

Macroinvertebrate assemblage response to highway crossings in forested wetlands: implications for biological assessment

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Abstract

Despite the mandate of the Clean Water Act to protect the physical, chemical, and biological integrity of the USA's wetlands, the use of biota to assess wetland condition has not been well explored. During June, 1996, we evaluated the response of macroinvertebrate assemblages to fill-culvert highway crossings in two bottomland forested wetlands in North Carolina. Our objective was to apply biological assessment methods and metrics that have been effectively used in streams to explore their applicability in forested wetlands. We found significant changes in several metrics as a function of distance from the highway crossings. Areal and numerical taxon richness increased within at least 40 m of highway when compared to control locations. Percent dominant taxon values were lowest within 10 m of the highway. Percent herbivores also increased significantly within at least 40 m of the highway, reflecting the lower % crown closure and associated shift in primary production from trees to herbaceous macrophytes and algae. The North Carolina Biotic Index, a metric of tolerance, did not reflect assemblage changes near the highway. Ordination and permutation tests revealed that assemblage composition was significantly different from controls at 10 and 40 m distances from the highway crossings. In particular, algal grazers such as the mayflies *Caenis* sp. and *Callibaetis* sp. responded positively and the damselflies *Ischnura* spp. and the fingernail clams *Sphaerium* spp. responded negatively to the crossings. Favorable algal and herbaceous detrital resources, greater patchiness and habitat complexity, and overall high tolerance to natural stressors probably contributed to the increase in taxon richness near the highway. However, significant deviation from control locations indicated the highway was a source of perturbation. Our findings illustrate the potential utility of macroinvertebrate assemblages for wetland assessment, but suggest the importance of defining the reference condition as well as the need for development of metrics for specific classes of wetlands.

Introduction

The United States Federal Highway Administration (FHWA) is charged with providing safe and efficient transportation systems, yet is also responsible for protecting water resources from highway-related perturbation. In 1978, the Department of Transportation (DOT) declared Order 5660.1A, which commits the FHWA to the protection, preservation, and enhancement of the USA's wetlands to the fullest extent practicable during the planning, construction, and operation of highway facilities (Rossiter and Crawford,

1983). However, aside from a few documents describing general guidelines for impact assessment (e.g., Adamus, 1983; Adamus and Stockwell, 1983; Kobriger et al., 1983; Thrasher, 1983), no methodology exists to quantify the effects of highway construction and operation on wetlands. Additionally, little research has been conducted to estimate the magnitude and extent that highway crossings may affect wetlands.

Wetland biota, such as macroinvertebrates, may be useful components of a wetland impact assessment system. Resident biota in a water body are natural

monitors of environmental quality and can reveal the effects of episodic as well as cumulative pollution and habitat alteration (Rosenberg and Resh, 1993). For example, use of macroinvertebrates as bioindicators, also known as biological assessment, has been shown to be one of the most reliable and cost-effective assessment tools of water and habitat quality in streams throughout the world (e.g., Kerans and Karr, 1994; Grows et al., 1997). Scientific literature is rich with information from lotic systems regarding macroinvertebrate species assemblages and their sensitivity to anthropogenic stressors (Hilsenhoff, 1987; Lenat, 1988; Lenat, 1993), which has led to robust, well-defined sampling protocols and associated metrics of human influence (e.g., Plafkin et al., 1989; Karr and Chu, 1997). However, the ecology of macroinvertebrates from low- to non-flowing systems, such as forested wetlands, has received minimal study and is poorly understood, particularly in a context of population- and community-level responses to perturbations (Batzer and Wissinger, 1996). Accordingly, development of biological assessment methods for wetlands has all but been ignored until recently (USEPA, 1997a), potentially leaving wetlands years behind the volumes published from streams. Most wetland assessment techniques in use today are based on ‘functional indicators’ (e.g., Brinson and Rheinhardt, 1996) and generally do not include metrics based on attributes of biotic communities despite the mandate of the Clean Water Act (CWA) to protect the physical, chemical, and biological integrity of the USA’s wetlands. This inconsistency with federal legislation has led to vocal dissatisfaction with current wetland assessment methods (e.g., Kusler and Niering, 1998) and a sudden increase in interest in incorporating biological components, such as macroinvertebrate assemblages, into assessment protocols (USEPA, 1997a).

In one of the few studies related to highway impacts on wetland ecosystems, Nunnery (1997) suggested that fill-culvert crossings, often used over wetlands in the southeastern USA, may have caused alteration to flow patterns, loss of tree canopy and basal area, increases in herbaceous macrophyte coverage and water temperature, and reductions in dissolved oxygen in two forested wetlands in North Carolina. These observed changes in water and habitat quality provided an impetus to evaluate macroinvertebrate assemblage response at these same wetlands. Since no accepted biological assessment sampling protocols or metrics have been developed specifically for freshwater wetlands, our primary objective was to apply sampling

methods and metrics developed from streams to explore their applicability in wetland habitats. We hypothesized that since these highway crossings had induced environmental gradients within the wetlands, attributes of macroinvertebrate assemblages should exhibit a dose-response relationship along those gradients which, in turn, could be used to assess magnitude and areal extent of highway impacts.

Methods

Study area

Our study wetlands, Beaverdam and Kill Swamps, are third-order watersheds located on Interstate-40 in Sampson Co., North Carolina, USA ($35^{\circ}14'N$, $78^{\circ}21'W$; Figure 1). Both are low- to non-flowing cypress-gum wetlands that are part of the Cape Fear River watershed and within the Middle Atlantic Coastal Plain Ecoregion (Omernik, 1987). Nunnery (1997) originally selected these sites because both (1) are bisected by fill-culvert type bridges, where fill dirt rather than pilings is used to support the road over the floodplain, (2) have similar watershed areas upstream of the crossings (24.1 and 18.4 km^2 for Beaverdam and Kill Swamps, respectively), (3) have similar 50 y flood stream flows ($31.75\text{ m}^3\text{s}^{-1}$ and $29.13\text{ m}^3\text{s}^{-1}$ for Beaverdam and Kill Swamps, respectively), (4) are on Bibb-Johnston association soils (hydric), and (5) have similar land-uses within their watersheds. Both sites also have areas of stagnant surface water adjacent to upstream and downstream sides of the highway fill, as well as ponding upstream of the crossings during periods of high flow (Nunnery, 1997; Richardson and Nunnery, 1998). Highway crossings, which were constructed in 1989, are separated by 1.8 km . Width of permanently-flooded wetland habitat at the highway crossings is 200 and 180 m for Beaverdam and Kill Swamps, respectively. We established areas of study as 200 m upstream and downstream from the highway crossings.

