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Macroinvertebrate Responses to a Gradient of Long-Term Nutrient Additions, Altered Hydroperiod, and Fire

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11.1 Introduction

The Everglades has been the sentinel ecosystem for illustrating the deleterious effects of agricultural land practices, fire suppression, and hydrological alterations on freshwater wetlands. Numerous studies have illustrated that Everglades biota are adapted for survival under highly oligotrophic conditions (e.g., Browder 1982; Steward and Ornes 1975a,b; Swift and Nicholas 1987; Richardson et al. 1999) and are strongly P limited (reviewed by Noe et al. 2002). Moreover, the natural Everglades ecosystem has evolved under dynamic hydrological conditions, with strong annual wet–dry cycles that are critically coupled with large, periodic fires (e.g., Davis 1994). Thus, it is not surprising that anthropogenic modifications to the natural nutrient, hydrological, and fire regimes of the Everglades during the past few decades have had remarkable effects on biota across all levels of ecological organization (Davis and Ogden 1994).

Although a variety of other human influences have been indicated as stressors to the Everglades, P-enriched runoff from the Everglades Agricultural Area (EAA) has been targeted as the chief offender (SFWMD 1992; Davis and Ogden 1994). The extensive canal and levee system that has compartmentalized the remnant Everglades has served as a conduit for P from the EAA and Lake Okeechobee, and water-control structures have been point sources of P to diked portions of the fen (SFWMD 1992, 2003, 2004, 2005, 2006).

In areas near water-control structures, P is primarily responsible for the transformation of the natural pattern of *Cladium jamaicense* Crantz (sawgrass) stands and open-water sloughs to dense stands of invasive *Typha domingensis* Pers. (cattail) and other invasive vegetation (Davis 1991; Urban et al. 1993; Newman et al. 1998; Richardson et al. 1999). Phosphorus inputs have also had profound effects on other Everglades biota, including microbes (Grimshaw et al. 1997; Qualls and Richardson 2000), periphyton (Vymazal et al. 1994; McCormick and O'Dell 1996; McCormick et al. 1996; Pan et al. 2000), invertebrates (Rader and Richardson 1994; King and Richardson 2002, 2003), vegetation (Richardson et al. 1999; King et al. 2004), and fish (Jordan 1996; Turner et al. 1999). The question is not whether the Everglades is changed by nutrient additions and hydrologic shifts but rather if current indices

or metrics of biotic response can provide an early detection system, especially for macroinvertebrates, which have been used as a fundamental index of ecosystem degradation and habitat loss in aquatic ecosystems (e.g., Rosenberg and Resh 1993; Karr and Chu 1997).

Bioassessment using macroinvertebrates as ecosystem indicators has become a widely accepted technique for monitoring water quality and ecological health of aquatic systems (Rosenberg and Resh 1993). Attributes of macroinvertebrate assemblages provide considerable information regarding levels and sources of impairment imposed by human influence (e.g., Karr and Chu 1997). Bioassessment is especially effective in lotic systems and is used to monitor environmental quality in streams throughout the world (e.g., Reynoldson et al. 1995; Zamora-Muñoz and Alba-Tercedor 1996; Bailey et al. 1998; Barbour et al. 1999; Moss et al. 1999; Smith et al. 1999). Until recently, however, the use of biota to assess ecological condition of lentic habitats like wetlands had not received much attention (US EPA 1997a). In the USA, several states (e.g., Apfelbeck 1999; Gernes and Helgen 1999) along with the US Environmental Protection Agency (Danielson 1998) have recognized the need for biologically grounded wetland assessment methods. Most wetland assessment techniques in use today are based on functional indicators that do not explicitly measure biological condition (e.g., Brinson and Rheinhardt 1996) despite the mandate of Section 101(a) of the Clean Water Act to restore and maintain the chemical, physical, and biological integrity of the USA's waters, which include wetlands. Such inconsistency with federal legislation has led to dissatisfaction with current wetland assessment methods (Kusler and Niering 1998) and a call for the development of methods that incorporate biological components, like macroinvertebrate assemblages, into assessment protocols (US EPA 1997a; King et al. 2000).

Although interest in wetland bioassessment is currently high, no accepted assessment protocols for wetlands have been developed and published like those that exist for streams (e.g., Barbour et al. 1999). Wetlands are, however, structurally and functionally very different from streams (e.g., Richardson 1999). Even the definition of what constitutes a wetland is a source of confusion and contention (e.g., Cowardin et al. 1979; USACE 1987). While there are exceptions, typical stream and wetland habitats differ markedly in permanence of surface water (predominantly permanent in streams vs. seasonal/semipermanent in wetlands), hydrologic gradient (high in streams vs. low-to-none in wetlands), sources of energy (mostly allochthonous in streams vs. autochthonous in wetlands), habitat structure (riffle/pool segments in streams vs. vegetated/unvegetated patches in wetlands), and water chemistry dynamics (comparatively stable water temperatures and dissolved oxygen (DO) in streams, dynamic fluxes in temperatures, and DO in wetlands) – many other differences can be added to this list. Thus, it is intuitive that structure and function of wetland invertebrate communities would differ from streams accordingly (Sharitz and Batzer 1999). Thus many of the models used to describe invertebrate community dynamics and bioassessments in streams have limited applicability to wetlands or, at least, require significant reevaluation before they can be used in the Everglades.

