Pennyroyal Plain Parulids: Characterizing Louisiana Waterthrush occupied stream reaches in an agriculturally-dominated landscape using a standardized bioassessment

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Nicole I. Santoyo

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Nicole I Santoyo　　　　23 May 2020
Date

DEDICATION

For Devon, the California boy in my life.

It’s not a poem but it’ll have to do.

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Each and every person with a hand in helping me get to this point – you rock. To my advisor and fellow birder, Dr. Steve Hamilton, thanks for taking me on as a graduate student and giving me a chance to execute this project. It means a lot that you trusted me to get stuff done. Thanks to Dr. Joe Schiller and Dr. Stefan Woltmann, for serving on my committee and the sundry advice along the way. Thanks to Sylvia Powell, Sandra Bojić, and Aaron Hite, fellow graduate students, for helping with conducting my bioassessments and keeping track of the woebegone densiometer. Faith Hudgens, our wonderful undergraduate lab member, helped quite a bit in the initial sorting of macroinvertebrates, for which she has my thanks. The Office of Student Research and Innovation at APSU helped fund my summer work and conference travel. All the landowners at my sites have my sincere gratitude for letting me and my team go tromping around on their property to look for bugs and the small brown warblers that eat them. What of my parents? Thanks for letting me be this way and giving me a place to live during my relatively protracted education.

ABSTRACT

The Western Pennyroyal Karst Plain (U.S. Environmental Protection Agency Ecoregion 71e, often shortened to "Pennyroyal Plain") historically was composed of open oak savanna and tallgrass prairie, but its relatively flat topography and abundance of treeless terrain lent to its conversion to predominantly agricultural land. This change in the landscape is to the detriment of instream and riparian community composition, as agriculture results in increases in sedimentation, nutrient input, alterations in stream flowpaths, and riparian zone deforestation. A riparian-obligate songbird, the Louisiana Waterthrush (*Parkesia motacilla,* family Parulidae) has been proposed as a bioindicator due to its reliance on relatively high-quality stream and riparian habitat to feed itself and its nestlings. On the Pennyroyal Plain, the waterthrush is met by seemingly unfavorable conditions of narrow riparian forest buffers and few aboveground streams, many of which are impacted by intensive row crop agriculture. In order to characterize the conditions in which waterthrush may be found in this ecoregion, I surveyed for waterthrush, sampled instream macroinvertebrates in accordance to Tennessee Department of the Environment and Conservation bioassessment protocols, and performed analyses of forested buffer width, land cover, and minor tributary confluences using GIS. Waterthrush were found at all sites during either surveys or during macroinvertebrate sampling. Bioassessment results classified sites as having impacts including bank sloughing, reduction of riparian vegetation, and reduced forested buffer width. Biotic index scores exceeded target scores for four out of seven sites, though even at those sites, some taxa and functional groups were not well represented. Forested buffer widths regularly reached 60-100m, but were not consistently as wide as 200m. Land cover in the surveyed watersheds revealed predominantly agricultural land use, and low

percentages of residential and commercial development. The conditions in which these waterthrush have been found suggest some ability for adults to tolerate agriculturally impacted streams, though their presence does not necessarily indicate nesting success. Further work is needed to ascertain what habitat details are most important to waterthrush – whether singly, as pairs, or to support nestlings.

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CHAPTER I. INTRODUCTION

In agriculture-dominated landscapes, original land cover is replaced with crops and pastures, which also affects rivers and streams. Since water is necessary for crops, it is often rerouted to provide irrigation or channelized to direct water off the land faster and prevent flooding (Brooker 1985). When crops are planted where there was once forest or grassland, the infiltration of water into the ground is reduced, and water runs into the surrounding streams more rapidly than if natural groundcover was present (Naiman and Décamps 1997). The conversion to cropland also promotes erosion and resultant increased sediment and nutrient (Lowrance et al. 1984) transport within the stream, which is facilitated by the reduction in width of riparian buffers (Fierro et al. 2017).

When stream condition is degraded, instream community composition changes as aquatic habitat is altered in ways that reduce its suitability to its natural community (Lau et al. 2006). To remediate waterways which have been damaged by human use, the Clean Water Act was implemented by the United States Environmental Protection Agency (USEPA) to restore running waters to their natural state (U.S. Environmental Protection Agency 1972). Internationally, governments often require water quality testing using indicator organisms as well as chemical and bacteriological testing. In the United States, streams are regulated in terms of their designated uses including drinking, recreation, navigation, and irrigation (U.S. Environmental Protection Agency 2012). Regulators realize that streams fulfill roles beyond human needs and recreation, that is, their support of aquatic life (U.S. Environmental Protection Agency 2012). To assess how streams meet these uses, various forms of chemical, physical, bacteriological, and ecological analyses are conducted (U.S. Environmental Protection Agency 2012). Terms like stream "health" and assessments of chemical and physical properties do not encompass the suite of ecosystem processes occurring in the stream, so the concept of biotic integrity was developed to address the maintenance of natural conditions in the stream. Biotic integrity refers to the stream's maintenance of natural conditions within their biogeographic and evolutionary context (Karr and Chu 1999). Biotic indices are often used as assessments of the structural and functional completeness of a community and are calibrated relative to reference conditions – in other words, locations where human impacts are relatively small and conditions are as close to natural as can be attained (Karr 1991). Ideally, the biometrics used by an index encompass the structural and functional traits of the instream community, while being sensitive to human-mediated impacts and limiting sensitivity to natural variations in communities. For instance, the macroinvertebrate bioassessment used by the Tennessee Department of Environment and Conservation (Tennessee Department of Environment and Conservation 2017) consists of several taxa richness metrics as well as percent abundances for both sensitive and tolerant organisms. When index values or their component biometrics are below specified values (these vary by jurisdiction), agencies may develop plans for their remediation.

In the region of this study, the Western Pennyroyal Karst (USEPA Level IV Ecoregion 71e), Louisiana Waterthrushes are met with stream and riparian communities impacted by agriculture, the dominant land use in the ecoregion, in the form of intensive row crops with some pasture land. These impacts affect the instream biota through various forms of pollution (Elbrecht et al. 2016), and reduction or destruction of the riparian zone (Fierro et al 2017). Instream macroinvertebrate communities may change in composition to reflect altered conditions where more pollution-intolerant taxa become less abundant. Terrestrial invertebrates may also be affected by habitat alteration and loss of plants on which they feed and seek shelter (Lyons et al. 2000). The Louisiana Waterthrush feeds on both instream and riparian terrestrial macroinvertebrates (Eaton 1958, Craig 1984), so the biotic integrity of both habitats is important for its survival. Impairments of the stream and riparian habitats occupied by breeding waterthrush may impact occupation by a single bird, pairs, or the ability of a pair to successfully fledge nestlings.

The Louisiana Waterthrush

The Louisiana Waterthrush, *Parkesia motacilla* (Vieillot), (Passeriformes: Parulidae) is a Neotropical migratory songbird wintering in southern North America, northern Central America, and the Caribbean, and breeding in the eastern United States north to southern Canada. They are riparian-obligates, occupying linear stretches of small streams and the surrounding riparian corridor (Eaton 1958). Both their breeding and nonbreeding habitats are similar (Eaton 1958, Hallworth et al. 2011). The biotic integrity of the riparian habitat (Prosser and Brooks 1998) as well as the stream itself (Mattson and Cooper 2006) is important, as the birds feed both in and around the water (Craig 1987, Craig 1984, Eaton 1954). Within the genus *Parkesia*, there is one other species, the Northern Waterthrush (*P. noveboracencis*), which occupies territories encompassing shallow wooded ponds and backwaters, its territories sometimes overlapping with its congener during their breeding season in the American Northeast (Craig 1984). Throughout the rest of this manuscript I will employ the American Ornithological Union 4-letter Alpha Codes when referring to these species. The Louisiana Waterthrush's Alpha Code is LOWA and Northern Waterthrush's is NOWA.

Habitat Use

Prosser and Brooks (1998) created a habitat suitability index for the LOWA based on a consultation of literature and field testing to determine waterthrush presence. Their model incorporated features noted in publications as important, such as riparian forest coverage, shrub height, stream topography, and land use surrounding the survey area. They concluded that the waterthrush favors headwater streams with well-developed pools and riffles. Tirpak et al. (2009) also created a habitat suitability index for the LOWA, finding the waterthrush associated with high percent canopy cover, large forest patches, and high forested landscape composition within 1km. Nonetheless, waterthrush may be found in suboptimal habitats such as catchments impacted by the effects of cattle grazing and urbanization (Mattson and Cooper 2006).

Greater riparian forest width can contain a greater variety of birds according to Peak and Thompson (2006), including those that are area-dependent with regards to interior forest habitat. For the waterthrush, however, Peak and Thompson’s (2006) model including width was not the most-supported, or even second-most supported, with the null model having the most support, meaning variables other than forest width explain their population density. The width of riparian forest recommended in management varies from 40-500m (Fischer 2000), depending on management goals. In one instance, the waterthrush was observed only on sites where riparian buffers were wider than 26m (Chapman et al. 2015).

Life History

Though the LOWA is increasingly well studied, there are still gaps in our understanding of the species, especially regarding what impacts its ability to occupy areas and successfully raise young. Eaton (1958) published a life history study of the Louisiana Waterthrush, forming the first in-depth inquiry into the bird's life. His study focuses on individuals occupying territories along 10 tributaries to Cayuga Lake near Ithaca, New York, as well as a short period of observations of waterthrush wintering in Cuba over a three-year period. He concludes that the differences in timing and duration of reproductive and developmental events compared to the Ovenbird (which at the time was placed in the same genus as the waterthrushes) were due to differences in habitat use and food source; that is, Ovenbirds are interior forest birds occupying polygonal territories, while Louisiana Waterthrush occupy linear territories along a stream, hunting both terrestrial and aquatic invertebrates.

Waterthrush males arrive on their breeding grounds in advance of females, typically from mid-March to mid-April depending on the latitude (Eaton 1958, Stucker and Cuthbert 2000). Males tend to have higher site fidelity than females returning to the same territory year after year, which is speculated to be due to the importance of choosing a high-quality territory to raise nestlings (Frantz 2019). Nests are constructed on sloping banks under overhanging vegetation, roots, and humus, and occasionally on ledges of shale (Eaton 1958, Stucker and Cuthbert 2000, pers. obs.). Females typically begin laying eggs in April or May (Eaton 1958, Mulvihill et al. 2009). Incubation is completed after approximately 12 days, and young leave the nest 10 days after hatching (Eaton 1958). Across latitudinal gradients, individuals at more northerly latitudes have a greater probability of re-nesting if a nest fails and having larger clutch size in their replacement broods, though fecundity between high and low latitude birds may be similar (Mulvihill 2011). Breeding birds and independent young begin to leave breeding territories in August and reach their nonbreeding territories by the end of the month (Eaton 1958). The timing of waterthrush arrival and nesting corresponds to emergence periods of aquatic insects, an important food resource (Eaton 1958).

