpublished data). Additionally, only a few mature individuals (based on presence of tubercles on males or expression of gametes) were collected in these streams during the spawning period. Given these observations we expected fewer individuals breeding within populations, and increased inbreeding, which has been observed in other species (e.g., O'Leary et al. 2013; Stock et al. 2023). There is limited information on sandhills chub reproduction, other than description of their pit-ridge nests and timing of spawning (Maurakis et al. 1990), so there is potentially an unknown mating behavior that may better explain the lack of inbreeding while existing in relatively isolated populations with low gene flow among populations. For example, individuals in isolated populations of brook trout were observed to be polygamous (Kanno et al. 2011), and both sexes of creek chub were observed to have multiple partners during spawning (Reighard 1910). Mating with multiple partners during a given spawning season reduces the probability a given mating event is with a full-sibling, potentially reducing the number of offspring that are inbred. Future research into kinship may provide insight about sandhills chub resistance to inbreeding depression through reproductive behavior.

The observed *G-W* values were less than 0.68 at all sites (values <0.68 indicate reduced population sizes and historical bottlenecks; Garza and Williamson, 2001). Bottlenecks can reduce genetic variation and adaptive potential of species (Allendorf et al. 2022), which could be of concern for sandhills chub. Although this study indicated a subtle relationship between *G-W* values and anthropogenic fragmentation, other studies have observed stronger relationships between population bottlenecks and habitat fragmentation (Neville et al. 2006; Fluker et al. 2019). For example, Fluker et al. (2019) observed bottlenecks in blueface darter (*Etheostoma cyanoprosopum*), an endemic headwater stream fish, that were related to impoundments and isolation. However, the Garza-Williamson index has the potential to be a function of small founding populations (Wolnicki et al. 2024) and may be indicative of the biogeographic history of headwater specialists and limited dispersal.

Although genetic diversity of sandhills chub was moderate, their populations are vulnerable to further habitat loss and anthropogenic disturbances, especially in the Wateree basin where sandhills chub exist in a limited number of streams and are more isolated than populations in the Great Pee Dee River basin. A challenge for studies investigating relationships between genetic health and habitat condition or fragmentation is that sites with small populations are often excluded from investigations because sample sizes are too small for robust statistical analyses. Our study sites tended to include areas with good habitat and a greater number of individuals, but we sampled fifteen sites that were less densely populated, which may be at greater risk from random events or anthropogenic disturbance (Fischer and Lindenmayer 2007). Therefore, our results may be indicative of relatively healthy sandhills chub populations, which seemingly occur regardless of the level of habitat fragmentation. Considering the projected increase in urbanized land in the southeastern U.S. (Terando et al. 2014), and large number of dams fragmenting stream networks across the United States (Cooper et al. 2017), management of fragmented and isolated species is of increasing importance. More broadly, this study is applicable to other species existing in isolated populations or fragmented landscapes and supports other studies which did not see reduced genetic diversity as a function anthropogenic fragmentation (Hou and Lou 2011; Sunny et al. 2014). As demonstrated here, species that exist in naturally fragmented landscapes may have ecological adaptations that preclude consequences from anthropogenic habitat fragmentation (Chiucchi and Gibbs 2010; Matesanz et al. 2018). Thus, our project results provide important baseline data and ecological understanding for monitoring sandhills chub populations given expected increases in anthropogenic habitat fragmentation and alteration via urbanization. Additionally, the realization that conservation of isolated headwater stream specialists in general will be complex and requires co-investigations of population genetics, behavior, dispersal, life history, and natural and anthropogenic forms of fragmentation is important for successful management of these unique species.

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Authorship

RWP, DPC, BJ, TLD, MS, JJH, JR., CB and RH designed the study. RWP and ZAR conducted field sampling. All genetics work was performed and overseen by RH and TLD. RWP and DPC analyzed the data. Data interpretation, writing and revising of manuscript were performed by all authors.

Data Sharing and Data Availability

Matrices and model code available upon reasonable requests.

Benefit-Sharing Statement

Benefits from this research have been shared with the appropriate agencies and partners, as well as those within the scientific community.

Ethical Treatment Statement

All sampling was approved and conducted under Coastal Carolina University IACUC protocol number 2021.003. Sampling was conducted in coordination with and authorized by the South

Carolina Department of Natural Resources, North Carolina Wildlife Resources Commission, and the U.S. Department of Defense.

Tables

Table 1. Average pairwise F_{ST} values ($\mu \pm SD$) for each HUC-8 sub-watershed comparison in the Great Pee Dee basin (R.Harrington, SCDNR, unpublished data). Average Euclidean distance between sites (km), average stream distance between sites (km), and average number of dams between sites for each HUC-8 sub-watershed comparison.

Comparison	*n* pairs	F_{ST}	Euclidean Distance (km)	Stream Distance (km)	Dams
Little Pee Dee - Little Pee Dee	1	0.064 ± NA	5.85	44.93	2.00
Little Pee Dee - Lower Pee Dee	15	0.319 ± 0.075	35.07	495.14	3.27
Lower Pee Dee - Lower Pee Dee	21	0.206 ± 0.095	40.47	82.11	3.14
Lumber - Little Pee Dee	14	0.179 ± 0.040	28.68	379.19	1.29
Lumber - Lower Pee Dee	48	0.258 ± 0.071	40.53	588.70	2.73
Lumber - Lumber	21	0.046 ± 0.025	12.21	31.67	0.57
Lumber - Lynches	56	0.186 ± 0.026	106.03	595.40	0.66
Lynches - Little Pee Dee	16	0.239 ± 0.026	86.09	499.27	1.38
Lynches - Lower Pee Dee	56	0.248 ± 0.053	68.34	465.47	2.82
Lynches - Lynches	28	0.054 ± 0.022	9.06	53.30	0.68

NA - Only one comparison

Table 2. Leave-one-out cross validation outputs for pairwise F_{ST} models. Difference in expected log pointwise predictive density (ΔELPD) of zero indicates the most supported model. Models with ΔELPD less than four have comparable model performance to top model.

Model	ΔELPD	ΔSE
~Dams + WS	0†	0.000
~Dams + Eudistance + WS + (Eudistance x dams)	-0.927†	0.199
~Dams + Eudistance + WS	-1.585†	0.393
~WS	-2.026†	2.603
~Eudistance + WS	-2.953†	2.763
~Sdistance + dams + (Sdistance x dams)	-10.096	4.638
~Sdistance + dams	-10.730	4.785
~Sdistance	-13.142	4.594
~Dams + Sdistance + WS + (Sdistance x dams)	-40.460	10.712
~Eudistance + dams + (Eudistance x dams)	-40.495	10.738
~Eudistance + dams	-48.393	10.783
~Eudistance	-56.592	10.986
~Dams	-67.110	12.459
~Intercept	-73.144	13.107

† *Denotes models with lowest ΔELPD (< 4) favoring support*

Dams = number of dams between site pairs.

WS = if site pairs were within the HUC-8 same-subwatershed.

Eudistance = Euclidean distance between sites (km).

Sdistance = stream distance between sites (km).

Table 3. Root mean square errors (RMSE) from 10-fold cross-validation for pairwise F_{ST} of site pairs within the same HUC-8 sub-watershed. Smaller RMSE indicates better model support. Mean percent predicted error = $(exp(RMSE) - 1)\ x\ 100$.

