2021

A Spatiotemporal Assessment of Fish Assemblage Response to Land-Use Change and the Evaluation of eDNA Metabarcoding for Describing Diverse Fish Communities

Timothy M. Owen

A Spatiotemporal Assessment of Fish Assemblage Response to Land-Use Change and the Evaluation of eDNA Metabarcoding for Describing Diverse Fish Communities

November 9th, 2021

A Thesis
Submitted in Partial Fulfillment of the Requirements for the
Degree of Master of Science
with a
Major in Environmental Studies
at
Virginia Commonwealth University

By
Timothy M. Owen

Major Professor: Stephen P. McIninch, Ph.D.

Committee Members: Edward R. Crawford, Ph.D.; Greg C. Garman, Ph.D.

Virginia Commonwealth University
Richmond, Virginia
November, 2021

Acknowledgements

I would first like to thank the VCU Rice Rivers Center for providing the financial support to make this research possible. For asking the right questions along the way, providing resources that have aided in my development as a researcher, and being exceptional well-rounded mentors, I'd like to thank my committee: Dr. Stephen McIninch, Dr. Greg Garman, and Dr. Edward Crawford. For his expertise in environmental DNA, his patience as a teacher, and for his direct contributions to the second chapter of this manuscript, I thank Dr. Aaron Aunins of the United States Geological Survey. I also want to thank David Hopler and Stephen Kaliris of VCU for helping coordinate and conduct the fisheries surveys performed in this study. Alan Weaver of the Virginia Department of Wildlife Resources was essential in providing logistical support, sampling equipment, and manpower throughout the study. I appreciate the additional field help given by Brycen Boettcher, Brady Donovan, Mathias Gaffney, Peter Grap, and Jonathan Harris. I'd like to think Curtis Roth, Mike Lien, Kevin Poole and other past colleagues at the Friends of the Teton River, the Idaho Department of Fish and Game, the U.S. Forest Service, and the Virginia Department of Wildlife Resources. Thank you for helping me develop the skills I needed to complete this project, I know I learned something by working with each of you. Lastly, I owe everything to my parents Michael and Sherry for cultivating my curiosity in the natural world, and my wife Kristin for her unconditional support and encouragement.

Table of Contents

List of Figures

Chapter 1

Chapter 2

List of Tables

Chapter 1

Chapter 2

Abstract

A SPATIOTEMPORAL ASSESSMENT OF FISH ASSEMBLAGE RESPONSE TO LAND-USE CHANGE AND THE EVALUATION OF EDNA METABARCODING FOR DESCRIBING DIVERSE FISH COMMUNITIES

By Timothy M. Owen, M.S. Environmental Studies

A thesis submitted in partial fulfillment of the requirements for the
degree of Master of Science
at Virginia Commonwealth University

Virginia Commonwealth University, 2021
Major Advisor: Stephen P. McIninch Ph.D.

Fish assemblages are often assessed as a biological proxy for environmental health. While humans value healthy environments for the ecosystem services and recreational opportunities they provide, it is increasingly evident that such resources can be paradoxically degraded by anthropogenic activities. In this investigation, we studied the relationship between different intensities of anthropogenic land-use change and habitat-driven fish assemblage response across multiple spatiotemporal scales. Secondarily, we explored the efficacy of eDNA metabarcoding against conventional electrofishing techniques for the purpose of describing complete fish communities. This study was conducted in the Tuckahoe Creek basin near Richmond, Virginia. This James River tributary serves as an optimal case-study due to a myriad of land-use changes that have continued to occur throughout the basin, in conjunction with a diverse fish assemblage that has been studied across a unique fisheries dataset that originated in 1869. Our findings indicate that fish assemblage dynamics are driven by localized, low-intensity development, and are therefore longitudinally discontinuous throughout the Tuckahoe Creek basin. Further, we observed that eDNA metabarcoding outperformed electrofishing in determining fish biodiversity throughout the system.

Vita

Timothy Michael Owen was born on May 25th, 1990, in Radford, Virginia. Timothy received a Bachelor of Science in Wildlife and Fisheries Resources from West Virginia University in 2012. During his tenure at West Virginia University, he was a seasonal fisheries technician for the United States Forest Service and assisted on Brook Trout conservation projects throughout the Monongahela National Forest. Timothy later aided in habitat rehabilitation and population monitoring of Lahontan Cutthroat trout in the Sierra Nevada Mountains as an employee of the High Sierra Ranger District. In 2013, Timothy moved to Idaho to work on the recovery of Idaho's Steelhead, Salmon, and Bull Trout populations with the Idaho Department of Fish and Game. Timothy became the fisheries lead at Friends of the Teton River Inc. in 2015, and helped orchestrate basin-wide population monitoring, fish passage projects, and fish migration studies concerning Yellowstone Cutthroat Trout. Timothy joined the Virginia Department of Wildlife Resources in 2017, assisting with statewide fish passage projects with an emphasis on the conservation of Virginia's anadromous fish species.

Chapter 1 : Localized Low-Intensity Anthropogenic Land-Use Change Drives Heterogeneous Response in Fish Assemblage Diversity and Distribution

A manuscript formatted for publication in the North American Journal of Fisheries Management

Timothy M. Owen, Stephen P. McIninch, Greg C. Garman

Abstract

Although intact native fish communities are valued for their ecosystem services, economic value, and recreational opportunities, they are often paradoxically degraded by increasing levels of anthropogenic activity. While studies investigating fish assemblage response to anthropogenic land-use change have often documented results consistent with the urban stream syndrome, others have resulted in findings to the contrary. In this study, we investigated the relationship between anthropogenic land-use changes and habitat driven fish assemblage response across multiple temporal and spatial scales. This case study was conducted at established sampling locations within Tuckahoe Creek, a Chesapeake Bay watershed near Richmond, Virginia. Tuckahoe Creek contains a diverse fish assemblage that is associated with a unique set of fisheries datasets that span up to sixty-two years. We found fish assemblage response to land cover change is best predicted by low intensity development quantified at smaller spatial scales ($r^2 = 0.937$; $p < 0.01$). While some sites we observed exhibited symptoms of urban stream syndrome, we found that fish assemblage changes were longitudinally discontinuous throughout nested sampling sites in the watershed, and at least partially correlated to the habitat needs of each site's baseline assemblage. Our results indicate that assessing fish diversity in systems subject to anthropogenic land-use change may benefit from higher sampling intensities.

Introduction

Healthy native fish communities are a key indicator of a functioning aquatic ecosystem. These natural resources are highly valued by an array of stakeholders for their environmental services, as well as the recreational opportunity they provide (Cooke et al., 2020). As human populations continue to shift toward more condensed areas, anthropogenic land-use activities are increasingly encroaching on natural environs (Sala et al., 2000). As such, anthropogenic land-use changes (ALUC) are considered a paramount threat to ichthyofaunal diversity and the natural function of the aquatic ecosystems they inhabit (Sala et al., 2000; Marchetti et al., 2006; Giacomozo et al., 2020; Pugh et al., 2020).

Fish assemblage response observed in lotic systems affected by ALUC include aquatic habitat degradation and a decrease in total fish species richness (Lodge et al., 2012). These degradations may result from hydrologic volatility, decreased recruitment of woody debris to the stream channel, increased substrate embeddedness, or disruption of the system's natural thermal regime. While these factors are certainly influenced by stochastic events, such as climactic conditions, ALUC can exacerbate these factors through increased coverage of impervious surfaces, loss of proximal terrestrial vegetation, and increased soil compaction (Phelan et al., 2017; Rapp et al., 2017).

Although many studies have assessed land-use change – fish response (LUCFR) dynamics, specific outcomes vary throughout the literature (Scott and Helfman 2001; Walters et al., 2003; Walsh et al., 2005; Burcher et al., 2007; Armstrong et al., 2011; Booth et al., 2016). These studies often encompass dissimilar study areas, chronological timelines, sampling intensities, spatial lenses, or fish communities (Weaver and Garman 1994; Smith et al., 2014; Cervantes-Yoshida et al., 2015; Le Pichon et al., 2017). As ALUC often occurs throughout a

continuum of timing and duration, along a spectrum of intensity and proximity, the reproduction of results concerning LUCFR research is innately difficult. Such confounding variables may be exacerbated in long-term studies, of which there is a scarcity of opportunity.

While many LUCFR studies have quantified trends by extrapolating relationships from probabilistically generated sampling locations, a growing number of studies have shown a significant connection between more localized, site-specific environmental factors and the observed fish assemblage response (Strayer et al. 2003; Hawkins et al., 2015; Patterson et al., 2017). This may indicate that localized ALUC drives fish response semi-independently of dynamics occurring elsewhere in a given system.

In the present study, we conducted a LUCFR investigation at established survey locations within the Tuckahoe Creek basin, located in Henrico County, Virginia. Our research builds upon a series of historical investigations that occurred within the basin in 1958, 1990, and 2014 (Flemer and Woolcott 1966; Weaver and Garman 1994; Stickley 2015). Weaver and Garman (1994) first described LUCFR dynamics within the Tuckahoe Creek basin by quantifying a long-term relationship (32 years; $r^2 = -0.84$, $P < 0.05$) between community-level fish diversity and the percentage of anthropogenic development within the riparian area of each survey location. Stickley (2015) later documented a similar relationship by indicating fish diversity from 1958 to 2014 was significantly affected by the coverage of impervious surfaces within the stream's riparian area, but found no significant relationship between the two variables had occurred between 1990 and 2014.

Although previous studies within the Tuckahoe Creek basin collectively describe long-term fish assemblage responses to ALUC, our objective was to investigate LUCFR dynamics at spatiotemporal scales that haven't been previously assessed. Further, we believe LUCFR

throughout the basin is longitudinally discontinuous, and attribute such effects to a combination of heterogeneity in the habitat needs of site-specific fish communities, and variation in the timing, intensity, and proximity of ALUC being assessed. Lastly, we theorize that many LUCFR dynamics within the basin have gone undocumented, and attribute this to the range of ALUC present at established survey sites versus that which has occurred at unobserved locations throughout the watershed.

Methods

Study Area

Tuckahoe Creek is a third order tributary of the James River, a major artery to the Chesapeake Bay, and its catchment spans the counties of Goochland, Hanover, and Henrico near Richmond, Virginia (Fig. 1.1). The stream system transcends a single geophysical province, encompassing characteristics of both the Virginia Piedmont and Virginia Coastal Plain throughout its 28-kilometer length.

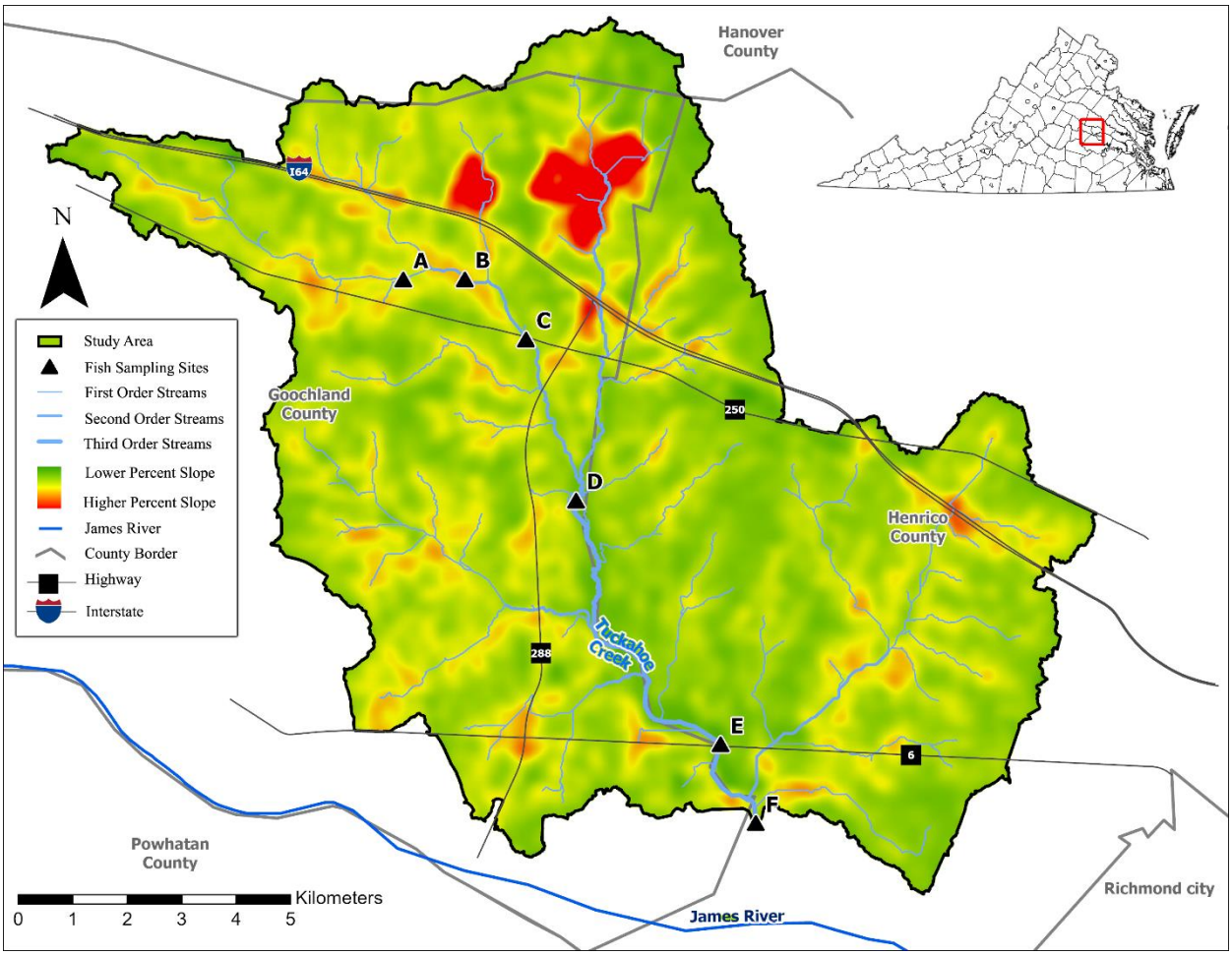

Figure 1.1 Study Area – Tuckahoe Creek watershed and region

Sites A and B are located at medium-gradient portions of the basin and are comprised of characteristics typical of Virginia Piedmont systems. Sites C-F are lower gradient, and are characterized by sprawling swampy habitats more commonly associated with Virginia Coastal Plain systems (Flemer and Woolcott 1966). Our sampling locations contained first order (Site A), second order (Sites B and C), and third order (Sites D-F) segments of the stream network (Table 1.1). The total study area is relatively similar in elevation, consisting of about a twenty-meter elevation difference from the most upstream site (Site A) to the most downstream site (Site F; Table 1.1).

Table 1.1 Physical characteristics of Tuckahoe Creek sampling locations.

Site	Stream Order	Elevation(m)	Mean Wetted Width (m) Flemer and Woolcott 1966
A	1	58.6	2.4
B	2	52.5	6.1
C	2	49.1	4.6
D	3	44.0	18.3
E	3	38.8	4.6
F	3	37.5	6.1

Dynamic land cover changes have occurred within the basin throughout the study period, with the majority of changes being indicative of a system experiencing long-term urbanization (Weaver and Garman 1994; Stickley 2015). Although much of the watershed has experienced an increase in anthropogenic activity, there are exceptions, where patchy areas of the basin have transitioned between various natural ecosystems and agricultural use. From 1953 to 2014, the area of natural land cover within the basin decreased from 73% to 33%, while land classified as impervious surface increased from 3% to 47%, and agricultural lands remained relatively constant decreasing from 24% to 20% (Stickley 2015).

Historical Fisheries Dataset

A distinctive long-term dataset of the Tuckahoe Creek fish assemblage originated with qualitative fisheries observations in 1869 (Cope 1869) and 1937 (Raney 1950). Subsequent quantitative fisheries surveys were conducted in 1958 with a seine (Flemer and Woolcott 1966), 1990 via seine and backpack electrofishing (Weaver and Garman 1994), and 2014 by backpack electrofishing (Stickley 2015). While the exact survey locations for the 1869 and 1937 observations are not known, each of the 1958, 1990, and 2014 fisheries surveys were conducted within the same established observation sites (Fig. 1.1).

