ABSTRACT

VANDER PLUYM, JENNIFER LYNNE. Impact of Bridges and Culverts on Stream Fish Movement and Community Structure. (Under the direction of David B. Eggleston).

This study was part of a larger, more comprehensive project assessing the effects of culvert designs on freshwater mussel habitat. Because many freshwater mussels depend on an obligate relationship with certain fish hosts to complete their life cycle as well as sole mechanism for dispersal, it is critical to identify obstacles to fish movement that, in turn, could negatively impact dispersal success of mussels. The primary goal of our study was to quantify the impact of four commonly used road crossings (bridge, arch culvert, box culvert, and pipe culvert) on stream fish abundance and diversity, as well as movement. We conducted a mark-recapture study in 16 streams located in the Piedmont region of the Cape Fear River Basin of North Carolina during the summer of 2004. Following electrofishing surveys, all fish were identified to species and measured to the nearest millimeter. Fish $\leq$ 30 mm total length (TL) were individually marked elastomer paint. These procedures were repeated four, eight, and 12 weeks after the initial sampling period. With the exception of species richness, all response variables: estimates of population size, species diversity, fish index of biotic integrity (FIBI), and Conditional Percent Movement (CPM) did not vary significantly with crossing type, position (upstream and downstream), or month. Downstream reaches of box culverts contained significantly higher species richness of stream fish than other crossing types. High diversity of stream fish downstream of box culverts may have been due to a scouring effect common below box and pipe culverts which results in pool formation and a possible change from benthic to pool fish species on a local level. The general lack of stream fish abundance and diversity responses to road crossings may be due to: the insensitivity of stream fish community variables (FIBI and diversity index) to anthropogenic effects, the overall resilience of fish communities, or the shifting baseline theory--fish communities having shifted to an impacted community prior to sampling. There were extremely low numbers of individuals that moved between stream reaches in the first study, therefore we conducted a second mark-recapture study using Passive Integrated Transponder (PIT) tags with remote antenna arrays on six streams, three streams with bridges and three streams with box culverts, during the summer of 2005. We surveyed each stream using electrofishing and marked all fish measuring $\geq$ 60 mm TL with an ISO PIT tag. Custom built antenna arrays, with weir nets to direct fish passage through the antenna loop, were installed in each stream either upstream or downstream of a given crossing and detected tagged fish continuously for 30 days. Estimates of mean percent movement of fish through box culverts (28.27% ± 12.24% SE) was almost half that of bridges (44.35% ± 8.77% SE); however, the percent tagged fish detected by the antenna for bridges and culverts showed no significant difference between the two crossing types. These results suggest that a larger study might detect a significant difference in fish movement through culverts as opposed to bridges. This application of PIT tags and remote antenna arrays proved a more effective and efficient use of research funding to assess stream fish movement through culverts and we recommend the antenna systems for further non-game fish research.

IMPACT OF BRIDGES AND CULVERTS ON STREAM FISH MOVEMENT AND COMMUNITY STRUCTURE

by

JENNIFER LYNNE VANDER PLUYM

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DEDICATION

To my family for having the strength to let me pick myself up after falling down and the kindness to question my madness in an encouraging way.

To my husband, Warren Mitchell, for everything.

BIOGRAPHY

Jenny Vander Pluym was born on July 4, 1974 in St. Louis, MO where she spent her childhood as far away from the ocean as one can physically get while remaining in the continental United States. While watching Jacques Cousteau on television, Jenny decided marine biology would be an excellent profession. After graduating high school in 1992, she headed off to the University of Wisconsin in Madison, WI in hopes of learning more, oddly enough, about the world of marine science. During her time in the cultural Mecca of the Midwest, she was exposed to many new things, but continued her interest in aquatic sciences. Jenny's life changed forever after spending a year abroad in Madrid, Spain, during which she found out what it was like to be independent and an adult. Upon returning from a year of wanderlust, she stalked the only marine scientist on campus, Dr. Dianna Padilla, who, after some negotiating, agreed to be Jenny's advisor, although, very quickly became her mentor. Dr. Padilla encouraged Jenny to pursue an opportunity working as a marine educator at Newfound Harbor Marine Institute on Big Pine Key, FL. The Florida Keys allowed Jenny to observe live marine organisms for the first time in her life that she had only seen frozen under a microscope, get SCUBA certified, and meet her future husband—Warren Mitchell. This began a new chapter of Jenny's life which centered on hands-on teaching, children, and living in amazing places such as: the Florida Keys, the San Gabriel Mountains, the San Bernadino Mountains, and Catalina Island. After a fulfilling and fun-filled career in outdoor education, Jenny decided to try getting back into performing science instead of teaching it. She worked in many different areas of fisheries on land in Southern Florida, and on the sea as an observer in Hawaii and Alaska. During all of these experiences, Jenny always knew she would return to school for a graduate degree in Marine Science. After a satellite call from the Bering Sea to Dr. David Eggleston at North Carolina State University, Jenny decided to pursue her graduate studies in Raleigh, NC in August of 2003. This was yet another new place for Jenny to enjoy, especially due to the world famous Carolina barbeque. While at North Carolina State University, Jenny has gotten involved with the American Fisheries Society as member of the North Carolina chapter and served as co-president of the Student Subunit during 2005. She has also enjoyed being the oldest member of the NCSU Club Field Hockey team for three years in a row.

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Above all, I would like to thank my advisor, Dave Eggleston, for his invaluable support, guidance, and limitless patience throughout this experience. Because of Dave's varied research interests, I was given numerous opportunities to conduct marine research in amazing places. In addition, Dave found support for me so that I could finish my thesis without pressure. Thank you.

I would also like to thank my committee members: Ken Pollock, Jay Levine, Dave Genereux, and Jim Gilliam for taking time to continuously improve this project with valuable discussions and suggestions.

This achievement would not have been possible without the love, support, and patience of my husband Warren Mitchell. I would also like to acknowledge my friends and many family members who encouraged me through this experience even though they have no idea why someone would dedicate 2.5+ years to injecting fish with various foreign objects!

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CHAPTER 1

IMPACT OF BRIDGES AND CULVERTS ON STREAM FISH MOVEMENT AND COMMUNITY STRUCTURE: TRADITIONAL MARK-RECAPTURE METHODS

ABSTRACT

Alteration of streams by construction of road crossing structures can degrade stream habitat leading to: a loss of fish spawning sites, smothering endangered mussel habitat, and an overall reduction of species richness and diversity. Structures of particular interest to ecologists, managers, and the Department of Transportation (NCDOT), are bridges and culverts. Culverts are typically the most economically feasible road crossing and potentially the most damaging to biota, stream morphology, and hydraulics.

The primary goal of our study was to quantify the impact of four commonly used road crossings (bridge, arch culvert, box culvert, and pipe culvert) on stream fish abundance, diversity, and movement. This study was part of a larger, more comprehensive study assessing the effects of culvert designs on freshwater mussel habitat. Many freshwater mussels depend on an obligate relationship with certain fish hosts to complete their life cycle and for dispersal. Because there is no other mechanism for dispersal documented for these mussels, it is critical to identify obstacles to fish movement that, in turn, could negatively affect dispersal success of mussels.

We conducted field surveys of stream fish and a mark-recapture study in 16 streams located in the Piedmont region of the Cape Fear River Basin of central North Carolina during the summer of 2004. Stream reaches 50 m above and below a given road crossing, or pseudo-crossing in the case of the control stream reaches without crossings, were blocked off and sampled using a combination of seining and triple-pass electrofishing. All fish were identified to species and measured to the nearest millimeter. Specimens larger than 30 cm total length (TL) were individually marked subcutaneously

with elastomer paint tags. These procedures were repeated four, eight, and 12 weeks after the initial sampling period.

All response variables: (1) estimates of population size, (2) species richness, (3) species diversity, (4) fish index of biotic integrity (FIBI), (5) Conditional Percent Movement (CPM), and (6) interaction terms were analyzed using split-plot, repeated measures ANOVA models with crossing type (bridge, arch culvert, box culvert, pipe culvert, control) as the main factor, position (upstream and downstream of the crossing) as the sub-plot factor, and month as the repeated measure. All response variables showed no month effect; therefore the data were pooled across time and reanalyzed with a split-plot ANOVA as described above. With the exception of species richness, all response variables did not vary significantly with crossing type or position (upstream and downstream). Downstream reaches of box culverts contained significantly higher species richness of stream fish than other crossing types. High diversity of stream fish downstream of box culverts may have been due to a scouring effect common below box and pipe culverts which results in pool formation and a possible change from benthic to pool fish species on a local level. The general lack of stream fish abundance and diversity responses to road crossings may be due to: the insensitivity of stream fish community variables (FIBI and diversity index) to anthropogenic effects, the insensitivity of fish communities to the presence of crossings, the overall resilience of fish communities, or the shifting baseline theory--fish communities having shifted to a different community prior to sampling. Fish abundance and diversity did not vary significantly with continuous stream habitat characteristics such as stream velocity (m/sec) or percent run, riffle, and pool habitats within a stream reach. Because low numbers of individuals were detected as having moved between stream reaches, no conclusions can be made on the effects of road crossings on stream fish movement. A possible explanation for low CPM is the inability of the small spatial scale of this study (100 m reach surrounding each road crossing) to encompass known ranges of some fish species coupled with the length of time between recapture events (four weeks). We recommend the use of Passive Integrated Transponder tags with remote antenna arrays as a potentially more effective mark-recapture method to assess road crossing impacts on stream fish movements.

Introduction

The degradation of critical stream habitat by the construction of road crossings has been documented throughout the world (Walling 1970; Peterson and Nyquist 1972; Duck 1985; QDPI 1998). Increased sedimentation linked with bridge and culvert construction (Hainly 1980; Waters 1995) can lead to a loss of fish spawning sites (Dane 1978; Muncy et al. 1979), smothering of endangered mussel habitat (Ellis 1936; Marking and Bills 1979), and cause an overall reduction of species richness and diversity (Barton 1977).

Bridges appear to have fewer effects on stream communities than some culverts (Gosse et al. 1998; Warren and Pardew 1998). A culvert is defined as a drain or waterway passage built so that a road may cross a body of water without stopping its flow. The most common culverts are: (1) arch, a concrete archway with natural stream bottom; (2) box, a series of two or three square concrete structures allowing flow; and (3) pipe, a series of two or three corrugated steel pipes (Fig 1). Culverts with the least alteration of flow through the crossing may also be the least obstructive to fish movement (Warren and Pardew 1998).

One consequence of culvert and bridge designs is a reduction in cross sectional area for water flow, leading to increased stream velocities at certain times to levels that exceed the swimming ability of small fish and prevent their upstream movement (Orth and White 1993; Gosse et al. 1998; Warren and Pardew 1998). This alteration of water flow can disrupt movement patterns that are essential for fish growth, survival, and reproduction (Evans and Johnston 1980), as well as maintenance of community structure (Porto et al. 1999). There must also be enough water to maintain a minimum depth in the culvert to allow relatively large fish passage through the culvert during periods of low water depths (Dryden and Stein 1975). It is thought that circular and elliptical culverts are preferable over flat-bottomed designs because of their greater depth of flow per unit discharge (Dane 1978). Fish passage through culverts has been extensively studied for anadromous fishes (Bates and Powers 1998; Kayler and Quinn 1998, United States General Accounting Office 2001), but not warm-water stream fish, and relatively little information exists as to which road crossing structures impede movement of non-commercial species (Jungwirth et al. 1998).

A loss of natural structural complexity in the stream bottom is another side effect of the presence of road crossings. When culverts are installed, natural stream bottoms are physically replaced by the uniformity of a metal pipe or concrete enclosure that alter fish habitat and change the hydraulic capacity of the waterway, with riffle habitat most commonly replaced by the culvert (Dane 1978; Gosse et al. 1998). Further degradation of the stream bottom is caused downstream of crossings from the increased velocity of water through the crossing resulting in deep scour pools (Wellman et al. 2000) which alters localized riffle-run-pool ratios. Structural complexity, specifically pool-riffle-run ratios, is critical to fish interactions with their physical and biological environment and, therefore, critical to the health of the entire fish community (Angermeier and Schlosser 1988). Structurally diverse natural streams typically have a great deal of buffering capacity: meanders tend to moderate the effects of floods, pools offer excellent refuges for fishes during dry periods, and riffles act as rearing and spawning grounds for many fish species (Karr and Schlosser 1977; Schlosser 1987a). Stream habitat complexity can regulate biodiversity and production levels in the stream channel (Zalewski et al. 1998).

Stream crossings are known to increase sediment inputs and disturb the natural sedimentation of the stream ecosystem (Harper and Quigley 2000; Wellman et al. 2000). Excessive levels of sedimentation have been considered the most common pollutant in streams and rivers (Kohler and Soluk 1997) and can affect the physiology and ecology of fish communities by retarding growth caused by reduced visual feeding efficiency, clogging gills leading to suffocation, reducing disease tolerance, and altering community structure (Wallen 1951; Waters 1995). Fish with complex patterns of reproductive behavior are vulnerable to interference by suspended solids during spawning processes and can be replaced by more adaptive species (Muncy et al. 1979). Pollutant and turbidity-tolerant fish species may displace other more sensitive species (Karr 1981). Thus, increased sedimentation from scour can decrease or change the adult fish community composition and populations of some species.

Road crossings may also negatively impact populations of threatened and endangered freshwater mussels (eg. *Fusconaia masoni* (Atlantic pigtoe), *Alasmidonta varicosa* (brook floater), *Villosa vaughaniana* (Carolina creekshell), *Lampsilis cariosa* (yellow lampmussel)). There is ongoing research to use mussels as biological indicators because their sessile lifestyle exposes them to contaminants in the stream system through respiration by filter feeding as well as prolonged periods buried in sediments. Scientists use pollutant levels in the tissue of mussels as well as the overall health of the organism itself to gauge water quality of a system (Goldberg et al. 1978; Chase et al. 2001). To support populations of freshwater mussels, streambeds must contain a sufficient depth of coarse material such as sand or gravel, which allows for mussel burrowing, but which remains stable during high flows (Layzer and Madison 1995). High scour and sheer stress in streams can reduce mussel abundance by stripping the streambed of sediments necessary for mussels to persist (Johnson and Brown 2000; Hardison and Layzer 2001). Like many benthic organisms, mussels have a planktonic larval phase that has many stages. The glochidial phase, when the juvenile mussel attaches to the gills of many different species of freshwater fish, is considered the dispersal phase that is followed by settlement once the matured glochidia releases from the host fish (Weiss and Layzer 1995; Haag and Warren 1997; Haag et al. 1999). This obligate relationship between freshwater mussels and fish populations makes freshwater mussels particularly susceptible to changes in the movement patterns and diversity of their host fish (Bogan 1993).

There have been very few studies of the effects of culverts on warmwater stream fish, and none conducted in North Carolina. We used field surveys and traditional mark-recapture methods to quantify the impact of four commonly used road crossings (bridge, arch culvert, pipe culvert, box culvert) on the stream fish communities by quantifying: (1) fish population size, (2) fish species richness, (3) fish species diversity, (4) fish index of biotic integrity (FIBI), and (5) conditional percent movement (CPM). This study was part of a larger, more comprehensive study that assessed long-term effects of road-crossings on distribution of freshwater mussels. We focused on disruption of fish movement and possible shifts in fish community structure as a function of presence/absence of road crossings and crossing type. The traditional mark-recapture approach employed in this study is the most commonly used methodology to assess impacts of crossings on stream fish movement and community structure (Warren and Pardew 1998; Gagen and Landrum; Wellman et al. 2000). There are potential weaknesses associated with using these methods; therefore, we also quantified fish movement using a different tag and recapture technique which is described in Chapter 2.

Methods

<u>Site selection</u>

A total of 16 sites were selected in either a random or directed manner from a total of 50 possible sites harboring mussel populations (Fig 2). Initially, all sites were located within the Cape Fear River Basin, North Carolina, to reduce variance in stream fish community. Only two out of four arch culvert sites in the Cape Fear River Basin were viable study sites because beaver (*Castor canadensis*) dams had been built within the study reaches of two sites. To maintain a balanced study design containing a sample size of three for each road crossing type or control, a third arch culvert site was added from the Neuse River Basin, North Carolina (Fig 2). Other crossing-type sites had more than enough streams to allow a random selection. Control streams do not refer to the engineering definition of a hydraulically controlled stream, but to streams without crossings. Habitat characteristics (as outlined by the North Carolina Department of Environment and Natural Resources) such as: (1) stream width measured by a tape measure, (2) stream depth measured by a meter stick, (3) predominate substrate type (bedrock, boulder, cobble, and sand), (4) percentage of habitat type (pond, riffle, and run), (5) bank stability distinguishing between right and left banks (a scale from 1-10 with a score of 1 equivalent to "100% eroded bank" and a score of 10 equivalent to "less than 5% eroded bank"), and (6) width of riparian zone distinguishing between right and left banks (a scale from 1-10 with a score of 1 equivalent to "less than 6 m of riparian vegetation" and a score of 10 representing "greater than 18 m of riparian vegetation") were quantified at 10 m intervals for 50 m above and below each crossing. Stream reach volume and area were calculated using the average of the widths and depths for each stream reach, and multiplied by the length of each reach: 50 m. There was no predominance of a given habitat type within streams with culverts as compared to those with bridges as compared to control streams (Appendix Table 1).