Site Pairs within the Same Sub-watershed		
Model	log-scale RMSE	Mean Percent Predicted Error
Euclidean Distance + Dam Presence + Euclidean Distance x Dam Presence + Watershed	0.419	52.0%
Euclidean Distance + Dam Presence + Watershed	0.426	53.1%
Stream Distance + Dam Presence + Stream Distance x Dam Presence + Watershed	0.444	55.9%
Stream Distance + Dam Presence + Watershed	0.457	57.9%
Stream Distance + Watershed	0.462	58.6%
Euclidean Distance + Watershed	0.472	60.3%
Dam Presence + Watershed + Dam Presence x Watershed	0.509	66.4%
Euclidean Distance + Dam Presence	0.519	68.0%
Intercept + Watershed	0.538	71.2%
Euclidean Distance + Dam Presence + Euclidean Distance x Dam Presence	0.543	72.1%
Stream Distance + Dam Presence + Stream Distance x Dam Presence	0.560	75.0%
Stream Distance + Dam Presence	0.564	75.8%
Euclidean Distance	0.612	84.5%
Stream Distance	0.671	95.6%
Dam Presence	0.693	100.0%
Null	0.819	126.8%

† Lowest RMSE and re-ran with all 71 observations to get mean posteriors and credible intervals

Table 4. Root mean square errors (RMSE) from 10-fold cross-validation for pairwise F_{ST} of site pairs not within the same HUC-8 sub-watershed. Smaller RMSE indicates better model support. Mean percent predicted error = $(exp(RMSE) - 1)\ x\ 100$. RMSE for quadratic model is in pairwise F_{ST} scale.

Site Pairs in Different Sub-watersheds			
Model	Log-scale RMSE	Mean Percent Predicted Error	RMSE
Stream Distance + Dam Presence + Stream Distance x Dam Presence	0.257	29.3%	
Stream Distance	0.258	29.5%	
Stream Distance + Dam Presence	0.258	29.4%	
Euclidean Distance + Dam Presence + Euclidean Distance x Dam Presence	0.258	29.4%	
Dam Presence	0.261	29.9%	
Euclidean Distance	0.262	30.0%	
Euclidean Distance + Dam Presence	0.263	30.0%	
Null	0.262	30.0%	
Euclidean Distance + Euclidean Distance^2			0.0482

† Lowest RMSE and re-ran with all 205 observations to get mean posteriors and credible intervals

Table 5. Sites where sandhills chubs were sampled in North and South Carolina, and their associated values of expected heterozygosity (H_E), observed heterozygosity (H_O), number of different alleles (N_A), inbreeding coefficient (F_{IS}), and the Garza-Williamson Index (*G-W*) (R. Harrington, SCDNR, unpublished data).

HUC-8	Site	*n*	H_E	H_O	F_{IS}	*G-W*	N_A
Deep River	DR1	30	0.459	0.461	0.001	0.270	4.000
Little Pee Dee	LPD1	30	0.379	0.357	0.022	0.224	3.522
Little Pee Dee	LPD2	30	0.294	0.271	0.055	0.265	3.043
Lower Pee Dee	LOPD1	30	0.464	0.45	0.082	0.219	4.609
Lower Pee Dee	LOPD2	30	0.459	0.482	-0.044	0.245	3.565
Lower Pee Dee	LOPD3	30	0.438	0.45	-0.028	0.223	3.783
Lower Pee Dee	LOPD4	30	0.433	0.421	0.025	0.213	4.217
Lower Pee Dee	LOPD5	30	0.216	0.198	0.109	0.254	1.826
Lower Pee Dee	LOPD6	22	0.245	0.241	0.009	0.295	1.913
Lower Pee Dee	LOPD7	30	0.279	0.299	-0.06	0.248	2.522
Lumber	LU1	28	0.474	0.454	0.064	0.256	4.217
Lumber	LU2	30	0.516	0.524	-0.006	0.238	5.783
Lumber	LU3	30	0.488	0.509	-0.043	0.238	4.826
Lumber	LU4	30	0.378	0.372	0.007	0.217	3.304
Lumber	LU5	30	0.503	0.508	-0.019	0.256	5.304
Lumber	LU6	30	0.451	0.455	-0.006	0.240	3.913
Lumber	LU7	30	0.495	0.474	0.06	0.222	4.826
Lynches	LY1	30	0.606	0.616	-0.018	0.222	5.826
Lynches	LY2	28	0.612	0.571	0.068	0.256	5.957
Lynches	LY3	30	0.586	0.566	0.064	0.200	5.478
Lynches	LY4	30	0.622	0.627	-0.003	0.266	5.826
Lynches	LY5	30	0.545	0.567	-0.048	0.269	5.217
Lynches	LY6	30	0.474	0.499	-0.056	0.286	3.87
Lynches	LY7	30	0.579	0.544	0.068	0.210	5.391
Lynches	LY8	30	0.585	0.546	0.114	0.229	5.739
Upper Cape Fear	UCF1	30	0.373	0.364	0.033	0.207	3.217
Upper Cape Fear	UC2	30	0.266	0.238	0.082	0.233	2.217
Upper Cape Fear	UCF3	30	0.393	0.37	0.097	0.212	3.652
Upper Cape Fear	UC4	30	0.379	0.394	-0.013	0.233	3.565
Wateree	WA1	29	0.389	0.429	-0.021	0.271	2.696
		Mean ± SD	0.446 ± 0.112	0.442 ± 0.113	0.020 ± 0.052	0.241 ± 0.025	4.127 ± 1.236
		Range	0.216 - 0.622	0.198 - 0.627	-0.06 - 0.114	0.199 - 0.296	1.826 - 5.957

Table 6. Root mean square errors (RMSE) for five-fold cross validation for H_E, F_{IS}, N_A, and *G-W*. Smaller RMSE values indicate greater model support.

H_E		N_A	
Model:	**RMSE**	**Model**	**RMSE**
Downstream dams	0.065†	Drainage area	0.926†
Null	0.073†	Null	0.973†
Downstream impoundments + free-flowing reach	0.074	Downstream dams	1.024
Free-flowing reach	0.074	Free-flowing reach	1.052
Drainage area	0.079	Downstream impoundments + free-flowing reach	1.064
Drainage area + free-flowing reach	0.080	Drainage area + downstream dams	1.069
Drainage area + downstream dams + free-flowing reach	0.082	Drainage area + downstream dams + free-flowing reach	1.079
Drainage area + downstream dams	0.089	Drainage area + free-flowing reach	1.141
F_{IS}		***G-W***	
Model	**RMSE**	**Model**	**RMSE**
Drainage area	0.050†	Downstream dams	0.024†
Drainage area + downstream dams + free-flowing reach	0.054	Downstream impoundments + free-flowing reach	0.025
Free-flowing reach	0.055	Drainage area + downstream dams + free-flowing reach	0.026
Downstream dams	0.055	Null	0.027†
Drainage area + free-flowing reach	0.056	Drainage area + downstream dams	0.027
Downstream impoundments + free-flowing reach	0.060	Drainage area	0.028
Drainage area + downstream dams	0.060	Free-flowing reach	0.028
Null	0.061†	Drainage area + free-flowing reach	0.037

† Smallest RMSE and re-ran with all 30 observations to get mean posteriors and credible intervals

Table 7. Mean posterior parameter estimates and 95% credible intervals for the best supported models for expected heterozygosity (H_E), observed heterozygosity (H_O), number of different alleles (N_A), inbreeding coefficient (F_{IS}), the Garza-Williamson Index (*G-W*), and pairwise F_{ST}.

Genetic Metric	Model	Variable	Posterior Mean Estimate (95% CI)
H_E	~ Intercept	Intercept	0.413 (0.303 −0.500)
F_{IS}	~ Intercept	Intercept	0.020 (-0.007 −0.046)
N_A	~ Intercept	Intercept	3.835 (2.738 −4.868)
G-W	~ Intercept	Intercept	0.241 (0.228 −0.256)
Pairwise F_{ST} (all Great Pee Dee comparisons)	~ Dams + Watershed Indicator		
		Intercept	0.100 (0.082 −0.118)
		Dams	0.011 (0.002 −00.019)
		Watershed Indicator	0.132 (0.112 −0.153)
log(Pairwise F_{ST}) (site pairs within same sub-watershed)	~ log(Euclidean Distance) + Dam Presence + Watershed ID		
		Intercept	-3.866 (-4.702 −-3.047)
		log(Euclidean Distance)	0.372 (0.255 −0.486)
		Dam Presence	0.449 (0.221 −0.678)
		Lower Pee Dee	0.476 (-0.317 −1.290)
		Lumber	-0.403 (-1.193 −0.418)
		Lynches	-0.193 (-0.967 −0.598)
log(Pairwise FST) (site pairs in different sub-watersheds)	~ Intercept		
		Intercept	-1.492 (-1.529 −-1.454)

Figures

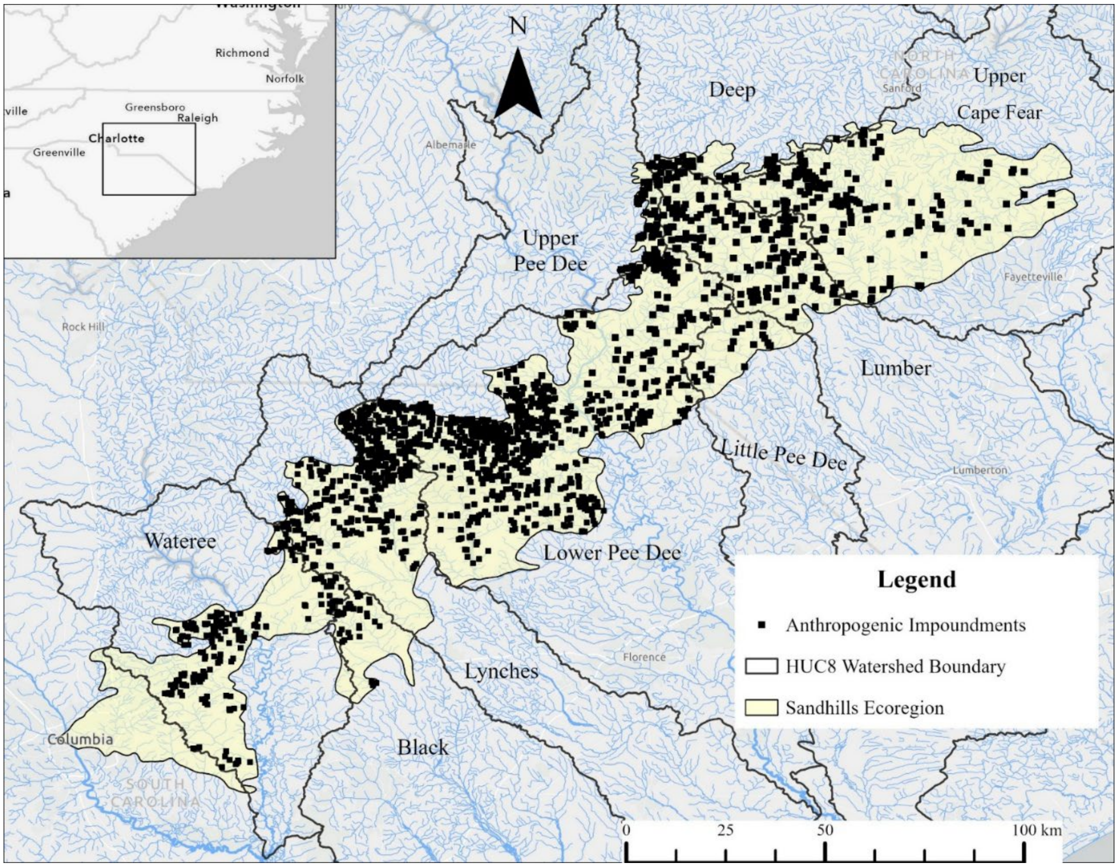

Figure 1. Dams in the Sandhills ecoregion of North Carolina and South Carolina. Basemaps via Esri 2025, Watershed boundary via USGS 2024, flowlines from USGS and USEPA NHDPlusV2, and ecoregion from USEPA 2012.

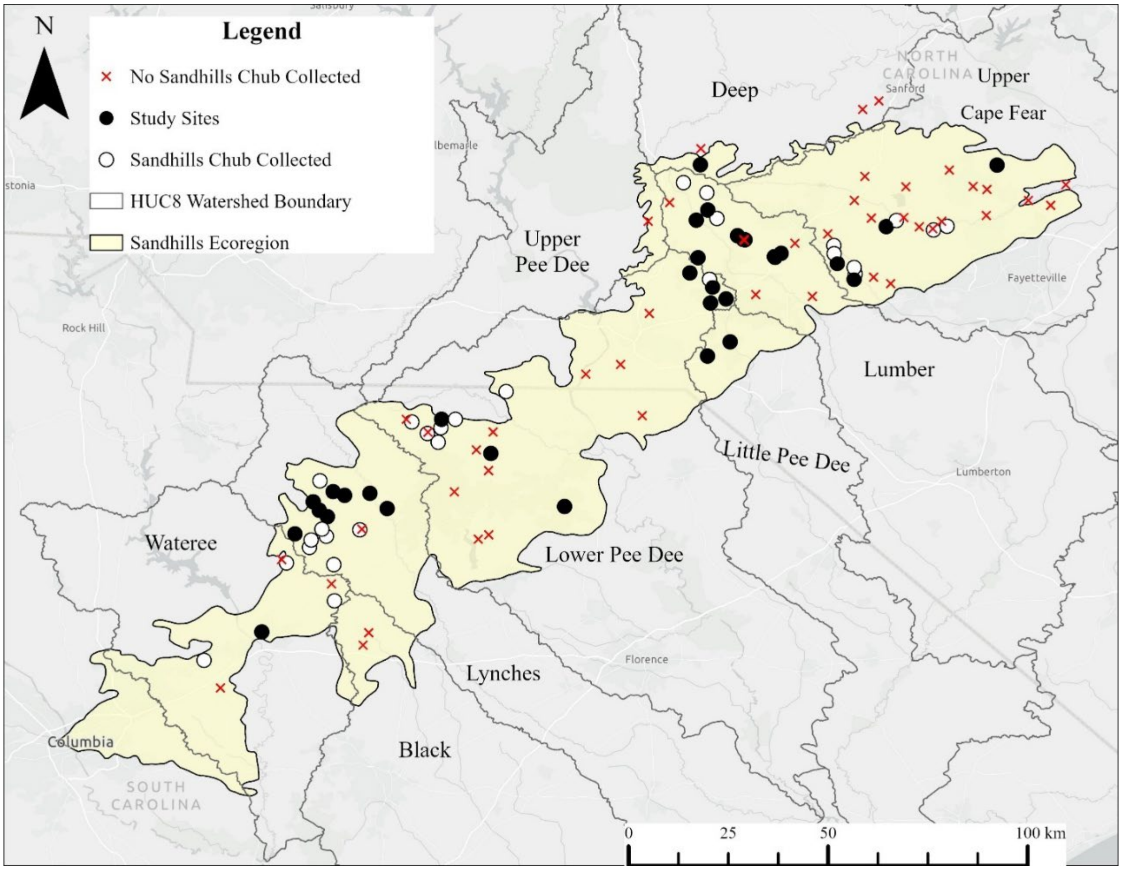

Figure 2. Locations where at least one sandhills chub was collected (hollow circle with black outline), or where no sandhills chubs were collected (red x's), during 2022−2024. Sites included in the genetic analyses (black points; 22−30 samples per site) were primarily sampled during 2022−2024, but archived samples from 2020 were used to increase sample sizes for some locations in South Carolina (10 sites). Basemap layer via Esri 2025, water boundary from USGS 2024, and ecoregion layer from USEPA 2012.