Although the sampling gears used in the past investigations of the Tuckahoe Creek fish assemblage varied with contemporary practices, each of the quantitative surveys was performed with the intent of capturing a complete representative community fish sample at each of the sites (Flemer and Woolcott 1958; Weaver and Garman 1994; Stickley 2015). Collectively, these surveys have resulted in documenting the presence, relative abundance, and distribution of 38 species of fish. Site E (Fig. 1.1) had to be excluded from this study, as it was unable to be sampled in 2014 and 2020 due to drastically changing site conditions and increased water depth (Stickley 2015).

Land Cover Change

Our study period spanned seven decades, and therefore it was necessary to obtain land-use data using a variety of methods across different resolutions (Table 1.2). Land cover classification definitions were examined from each of the datasets, and aggregated into either Natural Cover, Agricultural Cover, or Anthropogenic Development. Anthropogenic Development was then partitioned into Low Intensity, Medium Intensity, or High Intensity development for each of the study periods, based on the available descriptions within the land cover metadata (Table 1.3). The Open Water cover type was emitted from all datasets, as this classification fluctuated between describing natural areas of still water, and artificially dammed waterways that didn't fall into a single development classification.

Table 1.2 Datasets used for landscape analysis

Data Type	Description	Source	Associated Fish Sample
Land-Use Data	1953 Tuckahoe Creek Land Cover Classifications	Stickley 2015	1958
Land-Use Data	1992 Tuckahoe Creek Land Cover Classifications	NLCD: Multi-Resolution Land Characteristics Consortium	1990
Land-Use Data	2013 Tuckahoe Creek Land Cover Classifications	NLCD: Multi-Resolution Land Characteristics Consortium	2014
Land-Use Data	2016 Tuckahoe Creek Land Cover Classifications	NLCD: Multi-Resolution Land Characteristics Consortium	2020
Digital Elevation Model	2014 Virginia LiDAR Dataset	Virginia GIS Clearinghouse	ALL

All spatial analyses were performed in ArcGIS Pro v10.2 (ArcPro). Percentages of each land cover classification were analyzed for sites A, B, C, D, and F across three relevant spatial scales (Fig. 1.2; Wang et al., 2001). The catchment scale (Fig 1.2a) represented the largest spatial lens by area, and was generated using the Spatial Analyst Toolset in conjunction with Digital Elevation Model (DEM) data obtained from Virginia GIS Clearinghouse (VGIN). The riparian corridor scale (Fig 1.2b) was delineated by generating streamlines from DEM data using the Hydrology Toolset, and buffering streamlines by 100 meters, as recommended by the United States Environmental Protection Agency (Mayer et al., 2005), on both sides of the stream. Consistent with other contemporary land-use studies (Wang et al., 2001; Cervantes-Yoshida et. al, 2015), a local 3-kilometer site-catchment lens (Fig 1.2c) was generated by creating a 3-km buffer circle from the centroid of each of the sample sites and then intersected with that of the site's total catchment geometry. Land cover composition was assessed at each of the spatial scales by performing Tabulate Area with every combination of spatial scale and land cover dataset. Changes were derived by calculating percent composition differences for each of the designated land cover types between each study period.

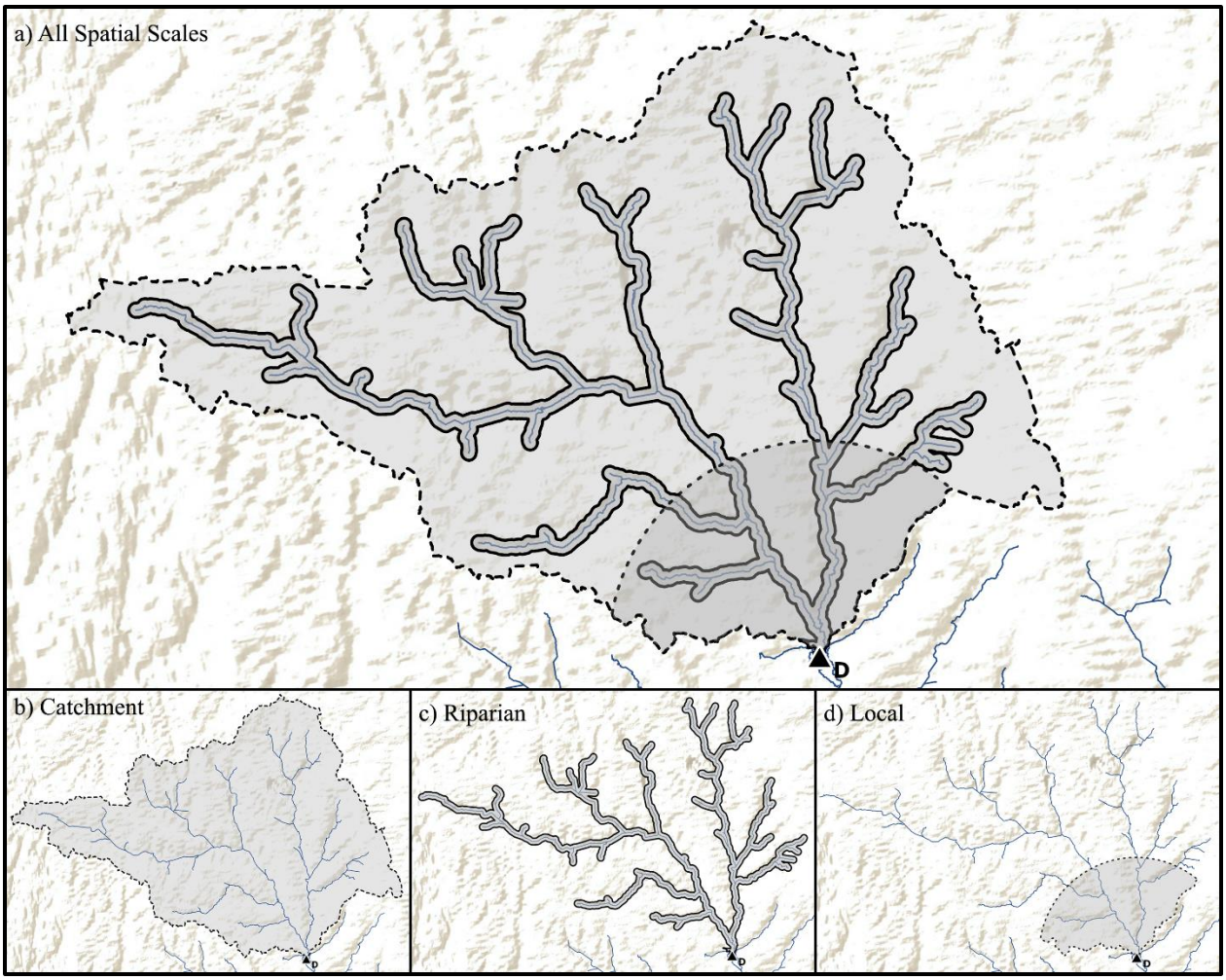

Figure 1.2 Spatial scales of land-use change assessed within the study.

Fisheries Sampling

Single-pass electrofishing surveys were performed at sites A, B, C, D, and F in July, August, and September of 2019 and 2020 (Fig. 1.1). These investigations took place within the same season, and at the same locations previously sampled in 1958 (Flemer and Woolcott 1966), 1990 (Weaver and Garman 1994), and 2014 (Stickley 2015). Electrofishing was completed using either one or two SmithRoot LR-30 backpack electrofishing units, and three to six netters, as needed for accurate catch efficiency.

Each electrofishing survey was performed in an upstream manner, following the same Environmental Protection Agency approved protocol that was utilized in the 2014 sampling effort (Stickley 2015). Fishes were netted and held in aerated containers throughout the duration of each sampling effort. Collected fish were identified to species, enumerated, and released back into the transect at survey completion. At each of the sites, a small sub-sample of collected specimens were treated with a lethal dose of MS-222, and preserved for a separate investigation (following IACUC protocol #AD10000441).

Fish Assemblage Change Analyses

All data entry was completed in Microsoft Excel and imported to R Studio V3.62 (R) for statistical analysis and display. Fish assemblage metrics were quantified by enumerating each species observed throughout each of the survey periods. Individual sampling efforts were deemed non-independent, and were therefore aggregated by site into either 1958, 1990, 2014, or 2020 observations. Each observed species was categorized by spawning habitat guild based on descriptions from Jenkins and Burkhead (1994). In the few cases where multiple spawning habitats preferences were documented, only the primary spawning habitat preference was listed for guild classification. Changes in relative abundance of each species and habitat-guild category were calculated by dividing the number of individuals in a category by the total number of species in all categories.

Fish assemblages at each site were categorized by species and spawning habitat guild, and assemblage changes between sites and survey periods were calculated by deriving Bray-Curtis Dissimilarity coefficients (BCD). Species-level LUCFR relationships were calculated using multiple linear regression analysis of the average sample-period BCD coefficient against percent land cover change across each combination of sample period (1958-1990, 1958-2014,

1958-2020, 1990-2014, 1990-2020, 2014-2020) and spatial scale (Catchment, Riparian, Local). The Akaike information criterion (AIC) tool in R was used to identify the model best fit for describing land-use driven fish assemblage changes.

Principal Component Analysis (PCA) was used to visualize the site-specific fish assemblage changes in species occupancy when grouped by their spawning habitat guilds. Change in representation of each spawning habitat guild was defined by the BCD for that time period, and grouped by sample site.

Basin-Wide Assessment of Observed LUCFR Dynamics

Upon analysis of the LUCFR dynamics observed at our long-term survey locations, additional spatial analysis was conducted to assess the heterogeneity of localized low-intensity development throughout the entire stream continuum. This was completed by following the ArcPro process detailed in section 2.3 and repeated for points that were generated every one-hundred meters, longitudinally, throughout the entirety of the Tuckahoe Creek mainstem corridor. Lastly, we compared the representativeness of localized land cover changes within the established long-term survey locations to those in unobserved areas of the watershed using a violin plot to indicate site-specific ALUC, relative to the basin-wide distribution of ALUC.

Results

Land Cover Change

The Tuckahoe Creek watershed has continued to experience land cover loss in natural and agricultural land cover types (Table 1.3) consistent with previously conducted studies within the basin (Weaver and Garman 1994; Stickley 2015). These land cover types were largely converted into low, medium, or high intensity anthropogenic development. Although land cover

changes varied extensively by site throughout each observation period, the increase in low intensity development was the most common change observed at the basin-wide lens.

Table 1.3 Land cover change throughout each iterative study period.

Land Cover	1953	1992	2013	2016	Most Common Attribute	Description
Natural	73.26	52.06	43.64	42.46	Deciduous Forest	All Natural Cover Types
Agricultural	24.32	19.45	11.47	11.17	Pasture	All Agricultural Cover Types
Low Intensity Development	2.42	17.10	37.12	37.56	Golf Course / Residential	<50% Impervious Surface Coverage
Med Intensity Development	0.00	4.21	6.23	7.08	Residential Housing Units	50-79% Impervious Surface Coverage
High Intensity Development	0.00	7.17	1.53	1.73	Commercial / Industrial	80-100% Impervious Surface Coverage

Fish Assemblage Dynamics

A total of thirty-nine species of fish, comprising 4,667 individuals were collected across fisheries surveys completed in 1958, 1990, 2014, and 2020. Bray-Curtis dissimilarity coefficients for each combination of the sample periods observed indicate that species diversity in the 1958 and 2014 fish communities were most different, while 2014 and 2020 were most similar (Table 1.4). In general, fish assemblage dissimilarity had a positive correlation to the time between sampling events. Total taxa represented within the basin was observed at 32 (1958), 27 (1990), 32 (2014), and 25 (2020) species.

Table 1.4 Bray-Curtis Dissimilarity Coefficients for relative abundance of fish species change between all observation periods. Higher numbers indicate greater degree of species-level change.

Observation Period	Time Between Observations	Mean Bray-Curtis Dissimilarity
1958-1990	32 Years	58
1958-2014	56 Years	73
1958-2020	62 Years	69
1990-2014	24 Years	64
1990-2020	30 Years	62
2014-2020	6 Years	48

Multiple linear regression analysis of BCD coefficients for relative species abundance showed that percent change in low intensity development at the local scale (ΔLIDL) was the best fit predictor of fish assemblage change within the basin (R^2=0.937, P=0.0015; Fig. 1.3).

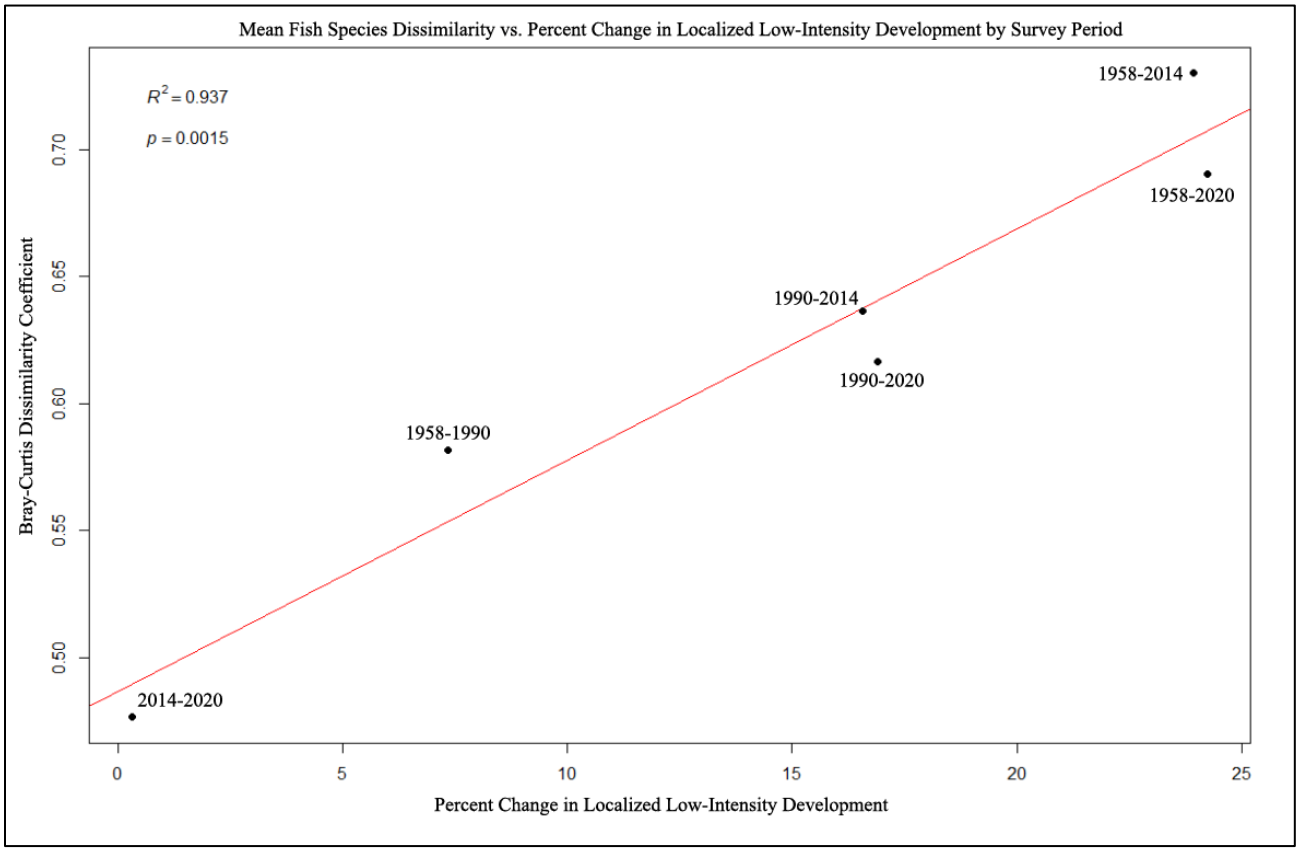

Figure 1.3 Simple linear regression of Bray-Curtis dissimilarity and percent change in low-intensity development at the local spatial scale.

Throughout the aggregate of sample periods, mean species dissimilarity was significantly correlated to mean spawning habitat dissimilarity when grouped by sample site (Pearson Product Correlation = 0.83; Table 1.5). For both categories of fish assemblage change (Species and Spawning Habitat), Site F was found to have experienced the most fish assemblage change between sample periods, while Site D was found to have changed the least.

Table 1.5 Spatial distribution of fish assemblage dissimilarity with fishes grouped by species and spawn-habitat guilds (Pearson Product Correlation = 0.83).