Diet

LOWA are capable of foraging in the water, from the air, and in riparian vegetation (Craig 1984, 1987). They prefer prey items up to 3 cm in length (Craig 1984), with the majority between 13-19 cm (Craig 1987) and choose foraging sites along streams more often, than the NOWA (Craig1984). According to Eaton (1958), major sources of food for waterthrush were chironomid (non-biting midges) larvae, followed by coleopteran (beetle) adults, also including a variety of other aquatic dipteran (true fly) larvae. He observed that waterthrush also fed on emergent insects such plecopteran and ephemeropteran (mayfly) adults frequently during April and May. Craig's (1984, 1987) findings were similar, with chironomids and trichopterans being the most observed prey item, followed by ephemeropterans and oligochaetes. Craig (1987) also noted the LOWA's predation on isopods, odonate (damselfly and dragonfly) nymphs, dytiscid (predatory diving beetle) larvae, tipulid (cranefly) larvae, and diplopods (millipedes). While earlier diet studies such as those of Craig (1984, 1987) focus most on both waterthrush species' acquisition of aquatic prey, a molecular diet analysis of the LOWA by Trevelline et al. (2016) found terrestrial insects were important to the nestling's diet. They found aquatic macroinvertebrates, while still an important contributor to nestling diets, were less frequently found than lepidopterans and spiders, suggesting that riparian forest is another important source of food for nestlings.

Phylogeny and Evolutionary History

It has been argued that the phylogeny is important to conservation; the conservation of current biodiversity is crucial to preserving the potential for future biodiversity (Vázquez and Gittleman 1998). When evaluating conservation priority, more evolutionary time is preserved within lineages with one or two species (Faith 1996). The genus *Parkesia* consists of two, and thus contains considerably greater amounts of evolutionary history relative to those with more extant species, such as *Setophaga*, a genus with numerous species. Phylogeny tends also to constrain the ecological role of organisms and influence their niche (Webb et al. 2002), evident in the similar habitat choice and feeding strategies of the two waterthrush (Craig 1984). This section briefly describes their ancestral traits within the family Parulidae.

The family Parulidae consists of migrant and non-migrant tropical species, and those with monochromatic or dichromatic sexes. The Louisiana Waterthrush is a monochromatic, migrant warbler, and phylogenetic analysis has placed it as an ancestral member of Parulidae, along with the Northern Waterthrush, Ovenbird (*Seiurus aurocapilla*), and Worm-eating Warbler (*Helmitheros vermivorum*) (Lovette et al. 2010). Both migration (Winger et al. 2012) and monochromatic sexes (Simpson 2015) are thought to be basal parulid traits.

Sensitivity to Ecological Disturbance

Waterthrush territory length is thought to be a proxy for habitat quality, as birds may expand their territories to compensate for poor resource availability (Wood et al. 2016). Mattson and Cooper (2006) found waterthrush to be an indicator of instream biotic integrity as determined by a bioassessment and proposed them as a charismatic, easy-to-survey “early warning system” for habitat disturbance.

Louisiana Waterthrush are not well surveyed by traditional bird survey methods because waterthrush are not often found away from the riparian corridor. For instance, the Breeding Bird Count involves surveying along roads, introducing a bias towards easily detectable birds along roadside habitats (Rosenberg et al. 2017), under-representing waterthrush and other riparian songbirds. This and other methods that do not follow the linear path of the stream may not capture much information about occupancy because it is unlikely to see more than one pair at any one point along the stream.

Aquatic Macroinvertebrates

Macroinvertebrates are widely used as bioindicators of instream biotic integrity because of the ease of collection, predictability of changes in community composition due to pollution, and their range of generation times, which can be several weeks to a year or more (U.S. Environmental Protection Agency 1999). This is in contrast to the use of chemical and physical water sampling, which only assesses water conditions at one point in time, which may not reveal impacts occurring over time or during different times of day, or structural changes in stream habitat (Karr and Chu 1999).

Members of the insect orders Ephemeroptera, Plecoptera and Trichoptera (EPT), or mayflies, stoneflies and caddisflies, have been shown to be prominent prey items for waterthrush, along with dipteran family Chironomidae, or non-biting midges (Eaton 1958, Craig 1984, Craig 1987). EPT in particular tend to be intolerant to pollution (Lenat, 1993), and relative to unimpacted streams, percent abundance of EPT and total generic richness are much lower in those heavily impacted by landscape changes such as mountaintop removal mining (Pond et al. 2008). Mountaintop removal mining introduces large amounts of sediment and conductivity-increasing ions, causing the absence of heptageniids and ephemerellids in the most impacted streams in (Pond et al. 2008).

Though mine spoil as in the Pond et al. (2008) study is an extreme example of habitat quality impairment, other forms of degradation, such as agriculturally-associated impacts, commonly impair water quality effecting benthic macroinvertebrate communities and lead to structural and functional shifts therein (Elbrecht et al. 2016). Agriculture results in warmer temperatures, higher nutrient concentrations, lower dissolved oxygen, increased sedimentation, and altered macroinvertebrate communities relative to less intensively farmed and forested sites (Harding and Winterbourn 1995, Hagen et al. 2006, Kyriakeas and Watzin 2006).

Combinations of disturbances caused by agriculture (sedimentation, nutrient enrichment, and dissolved oxygen reduction, for example) yield an additive effect on community composition (Elbrecht et al. 2016); in other words, two or more of these factors occurring together in a stream are more deleterious for intolerant species than one. Agriculturally mediated impacts may affect landscapes and the macrobenthos long after farming ceases (Harding at al. 1998). Cattle grazing in particular can affect streams significantly by cropping vegetation and trampling streambanks, increasing bank instability and sedimentation, and ultimately resulting in reductions in total numbers of macroinvertebrates and percent abundances of elmid beetles and EPT (McIver and McInnis 2007). Taxa are affected differentially by different forms of agriculture; for instance, trichopterans, such as *Hydropsyche* and *Cheumatopsyche*, often persist in disturbed watersheds, while cattle access leads to the loss of the more intolerant plecopterans (Kyriakeas and Watzin 2006).

Macroinvertebrates are often sampled during waterthrush studies in order to learn about their relationship to the macrobenthos and instream biotic integrity. Waterthrush presence, especially pair presence, was seen to be an indicator of relatively high percent abundance of EPT (Mattson and Cooper 2013). Where shale gas drilling occurs, benthic macroinvertebrate communities are affected, abundances of EPT and larger macroinvertebrates decrease, and habitat assessments also note decreased suitability for waterthrush. In response to these disturbances, waterthrush expanded their territories to compensate for poor resource availability (Wood et al. 2016).

The Western Pennyroyal Karst Plain

Ecoregions are delineated by a combination of geological, climatic, soil, and vegetation traits (Omernik 1987), with increasing nuance for Level IV ecoregions (Omernik and Griffith 2014). The Western Pennyroyal Karst, Level IV ecoregions 71e, is mostly underlain by the St. Louis and Ste. Genevieve limestone formations (Dicken 1935, White 1977), which are porous and rich with chert nodules. These limestones are part of the Mammoth Cave series within the Meramecian series (Dicken 1935). Limestone-derived substrates contain solutes that buffer pH, leading to values above 7 in many streams. In the Mammoth Cave Series, the Ste. Genevieve is most soluble, leading to some of the karstic qualities found in the Western Pennyroyal Karst. Sinkholes, underground streams, and sinking springs are common (Dicken 1935).

The Western Pennyroyal Karst, often shortened to "Pennyroyal Plain," was historically sparsely forested, and dominated with "barrens" maintained by natural and anthropogenic fire, as well as drought (Baskin et al. 1994). These conditions kept trees from overtaking soils that often had "pans," dense layers of soil that prevented deep root penetration. Fire suppression has eliminated much of the fire-dependent savannas and other early successional habitats, causing them to progress into dense forest where they have not been converted to pasture or row crops (Baskin et al. 1994, Baskin et al. 1997). Even though forests are more common, riparian areas are not well-forested and may consist of narrow strips of trees adjacent to row crop and pasture land. In nature, riparian areas often retain forest even in fire-dependent areas, as streams and incised floodplains can serve as a barrier to fire, even though they may be subject to heavy loads of sediment after fire events (Petit and Naiman 2007).

Objectives

I attempted to characterize breeding season habitat of the Louisiana Waterthrush as it occurs on the Western Pennyroyal Karst in a way that is easy to replicate and for which standardized methodology exists. This was in order to both a) assess the waterthrush as a as a proxy for macroinvertebrate community bioassessments, and b) allow for comparison to sites where macroinvertebrate bioassessment has already been performed by the state. Additionally, macroinvertebrates are widely used by environmental agencies of other states, so similar biometrics (%EPT is a common metric, for instance) can be compared even when other variables measured may differ. I also attempted to compare number of minor tributary confluences and land use traits of my study reaches, as watershed and reach-level variables may impact macroinvertebrates, waterthrush foraging effort, and nesting success. However, because my sites did not include locations where waterthrush were absent, I was not able to address such comparisons.

CHAPTER II. METHODS

Site selection was aided by satellite imagery. When sites were selected and permission was granted by landowners, I conducted the macroinvertebrate bioassessment and waterthrush survey. The bioassessment was performed according to Tennessee state protocols, requiring the collection of macroinvertebrates from two riffles within the stream and visual scoring of habitat features. To survey waterthrush, researchers performed a visual and aural search for at least 30 minutes during either the date the bioassessment occurred or at a separate survey date. For the bioassessment, habitat variables were scored, chemical data for the stream was collected, and instream macroinvertebrates were collected. In addition to on-ground data collection, GIS analysis of the sites was performed.

Site Selection

An initial search was conducted via Google Earth (Google, Mountain View, CA) using the National Hydrography Dataset superimposed on satellite imagery to identify first through third order stream flowpaths and potential access points. I attempted to select both state reference streams (Tennessee Department of Environment and Conservation 2017) and those without that designation in Ecoregion 71e. Landowners were identified and contacted for permission to access their property. My selections for the assessments were as follows: Passenger Creek (Montgomery Co., TN), Calebs Creek (Robertson Co., TN), Buzzard Creek (Robertson Co., TN) and Elk Fork (Todd Co., KY, and Robertson Co., TN). Four sites were located on Elk Fork where downstream sites include the watershed of upstream sites in their drainage area. Thus, they are subject to some of the same conditions. I acknowledge there can be concerns of pseudoreplication when sites are along the same stream (Hurlbert 1984). However, the length of Elk Fork sampled provides a gradient of conditions across which my observational units (individual stream reaches and their watersheds) are placed, and although the downstream sites are interdependent with upstream locations, they provide a vignette into separate sets of conditions that may be experienced by the waterthrush. That is, individual sites, even along one stream, contain their own riparian characteristics and impacts localized to those particular sites. Studies where sites are not randomly chosen, as here, do not lose all their utility and can be used as preliminaries for work where more rigorous statistical testing will be performed (U.S. Environmental Protection Agency 2002). Thus, this study can be seen as a series of preliminary observations of Pennyroyal Plain's habitat characteristics as they relate to the Louisiana Waterthrush, providing background for potential future investigations.

