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Habitat Selection and Partitioning Among Darters in Two Tributaries of the Clinch River, and Stream Restoration Effects on Substrate Profile

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To the Graduate Council:

I am submitting herewith a thesis written by Daniel James Walker entitled "Habitat Selection and Partitioning Among Darters in Two Tributaries of the Clinch River, and Stream Restoration Effects on Substrate Profile." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

J. Larry Wilson, Major Professor

We have read this thesis and recommend its acceptance:

Arnold Saxton, Raymond Albright

Accepted for the Council:
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Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Habitat Selection and Partitioning Among Darters in Two Tributaries of the Clinch River, and Stream Restoration Effects on Substrate Profile

A Thesis Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Daniel James Walker

May 2014

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Abstract

The group of fish referred to as darters is extremely biodiverse. Often, several closely related species will inhabit the same areas of streams, and prior research has investigated how these species may be partitioning the resources in low-order streams in which they are sympatric. The habitat partitioning of darters in two streams in the Clinch River system, Coal and Cove Creeks, was investigated. The study sites were picked due to their involvement in both physical and biological stream restoration efforts within the last several years, including the collection, translocation, and reintroduction of rainbow darters (*Etheostoma caeruleum*) from reference sites in Cove Creek to the impacted and restored sites in Coal Creek. Snorkel surveys were used to identify adult darters and mark their locations, and the substrate in the darters' immediate surroundings was quantified. These measurements were compared to transect survey data to assess whether darters were selecting for habitat, and then compared among the subpopulations of darters encountered. The numbers of adult redline (*Nothonotus ruffilineatum*), snubnose (*E. simoterum*), and rainbow darters encountered were great enough to proceed with analyses of habitat usage. It was determined that darters were selecting for habitat nonrandomly, and that all three of the species most encountered associated with coarse rocky substrate. Differences in habitat associations among species appeared to be driven by the environmental factors each species encountered at the site scale.

In a secondary study, effects of stream restoration were assessed by comparing substrate profiles of the sites where darter surveys were conducted. Each site was classified as either natural, impacted, or restored. Then, the collected substrate data from transect surveys at each site were compared. The substrate profile of the most upstream site in Coal

Creek, a site that has undergone stream restoration efforts, most closely resembled those of the ‘natural’ reference sites in Cove Creek. Substrate profiles of the more downstream sites of Coal Creek did not resemble those of the reference sites as closely, regardless of stream restoration effort.

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CHAPER I: INTRODUCTION

During the international Convention on Biological Diversity in 2002, world leaders committed to reducing the rate of global biodiversity loss significantly by the year 2010. However, Butchart et al. (2010) found that when 31 various indicators of biodiversity were assessed eight years later, nearly all showed evidence of further decline. The indicators of biodiversity the authors examined included extinction risk, habitat extent and composition, and community structure. Moreover, indicators of pressures on global biodiversity, such as human resource consumption, spread of invasive alien species, nitrogen pollution, and climate change, displayed evidence of increasing rates. It seems that eight years following the international agreement and subsequent endorsement by the United Nations, little change had occurred on a global scale in spite of some successes at the local scale, and indeed the planet appeared to be continually suffering a widespread loss of biodiversity.

Anthropogenic change to the environment can lead to extensive declines in one of the important indicators of environmental health: aquatic biodiversity. Clausen and York (2008) found that there was a strong correlation between increasing regional economic development and loss of aquatic environmental health. Various factors have been studied to assess the mechanisms driving changes to watersheds and their associated biodiversity that correlate with increasing human activity in catchment areas. Newcombe and MacDonald (1991) reviewed several studies of the impacts of increased suspended sediment exposure on various aquatic organisms. They found that there

was an apparently robust, general trend of biodiversity loss associated with exposure to suspended sediments. Angradi (1999) found that as fine sediment (≤ 2 mm) loads were experimentally increased in Appalachian streams in West Virginia, the Ephemeroptera-Plecoptera-Tricoptera (EPT) benthic macroinvertebrate indicator taxa group utilized as an indicator of aquatic health declined significantly. These results were evident even in treatments of river substrate with very low (≤ 10%) additions of fine sediment. Though sedimentation is a naturally occurring phenomenon in river systems, various human activities worldwide can have variable impacts on the fine sediment loads of nearby lotic systems. Wood and Armitage (1997) found that there was a strong correlation between human activities in watersheds and increased fine sedimentation, leading to a loss of aquatic flora and fauna. These authors listed agriculture as a major source of fine sedimentation, as it demands land clearing, which speeds sediment transport from the terrestrial system to the aquatic.

Diamond et al. (2002) found a very strong negative correlation between human land use in the catchments of the Clinch and Powell Rivers, Tennessee, and various indicators of aquatic biological health (e.g., fish species complex IBI, the EPT indicator benthic macroinvertebrates, and mussel species richness). These findings are important because the Clinch River watershed is a regional biodiversity hotspot: water quality in this area has been historically classified as very high, and the Clinch River has been identified as retaining outstanding aquatic biodiversity especially in the upper reaches where it remains one of the last free flowing rivers on the eastern coast of the United States (Brahana et al. 1986; Jenkins & Burkhead 1994). Streams in this drainage in

Tennessee are thought to be the only refuges for some endemic darter species (Percidae), where in other regions these same fish are much more successful (Etnier & Starnes 1993). Darters, small benthic species of fish from several genera, are unique among other local fishes in that they have very rapidly diversified (*Etheostoma* is considered the most speciose genus of freshwater fish in North America) and that most species have developed very advanced methods of reproduction, including specific site-selection for egg deposition and the displays of brilliant coloration by males of some of the species (Etnier & Starnes 1993). The state of Tennessee is home to 90 species of darter, making this group of fish the most diverse group in the state.

The Coal Creek Watershed Foundation (CCWF) is an activist group organized in 2000 with the purpose of improving the quality of the Coal Creek watershed, a 4th order tributary of the Clinch River in Anderson County, Tennessee. This group organized both physical and biological restoration efforts within the Coal Creek watershed. The Tennessee Valley Authority (TVA) conducted periodic sampling of the fish community found in Coal Creek. The darter species found in these surveys included the greenside darter (*Etheostoma blennioides*), the redline darter (*Nothonotus ruffilineatum*), the snubnose darter (*E*. *simoterum*) (Harrington & Near 2012), the stripetail darter (*E. kennicotti*), the logperch (*Percina* caprodes) and the blueside darter (*E. jessiae*). In conjunction with the CCWF and TVA, Schiding (2009) conducted fish reintroductions to restore species thought to be historically present in Coal Creek but missing from those TVA surveys, taking individuals from five species of nongame fishes, including the rainbow darter (*E. caeruleum*), from Cove Creek and releasing them into the Coal Creek

system.

The Coal Creek and Cove Creek watershed dichotomy provided a unique research opportunity. The Coal Creek system has undergone both physical and biological restoration efforts. Additionally, the Cove Creek system provided a natural system to use as a comparison to Coal Creek. The ecological interactions among the darter species that reside in these two watersheds could be compared. Furthermore, this system of neighboring watersheds was well suited to investigate the effects of biological and physical stream restoration effects on the Coal Creek system, and the ecological interactions of darters. The following study was conducted to achieve these specific objectives:

1) determine whether darter habitat usage was nonrandom;
2) determine species habitat selection;
3) assess how reintroduction of rainbow darters affected their habitat selection; and
4) determine the effects of stream restoration efforts on the substrate profiles of the streams.

CHAPTER II: LITERATURE REVIEW

Darter Ecology Research

Several predominant themes emerge in the literature regarding the study of darter ecology and interactions among sympatric populations of darter species. These major themes usually fall into one of the following general categories:

1) there is often a great deal of overlap in the ecological niche of various species in darter communities;
2) morphological differences among darter species may explain ecological differences; or
3) darter habitat selections appear to be tied closely to prey availability, depth, current velocity, and substrate type.

Additionally, less prominent themes can be found underlying many of the studies on darter ecology. Primarily, for every group of researchers investigating darter microhabitat use, it appears that there is a corresponding unique method for quantifying the substrate surrounding the darters, usually involving some sort of estimation when categorizing substrate particles. These themes reoccur throughout several decades of research on the ecology of darters

Prior to the publication by Matthews et al. (1982), the bulk of the literature on darters revolved around basic biological and life history descriptions, but little research had been published which quantified the ecological niche relationships of syntopic darters. In their study, the authors investigated the partitioning of diet and microhabitat

among *Etheostoma flabellare* (fantail darter), *E. podostemone* (riverweed darter), and *Percina roanoka* (Roanoke darter) in the Upper Roanoke River Drainage, Virginia. To quantify microhabitat use, researchers visually estimated the predominant substrate diameter to the nearest 2.5 cm in 2-m^2 areas of riffle for kick-seine collections, estimated dominant substrate type in mesohabitat sections for electrofishing collections, and then collected darters in each respective area. To assess diet, the authors sacrificed darters collected from two sample locations, removed the digestive tract of the sacrificed individuals, and identified the stomach contents to the lowest feasible taxon. Their results highlighted the first of the themes to be found throughout the literature, that is, they found only partial ecological segregation of these species in sympatry, with strong overlap among the three species in their overall use of resources. Furthermore, they described the riverweed darter as a trophic specialist, feeding on larvae of Chironomidae almost exclusively (over 80% of its diet); alternatively, the Roanoke and fantail darters displayed more generalist feeding approaches. Even then, the distinction was subtle, as chironomids consistently ranked as either the primary or secondary source of prey for all three species across all seasons.

To quantify microhabitat use, Matthews et al. (1982) created a substrate-size-by-current-velocity metric. With this metric, there was again more overlap among species, ranging from the lowest similarity of 78.4% overlap to over 90% in species-pair assessments. Only by combining current speed and prey size into one metric did significant differences among some of the species become apparent: the riverweed darter utilized the smallest foods and slowest microhabitats, and the Roanoke darter

preferred the swiftest current velocities. The authors concluded that these differences could be attributed to morphological differences among species, and most importantly gape width and body depth.

In contrast to the murky ecological separation presented by Matthews et al. (1982), Paine et al. (1982) presented more definitive evidence of ecological partitioning among four darter species in Irvine Creek, Wellington County, Ontario. This study assessed the resource partitioning of *E. microperca* (least darter), *E. nigrum* (Johnny darter), *E. flabellare* (fantail darter), and the *E. caeruleum* (rainbow darter). In this system, the four species were found to segregate themselves initially by habitat and then by food utilization. The researchers first categorized mesohabitat as riffle or weed bed, then collected darters using kick-seines in the riffles and run-seines through the weed beds.

Ten to fifteen individuals collected in each sampling effort were sacrificed for diet assessment. Importantly, where Matthews et al. (1982) had suggested that the differences in darter resource use may be linked to morphological traits, Paine et al. (1982) performed analyses of nine basic morphological characteristics on the individuals sacrificed for diet assessment. The characteristics analyzed were related to body form (including relative caudal peduncle length, relative pectoral fin length, and 'flatness' or lateral compression index) and mouth structure (relative mouth height, mouth position, and snout protrusibility). The authors found that the rainbow and fantail darters were most numerous in the riffles, while the Johnny and least darters were mostly found in weed beds. Interestingly, while fantail and rainbow darters were fairly

ubiquitous in both habitat types, the Johnny and least darters were rare or absent from riffles. The division of habitat among the four species was explained as containing one larger darter species (the rainbow and the Johnny darter) and one smaller species (the fantail and the least darter), respectively.

Within each habitat type, the macroinvertebrate prey base was partitioned among the two species present, but Paine et al. (1982) stated that this partitioning was likely due to morphological differences between the two species dominant in each habitat type. In both cases, one species had a subterminal mouth suitable for feeding on prey below the fish (the Johnny darter in the weed beds and the rainbow darter in the riffles), and the other had a smaller body and was better able to pursue swifter prey in tighter spaces (the least darter chasing more motile prey in and on plants in the weed bed, and the fantail darter pursuing prey above and around it in crevices in the riffle substrate).

The contents of the diet of each species appeared to back up these conclusions. For the weed bed species, the Johnny darter fed mostly on *Caenis*, a sprawling macroinvertebrate, and tube-dwelling Chironomini, while the least darter fed on macroinvertebrates such as Baetidae nymphs and Tanytarsini that clung to plant stalks. In riffle species, the diet of rainbow darters consisted mostly of macroinvertebrates such as Tanytarsini, Orthocladiinae, and Hydroptilidae, that were found on the tops of substrate particles, while the fantail darter diet was comprised of macroinvertebrates such as Chironomini, Diamesinae, and Ephemeroptera, that sprawled on or burrowed among the substrate particles. The clear division among these species in habitat use appeared to be driven by morphology and diet, but the authors could not state

conclusively which of the ecological characteristics was primary over the others.

The two riffle species, rainbow and fantail darters, were the focus of a more intense investigation of interspecific interaction by Schlosser and Toth (1984). These species had been shown to share the same general mesohabitat, and had generally overlapping diets that appeared to be partitioned by the habitat of the prey species. The study included field observations by snorkel survey of the behavior of these darters and experimental habitat preference tests. Direct observation of the darters *in situ* suggested that the rainbow darter foraged for prey along the top surface of substrate particles, while the fantail darter foraged among particles in crevices. This conclusion was further substantiated by the experimental data: rainbow darters demonstrated preference for particles with sizes >50 mm in diameter, and fantail darters preferred crevices >25 mm wide. Moreover, no significant differences in habitat preference occurred with the addition of congeneric competition. Again, the larger-bodied rainbow darter remained above the substrate, while the more flexible fantail darter remained within the rock crevices; the authors credit the fantail darter's smaller scale size and shallower body depth for allowing it to exploit the rock crevices. Additionally, the authors assessed how the darters reacted to changing scarcity of food resources. They found that in times of scarcity, food resource partitioning decreased, while during seasons of plentiful prey these two species utilized prey bases that more clearly differentiated from one another.

The preceding studies examined prey utilization in some detail, while only investigating habitat use in a cursory manner. All habitat quantification was generalized

into broad categories. In each case, some difference in prey utilization was attributed to morphological differences in darter species, but there were few hard conclusions drawn as the overlap in macroinvertebrate species that make up the diets of sympatric species was too great.

Chipps et al. (1994) focused their efforts on more rigorous quantification of the physical attributes of darter microhabitat use. Snorkel surveys were used to document the locations of four species of darter in the Kanawha River system of Virginia and West Virginia: *E. osburni* (candy darter, now the finescale saddled darter), *E. blennioides* (greenside darter), *P. gymnocephala* (Appalachian darter), and the fantail darter. To quantify microhabitat use by these darters, the authors used snorkel surveys to survey reaches of several streams, marking the location of observed darters with numbered flagging attached to lead weights. Snorkelers recorded the species of the darter and its corresponding flag number on writing slates attached to their wrists. This method proved to be minimally invasive to fish being observed, as the behavior of darters appeared undisturbed by the observers.

After snorkeling, the researchers measured depth and current velocity at each darter location, then quantified habitat by placing a 1-m^2 grid, divided into 20-cm^2 boxes, onto the stream bed and centered over each marker onto the substrate. The dominant substrate in each square was recorded, classified by Wentworth scale category. The benefit to this method was that it removed some bias from the substrate quantification. Prior studies had involved approximating the dominant substrate to certain measurements visually, while this method standardized that procedure to an extent by

utilizing the Wentworth scale to classify substrate. The results indicated a complex pattern of segregation among different axes—substrate type, depth, or velocity, depending on the species being compared. Again, the fantail darter was found to prefer crevices relative to the more deeply-bodied and less flexible finescale saddled darter, much like the relationship found with the rainbow darter in Schlosser and Toth (1984). The Appalachian darter was found to segregate from species of *Etheostoma* along a depth gradient, preferring deeper pools and runs; the *Percina* species also utilized more of the water column and was not restricted to the benthos, likely due to its larger size, cryptic coloration, and better swimming ability.

The separation of *Percina* species from *Etheostoma* species within the same community along a gradient defined by depth is mirrored in the findings of Stauffer et al. (1996). The *Percina* species in the darter community under study, *P. caprodes* (logperch) and *P. copelandi* (channel darter), were differentiated by their preference for deeper water. This study differed from the previously discussed studies in that there was a larger scope of its analysis of the ecological interactions among 11 species of darters in the Allegheny River, Pennsylvania. The results indicated that while overlapping ecological niches again defined the community, certain species were significantly different in their ecology. Of particular interest was the finding that during the month of June, the rainbow darter utilized habitat that was indistinguishable from the habitat of the greenside darter, and the rainbow darter used habitat with significantly lower velocities than *E. zonale* (banded darter) and *E. variatum* (variegate darter). In September, habitat characteristics of the rainbow darter were similar to those of the

three other species. In July, however, rainbow darters utilized significantly different habitat from all three species.

To quantify the habitat use of darters, the authors introduced another method for quantifying darter microhabitat use: following location and identification by snorkel survey, researchers placed a 0.25-m^2 acrylic sheet on the river bed. The sheet was divided into 5-cm^2 grids. Particles that covered more than 50% of each grid were considered the dominant substrate, and approximate measurements of the size of individual substrate particles were obtained by counting the number of grids that they covered. These measurements were then fit into another classification scheme, this time with four more broadly-defined categories than the Wentworth scale used by Chipps et al. (1994).

The effect of water current velocity on habitat selection by rainbow darters was further investigated by Harding et al. (1998). Stauffer et al. (1995) mentioned their inability to precisely quantify the velocity of water at the smallest scales around substrate particles, and instead relied on measurements of velocity along the surface of the substrate. Harding et al. (1998) quantified both the water velocity in the microhabitats utilized by rainbow darters as well as control velocity measurements taken from microhabitats not associated with rainbow darter presence. Velocity measurements were conducted using a hot bead thermistor probe, which recorded voltage changes generated by the flowing water and compared them to the voltage produced by known water velocities taken from two reference sites of laminar flow. The small size of this probe allowed researchers to measure water velocity at the exact

location of the observed darters, as well as in the middle of the water column and at the surface. These measurements were compared to measurements taken along transects that included sampling points representative of various habitat types in the stream reach.

The results of this method indicated that the locations of the rainbow darters were consistently in areas of lower water velocities, averaging around 3-5 cm-s^{-1} slower than the surrounding habitat, both at the microscale (relative to the other two measurements taken in the water column at each darter location) and the macroscale (the aggregated measurements taken at the transect points not associated with darter observations). The study also suggested that coarse water velocity measurements at the macroscale may not accurately represent the flow regime experienced by rainbow darters (or other benthic biota) at the microscale. Given the many descriptions of rainbow darters feeding along the top of substrate particle surfaces, the utilization of velocity shelters was likely tied to energy conservation and not to other reasons (e.g., foraging).

Darters are considered to be carnivorous, and previously mentioned studies described their generally insectivorous diet. In another study of the darter species-dense Allegheny River system, Van Snik Gray et al. (1997) assessed food resource partitioning among nine species of darter. The effects of season, ontogeny, and sex with relation to diet were examined. There was a general pattern of overlap in diets of the darters, and some variations were easily explained by morphological differences among species or among the life stages. Significant differences in diets of juvenile darters of both prey species size and type (smaller prey, more chironomid larvae) were attributed to the smaller size of juvenile darters relative to their adult counterparts. Both the

greenside darter and the banded darter were found to be trophic specialists, consuming 2-3 taxa of benthic macroinvertebrates. Conversely, several species including *E. maculatum* (spotted darter), variegate darter, fantail darter, and logperch consumed both larger (up to 13 mm in length) and more diverse prey species (7-10 taxa). However, during spring when prey sources were scarce, there was greater partitioning of the prey base than in summer when prey was more abundant. This was in direct opposition to the findings of Schlosser and Toth (1984) which suggested that there may a density-dependent effect on the results of ecological studies of darter communities, which is supported by the results of studies that are inconclusive unless specific species-pair contrasts are drawn.

As the evidence presented above suggests, ecological differences among sympatric species are subtle in species-rich darter communities. It can be said that darters are, as a rule, ecologically very similar, so much so that the introduction of a non-native species would likely result in clear competition between species. Van Snik Gray et al. (2005) documented how two darter species interacted when one was a non-native species that colonized the habitat of the other. In the Susquehanna River drainage, *E. olmstedi* (tessellated darter) is native to the system. The banded darter is native to the nearby Allegheny system, and its introduction to the Susquehanna was attributed to bait-bucket releases sometime in the 1960's. It has since become the most prevalent darter in many reaches of the Susquehanna. It is related to the tessellated darter so closely that hybrids had been documented, so it stands to reason that there could be direct competition between the two species.

Using a population of allopatric tessellated darters as a control group, the authors documented habitat characteristics of sympatric banded and tessellated darters in order to document a potential habitat shift in the presence of a non-native competitor. Microhabitat preferences of observed darters were again quantified with the use of the 0.25-m^2 acrylic sheet with 5-cm^2 grid. Habitat availability was quantified with systematic transect sampling, and substrate was measured at five equidistant points across each transect to characterize available habitat. This study documented a habitat shift by tessellated darters that lived in sympatry with the banded darters. In locations where the two species occurred, tessellated darters were absent from riffles and runs they inhabited when the banded darters were absent, and were instead relegated to marginal pool and bank habitats. This evidence, more so than that presented by studies of sympatric native species communities, suggested that there was the potential for strong interaction between darter species over habitat resources.

The streams that darters inhabit across the southeastern United States vary in size and description, an expectable consequence of the wide dispersal of this diverse group of species. Streams themselves are dynamic systems, changing (sometimes drastically) in physical composition in relation to environmental variables. For smaller streams in Georgia, a critically important environmental factor affecting stream composition is rainfall and drought. Increasing rainfall and subsequently increasing stream flow will generally lead to an increase in available habitat of a stream. Henry and Grossman (2008) assessed how this type of change affected the habitat selection of three darters—*P. nigrofaciata* (blackbanded darter), *E. inscriptum* (turquoise darter), and

tessellated darter—in a tributary of the Broad River, Georgia. During the 2001-2002 seasons that the study was conducted, rainfall and stream flow ranged from drought conditions (least available habitat) to flood conditions (most available habitat).