Site	Mean Species Bray-Curtis Coefficient	Mean Spawning Habitat Guild Bray-Curtis Coefficient
A	60	54
B	59	43
C	67	48
D	55	41
F	70	59

The relative abundance of the extant habitat spawning guilds varied temporally (Fig. 1.4), although some generalized basin-level trends did occur. Species reliant on pool habitats for spawning activity continued to trend toward higher relative abundance, while those spawning in riffle-run habitats experienced the largest relative decline. Backwater and pool-run spawning species have appeared to stabilize in abundance after initially displaying a significant decline in the earlier surveys. Riffle spawning species were most uncommon in the baseline 1958 fisheries surveys, and therefore had the least ability to exhibit a decrease in relative abundance, however, they have remained relatively constant.

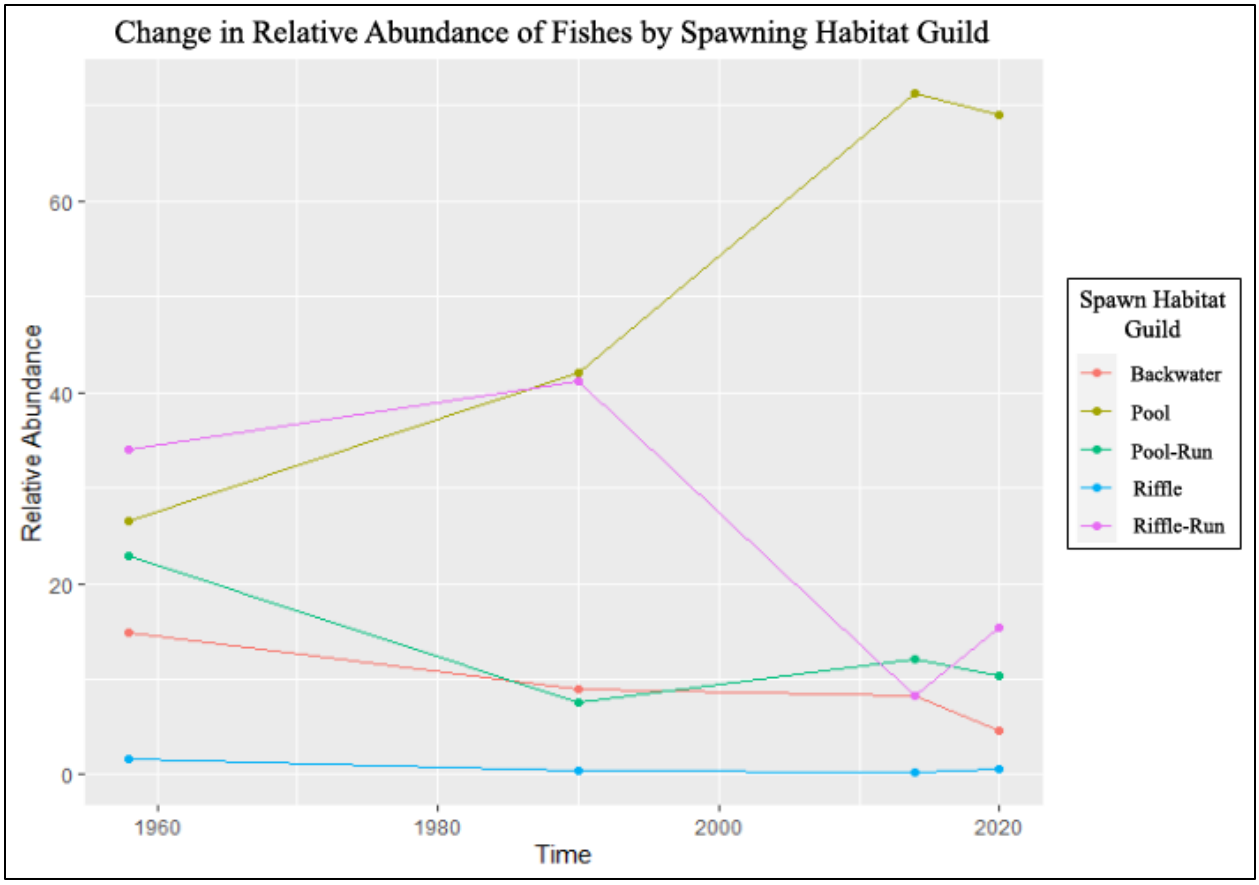

Figure 1.4 Change in relative abundance of fishes by spawning habitat guild by sample period.

The PCA results suggest that the observed trends are spatially distinct responses (Fig. 1.5). PC1 is indicative of the range of habitat-grouped fish community changes that have occurred throughout the study period at each location. PC2 is representative of the site-specific variance in habitat-grouped fish diversity within the same timeframe. The highest amount of total fish assemblage change was observed at Site F, the most downstream site in the drainage. Site F is also the second-most homogenous location and is dominated by pool dependent species. In contrast, Site D, the most homogenous location, exhibited the least amount of total change and the least amount of variation. Site C exhibited the highest level of assemblage diversity in regard

to spawning habitat requirements. Sites B and A are responsible for the largest declines in riffle-run, pool-run, and riffle dependent species of fish.

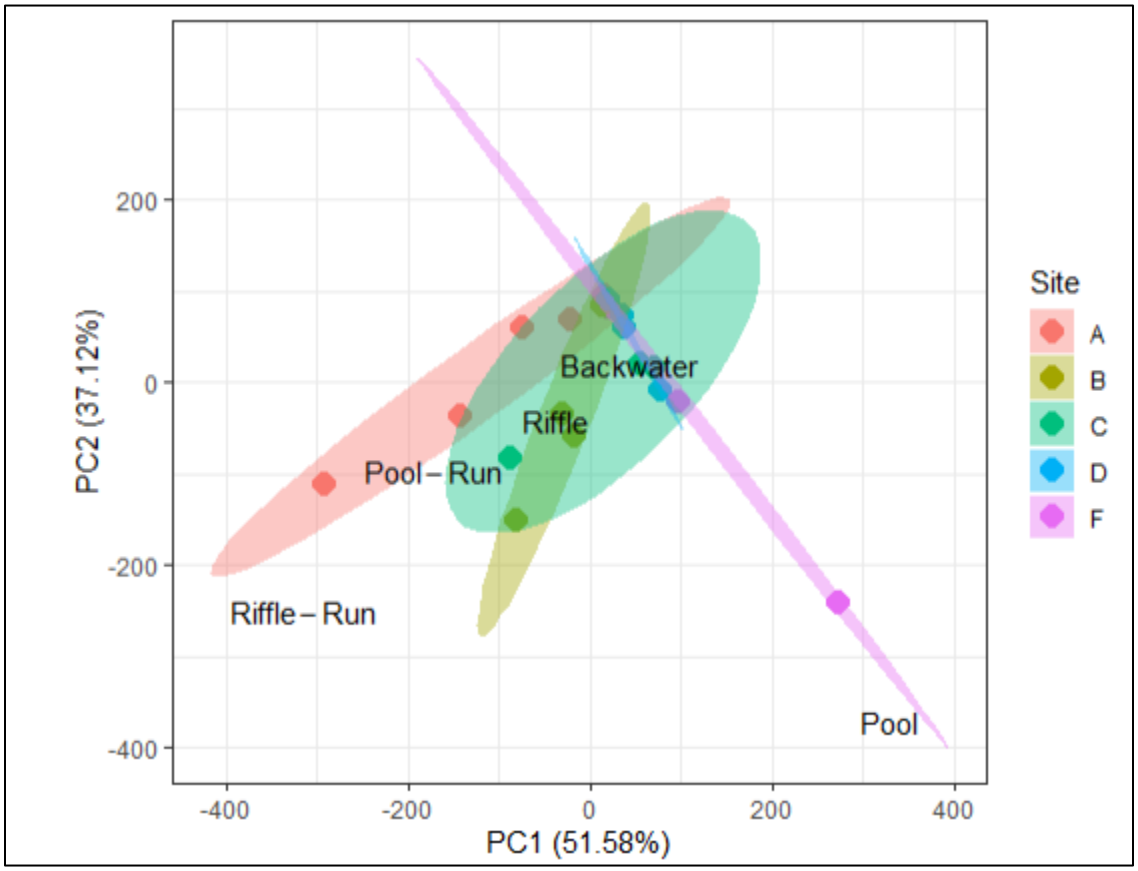

Figure 1.5 PCA of change in habitat guild species between sites over all sampling periods.

Basin-Wide Assessment of Observed LUCFR Dynamics

Extrapolating the results of our linear regression analysis, ΔLIDL at unobserved locations within the watershed displays high variability throughout the basin. This is best visualized over the longest chronological period of land cover changes we observed (1953-2016; Figures 1.6, 1.7 respectively). The areas least affected by ΔLIDL exist above Sites A and B. Locations near Site

C are subject to moderate levels of localized development, and large levels of ΔLIDL from 1953 to 2016 occurred between sites D and F.

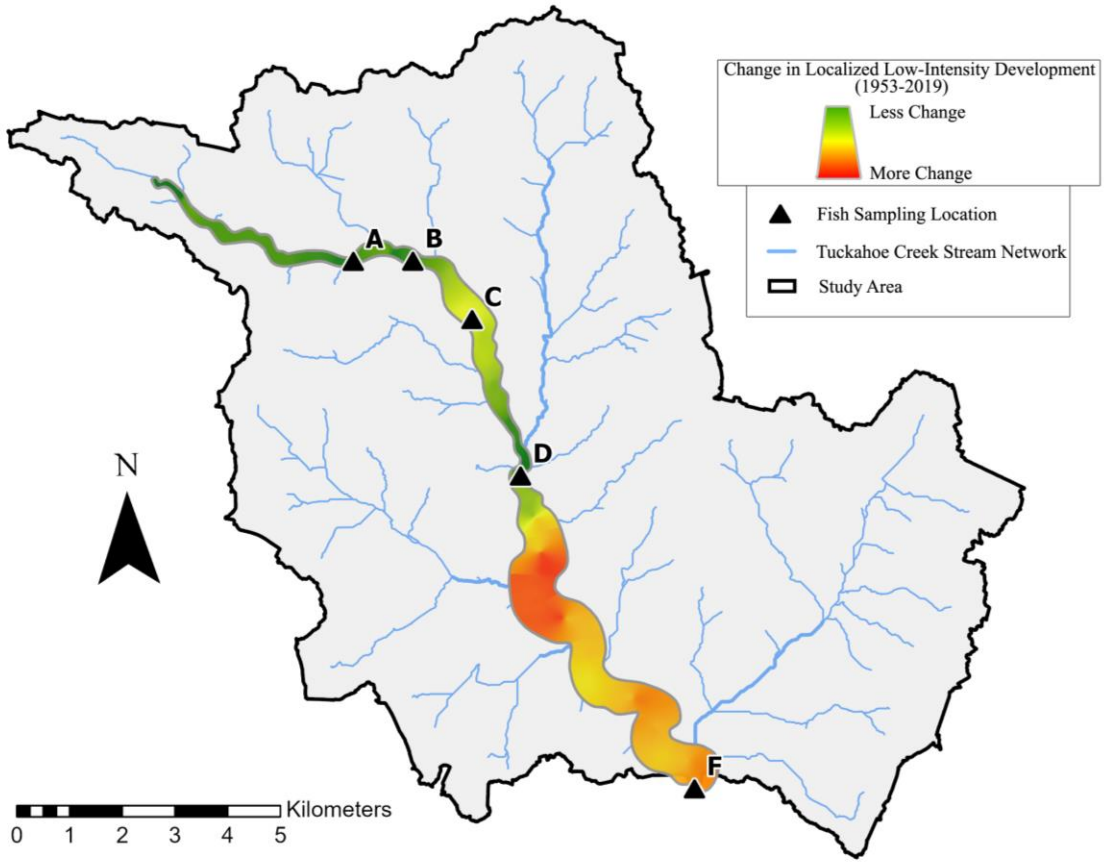

Figure 1.6 Variability of delta low intensity development (local scale) from 1953-2019.

Further, the intensity of ΔLIDL acting upon our sampling locations did not encompass the range of ΔLIDL intensity throughout basin within any of the survey periods (Fig. 1.7). Longer survey periods (e.g., 1958-2020) were associated with higher quantities, and greater levels of heterogeneous distribution of ΔLIDL, and therefore resulted in less site representativeness. Shorter survey periods (e.g., 2012-2020) indicate a smaller range of ΔLIDL

heterogeneity, and as a consequence, the observed sites encompassed a higher degree of representativeness.

Figure 1.7 Distribution and site representation of the change in low intensity development (local scale) throughout the Tuckahoe Creek system.

Discussion

The results presented herein indicate that increasing levels of ALUC has been a primary driver of fish assemblage change within the Tuckahoe Creek watershed. Further, our findings reveal that fish assemblage shifts are at least partially due to habitat alterations resulting from ALUC, and that localized low-intensity development is more significantly driving LUCFR dynamics than the other predictor variables tested. In addition, while basin-level metrics show a general trend of increased ALUC throughout the Tuckahoe Creek watershed, there were exceptions to this trend when examined at different spatiotemporal scales.

Basin-wide fish assemblage changes occurred across the entire study period, our findings however, suggest that LUCFR is longitudinally discontinuous, and that the observed heterogeneity in fish response is not solely a result of geomorphological differences (i.e., elevation, stream order, ecoregional designation). These conclusions are highlighted by results observed for sites D and F, which are most similar in baseline fish assemblage, stream order, elevation, and slope, yet the fish assemblage at Site D was found to have changed the least, and Site F exhibits the most fish assemblage dissimilarity between any of the observed locations and timeframes.

Our study shows the relationship between fish assemblage composition and low intensity disturbance within the basin is best quantified at a local catchment scale, and was less connected to disturbance at the other spatial lenses we assessed. We suggest that future efforts aimed at describing fish assemblage diversity in watersheds subject to ALUC would benefit from implementing higher sampling intensities than those conducted in watersheds comprised of homogenous landscapes. This is further supported by the ΔLIDL analysis of unobserved locations throughout the Tuckahoe Creek stream corridor, which provided evidence that the established long-term survey locations were not fully representative of the spectrum of ALUC intensity, and the corresponding spectrum of LUCFR, present within the study area.

Further reinforcing our findings of longitudinal stream discontinuity in fish response, our results indicate that the Tuckahoe Creek ecosystem possesses site-specific resistance, or the ability of a location to resist change, and site-specific resilience, the ability to recover from previous disturbances. Site resistance is best observed at site D, where dissimilarity scored lowest between all temporal lenses. This is in stark contrast to upstream locations, Sites A, B, and C, which were more dissimilar within the same periods of time (Table 1.5). Site resilience

was most evident at Site C, where the Bray-Curtis Dissimilarity Coefficient between the 1958 fish assemblage decreased by six from 1990 to 2020. Similarly, resilience was evident at the basin level as shown by the mean Bray-Curtis Dissimilarity Coefficients decreasing between the 1958 fish assemblage from 1990 to 2020

We conclude that variability in ΔLIDL should be considered when determining the sampling intensity necessary to describe fish assemblages at a basin-wide scale. The lack of a longitudinal pattern in fish assemblage dissimilarity within our results allows us to conclude that sampling locations are spatially unique and possess an array of characteristics that either exacerbate or alleviate the degradation factors of disturbances within the stream continuum. Additionally, the scale at which these locations exhibit discontinuity may be smaller than previously considered. Temporal fluctuations in fish presence and absence throughout our study period, either by individual taxon or when grouped by habitat guilds, suggests that individuals emigrate degraded sites in search of locations that are more optimally suited for their ecological needs. Our findings imply that our sampling efforts have adequately described the fish assemblage changes within the sampled transects, but due to the spectrum of ALUC throughout the Tuckahoe Creek watershed, additional sampling efforts would be necessary to accurately characterize basin-wide fish diversity.

Acknowledgements

Funding for this research was generously provided by the Rice Rivers Center at Virginia Commonwealth University, and the Virginia Department of Conservation and Recreation's Healthy Waters Program. Additional support was given by the U.S. Geological Survey, and the Virginia Department of Wildlife Resources. We specifically thank A. Weaver, B. Boettcher, B.

Donovan, J. Harris, D. Hopler, M. Gafney, P. Grap, and S. Kaliris for their assistance with field work. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement. This study was conducted under the Virginia Commonwealth University Animal Care and Use Committee Protocol #AD10000441.

References

Armstrong, D. S., T. a. Richards, and S. B. Levin. 2011. Factors influencing riverine fish assemblages in Massachusetts. U.S. Geological Survey Scientific Investigation Reports 5193:59.