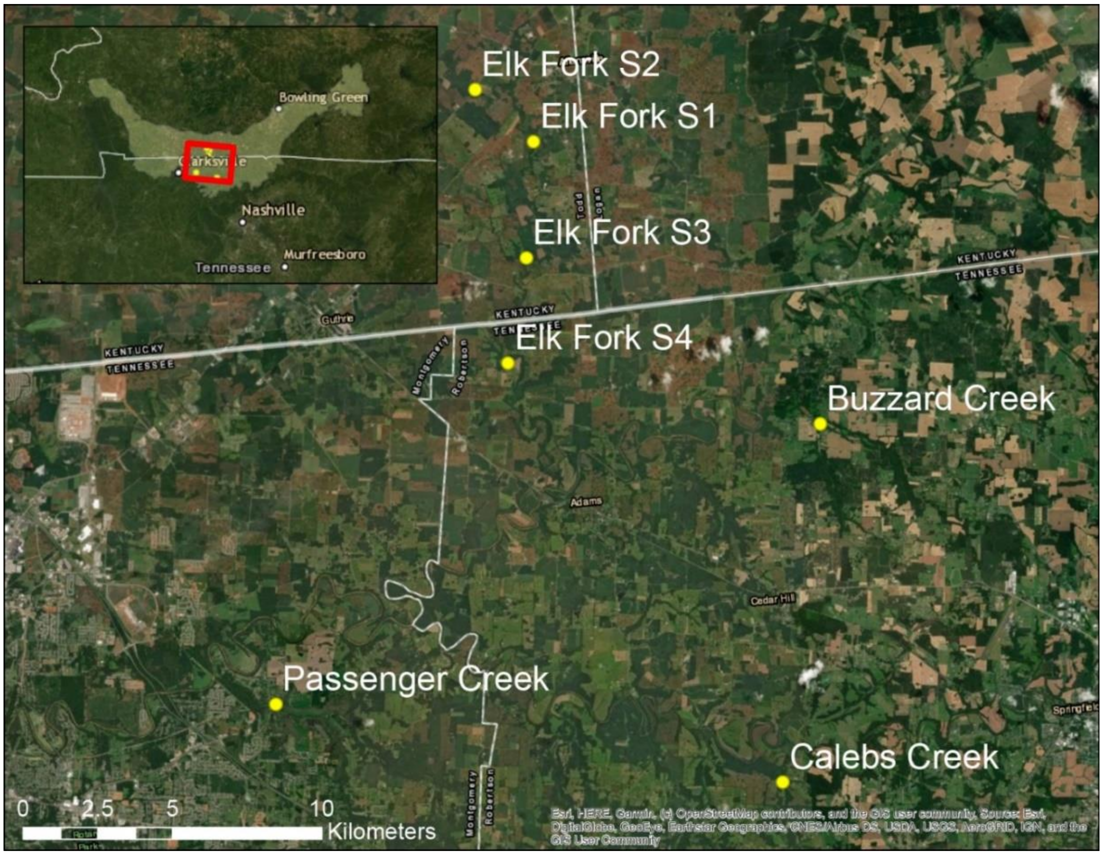

Figure 1. Site locations in the Pennyroyal Plain, Todd County, Kentucky, and Montgomery and Robertson counties, Tennessee. Elk Fork Creek sites are located along the same stream and are numbered in chronological order based on the bioassessment date. The Pennyroyal Plain designated as Ecoregion 71e is shown in the inset as a highlighted shape.

Waterthrush Survey

Waterthrush surveys were conducted concurrently with or before the date of the macroinvertebrate bioassessment in the spring and summer of 2018. For a 30-minute period, surveyors searched both aurally and visually for Louisiana Waterthrush. Recordings of waterthrush songs and calls (Macaulay Library, Cornell Lab of Ornithology, Ithaca, NY, recording ML112695 and ML87901) played back from an iPhone SE (Apple, Cupertino, CA) were used to provoke singing if no waterthrush were immediately visible or no singing or calling was heard. If no waterthrush were found during an initial visit, a second visit was planned during the same breeding season. If the second visit yielded no waterthrush observations, up to two more visits were made during the next breeding season, spring 2019. Waterthrushes are thought to have high site fidelity and will return to the same breeding territory for multiple seasons, so areas where waterthrush are present one year are likely to be occupied the next, often by the same individual (Frantz 2019, Bryant 2018, Goodpasture 1977, Stefan Woltmann, pers. comm.). Their high site fidelity means that it is likely that reaches where male waterthrush were seen in one sampling season have the same occupancy status from year to year, thus I have established their presence for the purposes of this study at these sites based on my observations during either 2018 or 2019.

Macroinvertebrate Sampling

Between May and July 2018, macroinvertebrate samples and habitat biometrics were collected according to the Tennessee Department of Environment and Conservation's Standard Operating Procedures for Macroinvertebrate Surveys (Tennessee Department of Environment and Conservation 2017), following protocols for the Semi-Quantitative Riffle Kick (SQKICK). A 1 m^2 kick net with 500 µm mesh was used to collect aquatic macroinvertebrates in wadable riffles of target streams. Two riffles within an approximately 100 m-long reach were chosen for sampling. This was accomplished with two participants, one holding the net downstream of another, the upstream participant kicking the substrate and overturning rocks to dislodge macroinvertebrates. Substrate and macroinvertebrates drift into the net, where they are collected when both participants lift the net from the water and transfer the contents to a sieve-bottomed bucket.

The sieve-bottomed bucket was washed with 70% isopropanol to dislodge organisms into 1 L Nalgene bottles. Additional 70% isopropanol was added as necessary to cover the substrate and preserve the organisms. A label with the site name and sampling date were placed into the sample bottles.

The organisms in each sample were subsampled to a target quantity of approximately 200 (± 40) individuals after being poured into a sorting tray with twenty-eight 2 in^2 grids. Four grids were randomly selected and all the material from each grid square was closely examined, with the macroinvertebrates found therein counted and separated from the substrate. If fewer than 160 individuals were obtained from the four initial grids, random grids were selected until the target was achieved. If more than 240 organisms were found in the initial four grids of the sorting tray, the subsample was placed in a gridded tray for further subsampled until the target quantity was obtained (200±40). Organisms were temporarily stored in a sorting dish containing 70% isopropanol awaiting further coarse sorting (order, family) and placement in isopropanol-filled vials based on those sorts. Chironomid (non-biting midge) larvae were mounted on microscope slides using CMC-10 (MS-222, Masters Company, Inc., Wood Dale, IL). Slides were labeled with the sample site and their number within the series for that site.

All arthropods were identified, using dissection (stereo) and compound microscopes, to the lowest practical taxa, typically genus, using relevant taxonomic keys (Merritt et al. 2008, Morse et al. 2017, Wiederholm 1983). If organisms lacked features that would allow for a genus-level identification due to size or missing parts (gills, antennae, etc.), they were identified to family. All insect pupae were identified to family only. Other invertebrates, such as oligochaetes, gastropods, Platyhelminthes, were identified no further. All organisms were stored in vials labeled with their collectors, collection date, site location, taxon, and the individual responsible for identification of those organisms.

For each site, seven biometrics were calculated as established in the TDEC protocols (Tennessee Department of Environment and Conservation 2017). The biometrics are Taxa Richness (TR), EPT Richness (EPT TR), Percent of EPT minus *Cheumatopsyche* (%EPT-CHEUM), Percent Oligochaetes and Chironomids (%OC), Percent Clingers minus *Cheumatopsyche* (%Clingers-Cheum), Percent Tennessee Nutrient Tolerant Taxa (%TNUTOL), and North Carolina Biotic Index value (NCBI). Taxa Richness measures are a count of all identified genera, or higher taxonomic categories for non-arthropods and damaged specimens. EPT Richness refers to a tally of all EPT genera minus the pollution tolerant caddisfly genus *Cheumatopsyche.* Percent EPT-*Cheumatopsyche* is the percentage of the sample consisting of non-*Cheumatopsyche* EPT. Similarly, Percent Oligochaetes and Chironomids is the portion of the sample comprised of oligochaete worms and midge larvae and pupae. Percent Nutrient Tolerant Taxa is the proportion of the sample represented by gastropods and the arthropod genera *Cheumatopsyche, Cricotopus/Orthocladius, Stenelmis*, *Polypedilum, Caenis*, and *Lirceus*. The North Carolina Biotic Index is a weighted average community tolerance based on established pollution tolerances of organisms in the community, with 0 being the most intolerant and 10 being most tolerant.

The seven metrics are also scored in terms of their amount of impact as determined from biocriteria values according to the ecoregion (Tables A-2 and A3). Scores range from 0-6 for each metric raw value, with 0 being severely impaired and 6 being least impaired. The scores given to values varies depending on what is expected for each ecoregion and, in some cases, for the time of year. The sum of these scores equals the Tennessee Macroinvertebrate Index (TMI) score, for which the highest possible score is 42. Perfect scores are not anticipated, and the "target score" for Ecoregion 71e is 32, meaning that the stream is meeting its designated use criteria established by the state for supporting aquatic life; meeting these criteria determine that stream habitat conditions do not signal a need to develop a remediation plan according to state guidelines (Tennessee Department of Environment and Conservation 2017). However, meeting target scores does not imply that functional or structural shifts in the instream macroinvertebrate communities consistent with agriculture-mediated habitat changes have not occurred.

A YSI 600 QS multi-parameter meter (YSI, Yellow Springs, OH) was used to determine temperature (°C), pH (SU), specific conductivity (mS/cm), and dissolved oxygen (mg/L & % sat.). A LaMotte 2020 (LaMotte Company, Chestertown, MD) nephelometric turbidity meter was used to measure turbidity (NTU). Canopy cover was measured using a convex spherical densiometer (Forestry Suppliers, Jackson, MS), which was read at the approximate midpoint of sampled riffles.