Fish sampling

During May, June, July, and August of 2004, we conducted field sampling of fish assemblages and a mark-recapture study on the 16 selected streams to determine the potential impact of road crossings on fish abundance, diversity, and movement. Three techniques were used to capture fish for determining relative abundance and species richness, as well as to conduct a mark-recapture study: (1) block nets measuring 13.72 m by 1.83 m with 0.48 cm mesh to enclose 50 m reaches above and below the road crossing, (2) seine nets measuring 4.57 m by 1.22 m and 6.09 m by 1.22 m with 0.48 cm mesh to sample large pool and run habitats more effectively, and (3) electrofishing using a 12A Smith-Root back pack unit to capture fish for tagging.

All sampling periods used block-nets to enclose 50 m reaches of each stream immediately upstream and downstream of a road crossing. For control streams, we sampled in an area 50 m upstream and downstream of an imaginary road crossing measuring 15 m in length. A length of 15 m was based on the average width of road crossings in our study (Appendix Table 1). Once enclosed, stream fish in the upstream and downstream reaches of each stream were sampled using a combination of seining and backpack electrofishing; triple-pass depletion methods were used to maximize recapture rates and effort (Seber and Lecren 1967; Lyons and Kanehl 1993; Lockwood and

Schneider 2000; Meador et al. 2003). Fish were removed from the study reaches after each collecting pass and kept in pop-up laundry hampers located directly in the stream flow until all of the sampling was completed. All fish were identified and measured to the nearest 1.0 mm total length (TL).

Prior to tagging, fish were anaesthetized using clove oil in place of MS-222 due to its lack of carcinogenic compounds, effectiveness, low cost, and high survival of fish (Iverson et al. 2003; Pirhonen and Schreck 2003). We used a 1:10 solution of 100% clove oil to ethanol solution and mixed 2.5 ml of the solution with 5 liters of stream water (Pirhonen and Schreck 2003). Aerators were constantly run in all buckets during the tagging process and water was changed on the half hour to maintain ambient temperature for the captured fish.

Once fish were anaesthetized, we subcutaneously injected an elastomer tag (Northwest Marine Technologies, Shaw Island, Washington) of fluorescent red, orange, green, or yellow into fish measuring > 30 mm TL along the dorsal and anal fin regions of a fish, with specific combinations of colors and tag locations to denote location (upstream or downstream) and individual (Lotrich and Meredith 1974; Warren and Pardew 1998). Fish were released into the study reach in which they were collected after the block nets were removed.

This entire mark-recapture procedure was repeated four, eight, and 12 weeks after initial sampling to assess temporal variability in fish movement and species composition. There was no tagging during the final sampling period in August because there were no more recapture events. Fish were identified, checked for marks using an LED flashlight that illuminated the elastomer marks (Northwest Marine Technologies), and tagged if necessary before release. The day following our first recapture event in June, a bridge was removed by the NCDOT at one of our study streams, (Little Brush Creek located in Chatham County NC; Fig 2) and was replaced by an arch culvert. A similar bridge site, Brush Creek, was chosen based on its proximity and similarity to Little Brush Creek (Fig 2). The data for these two bridges were combined for all response variables (see below).

To estimate potential fish emigration from the 50 m study reaches, we also sampled an additional 50 m stretch of stream above and below the original study reaches using the exact same protocol as described above; however, this additional sampling was conducted only once at a given site and unmarked fish were not tagged. During this "emigration sampling", fish were identified and measured only if they had an elastomer tag.

Environmental data

To account for potential relationships between fish movement, species composition, and physicochemical parameters, we collected abiotic information for each stream during each monthly sampling period. Stream depth was measured using a meter stick below the road crossing. Water velocity was measured using a General Oceanics flowmeter that was held with a rod just above the streambed adjacent to the downstream portion of a road crossing for 60 seconds. Some streams had such low flows that it would not turn the flowmeter rotor. In these cases, a neutrally buoyant object was timed as it traveled a distance of 1m. Stream depth and high flow conditions were recorded using a crest gauge that recorded high flows during non-sampling periods (Pritchard 1995). We measured water temperature, dissolved oxygen and conductivity using a hand-held YSI model 85 water quality instrument equipped with turbidity and DO probes. We measured pH using a portable pH meter. The water quality instruments were cleaned and calibrated between each sampling period.

<u>Response variables and hypotheses</u>

A total of three general stream fish response variables were calculated: (1) population size, (2) community structure, and (3) conditional percent movement (CPM). We hypothesized that all response variables would be lowest in streams with pipe culverts followed by box culverts, arch culverts, and bridges, and highest in control streams, irrespective of time.

Population size

Estimates of fish population size, standard errors, and capture probabilities for each stream reach (upstream and downstream) at each monthly sample period were calculated from the triple pass depletion data using CAPTURE software accessed on the USGS website www.mbr-pwrc.usgs.gov/software.html#a. To calculate an overall population size estimate for each stream reach, triple pass fish data were also pooled over time for each stream reach and divided by four, the number of sampling periods. These results were also analyzed with the CAPTURE software. Estimates of population size were also calculated using the combined upstream and downstream data for each sample period and across time. All estimates of population size were adjusted by the volume of the stream reach in which the fish were sampled. The three pass method of estimating population size also produces standard error values for each population estimates.

Community-level response

A total of three community-level response variables were calculated: (1) species richness, (2) species diversity index, and (3) fish index of biotic integrity (FIBI). Species richness, the number of fish species sampled, was calculated for each time period, position (upstream and downstream), as well as an overall value of species richness was calculated for each site. Each species richness value was standardized by the corresponding stream reach volume (Appendix Table 1), which was calculated from the habitat data collected at the beginning of the sampling season. We also standardized fish species richness by stream area; however, we found similar results between species richness standardized by stream volume and stream area, so we only consider species richness standardized by stream volume (species richness/m^3) in the remainder of this paper.

Stream fish species diversity was calculated for each stream reach at each sampling period using the Shannon-Weiner (SW) diversity index, which is based on the equation $H = -\sum P_i \times \ln P_i$, where P_i is the proportion of i species relative to the total number of species, and $\ln P_i$ is the natural logarithm of this proportion with the base-10 (Sanders 1968). The SW diversity index is commonly used to measure diversity and accounts for variation in abundance and evenness (Magurran 1988). Stream fish species diversity was also calculated for each stream reach across time.

We used a fish index of biotic integrity (FIBI) developed by Karr (1981) and Karr et al. (1986), and subsequently modified and employed by the North Carolina Department of Environment and Natural Resources (NCDENR). Due to differences in stream reach length in our study and the protocol for estimating the NC FIBI, we chose nine out of 12 metrics calculated for the Cape Fear River Basin, NC: (1) species richness, (2) number of darter (*Etheostoma* and *Percina*) species, (3) number sunfish (*Centrarchidae*) species, (4) number species suckers (*Catostomidae*) , (5) number intolerant species, (6) % tolerant individuals, (7) % omnivorous and herbivorous individuals, (8) % insectivorous individuals, and (9) % piscivorous individuals. We tabulated FIBI scores for each reach and stream for all four sampling periods as well an overall score. These scores were meant to represent overall health of the fish community based on the FIBI utilized by the state of North Carolina. The NC Division of Water Quality (NCDWQ) published the most recent version of the index in August of 2004. Sampling for the 2004 NCDWQ FIBI was conducted during 2003 (B. Tracy, NCDWQ, pers. comm.).

Movement response

Conditional percent fish movement (CPM), was calculated for each stream, position (upstream and downstream), and time period. CPM was calculated by taking the number of fish that moved downstream divided by the sum of the fish that moved downstream and fish recaptured upstream (K.Pollock, NCSU, pers.comm.). The same calculation was performed for fish that moved upstream. This number represents how many fish moved out of the total number recaptured from the fish marked in a given stream reach. Fish that are designated as having moved are a subset of individuals that were recaptured; only they were not recaptured in the study reach within which they were originally tagged. This percentage is conditional on recapture at a given event and assumes a constant recapture rate for all species.

<u>Sampling design and statistical analyses</u>

All response variables: population size, species richness, species diversity index, FIBI, CPM, and interaction terms were analyzed using split-plot repeated measures ANOVA models with crossing type as the main factor, position (upstream and downstream) as the sub-plot factor, and month as the repeated measure. All response variables showed no month effect; therefore the data were pooled across time and reanalyzed as described above. SAS PROC MIXED was chosen over PROC GLM due to, in some cases, the violation of certain assumptions (i.e., constant variance) necessary for the use of ANOVA analysis in GLM (SAS Institute 2003). PROC MIXED uses a restricted maximum likelihood-based estimation routine (REML) based on normal distribution theory and therefore does not compute nor display sums of squares nor mean square as errors. SAS PROC MIXED also allows for heterogeneous variances across groups. In rare cases, the data were not normally distributed; therefore F statistics were used as indicators of significance, as F statistics are robust to departures of normality (Scheiner and Gurevitch 2001). Scheffe's Multiple Comparison tests were used to determine if the response variables differed between road crossings (pooled) and controls.

Lastly, linear least-squares regressive models (PROC CORR, SAS Institute 2003) tested whether or not there was a significant relationship between the response variables and continuous stream habitat characteristics such as stream flow and percent run, riffle, and pool.

Results

A total of 7,500 meters of stream reach were sampled over the four-month field season. We marked 9,594 individual fish representing 43 species and 12 families of fish (Appendix Tables 2 and 3). The number of individual fish that moved within our study scale was very low, and ranged from 0 % to 3.01% per month (Table 1). Mean percent recapture was also relatively low, and ranged from 1.91% to 9.96% per month for the study reaches (upstream and downstream; Table 1) and improved considerably (2.96% to 21.7%) when the reaches within streams were pooled (Table 2).

Fish population patterns

Estimates of population size were calculated at the family level due to low numbers of individual species. Analysis of a time effect was not possible because no family was represented at every sampling period for each stream. When the population data were pooled across time, one family, Percidae, was present in all study reaches; Centrarchidae was present in 29 out of 30 study reaches and Cyprinidae was present in 27 out of 30 study reaches. Split-plot ANOVA models assessed the effects of crossing type and position of stream reach on all three families: Centrarchidae, Cyprinidae, and Percidae. Regardless of fish family, estimates of population size adjusted by stream reach volume did not differ significantly with crossing type (Split plot ANOVA, all $F < 1.10$ and $p > 0.41$, Table 3) or position of stream reach (Split plot ANOVA, all $F < 1.36$ and $p > 0.27$, Table 3). There was no statistically significant effect of crossing type on overall estimates of population size for any of the families: Centrarchidae, Cyprinidae, or Percidae (One way ANOVA, all $F < 1.85$ and $p > 0.15$, Table 4).

Fish community patterns

Species richness adjusted by stream reach volume did not vary with crossing type (Culverts: arch, box, and pipe, bridge and control), position (upstream and downstream), nor according to time (split-plot, repeated measures ANOVA; all $p > 0.31$, Fig 3); however, there was a significant crossing type by position interaction effect (subplot error df = 4, 25, $F = 3.80$, $p = 0.0074$). The crossing type by position interaction effect was due to downstream species richness being significantly higher than the upstream section of box culvert reaches than for other crossing types or the control streams; and upstream species richness being significantly higher in control streams than streams with crossings

(Scheffe's multiple comparisons test, Fig 3). The difference of species richness means for downstream reaches of box culverts could be linked with the scour effects common to box culverts that result in a pool habitat just below the culvert (Wellman et al. 2000); however, we found no difference in percent pool between upstream and downstream reaches nor by crossing (split-plot ANOVA; all $p > 0.14$, $F < 1.94$, Fig 4). Mean fish species diversity did not vary according to crossing type or position (split-plot, repeated measures ANOVA; all $p > 0.54$, Tables 5 and 6). None of the interaction terms were significant (Tables 5 and 6).

Fish health, as represented by FIBI scores, did not vary significantly with position (split-plot, repeated measures ANOVA, all $p > 0.17$; Fig 5, Table 7); however, FIBI scores did vary significantly with crossing type (df = 4, F= 2.53, p = 0.048). A subsequent Scheffe's multiple comparisons test was unable to identify which crossing types were significantly different (df = 4, F = 1.41, p = 0.26). The significant crossing effect on FIBI was likely due to relatively low FIBI scores for stream fish near bridges compared to other crossing types (Fig 5, Table 8).

<u>Fish movement patterns</u>

Conditional percent fish movement (CPM) did not vary according to road crossing type nor position (split-plot, repeated measures ANOVA; all $p > 0.22$, Fig 6). None of the interaction terms were significant. CPM, species richness, species diversity, and FIBI showed no correlations with continuous stream habitat characteristics such as: stream flow, depth, area, volume, percent riffle and percent pool (Pearson correlation coefficients, all $-0.21 < r < 0.31$, $p > 0.09$); however, CPM demonstrated a significant negative correlation with percent run (Pearson correlation coefficients, $r = -0.39$, $p = 0.03$, Fig 7).

Habitat characteristics

Stream width ranged from 4.7 to 10 m, but was relatively similar across road crossing types (Appendix Table 1). Similarly, stream depth ranged from 0.178 to 0.685 m and was quite varied among each crossing type. Neither percent pool nor percent run varied significantly between upstream and downstream reaches or with crossing types (split-plot ANOVA, all $p > 0.06$, Fig 4).

Discussion

The results from this study suggest that road crossings have little to no impact on the fish communities of the 16 streams sampled in the Piedmont region of North Carolina, at a 100 m spatial scale and a monthly time scale. These findings support those of a study of long-term impacts of bridge and culvert construction on fish communities in Tennessee where there was no statistical difference in measurements of fish diversity, abundance, and richness between stream reaches with bridges, culverts, or without crossings (Wellman et al. 2000). Moreover, we found no difference in fish community structure between upstream reaches and downstream reaches of crossings within a stream. Conversely, Gagen and Landrum (2000) reported an almost two-fold increase in mean stream fish species richness in stream reaches downstream from bridges than stream reaches upstream from bridges (control) on upland tributaries of the Oachita River, Arkansas.

Because there were extremely low numbers of individual fish that moved between upstream and downstream reaches in this study, no strong conclusions can be made on the effects of road crossings on stream fish movement. Stream fish movement through culverts in the Oachita Mountains of west-central Arkansas was an order of magnitude lower than through other crossing types, although there was little difference in stream fish movement between natural reaches and open box culverts (Warren and Pardew 1998). One main difference between the Warren and Pardew (1998) study and this one is in our definitions of culvert types. According to their study, only pipe culverts were in the category “culvert”, and two out of the four culverts sampled were perched 5-8 cm above the downstream reaches during some part of the study, creating a physical barrier to stream fish movement (Warren and Pardew 1998). Our study did not include any streams with perched crossings or those that were dry throughout the summer of 2004. It is possible that the inclusion of perched crossings in the Warren and Pardew (1998) study biased their findings towards negative impact of culverts on fish movement relative to this study. Conversely, crossings classified as “open-box” in the Warren and Pardew (1998) study were similar to our definition of box culverts, which would make the results from both studies comparable because there was no effect of box culverts (this study) and open box (Warren and Pardew 1998) on stream fish movement. The Warren and Pardew (1998) study also used sample reaches that were 100-150 m long, which may have improved their chances of detecting negative impacts of road crossings on stream fish.

A potential problem with using community structure as an indicator of ecosystem health is the resilience, or the ability of an ecosystem or community to recover after a disturbance, of warm-water stream fish. Fish communities can recover from construction activities within one year (Barton 1977; Peterson and Nyquist 1972). All of the crossings included in this study were over 30 years old giving the stream fish communities ample time to recover or re-equilibrate after construction of the crossings. Wellman et al. (2000) compared fish community with sediment deposition below culverts and bridges and documented sediment as having little effect on fish community structure on the short term (one year), but concluded prolonged sediment addition to downstream reaches would be enough to impair spawning activities of rare species with limited habitats.

Long term exposure to anthropogenic effects such as sedimentation from crossings, bank erosion resulting from clear cutting, and agricultural run-off, could weaken the resilience of a fish community to natural and human induced perturbations causing a shift to an alternative stable state, such as a more tolerant community (Scheffer et al. 2001; Carpenter 2002). Scheffer et al. (2001) further states, “feedbacks that stabilize different states involve both biological and physical and chemical mechanisms.” Thus, in stream ecosystems, consistent sediment loading, scouring, and flow alteration potentially caused by culverts could not only lead to a shift in stream fish communities, but could further insure the resilience of the potentially new, degraded stable state. The fish communities that we sampled could have shifted long ago and are now the assemblages maintained by these altered streams.