Booth, D. B., A. H. Roy, B. Smith, and K. A. Capps. 2016. Global perspectives on the urban stream syndrome. Freshwater Science 35:412–420.

Burcher, C. L., H. M. Valett, and E. F. Benfield. 2007. The land-cover cascade: Relationships coupling land and water. Ecology 88:228–242.

Cervantes-Yoshida, K., R. A. Leidy, and S. M. Carlson. 2015. Contemporary land change alters fish communities in a San Francisco Bay Watershed, California, U.S.A. PLoS ONE 10:1–19.

Cooke, S. J., R. J. Lennox, B. Cantrell, and A. J. Danylchuk. 2020. Micro-Fishing as an Emerging Form of Recreational Angling: Research Gaps and Policy Considerations. Fisheries 45:517–521.

Cope, E. D. 1869. On the distribution of fresh- water fishes in the Allegheny region of south-western Virginia. J. Acad. Nat. Sci. Phila. Ser. 2, 0:207-247.

Flemer, D. A., and W. S. Woolcott. 1966. Food habits and distribution of the fishes of Tuckahoe Creek, Virginia, with special emphasis on the bluegill, Lepomis m. macrochirus rafinesque. Chesapeake Science 7:75–89.

Giacomazzo, M., A. Bertolo, P. Brodeur, P. Massicotte, J. O. Goyette, and P. Magnan. 2020. Linking fisheries to land use: How anthropogenic inputs from the watershed shape fish habitat quality. Science of the Total Environment 717:135377. Elsevier B.V.

Hawkins, C. P., H. Mykrä, J. Oksanen, and J. J. Vander Laan. 2015. Environmental disturbance can increase beta diversity of stream macroinvertebrate assemblages. Global Ecology and Biogeography 24:483–494.

Jenkins, R.E. and N.M. Burkhead, 1994. Freshwater Fishes of Virginia. American Fisheries Society, Bethesda, Maryland, ISBN 13: 9780913235874.

Le Pichon, C., É. Tales, J. Belliard, and C. E. Torgersen. 2017. Spatially intensive sampling by electrofishing for assessing longitudinal discontinuities in fish distribution in a headwater stream. Fisheries Research 185:90–101. Elsevier B.V.

Lodge, D. M., C. R. Turner, C. L. Jerde, M. A. Barnes, L. Chadderton, S. P. Egan, J. L. Feder, A. R. Mahon, and M. E. Pfrender. 2012. Conservation in a cup of water: Estimating biodiversity and population abundance from environmental DNA. Molecular Ecology 21:2555–2558.

Marchetti, M. P., J. L. Lockwood, and T. Light. 2006. Effects of urbanization on California's fish diversity: Differentiation, homogenization and the influence of spatial scale. Biological Conservation 127:310–318.

Mayer, P. M., S. K. Reynolds, and T. J. Canfield. 2005. Riparian buffer width, vegetative cover, and nitrogen removal effectiveness: a review of current science and regulations. EPA. 118:1–40.

Patterson, L., J. Phelan, C. Goudreau, and R. Dykes. 2017. Flow-Biology Relationships Based on Fish Habitat Guilds in North Carolina. Journal of the American Water Resources Association 53:56–66.

Phelan, J., T. Cuffney, L. Patterson, M. Eddy, R. Dykes, S. Pearsall, C. Goudreau, J. Mead, and F. Tarver. 2017. Fish and Invertebrate Flow-Biology Relationships to Support the Determination of Ecological Flows for North Carolina. Journal of the American Water Resources Association 53:42–55.

Pugh, M. W., G. Pandolfi, T. Franklin, and M. M. Gangloff. 2020. Influences of in-stream habitat and upstream land-use on site occupancy of the Kanawha darter (Etheostoma kanawhae): A narrowly distributed species from the New River (Upper Kanawha Basin) (July):1–10.

Raney, E. 1950. Freshwater Fishes. Care and Handling of Australian Native Animals (June 1946):7–16.

Rapp, J. L., and P. A. Reilly. 2017. Virginia Flow-Ecology Modeling Results : An Initial Assessment of Flow Reduction Effects on Aquatic Biota. US Geological Survey, Open-File Report:68.

Sala, O. E., F. S. Chapin, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. R. Poff, M. T. Sykes, B. H. Walker, M. Walker, and D. H. Wall. 2000. Global biodiversity scenarios for the year 2100. Science 287:1770–1774.

Scott, M. C., and G. S. Helfman. 2001. Native Invasions, Homogenization, and the Mismeasure of Integrity of Fish Assemblages. Fisheries 26:6–15.

Smith, C. D., J. R. Fischer, and M. C. Quist. 2014. Historical changes in Nebraska's lotic fish assemblages: Implications of anthropogenic alterations. American Midland Naturalist 172:160–184.

Stickley, S. F. 2015. Long-Term Effects of Land Cover Change on Fish Assemblage Structure in the Piedmont and Coastal Plain Regions of Virginia Long-Term Effects of Land Cover Change on Fish. Virginia Commonwealth University (Thesis).

Strayer, D. L., R. E. Beighley, L. C. Thompson, S. Brooks, C. Nilsson, G. Pinay, and R. J. Naiman. 2003. Effects of land cover on stream ecosystems: Roles of empirical models and scaling issues. Ecosystems 6:407–423.

Walsh, C. J., A. H. Roy, J. W. Feminella, P. D. Cottingham, P. M. Groffman, and R. P. Morgan. 2005. The urban stream syndrome : current knowledge and the search for a cure Source : Journal of the North American Benthological Society , Vol . 24 , No . 3 (Sep ., 2005), Published by : The University of Chicago Press on behalf of the Society for Freshwate. The North American Benthological Society 24:706–723.

Walters, D. M., D. S. Leigh, and A. B. Bearden. 2003. Urbanization, sedimentation, and the homogenization of fish assemblages in the Etowah River Basin, USA. Hydrobiologia 494(2000):5–10.

Wang, L., J. Lyons, P. Kanehl, and R. Bannerman. 2001. Impacts of urbanization on stream habitat and fish across multiple spatial scales. Environmental Management 28:255–266.

Weaver, L. A., and G. C. Garman. 1994. Urbanization of a Watershed and Historical Changes in a Stream Fish Assemblage. Transactions of the American Fisheries Society 123:162–172.

Chapter 2 : Surface Water eDNA Metabarcoding Outperforms Simultaneous Electrofishing Efforts in Assessing Fish Diversity and Distribution

A manuscript formatted for publication in the *North American Journal of Fisheries Management*

Timothy M. Owen, Aaron W. Aunins, Stephen P. McIninch, Greg C. Garman

Abstract

Electrofishing is currently the most common method used to assess freshwater fish communities. Although conventional electrofishing practices are considered effective, there are inherent limitations. A complimentary fish detection tool known as eDNA sequencing has gained popularity in recent years, however its use across an array of fisheries applications remains novel. The purpose of this study was to compare results derived from simultaneously conducted eDNA metabarcoding and capture-based electrofishing surveys. Results were also assessed against historical observations that originated from the same watershed beginning in 1869. This study was conducted on a species rich fish assemblage spanning a variety of abiotic habitats to better assess the efficacy of metabarcoding technology across a range of environs and fish assemblages. The results of this study indicate that metabarcoding outperforms electrofishing in determining community-level fish diversity. Metabarcoding was most advantageous in detecting numerically uncommon species of fish and may have future utility in quantifying relative species abundance.

Introduction

Community level fish diversity is often assessed to better inform fisheries management decisions, but also functions as an optimal proxy for tracking changes in the ecological condition of aquatic ecosystems (Bunn and Arthington 2002; Reid et al., 2009). In freshwater applications, capture-based sampling methods, such as electrofishing, are frequently used to conduct these

assessments (Dunham et al., 2009). The continuous refinement of this gear has resulted in contemporary practices that are ultimately considered effective, safe, and conventionally ethical (Bennett et al., 2016). Despite advancements, there are still innate risks, biases, and other limitations associated with electrofishing that may be suboptimal in some applications (Bohlin et al., 1989; Niemelä et al., 2000; Snyder 2003; Quist et al., 2009).

An emerging fish sampling technique, derived from the analysis of environmentally sourced organismal deoxyribonucleic acid (DNA), provides an alternative non-invasive method of fish assemblage characterization (Lodge et al., 2012; McDevitt et al., 2019). All living organisms, including fish, continuously release DNA into their environment through cell regeneration, waste excrement, spawning, and a plethora of other natural metabolic processes (Bergman et al., 2016). The collection and analysis of this shed genetic material via Polymerase Chain Reaction amplification (PCR), for the purpose of species level detections, is known as genotyping (Tillotson et al., 2018). By analyzing environmentally sourced DNA samples (eDNA) via high-throughput multi-species genotyping, it may be advantageous to conduct community-level fish assemblage assessments using a technique known as eDNA metabarcoding (Jerde et al., 2019).

While reducing risk to study subjects and their environment is a primary consideration for fisheries investigations, non-invasive sampling methods, such as eDNA sampling, may be particularly advantageous when studying rare and sensitive fishes. This is compounded when target fishes are present alongside other endemic organisms of concern such as mussels, amphibians, or invertebrates. Conventional fish sampling gears can result in knowledge gaps that stem from limited human resources, insufficient spatial coverage (Foley et al., 2015), unsuitable in-situ sampling conditions, or inadequate sampling frequency. In contrast, previous studies have

demonstrated that eDNA sampling can be performed at a higher frequency, with fewer personnel, and at an array of environs. Studies utilizing eDNA often result in higher species richness than those derived from traditional sampling alone, however, there are exceptions (Perez et al. 2017; Ulibarri et al. 2017). Such research has been particularly successful in investigations aimed at morphologically small, and numerically uncommon fishes (Thomsen et al. 2012; McKelvey et al., 2016; Valentini et al., 2016; Wilcox et al., 2016).

Despite its advantages and the increasing use of metabarcoding within the fisheries discipline, many methodological constraints still exist, and further research is required to progress its applicability in fisheries science. For instance, the representation of metabarcoding research performed on species-rich fish assemblages in complex and dynamic natural systems is rather limited, with many studies focusing on relatively few fish species in artificial environments. Additionally, these studies often vary in collection gear and sampling mediums (Goldberg et al., 2011; Turner et al., 2015). Finally, less is known about DNA's environmental dispersion dynamics, spatiotemporal decay, and variation in shed rates by species, age, sex, and individual activity level. As such, using eDNA to quantify relative species abundance is not unanimously accepted (Strickland and Roberts 2019; Zhang et al., 2020; Sales et al., 2021).

In the present study, we assessed fish assemblage diversity and distribution at nested sampling locations through the implementation of simultaneously conducted eDNA metabarcoding and electrofishing (SCEME) techniques. In addition to SCEME, this study compares our metabarcoding effort against historical capture-based fisheries surveys that occurred within the same study area beginning in 1869 (Flemer and Woolcott 1966; Weaver and Garman 1994). We fortified our metabarcoding efforts by conducting this analysis using two metabarcoding detection primers (cytb and 12S;) across intra-site replicates with two

independent sampling mediums (surface water and sediment;). Specifically, this study sought to (1) compare the efficacy of eDNA metabarcoding for characterizing the presence, absence, and relative species abundance of a diverse fish assemblage within a complex aquatic ecosystem, and (2) assess surface water and stream substrate as mediums for conducting additional metabarcoding investigations.

Methods

Study Area and Historical Dataset

This study was conducted in Tuckahoe Creek, a Chesapeake Bay Watershed located in the western portion of the metropolitan area of Richmond, Virginia. The Tuckahoe Creek basin is unique in that it is located within the geological Fall zone, and therefore encompasses abiotic characteristics in its upper sections that define the Virginia Piedmont, and downstream areas that are more similar to the Atlantic Coastal Plain (Figure 2.1). As the system flows across this geological gradient towards its confluence with the James River, a multiplicity of abiotic characteristics is formed, and consequently, the system consists of an equally rich fish assemblage that is complex in ecological function.

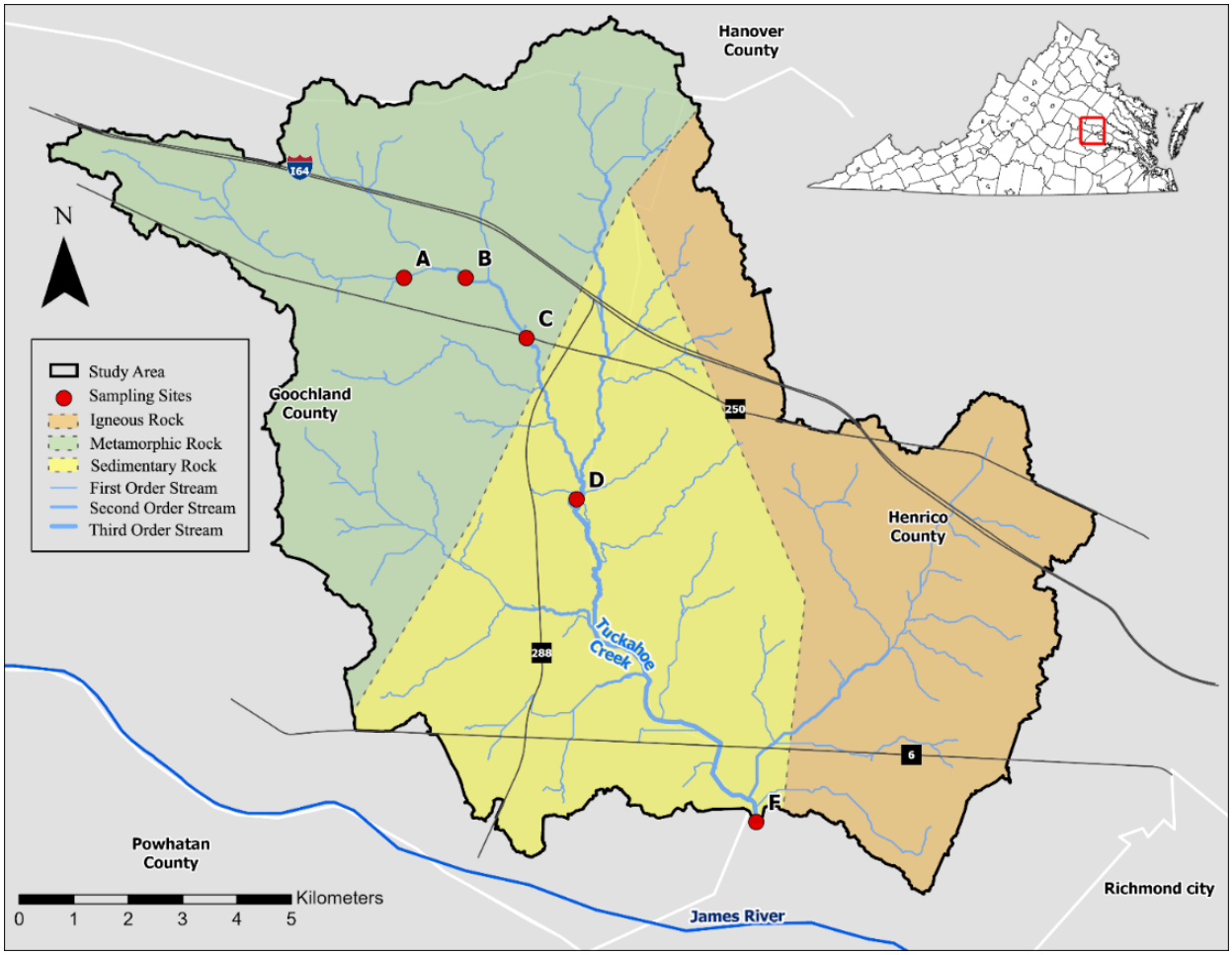

Figure 2.1 The Tuckahoe Creek study area. Geologic gradients are a proxy for geophysical province transition and basin heterogeneity.

The Tuckahoe Creek fish assemblage is described by a series of historical surveys that span over one-hundred-and-fifty-years. The first fishes identified within the basin were documented at unspecified locations in 1869 and 1937 (Cope 1869; Raney 1950). Later, capture-based surveys were conducted in 1958, 1990, 2014, and 2019 (Flemer and Woolcott 1966; Raney 1950; Weaver and Garman 1994; Stickley 2015). Sampling gears varied in these historical surveys, defined by the conventional norms of each respective sampling period. Collections in 1958 were the result of seining, while a combination of seining and electrofishing was used in 1990. Electrofishing was used as the single gear in 2014 and 2019. Each of these historical

investigations were repeated at the same established survey transects, within the same season, and with the intent of describing each site's comprehensive fish assemblage.

