CTE / NCDOT

Joint Environmental Research Program

Final Report

Functional Assessment of the Effects of Highway Construction on Coastal North Carolina Wetlands: Comparison of Effects Before and After Construction–Phase II (Construction)

Prepared By

Curtis J. Richardson
Neal A. Flanagan
Ryan S. King

Duke University Wetland Center
Nicholas School of the Environment
and Earth Sciences
Durham, NC 27708-0333

June 2003

Technical Report Documentation Page

1. Report No. **FHWA/NC/2002-016**	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle **Functional Assessment of the Effects of Highway Construction on Coastal North Carolina Wetlands: Comparison of Effects Before and After Construction–Phase II (Construction)**		5. Report Date **June 2003** 6. Performing Organization Code
7. Author(s) **Curtis J. Richardson, Neal A. Flanagan, and Ryan S. King**		8. Performing Organization Report No.
9. Performing Organization Name and Address **Duke University Wetland Center** **Nicholas School of the Environment and Earth Sciences** **Box 90333** **Durham, NC 27708-0333**		10. Work Unit No. (TRAIS) 11. Contract or Grant No.
12. Sponsoring Agency Name and Address **U.S. Department of Transportation** **Research and Special Programs Administration** **400 7th Street, SW** **Washington, DC 20590-0001**		13. Type of Report and Period Covered **Final Report** **April 1, 1999-December 31, 2001** 14. Sponsoring Agency Code **1999-07**

15. Supplementary Notes:
This project was supported by a grant from the U.S. Department of Transportation and the North Carolina Department of Transportation, through the Center for Transportation and the Environment, NC State University.

16. Abstract

A major challenge in environmental monitoring is differentiating of true impacts from changes due to natural variation or cycles in ecosystem function. In our study the use of the BACI sampling design has allowed for discrimination of construction impact from natural variation. Impacts have been detected in salinity, sediment accretion, D.O., phosphorus concentration, macrophyte community composition, algal productivity as well as macroinvertebrates and fish. These changes are likely the result of construction of the highway bypass of Jacksonville, NC. It is impossible to say whether these impacts will prove to be short-term or persist beyond the completion of the highway since data collection after construction was discontinued due to a lack of funding. It appears the impacts resulting from construction phase increased rates of runoff from the watershed due to road clearing, impeded fluxes of water from floods and importantly tides due to the presence of temporary culverts at the site. Changes in soil surface elevation due to sediment displacement during road fill placement, and increased sediment flux from road fill and clearing also occurred. These impacts should be temporary, and the system may return to its normal state after several growing seasons, provided sediment and nutrient changes do not remain altered. Of concern, however, is the impact of reduced salinity on the long-term biota of Wilson Creek. Unfortunately, the study has not been continued so it is impossible at this stage to assess the recovery of the site and determine if the biota have returned to conditions near the reference conditions. Fortunately, the design of the study will allow for a follow up study to assess recovery.

17. Key Words environmental impacts, road construction, wetlands, wetland conservation	18. Distribution Statement

19. Security Classif. (of this report) Unclassified	20. Security Classif. (of this page) Unclassified	21. No. of Pages 66	22. Price

Form DOT F 1700.7 (8-72) **Reproduction of completed page authorized**

DISCLAIMER

ACKNOWLEDGMENTS

We thank the following individuals or organizations for their assistance in this project: Jeff McCreary (invertebrate sample sorting); Jim Cooper (invertebrate sample sorting, field assistance); Karen Muldowney (invertebrate sample sorting); Matt Hanchey (field assistance);
Sarah Watts (invertebrate sample sorting); Evie Turley (invertebrate sampling sorting); Franklin Industrial Minerals (donation of feldspar); Dave Meyer, NOAA-Beaufort (loaning of fyke nets); Scott Van Horn, NCWRC-Durham (loaning of minnow traps); John Epler, expert taxonomist (verification of some species identifications); Mike Milligan, expert taxonomist (verification of some species identifications); Jerrell Daigle, expert taxonomist (verification of some species identifications); Randy Neighbarger (editing and formatting). Support for this project was provided by the U.S. Department of Transportation and the North Carolina Department of Transportation through the Center for Transportation and the Environment, NC State University.

SUMMARY

Our results clearly demonstrate that biological indicators like macrophytes, macroinvertebrates, and fish communities should be an integral component of a highway impact assessment program. Biota are excellent integrators of a variety of potential stressors imposed upon wetland systems by highway construction. Results from this study and our previous study (King *et al.* 2000) have shown that wetland biota are sensitive to disturbances associated with construction and operation of highways, and are better indicators of environmental impacts than conventional water chemistry or habitat surveys (*e.g.*, HGM). Although most attributes of biotic assemblages are not direct measures of wetland ecosystem processes per se, changes in biotic assemblages in response to human activities are indicative of both structural and functional changes in a wetland, and thus are linked to wetland ecosystem processes (Richardson 1994). Moreover, §101(a) of the Clean Water Act mandates the restoration and maintenance of biological integrity of the USA's streams, lakes, and wetlands, an unduly neglected aspect of wetland assessment (Karr and Chu 1997, Kusler and Niering 1998). Thus, biotic attributes are indeed functional indicators, and should be included in a functional assessment system for wetlands. Importantly, our BACI approach allowed for a clear test of the effects of the highway construction on biotic response and we were also able to eliminate the affect of environmental variation by the use of reference systems as well as before and after data collection comparisons.

One potential criticism of bioassessment is that it is laborious relative to rapid procedures like HGM. While our assessments were relatively intensive, use of the USEPA's Rapid Bioasessment Protocol for macroinvertebrates produced results that were equally, if not more informative than the laborious quantitative coring technique used to sample benthic macroinvertebrates. It is our recommendation that this rapid assessment procedure be considered over more quantitative sampling approaches, possibly using a composite sample from all available habitats as commonly done in many state biomonitoring programs (*e.g.*, FDEP 1996, Maxted *et al.* 2000). Since most of the useful information lies within species composition rather than in density estimates, rapid approaches like RBP are cost-effective techniques for generating species lists and semi-quantitative abundance estimates that serve well in assigning an impact rating to a site.

Highway construction in environmentally dynamic habitats like coastal wetlands may pose the most significant threat to biota through the loss of connectivity between areas upstream and downstream of highway crossings. While we do not have long-term post-construction data to evaluate recovery of the impacted site, short-term disturbance from construction caused significant alteration to species composition of both macroinvertebrates and fish as well as macrophytes and water chemistry. This is particularly important considering that water quality at all sites was considered poor prior to construction, as indicated by water-chemistry monitoring and the Estuarine Biotic Index. Thus, it should not be assumed that impaired sites like Edwards Creek are not susceptible to further impact, as our results have demonstrated that they can be. Our data suggest that the culverts installed in the extension pads and the temporary causeway were insufficient for allowing adequate flushing of tidal water upstream of the crossing. Our recommendation is that greater attention be directed toward minimizing the obstruction of tidal creeks (*i.e.* changes in salinity) during the construction phase, which may help reduce short-term

impacts to the biota and associated ecosystem processes of coastal wetlands.

Finally, post-construction phase data are needed to assess long-term impacts at this highway construction site and future studies at this site should utilize the existing reference sites and BACI comparison approach.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLE

I. INTRODUCTION

The development of the highway systems across the United States has created a need for a methodology to quantitatively detect impacts of highway construction on wetland ecosystem functions. President Carter's Executive Order 119990 (1977) required all federal agencies to minimize the destruction, loss or degradation of wetlands. In response, DOT issued order 55660.1A that commits the Federal Highway Administration (FHWA) to protect preserve and enhance the nations wetlands to the fullest extent possible during the construction and operation of highway facilities (Rossiter and Crawford, 1983). This leaves FHWA with a need for a methodology to assess the impacts of highway construction and operation on wetlands. Several studies have proposed general guidelines for qualitative assessment of highway impacts on the hydrology, biota, and water quality of wetland ecosystems (Darnell *et al.* 1976; Shuldiner *et al.* 1979A, 1979b; Adamus 1983; Adamus and Stockwell 1983). The Hydrogeomorphic (HGM) assessment procedure is a qualitative or semiquantitative procedure for rapid assessment of wetland function (Brinson *et al.* 1995; Smith *et al.* 1995; Rheinhardt *et al.* 1997). This approach differs from other approaches in that it requires wetlands be classified according to their common hydrologic, soil and vegetative characteristics into a narrowly defined regional subclass, and it requires the use of information from other reference wetlands of the same subclass to develop standards for assessment. The HGM procedure relies upon biotic and abiotic parameters that can be rapidly assessed in the field. These parameters are then indexed relative to measurement made from a group of substantially unimpacted reference wetlands. Potential shortcomings of HGM are its reliance upon somewhat subjective categorical or qualitative data and as few as one sampling date required to perform assessments.

The primary motivation for developing quantitative functional assessment techniques is the need to predict the effects of anthropogenic alterations of wetlands and to assess the spatial extent of impacts to determine mitigation requirements (Committee on Characterization of Wetlands, 1995). Two studies (Richardson 1995, Richardson and Nunnery 1997) point out that no such quantitative methodology currently exists. Richardson (1995) and Richardson and Nunnery (1997, 2001) propose a functional assessment framework for wetlands that uses carefully chosen parameters as key indicators of ecosystem level functions. Wetland functions are grouped into five ecosystem-level categories including hydrologic flux and storage, biological productivity, biogeochemical cycling and storage, decomposition, and community/wildlife habitat. Much like HGM (Brinson and Rheinhardt 1996), key indicator values obtained in the field from the impact wetland are scaled against those from reference wetlands and an ecological functional assessment (EFA) is completed (Richardson and Nunnery 2001). The scaled key indicator value from the impact wetland is plotted on the appropriate functional axis to create an ecosystem response surface (Figure 1). An EFA is then developed by measuring ecological responses across 5 functional groups to determine the percent change (+ or -) from reference conditions (Richardson and Nunnery 2001).

We have simultaneously collected data in one impacted and two nearby reference wetlands to test a before and after approach for determining the effects of highway construction on wetland functions. This approach is based on the Before After Control Impact (BACI) design of Green (1979); Stewart Oaten *et al.* (1986,1992, 1996); and Underwood (1991, 1992, 1994). Our research also represents an unprecedented opportunity for a controlled before and after study that assesses the impacts of highway crossings upon coastal wetland systems. The objectives of this study are to differentiate changes in ecosystem function that result in highway construction from changes due to regional natural variation and to present an integrated assessment of ecosystem functional responses effected by highway crossings.

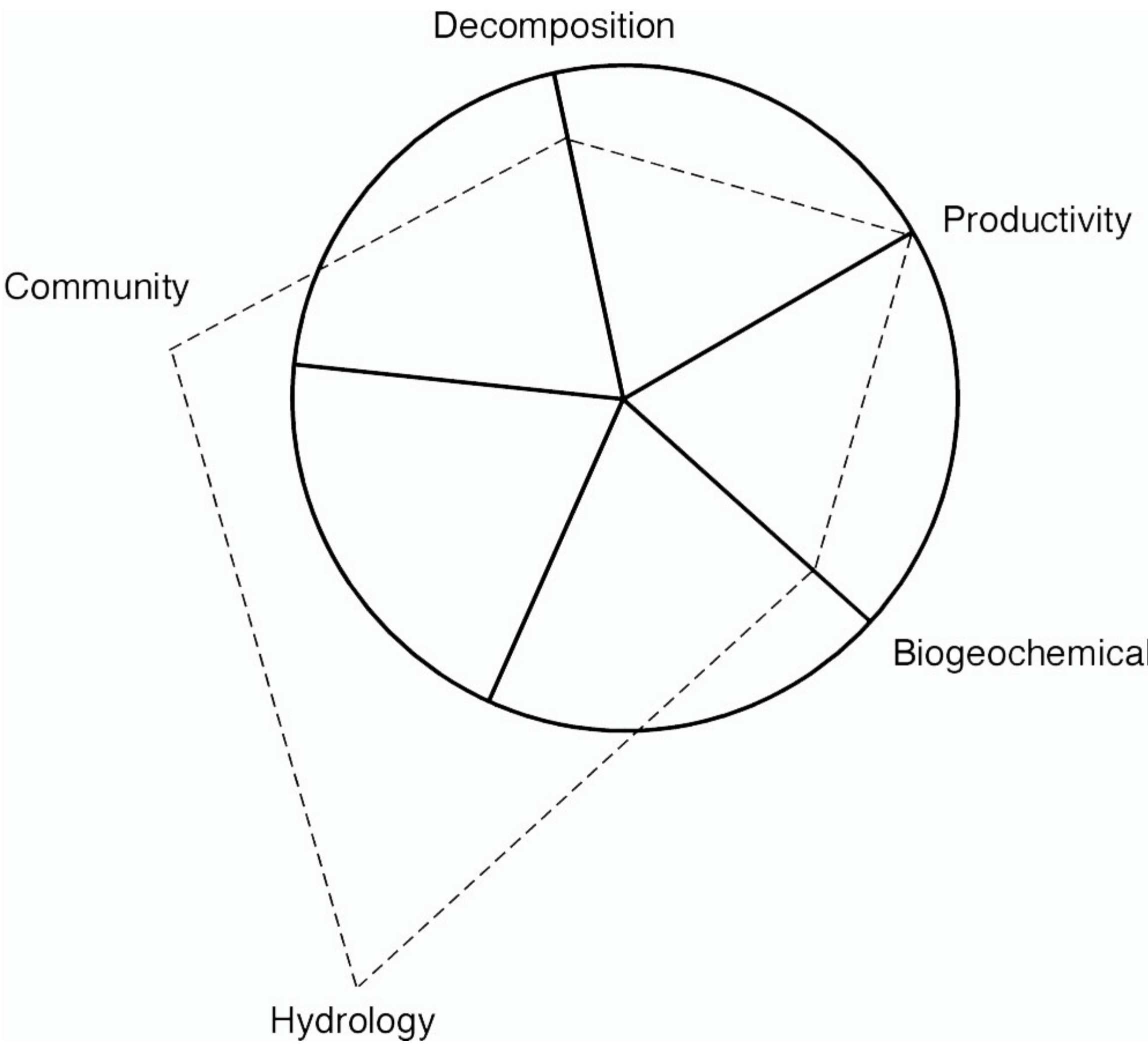

Figure 1. Ecosystems response surface showing ecosystem functions at a theoretical impact site (dashed line) scaled to levels found at a reference site.

II. METHODS

An implicit goal of most impact studies is to compare two states of a natural system: the state of the system in the presence of an impact and the state the system in the absence of the impact (Osenberg and Schmitt, 1996). We now present a methodology to predict and compare the impacted and unimpacted states of a given wetland and assess if significant alterations in ecosystem function result from highway construction.

A. Experimental Design

The experimental design of this study utilized a modified form of the Before After Control Impact (BACI) design called the "beyond BACI design" (Green 1979; Stewart Oaten *et al.* 1986,1992, 1996; Underwood 1991, 1992, 1994). BACI is designed to differentiate changes caused by human activity and those caused by natural temporal and spatial variation. The simplest BACI design calls for an impact site and a control site to be sampled once before and once after a given anthropogenic activity. The variability of samples taken from within a site is the error term used to test for impact and to look for interactions between time and location effects. This design is confounded by fluctuations of natural origin that may occur at one site and not at the other (Osenberg and Schmitt 1996). Stewart-Oaten (1986) proposed the BACI Paired Series (BACIPS) design to overcome this limitation. This design uses a time series of data points collected before and after an impact begins. For each date in the time series differences between the control and impact sites are calculated (these difference will henceforth be referred to as deltas). Stewart-Oaten (1996) suggest development of a model relating the behavior of the control and impacts sites prior to alteration. This model may be used to predict the hypothetical behavior of the impact site had the alteration never occurred by using post-impact control site data as a model input or covariant. Significant differences between predicted impact site behavior and observed behavior are indicative of environmental impact. Underwood (1992, 1996) points out that natural divergences in the state of two systems can occur stochastically without an impact, possibly resulting in false detection of impact. The "beyond BACI" design assumes the average behavior of a group of reference systems is less prone to random or stochastic fluctuations. Given the high variability seen in the brackish wetlands of coastal North Carolina, the "beyond BACI" design was chosen for this study. In this study, the definition of impact relies on comparison of deltas before and after a potential impact event. In this approach, the post-impact states of the reference sites are used to predict the state of the "impact" site in the absence of the impact event. In this study, the designation of a "significant impact" is based upon a statistical comparison of pre-impact deltas and post-impact deltas.

B. Field methods

1. Site Descriptions

The impacted site in this study is wetland located on Edwards Creek, a coastal wetland system situated on the Camp Lejeune military reserve near Jacksonville, NC (Figure 2). A bypass of Jacksonville was being built to cross Edwards Creek and associated wetland areas. (See Appendix A for site photos.) The system is as a tidally influenced brackish creek with substantial freshwater runoff from the surrounding watershed. There are daily tidal influxes of brackish water from the adjacent estuary that expose organisms in the Edwards Creek system to a wide range in salinity. The state of North Carolina has classified Edwards Creek as important areas for fish and wildlife propagation. Edwards Creek is classified as a high quality, nutrient sensitive water body (NC Division of Water Quality 1997a). The Edwards Creeks watershed covers an area of approximately 2.6 km^2 (1.0 mi^2) and is predominantly forested, though Camp Geiger occupies a large portion of the upper reaches of the watershed. A small gravel causeway crosses the creek mouth. Originally, a single 1-m diameter culvert allowed flow through the causeway. In the spring of 1998 this culvert was replaced with three similarly sized culverts. The wetland consists of a permanently flooded creek channel that is fringed by a band of emergent macrophytes of variable width that is dominated by the following taxa: *Spartina* sp., *Typha* sp. and *Scirpus* sp. Another zone is dominated by woody species: loblolly pine (*Pinus taeda*), eastern red cedar (*Juniperus virginiata*), bald cypress (*Taxodium distichum*), Sweet gum (*Liquidambar styraciflua*), tulip tree (*Liriodendron tulipifera*), green ash (*Fraxinus pennsylvanica*), red maple (*Acer rubrum*), sweet bay (*Magnolia virginiana*), Red bay (*Persea palustrus*), American Holly (*Ilex opaca*), and Dahoon (*Ilex cassine*). Soils found at the Edwards Creek site are predominantly Dorvonian Muck and Muckalee Loam. Soils in the Dorvonian series are poorly drained organic soils with several feet of brown to reddish brown muck overlying dark gray sandy loam. The Muckalee series is characterized by poorly drained grayish brown sandy loam (USDA, NRCS 1992). Edwards Creek appears typical of brackish wetlands adjacent to the New River Estuary. Despite human development within the Edwards Creek watershed, this system appears to be important as fish and wildlife habitat.

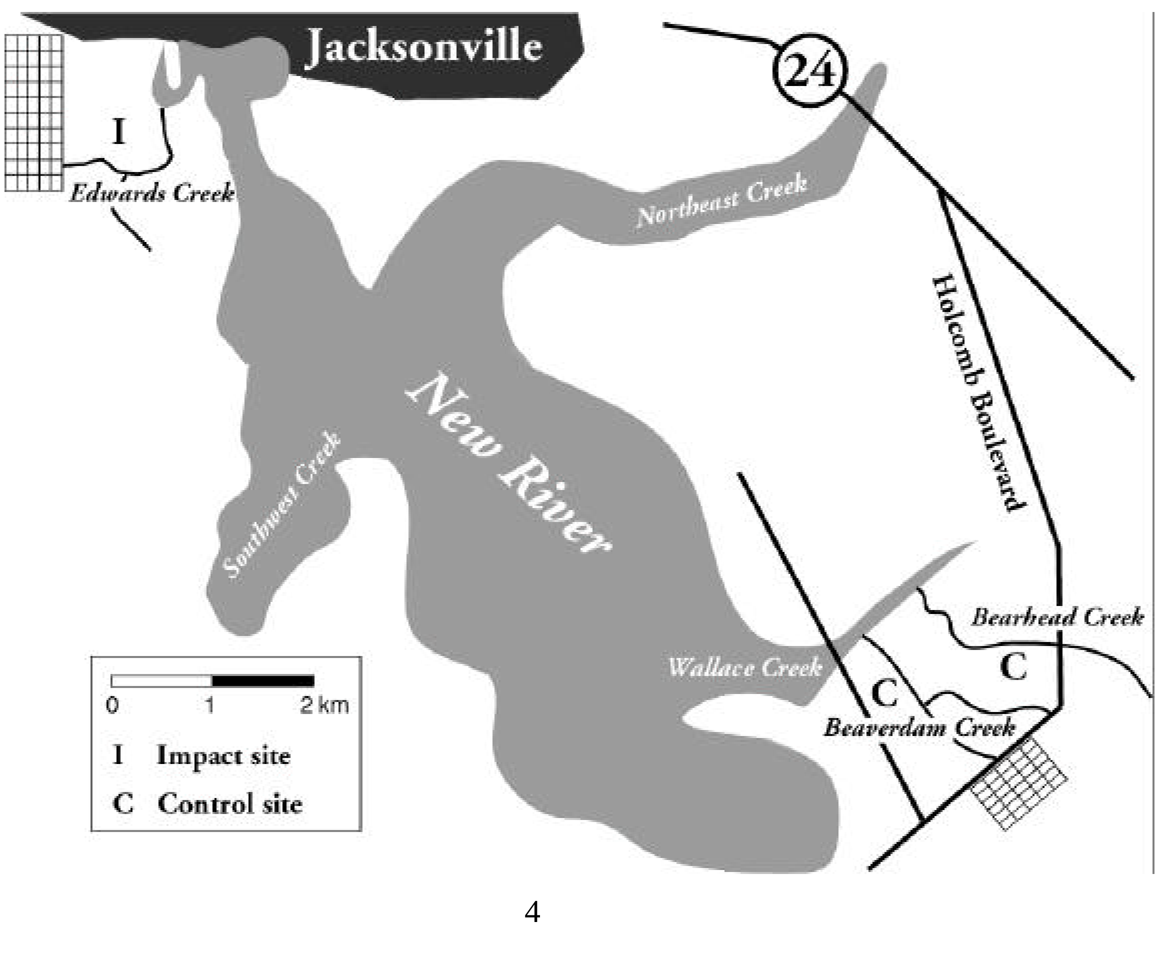

Figure 2. Relative location of impact and control wetlands around the New River Estuary, Jacksonville, NC.

Two control sites were continually monitored. Both reference wetlands are located within the boundary of Camp Lejeune (Figure 2). Both sites are tidal brackish wetlands with plant communities that are very similar to those of Edward's Creek. Beaverdam Creek (BD) and Bearhead (BH) Creek are two small watersheds that are immediately adjacent to each other. Both creeks are tributaries of Wallace Creek that is in turn a tributary of the New River Estuary. The watersheds of Beaverdam Creek and Bearhead Creek occupy an area of approximately 1.29 km^2 (0.5 mi^2) and 3.2 km^2 (1.25 mi^2) respectively. The soils of both Beaverdam creek and Bearhead creek are classified as Muckalee sandy loams (USDA, NRCS 1992). Land use patterns in the watersheds of the control and impact wetlands are similar. All watersheds are predominantly forested and have areas of human development associated with Camp Lejeune.