The lack of a road crossing effect on stream fish diversity may have also been due, in part, to metrics used to assess community structure. The Shannon-Weiner index incorporates richness, abundance, and evenness of species while giving importance to rare species (Pielou 1975), but lacks attributes of function (trophic level) or community structure (Brooks 2003; Roy et al. 2004); thereby, giving an incomplete measure of the fish community as a whole. Species richness can also be a misleading measurement of a fish assemblage. For example, when fish species richness was compared against levels of urbanization in the Eastern Piedmont and Coastal Plain regions of Maryland, obvious shifts from sensitive to tolerant fish species were observed, whereas fish species richness and abundance remained unchanged (Morgan and Cushman 2005). The use of species richness to detect changes in fish communities due to habitat destruction and species introduction was found to be misleading because of the inclusion of invasive species, whether native or endemic, in the species richness value (Scott and Helfman 2001). Alternatives to species richness as community structure measurements are indices of biotic integrity, which may be a more comprehensive and sensitive litmus to changes in organismal communities (Scott and Helfman 2001).

Much effort has been put into developing regional indices of biotic integrity to assess the health of stream ecosystems (Karr et al. 1986; Fausch et al. 1990; Roth et al. 1996), as well as in detecting the ecological impacts of human induced disturbances (Steedman 1988; Schulz et al. 1999; Teels et al. 2004). Although acceptance and use of these indices is prevalent in stream ecosystem literature (Hughes et al. 1990), recent studies have found that FIBI scores can be insensitive to known anthropogenic disruptions. For example, abundance is a more sensitive metric of population health for common and rare fish species in a given stream system than is percent occurrence between impacted and reference streams (Pirhalla 2004). The North Carolina FIBI has one metric of abundance for tolerant species, but uses only a percent occurrence of intolerant species. In a comprehensive study aimed at identifying indicators of urbanization effects on streams, abundance of sensitive fish species was a consistent response to urban impacts, whereas overall fish abundance and that of tolerant species were inconsistent responses (Walsh et al. 2005). When used to detect anthropogenic effects on lakes in Florida, FIBI scores were unreliable as higher scores were recorded for the lakes most impacted by human presence (Schulz et al. 1999). FIBI scores can be effective indicators of short term fish community recovery after disturbance, but ineffective as indicators of long term disturbance (Paller et al. 2000).

When examining ecosystems for changes due to anthropogenic influences, it is imperative to have natural benchmarks with which the data can be compared (Pauly 1995; Tegner and Dayton 1998). This is a major tenet of the 'shifting baseline syndrome' where each new generation of observers accepts, for example, the species composition and fishery stock size at the beginning of their careers as baseline, which results in inappropriate reference points for evaluating disturbances and establishing objectives for restoration. All indices of stream fish biotic integrity use a scale relative to the healthiest stream of a system (the reference stream), such that if that reference stream is also impacted and currently hosting a degraded community, the scores might indicate good stream health erroneously. It is also possible that a mobile fish community is not sensitive to alterations to stream characteristics so it is not a suitable indicator of stream health.

Regional environmental conditions, such as habitat ratios (riffle, run, pool) and sedimentation rates, are important in structuring fish communities (Maret et al. 1997; Waite and Carpenter 2000); however, it is possible that the natural variation of these fish communities may mask anthropogenic effects on stream fish assemblages (Grossman et al.1990; Fitzgerald et al. 1998; Grossman et al. 1998). For example, similar fish assemblages dominated by cosmopolitan species relative to endemic species were associated with stream reaches with high percent urban cover (Roy et. al. 2005), as well as correlated with stream reaches of decreased slope with less percent urban cover (Walters et. al. 2003b) on the Etowah River, Georgia. It is possible that any community changes due to road crossings in our study streams were indecipherable from the backdrop of the natural variation of that fish assemblage.

An ideal method to assess changes in a community due to anthropogenic impacts is that of a Before-After-Control-Impact (BACI) study (Underwood 1996). Extreme foresight and funding is needed for this approach since the study must take place prior to and after construction of a road crossing. This approach was not possible for our study since the road crossings were constructed 30 years or more ago; however, we suggest that future studies assessing the impacts of road crossings on fish community structure strive to employ BACI designs whenever feasible. For studies that include older crossings, we suggest that a more sensitive organism or community index, such as mussels or insects, be used to assess stream ecosystem health.

The practical difficulties of tracking large numbers of organisms through space and time are common in ecological field studies, resulting in a paucity of empirical information on taxa, specifically non-commercially important taxa (Okubo 1980; Turchin 1998 *in* Skalski and Gilliam 2000). The low number of fish that moved (Mean 0%-2.06% of fish tagged) within our study reaches indicates either a flaw with the spatial and temporal scale of the study, or a fish community dominated by sedentary members. It is possible that sampling 50 m above and below the road crossing was not a large enough area to capture the movement patterns of stream fish using mark-recapture methods in this study. When assessing distribution patterns and community organization of an assemblage, sampling should include the minimum home-range sizes of the dominant species (Grossman 1982; Grossman et al. 1982). Skalski and Gilliam (2000) found that while most individuals remained within 10-100 meters of the initial tagging site, four freshwater fish species (blue head chub, creek chub, redbreast sunfish, and rosyside dace; see Appendix Table 2 for scientific names), which were also the most common species across all 16 streams of our study, were able to travel distances up to 200 meters upstream and downstream over a five-month period. Other mark-recapture studies of stream fish report similar findings, whereby the fish populations were comprised of mostly 'stayers' that occupy limited areas and a few 'movers' that roam larger areas (Gerking 1959; Heggenes et al. 1991; Freeman 1995). The majority of recaptures over an 18 month period of juvenile redbreast sunfish and adult blackbanded darter were within 33 m of the original capture location (Freeman 1995). It is possible that the majority of stream fish in our study communities remained in the sample area and the lack of movement between study reaches in our study was due to small home ranges and not the 100 m spatial scale of sampling.

The spatial scale of sampling was expanded to 200 m once for each stream in this study to assess potential fish emigration from our 50 m study reaches after the initial tagging. Even with this expanded spatial resolution, only four streams had any fish recaptured from the extended sample reaches. Thus, one could assume that either the fish are staying in our reaches and electrofishing is not an effective way to sample them, or fish are moving out of both the sample 50 m reaches and the extended "emigration reaches." The latter is a more likely explanation, as electrofishing is an effective and common method to sample wadeable streams.

The time between recapture events might also have been a factor in our inability to capture potential movers within our study design. For example, in a similar study conducted by Warren and Pardew (1998), a smaller number of stream reaches were sampled than in our study with two-pass rather than triple-pass depletion sampling, which allowed for less time (12-17 days) between recapture events, as opposed to 30 days in our study. Monthly sampling intervals, however, were used by Skalski and Gilliam (2000) in a mark-recapture study of stream fish movements, but the area sampled ranged from 400-660 meters of one continuous stream reach. The use of mark-recapture alone may not have been effective at capturing patterns of fish movement at this temporal scale. Redbreast sunfish, a dominant fish in our study reaches, has been documented to travel 95 m within 24 hours of initial capture (Freeman 1995). Stream fish studied in Illinois have demonstrated rapid movement into defaunated sections of study streams within 60-140 hours after removing block nets (Peterson and Bayley 1993). Ideally, a combination of mark-recapture and telemetry sampling would give a conclusive picture of fish movement through road crossings (Murphy and Willis 1996).

Although designed using the best available and most commonly utilized approach, this study highlights problems with traditional mark-recapture methods used to assess fish movements through space and time. We recommend the use of PIT tags and remote antenna arrays, also called gates, for 24 hour monitoring of fish movement through a designated area (Morhardt et al. 2000; Barbin Zydlewski et al. 2001). This system places an antenna in the stream that will detect any fish carrying a PIT tag as it passes through the array while an electronic reader housed on shore downloads and stores all of the tag codes. The PIT tag method has the potential to increase sample sizes and use man-power more efficiently and effectively by reducing: (1) the number of sampling events, (2) sampling bias due to fright response, (3) recording error, and (4) handling time of fish, since individual fish are not disturbed upon recapture (Gibbons and Andrews 2004). Tag dimensions (12 mm) would restrict the size of fish that could be tracked to individuals greater than 60 mm TL, but would give a more accurate evaluation of the numbers of fish moving through crossings versus control areas because of the increased recapture rates (95-100% read efficiency), as well as the ability to monitor fish movements 24 hours a day and seven days a week (Gibbons and Andrews 2004).

This study was meant to produce scientific evaluations of culvert designs based on fish movement and community structure, as opposed to studies based on structural viability and cost. This is the first time the state of North Carolina has funded an ecology-based investigation of NCDOT crossing designs. Modification of culverts does not have to be limited to just minimizing ecosystem impacts of the structure, but can also be designed to the enhance habitat of the ecosystem. In Slawski and Ehlinger's (1998) groundbreaking study, they looked at the possibility of altering culvert design so that the culvert itself could be a habitat for fish. By elaborating on the principle that roughening the bottom of the culvert as means to slow flow and ease fish passage (Bates and Powers 1998), they modified culverts using baffles to increase habitat heterogeneity within the culvert.

Conclusions

The results of this study suggest that mobile stream fish communities may not be sensitive to stream ecosystem degradation and therefore, may not be the best organisms to use as indicators of stream health. The use of stream fish species richness and FIBI scores may not be an accurate measurement of long-term and consistent anthropogenic impacts on stream systems. The need for more sensitive measures to distinguish natural changes in an ecosystem from those caused by humans is highlighted in our results. Our study also points to the need to critically evaluate sampling designs for studies that assess the impacts of culverts as well on fish abundance, diversity, and movement. There is an inherent trade-off between more fish captured and more precise population estimates when more stream reach is sampled with fewer passes. Depletion methods as well as mark-recapture studies rely on multiple passes for population estimates. Future areas of research would be to further use our data to calculate cost-benefit analysis for using triple-pass versus double-pass depletion methods when planning future field studies. We recommend the use of PIT tags and remote antenna arrays for 24 hour monitoring of fish movement through a designated area. The PIT tag approach would restrict the size of fish that could be tracked, but would give a true evaluation of numbers of fish moving through a road crossing versus a control area. As more bridges are displaced by culverts it is imperative to understand the impacts of these crossings. Further research should be done to assess larger scale influence of culverts on stream ecosystems.

The collaborative nature of this study has produced a comprehensive amount of site-specific information on streams located in the Piedmont of North Carolina, which should facilitate ecosystem restoration. Using state funds to support an ecologically motivated study shows the dedication of NCDOT to the health of fresh-water streams. Once new designs for road crossings are initiated that attempt to minimize impacts, attention can be diverted to how to alleviate the previously impacted streams. This could lead to policy and restoration methods specific to culvert designs. North Carolina can be an example to other states and countries that a partnership between government and science can result in universal benefit.

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Table 1: Number of individual stream fish that moved upstream or downstream, overall % individuals that moved out of all fish that were tagged, mean % recaptured of tagged individuals over all three recapture periods (N=3), and overall Conditional Percent Movement (CPM) by individual stream reach.

Crossing	Creek	Position	Fish Moved	% Moved	% Recapture (±SE)	CPM
Arch	Horse	D	2	0.55%	3.17% (0.09)	5.00
	Horse	U	1	0.28%	3.54% (0.20)	3.70
	Rock	D	0	0.00%	2.85% (1.48)	16.67
	Rock	U	7	2.70%	7.80% (2.70)	41.18
	Terrells	D	3	0.48%	4.37% (0.36)	10.18
	Terrells	U	14	3.01%	8.09% (0.27)	23.33
Box	Marys	D	2	0.90%	7.47% (2.14)	4.21
	Marys	U	2	0.49%	6.71% (1.84)	3.28
	Poppaw	D	2	0.67%	6.40% (1.57)	9.68
	Poppaw	U	7	2.08%	6.66% (1.52)	14.00
	Wet	D	1	0.49%	4.15% (1.55)	10.26
	Wet	U	3	1.26%	4.37% (2.20)	12.50
Bridge	Brush	D	5	0.73%	5.90% (2.96)	13.85
	Brush	U	4	1.03%	3.27% (1.96)	10.00
	Little	D	1	0.22%	4.32% (0.79)	4.23
	Little	U	2	1.37%	6.34% (1.98)	12.50
	Polecat	D	0	0.00%	8.53% (2.23)	0.00
	Polecat	U	0	0.00%	4.62% (1.95)	0.00
Pipe	Dry	D	4	1.43%	1.91% (0.87)	5.30
	Dry	U	5	2.00%	9.20% (3.17)	6.25
	Reed	D	8	2.09%	9.96% (0.58)	7.27
	Reed	U	2	0.51%	6.62% (2.62)	4.76
	Rock	D	10	3.53%	8.83% (1.56)	4.41
	Rock	U	2	0.63%	9.21% (2.91)	0.00
Control	Brooks	D	3	0.92%	9.37% (1.54)	14.52
	Brooks	U	5	1.36%	9.78% (2.72)	27.78
	Flat	D	3	0.82%	4.43% (1.81)	6.80
	Flat	U	1	0.34%	3.62% (1.13)	2.38
	N_Prong	D	3	0.83%	6.83% (3.93)	8.82
	N_Prong	U	0	0.00%	5.46% (2.12)	2.78

Table 2: Mean percent stream fish that moved between study reaches within a stream regardless of direction and percent stream fish recaptured for each stream across all sampling periods (N = 6 for all results).

Crossing	Creek	% Moved	% Recaptured
Arch	Horse	0.41	7.80
	Rock	1.61	8.90
	Terrells	1.55	12.60
Box	Marys	0.68	15.50
	Poppaw	1.63	14.50
	Wet	1.08	9.18
Bridge	Brush	1.14	2.96
	Little	0.51	12.10
	Polecat	0.00	14.80
Pipe	Brooks	1.72	21.70
	Flat	1.29	7.5
	North Prong	2.06	11.05
Control	Dry	1.18	10.30
	Reed	0.62	18.00
	Rock	0.51	21.30

Table 3: Mean estimates of population size adjusted by stream reach volume for the three dominant fish families captured in NC Piedmont streams: Percidae, Centrarchidae, and Cyprinidae, in downstream and upstream (D and U) reaches in streams with crossing types (Culverts: Arch, Box, Bridge, Pipe, and Control). Estimates were calculated using CAPTURE software to analyze triple pass depletion data pooled across the 4 sample periods for each stream reach. Population means and standard errors were calculated for each position within a crossing type (N=3). (*N=2)

Family	Crossing	Position	Pop Mean/m^3	SE
Percidae	Arch	D	0.381	0.209
	Arch	U	0.439	0.317
	Box	D	0.115	0.049
	Box	U	0.274	0.146
	Bridge	D	0.361	0.070
	Bridge	U	0.354	0.273
	Pipe	D	0.123	0.085
	Pipe	U	0.186	0.054
	Control	D	0.121	0.012
	Control	U	0.374	0.209
Centrarchidae	Arch	D	0.575	0.083
	Arch	U	0.345	0.060
	Box	D	0.506	0.200
	Box	U	0.519*	0.071
	Bridge	D	0.428	0.184
	Bridge	U	0.450	0.296
	Pipe	D	0.671	0.261
	Pipe	U	0.526	0.103
	Control	D	0.491	0.150
	Control	U	0.726	0.277
Cyprinidae	Arch	D	0.446	0.251
	Arch	U	0.715	0.348
	Box	D	0.862*	0.058
	Box	U	1.053*	0.304
	Bridge	D	1.277	0.805
	Bridge	U	1.138	0.581
	Pipe	D	0.630	0.471
	Pipe	U	0.932	0.372
	Control	D	0.306	0.246
	Control	U	0.804	0.276

Table 4: Mean population size estimates for three dominant fish families: Percidae, Centrarchidae, and Cyprinidae, for all crossing types (Culverts: Arch, Box, Bridge, Pipe, and Control) pooled across position (Downstream and Upstream), creek (3 streams with each crossing type) and sample periods (4 samples).

Family	Crossing	Pop Mean	SE	N
Percidae	Arch	0.410	0.170	6
	Box	0.195	0.078	6
	Bridge	0.358	0.126	6
	Pipe	0.155	0.047	6
	Control	0.248	0.109	6
Centrarchidae	Arch	0.460	0.069	6
	Box	0.511	0.112	5
	Bridge	0.439	0.156	6
	Pipe	0.599	0.129	6
	Control	0.609	0.150	6
Cyprinidae	Arch	0.581	0.201	6
	Box	0.958	0.138	4
	Bridge	1.208	0.445	6
	Pipe	0.781	0.277	6
	Control	0.555	0.199	6

Table 5: Mean Shannon Weiner species diversity index score, standard error, and number of stream reaches (N) per crossing type (Culverts: Arch, Box, Bridge, Pipe; and Control) and position (Downstream and Upstream). See text for results of statistical analyses of means.

Crossing	Position	Mean Div Index	SE	N
Arch	D	2.20	0.09	3
Arch	U	2.30	0.14	3
Box	D	2.16	0.07	3
Box	U	2.18	0.11	3
Bridge	D	2.08	0.15	3
Bridge	U	2.07	0.08	3
Pipe	D	2.01	0.26	3
Pipe	U	2.27	0.18	3
Control	D	2.20	0.13	3
Control	U	2.22	0.04	3

Table 6: Mean Shannon Weiner species diversity index score, standard error, and number of stream reaches (N) per crossing type (Culverts: Arch, Box, Bridge, Pipe; and Control). See text for results of statistical analyses of means.

Crossing	Mean Div Index	SE	N
Arch	2.25	0.08	6
Box	2.17	0.06	6
Bridge	2.08	0.08	6
Pipe	2.14	0.15	6
Control	2.21	0.06	6

Table 7: Mean fish index of biotic integrity (FIBI), standard error, and number of stream reaches (N) per crossing type (Culverts: Arch, Box, Bridge, Pipe; and Control) and position (Downstream and Upstream). See text for statistical analyses of means.

Crossing	Position	Mean FIBI	SE	N
Arch	D	40.79	3.58	3
Arch	U	46.99	3.20	3
Box	D	43.23	4.30	3
Box	U	37.46	4.88	3
Bridge	D	37.24	4.37	3
Bridge	U	37.46	5.91	3
Pipe	D	42.12	4.83	3
Pipe	U	41.90	4.64	3
Control	D	43.45	3.97	3
Control	U	43.00	3.49	3

Table 8: Mean fish index of biological integrity (FIBI), standard error, and number of stream reaches (N) per crossing type (Culverts: Arch, Box, Bridge, Pipe; and Control). See text for results of statistical analyses of means.

Crossing	Mean IBI	SE	N
Arch	43.89	1.85	6
Box	40.34	2.36	6
Bridge	37.35	2.57	6
Pipe	42.01	2.47	6
Control	43.23	1.63	6

Figure 1: Examples of the crossing types assessed in this study (clockwise from top left): bridge, arch culvert, box culvert, and pipe culvert (Photographs by Chris Eads).

Figure 2: Study sites located west and north of Raleigh, NC, in the Cape Fear River basin. Each crossing type is represented by a different symbol. The letters inside each symbol correspond to an individual appendix table for each stream.

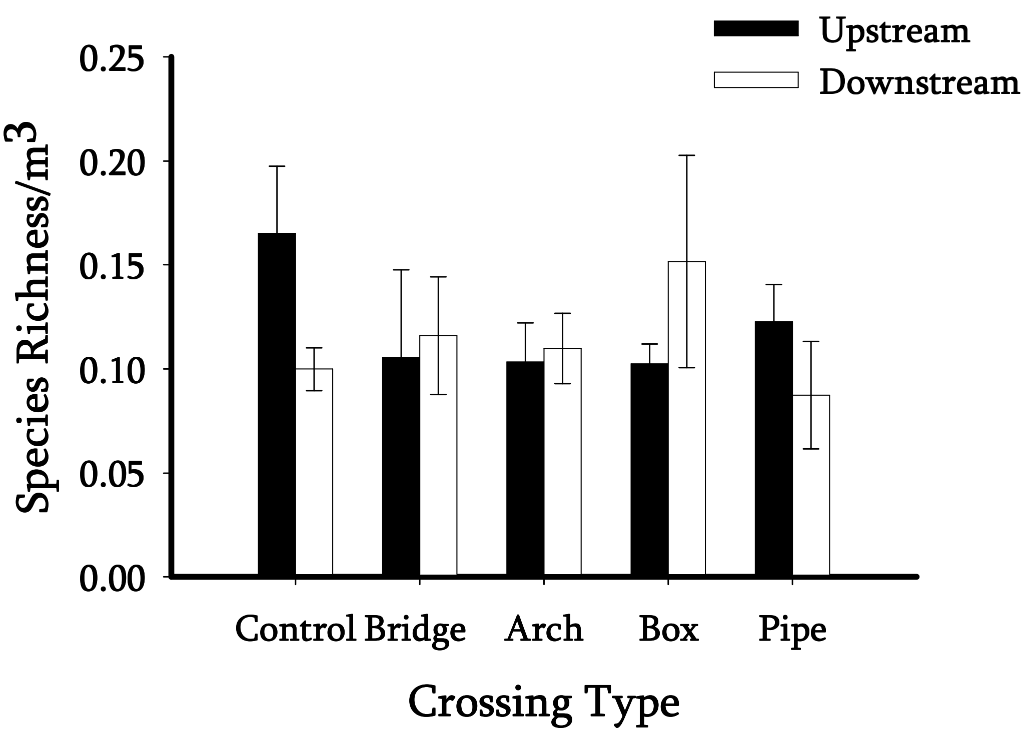

Figure 3: Mean stream fish species richness per m^3 (± SE) for each crossing type (bridge, culverts: arch, box, pipe, and control) and position (upstream and downstream), N = 3. See text for results of statistical analyses.

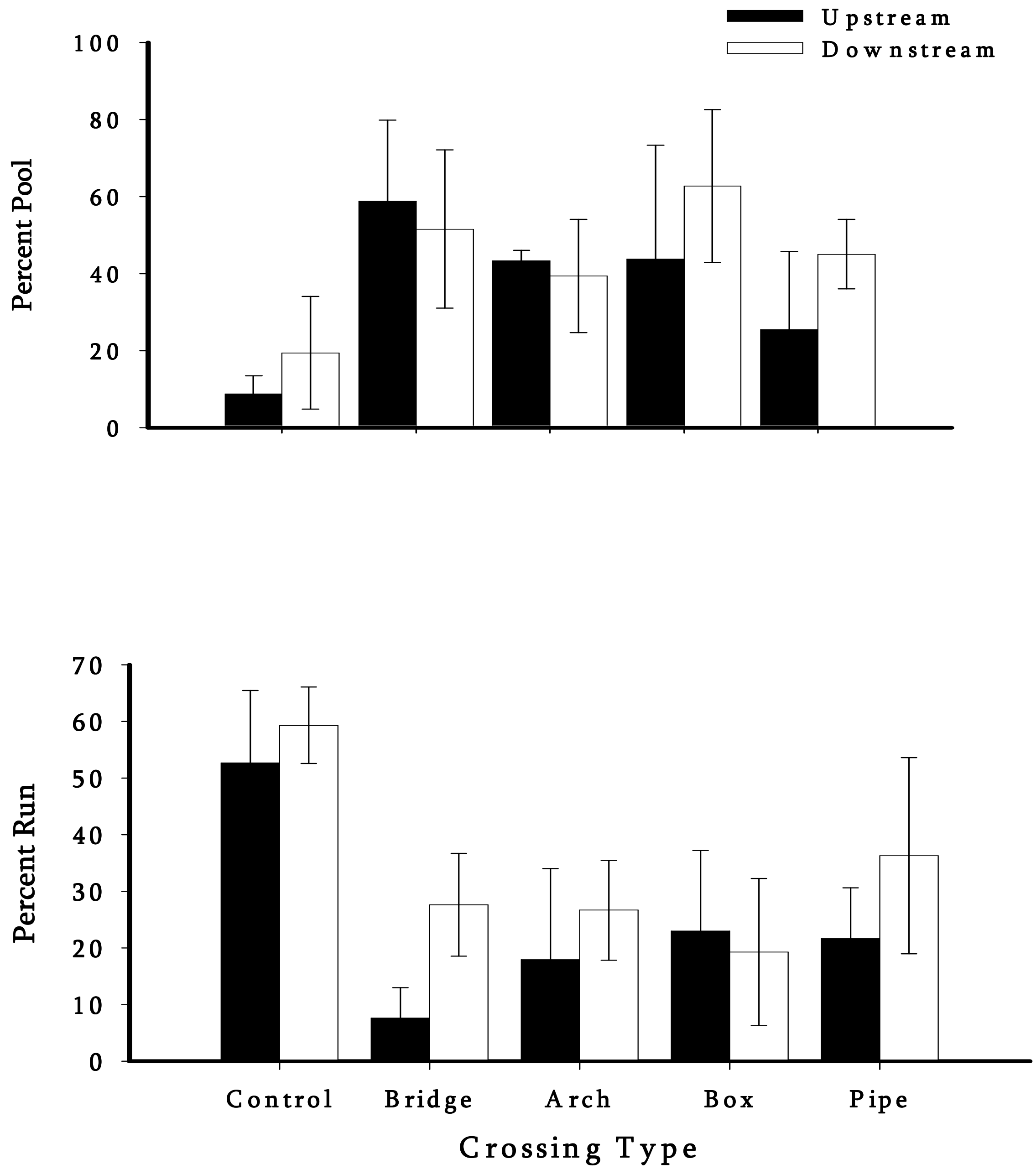

Figure 4: Mean percent pool (± SE) and mean percent run (± SE) of stream reach (50 m) by crossing type (bridge, culvert: arch, box, pipe, and control) and position (upstream and downstream), N = 3. See text for statistical analyses.

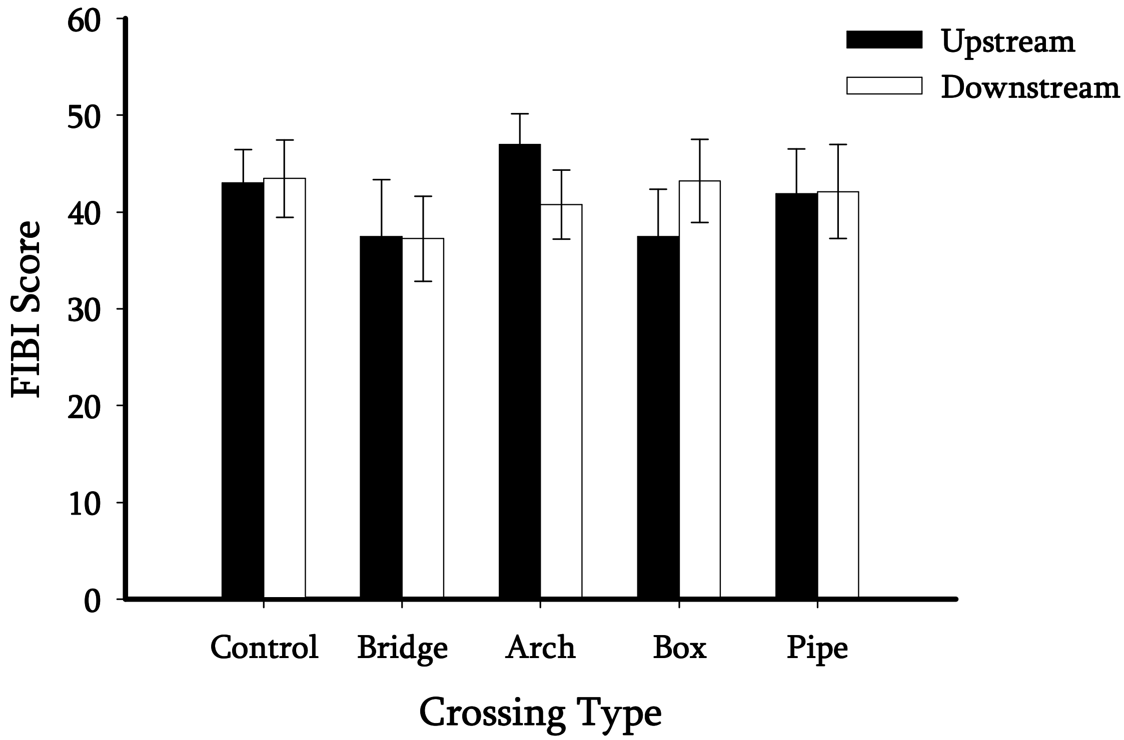

Figure 5: Mean fish index of biotic integrity (FIBI) score (± SE) of species for each crossing type (bridge, culvert: arch, box, pipe, and control) and position (upstream and downstream), N = 3. See text for results of statistical analysis.

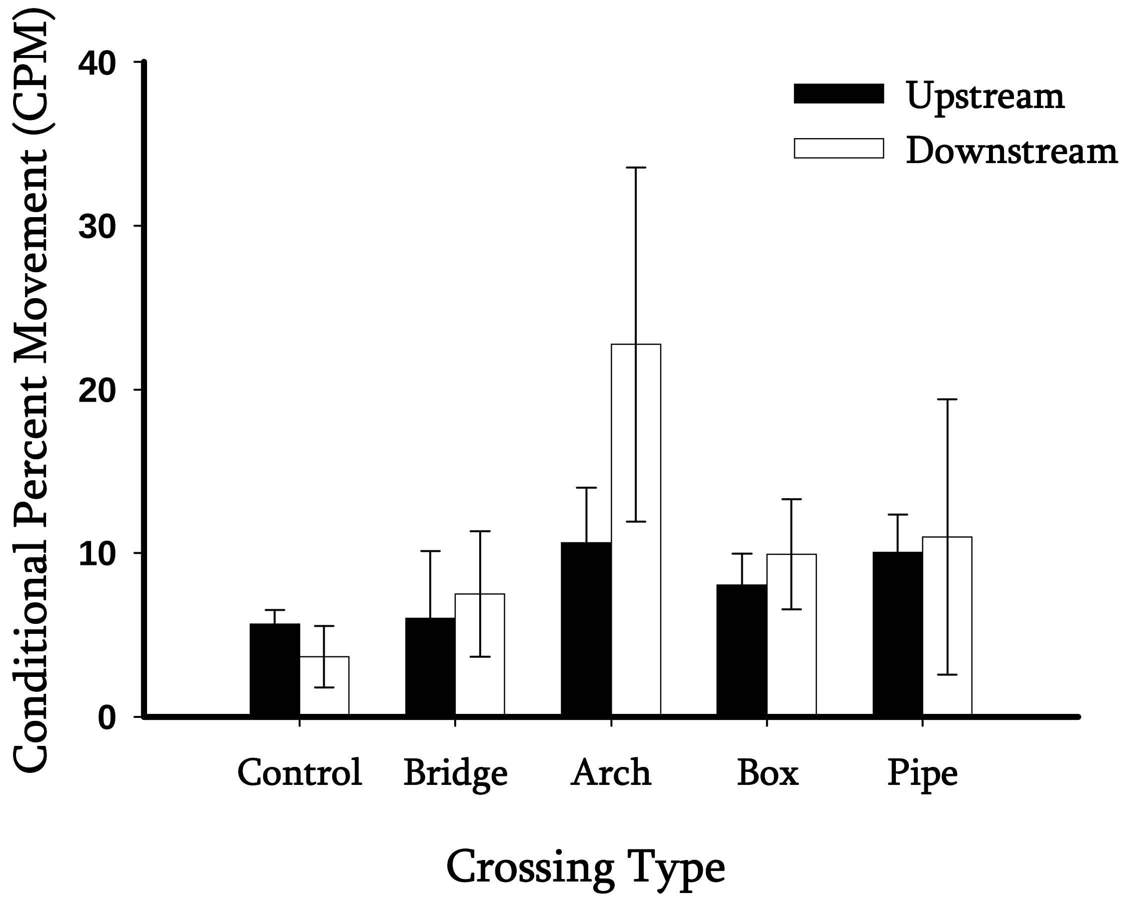

Figure 6: Mean stream fish conditional percent movement (± SE) by crossing type (bridge, culvert: arch, box, pipe, and control) and position (upstream and downstream), N = 3. See text for statistical analyses.

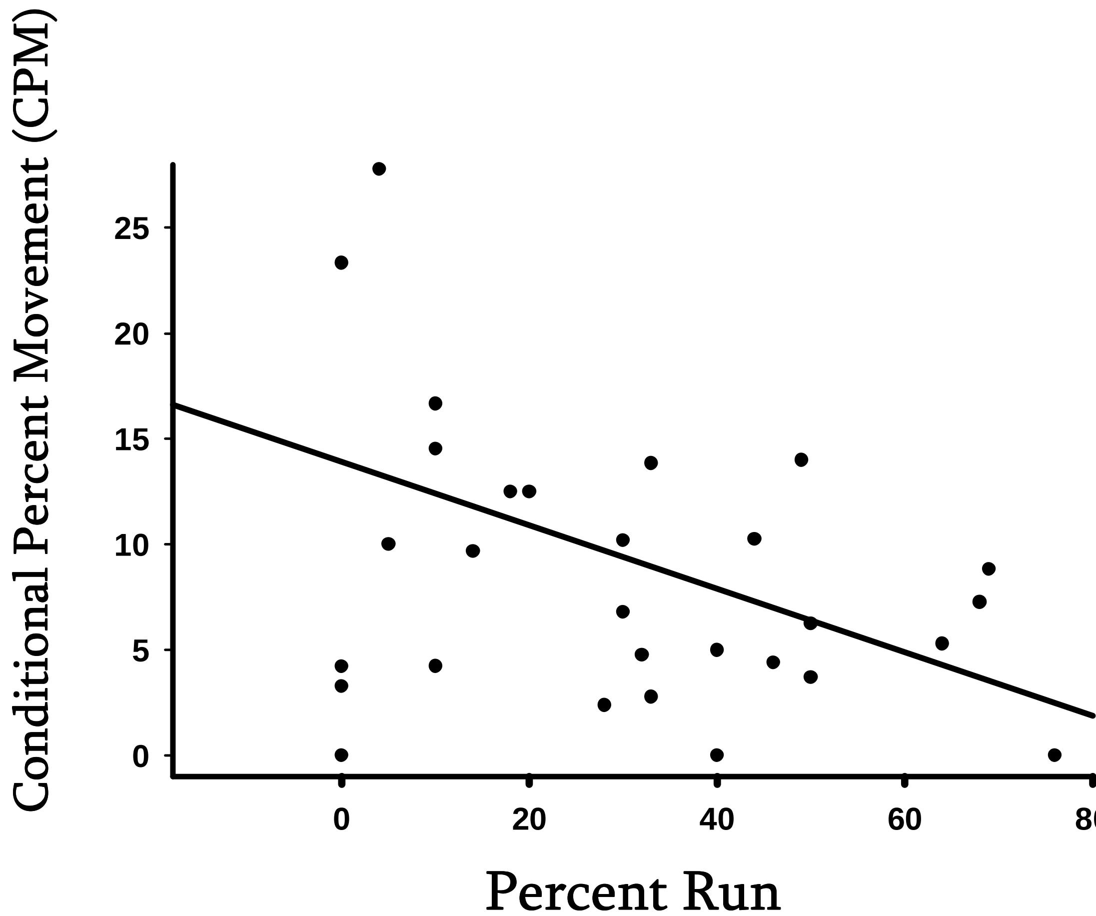

Figure 7: Conditional percent movement (CPM) vs. percent run by stream reach. CPM is negatively correlated with percent run (Correlation coefficient = -0.39, p = 0.03), N = 30. See text for statistical analysis.