We conducted our research at five of the established fisheries survey locations. Although a sixth location, Site E, was sampled near Virginia State Highway 6 in 1958 and 1990, it was excluded from our investigation due to site changes that prevented it from being sampled in 2014, 2019, and 2020. In addition, Site E historically contained the same species observations and abiotic characteristics present at other established transects (Flemer and Woolcott 1966). The upper-most sampling location investigated, Site A, is representative of the dendritic network of first-order streams that comprise the basin's medium-gradient, gravel-dominated headwaters. As Tuckahoe Creek grows into a second-order stream (Sites B and C), the system becomes progressively lower in gradient, and the stream substrate gradually changes from gravel to sand. Just upstream of its confluence with the James River, Tuckahoe Creek is a third-order stream. In this reach, Sites D and F are characterized by numerous slow-moving swamps and beaver complexes. Here, the stream is low-gradient, and substrate consists almost exclusively of silt and clay.

Fish Sampling Procedures

Our fisheries investigation consisted of simultaneously performed eDNA metabarcoding and electrofishing surveys in July of 2020. In an effort to minimize sample contamination and increase the confidence in our findings, each of the survey components was completed in an upstream manner, beginning at the most downstream sampling location, Site F, and ending at Site A, the most upstream sampling location (Figure 2.1). At the downstream terminus of each survey transect, a total of seven eDNA samples were filtered prior to initiating electrofishing. These consisted of three independent surface water samples and three independent sediment

samples. In an effort to account for highly localized occurrences of uncommon species, each of the surface water and sediment samples were collected at approximately 25%, 50%, and 75% of the wetted width, respectively. The seventh and final eDNA sample, a negative field control, was filtered in-situ from double-distilled bottled water.

Surface water samples were collected using a Smith-Root ® eDNA system (i.e., ANDe backpack in conjunction with Smith-Root single-use 5 micrometer filters;). Pressure settings for the ANDe unit was placed at ten pounds per square inch, with a one liter per minute flow rate, and a target filtration volume set to four liters. At the conclusion of each filtration, which was initiated by obtaining either the target volume, or a low-pressure pump alarm, each filter was air dried for one minute by allowing the ANDe pump to draw in air. The entire filter housing was then removed from the ANDe system, and stored within double-layered, sterile whirl-packs. Sediment samples were obtained using single-use plastic sterile scoops to obtain a mass of at least one-hundred grams of stream substrate. Sediment was then poured into sterile double-layered whirl-packs. All eDNA samples were labeled in a coded sequence for the purpose of blinding the processing lab to site location, as well as positive and negative field samples. All samples were immediately placed within an iced cooler in the field, held under the same storage conditions prior to being transported on dry ice, and stored at -20°C until DNA extraction.

Field contamination was further mitigated throughout the investigation using USGS approved sampling procedures. During all eDNA sampling procedures, researchers abstained from entering the stream when possible. At sites where this proved infeasible, a single collector entered the water downstream of the collection zone and allowed natural streamflow to clear the sampling area for one minute before collecting a sample. Latex gloves were worn by all personnel during eDNA collections, and gloves were changed between each of the individual

samples. Negative field controls were filtered last at each site, in theory, to test for any cumulative contamination that occurred within the previous six eDNA collections.

At each survey location, single-pass electrofishing surveys immediately proceeded the conclusion of eDNA filtering. Consistent with previous historical surveys, each electrofishing effort was conducted in an upstream manner, with the intent of collecting a representative sample of the entire fish assemblage present within each site (Weaver and Garman 1994). Electrofishing was completed using one or two Smith Root LR-30 backpack electrofishing units, and three to six dip-netters, as required for efficient fish capture. Fish were netted and placed into aerated holding tanks until the completion of each sample, at which time each individual was identified to species, enumerated, and released back into the sampling area. A small number of individuals were photographed, and received non-fatal caudal fin clips, which were later used to generate genomic sequences for species-specific 12S markers that were absent from any public genomic database during our initial query.

Molecular Analysis

All molecular methods and laboratory procedures were performed as designed by a USGS approved protocol, and are further detailed in appendix 1, and a separate, ongoing molecular study.

DNA Extraction

The extraction, amplification, and analysis of DNA from each of the eDNA samples was performed within a project-isolated eDNA laboratory, under a laminar flow hood, separate from any PCR product handling, at the United States Geological Survey (USGS) Eastern Ecological Science Center (EESC). Samples were removed from -20°C storage and allowed to thaw for fifteen minutes. For surface water samples, half of each filter membrane was placed in a 5 mL

screw-cap tube for subsequent DNA extraction using the materials and procedures provided with the Qiagen DNEasy Blood and Tissue Kit. For sediment samples, 250mg of material was taken from each of the samples, and subjected to the extraction procedures provided in the Qiagen Powersoil extraction kit.

Reference Library Construction and Sequencing

For the purpose of increasing confidence in species-level detections, we chose two metabarcoding primer pairs. One primer pair targets a ~224 base pairs (bp) portion of the mitochondrial 12S gene in fishes (Miya et al., 2015). The second primer pair targets a ~209 bp portion of the mitochondrial *cyt*b gene in fishes (Snyder and Stepien 2020). Preparation of sequencing libraries generally followed Illumina (2016). For the *cyt*b and 12S amplicons, all complete and partial mitogenomic sequences were downloaded from the Mitofish database (Iwasaki et al. 2013). The original taxonomic annotation by the sequence authors was assumed to be correct for each sequence. Each sequence accession number was then used to retrieve the corresponding taxid from GenBank. All species of fish previously observed within the study area had a cytb reference sequence, however multiple species of interest were missing a 12S reference (Appendix 1). Therefore, tissue samples of each missing specimen with a historical presence in Tuckahoe Creek were provided to USGS-LSC for DNA extraction and 12S reference sequencing.

Index Hopping and Bioinformatics

Recent metabarcoding literature has demonstrated that there is a low level of "index-hopping" in MiSeq sequencing runs, where reads (i.e., the detection of species-specific DNA) from one library are assigned to the wrong library at the demultiplexing stage (Snyder and Stepien 2020). As a result of this phenomenon, the number of mis-assigned reads in a library is

assumed to be approximately 0.1%. This can be problematic when trying to determine if a rare organism is present in a sample or not. To empirically determine the level of read misassignment in our study, and to establish a threshold for counting a species as present, we created a pooled library of marine fishes not expected to be observed in Tuckahoe Creek, a freshwater system. Using a Qiagen DNEasy Kit, DNA was extracted, amplified, and indexed (Illumina 2016) from tissue samples of six deep-sea marine fishes collected from the Atlantic Ocean.

To apply the results of the marine mock community for determining the threshold for presence of a taxon in the eDNA samples, all taxonomic assignments to a marine fish were identified, and the sum of the marine species reads in each sample was divided by the total number of reads in the sample to get a 'percent of marine representation'. The average of the marine species representation was taken across all samples and applied as the threshold for species-level detections in the Tuckahoe Creek eDNA assessment. In order to enumerate the quantity of reads of each species observed at each site, the number of reads per sequence was summed across each of the three site replicates, and divided by the total number of reads among all species at each respective location. For a species to be deemed present, this percentage, or the concentration of species specific DNA, needed to exceed the threshold determined from the marine mock community.

Fish Dataset Analysis

Fisheries data from historical capture-based surveys conducted in 1958, 1990, 2014, and 2019 were compiled with our original 2020 electrofishing and eDNA sampling data using Microsoft Excel. Fisheries survey data collected during the 1869 and 1937 Tuckahoe Creek investigations were not independently compared in this study, as these surveys did not contain any unique fish species. All data analysis and display outputs were generated in R Studio V3.62.

Each of the observed fishes from our study were categorized as either “Established”, “Intermittent”, or “Uncommon” species. These species-level occupancy categorizations were determined by their historical frequency of occurrence within the Tuckahoe Creek Watershed (Table 2.1). This categorical structure allowed us to contrast eDNA metabarcoding to conventional methods in distribution and detection sensitivity across different historical concentrations of species composition throughout the Tuckahoe Creek basin.

Table 2.1 Categorization of fish species observed throughout the Tuckahoe Creek watershed.

Species Category	Description	Number of Species	Number of Families
Established	Species of fish which have appeared in 4 of 4 historical investigations	18	9
Intermittent	Species of fish which have appeared in 2 or 3 of the 4 historical investigations	14	7
Uncommon	Novel fish species, or species of fish which have appeared in 1 of 4 historical investigations	15	8

For each species category designation, presence or absence was noted for each species, sampling event, and sampling location. Species detections for eDNA samples was attributed to the sample-specific quantity of species independent reads surpassing the threshold of necessary reads for either of the 12S or cytb markers, as detailed in section 2.7. Quantities of species-specific DNA within the samples that did not reach the minimum threshold of reads was considered null, and the associated species was designated as absent from that sample. The proportion of species-specific DNA within each eDNA was calculated by summating the number of species-specific reads from both of the 12S and cytb pairs, standardizing the resulting value by sample filtration volume, and dividing the product by the total number of reads for all species at each respective location. Similarly, percent composition for electrofishing was calculated by dividing number of individuals observed for a particular species by the total number of individuals observed at both the basin-wide and site-specific spatial scale.

Results

Fisheries Dataset

Electrofishing surveys conducted in July, 2020 resulted in an aggregate of twenty-seven species of fish collected throughout the basin's five established sampling locations. Each of the species collected during electrofishing had been previously documented as one of the thirty-nine extant species known to exist within the same locations in the 1958, 1990, 2012, or 2019 investigations.

In contrast, our eDNA metabarcoding effort detected the presence of forty-seven species of fish. Each of these detections was derived from the surface water component of the eDNA survey. Sediment samples were found to contain only trace levels of fish DNA, and thus were not examined further. Although additional investigation may be needed to investigate the role of PCR inhibition in this outcome, this is not a likely explanation, as our extraction process contained an inhibitor removal step. Qubit values indicated the absence of any DNA within our negative field controls, and these samples were not sequenced. Similarly, lab controls showed very small numbers of reads, suggesting there was not a contamination issue within our analysis.

Thirty-one of the species detected by metabarcoding were observed by both the 12S and cytb detection primers (Table 2.2). Six species detections were unique to the 12S primer, while nine were detected by only the cytb primer. Of these primer-specific disparities in species detections, Longnose Gar *Lepisosteus osseus* was the only aberration resulting from an unavailable primer, in which a cytb sequence was not available for our reference library at the time of sequencing. One disparity in primer-specific species detection occurred within the darter family, where the cytb marker detected Johnny Darter *Etheostoma nigrum* and Tessellated Darter

Etheostoma olmstedi, whereas the 12S marker only detected the latter species. We also observed a latitudinal variation within our same-site replicates (Table 2.2), with the highest concentration of detections being collected from the 25% wetted width samples, regardless of species occupancy category.

Table 2.2 Species occupancy results by method and latitudinal sampling location.

	SPECIES DETECTION BY METHOD (2020)			LATITUDINAL DETECTION RATES (EDNA)		
SPECIES OCCUPANCY CATEGORY	12S	CYTB	ELECTROFISHING	25% WETTED WIDTH	50% WETTED WIDTH	75% WETTED WIDTH
Established	17/18	18/18	18/18	22.8	15.4	21.6
Intermittent	14/14	12/14	9/14	11.2	7.4	9.3
Uncommon	7/15	10/15	0/15	5.5	3.2	3.6

Unsurprisingly, we also found surface water eDNA samples contained quantifiable DNA from non-target organisms, and species that were not previously defined in our reference genomic library. These genetic sequences were manually blasted against the National Center for Biotechnology Information (NCBI) database, and indicated that DNA from Cattle *Bos taurus*, Whitetail Deer *Odocoileus virginianus*, Common Snapping Turtle *Chelydra serpentina*, and two-lined salamander *Eurycea wilderae* was also present in the water column during the time of our collection. Two-lined salamanders were physically observed and noted during electrofishing surveys at the same locations that DNA was detected, while each of the other three non-target detections are locally present, but were not specifically noted during the collection events.

Species Diversity and Distribution

The detection and distribution of fishes derived from surface water metabarcoding generally outperformed the simultaneously conducted electrofishing efforts (Positive Control) across each of the species occupancy categories (Table 2.1). For the established fish species, which represents the most stable populations of fishes within the Tuckahoe Creek watershed,

eDNA and electrofishing successfully detected the presence of all eighteen species of fish (Table 2.3).

Table 2.3 Basin-wide detection status of Established Fish Species by sampling event.

	HISTORICAL CAPTURE-BASED SURVEYS				SIMULTANEOUS 2020 SURVEYS	
SPECIES	1958	1990	2014	2019	ELECTROFISHING	EDNA
Ameiurus nebulosus	X	X	X	X	X	X
Anguilla rostrata	X	X	X	X	X	X
Catostomus commersonii	X	X	X	X	X	X
Centrarchus macropterus	X	X	X	X	X	X
Enneacanthus gloriosus	X	X	X	X	X	X
Erimyzon oblongus	X	X	X	X	X	X
Esox niger	X	X	X	X	X	X
Etheostoma nigrum	X	X	X	X	X	X
Gambusia holbrooki	X	X	X	X	X	X
Lepomis auritus	X	X	X	X	X	X
Lepomis gulosus	X	X	X	X	X	X
Lepomis macrochirus	X	X	X	X	X	X
Luxilus cornutus	X	X	X	X	X	X
Micropterus salmoides	X	X	X	X	X	X
Nocomis leptocephalus	X	X	X	X	X	X
Notemigonus crysoleucas	X	X	X	X	X	X
Rhinichthys atratulus	X	X	X	X	X	X
Thoburnia rhothoeca	X	X	X	X	X	X

At a site-specific lens, eDNA detected a higher number of established species at each of the sampling locations (Table 2.4). Fifty-five presence or absence designations matched between the simultaneously conducted eDNA and electrofishing surveys for species in the established classification, and none of the species observed during the 2020 electrofishing effort were undetected by eDNA within the same sample site. In twenty-seven instances, established species of fish observed within the eDNA samples were absent from that locale's electrofishing survey, but had been previously observed at that site in at least one of the historical investigations (e.g.,

American Eel *Anguilla rostrata*, Site A). In just four instances, species-specific eDNA was detected at a location without any previous historical or simultaneous capture-based observation (1. Creek Chubsucker *Erimyzon oblongus*, Site F; 2. Bluehead Chub *Nocomis leptocephalus*, Site D and F; 3. Eastern Blacknose Dace *Rhinichthys atratulus*, Site C and F; 4. Torrent Sucker *Thoburnia rhothoeca*, Site C; Table 2.4).

Table 2.4 Site-specific community-level presence and absence of Established Fish Species by sampling event. HP= Historical Presence (1958, 1990, 2014, and 2019 cumulative), PC = Positive Control, EDNA= Surface Water eDNA.

	SAMPLE SITE A			SAMPLE SITE B			SAMPLE SITE C			SAMPLE SITE D			SAMPLE SITE F		
SPECIES	HP	PC	EDNA	HP	PC	EDNA	HP	PC	EDNA	HP	PC	EDNA	HP	PC	EDNA
Ameiurus nebulosus		X	X	X	X	X	X		X	X		X	X		X
Anguilla rostrata	X		X	X		X	X	X	X	X	X	X	X	X	X
Catostomus commersonii	X		X	X	X	X	X		X	X					
Centrarchus macropterus				X		X	X	X	X	X		X	X		X
Enneacanthus gloriosus				X			X		X	X	X	X	X		X
Erimyzon oblongus	X			X			X		X	X	X	X			X
Esox niger							X	X	X	X	X	X	X		X
Etheostoma nigrum	X	X	X	X	X	X	X	X	X	X		X	X		X
Gambusia holbrooki	X		X	X	X	X	X	X	X	X		X	X	X	X
Lepomis auritus	X		X	X		X	X	X	X	X	X	X	X		X
Lepomis gulosus				X		X	X	X	X	X		X	X		X
Lepomis macrochirus	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Luxilus cornutus	X		X	X	X	X									
Micropterus salmoides	X		X	X	X	X	X	X	X	X		X	X	X	X
Nocomis leptocephalus	X	X	X	X	X	X	X	X	X			X			X
Notemigonus crysoleucas	X			X		X	X	X	X	X	X	X	X	X	X
Rhinichthys atratulus	X	X	X		X	X			X						X
Thoburnia rhothoeca	X	X	X	X	X	X			X						

All fourteen of the intermittent species of fish were detected by eDNA, while just nine of these species were collected during the simultaneous electrofishing sample (Table 2.5). Unique eDNA detections within this category included the Swamp Darter *Etheostoma fusiforme*, which

had not been observed by any of the capture-based surveys throughout the basin in over thirty years.