A reference study area was established in a relatively undisturbed area of Beaverdam Swamp upstream from the highway crossing. This area began 350 m upstream from the highway crossing and extended for an additional 80 m upstream. To help verify that the selected reference area and the highway crossing areas were similar before highway construction began, aerial photographs taken in 1978 (11 y before construction was completed) were examined to estimate percent crown closure for each area. Using post-construction photographs, position of the crossings

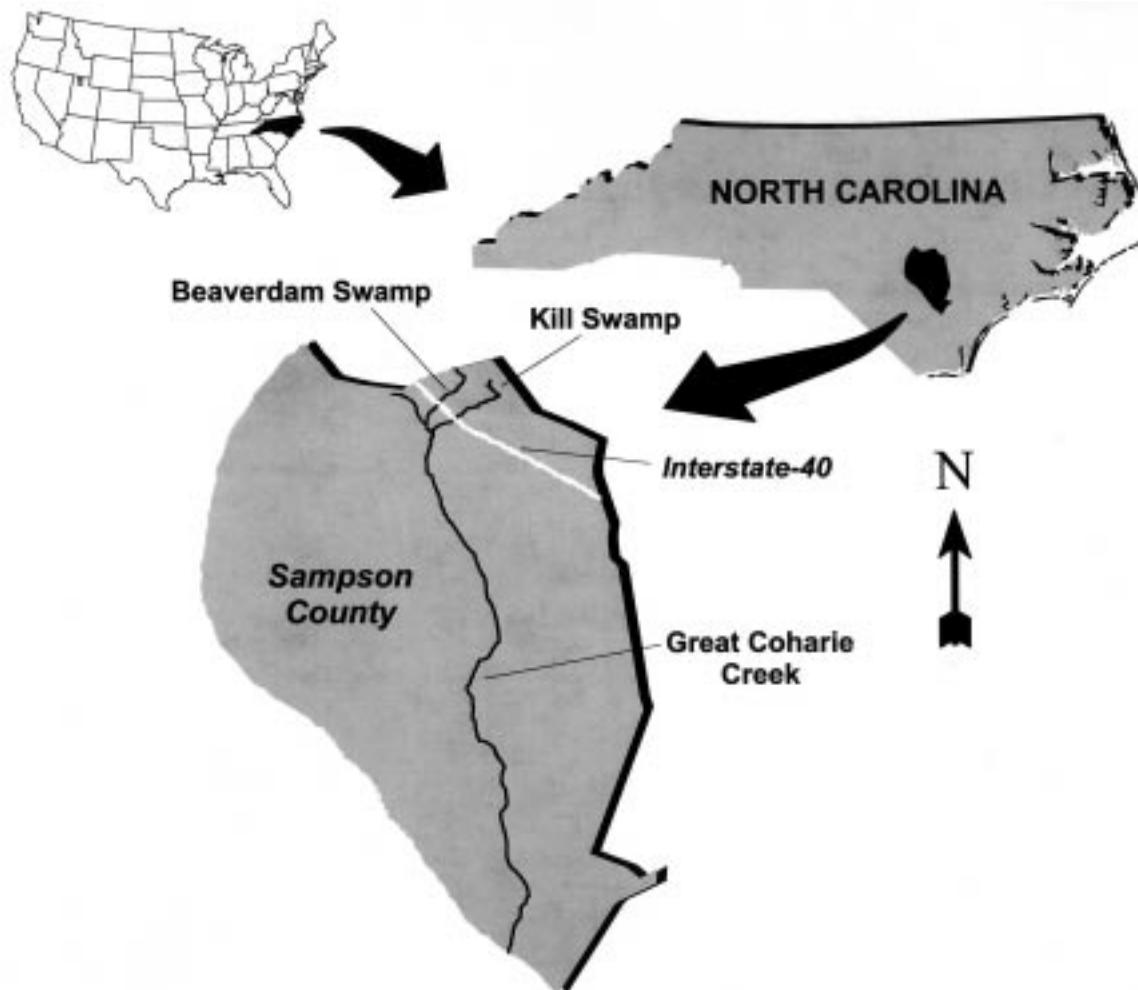


Figure 1. Location of the study wetlands in the coastal plain region of North Carolina, USA.

was transferred to pre-construction photos. Highway and reference areas were delineated from the crossings and percent crown closure was estimated following Paine (1981). Percent crown closure for highway areas ranged from 77–82%, while the reference area was 78%, suggesting that forest structure of the highway areas was very similar to the reference area before highway construction began.

Vegetation of the study wetlands was typical of bottomland forested wetlands found throughout the southeast USA (Clark and Benforado, 1981; Rheinhardt et al., 1998). Trees were primarily bald cypress (*Taxodium distichum* (L.)) and swamp tupelo (*Nyssa sylvatica* var. *biflora* Marshall). Herbaceous macrophyte assemblages were dominated by the invasive Asian spiderwort (*Murdannia keisak* (Hassk.) Hand.-

Mazz.) and cutgrass (*Leersia oryzoides* (L.) Swartz). Duckweed (*Spirodela polyrrhiza* (L.) Schlid.) was also abundant in open-canopy areas near the highway crossings.

Study design

Estimation of areal extent, as well as magnitude, of impact to a wetland is important for purposes of mitigation. We assumed that, if the highway crossings had disturbed these wetlands, the disturbance would manifest itself along a gradient. Greatest disturbance would be immediately adjacent to the highway fill, and would decrease in magnitude with increasing distance from the crossings. Therefore, our study design was established not only to detect a highway effect, but also to assess the spatial extent of the effects.

Transects were marked parallel to the highway crossings across the full width of the wetlands. Transects were placed at 10-, 40-, and 200-m distances on both sides of the crossings at both wetlands. In environmental impact assessment, gradient sampling designs have been shown to be more sensitive and powerful statistically than designs based on random allocation of samples (Ellis and Schneider, 1997). Thus, these three distance categories were considered statistical ‘treatments’ with each transect representing a replicate ($n = 4$ per distance treatment). In similar fashion, we also marked transects in the reference area. ‘Control’ transects ($n = 3$) were spaced 40-m apart and spanned the width of permanently-flooded wetland habitat.