APPENDICES

Appendix Table 1. Habitat characteristics measured 50 m downstream and upstream of each road crossing. Mean width, depth, % pool, % riffle, and % run were calculated from measurements collected every 10 m in length in each stream reach. Substrate refers to prominent bottom make-up for each reach.

Crossing	Creek	Position	Width (m)	Area (m^2)	Vol (m^3)	Depth (m)	% Pool	% Riffle	% Run	Substrate
Arch	Horse	Down	6	300	125.4	0.418	10	50	40	Gravel, cobble
	Rock		6.2	310	145.7	0.47	56	34	10	Gravel, sand, boulder
	Terrells		7.2	360	124.56	0.346	52	18	30	Cobble, boulder
	Horse	Up	10	500	251	0.502	46	4	50	Boulder, cobble
	Rock		7.75	387.5	113.92	0.294	38	58	4	Cobble, sand
	Terrells		6	300	111.6	0.372	46	54	0	Cobble, gravel, debris
Box	Marys	Down	5.5	275	119.35	0.434	100	0	0	Cobble, sand
	Poppaw		5.9	295	117.41	0.398	32	44	14	Cobble
	Wet		8.2	410	210.74	0.514	56	0	44	Bedrock, sand
	Marys	Up	5.8	290	149.64	0.516	100	0	0	Boulder, silt, cobble
	Poppaw		5.6	280	56	0.2	31	20	49	Cobble
	Wet		6.9	345	81.42	0.236	0	80	20	Bedrock, sand
Bridge	Brush	Down	6	300	93.36	0.3112	35	25	40	Bedrock, boulder, cobble
	Little Brush		5.24	262	85.94	0.328	54	20	26	Cobble, sand
	Little		7.3	365	153.3	0.42	90	0	10	Cobble, boulder
	Polecat		5.8	290	81.2	0.28	20	40	40	Cobble, gravel
	Brush	Up	6.2	310	166.78	0.538	50	40	10	Boulder, cobble
	Little Brush		4.7	235	68.15	0.29	42	58	0	Cobble
	Little		6.1	305	93.94	0.308	30	52	18	Cobble, boulder
	Polecat		7.5	375	256.87	0.685	100	0	0	Sand, gravel
Pipe	Dry	Down	7.3	365	206.59	0.566	54	36	10	Cobble, sand
	Reed		5.9	295	99.12	0.336	27	43	30	Cobble, sand, gravel
	Rock		7.7	385	212.52	0.552	54	13	33	Sand, silt
	Dry	Up	6.6	330	102.96	0.312	4	0	96	Sand, gravel
	Reed		6	300	103.8	0.346	66	6	28	Boulder, cobble
	Rock		7	350	120.4	0.344	6.00	25.00	69.00	Sand, silt

Appendix Table 1.---Extended.

Crossing	Creek	Position	Width (m)	Area (m^2)	Vol (m^3)	Depth (m)	% Pool	% Riffle	% Run	Substrate
Control	Brooks	Down	7.1	355	132.77	0.374	0	36	64	Cobble, boulder
	Flat		7.8	390	158.34	0.406	10	22	68	Cobble
	N. Prong		5.4	270	105.3	0.39	48	6	46	Cobble, gravel
	Brooks	Up	7.8	390	102.18	0.262	10	40	50	Cobble, boulder
	Flat		6.1	305	54.29	0.178	16	52	32	Cobble, boulder, gravel
	N. Prong		5.2	260	109.2	0.42	0	24	76	Cobble, gravel

Appendix Table 2. Comprehensive list of fish families and species collected by a combination of seining and backpack electrofishing during summer 2004 in the Cape Fear and Neuse River Basins, North Carolina.

Family	Scientific Name	Common Name
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch
Catostomidae	*Erimyzon oblongus*	Creek chubsucker
	Hypentelium nigricans	Northern hog sucker
	Moxostoma collapsum	Notchlip redhorse
Centrarchidae	*Lepomis auritus*	Redbreast sunfish
	Lepomis cyanellus	Green sunfish
	Lepomis gibbosus	Pumpkinseed
	Lepomis gulosus	Warmouth
	Lepomis macrochirus	Bluegill
	Lepomis microlophus	Redear sunfish
	Micropterus salmoides	Largemouth bass
	Pomoxis nigromaculatus	Black crappie
Clupeidae	*Dorosoma cepedianum*	Gizzard shad
Cyprinidae	*Clinostomus funduloides*	Rosyside dace
	Cyprinella analostana	Satinfin shiner
	Cyprinella spiloptera	Spotfin shiner
	Cyprinella nivea	Whitefin shiner
	Luxilus albeolus	White shiner
	Nocomis leptocephalus	Bluehead chub
	Notemigonus crysoleucas	Golden shiner
	Notropis alborus	Whitemouth shiner
	Notropis altipinnis	Highfin shiner
	Notropis chiliticus	Redlip shiner
	Notropis hudsonius	Spottail shiner
	Semotilus atromaculatus	Creek chub
Esocidae	*Esox americanus americanus*	Redfin pickerel
	Esox niger	Chain pickerel
Fundulidae	*Fundulus rathbuni*	Speckled killifish
Ictaluridae	*Ameiurus brunneus*	Snail bullhead
	Ameiurus nebulosus	Brown bullhead
	Ameiurus platycephalus	Flat bullhead
	Noturus insignis	Margined madtom
Moronidae	*Morone americana*	White perch
Percidae	*Etheostoma flabellare*	Fantail darter
	Etheostoma nigrum	Johnny darter
	Etheostoma olmstedi	Tessellated darter
	Etheostoma serrifer	Sawcheek darter
	Etheostoma vitreum	Glassy darter
	Perca flavescens	Yellow perch
	Percina crassa	Piedmont darter
	Percina roanoka	Roanoke darter
Poeciliidae	*Gambusia sp.*	Mosquitofish

Appendix Table 3(a). Fish families and species for Horse Creek, a stream with an arch culvert, collected by a combination of seining and backpack electrofishing during summer 2004. Horse Creek was sampled in Wake County, NC (Lat: 35 58° 25 N, Long: 78 33° 40 W), and was accessed from SR 1923.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	1
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	4
	Hypentelium nigricans	Northern hog sucker	7
	Moxostoma collapsum	Notchlip redhorse	9
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	60
	Lepomis cyanellus	Green sunfish	2
	Lepomis gibbosus	Pumpkinseed	3
	Lepomis gulosus	Warmouth	3
	Lepomis macrochirus	Bluegill	257
	Lepomis microlophus	Redear sunfish	3
	Pomoxis nigromaculatus	Black crappie	6
Clupeidae	*Dorosoma cepedianum*	Gizzard shad	14
Cyprinidae	*Cyprinella analostana*	Satinfin shiner	19
	Cyprinella spiloptera	Spotfin shiner	10
	Luxilus albeolus	White shiner	249
	Nocomis leptocephalus	Bluehead chub	293
	Notropis alborus	Whitemouth shiner	14
	Notropis altipinnis	Highfin shiner	2
	Semotilus atromaculatus	Creek chub	1
Ictaluridae	*Ameiurus brunneus*	Snail bullhead	1
	Ameiurus nebulosus	Brown bullhead	2
	Ameiurus platycephalus	Flat bullhead	46
	Noturus insignis	Margined madtom	51
Moronidae	*Morone americana*	White perch	19
Percidae	*Etheostoma flabellare*	Fantail darter	91
	Etheostoma nigrum	Johnny darter	8
	Etheostoma serrifer	Sawcheek darter	2
	Etheostoma vitreum	Glassy darter	2
	Perca flavescens	Yellow perch	6
	Percina crassa	Piedmont darter	2
	Percina roanoka	Roanoke darter	12
Poeciliidae	*Gambusia sp.*	Mosquitofish	1

Appendix Table 3(b). Fish families and species for Rock Creek, a stream with an arch culvert, collected by a combination of seining and backpack electrofishing during summer 2004. Rock Creek was sampled in Guilford County, NC (Lat: 36 03° 54 N, Long: 79 35° 57 W), and accessed from US 70.

Family	Scientific Name	Common Name	Individuals
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	74
	Lepomis cyanellus	Green sunfish	60
	Lepomis gibbosus	Pumpkinseed	2
	Lepomis macrochirus	Bluegill	151
	Lepomis microlophus	Redear sunfish	9
	Micropterus salmoides	Largemouth bass	42
Clupeidae	*Dorosoma cepedianum*	Gizzard shad	20
Cyprinidae	*Cyprinella analostana*	Satinfin shiner	6
	Nocomis leptocephalus	Bluehead chub	67
	Notropis alborus	Whitemouth shiner	5
	Notropis hudsonius	Spottail shiner	25
	Semotilus atromaculatus	Creek chub	15
Fundulidae	*Fundulus rathbuni*	Speckled killifish	29
Ictaluridae	*Ameiurus brunneus*	Snail bullhead	2
	Ameiurus nebulosus	Brown bullhead	5
	Ameiurus platycephalus	Flat bullhead	19
	Noturus insignis	Margined madtom	38
Percidae	*Etheostoma flabellare*	Fantail darter	26
	Etheostoma olmstedi	Tessellated darter	38
	Etheostoma serrifer	Sawcheek darter	7
	Perca flavescens	Yellow perch	17
	Percina crassa	Piedmont darter	2

Appendix Table 3(c): Fish families and species for Terrell's Creek, a stream with an arch culvert, collected by a combination of seining and backpack electrofishing during summer 2004. Terrell's Creek was sampled in Chatham County, NC (Lat: 35 49° 18 N, Long: 79 15° 20 W), and accessed from NC 87.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	80
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	26
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	179
	Lepomis cyanellus	Green sunfish	18
	Lepomis gibbosus	Pumpkinseed	2
	Lepomis macrochirus	Bluegill	30
	Micropterus salmoides	Largemouth bass	8
Cyprinidae	*Cyprinella nivea*	Whitefin shiner	1
	Nocomis leptocephalus	Bluehead chub	166
	Notropis alborus	Whitemouth shiner	32
	Notropis altipinnis	Highfin shiner	264
	Semotilus atromaculatus	Creek chub	57
Esocidae	*Esox niger*	Chain pickerel	9
Ictaluridae	*Ameiurus platycephalus*	Flat bullhead	50
	Noturus insignis	Margined madtom	227
Percidae	*Etheostoma flabellare*	Fantail darter	1
	Etheostoma olmstedi	Tessellated darter	547
	Etheostoma serrifer	Sawcheek darter	12
	Percina crassa	Piedmont darter	31

Appendix Table 3(d): Fish families and species for Mary's Creek, a stream with a box culvert, collected by a combination of seining and backpack electrofishing during summer 2004. Mary's Creek was sampled in Alamance County, NC (Lat: 35 56° 00 N, Long: 79 19° 50 W), and accessed from NC 87.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	45
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	122
	Moxostoma collapsum	Notchlip redhorse	3
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	202
	Lepomis cyanellus	Green sunfish	30
	Lepomis gibbosus	Pumpkinseed	56
	Lepomis gulosus	Warmouth	20
	Lepomis macrochirus	Bluegill	60
	Lepomis microlophus	Redear sunfish	2
	Micropterus salmoides	Largemouth bass	21
Cyprinidae	*Luxilus albeolus*	White shiner	73
	Nocomis leptocephalus	Bluehead chub	9
	Notropis alborus	Whitemouth shiner	3
	Notropis altipinnis	Highfin shiner	217
	Semotilus atromaculatus	Creek chub	4
Esocidae	*Esox americanus americanus*	Redfin pickerel	8
	Esox niger	Chain pickerel	8
Ictaluridae	*Ameiurus platycephalus*	Flat bullhead	4
	Noturus insignis	Margined madtom	4
Percidae	*Etheostoma olmstedi*	Tessellated darter	53
Poeciliidae	*Gambusia sp.*	Mosquitofish	23

Appendix Table 3(e): Fish families and species for Poppaw Creek, a stream with a box culvert, collected by a combination of seining and backpack electrofishing during summer 2004. Poppaw Creek was sampled in Alamance County, NC (Lat: 35 57° 35 N, Long: 79 31° 39 W), and accessed from SR 1113.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	29
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	1
	Hypentelium nigricans	Northern hog sucker	1
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	54
	Lepomis cyanellus	Green sunfish	5
	Lepomis gibbosus	Pumpkinseed	2
	Lepomis gulosus	Warmouth	2
	Lepomis macrochirus	Bluegill	177
	Lepomis microlophus	Redear sunfish	4
	Micropterus salmoides	Largemouth bass	14
	Pomoxis nigromaculatus	Black crappie	1
Cyprinidae	*Luxilus albeolus*	White shiner	30
	Nocomis leptocephalus	Bluehead chub	342
	Notropis alborus	Whitemouth shiner	9
	Semotilus atromaculatus	Creek chub	105
Fundulidae	*Fundulus rathbuni*	Speckled killifish	1
Ictaluridae	*Ameiurus platycephalus*	Flat bullhead	26
	Noturus insignis	Margined madtom	195
Percidae	*Etheostoma flabellare*	Fantail darter	4
	Etheostoma olmstedi	Tessellated darter	144
	Etheostoma serrifer	Sawcheek darter	2
	Percina crassa	Piedmont darter	3
Poeciliidae	*Gambusia sp.*	Mosquitofish	39

Appendix Table 3(f): Fish families and species for Wet Creek, a stream with a box culvert, collected by a combination of seining and backpack electrofishing during summer 2004. Wet Creek was sampled in Moore County, NC (Lat: 35 23° 25 N, Long: 79 38° 27 W), and accessed from NC 2427.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	52
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	199
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	65
	Lepomis cyanellus	Green sunfish	10
	Lepomis macrochirus	Bluegill	21
	Micropterus salmoides	Largemouth bass	1
Cyprinidae	*Clinostomus funduloides*	Rosyside dace	5
	Luxilus albeolus	White shiner	1
	Nocomis leptocephalus	Bluehead chub	61
	Notropis alborus	Whitemouth shiner	1
	Notropis altipinnis	Highfin shiner	203
	Notropis chiliticus	Redlip shiner	27
	Semotilus atromaculatus	Creek chub	62
Esocidae	*Esox americanus americanus*	Redfin pickerel	8
	Esox niger	Chain pickerel	5
Ictaluridae	*Noturus insignis*	Margined madtom	121
Percidae	*Etheostoma olmstedi*	Tessellated darter	72
	Percina crassa	Piedmont darter	12
Poeciliidae	*Gambusia sp.*	Mosquitofish	1

Appendix Table 3(g). Fish families and species for Brush Creek, a stream with a bridge, collected by a combination of seining and backpack electrofishing during summer 2004. Brush Creek was sampled in Chatham County, NC (Lat: 35 42° 33 N, Long: 79 32° 25 W), and accessed from SR 1102.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	5
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	8
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	139
	Lepomis cyanellus	Green sunfish	120
	Lepomis macrochirus	Bluegill	15
	Micropterus salmoides	Largemouth bass	3
Cyprinidae	*Clinostomus funduloides*	Rosyside dace	1
	Luxilus albeolus	White shiner	211
	Nocomis leptocephalus	Bluehead chub	345
	Notemigonus crysoleucas	Golden shiner	1
	Notropis altipinnis	Highfin shiner	31
	Notropis alborus	Whitemouth shiner	13
	Semotilus atromaculatus	Creek chub	9
Esocidae	*Esox niger*	Chain pickerel	11
Ictaluridae	*Ameiurus platycephalus*	Flat bullhead	103
	Noturus insignis	Margined madtom	40
Percidae	*Etheostoma olmstedi*	Tessellated darter	71
	Percina crassa	Piedmont darter	18
Poeciliidae	*Gambusia sp.*	Mosquitofish	1

Appendix Table 3(h). Fish families and species for Little Brush Creek, a stream with a bridge, collected by a combination of seining and backpack electrofishing during summer 2004. Little Brush Creek was sampled in Chatham County, NC (Lat: 35 38° 53 N, Long: 79 31° 23 W), and sampled from SR 1100.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	2
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	56
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	72
	Lepomis cyanellus	Green sunfish	67
	Lepomis gulosus	Warmouth	5
	Lepomis macrochirus	Bluegill	31
	Micropterus salmoides	Largemouth bass	4
	Pomoxis nigromaculatus	Black crappie	1
Cyprinidae	*Clinostomus funduloides*	Rosyside dace	2
	Cyprinella nivea	Whitefin shiner	1
	Luxilus albeolus	White shiner	51
	Nocomis leptocephalus	Bluehead chub	156
	Notropis alborus	Whitemouth shiner	51
	Notropis altipinnis	Highfin shiner	116
	Semotilus atromaculatus	Creek chub	29
Esocidae	*Esox niger*	Chain pickerel	2
Ictaluridae	*Ameiurus nebulosus*	Brown bullhead	1
	Noturus insignis	Margined madtom	8
Percidae	*Etheostoma olmstedi*	Tessellated darter	54
Poeciliidae	*Gambusia sp.*	Mosquitofish	4

Appendix Table 3(i). Fish families and species for Little Creek, a stream with a bridge, collected by a combination of seining and backpack electrofishing during summer 2004. Little Creek was sampled in Randolph County, NC (Lat: 35 32° 45 N, Long: 79 41° 18 W), and sampled from SR 2870.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	32
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	2
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	72
	Lepomis cyanellus	Green sunfish	34
	Lepomis gulosus	Warmouth	1
	Lepomis macrochirus	Bluegill	9
	Micropterus salmoides	Largemouth bass	5
Cyprinidae	*Clinostomus funduloides*	Rosyside dace	58
	Luxilus albeolus	White shiner	6
	Nocomis leptocephalus	Bluehead chub	78
	Notemigonus crysoleucas	Golden shiner	5
	Notropis alborus	Whitemouth shiner	44
	Notropis altipinnis	Highfin shiner	168
	Notropis chiliticus	Redlip shiner	2
	Semotilus atromaculatus	Creek chub	130
Esocidae	*Esox niger*	Chain pickerel	2
Ictaluridae	*Ameiurus platycephalus*	Flat bullhead	10
	Noturus insignis	Margined madtom	1
Percidae	*Etheostoma olmstedi*	Tessellated darter	354
	Percina crassa	Piedmont darter	2
Poeciliidae	*Gambusia sp.*	Mosquitofish	1

Appendix Table 3(j). Fish families and species for Polecat Creek, a stream with a bridge, collected by a combination of seining and backpack electrofishing during summer 2004. Polecat Creek was sampled in Randolph County, NC (Lat: 35 55° 10 N, Long: 79 47° 47 W), and accessed from NC 62.

Family	Scientific Name	Common Name	Individuals
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	41
	Lepomis gibbosus	Pumpkinseed	2
	Lepomis macrochirus	Bluegill	73
	Lepomis microlophus	Redear sunfish	5
	Micropterus salmoides	Largemouth bass	52
Cyprinidae	*Clinostomus funduloides*	Rosyside dace	14
	Nocomis leptocephalus	Bluehead chub	89
	Notropis alborus	Whitemouth shiner	19
	Notropis altipinnis	Highfin shiner	26
	Notropis chiliticus	Redlip shiner	11
	Semotilus atromaculatus	Creek chub	64
Fundulidae	*Fundulus rathbuni*	Speckled killifish	40
Ictaluridae	*Ameiurus platycephalus*	Flat bullhead	8
	Noturus insignis	Margined madtom	37
Percidae	*Etheostoma olmstedi*	Tessellated darter	95
	Percina crassa	Piedmont darter	6

Appendix Table 3(k): Fish families and species for Brooks Creek, a control stream, collected by a combination of seining and backpack electrofishing during summer 2004. Brooks Creek was sampled in Chatham County, NC (Lat: 35 46° 33 N, Long: 79 10° 05 W), and accessed from SR 1522.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	77
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	2
	Moxostoma collapsum	Notchlip redhorse	1
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	85
	Lepomis cyanellus	Green sunfish	51
	Lepomis gibbosus	Pumpkinseed	2
	Lepomis macrochirus	Bluegill	4
	Micropterus salmoides	Largemouth bass	17
Cyprinidae	*Cyprinella analostana*	Satinfin shiner	1
	Luxilus albeolus	White shiner	128
	Nocomis leptocephalus	Bluehead chub	333
	Notropis alborus	Whitemouth shiner	3
	Notropis altipinnis	Highfin shiner	24
	Semotilus atromaculatus	Creek chub	23
Fundulidae	*Fundulus rathbuni*	Speckled killifish	31
Ictaluridae	*Ameiurus brunneus*	Snail bullhead	1
	Ameiurus platycephalus	Flat bullhead	4
	Noturus insignis	Margined madtom	243
Percidae	*Etheostoma olmstedi*	Tessellated darter	71
	Perca flavescens	Yellow perch	4
	Percina crassa	Piedmont darter	7

Appendix Table 3(l). Fish families and species for Flat Creek, a control stream, collected by a combination of seining and backpack electrofishing during summer 2004. Flat Creek was sampled in Moore County, NC (Lat: 35 33° 27 N, Long: 79 34° 31 W), and accessed from SR 2876.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	33
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	116
	Moxostoma collapsum	Notchlip redhorse	1
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	87
	Lepomis cyanellus	Green sunfish	161
	Lepomis gibbosus	Pumpkinseed	8
	Lepomis gulosus	Warmouth	7
	Lepomis macrochirus	Bluegill	96
	Micropterus salmoides	Largemouth bass	5
Cyprinidae	*Luxilus albeolus*	White shiner	37
	Nocomis leptocephalus	Bluehead chub	25
	Notemigonus crysoleucas	Golden shiner	12
	Notropis alborus	Whitemouth shiner	2
	Notropis altipinnis	Highfin shiner	2
	Semotilus atromaculatus	Creek chub	1
Esocidae	*Esox americanus americanus*	Redfin pickerel	3
	Esox niger	Chain pickerel	40
Ictaluridae	*Ameiurus platycephalus*	Flat bullhead	50
	Noturus insignis	Margined madtom	10
Percidae	*Etheostoma olmstedi*	Tessellated darter	189
	Etheostoma serrifer	Sawcheek darter	1
	Percina crassa	Piedmont darter	6
Poeciliidae	*Gambusia sp.*	Mosquitofish	4

Appendix Table 3(m). Fish families and species for North Prong of Stinking Quarter Creek, a control stream, collected by a combination of seining and backpack electrofishing during summer 2004. North Prong Creek was sampled in Alamance County, NC (Lat: 35 59° 37 N, Long: 79 30° 53 W), and accessed from SR 1129.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	13
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	6
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	67
	Lepomis cyanellus	Green sunfish	191
	Lepomis gibbosus	Pumpkinseed	6
	Lepomis gulosus	Warmouth	4
	Lepomis macrochirus	Bluegill	50
	Micropterus salmoides	Largemouth bass	17
Cyprinidae	*Luxilus albeolus*	White shiner	167
	Nocomis leptocephalus	Bluehead chub	153
	Notropis alborus	Whitemouth shiner	35
	Notropis altipinnis	Highfin shiner	37
	Semotilus atromaculatus	Creek chub	30
Esocidae	*Esox niger*	Chain pickerel	1
Fundulidae	*Fundulus rathbuni*	Speckled killifish	8
Ictaluridae	*Ameiurus platycephalus*	Flat bullhead	15
	Noturus insignis	Margined madtom	38
Percidae	*Etheostoma flabellare*	Fantail darter	7
	Etheostoma olmstedi	Tessellated darter	74
	Percina crassa	Piedmont darter	4

Appendix Table 3(n). Fish families and species for Dry Creek, a stream with a pipe culvert, collected by a combination of seining and backpack electrofishing during summer 2004. Dry Creek was sampled in Chatham County, NC (Lat: 35 23° 50 N, Long: 79 37° 33 W), and accessed from SR 1276.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	49
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	81
	Moxostoma collapsum	Notchlip redhorse	1
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	64
	Lepomis cyanellus	Green sunfish	43
	Lepomis gibbosus	Pumpkinseed	1
	Lepomis gulosus	Warmouth	4
	Lepomis macrochirus	Bluegill	20
	Micropterus salmoides	Largemouth bass	16
Cyprinidae	*Clinostomus funduloides*	Rosyside dace	4
	Luxilus albeolus	White shiner	2
	Nocomis leptocephalus	Bluehead chub	33
	Notropis alborus	Whitemouth shiner	101
	Notropis altipinnis	Highfin shiner	184
	Semotilus atromaculatus	Creek chub	13
Esocidae	*Esox americanus americanus*	Redfin pickerel	3
	Esox niger	Chain pickerel	8
Fundulidae	*Fundulus rathbuni*	Speckled killifish	6
Ictaluridae	*Ameiurus platycephalus*	Flat bullhead	5
	Noturus insignis	Margined madtom	47
Percidae	*Etheostoma olmstedi*	Tessellated darter	108
	Percina crassa	Piedmont darter	15

Appendix Table 3(o). Fish families and species for Reed Creek, a stream with a pipe culvert, collected by a combination of seining and backpack electrofishing during summer 2004. Reed Creek was sampled in Randolph County, NC (Lat: 35 44° 46 N, Long: 79 37° 12 W), and accessed from SR 2626.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	85
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	73
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	126
	Lepomis cyanellus	Green sunfish	39
	Lepomis gibbosus	Pumpkinseed	12
	Lepomis macrochirus	Bluegill	70
	Micropterus salmoides	Largemouth bass	5
Cyprinidae	*Clinostomus funduloides*	Rosyside dace	138
	Luxilus albeolus	White shiner	5
	Nocomis leptocephalus	Bluehead chub	162
	Notropis alborus	Whitemouth shiner	15
	Notropis altipinnis	Highfin shiner	60
	Notropis chiliticus	Redlip shiner	7
	Semotilus atromaculatus	Creek chub	238
Ictaluridae	*Ameiurus platycephalus*	Flat bullhead	19
	Noturus insignis	Margined madtom	68
Percidae	*Etheostoma olmstedi*	Tessellated darter	113
	Percina crassa	Piedmont darter	1
Poeciliidae	*Gambusia sp.*	Mosquitofish	7

Appendix Table 3(p). Fish families and species for Rock Creek, a stream with a pipe culvert, collected by a combination of seining and backpack electrofishing during summer 2004. Rock Creek was sampled in Alamance County, NC (Lat: 35 58° 39 N, Long: 79 27° 14 W), and accessed from SR 1130.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	16
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	8
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	35
	Lepomis cyanellus	Green sunfish	103
	Lepomis gulosus	Warmouth	3
	Lepomis macrochirus	Bluegill	481
	Lepomis microlophus	Redear sunfish	1
	Micropterus salmoides	Largemouth bass	26
Cyprinidae	*Nocomis leptocephalus*	Bluehead chub	44
	Notropis alborus	Whitemouth shiner	3
	Semotilus atromaculatus	Creek chub	103
Fundulidae	*Fundulus rathbuni*	Speckled killifish	91
Ictaluridae	*Ameiurus platycephalus*	Flat bullhead	3
	Noturus insignis	Margined madtom	4
Percidae	*Etheostoma olmstedi*	Tessellated darter	86
Poeciliidae	*Gambusia sp.*	Mosquitofish	2

CHAPTER 2

IMPACT OF BRIDGES AND CULVERTS ON STREAM FISH MOVEMENT: PIT-TAGGING

ABSTRACT

We assessed unidirectional stream fish movement through two types of crossings, box culverts and bridges, using passive integrated transponder (PIT) tags and remote antenna arrays to further assess the potential impact of these two crossing types on stream fish in the Piedmont of North Carolina. The main goal of this study was to assess the movement of stream fish through crossings as a follow-up to a previous, more traditional mark-recapture study conducted in 2004 (Vander Pluym Chapter 1). We conducted electrofishing surveys of fish on six streams located in the Piedmont region of the Cape Fear River Basin, North Carolina during the Summer and early Fall of 2005. All fish measuring ≥ 60 mm TL were injected with an ISO PIT tag with a 12-gauge needle. Custom built antenna arrays, with weir nets to direct fish passage through the antenna loop, were installed in each stream either upstream or downstream of a given crossing. PIT tag reader systems (FS2001 Biomark, Inc.) were running continuously for 30 days with each system maintained by battery switches and data downloads every 7-10 days.

Results of a sign test of percent tagged fish, detected by the antenna for bridges and culverts, showed no significant difference between crossing types (df = 2, p = 0.125); although, mean percent movement of fish through box culverts (28.27% ± 12.24% SE) was almost half that of bridges (44.35% ± 8.77% SE). These results suggest that a larger study could detect a significant difference in fish movement through culverts as opposed to bridges; therefore, box culverts may impede natural fish movement in a given stream reach. Because this application of PIT tags and remote antenna arrays proved a more effective and efficient use of research funding to assess stream fish movement through culverts, we recommend the antenna systems for further non-game fish research.

Introduction

Tagging methods to study fish movement through space and time have been used since the 17th century when Izaak Walton attached ribbons to the caudal fins of Atlantic salmon to test the theory of natal site fidelity (Walton 1983). Technological advances since then have expanded the range and accuracy of methods used to monitor fish mobility in fresh and salt water environments, from the ability of a tag to help gather small-scale habitat use of a marine damsel fish *Pomacentrus amboinensis* (McCormick and Smith 2004), to being able to store many months worth of specific temperature and depth information of an individual pelagic tuna that is later uplinked via satellite to a web-based database (Schaefer and Fuller 2005). Data collected by tagging fish is not only integral to scientific research, but it also serves as the base of fisheries management and conservation decisions (Lucas and Baras 2000).

Trade-offs exist for all types of tags between the accuracy of the data gathered, the length of the study, the number of individuals that can be tagged, the amount of stress experienced by the fish from sampling and tagging methods, and the extent of resources available (Lucas and Baras 2000). The passive integrated transponder (PIT) tag is an internal marker that has become an essential tool for studying movement, behavior, and survival of a variety of fish species (Gibbons and Andrews 2004). There are many advantages to using PIT tags such as minimal injury of fish, high retention rate, small size (12 mm long x 2.1 mm diameter), no reliance on battery power, individual identification code, and little effect on behavior of fish (Prentice et al. 1990a). The tag consists of an integrated circuit chip, capacitor and antenna coil encapsulated in a glass cylinder, and its operation requires an external energy source (Prentice et al. 1990a; b), interrogated within the field of an induction coil which energizes and causes a tag to retransmit its code to the reader. Recent advances in remote antenna arrays, which are used to detect PIT tags, have expanded the utility of PIT tags to continuously monitor the movements of Atlantic salmon *Salmo salar* by placing permanent antennae at strategic points along the paths they use (Zydlewski et al. 2001), culvert passage of juvenile salmonids in Oregon (Hansen and Furniss 2003), salmonid use of discrete refugia (Burns et al. 1997), and recently in small stream fish (Cucherousset et al. 2005).

The majority of work conducted using PIT tag and antenna technology has been on salmonids, with only a few studies on non-game stream fish (Roussel et. al. 2000; Cuchrosset et al. 2005). Traditionally, the home ranges and movements of non-game stream fish have been studied using mark-recapture methods involving subcutaneous paint tags or fin-clips, which are often challenged by methodological problems that decrease recapture rates and bias movement distance distributions due to a limited area of recapture (Lucas and Baras 2000). PIT tags are a much more effective yet expensive alternative; however, the tag size, which is small relative to other tag types, restricts the size of taggable fish to those measuring $\geq$ 60 mm total length (TL) (Ombredane et al. 1998; Columbia Basin Fish and Wildlife 1999).

We assessed unidirectional stream fish movement through two types of road crossings, box culverts and bridges, using PIT tags and remote antenna arrays to further assess the potential impact of these two crossing types on stream fish movement in the Piedmont of North Carolina. The advantages of PIT tags and remote antenna arrays over more traditional mark-recapture methods, such as fin clips and elastomer paint tags, are: (1) increased recapture rates because of a 95-100% read efficiency of the antenna system,

(2) increased recapture rates due to the ability to constantly monitor fish movements, (3) reduced sampling effort due to elimination of recapture sampling, (4) reduced sampling bias due to fright response of more invasive capture methods, (5) reduced recording error, and (6) reduced handling time of fish, which can also lead to reductions in fish mortality (Gibbons and Andrews 2004).

