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Final Report

Distribution of Freshwater Mussel Populations in Relationship to Crossing Structures

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16. Abstract

The goals of this study were to evaluate the potential impact of road crossings on freshwater mussels in North Carolina and determine the factors that contribute to any impact. We visually surveyed mussels in the 300-meter reaches upstream and downstream of 80 road crossings in the upper Neuse and Cape Fear River Basins. We characterized the habitat at each site by estimating the percentage of riffle, run, and pool in 25-meter increments, and we used standardized criteria to score stream habitat quality. Aquatic insect samples were collected at 44 sites and diversity and tolerance values were compared upstream and downstream of the road. No differences between upstream and downstream were found using aquatic insects. Bank stability scores were highest near the crossing structures, especially within 50-75 meters upstream, but overall bank stability decreased with distance from the bridge downstream. Upstream bank stability scores were significantly higher than downstream bank stability scores (*p-value* > 0.05) and the most downstream 100-meter reach had the lowest scores. There was a possible trend of decreased pool habitat within 100 meters upstream and downstream of road crossings as pool habitat seemed to decrease with decreasing distance from the road ($p = 0.099$). These results may be indicators that some road crossings are altering stream habitat for some distance upstream and downstream of the road. Overall, several analyses show decreased relative abundance of the most common mussel species, *Elliptio complanata*, in the 50 meters immediately downstream of the road. Also, mean length of *E. complanata* was lower downstream than upstream ($p < 0.05$). No evidence of effects on other species was found, but no definitive conclusions can be made due to the rarity of these other species. We attribute declines in abundance just downstream of crossings to channel constriction in some bridge and culvert designs as well as the effects of recent construction.

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Acknowledgments

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Executive Summary

As transportation agencies continue to build roads and bridges, the need to preserve important natural resources becomes more pressing. Among North Carolina's most important aquatic natural resources are freshwater mussels (Unionidae), and this group of animals continues to be among the most endangered. When state biologists observed that some road crossings over streams had reduced mussel abundance near the structure, this study was initiated by the North Carolina Department of Transportation and the Institute for Transportation Research and Education at North Carolina State University. The goals of this study were to evaluate the potential impact of road crossings on freshwater mussels in North Carolina and determine the factors that contribute to any impact. We visually surveyed mussels in the 300-meter reaches upstream and downstream of 80 road crossings in the upper Neuse and Cape Fear River Basins. We characterized the habitat at each site by estimating the percentage of riffle, run, and pool in 25-meter increments, and we used standardized criteria to score habitat stream habitat quality. Aquatic insect samples were collected at 44 sites and diversity and tolerance values were compared upstream and downstream of the road. No differences between upstream and downstream were found using aquatic insects. Bank stability scores were highest near the crossing structures, especially within 50-75 meters upstream, but overall bank stability decreased with distance from the bridge downstream. Upstream bank stability scores were significantly higher than downstream bank stability scores (*p-value* < 0.001) and the most downstream 100-meter reach had the lowest scores. There was a possible trend of decreased pool habitat within 100 meters upstream and downstream of road crossings as pool habitat seemed to decrease with decreasing distance from the road ($p = 0.099$). These results may be indicators that some road crossings are altering stream habitat for some distance upstream and downstream of the road. Overall, several analyses show decreased relative abundance of the most common mussel species, *Elliptio complanata*, in the 50 meters immediately downstream of the road. Also, mean length of *E. complanata* was lower downstream than upstream in both the Neuse and Cape Fear study areas ($p < 0.05$). No evidence of effects on other species was found, but no definitive conclusions can be made due to the rarity of these other species. We attribute declines in abundance just downstream of crossings to channel constriction in some older bridge and culvert designs as well as the effects of recent construction.

Table of Contents

LIST OF FIGURES

Page

Page

LIST OF TABLES

Page

Page

Introduction

Transportation agencies continue to promote economic growth through infrastructure development; however, with this comes the responsibility of preserving important natural resources. When road and bridge construction is proposed, an environmental impact assessment is conducted to determine the potential threat that a given project has on sensitive species or ecological areas. Wildlife agencies are especially concerned when construction of road-crossings over streams is proposed because of the variety of potential impacts those activities can cause on the aquatic environment. Sedimentation, channelization, and stream bank modifications are all possible results of bridge and culvert construction that can be detrimental to the local aquatic fauna (Little and Mayer 1993; Forman and Alexander 1998). A high priority is often placed on determining potential impacts to freshwater mussels (Unionidae) when development projects encroach upon or cross surface waters. This priority is placed because Unionids are among the most endangered groups of animals in North America. In North Carolina, 34 of the 56 mussel species are listed as being endangered, threatened, of special concern or significantly rare, and an additional 9 of those species are believed to be extirpated from the state. So approximately 77% of the state's mussel species are either already gone or are in a state of imperilment. Across the continent, approximately 70% of the nearly 300 species of freshwater mussels are considered to be in some state of imperilment (Williams et al. 1993). Declines in freshwater populations likely began in the 1800s with mass deforestation (Hughes and Parmalee 1999) and overharvest (Anthony and Downing 2001). This still continues today with construction of impoundments on streams, poor agricultural practices, urban development, and other human activities (Bogan 1993).

Short-term effects of bridge and culvert construction activities have been documented to impact stream insects (Ogbeibu and Victor 1989) and fish (Barton 1977). Sedimentation, a potential consequence of bridge construction, has been shown to be detrimental to mussel populations (Ellis 1936, Marking and Bills 1979); however, long-term effects of the presence of road-crossings on mussels is somewhat unknown. Storm events may eventually flush construction-related sediments from a site (Taylor and Roff 1986), but a crossing structure may permanently alter the local habitat through channelization, blockage of stream meander, and channel constriction (Little and Mayer 1993; Forman and Alexander 1998). These effects have great potential to alter the benthic habitat where mussels reside. State biologists from the North Carolina Wildlife Resources Commission (NCWRC) and the North Carolina Department of Transportation (NCDOT) have observed lower abundances of freshwater mussels (Unionidae) downstream of certain road-crossings over streams. These observations led to the funding of this study. The primary goal was to determine the potential impact of crossing-structures on freshwater mussel populations. **Specific objectives of the study were**:

1. **To determine the relative abundance, diversity and spatial distribution of freshwater mussel populations near crossing-structures, and**

2. **To identify specific attributes about road-crossings, adjacent land use or other variables that may be altering the relative abundance, diversity and spatial distribution of freshwater mussels.**

To accomplish these objectives, we focused on evaluating as many road-crossings as possible within the two-year study. To reduce the risk of extraneous factors masking potential bridge-effects, we chose two study areas in the Neuse and Cape Fear River Basins that had relatively good habitat and water quality as well as abundant mussel fauna. We characterized the physical and chemical nature of all study sites, surveyed mussels, and collected aquatic insects. A subset of sites was used to evaluate the effect of road-crossings on channel shape and substrate characteristics. A Geographical Information System (GIS) was used to evaluate land use around sites and in the upstream watersheds. Our results show that mussel fauna are influenced by a complex set of variables.

Chapter 1

Study Area and Site Characterization

STUDY AREA AND SITE SELECTION

Selection of Study Area

To focus our assessment on the impact of road crossings on mussel fauna, we chose a study area with viable mussel populations and relatively good habitat and water quality. In coordination with NCDOT and NCWRC biologists, two areas of the North Carolina piedmont were chosen that met our criteria. To minimize species differences between sites, we kept all sites in each of the two study areas in the same sub-basins. Areas with federally endangered species were eliminated to avoid damaging sensitive habitats and to avoid the need for special federal permits. Areas with the highest water quality were identified using Basinwide Water Quality Plans of the Neuse River and Cape Fear River basins (NCDENR 1998; NCDENR 2000). We determined that the overall land use of the study area should be at least 60% forested to minimize stream impacts by agricultural and urban areas that could potentially complicate or mask the effects of a bridge or culvert. Land use and land cover data for the Neuse River basin were obtained from the Environmental Protection Agency's (EPA) Neuse River Land Use/Land Cover GIS layer. The 30 m resolution grid was derived from several Landsat 7 ETM+ scenes ranging in dates from October 1998 to March 1999 (EPA, 2000). EPA's National Land Cover Data (NLCD) was the source of the land use data for the Cape Fear study area. This 30-m resolution grid was derived from several Landsat 7 ETM+ scenes ranging in dates from 1991 to 1993 (Vogelmann *et al.*, 2001).

Description of Study Area

Two study areas were chosen in the piedmont of North Carolina (Figure 1.1). In the Neuse River basin, the area that drains into the upper portions of Falls Lake was chosen. This region is 1685.65 km^2 in area and covers portions of Orange, Durham, Person, Granville, and Wake Counties in North Carolina. The main drainages in the area are the Eno, Little, and Flat River watersheds, but several other smaller watersheds feed directly into Falls Lake from Granville and Wake Counties. The geology in this area results in variety of stream types from rocky to sandy, so a variety of stream channel types are represented in this relatively small portion of the piedmont. Durham, Hillsboro, Creedmoor and Butner are the primary municipalities in the region with Durham being the largest. The dominant land uses within the subbasin included forested (61%), urban (16%), and agriculture (18%). Various wetland types comprised 4% of the land cover, and other land uses (0.2%) were combined and consisted of barren and herbaceous cover types (Figure 1.2).

In the Cape Fear River basin, tributaries of the middle section of the Deep River were chosen from Polecat Creek in Guilford and Randolph County downstream to the Bear Creek watershed in Moore County. This region is 1570.29 km^2 in total area and covers portions of Guilford, Randolph, Chatham, Moore and Montgomery Counties in North Carolina. Asheboro and Ramseur are the main municipalities there, but the outskirts of Greensboro (Pleasant Garden area) lie within the uppermost portions of the Polecat Creek watershed. Like the study area in the Neuse basin, this part of the Cape Fear basin also has a variety of geologic formations and streambed types ranging from bedrock and boulder to very sandy. The land use consisted of forested (74%), agriculture (21%), and urban (3%). Other land types (2%) were combined and included water, barren, and wetlands (Figure 1.3).

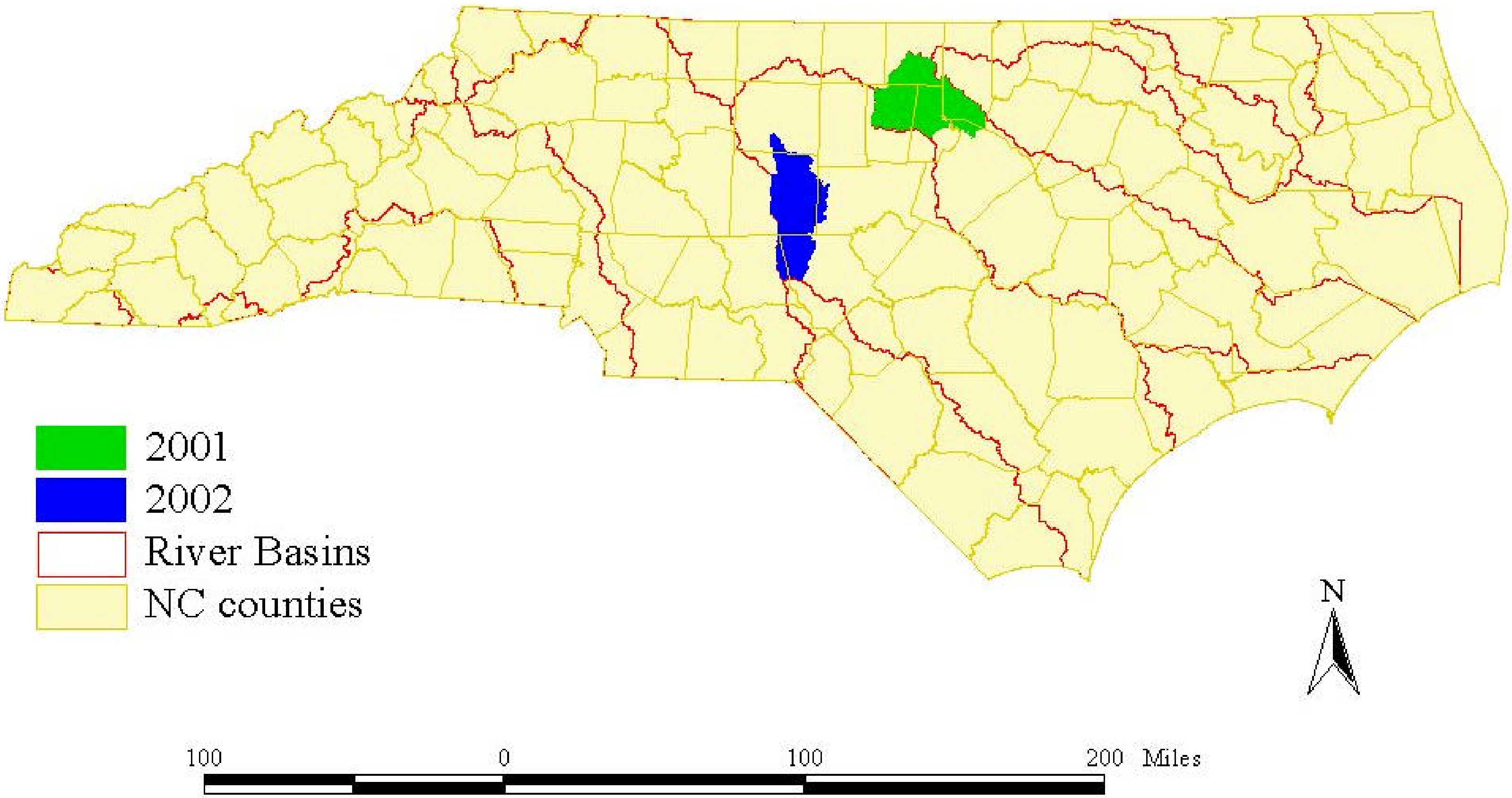

Figure 1.1. *Study areas chosen in 2001 and 2002 in the North Carolina piedmont.*

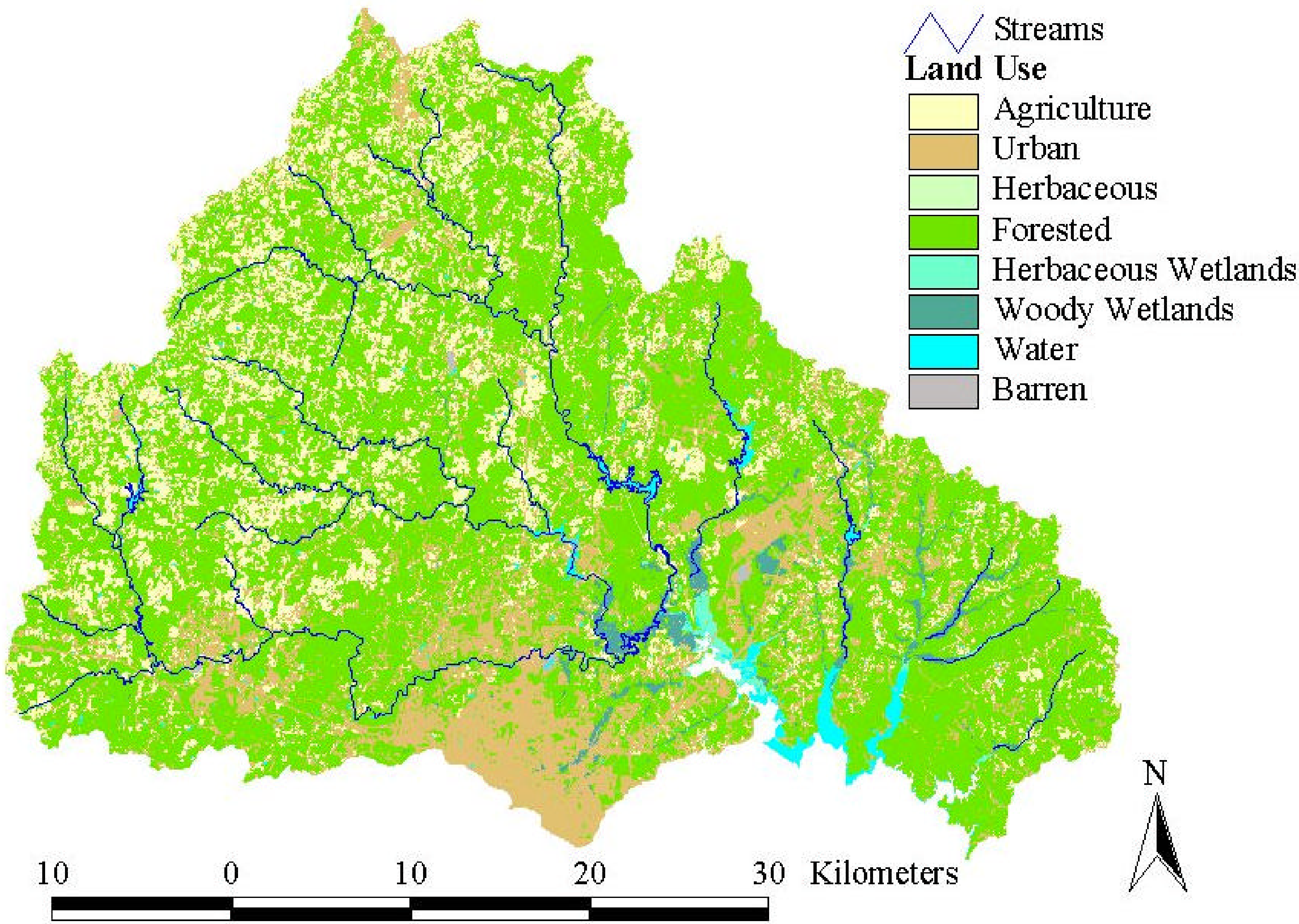

Figure 1.2. *Land use in the Neuse study area as determined by the EPA's Neuse River Land Use/Land Cover data. The 30-meter resolution grid was derived from several Landsat 7 ETM+ scenes ranging in dates from October 1998 to March 1999 (EPA, 2000).*

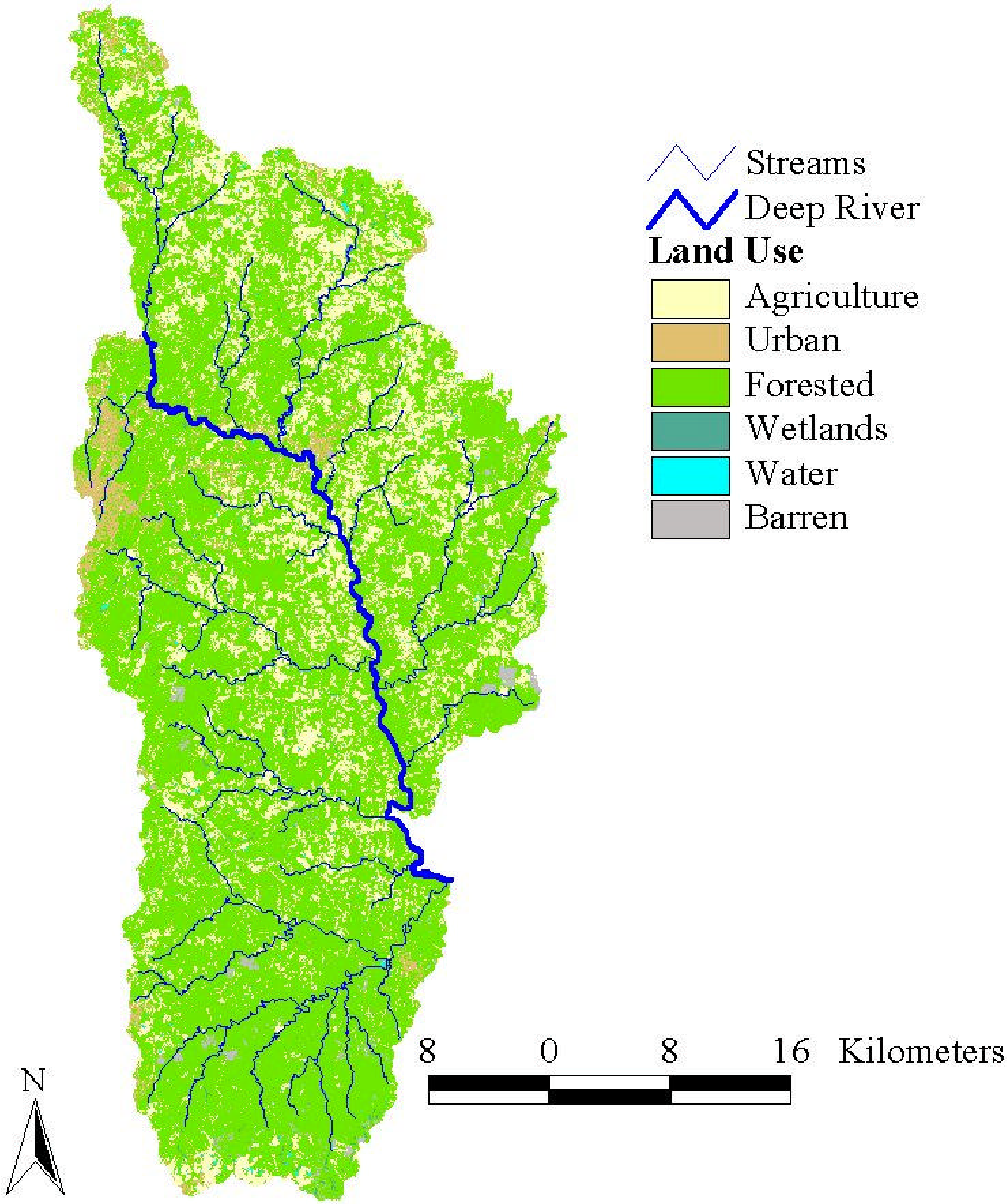

Figure 1.3. *Land use in the Cape Fear study area as derived by EPA's National Land Cover Data (NLCD). This 30-m resolution grid was derived from several Landsat 7 ETM+ scenes ranging in dates from 1991 to 1993 (Vogelmann et al., 2001).*

Site Selection

To account for the large amount of variation in mussel populations in nature, we chose to sample a large number of sites rather than more intensively monitor a small number of sites. By visiting many road crossings, we sought to separate differences in mussel distribution attributed to chance and the natural environment from differences actually caused by crossing structures. We also wanted to be able to sample around several different types of structures to determine potential differences in the structure's effect. A sampling site included the 300-meter stream reaches immediately upstream and downstream of the crossing as well as under or within the structure. This large distance was chosen to encompass a potential recovery zone downstream as well as establish good control data upstream of each crossing.

To select sites, a GIS data layer of all North Carolina crossing structures was obtained from NCDOT and was clipped according to the study area boundaries defined above. We then visited all identified road crossings over streams in the study area to determine if they would serve as viable study sites. We visited 123 crossing structures in the Neuse study area in March and April 2001 and determined that 44 sites (Figure 1.4, Table 1.1) met our criteria to serve as sampling locations. In the Cape Fear study area we visited 128 crossing structures and located 38 viable study sites; however, upon revisiting sites at the time of sampling, 2 of these sites were eliminated due to lack of water to leave a total of 36 sites (Figure 1.5, Table 1.2).
To serve as a study site, a location must have met the following criteria:

1. The stream and surrounding land had to be accessible to sampling. Access was restricted by the landowner at a few sites.
2. The stream had to be free flowing for 300 meters upstream and downstream of the road crossing. It could not be excessively dammed by humans or beavers.
3. The stream had to have a mussel population. If we found live freshwater mussels in a 30-60 minute search by 2-3 people, the site was considered to meet this criterion.
4. Macrohabitat had to be similar upstream and downstream of the road crossing. Large differences in stream gradient upstream and downstream would likely result in inherent differences in the mussel community and effects of the crossing structure would be difficult to determine.

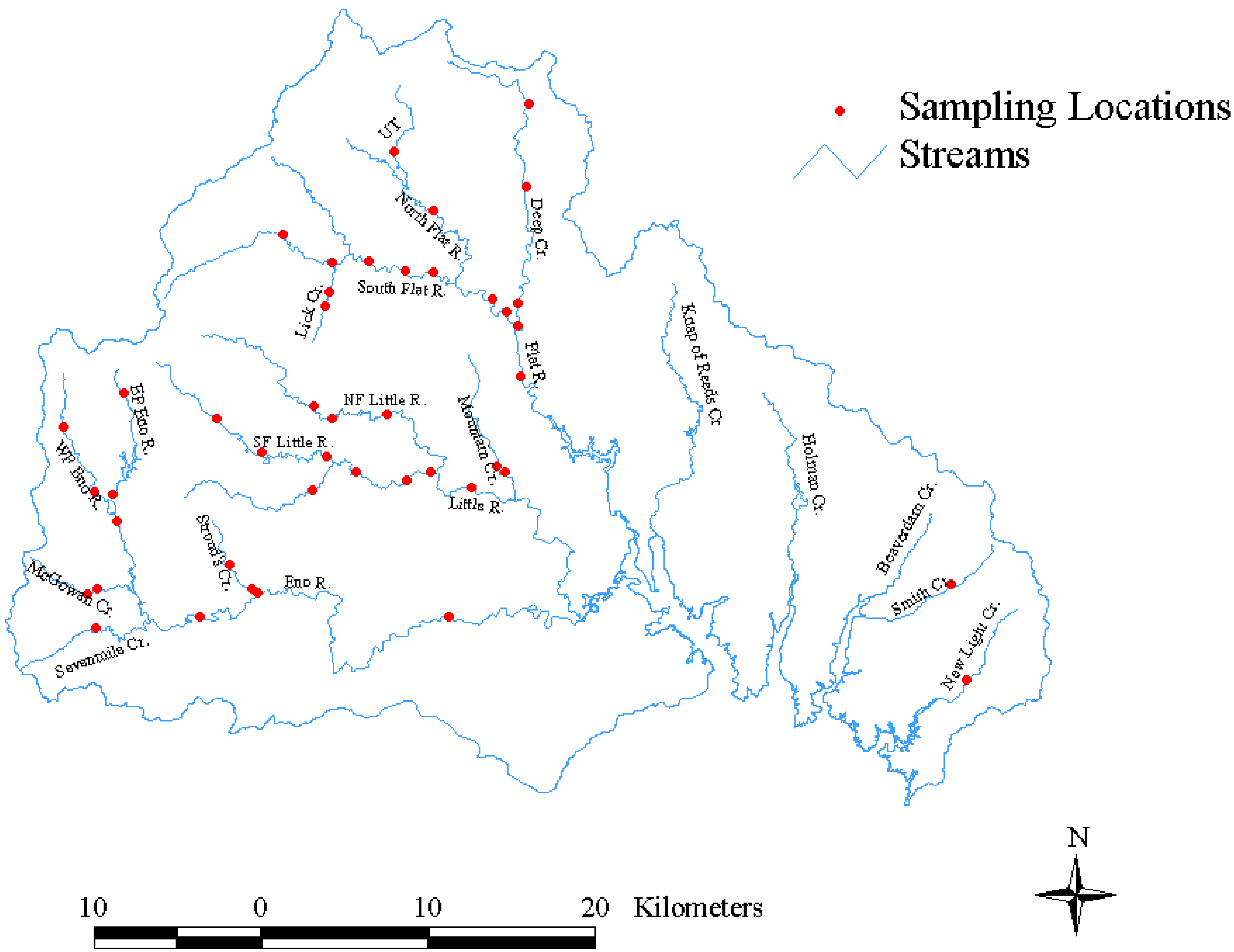

Figure 1.4. *Sampling locations in the Neuse study area.*

Table 1.1. A list of the 44 study sites selected in the Neuse River basin in 2001.

County	Bridge Number	Road Number	Stream
Durham	5	SR 1793	Mountain Creek
Durham	6	SR 1617	Mountain Creek
Durham	8	SR 1602	Flat River
Durham	9	SR 1471	Flat River
Durham	50	NC 157	Eno River
Durham	56	NC 157	South Fork Little River
Durham	57	SR 1461	South Fork Little River
Durham	64	SR 1461	Little River
Durham	151	SR 1614	Flat River
Granville	25	SR 1710	Smith Creek
Orange	4	SR 1004	West Fork Eno River
Orange	6	US 70 Bus.	Eno River
Orange	11	SR 1336	Eno River
Orange	12	SR 1332	East Fork Eno River
Orange	13	NC 57	South Fork Little River
Orange	30	NC 57	North Fork Little River
Orange	43	SR 1120	Sevenmile Creek
Orange	53	SR 1538	North Fork Little River
Orange	54	NC 157	North Fork Little River
Orange	55	SR 1540	South Fork Little River
Orange	57	SR 1538	South Fork Little River
Orange	64	SR 1561	Eno River
Orange	66	SR 1002	Stroud's Creek
Orange	67	SR 1324	McGowan Creek
Orange	114	SR 1548	Forrest Creek
Orange	126	SR 1526	Lick Creek
Orange	136	SR 1544	South Fork Little River
Orange	173	SR 1353	East Fork Eno River
Orange	200	SR 1555	Stroud's Creek
Orange	242	SR 1004	West Fork Eno River
Orange	251	SR 1004	McGowan Creek
Person	10	SR 1567	Deep Creek
Person	18	US 501	South Flat River
Person	21	SR 1715	North Flat River
Person	22	SR 1708	Unnamed tributary: North Flat River
Person	23	NC 157	South Flat River
Person	33	SR 1125	South Flat River
Person	36	SR 1123	South Flat River
Person	38	SR 1121	Lick Creek
Person	80	SR 1734	Deep Creek
Person	127	SR 1723	Deep Creek
Person	130	SR 1737	Flat River
Person	205	SR 1120	South Flat River
Wake	119	SR 1912	New Light Creek

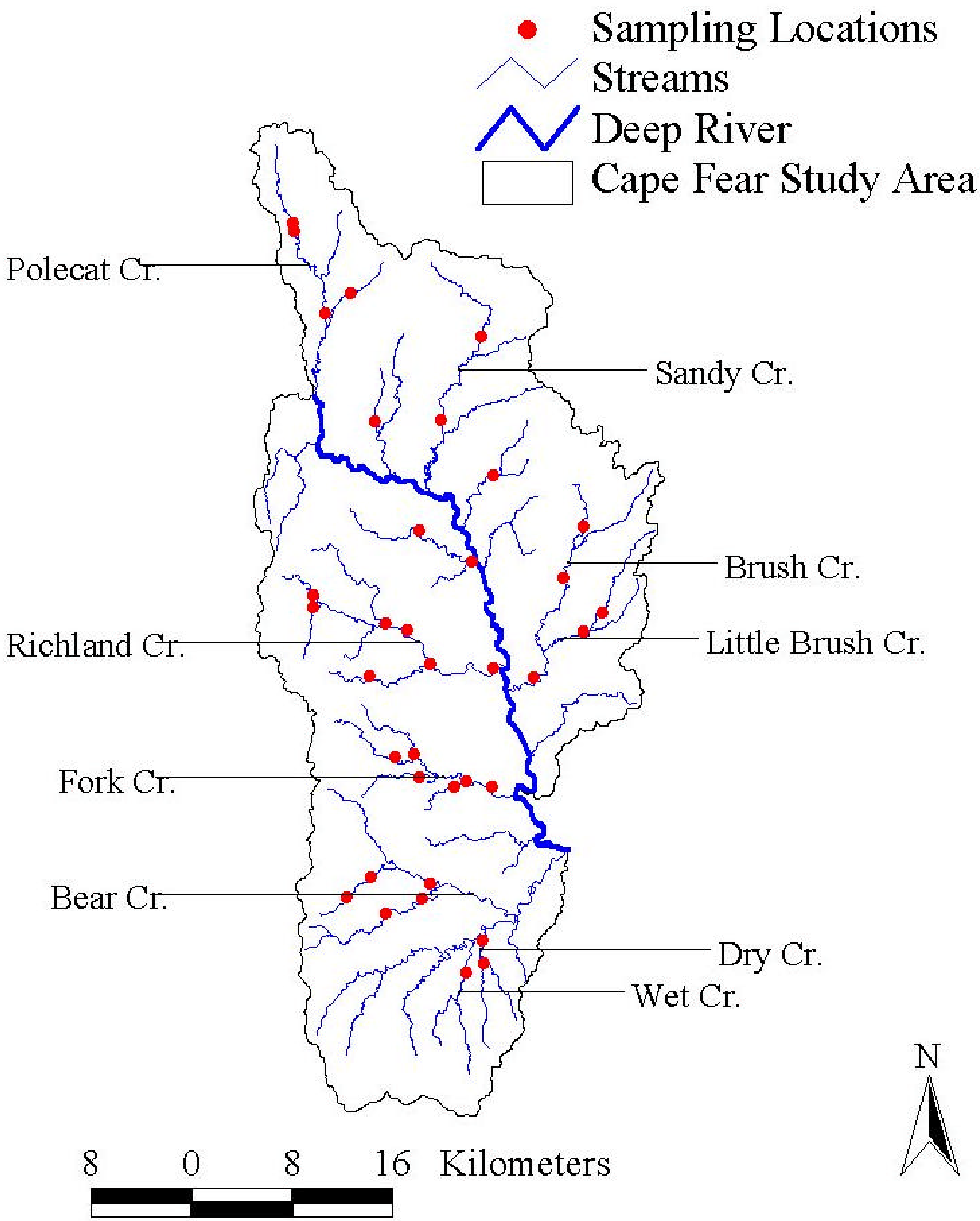

Figure 1.5. *Sampling locations in the Cape Fear study area.*

Table 1.2. A list of the 36 study sites selected in the Cape Fear River basin in 2002.

County	Bridge Number	Road Number	Stream
Chatham	245	SR 1148	Little Brush Creek
Chatham	247	SR 1100	Little Brush Creek
Chatham	251	SR 1104	Brush Creek
Guilford	23	SR 3433	Polecat Creek
Guilford	72	NC 62	Polecat Creek
Moore	12	NC 24/27	Wet Creek
Moore	28	NC 24/27	Dry Creek
Moore	127	SR 1428	Bear Creek
Moore	173	SR 1403	Wolf Creek
Moore	174	SR 1403	Williams Creek
Moore	184	SR 1404	Williams Creek
Moore	212	SR 1276	Dry Creek
Moore	225	SR 1275	Wolf Creek
Randolph	109	SR 2113	Polecat Creek
Randolph	110	NC 22/42	Brush Creek
Randolph	149	SR 2141	Unnamed tributary: Bush Creek
Randolph	175	SR 2614	Mill Creek
Randolph	188	SR 2657	Mill Creek
Randolph	199	SR 2873	Richland Creek
Randolph	208	SR 1003	Fork Creek
Randolph	210	SR 2869	Meadow Branch Creek
Randolph	211	SR 2863	Fork Creek
Randolph	214	SR 2900	Bachelor Creek
Randolph	218	SR 2845	Richland Creek
Randolph	220	SR 2849	Bachelor Creek
Randolph	228	SR 2834	Richland Creek
Randolph	257	SR 2824	Vestal Creek
Randolph	260	SR 2636	Brush Creek
Randolph	339	SR 2867	Reedy Creek
Randolph	349	SR 2870	Little Creek
Randolph	359	SR 2911	Richland Creek
Randolph	374	SR 2481	Sandy Creek
Randolph	415	SR 2873	Fork Creek
Randolph	443	SR 2261	Sandy Creek
Randolph	459	SR 2626	Reed Creek
Randolph	463	SR 2114	Little Polecat Creek

CHEMICAL AND PHYSICAL SITE CHARACTERIZATION

Introduction

An important aspect of assessing the impact of the bridge on the mussel fauna is evaluating the chemical and physical condition of the streams upstream and downstream of the road crossing. Extremely high or low values in routine water chemistry parameters would be an indicator of poor water quality at the site and may indicate other factors have more influence on the mussel population than any potential bridge effect. Poor physical habitat adjacent to a road crossing could be a result of the presence of the structure or a factor of a history of poor land use practices in the watershed or at the particular site. The goal of this portion of the study was to determine the inherent health of the stream habitat and water quality regardless of the crossing structure and to determine if the road has caused noticeable changes to the chemical or physical nature of the site. The specific objectives were to:

1. measure water quality with routine water chemistry parameters at the time of sampling upstream and downstream of the road crossing,
2. characterize habitat types throughout the sampled reach at each site,
3. assess the health of the physical habitat in the channel and in the riparian zone.

Methods

<u>Water Quality</u>: At each site, at the time when mussel surveys were done, we measured routine water chemistry parameters approximately 100 meters upstream and downstream of the road crossing. A handheld YSI model 63 was used to measure pH, conductivity (μS), and temperature (°C), and a YSI model 55 was used to measure dissolved oxygen (mg/L). However due to equipment failure pH measurements were not take at all Cape Fear location sites. Turbidity (NTU) was measured with a HF Scientific DRT-15CE model turbidimeter. These measurements were taken near the same approximate time of the day (late afternoon) at all sites.

Data normality was tested using a Ryan-Joiner test. Values from the two basins were compared using either a t-test (normal data) or a Mann Whitney test (non-normal data). Data from the two basins were then pooled, and a paired t-test was used to compare upstream and downstream values of each of the parameters to test for a possible crossing-structure effect.

<u>Habitat Characterization</u>: The two 300-meter reaches at each site were divided into 25-meter cross-sections by measuring down the center of the stream and putting survey flags on each bank to mark divisions. Cross-sections were then numbered consecutively from 1-24 with number 1 being at the most downstream end of the site, and number 24 being at the most upstream end, and the crossing structure being located between cross-sections 12 and 13 (Figure 1.6). We estimated the percentage of the habitat in each cross-section that was either pool, riffle, or run (total of 100%), and noted the apparent dominant substrate in each of those habitat types. The dominant substrate in the most abundant habitat type in a given cross-section was considered to be the dominant substrate for that cross-section. To maximize consistency, the same person conducted habitat characterization for all sites in both the Neuse and the Cape Fear basins. In the Cape Fear study area, we measured bank height and bankfull width every 25 meters.

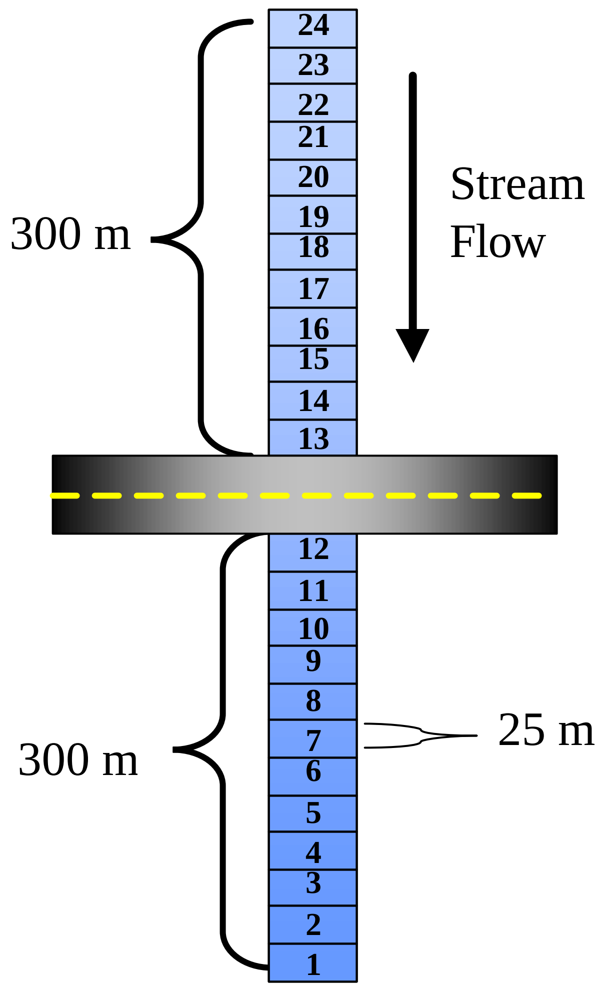

Figure 1.6. *A diagram of a sampling site, which included the 300-meter reaches of stream immediately upstream and downstream of the road crossing. Cross-sections were numbered consecutively from 1-24 with 1 being at the most downstream end of the site and 24 being at the most upstream end.*

Habitat Quality Evaluation: In the Neuse River basin in 2001, habitat in each 100-meter reach with sites was scored using EPA's Rapid Bioassessment Protocols (RBP) for wadeable streams (Barbour et al. 1999). Standardized criteria were used to rate habitat health in the channel, on the banks and in the riparian zone on a scale of 0-20 with 0 being poor and 20 being optimal (Appendix I-1,2). A total of 10 habitat metrics were rated including substrate embeddedness, sediment deposition, bank stability and riparian vegetative zone width. The same person evaluated all reaches and all sites sampled to maximize consistency of scoring.

In 2002 in the Cape Fear River basin, we evaluated habitat health based on the 25-meter cross-sections rather than the 100-meter reaches for which the protocol was developed. We also dropped most of the parameters to be evaluated and only rated bank stability and riparian zone vegetative width. These features could be more accurately assessed in 25-meter increments and represented the most important aspects of the habitat we wanted to evaluate. This also eliminated several metrics that where highly variable based on the inherent nature of the stream. Although the person who scored the Cape Fear sites did not score the sites in the Neuse, all sites in the Cape Fear in 2002 were assessed by the same person.

For all data, normality was tested with a Ryan-Joiner test (Dekker 1986) and upstream and downstream differences were tested with a Mann-Whitney test. Differences between 100-meter or 25-meter reaches were tested using a Kruskal-Wallis test (Noether 1991).

Results

Water Quality: Water quality parameters (Appendices I-3 – I-4) at the Upper Neuse and Cape Fear sites were similar (the exception of temperature; there was a significant difference (p = 0.031) in stream temperature values obtained in the basins. Mean temperature in the Neuse sites in 2001 from 24 April – 18 July was 22.3 °C (Standard Error (SE) = 0.6), and mean temperature in the Cape Fear sites in 2002 sampled from 23 April – 17 July was 20.2 °C (SE = 0.7). Streams tended to be colder from mid-May to mid June in 2002 compared to 2001 (Figure 1.7). This coincided with cooler air temperatures in 2002 compared to 2001 (Figure 1.8). To test if stream size was a factor in stream temperatures, site drainage area was compared between the two basins, and no significant difference was found (Mann-Whitney test *p-value (p)* = 0.67). The Neuse basin did have a few sites with larger drainage areas than were sampled in the Cape Fear study area, but a majority of the streams were of similar size.

Overall, there were no significant differences in upstream versus downstream values in any of the parameters tested (Table 1.3); however, testing of these parameters did identify sites where there may have been water quality problems. In the Neuse study area, 3 sites had at least one D.O. measurement below 4 mg/L, and in the Cape Fear study area, 10 sites had at least one D.O. measurement below 4 mg/L (Table 1.4). Three of those sites in the Cape Fear had D.O. values below 2 mg/L. Three sites in the Neuse basin had particularly high conductivity values, and four sites in the Cape Fear had high conductivity values (Table 1.5).

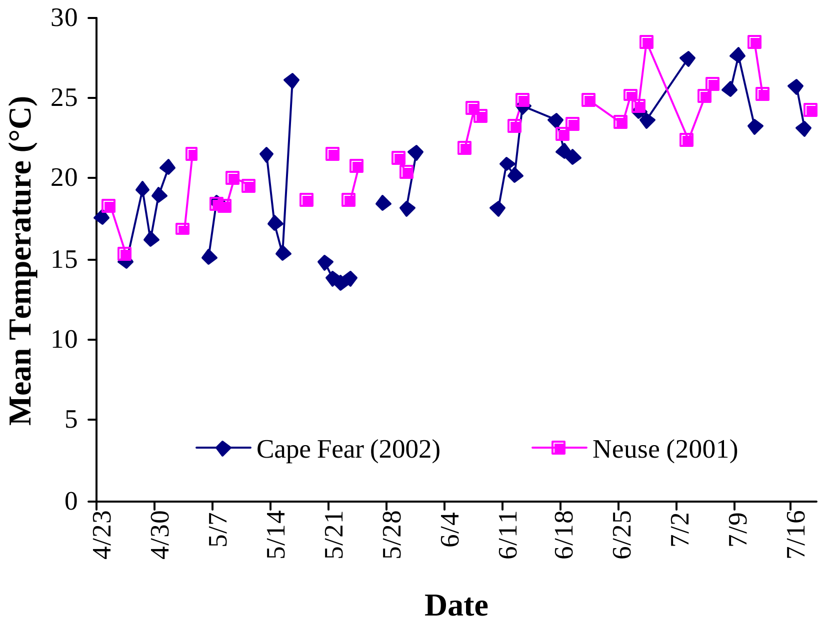

Figure 1.7. *Mean water temperatures (°C) (N = 2) at sampling sites in the Neuse (2001) and Cape Fear (2002) study areas.*

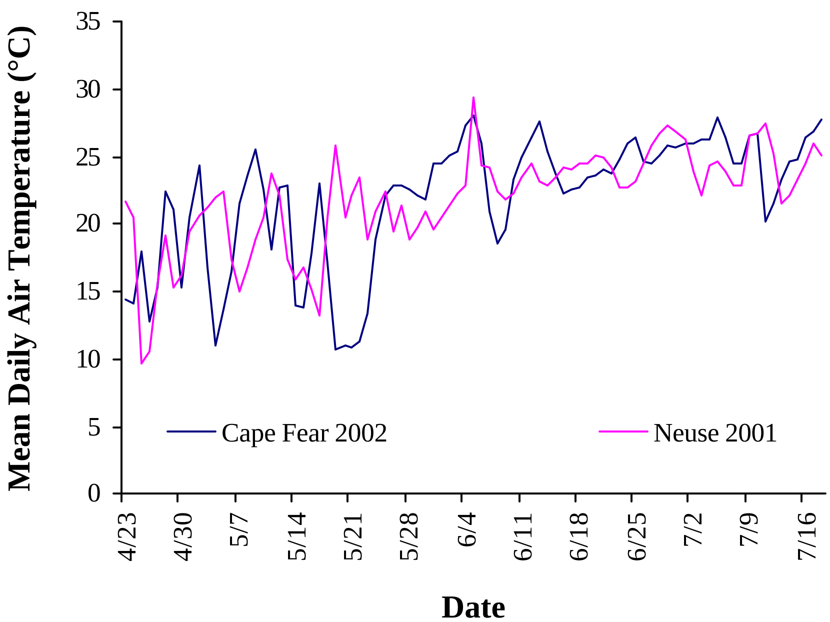

Figure 1.8. *Mean daily air temperatures (°C) obtained from weather stations nearest the Neuse (2001) and Cape Fear (2002) study areas.*

Table 1.3. Results of paired t-tests comparing upstream and downstream values of routine water chemistry parameters at sites in the Neuse and Cape Fear River basins.

Parameter	N	Upstream Mean	Upstream SE	Downstream Mean	Downstream SE	*p*-value
pH	53	7.29	0.25	7.34	0.35	0.209
Dissolved Oxygen (mg/L)	79	6.29	0.21	6.41	0.20	0.435
Temperature (°C)	79	21.8	0.45	22.0	0.46	0.156
Conductivity (μS)	77	91.6	2.92	92.2	2.94	0.779
Turbidity (NTU)	71	12.3	0.81	16.2	2.79	0.153

Table 1.4. Sites in the Neuse and Cape Fear study areas with at least one dissolved oxygen (D.O.) measurement below 4 mg/L.

River Basin	Bridge Number	Date Sampled	Stream	Lowest D.O. measurement (mg/L)
Neuse	O-242	7/3/01	West Fork Eno River	3.30
	O-12	7/6/01	East Fork Eno River	2.80
	O-64	8/21/01	Eno River	3.58
Cape Fear	R-214	4/23/02	Bachelor Creek	3.40
	C-245	5/30/02	Little Brush Creek	2.85
	M-28	6/10/02	Dry Creek	3.01
	R-211	6/12/02	Fork Creek	1.51
	R-210	6/13/02	Meadow Branch Creek	3.4
	R-208	6/17/02	Fork Creek	3.30
	C-251	6/19/02	Brush Creek	3.59
	M-127	6/27/02	Bear Creek	3.10
	R-260	6/28/02	Brush Creek	0.62
	R-109	7/17/02	Polecat Creek	1.93

Table 1.5. Sites in the Neuse and Cape Fear study areas with relatively high conductivity values relative to the mean for the other sites in the basin.

River Basin	Bridge Number	Date Sampled	Stream	Mean conductivity measurement (mS)
Neuse	O-200	5/24/02	Stroud's Creek	146
	D-50	7/11/01	Eno River	130
	O-64	8/21/01	Eno River	152
Cape Fear	G-23	5/6/02	Polecat Creek	147
	G-72	5/7/02	Polecat Creek	162
	R-210	6/13/02	Meadow Branch Creek	137
	R-260	6/28/02	Brush Creek	133

<u>Habitat Characterization</u>: Overall habitat at sites in the Cape Fear was slightly different than at the Neuse sites. Sites in the Neuse tended to have more boulder and sand, and sites in the Cape Fear tended to have more bedrock (Figure 1.9). The Neuse study area also tended to have more run habitat, whereas the Cape Fear study area had more pool habitat (Figure 1.10).