Habitat Assessment

Habitat assessments were performed using the Habitat Assessment Field Sheet for Moderate to High Gradient Streams (Tennessee Department of Environment and Conservation 2017, reproduced as figures A-5 and A-6). There are 10 categories on which streams are assessed which are associated with the level of potential anthropogenic impact experienced (i.e., epifaunal substrate/available cover, embeddedness, riparian zone width). Categories were scored from 1 to 10, with 1 being the lowest score indicating very poor conditions, and 10 being the highest score, for best possible conditions. Habitat Assessment Scores were determined for approximately 100 meter and may not represent the entire 500m reach used for the LOWA assessment. Participants in fieldwork contributed to the habitat assessment by conferring about the score to be given to each habitat category.

Buffer Analysis

Buffer analysis is used to characterize the area surrounding either a point or linear path, with analyses performed on the area within a boundary radius surrounding the point or line. In this study, I analyzed the buffer composition surrounding the stream. My buffer analyses were performed in ArcMap (Version 10.5, ESRI, Redlands, CA) to determine the completeness of forest cover over three different widths within a range of those indicated by Fischer (2000). 60, 100, and 200m. The stream's path was determined using flowlines found within the USGS National Hydrography Dataset Plus High Resolution (NHD, United States Geological Survey, https://www.usgs.gov/core-science-systems/ngp/national-hydrography/nhdplus-high-resolution). Terrain and stream reaches within a 500m radius were used for buffer analyses. This radius was chosen to encompass at least 500m of stream either up- or downstream of the macroinvertebrate sampling location. Waterthrush territories are between 300-500m in length (Eaton 1958), so ideally, the entirety of at least one territory would be contained within the zone of analysis.

Area analysis was aided by in-application geometry tools. The buffer zones surrounding the stream were drawn with the Buffer tool to create 60, 100, and 200m-wide buffers surrounding the stream. The polygon tool was used to delineate areas of riparian forest. Forest not contiguous with the riparian zone (i.e., separated from forest surrounding the stream by a field or road) was excluded from the polygon shapefile used for width analysis as it is unlikely that riparian obligate songbirds would use forest discontinuous with the stream. The buffer tool was used to draw the three buffer widths, and erased to find the forested area, which was then converted into a percentage of the area of the buffer within the radius.

Watershed Analysis

Land cover was assessed for watersheds containing my sites to characterize the surrounding land use for my sites. The catchment area, or land area draining into the stream at a given point, was determined using the USGS National Hydrography Dataset described above and delineated using the USGS application StreamStats (https://streamstats.usgs.gov/ss/). National Land Cover Database (NLCD, https://www.mrlc.gov/data) was superimposed on the catchments. Raster overlays of the catchment's land cover were created for the catchments using ArcMap's Extract to Mask function. Tabulate Area was then used to obtain the area of the catchments consisting of various forms of cover, and subsequently converted into a percentage. StreamStats was again used to find flowlines for minor tributaries which connect to the main stem sites measured by the bioassessment. All minor tributaries confluent with the mainstem within the 500m-radius circle delineated during the buffer analysis were counted, as such tributaries may serve as valuable nesting habitat when main stem conditions are less suitable or predation more likely (Vander Haegen and Degraaf 1995, Sylvia Powell, pers. comm.). Additionally, the existence of minor tributaries on a stream may provide more hydrologic stability for nesting habitat compared to the main stem, as streams with flashy hydrographs may be subject to flooding events that destroy nests (Frantz 2019).

CHAPTER III: RESULTS

Waterthrush Survey

At six sites, waterthrush presence was determined during Spring/Summer 2018. For five of those sites, waterthrush were detected during sampling or the first survey. At Elk Fork S2, a waterthrush was seen after an additional surveying occasion. At Calebs Creek, waterthrush presence was not determined until two more visits were conducted in spring 2019.

A field assistant (S. Powell) and I opportunistically observed adults flying into a minor tributary at Elk Fork S1 and suspected a pair had nested in the tributary and noted the presence of fledglings. I did not otherwise observe or seek to identify nesting pairs at my sites. This instance of fledging suggests nesting success may be possible in this stream, and potentially, those with similar conditions.

Physical and Chemical Data

All sites had pH values above 7 (range = 7.52 – 8.15), typical of this ecosystem where streams are well-buffered by the calcium carbonate dissolved from the limestone bedrock. Water temperature ranged from 17.0 to 20.9˚C. Buzzard Creek, the coldest site, emerges from a large spring upstream of the sampling site, contributing to its colder temperature. DO % saturation had a wider range (57.5 – 98.7%) which could be attributed to differences in land use and consequent nutrient input increasing the abundance of heterotrophs at less oxygenated sites. Elk Fork S4, with the lowest %DO, was the furthest downstream of the Elk Fork Sites and receives inputs from a larger watershed than the other three sites. In addition to this, it was relatively deep and fast moving with few riffles. Water chemistry and temperature experiences diel and seasonal changes, so these measurements, taken in the late morning and afternoon, may not be consistent with those taken earlier in the morning or later in the afternoon or evening, and at different times of the year (Tennessee Department of Environment and Conservation 2005).

Canopy cover varied greatly by site and reflected stream width as well as riparian forest density. Calebs Creek, with a narrow tree row, had the lowest percent canopy cover (19%), followed by Elk Fork S4 with a similarly deforested condition on one side, compounded by its larger width. Passenger Creek, with 78% cover, was narrower and more or less surrounded by overhanging trees even though it bordered a gravel driveway along the sampling location.

Table 1. Physical and chemical data collected as part of the bioassessment protocols for the seven sampling sites in Todd County, KY and Montgomery and Robertson counties, TN were pH, temperature (Temp,°C), dissolved oxygen (DO, % saturation) turbidity (Nephelometric Turbidity Units, NTU), specific conductivity (mS/cm), and percent canopy cover. Also shown is the number of tributary confluences within the named study reach.

Site Name	pH	Temp. (°C)	DO (%)	Turbid. (NTU)	Spec. Cond. (mS/cm)	Canopy Cover (%)	Confluences
Buzzard Cr.	7.83	16.98	98.7	2.05	0.421	31.3	3
Calebs Creek	8.06	20.34	79.0	0.00	0.447	19.3	5
Elk Fork S1	7.79	19.34	86.4	0.79	0.451	59.4	2
Elk Fork S2	7.55	19.35	66.7	1.68	0.452	36.7	2
Elk Fork S3	7.52	18.69	86.2	1.13	0.345	35.2	3
Elk Fork S4	7.76	19.25	57.5	4.87	0.493	19.8	3
Passenger Cr.	8.14	20.89	92.6	0.49	0.468	77.9	5

Habitat Assessment Scores

For Epifaunal substrate and Embeddedness, all sites exceeded regional expectations. Several sites had lower scores for Bank Stability, Vegetative Protection, and Riparian Vegetation than the expectations. Calebs and Buzzard creeks had the lowest Riparian Vegetation scores overall. Total scores for all sites exceeded those considered to meet regional guidelines (From Tennessee Department of Environment and Conservation 2017, Table A-1).

Table 2. The 10 criteria (metrics for which right and left bank are measured count as one criterion) for which the seven sampling sites in Todd County, KY and Montgomery and Robertson counties, TN, were scored. Epifaunal = Quality of epifaunal habitat; Embeddedness = Embeddedness of rock substrate within silt, Velocity = flow velocity/depth regimes present, SedimentDep = sediment depositional status, ChannelFlow = Water level within the channel, Channel Alt = degree of alterations to the channel, Reox Zones = frequency of reoxygenation zones in stream, BankStab = stability of bank substrate, VegProtect = Quality and classes of vegetation present, RiparianWidth = Width of riparian zone. "LB" and "RB" denote left and right bank parameters, respectively.

Site	Epifaunal	Embeddedness	Velocity	SedimentDep	ChannelFlow	ChannelAlt	Reox Zones	Bank StabLB	Bank StabRB	Veg ProtectLB	Veg ProtectRB	RiparianWidthLB	RiparianWidthRB	Total
Buzzard Cr	16	13	15	13	19	16	19	4	4	3	4	1	3	130
Calebs Cr	18	13	17	14	18	7	18	5	2	6	1	3	1	123
Elk Fork S1	12	11	12	8	19	20	19	5	2	4	2	10	9	133
Elk Fork S2	13	14	10	6	15	10	16	8	0	3	3	8	9	115
Elk Fork S3	19	19	17	20	20	18	17	8	5	4	7	2	10	166
Elk Fork S4	18	13	14	18	20	9	19	5	3	7	6	10	4	146
Passenger Cr	18	16	15	11	19	10	16	2	5	1	5	1	5	124

Macroinvertebrate Bioassessment

According to TMI values, sites experienced either no or mild impairment of biotic integrity (Table 4). All samples except those from Elk Fork S1 and S2 consisted of over 50% EPT minus *Cheumatopsyche.* Metrics that generally scored lower were %Clingers, %OC, and %EPT. Elk Fork S4 had the highest TMI value, owing to a high abundance of *Glossosoma* caddisfly larvae, a pollution-intolerant taxon. The lowest TMI value was calculated from the Elk Fork S2 sample, with all biometrics showing some impairment. The %OC was highest at Elk Fork Creek S2. The %Clingers-*Cheum* metric was impacted at all sites except Elk Fork S4, again due to *Glossosoma* abundance at that site.

Table 3. The seven biometrics for the seven sampling sites in Todd County, KY and Montgomery and Robertson counties, TN; TR = Taxa Richness, EPT TR = EPT Taxa Richness, %EPT-Cheum = Percent EPT minus *Cheumatopsyche*, %OC = Percent Oligochaetes and Chironomids, %Clingers-Cheum = Percent Clingers minus *Cheumatopsyche*, %TNUTOL = Percent Tennessee Nutrient Tolerant Organisms, and NCBI = North Carolina Biotic Index score.

Site	TR	EPT TR	%EPT-Cheum	%OC	%Clingers-Cheum	%TNUTOL	NCBI
Buzzard Cr	29	7	59.2	17.1	21.7	17.2	5.5
Calebs Cr	23	8	50.9	35.6	11.1	28.7	5.4
Elk Fork S1	20	6	49.6	26.3	24.4	48.7	5.5
Elk Fork S2	22	7	25.3	51.1	18.1	43.2	6.2
Elk Fork S3	18	8	54.8	10.6	58.8	29.6	5.0
Elk Fork S4	19	7	68.2	8.6	63.1	25.3	3.9
Passenger Cr	23	8	50.9	8.0	19.9	28.7	5.1

Table 4. Biocriteria scores assigned to biometric values for the seven sampling sites in Todd County, KY and Montgomery and Robertson counties, TN: TR = Taxa Richness, EPT TR = EPT Taxa Richness, %EPT-Cheum = Percent EPT minus *Cheumatopsyche*, %OC = Percent Oligochaetes and Chironomids, %Clingers-Cheum = Percent Clingers minus *Cheumatopsyche,* %TNUTOL = Percent Tennessee Nutrient Tolerant Organisms, and NCBI = North Carolina Biotic Index score.

