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Re-Examining Endemic Smoky Dace As An Indicator Species In The Upper Little Tennessee River Index Of Biological Integrity

Tayton C. Alvis
University of North Carolina at Chapel Hill, tcalvis@crimson.ua.edu

William O. McLarney
Mainspring Conservation Trust, billmclarney@gmail.com

Radmila Petric
University of North Carolina at Chapel Hill, rpetric@unc.edu

Jason P. Love
Western Carolina University, jlove@wcu.edu

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Abstract

Fish communities can be used to evaluate water quality and stream ecosystem health, helping resource managers understand the effects of anthropogenic disturbance. Fish species inform indexes of biological integrity through their ecological traits and tolerance to stressors (e.g. tolerant of disturbance, intolerant of disturbance), but these designations are difficult to determine without localized, species-specific information. The Upper Little Tennessee River watershed is subject to long-term aquatic biomonitoring, applying an index of biological integrity tailored to the region. In this index, the undescribed endemic Smoky Dace (Clinostomus sp.) is classified as intolerant based on a previous designation for the Rosyside Dace (Clinostomus funduloides). However, Smoky Dace have frequently been collected from impaired urban streams, warranting a re-examination of their status as an intolerant species. This study evaluated Smoky Dace's intolerance designation in the Upper Little Tennessee watershed by assessing the relationship between smoky dace and catchment land cover, riparian zone quality, biological integrity scores, and water quality. These relationships were compared to those of Creek Chub, a well-established tolerant species, and Yellowfin Shiner (Hydrophlox lutipinnis), an introduced tolerant species that competes with Smoky Dace. To supplement these comparisons, historic fish community data was examined using non-metric multidimensional scaling to understand the relationship between fish community structure and index of biological integrity scores, and habitat surveys were performed to determine species habitat preferences. Smoky Dace and Creek Chub preferred small streams with fine substrates, and frequently co-occurred at similar densities. Yellowfin Shiner were present at a low number of sites and had no relationship with Smoky Dace, but were associated with sites with high percent agricultural land cover and low percent forest cover. Creek Chub and Yellowfin Shiner strongly correlated with multiple indicators of stream degradation, but Smoky Dace exhibited few significant correlations with any indicators of stream health and clustered alongside known tolerant species in ordinal space, failing to support their status as an intolerant species. This study illustrates how endemism does not necessarily equate to intolerance, and emphasizes the importance of integrating ecological and geographic contexts when assigning the tolerance designations.

Keywords

Smoky Dace, Endemic, Biomonitoring, Fish Assemblage, Index of Biological Integrity, Little Tennessee River

Cover Page Footnote

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INTRODUCTION

Since the inception of the Index of Biological Integrity (IBI; Karr, 1981), fish biomonitoring has been widely applied to quantify stream health (Leonard & Orth**,** 1986; Saylor & Ahlstedt, 1990; Barbour et al., 1999; Fitzgerald et al., 1999). Fish IBI evaluates stream ecological health by examining fish community structure, with metrics quantifying species richness, composition, and abundance of different functional groups (Karr, 1981). Each adaptation of IBI needs to be regionally focused to account for variability in environmental characteristics and species composition across different watersheds (Karr et al., 1986; Ogren & Huckins, 2015). Protocols also need to adjust metrics to account for ecological differences that occur with stream size (Karr et al., 1986; Rahel & Hubert, 1991).

Many indexes of biological integrity utilize intolerant and tolerant species as indicators of ecological health. Intolerant species are particularly sensitive to human impacts, and are some of the first taxa to decline or disappear when disturbance occurs (Karr, 1981). Conversely, high densities of tolerant taxa can indicate poor water quality or habitat degradation, especially when more sensitive species have also declined (Karr, 1981; Karr et al., 1986). Karr (1981) emphasized the importance of selecting indicator species using long-term monitoring data and regional knowledge of declines. However, when information is lacking, selections are often made based on general ecological or taxonomic traits (Hilburn et al., 2023) which can sometimes result in improper designations. Indicator species misclassified as intolerant can interfere with the accuracy of IBI calculations (Scott & Helfman, 2001; Hilburn et al., 2023), resulting in improper evaluations of stream health.

The Upper Little Tennessee River basin is a freshwater biodiversity hotspot (Elkins et al., 2019) with a long history of aquatic biomonitoring. The Upper Little Tennessee River Stream Biomonitoring Program utilizes an IBI adapted by the Tennessee Valley Authority (TVA; Saylor & Ahlstedt, 1990) that was tailored to the Upper Little Tennessee watershed by McLarney (1995). Since 1990, this program has sampled stream fish assemblages throughout the Upper Little Tennessee watershed to monitor the ecological health of the region's streams. This IBI protocol adopted the use of numerous intolerant species from the TVA's IBI (Saylor & Ahlstedt, 1990), including Rosyside Dace (*Clinostomus funduloides*).

Rosyside Dace is a widespread taxon consisting of morphologically distinct geographic subspecies (Deubler, 1955) that may warrant elevation to species (Weyand & Piller, 2020). One such undescribed species, Smoky Dace (*Clinostomus* sp.), is endemic to the Upper Little Tennessee and Tuckasegee watersheds, and is classified as a species of special concern in North Carolina (North Carolina Administrative Code, 2008) due to population declines (Pringle, 2015) and narrow range of occurrence. During the Upper Little Tennessee IBI protocol's development, Smoky Dace was not widely recognized as an undescribed species, and was assumed to be intolerant based on previous classifications for the Rosyside Dace. However, biomonitoring data collected over the course of the program suggests that Smoky Dace are thriving in impaired urban tributaries (NCDWQ, 2012) of the Upper Little Tennessee (W.O. McLarney, pers. obs.), warranting a re-examination of the taxon's status as an indicator species.

Creek Chub (*Semotilus atromaculatus*) is a widely distributed, generalist species (Etnier & Starnes, 1993) typically sampled alongside Smoky Dace at many tributary sites in the Upper Little Tennessee watershed (W. O. McLarney, pers. obs.). Creek Chub is a well-established tolerant species in fish biomonitoring indices throughout the United States (Leonard & Orth, 1986; Saylor & Ahlstedt, 1990; Barbour et al., 1999; Fitzgerald et al., 1999) including the Upper Little Tennessee IBI (McLarney**,** 1995), and often occur in streams that have been heavily altered by urban or agricultural activity (Nagrodski et al., 2013). Because Creek Chub is a widely accepted tolerant species, frequently co-occurs with Smoky Dace, and occupies a similar functional feeding group, it is a useful comparison species for understanding how known indicator taxa respond to stream degradation.