Eight permanent transects were established at the impact site to determine ecological conditions (Figure 3). These transects are located upstream and downstream of the highway right-of-way at 25, 50, 100, 300 meter intervals as measured along the stream channel. Transects are labeled according to location downgradient (D) or upgradient (U) of the highway, and numbered according to their distance from the highway (low numbered transects are closer to the highway).

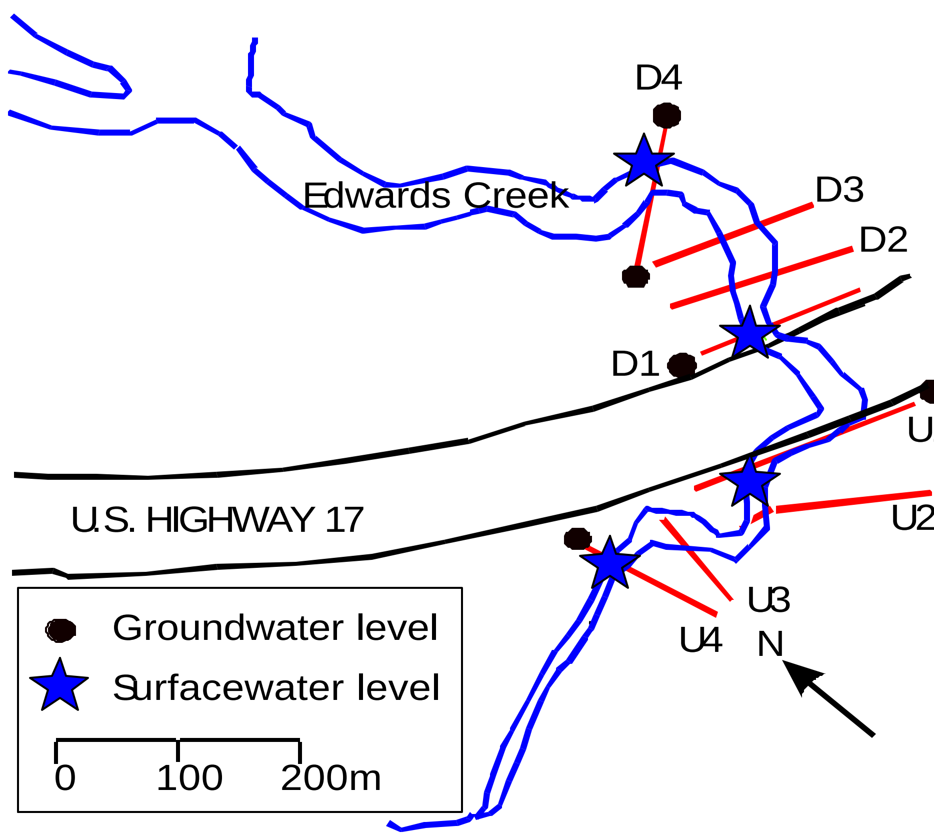

Figure 3. Transect locations upstream (U) and downstream (D) from the highway construction site over Edwards Creek.

Transect spacing is intended to capture impact gradients upstream or downstream of the highway crossing, and thus delineate the boundary between impacted and unimpacted wetland areas. This will allow for the quantification of the spatial extent of any impacts and resulting mitigation requirements. Two transects were established at each control site. Transects at Bearhead Creek are designated BHUS (Bearhead upstream) and BHDS (Bearhead downstream), and those at Beaverdam Creek are designated BDUS (Beaverdam upstream) and BDDS (Beaverdam downstream). To minimize the variability associated with a lateral elevation gradient occurring between the stream channel and surrounding upland areas, the sampling design in this study is stratified into three sampling blocks. These blocks correspond to zones consisting of a central channel of open water, a band of emergent macrophytes immediately adjacent to the channel (marsh), and a band dominated by woody species (Figure 4). Each transect is divided into segments corresponding to the sample blocks. Permanent reference points were chosen in each block, and five sampling quadrats were established at random distances and compass headings from each reference point.

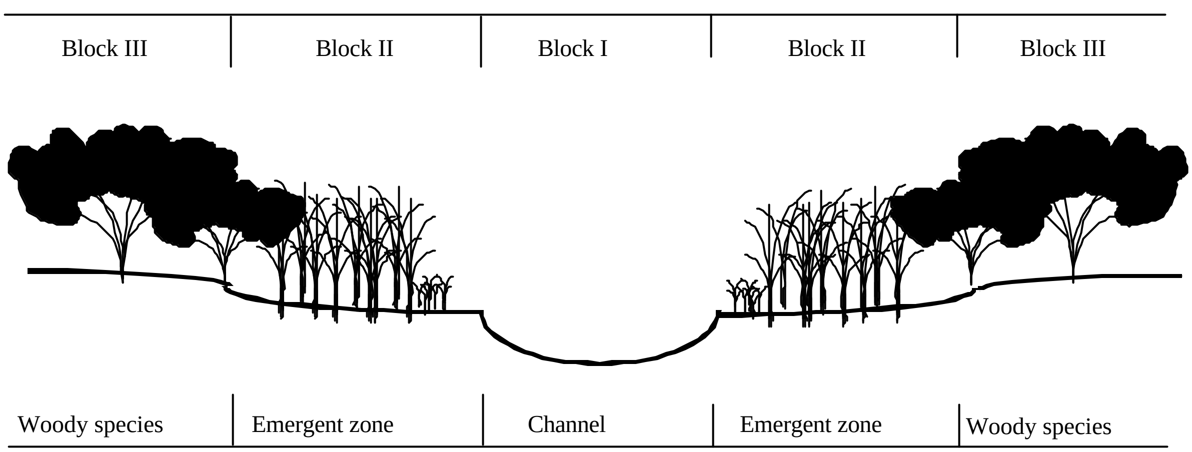

Figure 4. Cross-sectional view of the vegetation zones along the estuary channels. The sampling areas are marked as blocks I, II, and III.

2) Hydrologic flux and storage

The hydroperiod of wetlands is often regarded as the most important abiotic factor determining the structure and function of wetland systems (Mitsch and Gosselink 1993, Committee on Characterization of Wetlands 1995). From 1997 through 1998 water levels in the study wetlands were monitored using Remote Data Systems model WL40 digital data loggers (See Appendix A).

Equipment failures were detected in late 1999 after several hurricane events. The malfunctioning RDS units were subsequently replaced with Telog WLS-2901e data loggers in February 2000. At the impact site, water level recorders were placed 25 and 300 meters upstream and downstream of the highway crossing. Relative elevations of the recorders were found with laser level equipment to allow calculation of water surface elevation relative to a single reference datum.

3 Biogeochemistry

The water quality of the impact and control sites was monitored throughout the study period. YSI model 6920 sondes monitored water quality at 1-hour intervals. The sondes monitored pH, dissolved oxygen, conductivity, salinity, temperature and turbidity. At the impact site, sondes were installed in the creek channel 25 m upstream and 25 m downstream of the highway corridor. At the control sites single sondes were installed at random sites within the creek channel. Due to problems with probe fouling by biofilms and sediment, only the first week of data from each month was used in statistical analyses. (*N.B.* A check on the data indicated that accurate readings occurred for 12 days after placement of the probes.) Water sampling stations were established at transects DS4, DS1, US1 and US4 at the impact site and at two transects at each of the control sites. Water samples were collected monthly from two depths (10 and 50-cm below the surface) at each station. Subsamples were filtered through a 0.45 µ membrane or acidified to pH < 2 in the field and stored on ice. Water samples were analyzed for ortho-P, total P, NH_4^+-N and NO_2-NO_3-N.

4) Productivity

Assessing the productivity of the study wetlands required different methods be used in each sampling block. In the open water block, growth of periphyton (algae) on artificial substrates was used as an index of productivity. Productivity of the Emergent zone was estimated with peak standing biomass of ten dominant emergent macrophyte species. Peak biomass was estimated using regression models of stem biomass verses stem height and basal diameter. Regression models were developed using samples gathered from the study sites. Periphyton chlorophyll A content was used as an indicator of productivity and was being assessed by placing ten acrylic rods (3/8" diameter) in the channel near each transect. Rods were placed vertically by inserting approximately one-foot portion into the substrate while the remaining two-foot portion extends into the overlying water column. Periphyton were allowed to colonize the artificial substrate for one month. Chlorophyll A was measured by extracting samples with 90% alkalized acetone and measuring absorbance using a spectrometer.

5) Plant Communities

Plant communities were assessed along the transects at each location by measuring the number of stems in a meter square for each species or by doing tree counts along the transects using the line intercept method. A species list of all macrophytes was compiled for each transect before and after the highway was constructed. Plant biomass at each site was measured by harvesting aboveground material in meter-square plots at the peak of the growing season. Plant biomass dry weights were determined after drying the material (80^o C) to a constant weight.

6) Macroinvertebrates

Macroinvertebrates were sampled from the benthos of the creek channels using a 10-cm diameter acrylic coring tube (Murkin *et al.* 1994). Core samples included a sample of the water column. The top 10 cm of each core was extracted along with the surface water into a 0.5-mm mesh sieve bucket. Cores were rinsed to remove fine particles, placed in storage bags, and preserved in 5% formalin stained with rose bengal. A total of 8 cores were collected per transect on each date.

Macroinvertebrates were also sampled along the interface of the creek channel and the fringing marsh community. Here, ten 0.5-m length sweeps using a D-framed dip net (0.5-mm mesh) were collected along these macrophytes and composited into a sieve bucket. Each composite sample represented approximately 1.5 m^2 of total surface area. This was repeated at each transect on every date of sampling. Sampling was patterned after rapid assessment procedures used by the USEPA (1997), Barbour *et al.* (1999), and Maxted *et al.* (2000). Macrophyte sweep samples were preserved in 5% formalin stained with rose bengal.

Macroinvertebrates were initially sampled during 1997 on a quarterly basis to determine the optimal sampling window for annual assessment (Barbour *et al.* 1999). Spring (first 2 weeks of March) was chosen for annual sampling because this period approximated peak standing-stock biomass prior to mass emergence of many insect species, and typically coincided with an extended period of relatively stable salinity prior to the highly dynamic salinities of summer and fall (R. S. King, unpublished data). By sampling during this optimal index period, we were able to sample intensively and thus produce more reliable estimates of composition than if we had sampled more frequently but a lower level of intensity.

In the laboratory, core samples were sorted to separate invertebrates from sediment and detritus. All invertebrates were removed from every core. Surface areas of cores were used to convert counts of individual invertebrate species into densities (no./m^2). Macroinvertebrates were identified to the lowest practical taxonomic unit, usually species. Most species identifications were verified by expert taxonomists (see Acknowledgments).

Macrophyte sweep samples were processed according to the USEPA's Rapid Bioassessment Protocol for macroinvertebrates (Barbour *et al.* 1999). Material from each sample was evenly dispersed within a 20 x 45 cm gridded sorting pan, with 36 cells of 2 x 2 cm in size. A fixed count of 200 individuals were removed from each sample by randomly selecting cells with a random number table and separating specimens from material in each cell until a total of at least 200 was reached. The total number of grid cells removed was used to convert raw counts into densities (no./m^2) (King and Richardson, in press). Taxonomic identification procedures were identical to those used for core samples.

7) Fish and Large Crustaceans

Mobile macrofauna were sampled using fyke nets (1.2 x 1.2 m front-end opening, 3-m length wings and lead, 4-mm mesh netting). Fyke nets are passive sampling devices that function as large funnel traps (Hubert 1996). Fyke netting has been shown to be one of the most effective techniques for shallow, wetland habitats (*e.g.*, Brazner 1997).

One fyke net was deployed facing downstream at each site (Beaverdam, Bearhead, Edwards Creek downstream, Edwards Creek upstream). Nets were deployed for at least 3 consecutive 24 h periods during each sampling event. Fish and crustaceans were removed from nets at the end of each 24 h period, identified, counted, measured, weighed (at least 10 individuals of each species on each day), and noted for overall condition (*e.g.*, abnormalities). At least 1 individual of each species was retained as a voucher specimen to confirm identification; all others individuals were released unharmed approximately 50 m downstream of each net.

Fyke net sampling was conducted during mid-summer (July), a period coinciding with peak abundance of transient marine fishes, particularly juveniles. Sampling was also conducted during fall (October), but catches were very low and influenced by post-hurricane flooding during 2 different years. Thus, data from only summer catches were analyzed. Sampling occurred from 1997-2000. No samples were collected in 1998.

8) Macroinvertebrate Data Analysis

Macroinvertebrate data from benthic and macrophyte habitats were analyzed separately. Several attributes of the macroinvertebrate assemblage were evaluated for changes due to highway construction. Metrics based on compositional attributes rather than total densities or biomass have been shown to be the more effective in detecting impairment (Karr and Chu 1997), particularly in wetlands (*e.g.*, King *et al.* 2000, King and Richardson, in press). Thus, we de-emphasized changes in densities, which were highly variable, and focused on structural features of the assemblages.

Eaton (2001) identified 2 metrics that were reliable indicators of disturbance in estuarine waters of North Carolina. The first was an index based on tolerances or sensitivities of individual taxa to pollution. Termed the Estuarine Biotic Index (EBI) it is calculated as a weighted average of estuarine sensitivity values (ESV; see Appendix B) among all taxa. ESVs are weighted by qualitative abundance values. Since count data in this study were standardized to quantitative densities, we used log-transformed densities as the weighting factors. The index is scaled from 1-5, with 5 representing the best water and habitat quality.

The second metric found to be effective by Eaton (2001) was taxonomic richness, or the total number of taxa. This is the most widely used diversity metric in bioassessment today (Karr and Chu 1997). Numbers of taxa are expected to decline in the presence of pollution, although nutrients or habitat alterations may actually increase richness in some cases (Growns *et al.* 1992, King *et al.* 2000).

A third metric was Bray-Curtis dissimilarity, a multivariate distance measure ideal for macroinvertebrate community data (Faith *et al.* 1987, Legendre and Legendre 1998). Bray-Curtis dissimilarity was calculated using log-transformed densities of each individual taxon to decrease the weight of the most abundant taxa. This dissimilarity index is expressed as the % dissimilarity between pairs of samples.

Initial perusal of temporal fish data indicated that individual species abundances were too variable over time to be reliably compared with statistics. However, considered in aggregate, fish community structure appeared to be affected by the highway construction. Thus, we used Bray-Curtis dissimilarity as a metric of changes in the fish and crustacean assemblages over time.

Univariate metrics (EBI, number of taxa) were analyzed using repeated-measures ANOVA following a beyond-BACI design described by Underwood (1992). We first considered that the highway crossing might affect the wetland most noticeably adjacent to the highway, with diminishing effects with greater distance. However, preliminary analysis suggested that, in cases when the highway crossing appeared to affect biota, the effect was upstream-downstream rather than a distance effect. Thus, transects were used as replicates for the upstream and downstream areas, respectively. These were considered separate "impacted" levels of the control/impact main effect in the model. Transects from the control sites were used as replicates for the "control" level of the control/impact main effect. Collection dates prior to highway construction were identified as "before" level in the before/after main effect, and during or post-construction were labeled as an "after" level. A significant control/impact-before-after interaction term was the test statistic of interest, as this would indicate a disturbance effect related to highway construction and independent of natural temporal processes or changes observed in nearby control locations. Levels of a significant interaction were contrasted using and LSD multiple comparison test.

The Bray-Curtis dissimilarity metric was evaluated using nonmetric multidimensional scaling (nMDS), an ordination technique. NMDS projected samples into a 2-dimensional space to represent their interpoint distances (dissimilarity) in a manner analogous to constructing a map based on distances among cities (Clarke 1993). This approach was ideal for our study since we were interested in examining the trajectories of species assemblages through time at control and impact sites. For example, if the highway had an effect on species composition, we expected to see a change in the direction and/or magnitude of the successional trajectories at the impacted sites relative to the controls. If there was no effect, then the impacted assemblages should behave similarly to the control sites through time.

While the nMDS approach provided a visual assessment of possible highway-related effects on species composition through time, it was not useful for assigning p-values to a before-after/control-impact interaction term like RMANOVA. There are a few multivariate approaches capable of such a test (*e.g.*, NPMANOVA; Anderson 2001); however, these approaches require a balanced design, a feature not demonstrated with our data. Thus, we used a distance-based procedure designed to test for differences between 2 groups and assign bootstrapped 95% confidence limits to a test statistic. This statistic is an index of relative difference between groups, scaled from 0 to 1, and can therefore be used to compare differences among impacted and control locations before and after construction. Here, differences in the test statistics among locations that lay outside the 95% confidence limits were assigned as significant. For example, control and impact locations might differ significantly even before construction; however, if the magnitude of the difference between controls and impacted sites increased significantly (beyond 95% confidence limits) after construction, this would suggest that the highway had caused a significant change relative to what might be expected at the control locations over time. In conjunction with nMDS, these approaches were complementary and provided strong evidence of the presence or absence of highway-related disturbance to the impacted site.

III. RESULTS AND DISCUSSION

The main challenge of environmental impact assessment studies is to identify changes in system behavior that result from anthropogenic influence rather than from natural patterns of variation. Additional challenges arise when trying to assess the influence of a specific human impact to a system that may experience multiple impacts. The "Beyond BACI" experimental design can be a powerful tool for differentiating human induce changes in ecosystem from change that result from natural variation or from region scale processes. The design does not require identical functional characteristics or community composition at the control and impact sites. Rather, a simple variance measure of dissimilarity (Delta) between the Edwards Creek (impacted) site and two reference sites is used to model the similarity of the systems before and after an impact. A statistically significant change in the magnitude of the deltas is indicative of an impact. We tested the alternative hypothesis that the magnitude of these between site deltas would be significantly greater during highway construction than prior to construction. By accepting this alternative hypothesis we demonstrate a functional divergence of the impacted site from the reference site.

A. Hydrologic Flux and Storage

Hydrologic flux, the patterns of inundation and drawdown, is a primary factor influencing the structure and function of wetland systems. The hydrologic flux of both Edwards Creek and the two reference wetland systems was assessed using automatic water level recorders. The daily maximum depth of inundation is affected by several factors including tidal amplitude and runoff from upland areas. Figure 5a shows boxplots daily maximum stage at Edwards Creek and at the two reference wetlands. All sites show a significant decease in the mean stage height in the post construction (after) study period. This study was initiated during extremely wet climatic conditions following Hurricane Fran in 1996, and additional hurricanes in the pre-construction period in the fall of 1997 and 1998. The post construction phase of this study corresponds to an extended period of drought in North Carolina that continues in 2002. Monitoring stations located in upstream areas with relatively small tidal influence, and narrow stream, and large watershed areas had a disproportionate influence of upland runoff on hydrologic patterns. This effect was most noticeable in the upstream areas of Edwards Creek (station U4) prior to construction where the stage heights were typically high relative to the downstream areas of Edwards Creek which has a substantially wider channel with little increase in drainage area.

Figure 5b shows the "deltas" between reference stations (BH and BD) and the Edwards Creek stations (U4 and D4). With two exceptions the deltas show a trend toward zero when moving from the "before" period to the "after" indicating the hydrology of the Edwards Creek and the reference stations are become more similar. One exception to this trend is seen in comparing upstream areas of Edwards Creek to the Bear Head Creek reference wetland (BH_U4). These systems remain similar, with relatively high daily maximum stages despite the development of drought conditions. This could be the result of placement of cofferdams at the Edwards Creek site inhibiting the movement of upland runoff from areas upgradient of the bridge crossing. This would be consistent with observations of reduced salinity in this area (shown later). The second exception of the trend to smaller deltas is seen when comparing the downgradient area of Edwards Creek to the Beaver Dam Creek reference station (BD_D4). In this comparison we see greater deltas due to increases in the maximum stages in the downgradient areas of Edwards Creek (D4). This may be due to mitigation replacement of a small single culvert through a road causeway at the mouth of Edwards Creek with three much larger culverts designed to increase tidal flushing and wildlife access to Edwards Creek. Because of this increase in tidal action on the lower portion of Edwards Creek, drought conditions caused relatively small changes in daily maximum depths relative to those seen at reference sites.

In summary, the highway project seems to have had both positive and negative effects on the hydrology of Edwards Creek. Unfortunately, the nearly simultaneous initiation of mitigation projects (culvert replacement) and bridge construction, along with hurricane effects, have confounded efforts to assign hydrologic impacts solely to road construction activity alone although direct changes are clearly evident when comparing the before and after period of activity.

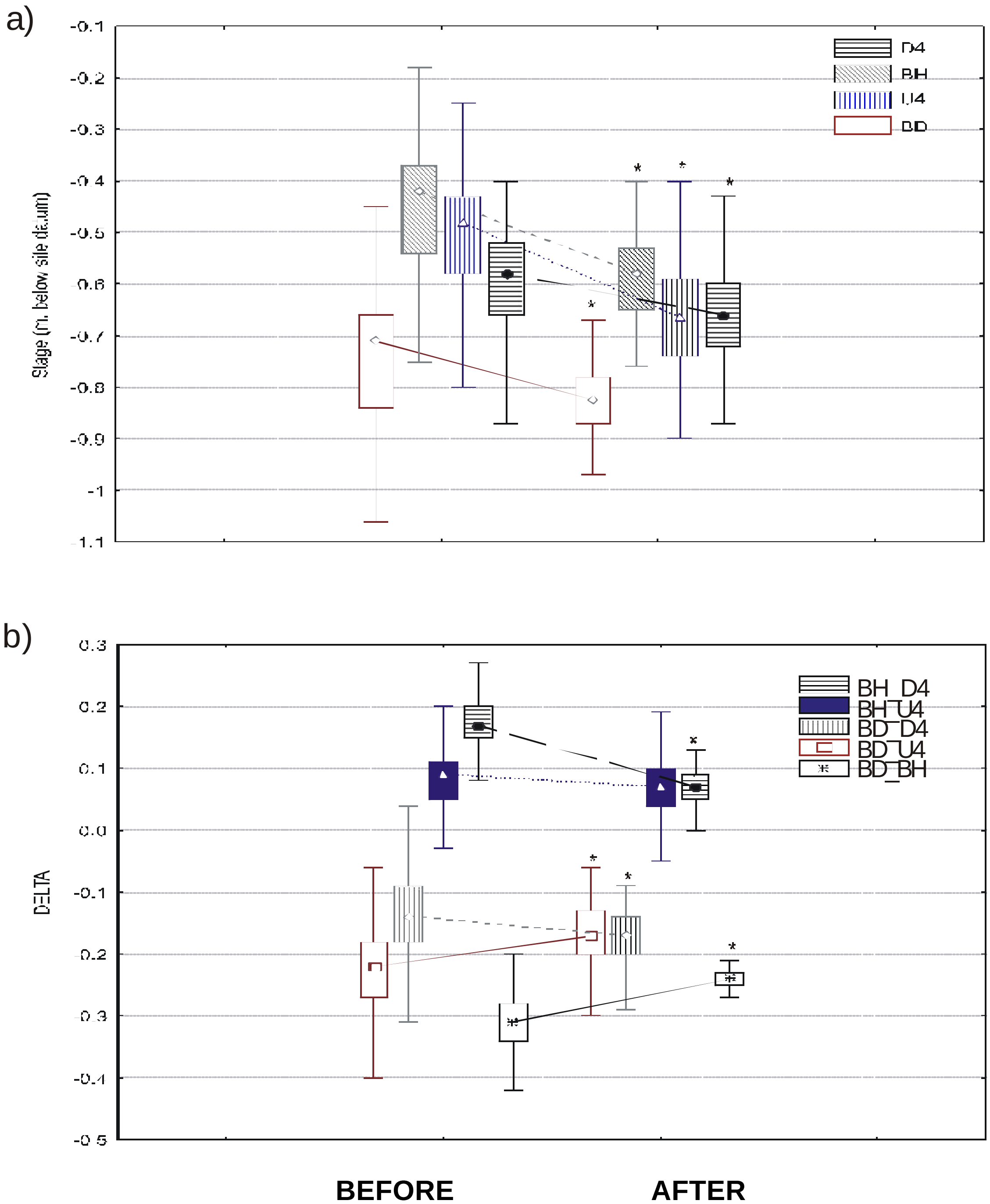

Figure. 5a. Boxplot of maximum daily water levels at stations upgradient (U4) and downgradient (D4) of a highway construction site on Edwards Creek, and at two reference wetlands (BD and BH) before (B) and after (A) the onset of construction. 5b. Boxplot of daily differences (deltas) between reference stations and Edwards Creek stations before and after construction onset. * denotes significant ($p < 0.05$) difference between before and after construction using Mann-Whitney *U* test. BD = Beaverdam

B. Biogeochemistry

Many studies have examined changes in water chemistry that result from disturbances. Likens *et al.* (1970) examined the effects of forest cutting and herbicide treatment on nutrient, chemical and sediment fluxes from experimental watersheds by monitoring stream flows and water chemistry at sampling stations located at the terminus of the experimental watershed. Uddameri *et al.* (1994) used a similar approach to study the response of a watershed in Maine to artificial acidification with the objectives of identifying the major processes controlling surface water acidity, and of assessing qualitative and quantitative watershed level responses to artificially increased levels of acidic deposition. By measuring several water quality parameters we assessed the biogeochemical functions of the impact and control wetlands that are the result of highway construction. Salinity is a major indicator of ecosystem function in brackish wetlands. A time series of maximum daily salinity values are presented in Figure 6.