Site	TR	EPT TR	%EPT-CHEUM	%OC	%Clingers-Cheum	%TNUTOL	NCBI	TMI
Buzzard Cr	6	4	6	6	2	6	4	34
Calebs Cr	4	4	6	4	0	6	6	30
Elk Fork S1	4	2	6	6	2	4	4	28
Elk Fork S2	4	4	2	4	2	4	4	24
Elk Fork S3	2	4	6	6	4	6	6	34
Elk Fork S4	4	4	6	6	6	6	6	38
Passenger Cr	4	4	6	6	2	6	6	34

Buffer Analysis

For the 60m buffers, all sites were above 70% forested (Fig. 2). Only Elk Fork S1, S2, and S3 were above 90% forested at the 60m buffer width. Calebs Creek was the least forested across all 3 buffer widths and had the greatest overall decrease in present forest cover from 600-200m, a change of 35.1%. Additionally, Calebs Creek had significant reduction of bankside vegetation along much of the measured length attributed to cattle access. On average, sites lost 6.9% of forest cover from 60-100m, 18.1% from 100-200, and 25% overall.

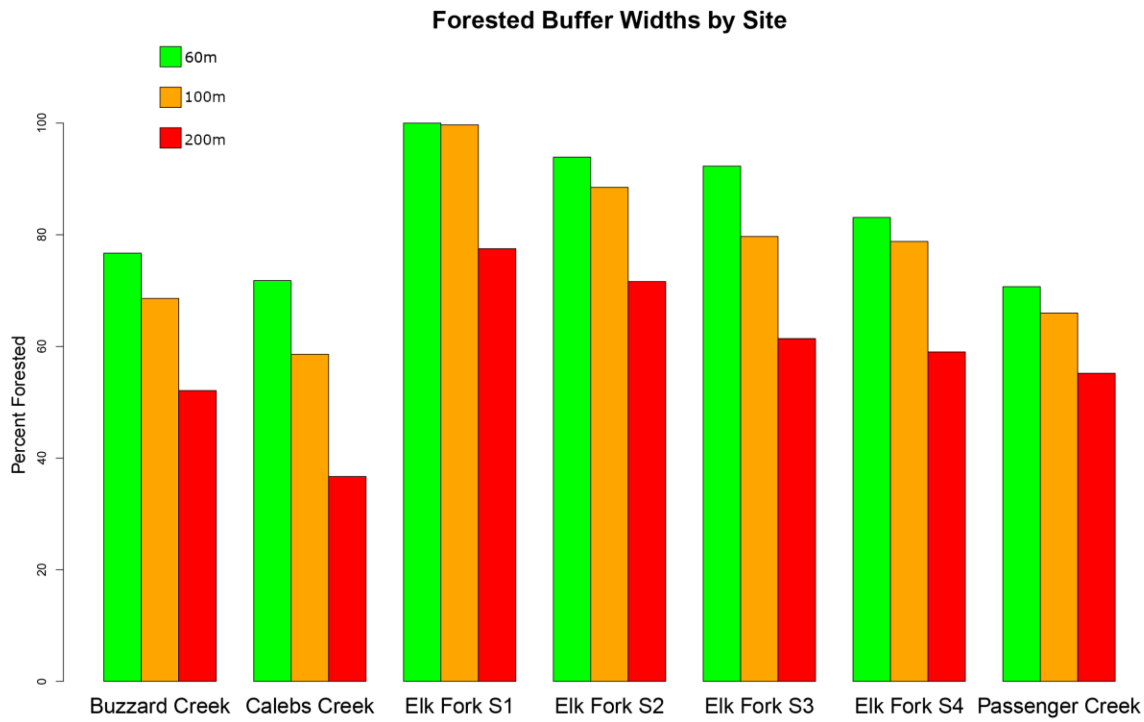

Figure 2. Bar charts of percentages of forested buffer width for sites at 60, 100, and 200m-wide buffers for the seven sampling sites in Todd County, KY and Montgomery and Robertson coutnies, TN.

Watershed Analysis

For all sites, either “Cultivated Crops” or “Hay/Pasture” land use were the most prevalent within the watershed (Table 5). “Deciduous Forest” was the next most common, covering more than a quarter of the Calebs Creek and Passenger Creek catchments. Classes of “Developed” land cover, when summed, were less than 10% at all sites. All Elk Fork catchments were a majority “Cultivated Crops,” where Buzzard, Calebs, and Passenger creeks each had a higher prevalence of “Hay/Pasture.” It should be noted, however, that all Elk Fork sites occur within the same, confluent waterbody, and there is some overlap in the watershed areas drained by each individual reach sampled. All site radii included at least two minor confluences, potential waterthrush nesting habitat, with an average of 3.29 confluences. Calebs and Passenger creeks each had five confluences, the most found in this study. Confluences provide more stream area along which to forage and construct nests, so their presence may be associated with nesting success.

Table 5. Land cover class abundance for the seven sampling sites in Todd County, KY and Montgomery and Robertson coutnies, TN. All values are percentages.

Cover Class	Buzzard Cr	Calebs Cr	Elk Fork S1	Elk Fork S2	Elk Fork S3	Elk Fork S4	Passenger Cr	Average
Cultivated Crops	35.50	8.67	63.58	55.03	65.94	65.7	16.17	44.37
Hay/Pasture	39.14	48.98	14.30	18.88	12.83	12.74	36.19	26.15
Deciduous Forest	17.30	28.54	14.17	17.11	13.24	13.58	30.27	19.17
Developed, Open Space	3.45	5.60	4.36	4.51	4.33	4.3	6.6	4.74
Mixed Forest	3.07	6.29	1.41	1.6	1.38	1.49	7.43	3.24
Developed, Low Intensity	0.62	0.45	0.86	1.14	0.74	0.72	1.98	0.93
Herbaceous	0.31	0.62	0.28	0.42	0.26	0.25	0.33	0.35
Developed, Medium Intensity	0.20	0.15	0.42	0.61	0.34	0.32	0.35	0.34
Open Water	0.10	0.24	0.24	0.21	0.23	0.22	0.22	0.21
Woody Wetlands	0.02	0.09	0.06	0.08	0.4	0.38	0.00	0.15
Shrub/Scrub	0.02	0.17	0.11	0.16	0.11	0.11	0.17	0.12
Evergreen Forest	0.01	0.20	0.10	0.12	0.08	0.07	0.25	0.12
Developed, High Intensity	0.20	0.01	0.09	0.13	0.07	0.07	0.03	0.09
Barren Land	0.04	0.00	0.01	0.01	0.02	0.01	0.00	0.01
Emergent Herbaceous Wetlands	0.00	0.00	0.01	0.00	0.04	0.04	0.00	0.01

CHAPTER IV. DISCUSSION

I attempted to find habitat variables associated with waterthrush occupancy by using a state macroinvertebrate bioassessment protocol coupled with GIS analysis of landscape details. At my sites, overall habitat scores passed guideline values. Tennessee Macroinvertebrate Index scores surpassed target values (Tennessee Department of Environment and Conservation 2017) at four out of seven sites. As waterthrush were found and observed singing on all seven sites examined, I can conclude these sites were acceptable for at least brief occupancy by a male waterthrush.

Physical and chemical data did not reveal impacts of a magnitude surpassing those noted to be of concern as described in the Tennessee Ecoregion Project (Tennessee Department of Environment and Conservation 2000). These metrics are not scored by biocriteria by the Tennessee QSSOP (Tennessee Department of Environment and Conservation 2017) so do not have a "pass/fail" threshold for contributing to habitat quality. These values alone are often not enough to form a basis for describing the functional impairments to a waterbody, as they can fluctuate rapidly depending on rainfall and discharge events, and do not reflect changes to stream morphology or habitat (Karr and Chu 1999). Sensitive taxa require low levels of pollution and their populations will be reduced or absent from more impaired waterbodies (Lenat 1993). Again, factors affecting these communities may not be apparent from the physical and chemical attributes of the water alone – thus other components of the waterbody are assessed by bioassessments used by monitoring agencies.

Habitat assessment scoring revealed many instances of eroded banks and missing classes of vegetation, as scored by the Tennessee Department of Environment and Conservation (2017) biocriteria, which can be attributed in part to surrounding agricultural land use. At all sites, hayfields, cropland, or cattle pastures were visible through the riparian vegetation on at least one side of the stream if the stream itself did not form the border for the agricultural land. All sites exceeded the target value of 113 needed to meet regional guidelines for habitat quality. Since riparian vegetation harbors a variety of terrestrial insects including lepidopterans (butterflies and moths), Araneae (spiders) and emergent insects with aquatic immature forms, the state of the riparian zone can be of importance to riparian-obligate birds and provide a subsidy for other insectivorous forest birds (Trevelline 2016, Trevelline 2019). Complex riparian vegetation can also provide emergence locations for odonates (dragonflies and damselflies) and increase their survivorship (Tavares et al. 2017). Odonates are relatively large-bodied prey that are most available to waterthrush around the time of their emergence, along with other emergent insects (Eaton 1958). The occurrence of bank instability and sloughing may present suboptimal nesting conditions because waterthrush place their nests in concealed locations near overhanging roots and herbaceous vegetation (Eaton 1958, Bent 1963), the or, as described by Mattson and Cooper (2009), destroy existing nests. Additionally, finer scale riparian vegetation assessment, such as percentage and height of shrub and herbaceous cover and ratio of deciduous to coniferous overstory cover (Prosser and Brooks 1998) may offer a more comprehensive depiction of variables important to waterthrush habitation.

The biometric values failing to meet biocriteria guidelines for this ecoregion, and the impacted habitat that caused this, may have impacted the waterthrush's ability to find certain prey items, but since they do not narrowly feed on the most intolerant EPTs (Eaton 1958, Craig 1984), they may prey upon common taxa with higher NCBI values, i.e., *Baetis* (Order Ephemeroptera) or *Cheumatopsyche* (Order Trichoptera). Though EPT abundance is not a direct association to biomass of larger-bodied taxa, the decreased abundance of prey in the waterthrush's favored size class (Craig 1984) may result in less successful instream feeding (Wood 2016). Some sites were above the target TMI of 32, suggesting the lowest impairment levels. However, high TMI values do not mean there has been no impact to the macroinvertebrate community from anthropogenic activity, but only that observed percent compositions of sampled taxa are more-or-less within the TDEC-established tolerances for its biocriteria.

Limitations of using the TDEC bioassessment protocols include that it does not comprise an analysis of biomass and only one microhabitat class (riffles) is sampled for macroinvertebrates. The greater breadth of sampling provided by a "Biorecon" (Tennessee Department of Environment and Conservation 2017) may provide a general assessment of multiple habitats, although it is not comparable to the biocriteria used in the SQKICK method. Biorecon may thus provide a better understanding of macroinvertebrate communities upon which waterthrush feed that are not sampled by the bioassessment using the SQKICK sampling.