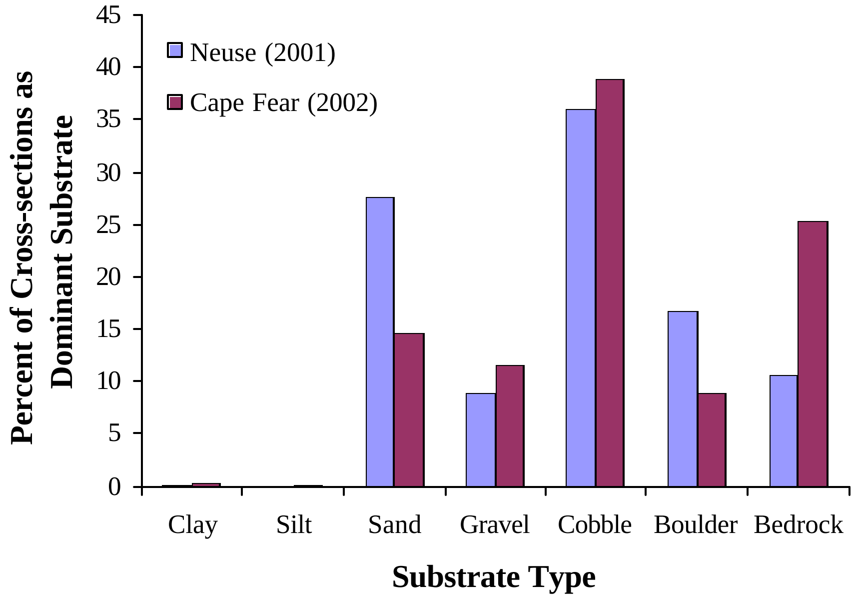

Figure 1.9. *The percentage of all cross-sections in each basin with a dominated by different substrate sizes. A total of 1060 cross-section were surveyed in the Neuse study area, and 883 were surveyed in the Cape Fear study area.*

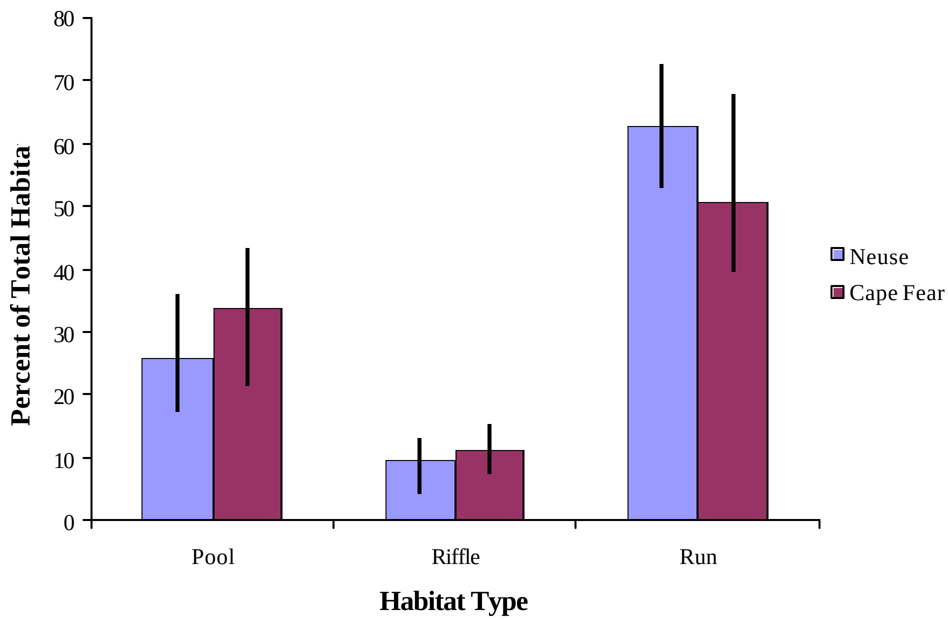

Figure 1.10. *Median percent of each habitat type in the Neuse and Cape Fear River basin sampling sites. Error bars represent 25 and 75% quartiles. For the Neuse sites, N=44. For the Cape Fear sites, N=36.*

With respect to the 12 culverts evaluated over the two years, no trends were seen in habitat composition longitudinally along the stream in percent pool, riffle or run; however, a trend was seen with respect to bridges. There tended to be less pool habitat within 50 -100 meters upstream and downstream of bridges. In the place of these lost pools were some riffles, but mostly an increase in run habitat was seen. Differences between cross-sections in percent pool and percent run were not significant at the $\alpha = 0.05$ level (Kruskal-Wallis test, $p = 0.099$ and $p = 0.160$ respectively); however a clear trend was seen of decreased pool habitat (Figure 1.11) near the road on both the upstream and downstream sides.

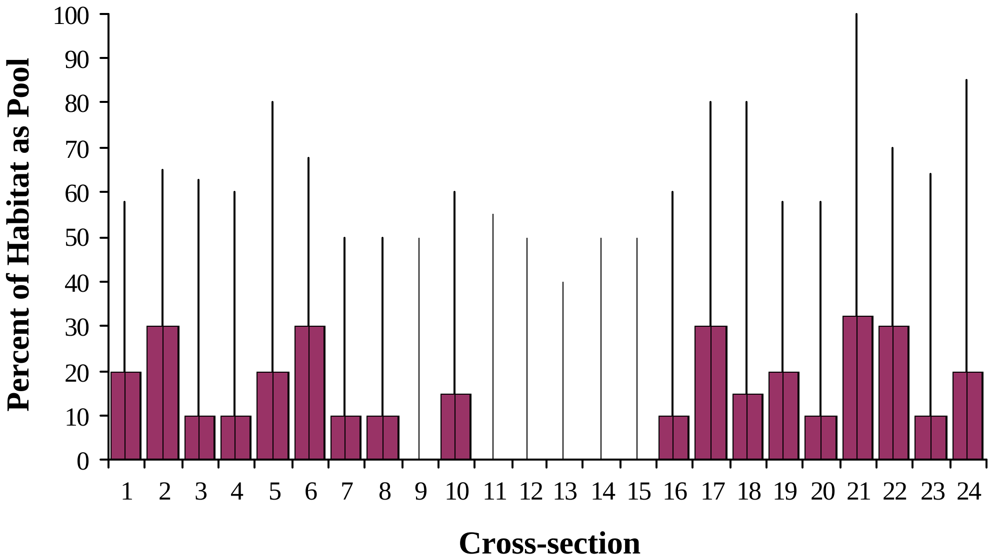

Figure 1.11. *The mean and median percentage of habitat as pool in each cross section. Error bars represent 25 and 75% quartiles (N=69). Cross-sections were numbered consecutively from downstream to upstream and the road crossing was between numbers 12 and 13.*

Habitat Quality Evaluation: In the Neuse River basin, total RBP scores at sites ranged from 98.2 to 174.7 out of a possible 200 (Appendix I-5). There were statistical differences between 100-meter reaches in channel alteration ($p = 0.039$), but no other statistical differences were found in total scores or individual metrics between reaches or between upstream and downstream of the crossing (Kruskal-Wallis test, $p > 0.05$, Appendix I-6). Scores for most metrics were very similar for most sites, but naturally sandy streams tended to score lower than rockier streams on several metrics. Bank stability and riparian zone vegetative cover had the greatest variation between sites and likely provided the most accurate representation of habitat quality (Figure 1.12).

In the Cape Fear River Basin, total riparian zone scores at sites ranged from 5.2 to 19.2, and no statistical differences were found between 25-meter cross-sections or between upstream and downstream ($p = 0.174$). There was a longitudinal trend in bank stability scores along streams in the Cape Fear study sites (Figure 1.13) and there were highly significant differences between cross-sections (Kruskal-Wallis test, $p < 0.001$). Overall upstream bank stability scores were also significantly higher than downstream bank stability scores (Mann-Whitney test, $p < 0.0001$). Bank stability was highest near the crossing, and for 50-75 meters upstream of the crossing, bank stability scores generally increased as distance from the structure decreased. As distance from the bridge increased downstream, bank stability scores decreased with the lowest scores falling in the most downstream 100 meters. At a few sites (Appendix VI), increased stream incision led to decreased bank stability, but we found no overall differences between cross-sections in bank height (p = 0.993) or bankfull width (p = 0.943).

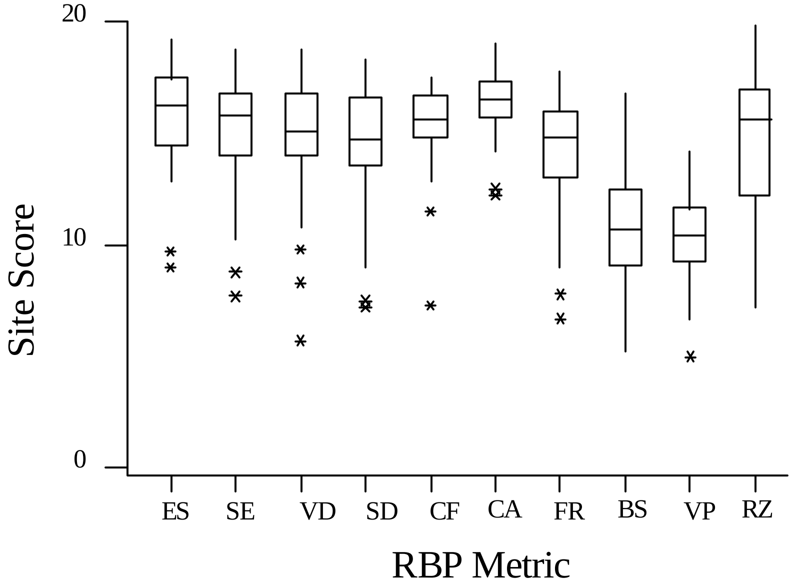

Figure 1.12. *Boxplots for the 10 Rapid Bioassessment Protocol habitat metrics for 44 sites in the Neuse study area. The metrics are epifaunal substrate cover (ES), substrate embeddedness (SE), velocity-depth regime (VD), sediment deposition (SD), channel flow status (CF), channel alteration (CA), frequency of riffles (FR), bank stability (BS), bank vegetative protection (VP), and riparian zone vegetative cover (RZ).*

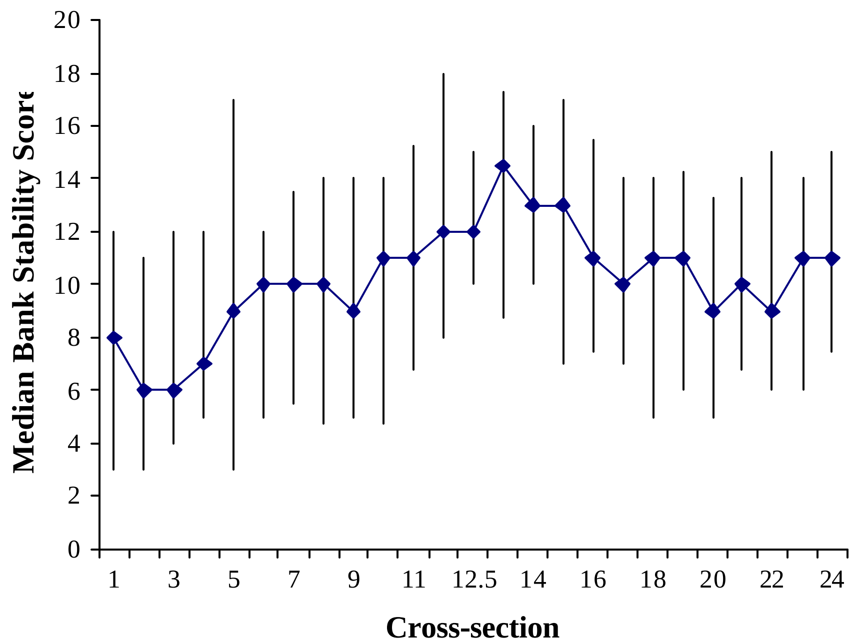

Figure 1.13. *Median bank stability scores for all 25-meter-long cross-sections at 36 sites in the Cape Fear River Basin. Error bars represent 25 and 75% quartiles. The section of stream under or within the crossing structure is represented by the number 12.5.*

Discussion

Water Quality: Cooler water temperatures in the Cape Fear basin in portions of 2002 compared to 2001 was likely due partially to cool air temperatures in May 2002. Other reasons for this difference are unknown, but since the data were taken in two different years as well as in different locations, no concrete conclusions can be drawn about long-term temperature regimes in these watersheds. The fact that there were no differences when upstream samples were compared to downstream in any of the parameters measured was expected. This snapshot water chemistry data was primarily meant to identify sites with very noticeable water problems. Any potential changes in water chemistry from upstream to downstream of a road crossing would likely only be detected during a rainstorm when runoff enters at the road and may not be detected at all with the routine parameters we measured. A more in depth study with continuous monitoring devices and storm surge samplers would be needed to enhance the resolution of the data collected, but this type of equipment and labor intensive sampling was beyond the scope of the funding allocated.

We did identify several sites with potential water quality problems. Low D.O. values were typically found in low gradient, sandy areas of the stream with little flow. The lack of rainfall during the sampling season of 2002 caused low flow and stagnant water in most streams in the Cape Fear basin. This led to the increase in number of sites with low D.O values in that basin. We believe that the low D.O. values likely do not represent substantial nutrient enrichment of these streams, but are only an indicator of the stream's natural ability to oxygenate itself. Of the sites with elevated conductivity values, these primarily occurred in urban areas. Of the two sites on the Eno River, one was downstream of Hillsborough and the other was in Durham at NC 157. The two sites on Polecat Creek with elevated conductivity are located downstream of the outskirts of Greensboro. The other three sites identified with higher conductivity occurred in more agricultural areas. These increased values are likely and indicator of an anthropogenic effect on water quality.

Habitat Characterization: Overall differences in habitat between the Neuse and Cape Fear river basins were slight, and streams varied in habitat as much within basins as they did between the two basins. For instance, several sites in the Flat River watershed within the Neuse contained a large amount of sand while portions of the adjacent Little River drainage in the Neuse were dominated by bedrock and boulder. The same differences occurred in the Cape Fear study area as Polecat and Sandy Creeks were highly sandy, but Richland and Brush Creeks contained a large amount of boulder and bedrock. Indeed this variation occurred within smaller watersheds and even within the same sampling sites in both basins, so we believe pooling habitat data between basins is acceptable for analysis.

When habitat was analyzed with respect to culverts, no trends in dominant substrate or pool-riffle-run composition and were seen. With bridges, differences in percent pool between cross-sections were not statistically significant ($p = 0.099$); however, the apparent trend still may be ecologically significant. Although the habitat alterations were not drastic overall, we believe this trend does represent a bridge-related change in the stream habitat at some sites. Bridge construction often results in channelization through bank stabilization, and widening or straightening of the channel (Little and Mayer 1993; Forman and Alexander 1998). The resulting channelization can alter habitat not only at the bridge but upstream and downstream as well including increased stream velocity, alteration of pool-riffle formations, uniformity of depth, and a shift in substrate characteristics (Hubbard et al. 1993).

Habitat Quality Evaluation: Several of the 10 RBP metrics used to score habitat in the Neuse study area such as frequency of riffles, velocity-depth regime, and epifaunal substrate cover were highly variable based on surface geology and stream slope. We felt this system was not adequate to describe streams of such a variable nature and did not provide enough resolution to look at differences longitudinally along the stream. We then refined the habitat quality assessment in 2002 for the Cape Fear River Basin to use only the most relevant parameters to rating habitat health across a variety of stream types. Rating bank stability and riparian zone vegetative cover in 25-meter increments also allowed for a more accurate assessment of the habitat rather than trying to judge 100-meter reaches.

The differences in bank stability scores upstream and downstream of the crossing were driven by high scores just upstream of the road crossing as well as especially low scores at the most downstream end of the sampling site. High bank stability in the cross-sections adjacent to the crossing structure is derived partially from the extensive vegetation that grows on the banks there due to the loss in canopy cover around the road. Also, banks around the structure have also been sloped to maximize stability at many crossings, and in some cases riprap was also used in these areas. Crossings that constrict the channel could also potentially cause more stable banks upstream by slowing flows during flood events and reducing the stream's erosive forces in that area. The reason for the lower bank stability scores downstream is unknown. If bridges have caused a loss in pool habitat as described above, there may truly be extensive, but subtle changes in bedload movement for a considerable distance downstream of bridges. This could potentially cause changes in bank erosion downstream. Although bank stability scoring was based on EPA's standardized criteria, it was still somewhat subjective. However, due to the large number of sites (N = 36) and cross-sections (N = 864) scored, the trend we saw in decreased stability downstream still may represent a real effect. We currently have no definitive explanation as to whether this represents a real crossing structure effect. More intensive and conclusive research is needed in this area to determine crossing-structure effects on bedload movement and bank erosion.

Summary of Findings

1. There were no differences in routine water chemistry parameters (pH, D.O., temperature, conductivity, and turbidity) between upstream and downstream of road-crossings when snapshot measurements were taken at normal flow.

2. We did identify 5 sites in the Neuse study area and 12 sites in the Cape Fear study area that have potential water quality problems due to either low D.O. or high conductivity levels. Biological data should be considered in light of this data.

3. Habitat in the Cape Fear study area was slightly different than that in the Neuse basin, but even larger variation was seen between streams within basins and even within sampling sites. Therefore, we feel confident in pooling habitat data between the two basins.

4. A loss in pool habitat around bridges and decrease in bank stability scores downstream may represent extensive, but subtle changes in stream bedload movement; however, more conclusive research is needed.

Chapter 2

Mussel and Aquatic Insect Survey

Introduction

Methodology for using stream biota to assess stream health is well established, and both fish and aquatic insects are widely used for this purpose (Barbour et al. 1999). Freshwater mussels have been used as biomonitors to assess certain types of water quality problems (Foe and Knight 1987; Goudreau et al. 1993), but they may also serve as good indicators of physical habitat disturbance. Because of their intolerance to sedimentation (Marking and Bills 1979), and need for stable substrate (Strayer 1999) and low sheer stress (Hardison and Layzer 2001), a wide variety of physical alterations to the stream environment may result in mussel declines. It is the drastic mussel declines across the country that have led to heightened interest in their conservation by wildlife agencies, and this study was initiated because of the specific concern over potential mussel loss around road crossings. However, results of this study not only have bearing on mussels but on other stream fauna as well. The goal of this portion of the study was to measure the effect of road crossings on freshwater mussels. Specific objectives were to:

1. **Use a rapid assessment technique to evaluate the effect of crossings using the aquatic insect community upstream and downstream of road crossings, and**

2. **Obtain relative abundance and diversity data for freshwater mussels upstream and downstream of road crossings in 25-meter cross-sections.**

Methods

<u>Freshwater Mussel Surveys</u>: Original site surveys in the Neuse study area were sampled from 24 April – 21 August 2001 (Appendix II-1), and the Cape Fear study area was sampled from 19 April – 17 July 2002 (Appendix II-2). To better understand the temporal variation in our data we resurveyed a total of 15 sites at either another time of year or at the same approximate date the following year (Appendix II-3). Five sites in each basin were surveyed at the beginning and end of the sampling seasons, and five sites from the Neuse study area were resurveyed in 2002 at approximately the same date they were surveyed in 2001. The same survey technique was used at all sites.

At each site, three surveyors each searched 1-meter-wide linear transects (one next to each bank and one in the center of the stream) using view scopes and snorkeling to visually locate mussels. These transects were searched in an upstream direction for the entire 600 meters of stream surveyed at each site and under the crossing structure. The 1-meter width was standardized on each surveyor by measuring against their armspan giving each person a reference point on their body by which to measure, and no mussels were included in the survey that fell outside this 1-meter width. As surveyors moved upstream, the 1-meter transects on each bank were measured from the water's edge using the reference point on their armspan, and the transect in the center of the stream was measured from the centerline of the surveyor's body. The same surveyor surveyed the same linear transect (left bank, middle, or right bank) for an entire site, and a standard rotation was used between sites. In larger, more diverse streams, we used 1-2 extra surveyors to qualitatively search areas between the three linear transects to try to find species not accounted for in the transects. The qualitative searches also yielded extra data on sex ratios and gravidity of sexually dimorphic species (Lampsilines). Most sites were completed within the same day, but five sites in the Neuse and two sites in the Cape Fear required two days to complete. All sites that required two days were completed in consecutive days, and no substantial weather changes or rain occurred between those days.

Only visual surveys were done to maximize consistency through time and between surveyors, and no excavation or rock flipping was used to locate mussels. Tactile searching was used occasionally as necessary when murky water, debris piles, or undercut banks made visual searches difficult; however, only mussels felt on the sediment surface were taken. Mussels were identified, and length was measured to the nearest millimeter using calipers on the first 15 of each species collected from each cross-section. We recorded the cross-section number (Chapter 1) and linear transect (left bank, middle, right bank) in which the mussel was located. Lampsilines were classified as male or female, and we checked for gravidity (presence of mussel larvae) of all known females. Mussels were returned to original life position as soon as data was recorded for each individual.

Two specific measures were taken in the field for quality assurance. Between sites we alternated between starting the survey at two different points within the reach to be sampled. At half of the sites, we started the survey at the most downstream end of the site and moved in an upstream direction to sample the entire reach. At the other sites, we started at the road crossing surveying the upstream reach first then going the downstream end and searching up to the road crossing. This was done to guard against a time bias with respect to the road crossing, so the same portion of stream was not always sampled at the same time of day. Also, a measure of detectability was taken at each site in a predetermined 75-meter reach by removing all mussels found in the bank transects and using a second pass by the field supervisor to locate any mussels missed. This provided a measure of variation in mussel detection between days and between surveyors. Detectability percentage was calculated as the number of mussels found in the first pass divided by the total number found in the first and second passes.

All data were tested for normality using a Ryan-Joiner test. We then analyzed the data in a variety of ways to assess potential differences in relative mussel abundance and diversity in relation to the road crossing. To equally weight all sites, relative abundance was calculated as the percent of mussels at a site occurring in a given cross-section. We calculated the Shannon-Weiner Diversity Index (Campbell et al. 1986; Thrush et al. 1998) as a measure of diversity for individual 25-meter cross-sections as well as the entire upstream and downstream reaches at all sites using the following formula :

$$\textbf{Shannon-Weiner Index} = \sum_{i-1}^{n} -\left(\frac{\text{Number of i}^{\text{th}}\text{ species found}}{\text{Total mussels found}} \times \log\left(\frac{\text{Number of i}^{\text{th}}\text{ species found}}{\text{Total mussels found}}\right)\right)$$

Other measures of diversity used included the number of species found other than *Elliptio complanata* (the most abundant species) and the number of individuals found of these other species. Differences between upstream and downstream were tested with a Wilcoxon Signed-Rank test. Differences between 25-meter cross-sections were tested with a Kruskal-Wallis test. A proportion was also used to test whether the percentage of bridges with more mussels upstream was significantly different than 50%. Mussel length was also assessed using a Kruskal-Wallis test to compare length between cross-sections and a Mann-Whitney test (Noether

1990) to compare between basins and between the entire upstream and downstream 300-meter reaches.

Aquatic Insect Collection: An aquatic sample was taken at each site in the Neuse study area at the first riffles upstream and downstream of the road crossing. Sampling was done on the same day that mussels were surveyed at a given site. At both riffles sampled, a 1-meter-wide kicknet was used with one person standing approximately 1 meter upstream of the net and kicking into the substrate overturning and cleaning rocks allowing insects to drift into the kicknet (NCDENR 2001). The net was then removed from the water and the insects were placed in 95% ethanol for preservation. Two people then each spent 10 minutes collecting insects by visually inspecting and overturning rocks, woody debris and other special habitat to collect taxa potentially missed by the kicknet sample. Any insects collected were added to the kicknet sample in ethanol. The sample was then labeled with the bridge number, date, collectors' names, and whether the sample was collected upstream or downstream of the crossing. Ephemeroptera, Plecoptera, and Trichoptera (EPT) were identified in the laboratory to the lowest possible taxa. The EPT taxa richness was calculated for each sample, and each taxon was given a pollution tolerance score using standards developed by the North Carolina Department of Environment and Natural Resources (NCDENR 2001). Data were tested for normality using a Ryan-Joiner test, and a paired t-test was used to test for differences in the number of EPT taxa and mean tolerance values upstream versus downstream.

Results

Freshwater Mussel Surveys: Between the two basins, we surveyed a total of 80 sites encompassing over 48 km of streams and collected a total of almost 45,000 mussels representing 16 species (Appendices II-7, II-8). In the Neuse basin, the number of mussels found at a site ranged from 2 to 3377 with a median of 307, and in the Cape Fear basin the number of mussels found ranged from 43 to 1866 with a median of 412 (Figure 2.1). The Neuse did contain a few more sites with an especially abundant mussel fauna, but the two basins were statistically similar in relative mussel abundance ($p = 0.826$). Some species were found in both the Cape Fear and Neuse study areas, and some species were found only in one basin or the other. However, *E. complanata* comprised approximately 96 and 97% of all mussels found in the two basins respectively (Table 2.1). Mussels in the Cape Fear basin were generally smaller than those in the Neuse study area ($p < 0.0001$, Figure 2.3).

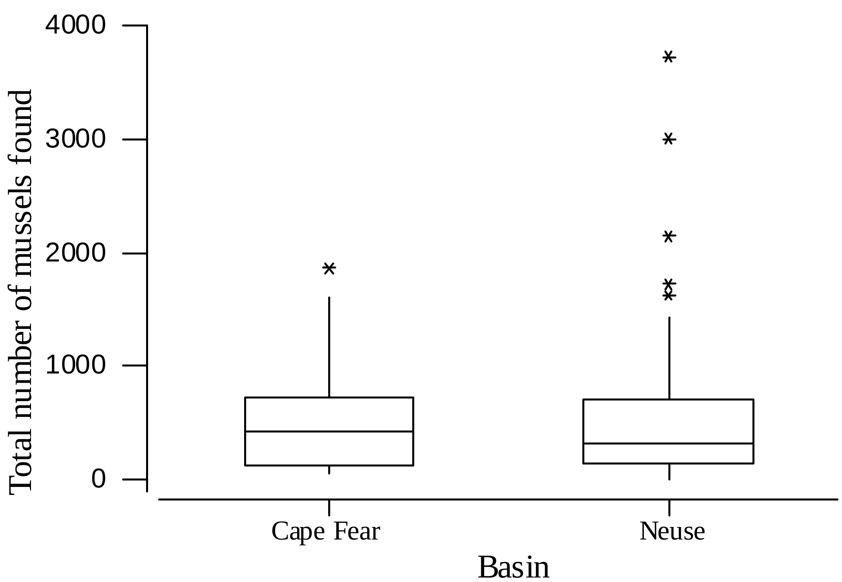

Figure 2.1. *Boxplot of the number of mussels found at sites in the Cape Fear and Neuse study areas.*

Table 2.1. A summary of the number of each species found in the two basins along with the status. Federal status is in parentheses (SoC = Species of Concern).

Species	Number Found: Neuse	Number Found: Cape Fear	Current Species Status
Alasmidonta undulata	0	2	State Threatened (SoC)
Alasmidonta varicosa	0	8	State Endangered (SoC)
Elliptio complanata	24,836	18,004	Stable
Elliptio icterina	0	180	Stable
Elliptio sp. (lanceolate)	0	2	Stable
Fusconaia masoni	31	4	State Endangered (SoC)
Lampsilis cariosa	45	1	State Endangered (SoC)
Lampsilis radiata	54	0	State Threatened
Lampsilis sp.	37	0	N/A
Lasmigona subviridis	2	0	State Endangered (SoC)
Pyganodon cataracta	164	84	Stable
Strophitus undulatus	191	30	State Threatened
Toxolasma pullus	0	8	State Endangered (SoC)
Villosa constricta	189	92	State Special Concern
Villosa delumbis	0	127	Significantly Rare
Villosa vaughaniana	0	189	State Endangered (SoC)
Utterbackia imbecillis	1	0	Stable

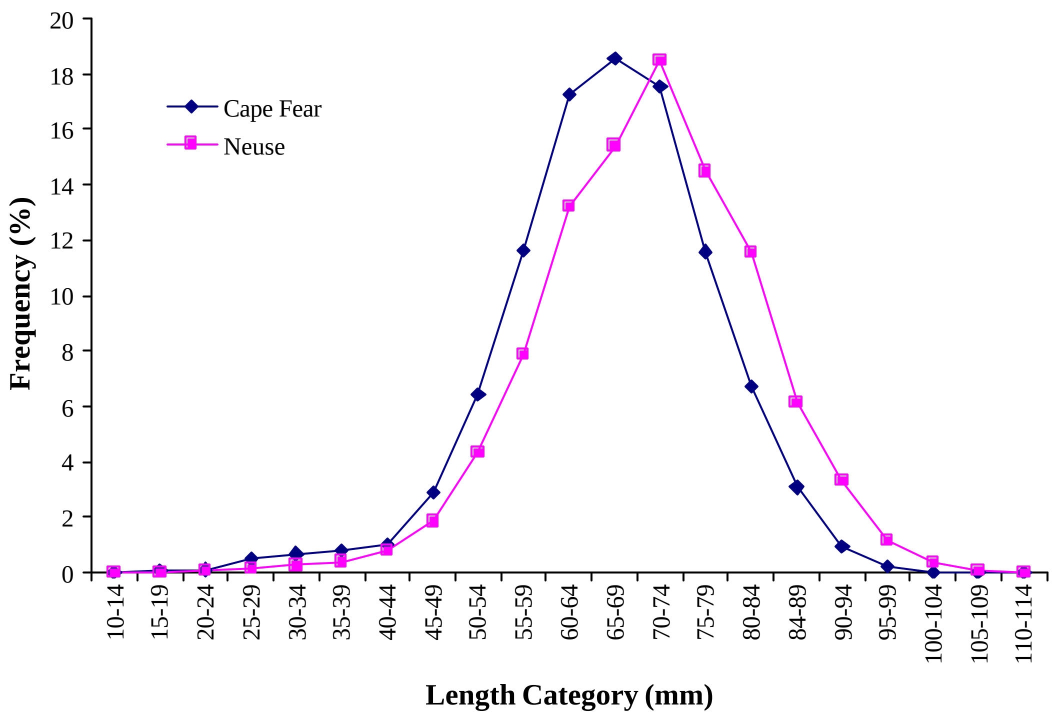

Figure 2.2. *Frequency histogram of length of Elliptio complanata in the Neuse and Cape Fear study areas.*

Bank transects (lanes) tended to have greater mussel density because of the abundance of *E. complanata.* Only 22% of all *E. complanata* were found in the center lane, whereas 33.3% would be expected if mussels were evenly distributed. Other species were more likely to be found in the middle of the stream (Table 2.3), and that transect tended to have a slightly greater diversity of species. In the Neuse basin, 98.2% of mussels found along banks were *E. complanata*, but only 94.0% of mussels in the middle lane were of this species. In the Cape Fear study area, *E. complanata* comprised 96.9% of mussels along banks and only 93.5% of mussels in the middle. In both basins, *E. complanata* in the middle lane were also significantly larger than those along the banks ($p < 0.001$). In the Neuse study area, *E. complanata* had a mean length of 72.4 mm (SE = 0.23) in the middle lane and 69.9 mm (SE = 0.15) along the banks (Appendix II-9). In the Cape Fear basin, this species had a mean length of 68.2 mm (SE = 0.29) in the middle of the stream and a mean length of 65.7 mm (SE = 0.16) along the banks (Appendix II-10). Qualitative data taken between lanes yielded only a few additional species at sites not found in linear transects. Of the 20 sites where qualitative searches were done in the Neuse study area, 1 additional species was found at 6 of those sites and 2 additional species were found at 2 sites. All additional species found in qualitative searches in the Neuse basin were represented by only one individual. In the Cape Fear basin, qualitative data was taken at 14 sites, and an additional species was found in 4 of those sites. Additional species were represented by only one individual except at one site where two individuals of an additional species were found. Overall, results of qualitative searches between lanes indicated data in linear transects were fairly representative of the mussel fauna at each site.

Table 2.2. Total numbers found of species found in both the Neuse and Cape Fear basins most likely to be found in the linear transect (lane) in the center of the stream rather than in bank transects.

Species	Basin	Number found			Percent of individuals in middle lane
		Left Lane	Middle Lane	Right Lane	
Fusconaia masoni	Neuse	11	16	5	50.0
	Cape Fear	0	3	1	75.0
Lampsilis cariosa	Neuse	4	33	9	71.7
	Cape Fear	0	1	0	100.0
Strophitus undulatus	Neuse	41	114	33	60.6
	Cape Fear	7	16	7	53.3
Villosa constricta	Neuse	42	113	35	53.8
	Cape Fear	14	55	22	60.4

Overall detectability was good (Figure 2.3); however, it was somewhat dependent on the number of mussels in the sampled reach. Median detectability for individual measurements was 90.9% with 25 and 75% quartiles of 81.7 and 100% respectively. There were no significant differences in detectability between surveyors ($p = 0.371$). Of the 16 times (10.0% of the time) where surveyors found no mussels in the first pass, a mussel was found on the second pass only 3 times. When there were fewer than 10 mussels in the 75-meter detectability reach, chances of having poor detection percentages increased (Figure 2.4). Of the 23 times (14.4%) that individual detectability was below 75%, fewer than 10 total mussels were found 14 of those times.

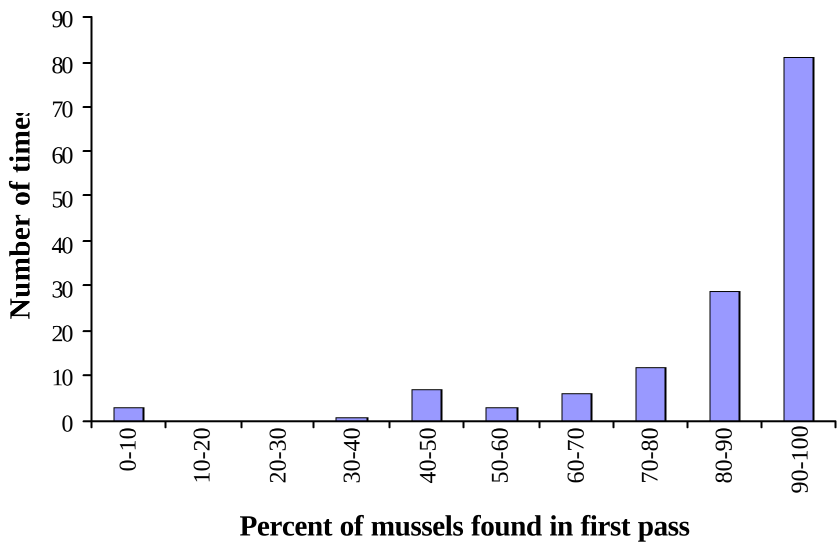

Figure 2.3. *Frequency histogram of individual detectability measurements. Percent of mussels found in the first pass was calculated by dividing the number found in the first pass by the total found in the two passes.*

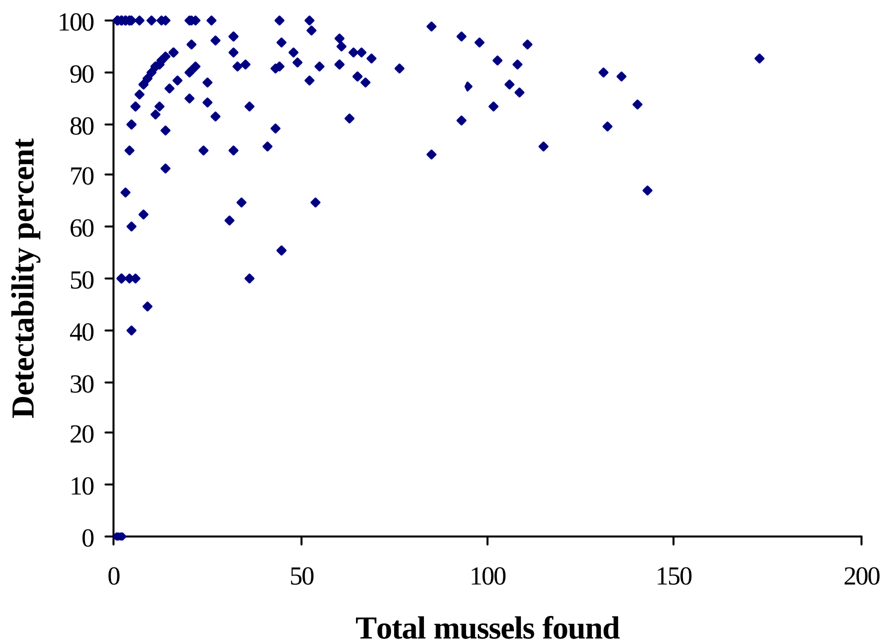

Figure 2.4. *Detectability success as a function of total number of mussels found.*

The Cape Fear River basin contained eight sites with milldam remains within the upstream 300-meter reach, and no milldam remains were found in the downstream reach at any site. The dam was still somewhat functional at cross-section 19 at one of those sites, and we did not survey the cross-sections upstream of it because of the deep, impounded water. Milldam remains represent impoundment of the stream and subsequent destruction of the impoundment at some time in the past. Reaches where remains were found were typically scoured to bedrock, high in gradient, and contained few mussels (Figure 2.5). In fact, of the eight sites with milldams, seven of them had substantially more mussels in the downstream reach compared to upstream, and the median percentage of mussels found in the upstream half of the sites was only 15.4% (Table 2.3). At the lone milldam site with more mussels in the upstream half, the milldam was located in the most upstream 50 meters of the sampling site. The number of mussels found downstream of the road was significantly greater than the number found upstream at these eight sites using a paired t-test ($p = 0.006$). Because of these large differences associated with the milldams, these sites were not included in our analyses of the effect of the road crossings.

Table 2.3. Summary of the number of mussels found in the upstream and downstream reaches at eight sites in the Cape Fear study area that contained milldam remains.

County	Bridge Number	Number of mussels found upstream	Number of mussels found downstream	Percent of mussels in the upstream reach
Chatham	251	19	97	16.4
Guilford	72	195*	796*	19.7*
Moore	174	81	68	54.4
Randolph	110	43	380	10.2
Randolph	208	209	1396	13.0
Randolph	218	166	983	14.4
Randolph	359	450	1412	24.2
Randolph	374	66	835	7.3

Figure 2.5. *Remains of a milldam.*

When the remaining 72 sites from both basins were assessed, relative abundance in upstream and downstream 300-meter reaches was similar ($p = 0.364$); however, there tended to be fewer mussels under the structure as well as in the 50-meters downstream of the road crossings. The number of mussels found under crossing structures is not directly comparable to numbers found in 25-meter cross-sections because the stream length under crossing structures averaged 9.6 m (SE = 0.7). However, there were very few mussels found under bridges. No mussels were found under 35 of the 72 crossings (48.6%), and we found only 5 or more mussels under crossings 7 times (9.7%); A Kruskal-Wallis test revealed highly significant differences between cross-sections in the percentage of mussels at a site occurring in each cross-section ($p < 0.001$, Table 2.4). In general fewer mussels were found in the first 25-50 meters immediately downstream of the crossing structure (Figure 2.6). When the 24 most abundant sites were analyzed, this trend of reduced relative abundance was clear (Figure 2.7), and highly significant ($p < 0.001$) differences existed between cross-sections. There was no difference between cross-sections in the percentage of mussels that occurred in the middle of the stream as opposed to the bank transects ($p = 0.923$), indicating the crossings affected both banks and the center of the stream equally.

We found more than 50 mussels in the two cross-sections immediately below the road 2-3 times less compared to other cross-sections (Figure 2.8). Cross-sections 11 and 12 had no mussels more often than other transects, and cross-section 12 had 5 or fewer mussels more than all other cross-sections (Figure 2.9). When the number of mussels in individual downstream cross-sections was compared to the median number found in the 12 upstream cross-sections, 48 out of the 72 crossings (66.7%) had fewer mussels in 25-meter reach immediately downstream of the road (Figure 2.10). This number of bridges was significantly higher than 50% ($p = 0.006$). The number of crossings with a higher cumulative number of mussels upstream versus downstream was also significantly higher than 50% at 50, 75, and 100 meters from the crossing structure ($p < 0.05$, Figure 2.11). Although fewer mussels were found in cross-section 12 than in number 11, the percent of bridges with more mussels in the first 25 meters upstream than downstream was not significantly different than 50%. This was an indicator that there may be fewer mussels in the 25 meters immediately upstream at some road crossings. The trend of fewer mussels immediately below the crossing was especially evident when the area near the road was analyzed at the 24 sites where mussels were most abundant (Figure 2.12, Table 2.5).

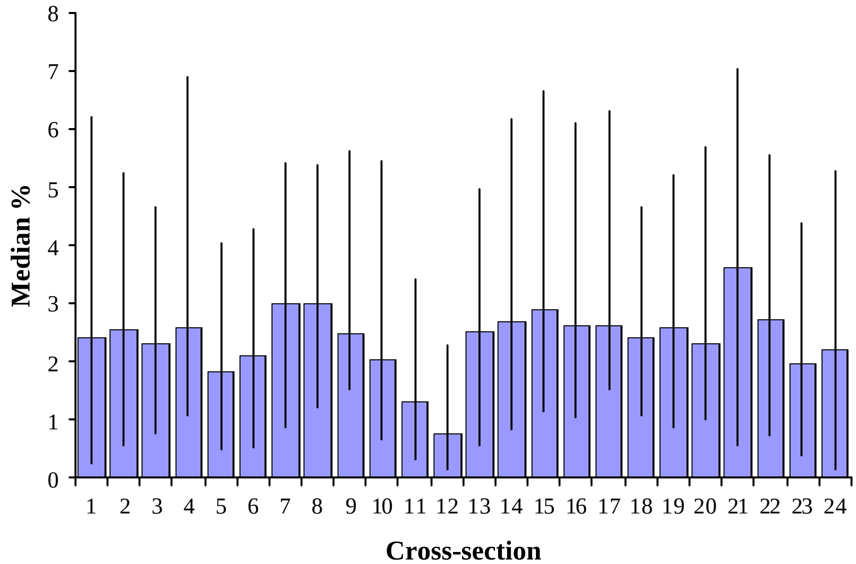

Figure 2.6. *Median percent of mussels at a site found in a given 25-meter cross-section with 25% quartile error bars. Cross-sections were numbered consecutively from downstream to upstream, and the road crossing occurred between cross-sections 12 and 13.*

Table 2.4. Results of the Kruskal-Wallis test comparing the percentage of mussels at a site found in the different 25-meter cross-sections (*p* < 0.001). Cross-sections were numbered consecutively from downstream to upstream, and the road crossing was in the middle of the site.

Cross-section	Median	Average Rank	Z
1	2.3	867.0	0.25
2	2.6	870.0	0.31
3	2.3	832.7	-0.35
4	2.6	937.9	1.49
5	1.8	759.3	-1.63
6	1.7	794.0	-1.02
7	2.5	884.8	0.57
8	3.0	931.7	1.39
9	2.4	948.1	1.67
10	2.0	841.3	-0.20
11	1.2	663.7	-3.30
12	0.7	530.6	-5.63
13	2.2	824.5	-0.49
14	2.7	915.8	1.11
15	2.5	938.0	1.50
16	2.6	927.8	1.32
17	2.7	955.3	1.80
18	2.3	872.0	0.34
19	2.6	873.7	0.37
20	2.3	886.9	0.60
21	3.4	947.3	1.66
22	2.6	855.5	0.05
23	1.7	788.1	-1.13
24	2.1	814.0	-0.67

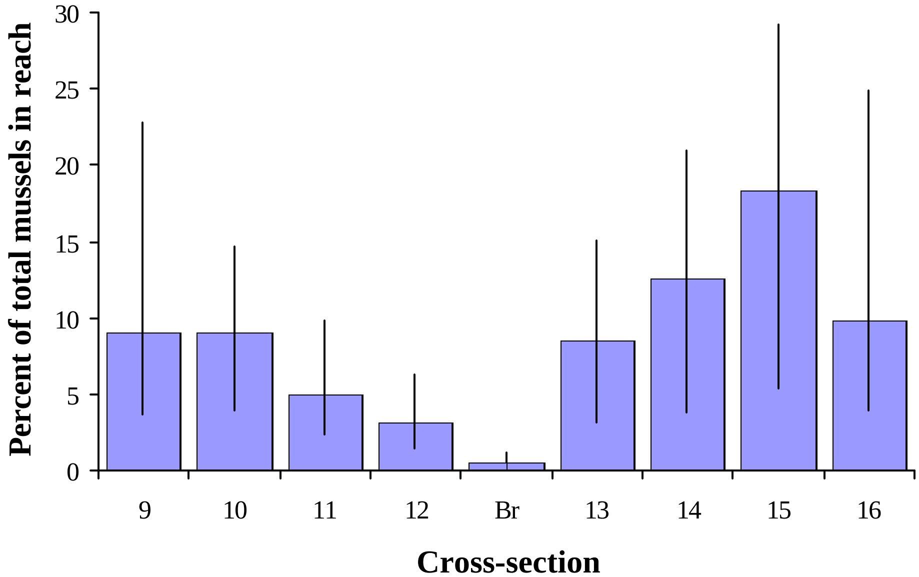

Figure 2.7. *Median percent of mussels within 100 meters of the road crossing found in a given 25-meter cross-section with 25% quartile error bars. This analysis used only sites with at least 100 mussels in either of the 100-meter reaches immediately upstream or downstream of the road crossing (N=24). Cross-sections were number consecutively from downstream to upstream with the road crossing (Br) being in the middle of the site.*

Table 2.5. Results of the Kruskal-Wallis test comparing the percentage of mussels within 100 meters of the road crossing found in the different 25-meter cross-sections ($p < 0.001$). This analysis used only the 24 sites with at least 100 mussels in either of the 100-meter reaches immediately upstream or downstream of the crossing structure. Cross-sections were numbered consecutively from downstream to upstream, and the road crossing was in the middle of the site.