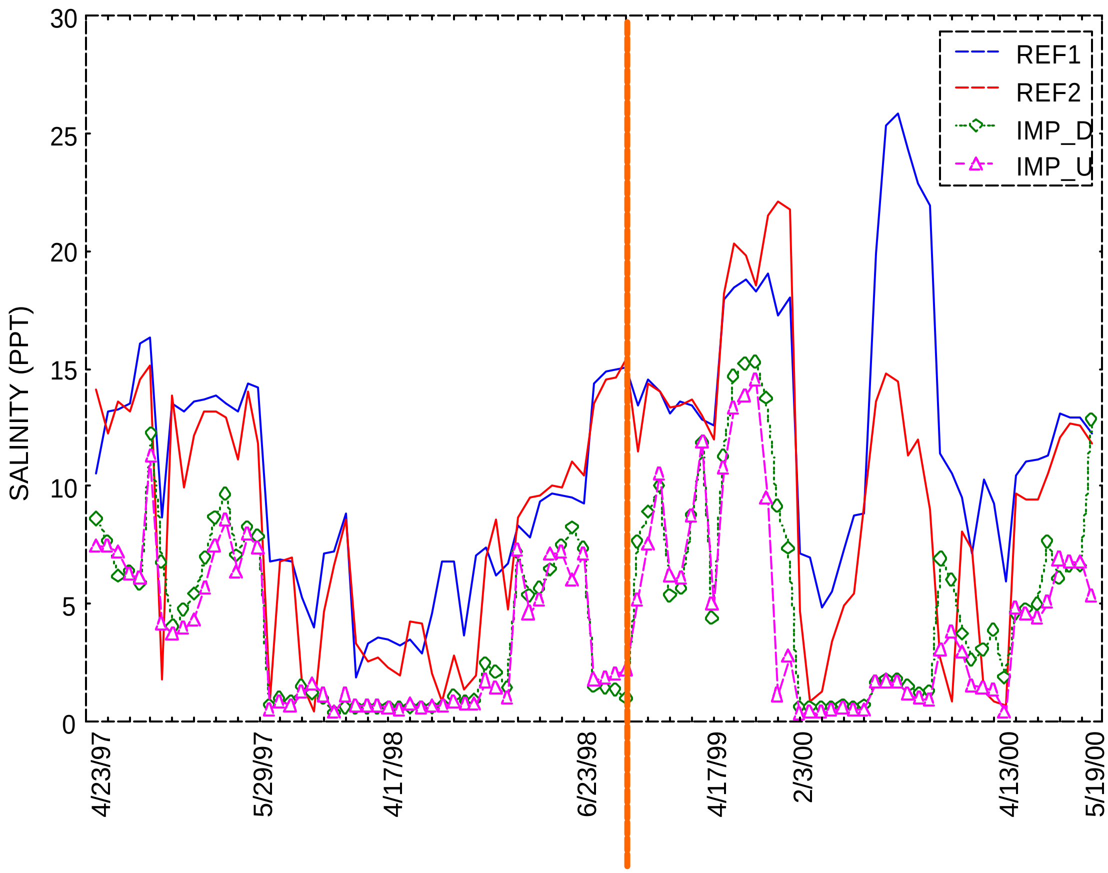

Figure 6. Time series of salinity readings in reference sites (REF1, REF2) and upstream (U) and downstream (D) of the Edwards Creek construction site (IMP). The vertical line depicts the start of construction activity.

Figure 6 shows the median salinity values (IMP D, U) at the Edwards Creek wetland are lower than at the control wetlands. However, higher median salinities at the control sites are probably due to their closer proximity to the mouth of the New River Estuary. Salinity ranges are similar at the impact and control wetlands with values ranging from less than 0.5 to 15.8 ppt at Edwards Creek, 0.8 to 20.0 at Bearhead Creek and 0.1 to 19.8 and Beaverdam Creek. Between-site deltas for salinity levels observed at the study sites during the 1997 field season prior to construction (before) as compared to post-construction levels (after) are shown in Figure 7. Prior to highway construction, salinity readings at the Edwards Creek displayed a high level of concordance with reference site readings as demonstrated by the smaller error bars on the boxplots of preconstruction intersite deltas. The results of Mann-Whitney U test presented in Table 1 indicate significant differences were found between before and after deltas for each Impact-reference contrast, with deltas changing from – 4 to – 8. However there was not a significant difference in reference–reference contrasts as delta values remained near 0. This shows the divergence of the reference wetlands from the impact wetland with regard to salinity, and suggests highway construction has impeded the movement of saline water into the Edwards Creek system. Daily minimum dissolved oxygen saturation data are summarized as box plots in Figure 8. Daily minimum saturation levels increased at sampling stations, except for Edwards Creek impacted station, which is located down gradient of the construction area at Edwards Creek (Figures 8, 9). The reference sites showed similar DO values before and after the construction, while the downstream impacted site showed a significant drop in oxygen (Figure 8) and a significant change in the delta value (Figure 10). A trend of increased sedimentation was found with rates of sediment accretion increasing at all sites except the reference sites (Figure 9). This suggests increased suspended sediments downstream of the construction site may be inhibiting photosynthesis in the water column and causing reduction in oxygen during construction at the downstream site (Figure 8).

Water temperature medians and ranges are similar at all sample points (data not shown). A time series of turbidity data summarized in Figure 11 suggests similar median turbidity values at all sites. There are some notable differences in peak turbidity values at the sites after construction, where turbidity decreased at all sites (Figure 11, 12). The highest turbidity values are associated with storm events. Peak turbidity values at transect Edwards Creek D are slight higher than those at U, probably due to the confluence of a small tributary with Edwards Creek between transects U and D (Figure 12). Turbidity values at the Bearhead Creek control wetland are quite similar to those at the impact wetland. Daily maximum peak turbidity values at the Beaverdam Creek control wetlands are considerably higher than those observed in the other wetlands prior to construction (Figure 12). Field observations of fine clay sediments in the creek channel and observations of high current velocities during storm events may explain this. A BACI comparison of before and after inputs surprisingly shows that there is no significant impact of construction on turbidity (Figure 13). Figure 14 summarizes ortho-phosphorus concentrations (PO_4-P) from monthly water samples taken from two depths at stations in the impact and control wetlands. Ortho-P concentrations at Edwards Creek are higher than those of the reference wetlands both in surface water samples and in samples taken at a depth of 50 cm (Figure 14). Downstream PO_4-P values decreased significantly after the highway was under construction (Figure 15). This may be due to the increased sediment load added to the water column, which would likely precipitate and remove P from the water column. Similar results are observed with total P concentrations (Table 1). Median total nitrogen concentrations are more similar at the impact and control wetlands (data not shown). However, high TN values (> 10,000 ug/L) in several samples from Edwards Creek resulted in much larger ranges in total nitrogen at the impact site. The explanation of these results is uncertain, but may be related to the presence of a wastewater treatment facility less than 1 km from the mouth of Edwards Creek.

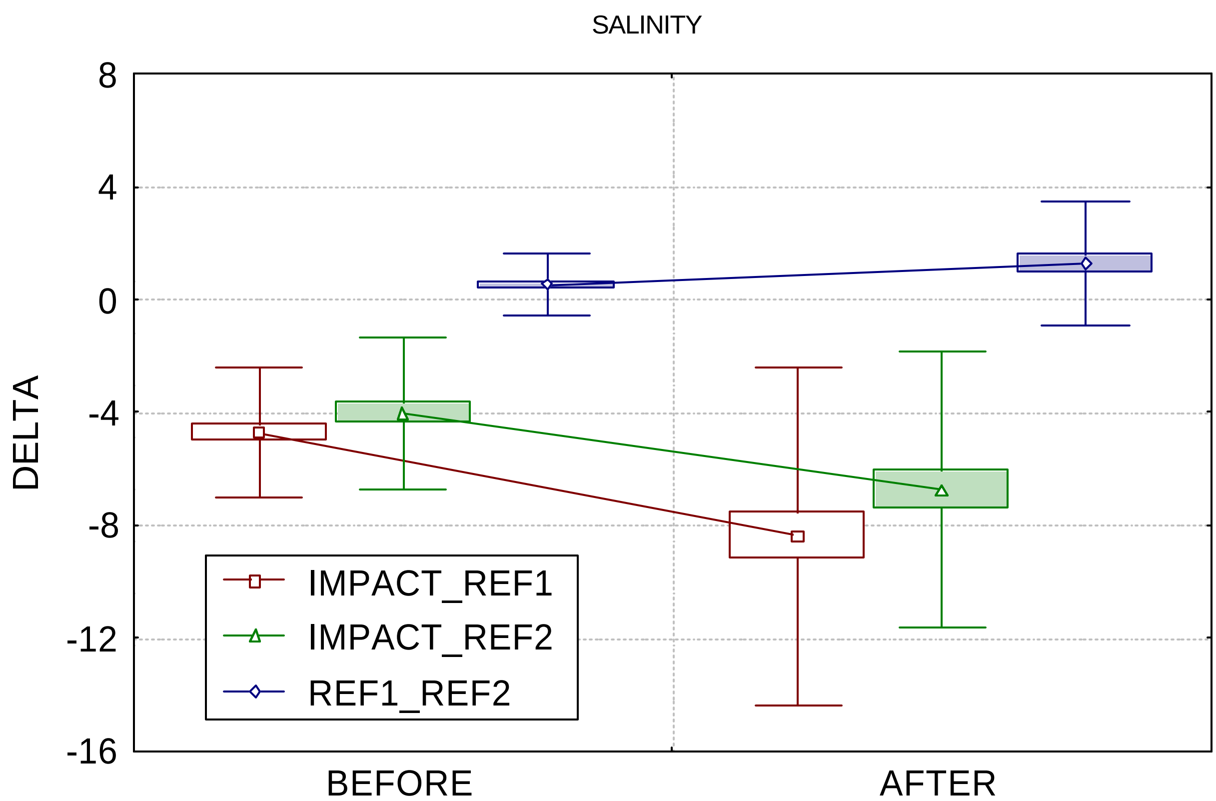

Figure 7. Salinity delta changes in impacted versus reference sites before and after highway construction (IMPACT = construction site at Edwards Creek, REF1 = reference site at Beaverdam Creek, REF2 = reference site at Bearhead Creek)

Table 1. Mann-Whitney U tests for parameters for each impact-reference contrast.

Parameter	DELTA y	p-value	N before	N after
Salinity max	IMP vs. DS REF-1	0.0004 *	46	47
	IMP vs. DS REF-2	0.0093 *	46	47
	IMP vs. US REF-1	0.0000 *	46	47
	IMP vs. US REF-2	0.0045 *	46	47
	REF-1 vs. REF-2	0.2685	46	47
Sedimentation	IMP vs. DS REF-1	0.0636	7	7
	IMP vs. US REF-2	0.0046 *	12	17
	REF-1 vs. REF-2	0.2010	13	12
D.O. % min	IMP vs. DS REF-1	0.0001*	39	38
	IMP vs. DS REF-2	0.0002*	39	38
	IMP vs. US REF-1	0.5276	39	38
	IMP vs. US REF-2	0.4297	39	38
	REF-1 vs. REF-2	0.9188	39	38
PO_4-P	IMP vs. DS REF-1	0.0086*	11	14
	IMP vs. DS REF-2	0.0266*	11	14
	IMP vs. US REF-1	0.6614	11	14
	IMP vs. US REF-2	0.0897	11	14
	REF-1 vs. REF-2	0.6029	11	14
Total P	IMP vs. DS REF-1	0.0261*	10	14
	IMP vs. DS REF-2	0.0224*	10	14
	IMP vs. US REF-1	0.0895	10	14
	IMP vs. US REF-2	0.0790	10	14
	REF-1 vs. REF-2	0.3632	10	14
Periphyton Chlorophyll a	IMP vs. DS REF-1	0.1800	7	7
	IMP vs. DS REF-2	0.1100	7	7
	IMP vs. US REF-1	0.0130*	7	7
	IMP vs. US REF-2	0.0030*	7	7
	REF-1 vs. REF-2	0.4820	7	7

y DS = downstream, US = upstream, REF = reference sites, IMP = impacted sites
* = significance P<0.05

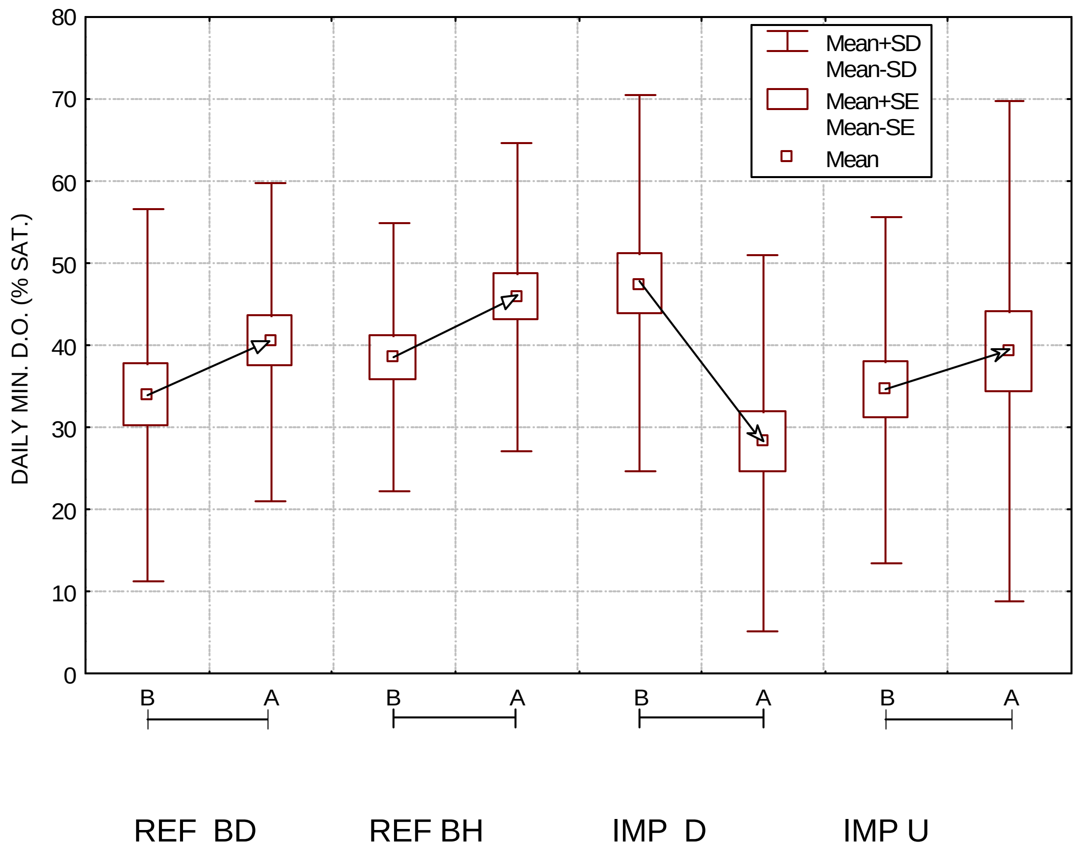

Figure 8. Box plots of daily minimum DO saturation before (B) and after (A) highway construction. (REF BD = reference site at Beaverdam Creek, REF BH = reference site at Bearhead Creek, IMP D = Edwards Creek downstream, IMP U = Edwards Creek upstream)

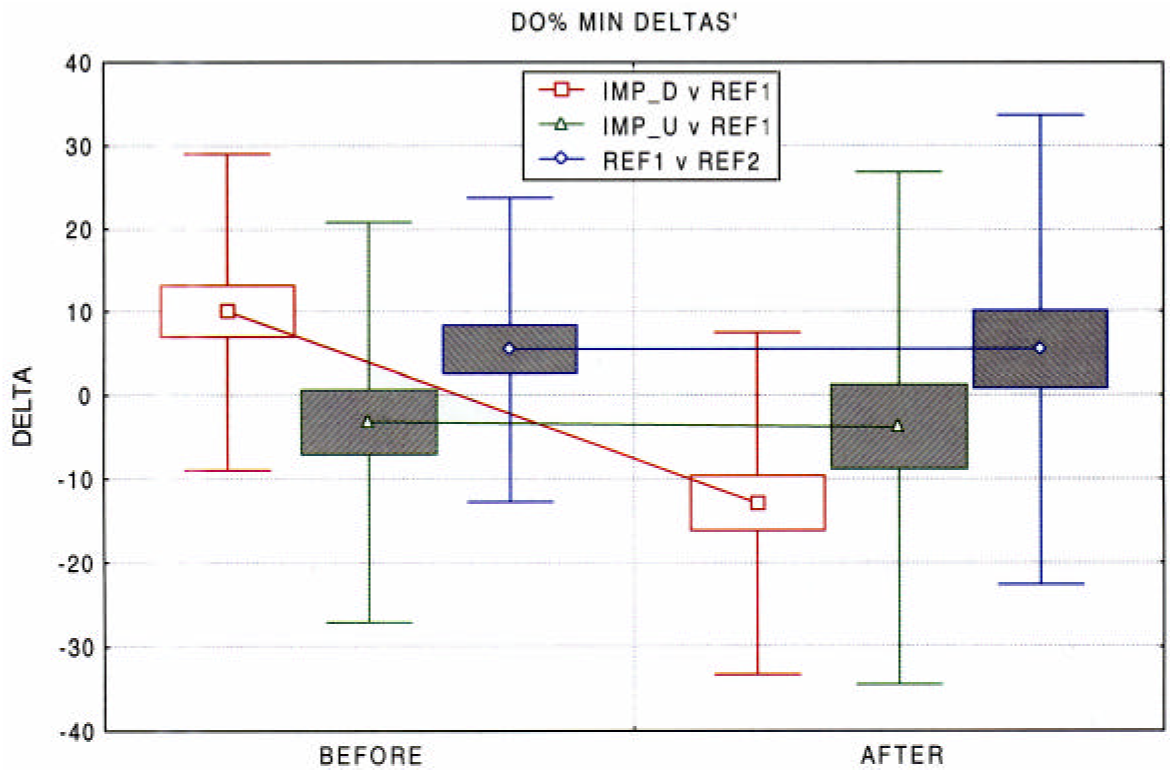

Figure 9. DO delta changes in impacted versus reference sites before and after highway construction. (IMP_D = Edwards Creek downstream, IMP_U = Edwards Creek upstream site, REF1 = reference site at Beaverdam Creek, REF2 = reference site at Bearhead Creek)

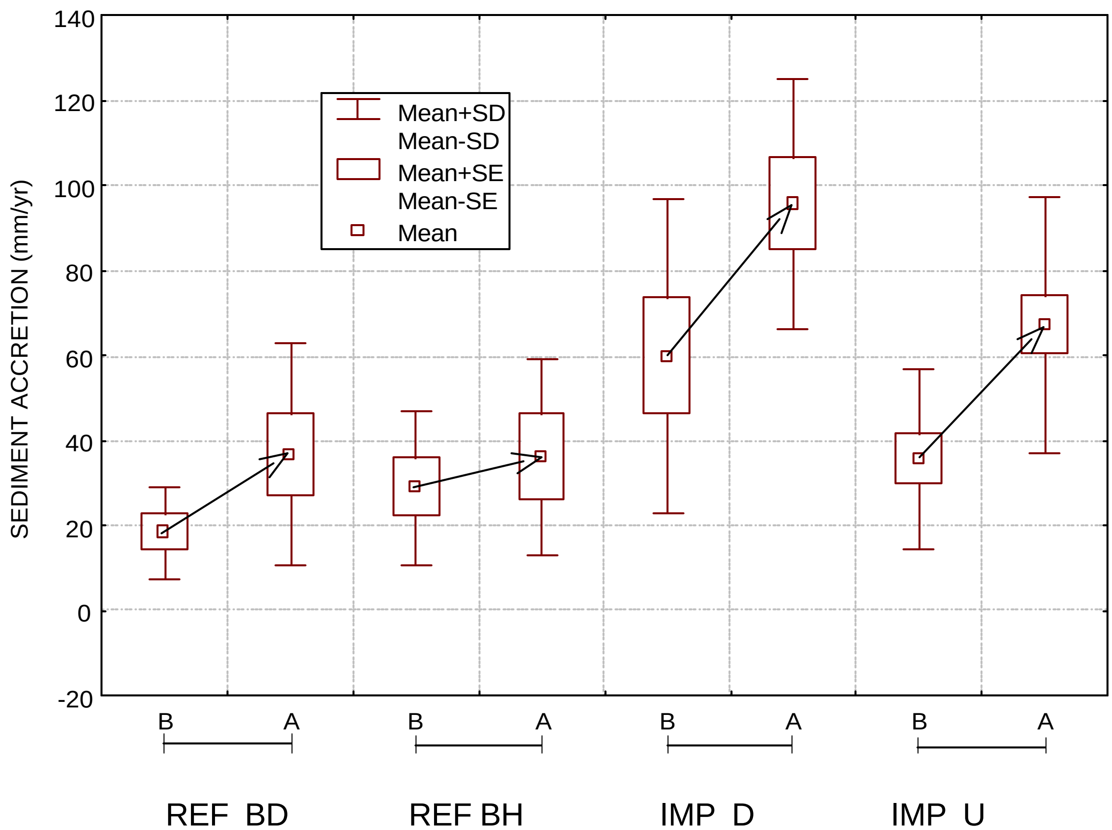

Figure 10. Box plots of sediment accretion rates before (B) and after (A) highway construction. (REF BD = reference site at Beaverdam Creek, REF BH = reference site at Bearhead Creek, IMP D = Edwards Creek downstream, IMP U = Edwards Creek upstream)