Since the purpose of this macroinvertebrate survey is to convey information about aquatic habitat and its biotic integrity, information about riparian habitat important to riparian birds may not be captured. Though waterthrush presence and density has been correlated with habitat quality and biotic integrity, the aquatic prey they take are not solely pollution intolerant and include taxa such as chironomids (Craig 1984) and odonate nymphs (Eaton 1958). Examining the quality of riparian habitat as it relates to the birds that use it requires a finer scale analysis of terrestrial habitat than can be provided by a stream-focused survey. Louisiana Waterthrush-specific habitat suitability indices such as those developed by Prosser and Brooks (1998) and Tirpak et al. (2009) include terrestrial habitat metrics such as vegetation height and composition and landscape characteristics not measured by the TDEC macroinvertebrate survey protocols used here.

Waterthrush occupation did not seem to be impeded by some of the narrower buffer widths observed in this study, and the occasional fragmentation of surrounding forest. The presence of waterthrush at all seven sites despite differences in forested cover available may indicate that wide buffers are not required to sustain at least a single adult bird, if not a pair and potentially nestlings. Other characteristics of the riparian forest or stream morphology may be better determinants of waterthrush occupancy. However, greater riparian width has the advantage of excluding edge-exploiting species such as the Brown-headed Cowbird (*Molothrus ater*) (Stucker and Cuthbert 2000), which has been observed to parasitize waterthrush nests (Eaton 1958). Cowbirds have been seen to frequent forest as far as 350 m from the edge of row crop, feedlot and grassland habitats (Howell et al. 2007), so riparian buffers narrower than this width may expose waterthrush to increasing risk of nest parasitism by cowbirds. Wider forested buffers also have the potential to reduce nest predation (Vander Haegen and Graaf 1996). Other birds stand to benefit from intact riparian zones as well; riparian zones at least 75 m wide have been observed to support all but the most sensitive interior-forest birds in Illinois (Chapman et al. 2015). Forest-dwelling birds besides LOWA have also been found to provision nestlings with emergent aquatic insects (Trevelline et al. 2018), emphasizing the importance of the macrobenthos not only to the waterthrush, but other songbirds as well.

The watersheds containing my sites consisted mostly of either crop, hay, or pasture lands, and there were little or no built-up residential or industrial areas (Table 5). Most developed land was in the form of roads and highways. Though the waterthrush is sometimes categorized as an interior forest bird, these watersheds contained relatively little forest. At the very least, male waterthrush seem to be able to use these sites and set up territories. Occasionally, waterthrush were observed to use housing developments as foraging locations (Hallworth et al. 2014) on their nonbreeding ground; while the riparian zone is an important habitat feature to them, their plasticity in terms of feeding may mean they are able to forage in disturbed areas. However, thin riparian buffers and adjacent fields may harbor nest parasites and predators that decrease the birds' nesting success (Mattson and Cooper 2009). The small number of confluences at some sites could be attributed to the nature of the karst bedrock; that is, more tributaries to the stream could exist below ground. In sites located in the southern margin of the ecoregion, streams may be less typical of the low-relief, lower-gradient streams found mid-Pennyroyal Karst Plain and share more characteristics with the higher-gradient, more incised streams of the Western Highland Rim (Tennessee Department of Environment and Conservation 2005).

The presence of a male does not necessarily translate to female presence, and certainly not to nest success, so that the occurrence of waterthrush at all these sites may not indicate the conditions there were suitable for raising nestlings, only that an adult can occupy these sites and establish territories. Since this study does not formally address territory density, I cannot make inferences about whether the waterthrush observed is nesting. Adult waterthrush may appear to tolerate a range of conditions and give the appearance of thriving despite expanding territories to make up for suboptimal habitat (Wood 2016, Frantz 2019), but whether this translates to nest success is unknown. Waterthrush nestlings, as altricial young, present as the most vulnerable life stage and require considerable effort to raise (Mattson and Cooper 2007). The most important life history component determining reproductive success in these birds is nest survival (the ability of the nest to hatch at least one egg) and the survivorship of the fledglings (Mattson and Cooper 2007), thus the retention of habitat features necessary to support nesting and fledglings is important to maintaining the LOWA populations.

The waterthrush has been characterized as relying on pristine, well-forested streams (Prosser and Brooks 1998), but this study illustrates their use of a different kind of habitat, streams running through intensively farmed areas with interrupted or thin forest buffers, and, in some cases, far less than pristine water quality potentially expected for the focal ecoregion. This suggests waterthrush niche breadth may include a wider variety of stream habitats than suggested by some earlier research, and compounds on later work finding waterthrush in more modified habitats (Hallworth et al. 2011, Mattson and Cooper 2006, Mattson and Cooper 2009). Additionally, my study may add to our understanding of LOWA niche breadth and ability to occupy suboptimal habitat. Because these birds can rely on a variety of aquatic and terrestrial resources to feed (Eaton 1958, Craig 1984, Craig 1987) and appear to expand their territories in response to poor-quality resources (Wood et al 2016), one can infer both riparian and aquatic habitats are important to their survival and reproductive success. Despite finding waterthrush territories along impacted stream reaches, the bird’s presence remains correlated to instream and riparian habitat biotic integrity (Prosser and Brooks 1998, Mattson and Cooper 2006), and thus, a reasonably robust riparian and instream macroinvertebrate community is needed to support their populations.

Future Research Implications

This study attempted to find correlations between waterthrush detectability and habitat quality on an agriculturally impacted landscape, but since waterthrush were found on all study sites, I refrain from making sweeping inferences on what conditions may cause them to be apparently absent from a site based on my findings.

I assessed habitat and macroinvertebrate community characteristics by means described in the TDEC protocols in addition to GIS landscape analysis. The inclusion of other habitat variables in future work – distance from stream, estimated canopy cover, shrub height, landscape classification (Prosser and Brooks 1998, Tirpak et al 2009), for instance – may result in a more nuanced characterization of waterthrush-occupied areas in this ecoregion. Stucker and Cuthbert (2000) use macroinvertebrates observed to be important food items for the LOWA to form indexes based on Eaton's (1958) and Craig's (1987) conclusions, as well as findings collected by Robinson in *The Birds of North America* (1995). These, coupled with the multiple-habitat approach of Biorecon assessments (Tennessee Department of Environment and Conservation 2017) provide a suite of information about multiple instream and riparian habitat variables that can be applied to future habitat suitability investigations.

Further research is needed to determine nesting success of the Louisiana Waterthrush in the Western Pennyroyal Karst Plain ecoregion, and more sites should be included to encompass a wider gradient of disturbance conditions in order to find conditions that waterthrush will not occupy. Waterthrush individual versus pair presence can yield information about habitat quality that may not be resolved with presence-only determinations as pair presence, together with a visual habitat analysis has a closer relationship to instream biotic integrity than male-only presence (Mattson and Cooper 2006). Also, females have been observed to abandon disturbed sites (Frantz 2019). A more detailed analysis of nest success within agriculturally-impacted areas is needed to ascertain whether waterthrush can fledge young within reaches subject to riparian deforestation and cattle access. Despite their utility as an “early warning system” (Mattsson and Cooper 2006) for disturbances to stream biotic integrity, there are still many gaps in our knowledge about Louisiana Waterthrush ecology within the Pennyroyal Plain and agriculture-dominated areas in general, a habitat in which they are able to breed and fledge young despite the land use patterns surrounding its streams.

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APPENDIX

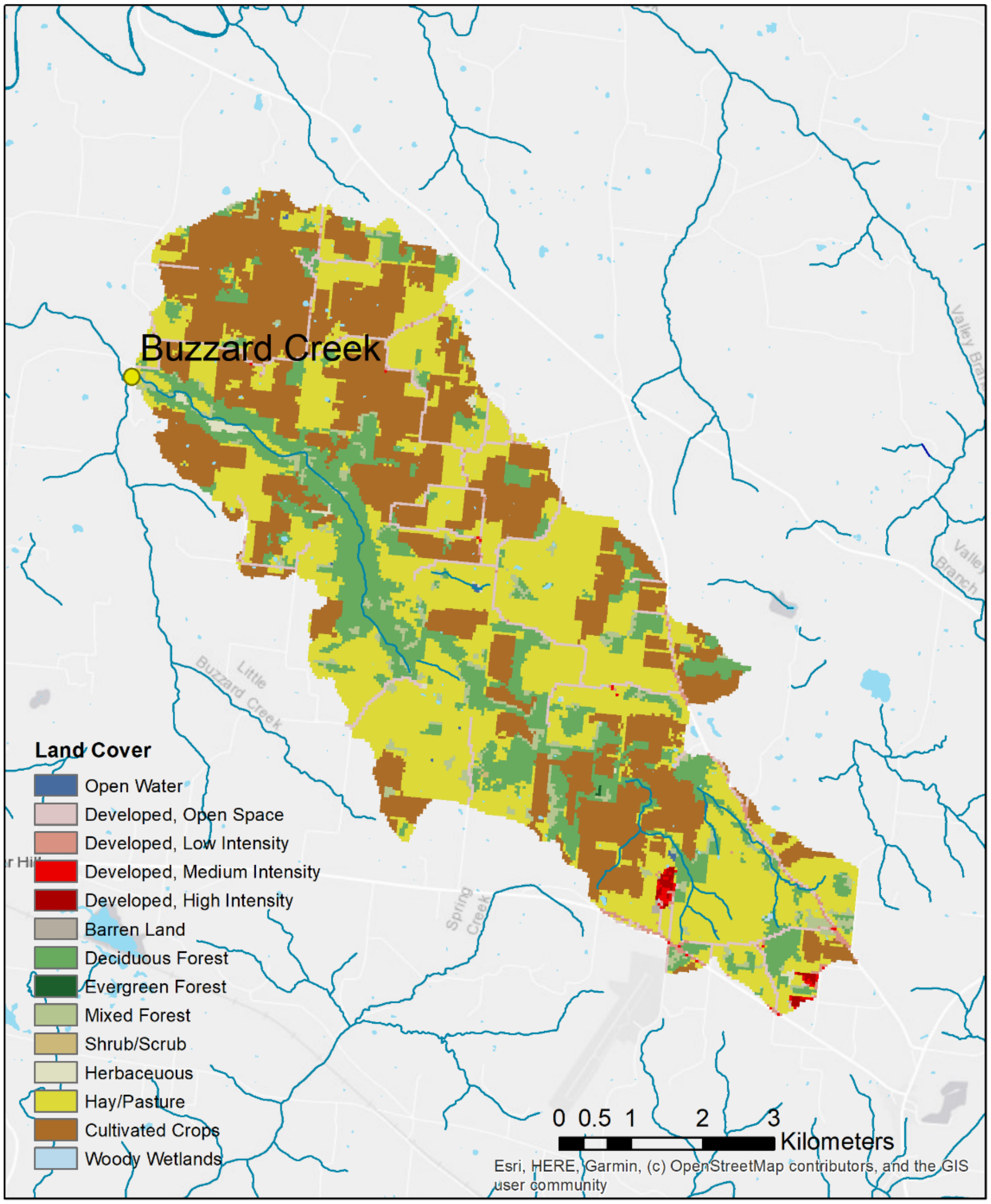

Figure A-1. Buzzard Creek watershed upstream of sampling location in Robertson County, Tennessee. Land cover classes from National Land Cover Database are shown for the watershed.