Cross-section	Median Percent of mussels found	Average Rank	Z
9	9.0	129.5	1.75
10	9.0	119.7	0.93
11	5.0	97.1	-0.95
12	3.1	74.1	-2.86
Road Crossing	0.5	34.0	-6.19
13	8.5	117.3	0.73
14	12.6	129.1	1.71
15	18.3	146.5	3.16
16	9.8	129.2	1.72

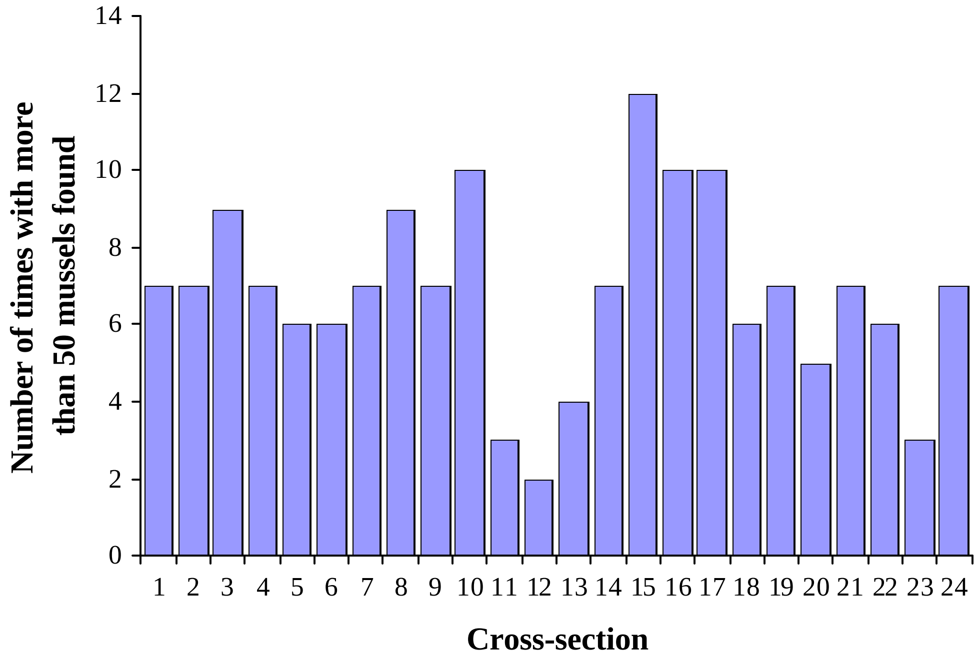

Figure 2.8. *The number of times out of 72 sites sampled where more than 50 mussels were found in the individual cross-sections. Cross-section 1 was at the most downstream end of the study sites and cross-section 24 was at the most upstream end. The road crossing occurred between cross-sections 12 and 13.*

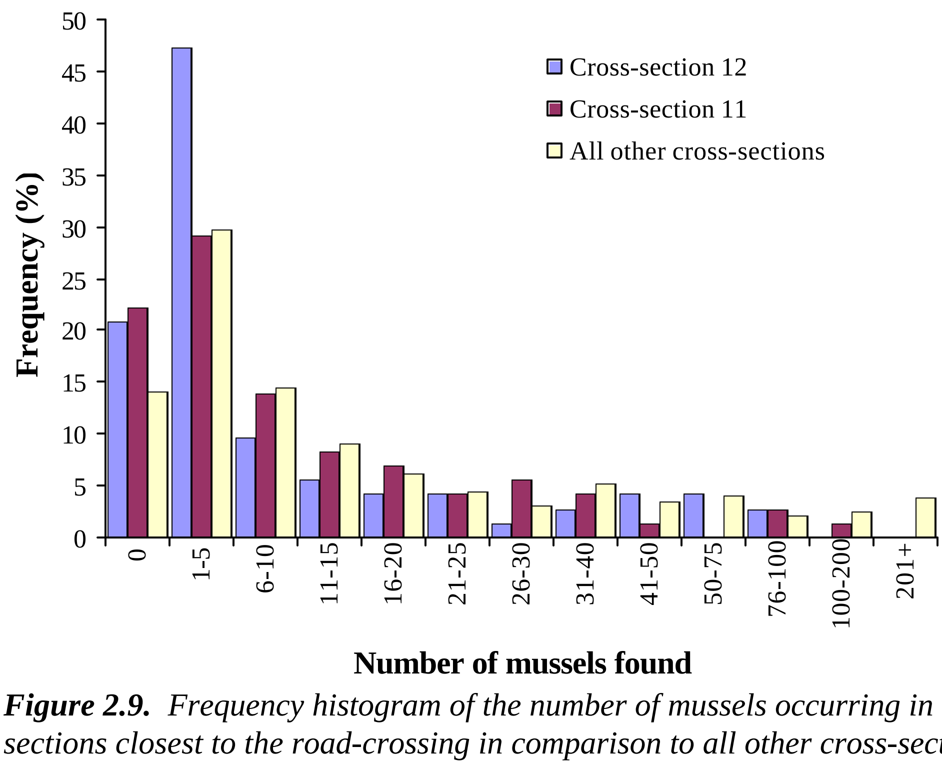

Figure 2.9. *Frequency histogram of the number of mussels occurring in the 25-meter cross-sections closest to the road-crossing in comparison to all other cross-sections. Cross-section 12 is immediately adjacent to the crossing and cross-section 11 is downstream of 12.*

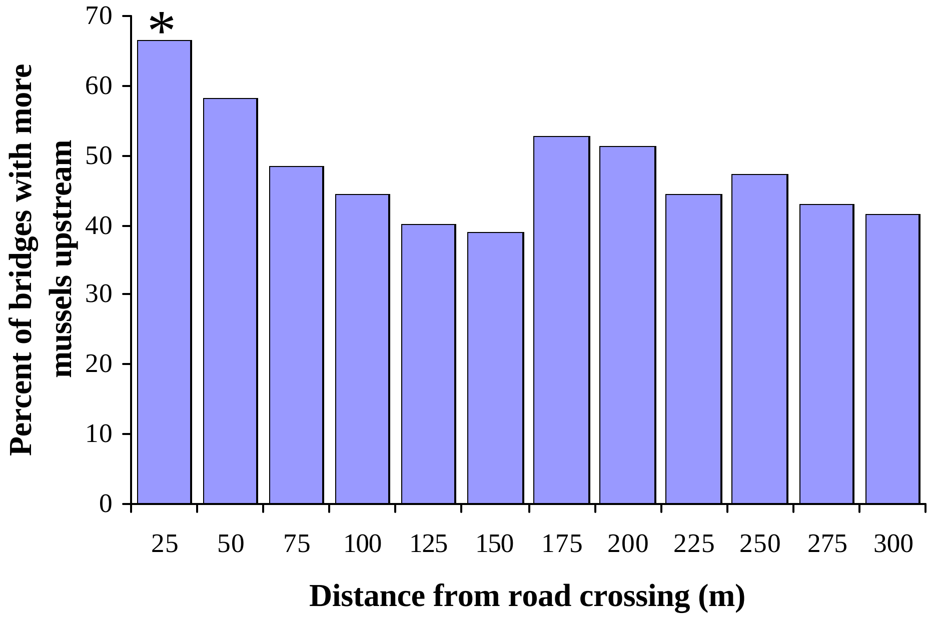

Figure 2.10. *The percent of bridges with fewer mussels in individual cross-sections downstream when compared to the median number of mussels per cross-section upstream. An asterisk(*) represents the cross-sections where the percentage was significantly different than 50% (p < 0.05, N=72)*

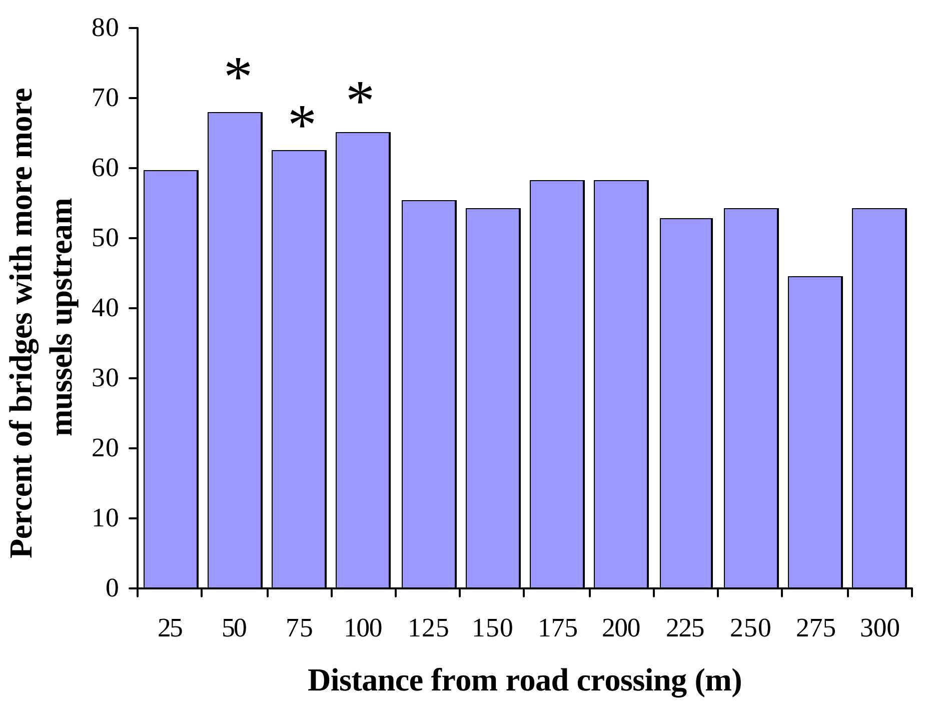

Figure 2.11. *The percent of bridges with more cumulative mussels upstream than downstream when calculated at varying reach lengths. An asterisk(*) represents those reach lengths where the percentage was significantly different than 50% (p < 0.05, N=72).*

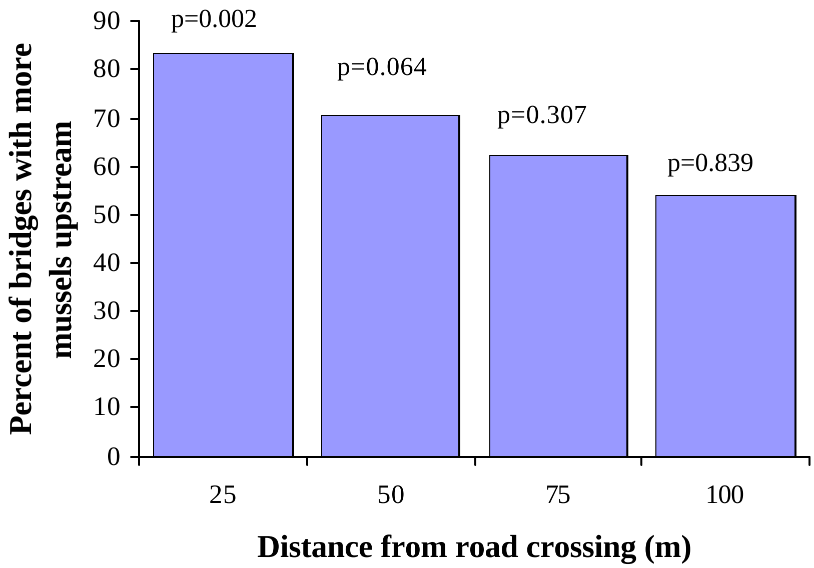

Figure 2.12. *The percent of bridges with fewer mussels in individual cross-sections downstream when compared to the median number of mussels per cross-section upstream. This analysis used only sites with at least 100 mussels in either of the 100-meter reaches immediately upstream or downstream of the road crossing (N=24). The p-values for the proportion test that the percent of bridges with more mussels upstream equals 50% are included for each cross-section.*

Median length of *E. complanata* was significantly greater in the Neuse basin (N = 8794, median = 71.0 mm) than in the Cape Fear basin (N = 6578, median = 67.0 mm) using a Mann-Whitney test ($p < 0.0001$). There was a trend in decreased *E. complanata* length with increasing drainage area in both basins; however, there was greater variation in this trend in the Cape Fear study area (Figure 2.13). Both the Neuse and Cape Fear study areas had significant differences in *E. complanata* length between transects ($p < 0.001$), but no clear trends were seen with distance from the bridge. However, *E. complanata* in the upstream reaches were significantly larger than in the downstream reaches ($p < 0.0001$). Although we found a similar number of very small individuals (< 40 mm) upstream and downstream, downstream reaches had more intermediate sized mussels (55 – 70 mm), and upstream reaches had more large mussels in both basins (Figures 2.14, 2.15).

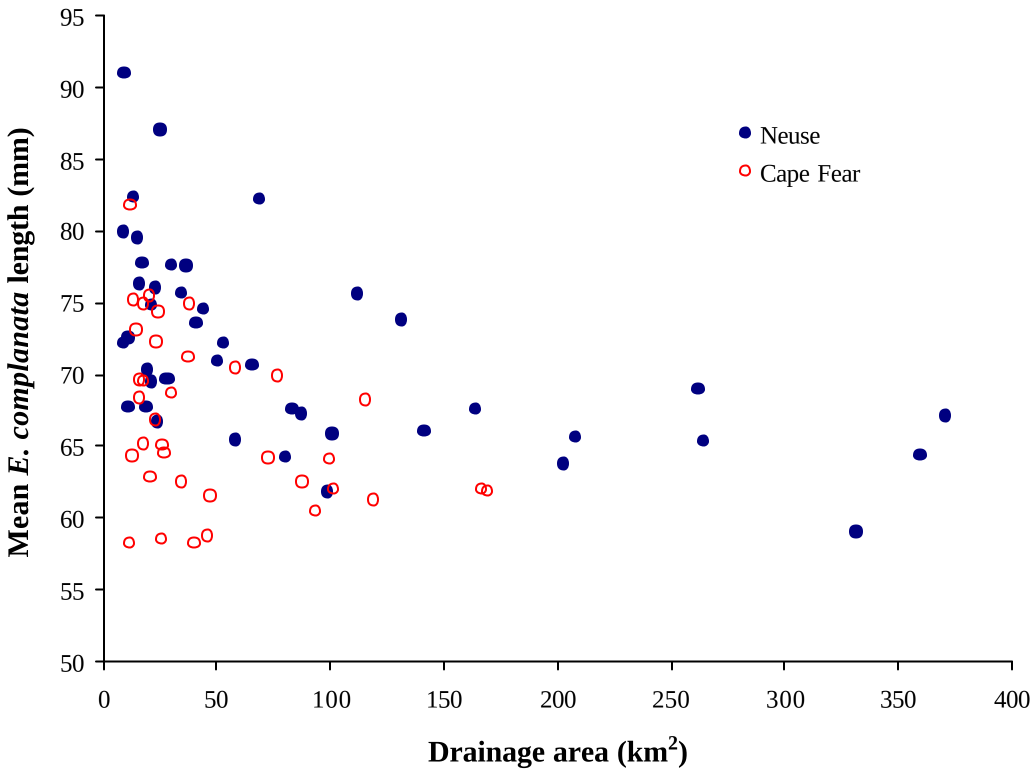

Figure 2.13. *Mean length of Elliptio complanata at all sites sampled versus drainage area of the site.*

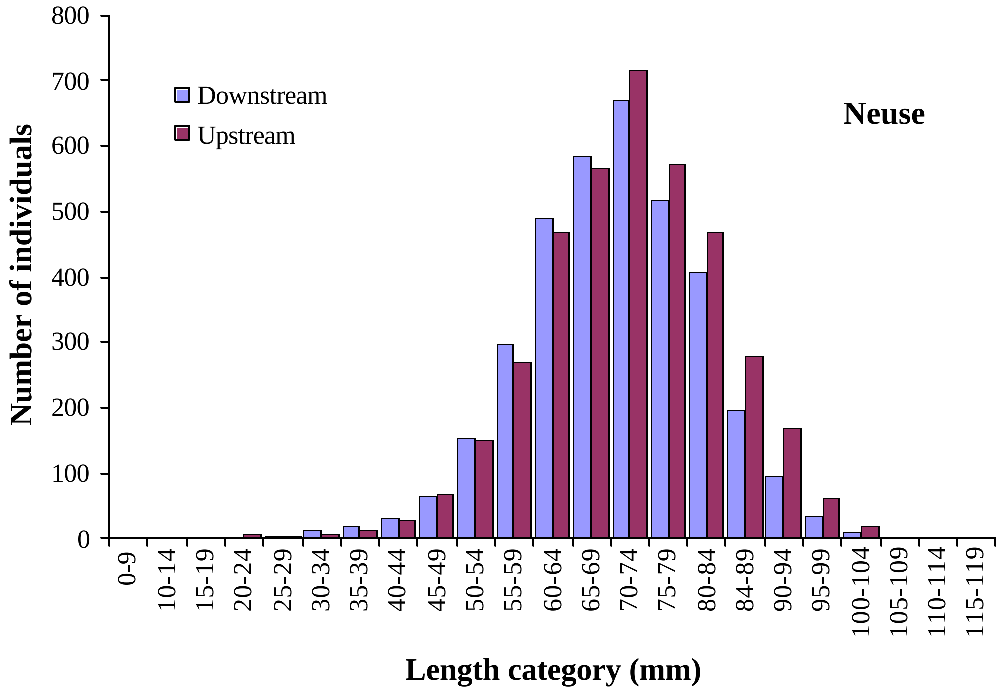

Figure 2.14. *Frequency histogram of Elliptio complanata length in upstream and downstream reaches in the Neuse study area.*

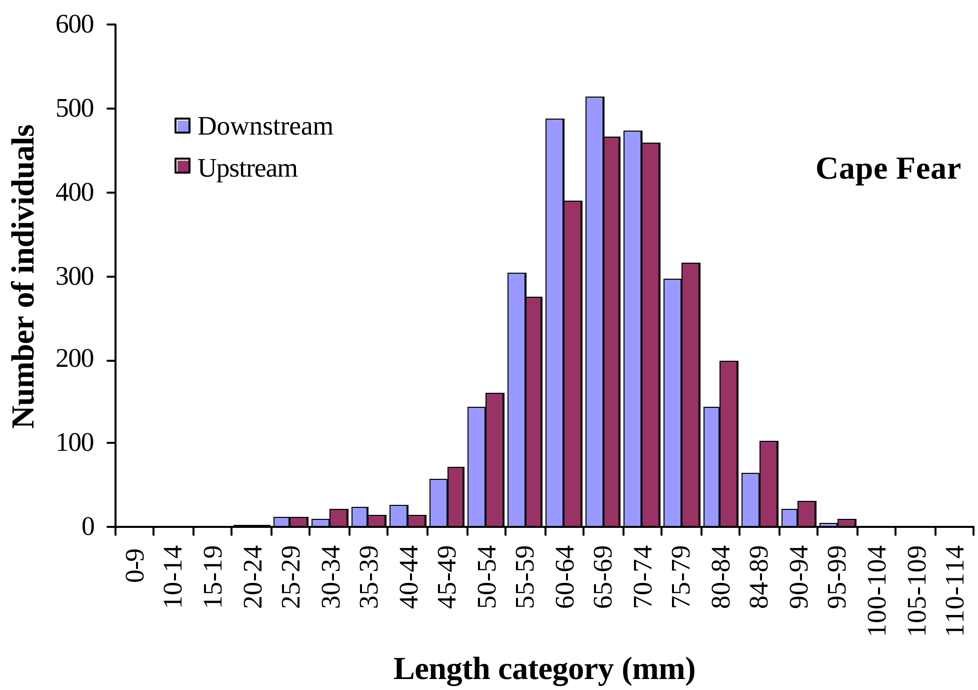

Figure 2.15. *Frequency histogram of Elliptio complanata length in upstream and downstream reaches in the Cape Fear study area.*

We found no evidence of an effect on species other than *E. complanata* either between upstream and downstream reaches or between 25-meter cross-sections. There were no differences in the Shannon-Weiner Diversity Index, number of other species besides *E. complanata*, or number of individuals from those other species ($p > 0.05$, Appendix II-11). The total number of individuals found of some non-*Elliptio* species initially appeared to have greater numbers either downstream or upstream (Appendix II-8); however, this was driven by distribution unrelated to the bridge at a small number of sites. No species was significantly more likely to found either upstream or downstream of the crossing structure. There were also no trends in sex ratios or gravidity of Lampsiline species (*Lampsilis*, *Villosa*, *Toxolasma*) in relation to road crossings (Table 2.6). Neither percent of individuals as female ($p = 0.710$) nor percent of females as gravid ($p = 0.122$) was significantly different between upstream and downstream.

Table 2.6. Summary of Lampsiline (sexually dimorphic species) sex ratios and gravidity percentages by cross-section. All individuals collected in the genera *Lampsilis, Villosa*, and *Toxolasma* are included in this data.

Cross-section	Number of males found	Number of females found	Percent of individuals as female	Number of females checked for gravidity	Number gravid	Percent of females as gravid
1	20	17	45.9	14	1	7.14
2	36	17	32.1	16	3	18.8
3	31	10	24.4	9	3	33.3
4	35	10	22.2	10	1	10.0
5	35	14	28.6	12	1	8.3
6	31	14	31.1	11	4	36.4
7	27	9	25.0	7	2	28.6
8	39	21	35.0	20	5	25.0
9	21	12	36.4	12	7	58.3
10	24	11	31.4	10	5	50.0
11	30	10	25.0	10	4	40.0
12	12	11	47.8	11	2	18.2
Road Crossing	13	6	31.6	3	1	33.3
13	33	13	28.3	12	1	8.3
14	31	17	35.4	15	1	6.7
15	19	16	45.7	16	4	25.0
16	14	5	26.3	3	0	0.0
17	24	6	20.0	4	0	0.0
18	19	9	32.1	8	1	12.5
19	21	12	36.4	10	0	0.0
20	18	11	37.9	6	2	33.3
21	24	8	25.0	2	1	50.0
22	12	7	36.8	5	2	40.0
23	8	3	27.3	1	1	100.0
24	19	10	34.5	6	3	50.0
Downstream	**341**	**156**	**31.4**	**142**	**38**	**26.8**
Upstream	**242**	**117**	**32.6**	**88**	**16**	**18.2**

When sites were resurveyed at a later date, varying degrees of temporal variation were found (Table 2.7). Across the 15 sites, the number of mussels found during the second survey as a percentage of the original number found ranged from 29% to 275%, but distribution of mussels within sites remained fairly constant. Overall, the most abundant reaches within a site during the first survey were usually the most abundant reaches during the second survey; however there were some notable within-site and between site differences. In September 2001, we consistently found approximately one-third of the mussels at sites originally surveyed in April and May of that year (Appendices II-12 – II-16). One exception (Orange County – 136) had a relatively similar number of mussels downstream during the second survey, yet there was a substantial decrease in the number of mussels found upstream. In September 2002, we found more mussels at three of the five sites originally surveyed in April and May of that year (Appendices II-17 – II-21). At the other two sites, greater than 90% of the total number of mussels found in the first survey were found in the second survey; however, one of those sites (Randolph Co. – 459) had a great change in distribution of relative abundance within the site. There was a substantial increase in the numbers of mussels found downstream during the second survey, and there was a substantial decrease in the numbers of mussels found upstream. In July 2002, 3 of the 5 sites resurveyed in the Neuse one year later had very similar distribution in the numbers and species found across the site (Appendices II-22 – II-28). One of the other two sites (Person Co.– 18) had a substantial decrease in number found across all species. The other site (Orange – 6) had a substantial increase in the number found across most species.

Table 2.7. Summary of the total numbers of individuals and species found in 15 sites resurveyed.

		Date		Total Number of mussels found			Number of Species found	
County	Bridge Number	First Survey	Second Survey	First Survey	Second Survey	Second/First (%)	First Survey	Second Survey
Orange	114	Apr/May 2001	Sept 2001	467	145	31.0	2	1
Orange	136	Apr/May 2001	Sept 2001	971	440	45.3	2	2
Person	10	Apr/May 2001	Sept 2001	129	38	29.5	1	1
Person	23	Apr/May 2001	Sept 2001	41	12	29.3	4	3
Person	127	Apr/May 2001	Sept 2001	610	204	33.4	5	4
Orange	13	July/Aug 2001	July/Aug 2002	695	507	72.9	3	4
Orange	55	July/Aug 2001	July/Aug 2002	327	387	118.3	3	3
Orange	6	July/Aug 2001	July/Aug 2002	1194	2171	181.8	4	6
Person	18	July/Aug 2001	July/Aug 2002	2216	1420	64.1	7	7
Person	21	July/Aug 2001	July/Aug 2002	848	793	93.5	4	3
Guilford	23	Apr/May 2002	Sept 2002	116	320	275.9	2	2
Randolph	149	Apr/May 2002	Sept 2002	250	346	138.4	2	2
Randolph	349	Apr/May 2002	Sept 2002	122	198	162.3	4	3
Randolph	459	Apr/May 2002	Sept 2002	398	366	92.0	2	3
Randolph	463	Apr/May 2002	Sept 2002	77	70	90.9	2	2

Aquatic Insect Collection: A total of 16 species of Ephemeroptera, Plecoptera, or Trichoptera were collected between all sites with 8 species being the highest number collected at an individual site (Appendix II-4). The time of year the samples were collected affected the content of the samples, and fewer taxa were collected as the summer progressed (Table 2.1, Appendix II-5,6). There were no significant differences between upstream and downstream number of taxa ($p = 0.494$) or mean tolerance values ($p = 0.416$).

Table 2.8. Results of correlation test of time and number of EPT taxa and individuals collected.

Parameter	Pearson Correlation Coefficient	*p*-value
Number of taxa collected		
Ephemeroptera	-0.338	0.036 *
Plecoptera	-0.330	0.040 *
Trichoptera	0.050	0.760
Overall	-0.329	0.041 *
Number of Individuals collected		
Ephemeroptera	0.161	0.328
Plecoptera	-0.390	0.014 *
Trichoptera	0.179	0.274
Overall	0.096	0.561

* = Significant at $\alpha = 0.05$

Discussion

The mussel surveys conducted provide a relatively robust opportunity to examine the potential impact of crossing structures on freshwater mussel populations. There were no significant differences in detectability between surveyors, and surveyors constantly rotated lanes between sites. Although most detectability evaluations found a high percentage of mussels, sites with higher densities of mussels may have a lower degree of error associated with the data.

We observed some differences in species composition and mean length between basins and between the middle and bank transects. Differences in length between the middle of the stream and banks was attributed partially to the lack of small mussels in the middle lane. Juveniles may prefer the protected habitat along the bank that often has finer substrate. Since mussel densities and sediment grain size has been shown to affect mussel growth (Kat 1982), growth rates could also be greater in the center of a stream. Differences in species composition between bank transects and the center of the stream is due to the different types of habitat encountered in the different lanes. Streams will generally have lower current velocities and finer sediment size along banks compared to the center of the stream. The center lane was more often associated with the channel thalweg, which would cause greater velocities and larger substrate particle size. The species we found more often in the center lane (*S. undulatus*, *F. masoni*, *V. constricta*, and *L. cariosa*) are known to prefer cleaner, coarser substrates in faster moving waters (Johnson 1970). Differences in mussel data between the Neuse and Cape Fear study areas were minimal. Streams in both basins were identically dominated by *E. complanata*, and several species were found in both basins. The reason for the difference in *E. complanata* length between the two basins is unknown. It could be due to potential genetic differences within the *E. complanata* complex, surface geology, temperature differences (See Chapter 1), dietary differences or some other unknown factor.

Sites with milldams were removed from analyses because they were shown to have skewed mussel distributions with few mussels being found near where the dam was constructed. While the dams were functional they impounded water trapping fine sediment and likely greatly reducing the mussel population on the upstream side. Impoundment has been linked to declining mussel populations (Hughes and Parmalee 1999), and dam removal has been shown to alter invertebrate communities and sediment characteristics (Stanley et al. 2002). Impoundment and subsequent destruction of milldams may have caused reduced mussel populations in these areas through long-term alteration of flows and sediment dynamics upstream followed by a single event of high flow and benthic scour. Also, these dams were often built in areas of high gradient to maximize the energy produced by the dam. These high gradient reaches were dominated by bedrock and boulder habitat and usually make poor mussel habitat because of the lack of fine sediment. The damage done by construction and removal of a dam as well as the natural lack of mussels in high gradient areas probably had a far greater impact on mussel distribution than the road crossing at those sites.

Mussel surveys did reveal an effect of road crossings on streams. Although the overall effects of these structures on mussel abundance were not seen over the entire sampled reach, there seemed to be a clear local impact at several sites. The primary effect seen was the loss of *E. complanata* under the crossing as well as immediately downstream of the road. Many crossings likely reduce abundance in the first 25 meters downstream and some impact the first 50 meters downstream. Several different analyses confirmed this result. The two 25-meter cross-sections immediately downstream of the bridge had the lowest relative abundance in comparisons using all 72 sites as well as the 24 most abundant sites. A significant proportion ($p < 0.05$) of the study sites had more mussels upstream in the first 25-50 meters. Although the fewest mussels were found in cross-section 12 (immediately downstream of the road), we found differences between the 25-meters on either side of the crossing less often than we found differences in the 50 meters on either side of the road. This was an indicator that there may be fewer mussels in the 25 meters immediately upstream at some road crossings.

Differences in length of *E. complanata* between upstream and downstream may reflect an impact of the bridge. Although there was a trend of decreased length at downstream sites, this occurred over kilometers between sites and may not sufficiently explain a change in length over only 300 meters. Differences within sites did not appear to be the result of increased recruitment, because the number of individuals under 40 mm was similar upstream and downstream in both basins. If these differences represent a true effect of the road crossing, it could either be due to increased longevity upstream or simply decreased growth rates downstream. Differences in substrate type have been shown to affect growth of this species (Kat 1982), and this study did find subtle habitat differences downstream of road crossings. An age and growth study would be required to begin to answer the question of whether decreased length downstream represents a true effect of the bridge or culvert.

We found no evidence of an effect of road crossings on other species. This may be due to the rarity of these species and consequent lack of data, or it may indicate that the crossings only affect some species. Species such as *V. constricta*, which prefers faster water and coarser substrate compared to *E. complanata*, may not be affected as severely by most crossings. In fact, even though this was the second most abundant species, we still found several downstream within in a short distance of crossing structures. Additionally, male-female ratios and percent of females as gravid were similar upstream and downstream, and no notable differences were seen near the road crossing. At the outset of this study, there was concern about vibration of the streambed from vehicles passing over bridges reducing gravidity of female mussels. Although few gravid female Lampsilines were seen near road crossings, a similar result was found in most cross-sections throughout the sites.

Although *E. complanata* was by far the most abundant species in the areas sampled, this localized decrease in abundance gives cause for concern for multiple reasons. This group is considered a species complex, and there may be several species or subspecies currently grouped under the name of *E. complanata.* Indeed, we found several different shell forms of this species group in our surveys, and some of these forms were quite rare. If there are indeed rare species or subspecies currently in this group, loss of these animals may have greater significance in the future as the scientific community refines its ability to differentiate between these species. Although *E. complanata* is primarily considered to be a habitat generalist, they may have a slight preference for sand and smaller substrate particle sizes (Johnson 1970). Their abundance along stream banks in this study supports a preference for highly stable habitat with finer sediment. Loss of this animal around bridges and culverts may be an indicator that other rare mussel species with similar habitat and ecological needs may also be lost. Finally, loss of the most abundant mussel species may cause localized shifts in a stream's ecology because of the large percentage of bivalve biomass lost from the system. Balfour and Smock (1995) found that *E. complanata* comprised approximately 68% of the invertebrate biomass in a stream in Virginia, and the abundance of this species in our study suggests it also makes up a majority of the invertebrate biomass in many North Carolina streams.

The variation in the data over time at sites that were resurveyed was likely due to the vertical migration of mussels rather than actual changes in the population at a site. If mussels burrow down below the surface of the sediment, they would be unavailable to our survey techniques. There are likely multiple factors that affect the burrowing of these species. *Elliptio complanata* and other species have been shown to migrate vertically through the substrate in seasonal patterns (Amyot and Downing 1997; Watters 2001). The consistent reduction in the number of *E. complanata* found in September 2001 relative to the spring of that year follows the pattern found by Balfour and Smock (1995) in Virginia. They found that most *E. complanata* were on the surface of the substrate during April, and more individuals buried themselves beneath the sediment surface over the course of the summer. The time of year when this species is on the sediment surface corresponds to their time of spawning (Matteson 1948), and this is the proposed reason for their vertical migration (Balfour and Smock 1995; Amyot and Downing 1998). When these resurveys were repeated at five sites in the Cape Fear basin, results were vastly different. Instead of a decrease in the number of mussels found in September, there was an overall increase. If the seasonal migration pattern seen by other researchers is true of the species in North Carolina, there are factors other than time of year that affect vertical movement. In 2002, the extreme drought may have altered this pattern. In July and August of that year, many streams in the Cape Fear study area were observed to be dry, and although some mussel mortality was seen, the majority of mussels must have been buried beneath the substrate surface. Subsequent rains in early September 2002 refilled the streams, and mussels were again up on the sediment surface available to our resurveys. The extended time they spent buried beneath a dry streambed may have altered their otherwise seasonal behavior pattern causing a large percentage of them to again resurface. Large changes in distribution between April and September surveys within two sites (Orange Co. – 136, and Randolph Co. - 459) may indicate other factors affected this behavior as well. Also, while 3 sites resurveyed on the same approximate date one year later had very similar results to the original survey, one had a substantial increase, and the other had a substantial decrease in the number of mussels found. Seasonal behavior would not account for these results. Perhaps mass burrowing in response to a single weather event, or differences in water quality or substrate could account for the results of resurveys at some sites. Kat (1982) found differences in horizontal migration of *E. complanata* between different substrate types, so this may also hold true for vertical migration. Our survey protocol was primarily designed to compare relative abundance and diversity in reaches within sites and cover a long stretch of stream at many sites. Because of these vertical migration patterns, other researchers have proposed excavation of sediment in quadrats to monitor mussel populations (Smith et al. 2001). This would have greatly reduced the amount of stream that could be surveyed at an individual site as well as the number of sites surveyed. This practice is also destructive to the habitat and is generally not encouraged by North Carolina wildlife managers. Our data are highly useful for analyzing many sites, but caution should be taken when using the numbers presented here to make conclusions about the effect of a single bridge or culvert.

Aquatic insects have been widely used to provide a measure of biological integrity in streams (Barbour et al. 1998). Our data revealed no differences upstream and downstream of the crossing structures but still may yield some important information. We found a correlation in number of EPT taxa collected with time of year due to emergence of insect taxa from spring to summer. Once nymphs reach maturity during this time of the year they emerge from the streams as adults and would be unaccounted for in late summer samples. Plecoptera are especially more abundant in spring (NCDENR 2001), and more individuals of this group were collected during the spring portion of the study. The impact these seasonal patterns had on the upstream and downstream comparisons is unknown, but both samples at a given site were taken at the same time. One additional variable that could not be accounted for by our survey may have affected the content of individual samples. If a sample was taken especially close to the road crossing, the overhead canopy was likely diminished. Several studies have shown that differences in overhead shading will affect the aquatic insect community by altering the local food supply (Hawkins et al. 1982, Behmer and Hawkins 1986; Clenaghan et al. 1998). So differences in shading where upstream and downstream samples were taken may have affected the taxa at that point. Also, the more qualitative nature of our survey makes upstream and downstream comparisons somewhat less meaningful. Smith and Kaster (1983) used a rigorous quantitative method over the course of one year upstream and downstream of a road crossing and found no substantial differences in the insect community. Other surveys during road construction on streams have found changes in the insect fauna downstream but they stated that streams quickly recovered (Peterson and Nyquist 1972; Barton 1977). Taylor and Roff (1986) did find some taxa differences downstream of a highway remained years after construction and that abundance was much higher downstream as a result of nutrient input. Perhaps more rigorous quantitative sampling in our study would have revealed at differences at a few sites, but this was beyond the scope of the study. Although our surveys found no differences upstream versus downstream of the road crossings surveyed, the data aid in the identification of impaired sites. Biological integrity on a location may be impaired where the number of taxa collected deviates greatly from the average over time.

Summary of Findings

1. Eight sites in the Cape Fear basin were eliminated from analyses because of the presence of milldam remains, which were shown to be highly correlated with skewed mussel distribution.

2. Detectability increased when mussels in a linear transect were at a density of greater than 10 per 75-meter reach, indicating more abundant sites have a smaller degree of error associated with the data.

3. In general there were fewer *E. complanata* under crossing structures and in the first 50 meters downstream of the road crossings. There was rarely an abundant mussel bed found in this area. At a few crossings there may be some reduction in mussel abundance in the 25-meter reach immediately upstream of the road.

4. This effect was not seen at all crossings. Many crossings affected *E. complanata* abundance in the first 25 meters downstream and some affected the first 50 meters.

5. Mean *E. complanata* length was slightly, but significantly greater upstream than downstream of roads. A significant trend of decreased length with increased stream size was seen between sites, but this may not completely explain length changes in a short 300-meter reach within a site. An age and growth study is required to answer this question.

6. No effect was seen on species other than *E. complanata.* This may be due to a lack of data because of the rarity of these species or may truly indicate that some species are influenced less than *E. complanata.*

7. Resurveys at 15 sites reveal that distributional trends according to visual surveys (no excavation) within sites usually remain consistent over time, although the total number of mussels found will change between surveys. Although seasonal mussel behavior likely plays a role in this, other unknown environmental factors may have also affected the number of mussels available to our protocol at some sites.

8. Because of the temporal variation inherent in data collected using visual surveys, caution should be taken when using numbers in this report to assess the effect of an individual bridge on the local mussel fauna.

9. There were no differences detected in aquatic insect communities upstream versus downstream of the road crossings using a qualitative method. Existing literature suggests that rigorous quantitative sampling may detect differences at some sites.

Chapter 3

Land Use Analysis

Introduction

Despite the continual decline of mussel populations, little is known about the factors affecting their distribution. Few studies have successfully described the instream physical habitat requirements of mussel species (Strayer and Ralley, 1993; Layzer and Madison, 1995). Stream size, tidal influence, surface geology, and hydrologic regimes have been observed to affect mussel species composition and abundance (Strayer, 1983; Di Maio and Corkum, 1995; and Strayer, 1993).

This portion of the study examines the relationship between land use and mussel populations in the two study areas. Although numerous macroinvertebrate and fish community studies have documented changes in populations due to agricultural and urban land uses (Lenat and Crawford, 1994; Richards and Host, 1994; Richards *et al*., 1996), few investigations have focused on freshwater mussel distributions. The type of riparian buffer zone surrounding the river has been shown to effect mussel assemblages (Morris and Corkum, 1996). Some unionid species were more abundant in grassy riparian corridors, while others were more frequent in forested riparian zones. The two riparian types were associated with large-scale habitat factors such as shading, solar radiation, and concentrations of nutrients. Grass buffers had greater temperature variability and higher concentrations of ammonia and nitrogen than forested riparian zones.

The association of land use types with mussel populations was investigated in central Alabama in three watersheds (Howard, 1997). A GIS was used to characterize the land use in three spatial scales: upstream drainage area, upstream floodplain reach, and local floodplain reach, extending 1 km upstream from sample site. Spearman's rank correlation tests indicated that at the upstream drainage level, significant negative correlations existed between mussel assemblages and logging, mining, pine monoculture, bare, and urban land use types and a significant positive relationship with pine hardwood. At the upstream floodplain reach, the relationships were stronger and the negative relationships were the same as those as the upstream drainages.

The goal of our study was to investigate potential relationships between mussel populations and land use and other environmental variables. Specific objectives included:

1. characterizing land use within multiple spatial areas including upstream watershed, upstream riparian buffers, and local riparian buffers immediate to the sample sites.

2. identifying potential correlation between land use and biological, chemical and physical variables at the study sites

Methods

Land Use Within Study Areas

Upper Neuse Study Area: Land use and land cover for the Neuse study was obtained from the Environmental Protection Agency's (EPA) Neuse River Land Use/Land Cover GIS layer. The grid was clipped to the extent of the Neuse 1 subbasin and reclassified. The 30-meter resolution grid was derived from several Landsat 7 ETM+ scenes ranging in dates from October 1998 to March 1999 (EPA, 2000). The original EPA classification contains 48 level 3 classes that were combined into 9 level 1 classes (Appendix III-1). The final classification consisted of urban, row crop agriculture, non-row crop agriculture, forest, herbaceous, water, herbaceous wetlands and woody wetlands, and barren land cover types (Figures 3.1). The dominant land uses within the subbasin included forested (63.6%), urban (16.5%), and agriculture (18.5%). Other land uses (1.6%) were combined and consisted of barren, herbaceous, and water cover types.

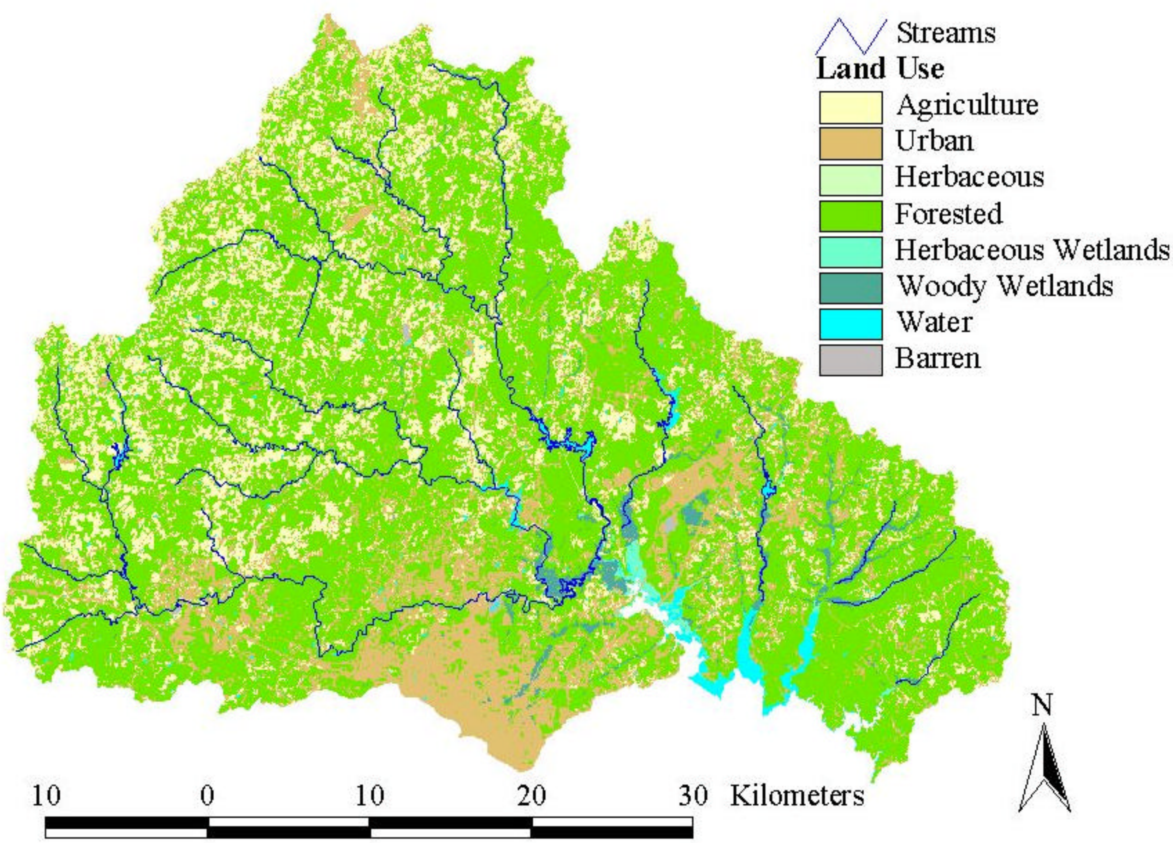

Figure 3.1. *Land use in the Neuse study area as determined by EPA satellite imagery (2000).*

Cape Fear Study Area: EPA's National Land Cover Data (NLCD) was the source of the land use data for the Cape Fear study site. This 30-m resolution grid was derived from several Landsat 7 ETM+ scenes ranging in dates from 1991 to 1993 (Vogelmann *et al.*, 2001). The land use classes consisted of urban, agriculture, forest, water, barren, and wetlands (Figure 3.2). The land uses consisted of forested (74%), agriculture (21%), and urban (3%). Other land types (2%) were combined and included barren, hebaceous and water cover types.

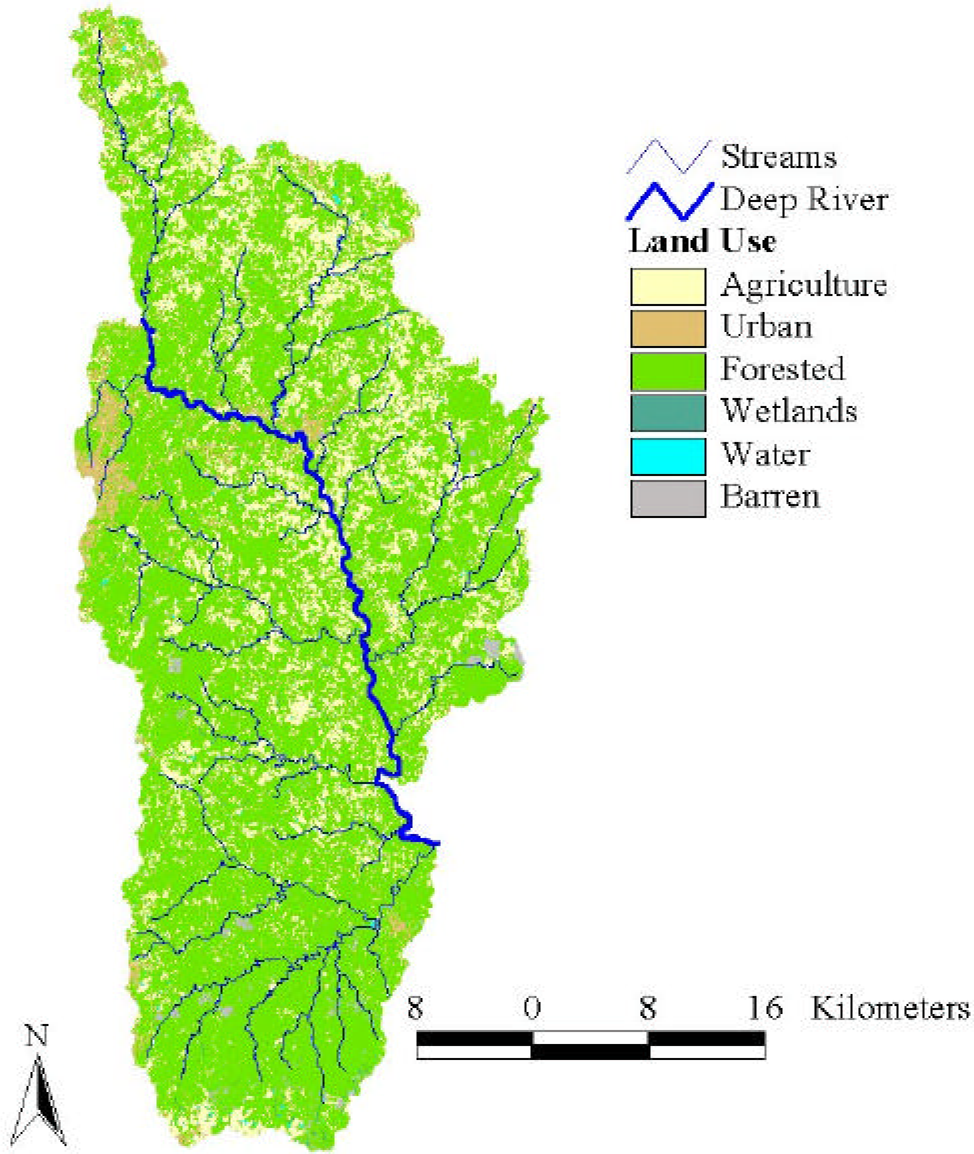

Figure 3.2. *Land use in the Cape Fear study area as determined by EPA satellite imagery (1991-1993).*

Land Use/Land Cover Analysis

The land use/land cover was characterized using GIS within 3 spatial areas: upstream catchment above each sample site; upstream riparian buffers (100-meter and 250-meter widths) extending from each sample site to the upstream most point; and local 100-meter and 250-meter width riparian buffers immediately adjacent to the sample sites. In the Cape Fear study, land use was determined within 2 spatial areas: upstream riparian buffers and local riparian buffers (100-meter and 250-meter widths).

Land Use Within Upstream Watersheds: The upstream catchment of each sample site was delineated using an extention to ArcView 3.2, the CRWR-PreProcessor (CRWR-PrePro) (ESRI, 1999). The CRWR-PrePro is a customized ArcView project built on the ArcView Spatial Analyst Extension to delineate watersheds and stream networks using Digital Elevation Models (DEMs).

DEMs were acquired from the North Carolina State University Soils Department, which obtained the United States Geological Survey's (USGS) National Elevation Dataset DEMs with 30-meter resolution tiled by county. The six counties comprising the study area were mosaiced using ArcInfo Workstation. The CRWR-PrePro project was used to perform hydrological modeling functions. The process involved filling sinks in the DEM, determining the flow direction, and generating the flow accumulation. A shapefile containing the location of the sample sites was used as outlets on the stream grid. The subwatersheds were then delineated above each sample site. In the Neuse study area, 44 watersheds were delineated and in the Cape Fear, 36 watersheds were defined.

The watershed polygon shapefiles created in the watershed delineation procedure were overlaid with the land use/land cover layer in ArcView. The "tabulate areas" function was used to determine the land use within each of the watersheds upstream of each of the sample sites. The area (m^2) of each land use type is reported in a table for each of the catchments. The resulting tables were exported into Excel and were then combined into one spreadsheet. The percentage of the area of each land cover type within the watersheds was calculated. The 9 land classes were combined into the 5 major land cover classes: urban, row crop agriculture, non-row crop agriculture, forest, and other. The other category combined the herbaceous, water, herbaceous wetlands and woody wetlands, and barren classes, which together comprise only 0.1% of the total study area.

Upstream Riparian Buffers: Buffer analysis of the modified stream link shapefiles created in the watershed delineation process were used to extract land use/land cover data for a region 100-meter and 250-meter on each side of the river (Richards *et al.*, 1996; Richards and Host, 1994) (Figure 3.3). These widths were used because a variety of stream functions respond to buffers at least 100-meter distance from the stream (Large and Petts, 1996).

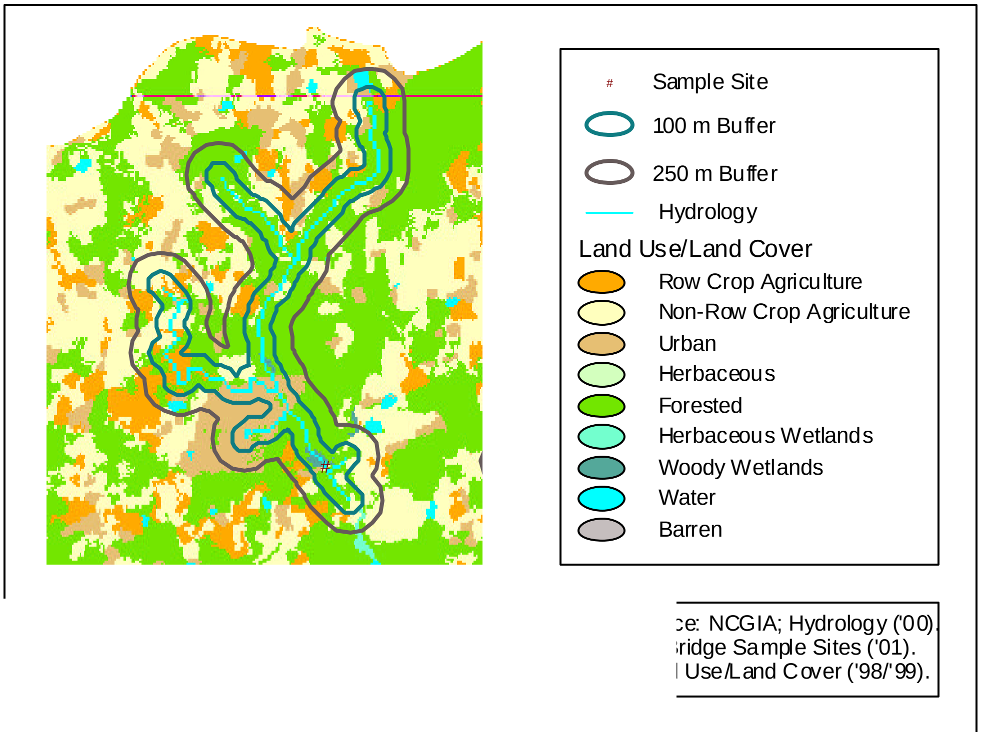

Figure 3.3. *Upstream Riparian Buffers*

A script, "buffer with attribute" (ESRI, 2000), was used to label each buffered stream section with the sample site number. The script selected the sample site number field in the modified stream links shapefiles. The "tabulate areas" function was used to calculate the area of land cover types within each of the upstream riparian buffers. The resulting tables for the 100-meter and 250-meter buffers were exported and combined in Excel. The land covers were combined into the five classes and expressed as the percentage of the total area.

<u>Local Riparian Buffers</u>: Buffer analysis was performed on the 600-meter stream reaches sampled at each of the sites. The bridge shapefile was overlaid with the stream network grid and a new shapefile was created by digitizing the stream segments 300 meters upstream and downstream of the bridge. The sample site number was added as a new field to the attribute table. Buffers with widths of 100-meter and 250-meter distances were created using the buffer with attribute script such that each buffered section was labeled with the site number. The tabulate areas procedure was used to calculate the area of each land class within each of the buffered regions. The land classes were combined into the five classes and the percentage of the land cover was calculated.

Other Environmental Variables

In the Upper Neuse study, other environmental variables were quantified using GIS. Road density was derived from NCDOT's road layer, which included primary and secondary roads tiled by county. The road coverages were merged and clipped to the extent of the study area. The road density (km/ km^2) was determined for each upstream watershed by summing the lengths (km) of the roads divided by the area (km^2) of the watershed. The mean stream slope was determined for the 600-meter stream reach of the sample sites. A slope grid was created from the original DEM using the Derive Slope function of Spatial Analyst Extension (ESRI). Zonal statistics were performed to generate a table of the mean slope of the 600-meter segment of each site. Habitat quality assessment data and water chemistry variables were also included in the Neuse study area.

Statistical Methods

Upper Neuse Study: Detrended Correspondence Analysis (DCA) and Nonmetric Multidimensional Scaling (NMS) indirect ordination techniques were used to examine the community structure and possible gradients using PC-ORD software. DCA uses eignenvalue analysis of chi-square distances among sites and is based on unimodal distributions of species. In contrast, NMS works on a matrix of ranked distances among the sites and is distribution-free, unaffected by non-normality and non-linearity in the data. Similar results of the two techniques result when robust relationships or gradients are present and can be used as confirmation of each other. Different results from the NMS and DCA techniques imply weak or apparent but non-existent relationships (Minchin, 1987; Kent and Coker, 1992).

An abundance matrix of 7 individual species by sample site was log (x + 1.1) transformed. *Utterbackia imbecillis* and *Lasmigona subvirdis* were not included in the matrix because they were only found at one or two sites respectively (less than 5% of the total sites). *Elliptio complanata* was also not included because it occurs in all sites and does not contribute to the community structure. Four outlier sites were eliminated with the two lowest and two highest abundances.

The variation accounted for in each ordination was calculated using the Relative Euclidian measure. Ordination analysis was used to characterize the community types present by examining species composition relative to each axis. Sites close together in ordination space represent sites with similar species composition.

Biplot overlays with an environmental matrix containing the percentage of land cover types within the multiple spatial areas and the other environmental variables were used to identify gradients of the community structure data. Strong correlations ($r > 0.5$) of the environmental variables with each axis of ordination were used to indicate environmental gradients.

Cape Fear Study: The relationship between total mussel abundance of each site and the percentage of land use within the upstream watersheds and local riparian buffers was examined using Spearman's Rank Correlation and Kendall's Tau Correlation using JMP software. These two nonparmetric techniques are ordinal measures of association that estimate the monotonic increasing or decreasing function between two variables. The correlations do not measure causation, but instead measure the covariation between variables (Burt and Barber, 1996).