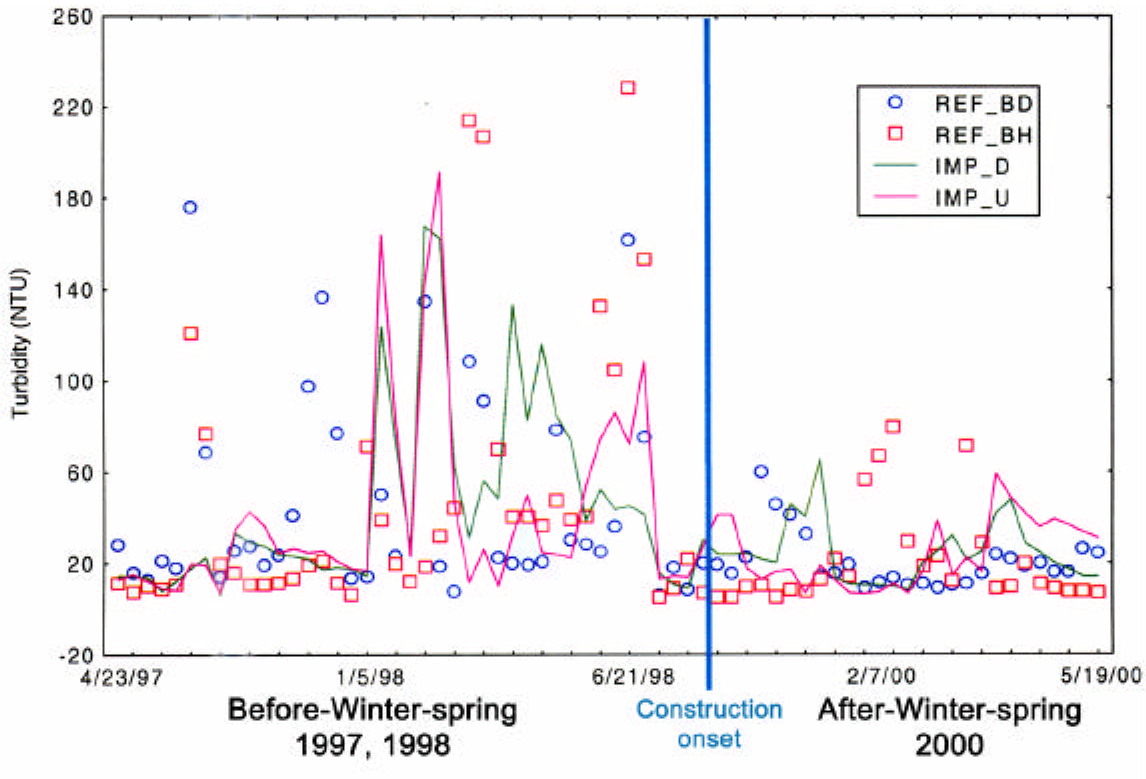

Figure 11. A time series of turbidity values before and after highway construction. The vertical line indicates the start of construction activity. (REF_BD = reference site at Beaverdam Creek, REF_BH = reference site at Bearhead Creek, IMP_D = Edward Creek downstream, IMP_U = Edward Creek upstream)

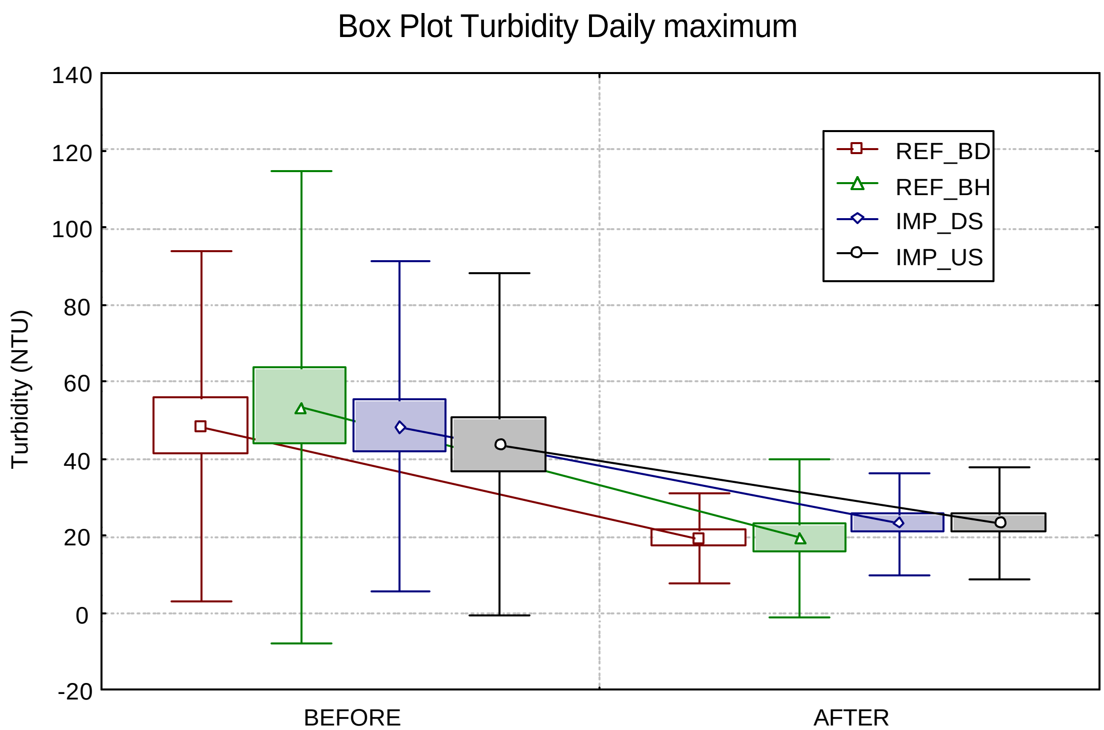

Figure 12. Turbidity box plots of reference and impacted sites before and after highway construction. (REF_BD = reference site at Beaverdam Creek, REF_BH = reference site at Bearhead Creek, IMP_D = Edwards Creek downstream, IMP_U = Edwards Creek upstream)

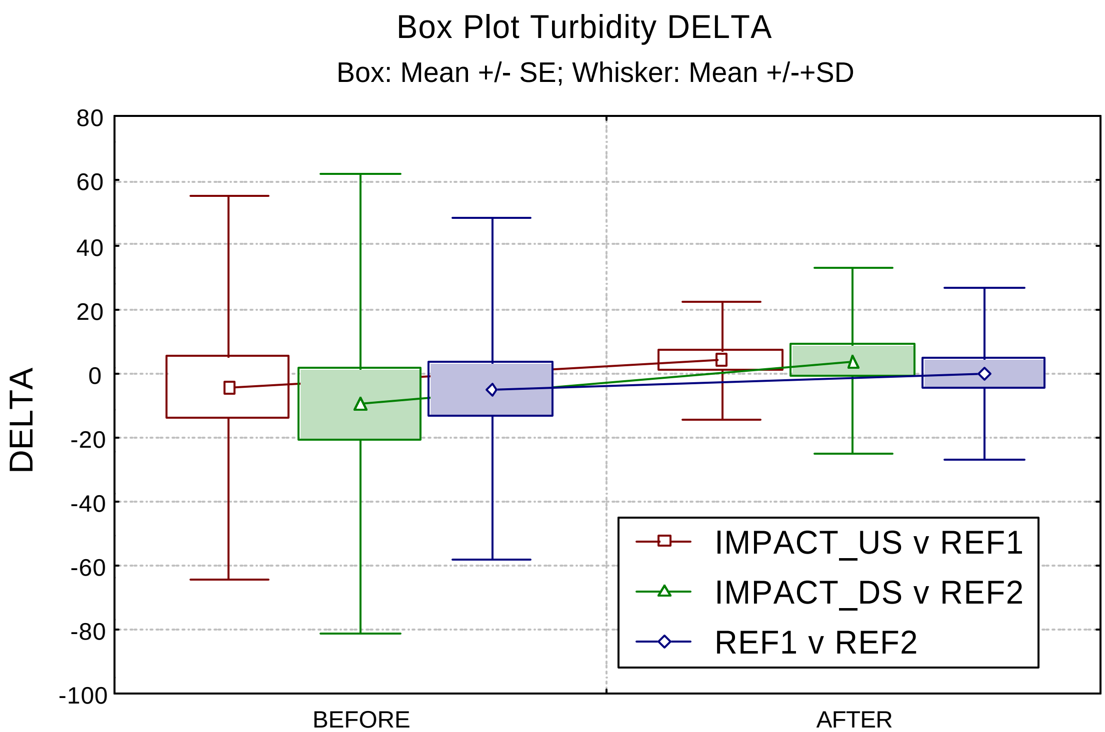

Figure 13. Box plot of turbidity DELTA values before and after highway construction. (REF_1 = reference site at Beaverdam Creek, REF_2 = reference site at Bearhead Creek, IMPACT_US = Edwards Creek upstream, IMPACT_DS = Edwards Creek downstream)

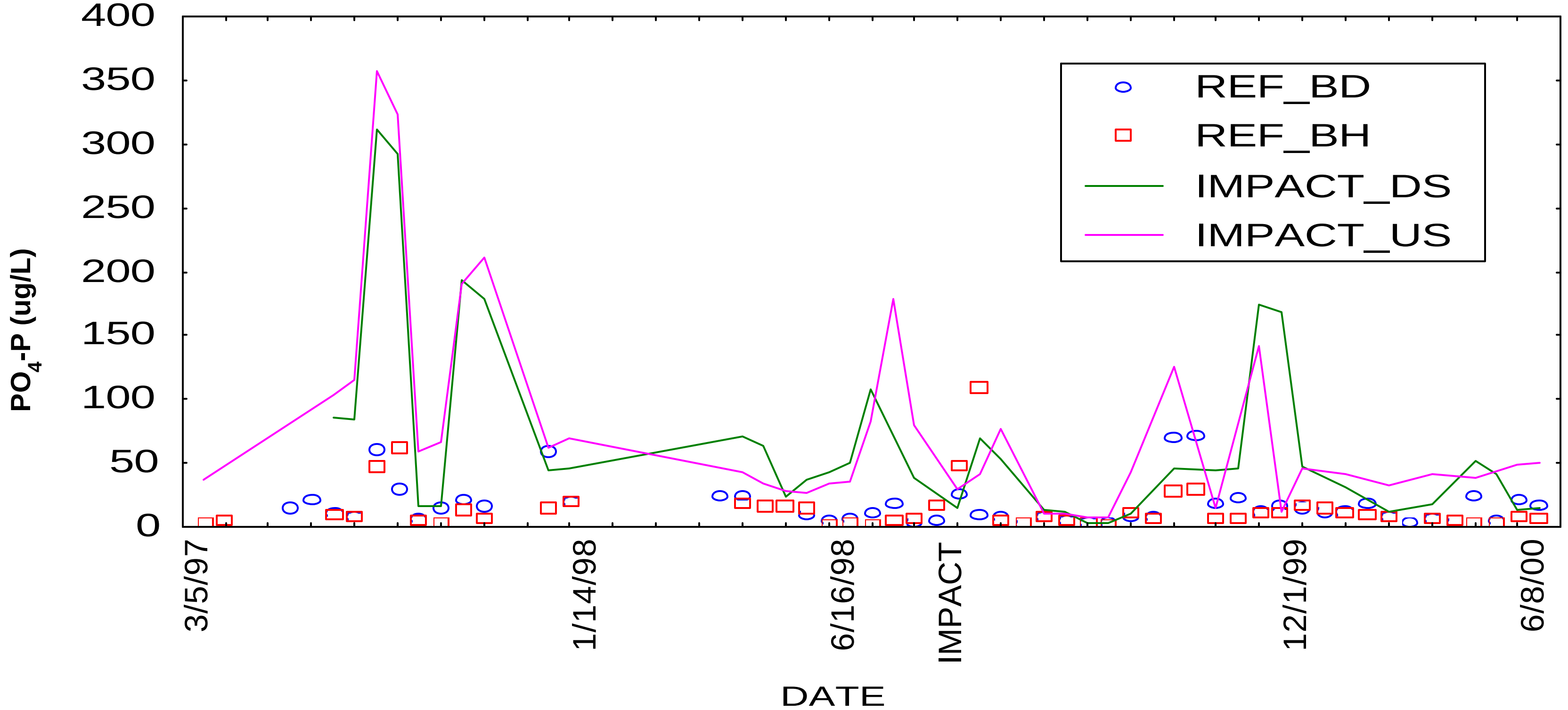

Figure 14. Ortho-phosphorus concentration before and after highway construction. The date of construction is labeled as impact on the graph. (REF_BD = reference at Beaverdam Creek, REF_BH = reference site at Bearhead Creek, IMPACT_DS = Edward Creek downstream, IMPACT_US = Edward Creek upstream)

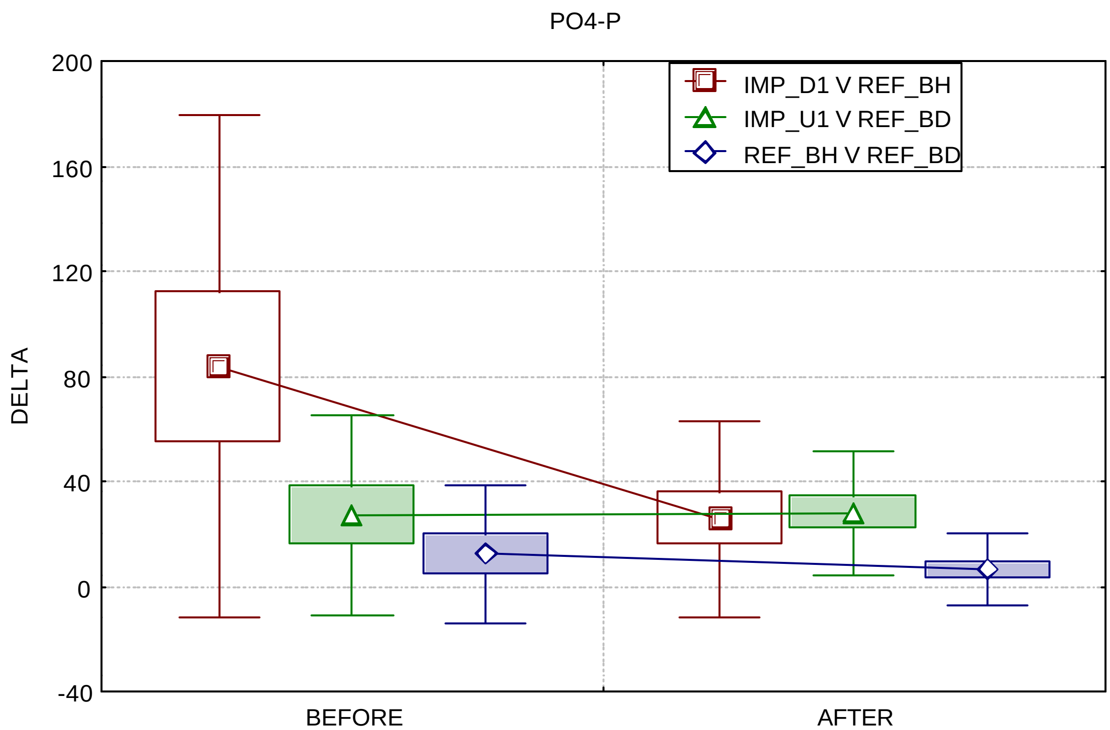

Figure 15. Ortho-phosphorus DELTAs before and after highway construction. (IMP_D1 = Edward Creek downstream, IMP_U1 = Edward Creek upstream,

In summary, water quality data suggest the construction and reference wetlands are very similar in term of physical aspects of water quality (DO, salinity, temperature, turbidity). However, Edwards Creek appears to be influenced by an unidentified source of nutrient enrichment. The site appears to be enriched with phosphorus to a greater degree than nitrogen. Several authors have suggested that coastal marsh vegetation is nitrogen limited (Valiela and Teal, 1974; Smart and Barko, 1980; Mitsch and Gosselink, 1993). Therefore, nutrient enrichment at Edwards Creek may not cause large differences in the productivity of the impact and control wetlands. This idea seems to be supported by the macrophyte and periphyton data collected (shown later). By contrast, the before and after BACI analysis showed a clear effect of highway construction activity on salinity maximums as compared to all reference sites. Significant effects were also found for DO, PO_4-P, and TP.

C. Productivity

Primary productivity is a key function of wetlands and all ecosystems. The primary productivity of a wetland system in large part determines the systems ability to support secondary productivity (fish, waterfowl, *etc.*) and influences other wetland functions such as nutrient storage; chemical transformation reactions (denitritification); and the accumulation and/or export of carbon (Mitsch and Gosselink, 1993; Richardson, 1994). The productivity of the marsh community was assessed using peak standing biomass as a functional indicator. Figure 16 presents means, standard errors, and standard deviations of standing stock calculated for 5 quadrats from each transect. No discernible patterns were seen in comparing the standing stock of the study wetlands. Mean values at Edwards Creek ranged from 220 to 556 $g.m^{-2}$ DW. In the control wetlands mean standing stock ranged from 338 to 488 $g.m^{-2}$ DW. Within the creek channel, chlorophyll a concentrations in periphyton used to assess productivity showed that highway construction sites increased in value (Figure 17) with the upstream impacted sites showing a significant increase over reference sites (Table 1). By contrast, the reference sites both displayed a decrease in chlorophyll a after the construction.

D. Plant Communities

The emergent plant communities at the impact and reference sites were indistinguishable prior to construction in 1997. An ordination of the survey data from 1997 is presented in figure 18. Figure 18 shows an ordination of macrophyte data using non-metric multidimensional scaling. The figure shows a lack of clustering that is related to site location of sample quadrats. This suggests the plant communities of the impact and reference sites cannot be distinguished prior to the onset of highway construction. With the onset of highway construction in 1998 we begin to see the reference sites (specifically Bearhead Creek) clustering separately from the other sites (Figure 19). Ordination of data from the 1999 growing season show a clustering of quadrats from the reference wetlands (Figure 19) that suggests a divergence of the reference wetland macrophyte communities from the communities at the impact site after highway construction. One year following construction, both reference sites were showing a pattern of clustering separately from the impact site. *Scirpus robustus* was present on all transects and was frequently dominant. Other dominant species include *Typha glauca*, *Typha angustifolia*, *Lythrum lineare*, *Kosteletzkya virginica*, and *Spartina cynosyroides*. *Cladium jamaicense* occurred in narrow bands along stream channels at all sites.

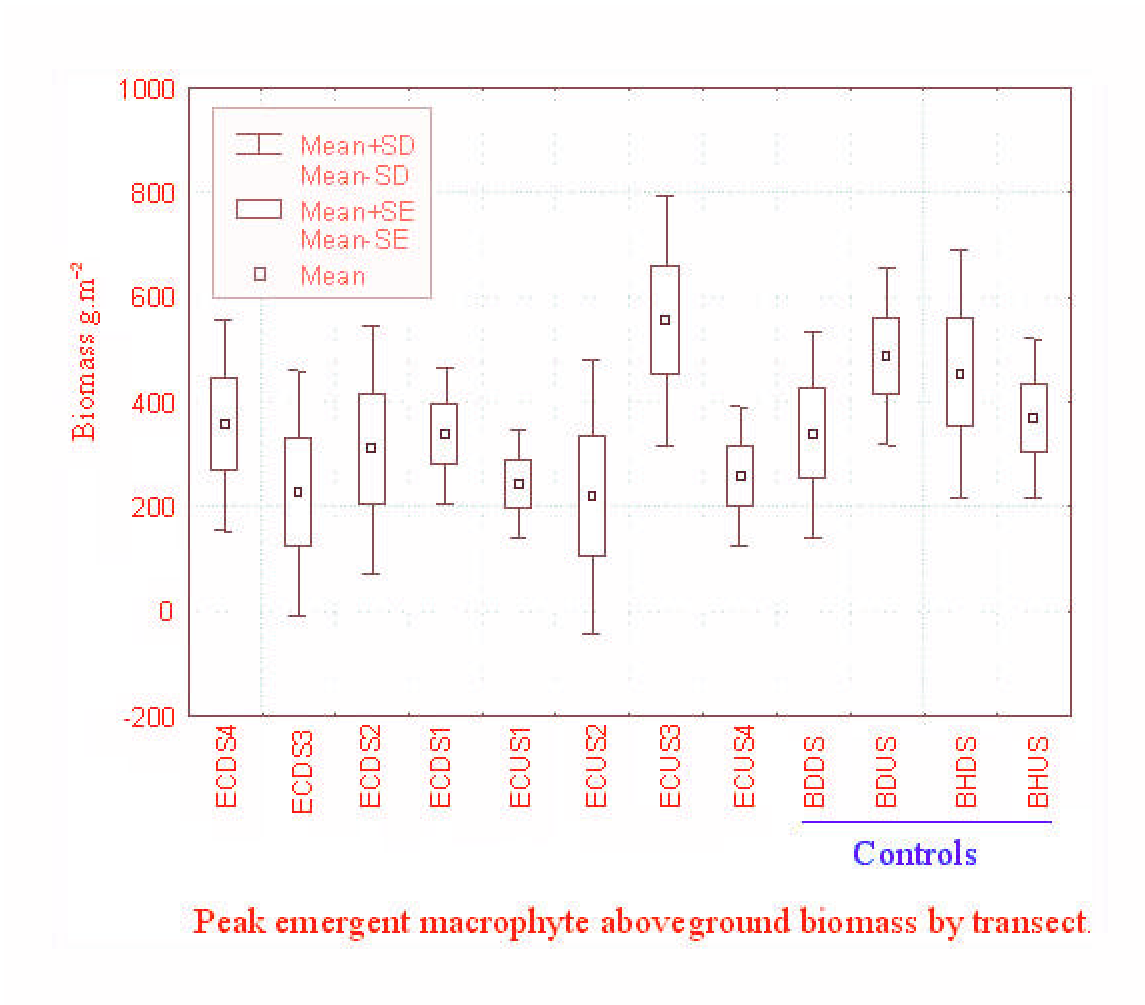

Figure 16. Peak emergent macrophyte aboveground biomass by transect. ECD = Edwards Creek downstream, ECU = Edwards Creek upstream, BDDS = Beaverdam Creek downstream, BDDU = Beaverdam Creek upstream, BHDS = Bearhead Creek downstream, BHUS = Bearhead Creek upstream.