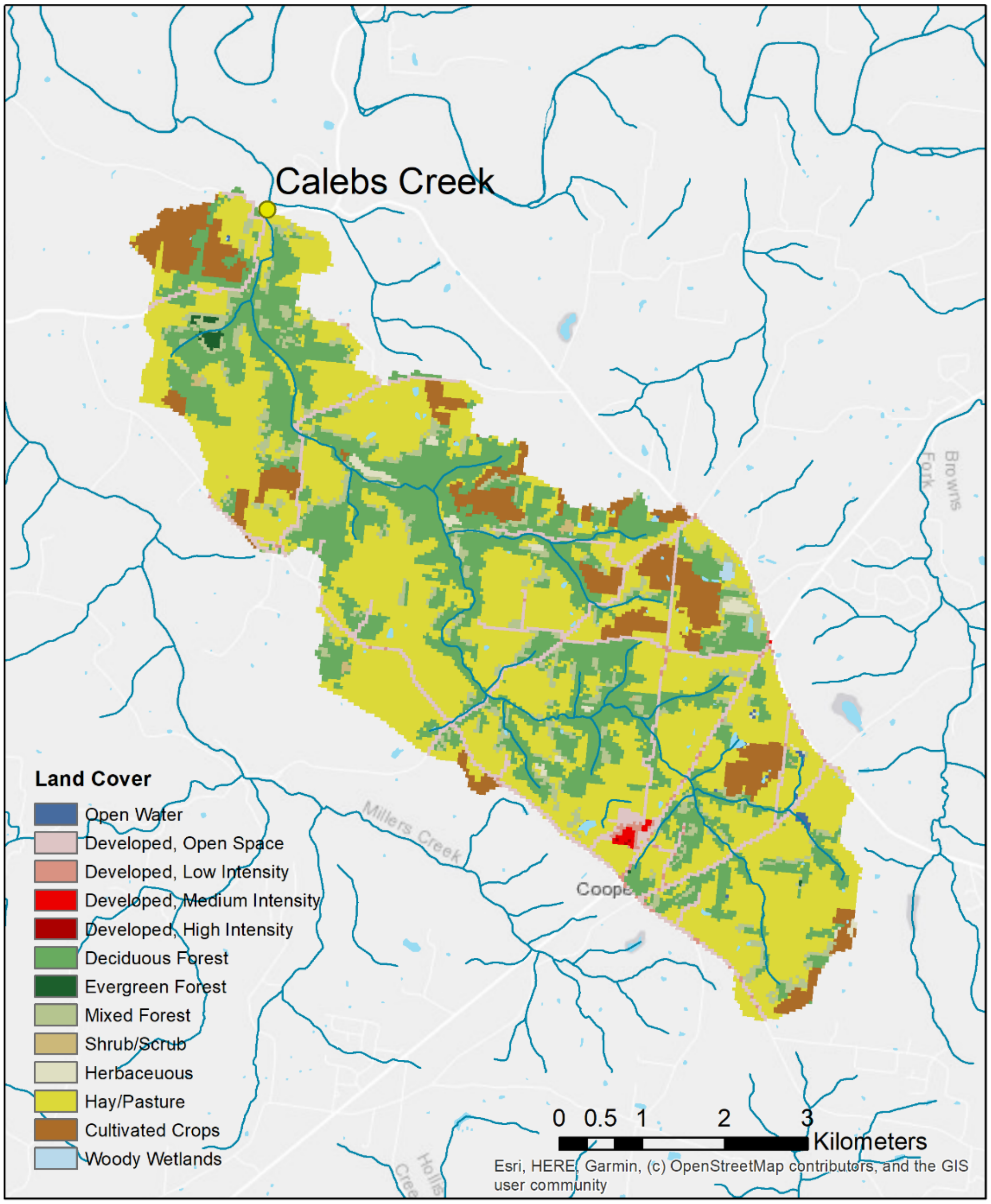

Figure A-2. Calebs Creek watershed upstream of sampling location in Robertson Co., Tennessee. Land cover classes from National Land Cover Database are shown for the watershed.

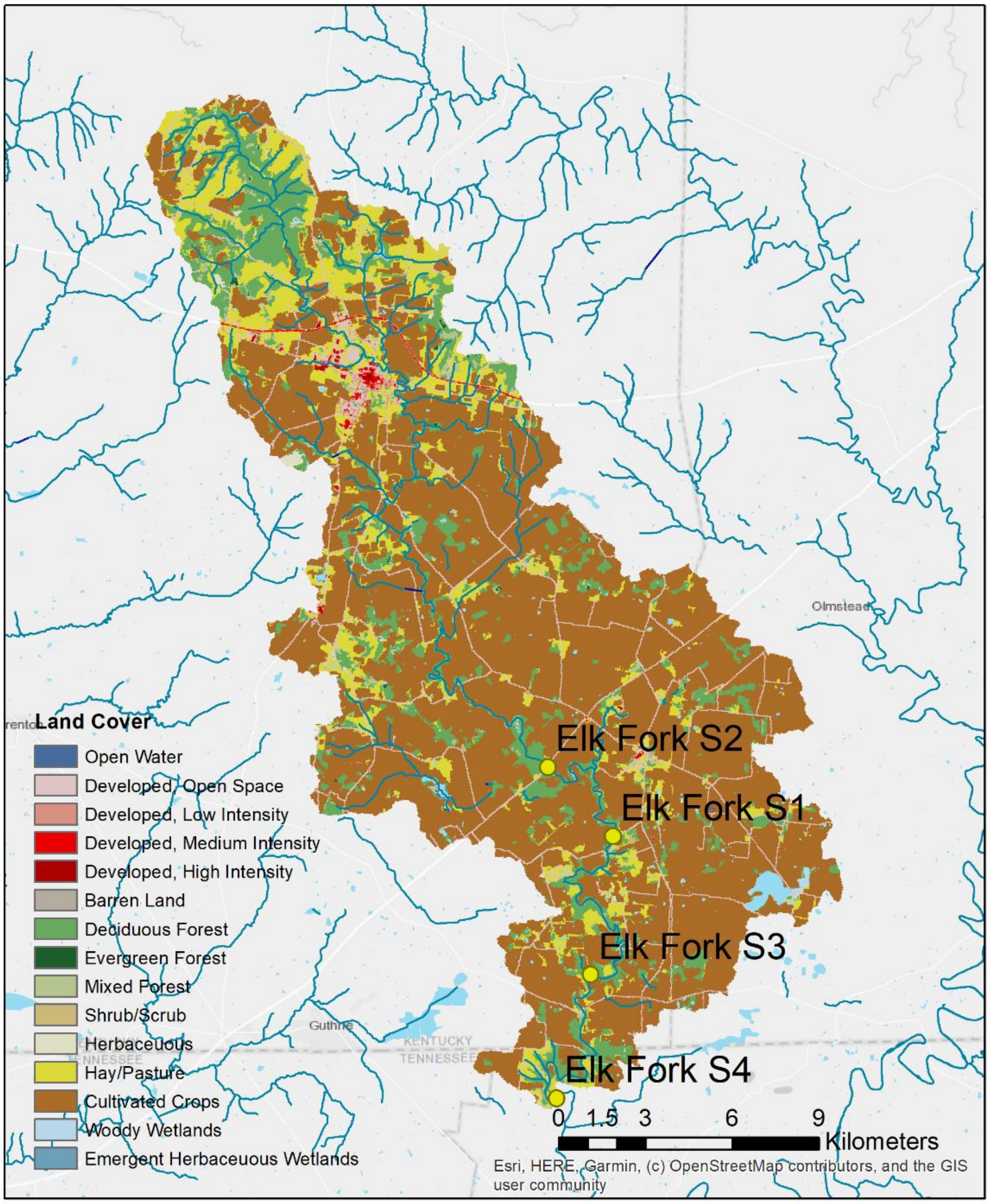

Figure A-3. Elk Fork watershed upstream of sampling location in Todd Co., Kentucky. Land cover classes from National Land Cover Database are shown for the watershed.