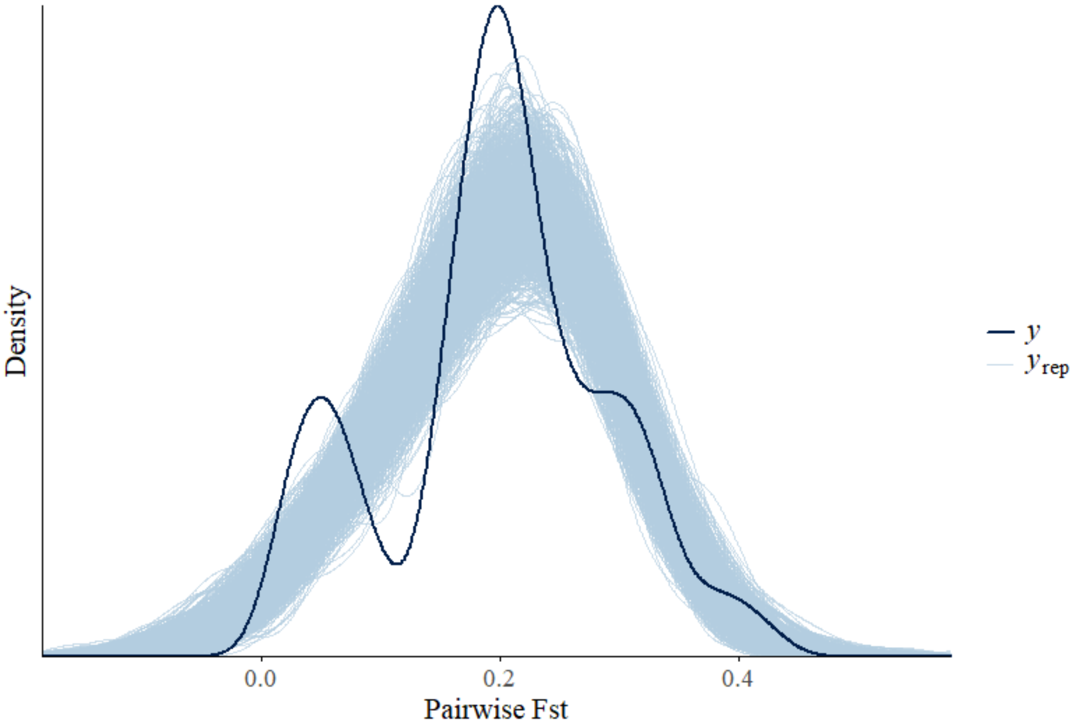

Figure 3. Posterior predictions of pairwise F_{ST} (blue lines) overlayed with the observed pairwise F_{ST} (black line) for the model including the number of dams between site pairs and an indicator variable denoting if site pairs were within the same sub-watershed.

Model : Pairwise $F_{\mathrm{ST}} = \beta_0 + \beta_{dams} \times dams_i + \beta_{sub-watershed\,indicator} \times sub-watershed_i$

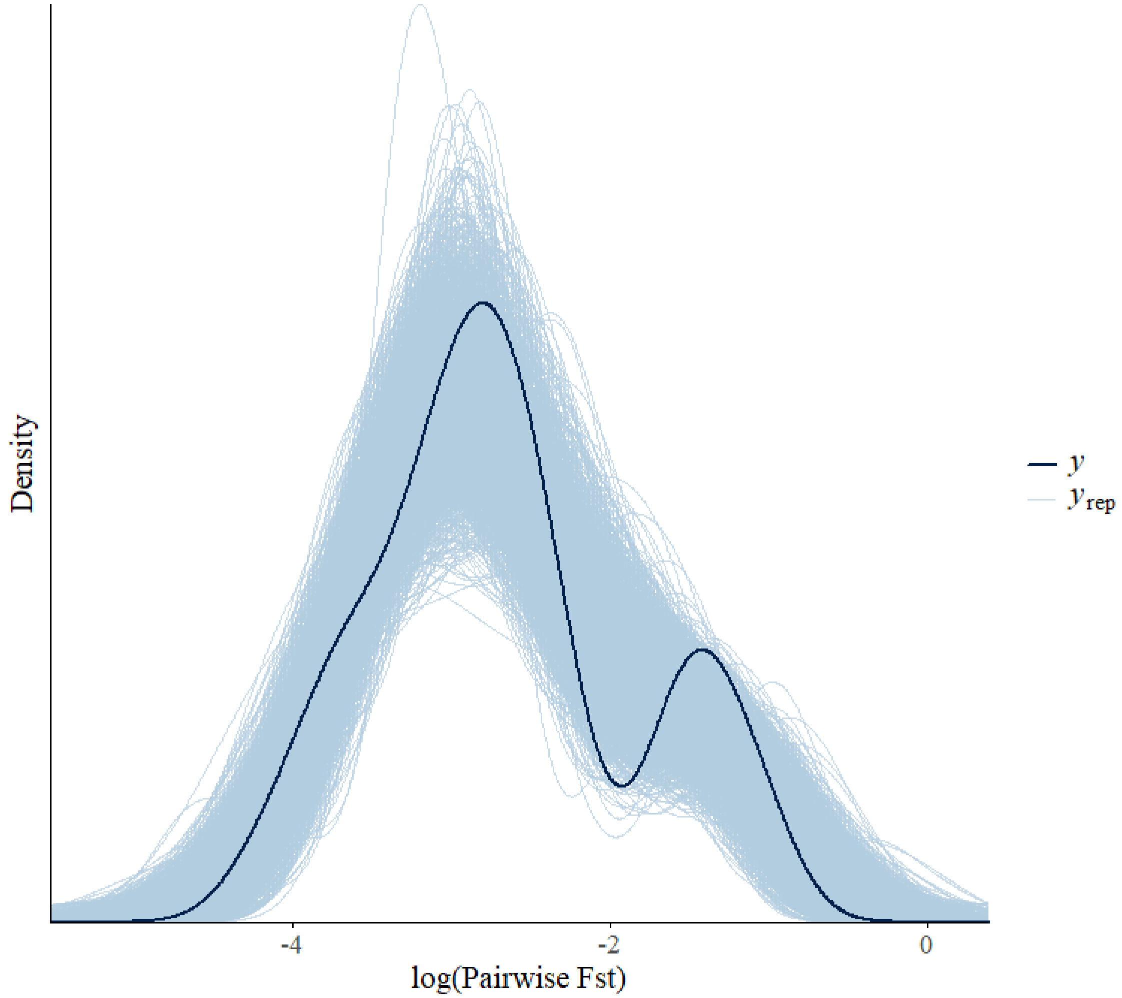

Figure 4. Posterior predictions of log-transformed pairwise F_{ST} (blue lines) overlayed with observed values (black line) for the model with smallest RMSE for site pairs within the same HUC-8 sub-watershed. Model: log(pairwise F_{ST}) = $\beta_0 + \beta_{log(Euclidean\ distance)} \times log(Euclidean\ distance)_i + \beta_{Dam\ presence} \times Dam\ Presence_i + \beta_{Watershed\ ID} \times Watershed_i$

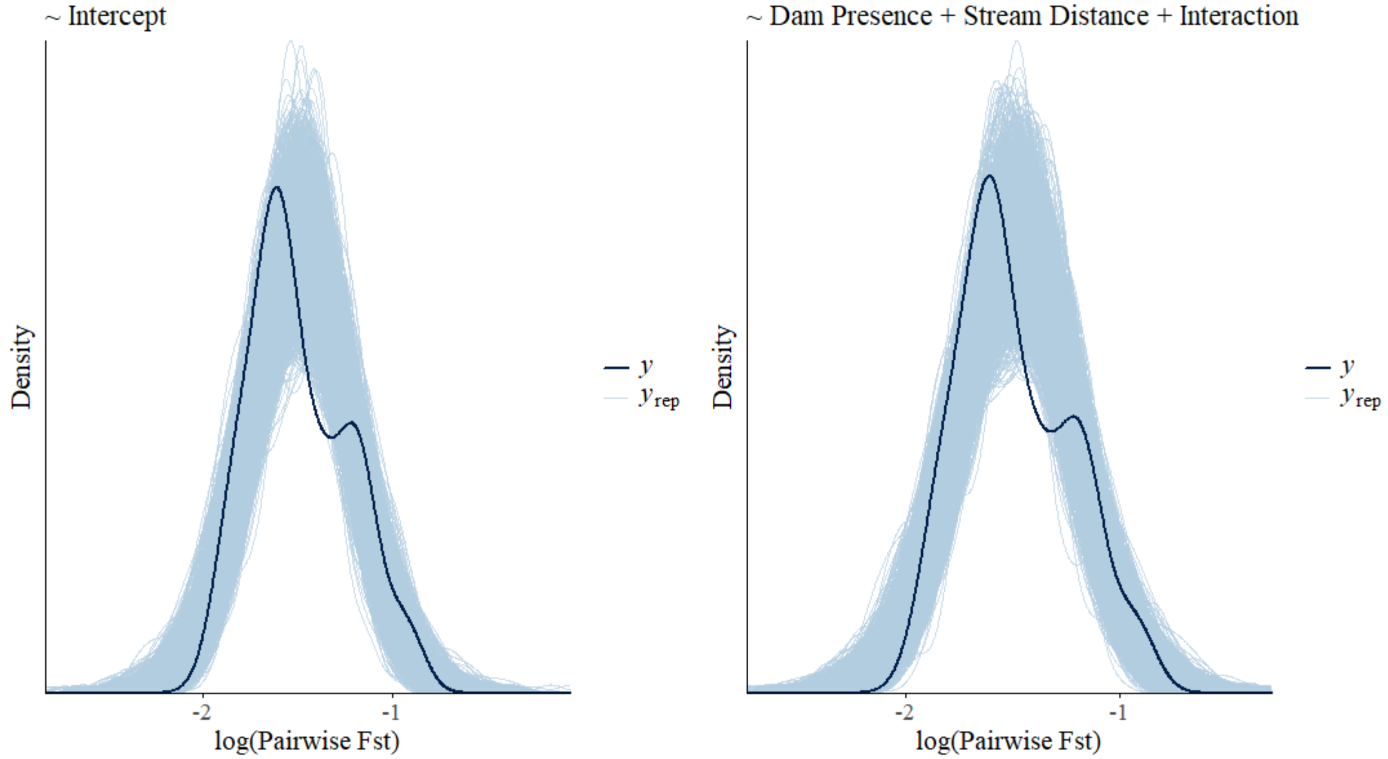

Figure 5. Posterior predictions of log-transformed pairwise F_{ST} (blue lines) overlayed with observed values (black line) for the null model (left) and model with smallest RMSE (right) for site pairs that were not within the same HUC-8 sub-watershed. Left model: log(pairwise F_{ST}) = β_0. Right model : log(pairwise F_{ST}) = $\beta_0 + \beta_{Dam\ presence} \times Dam\ presence_i + \beta_{log\ (stream\ distance)} \times log\ (stream\ distance)_i + \beta_{Interaction} \times log\ (Stream\ distance)_i \times Dam\ Presence_i$.

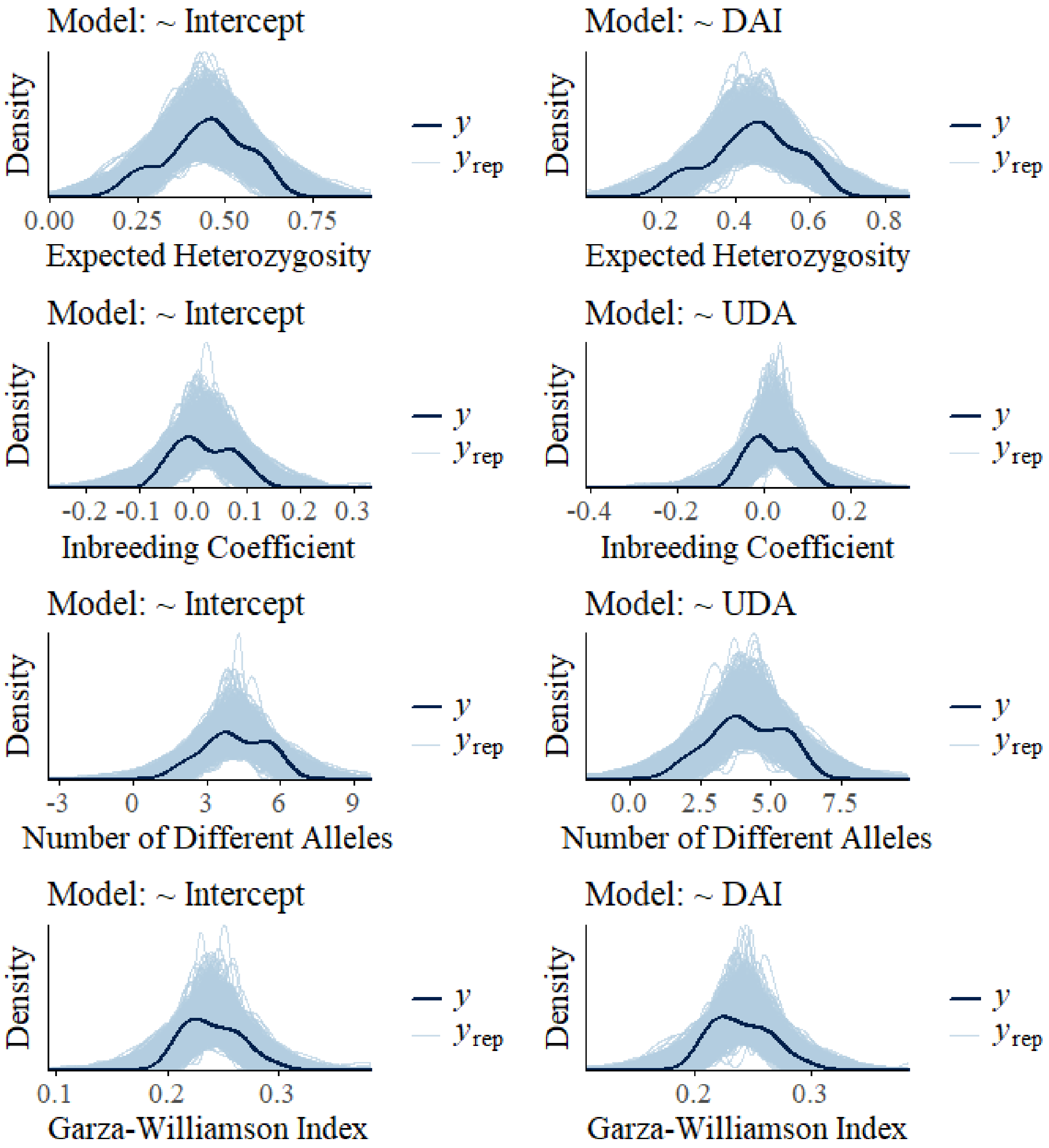

Figure 6. Posterior predictions of H_E, F_{IS}, N_A, and *G-W* (blue lines) overlayed with observed H_E, F_{IS}, N_A, and *G-W* values (black line) for models with smallest RMSE which indicates better model performance. Model names are listed at the top corner of each plot. UDA is the upstream drainage area (km^2) and DAI is the number of dams downstream of a site.

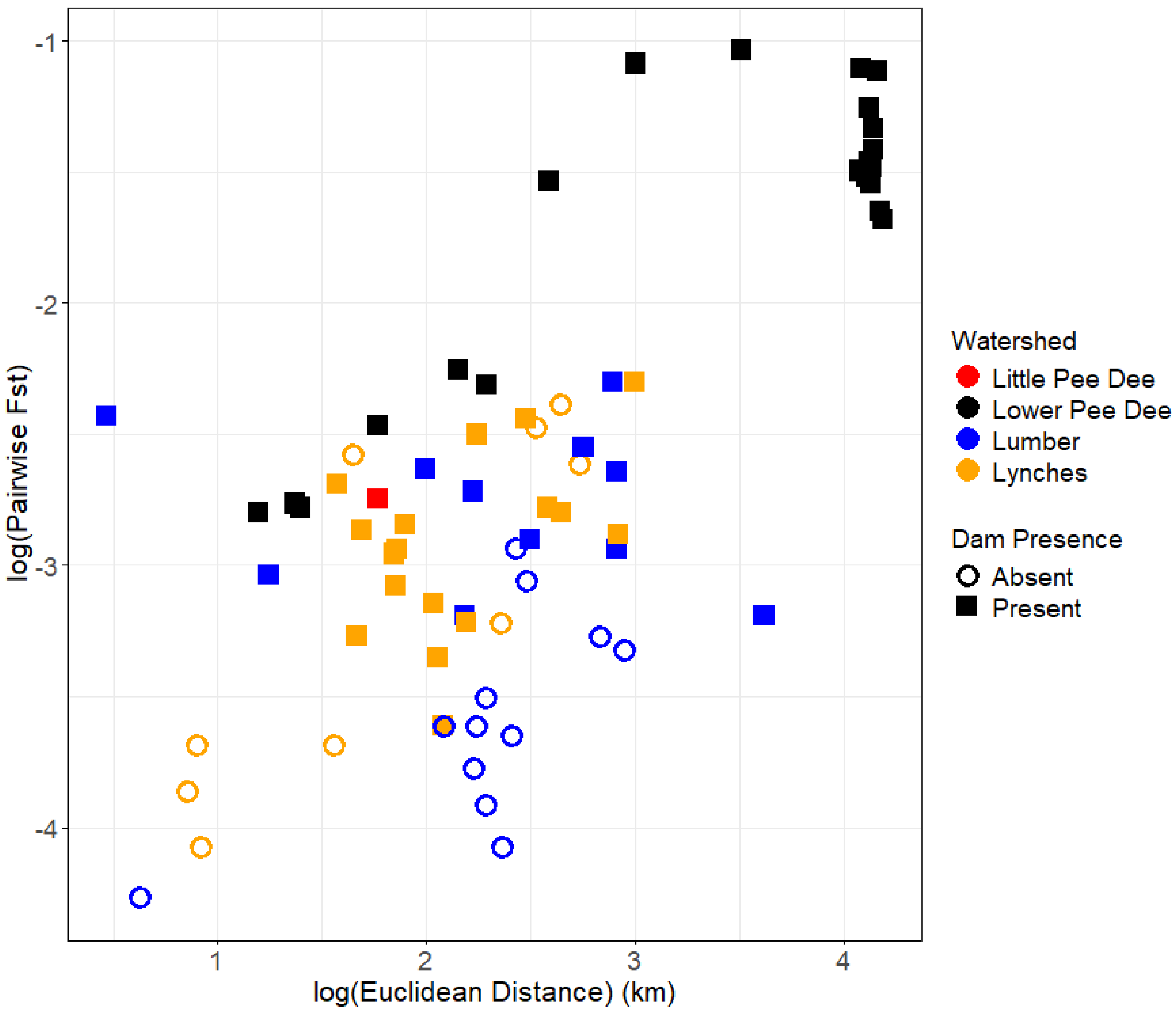

Figure 7. Scatterplot of log-transformed Euclidean distance (km) on x-axis with log-transformed pairwise F_{ST} for site pairs within the same HUC-8 sub-watershed on the y-axis. Dot color represents the HUC-8 sub-watershed a site pair is within. Dot shape represents whether there is a dam present between a site pair.

Appendix

Table A.1. Sites where sandhills chub were sampled in North and South Carolina, and their associated stream site values for upstream drainage area (km^2), number of dams downstream, and length of free-flowing stream distance nested within a site (km).

HUC-8	Site	n	Upstream Drainage Area (km^2)	Dams Downstream	Free-Flowing Stream Length (km)
Deep River	DR1	30	7.97	2	14.22
Little Pee Dee	LPD1	30	2.42	2	3.22
Little Pee Dee	LPD2	30	4.59	0	33.54
Lower Pee Dee	LOPD1	30	2.94	3	56.60
Lower Pee Dee	LOPD2	30	2.65	1	10.16
Lower Pee Dee	LOPD3	30	2.97	4	12.11
Lower Pee Dee	LOPD4	22	8.45	0	5.23
Lower Pee Dee	LOPD5	30	24.20	1	54.97
Lower Pee Dee	LOPD6	30	4.04	4	3.37
Lower Pee Dee	LOPD7	30	8.18	4	25.70
Lumber	LU1	28	4.26	1	101.38
Lumber	LU2	30	2.26	0	129.59
Lumber	LU3	30	1.89	1	1.68
Lumber	LU4	30	3.73	0	51.45
Lumber	LU5	30	0.83	0	10.67
Lumber	LU6	30	1.69	1	2.40
Lumber	LU7	30	2.31	0	103.10
Lynches	LY1	30	3.43	0	143.64
Lynches	LY2	30	5.91	1	30.45
Lynches	LY3	30	7.71	0	128.52

Lynches	LY4	30	2.51	0	93.98
Lynches	LY5	28	1.46	0	7.18
Lynches	LY6	30	2.09	1	37.17
Lynches	LY7	30	5.67	1	3.82
Lynches	LY8	30	2.17	0	22.30
Upper Cape Fear	UCF1	30	5.75	2	131.57
Upper Cape Fear	UCF2	30	4.56	2	131.57
Upper Cape Fear	UCF3	30	2.51	4	448.05
Upper Cape Fear	UCF4	30	2.50	2	6.08
Wateree	WA1	29	2.26	2	5.12
		Mean	4.46	1.30	60.29
		Range	0.83 - 24.20	0 - 4	1.68 - 448.05

Table A.2. Ranges and mean values for continuous predictor variables in F_{ST} analysis, separated by if a site pair was within the same sub-watershed, as well as total values for all 276 comparisons.

	Mean PWB (#)	PWB Range (#)	Euclidean Distance (km)	Euclidean Distance Range (km)	Stream Distance (km)	Stream Distance Range (km)
Across	2.02	0 - 6	68.37	4.18 - 122.25	528.7	367.18 - 617.79
Within	1.39	0 - 5	19.24	1.59 - 65.79	55.3	2.79 - 129.25
Total	1.86	0 - 6	55.73	1.59 - 122.25	406.95	2.79 - 617.79

PWB is the number of dams between sites.

Table. A3. Mean (± SD) values (excluding Deep River and Wateree where n = 1) of expected heterozygosity (H_E), observed heterozygosity (H_O), number of different alleles (N_A), inbreeding coefficient (F_{IS}), and the Garza-Williamson Index (*G-W*) for each HUC-8 sub-watershed (R. Harrington, SCDNR, unpublished data).

HUC-8	n	H_E	H_O	F_{IS}	N_A	*G-W*
Deep River	1	0.459	0.461	0.001	4	0.27
Little Pee Dee	2	0.336 (± 0.060)	0.314 (± 0.061)	0.038 (± 0.023)	3.282 (± 0.338)	0.244 (± 0.029)
Lower Pee Dee	6	0.362 (± 0.110)	0.363 (± 0.115)	0.013 (± 0.064)	3.205 (± 1.118)	0.242 (± 0.028)
Lumber	7	0.472 (± 0.046)	0.471 (± 0.052)	0.008 (± 0.040)	4.596 (± 0.846)	0.238 (± 0.015)
Lynches	8	0.576 (± 0.048)	0.567 (± 0.041)	0.024 (± 0.063)	5.413 (± 0.673)	0.242 (± 0.031)
Upper Cape Fear	4	0.353 (± 0.058)	0.342 (± 0.070)	0.05 (± 0.050)	3.163 (± 0.658)	0.221 (± 0.014)
Wateree	1	0.389	0.429	-0.021	2.696	0.271

n is the number of sites in a given HUC-8 sub-watershed

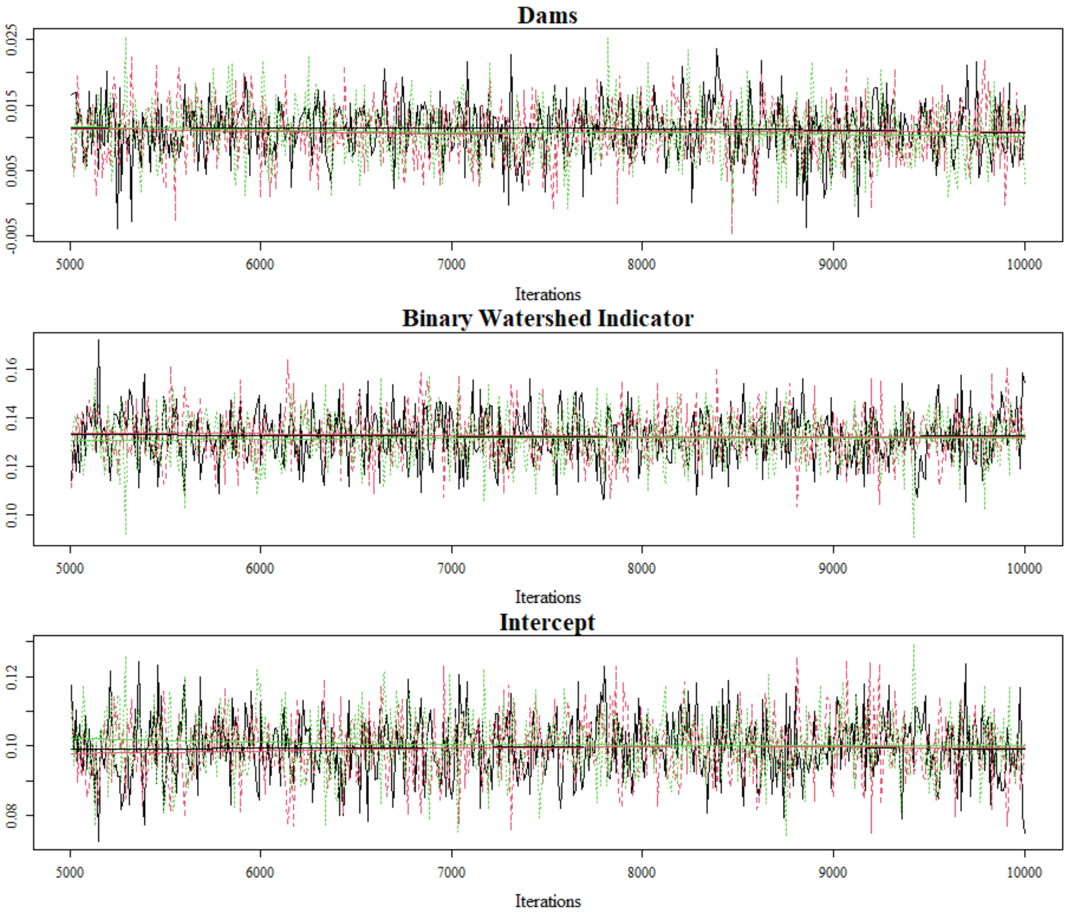

Figure A.1. Traceplots of MCMC simulations for parameters in the best supported model for pairwise F_{ST} indicating model convergence.

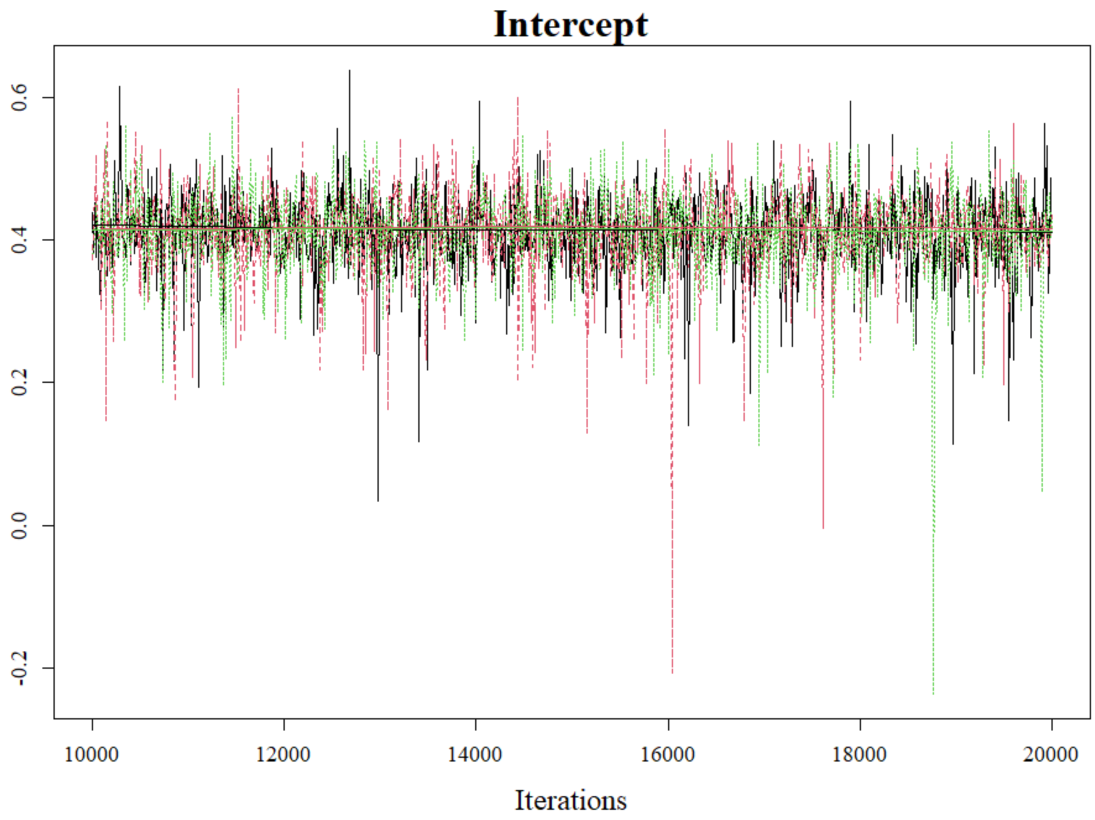

Figure A.2. Traceplot of MCMC simulations for intercept in the best supported model for expected heterozygosity, which indicates model convergence.