Threats to stream integrity in the Upper Little Tennessee watershed include urban and agricultural development, which influences fish assemblage structure and presence of sensitive species (Sutherland et al., 2002) and introduced species such as Yellowfin Shiner (*Hydrophlox lutipinnis*) that colonize disturbed streams (W. O. McLarney, pers. obs.) and compete with Smoky Dace for forage (Hazelton & Grossman, 2009) and spawning habitat (Johnston et al., 1995). Many IBI sites in the Upper Little Tennessee watershed have also been assessed by additional stream health indices that provide insight into ecological health. These include the Stream Visual Assessment Protocol (SVAP) (Bjorkland et al., 2001) which rapidly assesses reach-scale stream and riparian habitat quality, and Stream Monitoring Information Exchange (SMIE) biotic integrity (Traylor, 2014), that scores a site based on the abundance and diversity of aquatic macroinvertebrates. By leveraging multiple indices that assess site quality by different means, it is possible to understand how species fare under a multitude of disturbance pressures and provide a more accurate evaluation of species tolerance.

This study aimed to evaluate the intolerance classification of Smoky Dace by evaluating their relationship with multiple indicators of stream health and relating those trends to Creek Chub (a well-established tolerant species) and Yellowfin Shiner (an introduced tolerant species and direct competitor with Smoky Dace). Additionally, site habitat data was collected to compare species habitat preferences and aid future sampling efforts and conservation actions directed toward Smoky Dace.

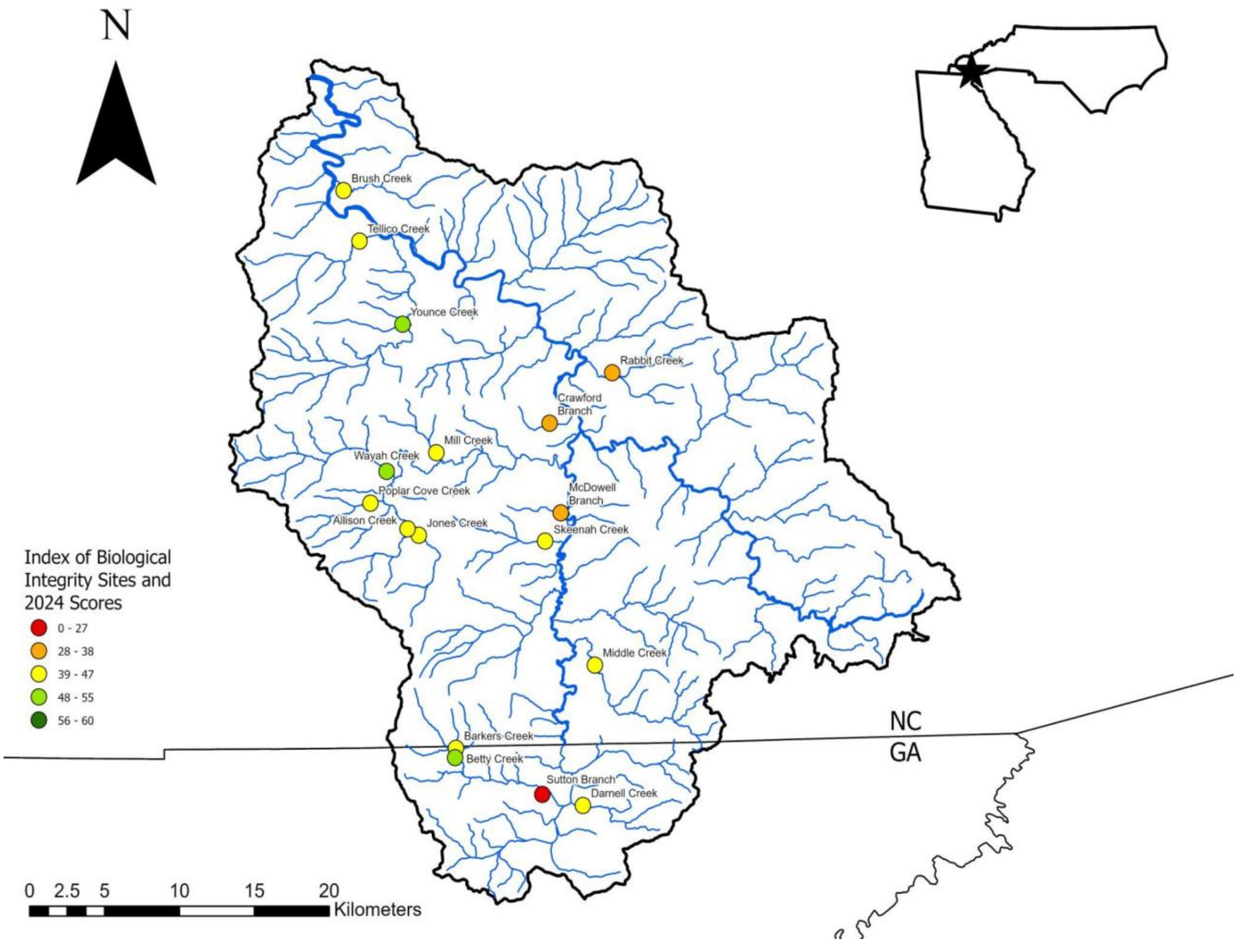
METHODS

Study Area

Field work was conducted in the Upper Little Tennessee River watershed (HUC 06010202) upstream of Fontana reservoir in North Carolina and Georgia, USA (~ 1,145 km^2 drainage area) (Figure 1A, 1B). The Little Tennessee River flows north from its headwaters in Rabun County, GA, into Macon and Swain Counties, NC, before reaching Fontana reservoir, a major impoundment. The watershed is predominantly forested, but has seen an increase in developed land cover in the past 20 years, with some tributary watersheds rising by as much as 6% from 2001-2019 (Gibbs et al., 2024). The Upper Little Tennessee River is separated into two distinct sections by Lake Emory Dam, with the stretch below the dam regarded as one of the most ecologically intact warmwater rivers in the Southern Blue Ridge (NCDEQ, 2018). The section upstream of the dam is heavily sedimented and subject to water quality impairment (NCDWQ, 2012).

Site Selection

Seventeen sites were selected to sample during May - July 2024 that historically yielded Smoky Dace and encompassed a range of conditions where the taxon occurs (Figure 1A). Sites in the Cullasaja, Cowee, and Watauga basins (Figure 1B) were omitted due to a notable lack of occurrences in those watersheds. Site selection was informed by historic fish assemblage data collected by the Upper Little Tennessee River Stream Biomonitoring Program (McLarney, 1995), spanning over 3 decades (May - August 1990 - 2023) across 182 unique sites.