Another method of quantifying habitat was employed, i.e., visual estimation of the percent composition of eight substratum classes based on maximum linear dimension in an area of 20 cm^2. These measurements were done in both transect surveys to quantify available habitat as well as in conjunction with darter observations from snorkel surveys. Furthermore, the relative macroinvertebrate abundances associated with the habitats measured were quantified. Results revealed that the darters use of habitat was strongly associated with environmental factors governing this system, and more so than the prey abundances. While principal components analysis had to group the physical components of the measured habitat into unwieldy axes to extract significant results, the general trends were, that during low flows when habitat was most restricted, there was greater partitioning among species, and during the highest flows there was greater overlap. Even though these results apply to physical habitat characteristics and not the prey utilization assessed in earlier studies, they add to the argument that ecologically similar species will partition resources more during times of scarcity (Van Snik Gray et al. 1997) and will increase their ecological overlap during times of plenty.

Across more than three decades of research, the study of darter ecology has progressed from basic biological descriptions of species to intensive studies of communities comprised of many species. At present, it appears to have converged on

several issues into defined and coherent positions. First, darters in general are usually so closely related that significant distinctions in their respective niches will be subtle, and thus warrant intense scrutiny. Second, it has been established that benthic macroinvertebrates are critical resources for darter communities, and that species differences in prey can be found. In establishing methods for studying the habitat preferences of darters, it is necessary to establish rigorous categorical guidelines for describing the physical habitat utilized by the darters, and representative samples of the available habitat must be taken to determine overall habitat selection patterns and habitat availability. As a final note, the various discrepancies in the results of studies of darters justify investigation of the ecology of this extremely diverse group of organisms on a community-by-community basis. If disparate findings can be generated by the study of seemingly comparable populations and species, there must be inherent differences unique to each system to drive the various conclusions that this field of research has drawn.

Stream Restoration Research

In 1992, the National Research Council (NRC) defined aquatic ecosystem restoration as returning an ecosystem to a close approximation of its condition prior to disturbance (NRC 1992). This deceivingly simple definition understates the complexity inherent in any attempt to remediate anthropogenic effects on an aquatic system. Aquatic systems do not exist in a vacuum, and flowing water systems tie many human communities together. This complicates stream restoration efforts, as many

communities recognize the need for ecological remediation in their aquatic systems, yet there is a lack of general unifying oversight to remediation projects. The literature of the field of aquatic restoration has described three steps for successful, sustainable stream restoration projects:

1) study the system for both current and historical characteristics (e.g., biological, geomorphological);
2) design and execute the restoration plan; and
3) evaluate the success of the restoration for at least 10 years.

In the execution of a stream restoration project, the ecological context of every action should be remembered, and the consequences of each action may not be readily clear. It should be foremost in the minds of all involved that stream systems are the result of an infinite number of interactions among the geomorphology, hydrology, and biota of the system (Kauffman et al. 1997).

The first step in stream restoration is alluded to in the phrase ‘condition prior to disturbance’ in the definition above. This phrase implies that there is a goal for stream restoration projects, i.e., to return the condition of the system to that of a historical un-impacted state. Thorough study of the system prior to the design and implementation of a restoration project is necessary for two principal reasons:

1) it may provide insight into the historical condition of the stream and thus influence the goals of the restoration project; and
2) it will lead to an understanding of the forces at work in the system, which will ensure that the restoration project is likely to account for those forces

(Kondolf 1995; Kondolf & Micheli 1995).

The findings of the initial study of the system will drive each following step in the stream restoration process. The use of standardized reaches and unified classification schemes will facilitate better comparisons among impacted and control reaches (Kondolf 1995). Additionally, the process of historical and current surveys of a stream prior to restoration will provide baseline data that are often lacking in other stream restoration projects, and which can be used to assess the effectiveness of the project (Kondolf & Micheli 1995). Overall, rigorous scientific data collection principles should be applied to ensure success of a stream restoration project, and to enable those involved to compare the results of projects across sites and systems.

The primary adjective that should describe a stream restoration project is “sustainable”. The significant resources often required to perform a stream restoration project can represent a large investment by the party involved (Muotka et al. 2002; CCWF 2013). The size of this investment would naturally require its protection, and the dynamism of stream systems mean that planning the sustainability of a restoration project is no small feat. In many cases a tension exists between the desired flexibility in project design needed to meet ecological objectives and erosion and flood control interests (Shields et al. 2003). Therefore, great care must be taken during the development stages of a stream restoration project. Often, specific goals of stream restorations are biological, such as the restoration of a particular sport fishery (Muotka et al. 2002; CCWF 2013). By focusing on restoring the integrity of ecological processes and function of the system as a whole, however, the restoration project is more likely to

successfully attain the restoration of both habitat and species of interest (Kauffman et al. 1997). A holistic focus on the ecological soundness of the system is more likely to generate positive outcomes. Stream restoration projects can either be passive, only involving the cessation of anthropogenic activities that are causing degradation or preventing recovery, or active, usually involving in-stream or riparian zone construction and engineering (Kauffman et al. 1997).

Passive stream restoration projects can mean the cessation of nearly any anthropogenic input to a stream system, such as the removal of point-source pollution. The benefits to this type of restoration project seem fairly self-evident, so it may be more illustrative to focus on the results of two case studies of active stream restoration. Muotka et al. (2002) investigated the potential for unintended consequences of stream restoration in forest streams of Finland, which had been drastically manipulated to facilitate log transport in the 1950s and 1960s, and which had subsequently been the focus of restoration efforts intended to increase the production of the sport fishery. One aspect of the major restoration effort had been the use of heavy machinery in and around the small- to medium-sized streams to reintroduce boulder dams and flow deflectors to enhance spawning habitat for brown trout (*Salmo trutta*) (Muotka & Syrjanen 2007). These impediments to log transport had been removed during the channelization process. However, the use of the large treaded machinery had incidentally led to widespread death of aquatic mosses, which provided important habitat for benthic macroinvertebrates not explicitly targeted by the stream restoration activities. It was determined that while the physical habitat quality for brown trout was

nearly immediately improved by stream restoration efforts, there was a 6-8 year lag in the restoration of the macroinvertebrate community, as the mosses they depended on slowly recolonized the streams from which they had been unintentionally removed. This affected the overall success of restoration efforts, as the sport fishery depended on macroinvertebrates for prey, and thus the increase in fishery quality was slower than would have been expected given the new high quality habitat available to the fish.

Examples of active stream restoration efforts can also be found in the Coal Creek Watershed, Anderson County, Tennessee. There, a local activist group (the Coal Creek Watershed Foundation) was formed in 2000 with the initial goal of improving the quality of this 4th order stream so that it would provide spawning habitat for the naturalized rainbow trout (*Oncorhynchus mykiss*) of the Clinch River (CCWF 2013). The activities of the CCWF have grown to include the organization of general stream restoration efforts, with goals including flood management and erosion control.

The former goal drove restoration efforts of the CCWF conducted at the Fraterville Bridge of Highway 116, where in 2009 an excavator was used to widen a channelized portion of Coal Creek, thereby reconnecting the stream with its floodplain to allow for the dispersal of floodwaters that were being constricted by the narrowed stretch of the stream. The latter goal drove restoration efforts that were conducted at the confluence of Coal Creek and its tributary, Slatestone Creek, at Briceville Elementary School. Downed trees from a tornado in 2002 had rerouted the flow of water at the confluence into the stream bank, leading to swift erosion of the left descending bank. This instability of the stream bank was threatening areas frequented by students of the

school. Again, the deepening stream bank was excavated so that a more natural slope into the floodplain was recreated, and the bank was reinforced with riprap and riparian vegetation planting.

The CCWF has also coordinated biological restoration efforts. In 2008, the CCWF worked with the University of Tennessee to reintroduce nongame species of fish that were thought to be native to Coal Creek but had been missing from annual surveys of the fish biota. These species were the rainbow darter, the fantail darter, the telescope shiner (*Notropis telescopus*), the Tennessee shiner (*N. leucoides*), the warpaint shiner (*Luxilus coccogenis*), and the whitetail shiner (*Cyprinella galactura*) (Schiding 2009).

The final step to ensuring the success of stream restoration efforts alluded to in the literature is to periodically assess the effects of a project. The study by Muotka et al. (2002) represents the kind of follow-up investigation that is often lacking from stream restoration projects. Post-project evaluation is a crucial step but is often marginalized, and is usually sacrificed first in cases of dwindling funding, when it may be that reevaluation studies should continue for the next decade after completion of a project (Kondolf & Micheli 1995). There is evidently a knowledge gap surrounding the medium- to long-term effects of many restoration efforts, justifying more thorough research into the effects of various stream restoration projects. Cases such as the Coal Creek watershed illustrate how the investigation of restoration efforts can become increasingly complex, as multiple restoration and mitigation efforts have been conducted in the same small watershed and may have influence over each other. Furthermore, there have yet to be any follow up studies assessing the success of the various physical stream

restoration efforts within the Coal Creek system. There have been some assessments of the biological community following the efforts of Schiding (2009), but those have been sporadic and infrequent.

Stream restoration projects are generally met with the same impediments to success: species extinction, introduction of non-native predators or competitors, loss of hydrologic function, and fundamental alteration of geomorphic features (Kauffman et al. 1997). Furthermore, there appears to be a changing trend of focusing stream restoration projects not on general ecological restoration, but on the reestablishment or initiation of specific ecosystem services provided by the aquatic system (Palmer et al. 2013). However, the body of research suggests that thorough study of stream restoration effectiveness and ecological impacts is necessary to ensure sustainable outcomes of stream restoration projects. Restoration of degraded riparian zones and the subsequent conservation efforts after recovery require knowledge of the ecosystems as they function, and the attributes at work generating their composition, structure, and productivity (Kauffman et al. 1997).

To attain the goal of restoration is to facilitate a sustainable restoration of the linkages among the terrestrial, riparian, and aquatic ecosystems (Kauffman et al. 1997). To accomplish this requires communication among the parties involved and public education about the restoration efforts, and the guidance of rigorous quantifiable information (Kondolf 1995). It is imperative to minimize the potential damage of any stream restoration efforts, as stream communities evolved with disturbances but anthropogenic restoration efforts are evolutionarily novel (Muotka et al. 2002).

CHAPTER III: METHODS

Study Sites

Coal Creek is a 4th order tributary of the Clinch River located in Anderson County, Tennessee (Figure 1A). Its headwaters flow east from the highlands located to the west of Briceville, Fraterville, and Lake City. The main stem meets with the Clinch River immediately downstream of Norris Dam (Figure 1C). Coal Creek has been the focus of multiple stream restoration efforts, encompassing both physical restoration projects, mostly in the form of bank stabilization, and biological restoration efforts (Schiding 2009). This stream presented an optimum location for the investigation of the interactions among sympatric darter species and the effects of stream restoration efforts on the physical and biological characteristics of a small stream system. For the analytical purposes of this study, the three reaches surveyed in the Coal Creek system (located on the mainstem, going from downstream-most to upstream: The Wye, Fraterville Bridge, and Briceville Elementary School) and the subsequent data collected in those surveys have been treated as the experimental dataset.