Table 2.5 Basin-wide detection status of Intermittent Fish Species by sampling event.

	HISTORICAL CAPTURE-BASED SURVEYS				SIMULTANEOUS 2020 SURVEYS	
SPECIES	1958	1990	2014	2019	ELECTROFISHING	EDNA
Ameiurus natalis	X		X		X	X
Aphredoderus sayanus	X	X	X		X	X
Chrosomus oreas	X	X		X	X	X
Clinostomus funduloides	X	X	X		X	X
Dorosoma cepedianum			X	X		X
Etheostoma fusiforme	X	X				X
Hybognathus regius	X		X		X	X
Lepomis cyanellus		X	X	X	X	X
Lepomis gibbosus	X	X	X	X		X
Lepomis microlophus			X	X	X	X
Noturus insignis	X	X	X			X
Pomoxis nigromaculatus	X		X			X
Semotilus atromaculatus		X	X	X	X	X
Umbra pygmaea	X	X	X		X	X

For intermittent species, surface water metabarcoding resulted in forty-one site-specific detections (Table 2.6). This was substantially higher than the eighteen observations derived from electrofishing alone. Forty-two observations within this category were congruent between the two gears. There were three instances of species-specific DNA being detected at locations not previously documented by capture-based surveys (e.g., Black Crappie *Pomoxis nigromaculatus*, Site C and D. In contrast, there are nine instances in which historical records indicate a species was present, but the species was not detected during SCEME, which further demonstrates the complexity and dynamic nature of the fish assemblage within the study area.

Table 2.6 Site-specific community-level presence and absence of Intermittent Fish Species by sampling event. HP= Historical Presence (1958, 1990, 2014, and 2019 cumulative), PC = Positive Control, EDNA= Surface Water eDNA.

	SAMPLE SITE A			SAMPLE SITE B			SAMPLE SITE C			SAMPLE SITE D			SAMPLE SITE F		
SPECIES	HP	PC	EDNA	HP	PC	EDNA	HP	PC	EDNA	HP	PC	EDNA	HP	PC	EDNA
Ameiurus natalis							X	X	X	X	X	X			X
Aphredoderus sayanus				X			X	X	X	X		X	X	X	X
Chrosomus oreas	X	X	X	X			X		X						
Clinostomus funduloides	X	X	X	X	X	X	X		X						X
Dorosoma cepedianum							X					X	X		X
Etheostoma fusiforme										X		X	X		
Hybognathus regius				X		X	X		X	X	X		X		
Lepomis cyanellus	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lepomis gibbosus	X			X		X	X		X	X		X			X
Lepomis microlophus				X			X		X	X			X	X	X
Noturus insignis	X		X	X		X	X		X						
Pomoxis nigromaculatus							X					X	X		X
Semotilus atromaculatus	X	X	X	X	X	X			X						X
Umbra pygmaea			X	X	X	X	X	X	X	X		X	X		

The largest disparity in species detections between sampling gears during SCEME occurred within the uncommon species occupancy category. None of the fifteen fishes within this classification were observed in the simultaneous electrofishing component of the survey, but fourteen were observed within the metabarcoding effort (Table 2.7). Detections for uncommon species included three species that were last observed in 1958, the Satinfin Shiner *Cyprinella analostana*, Roseface Shiner *Notropis rubellus*, and Fallfish *Semotilus corporalis*. Stripeback Darter *Percina notogramma*, was absent from both components of SCEME. Only two Stripeback Darter individuals have been observed within the basin, with the last observation occurring during the 1958 investigation.

Table 2.7 Basin-wide detection status of Uncommon Fish Species by sampling event.

	HISTORICAL CAPTURE-BASED SURVEYS				SIMULTANEOUS 2020 SURVEYS	
SPECIES	1958	1990	2014	2019	ELECTROFISHING	EDNA
Ameiurus catus			X			X
Amia calva			X			X
Cyprinella analostana	X					X
Cyprinus carpio						X
Etheostoma olmstedi						X
Gambusia affinis						X
Ictalurus furcatus						X
Ictalurus punctatus						X
Lepisosteus osseus						X
Micropterus floridanus						X
Notropis rubellus	X					X
Perca flavescens				X		X
Percina notogramma	X					
Pylodictis olivaris						X
Semotilus corporalis	X					X

Similar to the trend observed at the basin-wide lens, the most disparity in site-specific detections existed within the uncommon fish species classification. Although the two SCEME gears aligned on fifty "absent" designations, all twenty-three "present" site occupancy observations resulted solely from the metabarcoding component of SCEME (Table 2.8). Among the six uncommon fishes previously documented in Tuckahoe Creek, three eDNA detections occurred at the same sample site described by the historical capture-based surveys (e.g., Bowfin *Amia calva*, Site F).

Table 2.8 Site-specific community-level presence and absence of Uncommon Fish Species by sampling event. HP= Historical Presence (1958, 1990, 2014, and 2019 cumulative), PC = Positive Control, EDNA= Surface Water eDNA.

	SAMPLE SITE A			SAMPLE SITE B			SAMPLE SITE C			SAMPLE SITE D			SAMPLE SITE F		
SPECIES	HP	PC	EDNA	HP	PC	EDNA	HP	PC	EDNA	HP	PC	EDNA	HP	PC	EDNA
Ameiurus catus							X					X			X
Amia calva							X			X			X		X
Cyprinella analostana									X				X		
Cyprinus carpio															X
Etheostoma olmstedi			X			X			X			X			X
Gambusia affinis						X									
Ictalurus furcatus									X						
Ictalurus punctatus															X
Lepisosteus osseus															X
Micropterus floridanus			X			X			X			X			X
Notropis rubellus				X			X		X						
Perca flavescens										X					X
Percina notogramma										X					
Pylodictis olivaris															X
Semotilus corporalis	X						X		X				X		

Relationship between Relative Species Abundance and Proportional Species-Species DNA

Consistent with previous investigations of the Tuckahoe Creek fish assemblage, both SCEME techniques concurred that Bluegill *Lepomis macrochirus* remained the most abundant species of fish within the basin (Figure 2.2). For fishes within the established species category, the variance in basin-wide relative abundance estimates derived from electrofishing, and the proportion of corresponding species-specific DNA present at each sampling location was within five percent for all eighteen species. When ranked by order of relative abundance, three species in the established category shared the same ordered rank, which includes the most and least abundant species, *Lepomis macrochirus* and *Centrarchus macropterus*, respectively. Electrofishing derived relative abundance and proportional species-specific DNA had a higher deviation in the intermittent (Figure 2.3) and uncommon (Figure 2.4) classifications of fish. The

ordered rank of percent occupancy in intermittent species was congruent in just two species, *Lepomis cyanellus* and *Semotilus atromaculatus*. Because the electrofishing component of SCEME failed to capture any of the uncommon species, no comparisons for this occupancy category could be made.

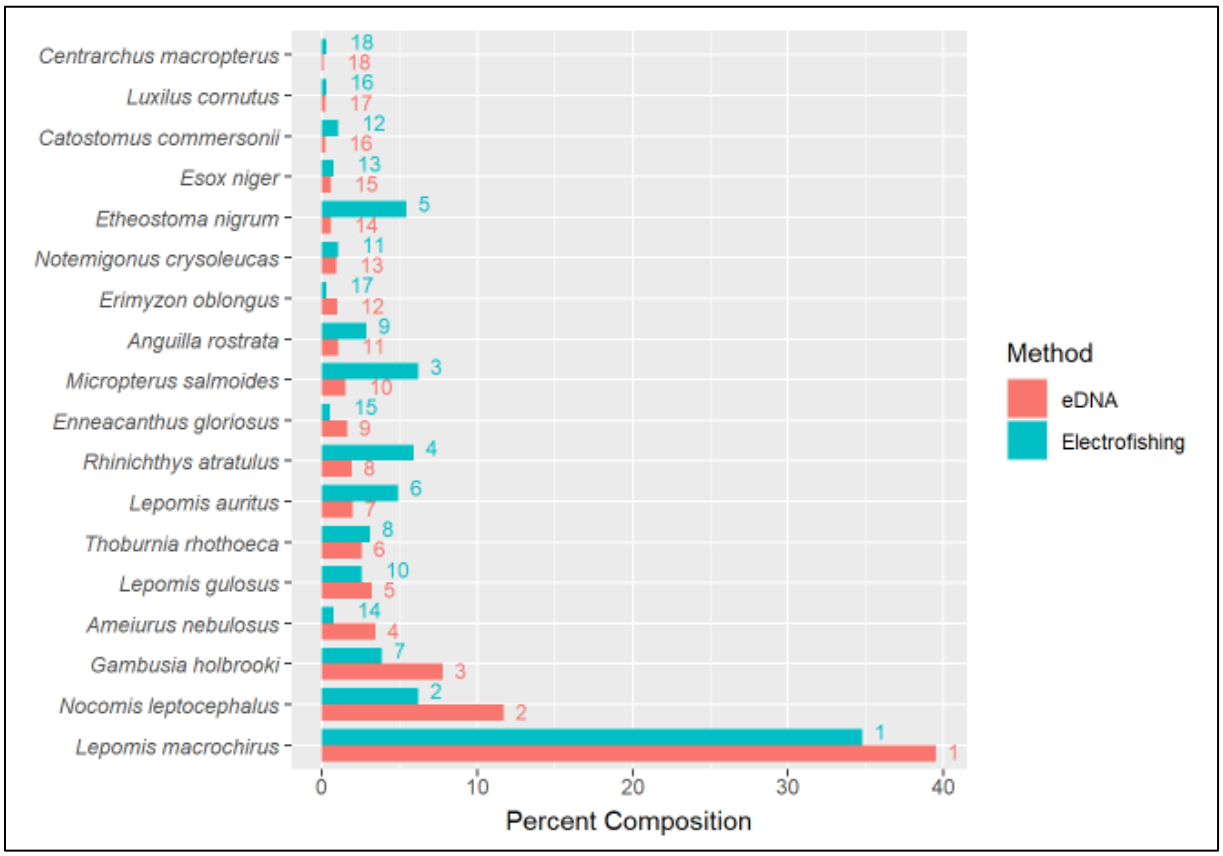

Figure 2.2 Basin-wide relative abundance of Established Fish Species observed by 2020 survey method; numbers denote the ranked order determined by relative abundance for each method.

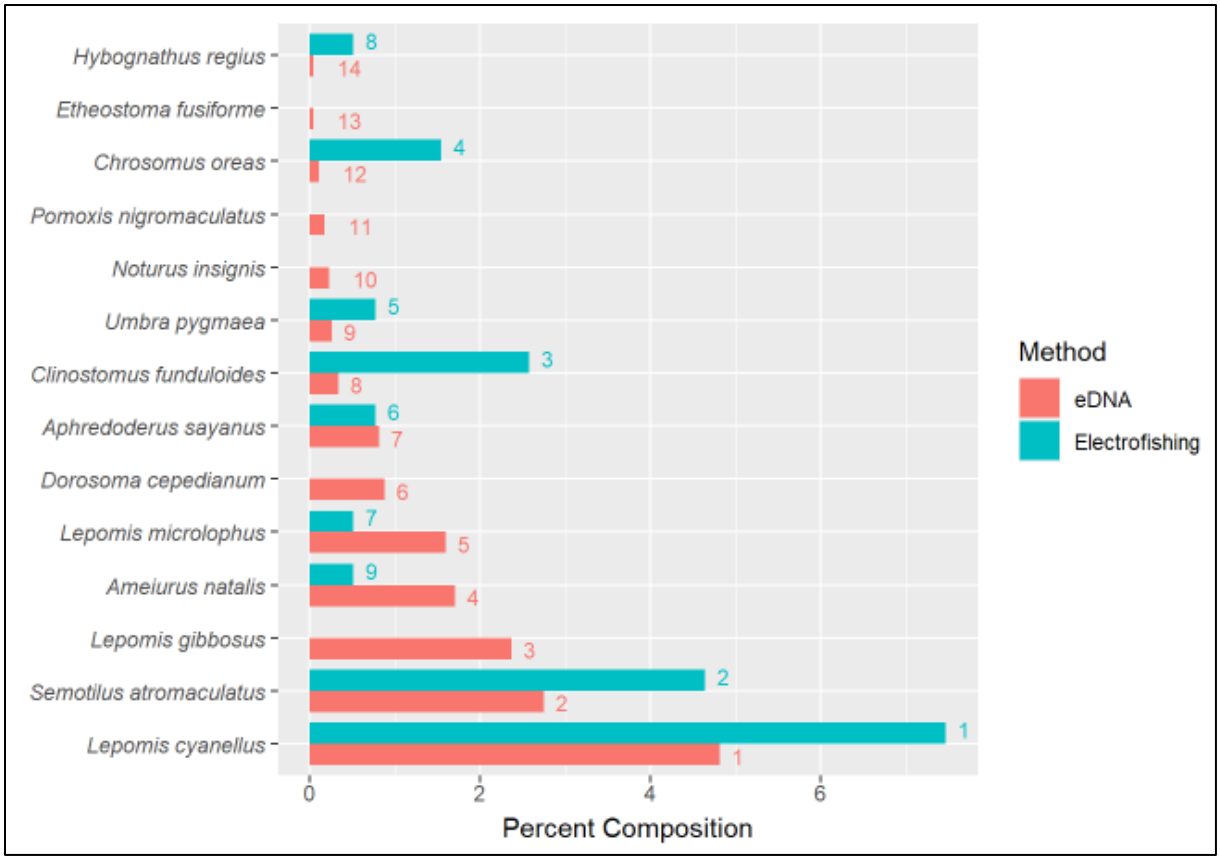

Figure 2.3 Basin-wide relative abundance of Intermittent Fish Species observed by 2020 survey method; numbers denote the ranked order determined by relative abundance for each method.

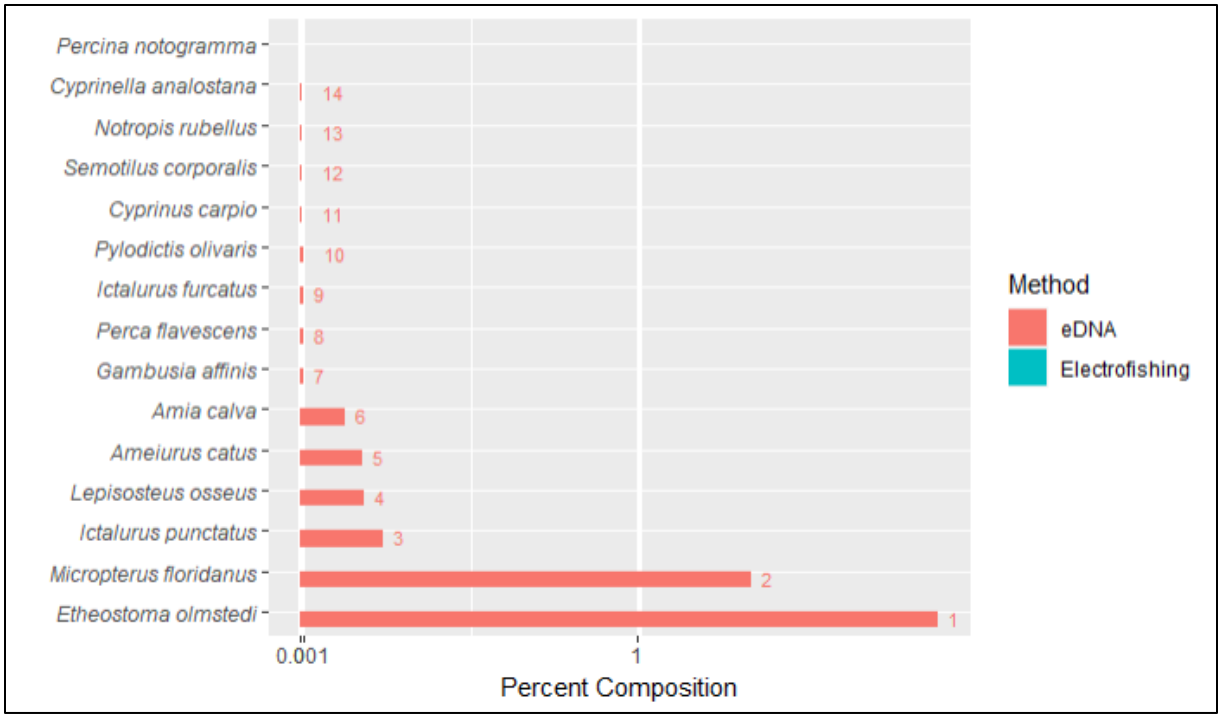

Figure 2.4 Basin-wide relative abundance of Uncommon Fish Species observed by 2020 survey method; numbers denote the ranked order determined by relative abundance for each method.

Site-specific estimates of relative abundance reflect a unique fish community at each of the locations sampled. This was evident regardless of sampling gear. Quantities of species-specific eDNA as described by percent composition did not indicate a strong downstream accumulative pattern of DNA throughout the continuum of nested sites for either of the categorical occupancy designations (e.g., Bluehead Chub *Nocomis leptocephalus* Figure 2.5, Green Sunfish *Lepomis cyanellus* Figure 2.6, Tessellated Darter *Etheostoma olmstedi* Figure 2.7).

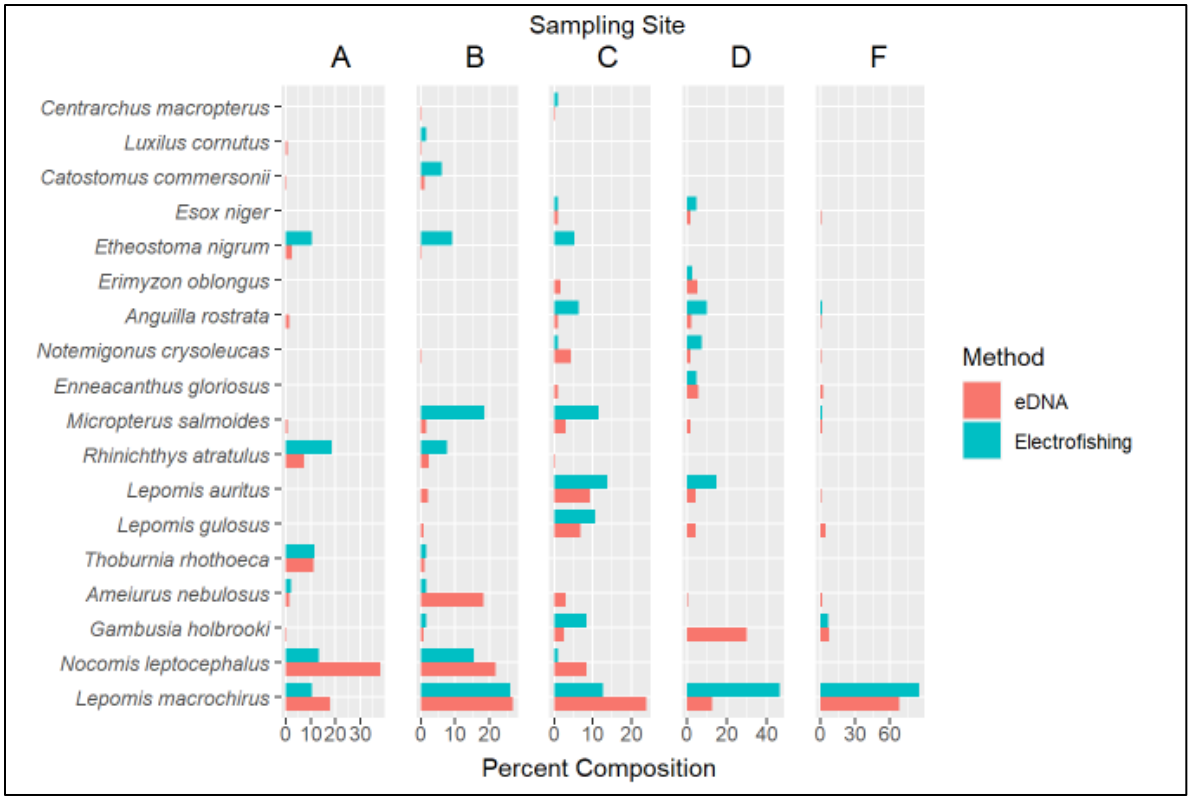

Figure 2.5 Site-specific relative abundance of Established Fish Species observed by 2020 survey method.

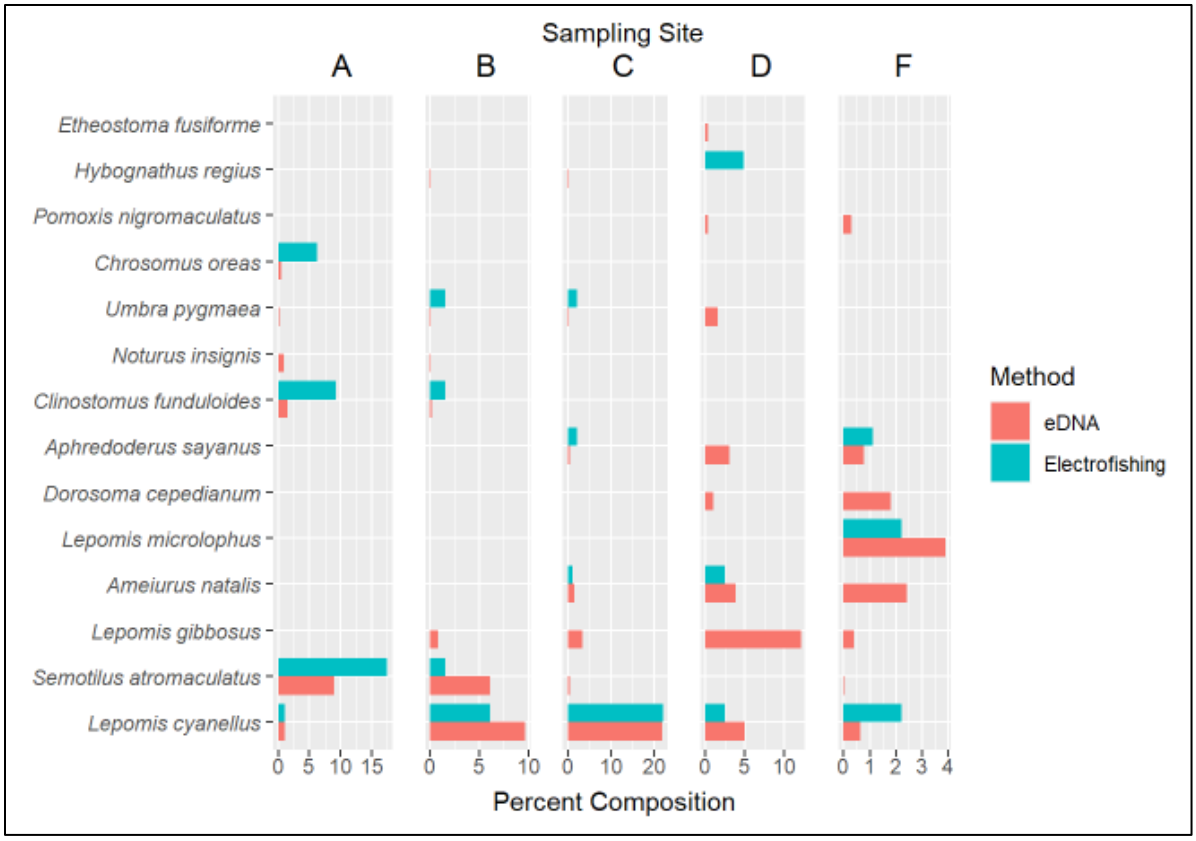

Figure 2.6 Site-specific relative abundance of Intermittent Fish Species observed by 2020 survey method.

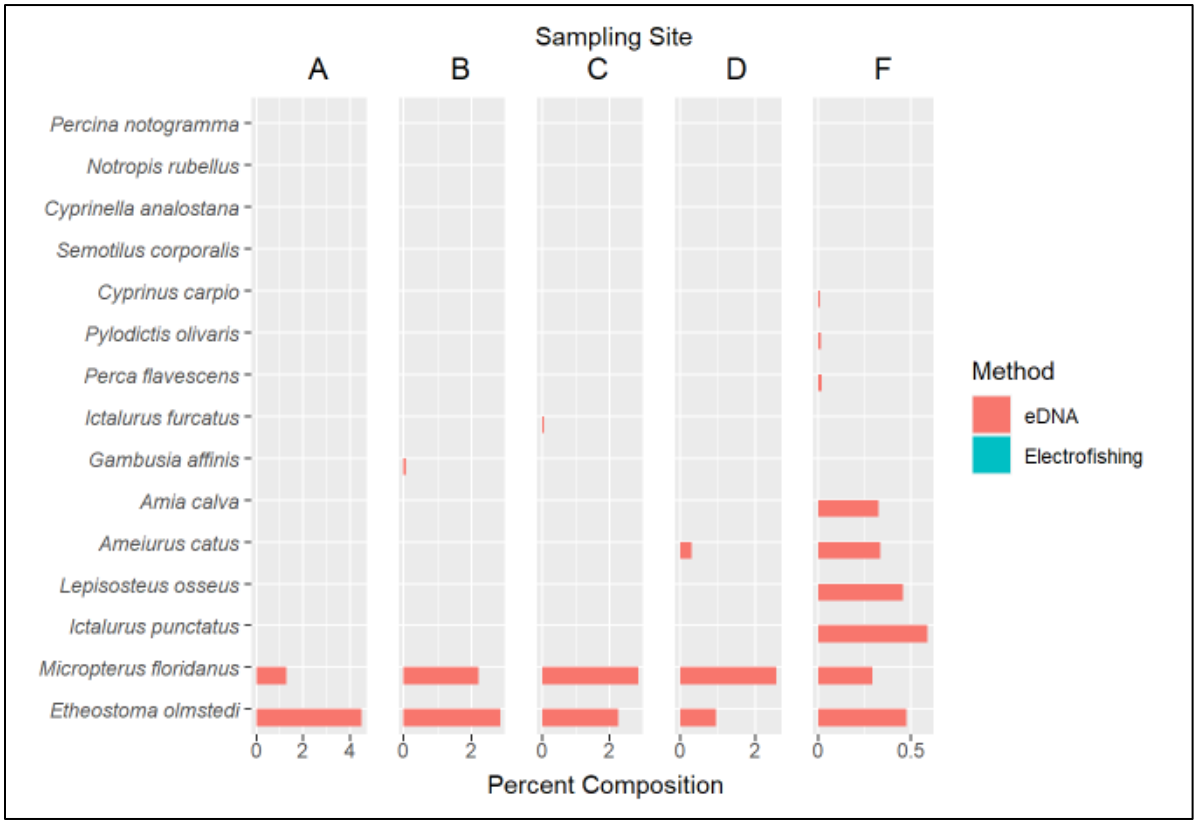

Figure 2.7 Site-specific relative abundance of Uncommon Fish Species observed by 2020 survey method.

Discussion

The metabarcoding component of SCEME in this study has resulted in a more comprehensive fish community dataset for the Tuckahoe Creek watershed than had previously been accomplished by historical capture-based surveys originating in 1869. This is highlighted by the added documentation of eight novel species of fish within the basin. Based on the metabarcoding techniques we assessed against the simultaneously conducted electrofishing survey, in addition to the historical capture-based surveys, our findings indicate that eDNA metabarcoding outperforms traditional sampling methods in describing both site-specific and basin-wide fish diversity. Through evaluating SCEME on a species rich assemblage, within a

natural system that is both dynamic and exhibits a plethora of abiotic characteristics, our study indicates that metabarcoding is an efficacious tool that has application across many fish communities, aquatic habitats, and environmental circumstances.

By performing SCEME across nested sites within the same basin, our results indicate that eDNA metabarcoding has the potential to be spatially discrete within the same watershed, which could further aid in describing longitudinal species-level distributions. Localized detection sensitivity of metabarcoding was also apparent in our study where species-level detection varied on a latitudinal scale among same-site replicates. This result coincides with other eDNA research which has shown highly varied DNA dispersal and decay qualities, which may be dependent on the preferred habitats of target species, in combination with site-specific abiotic characteristics such as streamflow dynamics. Although sediment sourced eDNA ultimately proved inadequate in our study, we believe additional research is warranted to investigate the cause of DNA degradation in this medium, and if different techniques can improve upon its utility in the future.

Although we aimed to compare the relative abundance of fishes between metabarcoding and electrofishing in this study, and generally found a pattern of similarity, it is likely that different communities were being simultaneously assessed by each SCEME technique. While the species compositions derived from electrofishing directly reflect the assemblage of the corresponding transect, and no additional area, the spatial extent of the fishery being assessed by each of the eDNA samples is unknown in our study. Based on findings in related literature that show eDNA dispersal and decay to be situationally varied, it is likely that the eDNA samples in our study reflect fish communities over varying longitudinal scales. However, given our exhaustive electrofishing efforts within the site-specific transects, we conclude that metabarcoding characterized the fish community across a greater upstream longitudinal distance

than each respective electrofishing survey. Under this assumption, in addition to the known species-specific biases associated with electrofishing, it is feasible that relative abundance as characterized by the proportion of species-specific DNA within the metabarcoding analysis in our study describes fish community composition at a higher accuracy than electrofishing, particularly at spatial scales not feasible for capture-based methods (i.e., basin-wide).

Of the eight novel species detected by metabarcoding in our study, Florida Largemouth Bass *Micropterus floridanus*, Tessellated Darter *Etheostoma olmstedi*, and Western Mosquitofish *Gambusia affinis*, may have been recently introduced to the system, as they are commonly spread by recreational anglers for bait or sport. Another possibility is that these species were misidentified in previous capture-based surveys due to sharing morphologically indiscriminate features with the similar group of previously observed species, Largemouth Bass *Micropterus salmoides*, Johnny Darter *Etheostoma nigrum*, and Eastern Mosquitofish *Gambusia holbrooki*, respectively. The final possibility is that these species have integrated and resulting hybrids may exhibit more phenotypical expression of characteristics associated with the latter set of species (Jenkins and Burkhead 1994).

The remaining novel fishes, the Common Carp *Cyprinus carpio*, Longnose Gar *Lepisosteus osseus*, Blue Catfish *Ictalurus furcatus*, Channel Catfish *Ictalurus punctatus*, and Flathead Catfish *Pylodictis olivaris*, were documented as having a low abundance as determined by the proportion of their DNA within the metabarcoding analysis. Finally, while each of the eight novel species have been previously described throughout Tuckahoe Creek's parent watershed, the James River basin, only the Tessellated Darter and Longnose Gar are considered native. This alludes to the possibility that metabarcoding is an extremely useful tool for locating

undesirable or introduced species (Baudry et al., 2021), allowing for more expedient management responses.

In addition to data-centric advantages made possible by metabarcoding, as evidenced in this study, a number of ethical and logistical considerations also exist. With the well-being of the study subjects in mind, metabarcoding may be ethically advantageous for assessing fish communities during periods of drought or elevated water temperatures, as well as during vulnerable life history phases that can include migration, active reproduction, or thermal refugia. Further, eDNA sampling can be replicated across higher spatial intensities at a faster rate than conventional sampling techniques (Civade et al., 2016), and sampling frequency likely has little or no effect on the fishery or an ecosystem's abiotic components. Our study also indicates that samples may contain DNA from more distant upstream sources, potentially allowing for an increased flexibility in logistics due to site access, as well as water quality parameters such as depth, conductivity, depth, temperature, and turbidity. Lastly, metabarcoding results are not biased by an individual surveyor's ability to see, net, or correctly identify specimens. As a result, metabarcoding is highly duplicable regardless of the individuals performing the collection.

Along with using a combination of complimentary detection primers, we increased the validity of our metabarcoding findings by developing and abiding by a structured field and laboratory protocol. While additional measures may paradoxically increase the cost of laboratory analysis, we believe this practice is invaluable to the metabarcoding process. Additional legitimacy in our results can be attributed to the partial blinding measures that were undertaken from the inception of the study. Laboratory analysts received only coded identifiers for each sample, which were absent of descriptive information, and were given an exhaustive list of plausible species that far exceeded the expected detections with which to build the reference

database. Lab personnel also remained blind to the capture-based results until the study was concluded.