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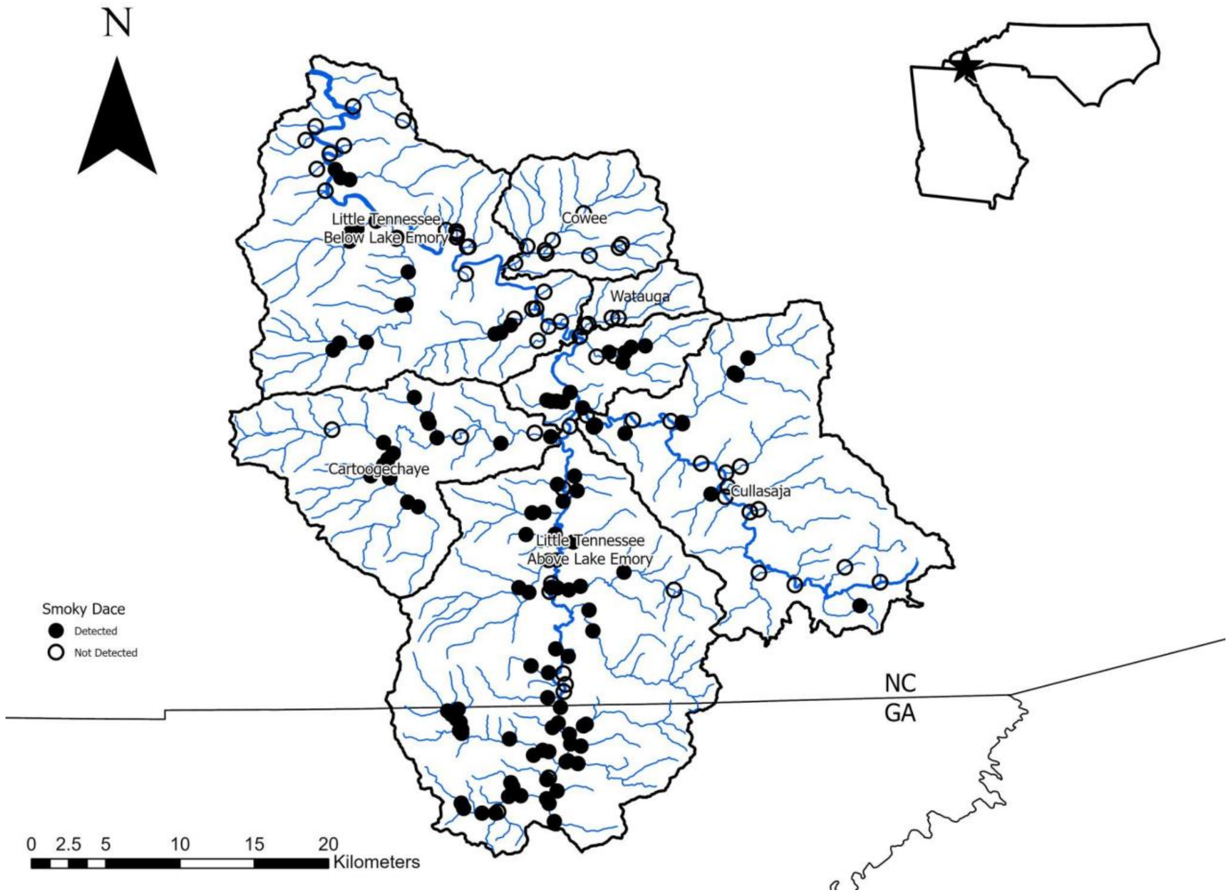

B

Figure 1. (A) Sites and corresponding 2024 Fish Index of Biological Integrity scores (binned by score classification) in the Little Tennessee watershed, located in western North Carolina and north Georgia. **(B)** Distribution of Smoky Dace across historic sampling sites in the Upper Little Tennessee watershed (1999-2024). Filled circles indicate sites where Smoky Dace were detected. Smoky Dace were notably absent from the Cowee and Watauga basins, and occurred more often above Lake Emory.

Stream Health Metrics

Site health was assessed using a combination of catchment land cover (% forested, % developed, % agricultural), bioassessment indices (IBI, SMIE), and habitat indices (SVAP, % riffle embeddedness, water quality) (Table 1). To quantify catchment land use at each site, Streamstats v4.28.0 (USGS, 2019) was used to delineate watersheds and calculate total drainage area for each site, and the National Land Cover Database (NLCD) 2024 annual release (USGS, 2024) was used to determine the percentage of land cover categorized as forested (classes 41-43), developed (classes 21-24), and agricultural (classes 71, 81 & 82). Percent land cover was calculated by dividing the proportion of land categorized as each respective land use class (forested, developed, agricultural) by the total area of each study watershed.

Table 1. Integrity classes for each selected stream health metric (fish Index of Biological integrity (IBI) (McLarney, 1995), Stream Visual Assessment Protocol (SVAP) (Bjorkland et al., 2001), Stream Monitoring Information Exchange (SMIE) macroinvertebrate biological integrity (Traylor, 2014)) Note that not all metrics have explicit scores for each class.

	Metric		
Integrity Class	**IBI**	**SVAP**	**SMIE**
Very Poor	12-24	1.0-1.8	
Poor - Very Poor	25-28		
Poor	28-35	1.9-2.5	>5.22
Fair-Poor	36-38		
Fair	39-44	2.6-3.1	4.11-5.21
Good-Fair	45-47		3.57-4.10
Good	48-55	3.2-3.6	3.10-3.56
Good-Excellent	56-57		
Excellent	58-60	3.7-4.0	<3.09

IBI Sampling & Calculation

Sites were divided into subsamples based on natural breaks in habitat (pool, riffle, run). Each subsample measured for length and width and individually sampled using a combination of HT2000 backpack electrofishers, dip nets, and seine nets (4 × 10 ft; 3.2 mm mesh). After securing the seine at the downstream end of the subsample, electrical shocks were administered while moving upstream, and stunned fishes were collected using dip nets and transferred into 5-gallon buckets for recovery. Following a second downstream pass, collected fishes were identified to species, counted, and released below the sampled reach. This process was repeated for each successive upstream subsample until a length of reach 15 times the average reach width was surveyed. Fish count data were used to calculate an IBI rating for each site, with individual metrics designed to evaluate different aspects of the fish community (see supplemental materials, Tables 1, 2, 3, 4, 5).

SMIE Sampling & Calculation

To calculate SMIE, macroinvertebrates were sampled from a section of riffle habitat using a kick net (3 × 3 ft; 500 µm mesh), and dip nets were used to sample leaf packs and stream bank habitats. Sampled macroinvertebrates were counted and identified using field keys (Traylor, 2014) that grouped macroinvertebrates into 43 unique categories, with each category having a corresponding tolerance value (see supplemental materials, Table 6) used to calculate an SMIE score using the following formula (Traylor, 2014):

$$\text{SMIE BI} = \sum \text{Abundance} \times \text{Tolerance Values} / \text{Total Abundance}$$

Where abundance is the number of individuals from each category present, tolerance values are given for each taxon, and total abundance is the total number of macroinvertebrates sampled.

SVAP and Habitat Surveys

In-stream and riparian habitat quality were assessed using SVAP (Bjorkland et al., 2001) by making visual observations of bank condition, vegetation cover, overstory shading, habitat diversity/availability, pollution (including livestock access in agricultural areas), and potential barriers to fish movement. The total score averages 11 individual elements that each receive a rating from 1-4 (see supplemental materials, Table 7).

Reach-scale habitat surveys were conducted at each site using a modified protocol developed by the United States Forest Service (Platts et al., 1983). Sites were divided into 10 m transects along the length of the reach sampled during IBI surveys (n = 7-10), measured for wetted width, and categorized as a major habitat type (riffle, run, pool). Each transect was sampled at 1%, 25%, 50%, 75%, and 99% of the total wetted width (n = 5 per transect). At each sampling point, depth and flow were measured using a top setting wading rod and FH950 portable velocity meter (0.6 depth from water's surface). Additionally, pebbles were measured to quantify substrate size, and percentages of the two dominant substrate types (sand, gravel, cobble, boulder, bedrock) were visually estimated. To assess riffle embeddedness, 10 stones were collected from riffles throughout the reach and measured for total height (as oriented in the stream) and height at which biofilm growth was observed, reflecting the proportion of stone embedded in the benthos.

Water Quality

Point measurements of dissolved oxygen and conductivity were collected using a YSI multiprobe. Hobo Water Temperature Pro v2 Data loggers were deployed to collect hourly temperature data at a subset of sites (n = 9) from 4 to 27 July 2024.

Statistical Analysis

Statistical analysis and data visualizations were conducted using R-Studio 4.4.2. (R Core Team, 2024) ($\alpha = 0.05$). Prior to analysis, fish count data were standardized as catch/100 m^2. Spearman’s rank-order correlation was performed using the R *Hmisc* package (Harrell Jr., 2025) to evaluate the monotonic relationships between reach averages of measured habitat variables, stream health metrics, and densities of each species of interest.

Historic biomonitoring data from the Upper Little Tennessee spanning 107 sites from 2010-2022 (n = 335 sampling events) was utilized to assess the relationship between fish assemblages and IBI scores using Non-metric Multidimensional Scaling (NMDS) with the Bray-Curtis dissimilarity index. This dataset was filtered to only include species present in greater than 5% of sampling events. NMDS was performed with the R *vegan* package (Oksanen et al., 2025). Sites were grouped by IBI score class as defined in McLarney’s IBI index (see supplemental materials, Table 4) and confidence ellipses were calculated for each class to visualize how site scores clustered by IBI score. Species scores were overlaid onto the ordination to visualize the contributions of each species to NMDS axes, with tolerance designations included for species explicitly classified in the Upper Little Tennessee IBI (see supplemental materials, Table 5).

RESULTS

Sampling yielded 7,291 individuals of 31 species from 17 sites during May - July 2024. Smoky Dace were the 5th most abundant species (352 individuals sampled) with an average catch/100 m^2 of 9.1, ranging from 0 individuals/100 m^2 at Tellico Creek to 29.2 individuals/100 m^2 at Crawford Branch. Creek Chub were the 8th most abundant species (235 individuals sampled) with an average catch/100 m^2 of 6.7, ranging from 0.1 individuals/100 m^2 at Wayah Creek to 42.3 individuals/100 m^2 at Crawford Branch. Yellowfin Shiner were present at 7 of the 17 sampled sites and were most abundant at Rabbit Creek (55 individuals/100 m^2) Across sites, the mean IBI score was 43 and ranged from 26 to 55, mean SVAP score was 3.22 and ranged from 2.45 to 3.70, and mean SMIE score was 3.51 and ranged from 4.73 to 2.48 (Table 2). Developed land cover across sites ranged from 1.1 to 64.2%, agricultural from 1.1 to 23.2%, and forested from 30.4 to 97.1%, with means of 10.9, 6.8, and 82%, respectively (Table 2).

Table 2. Site information and stream health scores from each site sampled in 2024 including stream name, coordinates, catchment area, stream health metric scores, and land cover percentages.

Stream	Latitude	Longitude	Catchment Area (km^2)	IBI	SVAP	SMIE	%Developed	%Agricultural	%Forested
Allison Creek	35.1179715	-83.47334997	6.91	42	3.48	3.44	89.2	6.1	4.7
Barkers Creek	34.99260311	-83.44279853	2.43	46	3.15	-	92.6	4.7	2.3
Betty Creek	34.98669	-83.44372	17	55	3.7	3.13	88	4.8	6.5
Brush Creek	35.31443902	-83.51368331	10	47	3.48	3.07	93.1	4.4	2.5
Crawford Branch	35.17969613	-83.38906161	2.35	35	2.45	4.73	30.4	64.2	5
Darnell Creek	34.95984	-83.37037	5.71	47	2.89	3.69	97.1	1.1	1.5
Jones Creek	35.11523157	-83.46695884	7	42	3.23	3.17	91.6	3.2	4.5

McDowell Branch	35.1287803	-83.38279795	1.37	36	3.07	3.58	61.6	26.8	11.4
Middle Creek	35.04016	-83.36127	14	47	3.48	3.14	82.8	8	8.9
Mill Creek	35.18140793	-83.46916989	3.95	39	3.11	4.45	85.5	11.6	2.6
Poplar Cove Creek	35.13392868	-83.49560735	3.94	48	3.31	3.39	90.1	6.7	2.8
Rabbit Creek	35.21262551	-83.3295214	10	28	2.8	3.6	65.5	13.5	20.7
Skeenah Creek	35.11187	-83.39091	6.97	39	2.8	-	71.2	15.6	12.5
Sutton Branch	34.96790161	-83.39164265	0.49	26	2.95	-	72.4	3.3	23.2
Tellico Creek	35.28334	-83.50397	12.1	41	3.43	3.05	96.7	1.7	1.1
Wayah Creek	35.15408726	-83.48786832	14.2	52	3.23	3.08	94.6	1.8	1.8
Younce Creek	35.23779013	-83.47420967	3.7	48	3.34	2.71	92.1	3.6	4.1

Results of Spearman Correlation analysis are summarized in the following correlogram (Figure 2) generated using R *Corrplot* (Wei & Simko, 2024). Smoky Dace density had significant, positive correlations with Creek Chub density (r_s(15) = 0.52, $p < 0.05$), SMIE scores (r_s(12) = 0.81, $p < 0.01$), and percent gravel substrate (r_s(14) = 0.64, $p < 0.01$), and significant, negative correlations with watershed area (r_s(15) = -0.84, $p < 0.001$) and percent boulder substrate (r_s(14) = -0.58, $p < 0.05$). There was a negative association between SVAP scores and Smoky Dace density (r_s(15) = -0.43, p = 0.0853). Creek Chub density positively correlated with percent sand substrate (r_s(14) = 0.59, $p < 0.05$) and percent gravel substrate (r_s(14) = 0.57, $p < 0.05$), and negatively correlated with IBI score (r_s(15) = -0.78, $p < 0.001$), watershed area (r_s(15) = -0.75, $p < 0.001$), percent cobble substrate (r_s(14) = -0.72, $p < 0.01$), SVAP score (r_s(15) = -0.59, $p < 0.05$), and percent forested land cover (r_s(15) = -0.52, $p < 0.05$). There was a positive association between Creek Chub density and percent riffle embeddedness (r_s(15) = 0.45, p =

0.0672). Neither Smoky Dace nor Creek Chub density correlated with Yellowfin Shiner density, though Yellowfin Shiner were limited in occurrence at sampled sites (n = 7). Yellowfin Shiner density positively correlated with percent agricultural land cover ($r_s(17) = 0.68$, $p < 0.01$) and percent sand substrate ($r_s(16) = 0.53$, $p < 0.05$) and negatively correlated with percent forested land cover ($r_s(17) = -0.51$, $p < 0.05$).