Cove Creek is another tributary of the Clinch River located in Campbell County, Tennessee (Figure 1B). Cove Creek is a 3rd order stream until its impoundment near Cove Lake State Park, Caryville, Tennessee, upstream of Norris Dam. The Cove Creek Watershed is contained almost entirely within the Royal Blue subunit of the Cumberland Wildlife Management Area.

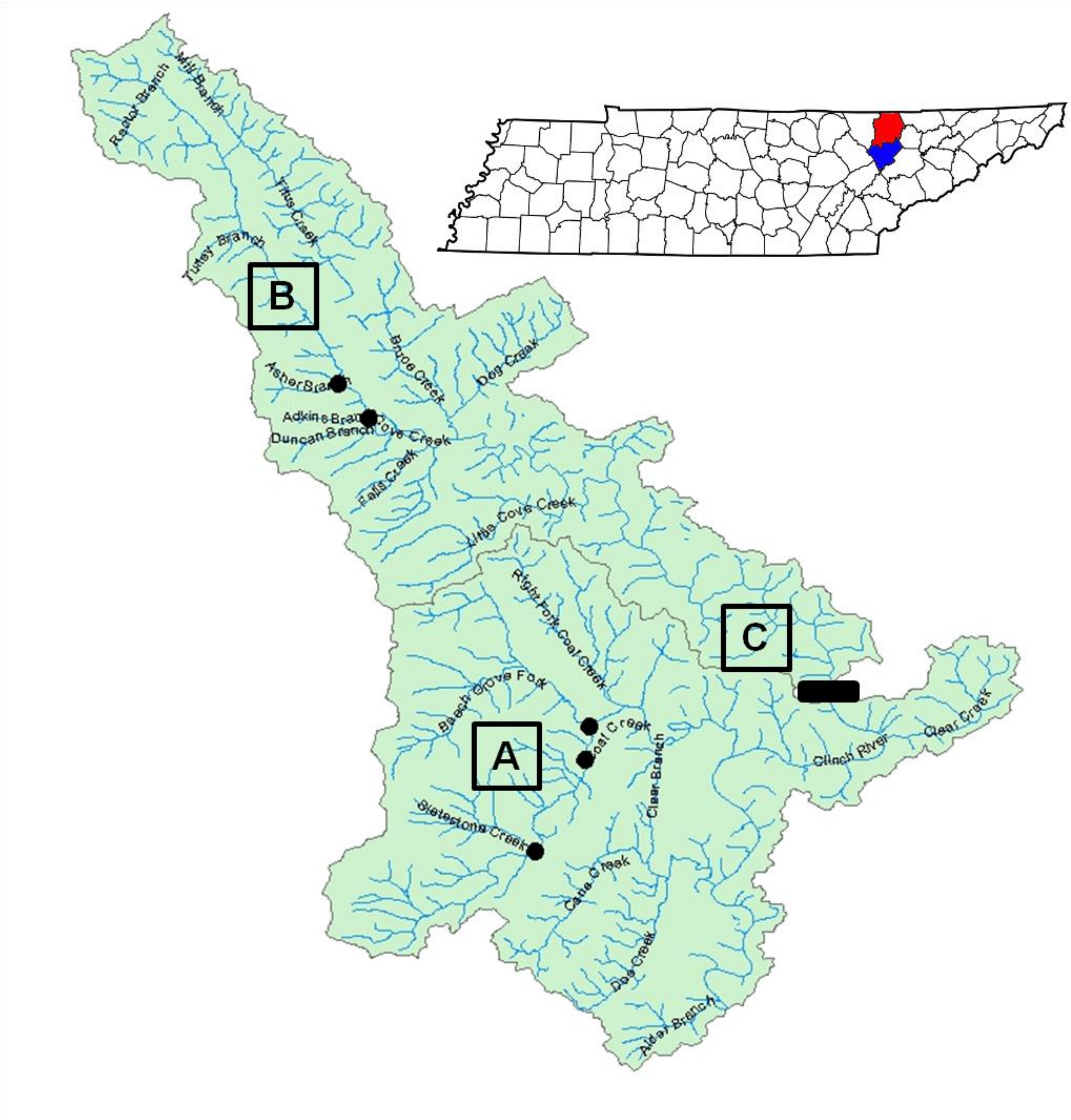

Figure 1. Watershed map of Clinch River system near Norris Dam. A) Coal Creek and sites surveyed. B) Cove Creek and sites surveyed. C) Norris Dam. The inset identifies the map location in Tennessee.

Cove Creek is home to the source population of rainbow darters collected by Schiding (2009) which were reintroduced into the Coal Creek system and subsequently established a self-sustaining population after reintroduction. Therefore, the two reaches surveyed in Cove Creek (Red Ash and Highway 63 Bridge) were treated in analyses as the control groups, as they were considered to represent a more natural state that streams of this size in this region may have exhibited prior to extensive human settlement and manipulation of the landscape. In both Coal Creek and Cove Creek, the study reaches were selected either due to their use in previous research, or for ease of access.

Habitat Surveys

To collect the data necessary to assess darter habitat selection, use, and partitioning, a novel method was employed to survey the defined reaches. Each reach was measured into 10 sections, each 15 m in length. This length was chosen after a preliminary survey effort, and was found to contain the various mesohabitats available at each site. It also allowed for surveys to be completed in an 8-hour work day and thus limit diurnal effects on darter behavior. At the downstream base of each section, a transect perpendicular to the thalweg was surveyed with three equidistant quadrats (Harding et al. 1998). Each quadrat measured 0.25 m^2, and was divided into a grid measuring 12.5 cm^2. The quadrats nearest each bank were placed 1.0-1.5 m within the total wetted width of the stream, and the third was placed in the middle of the stream equidistant from the two bank-quadrat placements. The placement of the quadrats

ensured that the substrate quantified was likely to represent habitat available for exploitation by the darters in the reach by avoiding marginal habitat along the banks which would be more likely to be excluded from the stream due to seasonal fluctuations in flow.

A measurement of depth of the stream was taken from the center of the quadrat, and then the substrate was described. First, a researcher would assess the substrate in the quadrat in each of the quarters. Using visual and tactile assessment, the researcher determined the dominant and second-dominant substrate of each quarter. The dominant substrates were defined as those that covered ≥ 50% of the surface area of the 12.5-cm^2 quarter being assessed, while the second-dominant substrate was defined as covering ≥ 50% of the remaining area not covered by the dominant substrate particle. If the substrate particles defined as dominant or second-dominant had diameters along the B-axis of 1.0 – 25.6 cm, the researcher removed the particle from the stream bed and measured its diameter along the B-axis; otherwise, the researcher categorized the substrate according to a modified version of the Wentworth scale (Barbour et al. 1999, Bunte & Abt 2001). The scale used to categorize substrate from measurements of particle diameter and visual-tactile assessment was based on the following:

1)	Organic detritus	leaf litter, woody debris;
2)	Silt	inorganic particles small enough to suspend in the water column when disturbed;
3)	Sand	inorganic particles too large to suspend in the water column to ≤ 1.0 cm in diameter;

4)	Medium gravel	rocky substrate 1.0 – 1.5 cm in diameter;
5)	Coarse gravel	1.6 – 3.2 cm;
6)	Very coarse gravel	3.3 – 6.4 cm;
7)	Small cobble	6.5 – 12.8 cm;
8)	Large cobble	12.9 – 25.6 cm;
9)	Boulder	> 25.6 cm; and
10)	Bedrock	exposed underlying rock layer with no discernible edges.

The aggregated data from the 27-33 quadrats measured at each site were used to describe the available habitat profile for the reach.

To describe the habitat utilized by the darters in each reach, snorkel surveys were conducted in the sections between each transect. The method utilized here was most similar to that employed by Chipps et al. (1994). A preliminary effort was made to verify the accuracy of identification of darters encountered by snorkelers, and to encourage accurate identification only adult darters (≥ 40 mm TL) were recorded for observations. All snorkelers had successfully completed a university-level ichthyology course which required training in fish identification.

For each snorkel survey, two or three snorkelers swam upstream from the downstream-most point of each reach. Each snorkeler was assigned an alley of equal width to survey, and care was taken so that the reaches were evenly surveyed. Snorkelers communicated movements of specific darters to avoid recording multiple observations for individuals. When a darter had been positively identified to species, the

snorkeler would mark the location of the darter as it was when initially observed with a 57-g lead fishing weight. Attached to the lead weight was high-visibility surveyor's tape marked with a number. The snorkeler would then record the species of darter seen at the site on a wrist slate.

Once the snorkelers had moved upstream beyond the placed markers, another researcher would conduct quadrat assessments of the substrate associated with the darter observation, following the same procedure as was used in the transect surveys. Each quadrat was centered on the position of the lead weight. The habitat quantification was then conducted, with the number on the flagging identifying each set of eight substrate particle descriptions. At the end of each 10-m section survey, snorkelers would provide the species associated with each number, and then the survey of the next section would begin. The data collected by this snorkel survey were used to describe observed habitat usage by the various darter species encountered at each site.

Analyses

For each quadrat that was assessed, there were eight quantifications of substrate particles. Each of the eight observations was classified into one of 10 categories based on the Wentworth scale of classification (Bunte & Abt 2001). All subsequent tests of these data relied on a significance level of $\alpha = 0.05$.

Analysis of variance (ANOVA, Proc GLIMMIX, SAS 9.3, SAS Inc., Cary, North Carolina) of the frequencies of occupied substrate versus frequency of habitat from the transect surveys was used to assess whether darter habitat usage differed

from the available habitat, and how this changed across sites. Data were tested for normality, and means compared using LSD mean separation. Then a “site*species” (a combined variable for differentiating among species found at different locations) habitat dataset was used to generate frequency tables (Proc Freq, SAS 9.3) of the occurrence of each substrate with each “site*species”. The substrate occurrence frequencies were then analyzed for similarity with chi-square tests, and correspondence analysis (Proc Corresp, SAS 9.3) was used to visualize associations between each darter species at each site and the various substrate categories.

For the purposes of this study, the habitat usage data collected for similar species at different sites was treated separately. While it may be that these fish are part of continuous, watershed-wide populations, it seemed appropriate to treat the species at each site as separate groups. Given the relatively short time span within which the snorkel surveys were conducted and the generally territorial nature of the species encountered, it is possible but not likely that the same fishes were observed at different reaches. Therefore, any potential environmental influences on their behavior would be adjusted for by comparing the species present at each site, functionally describing each “site*species” as a distinct subpopulation of the species within the watershed. Codes used to distinguish each “site*species” were all combinations of the common names of the species (RAINbow, SNUBnose, REDline) and the site name (63 Bridge, Red Ash, Briceville, Fraterville, and The Wye).

To identify trends in the data described in “site*species” groups, resource matrices were generated (adapted from Colwell & Futuyama 1971) and several

comparisons were made using a heat map coloration scheme. In these heat maps, red coloration corresponded with positive association of that “site*species” with the substrate category, and blue coloration corresponded with negative association. The depth of shading corresponded with increasing cell chi-square value.

To assess substrate profile data, histograms were used to describe the substrate profiles of the sites in this study. Heat map coloration was applied to the raw data to assess trends in particle occurrences across sites. This process resulted in tables where darker coloration of the data cell indicated greater occurrence of that particular substrate in the quadrats measured.

CHAPTER IV: RESULTS

The surveys of all five sites were completed between 19 June 2013 and 26 July 2013. Snorkel surveys were conducted between 0900 and 1600 each day, and were conducted when visibility was deemed sufficient to allow for identification of darters to species. In addition to the rainbow, redline, and snubnose darters that were observed in quantities great enough for analysis, snorkelers also encountered the benthic species *Rhinichthys atratulus* (blacknose dace), blueside darter, logperch, fantail darter, and greenside darter.

Nonrandom Habitat Selection

To compare the habitat profile occupied by darters at each site to the available habitat quantified from transect surveys, the occurrence of each of the 10 substrate categories in the darter habitat assessments and transect surveys were calculated. These data (n = 100: five sites with 10 occupied habitat categories and 10 unoccupied [available] habitat categories each) were tested with a two-way ANOVA, which found significant differences between the habitat profile utilized by the darters, and the habitat available in their site-scale environment ($P < 0.0001$, $F = 28.69$, $df = 27$). Results from this test suggested that darters had nonrandomly selected for specific substrate types, which justified further analysis into specific habitat selection among species. Post-hoc testing of means separation using least significant differences found significant differences in the counts of the substrate categories between the occupied and

available habitat types (mean = 33.90, $P < 0.0001$, $t = 5.60$).

Species Habitat Partitioning

A correspondence analysis was performed which visualized the associations of various “site*species” encountered with substrate categories (Figure 2). The “site*species” points are clustered in a cloud that appears driven by association with the sand to boulder substrate categories (3 to 9 on the scale).The data were then modified by dropping any “site*species” with less than five observations ($n < 5$), and the correspondence analysis was performed again (Figure 3). The “site*species” were grouped by site with fairly high fidelity for all sites except The Wye.

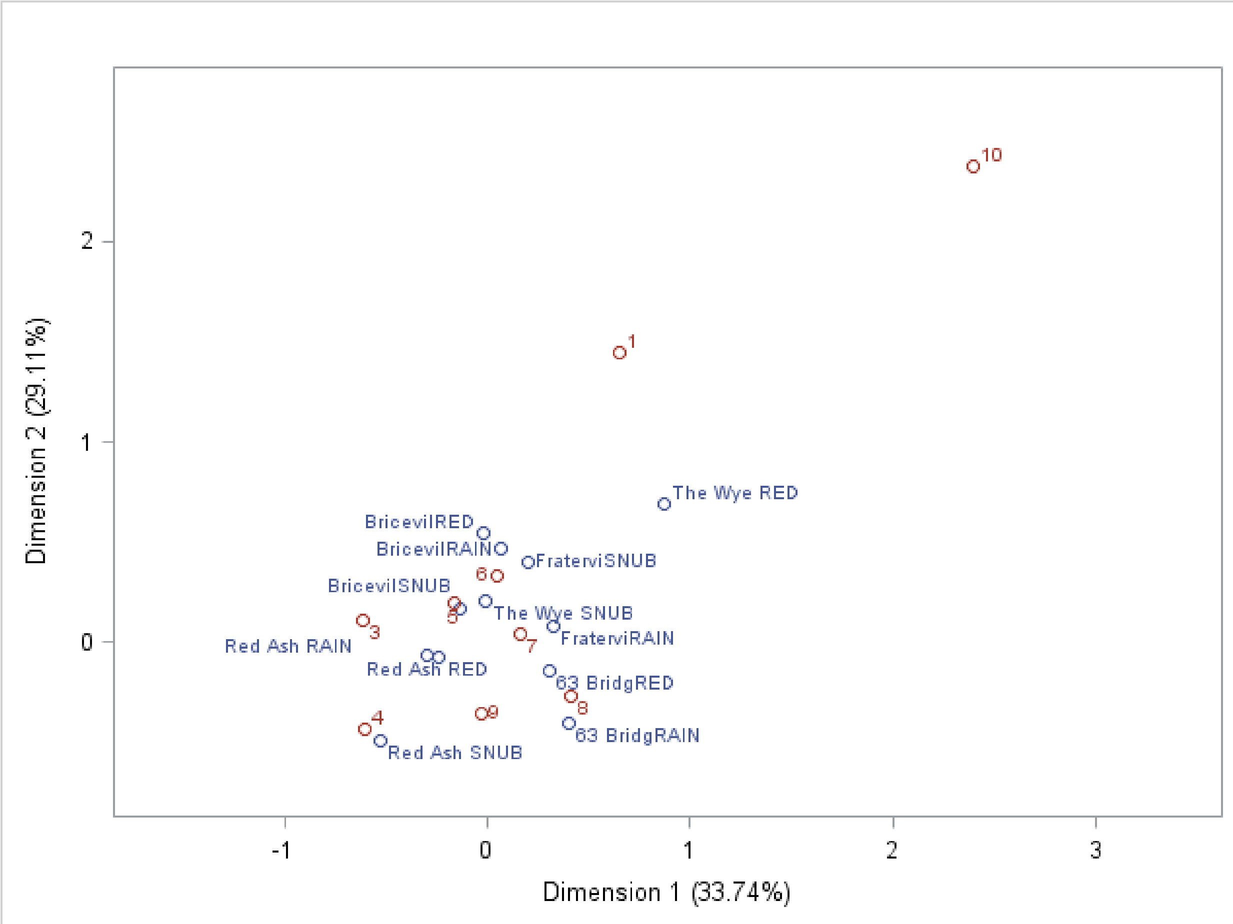

Figure 2. Correspondence analysis of habitat association data for all site*species observed. The site*species points appear to cluster around substrate categories 3 through 9 (sand to boulder).

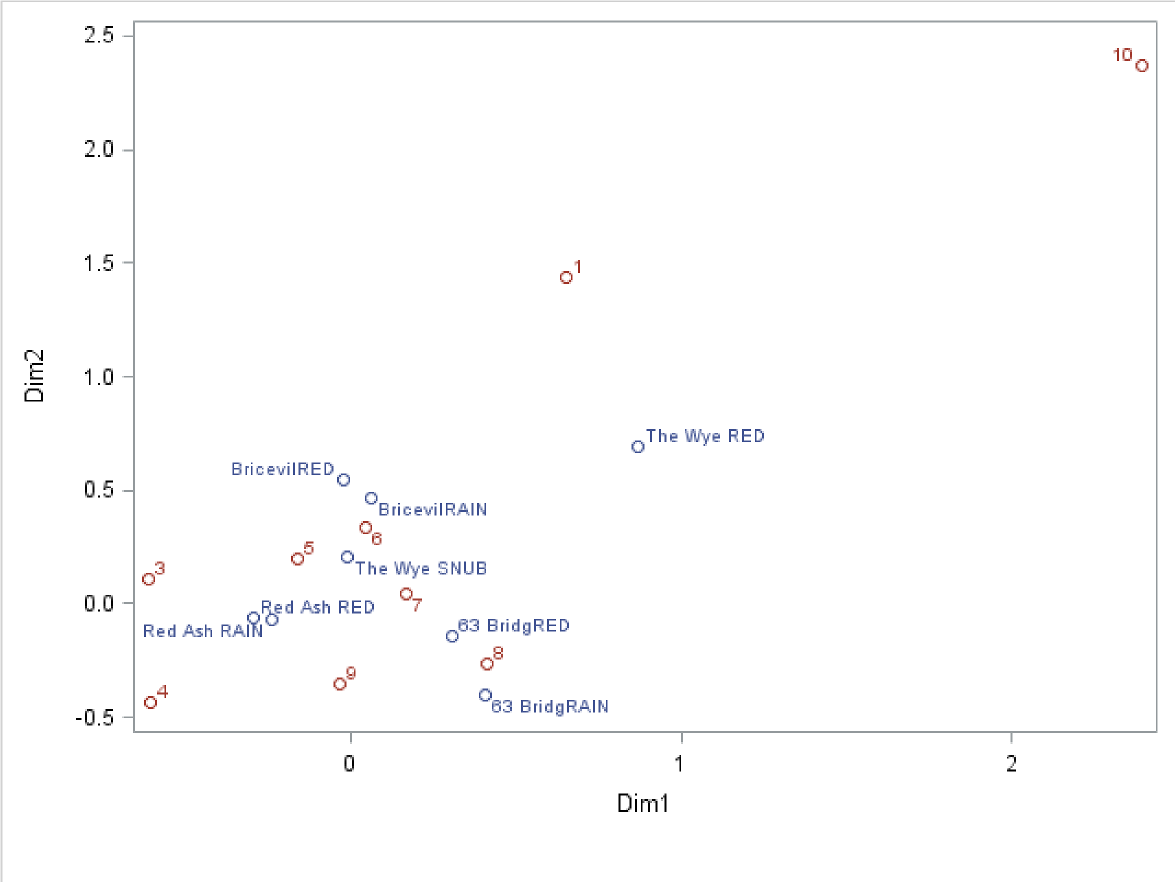

Figure 3. Correspondence analysis of habitat associations of all site*species where n ≥ 5.

Chi-square tests for association were performed to determine significant associations among the “site*species” variables and each substrate category (n = 330). The chi-square tests found significant associations among all “site*species” pairs and the substrate categories 5, 6, 7, 8, and 9 (likelihood ratio chi square, Fisher’s exact test for each $P < 0.0001$). These categories represented the coarse gravel through cobble and boulder substrate gradations, which lack the additional sand and medium gravel points that enhanced the clustering observable in the correspondence analysis. The cell chi-square values and results of the chi-square tests for association are reported in Table 1.

A heat map color scheme was applied to Tables 1, 2, and 3, which display the cell chi-square values form the frequency tables. In Table 1, there do not appear to be any species-specific trends in habitat use. The heat map in Table 2 indicated no readily-visible trends in habitat use of the species across sites. However, when “site*species” combinations with less than five darters observed ($n < 5$) were dropped, and comparisons were made among sympatric species at a site, trends indicated partitioning of the habitat, particularly at the Red Ash and Briceville sites (Table 3). This was evidenced by a distinct lack of matching patterns in the heat map coloration of Table 3, which may suggest differing utilizations of habitat among species at those sites.

Table 1. Cell chi-square values, a measure of association between "site*species" and the 10 substrate categories. Column totals for each substrate category (1 – 10) were not calculated for those substrate categories not absent from any site*species locations. Blue shading indicates negative association, while red coloration indicates positive association. Depth of shading increases with increasing cell chi-square values. P-values calculated with Fisher's exact test.

Species*Site	1	2	3	4	5	6	7	8	9	10	N
63 Bridge RAIN	0	0	0	0.188	0.802	4.5448	18.514	5.8178	2.3911	0	55
Briceville RAIN	0	0	21.307	0	11.368	31.768	21.37	6.7866	36.614	0	5
Fraterville RAIN	0	0	2.7379	0.7368	13.169	17.05	17.042	10.265	3.1519	0	2
Red Ash RAIN	0	0	15.511	0.0038	19.952	56.74	29.27	5.8667	6.2464	0	118
63 Bridge RED	0	0	0	0.2105	4.0281	3.561	0.1482	0.0115	0.0951	0	21
Briceville RED	0	0	0.5809	0	4.0804	12.944	0.1587	0.0129	0.8542	1	17
Red Ash RED	0	0	0.1633	0.0592	0.0958	6.1553	0.0975	0.2133	0.2861	0	34
The Wye RED	0	0	2.1613	0	0.8083	2.2069	3.1849	8.0903	0.5661	5	10
Briceville SNUB	0	0	46.503	0	6.0981	11.511	39.069	22.217	195.78	0	26
Fraterville SNUB	0	0	17.217	4.4912	49.431	103.16	82.037	71.368	46.69	0	21
Red Ash SNUB	0	0	47.957	0.1096	28.944	50.448	55.725	16.049	30.691	0	1
The Wye SNUB	0	0	69.49	0	18.321	19.282	66.353	24.937	57.415	0	20
Likelihood Ratio χ^2	N/A	N/A	N/A	N/A	213.6387	458.3687	525.2826	227.0806	334.9113	N/A	
Column P-value	N/A	N/A	N/A	N/A	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	N/A	

Table 2. Habitat associations compared within each species though the application of heat map color scheme, when n ≥ 5. Blue shading indicates negative association, while red coloration indicates positive association. Depth of shading increases with increasing cell chi-square values.

Species*Site	1	2	3	4	5	6	7	8	9	10	N
63 Bridge RAIN	0	0	0	0.188	0.802	4.5448	18.514	5.8178	2.3911	0	55
Briceville RAIN	0	0	21.307	0	11.368	31.768	21.37	6.7866	36.614	0	5
Red Ash RAIN	0	0	15.511	0.0038	19.952	56.74	29.27	5.8667	6.2464	0	118
63 Bridge RED	0	0	0	0.2105	4.0281	3.561	0.1482	0.0115	0.0951	0	21
Briceville RED	0	0	0.5809	0	4.0804	12.944	0.1587	0.0129	0.8542	1	17
Red Ash RED	0	0	0.1633	0.0592	0.0958	6.1553	0.0975	0.2133	0.2861	0	34
The Wye RED	0	0	2.1613	0	0.8083	2.2069	3.1849	8.0903	0.5661	5	10
Briceville SNUB	0	0	46.503	0	6.0981	11.511	39.069	22.217	195.78	0	26
Fraterville SNUB	0	0	17.217	4.4912	49.431	103.16	82.037	71.368	46.69	0	21
The Wye SNUB	0	0	69.49	0	18.321	19.282	66.353	24.937	57.415	0	20

Table 3. Comparison of cell chi-square values calculated for sympatric groups of species when n ≥ 5, analogous to the information in Figure 3. Blue shading indicates negative association, while red coloration indicates positive association. Depth of shading increases with increasing cell chi-square values.

Watershed	Species*Site	1	2	3	4	5	6	7	8	9	10	n
Cove	63 Bridge RAIN	0	0	0	0.188	0.802	4.5448	18.514	5.8178	2.3911	0	55
	63 Bridge RED	0	0	0	0.2105	4.0281	3.561	0.1482	0.0115	0.0951	0	21
Cove	Red Ash RAIN	0	0	15.511	0.0038	19.952	56.74	29.27	5.8667	6.2464	0	118
	Red Ash RED	0	0	0.1633	0.0592	0.0958	6.1553	0.0975	0.2133	0.2861	0	34
Coal	Briceville RAIN	0	0	21.307	0	11.368	31.768	21.37	6.7866	36.614	0	5
	Briceville RED	0	0	0.5809	0	4.0804	12.944	0.1587	0.0129	0.8542	1	17
	Briceville SNUB	0	0	46.503	0	6.0981	11.511	39.069	22.217	195.78	0	26
Coal	The Wye RED	0	0	2.1613	0	0.8083	2.2069	3.1849	8.0903	0.5661	5	10
	The Wye SNUB	0	0	69.49	0	18.321	19.282	66.353	24.937	57.415	0	20

Rainbow Darter Habitat Use

A rainbow darter population in Coal Creek has been established since the efforts of Schiding (2009). In the intervening years, rainbow darters have been found with some regularity in annual or bi-annual survey efforts (CCWF, unpublished data). During the course of this study, nine rainbow darters were found across the three sites in Coal Creek. While those “site*species” with less than five observations were dropped from previous analyses, they were included to assess habitat use of rainbow darters across the watersheds. A correspondence analysis placed rainbow darters, encountered at four of the five sites, in relation to the substrate categories (Figure 4). The arrangement of the “site*species” points of the rainbow darters in Figure 4, and the trend highlighted in Table 2, indicates that the population of rainbow darters at the Briceville site in Coal Creek appear to be utilizing habitat differently than rainbow darters in Cove Creek (the source population).

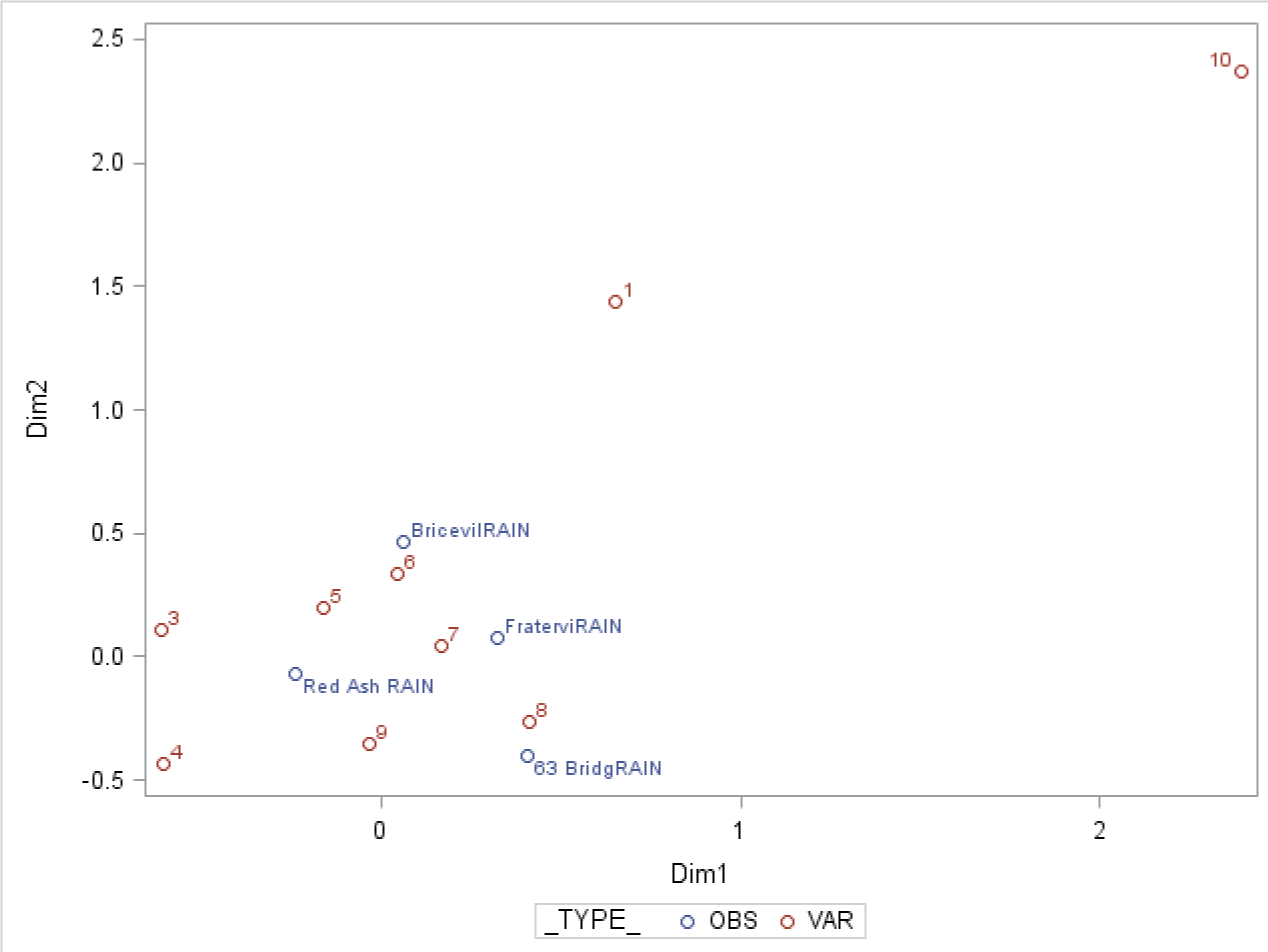

Figure 4. Correspondence analysis of the habitat associations of rainbow darters in Coal and Cove Creeks.

Effects of Stream Restoration

Stream restoration efforts were known to occur at two of the Coal Creek sites surveyed: Fraterville and Briceville Elementary (CCWF 2013). To assess how those efforts may have affected the substrate profile at those sites, data collected during transect surveys were used to describe the overall habitat of each of the sites. Each site was rated as either natural, restored, or impacted prior to substrate surveys based on visual assessment of physical characteristics of the reach (e.g., degree of channelization, mesohabitat heterogeneity, history of stream restoration effort) and transect surveys of substrate were conducted. Both sites in Cove Creek were considered natural. The Briceville and Fraterville sites in Coal Creek were considered restored, and the site at The Wye was considered impacted.

Frequency tables and chi-square tests compared occurrences of each of the 10 substrate categories at each of the sites. Significant differences in the occurrence of substrate categories were present among sites ($P < 0.001$). These results were likely driven by the occurrence patterns seen in Table 4. The greatest levels of occurrence of cobble and boulder substrate were in Cove Creek at the Highway 63 Bridge, possibly the least disturbed site of the study (Figure 5). The site at Briceville Elementary displayed similar patterns to those evident in both sites of Cove Creek, with the greatest occurrence of small cobble of the sites in Coal Creek (Figure 6). On the other hand, The Wye and Fraterville both displayed greater occurrences of sand particles (Figures 7 and 8). Both Red Ash and The Wye had the highest number of occurrences of bedrock, and The Wye contained the most silt found at any site.

Table 4. Occurrences of each substrate category at each site, out of a typical 8 observations * 3 quadrats * 10 transects = 240 observations. Sites marked ‘α’ are considered natural, sites with ‘β’ have undergone stream restoration efforts, and ‘γ’ denotes impacted state. Deeper shading indicates greater occurrences of a particle type at a site.

Site	1	2	3	4	5	6	7	8	9	10	n
63 Bridge	0	0	5	5	12	32	80	28	102	0	264
Red Ash	1	2	5	0	13	35	82	41	27	34	240
Briceville	1	0	23	3	14	63	98	24	38	0	264
Fraterville	2	1	64	29	23	42	35	25	17	0	238
The Wye	10	0	57	16	0	38	42	37	8	22	230

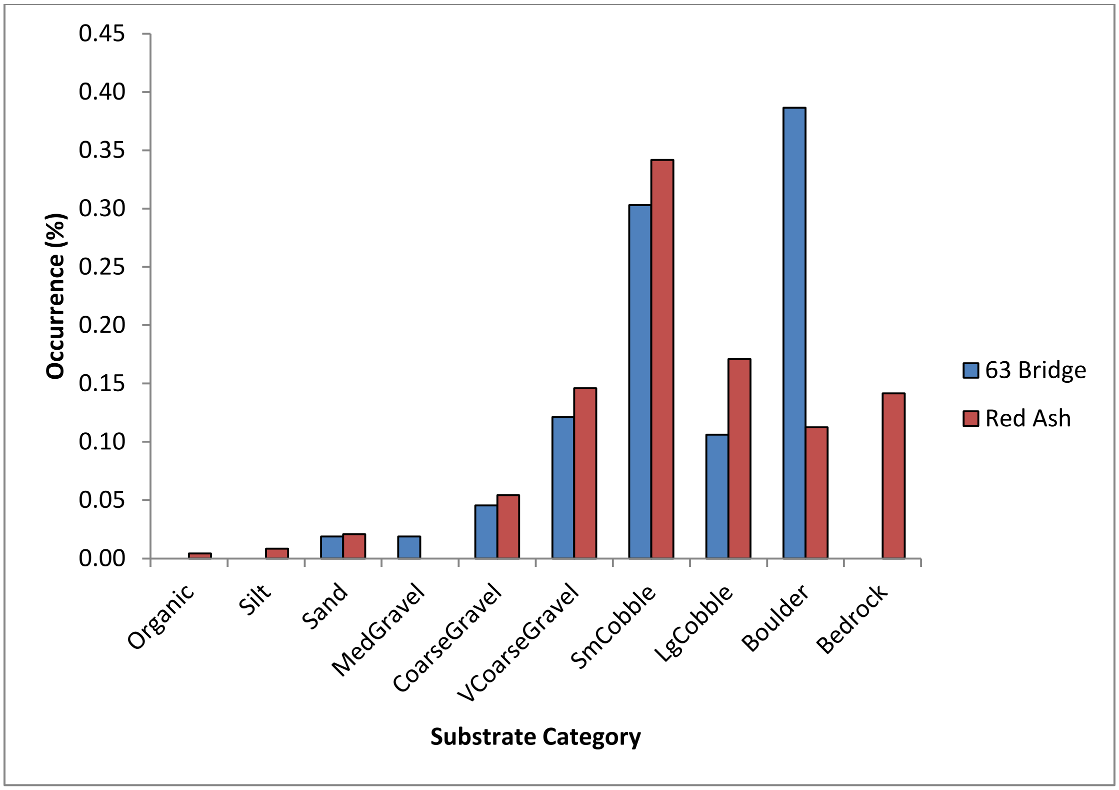

Figure 5. Substrate profile for the Cove Creek sites. Profile generated by plotting occurrences of each substrate category encountered during transect surveys of available habitat at each site. While there appears to be a general trend of similarity between the substrate profiles at each of these sites, frequency table analysis indicated significant differences (chi-square = 87.3081, $p < 0.0001$).

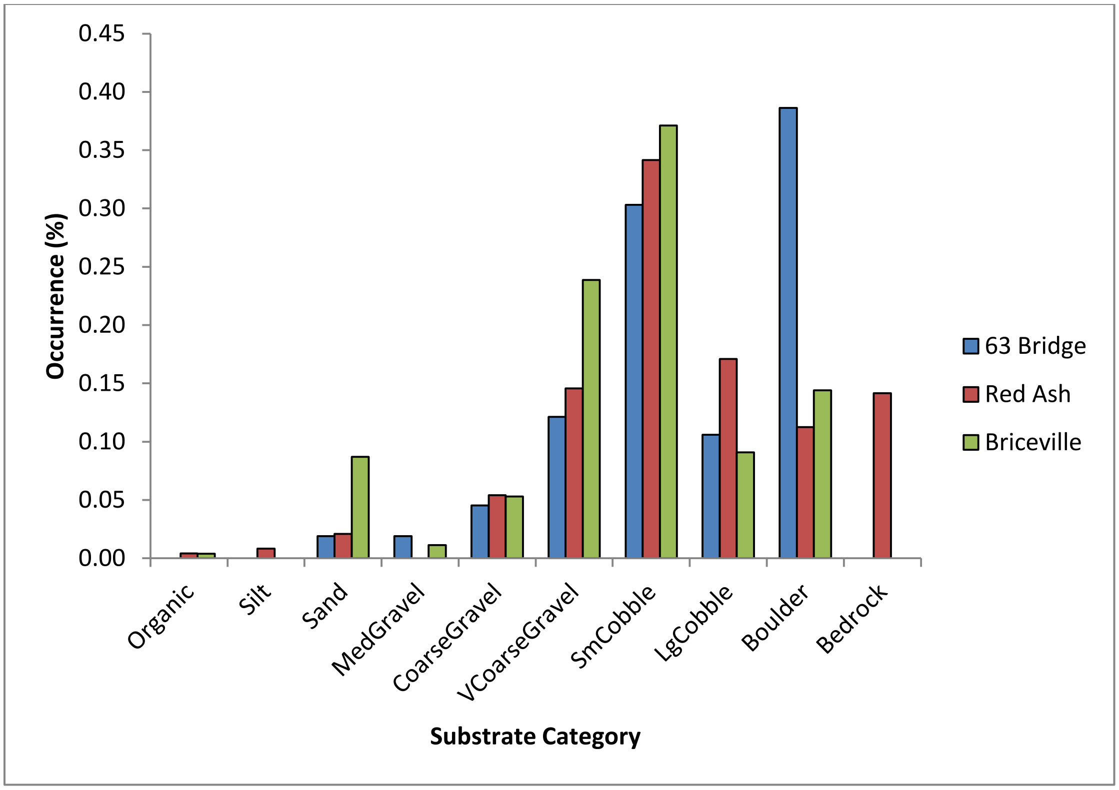

Figure 6. Substrate profiles of the Coal Creek site Briceville and those of the natural sites in Cove Creek differed (chi-square = 1777.5558, $p < 0.001$).

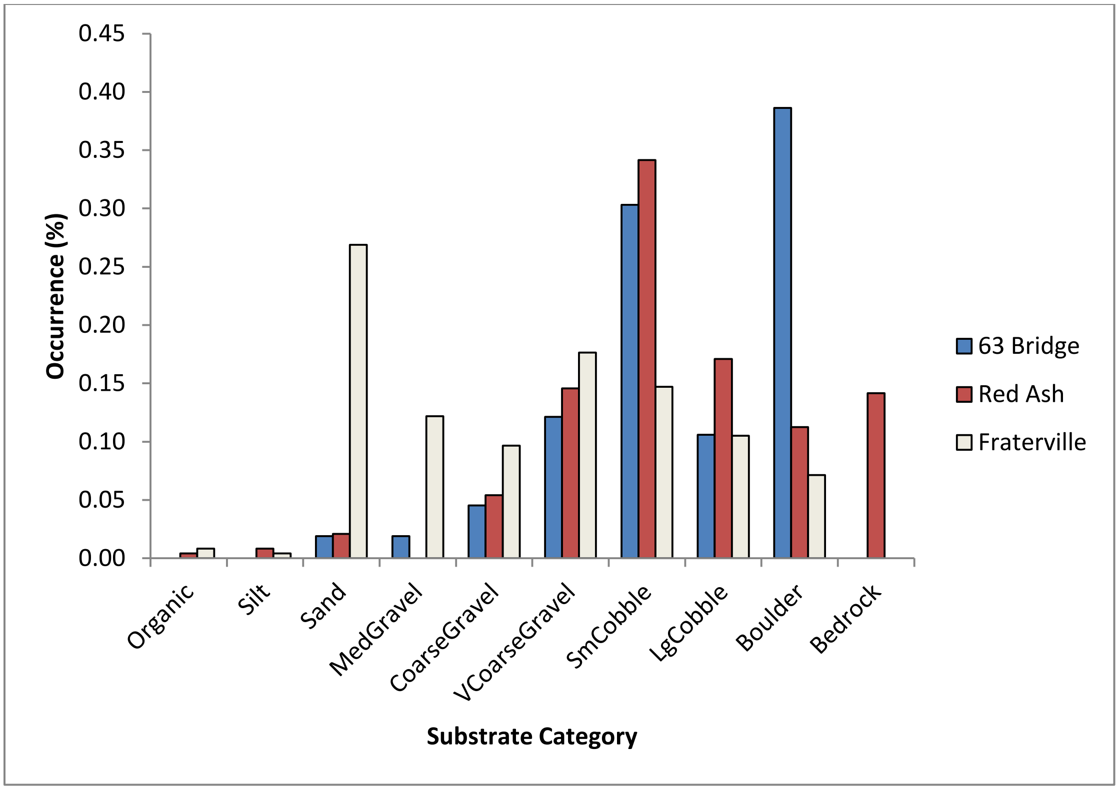

Figure 7. Comparison of the substrate profiles of the Coal Creek site at Fraterville and those of the natural sites in Cove Creek (chi-square = 330.0606, $p < 0.0001$).

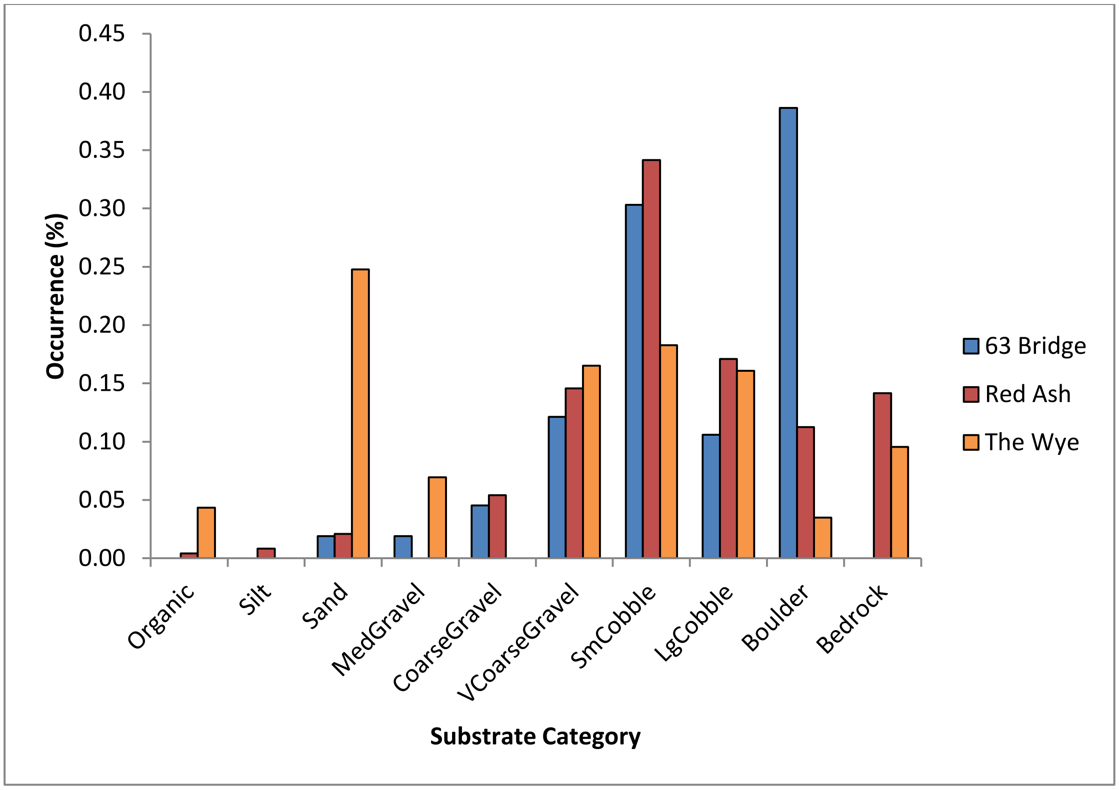

Figure 8. Comparison of the substrate profiles of the Coal Creek site at The Wye and those of the natural sites in Cove Creek (chi-square = 288.6254, $p < 0.0001$).

CHAPTER V: CONCLUSIONS

Habitat Partitioning

The first two research objectives investigated in this study, to determine if the darters were selecting habitat nonrandomly and if so, what habitat they were associated with across all sites, were pursued to determine how sympatric darters in Coal and Cove Creeks may have partitioned their habitat if partitioning was indeed occurring. To attempt to draw any conclusions about darter habitat usage in this system, evidence first needed to be presented that the darters were selecting certain habitats, and not utilizing the substrate in a pattern similar to occurrence patterns of habitat at each site. Testing indicated that darters were selecting for certain substrate types, as the ANOVA results suggested a significant difference between the habitat associated with the darters and the habitat profiles constructed for each reach from the transect survey data. It should be noted that substrate category 2 (silt) was only found in transect surveys, and never associated with the three darters analyzed in this study.

The textbook description of habitat preferences of these darter species is described generally as clean (lacking fine sediment accumulation), gravel and cobble (Etnier & Starnes 1993). However, the data did not support a narrowed description of habitat preferences for these three species, as the chi-square test for association found significant associations among the three species assessed and the substrate categories 5 through 9 (medium gravel to boulder). The textbook definition was supported by the data, as darters were associated with gravel, cobble, and boulder, and were never

associated with silt sedimentation. The broad range of habitat preferences may be one factor driving the success of these species, which are widely dispersed across the state of Tennessee.

When comparisons were made within each species by the use of heat map coloration in Table 2, the only readily noticeable difference occurred among rainbow darter populations, namely the difference in habitat use by the Briceville population of rainbow darters. There do not appear to be strong distinctions within the other two species. Furthermore, the readily apparent trends in Table 3 suggested that at the site level, syntopic subpopulations of different species were utilizing habitat in different ways, effectively partitioning that resource. The differences in habitat utilization could be attributed to either specialist (exhibiting strong associations with certain substrate types) or generalist (lacking strong association with any substrate types over the others) habitat use patterns.

At the Highway 63 Bridge site, rainbow darters were strongly associated with small cobble substrate, while redline darters at that site displayed a more generalist approach to their habitat utilization. This pattern of specialization in the rainbow darters and generalization in the redline darter data was apparent at the Red Ash site, also in Cove Creek, though rainbow darters there were most strongly associated with the very coarse gravel substrate category. Indeed, the redline darters at that site displayed avoidance of the substrate category preferred by the rainbow darters (category 6, very coarse gravel), while not strongly preferring any type. The close proximity by dimension of the two substrate categories used by rainbow darters in Cove Creek should be

illustrative of the apparent pattern seen in the reference sites.

Outside the reference sites, the redline darters again displayed generalized patterns of habitat association, with no apparently strong associations with substrate at any of the Coal Creek sites. There were not enough darters of any species observed at Fraterville to warrant attempts to describe habitat partitioning. A pattern of generalist cohabiting with a specialist in a two-species system was found at the site The Wye, where snubnose darters (specialists) were strongly associated with sand, small cobble, and boulder substrates, and the redline darter (generalists) lacked any strong associations. The results at Briceville stand apart, where the rainbow darters were found in fewer quantities than would be expected across the typical preferred substrate categories (sand and coarse gravel to boulder).

The trends mentioned above are derived from the heat maps of Tables 2 and 3. However, these trends do not appear as clearly in the correspondence analysis results. For most sites, there was more similarity among the various species observed at each site than there was among the species across sites, which was evident in the grouping of the “site*species” points in the output. Contrastingly, the redline and snubnose darter subpopulations encountered at The Wye were fairly distinct in their locations in the graph. Regardless, the correspondence analysis showed a general trend of grouping darter subpopulations by site (as seen in the locations of the Briceville, Red Ash, and Highway 63 Bridge “site*species” points). This result may be an artifact of the habitat choices available to the darters being dominated by site effects. When considered with the heat map trends, it may then be the case that any habitat partitioning by these

darters is first determined by the habitat available at the mesoscale. The syntopic species then appear to partition the habitat within the site, and their habitat use is not dictated by stronger, species-wide imperatives.

These results were similar to those from previous studies of darter ecology, which often mention the broad overlap in the ecological niches utilized by sympatric darter species, and describe only subtle differences in various characteristics of darter resource use. Specifically, Paine et al. (1982) described a similar two-tiered resource division pattern among four species of darters, first by mesohabitat, and then by prey base (which apparently drove substrate associations).

Reintroduction and Habitat Use

This study, like other sampling efforts in Coal Creek, found rainbow darters in several locations. Given the life-span of these fish (2-3 years), it is suggested that the translocation efforts of rainbow darters from Cove Creek to Coal Creek four years prior to this study successfully established a reproducing population of rainbow darters in Coal Creek. From habitat partitioning analysis, it is evident that the source population of rainbow darters in Cove Creek exhibit a general association with several categories of rocky substrate, and the strength of those associations can be varied, depending on the community interactions between rainbow darters and the species they encounter at the reach scale. This mechanism, where rainbow darters may be able to select from a pool of suitable habitat types, could lend flexibility in the functional niche utilized by this species, with obvious evolutionary benefits. However, within the Coal Creek system at

the only site where sufficient numbers of rainbow darters were located, Briceville, it appears that the reintroduced population has deviated in its habitat use, as indicated in Table 3. At this site, the redline darters are exhibiting a generalist approach in their habitat utilization. The snubnose darters appear to be strongly associating with the types of substrate that the rainbow darters are found associated with in Cove Creek. This suggests that the rainbow darters may be unable to utilize the habitat that the source population utilizes. The rainbow darters at the Briceville site may be undergoing competitive exclusion by the snubnose darters from their preferred habitat.

Stream Restoration and Substrate Profile

The analyses of substrate profiles were cursory, and there were significant differences between the substrate profiles of all five sites. However, there appeared to be some trends of note occurring, as visualized in Table 4. The Wye was considered the most impacted site, due to deep channelization (vertical bank profiles of height > 3 m) and a homogenous mesohabitat profile (only run-pool mesohabitat observed). The results of the transect surveys indicated that this site also had the highest occurrences of sand and silt particles. In these low-order Appalachian-region streams, an increase in fine sedimentation may be the result of anthropogenic effects on hydrologic processes governing the system. The Wye could be considered the negative control in this study, and its substrate profile the consequence of unmitigated alteration to the reach by human activity. On the other hand, the sites in Cove Creek both contained the highest occurrences of small cobble substrate. These sites were visually assessed to be

relatively unimpacted, as they did not display symptoms of channelization, supported diverse mesohabitat profiles, and maintained robust riparian vegetation communities. The habitat found in these two sites that could potentially support benthic aquatic communities tended towards larger rocky substrate particles, which have been assumed, for the purposes of this study, to be the more natural condition for streams in this area.

The sites where stream restoration efforts had been conducted had various results in the survey of their substrate (and therefore potential darter habitat) profiles. The Fraterville site had a high level of sand particles, more similar to The Wye than to the reference sites in Cove Creek. Briceville, on the other hand, more closely resembled the cobble-dominated substrate profiles of the Cove Creek sites. There may be several factors that explain these results. First, the Fraterville site is downstream of the Briceville site, closer to The Wye, and not in the headwaters region of the watershed. The Fraterville site was selected due to the stream bank restructuring that occurred in 2009, when heavy machinery was used to excavate a bench in the stream bank where the land had previously been graded, which led to channelization occurring in the stream. The excavation essentially reconstructed the floodplain of the creek at that site, allowing flood waters to disperse where they had been impounded before. The impoundment of the stream during flood events had resulted in an increase in the deposition of sediment at that site. It may be that the sediment that had been deposited prior to the restoration effort has not yet been washed downstream, if the excavated floodplain had restored the sediment carrying capacity of the stream at this site

(Fenneman & Johnson 1948, Brahana et al. 1986).

While defining the mechanisms governing the substrate profiles of the sites in Coal Creek may be beyond the scope of this study, it was evident that the success of patchwork stream restoration efforts should be closely monitored in this system and others. To protect the investment of the resources necessary to reduce or mitigate the effects of anthropogenic activities on a watershed, proper resources should be allocated for follow-up studies of the system. Furthermore, the cascading implications of any stream restoration effort should be considered. As the case at the Fraterville site demonstrates, projects undertaken with one or few goals in mind (the end of impoundment of flood waters in this instance) may have some positive or negative benefits for the broader stream ecosystem at that site. The floodwaters may be managed for human needs, but it is yet to be shown how the habitat relied upon by the darters, and the rest of the stream biota, has been affected by the stream restoration effort. Further study would be necessary to track changes in the substrate profile at this site to fully quantify the relative success of the restoration effort.

CHAPTER VI: SUMMARY

1) The three darter species analyzed in this study, the redline, snubnose, and rainbow darters, are using habitat in patterns that differ significantly from habitat availability patterns for each site, indicating nonrandom selection by the darters is occurring.
2) There is strong overlap in the preferred habitat profiles of the three species: all three species are significantly associated with coarse gravel to boulder substrate.
3) There appears to be a tiered system of habitat association, where partitioning is first influenced by availability at the site scale, and then habitat is partitioned among the syntopic subpopulations.
4) Redline darters are habitat generalists, not associating with any of the coarse gravel to boulder substrates over the others.
5) Rainbow darters in two-species dominated sites specialized towards coarse gravel and small cobble substrates, while snubnose darters in two-species sites specialized towards sand, small cobble, and boulder substrates.
6) At the Coal Creek site Briceville, there is evidence to suggest that the reintroduced rainbow darters are being restricted from their preferred habitat by the snubnose darters they encounter at that site.
7) Stream restoration efforts may have influenced the fine sediment carrying capacity at two sites in Coal Creek, but long-term data of substrate profile will be necessary to determine the lasting effects of stream restoration.

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APPENDIX

Appendix 1. Counts of all benthic species observed during snorkel surveys at each site.

Watershed	Site	Species	Count
Cove	63 Bridge	Rainbow	55
		Redline	21
		Greenside	1
		Blueside	1
		Logperch	19
Cove	Red Ash	Rainbow	118
		Fantail	1
		Redline	34
		Snubnose	1
		Blueside	2
		Logperch	26
Coal	Briceville	Rainbow	5
		Redline	17
		Snubnose	26
		Greenside	4
		Logperch	3
Coal	Fraterville	Rainbow	2
		Snubnose	21
		Fantail	1
		Greenside	4
		Blueside	6
		Logperch	3
		Blacknose Dace	10
Coal	The Wye	Redline	10
		Snubnose	20
		Blueside	5
		Logperch	9

VITA

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