This study builds upon a growing body of evidence that suggests surface water metabarcoding is an optimal technique for assessing fish assemblage diversity. We found the advantage of metabarcoding was particularly evident when looking at species of fish that we categorized as intermittent or uncommon within the study area. As contemporary fisheries investigations increasingly prioritize the monitoring and conservation of rare, sensitive, and threatened species, it is likely that the application of eDNA techniques will see increased usage in fisheries science. Despite limitations, our study demonstrates that eDNA metabarcoding is a tool that must be considered for describing fish assemblages in dynamic environments. We believe and advocate for additional research using SCEME techniques, which will advance the understanding of metabarcoding and benefit fisheries science at a disciplinary level.

Acknowledgements

Funding for this research was generously provided by the Rice Rivers Center at Virginia Commonwealth University. Further support was provided by the United States Geological Survey: Eastern Ecological Science Center. We thank A. Weaver, B. Boettcher, B. Donovan, J. Harris, D. Hopler, M. Gafney, P. Grap, and S. Kaliris for their assistance with field work. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement. This study was conducted under the Virginia Commonwealth University Animal Care and Use Committee Protocol #AD10000441.

References

Baudry, T., Q. Mauvisseau, J. P. Goût, A. Arqué, C. Delaunay, J. Smith-Ravin, M. Sweet, and F. Grandjean. 2021. Mapping a super-invader in a biodiversity hotspot, an eDNA-based success story. Ecological Indicators 126.

Bennett, R. H., B. R. Ellender, T. Mäkinen, T. Miya, P. Pattrick, R. J. Wasserman, D. J. Woodford, and O. L. F. Weyl. 2016. Ethical considerations for field research on fishes. Koedoe 58:1–15.

Bergman, P. S., G. Schumer, S. Blankenship, and E. Campbell. 2016. Detection of adult green sturgeon using environmental DNA analysis. PLoS ONE 11:1–8.

Bohlin, T., S. Hamrin, T. G. Heggberget, G. Rasmussen, and S. J. Saltveit. 1989. Electrofishing - Theory and practice with special emphasis on salmonids. Hydrobiologia 173:9–43.

Bunn, S. E., and A. H. Arthington. 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environmental Management 30:492–507.

Cope, E. D. 1869. On the distribution of fresh- water fishes in the Allegheny region of south-western Virginia. J. Acad. Nat. Sci. Phila. Ser. 2, 0:207-247.

Civade, R., T. Dejean, A. Valentini, N. Roset, J. C. Raymond, A. Bonin, P. Taberlet, and D. Pont. 2016. Spatial Representativeness of Environmental DNA Metabarcoding Signal for Fish Biodiversity Assessment in a Natural Freshwater System. PLoS ONE 11:1–19.

Dunham, J. B., Rosenberger, A. E., Thurow, R. F., Dolloff, C. A., & Howell, P. J. 2009. Coldwater Fish in Wadeable Streams: Chapter 8. In S. A. Bonar, W. A. Hubert, & D. W. Willis, Standard Methods for Sampling North American Freshwater Fishes. 119–138. American Fisheries Society, Bethesda, MA.

Flemer, D. A., and W. S. Woolcott. 1966. Food habits and distribution of the fishes of Tuckahoe Creek, Virginia, with special emphasis on the bluegill, Lepomis m. macrochirus rafinesque. Chesapeake Science 7:75–89.

Foley, K., A. Rosenberger, and F. Mueter. 2015. Effectiveness of singlepass backpack electrofishing to estimate juvenile Coho Salmon abundance in Alaskan headwater streams. Fisheries Science 81:601–610

Goldberg C.S., D. S. Pilliod, R. S. Arkle, and L. P. Waits. 2011. Molecular detection of vertebrates in stream water: a demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. PLoS ONE 6: e22746.

Iwasaki W., T. Fukunaga, R. Isagozawa, K. Yamada, Y. Maeda, T.P. Satoh, T. Sado, K. Mabuchi, H. Takeshima, M. Miya, and M. Nishida. 2013. MitoFish and MitoAnnotator: a mitochondrial genome database of fish with an accurate and automatic annotation pipeline. Molecular Biology and Evolution 30: 2531–2540.

Jenkins, R.E., and N.M. Burkhead. 1994. Freshwater Fishes of Virginia. American Fisheries Society, Bethesda, Maryland.

Jerde, C. L., E. A. Wilson, and T. L. Dressler. 2019. Measuring global fish species richness with eDNA metabarcoding. Molecular Ecology Resources 19:19–22.

Lodge, D. M., C. R. Turner, C. L. Jerde, M. A. Barnes, L. Chadderton, S. P. Egan, J. L. Feder, A. R. Mahon, and M. E. Pfrender. 2012. Conservation in a cup of water: Estimating biodiversity and population abundance from environmental DNA. Molecular Ecology 21:2555–2558.

McDevitt, A. D., N. G. Sales, S. S. Browett, A. O. Sparnenn, S. Mariani, O. S. Wangensteen, I. Coscia, and C. Benvenuto. 2019. Environmental DNA metabarcoding as an effective and rapid tool for fish monitoring in canals. Journal of Fish Biology 95:679–682.

Mckelvey, K. S., M. K. Young, W. L. Knotek, K. J. Carim, T. M. Wilcox, T. M. Padgett-Stewart, and M. K. Schwartz. 2016. Sampling large geographic areas for rare species using environmental DNA: A study of bull trout Salvelinus confluentus occupancy in western Montana. Journal of Fish Biology 88:1215–1222.

Miya, M., Y. Sato, T. Fukunaga, T. Sado, J. Y. Poulsen, K. Sato, T. Minamoto, S. Yamamoto, H. Yamanaka, H. Araki, M. Kondoh, and W. Iwasaki. 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. Royal Society Open Science 2:150088.

Niemelä, E., M. Julkunen, and J. Erkinaro. 2000. Quantitative electrofishing for juvenile salmon densities: Assessment of the catchability during a long-term monitoring programme. Fisheries Research 48:15–22.

Sales, N. G., O. S. Wangensteen, D. C. Carvalho, K. Deiner, K. Præbel, I. Coscia, A. D. McDevitt, and S. Mariani. 2021. Space-time dynamics in monitoring neotropical fish communities using eDNA metabarcoding. Science of the Total Environment 754:142096. Elsevier B.V.

Perez, C. R., S. A. Bonar, J. J. Amberg, B. Ladell, C. Rees, W. T. Ste- wart, C. J. Gill, C. Cantrell, and A. T. Robinson. 2017. Comparison of American Fisheries Society (AFS) standard fish sampling tech- niques and environmental DNA for characterizing fish communities in a large reservoir. North American Journal of Fisheries Manage- ment 37:1010–1027.

Quist, M.C., Bonvechio, K.I. & Allen, M.S. 2009. Statistical analysis and data management. Standard Methods for Sampling North American Freshwater Fishes. 171–194. American Fisheries Society, Bethesda, MA.

Raney, E. 1950. Freshwater Fishes. Care and Handling of Australian Native Animals (June 1946):7–16.

Reid, S. M., G. Yunker, and N. E. Jones. 2009. Evaluation of single-pass backpack electric fishing for stream fish community monitoring. Fisheries Management and Ecology 16:1–9.

Snyder, D.E., 2003, Electrofishing and its harmful effects on fish, Information and Technology Report 2003-0002, U.S. Geological Survey, Denver, CO.

Snyder, M.R., and C.A. Stepien. 2020. Increasing confidence for discerning species and population compositions from metabarcoding assays of environmental samples: case studies of fishes in the Laurentian Great Lakes and Wabash River. Metabarcoding and Metagenomics 4: 47-64.

Stickley, S. F. 2015. Long-Term Effects of Land Cover Change on Fish Assemblage Structure in the Piedmont and Coastal Plain Regions of Virginia Long-Term Effects of Land Cover Change on Fish. Thesis. Virginia Commonwealth University, Richmond, VA

Strickland, G. J., and J. H. Roberts. 2019. Utility of eDNA and occupancy models for monitoring an endangered fish across diverse riverine habitats. Hydrobiologia 826:129–144. Springer International Publishing.

Thomsen, P. F., J. Kielgast, L. L. Iversen, C. Wiuf, M. Rasmussen, M. T. P. Gilbert, L. Orlando, and E. Willerslev. 2012. Monitoring endangered freshwater biodiversity using environmental DNA. Molecular Ecology 21:2565–2573.

Tillotson, M. D., R. P. Kelly, J. J. Duda, M. Hoy, J. Kralj, and T. P. Quinn. 2018. Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales. Biological Conservation 220:1–11.

Turner, C. R., K. L. Uy, and R. C. Everhart. 2015. Fish environmental DNA is more concentrated in aquatic sediments than surface water. Biological Conservation 183:93–102. Elsevier Ltd.

Ulibarri, R. M., S. A. Bonar, C. Rees, J. Amberg, B. Ladell, and C. Jack- son. 2017. Comparing efficiency of American Fisheries Society standard snorkeling techniques to environmental DNA sampling techniques. North American Journal of Fisheries Management 37:644–651.

Valentini, A., P. Taberlet, C. Miaud, R. Civade, J. Herder, P. F. Thomsen, E. Bellemain, A. Besnard, E. Coissac, F. Boyer, C. Gaboriaud, P. Jean, N. Poulet, N. Roset, G. H. Copp, P. Geniez, D. Pont, C. Argillier, J. M. Baudoin, T. Peroux, A. J. Crivelli, A. Olivier, M. Acqueberge, M. Le Brun, P. R. Møller, E. Willerslev, and T. Dejean. 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Molecular Ecology 25:929–942.

Weaver, L. A., and G. C. Garman. 1994. Urbanization of a Watershed and Historical Changes in a Stream Fish Assemblage. Transactions of the American Fisheries Society 123:162–172.

Wilcox, T. M., K. S. McKelvey, M. K. Young, A. J. Sepulveda, B. B. Shepard, S. F. Jane, A. R. Whiteley, W. H. Lowe, and M. K. Schwartz. 2016. Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char Salvelinus fontinalis. Biological Conservation 194:209–216. The Authors.

Zhang, S., Q. Lu, Y. Wang, X. Wang, J. Zhao, and M. Yao. 2020. Assessment of fish communities using environmental DNA: Effect of spatial sampling design in lentic systems of different sizes. Molecular Ecology Resources 20:242–255.

Appendix

Table 2.9 Presence and absence of genetic sequence availability for final reference database (* denotes that the sequence was generated in this study).

SPECIES	COMMON NAME	DOCUMENTED IN VIRGINIA	HISTORICALLY PRESENT IN TUCKAHOE CREEK	CYTB SEQUENCE	12S SEQUENCE
Alosa sapidissima	American Shad	Y		Y	Y
Ambloplites rupestris	Rock Bass	Y		Y	Y
Ameiurus catus	White Bullhead	Y	Y	Y	Y
Ameiurus natalis	Yellow Bullhead	Y	Y	Y	Y
Ameiurus nebulosus	Brown Bullhead	Y	Y	Y	Y
Amia calva	Bowfin	Y	Y	Y	Y
Anguilla rostrata	American Eel	Y	Y	Y	Y
Aphredoderus sayanus	Pirate Perch	Y	Y	Y	Y
Campostoma anomalum	Central Stoneroller	Y		Y	Y
Carassius auratus	Goldfish	Y		Y	Y
Carpoides cyprinus	Quillback	Y		Y	
Catostomus commersoni	White Sucker	Y	Y	Y	Y
Centrarchus macropterus	Flier	Y	Y	Y	Y*
Channa argus	Northern Snakehead	Y		Y	Y
Chrosomus oreas	Mountain Redbelly Dace	Y	Y	Y	Y*
Clinostomus funduloides	Rosyside Dace	Y	Y	Y	Y*
Ctenopharyngodon idella	Grass Carp	Y		Y	Y
Cyprinella analostana	Satinfin Shiner	Y	Y	Y	Y*
Cyprinus carpio	Common Carp	Y		Y	Y
Dorosoma cepedianum	Gizzard Shad	Y	Y	Y	Y
Enneacanthus gloriosus	Bluespotted Sunfish	Y	Y	Y	Y*
Enneacanthus obesus	Banded Sunfish	Y		Y	
Erimyzon oblongus	Creek Chubsucker	Y	Y	Y	Y
Esox masquinongy	Muskellunge	Y		Y	Y
Esox niger	Chain Pickerel	Y	Y	Y	Y
Etheostoma flabellare	Fantail Darter	Y		Y	Y
Etheostoma fusiforme	Swamp Darter	Y	Y	Y	Y*
Etheostoma longimanum	Longfin Darter	Y		Y	
Etheostoma nigrum	Johnny Darter	Y	Y	Y	Y
Etheostoma olmstedi	Tessellated Darter	Y		Y	Y
Etheostoma vitreum	Glassy Darter	Y		Y	Y
Gambusia holbrooki	Eastern Mosquitofish	Y	Y	Y	Y
Hybognathus regius	Eastern Silvery Minnow	Y	Y	Y	Y*
Hypentelium nigricans	Northern Hogsucker	Y		Y	Y

Table 2.9 (cont.)

Ictalurus furcatus	Blue Catfish	Y		Y	Y
Ictalurus punctatus	Channel Catfish	Y		Y	Y
Lampetra aepyptera	Least Brook Lamprey	Y			Y
Lepisosteus osseus	Longnose Gar	Y			Y
Lepomis auritus	Redbreast Sunfish	Y	Y	Y	Y
Lepomis cyanellus	Green Sunfish	Y	Y	Y	Y
Lepomis gibbosus	Pumpkinseed	Y	Y	Y	Y
Lepomis gulosus	Warmouth	Y	Y	Y	Y
Lepomis macrochirus	Bluegill	Y	Y	Y	Y
Lepomis megalotis	Longear Sunfish	Y		Y	Y
Lepomis microlophus	Redear Sunfish	Y	Y	Y	Y*
Lethenteron appendix	American Brook Lamprey	Y			Y
Luxilus cornutus	Common Shiner	Y	Y	Y	Y
Lythrurus ardens	Rosefin Shiner	Y		Y	Y
Micropterus dolomieu	Smallmouth Bass	Y		Y	Y
Micropterus henshalli	Alabama Bass	Y		Y	
Micropterus punctulatus	Spotted Bass	Y		Y	Y
Micropterus salmoides	Largemouth Bass	Y	Y	Y	Y
Morone americana	White Perch	Y		Y	Y
Morone saxatilis	Striped Bass	Y		Y	Y
Moxostoma cervinum	Black Jumprock	Y		Y	Y
Moxostoma erythrurum	Golden Redhorse	Y		Y	
Moxostoma macrolepidotum	Shorthead Redhorse	Y		Y	
Nocomis leptocephalus	Bluehead Chub	Y	Y	Y	Y
Nocomis raneyi	Bull Chub	Y			
Notemigonus crysoleucas	Golden Shiner	Y	Y	Y	Y
Notropis amoenus	Comely Shiner	Y		Y	
Notropis hudsonius	Spottail Shiner	Y		Y	Y
Notropis procne	Swallowtail Shiner	Y		Y	
Notropis rubellus	Rosyface Shiner	Y	Y	Y	Y*
Notropis telescopus	Telescope Shiner	Y		Y	Y
Noturus insignis	Margined Madtom	Y	Y	Y	Y*
Oncorhynchus mykiss	Rainbow Trout	Y		Y	Y
Perca flavescens	Yellow Perch	Y	Y	Y	Y
Percina notogramma	Stripeback Darter	Y	Y	Y	Y*
Percina peltata	Shield Darter	Y			
Percina roanoka	Roanoke Darter	Y		Y	

Table 2.9 (cont.)

Petromyzon marinus	Sea Lamprey	Y			Y
Pimephales notatus	Bluntnose Minnow	Y		Y	Y
Pimephales promelas	Fathead Minnow	Y		Y	Y
Pomoxis annularis	White Crappie	Y		Y	Y
Pomoxis nigromaculatus	Black Crappie	Y	Y	Y	Y
Pylodictis olivaris	Flathead Catfish	Y		Y	Y
Rhinichthys atratulus	Blacknose Dace	Y	Y	Y	Y
Rhinichthys cataractae	Longnose Dace	Y		Y	Y
Salmo trutta	Brown Trout	Y		Y	Y
Salvelinus fontinalis	Brook Trout	Y		Y	Y
Sander vitreus	Walleye	Y		Y	Y
Semotilus atromaculatus	Creek Chub	Y	Y	Y	Y
Semotilus corporalis	Fallfish	Y	Y	Y	Y*
Thoburnia rhothoeca	Torrent Sucker	Y	Y	Y	Y
Umbra pygmaea	Eastern Mudminnow	Y	Y	Y	Y