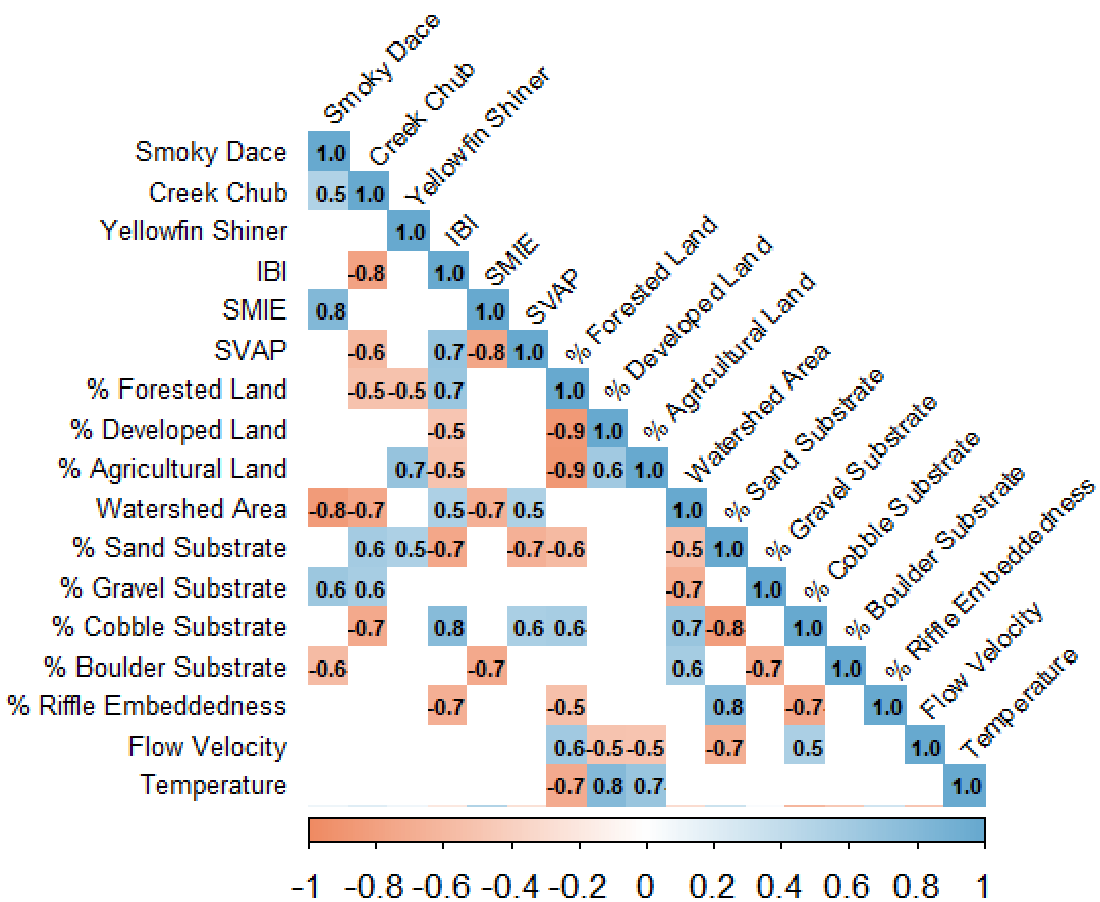

Figure 2. Spearman correlogram showing the strength and direction of correlations between Smoky Dace, Creek Chub, and Yellowfin Shiner density, habitat variables, stream health metrics (fish index of biological integrity (IBI), stream visual assessment protocol (SVAP), stream monitoring information exchange macroinvertebrate biological integrity (SMIE)), and land cover percentages. Blank squares indicate non-significant relationships using a significance threshold of $\alpha = 0.05$. Smoky Dace and Creek Chub density shared similar correlation patterns with habitat variables. Creek Chub density negatively correlated with multiple indicators of disturbance (% forested land, SVAP, IBI). Smoky Dace density did not readily correlate with indicators of disturbance.

Bray-Curtis NMDS provided a fair representation of the assemblage data (stress = 0.183) (Figure 3). Although considerable overlap occurred among IBI classes (particularly between fair and good categories), sites showed partial separation along NMDS2. Intolerant species tended to drive site scores in the positive direction along NMDS1 and the negative direction of NMDS2, and tolerant species tended to drive site scores in the positive direction of NMDS2. Species scores for intolerant taxa tended to cluster around sites classified as good and excellent, with the exception of Smoky Dace, which fell outside of the fair class. Variation along the ordination axes appeared to be influenced by Smoky Dace and Creek Chub, which were associated with sites classified as fair and poor, respectively. Yellowfin Shiner were also associated with sites classified as poor, but did not fall alongside Creek Chub or Smoky Dace, instead clustering with other introduced tolerant species such as Redbreast Sunfish (*Lepomis auritus*).