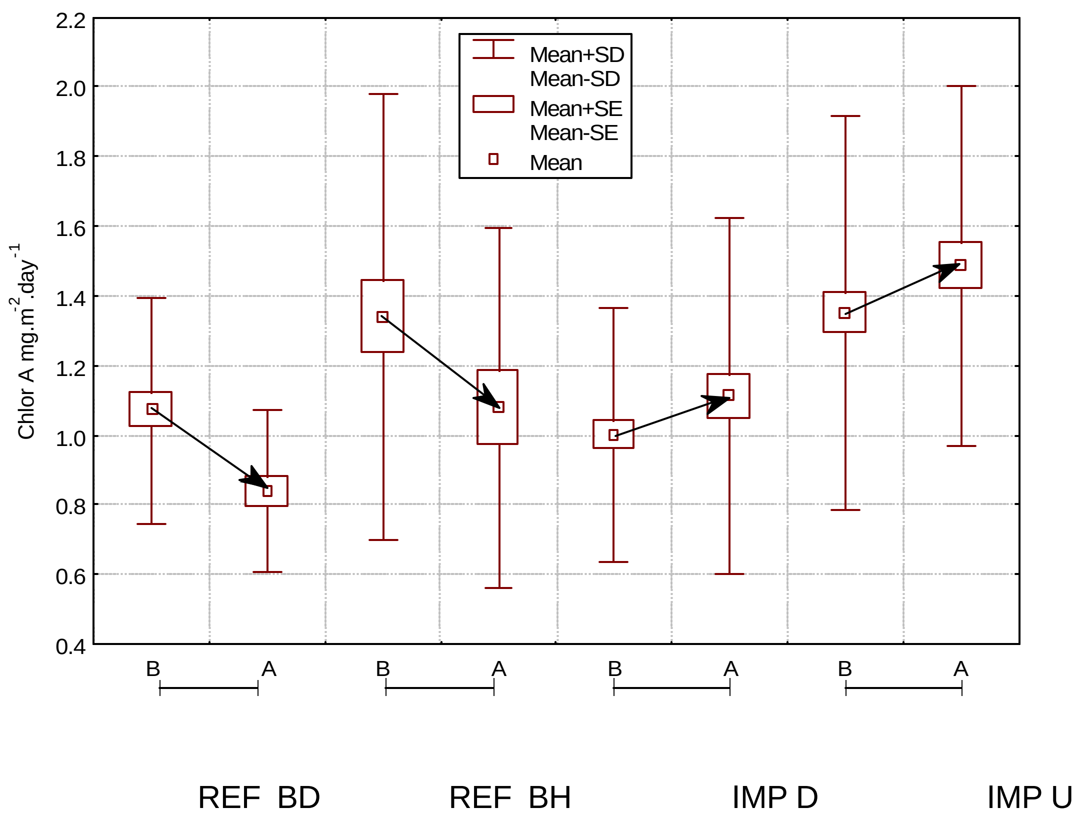

Figure 17. Box plots of mean daily chlorophyll *a* values before and after highway construction. (REF BD = reference at Beaverdam Creek, REF BH = reference site at Bearhead Creek, IMP D = Edwards Creek downstream, IMP U = Edwards Creek upstream)

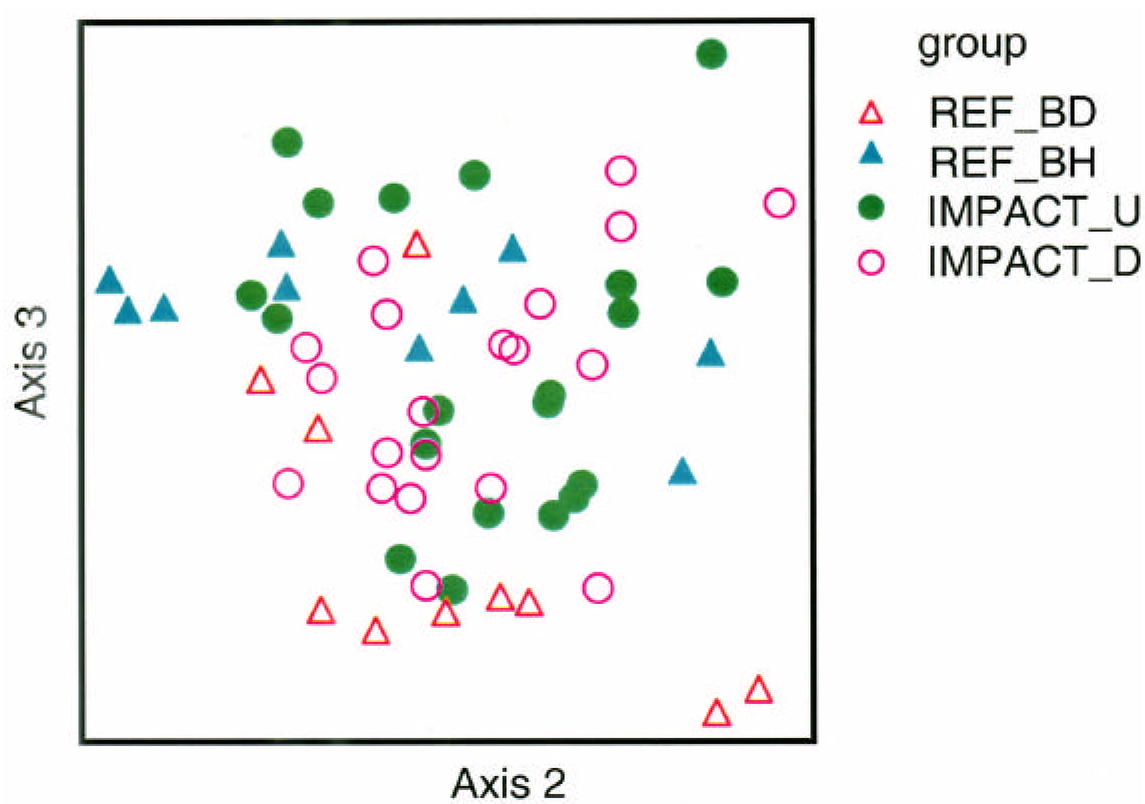

Figure 18. An ordination of stem counts of plant species before construction (1997) at the transect sties around Edwards Creek near the New River Estuary.
(REF_BD = reference site at Beaverdam Creek, REF_BH = reference site at Bearhead Creek, IMPACT_D = Edwards Creek downstream, IMPACT_U = Edwards Creek upstream)

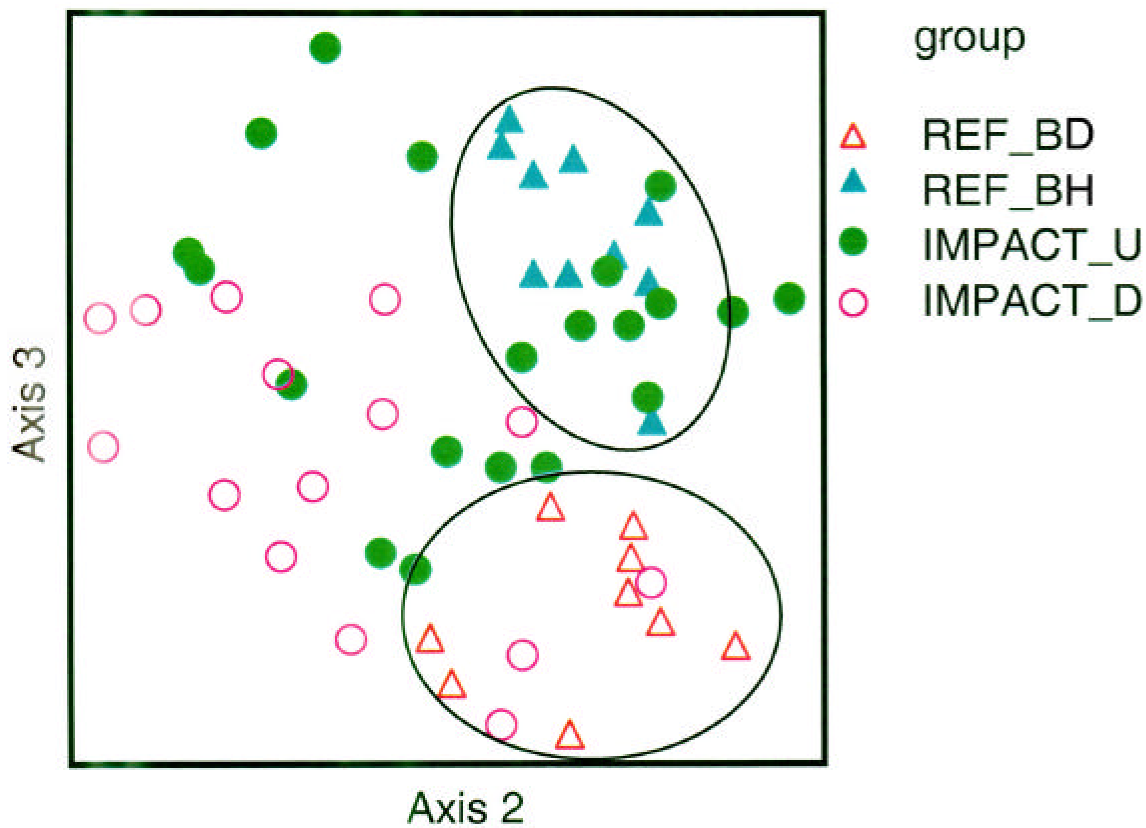

Figure 19. An ordination of stem counts of plant species after the highway construction on Edwards Creek near the New River Estuary.
(REF_BD = reference site at Beaverdam Creek, REF_BH = reference site at Bearhead Creek, IMPACT_D = Edward Creek downstream, IMPACT_U = Edward Creek upstream)

Also, synoptic surveys of submergent vegetation found that *Potomogeton pectinatus* was the sole vascular plant species present in the open water areas of Edwards Creek during 1997 and 1998. Beginning in the summer of 1999 after construction, floating mats of *Alternanthera philoxeroides* were observed in the areas upstream of the highway causeway and culvert at Edwards Creek. By late summer 2000 *Alternanthera philoxeroides* mats covered approximately 40% of the upstream open water areas of Edwards Creek. These floating mats were not observed in the downstream areas of Edwards Creek or at the reference wetlands at anytime during the study. The salinity intolerance of *Alternanthera philoxeroides* (USDA, NRCS 1999), and the absence of the taxa in downstream areas, suggests the causeway and culverts found in the highway corridor have reduced salinity in the upstream areas of Edwards Creek compared to the downstream area (Figures 6, 7), which may account for the change in aquatic vegetation upstream of the new highway.

E. Macroinvertebrates

A total of 120 macroinvertebrate taxa were identified during 1997-1999 (Appendix B). Densities were variable over time, but typically ranged between 5,000-35,000 individuals/m^2, illustrating the significant contribution of macroinvertebrates to secondary productivity of the study wetlands.

The Estuarine Biotic Index (EBI) suggested that macroinvertebrate assemblage composition did not shift toward species that were more pollution tolerant in response to highway construction in either the benthic or macrophyte habitats (Figures 20 and 21). Eaton (2001) defined a score of < 1.9 as an indication of severe water-quality impairment—all observations fell below this threshold. Variability in the index at the control sites prior to construction was greater than changes at the impact sites after construction, yielding an insignificant before-after/control-impact interaction term in RMANOVA models (P>0.05). EBI scores were all relatively low, regardless of construction. Thus, results from the index suggest that water-quality problems existed at all the sites prior to the initiation of our study. This is not to say that macroinvertebrates were insensitive to any potential disturbances presented by the highway, but simply that most taxa at the sites were already indicative of pollution problems before construction began.

Diversity of macroinvertebrates in the benthos, expressed as the total number of macroinvertebrate taxa, was not significantly affected by the highway crossing (Figure 22). Little change in diversity occurred between 1998 and 1999 at control and impacted sites. Changes in diversity were more apparent between the 2 years prior to construction, with a sharp decrease in number of taxa at the impacted sites due to greater salinity in 1998. However, number of taxa collected in the macrophyte/edge habitat did significantly change in response to the highway construction activity (Figure 23). A significant (P=0.0327) before-after/ impact-control interaction term showed that diversity at transects upstream of the highway crossing increased relative to patterns at downstream and control transects. This increase in diversity may have been due to a combination of factors directly related to obstruction of flow upstream of the temporary causeway. We documented lower salinities after construction (Table 1, Figure 7) and some minor ponding in this upstream area, which were accompanied by an expansion of alligatorweed (*Alternanthera philoxeroides*), a salt-intolerant, floating, creeping macrophyte. Mats of alligatorweed provided a new habitat for macroinvertebrates, and allowed many taxa to live at or near the water surface where freshwater overlaid heavier, saltier water from the estuary. Increased habitat complexity has been shown to yield increased diversity in many aquatic systems (*e.g.*, Brown *et al.* 1988, O'Connor 1991). In our previous highway study, increased macrophyte habitat resulting from highway crossings in forested wetlands of the coastal plain also increased diversity immediately adjacent to the crossings relative to control areas (King *et al.* 2000).

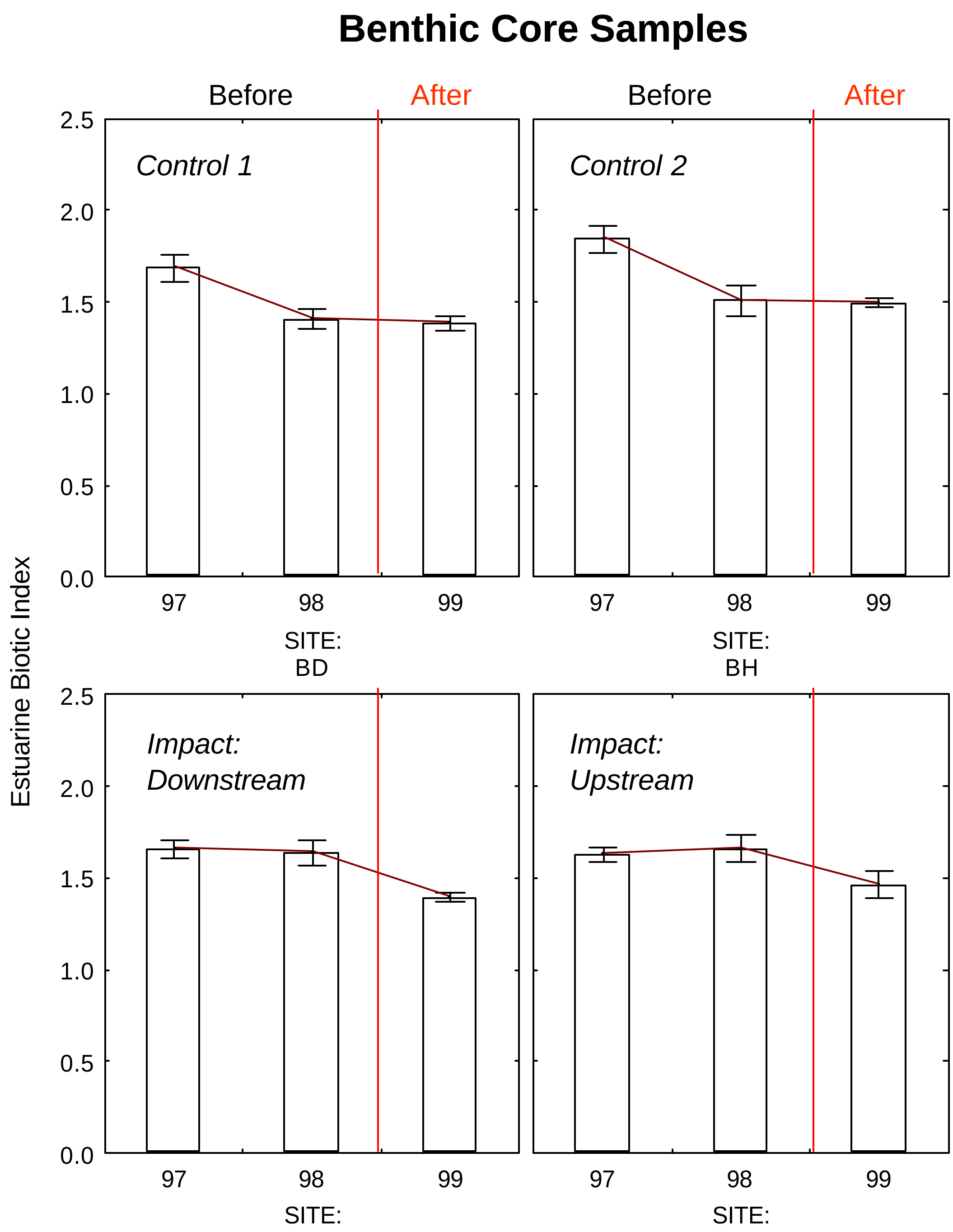

Figure 20. Mean (± 1 SE) Estuarine Biotic Index (EBI) values at control (BD and BH) and impact (D and U) sites, before (1997 and 1998) and after (1999) highway construction.

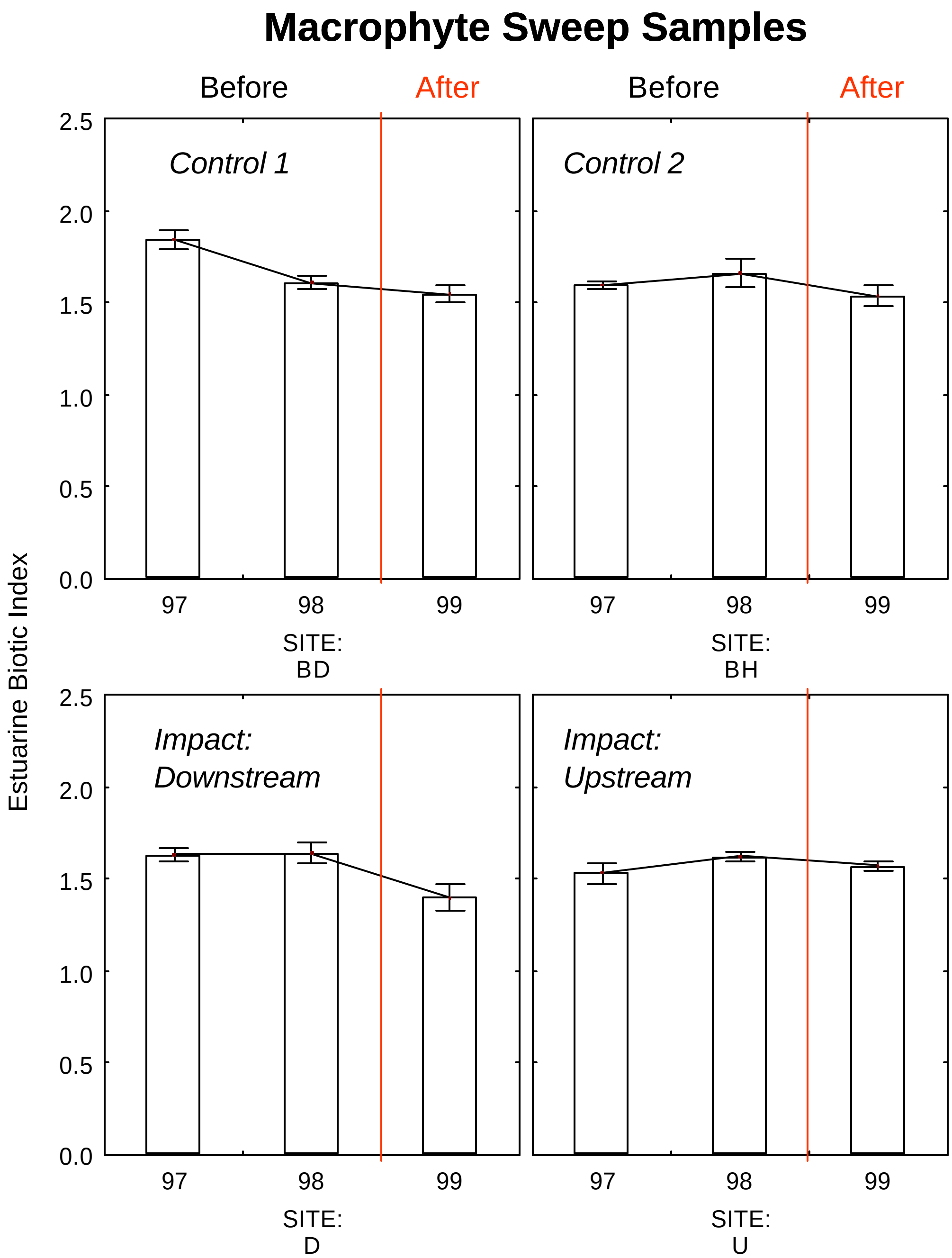

Figure 21. Mean (± 1 SE) Estuarine Biotic Index (EBI) values at control (BD and BH) and impact (D and U) sites, before (1997 and 1998) and after (1999) highway construction.

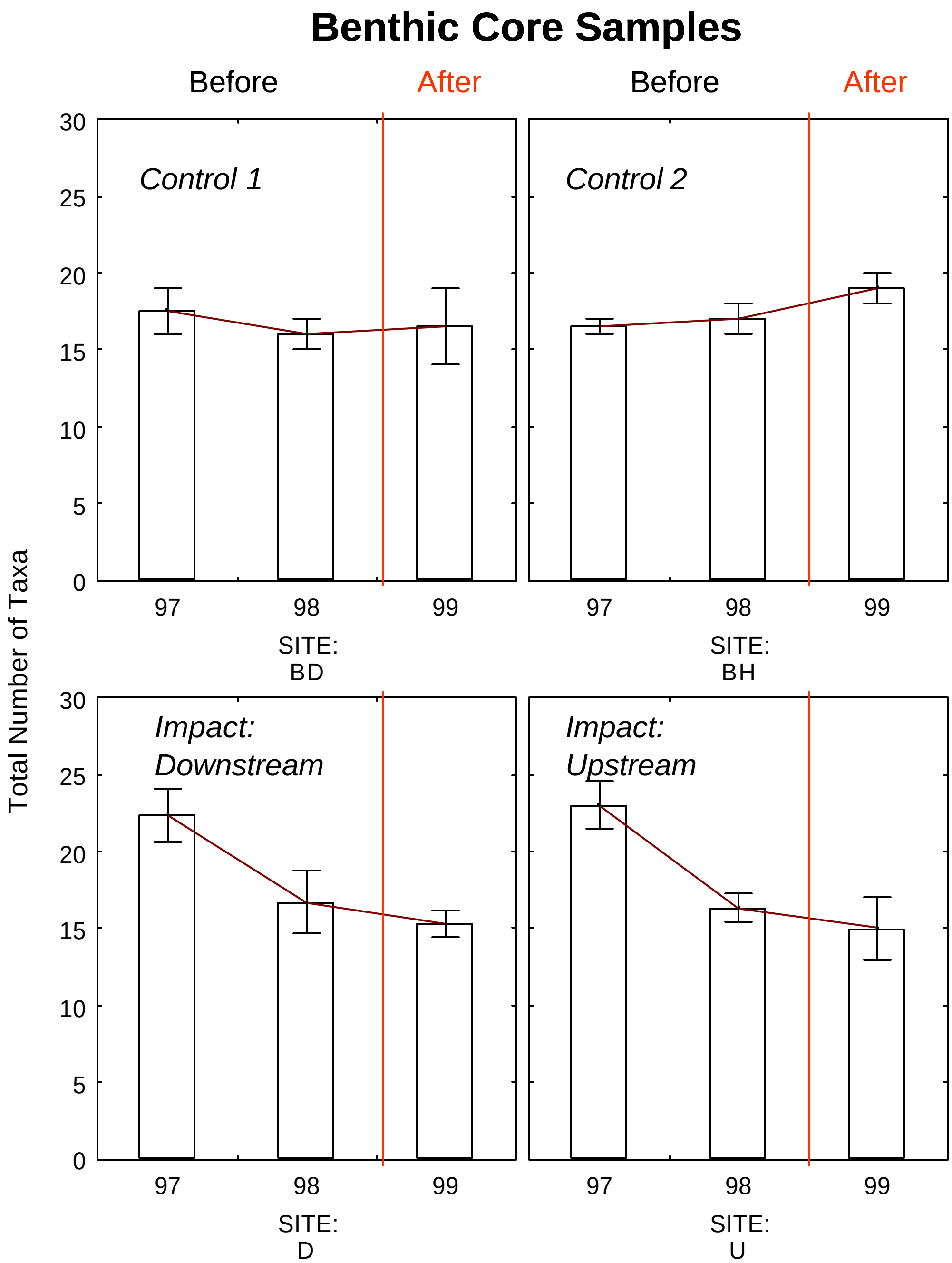

Figure 22. Mean (± 1 SE) number of macroinvertebrate taxa at control (BD and BH) and impact (D and U) sites, before (1997 and 1998) and after (1999) highway construction.