Results

Neuse Study

Effect of Land Cover and Other Environmental Variables: NMS and DCA ordination techniques were used to examine the mussel community structure and to identify potential environmental gradients. The environmental matrix consisted of the land cover data and the other variables. The percentage of each land cover type within the upstream watersheds, upstream riparian buffers (100-meter and 250-meter widths), and local buffers (100-meter and 250-meter widths) was determined for urban, forest, row crop, non-row crop agriculture, and other categories (Appendices III-2 – III-6). The other environmental variables were the mean for each sample site of the water chemistry data, habitat quality assessment factors, road density, stream slope, and drainage area (Appendix III-7).

DCA Ordination

In the DCA ordination of the log-transformed abundance data, axis 1 accounted for 47.5% of the variation in the distance matrix, and axis 2 for 19.8%, a total of 67.3% (Table 3.1). Correlation analysis of DCA axes with environmental variables showed strong associations ($r > 0.5$) of drainage area, habitat quality index, and urban land cover in the local 100-meter width buffer with the first axis (Figure 3.4). Other environmental variables including pH, bank stability, stream slope, and temperature had milder associations with axis 1 ($r > 0.45$) (Appendix III-11). Axis 2 was highly associated with conductivity ($r = -0.574$) (Figure 3.5, Appendix III-11).

Table 3.1. Correlation Coefficients of Ordination Axes for DCA and NMS Techniques

Ordination Type		R-Square	Cumulative
DCA	Axis 1	0.475	0.475
	Axis 2	0.198	0.673
NMS	Axis 1	0.298	0.298
	Axis 2	0.161	0.459

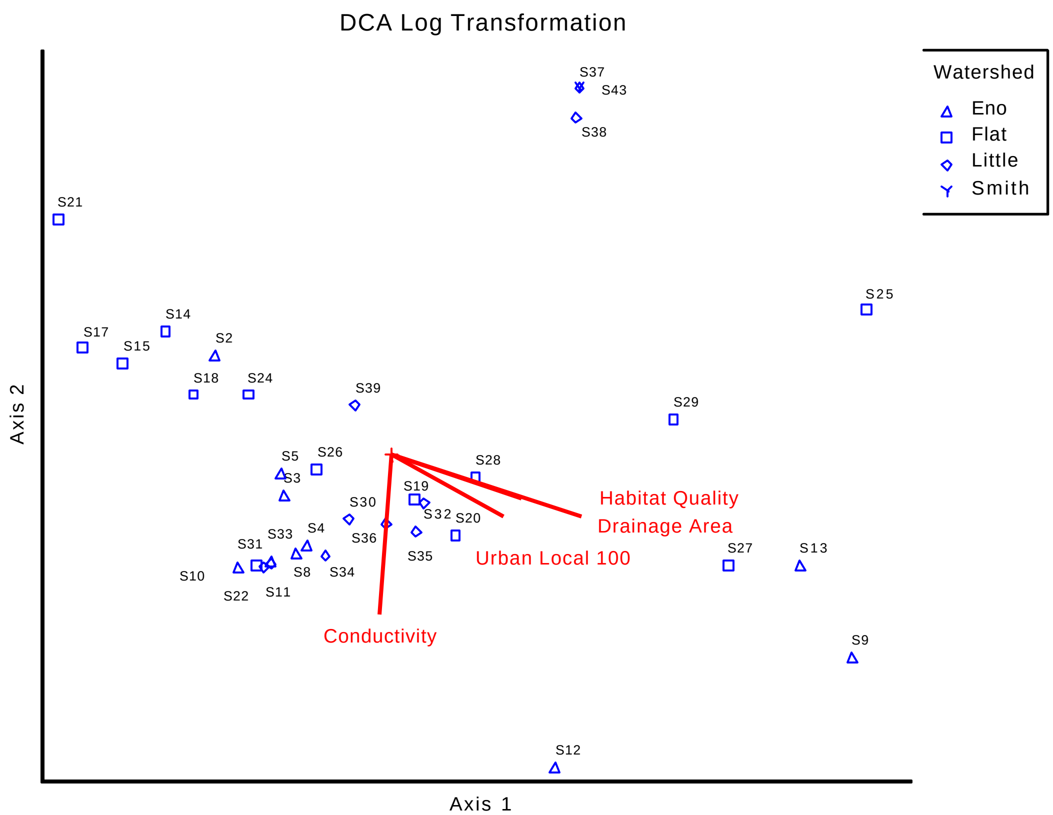

Figure 3.4. *DCA Ordination with Environmental Gradients*

Two species, *Strophitus undulatus* and *Pyganodon cataracta*, were strongly associated with axis 1 (r < -0.5), but no species were highly correlated with axis 2 (Appendix III-12). *Strophitus undulatus* (Figure 3.5) and *Pyganodon cataracta* (Figure 3.6) were found in sites characterized by small drainage areas, low habitat index scores, and were not heavily influenced by nearby urban areas.

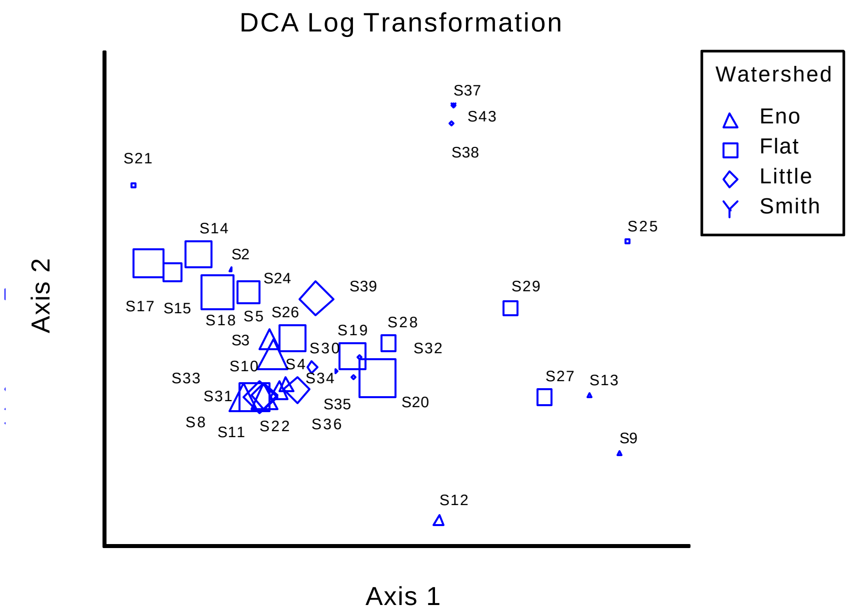

Figure 3.5. *Scatterplot of Strophitus undulatus Composition Among Sites from DCA Ordination. Symbol size corresponds to abundance within each site.*

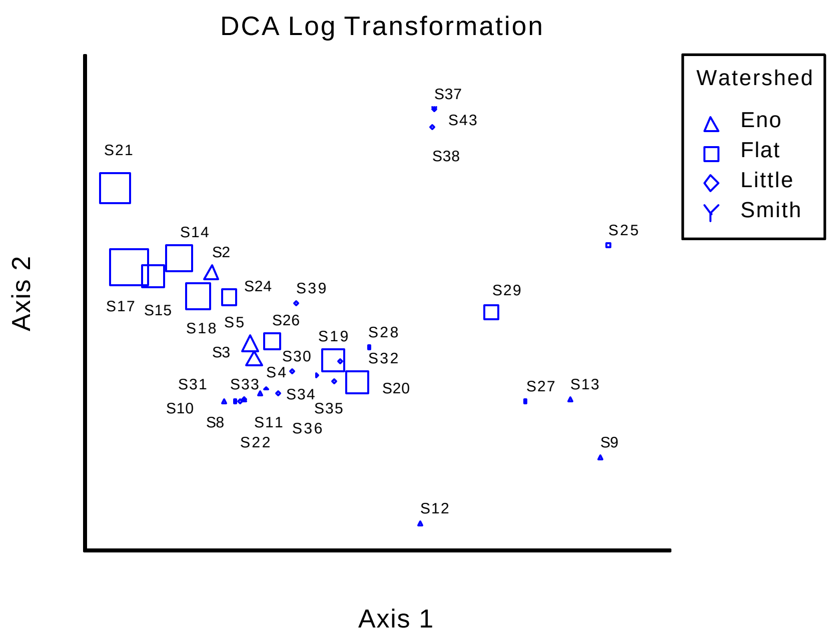

Figure 3.6. *Scatterplot of Pyganodon cataracta Composition Among Sites from DCA Ordination. Symbol size corresponds to abundance within each site.*

NMS Ordination

The NMS ordination of the sample sites revealed a similar species composition as the DCA (Figure 3.8). Axis 1 accounted for 29.8% of the variation accounted for by the distance matrix and axis 2 was 16.1%, a total of 45.9% (Table 3.2). Environmental variables strongly correlated with axis 1 were drainage area, habitat quality assessment, and urban land use in the local buffers (r > 0.5) (Figure 3.7). Weaker associations with axis 1 (r > 0.45) include pH, embeddedness, and sediment deposition. Axis 2, however, did not have strong correlations with any of the environmental factors (Appendix III-13).

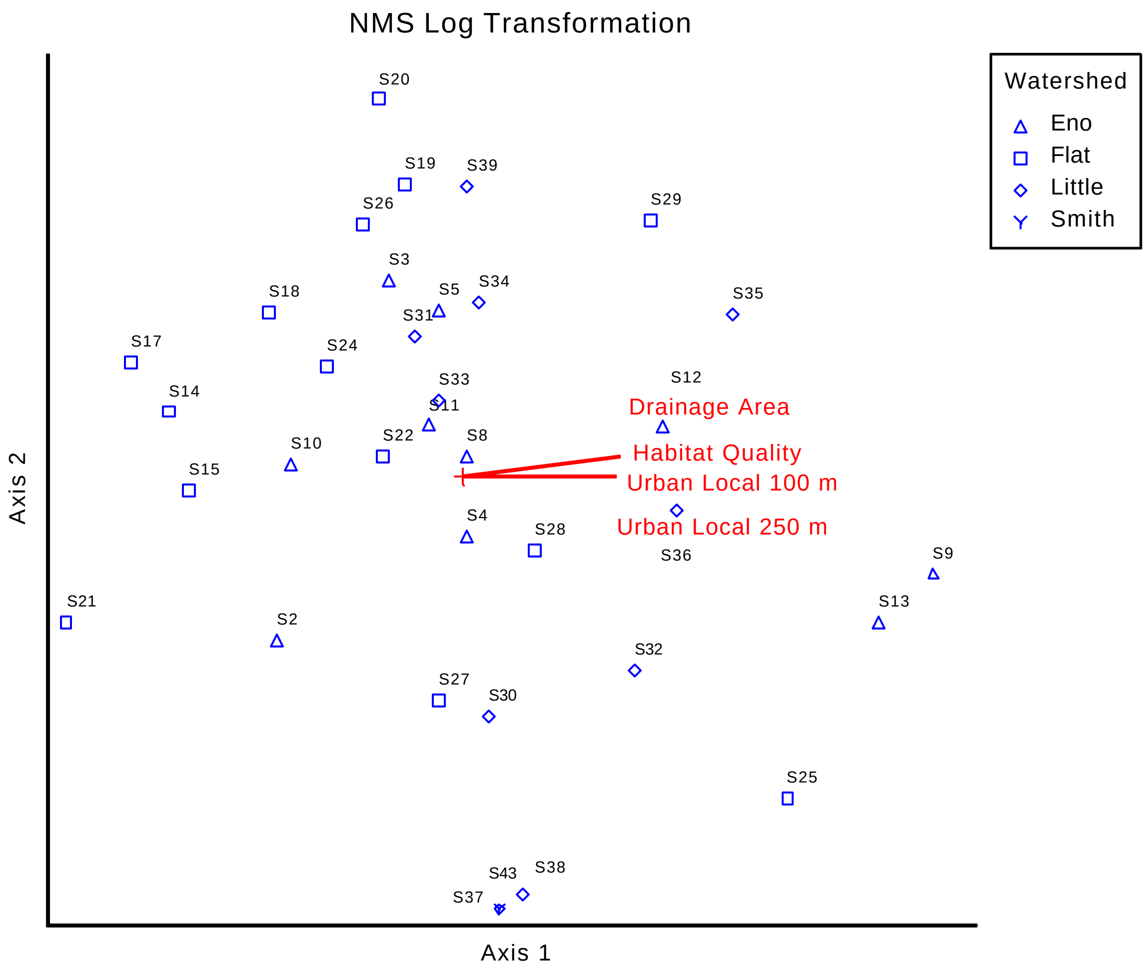

***Figure 3.7**. NMS Ordination with Environmental Gradients*

Species correlations with NMS ordination axes resulted in strong associations between *S. undulatus* and *P. cataracta* (r < -0.5) (Appendix III-14). Axis 2 was highly correlated with *S. undulatus*, *Villosa constricta*, and *Fusconaia masoni* (r > 0.5). Sites with high abundance of *S. undulatus* (Figure 3.8) and *P. cataracta* (Figure 3.9) occurred on the lower end of the gradient and were characterized by small drainage areas, low habitat quality values, and smaller percentages of urban land use in the immediate area.

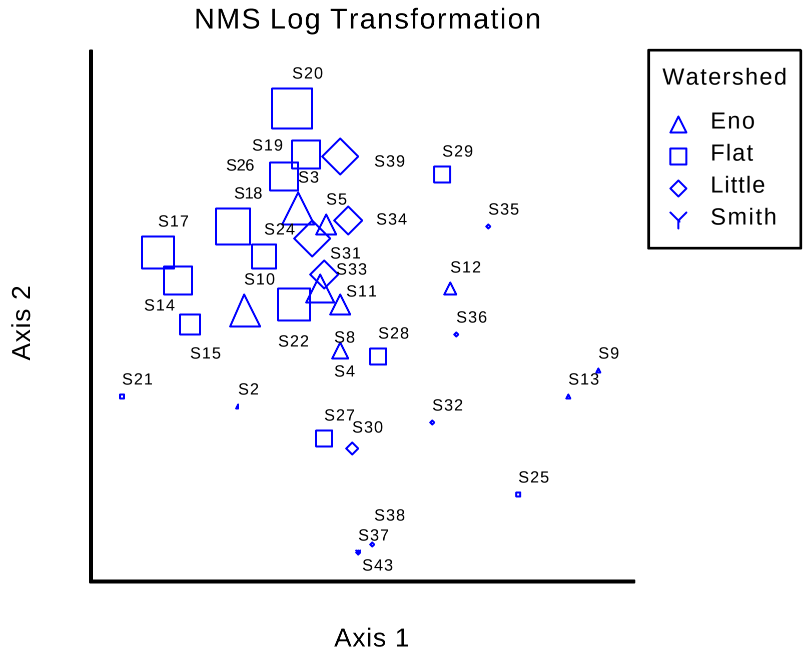

Figure 3.8. *Scatterplot of Strophitus undulatus Composition Among Sites from NMS Ordination. Symbol size corresponds to abundance within each site.*

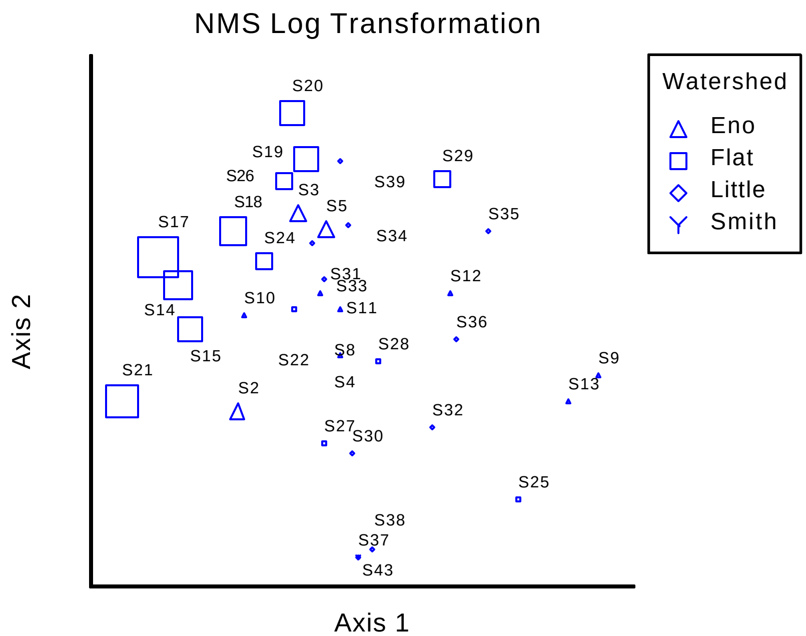

Figure 3.9. *Scatterplot of Pyganodon cataracta Composition Among Sites from NMS Ordination. Symbol size corresponds to abundance within each site.*

A. Cape Fear Study: Relationship Between Land Use and Mussel Abundance

Results of the the Spearman's Rho and Kendall's Tau correlations revealed only two significant relationships between total mussel abundance and percent of land uses (Table 2, Appendix III, Tables 8-10). A significant positive correlation existed between mussel abundance and proportion of forested land within the 100-m buffer (rho = 0.36, p = 0.03; tau = 0.26, p = 0.03). A significant negative correlation was found between mussel abundance and percent of agricultural land within the 100-m buffer (rho = -0.39, p = 0.02; tau = -0.28; p = 0.02).

Table 3.2. Spearman's Rank Coefficients (Rho) and Kendall's Rank Coefficients (Tau). Correlations between total mussel abundance of sample sites and proportion of land use within multiple spatial scales (upstream watershed and local buffers).

Land Use	Rho	P-value	Tau	P-value
Upstream Watershed				
Forest	0.1158	0.5011	0.0827	0.4787
Urban	0.2702	0.1110	0.2130	0.0698
Agriculture	-0.1346	0.4337	-0.1081	0.3542
Other	0.1063	0.5371	0.0827	0.4787
250-meter Local Buffer				
Forest	0.2478	0.1451	0.1844	0.1140
Urban	-0.1772	0.3012	-0.1422	0.3036
Agriculture	-0.2069	0.2261	-0.1448	0.2150
Other	-0.0667	0.6990	-0.0329	0.7927
100-meter Local Buffer				
Forest	0.3634	0.0294	0.2602	0.0285
Urban	-0.1705	0.3201	-0.1393	0.3169
Agriculture	-0.3903	0.0186	-0.2798	0.0198
Other	-0.0262	0.8795	-0.0201	0.8804

Discussion

A. GIS Techniques

The use of GIS-based methods was highly effective for this study. GIS technology allows the quantitative characterization of landscapes at local, watershed and regional scales. Environmental factors such as land cover, stream slope, drainage area, road density were calculated using GIS tools. Buffer analysis in GIS has become a useful tool in studies investigating riparian buffers (Johnson and Gage, 1997). Very few mussel studies to date have used GIS to quantify land use and investigate its relationship with mussel populations. Although no clear relationships between mussel abundance and land-use characteristics were observed, the GIS actually validated our study design. The primary focus of the studies was the impact of crossing structures on freshwater mussel populations. The GIS effort confirms that our design was effective in eliminating other variables that may be impacting mussel populations.

The watershed delineation procedure using the CRWR-PrePro was successful at delineating the upstream watershed above each sample site, but was a time-consuming, multi-step process. A newer version, HEC-GeoHMS (Geospatial Hydrologic Modeling Extension), developed by the US Army Corps of Engineers (USACE, 2000) is an ArcView extension that allows more functionality than the CRWR-PrePro. HEC-GeoHMS follows the same steps of hydrological modeling as CRWR-PrePro extension but allows the terrain processing to be done in batch mode. This includes all of the steps from filling sinks in the DEM to delineating the watersheds. The ability to perform all the steps in a batch allows for more efficient use of analyst's time. After the terrain processing has been performed, the user is able to modify the watershed grid. Basins may be merged, split at the confluences, and new outlet points can be added. New outlet points can be added as batch points, which also speeds the delineation process. Other new procedures include deriving stream and watershed characteristics such as river length, river slope, and flow path distance that could be useful in future studies (USACE, 2000).

A new Arc Hydro data model has been developed by ESRI to perform hydrological modeling functions. The model provides a data structure for a large variety of water resource features including stream networks, drainage features, and hydrography. It contains the terrain processing capabilities of CRWR-PrePro or HEC-GeoHMS extensions but allows for more hydrological analysis and modeling. The geometric stream network allows the connectivity of rivers and streams and performs flow path analyses and calculations. Arc Hydro also connects rivers to time series recorded by gauging devices (Maidment, 2001). Future studies may benefit from the many hydrological modeling tools and features of the Arc Hydro model.

By using GIS we were able to quantify several environmental variables including stream slope, road density, drainage area, and land use/land cover. GIS also has the ability to characterize such factors within multiple spatial scales. The proportion of land cover was calculated for upstream catchment, upstream riparian buffers, and regional buffers immediate to the sample site. The use of GIS also validated the study design by eliminating factors other than the bridges. Future mussel studies could greatly benefit from the ability of GIS-based techniques to explore macrohabitat variables at both landscape and regional scales.

B. Upper Neuse Study: Mussel Community Structure and Environmental Gradients

No trends were seen in land use at any scale with the number of mussels or species found at a site. Our survey protocols did not produce true abundance (census) data, so this may have complicated potential links between land use and actual mussel populations. Another contributing factor to this was the relative uniformity of low-impact land use across all sites in the study area. Even sites with the greatest amount of agricultural land cover had relatively low impact land use. This may mean that the assessment of bridge and culvert impacts in this area is particularly valid because land use likely did not complicate or mask any bridge effect. Also, mussels are likely impacted by a large number of environmental variables, so perhaps other variables played a larger role in mussel abundance than did land use in this basin. The DCA and NMS ordinations revealed very similar species composition structures and environmental gradients. The DCA ordination displayed more overlap and species similarity between sites than the NMS. Axis 1 in both techniques was highly correlated with drainage area, habitat quality index, and urban land cover immediate to the sample site. In the DCA, only urban area within the 100-meter local buffer was highly correlated with the first ordination axis. However, in the NMS analysis urban land cover in both the 100-meter and 250-meter local buffers was strongly associated with axis 1. Both analyses also had a low correlation between pH and axis 1. No environmental variables were strongly correlated with axis 2 in the NMS, but conductivity was highly associated with the second DCA ordination axis.

The observed environmental gradients helped explain the distribution of two species, *S. undulatus* and *P. cataracta*, in both ordinations. In the NMS and DCA, these species occurred in similar areas of the ordination space and were associated with sites with small drainage areas, low habitat quality indices, and small percentages of urban land cover immediate to the area. The species tended to occur at sites with lower pH, smaller stream slopes, cooler temperatures, and lower scores for embeddedness, sediment deposition, and bank stability. The low values for embeddedness and sediment deposition indicate sites with higher amounts of fine sediments. This result would be expected more of *P. cataracta* than of *S. undulatus* because *S. undulatus* has a greater affinity for swifter water and coarser substrate (Johnson 1970). Although several species, including *S. undulatus*, were highly correlated with the second NMS ordination axis, no environmental variables were strongly associated with it. Axis 2 of the DCA technique indicated a conductivity gradient, but it did not sufficiently explain the distribution of any of the species. Further study is needed to determine the factors determining the abundance of other mussel populations.

The drainage area gradient found in the NMS and DCA ordination is consistent with other mussel studies. Stream size, measured as a function of drainage area, has been found to account for the variability in mussel community structure. Stream size can indirectly affect mussel populations by affecting temperature, current speed, and substrate particle size (Strayer, 1983; Strayer, 1993). This could help explain the other associations observed with axis 1 including temperature, habitat quality, embeddedness, sediment deposition, and bank stability.

The headwaters of the Neuse River basin is a high water quality region as evidenced by the numerous sample sites with high mussel abundance and species richness. This finding has been supported by good and high water quality scores found in fish and macroinvertebrate sampling in the region (NCDWQ, 2001). A stronger relationship between urbanization and mussel abundance may have been observed if more highly urbanized sites were investigated. For example, streams near Durham such as Ellerbe and Knapp of Reeds creeks, have historically been areas of low water quality. Macroinvertebrate studies have indicated Poor and Fair water quality in these regions (NCDWQ, 2001). Inclusion of such urbanized regions could more fully describe the relationship between urban land use and mussel populations in future studies.

B. Cape Fear Study: Relationship Between Land Use and Mussel Populations

Significant correlations between total number of mussels found and land use occurred only within the local 100-meter buffers. Two significant relationships were observed with forest and agricultural land uses immediate to the sample site. As the proportion of forested area within the 100-meter buffer increased, the total mussel abundance also increased. However, as the percentage of agricultural land use increased, the mussel abundance decreased. Although the number of mussels found at a site in our study cannot be directly tied to a population abundance value, these results may still indicate the riparian zone cover plays an important role in mussel abundance. No conclusion can be made about why such a significant trend was found in the Cape Fear but not in the Neuse study area. Although the Cape Fear generally had a lower percentage of land devoted to agriculture, the impact seemed to be greater in the Cape Fear study area where it did occur. We noticed livestock access to some streams that we did not see in the Neuse. We observed destabilized banks as well as a few elevated conductivity values in agricultural areas in the Cape Fear. Riparian buffer strips have been shown by other researchers to greatly affect a variety of physical, chemical and biological variables in streams (Davies and Nelson 1994; Nerbonne and Vondracek 2001), so this may represent a real effect of land use on the mussel fauna. Because riparian zone management is such an important issue in land use planning, more research should be done on the link between buffer strips and mussel populations.

Summary of Findings

1. The Neuse study area had relatively uniform, low-impact land use across the study area. This may mean that assessment of bridge and culvert effects in this area are particularly valid

2. Distribution of *S. undulatus* and *P cataracta* was correlated with certain environmental variables; however, several other unknown variables likely still play a large role in these species.

3. A positive correlation between percent of forested land use and the number of mussels found in the Cape Fear area may be due to more intensive land use effects in agricultural areas there.

Chapter 4

Geomorphological Assessment and Mussel Habitat

Introduction

While some attributes concerning the declines of aquatic habitat quality, mussel populations and other benthic organisms in streams is well documented (Bovee and Milhous, 1978; Huehner, 1987; Statzner and Gore, 1988; Carling, 1992; and others), there is limited understanding of how channel dynamics affect habitat preferences of freshwater mussels. Due to their complex life history and sometimes species-specific habitat needs, there may be marked variable differences in how channel dynamics and physical difference impact mussel populations. Mussels are relatively sessile inhabitants of the streambed for most of their lives, and subject to the vagaries of processes occurring in fluvial systems. Variability in stream discharge and sediment transport mechanics contribute to the suitability of a given stream bed for mussel habitat and is affected by both natural and anthropogenic processes. Stream crossing structures, such as bridges and culverts, may alter stream flow dynamics and ultimately affect the steam bed and banks, and possibly, freshwater mussels.

Suitable habitat in streams is one of the key factors in the life history of freshwater mussels. Generally, their preference is to "well-oxygenated shallow water with stable coarse sand or sand gravel mixture beds" (Lee and DeAngelis 1997). The influence of hydrologic and hydraulic characteristics of the channel play a major role in producing and maintaining the physical environment preferred by benthic populations (Hynes 1970; Resh et al. 1988; Poff 1992; Carling 1992; Robinson 1993). Giberson and Cobb (1995) suggest that extreme flow events constitute disturbance for macroinvertebrates only if the substrate moves. Furthermore, several studies have been conducted specifically for freshwater mussel distribution and hydrologic conditions (Salmon and Green 1983; Statzner et al. 1988; Holland-Bartels 1990; Strayer and Ralley 1993; Di Maio and Corkum 1995; Strayer 1999). In these studies, mussel distribution was correlated to multi-scaled descriptors of stream flow, such as Reynolds number, Froude number, flood frequency, drainage area, velocity and water depth. Substrate size and stability were found to be an important factor in several studies, however, studies specifically conducted in an effort to qualify and quantify substrate size preference for different mussel species have reported little correlation (Balfour and Smock 1995, Layzer and Madison 1995). Di Maio and Corkum (1995) showed no significant differences between *Elliptio complanata* distribution and the percentage of substrate in motion at bankfull flows. They suggest this finding may be due to evidence that *E. complanata* burrow deep into the substrate in late autumn and re-emerge in spring (Amyot and Downing 1991). Mussels may be able to avoid dislodgment from the stream bed by burrowing deeper into the substrate, suggesting that stability of surface sediments alone may be inadequate in describing distribution (Di Maio and Corkum 1995). Additional research efforts examining mussel habitat preference linked to channel processes, rather than channel descriptors only may help to understand more about freshwater mussel habitat.

This portion of the study focused on the physical processes occurring in sites in the Neuse study area. The objectives to the study were to:

1. Assess the physical effect of crossing structures on stream channels and their processes and how they effect freshwater mussels and their habitat, and

2. Describe habitat preferences of freshwater mussels and attempt to discover the channel processes that contribute to that habitat.

The objectives of this portion of our work are intrinsically linked since an understanding of freshwater mussel habitat is required prior to understanding any effect a crossing structure may have on that habitat.

Initial Observations and Reconnaissance

Field reconnaissance at sites in the Neuse study area was conducted during the fall of 2001 to observe mussel distribution and the physical characteristics of their habitat at and around stream crossings, as well as upstream and downstream of crossings. Generally, we believed larger populations of mussels were found in areas where the substrate was relatively deep (appx. > 20cm), though there were a few exceptions. There appeared to be no obvious pattern in substrate size preference, thalweg preference over channel margins, or channel width and depth. In the Upper Neuse basin, most channels are underlain by metamorphic and igneous rocks that produce varying sediment sizes ranging from silt, sand and gravel to boulder and cobble. Bedrock outcrops are also plentiful throughout the study area, and several stream crossing sites were constructed at or near bedrock dominated streams. On the extreme ends of the habitat spectrum, few mussels populated bedrock dominated reaches while abundant populations were sometimes found in sand-bed reaches. However, the majority of mussels populated areas between these two extremes. Locations with relatively high populations tended to have a sediment supply capable of producing pool-riffle or run-riffle channel morphologies where sediment size varies, is available for transport, and stream discharge is capable of sorting regardless of the presence of a stream crossing. These characteristics are commonly associated with stable channels, that is, where sediment supply and transport is in balance with discharge. However, not all of the streams among the sample sites would be considered stable.

Based on these preliminary observations, it appeared that mussel abundance is a function of past and current channel condition and dynamics, and not only a function of stream crossings. Stream crossing structures may be one of many factors contributing to stream dynamics, and ultimately freshwater mussel populations. North Carolina streams have a long history of disturbance from land use and adjustment to changes in sediment and water flux. The initial construction of a bridge, along with its physical presence and attributes with respect to channel process is likely a factor to be included in the "past land use" category. However, since the history of land use in the North Carolina Piedmont is about 150-200 years, and current stream crossings studied in the Upper Neuse are about 1-60 years old, it is unlikely that the presence of crossing structures alone are affecting mussel populations. Instead, the presence or absence of freshwater mussels around crossing structures is a combination of channel condition and evolution, underlying geology, and the structure itself. The following analysis attempts to clarify the contribution of these variables with respect to freshwater mussels.

One of the important factors observed during field reconnaissance was the link between presence of mussels and the availability of a suitable substrate quantity. The depth of the channel substrate provides a more comprehensive explanation of mussel habitat preference, and stream-crossing structures may influence substrate depth. It is likely mussels prefer an optimal substrate depth in order to bury themselves as was observed in this study. Although no obvious trend was observed during reconnaissance, substrate size has been linked to mussel habitat (Balfour and Smock 1995, Layzer and Madison 1995). Substrate texture, rather than size only, may play a key role in the ability of mussels to burrow, the availability of suitable food sources, the ability to filter and feed at various life stages, as well as the degree of oxygen exchange. Substrate texture may also be a key factor in the ability of mussels to burrow since some substrates may be easier to maneuver through than others. In fact, Kat (1982) observed differences in horizontal migration between substrate types. Stream crossing structures constructed below bankfull, or top of bank flows, and/or structures having piers, vertical abutments and guide walls potentially constrict channel flow resulting in removal of the finer fraction of the substrate sediments (relative to discharge). Some channel constriction was observed at several stream crossing sites including both bridges and culverts. Additionally, bank erosion was observed downstream of several road crossings, though not all bank erosion was associated with stream crossing structures or constriction, suggesting other variables also affect this.

Channel Scour and Stream Crossings

Channel scour around bridge piers and abutments is the most common problem arising from the presence of bridge crossing structures and is the leading cause of bridge failure in the United States (Richardson and Davis, 2001). Scour is defined as erosion of the streambed or bank material due to flowing water (Richardson and Davis, 2001). Because of the need to provide safe, reliable transportation, evaluation of channel scour dynamics has been and is currently extensively researched (Murillo, 1987; Mueller, et al., 1994; Landers and Mueller, 1996; Chiew, 1987; Lim and Cheng, 1998; Cardoso and Bettess, 1999 and others). Within the context of channel or streambed scour at bridges, scour is separated into three components. They are long-term aggradation and degradation, contraction and general scour, and local scour (Richardson et al., 1991; Richardson et al., 1993). For the purposes of this time-limited study, long-term aggradation and degradation could not be considered. However, contraction and local scour are considered since both are more directly linked to stream crossing structures. Scour may occur at various stream flows, but is most commonly observed during low frequency, high magnitude flows. Streambed scour may contribute to disturbance by removal of substrate sediment quantity and texture required for mussel survival.

Methods

Stream Crossing Structures: Each site was evaluated for the potential and/or existence of scour at crossing structures, general stability and geomorphic features. Digital photos were also taken of the structures, as well as upstream and downstream at each site. This data was then used to develop an instability index for each site (Simon and Downs 1995) to produce a systematic method for stream crossing evaluation. All stability criteria used (Appendix IV-1) were adopted from Simon and Downs (1995) methodology for channel stability around bridges denoting more instability with increasing index. The following parameters were used to develop the index:

1. Bed Material
2. Bed Protection
3. Stage of Channel Evolution
4. Percent of channel constriction
5. Number of Piers in channel
6. Percent blockage (from debris)
7. Bank erosion for each bank
8. Meander impact point from bridge (meters)
9. Pier Skew for each pier
10. Mass wasting at pier
11. High-flow angle of approach (in degrees)
12. Percent Woody Vegetation Cover

Site Selection for Additional Data Collection at Stream Crossings

A sample of 10 bridges was selected for detailed examination (Table 4.1). These sites were chosen to cover a wide range of the following attributes: low, moderate, or high mussel abundance upstream and downstream of the bridge, drainage size, dominant grain size, year bridge was built, channel confinement and location relative to other sites.

Cross Sections: Conventional survey equipment was used to measure at least 2 cross sections upstream and downstream of the 10 crossing structures. The cross-section numbers corresponded to cross-sections used for mussel surveys (See Chapter 1). Rod readings were recorded to the nearest .01 meters. Cross-section data was used to obtain channel geometry and to calculate channel bed slope, boundary shear stress, critical shear stress and velocity (equations in Appendix IV-2)

Pebble Counts: Wolman (1957) pebble counts were performed for each cross sectional area upstream and downstream of bridge. A minimum of 100 counts and maximum of 400 counts were recorded depending on channel size and the degree of the smaller fraction (<8mm) of sediment in the substrate (Petrie and Diplas 2000; Rice and Church 1996). Pebble counts were used to produce grain size distribution graphs for determining D_{10}, D_{16}, D_{50}, D_{84} and D_{90}.

Table 4.1: Site descriptions for the 10 sites used in intensive channel surveys. Bridge numbers use the first number of the county in which they area located at the NCDOT structure ID number. Orange (O) and Person Counties contained the sites used.

Bridge Number	Year Built	Channel Confinement	Substrate	Drainage Area (km^2)	Other
O43	1961	moderate	poorly sorted boulder, bedrock, cobble	80.5	More mussels upstream than downstream. Bedrock control.
O66	1953	low	cobble, bedrock	23.8	More mussels upstream than downstream, but many under bridge. Minor erosion on banks.
O67	1953	low-moderate	full range	11.3	More mussels upstream than downstream. Reach is downstream but relatively close to granite unit in upper watershed. May be the primary source of sediment. Bridge abutment confines channel.
O251	1950	low	gravel, sand	8.43	Box culvert, lots of mussels. Many more upstream.
P10	1992	moderate	coarse sand and gravel over bedrock	23	Mussel numbers low with little difference upstream and downstream of bridge.
P18	1985	high	sand over bedrock	141.6	Mussel abundance and diversity very high under the bridges.
P23	1961	low	cobble, gravel	43.5	Few mussels found both upstream and downstream of bridge.
P33	1970	high	bedrock upstream, sand/ gravel downstream	130.8	More mussels upstream than downstream.
P80	1999	moderate-high	coarse sand and gravel	80.5	Large boulder dam in front of LWD jam (upstream) storing mobile load of gravel and sand. Lots of mussels except around bridge possibly due to recent construction.
P127	1998	high	cobble, gravel over bedrock	50.3	More mussels upstream out of direct influence of bridge.

Channel Characteristics and Mussel Habitat

<u>Site Selection</u>: Two streams within the Upper Neuse basin were chosen for field sites to evaluate channel characteristics and mussel habitat. Sites were chosen based on mussel surveys indicating variability of mussel relative abundance between each reach and stream. Figures 4.1 and 4.2 illustrate the results of the mussel counts for the streams chosen for study. These streams were chosen due to the large variation of mussels between segments and sites, substrate distribution, and channel size. Reaches from each stream were selected for detailed mapping of substrate depth and topography. Reaches represent stream locations where the numbers of mussels vary, but channel type is relatively constant and the channel remains relatively straight over a 50-meter distance. Furthermore, these segments were chosen because they do not have bedrock dominated substrates, debris jams and other obstructions that may influence sediment transport and deposition.

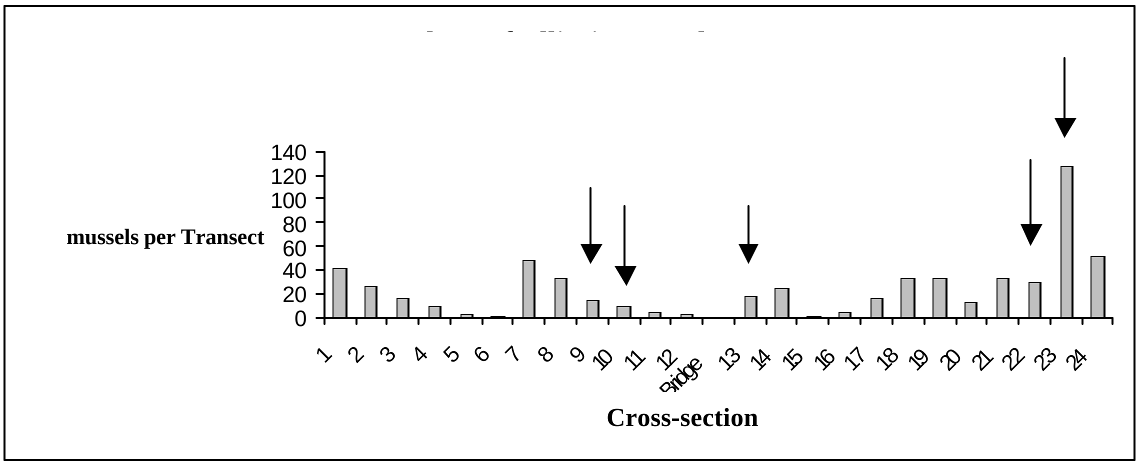

Figure 4.1. *Number of Elliptio complanata found in each cross-section during surveys at Person County bridge number 127 in 2001. Arrows indicate selected study reaches.*

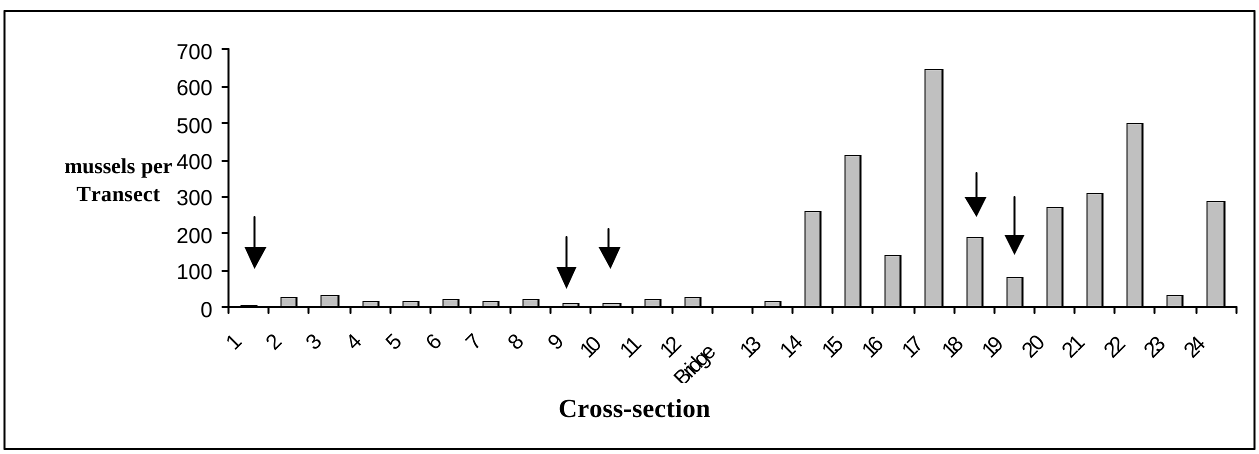

Figure 4.2. *Number of Elliptio complanata found in each cross-section during surveys at Orange County bridge number 67 in 2001. Arrows indicate selected study reaches.*

Channel Cross Sections and Substrate Depth: Conventional survey equipment was used to measure channel geometry, and channel substrate thickness. The area of detailed measurement for each site included the channel and adjacent floodplain and extended up and downstream at least several channel widths or channel units. For example, at least two pool/riffle sequences define the length of the reach with pool/riffle morphology. Since riffle/run channel morphology is not as easily discerned, length was a minimum of 3 bankfull widths, unless the riffle and run could be identified and then their lengths governed the total reach length. Once the reach study area was established, a series of cross sections separated by approximately 3-5 meters was surveyed. Rod readings were taken at 20cm intervals and recorded to the nearest .01 meters. A 0.5-inch diameter graduated steel rod was hammered into the substrate until a non-permeable layer was reached to measure substrate depth. Depth was measured to the nearest .01 meters.

Mussel Habitat Substrate Depth: The entire area of the study reaches were visually re-surveyed to attempt to find all mussels. Two passes were used for each reach to ensure a large majority of mussels were found. The location of each individual was flagged using standard survey flags, and substrate depth was measured at each location with a 0.5 inch diameter rod. If a large number of mussels were found clustered together in a small area, the mussels were counted and several depth measurements were taken at the location of the cluster to achieve an average substrate depth for that location.

Pebble Counts and Substrate Texture: Wolman (1957) pebble counts were performed for each area of the bed. A minimum of 100 counts and maximum of 400 counts were counted depending on channel size and the degree of the smaller fraction (<8mm) of sediment in the substrate (Petrie and Diplas 2000; Rice and Church 1996). Additionally, a qualitative estimate of dominant and sub-dominant substrate texture (clay, silt, sand, gravel, cobble, boulder, bedrock) was determined at each 20cm interval point along the cross section during measurement and again at each mussel location. These qualitative estimates were based on the standard Wentworth grain size scale (Doeglas 1968).

Substrate Depth Distribution Maps: Channel cross sections, substrate depth within the channel, and substrate depth at each mussel location were completed in March 2002. Data of substrate depth at mussel locations were entered into a spreadsheet and histograms were developed for each reach and the total site to determine the distribution of depths. Depth distributions indicated normal breaks in preferred mussel substrate depth. These breaks were used to classify depths and were subsequently analyzed using a geographic information system (GIS). Grid themes were interpolated from depth point measurements and reclassified to reflect the substrate depth classes. The number of cells within each class was counted and percentages were calculated to illustrate the amount and distribution of substrate depths within each reach. The same analysis was conducted with the qualitative substrate composition data using the dominant substrate size. Because this data is qualitative, grid themes could not be developed. Instead, substrate size class percentages were calculated using only the data collected at each point along the cross sections.

Analysis and Results

Stream Crossing Structures: Several types of analysis were conducted to examine the effect of stream crossing structures on freshwater mussel relative abundance. The total number of mussels within 50 meters upstream and downstream of the structure was compared to the instability index derived for each site. The instability index was calculated for the total index, the index for the channel component only, and the index for the bridge component only (Table 4.2). Multiple plots of the total, upstream and downstream mussel numbers and total, channel only and bridge only instability indices were conducted to look for overall trends in the data. No overall trends were detected for the combined sites possibly due to large variances in the mussel relative abundance and the relatively small number of sites. Furthermore, analysis of the differences between pier presence/absence in the channel, pier shape and position relative to mussels did not yield useful information. However, several notable observations were found in the data.

Site D6 has the highest instability index of 21 and, at the time of the mussel survey, no mussels were found within 50 meters up and downstream of the bridge. Simon and Downs (1995) suggest an index greater than 20 can be considered as having substantial potential for "critical" instability than could threaten bridges and land adjacent to the channel. Additionally, the bridge only component of the index is 13 while the channel only component is 8, suggesting that at this site, the bridge itself may be contributing more to the overall instability.

Table 4.2: Channel Stability Index and relative mussel abundance at each site in the Neuse study area. The bridge ID used the first letter of the county (Durham, Granville, Orange, Person, or Wake) with the NCDOT structure number.

Bridge ID (Culverts in Bold Text)	Total Stability Index	Total number of mussels found within 50 meters of structure (up and downstream)	Number of mussels found in 50 meters downstream	Number of mussels found in 50 meters upstream	Stability Index without Bridge Variables	Stability Index Bridge Variables Only	Bridge Variable Percent of Total Index
O13	5.5	34	9	25	4.5	1	18
P130	5.5	0	0	0	4.5	1	18
O55	6	34	9	25	5	1	17
P21	6.5	61	42	19	4.5	2	31
P23	6.5	6	4	2	4.5	2	31
P33	7	248	130	118	6	1	14
P80	7.5	197	146	51	7.5	0	0
D50	8	0	0	0	5	3	38
D9	8	24	23	1	7	1	13
P205	8	8	7	1	7	1	13
D57	8.5	25	18	7	8.5	0	0
O126	8.5	31	18	13	6.5	2	24
O200	8.5	22	21	1	6.5	2	24
P22	8.5	11	8	3	7.5	1	12
P36	10	90	42	48	8	2	20
O114	10.5	20	15	5	4.5	6	57
O4	10.5	15	2	13	7.5	3	29
O66	10.5	31	26	5	8.5	2	19
D151	11	43	42	1	7	4	36
D56	11	157	122	35	9	2	18
G25	11	43	9	34	7	4	36
O53	11	174	24	150	10	1	9
O64	11	37	11	26	9	2	18
P127	11	50	43	7	11	0	0
O251	11.5	219	112	107	8.5	3	26
O30	11.5	145	122	23	8.5	3	26
O43	11.5	15	13	2	5.5	6	52
O6	11.5	126	63	63	9.5	2	17
O67	11.5	324	278	46	8.5	3	26
O173	12	2	2	0	9	3	25
P10	12	18	7	11	10	2	17
P18	12	722	633	89	10	2	17
D8	12.5	11	11	0	7.5	5	40
O12	13	42	39	3	9	4	31
W119	13	0	0	0	10	3	23
O54	13.5	3	3	0	10.5	3	22
O57	14	50	45	5	10	4	29
O242	14.5	7	4	3	5.5	9	62
D64	15	0	0	0	6	9	60
O11	15	141	131	10	8	7	47
P38	15	86	50	36	10	5	33
O136	17	137	85	52	7	10	59
D5	18	34	27	7	10	8	44
D6	21	0	0	0	8	13	62

Site evaluation and stability index criteria reveal bridge D6 to have 2 skewed piers within the bankfull channel, a high-flow angle of approach between 26-40 degrees, an influence of a meander upstream of the bridge within 10 meters of the bridge, 6-25% channel constriction, and a debris blockage at the piers. These criteria along with channel bank erosion by fluvial and mass wasting processes (degradational stage) suggest the index is within reason, and the channel condition does not represent optimal habitat for freshwater mussels. On the other hand, sites O67 and P18 have the greatest relative abundance of mussels around the bridge, and overall instability indices of 11.5 and 12, respectively. Both streams at these sites have much greater mussel numbers upstream of the bridge than downstream, but the bridge component of the index suggests only a small influence relative to the channel stability. The channel surveyed for mussels upstream and downstream of bridge O67 indicated the greatest numbers of mussels for all surveyed channels within the Upper Neuse basin. Observations made during field work suggest this channel to be relatively stable along with having a constant sand and gravel source from an upstream granitic geological unit. This combination of supply size and quantity, and stability suggest ideal conditions for freshwater mussels. The bridge itself is relatively old (built in 1953), and constricts the channel by about 20 percent. This constriction may contribute to the lower mussel counts immediately downstream by removal of substrate sediments by contraction and/or local scour.

Site P18, a bridge on US 501 over the South Flat River, has large numbers of mussels upstream of the bridge, under the bridge, with decreasing numbers downstream of the bridge. This structure is relatively new (1985) and does not constrict the channel. Its piers are at the bankfull channel margins and may be associated with local scour during high flows. The difference in upstream and downstream relative abundance of mussels may be explained by the differences in substrate material. The upstream reaches contain deep (up to 80 cm) deposits of coarse sand overlying shallow bedrock, while the downstream reaches lack the depth of sand deposits and are dominated by shallow bedrock and large boulders for about 100m. The differences in substrate may be the result of general scour resulting from the presence of the bridge, but may also be the result of elevation differences in the bedrock itself. Finally, site D50 has a relatively low instability index of 8, but has no mussels around the bridge. This may be explained by the dominance of bedrock and boulder substrate. Since these materials are more resistance to erosion and transport, the instability is expected to be low. At the same time, bedrock is not an optimal substrate for freshwater mussels, and a low relative abundance is also expected.