Methods

Site selection

A total of six sites were selected in a directed manner from a total of 42 possible sites harboring mussel populations (Fig 1). All sites were located within the Cape Fear River Basin, North Carolina, to reduce variance in measures of stream fish community. Because of drought conditions during summer 2005, and to avoid culvert perching or other physical barriers to stream fish movement (dry stream bed), we could only use one site from our 2004 sampling: Mary's Creek (Fig 1). For a balanced design, we chose three sites for each crossing type: box culvert and bridge. Habitat characteristics (as outlined by the North Carolina Department of Environment and Natural Resources) such as: (1) stream width measured by a tape measure, (2) stream depth measured by a meter stick, (3) predominate substrate type (bedrock, boulder, cobble, and sand), (4) percentage of habitat type (pond, riffle, and run), (5) bank stability distinguishing between right and left banks (a scale from 1-10 with a score of 1 equivalent to "100% eroded bank" and a score of 10 equivalent to "less than 5% eroded bank"), and (6) width of riparian zone distinguishing between right and left banks (a scale from 1-10 with a score of 1 equivalent to "less than 6 m of riparian vegetation" and a score of 10 representing "greater than 18 m of riparian vegetation"), were quantified at 10 m intervals along a distance of 150 m above and below each crossing. Stream reach volume and area were calculated using the average of the widths and depths for each stream reach, and multiplied by the length of each reach, 150 m. Stream width and depth directly above and below the crossing were the most important measurements considered when choosing a site because this area had to accommodate the PIT tag antenna array (see below for more detail) and maximize fish passage through the antenna.

Antenna and reader configuration

ISO PIT tags measuring 12.45 mm long by 2.02 mm wide (Biomark, Inc.) and operating at 134.2 kHz were matched to a full-duplex FS2001 FR-ISO reader and tuning box (Biomark, Inc.) to operate the complete PIT tag system. Full-duplex tags can only be read by ISO readers and were the best choice for this study because they were the smallest PIT tag available. The reader and tuning box were connected to an open loop inductor antenna that generated both an energizing electromagnetic field and received transmitted signals from a PIT tag as the tagged animal passed through the field. The reader stored all tag information with internal memory until it was downloaded with a laptop computer. The antenna was constructed using 14-gauge insulated Thermoplastic High Heat Resistant Nylon coated (THHN) copper wire which was wound in a square loop (11 wraps) measuring 1.22 m wide by 0.46 m tall and housed in square PVC-pipe framing built with pipe measuring 2.54 cm in diameter and reinforced with PVC cement at the elbow connections. A bank of tuning capacitors (1600v metal polypro 1000-4700 uf, DIGI-Key, Corp.) was soldered to the loop and housed in the PVC-pipe framing between the coil and the cable. Combinations of capacitors allowed the antenna circuit to be tuned to the resonant frequency (natural frequency of vibration determined by the physical parameters of the vibrating object, in this case, the tag at 134.2 kHz) to yield a target current of 2.6-4.3 Amps through the reader (Biomark “Tuning instructions for custom antennas”, www.biomark.com/manuals.htm). Electronic shielded Twinax cable (Belden part no. 9815, Hagemeyer North America) connected the antenna, which was located in the stream, to the tuning box and reader system on shore. The entire system was powered by two 12-V, marine deep cycle 630 cca batteries connected in series to the reader. The reader, tuning box, and batteries were housed in heavy-duty, water-tight plastic containers on shore. All spots of possible leaking on the PVC-pipe frame and containers on shore were sealed with aquarium sealant (Fig 2).

Each antenna was tuned and tested in a local forest and stream (Schenck Forest, Raleigh, NC) before deploying to the study stream. One day prior to sampling a given stream, the antenna was tested and retuned at the research stream to account for environmental factors such as other antennae, power lines, or structures with embedded reinforced steel (bridges and culverts included). Due to potential electrical interference, the antenna had to be located at least 0.61 m away from the crossing. Because warmwater centrarchids favor upstream movement during spring and summer periods (Gatz and Adams 1994), we initially decided to measure only stream fish movement upstream. Excess electrical interference, presumably due to nearby transformers, forced us to place the antenna system of two streams (Mary’s Creek and Vestal Creek, Fig 1) downstream of the crossings. Antenna systems for the remaining four streams were successfully placed upstream of the crossings. Thus, two streams had reader systems placed downstream of the crossings and four streams had reader systems placed upstream of the crossings. All reader and antenna systems were tested for the distance over which the antenna could read a tag, which varied according to tag orientation from 15-30 cm directly upstream and downstream of the antenna.

Each antenna was secured in a given stream to iron rebar; the rebar was driven into the streambed as deep as possible and located 1.3 m apart. One piece of weighted nylon netting with 0.48 cm mesh size was stretched from each side of the antenna to iron rebar driven into the dry bank in order to restrict fish passage to only the open space provided by the antenna loop (Fig 2). The bottom of the netting was further weighted with rocks to ensure its effectiveness as a fish weir. The reader was then turned on and left running until subsequent battery changes and data downloads, which was every 7-10 days.

Fish sampling

Three techniques were used to capture fish for PIT-tagging in this study: (1) block nets measuring 13.72 m long x 1.83 m tall with 0.48 cm mesh to enclose three 50 m reaches above or below a road crossing, (2) seine nets measuring 4.57 m long x 1.22 m tall and 6.09 m long x 1.22 m tall with 0.48 cm mesh to sample large pool and run habitats more effectively, and (3) electrofishing using a 12A Smith-Root back pack unit to capture fish for tagging. We only sampled the fish on the side of a given crossing opposite of the antenna system to measure one direction of fish movement. For example, if an antenna was placed upstream of a crossing then only the fish in 150 m downstream of the crossing were sampled, and vice versa. All fish sampling used block-nets to enclose three adjacent 50 m reaches of each stream immediately upstream or downstream of a road crossing. We chose to partition the 150 m sample reach into adjacent 50 m sections in an effort to reduce the time over which fish were being held which, in turn, reduced mortality. Once enclosed, stream fish in the upstream or downstream reaches of each stream were sampled using a combination of seining and backpack electrofishing; double-pass depletion methods were used to maximize the number of fish sampled measuring 60 mm TL and larger. After analyzing capture rates of fish measuring ≥ 60 mm from the 2004 triple pass depletion methods across 16 streams (Chapter 1), we determined that increasing sample reach size while decreasing pass numbers from three to two would increase our expected number fish within the target fish size range of ≥ 60 mm (Table 1). Fish were removed from the study reaches after each collecting pass and kept in pop-up laundry hampers located directly in the stream flow. After each 50 m section was sampled with double pass depletion methods, we tagged (see tagging methods below) the fish from that section to decrease holding time and handling mortality, and then released them near the original site of capture.

Prior to tagging, fish were anaesthetized using clove oil in place of MS-222 due to its lack of carcinogenic compounds, high effectiveness, low cost, and high survival of fish (Iverson et al. 2003; Pirhonen and Schreck 2003). We used a 1:10 ratio of 100% clove oil to ethanol solution and mixed 2.5 ml of the solution with 5 liters of stream water (Pirhonen and Schreck 2003). Aerators were constantly run in all buckets during the tagging process and water was changed on the half hour to maintain ambient temperature and DO levels for captured fish. Once fish were anaesthetized, we then inserted a scanned PIT tag into the ventral area of the abdominal cavity of fish measuring ≥ 60 mm TL with a 12-gauge veterinary needle (Biomark, Inc.) following procedures outlined by Columbia Basin Fish and Wildlife Authority (PIT Tag Steering Committee Version 2.0). For each individual fish that was tagged, we recorded the tag number, species and length to the nearest 1.0 mm (TL). The point of injection was then swabbed with a mixture of Vaseline and betadine to stop infection and advance healing. Tagged fish were placed in oxygenated buckets for recovery. Once a fish recovered, as evidenced by alertness and opercular movement, they were released into the stream reach section from which they were collected. Block nets were not removed from any of the three sections until all 150 m of a given stream was sampled, and the antenna system was functioning properly.

Fish were sampled using the PIT tag approach from June 22 to October 2, 2005. Only three streams were sampled and running at a given time. Two readers were flooded resulting in one damaged beyond repair and needing a replacement. Turn around of replacement and repaired equipment caused a lag in data collection in two of the streams (Fork Creek and Mary's Creek, Fig 1), as well as multiple delays in redeployment of the reader systems to the second set of three streams until later that summer and into the fall.

<u>PIT-tagging systems</u>

Streams were monitored for 30-43 days during which antenna systems were serviced on a cycle of 7-10 days. Servicing included changing batteries, downloading tag codes with a laptop computer, and clearing net weirs of debris and repairing nets as needed. Tag read range and current strength was tested at each visit, followed by any fine tuning needed to maximize read range and current strength. All systems maintained at least a 0.30 m tag read range directly upstream and downstream of the antenna at 2.6 Amps of current or higher; although one stream system, Vestal Creek (Fig. 1), maintained the aforementioned read range with only 1.4 Amps of current. Tag data, including time and date stamps for each detection, were entered and managed in a relational database.

Response variable and hypothesis

We calculated the number of fish that passed through each crossing by counting each unique tag number once during the entire monitoring period. We did not try to reconstruct multiple passes of one individual because once a fish had passed through the antenna, it was possible that the antenna could detect the fish again within its read range without the fish actually passing through the crossing in the opposite direction. Without an antenna system on each side of a given crossing, it was impossible to conclusively reconstruct movement history of a fish detected more than once. Because we tagged only individuals on the opposite side of the crossing from the antenna, it is certain that fish detected by the antenna had to pass through each crossing to be detected. We hypothesized that a significantly larger proportion of tagged fish would be detected swimming through the antenna array installed near bridges than those installed near box culverts, because summer draw down of water in stream reaches near box culverts can create barriers to stream fish movement due a scour pool-perch effect created just downstream of the culvert (Dane 1978).

Sampling design and statistical analyses

Movement data was analyzed using a sign test approach for two independent samples: (1) the proportion of tagged fish that were detected with the antenna array for box culverts relative to the number of fish tagged, and (2) the proportion of tagged fish that were detected with the antenna array for the streams with bridges relative to the number of fish tagged. Recapture data was standardized to a 30 day recapture period at all sites. Because low sample sizes, as in this study (N = 3), reduce the power of the equal variance test resulting in failure to reject the null hypothesis of equal variances

(Cody and Smith 1997), which is an assumption of parametric comparison tests, we conducted a nonparametric sign test (Zar 1984). Difference in mean stream fish movement relative to crossing type was analyzed using a non-parametric sign test pairing streams by position of antenna (upstream or downstream of the crossing) and stream depth (Appendix Table 1).

Results

A total of 681 fish measuring and representing 19 species and seven families of fish ≥ 60 mm were captured and tagged with PIT tags (Appendix Tables 2 and 3). Out of 681 tagged individuals, 258 stream fish were detected at least once by antenna systems during a 30 day running period in six streams (Table 2). The proportion of tagged fish that travelled through the crossing on each stream ranged from 3.95% to 55.97% with the mean proportion of movers 28.27% ± 12.24% (SE) for streams with box culverts, and 44.35% ± 8.77% (SE) for streams with bridges (Tables 2 and 3).

The mean proportion of tagged stream fish that traveled through a crossing was nearly twice as high near bridges (44.35%) than box culverts (28.27%, Fig 3) suggesting that fish movement may be negatively affected by the presence of box culverts; however, the trend was not statistically significant (sign test, df = 2, p = 0.125). The low number of streams (N = 3) sampled for each crossing type and resulting high variance (Fig. 3) is the likely reason for a non-significant p-value. For example, assuming a similar difference in the number of stream fish that moved between bridges and box culverts (Table 3), if sample size was increased to N = 5, then the sign test would have produced a significant p-value of 0.031 (Zar 1984).

All fish tagged during the 2005 field season using PIT-tags measured ≥ 60 mm TL, whereas those tagged externally in 2004 were smaller, which could have biased the results between the two studies in favor of finding more fish movement in 2005 with larger fish (i.e., larger fish may have a greater tendency to move). During 2004, 75% of the fish tagged measured ≥ 60 mm and 67% of the fish that moved measured ≥ 60 mm (Table 4). This suggests that larger fish do not have a greater tendency to move than smaller fish in these warm-water stream systems.

The methods used during the 2005 field season proved to be more effective in detecting fish movement through road crossings and a more efficient use of research funds than those employed during the 2004 field season described in Chapter 1 (Table 4). The PIT tag and remote antenna array systems detected 37.89% of fish tagged in 30 days of monitoring six streams as opposed to the traditional mark-recapture methods detection of 1.06% of tagged fish in 120 days of monitoring 16 streams (Table 4).

Discussion

The results from this study suggest that there is no significant difference between fish movement through bridges and box culverts in streams of the Piedmont of North Carolina. These findings support those from a more extensive study conducted on 16 streams in the Piedmont of North Carolina using more traditional mark-recapture methods as described in Chapter 1, which found no significant difference in conditional percent movement of stream fish between four different crossing types (bridges, arch culvert, box culvert, and pipe culvert) and control streams. A similar, previous assessment of fish movement across crossing types, which included perched culverts, also found no significant difference in fish movement through bridges and box culverts

(Warren and Pardew 1998, see Chapter 1 for a more thorough review of relevant literature).

The main difference between this study and those mentioned above was the effectiveness of the methods used. Both studies (Warren and Pardew 1998; Vander Pluym Chapter 1) used traditional methods of tagging fish with subcutaneous elastomer paint and conducting multiple electrofishing events aimed at recapturing individuals. This approach, although common, appears much less effective and more labor intensive than the PIT-tag approach used in this study. Warren and Pardew (1998) reported recapture rates of 18% during spring sampling and 21% during summer sampling, with a range of 12-17 days between recapture events. Vander Pluym (Chapter 1) reported lower recapture rates that ranged from 2.96% to 21.7% during summer sampling, with 30 days in between recapture events. With the PIT-tagging approach, recapture rates ranged from 3.95% to 55.95% because of the stationary antenna arrays deployed at each site, with continuous tag detection over 30 days and no re-sampling needed. Not only did the PIT-tag methods have a much greater recapture rate (3.95-55.95% vs. 2.96-21.7%), but it also assessed movement more effectively. For example, this study detected 258 fish out of 681 tagged individuals (37%) having moved through crossings in 30 days of sampling in comparison to 102 fish out of 9,594 individuals tagged (1.06%) in four months of sampling during the initial study described in Chapter 1.

It is possible that the use of the larger tag selected for fish that are more apt to move due to the fact that only fish measuring ≥ 60 mm could be tagged. When comparing the 2005 PIT tag study data to that from Chapter 1, we found that approximately the same percentage of fish measuring ≥ 60 mm that were tagged with the smaller, less size-selective methods moved through the crossings as were tagged (75% vs. 67%). Thus, we do not believe that the size restriction of the PIT tag biased our results towards fish more prone to move.

The difference in methods between Chapter 1 and this study are reflected in the interpretability of the data. In the initial study, there were so few fish detected as moving through the different crossing types that we were unable to draw strong conclusions regarding the effects of crossing type on stream fish movement. Although the recapture success of tagged fish was vastly improved using the PIT-tagging approach compared to the subcutaneous paint marking approach, the PIT-tagging study suffered from relatively low replication (N = 3 streams), which likely reduced the statistical power to detect a significant difference in movement rates, even though movement rates were nearly twice as high through bridges than box culverts.

The increased efficiency and effectiveness of the PIT-tag and antenna array methodology used in this study, compared to previous studies using traditional mark-recapture methods used in past studies (Warren and Pardew 1998; Skalski and Gilliam 2000) illustrates the benefits of reassessing commonly used methods to investigate an ecological question more thoroughly. PIT-tags and remote antenna arrays appear to be an effective way to monitor warmwater stream fish movements through culverts and bridges. These methods have been used in Oregon to assess salmonid passage through culverts (Hanson and Furniss 2003), salmonid use of nature-like bypass channels associated with a dam in Denmark (Aarestrup et al. 2003), and bypass pipes at hydroelectric dams on the Columbia River (Axel et al. 2005). Currently, research on small stream fish is expanding to the use of these technological advances in fish tracking

(Roussel et al. 2000; Cucherousset et al. 2005); although, budgetary restraints often hinder research on non-commercially important species.

Ways to increase the detection ability of the antenna system and decrease the overall cost is to use a different type of PIT-tag, the half-duplex tag, which is detected by reader systems that can be custom built by the researcher from commercially available parts from Texas Instruments. Because the antenna size is not restricted by a manufactured reader, the researcher can customize the entire system to the environment the system will be placed in. The one drawback to this custom is that the size of the PIT-tag is twice the size of the ISO tag (23 mm long, 4 mm diameter), which likely restricts the size of the fish that can be tracked even more.

Conclusions

This study assessed warmwater stream fish movement through bridges versus box culverts using PIT-tagging. Our results showed a trend towards greater fish movement through bridges and culverts; however, the trend was not statistically significant. We recommend the use of the full-duplex PIT-tags in concert with remote antenna arrays for tracking small fish in wadeable streams. We also recommend exploration of the half-duplex tag system for larger individuals as a more flexible and affordable alternative to ISO tag systems. The nature of this study points to a need to re-evaluate traditional mark-recapture methods that are commonly used when assessing the impacts of road crossings on movement of stream fish. The only way fisheries research can continue to produce reliable data upon which to base management decisions is by constantly assessing the reliability of the methods used.

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Table 1: Triple-pass depletion capture analysis of fish measuring ≥ 60 mm TL from a previous study of 16 streams (Chapter 1). Data were pooled across four sampling periods and described as a function of 50 m and 100 m reaches located in the Piedmont of NC. The greatest percentage (82%) of large stream fish was caught in the first and second pass. By extending the reach to 150 m and using only double pass rather than triple pass depletion methods, we estimated capturing 160.77 large stream fish as opposed to 131.5 in 100 m using triple pass depletion.

Pass	Total Fish Captured	Average 50 m	Average 100 m	Percent
1	4142	34.50	69.03	53%
2	2291	19.09	38.18	29%
3	1457	12.15	24.30	18%
Total	7890	65.75	131.5	100%

Table 2: Number of individual stream fish that moved through the crossing, their direction of movement, number of individuals tagged initially, and overall % individuals that moved out of all fish that were tagged in 30 days of PIT-tag monitoring (N = 3 for all results).

Crossing	Creek	Direction	Fish Moved	Fish Tagged	% Moved
Box	Marys	D	3	76	3.95%
	Little Polecat	U	57	133	42.85%
	Rocky	U	76	200	38%
		Total	136	409	33.25%
Bridge	Vestal	D	26	96	27.08%
	Fork	U	21	42	50.00%
	Williams	U	75	134	55.97%
		Total	122	272	44.85%

Table 3: Results of sign test of proportion of tagged fish moved through the crossing, bridge or box culvert, for three pairs of streams.

Pair	Creek	Crossing	Direction	% Moved	Difference	P value
1	Vestal	Bridge	D	27.08%	+23.13	
	Marys	Culvert	D	3.95%		
2	Fork	Bridge	U	50.00%	+7.15	0.125
	Little Polecat	Culvert	U	42.85%		
3	Williams	Bridge	U	55.97%	+22.72	
	Rocky	Culvert	U	33.25%		

Table 4: Cost effective comparison of the two field seasons during which movement of fish through crossings was assessed broken down by number of fish tagged, total number of fish moved through the crossings, number of fish measuring ≥ 60 mm that moved, overall percent of tagged fish that moved, number of streams sampled in each field season, days of fish movement monitored, hours worked in the field, and cost in dollars of each field season. *The 2004 field season lasted for four months with each site sampled once each month and the 2005 field season lasted for one month with each site sampled once overall.

Field Season	Fish Tagged	Fish Moved	≥ 60 mm Moved	Total % Moved	Streams	Days	Hours	Cost ($)
2004	9594	102	68	1.06	16	120*	2560	23,000
2005	681	258	258	37.89	6	30	300	26,330

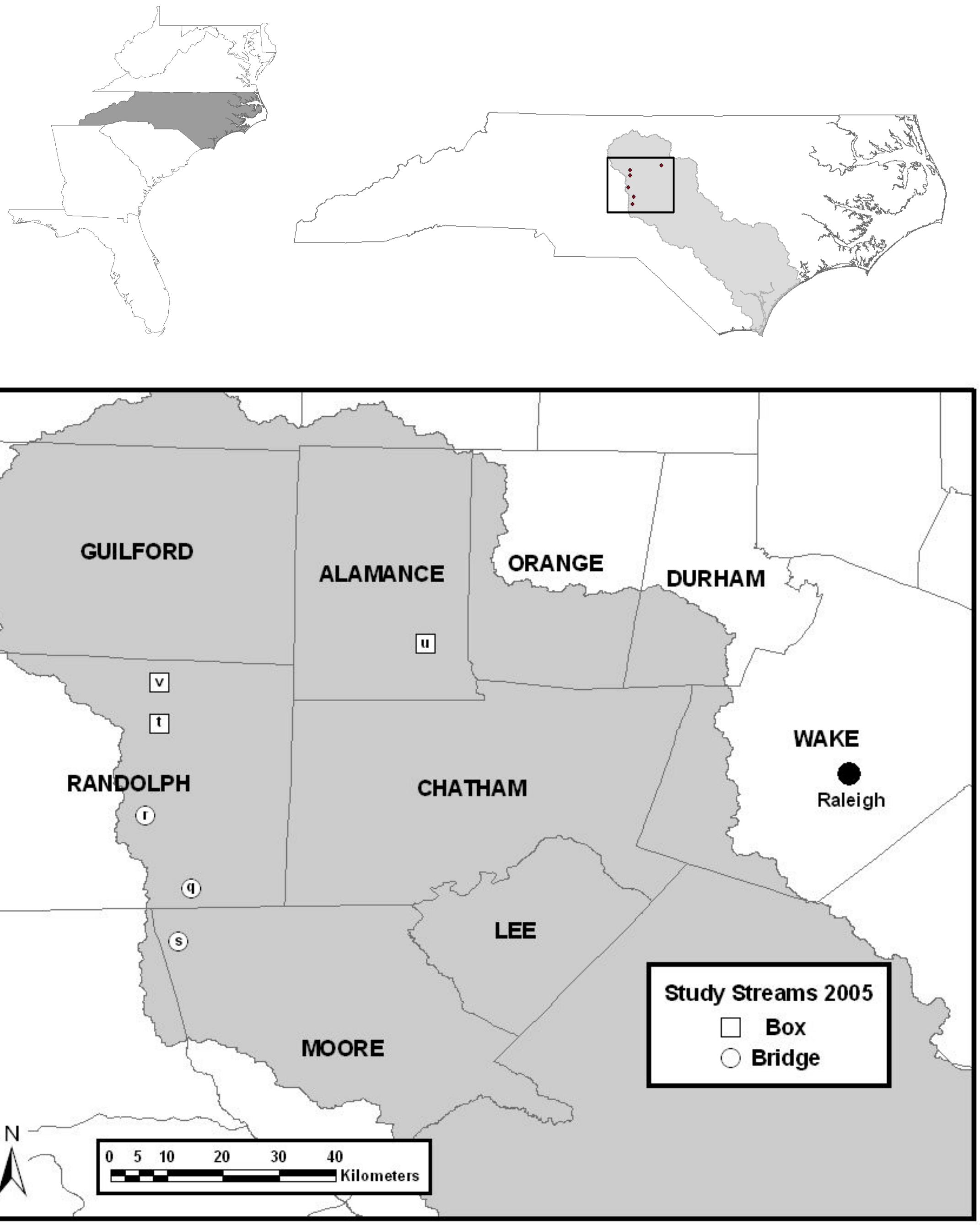

Figure 1: Study sites located west of Raleigh, NC, in the Cape Fear River basin. Each crossing type is represented by a different symbol. The letters inside each symbol correspond to an individual appendix table for each stream.

Figure 2: Remote antenna array complete with net weirs, shielded cable, FS2001 reader, tuning box, and batteries in place downstream of the box culvert in Mary's Creek (Photographs by Jenny Vander Pluym).

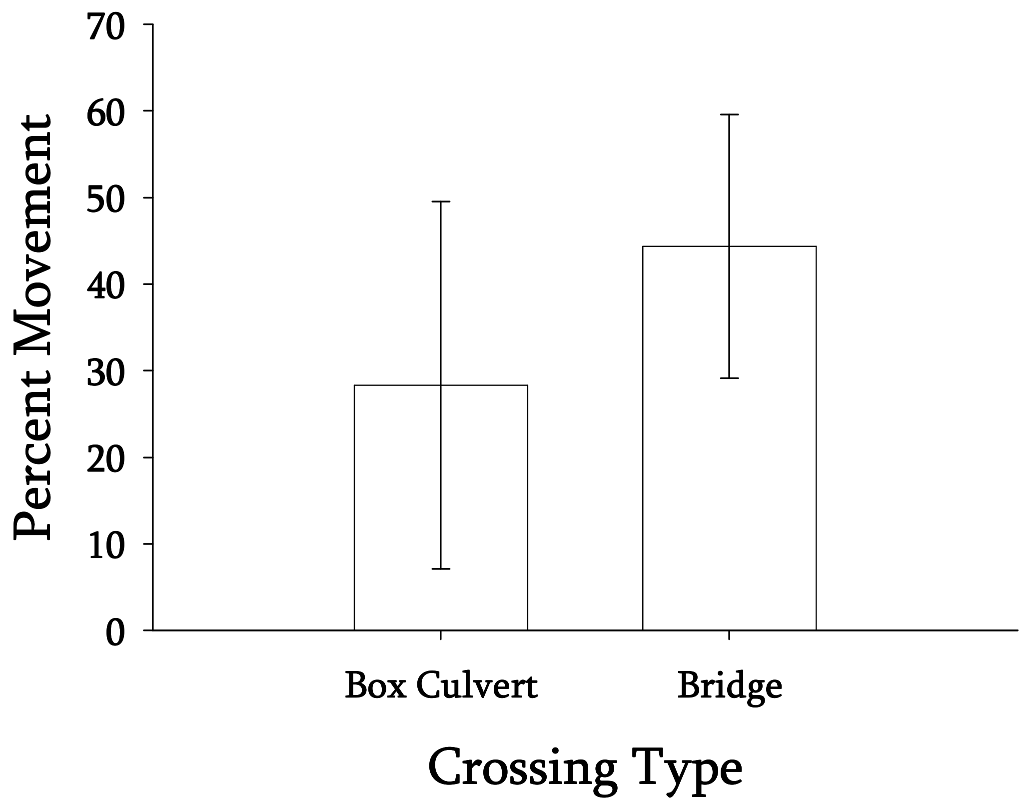

Figure 3: Mean percent stream fish movement (± SE) by crossing type (box culvert and bridge) over 30 days of monitoring (N=3). See text for results of statistical analysis.

APPENDICES

Appendix Table 1: Habitat characteristics measured 150 m downstream or upstream (the opposite side of the crossing from the antenna) of each road crossing. Mean width, depth, % pool, % riffle, and % run were calculated from measurements collected every 10 m in length in each stream reach. Substrate refers to prominent bottom make-up for each reach.

Crossing	Creek	Position	Width (m)	Depth (m)	Area (m^2)	Vol (m^3)	% Pool	% Riffle	% Run	Substrate
Culvert	Marys	U	4.683	0.415	702.45	316.103	93	7	0	Sand, boulder, mud
	Little Polecat	D	5.24	0.323	786	253.878	67	10	23	Sand, cobble
	Rocky	D	5.553	0.157	832.95	130.773	0	9	91	Sand, cobble, gravel
Bridge	Vestal	U	7.203	0.365	1080.45	394.364	49	25	26	Gravel, boulder, sand
	Fork	D	6.846	0.609	1026.9	625.382	74	1	25	Gravel, sand, boulder
	Williams	D	6.833	0.349	1024.95	357.707	53	15	32	Boulder, cobble, sand

Appendix Table 2: Comprehensive list of fish families and species collected by a combination of seining and backpack electrofishing during summer 2005 in the Cape Fear River Basin, North Carolina.

Family	Scientific Name	Common Name
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch
Catostomidae	*Erimyzon oblongus*	Creek chubsucker
	Moxostoma collapsum	Notchlip redhorse
Centrarchidae	*Lepomis auritus*	Redbreast sunfish
	Lepomis cyanellus	Green sunfish
	Lepomis gibbosus	Pumpkinseed
	Lepomis gulosus	Warmouth
	Lepomis macrochirus	Bluegill
	Micropterus salmoides	Largemouth bass
Cyprinidae	*Clinostomus funduloides*	Rosyside dace
	Luxilus albeolus	White shiner
	Nocomis leptocephalus	Bluehead chub
	Semotilus atromaculatus	Creek chub
Esocidae	*Esox americanus americanus*	Redfin pickerel
	Esox niger	Chain pickerel
Fundulidae	*Fundulus rathbuni*	Speckled killifish
Ictaluridae	*Ameiurus brunneus*	Snail bullhead
	Ameiurus platycephalus	Flat bullhead
	Noturus insignis	Margined madtom

Appendix Table 3(q): Fish families and species, measuring ≥ 60 mm TL, for Fork Creek, a stream with a bridge, collected by a combination of seining and backpack electrofishing on August 28, 2005. Fork Creek was sampled in Randolph County, NC (Lat: 35 32° 38 N, Long: 79 42° 15 W), and accessed from SR 2862.

Family	Scientific Name	Common Name	Individuals
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	4
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	22
	Lepomis cyanellus	Green sunfish	4
	Lepomis gibbosus	Pumpkinseed	2
	Lepomis gulosus	Warmouth	1
	Lepomis macrochirus	Bluegill	3
	Micropterus salmoides	Largemouth bass	1
Esocidae	*Esox niger*	Chain pickerel	1
Ictaluridae	*Ameiurus platycephalus*	Flat bullhead	4

Appendix Table 3(r): Fish families and species, measuring ≥ 60 mm TL, for Little Polecat, a stream with a box culvert, collected by a combination of seining and backpack electrofishing on September 24, 2005. Little Polecat was sampled in Randolph County, NC (Lat: 35 52° 19 N, Long: 79 45° 16 W), and accessed from SR 2106.

Family	Scientific Name	Common Name	Individuals
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	8
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	26
	Lepomis gibbosus	Pumpkinseed	8
	Lepomis gulosus	Warmouth	4
	Lepomis macrochirus	Bluegill	32
	Micropterus salmoides	Largemouth bass	2
Cyprinidae	*Luxilus albeolus*	White shiner	5
	Nocomis leptocephalus	Bluehead chub	34
	Semotilus atromaculatus	Creek chub	12
Fundulidae	*Fundulus rathbuni*	Speckled killifish	1
Ictaluridae	*Noturus insignis*	Margined madtom	1

Appendix Table 3(s): Fish families and species, measuring ≥ 60 mm TL, for Mary's Creek, a stream with a box culvert, collected upstream of the crossing by a combination of seining and backpack electrofishing on June 22, 2005. Mary's Creek was sampled in Alamance County, NC (Lat: 35 56° 00 N, Long: 79 19° 50 W), and accessed from NC 87.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	3
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	15
	Moxostoma collapsum	Notchlip redhorse	3
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	30
	Lepomis cyanellus	Green sunfish	2
	Lepomis gibbosus	Pumpkinseed	7
	Lepomis gulosus	Warmouth	3
	Lepomis macrochirus	Bluegill	3
	Micropterus salmoides	Largemouth bass	1
Cyprinidae	*Luxilus albeolus*	White shiner	2
	Nocomis leptocephalus	Bluehead chub	3
Esocidae	*Esox americanus americanus*	Redfin pickerel	1
	Esox niger	Chain pickerel	1
Ictaluridae	*Ameiurus platycephalus*	Flat bullhead	4
	Noturus insignis	Margined madtom	4

Appendix Table 3(t): Fish families and species, measuring ≥ 60 mm TL, for Vestal Creek, a stream with a bridge, collected by a combination of seining and backpack electrofishing on June 25, 2005. Vestal Creek was sampled in Randolph County, NC (Lat: 35 39° 34 N, Long: 79 46° 37 W), and accessed from SR 2824.

Family	Scientific Name	Common Name	Individuals
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	10
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	39
	Lepomis gulosus	Warmouth	7
	Lepomis macrochirus	Bluegill	6
	Micropterus salmoides	Largemouth bass	1
Cyprinidae	*Nocomis leptocephalus*	Bluehead chub	24
	Semotilus atromaculatus	Creek chub	3
Ictaluridae	*Ameiurus platycephalus*	Flat bullhead	4
	Noturus insignis	Margined madtom	4
	Ameiurus brunneus	Snail bullhead	1

Appendix Table 3(u): Fish families and species, measuring ≥ 60 mm TL, for Rocky River, a stream with a box culvert, collected by a combination of seining and backpack electrofishing on October 2, 2005. Rocky River was sampled in Chatham County, NC (Lat: 35 48° 26 N, Long: 79 31° 40 W), and accessed from SR 1300.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	1
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	25
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	16
	Lepomis cyanellus	Green sunfish	4
	Lepomis gibbosus	Pumpkinseed	5
	Lepomis macrochirus	Bluegill	5
Cyprinidae	*Nocomis leptocephalus*	Bluehead chub	91
	Semotilus atromaculatus	Creek chub	51
Ictaluridae	*Noturus insignis*	Margined madtom	2

Appendix Table 3(v): Fish families and species, measuring ≥ 60 mm TL, for William's Creek, a stream with a bridge, collected by a combination of seining and backpack electrofishing August 26, 2005. William's Creek was sampled in Moore County, NC (Lat: 35 27° 31 N, Long: 79 43° 28 W), and accessed from SR 1403.

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	2
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	4
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	8
	Lepomis cyanellus	Green sunfish	82
	Lepomis gulosus	Warmouth	3
	Lepomis macrochirus	Bluegill	13
Cyprinidae	*Clinostomus funduloides*	Rosyside dace	2
	Nocomis leptocephalus	Bluehead chub	9
	Semotilus atromaculatus	Creek chub	4
Esocidae	*Esox americanus americanus*	Redfin pickerel	8
Ictaluridae	*Ameiurus platycephalus*	Flat bullhead	2
	Noturus insignis	Margined madtom	5