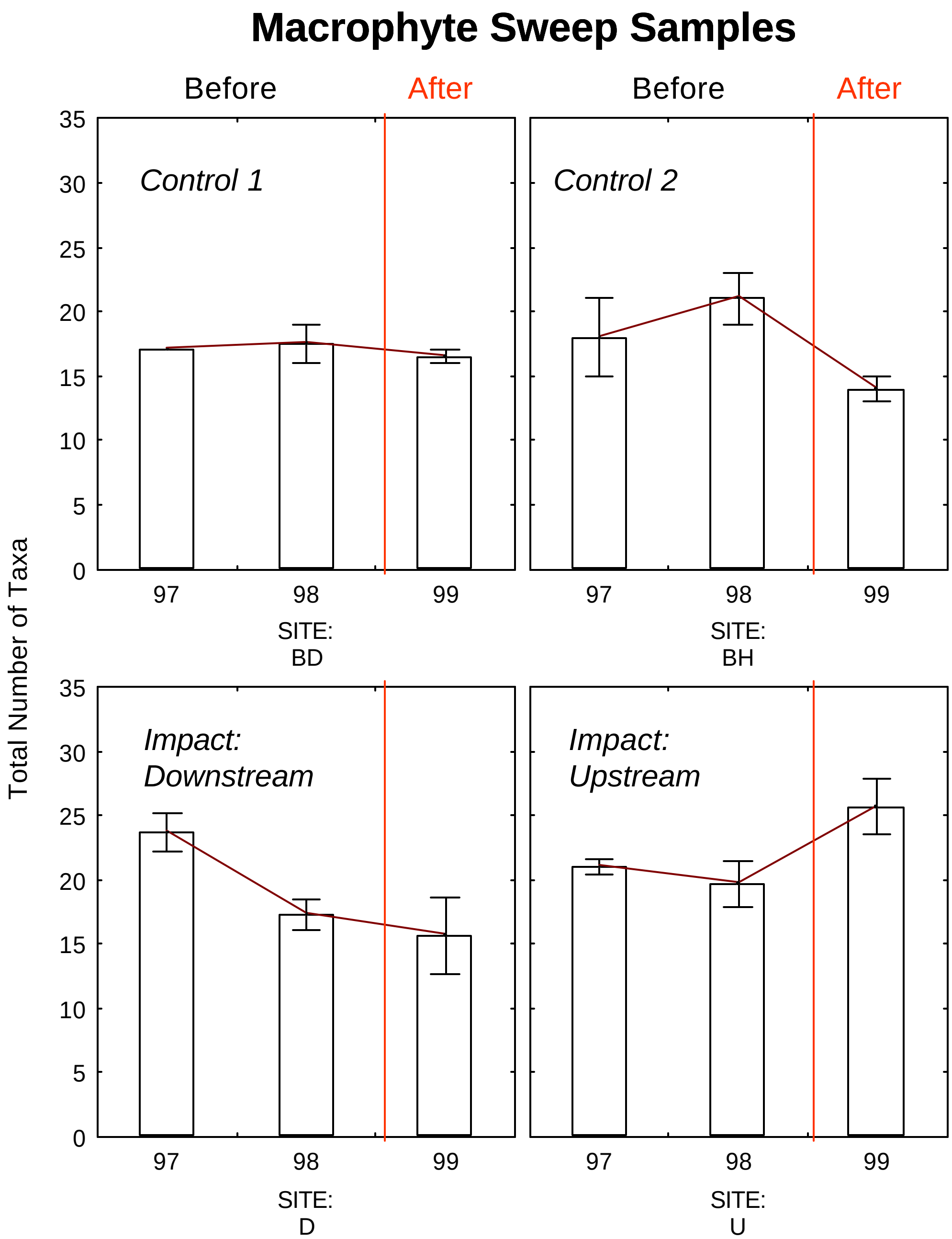

Figure 23. Mean (± 1 SE) number of macroinvertebrate taxa at control (BD and BH) and impact (D and U) sites, before (1997 and 1998) and after (1999) highway construction.

To further evaluate the hypothesis that a reduction of saline water upstream of the highway was responsible for the highway effect, we tested whether or not the percentage of salinity-indicator taxa in the benthos decreased significantly relative to downstream and control locations. Although a before-after/control-impact interaction term was not significant, the percentage of salinity indicators at the upstream area diverged from the trends at the downstream and control sites (Figure 24). Means increased between 1998 (pre-construction) and 1999 (post construction) at all downstream and control sites, but decreased in the upstream area.

Response of Gastropoda (aquatic snails) in the macrophyte habitat also indicated a significant highway effect upstream of the causeway (P(0.0001). The percentage of gastropods dramatically increased upstream after construction while showing little or no change at the downstream and control locations (Figure 25). The gastropods that increased the most were freshwater species (*Physella* spp., *Planorbella* sp), implying that decreased salinity played a role. Gastropods are also grazers of periphyton (algae and microbes) and are often excellent indicators of organic pollution, particularly the genus Physella (North Carolina Division of Water Quality 1997b). Thus, in addition to the decrease of salinity and increase in macrophyte habitat, the obstruction of flow by the causeway may have also diminished the flushing of nutrients from the watershed, which may have subsequently increased the productivity and nutrient content of periphyton in this area.

Macroinvertebrate species composition, expressed as Bray-Curtis dissimilarity, varied significantly over time but also was affected by the highway construction (Figures 26 and 27). First, between 1997 and 1998 (pre-construction), species composition at all transects succeeded from assemblages indicative of freshwater to ones indicative of brackish conditions. Although this successional process suggested that composition was highly variable over time, transects from both pre-impact and control sites followed nearly identical trajectories. This validated that our control sites were indeed “controls” by demonstrating that temporal variation in the impacted and control sites was controlled by the same organizational factors (*e.g.*, temporal changes in salinity, precipitation, *etc*.) because they behaved similarly over time. In particular, this pre-construction variation was likely due to annual differences in salinity. The summer of 1996 was one of the wettest in recent history in coastal North Carolina, with 2 major hurricanes (Bertha and Fran) and numerous other storms. Freshwater runoff from these hurricanes pushed back the salinity “wedge” that typically infiltrates estuaries during the summer months due to low flow and increased evapotranspiration. Low salinity, particularly at the impact site (which was on the edge of tidal freshwater and oligohaline during the winter/spring months), allowed a relatively diverse array of freshwater wetland species to colonize the site. Insects, in particular, are sensitive to salinity and few can tolerate salinities above 2-5 ppt for extended periods (Williams and Williams 1998). Consequently, benthic aquatic insects such as chironomids (midges) were more diverse in spring of 1997 than 1998. Contrary to precipitation patterns the previous year, summer and fall of 1997 were exceptionally dry. Low freshwater flows in the New River estuary in months prior to the 1998 collection resulted in high salinities and a reduction in salt-intolerant benthic macroinvertebrates.

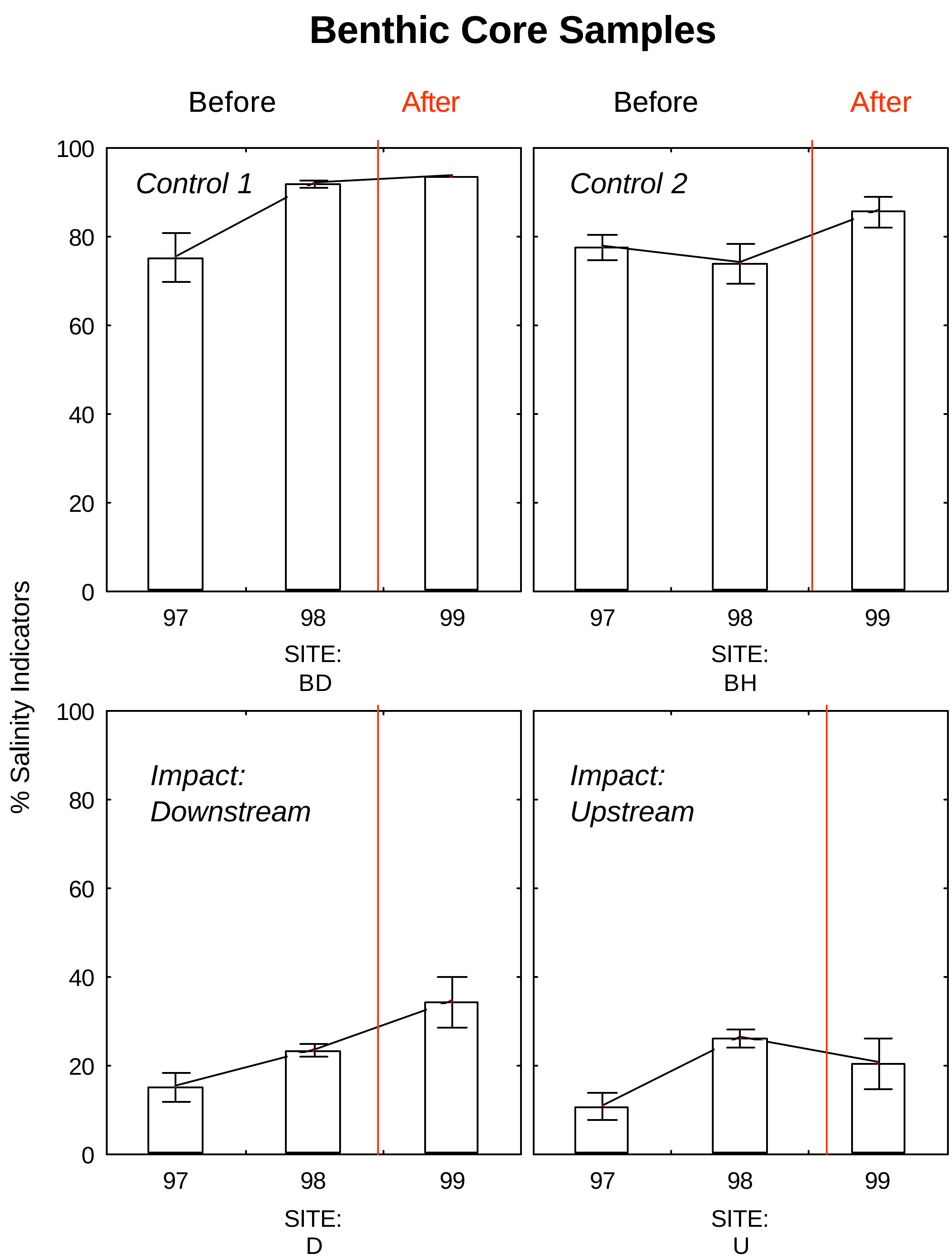

Figure 24. Mean (± 1 SE) % salinity indicator individuals at control (BD and BH) and impact (D and U) sites, before (1997 and 1998) and after (1999) highway construction.

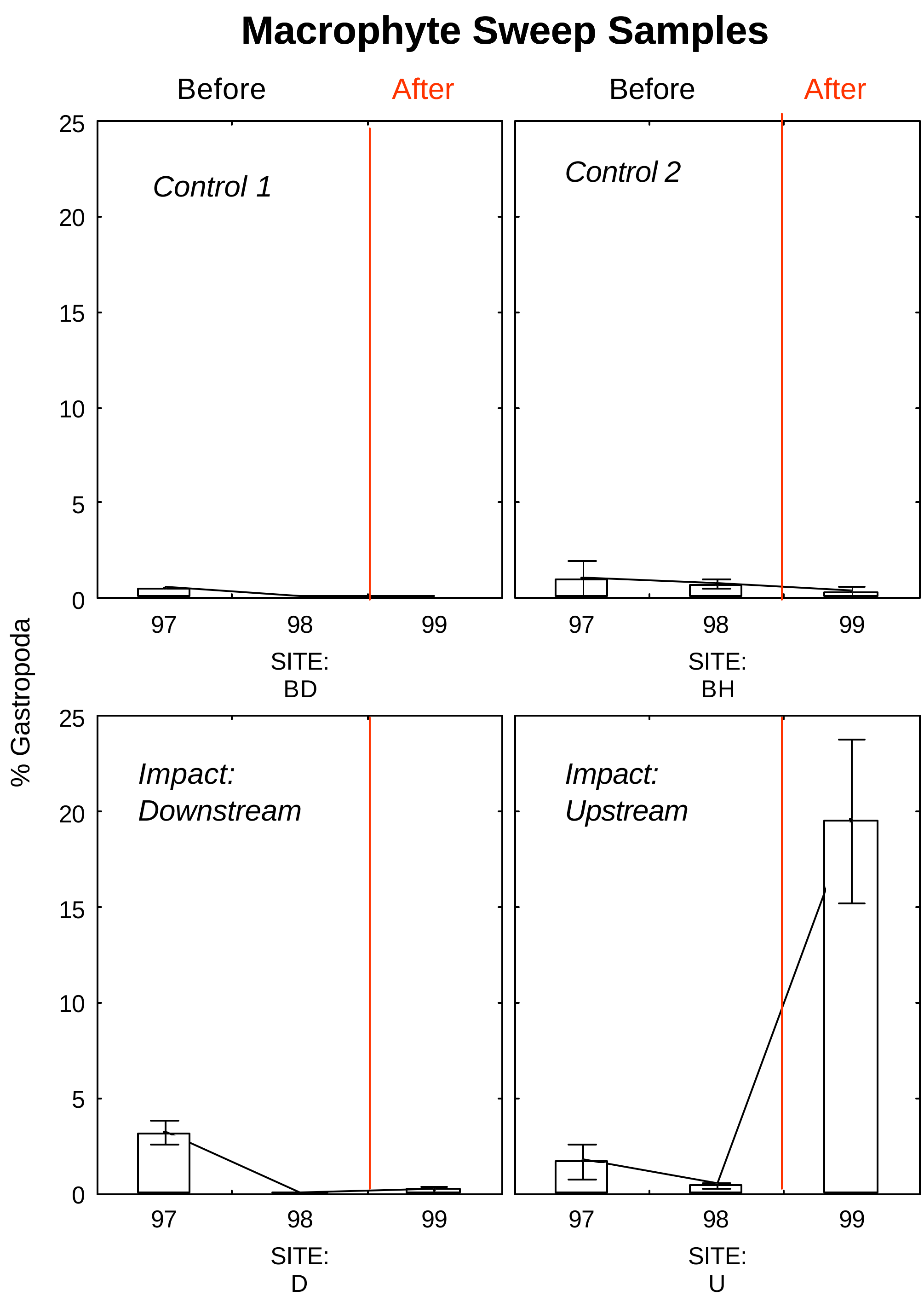

Figure 25. Mean (± 1 SE) % Gastropoda (aquatic snails) at control (BD and BH) and impact (D and U) sites, before (1997 and 1998) and after (1999) highway construction.

Despite the highly dynamic patterns of composition over time, species composition at the impacted sites clearly diverged from patterns at the control sites after construction began (Figures 26 and 27). Most notable was the divergence between upstream and downstream locations at the impacted site. Differences between upstream and downstream areas were minimal prior to construction during both 1997 and 1998. However, differences between assemblages after construction were significantly greater (95% CI) than before construction, particularly in the macrophyte habitat (Figure 27). Interestingly, the downstream assemblage became significantly more similar to the control assemblages after highway construction (Figure 27)—it is difficult to interpret this as an “impact” but it clearly indicated a change over time due to the highway. Finally, differences between control-site assemblages (Beaverdam and Bearhead) did not change over time. Thus, the divergence of assemblages upstream and downstream of the highway was consistent with the pattern of decreased salinity upstream (Figure 7), and a shift toward an assemblage characteristic of fresh rather than brackish water.

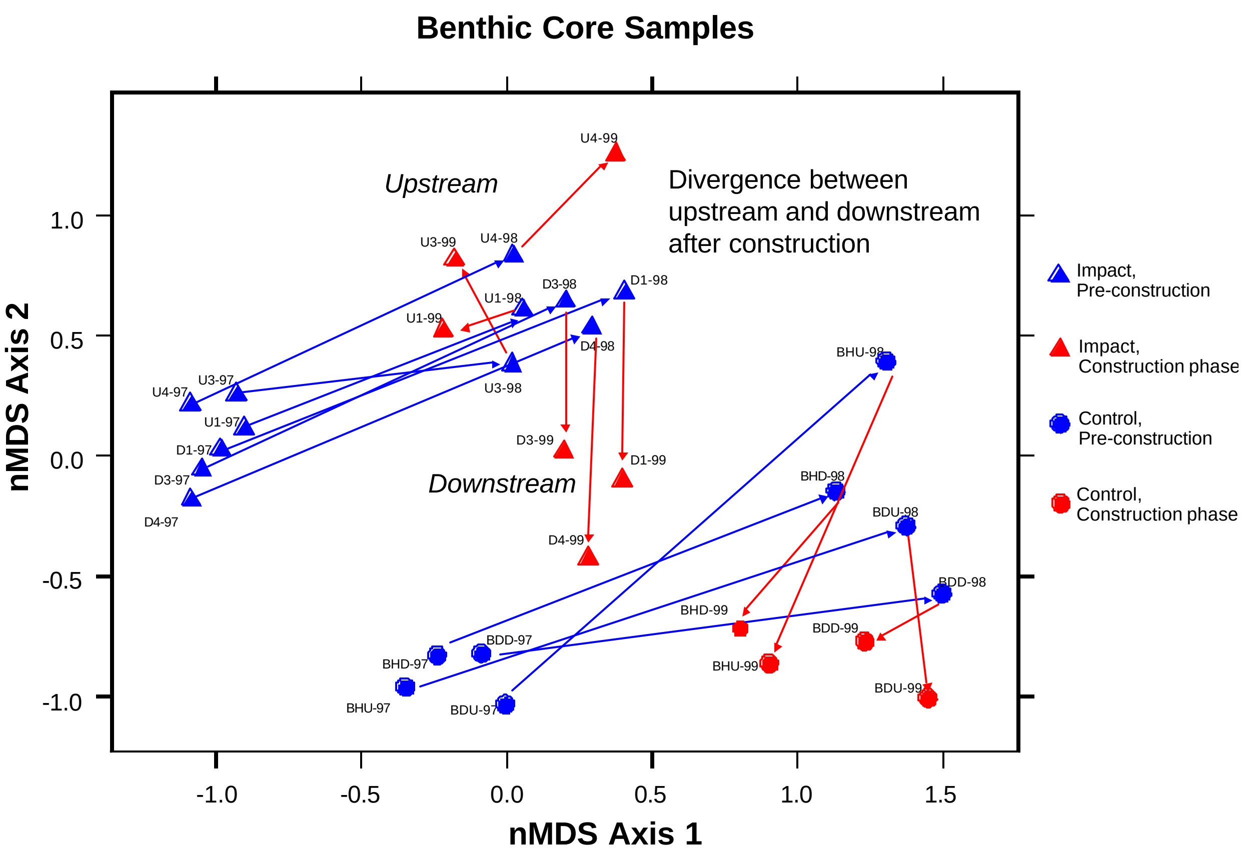

Figure 26. Nonmetric multidimensional scaling (nMDS) ordination of macroinvertebrate species composition from benthic habitats at impacted (upstream and downstream) and control sites, before (1997 and 1998) and after (1999) highway construction. Arrows indicate the trajectories of assemblage composition at each transect through time. Distances among points in the 2-dimensional space are proportional to their differences in species composition (Bray-Curtis dissimilarity). Stress =0.153. Codes for transects: U=upstream, impacted; D=downstream, impacted; BH=Bearhead, control; BD=Beaverdam, control; 97=1997; 98=1998; 99=1999

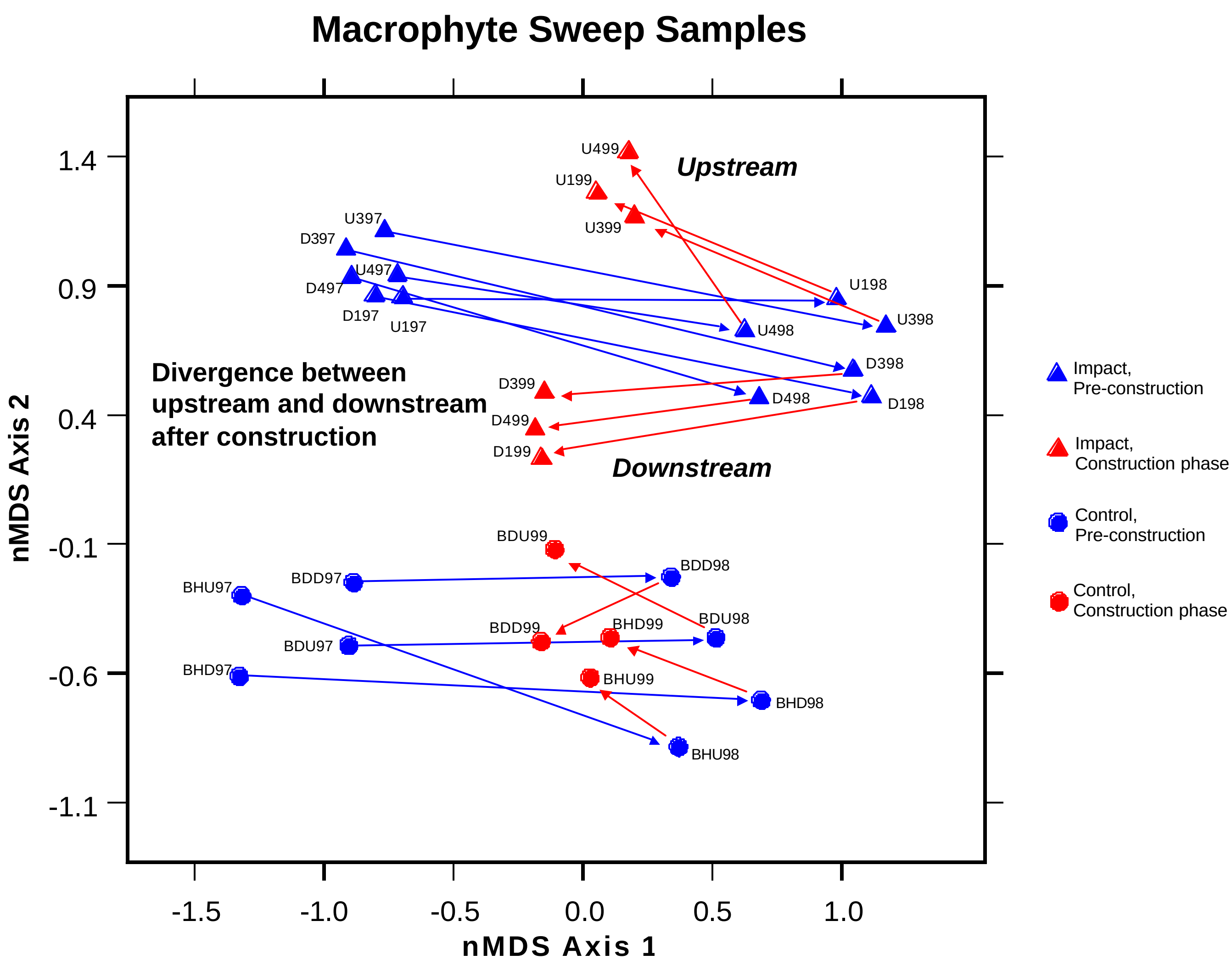

Figure 27. Nonmetric multidimensional scaling (nMDS) ordination of macroinvertebrate species composition from macrophyte habitats at impacted (upstream and downstream) and control sites, before (1997 and 1998) and after (1999) highway construction. Arrows indicate the trajectories of assemblage composition at each transect through time. Distances among points in the 2-dimensional space are proportional to their differences in species composition (Bray-Curtis dissimilarity). Stress = 0.134.
Codes for transects: U = upstream, impacted; D = downstream impacted; BH = Bearhead, control; BD = Beaverdam, control; 97=1997; 98 = 1998; 99 = 1999.

F. Fish and Large Decapods

Approximately 40 species of fish and large decapod crustaceans were collected while sampling with fyke nets during 1997-2000 (Appendix B). The majority of these fishes were transient marine or estuarine fishes using the study wetlands as nursery habitat, a critical function of coastal wetland ecosystems (Brazner 1997). At least 9 of the species collected were considered commercially valuable (southern flounder, spotted seatrout, spot, Atlantic croaker, menhaden, bay anchovy, blue crab, white shrimp, brown shrimp) and several others were important recreational sport fish (juvenile tarpon, ladyfish, jack, white catfish, pumpkinseed, warmouth, bluegill).

Abundances of individual fish and crustacean species were highly variable over time. Although abundance of several species appeared to be affected by the highway construction, none exhibited patterns that were consistent enough to be detected statistically. However, on an assemblage level (considering all species simultaneously, as a community), species composition at the impacted sites diverged from the patterns observed at the control sites after construction (Figure 28). In 1997, before construction, assemblages at the impacted and control sites were relatively similar. Upstream/downstream assemblages were somewhat different prior to construction, but the magnitude of this difference increased significantly (95% CI) once construction began in 1999. Here, assemblages upstream of the causeway diverged markedly from the downstream area, as well as the control sites. The downstream assemblage also changed relative to patterns at the control sites, but to a lesser degree.

The upstream/downstream effect appeared to be related to changes in salinity and an overall loss of connectivity between the upstream and downstream areas due to the highway crossing. Small culverts were all that allowed fish to move upstream past the temporary fill crossing, and this appeared to affect passage of some estuarine fishes. In particular, fish and crustaceans characteristic of freshwater lakes and wetlands (golden shiner, eastern mud minnow, pumpkinseed, warmouth, white catfish, crayfish) became more abundant upstream after construction while not increasing in abundance downstream or at controls (Figure 28), further indicating a separation between areas within the wetland. Thus, these results suggest that the highway crossing had a significant effect on the fish and crustacean assemblages of Edwards Creek due to the effects of highway construction on water movement from upstream to downstream areas. The long-term effects of this are unknown but may be reduced due to the removal of the temporary fill crossing.

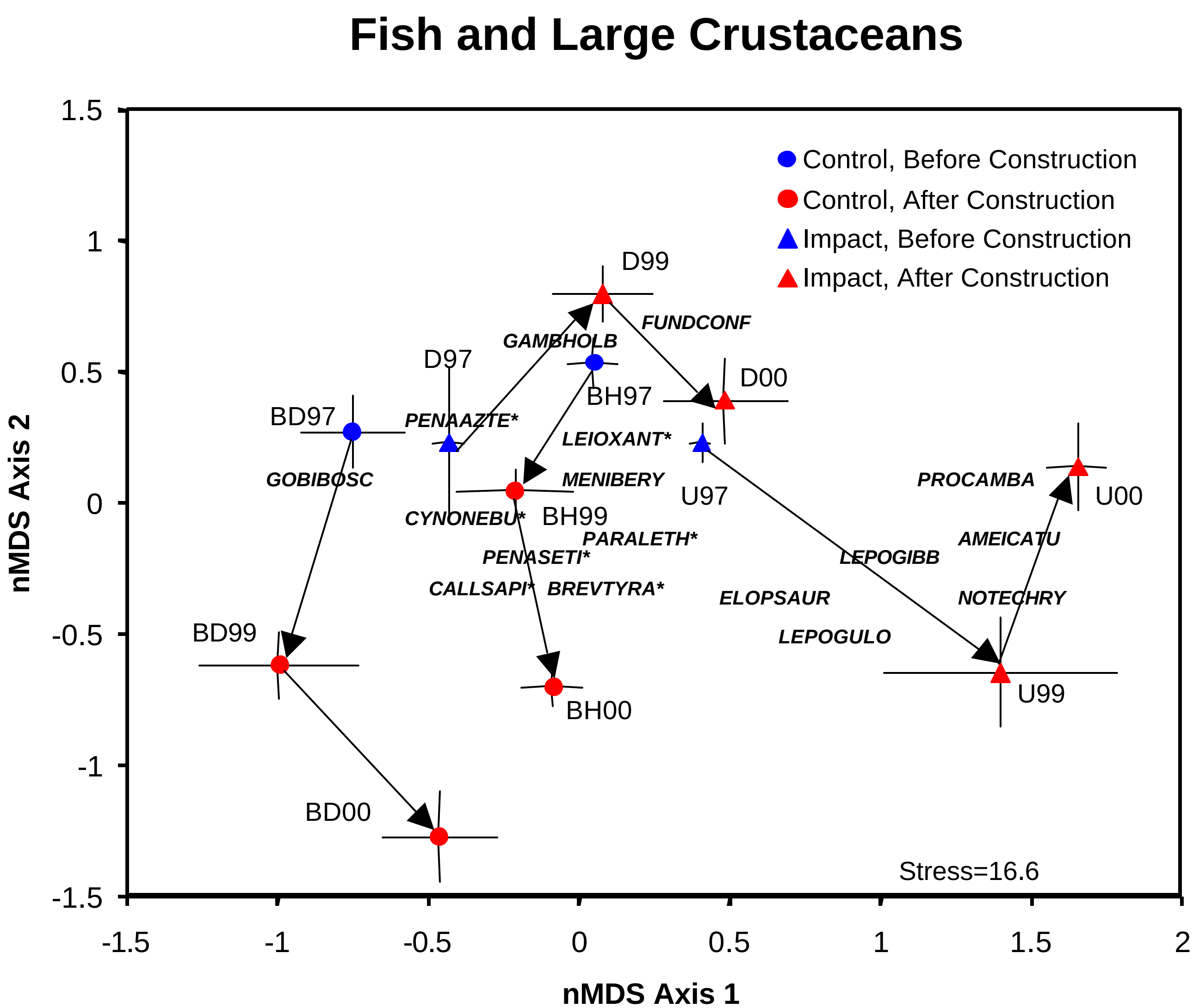

Figure 28. Nonmetric multidimensional scaling (nMDS) ordination of fish and large crustacean species composition at impacted (upstream and downstream) and control sites, before (1997) and after (1999 and 2000) highway construction. Error bars ± 1 SE among replicates at each location. Stress =0.134. Codes for transects: U=upstream, impacted; D=downstream, impacted; BH=Bearhead, control; BD=Beaverdam, control; 97=1997; 99=1999, 00=2000. See Appendix 2 for species codes (species codes indicate the centroids of species in the ordination). *Commercially valuable species.

G. Model Analysis

The mantel ecosystem response surface model represents a comprehensive summary of the degree of dissimilarity between the impact and reference sites before and after construction (Figure 29). The Mantel statistic is a non-parametric describing the correlation between two matrices (Mantel 1967, Legendre and Fortin 1989). One matrix represents group membership of sampling points and the other matrix represents the similarity of sampling points in a given time period for a given parameter. A randomized permutation routine is used to evaluate the statistical significance of this correlation. The test looks for relations between site similarity and group membership. Sites were placed into two groups: reference (Bearhead Creek, and Beaverdam Creek) and impact (Edwards Creek). The null hypothesis is that group membership is not predictive of the similarity of two sites. The test was performed before and after the onset of road construction. Impacts are indicated when there is not a significant Mantel statistic prior to construction, but after construction a significant Mantel statistic is observed.

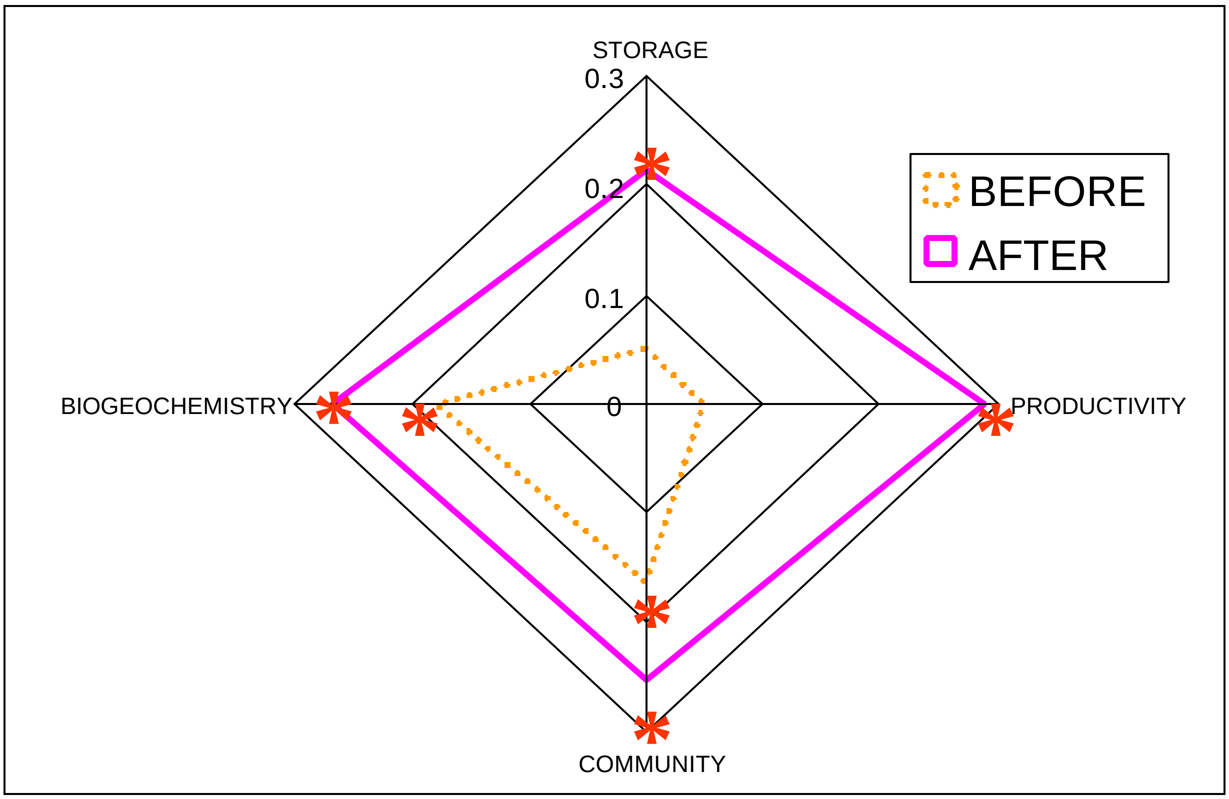

Figure 29. A mantel correlogram showing the similarity of stations on impact and reference sites both before and after construction. A small and/or insignificant mantel statistics suggest impact and reference sites are indistinguishable.

***- Indicates Mantel statistic is significant (p<0.050).**

Mantel statistics plotted as a polar diagram produced a Mantel correlogram where each axis represents a specific functional indicator (Figure 29). Here, two sets of Mantel statistics are plotted and connected with lines. These sets represent data collected before construction and after construction. Four axes on this figure represent four indicators of ecosystem function: (1) plant productivity (biomass), (2) biogeochemical cycling (total phosphorus), (3) community structure (plant community composition), and (4) sediment storage (sediment accretion). The most significant changes in function are shown for productivity and storage, where no significant relationships were seen prior to construction. This suggests that the highway construction activity resulted in a significant change in both community structure and the amount of sediment released.

The mantel correlogram also suggests a divergence in ecosystem community structure and water biogeochemistry after the highway construction was begun. The amount of time that this effect remains in place is unknown, since monitoring was discontinued after construction due to limited funding.

IV. CONCLUSIONS AND RECOMMENDATIONS

A major challenge in environmental monitoring is differentiating of true impacts from changes due to natural variation or cycles in ecosystem function. In our study the use of the BACI sampling design has allowed for discrimination of construction impact from natural variation. Impacts have been detected in salinity, sediment accretion, D.O., phosphorus concentration, macrophyte community composition, algal productivity as well as macroinvertebrates and fish. These changes are likely the result of construction of the highway bypass of Jacksonville, NC. It is impossible to say whether these impacts will prove to be short-term or persist beyond the completion of the highway since data collection after construction was discontinued due to a lack of funding. It appears the impacts resulting from construction phase increased rates of runoff from the watershed due to road clearing, impeded fluxes of water from floods and importantly tides due to the presence of temporary culverts at the site. Changes in soil surface elevation due to sediment displacement during road fill placement, and increased sediment flux from road fill and clearing also occurred. These impacts should be temporary, and the system may return to its normal state after several growing seasons, provided sediment and nutrient changes do not remain altered. Of concern, however, is the impact of reduced salinity on the long-term biota of Wilson Creek. Unfortunately, the study has not been continued so it is impossible at this stage to assess the recovery of the site and determine if the biota have returned to conditions near the reference conditions. Fortunately, the design of the study will allow for a follow up study to assess recovery.

Importantly, our results clearly demonstrate that biological indicators like macrophytes, macroinvertebrates, and fish communities should be an integral component of a highway impact assessment program. Biota are excellent integrators of a variety of potential stressors imposed upon wetland systems by highway construction. Results from this study and our previous study (King *et al.* 2000) have shown that wetland biota are sensitive to disturbances associated with construction and operation of highways, and are better indicators of environmental impacts than conventional water chemistry or habitat surveys (*e.g.*, HGM). Although most attributes of biotic assemblages are not direct measures of wetland ecosystem processes per se, changes in biotic assemblages in response to human activities are indicative of both structural and functional changes in a wetland, and thus are linked to wetland ecosystem processes (Richardson 1994). Moreover, §101(a) of the Clean Water Act mandates that the restoration and maintenance of biological integrity of the USA's streams, lakes, and wetlands, an unduly neglected aspect of wetland assessment (Karr and Chu 1997, Kusler and Niering 1998). Thus, biotic attributes are indeed functional indicators, and should be included in a functional assessment system for wetlands. Importantly, our BACI approach allowed for a clear test of the effects of the highway construction on biotic response and we were also able to eliminate the affect of environmental variation by the use of reference systems as well as before and after data collection comparisons.

One potential criticism of bioassessment is that it is laborious relative to rapid procedures like HGM. While our assessments were relatively intensive, use of the USEPA's Rapid Bioasessment Protocol for macroinvertebrates produced results that were equally, if not more informative than the laborious quantitative coring technique used to sample benthic macroinvertebrates. It is our recommendation that this rapid assessment procedure be considered over more quantitative sampling approaches, possibly using a composite sample from all available habitats as commonly done in many state biomonitoring programs (*e.g.*, FDEP 1996, Maxted *et al.* 2000). Since most of the useful information lies within species composition rather than in density estimates, rapid approaches like RBP are cost-effective techniques for generating species lists and semi-quantitative abundance estimates that serve well in assigning an impact rating to a site.

Highway construction in environmentally dynamic habitats like coastal wetlands may pose the most significant threat to biota through the loss of connectivity between areas upstream and downstream of highway crossings. While we do not have long-term post-construction data to evaluate recovery of the impacted site, short-term disturbance from construction caused significant alteration to species composition of both macroinvertebrates and fish as well as macrophytes and water chemistry. This is particularly important considering that water quality at all sites was considered poor prior to construction, as indicated by water-chemistry monitoring and the Estuarine Biotic Index. Thus, it should not be assumed that impaired sites like Edwards Creek are not susceptible to further impact, as our results have demonstrated that they can be. Our data suggest that the culverts installed in the extension pads and the temporary causeway were insufficient for allowing adequate flushing of tidal water upstream of the crossing. Our recommendation is that greater attention be directed toward minimizing the obstruction of tidal creeks (*i.e.* changes in salinity) during the construction phase, which may help reduce short-term impacts to the biota and associated ecosystem processes of coastal wetlands.

Finally, post-construction phase data are needed to assess long-term impacts at this site and future studies at this site should utilize the existing reference sites and BACI comparison approach.

Appendix A. Site photos.

Edwards Creek Site Before Construction

Edwards Creek Site During Clearing

Edwards Creek Site During Construction

Reference Site at Beaverdam Creek

Reading from Water Level Recorder

Water Sampling for Chemical Analyses

Appendix B. List of invertebrate taxa collected from benthic cores and macrophyte sweeps during 1997-1999.

GROUP	FAMILY	TAXON	CODE	1997	1998	1999	Habitat	Trophic	Feeding	ESV
Amphipoda	Corophiidae	*Corophium lacustre* Vanhoffen	COROPHIU		R	R	B, M	D	C	2.00
Amphipoda	Gammaridae	*Gammarus tigrinus/daiberi*	GAMMARUS	A	A	A	M, B	D	C	2.50
Amphipoda	Talitridae	*Uhlorchestia uhleri* (Shoemaker)	ORCHESTI	C	C	C	M	D	C	2.00
Cladocera	Chydoridae	Chydoridae	CHYDORID		R	R	B, M	H	F, G	
Cladocera	Daphnidae	*Ceriodaphnia* sp.	CERIODAP	A	A	A	B, M	H	F	
Cnidaria	Hydridae	*Hydra* sp.	HYDRA			R	B	?	?	
Coleoptera	Carabidae	Carabidae	CARABIDA	R			M	P	Eng	
Coleoptera	Dytiscidae	*Agabus* sp.	AGABUS		R		M	P	Prc	1.35
Coleoptera	Dytiscidae	*Ilybius* sp.	ILYBIUS	R	C	C	M, B	P	Prc	
Coleoptera	Dytiscidae	*Neoporus* cf. *carolinus* (Fall)	NEOPORUS	C	A	C	B, M	P	Prc	1.48
Coleoptera	Haliplidae	*Haliplus* sp.	HALIPLUS	R	R	R	M	H	Sh, Prc	1.45
Coleoptera	Haliplidae	*Peltodytes* sp.	PELTODYT	C	C	C	M, B	H, P	Sh, Prc	1.44
Coleoptera	Hydrophilidae	*Berosus* sp.	BEROSUS			R	M, B	H, P	Prc, C	1.55
Coleoptera	Hydrophilidae	*Tropisternus blatchleyi* d' Orchymont	TROPBLAT	R	R	R	M	H	Prc, C	1.11
Coleoptera	Lampyridae	Lampyridae	LAMPYRID	R		R	M	P	Eng	
Coleoptera	Noteridae	*Suphisellus* sp.	SUPHISEL	R			M	P	Eng	
Coleoptera	Staphylinidae	Staphylinidae	STAPHYLI	R			M	P	Eng	
Copepoda	Calanoida	Calanoida	CALANOID		C	C	B, M	H	F, C	
Copepoda	Cyclopoida	Cyclopoida	CYCLPOID	A	A	A	B, M	H, P	F, C	
Decapoda	Cambaridae	*Procambarus* sp	PROCAMBA			R	B, M	D, H	C	
Decapoda	Palaemonidae	*Palaemonetes pugio* Holthuis	PALAEPUG	C	C	C	M	D, H, P	C	2.50
Decapoda	Portunidae	*Callinectes sapidus* Rathbun	CALLSAPI		R	R	M	P, H, D	C	2.00
Diptera	Ceratopogonidae	*Bezzia/Palpomyia* (complex)	BEZZIA	C	C	C	B, M	P	Eng	2.30

Diptera	Ceratopogonidae	*Ceratopogon* sp.	CERATOPO			R	M, B	P	Eng	2.30
Diptera	Ceratopogonidae	*Culicoides* sp.	CULICOID			R	M	P	Eng	2.30
Diptera	Ceratopogonidae	*Dasyhelea* sp.	DASYHELE		R	R	M	D, H	C, G	2.80
Diptera	Chaoboridae	*Chaoborus punctipennis* (Say)	CHAOBPUN	R	R	R	B	P	Eng	1.40
Diptera	Chironomidae	*Chironomus* sp. 1	CHMUSSP1	A	A	A	B, M	D, H	C, Sh	1.00
Diptera	Chironomidae	*Chironomus stigmaterus* (Say)	CHIRSTIG	C	C	C	B	D, H	C, Sh	1.00
Diptera	Chironomidae	*Cladopelma* sp.	CLADOPEL	R		R	M, B	D	C	3.28
Diptera	Chironomidae	*Cladotanytarsus*	CLADOTAN			R	M	H, D	C	4.50
Diptera	Chironomidae	*Clinotanypus pinguis* (Loew)	CLINPING	R			B	P	Eng	1.30
Diptera	Chironomidae	*Cricotopus bicinctus* Meigen	CRICBICI	R		R	M	H	Sh, G	1.51
Diptera	Chironomidae	*Cricotopus sylvestris* (Fabricius) gr.	CRICSYLV	A	C	C	M, B	H	Sh, G	
Diptera	Chironomidae	*Cryptochironomus* sp.	CRYPTOCH	R	R	R	B	P	Eng	1.00
Diptera	Chironomidae	*Dicrotendipes modestus* (Say)	DICROMOD	A	C	C	M, B	D, H	C, F, Sh	2.80
Diptera	Chironomidae	*Einfeldia natchitochae* Sublette	EINNATCH	A			B, M	D	C	2.02
Diptera	Chironomidae	*Endochironomus nigricans* (Johannsen)	ENDONIGR		R		M	H	Sh	1.10
Diptera	Chironomidae	*Goeldichironomus devineyae* (Beck)	GOELDDEV		A	R	M, B	D	C	
Diptera	Chironomidae	*Goeldichironomus holoprasinus* (Goeldi)	GOELDHOL		R	C	M, B	D	C	
Diptera	Chironomidae	*Hydrobaenus* sp.	HYDROBAE		R		M	H, D	G, C	1.16
Diptera	Chironomidae	*Kiefferulus dux* (Johannsen)	KIEFFDUX	R	R	C	M, B	D	C	
Diptera	Chironomidae	*Larsia decolorata* (Malloch)	LARSDECO	C	R	R	B, M	P	Eng	1.24
Diptera	Chironomidae	*Limnophyes* sp.	LIMNOPHY	A		R	M	D	C	
Diptera	Chironomidae	*Nanocladius crassicornus/rectinervis* gr.	NANOCLAD		R	R	B, M	D	C	2.02
Diptera	Chironomidae	*Parachironomus directus* (Dendy & Sublette)	PARACSP1	R			B	P, D	Eng, C	1.10
Diptera	Chironomidae	*Parachironomus* sp. 2	PARACSP2	R		R	B, M	P, D	Eng, C	1.10
Diptera	Chironomidae	*Parakiefferiella* sp.	PARAKIEF		R		M	D	C	4.10
Diptera	Chironomidae	*Paratanytarsus* sp. A Epler	PARASPA		R		M	D	C	1.54