Although no absolute trends can be determined from the stability index and criteria data and the number of mussels around the bridge, this data does prove useful in suggesting that other channel process-driven factors are likely involved. Figure 4.3 illustrates the total relative abundance of mussels for the entire 600-meter length of mussel survey by site, and the instability index by site. Approximately 50% of all sites have less than 306 mussels, 95% have less than 1725, and the remaining sites (2) have over 2900. Generally, channels with an instability index greater than 13 have lower mussel numbers, all of which fall into the 50^{th} percentile of mussel number distribution. The two sites with the greatest number of mussels are site O251 (box culvert) and O67 (bridge), both in the Eno River watershed in McGowan Creek. Site O251 is upstream of site O67 and both are located downstream of the large granitic geological formation previously mentioned. The instability index for both sites is 11.5, and field observations indicated that bankfull flow dimensions are coincident with top of bank flow dimensions, suggesting relative stability. The granitic unit provides a nearby source of fine-to-coarse sand

and gravel to these reaches while the stability of the channel provides balance between supply, transport and deposition allowing substrate development suitable for mussel habitat.

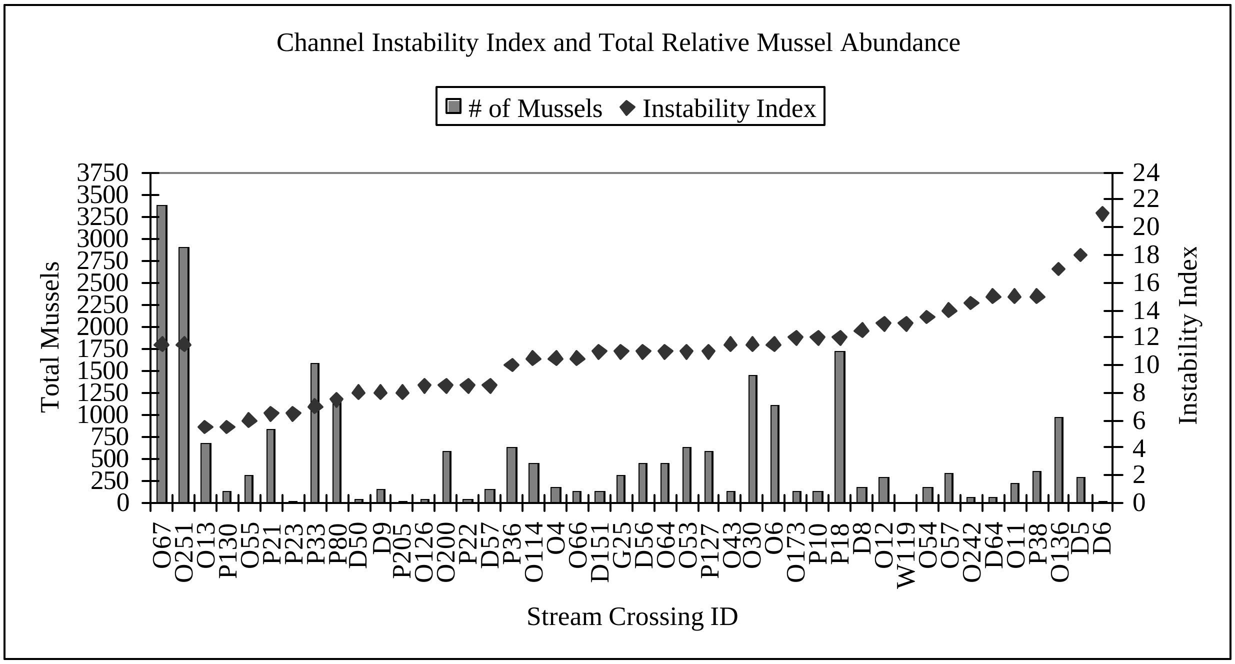

Figure 4.3. *Channel Instability Index and the total number of mussels found in the entire 600 meters surveyed.*

<u>Channel Geometry and Channel Process Indices</u>: Cross-section data collected from the ten sub-sample sites was used to calculate the top of bank channel width, mean depth, maximum depth, bed slope and hydraulic radius for each cross section at each site. Top of bank dimensions were used rather than bankfull dimensions due to the inconsistencies in bankfull determination from field observations. Since the top of bank would be roughly equal to the bankfull geometry given a stable channel, the difference between the two indicates the degree of channel instability and incision. Channel geometry values were averaged separately for upstream and downstream of the stream crossing. Pebble count data was plotted to obtain grain size distribution for each reach at each site upstream and downstream of the crossing and the 10^{th}, 16^{th}, 50^{th}, 84^{th} and 90^{th} percentiles were selected for analysis. Geometry and grain size data were used to calculate boundary shear stress, critical shear stress (from Shields Diagram) for each percentile particle size (Equations are listed in Appendix IV-2). Velocity was estimated using Manning's equation with n = 0.035 representing natural channels with sinuosity, pools and riffles, in-channel vegetation and coarse bed material (Chisolm and Tsang 1971). Estimated values were used only as indicators for entrainment thresholds and current channel condition.

The data associated with the 50-meter reaches upstream and downstream of the road crossings indicate several differences between boundary shear stress (T_b), and in the threshold of critical grain sizes relative to boundary shear stress (T_b) (Tables 4.3, 4.4, & 4.5). For example, T_b increases from the upstream reach of the bridge to the downstream reach at sites P23 and O67 as do the relative mussel counts. However, the relative numbers of mussels up and downstream of site P23 are 4 and 2 respectively and 20 for the total 600-meter survey distance. The overall influence of the bridge at P23 is unclear since there is little difference between mussel relative abundance around the bridge and the entire survey distance. The decrease in mussel relative abundance 50 meters up and downstream of site O67 is more substantial, i.e., 278 and 46 respectively and the threshold grain size upstream is 2mm (D50), and downstream is 8mm (D50). The top-of-bank estimated discharge is very close to predicted bankfull discharge (Figure 4.4. and Table 4.6). Critical grain size relative to boundary shear stress indicates that at the maximum flow confined within the channel banks, only the smaller fraction of sediment is entrained and is probably transported. Since this reach has an ample supply of gravel and sand from the upstream granitic geological unit, sediment transported during bankfull flow does not exceed deposition during receding flow. This condition promotes development and maintenance of favorable mussel habitat by allowing for optimal substrate size and depth. The bridge and instability analysis reported earlier in this document suggest that McGowan Creek, where site O67 is located, is a relatively stable stream and the geometry and hydraulic indicators support this finding. However, the bridge may have an effect on the streambed as a result of contraction, local and/or general scour. Site O67 is one of the older bridges built with a vertical concrete abutment located within the bankfull channel banks.

The geometry data and transport indicators for Site P18 suggest the same findings as with the bridge and instability analysis as T_b and mean depth decrease from upstream to downstream while the 50^{th} percentile grain size increases. This condition reflects the presence of shallow bedrock in the downstream reach. Furthermore, the higher elevation of bedrock directly downstream of the bridge may be aiding in trapping the finer fraction of sediment and allowing for its deposition under and upstream of the bridge.

Table 4.3: Channel geometry of 10 sites surveyed in the Neuse Study area.

Bridge Number	50 meter Reach	Width (m)	Maximum Channel Depth (m)	Mean Channel Depth (m)	Area (m^2)	Slope	Number of mussels found in reach
P10	upstream	10.40	1.30	1.00	10.40	0.052	7
P10	downstream	10.55	1.70	0.90	9.50	0.055	11
P23	upstream	13.50	1.00	1.00	13.50	0.004	4
P23	downstream	14.30	2.80	1.60	22.88	0.007	2
P127	upstream	11.40	2.00	1.55	17.67	0.002	43
P127	downstream	14.05	1.90	1.55	21.78	0.007	7
P33	upstream	17.90	1.60	1.00	17.90	0.004	130
P33	downstream	21.05	2.00	1.65	34.73	0.002	118
P18	upstream	16.85	2.90	2.65	44.65	0.002	633
P18	downstream	16.35	1.80	1.95	31.88	0.002	89
O67	upstream	9.85	1.70	0.95	9.36	0.001	278
O67	downstream	12.00	1.80	1.10	13.20	0.002	46
P80	upstream	15.60	2.30	1.74	27.14	0.018	146
P80	downstream	12.93	2.00	1.49	19.31	0.015	51
O66	upstream	9.55	0.90	0.85	8.12	0.020	26
O66	downstream	14.80	0.90	0.80	11.84	0.013	5
O251	upstream	8.70	1.50	0.85	7.40	0.010	112
O251	downstream	11.80	1.20	0.90	10.62	0.017	107
O43	upstream	10.60	2.20	4.47	47.35	0.018	13
O43	downstream	14.40	1.50	0.80	11.52	0.013	2

Table 4.4: Shear stress Variables used as Channel Condition Indicators

Site	50 meter Reach	D10 (mm)	Tc (N/m2) D10	D16 (mm)	Tc (N/m2) D16	D50 (mm)	Tc (N/m2) D50	D84 (mm)	Tc (N/m2) D84	D90 (mm)	Tc (N/m2) D90	Tb (N/m2) TOB	Mussel Relative Abundance 50 meter Reach	Mussel Relative Abundance 300 meter Reach	Mussel Relative Abundance for total 600m Survey Distance
P10	upstream	0.0625	0.092	64	62.093	2048	1986.970	bedrock	na	bedrock	na	448.532	7	42	
P10	downstream	0.5	0.283	2	1.940	128	124.186	2048	1986.970	2048	1986.970	443.695	11	87	129
P23	upstream	0.25	0.186	0.25	0.243	2	1.940	64	62.093	128	124.186	30.678	4	5	
P23	downstream	8	7.503	16	15.523	128	124.186	256	248.371	256	248.371	99.086	2	15	20
P127	upstream	0.25	0.186	1	0.970	8	7.762	2048	1986.970	bedrock	na	308.021	43	386	
P127	downstream	0.5	0.283	2	1.940	8	7.762	64	62.093	128	124.186	96.395	7	212	598
P33	upstream	0.5	0.283	4	3.881	64	62.093	128	124.186	2048	1986.970	37.421	130	231	
P33	downstream	0.25	0.186	0.5	0.485	32	31.046	64	62.093	128	124.186	29.655	118	1355	1586
P18	upstream	4	3.040	4	3.881	1	0.970	256	248.371	bedrock	na	44.616	633	1236	
P18	downstream	0.25	0.186	4	3.881	64	62.093	bedrock	na	bedrock	na	34.310	89	489	1725
O67	upstream	0.0625	0.092	0.0625	0.060	2	1.940	128	124.186	256	248.371	9.963	278	3155	
O67	downstream	0.0625	0.092	2	1.940	8	7.762	128	124.186	256	248.371	16.827	46	220	3375
P80	upstream	4	3.040	1	0.970	16	15.523	64	62.093	128	124.186	263.689	146	480	
P80	downstream	0.5	0.283	2	1.940	16	15.523	64	62.093	128	124.186	187.701	51	1290	1770

Mussel relative abundance is low around site P10, but this is expected given the overall coarseness of the bed (boulder upstream, cobble downstream) and the steeper stream gradient (~0.05). All of the indices suggest very little deposition of the smaller fraction of sediment size occurs there, except possibly to fill interstices between boulders and large cobble. Since the T_b indicator, as well as the channel width and depth, is about the same upstream and downstream, a physical effect from the presence of the bridge is unlikely.

Table 4.5: Peak Flow Estimates for Sub-sample Sites.

Equation Source	R.I.	P80	P10	P23	P33	P18	O43	O251	O67	O66	P127
(Harman et.al.,1999)	Bankfull	29.94	12.16	19.22	42.46	44.97	11.05	5.90	7.29	12.46	21.33
USGS (1993)	Q2	43.83	18.45	28.65	61.29	64.76	16.84	9.21	11.30	18.89	31.66
USGS (1993)	Q5	70.22	30.36	46.50	97.21	102.54	27.78	15.48	18.86	31.06	51.23
USGS (1993)	Q10	92.96	40.44	61.75	128.38	135.36	37.03	20.73	25.22	41.36	67.98
USGS (1993)	Q25	129.98	56.54	86.34	179.50	189.26	51.78	28.98	35.26	57.83	95.05
USGS (1993)	Q50	153.59	68.07	102.96	210.56	221.74	62.46	35.42	42.91	69.60	113.11
USGS (1993)	Q100	185.55	82.96	124.93	253.51	266.83	76.20	43.48	52.55	84.80	137.09

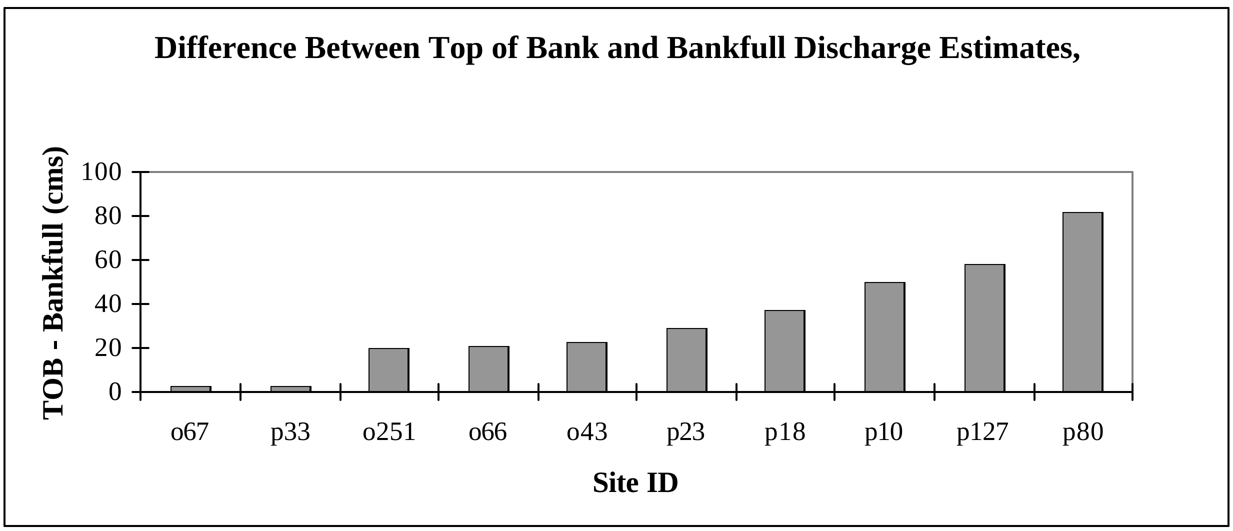

Figure 4.4. *Difference in top of bank and bankfull discharge elevations for sub-sample sites.*

Comparisons between estimated top-of-bank (TOB) and bankfull discharge indicate minor differences between these discharges for channels at sites O67 and P33, both of which have relatively high numbers of freshwater mussels. The large disparity between TOB and bankfull flows at the remaining sites suggest channels at these sites are either in a stage of degradation, or at the early stage of aggradation, but does not explain the variability in mussel relative abundance.

All the data presented above suggest complicated interactions between multiple variables better explain the relative abundance of mussels, their habitat needs, and the influence of stream crossing structures. The following section addresses mussel habitat and channel characteristics that likely account for mussel distribution in a more general sense, and that will provide more insight into the influence of stream crossings.

Channel Characteristics of Mussel Habitat: The following analysis and results refers to the mussel and channel substrate depth and texture data collected at 3 reaches in McGowan Creek, up and downstream of site O67, and 3 reaches in Deep Creek up and downstream of site P127. Substrate depth and texture data was collected for a total of 935 mussels in the combined reaches. Figure 4.5 shows the distribution of the substrate depth data (mean = 0.151, median = 0.139, std = 0.091). All of the values that extend beyond the upper bound fall within the largest depth class (> 0.40m) so do not bias the overall depth distribution analyses.

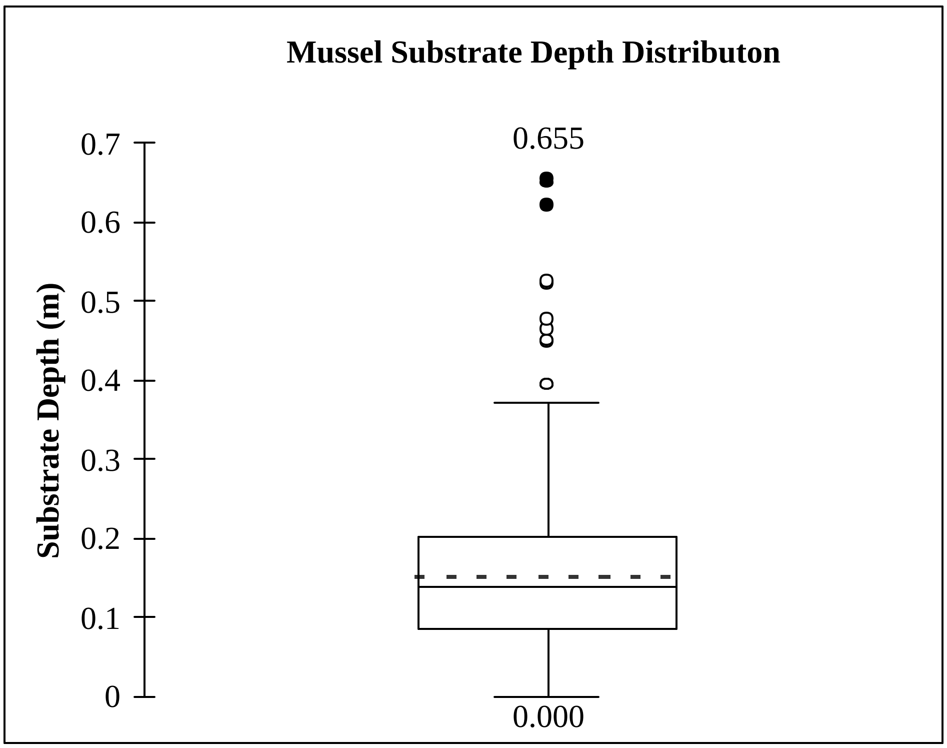

Figure 4.5: *Boxplot of substrate depth measured where mussels were found on the surface of the substrate (N = 935).*

The overall results of mussels found in the study reaches during April 2002 indicate the greatest number of mussels per square meter were observed in reach 19 of McGowan Creek while the lowest was observed in reach 13 in Deep Creek (Table 4.7). Examination of the substrate depth (Figure 4.6) of each reach indicates that 73% of mussels are found in substrate depths ranging from 5cm to 30cm, and 34% found in substrate depths of 10cm to 20cm. In McGowan Creek most mussels prefer sand while in Deep Creek, most mussels prefer cobble.

Contingency tables for all depth and size data were constructed for the percent of mussels found in all combinations of substrate depth and dominant sediment size to test for independence of the variables and to reveal additional trends in the data. The data are statistically independent ($p < 0.0001$), but the percentages did not indicate any useful trends. The data was further stratified by stream, but this yielded similar results (Figure 4.6). Contingency tables were constructed again separately for each stream, but only indicated the same results as shown in Figure 4.6. The substrate depth preference is similar in both McGowan Creek and Deep Creek, but the dominant sediment size preference is very different. Dominant sediment size data was collected for surface sediment only, so does not adequately describe substrate texture preference of freshwater mussels. The dominant cobble in Deep Creek likely covers and hides a large quantity of finer sediment in the subsurface that is not accounted for in the surface sediment data. Further investigation into subsurface and surface sediment texture is required to better understand sediment preferences.

Table 4.6: Total mussels by area within each reach and site obtained from survey data completed 15 April 2002.

Site	Reach	Total Mussels Found	Total Area Surveyed (m^2)	# of Mussels/m^2
McGowan Creek	19	321	152	6.17
McGowan Creek	17	63	36	1.75
McGowan Creek	10-11	23	121	0.19
Total		407	309	1.32
Deep Creek	23-24	486	289	1.7
Deep Creek	13	6	168	0.04
Deep Creek	10-11	36	101	0.36
Total		528	558	0.94

Further examination of the substrate depth and substrate texture, independent of mussels (Figure 4.5, Tables 4.8 and 4.9), show the percentage of substrate depth and dominant sediment size within a reach is coincident with the variation in mussel locations. For example, in reach 19 of McGowan Creek, 35% of the substrate depth is between 10cm and 20cm where most of the mussels were located. Conversely, reach 13 of Deep Creek consist primarily of a very shallow substrate, that is, less than 5 cm, and very few mussels were found in that reach. The same situation exists with respect to dominant sediment size in that the majority of the reach contains the sediment size where the majority of mussels were found. This finding suggests that mussels are limited by the availability of substrate depth and texture comprising their habitat. Adequate combinations of depth and texture are controlled by channel processes. Since the channels in the Upper Neuse are all experiencing varying levels of evolution, some channels may be receiving sediment beyond the transport capacity, while others may be sediment limited. Further study of this concept, with the inclusion of subsurface sediment analyses would likely prove useful in understanding mussel distribution.

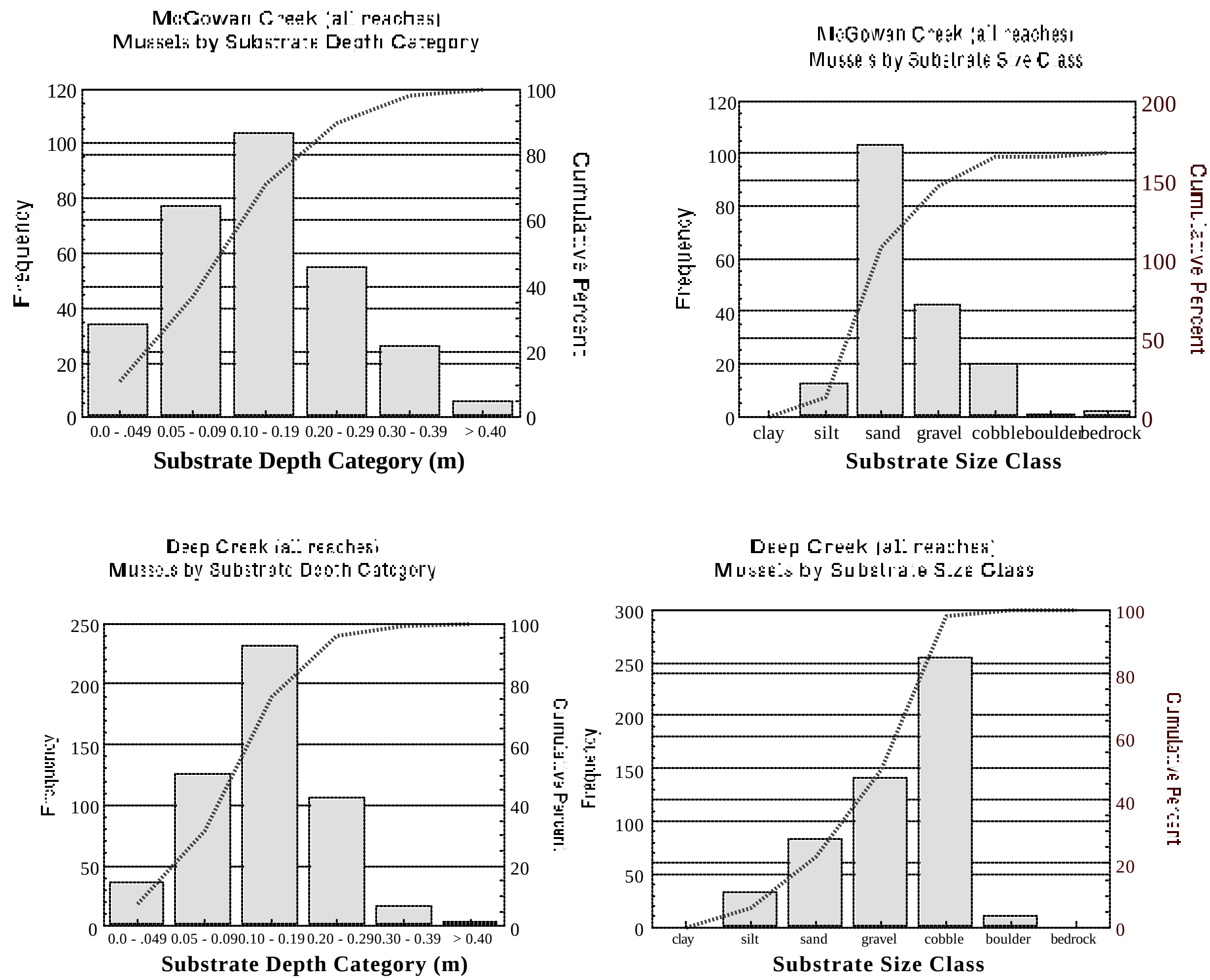

Figure 4.6. *Mussel frequency and cumulative percent by substrate depth category and substrate size class for each site.*

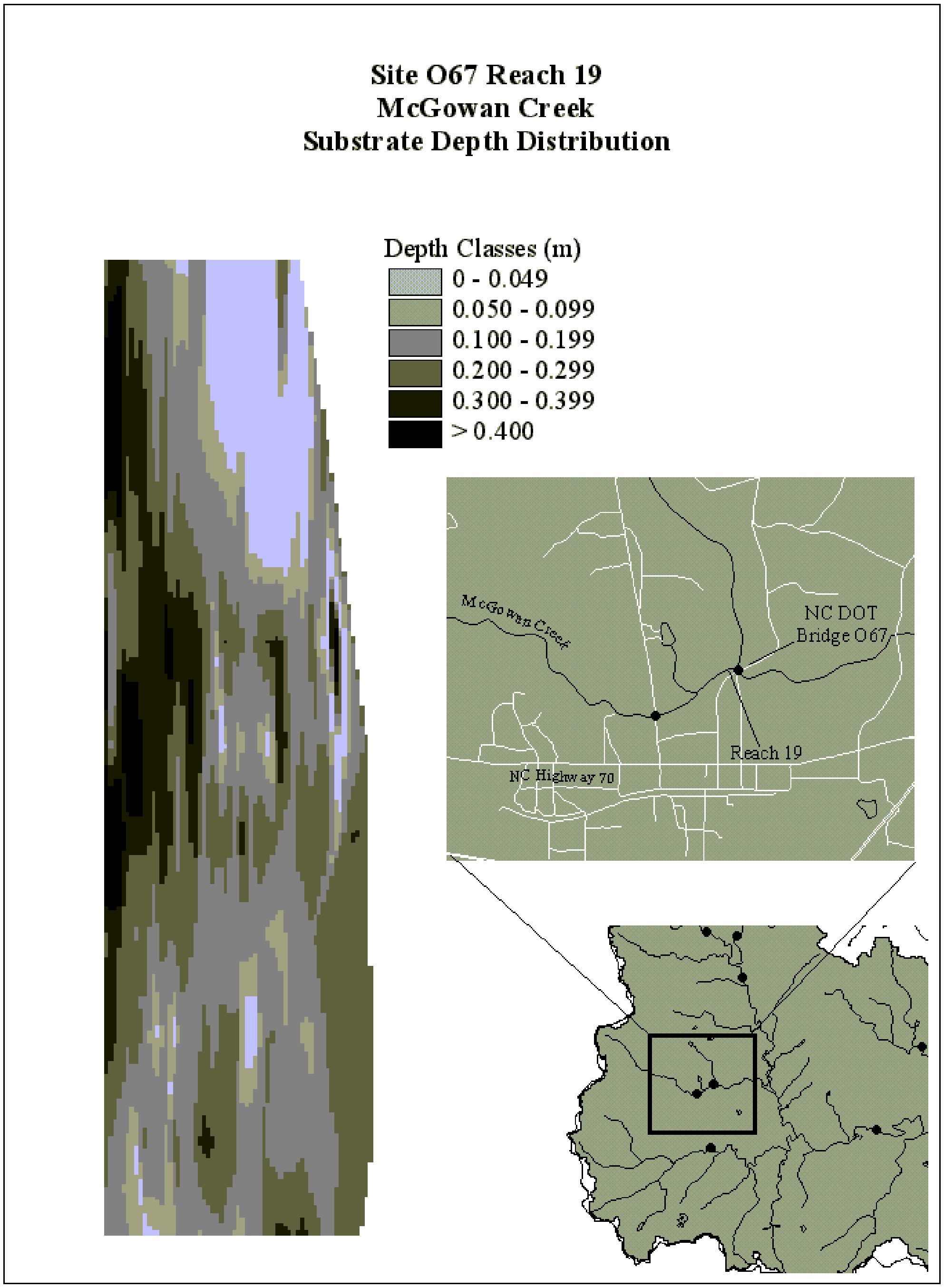

Figure 4.7. *Example of Substrate Depth Distribution Map Developed in Using GIS.*

Table 4.7: Percentages of channel substrate depth class by reach and site.

Site	Reach		Substrate Depth Category (m)				
		0 - 0.049	0.05 - 0.09	0.10 - 0.19	0.20 - 0.29	0.30 – 0.39	> 0.4.0
McGowan Creek	19	15.52%	7.86%	35.13%	29.47%	10.78%	1.10%
McGowan Creek	17	25.53%	55.79%	14.75%	2.14%	1.43%	0.33%
McGowan Creek	1011	29.41%	36.20%	19.86%	5.07%	1.87%	0.76%
Total		24.59%	44.19%	19.09%	7.23%	3.07%	0.53%
Deep Creek	2324	13.23%	17.75%	39.84%	15.68%	1.87%	0.41%
Deep Creek	13	46.22%	40.74%	10.47%	0.48%	0.00%	0.00%
Deep Creek	1011	8.20%	32.38%	46.09%	8.81%	2.46%	0.13%
Total		9.93%	28.34%	44.04%	10.67%	2.27%	0.21%

Table 4.8: Percentages of channel substrate size class by reach and site.

			Substrate Size Class				
Site	Reach	clay	silt	sand	gravel	cobble	boulder
McGowan Creek	19	0.00%	4.97%	48.68%	23.84%	12.25%	0.00%
McGowan Creek	17	1.05%	21.05%	28.42%	22.11%	25.26%	1.05%
McGowan Creek	1011	0.58%	11.05%	9.88%	27.33%	33.14%	2.33%
Total		0.35%	9.49%	33.57%	24.60%	20.74%	0.88%
Deep Creek	2324	0.00%	1.90%	7.59%	15.72%	65.58%	2.71%
Deep Creek	13	0.00%	4.96%	19.15%	27.66%	21.28%	0.00%
Deep Creek	1011	0.00%	12.41%	20.69%	35.17%	26.90%	4.83%
Total		0.00%	4.89%	12.98%	22.60%	47.48%	2.60%

Discussion

Analysis of channel stability indices yielded no clear-cut relationships between structure-induced instability and differences in the number of mussels found upstream and downstream. Differences may be seen in stream crossings having in-channel structures that allow channel constriction and bed scour downstream of the constriction, e.g., Site O67, but the quantity of these is low and therefore insufficient to determine a statistical relationship. Less obvious stream crossing influences were observed for sites D5, D6, D8, D50, D64, D151, O11, O12, O30, O57, O114, O136, O242, O251, and P38 based on the instability index and relative contribution of bridge attributes to the index. The majority of the bridges at these sites were built in the 1950s and 1960s or were culverts (O30, O242, O251 and P38). Older bridges were found to have the attributes affecting channel processes, such as constriction from abutments and bridge piers that potentially result in contraction scour, and to have bridge pier presence that potentially results in local scour. These sites account for 34% of all examined crossing sites. Instability indices suggest the remaining 66% of stream crossing sites do not indicate evidence to support a major influence of the crossing. This suggests that the physical relationship between relative abundance of freshwater mussels upstream and downstream of road crossings at these sites may be explained more by channel characteristics at specific sites and less by the presence of the road crossing. This observation does not exclude the potential for a chemical influence of road runoff from crossings on mussel populations. Although a physical influence from the stream crossing may be present immediately around the structure, the abundance of bedrock and boulder dominated reaches, sediment supply and the specific evolutionary stage of the channel all contribute to the balance, or lack of balance, between transport and deposition, as well as likely provide primary controls on mussel habitat. Instead, the influence lies more in the varying attributes of the channel. The channel is a function of the natural and anthropogenic environment around it, that is, the underlying and surrounding geology, along with climate, stream discharge, and sediment supply. These variables provide the primary control of the quantity, quality and size distribution of the sediment for a specific channel. Alterations in sediment supply and stream discharge may be a result of urban development and agricultural practices and would likely cause the greatest harm to mussels that require stable sediment of adequate supply.

Current land use reflects many of the environmental efforts made in the past 50-75 years, including soil conservation practices and best management practices associated with agricultural and urban development. While stream adjacent lands may show recovery in terms of vegetation cover, hydrologic function, and soil and water runoff, the effect of land use practices on channel processes prior to the 1930s may still be present. The differences in the rates of land and channel adjustment may account for the lack of more correlation between relative mussel abundance and land use variables presented in Chapter 3. An additional complication presents itself in the case of recent urban development since the channel recovery may have been interrupted resulting in another phase of adjustment. Channel evolution as a result of land use practices specifically for the North Carolina Piedmont must be added to the overall equation in order to understand current channel processes and related freshwater mussel habitat.

Channel Evolution and Geomorphic Control on Mussel Habitat

The task of understanding North Carolina streams is not trivial due to the complexity of stream systems along with a relatively long history of settlement and land use. The Piedmont region of Southern United States has experienced accelerated sediment erosion and subsequent delivery to stream channels for over 200 years concurrent with population growth and settlement rates (Trimble 1974). The majority of this sediment is associated with European settlement agricultural practices beginning in the mid-1700s (Trimble 1974). According to Trimble (1974), sediment erosion from agricultural lands in North Carolina was at its peak between 1860 and 1920. During the next 50 years, erosion rates decreased due to the decline in agriculture, conversion of cropland to pasture and forest, and the implementation of soil conservation practices. The effects of pre-settlement and post-settlement sediment supply and deposition in the eastern and Midwestern United States has been documented in the recent past (Trimble 1974; Wilson 1983; Jacobson and Coleman 1987). Jacobson and Coleman (1987) used floodplain stratigraphy to identify three distinct statrographic units deposited during three separate periods of different sediment supplies and hydrology in the Maryland Piedmont. These periods are described as pre-settlement, post-settlement, and post-1930 representing agricultural land use for each time period. According to Jacobson and Coleman (1987), prior to European settlement, floodplains consisted of relatively thin, fine, overbank sediments deposited on top of thin laterally accreted sand and gravel.   With the increase in settlement and agricultural use between 1730 and 1930, sediment supply and runoff also increased, although sediment supply increased at a faster rate. These increases resulted in thick, fine overbank deposits and thin lateral accretion sands, and channel aggradation is proposed. After 1930, farm abandonment and soil conservation practices resulted in a 70% decrease in sediment supply but only a 24% decrease in water yield, suggesting a channel scenario for increased sediment transport and remobilization of previous deposits. In several cases, Jacobson and Coleman (1987) found distinct new floodplain surfaces being created, likely sourced from upstream agricultural sediment. Additionally, they showed an increase in the proportion of sand and gravel deposits in very recent time as compared to the agricultural period. This finding is consistent with other studies (Costa 1975; Knox 1977) where small streams in the Maryland Piedmont were shown to be incising due to lack of sediment load, and most recent deposits in Wisconsin consisted of bedload transported sand and gravels.

The general belief is that sediment eroded in the Piedmont region is being delivered to the coastal plain; however, Phillips (1998) suggested most Piedmont derived sediment is currently in storage and is contributing very little to the coastal sediment budget. He specifically emphasized the lack of connectivity between Piedmont and Coastal Province channels. On the other hand, Richter and Nau (1995) suggested active erosion in the Yadkin Basin from suspended sediment records, noting the expected magnitude of sediment decreases following soil conservation practices is not occurring. If water yield has not decreased as fast as sediment yield, the sediment source in the Yadkin basin may be the result of bank erosion. This scenario is similar to the one previously described by Jacobson and Coleman (1987)

Research describing sediment supply changes in the Maryland Piedmont, the southern Piedmont and Wisconsin can be reasonably applied to North Carolina due to similarities in settlement patterns and agricultural practices. Many of the features described by Trimble, Jacobson and Coleman have been observed in North Carolina Piedmont streams. When applied to North Carolina, a general scenario can be presented where channelization, sediment and water yield increased over time overwhelming sediment transport capacity of streams. As sediment volumes decreased, channels began incising though previous deposits, leaving residual terraces that very rarely receive flood waters. If sediment yield decreased at a faster rate than water yield, incision beyond the pre-settlement bed elevation would be expected given the absence of grade control. As bed degradation continues, the height and angle of channel banks increase by bank erosion. At some critical height and angle, banks would begin to fail, subsequently introducing more sediment into the channel until another equilibrium threshold is met. Each of the channel phases described fit the general model of channel evolution researched and reported by Simon (1989) that reflects shifting dominance of channel processes over time.

Channel evolution and the associated channel processes provide the framework for understanding geomorphic control for mussel habitat. Substrate depth and texture are two important physical indicators linked to current channel condition as a result of past practices and ultimately are linked to freshwater mussel habitat. The presence or absence of adequate substrate depth and texture for mussels is a function of water-sediment interactions, more specifically, a readily sediment supply must be available, as in McGowan Creek. Based on the results of the land cover analysis in Chapter 3 and evaluation of land use history, the primary source of sediment and the majority of sediment is likely not from adjacent land, but from bank erosion within the channel. It is this source controls the depth and texture of the channel substrate. Research is currently being conducted by this author to examine this hypothesis.

Summary of Findings

1. No overall trends were seen in the number of mussels found at a site and the channel Instability Index; however, the sites with Instability scores over 13 were in the lower 50^{th} percentile of number of mussels found at a site. This supports the idea that mussels require stable channels.

2. Road crossings contributing the most to the structure portion of the instability index were primarily built between 1950 and 1969 or were culverts. These structures had the greatest likelihood to constrict the channel at the road. Bridges constructed after 1970 were less likely to contribute significantly to the Instability Index.

3. From the Instability Index, the following bridges were found to contribute the most to channel instability in the Neuse study area:

County	Structure Number	County	Structure Number
Durham	5	Orange	30
Durham	6	Orange	57
Durham	8	Orange	114
Durham	50	Orange	136
Durham	64	Orange	242
Durham	151	Orange	251
Orange	11	Person	38
Orange	12		

At the other sites studied, mussel distribution in relation to the road crossing is more likely associated with site-specific natural conditions rather than the presence of the crossing structure.

4. Mussels prefer substrate of a greater depth (> 10 cm). We believe this is needed to accommodate the burrowing behavior that mussels exhibit.

5. Channel geometry and grain size analysis suggest that mussel distribution is likely controlled by the complex interactions of a large number of variables.

6. Correlations between present-day land use and mussel abundance may be weak because historical land use practices have greatly influenced channel stability and sediment supply in North Carolina's piedmont streams.

Chapter 5

Crossing Structure Attributes

Introduction

The main goal of this study was to determine what effect road crossing structures have on freshwater mussel populations. In this study we have found that the main effect of crossings on the streams studied is that *Elliptio complanata* abundance is reduced immediately downstream of road crossings. The final objective of this study was to determine what attributes of these structures influenced the effect that structure has on the mussel population. The attributes of most concern in this study were the age and design of the structure, whether the structure constricts the channel at high flows, the average daily traffic (ADT) on the road above, and the construction materials. In addition to assessing how these variables play a role in the decline of mussels around the road, we wanted to identify those bridges with relatively abundant mussel beds immediately downstream of the crossing structure and determine what about those structures allowed those mussel beds to exist near the road.

The year in which the structure was built may affect the stream in two ways. Effects of recent construction would be seen in the most recently built bridges and culverts. Also, as structural design has changed through the years, a certain time period may have produced a greater number of crossings with a design detrimental to the stream. The primary design concern is the comparison between a bridge and a culvert. Natural resource managers recommend the use of a bridge rather than a culvert, because it is perceived that culverts due more damage to stream habitat and biota through channel constriction. However, for the transportation agency, culverts are more cost-effective because they are often less expensive to install, require less maintenance, and have a longer effective life. Also, the specific design of the bridge or culvert will influence the structure's effect on stream hydraulics. Culverts can produce scour on the downstream end of the structure by narrowing the channel at that point, therefore increasing stream velocities and bedload movement. Cross-sectional area, slope, and shape of culverts have all been shown to affect the amount of scour produced (Abt et al. 1985; Abt et al. 1987). However, making a blanket statement that culverts create more scour than bridges ignores several variables. Some older bridge designs with wall-like abutments have a cross-sectional area similar to that of a box culvert, and some culvert designs have a very large cross-sectional area with sufficient flow capacity for the channel where they are located. Also, many older bridges had piers and supports in the channel that would increase contractual scour as well as local pier scour. Other bridges have riprap and earthen fill installed up to the channel also causing constriction. Some culverts are designed with very large openings and can have sufficient capacity for overbank flows. Regardless of whether a structure is a bridge or a culvert, the amount of channel constriction must be assessed.

The ADT on the road above must also be considered as a potential factor because increased traffic would increase the pollutants on the road surface. Runoff from impervious surface can be one of the main carriers of these pollutants to surface waters, and a wide range of organic and heavy metal contamination has been detected in waters adjacent to paved roads (Federal Highway Administration 1981; Hoffman et al. 1985). We also wanted to test populations downstream of wooden bridges because they are treated with creosote, which can have adverse effects aquatic life (Hyotylainen and Oikari 1999; Sibley et al. 2001). Freshwater mussels are long-lived, sessile animals that spend their lives burrowed in sediment and filtering water for respiration and feeding. These characteristics mean mussels are susceptible to accumulation of a variety of contaminants in the water column, sediments, or adsorbed to particulates in the water column (Foster and Bates 1978; Hartley and Johnston 1983).

Methods

A database of crossing structure attributes was acquired from the NCDOT, and this provided information on the year the structure was built and the estimated ADT. For structures with the different attributes to be tested, we used a variety of analyses to see which attributes had the greatest effect on relative mussel abundance. Relative abundance of *E. complanata* was compared in upstream versus downstream 300-meter reaches using a t-test to compare the mean percent of mussels at a site upstream of the road with 50%. Differences between 25-meter cross-sections were tested using a Kruskal-Wallis Test. The number of bridges with more mussels upstream than downstream at various distances from the bridge was compared to 50% with a proportion test.

<u>Structure Age</u>: Structure age was initially grouped by decade to evaluate if particular eras produced more bridges with fewer mussels downstream. Crossings built in decades where trends were seen were analyzed as described above to see if there were differences in relative mussel abundance between upstream and downstream of the bridge as well as between 25-meter cross-sections.

<u>Deck Drains</u>: All structures were checked to see what type of deck drainage they had. Comparisons between different drainage designs were difficult to make because only two bridges of the 68 surveyed diverted water off the bridge to the vegetation on the side of the road. The other bridges drained directly to the stream below.

<u>Bridges versus culverts</u>: Mussel data for the 12 culvert sites were analyzed separately from data from bridge sites to determine if mussel distribution in relation to the crossing structure differed between the two types of structures.

<u>Channel constriction</u>: All bridges and culverts with walls, earthen fill, or abutments in or very near the channel were identified and used to test whether these attributes affected mussel abundance near the bridge.

<u>Average Daily Traffic</u>: We performed a simple linear regression using the estimated ADT from the bridge database as the independent variable and the percentage of mussels at a site that were found upstream of the bridge. Linear regression was also used to test for trends in ADT and the percentage of mussels at a site occurring in cross-sections adjacent to the bridge. In all tests, traffic data was log-transformed to achieve normality. Percent of mussels at a site in cross-sections 12 and 13 were square-root transformed to achieve normality.

<u>Construction Materials</u>: We compared relative abundance upstream and downstream 300-meter reaches, within 50 meters of the bridge, and between cross-sections using only bridges with wood piers or abutments in the channel.

<u>Crossings with the least impact</u>: Sites with relatively abundant mussel beds were evaluated to determine what characteristics created relatively abundant mussel beds in cross-section 12. A site was considered to have a low impact on the mussels immediately downstream of the bridge

(cross-section 12) if there were at least 20 *E. complanata* found in the cross-section and this number exceeded the mean number of mussels per cross-section at that site (4.17%).

Results

<u>Structure Age</u>: Of the 72 crossing structures included in analyses, 44 of them (61%) were constructed between 1950 and 1969. At these sites, a mean of 55.9% (SE=3.2) of mussels were found upstream of the crossing structures, (Figure 5.1), and this was statistically greater than 50% (p = 0.036). More mussels were found in the upstream 300-meter reach than in the downstream reach at 26 of the 44 crossings constructed in that era (59.1%), but this was not significantly greater than 50% (p = 0.146). All other sites had a mean of only 44.9% (SE=5.0) of mussels upstream of the road, and this was statistically similar to 50% (N=28, p = 0.313). Of the 44 structures constructed in the 50s and 60s, 31 of them (70.5%) had more mussels in the first 50 meters upstream of the bridge compared to the first 50 meters downstream. This percentage was significantly greater than 50% using a proportion test (p = 0.010). Of the 19 structures built after 1970, 12 of them (63.2%) had more mussels in the 50 meter-reach upstream compared to the 50-meter reach downstream, but this was not statistically significant (p = 0.359) than 50%.

Of the 10 crossings built since 1990, we found more mussels in the upstream 300-meter reach at 5 of them (50%); however, there may still be some evidence of lasting effects of construction. Of the 7 structures constructed since 1996, all of them had more mussels upstream within 75 meters of the road. At these 7 sites, a mean of 79.2% (95% CI = 66.9, 91.9) of mussels within 75 meters of the road were found on the upstream side.

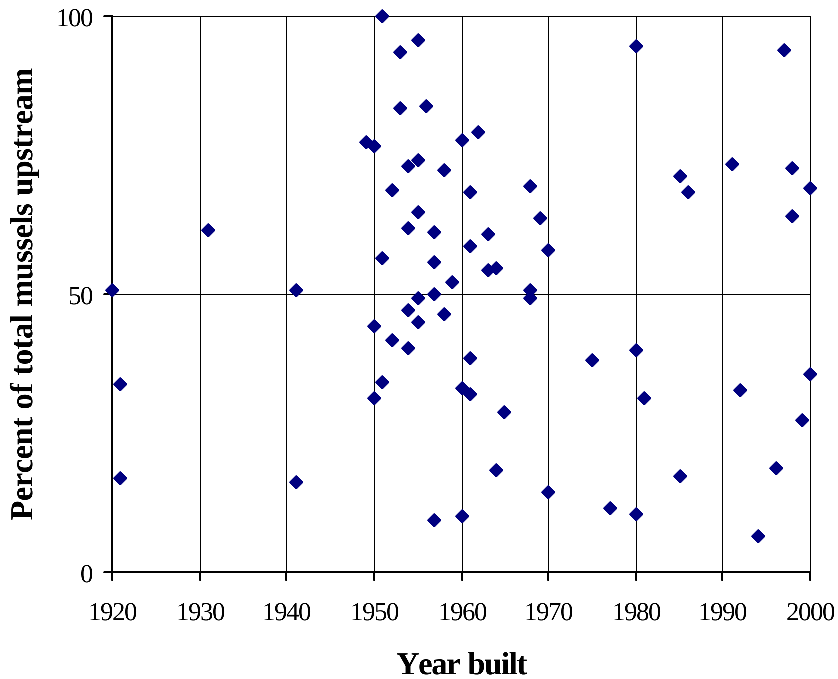

Figure 5.1. *Percent of mussels found at each site found upstream of the bridge by the year the bridge was constructed.*

Bridges versus culverts: Of the 80 sites surveyed during the study, only 12 of those were culverts of various designs. No culverts were included in the eight sites that were dropped from analyses due to the presence of a milldam. There were five box culverts, four corrugated pipe culverts and two large arch culverts (Table 5.1). One of the main differences between culverts and bridges was the actual physical footprint of the structure. Mean length of stream under bridges was 7.7 m (95% CI = 7.3, 8.1) and mean length of stream under all culverts (all designs pooled) was 18.0 m (95% CI = 14.7, 21.3). The mean percentage of mussels at culvert sites found in the upstream 300-meter reach was 51.5% (95% CI = 34.3, 64.6), and 6 of the 12 culverts (50%) had more mussels upstream than downstream. There were no differences between cross-sections in the percent of mussels at a site occurring in those cross-sections (Figure 5.2, $p = 0.289$). The number of culverts with more mussels upstream at various distances was statistically similar to 50% ($p > 0.05$); however, 9 of the 12 culverts did have more mussels upstream from 50 meters to 125 meters (Table 5.2).

Table 5.1. The design and length of stream within culverts sampled during the study.

County	Structure Number	Design	Length of stream within structure (m)
Orange	13	Large Arch	11.5
Moore	225	Large Pipe Arch[1]	17.4
Orange	251	Box	15.5
Orange	30	Box	15.2
Orange	242	Box	15.6
Moore	12	Box	19.6
Randolph	339	Box[2]	17.0
Randolph	463	Box	13.8
Person	38	Corrugated Pipe	23.7
Person	22	Corrugated Pipe[3]	20.5
Moore	212	Corrugated Pipe	28.9
Randolph	459	Corrugated Pipe	16.7

[1] = This structure consisted of 3 large arches (1 supported base flow, 2 were for over-bank flow).
[2] = This box culvert had only one cell (no divisions).
[3] = This culvert had an open bottom.

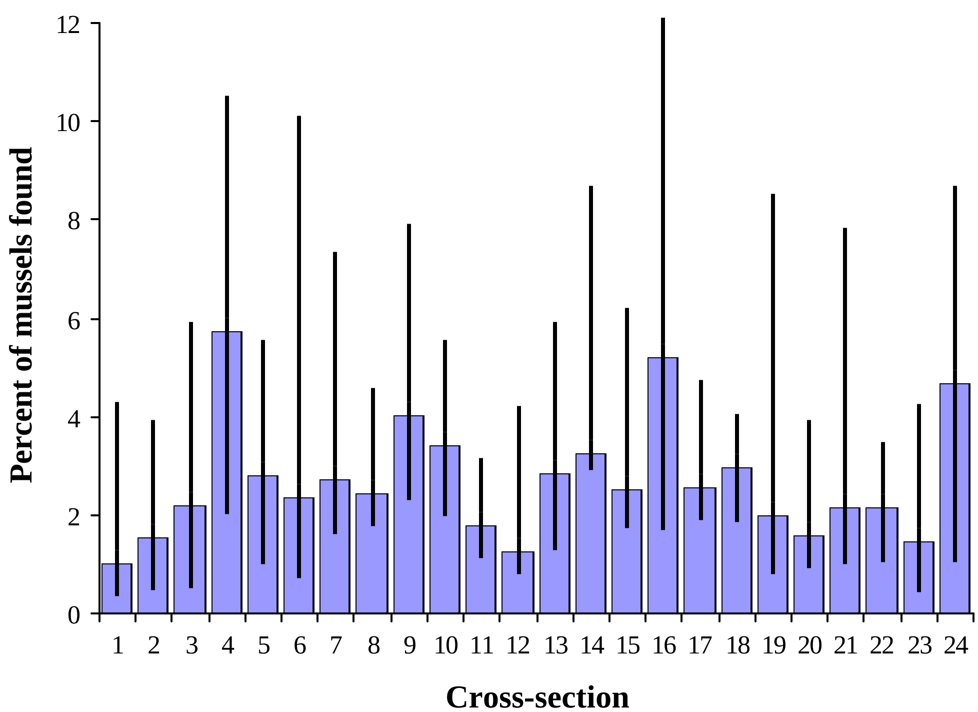

Figure 5.2. *Median percentage of mussels found in a given cross-section with 25 and 75% quartile error bars at 12 sites with culvert road crossings. Cross-sections were numbered*

consecutively from downstream to upstream, and the culvert occurred between numbers 12 and 13.

Table 5.2. Summary of number of crossings with more mussels upstream at various distances from the road. The first percentage is calculating by comparing the total number of mussels found upstream and downstream within a given distance of the road (Cumulative). Another comparison uses individual downstream cross-sections at different distances from the bridge and compares to the median number of mussels found in the upstream cross-sections (Upstream Median).

Comparison	Distance from road (m)	Number of crossings with more upstream	Percent of crossings with more upstream	95% CI (% of crossings)
Cumulative	25	5	41.7	15.2, 72.3
	50	9	75.0	42.8, 92.5
	75	9	75.0	42.8, 92.5
	100	9	75.0	42.8, 92.5
	125	9	75.0	42.8, 92.5
	150	6	50.0	21.1, 78.9
	175	6	50.0	21.1, 78.9
	200	6	50.0	21.1, 78.9
	225	6	50.0	21.1, 78.9
	250	5	41.7	15.2, 72.3
	275	5	41.7	15.2, 72.3
	300	6	50.0	21.1, 78.9
Upstream Median	25	7	58.3	27.7, 84.8
	50	8	66.7	34.9, 90.1
	75	6	50.0	21.1, 78.9
	100	5	41.7	15.2, 72.3
	125	6	50.0	21.1, 78.9
	150	4	33.3	9.9, 65.1
	175	7	58.3	27.7, 84.8
	200	6	50.0	21.1, 78.9
	225	4	33.3	9.9, 65.1
	250	7	58.3	27.7, 84.8
	275	7	58.3	27.7, 84.8
	300	8	66.7	34.9, 90.1

Channel Constriction: Of the 80 crossings surveyed, 44 were judged to have the potential for channel constriction (Appendix V-1), and none of the sites eliminated because of milldam remains were among these 44. Thirty of these 44 structures (68.2%) were built between 1950 and 1969; whereas crossings in this time frame represented 61.1% of the 72 bridges used in analyses. These proportions were not statistically different from one another ($p = 0.436$). There were significant differences between cross-sections in the percent of mussels at a site occurring in those cross-sections ($p = 0.004$, Appendix V-2), and the two 25-meter reaches immediately downstream of the road had the road had the lowest abundance (Figure 5.3). The mean percentage of mussels at the site found in the upstream 300-meter reach was 55.5% (95% CI = 49.9, 61.0), and 50% was slightly within the 95% confidence interval ($p = 0.053$). The number of crossings with constriction with more mussels in the upstream 300-meter reach was 26 (59.1%, 95% CI = 43.2, 73.7). The number of crossings with constriction having fewer mussels in the first 50 meters downstream was significantly higher than 50% ($p < 0.05$, Table 5.3).

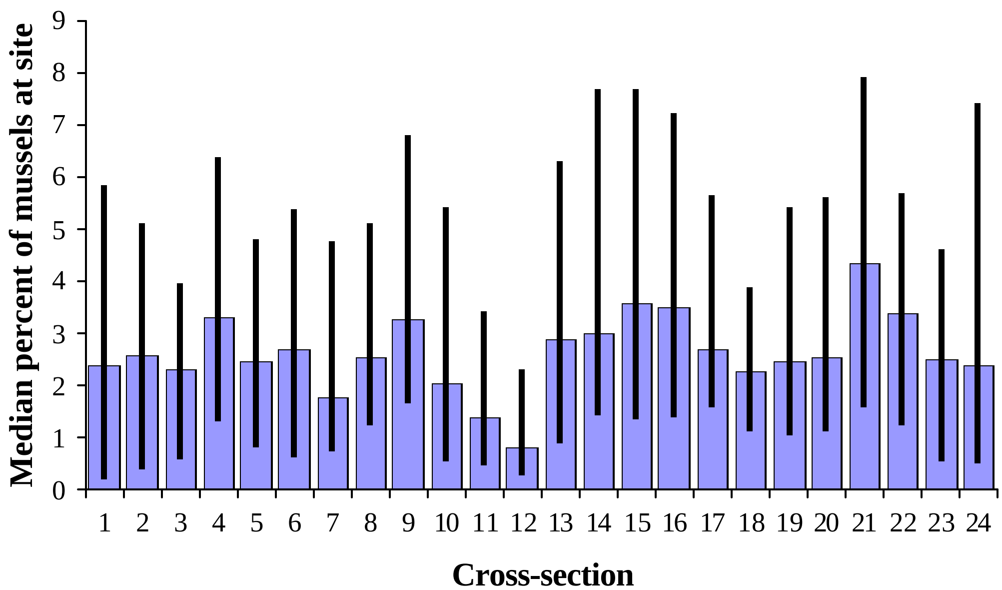

Figure 5.3. *Median percent of mussels at a site occurring in a given 25-meter cross-section using only the 44 sites which were deemed to have the potential for scour. Error bars represent 25 and 75% quartiles. Cross-sections were numbered consecutively from downstream to upstream, and the road crossing was between 12 and 13.*

Table 5.3. Summary of number of crossings with a constriction with more mussels upstream at various distances from the road. The first percentage is calculating by comparing the total number of mussels found upstream and downstream within a given distance of the road (Cumulative). Another comparison uses individual downstream cross-sections at different distances from the bridge and compares to the median number of mussels found in the upstream cross-sections (Upstream Median). An asterisk indicates the percentage is significantly different than 50% (α =0.05).

Comparison	Distance from road (m)	Number of crossings with more upstream	Percent of crossings with more upstream	95% CI (% of crossings)
Cumulative	25	31	70.5*	54.8, 83.2
	50	34	77.3*	62.2, 88.5
	75	32	72.7*	57.2, 85.0
	100	31	70.5*	54.8, 83.2
	125	29	65.9	50.1, 79.5
	150	26	59.1	43.2, 73.7
	175	27	61.4	45.5, 75.6
	200	27	61.4	45.5, 75.6
	225	27	61.4	45.5, 75.6
	250	26	59.1	43.2, 73.7
	275	26	59.1	43.2, 73.7
	300	26	59.1	43.2, 73.7
Upstream Median	25	32	72.7*	57.2, 85.0
	50	31	70.5*	54.8, 83.2
	75	24	54.5	38.8, 69.6
	100	21	47.7	32.5, 63.3
	125	23	52.3	36.7, 67.5
	150	22	50.0	34.6, 65.4
	175	24	54.5	38.8, 69.6
	200	25	56.8	41.0, 71.7
	225	21	47.7	32.5, 63.3
	250	25	56.8	41.0, 71.7
	275	23	52.3	36.7, 67.5
	300	22	50.0	34.6, 65.4

Figure 5.4. *Photograph of a bridge constructed in 1957 with wall-like abutments in the channel.*

Figure 5.5. *Photograph of a bridge constructed in 1961 with wall-like abutments in the channel.*

Average daily traffic: Overall, traffic in both study areas was relatively low, and very few sites had high ADT (Figure 5.6). There were no significant trends in ADT and mussel abundance in any test (p > 0.05, Table 5.4)

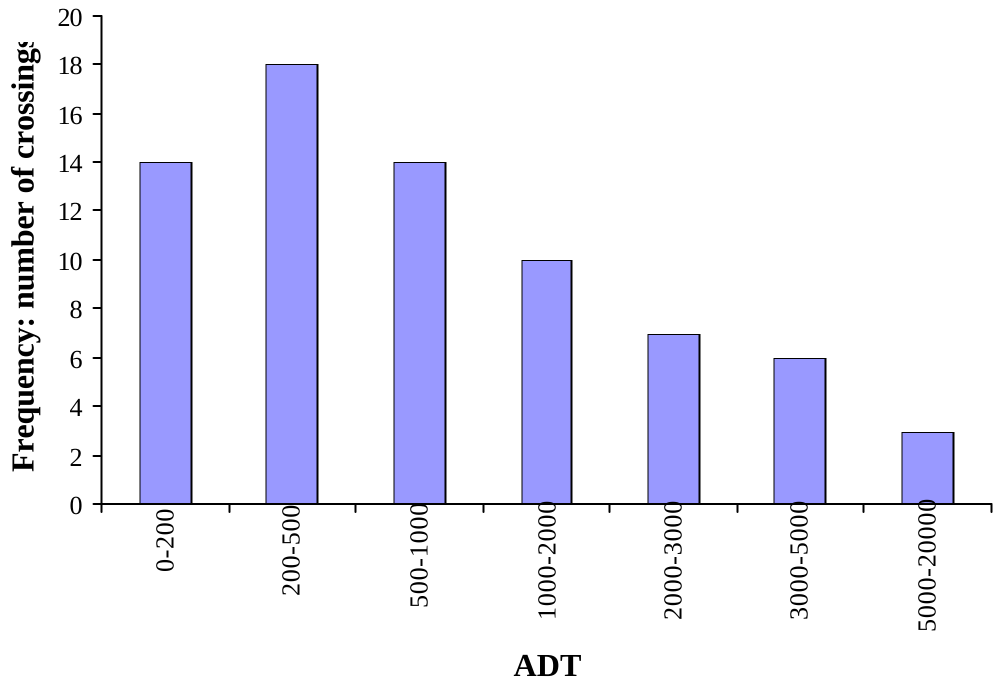

Figure 5.6. *Frequency histogram of the number of crossings with different average daily traffic (ADT) amounts*

Table 5.4. Results of linear regression using log-transformed average daily traffic as the independent variable.

Dependent Variable	r^2	p-value
Percent of mussels upstream	0.0%	0.871
Percent of mussels in CS-12	1.8%	0.255
Percent of mussels in CS-13	0.0%	0.936

Construction Materials: Of the 80 road-crossings surveyed, 29 (36.3%) had wooden construction materials in the channel of the stream. Two of sites were among the milldam sites that were removed from analysis. Of the remaining 27 bridges with wooden supports, 25 of those (92.6%) were constructed during the 50s and 60s, so there is a high correlation between bridge age and having wooden supports in the channel. The mean percentage of mussels at a site found in the upstream 300-meter reach was 55.3%, and this was not statistically greater than 50% ($p = 0.104$). There were significant differences between cross-sections in the percent of mussels at a site occurring in those cross-sections ($p = 0.001$, Appendix V-3), and the two 25-meter reaches immediately downstream of the road had the road had the lowest abundance (Figure 5.7). The median value for cross-section 13 was lower in then analysis than in others.

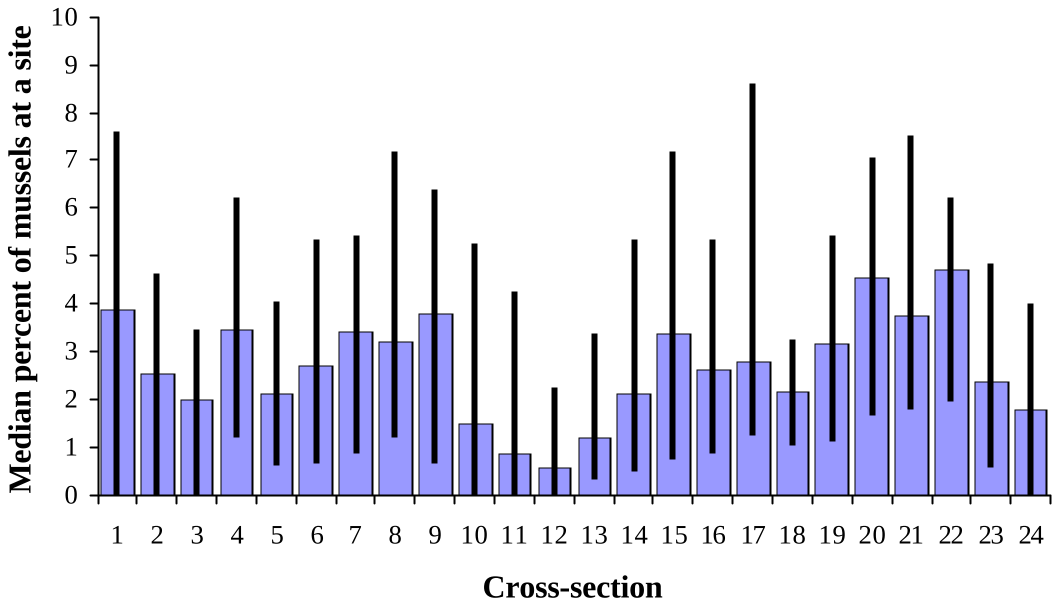

Figure 5.7. *Median percent of mussels at a site occurring in a given 25-meter cross-section using only the 27 sites with wooden bridge supports or abutments in the channel. Error bars represent 25 and 75% quartiles. Cross-sections were numbered consecutively from downstream to upstream, and the road crossing was between 12 and 13.*

Crossings with the least impact: A total of only four crossings met the criteria we selected to determine the structures with the least impact (Table 5.5, Figure 5.8). Three of these structures were bridges, and one was a culvert. Estimated ADT on these structures was highly variable and ranged from 90 to 19000. The common thread across the four structures (Figures 5.8 and 5.9) seemed to be that they did not constrict the channel and allowed for over bank flows. Across the 72 sites sampled, the 23 other cross-sections besides number 12 met these criteria far more often ranging from 8 – 19 times.

Table 5.5. The four road crossings that met our criteria as having the least impact on relative mussel abundance in cross-section (CS) 12 (immediately downstream of the crossing).

County	Bridge Number	Structure type	Average Daily Traffic	Number of mussels in CS 12	Percent of mussels at site in CS 12
Orange	6	Normal Bridge	19000	49	4.4
Orange	53	Normal Bridge	1215	29	4.2
Moore	225	Arch culvert, multicell	450	73	8.4
Randolph	260	Normal Bridge	90	38	6.6

Orange – 6:

This bridge over the Eno River in Hillsborough, NC uses square piers up on the banks, and spans the flood plain by a great distance.

Orange – 53: This bridge over the North Fork of the Little River used steel posts at the top of the bank. Riprap fill was not to the edge of the bank leaving room for over-bank flows.

Figure 5.8. *Photographs of the two road crossings in the Neuse study area that met our criteria of having the least impact on relative mussel abundance immediately downstream of the structure.*

Moore – 225: This large arch culvert is made of three cells. The cell in this picture carries the entire base flow, and the two cells two the left carry over-bank flows. More mussels were found in cross-section 12 (first 25 meters) here than at any other site in the study.	
Randolph – 260: This bridge has a round pier in the channel that may cause minor scour locally, but the channel is widened at the bridge. Deposition is seen around the bridge, and a stable riffle-run just downstream of the structure contained a good mixture of cobble gravel and sand.	

Figure 5.9. *Photographs of the two road crossings in the Cape Fear study area that met our criteria of having the least impact on relative mussel abundance immediately downstream of the structure.*

Discussion

By looking at the crossing structure attributes of main concern, we were able to gain a better understanding of the probable mechanisms by which these structures impact the mussel population in the first 25-50 meters downstream of the road. The age of the structure appeared to be very important for two reasons. Structures built in the 1950s and 1960s had significant local impacts, and the newest bridges (dating back to 1996) all had fewer mussels in the 75-meter reach just downstream of the structure. Impacts from new construction may be caused by the presence of heavy machinery in the channel, construction of causeways, or localized impacts from bank modifications. Several studies have documented the increases in sediment deposition (Barton 1977; Hainly 1980) and deleterious effects on biota downstream during bridge and culvert construction (Barton 1977; Ogbeibu and Victor 1989). Of the structures built in the 50s and 60s, approximately 70% of them had fewer mussels in the first 50 meters downstream compared to the similar reach upstream. These are the crossings that were driving the overall result of decreased abundance immediately downstream of roads. This time frame was the period in which approximately 60% of the crossings in the study were built, and a large percentage of these bridges were designed with wooden, wall-like abutments. Bridges built during this period were more likely to constrict high flows and have wooden supports or abutments in or immediately adjacent to the channel. We suspect that the primary mechanism behind these local decreases was channel constriction rather than toxic effects from the presence of creosote-treated wood in the channel. Structures built after 1970 did not show significant decreases in mussel abundance immediately downstream of the road.

Other researchers have found that scour of substrate is associated with reduced mussel abundance (Strayer 1999; Johnson and Brown 2000). Hardison and Layzer (2001) found that substrate shear stress was also negatively correlated with mussel abundance. If a crossing narrows a channel during high flow events, shear stress and scour would be expected in the area immediately downstream of the bridge or culvert. Although the effects of the creosote on wooden bridges is unknown, we suspect that any creosote that did leach into the water column would be highly diluted. Also, any toxic effect of a bridge would likely be spread out over a longer distance rather than just locally around the road. There were slightly more mussels in the upstream 300-meter reach compared to the downstream reach at crossings built in this period, indicating that some of these structures may have an impact beyond 50 meters. However, the ecological significance of this result is unknown. Intensive chemical and tissue sampling would be required to determine if any road-related toxicological effects were subtle declines in mussel abundance further downstream.

Analyses done on those crossings that constricted the channel show clear declines in the 50 meters downstream of the road. In fact, in no other analysis had such a highly significant result when comparing abundance of cross-section 11 (50 meters downstream) to the median number of mussels found in upstream cross-sections. Crossings built from 1950-1969 made up approximately 70% of this group. Because these structures had such a significant decrease in relative mussel abundance just downstream of the road, we believe this is the primary mechanism by which the structures impact the mussels in this area. Culverts were expected to be among the crossings that caused the greatest decreases just downstream of the road, but we found no significant trends in mussel distribution with culverts. Perhaps a larger sample size of culverts would produce roughly the same mussel distribution patterns as those seen in the analysis using the 44 crossings that cause constriction. Some culverts had caused obvious scour pools on the downstream end than others, and there tended to be few mussels in those pools.

There was no correlation with average daily traffic and either the percent of mussels at a site in the upstream half or in abundance in the cross-sections near the road. Most sites surveyed during the study were in a very rural area, and few had high levels of traffic. Most sites were well off of primary roads and likely receive much lower levels of pollutants from cars, and are likely not salted during winter storms. Although sample size of certain design types was not adequate to address the effects of deck drains, Yousef et al (1983) found that sediment collected below bridges with scupper drains had significantly higher heavy metal concentrations than under bridges without scupper drains. Also, Yousef et al (1984) found that heavy metals could become fixed in vegetated floodplains when highway runoff was diverted there rather than directly to the stream.

Only 4 structures out of 72 analyzed met the criteria of having at least 20 mussels and above average relative abundance for that particular site in cross-section 12. This is another indicator of the rarity of abundant mussel beds just downstream of a crossing structure. These four structures generally did not constrict the channel and even had some deposition on the downstream end. The three bridges did not have earthen fill or abutments up to the edge of the channel. The channel was actually widened at one bridge (Randolph Co. – 260). Another (Orange Co. – 53) had some distance between the top of the bank and the earthen and riprap fill. The last bridge was very high and spanned the channel by a great distance allowing overbank flows to dissipate rather than constrict at the road. The one culvert in this group had a design that greatly decreased the erosive forces on mussel beds during high flows. When culverts are installed, sufficient capacity should be included for overbank flows to reduce this local scour.

Summary of Findings

1. Approximately 60% of all structures surveyed were constructed from 1950-1969. These road crossings had significantly fewer mussels in the 50 meters immediately downstream of the bridge and drove the overall result of the decrease in abundance just downstream of the road.

2. Crossing structures built in the 1950s and 1960s had significantly fewer mussels in the 300-downstream reach, but the cause and ecological significance of this is unknown. This may indicate that these more constricting structures have an impact beyond 50 meters.

3. Although many structures constructed in the 1950s and 1960s used creosoted treated wood in the stream channel, we believe channel constriction is the main reason the structures caused declines in mussel abundance near the road.

4. No statistically significant decrease in relative mussel abundance was found downstream of structures built after 1970.

5. No significant differences were seen in mussel distribution between culverts and bridges; however, a larger sample size of culverts is needed to truly determine the difference in impacts between these types of structures.

6. There was no correlation between average daily traffic and mussel abundance downstream or in cross-sections near the road.

7. The structures with the best mussel populations within 25 meters downstream of the road constricted the channel very little in relation to other structures.

Overall Study Conclusions and Recommendations

Conclusions

The following represents our main conclusions specifically regarding road crossings:

1. On average, bridges on streams of this size may decrease pool habitat within 50-100 meters upstream and 50-100 meters downstream of bridges.

In the place of these lost pools were some riffles, but mostly an increase in run habitat was seen. This is a common result of channelization as stream habitat looses its complexity (Hubbard et al. 1993), but may also be due to other changes in bedload movement. If this trend of decreased pool habitat represents a true bridge effect, it was not the driving force behind the mussel declines just downstream of the bridge; however, the habitat changes and mussel declines may have the same root cause in hydrologic changes at the crossing structure.

2. The presence of road crossings can cause some changes in bank stability both upstream and downstream.

Bank stability was highest in the cross-sections immediately upstream and downstream of the road because of herbaceous vegetation along the banks due to the artificial sloping and loss in canopy cover at the road. Upstream of the road, bank stability remained slightly above average 75-100 meters upstream. We attribute this to channel constriction at the structure reducing velocities of high flows in this reach; consequently, the erosive forces during storm events would be reduced. Downstream of crossings, bank stability steadily declined to below upstream stability levels with increasing distance from the road, and the lowest stability scores were found in the first 100 meters downstream. Further study is required to determine if this effect 300 meters downstream is truly an effect of road crossings at some sites. No statistical correlation was seen between bank stability and the number of mussels found in a cross-section; however, a few sites (Appendix VI) were found to have fewer mussels in areas of high channel incision and low bank stability.

3. The most notable effect that road crossings have on mussels is that they decrease abundance of *E. complanata* under as well as approximately 50 meters downstream.

We believe this result was primarily caused by channel constriction in bridges constructed between 1950 and 1969, which often had wall-like abutments in or adjacent to the channel. Channel constriction, as well as channelization, leads to localized scour and destabilization of the substrate. Also, all seven structures built since 1996 showed this trend and indicate there are some lasting impacts of construction. Localized habitat alterations (e.g. stream bed compaction) or sedimentation during construction are likely residual effects of construction. The length of stream that is subject to these effects of constriction and construction is likely proportional to the area of stream that is within the hydrologic influence of the structure. We believe that as stream size increased the linear distance of stream that was affected by that structure would increase.

4. The main structural design difference between crossings with an impact and crossings without an impact is channel constriction.

The crossings with the least impact generally sufficiently spanned the channel and had sufficient capacity for over-bank flows. Structures built in the 1950s and 1960s generally had the greatest channel constriction and had the greatest impact on mussel abundance. Channel constriction is one of the greatest concerns with culverts, and we did find some culverts in the study area with significant scour pools and few mussels on the downstream end. However we found no significant differences in mussel distribution between culverts and bridges in this study, and a larger sample size of culverts is needed to make definitive conclusions about these two structure types.

5. There is evidence that mussel length is affected downstream of road crossings, but more research is needed in this area.

We also found a general decrease in *E. complanata* length with increasing drainage area, but this may not explain a change in mean length within sites from upstream to downstream of the road crossing. The ecological significance and cause of this result is unknown and would require further study. From the present study, we cannot determine whether this indicates a crossing-structure effect further downstream than 50 meters.

6. Some species may be less affected than *E. complanata*, but the rarity these of other species precludes many concrete conclusions.

No trends were found in the relation to road crossings in sex ratios, gravidity, diversity or abundance of other species. In the cases of some species, this is due to a lack of data, but other species may truly not be as affected by the habitat alterations at road crossings. Although *E. complanata* is the most abundant species in North Carolina, loss of this species should be guarded against for several reasons. There may be rare species currently grouped under the complex called *E. complanata* because of their similarity in appearance. Indeed, we found several different shell shapes of this group in our study, and some of those were rare. Other rare species with similar ecological niches to *E. complanata* may be affected by road crossings. Also, loosing the most abundant species of bivalve in a reach or stream could cause localized shifts in stream ecology.

7. Mussel distribution is likely driven by the complex interaction of many environmental variables.

Some trends in current land use and mussel population health may be found, but the mussel fauna of North Carolina is likely still greatly affected by the destabilization of streams due to poor land use long ago. Crossing structures can have this type of effect locally around the bridge, but there is tentative evidence warranting further study that suggests some crossings may have effects over distances greater than 50 meters.

Recommendations

1. Stabilization of banks around crossings structures should not come at the expense of destabilization of other areas of the stream channel. Excessive bank alterations or riprap fill should be avoided.

2. Substantial changes in water flow or sediment supply should be avoided during road and bridge construction because they would alter bedload dynamics and consequently would be detrimental to mussel fauna.

3. To avoid localized loss of mussel habitat immediately downstream of the road, best management practices should be followed during bridge and culvert construction by minimizing machinery movement in the channel, sediment runoff, channelization and severe bank alterations. Stream channels should not be straightened, widened or narrowed.

4. To maintain habitat near crossing structures after construction, structural designs should allow for sufficient over-bank flows during low-frequency flood events to prevent contractual scour. Overall design of crossing structures since 1970 has consistently improved in terms of reducing channel constriction, but the degree of fill and constriction on the floodplain varies between sites.

5. New bridges and culverts should be placed in areas where channel stability and bedload movement would be least affected.

6. Future research is recommended in the following areas:
   - A. Differences between culverts and bridges on mussel habitat and population health.
   - B. Comparison of the impacts on channel stability between a wide variety of structural designs.
   - C. Changes in bedload movement and channel stability over a substantial distance after bridge and culvert installation.
   - D. Assessment of age and growth of freshwater mussels downstream of road crossings.
   - E. Additional studies should be conducted to assess the chemical and physical impact of riprap on stream fauna.
   - F. Genetic characterization of the various forms of *Elliptio complanata* found during the course of these studies to ensure that they are not distinct, potentially imperiled species.
   - G. Assessment of the potential impact of crossing structures on small stream fish movement.

Implementation and Technology Transfer

During this study, we have determined that the main factor in loss of mussel populations around road crossings is channel constriction and destabilization near the structures. We pointed out some of the designs that are most responsible for these problems and provide suggestions on what might be done to alleviate detrimental effects to the stream environment. Issues raised in this report should be brought to the attention of stream ecologists, bridge design engineers, and construction managers. Those who act as intermediaries between these groups at NCDOT and other transportation agencies should be informed regarding the ecological effects of channel constriction and destabilization. Ecological, economical, and safety aspects of different crossing structure designs should be discussed among these groups to make the best possible decisions regarding interactions between roads and surface waters. Best Management Practices in bridge and culvert construction to minimize channelization, sedimentation and other habitat changes around project sites should constantly be researched.

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Appendix I

Site Selection and Characterization Appendices

Parameters to be evaluated in sampling reach

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
1. Epifaunal Substrate/ Available Cover	Greater than 70% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient).	40-70% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	20-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. Embeddedness	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. Velocity/Depth Regime	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow). (Slow is < 0.3 m/s, deep is > 0.5 m.)	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/ depth regime (usually slow-deep).
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Appendix I-1. *Rapid Bioassessment Protocols habitat assessment criteria (front of data sheet) used for scoring habitat. Source: Barbour, MT, J Gerritsen, BD Snyder, and JB Stribling. 1999. Rapid Bioassessment Protocols for use in streams and wadeable rivers: Periphyton, Benthic Macroinvertebrates, and Fish, Second Edition. EPA 841-B-99-002. Environmental Protection Agency; Office of Water; Washington D.C.*

HABITAT ASSESSMENT FIELD DATA SHEET—HIGH GRADIENT STREAMS (BACK)

Parameters to be evaluated broader than sampling reach

Habitat Parameter	Condition Category: Optimal	Suboptimal	Marginal	Poor
6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7. Frequency of Riffles (or bends)	Occurrence of riffles relatively frequent; ratio of distance between riffles divided by width of the stream <7:1 (generally 5 to 7); variety of habitat is key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is a ratio of >25.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. Bank Stability (score each bank) Note: determine left or right side by facing downstream.	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
9. Vegetative Protection (score each bank)	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

Appendix I-2. *Rapid Bioassessment Protocols habitat assessment criteria (back of data sheet) used for scoring habitat. Source: Barbour, MT, J Gerritsen, BD Snyder, and JB Stribling. 1999. Rapid Bioassessment Protocols for use in streams and wadeable rivers: Periphyton, Benthic Macroinvertebrates, and Fish, Second Edition. EPA 841-B-99-002. Environmental Protection Agency; Office of Water; Washington D.C.*

Appendix I-3. Raw water chemistry data for all sites in the Neuse study area.

County	Bridge Number	Date Sampled	Upstream pH	Downstream pH	Mean pH	Upstream D.O. (mg/L)	Downstream D.O (mg/L)	Mean D.O. (mg/L)	Upstream Temperature (°C)	Downstream Temperature (°C)	Mean Temperature (°C)	Upstream Conductivity (mS)	Downstream Conductivity (mS)	Mean Conductivity (mS)	Upstream Turbidity (NTU)	Downstream Turbidity (NTU)	Mean Turbidity (NTU)
Durham	151	6/28/01	7.6	7.9	7.7	6.2	6.9	6.6	28.2	28.8	28.5	91.6	85.6	88.6	9.2	15.3	12.2
Durham	5	6/8/01	7.3	7.4	7.4	5.9	6.8	6.3	23.8	23.8	23.8	114.3	85.8	100.1	15.5	7.8	11.6
Durham	50	7/11/01	7.5	7.8	7.6	7.5	8.0	7.7	28.2	28.7	28.5	116.0	143.2	129.6	4.0	5.0	4.5
Durham	56	6/26/01	7.6	7.7	7.7	7.2	6.8	7.0	24.8	25.5	25.2	100.8	100.4	100.6	8.9	8.3	8.6
Durham	57	8/14/01	7.4	7.4	7.4	7.6	6.3	7.0	26.8	27.3	27.1	82.1	101.0	91.6	4.7	7.5	6.1
Durham	6	5/8/01	7.4	7.6	7.5	8.7	8.4	8.6	18.4	18.2	18.3	79.9	99.3	89.6	6.2	5.6	5.9
Durham	64	5/21/01	7.5	7.5	7.5	7.5	7.7	7.6	21.5	21.4	21.5	86.7	71.1	78.9	5.0	4.9	5.0
Durham	8	8/8/01	7.4	7.3	7.3	6.9	7.4	7.2	28.6	28.7	28.7	95.0	96.0	95.5	12.1	14.4	13.3
Durham	9	8/7/01	7.2	7.5	7.3	7.4	6.1	6.8	28.2	29.4	28.8	90.2	70.5	80.4	14.8	14.2	14.5
Granville	25	6/18/01	7.3	7.3	7.3	7.3	7.0	7.1	22.7	22.8	22.8	71.7	55.5	*	18.8	18.4	18.6
Orange	11	6/27/01	7.2	7.1	7.2	5.8	5.8	5.8	24.3	24.7	24.5	89.4	90.6	90.0	20.4	31.1	25.8
Orange	114	4/24/01	7.1	7.1	7.1	7.8	7.8	7.8	18.2	18.4	18.3	87.6	89.1	88.4	13.7	12.1	12.9
Orange	12	7/6/01	7.0	7.1	7.1	6.2	2.8	4.5	25.8	25.8	25.8	82.1	83.7	82.9	6.5	12.0	9.3
Orange	126	6/6/01	7.3	7.2	7.2	7.3	7.9	7.6	21.2	22.5	21.9	79.4	70.2	74.8	21.1	18.9	20.0
Orange	13	7/19/01	7.0	6.8	6.9	5.3	7.0	6.1	23.7	23.7	23.7	91.7	64.0	77.9	8.6	11.0	9.8
Orange	136	5/3/01	7.1	7.1	7.1	7.0	6.8	6.9	17.1	16.6	16.9	*	74.9	74.9	8.8	10.4	9.6
Orange	173	5/29/01	7.4	7.4	7.4	6.6	7.1	6.9	21.6	20.9	21.3	96.7	98.3	97.5	*	*	*
Orange	200	5/24/01	7.3	7.3	7.3	7.1	6.3	6.7	20.7	20.7	20.7	147.8	143.7	145.8	15.9	8.0	12.0
Orange	242	7/3/01	6.8	6.5	6.6	3.8	3.3	3.5	21.6	23.2	22.4	101.9	108.1	105.0	24.8	15.1	20.0
Orange	251	5/11/01	7.2	7.3	7.2	5.6	6.2	5.9	19.9	19.2	19.6	85.0	92.5	88.8	9.0	6.5	7.7
Orange	30	5/23/01	7.1	7.5	7.2	5.4	5.9	5.6	18.7	18.7	18.7	78.0	78.4	78.2	13.2	11.7	12.5
Orange	4	8/20/01	7.0	7.1	7.0	5.6	4.7	5.1	25.4	25.3	25.4	67.3	*	67.3	3.3	7.9	5.6
Orange	43	8/16/01	6.8	7.8	7.0	4.7	4.2	4.4	24.5	25.0	24.8	76.8	80.5	78.7	13.5	7.3	10.4
Orange	53	7/12/01	7.0	6.5	6.7	5.3	4.7	5.0	25.1	25.4	25.3	80.8	82.3	81.6	23.4	27.3	25.4
Orange	54	6/19/01	7.2	7.0	7.1	4.9	5.5	5.2	23.7	23.1	23.4	86.2	81.7	84.0	14.5	20.8	17.7
Orange	55	7/23/01	7.0	7.1	7.0	6.1	7.2	6.6	23.1	23.6	23.4	103.5	103.4	103.5	8.3	8.0	8.1
Orange	57	6/25/01	7.4	7.6	7.5	6.0	5.9	6.0	23.5	23.4	23.5	95.6	95.6	95.6	13.0	19.9	16.5
Orange	6	8/3/01	7.3	7.5	7.3	6.9	6.6	6.7	23.8	23.8	23.8	94.6	120.8	107.7	14.0	23.5	18.8
Orange	64	8/21/01	7.4	7.3	7.4	3.6	4.6	4.1	25.4	25.9	25.7	152.1	151.9	152.0	6.8	7.8	7.3
Orange	66	5/18/01	7.3	7.6	7.4	8.0	8.5	8.3	18.4	18.9	18.7	103.7	104.7	104.2	10.4	12.5	11.5
Orange	67	5/31/01	7.5	7.5	7.5	7.4	7.3	7.3	20.3	20.4	20.4	81.6	84.1	82.9	6.0	8.2	7.1
Person	10	4/26/01	7.9	8.2	8.0	9.4	9.7	9.5	15.1	15.4	15.3	55.6	55.7	55.7	10.6	12.3	11.5
Person	127	5/4/01	7.4	7.6	7.5	8.5	9.2	8.8	21.4	21.6	21.5	69.4	69.5	69.5	7.9	6.6	7.3
Person	130	8/9/01	7.5	7.9	7.6	6.7	7.2	7.0	28.7	28.8	28.8	9.4	10.8	10.1	13.5	11.4	12.5
Person	18	7/18/01	7.0	6.8	6.9	4.3	4.8	4.6	24.1	24.2	24.2	95.5	83.8	89.7	17.2	30.3	23.8
Person	205	8/6/01	7.2	7.1	7.1	6.3	6.6	6.5	23.4	23.5	23.5	90.4	80.9	85.7	1.8	1.3	1.5
Person	21	7/25/01	7.7	6.7	7.0	5.9	6.3	6.1	25.8	25.5	25.7	96.1	95.0	95.6	12.0	15.0	13.5
Person	22	5/9/01	6.9	7.0	6.9	6.3	6.8	6.5	19.6	20.5	20.1	67.8	73.6	70.7	25.5	17.0	21.3
Person	23	5/7/01	7.3	7.3	7.3	9.1	8.7	8.9	18.1	18.6	18.4	86.8	90.0	88.4	20.0	24.0	22.0
Person	33	6/21/01	7.3	7.5	7.3	5.9	6.8	6.4	24.4	25.4	24.9	100.1	63.1	81.6	13.8	12.0	12.9
Person	36	7/5/01	6.9	7.0	7.0	5.0	5.9	5.5	25.0	25.2	25.1	106.5	107.4	107.0	14.7	19.5	17.1
Person	38	6/13/01	7.5	7.2	7.3	6.8	5.6	6.2	26.5	23.2	24.9	85.7	82.3	84.0	14.4	24.5	19.5
Person	80	6/12/01	7.5	7.4	7.4	7.6	7.7	7.6	22.9	23.6	23.3	59.8	56.5	58.2	12.0	12.0	12.0
Wake	119	6/7/01	7.3	7.4	7.3	7.4	8.1	7.8	23.9	24.8	24.4	42.3	78.3	60.3	14.2	20.0	17.1

Appendix I-4. Raw water chemistry data for all sites in the Cape Fear study area.

County	Bridge Number	Date Sampled	Upstream pH	Downstream pH	Mean pH	Upstream D.O. (mg/L)	Downstream D.O (mg/L)	Mean D.O. (mg/L)	Upstream Temperature (°C)	Downstream Temperature (°C)	Mean Temperature (°C)	Upstream Conductivity (mS)	Downstream Conductivity (mS)	Mean Conductivity (mS)	Upstream Turbidity (NTU)	Downstream Turbidity (NTU)	Mean Turbidity (NTU)
Chatham	245	5/30/02	*	*	*	2.9	3.0	2.9	18.2	18.2	18.2	89.0	88.5	88.8	31.7	19.3	25.5
Chatham	247	5/31/02	*	*	*	7.2	5.5	6.3	22.6	20.7	21.7	103.3	110.3	106.8	5.3	4.1	4.7
Chatham	251	6/19/02	*	*	*	3.6	5.4	4.5	20.8	21.9	21.4	90.8	127.4	109.1	5.6	5.0	5.3
Granville	23	5/6/02	7.4	7.8	7.5	7.7	7.8	7.8	15.3	15.0	15.2	134.9	158.3	146.6	*	*	*
Granville	72	5/7/02	7.2	7.8	7.4	6.6	7.0	6.8	18.4	18.7	18.6	162.9	161.5	162.2	13.3	14.0	13.7
Moore	12	5/20/02	*	*	*	9.3	9.3	9.3	15.2	14.4	14.8	34.3	29.7	32.0	*	7.2	7.2
Moore	127	6/27/02	*	*	*	3.1	4.5	3.8	23.4	24.9	24.2	107.9	130.6	119.3	3.4	2.9	3.2
Moore	173	5/21/02	*	*	*	8.3	8.3	8.3	13.7	13.9	13.8	56.5	56.4	56.5	8.6		8.6
Moore	174	5/13/02	5.9	5.5	5.7	5.7	4.9	5.3	22.0	21.0	21.5	84.9	81.7	83.3	15.1	15.4	15.3
Moore	184	5/15/02	*	*	*	8.7	6.7	7.7	15.7	15.1	15.4	70.5	68.0	69.3	10.0	12.8	11.4
Moore	212	6/11/02	*	*	*	5.5	6.9	6.2	21.6	20.3	21.0	82.9	77.8	80.4	13.6	23.0	18.3
Moore	28	6/10/02	*	*	*	3.0	5.3	4.2	18.2	18.1	18.2	87.1	95.9	91.5	12.8	6.3	9.6
Randolph	109	7/17/02	*	*	*	1.9	3.2	2.6	22.5	23.7	23.1	118.8	98.6	108.7	19.6	200.0	109.8
Randolph	110	7/3/02	*	*	*	10.0	5.9	7.9	28.2	26.7	27.5	102.9	111.0	107.0	3.9	4.6	4.2
Randolph	149	5/2/02	7.8	7.1	7.3	9.0	7.1	8.1	20.9	20.5	20.7	92.2	85.5	88.9	*	*	*
Randolph	175	5/14/02	*	*	*	6.0	6.0	6.0	17.5	17.0	17.3	78.4	72.6	75.5	14.6	13.7	14.2
Randolph	188	6/18/02	*	*	*	6.3	7.5	6.9	21.7	21.7	21.7	99.7	68.0	83.9	10.6	15.3	13.0
Randolph	199	7/11/02	*	*	*	6.6	5.4	6.0	23.4	23.1	23.3	93.9	92.5	93.2	7.1	13.9	10.5
Randolph	208	6/17/02	*	*	*	7.4	3.3	5.3	24.2	23.1	23.7	25.3	108.7	67.0	4.4	6.7	5.6
Randolph	210	6/13/02	*	*	*	6.9	3.4	5.1	25.7	23.3	24.5	157.7	115.9	136.8	14.3	15.5	14.9
Randolph	211	6/12/02	*	*	*	1.5	2.1	1.8	20.2	20.3	20.3	106.2	105.1	105.7	*	26.5	26.5
Randolph	214	4/23/02	7.0	7.2	7.1	3.5	4.6	4.0	17.6	17.7	17.7	90.9	93.1	92.0	16.9	31.3	24.1
Randolph	218	5/15/02	*	*	*	5.1	6.4	5.8	25.4	26.8	26.1	95.7	97.2	96.5	3.4	3.9	3.7
Randolph	220	4/19/02	7.2	7.1	7.1	7.8	7.4	7.6	23.2	23.2	23.2	67.9	74.1	71.0	0.9	40.0	20.5
Randolph	228	4/29/02	7.4	7.6	7.4	5.9	4.8	5.3	19.4	19.4	19.4	76.9	79.9	78.4	6.2	7.6	6.9
Randolph	257	5/23/02	*	*	*	6.6	8.4	7.5	13.6	14.1	13.9	103.5	107.7	105.6	13.0	7.3	10.2
Randolph	260	6/28/02	*	*	*	0.6	3.7	2.2	22.7	24.5	23.6	138.7	128.0	133.4	13.7	8.9	11.3
Randolph	339	5/22/02	*	*	*	7.2	10.3	8.8	11.8	15.3	13.6	92.6	96.7	94.7	11.8	6.0	8.9
Randolph	349	4/30/02	7.4	7.9	7.6	7.8	8.7	8.3	15.5	17.0	16.3	97.0	93.2	95.1	7.5	19.6	13.6
Randolph	359	7/9/02	*	*	*	5.4	7.7	6.5	27.2	28.1	27.7	108.9	106.4	107.7	2.5	5.3	3.9
Randolph	374	7/8/02	*	*	*	5.3	6.6	6.0	25.3	25.8	25.6	95.1	99.3	97.2	22.0	33.0	27.5
Randolph	415	7/16/02	*	*	*	4.2	5.3	4.7	25.3	26.2	25.8	99.3	98.1	98.7	28.3	7.2	17.8
Randolph	443	5/27/02	*	*	*	6.2	6.3	6.2	18.4	18.5	18.5	105.7	96.8	101.3	*	*	*
Randolph	459	5/1/02	7.5	7.7	7.6	7.2	7.6	7.4	19.0	19.0	19.0	115.1	122.3	118.7	*	*	*
Randolph	463	4/26/02	7.7	7.5	7.6	9.8	10.0	9.9	14.7	15.1	14.9	80.3	81.3	80.8	29.5	17.7	23.6

Appendix I-5. Mean RBP scores for all sites in the Neuse study area.

County	Bridge Number	Epifaunal substrate cover	Embeddedness	Velocity / depth regime	Sediment deposition	Channel flow status	Channel alteration	Frequency of riffles	Bank stability	Vegetative protection	Riparian zone	TOTAL
Durham	151	15.2	14.0	14.0	14.8	16.2	15.7	9.8	11.5	11.5	15.8	138.5
Durham	5	18.0	16.3	16.2	16.3	15.5	16.3	14.3	11.7	12.2	16.7	153.5
Durham	50	18.2	17.5	17.7	17.5	16.2	17.2	17.0	16.8	12.7	18.5	169.2
Durham	56	16.5	17.3	14.5	15.8	16.7	17.0	12.7	10.7	13.2	17.0	151.3
Durham	57	16.2	14.7	14.8	13.5	13.7	17.0	15.7	11.5	10.0	16.3	143.3
Durham	6	16.5	15.7	15.0	13.5	14.7	16.7	14.3	10.8	11.5	15.5	144.2
Durham	64	19.0	17.5	18.5	18.3	16.3	17.7	17.8	13.2	11.3	19.2	168.8
Durham	8	19.2	17.8	17.0	17.0	17.3	17.7	16.3	13.5	12.8	16.0	164.7
Durham	9	17.8	16.7	17.3	17.0	17.0	17.5	16.8	13.8	13.5	16.5	164.0
Granville	25	14.0	14.0	15.3	12.5	13.8	17.8	15.7	11.2	9.7	19.8	143.8
Orange	11	17.1	16.1	16.3	15.3	15.5	17.4	16.1	10.8	11.1	14.8	150.5
Orange	114	14.7	13.5	10.8	13.5	17.0	17.8	13.8	11.5	10.7	16.5	139.8
Orange	12	15.3	16.0	16.2	14.8	16.0	17.8	13.2	5.2	6.7	18.2	139.3
Orange	126	15.3	16.5	16.8	16.7	15.2	15.5	15.3	7.8	9.3	19.3	147.8
Orange	13	16.8	16.8	17.5	17.7	16.7	16.7	17.0	12.3	10.3	14.0	155.8
Orange	136	9.0	7.7	5.7	7.2	13.7	16.3	6.7	8.7	7.8	15.5	98.2
Orange	173	15.5	8.8	11.3	9.0	7.3	14.8	10.0	16.0	14.0	15.7	122.5
Orange	200	14.2	14.8	15.0	14.7	17.0	15.2	14.7	8.2	8.8	7.2	129.7
Orange	242	14.2	13.8	14.7	13.7	15.5	15.2	9.0	14.8	14.2	15.3	140.3
Orange	251	16.7	15.7	14.2	13.7	15.0	16.3	15.2	10.2	10.5	10.3	137.7
Orange	30	13.8	15.0	14.2	13.0	16.2	16.5	14.7	9.3	10.2	14.7	137.5
Orange	4	13.8	14.0	14.0	14.5	15.8	16.8	16.2	10.0	7.0	12.0	134.2
Orange	43	16.3	17.0	16.5	16.3	15.7	16.0	15.3	10.5	8.0	14.7	146.3
Orange	53	17.8	16.8	16.2	16.3	17.0	14.7	12.8	10.2	9.7	8.7	140.2
Orange	54	16.0	15.0	12.0	14.0	16.0	16.2	10.3	9.8	9.3	10.3	129.0
Orange	55	17.2	17.5	16.5	16.8	16.7	16.7	16.0	13.0	11.5	17.0	158.8
Orange	57	14.5	16.2	14.3	14.0	15.0	16.5	11.3	7.8	9.2	13.5	132.3
Orange	6	15.0	14.0	15.2	14.5	14.8	15.7	14.8	8.7	9.5	16.5	138.7
Orange	64	16.3	16.2	15.7	16.7	17.3	19.0	15.8	9.0	11.2	17.0	154.2
Orange	66	18.3	17.2	16.5	16.8	16.5	16.5	15.7	12.5	11.3	9.3	150.7
Orange	67	16.7	16.3	17.7	16.3	17.5	16.8	17.0	11.0	9.7	11.2	150.2
Person	10	17.5	14.8	14.5	14.8	14.8	16.8	13.8	12.7	13.0	12.0	144.8
Person	127	14.5	15.8	13.7	10.2	12.8	16.8	15.2	9.7	10.8	14.7	134.2
Person	130	19.2	18.8	18.8	17.8	17.3	18.2	17.2	15.2	12.3	19.8	174.7
Person	18	16.2	13.5	13.7	14.3	16.5	17.3	16.0	5.8	7.8	12.3	133.5
Person	205	13.7	10.8	11.0	10.3	11.5	12.2	12.5	5.3	5.0	12.2	104.5
Person	21	17.2	16.7	17.2	15.7	15.3	17.3	16.8	9.8	9.3	17.8	153.2
Person	22	13.0	12.2	9.8	12.2	14.2	16.3	13.0	10.7	11.7	15.2	128.2
Person	23	9.7	10.2	8.3	7.5	15.2	16.2	7.8	12.3	10.5	8.7	106.3
Person	33	18.8	18.2	17.0	17.8	17.0	14.2	15.8	14.0	12.3	16.5	161.7
Person	36	18.2	16.7	16.8	16.0	15.2	17.5	15.7	8.3	8.2	16.7	149.2
Person	38	14.5	13.8	14.2	14.7	15.0	15.7	14.7	8.8	10.0	15.8	137.2
Person	80	17.3	16.5	16.3	15.7	15.3	15.5	14.8	9.8	9.3	17.8	148.5
Wake	119	12.8	14.7	15.7	13.0	13.2	12.5	14.3	10.3	9.7	8.5	124.7

Appendix I-6. Results of Kruskal-Wallis test comparing each of the 10 RBP metrics in the Neuse study area between 100-meter reaches. Median value was calculated using all sites (N = 44). Z-scores are included in parentheses for the one parameter with statistical significance. Cross-sections were numbered consecutively from downstream to upstream, and the road crossing occurred between 12 and 13.

	25-meter cross-sections (Median value)						
Parameter	**1-4**	**5-8**	**9-12**	**13-16**	**17-20**	**21-24**	***p* - value**
Epifaunal substrate cover	17	16	16	16	17	16.5	0.693
Substrate embeddedness	16	16	16	16	16	16	0.849
Velocity depth regime	15	16	16	16	15	15.5	0.973
Sediment deposition	15	15	15	15	15	14	0.901
Channel flow status	16.5	16	16	16	16	15.5	0.973
Channel alteration	17 *(2.09)*	17 *(1.17)*	16.5 *(-1.57)*	17 *(-2.21)*	17 *(0.39)*	17 *(0.12)*	0.039
Frequency of riffles	16	15	15	15	16	15.5	0.926
Bank stability	11	10	12	10	10	10	0.676
Bank vegetative protection	10	10	11	11	9.5	10	0.558
Riparian zone vegetation	16	15.5	16	16	16	16	0.932

Appendix II

Mussel and Aquatic Insect Survey Appendices

Appendix II-1. Timetable of sites surveyed in the Neuse study area.

County	Bridge Number	Stream	State Route Number	Survey Date
Orange	114	Forrest Creek	1548	4/24/01
Person	10	Deep Creek	1567	4/26/01
Orange	136	South Fork Little River	1544	5/2/01
Person	127	Deep Creek	1723	5/4/01
Person	23	South Flat River	NC 157	5/7/01
Durham	6	Mountain Creek	1617	5/8/01
Person	22	Unnamed tributary: North Flat River	1708	5/9/01
Orange	251	McGowan Creek	1004	5/10/01
Orange	66	Stroud's Creek	1002	5/18/01
Durham	64	Little River	1461	5/21/01
Orange	30	North Fork Little River	NC 57	5/22/01
Orange	200	Stroud's Creek	1555	5/24/01
Orange	173	East Fork Eno River	1353	5/29/01
Orange	67	McGowan Creek	1324	5/30/01
Orange	126	Lick Creek	1526	6/6/01
Wake	119	New Light Creek	1912	6/7/01
Durham	5	Mountain Creek	1793	6/8/01
Person	80	Deep Creek	1734	6/11/01
Person	38	Lick Creek	1121	6/13/01
Granville	25	Smith Creek	1710	6/18/01
Orange	54	North Fork Little River	NC 157	6/19/01
Person	33	South Flat River	1125	6/20/01
Orange	57	South Fork Little River	1538	6/25/01
Durham	56	South Fork Little River	NC 157	6/26/01
Orange	11	Eno River	1536	6/27/01
Durham	151	Flat River	1614	6/28/01
Orange	242	West Fork Eno River	1004	7/3/01
Person	36	South Flat River	1123	7/5/01
Orange	12	East Fork Eno River	1332	7/6/01
Durham	50	Eno River	NC 157	7/11/01
Orange	53	North Fork Little River	1538	7/12/01
Person	18	South Flat River	US 501	7/18/01
Orange	13	South Fork Little River	NC 57	7/19/01
Orange	55	South Fork Little River	1540	7/23/01
Person	21	North Flat River	1715	7/25/01
Orange	6	Eno River	NC 86	8/2/01
Person	205	South Flat River	1120	8/6/01
Durham	8	Flat River	1602	8/8/01
Durham	9	Flat River	1771	8/9/01
Person	130	Flat River	1737	8/9/01
Durham	57	South Fork Little River	1461	8/14/01
Orange	43	Sevenmile Creek	1120	8/17/01
Orange	4	West Fork Eno River	1004	8/20/01
Orange	64	Eno River	1561	8/21/01

Appendix II-2. Timetable for 36 sites surveyed in the Cape Fear study area.

County	Bridge Number	Stream	Road Number	Survey Date
Randolph	220	Bachelor Creek	SR 2849	4/19/02
Randolph	214	Bachelor Creek	SR 2900	4/23/02
Randolph	175	Mill Creek	SR 2614	4/25/02
Randolph	463	Little Polecat Creek	SR 2114	4/26/02
Randolph	228	Richland Creek	SR 2834	4/29/02
Randolph	349	Little Creek	SR 2870	4/30/02
Randolph	459	Reed Creek	SR 2626	5/1/02
Randolph	149	UT to Bush Creek	SR 2141	5/2/02
Guilford	23	Polecat Creek	SR 3433	5/6/02
Guilford	72	Polecat Creek	NC 62	5/7/02
Moore	174	Williams Creek	SR 1403	5/13/02
Moore	184	Williams Creek	SR 1404	5/14/02
Moore	225	Wolf Creek	SR 1275	5/16/02
Moore	12	Wet Creek	NC 24/27	5/20/02
Moore	173	Wolf Creek	SR 1403	5/21/02
Randolph	339	Reedy Creek	SR 2867	5/22/02
Randolph	257	Vestal Creek	SR 2824	5/23/02
Randolph	443	Sandy Creek	SR 2261	5/28/02
Chatham	245	Little Brush Creek	SR 1148	5/30/02
Chatham	247	Little Brush Creek	SR 1100	5/31/02
Moore	28	Dry Creek	NC 24/27	6/10/02
Moore	212	Dry Creek	SR 1276	6/11/02
Randolph	211	Fork Creek	SR 2863	6/12/02
Randolph	210	Meadow Branch Creek	SR 2869	6/13/02
Randolph	208	Fork Creek	SR 1003	6/17/02
Chatham	251	Brush Creek	SR 1104	6/19/02
Randolph	188	Mill Creek	SR 2657	6/20/02
Moore	127	Bear Creek	SR 1428	6/27/02
Randolph	260	Brush Creek	SR 2636	6/28/02
Randolph	110	Brush Creek	NC 22/42	7/3/02
Randolph	374	Sandy Creek	SR 2481	7/8/02
Randolph	359	Richland Creek	SR 2911	7/9/02
Randolph	199	Richland Creek	SR 2873	7/11/02
Randolph	218	Richland Creek	SR 2845	7/15/02
Randolph	415	Fork Creek	SR 2873	7/16/02
Randolph	109	Polecat Creek	SR 2113	7/17/02

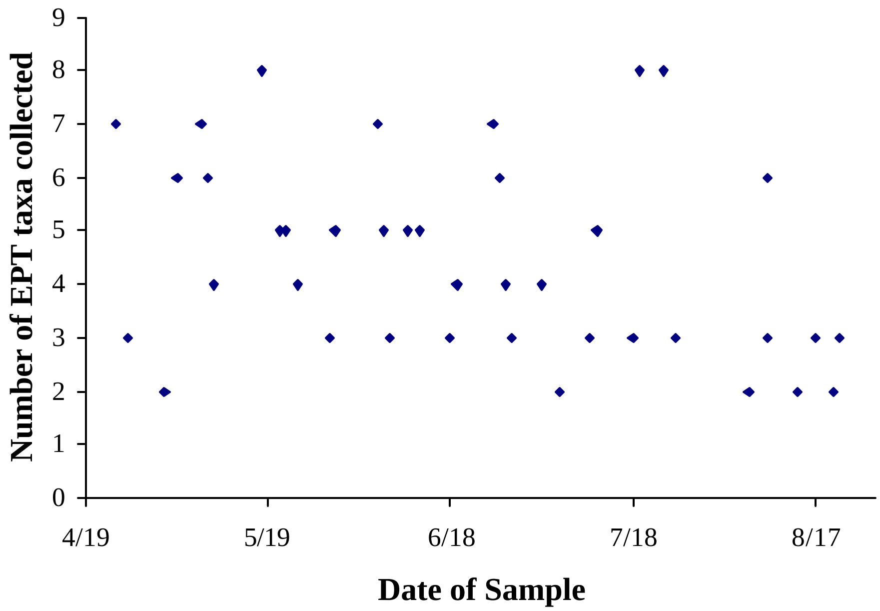
Appendix II-3. Timetable of the 15 sites surveyed twice during the study.

Basin	County	Bridge Number	Original Survey Date	Second Survey Date
Neuse	Orange	114	4/24/01	9/6/01
	Person	10	4/26/01	9/7/01
	Orange	136	5/2/01	9/11/01
	Person	127	5/4/01	9/4/01
	Person	23	5/7/01	9/12/01
Neuse	Person	18	7/18/01	7/29/02
	Orange	13	7/19/01	7/23/02
	Orange	55	7/23/01	7/24/02
	Person	21	7/25/01	7/30/02
	Orange	6	8/2/01	7/31/02
Cape Fear	Randolph	463	4/26/02	9/17/02
	Randolph	459	4/30/02	9/18/02
	Randolph	349	5/1/02	9/11/02
	Randolph	149	5/2/02	9/19/02
	Guilford	23	5/6/02	9/2302

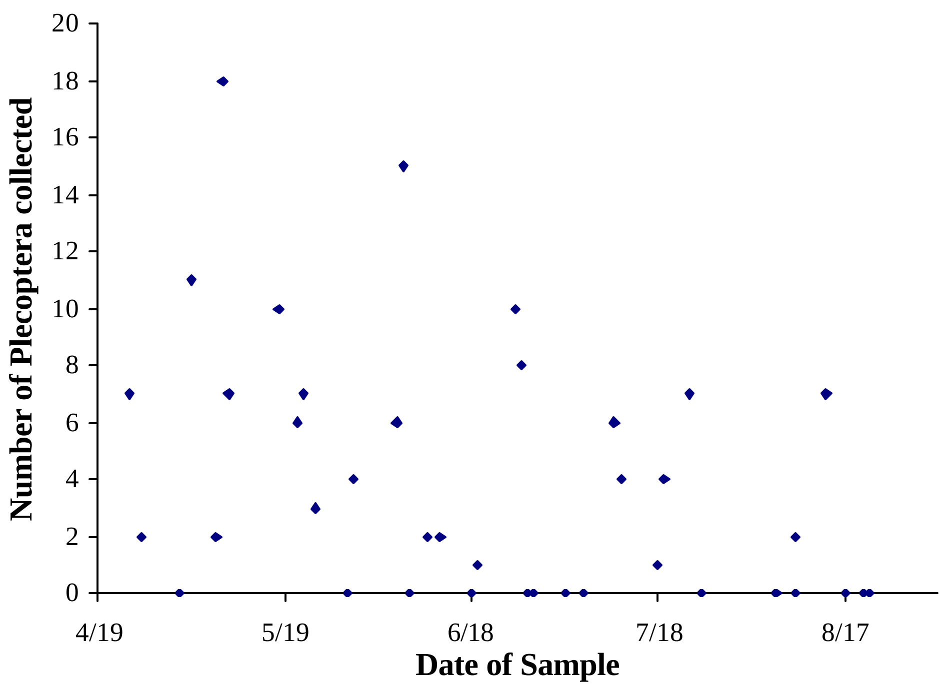
Appendix II-4. Aquatic collection raw data for each site. The site number is the first number of the county followed by the NCDOT bridge number. Durham (D), Granville (G), Orange (O), Person (P), and Wake (W) Counties are represented.

		Ephemeroptera										***Plecoptera***				***Trichoptera***		
Site Number	**Location**	***Baetis intercalaris***	***Caenis sp.***	***Ephemerella catawba***	***Eurylophella sp.***	***Isonychia sp.***	***Leucrocuta sp.***	***Stenonema modestum***	***Stenacron interpunctatum***	***Serratella deficiens***	***Timpanoga simplex***	***Acroneuria sp.***	***Eccoptura xanthenis***	***Neoperla sp.***	***Perlesta sp.***	***Cheumatopsyche sp.***	***Hydropsyche betteni***	**Total EPT Taxa**
D-5	Upstream							2	4									2
D-5	Downstream					1		5	2									3
D-50	Upstream					8												1
D-50	Downstream					1		4				6						3
D-56	Upstream	4				10		18				3		5			16	6
D-56	Downstream							16										1
D-6	Upstream	20	1			1			9						1		4	6
D-6	Downstream											1						1
D-64	Upstream							5		2				1	2			4
D-64	Downstream		2					2							3			3
D-9	Upstream			1		6			8			1					5	5
D-9	Downstream					2		8	10			1						4
O-11	Upstream							12									1	2
O-11	Downstream					2		12	3									3
O-12	Upstream							17										1
O-12	Downstream								9									1
O-126	Upstream							7	13				1		1			4
O-126	Downstream					2	1	4	2	5					4			6
O-13	Upstream					2						2					11	3
O-13	Downstream	2						4	2					1	1			5
O-173	Upstream																	0
O-173	Downstream	1	6						2									3
O-200	Upstream					2		7	9									3
O-200	Downstream					5									3		10	3
O-242	Upstream					1		2	15								3	4
O-242	Downstream							2									5	2
O-251	Upstream							13	12									2
O-251	Downstream							10	1			3			4			4
O-30	Upstream								8						6			2
O-30	Downstream			1				1	3						1		1	5
O-4	Upstream							1	7									2
O-4	Downstream							2	11									2
O-43	Upstream							3	18									2
O-43	Downstream					2		11	2									3
O-53	Upstream					16		4				2		1				4

Site Number	Location	*Ephemeroptera*										*Plecoptera*				*Trichoptera*		Total EPT Taxa
		Baetis intercalaris	*Caenis sp.*	*Ephemerella catawba*	*Eurylophella sp.*	*Isonychia sp.*	*Leucrocuta sp.*	*Stenonema modestum*	*Stenacron interpunctatum*	*Serratella deficiens*	*Timpanoga simplex*	*Acroneuria sp.*	*Eccoptura xanthenis*	*Neoperla sp.*	*Perlesta sp.*	*Cheumatopsyche sp.*	*Hydropsyche betteni*	
O-53	Downstream					15		2	1					1				4
O-55	Upstream	7				1		42	2			4				8	30	7
O-55	Downstream					6		16				4		1		9	5	6
O-57	Upstream					1		5	3									3
O-57	Downstream	8				2		2	5			3		7			8	7
O-64	Upstream					2		9										2
O-64	Downstream					1		3	2									3
O-66	Upstream		7					1	5					6				4
O-66	Downstream		2	1				2	7	1		1		2	1			8
O-67	Upstream							11	10		1							3
O-67	Downstream					4		11							4			3
P-10	Upstream							1							2			2
P-10	Downstream								5									1
P-127	Upstream	7		2		1		3									3	5
P-127	Downstream			2											11			2
P-130	Upstream			4					9								4	3
P-130	Downstream								13								1	2
P-18	Upstream							6	3			1						3
P-18	Downstream							1	11									2
P-205	Upstream							4	3									2
P-205	Downstream								2									1
P-21	Upstream					5		4	13									3
P-21	Downstream					1		5	10									3
P-22	Upstream		1						7									2
P-22	Downstream		2			10	8	4							18			5
P-38	Upstream					17		1	1						2			4
P-38	Downstream					10		2	1	1								4
P-80	Upstream					10		3	2									3
P-80	Downstream					3		5	6			1		1				5
W-119	Upstream					2		5	1						9			4
W-119	Downstream						1	7	2						6			4



Appendix II-5. *The number of EPT taxa collected at 44 sites over time. These two variables were significantly and negatively correlated (*r = *-0.329,* p = *0.041).*



Appendix II-6. *The number of individuals collected of the order Plecoptera collected at 44 sites over time. These two variable were significantly and negatively correlated (*r = *0.390,* p = *0.014).*

Appendix II-7. A summary of all mussels found at each of the 80 sites sampled during the study.

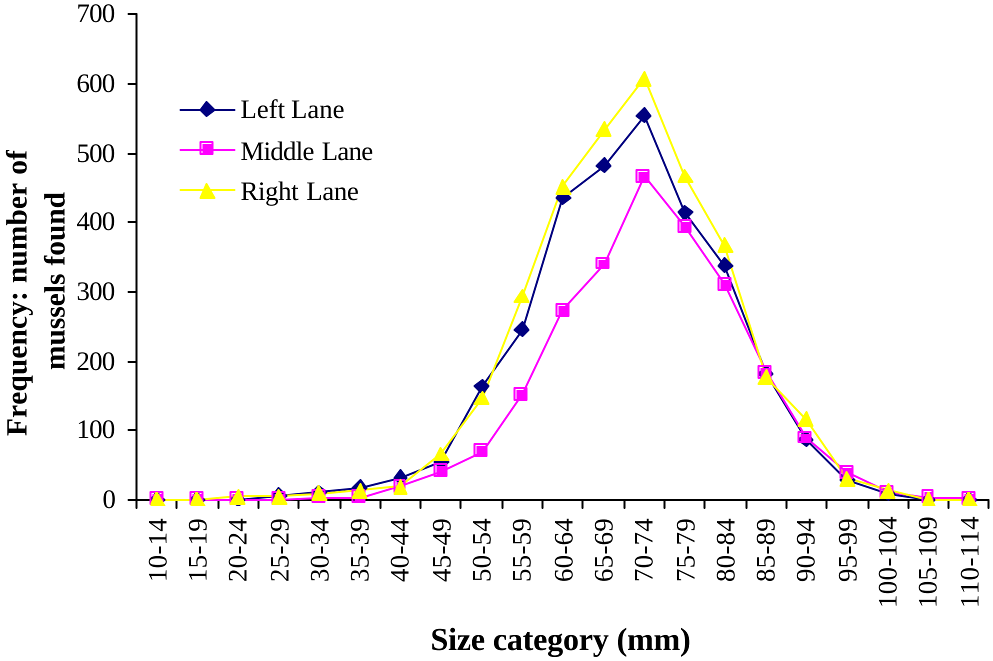
Basin	County	Bridge Number	*Elliptio complanata*	*Elliptio icterina*	*Elliptio sp. (lanceolate)*	*Fusconaia masoni*	*Pyganodon cataracta*	*Strophitus undulatus*	*Villosa constricta*	*Villosa delumbis*	*Villosa vaughaniana*	*Lampsilis cariosa*	*Lampsilis radiata*	*Lampsilis sp.*	*Alasmidonta undulata*	*Alasmidonta varicosa*	*Toxolasma pullus*	*Lasmigona subviridis*	*Utterbackia imbecillis*	Total Mussels
Neuse	Durham	151	146	0	0	2	2	2	3	0	0	5	44	2	0	0	0	0	0	206
	Durham	5	307	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	307
	Durham	50	38	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	40
	Durham	56	457	0	0	1	0	0	14	0	0	0	0	0	0	0	0	0	0	472
	Durham	57	148	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	150
	Durham	6	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15
	Durham	64	65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	65
	Durham	8	193	0	0	0	0	2	0	0	0	2	4	0	0	0	0	0	0	201
	Durham	9	168	0	0	0	0	2	1	0	0	1	1	0	0	0	0	0	0	173
	Granville	25	313	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	316
	Orange	11	226	0	0	1	2	3	5	0	0	0	0	0	0	0	0	0	0	237
	Orange	114	466	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	467
	Orange	12	304	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	306
	Orange	126	6	0	0	0	52	0	0	0	0	0	0	4	0	0	0	0	0	62
	Orange	13	674	0	0	0	0	14	7	0	0	0	0	0	0	0	0	0	0	695
	Orange	136	970	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	971
	Orange	173	130	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130
	Orange	200	579	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	592
	Orange	242	69	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	72
	Orange	251	2916	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2916
	Orange	30	1446	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	1449
	Orange	4	188	0	0	3	2	12	8	0	0	0	0	0	0	0	0	0	0	213
	Orange	43	141	0	0	0	0	3	2	0	0	0	0	0	0	0	0	0	1	147
	Orange	53	637	0	0	0	0	15	30	0	0	0	0	9	0	0	0	0	0	691
	Orange	54	169	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	171
	Orange	55	316	0	0	0	0	9	2	0	0	0	0	0	0	0	0	0	0	327
	Orange	57	333	0	0	1	0	11	6	0	0	0	0	0	0	0	0	0	0	351
	Orange	6	1189	0	0	0	0	0	0	0	0	2	2	0	0	0	0	1	0	1194
	Orange	64	451	0	0	0	0	0	2	0	0	4	0	0	0	0	0	0	0	457
	Orange	66	146	0	0	0	0	7	2	0	0	0	0	0	0	0	0	0	0	155
	Orange	67	3375	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	3377
	Person	10	129	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	129
	Person	127	598	0	0	0	2	4	4	0	0	0	0	1	0	0	0	0	0	609
	Person	130	143	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	146
	Person	18	2113	0	0	17	8	22	23	0	0	27	0	6	0	0	0	0	0	2216
	Person	205	22	0	0	0	6	4	0	0	0	0	0	0	0	0	0	0	0	32
	Person	21	834	0	0	0	0	12	1	0	0	0	0	1	0	0	0	0	0	848
	Person	22	52	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	70
	Person	23	21	0	0	0	12	7	0	0	0	0	0	1	0	0	0	0	0	41

Basin	County	Bridge Number	*Elliptio complanata*	*Elliptio icterina*	*Elliptio sp. (lanceolate)*	*Fusconaia masoni*	*Pyganodon cataracta*	*Strophitus undulatus*	*Villosa constricta*	*Villosa delumbis*	*Villosa vaughaniana*	*Lampsilis cariosa*	*Lampsilis radiata*	*Lampsilis sp.*	*Alasmidonta undulata*	*Alasmidonta varicosa*	*Toxolasma pullus*	*Lasmigona subviridis*	*Utterbackia imbecillis*	Total Mussels
	Person	33	1587	0	0	4	6	9	12	0	0	4	0	3	0	0	0	0	0	1625
Neuse	Person	36	630	0	0	0	15	16	5	0	0	0	0	1	0	0	0	0	0	667
	Person	38	354	0	0	0	36	11	0	0	0	0	0	0	0	0	0	0	0	401
	Person	80	1770	0	0	1	2	10	55	0	0	0	0	1	0	0	0	0	0	1839
	Wake	119	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Cape Fear	Chatham	245	154	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	172
	Chatham	247	253	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	334
	Chatham	251	76	4	0	1	0	2	0	3	2	0	0	0	0	0	0	0	0	116
	Guilford	23	110	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	116
	Guilford	72	1069	0	0	0	6	0	4	16	8	0	0	0	0	0	0	0	0	1223
	Moore	12	539	5	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	596
	Moore	127	438	11	0	0	16	0	6	5	10	0	0	0	0	0	0	0	0	668
	Moore	173	534	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	605
	Moore	174	111	0	0	0	1	0	13	0	17	0	0	0	0	0	0	0	0	157
	Moore	184	86	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	114
	Moore	212	744	3	0	0	1	0	24	21	45	0	0	0	0	0	0	0	0	892
	Moore	225	790	0	0	0	0	0	6	1	10	0	0	0	0	0	0	0	0	879
	Moore	28	323	0	0	0	0	0	1	5	9	0	0	0	0	0	0	0	0	382
	Randolph	109	1233	4	0	0	0	0	7	2	24	0	0	0	0	0	0	0	0	1471
	Randolph	110	362	5	1	1	0	2	6	1	0	0	0	0	2	7	0	0	0	427
	Randolph	149	229	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	250
	Randolph	175	102	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	145
	Randolph	188	39	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	47
	Randolph	199	568	17	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	694
	Randolph	208	1088	55	0	1	2	19	12	23	8	0	0	0	0	0	0	0	0	1606
	Randolph	210	14	0	0	0	3	0	0	17	8	0	0	0	0	0	0	0	0	43
	Randolph	211	492	1	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	583
	Randolph	214	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	128
	Randolph	218	1080	4	0	0	0	0	0	7	1	1	0	0	0	0	6	0	0	1149
	Randolph	220	411	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	442
	Randolph	228	579	0	0	0	1	0	0	3	2	0	0	0	0	0	0	0	0	746
	Randolph	257	72	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	82
	Randolph	260	438	4	0	0	6	2	1	0	0	0	0	0	0	1	0	0	0	577
	Randolph	339	23	0	0	0	30	0	0	0	0	0	0	0	0	0	0	0	0	58
	Randolph	349	108	0	0	0	1	0	0	4	2	0	0	0	0	0	0	0	0	122
	Randolph	359	1670	6	0	0	0	0	0	3	1	0	0	0	0	0	2	0	0	1866
	Randolph	374	696	52	0	0	0	0	10	3	2	0	0	0	0	0	0	0	0	902
	Randolph	415	182	4	1	0	0	3	2	0	1	0	0	0	0	0	0	0	0	222
	Randolph	443	418	3	0	0	1	0	0	5	0	0	0	0	0	0	0	0	0	442
	Randolph	459	315	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	398
	Randolph	463	54	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	77

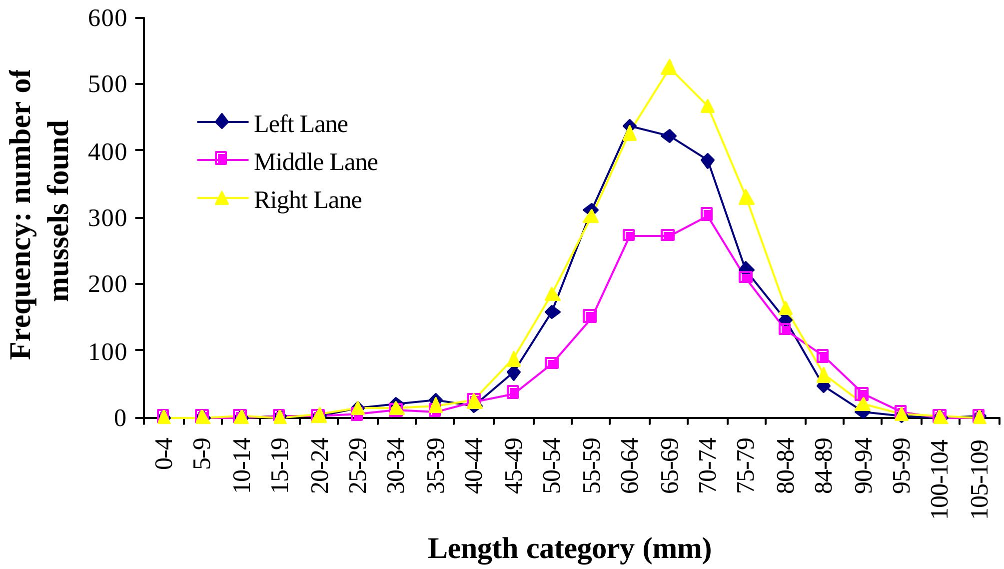
Basin	County	Bridge Number	*Elliptio complanata*	*Elliptio icterina*	*Elliptio sp. (lanceolate)*	*Fusconaia masoni*	*Pyganodon cataracta*	*Strophitus undulatus*	*Villosa constricta*	*Villosa delumbis*	*Villosa vaughaniana*	*Lampsilis cariosa*	*Lampsilis radiata*	*Lampsilis sp.*	*Alasmidonta undulata*	*Alasmidonta varicosa*	*Toxolasma pullus*	*Lasmigona subviridis*	*Utterbackia imbecillis*	Total Mussels
		TOTAL	42840	180	35	248	221	281	127	189	46	54	37	2	8	8	2	1	2	44281

Appendix II-8. The total number of each species found in the 24 cross-sections or under or within the crossing structures out of the 72 sites used for analysis. Distribution in the data of many of the more rare species was driven by mussel distribution at a small number of sites where they were most abundant.

Cross-section	*Elliptio complanata*	*Elliptio icterina*	*Elliptio sp. (lanceolate)*	*Fusconaia masoni*	*Pyganodon cataracta*	*Strophitus undulatus*	*Villosa constricta*	*Villosa delumbis*	*Villosa vaughaniana*	*Lampsilis cariosa*	*Lampsilis radiata*	*Lampsilis sp.*	*Alasmidonta varicosa*	*Lasmigona subviridis*	*Utterbackia imbecillis*
1	1997	2	0	2	3	12	11	1	8	0	1	2	1	0	0
2	1372	1	0	2	4	5	20	4	13	1	1	1	0	0	0
3	1225	0	0	0	4	15	12	2	7	0	0	2	0	0	0
4	1354	0	0	1	7	8	18	5	7	0	1	1	0	0	0
5	1191	3	0	4	13	6	5	7	19	0	2	2	0	0	0
6	1261	1	1	0	9	10	9	5	9	2	2	0	0	0	1
7	1314	0	0	0	8	10	7	0	5	2	2	1	0	0	0
8	1523	2	0	1	4	14	16	2	2	1	0	0	0	0	0
9	1588	1	0	2	8	9	4	8	12	3	0	1	0	0	0
10	1734	10	0	0	9	11	13	9	3	3	1	3	0	0	0
11	823	2	0	1	16	12	15	4	6	2	1	2	0	0	0
12	504	0	0	0	10	4	10	1	6	0	0	1	0	0	0
Road Crossing	543	0	0	6	5	7	1	1	1	4	0	0	0	0	0
13	1380	1	0	1	31	14	9	3	4	3	1	4	0	0	0
14	1538	6	0	0	16	15	17	5	7	3	3	2	0	0	0
15	2202	0	0	0	11	5	17	1	6	1	0	2	0	0	0
16	1535	5	0	2	7	8	3	1	5	1	2	1	0	1	0
17	2025	1	0	0	9	5	3	6	8	3	3	4	0	0	0
18	1305	4	0	0	11	3	5	0	2	2	4	1	0	0	0
19	1278	3	0	2	7	6	14	1	3	3	3	3	0	0	0
20	1408	2	0	1	8	5	7	0	5	2	7	1	0	0	0
21	2048	1	0	5	8	2	4	1	6	5	4	3	0	0	0
22	1439	0	0	2	11	2	3	2	3	2	5	0	0	0	0
23	1100	3	0	0	8	3	4	0	0	2	2	0	0	1	0
24	1465	2	0	0	5	5	8	2	3	0	9	0	0	0	0
Downstream Total	15886	22	1	13	95	116	140	48	97	14	11	16	1	0	1
Upstream Total	18723	28	0	13	132	73	94	22	52	27	43	21	0	2	0
Grand Total	**35152**	**50**	**1**	**32**	**232**	**196**	**235**	**71**	**150**	**45**	**54**	**37**	**1**	**2**	**1**



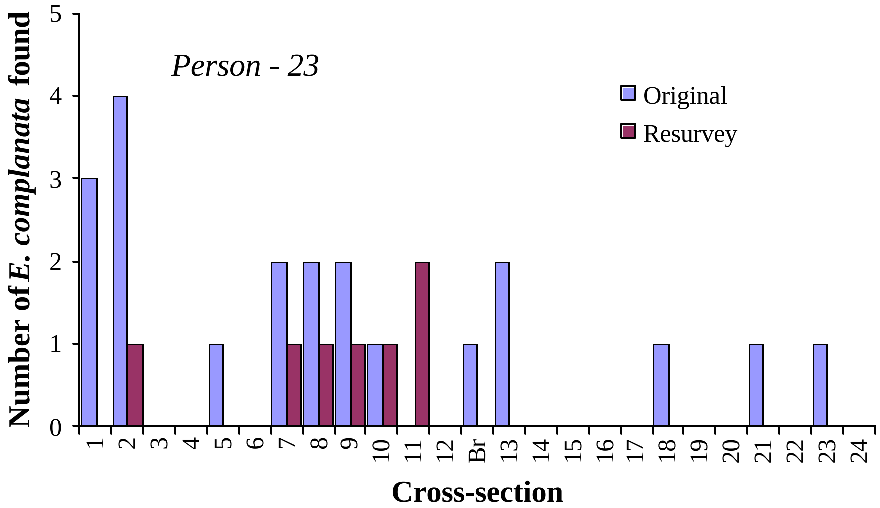
Appendix II-9. *Frequency histogram of the number of individuals found in different size categories in the Neuse study area in the three linear transects (left bank, middle, and right bank).*



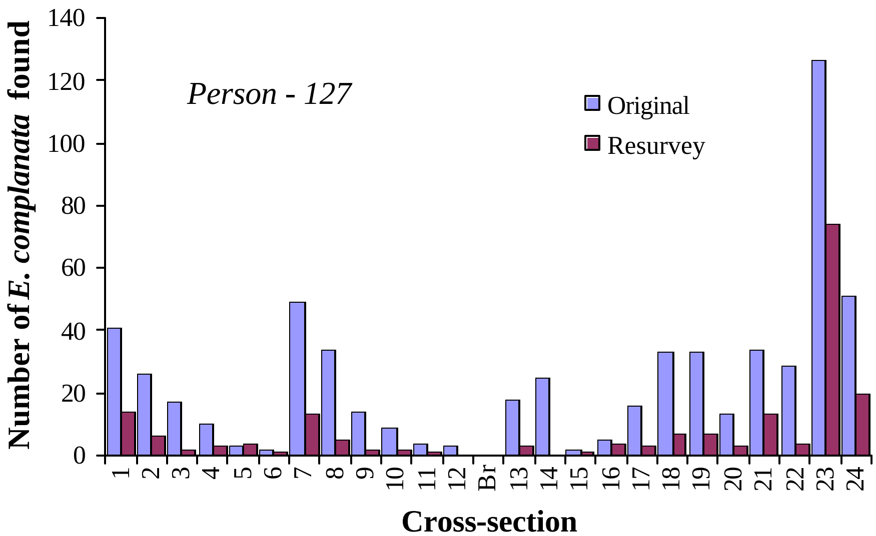
Appendix II-10. *Frequency histogram of the number of individuals found in different size categories in the Cape Fear study area in the three linear transects (left bank, middle, and right bank).*

Appendix II-11. Results of statistical tests analyzing mussel diversity in upstream versus downstream reaches and between 25-meter cross-sections.

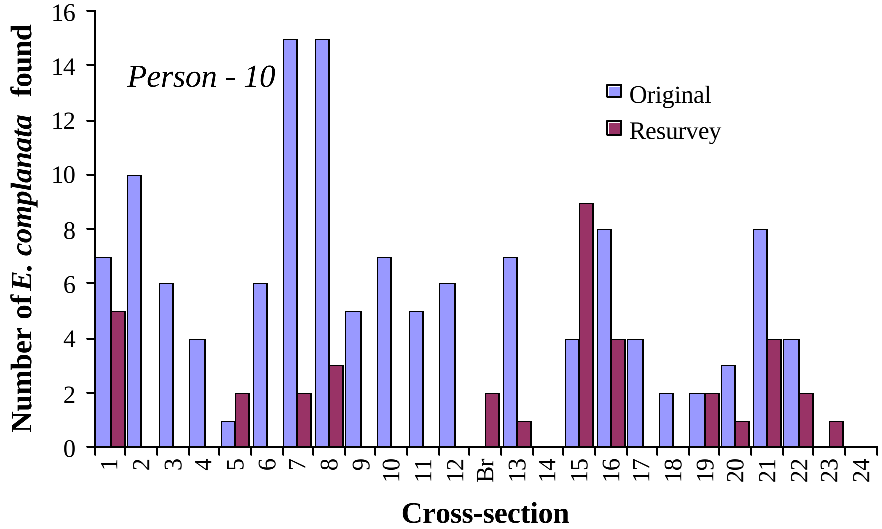
Parameter	**Comparison**	**Statistical Test**	***p*-value**
Shannon-Weiner Diversity Index	Upstream vs. Downstream	Signed-Rank	0.154
	Between cross-sections	Kruskal-Wallis	0.877
Number of Mussel Species other	Upstream vs. Downstream	Signed-Rank	0.786
than Elliptio	Between cross-sections	Kruskal-Wallis	0.546
Number of individuals of species	Upstream vs. Downstream	Signed-Rank	0.823
other than *Elliptio complanata*	Between cross-sections	Kruskal-Wallis	0.646



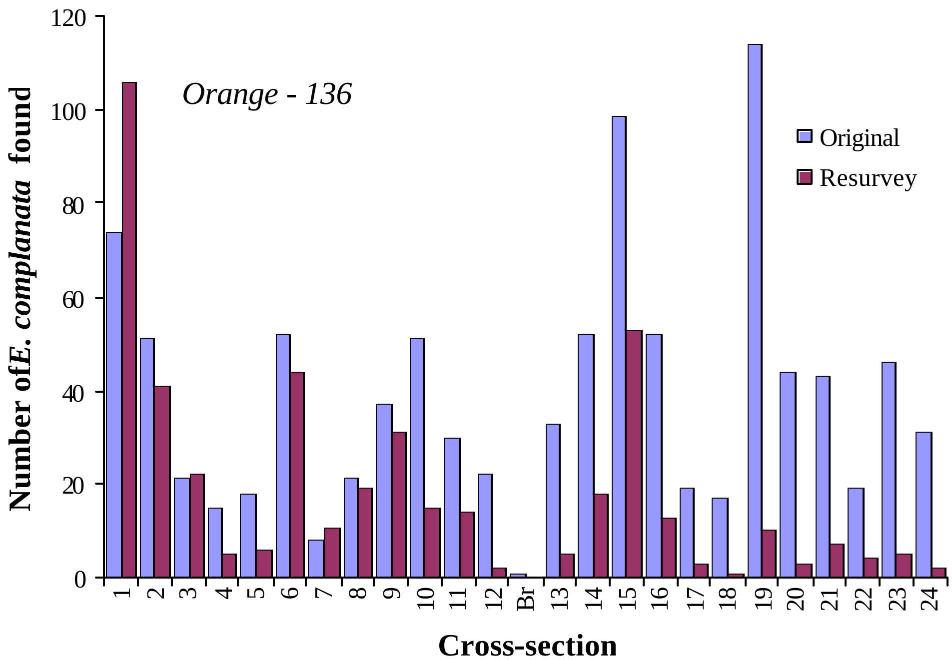
Appendix II-12. *Number of E. complanata found in each 25-meter cross-section in the original survey and resurvey at Person County bridge number 23 in the Neuse study area.*



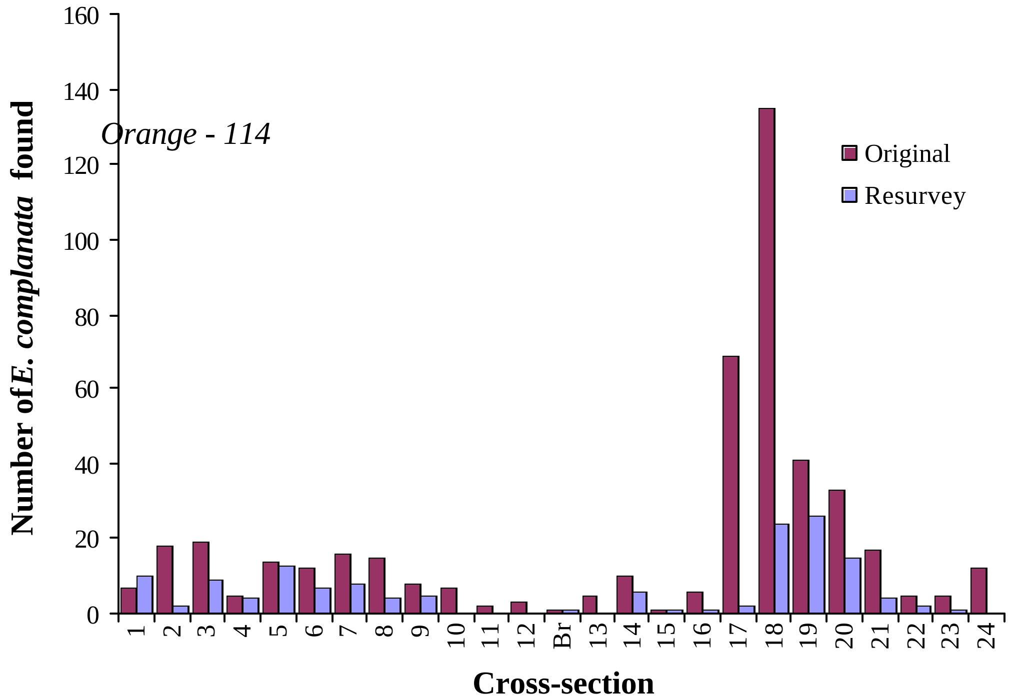
Appendix II-13. *Number of E. complanata found in each 25-meter cross-section in the original survey and resurvey at Person County bridge number 127 in the Neuse study area.*



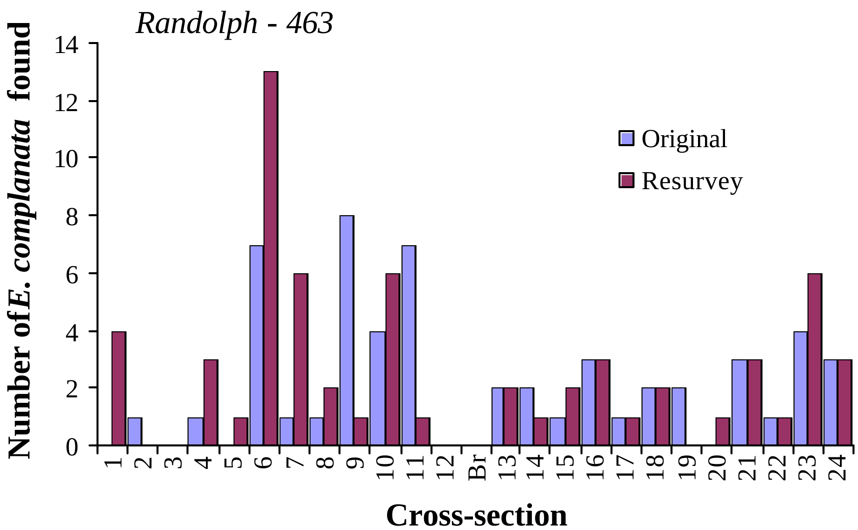
Appendix II-14. *Number of E. complanata found in each 25-meter cross-section in the original survey and resurvey at Person County bridge number 10 in the Neuse study area.*



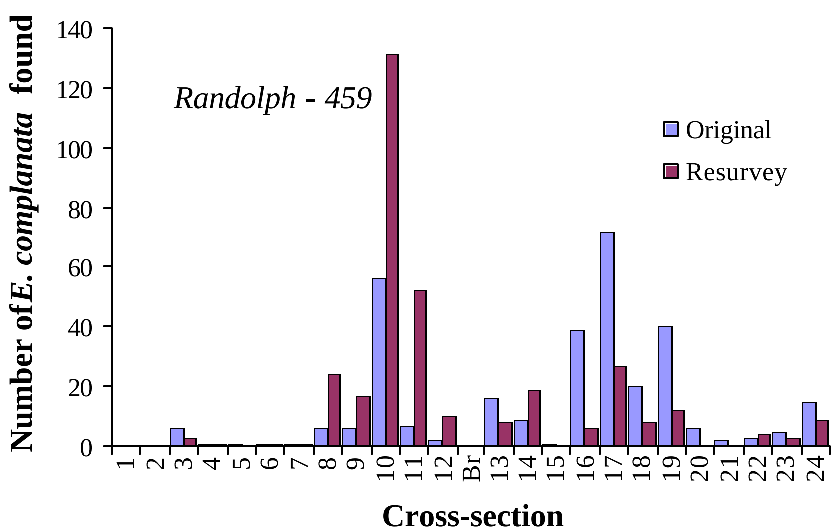
Appendix II-15. *Number of E. complanata found in each 25-meter cross-section in the original survey and resurvey at Orange County bridge number 136 in the Neuse study area.*



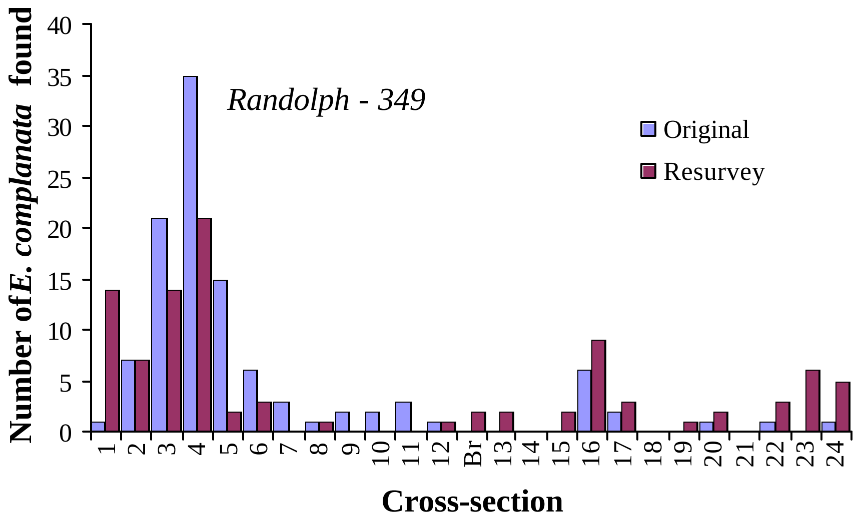
Appendix II-16. *Number of E. complanata found in each 25-meter cross-section in the original survey and resurvey at Orange County bridge number 114 in the Neuse study area.*



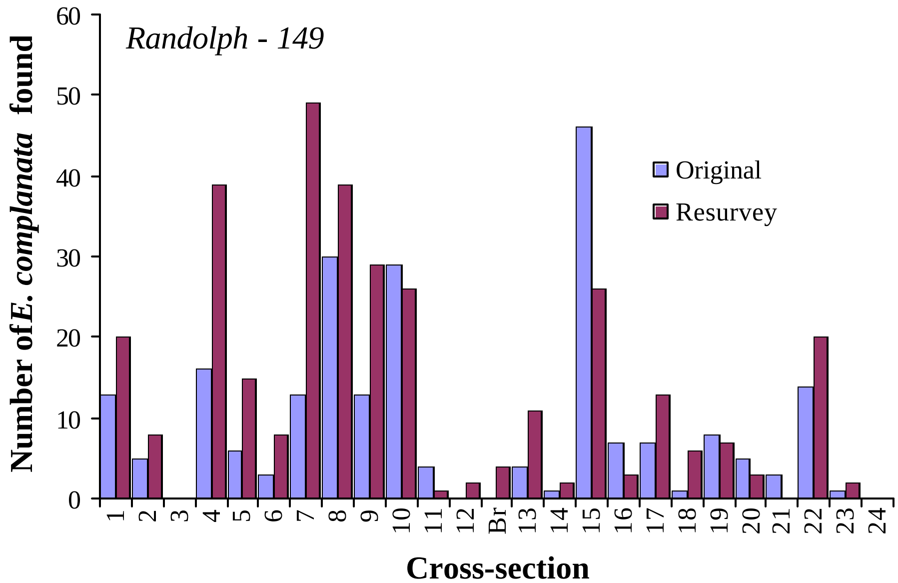
Appendix II-17. *Number of E. complanata found in each 25-meter cross-section in the original survey and resurvey at Randolph County bridge number 463 in the Cape Fear study area.*



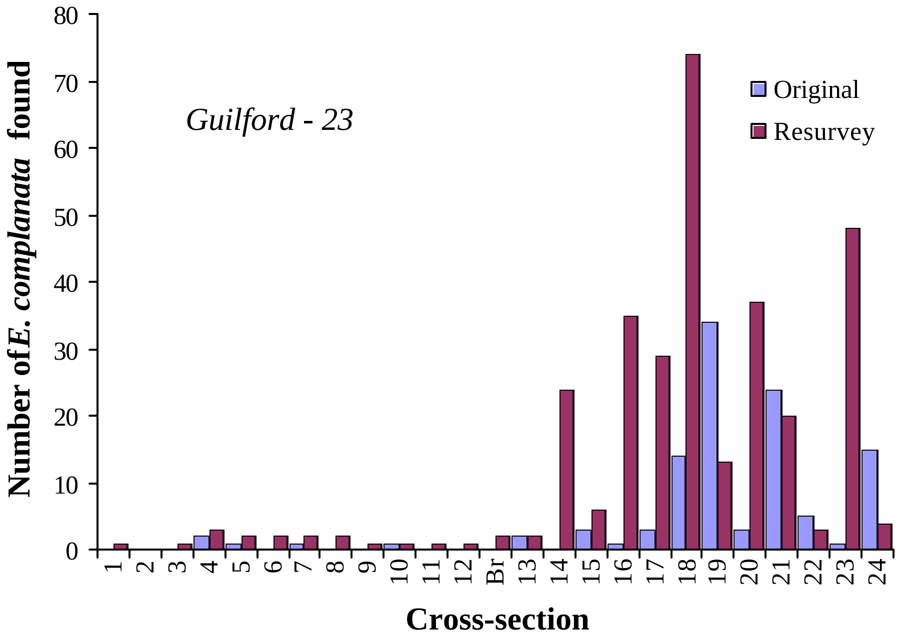
Appendix II-18. *Number of E. complanata found in each 25-meter cross-section in the original survey and resurvey at Randolph County bridge number 459 in the Cape Fear study area.*



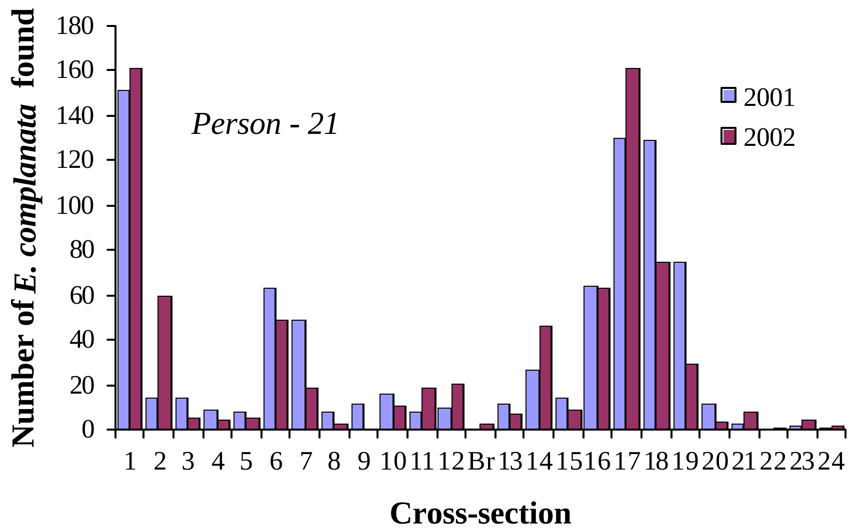
Appendix II-19. *Number of E. complanata found in each 25-meter cross-section in the original survey and resurvey at Randolph County bridge number 349 in the Cape Fear study area.*



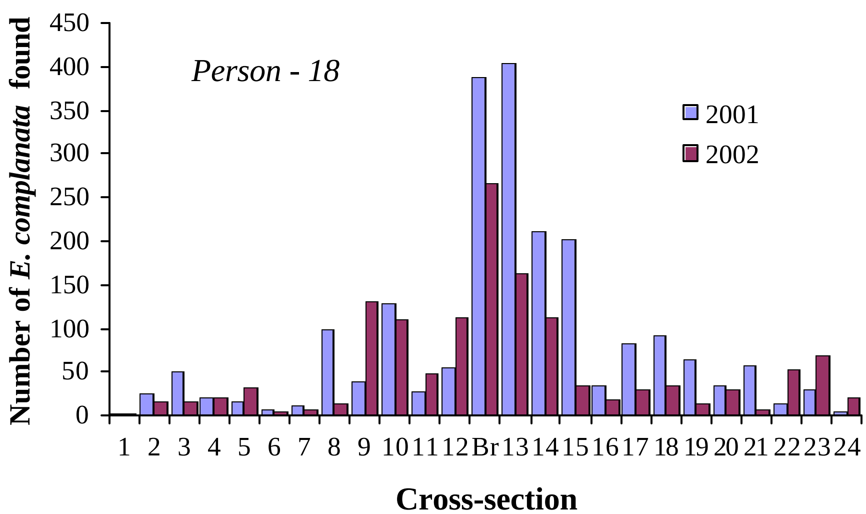
Appendix II-20. *Number of E. complanata found in each 25-meter cross-section in the original survey and resurvey at Randolph County bridge number 149 in the Cape Fear study area.*



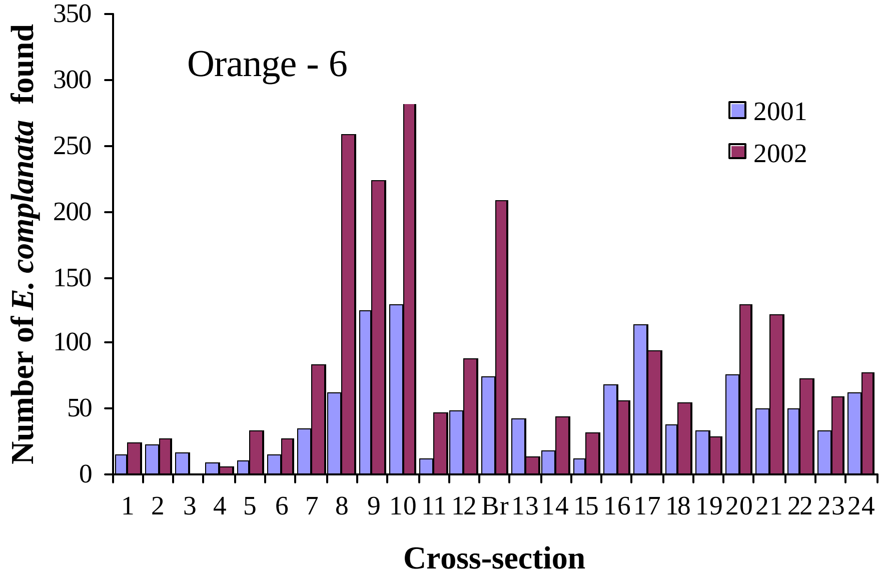
Appendix II-21. *Number of E. complanata found in each 25-meter cross-section in the original survey and resurvey at Guilford County bridge number 23 in the Cape Fear study area.*



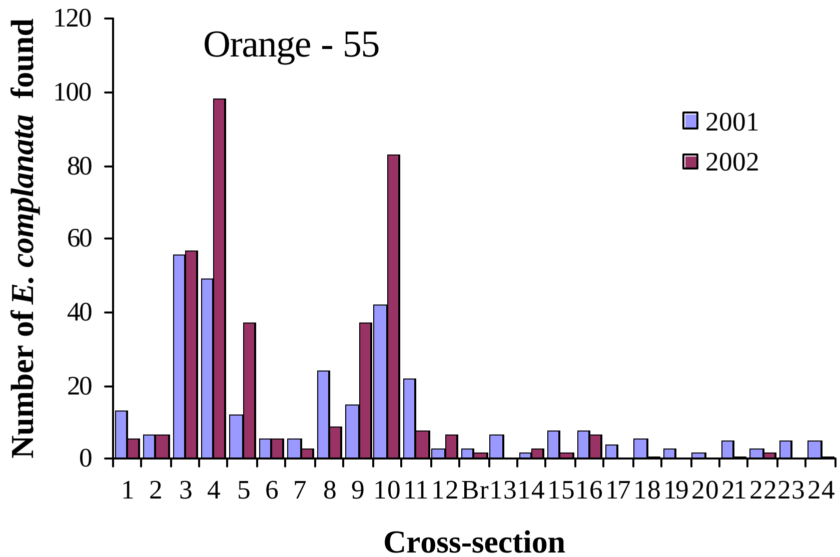
Appendix II-22. *Number of E. complanata found in each 25-meter cross-section in the original survey and resurvey one year later at Person County bridge number 21 in the Neuse study area.*



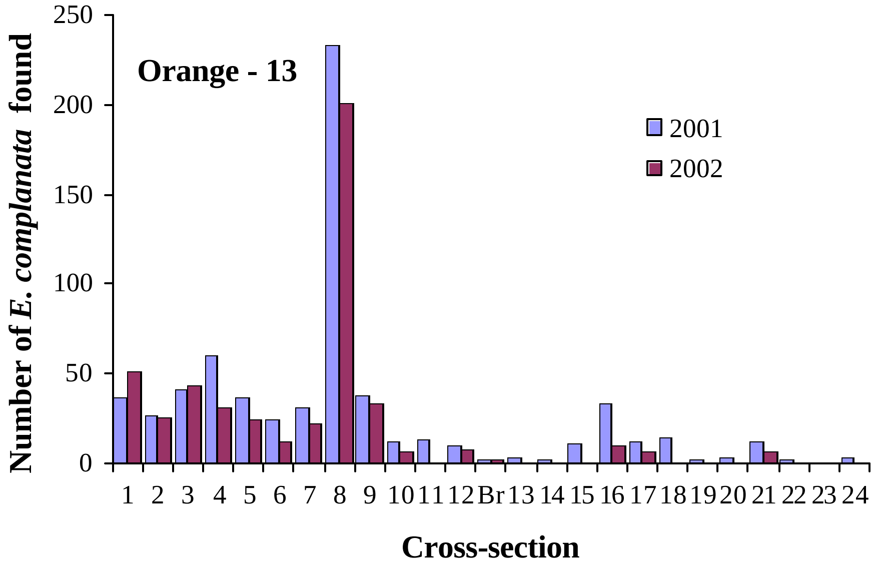
Appendix II-23. *Number of E. complanata found in each 25-meter cross-section in the original survey and resurvey one year later at Person County bridge number 18 in the Neuse study area.*



Appendix II-24. *Number of E. complanata found in each 25-meter cross-section in the original survey and resurvey one year later at Orange County bridge number 6 in the Neuse study area.*



Appendix II-25. *Number of E. complanata found in each 25-meter cross-section in the original survey and resurvey one year later at Orange County bridge number 55 in the Neuse study area.*



Appendix II-26. *Number of E. complanata found in each 25-meter cross-section in the original survey and resurvey one year later at Orange County bridge number 13 in the Neuse study area.*

Appendix III

Land Use Analysis Appendices

Appendix III-1. EPA's Neuse River Basin Land Use/Land Cover Classification

Code	Description
0	Unclassed
100	**Urban**
110	Urban - High Density
120	Urban - Medium Density
122	Urban - Medium - Agriculture
123	Urban - Medium - Woody
124	Urban - Medium - Herbaceous
125	Urban - Medium - Water
126	Urban - Medium - Wetland
127	Urban - Medium - Barren
128	Urban - Medium - Unknown/Other
130	Urban - Low Density
132	Urban - Low - Agriculture
133	Urban - Low - Woody
134	Urban - Low - Herbaceous
135	Urban - Low - Water
136	Urban - Low - Wetland
137	Urban - Low - Barren
138	Urban - Low - Unknown/Other
200	**Agriculture**
210	Agriculture - Row Crops
211	Agriculture - Row - Cotton
212	Agriculture - Row - Corn
213	Agriculture - Row - Soybean
214	Agriculture - Row - Tobacco
215	Agriculture - Row - Vegetables
216	Agriculture - Row - Other Row
217	Agriculture - Row - Wheat
218	Agriculture - Row - Other Grains
220	Agriculture - Pasture/Hay
230	Agriculture - Fallow
300	**Woody**
310	Woody - Deciduous
320	Woody - Evergreen
330	Woody - Mixed
400	**Herbaceous**
410	Herbaceous - Natural
420	Herbaceous - Maintained
500	**Water**
510	Water - Streams/Rivers
520	Water - Lakes
530	Water - Reservoirs
540	Water - Estuaries
550	Water - Ponds
600	**Wetlands**
610	Wetlands - Herbaceous
620	Wetlands - Woody
700	**Barren**
710	Barren - Non-vegetated
720	Barren - Transitional

Appendix III-2. Neuse study area upstream catchment land use (%) row crop agriculture (RC Ag.); non-row crop agriculture (NRC Ag.); total agriculture includes row crop and non-row crop agriculture (Ttl. Ag.).

Site	RC Ag.	NRC Ag.	Ttl Ag.	Urban	Forest	Other
1	10.6%	32.2%	42.7%	11.7%	44.1%	1.0%
2	11.0%	25.9%	36.9%	6.6%	54.4%	2.1%
3	7.5%	27.0%	34.5%	6.5%	57.9%	3.4%
4	6.0%	31.9%	38.0%	6.3%	52.4%	1.5%
5	6.5%	27.6%	34.2%	6.2%	57.5%	0.2%
6	2.3%	14.9%	17.1%	13.3%	67.9%	2.1%
7	2.8%	20.3%	23.1%	12.2%	63.4%	1.6%
8	1.9%	20.4%	22.3%	12.6%	64.5%	1.2%
9	3.9%	21.8%	25.7%	10.6%	62.3%	0.6%
10	2.7%	21.4%	24.1%	12.3%	63.4%	1.4%
11	2.1%	23.3%	25.4%	13.8%	60.7%	1.2%
12	3.4%	20.6%	24.0%	12.7%	62.1%	0.2%
13	2.3%	16.6%	19.0%	16.8%	63.2%	1.3%
14	6.4%	31.7%	38.1%	7.9%	53.4%	0.8%
15	6.8%	29.7%	36.4%	6.6%	56.5%	0.5%
16	8.0%	20.3%	28.3%	2.5%	69.0%	0.7%
17	7.3%	20.8%	28.1%	3.4%	68.2%	0.2%
18	6.6%	26.5%	33.1%	7.4%	59.1%	1.3%
19	7.4%	25.9%	33.3%	7.2%	59.1%	1.4%
20	7.2%	25.2%	32.4%	7.4%	59.8%	0.5%
21	6.7%	28.5%	35.1%	17.1%	47.4%	0.4%
22	8.0%	24.3%	32.3%	14.4%	52.7%	0.5%
23	4.3%	24.0%	28.3%	9.1%	61.3%	0.6%
24	8.4%	22.1%	30.5%	8.9%	59.2%	0.3%
25	7.1%	23.9%	31.0%	9.9%	58.6%	0.5%
26	6.4%	17.6%	24.0%	7.1%	67.7%	0.4%
27	7.1%	23.8%	30.9%	9.9%	58.7%	0.4%
28	6.8%	21.9%	28.6%	9.1%	61.5%	0.3%
29	6.6%	22.0%	28.6%	9.1%	61.5%	1.2%
30	8.0%	26.4%	34.4%	4.8%	59.4%	0.6%
31	6.5%	26.7%	33.2%	4.2%	61.5%	0.9%
32	3.8%	23.1%	26.9%	8.1%	63.3%	0.9%
33	5.9%	29.8%	35.6%	4.4%	59.2%	0.5%
34	5.1%	28.1%	33.1%	5.4%	60.5%	1.2%
35	4.7%	26.0%	30.7%	5.7%	62.7%	1.7%
36	4.6%	25.8%	30.5%	5.8%	62.9%	1.0%
37	7.6%	25.2%	32.8%	5.1%	60.8%	1.4%
38	7.4%	25.5%	32.9%	5.3%	60.5%	1.4%
39	6.3%	24.1%	30.4%	5.4%	63.2%	1.0%
40	4.5%	22.6%	27.1%	6.7%	65.1%	1.3%
41	2.0%	31.8%	33.8%	8.6%	57.0%	0.8%
42	1.8%	32.1%	34.0%	8.6%	56.9%	1.0%
43	2.0%	5.3%	7.3%	8.4%	81.6%	1.9%
44	4.1%	8.4%	12.5%	6.7%	78.8%	2.7%

Appendix III-3. Neuse study area upstream 250-meter riparian buffer land use (%) row crop agriculture (RC Ag.); non-row crop agriculture (NRC Ag.); total agriculture includes row crop and non-row crop agriculture (Ttl. Ag.).

Site	RC Ag.	NRC Ag.	Ttl. Ag.	Urban	Forest
1	10.5%	22.4%	32.8%	13.7%	51.5%
2	8.1%	20.6%	28.7%	5.8%	61.4%
3	5.2%	21.8%	27.1%	6.1%	64.4%
4	5.6%	19.1%	24.7%	6.0%	62.5%
5	5.3%	19.1%	24.4%	5.9%	65.4%
6	2.5%	10.3%	12.8%	9.7%	73.6%
7	2.5%	16.4%	18.9%	9.3%	69.1%
8	2.2%	21.3%	23.5%	8.7%	66.6%
9	3.2%	16.8%	20.0%	8.8%	68.6%
10	3.3%	26.9%	30.1%	9.1%	60.5%
11	2.7%	26.7%	29.3%	10.8%	59.7%
12	2.8%	17.2%	20.0%	10.5%	67.2%
13	2.0%	14.2%	16.1%	14.0%	68.0%
14	5.0%	25.0%	30.0%	5.8%	63.4%
15	5.8%	24.1%	29.9%	4.9%	64.4%
16	5.2%	14.9%	20.1%	1.8%	77.3%
17	4.8%	16.1%	20.8%	1.8%	76.7%
18	6.0%	23.1%	29.2%	5.7%	64.4%
19	7.2%	22.5%	29.6%	5.8%	63.9%
20	7.0%	21.7%	28.7%	6.2%	64.3%
21	6.7%	27.4%	34.1%	13.1%	52.3%
22	7.1%	21.9%	29.0%	11.8%	58.1%
23	4.5%	21.1%	25.6%	8.6%	63.1%
24	8.4%	20.2%	28.6%	8.9%	59.6%
25	6.8%	21.4%	28.3%	8.5%	62.3%
26	7.0%	17.1%	24.1%	7.1%	66.3%
27	6.8%	21.3%	28.1%	8.5%	62.4%
28	6.7%	19.9%	26.6%	8.0%	64.1%
29	6.5%	20.1%	26.6%	8.1%	63.9%
30	7.3%	20.7%	28.0%	2.7%	66.4%
31	5.7%	21.8%	27.4%	2.9%	67.6%
32	4.1%	21.0%	25.1%	6.2%	64.7%
33	5.1%	26.0%	31.1%	3.2%	64.2%
34	4.4%	24.8%	29.2%	4.1%	64.6%
35	4.1%	22.7%	26.7%	4.5%	66.9%
36	4.0%	22.6%	26.6%	4.6%	67.0%
37	7.1%	22.3%	29.4%	3.6%	64.4%
38	6.7%	22.7%	29.4%	4.0%	64.1%
39	5.6%	20.7%	26.3%	4.4%	67.5%
40	4.2%	19.4%	23.6%	5.5%	68.7%
41	1.0%	35.2%	36.2%	7.0%	55.6%
42	1.1%	35.6%	36.8%	7.1%	55.0%
43	2.6%	3.4%	6.0%	3.8%	84.3%
44	4.0%	6.8%	10.8%	3.1%	81.9%

Appendix III-4. Neuse study area upstream 100-meter riparian buffer land use (%) row crop agriculture (RC Ag.); non-row crop agriculture (NRC Ag.); total agriculture includes row crop and non-row crop agriculture (Ttl. Ag.).

Site	RC Ag.	NRC Ag.	Ttl. Ag.	Urban	Forest
1	8.8%	14.3%	23.1%	13.9%	60.8%
2	5.7%	12.3%	18.0%	4.8%	68.5%
3	4.0%	15.9%	20.0%	6.1%	68.8%
4	4.5%	11.4%	15.9%	5.5%	65.0%
5	3.9%	12.9%	16.9%	5.6%	69.0%
6	3.4%	7.7%	11.1%	8.4%	71.9%
7	2.3%	8.2%	10.5%	6.7%	76.8%
8	1.8%	17.1%	18.9%	8.4%	70.0%
9	2.4%	11.2%	13.6%	7.1%	74.3%
10	1.9%	21.4%	23.3%	7.6%	68.8%
11	1.6%	22.7%	24.3%	9.7%	65.8%
12	2.1%	12.0%	14.1%	8.8%	72.9%
13	1.5%	10.4%	11.9%	11.6%	73.1%
14	3.1%	15.5%	18.6%	3.7%	75.9%
15	3.8%	15.8%	19.6%	3.1%	75.8%
16	4.0%	12.9%	17.0%	0.7%	80.2%
17	3.5%	12.7%	16.2%	0.6%	81.4%
18	4.0%	15.5%	19.5%	3.9%	75.1%
19	4.5%	15.3%	19.8%	4.2%	74.7%
20	4.3%	14.7%	19.0%	4.6%	75.0%
21	5.4%	23.5%	28.9%	10.7%	59.5%
22	4.7%	15.8%	20.5%	9.9%	67.3%
23	2.9%	13.5%	16.4%	8.4%	68.9%
24	4.6%	12.7%	17.3%	9.5%	66.8%
25	4.4%	14.7%	19.2%	6.9%	72.0%
26	3.9%	10.4%	14.3%	7.1%	73.0%
27	4.4%	14.7%	19.1%	6.9%	72.1%
28	4.3%	13.3%	17.6%	6.8%	72.6%
29	4.1%	13.7%	17.8%	7.0%	72.2%
30	5.4%	13.5%	18.9%	1.7%	73.4%
31	3.9%	14.1%	18.0%	2.3%	75.4%
32	1.8%	14.4%	16.2%	4.8%	69.5%
33	3.5%	18.3%	21.8%	2.7%	72.4%
34	2.8%	17.4%	20.2%	3.1%	72.4%
35	2.5%	15.4%	17.9%	3.2%	75.1%
36	2.4%	15.5%	17.9%	3.3%	75.1%
37	4.8%	16.0%	20.8%	2.2%	71.3%
38	4.5%	16.1%	20.7%	2.5%	71.4%
39	3.7%	14.3%	18.0%	3.2%	74.6%
40	2.6%	13.0%	15.7%	4.2%	75.6%
41	0.7%	25.3%	26.0%	8.1%	63.1%
42	0.6%	24.7%	25.3%	8.0%	64.2%
43	2.0%	2.0%	4.0%	2.5%	80.4%
44	1.7%	4.4%	6.2%	2.1%	83.1%

Appendix III-5. Neuse study area local 250-meter riparian buffer land use (%) row crop agriculture (RC Ag.); non-row crop agriculture (NRC Ag.); total agriculture includes row crop and non-row crop agriculture (Ttl. Ag.).

Site	RC Ag.	NRC Ag.	Ttl. Ag.	Urban	Forest
1	10.4%	11.8%	22.1%	3.5%	69.8%
2	1.1%	14.6%	15.7%	6.2%	78.1%
3	0.0%	18.8%	18.8%	2.5%	78.7%
4	2.7%	11.2%	14.0%	8.1%	77.7%
5	4.7%	6.3%	11.0%	8.9%	79.8%
6	0.0%	6.5%	6.5%	5.4%	87.1%
7	1.8%	4.8%	6.6%	3.5%	89.9%
8	5.0%	13.9%	18.8%	12.2%	68.8%
9	0.0%	14.9%	14.9%	53.2%	31.4%
10	2.0%	64.5%	66.5%	10.6%	22.9%
11	0.0%	6.9%	6.9%	38.8%	53.9%
12	1.6%	6.9%	8.5%	22.0%	68.2%
13	0.0%	0.0%	0.0%	29.2%	69.8%
14	0.0%	22.8%	22.8%	12.4%	64.8%
15	0.8%	12.8%	13.6%	1.7%	82.6%
16	2.3%	11.7%	14.0%	2.7%	83.2%
17	1.3%	22.7%	23.9%	2.0%	74.1%
18	26.5%	21.4%	47.9%	2.5%	48.7%
19	0.0%	4.2%	4.2%	7.5%	88.3%
20	7.6%	13.8%	21.4%	17.1%	61.5%
21	4.0%	13.3%	17.3%	9.2%	70.3%
22	0.7%	12.3%	13.0%	2.6%	82.5%
23	3.7%	20.2%	23.8%	6.0%	68.0%
24	5.4%	11.2%	16.6%	10.9%	56.9%
25	0.0%	3.5%	3.5%	11.3%	82.1%
26	13.1%	14.7%	27.8%	8.7%	63.6%
27	1.0%	18.0%	19.0%	5.4%	73.0%
28	3.4%	18.5%	21.9%	24.4%	53.7%
29	0.0%	3.9%	3.9%	8.8%	85.3%
30	0.9%	24.7%	25.5%	0.2%	69.7%
31	7.2%	9.3%	16.5%	11.1%	72.4%
32	0.0%	42.1%	42.1%	9.7%	46.3%
33	3.8%	19.6%	23.4%	6.6%	67.6%
34	0.0%	10.3%	10.3%	3.7%	85.6%
35	0.0%	0.2%	0.2%	5.2%	94.6%
36	2.8%	29.5%	32.3%	11.2%	56.5%
37	0.0%	13.1%	13.1%	5.2%	81.7%
38	0.0%	43.6%	43.6%	17.5%	38.2%
39	4.6%	22.5%	27.1%	10.0%	62.4%
40	0.0%	3.7%	3.7%	11.5%	84.3%
41	0.0%	40.4%	40.4%	0.9%	58.7%
42	0.0%	31.3%	31.3%	9.1%	59.6%
43	0.0%	0.0%	0.0%	0.0%	75.1%
44	7.4%	20.1%	27.4%	5.0%	40.3%

Appendix III-6. Neuse study area local 100-meter riparian buffer land use (%) row crop agriculture (RC Ag.); non-row crop agriculture (NRC Ag.); total agriculture includes row crop and non-row crop agriculture (Ttl. Ag.).

Site	RC Ag.	NRC Ag.	Ttl. Ag.	Urban	Forest
1	0.0%	8.5%	8.5%	2.5%	75.7%
2	0.0%	1.0%	1.0%	8.3%	90.7%
3	0.0%	8.5%	8.5%	2.7%	88.8%
4	0.3%	2.2%	2.5%	5.4%	92.1%
5	0.8%	8.6%	9.5%	9.5%	81.1%
6	0.0%	6.7%	6.7%	0.0%	93.3%
7	0.0%	0.0%	0.0%	0.0%	100.0%
8	2.2%	9.4%	11.6%	3.7%	84.0%
9	0.0%	11.6%	11.6%	40.5%	47.9%
10	0.0%	63.9%	63.9%	5.9%	30.3%
11	0.0%	4.1%	4.1%	54.2%	41.6%
12	0.0%	0.0%	0.0%	24.9%	71.5%
13	0.0%	0.0%	0.0%	45.8%	54.2%
14	0.0%	24.7%	24.7%	10.7%	64.6%
15	1.2%	6.8%	8.0%	0.0%	88.8%
16	1.0%	2.5%	3.5%	0.0%	96.5%
17	0.3%	12.8%	13.1%	0.0%	86.9%
18	9.6%	14.8%	24.3%	0.0%	75.7%
19	0.0%	0.8%	0.8%	6.6%	92.5%
20	8.9%	11.2%	20.1%	16.8%	63.1%
21	2.8%	6.5%	9.3%	0.0%	84.5%
22	0.0%	5.6%	5.6%	1.7%	89.5%
23	0.3%	8.0%	8.3%	7.3%	78.2%
24	1.5%	2.4%	3.9%	2.4%	63.5%
25	0.0%	0.0%	0.0%	18.4%	73.3%
26	5.4%	12.9%	18.3%	9.6%	72.1%
27	0.0%	10.5%	10.5%	8.3%	75.4%
28	0.2%	3.0%	3.2%	25.5%	71.4%
29	0.0%	1.7%	1.7%	18.5%	74.0%
30	1.2%	3.5%	4.7%	0.5%	86.2%
31	0.0%	4.2%	4.2%	19.0%	76.8%
32	0.0%	6.4%	6.4%	4.4%	83.6%
33	0.0%	0.3%	0.3%	1.2%	98.5%
34	0.0%	34.8%	34.8%	5.3%	54.5%
35	0.0%	0.5%	0.5%	0.0%	99.5%
36	0.0%	26.8%	26.8%	14.5%	58.7%
37	0.0%	2.7%	2.7%	0.2%	97.1%
38	0.0%	41.9%	41.9%	14.7%	41.5%
39	2.4%	5.8%	8.1%	17.0%	73.7%
40	0.0%	0.2%	0.2%	15.1%	83.9%
41	0.0%	14.7%	14.7%	0.0%	85.3%
42	0.0%	10.0%	10.0%	13.2%	76.8%
43	0.0%	0.0%	0.0%	0.0%	49.3%
44	10.6%	11.1%	21.7%	10.6%	25.4%

Appendix III-7. Neuse study area mean water chemistry values.

Site	pH	DO (mg/L)	Temp. (°C)	Cond. (°mS)	Turb. (NTU)	Road Den. (km/km^2)	Slope (%)	Drain. Area
1	7.4	6.9	21.3	97.5	---	6.3	6.1	8.9
2	6.6	3.5	22.4	105.0	20.0	4.9	8.6	13.6
3	7.0	5.1	25.4	67.3	5.6	3.6	6.8	28.1
4	7.1	4.5	25.8	82.9	9.3	4.3	7.4	28.7
5	7.2	5.8	24.5	90.0	25.8	3.6	4.0	66.0
6	7.2	5.9	19.6	88.8	7.7	6.5	4.4	8.9
7	7.5	7.3	20.4	82.9	7.1	6.0	5.2	11.4
8	7.0	4.4	24.8	78.7	10.4	6.5	4.1	21.3
9	7.3	6.4	24.9	81.6	12.9	3.7	6.2	164.0
10	7.3	6.7	20.7	145.8	12.0	5.4	5.1	17.6
11	7.4	8.3	18.7	104.2	11.5	5.3	3.8	24.1
12	7.4	4.1	25.7	152.0	7.3	3.8	5.0	208.4
13	7.6	7.7	28.5	129.6	4.5	3.5	5.3	331.8
14	7.3	8.9	18.4	88.4	22.0	2.3	2.2	44.5
15	7.1	6.5	23.5	85.7	1.5	2.5	3.2	69.2
16	7.2	7.6	21.9	74.8	20.0	4.9	2.4	9.3
17	7.3	6.2	24.9	84.0	19.5	4.6	2.9	11.4
18	7.0	5.5	25.1	107.0	17.1	2.4	2.6	112.0
19	7.3	6.7	23.8	107.7	18.8	2.4	4.1	131.5
20	6.9	4.6	24.2	89.7	23.8	2.4	6.0	141.9
21	6.9	6.5	20.1	70.7	21.3	2.3	2.3	15.2
22	7.0	6.1	25.7	95.6	13.5	2.5	3.0	83.5
23	8.0	9.5	15.3	55.7	11.5	2.3	2.9	23.2
24	7.5	8.8	21.5	69.5	7.3	2.1	3.0	50.6
25	7.6	7.0	28.8	10.1	12.5	2.5	15.9	262.1
26	7.4	7.6	23.3	58.2	12.0	1.7	5.8	80.7
27	7.3	7.2	28.7	95.5	13.3	2.5	5.6	264.4
28	7.3	6.8	28.8	80.4	14.5	2.2	8.4	359.9
29	7.7	6.6	28.5	88.6	12.2	2.2	4.1	370.7
30	7.1	6.9	16.9	74.9	9.6	3.8	3.8	30.0
31	6.9	6.1	23.7	77.9	9.8	3.6	3.3	41.3
32	7.1	7.8	18.3	88.4	12.9	4.9	1.2	19.1
33	7.0	6.6	23.4	103.5	8.1	3.5	1.9	58.5
34	7.5	6.0	23.5	95.6	16.5	3.8	2.0	87.6
35	7.7	7.0	25.2	100.6	8.6	3.9	3.3	99.0
36	7.4	7.0	27.1	91.6	6.1	3.9	3.2	101.0
37	7.2	5.6	18.7	78.2	12.5	2.2	3.6	34.8
38	7.1	5.2	23.4	84.0	17.7	2.2	2.7	37.0
39	6.7	5.0	25.3	81.6	25.4	2.0	4.7	53.2
40	7.5	7.6	21.5	78.9	5.0	2.9	11.0	202.7
41	7.4	6.3	23.8	100.1	11.6	3.4	5.0	19.8
42	7.5	8.6	18.3	89.6	5.9	3.4	4.4	21.4
43	7.3	7.1	22.8		18.6	4.8	3.8	15.9
44	7.3	7.8	24.4	60.3	17.1	1.2	3.5	25.4

Appendix III-8. Land use in upstream watershed at sites in the Cape Fear study area.

Site	Forest	Urban	Agriculture	Other
18245	73.8%	0.5%	23.6%	2.0%
18247	81.2%	0.9%	15.9%	2.1%
18251	67.4%	0.5%	30.9%	1.2%
40023	59.3%	11.8%	26.8%	2.1%
40072	59.5%	11.2%	27.5%	1.9%
62012	86.4%	0.2%	6.9%	6.5%
62028	91.9%	0.0%	2.4%	5.7%
62127	82.9%	0.4%	16.2%	0.6%
62173	82.8%	1.5%	13.5%	2.2%
62174	85.0%	0.5%	14.2%	0.3%
62184	80.1%	0.9%	18.7%	0.4%
62212	92.3%	0.0%	2.5%	5.2%
62225	83.4%	1.1%	11.4%	4.1%
75109	62.6%	6.5%	29.3%	1.6%
75110	72.1%	0.9%	25.8%	1.2%
75149	78.9%	0.1%	19.8%	1.1%
75175	70.9%	5.1%	22.5%	1.6%
75188	65.2%	2.7%	30.6%	1.4%
75199	76.7%	4.4%	18.0%	0.9%
75208	80.0%	0.2%	17.5%	2.2%
75210	75.4%	0.0%	23.7%	0.9%
75211	81.7%	0.4%	13.9%	4.0%
75213	76.1%	6.4%	16.5%	0.9%
75214	82.3%	0.1%	16.3%	1.4%
75218	76.4%	7.6%	14.9%	1.0%
75220	85.9%	0.0%	11.5%	2.5%
75228	84.0%	3.0%	11.7%	1.3%
75257	60.4%	30.9%	7.9%	0.8%
75260	68.4%	1.4%	29.0%	1.2%
75339	83.9%	0.5%	13.0%	2.6%
75349	83.0%	0.0%	16.7%	0.3%
75359	76.8%	7.1%	15.1%	1.0%
75374	62.7%	2.1%	33.4%	1.8%
75415	77.9%	0.2%	19.9%	2.0%
75443	61.9%	3.5%	32.5%	2.2%
75459	57.5%	1.3%	39.8%	1.5%
75463	50.8%	1.8%	46.1%	1.3%

Appendix III-9. Local Riparian Buffer Land Use Types (250-meter width) in the Cape Fear study area.

Site	Forest	Agriculture	Urban	Other
18245	93.4%	5.7%	0.0%	0.9%
18247	96.3%	0.0%	0.0%	3.7%
18251	35.0%	65.0%	0.0%	0.0%
40023	71.7%	18.7%	0.0%	9.6%
40072	84.5%	15.5%	0.0%	0.0%
62012	90.4%	3.7%	0.0%	6.0%
62028	91.2%	8.8%	0.0%	0.0%
62127	74.5%	25.0%	0.0%	0.5%
62173	84.4%	6.0%	0.0%	9.6%
62174	86.2%	12.4%	0.0%	1.4%
62184	89.8%	10.2%	0.0%	0.0%
62212	99.1%	0.9%	0.0%	0.0%
62225	92.3%	7.7%	0.0%	0.0%
75109	92.2%	7.8%	0.0%	0.0%
75110	86.9%	10.7%	0.0%	2.3%
75149	71.7%	26.4%	0.5%	1.4%
75175	67.6%	30.1%	0.0%	2.3%
75188	46.3%	52.8%	0.0%	0.9%
75199	85.8%	14.2%	0.0%	0.0%
75208	93.0%	7.0%	0.0%	0.0%
75210	66.7%	33.3%	0.0%	0.0%
75211	72.6%	27.4%	0.0%	0.0%
75213	66.5%	32.1%	0.0%	1.4%
75214	47.7%	51.9%	0.0%	0.5%
75218	72.0%	27.1%	0.0%	0.9%
75220	66.4%	33.6%	0.0%	0.0%
75228	49.3%	50.7%	0.0%	0.0%
75257	70.8%	29.2%	0.0%	0.0%
75260	72.7%	27.3%	0.0%	0.0%
75339	85.4%	6.8%	0.0%	7.8%
75349	87.7%	11.8%	0.0%	0.5%
75359	70.8%	27.4%	0.0%	1.8%
75374	77.2%	22.8%	0.0%	0.0%
75415	96.2%	3.8%	0.0%	0.0%
75443	59.8%	31.5%	0.0%	8.7%
75459	52.1%	29.5%	17.5%	0.9%
75463	69.7%	28.0%	2.3%	0.0%

Appendix III-10. Local riparian buffer land use in the Cape Fear study area (100-meter width)

Site	Forest	Agriculture	Urban	Other
18245	100.0%	0.0%	0.0%	0.0%
18247	94.3%	0.0%	0.0%	5.7%
18251	80.0%	20.0%	0.0%	0.0%
40023	80.6%	8.3%	0.0%	11.1%
40072	96.9%	3.1%	0.0%	0.0%
62012	88.9%	0.0%	0.0%	11.1%
62028	94.3%	5.7%	0.0%	0.0%
62127	85.7%	14.3%	0.0%	0.0%
62173	82.9%	2.9%	0.0%	14.3%
62174	100.0%	0.0%	0.0%	0.0%
62184	94.1%	5.9%	0.0%	0.0%
62212	97.1%	2.9%	0.0%	0.0%
62225	94.1%	5.9%	0.0%	0.0%
75109	100.0%	0.0%	0.0%	0.0%
75110	93.9%	0.0%	0.0%	6.1%
75149	90.6%	9.4%	0.0%	0.0%
75175	97.2%	2.8%	0.0%	0.0%
75188	61.1%	36.1%	0.0%	2.8%
75199	97.1%	2.9%	0.0%	0.0%
75208	100.0%	0.0%	0.0%	0.0%
75210	73.5%	26.5%	0.0%	0.0%
75211	85.7%	14.3%	0.0%	0.0%
75213	67.6%	23.5%	0.0%	8.8%
75214	51.5%	48.5%	0.0%	0.0%
75218	100.0%	0.0%	0.0%	0.0%
75220	47.2%	52.8%	0.0%	0.0%
75228	59.5%	40.5%	0.0%	0.0%
75257	84.4%	15.6%	0.0%	0.0%
75260	85.3%	14.7%	0.0%	0.0%
75339	94.3%	2.9%	0.0%	2.9%
75349	83.3%	16.7%	0.0%	0.0%
75359	86.5%	8.1%	0.0%	5.4%
75374	100.0%	0.0%	0.0%	0.0%
75415	100.0%	0.0%	0.0%	0.0%
75443	61.1%	38.9%	0.0%	0.0%
75459	57.1%	22.9%	20.0%	0.0%
75463	65.7%	31.4%	2.9%	0.0%

Appendix III-11. Environmental matrix variables correlations with DCA axes.

	Axis 1			Axis 2		
Environmental Variable	**R**	**R-Square**	**Tau**	**R**	**R-Square**	**Tau**
Upstream Land Cover						
Row Crop	-0.23	0.053	-0.151	0.307	0.095	0.299
Non-Row Crop	-0.412	0.17	-0.323	-0.129	0.017	0.087
Total Ag.	-0.405	0.164	-0.303	0.004	0	0.175
Urban	0.184	0.034	0.131	-0.206	0.042	-0.138
Forest	0.308	0.095	0.232	0.109	0.012	-0.118
Other	0.002	0	0.064	0.181	0.033	0.064
100 m Buffer						
Row Crop	-0.175	0.031	-0.071	0.365	0.133	0.259
Non-Row Crop	-0.395	0.156	-0.232	-0.113	0.013	-0.01
Total Ag.	-0.423	0.179	-0.242	0.003	0	0.04
Urban	0.168	0.028	0.05	-0.331	0.11	-0.225
Forest	0.2	0.04	0.084	0.072	0.005	0.057
Other	0.11	0.012	0.148	0.193	0.037	0.081
250 m Buffer						
Row Crop	-0.148	0.022	-0.077	0.338	0.114	0.272
Non-Row Crop	-0.414	0.171	-0.296	-0.148	0.022	-0.013
Total Ag.	-0.405	0.164	-0.303	-0.008	0	0.121
Urban	0.218	0.048	0.101	-0.291	0.085	-0.229
Forest	0.235	0.055	0.195	0.134	0.018	-0.101
Other	0.125	0.016	0.188	0.148	0.022	0.047
Local 100 m Buffer						
Row Crop	-0.224	0.05	-0.28	0.035	0.001	0.202
Non-Row Crop	-0.168	0.028	-0.211	0.027	0.001	-0.005
Total Ag.	-0.203	0.041	-0.221	0.033	0.001	0.012
Urban	0.482	0.233	0.341	-0.357	0.127	-0.242
Forest	-0.283	0.08	-0.178	0.021	0	0.084
Other	0.139	0.019	0.149	0.405	0.164	0.161
Local 250 m Buffer						
Row Crop	-0.302	0.091	-0.311	-0.029	0.001	-0.069
Non-Row Crop	-0.231	0.053	-0.202	0.034	0.001	0.047
Total Ag.	-0.318	0.101	-0.215	0.021	0	0.047
Urban	0.438	0.192	0.222	-0.352	0.124	-0.195
Forest	-0.048	0.002	0	0.103	0.011	0.087
Other	0.109	0.012	0.106	0.395	0.156	0.038
Road Density	-0.148	0.022	-0.05	-0.295	0.087	-0.272
Stream Slope	0.463	0.215	0.303	-0.03	0.001	-0.121
pH	0.492	0.242	0.316	-0.103	0.011	-0.04
DO	0.063	0.004	0.071	0.065	0.004	0.03
Temperature	0.456	0.208	0.295	-0.259	0.067	-0.18
Conductivity	-0.157	0.025	-0.05	-0.574	0.33	-0.353
Turbidity	-0.146	0.021	-0.059	0.359	0.129	0.261
Embeddedness	0.411	0.169	0.253	-0.31	0.096	-0.345
Sediment Deposition	0.408	0.167	0.226	-0.409	0.167	-0.392
Bank Stability	0.449	0.201	0.231	-0.026	0.001	-0.044
Bank Vegetation	0.39	0.152	0.218	-0.045	0.002	-0.029
Riparian Width	0.363	0.132	0.247	-0.058	0.003	-0.07
Channel Alteration	0.264	0.07	0.231	-0.132	0.017	-0.101
Habitat Quality	0.52	0.271	0.279	-0.303	0.092	-0.293
Drainage Area	0.625	0.391	0.417	-0.359	0.129	-0.209
Diversity	-0.255	0.065	-0.276	0.165	0.027	0.148

Appendix III-12. Species abundance correlations with DCA axes

Species	Axis 1			Axis 2		
	R	R-Square	Tau	R	R-Square	Tau
S. undulatus	-0.501	0.251	-0.371	-0.287	0.082	-0.156
V. constricta	-0.182	0.033	-0.046	-0.284	0.081	-0.145
P. cataracta	-0.518	0.269	-0.435	0.292	0.085	0.395
L. cariosa	0.432	0.186	0.404	-0.333	0.111	-0.314
L. radiata	0.56	0.313	0.495	-0.05	0.003	-0.117
L. sp.	0.147	0.021	0.138	0.436	0.19	0.39
F. masoni	0.181	0.033	0.239	-0.207	0.043	-0.127

Appendix III-13. Environmental matrix variables correlations with NMS axes.

Environmental Variable	Axis 1 R	Axis 1 R-Square	Axis 1 Tau	Axis 2 R	Axis 2 R-Square	Axis 2 Tau
Upstream Land Cover						
Row Crop	-0.409	0.167	-0.276	0.067	0.004	0.017
Non-Row Crop	-0.303	0.092	-0.219	0.242	0.059	0.094
Total Ag.	-0.38	0.144	-0.293	0.215	0.046	0.061
Urban	0.125	0.016	0.04	-0.145	0.021	-0.104
Forest	0.307	0.094	0.31	-0.102	0.01	0.003
Other	0.056	0.003	0.087	-0.056	0.003	-0.057
100 m Buffer						
Row Crop	-0.403	0.163	-0.195	-0.048	0.002	-0.024
Non-Row Crop	-0.332	0.11	-0.188	0.169	0.028	0.037
Total Ag.	-0.432	0.187	-0.259	0.144	0.021	0
Urban	0.151	0.023	0.013	0.036	0.001	0.03
Forest	0.151	0.023	0.067	0.099	0.01	0.131
Other	0.201	0.04	0.219	-0.338	0.114	-0.168
250 m Buffer						
Row Crop	-0.377	0.142	-0.229	0.035	0.001	0.044
Non-Row Crop	-0.337	0.113	-0.252	0.245	0.06	0.04
Total Ag.	-0.42	0.176	-0.347	0.222	0.049	0.013
Urban	0.168	0.028	0.037	-0.034	0.001	-0.027
Forest	0.25	0.062	0.299	-0.103	0.011	0.034
Other	0.222	0.049	0.259	-0.325	0.106	-0.175
Local 100 m Buffer						
Row Crop	-0.309	0.095	-0.327	0.392	0.154	0.232
Non-Row Crop	-0.197	0.039	-0.279	0.001	0	0.127
Total Ag.	-0.245	0.06	-0.282	0.067	0.004	0.144
Urban	0.526	0.276	0.29	0.017	0	0.044
Forest	-0.206	0.042	-0.148	0.125	0.016	0.057
Other	0	0	0.128	-0.344	0.119	-0.251
Local 250 m Buffer						
Row Crop	-0.293	0.086	-0.318	0.362	0.131	0.263
Non-Row Crop	-0.226	0.051	-0.185	-0.148	0.022	-0.108
Total Ag.	-0.31	0.096	-0.232	-0.009	0	0
Urban	0.501	0.251	0.246	-0.039	0.002	-0.02
Forest	-0.06	0.004	0.003	0.136	0.018	0.087
Other	-0.02	0	0.132	-0.34	0.115	-0.283
Road Density	0.101	0.01	0.121	-0.137	0.019	-0.138
Stream Slope	0.321	0.103	0.151	-0.182	0.033	-0.04
pH	0.499	0.249	0.34	-0.049	0.002	-0.013
DO	0.051	0.003	0.054	-0.079	0.006	-0.138
Temperature	0.417	0.174	0.275	0.174	0.03	0.079
Conductivity	-0.011	0	0	0.328	0.107	0.151
Turbidity	-0.34	0.116	-0.201	0.133	0.018	0.069
Embeddedness	0.473	0.224	0.314	0.081	0.007	-0.017
Sediment Deposition	0.459	0.211	0.256	0.158	0.025	0.049
Bank Stability	0.441	0.195	0.285	-0.3	0.09	-0.214
Bank Vegetation	0.371	0.138	0.256	-0.191	0.036	-0.151
Riparian Width	0.404	0.163	0.285	-0.223	0.05	-0.138
Channel Alteration	0.279	0.078	0.241	-0.166	0.027	-0.142
Habitat Quality	0.545	0.297	0.323	-0.032	0.001	-0.071
Drainage Area	0.553	0.306	0.306	0.201	0.04	0.161
Diversity	-0.371	0.138	-0.32	0.374	0.14	0.391

Appendix III-14. Species abundance correlations with NMS axes

	Axis 1			Axis 2		
Species	**R**	**R-Square**	**Tau**	**R**	**R-Square**	**Tau**
S. undulatus	-0.524	0.274	-0.479	0.753	0.568	0.594
V. constricta	-0.023	0.001	-0.042	0.78	0.608	0.69
P. cataracta	-0.682	0.466	-0.592	0.371	0.138	0.343
L. cariosa	0.246	0.06	0.202	0.323	0.105	0.157
L. radiata	0.378	0.143	0.387	0.012	0	-0.201
L. sp.	-0.08	0.006	-0.082	0.183	0.034	0.226
F. masoni	0.106	0.011	0.112	0.558	0.312	0.494

Appendix IV

Geomorphological Asssessment and Mussel Habitat Appendices

Appendix IV-1. Variables, Diagnostic Criteria, and Assigned Values for Calculation of Channel Intability Index (Simon and Downs 1995)

Parameter	**Criteria and Score**					
Bed Material	0 = Bedrock	1 = boulder/cobble	2 = gravel	3 = sand	3.5 = unknown alluvium	4 = silt/clay
Bed Protection	0 = yes	1 = no	(with)	2 = 1 bank protected	3 = 2 banks protected	
Stage of Channel Evolution	0 = stage I	1 = stage II	2 = stage III	4 = stage IV	3 = stage V	1.5 = stage VI
Percent of Channel Constriction	0 = 0-5	1 = 6-25	2 = 26-50	3 = 51-75	4 = 76-100	
Number of Piers in Channel	0 = 0	1 = 1-2	2 = > 2			
Percent of Blockage	0 = 0-5	1 = 6-25	2 = 26-50	3 = 51-75	4 = 76-100	
Bank Erosion for Each Bank	0 = none	1 = fluvial	2 = mass wasting			
Meander Impact Point from Bridge (in meters)	3 = 0-10	2 = 11-20	1 = 21-35	0 = > 35		
Pier Skew for each Pier (sum for all piers in channel)	1= yes	0 = no				
Mass Wasting at Pier (calc. For each pier)	3 = yes	0 = no				
High Flow Angle Approach (in degrees)	0 = 0-10	1 = 11-25	2 = 26-40	2.5 = 41-60	3 = 61-90	
Percent Woody Vegetation Cover	3 = 0-15	2.5 = 16-30	2 = 31-60	1 = 61-99	0 = 100	

Appendix IV-2. Hydraulic formula equations.

Average Boundary Shear Stress: T_b = ? g R Se

where

? = the density of the fluid (water =1000kg/m^3)

g = gravitational acceleration (9.81m/s^2)

R = hydraulic radius, defined as channel cross-sectional area divided by wetted perimeter --- for wide channels, R is very close to mean water depth

Se = the "energy slope," which is the rate at which kinetic and potential energy decrease along the channel – for many channels it's very close to the channel bed slope, and in practice the bed slope is often used to calculate T_b

Critical Shear Stress from Shield's Curve: T_{ci} = T^* ($?_s$ – $?_w$) g D_i

where

T_{ci} = critical shear stress of particle D_i

T_* = Shield's Parameter from Shield's Diagram

ρ_s = the density of sediment particle, 2.65 g/cm^3

ρ_w = the density of water, 1.0 g/cm^3

g = gravitational acceleration

D_i is the bed particle size diameter

Manning's Equation: $U = k/n\ R^{2/3}\ S^{½}$

where

U is the discharge velocity (in m/s),
k is the unit conversion factor, document k=1
n is the Manning's n coefficient, n = 0.035
R is the hydraulic radius (in meters),
and S is the slope (in meter/meter).

Appendix V

Crossing Structure Attributes and Mussel Distribution Appendices

Appendix V-1. List of the 44 bridges in the two study areas deemed to have the potential for channel constriction.

Basin	County	Bridge Number
Neuse	Durham	151
Neuse	Durham	5
Neuse	Durham	56
Neuse	Durham	6
Neuse	Durham	8
Neuse	Orange	11
Neuse	Orange	114
Neuse	Orange	12
Neuse	Orange	126
Neuse	Orange	13
Neuse	Orange	136
Neuse	Orange	173
Neuse	Orange	242
Neuse	Orange	251
Neuse	Orange	30
Neuse	Orange	4
Neuse	Orange	57
Neuse	Orange	66
Neuse	Orange	67
Neuse	Person	10
Neuse	Person	22
Neuse	Person	23
Neuse	Person	33
Neuse	Person	36
Neuse	Person	38
Neuse	Wake	119
Cape Fear	Moore	12
Cape Fear	Moore	127
Cape Fear	Moore	173
Cape Fear	Moore	184
Cape Fear	Moore	212
Cape Fear	Moore	225
Cape Fear	Moore	28
Cape Fear	Randolph	149
Cape Fear	Randolph	175
Cape Fear	Randolph	210
Cape Fear	Randolph	211
Cape Fear	Randolph	214
Cape Fear	Randolph	220
Cape Fear	Randolph	228
Cape Fear	Randolph	257
Cape Fear	Randolph	339
Cape Fear	Randolph	459
Cape Fear	Randolph	463

Appendix V-2. Results of Kruskal-Wallis test comparing percentages of mussels occurring in each 25-meter cross-section in 44 sites judged to have the potential to produce scour through channel-constriction. Cross-sections were numbered consecutively from downstream to upstream and the road crossing was between 12 and 13.

Cross-section	N	Median	Average Rank	Z-score
1	44	2.3764	492.7	-0.8
2	44	2.5786	498.1	-0.67
3	44	2.3022	474.6	-1.2
4	44	3.2917	573.2	0.99
5	44	2.4438	507.9	-0.46
6	44	2.696	515.4	-0.29
7	44	1.7497	492.8	-0.79
8	44	2.511	533.4	0.11
9	44	3.2468	588.1	1.32
10	44	2.0422	510.8	-0.39
11	44	1.3961	412.9	-2.57
12	44	0.8174	340.3	-4.18
13	44	2.8571	557.5	0.64
14	44	3.009	593.9	1.45
15	44	3.5825	600.9	1.61
16	44	3.4923	599.4	1.58
17	44	2.6987	576.9	1.07
18	44	2.2482	501.9	-0.59
19	44	2.4487	534.7	0.14
20	44	2.5404	548.9	0.45
21	44	4.3273	629.8	2.25
22	44	3.3565	578.7	1.11
23	44	2.4848	502.2	-0.58
24	44	2.361	519.2	-0.21

Appendix V- 3. Results of Kruskal-Wallis test comparing percentages of mussels occurring in each 25-meter cross-section in 27 sites with wooden bridge supports. Cross-sections were numbered consecutively from downstream to upstream and the road crossing was between 12 and 13.

Cross-section	N	Median	Average Rank	Z-score
1	27	3.9	351.6	0.77
2	27	2.6	307.7	-0.48
3	27	2.0	267.6	-1.61
4	27	3.5	358.5	0.96
5	27	2.1	291.6	-0.93
6	27	2.7	316.4	-0.23
7	27	3.4	356.3	0.9
8	27	3.2	358.8	0.97
9	27	3.8	346.4	0.62
10	27	1.5	277.5	-1.33
11	27	0.9	252.5	-2.04
12	27	0.6	181.6	-4.05
13	27	1.2	267.8	-1.61
14	27	2.1	304.9	-0.56
15	27	3.4	362.3	1.07
16	27	2.6	334.9	0.3
17	27	2.8	372.7	1.37
18	27	2.2	313.5	-0.31
19	27	3.2	368.6	1.25
20	27	4.5	392.3	1.92
21	27	3.8	390.2	1.86
22	27	4.7	399.8	2.13
23	27	2.4	326.3	0.05
24	27	1.8	288.2	-1.03