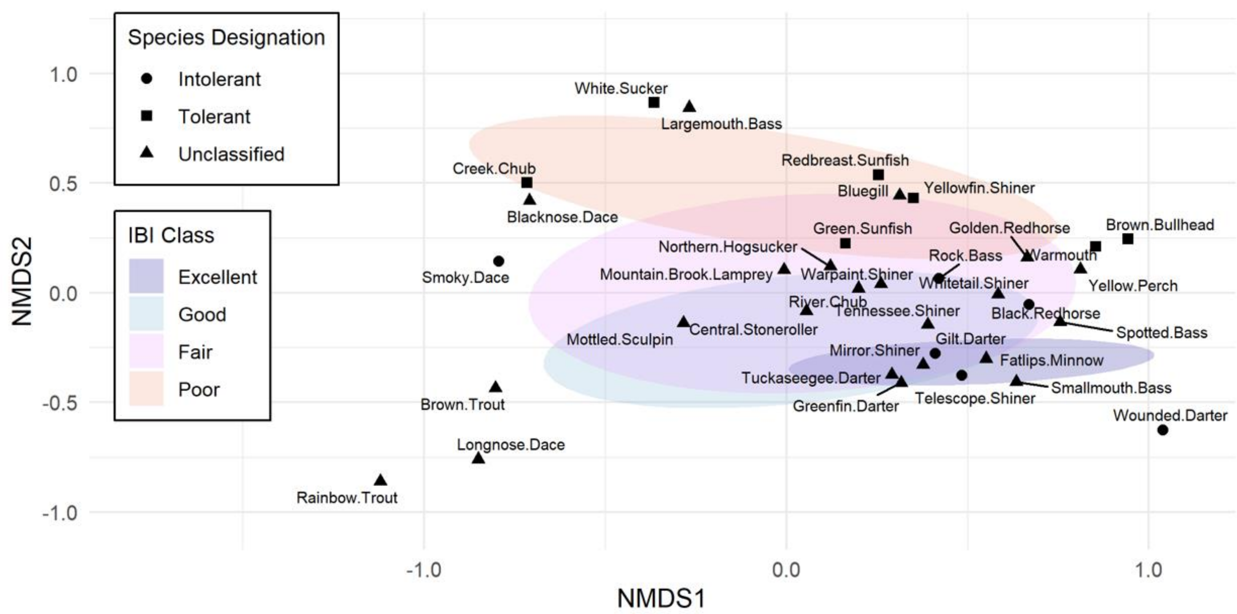

Figure. 3. Non-metric multidimensional scaling ordination using the Bray-Curtis dissimilarity index, generated using historic fish assemblage data at 107 sites (n = 335, 2010-2022) throughout the Upper Little Tennessee watershed. Species scores were overlaid onto confidence ellipses for each index of biological integrity score class to visualize the contribution of fish species to ordination axes. Species scores for tolerant and intolerant species generally fell in and around ellipses for poor and good/excellent scores, respectively, with the exception of Smoky Dace, which fell outside of the fair class.

DISCUSSION

Maintaining up-to-date and accurate IBI metrics is critical for understanding stream health, and is of particular importance in the Upper Little Tennessee watershed, where debris removal efforts associated with Hurricane Helene have impacted aquatic communities (J. Love, unpublished data). This work demonstrates that Smoky Dace may not be a suitable intolerant for IBI, as the species did not meaningfully correlate with any metrics of stream health examined in this study. Conversely, Creek Chub and Yellowfin Shiner significantly correlated with multiple indicators of stream degradation, demonstrating tolerance. It is likely that tolerant species more readily correlate with these metrics because they often surge in abundance as streams degrade, whereas intolerant species almost never dominate, but instead simply decline or disappear from sites after disturbance occurs. Though the responses of intolerant species generally do not mirror in magnitude that of tolerant species, it is still surprising that Smoky Dace persisted at sites scoring poorly across multiple indices. Additionally, NMDS revealed that Smoky Dace deviated significantly from all other intolerant species, implying that their abundance may be influenced by different factors. Smoky Dace was associated with lower IBI score classes (Fair, Poor) and clustered alongside a known tolerant species (Creek Chub) which may indicate a similar degree of sensitivity (Hilburn et al., 2023).

Smoky Dace and Creek Chub co-occurred in high numbers at many sites, possibly due to similar habitat preferences and associations in reproductive behavior between the two taxa. Creek

Chub create pit-ridge nests (Johnston & Page, 1992), of which *Clinostomus* are known nest associates, possibly supporting higher densities of Smoky Dace where Creek Chub are present. For example, Smoky Dace and Creek Chub were the dominant species at Crawford Branch, a low-order, urban stream on the EPA's 303-d impairment list (NCDWQ, 2012). Smoky Dace and Creek Chub were 2 of only 7 total taxa sampled from Crawford Branch in 2024, with Smoky Dace as the only nest associate. This lack of competition may give Smoky Dace increased access to spawning opportunities, resulting in their success at Crawford Branch and other similar sites.

Smoky Dace density did not clearly relate to IBI, SVAP, or catchment land use, but may exhibit stronger responses to other stressors not examined in this study. Pringle (2015) found that Smoky Dace were one of the few species declining in the Upper Little Tennessee watershed, and they have notably been extirpated from a number of streams that previously hosted populations over the course of the biomonitoring program (W. O. McLarney, pers. obs.). Indexes such as fish IBI, SVAP, and SMIE likely overlook local environmental factors and pollution sources that may influence the abundance of sensitive taxa. It is also important to note that some metrics used in this study (SVAP, SMIE) do not account for stream size, leading to discrepancies in scoring that potentially obscure relationships, especially considering that Smoky Dace are strongly associated with small streams.

Smoky Dace may have declined in larger streams while remaining abundant in small tributaries (W. O. McLarney, pers. obs.) and future surveys should prioritize low-order streams to better quantify Smoky Dace abundance and extent. These declines are possibly due to changes in temperature or the introduction of non-native species. Nelson et al. (2021) indicated that the closely related Rosyside Dace exhibited significantly lower thermal tolerance than Creek Chub and Blacknose Dace (*Rhinichthys atratulus*). Although there was no significant relationship between temperature and Smoky Dace in this study, loggers were only deployed at a limited number of sites (n = 9; see supplemental materials, Table 8). Despite a limited sample size, average temperature strongly correlated with land use, consistent with previous studies (Pluhowski, 1970; Burton & Likens, 1973). The lack of correlation between Smoky Dace and temperature may still be informative, but would benefit from additional site-level temperature data. Similarly, Yellowfin Shiner were present only at a limited number of sites (n = 7) and did not correlate with Smoky Dace density. However, they achieved highest densities at Rabbit and Skeenah Creeks, two degraded sites with declining numbers of Smoky Dace. Examination of historic trends in Smoky Dace abundance that account for introductions of Yellowfin Shiner may shed light into the relationship between this ongoing invasion and potential declines.

Numerous studies have associated endemic fish species with sensitivity to anthropogenic disturbances (Hopkins II, & Burr, 2009; Larentis et al., 2022; Scott, 2006). Pringle (2015) classified Smoky Dace as a highland endemic species, and Scott & Helfman (2001) noted that highland endemics were often the best indicator species in Southern Appalachian streams because they are adapted to cool waters with low nutrient input. Though the presence of endemic species can be an indicator of site health, this work demonstrates that endemic status does not always equate to intolerance, and highlights the problematic nature of generalizing tolerance designations across watersheds or to similar species.

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SUPPLEMENTAL MATERIALS

Table 1. Individual IBI metrics for watersheds draining 1-4 square miles (McLarney, 1995).

IBI Metric Scoring Criteria for the Upper Little Tennessee River Watershed for Streams Draining 1- 4 Square Miles			
		Score	
Metric	1.5	4.5	7.5
1. Total Ephemeroptera taxa	<3	3-5	>5
2. Total EPT taxa	<8	8-15	>15
3. Brook trout present/absent	Absent	Sympatric	Allopatric
4. Catch rate (mean number of individual fish per 5 min. of electrofisher time)	<5	5-9	>9
5. % of individuals with disease and/or other anomalies	>5%	2-5%	<2%
6. % of individuals as tolerant species	>20%	10-20%	<10%
7. % of individual fish as wild trout (all spp.)	Absent	0-10%	>10%
8. % of individual fish as omnivores and herbivores	>20%	10-20%	<10%

Table 2. Individual IBI metrics for watersheds draining 4-7 square miles (McLarney, 1995).

IBI Metric Scoring Criteria for the Upper Little Tennessee River Watershed for Streams Draining 4 -7 Square Miles			
		Score	
Metric	1.5	4.5	7.5
1. Total number of native species	<6	6-10	>10
2. Number of intolerant species	<2	2	>2
3. % of individuals as tolerant species	>20%	10-20%	<10%
4. % of individuals as omnivores and herbivores	>20%	10-20%	10%
5. % of individuals as specialized insectivores	<20%	20-45%	>45%
6. Catch rate per unit of effort *	<11	11-18	>18
7. % of individuals as darters and sculpins	<35%	35-65%	>65%
8. % of individuals with disease and/or other anomalies	>5%	2-5%	<2%
*If catch rate is less than 3, low scores should be automatically given for Metrics 8, 11 & 12			

Table 3. Individual IBI metrics for watersheds draining 7-15 square miles (McLarney, 1995).

IBI Metric Scoring Criteria for the Upper Little Tennessee River Watershed for Streams Draining 7-15 Square Miles			
		Score	
Metric	1.3	4.0	6.7
1. Total number of native species	<6	6-10	>10
2. Number of darter species	0	1-2	>2
3. Number of intolerant species	<2	2	>2
4. % of individuals as tolerant species	>20%	10-20%	<10%
5. % of individuals as omnivores and herbivores	>20%	10-20%	<10%
8. % of individuals as specialized insectivores	<20%	20-45%	>45%
6. Catch rate per unit of effort *	<11	11-18	>18
7. % of individuals as darters and sculpins	<35%	35-65%	>65%
12. % of individuals with disease and/or other anomalies	>5%	2-5%	<2%
* If catch rate is less than 3, low scores should be automatically given for Metrics 8,11, & 12			

Table 4. IBI classes with corresponding score ranges and descriptions (McLarney, 1995).

Biotic Integrity Classes		
Excellent	Comparable to the best situations without human impacts. Includes all expected species for the particular type and size of stream. All species, including the least tolerant, with full array of sizes and ages. Balanced trophic structure. Low incidence of diseases, parasites and anomalies.	58-60
Good	Species richness may be somewhat below expectations, especially due to loss of most intolerant forms. Some species with less than optimal abundance or size distribution. Trophic structure shows some signs of stress.	48-55
Fair	Fewer intolerant forms. More skewed trophic structure. In some cases older age classes for predators may be rare.	39-44
Poor	Dominance by pollution-tolerant species. Species with specialized habitat requirements scarce. Carnivores scarce. Diseases, parasites and anomalies common.	28-35

Very Poor	Fish may be scarce or over-abundant (in nutrient-enriched rivers). Tolerant species dominant. Diseases, parasites and anomalies common.	12-24

Table 5. Sampled fish taxa with corresponding diet and tolerance classifications utilized in calculation of IBI scores (McLarney, 1995). Note that not all species have a corresponding tolerance designation.

Species	Trophic Guild	Tolerant/Intolerant
Northern Hogsucker (*Hypentelium nigricans*)	Invertivore	
White Sucker (*Catostomus commersonii*)	Omnivore	Tolerant
Black Redhorse (*Moxostoma duquesnei*)	Invertivore	Intolerant
Golden Redhorse (*Moxostoma erythrurum*)	Invertivore	
Spotted Bass (*Micropterus punctulatus*)	Piscivore	
Largemouth Bass (*Micropterus salmoides*)	Piscivore	
Smallmouth Bass (*Micropterus dolomieu*)	Piscivore	
Rock Bass (*Ambloplites rupestris*)	Piscivore	Intolerant
Bluegill (*Lepomis macrochirus*)	Invertivore	
Green Sunfish (*Lepomis cyanellus*)	Invertivore	Tolerant
Redbreast Sunfish (*Lepomis auritus*)	Invertivore	Tolerant
Warmouth (*Lepomis gulosus*)	Invertivore	Tolerant
Mottled Sculpin (*Cottus bairdii*)	Invertivore	
River Chub (*Nocomis micropogon*)	Omnivore	
Creek Chub (*Semotilus atromaculatus*)	Omnivore	Tolerant
Blacknose Dace (*Rhinichthys atratulus*)	Generalist Feeder	
Longnose Dace (*Rhinichthys cataractae*)	Specialized Insectivore	

Smoky Dace (*Clinostomus* sp.)	Specialized Insectivore	Intolerant
Central Stoneroller (*Campostoma anomalum*)	Herbivore	
Tennessee Shiner (*Paranotropis leuciodus*)	Specialized Insectivore	
Warpaint Shiner (*Coccotis coccogenis*)	Specialized Insectivore	
Whitetail Shiner (*Cyprinella galactura*)	Specialized Insectivore	
Mirror Shiner (*Paranotropis spectrunculus*)	Specialized Insectivore	
Telescope Shiner (*Notropis telescopus*)	Specialized Insectivore	Intolerant
Fatlips Minnow (*Phenacobius crassilabrum*)	Specialized Insectivore	
Yellowfin Shiner (*Hydrophlox lutipinnis*)	Omnivore	Tolerant
Brown Bullhead (*Ameiurus nebulosus*)	Omnivore	Tolerant
Tuckasegee Darter (*Etheostoma gutselli*)	Specialized Insectivore	
Greenfin Darter (*Nothonotus chlorobranchius*)	Specialized Insectivore	
Wounded Darter (*Nothonotus vulneratus*)	Specialized Insectivore	Intolerant
Gilt Darter (*Percina evides*)	Specialized Insectivore	Intolerant
Yellow Perch (*Perca flavescens*)	Omnivore	
Mountain Brook Lamprey (*Ichthyomyzon greeleyi*)	Herbivore	
Brook Trout (*Salvelinus fontinalis*)	Invertivore	Intolerant
Brown Trout (*Salmo trutta*)	Piscivore	
Rainbow Trout (*Oncorhynchus mykiss*)	Invertivore	

Table 6. Taxonomic categories and corresponding tolerance values utilized in calculation of SMIE scores (Traylor, 2014).

Taxa #	Group	SMIE Taxa Name	Tolerance Value
1	Plecoptera	Giant Shredder	1.8
2	Plecoptera	Roach Shredder	1.3
3	Plecoptera	Quick Crawling Predator	1.3
4	Plecoptera	Fragile Detritivores	1.3
5	Ephemeroptera	Flattened Scraper	4
6	Ephemeroptera	Spiny Crawler	3.4
7	Ephemeroptera	Round Headed Swimmer	4.3
8	Ephemeroptera	Burrowing mayflies	4
9	Ephemeroptera	Spiny Turtle	3.2
10	Ephemeroptera	Filter Mayfly	3.6
11	Trichoptera	Net Spinning Caddis	4
12	Trichoptera	Small Head Caddis	1.5
13	Trichoptera	Stick Bait Caddis	2.5
14	Trichoptera	Square Log Cabin Caddis	2.2
15	Trichoptera	Sand and Stick Case Caddis	4
16	Trichoptera	Vegetated Case Caddis	2.9
17	Trichoptera	Gravel Coffin Case Caddis	0.8
18	Trichoptera	Sand Snail Shell Caddis	0
19	Trichoptera	Sand and Mineral Case Caddis	2.6
20	Coleoptera	Water Penny	2.3
21	Coleoptera	Predator Beetle larvae	6.4
22	Coleoptera	Adult Riffle Beetles	4.5
23	Coleoptera	Larval Riffle Beetles	3.2
24	Megaloptera	Hellgrammite	5.2
25	Megaloptera	Fishfly	5.3
26	Megaloptera	Alderfly	7
27	Oligochaete	aquatic worms	7
28	Oligochaete	Leech	7.1
29	Diptera	Water Snipe	1.8
30	Diptera	Water Worm	7.5
31	Diptera	Fat Headed Cranefly	3.5
32	Diptera	Chironomid Midge	6
33	Diptera	Red Midge	9.3
34	Diptera	Blackfly	4.9
35	Crustacea	Crayfish	6
36	Crustacea	Sowbug (Isopod)	7.4
37	Crustacea	Scud (Amphipod)	7.2
38	Gastopoda	Coiled Left Face Snail	8.7
39	Gastopoda	Coiled Right Face Snail	5.6
40	Gastopoda	Rounded Right Face Snail	6.6
41	Pelecypoda	Mussels and Clams	5.3

42	Odonata	Damselflies	7
43	Odonata	Dragonflies	4

Table 7. SVAP elements and criterion for scoring within each category. The three vegetation "types" (element 3) refer to trees, shrubs, and grasses. Riffle embeddedness (element 5) refers to the length of time it takes for the water to clear after performing a riffle kick test, in which an individual kicks up sediment in a riffle. Habitat "types" (element 10) include logs, small wood accumulations, large boulders, small boulder clusters, undercut banks, side channels, cobble riffles, leaf mats, macrophyte beds, overhanging vegetation, deep pools, and submerged roots (Bjorkland et al., 2001).

Element	4 (Excellent)	3 (Good)	2 (Fair)	1 (Poor)
(1) Bank Condition	Gently sloping and stable banks protected by roots, wood and natural rock. No man-made structures on bank Little erosion or bank failures.	Low to moderately steep banks protected by vegetation. < 25% man-made structures cover bank. Erosion and bank failures present with some re-growth or vegetation.	Steep banks with little protection from vegetation. 25-50% man-made structures cover bank. Livestock or recreational access are negatively impacting bank stability	Vertical banks with raw soil present and multiple bank failures and erosion are evident. >50% man-made structures Livestock or recreational access are negatively impacting bank.
(2) Streamside Vegetation Quantity	Undisturbed vegetation extends at least 2 stream widths.	Undisturbed vegetation extends 1 stream width.	Undisturbed vegetation extends half of the stream width. No more than 30% gaps in vegetation	Undisturbed vegetation extends a third or less of the stream width. >30% gaps in vegetation
(3) Streamside vegetation Quality	All 3 types well represented	2 types	1 type	No natural vegetation
(4) Canopy Cover	>75% of water surface shaded along the entire stream.	75-50% of water surface shaded along the entire stream.	50-25% of water surface shaded along the entire stream.	<25% of water surface shaded along the entire stream.
(5) Riffle Embeddedness	<1 second (or no visible sediment)	2-5 seconds	6-9 seconds	>10 seconds
(6) Trash and Garbage	No trash visible	Trash occasionally visible, minimal toxic concerns	One or another form of trash constantly in view, but with no large concentrations. Toxic or questionable materials present.	Large concentrations of trash nearly always in view and/or major toxic concerns.

(7) Non-Trash Pollution	No evidence of non-trash pollution in the stream.	Temporary sources such as animal carcasses or food waste. Dense human populations near the stream, diapers present.	Feedlots or animal pens poorly buffered, but without direct drainage. Unbuffered or barely buffered fertilized lawns or row crop fields.	Feedlots, trout farms or dog pens draining directly to the stream.
(8) Livestock	No livestock access more significant than wandering dogs.	Restricted access, such as exclosures, or very small numbers (e.g. 3 ducks, a single horse).	Livestock in pastures which are fenced away from the stream but with little or no vegetative buffer. Deduct half-point if: Livestock with full access to tributary streams and ditches draining directly into the stream.	Extensive, continual and uncontrolled access by livestock
(9) Pools	≥ 4 pools/500 ft. At least 1 “deep pool” Structure present in most pools.	2-3 pools/500 ft. Should have at least 1 “deep pool” and patchy structure.	1 pool / 500 ft.	No pools
(10) Available Habitat/Cover	Greater than 75% of stream reach contains at least 9 habitat types.	50-75% of stream reach contains 6-8 habitat types.	25-50% of stream reach contains 4-6 habitat types.	<25% of stream reach contains 3 or less habitat types.
(11) Barriers to Fish Movement	No artificial barriers are present.	Lack of habitat and canopy cover restrict movement of fish species.	Physical structures and/or water withdrawals restrict movement of fish species.	Physical structures and/or water withdrawals severely restrict movement of fish species.

Table 8. Summary statistics for temperature logger data (degrees Celsius) collected at 1 hour intervals from 4 to 27 July 2024 (n=9).

Stream	**Latitude**	**Longitude**	**Mean**	**Standard Deviation**	**Minimum**	**Maximum**
Betty Creek	34.98669	-83.44372	19.86	0.90	17.92	22.20
Crawford Branch	35.17969613	-83.38906161	20.47	0.98	17.82	25.04

Darnell Creek	34.95984	-83.37037	19.28043	0.745614	17.51	21.39
Jones Creek	35.11523157	-83.46695884	19.85479	1.281579	16.82	22.585
Middle Creek	35.04016	-83.36127	20.18751	0.810602	18.2	22.585
Skeenah Creek	35.11187	-83.39091	21.06148	1.226689	18.2	24.532
Tellico Creek	35.28334	-83.50397	19.31235	0.694648	17.296	21.318
Wayah Creek	35.15408726	-83.48786832	19.89516	1.21236	17.249	23.376
Younce Creek	35.23779013	-83.47420967	20.54	1.05	18.01	23.16