Diptera	Chironomidae	*Polypedilum illinoense* gr.	POLYILLI	R	R	R	M	H, D	Sh, C	1.30
Diptera	Chironomidae	*Polypedilum trigonus* Townes	POLYTRIG			R	M	H, D	Sh, C	1.30
Diptera	Chironomidae	*Procladius* sp.	PROCLAD	R	R	R	B, M	P, D	Eng, C	1.31
Diptera	Chironomidae	*Psectrocladius elatus* Roback	PSECTROC		R		M	D, H	C, Sh	3.24
Diptera	Chironomidae	*Rheotanytarsus* sp.	RHEOTANY		R		B	D, H	C, F	2.30
Diptera	Chironomidae	*Tanypus neopunctipennis* Sublette	TANYNEOP	A	C	C	B, M	P, H	P, C	1.00
Diptera	Chironomidae	*Tanytarsus limneticus* Sublette	TANYLIMN			R	B	D, H	C, F	2.50
Diptera	Chironomidae	*Tanytarsus* sp. 1 (NCDWQ)	TANYSP1	A	R	R	M, B	D, H	C, F	2.50
Diptera	Chironomidae	*Tanytarsus* sp. 10 (NCDWQ)	TANYSP10		R	R	B, M	D, H	C, F	2.80
Diptera	Chironomidae	*Tribelos jucundum* (Walker)	TRIBJUNC	R			B	D	C	2.29
Diptera	Ephydridae	Ephydridae	EPHYDRID			R	M	P	Eng	
Diptera	Muscidae	cf. *Limnophora* sp.	LIMNOPHO	R			M	P	Prc	
Diptera	Sciomyzidae	cf. *Sepedon* sp.	SEPEDON	R			M	P	Eng	
Diptera	Stratiomyiidae	*Odontomyia* sp.	ODONTOMY			R	M	D, H	C, F	1.70
Diptera	Stratiomyiidae	*Stratiomys* sp.	STRATIOM		R		M	D, H	C, F	1.67
Diptera	Tabanidae	*Chrysops* sp.	CHRYSOPS	C	R	R	M, B	P	Prc	2.14
Diptera	Tabanidae	*Tabanus* sp.	TABANUS	R			M	P	Prc	1.27
Diptera	Tipulidae	*Limonia*	LIMONIA			R	M	D	C	
Diptera	Tipulidae	*Tipula* sp.	TIPULA			R	M	H, D	Sh	
Ephemeroptera	Baetidae	*Callibaetis* sp.	CALLIBAE	R	R	R	B, M	H	C, G	1.10
Gastropoda	Hydrobiidae	Hydrobiidae	HYDROBII	C	R	R	M	H	G	
Gastropoda	Physidae	*Physella* sp. 1	PHYSSP1	C	R	A	M, B	H	G	1.30
Gastropoda	Physidae	*Physella* sp. 2	PHYSSP2		R	R	M, B	H	G	1.30
Gastropoda	Planorbidae	*Micromenetus dilatus* (Gould)	MICRDILA	R	R	R	B	H	G	1.62
Gastropoda	Planorbidae	*Planorbella* sp.	PLANBELL		R	C	B	H	G	2.11
Harpacticoidae	Harpacticoidae	Harpacticoida	HARPACTA			R	B	H,D	F,G	

Hemiptera	Belostomatidae	*Belostoma testaceum/lutarium*	BELOSTOM	R		R	M	P	Prc	1.07
Hemiptera	Corixidae	*Trichocorixa* sp.	TRICHCOR	C	R	C	M, B	P	Prc	1.35
Isopoda	Anthuridae	*Cyathura polita* Stimpson	CYATHURA	R	R	R	B	D	C	2.00
Isopoda	Asellidae	*Caecidotea* sp.	CAECIDOT	R	R	R	M	D	C	
Isopoda	Sphaeromidae	*Cassidinidea ovalis* (Say)	CASSOVAL	A	A	A	M, B	D	C	2.00
Lepidoptera	Pyralidae	*Acentria* sp.	ACENTRIA			R	M	H	Sh	
Nematoda	Mermithidae	Mermithidae sp.	MERMITHI	A	C	C	B	?	?	
Odonata	Aeshnidae	*Nasiaeschna pentacantha* (Rambur)	NASIPENT		R		M	P	Eng	1.65
Odonata	Coenagrionidae	*Enallagma* sp.	ENALLAGM	R	R	C	M, B	P	Eng	1.50
Odonata	Coenagrionidae	*Ischnura* sp.	ISCHNURA	C	C	C	M, B	P	Eng	1.17
Odonata	Lestidae	*Lestes inaequalis* Walsh	LESTINEQ		R		M	P	Eng	1.20
Odonata	Libellulidae	*Brachymesia gravida* (Calvert)	BRACGRAV	R		R	B, M	P	Eng	
Odonata	Libellulidae	*Erythemis simplicicollis* (Say)	ERYTHSIM	R		C	M	P	Eng	1.10
Odonata	Libellulidae	*Erythrodiplax berenice* (Drury)	ERYTHROD		R		M	P	Eng	
Odonata	Libellulidae	*Libellula needhami* Westfall	LIBENEED			R	B	P	Eng	1.10
Odonata	Libellulidae	*Libellula* sp.	LIBELLUL	R			M, B	P	Eng	1.13
Odonata	Libellulidae	*Pachydiplax longipennis* (Burmeister)	PACHLONG	C	C	C	M, B	P	Eng	1.05
Odonata	Libellulidae	*Perithemis* sp.	PERITHEM	C		R	B, M	P	Eng	1.00
Oligochaeta	Enchytraeidae	Enchytraeidae	ENCHYTRA	A	A	A	M, B	D	C	1.06
Oligochaeta	Naididae	*Chaetogaster diaphanus*(Gruithuisen)	CHAETDIA		R		B, M	P	Eng	
Oligochaeta	Naididae	*Dero* sp.	DEROSP		C	C	M, B	H, D	C	1.14
Oligochaeta	Naididae	*Nais communis/variabilis*	NAISCOMM	A	A	A	M, B	H, D	C	1.00
Oligochaeta	Naididae	*Paranais litoralis* (Muller)	PARANAIS	C	A	A	M, B	H, D	C	1.00
Oligochaeta	Naididae	*Pristina* sp.	PRISTINA	R	R	C	B, M	H, D	C	1.40

Oligochaeta	Tubificidae	*Ilyodrilus* sp.	ILYODRIL		R	R	B	D	C	1.00
Oligochaeta	Tubificidae	*Limnodrilus hoffmeisteri* Clarapede	LIMNHOFF	A	A	A	B, M	D	C	1.00
Oligochaeta	Tubificidae	Tubificidae imm.	TUBIFICI	A	A	A	B, M	D	C	1.00
Oligochaeta	Tubificidae	*Tubificoides* sp.	TUBICOID	R	C	C	B, M	D	C	1.00
Ostracoda	Cyprdopsidae	Cypridopsidae	CYPRIDOP			R	M	H, D	G	
Ostracoda	Cyprididae	Cyprididae	CYPRIDID	C	R	R	B	H, D	G	
Pelecypoda	Corbiculidae	*Rangia cuneata* (Conrad)	RANGCUNE	C	R	C	B	H, D	F	1.00
Pelecypoda	Mytilidae	*Mytilopsis leucophaeta* (Conrad)	MYTILEUC		R		M	H, D	F	1.00
Pelecypoda	Tellinidae	Tellinidae	TELLINID	R			B	H, D	F	
Polychaeta	Ampharetidae	*Hobsonia florida* (Hartman)	HOBSONIA	A	A	A	B, M	D	C	2.00
Polychaeta	Capitellidae	*Heteromastus filiformis* (Clarapede)	HETEFILI		A	A	B, M	?	?	1.00
Polychaeta	Nereidae	*Laeonereis culveri* (Webster)	LAEOCULV	R	A	A	B	D, P	C	1.00
Polychaeta	Nereidae	*Namalycastis abiuma* (Muller)	NAMALYCA	R	R	R	M	D, P	C	
Polychaeta	Nereidae	*Stenoninereis martini* Wesenberg-Lund	STENMART			C	B	D	C	
Polychaeta	Phyllodocidae	*Eteone heteropoda* Hartman	ETEOHETE		R	C	B	D	C	2.00
Polychaeta	Spionidae	*Polydora ligni*	POLYDORA	R	A	C	B, M	?	?	1.00
Polychaeta	Spionidae	*Streblospio benedicti* Webster	STREBENE			R	M	D, H	C	1.00
Trichoptera	Leptoceridae	*Oecetis* sp. A Floyd	OECETSPA	R			B, M	P, H	Eng, Sh	2.50
Triclidada	Planariidae	Planariidae	PLANARII			R	M	D, H	G	

Abbreviations:

Habitat: B=benthos, M=marsh/edge

Trophic: D=Detritivore, H=Herbivore, P=Predator

Feeding: C=Collector, G=Grazer, F=Filterer, Sh=Shredder, Eng=Engulfer, Prc=Piercer

Year: R=rare, C=common, A=abundant

ESV=Estuarine sensitivity value (1-5; 1=tolerant, 5=sensitive).

Appendix C. List of fish and large decapod crustacean species collected at impact and control sites during 1997-2000.

Code	Scientific name	Common name
AMEICATU	*Ameiurus catus*	white catfish
ANCHMITC	*Anchoa mitchilli*	bay anchovy*
ANGUROST	*Anguilla rostrata*	american eel
BAIRCHRY	*Bairdiella chrysoura*	silver perch
BREVTYRA	*Brevoortia tyrranus*	menhanden*
CALLSAPI	*Callinectes sapidus*	blue crab*
CARANXSP	*Caranx* sp.	jack
CYNONEBU	*Cynoscion nebulosus*	spotted seatrout*
CYPRVARI	*Cyprinodon variegatus*	sheepshead minnow
DIAPOLIS	*Diapterus olisthostomus*	irish pompano
DORMMACU	*Dormitator maculatus*	fat sleeper
ELEOPISO	*Eleotris pisonis*	spinycheek sleeper
ELOPSAUR	*Elops saurus*	ladyfish
ENNEGLOR	*Enneacanthus gloriosus*	bluespotted sunfish
ETHEFUSI	*Etheostoma fusiforme*	swamp darter
FUNDCONF	*Fundulus confluentus*	marsh killifish
FUNDHETE	*Fundulus heteroclitus*	mummichog
FUNDLUCI	*Fundulus luciae*	spotfin killifish
GAMBHOLB	*Gambusia holbrooki*	eastern mosquitofish
GOBIBOSC	*Gobiosoma bosci*	naked goby
LAGORHOM	*Lagodon rhomboides*	pinfish
LEIOXANT	*Leiostomus xanthurus*	spot*
LEPIOSSE	*Lepisosteus osseus*	longnose gar
LEPOGIBB	*Lepomis gibbosus*	pumpkinseed
LEPOGULO	*Lepomis gulosus*	warmouth
LEPOMACR	*Lepomis macrochirus*	bluegill
LUCAPARV	*Lucania parva*	rainwater killifish
MEGAATLA	*Megalops atlanticus*	tarpon
MENIBERY	*Menidia beryllina*	inland silverside
MICRUNDU	*Micropogonias undulatus*	atlantic croaker*
MUGICEPH	*Mugil cephalus*	striped mullet*
NOTECHRY	*Notemigonus chrysoloucas*	golden shiner
PALAPUGI	*Palaemonetes pugio*	grass shrimp
PARALETH	*Paralichthys lethostigma*	southern flounder*
PENAAZTE	*Penaeus aztecus*	brown shrimp*
PENASETI	*Penaeus setiferous*	white shrimp*
PROCAMBA	*Procambarus* sp.	crayfish
TRINMACU	*Trinectes maculatus*	hogchoker

UMBRPYGM *Umbra pygmaea* eastern mudminnow

*indicates commercially valuable species

CITED REFERENCES

Adamus, P.R. and L. T. Stockwell 1983. A method for wetland functional assessment. Volume II. U.S. Dept. of Transportation. Federal Highway Administration. Office of Research and Development. Report No. FHWA-IP-82-83. p.176.

Adamus, P.R. 1983. A method for wetland functional assessment. Volume I. U.S. Dept. of Transportation. Federal Highway Administration. Office of Research and Development. Report No. FHWA-IP-82-83. p.134.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26:32-46.

Barbour, M. T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish. U. S. Environmental Protection Agency, Office of Water. Washington, DC. EPA 841-0B-99-002.

Brazner, J. C. 1997. Regional, habitat, and human development influences on coastal wetland and beach fish assemblages in Green Bay, Lake Michigan. *Journal of Great Lakes Research* 23:36-51.

Brazner, J.C. and J.J. Magnuson. 1994. Patterns of fish species richness and abundance in coastal marshes and other nearshore habitats in Green Bay, Lake Michigan. Proceedings of the International Association for Theoretical and Applied Limnology 25:2098-2104.

Brinson, M.M., Rheinhardt, R, Hauer, R, Lee, L.C., Nutter, W.L., Smith, R.D. and D. Whigham. 1995. A Guidebook for Application of Hydrogeomorphic Assessments to Riverine Wetlands, Technical Report WRP-DE-11, U.S. Army Engineer Waterways Experimental Station, Vicksburg, MS.

Brinson, M.M., Rheinhardt, R. 1996. The role of reference wetlands in functional assessment and mitigation. *Ecological Applications* 6 (1):69-76.

Brown, C. L., T. P. Poe, J. R. French, and D. W. Schloesser. 1988. Relationships of phytomacrofauna to surface area in naturally occurring macrophyte stands. *J. N. Am. Benthol. Soc.* 7:129-139.

Clarke, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. *Austral. J. Ecol.* 18:117-143.

Committee on Characterization of Wetlands. 1995. *Wetlands: characteristics and boundaries*. National Academy Press, Washington, D.C. pp. 307.

Darnell, R.M., W.E. Pequegnat, B.M. James, F.J. Benson, and R.E. Defenbaugh. 1976. *Impacts of construction activities in wetlands of the United States*. U.S. Environmental Protection Agency. Ecological Research Series.

Eaton, L. 2001. Development and validation of biocriteria using benthic macroinvertebrates for North Carolina Estuarine Waters. *Marine Pollution Bulletin* 42:23-30.

FDEP (Florida Department of Environmental Protection). 1996. Standard operating procedures manual–benthic macroinvertebrate sampling and habitat assessment methods: 1. Freshwater streams and rivers. Florida Department of Environmental Protection, Tallahassee, Florida.

Faith, D. P., Minchin, P. R. and L. Belbin, L. 1987. Compositional dissimilarity as a robust measure of ecological distance. *Vegetatio* 69:57-68.

Green, R.H. 1979. *Sampling Design and Statistical Methods for Environmental Biologist*. John Wiley and Sons, New York, NY.

Growns, J. E., J. A. Davis, F. Cheal, L. G. Schmidt, R. S. Rosich, and S. J. Bradley. 1992. Multivariate pattern analysis of wetland invertebrate communities and environmental variables in western Australia. *Australian Journal of Ecology* 17:275-288.

Hubert, W.A. 1996. Passive capture techniques. In: B.R. Murphy and D.W. Willis (eds.) Fisheries *Techniques*, second edition, p. 157-182. American Fisheries Society, Bethesda, MD, USA.

Karr, J.R., and E.W. Chu. 1997. Biological monitoring and assessment: using multimetric indexes effectively. University of Washington, Seattle, WA. EPA 235-R97-001.

King, R. S., K. T. Nunnery, and C. J. Richardson. 2000. Macroinvertebrate assemblage response to highway crossings in forested wetlands: implications for biological assessment. *Wetlands Ecology and Management* 8:243-256.

King, R. S., and C. J. Richardson. In press. Evaluating subsampling approaches and macroinvertebrate taxonomic resolution for wetland bioassessment. *Journal of the North American Benthological Society.*

Kusler, J. and Niering, W. 1998. Wetland assessment: Have we lost our way? *National Wetlands Newsletter* 20:8-14.

Legendre, P. & M.-J. Fortin. 1989. Spatial pattern and ecological analysis. *Vegetatio* 80: 107-138.

Legendre, P., and L. Legendre. 1998. *Numerical ecology*, 2nd edition. Elsevier, Amsterdam, The Netherlands.

Likens, G.E., H. Bormann, N.M. Johnson, D.W. Fisher, and R.S. Pierce. 1970. Effects of forest cutting and herbicide treatment on nutrient budgets in the Hubbard Brook Watershed-Ecosystem. *Ecological Monographs*: 40:23-47.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res.* 27:209-220.

Maxted, J. R., M. T. Barbour, J. Gerritsen, V. Poretti, N. Primrose, A. Silvia, D. Penrose, and R. Renfrow. 2000. Assessment framework for mid-Atlantic coastal plain streams using benthic macroinvertebrates. *Journal of the North American Benthological Society* 19:128-144.

Mitsch, W.J. and J.G. Gosselink. 1993. *Wetlands*. 2nd edition. Van Nostrand Reinhold Company Inc. New York. 539p.

Murkin, H.R., D.A. Wrubleski, and F.A. Reid. 1994. Sampling invertebrates in aquatic and terrestrial habitats. In: T.A. Bookhout (ed.), *Research and Management Techniques for Wildlife and Habitats*, p. 349-369. The Wildlife Society, Bethesda, MD, USA.

North Carolina Division of Water Quality. 1997a. White Oak River Basinwide Water Quality Plan. North Carolina Department of Environment, Health and Natural Resources, Raleigh, NC, USA.

North Carolina Division of Water Quality. 1997b. Standard Operating Procedures, Biological Monitoring. North Carolina Department of Environment, Health, and Natural Resources, Raleigh, NC, USA.

O'Connor, N. A. 1991. The effect of habitat complexity on the macroinvertebrates colonising wood substrates in a lowland stream. *Oecologia* 85:504-512.

Osenberg, C.W. and R.J. Schmitt. 1996. Detecting Ecological Impacts Caused by Human Activity. In: Osenberg, C.W. and R.J. Schmitt (eds.). *Detecting Ecological Impacts: concepts and applications in coastal habitats.* Academic Press, New York.

Rheinhardt, R.D., Brinson, M.M., and P.M. Farley. 1997. Applying Wetland Reference Data to Functional Assessment, Mitigation and Restoration. *Wetlands* 17:2:195-215.

Richardson, C.J. 1994. Ecological functions and human values in wetlands: A framework for assessing forestry impacts. *Wetlands* 14:1:1-9.

Richardson, C.J., R.S. King, and K. Nunnery. 1997. A functional assessment of wetland ecosystem response to highways: Phase I macroinvertebrate community studies. Report to the Center for Transportation and the Environment, Raleigh, NC, USA. Duke Wetland Center Publication 97-02.

Richardson, C.J. 1995. Functional Assessment of the Effects of Highway Construction on Wetlands: Comparisons of Effects Before, During and After Construction. Proposal submitted to The Center for Transportation and the Environment, Raleigh, NC.

Richardson, C.J. 1994. Ecological functions and human values in wetlands: a framework for assessing forestry impacts. *Wetlands* 14:1-9.

Richardson, C.J., R. King, and K. Nunnery. March 1997. A functional assessment of wetland ecosystem response to highways: phase 1 macroinvertebrate studies. Duke Wetland Center publication 97-02. Nicholas School of the Environment, Duke University, Durham, NC.

Richardson, C.J., and K. Nunnery. 2001. Ecological functional assessment (EFA): A new approach to determining wetland health. Pp. 95-112. In: Vymazal, J. (ed.), *Transformations of Nutrients in Natural and Constructed Wetlands*. Backhuys Publishers, Leiden. 519 pp.

Rossiter J A and Crawford R D 1983 Evaluation of artificial wetlands in North Dakota: recommendations for future design and construction. *Transportation Research Record* 948:21-25.

Shuldiner, P.W., D.F. Cope and R.B. Newton. 1979a. Ecological effects of highway fills on wetlands--research report. *NCHRP Report* 218A p.34.

Shuldiner, P.W., D.F. Cope and R.B. Newton. 1979b. Ecological effects of highway fills on wetlands--users manual. *NCHRP Report* 218B p.99

Smart, R.M. and J.W. Barko. 1980. Nitrogen nutrition and salinity tolerance of *Distichlis spicata* and *Spartina alterniflora*. *Ecology* 61:630-638.

Smith, D.R., Ammann, A., Bartoldus, C., and M. Brinson. 1995. An Approach for Assessing Wetland Functions Using Hydrogeomorphic Classification, Reference Wetlands, and Functional Indices, Technical Report WRP-DE-9, U.S. Army Engineer Waterways Experimental Station, Vicksburg, MS.

Stewart Oaten, A. 1996. Goals in Environmental Monitoring. In: Osenberg, C.W. and R.J. Schmitt (eds.). *Detecting Ecological Impacts: concepts and applications in coastal habitats.* Academic Press, New York.

Stewart Oaten, A., J.R. Bench and C.W. Osenberg. 1992. Assessing the effects of unreplicated perturbation: no simple solution. *Ecology* 73:1396-1404.

Stewart Oaten, A., W.W. Murdoch and K.R. Parker. 1986. Environmental impact assessment: "pseudoreplication" in time? *Ecology*, 67:929-940.

U.S. Environmental Protection Agency. 1997. Field and laboratory methods for macroinvertebrate and habitat assessment of low gradient, nontidal streams. Mid-Atlantic Coastal Streams Workgroup, Region 3, Wheeling, WV, USA.

USDA, NRCS. 1992. Soil Survey of Onslow County, North Carolina. US Dept. of Agriculture-Natural Resources Conservation Service, Raleigh, NC, USA.

USDA, NRCS 1999. The PLANTS database (http://plants.usda.gov/plants). National Plant Data Center, Baton Rouge, LA 70874-4490 USA.

Uddameri, V., Norton, S.A., Kahl, J.S. and J.P. Scofield. 1995. Randomized Intervention Analysis of the Response of the West Bear Brook Watershed, Maine to Chemical Manipulation. *Water Air and Soil Pollution* 79:131-146.

Underwood, A. J. 1991. Beyond BACI: experimental designs for detecting human impacts on temporal variations in natural populations. *Australian Journal of Marine and Freshwater Research* 42:569-587.

Underwood, A. J. 1992. Beyond BACI: detection of environmental impacts populations in the real but variable world. *Journal of Experimental Marine Biology and Ecology* 161:145-178.

Underwood, A. J. 1994. On beyond BACI: sampling designs that might reliably detect environmental disturbance. *Ecological Applications* 4:1:3-15.

Valiela, I. and J.M. Teal. 1974. Nutrient limitation in salt marsh vegetation. Pp. 547-563 in: *Ecology of Halophytes*. Eds. R. Reinold and W. H. Queen. Academic Press. New York, NY, USA.

Williams, D.D., and N.E. Williams. 1998. Aquatic insects in an estuarine environment: densities, distribution, and salinity tolerance. *Freshwater Biology* 39:411-421.