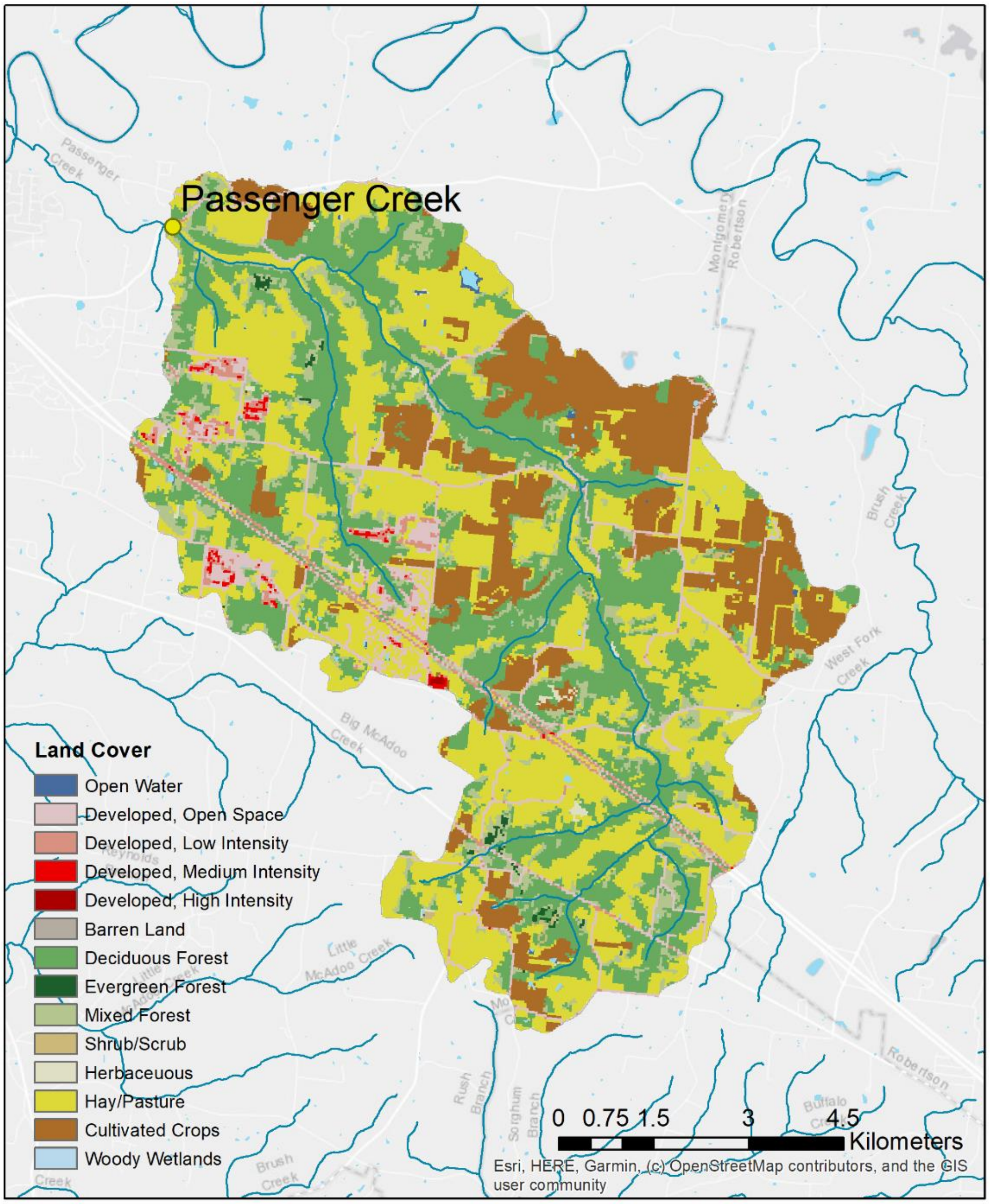

Figure A-4. Passenger Creek watershed upstream of sampling location in Montgomery County, Tennessee. Land cover classes from National Land Cover Database are shown for the watershed.

Table A-1. Expected scores for habitat values for Ecoregion 71e streams draining an area larger than 6.47 km2. Spring is January-June, and fall is July-December. Total scores for values considered to meet regional guidelines are also listed. Adapted from TDEC QSSOP for Macroinvertebrate Surveys (2017).

	Substrate	Embeddedness	Channel Substrate	Velocity/Depth	Pool Variability	Sediment Deposition	Flow Status	Channel Alteration	Riffle Frequency	Channel Sinuosity	Bank Stability	Vegetative Protection	Riparian Vegetation	Totals
Spring	12	11	N/A	14	NA	11	14	12	13	NA	5	5	4	113
Fall	11	10	NA	11	NA	10	13	12	12	NA	4	4	4	114

Table A-2. Scores given to biometrics falling within designated values, as calibrated to Ecoregion 71e. Reproduced from TDEC QSSOP for Macroinvertebrate Surveys (2017).

Bioregion 71e Season: January - June Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage: > 2.5 square miles Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 27	19 – 27	9 – 18	< 9
EPT Richness (EPT)	> 10	7– 10	4 – 6	< 4
% EPT-Cheum	> 38.6	25.8 – 38.6	12.9 – 25.7	< 12.9
% OC	< 30.0	30.0– 53.3	53.4 – 76.6	> 76.6
NCBI	< 5.50	5.50 – 6.99	7.00 – 8.50	> 8.50
% Clingers-Cheum	> 48.2	32.2 – 48.2	16.1 – 32.1	< 16.1
% TNutol	< 37.8	37.8 – 58.5	58.6 – 79.2	> 79.2

Table A-3. Scores given to biometrics falling within designated values, as calibrated to Ecoregion 71e. Reproduced from TDEC QSSOP for Macroinvertebrate Surveys (2017).

Bioregion 71e Season: July-December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage: > 2.5 square miles Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 24	17 – 24	8 – 16	< 8
EPT Richness (EPT)	> 7	6 – 7	3 – 5	< 3
% EPT-Cheum	> 44.9	30.0 – 44.9	15.0 – 29.9	< 15.0
% OC	< 26.0	26.0– 50.6	50.7 – 75.3	> 75.3
NCBI	< 5.53	5.53 – 7.01	7.02 – 8.51	> 8.51
% Clingers-Cheum	> 49.6	33.1 – 49.6	16.6 – 33.0	< 16.6
% TNutol	< 38.2	38.2 – 58.7	58.8 – 79.4	> 79.4

Figure A-5. Front page of habitat assessment sheet for moderate to high-gradient streams, used for scoring stream and riparian characteristics. Reproduced from TDEC QSSOP for Macroinvertebrate Surveys (2017).

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HABITAT ASSESSMENT FIELD SHEET- MODERATE TO HIGH GRADIENT STREAMS (FRONT)
(See Protocol E for detailed descriptions and rank information)

DWR Station ID:		**Habitat Assessment By:**	
Monitoring Location Name:		**Date:**	**Time:**
Monitoring Location:		**Field Log Number:**	
HUC:	WS Group:	Ecoregion:	QC: ☐ Duplicate ☐ Consensus

	Optimal	**Suboptimal**	**Marginal**	**Poor**
1. Epifaunal Substrate/ Available Cover	Over 70% of stream reach has natural stable habitat suitable for colonization by fish and/or macroinvertebrates. Four or more productive habitats are present.	Natural stable habitat covers 40-70% of stream reach. Three or more productive habitats present. (If near 70% and more than 3 go to optimal.)	Natural stable habitat covers 20 -40% of stream reach or only 1-2 productive habitats present. (If near 40% and more than 2 go to suboptimal.)	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
2.Embeddedness of Riffles	Gravel, cobble, and boulders 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space. If near 25% drop to suboptimal if riffle not layered cobble.	Gravel, cobble and boulders 25-50% surrounded by fine sediment. Niches in bottom layers of cobble compromised. If near 50% & riffles not layered cobble drop to marginal.	Gravel, cobble, and boulder s are 50-75% surrounded by fine sediment. Niche space in middle layers of cobble is starting to fill with fine sediment.	Gravel, cobble, and boulders are more than 75% surrounded by fine sediment. Niche space is reduced to a single layer or is absent.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
3. Velocity/ Depth Regime	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow).	Only 3 of the 4 regimes present (if fast-shallow is missing score lower). If slow-deep missing score 15.	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/depth regime. Others regimes too small or infrequent to support aquatic populations.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
4. Sediment Deposition	Sediment deposition affects less than 5% of stream bottom in quiet areas. New deposition on islands and point bars is absent or minimal.	Sediment deposition affects 5-30% of stream bottom. Slight deposition in pool or slow areas. Some new deposition on islands and point bars. Move to marginal if build-up approaches 30%.	Sediment deposition affects 30-50% of stream bottom. Sediment deposits at obstruction, constrictions and bends. Moderate pool deposition.	Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
5. Channel Flow Status. .	Water reaches base of both lower banks and streambed is covered by water throughout reach. Minimal productive habitat is exposed.	Water covers > 75% of streambed or 25% of productive habitat is exposed.	Water covers 25-75% of streambed and/or productive habitat is mostly exposed.	Very little water in channel and mostly present as standing pools. Little or no productive habitat due to lack of water.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				

Figure A-6. Back page of habitat assessment sheet for moderate to high-gradient streams, used for scoring stream and riparian characteristics. Reproduced from TDEC QSSOP for Macroinvertebrate Surveys (2017).

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HABITAT ASSESSMENT FIELD SHEET- MODERATE TO HIGH GRADIENT STREAMS (BACK)

DWR Station ID________________ Date______ Assessors____________

	Optimal	**Suboptimal**	**Marginal**	**Poor**
6. Channel Alteration	Channelization, dredging rock removal or 4-wheel activity (past or present) absent or minimal; natural meander pattern. NO artificial structures in reach. Upstream or downstream structures do not affect reach.	Channelization, dredging or 4-wheel activity up to 40%. Channel has stabilized. If larger reach, channelization is historic and stable. Artificial structures in or out of reach do not affect natural flow patterns.	Channelization, dredging or 4-wheel activity 40-80% (or less that has not stabilized.) Artificial structures in or out of reach may have slight affect.	Over 80% of reach channelized, dredged or affected by 4-wheelers. Instream habitat greatly altered or removed. Artificial structures have greatly affected flow pattern.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
7. Frequency of re-oxygenation zones. Use frequency of riffle or bends for category. Rank by quality.	Occurrence of re-oxygenation zones relatively frequent; ratio of distance between areas divided by average stream width <7:1.	Occurrence of re-oxygenation zones infrequent; distance between areas divided by average stream width is 7 - 15.	Occasional re-oxygenation area. The distance between areas divided by average stream width is over 15 and up to 25.	Generally all flat water or flat bedrock; little opportunity for re-oxygenation. Distance between areas divided by average stream width >25.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
8. Bank Stability (score each bank) Determine left or right side by facing downstream.	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion. If approaching 30% score marginal if banks steep.	Moderately unstable; 30-60 % of bank in reach has areas of erosion; high erosion potential during floods, If approaching 60% score poor if banks steep.	Unstable; many eroded area; raw areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
Comments				
9. Vegetative Protective (score each bank) includes vegetation from top of bank to base of bank. Determine left or right side by facing downstream	More than 90% of the bank covered by undisturbed vegetation. All 4 classes (mature trees, understory trees, shrubs, groundcover) are represented and allowed to grow naturally. All plants are native.	70-90% of the bank covered by undisturbed vegetation. One class may not be well represented. Disruption evident but not effecting full plant growth. Non-natives are rare (< 30%)	50-70% of the bank covered by undisturbed vegetation. Two classes of vegetation may not be well represented. Non-native vegetation may be common (30-50%).	Less than 50% of the bank covered by undisturbed vegetation or more than 2 classes are not well represented or most vegetation has been cropped. Non-native vegetation may dominate (> 50%)
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
Comments				
10. Riparian Vegetative Zone Width (score each bank.) Zone begins at top of bank.	Average width of riparian zone > 18 meters. Unpaved footpaths may score 9 if run-off potential is negligible.	Average width of riparian zone 12-18 meters. Score high if areas < 18 meters are small or are minimally disturbed.	Average width of riparian zone 6-11 meters. Score high if areas less than 12 meters are small or are minimally disturbed.	Average width of riparian zone <6 meters. Score high if areas less than 6 meters are small or are minimally disturbed.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
Comments				

Total Score ________ Comparison to Ecoregion Guidelines (circle): ABOVE or BELOW
If score is below guidelines , result of (circle): Natural Conditions or Human Disturbance
Describe: