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A comparison of the Impacts of Culverts versus Bridges on Stream Habitat and Aquatic Fauna

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16. Abstract

This project was an interdisciplinary look at the differences in impacts between culverts and bridges on stream habitat and stream fauna. There were four essential components:

1. Freshwater mussels: We took habitat measurements and conducted mussel surveys at 43 culverts across the piedmont in NC. Overall, habitat downstream of culverts was much more impacted than downstream of bridges. The reduction in mussel populations downstream of culverts was also more pronounced than at bridge sites. These effects were magnified in certain soil types that were more erodable.
2. Geomorphology: Detailed stream morphology and substrate measurements were taken at arch, pipe and box culverts and bridges. All crossing types were shown to increase stream cross-sectional area downstream by constricting flow at the crossing.
3. Toxicology: We conducted toxicity tests with polycyclic aromatic hydrocarbons (PAHs) on all life stages of freshwater mussels. We also assessed genetic damage due to PAH exposure. We found that PAHs are not acutely toxic to mussels but may possibly be contributing to long-term genetic damage.
4. Fish Passage: Fish community structure and passage was assessed at different crossing designs in the piedmont. There were no significant differences detected in community structure between crossing types. Though not statistically significant, data suggest a trend toward greater fish movement through bridges than culverts.

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Executive Summary:

This project was a multi-disciplinary approach to assessing the differences in impacts between bridges and culverts on stream habitat and stream fauna with an emphasis on freshwater mussels. Each discipline (geomorphology, freshwater mussels, toxicology, and fish passage) presents their research as a single chapter in this report.

Geomorphology: Culverts and bridges are necessary in order to cross waterways during road construction. However, these structures have detrimental affects on the hydrology and ecology of the streams they cross. The objective of this study was to investigate how these bridges and culverts alter stream hydrology and geomorphology by determining the effects on the upstream and downstream reaches of a road crossing on the cross sectional area, the hyporheic depth, on riffle habitat, and substrate types. Three types of culverts (arch, box, and pipe) and small bridges were evaluated. All four types of stream crossings were determined to increase the cross sectional area downstream of the structure. Crossing structures also affected hyporheic zone depths by decreasing average depths downstream of the structure. Finally, most mussels seemed to occur in substrates that were dominated by relatively large particles (gravel and cobble) that were less movable by sheer stress during higher flows. Each of the problems discovered with these structures is a result of the channel restriction and the increased flow velocity and turbulence scour that it creates. These detrimental conditions can be mitigated by providing for floodplain access for higher flows. It is recommended that culverts be designed for low flows and high flows. Oversizing culverts, compared to current design criteria will allow floodplain access and build bankfull benches in the extra openings to restrict low flows to a few openings. The use of bridges that span across the valley limiting fill and allowing floodplain access may even be more beneficial. When valley fill is necessary, then side culverts in the floodplain may alleviate degradation and allow more natural floodplain hydrology.

Freshwater Mussels: Freshwater mussels require stable habitat for persistence in streams, and anything that disrupts stream channel stability poses a threat to mussels. When bridges and culverts constrict stream channels, scour and bank erosion may generate channel instability that is detrimental to this faunal group. To follow up on an original study of road-crossings that primarily focused on bridges, we took habitat measurements and surveyed mussels at 43 culverts across the piedmont of North Carolina. We found that channels tended to be wider and deeper downstream of culverts compared to upstream. Scour holes were prevalent downstream of culverts and were especially prevalent downstream of pipe culverts. Mussel populations were reduced for the entire surveyed reach downstream (150 m) compared to upstream, and increased scour at the culvert was linked with decreasing mussel abundance downstream. Mean length, width, and height of *Elliptio complanata* were reduced downstream of culverts, but shell width seemed the most impacted. Both habitat changes and mussel population effects were more pronounced at culverts compared to bridges. Culverts did tend to stabilize sediments from 75-125 m upstream and actually increased mussel abundance in those areas. The overall effects of culverts were magnified in the northern and eastern edge of the North Carolina piedmont where soils are generally more erodable. We recommend bridges be used as the preferred crossing to allow flood plain access at road crossings and reduce scour. If culverts are constructed, additional openings on the flood plain would be highly beneficial. Because the northern and eastern edge of the piedmont is the home of two federally endangered species, special care should be used in bridge and culvert installation in these areas to avoid stream erosion and channel instability.

Toxicology: Freshwater mussels (Bivalvia: Unionidae) are among the most threatened of aquatic species in the world. One of the major issues implicated in this decline is water pollution. Polycyclic aromatic hydrocarbons (PAHs) are a suite of hydrophobic environmental pollutants common in terrestrial and aquatic ecosystems. These compounds are largely derived from petroleum related sources (e.g., gasoline, oil) and are of major concern from transportation-related runoff to aquatic systems due to the acute and chronic (e.g., mutagenic and carcinogenic) toxic properties of many members of this class. The effects of exposure to PAHs have been investigated in many species of bivalves; however, to date no comprehensive study of the effects of exposure to these compounds on all life stages of native freshwater mussels have been completed. The goals of this study therefore were to investigate the effects of exposure to PAHs on all life stages of freshwater mussels and to develop diagnostic tests that are rapid, accurate, inexpensive, and of minimal impact to the mussels. This study examined the acute (48 h) toxicity of PAHs to the glochidial (larval) and juvenile stages of mussels and the subacute (7 d) toxic effects on adult mussels. Additionally, the study examined the use of genetic damage as a biomarker of exposure of mussels to PAHs by utilizing the Comet assay to determine levels of DNA strand breakage following aqueous exposure. Finally, mussels were collected from areas of high and low environmental levels of PAHs and were analyzed to validate laboratory findings and to examine relations to previously obtained field PAH mussel, water, and sediment measurements. We found that there were no acute toxic effects of PAHs on glochidia or juveniles of the two species of freshwater mussels examined, up to concentrations approaching water solubility, and well exceeding those commonly measured in the streams of North Carolina. Experiments with adult *Elliptio complanata*, both in the laboratory and from the field, indicated that genetic damage due to PAH exposure was likely present, however the results were highly variable and the potential for biological, ecological, and toxicological consequences were uncertain. Further development and improvement of assay methods may reduce this variation. Generally, mussels from streams with higher average daily traffic counts (ADTC) exhibited greater levels of genetic damage compared to mussels from streams with lower ADTC values. Data obtained from the laboratory study generally showed increasing DNA damage relative to increasing PAH concentration. Based on the data generated, however, PAHs are not likely contributing to acute toxicity of mussels in North Carolina streams, but the chronic, long-term pervasive effect of PAHs on native freshwater mussels remain uncertain.

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

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

We conducted field surveys of stream fish and a mark-recapture study in 16 streams located in the Piedmont region of the Cape Fear River Basin of central North Carolina during the summer of 2004. Stream reaches 50 m above and below a given road crossing, or pseudo-crossing in the case of the control stream reaches without crossings, were blocked off and sampled using a combination of seining and triple-pass electrofishing. All fish were identified to species and measured to the nearest millimeter. Specimens larger than 30 cm total length (TL) were individually marked subcutaneously with elastomer paint tags. These procedures were repeated four, eight, and 12 weeks after the initial sampling period.

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

Passive integrated transponder (PIT) tags and remote antenna array systems have been used extensively on the west coast of the United States to monitor the movements of salmonids, but other studies have also implemented these systems to track eel migrations and bass habitat use. These antenna have been customized to monitor the passage of salmonids through culverts (Hansen and Furniss 2003), hydroelectric dams (Axel et al. 2005), and dam bypass regions (Aarestrup et al. 2003). We assessed unidirectional stream fish movement through two types of crossings, box culverts and bridges, using PIT tags and remote antenna arrays to further assess the potential impact of these two crossing types on stream fish in the Piedmont of North Carolina. The main goal of this study is to assess the movement of stream fish through crossings as a follow-up to a previous, more traditional mark-recapture study conducted in 2004 (Vander Pluym unpubl. thesis). We conducted electrofishing surveys of fish on six streams located in the

Piedmont region of the Cape Fear River Basin, North Carolina during the Summer and early Fall of 2005. All fish measuring ≥ 60 mm TL were injected with an ISO PIT tag with a 12-gauge needle. Custom built antenna arrays, with weir nets to direct fish passage through the antenna loop, were installed in each stream either upstream or downstream of a given crossing. PIT-tag reader systems (FS2001 Biomark, Inc.) were running continuously for 30 days with each system maintained by battery switches and data downloads every 7-10 days.

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

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CHAPTER 1:
The Effects of Culverts and Bridges on Stream Geomorphology

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Introduction

Culverts and bridges are constructed to accommodate road traffic over surface waters. (Hamill, 1999). These crossing structures can have a negative affect on the hydrology and ecology of the waterway (Wellman et al., 2000); (Gilvear et al., 2002; Gregory and Brookes, 1983). Culverts and bridges can increase stream velocities, turbulence of flow, aggradation, scour, and bank erosion downstream of the crossing structure (Richardson and Richardson, 1999). Changes in flow velocities and channel geomorphology may, in turn, result in stream habitat alteration and adverse effects on the stream biota. Channel hydraulic alterations can also cause channel incision, which disconnects the waterway from its floodplain, compounding the degradation of the ecology of the stream and riparian corridor (Philippi, 1996).

Thousands of stream crossing structures are present in the Piedmont of North Carolina. These culverts and bridges vary in age, type, and impact on the stream channel. As culverts age, and become structurally unsound, they are replaced. Culverts are less expensive to install and maintain, and where feasible preferred by NC DOT officials. However, natural resource and regulatory agencies have raised questions about the effect of current bridge and culvert design and installation practices on stream channel biota and aquatic habitat quality.

In this study we examined how culverts alter stream geomorphology. Stream cross sectional areas, hyporheic depths, and habitat types of the stream reaches upstream and downstream of the road crossings were measured and compared.

Bridges and Culverts and their Effects on Stream Morphology

For a road to cross a stream, engineers must design and construct a culvert or bridge. However, these crossings adversely affect stream habitat, hydrology, and floodplain connectivity. Fish, freshwater mussels and other invertebrates are adversely affected by crossing structure construction and consequent alternations in stream hydrology. Channel scour is one of the main issues to be addressed when a bridge or culvert is designed and constructed for a road crossing. Boulders and woody debris can alter a channel by causing turbulent flows that create scour (McKenney et al., 1995; Robert, 2003). In the same way, bridges and culverts can have an impact on channel scour and bed degradation.

Scour below bridges and culverts. Several different types of scour can occur around culverts and bridges during high flows. Local scour effects the bridge abutments and piers. Flow eddies and turbulent flow erosion can happen at these locations. Contraction scour occurs when the natural cross sectional flow area of a stream channel is reduced or constricted. As this area is reduced, water velocities increase. Increased velocity adds to the shear stress and thus exacerbates bed degradation at that site (Hamill, 1999; Richardson and Richardson, 1999; Simon and Johnson, 1999; Umbrell et al., 1998). Therefore the cross sectional area is expanded by scour and bank degradation to handle these flows as the stream tries to reach equilibrium. Furthermore, as the channel adjusts towards a lower state of energy by lowering bed elevation and channel widening, the bridge structure is compromised (Simon and Johnson, 1999). When the bridge is submerged by even greater flows, then this pressurized flow increases shear stress and creates scour (Jones et al., 1999).

Contraction scour can be further split into two types of scour. The first, live bed scour, occurs when sediment transported into the bridge area scours the stream bed. Secondly, clear water scour occurs during clear water stages and the increased flow velocities create higher shear stresses and thus scour the stream bed (Richardson and Richardson, 1999).

Scour can have a long term impact on bed degradation and affect entire channel reaches (Simon and Johnson, 1999). During high flows it has been recorded that bed degradation of 6 m can occur as a result of this contraction scour (Richardson and Richardson, 1999). These major channel scours are usually downstream of major channel constrictions, such as crossings, and check dams (Hooke and Mant, 2000). The narrow section at a bridge can cause backwater and a hydraulic jump through the bridge opening eventually causing the development of enormous scour holes just downstream. These scour holes ultimately migrate upstream through the bridge opening, posing a threat to the stability of the bridge (Darby, 1999). At some bridge sites, aggradation can occur that raises bed elevation and may bury macro fauna. Aggradation also increases the backwater effect and affects the pressure on the structure and passability of the bridge (Johnson et al., 2002). Bridges seem to more readily allow sediment transport than culverts and therefore have less accumulation up stream of the crossing (Wellman et al., 2000).

Culverts have similar effects on stream geomorphology and hydrology, but since most have artificial bottoms their bed effects usually stop at the structure. However these effects can have a greater impact on fish and other mobile aquatic species than bridges since they disconnect the upstream channel from the downstream channel once the culvert becomes perched from the degradation caused by increased velocities and turbulence (Hendrickson, 1964). A perched culvert has its downstream invert elevated above the channel bottom. Severely perched culverts have been especially problematic for anadromous fish, resident fish, and terrestrial species because they disrupt the connectivity of the stream channel (Castro, 2003). Severe erosion of the channel bottom is often the cause for culvert crossing failures. Culverts can also cause sediment accumulation in the channel upstream of their position. Wellman and coauthors (2000) found that box culverts caused the most sediment accumulation (Wellman et al., 2000). However it is noted that the degradation from culverts has a limited scope downstream (Corry et al., 1975).

Culvert design has usually focused on the criterion of passing normal to flood flows through a limited cross sectional space. Many adverse geomorphological effects have resulted including plugging of the culvert, aggradation, and the high flow velocities which have contributed to the channel bottom scour that elevates the downstream end of the culvert (Gregory and Brookes, 1983). Culverts that are undersized can be overtopped by high flows, resulting in erosion of the road surface and road fill. Culverts installed at an excessive gradient can also create downstream erosion by increasing flow velocities and turbulence at the culvert outlet (Adair et al., 2002). Culvert construction handbooks generally state that in higher gradient streams, providing for a spillway into a pool at the culvert outlet will reduce velocities and dissipate energy (American Concrete Pipe Association, 1964; Hendrickson, 1964).

In an effort to minimize costs and maintain flow velocity in the culvert, engineers sometimes, decrease stream sinuosity, divert flows, straighten reaches at the crossing, or perch culverts above the stream-bed. Purposefully perching of a culvert and establishing a plunge pool at the end during installation is stated to sometimes be “beneficial, for the sediment will settle out” (American Concrete Pipe Association, 1964). Corrective measures are usually taken by engineers to maintain stream velocity; in some cases by removing rocks, or by armoring or shaping the channel (American Concrete Pipe Association, 1964).

Many of the standard culvert installation practices have deleterious effects on stream habitat and stream hydrology. In the past, the only factors considered when a project was designed were structure cost, structure safety, flow capacity, and any economic disasters that may come about from excessive ponding or flooding, usually pertaining to businesses or crops in the adjacent floodplain (American Concrete Pipe Association, 1964; Hamill, 1999; Hendrickson, 1964).

Incision and Stream Morphology

The formation of a stream channel is dependent on a complex set of variables. Isolation of the effect of one of these variables can be difficult. On the short time scale, channel morphology may be regarded as controlled by the physical characteristics of the system and quantities of water and sediment supplied (Schumm et al., 1987). Most of the investigated streams in our study have been channelized or incised at the crossing site and or beyond. Such channel alterations have led to incision that disconnects the stream from its floodplain, and this instability can migrate through the whole system (Johnson et al., 2001). Channelization and incision removes habitat and leads to an unstable channel ecosystem that will continuously erode until it reaches a new equilibrium (Darby, 1999; Gregory and Walling, 1987). The scour that the culverts and bridges cause only compounds channel incision and habitat degradation problems. Channel degradation is a response to a disturbance in which there is an excess of flow energy, shear stress or stream power relative to the amount of sediment supplied to the stream (Darby, 1999). Gilvear etal. (2002) state that: "A river channel's geometry, planform, bed material size and levels of bed and bank stability are all controlled by river flow regime, both in terms of overall water yield, and the frequency and magnitude of flood events".

Incised streams are disturbed ecosystems. Since these streams are incised by the local scour and channelization, the response of these streams will begin with deepening and then transition to widening as bank undercutting and slumping occur (Darby, 1999). The increased cross sectional area creates reduced velocities, reducing the channel's sediment transport capacity and allowing sediment to settle out. Increased sedimentation rates can bury aquatic life and lead to mid channel bar formation, which can deflect flows towards the banks causing further bank erosion (Frizzell et al., 2004). Basically, any alteration or control on a natural stream system can modify channel size and shape and induce a range of geomorphological problems (Gilvear et al., 2002). Previous studies have shown that bridges have caused increased cross-sectional areas by two times or more up to 85 m downstream of a crossing (Gregory and Brookes, 1983). This widening process and bank erosion can cause large amounts of sediment to enter the system that can also bury any aquatic life downstream, and cause macroinvertebrate mortality. However, these instances of increased bank erosion and sediment movement are related to the type of structure at the crossing.

Floodplain Importance

In North America, up to 90% of floodplains may be in agricultural use and therefore some of the floodplain functions are lost. When developed or used in agriculture the natural hydrology is altered and natural forest ecosystems are lost (Tockner and Stanford, 2002). The ecological services that floodplains provide and the threats upon them make them one of the most endangered landscapes. The hydrology of a floodplain is the single most important aspect controlling the ecological functions of this ecosystem. The dense vegetation in these riparian areas increases Manning's "n" and retards flow and thus causes slower velocities of flood flows (Rodzenko et al., 1988). When high flows enter the floodplain, the travel time of the flood waves moving downstream are increased, and reduced peak flood flows result (Rodzenko et al., 1988). These slower controlled flood flows allow sediments to fall out into the floodplain.

Construction projects that alter the floodplain hydrology, may degrade or lead to the destruction of such ecosystems (Philippi, 1996). Structures that deprive floodplains of the flood pulse generate the most damage to the health of the riparian ecosystem (Philippi, 1996). Clearing, development, and channelization of floodplain ecosystems have adversely effected the wildlife habitat within them (Lovell et al., 1988). The Army Corps of Engineers found that in some areas, development encroachments of more than 15% of the natural floodplain resulted in more than a one foot rise in flood elevation; more than allowed by FEMA (Rodzenko et al., 1988). Bridge and culvert embankments that constrict flow may result in backwater upstream and thus alter floodplain functionality (Gilbert and Schnuck-Kolben, 1987).

The Hyporheic Zone and Mussel Habitat

"The hyporheic zone is composed of the shallow, saturated sediment below and to the sides of the stream bottom" (Schindler and Krabbenhoft, 1998). Its importance and influence is regulated by water movement, permeability, substrate particle size, resident biota, and physiochemical features (Boulton et al., 1998; Olsen and Townsend, 2003). River regulation, agriculture, urban, and industrial activities all have the potential to impair interstitial bacteria and invertebrate biota and disrupt the hydrological connections between the hyporheic zone and the stream, groundwater, riparian, and floodplain ecosystems (Hancock, 2002; Marshall and Hall Jr, 2004). The hyporheic zone is a key hydrological and biological component of most sand bed and gravel streams. Impacts on the hyporheic zone potentially jeopardize the water quality of streams and groundwater.

The hyporheic zone acts as a biological filter that is a refuge from the shear stress of the surface for macro and micro invertebrate fauna (Boulton et al., 1998; Hancock, 2002). An important interface hydrologically, chemically, and biologically for streams, the hyporheic zone can also act as a refuge for biota during dryer periods (Schindler and Krabbenhoft, 1998); (Del Rosario and Resh, 2000). However all these ecological functions of the hyporheic zone can change due to channel degradation.

In streams were there has been incision or scour, the biochemical processes of the hyporheic zone can change. Ammonification, nitrification and denitrification often occur in the hyporheic zones of shallow streams. Near the surface of the bottom substrate, constant mixing of interstitial water with the flowing aerated stream water maintains an aerated zone where ammonification and nitrification can occur. Deeper in the sediments is an anaerobic zone where denitrification can occur. (Shibato et al., 2004); (Boulton et al., 1998; Hinkle et al., 2001). The deeper parts of hyporheic zones can be a sink for dissolved organic carbon and organic nitrogen, as well as nitrate (Shibato et al., 2004), but shallow disturbed hyporheic zones can be a source of dissolved organic carbon, organic nitrogen, and nitrate (Schindler and Krabbenhoft, 1998).

The deeper the hyporheic zone, the larger the biochemical and ecological role it will have, especially where bedrock is farther below the channel bottom surface (Boulton et al., 1998). Where there is exposed bedrock from scour, mussels can not burrow into the hyporheic zone to flee from shear stress during higher flows (Frizzell et al., 2004). The hyporheos consists of fauna that reside in this ecosystem and is composed of surface and subsurface species (Boulton et al., 1998; Schindler and Krabbenhoft, 1998). Sediment composition and vertical hydrological exchange determine the composition, populations, and distribution of the hyporheos (Boulton et al., 1998; Olsen and Townsend, 2003). Hyporheic zone development and importance is greatest in intermediate stream reaches and less important in lowland rivers and headwater streams (Boulton et al., 1998; Hancock, 2002). Ultimately the significance of the hyporheic zone to the stream is a function of its activity, health, and extent of connectivity

(Boulton et al., 1998). Because of its ecological importance, managers must recognize the importance of links between the hyporheic zone and the surrounding habitats and incorporate hyporheic zone restoration or enhancement into their restoration and management plans (Hancock, 2002).

Mussels are part of this hyporheic zone but more related to the top layers. Research has pointed to the importance of the stability of substrate rather than the type of substrate that a stream contains for maintenance of mussel habitat. Streams with a good riparian zone and equal fractions of fine sediments, sands, gravels, and cobble seem to maintain normal mussel numbers (Poole and Downing, 2004). Some studies relate this provision of good mussel habitat to the larger substrate types and the resistance to movement of the larger particles by the shear stress generated by high flows (Strayer, 1999; Vannote and Minshall, 1982). Therefore mussel beds can be safely established in these “refuges” from shear stress and bed transport. In a study of mussels in the Salmon River Canyon in Idaho, mussel beds were mostly found in areas with cobble filled with gravel, or pockets of gravel behind boulders (Vannote and Minshall, 1982). These “refuge areas” are formed from local fluvial geomorphological processes.

Bridges and culverts can drastically affect the stable equilibrium of localized stream bottom areas that provide good mussel habitat. When scour or aggradation occurs from the road crossing affecting the local hydrologic processes, it can lead to mussel mortality (Box and Mossa, 1999; Vannote and Minshall, 1982). Mussel mortality rates reached over 90% for all species in one study when a silt layer began to cover the sand or gravel (Box and Mossa, 1999). These “refuge populations” are important for the long term recruitment in establishing populations in other parts of the channel (Vannote and Minshall, 1982).

The goal of this study was to determine how culverts and bridges affect stream geomorphology. Specific objectives were to determine if bridge or culvert road crossings have an impact upstream or downstream on:

1. Stream cross sectional area,
2. Hyporheic zone depth,
3. Riffle habitat or
4. Substrate types.

Methods

Experimental Design and Study Site Selection

The initial population of potential study sites was selected by The College of Veterinary Medicine (CVM) research team in the previous bridge study and the current culvert study of road crossing impacts on mussel populations. The choice of study sites was limited to those within 50 miles of Raleigh and that had mussel populations upstream of the road crossing. Most were in the Piedmont with a limited number in the Coastal Plain. Given this limited site database, we decided to limit our focus to one soil system in a single geologic region to minimize the natural variability among stream channels. Although several alternative study designs would have been more robust, site selection was limited by nonhydrologic design features.

A preliminary study was conducted to investigate the condition of each road crossing included in the master study and adjacent land-use. We measured bankfull widths, thalweg depths at first riffle above crossing, took pictures, and recorded dominant substrates. During this investigation we noticed a wide variation of stream widths, adjacent land uses, and substrate types, all of which can affect the hydrologic functions of a stream. We further noted many crossings had beaver dams or old mill-dams that can also affect the hydrologic functions of a stream, especially stream gradient. Therefore we attempted to minimize variability among stream crossing environments maximize our ability to detect significant impacts of the culverts and bridges on stream geomorphology.

A total of 14 stream crossing sites (six bridges and eight culverts) were selected for more intensive study. These sites are dispersed across seven Piedmont counties (See Table 1.1). These sites were selected with the following parameters to control the environment around the area and to minimize impacts on stream channel geomorphology from factors other than the road crossing itself. Sites selected were: 1) All in the Carolina Slate Belt, to control soil erodability factors and stream substrate materials; 2) Active agricultural areas and/or cattle pastures around potential sites that allowed cattle access to streams were omitted because of sedimentation and erodability effects that can cause channel incision and degradation of aquatic habitats and hydrology (Schumm et al., 1987). Where erosion rates are high, these agricultural lands can cause severe stream aggradation that could not be attributed to the constructed structure this study investigated (Johnson et al., 2001). 3) Potential study streams that had a stream confluence within the 70 m reach upstream or the 70 m reach downstream of the road crossing were omitted because of the resulting dynamic turbulent flows that create scour holes. This scour has an effect on the hyporheic zone and would cause an inconsistency in measurements (Robert, 2003). Streams that had control devices such as dams or sills, man-made, by beavers, or natural, in the vicinity of the road crossing were omitted because of their adverse effects on free flow and stream gradient. Larger rivers with bridges that had bankfull widths greater than the streams with culverts were also removed. Also, larger rivers or streams seemed to have bridges that allowed great amounts of floodplain access and thus were not comparable to the restricted flows of smaller bridges and culverts. 4) Sites with relatively high proportions of urbanization in the watersheds were omitted because urbanization can have negative impacts on the physical, chemical, and biological character of the streams (Henshaw and Booth, 2000); (Finkenbine et al., 2000). 5) Sites without owner granted access were omitted.

Table 1.1. Study Sites List

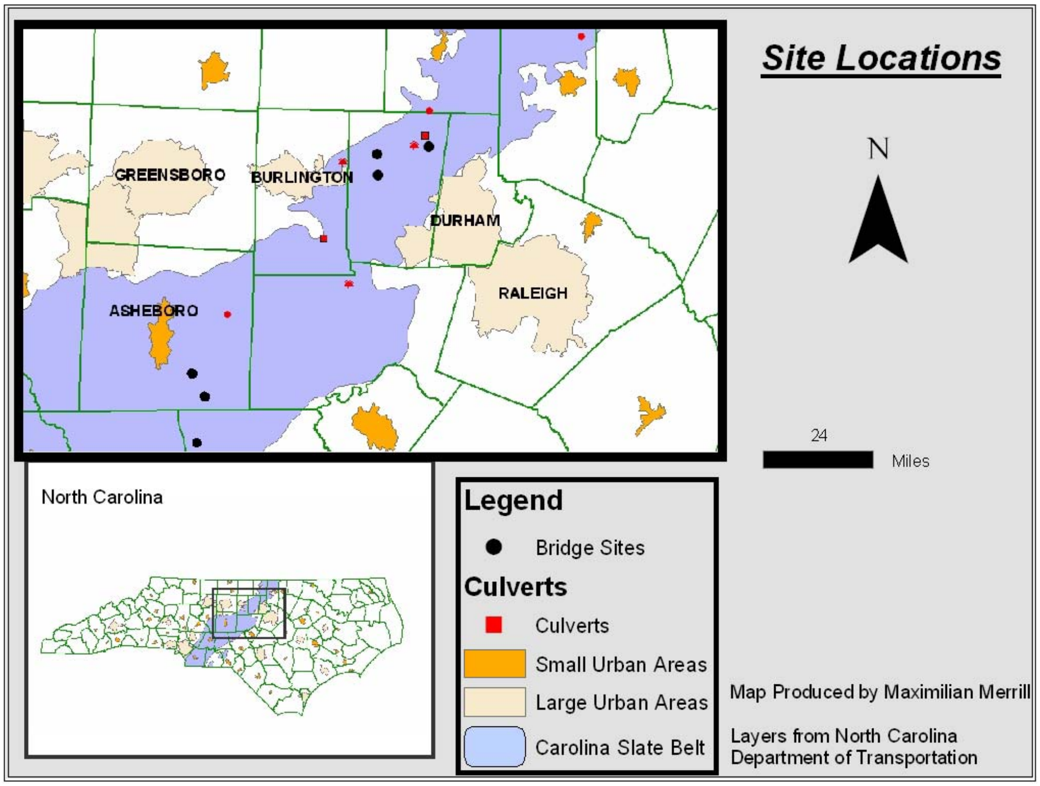
County and Site #	Type
Alamance 20	Box
Alamance 29	Arch
Chatham 12	Arch
Granville 217	Pipe
Moore 173	Bridge
Orange 13	Arch
Orange 30	Box
Orange 4	Bridge
Orange 55	Bridge
Orange 67	Bridge
Person 38	Pipe
Randolph 220	Bridge
Randolph 349	Bridge
Randolph 459	Pipe

This study was designed to compare the channel geomorphology upstream and downstream of culvert or bridge road crossings on streams with current mussel populations. We measured four factors: habitat areas (riffles, substrate types), hyporheic layer depths, channel gradient, and cross sectional areas. The channel section upstream of the road crossing is the control site for each comparison to downstream impacts. The purpose of this study is to provide information on how the road crossing is affecting stream geomorphology and how that may relate to mussel habitat near the crossing.

Geographic Location

All of the stream study sites are located in the Cape Fear and Neuse River Basins of North Carolina. The stream networks commonly have a dendritic drainage pattern and the study streams are all at least 2nd order and no greater than 4th order at the crossing (Thorne et al., 1997). The study sites are in Alamance, Chatham, Granville, Orange, Moore, Person, and Randolph counties, all which are in the Carolina Slate Belt soil system (Figure 1.1). This soil system has a longitudinal axis that is aligned in a northeast to southwest direction, from north of Raleigh to south of Asheboro. Topography in this part of the Piedmont in North Carolina is characterized by moderate to severe slopes. The valley sides can be very narrow. First and second order streams are common but very short in length (Daniels et al., 1999).

Figure 1.1. Site Location Map



Climate

The study area has a sub-humid and temperate climate with an average rainfall of 45.5 inches per year. The average high temperature is 70.0 degrees while the average low temperature is 47.0. The mean temperature is 58.6 degrees.

Geology and Soils

Study sites were chosen in one geological region with a limited range of soil types to limit the natural geomorphic variability among the study streams. Different soil types can produce different effects from disturbance (Schumm et al., 1987). Both large scale and local effects on movement of surface water exists because of geologic structure (Viessman et al., 1989). All soils in the Carolina Slate Belt system are formed from parent materials of gneiss, schist, phyllite, and volcanic igneous rocks along with slates. The less eroded soils are at least 30 percent silt plus very fine sand in the B horizon with silt surfaces. This high silt content separates these slate belt soils from those in other soils systems in North Carolina. Saprolite or bedrock is usually at the base of these shallow soils (Table 1.2). In our research area, which is mostly in the northern portion of the slate belt region; Georgeville and Herndon soils usually occur on the ridge tops while Nason and Tatum occur in the valleys. Georgeville and Badin are the most common soils in the study area and all soils in the region generally are moderately permeable (Daniels et al., 1999).

Land Use

Historically, a relatively high percentage of the forests in this region were clear cut for pasture or for row crop agriculture. Since the industrial revolution and immigration of the textile industry to North Carolina, many of the fields and pastures became fallow and now the region is mostly forested. Forests in these areas are dominated by hardwood, hardwood-pine mixed forests, or pine plantations, with agriculture lands sporadically placed along the hillsides and in the valleys

Table 1.2. Major Soils in the Carolina Slate Belt System (Daniels 1999)

Soil Series	B horizon color	B horizon texture	Major slope range (%)	Thickness >1 meter	Thickness < 1 meter	Comments
Herndon [2]	YR-YB	Clay	2-15	X		
Nason [3]	YR	Clay	2-15		X	
Misenheimer [2,4,5]	YB	Loamy	0-5		X	Level bedded slates
Goldston [2]	YB	Loamy	4-25		X	40-60% slate fragments
Georgeville [2]	R	Clay	6-12	X		
Tatum [3]	R	Clay	4-15		X	15-40% slate fragments
Badin [3,4]	R	Clay	4-25		X	10-35% slate fragments
Orange [5]	YB	Clay	0-7		X	Smectitic; Subsoil>35% base saturated
Lignum [3]	YB	Clay	2-7		X	Somewhat poorly drained

1. YR=Yellowish red; YB=Yellowish brown; R= Red
2. Kaolinitic clay mineralogy
3. Mixed clay mineralogy (more than 10% expanding 2:1 clays)
4. Less than 1 m to hard rock
5. Moderately well drained

Stream Geomorphology Measurements

All stream channel measurements were made with a Sokkia SET 30R total station using a prism reflector and 7.62m (25ft) long survey rod. Cross sections were surveyed both upstream and downstream of each stream crossing at 1, 5, 10, 20, and 50 m distances from the bridge or culvert along the thalweg (Castro, 2003; Gregory and Brookes, 1983; Hadley and Emmett, 1998). The cross sections were established from the structure edge with a 100 m tape. Survey pins were set at each cross section and a measuring tape was strung across the stream perpendicular to the flow. Permanent pins of rebar were set beside the survey pins in case a return visit was needed.

Between cross sections, the stream channel was surveyed to gain a planar image of the stream channel and how it ties into the crossing. Location measurements were made at points along bankfull, top of bank, water surface, thalweg, and across the upstream and downstream ends of the culvert and bridge (Castro, 2003). The stream points were measured to 70 m upstream and downstream of each culvert and bridge.

Hyporheic Zone Depth Measurements

At each channel cross section a piece of rebar was driven into the hyporheic zone to record the depth at 5 equal intervals from the left water surface edge to the right water surface edge (Wellman et al., 2000). Once bedrock or saprolite was reached the depth was recorded to the nearest 0.5 cm.

Habitat Measurements

Box stated that a simple ordinal index ranking average sediment sizes may be a useful substrate assessment approach for drawing inferences between mussel density and substrate composition (Box and Mossa, 1999). To measure substrate types upstream and downstream of the crossing, the dominant textural character of the substrate was evaluated at each point where there was a change of substrate in the stream channel. This study used substrate texture classes to characterize these substrate measurements (Table 1.3). Determination of substrate was from previous training using the USDA size classification (Table 1.4). One person did the ocular substrate analysis part on each site to establish continuity among measurements. Sands and silts (particles less than 2 mm) were grouped into the *sand* class. Pebbles and all sizes of gravels were grouped into one *gravel* class. Cobbles and boulders were grouped into one *cobble* class. Bedrock and saprolite were classified into one *bedrock* class.

Table 1.3. Substrate Classes

Class	Label
Predominately Bedrock	b
Predominately Bedrock w/ Cobble	b/c
Predominately Bedrock w/ Gravel	b/g
Predominately Bedrock w/ Sand	b/s
Predominately Cobble	c
Predominately Cobble w/ Bedrock	c/b
Predominately Cobble w/ Gravel	c/g
Predominately Cobble w/ Sand	c/s
Predominately Gravel	g
Predominately Gravel w/ Bedrock	g/b
Predominately Gravel w/ Cobble	g/c
Predominately Gravel w/ Sand	g/s
Predominately Sand	s
Predominately Sand w/ Bedrock	s/b
Predominately Sand w/ Cobble	s/c
Predominately Sand w/ Gravel	s/g

Riffle habitat locations and endpoints were also measured using the total station. These measurements established an area of riffles upstream and downstream of each crossing. The stream substrate habitats were measured to 70 m upstream and downstream of each culvert and bridge.

Table 1.4. USDA Particle Size Classes

Material	Size (mm)
Clay, total	<0.002
Silt, total	0.002 - 0.05
Silt, fine	0.002 - 0.02
Silt, coarse	0.02 - 0.05
Sand, total	0.05 - 2.00
Very fine sand	0.05 - 0.10
Fine sand	0.10 - 0.25
Medium sand	0.25 - 0.50
Coarse sand	0.50 - 1.00
Very coarse sand	1.00 - 2.00

Statistical Analysis

Each relationship measured was evaluated using the statistical package: JMP 5.1.1. Each of the upstream measurements was paired with its downstream location counterpart and compared using analysis of covariance (ANCOVA: multiple factors) because of the four crossing types studied. An initial full model was used to analyze the culvert sites by location of cross section and hyporheic depth, but was found not to be significant. All interaction terms were dropped because none were significant. Therefore the model used was:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2$$

where;

Y= Predicted Downstream

X1= Actual Upstream Measurement

X2= Type of Crossing.

This reduced model is the reason for the parallel regression lines for each type of crossing measured. All measurements were finally analyzed using this model. On each graph one should pay attention to the regression lines and where they cross the 1:1 slope line. When the regression line crosses the 1:1 line the effects of the crossing structure change.

RESULTS AND DISCUSSION

General Description of Sites

During the preliminary study of culvert sites, it was noted that many channel characteristics in the vicinity of the culvert may have resulted from the impacts of the stream crossing structures. Notes were taken at each culvert site to guide selection of parameters to be used to select the final intensive study sites (Table 1.5).

First and foremost, all culvert crossings were restricting the floodplain width to a narrow portion under the crossing. This constriction of the floodplain is probably the most important impact of these culvert crossings, thus affecting flow velocity, sediment transport, and channel erosion/sedimentation processes at high flows. At a majority of the culvert crossings, the stream appeared to be enlarged and incised downstream of the crossing compared to the upstream channel reach. Hupp and Simon (1991) would define these streams in stage IV of the evolution process. Thus these streams will continue to widen and degrade until aggradation starts and they form a new but smaller floodplain. Many trees were overhanging banks and the banks were slumping more often downstream of the crossing structure (Figure 1.2).

Figure 1.2. Photographs of Channel Widening, Incision, and Overhanging Trees

(looking downstream from culvert).

Table 1.5. Short Note Database for Potential Culvert Sites

ID #	Type	Year Built	Basin	County	Field Notes
4	Box	1934	Cape Fear	Alamance	water using 2 out of three boxes, slow water flow, ferry control devise downstream, small creek, downstream
20	Box	1930	Cape Fear	Alamance	incised below and above
29	Arch	1935	Cape Fear	Alamance	Highly incised, beaver dam, large amounts of debris downstream, narrow buffer
74	Pipe (Arch)	1997	Cape Fear	Alamance	braided up stream, deep pool below, incised more
158	Box	1997	Cape Fear	Alamance	slightly entrenched above, acting like a bridge, 1 box used, grass and shrubs on shoulder
204	Pipe	1978	Cape Fear	Alamance	slightly entrenched, bars upstream and down, deep pool below
338	Box	1960	Cape Fear	Alamance	slightly entrenched, 2 sides used third directly to floodplain
62	Box	1984	Dan	Caswell	greatly incised, slow moving, beaver activity
12	Arch	1933	Cape Fear	Chatham	bank protection needs minor repairs
18	Box	1968	Cape Fear	Chatham	incised more below, culvert in large pool
464	Box	1970	Cape Fear	Chatham	long pool after culvert, cows in creek above with bank erosion, very deep
470	Pipe	1971	Cape Fear	Chatham	culvert much wider than bankfull width, small creek
16	Box	1941	Tar	Franklin	deep hyporheic zone, more incised below but banks seem stable
62	Box	1973	Tar	Franklin	greatly incised, straightened, very deep could not get in
9	Pipe (Arch)	1989	Tar	Granville	slightly incised below less above, foot bridge above, almost flow to floodplain
28	Box	1931	Tar	Granville	greatly incised above and below, large water flow?, large log jam, large pool after culvert
29	Box	1950	Tar	Granville	greatly incised, straightened, 2 boxes only used
46	Box	1934	Dan	Granville	incised below less above, does not seem straightened
116	Pipe	1975	Dan	Granville	Slightly incised below less above, log jam.
217	Pipe (Arch)	1990	Dan	Granville	slightly incised below less above, more eroded downstream
254	Box	1960	Tar	Granville	Medium incised above with long rip rap, below highly incised, sand bar, exposed trees and roots.
268	Box	1991	Tar	Granville	slightly to medium incised
190	Arch	1930	Cape Fear	Guilford	deep pool below, beaver dam below
257	Pipe	1988	Cape Fear	Guilford	silt in pipes, more incised below, large pools above and below, culvert wider than BFW
608	Box	1938	Cape Fear	Guilford	Small creek, highly incised. 1 side used
26	Box	1991	Cape Fear	Harnett	incised downstream, sand in culvert, some scour downstream
2052	Box	1947	Neuse	Johnston	deep pool below, may be straightened, beaver dams up and down stream
27	Box	1967	Pee Dee	Montgomery	seems straightened above till rock face
44	Box	1931	Cape Fear	Montgomery	medium incised, deep pool above and below
12	Box	1931	Cape Fear	Moore	slightly incised, no cement floor
212	Pipe	1970	Cape Fear	Moore	slightly incised
220	Pipe (Arch)	1995	Cape Fear	Moore	highly incised, old bridge acting as deflector
225	Pipe (Arch)	1975	Cape Fear	Moore	slightly incised, not straightened, side culvert for swamp, 2 sides used
13	Arch	1941	Cape Fear	Orange	little influence, low incision, seemed to be normal riffle pool sequence, on bridge embankments in stream
30	Box	1941	Cape Fear	Orange	slightly incised above but less below, two sides used of box
242	Box	1950	Cape Fear	Orange	beaver activity
251	Box	1950	Cape Fear	Orange	banks beginning to slump, debris restrict channel slightly
263	Box	1986	Cape Fear	Orange	very long culvert, floodplain on each side
?	Arch	?	Cape Fear	Orange	highly incised below
22	Pipe (Arch)	1985	Cape Fear	Person	slightly incised , beaver dam upstream and maybe down, slow moving water
38	Pipe	1991	Cape Fear	Person	slightly incised , gravel bar below
211	Pipe	1994	Cape Fear	Person	banks slightly entrenched but stable
339	Box	2000	Cape Fear	Randolph	more incised below, new culvert, different than the rest
459	Pipe	1955	Cape Fear	Randolph	2 pipes being used, highly incised above and below
463	Box	1968	Cape Fear	Randolph	old cow fence above, maybe old pasture, 2 boxes used, 90 degree incision on banks, trib connection below
49	Box	1968	Cape Fear	Wake	incised banks 90 degrees, but stable
134	Box	1992	Cape Fear	Wake	greatly incised, bedrock and sediment in pools
135	Arch	1988	Cape Fear	Wake	slightly incised
372	Pipe	1993	Cape Fear	Wake	incised banks but vegetated, new culvert
561	Box	1926	Cape Fear	Wake	sinuous upstream and straight below, more entrenched downstream, sand in culvert

Because some of the culverts are oversized for low flows, mid-channel bars have formed (Figure 1.3). This is a definite sign that the channel cross section is too large and therefore normal sediment transport is not taking place. Furthermore it seems that over time some of these oversized culverts are forming bankfull benches in the culvert openings not readily used during low flows. These culverts with bankfull benches established inside, seemed to have the least impact on downstream conditions, and resulted in a more stable channel environment (Figure 1.4). In effect, where ample cross sectional flow area is available in a multi-opening box culvert, the stream has re-established a low flow channel in one or more openings and using the remaining openings as the bankfull channel. The larger multi-opening box culverts exhibited little perching (Figures 1.3 - 1.4). However, relatively small pipe culverts that severely restricted high flows often had an incised pool downstream of the culvert resulting in perching of the downstream end of the culvert. (Figure 1.5).

Figure 1.3. Photograph of Mid-Channel Bar Forming.	Figure 1.4. Bankfull Bench Forming in Culvert.
	

Figure 1.5. Perched Pipe Culvert.

Minimum Impact Example

Certain sites seemed to have the least amount of impacts on stream geomorphology. The Randolph 220 Bridge site was one of the crossings that had the least amount of impact on cross sectional areas, hyporheic depths, riffle habitat, and longitudinal profiles. The survey data illustrates that this crossing does not greatly increase cross sectional area downstream nor does it decrease hyporheic or riffle habitat downstream (Figure 1.6 and Table 1.6). The total change in riffle habitat came to about 15 m^2 which was the least amount of change measured (Appendix D). These results probably stem from the fact that this bridge allows larger flows to access the floodplain, thus minimizing the energy through the river channel. However other crossing types have a great impact on the stream geomorphology.

Table 1.6. Cross Section Areas and Hyporheic Depths of Randolph 220

Site Name		Ave Depth (cm) UpStrm	Ave Depth (cm) Dwn Strm	Difference
Rand 220 Bridge	X1	21.10	9.42	-11.68
	X5	19.16	9.81	-9.35
	X10	3.94	10.06	6.13
	X20	11.03	12.45	1.42
	X50	8.77	20.19	11.42
		X-Area (m^2)	**X-Area (m^2)**	
		UpStrm	***Dwn Strm***	***Difference***
	X1	6.41	2.41	-4
	X5	6.51	4.03	-2.48
	X10	5.51	5.3	-0.21
	X20	6.77	7.51	0.74
	X50	5.2	6.43	1.23

Figure 1.6. Cross Section Areas at 10m from crossing Randolph 220

(.21 m^2 difference)

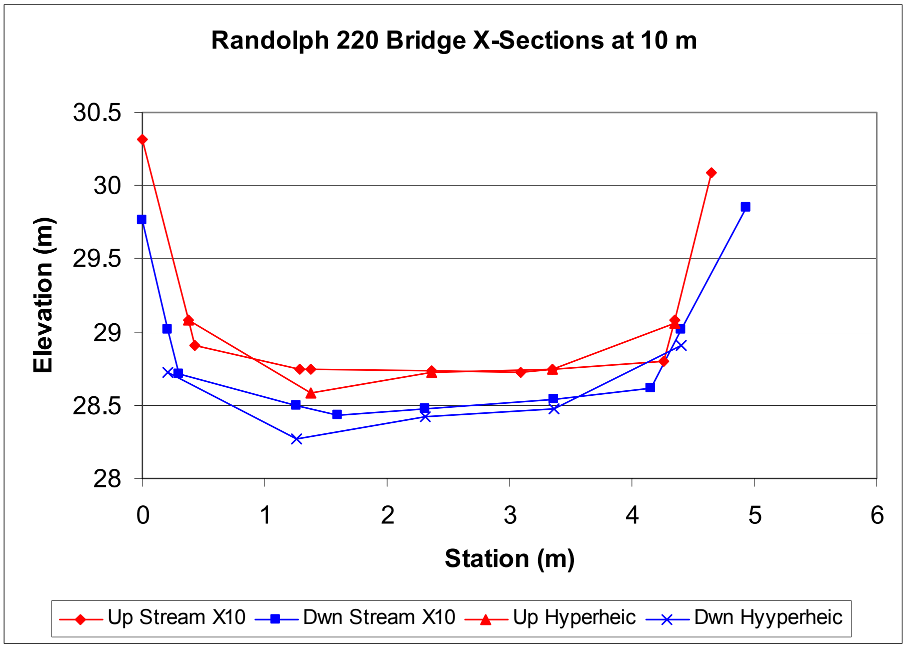
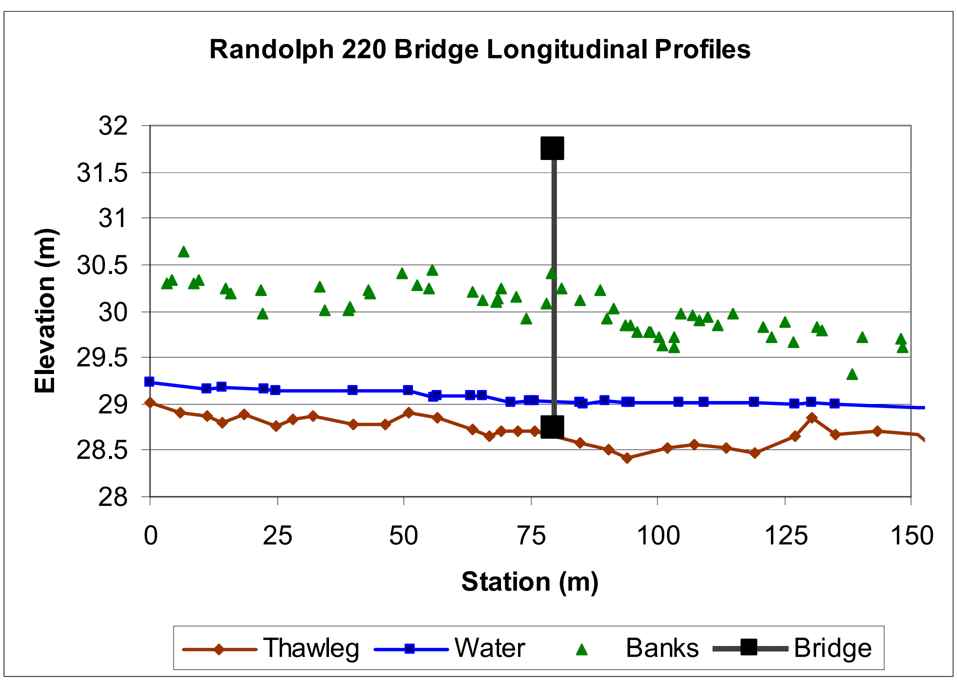


Figure 1.7. Longitudinal Profile with Very Little Change after Crossing Structure



Maximum Impact Example

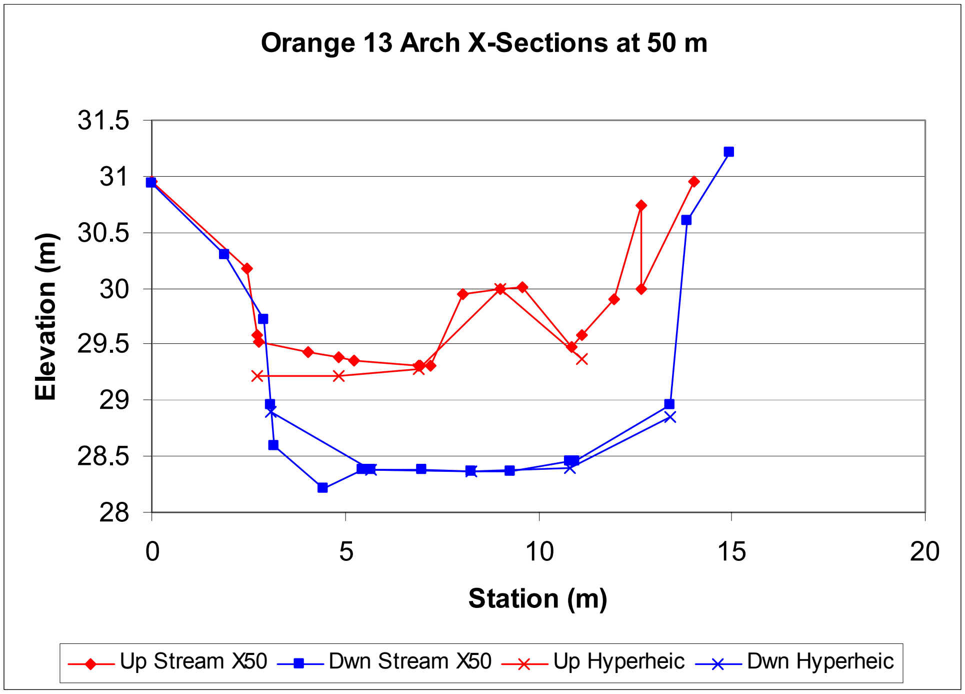
Arch culverts seem to have a great impact on stream geomorphology. Even though they may be good for fish passage other hydrology factors are being affected. The data for the cross sectional analysis and hyporheic zone analysis points to a decreasing of hyporheic zone depths downstream and an increase of cross sectional area in the same sections (Table 1.7 and Figure 1.8). This is probably due to the channel constriction of the floodplain. This arch culvert spans from bank to bank and fill is placed up to the culvert. Therefore absolutely no floodplain access is allowed. This site is especially sensitive to culvert effects because it is one of the streams in a high relief region and therefore the narrow floodplain present is even more valuable to slow and dissipate higher flows.

Table 1.7. Cross Section Areas and Hyporheic Depths of Orange 13

		Ave Depth (cm)	Ave Depth (cm)	
Site Name		*UpStrm*	*Dwn Strm*	*Difference*
	X1	10.52	9.42	14.19
	X5	30.32	8.26	-11.48
	X10	38.13	24.77	20.90
	X20	11.68	45.48	29.87
	X50	15.35	4.97	-13.10
Rand 220 Bridge		X-Are (m^2)	X-Area (m^2)	
		UpStrm	*Dwn Strm*	*Difference*
	X1	13.71	4.93	-8.77
	X5	19.94	25.30	5.36
	X10	23.56	23.94	0.38
	X20	20.09	34.49	14.40
	X50	14.86	27.92	13.06

Figure 1.8. Cross Section Areas at 50m from Crossing Orange 13

(13.06 m^2 difference)



Cross Section Area Effects

A total of 140 cross sections were measured at the 14 intensively studied culvert and bridge sites. The cross sectional areas ranged from 1.85 m^2 to 34.29 m^2. When comparing downstream segments with their upstream counterparts, most of the culverts and bridges tended to increase downstream cross sectional area. In Figure 9 is an example where the channel has significantly widened downstream of a stream crossing. Analysis of the 140 cross sections shows that there is a difference between box culverts and arch culverts in their downstream impacts on cross sectional area (Figure 1.10). The regression lines of upstream cross sectional area versus downstream cross sectional area have a slightly positive y intercept, meaning that the channels of the smaller streams are slightly greater in size downstream of the crossing than upstream.

All regression lines of upstream versus downstream cross-sectional areas for the different types of crossings have a slope at .861 and the overall R^2-value is .538. Cross section location was not a significant factor. There was also no statistical difference between regression lines when the data from all culvert types were pooled and compared to the bridges (Appendix D).

The statistical comparison of downstream versus upstream cross sections show that box culverts have less effect on increasing downstream cross sectional area than the other crossing types. This is concurrent with the observations in the field. While doing the preliminary study, it was noted that box culverts seemed to be often oversized compared to the other types. Box culverts do not restrict high flows as severely as do smaller culvert types that can create a back water affect. Many of these box culverts only used a few of the openings during low flows while bankfull benches were formed or forming in the other openings (Figure 1.4). This allowed for sediment transport during low flows and floodplain access during higher flows. All crossings tended to increase cross section areas downstream, except for the larger streams where the regression line crosses the 1:1 slope line, at which point the larger streams seem not have increased in cross sectional area downstream. This is probably due to the fact that the larger streams are crossed with large box culverts or bridges, which have less effect on the downstream cross sectional area (Figure 1.10). The culverts and small bridges used in this study restrict the floodplain hydrology causing channel scour and bank erosion that increases downstream cross sectional area and degrades mussel habitat.

Figure 1.9. Example of Upstream Cross Section VS Downstream Cross Section

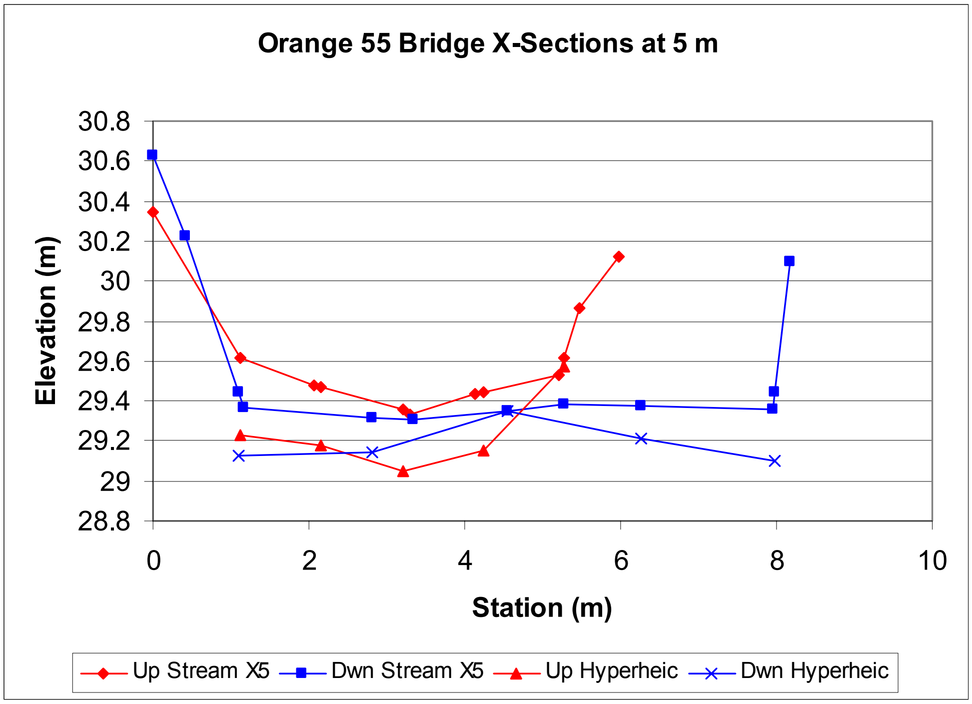
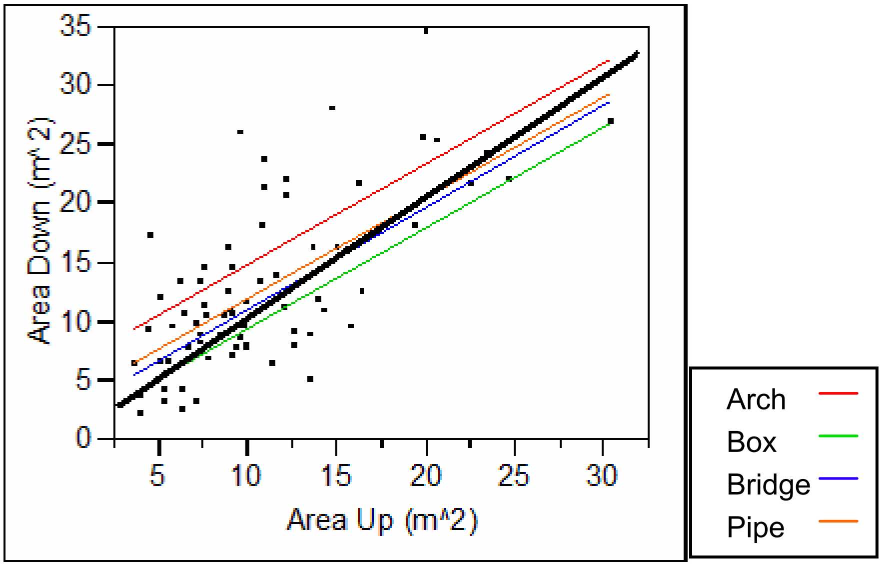


Figure 1.10. Regression Plot of all Crossing Types (Cross Section Areas)



***Black line shows 1:1 slope line**

Level			Least Sq Mean
Arch	A		15.595120
Pipe	A	B	12.530572
Bridge	A	B	11.800844
Box		B	10.148930

Levels not connected by same letter are significantly different

Hyporheic Zone Depth Effects

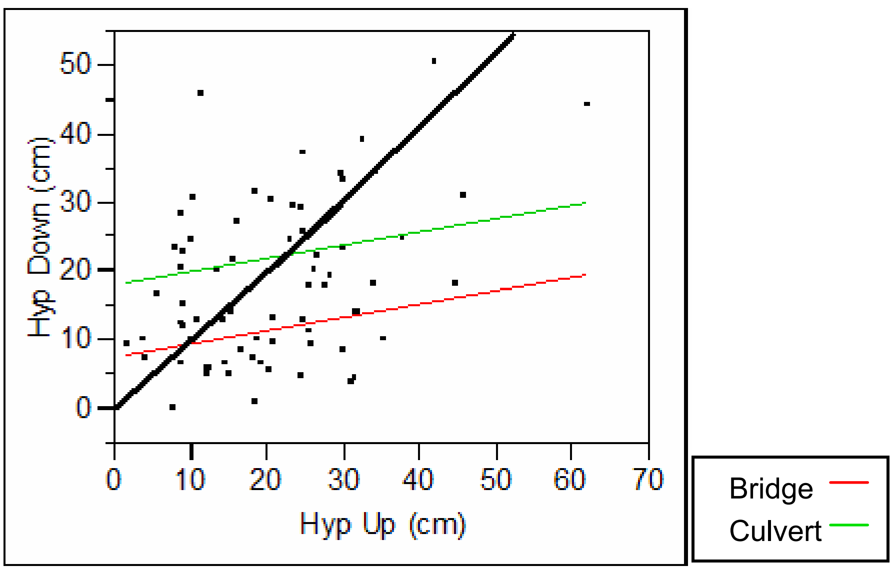
The average hyporheic zone depth for each cross section was determined from the five measurements made at each cross section location. Regression comparisons of upstream versus downstream depths were performed on the average hyporheic zone depths at the cross sections. Initial hyporheic zone depths ranged from 0 to 62.13 cm. The high variability among the study streams in types and depths of hyporheic zones resulted in regression equations that explained only about 30 % of that variation.

For the regression of bridges compared to the pooled culvert data, R^2-values are around 0.32 with the slopes of the regression lines at 0.19 (Figure 1.11).

Comparing the regression lines for all four different types of crossings, the regression for bridges was similar to arch culverts but significantly different from pipe culverts and box culverts. These slopes are .10 and the overall R^2 values are .345. No statistical difference was detected when comparing different cross section locations (Appendix D). However there seemed to be the least amount of change when comparing cross sections at 5m and a greater effect when comparing 1m locations.

All types of road crossings seem to have an effect on decreasing hyporheic zone depth downstream though the impact of the crossing bucked that trend at certain sites (Figure 1.12). Note the table of hyporheic zone depths in Appendix E. Of the 3 arch culverts, there was a definite decrease in hyporheic zone depths downstream of the culvert at Alamance 29, a definite increase in hyporheic zone depths downstream of the culvert at Chatham 12 and a mixed bag of effects at Orange 13. The general trend for decreased hyporheic zone depths downstream of the crossing is probably due to the scour that occurs as high velocity restricted flow is released into the channel. However, this trend for decreased hyporheic zone depths downstream of the crossings is only true for the larger depths. For the streams with shallow hyporheic depths, this trend is not as clear. Each regression line crosses the 1:1 slope line between 10 and 20 cm of hyporheic zone depth, showing that these effects are not as significant with streams that have shallow hyporheic zone depths. This is intuitive because if the stream is already degraded or has scoured the bottom sediments down close to a restrictive layer with very shallow hyporheic depths the scour will have less effect downstream. If the stream flows on bedrock upstream then it can not get much shallower in depth downstream.

Figure 1.11. Regression Plot for Bridges vs Culverts (Hyporheic Zone Depths).

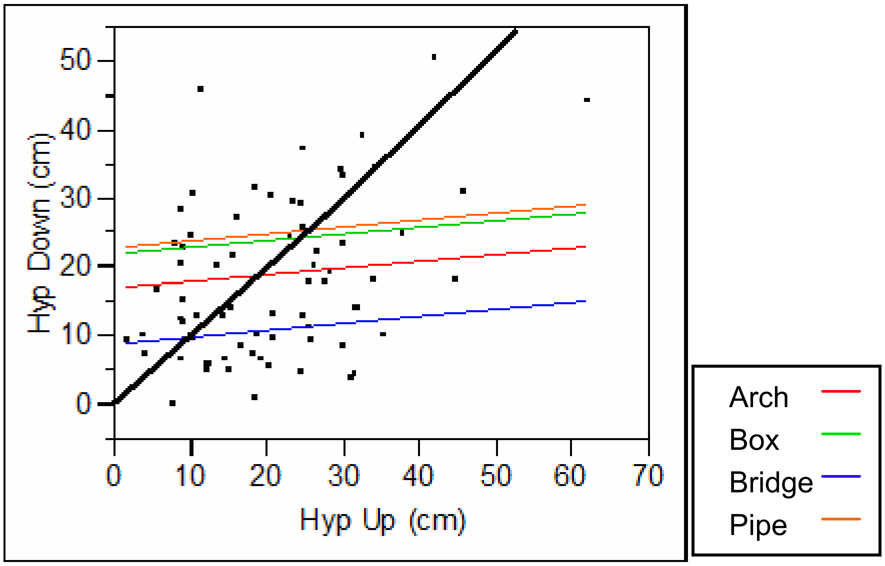


***Black line shows 1:1 slope line**

Level			Least Sq Mean
Culvert	A		22.136122
Bridge		B	11.774505

Levels not connected by same letter are significantly different

Figure 1.12. Regression plot for all crossing types (Hyporheic Zone Depths)



***Black line shows 1:1 slope line**

Level			Least Sq Mean
Pipe	A		25.076814
Box	A		24.040801
Arch	A	B	19.023061
Bridge		B	11.225796

Levels not connected by same letter are significantly different

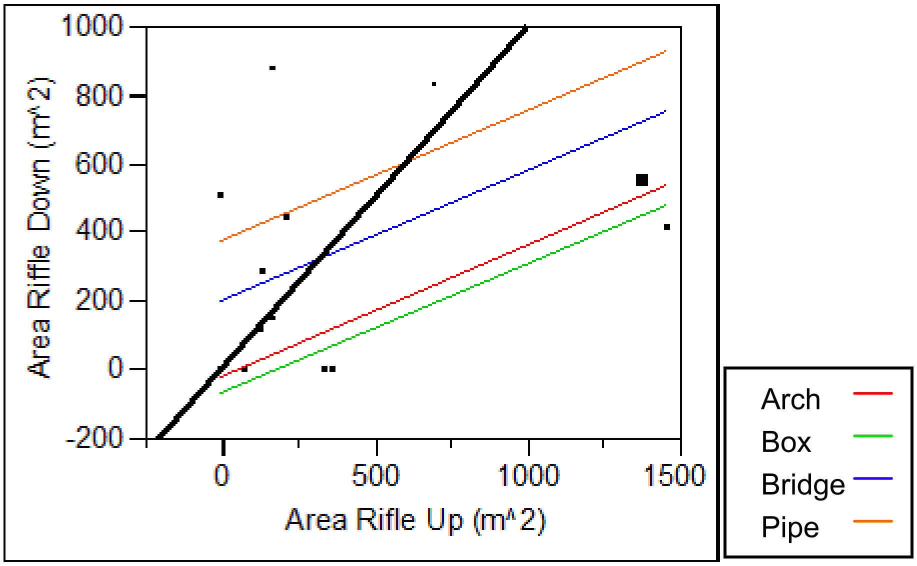
The results show that bridges have the greatest effect on hyporheic zone depths while pipes and box culverts have the least effect. This may be due to the fact that cross sectional areas at the pipes and boxes (with bankfull benches) allow for sediment transport in low flows because they keep their velocities. The other crossings are wider and thus create slower flow velocities upstream of the culvert. Therefore they settle out sediments and do not allow for as much sediment transport at these low flows. This causes downstream sections to be sediment starved. Scour may control the bed gradient but it seems that low flow transport may control hyporheic zone depth.

Riffle Area Effects

Each of the 14 sites was measured for area of riffles upstream of the culvert and downstream. The range of riffle areas was 0 to 1377.83 m^2. There was no significant difference in the regressions between the types of crossings or when bridges were compared with all culverts pooled. Each regression line in a reduced model had a slope of .37 and an overall R^2 value of .37 (Figure 1.13). The regression lines show a pattern that may point to arch and box culverts having a greater effect on downstream riffle areas than bridges or pipe culverts. However all crossing structures seem to reduce riffle area downstream within the study reach. Again, better detection of statistical differences was an issue because of the lack of sample size. Sample size could be increased by limiting the selection parameters and examining streams that did not have mussel populations. This would allow for more sites to be studied. However adjusting the selection parameters may increase variability and decrease R^2 values. Also future studies of this type should separate small bridges with wingwalls from the newer longer spanning cement bridges.

Because the sample size for comparing the impact of the crossings on riffle areas is so small, we can not draw any firm conclusions about crossing effects. However, if the data for all the crossings are pooled, the slope of the regression line is less than 1, thus pointing to an effect of structures on reducing area of downstream riffle habitat (See Appendix D). Therefore, there may be some sort of effect that the crossing has on downstream riffle habitats but more research will be needed to determine whether such an effect exists.

Figure 1.13. Regression Plot for all Crossing Types (Riffle Areas)



***Black line shows 1:1 slope line**

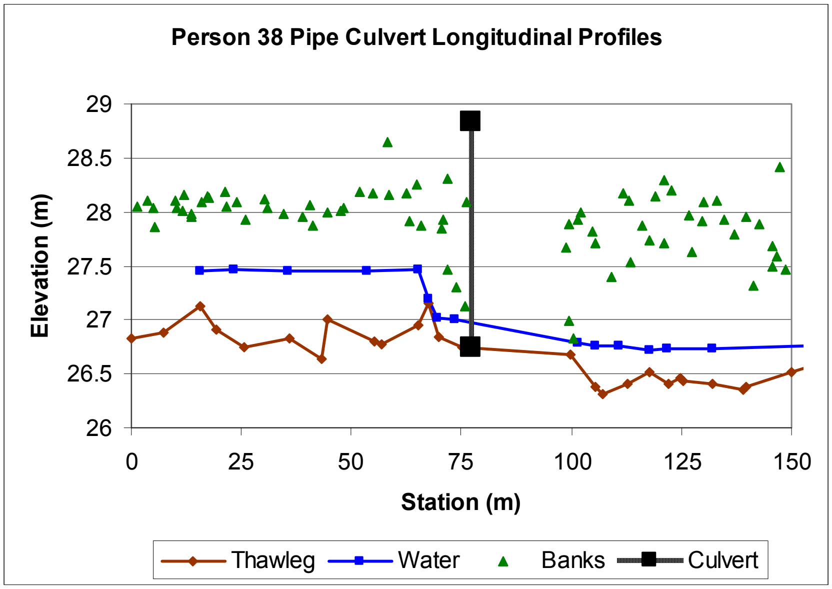
Level		Least Sq Mean
Pipe	A	517.55005
Bridge	A	342.36685
Arch	A	122.10017
Box	A	74.48411

Levels not connected by same letter are significantly different

Longitudinal Profiles Effects

All sites were surveyed along the thalweg, banks, and water surface to determine the longitudinal channel gradients through these stream reaches with crossings. The longitudinal profiles show that pipe culverts and one bridge (Orange 67) had the most influence on stream gradient below the crossing (Figure 1.14). Most bridges, arch culverts and box culverts seem not to cause a significant change in the stream elevation (See Appendix C).

Figure 1.14. Longitudinal Profile of Steep Gradient after Crossing Structure



Contraction scour is the factor that appears to cause the decrease in channel elevation along the thalweg downstream of the crossing. The longitudinal profiles of all the pipe culverts and Orange 67 bridge show a significant drop in bed elevation downstream of the crossing. This contraction creates scour and degrades bed levels. Even though these pipe culverts may be allowing low flow sediment transport, at higher flows they are causing scouring of bed levels. This is directly related to the floodplain restriction and thus all of the water's energy is focused through those pipes, when its energy would otherwise be dissipated in the floodplain. The Orange 67 bridge also had this same effect of a drop in bed elevation downstream of the structure because it is one of the smaller bridges with wingwalls in the study that is constricting higher flows and also scouring the downstream section.

CONCLUSIONS AND RECOMMENDATIONS

In review, the impacts of bridge and culverts on stream channels are that they:

1. increase channel cross sectional area downstream
2. decrease hyporheic zone depths downstream and
3. may decrease riffle habitat downstream.

The key to minimize adverse impacts on the stream channel in culvert and bridge design is to allow the stream to dissipate its energy into the floodplain during high flows. To counteract the typical flow restriction and scouring effects, it is recommended that culverts be designed to accommodate both low flows and high flows. Large multi-opening box culverts that are forming bankfull benches are mimicking the natural processes of sediment transport and deposition during high flows. Such large culverts allow for sediment transport during low flows and energy dissipation into the flood plain during higher flows. Also bridges that span across the valley limiting fill and allowing floodplain access have the same effect of providing for flow energy dissipation during high flows. When valley fill is necessary, then side culverts in the floodplain may provide for additional flood flow capacity and energy dissipation, thus alleviating degradation and allowing for more natural floodplain hydrology.

These design suggestions will allow for sediment transport during low flow and thus minimizing impacts on downstream hyporheic zone depths. Furthermore they will allow for maximum energy dissipation during higher flows that seem to degrade the banks and habitat downstream of the crossing.

References

Adair, S. et al., 2002. Management and Techniques for Riparian Restoration, United States Department of Agriculture- Forest Service, Fort Collins.

American Concrete Pipe Association, P.C. (Editor), 1964. Handbook of Concrete Culvert Pipe Hydraulics, Chicago.

Boulton, A.J., Findley, S., Marmonier, P., Stanely, E.H. and Valett, H.M., 1998. The Functional Significance of the Hyporheic Zone in Streams. Annual Review of Ecology and Systematics, 29: 59-81.

Box, J.B. and Mossa, J., 1999. Sediment, Land Use, and Freshwater Mussels: Prospects and Problems. Journal of North American Benthological Society, 18(1): 99-117.

Castro, J., 2003. Geomorphic Impacts of Culvert Replacement and Removal: Avoiding Channel Incision. United States Fish and Wildlife Service- Oregon Fish and Wildlife Office, Portland.

Corry, M.L., Thompson, P.L., Watts, F.J., Jones, J.S. and Richards, D.L., 1975. Hydraulic Design of Energy Dissipators for Culverts and Channels. In: U.S.D.o. Transportation (Editor). Federal Highway Administration.

Daniels, R.B., Buol, S.W., Kleiss, H.J. and Ditzler, C.A., 1999. Soil Systems in North Carolina. Soil Science Dept. North Carolina State University, Raleigh.

Darby, S.E. (Editor), 1999. Incised River Channels: Processes, Forms, Engineering and Management. John Wiley & Sons, West Sussex.

Del Rosario, R. and Resh, V.H., 2000. Invertebrates in Intermittent and Perennial Streams: is the Hyporheic Zone a refuge from Drying? Journal of North American Benthological Society, 19(4): 680-696.

Finkenbine, J.K., Atwater, J.W. and Mavinic, D.S., 2000. Stream Health After Urbanization. Journal of American Water Resources Association, 36(5): 1149-1159.

Frizzell, R.L., Zevenbergen, L.W. and Navarro, R., 2004. Stream Channel Restoration at Bridge Sites. Trimble Inc. 6445 Powers Ferrry road, Suite 100, Atlanta, GA 30339, pp. 1-9.

Gilbert, J.J. and Schnuck-Kolben, R.E., 1987. Effects of Proposed Highway Embankment Modifications on Water-Surface Elevations in the Lower Pearl River Flood Plain Near Slidell, Louisiana. 86-4129, U.S. Geological Survey, Baton Rouge.

Gilvear, D.J., Heal, K.V. and Stephen, A., 2002. Hydrology and the Ecological Quality of Scottish River Ecosystems. The Science of the Total Environment, 294: 131-159.

Gregory, K.J. and Brookes, A., 1983. Hydrogeomorphology Downstream from Bridges. Applied Geography, 3: 145-159.

Gregory, K.J. and Walling, D.E., 1987. Human Activity and Environmental Processes, John Wiley & Sons Ltd., West Sussex.

Hadley, R.F. and Emmett, W.W., 1998. Channel Changes Downstream from a Dam. Journal of American Water Resources Association, 34(3): 629-636.

Hamill, L., 1999. Bridge Hydraulics. Routledge, London.

Hancock, P.J., 2002. Human Impacts on the Stream-Groundwater Exchange Zone. Environmental Management, 29(6): 763-781.

Hendrickson, J.G.J., 1964. Hydraulics of Culverts. American Concrete Pipe Association, Chicago.

Henshaw, P.C. and Booth, D.B., 2000. Natural Restabilization of Stream Channels in Urban Watersheds. Journal of American Water Resources Association, 36(3): 1219-1235.

Hinkle, S.R. et al., 2001. Linking the Hyporheic Flow and Nitrogen Cycling near the Willamette River- A large River in Oregon. Journal of Hydrology, 244: 157-180.

Hooke, J.M. and Mant, J.M., 2000. Geomorphological Impacts of a Flood Event on Ephemeral Channels in Spain. Geomorphology, 34: 163-180.

Hupp, C.R. and Simon, A., 1991. Bank Acceration and the Development of Vegetated Depositional Surfaces Along Modified Alluvial Channels. Geomorphology, 4: 111-124.

Johnson, P.A., Hey, R.D., Brown, E.R. and Rosgen, D.L., 2002. Stream Restoration at Bridge Sites. Journal of American Water Resources Association, 38(1): 55-67.

Johnson, P.A., Hey, R.D., Horst, M.W. and Hess, A.J., 2001. Aggradation at Bridges. Journal of Hydraulic Engineering, 127(2): 154-157.

Jones, J.S., Bertolidi, D.A. and Umbrell, E.R., 1999. Interim Procedure for Pressure Flow Scour. In: Richardson and P.F. Lagasse (Editors). Stream Stability and Scour at Highway Bridges. American Society of Civil Engineering.

Levine, J.F. et al., 2003. Distribution of Freshwater Mussel Populations in Relationship to Cossing Structures, North Carolina State University, Raleigh.

Lovell, T.L., Griffith, R.S. and Killen, R., 1988. Changes in Floodplain Management Philosophy and Policy Resulting from the Trinity River Regional Environmental Impact Statement. In: D. Accurti (Editor), Floodplain Harmony. Natural Hazards Research and Applications Center, Nashville, Tenessee, pp. 73-78.

Marshall, M.C. and Hall Jr, R.O., 2004. Hyporheic Invertebrates Affect the N Cycling and Respiration in Stream Sediment Microcosms. Journal of The North American Benthological Society, 23(3): 416-428.

McKenney, R., Jacobson, R.B. and Wetheimer, R.C., 1995. Woody Vegetation and Channel Morphogenesis in Low Gradient Gravel-Bed Streams in the Ozark Plateaus, Missouri and Arkansas. Geomorphology, 13: 175-198.

Olsen, D.A. and Townsend, C.R., 2003. Hyporheic Community Composition in a Gravel-Bed Stream: Influence of vertical Hydrological Exchange, Sediment Structure and Physicochemistry. Freshwater Biology, 48: 1363-1378.

Philippi, N.S., 1996. Floodplain Management-Ecologic and Economic Perspectives. R.G. Landes Company, Chicago.

Poole, K.E. and Downing, J.A., 2004. Relationship of Declining Mussel Biodiversity to Stream Reach and Watershed Characteristics in an Agricultural Landscape. Journal of North American Benthological Society, 23(1): 114-125.

Richardson, E.V. and Richardson, J.R., 1999. Determining Contraction Scour. In: E.V. Richardson and P.F. Lagasse (Editors), Stream Stability and Scour at Highway Bridges, American Society of Engineers, pp. 483-490.

Robert, A., 2003. River Processes. Arnold, London, 214 pp.

Rodzenko, G., Tram, J.J. and Plasencia, D.J., 1988. Loss of Overbank Storage in Floodplain Management. In: D. Accurti (Editor), Floodplain Harmony. Natural Hazards Research and Applications Center, Nashville, Tennessee, pp. 239-247.

Schindler, J.E. and Krabbenhoft, D.P., 1998. The Hyporheic Zone as a Source of Dissolved Organic Carbon and Carbon Gases to a Temperate Forested Stream. Journal of Biochemistry, 43: 157-174.

Schumm, S.A., Mosley, M.P. and E, W.W., 1987. Experimental Fluvial Geomorphology. John Wiley & Sons, West Sussex.

Shibato, H. et al., 2004. Nitrogen Dynamics in the Hyporheic Zone of the Forested Stream During a Small Storm, Hokkaido, Japan. Biochemistry, 69: 83-104.

Simon, A. and Johnson, P.A., 1999. Relative Roles of Long Term Channel Adjustments Processes and Scour on the Reliability of Bridge Foundations. In: E.V. Richardson and P.F. Lagasse (Editors), Stream Stability and Scour at Highway Bridges. American Society of Civil Engineers, pp. 151-165.

Strayer, D.L., 1999. Use of Flow refuges by Unionid Musssels in Rivers. Journal of North American Benthological Society, 18(4): 468-476.

Thorne, C.R., Hey, R.D. and Newson, M.D. (Editors), 1997. Applied Fluvial Geomorphology for River Engineering and Management. John Wiley 7 Sons Ltd, West Sussex, 370 pp.

Tockner, K. and Stanford, J.A., 2002. Riverine Flood Plains: Present State and Future Trends. Environmental Conservation, 29(3): 308-330.

Umbrell, E.R., Young, G.K., Stein, S. and Jones, J.S., 1998. Clear-Water Contraction Scour under Bridges in Pressure Flow. Journal of Hydrologic Engineering: 236-240.

Vannote, R.L. and Minshall, G.W., 1982. Fluvial Processes and Local Lithology Controlling Abundance, Structure, and Composition of Mussel Beds, National Academy of Sciences, pp. 4103-4107.

Viessman, W.J., Lewis, G.L. and Knapp, J.W., 1989. Introduction to Hydrology, Harper & Row, Publishers Inc., New York.

Wellman, J.C., Combs, D.L. and Cook, B., 2000. Long-Term Impacts of Bridge and Culvert Construction or Replacement on Fish Communities and Sediment Characteristics of Streams. Journal of Freshwater Ecology, 15(3): 317-328.

Yanes, M., Velasco, J.M. and Suarez, F., 1995. Permeability of Roads and Railways to Vertebrates: The Importance of Culverts. Biological Conservation, 71: 217-222.

CHAPTER 2:
A comparison of the effects of bridges and culverts on mussels and their habitat

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Introduction

The impacts of bridge and culvert construction on streams and stream biota - specifically fish and aquatic insects - have been well-documented in ecological literature. Short-term effects of bridge and culvert construction activities have been shown to immediately impact stream insects (Lenat et al. 1981; Ogbeibu and Victor 1989; Stout and Coburn 1989) and fish (Whitney and Bailey 1959; Barton 1977). The detrimental effects of sedimentation, a potential consequence of bridge construction, have been studied for decades (Ellis 1936; Chutter 1969; Bruton 1985; Wood and Armitage 1997). Storm events may eventually flush construction-related sediments from a site, and mobile biota such as fish and aquatic insects eventually recover from construction activities (Taylor and Roff 1986), but the long-term impacts of road crossings on freshwater mussels is relatively unknown. Observations by NCDOT and NC Wildlife Resources Commission biologists of decreased mussel abundance downstream of long-standing road crossings gave rise to concern over road-crossing impacts to this fauna.

Over 55 species of freshwater mussels (Bivalvia: Unionidae) inhabit the surface waters of North Carolina (Bogan 2002). Freshwater mussels are an integral part of aquatic ecosystems. They provide food for a variety of terrestrial and aquatic species, and they filter algae, bacteria, sediment, and fine particulate organic matter from the water. These living filters improve water quality and also serve as indicators of pollution and habitat degradation (Goudreau et al. 1993; Foe and Knight, 1987). We know mussels are impacted by sedimentation (Ellis 1936, Marking and Bills 1979), but the biology and ecology of mussels may make them especially susceptible to the long-term effects of bridges and culverts. Freshwater mussels are relatively sessile and will spend years buried in the sediment of a stream or lake actively moving only short distances. Mussels must endure any chemical or physical alterations to their habitat, and they cannot escape disturbances like more mobile fish or aquatic insects. The substrate must contain a sufficient depth of finer sediments, such as sand or gravel, for burrowing but must also be stable during high flows. Scour and shear stress on stream substrates have been associated with reduced mussel abundance (Strayer 1999; Johnson and Brown 2000; Hardison and Layzer 2001). Consequently, mussels are susceptible to any activities that disrupt stream hydrology and geomorphic processes.

Installation of crossing structures may permanently alter the local habitat through channelization, blockage of stream meander, and channel constriction (Little and Mayer 1993; Forman and Alexander 1998). In a study funded by the North Carolina Department of Transportation (NCDOT), we found localized impacts to stream habitat and mussel fauna near bridges in the piedmont of North Carolina (Levine et al. 2003). We found evidence of channel constriction and channelization and loss of pools near bridges and decreased mussel abundance within 50 meters downstream of road crossings. We attributed much of the impacts to channel-constricting bridges constructed in the 1950s and 1960s as well as some potential lingering impacts from the most recent construction. There was also an overall decrease in length of *Elliptio complanata*, a common mussel species, downstream of road crossings compared to upstream. Due to a relatively small number of culverts sampled during the study (N=12) compared to the number of bridges (N=68), we were unable to draw many conclusions about the relative impacts of culverts on mussels.

Culverts are often preferred by transportation agencies over bridges because they last longer and are usually cheaper to install; however, natural resource managers have typically been frowned on the use of culverts because of the potential they have to damage stream habitat. Reduction of the stream's cross-sectional area at culverts heightens downstream scour (Abt et al. 1984), and this can destabilize large reaches of stream and cause bed degradation (Richardson and Richardson 1999, Simon and Johnson 1999). These processes that degrade channel stability would likely impact mussels that rely on stable habitat. The goal of this study was to assess existing culverts and evaluate the degree to which they are affecting habitat and resident mussels, and to compare results with the original study that focused primarily on bridges. The original objectives of this project were to:

1. determine the impact of culverts on the relative abundance, diversity and spatial distribution of freshwater mussels in the North Carolina piedmont;
2. measure essential habitat characteristics to determine the physical impact of culverts;
3. compare newly acquired data to existing data gained in previous surveys of 68 bridges and 12 culverts of various designs, and
4. identify crossing structure design attributes or other factors which may alter the physical or biological impact of road-crossings on streams.

Methods

Site Selection

We used the NCDOT bridge database to identify all culverts on perennial streams within a 120 km radius of our base of operations in the College of Veterinary Medicine at NC State University. Culverts within the Coastal Plain or Sandhills ecoregion were eliminated to maximize similarities with the original study. We also eliminated sites within municipal boundaries to avoid complicating factors of urbanization. We then visited 325 identified culverts from July – September 2003 to determine whether these sites would serve as viable study sites. Culverts within the two study areas of the previous study (Levine et al. 2003) were not visited during this period since they had been scouted before. Viable culvert study sites from the previous study areas were resurveyed as part of this culvert project.

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 have a well defined channel and be free flowing for 150 m upstream and downstream of the road crossing. It could not be swampy habitat or wetland-like or be excessively dammed by humans or beavers.
3. The stream had to have a mussel population. If we found 10 live freshwater mussels in a 20-30 minute search by 2-3 people, the site was considered to meet this criterion. If there was no mussel population at a site, there would be no way to assess the impact of existing crossing structures on mussel populations.
4. Macrohabitat had to be comparable upstream and downstream of the road crossing. Large, naturally occurring differences in stream gradient and substrate upstream and downstream of the road would likely result in inherent differences in the mussel community, and effects of the crossing structure would be difficult to determine.
5. There could not be any obvious landuse practices that would significantly impact the adjacent stream and its fauna in a way that may mask any effect of the culvert.

We originally identified 50 culverts that would serve as viable study sites, but by the time the surveys were to begin in 2004 we had lost 3 sites to forest clear-cutting at the site, 3 sites where beavers had significantly altered the habitat and 1 site that went dry in 2004 and could not be surveyed. We were left with 43 study sites (Table 2.1, Fig. 2.1)

Table 2.1. List of Mussel Survey Study Sites

County	DOT Bridge Number	Culvert Type	Stream	Road Number	Road Name
Alamance	29	Arch	Mill Creek	NC 119	NC 119
Chatham	12	Arch	Terrell's Creek	NC 87	NC 87
Guilford	190	Arch	Rock Creek	US 70	US 70
Orange	13	Arch	South Fork Little River	NC 57	NC 57
Moore	225	Arch	Wolf Creek	SR 1275	Big Oak Church Rd
Wake	135	Arch	Horse Creek	SR 1923	Thompson Mill Rd
Alamance	4	Box	Lick Creek	NC 87	NC 87
Alamance	20	Box	Mary's Creek	NC 87	NC 87
Alamance	338	Box	Poppaw Creek	SR 1113	Foster Store Rd
Franklin	6	Box	Crooked Creek	US 401	US 410
Franklin	16	Box	Norris Creek	NC 39	NC 39
Franklin	62	Box	Fox Creek	NC 56	NC 56
Granville	26	Box	Shelton Creek	US 158	US 158
Granville	28	Box	North Fork Tar River	US 158	US 158
Granville	29	Box	Coon Creek	US 158 Bus.	US 158 Business
Granville	46	Box	Grassy Creek	NC 96	NC 96
Granville	254	Box	Coon Creek	SR 1195	Salem Rd
Granville	268	Box	Coon Creek	US 158	US 158
Guilford	608	Box	Big Alamance Creek	SR 3549	Liberty Rd
Halifax	61	Box	Rocky Swamp	NC 561	NC 561
Harnett	26	Box	Camels Creek	SR 1265	Cool Springs Rd.
Johnston	2052	Box	Buffalo Creek	NC 42	NC42
Montgomery	27	Box	West Fork Little River	NC 134	NC134
Nash	310	Box	Redbud Creek	SR 1321	Redbud Rd
Orange	30	Box	North Fork Little River	NC 57	NC 57
Orange	263	Box	New Hope Creek	I-40	I-40
Randolph	339	Box	Reedy Creek	SR 2867	Jugtown Rd.
Randolph	463	Box	Little Polecat Creek	SR 2114	Providence Church Rd
Wake	134	Box	Horse Creek	SR 1927	Kearney Rd
Wake	561	Box	Terrible Creek	US 401	US 401
Alamance	204	Pipe	Rock Creek	SR 1130	Friendship Patterson Rd
Granville	9	Pipe	Coon Creek	SR 1522	Horner Siding Rd
Granville	116	Pipe	Grassy Creek	SR 1323	Adcock Rd
Granville	177	Pipe	Shelton Creek	SR 1304	Sunset Rd
Granville	217	Pipe	UT Gill's Creek	SR 1515	Mountain Rd
Halifax	110	Pipe	Powell's Creek	SR 1338	Hollister-Glenview Rd
Moore	212	Pipe	Dry Creek	SR 1276	Alton Rd
Moore	220	Pipe	Big Governor's Creek	SR 1651	Old River Rd
Person	38	Pipe	Lick Creek	SR 1121	Willie Gray Rd
Person	211	Pipe	Mayo Creek	SR 1501	Mayo Lake Rd
Randolph	459	Pipe	Reed Creek	SR 2626	Lee Layne Rd
Wake	372	Pipe	Middle Creek	SR 1301	Sunset Lake Rd
Wilson	194	Pipe	Little Creek	SR 1123	Hawley Rd

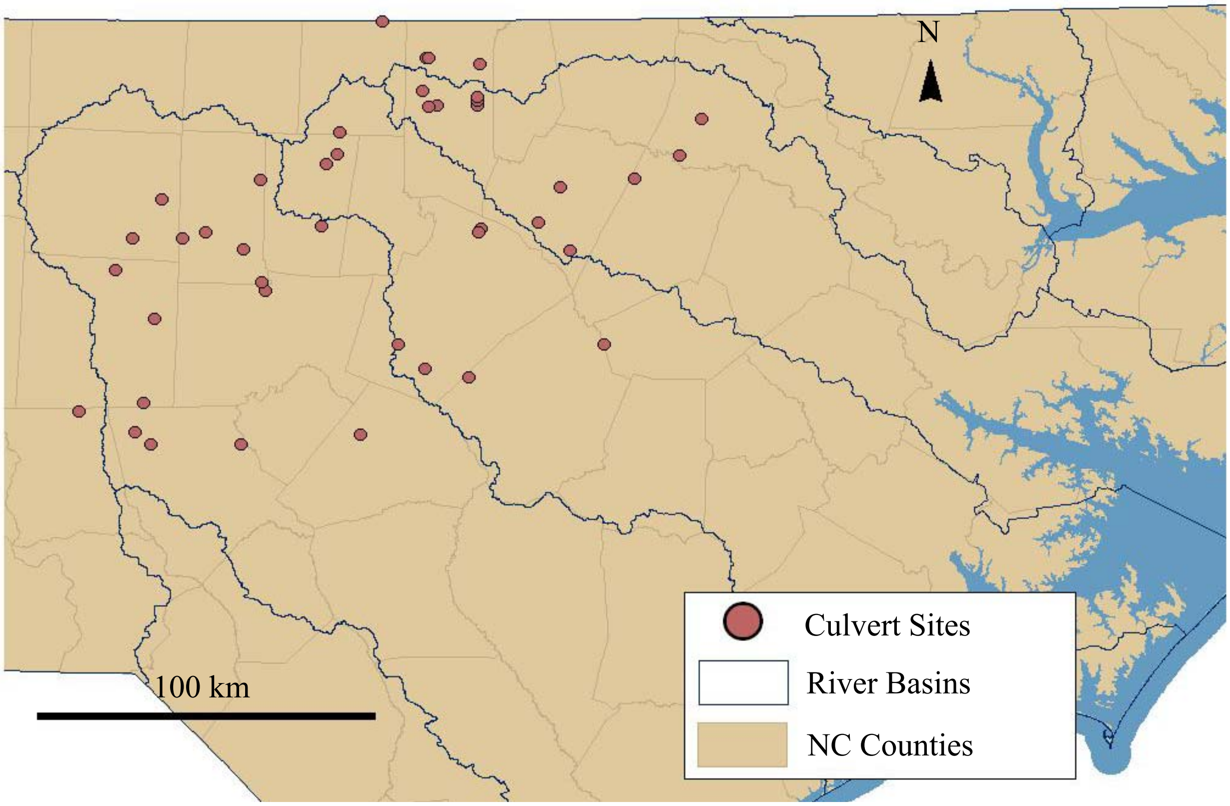

Figure 2.1. Map of Culvert Study Sites

Description of Study Sites

There were 4 sites in the Roanoke basin, 13 sites in the Tar-Pamlico, 9 in the Neuse, 16 in the Cape Fear, and 1 in the Yadkin-Pee Dee basin. When divided by soil system (Daniels et al. 1999), there were 9 sites in the Mixed Felsic and Mafic system, 20 in the Carolina Slate Belt, 5 in the Felsic Crystalline, 1 in the Triassic Basin, and 8 in the Upper Coastal Plain and Piedmont system (Fig. 2.2). We surveyed a total of 24 box culverts, 13 pipe culverts and 6 arch culverts. Median age of pipe culverts was 16 years (Quartiles: 12 and 40 years), while boxes (Median age = 59, Quartiles: 37 and 71 years) and arches (Median age = 67 years, Quartiles: 27 and 73 years) were much older. This correlation in culvert age and type prevented separation of the effects of these two variables in our analyses. Median bankfull width of all culvert sites was 6.9 m and ranged from 4.3 to 16.5 m. Median watershed area above all culverts was 23.6 km^2 and ranged from 2.0 to 143.7 km^2. Detailed descriptions of each site can be found in Appendix II.

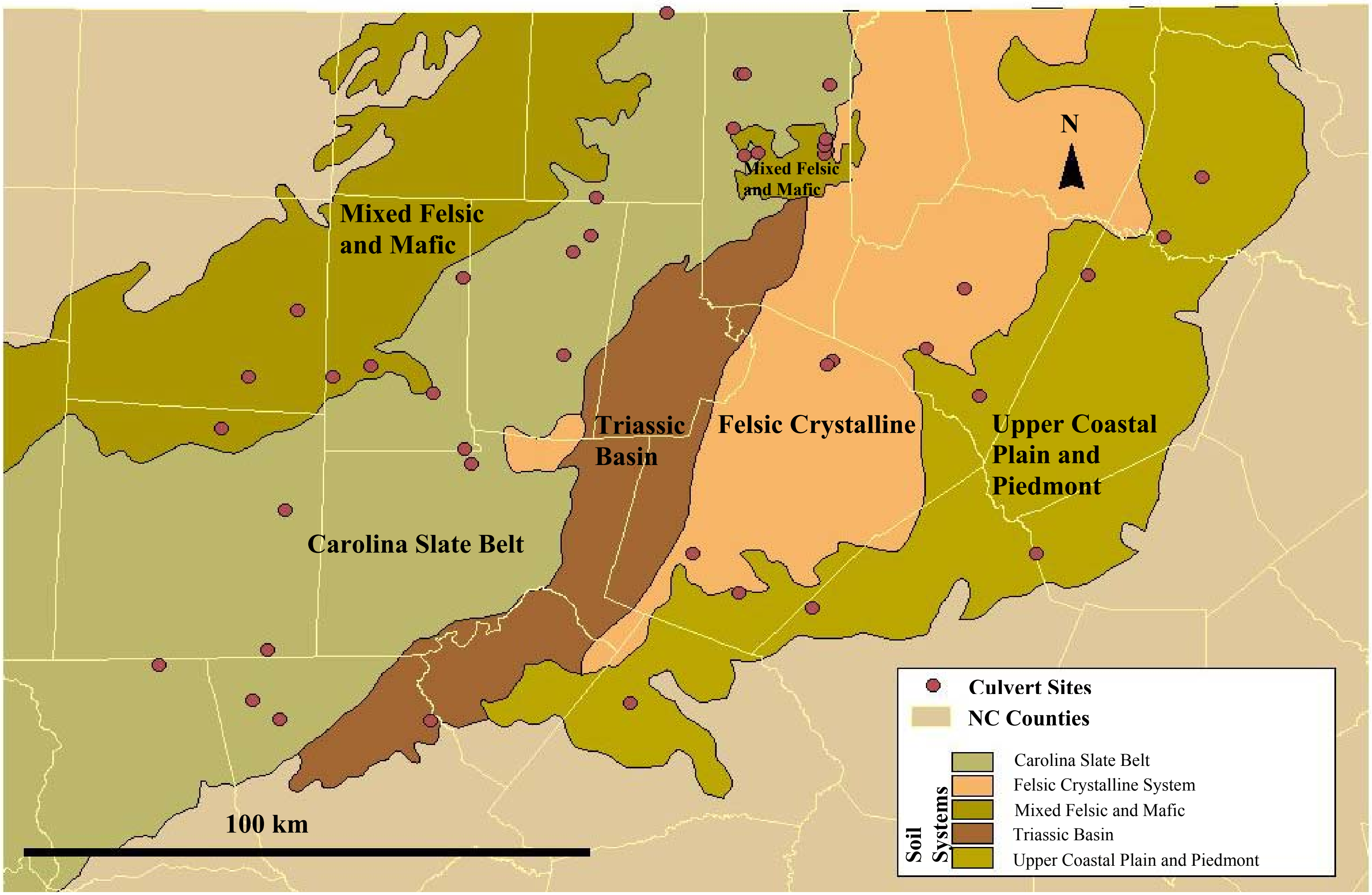

Figure 2.2. Map of Culvert sites and Soil Systems

Study Site Setup

In our previous study (Levine et al. 2003), we found that the detectable impacts of these road crossings were within 50 – 100 m from the structure. Because of this, study sites were shortened to encompass only the 150-m reaches immediately upstream and downstream of culverts. Before sites were surveyed for mussel and habitat data, this 300-m stream reach was divided into twelve 25-m sections and numbered from 1 to 12 with 1 being at the most upstream end, 12 at the most downstream end, and the culvert dividing sections 6 and 7 (Fig. 2.3). These sections were delineated by measuring down the middle of the channel with a measuring tape and flagging the banks at divisions between the sections.

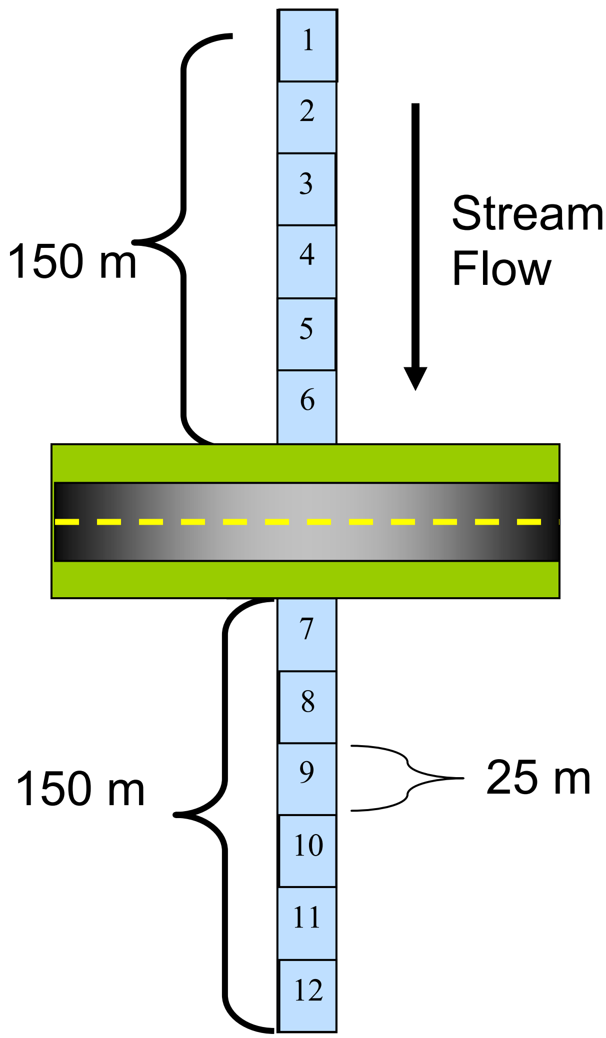

Figure 2.3. Diagram of study site layout.

Habitat

At each site, we estimated the percentage of each 25-m section that was either pool, riffle, or run, and we documented the dominant and subdominant substrate types (clay, silt, sand, gravel, cobble, boulder, or bedrock) in each habitat unit. At the delineating marks for each 25-m section, we measured bankfull width and bank height from the thalweg to the top of each bank. We also used EPA Rapid Bioassessment Protocols (Barbour et al. 1999) to score bank stability for each section. Photographs were taken of the upstream and downstream views of each culvert as well as the adjacent habitats above and below the structure. We noted the presence or absence of any obvious scour pool on the downstream end of the culvert and measured its length. Because the original bridge sites were primarily in the Carolina Slate Belt, we visited 11 randomly selected bridge sites in the Upper Coastal Plain and Piedmont soil system. We took photographs and made observations on potential habitat alterations around these bridges in another soil system.

Water Quality

At each site, on the day it was surveyed, we measured routine water chemistry parameters (temperature, pH, dissolved oxygen and conductivity) to document any potential serious water quality problems at the site.

Mussel Survey

Mussel surveys at culvert sites were conducted from 27 April – 24 August 2004 and from 27 April – 24 May 2005. In 2005, we resurveyed 4 randomly selected bridge sites and 4 randomly selected culvert sites that were surveyed in 2004. To conduct a survey, 3 people each searched 1-m-wide longitudinal 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 300-m stream reach at each site as well as within culverts that had enough natural light to allow searching. We standardized transect width by measuring against the arm-span of each surveyor to establish a reference point by which they would measure a 1-m width. Those searching along banks used this reference point to measure their lane from the water's edge, and surveyors in the center of the stream measured from the centerline of their body always moving upstream in a straight line.

We picked up all mussels located within the longitudinal transects, and no mussels were included in survey data that fell outside these transects. To maximize consistency through time and between surveyors, only visual searches were done, 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. When mussels were collected, we identified them and used calipers to measure length, width, and height to the nearest mm on the first 15 of each species collected from each 25-m section. We recorded the cross-section number and linear transect (left bank, middle, right bank) in which the mussel was located. Lampsilines were classified as male or female by shell shape, and we checked for gravidity in all known females. Mussels were then 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 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 to the downstream end and searching up to the road crossing. A measure of detectability was also taken in a predetermined 50-m reach at each site by removing all mussels found in the bank transects and using a 2nd 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 both passes.

Data Analysis

The statistical package Minitab 13.30 was used for all statistical tests. We reanalyzed mussel survey data from bridge sites in the original study. We only used sites (N = 51) with a watershed size within the range of watershed sizes in the culvert study. Because study sites in the original bridge study were twice as long, we eliminated data from the stream reaches that would fall outside of the culvert study site set up (upper and lowermost 150-m).

In addition to making comparisons between upstream and downstream and between 25-m sections, we used a combination of habitat and mussel data to designate all bridges and culverts as high impact, low impact or no detectable impact. Crossing structures designated as having high impact were those that had substantially fewer mussels and/or substantial habitat alteration downstream for greater than 75 m. We designated crossing structures as low impact if the apparent impact on habitat and relative mussel abundance was within 25 to 75 m of the structure. Where there were no obvious trends in mussel or habitat data that would indicate impact, we designated a site as having no detectable impact. In several cases, sites with an overall low abundance of mussels fell into this category because no trends could be detected that could be related to the crossing structure. These designations represent our best guess as scientists based on observation and a single survey.

To understand impacts on rare species, we examined trends in distribution in relation to *Elliptio sp.* at sites where 10 or more individuals of a rare species was found.

Results

Habitat

Habitat Type – Habitat within culverts was vastly different than habitat under bridges. Only 13 of the 43 culverts sampled (30.2%) had natural substrate within them, and no pipe culverts had natural substrate. The length of stream within culverts ranged from 10.1 to 82.9 m with a median of 18.3 m (25 and 75% quartiles = 15.4, 25.0).

There were no differences in dominant substrate between upstream and downstream or between 25-m sections. There were changes in habitat type at sampled streams that we could be attributed to culverts. Overall upstream and downstream reaches were not significantly different in amount of either pool ($p = 0.102$), riffle ($p = 0.976$), or run ($p = 0.104$) habitat (Paired T-test). There was, however, a clear trend of habitat modification around culverts. There was more pool immediately adjacent to culverts, and there tended to be an increase in riffle and run habitat 50 m downstream of the culverts (Fig. 2.4). In fact, there were significant differences in amount of run habitat between 25-m sections ($p = 0.025$, Friedman Test). Differences in pool ($p = 0.114$) and riffle ($p = 0.807$) between 25-m sections were not statistically different.

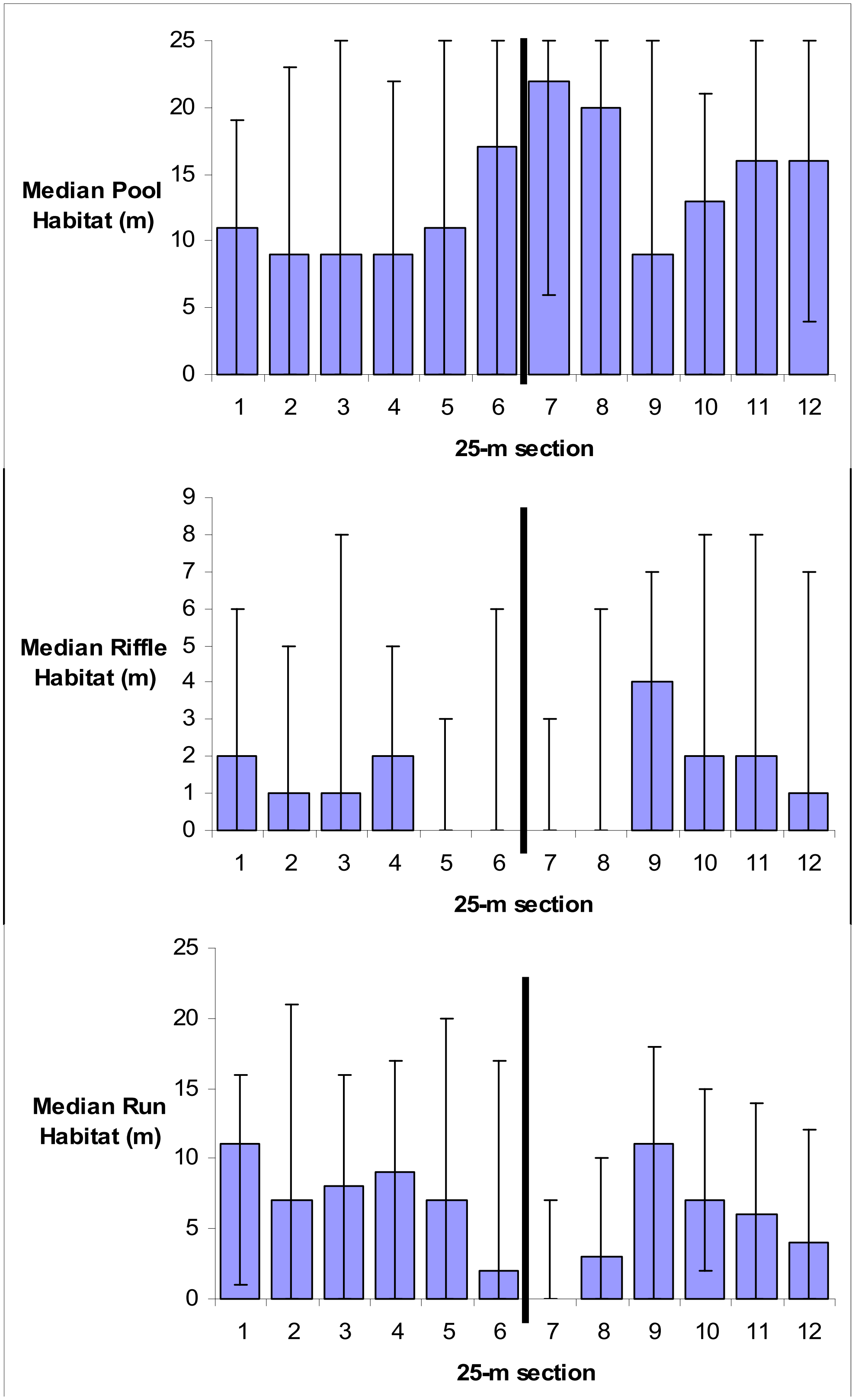

Figure 2.4. Median length of pool, riffle and run habitat in all 25-m sections (N=43). Error bars represent 25 and 75% quartiles. Section 1 is at the most upstream end of the sampling site and section 12 is at the most downstream end. The vertical line represents the location of the culvert.

Habitat Quality – Channel width downstream (mean = 7.57 m, SD = 2.6 m) was significantly greater than channel width upstream of culverts (mean = 7.28 m, SD = 3.0 m) (GLM). There were seven individual sites that had significantly wider banks downstream compared to upstream ($p < 0.05$) and only two sites with a significantly wider channel upstream ($p < 0.05$, Table 2.2). Four other additional sites had marginally significant ($0.05 < p < 0.10$) with 3 of those being wider upstream than down (Table 2.2). Of the 8 culverts sampled in the Upper Coastal Plain and Piedmont soil system, 6 of them (75%) had wider channels downstream of the culverts compared to upstream ($p < 0.10$). In addition, there were also significant differences in channel width between 25-m sections overall ($p = 0.005$, GLM) indicating a widening of the channel immediately below the culvert (Fig. 2.5).

Table. 2.2. Sites with significant differences in channel width between upstream and downstream of the culvert.

Site	Is the channel wider upstream or down?	Culvert Type	Soil System	*p*-value
Franklin 16	Downstream	Box	Upper Coastal/Piedmont	0.0217
Granville 254	Downstream	Box	Mixed Felsic/Mafic	0.006
Halifax 61	Downstream	Box	Upper Coastal/Piedmont	0.0449
Johnston 2052	Downstream	Box	Upper Coastal/Piedmont	0.0079
Moore 220	Upstream	Pipe	Triassic Basin	0.0073
Moore 225	Downstream	Pipe	Carolina Slate Belt	0.0281
Nash 310	Downstream	Box	Upper Coastal/Piedmont	0.0255
Randolph 459	Upstream	Pipe	Carolina Slate Belt	0.0073
Wake 561	Downstream	Box	Upper Coastal/Piedmont	0.0117
Marginally Significant				
Alamance 338 (up)	Upstream	Box	Carolina Slate Belt	*0.0881*
Chatham 12 (up)	Upstream	Arch	Carolina Slate Belt	*0.0742*
Harnett 26 (down)	Downstream	Box	Upper Coastal/Piedmont	*0.0865*
Wake 135 (up)	Upstream	Arch	Felsic Crystalline	*0.0531*

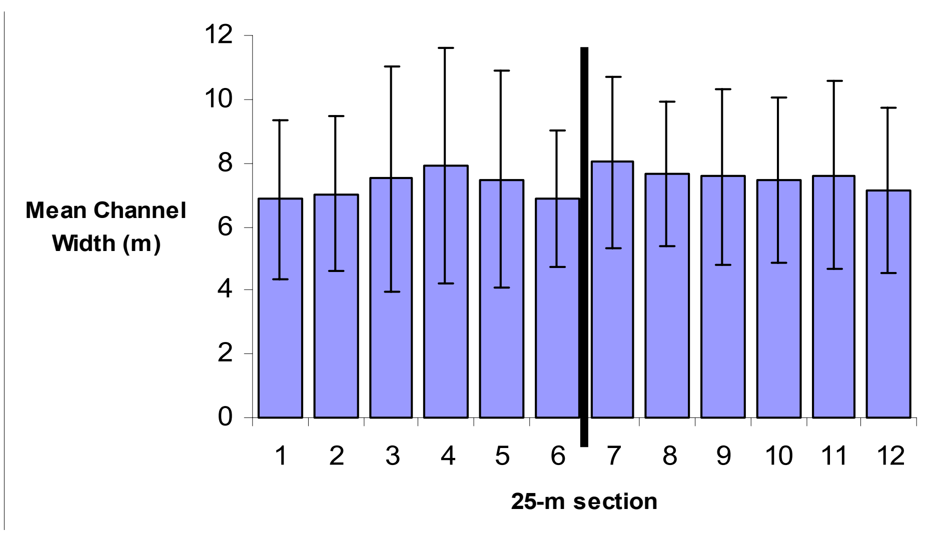

Figure 2.5. Mean Channel width ± SD at each 25-m section (N=43). Section 1 is at the most upstream end of the sampling site and section 12 is at the most downstream end. The vertical line represents the location of the culvert.

There were no significant differences in channel width at bridge sites from the previous study between upstream and downstream (p = 0.423, GLM) or between 25-m sections (0.611, GLM) (N=22). Of the 4 bridges that had significantly different channel widths between upstream and downstream (p<0.05), 2 had wider channels upstream and 2 had wider channels downstream.

Mean bank height downstream (1.72 ± 0.5 m) was also significantly different than bank height upstream (1.59 ± 0.6 m) (p = 0<0.001, GLM). There were 7 sites with statistically higher bank heights downstream and only 1 site with higher banks upstream (p < 0.10) (Table 2.3). There were also significant differences in bank height between cross-sections (p = 0.037, GLM) (Fig. 2.6). Bridge sites from the previous study (N=22) had no significant differences in bank height between upstream and downstream reaches (p = 0.356) or between 25-m sections (p = 0.292, GLM). Only one individual bridge site had significantly higher bank heights downstream than upstream (p = 0.011, t-test)

Table 2.3. Sites with significant differences in bank height between upstream and downstream of the culvert.

Site	Are the banks higher upstream or down?	Culvert type	Soil System	*p*-value
Chatham 12	Downstream	Arch	Carolina Slate Belt	0.0003
Franklin 6	Downstream	Box	Felsic Crystalline	0.0066
Granville 217	Downstream	Pipe	Carolina Slate Belt	0.0107
Moore 212	Downstream	Pipe	Carolina Slate Belt	0.0078
Wake 135	Upstream	Arch	Felsic Crystalline	0.0033
Marginally significant				
Granville 46	Downstream	Box	Carolina Slate Belt	0.0538
Orange 13	Downstream	Arch	Carolina Slate Belt	0.0526
Person 38	Downstream	Pipe	Carolina Slate Belt	0.0526

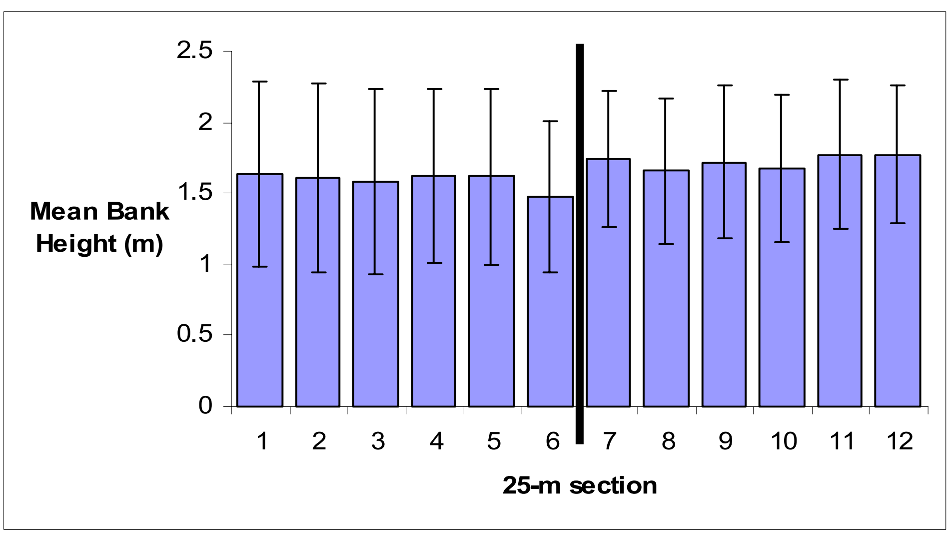

Figure 2.6. Mean Bank Height ± SD at each 25-m section (N=43). Section 1 is at the most upstream end of the sampling site and section 12 is at the most downstream end. The vertical line represents the location of the culvert.

Bank stability scores were statistically higher (p = 0.045, Friedman) upstream (median = 4.0, 25 and 75% quartiles = 2.5 and 5.5) than downstream (median = 3.5, 25 and 75% quartiles = 2.5 and 5.0). There were also significant differences (p < 0.001, Friedman) between 25-m sections with the highest bank stability scores being adjacent to the culvert and the lowest scores being in the most downstream reaches of the study site (Fig. 2.7).

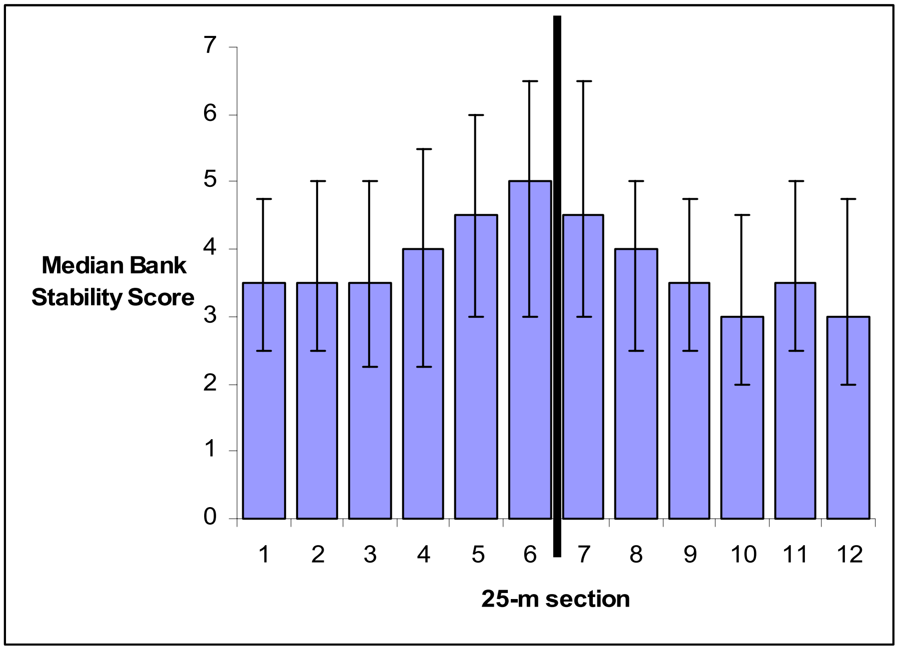

Figure 2.7. Median bank stability scores for each 25-meter section. Error bars represent 25 and 75% quartiles. Section 1 is at the most upstream end of the sampling site and section 12 is at the most downstream end. The vertical line represents the location of the culvert.