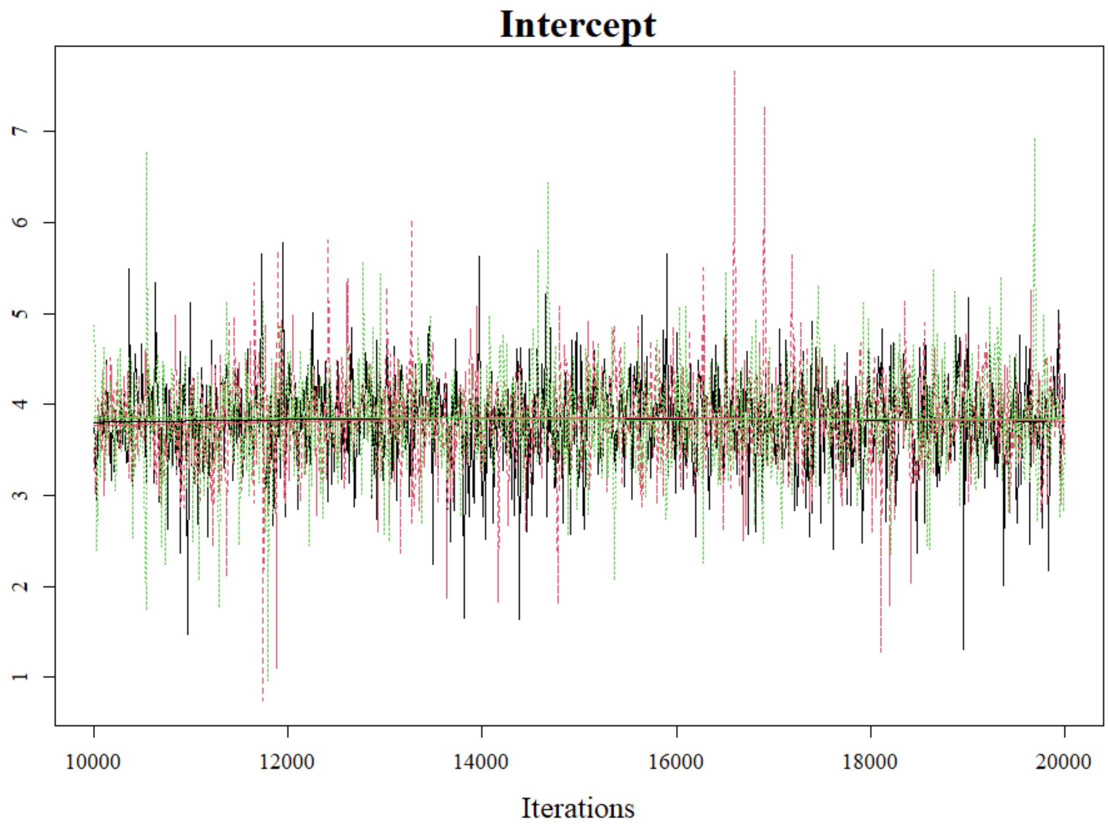

Figure A.3. Traceplot of MCMC simulations for intercept in the best supported model for the number of different alleles, which indicates model convergence.

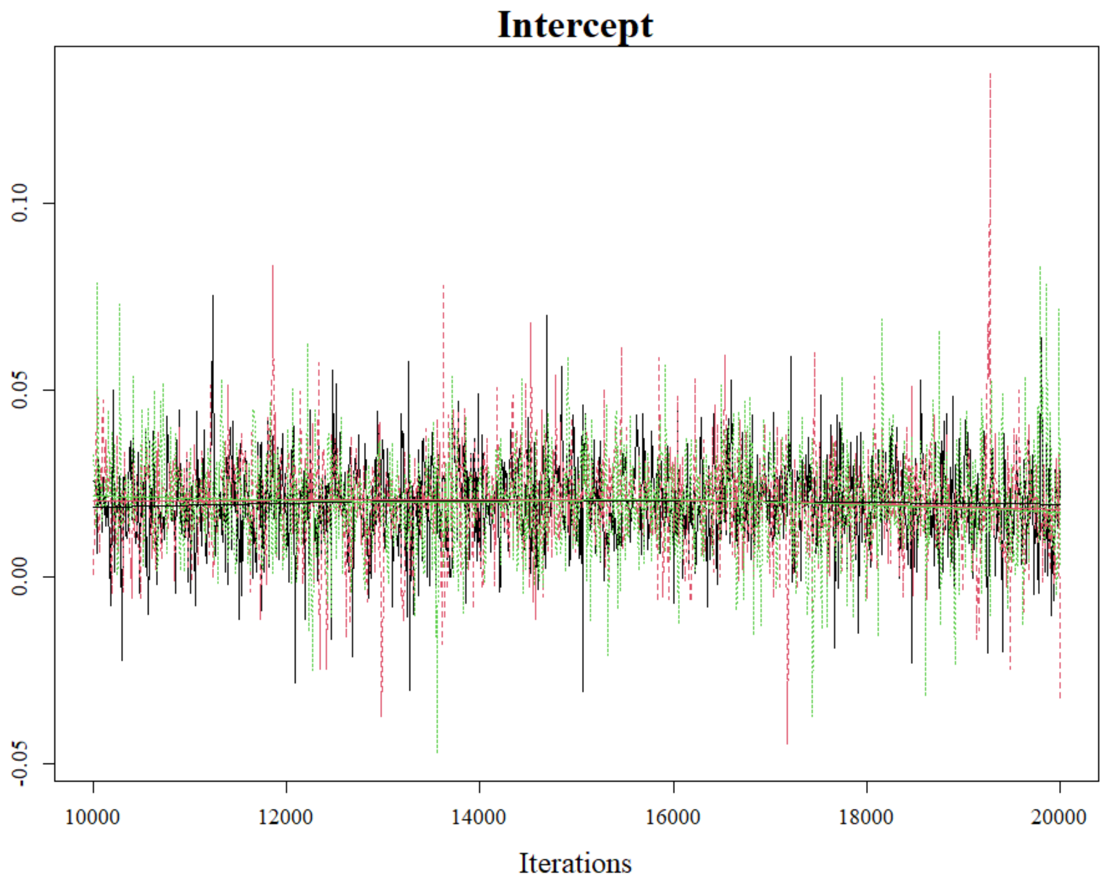

Figure A.4. Traceplot of MCMC simulations for the intercept in the best supported model of inbreeding coefficient, which indicates model convergence.

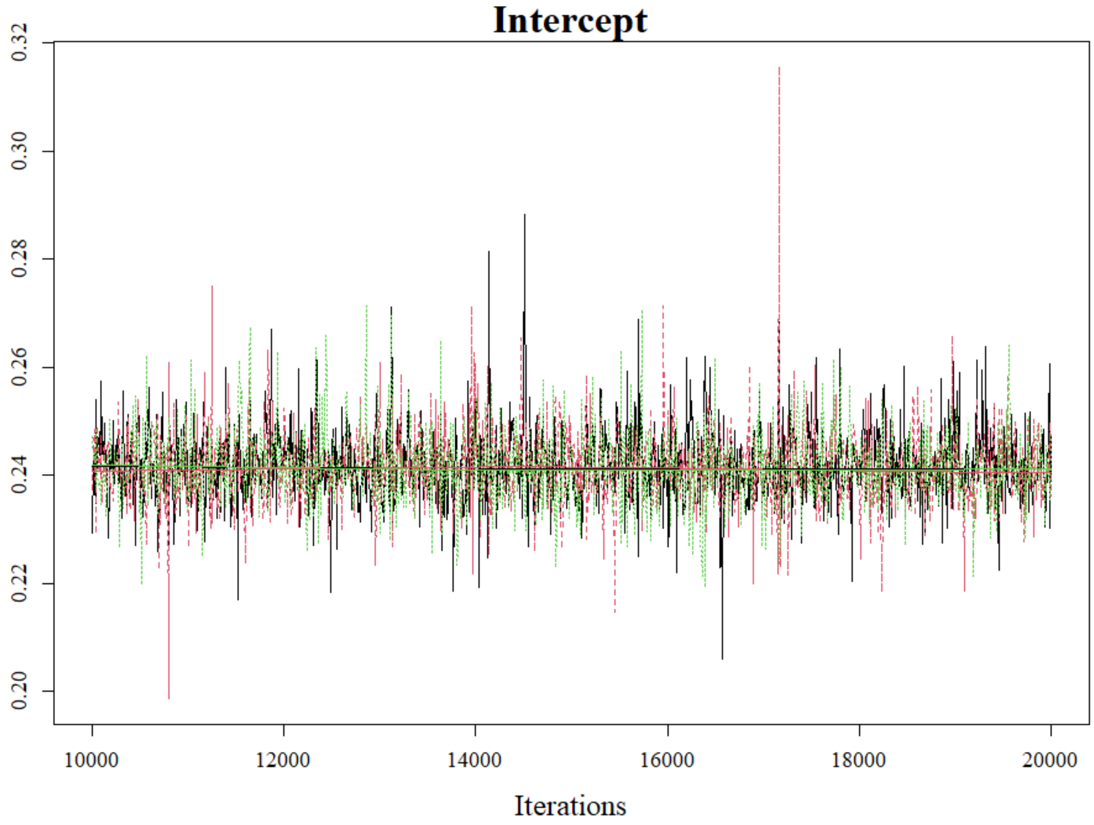

Figure A.5. Traceplot of MCMC simulations for the intercept in the best supported model for the Garza-Williamson index, which indicates model convergence.