Along each transect, we placed three 5-m radius sub-plots spaced 40 m apart. The middle sub-plot was placed at the lowest elevation adjacent to, but not within, the main channel. The remaining two plots flanked the median plot, which helped standardize comparisons among transects. All plots were permanently flooded (Nunnery, 1997) and inundated with at least 5 cm of surface water at the time of sampling (most depths were 10–15 cm).

Environmental characterization

Water depth (cm), dissolved oxygen (mg L⁻¹), water temperature (°C), tree basal area (m²ha⁻¹), crown closure (%), volume of downed coarse woody debris (m³ha⁻¹), and long-term sediment accretion (mm y⁻¹; ¹³⁷Cs marker technique) were measured in all sub-plots along each transect. Herbaceous macrophyte biomass (g m⁻²) and herbaceous species richness (total number of species) also were measured but only in two of the three sub-plots (standardized among transects). Water depth, dissolved oxygen, water temperature, and sediment accretion was measured in an open-water area nearest to each sub-plot centroid. Herbaceous biomass was estimated using two 0.25 m² clip quadrats within each sub-plot, distributed using a random bearing and distance from the sub-plot centroid. The remaining variables were estimated for the entire sub-plot. Values of each environmental variable at each sub-plot were averaged for the entire transect. Further details for environmental sampling methods are provided in Nunnery (1997) and Richardson and Nunnery (1998). Environmental variables were measured within one week of macroinvertebrate sampling, except for basal area, crown closure, woody debris, and sediment accretion, which were measured the previous year (May-June 1995).

Macroinvertebrate sampling

We based our macroinvertebrate sampling on multi-habitat protocols developed by the North Carolina Division of Water Quality (NCDWQ, 1997) and the Mid-Atlantic Coastal Streams Workgroup (USEPA, 1997b) for low-gradient coastal plain streams. In these protocols, a composite sample is produced by taking subsamples from multiple habitats within a site. We sampled the four most abundant habitats present in each area: (1) herbaceous macrophytes, (2) bald cypress and swamp tupelo trunks, (3) benthos (sediments), and (4) submerged coarse woody debris (snags). Habitat samples were collected by ‘jabbing’ a D-framed sweep net (0.3-m wide, 600-μm mesh) into the target area for a distance of 0.5 m (Cheal et al., 1993; FDEP, 1996; USEPA, 1997b; Turner and Trexler, 1997). A sample from each habitat was collected in all three sub-plots along each transect (12 total habitat jabs per composite sample). Samples from each habitat were emptied into a sieve bucket (600-μm mesh), washed, and preserved in 95% ethanol stained with rose bengal for laboratory processing. We collected all macroinvertebrate samples from 17–28 June 1996. One transect from each distance category was sampled on each date to avoid potential confounding effects of time since all transects could not be sampled on one day.

In the laboratory, macroinvertebrates were separated from detritus and identified to the lowest taxon possible. When species identifications were not possible, we separated taxa based on ‘morphospecies,’ a level of taxonomy that identified probable species but did not attach specific names (Oliver and Beattie, 1996). Identifications were conducted using Brigham et al., 1982; Brinkhurst, 1986; Thorp and Covich; 1991; Epler, 1995; Epler, 1996; and Merritt and Cummins, 1996, and verified by expert taxonomists (see Acknowledgements).

Metrics and data analyses

Macroinvertebrate metrics of human influence have not been specifically developed and tested for forested wetlands (e.g., USEPA, 1997a). Therefore, we selected a suite of metrics that have been shown to be robust in streams, particularly low-gradient streams in the southeastern USA, or had potential as metrics based on wetland invertebrate literature or personal communication with other researchers. Metrics were selected from four general classes: (1) taxonomic richness and

diversity, (2) feeding ecology, (3) tolerance, and (4) multivariate measures of taxonomic dissimilarity.

We included two measures of total taxonomic richness: areal and numerical richness (e.g., Larsen and Herlihy, 1998). Areal richness was the total number of taxa collected per transect. We used Hurlbert's (1971) rarefaction equation for numerical richness. We calculated numerical richness based on 100 individuals (NR100), a widely-used fixed-count for subsampling benthic samples (e.g., Plafkin et al., 1989; Barbour and Gerritsen, 1996). Percent dominant taxon also is commonly used as a diversity measure, as degraded aquatic systems often are characterized by one or a few highly dominant taxa (Karr and Chu, 1997). We also assessed number of odonate (damselflies and dragonflies) and chironomid (midges) taxa as potential metrics since they often represent a large proportion of the taxa in wetlands and have been shown to be promising indicators of perturbation in low-gradient streams in Florida (Barbour et al., 1996; R. Frydenborg [FDEP] pers. comm.).

We used feeding group metrics with reservation since most designations are based on mouthpart morphology rather than specific food resources (Merritt and Cummins, 1996), an assumption that has led to criticism regarding their reliable use in biological assessment (e.g., Karr and Chu, 1997). Feeding ecology metrics were limited to those taxa that we felt confident exhibit relatively specific modes of food acquisition or food sources. We included % herbivores (mostly algal grazers but a few herbaceous macrophyte piercers/miners), % predators (taxa that are predominantly secondary or tertiary consumers), and % filterers (taxa that filter particulates from the water column). A large number of taxa collected were classified as collector-gatherers, which vary dramatically in types of food consumed, or had poorly-known life histories. These taxa were excluded from feeding group analyses.

Biotic indices are the most commonly used metrics of assemblage tolerance in biological assessment (Rosenberg and Resh, 1993). Although calculated using taxon-specific tolerance values that are often subjectively assigned, biotic indices nevertheless have been shown to have merit in stream assessment (Hilsenhoff, 1987; Plafkin et al., 1989; Lenat, 1993). We used the North Carolina Biotic Index (NCDWQ, 1997) as our metric of tolerance. Possible values for this metric range from 0 to 10, with low scores indicating a very intolerant, sensitive assemblage, while high values indicate a tolerant one.

Our only multivariate metric was Bray-Curtis dissimilarity, a coefficient expressed as taxonomic dissimilarity between pairs of assemblages. Bray-Curtis dissimilarity is a function of shared abundances among taxa between sample pairs, with identical taxa and identical abundances among taxa resulting in 0% dissimilarity, while no common taxa result in 100% dissimilarity. We selected Bray-Curtis dissimilarity because it has been shown to be one of the most robust and ecologically interpretable distance measures for multivariate species data (Faith et al., 1987). Metrics were calculated based on a composite sample for each transect. We considered data from each transect equivalent to a 'site' assessment. Thus transects, not sub-plot samples, were replicates within highway distance (10-, 40-, and 200-m; n = 4) or control treatments (n = 3). With the exception of taxonomic dissimilarity, variation in environmental and metric values among treatments were evaluated using one-way analysis of variance (ANOVA). We inspected metrics for normality using normal-probability residual plots and tested variance homogeneity using Bartlett's test ($p < 0.05$). All data, except crown closure and herbivores, met the assumptions of normal residuals and homogeneity of variance for ANOVA (Bartlett's test, $0.0650 < p < 0.9456$). Percent crown closure was arcsine-transformed, while % herbivore data required a $\log_{10}(x)$ transformation, which normalized residuals and homogenized variances (Bartlett's test, $p = 0.441$ and 0.693 , respectively). We used Tukey's HSD to compare means of metric values that significantly differed among treatments, as deemed by ANOVAs. All differences were considered significant when $p < 0.05$. ANOVAs were conducted using Statistica (StatSoft, Inc., 1998).

Since ANOVA is not appropriate for taxonomic dissimilarity, we used two multivariate procedures to evaluate assemblage response to the highway crossings. First, we tested the hypothesis that macroinvertebrate assemblage composition differed among highway distance treatments using multi-response permutation procedure (MRPP; Mielke et al., 1981; Zimmerman et al., 1985). We used Bray-Curtis dissimilarity as the distance metric. Differences among groups were considered significant when $p < 0.05$. If the overall test among groups was deemed significant, pairwise comparisons were made to determine which groups differed. MRPP was performed using PC-ORD (McCune and Mefford, 1997).

Second, we assessed gradients in taxonomic composition among transects and their relationship

Table 1. Results from ANOVA on environmental variable data among highway distance categories. Mean (\pm 1 S.E.; n = 4 for 10, 40, and 200 m; n = 3 for control) environmental characteristics are reported by highway distance categories. Significant F ratios and associated P values are shown in bold. Means with the same superscript letters do not differ ($p > 0.05$, Tukey's HSD test). Percent crown closure were arcsine-transformed for analysis but means below were calculated using untransformed data.

Environmental variable	F _(3,11)	P	10 m	40 m	200 m	Control
Crown closure (%)	3.51	0.049	46.5 (16.0) ^B	54.9 (10.0) ^B	77.3 (10.4) ^{AB}	91.8 (8.17) ^A
Dissolved oxygen (mg l ⁻¹)	2.56	0.109	1.48 (0.14)	1.90 (0.21)	2.10 (0.43)	2.60 (0.18)
Downed woody debris (m ³ ha ⁻¹)	2.49	0.114	10.19 (1.01)	8.78 (0.80)	7.22 (0.10)	4.53 (0.93)
Herbaceous biomass (g m ⁻²)	4.90	0.021	87.0 (13.3) ^A	98.7 (28.9) ^A	23.6 (15.9) ^B	13.6 (6.50) ^{BC}
Number of herb taxa	0.91	0.465	6.50 (0.50)	6.75 (1.44)	5.75 (1.18)	4.33 (0.66)
Sediment accretion (mm y ⁻¹)	2.67	0.099	5.70 (0.75)	3.71 (0.72)	2.85 (0.55)	3.06 (0.98)
Temperature (°C)	6.64	0.008	24.1 (0.46) ^A	24.3 (0.61) ^A	24.2 (1.25) ^A	19.8 (0.09) ^B
Tree basal area (m ² ha ⁻¹)	3.88	0.041	12.5 (2.47) ^B	13.6 (4.61) ^B	19.9 (4.40) ^{AB}	31.3 (6.08) ^A
Water depth (cm)	3.46	0.054	12.6 (2.56)	12.2 (1.65)	14.7 (2.12)	8.04 (0.91)

to measured environmental variables using non-metric multi-dimensional scaling (NMDS; Ludwig and Reynolds, 1988; Clarke, 1993). NMDS arranges samples (in this case, transects) so that ranked dissimilarities among points match as closely as possible to Euclidean distance in ordination space. A stress coefficient is calculated for an ordination configuration, which reflects the relative agreement between rank dissimilarities in the distance matrix and distances among points in ordination space. We selected an appropriate number of dimensions (axes) for each ordination based on marginal reductions in stress coefficients with each additional axis added. We used Bray-Curtis dissimilarity as a basis for sample ranks, thus complementing the MRPP analysis. Dissimilarities were based on abundance (relativized by each taxon maximum abundance to give equal weight among taxa) of taxa collected along each transect.

To assess whether measured environmental (habitat and water quality) variables were related to gradients in assemblage composition, we calculated maximum Pearson product-moment correlation coefficients of environmental variables in ordination space. Environmental gradients are not necessarily parallel to ordination axes, thus we searched in all directions in the space for vectors of maximum correlation with any environmental variable. We used means as variable values for each transect, with the exception of herbaceous species richness, which was the total number of species per transect. The statistical significance of environmental correlations was tested using Monte-Carlo permutation tests rather than parametric tests since samples in ordination space are not independent. We computed 1000 random permutations

and considered variables significant when $p < 0.05$. We also conducted this analysis for taxon variables to help explain which taxa were most related to the transect configurations in ordination space. Only taxa collected in at least five of the fifteen transects were included in the vector fitting analysis, although all taxa were used to generate the ordinations. Number of individuals of each taxon per transect were used in the analysis. NMDS and vector fitting were performed using DECODA (Minchin, 1994).

Results

Environmental characterization

Mean values of several environmental variables changed as a function of distance from the highway crossings (Table 1). Sedimentation rates, which were based on ¹³⁷Cs dating in soil cores, were generally highest immediately adjacent to the highway fill (10 m) but similar beyond 40 m. Most noticeable was the significant decrease in crown closure and tree basal area with decreasing distance from the highway. With the exception of water depth, which was similar among highway transects but slightly lower at the controls, the remaining variables showed responses to the highway crossings that likely were inter-correlated with the change in forest structure (Table 1). Means for downed coarse woody debris were as over twice as great 10-m from the highway than at control transects; however, this trend was not significant (Table 1). Herbaceous biomass was several times greater at 10- and 40-m distances than at 200-m or controls. Gen-

erally more herb taxa were also found near the highway. Dissolved oxygen was quite low throughout the wetlands but was generally highest at controls. Conversely, water temperature was significantly lower at the heavily-canopied control transects.

Macroinvertebrate assemblage metrics

We collected and identified a total of 67 taxa (Table 2). Total number of individuals (abundance) collected was not different among highway distance categories (Table 3). Among the nine additional univariate metrics compared using ANOVA, four differed as a function of distance from the highway crossings (Table 3). Areal and numerical richness (NR100) increased with decreasing distance from the highway. Areal richness was higher at 10- and 40-m transects than the controls, while NR100 at 10 m was greater than both 200-m and control transects (Table 3). Conversely, % dominant taxon was significantly higher at the controls than 10 m and 40 m (Table 3). One feeding group metric, % herbivores, also was influenced by the highway crossings. It showed a negative response with increasing distance from the highway, with significantly greater values at 10 m than 200 m or controls (Table 3). Other metrics did not discriminate among distance transects, particularly the North Carolina Biotic Index (NCBI). NCBI values were all high, indicating ‘tolerant’ assemblages based on the stream-derived tolerance values, but showed no response to the highway crossings (Table 3).

Considering multivariate taxon data, the Bray-Curtis dissimilarity metric differed among highway distance categories (MRPP, $p = 0.032$). Assemblages at both 10- and 40-m distances differed from controls ($p < 0.05$). NMDS ordination projected this trend, with 10- and 40-m transects clearly separated from controls (Figure 2, two-dimension stress = 0.1615). Assemblages at the 200-m distances were variable but generally more similar to controls than to 10 or 40 m (Figure 2).

Vector fitting of environmental data to NMDS ordination configurations showed that several variables were correlated to gradients in macroinvertebrate assemblage composition (Figure 2a, Table 4). Vectors for herbaceous biomass, downed coarse woody debris, and water temperature all were directed toward transects marked near the highway crossings, while basal area, crown closure, and dissolved oxygen were negatively related to highway transects and had vectors directed toward the control transect assemblages (Fig-

ure 2a, Table 4). Vector fitting of taxon data indicated at least 10 taxa that were significantly related to transect configurations in ordination space (Figure 2b, Table 4). The strongest relationship was for the odonates *Ischnura* spp., which had a high affinity for 200-m and control transects (Tables 2 and 4). *Sphaerium* spp. also showed a negative response to the highway crossings, reaching greatest abundance at the controls (Tables 2 and 4). The remaining taxa had vectors of maximum correlation directed toward transects near the highway (Figure 2b, Table 4). Among these taxa that were positively correlated to highway crossing transects, the most notable were the only two mayfly taxa, *Callibaetis* sp. and *Caenis* sp. (Figure 2b, Table 4). These mayflies are primarily algal grazers (Table 2) and their abundance near the highway contributed to the increase in % herbivores detected in the univariate metric analysis (Table 3). The gastropods *Physella* spp. and *Pseudosuccinea columella*, also algal grazers, were generally more abundant near the highway than at controls, but were not significantly correlated to the NMDS configuration (Table 2).

Discussion

The results of this study support the notion that biological assessment using macroinvertebrate assemblages can be effective for detecting highway influence in bottomland forested wetlands. A dose-response relationship along the highway distance gradient for several univariate metrics and multivariate assemblage composition provides strong evidence that fill-culvert highway crossings can have a profound influence on macroinvertebrates in wetlands. However, the mechanisms often responsible for degrading biological integrity in streams, such as organic enrichment, low dissolved oxygen, and high water temperatures (Rosenberg and Resh, 1993), may not have played a significant role in our study wetlands on an assemblage level. This was reflected in the very high tolerance values, which were developed by the NCDWQ (1997) for streams in North Carolina, among our macroinvertebrate taxa. The taxa present in our study wetlands would be characteristic of highly polluted lotic habitats based on these values due to their high tolerance to environmental conditions regarded as stressful to taxa in high-quality, undisturbed streams. Although we documented lower dissolved oxygen and higher temperatures near the highway when compared to our control transects, the NCBI was insensitive to

Table 2. Taxa collected among highway distance transects at Beaverdam and Kill Swamps, NC during June 1996, shown by rank abundance (taxa with equal abundances given equal ranks), transects where collected (10-, 40-, 200-m and control (C) distance categories; categories in rank order of mean abundance transect-1), North Carolina Tolerance Value (NCTV; NCDWQ 1997), and probable feeding group category (F = filterer, H = herbivore, P = predator, and * = highly variable in feeding habits or unknown).

Class/Order	Family	Genus/Species	Abundance	Transect	NCTV	Feeding Group
Amphipoda	Crangonyctidae	<i>Crangonyx</i> sp.	45	200	7.87	*
Coleoptera	Dytiscidae	<i>Celina angustata</i> Aubé	30	10=40>200	8.04	P
		<i>Celina slossoni</i> Mutchler	32	10>200>40	8.04	P
		<i>Copelatus caelatipennis princeps</i> Young	45	10	—	P
		<i>Coptotomus venestus</i> (Say)	53	10	9.26	P
		<i>Cybister fimbriolatus</i> Wilke	35	200=40>10	—	P
		<i>Ilybius oblitus</i> Sharp	45	40	—	P
		<i>Neoporush carolinus</i> (Fall)	37	C	8.62	P
		<i>Neoporush undulatus</i> Say	53	40>C>10	8.62	P
	Gyrinidae	<i>Dineutus emarginatus</i> (Say)	53	10	5.54	P
	Halaplidae	<i>Peltodytes dunavani</i> Young	45	C>10	8.73	H
	Hydrophilidae	<i>Berosus</i> sp. (adults)	32	10>40>C=200	8.43	H
		<i>Enochrus ochraceus</i> (Melsheimer)	53	200	—	*
		<i>Tropisternus collaris striolatus</i> (LeConte)	53	40	9.68	*
		<i>Tropisternus blatchleyi</i> d'Orchymont	23	C>200>10>40	9.68	*
	Noteridae	<i>Hydrocanthus iricolor</i> (Say)	10	40>10>200>C	7.14	P
Decapoda	Cambaridae	<i>Procambarus</i> sp.	45	40>10	7.50	H
Diptera	Ceratopogonidae	<i>Bezzia/Palpomyia</i> (complex) sp.1	14	10>200>40	6.86	P
		<i>Bezzia/Palpomyia</i> (complex) sp.2	24	10>C>40=200	6.86	P
	Chironomidae	<i>Ablabesmyia peleensis</i> (Walley)	4	40>10>200>C	9.67	P
		<i>Chironomus</i> spp.	3	40>10>200>C	9.63	*
		<i>Clinotanypus pinguis</i> (Loew)	53	200	8.74	P
		<i>Dicrotendipes modestus</i> (Say)	19	10=200>40	8.73	*
		<i>Glyptotendipes</i> sp.	53	40	9.47	F
		<i>Paratanytarsus</i> sp.	37	200>C	8.45	*
		<i>Polypedilum illinoense</i> (Malloch)	27	10>40=200>C	9.00	*
		<i>Polypedilum tritum</i> (Walker)	26	10	—	*
		<i>Procladius</i> spp.	6	200>C>10>40	9.10	P
		<i>Rheotanytarsus</i> sp.	53	10	—	*
		<i>Stictochironomus</i> sp.	53	10	6.52	*
		<i>Tanypus stellatus</i> Coquillet	17	200>40>10	9.19	P
		<i>Tanytarsus</i> spp.	12	200>10>C>40	6.76	*
		<i>Tribelos</i> sp.	53	C	6.31	*
	Culicidae	<i>Mansonia perturbans</i> (Walker)	53	40	8.50	F
		<i>Uranotaenia sappharina</i> (Osten Sacken)	32	10>40>200	8.50	F
	Ptychopteridae	<i>Bittacomorpha clavipes</i> (Fabricius)	25	200>C	—	*
	Stratiomyidae	<i>Stratiomys</i> sp.	45	200	8.08	*
	Tabanidae	<i>Chrysops</i> sp.	39	C>200=10	6.73	*
	Tipulidae	<i>Limonia</i> sp.	45	C>10	—	*
Ephemeroptera	Baetidae	<i>Callibaetis</i> sp.	16	40>10>C>200	9.84	H
	Caenidae	<i>Caenis</i> sp.	15	10>40>200	7.41	H
Gastropoda	Lymnaeidae	<i>Pseudosuccinea columella</i> (Say)	12	10>200>40>C	7.65	H
	Physidae	<i>Physella</i> spp.	8	40>10>200>C	8.84	H
	Planorbidae	<i>Micromenetus dilatatus</i> (Gould)	45	40>200	8.23	H
Hemiptera	Belostomatidae	<i>Belostoma lutarium</i> (Stål)	9	200>C>40>10	9.80	P
	Corixidae	<i>Hesperocorixa brimleyi</i> (Kirkaldy)	28	40>10	9.00	P

Table 2. Continued.

Class/Order	Family	Genus/Species	Abundance	Transect	NCTV	Feeding Group
Hirudinea	Naucoridae	<i>Hesperocorixa lucida</i> (Abbott)	5	40>C>200>10	9.00	P
		<i>Trichocorixa louisianae</i> Jaczewski	45	10	9.00	P
	Nepidae	<i>Pelocoris femoratus</i> (Palisot-Beauvois)	7	10>40>200	7.01	P
		<i>Ranatra</i> sp.1	39	C>40=10	7.82	P
	Notonectidae	<i>Ranatra</i> sp.2	53	10	7.82	P
		<i>Notonecta irrorata</i> Uhler	53	40	8.71	P
	Erpobdellidae	<i>Erpobdella/Mooreobdella</i> sp.	30	10>200>40	8.33	P
	Glossiphoniidae	<i>Batracobdella phalera</i> (Graf)	30	10=40=200	9.10	P
Megaloptera	Corydalidae	<i>Chauliodes rastricornis</i> Rambur	39	10=40=200	8.42	P
Odonata	Coenagrionidae	<i>Ischnura</i> spp. (<i>I. posita</i> & <i>I. prognata</i>)	1	C>200>40>10	9.52	P
Oligochaeta	Corduliidae	<i>Nehelennia irene</i> (Hagen)	39	40	—	P
		<i>Epitheca (Tetragoneuria)</i> sp.	35	10>C>40	8.57	P
	Libellulidae	<i>Erythemis simplicicollis</i> (Say)	11	40>10>200	9.72	P
		<i>Libellula</i> sp.	53	200	9.64	P
	Lumbriculidae	<i>Pachydiplax longipennis</i> (Burmeister)	19	10>200>C>40	9.86	P
		<i>Eclipidrilus</i> sp.	17	40>10>200	7.03	*
	Naididae	<i>Dero</i> spp.	28	10>200>40	—	*
		<i>Nais</i> spp.	19	40>10>200>C	8.88	*
	Tubificidae	<i>Limnodrilus hoffmeisteri</i> Clarapede	19	200>C>10	7.11	*
Pelecypoda	Sphaeriidae	<i>Sphaerium</i> spp.	2	C>40>200>10	7.58	F
Trichoptera	Leptoceridae	<i>Oecetis</i> sp. A Floyd	53	200	2.00	P

this disparity. These water quality values, even at the control transects, likely were beyond a threshold for most sensitive taxa. Large deposits of organic matter, low dissolved oxygen, and high water temperatures are typical of even the most pristine wetlands, particularly in the southeast USA during much of the year, thus ‘tolerance’ to such conditions may convey little information in the context of wetland biological assessment. However, much more data are needed from a wide range of wetland types and levels of ecological impairment before an adequate assessment of the utility of tolerance can be made.

Habitat structure potentially was an important forcing factor for macroinvertebrate assemblage composition. In particular, our data suggest that forest canopy may play an especially important role in structuring assemblages in forested wetlands. Loss of canopy within at least 40 m of the highway crossings was coincident with a reduction in tree basal area and an increase in downed woody debris, likely due to increased tree mortality due to surface water ponding, altered flow patterns, beavers, and other potential factors (Nunnery, 1997; Richardson and Nunnery, 1998). This reduction in crown closure also allowed

much greater sunlight penetration to the water surface, which resulted in higher water temperatures and lower dissolved oxygen during our sampling. Herbaceous macrophytes also showed a positive response to decreased crown closure in areas near the highway. Thus, a canopy-driven cascade of changes in water and habitat quality variables likely occurred which also was concomitant with changes in macroinvertebrate assemblage composition. The significant increase in herbivorous taxa, particularly algal grazers, is highly consistent with this observed shift in primary production from trees to macrophytes and algae near the highway crossings. While feeding group metrics have not been shown to provide a consistent signal of human influence in stream biological assessment (e.g., Karr and Chu, 1997), our data suggest that % herbivores may be useful in forested systems that exhibit dense canopy and/or have relatively little algal and herbaceous macrophyte production in their undisturbed reference condition.

Canopy also may have been responsible for the highly negative relationship between the highway crossings and the damselflies *Ischnura* spp. *Ischnura prognata*, one of the two *Ischnura* species identi-

Table 3. Results from ANOVA on macroinvertebrate assemblage metrics among highway distance categories. Significant F ratios and associated P values are shown in bold. Means (± 1 S.E.; n = 4 for 10, 40, and 200 m; n = 3 for control) for each metric are reported by highway distance categories. Means with the same superscript letters do not differ ($p > 0.05$, Tukey's HSD test). Percent herbivore data were log-transformed for analysis but means below were calculated using untransformed data.

Metric	F _(3,11)	P	10-m	40-m	200-m	Control
No. of individuals	0.25	0.858	151.5 (27.7)	185.3 (42.6)	161.3 (25.6)	155.0 (7.94)
Areal richness (no. of taxa)	3.64	0.048	27.0 (2.61) ^A	24.3 (2.66) ^A	20.3 (2.25) ^{AB}	17.0 (0.57) ^B
Numerical richness (NR100)	5.47	0.015	23.6 (0.94) ^A	19.6 (2.05) ^{AB}	17.4 (0.26) ^B	14.5 (0.71) ^B
% dominant taxon	6.68	0.008	19.7 (2.62) ^B	24.3 (5.98) ^B	38.9 (5.91) ^B	48.2 (3.19) ^A
No. of odonate taxa	3.11	0.071	3.50 (0.28)	3.25 (0.63)	1.75 (0.48)	2.33 (0.33)
No. of chironomid taxa	1.43	0.287	6.50 (0.65)	5.00 (0.82)	5.00 (0.71)	4.67 (0.33)
% predators	1.41	0.291	43.2 (2.3)	39.5 (5.25)	50.7 (5.57)	52.7 (7.31)
% herbivores	6.05	0.012	11.8 (2.4) ^A	9.63 (3.96) ^{AB}	4.78 (1.43) ^B	2.92 (1.26) ^B
% filterers	1.14	0.375	9.54 (3.69)	10.0 (3.82)	9.72 (4.23)	22.3 (10.3)
NC Biotic Index	0.23	0.873	8.73 (0.13)	8.79 (0.055)	8.88 (0.25)	8.89 (0.04)

Table 4. Maximum correlations (r) of significant environmental and taxon vectors in NMDS ordination space for macroinvertebrate assemblage data (see Figure 2). Probabilities (P) were calculated using 1000 random permutations.

Variable	r	P
Environmental		
Coarse woody debris	0.796	≤ 0.001
Dissolved oxygen	0.772	0.006
Herbaceous biomass	0.763	0.003
Crown closure	0.727	0.019
Tree basal area	0.659	0.034
Temperature	0.634	0.041
Taxon		
<i>Ischnura</i> spp.	0.894	≤ 0.001
<i>Callibaetis</i> sp.	0.714	0.008
<i>Caenis</i> sp.	0.699	0.013
<i>Erythemis simplicicollis</i>	0.684	0.014
<i>Celina angustata</i>	0.667	0.031
<i>Tanyptus stellatus</i>	0.666	0.010
<i>Pelocoris femoratus</i>	0.664	0.026
<i>Eclipidrilus</i> sp.	0.652	0.039
<i>Hydrocanthus iricolor</i>	0.642	0.039
<i>Sphaerium</i> spp.	0.618	0.049

fied (not separable from *I. posita* at early instars), is known to inhabit heavily-canopied swamps (Wharton et al., 1981; Huggins and Brigham, 1982) and is infrequently collected outside of shaded habitat (J. Daigle [FDEP], personal communication). This species may be sensitive to alterations in forest structure such as

the reduced crown closure near the highway crossings. Adult odonates in general are also attracted to lightly-colored surfaces where prey insects, such as Chironomidae, often gather in mating swarms (Titmus, 1979). While speculative, it seems reasonable that highway traffic could contribute to increased mortality in odonate adults feeding above the light gray, reflective road surface. Reduced adult survivorship and associated reproduction could reduce abundance of immatures (nymphs) in the surrounding wetlands. The effects of highway traffic on flying adult stages of aquatic insects needs further investigation.

Sphaerium spp. also appeared to respond negatively to the highway crossings. *Sphaerium* spp. and other taxa of fingernail clams such as *Musculium*, *Pisidium*, and *Eupera* are well documented from bottomland forested wetlands across the southeastern USA (reviewed by Wharton et al., 1981). These clams are often present in enormous numbers (Parsons and Wharton, 1978) as they filter the fine particulate organic matter plentiful in these systems (Beck, 1977). The mechanism(s) responsible for the apparent decrease in fingernail clam abundance near our highway crossings is not clear; however, greater sediment accretion, altered flow patterns due to the highway fill, and reduced dissolved oxygen all may have contributed. Cooper (1987) indicated that *Sphaerium rhomboideum* abundance was adversely impacted by sedimentation associated with agricultural runoff in a low-gradient southeast-USA stream. Hayes and Markarewicz (1982) also suggested that suspended sediment resulting from dredging activity in the St. Lawrence River, USA, was responsible for significant

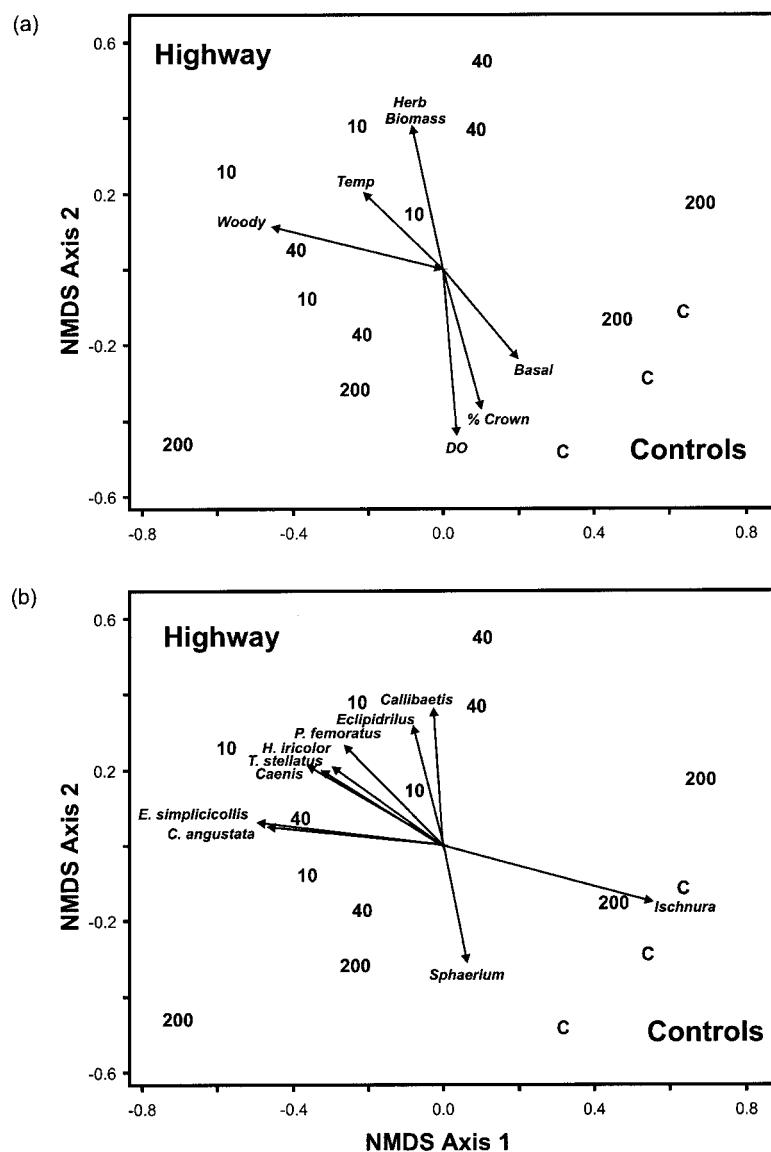


Figure 2. Nonmetric multi-dimensional scaling (NMDS) ordination of macroinvertebrate assemblage data collected among the 15 highway distance transects in Beaverdam and Kill Swamps, North Carolina. Characters reflect the highway distance category, where 10 = 10 m, 40 = 40 m, 200 = 200 m, and C = control. Arrows indicate significant vectors of maximum linear correlations between: (a) environmental variables and the configuration of transects based on assemblage composition, and, (b) taxon variables and the configuration of transects based on assemblage composition (see Table 4).

ant reductions in *Sphaerium corneum*. An additional study documented declining populations of fingernail clams related to organic pollution (and presumably lower dissolved oxygen) and altered, stagnant flow in the Mississippi River (Wilson et al., 1995). These results identify fingernail clams as sensitive to some types of human influence and suggest that they may be characteristic of high-quality forested wetlands.

Taxonomic richness, or the total number of unique taxa per unit area or number of individuals, is the most widely used metric in biological assessment protocols (e.g., Plafkin et al., 1989; Rosenberg and Resh, 1993). Using a reference site approach in the context of stream assessment, taxon richness values are generally expected to decrease with increasing levels of perturbation resulting from human influence (Karr

and Chu, 1997), although exceptions exist, particularly in unproductive habitats (Plafkin et al., 1989). We observed an increase in both areal and numerical richness as well as a decrease in % dominant taxon with decreasing distance from the highway crossings. Several factors may have been responsible for this phenomenon. We documented greater macrophyte biomass and volume of downed woody debris near the highway crossings, both of which provide ideal substrate for colonization. Woody snags have been well documented as critical habitat for invertebrate production in low-gradient coastal plain streams of the southeastern USA (Benke et al., 1985; Smock et al., 1985; Smock et al., 1989). Increased habitat complexity and patchiness among habitats, in terms of both macrophytes and woody debris, also has been shown to result in greater macroinvertebrate richness given the same surface area (e.g., Brown et al., 1988; O'Connor, 1991). These factors coupled with the increased algal resources and the high inherent tolerance to natural stressors present in wetland assemblages likely resulted in a subsidy effect (Odum et al., 1979) rather than a stress to the assemblage as a whole. This illustrates the importance of clearly defining the reference condition in wetland assessment (Brinson and Rheinhardt, 1996). We defined our reference condition as an area which most closely approximated the highway study areas before construction based on physical characteristics of the watersheds and pre-highway aerial photography. Reference did not necessarily connote 'pristine' or 'unimpacted' in our study, but rather a baseline condition from which potential highway impacts could be assessed. Although we observed significantly higher richness within 40 m of the highway, our supposition was that any significant deviation from reference was perturbation. Thus, taxon richness was an effective metric but potentially misleading if not carefully interpreted in the context of appropriate reference condition. Use of a robust composition metric in conjunction with ordination, such as NMDS, may aid in visualization and interpretation of richness metrics since assemblage dissimilarity incorporates changes in composition rather than just the number of taxa (Reynoldson et al., 1997). While lower or higher taxon richness values will be generally be reflected as an increase in dissimilarity from reference condition, dissimilarity will also increase with a turnover of taxa (β -diversity) that does not result in a change in overall richness.

The results of this study are encouraging from a biological assessment perspective and provide evi-

dence that macroinvertebrate assemblages can be used to detect environmental gradients and potentially diagnose sources of perturbation in wetlands. While our study was limited by a relatively small sample size and one reference area, our results suggest that the use of macroinvertebrates for wetland assessment warrants further exploration. Many state, federal, and other organizations cost-effectively employ biological monitoring programs in streams in conjunction with conventional water and habitat quality assessments. This complementary approach results in better management of water resources and is necessary to comply with the Clean Water Act. Biological and functional assessment approaches could likely be integrated in a similar complementary manner to increase our ability to preserve and protect the integrity of wetland ecosystems. Future macroinvertebrate research from additional forested wetlands as well as from other wetland classes is clearly needed, however, to help identify robust, diagnostic metrics of human influence.

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