A total of 21 of the 43 culverts (48.8%) were found to have obvious scour pools downstream of the culvert. We found scour at 76.9% of pipe culverts (N=13) and only 44.0% of box culverts (N=25). These proportions were significantly different (p = 0.032, proportion test). None of the 6 arch culverts tested had obvious scour pools downstream. Scour was quite prevalent in the Upper Coastal Plain and Piedmont soil system with 7 of the 8 culverts tested showing significant scour (Table 2.4). Scour pools in this soil system ranged from 20 to 50 m and had a mean of 30.3 ± 11.1 m. Overall, scour pools ranged from 5 to 50 m in length with a mean of 22.3 ± 12.6 m.

Table 2.4 Number of Culverts with downstream scour in each soil system.

Soil Type	Number of Culverts with scour downstream	Total Number of Culverts Sampled	Percentage of Culverts with scour downstream
Mixed Felsic and Mafic	2	9	22.2%
Carolina Slate Belt	6	20	30%
Triassic Basin	1	1	100%
Felsic Crystalline	3	5	60%
Upper Coastal Plain and Piedmont	7	8	87.5%

<u>Bridges in the Upper Coastal Plain and Piedmont Soil System</u> – Overall, we observed less habitat alteration at bridges compared to culverts in the Upper Coastal Plain and Piedmont soil system in the vicinity of our culvert sites (Figs. 2.8 – 2.11). However, bridges that were older and overly narrow tended to cause downstream scour.

Figure 2.8. A well constructed bridge in the Upper Coastal Plain/Piedmont soil system that does not constrict channel. Downstream habitat appears very similar to upstream.

Figure 2.9. A widened channel at this bridge in the Upper Coastal Plain/Piedmont soil system creates some local deposition but does not constrict the channel and scour downstream habitat.

Figure 2.10. This older constricting bridge has caused significant scour downstream

Fig. 2.11. The channel is much shallower and more narrow in the upstream reach.

Mussel Survey

Mussel Abundance – During this culvert study, we found a total of 30,059 mussels and 29,310 of them (97.5%) were *Elliptio spp.* (almost all *E. complanata* complex). Therefore, relative mussel abundance analyses are almost entirely driven by the number of *E. complanata* found. There were significantly more mussels in the upstream reach (median = 135, 25 and 75% quartiles = 52 and 553) than in the downstream reach (median = 98, 25 and 75% quartiles = 24 and 393) ($p = 0.026$, Wilcoxon Signed Rank Test). Of the 43 culverts sampled, we found more mussels upstream than downstream at 28 of them (65.1%). There were also significant differences ($p < 0.001$, Friedman test) between 25-m sections with the most mussels being found between 75 and 125 m upstream of the culverts and the fewest being found just downstream of the culverts (Fig. 2.12). We designated a total of 15 culverts as high impact, 15 as low impact and 13 as having no detectable impact.

We found no significant difference between the number of mussels found upstream and downstream of bridge sites ($p = 0.353$, Wilcoxon Signed Rank Test). There were differences between 25-m sections at bridge sites ($p < 0.001$, Friedman test), but the differences were much less dramatic than at culvert sites (Fig. 2.13). We designated a total of 4 bridges as having high impact 19 as having low impact and 28 as having no detectable impact. Overall, the impact of our culvert sites on mussel distribution was much greater than that of our bridge sites.

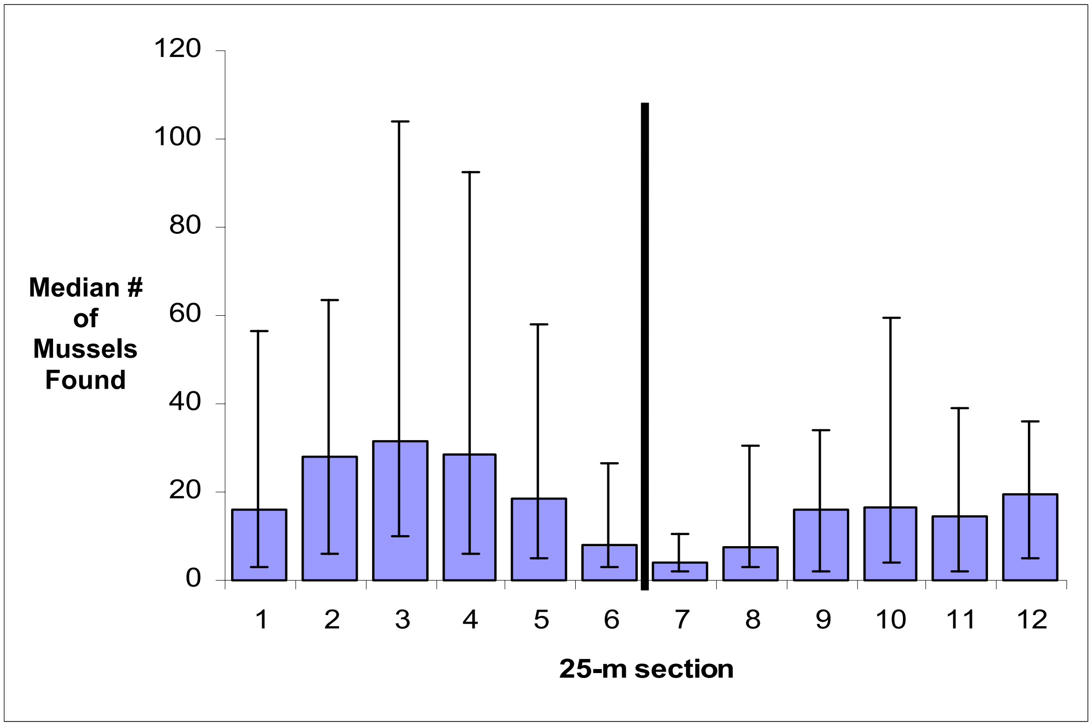

Figure 2. 12. Median number of mussels found in each 25-m section of culvert sites (N=43). Error bars represent 25 and 75% quartiles. Section 1 is at the most upstream end of the sampling site and section 12 is at the most downstream end. The vertical line represents the location of the culvert.

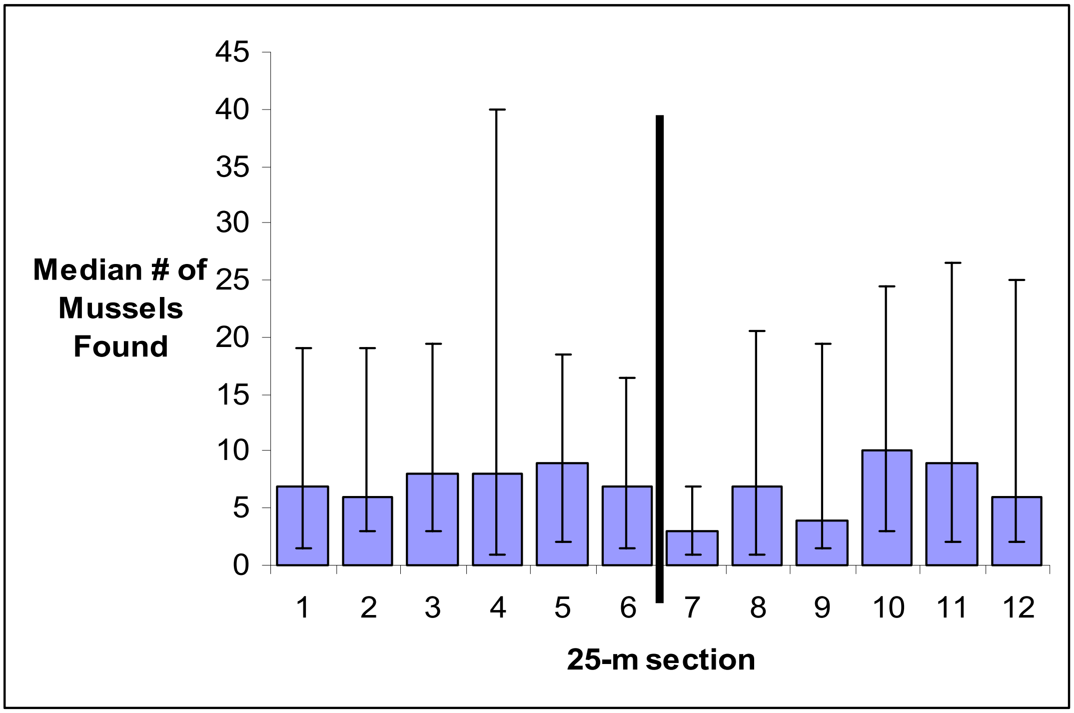

Figure 2.13. Median number of mussels found in each 25-m section at bridge sites in original study (N=51). Error bars represent 25 and 75% quartiles. Section 1 is at the most upstream end of the sampling site and section 12 is at the most downstream end. The vertical line represents the location of the bridge.

Soil System Effects on Mussel Distribution – Mussel distribution at culvert sites was greatly affected by the Soil System in which the site was located. Culvert sites in the Upper Coastal Plain and Piedmont Soil System tended to have substantially fewer mussels downstream of the culvert, and we designated 7 of the 8 culverts in this soil system to be in the high impact category.

Overall, the Carolina Slate Belt seemed to be least impacted by the culverts (Table, 2.5, Fig. 2.14), but there a high amount of variation in impact within this soil system. Within the Slate Belt, 4 of the 6 sites in Granville County were designated as high impact, and the other 2 were designated as low impact. Outside of Granville County within this soil system, we designated 1 site as high impact, 6 as low impact and 7 as no impact detected. The original bridge study was located almost entirely in the Carolina Slate Belt with only 4 of the 51 comparable sites to this study falling outside this soil system. There was only one bridge site located in Granville County and it was located in the Felsic Crystalline System. Culvert impacts on mussel populations seemed relatively similar to impacts at existing bridges in the Slate Belt outside of Granville County.

Table 2.5. Number of culverts designated as high impact, low impact or no detected impact in each of the soil systems in the study.

Soil System	High Impact	Low Impact	No Detected Impact
Carolina Slate Belt	5	8	7
Mixed Felsic and Mafic	2	2	5
Triassic Basin	0	0	1
Felsic Crystalline	1	4	0
Upper Coastal Plain/Piedmont	7	8	0

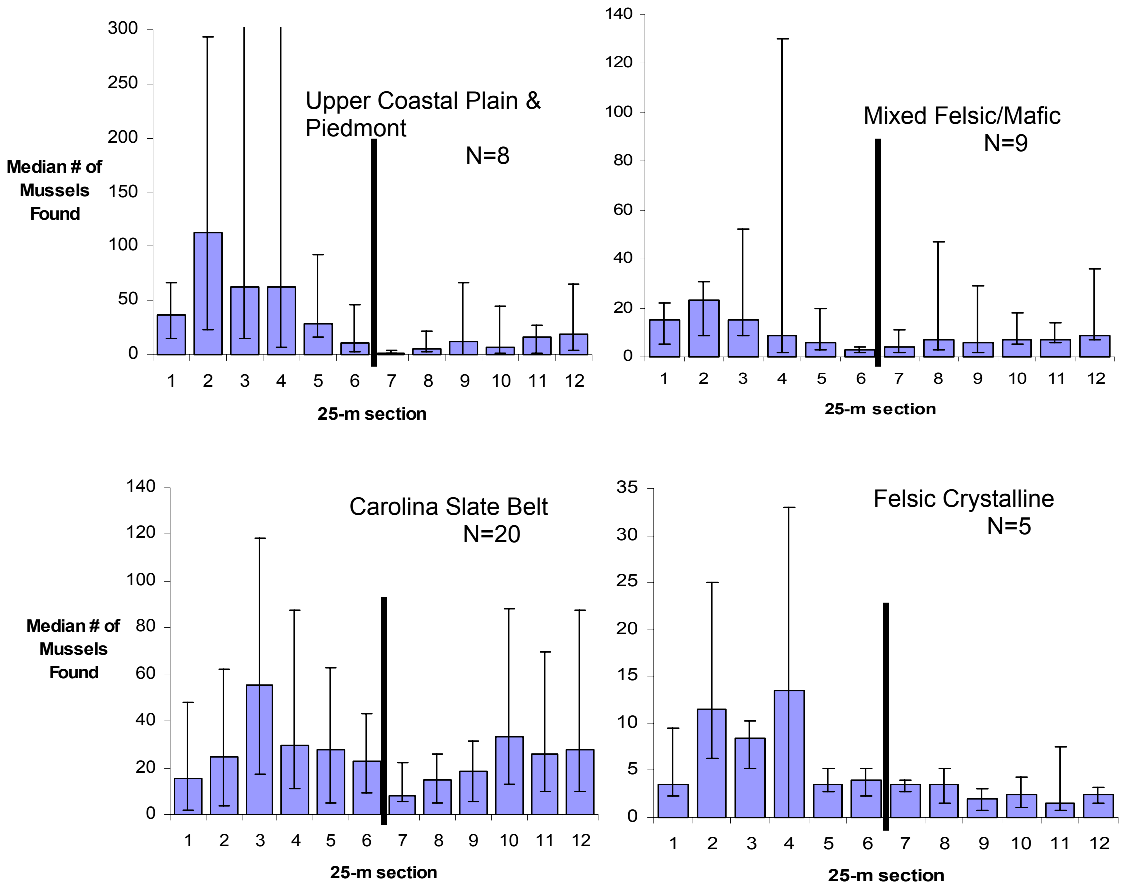

Figure 2.14. Median number of mussels found in each 25-m section in the various soil systems represented in the study. Error bars represent 25 and 75% quartiles. Section 1 is at the most upstream end of the sampling site and section 12 is at the most downstream end. The vertical line represents the location of the culvert. Because of the low sample size, we did not graph the one site in the Triassic Basin soil system.

<u>Scour Effects on Mussel Distribution</u> – The amount of scour downstream was related to the number of mussels downstream. There were 15 sites that had scour pools 19 m in length or greater, and 14 of those (93.3%) had more mussels upstream than downstream. We noted 6 sites that had smaller scour pools (< 19 m), and only 1 of those (16.7%) had more mussels upstream than downstream. Of the 22 other sites that had not obvious scour pool, 13 of them (59.1%) had more mussels upstream.

<u>Culvert Design Effects of Mussel Distribution</u> – We found that 19 of the 24 box culverts (79.2%) had more mussels upstream than downstream. Only 7 of the 13 pipe culverts (53.8%) had more mussels upstream than downstream, but these proportions were not significantly different from each other ($p = 0.116$, proportion test). Of the 6 arches tested, 4 of them (66.7%) had more mussels upstream. The mean percentage of mussels at a site that were found upstream of the culvert was 65.1 ± 21.3% for boxes, 50.9 ± 28.7% for pipes, and 49.4 ± 18.0% for arches, but none of these were statistically different ($p = 0.120$, Kruskall-Wallis).

Mussel Diversity –As in the previous bridge study, we found very few individuals of species other than *E. complanata* (total of 749). Because of this small sample size, we could not conduct direct comparisons of abundance of rare species upstream and downstream of culverts or between 25-m sections. We did examine distribution of these rare species in relation to the common *E. complanata* at sites where we found more than 10 individuals of a given species. At 5 of the 7 sites where it was more abundant, *Pygandon cataracta* distribution was opposite that of E. complanata in relation to the culvert (Table 2.6). *Alasmidonta heterodon* was the only other species that ever exhibited a distribution in relation to the culvert different than that of E. complanata. However, at 1 of the 2 sites where *A. heterodon* was abundant, distribution very strongly mirrored that of *E. complanata*. At the other site, both species were fairly evenly distributed above and below the culvert with only small differences in their distribution.

Table 2.6. Distribution of species other than *Elliptio complanata* at sites where there were 10 or more individuals of that species found. We compare distributions of these species to that of *E. complanata* at the given study site.

Species	Site where it was abundant	Number found (upstream / downstream)	Number of *E. complanata* found (upstream / downstream)	Was the species more abundant in a different reach than *E. complanata*?
Alasmidonta heterodon	Halifax 61	92/1	4081/89	No
Alasmidonta heterodon	Halifax 61	16/27	576/518	Yes
Lampsilis sp. (Tar/Neuse)	Granville 177	66/9	1209/72	No
Pyganodon cataracta	Chatham 12	6/16	304/94	Yes
Pyganodon cataracta	Orange 263	4/28	639/490	Yes
Pyganodon cataracta	Randolph 339	2/14	19/9	Yes
Pyganodon cataracta	Randolph 459	13/4	256/355	Yes
Pyganodon cataracta	Person 38	14/5	553/539	No
Pyganodon cataracta	Alamance 338	1/11	582/316	Yes
Pyganodon cataracta	Granville 29	8/3	52/25	No
Strophitus undulatus	Granville 26	15/10	576/518	No
Villosa constricta	Alamance 29	4/10	1025/1939	No
Villosa constricta	Moore 212	39/14	341/260	No
Villosa delumbis	Alamance 29	2/10	1025/1939	No
Villosa delumbis	Moore 212	15/15	341/260	Even split
Villosa delumbis	Orange 263	11/8	639/490	No
Villosa delumbis	Randolph 459	3/7	256/355	No
Villosa vaughaniana	Alamance 29	12/33	1025/1939	No
Villosa vaughaniana	Moore 212	46/29	341/260	No

Mussel Length, Width and Height - There were small - but statistically significant - differences in length, width, and height of *E. complanata* between upstream and downstream and between 25-m sections ($p > 0.05$, GLM, Table 2.7). In each case, the 25-m reach immediately downstream of the culvert had the lowest means of these metrics (Figs. 2.15-2.17). There also seemed to be an overall gradient effect in these metrics for 50-75 m upstream of the culverts. Width seemed to be most affected by the culvert with a clear downstream effect as well as the upstream effect. Additionally, length/width ratios were significantly higher downstream than upstream meaning that on average, mussels were wider upstream than downstream (Fig. 2.18).

Table 2.7. Mean length, width and height of *Elliptio complanata* upstream and downstream of culvert sites. The p-values presented are the result of a GLM test comparing upstream and downstream values blocked by site.

Metric	Overall Upstream Mean ± SD (mm)	Overall Downstream Mean ± SD (mm)	*p*-value
Length	73.98 ± 14.49	73.99 ± 14.70	0.016
Width	23.49 ± 4.93	23.27 ± 4.89	< 0.001
Height	41.98 ± 8.54	41.91 ± 8.42	0.010
Length/Width ratio	3.18 ± 0.40	3.22 ± 0.41	< 0.001

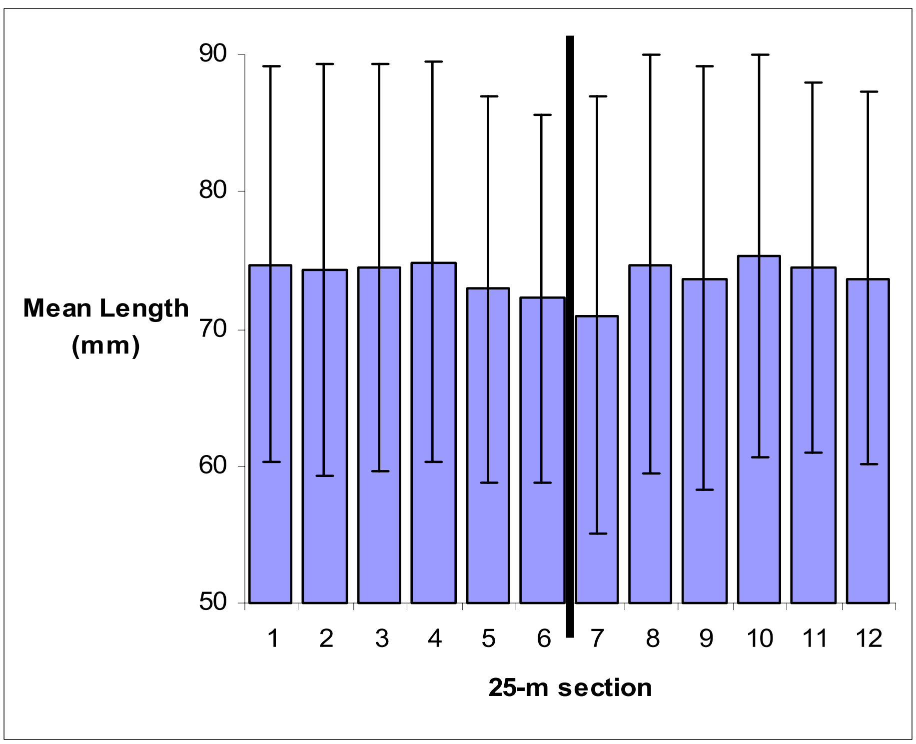

Figure 2.15. Mean length ± SD of *Elliptio complanata* in each 25-m section. Note that the Y-axis begins at 50 mm. Section 1 is at the most upstream end of the sampling site and section 12 is at the most downstream end. The vertical line represents the location of the culvert.

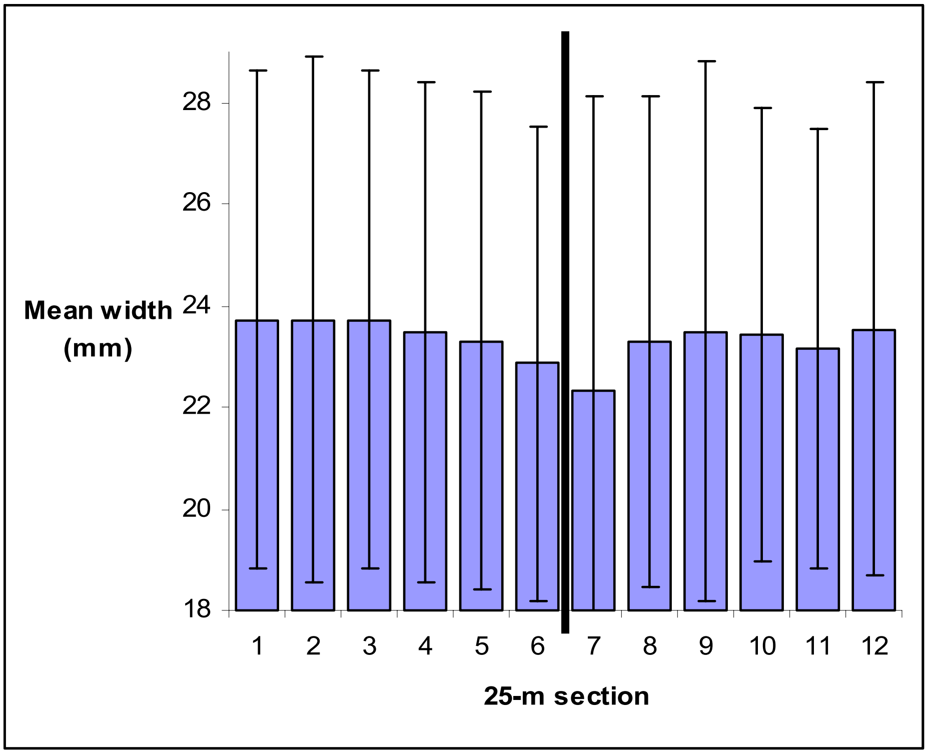

Figure 2.16. Mean width ± SD of *Elliptio complanata* in each 25-m section. Note that the Y-axis begins at 18 mm. Section 1 is at the most upstream end of the sampling site and section 12 is at the most downstream end. The vertical line represents the location of the culvert.

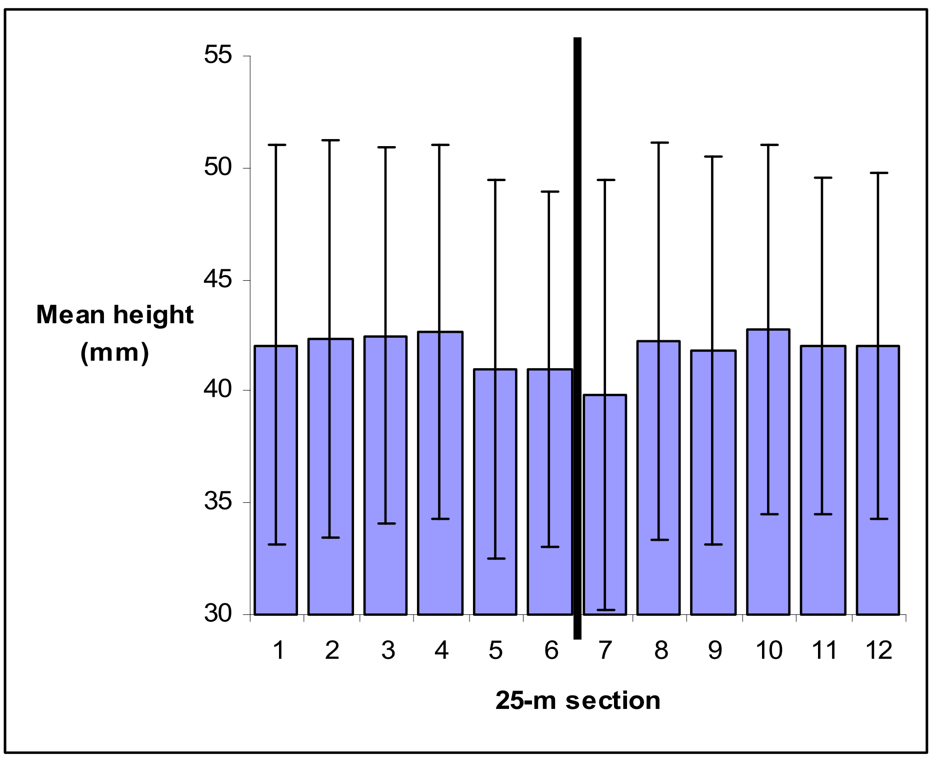

Figure 2.17. Mean height ± SD of *Elliptio complanata* in each 25-m section. Note that the Y-axis begins at 30 mm. Section 1 is at the most upstream end of the sampling site and section 12 is at the most downstream end. The vertical line represents the location of the culvert.

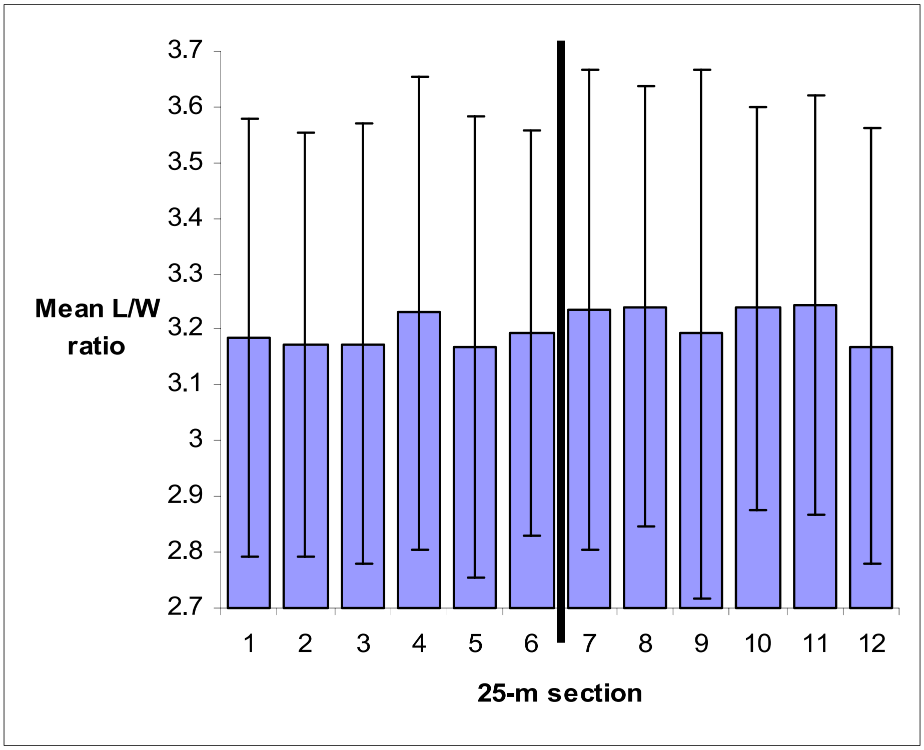

Figure 2.18. Mean Length/Width ratios ± SD of *Elliptio complanata* in each 25-m section. Note that the Y-axis begins at 2.7. Section 1 is at the most upstream end of the sampling site and section 12 is at the most downstream end. The vertical line represents the location of the culvert.

Data Quality Assurance

Detectability - Overall detectability was good during the culvert study (median = 90.0%, 25 and 75% quartiles = 80.0 and 100.0%). There were no significant differences between individual surveyors ($p = 0.410$, Kruskall-Wallis), and median detectability for individuals ranged from 84.1% (25 and 75% quartiles = 66.7 and 100%) to 91.1% (25 and 75% quartiles = 82.6 and 100.0%). Detectability was also similar to that in the original bridge study, and there were no significant differences between all surveyors from both studies ($N = 10$, $p = 0.529$, Kruskall-Wallis).

Site Resurveys – We saw a high degree of repeatability in our bridge and culvert sites that we resurveyed. Within the bridge sites, which were surveyed 4 years after the original survey, 3 of the 4 had very similar distribution in relation to the structure in both surveys (Fig. 2.19). One bridge site (Person 80) had experienced some habitat changes, and there were more mussels immediately downstream of the bridge in 2005 compared to the original survey 4 years earlier. All culvert sites had very similar distribution between years (Fig. 2.20)

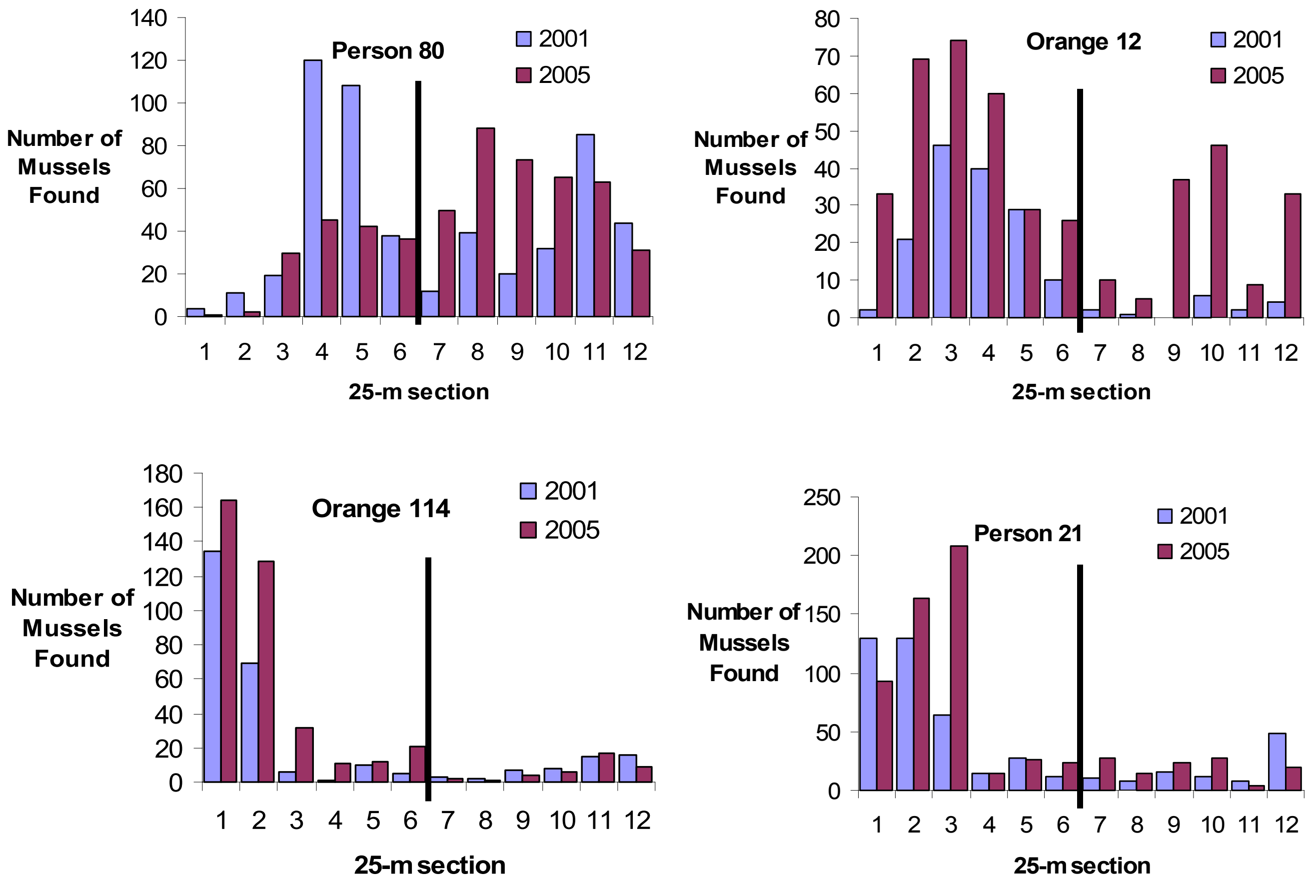

Figure 2.19. Number of mussels found in each 25-m section in surveys of 4 randomly selected bridge sites in 2001 and in 2005. Section 1 is at the most upstream end of the sampling site and section 12 is at the most downstream end. The vertical line represents the location of the bridge.

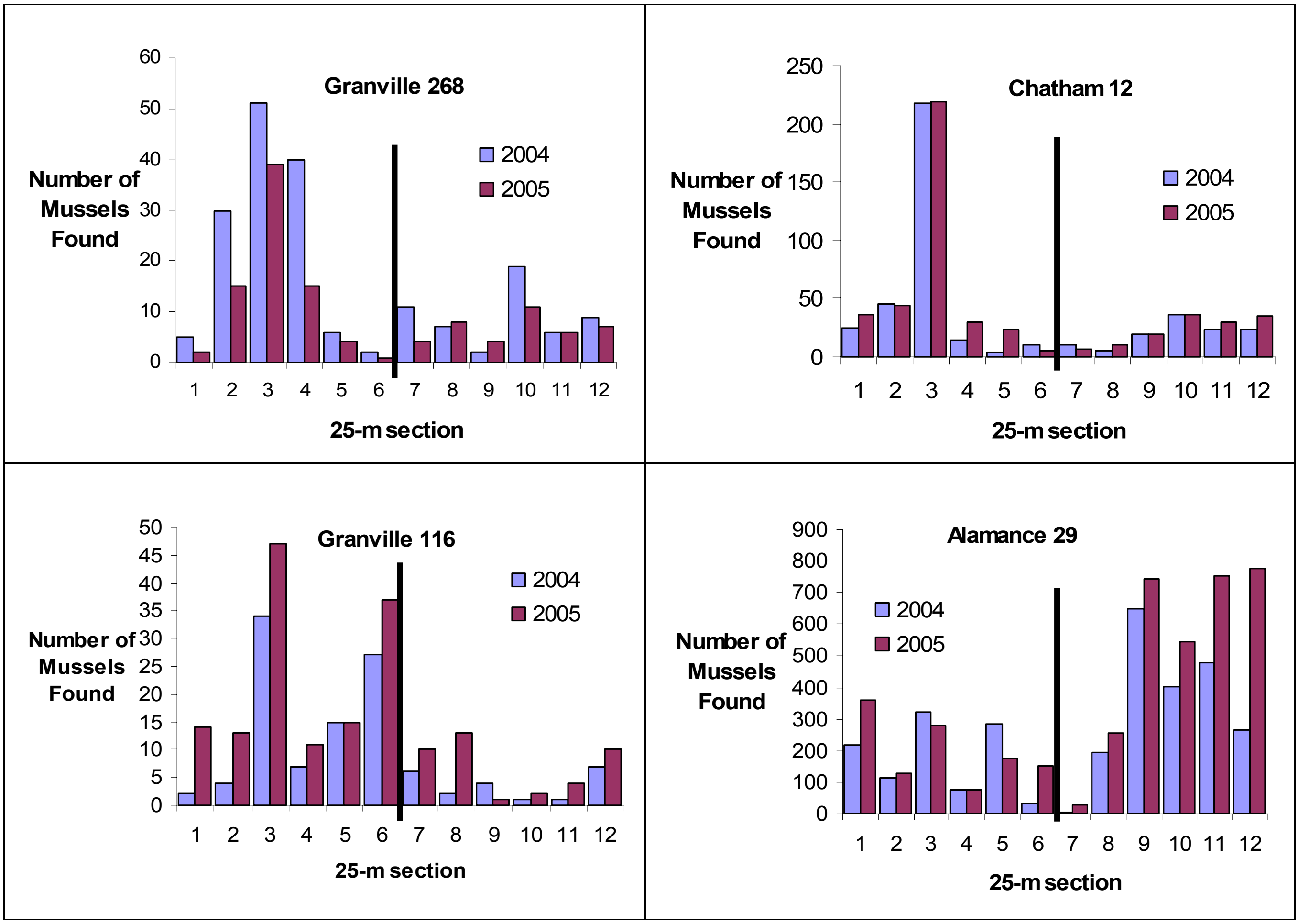

Figure 2.20. Number of mussels found in each 25-m section in surveys of 4 randomly selected culvert sites in 2004 and in 2005. Section 1 is at the most upstream end of the sampling site and section 12 is at the most downstream end. The vertical line represents the location of the culvert.

Discussion

The initial difference between bridges and culverts that stood out was the footprint of the structures and the habitat within those structures. Bridges covered less than half of the amount of stream that culverts did (Levine et al. 2003), and a relatively small percentage of culverts (30.2%) had a natural stream bottom. But beyond the actual footprint, culverts had a greater impact on streams for long distances both upstream and downstream of the structures. While the effect of bridges was very localized and seemed to be within 50 m of the bridge (Levine et al. 2003), we saw an overall effect of culverts extend for almost the entire sampled reach both upstream and down. We attribute our results to the channel-constricting hydrologic influence of culverts.

During channel-forming storm events, under-sized culverts restrict flow upstream but cause increased velocity downstream. This increased energy will scour and destabilize the stream channel downstream (Hamill, 1999; Richardson and Richardson, 1999; Simon and Johnson, 1999; Umbrell et al., 1998). Our habitat data clearly showed a trend of scour and bank erosion downstream of existing culverts in the piedmont of North Carolina. There were no statistical differences in substrate size along the stream, but habitat type noticeably changed overall in response to the presence of the culvert. There tended to be an increase in pool habitat due to scour in the first 50 m downstream of culverts, and a decrease in riffle and run habitat. At many sites, bank height and channel width was at its greatest in this reach just below the culvert, indicating degradation of banks and downcutting of the channel. From 50-75 meters downstream, there was a depositional area of material that had been scoured by the culvert causing relatively high amount of riffle and run habitat. Although the size of the scour pool and amount of deposition varied between sites, this phenomenon of scour and deposition is commonly seen at culverts (Abt et al. 1984). We found scour to be most prevalent at pipe culverts and least prevalent at arch culverts. Box and arch culverts have been shown to reduce scour compared to pipe culverts when cross-sectional area of a culvert is normalized (Abt et al. 1984, Abt et al. 1987). Although the greatest effect of culverts was within the first 50 m downstream, the overall effects on habitat extended for the entire 150-m reach sampled. Both bank height and channel width were affected for the entire reach.

In this study, we found that the habitat damage done to streams is negatively impacting mussel populations below culverts. Because mussels are so dependent on stable substrates (Strayer 1999; Johnson and Brown 2000), relative mussel abundance was lower downstream than upstream with the heaviest impacts being in the scoured reach immediately downstream of the culvert. Although 97.5% of mussels we found were *E. complanata*, distribution of all other species except for *P. cataracta* corresponded well with that of *Elliptio* in relation to the culvert. From this, we believe that although the overall results are driven by a single species, this species is a good representative for the rare species in the North Carolina piedmont.

By measuring length, width and height of mussels, we found the greatest difference in upstream and downstream to be in width. Mussel width may be tied to overall fitness (Lobel et al. 1991; Robert et al. 1993; Arrieche et al. 2002) but also may simply be a morphological response to changes in habitat (Green 1972; Bailey and Green 1988). The ecological significance of this difference unknown but it does represent a sublethal effect of culverts that extends at least 150 m downstream.

We also saw an upstream effect at culvert sites. The 25-m reach immediately upstream was generally heavily impacted, and there tended to be fewer mussels in this reach. During storm events, water comes down to undersized culverts faster than it can get through, and water level rises on the upstream side. Upstream scour pools were observed in this reach, and we saw an overall increase in pool habitat. Consequently, relative mussel abundance was also reduced. Farther upstream (75-125 m above the culverts, there seemed to be an increase in the number of mussels found over what would be naturally found there if there were no culvert. We hypothesize that culverts are acting to stabilize the upstream sediments by slowing down flows during storm events in this area and reducing erosive forces upstream. This increases substrate stability in a short reach of stream and mussels are thriving in this localized area. Does this mean culverts are beneficial? Does the stability provided upstream offset the instability created downstream? We believe not. What this represents is an alteration of natural sediment transport, the hyporheic zone, and the stream ecosystem and as a whole. The hyporheic zone is very important to the stream ecosystems as a whole, and disruption of the hyporheic zone can disrupt important chemical, physical, and biological processes in the ecosystem (Boulton et al. 1998; Schindler and Krabbenhoft 1998; Del Rosario and Resh 2000; Hancock 2002). What may be beneficial is that this information could be used in stream restoration to create mussel habitat and refugia of stable substrate.

We found that these culvert effects are magnified at some sites and minimized at other sites. In addition to whether the culvert is sized and installed properly, local soil type and geology likely greatly determines local impacts. Sites in the Upper Coastal Plain and Piedmont soil system were especially susceptible to scour, channel widening and general channel degradation. Although this soil system is a broad generalization of soil types (Daniels et al. 1999), and there are physical differences within this system, this soil system is generally characterized by unconsolidated soils that are more susceptible to erosion (Kleiss, pers. comm.). Mussel populations there were also highly affected by these habitat changes having far more mussels upstream of the culvert than downstream. Bridges we observed in this area tended to show much less habitat impact than culverts. Scour and channel widening was much less prevalent, and wider the flood plain access, the less scour was seen. Culvert sites in the northern portion (Granville County) of the Carolina Slate Belt were also more heavily impacted than sites further south or in other soil systems.

Unfortunately, these heavily impacted areas coincide with rare mussel species that reside in the piedmont. These two areas together contain a great deal of the known range in North Carolina of two federally endangered mussels, *A. heterodon* (dwarf wedgemussel) and *Elliptio steinstansana* (Tar spinymussel), and one relatively rare undescribed species, *Lampsilis sp.* of the upper Tar and Neuse basins. Because these species reside in areas of highly erodable soils, special care should be taken in the region when constructing or replacing bridges or culverts. Of primary importance is the elimination of scour and channel destabilization.

The overall effects seen at culverts are much greater than those seen at bridges. Habitat alteration was much more subtle around bridges. We saw a general trend of decreased pool habitat for 50-100 m on either side of the bridge that we attribute to channelization at the crossing (Levine et al. 2003). The decrease in relative mussel abundance was very localized and was limited to 25-50 m downstream of bridges. At culvert sites, the overall trend showed decreased mussel abundance, and increased bank height and channel width for the entire 150-m reach sampled downstream. The bridges that had the greatest impact on mussels were those built from 1950-1970, which tended to reduce or eliminate flood plain access (Levine et al. 2003). Because almost all bridges in the original study were located in the Carolina Slate Belt, the overall differences between those data and this culvert analysis must be tempered somewhat with this fact. Within the southern portion of the Slate Belt, culvert impacts were more similar to bridges than in other areas of the state with more erodable soils. However, bridges in the Upper Coastal Plain and Piedmont soil system that spanned the channel and allowed some flood plain access had obviously less impact on habitat than culverts in this region. Also, even the lower portion of the Slate Belt had culverts (e.g. Alamance 20 and Moore 220) that created obvious habitat damage not seen at any bridge. As in the previous study, the least impact on mussels was seen at a site with an arch culvert that had 2 extra cells allowing flood plain access.

Conclusions and Recommendations:

1. **Existing culverts have a greater impact on stream habitat and mussel fauna than do bridges.** In general, there is more scour and widening of the channel downstream and retention of sediments upstream. Mussel abundance is affected by these habitat changes.
2. **The hydrological, geomorphological and biological effects of culverts are magnified in areas where soils and stream substrates are easily eroded.** If a culvert is being considered for a structure replacement. A thorough evaluation of erodability of stream banks and stream substrate should be conducted. Results of these assessments should be factored into the design of the crossing structure to prevent widening, deepening, or general destabilization of the stream channel. Habitat impacts of culverts are more similar to bridges in areas that are less likely to be eroded, like much of the Carolina Slate Belt.
3. **In general, we recommend culverts not be used in streams with mussel populations.** Bridges that span the channel, allow flood plain access and do not contain supports in the channel should be used instead.
4. **We strongly recommend bridges be used instead of culverts in areas that contain rare species and areas that are especially susceptible to erosion.** Examples from our study include the northern part of the Carolina Slate belt and Upper Coastal Plain/Piedmont soil system. This area is not only highly susceptible to scour but also contains two federally endangered mussels.
5. **If a culvert is to be used for a crossing structure, we recommend the use of extra culvert cells in the flood plain to reduce scour.**
6. **Because many culverts have helped create stable mussel habitat upstream of the crossing, we recommend extreme caution in avoiding destabilizing these sediments when culverts are eventually replaced.** Upstream mussel populations should be considered in environmental risk assessments of culvert replacements, and action should be taken to conserve rare species.

References

Abt, SR, JF Ruff, and C Mendoza. 1984. Scour at culvert outlets in multibed materials. Transportation Research Record. 948:55-62.

Arrieche, D, B Licet, N Garcia, C Lodeiros, and A Prieto. 2002. Condition index, gonadic index and yield of the brown mussel *Perna perna* (Bivalvia: Mitilidae) from the Morro de Guarapo, Venezuela. Interciencia 27(11):613-619.

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.

Bailey, RC, and RH Green. 1988. Within-basin variation in the shell morphology and growth rate of a freshwater mussel. Canadian Journal of Zoology. 66:1704-1707.

Bogan, AE 1993. Freshwater bivalve extinctions (Mollusca: Unionoida): a search for causes. American Zoology 33: 599-609.

Bogan, AE 2002. Workbook and key to the freshwater bivalves of North Carolina. NC Museum of Natural Sciences, Raleigh, NC.

Bruton, MN. 1985. The effects of suspensoids on fish. Hydrobiologia. 125:221-241.

Chutter, FM. 1969. The effects of silt and sand on the invertebrate fauna of streams and rivers. Hydrobiologia. 34:57-76.

Daniels, R.B., Buol, S.W., Kleiss, H.J. and Ditzler, C.A., 1999. Soil Systems in North Carolina. Soil Science Dept. North Carolina State University, Raleigh. 118 pp.

Del Rosario, R. and Resh, V.H., 2000. Invertebrates in Intermittent and Perennial Streams: is the Hyporheic Zone a refuge from Drying? Journal of North American Benthological Society, 19(4): 680-696.

Ellis MM. 1936. Erosion silt as a factor in aquatic environments. Ecology. 17:29-42.

Foe, C, and A Knight. 1987. Assessment of the biological impact of point source discharges employing Asiatic clams. Archives of Environmental Contamination and Toxicology. 16:39-51.

Forman, RTT and LE Alexander. 1998. Roads and their major ecological effects. Annual Review of Ecology and Systematics. 29:207-231.

Goudreau SE, RJ Neves, and RJ Sheehan. 1993. Effects of wastewater treatment plant effluents on freshwater mollusks in the upper Clinch River, Virginia, USA. Hydrobiologia. 252: 211-230.

Green, RH. 1972. Distribution and morphological variation of *Lampsilis radiata* (Pelecypoda, Unionidae) in some central Canadian lakes: a multivariate statistical approach. Journal of the Fisheries Research Board of Canada. 29:1565-1570.

Hardison, BS, and JB Layzer. 2001. Relations between complex hydraulics and the localized distribution of mussels in three regulated rivers. Regulated Rivers: Research and Management. 17:77-84.

Johnson, PD and KM Brown. 2000. The importance of microhabitat factors and habitat stability to the threatened Louisiana pearl shell, Margaritifera hembeli. Canadian Journal of Zoology. 78:271-277.

Kleiss, JH. Professor and Academic Coordinator. Department of Soil Science. North Carolina State University.

Little, JD and JJ Mayer. 1993. Bridge and road construction. In: CF Bryan and DA Rutherford, editors, Impacts on Warmwater Streams: Guidelines for Evaluation. Southern Division of the American Fisheries Society, Warmwater Streams Committee.

Levine, JF, AE Bogan, PA Russell, EF Andersen, and CB Eads. 2003. Distribution of freshwater mussel populations in relationship to crossing structures. Final report submitted to the North Carolina Department of Transportation (HWY-2003-02). 182 pp.

Marking, LL, and TD Bills. 1979. Acute effects of silt and sand sedimentation on freshwater mussels. Pp. 204-211 in JL Rasmussen, ed. Proc. Of the UMRCC symposium on the Upper Mississippi River bivalve mollusks. UMRCC. Rock Island IL. 270 pp.

Lenat, DR, DL Penrose, and KW Eagleson. 1981. Variable effects of sediment addition on stream benthos. Hydrobiologia. 79:187-194.

Lobel, PB, CD Bajdik, SP Belkode, SE Jackson, and HP Longerich. 1991. Improved protocol for collecting mussel watch specimens taking into account sex, size, condition, shell shape, and chronological age. Archives of Environmental Contamination and Toxicology. 21(3):409-414.

Ogbeibu, AE, and R Victor. 1989. Effects of Road and Bridge Construction on the Bank-Root Macrobenthic Invertebrates of a Southern Nigerian Stream. Environmental Pollution. 56(2): 85-100.

Robert, R, G Trut, M Borel, D Maurer. 1993. Growth, fatness, and gross biochemical-composition of the Japanese oyster, Crassostrea-gigas, in Stanway cylinders in the bay of Arcachon, France. Aquaculture. 110(3-4):249-261.

Stout, BM, III, and CB Coburn, JR. 1989. Impact of highway construction on leaf processing in aquatic habitats of eastern Tennessee. Hydrobiologia. 178:233-242.

Strayer, DL. 1999. Use of Flow refuges by Unionid Mussels in Rivers. Journal of North American Benthological Society. 18(4):468-476.

Umbrell, E.R., Young, G.K., Stein, S. and Jones, J.S., 1998. Clear-Water Contraction Scour under Bridges in Pressure Flow. Journal of Hydrologic Engineering: 236-240.

Whitney, AN, and JE Bailey. 1959. Detrimental effects of highway construction on a Montana stream. Transactions of the American Fisheries Society. 88:72-73.

Wood, PJ and PD Armitage. 1997. Biological effects of fine sediment in the lotic environment. Environmental Management. 21:203-217.

CHAPTER 3:
Impact of Bridges and Culverts on Stream fish Movement and Community Structure

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Introduction

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

Bridge construction appears to have fewer effects on stream communities than certain designs of culverts (Gosse et al. 1998; Warren and Pardew 1998). A culvert is defined as a drain or waterway passage built so a road may cross a body of water without stopping its flow. The most common culverts are: (1) arch, a cement archway with natural stream bottom; (2) box, a series of two or three square cement structures allowing flow; and (3) pipe, a series of two or three corrugated steel pipes (Fig 3.1). Culverts with the least alteration of flow through the crossing should also be the least obstructive to fish movement (Warren and Pardew 1998).

A major deficiency in culvert design is a reduction in cross sectional area for water flow, leading to increased stream velocities at certain times to levels that exceed the swimming ability of small fish and prevent their upstream movement (Gosse et al. 1998; Warren and Pardew 1998; Wellman et al. 2000). This alteration of water flow can disrupt movement patterns that are essential for fish growth, survival, and reproduction (Evans and Johnston 1980), as well as maintenance of community structure (Porto et al. 1999). Jungwirth et al. (1998) report that relatively little information exists as to which structures are effectively impassable for non-commercial fish species. Fish passage through culverts has been studied heavily for anadromous fishes, but not warmwater stream fish.

Regardless of its velocity, there must be enough water to maintain a minimum depth in the culvert to allow the larger fish to travel through the culvert during periods of low water depths (Dryden and Stein 1975). It is thought that circular and elliptical culverts are preferable over flat-bottomed designs because of their greater depth of flow per unit discharge (Dane 1978).

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

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

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

There have been very few studies of the effects of culverts on warmwater stream fish, and none conducted in North Carolina. We quantified the impact of four commonly used road crossings (bridge, arch culvert, pipe culvert, box culvert) on the stream fish communities beneath them by comparing six response variables: (1) estimates of population size, (2) species richness, (3) species diversity, (4) fish index of biotic integrity (FIBI), (5) conditional percent movement (CPM), (6) interaction terms, of control streams without crossings to streams with crossings. This study is part of a larger, more comprehensive study that is assessing long-term effects of road-crossings on distribution of freshwater mussels that are likely determined by the design of the structure. We focused on disruption of fish movement and possible shifts in fish community structure as a function of presence/absence of road crossings and crossing type.

Methods

<u>Site selection</u>

A total of 16 sites were selected in either a random or directed manner from a total of 50 possible sites harboring mussel populations (Fig 3.2). Initially, all sites were located within the Cape Fear River Basin, North Carolina, to reduce variance in stream fish community. Only two out of four arch culvert sites in the Cape Fear River Basin were viable study sites because beaver (*Castor canadensis*) dams had been built within the study reaches of two sites. To maintain a balanced study design containing a sample size of three for each road crossing type or control, a third arch culvert site was added from the Neuse River Basin, North Carolina (Fig 3.2). Other crossing-type sites had more than enough streams to randomly choose from. Habitat characteristics (as outlined by the North Carolina Department of Environment and Natural Resources) such as: (1) stream width measured by a tape measure, (2) stream depth measured by a meter stick, (3) predominate substrate type (bedrock, boulder, cobble and sand), (4) percentage of habitat type (pond, riffle, and run), (5) bank stability distinguishing between right and left banks (a scale from 1-10 with a score of 1 equivalent to "100% eroded bank" and a score of 10 equivalent to "less than 5% eroded bank"), and (6) width of riparian zone distinguishing between right and left banks (a scale from 1-10 with a score of 1 equivalent to "less than 6 m of riparian vegetation" and a score of 10 representing "greater than 18 m of riparian vegetation") were quantified at 10 m intervals for 50 m above and below each crossing. Stream reach volume and area were calculated using the average of the widths and depths for each stream reach, and multiplied by the length of each reach: 50 m. There was no predominance of a given habitat type within streams with culverts as compared to those with bridges as compared to control streams (Appendix Table 3.1).

<u>Fish sampling</u>

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

All sampling periods used block-nets to enclose 50 m reaches of each stream immediately upstream and downstream of a road crossing. For control streams, we sampled in an area 50 m upstream and downstream of an imaginary road crossing measuring 15 m in length. A length of 15 m was based on the average width of road crossings in our study (Appendix Table 3.1). Once enclosed, stream fish in the upstream and downstream reaches of each stream were sampled using a combination of seining and backpack electrofishing; triple-pass depletion methods were used to maximize recapture rates and effort (Seber and Lecren 1967; Lyons and Kanehl 1993; Lockwood and Schneider 2000; Meador 2000). Fish were removed from the study reaches after each collecting pass and kept in pop-up laundry hampers located directly in the stream flow until all of the sampling was completed. All fish were identified and measured to the nearest 1.0 mm total length (TL).

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

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

This entire mark-recapture procedure was repeated four, eight, and 12 weeks after initial sampling to assess temporal variability in fish movement and species composition. There was no tagging during the final sampling period in August because there were no more recapture events. Fish were identified, checked for marks using an LED flashlight that illuminated the elastomer marks (Northwest Marine Technologies), and tagged if necessary before release. The day following our first recapture event in June, a bridge was removed by the NCDOT at one of our study streams, (Little Brush Creek located in Chatham County NC; Fig 3. 2) and was replaced by an arch culvert. A similar bridge site was chosen based on its proximity and similarity to Little Brush Creek, surprisingly, it was called Brush Creek (Fig 3. 2). The data for these two bridges were combined for all response variables (see below).

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

Environmental data

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

Response variables and hypotheses

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

Population size

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

Community-level response

A total of three community-level response variables were calculated: (1) species richness, (2) species diversity index, and (3) fish index of biotic integrity (FIBI). Species richness, the number of fish species sampled, was calculated for each time period, position (upstream and downstream), as well as an overall value of species richness was calculated for each site. Each species richness value was standardized by the corresponding stream reach volume (Appendix Table 3.1), which was calculated from the habitat data collected at the beginning of the sampling season. We also standardized fish species richness by stream area; however, we found similar results between species richness standardized by stream volume and stream area, so we only consider species richness standardized by stream volume (species richness/m^3) in the remainder of this paper.

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

We used a fish index of biotic integrity (FIBI) developed by Karr (1981) and Karr et al. (1986), and subsequently modified and employed by the North Carolina Department of

Environment and Natural Resources (NCDENR). Due to differences in stream reach length in our study and the protocol for estimating the NC FIBI, we chose nine out of 12 matrices calculated for the Cape Fear River Basin, NC: (1) species richness, (2) no. darter (*Etheostoma* and *Percina*) species, (3) no. sunfish (*Centrarchidae*) species, (4) no. species suckers (*Catostomidae*) , (5) no. intolerant species, (6) % tolerant individuals, (7) % omnivorous and herbivorous individuals, (8) % insectivorous individuals, and (9) % piscivorous individuals. We tabulated FIBI scores for each reach and stream for all four sampling periods as well an overall score. These scores were meant to represent overall health of the fish community based on the FIBI utilized by the state of North Carolina. The NC Division of Water Quality (NCDWQ) published the most recent version of the index in August of 2004. Sampling for the 2004 NCDWQ FIBI was conducted during 2003 (B. Tracy, NCDWQ, pers. comm.).

Movement response

Conditional percent fish movement (CPM) was calculated for each stream, position (upstream and downstream), and time period. CPM was calculated by taking the number of fish that moved downstream divided by the sum of the fish that moved downstream and fish recaptured upstream (K.Pollock, NCSU, pers.comm.). The same calculation was preformed for fish that moved upstream. This number represents how many fish moved out of the total number recaptured from the fish marked in a given stream reach. This percentage is conditional on recapture at a given event and assumes a constant recapture rate for all species.

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

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

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

Results

A total of 7500 meters of stream reach were sampled over the four-month field season. We marked 9,300 individual fish representing 43 species and 12 families of fish (Appendix Tables 3.2 and 3.3). The number of individual fish that moved within our study scale was very low, and ranged from 0 % to 3.01% per month (Table 3.1). Mean percent recapture was also relatively low, and ranged from 1.91% to 9.96% per month for the study reaches (upstream and downstream; Table 3.1) and improved considerably (2.96% to 21.7%) when the reaches within streams were pooled (Table 3.2).

Fish population patterns

Estimates of population size were calculated at the family level due to low numbers of individual species. Analysis of a time effect was not possible because not one family was represented at every sampling period for each stream. When the population data was pooled across time, one family, Percidae, was present in all study reaches; Centrarchidae was present in 29 out of 30 study reaches and Cyprinidae was present in 27 out of 30 study reaches. Split-plot ANOVA models assessed the effects of crossing type and position of stream reach on all three families: Centrarchidae, Cyprinidae, and Percidae. Regardless of fish familiy, estimates of population size adjusted by stream reach volume did not differ significantly with crossing type (Split plot ANOVA, all $F < 1.10$ and $p > 0.41$, Table 3.3) nor position of stream reach (Split plot ANOVA, all $F < 1.36$ and $p > 0.27$, Table 3.3). There was no statistically significant effect of crossing type on overall estimates of population size for any of the families: Centrarchidae, Cyprinidae, or Percidae (One way ANOVA, all $F < 1.85$ and $p > 0.15$, Table 3.4).

Fish community patterns

Species richness adjusted by stream reach volume did not vary with crossing type (Culverts: arch, box, and pipe, bridge and control), position (upstream and downstream), nor according to time (split-plot, repeated measures ANOVA; all $p > 0.31$, Fig 3.3); however, there was a significant crossing type by position interaction effect (subplot error df = 4, 25, $F = 3.80$, $p = 0.0074$). The crossing type by position interaction effect was due to downstream species richness being significantly higher in the upstream section of box culvert reaches than for other crossing types or the control streams; and upstream species richness being significantly higher in control streams than streams with crossings (Scheffe's multiple comparisons test, Fig 3.3). The difference of species richness means for downstream reaches of box culverts could be linked with the scour effects common to box culverts that result in a pool habitat just below the culvert (Wellman et al. 2000); however, we found no difference in percent pool between upstream and downstream reaches nor by crossing (split-plot ANOVA; all $p > 0.14$, $F < 1.94$, Fig 3.4). Mean fish species diversity did not vary according to crossing type or position (split-plot, repeated measures ANOVA; all $p > 0.54$). None of the interaction terms were significant (Tables 3.5 & 3.6).

Fish community health, as represented by FIBI scores, did not vary significantly with position (split-plot, repeated measures ANOVA, all $p > 0.17$; Fig 3.5, Table 3.7); however, FIBI scores did vary significantly with crossing type (df = 4, F= 2.53, p = 0.048). A subsequent Scheffe multiple comparisons test was unable to identify which crossing types were significantly different (df = 4, F = 1.41, p = 0.26). The significant crossing effect on FIBI was likely due to relatively low FIBI scores for stream fish near bridges compared to other crossing types (Fig 3.5, Table 3.8).

<u>Fish movement patterns</u>

Conditional percent fish movement (CPM) did not vary according to road crossing type nor position (split-plot, repeated measures ANOVA; all $p > 0.22$, Fig 3.6). None of the interaction terms were significant. CPM, species richness, species diversity, and FIBI showed no correlations with continuous stream habitat characteristics such as: stream flow, depth, area, volume, percent riffle and percent pool (Pearson correlation coefficients, all $-0.21 < r < 0.31$, $p > 0.09$); however, CPM demonstrated a significant negative correlation with percent run (Pearson correlation coefficients, r = -0.35, p = 0.05).

<u>Habitat characteristics</u>

Stream width ranged from 4.7 to 10 m, but was relatively similar across road crossing types (Appendix Table 3.1). Similarly, stream depth ranged from 0.178 to 0.685 m and was quite varied among each crossing type. Neither percent pool nor percent run varied significantly between upstream and downstream reaches nor with crossing types (split-plot ANOVA, all $p > 0.06$, Fig 3.4).

Discussion

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

Because there were extremely low numbers of individual fish that moved between upstream and downstream reaches in this study, no conclusions can be made on the effects of road crossings on stream fish movement. Stream fish movement through culverts in the Oachita Mountains of west-central Arkansas was an order of magnitude lower than through other crossing types; although, there was little difference in stream fish movement between natural reaches and open box culverts (Warren and Pardew 1998). One main difference between the

Warren and Pardew (1998) study and this one is in our definitions of culvert types. According to their study, only pipe culverts were in the category "culvert", and two out of the four culverts sampled were perched 5-8 cm above the downstream reaches during some part of the study, which created a physical barrier to stream fish movement. Our study did not include any streams with perched crossings or those that were dry throughout the summer of 2004. It is possible that the inclusion of perched crossings in the Warren and Pardew (1998) study biased their findings towards negative impact of culverts on fish movement relative to this study. Conversely, crossings classified as "open-box" in the Warren and Pardew (1998) study were similar our definition of box culverts, which would make the results from both studies comparable because there was no effect of box culverts (this study) and open box (Warren and Pardew 1998) on stream fish movement. The Warren and Pardew (1998) study also used sample reaches that were 100-150 m long, which may have improved their chances of detecting negative impacts of road crossings on stream fish, and sampled using double pass as opposed to triple pass depletion.

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

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

When examining ecosystems for changes due to anthropogenic influences, it is imperative to have natural benchmarks with which the data can be compared (Pauly 1995; Tegner and Dayton 1998). This is a major tenet of the 'shifting baseline syndrome' where each new generation of observers accepts, for example, the species composition and fishery stock size at the beginning of their careers as baseline, which results in inappropriate reference points for evaluating disturbances and establishing objectives for restoration. All indices of stream fish biotic integrity use a scale relative to the healthiest stream of a system (the reference stream), such that if that reference stream is also impacted and currently hosting a degraded community, the scores might indicate good stream health erroneously. It is possible that the stream fish communities shifted 30 years ago when the culverts were put in place; therefore, no significant differences in FIBI scores were found among our study streams.

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

Much effort has been put into developing regional indices of biotic integrity to assess the health of stream ecosystems (Karr et al. 1986; Fausch et al. 1990; Roth et al. 1996), as well as in detecting the ecological impacts of human induced disturbances (Steedman 1988; Schulz et al. 1999; Teels et al. 2004). Although acceptance and use of these indices is prevalent in stream ecosystem literature (Hughes et al. 1990), recent studies have found FIBI scores insensitive to known anthropogenic disruptions. For example, abundance is a more sensitive metric of population health for common and rare fish species in a given stream system than is percent occurrence between impacted and reference streams (Pirhalla 2004). The North Carolina FIBI has one metric of abundance for tolerant species, but uses only a percent occurrence of intolerant species. In a comprehensive study aimed at identifying indicators of urbanization effects on streams, abundance of sensitive fish species was a consistent response to urban impacts, whereas overall fish abundance and that of tolerant species were inconsistent responses (Walsh et al. 2005). When used to detect anthropogenic effects on lakes in Florida, FIBI scores were unreliable as higher scores were recorded for the lakes most impacted by human presence (Schulz et al. 1999). FI BI scores can be effective indicators of short term fish community recovery after disturbance, but ineffective as indicators of long term disturbance (Paller et al. 2000). A possible explanation of the inadequacies of FIBI is the impossibility of an FIBI to distinguish between the natural variations in fish assemblages and fish community shifts due to anthropogenic impacts (Bramblett and Fausch 1991).

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

An ideal method to assess changes in a community due to anthropogenic impacts is that of a Before-After-Control-Impact (BACI) study (Underwood 1996). Extreme foresight and funding is needed for this approach since the study must take place prior to and after a disturbance. This approach was not possible for our study since the road crossings were constructed 30 years or more ago; however, we suggest that future studies assessing the impacts of road crossings on fish community structure strive to employ BACI designs whenever feasible. For studies that include older crossings, we suggest that a more sensitive organism or community, such as mussels or insects, be used to assess stream ecosystem health.
The practical difficulties of tracking large numbers of organisms through space and time are common in ecological field studies, resulting in a paucity of empirical information on taxa, specifically non-commercially important taxa (Okubo 1980; Turchin 1998; Skalski & Gilliam 2000). The low number of fish that moved (Mean 0%-2.06% of fish tagged) within our study reaches indicates either a flaw with the spatial and temporal scale of the study, or a fish community dominated by sedentary members. It is possible that sampling 50 m above and below the road crossing was not a large enough area to capture the movement patterns of stream fish using mark-recapture methods in this study. When assessing distribution patterns and community organization of an assemblage, sampling should include the minimum home-range sizes of the dominant species (Grossman 1982; Grossman et al. 1982). Skalski & Gilliam (2000) found that while most individuals remained within 10-100 meters of the initial tagging site, four freshwater fish species (blue head chub, creek chub, redbreast sunfish and rosyside dace; see Appendix Table 3.2 for scientific names), which were also the most common species across all 16 streams of our study, were able to travel distances up to 200 meters upstream and downstream over a five-month period. Other mark-recapture studies of stream fish report similar findings, whereby the fish populations were comprised of mostly ‘stayers’ that occupy limited areas and a few ‘movers’ that roam larger areas (Gerking 1959; Hegenes et al. 1991; Freeman 1995). The majority of recaptures over an 18 month period of juvenile Redbreast sunfish and adult Blackbanded darter were within 33 m of the original capture location (Freeman 1995). It is possible that the majority of stream fish in our study communities remained in the sample area and the lack of movement between study reaches in our study was due to small home ranges and not the 100 m spatial scale of sampling.

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

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

This study highlights problems with traditional mark-recapture methods used to assess fish movements through space and time. We recommend the use of PIT tags and remote antenna arrays, also called gates, for 24 hour monitoring of fish movement through a designated area (Morhardt et al. 2000; Barbin Zydlewski et al. 2001). This system places an antenna in the stream that will detect any fish carrying a PIT tag as it passes through the array while an electronic reader housed on shore downloads and stores all of the tag codes. The PIT tag method has the potential to increase sample sizes and use man-power more efficiently and effectively by reducing the number of sampling events, sampling bias due to fright response, recording error, and handling time of fish, since individual fish are not disturbed upon recapture (Gibbons and Andrews 2004). Tag dimensions (12 mm) would restrict the size of fish that could be tracked to individuals greater than 60 mm TL, but would give a more accurate evaluation of numbers of fish moving through crossings versus control areas because of the increased recapture rates (95-100% read efficiency), as well as the ability to monitor fish movements 24-7 (Gibbons and Andrews 2004).

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

Conclusions

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

The collaborative nature of this study has produced a comprehensive amount of site-specific information, which should facilitate ecosystem restoration. Once new road crossing designs for reduced impact are initiated, attention can be diverted to how to alleviate the previously impacted streams. This could lead to policy and restoration methods specific to culvert designs. North Carolina can be an example to other states and countries that a partnership between government and science can result in universal benefit.

References

Angermeier, P.L. and I.J. Schlosser. 1989. Species-area relationships for stream fishes. Ecology 70: 1450-62.

Barbin Zydlewski, G., A. Haro, K.G. Whalen, and S.D. McCormick. 2001. Performance of stationary and portable passive transponder detection systems for monitoring of fish movements. Journal of Fish Biology 58: 1471-1475.

Barton, B.A. 1977. Short term effects of highways construction on limnology of a small stream in Southern Ontario. Freshwater Biology 7: 99-108.

Bates, K. and P. Powers. 1998. Upstream passage of juvenile Coho Salmon through roughened culverts. Fish Migration and Fish Bypasses. Department of Hydrobiology, Fisheries and Aquaculture, University of Agricultural Sciences, Vienna, Austria. 192-202 pp.

Bogan, A. E. 1993. Freshwater bivalve extinctions (Mollusca: Unionoida): a search for causes. American Zoologist 33: 599-609.

Bramblett, R.G. and K.D. Fausch. 1991. Variable fish communities and the index of biotic integrity in a western great plains river. Transactions of the American Fisheries Society 120: 752-769.

Brooks Jr., P.F. 2003. Three great challenges for half century old computer science. Journal of the Association for Computing Machinery 50(1): 25-26.

Burnham, K.P., Anderson, D.R., White, G.C., Brownie, C., Pollock, K.H. 1987. Design and analysis methods for fish survival experiments based on release-recapture. American Fisheries Society Monographs 5: 1-437.

Carpenter, S.R. 2002. Ecological futures: building an ecology of the long now. Ecology 83: 2069-2083.

Chase, M.E., Jones, S.E., Hennigar, P., Sowles, J., Harding, G.C.H., Freeman, K., Wells, P.G., Krahfrost, C., Coombs, K., Crawford, R., Pederson, J., Taylor, D. 2001. Gulfwatch: monitoring spatial and temporal patterns of trace metals and organic contaminants in the Gulf of Maine (1991-1997) with the blue mussel, *Mytilus edulis* L. Marine Pollution Bulletin 42: 491-505.

Dane, B. G. 1978. A review and resolution of fish passage problems at culvert sites in British Columbia. Fisheries and Marine Service Technical Report no. 810. 126 p.

Dryden, R.L. and C. S. Jessop. 1974. Impact analysis of the Dempster Highway culvert on the physical environment and fish resources of Frog Creek. Environment Canada, Fisheries and Marine Service. Technical Report Series CEN/T-71-5 59 p.

Dryden, R. L. and J. M. Stein. 1975. Guidelines for the protection of the fish resources of the Northwest Territories during highway construction and operation. Department of the Environment, Fisheries and Marine Service, Technical Report Series No. CEN/T-75-1.

Duck, R. W. 1985. The effects of road construction on sediment deposition in Lock Earn, Scotland. Earth Surface and Landforms 10: 401-406.

Ellis, M. M. 1936. Erosion silt as a factor in aquatic environments. Ecology. 17: 29-42.

Evans, W. A., and B. Johnston. 1980. Fish migration and fish passage: a practical guide to solving fish passage problems. U. S. Forest Service, Washington, D.C.

Fausch, K.D., J. Lyons, J.R. Karr, and P.L. Angermeier. 1990. Fish communities as indicators of environmental degradation. American Fisheries Society Symposium 8: 123-144.

Freeman, M.C. 1995. Movements by two small fishes in a large stream. Copeia 2: 361-367.

Fitzgerald, D.G., E. Kott, R.P. Lanno and D.G. Dixon. 1998. A quarter century of change in the fish assemblages of three small streams modified by anthropogenic activities. Journal of Aquatic Ecosystem Stress and Recovery 6:111-127.

Gagen, C.J. and C.Landrum. 2000. Response of warm-water fish to road crossings on Oachita Mountain streams. United States Department of Agriculture, Forest Service, Ouachita National Forest, Interim Report February 2000. 13 pp.

Gatz, A.J. and S.M. Adams. 1994. Patterns of movement of centrarchids in two warmwater streams in eastern Tennessee. Ecology of Freshwater Fish 3: 35-48.

Gerking, S.D. 1959. The restricted movement of fish populations. Cambridge Biological Society Biological Review 34: 221-242.

Gibbons, J.W. and K.M. Andrews. 2004. PIT tagging: simple technology at its best. Bioscience 54(5): 447-454.

Goldberg, E.D., Bowen, V.T., Farrington, J.W., Harbey, G., Martin, J.H., Parker, P.L., Risebrough, R.W., Roberton, W., Schneider,E., Gamble, E. 1978. The mussel watch. Environmental Conservation 5: 101-125.

Gosse, M.M., A.S. Power, D.E. Hyslop, and S.L. Pierce. 1998. Guidelines for protection of freshwater fish habitat in Newfoundland and Labrador. Fisheries and Oceans, St. John’s, N.F.

Grossman, G.D. 1982. Dynamics and organization of a rocky intertidal fish assemblage: the persistence and resilience of taxocene structure. American Naturalist 119: 611-637.

Grossman, G.D., P.B. Moyle, and J.O. Whitaker, Jr. 1982. Stochasticity in structural and functional characteristics of an Indiana stream fish assemblage: a test of community theory. American Naturalist 120: 423-453.

Grossman, G.D., J.F. Dowd, and M. Crawford. 1990. Assemblage stability in stream fishes: a review. Environmental Management 14(5): 661-671.

Grossman, G.D., R.E. Ratajczak, M. Crawford, and M.C. Freeman. 1998. Assemblage organization in stream fishes: effects of environmental variation and interspecific interactions. Ecological Monographs 68: 395-420.

Haag, W.R. and M.L. Warren. 1997. Host fishes and reproductive biology of freshwater mussel species from the Mobile Basin, USA. Journal of the North American Benthological Society 16(3): 576-585.

Haag, W. R., Warren, M.L., Shillingsfor, M. 1999. Host fishes and host-attracting behavior of *Lampsilis altilis* and *Villosa vibex* (Bivalvia: Unionidae). American Midland Naturalist 141(1): 149-157.

Hardison, B.S. and J.B. Layzer. 2001. Relations between complex hydraulics and the localized distribution of mussels in three regulated rivers. Regulated Rivers: Research and Management. 17: 77-84.

Hainly, R. A. 1980. The effects of highway construction on sediment discharge into Blockhouse Creek and Stream Valley Run, Pennsylvania. U. S. Geological Survey, Water Resource Investigation 80-86.

Harper, D.J., and J.T. Quigley. 2000. No net loss of fish habitat: an audit of forest road crossings of fish-bearing streams in British Columbia, 1996-1999. Canadian Technical Report of Fisheries and Aquatic Sciences 2319.

Heggenes, J., T.G. Northcote, and A. Peter. 1991. Spatial stability of cutthroat trout (*Oncorhynchus clarki*) in a small, coastal stream. Canadian Journal of Fisheries and Aquatic Sciences 48: 757-762.

Hughes, R.M., T.R. Whittier, and C.M. Rohm. 1990. A regional framework for establishing recovery criteria. Environmental Management 14: 673-683.

Iverson, M., Finstad, B., McKinley, RS, Eliassen, RA. 2003. The efficacy of metomidate, clove oil, Aqui-S registered and Benzoak as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts, and their potential stress-reducing capacity. Aquaculture 221(1-4): 549-56.

Johnson, P.D. and K.M. Brown. 2000. The importance of microhabitat factors and habitat stability to the threatened Louisiana pearl shell, *Margaritifera hembeli*. Canadian Journal of Zoology 78: 271-277.

Jungwirth, M., Schmutz, S., and S. Weiss. 1998. Fish migration and fish bypasses. Oxford, Malden, MA.

Karr, J.R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6: 21-27.

Karr, J.R. and I.J. Schlosser. 1977. Impact of nearstream vegetation and stream morphology on water quality and stream biota. Ecological Research Series EPA-600/3-77-097. United States Environmental Protection Agency, Athens, Georgia, USA.

Karr, J. R., Fausch, K.D., Angermeier, P.L., Yant, P.R., and I.J. Schlosser. 1986. Assessing biotic integrity in running water: a method and its rationale. Illinois Natural History Special Publication No. 5, Urbana, IL.

Kohler, S.L. and D.A. Soluk. 1997. Effects of sedimentation on stream communities. Illinois Natural History Survey Reports, No. 345.

Layzer, J.B. and L.M. Madison. 1995. Microhabitat use by freshwater mussels and recommendations for determining their instream flow needs. Regulated Rivers: Research and Management 10: 329-345.

Lebreton, J.D., Burnham, K.P., Clobert, J., Anderson, D.R. 1992. Modeling survival: testing biological hypotheses using marked animals: a unified approach with case studies. Ecological Monographs 62: 67-118.

Little, J. D. and J. J. Mayer. 1993. Bridge and road construction. *In*: C. F. Bryan and D. A. Rutherford, editors, Impacts on Warmwater Streams: Guidelines for Evaluation. Southern Division of the American Fisheries Society. Warmwater Streams Committee.

Lockwood, R. N. and Schneider, J. C. Stream fish population estimates by mark and recapture and depletion methods. Chapter 7 in Schneider, J. C. (ed.) 2000. Manual of fisheries survey methods II: with periodic updates. Michigan Department of Natural Resources, Fisheries Special Report 25, Ann Arbor.

Lotrich, V. A., and W. H. Meredith. 1974. A technique and the effectiveness of various acrylic colors for subcutaneous marking of fish. Transactions of the American Fisheries Society 103: 140-143.

Lyons, J., and P. Kanehl. 1993. A comparison of four electroshocking procedures for assessing the abundance of smallmouth bass in Wisconsin streams. U. S. Department of Agriculture, Forest Service, General Technical Reports NC-159, St. Paul, Minnesota.

Magurran, A.A. 1988. Ecological Diversity and its Measurement. Princeton University Press, Princeton, NJ.

Maret, T.R., C.T. Robinson, and G.W. Minshall. 1997. Fish assemblages and environmental correlates in least-disturbed streams of the upper Snake River basin. Transaction of the American Fisheries Society 126: 200-216.

Marking, L. L., and T. D. Bills. 1979. Acute effects of silt and sand sedimentation on freshwater mussels. Pp. 204-211 in J. L. Rasmussen, ed. Proc. of the UMRCC symposium on the upper Mississippi River bivalve mollusks. UMRCC. Rock Island, IL. 270 pp.

Meador, M.R. and J.P. McIntyre. 2003. Effects of electrofishing gear type on spatial and temporal variability in fish community sampling. Transactions of the American Fisheries Society 132(4): 709-716.

Morgan, R.P. and S.F. Cushman. 2005. Urbanization effects on stream fish assemblages in Maryland, USA. Journal of the North American Benthological Society 24: 643-655.

Morhardt, J.E., D. Bishir, C.I. Handlin, and S.D. Mulder. 2000. A portable system for reading large passive integrated transponder tags from wild trout. North American Journal of Fisheries Management 20: 276-283.

Muncy, R. J., G. J. Atchison, R. V. Bulkley, B. W. Menzel, L. G. Perry, and R. C. Summerfelt. April 1979. Effects of suspended solids and sediment on reproduction and early life of warmwater fishes: a review. Ecol. Res. Series U. S. Environmental Protection Agency. 110 pp.

Murphy, B.R. and D.W. Willis. 1996. Fisheries Techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

North Carolina Department of Environment, Health and Natural Resources. 1997. Fish Community Structure *In*: Standard Operating Procedures Biological Monitoring. Division of Water Quality, Water Quality Section, January.

Okubo, A. 1980. Diffusion and ecological problems: mathematical models. Springer-Verlag, New York, New York, USA.

Orth, D.J. and R.J. White. 1993. Stream habitat management. In: C.Kohler and W.A. Hubert, editors, Inland Fisheries, Management in North America. American Fisheries Society, Bethesda, Maryland.

Paller, M.H., M.J.M. Reichert, and J.M. Dean. 1996. Use of fish communities to assess environmental impacts in South Carolina coastal plain streams. Transactions of the American Fisheries Society 125(6): 633-644.

Paller, M.H., M.J.M. Reichert, J.M. Dean, and J.C. Seigle. 2000. Use of fish community data to evaluate restoration success of a riparian stream. Ecological Engineering 15: S171-S187.

Peterson, J.T. and P.B. Bayley. 1993. Colonization rates of fishes in experimentally defaunated warmwater streams. Transactions of the American Fisheries Society 122: 199-207.

Pirhonen, J. and C.B. Schreck. Effects of anaesthesia with MS-222, clove oil and CO sub(2) on feed intake and plasma cortisol in steelhead trout (*Oncorhynchus mykiss*). Aquaculture 220 (1-4): 507-514.

Peterson, L. A. and D. Nyguist. 1972. Effects of highway construction on a subarctic stream. North, Eng. 4: 18-20.

Pielou, E.C. 1975. Ecological Diversity. Wiley-Interscience Publication, New York. 165 pp.

Pirhalla, D.E. 2004. Evaluating fish-habitat relationships for refining regional indices of biotic integrity: development of a tolerance index of habitat degradation for Maryland stream fishes. Transactions of the American Fisheries Society 133: 144-159.

Porto, L.M., R.L. McLaughlin, and D.L.G. Noakes. 1999. Low-head barrier dams restrict the movements of fishes in two Lake Ontario streams. North American Journal of Fisheries management 19(4): 1028-1036.

Pritchard, K. 1995. Combination Staff Gauge/Crest Gauge. The Volunteer Monitor 7 (2): Fall.

Queensland Department of Primary Industries, Brisbane. 1998. Fish passage in streams: fisheries guidelines for design of stream crossings. Fish Habitat Guidelines Dep. Prim. Ind. Qld. No. 001: 37 pp.

Riley, A.L. and M. Mcdonald. 1996. Urban waterways restoration training manual for youth service and conservation corps. California salmonid stream habitat and restoration manual, 2nd edition. California Department of Fish and Game, Sacramento.

Roth, N.E., J.D. Allen, and D.E. Erickson. 1996. Landscape influences on stream biotic integrity assessed at multiple spatial scales. Landscape Ecology 11: 141-156.

Roth, N., M. et al. 1998. Maryland Biological Stream Survey: Development of a fish index of biotic integrity. Environmental Monitoring and Assessment. 51: 89-106.

Roy, A., S.K. Tripathi, and S.K. Basu. 2004. Formulating diversity vector for ecosystem comparison. Ecological Modelling 179: 499-513.

Scheffer, M., S. Carpenter, J.A. Foley, C. Folke, and B. Walker. 2001. Catastrophic shifts in ecosystems. Nature 413: 591-596.

Scheiner, S.M. and J. Gurevitch. 2001. Design and analysis of ecological experiments, 2nd edition. Oxford University Press, New York.

Schlosser, I.J. 1987(a). The role of predation in age- and size-related habitat use by stream fishes. Ecology 66: 651-59.

Schulz, E.J., M.V. Hoyer, and D.E. Canfield Jr. 1999. An Index of Biotic Integrity: a test with limnological and fish data from sixty Florida lakes. Transactions of the American Fisheries Society 128: 564-577.

Scott, M.C. and G.S. Helfman. 2001. Native invasions, homogenization, and the mismeasure of integrity of fish assemblages. Fisheries 26(11): 6-15.

Seber, G.A.F. 1982. The Estimation of Animal Abundance. Macmillon Publishing: New York. 654 pp.

Seber, G. A. F., and E. D. Le Cren. 1967. Estimating population parameters from catches large relative to the population. Journal of Animal Ecology 36: 631-643.

Skalski, G.T. and J.F. Gilliam. 2000. Modeling diffusive spread in a heterogeneous population: A movement study with stream fish. Ecology 81(6): 1685-1700.

Slawski, T. M., and T. J. Ehlinger. 1998. Fish habitat improvement in box culverts: management in the dark? North American Journal of Fisheries Management 18: 676-685.

Schwartz, C. J. 2001. The Jolly-Seber model: more than just abundance. Journal of Agricultural, Biological and Environmental Statistics 6: 175-185.

Steedman, R.J. 1988. Modification and assessment of an index of biotic integrity to quantify stream quality in southern Ontario. Canadian Journal of Fisheries and Aquatic Sciences 45: 492-501.

Teels, B.M., L.E. Mazanti, and C.A. Rewa. 2004. Using an IBI to assess effectiveness of mitigation measures to replace loss of a wetland-stream ecosystem. The Society of Wetland Scientists 24(2): 375-384.

Turchin, P. 1998. Quantitative analysis of movement and spatial population dynamics. John Wiley, New York, New York, USA.

Vaughan, D.S. and W. Van Winkle. 1982. Corrected analysis of the ability to detect reductions in year-class strength of the Hudson River white perch (*Morone Americana*) population. Canadian Journal of Fisheries and Aquatic Sciences 39: 782-785.

Waite, I.R. and K.D. Carpenter. 200. Associations among fish assemblage structure and environmental variables in Willamette basin streams, Oregon. Transactions of the American Fisheries Society 129: 754-770.

Wallen, E.I. 1951. The direct effect of turbidity on fishes. Bulletin of Oklahoma Agrucultural and Mechanical College, Stillwater, Oklahoma. Arts and Sciences Studies, Biological Series No. 2 48: 1-27.

Walling, D. E. and K. J. Gregory. 1970. The measurement of the effects of building construction on drainage basin dynamics. Journal of Hydrology 11:129-144.

Walsh, C.J., A.H. Roy, J.W. Feminella, P.D. Cottingham, P.M. Groffman, and R.P. Morgan II. 2005. The urban stream syndrome: current knowledge and the search for a cure. Journal of the North American Benthological Society 24(3): 706-723.

Warren, M. L., and M. G. Pardew. 1998. Road crossings as barriers to small-stream fish movement. Transactions of the American Fisheries Society 127: 637-644.

Walters, D.M., D.S. Leigh, M.C. Freeman, B.J. Freeman, and C.M. Pringle. 2003b. Geomorphology and fish assemblages in a Piedmont river basin, USA. Freshwater Biology 48: 1950-1970.

Waters, T. F. 1995. Sediment in streams: sources, biological effects and control. American Fisheries Society Monograph 7.

Weiss, J.L. and J.B. Layzer. 1995. Infestations of glochidia on fishes in the Barren River, Kentucky. American Malacological Bulletin 11(2): 153-159.

Wellman, J.C. 1999. Fish community and sediment assessment following bridge construction/replacement in the Valley and Highland Rim provinces of Tennessee. Biology. Cookeville, Tennessee Technological University: 233.

Wellman, J. C., D. L Combs, and S. B. Cook. 2000. Long-term impacts of bridge and culvert construction or replacement of fish communities and sediment characteristics of streams. Journal of Freshwater Ecology. 15(3): 317-328.

White, G. C. and K. P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. Bird Study 46: supplement 120-138.

Zalewski, M., Bis, B., Lapinska, M., Frankiewicz, P., and W. Puchalski. 1998. The importance of the riparian ecotone and river hydraulics for sustainable basin-scale restoration scenarios. Aquatic Conservation-Marine and Freshwater Ecosystems 8(2): 287-307

Chapter 3: Tables and Figures

Table 3.1: Number of individual stream fish that moved upstream or downstream, overall % individuals that moved out of all fish that were tagged, mean % recaptured of tagged individuals over all three recapture periods, and Standard Error (N = 3 for all results).

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

Table 3.2: Mean percent stream fish that moved between study reaches within a stream regardless of direction and percent stream fish recaptured for each stream across all sampling periods (N = 6 for all results).

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

Table 3.3: Mean estimates of population size adjusted by stream reach volume for the three dominant fish families captured in NC Piedmont streams: Percidae, Centrarchidae, and Cyprinidae, in downstream and upstream (D & U) reaches in streams with crossing types (Culverts: Arch, Box, Bridge, Pipe, and Control). Estimates were calculated using CAPTURE software to analyze triple pass depletion data pooled across the 4 sample periods for each stream reach. Population means and standard errors were calculated for each position within a crossing type (N=3). (*N=2)

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

Table 3.4: Mean population size estimates for three dominant fish families: Percidae, Centrarchidae, and Cyprinidae, for all crossing types (Culverts: Arch, Box, Bridge, Pipe, and Control) pooled across position (Downstream and Upstream), creek (3 streams with each crossing type) and sample periods (4 samples).

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

Table 3.5: Mean Shannon Weiner species diversity index score, standard error, and number of stream reaches (N) per crossing type (Culverts: Arch, Box, Bridge, Pipe; and Control) and position (Downstream and Upstream). See text for results of statistical analyses of means.

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

Table 3.6: Mean Shannon Weiner species diversity index score, standard error, and number of stream reaches (N) per crossing type (Culverts: Arch, Box, Bridge, Pipe; and Control). See text for results of statistical analyses of means.

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

Table 3.7: Mean fish index of biotic integrity (FIBI), standard error, and number of stream reaches (N) per crossing type (Culverts: Arch, Box, Bridge, Pipe; and Control) and position (Downstream and Upstream). See text for statistical analyses of means.

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

Table 3.8: Mean fish index of biological integrity (FIBI), standard error, and number of stream reaches (N) per crossing type (Culverts: Arch, Box, Bridge, Pipe; and Control). See text for results of statistical analyses of means.

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

Figure 3.1: Examples of the crossing types assessed in this study (clockwise from top left): bridge, arch culvert, box culvert, and pipe culvert (Photographs by Chris Eads).

Figure 3.2: Study sites located west and north of Raleigh, NC, in the Cape Fear River basin. Each crossing type is represented by a different symbol. The letters inside each symbol correspond to an individual appendix table for each stream.

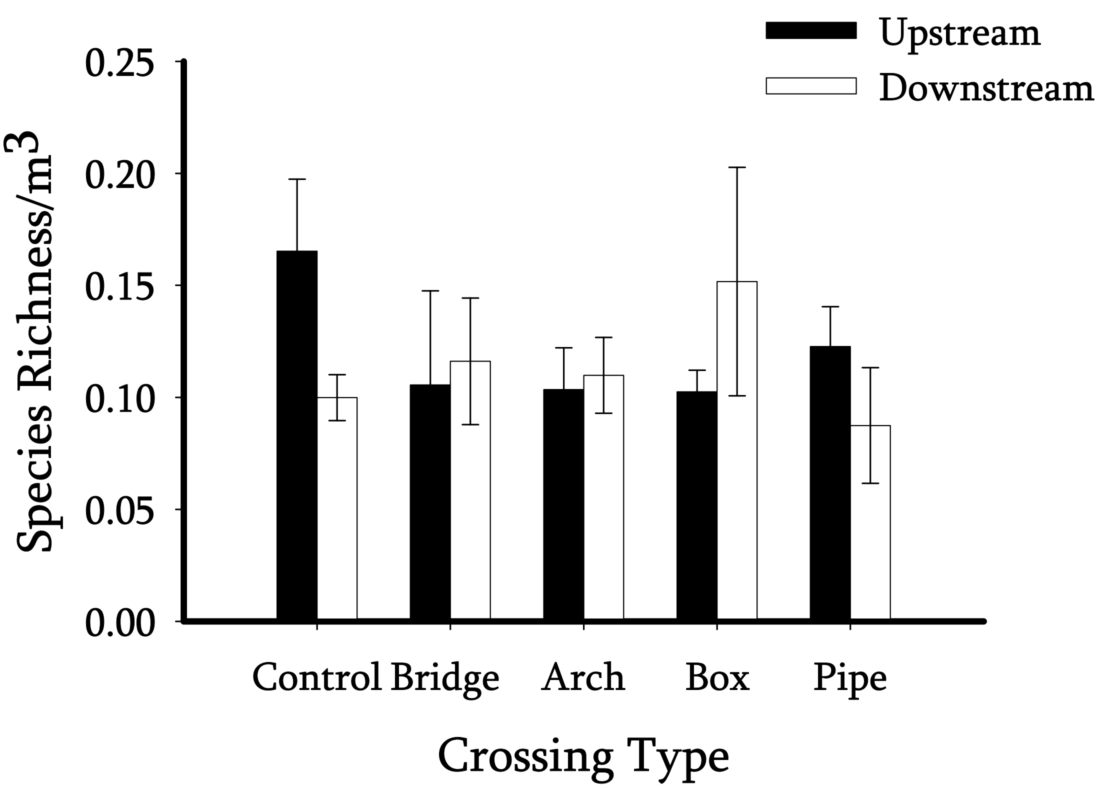

Figure 3.3: Mean stream fish species richness per m^3 (± SE) for each crossing type (bridge, culverts: arch, box, pipe, and control) and position (upstream and downstream), N = 3. See text for results of statistical analyses.

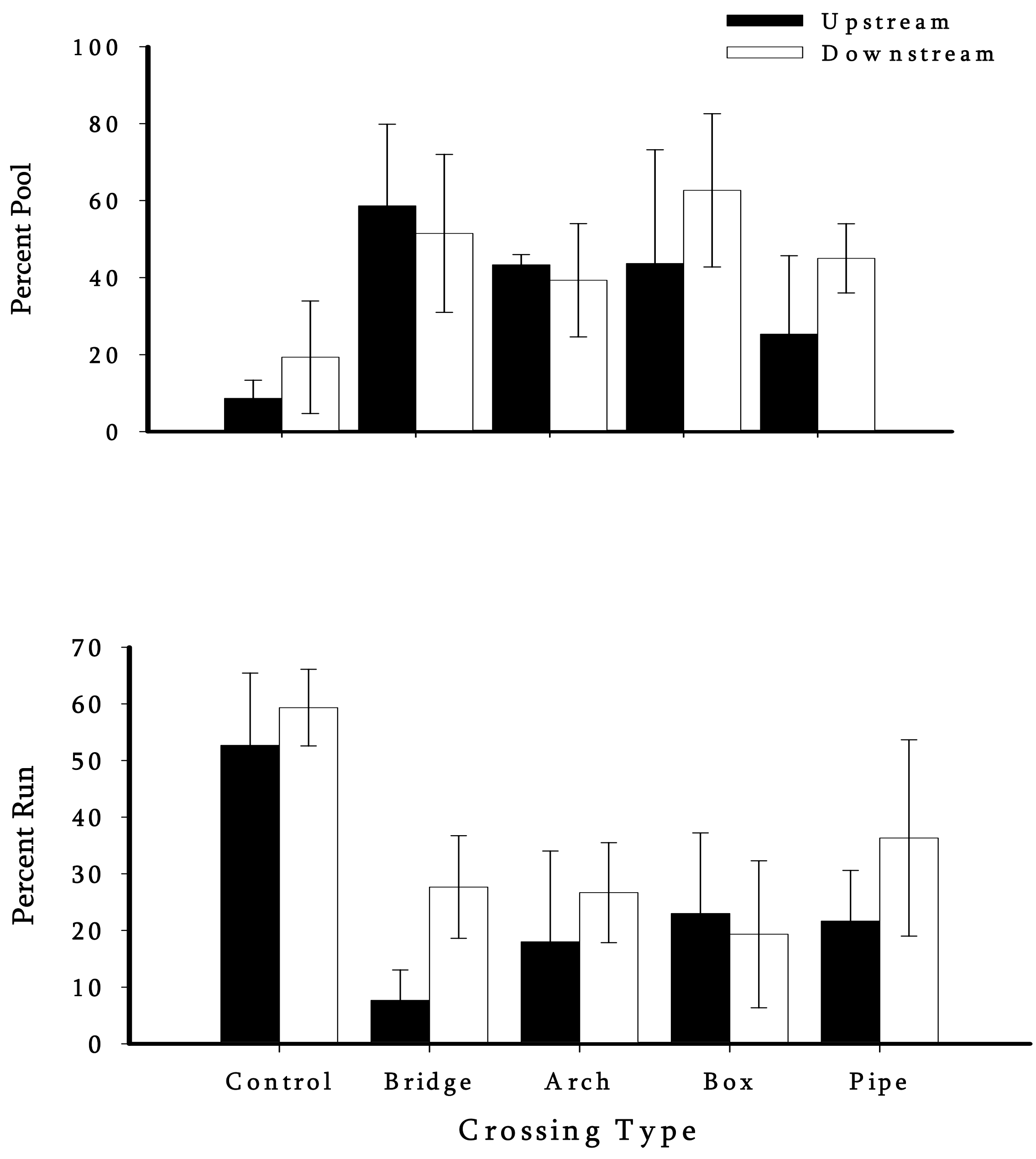

Figure 3.4: Mean percent pool (± SE) and mean percent run (± SE) of stream reach (50 m) by crossing type (bridge, culvert: arch, box, pipe, and control) and position (upstream and downstream), N = 3. See text for statistical analyses.

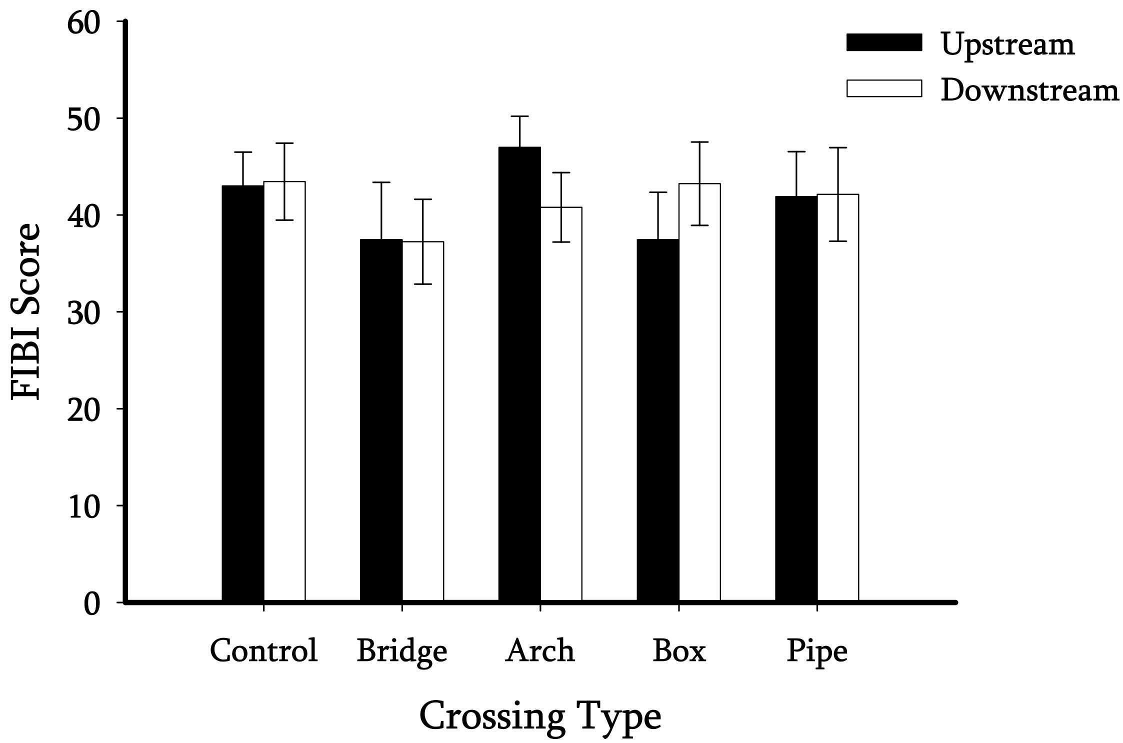

Figure 3.5: Mean fish index of biotic integrity (FIBI) score (± SE) of species for each crossing type (bridge, culvert: arch, box, pipe, and control) and position (upstream and downstream), N = 3. See text for results of statistical analysis.

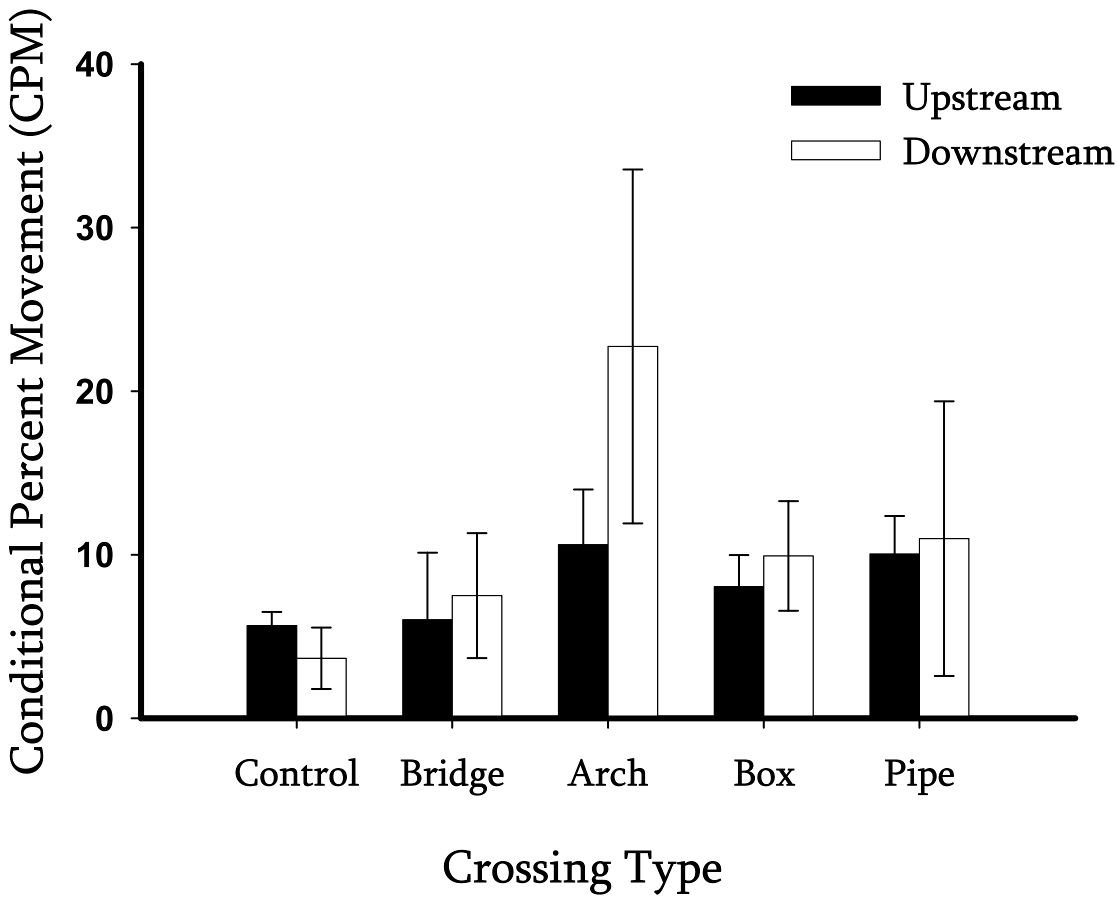

Figure 3.6: Mean stream fish conditional percent movement (± SE) by crossing type (bridge, culvert: arch, box, pipe, and control) and position (upstream and downstream), N = 3. See text for statistical analyses.

CHAPTER 4:
Impact of Bridges and Culverts on Stream Fish Movement: PIT-tagging methods.

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Introduction

Tagging methods to study fish movement through space and time have been in use since the 17th century when Izaak Walton attached ribbons to the caudal fins of Atlantic salmon to test the theory of natal site fidelity (Walton 1983). Technological advances since then have expanded the range and accuracy of methods used to monitor fish mobility in fresh and salt water environments from the ability of a tag to help gather small-scale habitat use of a damsel fish (McCormick and Smith 2004) to being able to store many months worth of specific temperature and depth information of an individual pelagic tuna that is later transmitted via satellite (Schaefer and Fuller 2005). Fish marking data is not only integral to scientific research but it also serves as the base of fisheries management and conservation decisions (Lucas and Baras 2000).
Trade-offs exist for all types of tags between the accuracy of the data gathered, the length of the study, the number of individuals that can be tagged, the amount of stress experienced by the fish from sampling and tagging methods, and the extent of resources available (Lucas and Baras 2000). The passive integrated transponder (PIT) tag is an internal marker that has become an essential tool for studying movement, behavior, and survival of a variety of fish species (Gibbons and Andrews 2004). There are many advantages to using PIT tags such as minimal injury of fish, high retention rate, small size (12 mm long x 2.1 mm diameter), no reliance on battery power, individual identification code, and little effect on behavior of fish (Prentice et al. 1990a). The tag consists of an integrated circuit chip, capacitor and antenna coil encapsulated in a glass cylinder, and its operation requires an external energy source (Prentice et al. 1990a; b), interrogated with the field of an induction coil, energizes and causes a tag to retransmit its code to the reader. Recent advances in remote antenna array, which are used to detect PIT tags, have expanded the utility of PIT tags to continuously monitoring the movements of Atlantic salmon *Salmo salar* by placing permanent antennae at strategic points along the paths they use (Zydlewski et al. 2001), culvert passage of juvenile salmonids in Oregon (Hansen and Furniss 2003), salmonid use of discrete refugia (Burns et al. 1997), and recently in small stream fish (Cucherousset et al. 2005).

The majority of work conducted using PIT tag and antenna technology has been on salmonids with only a few studies on non-game stream fish (Roussel et. al. 2000; Cuchrosset et al. 2005). Traditionally, non-game stream fish home ranges and movements have been studied using mark-recapture methods involving subcutaneous paint tags or fin-clips, which are often challenged by methodological problems that decrease recapture rates and bias movement distance distributions due to a limited area of recapture (Lucas and Baras 2000). PIT tags, however, are a much more effective yet expensive alternative; although, the tag size, small relative to other tag types, restricts the taggable fish to those measuring ≥ 60 mm (Ombredane et al. 1998, Columbia Basin Fish and Wildlife 1999).
We assessed unidirectional stream fish movement through two types of crossings, box culverts and bridges, using PIT tags and remote antenna arrays to further assess the potential impact of these two crossing types on stream fish in the Piedmont of North Carolina. The advantages of PIT tags and remote antenna arrays over more traditional mark-recapture methods, such as fin clips and elastomer paint tags, are: (1) increased recapture rates because of a 95-100% read efficiency of the antenna system, (2) increased recapture rates due to the ability to constantly

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

Methods

Site selection

A total of six sites were selected in a directed manner from a total of 42 possible sites harboring mussel populations (Fig 4.1). All sites were located within the Cape Fear River Basin, North Carolina, to reduce variance in measures of stream fish community. Because of drought conditions during summer 2005, and to avoid culvert perching or other physical barriers to stream fish movement (dry stream bed), we could only use one site from our 2004 sampling: Mary's Creek (Fig 4.1). For a balanced design, we chose three sites for each crossing type: box culvert and bridge. Habitat characteristics (as outlined by the North Carolina Department of Environment and Natural Resources) such as: (1) stream width measured by a tape measure, (2) stream depth measured by a meter stick, (3) predominate substrate type (bedrock, boulder, cobble and sand), (4) percentage of habitat type (pond, riffle, and run), (5) bank stability distinguishing between right and left banks (a scale from 1-10 with a score of 1 equivalent to "100% eroded bank" and a score of 10 equivalent to "less than 5% eroded bank"), and (6) width of riparian zone distinguishing between right and left banks (a scale from 1-10 with a score of 1 equivalent to "less than 6 m of riparian vegetation" and a score of 10 representing "greater than 18 m of riparian vegetation"), were quantified at 10 m intervals along a distance of 150 m above and below each crossing. Stream reach volume and area were calculated using the average of the widths and depths for each stream reach, and multiplied by the length of each reach, 150 m. Stream width and depth directly above and below the crossing were the most important measurements considered when choosing a site because this area had to accommodate the PIT tag antenna array (see below for more detail) and maximize fish passage through the antenna.

Antenna and reader configuration

ISO PIT tags measuring 12.45 mm long by 2.02 mm wide (Biomark, Inc.) and operating at 134.2 kHz were matched to a full-duplex FS2001 FR-ISO reader and tuning box (Biomark, Inc.) to operate the complete PIT tag system. Full-duplex tags can only be read by ISO readers and were the best choice for this study because they were the smallest PIT tag available. The reader and tuning box were connected to an open loop inductor antenna that generated both an energizing electromagnetic field and received transmitted signals from a PIT tag as the tagged animal passed through the field. The reader stored all tag information with internal memory until it was downloaded with a laptop computer. The antenna was constructed using 14-gauge insulated Thermoplastic High Heat Resistant Nylon coated (THHN) copper wire which was wound in a square loop (11 wraps) measuring 1.22 m wide by 0.46 m tall and housed in square PVC-pipe framing built with pipe measuring 2.54 cm in diameter and reinforced with PVC cement at the elbow connections. A bank of tuning capacitors (1600v metal polypro 1000-4700 uf, DIGI-Key, Corp.) was soldered to the loop and housed in the PVC-pipe framing between the coil and the cable. Combinations of capacitors allowed the antenna circuit to be tuned to the resonant frequency (natural frequency of vibration determined by the physical parameters of the vibrating object, in this case, the tag at 134.2 kHz) to yield a target current of 2.6-4.3 Amps through the reader (Biomark "Tuning instructions for custom antennas", www.biomark.com/manuals.htm). Electronic shielded Twinax cable (Belden part no. 9815, Hagemeyer North America) connected the antenna, which was located in the stream, to the tuning box and reader system on shore. The entire system was powered by two 12-V, marine deep cycle 630 cca batteries connected in series to the reader. The reader, tuning box, and batteries were housed in heavy-duty, water-tight plastic containers on shore. All spots of possible leaking on the PVC-pipe frame and containers on shore were sealed with aquarium sealant (Fig 4.2).

Each antenna was tuned and tested in a local forest and stream (Schenck Forest, Raleigh, NC) before deploying to the study stream. One day prior to sampling a given stream, the antenna was tested and retuned at the research stream to account for environmental factors such as other antennae, power lines, or structures with embedded reinforced steel (bridges and culverts included). Due to potential electrical interference, the antenna had to be located at least 0.61 m away from the crossing. Because warmwater centrarchids favor upstream movement during spring and summer periods (Gatz and Adams 1994), we initially decided to measure only stream fish movement upstream. Excess electrical interference, presumably due to nearby transformers, forced us to place the antenna system of two streams (Mary's Creek and Vestal Creek, Fig 4.1) downstream of the crossings. Antenna systems for the remaining four streams were successfully placed upstream of the crossings. Thus, two streams had reader systems placed downstream of the crossings and four streams had reader systems placed upstream of the crossings. All reader and antenna systems were tested for the distance over which the antenna could read a tag, which varied according to tag orientation from 15-30 cm directly upstream and downstream of the antenna.

Each antenna was secured in a given stream to iron rebar; the rebar was driven into the streambed as deep as possible and located 1.3 m apart. One piece of weighted nylon netting with 0.48 cm mesh size was stretched from each side of the antenna to iron rebar driven into the dry bank in order to restrict fish passage to only the open space provided by the antenna loop (Fig 4.2). The bottom of the netting was further weighted with rocks to ensure its effectiveness as a fish weir. The reader was then turned on and left running until subsequent battery changes and data downloads, which was every 7-10 days.

<u>Fish sampling</u>

Three techniques were used to capture fish for PIT-tagging in this study: (1) block nets measuring 13.72 m long x 1.83 m tall with 0.48 cm mesh to enclose three 50 m reaches above or below a road crossing, (2) seine nets measuring 4.57 m long x 1.22 m tall and 6.09 m long x 1.22 m tall with 0.48 cm mesh to sample large pool and run habitats more effectively, and (3) electrofishing using a 12A Smith-Root back pack unit to capture fish for tagging. We only sampled the fish on the side of a given crossing opposite of the antenna system to measure one direction of fish movement. For example, if an antenna was placed upstream of a crossing then only the fish in 150 m downstream of the crossing were sampled, and vice versa. All fish sampling used block-nets to enclose three adjacent 50 m reaches of each stream immediately upstream or downstream of a road crossing. We chose to partition the 150 m sample reach into adjacent 50 m sections in an effort to reduce the time over which fish were being held which, in turn, reduced mortality. Once enclosed, stream fish in the upstream or downstream reaches of each stream were sampled using a combination of seining and backpack electrofishing; double-pass depletion methods were used to maximize the number of fish sampled measuring 60 mm TL and larger. After analyzing capture rates of fish measuring ≥ 60 mm from the 2004 triple pass depletion methods across 16 streams (Chapter 3), we determined that increasing sample reach size while decreasing pass numbers from three to two would increase our expected number fish within the target fish size range of ≥ 60 mm (Table 3.1). Fish were removed from the study reaches after each collecting pass and kept in pop-up laundry hampers located directly in the stream flow. After each 50 m section was sampled with double pass depletion methods, we tagged (see tagging methods below) the fish from that section to decrease holding time and handling mortality, and then released them near the original site of capture.

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

Fish were sampled using the PIT tag approach from June 22 to October 2, 2005. Only three streams were sampled and running at a given time. Two readers were flooded resulting in one damaged beyond repair and needing a replacement. Turn around of replacement and repaired equipment caused a lag in data collection in two of the streams (Fork Creek and Mary's Creek, Fig 4.1), as well as multiple delays in redeployment of the reader systems to the second set of three streams until later that summer and into the fall.

PIT-tagging systems

Streams were monitored for 30-43 days during which antenna systems were serviced on a cycle of 7-10 days. Servicing included changing batteries, downloading tag codes with a laptop computer, and clearing net weirs of debris and repairing nets as needed. Tag read range and current strength was tested at each visit, followed by any fine tuning needed to maximize read range and current strength. All systems maintained at least a 0.30 m tag read range directly upstream and downstream of the antenna at 2.6 Amps of current or higher; although one stream system, Vestal Creek (Fig. 4.1), maintained the aforementioned read range with only 1.4 Amps of current. Tag data, including time and date stamps for each detection, were entered and managed in a relational database.

Response variable and hypothesis

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

Sampling design and statistical analyses

Movement data was analyzed using a sign test approach for two independent samples: (1) the proportion of tagged fish that were detected with the antenna array for box culverts relative to the number of fish tagged, and (2) the proportion of tagged fish that were detected with the antenna array for the streams with bridges relative to the number of fish tagged. Recapture data was standardized to a 30 day recapture period at all sites. Because low sample sizes, as in this study (N = 3), reduce the power of the equal variance test resulting in failure to reject the null hypothesis of equal variances (Cody and Smith 1997), which is an assumption of parametric comparison tests, we conducted a nonparametric sign test (Zar 1984). Difference in mean stream fish movement relative to crossing type was analyzed using a non-parametric sign test pairing streams by position of antenna (upstream or downstream of the crossing) and stream depth (Appendix Table 4.1).

Results

A total of 681 fish measuring and representing 19 species and seven families of fish ≥ 60 mm were captured and tagged with PIT tags (Appendix Tables 4.2 and 4.3). Out of 681 tagged individuals, 258 stream fish were detected at least once by antenna systems during a 30 day running period in six streams (Table 4.2). The proportion of tagged fish to travel through the crossing on each stream ranged from 3.95%- 55.97% with the mean proportion of movers 28.27% ± 12.24% (SE) for streams with box culverts, and 44.35% ± 8.77% (SE) for streams with bridges (Tables 4.2 and 4.3).

The mean proportion of tagged stream fish that traveled through a crossing was nearly twice as high near bridges (44.35%) than box culverts (28.27%, Fig 4.3); however, the trend was not statistically significant (sign test, df = 2, p = 0.125). The low number of streams (N=3) sampled for each crossing type and resulting high variance (Fig 4.3) is the likely reason for a non-significant p-value. For example, assuming a similar difference in the number of stream fish that moved between bridges and box culverts (Table 4.3), if sample size was increased to N = 5, then the sign test would have produced a significant p-value of 0.031 (Zar 1984).

Discussion

The results from this study suggest that there is no significant difference between fish movement through bridges and box culverts in these six streams of the Piedmont of North Carolina. With such a small sample size, no definite conclusions can be made; although, the almost two-fold difference in mean movement between bridges and culverts suggests a trend that a larger study could prove to be statistically significant. A similar, previous assessment of fish movment through crossing types, which included perched culverts, also found no significant difference in fish movement through bridges and box culverts (Warren and Pardew 1998, see Chapter 3 for a more thorough review of relevant literature).

The main difference between this study and those mentioned above was the effectiveness of the methods used in a mark-recapture study. Both studies (Warren and Pardew 1998; Vander Pluym Chapter 3) used traditional methods of tagging fish with subcutaneous elastomer paint and conducting multiple electrofishing events aimed at recapturing individuals. This approach, although common, appears much less effective and more labor intensive than the PIT-tag approach used in this study. Warren and Pardew (1998) reported recapture rates of 18% during spring sampling and 21% during summer sampling with a range of 12-17 days between recapture events. Vander Pluym (Chapter 3) reported somewhat lower recapture rates, ranging from 2.96% to 21.7% during summer sampling with 30 days in between recapture events. Because of the stationary antenna arrays deployed at each site, with the PIT-tagging approach recapture rates ranged from 3.95% to 55.95% with continuous tag detection over 30 days and no re-sampling necessary. Not only did the PIT-tag methods have a much greater recapture rate, but it also assessed movement more effectively. For example, this study detected 258 fish out of 681 tagged individuals having moved through crossings in 30 days of sampling, in comparison to 102 fish out of 9,300 (0.01%) individuals tagged in four months of sampling during the initial study described in Chapter 3.

The difference in methods between Chapter 3 and this study are reflected in the interpretability of the data. In the initial study, there were so few fish detected as moving through the different crossing types that we were unable to draw strong conclusions regarding the effects of crossing type on stream fish movement. Although the recapture success of tagged fish was vastly improved using the PIT-tagging approach compared to the subcutaneous ink marking approach, the PIT-tagging study suffered from relatively low replication (N=3) streams, which likely reduced the statistical power to detect a significant difference in movement rates, even though movement rates were nearly twice as high through bridges than box culverts. Using hypothetically similar results but with a sample size of 5 for each crossing type would have yielded a significant p-value (Zar 1984).

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

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

Conclusions

This study assessed warmwater stream fish movement through bridges versus box culverts using PIT-tagging. Our results showed no significant difference between fish movement through bridges and culverts; however, they do suggest with an increased number of study streams movements through box culverts could be significantly lower than through bridges. We recommend the use of the full-duplex PIT-tags in concert with remote antenna arrays for tracking small fish in wadeable streams. We also recommend exploration of the half-duplex tag system for larger individuals as a more flexible and affordable alternative to ISO tag systems. The nature of this study points to a need to reevaluate traditional mark-recapture methods that are commonly used when assessing the impacts of road crossing on movement of stream fish. The only way fisheries research can continue to produce reliable data upon which to base management decisions is by constantly assessing the reliability of the methods used.

References

Aarestrup, K., Lucas, M.C., and J.A. Hansen. 2003. Efficiency of a nature-like bypass channel for sea trout (*Salmo trutta*) ascending a small Danish stream studied by PIT telemetry. Ecology of Freshwater Fish 12: 160-168.

Axel, G.A., Prentice, E.F., and B.P. Sandford. 2005. PIT-tag detection system for large-diameter juvenile fish bypass pipes at Columbia River Basin hydroelectric dams. North American Journal of Fisheries Management 25: 646-651.

Burns, M.D., Fraser, N.H.C., and N.B. Metcalfe. 1997. An automated system for monitoring fish activity patterns. Transactions of the American Fisheries Society 126: 1036-1040.

Cody, R.P. and J.K. Smith. 1997. Applied statistics and the SAS programming language. Prentice Hall, Upper Saddle River, New Jersey.

Columbia Basin Fish and Wildlife Authority, PIT Tag Steering Committee. 1999. PIT tag marking procedures manual. Version 2.0. www.dfw.state.or.us/ODFWhtml/springfield/PIT_tag_Marking_Procedures_Manual.pdf

Cucherousset, J., Roussel, J.-M., Keeler, R., Cunjack, R.A., and R. Stump. 2005. The use of two new portable 12-mm PIT tag detectors to track small fish in shallow streams. North American Journal of Fisheries Management 25: 270-274.

Dane, B. G. 1978. A review and resolution of fish passage problems at culvert sites in British Columbia. Fisheries and Marine Service Technical Report no. 810. 126 p.

Gatz, A.J. and S.M. Adams. 1994. Patterns of movement of centrarchids in two warmwater streams in eastern Tennessee. Ecology of Freshwater Fish 3: 35-48.

Gibbons, J.W. and K.M. Andrews. 2004. PIT tagging: simple technology at its best. BioScience 54(5): 447-454.

Hansen, B. and M.J. Furniss. 2003. Effectiveness monitoring of culverts replaced or retrofitted for fish passage. 2003 Progress Report, Aquatic and Land Interactions Program, Corvallis Forestry Sciences Laboratory.

Iverson, M., Finstad, B., McKinley, RS, Eliassen, RA. 2003. The efficacy of metomidate, clove oil, Aqui-S registered and Benzoak as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts, and their potential stress-reducing capacity. Aquaculture 221(1-4): 549-56.

Lucas, M.C. and E. Baras. 2000. Methods for studying spatial behaviour of freshwater fishes in the natural environment. Fish and Fisheries 1: 283-316.

McCormick, M.I. and S. Smith. 2004. Efficacy of passive integrated transponder tags to determine spawning-sit visitations by a tropical fish. Coral Reefs 23: 570-571.

Ombredane, D., Bagliniere, J.L., and F. Marchand. 1998. The effects of passive integrated transponder tags on survival and growth of juvenile brown trout (*Salmo trutta* L.) and their use for studing movement in a small river. Hydrobiologia 371/372: 99-106.

Prentice, E.F., Flagg, T.A., and C.S. McCutcheon. 1990a. Feasibility of using implantable passive integrated transponder (PIT) tags in salmonids. Pages 317-322 *in* N.C. Parker, A.E. Giorgi, R.C. Heidinger, D.B. Jester, Jr., E.D. Prince, and G.A. Winans. Fish-marking techniques. American Fisheries Society, Symposium 7, Bethesda, Maryland.

Prentice, E.F., Flagg, T.A., McCutcheon, C.S., and D.F. Brastow. 1990b. PIT-tag monitoring system for hydroelectric dams and fish hatcheries. Pages 323-334 *in* N.C. Parker, A.E. Giorgi, R.C. Heidinger, D.B. Jester, Jr., E.D. Prince, and G.A. Winans. Fish-marking techniques. American Fisheries Society, Symposium 7, Bethesda, Maryland.

Roussel, J.-M., Haro, A., and R.A. Cunjack. 2000. Field test of a new method for tracking small fishes in shallow rivers using passive integrated transponder (PIT) technology. Canadian Journal of Fisheries and Aquatic Sciences 57: 1326-1329.

Schaefer, K.M. and D.W. Fuller. 2005. Behavior of bigeye (*Thunnus obesus*) and skipjack (*Katsuwonus pelamis*) tunas within aggregations associated with floating objects in the equatorial eastern Pacific. Marine Biology 146 (4): 781-792.

Scheiner, S.M. and J. Gurevitch. 2001. Design and analysis of ecological experiments, 2nd edition. Oxford University Press, New York.

Skalski, G.T. and J.F. Gilliam. 2000. Modeling diffusive spread in a heterogeneous population: A movement study with stream fish. Ecology 81(6): 1685-1700.

Walton, I. 1983. The Compleat Angler 1653-1676, with an introduction and commentary by J. Bevan. Oxford University Press, New York.

Warren, M. L., and M. G. Pardew. 1998. Road crossings as barriers to small-stream fish movement. Transactions of the American Fisheries Society 127: 637-644.

Zar, J.H. 1984. Biostatistical Analysis, 2nd ed. Prentice Hall, Upper Saddle River, New Jersey.

Zydlewski, G.B., Haro, A., Whalen, K.G., and S.D. McCormick. 2001. Performance of stationary and portable passive transponder detection systems for monitoring of fish movements. Journal of Fish Biology 58: 1471-1475.

Chapter 4: Tables and Figures

Table 4.1: Triple-pass depletion capture analysis of fish measuring ≥ 60 mm TL from a previous study of 16 streams (Chapter 3). Data were pooled across four sampling periods and described as a function of 50 m and 100 m reaches located in the Piedmont of NC. The greatest percentage (82%) of large stream fish was caught in the first and second pass. By extending the reach to 150 m and using only double pass rather than triple pass depletion methods, we estimated capturing 160.77 large stream fish as opposed to 131.5 in 100 m using triple pass depletion.

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

Table 4.2: Number of individual stream fish that moved through the crossing, their direction of movement, number of individuals tagged initially, and overall % individuals that moved out of all fish that were tagged in 30 days of PIT-tag monitoring (N = 3 for all results).

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

Table 4.3: Results of sign test of proportion of tagged fish moved through the crossing, bridge or box culvert, for three pairs of streams.

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

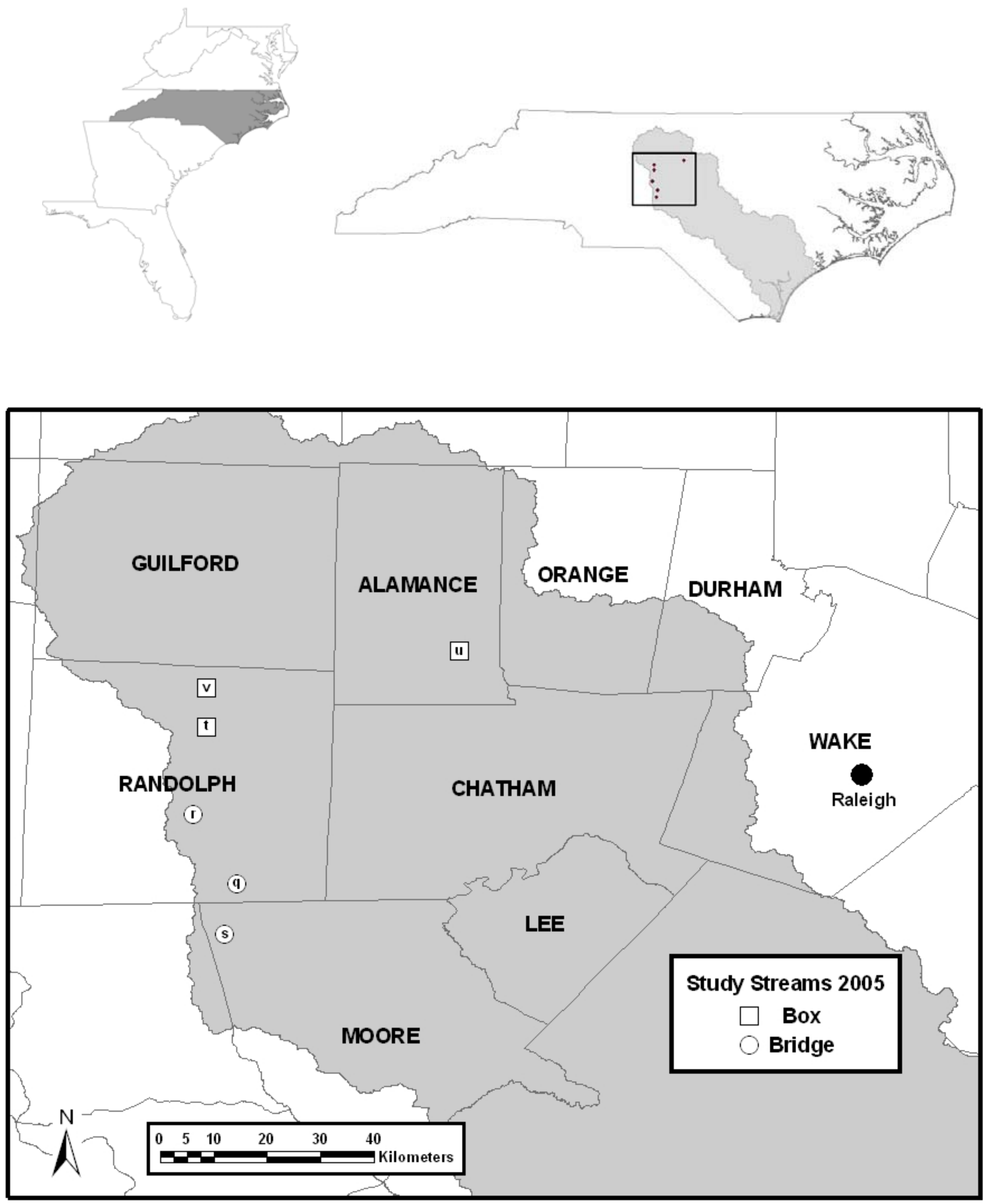

Figure 4.1: Study sites located west of Raleigh, NC, in the Cape Fear River basin. Each crossing type is represented by a different symbol. The letters inside each symbol correspond to an individual appendix table for each stream.

Figure 4.2: Remote antenna array complete with net weirs, shielded cable, FS2001 reader, tuning box, and batteries in place downstream of the box culvert in Mary's Creek (Photographs by Jenny Vander Pluym).

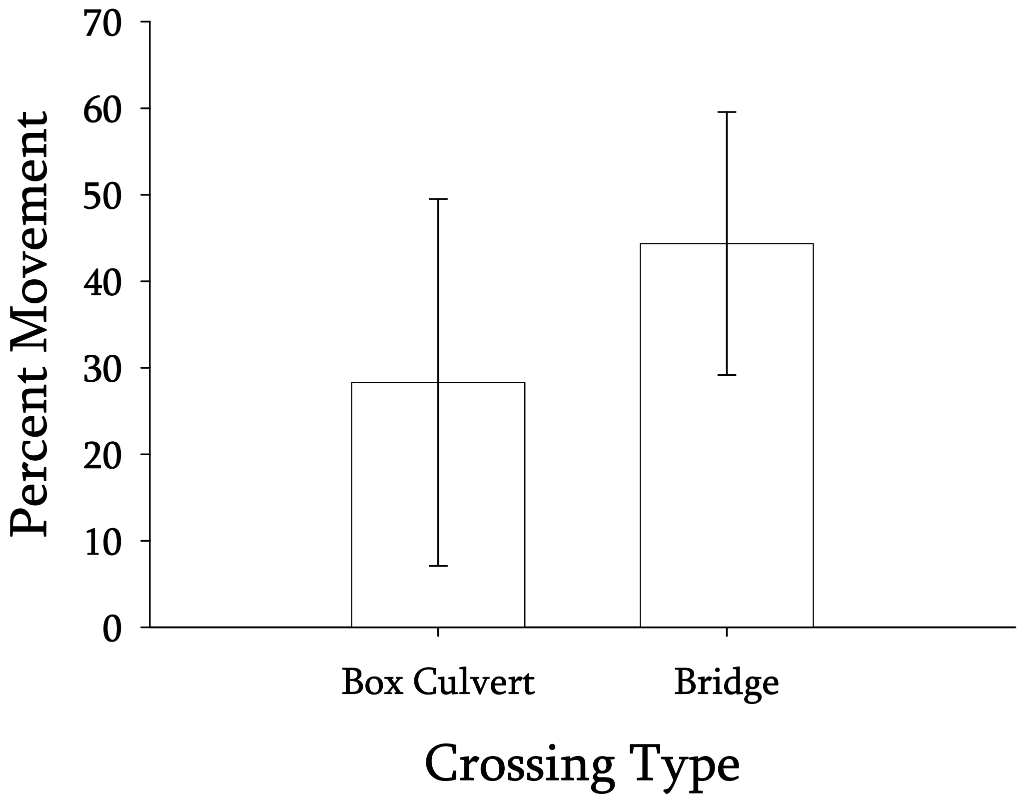

Figure 4.3: Mean percent stream fish movement (± SE) by crossing type (box culvert and bridge) over 30 days of monitoring (N=3). See text for results of statistical analysis.

CHAPTER 5:
Effects of Polycyclic Aromatic Hydrocarbon Exposure on Three Life Stages of Freshwater Mussels (Bivalvia: Unionidae)

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Introduction

Polycyclic aromatic hydrocarbon (PAH) compounds are a class of hydrophobic environmental pollutants widespread in terrestrial and aquatic environments. Many of the compounds in this group are of major concern to environmental agencies and researchers worldwide due to their mutagenic and carcinogenic properties (Baumard et al., 1999). Polycyclic aromatic hydrocarbons enter the environment via natural (biogenic) processes (e.g., forest or grass fires, natural petroleum seeps, etc.), and anthropogenic processes, including accidental spills or releases of petroleum compounds into the environment (e.g., tanker spills, oil platform releases) and high temperature combustion (petrogenic and pyrolitic) processes (e.g., the burning of fossil fuels, industrial activities) (Eisler, 1987; Fernandes et al., 1997; Baumard et al., 1998; Piccardo et al., 2000). Higher molecular weight PAH compounds are mainly generated by high temperature combustion of organic matter, therefore anthropogenic activities are generally considered to be the major source of higher molecular weight PAH environmental contamination (Piccardo et al., 2001). Low molecular weight PAH compounds may be produced by fossil fuel combustion, but are also major components of petroleum products (Fernandes et al., 1997), and natural processes (Eisler, 1987).

Polycyclic aromatic hydrocarbon compounds are considered to be highly hazardous to the environment and to human health. Four- to seven-ring PAHs are highly mutagenic and carcinogenic, and two- to three-ring PAHs, although less mutagenic, can be highly toxic (Eisler, 1987). Lower molecular weight PAH compounds are less mutagenic, but can be highly toxic (Fernandes et al., 1997). In many cases the parent compounds are relatively inert, but the metabolites exert toxicity. Low molecular weight PAHs, dominant in fossil fuel assemblages, are more labile and readily volatilize into the atmosphere from the air/water interface. With increasing molecular weight comes decreasing water solubility, with the result that lower molecular weight PAHs are preferentially adsorbed to particles in the water column (Baumard et al., 1999). Lower molecular weight PAH compounds in the water column may therefore be more bioavailable to organisms. Polycyclic aromatic hydrocarbons can interact with cells to produce toxic responses by binding to lipophilic sites in cells and interfering with cellular processes (Neff, 1979). In light of the toxicity and carcinogenicity issues to terrestrial organisms, aquatic organisms, and to humans, the monitoring of PAH contamination in the environment is critical.

Polycyclic aromatic hydrocarbons enter aquatic environments by many routes, including domestic and industrial effluents, surface runoff from land, atmospheric deposition, and spillage from petroleum operations (Eisler, 1987; Piccardo et al., 2001). 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). Maltby and co-workers (1995) demonstrated that stormwater runoff from a motorway in the United Kingdom was toxic to the benthic amphipod *Gammarus pulex*. Heavy metals and PAHs at levels that could significantly impact aquatic biota were detected in runoff from a bridge in Canada (Marsalek et al., 1997). Beasely and Kneale (2002) noted that there is considerable evidence that heavily trafficked roads are an important source of toxicants to streams. Hallhagen (1973) and Wakeham (1977) indicated that urban storm water runoff was responsible for a significant level of hydrocarbon contamination to aquatic systems. Data from a previous study of roadway crossing structures in North Carolina found that there were elevated levels of PAH compounds downstream of 18 bridges and 2 culverts, and that this increase in contaminant levels correlated to decreased freshwater mussel abundance in stream reaches directly downstream of the crossing structure (Shea et al. 2004).

Biomonitoring of environmental conditions, especially in relation to measurements of hydrophobic contaminants, is more cost effective and accurate compared to direct environmental sampling. For instance, direct analyses of water samples for PAH contaminations are time-consuming, require large sample sizes (and are therefore innately more expensive), and do not necessarily represent the bioavailable fraction present in the water column (Gewurtz et al., 2002). Beasely and Kneale (2002) state that "snapshot" monitoring represents conditions only at a single point in time and may imply a greater grade of water quality than actually present. Additionally, these types of samples may miss periods of high contamination due to pulsed events (i.e., storm runoff). Sentinel organisms, however, concentrate contaminants within their tissues making trace levels of contaminants easier to monitor (Baumard et al., 1998). According to Pereira et al. (1996), bed sediments and lipid tissues of aquatic organisms integrate hydrophobic contaminants over seasonal or yearly timeframes, indicating that the biota of a stream may be more effective as monitors of water quality than the water in which they reside. However, this accumulation of organic contaminants is a complex function of the physiochemical properties of the contaminant, its distribution within the system, and the feeding behavior and metabolism of the aquatic organism used as biomonitors.

Due to their primarily sessile lifestyles and filter feeding activities, bivalve mollusks are among the most sensitive aquatic species to environmental contamination (Dame, 1996). In fact, some mollusks may be more susceptible to the effects of PAHs compared to vertebrate species due to the lack of efficient enzymes for metabolizing and detoxifying PAH compounds and metabolites (Eisler, 1987). The Cytochrome P-450 (CYP450) monooxygenase system is an apparently universally distributed system involved in the metabolism of xenobiotics, including PAH compounds. Increases in the activity of the CYP450 system are routinely used as a means of detecting exposure to PAHs and other pollutants in fish, although the response in bivalves is less obvious. Porte and co-workers (2001) attempted to develop an integrated monitoring strategy using CYP450 activity, benzo(a)pyrene hydroxylase (BPH) activity, and stress-70 proteins. They found that exposure to PAH-contaminated environments did not elicit a CYP450 response, but there was a clear induction of stress proteins (stress-70). It is important to note, however, that stress proteins are induced by a great number of environmental factors (e.g., UV light, salinity, temperature, oxidizing agents, etc.) in addition to contaminants (Sanders, 1993). In one study, no clear evidence of changes in activity of respiratory enzymes due to exposure to petroleum hydrocarbons were observed in the blue mussel (*Mytilus edulis planatus*) (Long et al., 2003).

The use of bivalves as sentinel organisms in aquatic environments has proved to be an effective method of monitoring chemical contaminant levels. Oysters (*Crassostrea sp.*) and mussels (*Mytilus sp.*) are commonly used sentinel organisms in marine ecosystems (Baumard et al., 1999; Piccardo et al., 2001; Geffard et al., 2002). For many years freshwater bivalves have been used as biomonitors of pollutant contamination in waterways (Renaud et al., 1995; Gagné et al., 2002; Gewurtz et al., 2002). Cataldo and co-workers (2001) used juvenile *Corbicula fluminea* survival to monitor sediment pollutant levels in Argentina. They determined that *C. fluminea* mortality rates corresponded well with sediment pollutant levels. Gewurtz and co-workers (2002) used the mussel *Elliptio complanata* to perform quantitative biomonitoring of PAHs. In another study, *E. complanata* and *Dreissena polymorpha* were used to study the effects of exposure to pollutants dispersed in a municipal effluent plume (Gagné et al., 2002). Assays of tissue body burden conducted on *Mytilus sp*., *Anodonta anatina, Unio tumidus*, and *E. complanata* have demonstrated that mussels bioaccumulate PAHs and are reliable sentinel organisms (Cossu et al., 1997; Anderson et al., 1999; Gewurtz et al., 2002; Hyötyläinen et al., 2002; Thorsen et al., 2004).

A major objective of toxicology-related epidemiological testing is to provide reliable and specific information concerning the effects of exposure to a particular agent. The possibility of using biomarkers of exposure to substitute for classical endpoints (e.g., disease incidence or mortality) in molecular epidemiological studies is promising (Bonassi and Au, 2002). However, documentation of exposure may be non-existent or difficult to obtain in many cases. Bonassi and Au (2002) suggested that the use of biomarkers of exposure may provide a more precise method of obtaining that information, and that the data, if correctly collected, may be utilized to calculate the internal exposure doses and to determine the dose-response relationship. According to Porte et al. (2001), mussels exhibit a series of sublethal biochemical responses to pollutants, making them excellent choices for pollution monitoring studies of chemical analysis of tissue burden and biomarkers of exposure. In instances of non-lethal exposures, biomarkers may be used to determine exposure level of sentinel organisms.

The primary routes of exposure for bivalves are across the gill and digestive gland membranes. Biomarkers of PAH exposure for bivalves have included growth and development (Geffard et al., 2002; Widdows et al., 2002), CYP450 induction (Anderson et al., 1999; Porte et al., 2001; Gagné et al., 2002), respiratory enzymes (Long et al., 2003), embryogenisis and larval development (His et al., 1997; Geffard et al., 2002), hemocyte phagocytosis (Fournier et al., 2000; Blaise et al., 2002), antioxidant enzymes, glutathione and lipid peroxidation (Cossu et al., 1997; Doyette et al., 1997), and DNA damage in hemocytes, digestive tissues and somatic cells (Sasaki et al., 1997; Wilson et al., 1998; Pavlica et al., 2001; Coughlan et al., 2002; Hamoutene et al., 2002; Large et al., 2002; Rank and Jensen, 2003; Klobučar et al., 2003; Siu et al., 2004). In one study, RNA arbitrarily primed PCR was used to look for genomic aberrations in digestive tissues of *Unio tumidus* exposed to effluent from a cokery plant on the Fensch River (France) known to be responsible for PAH contamination (Rodius et al., 2002).

Previous studies of biomarkers of contaminant exposure have utilized gill and digestive gland dissected from mussels (Cossu et al., 1997; Doyotte et al., 1997; Long et al., 2003) or whole body analyses (Anderson et al., 1999; Porte et al., 2001) for CYP450 and stress-70 protein induction (Porte et al., 2001), respiratory enzyme activity (Long et al., 2003), and hemocyte phagocytosis (Fournier et al., 2000; Blaise et al., 2002) in mussels exposed to contaminants. However, the results of some of these experiments have been inconclusive. Cytochrome P450 activity in bivalves is believed ineffective in relation to the metabolism of PAH compounds, hence the propensity of these chemicals to bioaccumulate in mollusks (Eisler, 1987). Stress-70 proteins are non-specific (i.e., elicited by a variety of stressors) and are thus offer little predictive value in determining exposure to specific contaminants (Porte et al., 2001). Additionally, the use of respiratory enzymes has proved to be an unreliable measure of contaminant exposure in bivalve mollusks (Long et al., 2003). A comparison of lethal and non-lethal biomarker techniques is needed. One such non-lethal technique, the sampling of bivalve hemocytes may yield reliable results with minimal impact on the animals (Gustafson, et al., 2005). Hemocytes may be sampled from the hemolymph extracted from the adductor muscle of bivalves with minimal effort and adverse impact to the animal.

Bivalve mollusks are vital members of aquatic ecosystems. They function as living filters, trapping food and particles in the water column as they filter-feed (Dame, 1996). Particles not ingested are excluded in pseudo-feces and effectively removed from the water column, enhancing the removal of particle-associated contaminants from the system. Native freshwater mussels (Bivalvia: Unionidae) filter large volumes of water on a daily basis, removing suspended particles and pollutants at a rate faster than accounted for through normal settling (Aldridge, 1999). Furthermore, freshwater mussels are important components of aquatic food webs, forming a major portion of the diet of muskrats, otters, raccoons, and other carnivorous animals that use rivers and streams as feeding areas. Biomonitoring of freshwater mussels may provide an early detection of potential problems arising from exposure to environmental contaminants. This would allow a potential pollution problem to be addressed prior to reaching levels within the system that would pose a threat to humans, agricultural animals, and other wildlife.

As a group, native freshwater mussels are among the most threatened aquatic animal species in North America (Peacock, et al., 2005). The National Native Mussel Conservation Committee (1998) estimated that 67% of the nearly 300 species of native North American mussels are either vulnerable to extinction or are already extinct, and recognized water pollution as a major factor in unionid decline. Despite this speculation, little documentation of the effects of major aquatic pollutants on these animals exists (Moulton et al., 1996). Major sources of water pollution in streams and rivers home to freshwater mussels include agricultural runoff containing various pesticides and chemicals, roadway runoff, municipal wastewater treatment plants, and industrial effluent. In particular, roadway runoff and municipal wastewater discharges can carry heavy loads of PAHs into an aquatic system. This may be particularly hazardous to mussels during their reproductive period.

Freshwater mussels have a stage of development during which they are obligate parasites on fish (Huebner and Pynnönen, 1992; Pynnönen, 1995; McMahon and Bogan, 2001). The female mussel broods her larvae, called glochidia, inside specially adapted chambers within her gills known as marsupia. When mature, the female mussel will either release the glochidia into the water in a mucosal conglutinate packet or attempt to attract an appropriate host organism using a section of her mantle as a lure designed to mimic a prey item (Jacobson et al., 1997; McMahon and Bogan, 2001) depending on the species of mussel. When the glochidia come into contact with a potential host organism they rapidly snap their valves together and attach themselves to either a fin or to the gills of the host. Once attached, the host rapidly forms a cyst around the glochidia. The period of encystment on the host varies between species and many species of mussel have a specific suite of fish hosts. Upon encystment on the host fish, the mussel glochidia are assumed to be well protected from stressful environmental conditions.

However, in the period of development prior to or just after release into the environment, glochidia may be at risk of exposure to toxic compounds in the water column.

Experiments utilizing the glochidia of freshwater mussels have demonstrated sensitivity of glochidia to many toxic compounds, including PAHs. Huebner and Pynnönen (1992), Pynnönen (1995), and Hanstén et al. (1996) demonstrated that glochidia of *Anodonta sp.* were sensitive to sub-lethal exposure concentrations of heavy metals and low pH, and that these exposures could significantly impact viability and survival. Keller et al. (1998) tested the toxicity of diesel fuel contaminated sediments on the glochidia of *Lampsilis siliquoidea* and *Lasmigona costata* and juvenile *Villosa villosa*, with ambiguous results. It should be noted, however, that the contaminant levels in these experiments were below the documented 'lowest effects level' from the literature. Weinstein (2000) tested the glochidia of *Utterbackia imbecillis* to characterize the acute toxicity of photo-activated fluoranthene. He found that the glochidia rapidly accumulated the contaminant within their tissues and the presence of low UV intensities made the glochidia >45 times more sensitive to fluoranthene. In 2001, Weinstein and Polk repeated this experiment with anthracene and pyrene on the same species of mussel with similar results. Tests on juveniles of many bivalve species have produced comparable results (McKinney and Wade, 1996; Ahrens et al., 2002). However, little is known about the toxicity of PAHs found at relatively low levels in streams with little urbanization in the watersheds.

This study was conducted as part of a larger study funded by the NC Department of Transportation examining the impact of crossing structures on freshwater mussels and their habitat. The primary goal of this effort was to examine the effects of exposure to PAHs on various life stages (glochidia, juvenile, and adult) of freshwater bivalves. Mussels at each of the three different life stages were analyzed to assess toxicity and to evaluate biomarkers of exposure and genotoxic effects resulting from exposure to PAH compounds. The secondary goal of this study was to explore and develop non-lethal sampling regimes and test procedures for working with this rapidly declining group of aquatic macro-invertebrates. The specific objectives of this study were:

1) To quantify in the laboratory and the field the effects of exposure to PAH compounds on all life stages of freshwater mussels;

2) Develop non-lethal techniques useful in determining exposure history of freshwater bivalves to PAH compounds that are accurate, rapid, inexpensive, and have minimal adverse impact on the animals being sampled.

Methods

Study organisms

Three species of unionid mussels (*Elliptio complanata*, *Lampsilis fasciola*, and *Lampsilis siliquoidea*) were used in glochidia, juvenile, and adult tests. *Lampsilis fasciola* and *Lampsilis siliquoidea* were used in glochidia and juvenile tests, and *Elliptio complanata* was used in adult tests. *Lampsilis fasciola* glochidia were obtained from gravid females collected from the Little Tennessee River near Franklin, NC. Juvenile *L. fasciola* were obtained from individuals transformed in the Freshwater Mussel Rearing Facility at the NCSU College of Veterinary Medicine. *Lampsilis siliquoidea,* an interior drainage mussel found in the Midwestern United States, glochidia and juveniles were obtained from Dr. Chris Barnhart at Missouri State University in Springfield, MO. *Elliptio complanata* is a common mussel found in many Atlantic slope drainage streams in North Carolina, and is a tachytictic brooder (Bogan, 2002). *Lampsilis siliquoidea* and *L. fasciola* are sexually dimorphic and are bradytictic brooders. *Elliptio complanata* and *L. fasciola* represent different reproductive strategies and mussel habitats found in North Carolina.

Collection of study organisms

For glochidial tests, gravid mussels were collected by hand and kept damp, cool, and dark for transport to the laboratory where they were placed in an indoor closed, recirculating holding facility at an ambient air temperature of 21ºC and a 12:12 light:dark cycle. Gravid mussels brought into the laboratory were placed into a tank equipped with a chiller system to maintain a water temperature of 12ºC to reduce the possibility of premature release of glochidia prior to use. Collection time of gravid mussels varied between species, based on the time of year for the maturation of glochidia within the marsupia.

All adult mussels brought into the lab were measured (total length to the nearest mm), weighed (to the nearest g), and marked with an identifying number with a rotary grinding tool. Mussels were held in the laboratory prior to testing in closed, re-circulating tanks with aerated tap water from the City of Raleigh conditioned with sodium thiosulfate to remove chloramine ions. Mussels in the laboratory were fed a diet of *Chlorella sp.* cultured at our facility in 150L batches. Glochidia used for testing were flushed directly from the marsupia of gravid females collected from the field after the depuration period. Female mussels were returned to their native stream following extraction of glochidia.

Eastern elliptio mussels (*E. complanata*) collected for use in the adult PAH toxicity test were obtained from a relatively uncontaminated stream in Central North Carolina (based on data obtained in a previous NCDOT funded study) and transported to the laboratory in a 45.5L cooler filled approximately half full with ambient water from their native stream. The mussels were acclimated by replacing roughly half the volume of ambient water with ASTM moderately hard re-constituted water (ASTM, 1993) every hour until the entire volume had been replaced. Mussels were weighed and measured as previously described, and their shells were scrubbed to remove attached debris. The mussels were randomly assigned a number (I or II) and following acclimation overnight, one mussel from each number group was randomly distributed to an experimental unit (test chamber).

Field Study Site selection

Twenty streams in North Carolina were randomly selected from the 50 streams utilized in the NCDOT funded study for use in the Toxicology portion of the study. The sites for the intensive field study were a subset of 6 randomly selected streams out of the 20 used in the Toxicology portion of the study (Table 5.1). At all 20 sites, passive sampling devices (PSDs) were deployed upstream and downstream of the crossing structure to determine baseline levels of stream contamination with PAH compounds. Passive sampling devices have been shown to be a good surrogate for mussel tissues in determining PAH contaminant levels within a stream (Shea et al., 2004). Toxicity data was compared to that obtained from 18 bridges and 2 culverts in a previous study.

2.4 Test solutions and supplies

Baseline data from a previous study funded through NCDOT was used to determine polycyclic aromatic hydrocarbon (PAH) levels in test solutions (Table 5.2). The test concentrations were based on the mean PAH levels measured at relatively uncontaminated sites (agricultural/rural/forested) and highly contaminated sites (urban) in a previous study, and designed to cover a range of potential contaminant levels (Shea, et al., 2004), up to solubility of most of the higher molecular weight PAH compounds in water. The stock PAH test solutions were prepared using a mixture of Alaskan North Slope crude oil and creosote dissolved in dichloromethane (DCM). Test concentrations consisted of stock solutions diluted with ASTM moderately hard re-constituted water (ASTM, 1993). Controls consisted of ASTM water and 200µl DCM + ASTM water. Positive control treatments consisted of 4-Nitroquinoline-N-oxide + ASTM water. All test treatments were conducted in triplicate.

Test containers were borosilicate glass dishes washed with HPLC grade reverse-osmosis water, acetone-rinsed, and oven-baked between trials to remove organic residues and other contaminants. Glass containers were used to minimize loss of PAH compounds due to adsorption onto the surface of containers. Test containers consisted of 120 x 90mm dishes for glochidia and juvenile tests, and 3L glass jars for adult trials.

Glochidia were gently flushed from one marsupia of each female mussel using a 50cc hypodermic syringe with a 10-gauge needle and ASTM water. Glochidia and juvenile experiments were conducted at 21^{o}C with a 12:12 light:dark cycle. Adult experiments were conducted at 20^{o}C ambient air temperature and aerated gently with a 16:8 light:dark cycle. Water quality variables (temperature, dissolved oxygen, pH, and conductivity) were measured daily in each test chamber. Mussels were not fed during any of the experiments.

Test protocols

<u>Glochidial tests</u> - Acute (48h) toxicity tests were conducted on glochidia during summer 2004, depending on the mussel species and availability of glochidia. Each brood was tested for viability with the addition of 2-3 drops of saturated NaCl solution to a sub-sample of the brood. When exposed to NaCl solution glochidia snap closed, viability is determined based on the percent of glochidia within the sub-sample that close following NaCl exposure. Broods with less

than 90% viability were not used in experiments. Once viability was determined the broods were pooled to minimize any between animal associated bias and about 150 glochidia were added to each test chamber. Glochidia were added to the test containers by gently swirling the holding container and withdrawing ~0.5cc into a borosilicate glass pipette to obtain a random sample. At 24 and 48h of exposure to PAHs, a sub-sample of ~50 glochidia was tested for viability using the NaCl method, and the test solutions were renewed ($^2/_3$ volume) with new stock solution in ASTM water. *Lampsilis fasciola* glochidia not used in the acute toxicity tests were used to infest fish hosts (largemouth bass, *Micropterus salmoides*) to obtain laboratory-reared juveniles.

Largemouth bass were infested with glochidia by either pipetting glochidia directly onto the gills or by placing the fish with glochidia in a 10-gallon aquarium rapidly aerated to mix the water well. The period of encystment of the glochidia on the host fish varies per species of mussel, but lasts only a few weeks (http://news.fws.gov/mussels.html). During the encystment period the fish hosts were maintained in recirculating 10-gallon aquaria in the Mussel Barn at the NC State University College of Veterinary Medicine. Aquaria were siphoned daily beginning one week post-infestation to collect transformed juvenile mussels.

Juvenile tests - Acute toxicity testing was performed on recently (<30 day old) transformed mussels of both species, depending on transformation success from fish hosts and availability of juveniles from the supplier, during summer 2004, and on >60d old *L. siliquiodea*. Test duration was 96h and viability assessment was conducted at 48 and 96h of exposure. Viability was determined during a 5-minute observation period and based on foot movement inside or outside of the shell. Seven juvenile mussels were used per replicate, and all PAH treatments were conducted in triplicate. PAH test solutions were renewed daily ($^2/_3$ volume) with new stock solution in ASTM water.

Adult positive control tests - Adult *E. complanata* (N=4) were sampled from a relatively uncontaminated reference site (Richland Creek, Wake County, NC) on 16 Feb 2005. Approximately 0.5ml of hemolymph was drawn from the anterior adductor muscle of each mussel (Gustafson et al., 2005). Hemolymph samples were pooled to account for between animal variation, and allocated in 100µl aliquots to 4 tubes for a positive control experiment using 4-nitroquinoline-N-oxide, a known genotoxic compound (Le Pennec and Le Pennec, 2001; Conners and Black, 2004). Treatments consisted of 2 control (100µl untreated hemolymph) tubes and 2 treatment tubes (100µl hemolymph + 10µl 0.25mg/ml 4-nitroquilonline-N-oxide) and placed in a refrigerator. One tube from each treatment was sampled after 4h exposure and the second tube was sampled at 24h. Two samples were taken from each tube for comet assay analysis.

Adult Laboratory Exposure Study - Adult *E. complanata* (N=62, 6 per treatment, 8 treatments, plus an additional 13 to obtain baseline data) collected on 03 March 2005 from a relatively uncontaminated reference site on the Eno River were exposed in the laboratory to the PAH test concentrations (Table 5.2) for 14d following a 24h depuration and acclimation period in the laboratory. Pre-exposure hemolymph samples were taken to determine baseline levels of genetic damage in the population. Three mussels were sampled in the field to determine pre-acclimation levels of genetic damage. Hemolymph was removed from the anterior adductor muscle and placed in 1ml plastic tubes and stored dark and cold for transport to the laboratory. Once in the lab, these samples were processed immediately for the Comet assay to minimize loss due to cellular degradation. The remaining mussels were acclimated to laboratory conditions for 24h prior to use. Mussels were scrubbed with a soft bristled brush to remove particulate matter attached to the shells to prevent particle adsorption of test solutions and randomly labeled with either an "I" or "II" for allocation to treatments. Following the 24h acclimation period, one mussel from each group was randomly allocated to each of the treatments (control, positive control, solvent control, PAH 1-200µg/L). Hemolymph (0.25ml) was drawn from 10 mussels post-acclimation and processed immediately for Comet assay. These same 10 mussels were removed from their shells, and the tissues frozen at –80^{o}C for later tissue PAH analysis. Hemolymph from each of the experimental mussels was repeatedly sampled on days 3, 7, and 14. On d14 all experimental mussels were removed from their shells, and frozen at –80^{o}C for PAH tissue analysis. Test solutions of PAH were renewed daily ($^{2}/_{3}$ volume) with the exception of the positive control which was only renewed on d7. Water quality measurements (temperature, dissolved oxygen, and pH) were taken daily in each test chamber and water in the test containers was completely changed on d7. Composite waters samples (100ml per treatment) were taken for PAH analysis on d0, d7, and d14.

Adult Field Study - Adult *E. complanata* (N=6 per stream, 36 total) were collected from 6 streams (Table 1) out of the 20 chosen for study in a NC Department of Transportation (NCDOT) funded study examining the effects of culvert style crossing structures on freshwater mussels. Mussels were collected between 25 – 50m upstream and downstream of each of the road crossing structures from 15–17 December 2004. Two streams were considered reference sites and corresponded to a low average daily traffic count (e.g., <500 vehicles). Two streams were from suburban areas and corresponded to moderate average daily traffic volume (e.g., 500-1000 vehicles). The remaining 2 streams were from high traffic areas (>10,000 ADTC): one stream passed beneath Interstate 40 between Raleigh and Research Triangle Park, the other passed beneath Interstate 40 at Raleigh Durham International Airport and is directly beneath the runway flight path of the airport. Streams chosen for this portion of the study were matched as closely as possible regarding geomorphological structure (e.g., drainage area, flow, size, substrate composition) to minimize potential variation due to non-contaminant related variables. Mussels collected from these streams were processed immediately for testing. Mussels were weighed and measured as previously described, and ~1.0ml of hemolymph was drawn to obtain hemocytes for use in the Comet assay.

Passive sampling devices (PSDs) were deployed at these study sites upstream and downstream of the crossing structure following the methods of Shea et al. (2004). Briefly, PSDs were constructed using approximately 12.7µm thick low-density polyethylene (PE) tubing, containing no plasticizers or additives. The PE tubing (5cm x 30cm, surface area of 300cm^2) was pre-extracted with hexane for 48h prior to use and fixed inside a protective polyethylene cage. Two PSDs were placed in each cage and deployed within a 50m zone upstream and downstream from the crossing structure and retrieved approximately 30d later. Previous work has demonstrated that a 30d deployment time allows the 12.7µm PE to reach equilibrium with water. Following deployment, one of the PSDs was archived at -20ºC and the second was cleaned with de-ionized water and a soft brush, followed by a rinse in acetone to rigorous solvent remove material from the surface of the LDPE prior to extraction. Data collected from the PSDs, directly related to PAH contaminant levels found within the streams, was used for comparison with DNA damage levels in adult mussels sampled from the same stream.

Test procedures

This study utilized acute toxicity and DNA strand breakage to explore the effects of PAH exposure on the glochidial, juvenile, and adult life stages of freshwater mussels and to explore the use of non-traditional tissue types (i.e., hemocytes) for use in the Comet assay for determination of levels of genetic damage in relation to exposure level. The goal was to develop accurate, rapid, and cost effective non-lethal sampling procedures to determine effects of exposure of mussels to PAHs.

Hemolymph was drawn from the anterior adductor muscle of adult mussels. Following hemolymph extraction, mussels were dissected and the tissue frozen at -80^{o}C for tissue body burden analysis in the Environmental Toxicology Laboratory at NCSU.

<u>Comet Assay</u> - The single-cell gel electrophoresis assay (Comet assay) was performed to determine the extent of genetic damage due to exposure to PAHs. This assay measures the level of DNA damage in single cells and has been reliably used on a variety of organisms (Cotelle and Férard, 1999). Slides were prepared using an adaptation of the methods outlined by Woods et al. (1999) and Coughlan et al. (2002). Microscope slides were prepared by dipping each slide in 1.5% normal-melting agarose in phosphate buffered saline followed by air-drying and storage in a desicator until use. All of the following steps were conducted under low light conditions to prevent confounding DNA damage due to ultra-violet radiation exposure. To prepare the sample, 100µl of mussel hemolymph was mixed with 100µl of 1.3% low melting point agarose (LMPA). The tubes were vortexed gently to mix the sample then 100µl were drawn off and placed on the slide, a 40 x 60 mm coverslip added, and the gels allowed to set on ice. Once the cell layer had set, the coverslips were removed, a third layer of 1.5% NMA was added and allowed to set as before.

Once the gels were set, the cover slips were removed and the cells lysed in a high salt buffer (2.5 M NaCl, 10 mM Tris, 100 mM EDTA, 1% (v/v) Triton X-100, and 10% (v/v) DMSO, pH 10.0) for at least 90 min to 8h in coplin jars at 4ºC in the dark. Following the lysis period, the slides were rinsed 3 times with DI water for 5 minutes and gently placed in a horizontal electrophoresis tank and covered with an alkaline solution (0.3 M NaOH, 1 mM EDTA; pH >12) for 15 min at 4ºC in the dark to allow for unwinding of the DNA. Without changing the electrolysis solution, a 25 V, 300 mA current was applied for 15 min, followed by neutralization three times with Tris buffer (0.4 M Tris-HCl; pH 7.4) at 5-minute intervals followed by rinsing with cold EtOH. Slides were then stored in a desicator until visual microscopic analysis. When ready to be read, slides were stained with 2–3 drops of ethidium bromide for 5 min, the coverslips were replaced and randomly selected nucleoids were photographed at 100x magnification using an Olympus BH-2 epifluorescence microscope fitted with a Fuji Finepix S5100 digital camera. DNA damage was expressed in terms of tail moment (TM, determined as the product of the tail length and the fraction of DNA in the tail) and olive moment (OM, the summation of Tail Intensity profile values multiplied by their relative distances to the Head Center, divided by Total Comet Intensity).

Contaminant Analysis

Mussel and PSD samples were extracted for PAH analysis as described by Thorsen et al. (2004) and Luellen and Shea (2002). Samples were shaker-extracted (200 rpm) for 24-h using dichloromethane (DCM) for mussels and PSDs. Concentrated extracts were fractionated using high performance gel permeation chromatography to remove high molecular weight matrix components (e.g., lipids, polyethylene waxes). The extracts were solvent exchanged into hexane and then further purified on a 3-g silica column. Mussel lipid content was determined by passing extracts through a gel permeation chromatography (GPC) column, collecting the lipid fraction, evaporating and weighing. Samples were analyzed for 48 PAH analytes including the 16 USEPA priority PAHs.

Instrumental analysis was conducted following the methods outlined in Shea et al. (2004). Briefly, the purified extracts were analyzed for total PAHs using an Agilent 6890 gas chromatograph (GC) connected to an Agilent 5973N MSD utilizing a Restek 30m x 0.25mm Rtx-5 (film 5 thickness 0.25 µm) MS w/Integra-Guard column. The pressure was ramped to 40 psi before injection with a 1-min hold time. The flow was then dropped to give a constant flow of 1mL/min for the duration of the run. The temperature program for PAH analysis was as follows: initial temperature 40 °C for 1 min with a ramp of 6 °C /min to 290 °C and a final hold time of 30 min; injector temperature 300 °C, detector temperature 280 °C. Selected ion monitoring (SIM) was used for analysis.

Statistical Analyses

<u>Acute Toxicity Tests on Glochidia</u> - Data from the 48h acute toxicity tests on glochidia were used to determine "No Observed Effects Concentration" (NOEC) and "Lowest Observed Effects Concentration" (LOEC) curves using PROC PROBIT in SAS based on 48h survival. Additionally, ToxStat software (Gulley and WEST, Inc., 1994) was used to determine LC_{50} values using the Spearman-Karber method and 95% confidence intervals. Tests were considered valid if mortality was <20% in the controls during the duration of the test.

<u>Juvenile Tests</u> - Data from the 96h acute toxicity tests on juvenile mussels were used to determine NOEC and LOEC curves using PROC PROBIT in SAS and the Spearman-Karber method in ToxStat (Gulley and WEST, Inc., 1994) based on 96h survival, as stated previously.

<u>Comet Assay</u> - Comet images were analyzed using CometScore™ software (TriTek Corporation, http://tritekcorp.com). Data were exported from CometScore into Microsoft Excel and then to JMP (SAS Corporation, Cary, NC) for statistical analysis. The average distance of strand migration of ~50 nuclei per slide were used in data analysis using a one-way analysis of variance (ANOVA). When conducting the comet assay, the slides, not the individual cells on the slide, were considered the least unit of measure (i.e. the means of all of the cells measured on a given slide are used for analysis, not the individual cells). The Tukey-Kramer HSD method for pairwise comparisons of means between treatments was used for statistical analysis. This procedure requires a single value for judging the significance of differences between measured parameters. Statistical significance was considered at $p < 0.05$. Data were normalized by logarithmic transformation, where necessary.

Results

Glochidial Tests

In repeated tests, no significant mortality was observed in glochidia exposed to any of the experimental concentrations of PAHs. Experimental exposures with *L. fasciola* and *L. siliquoidea* indicated LC_{50} values greater than solubility of most PAH compounds in water (Table 5.3).

Juvenile Tests

Tests with *L. fasciola* and *L. siliquoidea* juveniles indicated no acute toxicity to any PAH treatment after 96h of exposure. Tests indicated LC_{50} values greater than solubility of most PAH compounds in water (Table 5.4). Although not quantified as a test endpoint, some lethargy was observed in mussels exposed to the greatest PAH concentration (200µg/L). Based on the data, however, any LOEC and NOEC concentrations appear to be well above solubility of most PAH compounds in water.

Adult tests

Adult Positive Control Experiment - Hemocytes exposed to 4-NQO for only 4 hours exhibited significantly greater levels of genetic damage compared to controls (Fig. 5.1) expressed in terms of tail moment and olive moment (as defined previously). This trend continued at 24h, although levels of genetic damage in both the control and treatment samples were reduced.

Adult Mussel PAH Experiment - Samples of hemolymph (1.0ml) were taken from test mussels on days 3, 7, and 14. However, the first set of slides made from the d14 samples was compromised when nearly all of the gels slipped off of the slides during the 24h lysis period. The slides were immediately remade, however the cells appeared to have degraded and, therefore, the data obtained from the d14 samples has not been reported. Most comet parameters at d7 showed distinct trends towards increasing levels of DNA damage with increasing PAH exposure levels. Trends in tail moment and olive moment increased with increasing PAH concentration over time, compared to control values (Fig. 5.2). The data were highly variable resulting in low levels of statistical significance in both comet parameters.

Solvent control treatments did not exhibit any significant difference from control treatments. Levels of DNA damage in the positive control treatments did vary significantly from control treatments, particularly in samples from d3.

Other comet parameters demonstrated similar increasing trends with exposure level and time. Most notably, the percent DNA in the comet tails (%DNA in Tail) increased over time compared to controls (Fig. 5.3).

Adult Field Study - Data obtained from PSDs deployed upstream and downstream of the 20 crossing structures indicated a general trend towards increasing contamination level with increasing average daily traffic count (Fig. 5.4). Stream G29 was omitted from the analysis of the PSD data because sewer line construction and paving in the vicinity lead to concentrations of PAHs that were unusually high compared to other streams with similar traffic loads. When site G29 was included in the analysis the regression equation was:
$y = 0.0804x + 3316.1$ ($R^2 = 0.1254$). There was no significant difference between petrogenic and pyrogenic PAHs between low, medium, or high ADTC groups of streams, although there were differences between individual streams, even within ADTC groups (Fig. 5.6).

Levels of genetic damage in mussel hemocytes from field-collected mussels generally increased with average daily traffic count (ADTC) (Fig. 4), measured as vehicle crossings per day. As in the laboratory adult mussel PAH exposure study, the data were highly variable, but the trend towards increasing genetic damage in relation to water column PAH concentration was distinct. The lone exception to this trend was stream A338. This stream represented the least average daily traffic volume of any site in the field study (Table 5.1), but the PSD data indicated an extremely high level of PAH contamination relative to streams of comparable ADTC (Table 5). Despite the high PAH contamination at this site, mussels sampled from A338 exhibited the lowest levels of DNA damage measured in the field study. Stream O263 had the second highest ADTC of the streams in this study (Table 5.1), yet the PSD data indicated that the PAH levels were slightly less than streams with significantly lower ADTC values (Table 5.5). Levels of DNA damage in mussels sampled at this location, however, reflected the trend of increasing levels of genetic damage with increasing ADTC.

Discussion

Data from the glochidial and juvenile tests appeared to contradict previous published information, however these studies were conducted with other freshwater mussel species. Weinstein (2000) and Weinstein and Polk (2001) reported high sensitivity and mortality of *U. imbecillis* glochidia to relatively low levels of several different PAH compounds (fluoranthene, pyrene, and anthracene) following photoactivation with ultraviolet light. This study utilized total PAHs and used a 16:8 light/dark cycle with no UV photoactivation of the PAHs. The levels of the individual PAHs were therefore considerably lower than the concentrations reported by the previous works. It is likely that this study presents a more natural scenario (i.e., more like the naturally occurring conditions) than the Weinstein studies.

The experiments with glochidia did not yield any evidence of acute toxicity to PAHs and suggested that LC_{50} levels for total PAHs may be above solubility of the compounds in water. The measured endpoint, however, was simply survival of glochidia during a 48h exposure. It is possible that sub-lethal effects occurred due to exposure, although no quantification attempts were made. No attempt at measuring single strand DNA breaks using the Comet assay with glochidia or juveniles was successful. In methods development trials with *U. imbecillis,* attempts were made to duplicate the methods utilized by Conners and Black (2004) to test for genetic damage with limited success. Further work in refining methods of removing tissue from the minute shell fragments of the glochidia and juveniles will present greater opportunities for determining genotoxic effects on these life stages of mussels.

Experiments with juvenile mussels did not yield any evidence of acute toxicity of PAHs. Mortality in PAH treatments was not significantly different from that of controls. Although some lethargy was observed, no quantification of this endpoint was made in the tests. A direct method of quantification of sub-lethal effects due to exposure would be to measure time to first movement. Lethargy could thereby be quantified and related to exposure level. In the wild, lethargic responses due to exposure to contaminants could directly impact the survival of juvenile mussels by delaying closing response initiated by the proximity of a potential predator.

The data obtained from the positive control experiment indicate that mussel hemocytes may be affected by exposure to environmental genotoxic contaminants and therefore may be a viable alternative to traditionally sampled tissue types such as gill or digestive gland tissues from mussels. The decrease in levels of genetic damage over the 24h period of the positive control experiment may be due to a reduction in cell viability over time. The data concur with the findings of Siu et al. (2004) and Klobučar et al. (2003). Both of these studies found that hemocytes were sensitive to genotoxins and that the use of hemocytes was a sensitive and valuable tool in monitoring of these compounds in the environment. Additionally, hemocytes are rapidly and easily sampled with minimal impact on the organism. During the laboratory portion of this study, 0.25ml of hemolymph was sampled from mussels 3 times during a 2-week period. No mussels died during the experiment, suggesting that repeated sampling of small amounts of hemolymph is not detrimental to short-term survival of the mussel.

The PAH exposure study with adult eastern elliptio demonstrated clear time and concentration dependant effects on levels of genetic damage in mussel hemocytes, although the results exhibited a high degree of variation. Previous in vivo studies (Siu et al., 2004; Rank and Jensen, 2003; Klobučar et al., 2003) have found that mussel hemocytes withdrawn from exposed mussels are as sensitive as tissues (gill, digestive gland, etc.) in detecting DNA damage in the mussels. This indicates that rapid, cost effective, and non-lethal hemolymph sampling (Gustafson et al, 2005) may be a viable alternative to whole mussel or tissue sampling methods for assessing the effects of genotoxic compounds.

Data obtained from the field portion of the study demonstrated a distinct trend in increasing levels of genetic damage in relation to average daily traffic load, and thus presumably PAH exposure. Generally, ADTC on a roadway corresponded well to PAH concentrations within the stream. The exceptions to this relationship were likely due to other factors such as land use patterns in the watershed, atmospheric deposition influenced by regional weather patterns, or other anthropogenic activities upstream of the crossing structure. Therefore, based on the data, sampling and analysis of mussel hemocytes for genotoxic compounds may yield important information about contaminant loading in a stream and its effects on the biota within the stream.

Although much of the data obtained from the Comet assay in this study were highly variable, the positive control exposure experiment indicates that mussel hemocytes present a potential alternative to lethal methods of testing. The data obtained from the laboratory and field portions of this study indicate that mussel hemocytes are sensitive to PAH exposure in the environment. However, methods need to be refined and attempts made to reduce variability. Although additional testing is required to refine assay methods, this study indicates that the methods are robust and that PAH contamination in streams may be negatively affecting freshwater mussels.

Conclusions

Overall, we found that there were no acute toxic effects of PAHs on glochidia or juveniles of the two species of freshwater mussels examined, up to concentrations approaching water solubility, and well exceeding those commonly measured in the streams of North Carolina. Experiments with adult *Elliptio complanata*, both in the laboratory and from the field, indicated that genetic damage due to PAH exposure was likely present, however the results were highly variable and the potential for biological, ecological, and toxicological consequences were uncertain. Further development and improvement of assay methods may reduce this variation. Generally, mussels from streams with higher average daily traffic counts (ADTC) exhibited greater levels of genetic damage compared to mussels from streams with lower ADTC values. Data obtained from the laboratory study generally showed increasing DNA damage relative to increasing PAH concentration. Based on the data generated, however, PAHs are not likely contributing to acute toxicity of mussels in North Carolina streams, but the chronic, long-term pervasive effect of PAHs on native freshwater mussels remain uncertain.

References

Ahrens MJ, Nieuwenhuis R, Hickey CW (2002) Sensitivity of Juvenile *Macomona liliana* (Bivalvia) to UV-Photoactivated Fluoranthene Toxicity. *Enviro Tox*, 17: 567 – 577.

Altman SA, Randers L, Rao G (1999) Comparison of trypan blue dye exclusion and fluorometric assays for mammalian cell viability determinations. *Biotechno Prog*, 9: 671 – 674.

American Society for Testing and Materials (ASTM) (2002) Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians (ASTM E729-96). ASTM annual book of standards volume 11.05, ASTM, West Conshohocken, PA.

Anderson JW, Jones JM, Steinert S, Sanders B, Means J, McMillin D, Vu T, Tukey R (1999) Correlation of CYP1A1 induction, as measured by the P450 RGS biomarker assay, with high molecular weight PAHs in mussels deployed at various sites in San Diego Bay in 1993 and 1995. *Mar Enviro Res,* 48: 389 – 405.

Baumard P, Budzinski H, Garrigues P (1998) PAHs in Arcachon Bay, France: Origin and Biomonitoring with Caged Organisms. *Mar Poll Bull,* 36 (8): 577 – 586.

Baumard P, Budzinski H, Garrigues P, Narbonne JF, Burgeot T, Michel X, Bellocq J (1999) Polycyclic aromatic hydrocarbon (PAH) burden of mussels (*Mytilus sp*.) in different marine environments in relation with sediment PAH contamination, and bioavailability. *Mar Enviro Res*, 47: 415 – 439.

Beasley G, Kneale P (2002) Reviewing the impact of metals and PAHs on macroinvertebrates in urban watercourses. *Prog Phys Geo*, 26(2): 236 – 270.

Blaise C, Trottier S, Gagné F, Lallement C, Hansen P-D (2002) Immunocompetence of Bivalve Hemocytes as evaluated by a Miniaturized Phagocytosis Assay. *Enviro Tox*, 17: 160 – 169.

Bogan AE (2002) *Workbook and key to the freshwater bivalves of North Carolina*. North Carolina Museum of Natural Sciences, Raleigh, NC, 101 pp, 10 color plates.

Bonassi S, Au WW (2002) Biomarkers in molecular epidemiology studies for health risk prediction. *Mut Res*, 511: 73 – 86.

Cataldo D, Columbo JC, Boltovskoy D, Bilos C, Landoni P (2001) Environmental toxicity assessment in the Parana River delta (Argentina): simultaneous evaluation of selected pollutants and mortality rates of *Corbicula fluminea* (Bivalvia) early juveniles. *Enviro Poll,* 112: 379 – 389.

Conners DE, Black MC (2004) Evaluation of lethality and genotoxicity in the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care. *Arch Enviro Cont Tox*, 46(3): 362-371.

Cossu C, Doyette A, Jacquin MC, Babut M, Exinger A., Vasseur P (1997) Glutathione Reductase, Selenium-Dependent Glutathione Peroxidase, Glutathione Levels, and Lipid Peroxidation in Freshwater Bivalves, *Unio tumidus*, as Biomarkers of Aquatic Contamination in Field Studies. *Ecotox Enviro Safety*, 38: 122-131.

Cotelle S, Férard JF (1999) Comet Assay in Genetic Ecotoxicology: A Review. *Enviro Mol Mut*, 34: 246 – 255.

Coughlan BM, Hartl MGJ, O'Reilly SJ, Sheehan D, Morthersill C, van Pelt FNAM, O'Halloran J, O'Brien NM (2002) Detecting genotoxicity using the Comet assay following chronic exposure of Manila clam *Tapes semidecussatus* to polluted estuarine sediments *Mar Pol Bul*, 44: 1359 – 1365.

Dame RF (1996) *Ecology of Marine Bivalves: an Ecosystems Approach*. CRC Press, Boca Raton, FL.

Doyette A, Cossu C, Jacquin M-C, Babut M, Vasseur P (1997) Antioxidant enzymes, glutathione, and lipid peroxidation as relevant biomarkers of experimental of field exposure in the gills and the digestive gland of the freshwater bivalve *Unio tumidus*. *Aqua Tox,* 39: 93 – 110.

Eisler R (1987) Polycyclic aromatic hydrocarbon hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish Wildlife Service Biological Report 85(1.11). 81 pp.

Federal Highway Administration (1981) Constituents of highway runoff. Vol. VI. Executive Summary Final Report. Report No. FHWA/RD-G1/047. Environmental Division, Federal Highway Administration, Washington, DC.

Fernandes MB, Sicre M-A, Boireau A, Tronczynski J (1997) Polyaromatic Hydrocarbon (PAH) Distributions in the Seine River and its Estuary. *Mar Pol Bul*, 34(11); 857 – 867.

Fournier M, Cyr D, Blakely B, Boermans H, Brousseau P (2000) Phagocytosis as a Biomarker of Immunotoxicity in Wildlife Species Exposed to Environmental Xenobiotics. *Amer Zoo*, 40: 412 – 420.

Gagné F, Blaise C, Aoyama I, Luo R, Gagnon C, Couillard Y, Cambell P, Salazar M (2002) Biomarker Study of a Municipal Effluent Dispersion Plume in Two Species of Freshwater Mussels. *Enviro Tox*, 17: 149 – 159.

Geffard O, Budzinski H, His E (2002) The Effects of Elutriates from PAH and Heavy Metal Polluted Sediments on *Crassostrea gigas* (Thunberg) Embryogenesis, Larval Growth, and Bioaccumulation by the Larvae of Pollutants from Sedimentary Origin. *Ecotox*, 11: 403 – 416.

Gewurtz SB, Drouillard KG, Lazar R, Haffner GD (2002) Quantitative Biomonitoring of PAHs Using the Barnes Mussel (*Elliptio complanata*). *Arch Enviro Cont Tox*, 43: 497 – 504.

Gulley DD, WEST, Inc. (1994) TOXSTAT 3.4. WEST, Inc., Cheyenne, WY.

Gustafson LL, Stoskopf MK, Bogan AE, Showers W, Kwak TJ, Hanlon S, Levine JF (2005) Evaluation of a nonlethal technique for hemolymph collection in *Elliptio complanata*, a freshwater bivalve (Mollusca: Unionidae). *Dis Aqua Org*, 65: 159-165.

Hallhagan A (1973) Survey of present knowledge and discussion of input of petroleum to the marine environment in Sweden. Paper presented at workshop on Inputs, Fates, and Effects of Petroleum in the Marine Environment, National Academy of Sciences.

Hamoutene D, Payne JF, Rahimtula A, Lee K (2002) Use of the Comet assay to assess DNA damage in hemocytes and digestive gland cells of mussels and clams exposed to water contaminated with petroleum hydrocarbons. *Mar Enviro Res*, 54: 471 – 474.

Hanstén C, Heino M, and Pynnönen K (1996) Viability of glochidia of *Anodonta anatina* (Unionidae) exposed to selected metals and chelating agents. *Aqua Tox*, 34: 1 – 12.

Hoffman EJ, Latimer JS, Hunt CD, Mills GL, Quinn JG (1985) Stormwater runoff from highways. *Water, Air, and Soil Pol*, 25: 349 - 364.

Huebner JD, Pynnönen KS (1992) Viability of glochidia of two species of *Anodonta* exposed to low pH and selected metals. *Can J Zoo*, 70: 2348 – 2355.

Hyötyläinen T, Karels A, Oikari A (2002) Assessment of bioavailability and effects of chemicals due to remediation actions with caging mussels (*Anodonta anatina*) at a creosote-contaminated lake sediment site. *Water Res*, 36: 4497 – 4504.

Jacobson PJ, Neves RJ, Cherry DS, Farris JL (1997) Sensitivity of glochidial stages of freshwater mussels (Bivalvia: Unionidae) to copper. *Enviro Tox Chem*, 16(11): 2384 – 2392.

Keller AE, Ruessler DS, Chaffee CM (1998) Testing the toxicity of sediments contaminated with diesel fuel using glochidia and juvenile mussels (Bivalvia: Unionidae). *Aqua Eco Health Manage*, 1: 37 – 47.

Klobučar GIV, Pavlica M, Erben R, Papeš D (2003) Application of the micronucleus and comet assays to mussel *Dreissena polymorpha* haemocytes for genotoxicity monitoring of freshwater environments. *Aqua Tox*, 64: 15 – 23.

Large AT, Shaw JP, Peters LD, McIntosh AD, Webster L, Mally A, Chipman JK (2002) Different levels of mussel (*Mytilus edulis*) DNA strand breaks following chronic field and acute laboratory exposure to Polycyclic aromatic hydrocarbons. *Mar Enviro Res*, 54: 493 – 497.

Le Pennec G, Le Pennec M (2001) Evaluation of the toxicity of chemical compounds using the digestive acini of the bivalve mollusk *Pecten maximus* L. maintained alive in vitro. *Aqua Tox*, 53: 1 – 7.

Long SM, Ryder KJ, Holdway DA (2003) The use of respiratory enzymes as biomarkers of petroleum hydrocarbon exposure in *Mytilus edulis planatus*. *Ecotox Enviro Saf*, 53: 261 – 270.

Lorenzen A, Kennedy S (1993) A fluorescence-based protein assay for use with a microplate reader. *Anal. Biochem*, 243: 229 – 243.

Luellen DR, Shea D (2003) Semipermeable membrane devices accumulate conserved ratios of sterane and hopane petroleum biomarkers. *Chemo* 53:705-713.

Maltby L, Alistair ABA, Boxall BA, Forrow DM, Calow P, Betton CI (1995) The effects of motorway runoff on freshwater ecosystems: 2. Identifying major toxicants. *Enviro Tox Chem*, 14(6): 1093 – 1101.

Marsalek J, Brownlee B, Mayer T, Lawal S, Larkin GA (1997) Heavy Metals and PAHs in Stormwater Runoff from the Skyway Bridge, Burlington, Ontario. *Water Qual Res J Canada*, 32(4): 815-827.

McKinney AD, Wade DC (1996) Comparative response of *Ceriodaphnia dubia* and juvenile *Anodonta imbecillis* to pulp and paper mill effluents discharged to the Tennessee River and its tributaries. *Enviro Tox*, 15(4): 514 – 517.

McMahon RF, Bogan AE (2001) Mollusca: Bivalvia. In Ecology and Classification of North American Freshwater Invertebrates, 2nd Edition. Academic Press, pp. 331 - 430.

Moulton CA, Fleming WJ, Purnell CE (1996) Effects of two cholinesterase-inhibiting pesticides on freshwater mussels. *Enviro Tox Chem,* 15(2): 131-137.

National Native Mussel Conservation Committee (1998) National Strategy for the Conservation of Native Freshwater Mussels. *J Shell Res*, 17(5): 1419-1428.

Neff JM (1979) Polycyclic Aromatic Hydrocarbons in the Aquatic Environment: Sources, Fates, and Biological Effects. Applied Science, Barking, Essex.

Pavlica M, Klobučar GIV, Mojaš N, Erben R, Papeš D (2001) Detection of DNA damage in haemocytes of zebra mussel using comet assay. *Mut Res*, 490: 209 – 214.

Peacock E, Haag WR, Warren Jr. ML (2005) Prehistoric decline in freshwater mussels coincident with the advent of maize agriculture. *Cons Bio*, 19(2): 547-551.

Pereira WE, Domagalski JL, Hostettler FD, Brown LR, Rapp JB (1996) Occurrence and accumulation of pesticides and organic contaminants in river sediment, water, and clam tissues from the San Joaquin River and tributaries, California. *Enviro Tox Chem*, 15(2): 172 – 180.

Piccardo MT, Coradeghini R, Valerio F (2001) Polycyclic Aromatic Hydrocarbon Pollution in Native and Caged Mussels. *Mar Pol Bul*, 42(10): 951 – 956.

Porte C, Biosca X, Solé M, Albaigés J (2001) The integrated use of chemical analysis, cytochrome P450 and stress proteins in mussels to assess pollution along the Galician coast (NW Spain*). Enviro Pol*, 112: 261 – 268.

Pynnönen K (1995) Effect of pH, hardness and maternal pre-exposure on the toxicity of Cd, Cu, and Zn to the glochidial larvae of a freshwater clam Anodonta cygnea. *Water Res*, 29(1): 247 – 254.

Rank J, Jensen K (2003) Comet assay on gill cells and hemocytes from the blue mussel *Mytilus edulis*. *Ecotox Enviro Saf*, 54: 323-329.

Renaud CB, Kaiser KLE, Comba ME, Metcalfe-Smith JL (1995) Comparison between lamprey ammocoetes and bivalve molluscs as biomonitors of organochlorine contaminants. *Ca. J Fish Aquat Sc*., 52: 276-282.

Roduis F, Hammer C, Vasseur V (2002) Use of RNA Arbitrarily Primed PCR to Identify Genomic Alterations in the Digestive Gland of the Freshwater Bivalve *Unio tumidus* at a contaminated site. *Enviro Tox*, 17: 538 – 546.

Sanders BM (1993) Stress proteins in aquatic organisms: an environmental perspective. *Crit Revs Tox*, 23: 49 – 75.

Sasaki YF, Izumiyama F, Nishidate E, Ishibashi S, Tsuda S, Matsusaka N, Asano N, Saotome K, Sofuni T, Hayashi M (1997) Detection of genotoxicity of polluted seawater using shellfish and the alkaline single-cell gel electrophoresis (SCE) assay: a preliminary study. *Mut Res*, 393: 133 - 139.

Shea D, Cope WG, Lazaro P, Thorsen W, Forestier D (2004) Highway Runoff as a Source of Contaminants to Freshwater Mussels. Final report to NC Dept. of Transportation. NCSU Dept. of Environmental and Molecular Toxicology, 59 pp.

Siu WHL, Cao J, Jack RW, Wu RSS, Richardson BJ, Xu L, Lam PKS (2004) Application of the comet and micronucleus assays to the detection of B[*a*]P genotoxicity in haemocytes of the green-lipped mussel (*Perna viridis*). *Aqua Tox*, 66: 381- 392.

Thorsen WA, Cope WG, Shea D (2004) Bioavailability of PAHs: Effects of Soot Carbon and PAH Source. *Environ Sci Technol.* 38: 2029-2037.

Wakeham SG (1977) A characterization of the sources of petroleum hydrocarbons in Lake Washington. *J Water Pol Control Fed*, 49: 1680 – 1689.

Weinstein JE (2000) Characterization of the acute toxicity of photoactivated fluoranthene to glochidia of the freshwater mussel, *Utterbackia imbecillis*. *Environ Tox Chem*, 20(2): 412 – 419.

Weinstein JE, Polk KD (2001) Phototoxicity of anthracene and pyrene to glochidia of the freshwater mussel *Utterbackia imbecillis*. *Environ Tox Chem*, 20(9): 2021 – 2028.

Widdows J, Donkin P, Staff FJ, Matthiessen P, Law RJ, Allen YT, Thain JE, Allchin CR, Jones BR (2002) Measurement of stress effects (scope for growth) and contaminant levels in mussels (Mytilus edulis) collected from the Irish Sea. *Mar Environ Res*, 53: 327-356.

Wilson JT, Pascoe PL, Parry JM, Dixon DR (1998) Evaluation of the comet assay as a method for the detection of DNA damage in the cells of a marine invertebrate, *Mytilus edulis* L. (Mollusca: Pelecypoda). *Mut Res*, 399: 87 – 95.

Woods JA, O'Leary KA, McCarthy RP, O'Brien NM (1999) Preservation of comet assay slides: comparison with fresh slides. *Mut Res*, 429: 181 – 187.

Chapter 5: Tables and Figures

Table 5.1: Sites selected for use in this study. Sites where mussels were sampled for Comet assay are highlighted in gray.

River Basin	County	Bridge Number	Creek	Road	Average Daily Traffic Volume (vehicles/day
Cape Fear	Alamance	74	UT to Back Creek	Jimmie Kerr Road	55
Cape Fear	Randolph	339	Reedy Creek	Jugtown Rd	90
Dan	Person	211	Mayo Creek	Mayo Lake Rd.	90
Cape Fear	Alamance	338	Poppaw Creek	Foster's Store Rd	100
Neuse	Person	38	Lick Creek	Willie Gray Road	130
Tar	Granville	177	Shelton Cr	Sunset Rd	< 500
Tar	Granville	9	Coon Creek	Mountain Road (Horner Siding Rd)	440
Cape Fear	Randolph	459	Reed Creek	Low Bridge Road	1100
Cape Fear	Alamance	204	Rock Creek	Friendship Patterson Rd (Walt Shoe Rd)	1500
Cape Fear	Chatham	12	Terrell's Creek	NC 87	2300
Cape Fear	Chatham	18	Dry Creek	NC 87	2550
Cape Fear	Alamance	20	Mary's Creek	NC 87	3500
Tar	Granville	28	North Fork	US 158	3500
Neuse	Orange	30	North Fork Little River	NC 57	3600
Tar	Franklin	62	Fox Creek	NC 56	10000
Neuse	Johnston	2052	Buffalo Creek	NC 42	13000
Tar	Granville	29	Coon Creek	Business 158 (in Oxford)	13000
Neuse	Wake	561	Terrible Creek	US 401	24000
Cape Fear	Orange	263	New Hope Creek	I-40	56000
Neuse	Wake	49	Brier Creek	I-40	126000

Table 5.2: PAH test concentrations.

PAH1	PAH2	PAH3	PAH4	PAH5
1µg/L	10µg/L	50µg/L	100µg/L	200µg/L

Table 5.3. Acute (48h) LC_{50} values for acute toxicity tests with glochidia.

	S-K LC_{50} (95% CI)	
Species	24 h	48 h
L. fasciola	>200 µg/L	>200 µg/L
L. siliquoidea	>200 µg/L	>200 µg/L

Table 5.4. Acute (96h) LC_{50} values for acute toxicity tests with juvenile mussels.

Species	Life Stage	PAH (µg/L)
L. fasciola	juvenile	> 200
L. siliquoidea	juvenile	> 200
L. siliquoidea	juvenile (2 mo.)	> 200

Table 5.5. Average sum of PAH contamination measured from PSDs deployed above and below the crossing structure for streams where mussels were sampled for Comet assay testing. Streams are listed in order of ADTV.

Stream	Ave. Sum PAH (ng/sampler)
A338	9011.087
G177	753.280
C12	1893.204
O30	1763.858
O263	1464.326
W49	14591.448

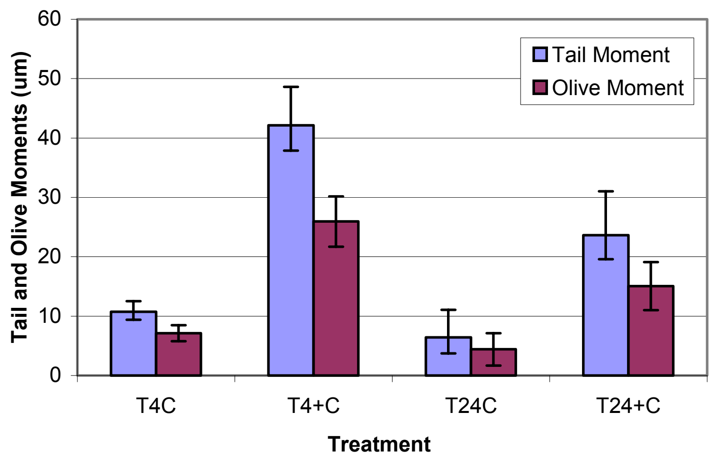


Fig. 5.1. Tail and Olive Moment values for *E. complanata* hemocytes exposed for 4 and 24 hours to 4-NQO compared to unexposed cells, with 95% confidence intervals. There are no units associated with tail or olive moment measurements. (C = Control sample, +C = Positive Control)

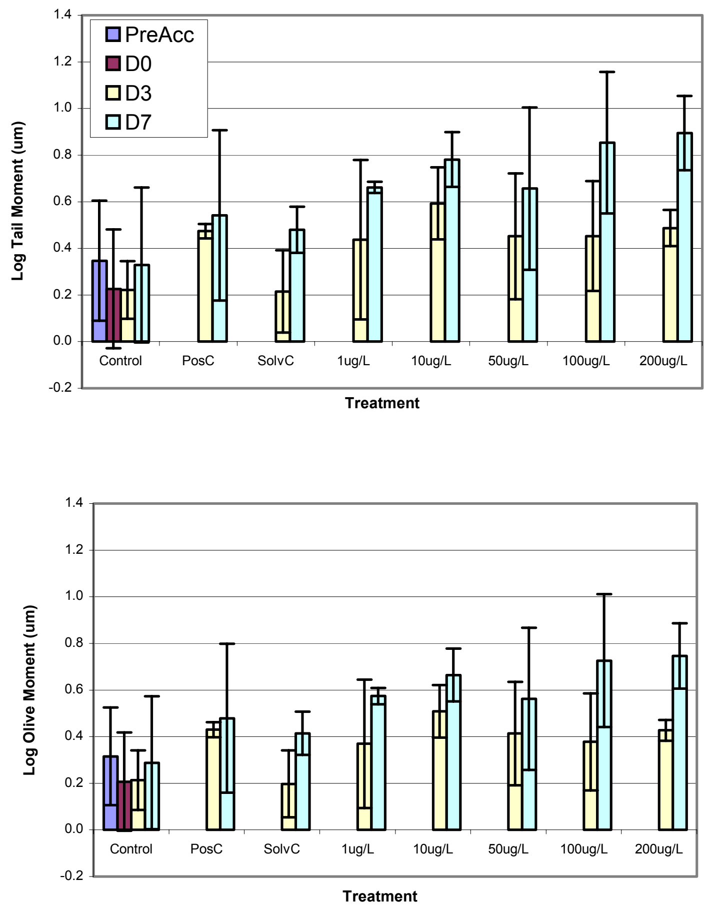


Fig. 5.2. Tail and Olive Moment values for laboratory study, logarithmically transformed with 95% confidence intervals.

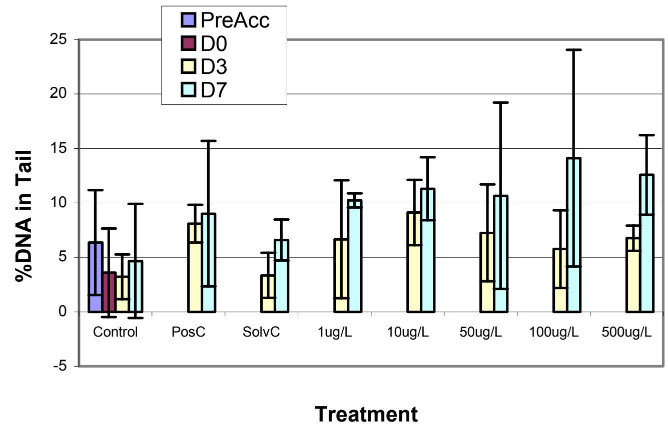


Fig. 5.3. Percent DNA in comet tails per treatment, with 95% confidence intervals from the laboratory study.

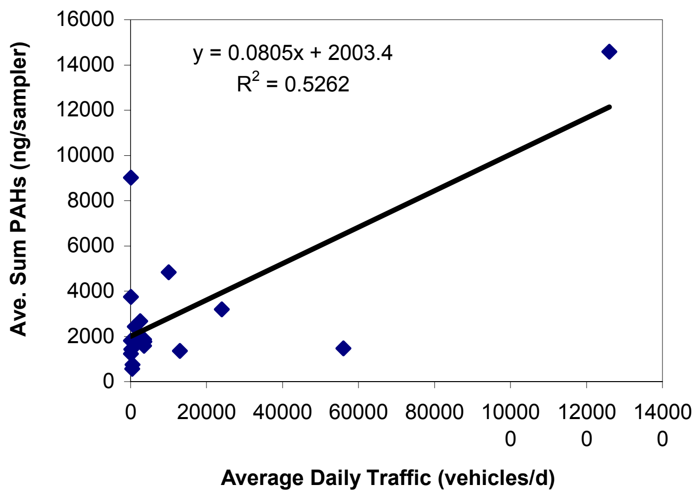


Fig. 5.4. Regression of Average Daily Traffic Count versus PAH concentration on PSDs. Stream G29 was omitted from this regression.

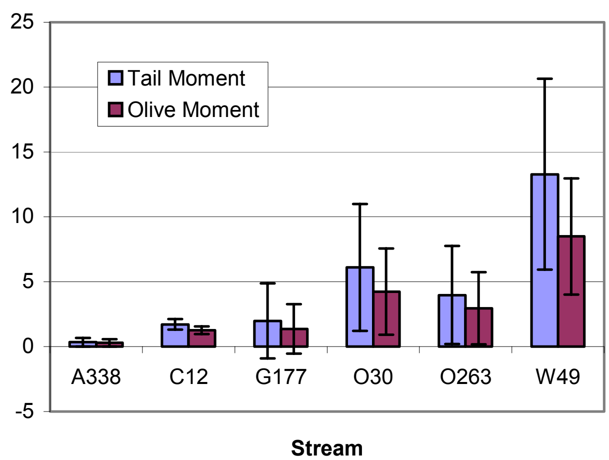


Fig. 5.5. Tail and Olive Moment (um) values for the streams sampled for the field portion of the study, with 95% confidence intervals.

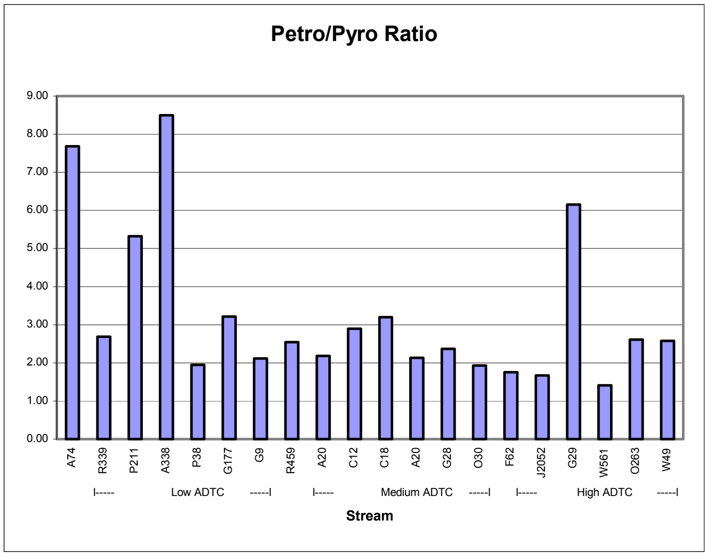


Fig. 5.6. **Petrogenic/pyrogenic PAH ratio in the 20 streams used in this study. Streams are categorized from lowest ADTV to highest. There is no**

APPENDIX I:
The Effects of Culverts and Bridges on Stream Geomorphology

Appendix I-A: Preliminary Study Data Sheets and Photos

This list of definitions will help you understand each site.

Site : My ID #
BR# : DOT database #
CO : County
BASIN : River Basin

BFW= Bankfull width at first riffle above culvert
THD= Thawleg depth from top of bankfull
CW= Width of culvert from one side of the road to the next
CT= Culvert type
Built= Year culvert was built
Substrate= stream bottom material seen at visit
Channel cond= DOT database information on channel see table on next page
Scour status= DOT database information on scour around culvert see table on next page
Conditions= My notes of stream condition during each visit

Opinion= My opinion of site due to conditions

Site 11
BR#12
CO Chatham
BASIN Cape Fear

BFW=’
THD= ’
CW= 45’
CT= Single 38’18’ RC Arch
Built=1933
Substrate= cobble, gravel, bedrock
Channel cond= bank protection needs minor repairs
Scour Status= scour above top of footing

Conditions= slightly incised both up and downstream, much more downstream, more rocky upstream
Land Use= Wooded?
Opinion= OK Site

*** Pic 1 of arch culvert looking upstream**
****Pic 2 looking upstream from top of culvert**

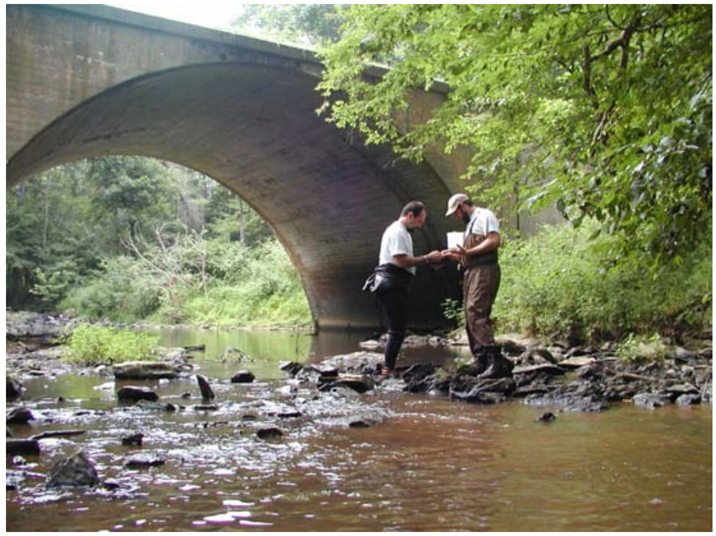
Site 36
BR#38
CO Person
BASIN Neuse

BFW=25’
THD= 2’5’’
CW= ’80
CT= Double 117’’x79’’ corrugated pipe
Built= 1991
Substrate= rocky and sandy pools,
Channel cond=banks well protected or well vegetated, no control devise needed
Scour status= scour above top of footing

Conditions= slightly incised , gravel bar below
Land Use= wooded?
Opinion=Ok site

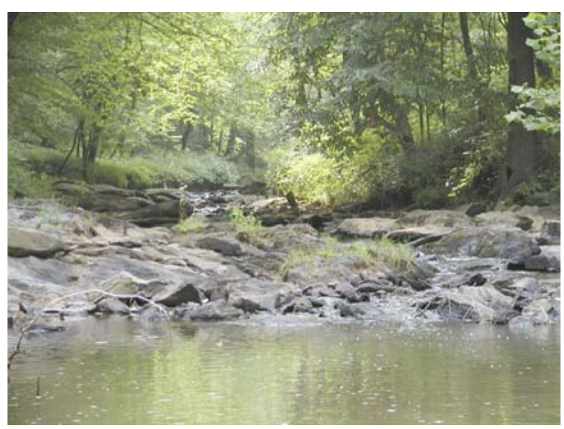
***Pic 1 looking upstream into arch culvert**
****Pic 2looking upstream from top of culvert**

Site 34
BR#13
CO Orange
BASIN Neuse



BFW=28’5’’
THD= 2’2’’
CW= 37’7’’
CT= 51’4’’ Cement Arch
Built =1941
Substrate= rocky, sandy pools
Channel cond= banks beginning to slump, debris restricting channel slightly
Scour status= scour above top of footing

Conditions= little influence, low incision, seemed to be normal riffle pool sequence, on bridge embankments in stream
Land Use= wooded?
Opinion= Good site because of substrate bottom



***Pic 1 looking upstream at culvert**
****Pic 2 looking upstream from culvert**

Site 02
BR#20
CO Alamance
BASIN Cape Fear

BFW=23.6'
THD= 2.4'
CW= 58. 6'
CT= Quadruple 8x9 RC box
Built=1930
Substrate= sand and gravel
Channel cond= bank beginning to slump , debris restrict channel
Scour Status= scour above top of footing

Condition notes= incised below and above
Land Use= Wooded, grassy bank
Opinion= not a good site

***Pic 1 looking downstream at the culvert**
****Pic 2 looking upstream from culvert**

Site 23
BR#459
CO Randolph
BASIN Cape Fear

BFW=17.2'
THD=1.3'
CW=54'6''
CT= Triple 120'' Corrugated Pipe
Built 1955
Substrate= rock above, sand and gravel bars below
Channel Cond= bank beginning to slump, debris restricts channel slightly
Scour Status= scour above top of footing

Conditions= 2 pipes being used, highly incised above and below
Land Use= pasture above and below
Opinion= not a good site..cows

***Pic 1 looking downstream at culvert**
****Pic 2 looking upstream at culvert**

Site 28
BR#217
CO Granville
BASIN Dan

BFW=17.2’
THD=1.0 ’
CW= 92’6’’
CT= Triple 142x 91 Corrugated Pipe arch
Built= 1990
Substrate= rocky
Channel cond= bank protection needs minor repairs
Scour status= scour above top of footing

Conditions= slightly incised below less above, more eroded downstream
Land Use= Wooded
Opinion= Ok site!

***Pic 1 looking downstream at culvert**
****Pic 2 looking upstream from culvert**

Site 03
BR#29
CO Alamance
BASIN Cape Fear

BFW=25' 6''
THD= 4'6''
CW= 37' 3''
CT= Single RC 39'6''x20 Arch
Built= 1935
Substrate= Sandy
Channel cond= bank protection needs repairs
Scour status= scour above top of footing

Conditions= Highly incised, beaver dam, large amounts of debris downstream, narrow buffer
Land use= New golf course upstream
Opinion= Not a good site because of constricted flow by dam and golf course.

***Pic 1 looking upstream from bank near culvert**
****Pic 2 looking downstream through culvert**

Site 03
BR#67
CO Orange
BASIN Neuse

BFW= 12.2’
THD= 1.2’
CW=
CT=
Built= 1953
Substrate= Cobble and gravel
Channel cond= unknown
Scour status= unknown
Conditions= Extreme erosion on banks downstream
Land use= Wooded some nearby houses
Opinion= OK site

***Pic 1 looking upstream at bridge**
****Pic 2 looking downstream from bridge**

Site 03
BR#4
CO Orange
BASIN Neuse

BFW=19.7'
THD= .9'
BW=
BT= Cement and Metal
Built= 1949
Substrate= Sand and Cobble
Channel cond=unknown
Scour status=unknown

Conditions=Incision upstream and downstream
Land use= Wooded, past farmland
Opinion= Larger site but OK

***Pic 1 looking up stream at bridge**
****Pic 2 looking upstream from under the bridge**

Site 03
BR#55
CO Orange
BASIN Neuse

BFW=16.7’
THD=1.2’
BW=
BT= Metal and wood
Built=1964
Substrate= Cobble and gravel
Channel cond=unknown
Scour status=unknown

Conditions=slight incision upstream, one side being used during low flows
Land use= Wooded but a pasture near stream
Opinion= Good Site!

***Pic 1 looking up stream at bridge**
****Pic 2 looking upstream from bridge**

Site 03
BR#30
CO Orange
BASIN Neuse

BFW=24.8’
THD=1.6’
CW= 24’
CT= Triple Box
Built=1941
Substrate= Cobble and Sand
Channel cond=unknown
Scour status=unknown
Conditions=Bankfull forming in one side, Incision upstream and down
Land use= Wooded but a lawn near stream
Opinion= Good Site!

***Pic 1 looking upstream from culvert**
****Pc 2 looking upstream at culvert**

Site 10
BR#12010
CO Randolph
BASIN Cape Fear
WB= BACHELOR CREEK
BFW=15'9''
THD= 2'7''
BW= 159
BT= Wood and Metal
Built= 1954
Substrate=Sand and Gravel, bedrock below
Channel cond= stable banks
Conditions= Wooded w Ag

Opinion= half a wing wall on each side

***Pic 1 looking downstream at bridge**
****Pic 2 looking downstream from under the bridge**

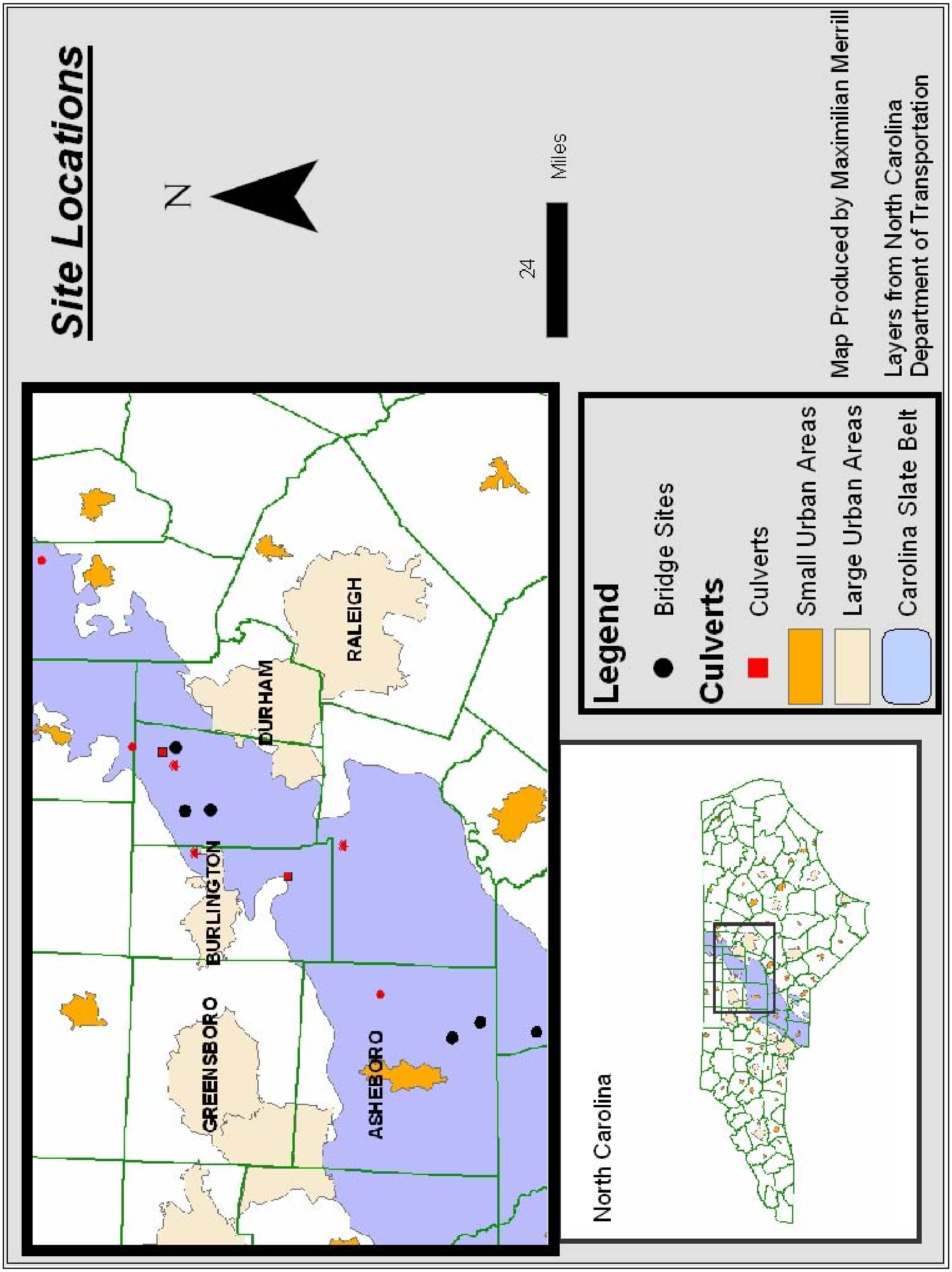
Site 22
BR#12032
CO Randolph
BASIN Cape Fear
WB= LITTLE CREEK
BFW=25'1''
THD= 3'1''
BW= 220
BT= Metal
Built= 1955
Substrate= cobble, gravel, sand
Channel cond= downstream straightened
Conditions= Wooded and Ag
Opinion= OK site!

***Pic 1 looking downstream at bridge**
****Pic 2 looking upstream from bridge**

Site 25
BR#173
CO Moore
BASIN Cape Fear
WB=WILLIAMS CREEK
BFW=14’3
THD= 3’4”
BW= 193
BT= Wood w/wingwalls
Built= 1955
Substrate= cobble, gravel, bedrock
Channel cond= highly incised
Conditions= Old Ag
Opinion= Good Site

***Pic 1 looking at bridge abutment**
****Pic 2 looking upstream from bridge**

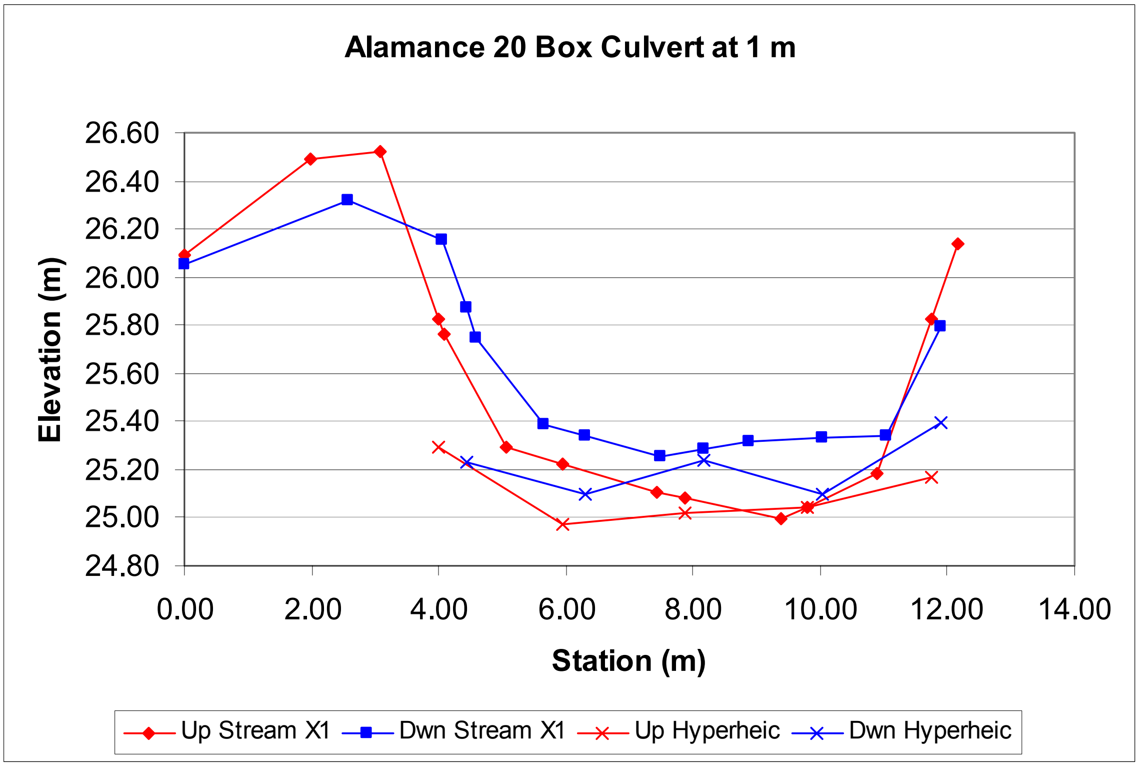
Appendix II-B: Site Location Map

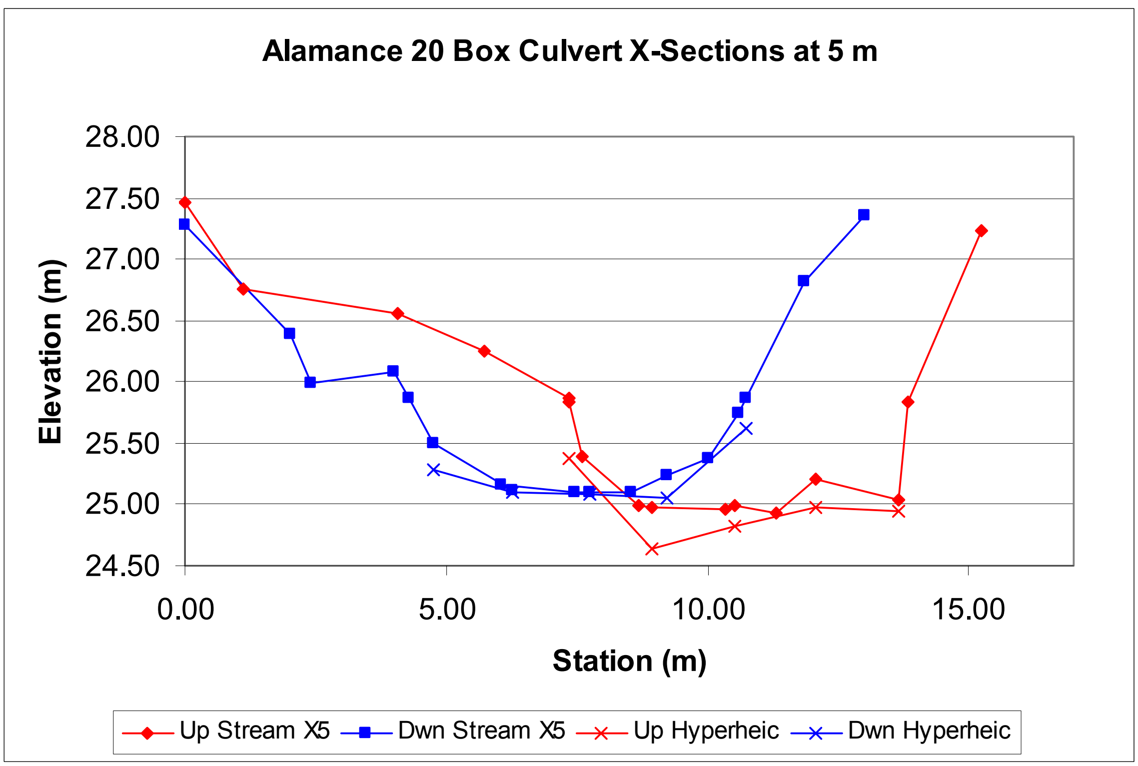


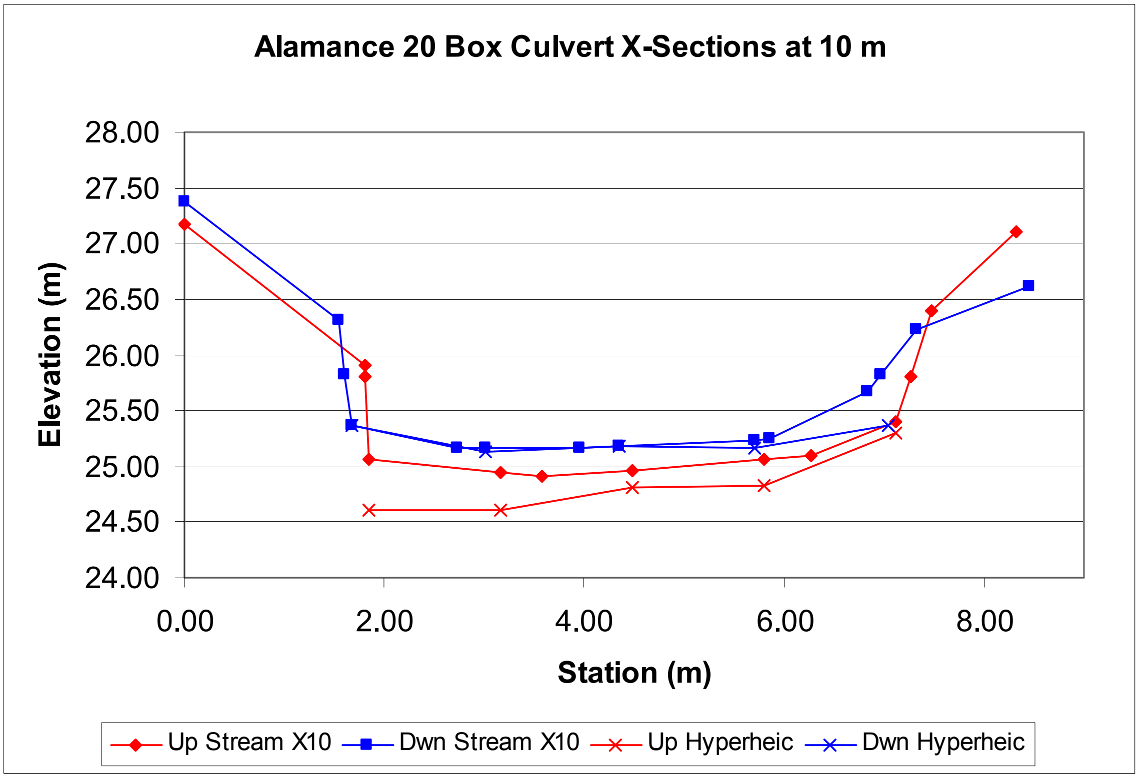
Site Locations
N
24
Miles
Map Produced by Maximilian Merrill
Layers from North Carolina Department of Transportation
Legend
Bridge Sites
Culverts
Culverts
Small Urban Areas
Large Urban Areas
Carolina Slate Belt
RALEIGH
DURHAM
BURLINGTON
GREENSBORO
ASHEBORO
North Carolina

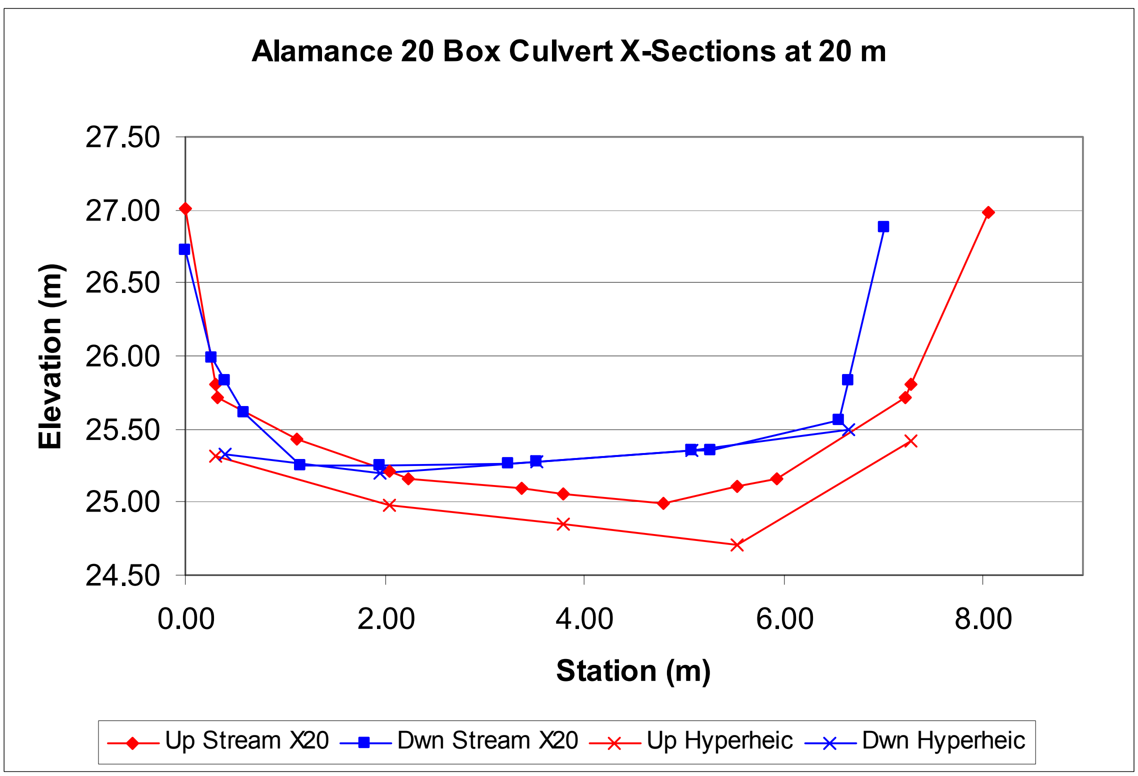
Appendix C: Cross Sectional Area, and Longitudinal Profile. (In Alphabetical Order)

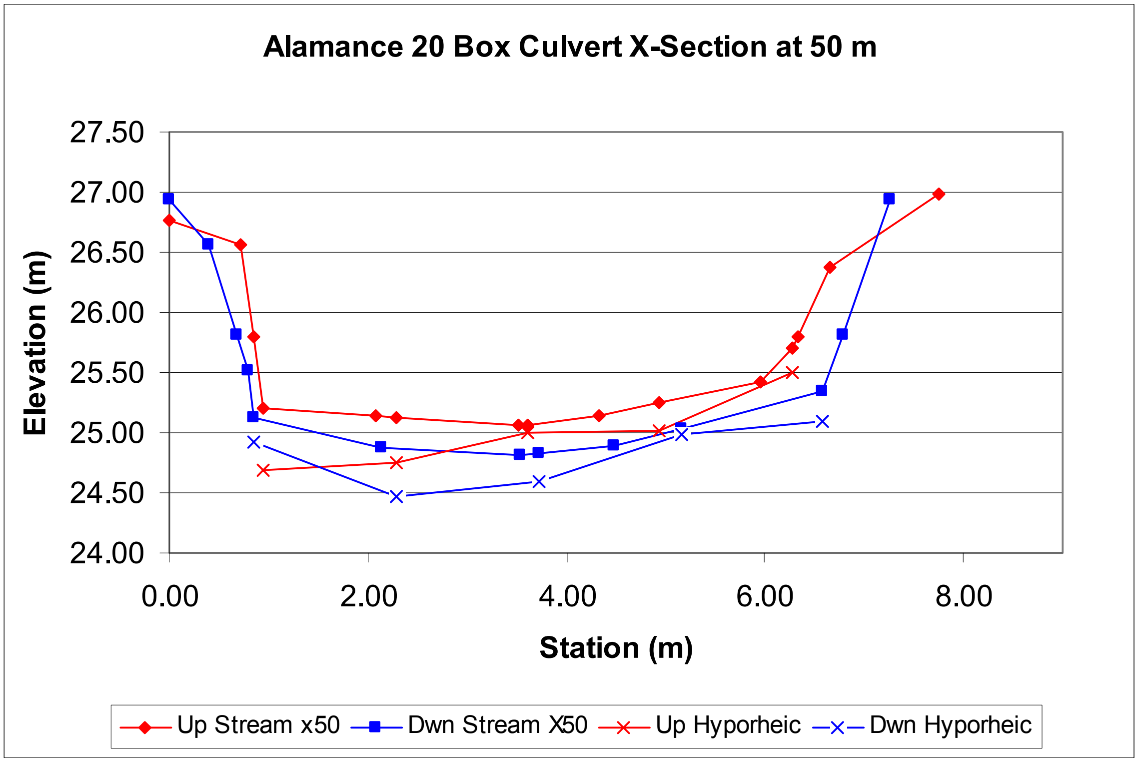
Alamance 20 Cross Section and Longitudinal Profile

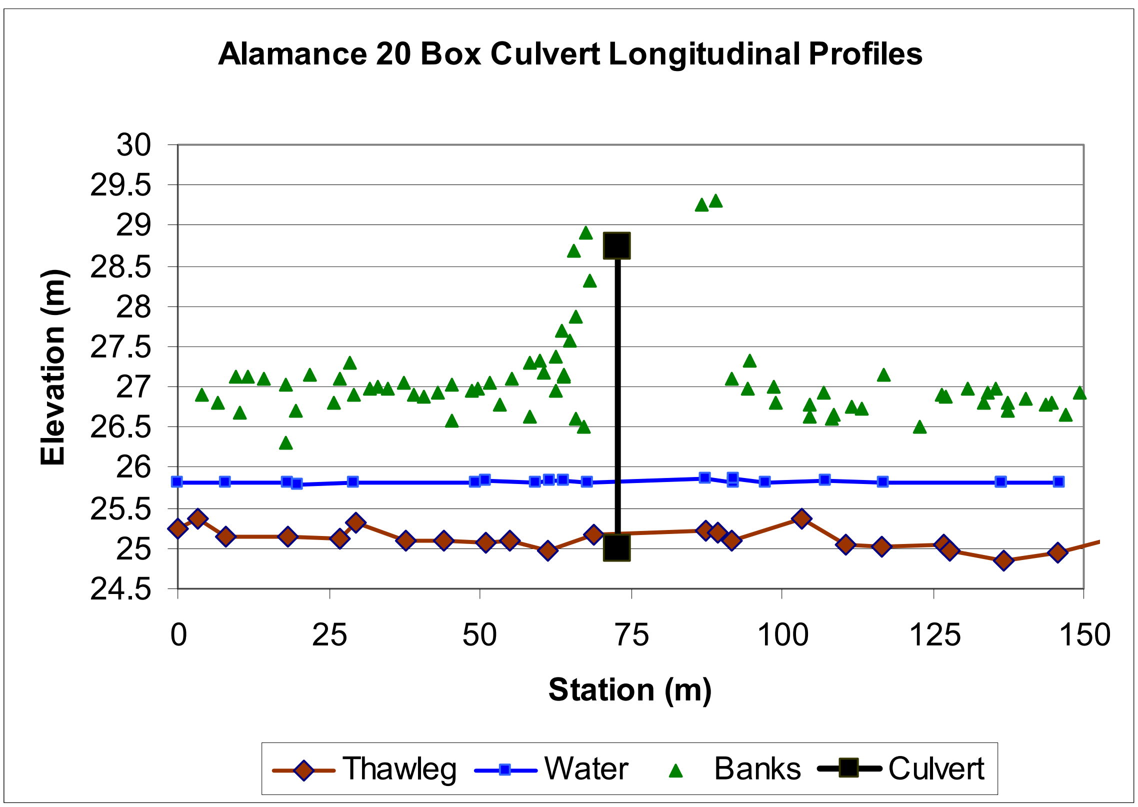




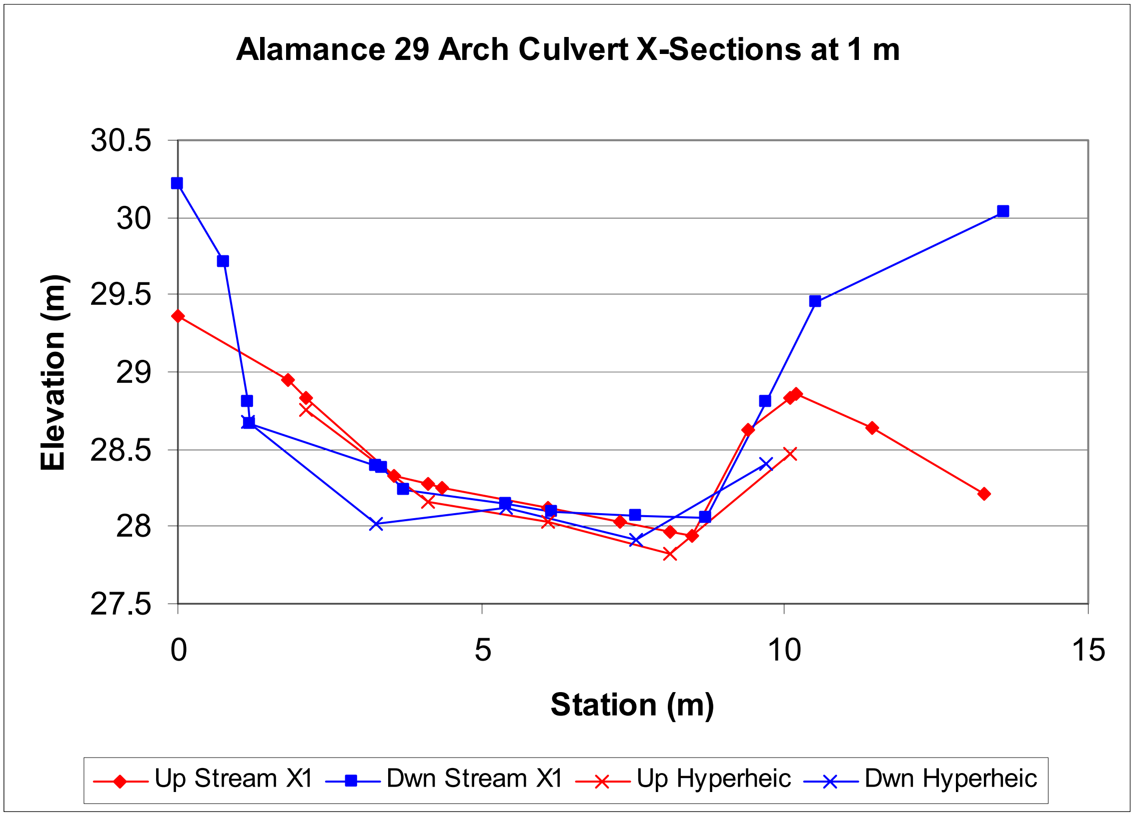

Alamance 20 Box Culvert X-Sections at 10 m
Elevation (m)
28.00
27.50
27.00
26.50
26.00
25.50
25.00
24.50
24.00
0.00
2.00
4.00
6.00
8.00
Station (m)
Up Stream X10
Dwn Stream X10
Up Hyperheic
Dwn Hyperheic

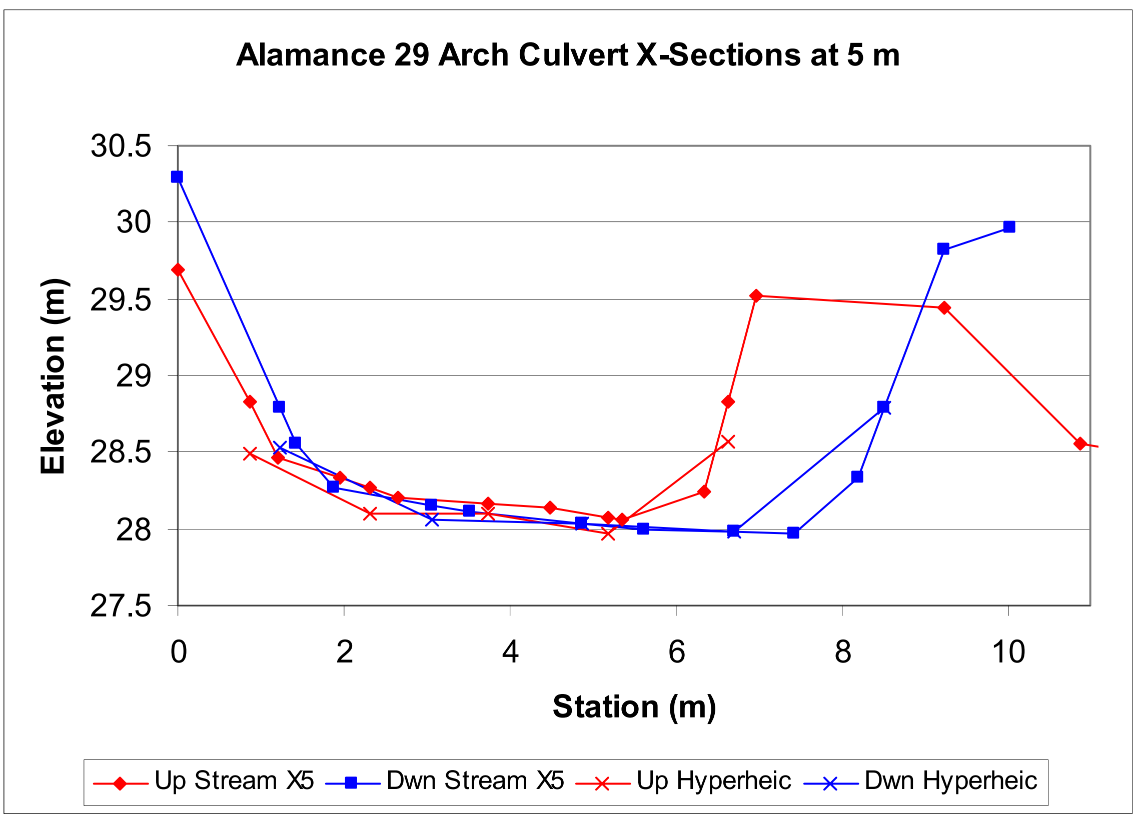

Alamance 20 Box Culvert X-Sections at 20 m
Elevation (m)
27.50
27.00
26.50
26.00
25.50
25.00
24.50
0.00
2.00
4.00
6.00
8.00
Station (m)
Up Stream X20
Dwn Stream X20
Up Hyperheic
Dwn Hyperheic

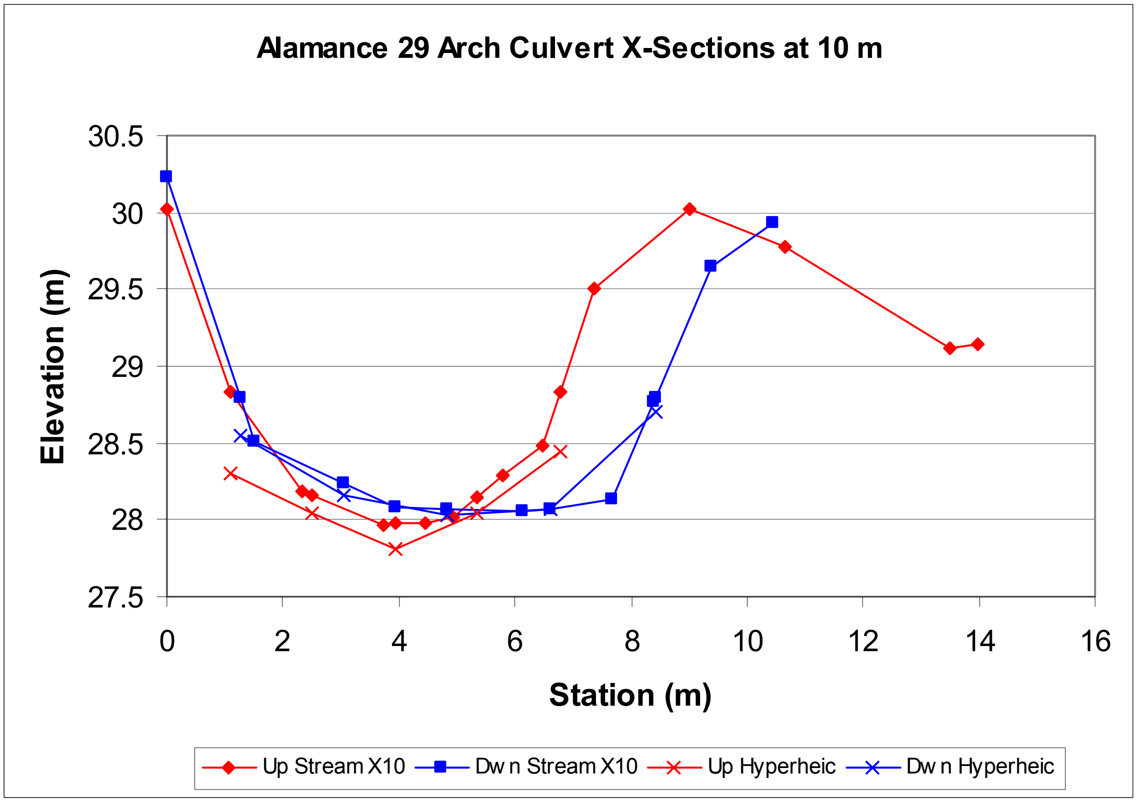

Alamance 20 Box Culvert X-Section at 50 m
Elevation (m)
Station (m)
27.50
27.00
26.50
26.00
25.50
25.00
24.50
24.00
0.00
2.00
4.00
6.00
8.00
Up Stream x50
Dwn Stream X50
Up Hyporheic
Dwn Hyporheic

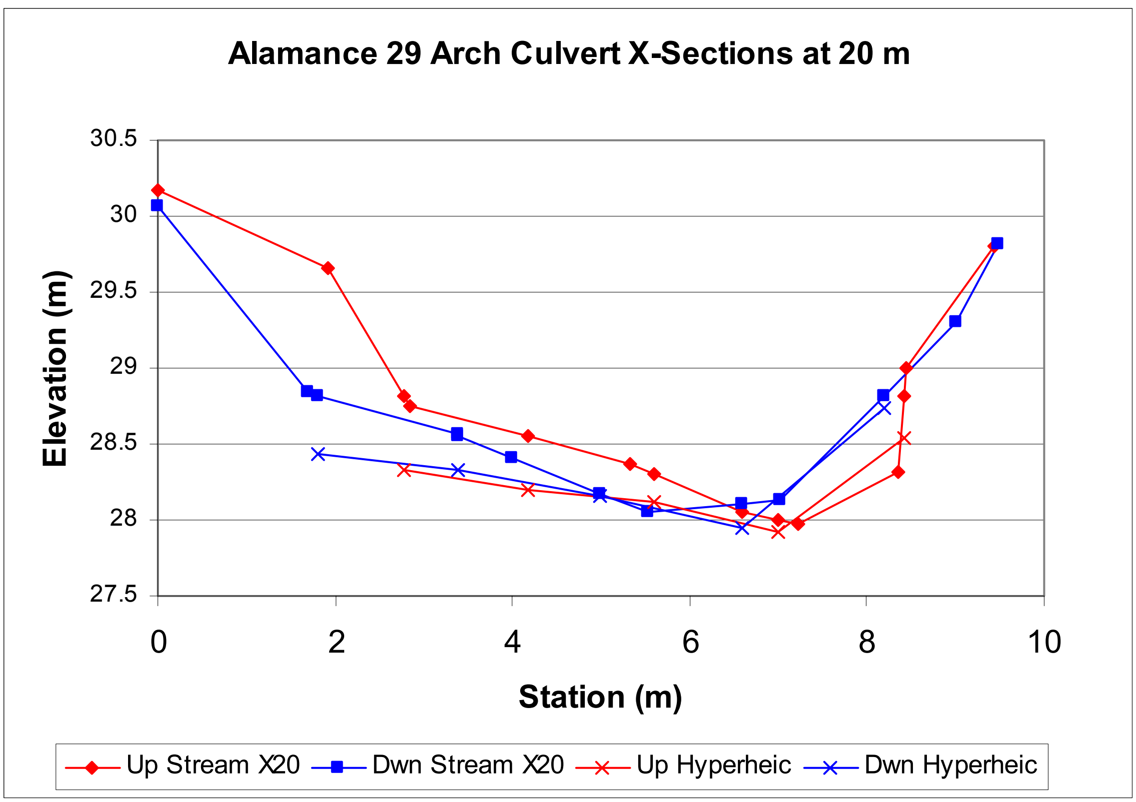

Alamance 20 Box Culvert Longitudinal Profiles
Elevation (m)
Station (m)
30
29.5
29
28.5
28
27.5
27
26.5
26
25.5
25
24.5
0
25
50
75
100
125
150
Thawleg
Water
Banks
Culvert

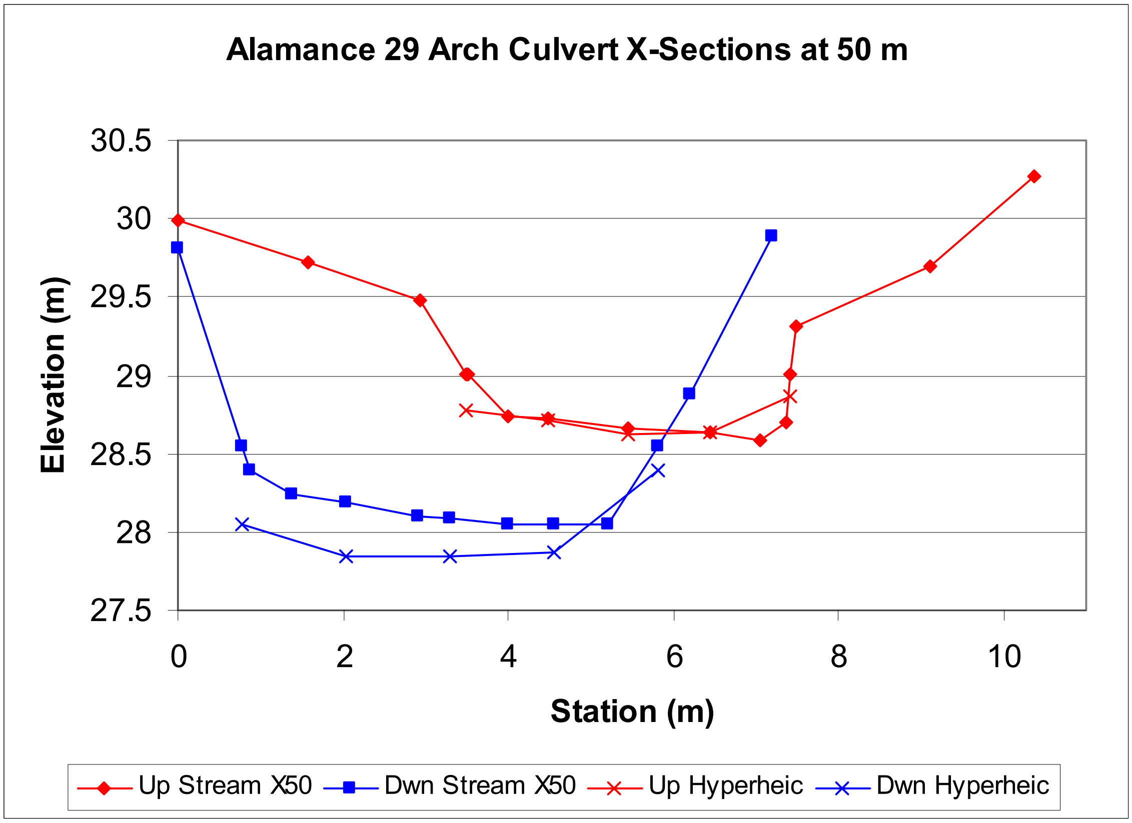
Alamance 29 Cross Section and Longitudinal Profile

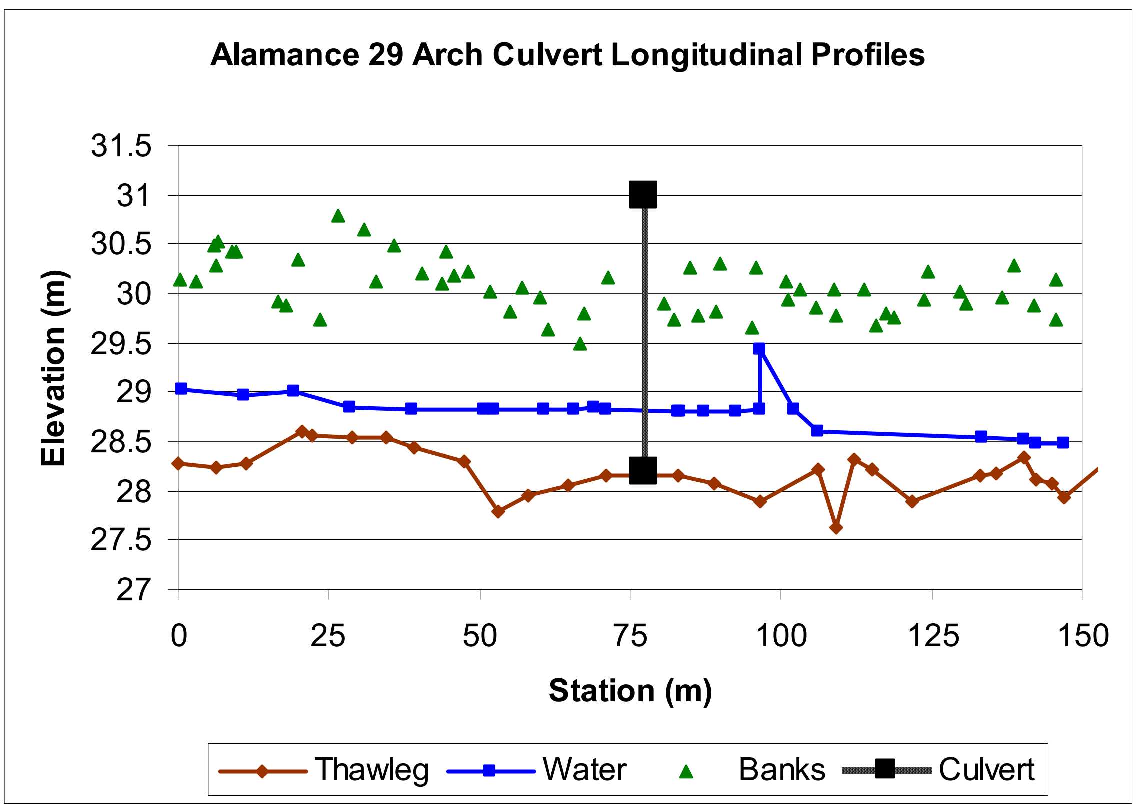




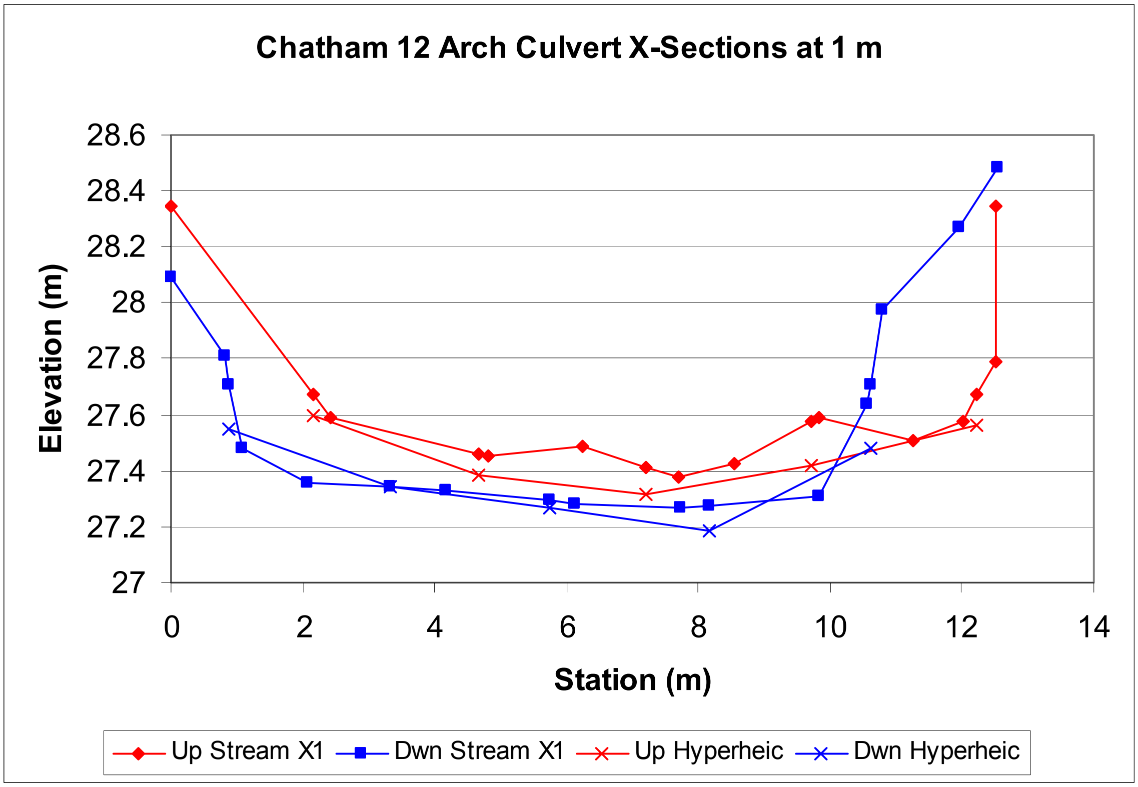

Alamance 29 Arch Culvert X-Sections at 10 m
Elevation (m)
30.5
30
29.5
29
28.5
28
27.5
0
2
4
6
8
10
12
14
16
Station (m)
Up Stream X10
Dw n Stream X10
Up Hyperheic
Dw n Hyperheic

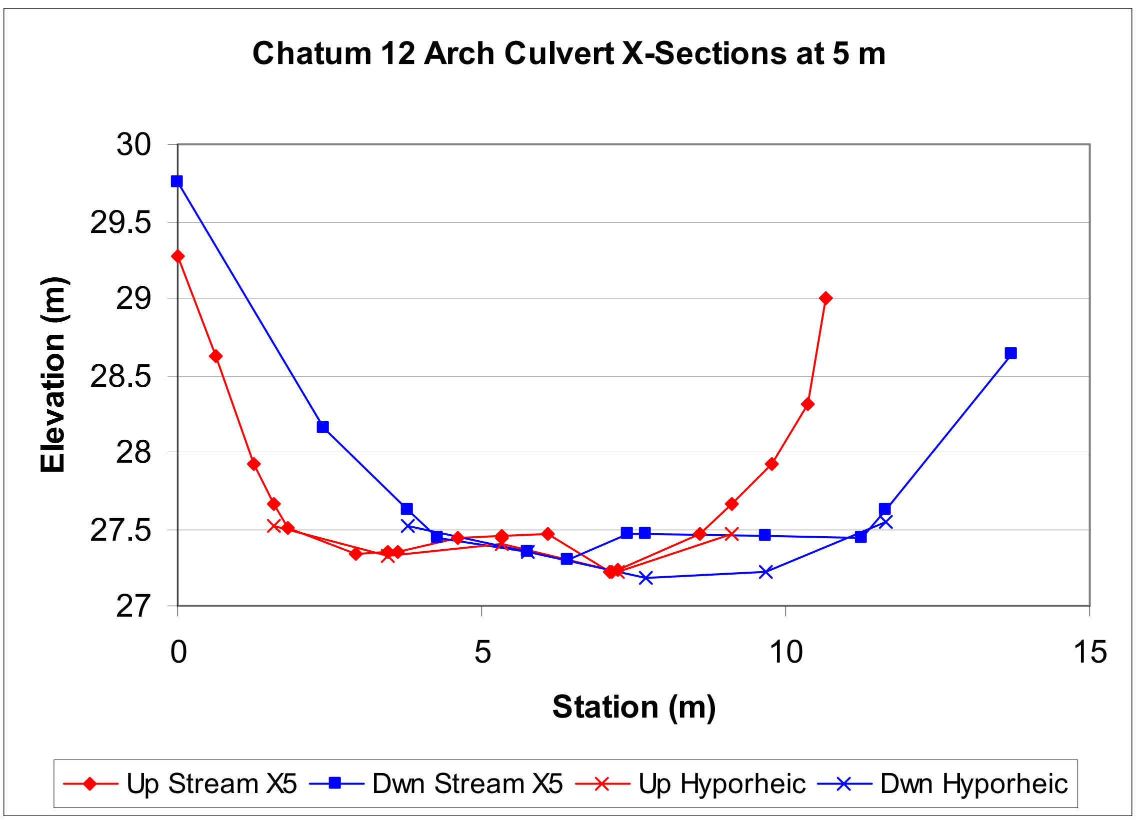

Alamance 29 Arch Culvert X-Sections at 20 m
Elevation (m)
30.5
30
29.5
29
28.5
28
27.5
0
2
4
6
8
10
Station (m)
Up Stream X20
Dwn Stream X20
Up Hyperheic
Dwn Hyperheic

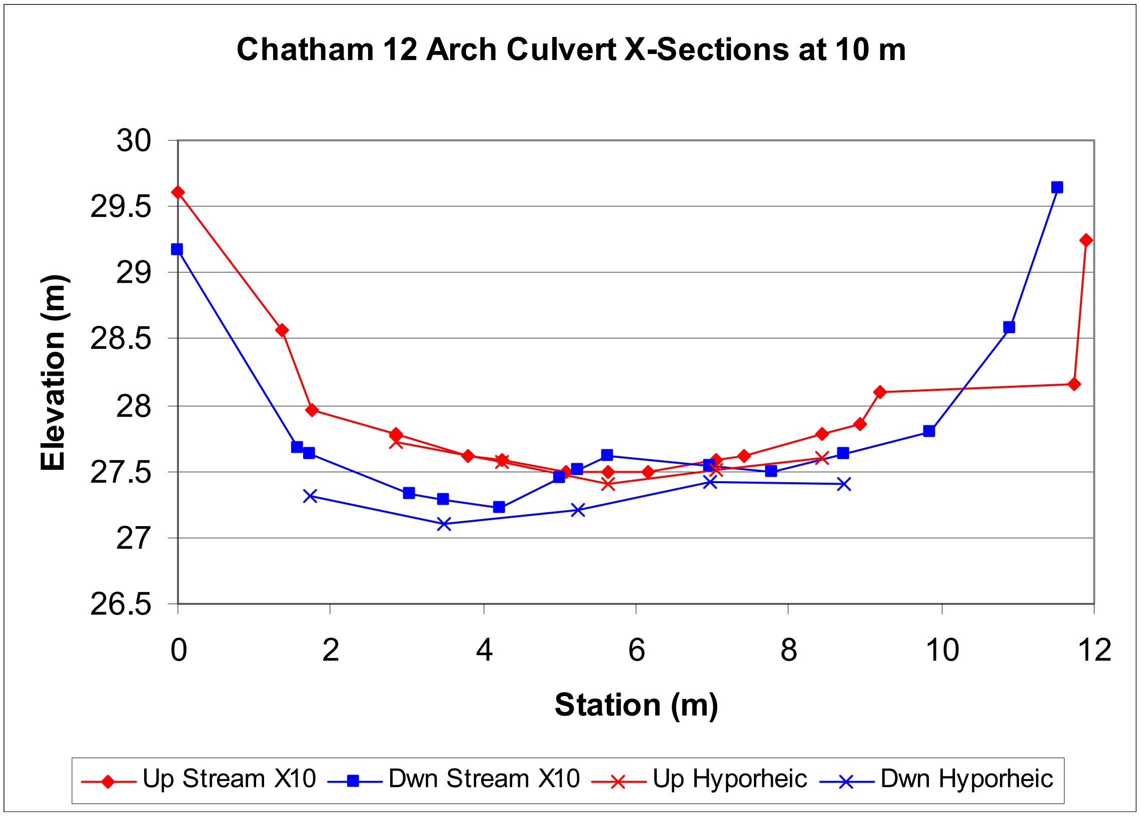

Alamance 29 Arch Culvert X-Sections at 50 m
Elevation (m)
30.5
30
29.5
29
28.5
28
27.5
0
2
4
6
8
10
Station (m)
Up Stream X50
Dwn Stream X50
Up Hyperheic
Dwn Hyperheic


Alamance 29 Arch Culvert Longitudinal Profiles
Elevation (m)
31.5
31
30.5
30
29.5
29
28.5
28
27.5
27
0
25
50
75
100
125
150
Station (m)
Thawleg
Water
Banks
Culvert

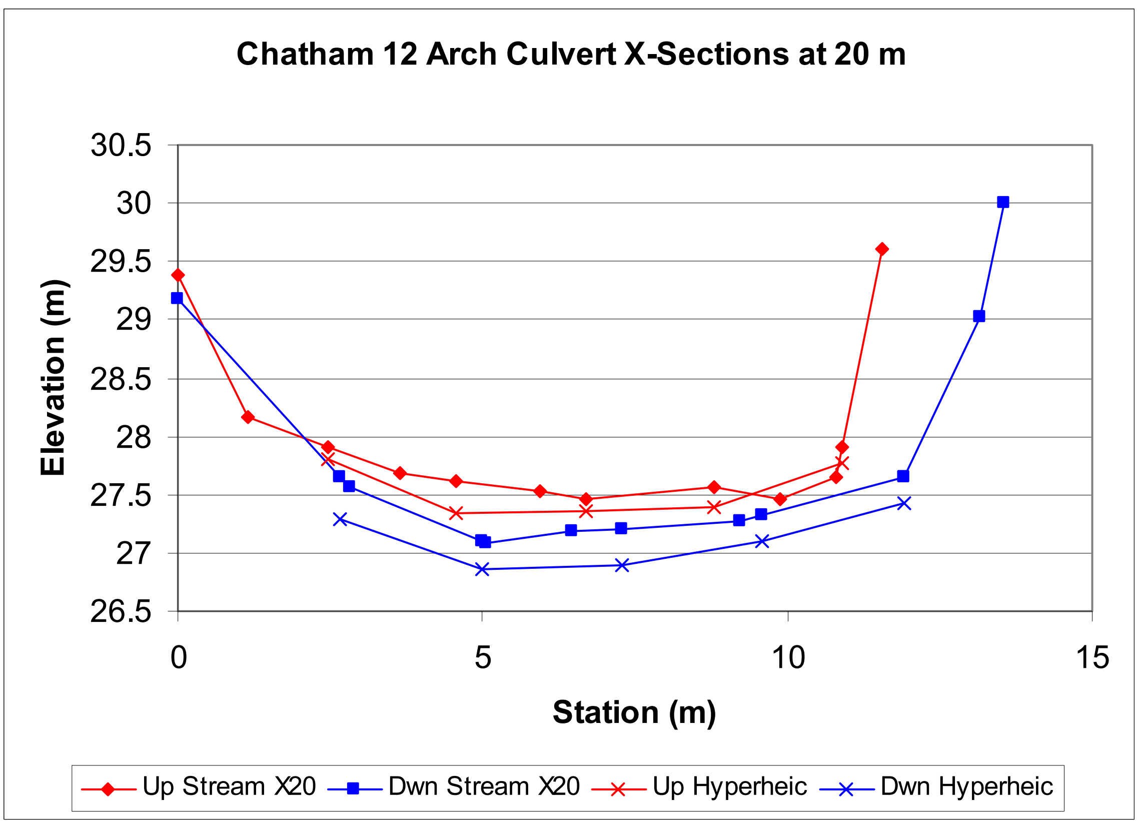
Chatham 12 Cross Section and Longitudinal Profile



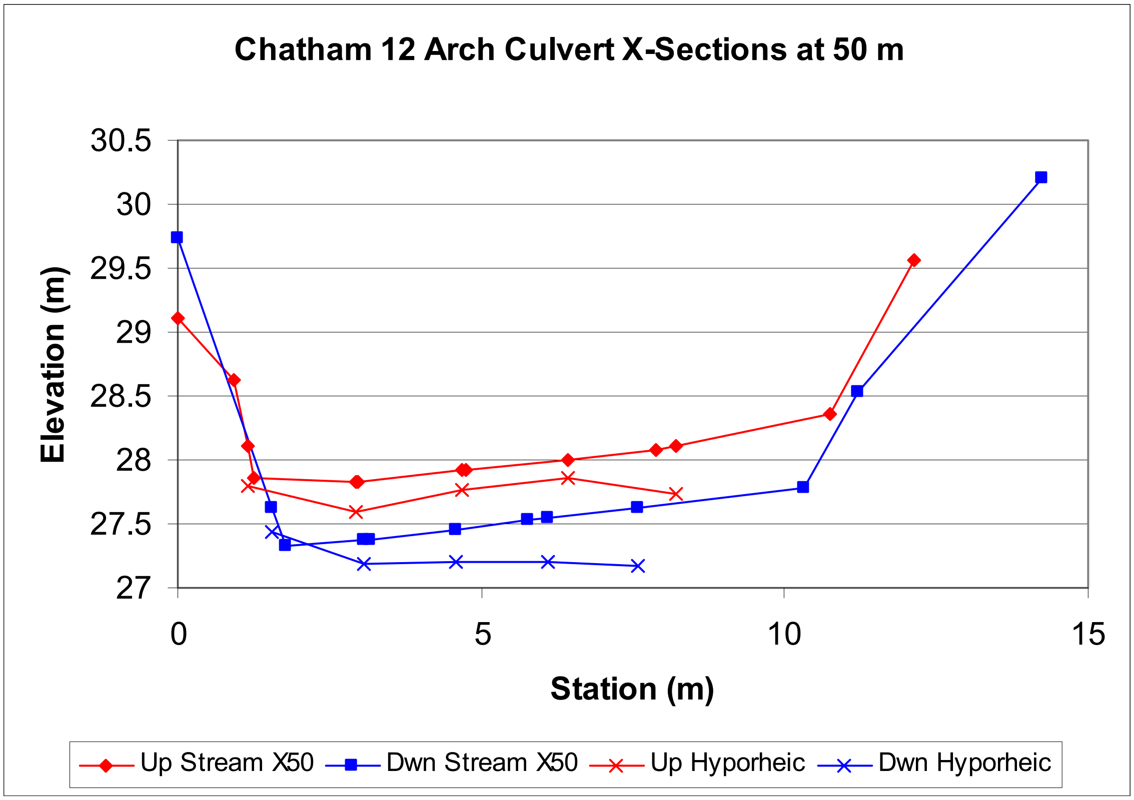


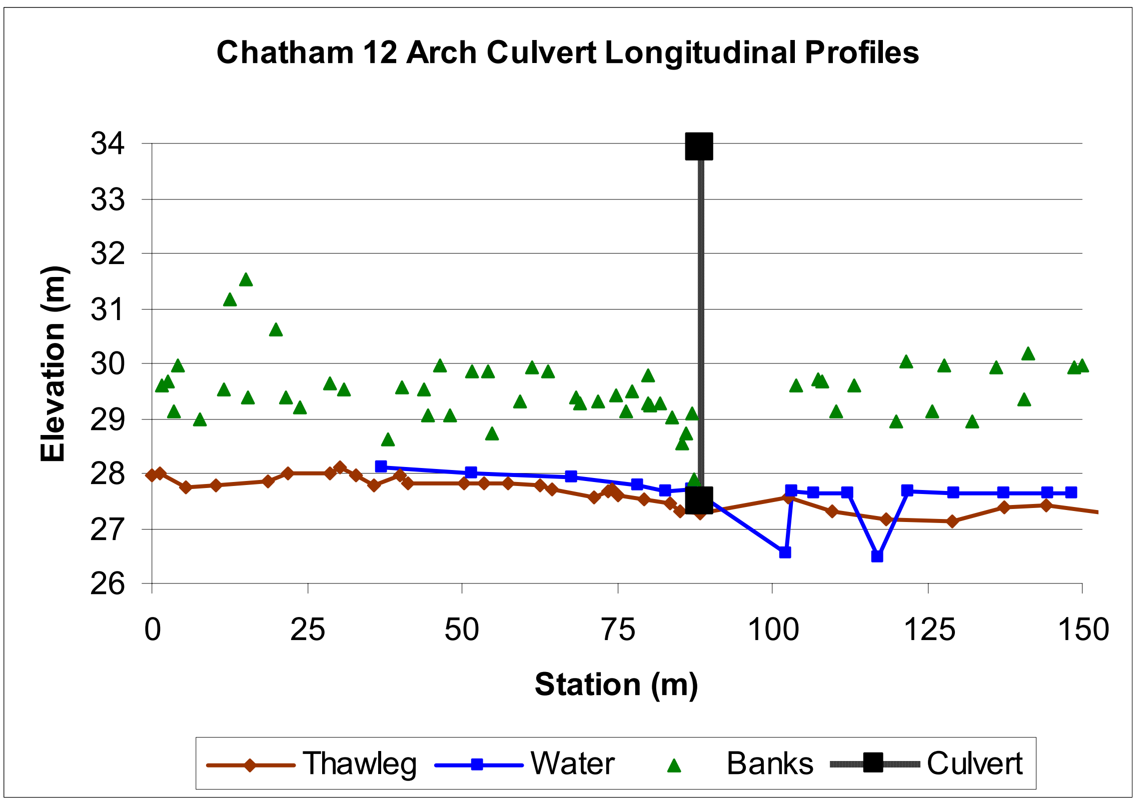


Chatham 12 Arch Culvert X-Sections at 10 m
30
29.5
29
28.5
28
27.5
27
26.5
Elevation (m)
0
2
4
6
8
10
12
Station (m)
Up Stream X10
Dwn Stream X10
Up Hyporheic
Dwn Hyporheic

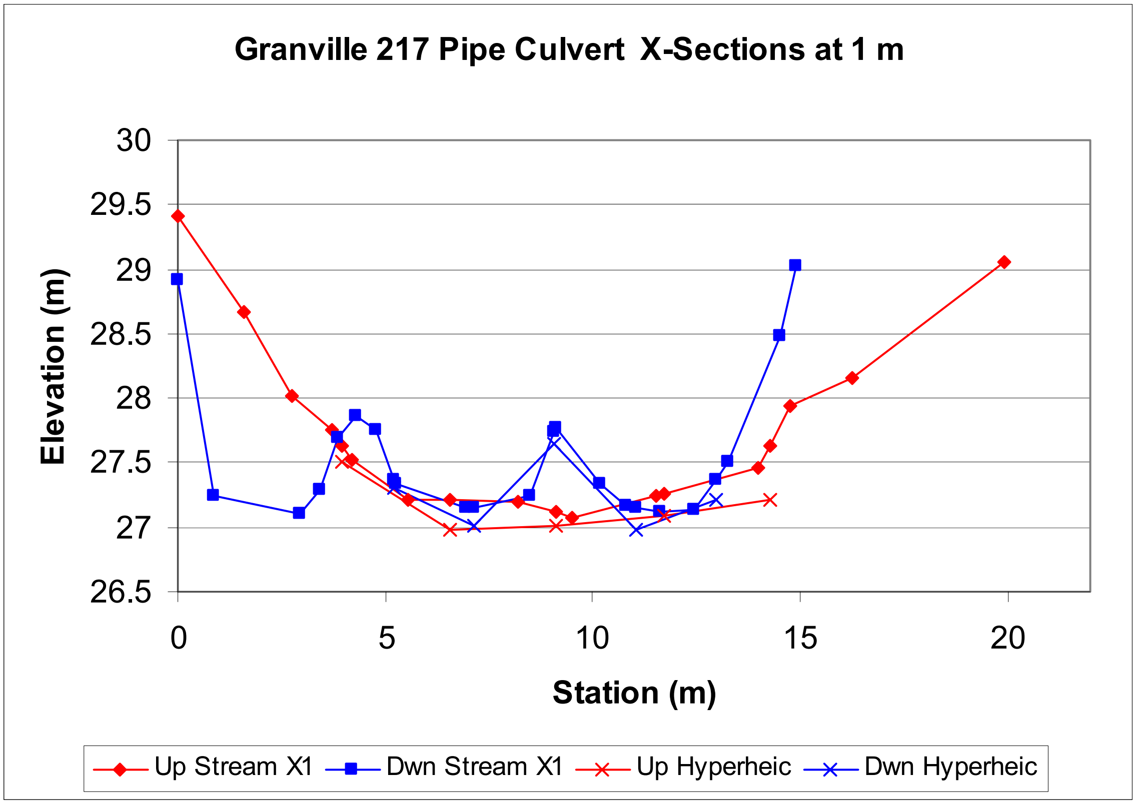


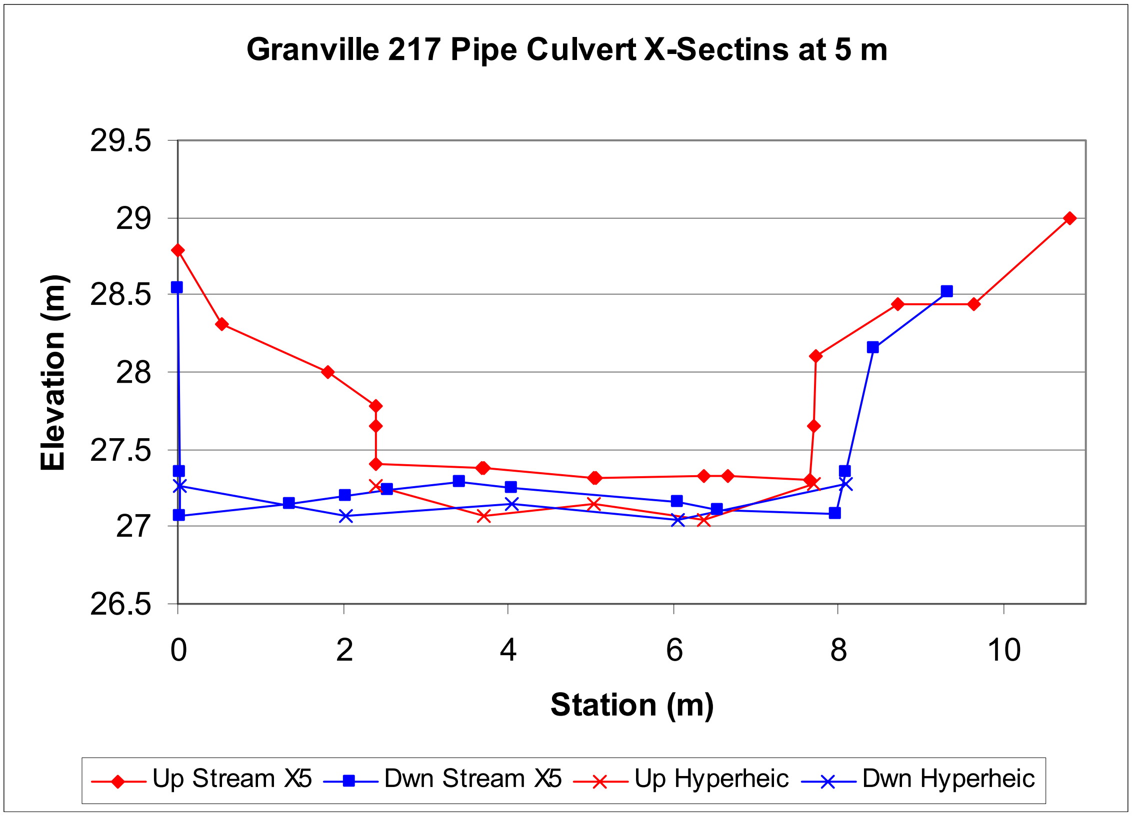
Chatham 12 Arch Culvert X-Sections at 20 m
30.5
30
29.5
29
28.5
28
27.5
27
26.5
Elevation (m)
0
5
10
15
Station (m)
Up Stream X20
Dwn Stream X20
Up Hyperheic
Dwn Hyperheic

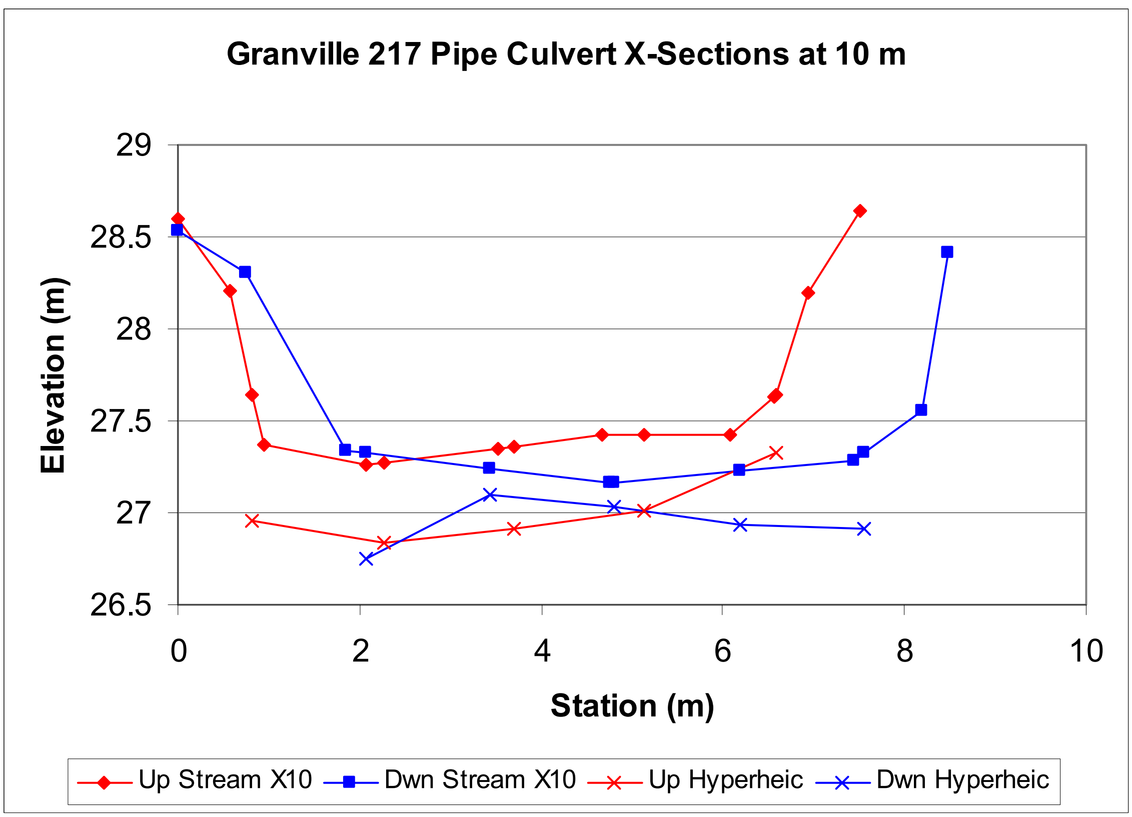

Chatham 12 Arch Culvert X-Sections at 50 m
Elevation (m)
30.5
30
29.5
29
28.5
28
27.5
27
0
5
10
15
Station (m)
Up Stream X50
Dwn Stream X50
Up Hyporheic
Dwn Hyporheic

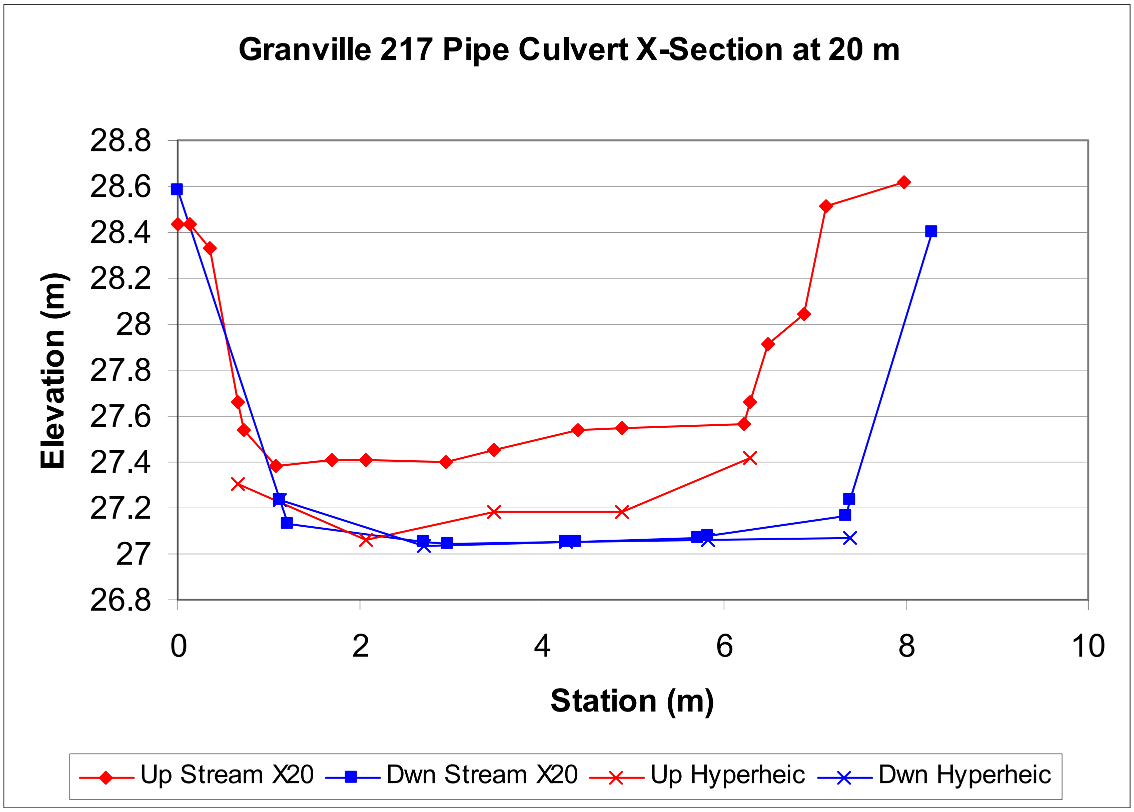

Chatham 12 Arch Culvert Longitudinal Profiles
Elevation (m)
34
33
32
31
30
29
28
27
26
0
25
50
75
100
125
150
Station (m)
Thawleg
Water
Banks
Culvert

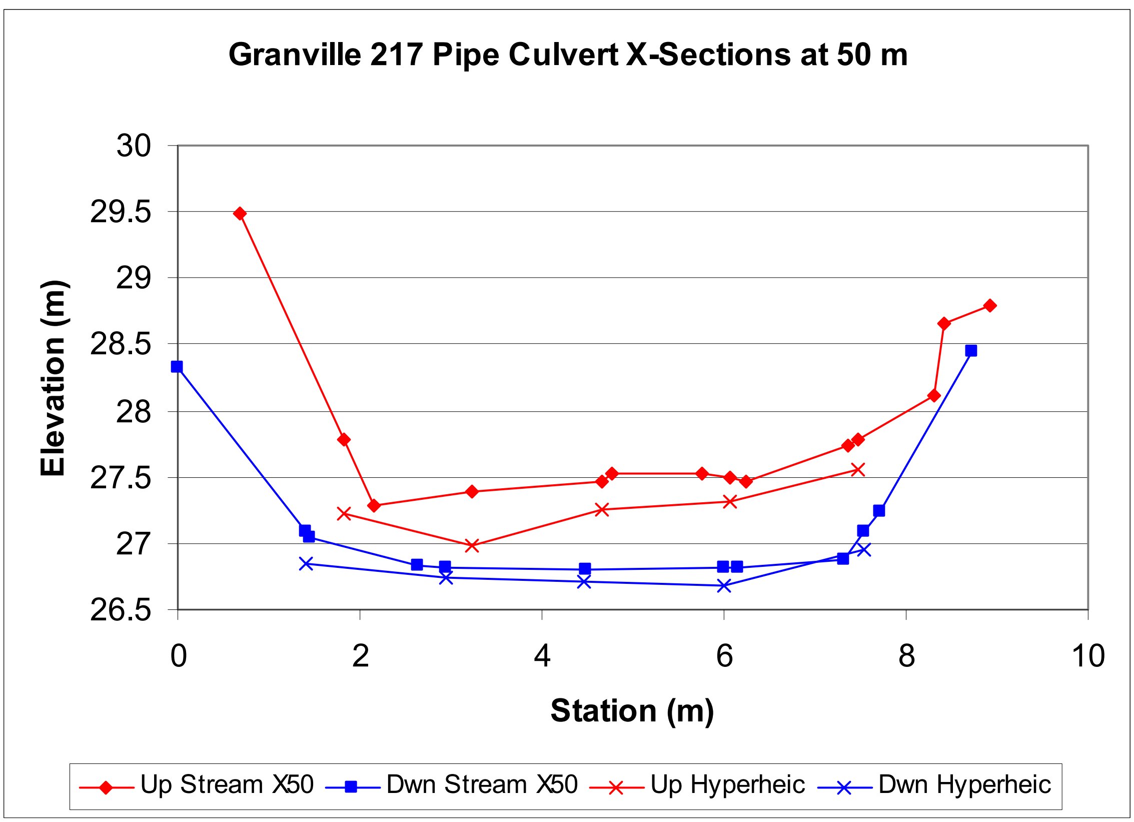
Granville 217 Cross Section and Longitudinal Profile

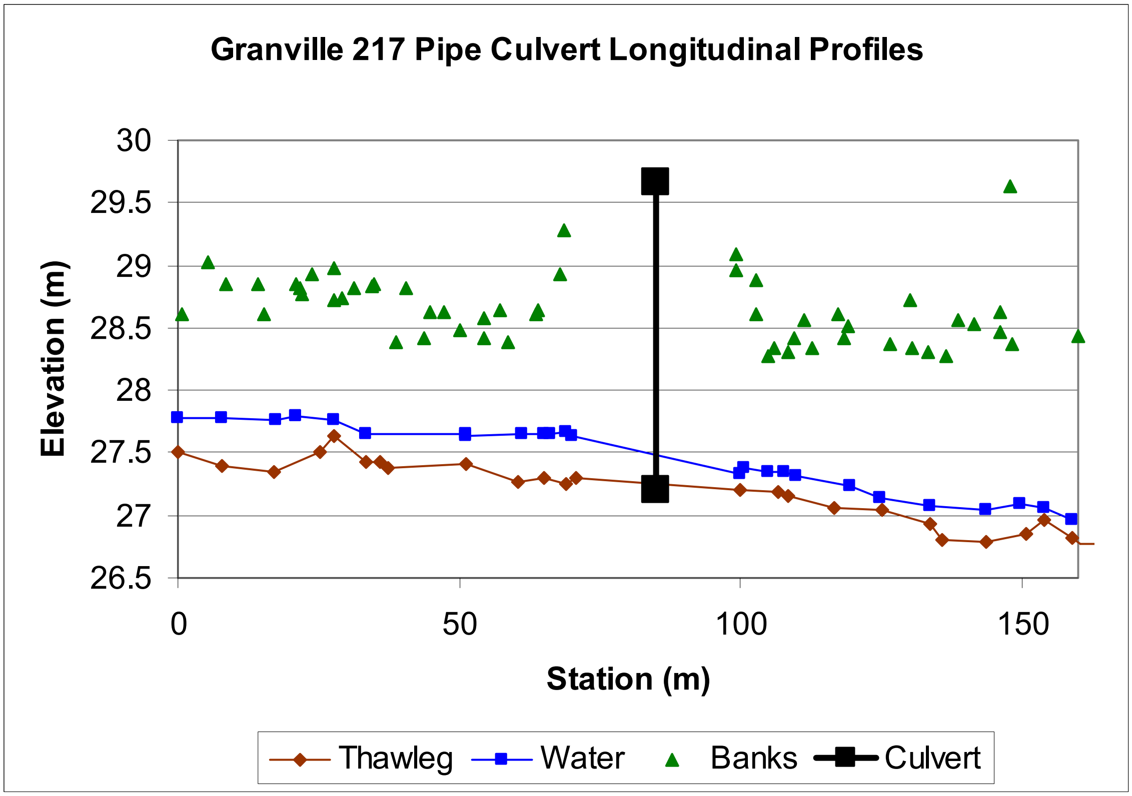



Granville 217 Pipe Culvert X-Sectins at 5 m
Elevation (m)
29.5
29
28.5
28
27.5
27
26.5
0
2
4
6
8
10
Station (m)
Up Stream X5
Dwn Stream X5
Up Hyperheic
Dwn Hyperheic


Granville 217 Pipe Culvert X-Sections at 10 m
Elevation (m)
29
28.5
28
27.5
27
26.5
0
2
4
6
8
10
Station (m)
Up Stream X10
Dwn Stream X10
Up Hyperheic
Dwn Hyperheic

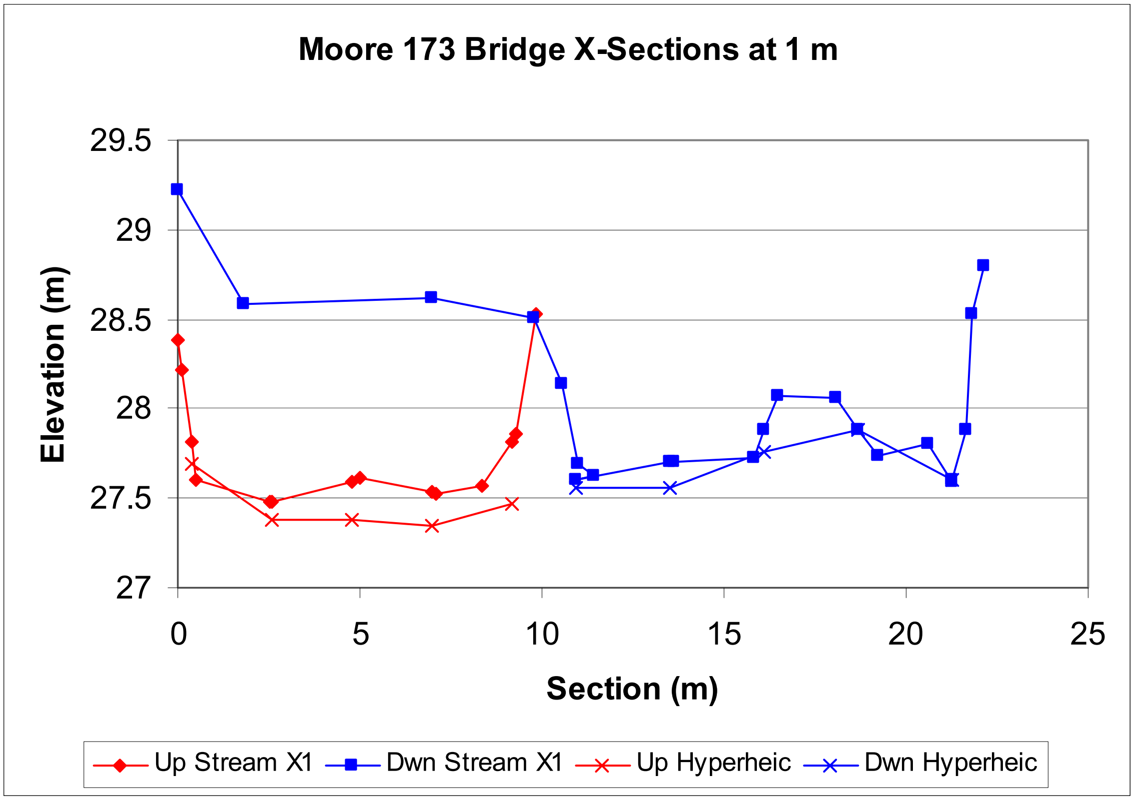

Granville 217 Pipe Culvert X-Section at 20 m
Elevation (m)
28.8
28.6
28.4
28.2
28
27.8
27.6
27.4
27.2
27
26.8
0
2
4
6
8
10
Station (m)
Up Stream X20
Dwn Stream X20
Up Hyperheic
Dwn Hyperheic

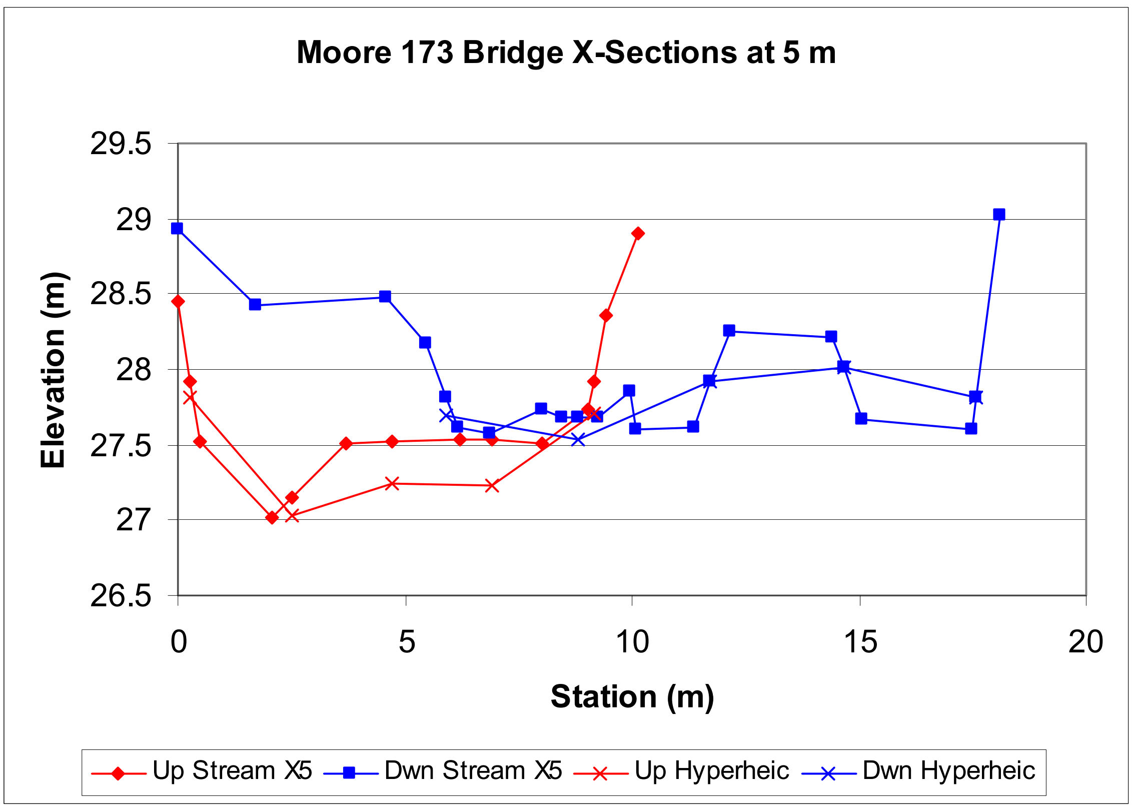

Granville 217 Pipe Culvert X-Sections at 50 m
Elevation (m)
30
29.5
29
28.5
28
27.5
27
26.5
0
2
4
6
8
10
Station (m)
Up Stream X50
Dwn Stream X50
Up Hyperheic
Dwn Hyperheic

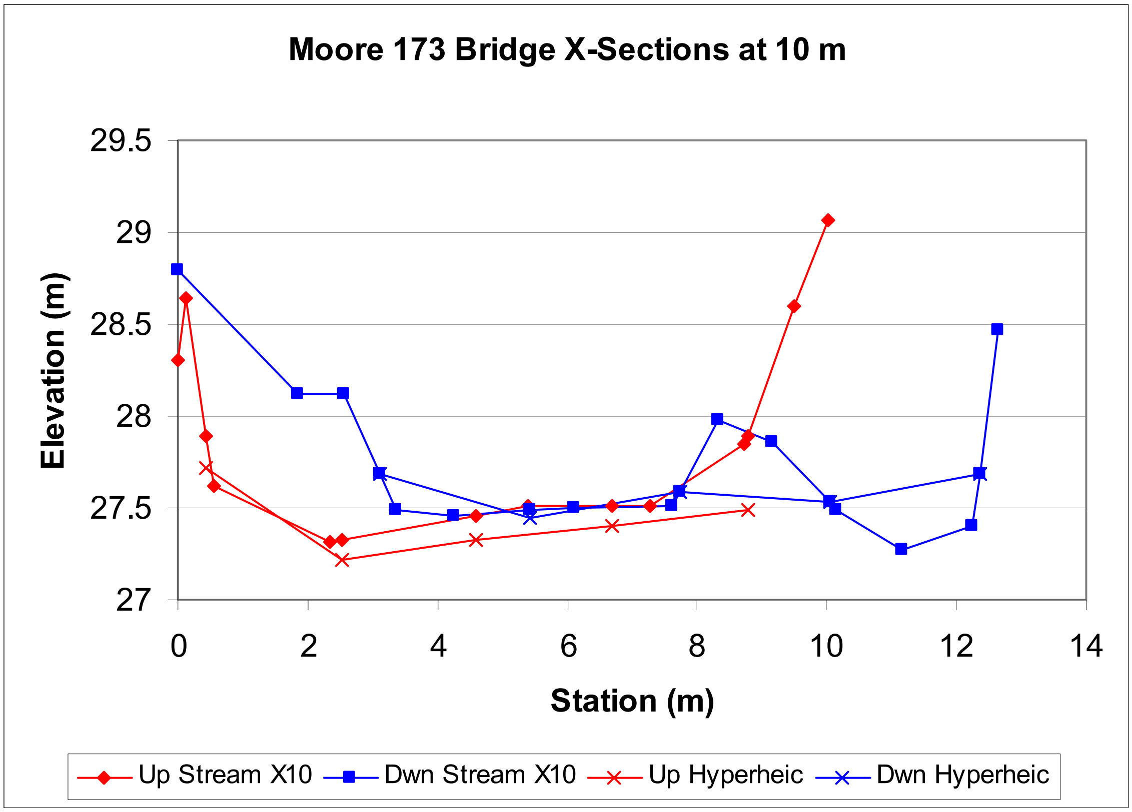


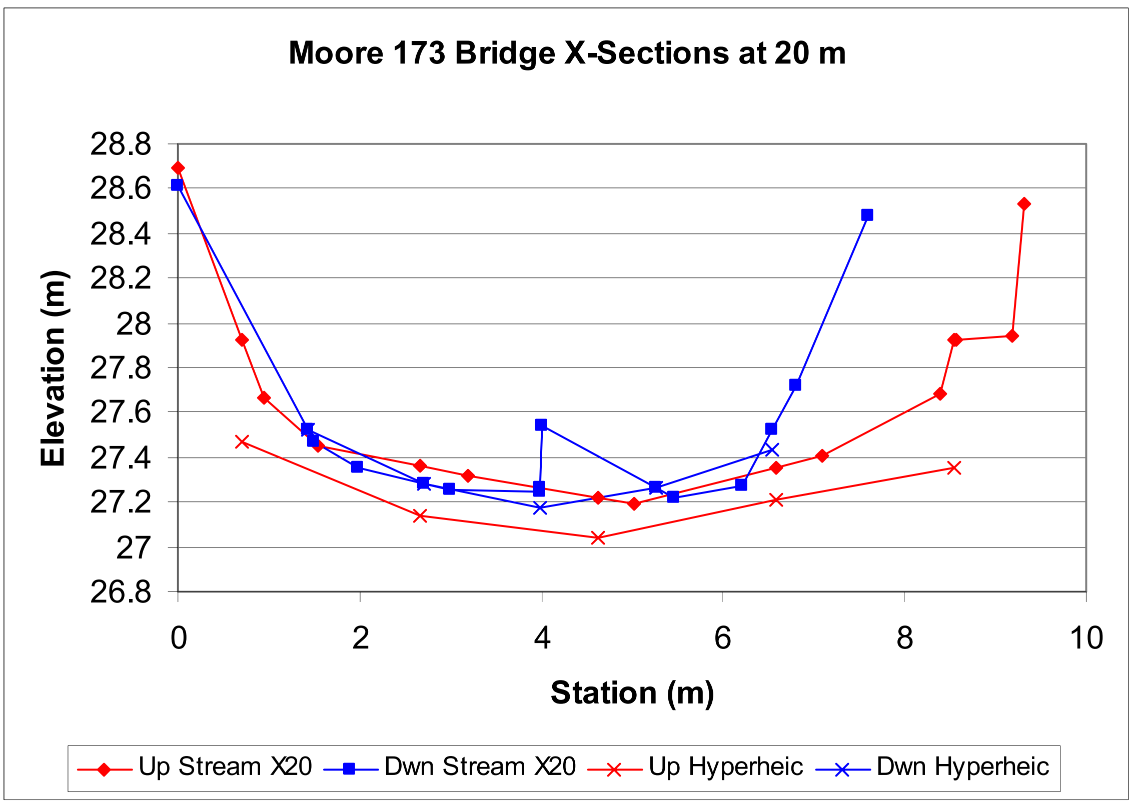
Granville 217 Pipe Culvert Longitudinal Profiles
Elevation (m)
30
29.5
29
28.5
28
27.5
27
26.5
0
50
100
150
Station (m)
Thawleg
Water
Banks
Culvert

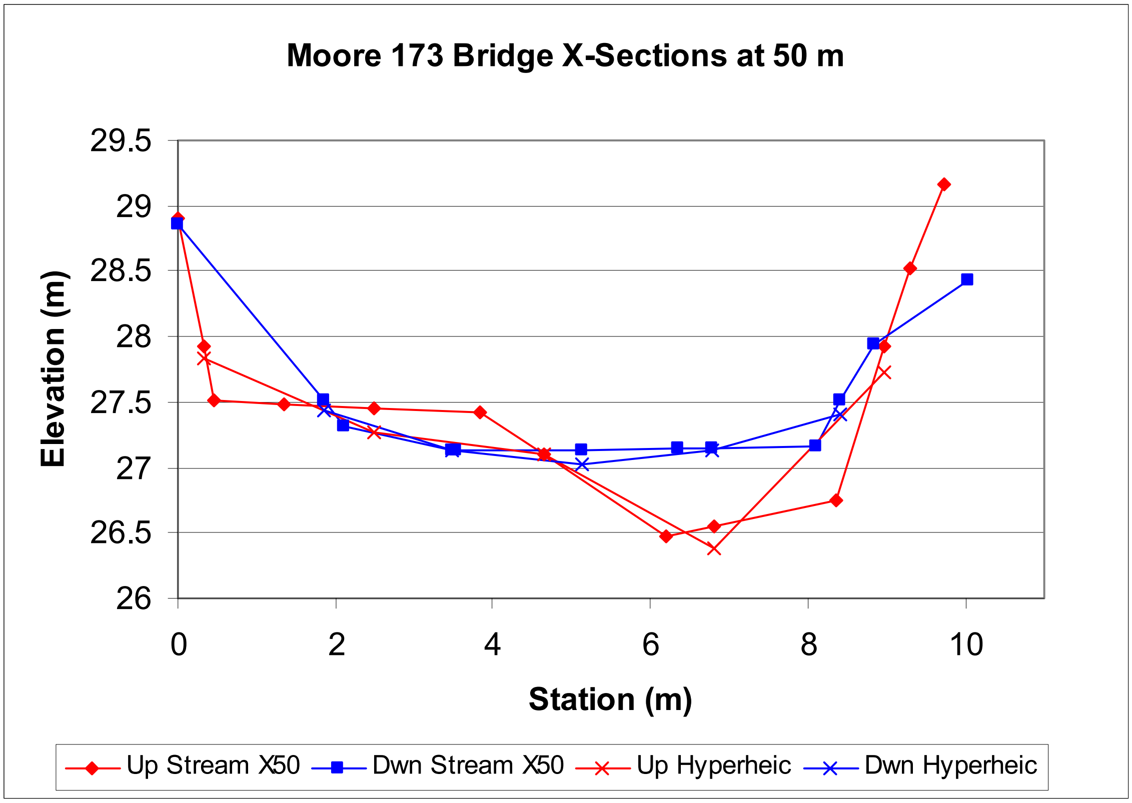
Moore 173 Cross Section and Longitudinal Profile

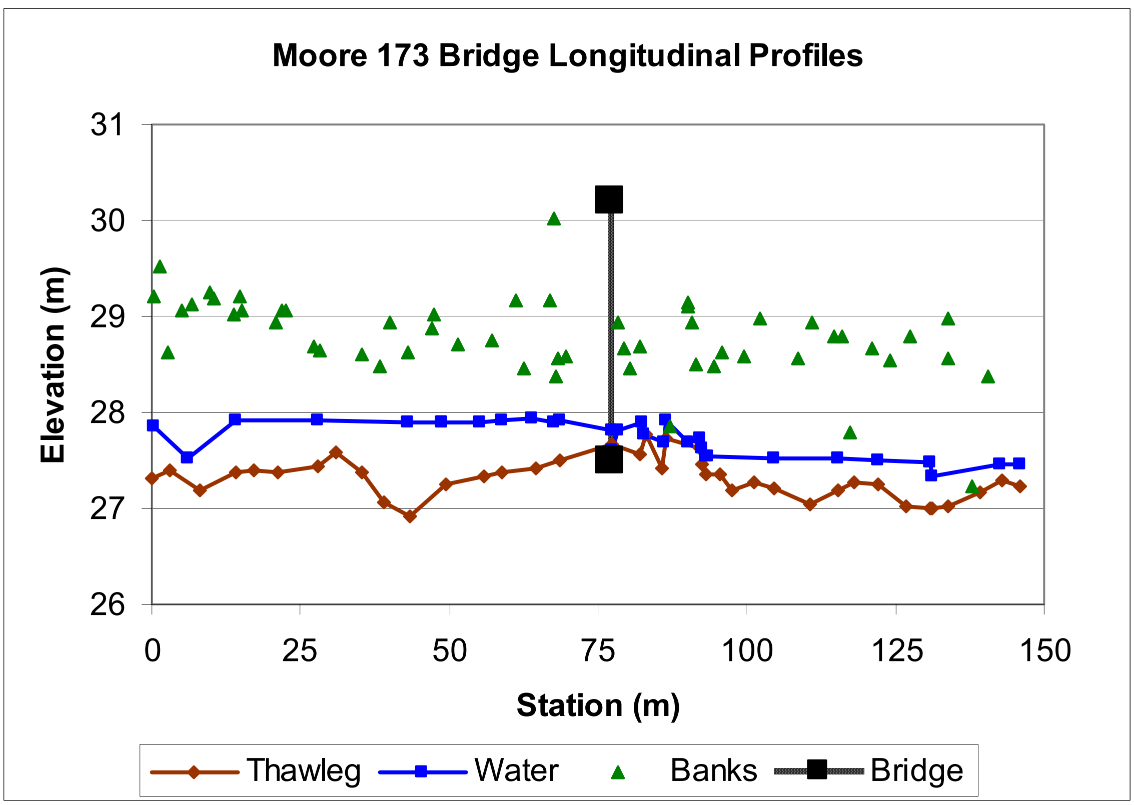




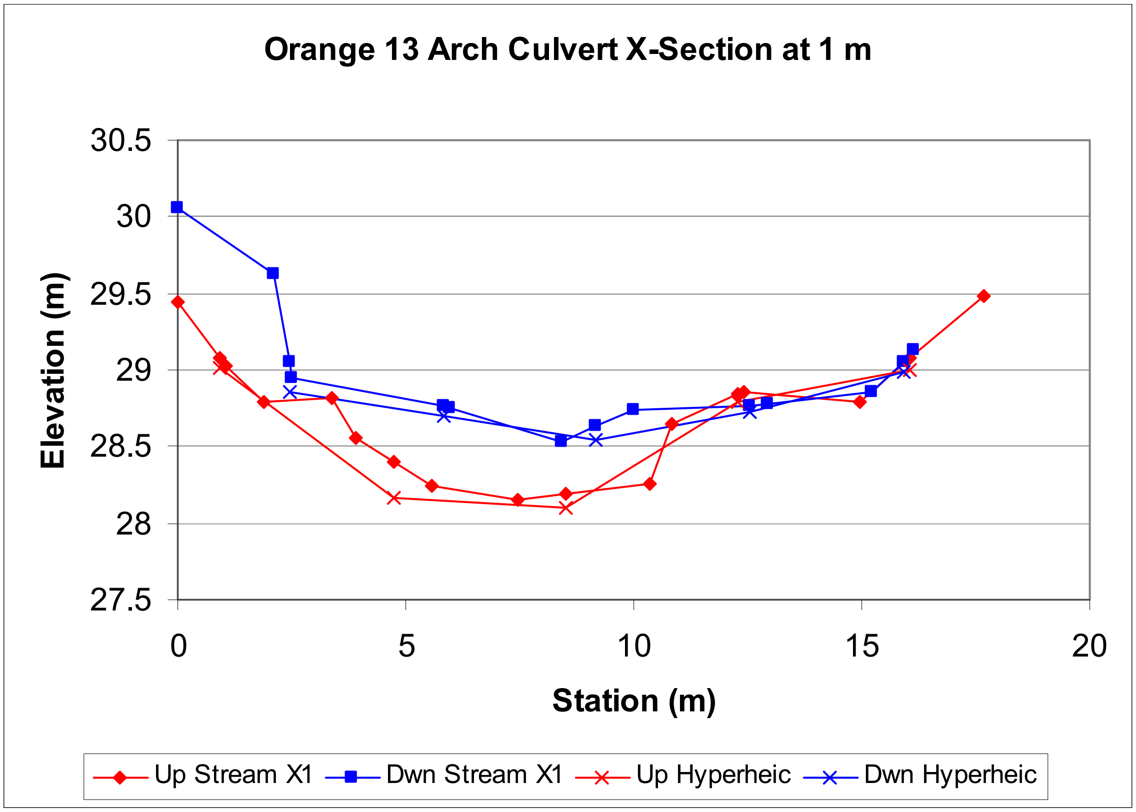

Moore 173 Bridge X-Sections at 10 m
29.5
29
28.5
28
27.5
27
0
2
4
6
8
10
12
14
Elevation (m)
Station (m)
Up Stream X10
Dwn Stream X10
Up Hyperheic
Dwn Hyperheic

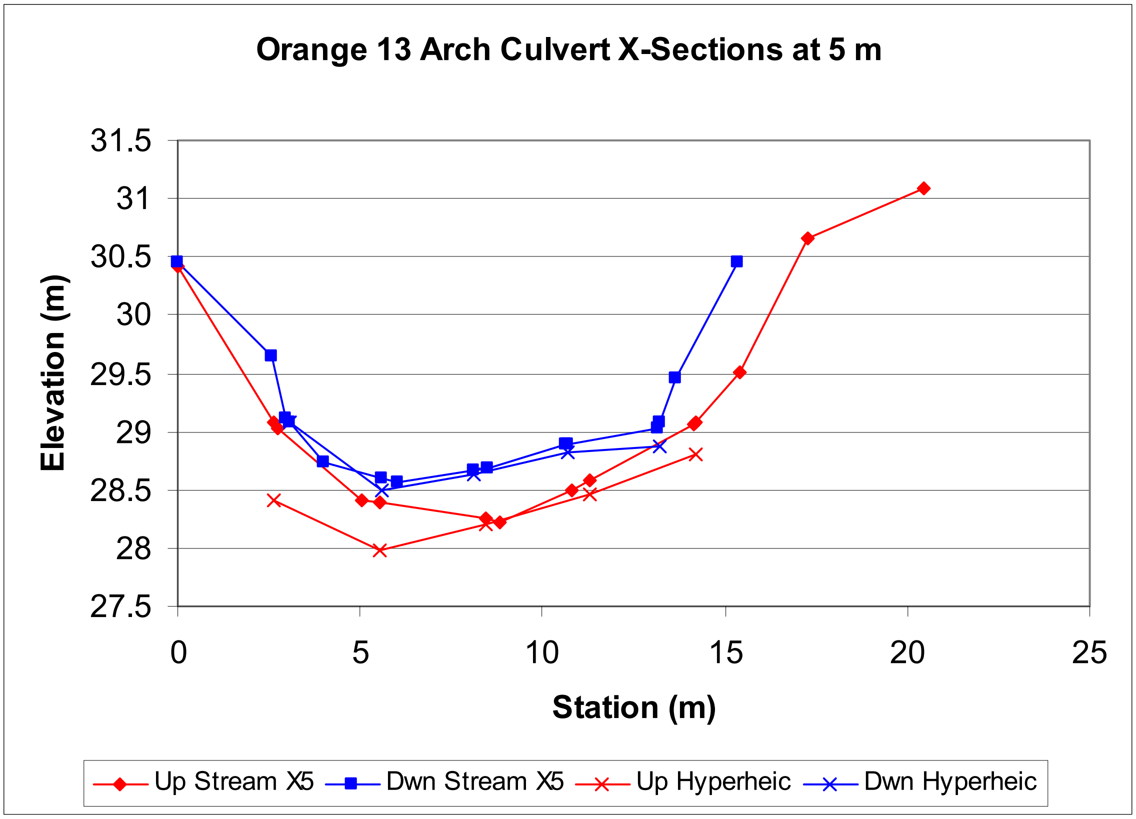

Moore 173 Bridge X-Sections at 20 m
28.8
28.6
28.4
28.2
28
27.8
27.6
27.4
27.2
27
26.8
0
2
4
6
8
10
Elevation (m)
Station (m)
Up Stream X20
Dwn Stream X20
Up Hyperheic
Dwn Hyperheic


Moore 173 Bridge X-Sections at 50 m
29.5
29
28.5
28
27.5
27
26.5
26
Elevation (m)
0
2
4
6
8
10
Station (m)
Up Stream X50
Dwn Stream X50
Up Hyperheic
Dwn Hyperheic

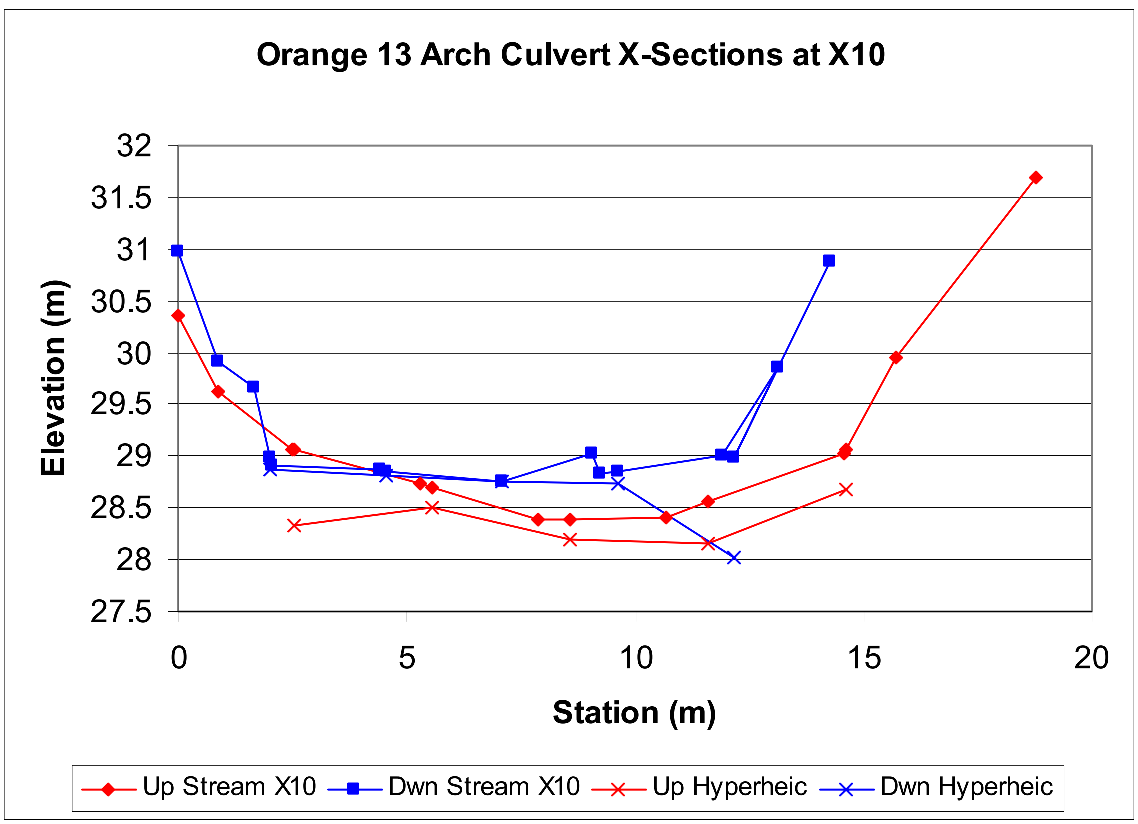

Moore 173 Bridge Longitudinal Profiles
31
30
29
28
27
26
Elevation (m)
0
25
50
75
100
125
150
Station (m)
Thawleg
Water
Banks
Bridge

Orange 13 Cross Section and Longitudinal Profile

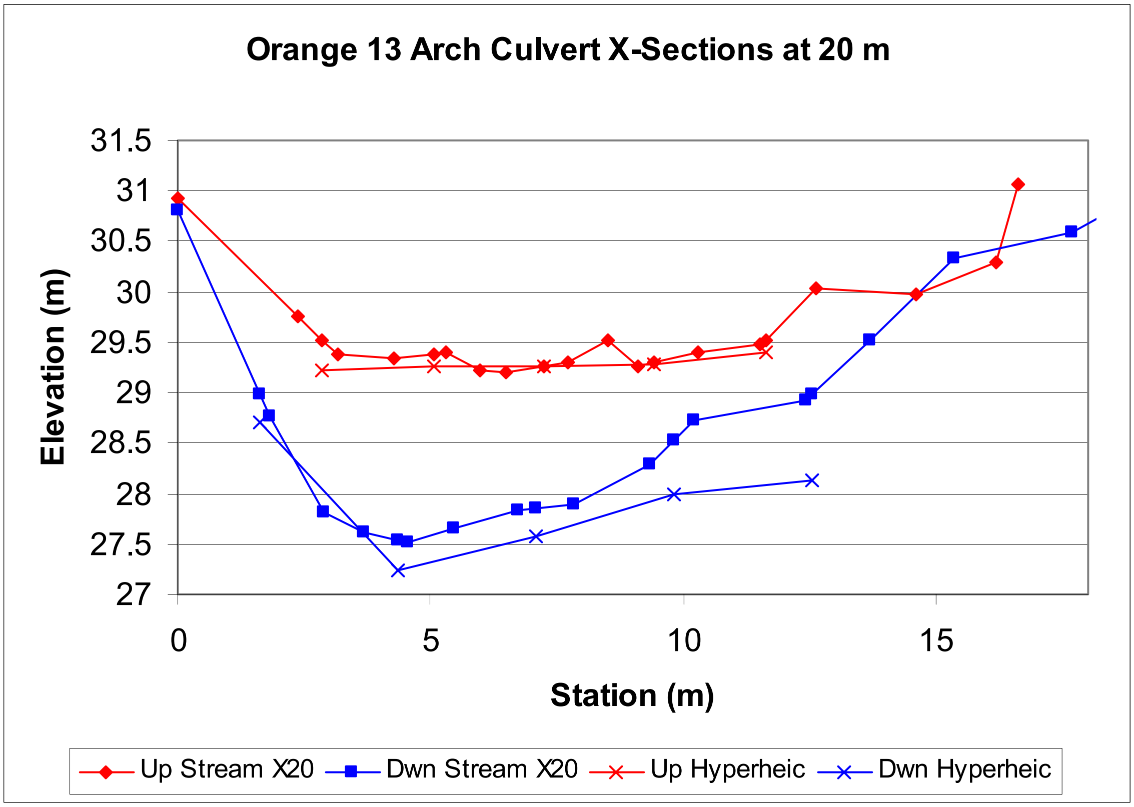


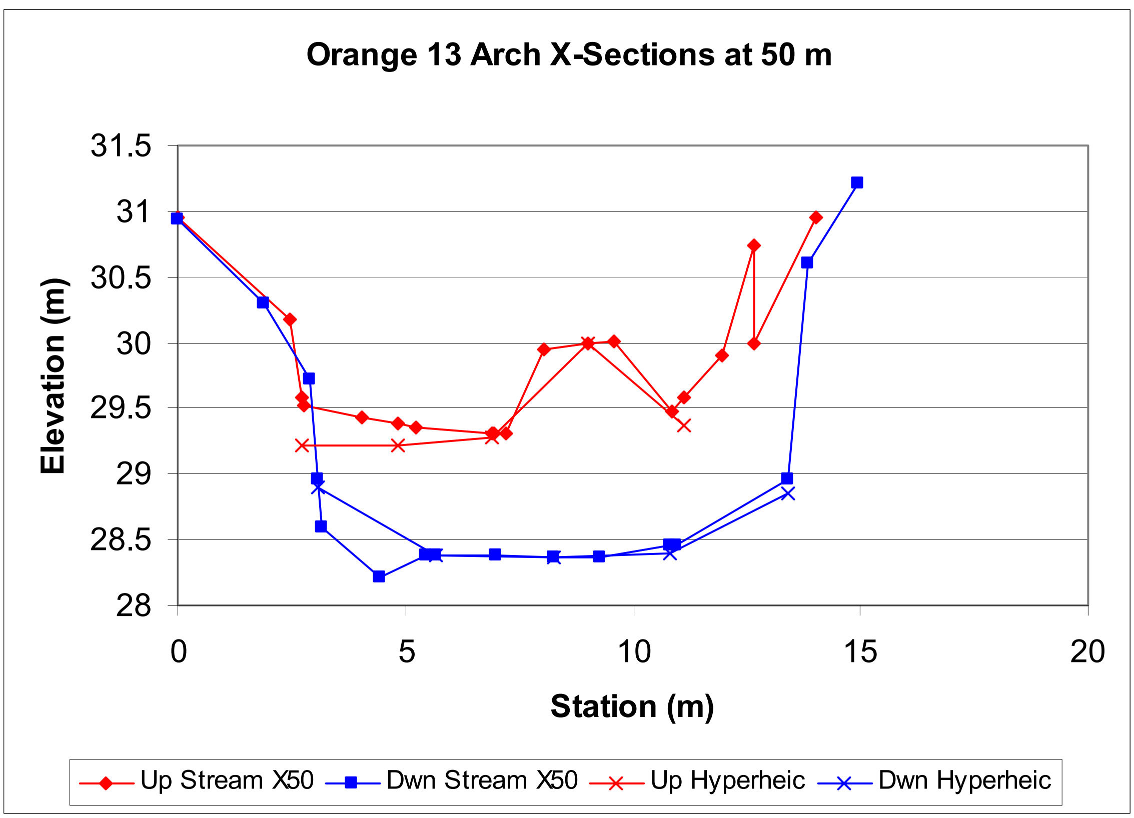


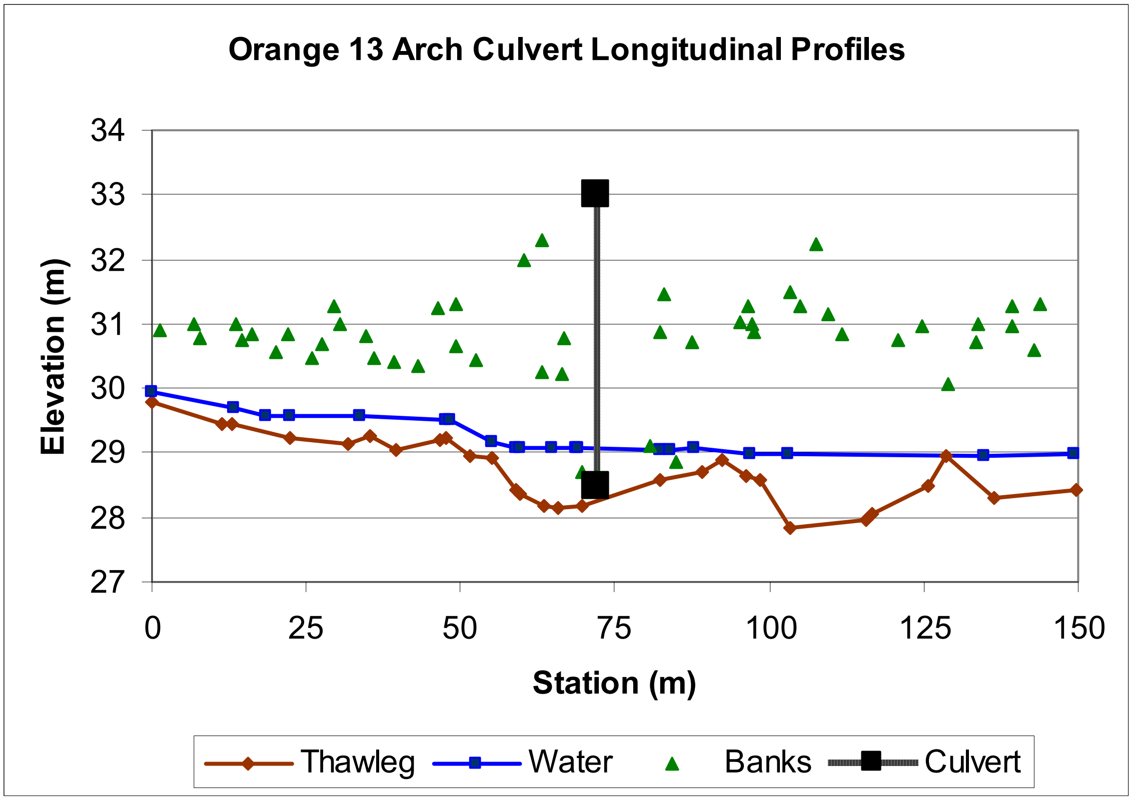
Orange 13 Arch Culvert X-Sections at 5 m
Elevation (m)
31.5
31
30.5
30
29.5
29
28.5
28
27.5
0
5
10
15
20
25
Station (m)
Up Stream X5
Dwn Stream X5
Up Hyperheic
Dwn Hyperheic



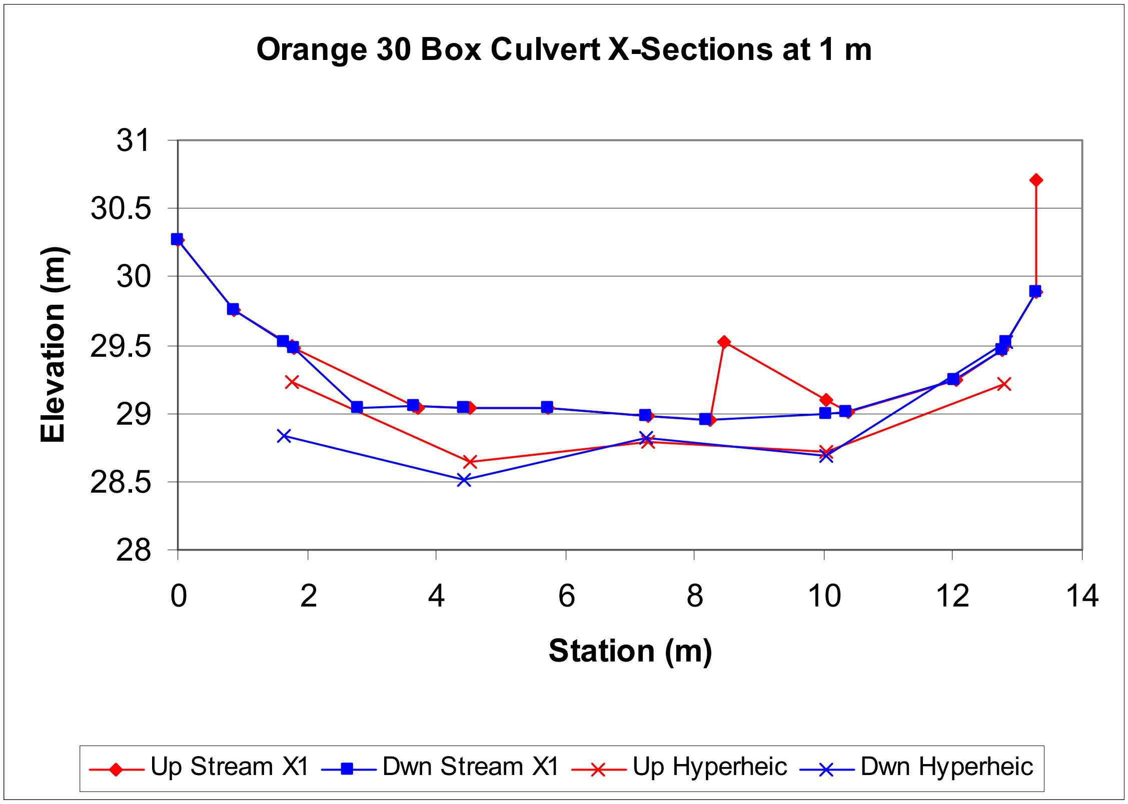
Orange 13 Arch Culvert X-Sections at X10
Elevation (m)
32
31.5
31
30.5
30
29.5
29
28.5
28
27.5
0
5
10
15
20
Station (m)
Up Stream X10
Dwn Stream X10
Up Hyperheic
Dwn Hyperheic

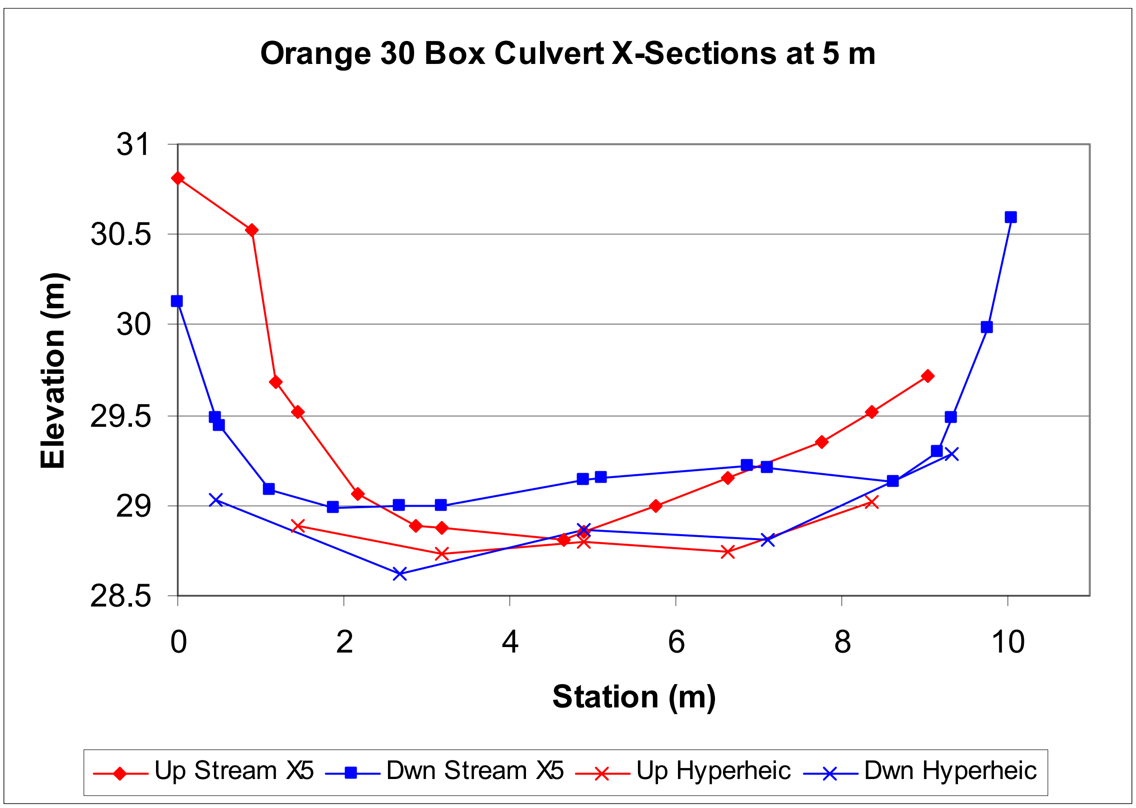

Orange 13 Arch Culvert X-Sections at 20 m
Elevation (m)
31.5
31
30.5
30
29.5
29
28.5
28
27.5
27
0
5
10
15
Station (m)
Up Stream X20
Dwn Stream X20
Up Hyperheic
Dwn Hyperheic

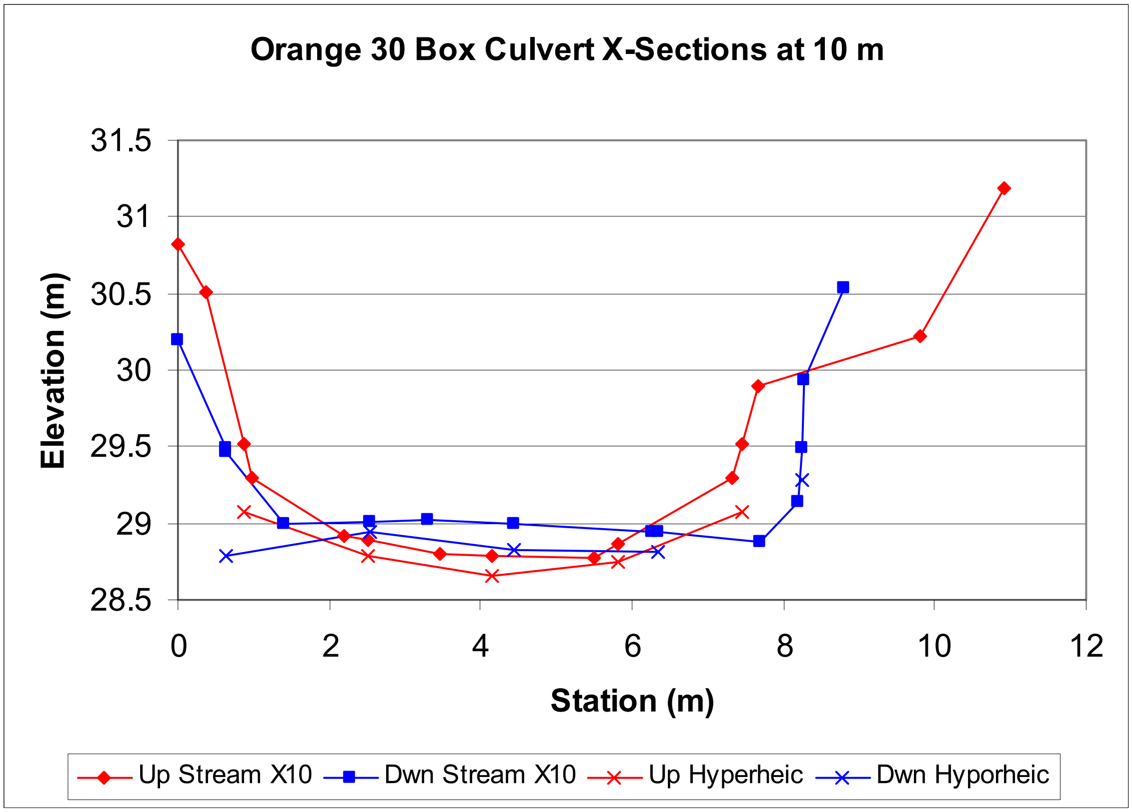

Orange 13 Arch X-Sections at 50 m
Elevation (m)
31.5
31
30.5
30
29.5
29
28.5
28
0
5
10
15
20
Station (m)
Up Stream X50
Dwn Stream X50
Up Hyperheic
Dwn Hyperheic

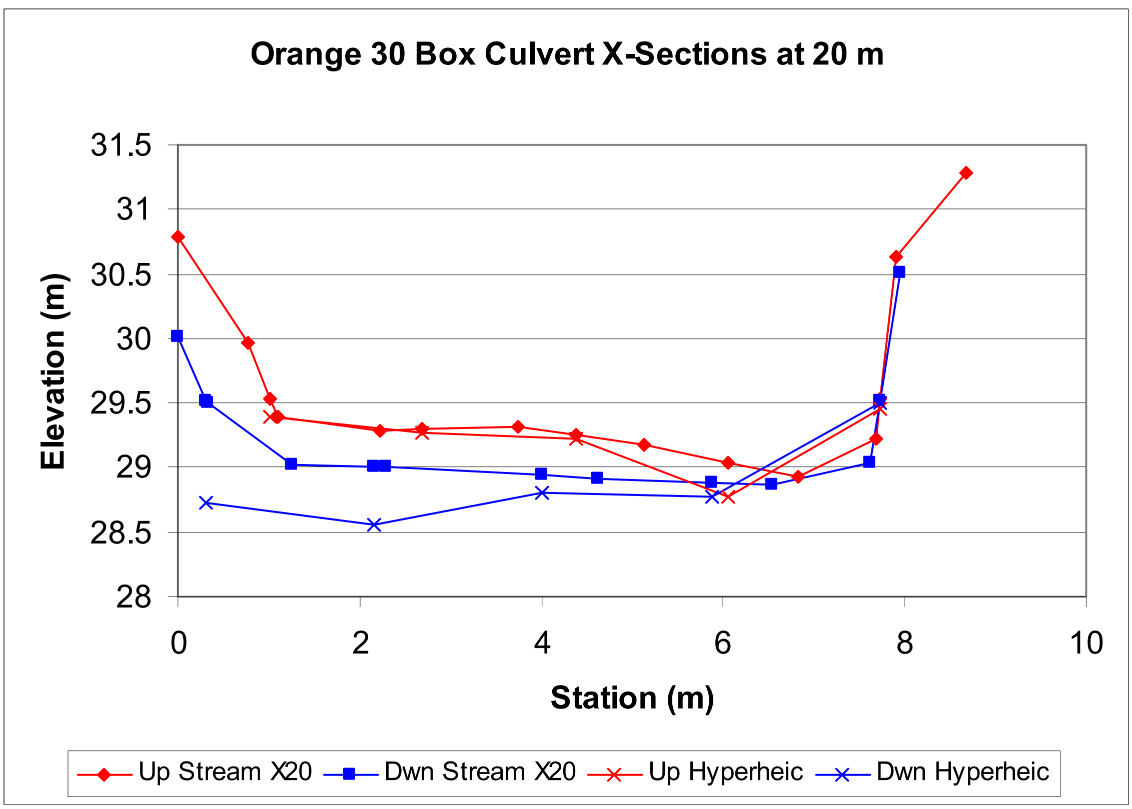

Orange 13 Arch Culvert Longitudinal Profiles
34
33
32
31
30
29
28
27
Elevation (m)
0
25
50
75
100
125
150
Station (m)
Thawleg
Water
Banks
Culvert

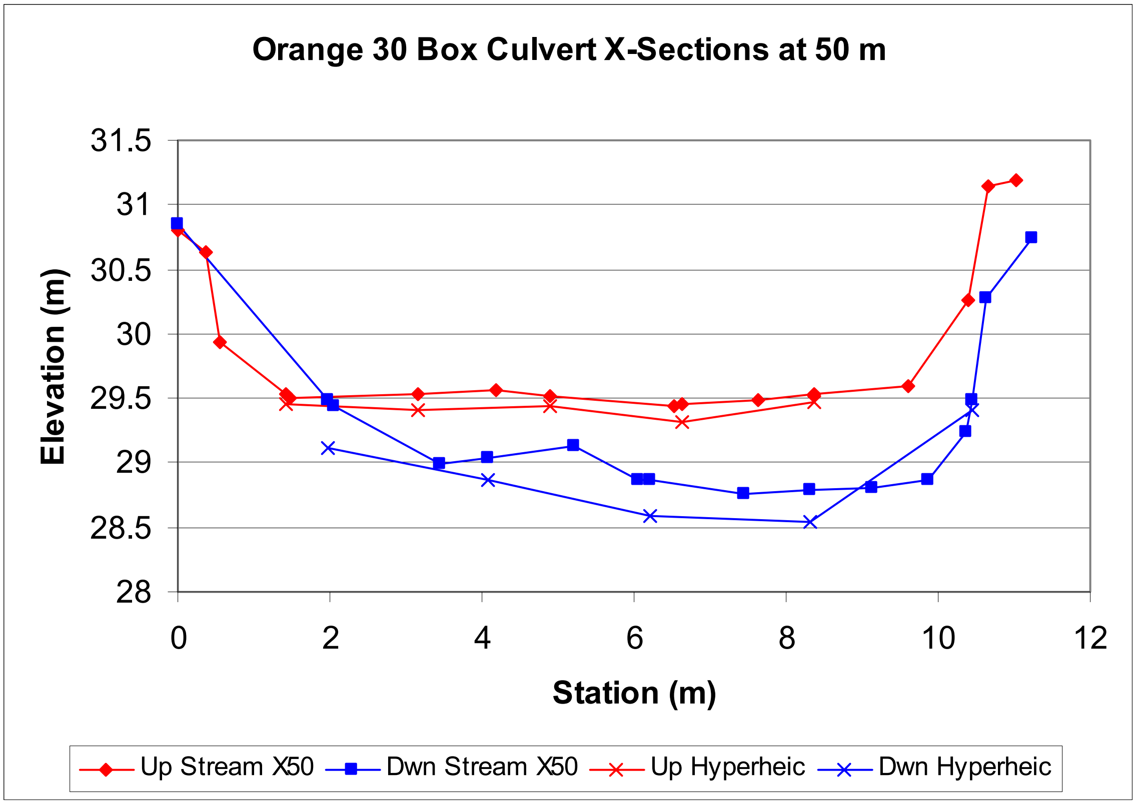
Orange 30 Cross Section and Longitudinal Profile

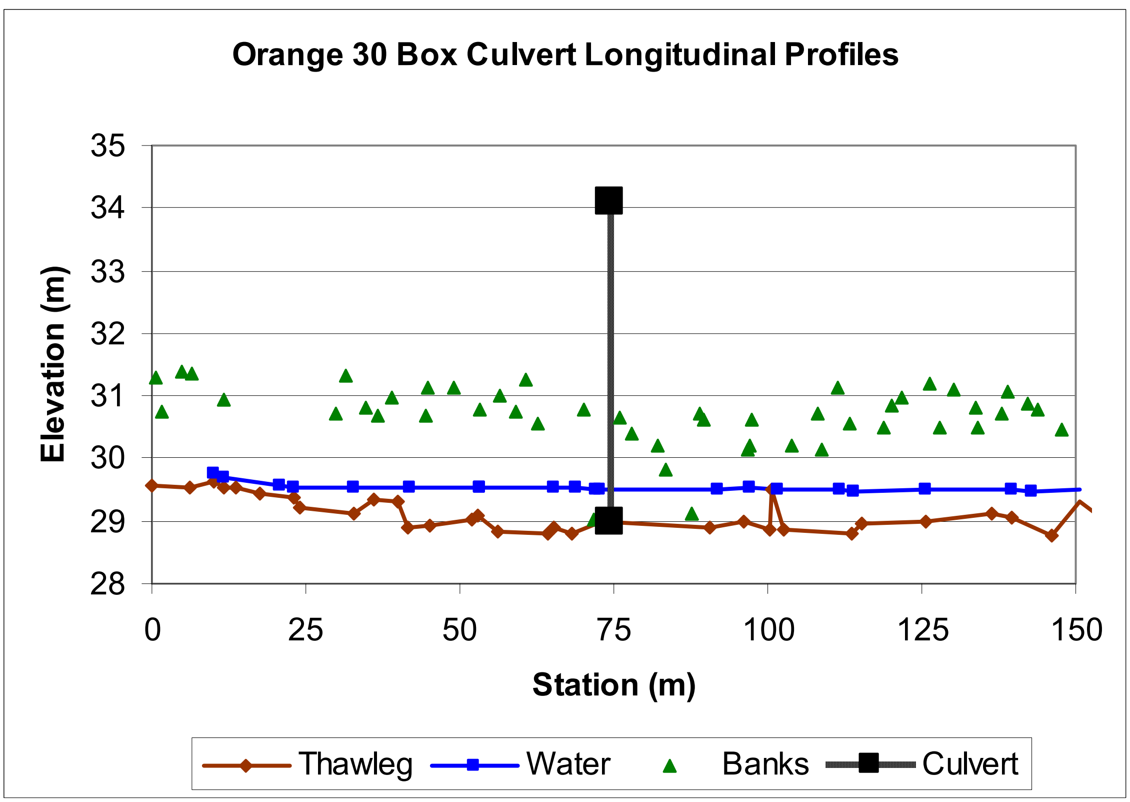




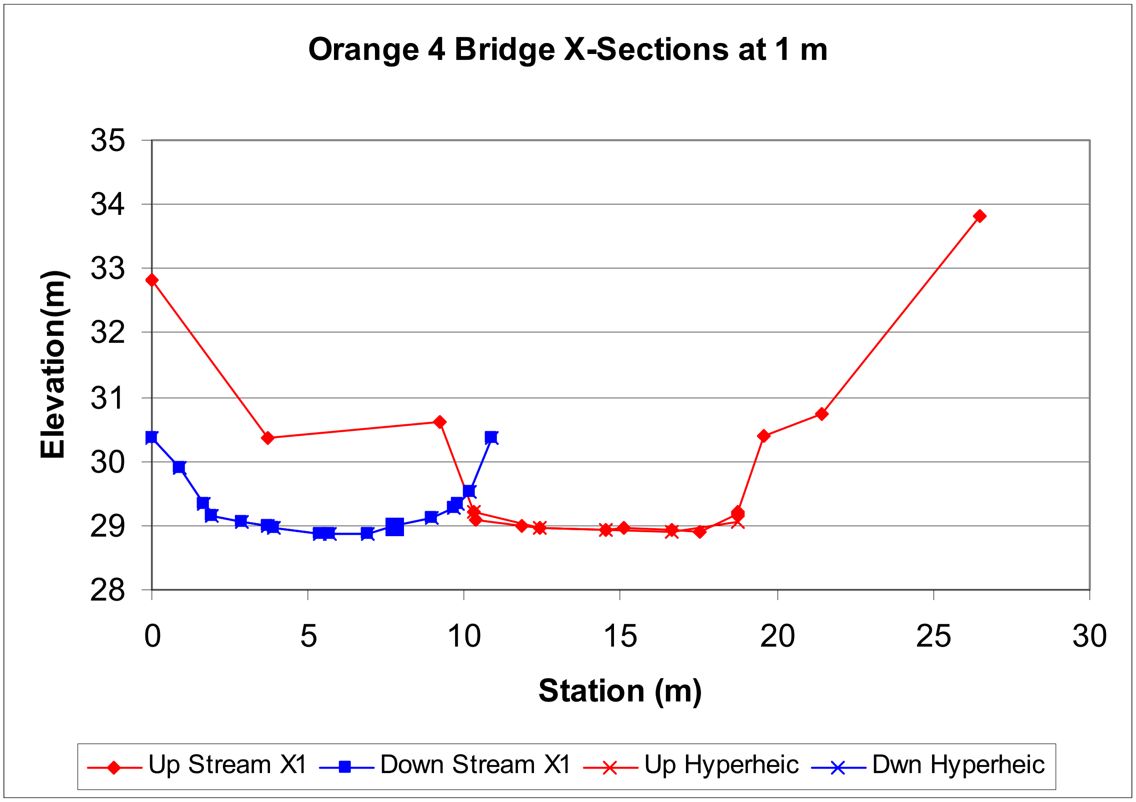

Orange 30 Box Culvert X-Sections at 10 m
Elevation (m)
31.5
31
30.5
30
29.5
29
28.5
0
2
4
6
8
10
12
Station (m)
Up Stream X10
Dwn Stream X10
Up Hyperheic
Dwn Hyporheic

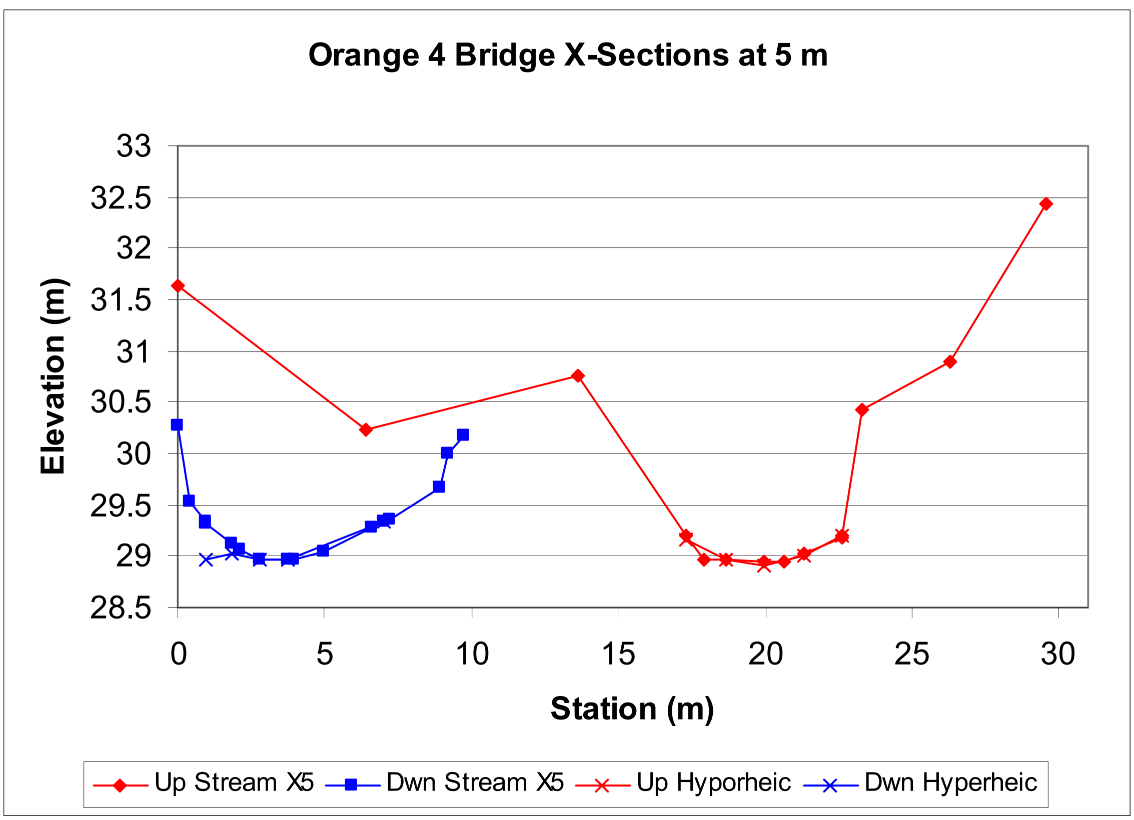

Orange 30 Box Culvert X-Sections at 20 m
Elevation (m)
31.5
31
30.5
30
29.5
29
28.5
28
0
2
4
6
8
10
Station (m)
Up Stream X20
Dwn Stream X20
Up Hyperheic
Dwn Hyperheic

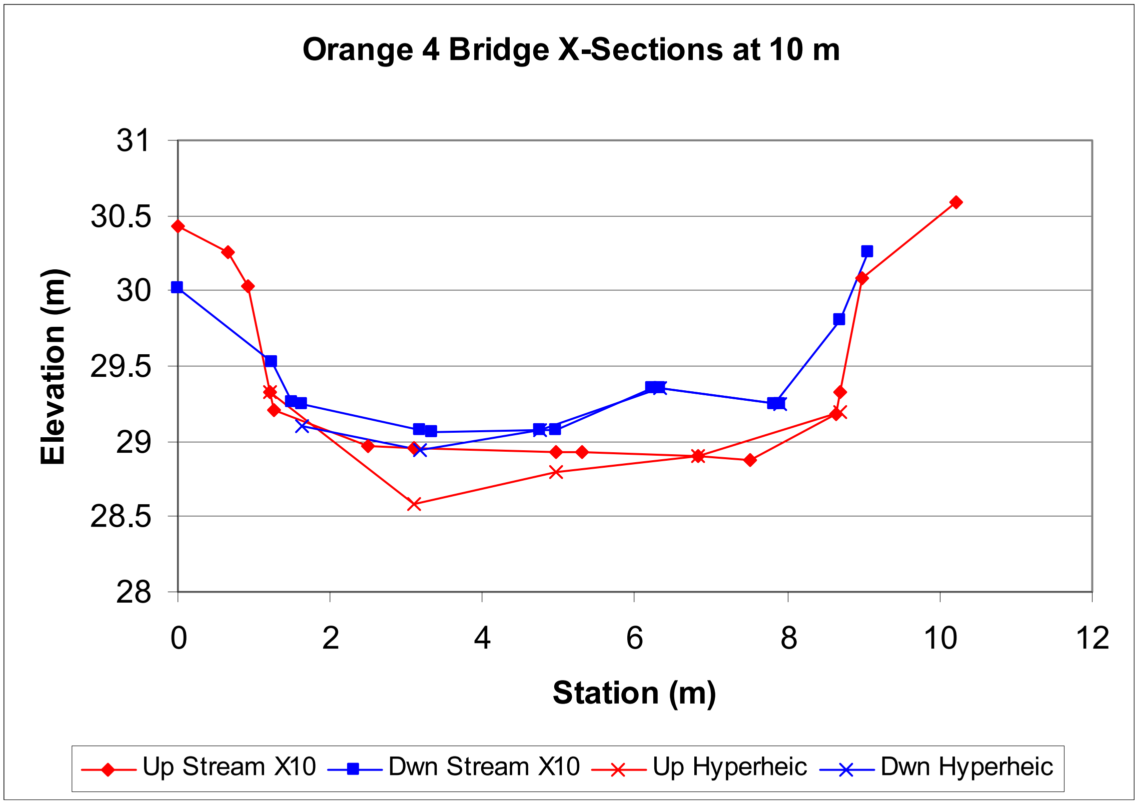

Orange 30 Box Culvert X-Sections at 50 m
Elevation (m)
31.5
31
30.5
30
29.5
29
28.5
28
0
2
4
6
8
10
12
Station (m)
Up Stream X50
Dwn Stream X50
Up Hyperheic
Dwn Hyperheic

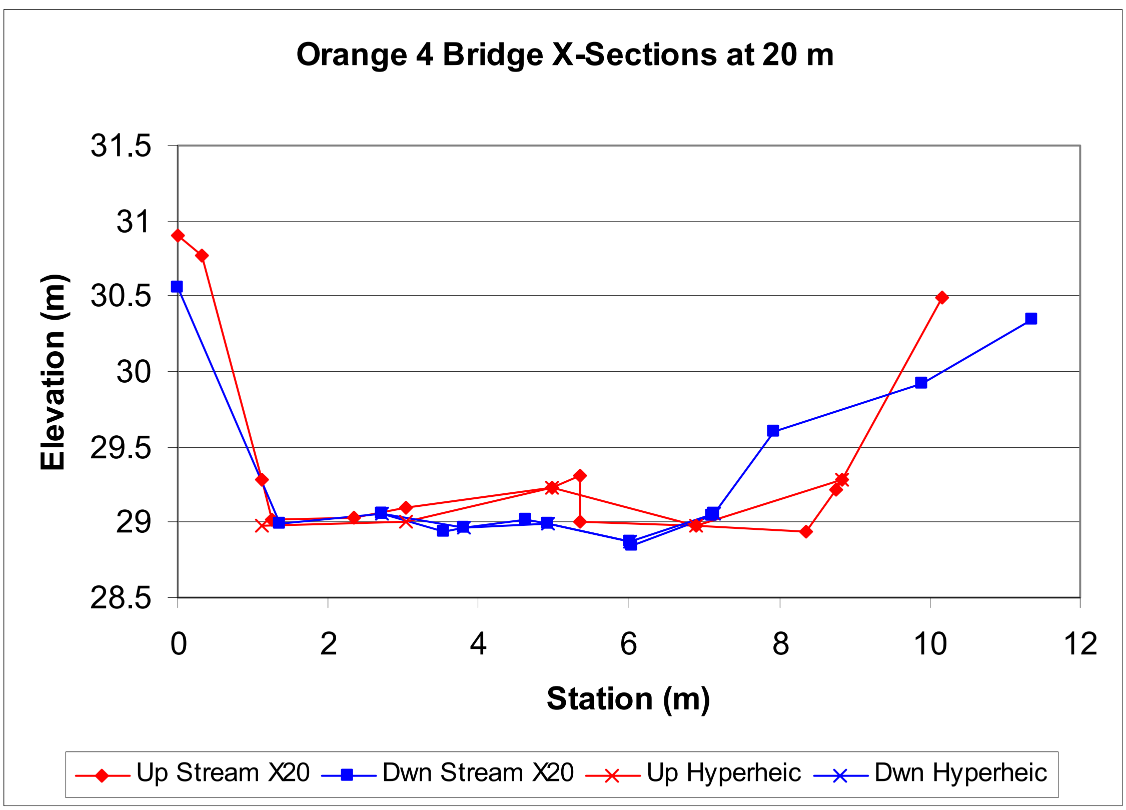

Orange 30 Box Culvert Longitudinal Profiles
Elevation (m)
35
34
33
32
31
30
29
28
0
25
50
75
100
125
150
Station (m)
Thawleg
Water
Banks
Culvert

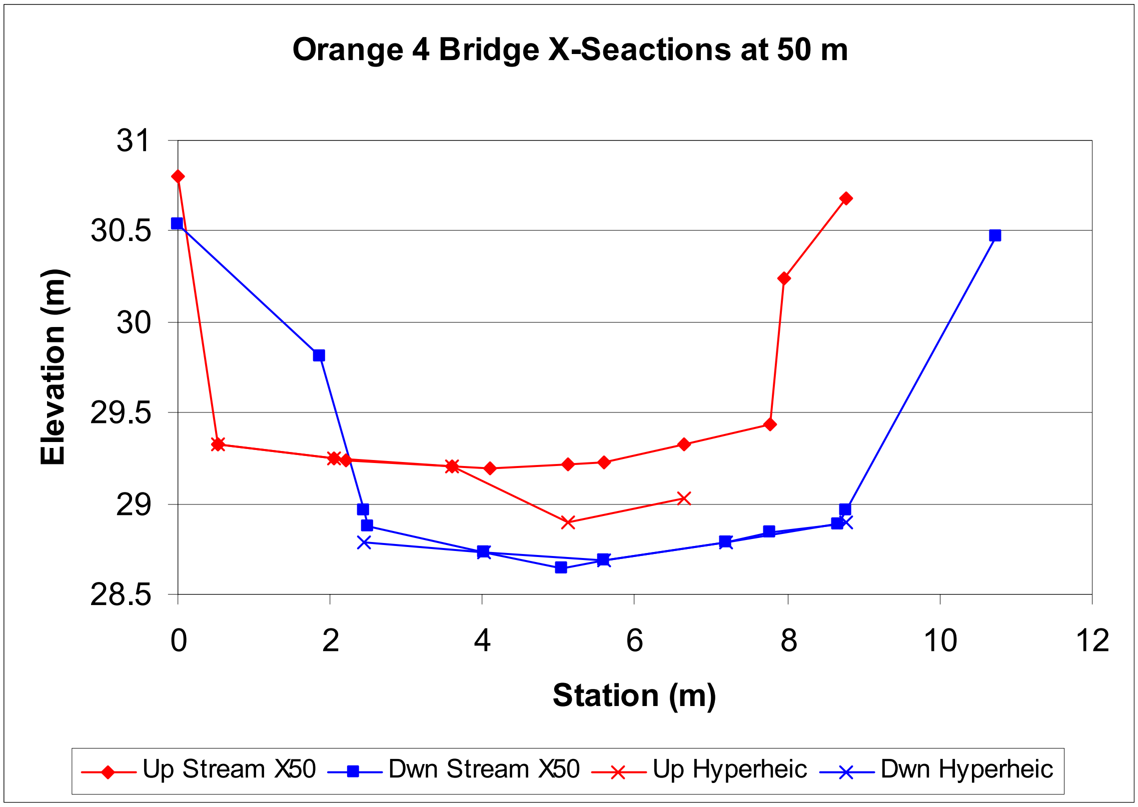
Orange 4 Cross Section and Longitudinal Profile

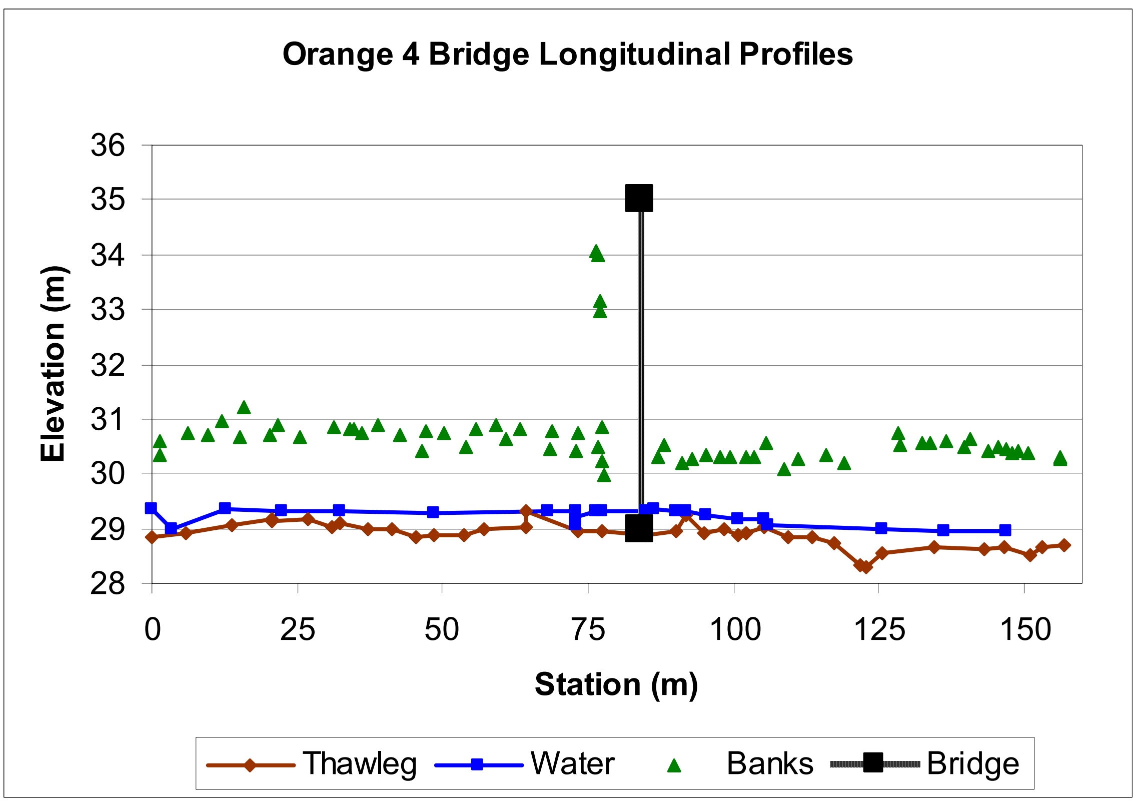




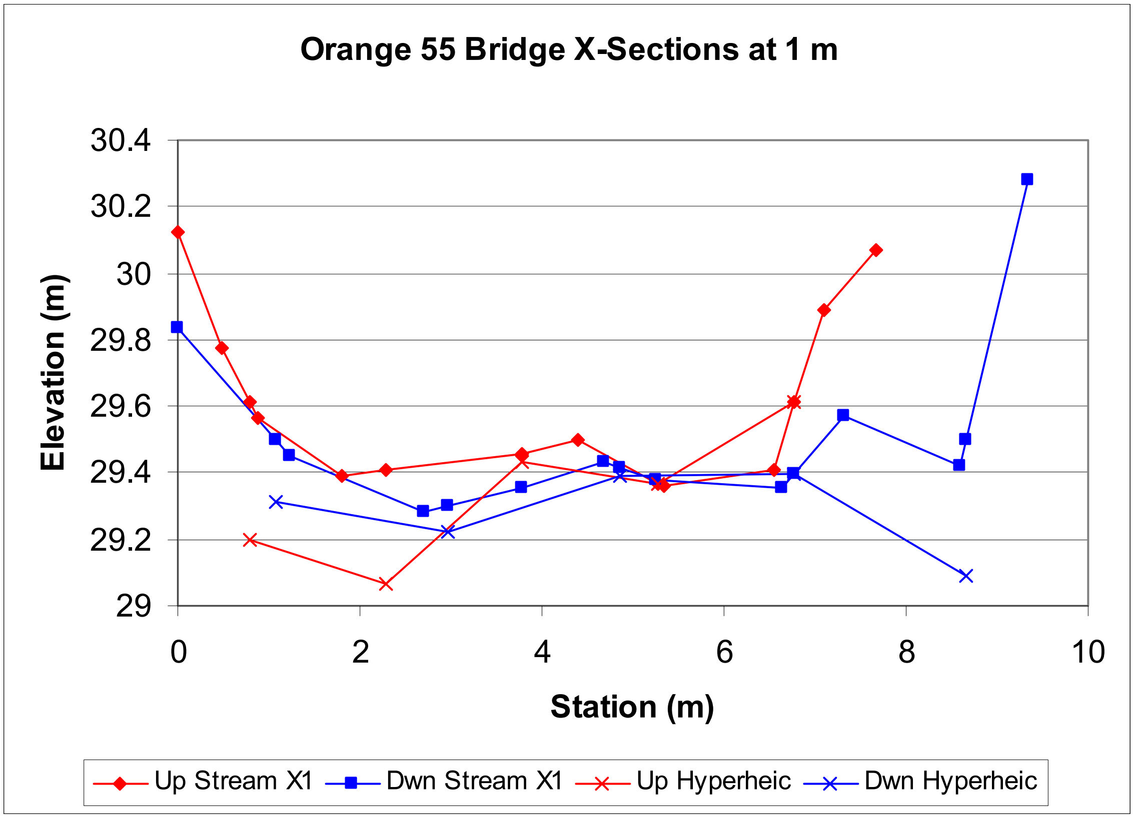

Orange 4 Bridge X-Sections at 10 m
Elevation (m)
31
30.5
30
29.5
29
28.5
28
0
2
4
6
8
10
12
Station (m)
Up Stream X10
Dwn Stream X10
Up Hyperheic
Dwn Hyperheic

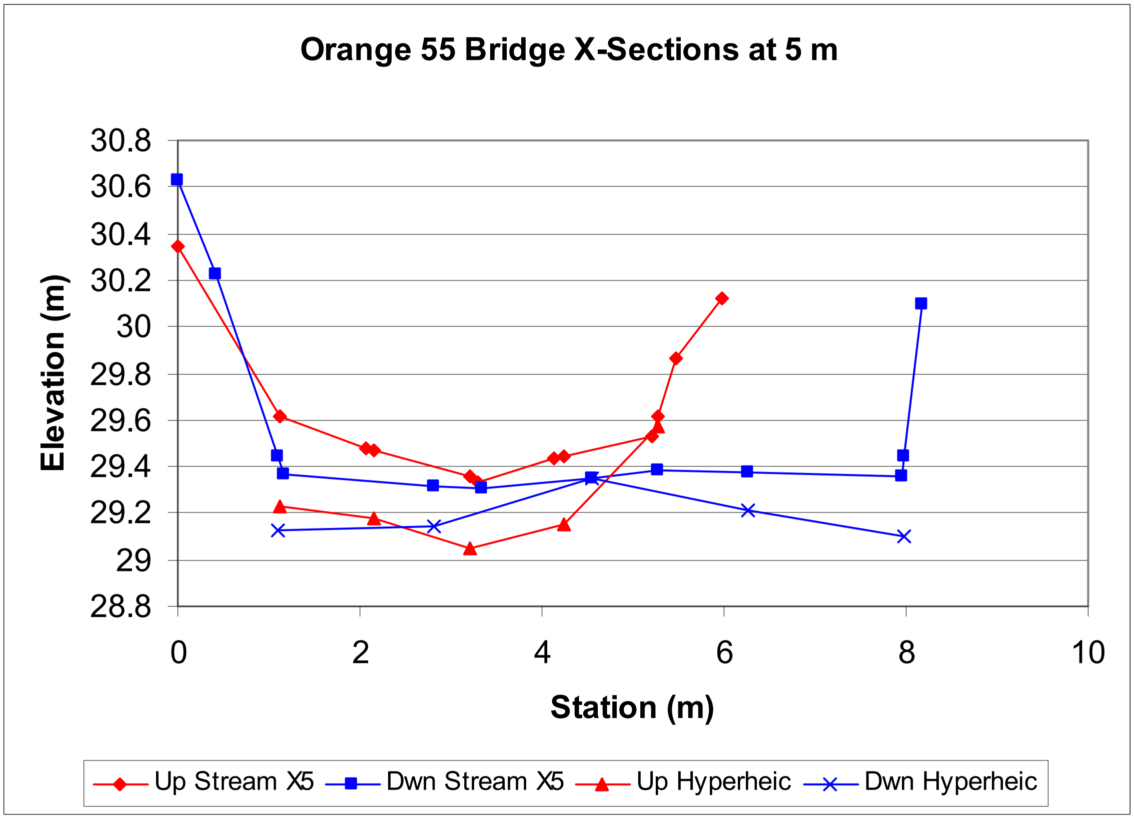

Orange 4 Bridge X-Sections at 20 m
Elevation (m)
31.5
31
30.5
30
29.5
29
28.5
0
2
4
6
8
10
12
Station (m)
Up Stream X20
Dwn Stream X20
Up Hyperheic
Dwn Hyperheic

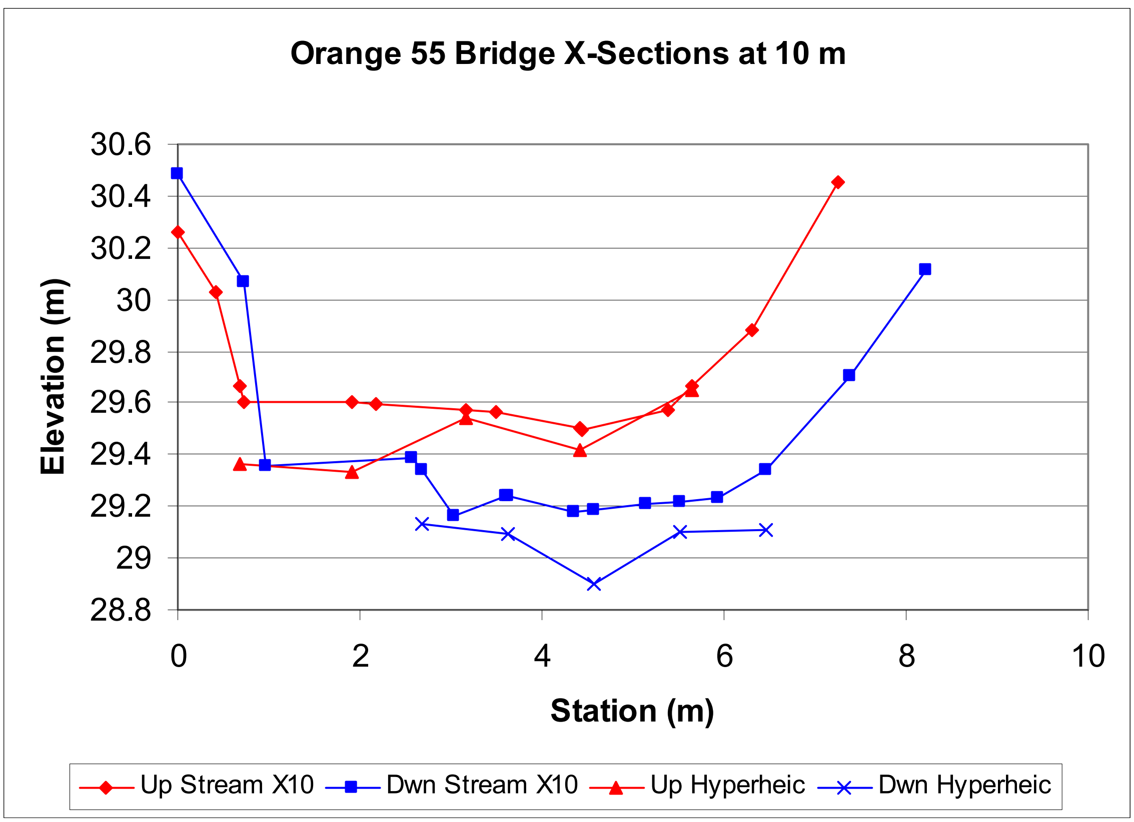

Orange 4 Bridge X-Seactions at 50 m
31
30.5
30
29.5
29
28.5
0
2
4
6
8
10
12
Elevation (m)
Station (m)
Up Stream X50
Dwn Stream X50
Up Hyperheic
Dwn Hyperheic

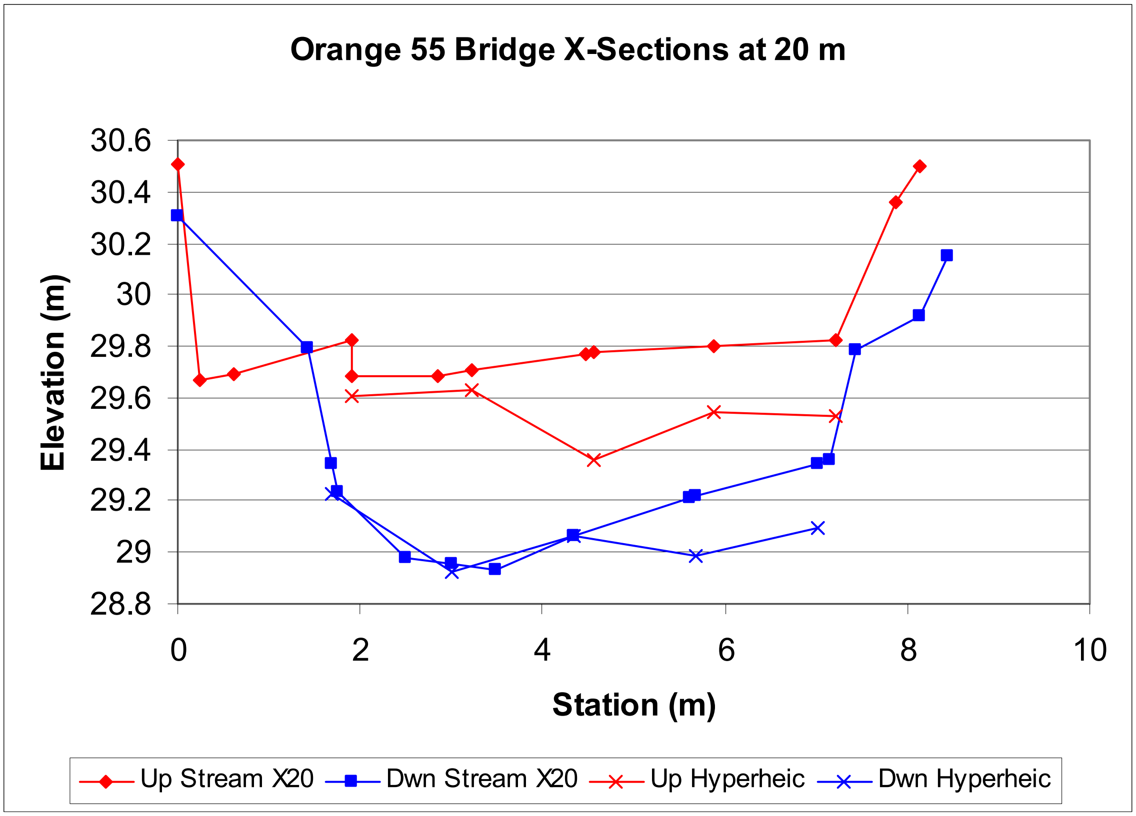

Orange 4 Bridge Longitudinal Profiles
36
35
34
33
32
31
30
29
28
0
25
50
75
100
125
150
Elevation (m)
Station (m)
Thawleg
Water
Banks
Bridge

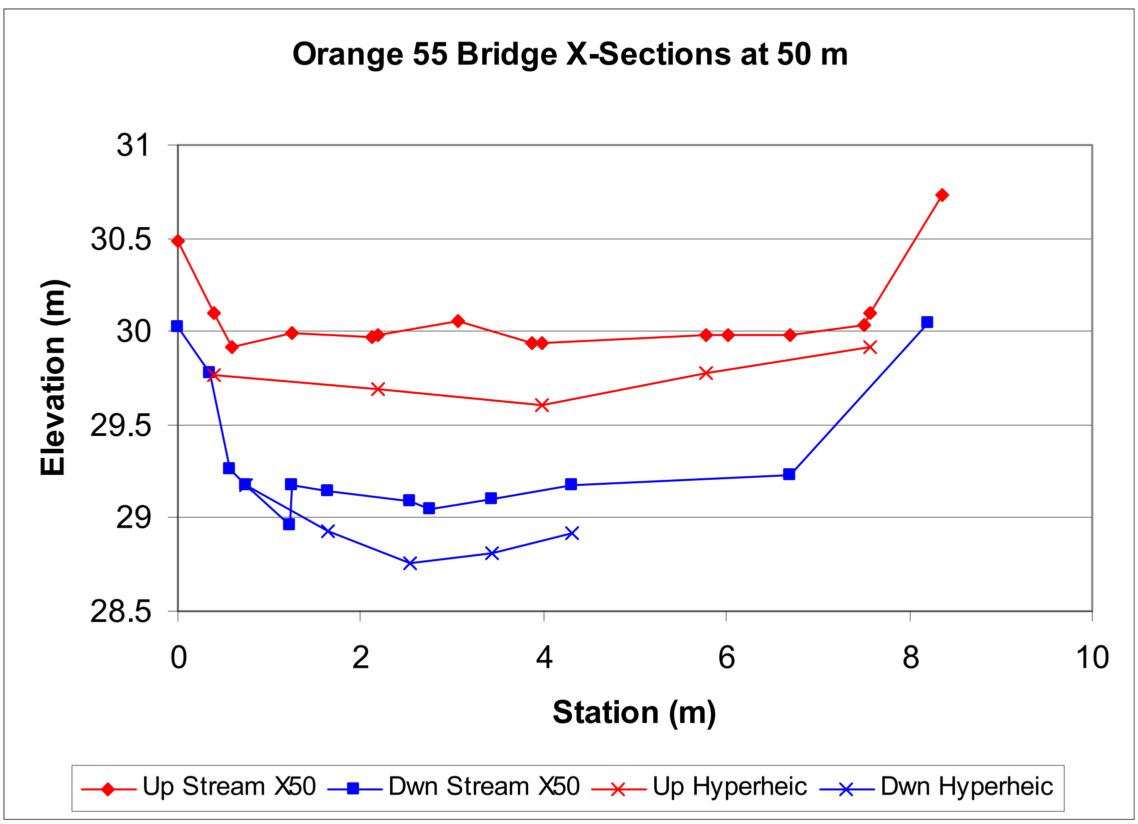
<u>**Orange 55 Cross Section and Longitudinal Profile**</u>

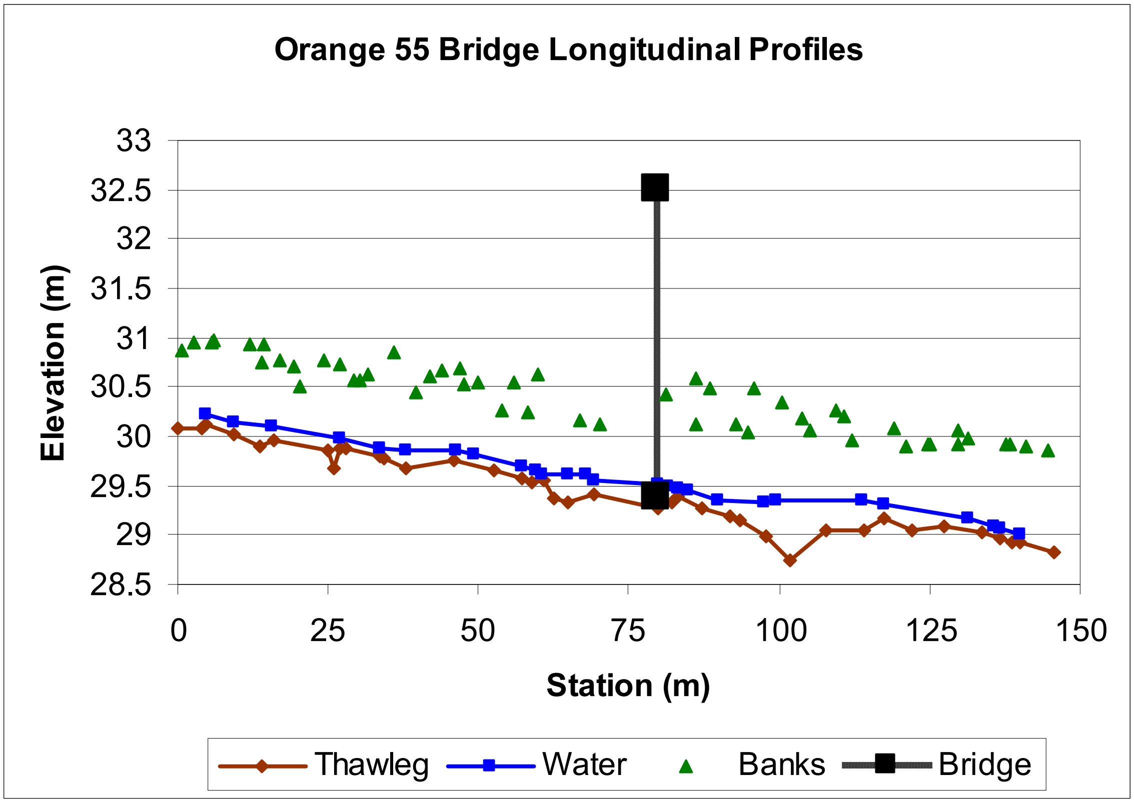



Orange 55 Bridge X-Sections at 5 m
30.8
30.6
30.4
30.2
30
29.8
29.6
29.4
29.2
29
28.8
Elevation (m)
0
2
4
6
8
10
Station (m)
Up Stream X5
Dwn Stream X5
Up Hyperheic
Dwn Hyperheic


Orange 55 Bridge X-Sections at 10 m
30.6
30.4
30.2
30
29.8
29.6
29.4
29.2
29
28.8
Elevation (m)
0
2
4
6
8
10
Station (m)
Up Stream X10
Dwn Stream X10
Up Hyperheic
Dwn Hyperheic

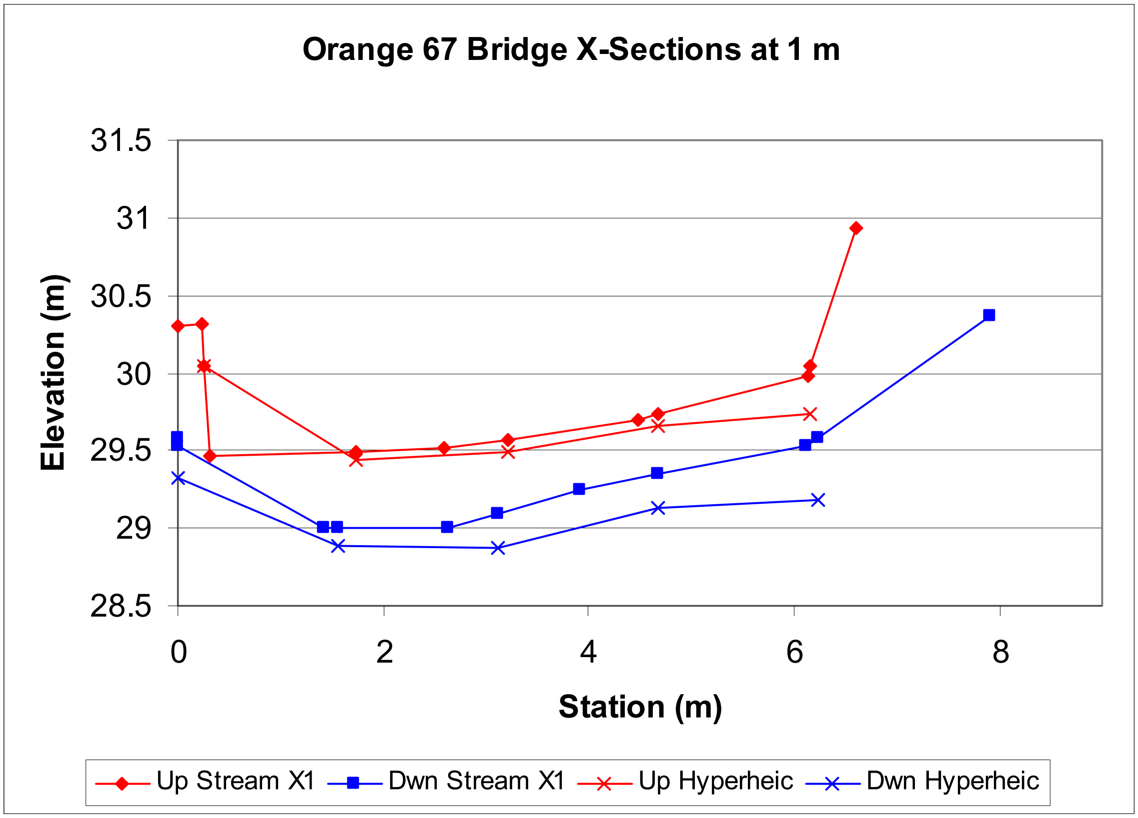

Orange 55 Bridge X-Sections at 20 m
Elevation (m)
30.6
30.4
30.2
30
29.8
29.6
29.4
29.2
29
28.8
0
2
4
6
8
10
Station (m)
Up Stream X20
Dwn Stream X20
Up Hyperheic
Dwn Hyperheic

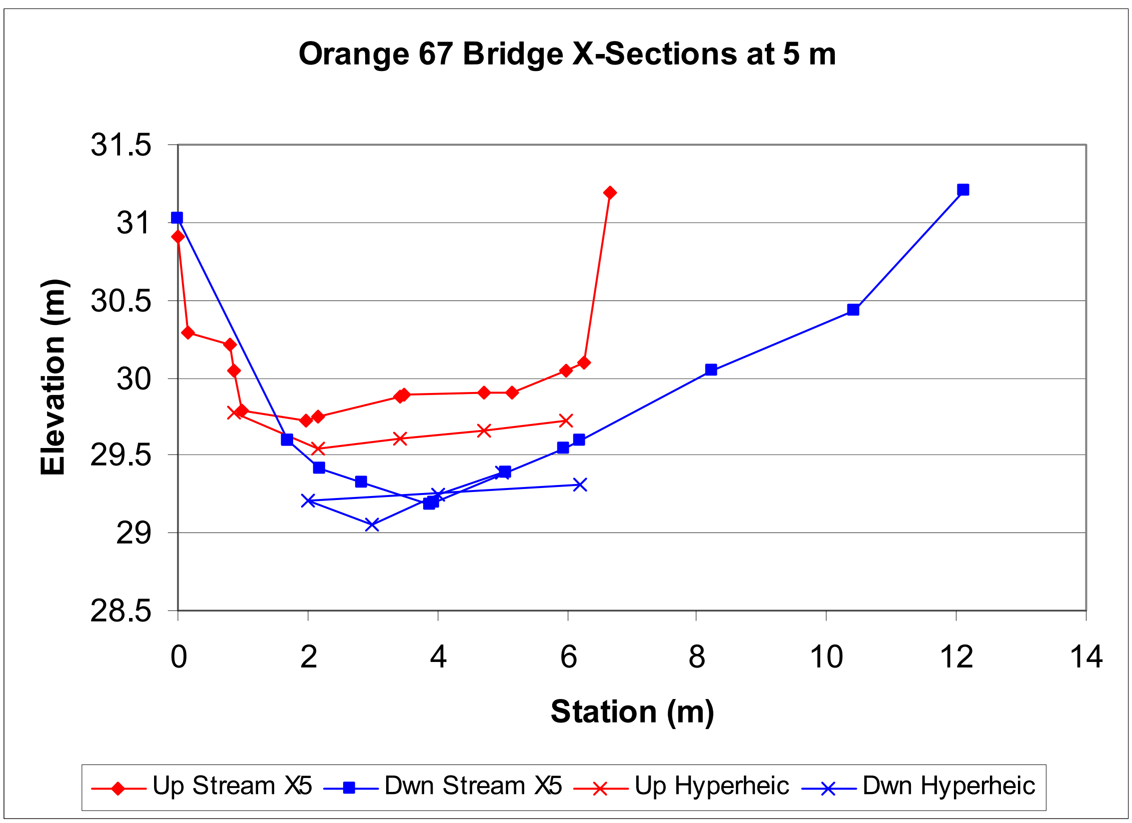

Orange 55 Bridge X-Sections at 50 m
Elevation (m)
31
30.5
30
29.5
29
28.5
0
2
4
6
8
10
Station (m)
Up Stream X50
Dwn Stream X50
Up Hyperheic
Dwn Hyperheic

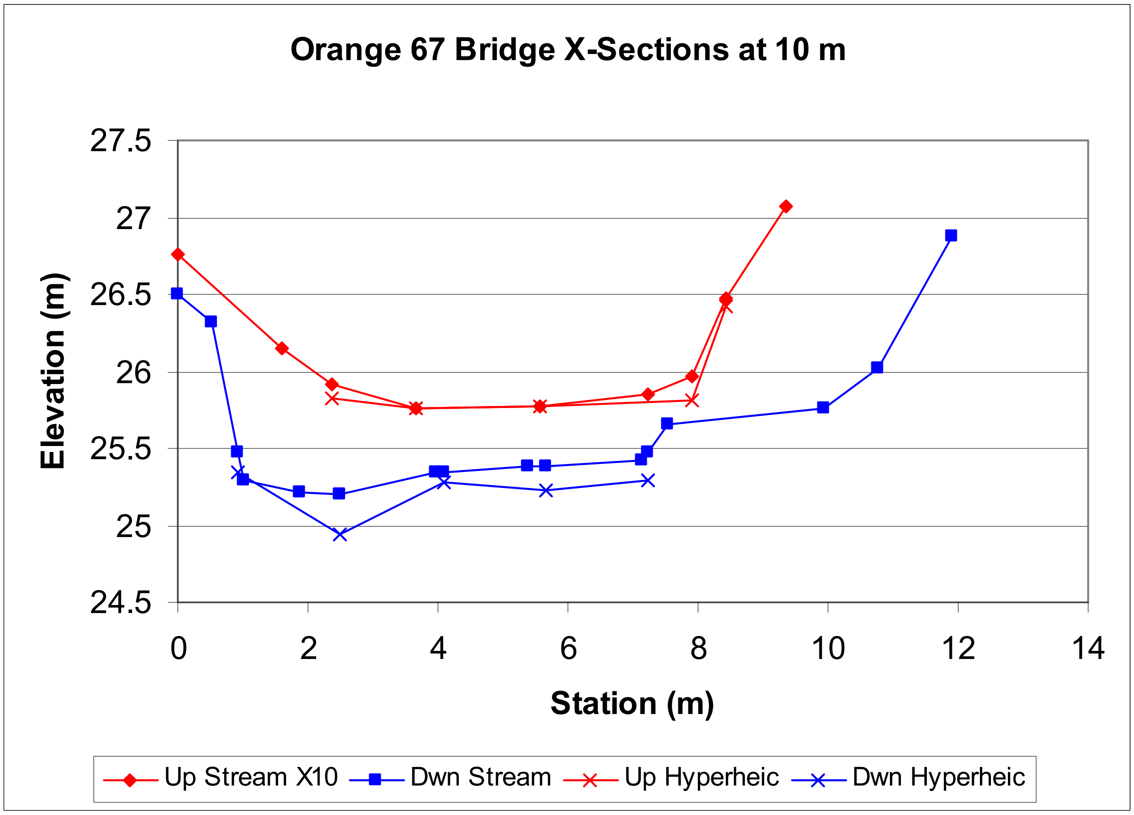


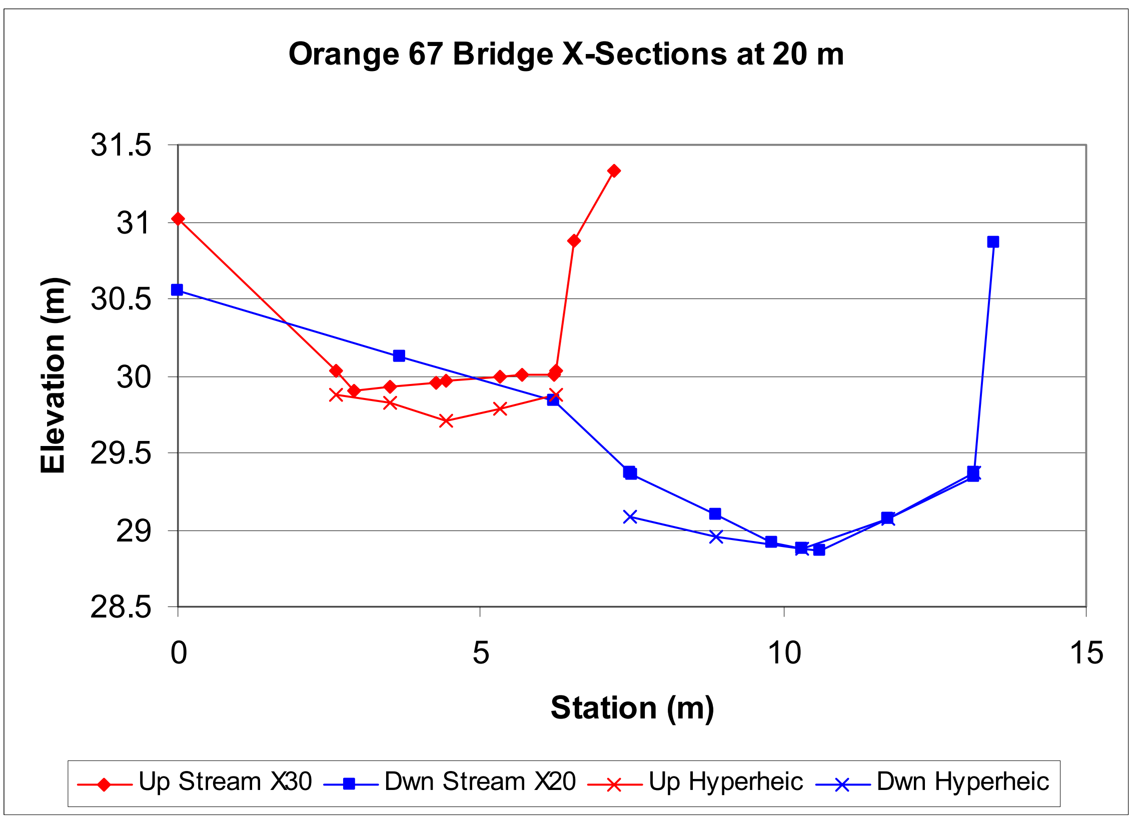
Orange 55 Bridge Longitudinal Profiles
Elevation (m)
33
32.5
32
31.5
31
30.5
30
29.5
29
28.5
0
25
50
75
100
125
150
Station (m)
Thawleg
Water
Banks
Bridge

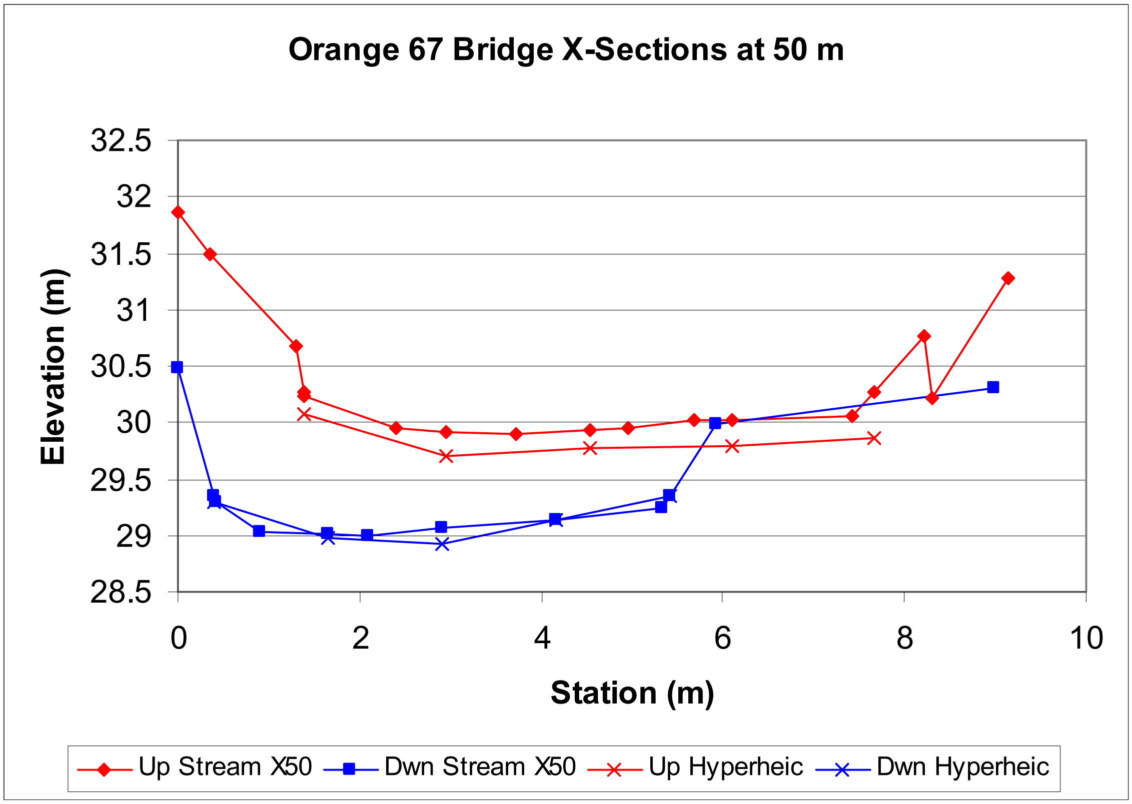
Orange 67 Cross Section and Longitudinal Profile

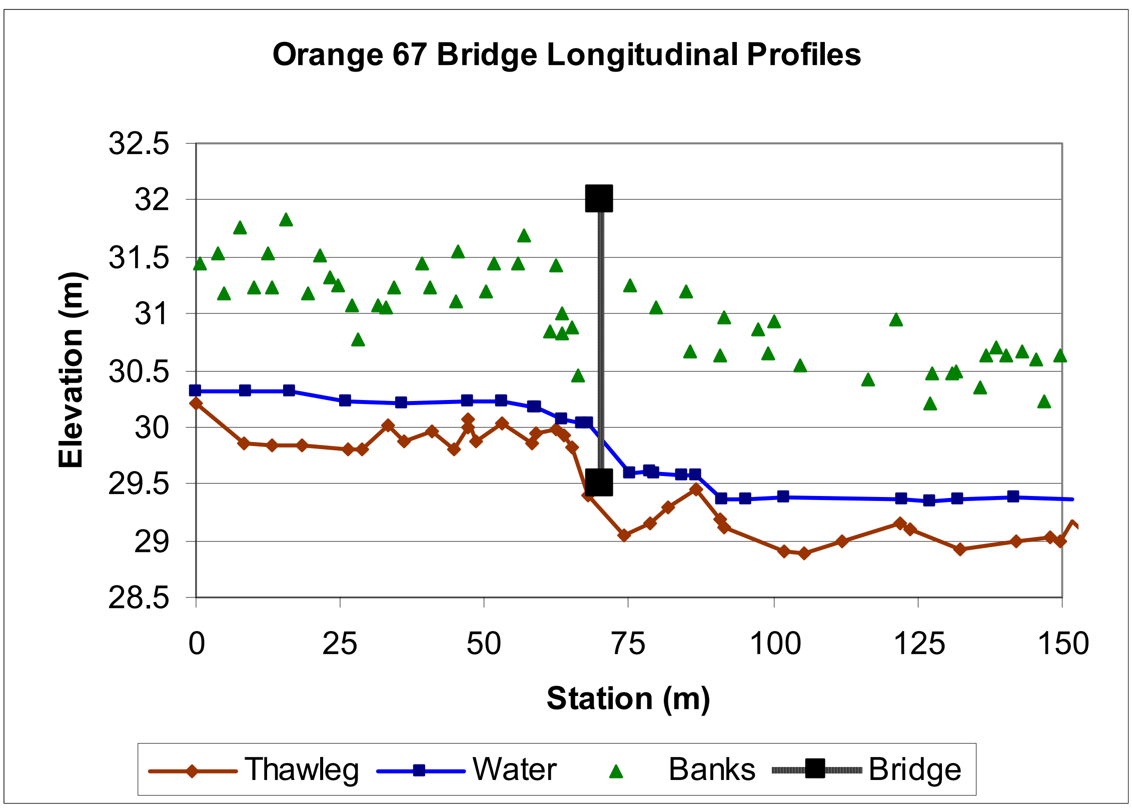




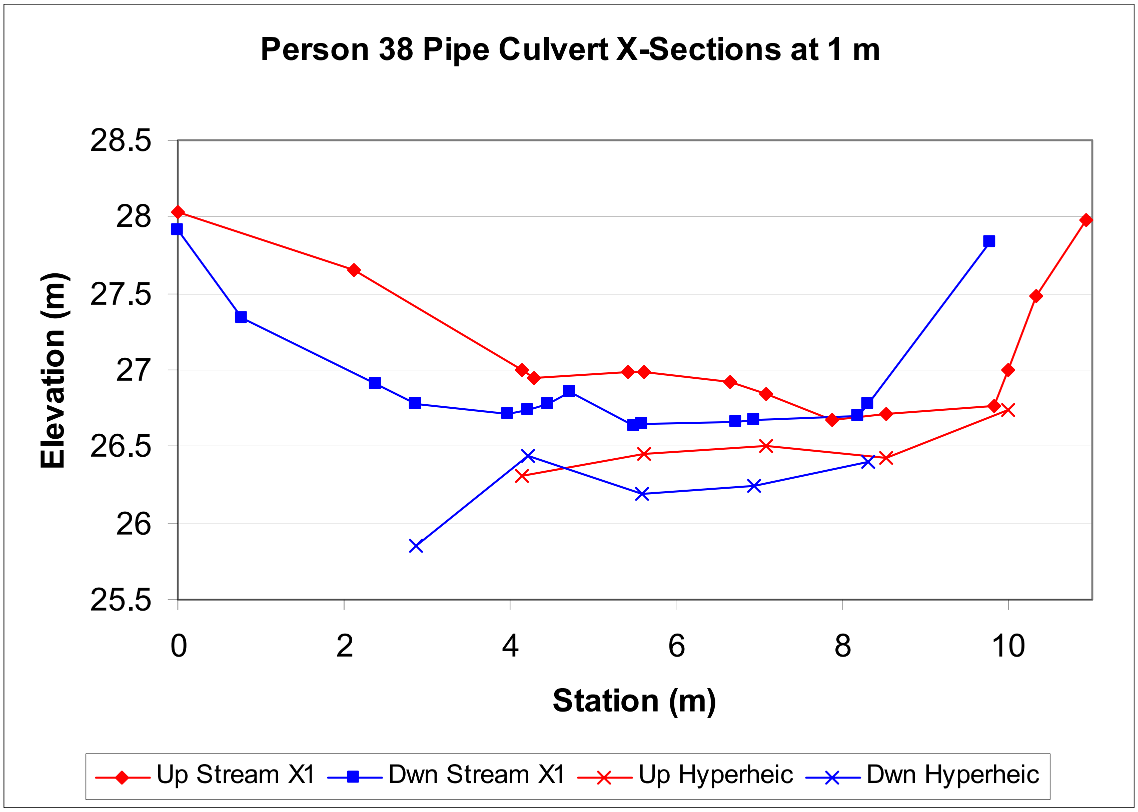

Orange 67 Bridge X-Sections at 10 m
Elevation (m)
27.5
27
26.5
26
25.5
25
24.5
0
2
4
6
8
10
12
14
Station (m)
Up Stream X10
Dwn Stream
Up Hyperheic
Dwn Hyperheic

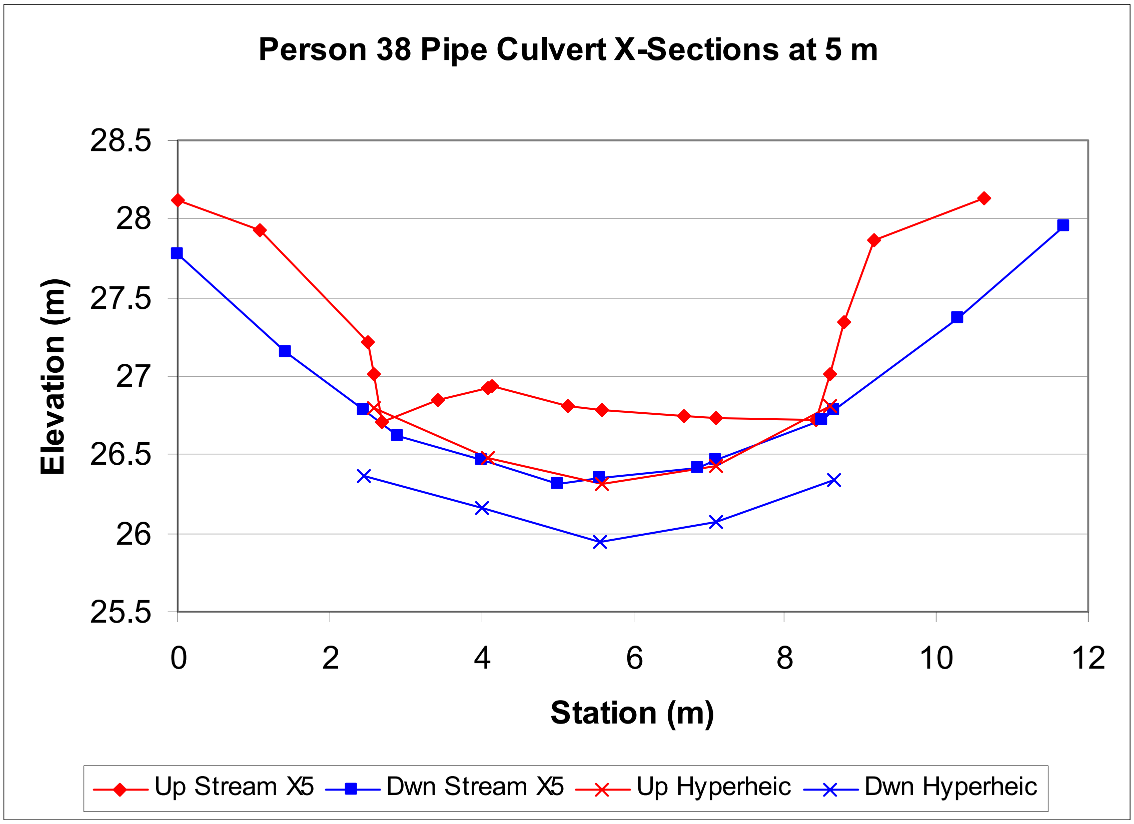

Orange 67 Bridge X-Sections at 20 m
Elevation (m)
31.5
31
30.5
30
29.5
29
28.5
0
5
10
15
Station (m)
Up Stream X30
Dwn Stream X20
Up Hyperheic
Dwn Hyperheic


Orange 67 Bridge X-Sections at 50 m
Elevation (m)
Station (m)
32.5
32
31.5
31
30.5
30
29.5
29
28.5
0
2
4
6
8
10
Up Stream X50
Dwn Stream X50
Up Hyperheic
Dwn Hyperheic

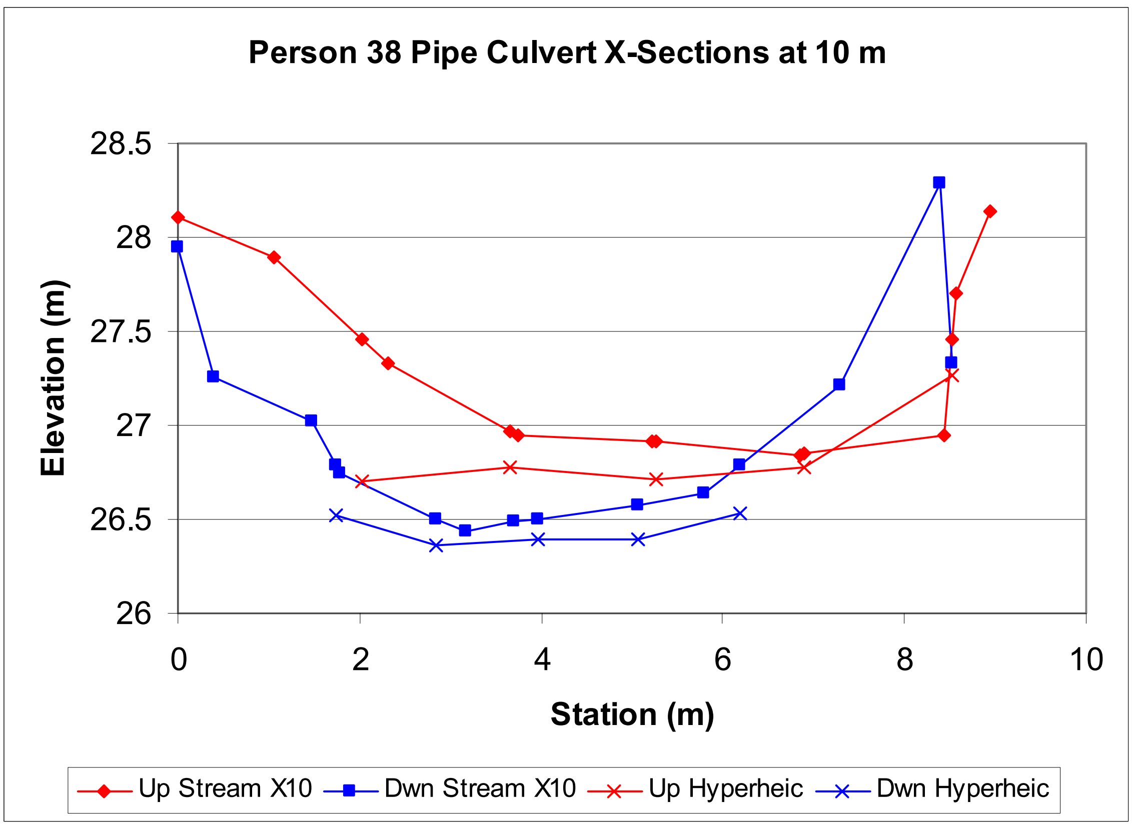

Orange 67 Bridge Longitudinal Profiles
Elevation (m)
Station (m)
32.5
32
31.5
31
30.5
30
29.5
29
28.5
0
25
50
75
100
125
150
Thawleg
Water
Banks
Bridge

Person 38 Cross Sections and Longitudinal Profile

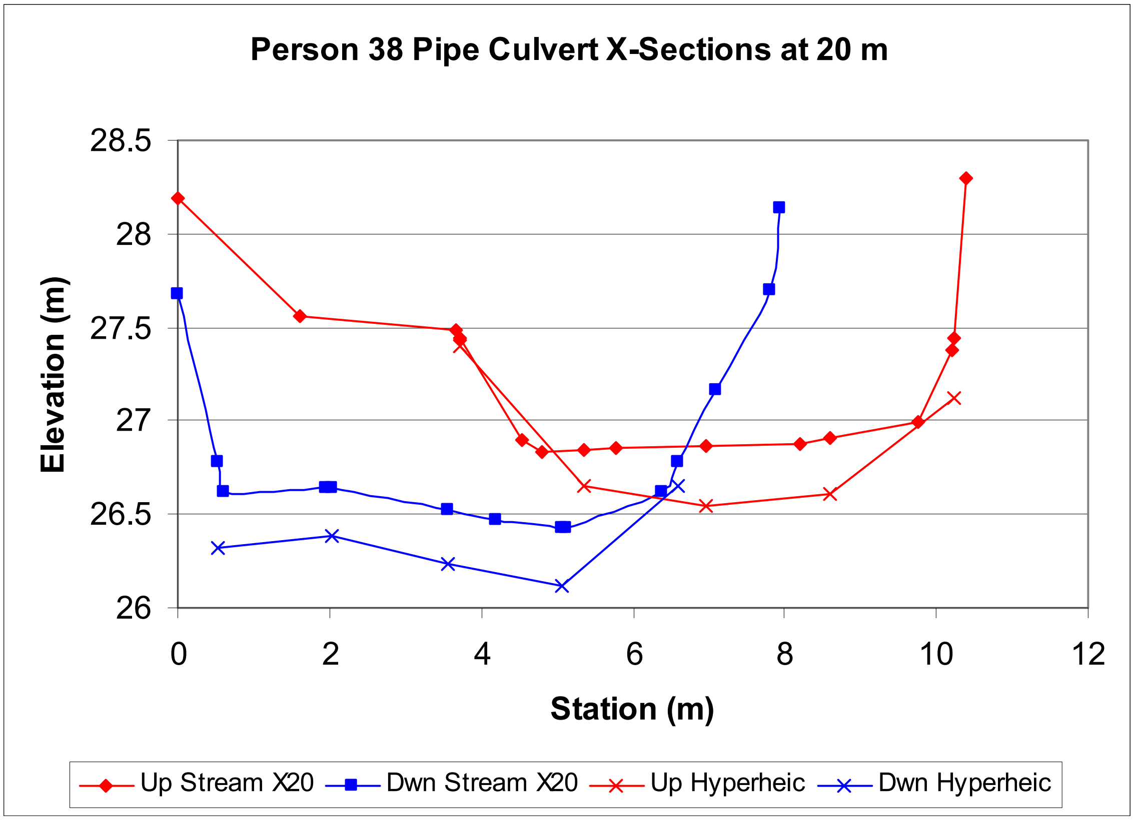


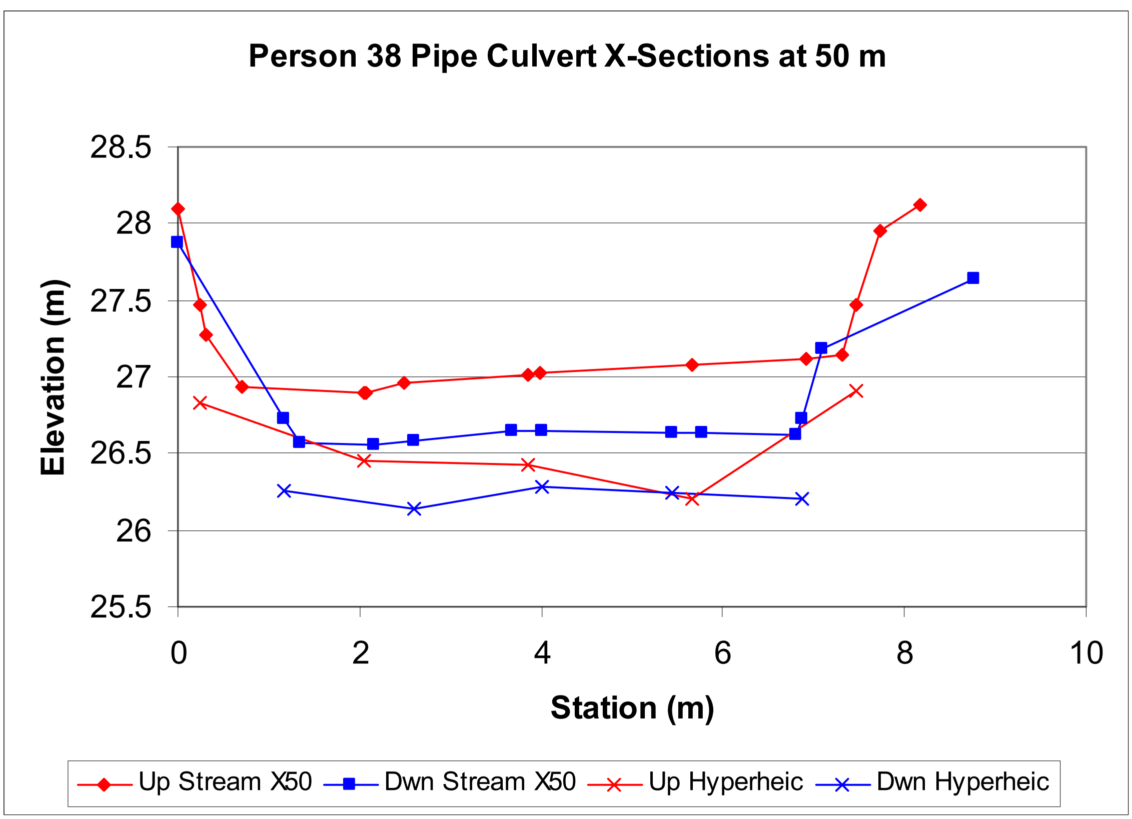


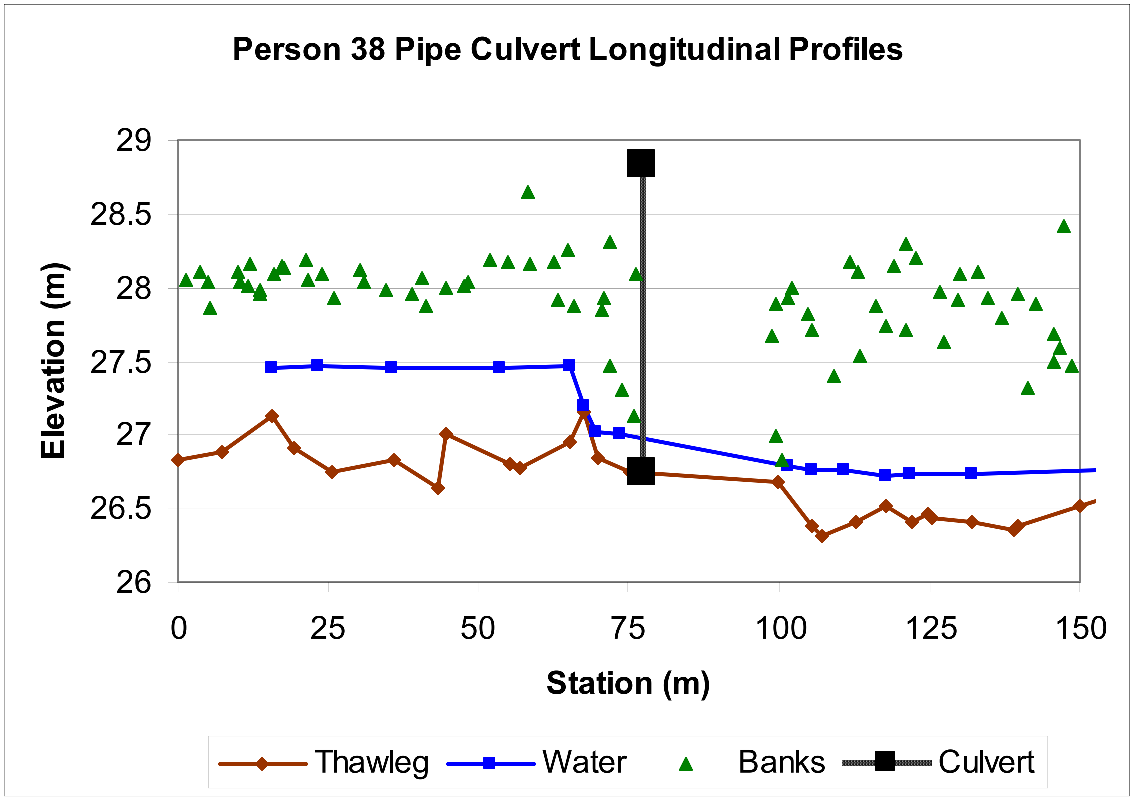
Person 38 Pipe Culvert X-Sections at 5 m
Elevation (m)
Station (m)
Up Stream X5
Dwn Stream X5
Up Hyperheic
Dwn Hyperheic



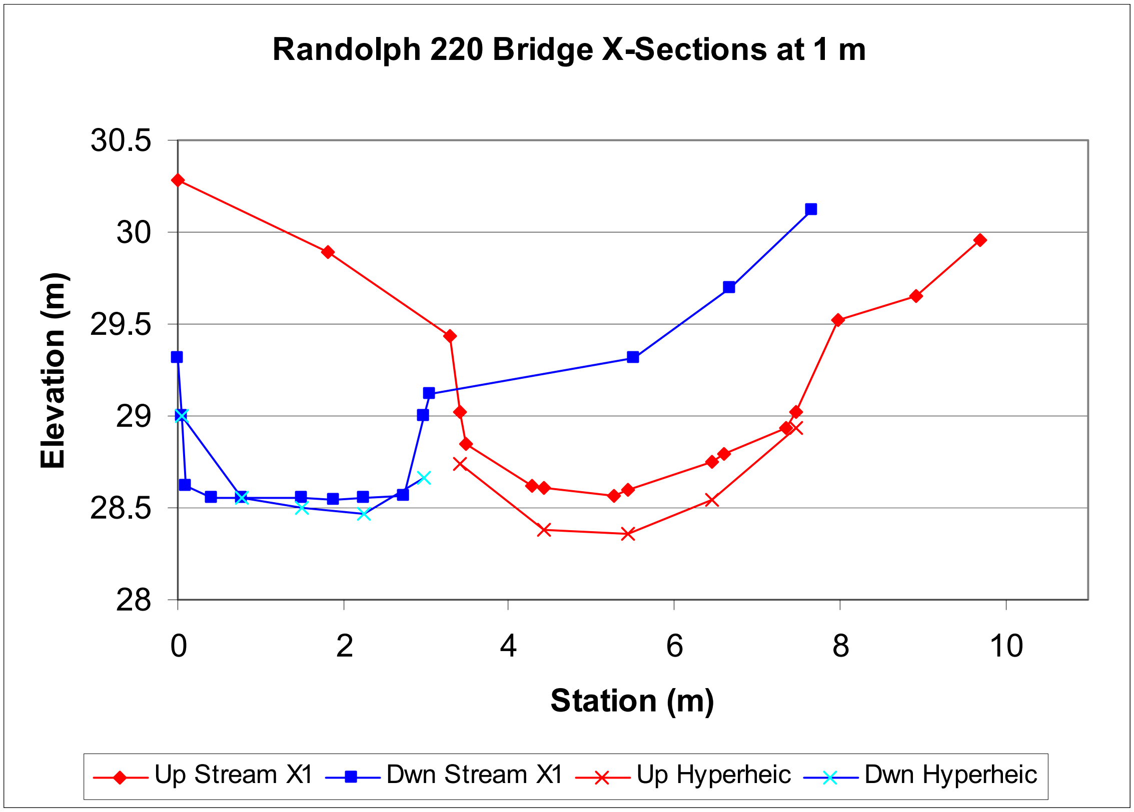
Person 38 Pipe Culvert X-Sections at 10 m
Elevation (m)
Station (m)
Up Stream X10
Dwn Stream X10
Up Hyperheic
Dwn Hyperheic

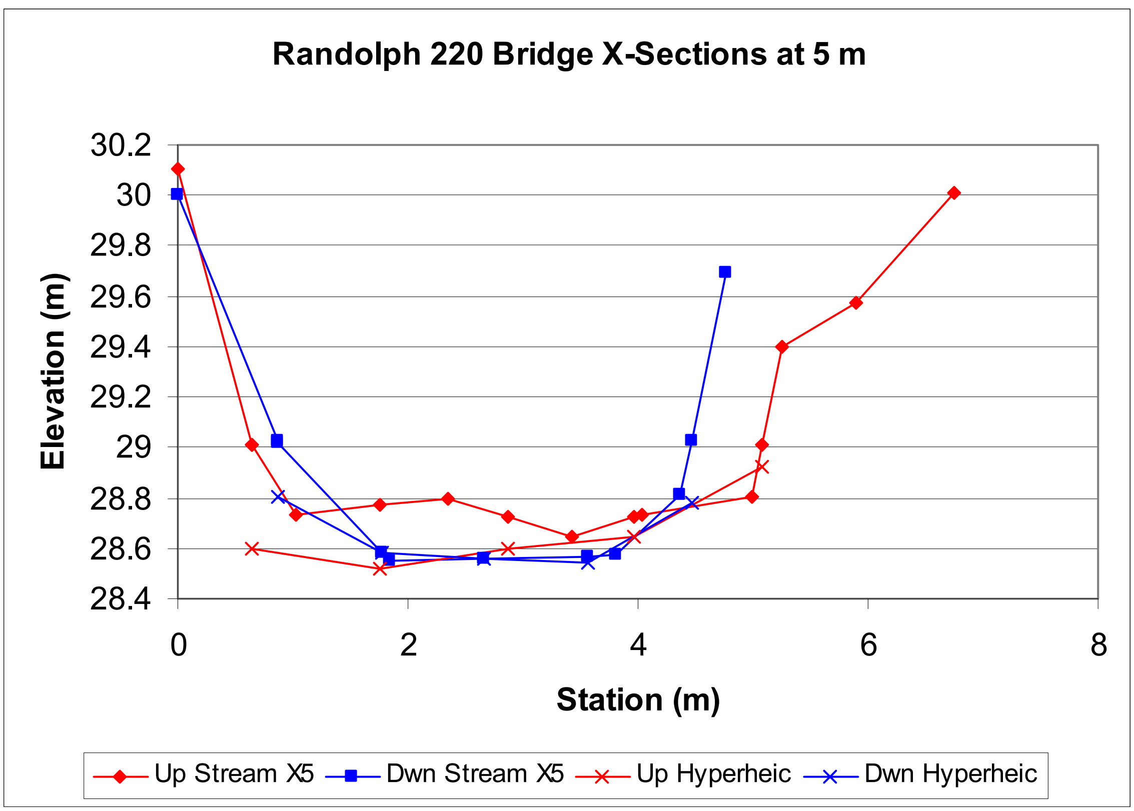

Person 38 Pipe Culvert X-Sections at 20 m
28.5
28
27.5
27
26.5
26
0
2
4
6
8
10
12
Elevation (m)
Station (m)
Up Stream X20
Dwn Stream X20
Up Hyperheic
Dwn Hyperheic

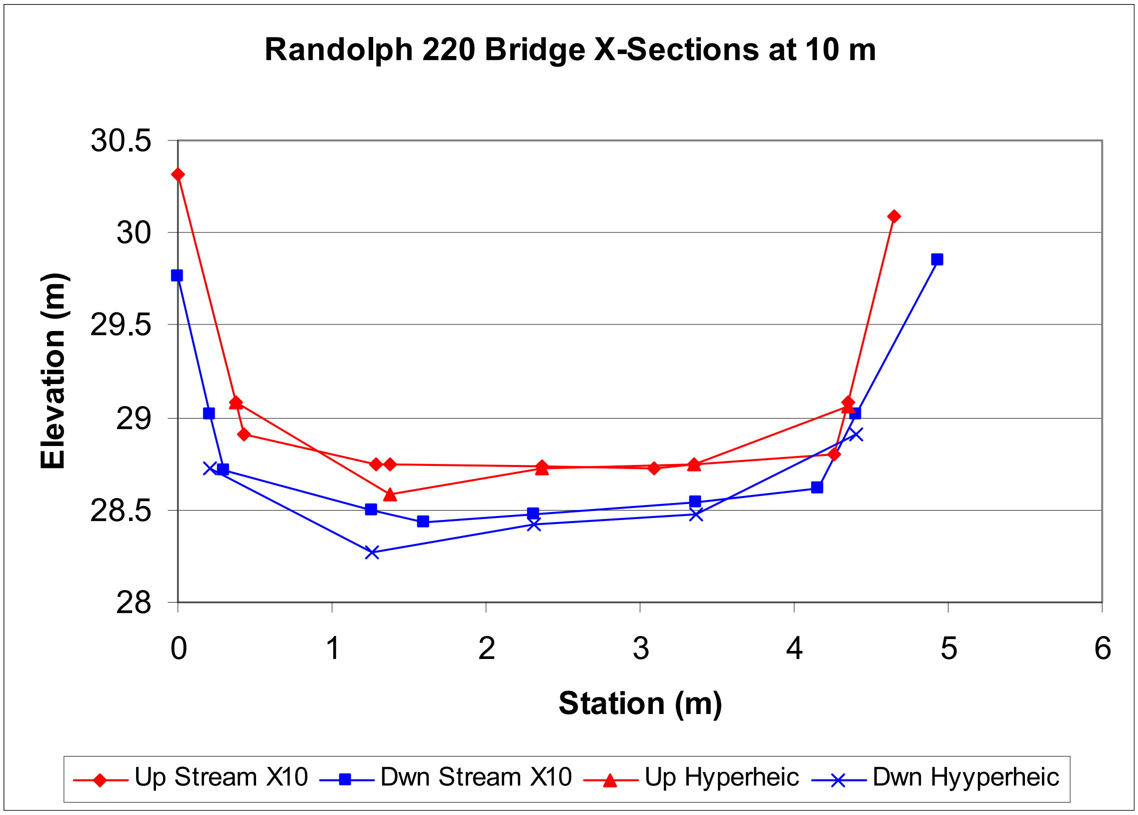

Person 38 Pipe Culvert X-Sections at 50 m
28.5
28
27.5
27
26.5
26
25.5
0
2
4
6
8
10
Elevation (m)
Station (m)
Up Stream X50
Dwn Stream X50
Up Hyperheic
Dwn Hyperheic

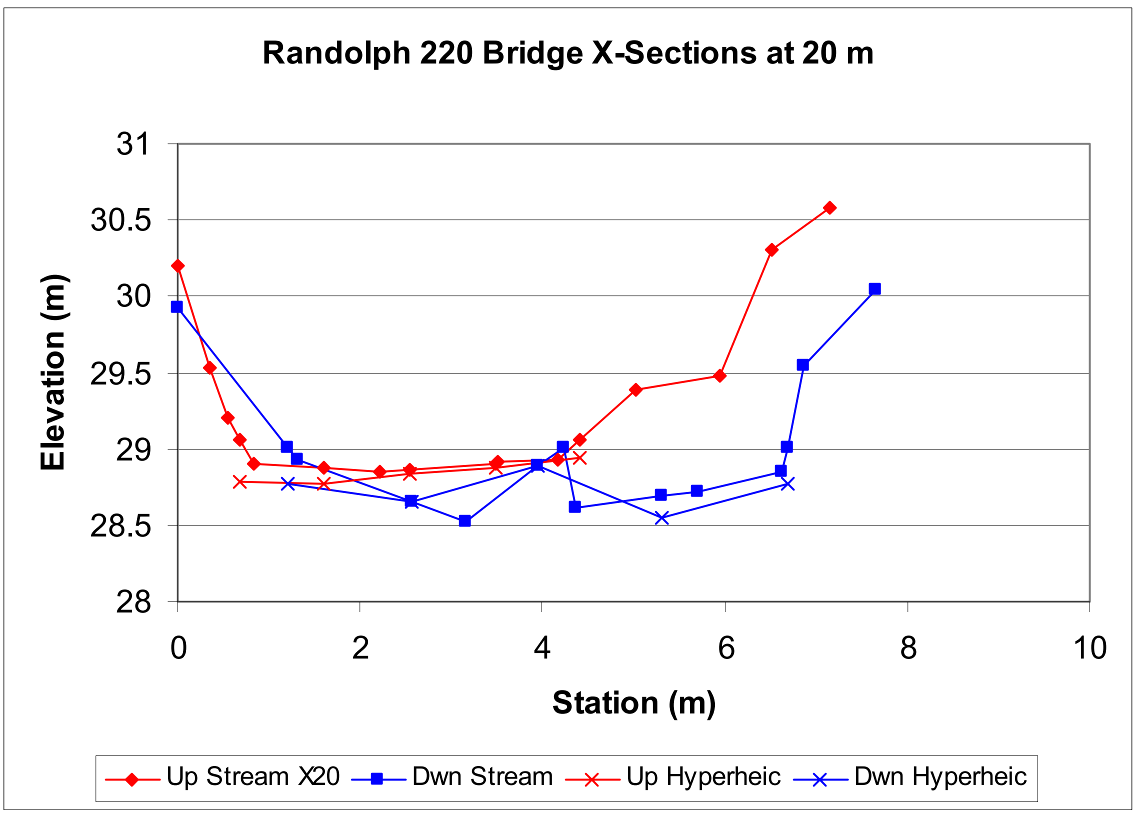

Person 38 Pipe Culvert Longitudinal Profiles
Elevation (m)
29
28.5
28
27.5
27
26.5
26
0
25
50
75
100
125
150
Station (m)
Thawleg
Water
Banks
Culvert

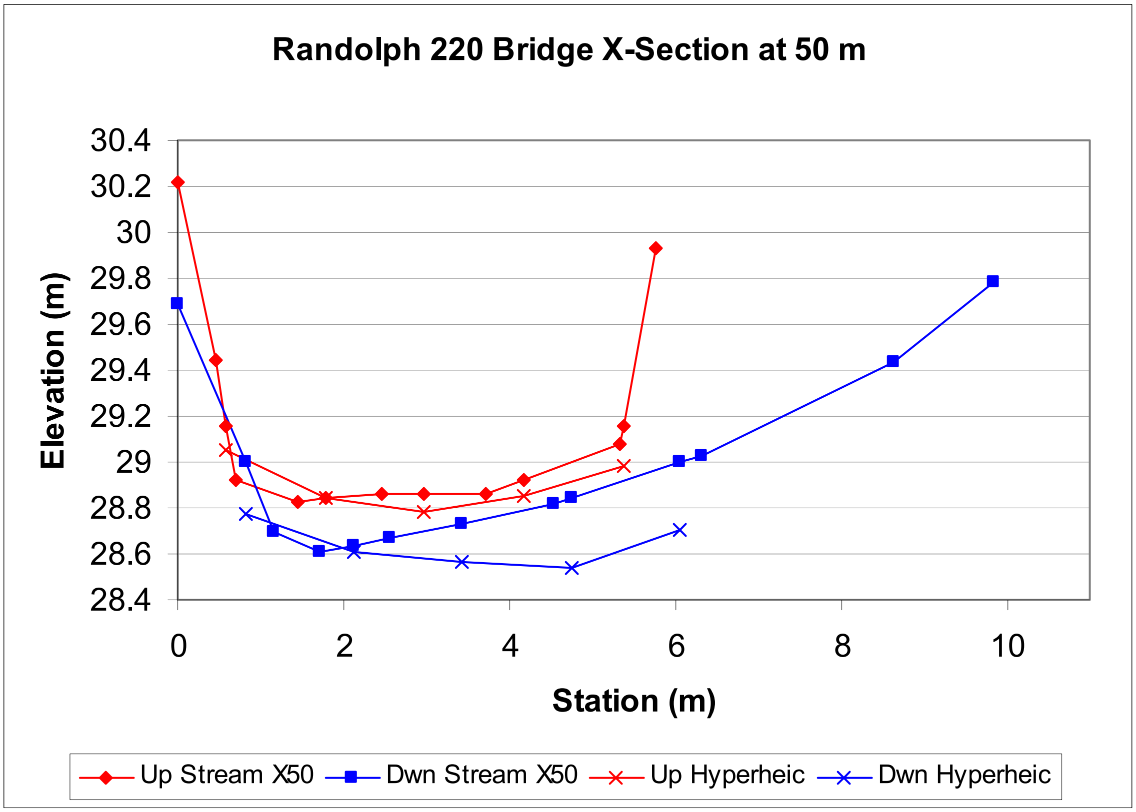
Randolph 220 Cross Section and Longitudinal Profile

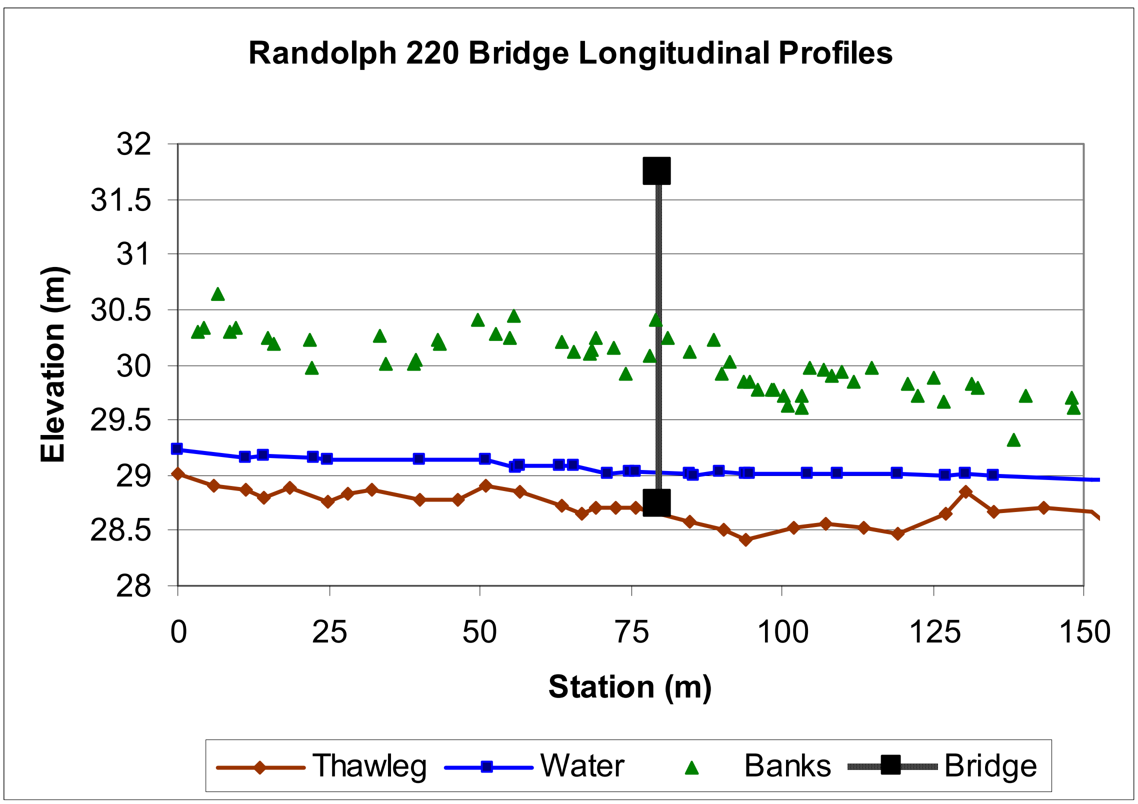




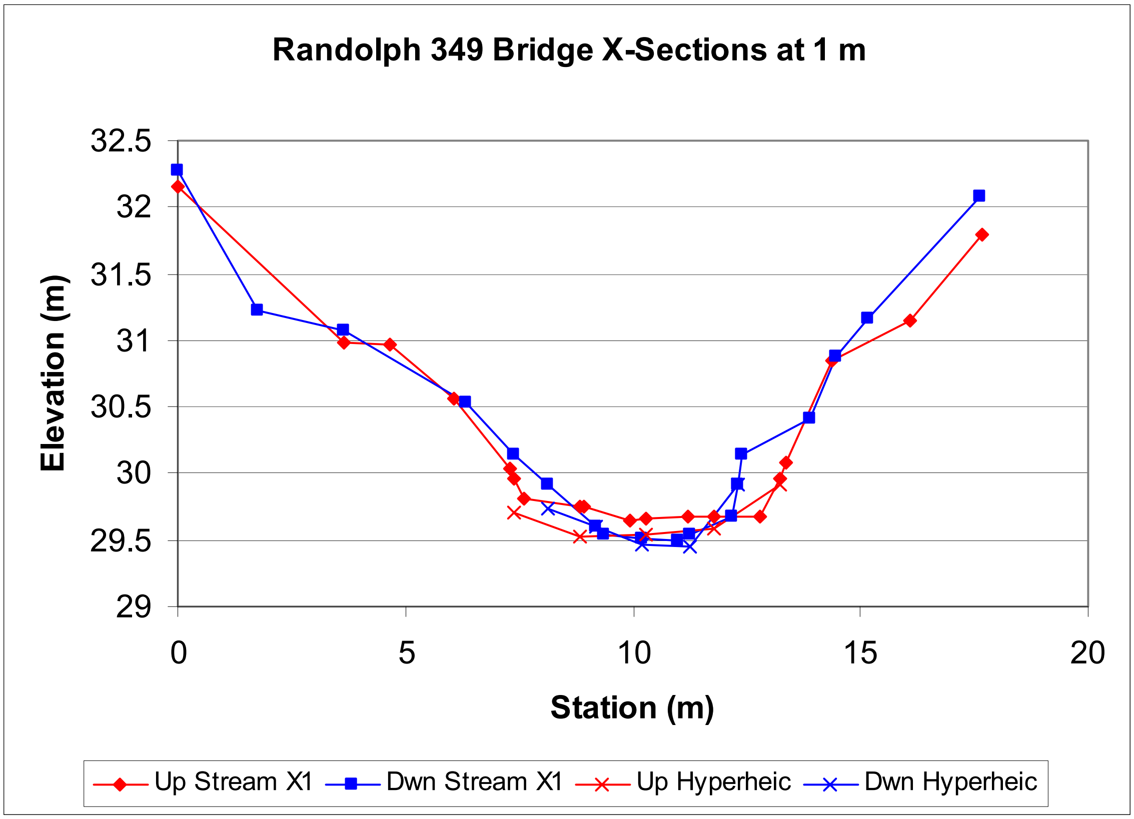

Randolph 220 Bridge X-Sections at 10 m
Elevation (m)
30.5
30
29.5
29
28.5
28
0
1
2
3
4
5
6
Station (m)
Up Stream X10
Dwn Stream X10
Up Hyperheic
Dwn Hyyperheic

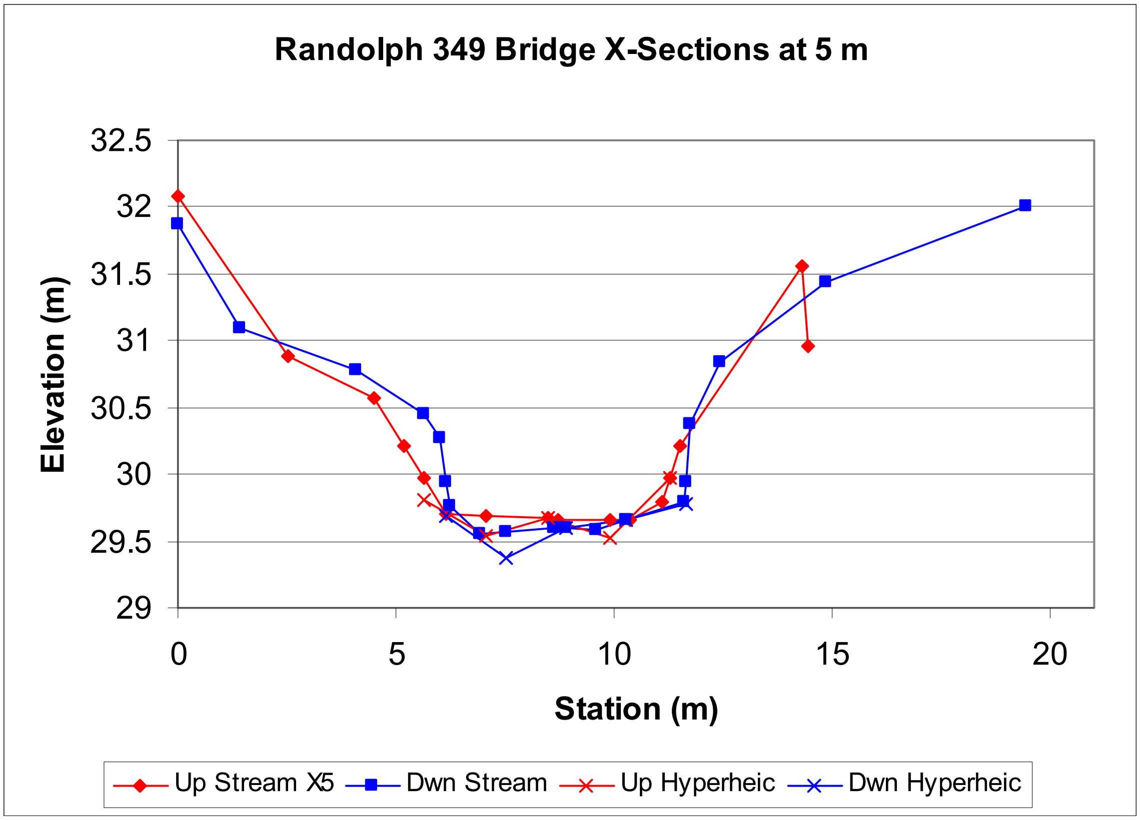

Randolph 220 Bridge X-Sections at 20 m
Elevation (m)
31
30.5
30
29.5
29
28.5
28
0
2
4
6
8
10
Station (m)
Up Stream X20
Dwn Stream
Up Hyperheic
Dwn Hyperheic

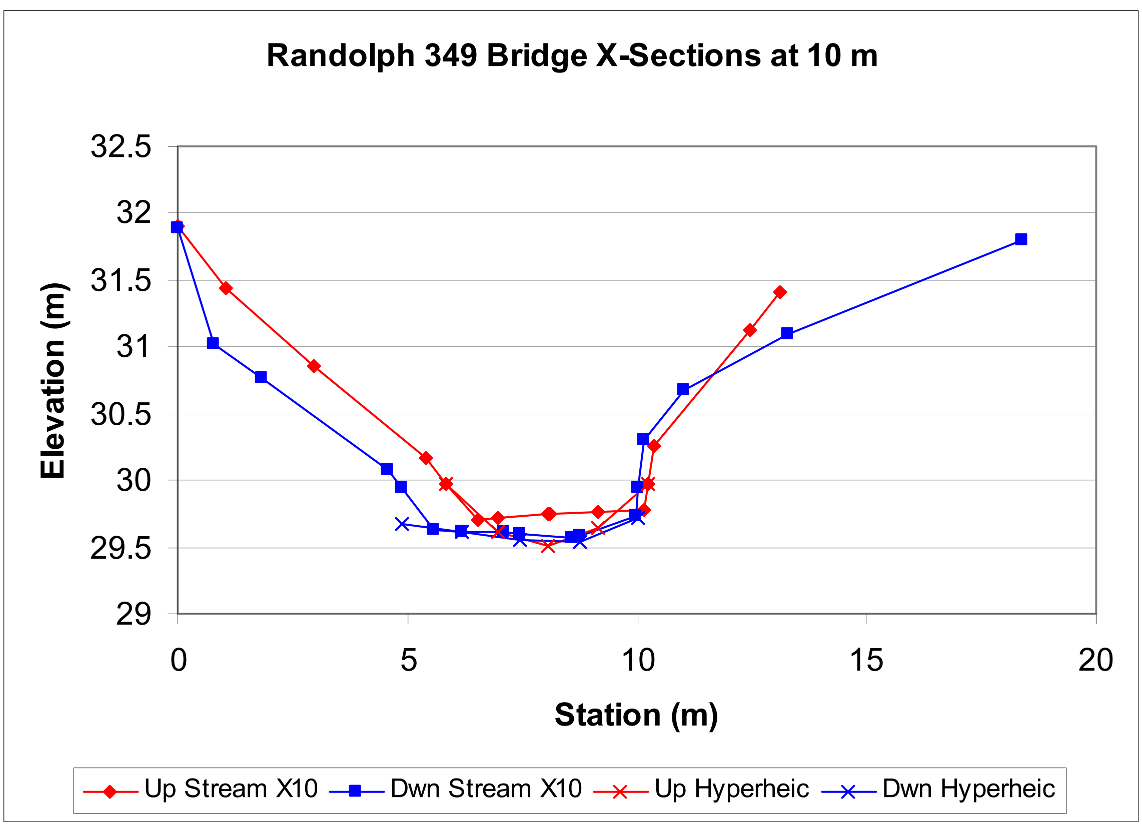

Randolph 220 Bridge X-Section at 50 m
30.4
30.2
30
29.8
29.6
29.4
29.2
29
28.8
28.6
28.4
Elevation (m)
0
2
4
6
8
10
Station (m)
Up Stream X50
Dwn Stream X50
Up Hyperheic
Dwn Hyperheic

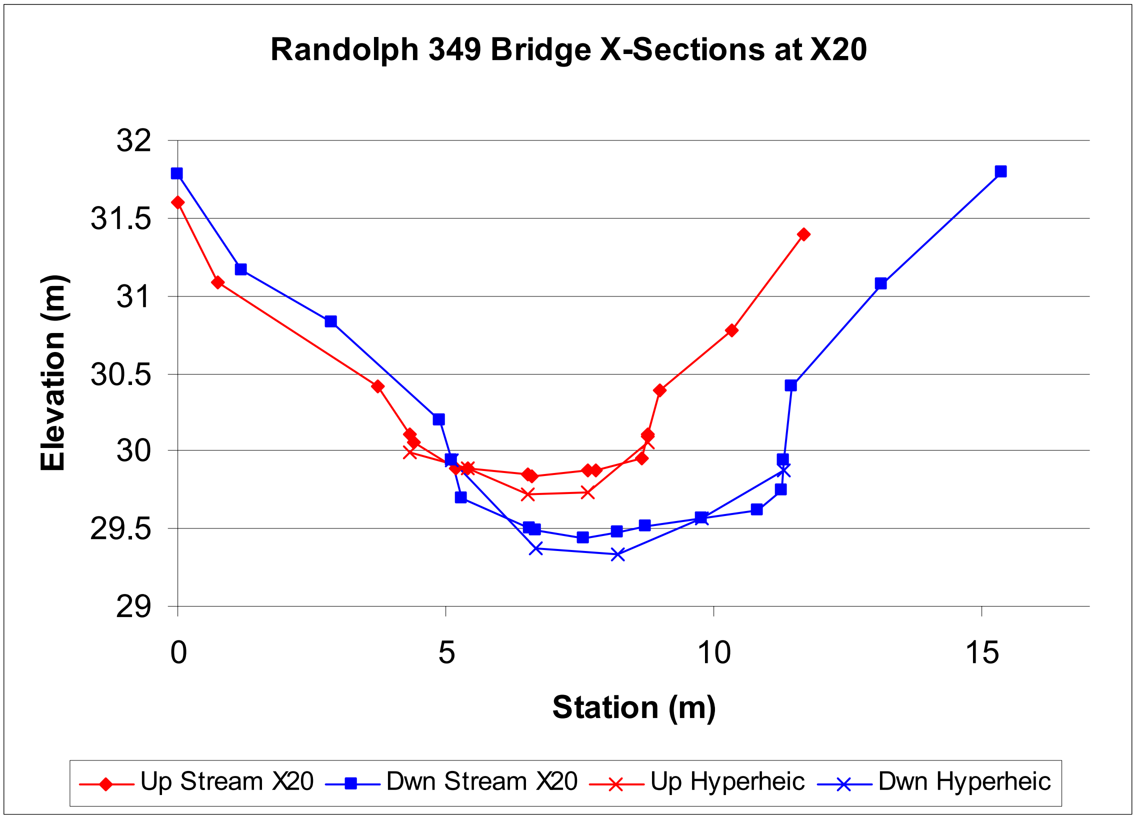

Randolph 220 Bridge Longitudinal Profiles
32
31.5
31
30.5
30
29.5
29
28.5
28
Elevation (m)
0
25
50
75
100
125
150
Station (m)
Thawleg
Water
Banks
Bridge

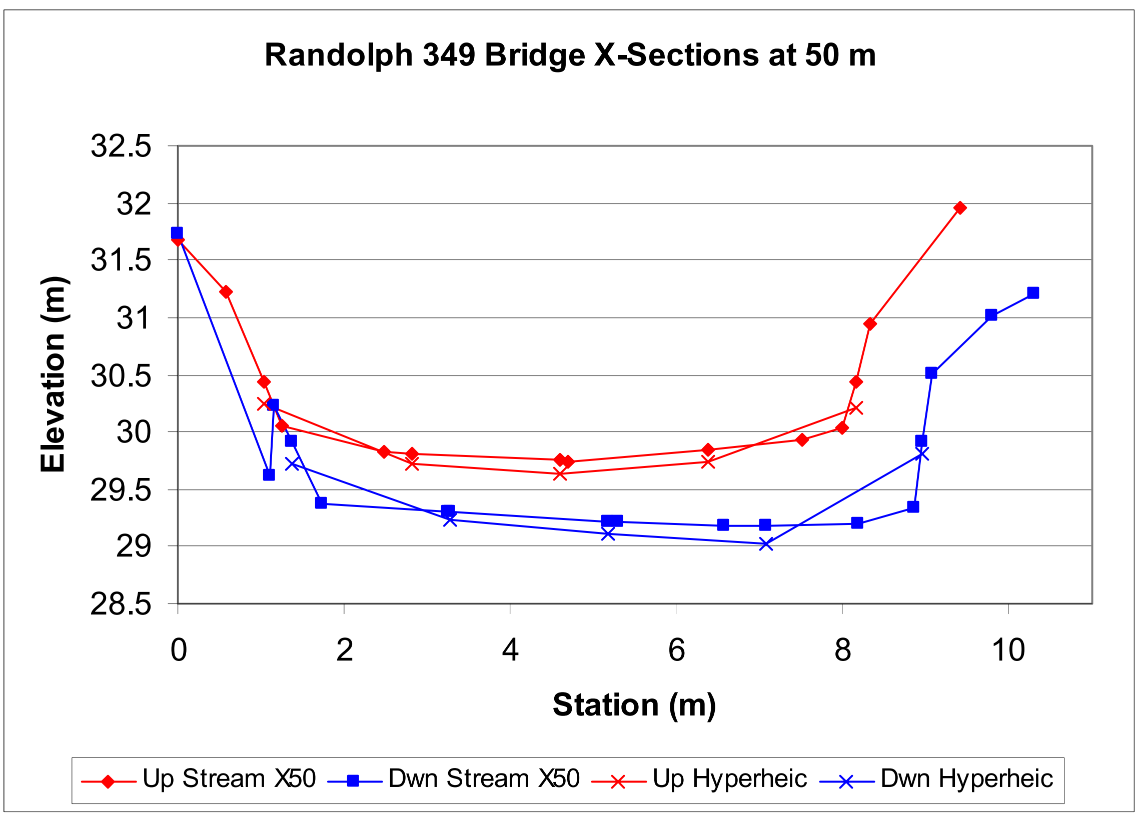
Randolph 349 Cross Section and Longitudinal Profile

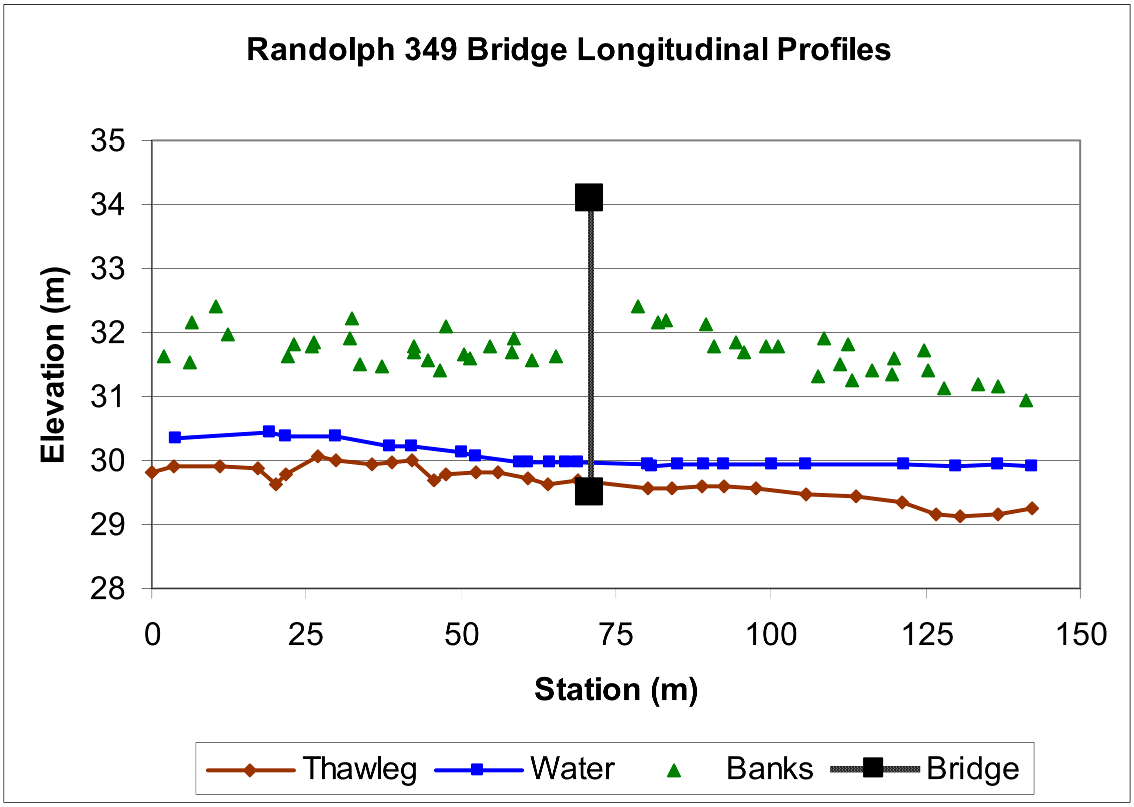



Randolph 349 Bridge X-Sections at 5 m
Elevation (m)
32.5
32
31.5
31
30.5
30
29.5
29
0
5
10
15
20
Station (m)
Up Stream X5
Dwn Stream
Up Hyperheic
Dwn Hyperheic


Randolph 349 Bridge X-Sections at 10 m
Elevation (m)
32.5
32
31.5
31
30.5
30
29.5
29
0
5
10
15
20
Station (m)
Up Stream X10
Dwn Stream X10
Up Hyperheic
Dwn Hyperheic

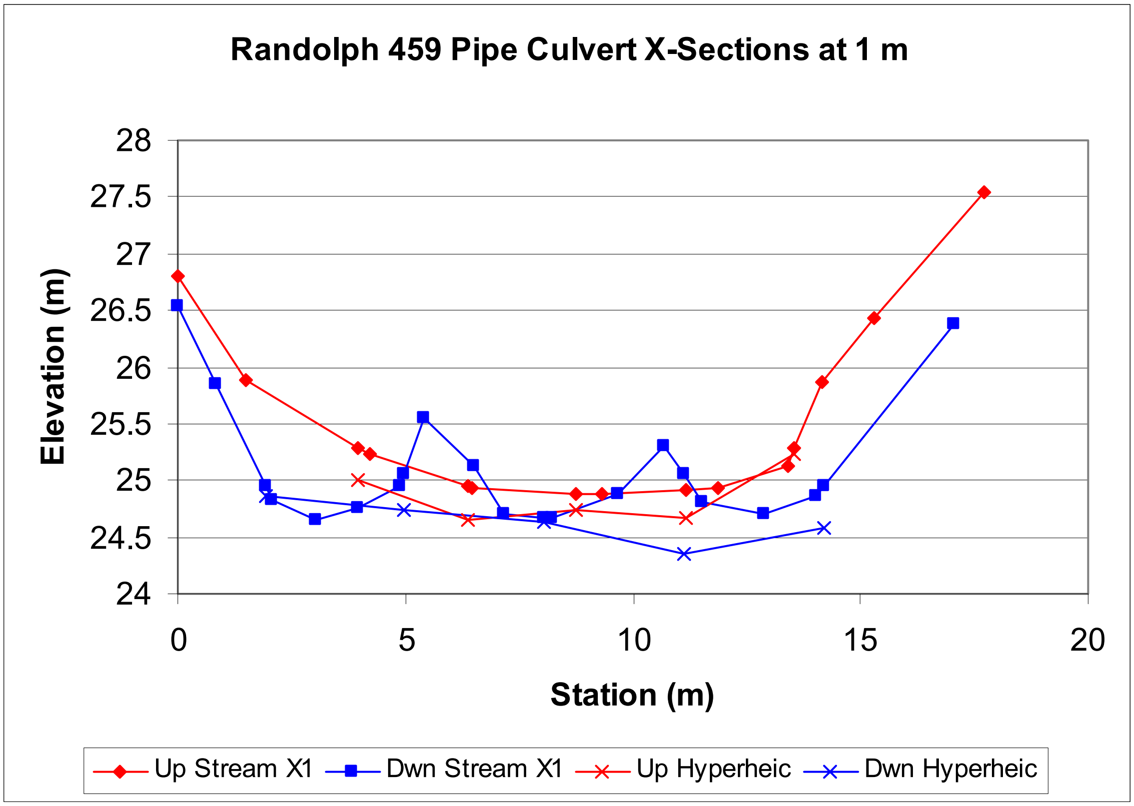

Randolph 349 Bridge X-Sections at X20
Elevation (m)
32
31.5
31
30.5
30
29.5
29
0
5
10
15
Station (m)
Up Stream X20
Dwn Stream X20
Up Hyperheic
Dwn Hyperheic

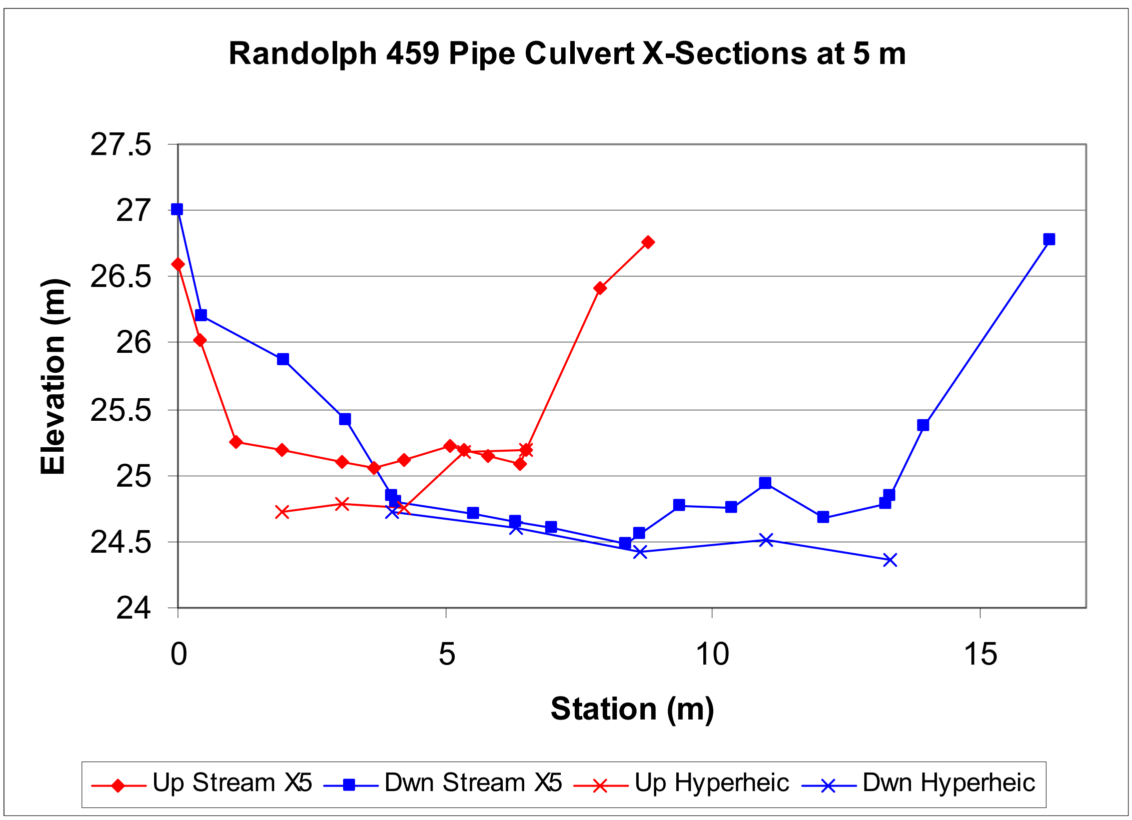

Randolph 349 Bridge X-Sections at 50 m
Elevation (m)
32.5
32
31.5
31
30.5
30
29.5
29
28.5
0
2
4
6
8
10
Station (m)
Up Stream X50
Dwn Stream X50
Up Hyperheic
Dwn Hyperheic

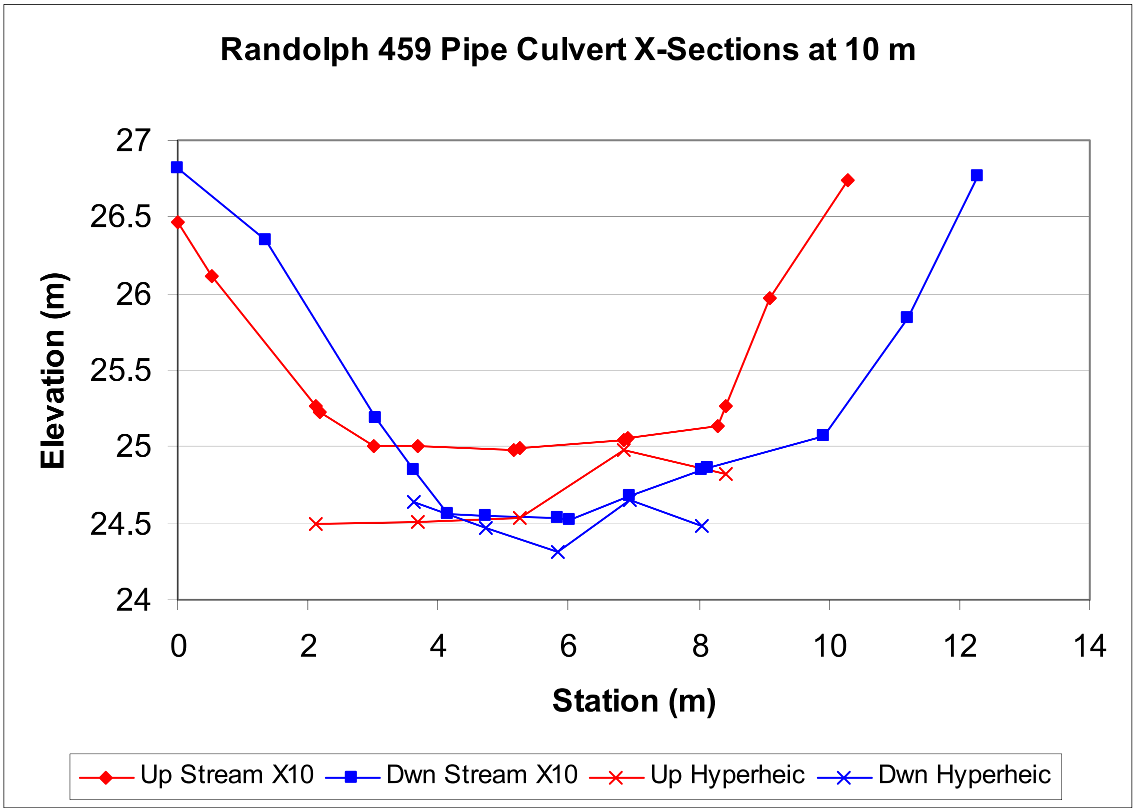


Randolph 349 Bridge Longitudinal Profiles
Elevation (m)
35
34
33
32
31
30
29
28
0
25
50
75
100
125
150
Station (m)
Thawleg
Water
Banks
Bridge

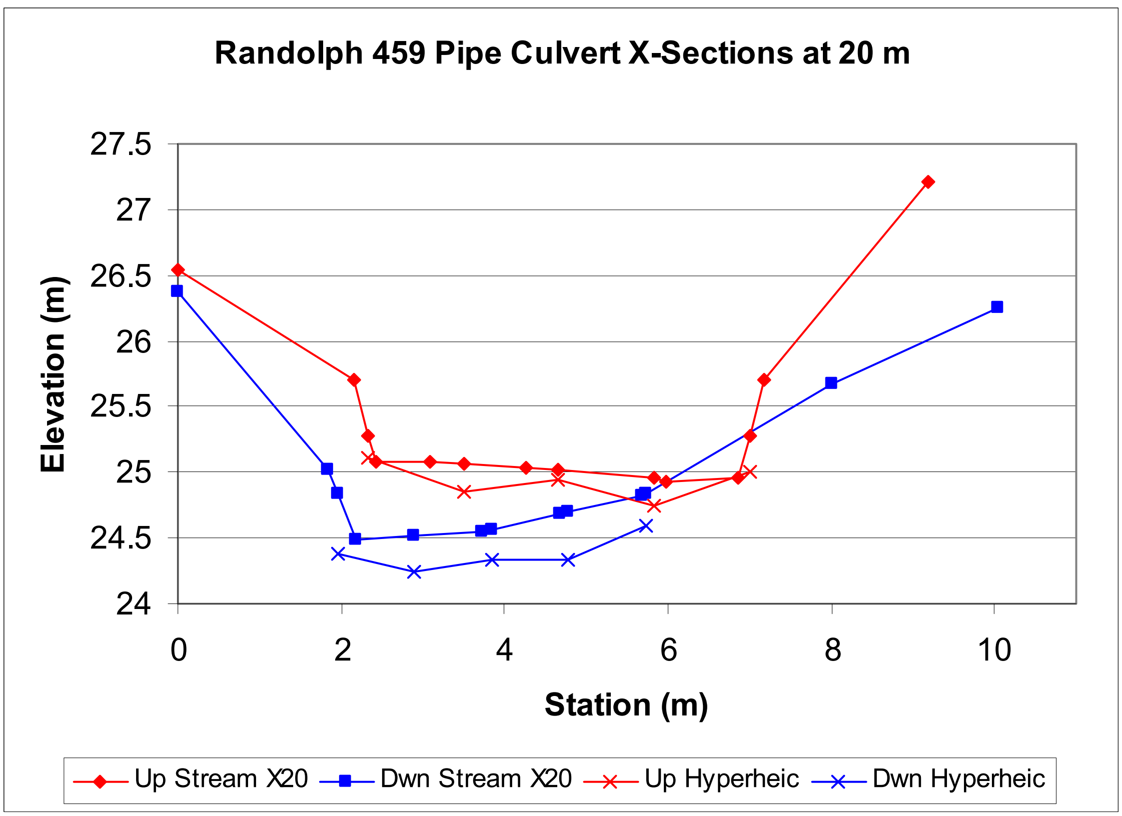
Randolph 459 Cross Section and Longitudinal Profile



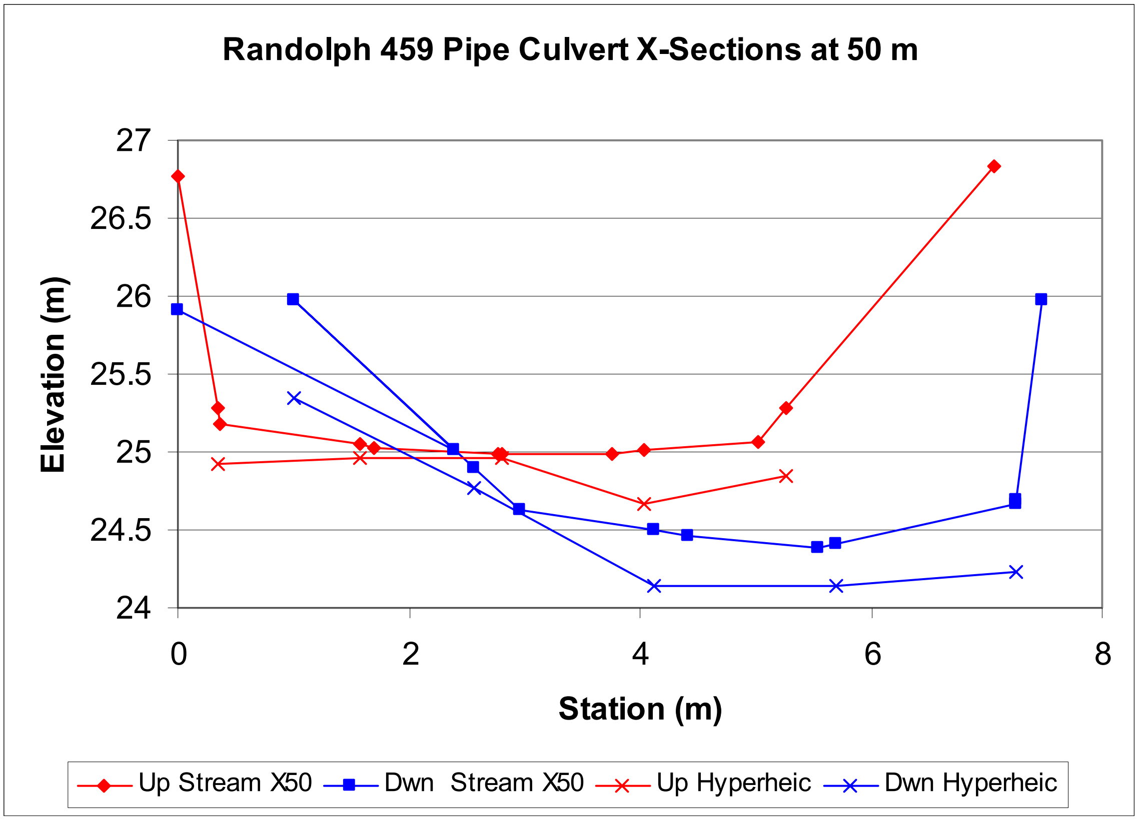




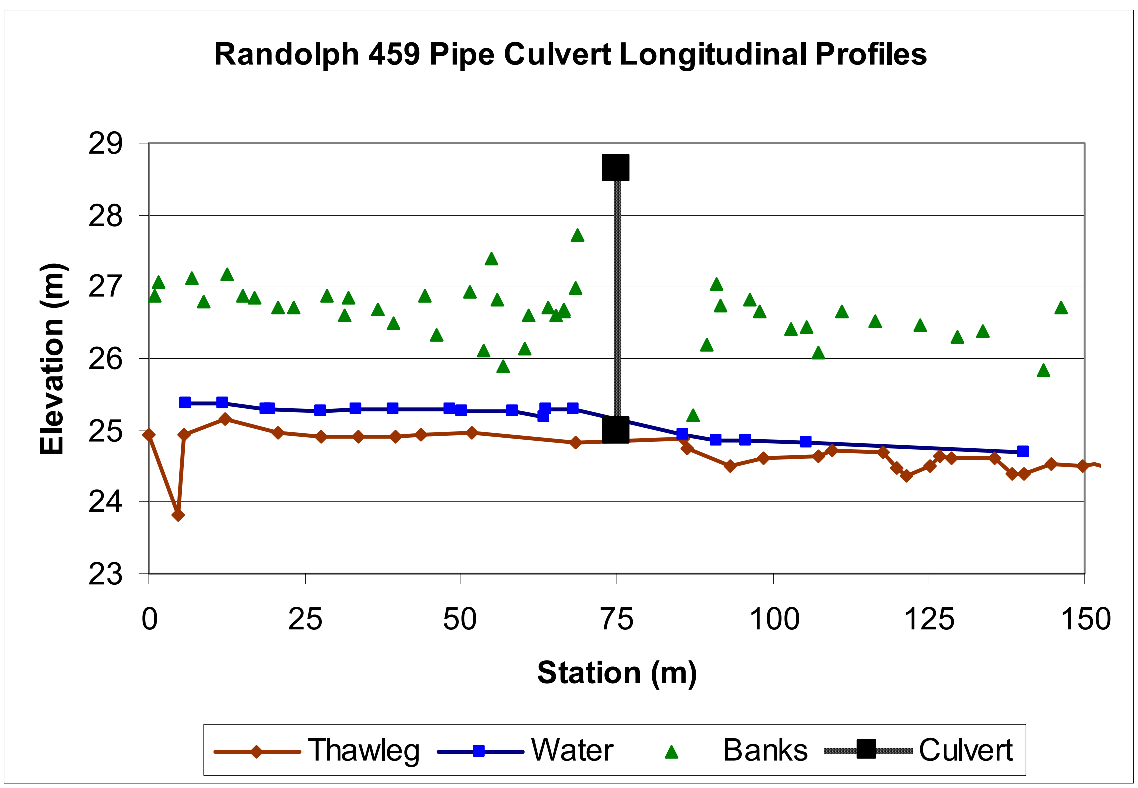
Randolph 459 Pipe Culvert X-Sections at 10 m
Elevation (m)
27
26.5
26
25.5
25
24.5
24
0
2
4
6
8
10
12
14
Station (m)
Up Stream X10
Dwn Stream X10
Up Hyperheic
Dwn Hyperheic



Randolph 459 Pipe Culvert X-Sections at 20 m
Elevation (m)
27.5
27
26.5
26
25.5
25
24.5
24
0
2
4
6
8
10
Station (m)
Up Stream X20
Dwn Stream X20
Up Hyperheic
Dwn Hyperheic



Randolph 459 Pipe Culvert X-Sections at 50 m
Elevation (m)
27
26.5
26
25.5
25
24.5
24
0
2
4
6
8
Station (m)
Up Stream X50
Dwn Stream X50
Up Hyperheic
Dwn Hyperheic



Randolph 459 Pipe Culvert Longitudinal Profiles
Elevation (m)
29
28
27
26
25
24
23
0
25
50
75
100
125
150
Station (m)
Thawleg
Water
Banks
Culvert

Appendix I-D: Statistical Tables and Graphs

Cross Section Area Statistics

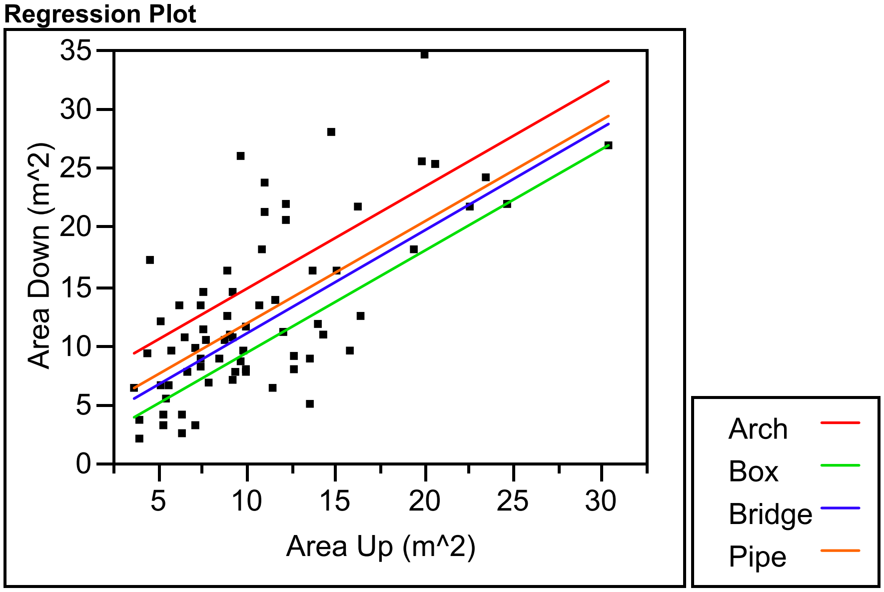
Hyporheic Depth Statistics

Habitat Area Statistics

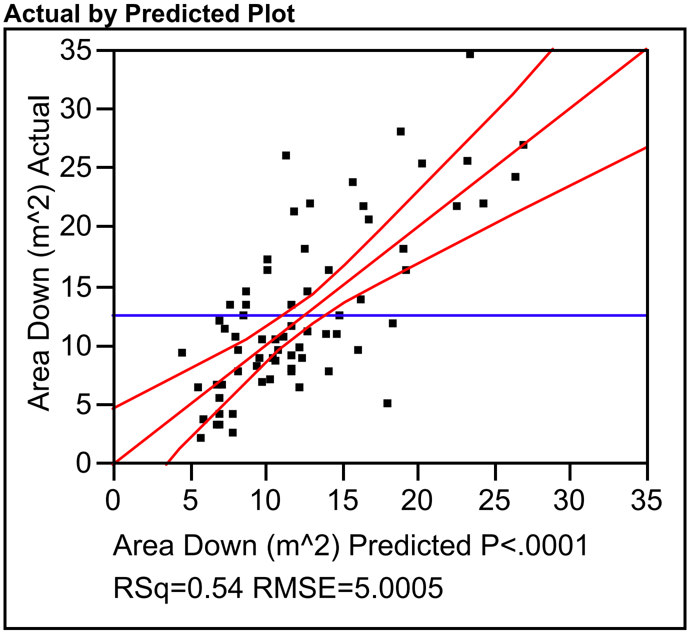
Cross Section Area Statistics

Comparison of All Crossing Types:

Response Area Down (m^2)
Whole Model
Regression Plot



Actual by Predicted Plot



Summary of Fit

RSquare	0.538971
RSquare Adj	0.5106
Root Mean Square Error	5.000476
Mean of Response	12.53429
Observations (or Sum Wgts)	70

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	1900.0888	475.022	18.9973
Error	65	1625.3095	25.005	Prob > F
C. Total	69	3525.3983		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	64	1618.1274	25.2832	3.5203
Pure Error	1	7.1821	7.1821	Prob > F
Total Error	65	1625.3095		0.4041
				Max RSq
				0.9980

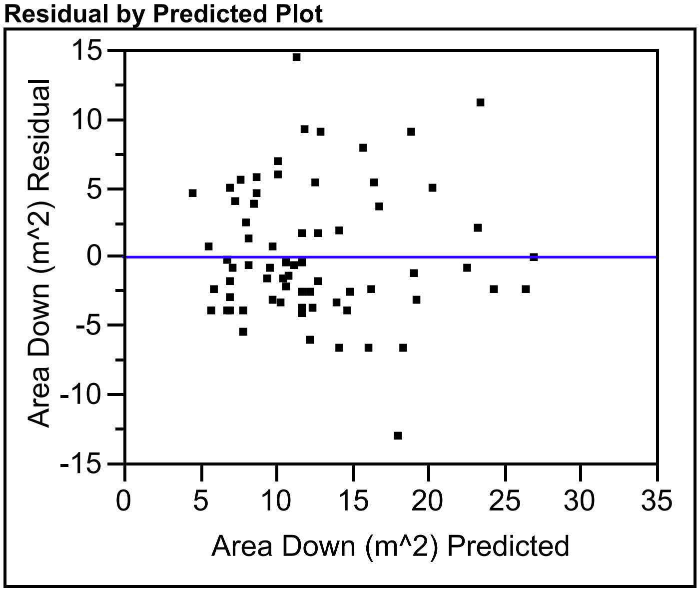
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	3.1376031	1.459145	2.15	0.0353
Area Up (m^2)	0.8616149	0.114871	7.50	<.0001
Type [Arch]	3.0762538	1.133326	2.71	0.0085
Type [Box]	-2.369936	1.296354	-1.83	0.0721
Type [Bridge]	-0.718023	0.941777	-0.76	0.4486

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Area Up (m^2)	1	1	1406.7895	56.2609	<.0001
Type	3	3	209.2605	2.7896	0.0474

Residual by Predicted Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Arch	15.595120	1.3137415	17.4160
Box	10.148930	1.5908621	11.4560
Bridge	11.800844	0.9292679	10.5007
Pipe	12.530572	1.2911755	12.4387

LSMeans Differences Tukey HSD

Alpha=
0.050 Q=
2.63676LSMean[i] By LSMean[j]

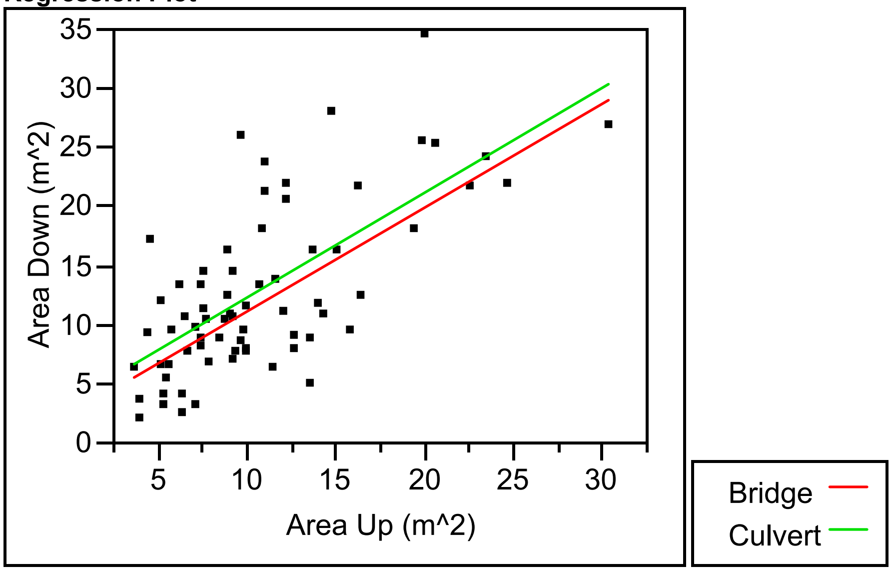
Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	Arch	Box	Bridge	Pipe
Arch	0 0 0 0	5.44619 2.04258 0.06038 10.832	3.79428 1.63512 -0.5171 8.10569	3.06455 1.84364 -1.7967 7.92578
Box	-5.4462 2.04258 -10.832 -0.0604	0 0 0 0	-1.6519 1.85871 -6.5529 3.24905	-2.3816 2.04994 -7.7868 3.02355
Bridge	-3.7943 1.63512 -8.1057 0.51714	1.65191 1.85871 -3.2491 6.55288	0 0 0 0	-0.7297 1.58947 -4.9208 3.46133
Pipe	-3.0645 1.84364 -7.9258 1.79668	2.38164 2.04994 -3.0236 7.78684	0.72973 1.58947 -3.4613 4.92079	0 0 0 0

Level			Least Sq Mean
Arch	A		15.595120
Pipe	A	B	12.530572
Bridge	A	B	11.800844
Box		B	10.148930

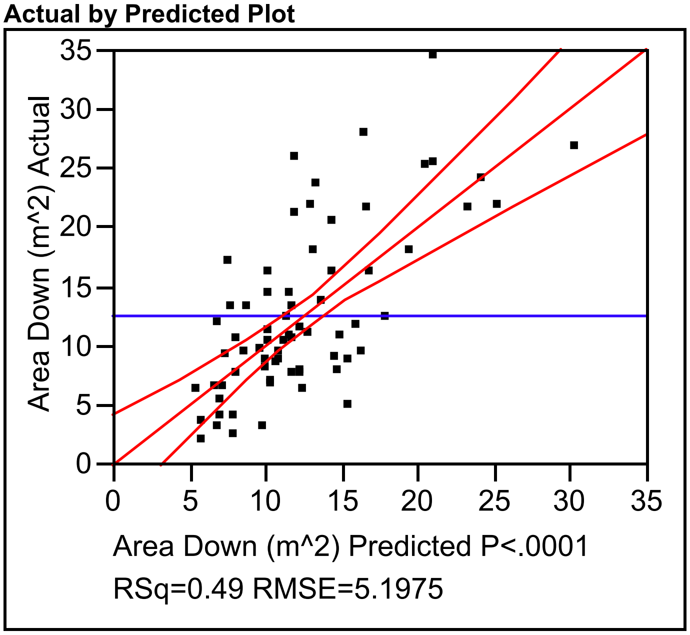
Levels not connected by same letter are significantly different (alpha =.05)

Comparison of Bridges vs Culverts:

Response Area Down (m^2)
Whole Model
Regression Plot



Actual by Predicted Plot



Summary of Fit

RSquare	0.486592
RSquare Adj	0.471266
Root Mean Square Error	5.197542
Mean of Response	12.53429
Observations (or Sum Wgts)	70

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	1715.4305	857.715	31.7502
Error	67	1809.9678	27.014	Prob > F
C. Total	69	3525.3983		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	66	1802.7857	27.3149	3.8032
Pure Error	1	7.1821	7.1821	Prob > F
Total Error	67	1809.9678		0.3902
				Max RSq
				0.9980

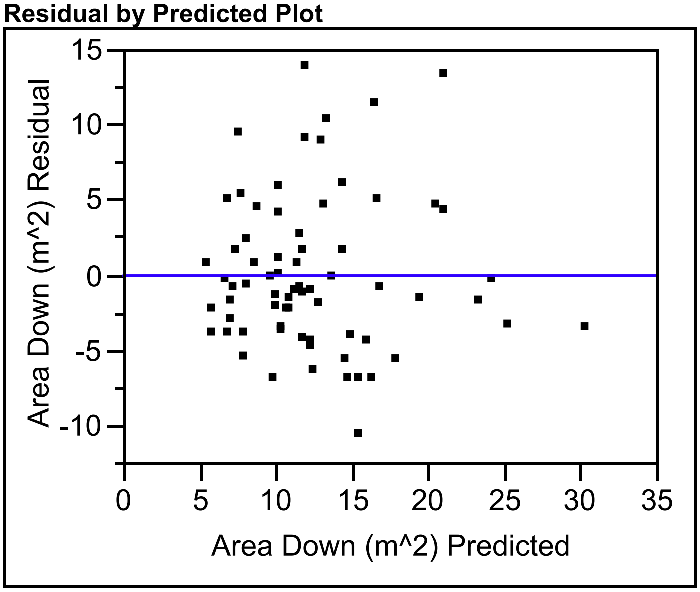
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.8624687	1.411758	2.03	0.0466
Area Up (m^2)	0.8802023	0.11819	7.45	<.0001
Bridge vs Culvert[Bridge]	-0.61722	0.646772	-0.95	0.3434

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Area Up (m^2)	1	1	1498.3112	55.4633	<.0001
Bridge vs Culvert	1	1	24.6022	0.9107	0.3434

Residual by Predicted Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Bridge	11.828892	0.96555143	10.5007
Culvert	13.063331	0.83261826	14.0595

LSMeans Differences Student's t

Alpha=
0.050 t=
1.99601LSMean[i] By LSMean[j]

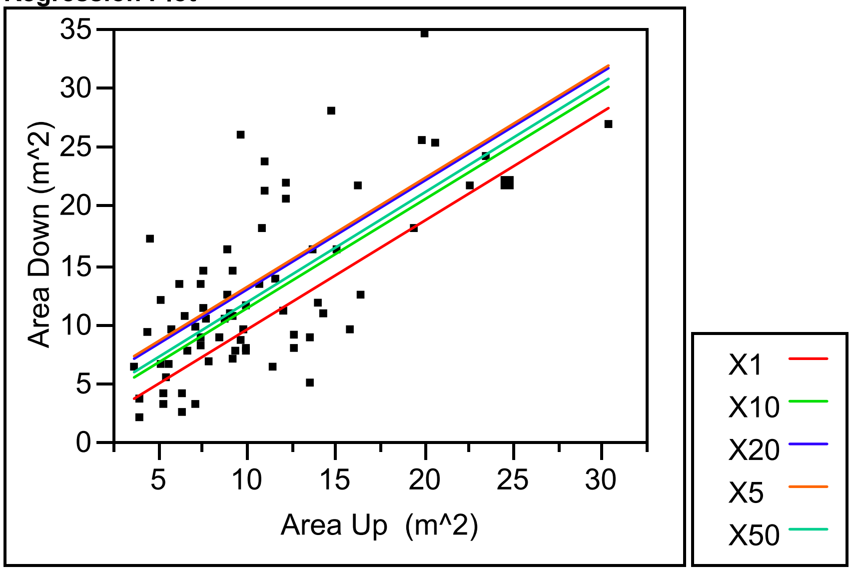
Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	Bridge	Culvert
Bridge	0 0 0 0	-1.2344 1.29354 -3.8164 1.34748
Culvert	1.23444 1.29354 -1.3475 3.81636	0 0 0 0

Level		Least Sq Mean
Culvert	A	13.063331
Bridge	A	11.828892

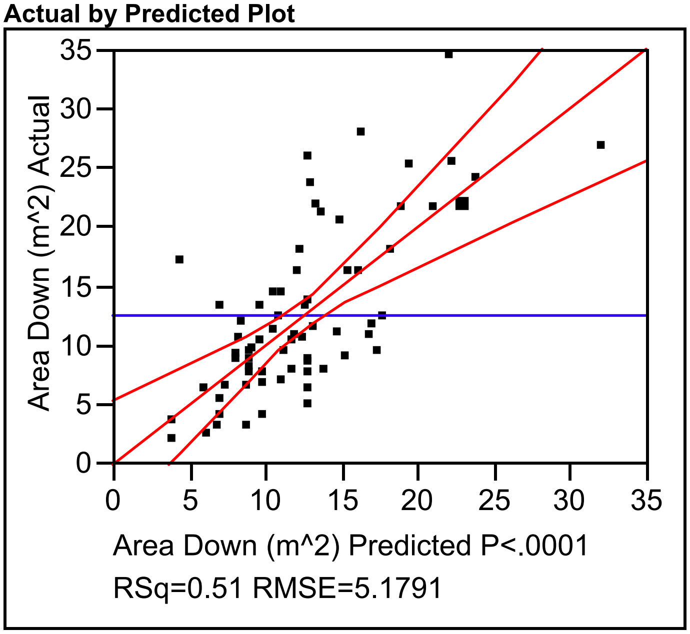
Levels not connected by same letter are significantly different

Comparison of Cross Section Locations:

Response Area Down (m^2)
Whole Model
Regression Plot



Actual by Predicted Plot



Summary of Fit

RSquare	0.513054
RSquare Adj	0.475011
Root Mean Square Error	5.179102
Mean of Response	12.53429
Observations (or Sum Wgts)	70

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	1808.7199	361.744	13.4863
Error	64	1716.6784	26.823	Prob > F
C. Total	69	3525.3983		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	63	1565.1244	24.843	0.1639
Pure Error	1	151.5541	151.554	Prob > F
Total Error	64	1716.6784		0.9838
				Max RSq
				0.9570

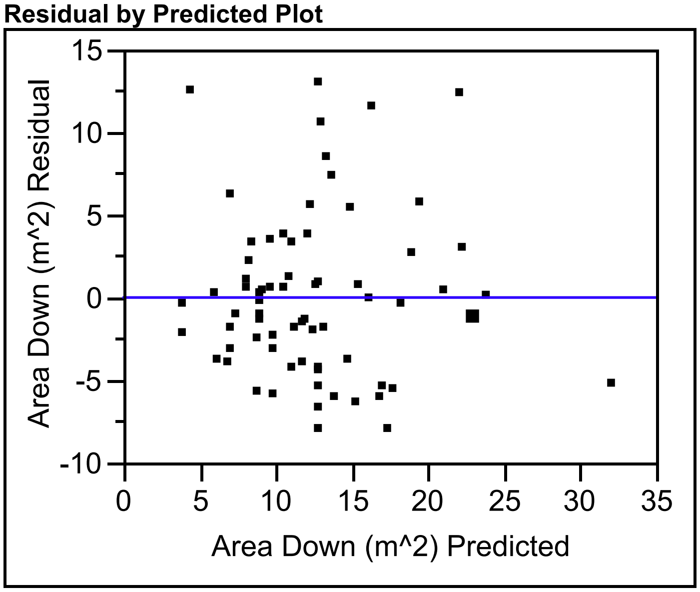
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.4709257	1.398767	1.77	0.0821
Area Up (m^2)	0.9242616	0.115204	8.02	<.0001
Station[X1]	-2.250884	1.243856	-1.81	0.0751
Station[X10]	-0.409562	1.23819	-0.33	0.7419
Station[X20]	1.103739	1.240863	0.89	0.3771
Station[X5]	1.4101441	1.239381	1.14	0.2595

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Area Up (m^2)	1	1	1726.4999	64.3662	<.0001
Station	4	4	117.8915	1.0988	0.3649

Residual by Predicted Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
X1	10.283402	1.3893760	11.2471
X10	12.124724	1.3843052	11.9714
X20	13.638025	1.3866969	12.9671
X5	13.944430	1.3853706	14.4064
X50	12.680848	1.3862027	12.0793

LSMeans Differences Tukey HSD

Alpha=
0.050 Q=
2.80707LSMean[i] By LSMean[j]

Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	X1	X10	X20	X5	X50
X1	0 0 0 0	-1.8413 1.96246 -7.3501 3.66744	-3.3546 1.96809 -8.8792 2.16994	-3.661 1.95852 -9.1587 1.83666	-2.3974 1.96722 -7.9196 3.12466
X10	1.84132 1.96246 -3.6674 7.35009	0 0 0 0	-1.5133 1.95858 -7.0112 3.98456	-1.8197 1.95902 -7.3188 3.67939	-0.5561 1.95831 -6.0532 4.941
X20	3.35462 1.96809 -2.1699 8.87919	1.5133 1.95858 -3.9846 7.01117	0 0 0 0	-0.3064 1.9626 -5.8156 5.20275	0.95718 1.95754 -4.5378 6.45211
X5	3.66103 1.95852 -1.8367 9.15871	1.81971 1.95902 -3.6794 7.3188	0.30641 1.9626 -5.2028 5.81556	0 0 0 0	1.26358 1.962 -4.2439 6.77105
X50	2.39745 1.96722 -3.1247 7.91955	0.55612 1.95831 -4.941 6.05324	-0.9572 1.95754 -6.4521 4.53776	-1.2636 1.962 -6.771 4.24389	0 0 0 0

Level		Least Sq Mean
X5	A	13.944430
X20	A	13.638025
X50	A	12.680848
X10	A	12.124724
X1	A	10.283402

Levels not connected by same letter are significantly different (alpha =.05)

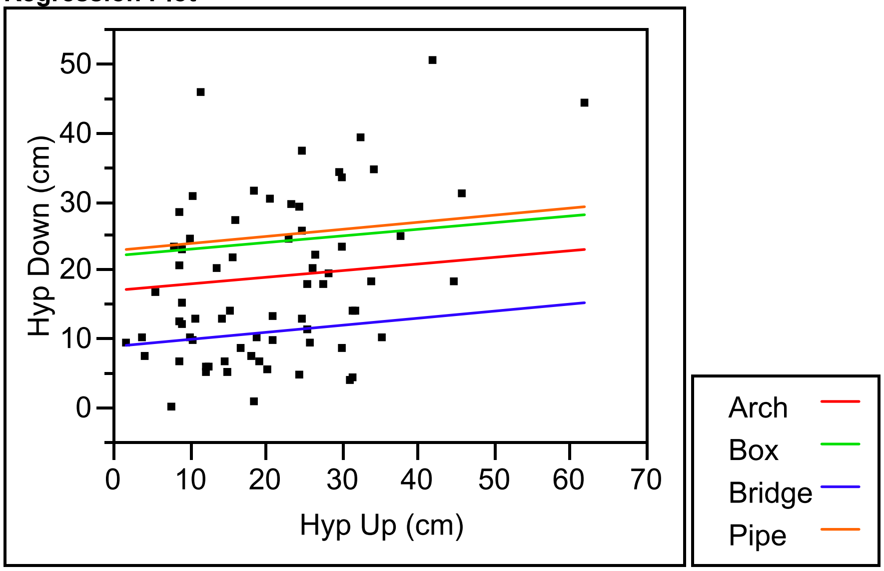
Hyporheic Zone Depth Statistics

Comparison of All Crossing Types:

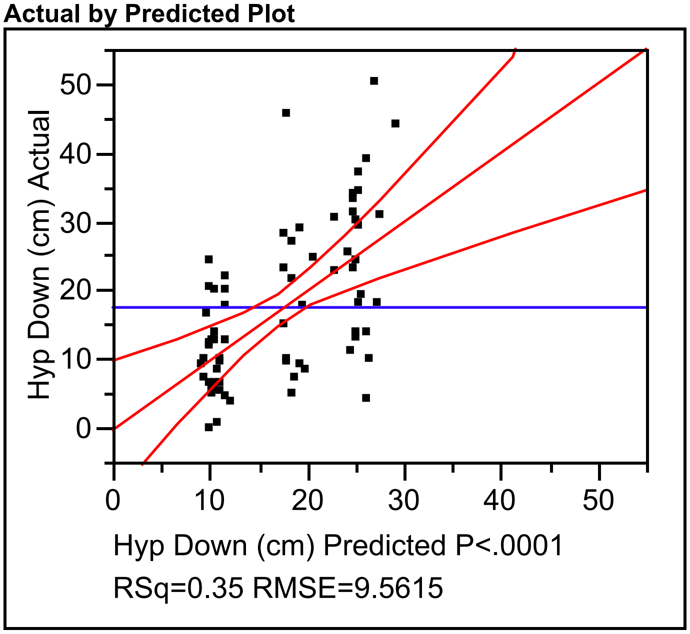
Response Hyp Down (cm)

Whole Model

Regression Plot



Actual by Predicted Plot



Summary of Fit

RSquare	0.34532
RSquare Adj	0.305032
Root Mean Square Error	9.56145
Mean of Response	17.69543
Observations (or Sum Wgts)	70

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	3134.3896	783.597	8.5713
Error	65	5942.3868	91.421	Prob > F
C. Total	69	9076.7763		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	64	5890.4687	92.0386	1.7728
Pure Error	1	51.9180	51.9180	Prob > F
Total Error	65	5942.3868		0.5446
				Max RSq
				0.9943

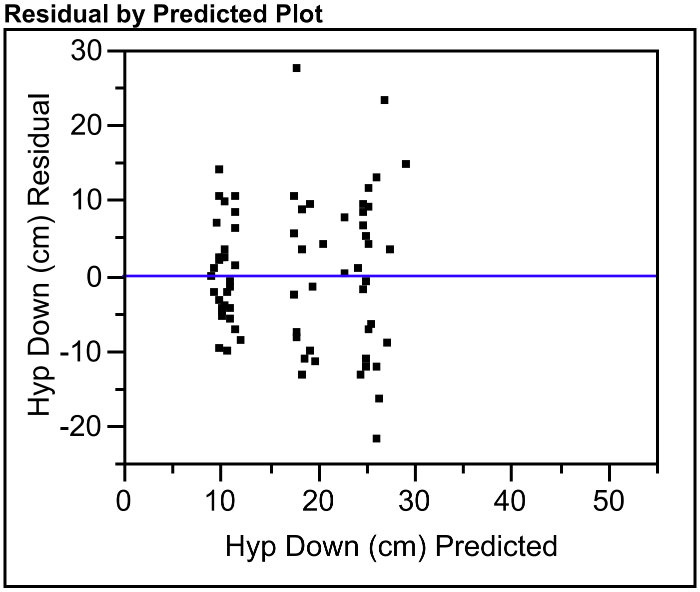
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	17.718798	3.174117	5.58	<.0001
Hyp Up (cm)	0.1007816	0.127313	0.79	0.4315
Type[Arch]	-0.818557	2.225495	-0.37	0.7142
Type[Box]	4.1991827	2.501256	1.68	0.0980
Type[Bridge]	-8.615822	2.010913	-4.28	<.0001

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Hyp Up (cm)	1	1	57.2884	0.6266	0.4315
Type	3	3	1707.3880	6.2253	0.0009

Residual by Predicted Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Arch	19.023061	2.4971139	18.7260
Box	24.040801	3.0915252	24.5510
Bridge	11.225796	1.9021808	10.6277
Pipe	25.076814	2.8665174	26.2300

LSMeans Differences Tukey HSD

Alpha=
0.050 Q=
2.63676LSMean[i] By LSMean[j]

Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	Arch	Box	Bridge	Pipe
Arch	0	-5.0177	7.79727	-6.0538
	0	4.03446	3.04742	3.94282
	0	-15.656	-0.2381	-16.45
	0	5.62015	15.8326	4.34252
Box	5.01774	0	12.815	-1.036
	4.03446	0	3.76162	3.98706
	-5.6202	0	2.89651	-11.549
	15.6556	0	22.7335	9.47691
Bridge	-7.7973	-12.815	0	-13.851
	3.04742	3.76162	0	3.74655

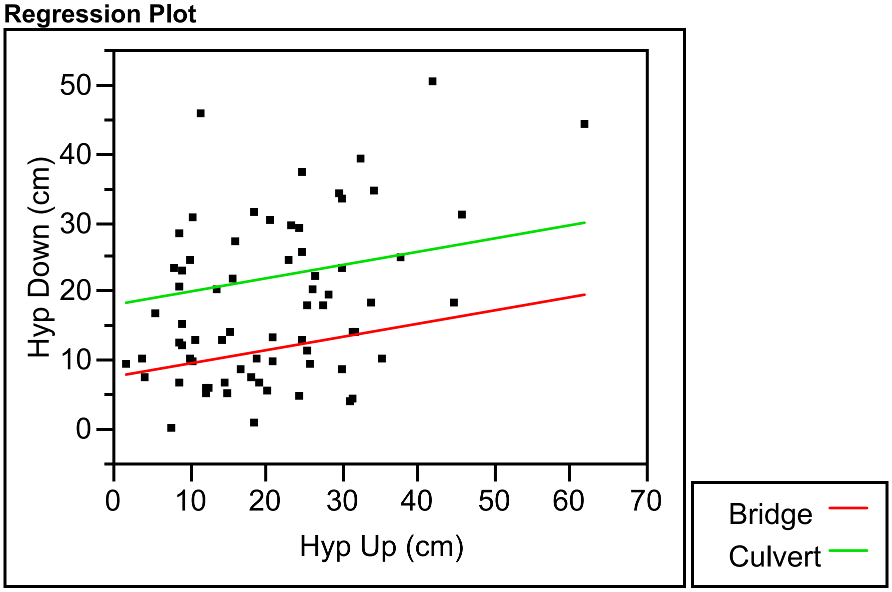
	-15.833	-22.733	0	-23.73
	0.23806	-2.8965	0	-3.9723
Pipe	6.05375	1.03601	13.851	0
	3.94282	3.98706	3.74655	0
	-4.3425	-9.4769	3.97226	0
	16.45	11.5489	23.7298	0

Level			Least Sq Mean
Pipe	A		25.076814
Box	A		24.040801
Arch	A	B	19.023061
Bridge		B	11.225796

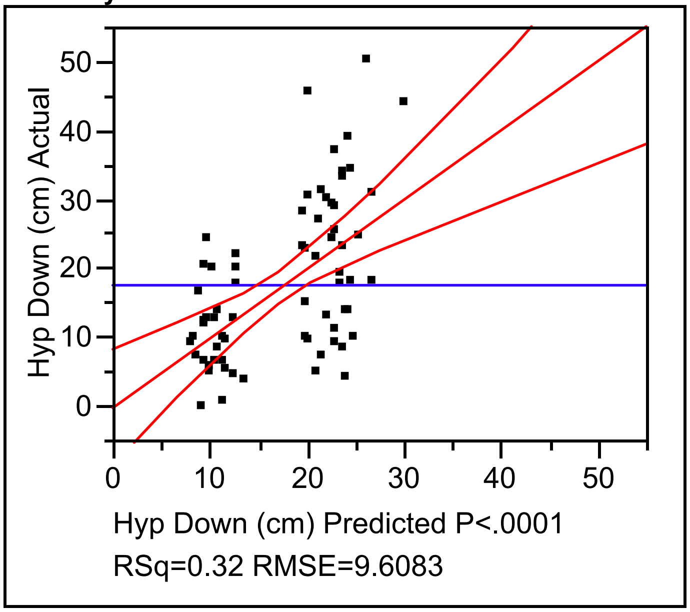
Levels not connected by same letter are significantly different (alpha =.05)

Comparison of Bridges vs Culverts:

Response Hyp Down (cm)
Whole Model
Regression Plot



Actual by Predicted Plot



Summary of Fit

RSquare	0.318542
RSquare Adj	0.2982
Root Mean Square Error	9.608332
Mean of Response	17.69543
Observations (or Sum Wgts)	70

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	2891.3340	1445.67	15.6593
Error	67	6185.4424	92.32	Prob > F
C. Total	69	9076.7763		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	65	6104.4921	93.9153	2.3203
Pure Error	2	80.9502	40.4751	Prob > F
Total Error	67	6185.4424		0.3483
				Max RSq
				0.9911

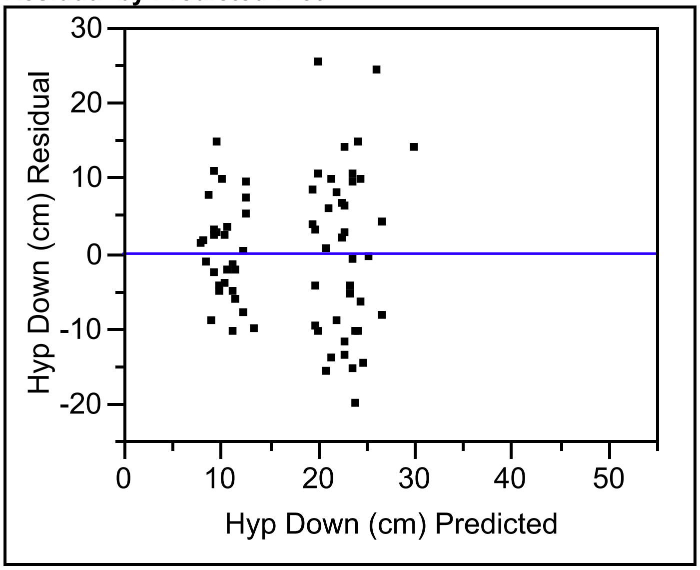
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	12.88507	2.577356	5.00	<.0001
Hyp Up (cm)	0.1932361	0.113248	1.71	0.0926
Bridge vs Culvert[Bridge]	-5.180808	1.300845	-3.98	0.0002

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Hyp Up (cm)	1	1	268.7879	2.9115	0.0926
Bridge vs Culvert	1	1	1464.3324	15.8615	0.0002

Residual by Predicted Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Bridge	11.774505	1.8785836	10.6277
Culvert	22.136122	1.6006579	22.9963

LSMeans Differences Student's t

Alpha=
0.050 t=
1.99601LSMean[i] By LSMean[j]

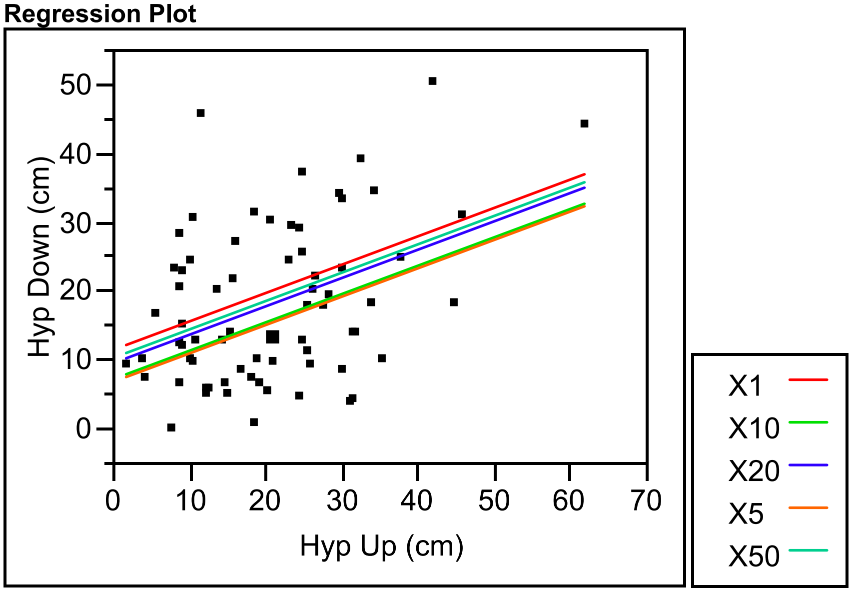
Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	Bridge	Culvert
Bridge	0 0 0 0	-10.362 2.60169 -15.555 -5.1686
Culvert	10.3616 2.60169 5.16862 15.5546	0 0 0 0

Level			Least Sq Mean
Culvert	A		22.136122
Bridge		B	11.774505

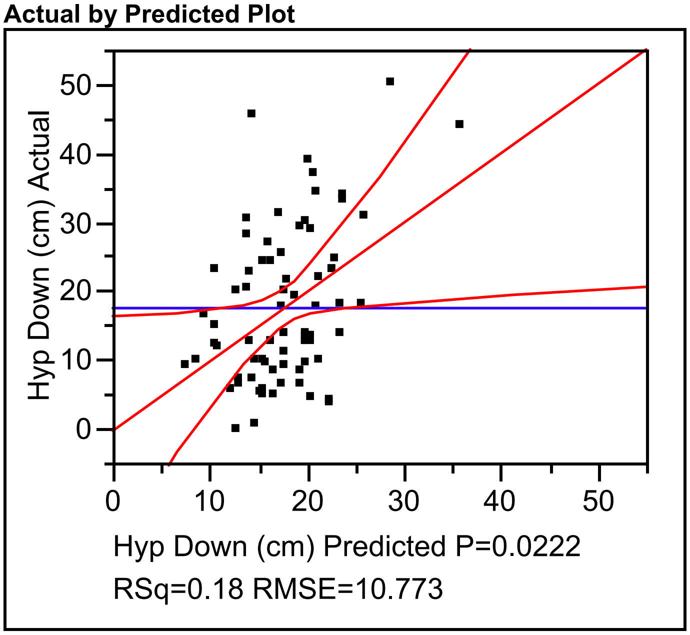
Levels not connected by same letter are significantly different (alpha= .05)

Comparison of Cross Section Locations:

Response Hyp Down (cm)
Whole Model
Regression Plot



Actual by Predicted Plot



Summary of Fit

RSquare	0.181724
RSquare Adj	0.117796
Root Mean Square Error	10.77273
Mean of Response	17.69543
Observations (or Sum Wgts)	70

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	1649.4666	329.893	2.8426
Error	64	7427.3097	116.052	Prob > F
C. Total	69	9076.7763		0.0222

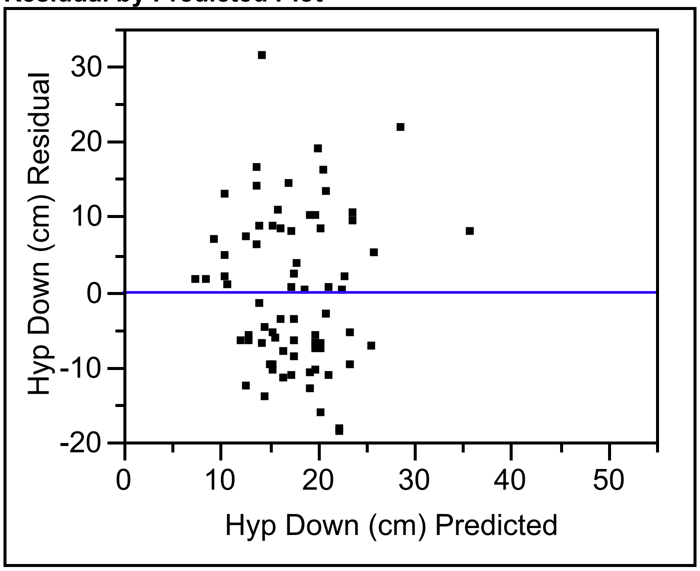
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	9.0250679	2.727244	3.31	0.0015
Hyp Up (cm)	0.4116282	0.114138	3.61	0.0006
Station[X1]	2.3196352	2.58523	0.90	0.3729
Station[X10]	-1.886788	2.576863	-0.73	0.4667
Station[X20]	0.4899755	2.579776	0.19	0.8500
Station[X5]	-2.252977	2.582005	-0.87	0.3862

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Hyp Up (cm)	1	1	1509.3837	13.0061	0.0006
Station	4	4	222.4650	0.4792	0.7508

Residual by Predicted Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
X1	20.015064	2.8881301	19.1936
X10	15.808641	2.8806435	16.1450
X20	18.185404	2.8832492	17.6300
X5	15.442451	2.8852436	16.1193
X50	19.025583	2.8808990	19.3893

LSMeans Differences Tukey HSD

Alpha=
0.050 Q=
2.80707LSMean[i] By LSMean[j]

Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	X1	X10	X20	X5	X50
X1	0 0 0 0	4.20642 4.08435 -7.2586 15.6715	1.82966 4.07238 -9.6018 13.2611	4.57261 4.09285 -6.9163 16.0615	0.98948 4.08495 -10.477 12.4562
X10	-4.2064 4.08435 -15.671 7.25862	0 0 0 0	-2.3768 4.07921 -13.827 9.07386	0.36619 4.0728 -11.066 11.7988	-3.2169 4.07172 -14.647 8.21264
X20	-1.8297 4.07238 -13.261 9.60178	2.37676 4.07921 -9.0739 13.8274	0 0 0 0	2.74295 4.08602 -8.7268 14.2127	-0.8402 4.07968 -12.292 10.6118
X5	-4.5726 4.09285 -16.062 6.9163	-0.3662 4.0728 -11.799 11.0664	-2.743 4.08602 -14.213 8.72678	0 0 0 0	-3.5831 4.07263 -15.015 7.84903

X50	-0.9895	3.21694	0.84018	3.58313	0
	4.08495	4.07172	4.07968	4.07263	0
	-12.456	-8.2126	-10.612	-7.849	0
	10.4773	14.6465	12.2921	15.0153	0

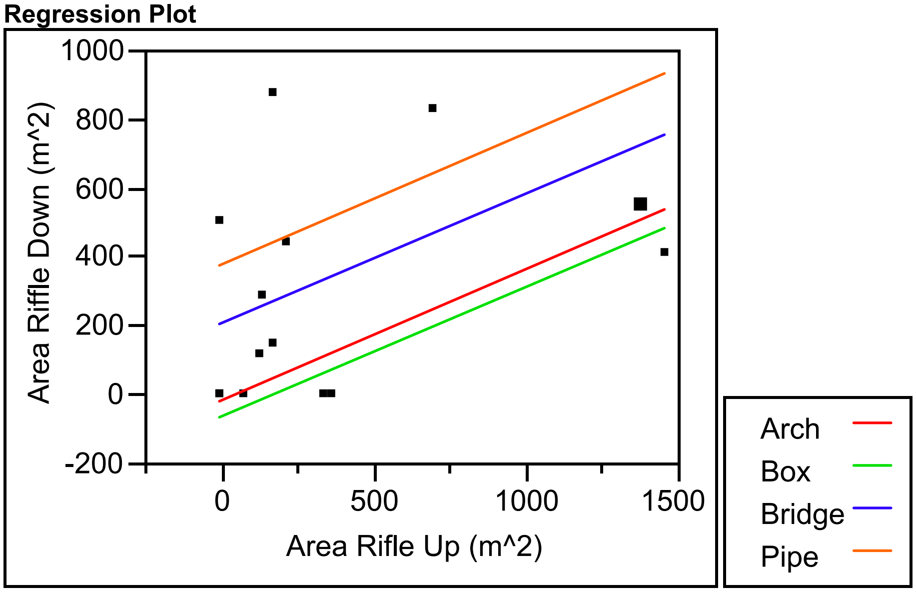
Level		Least Sq Mean
X1	A	20.015064
X50	A	19.025583
X20	A	18.185404
X10	A	15.808641
X5	A	15.442451

Levels not connected by same letter are significantly different (alpha = .05)

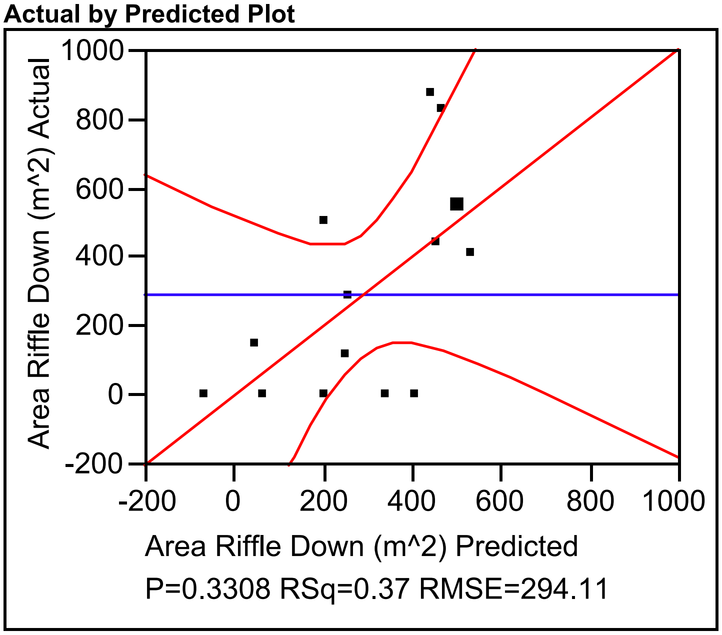
Habitat Area Statistics

Comparison of All Crossing Types:

Response Area Riffle Down (m^2)
Whole Model
Regression Plot



Actual by Predicted Plot



Summary of Fit

RSquare	0.371339
RSquare Adj	0.091934
Root Mean Square Error	294.1114
Mean of Response	294.4371
Observations (or Sum Wgts)	14

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	459854.4	114964	1.3290

Source	DF	Sum of Squares	Mean Square	F Ratio
Error	9	778513.8	86502	Prob > F
C. Total	13	1238368.2		0.3308

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	8	654511.83	81814	0.6598
Pure Error	1	124002.00	124002	Prob > F
Total Error	9	778513.83		0.7468
				Max RSq
				0.8999

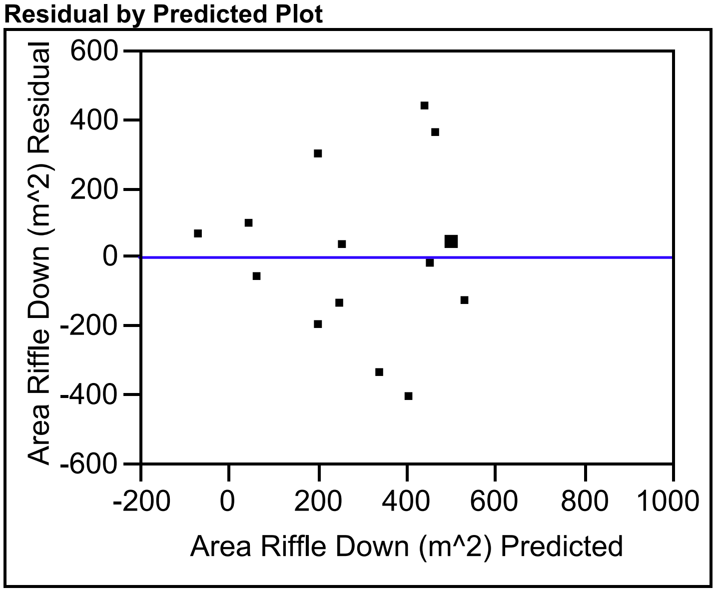
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	124.42875	126.7824	0.98	0.3520
Area Rifle Up (m^2)	0.3788447	0.242259	1.56	0.1523
Type[Arch]	-142.0251	209.3639	-0.68	0.5146
Type[Box]	-189.6412	177.7226	-1.07	0.3137
Type[Bridge]	78.241551	126.5575	0.62	0.5517

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Area Rifle Up (m^2)	1	1	211537.16	2.4455	0.1523
Type	3	3	288818.39	1.1130	0.3937

Residual by Predicted Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Arch	122.10017	229.13496	362.687
Box	74.48411	213.35277	0.000
Bridge	342.36685	125.11649	287.362
Pipe	517.55005	177.51470	436.630

LSMeans Differences Tukey HSD

Alpha=
0.050 Q=
3.12182LSMean[i] By LSMean[j]

Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	Arch	Box	Bridge	Pipe
Arch	0 0 0 0	47.6161 335.675 -1000.3 1095.53	-220.27 281.033 -1097.6 657.069	-395.45 316.127 -1382.3 591.44
Box	-47.616 335.675 -1095.5 1000.3	0 0 0 0	-267.88 240.464 -1018.6 482.801	-443.07 268.517 -1281.3 395.196

Bridge	220.267 281.033 -657.07 1097.6	267.883 240.464 -482.8 1018.57	0 0 0 0	-175.18 208.627 -826.48 476.113
Pipe	395.45 316.127 -591.44 1382.34	443.066 268.517 -395.2 1281.33	175.183 208.627 -476.11 826.48	0 0 0 0

Level		Least Sq Mean
Pipe	A	517.55005
Bridge	A	342.36685
Arch	A	122.10017
Box	A	74.48411

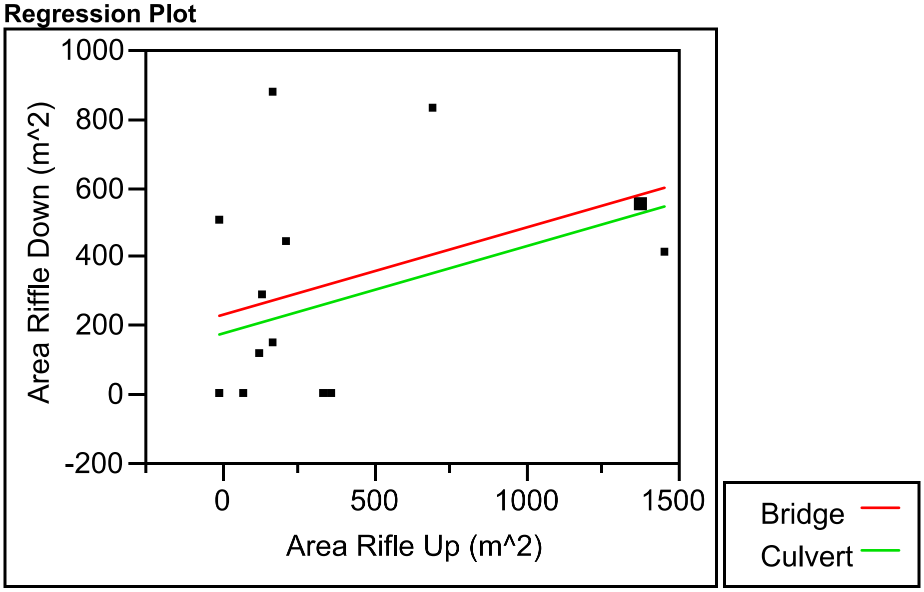
Levels not connected by same letter are significantly different (alpha =.05)

Comparison of Bridges vs Culverts:

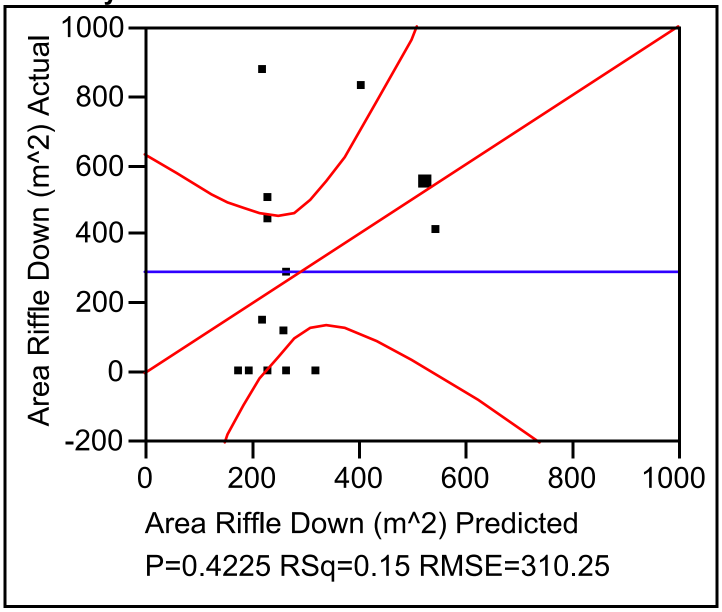
Response Area Riffle Down (m^2)

Whole Model

Regression Plot



Actual by Predicted Plot



Summary of Fit

RSquare	0.145008
RSquare Adj	-0.01044
Root Mean Square Error	310.2484
Mean of Response	294.4371
Observations (or Sum Wgts)	14

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	179573.8	89786.9	0.9328
Error	11	1058794.5	96254.0	Prob > F
C. Total	13	1238368.2		0.4225

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	10	934792.5	93479	0.7539
Pure Error	1	124002.0	124002	Prob > F
Total Error	11	1058794.5		0.7238
				Max RSq
				0.8999

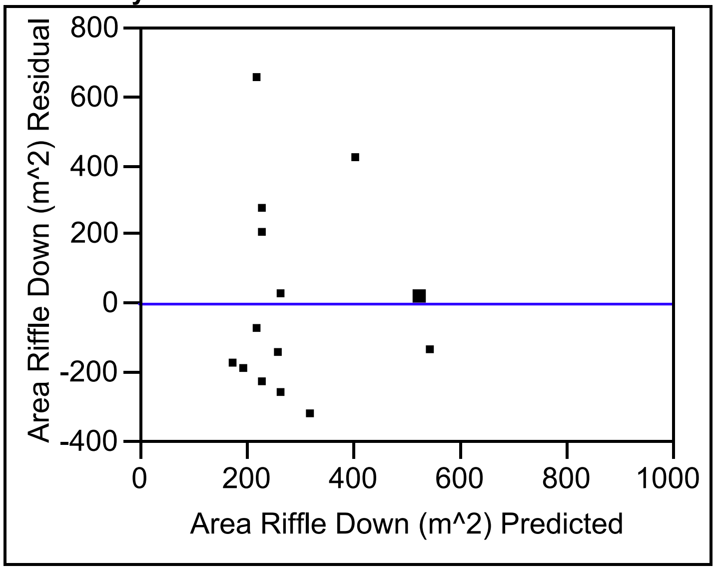
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	204.94486	106.0156	1.93	0.0794
Area Rifle Up (m^2)	0.2527359	0.185307	1.36	0.1999
Bridge vs Culvert[Bridge]	25.917268	87.02153	0.30	0.7714

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Area Rifle Up (m^2)	1	1	179048.13	1.8602	0.1999
Bridge vs Culvert	1	1	8537.76	0.0887	0.7714

Residual by Predicted Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Bridge	324.05688	129.48445	287.362
Culvert	272.22234	111.52999	299.744

LSMeans Differences Student's t

Alpha=
0.050 t=
2.20099LSMean[i] By LSMean[j]

Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	Bridge	Culvert
Bridge	0 0 0 0	51.8345 174.043 -331.23 434.901
Culvert	-51.835 174.043 -434.9 331.232	0 0 0 0

Level		Least Sq Mean
Bridge	A	324.05688
Culvert	A	272.22234

Levels not connected by same letter are significantly different (alpha = .05)

Appendix E: Data Tables

Cross Section Areas

Site Name		X-Area (m^2)	X-Area (m^2)	
		UpStrm	***Dwn Strm***	***Difference***
Chatham 12 Arch	X1	9.42	7.53	-1.89
	X5	14.20	11.62	-2.58
	X10	15.22	16.05	0.82
	X20	12.37	20.40	8.02
	X50	11.20	23.59	12.39
Granville 217 Pipe	X1	24.71	21.84	-2.87
	X5	10.11	11.36	1.25
	X10	7.46	7.93	0.47
	X20	5.90	9.34	3.44
	X50	7.76	10.38	2.62
Orange 4 Bridge	X1	19.53	17.88	-1.65
	X5	9.72	8.51	-1.21
	X10	11.61	6.16	-5.45
	X20	12.14	10.95	-1.19
	X50	10.82	13.31	2.48
Orange 30 Box	X1	13.73	8.63	-5.10
	X5	4.58	9.17	4.58
	X10	9.28	14.37	5.10
	X20	7.68	11.27	3.59
	X50	16.47	12.33	-4.14
Orange 67 Bridge	X1	4.06	1.85	-2.21
	X5	6.27	13.18	6.91
	X10	6.68	10.48	3.80
	X20	5.33	11.87	6.54
	X50	9.28	6.94	-2.34
Person 38 Pipe	X1	8.56	8.70	0.14
	X5	9.37	10.51	1.14
	X10	7.53	8.70	1.17
	X20	10.11	7.57	-2.55
	X50	7.92	6.73	-1.19
Rand 349 Bridge	X1	20.78	25.19	4.41
	X5	16.35	21.67	5.32
	X10	12.33	21.90	9.56
	X20	11.09	21.10	10.00
	X50	13.84	16.09	2.25
Alamance 20 Box	X1	7.23	3.02	-4.21
	X5	30.49	26.81	-3.68
	X10	12.78	7.79	-4.99
	X20	12.73	8.96	-3.77
	X50	9.08	12.21	3.14
Moore 173 Bridge	X1	7.45	13.28	5.83
	X5	9.04	16.10	7.06
	X10	9.96	9.41	-0.55

		X-Area (m^2)	X-Area (m^2)	
		UpStrm	***Dwn Strm***	***Difference***
	X20	14.35	10.81	-3.54
	X50	15.96	9.38	-6.58
Alamance 29 Arch	X1	4.66	17.02	12.36
	X5	7.74	14.42	6.68
	X10	11.70	13.69	1.99
	X20	9.17	10.67	1.49
	X50	7.18	9.67	2.49
Orange 55 Bridge	X1	4.11	3.54	-0.58
	X5	5.39	3.09	-2.31
	X10	5.45	4.02	-1.43
	X20	5.69	6.35	0.66
	X50	3.74	6.28	2.54
Orange 13 Arch	X1	13.71	4.93	-8.77
	X5	19.94	25.30	5.36
	X10	23.56	23.94	0.38
	X20	20.09	34.49	14.40
	X50	14.86	27.92	13.06
Rand 220 Bridge	X1	6.41	2.41	-4.00
	X5	6.51	4.03	-2.48
	X10	5.51	5.30	-0.21
	X20	6.77	7.51	0.74
	X50	5.20	6.43	1.23
Rand 459 Pipe	X1	22.67	21.64	-1.03
	X5	9.72	25.92	16.21
	X10	11.04	17.86	6.82
	X20	8.85	10.25	1.40
	X50	10.01	7.85	-2.16

Hyporheic Depth Measurements

Site Name		Ave Depth UpStrm	Ave Depth Dwn Strm	Difference
Chatham 12 Arch	X1	10.28	10.03	-0.25
	X5	9.14	15.11	5.97
	X10	8.38	23.24	14.86
	X20	16.26	26.92	10.66
	X50	24.64	28.70	4.06
Granville 217 Pipe	X1	21.21	12.83	-8.38
	X5	35.43	9.91	-25.52
	X10	45.85	30.86	-14.99
	X20	31.50	4.06	-27.44
	X50	32.00	13.72	-18.28
Orange 4 Bridge	X1	4.32	7.24	2.92
	X5	1.78	8.96	7.18
	X10	12.57	5.59	-6.98
	X20	8.00	0.00	-8.00
	X50	12.36	4.70	-7.66
Orange 30 Box	X1	29.95	34.04	4.09
	X5	34.42	34.16	-0.26
	X10	25.02	25.27	0.25
	X20	10.79	30.35	19.56
	X50	9.14	22.73	13.59
Orange 67 Bridge	X1	10.41	24.13	13.71
	X5	25.78	17.78	-8.00
	X10	5.72	16.38	10.66
	X20	16.99	8.52	-8.47
	X50	24.77	4.32	-20.45
Person 38 Pipe	X1	42.06	50.32	8.26
	X5	32.65	38.97	6.32
	X10	28.45	19.03	-9.42
	X20	23.74	29.10	5.35
	X50	62.13	43.94	-18.19
Rand 349 Bridge	X1	14.97	6.32	-8.65
	X5	9.03	12.32	3.29
	X10	9.29	11.68	2.39
	X20	8.90	6.45	-2.45
	X50	14.45	12.58	-1.87
Alamance 20 Box	X1	30.13	33.16	3.03
	X5	31.74	13.74	-18.00
	X10	25.68	11.03	-14.65
	X20	34.26	18.06	-16.19
	X50	30.13	**22.97**	-7.16
Moore 173 Bridge	X1	19.55	6.32	-13.23

		Ave Depth	Ave Depth	
		UpStrm	*Dwn Strm*	*Difference*
	X5	20.32	5.10	-15.23
	X10	18.65	0.77	-17.87
	X20	31.23	3.55	-27.68
	X50	12.71	5.74	-6.97
Alamance 29 Arch	X1	15.87	21.61	5.74
	X5	**18.52**	7.35	-11.16
	X10	25.94	9.16	-16.77
	X20	27.81	17.81	-10.00
	X50	8.90	28.06	19.16
Orange 55 Bridge	X1	15.74	13.87	-1.87
	X5	26.39	19.81	-6.58
	X10	13.87	20.06	6.19
	X20	25.16	12.71	-12.45
	X50	26.90	22.00	-4.90
Orange 13 Arch	X1	10.52	9.42	-1.10
	X5	30.32	8.26	-22.06
	X10	38.13	24.77	-13.35
	X20	11.68	**45.48**	33.81
	X50	15.35	4.97	-10.39
Rand 220 Bridge	X1	21.10	9.42	-11.68
	X5	19.16	9.81	-9.35
	X10	3.94	10.06	6.13
	X20	11.03	12.45	1.42
	X50	8.77	20.19	11.42
Rand 459 Pipe	X1	20.84	30.00	9.16
	X5	23.23	24.39	1.16
	X10	44.84	18.13	-26.71
	X20	18.65	31.36	12.71
	X50	25.01	36.83	11.82

Riffle Areas

Site	***Up/Dwn***	***Total (ft ^2)***	***Total (m^2)***
Chatham 12	Up	4520.59	1377.89259
Chatham 12	Dwn	1782.27	543.242502
Granville 217	Up	568.33	173.22909
Granville 217	Dwn	2866.66	873.768593
Orange 4	Up	1213.31	369.821385
Orange 4	Dwn	0	0
Orange 30	Up	1129.49	344.272738
Orange 30	Dwn	0	0
Orange 67	Up	464.13	141.468544
Orange 67	Dwn	942.39	287.243965
Person 38	Up	248.93	75.8747866
Person 38	Dwn	0	0
Rand 349	Up	0	0
Rand 349	Dwn	0	0
Alamance 20	Up	0	0
Alamance 20	Dwn	0	0
Moore 173	Up	0	0
Moore 173	Dwn	1633.83	497.99744
Alamance 29	Up	562.86	171.561814
Alamance 29	Dwn	461.78	140.752256
Orange 55	Up	2290.71	698.216898
Orange 55	Dwn	2697.61	822.241526
Orange 13	Up	4796.32	1461.93611
Orange 13	Dwn	1325.66	404.066081
Rand 220	Up	432.41	131.800171
Rand 220	Dwn	382.84	116.691051
Rand 459	Up	709.78	216.343575
Rand 459	Dwn	1430.82	436.119239

Substrate Areas

			T0		T1	
			Area	Type	Area	Type
Chatham 12	Up Strm	FT^2	2013.9	Bedrock w/Gravel	2566.02	Bedrock w/Sand
		M^2	613.8442		782.132	
	Dwn Strm	FT^2	1118.46	Gravel w/Cobble	2447.96	Cobble w/Gravel
		M^2	340.9108		746.147	
Granville 217Pipe	Up Strm	FT^2	371.6	Sand w/Cobble	495.59	Cobble w/Sand
		M^2	113.2651		151.058	
	Dwn Strm	FT^2	866.47	Sand w/Cobble	887.47	Cobble w/Gravel
		M^2	264.1033		270.504	
Orange 4 Bridge	Up Strm	FT^2	1393.15	Bedrock	2062.81	Bedrock w/Cobble
		M^2	424.6373		628.752	
	Dwn Strm	FT^2	1929.91	Bedrock w/Gravel	1839.57	Bedrock w/ Cobble
		M^2	588.2437		560.708	
Orange 30 Box	Up Strm	FT^2	994.09	Cobble w/Gravel	1098.02	Gravel w/Sand
		M^2	303.0023		334.681	
	Dwn Strm	FT^2	1039.88	Sand w/Cobble	1074.22	Cobble w/Gravel
		M^2	316.9593		327.426	
Orange 67 Bridge	Up Strm	FT^2	985.42	Bedrock w/Cobble	1029.07	Cobble w/Gravel
		M^2	300.3597		313.664	
	Dwn Strm	FT^2	326.19	Cobble w/Sand	980.16	Cobble w/Gravel
		M^2	99.42392		298.756	
Person 38	Up Strm	FT^2	887.05	Cobble w/Gravel	2070.61	Gravel w/Cobble
		M^2	270.3761		631.13	
	Dwn Strm	FT^2	750.68	Sand w/Gravel	1552.02	Gravel w/Cobble
		M^2	228.81		473.061	
Rand 349 Bridge	Up Strm	FT^2	900.11	Cobble w/Bedrock	458.92	Cobble w/Gravel
		M^2	274.3569		139.881	
	Dwn Strm	FT^2	5899.28	Bedrock w/Cobble	993.1	Bedrock w/Sand
		M^2	1798.122		302.701	
4 Box 87	Up Strm	FT^2	1027.64	Sand w/Cobble	585.78	Gravel w/Cobble
		M^2	313.2285		178.548	
	Dwn Strm	FT^2	285.9	Sand	3211.1	Cobble w/Gravel
		M^2	87.14338		978.755	
Moore 173	Up Strm	FT^2	2916.84	Gravel w/Sand	1561.19	Cobble w/Gravel
		M^2	889.0636		475.856	
	Dwn Strm	FT^2	19991.65	Cobble w/Bedrock	256.66	
		M^2	6093.529		78.2309	
Orange 29 Arch	Up Strm	FT^2	558.34	Bedrock	887.64	Bedrock w/Cobble
		M^2	170.1841		270.556	
	Dwn Strm	FT^2	2376.69	Sand	928.08	Cobble w/ Gravel
		M^2	724.4239		282.882	
Orange 55 Br	Up Strm	FT^2	449.99	Cobble w/Bedrock	308.2	Cobble
		M^2	137.1586		93.9405	
	Dwn Strm	FT^2	875.67	Cobbel w/Bedrock	3970.73	Cobble
		M^2	266.9075		1210.29	
Orange Cnt 57	Up Strm	FT^2	759.58	Cobble w/Sand	469.51	Bedrock w/Sand
		M^2	231.5228		143.108	
	Dwn Strm	FT^2	1702.32	Bedrock w/Gravel	862.11	Bedrock w/Cobble

		M^2	518.8734		262.774	
Rand 10 Brd	Up Strm	FT^2	1899.81	Bedrock w/Gravel	1068.05	Bedrock w/ Cobble
		M^2	579.0691		325.546	
	Dwn Strm	FT^2	974.78	Bedrock w/Gavel	360.43	Bedrock
		M^2	297.1166		109.86	
Rand 459	Up Strm	FT^2	3269.61		523.42	
		M^2	996.5892		159.54	
	Dwn Strm	FT^2	1833.75	Sand w/Gravel	1002.13	Gravel w/Sand
		M^2	558.9338		305.453	

Site Name			T2		T3	
			Area	**Type**	**Area**	**Type**
Chatham 12	Up Strm	FT^2	1112.98	Cobble w/Sand	1249.54	Gravel w/Cobble
		M^2	339.2404		380.864	
	Dwn Strm	FT^2	2077.73	Gravel w/Cobble	1930.85	Cobble w/Gravel
		M^2	633.2998		588.53	
Granville 217Pipe	Up Strm	FT^2	2076.22	Cobble	151.41	Cobble w/Gravel
		M^2	632.8396		46.1503	
	Dwn Strm	FT^2	733.17	Cobble w/Bedrock	1069.12	Cobble
		M^2	223.4729		325.872	
Orange 4 Bridge	Up Strm	FT^2	1994.74	Bedrock w/Sand	2626.35	Bedrock w/ Gravel
		M^2	608.0041		800.521	
	Dwn Strm	FT^2	116.29	Bedrock w/Gravel	298.69	Bedrock
		M^2	35.44562		91.0418	
Orange 30 Box	Up Strm	FT^2	659.49	Bedrock w/Gravel	795.54	Gravel w/Sand
		M^2	201.015		242.484	
	Dwn Strm	FT^2	3037.05	Cobble w/Bedrock	2662.69	Sand
		M^2	925.7041		811.598	
Orange 67 Bridge	Up Strm	FT^2	645.17	Cobble w/Sand	546.98	Sand w/Cobble
		M^2	196.6502		166.722	
	Dwn Strm	FT^2	2984.47	Cobble w/Bedrock	1278.6	Cobble w/Gravel
		M^2	909.6775		389.722	
Person 38	Up Strm	FT^2	3776.11	Gravel w/Sand		
		M^2	1150.972			
	Dwn Strm	FT^2	1164.7	Gravel w/Sand	819.96	Sand w/Gravel
		M^2	355.0049		249.927	
Rand 349 Bridge	Up Strm	FT^2	1637.67	Cobble w/Bedrock	2405.68	Bedrock w/Cobble
		M^2	499.1679		733.26	
	Dwn Strm	FT^2	2101.79	Bedrock		
		M^2	640.6334			
4 Box 87	Up Strm	FT^2	346.47	Cobble w/Gravel	571.05	Gravel w/Cobble
		M^2	105.6053		174.058	
	Dwn Strm	FT^2	866.18	Cobble w/Gravel	681.43	Sand
		M^2	264.0149		207.702	
Moore 173	Up Strm	FT^2	1288.88	Cobble w/Bedrock	1528.48	Cobble
		M^2	392.8554		465.886	
	Dwn Strm	FT^2	325.69		1296.1	
		M^2	99.27152		395.056	
Orange 29 Arch	Up Strm	FT^2	549.24	Bedrock w/Sand	711.96	Gravel w/Sand

		M^2	167.4104		217.008	
	Dwn Strm	FT^2	527.53	Cobble w/Sand	1192.65	Gravel w/Sand
		M^2	160.7931		363.524	
Orange 55 Br	Up Strm	FT^2	2123.88	Cobble w/Gravel	751.69	Cobble
		M^2	647.3665		229.118	
	Dwn Strm	FT^2	1100.99	Cobble w/Gravel		
		M^2	335.5858			
Orange Cnt 57	Up Strm	FT^2	1043.19	Sand	1764.49	Bedrock w/Cobble
		M^2	317.9682		537.823	
	Dwn Strm	FT^2	3316.38	Sand w/Cobble	2724.16	Bedrock w/Gravel
		M^2	1010.845		830.334	
Rand 10 Brd	Up Strm	FT^2	1637.89	Cobble w/Gravel		
		M^2	499.2349			
	Dwn Strm	FT^2	1252.39	Bedrock w/Sand	1210.08	Sand w/Gravel
		M^2	381.7331		368.837	
Rand 459	Up Strm	FT^2	1875.97		370.55	
		M^2	571.8026		112.945	
	Dwn Strm	FT^2	1475.83	Gravel	420.69	Sand
		M^2	449.8385		128.228	

			T4		T5	
Site Name			**Area**	**Type**	**Area**	**Type**
Chatham 12	Up Strm	FT^2	3281.64	Cobble w/Gravel		Cobble w/Bedrock
		M^2	1000.256			
	Dwn Strm	FT^2	2336.28	Gravel w/Cobble		
		M^2	712.1068			
Granville 217Pipe	Up Strm	FT^2	2992.29	Cobble w/Sand		
		M^2	912.0611			
	Dwn Strm	FT^2	1302.45	Cobble w/Sand	998.07	Gravel w/Cobble
		M^2	396.9916		304.215	
Orange 4 Bridge	Up Strm	FT^2				
		M^2				
	Dwn Strm	FT^2	1046.52	Bedrock w/Gravel	1282.68	Bedrock w/Sand
		M^2	318.9832		390.966	
Orange 30 Box	Up Strm	FT^2	1166.45	Bedrock w/Sand	583.38	Gravel w/Cobble
		M^2	355.5383		177.816	
	Dwn Strm	FT^2				
		M^2				
Orange 67 Bridge	Up Strm	FT^2	2358.7	Cobble w/Sand		Sand
		M^2	718.9405			
	Dwn Strm	FT^2	1463.32	Cobble w/ Bedrock	1137.41	Cobble w/Bedrock
		M^2	446.0254		346.687	
Person 38	Up Strm	FT^2				
		M^2				
	Dwn Strm	FT^2	1500.92	Sand w/Gravel		
		M^2	457.486			
Rand 349 Bridge	Up Strm	FT^2	498.84	Cobble	404.35	Sand w/Cobble
		M^2	152.0483		123.247	
	Dwn Strm	FT^2				

		M^2				
4 Box 87	Up Strm	FT^2	548.5	Sand w/Cobble	757.78	Gravel w/Cobble
		M^2	167.1848		230.974	
	Dwn Strm	FT^2	1120.71			
		M^2	341.5966			
Moore 173	Up Strm	FT^2				
		M^2				
	Dwn Strm	FT^2	834.24		757.95	
		M^2	254.2794		231.026	
Orange 29 Arch	Up Strm	FT^2	1222.71	Cobble w/Gravel	1877.6	Cobble w/Bedrock
		M^2	372.6865		572.299	
	Dwn Strm	FT^2	1594.75	Gravel		Gravel w/Cobble
		M^2	486.0857			
Orange 55 Br	Up Strm	FT^2	938.13	Gravel w/Cobble	612.14	Cobble
		M^2	285.9455		186.583	
	Dwn Strm	FT^2				
		M^2				
Orange Cnt 57	Up Strm	FT^2	2026.62	Bedrock w/Cobble	2451.12	Cobble w/Bedrock
		M^2	617.7213		747.11	
	Dwn Strm	FT^2	3978.68	Bedrock w/Sand		
		M^2	1212.716			
Rand 10 Brd	Up Strm	FT^2				
		M^2				
	Dwn Strm	FT^2	914.81	Bedrock w/Gravel		
		M^2	278.8375			
Rand 459	Up Strm	FT^2				
		M^2				
	Dwn Strm	FT^2	1022.59	Gravel	521.15	Sand w/Gravel
		M^2	311.6892		158.848	

Site Name			T6		T7	
			Area	**Type**	**Area**	**Type**
Chatham 12	Up Strm	FT^2				
		M^2				
	Dwn Strm	FT^2				
		M^2				
Granville 217Pipe	Up Strm	FT^2				
		M^2				
	Dwn Strm	FT^2	597.12			
		M^2	182.0044			
Orange 4 Bridge	Up Strm	FT^2				
		M^2				
	Dwn Strm	FT^2	738.9	Bedrock	386.78	
		M^2	225.2195		117.892	
Orange 30 Box	Up Strm	FT^2	445.26	Cobble w/Gravel	680.27	Gravel w/Cobble
		M^2	135.7169		207.349	
	Dwn Strm	FT^2				
		M^2				
Orange 67 Bridge	Up Strm	FT^2				

		M^2				
	Dwn Strm	FT^2	684.88	Cobble w/Gravel		Bedrock w/ Cobble
		M^2	208.754			
Person 38	Up Strm	FT^2				
		M^2				
	Dwn Strm	FT^2				
		M^2				
Rand 349 Bridge	Up Strm	FT^2	2121.52	Cobble w/Bedrock		
		M^2	646.6472			
	Dwn Strm	FT^2				
		M^2				
4 Box 87	Up Strm	FT^2	1347.78	Sand w/Cobble	554.54	Cobble w/Sand
		M^2	410.8083		169.026	
	Dwn Strm	FT^2				
		M^2				
Moore 173	Up Strm	FT^2				
		M^2				
	Dwn Strm	FT^2	1382.42			
		M^2	421.3667			
Orange 29 Arch	Up Strm	FT^2				
		M^2				
	Dwn Strm	FT^2				
		M^2				
Orange 55 Br	Up Strm	FT^2	836.25	Cobble w/Gravel		Cobble
		M^2	254.8921			
	Dwn Strm	FT^2				
		M^2				
Orange Cnt 57	Up Strm	FT^2	1856.86	Bedrock w/Cobble		
		M^2	565.9778			
	Dwn Strm	FT^2				
		M^2				
Rand 10 Brd	Up Strm	FT^2				
		M^2				
	Dwn Strm	FT^2				
		M^2				
Rand 459	Up Strm	FT^2				
		M^2				
	Dwn Strm	FT^2	829.61	Gravel		
		M^2	252.8682			

Site Name			**T8**		**Total**	
			Area	**Type**		
Chatham 12	Up Strm	FT^2			8210.18	FT^2
		M^2			**2502.493**	**M^2**
	Dwn Strm	FT^2			8792.82	FT^2
		M^2			**2680.084**	**M^2**
Granville 217Pipe	Up Strm	FT^2			5715.51	FT^2
		M^2			**1742.109**	**M^2**
	Dwn Strm	FT^2			5587.4	FT^2
		M^2			**1703.06**	**M^2**

Orange 4 Bridge	Up Strm	FT^2			6683.9	FT^2
		M^2			**2037.277**	**M^2**
	Dwn Strm	FT^2			5709.43	FT^2
		M^2			**1740.255**	**M^2**
Orange 30 Box	Up Strm	FT^2	571.19	Gravel/Cobble/Bedrock	5999.6	FT^2
		M^2	174.1		**1828.7**	**M^2**
	Dwn Strm	FT^2			6773.96	FT^2
		M^2			**2064.728**	**M^2**
Orange 67 Bridge	Up Strm	FT^2			4579.92	FT^2
		M^2			**1395.977**	**M^2**
	Dwn Strm	FT^2			8528.84	FT^2
		M^2			**2599.622**	**M^2**
Person 38	Up Strm	FT^2			5846.72	FT^2
		M^2			**1782.102**	**M^2**
	Dwn Strm	FT^2			5037.6	FT^2
		M^2			**1535.479**	**M^2**
Rand 349 Bridge	Up Strm	FT^2			7526.98	FT^2
		M^2			**2294.251**	**M^2**
	Dwn Strm	FT^2			3094.89	FT^2
		M^2			**943.3339**	**M^2**
4 Box 87	Up Strm	FT^2			4711.9	FT^2
		M^2			**1436.205**	**M^2**
	Dwn Strm	FT^2			5879.42	FT^2
		M^2			**1792.069**	**M^2**
Moore 173	Up Strm	FT^2			4378.55	FT^2
		M^2			**1334.598**	**M^2**
	Dwn Strm	FT^2			4853.06	FT^2
		M^2			**1479.231**	**M^2**
Orange 29 Arch	Up Strm	FT^2			5249.15	FT^2
		M^2			**1599.96**	**M^2**
	Dwn Strm	FT^2			4243.01	FT^2
		M^2			**1293.285**	**M^2**
Orange 55 Br	Up Strm	FT^2			5570.29	FT^2
		M^2			**1697.845**	**M^2**
	Dwn Strm	FT^2			5071.72	FT^2
		M^2			**1545.879**	**M^2**
Orange Cnt 57	Up Strm	FT^2			9611.79	FT^2
		M^2			**2929.709**	**M^2**
	Dwn Strm	FT^2			10881.33	FT^2
		M^2			**3316.67**	**M^2**
Rand 10 Brd	Up Strm	FT^2			2705.94	FT^2
		M^2			**824.7805**	**M^2**
	Dwn Strm	FT^2			3737.71	FT^2
		M^2			**1139.268**	**M^2**
Rand 459	Up Strm	FT^2			2769.94	FT^2
		M^2			**844.288**	**M^2**
	Dwn Strm	FT^2			5272	FT^2
		M^2			**1606.925**	**M^2**

APPENDIX II:
A comparison of the effects of bridges and culverts on mussels and their habitat:

Individual Site Summaries

Culvert Summaries:

The following represents a summary of each individual site surveyed during the culvert portion of our study from 2004 to 2005. We present mussel and habitat data for each site and offer our best estimate as to the amount of damage the culvert did to the stream channel and its mussel fauna. We roughly categorized stream alterations into high impact, low impact, and no detected impact. A culvert fell into the high impact category if it significantly impacted either habitat or relative mussel abundance in the downstream reaches for more than 50 meters. Low impact sites were those which we only detected obvious impact within 50 meters of the crossing structure or more subtle impacts over a greater distance. Culverts which fell into the no detected impact category may have either truly had no impact or overall stream conditions at the site made it difficult to know how much the structure impacted the site. These classifications are only best guesses based on 1 mussel survey and general habitat measurements and observations. The classification of any individual culverts should not be used to project potential impacts at other locations not surveyed. Each site is different and should be assessed separately. The sites are organized first by general soil system then alphabetically by county.

MIXED FELSIC AND MAFIC SYSTEM

Impact	County – Bridge Number	Culvert Type
High	Guilford 608	Box
Low	Granville 254 Granville 268 Granville 9	Box Box Pipe
None Detected	Alamance 204 Alamance 338 Granville 29 Guilford 190 Randolph 463	Pipe Box Box Arch Box

County:	Alamance	**Bridge Number:**	204
Road Crossing:	Friendship-Patterson Road	**Stream:**	Rock Creek
Date Sampled:	19 August 2004		

Mussels found at site			
Elliptio complanata	142	**Year Built:**	1978
V. constricta	1	**Number of Cells: (w/ base flow)**	3(1)
V. vaughaniana	1	**Obvious scour hole?**	No
Total mussels	**144**	**% of mussels upstream:**	29.8%

Summary:

Overall, habitat at this site is not well suited for mussels. It is characterized by coarse sand, and the gradient is relatively high for a sandy stream. This results in very little habitat complexity and not much in the way of stable microhabitats suitable for mussel colonization. Hence, mussel distribution is very patchy, and relies on localized natural stream features that can stabilize adjacent substrates. There were no obvious impacts to stream habitat or relative mussel abundance downstream.

Figure 1. habitat downstream

Figure 2. downstream view of the culvert

Figure 3. upstream view of the culvert

Figure 4. Upstream habitat

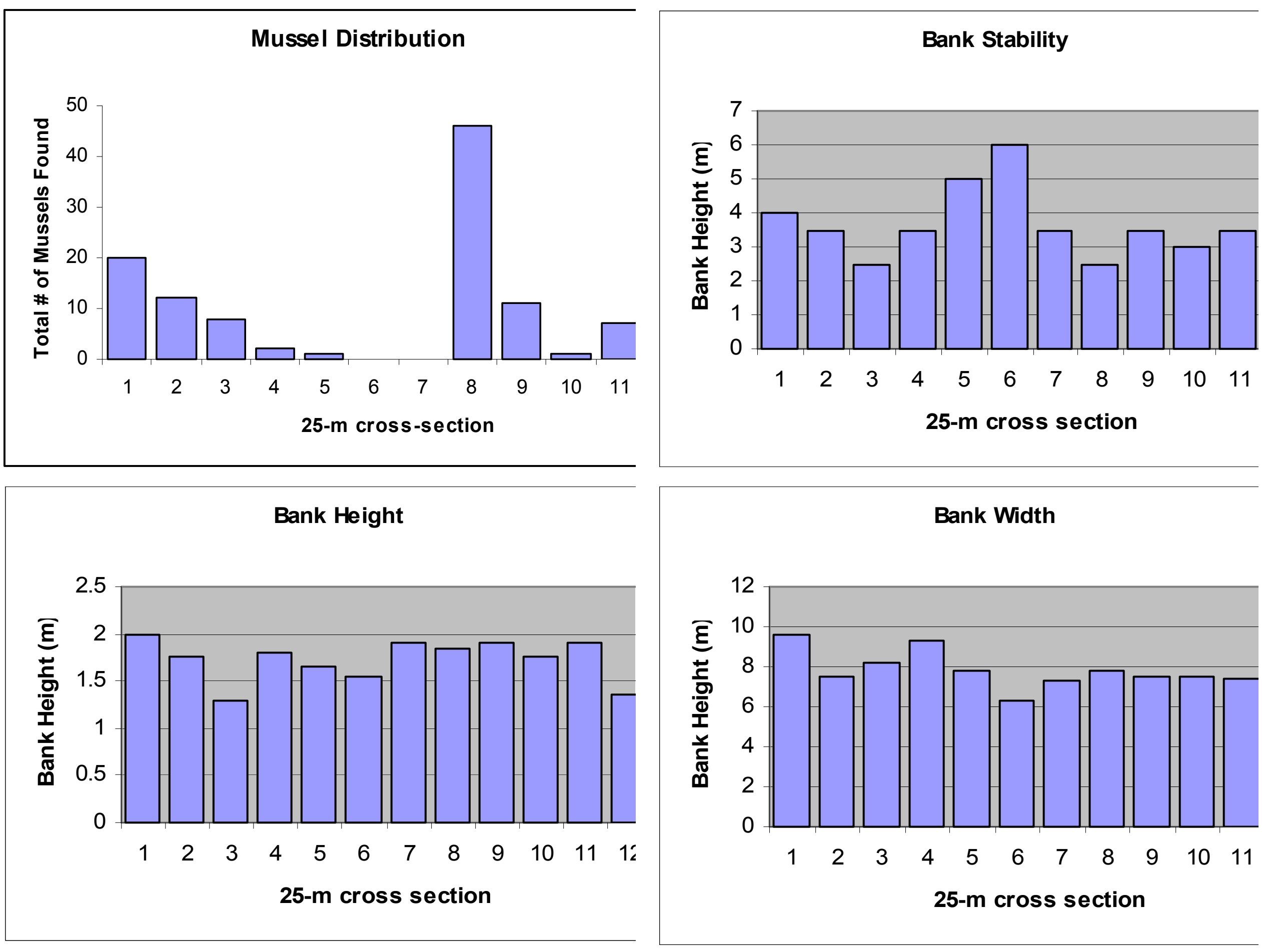


Figure 5. Mussel distribution and habitat data from this site.

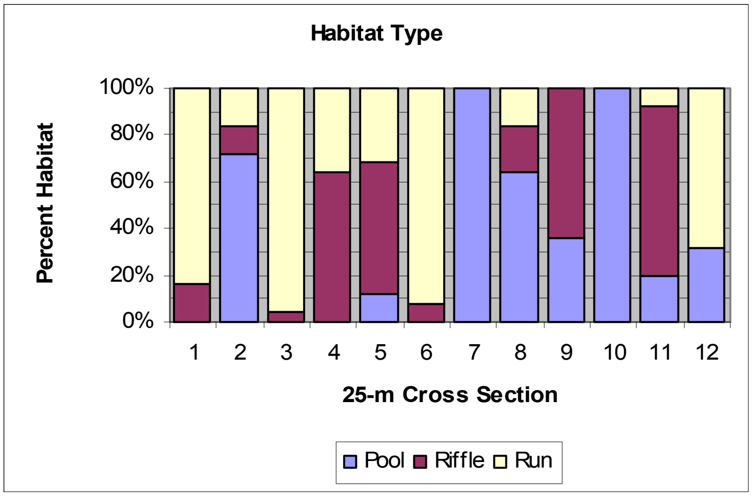


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Alamance	**Bridge Number:**	338
Road Crossing:	Foster's Store Rd.	**Stream:**	Pawpaw Creek
Date Sampled:	26 July 2004		

Mussels found at site			
Elliptio complanata	898	**Year Built:**	1960
P. cataracta	12	**Number of Cells: (w/ base flow)**	3(2)
S. undulatus	1	**Obvious scour hole?**	No
V. delumbis	1	**% of mussels upstream:**	64.0%
V. vaughaniana	1		
Total mussels	**913**		

Summary:

This is a very rocky stream with minimal fine substrate. A mill pond just upstream of the study site likely accounts for this. Banks are very stable, and the culvert seems to have little impact on the physical habitat.

Figure 1. Upstream

Figure 2. Upstream

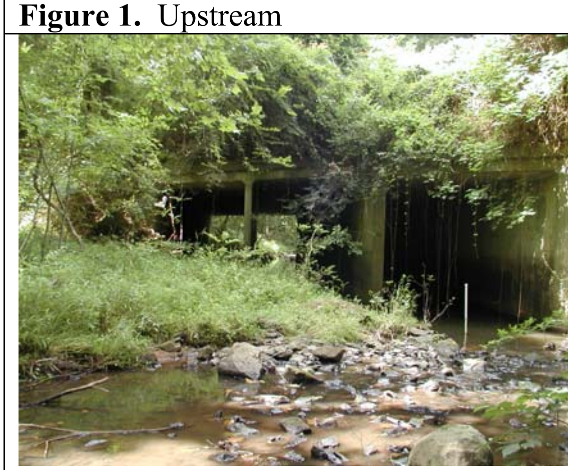


Figure 3. Upstream side of culvert

Figure 4. Downstream side of culvert

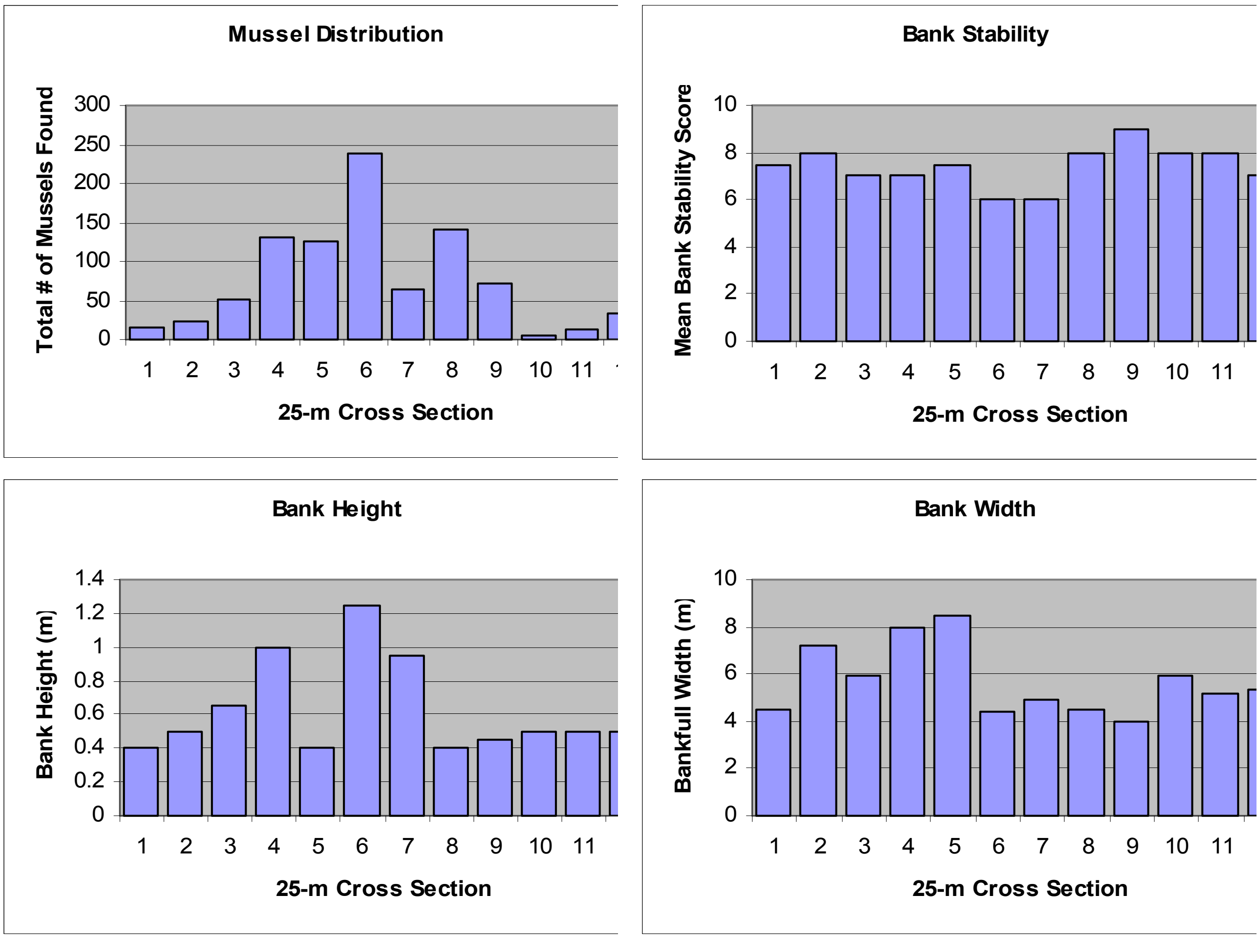


Figure 5. Mussel distribution and habitat data from this site.

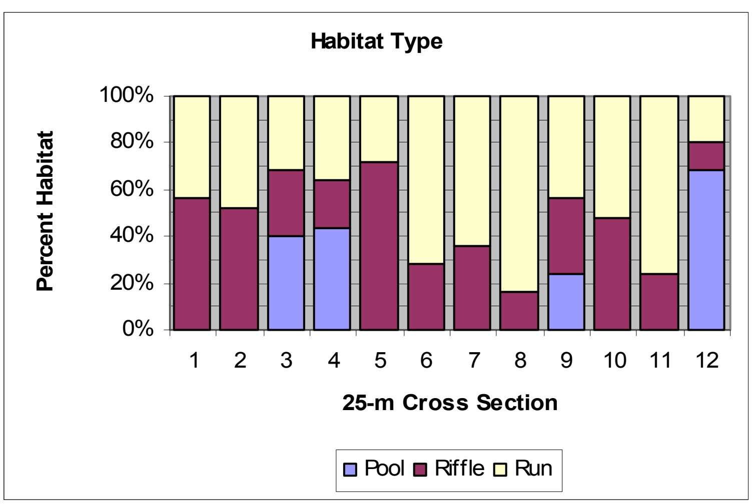


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Granville	**Bridge Number:**	254
Road Crossing:	Salem Road	**Stream:**	Coon Creek
Date Sampled:	16 July 2004		

Mussels found at site			
Elliptio complanata	103	**Year Built:**	1931
		Number of Cells: (w/ base flow)	3(3)
Total mussels	**103**	**Obvious scour hole?**	Yes
		% of mussels upstream:	27.2%

Summary:

This site had marginal habitat overall, so the impact of the culvert on mussel density was somewhat difficult to quantify. Bank width was significantly greater downstream (Fig. 5) and there was some evidence of erosion and deposition downstream. A majority of the mussels at the site were located in an area along the left descending bank that seemed to be somewhat of a refuge from high flow events. The erosion and deposition immediately downstream of the culvert seemed to alter the channel in a way that created refuge just downstream.

Figure 1. Upstream habitat

Figure 2. Debris upstream of culvert

Figure 3. Downstream habitat	**Figure 4.** Downstream bank erosion

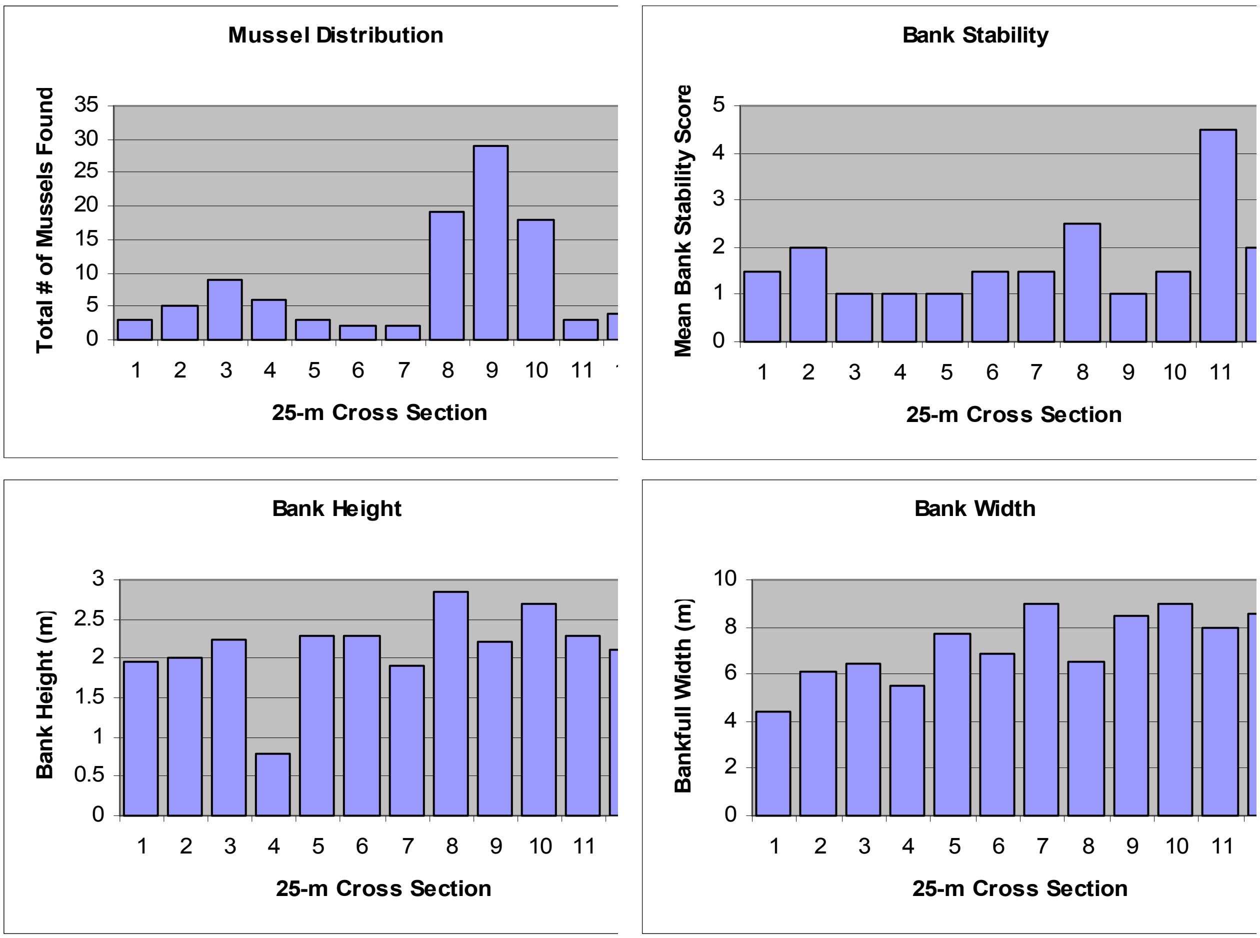


Figure 5. Mussel distribution and habitat data from this site.

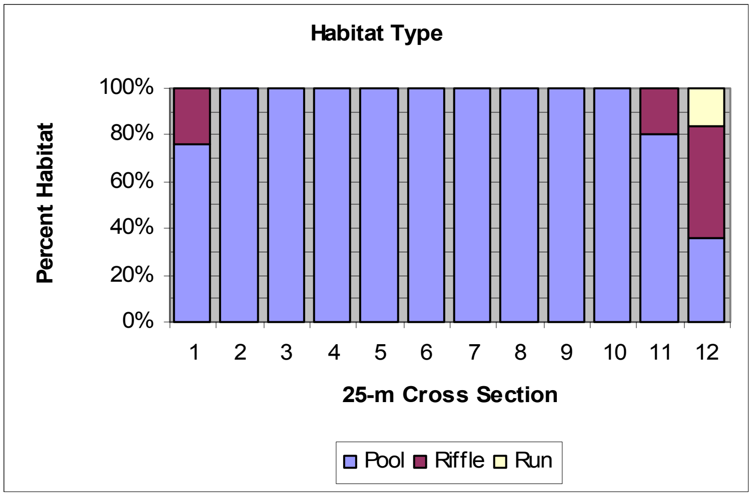


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Granville	**Bridge Number:**	268
Road Crossing:	US 158	**Stream:**	Coon Creek
Date Sampled:	10 June 2004 & 27 May 2005		

Mussels found at site (2004)			
Elliptio complanata	187	**Year Built:**	1931
P. cataracta	1	**Number of Cells: (w/ base flow)**	2(2)
Total mussels	**188**	**Obvious scour hole?**	Yes
		% of mussels upstream:	71.3%

Summary:

Mussel distribution at this site would indicate some disturbance downstream of the culvert, but our habitat metrics did not indicate any significant alterations to the channel. Since the site was characterized by shifting sands, mussels were patchy and primarily located in stable refugia in certain places along the bank. We generally observed few of these refugia downstream, but we cannot be certain it was due to the presence of the culvert. The area immediately downstream of the culvert seemed to be altered since trees had been cleared and the creek seemed somewhat channelized (Fig. 2), although this was not necessarily caused by the culvert itself but was likely just associated work (perhaps utility work) because of the presence of the road. We suspect the structure and these channel alterations downstream (Fig. 2) did contribute somewhat to the lack of habitat downstream.

Figure 1. Upstream habitat

Figure 2. Downstream side of culvert

Figure 3. Downstream habitat

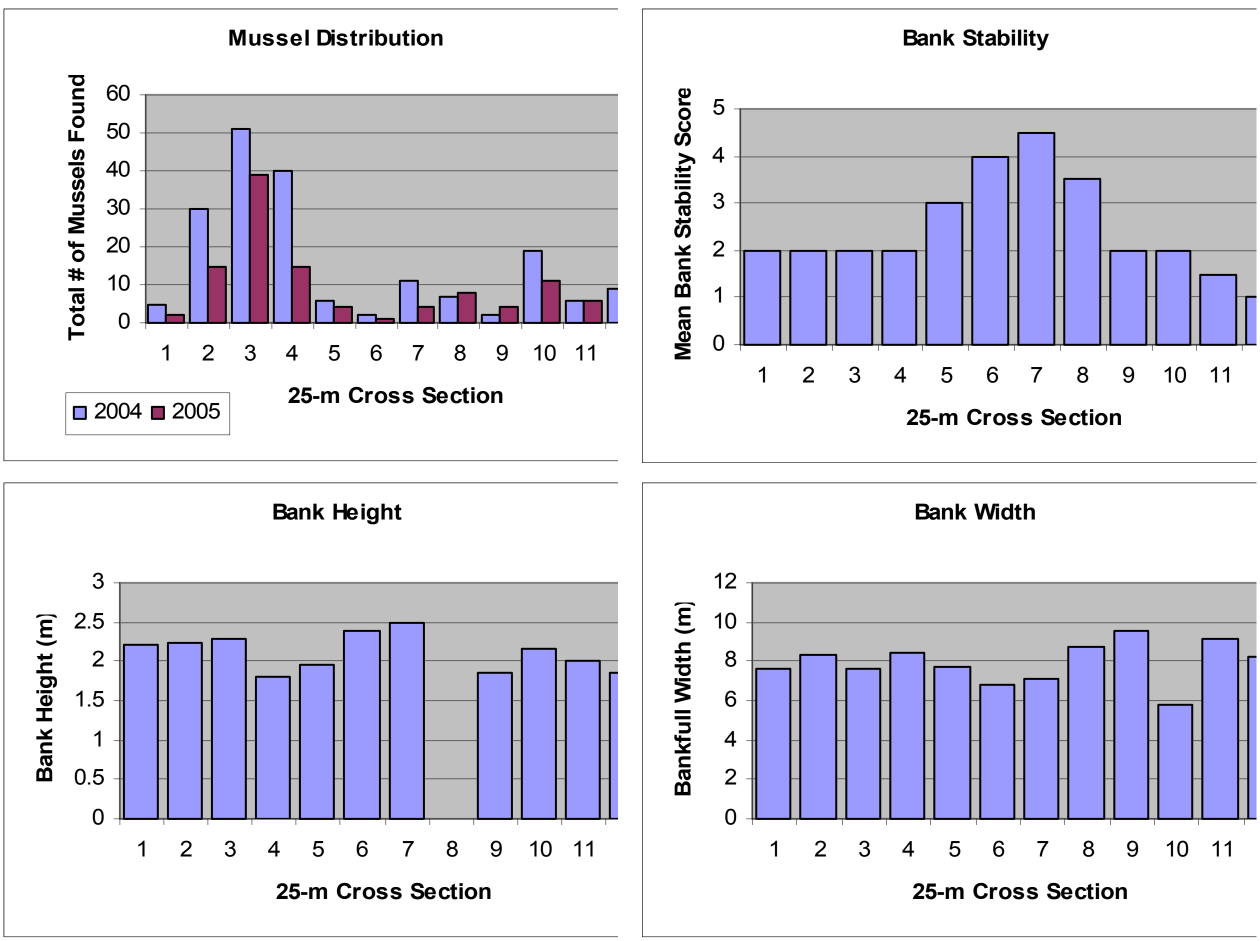


Figure 4. Mussel distribution and habitat data from this site.

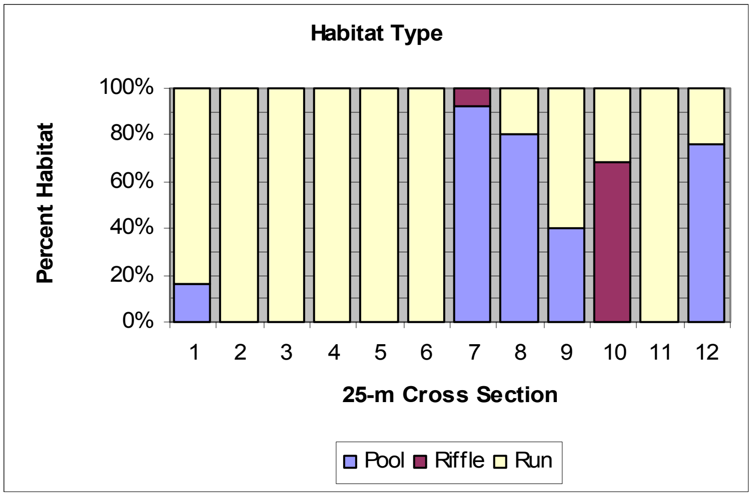


Figure 5. Percentage of habitat type within each 25-m cross section.

County: Granville **Bridge Number:** 29
Road Crossing: NC Business 158 **Stream:** Coon Creek
Date Sampled: 8 July 2004

Mussels found at site			
Elliptio complanata	77	**Year Built:**	1950
P. cataracta	11	**Number of Cells: (w/ base flow)**	6(3)
Total mussels	**88**	**Obvious scour hole?**	No
		% of mussels upstream:	68.2%

Summary:

The stream at this site generally had poor mussel habitat. There were only a few places along the banks that remained stable where mussels could live. The majority of the channel tended to be shifting sands. There were relatively few mussels near the culvert, but the general poor habitat at the site made it difficult to determine the full effects of the culvert.

Figure 1. Upstream habitat

Figure 2. Upstream side of culvert

Figure 3. Downstream side of culvert

Figure 4. Downstream habitat

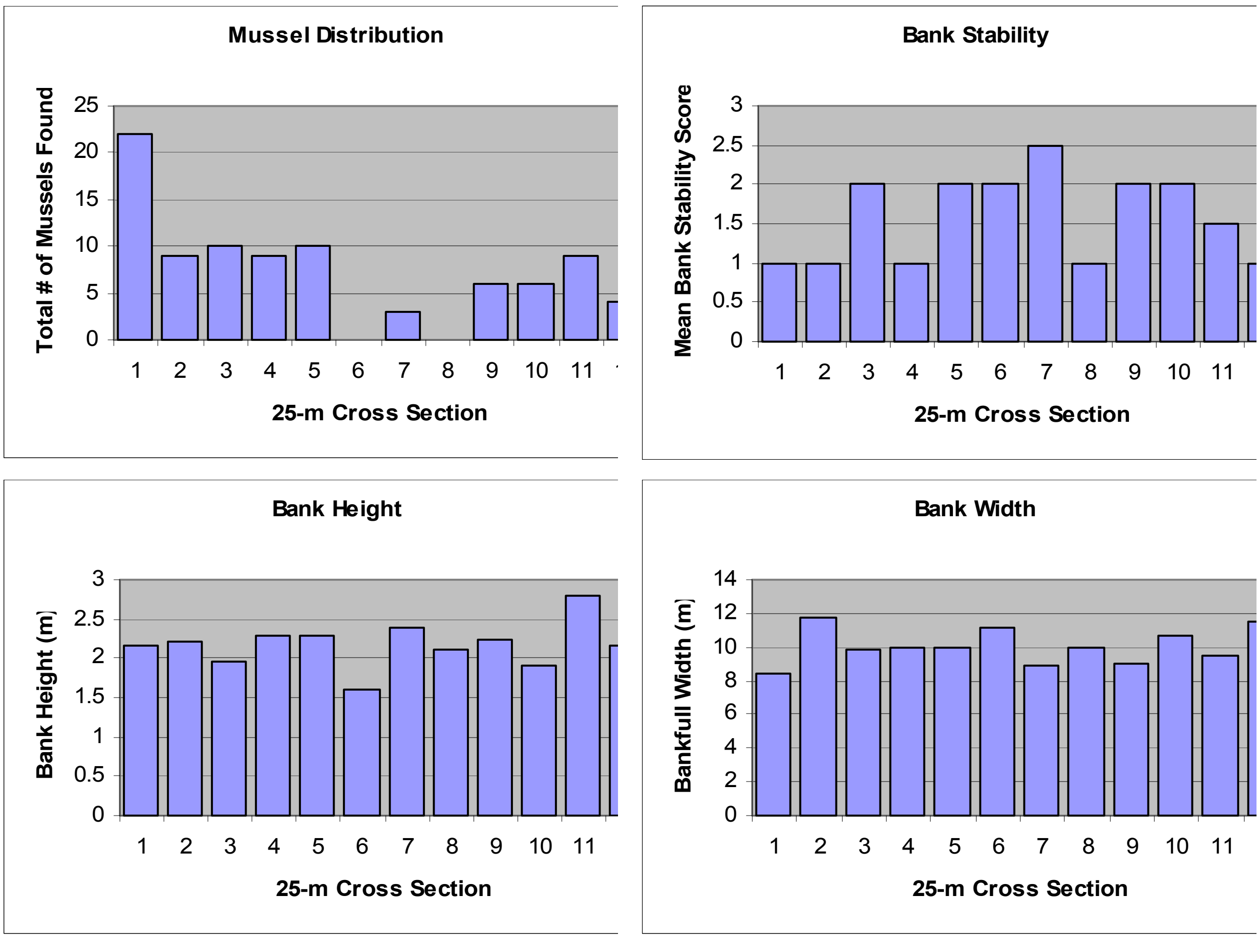


Figure 5. Mussel distribution and habitat data from this site.

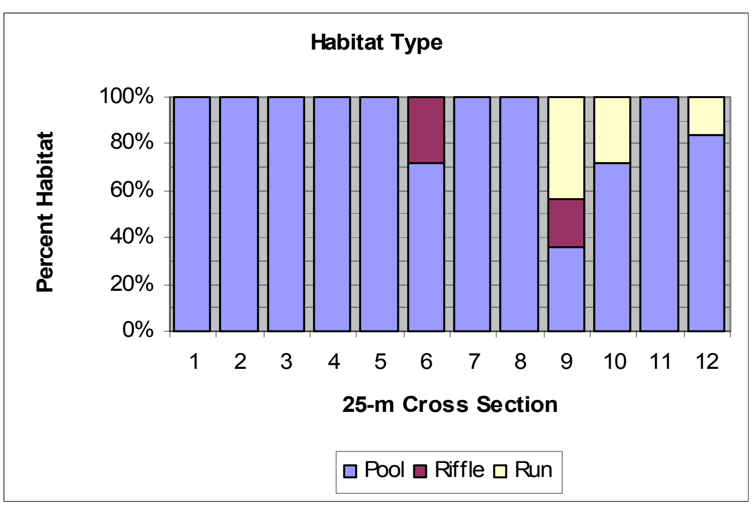


Figure 6. Percentage of habitat type within each 25-m cross section.

County: Granville **Bridge Number:** 9
Road Crossing: Mountain Road **Stream:** Coon Creek
Date Sampled: 7 July 2004

Mussels found at site			
Elliptio complanata	1297	**Year Built:**	1989
		Number of Cells: (w/ base flow)	3(1)
Total mussels	**1297**	**Obvious scour hole?**	Yes
		% of mussels upstream:	52%

Summary:

Mussels were fairly evenly distributed throughout this site except for the 25-meter reach immediately downstream of the culvert that contained few mussels. Channel width was greatly increased in the first 50 meters downstream of the culvert (Fig. 5). The culvert had caused significant scour in this area (Fig. 4). The rocky nature of the habitat overall (Figs. 1, 2) may have limited the habitat damage to the area near the culvert.

Figure 1. Upstream habitat

Figure 2. Upstream side of culvert

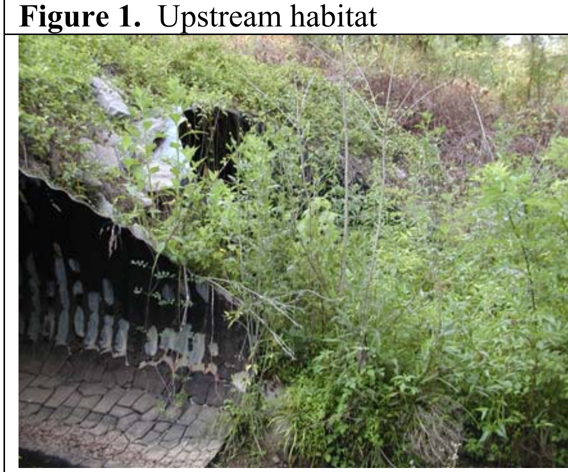


Figure 3. Downstream - perched culvert

Figure 4. Downstream scour hole

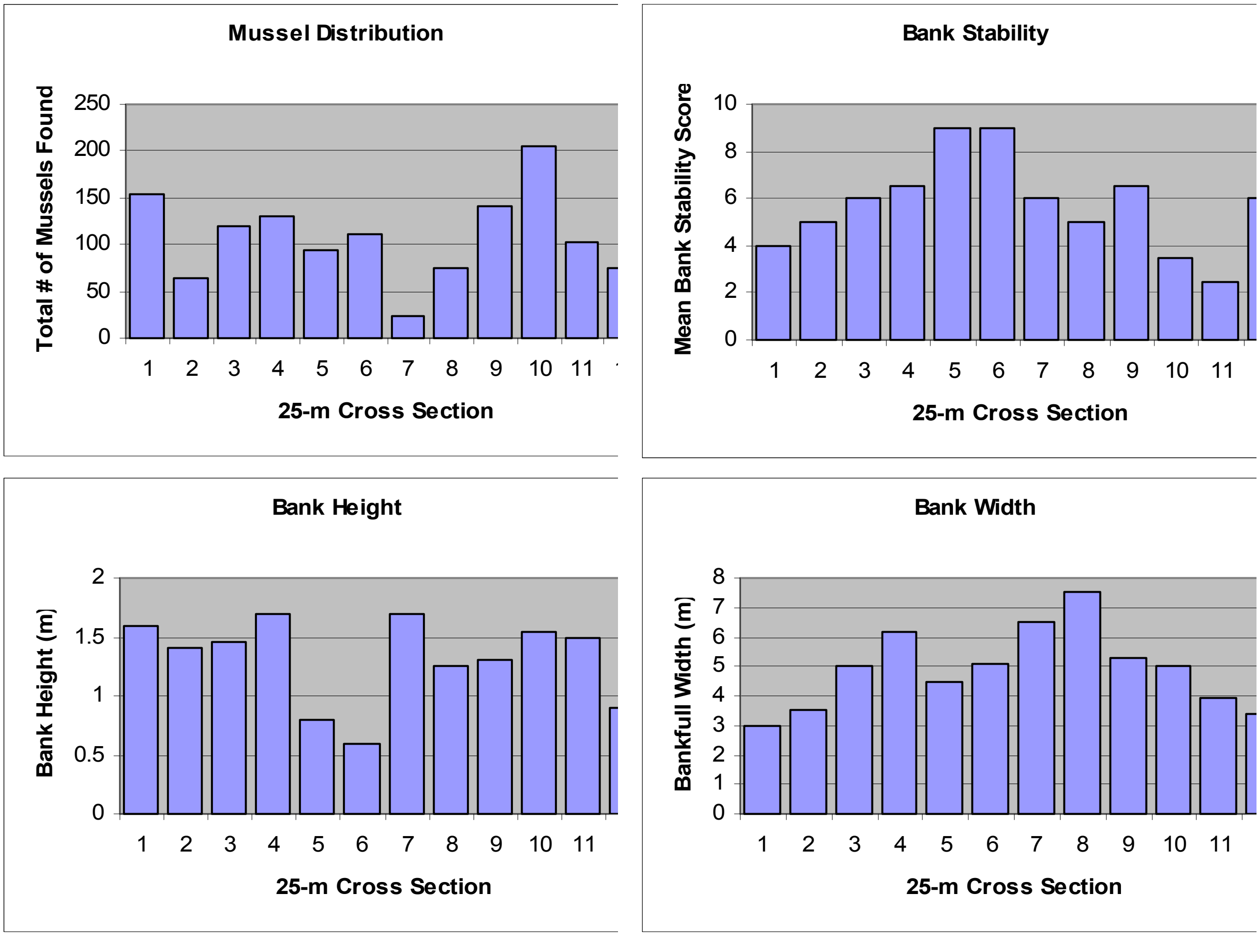


Figure 5. Mussel distribution and habitat data from this site.

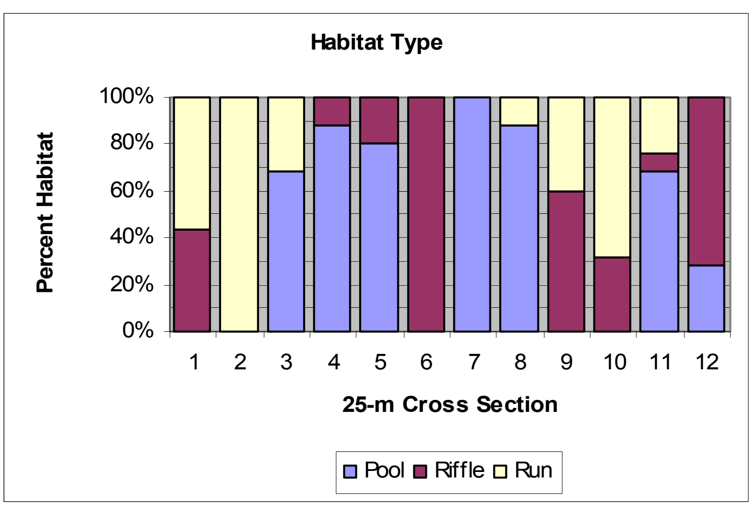


Figure 6. Percentage of habitat type within each 25-m cross section.

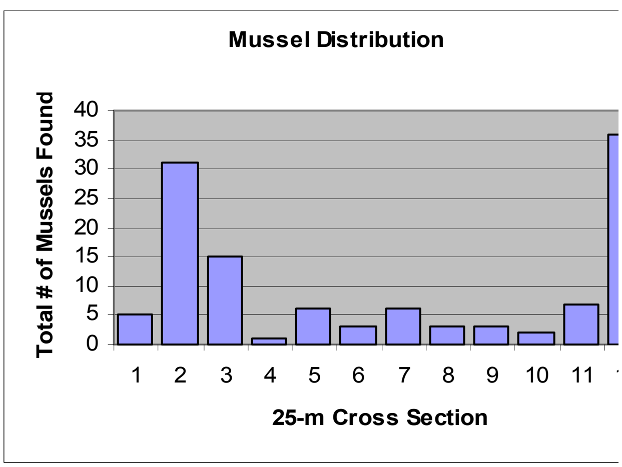
County: Guilford **Bridge Number:** 190
Road Crossing: NC 70 **Stream:** Rock Creek
Date Sampled: 27 April 2005

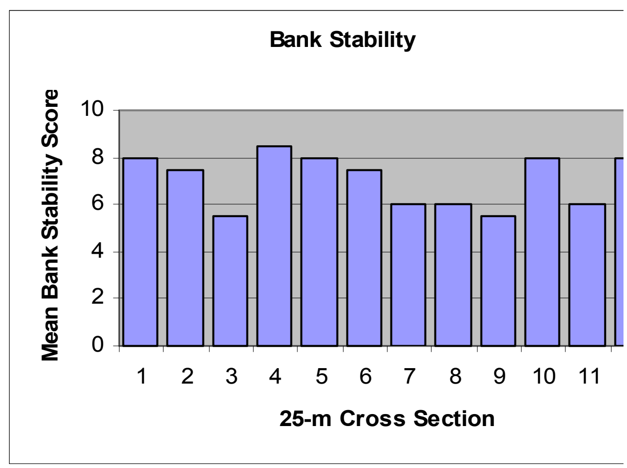
Mussels found at site			
Elliptio complanata	112	**Year Built:**	1930
V. constricta	6	**Number of Cells: (w/ base flow)**	1(1)
Total mussels	**118**	**Obvious scour hole?**	No
		% of mussels upstream:	51.7%

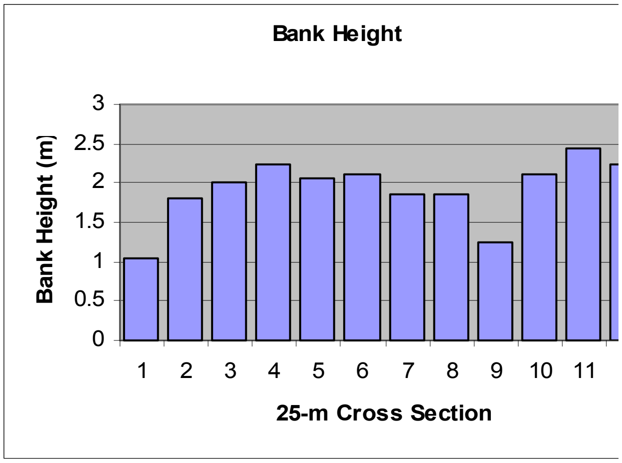
Summary:

Habitat alteration near the culvert seemed minimal. There are also no indications from the mussel data that this structure has caused much damage to the stream. Greater channel widths downstream are attributable to the confluence of a small tributary just upstream of the culvert. This arch culvert seemed relatively benign in its hydrological effects.

No pictures available







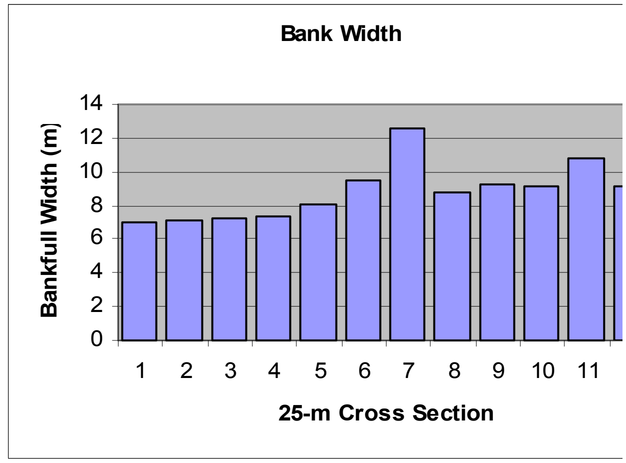


Figure 5. Mussel distribution and relevant habitat data from this site.

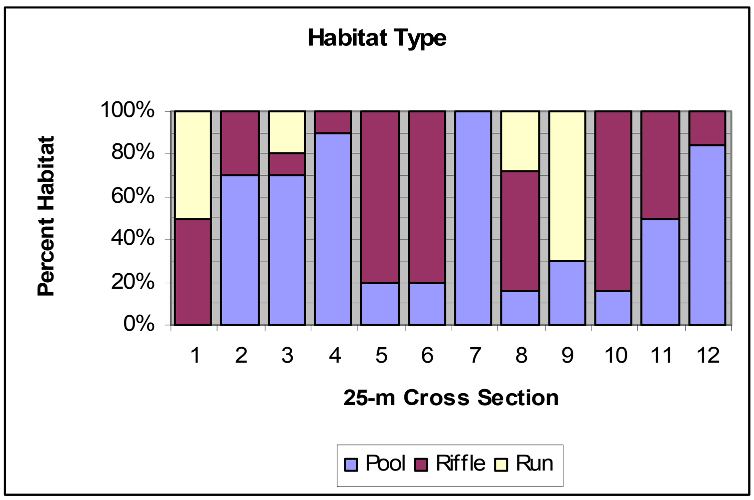


Figure 6. Percentage of habitat type within each 25-m cross section.

County: Guilford **Bridge Number:** 608
Road Crossing: Liberty Road **Stream:** Big Alamance Creek
Date Sampled: 11 August 2004

Mussels found at site			
Elliptio complanata	424	**Year Built:**	1938
P. cataracta	1	**Number of Cells: (w/ base flow)**	4(2)
V. delumbis	5	**Obvious scour hole?**	No
V. vaughaniana	2	**% of mussels upstream:**	91.9%
Total mussels	**432**		

Summary:

This culvert has likely caused significant damage to the mussel fauna at this site. The channel downstream tended to be slightly wider (Fig. 5) and there were more point bars formed in the channel there (Fig. 4). There were over 11 times as many mussels upstream compared to downstream.

Figure 1. Upstream habitat

Figure 2. Upstream side of culvert

Figure 3. Downstream side of culvert

Figure 4. Downstream habitat

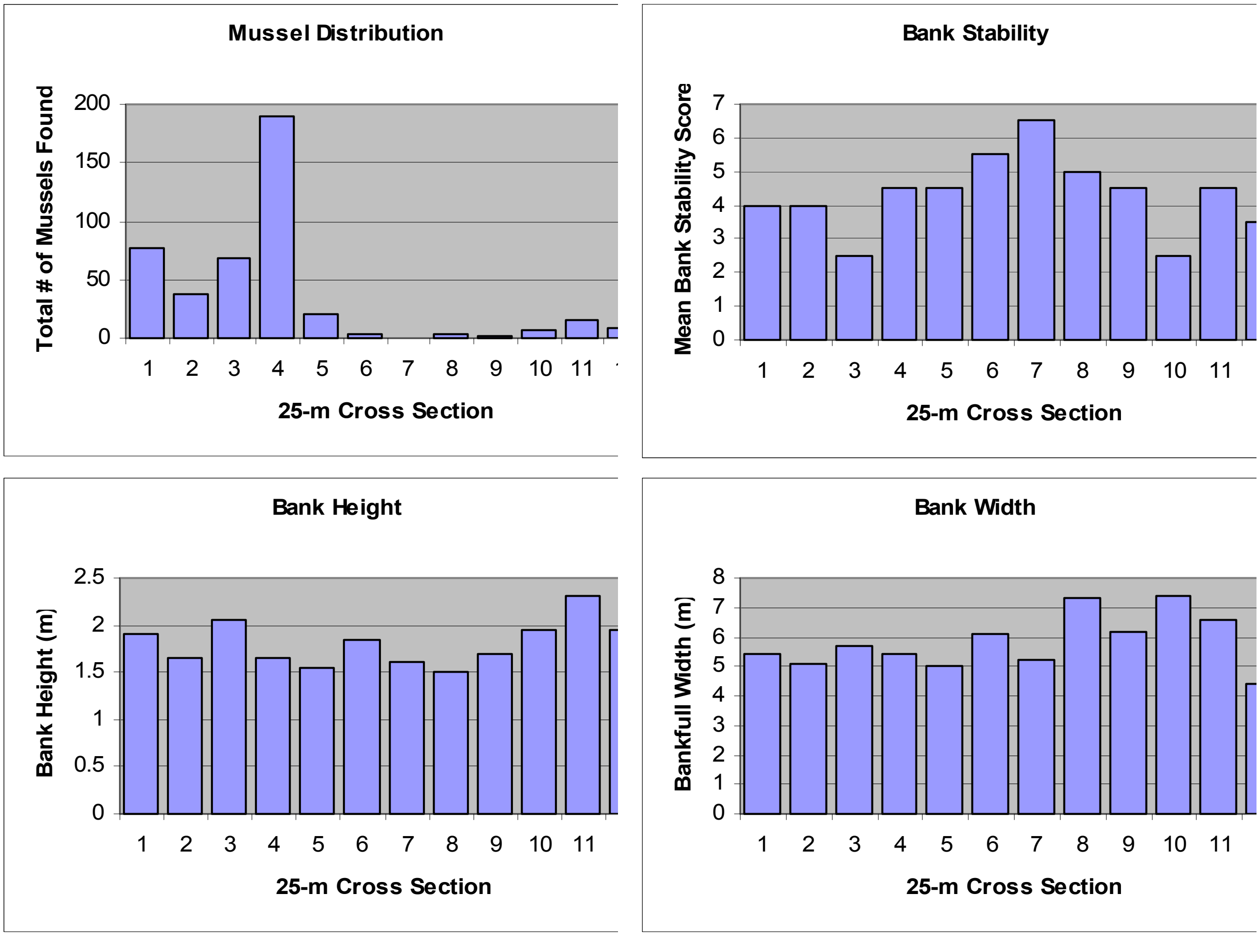


Figure 5. Mussel distribution and habitat data from this site.

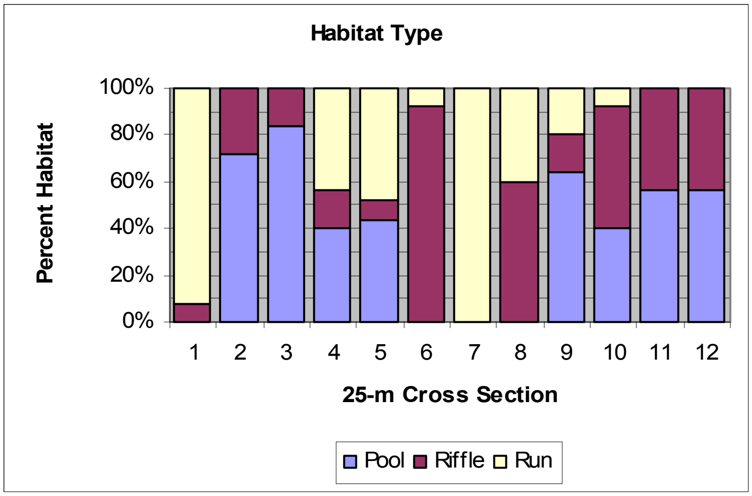


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Randolph	**Bridge Number:**	463
Road Crossing:	Providence Church Rd.	**Stream:**	Little Polecat Creek
Date Sampled:	24 May 2005		

Mussels found at site			
Elliptio complanata	21	**Year Built:**	1968
V. vaughaniana	8	**Number of Cells: (w/ base flow)**	3(1)
Total mussels	**29**	**Obvious scour hole?**	No
		% of mussels upstream:	20.7%

Summary:

This site overall is in poor condition. Banks are eroding, and the substrate is quite unstable. Mussels only exist in a few isolated patches of stable refugia along banks. With the already poor habitat, we could not attribute any specific impacts to this structure. There was no obvious scour or additional channel widening downstream compared to upstream.

Figure 1. Upstream habitat

Figure 2. Further upstream dam

Figure 3. Downstream side of culvert

Figure 4. Downstream habitat

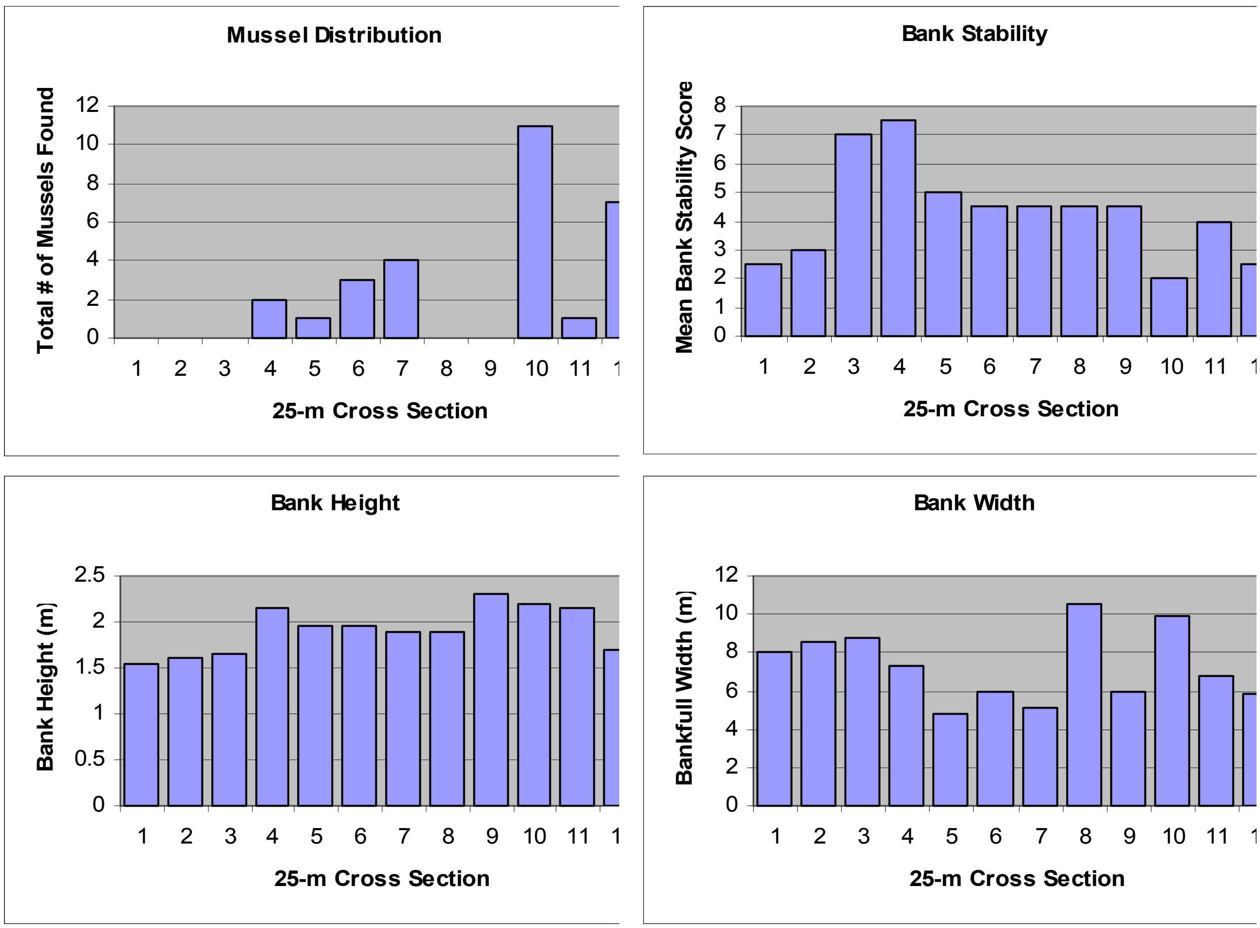


Figure 5. Mussel distribution and habitat data from this site.

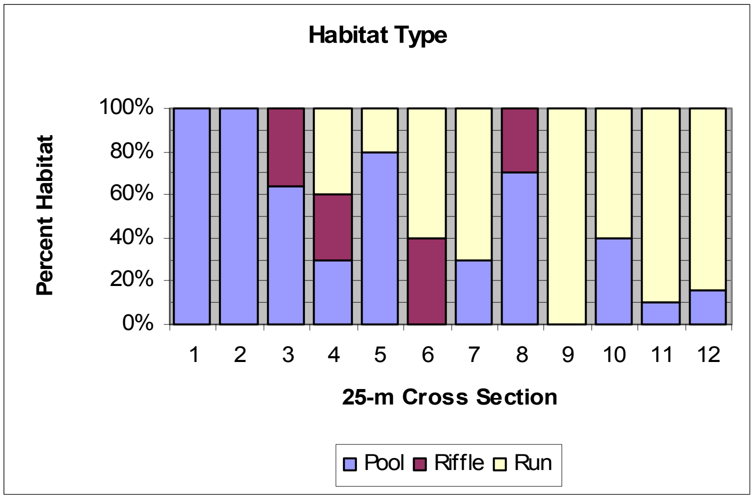


Figure 6. Percentage of habitat type within each 25-m cross section.

CAROLINA SLATE BELT

Impact	County – Bridge Number	Culvert Type
High	Alamance 20	Box
	Granville 116	Pipe
	Granville 217	Pipe
	Granville 46	Box
Low	Alamance 29	Arch
	Alamance 4	Box
	Granville 26	Box
	Granville 28	Box
	Orange 263	Box
	Orange 30	Box
	Person 211	Pipe
	Person 38	Pipe
None Detected	Chatham 12	Arch
	Montgomery 27	Box
	Moore 225	Pipe
	Orange 13	Arch
	Randolph 339	Pipe
	Randolph 459	Box

County: Alamance **Bridge Number:** 20
Road Crossing: NC 86 **Stream:** Mary's Creek
Date Sampled: 18 August 2004

Mussels found at site			
Elliptio complanata	968	**Year Built:**	1930
P. cataracta	5	**Number of Cells: (w/ base flow)**	4(2)
V. delumbis	3	**Obvious scour hole?**	No
Total mussels	**976**	**% of mussels upstream:**	45.0%

Summary:

Overall, this site is degraded, and past land use in the watershed likely has a great deal to do with the characteristics of the overall site. The stream is incised, and the banks are quite unstable. Bank stability scores were slightly lower downstream than upstream. The culvert has sediment deposition in two of the cells that is developing a bankfull bench (Fig. 1), and no obvious scour was seen downstream of the culvert. Bank heights were lower downstream, but the channel was wider downstream. However, there was an obvious decrease in mussel abundance immediately downstream of the culvert. We attribute this to initial widening of the channel evidenced by channel width measurements. Few trees stand along the bank, and in fact, several trees along the bank downstream have already fallen in or are in the process (Figs. 2 and 4); consequently, there is little stable habitat along the banks within 75 meters of the culvert. Additional habitat complexity further downstream helps create mussel habitat, and mussel abundance increases there (Fig. 5). Since the upstream reach has not been widened, more trees remain creating mussel habitat (Fig. 3). We believe this culvert has caused significant damage to the downstream habitat.

Figure 1. upstream view.

Figure 2. downstream habitat.

Figure 3. upstream habitat.

Figure 4. bank sloughing downstream.

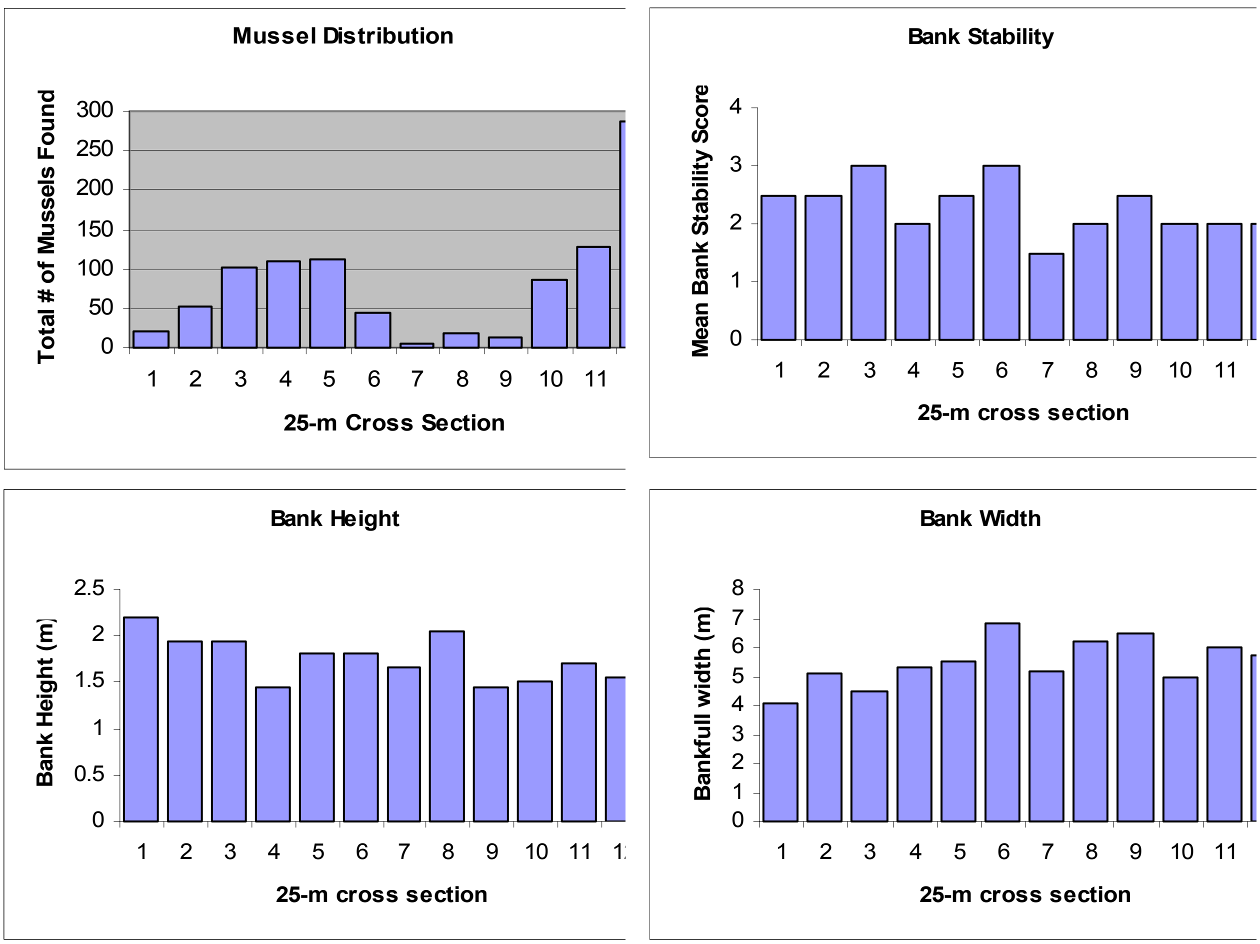


Figure 5. Mussel distribution and habitat data from this site.

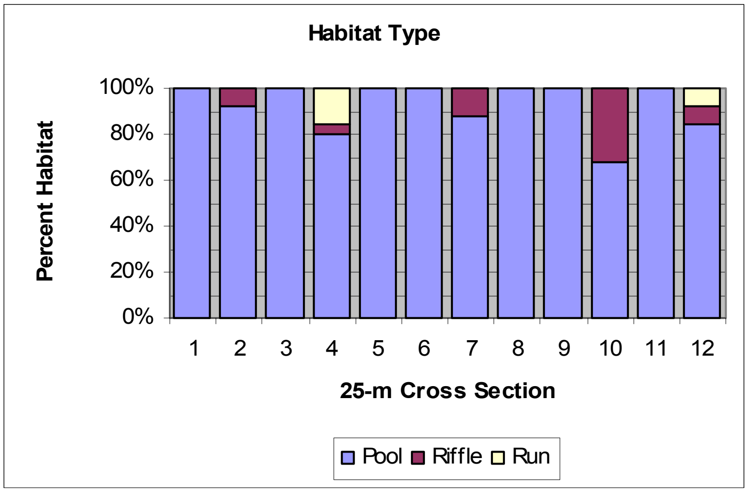


Figure 6. Percentage of habitat type within each 25-m cross section.

County: Alamance **Bridge Number:** 29
Road Crossing: NC 119 **Stream:** Mill Creek
Date Sampled: 8 August 2004 & 27 June 2005

Mussels found at site			
Elliptio complanata	2966	**Year Built:**	1935
P. cataracta	1	**Number of Cells: (w/ base flow)**	1(1)
U. imbecillis	2	**Obvious scour hole?**	No
V. constricta	14	**% of mussels upstream:**	34.3%
V. delumbis	12		
V. vaughaniana	45		
Total mussels	**3038**		

Summary:
The habitat immediately at the culvert is poor. The culvert is much wider than the stream itself; however, habitat quickly improves, and there are a larger number of mussels and fair diversity downstream. This Arch with a large opening doesn't appear to affect the habitat downstream, but a normal stream channel with a bankfull bench has not formed.

Figure 1. Arch Culvert

Figure 2. Upstream Habitat

Figure 3. Downstream Habitat	**Figure 4.** Debris

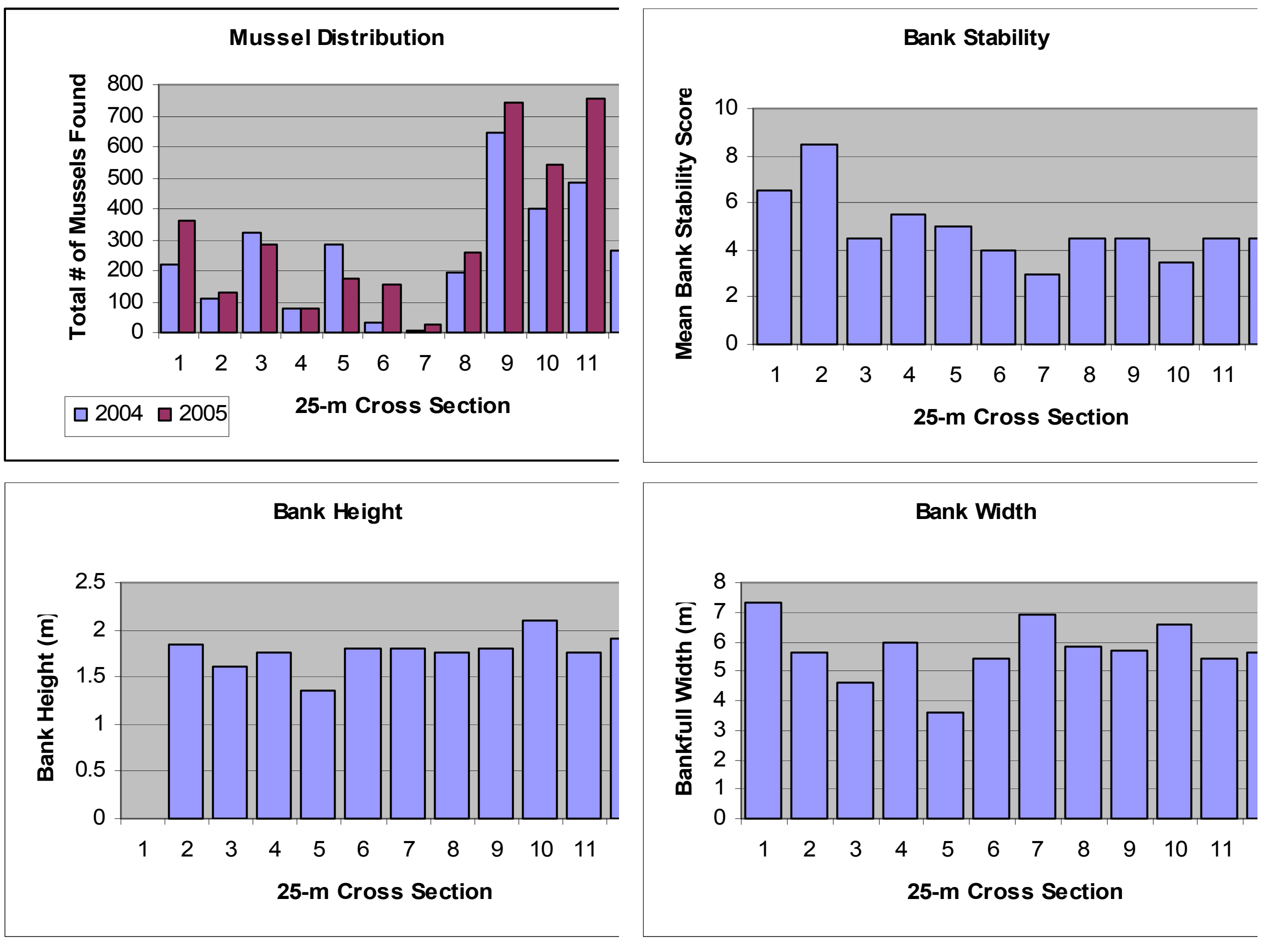


Figure 5. Mussel distribution and habitat data from this site.

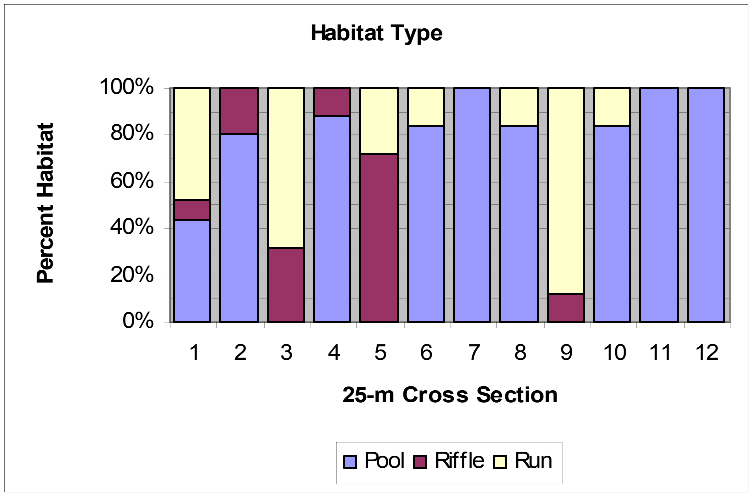


Figure 6. Percentage of habitat type within each 25-m cross section.

County: Alamance　　**Bridge Number:** 4
Road Crossing: NC 87　　**Stream:** Lick Creek
Date Sampled: 5 May 2005

Mussels found at site			
Elliptio complanata	20	**Year Built:**	1938
P. cataracta	4	**Number of Cells: (w/ base flow)**	3(1)
Total mussels	**24**	**Obvious scour hole?**	No
		% of mussels upstream:	66.7%

Summary:

With the total number of mussels in this stream so very low, it is difficult to determine how much of an impact the structure has on the stream. Banks tended to be a little higher downstream and habitat was more uniform, but any habitat alteration by the culvert was subtle.

Figure 1. Downstream

Figure 2. Downstream side of culvert

Figure 3. Upstream side of culvert

Figure 4. Upstream

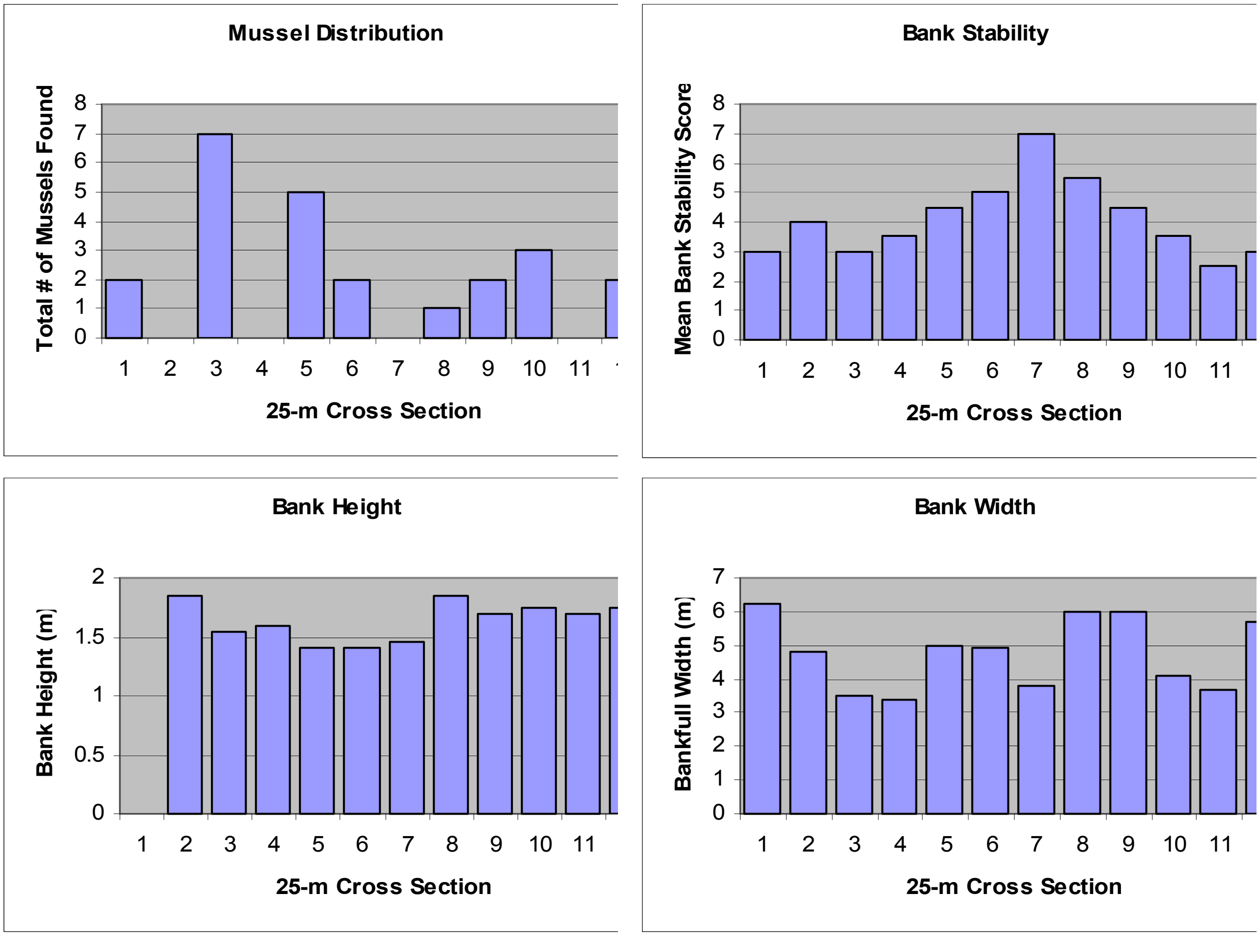


Figure 5. Mussel distribution and habitat data from this site.

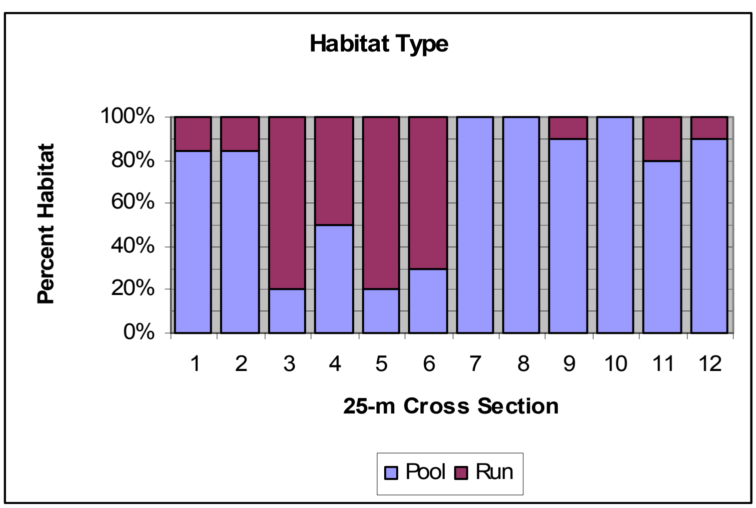


Figure 6. Percentage of habitat type within each 25-m cross section.

County: Chatham **Bridge Number:** 12
Road Crossing: NC 87 **Stream:** Terrell's Creek
Date Sampled: 2 August 2004 & 7 June 2005

Mussels found at site			
Elliptio complanata	403	**Year Built:**	1933
P. cataracta	22	**Number of Cells: (w/ base flow)**	1(1)
S. undulatus	6	**Obvious scour hole?**	No
V. delumbis	5	**% of mussels upstream:**	73.1%
Total mussels	**431**		

Summary:

Although habitat differs greatly from upstream to downstream of this culvert, mussel abundance is very similar except for one cross-section where mussels were abundant along the bank in a protected area upstream. Downstream habitat is much more homogeneous. Upstream habitat is rockier and steeper. Banks are significantly higher downstream. It is difficult to tell what differences in habitat are natural and what are caused by the culvert.

Figure 1. Upstream

Figure 2. Upstream side of culvert

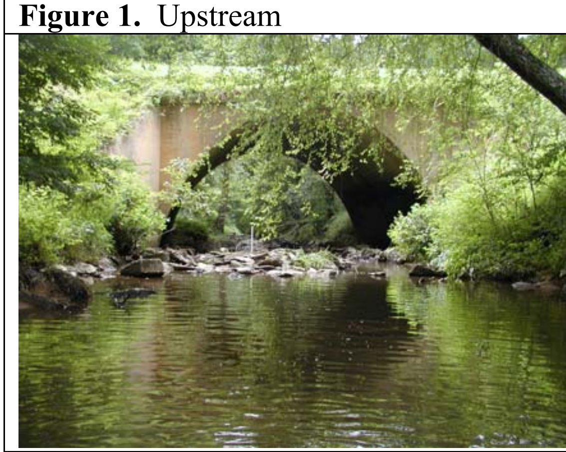


Figure 3. Downstream side of culvert

Figure 4. Downstream

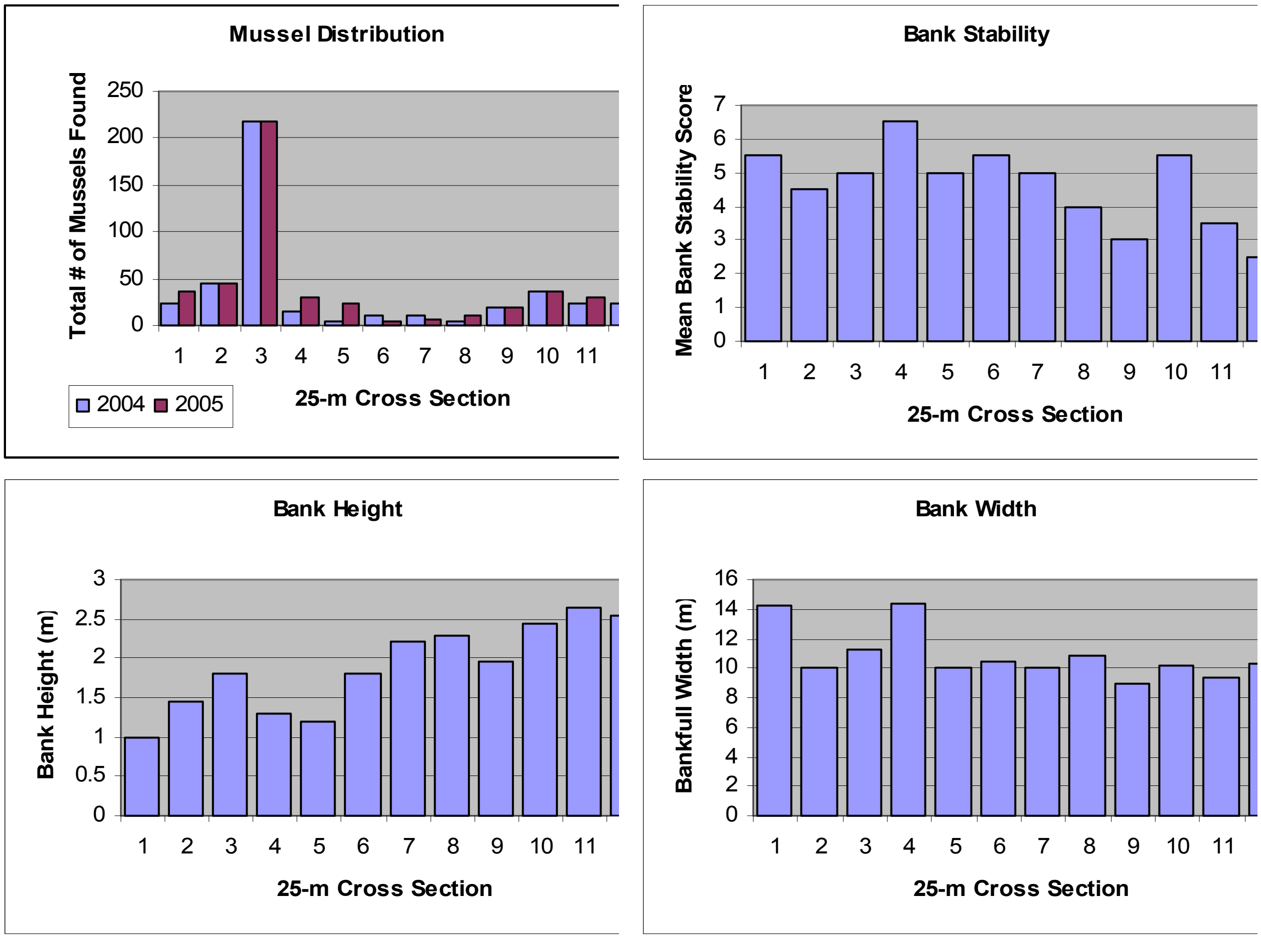


Figure 5. Mussel distribution and habitat data from this site.

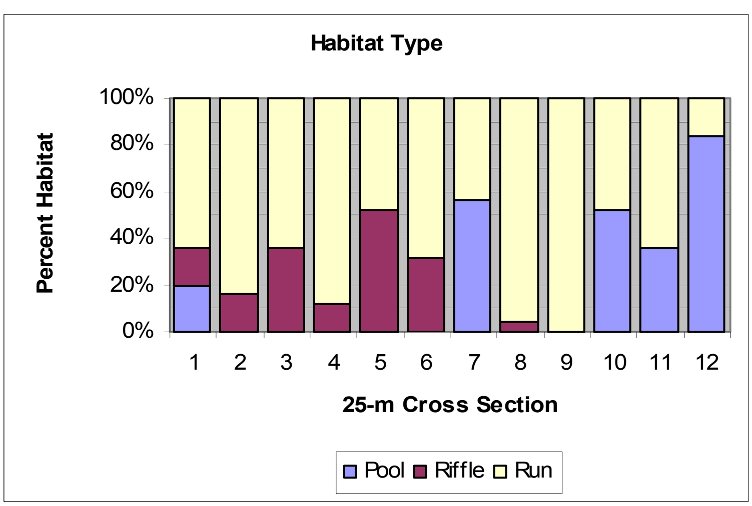


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Granville	**Bridge Number:**	116
Road Crossing:	Adcock Road	**Stream:**	Grassy Creek
Date Sampled:	27 April 2004 & 26 May 2005		

Mussels found at site (2004)			
Elliptio complanata	106	**Year Built:**	1978
P. cataracta	4	**Number of Cells: (w/ base flow)**	3(3)
Total mussels	**110**	**Obvious scour hole?**	Yes
		% of mussels upstream:	80.9%

Summary:

This culvert has obviously caused noticeable damage to downstream habitat. There is deep scour at the mouth of the culvert, and downstream banks were quite eroded compared to upstream. Mussel abundance was a product of habitat as there were 4 times as many mussels upstream as there were downstream.

Figure 1. Debris upstream of culvert

Figure 2. Upstream habitat

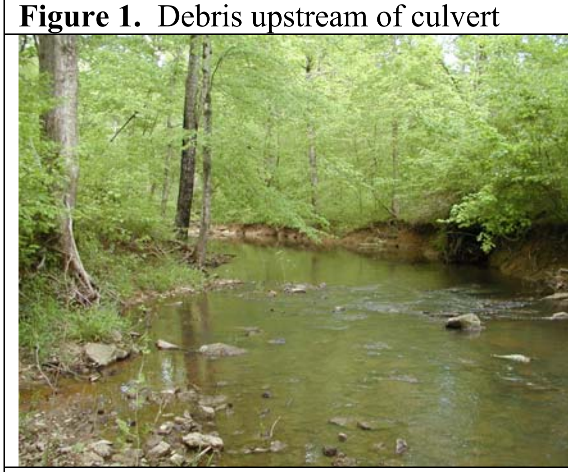


Figure 3. Downstream habitat

Figure 4. Downstream view of culvert

Figure 5. Downstream erosion

Figure 6. Downstream erosion

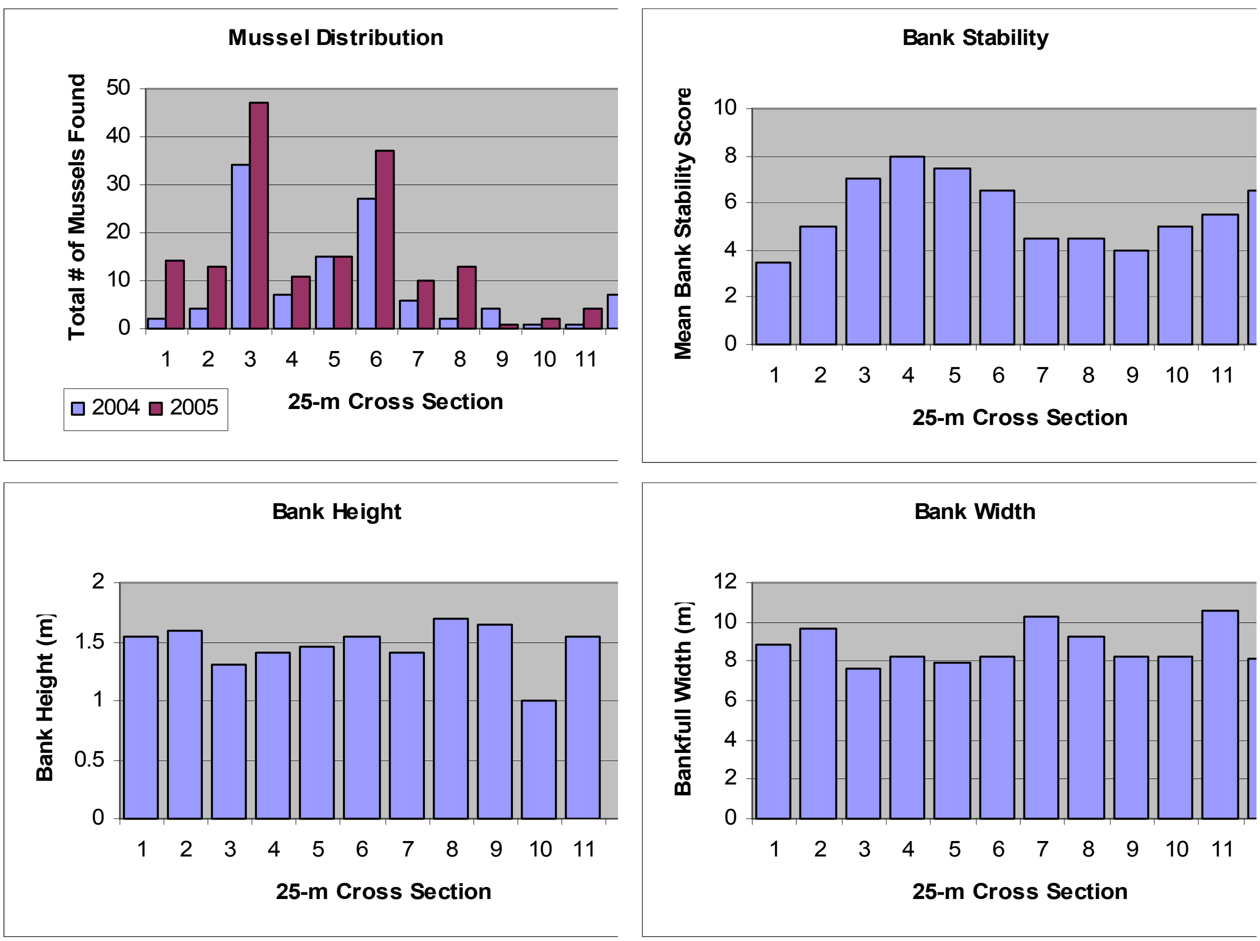


Figure 5. Mussel distribution and habitat data from this site.

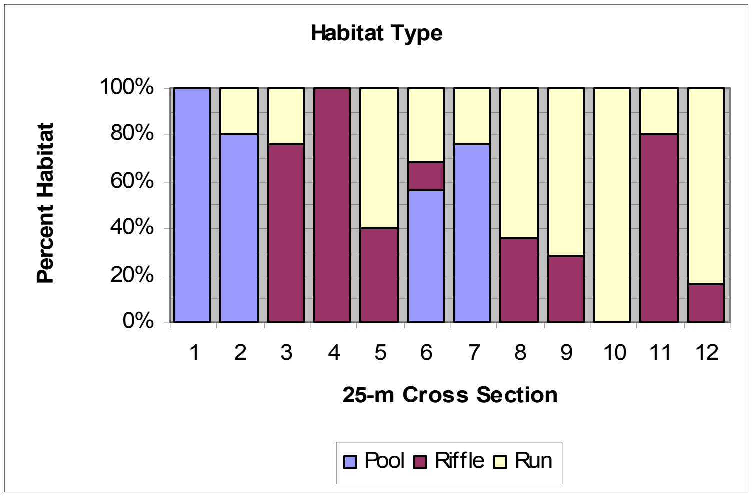


Figure 6. Percentage of habitat type within each 25-m cross section.

County: Granville **Bridge Number:** 177
Road Crossing: Sunset Road **Stream:** Shelton Creek
Date Sampled: 9 June 2004

Mussels found at site			
Elliptio complanata	1281	**Year Built:**	1960
L. oribatagalma	75	**Number of Cells: (w/ base flow)**	3(2)
Total mussels	**1356**	**Obvious scour hole?**	Yes
		% of mussels upstream:	94%

Summary:

Mussel abundance was much higher upstream of this culvert compared to downstream as there were over 23 times as many mussels upstream compared to downstream. There was obvious scour immediately downstream of the culvert, and much of the downstream reach was significantly deeper. Additionally, there were many more downed trees along the banks downstream where the banks had eroded. We believe this culvert has highly impacted this stream.

Figure 1. Upstream

Figure 2. Upstream side of culvert

Figure 3. Downstream side of culvert	**Figure 4.** Downstream habitat

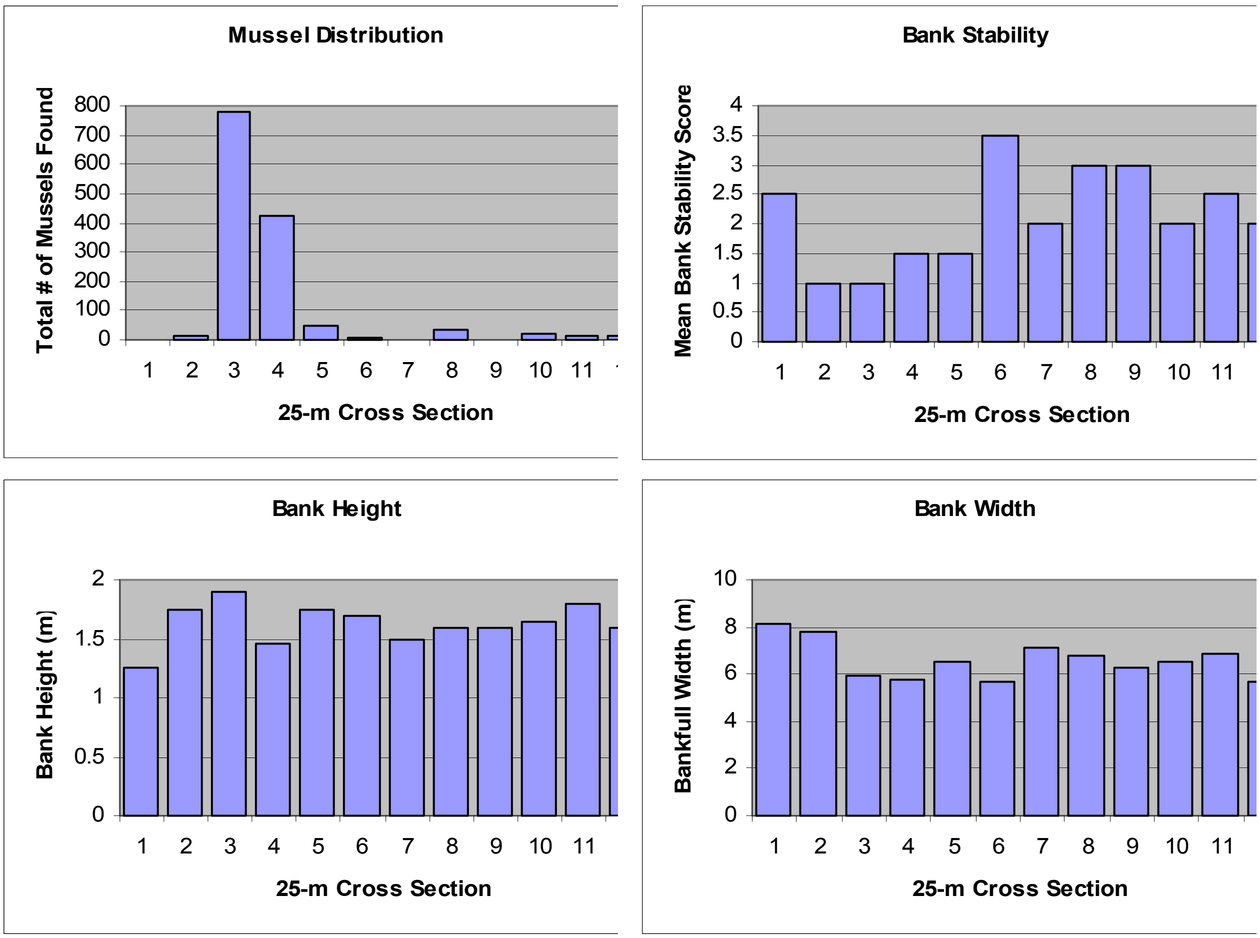


Figure 5. Mussel distribution and habitat data from this site.

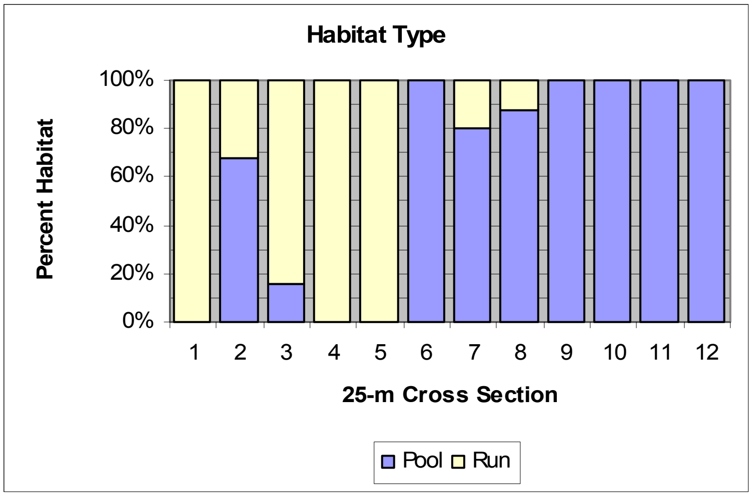


Figure 6. Percentage of habitat type within each 25-m cross section.

County: Granville **Bridge Number:** 217
Road Crossing: Mountain Road **Stream:** UT to Gills Creek
Date Sampled: 27 April 2004

Mussels found at site			
Elliptio complanata	407	**Year Built:**	1933
		Number of Cells: (w/ base flow)	3(2)
Total mussels	**407**	**Obvious scour hole?**	No
		% of mussels upstream:	74.9%

Summary:

This site represents another classic example of what an undersized pipe culvert can do to a piedmont stream. The downstream channel is incised and the banks are eroded (Figs.3, 5, and 6), and bank height was significantly greater downstream compared to upstream. There were 3 times as many mussels upstream as downstream, and mussel abundance gradually increased downstream with increasing distance from the culvert.

Figure 1. Upstream side of culvert

Figure 2. Downstream side of culvert

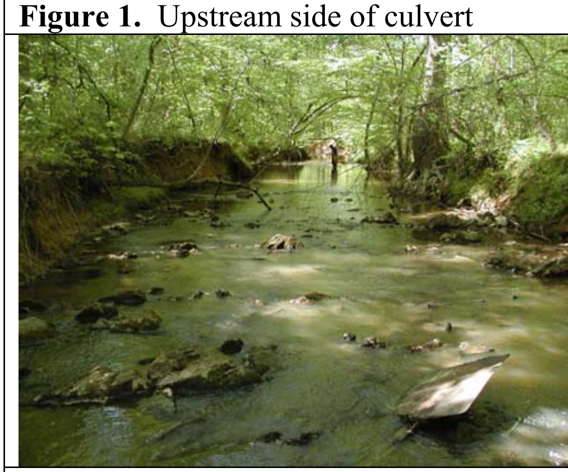


Figure 3. Downstream habitat

Figure 4. Upstream habitat

Figure 5. downstream bank erosion

Figure 6. downstream bank erosion

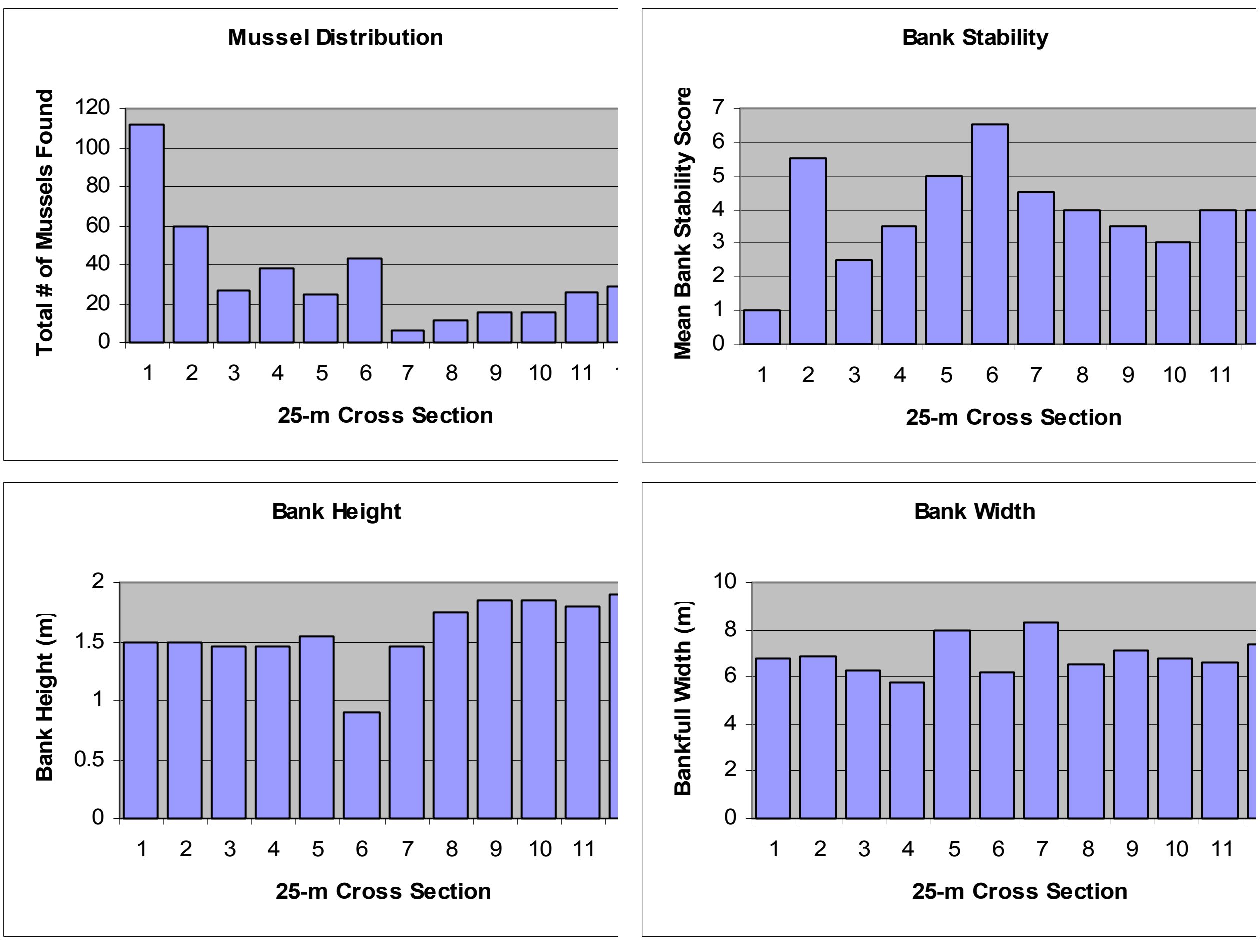


Figure 7. Mussel distribution and habitat data from this site.

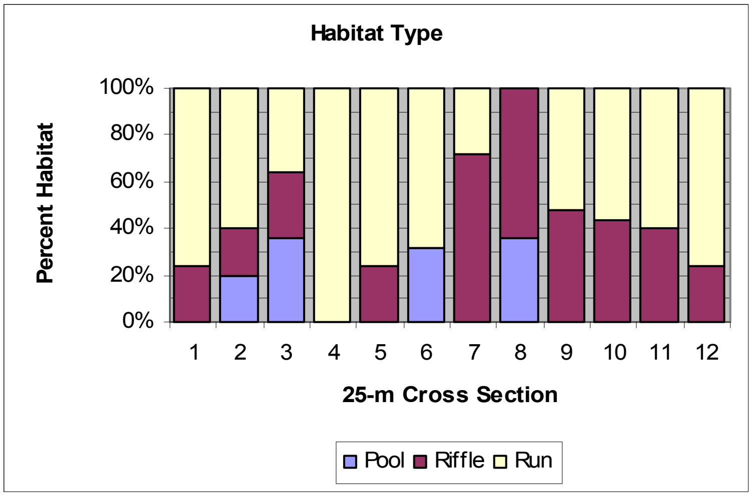


Figure 8. Percentage of habitat type within each 25-m cross section.

County:	Granville	**Bridge Number:**	26
Road Crossing:	US 158	**Stream:**	Shelton Creek
Date Sampled:	24 August 2004		

Mussels found at site			
Elliptio complanata	1094	**Year Built:**	1991
A. heterodon	43	**Number of Cells: (w/ base flow)**	4(4)
F. masoni	8	**Obvious scour hole?**	No
Lampsilis sp.	9	**% of mussels upstream:**	52%
S. undulatus	25		
Total mussels	**1179**		

Summary:

This site is quite diverse with a relatively large number of federally endangered dwarfwedge mussels (Alasmidonta heterodon) occurring there. The banks are eroding, but they were actually most eroded in the most upstream reaches. There was greatly reduced mussel abundance in the first 75 meters downstream of the culvert, but none of our habitat metrics or observations would fully explain why that was the case. It could be that the crossing structure that was previously at the site caused more damage than the current structure (the current culvert was constructed in 1991), but that is only a guess. The current structure is not causing obvious habitat problems with the site.

Figure 1. Upstream habitat

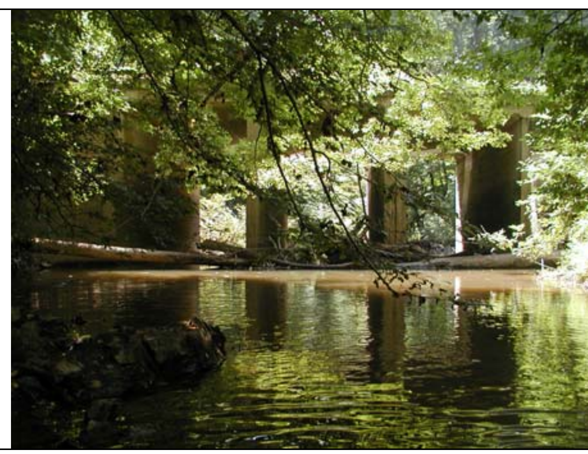


Figure 2. Upstream side of culvert

Figure 3. Downstream habitat

Figure 4. Bank erosion downstream

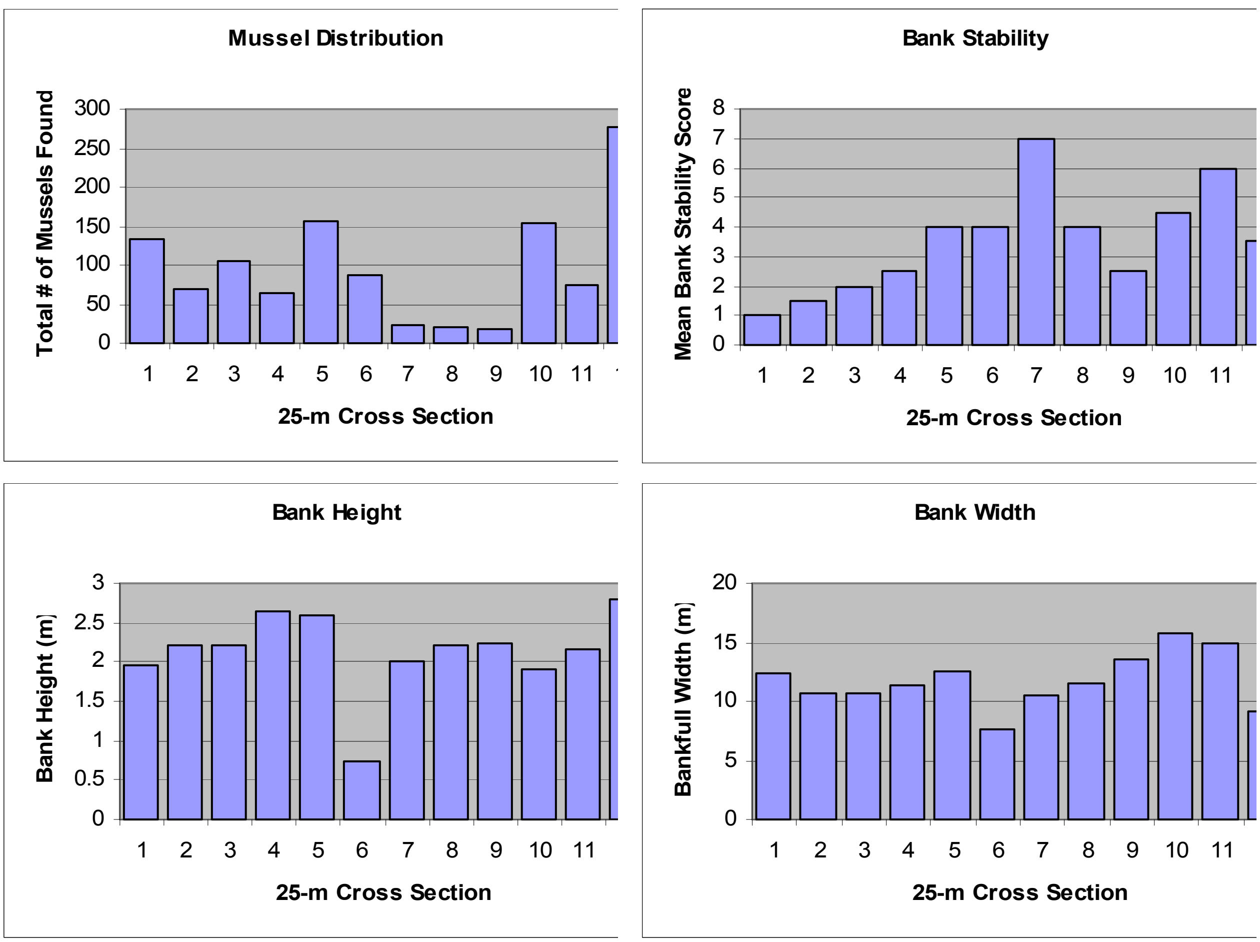


Figure 5. Mussel distribution and habitat data from this site.

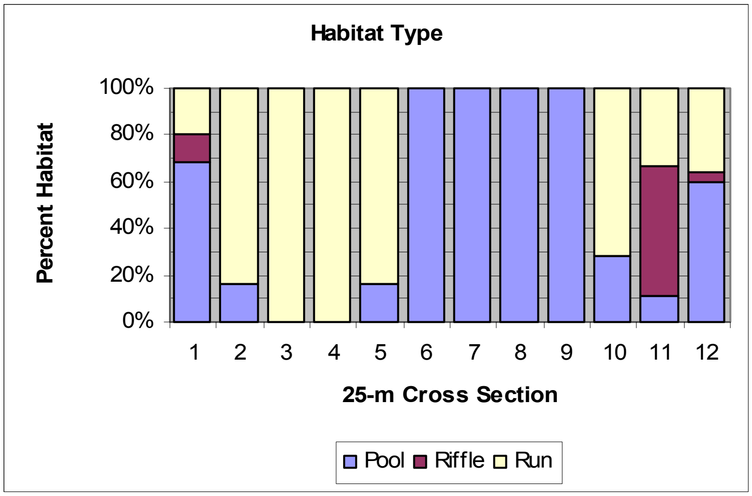


Figure 6. Percentage of habitat type within each 25-m cross section.

County: Granville **Bridge Number:** 28
Road Crossing: US 158 **Stream:** North Fork
Date Sampled: 22 July 2004

Mussels found at site			
Elliptio complanata	54	**Year Built:**	1935
P. cataracta	2	**Number of Cells: (w/ base flow)**	4(1)
S. undulatus	1	**Obvious scour hole?**	No
A. heterodon (1 shell only)		**% of mussels upstream:**	49.1%
Total mussels	**57**		

Summary:

Habitat at this site was poor. The channel was incised, the banks were unstable, there were sandbars in the channel, and there was little stable refugia for mussel colonization. The culvert here doesn't appear to do substantial damage to the stream. There is a mid-channel bar on the downstream side of the culvert (Fig. 3), and there was a very large debris jam on the upstream side of the culvert (Fig. 2). The one shell we picked up of the dwarfwedge mussel (*Alasmidonta heterodon*) was a new record for this stream.

Figure 1. Upstream

Figure 2. Debris upstream culvert

Figure 3. Downstream side of culvert

Figure 4. Downstream

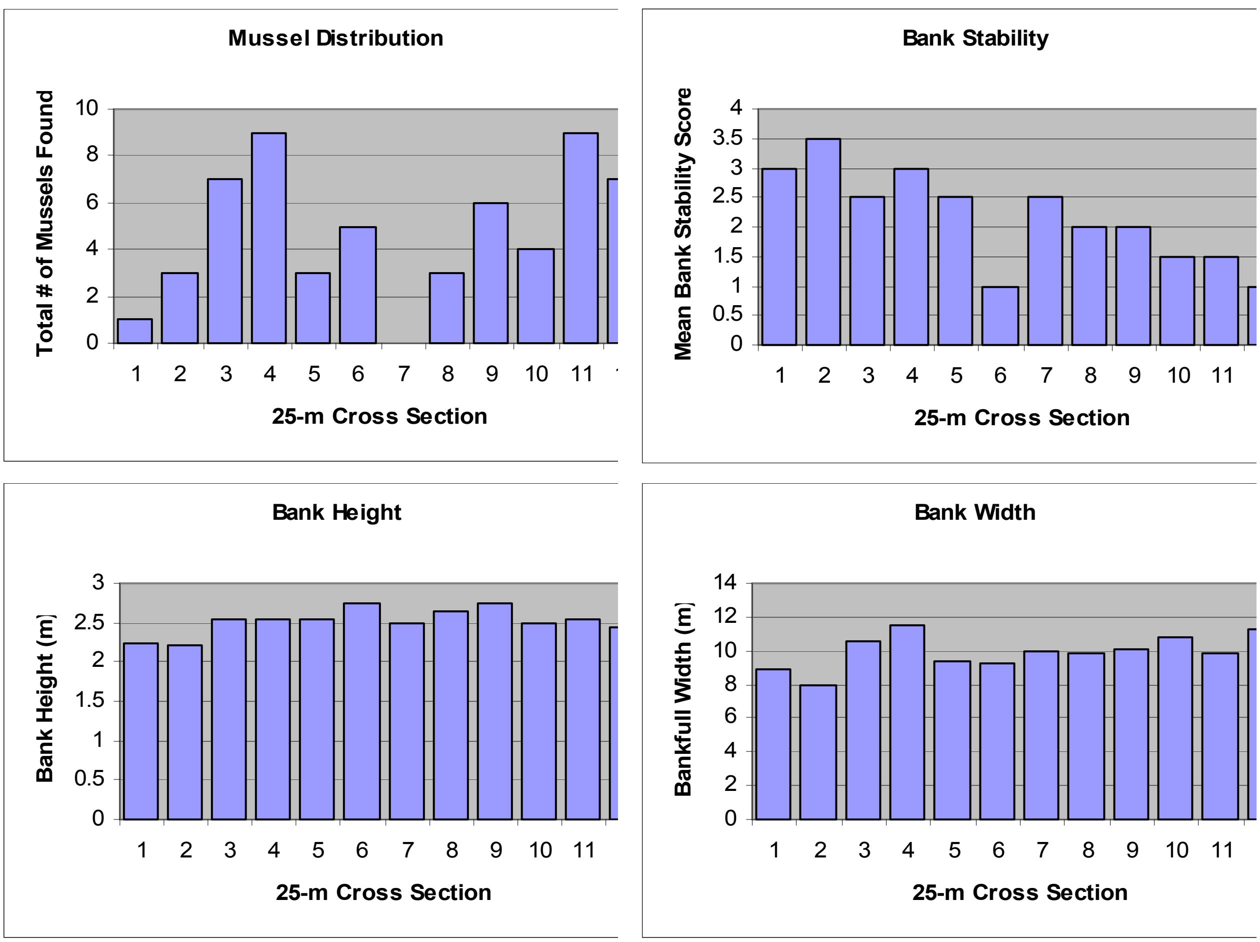


Figure 5. Mussel distribution and habitat data from this site.

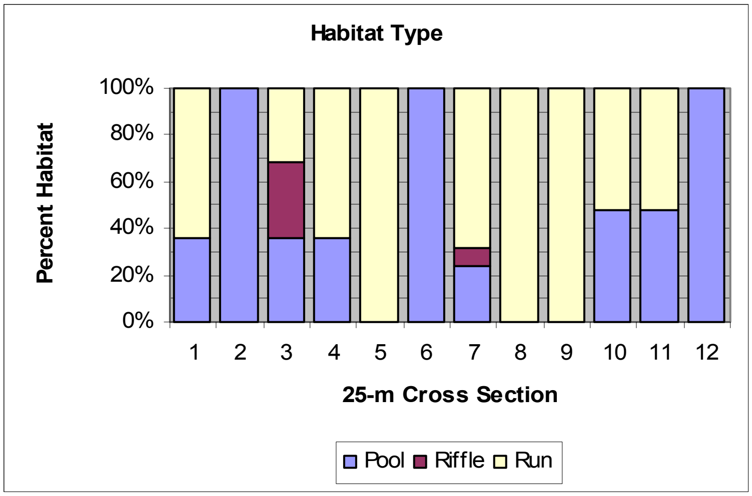


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Granville	**Bridge Number:**	46
Road Crossing:	NC 96	**Stream:**	Grassy Creek
Date Sampled:	29 April 2004		

Mussels found at site			
Elliptio complanata	210	**Year Built:**	1934
		Number of Cells: (w/ base flow)	4(4)
Total mussels	**210**	**Obvious scour hole?**	Yes
		% of mussels upstream:	43.8%

Summary:

Habitat type upstream of this culvert was much different than habitat downstream (Fig. 6). Stream banks lower and more stable (Fig. 1), and the channel is not as wide upstream (Fig. 5). The depth of the scour at the culvert is not very deep (Fig. 2), but downstream channel morphology seems degraded. We believe differences in upstream and downstream habitat are likely a combination of natural differences in slope as well as habitat destruction caused by constriction at the culvert. Mussel abundance was low immediately downstream of the culvert but recovered downstream. Parts of the upstream habitat were naturally rocky and not conducive to mussel colonization.

Figure 1. Upstream habitat

Figure 2. Downstream side of culvert

Figure 3. Logs downstream

Figure 4. Downstream habitat

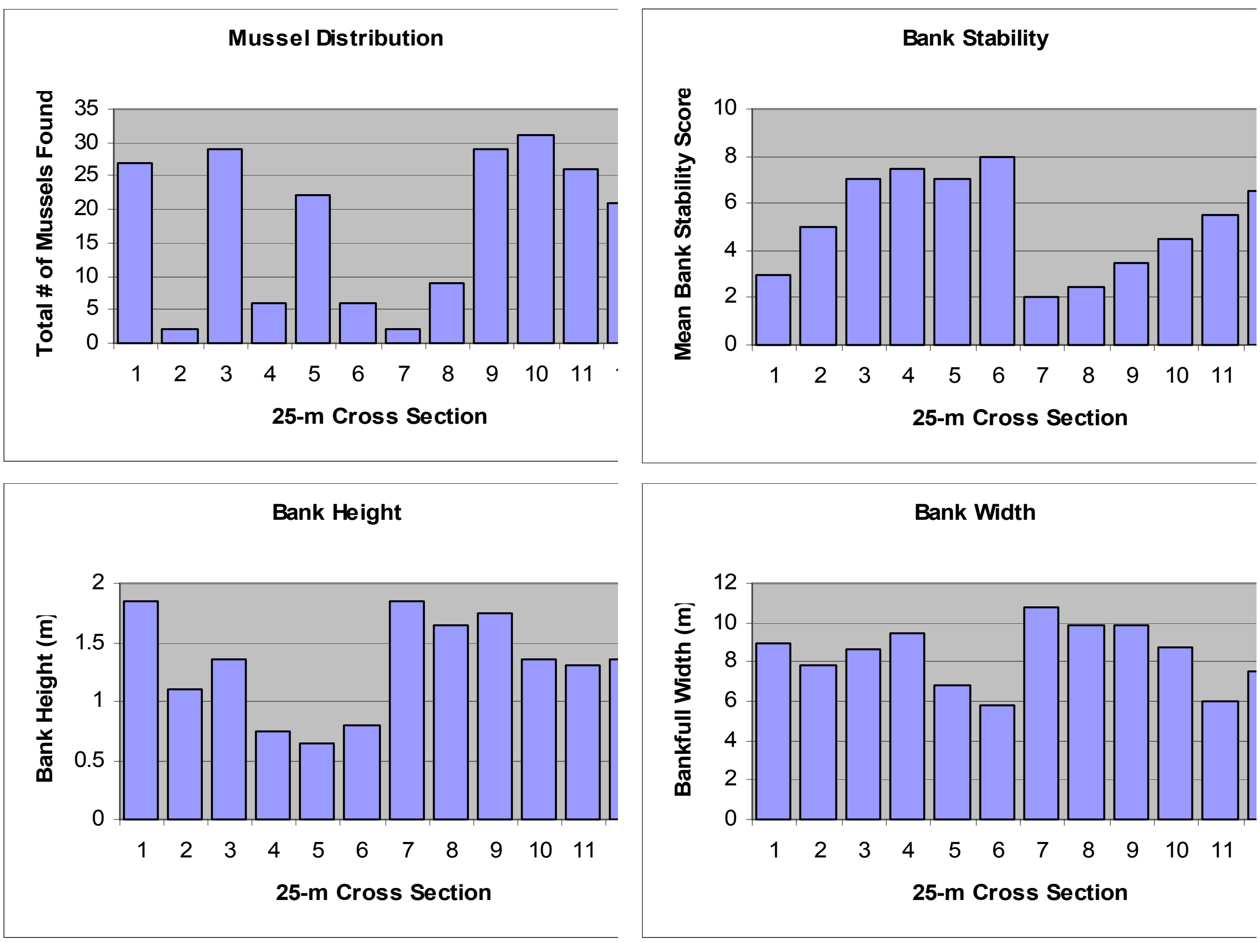


Figure 5. Mussel distribution and habitat data from this site.

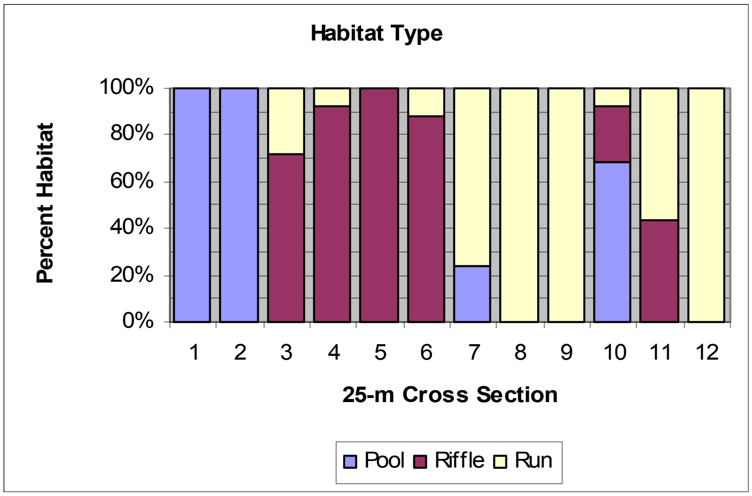


Figure 6. Percentage of habitat type within each 25-m cross section.

County: Montgomery **Bridge Number:** 27
Road Crossing: NC 134 **Stream:** West Fork Little River
Date Sampled: 19 May 2005

Mussels found at site			
Elliptio complanata	100	**Year Built:**	1967
A. varicosa	1	**Number of Cells: (w/ base flow)**	3(3)
V. constricta	4	**Obvious scour hole?**	No
V. delumbis	1	**% of mussels upstream:**	67.3%
V. vaughaniana	7		
Total mussels	**113**		

Summary:

This stream is located in the Uwharries area. It is dominated by boulder and bedrock. The culvert seemed to have little effect on the channel except for perhaps minimal effect immediately at the crossing structure. There were fewer mussels immediately downstream of the culvert compared to immediately upstream, but the lack of obvious channel alteration made it difficult to attribute this to the culvert. These rockier streams that won't erode as quickly may be more conducive to having culverts placed on them.

Figure 1. Downstream habitat

Figure 2. Downstream side of culvert

Figure 3. Upstream habitat

Figure 4. Further upstream habitat

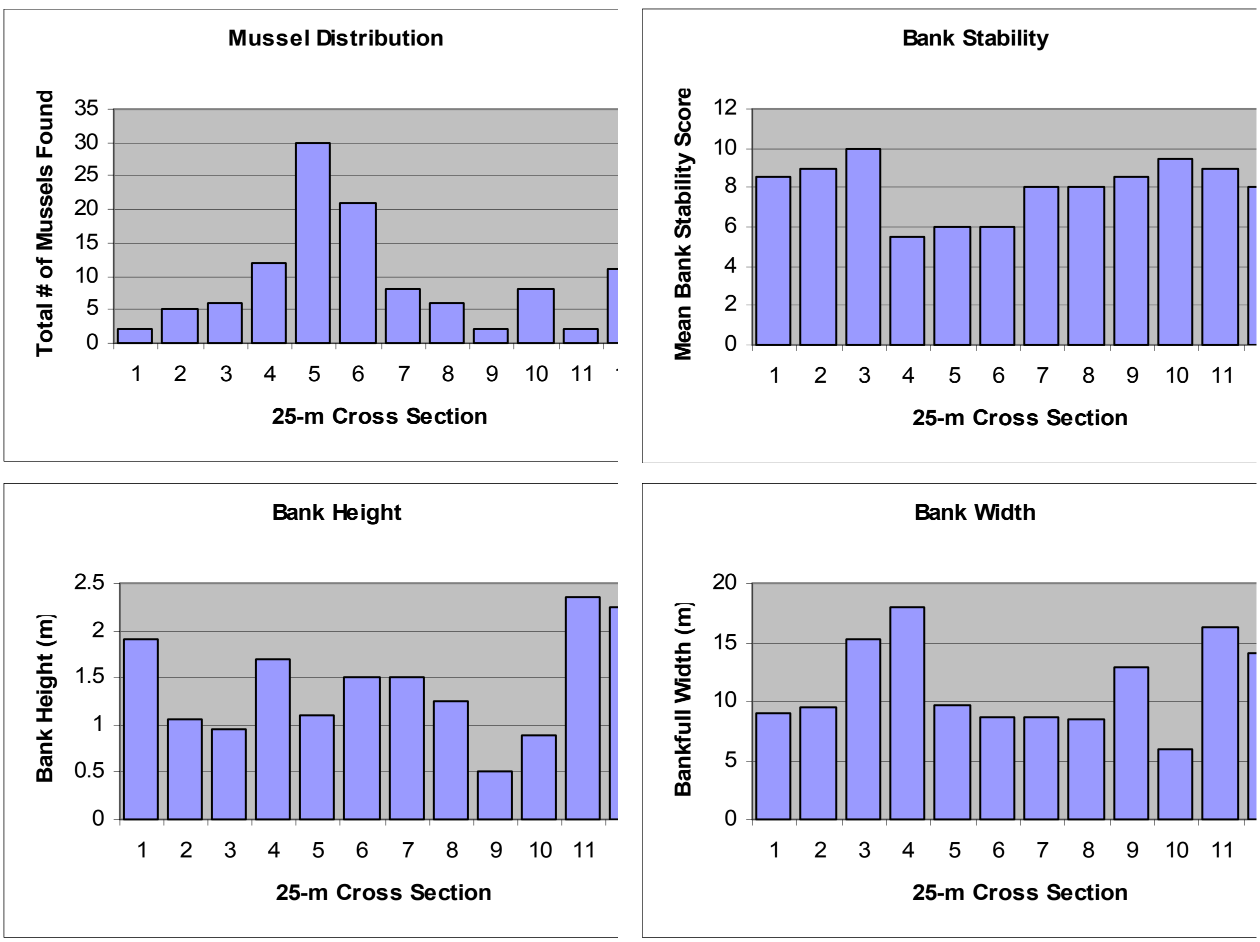


Figure 5. Mussel distribution and habitat data from this site.

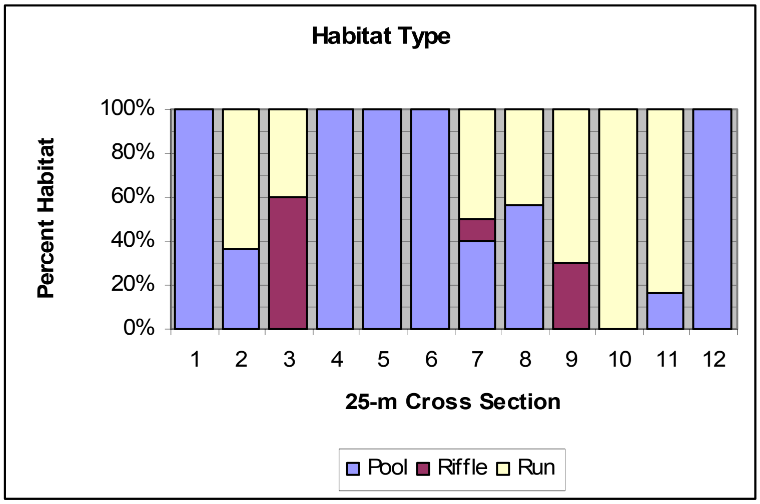


Figure 6. Percentage of habitat type within each 25-m cross section.

County: Moore **Bridge Number:** 212
Road Crossing: Alton Road **Stream:** Dry Creek
Date Sampled: 23 July 2004

Mussels found at site			
Elliptio complanata	601	**Year Built:**	1970
V. constricta	53	**Number of Cells: (w/ base flow)**	3(3)
V. delumbis	30	**Obvious scour hole?**	Yes
V. vaughaniana	75	**% of mussels upstream:**	58.1%
Total mussels	**759**		

Summary:

There was a decrease in mussel abundance in the first 50 meters downstream of the culvert. We attribute this to the scour caused by channel constriction. Additionally, bank height was significantly higher downstream and there were more gravel bars in the channel there. However, mussel fauna downstream past 50 meters from the structure was similar to upstream.

Figure 1. Downstream habitat

Figure 2. Downstream side of culvert

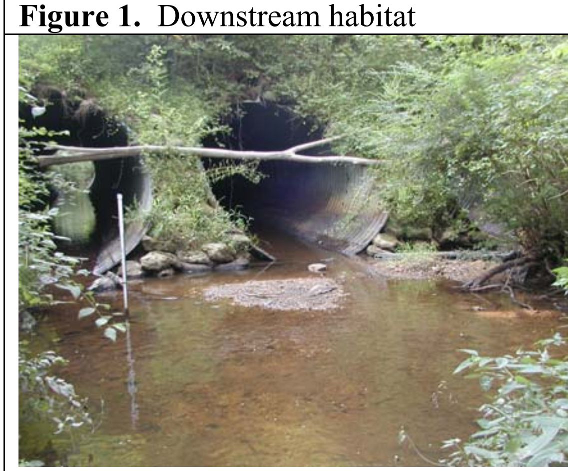


Figure 3. Upstream side of culvert

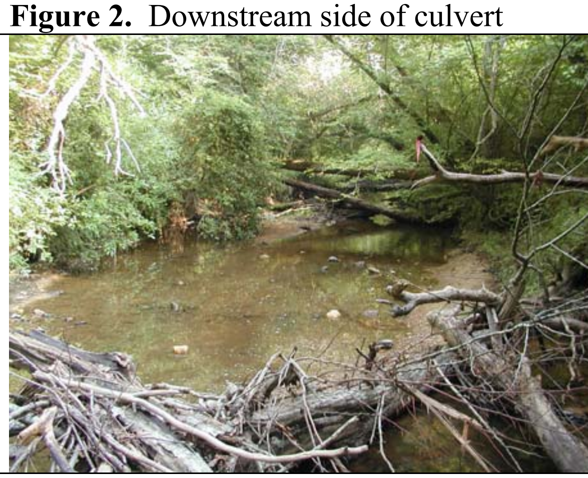


Figure 4. Upstream habitat

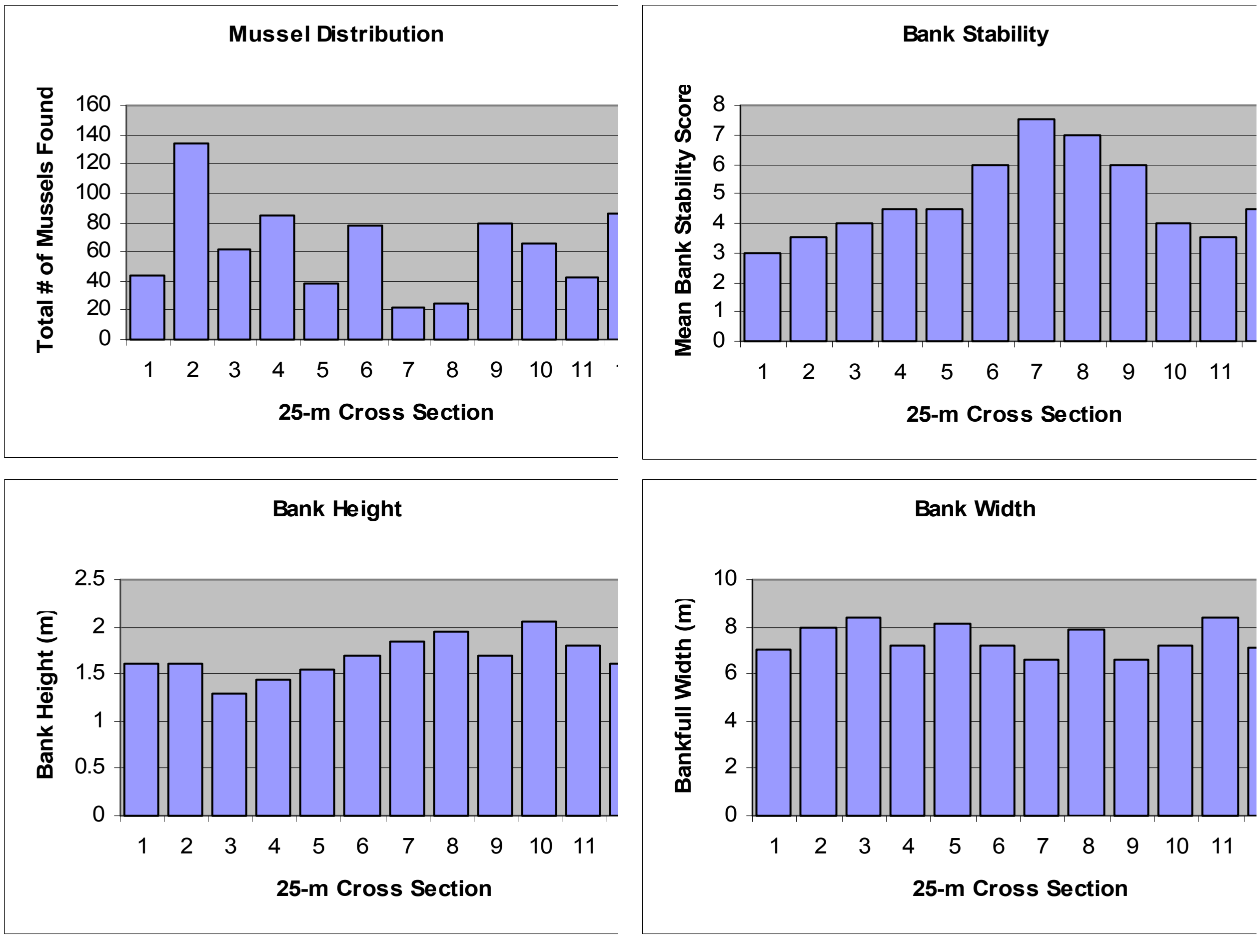


Figure 5. Mussel distribution and habitat data from this site.

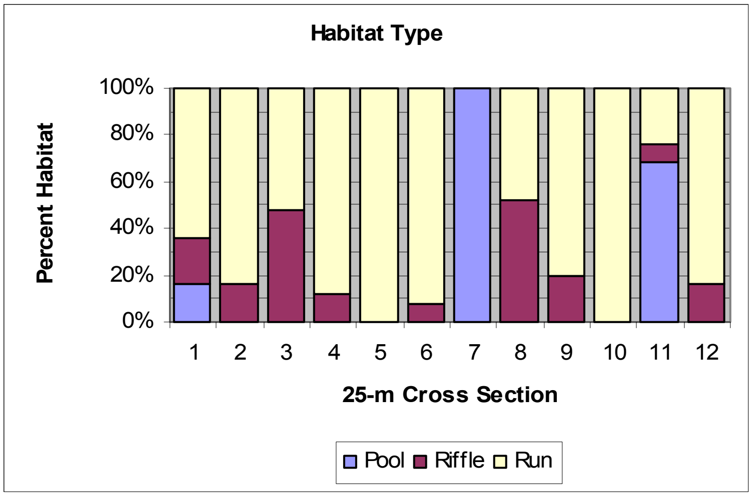


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Moore	**Bridge Number:**	225
Road Crossing:	Big Oak Church Rd.	**Stream:**	Wolf Creek
Date Sampled:	28 April 2005		

Mussels found at site			
Elliptio complanata	379	**Year Built:**	1975
V. constricta	1	**Number of Cells: (w/ base flow)**	3(1)
V. vaughaniana	4	**Obvious scour hole?**	No
Total mussels	**384**	**% of mussels upstream:**	56.3%

Summary:

Figure 1. Culvert

Figure 2. Vegetation near culvert

Figure 3. Upstream side of culvert

Figure 4. Downstream side of culvert

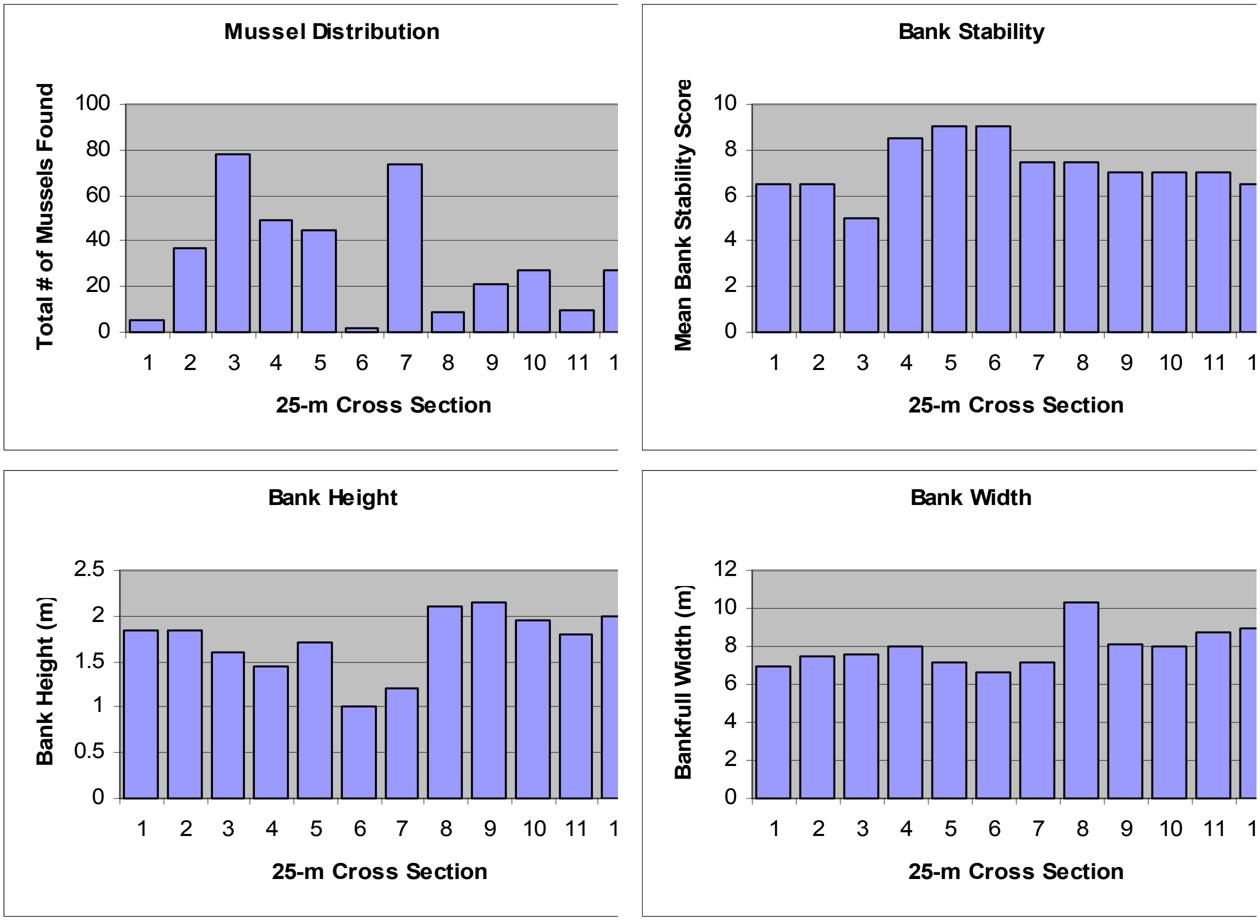


Figure 5. Mussel distribution and habitat data from this site.

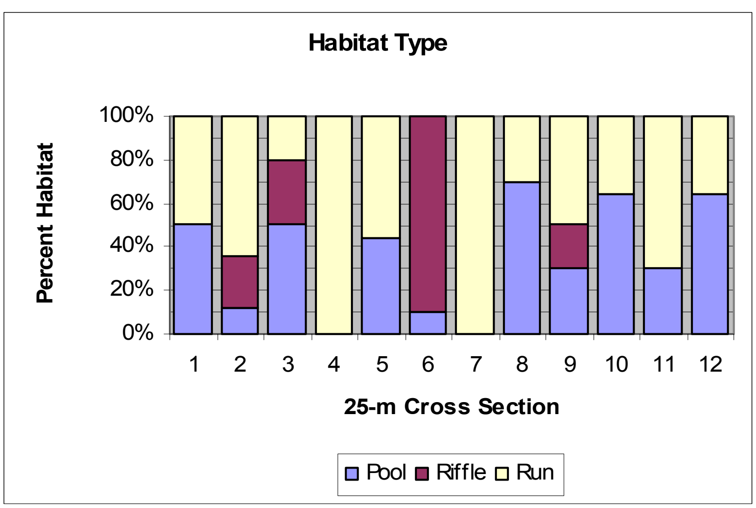


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Orange	**Bridge Number:**	13
Road Crossing:	NC 57	**Stream:**	South Fork Little River
Date Sampled:	13 May 2004		

Mussels found at site			
Elliptio complanata	233	**Year Built:**	1941
S. undulatus	2	**Number of Cells: (w/ base flow)**	1(1)
V. constricta	4	**Obvious scour hole?**	No
Total mussels	**239**	**% of mussels upstream:**	32.2%

Summary:

The culvert at this site seems to have minimal impact on this stream and the mussel fauna. Natural differences in substrate and slope between upstream and downstream likely play a greater role in mussel distribution. The upstream reach is dominated by boulder and bedrock (Fig. 2) and has a relatively high slope and high percentage of riffle (Fig. 6). The downstream reach is much flatter and composed of a mix of sand, gravel, and cobble. There doesn't seem to be much, if any, scour, channel widening, or channel incision because of the culvert.

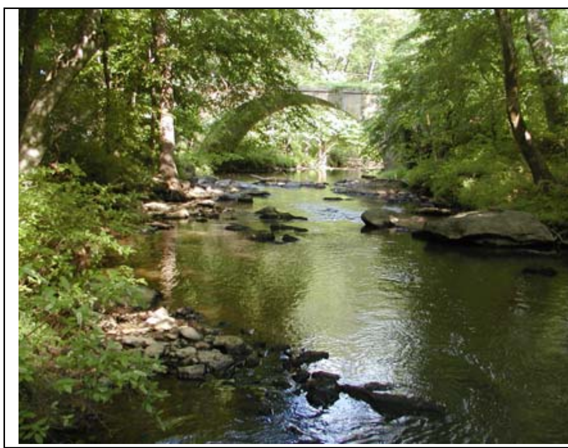


Figure 1. Culvert from upstream

Figure 2. Upstream riffle

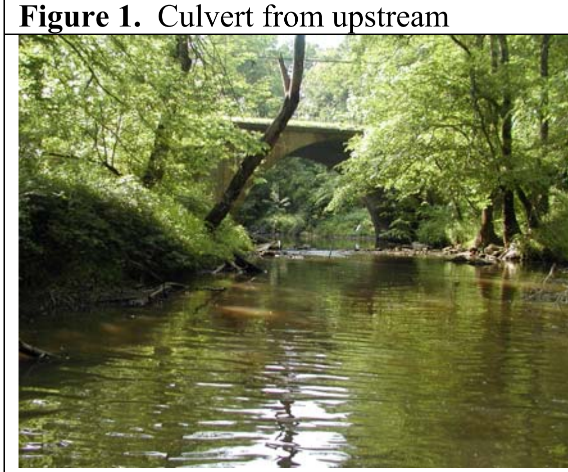


Figure 3. Downstream side of culvert	**Figure 4.** Downstream habitat

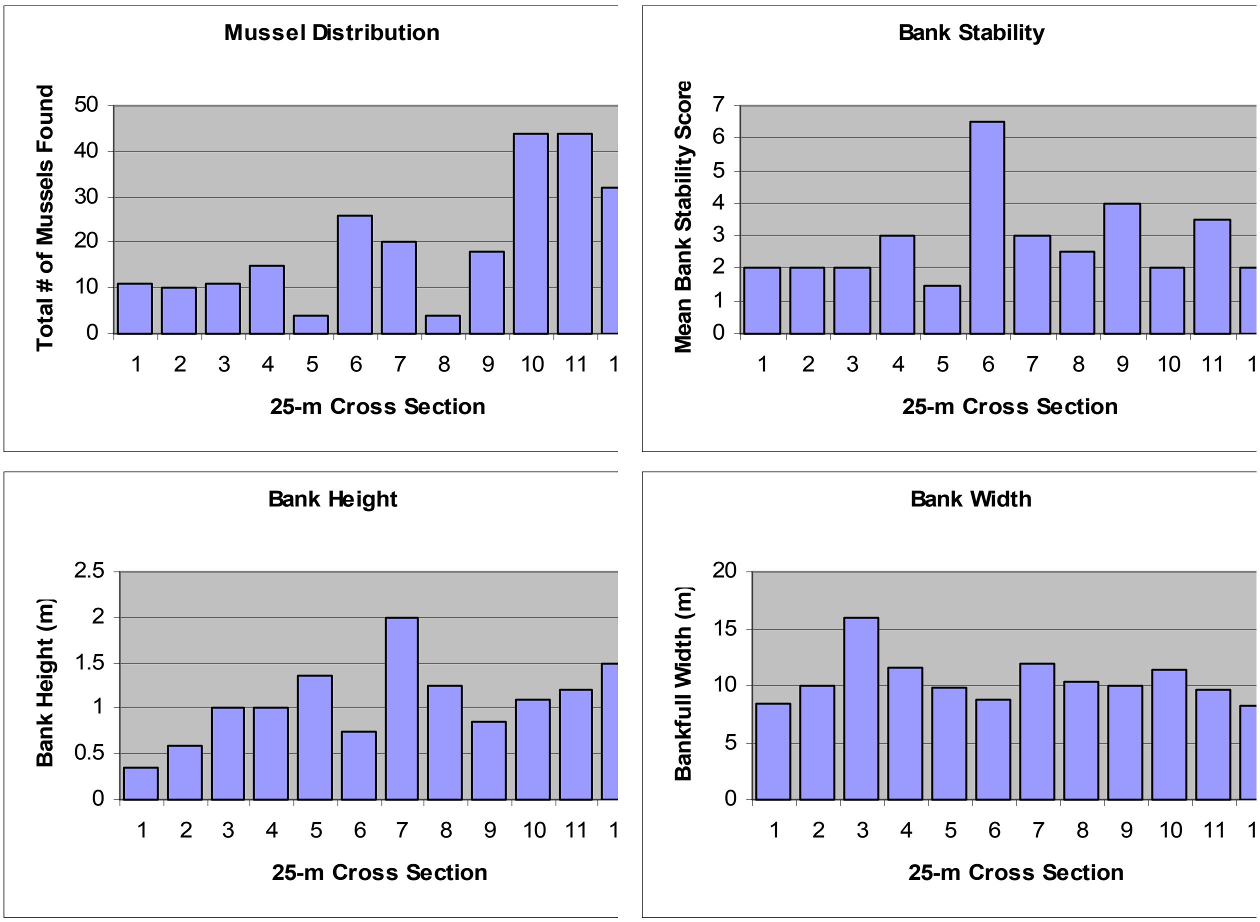


Figure 5. Mussel distribution and habitat data from this site.

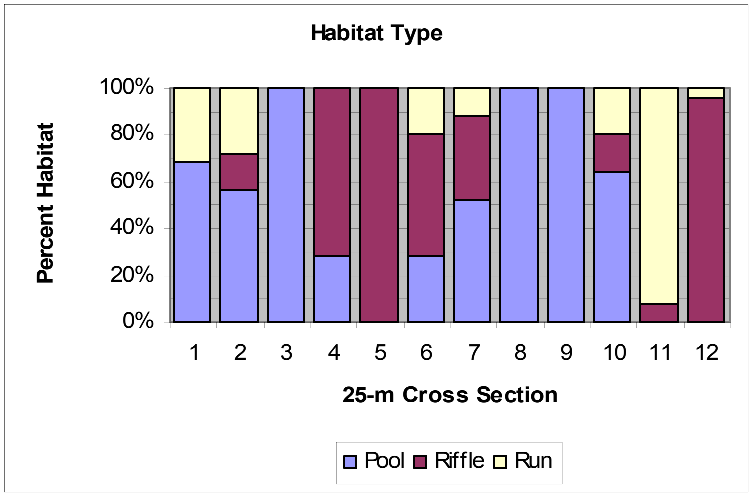


Figure 6. Percentage of habitat type within each 25-m cross section.

County: Orange **Bridge Number:** 263
Road Crossing: I-40 **Stream:** New Hope Creek
Date Sampled: 23 August 2004

Mussels found at site	
Elliptio complanata	1129
P. cataracta	32
V. constricta	4
V. delumbis	19
V. vaughaniana	2
Total mussels	**1186**

Year Built:	1986
Number of Cells: (w/ base flow)	4(4)
Obvious scour hole?	No
% of mussels upstream:	55.3%

Summary:

Relative mussel abundance is low near the culvert on both the upstream and downstream sides. We saw very little bank erosion or scour as a result of this structure, so we cannot determine the exact mechanism causing the lower mussel numbers. We suspect that the installation of a structure of this size (83 meters long) required a great deal of stream alteration during the construction. This may have had lasting effects to the channel and its fauna.

Figure 1. Upstream habitat

Figure 2. Upstream side of culvert

Figure 3. Downstream side of culvert

Figure 4. Downstream habitat

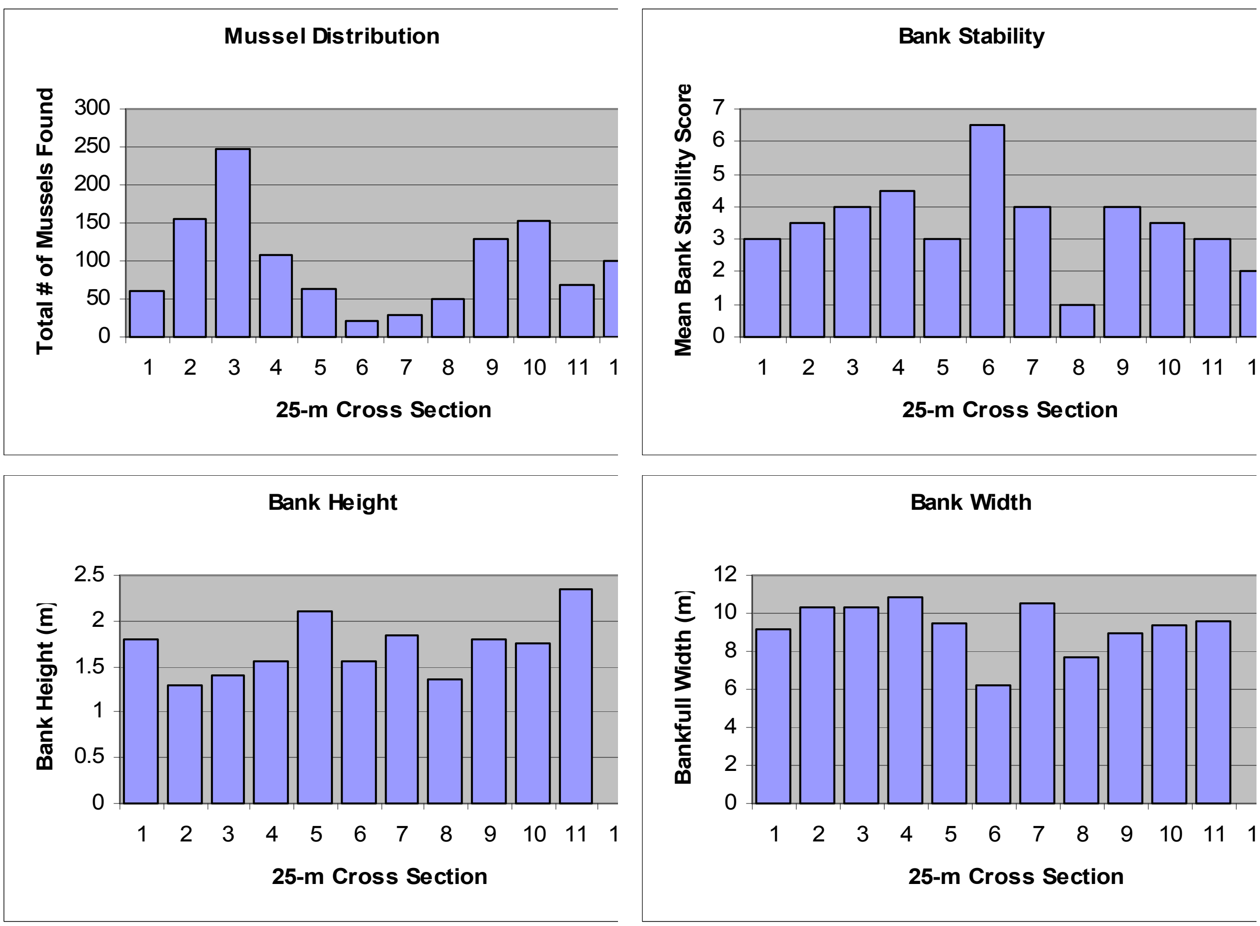


Figure 5. Mussel distribution and habitat data from this site.

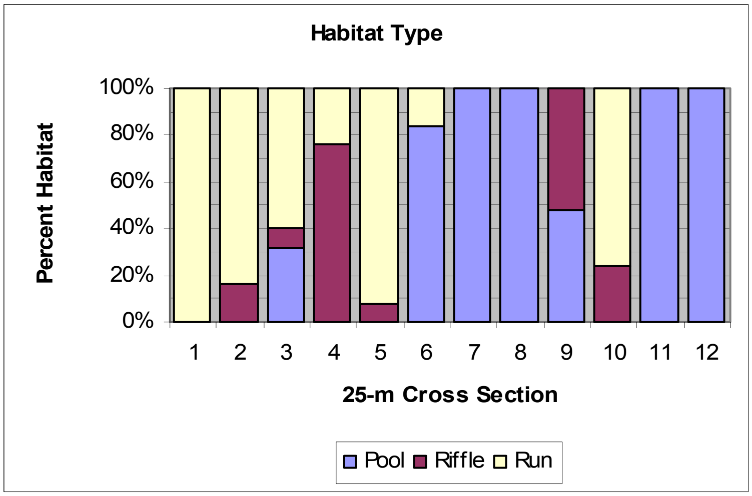


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Orange	**Bridge Number:**	30
Road Crossing:	NC 57	**Stream:**	North Fork Little River
Date Sampled:	18 May 2004		

Mussels found at site			
Elliptio complanata	794	**Year Built:**	1941
		Number of Cells: (w/ base flow)	3(2)
Total mussels	**794**	**Obvious scour hole?**	No
		% of mussels upstream:	43.6%

Summary:

We surveyed this site in 2001 as part of our original study, and we found similar results in this survey done three years later in 2004. There seemed to be a decrease in relative mussel abundance in the first 75 meters downstream of the culvert, but recovery was noted further downstream. Our habitat metrics did not reveal any obvious impacts from the culvert, but there was generally a lack of stable refugia in the adjacent downstream area where mussels were rare.

Figure 1. Upstream habitat

Figure 2. Upstream side of culvert

Figure 3. Downstream side of culvert

Figure 4. Downstream habitat

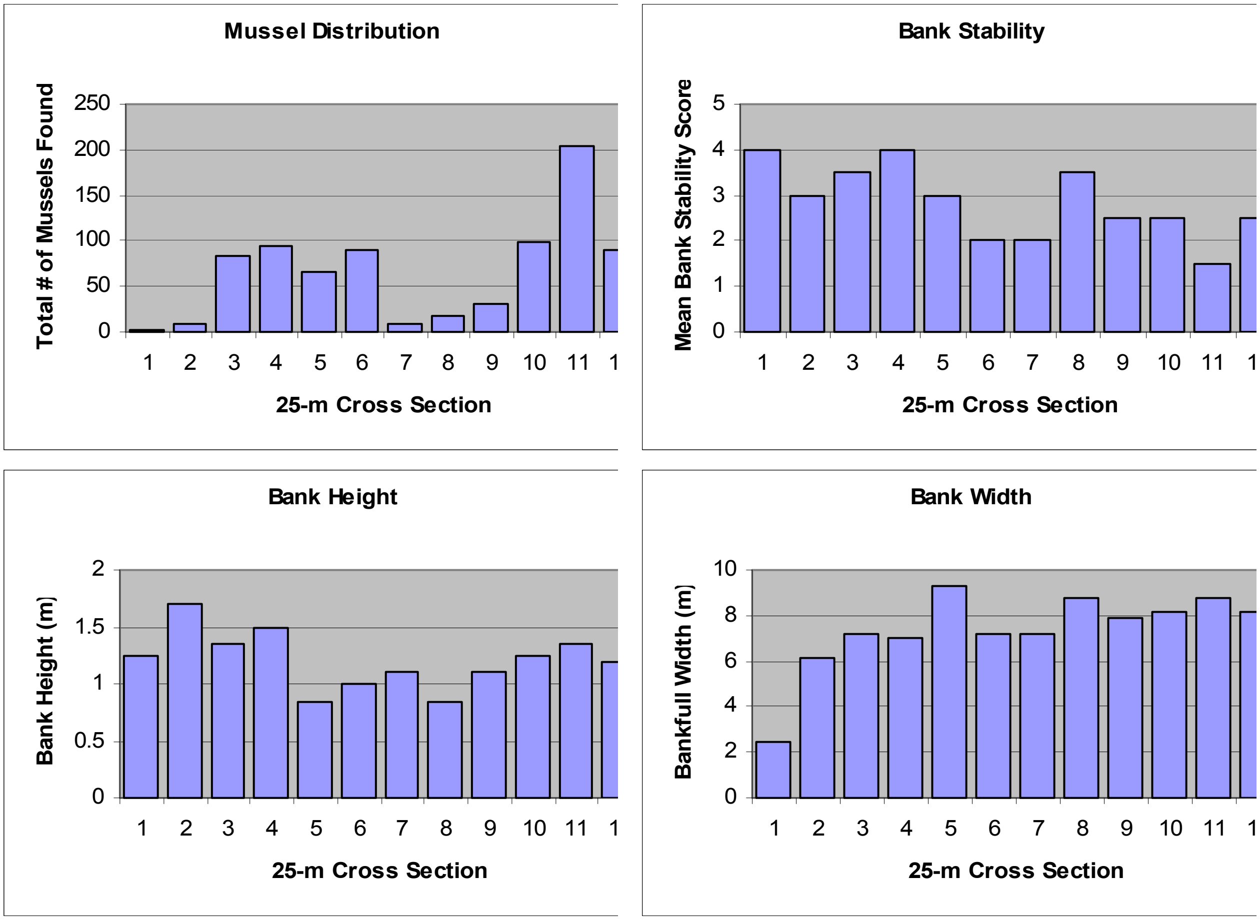


Figure 5. Mussel distribution and habitat data from this site.

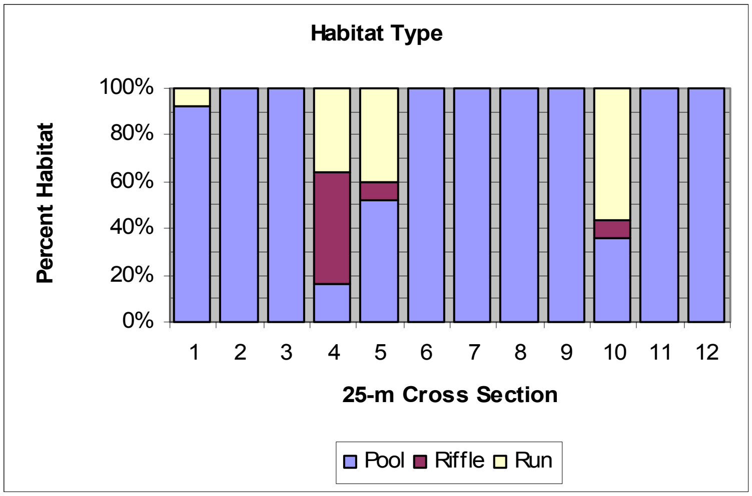


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Person	**Bridge Number:**	211
Road Crossing:	Mayo Lake Road	**Stream:**	Mayo Creek
Date Sampled:	2 June 2004		

Mussels found at site			
Elliptio complanata	558	**Year Built:**	1994
P. cataracta	4	**Number of Cells: (w/ base flow)**	3(3)
S. undulatus	1	**Obvious scour hole?**	Yes
Total mussels	**563**	**% of mussels upstream:**	8.7%

Summary:

This was by far the largest stream sampled in the culvert portion of the study. There was scour downstream of the culvert and few mussels found within 75 meters of the structure; however the lack of habitat and mussels upstream makes it difficult to truly assess the impacts of this culvert. Because of the scour downstream, we would not recommend culverts be placed on a stream this size.

Figure 1. Upstream gravel bar

Figure 2. Upstream habitat

Figure 3. Downstream of culvert

Figure 4. Downstream monitoring station

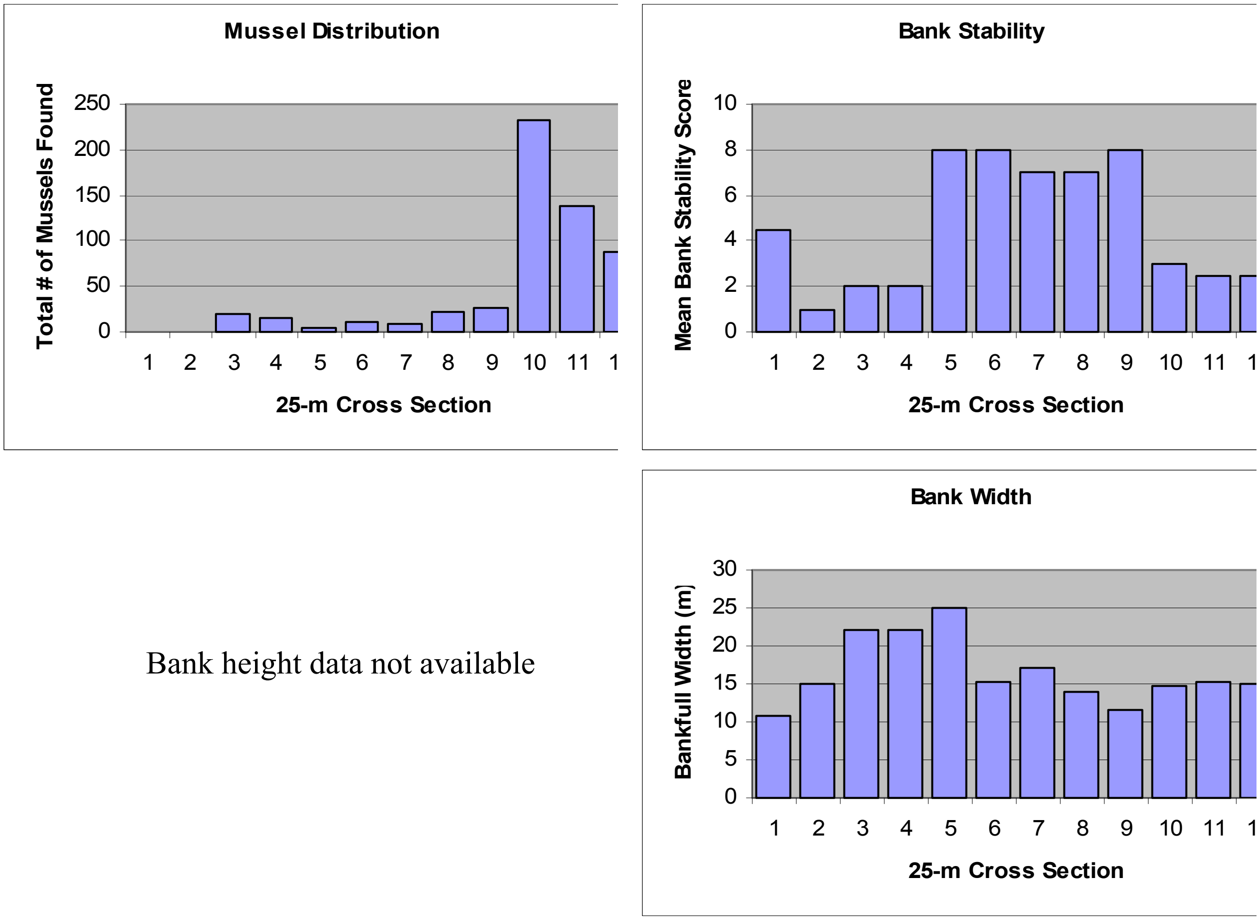


Figure 5. Mussel distribution and habitat data from this site.

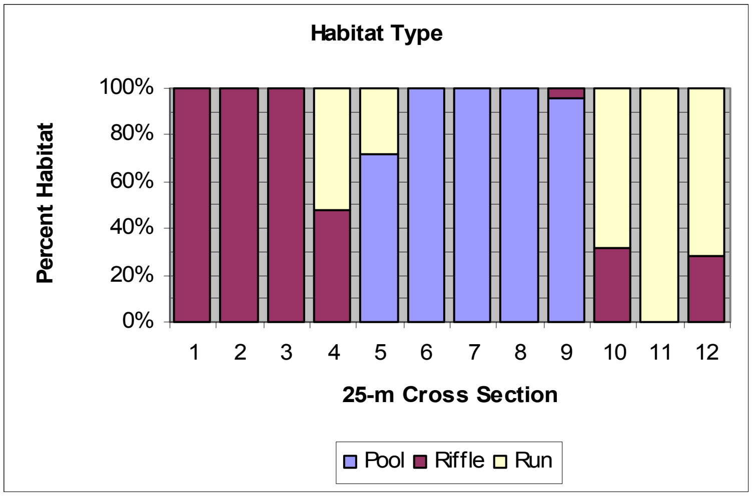


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Person	**Bridge Number:**	38
Road Crossing:	Willie Gray Road	**Stream:**	Lick Creek
Date Sampled:	3 June 2004		

Mussels found at site			
Elliptio complanata	1092	**Year Built:**	1991
P. cataracta	19	**Number of Cells: (w/ base flow)**	2(2)
S. undulatus	2	**Obvious scour hole?**	Yes
Total mussels	**1113**	**% of mussels upstream:**	51.0%

Summary:

We had surveyed this site in 2001, and found mussels at a much lower density than what we found in 2004. Surveys in 2004 yielded only 224 total mussels in the reach surveyed in 2004 compared to 1113 mussels found in 2004. The number of species other than *Elliptio complanata* remained fairly similar between surveys, but the number of *E. complanata* greatly increased. We do not know why this is the case. It may be that differences in water levels at the times of the two surveys caused the differences in the numbers found. The culvert lies at an angle to the downstream (Fig. 3) and most of the culverts energy is directed into the bank. This likely dissipates erosive forces during large storm events; however, banks were slightly higher on average downstream and there were more point bars and sand deposition there as well. Currently, this doesn't seem to be causing great damage to the mussel fauna there. The extreme angle of the culvert has caused some bank erosion (Fig. 5), but the bank seems to be holding for now. At some point in the future – perhaps many years from now - I suspect there will be failure of this bank. In that event, there would likely be great consequences to the mussel fauna in the downstream reaches.

Figure 1. Upstream habitat

Figure 2. Upstream side of culvert

Figure 3. Downstream side of culvert

Figure 4. Downstream habitat

Figure 5. Immediately downstream of culvert. Flow is directed into the bank.

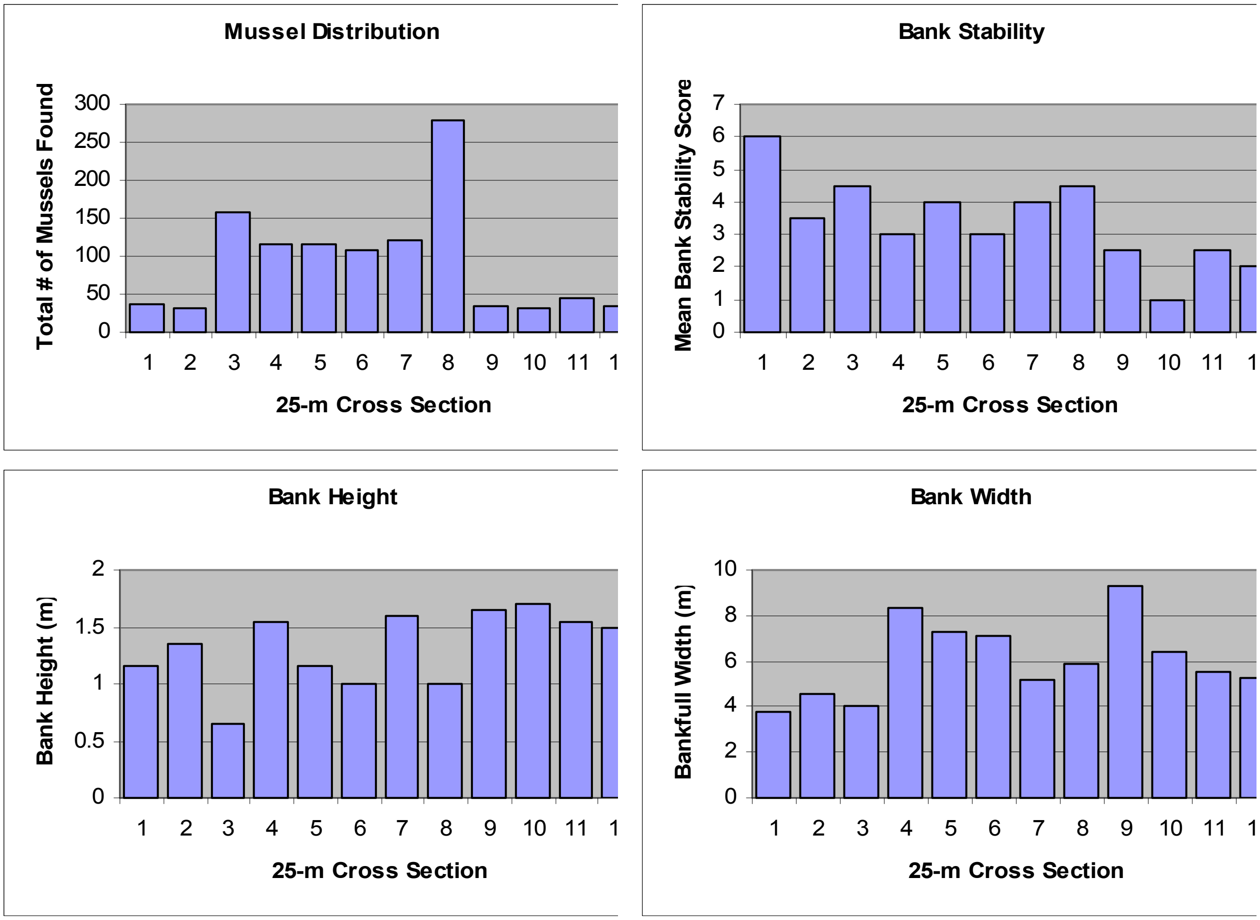


Figure 6. Mussel distribution and habitat data from this site.

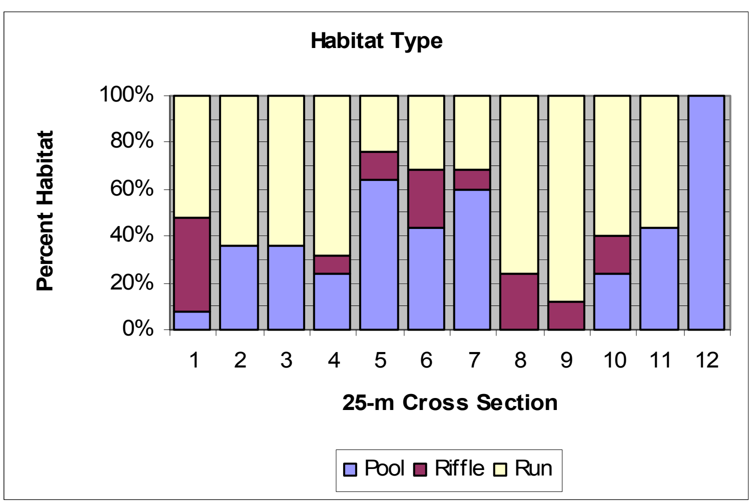


Figure 7. Percentage of habitat type within each 25-m cross section.

County:	Randolph	**Bridge Number:**	339
Road Crossing:	Jugtown Road	**Stream:**	Reedy Creek
Date Sampled:	27 July 2004		

Mussels found at site			
Elliptio complanata	28	**Year Built:**	2000
P. cataracta	16	**Number of Cells: (w/ base flow)**	1(1)
V. vaughaniana	2	**Obvious scour hole?**	No
Total mussels	**46**	**% of mussels upstream:**	45.7%

Summary:

This culvert seems to be having little impact on the stream and its mussel fauna. There were no differences in bank height or channel width upstream or down, and a large number of the mussels at the site were found within 25 meters of the structure. Naturally poor habitat in the other parts of the site likely accounts for this. The upper reaches of the site were dominated by bedrock and contained very little fine sediment. We surveyed this site previously in 2002 and found much of it to be dry while the area near the structure still contained water. This may also help explain the overall lack of mussels.

Figure 1. Upstream side of culvert

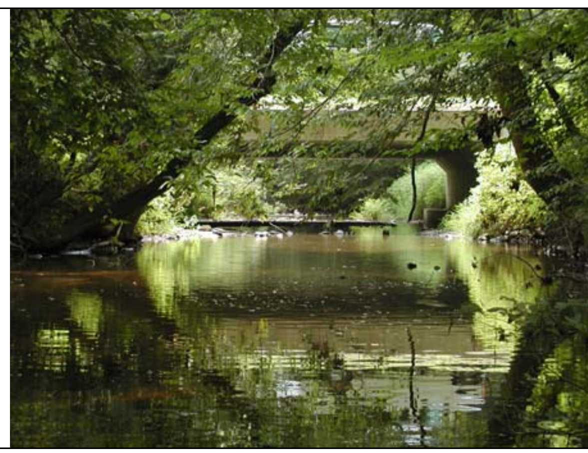


Figure 2. downstream side of culvert

Figure 3. upstream habitat

Figure 4. Grassy downstream habitat

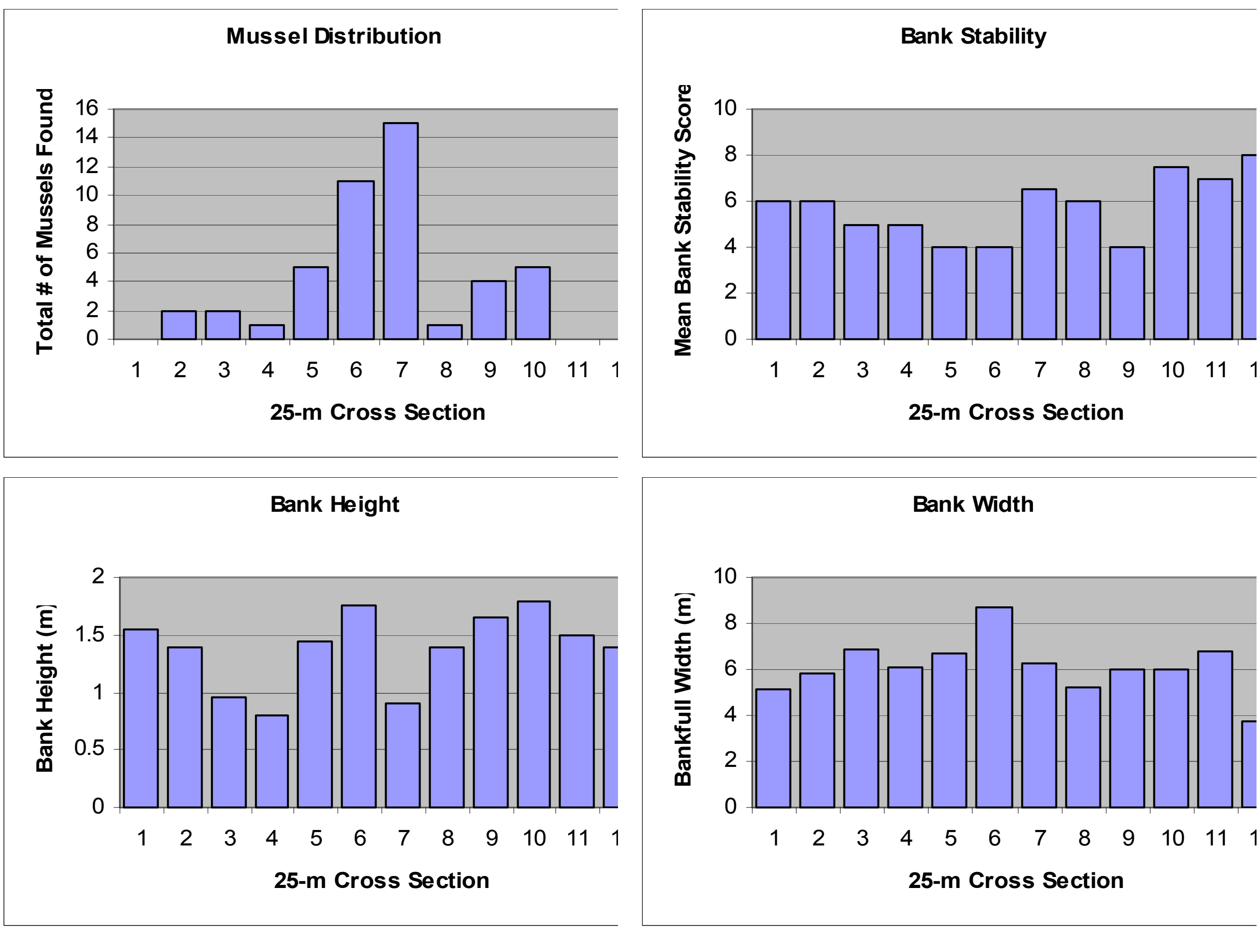


Figure 5. Mussel distribution and habitat data from this site.

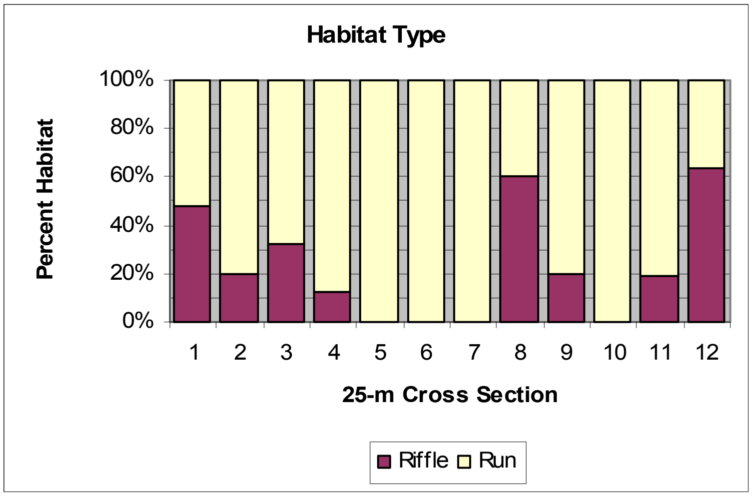


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Randolph	**Bridge Number:**	459
Road Crossing:	Low Bridge Rd.	**Stream:**	Reed Creek
Date Sampled:	17 August 2004		

Mussels found at site			
Elliptio complanata	621	**Year Built:**	1955
P. cataracta	17	**Number of Cells: (w/ base flow)**	3(2)
V. delumbis	10	**Obvious scour hole?**	No
Total mussels	**648**	**% of mussels upstream:**	42.0%

Summary:

There were no obvious impacts of this structure on the stream and its mussel fauna. In fact, more mussels were found downstream than upstream. We also did not detect any significant changes to the habitat around the culvert.

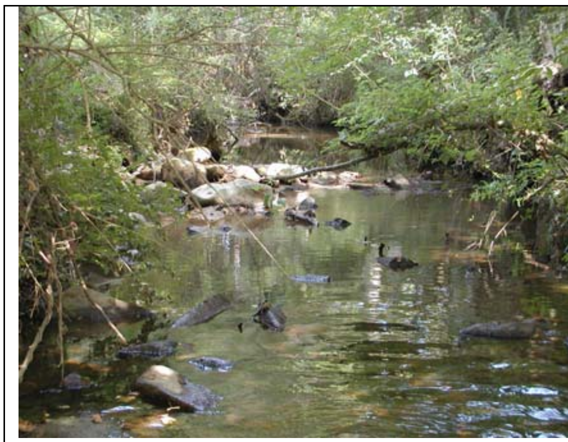


Figure 1. Upstream habitat

Figure 2. Upstream side of culvert

Figure 3. Downstream view of culvert

Figure 4. Downstream habitat

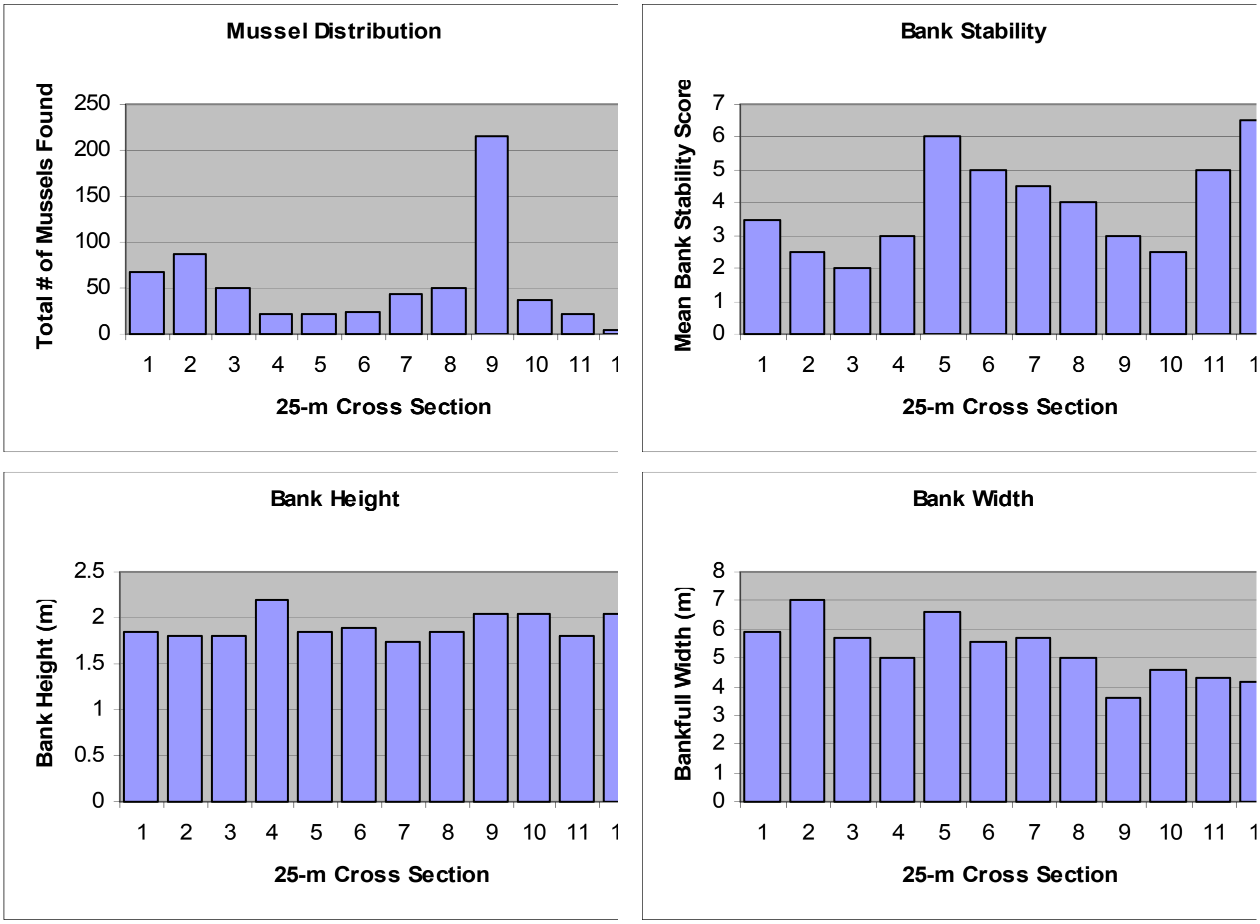


Figure 5. Mussel distribution and habitat data from this site.

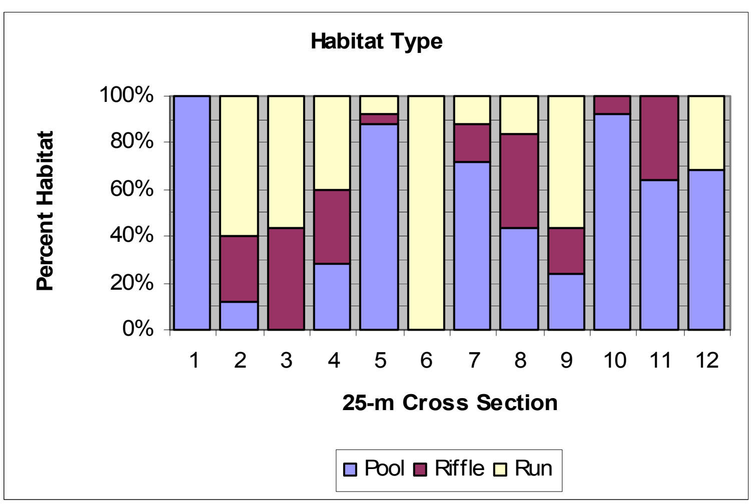


Figure 6. Percentage of habitat type within each 25-m cross section.

FELSIC CRYSTALLINE

Impact	County – Bridge Number	Culvert Type
High	Franklin 62 Franklin 6	Box Box
Low	Wake 134 Wake 135 Wake 372	Box Arch Pipe
None Detected		

County:	Franklin	**Bridge Number:**	6
Road Crossing:	US 401	**Stream:**	Crooked Creek
Date Sampled:	22 June 2004		

Mussels found at site			
Elliptio complanata	736	**Year Built:**	1955
U. imbecillis	1	**Number of Cells: (w/ base flow)**	3(3)
Total mussels	**737**	**Obvious scour hole?**	Yes
		% of mussels upstream:	58.5%

Summary:

This site lies in the Upper Coastal Plain Soil System and has the classic culvert affect from this area. There is a large, deep scour pool immediately downstream of the culvert, and few mussels were found in this area. Bank height was also significantly greater downstream of the culvert.

Figure 1. Upstream habitat

Figure 2. Upstream side of culvert

Figure 3. Downstream side of culvert

Figure 4. Downstream habitat

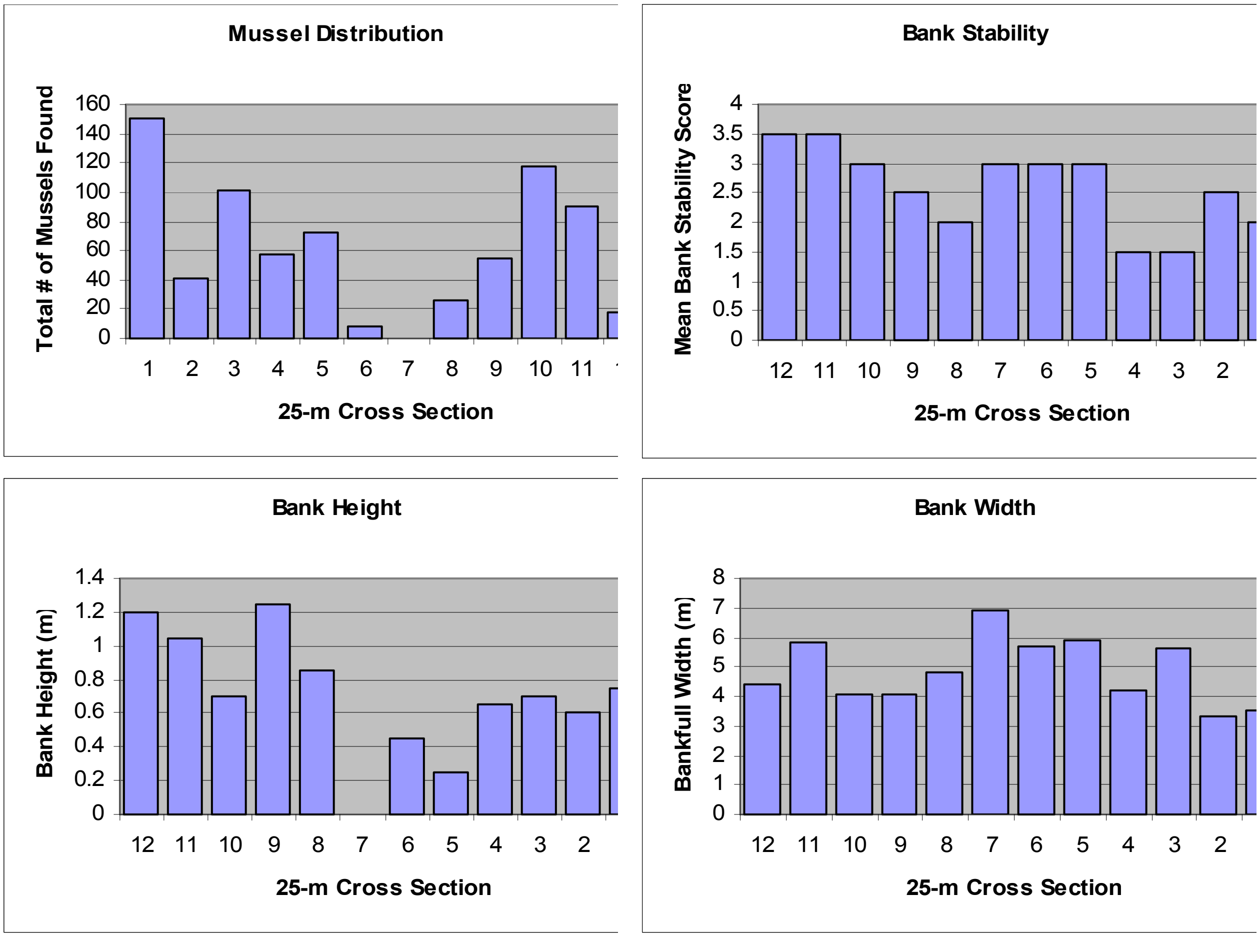


Figure 5. Mussel distribution and habitat data from this site.

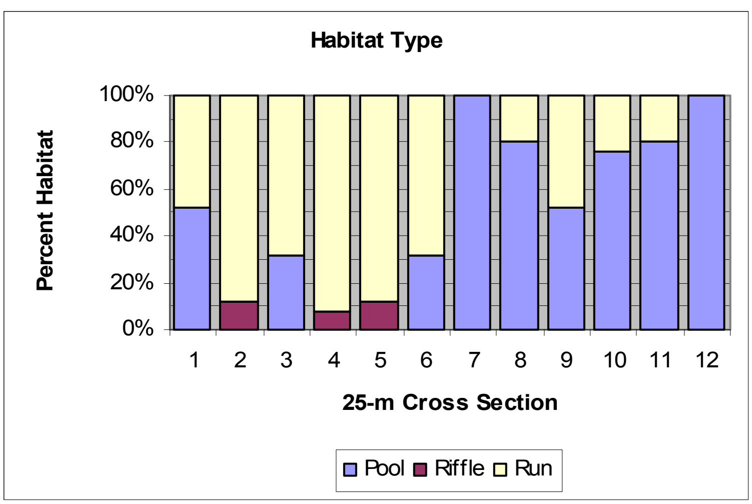


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Franklin	**Bridge Number:**	62
Road Crossing:	NC 56	**Stream:**	Fox Creek
Date Sampled:	1 July 2004		

Mussels found at site			
Elliptio complanata	128	**Year Built:**	1931
		Number of Cells: (w/ base flow)	3(1)
Total mussels	**128**	**Obvious scour hole?**	Yes
		% of mussels upstream:	96.9%

Summary:

Habitat downstream of the culvert was poor. The channel was very deep compared to the upstream, and banks were also less stable. In addition to the culvert, this was likely influenced by some amount of channelization downstream as it was adjacent to a gravel parking lot. There appeared to be some channel alteration done in the past, perhaps to accommodate the parking lot, so it was hard to separate damage done by this from that potentially done by the culvert. The culvert is likely influencing sediment movement to some degree by holding back sediment upstream creating shallower habitat and helping to scour the downstream. Mussel abundance was greatly reduced in the poor habitat downstream with 96.9% of all mussels found being upstream of the culvert.

Figure 1. Downstream

Figure 2. Downstream side of culvert

Figure 3. Upstream side of culvert

Figure 4. Upstream habitat

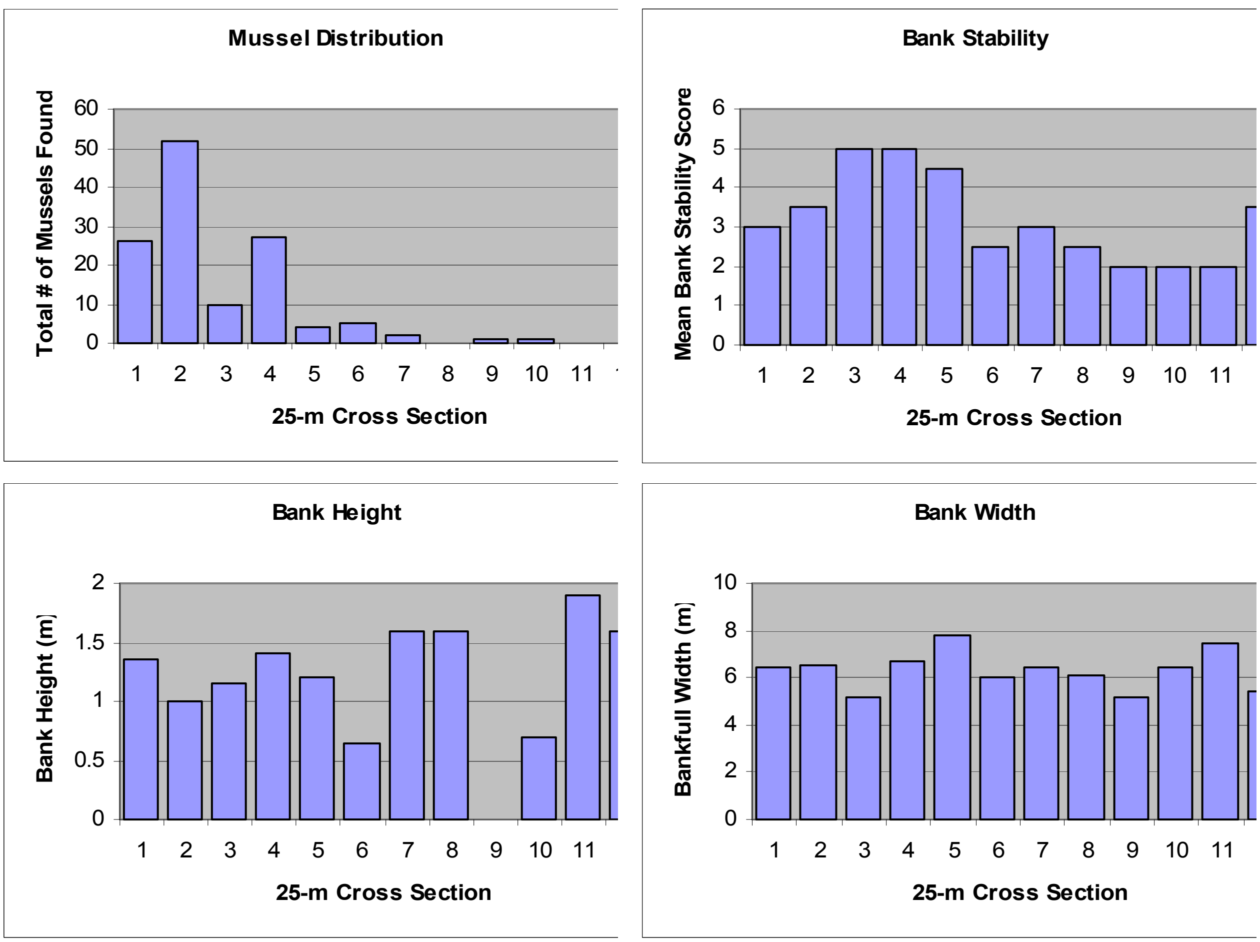


Figure 5. Mussel distribution and habitat data from this site.

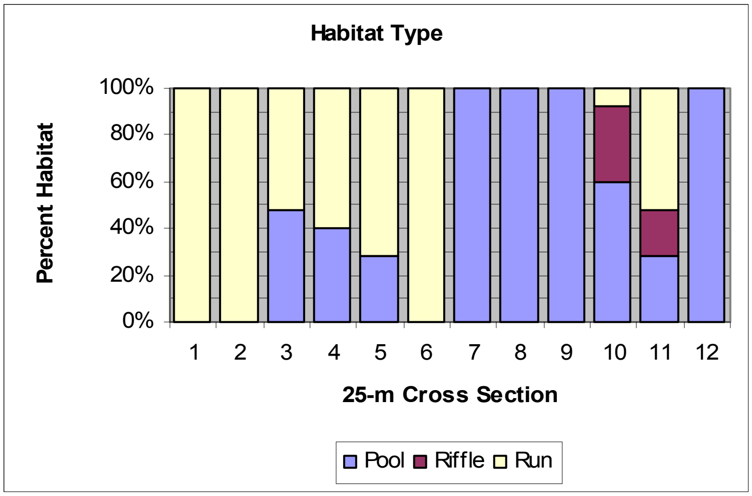


Figure 6. Percentage of habitat type within each 25-m cross section.

County: Wake **Bridge Number:** 134
Road Crossing: Kearney Road **Stream:** Horse Creek
Date Sampled: 5 May 2004

Mussels found at site			
Elliptio complanata	111	**Year Built:**	1992
		Number of Cells: (w/ base flow)	3(3)
Total mussels	**111**	**Obvious scour hole?**	No
		% of mussels upstream:	83.8%

Summary:

Although relative mussel abundance was much higher upstream compared to downstream, the habitat types were quite different due to natural conditions. The downstream reach was much steeper and dominated by riffle and rocky habitat. There was some bank destabilization downstream. We do not know how much of the lower relative mussel abundance and bank erosion can be attributed to this structure; however it is unfriendly to fish movement. This culvert was slightly perched – roughly 3-4 inches above the stream at base flow (Fig. 2).

Figure 1. Upstream habitat

Figure 2. Downstream side of culvert

Figure 3. Downstream habitat	**Figure 4.** Sharp bend downstream

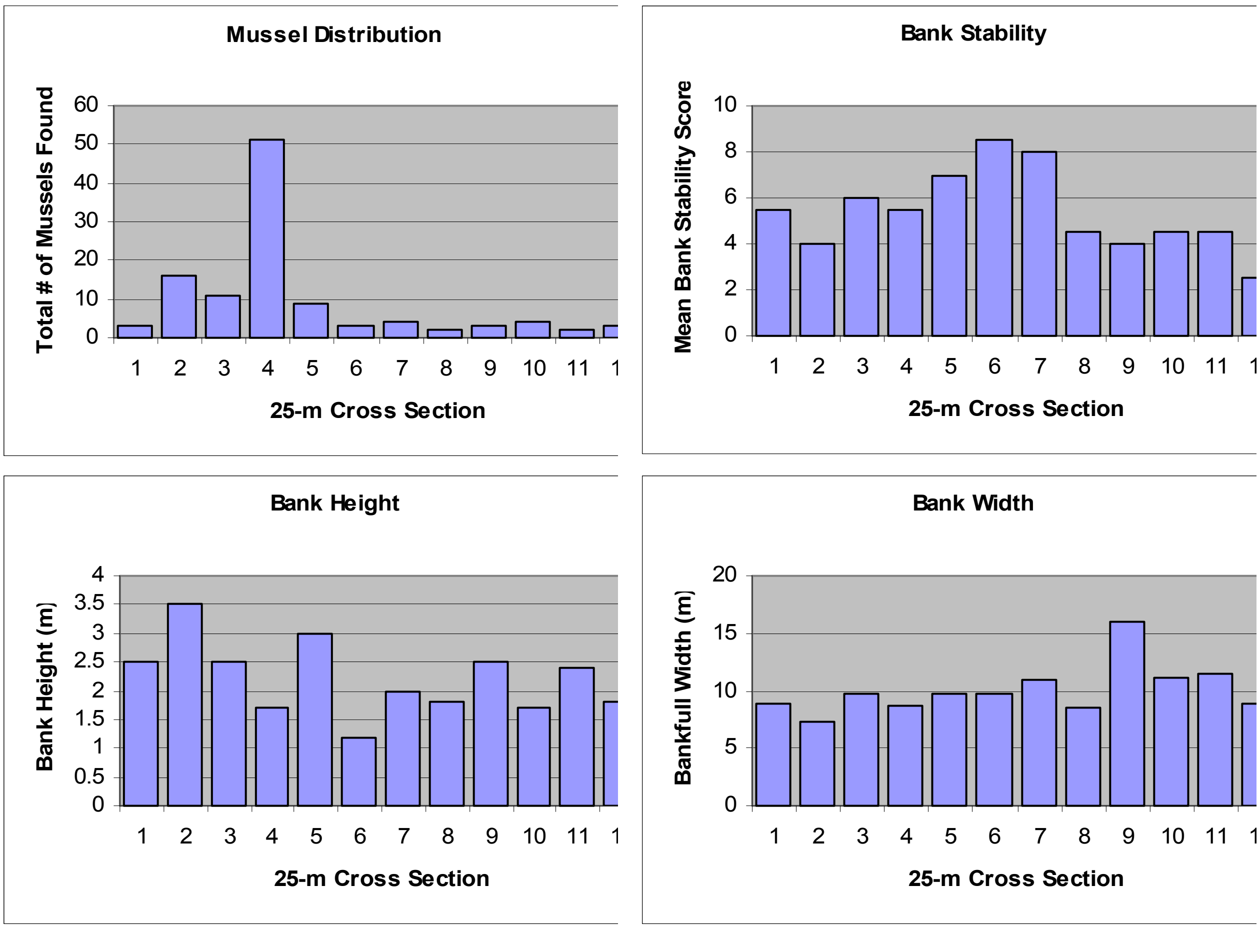


Figure 5. Mussel distribution and habitat data from this site.

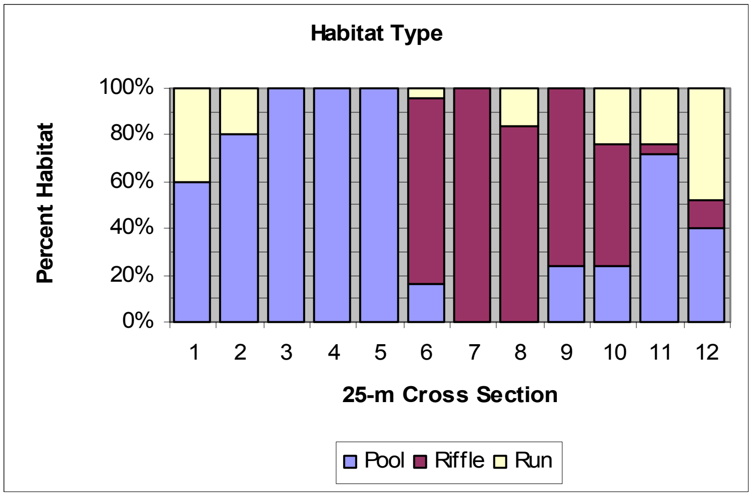


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Wake	**Bridge Number:**	135
Road Crossing:	Thompson Mill Rd.	**Stream:**	Horse Creek
Date Sampled:	1 June 2004		

Mussels found at site			
Elliptio complanata	28	**Year Built:**	1988
		Number of Cells: (w/ base flow)	1(1)
Total mussels	**28**	**Obvious scour hole?**	No
		% of mussels upstream:	53.6%

Summary:

This site had very poor mussel habitat, and we found a very low number of mussels. Portions of the banks were highly unstable, and there was a very large pile of trees that had fallen into the channel downstream. Bank height upstream was much higher than downstream with much of the upstream having banks approximately 4 meters high (Fig. 5). We cannot attribute any habitat damage or reduced mussel abundance to the current structure; however, because of the extreme incision upstream, it is a possibility that the installation of this arch in 1988 caused a head cut and significant down-cutting of the upstream channel. We would need to analyze data from the old structure to know if this was the case or not.

Figure 1. Upstream habitat

Figure 2. Upstream side of culvert

Figure 3. Downstream side of culvert

Figure 4. Downstream bank erosion

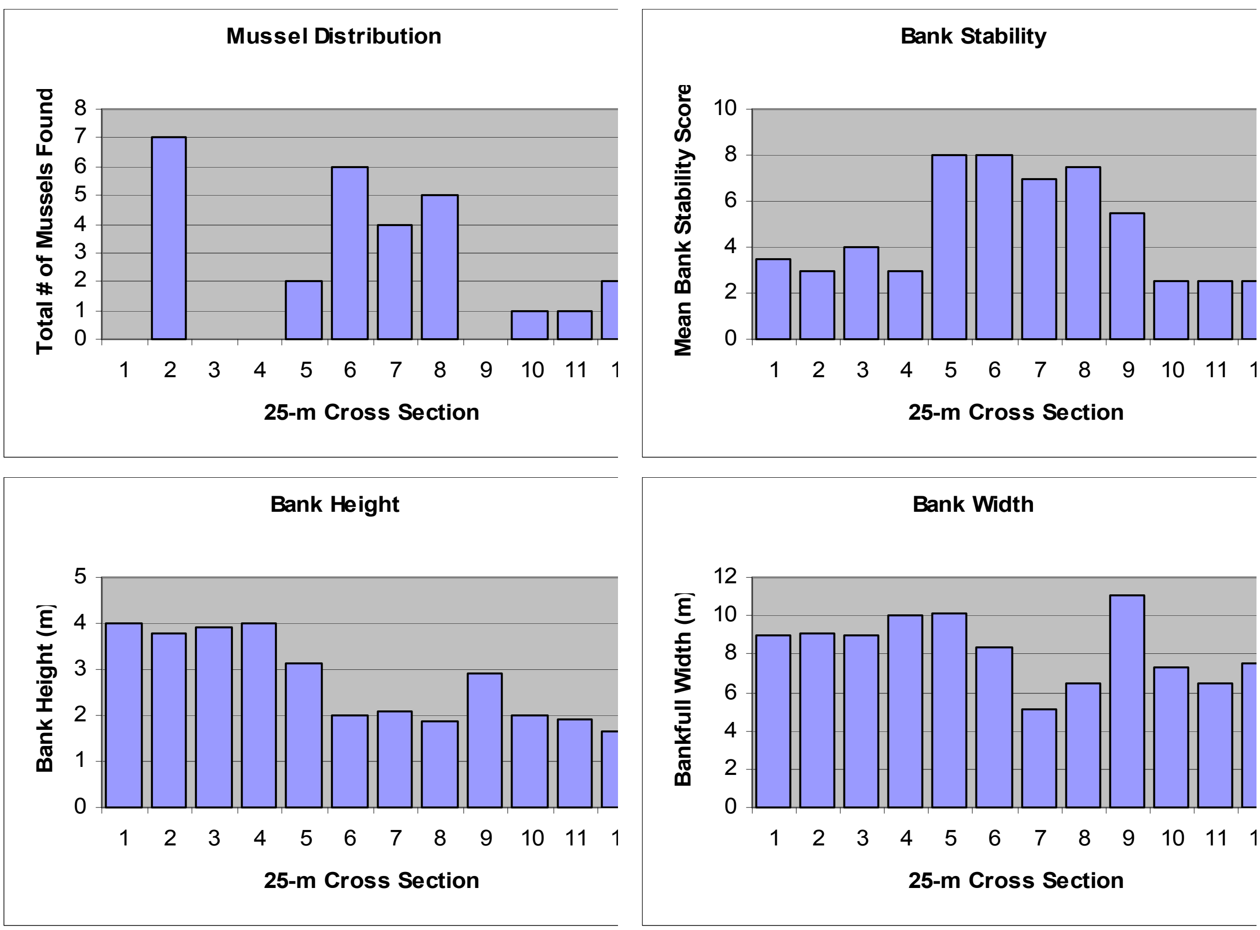


Figure 5. Mussel distribution and habitat data from this site.

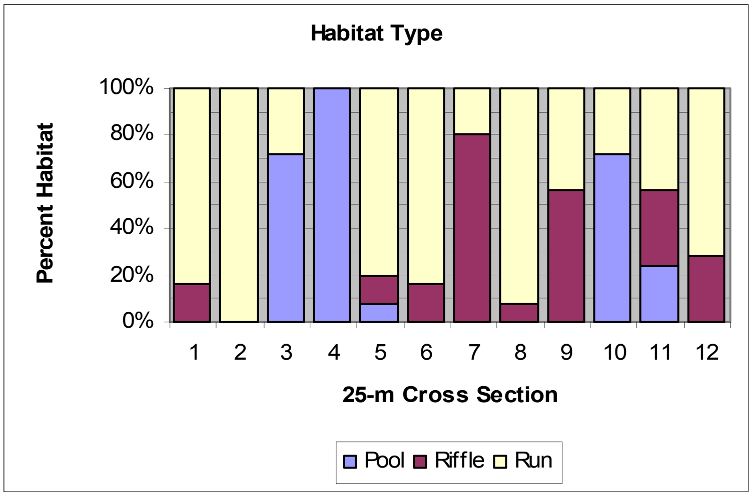


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Wake	**Bridge Number:**	372
Road Crossing:	Sunset Lake Rd.	**Stream:**	Middle Creek
Date Sampled:	17 May 2004		

Mussels found at site			
Elliptio complanata	62	**Year Built:**	1993
P. cataracta	1	**Number of Cells: (w/ base flow)**	2(2)
Total mussels	**63**	**Obvious scour hole?**	Yes
		% of mussels upstream:	28.6%

Summary:

This entire site is in poor condition. As urbanization has encroached upon this watershed, it has likely contributed to the destabilization of this channel. There are several trees that have fallen from the banks into the stream both upstream and downstream. We can attribute very little, if any, damage to mussel fauna and stream habitat to this structure.

Figure 1. Downstream habitat

Figure 2. Downstream side of culvert

Figure 3. Upstream side of culvert

Figure 4. Upstream habitat

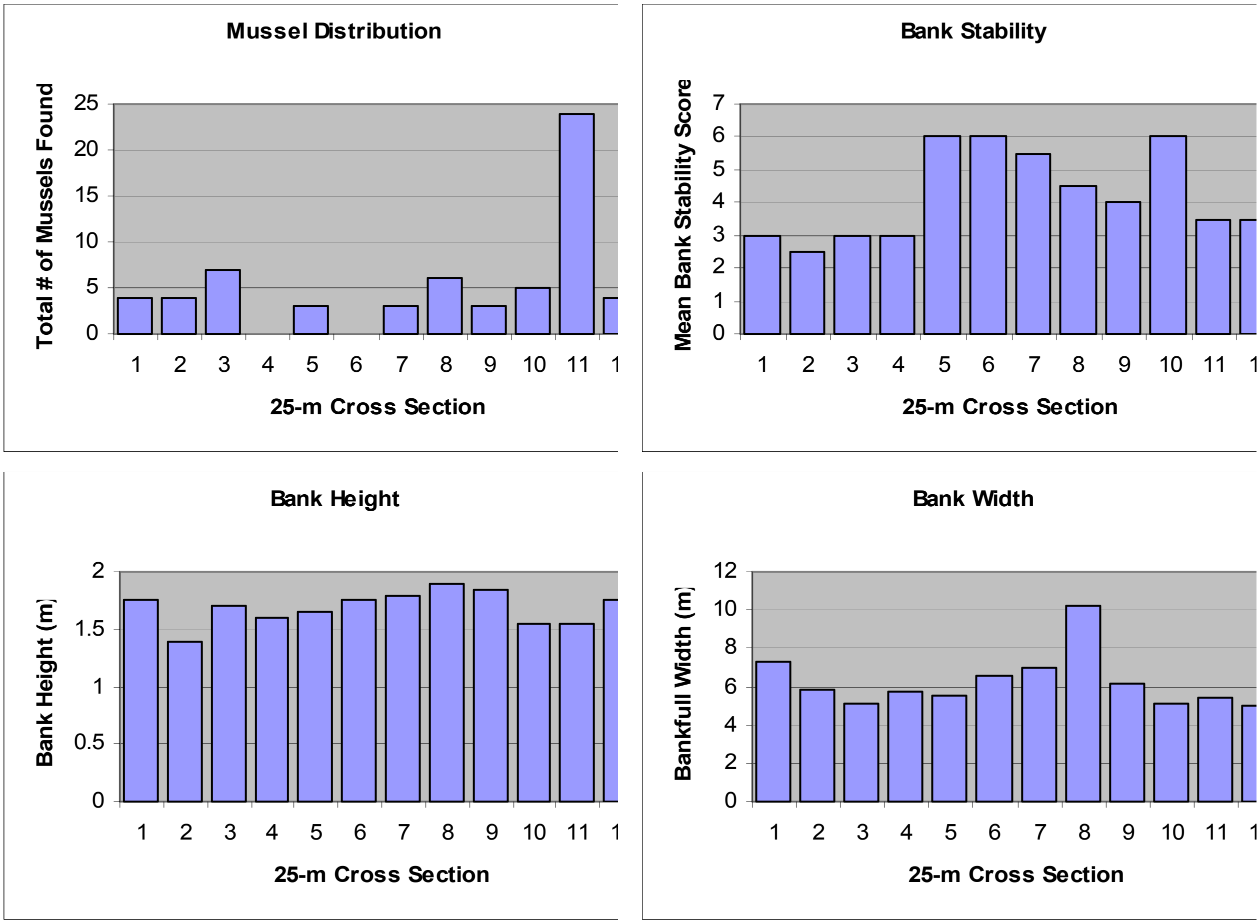


Figure 5. Mussel distribution and habitat data from this site.

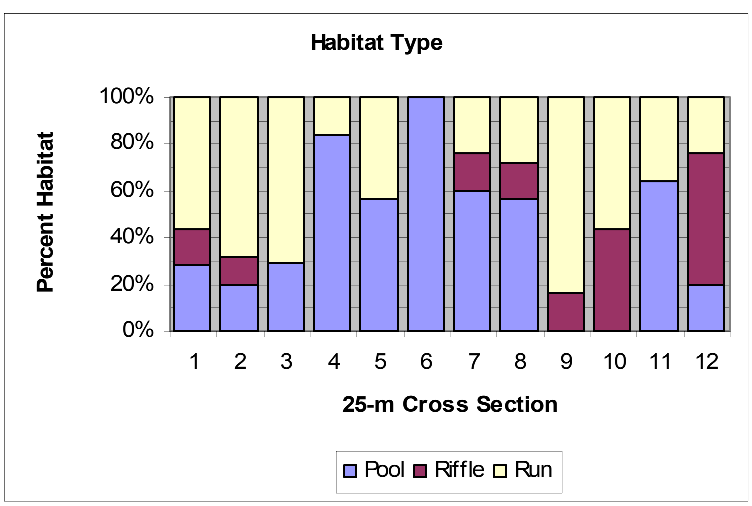


Figure 6. Percentage of habitat type within each 25-m cross section.

TRIASSIC BASIN

Impact	County – Bridge Number	Culvert Type
High		
Low	Moore 220	Pipe
None Detected		

County:	Moore	**Bridge Number:**	220
Road Crossing:	Old River Road	**Stream:**	Big Governors Creek
Date Sampled:	29 July 2004		

Mussels found at site			
Elliptio complanata	685	**Year Built:**	1995
V. delumbis	5	**Number of Cells: (w/ base flow)**	3(1)
V. vaughaniana	1	**Obvious scour hole?**	Yes
Total mussels	**691**	**% of mussels upstream:**	8.8%

Summary:

Strangely, habitat and mussel data at this culvert is contrary to that at most culverts. Only a small number of mussels were found upstream of the culvert compared to downstream. Mussel habitat upstream was generally poor. It was deep, and the channel was significantly wider upstream compared to downstream. There was also little in the way of refugia for mussels along the banks upstream. It would have been interesting to have habitat and mussel data before this current culvert was installed in 1995 to know if the structure has any affect on why the stream is in its current condition. There was a scour hole at the downstream mouth of the culvert and few mussels in that 25-meter reach.


Figure 1. Upstream habitat


Figure 2. Upstream side of culvert

Figure 3. Downstream side of culvert

Figure 4. Downstream habitat

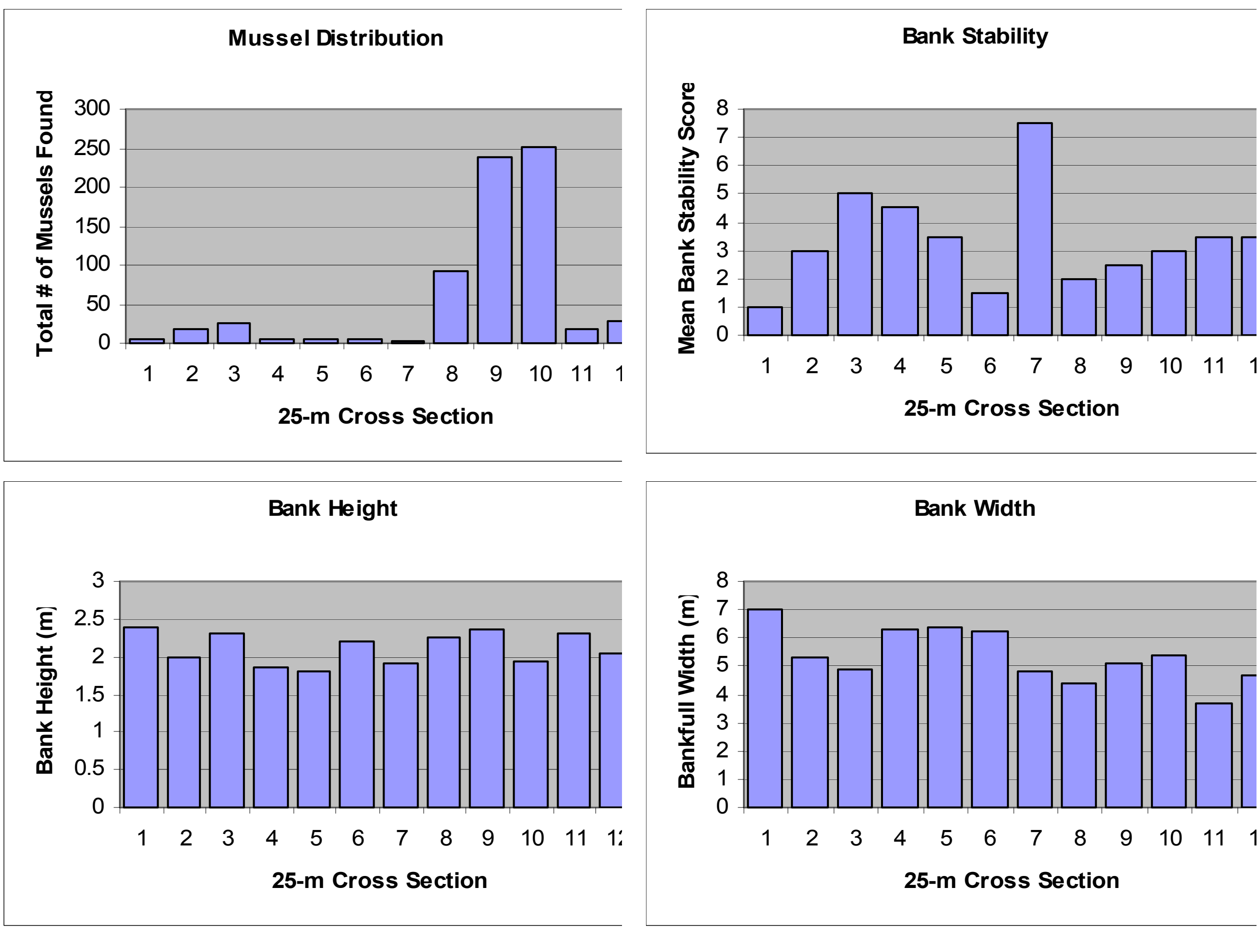


Figure 5. Mussel distribution and habitat data from this site.

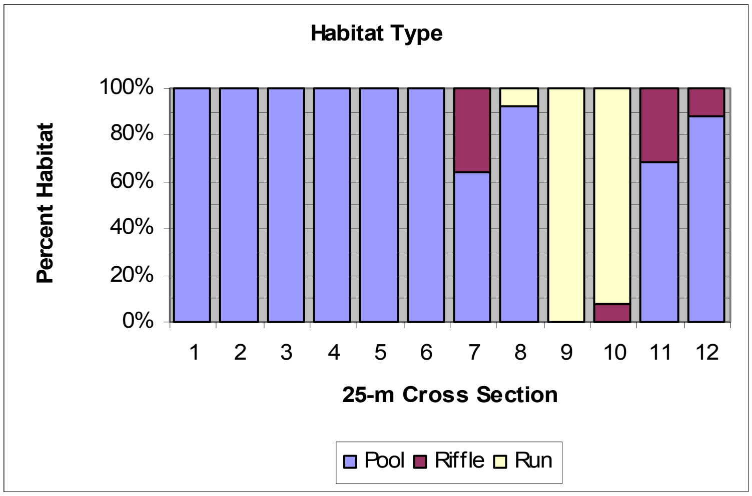


Figure 6. Percentage of habitat type within each 25-m cross section.

UPPER COASTAL PLAIN AND PIEDMONT

Impact	County – Bridge Number	Culvert Type
High	Halifax 61 Halifax 110 Johnston 2052 Nash 310 Wake 561 Wilson 194	Box Pipe Box Box Box Pipe
Low	Harnett 26 Franklin 16	Box Box
None Detected		

County: Franklin **Bridge Number:** 16
Road Crossing: NC 39 **Stream:** Norris Creek
Date Sampled: 21 June 2004

Mussels found at site			
Elliptio complanata	1366	**Year Built:**	1941
Utterbackia imbecillis	1	**Number of Cells: (w/ base flow)**	4(4)
Alasmidonta heterdon	1	**Obvious scour hole?**	No
Total mussels	**1366**	**% of mussels upstream:**	37.4%

Summary:

Channel width has been greatly increased just downstream of the culvert, and there was a subsequent lack of mussels in this area. This has caused a great deal of sand to settle out (Fig. 2). The stream seemed to recover somewhat after 50 meters (Fig. 3) where mussel abundance rebounded; however, channel width remained greater than that upstream of the culvert (Fig. 5). We found one individual of the dwarfwedge mussel (*Alasmidonta heterodon*), a federally endangered species. This was a new record for this creek.

Figure 1. Upstream side of culvert

Figure 2. Downstream side of culvert

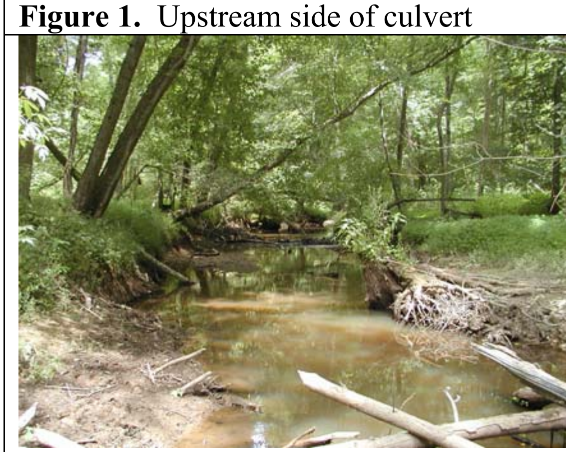


Figure 3. Downstream

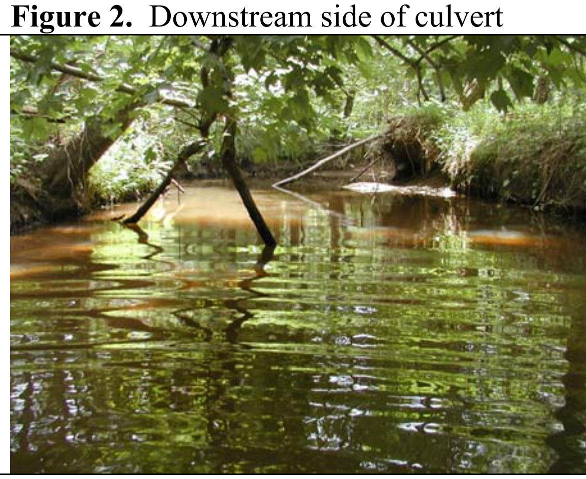


Figure 4. Upstream

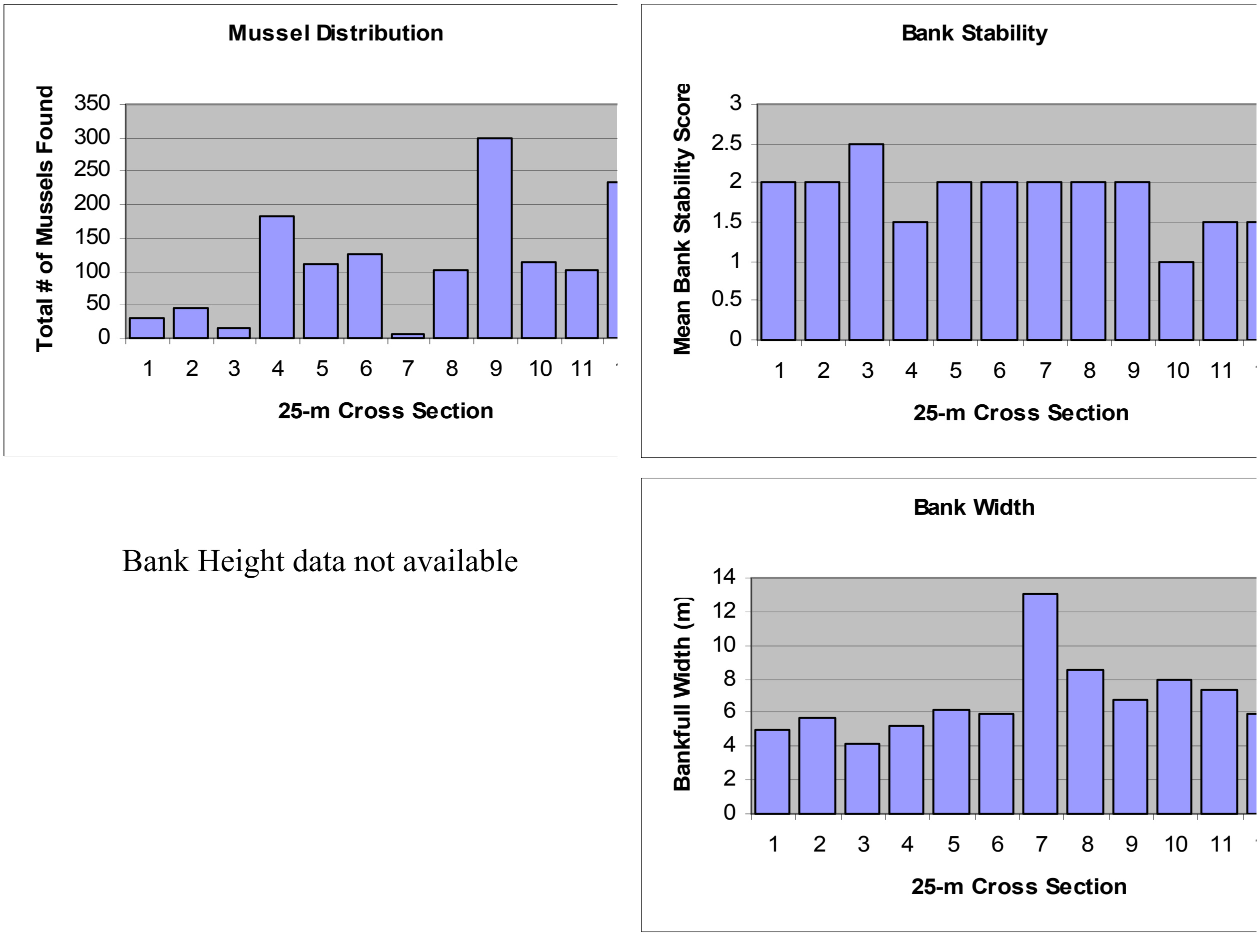


Figure 5. Mussel distribution and habitat data from this site.

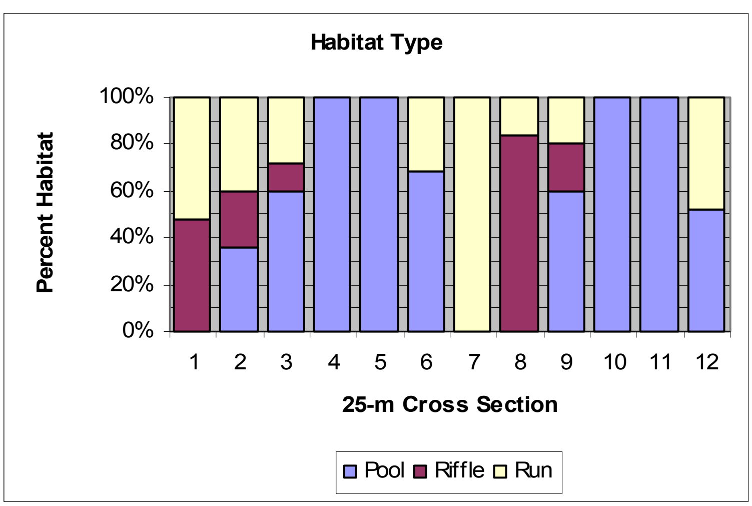


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Halifax	**Bridge Number:**	61
Road Crossing:	NC 561	**Stream:**	Rocky Swamp
Date Sampled:	29 June 2004		

Mussels found at site			
Elliptio complanata	4170	**Year Built:**	1945
A. heterodon	93	**Number of Cells: (w/ base flow)**	3(3)
Total mussels	**4263**	**Obvious scour hole?**	Yes
		% of mussels upstream:	97.9%

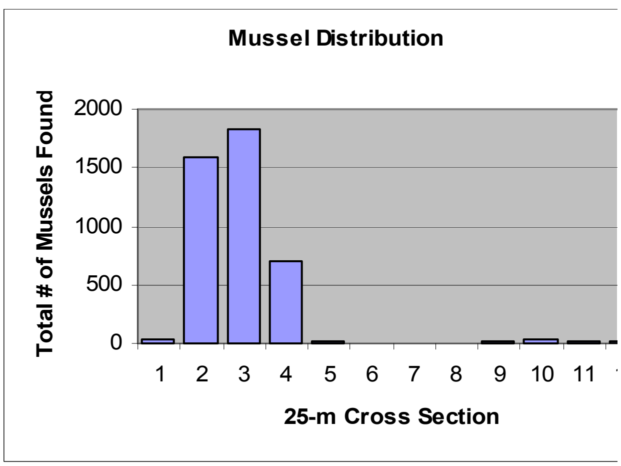
Summary:

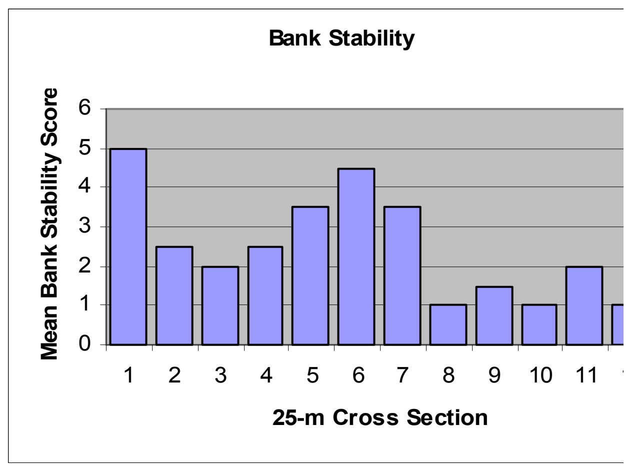
The culvert at this site obviously has a great deal of hydrologic influence on this stream. There is scour immediately upstream as well as immediately downstream of the culvert. In fact, some of the earthen material has even been eroded away from under the culvert on the upstream side. Further upstream of this scour, however, may be one of the most important habitats in North Carolina. We discovered a population of the federally endangered dwarfwedge mussel (Alasmidonta heterodon) that is the most dense population known in the state. We found over 90 individuals of this species in a 60-70 meter reach. Additionally, in this same reach, we found - by far - the most dense population of *Elliptio complanata* in the entire study with almost 3500 individuals (and many very young ones) found in 50 meters of stream. The downstream habitat is relatively poor for mussels and few were found compared to upstream. It would be easy to conclude that with the enormous amount of scour, greater channel widths, and greatly reduced mussel abundance downstream, that this culvert is detrimental; however, it could be that the hydrologic constriction provided by this structure may actually be beneficial to the upstream habitat. Could it be that this culvert, and other similar culverts help stabilize some upstream habitats during channel-forming storm events?

Figure 1. Downstream scour hole

Figure 2. Downstream habitat

Figure 3. *A.h.* habitat facing downstream	**Figure 4.** *A.h.* habitat facing upstream





Bank height data not available

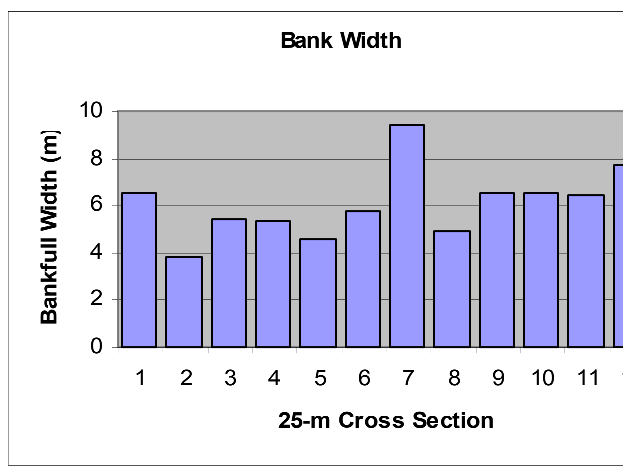


Figure 5. Mussel distribution and habitat data from this site.

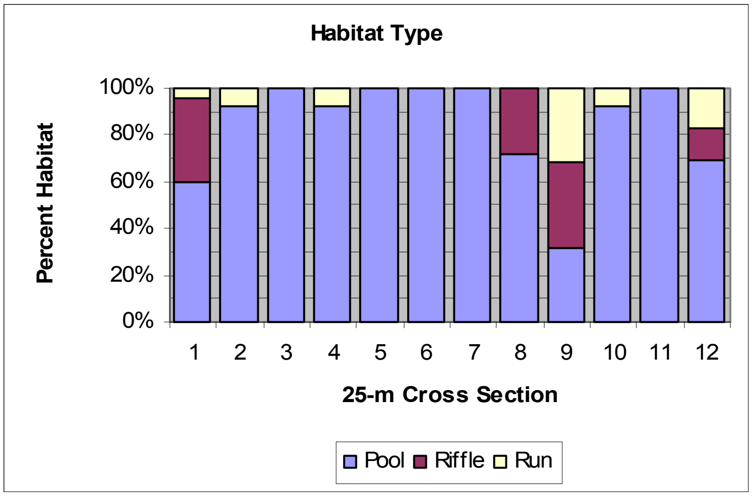


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Halifax	**Bridge Number:**	110
Road Crossing:	Hollister-Glenview Road	**Stream:**	Powell's Creek
Date Sampled:	28 June 2004		

Mussels found at site			
Elliptio complanata	765	**Year Built:**	1993
V. constricta	1	**Number of Cells: (w/ base flow)**	3(2)
Total mussels	**766**	**Obvious scour hole?**	No
		% of mussels upstream:	69.6%

Summary:

This culvert has created a blowout of the habitat immediately below the structure with a large scour hole. Downstream banks also appeared eroded (Fig. 4) compared to more stable banks upstream (Fig. 1), and downstream habitat was generally less stable. This culvert seemed to greatly decrease mussel abundance downstream because of this destabilization (Fig. 5).

Figure 1. Upstream habitat

Figure 2. Upstream side of culvert

Figure 3. Downstream perched culvert

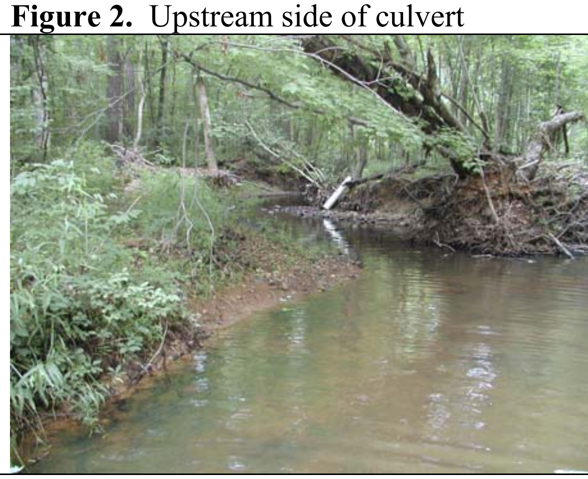
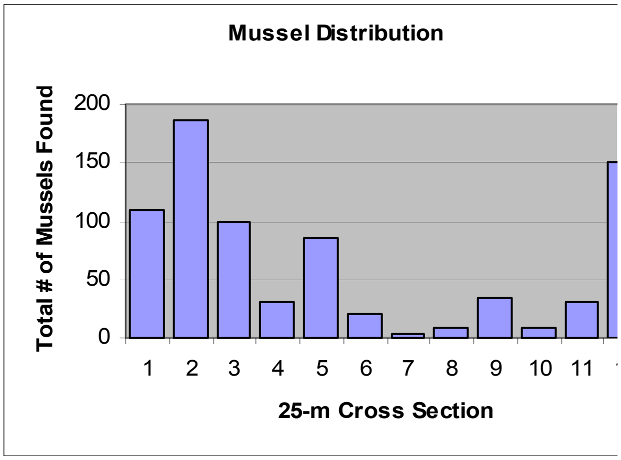
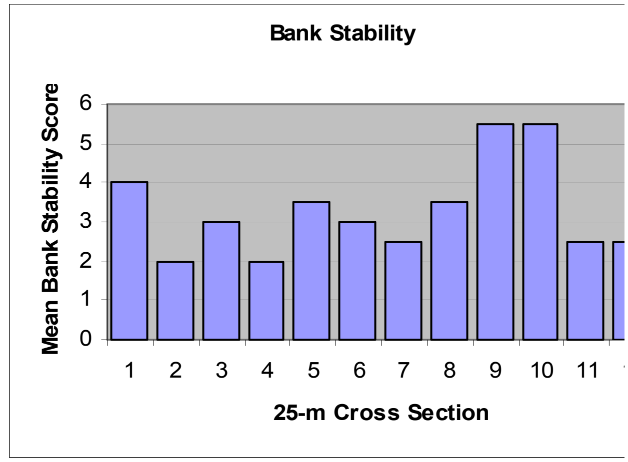


Figure 4. Downstream habitat





Bank Height data not available

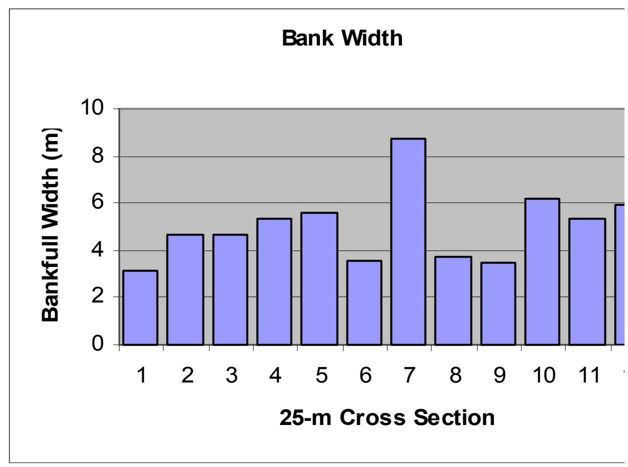


Figure 5. Mussel distribution and habitat data from this site.

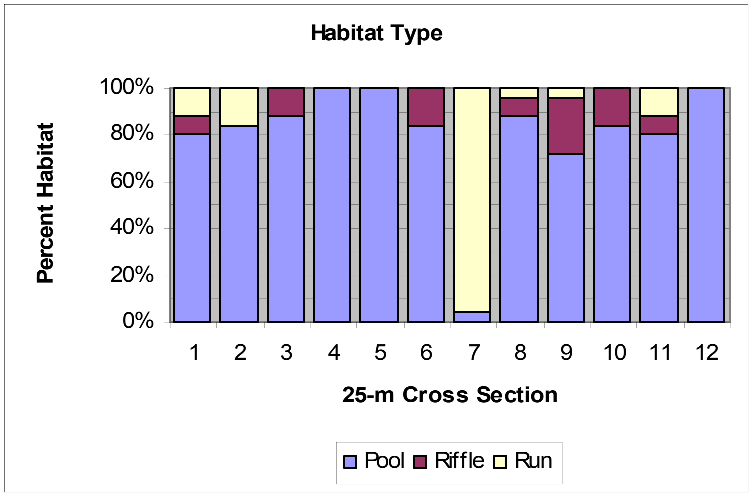


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Harnett	**Bridge Number:**	26
Road Crossing:	Cool Springs Rd.	**Stream:**	Camels Creek
Date Sampled:	28 July 2004		

Mussels found at site			
Elliptio complanata	78	**Year Built:**	1991
		Number of Cells: (w/ base flow)	3(1)
Total mussels	**78**	**Obvious scour hole?**	Yes
		% of mussels upstream:	69.2%

Summary:

This site had poor habitat and mussel abundance over all. And although there was evidence of channel destabilization and widening downstream, it is difficult to say how much this has impacted the mussels at the site. The overall low number of mussels found made it difficult to come to conclusions on this specific site.

Figure 1. Upstream habitat

Figure 2. Upstream side of culvert

Figure 3. Downstream habitat

Figure 4. Further downstream habitat

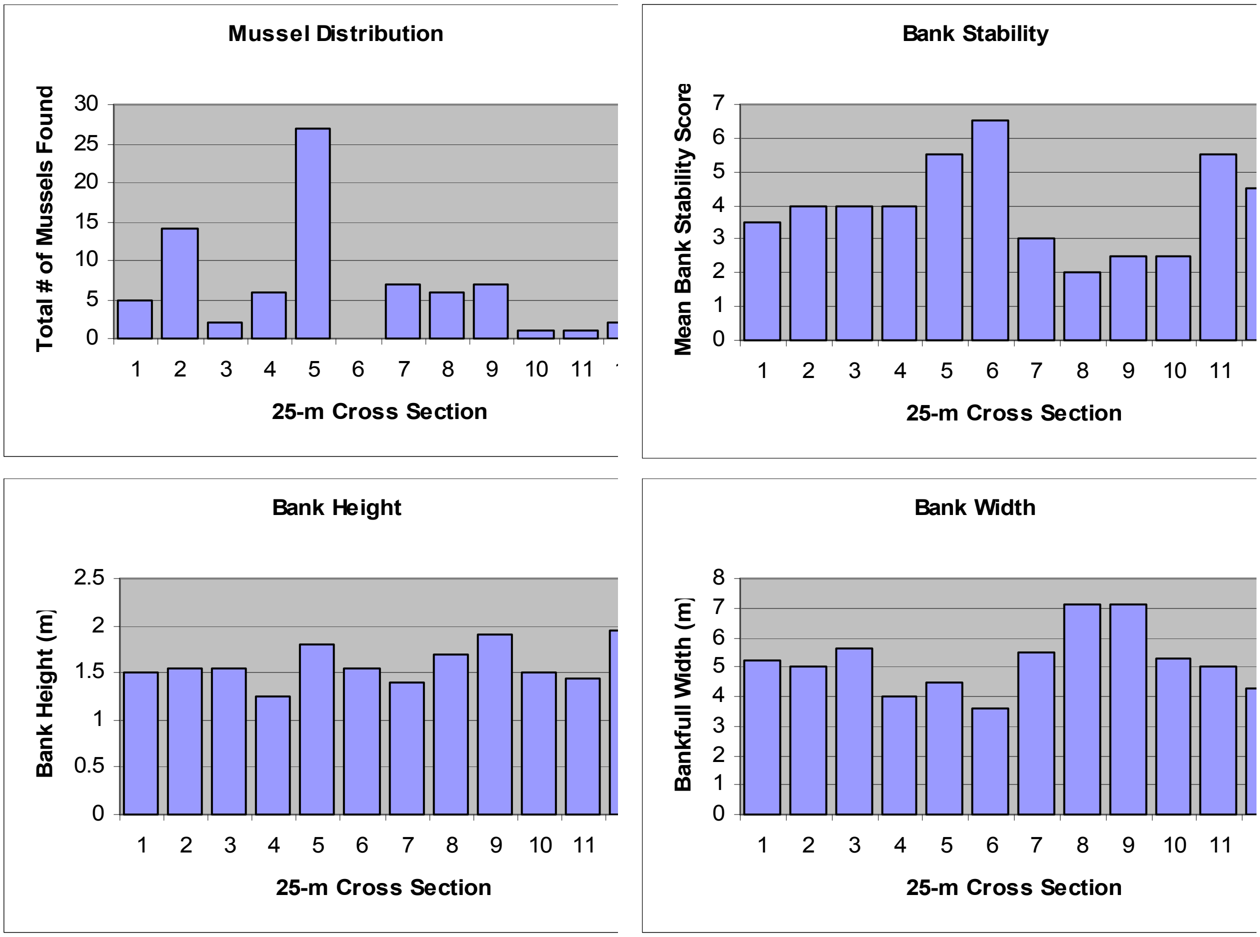


Figure 5. Mussel distribution and habitat data from this site.

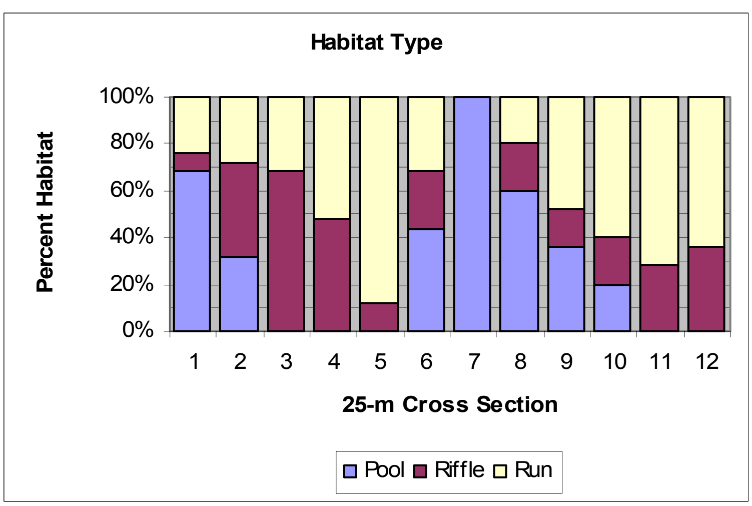


Figure 6. Percentage of habitat type within each 25-m cross section.

County: Johnston **Bridge Number:** 2052
Road Crossing: NC 42 **Stream:** Buffalo Creek
Date Sampled: 7 June 2004

Mussels found at site			
Elliptio complanata	603	**Year Built:**	1947
		Number of Cells: (w/ base flow)	2(2)
Total mussels	**603**	**Obvious scour hole?**	Yes
		% of mussels upstream:	99.3%

Summary:

This culvert has had a significant impact downstream. A very large scour pool was formed downstream of the culvert and the channel was significantly wider downstream. There were several trees falling into the stream from the banks and gravel bars were forming in the channel. An amazing 99.3% of the mussels at this site were upstream of the culvert. These culverts in the transitional areas from piedmont to coastal plain generally have a drastic effect on stream habitat and mussel fauna.

Figure 1. Upstream side of culvert

Figure 2. Downstream scour hole

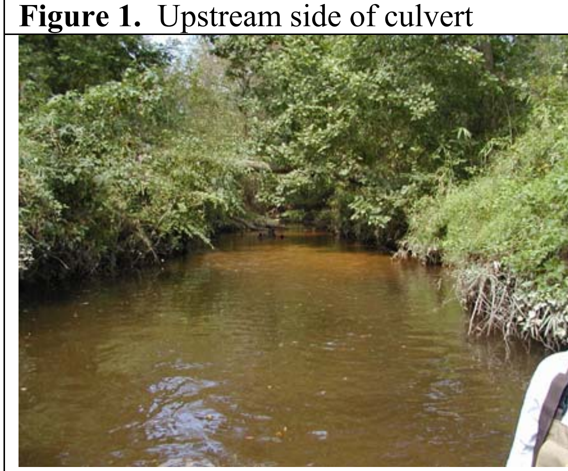


Figure 3. Upstream habitat	**Figure 4.** Habitat further downstream

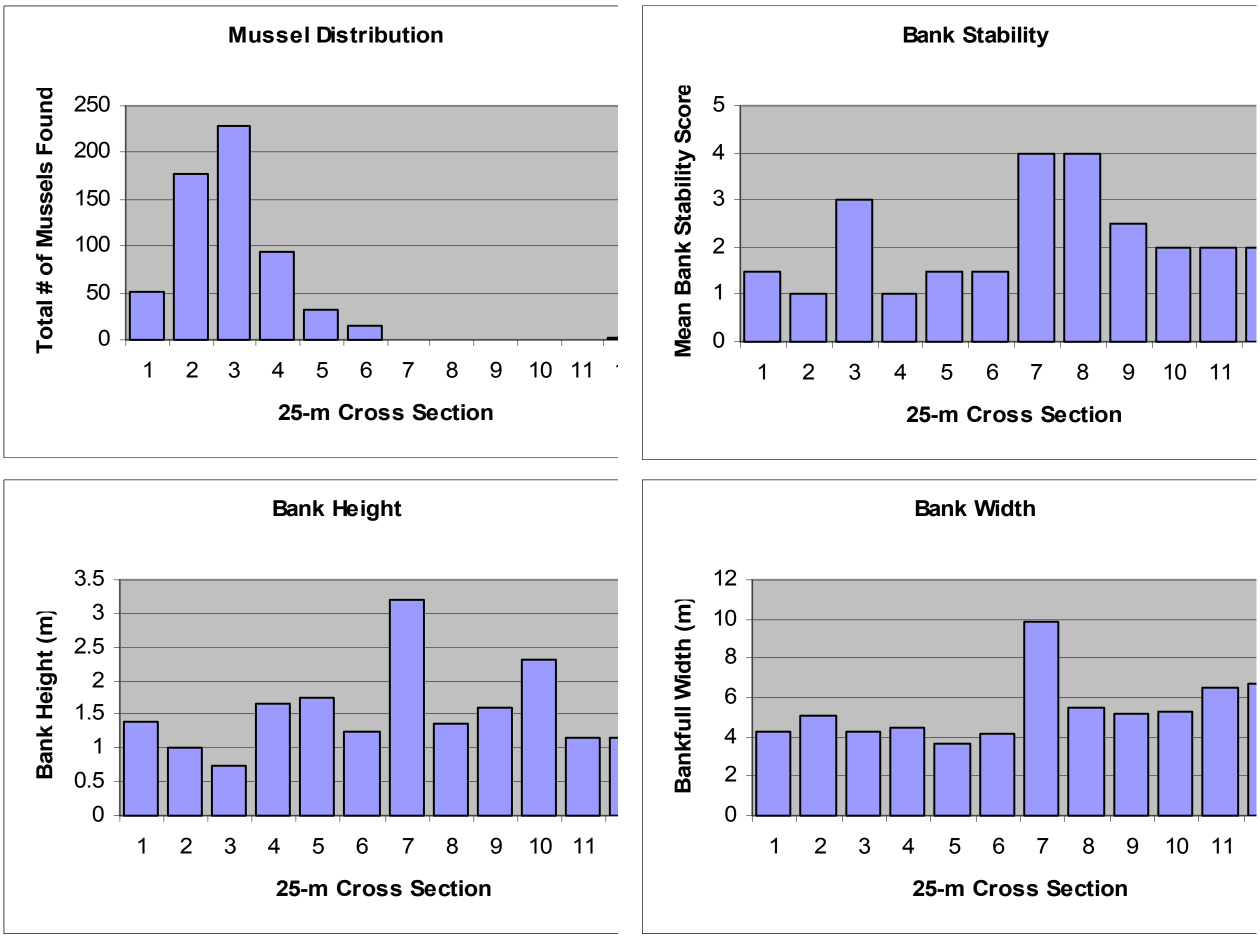


Figure 5. Mussel distribution and habitat data from this site.

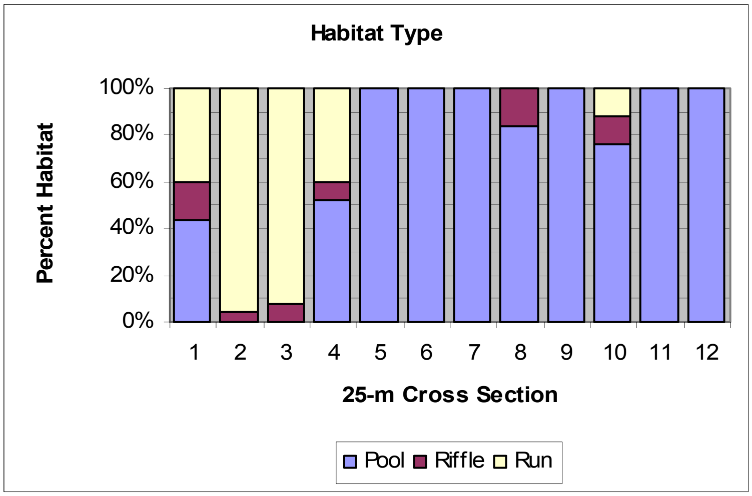


Figure 6. Percentage of habitat type within each 25-m cross section.

County: Nash **Bridge Number:** 310
Road Crossing: Redbud Road **Stream:** Redbud Creek
Date Sampled: 24 June 2004

Mussels found at site			
Elliptio complanata	109	**Year Built:**	1960
		Number of Cells: (w/ base flow)	3(3)
Total mussels	**109**	**Obvious scour hole?**	Yes
		% of mussels upstream:	80.7%

Summary:

This is another upper coastal plain site that has been greatly affected by the culvert. The channel downstream of this structure has been significantly widened (Figs. 1, 2, and 5), and mussel habitat is very poor in this area. In fact, the only mussels we found downstream were in the furthermost downstream section.

Figure 1. Downstream habitat

Figure 2. Downstream side of culvert

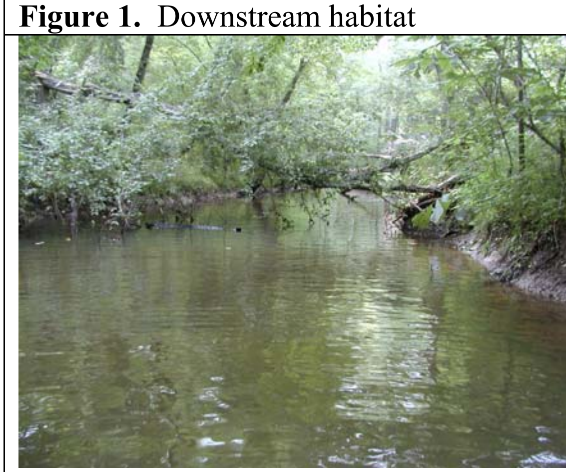


Figure 3. Upstream habitat

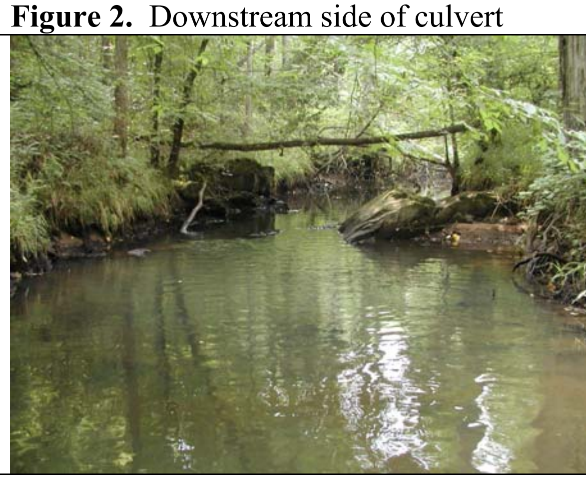
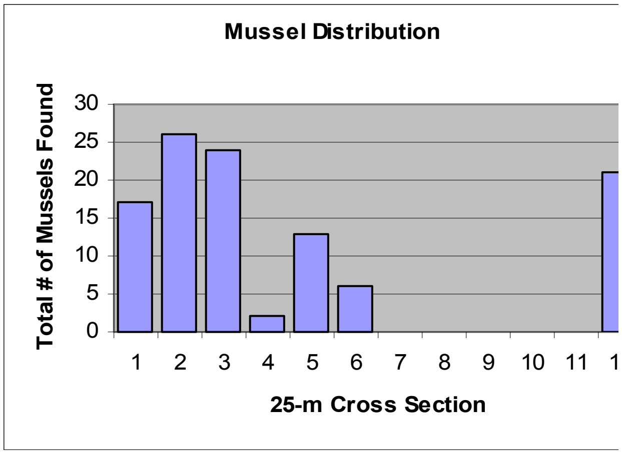
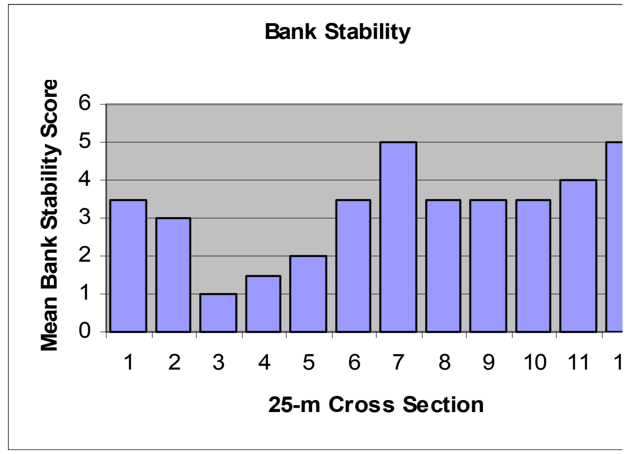


Figure 4. Further upstream habitat





Bank height data not available

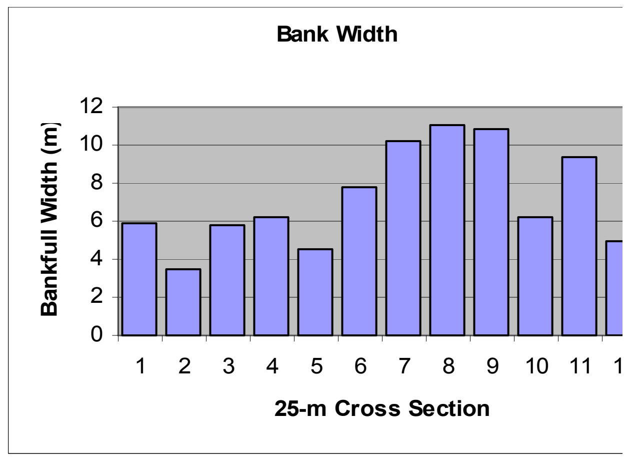


Figure 5. Mussel distribution and habitat data from this site.

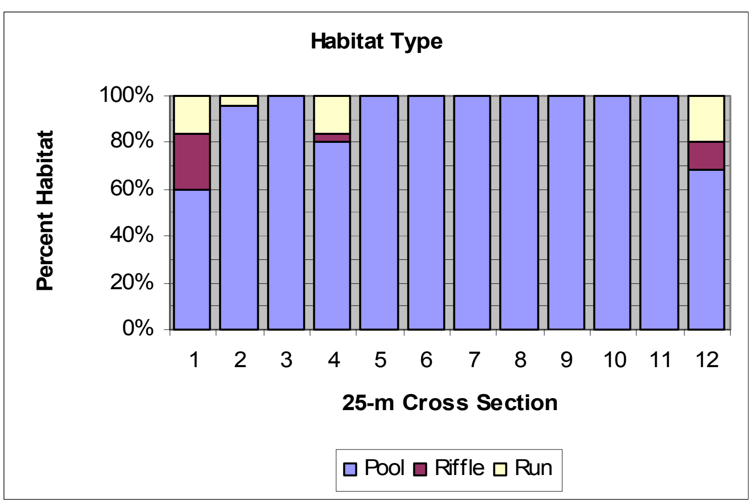


Figure 6. Percentage of habitat type within each 25-m cross section.

County: Wake **Bridge Number:** 561
Road Crossing: US 401 **Stream:** Terrible Creek
Date Sampled: 20 May 2004

Mussels found at site			
Elliptio complanata	63	**Year Built:**	1926
P. cataracta	1	**Number of Cells: (w/ base flow)**	3(2)
Total mussels	**64**	**Obvious scour hole?**	Yes
		% of mussels upstream:	65.6%

Summary:

This structure has had a significant impact on stream channel morphology. Immediately downstream of the structure, there was a very deep scour hole. The stream channel has significantly widened there, banks were less stable, and point bars had formed in the channel downstream. Mussel abundance was lower downstream but the overall low numbers of mussels at the site made it somewhat difficult to truly assess the impacts on the mussel fauna. The upstream habitat was also not especially conducive to mussel colonization as there were few patches of stable instream substrates.

Figure 1. Upstream habitat

Figure 2. Upstream side of culvert

Figure 3. Downstream scour hole

Figure 4. Downstream habitat

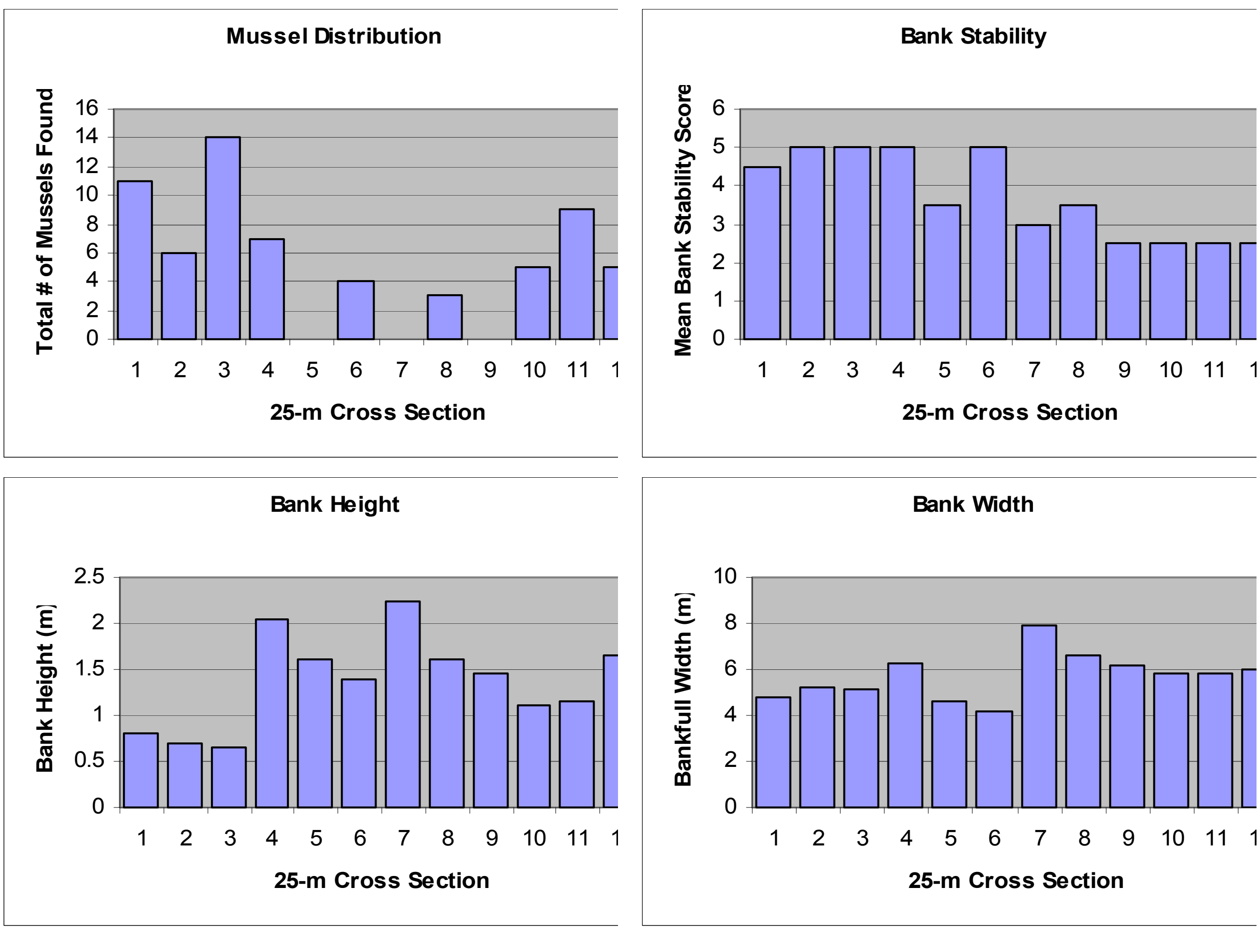


Figure 5. Mussel distribution and habitat data from this site.

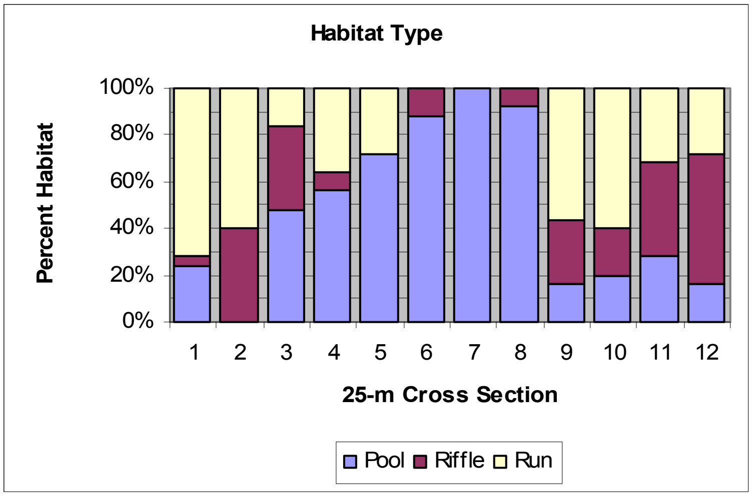


Figure 6. Percentage of habitat type within each 25-m cross section.

County:	Wilson	**Bridge Number:**	194
Road Crossing:	Hawley Road	**Stream:**	Little Creek
Date Sampled:	26 May 2004		

Mussels found at site			
Elliptio complanata	3881	**Year Built:**	1991
		Number of Cells: (w/ base flow)	2(2)
Total mussels	**3881**	**Obvious scour hole?**	Yes
		% of mussels upstream:	89.9%

Summary:

This was another example of a culvert in the upper coastal plain and piedmont soil system with highly stable habitat upstream and a high density of associated mussels with less stability and far fewer mussels downstream. There was also a large scour hole at the downstream mouth of the culvert and few mussels there. Culverts in this soil system tend to have very drastic effects on streams.

Figure 1. Upstream habitat

Figure 2. Upstream side of culvert

Figure 3. Downstream side of culvert

Figure 4. Downstream habitat

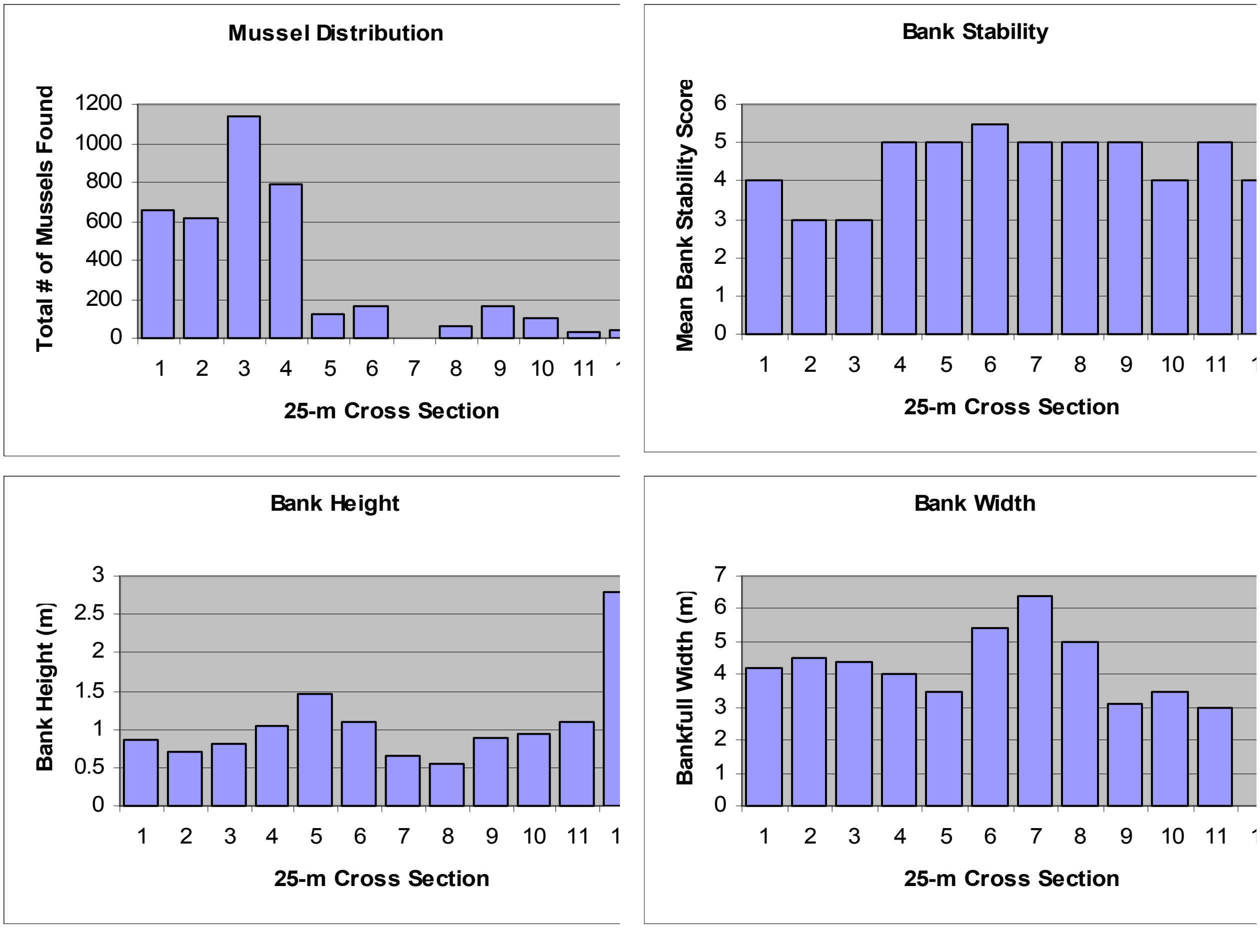


Figure 5. Mussel distribution and habitat data from this site.

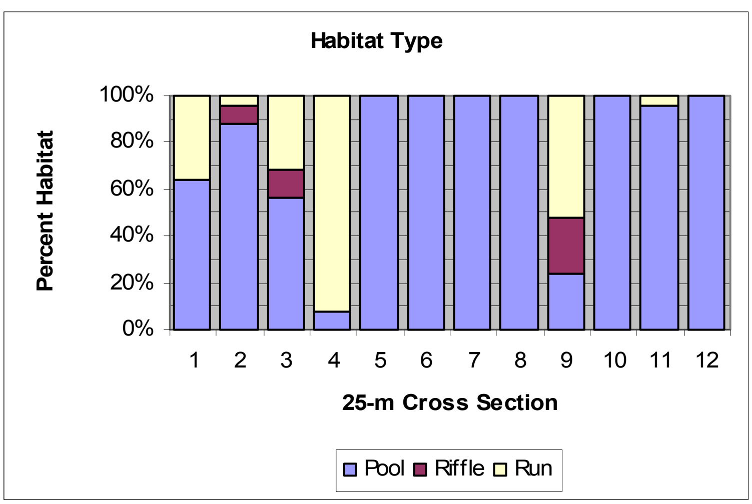


Figure 6. Percentage of habitat type within each 25-m cross section.

APPENDIX III:
Impact of Bridges and Culverts on Stream fish Movement and Community Structure

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

Crossing	Creek	Position	Width (m)	Area (m^2)	Vol (m^3)	Depth (m)	% Pool	% Riffle	% Run	Substrate
Arch	Horse	Down	6	300	125.4	0.418	10	50	40	Gravel, cobble
	Rock		6.2	310	145.7	0.47	56	34	10	Gravel, sand, boulder
	Terrells		7.2	360	124.56	0.346	52	18	30	Cobble, boulder
	Horse	Up	10	500	251	0.502	46	4	50	Boulder, cobble
	Rock		7.75	387.5	113.92	0.294	38	58	4	Cobble, sand
	Terrells		6	300	111.6	0.372	46	54	0	Cobble, gravel, debris
Box	Marys	Down	5.5	275	119.35	0.434	100	0	0	Cobble, sand
	Poppaw		5.9	295	117.41	0.398	32	44	14	Cobble
	Wet		8.2	410	210.74	0.514	56	0	44	Bedrock, sand
	Marys	Up	5.8	290	149.64	0.516	100	0	0	Boulder, silt, cobble
	Poppaw		5.6	280	56	0.2	31	20	49	Cobble
	Wet		6.9	345	81.42	0.236	0	80	20	Bedrock, sand
Bridge	Brush	Down	6	300	93.36	0.3112	35	25	40	Bedrock, boulder, cobble
	Little Brush		5.24	262	85.94	0.328	54	20	26	Cobble, sand
	Little		7.3	365	153.3	0.42	90	0	10	Cobble, boulder
	Polecat		5.8	290	81.2	0.28	20	40	40	Cobble, gravel
	Brush	Up	6.2	310	166.78	0.538	50	40	10	Boulder, cobble
	Little Brush		4.7	235	68.15	0.29	42	58	0	Cobble
	Little		6.1	305	93.94	0.308	30	52	18	Cobble, boulder
	Polecat		7.5	375	256.87	0.685	100	0	0	Sand, gravel
Control	Brooks	Down	7.1	355	132.77	0.374	0	36	64	Cobble, boulder
	Flat		7.8	390	158.34	0.406	10	22	68	Cobble
	N. Prong		5.4	270	105.3	0.39	48	6	46	Cobble, gravel
	Brooks	Up	7.8	390	102.18	0.262	10	40	50	Cobble, boulder
	Flat		6.1	305	54.29	0.178	16	52	32	Cobble, boulder, gravel
	N. Prong		5.2	260	109.2	0.42	0	24	76	Cobble, gravel
Pipe	Dry	Down	7.3	365	206.59	0.566	54	36	10	Cobble, sand
	Reed		5.9	295	99.12	0.336	27	43	30	Cobble, sand, gravel
	Rock		7.7	385	212.52	0.552	54	13	33	Sand, silt
	Dry	Up	6.6	330	102.96	0.312	4	0	96	Sand, gravel
	Reed		6	300	103.8	0.346	66	6	28	Boulder, cobble

Rock	7	350	120.4	0.344	6.00	25.00	69.00	Sand, silt

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

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

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

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

Appendix Table III-3(b). Fish families and species for Rock Creek, a stream with an arch culvert, collected by a combination of seining and backpack electrofishing during summer

2004. Rock Creek was sampled in Guilford County, NC (Lat: 36 03° 54 N, Long: 79 35° 57 W), and accessed from US 70.

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

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

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

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

Family	Scientific Name	Common Name	Individuals

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

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

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	29

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

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

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	52
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	199

Centrarchidae	*Lepomis auritus*	Redbreast sunfish	65
	Lepomis cyanellus	Green sunfish	10
	Lepomis macochirus	Bluegill	21
	Micropterus salmoides	Largemouth bass	1
Cyprinidae	*Clinostomus funduloides*	Rosyside dace	5
	Luxilus albeolus	White shiner	1
	Nocomis leptocephalus	Bluehead chub	61
	Notropis alborus	Whitemouth shiner	1
	Notropis altipinnis	Highfin shiner	203
	Notropis chiliticus	Redlip shiner	27
	Semotilus stromaculatus	Creek chub	62
Esocidae	*Esox americanus americanus*	Redfin pickerel	8
	Esox niger	Chain pickerel	5
Ictaluridae	*Noturus insignis*	Margined madtom	121
Percidae	*Etheostoma olmstedi*	Tessellated darter	72
	Percina crassa	Piedmont darter	12
Poeciliidae	*Gambusia sp.*	Mosquitofish	1

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

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	5
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	8
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	139
	Lepomis cyanellus	Green sunfish	120

	Lepomis macochirus	Bluegill	15
	Micropterus salmoides	Largemouth bass	3
Cyprinidae	*Clinostomus funduloides*	Rosyside dace	1
	Luxilus albeolus	White shiner	211
	Nocomis leptocephalus	Bluehead chub	345
	Notemigonus crysoleucas	Golden shiner	1
	Notropis altipinnis	Highfin shiner	31
	Notropis alborus	Whitemouth shiner	13
	Semotilus stromaculatus	Creek chub	9
Esocidae	*Esox niger*	Chain pickerel	11
Ictaluridae	*Ameiurus playcephalus*	Flat bullhead	103
	Noturus insignis	Margined madtom	40
Percidae	*Etheostoma olmstedi*	Tessellated darter	71
	Percina crassa	Piedmont darter	18
Poeciliidae	*Gambusia sp.*	Mosquitofish	1

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

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	2
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	56
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	72
	Lepomis cyanellus	Green sunfish	67
	Lepomis gulosus	Warmouth	5
	Lepomis macochirus	Bluegill	31

	Micropterus salmoides	Largemouth bass	4
	Pomoxis nigromaculatus	Black crappie	1
Cyprinidae	*Clinostomus funduloides*	Rosyside dace	2
	Cyprinella nivea	Whitefin shiner	1
	Luxilus albeolus	White shiner	51
	Nocomis leptocephalus	Bluehead chub	156
	Notropis alborus	Whitemouth shiner	51
	Notropis altipinnis	Highfin shiner	116
	Semotilus stromaculatus	Creek chub	29
Esocidae	*Esox niger*	Chain pickerel	2
Ictaluridae	*Ameiurus nebulosus*	Brown bullhead	1
	Noturus insignis	Margined madtom	8
Percidae	*Etheostoma olmstedi*	Tessellated darter	54
Poeciliidae	*Gambusia sp.*	Mosquitofish	4

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

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	32
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	2
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	72
	Lepomis cyanellus	Green sunfish	34
	Lepomis gulosus	Warmouth	1
	Lepomis macochirus	Bluegill	9
	Micropterus salmoides	Largemouth bass	5

Cyprinidae	*Clinostomus funduloides*	Rosyside dace	58
	Luxilus albeolus	White shiner	6
	Nocomis leptocephalus	Bluehead chub	78
	Notemigonus crysoleucas	Golden shiner	5
	Notropis alborus	Whitemouth shiner	44
	Notropis altipinnis	Highfin shiner	168
	Notropis chiliticus	Redlip shiner	2
	Semotilus stromaculatus	Creek chub	130
Esocidae	*Esox niger*	Chain pickerel	2
Ictaluridae	*Ameiurus playcephalus*	Flat bullhead	10
	Noturus insignis	Margined madtom	1
Percidae	*Etheostoma olmstedi*	Tessellated darter	354
	Percina crassa	Piedmont darter	2
Poeciliidae	*Gambusia sp.*	Mosquitofish	1

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

Family	Scientific Name	Common Name	Individuals
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	41
	Lepomis gibbosus	Pumpkinseed	2
	Lepomis macochirus	Bluegill	73
	Lepomis microlophus	Redear sunfish	5
	Micropterus salmoides	Largemouth bass	52
Cyprinidae	*Clinostomus funduloides*	Rosyside dace	14
	Nocomis leptocephalus	Bluehead chub	89
	Notropis alborus	Whitemouth shiner	19
	Notropis altipinnis	Highfin shiner	26

	Notropis chiliticus	Redlip shiner	11
	Semotilus stromaculatus	Creek chub	64
Fundulidae	*Fundulus rathbuni*	Speckled killifish	40
Ictaluridae	*Ameiurus playcephalus*	Flat bullhead	8
	Noturus insignis	Margined madtom	37
Percidae	*Etheostoma olmstedi*	Tessellated darter	95
	Percina crassa	Piedmont darter	6

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

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	77
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	2
	Moxostoma collapsum	Notchlip redhorse	1
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	85
	Lepomis cyanellus	Green sunfish	51
	Lepomis gibbosus	Pumpkinseed	2
	Lepomis macochirus	Bluegill	4
	Micropterus salmoides	Largemouth bass	17
Cyprinidae	*Cyprinella analostana*	Satinfin shiner	1
	Luxilus albeolus	White shiner	128
	Nocomis leptocephalus	Bluehead chub	333

	Notropis alborus	Whitemouth shiner	3
	Notropis altipinnis	Highfin shiner	24
	Semotilus stromaculatus	Creek chub	23
Fundulidae	*Fundulus rathbuni*	Speckled killifish	31
Ictaluridae	*Ameiurus brunneus*	Snail bullhead	1
	Ameiurus playcephalus	Flat bullhead	4
	Noturus insignis	Margined madtom	243
Percidae	*Etheostoma olmstedi*	Tessellated darter	71
	Perca flavescens	Yellow perch	4
	Percina crassa	Piedmont darter	7

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

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	33
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	116
	Moxostoma collapsum	Notchlip redhorse	1
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	87
	Lepomis cyanellus	Green sunfish	161
	Lepomis gibbosus	Pumpkinseed	8
	Lepomis gulosus	Warmouth	7
	Lepomis macochirus	Bluegill	96
	Micropterus salmoides	Largemouth bass	5
Cyprinidae	*Luxilus albeolus*	White shiner	37
	Nocomis leptocephalus	Bluehead chub	25
	Notemigonus crysoleucas	Golden shiner	12
	Notropis alborus	Whitemouth shiner	2

	Notropis altipinnis	Highfin shiner	2
	Semotilus stromaculatus	Creek chub	1
Esocidae	*Esox americanus americanus*	Redfin pickerel	3
	Esox niger	Chain pickerel	40
Ictaluridae	*Ameiurus playcephalus*	Flat bullhead	50
	Noturus insignis	Margined madtom	10
Percidae	*Etheostoma olmstedi*	Tessellated darter	189
	Etheostoma serrifer	Sawcheek darter	1
	Percina crassa	Piedmont darter	6
Poeciliidae	*Gambusia sp.*	Mosquitofish	4

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

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	13
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	6
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	67
	Lepomis cyanellus	Green sunfish	191
	Lepomis gibbosus	Pumpkinseed	6
	Lepomis gulosus	Warmouth	4
	Lepomis macochirus	Bluegill	50
	Micropterus salmoides	Largemouth bass	17
Cyprinidae	*Luxilus albeolus*	White shiner	167
	Nocomis leptocephalus	Bluehead chub	153
	Notropis alborus	Whitemouth shiner	35
	Notropis altipinnis	Highfin shiner	37
	Semotilus stromaculatus	Creek chub	30
Esocidae	*Esox niger*	Chain pickerel	1

Fundulidae	*Fundulus rathbuni*	Speckled killifish	8
Ictaluridae	*Ameiurus playcephalus*	Flat bullhead	15
	Noturus insignis	Margined madtom	38
Percidae	*Etheostoma flabellare*	Fantail darter	7
	Etheostoma olmstedi	Tessellated darter	74
	Percina crassa	Piedmont darter	4

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

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	49
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	81
	Moxostoma collapsum	Notchlip redhorse	1
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	64
	Lepomis cyanellus	Green sunfish	43
	Lepomis gibbosus	Pumpkinseed	1
	Lepomis gulosus	Warmouth	4
	Lepomis macochirus	Bluegill	20
	Micropterus salmoides	Largemouth bass	16
Cyprinidae	*Clinostomus funduloides*	Rosyside dace	4
	Luxilus albeolus	White shiner	2
	Nocomis leptocephalus	Bluehead chub	33
	Notropis alborus	Whitemouth shiner	101
	Notropis altipinnis	Highfin shiner	184
	Semotilus stromaculatus	Creek chub	13
Esocidae	*Esox americanus americanus*	Redfin pickerel	3

	Esox niger	Chain pickerel	8
Fundulidae	*Fundulus rathbuni*	Speckled killifish	6
Ictaluridae	*Ameiurus playcephalus*	Flat bullhead	5
	Noturus insignis	Margined madtom	47
Percidae	*Etheostoma olmstedi*	Tessellated darter	108
	Percina crassa	Piedmont darter	15

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

Family	Scientific Name	Common Name	Individuals
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	85
Catostomidae	*Erimyzon oblongus*	Creek chubsucker	73
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	126
	Lepomis cyanellus	Green sunfish	39
	Lepomis gibbosus	Pumpkinseed	12
	Lepomis macochirus	Bluegill	70
	Micropterus salmoides	Largemouth bass	5
Cyprinidae	*Clinostomus funduloides*	Rosyside dace	138
	Luxilus albeolus	White shiner	5
	Nocomis leptocephalus	Bluehead chub	162
	Notropis alborus	Whitemouth shiner	15
	Notropis altipinnis	Highfin shiner	60
	Notropis chiliticus	Redlip shiner	7
	Semotilus stromaculatus	Creek chub	238
Ictaluridae	*Ameiurus playcephalus*	Flat bullhead	19
	Noturus insignis	Margined madtom	68

Percidae	*Etheostoma olmstedi*	Tessellated darter	113
	Percina crassa	Piedmont darter	1
Poeciliidae	*Gambusia sp.*	Mosquitofish	7

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

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

APPENDIX IV:
Impact of Bridges and Culverts on Stream Fish Movement: PIT-tagging methods.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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