In addition to poorly known sensitivities to anthropogenic stressors (Batzer and Wissinger 1996) and relatively few established metrics of human influence (Lemly and King 2000), wetland macroinvertebrate assemblages present difficulties in sampling and sample processing that are less prevalent in lotic bioassessments. However, detailed studies of the methods used to evaluate and assess macroinvertebrate communities and develop bioassessment sampling criteria in the Everglades are presented in King and Richardson (2002, 2003). In this chapter, we highlight some of these key findings and synthesize macroinvertebrate responses to nutrient additions and other environmental variables such as hydrology and fire.

The three primary objectives of this phase of the research were to (1) evaluate the utility of wetland macroinvertebrate assemblages as an indicator group for bioassessment in the Everglades, with emphasis on the implications of differing laboratory methods of sample processing and levels taxonomic identification; (2) quantify the response of macroinvertebrate community biomass and species richness to P enrichment; and (3) identify major dimensions of community structure and the primary environmental factors controlling community organization. To address these objectives, two conceptual frameworks were used to guide the design of experiments and testing of hypotheses.

11.1.1 Subsidy–Stress Model

Odum et al. (1979) developed a conceptual model to describe ecological responses to system inputs (Fig. 11.1). These inputs may be usable (e.g., nutrients or energy) or acutely toxic (e.g., herbicides). Those inputs that are usable are initially hypothesized to result in a subsidy effect – a deviation above the system's normal operating range – while those that are acutely toxic, a stress effect. However, increasing concentrations or levels of usable inputs may eventually result in a decrease in system performance. Termed the "subsidy–stress gradient," this conceptual model may be usefully applied to predict community or ecosystem-level responses to P inputs in the Everglades since P is limiting and, therefore, represents a usable system input.

How might inputs of P affect wetland invertebrate assemblages in an unproductive, naturally dynamic environment? Most taxa documented in the Everglades (Rader and Richardson 1992, 1994) and most wetland ecosystems are adapted for harsh, often temporary conditions, hence have limits of tolerance (sensu Shelford 1913) that may far exceed stress presented by changes such as depressed dissolved oxygen due to eutrophication. Intuitively, stimulation of primary producers (i.e., food resources) by P inputs would initially result in a subsidy effect for invertebrate assemblages on a community level (e.g., biomass, species richness). However, this response may not hold true at high concentrations of P if such inputs result in the expansion of dense, invasive vegetation and a reduction in quantity of high-energy food resources such as periphyton. Here, a stress response may be expected. At population (species) levels, however, P could result in a subsidy, stress, or subsidy–stress effect depending on a variety of factors such as niche breadth and opportunistic

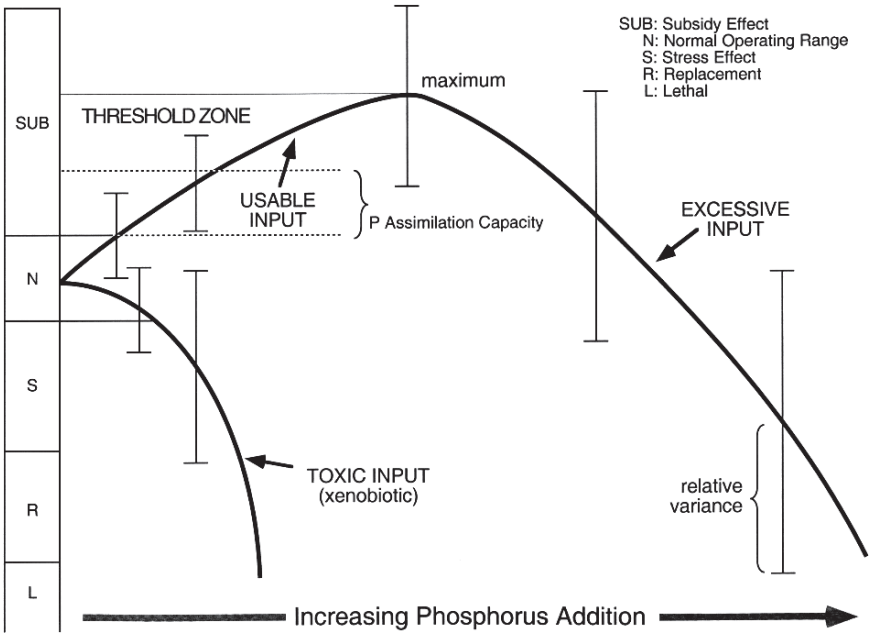

Fig. 11.1 Conceptual diagram of the subsidy–stress model illustrating hypothetical P–response relationship for invertebrate assemblages in the Everglades (modified from Odum et al. 1979)

nature of each taxon (e.g., specialist vs. generalist; Mihuc 1997). Thus, the subsidy–stress model is specific enough to be useful, but general enough to be inclusive of different types of perturbations that depend upon level of organization (Odum et al. 1979).

11.1.2 Hierarchy Theory

Hierarchy theory is a broad theory about the relationships between ecological processes and spatial and temporal scales and patterns observed across landscapes (Allen and Star 1982; O'Neill et al. 1986). More specifically, it is a conceptual framework that describes the ecological coupling of pattern at multiple scales – how pattern at lower-level scales can interact to give rise to pattern at higher levels. In a hierarchical system, lower-level units (e.g., patches of vegetation) can be thought of as small and relatively fast moving entities through time and space, while higher-level patterns are larger and slower (Urban et al. 1987). Lower-level units integrate to generate higher-level pattern, but higher-level pattern controls those at lower levels.

In the Everglades, vegetation pattern in low-P, unimpacted areas has been described as a mosaic that varies markedly at fine scales but relatively little at coarse scales across the landscape (Jordan et al. 1997; King et al. 2004). This mosaic pattern undoubtedly plays a significant role in ecological processes and vice versa (Watt 1947). For example, fine-scale heterogeneity may be important for seasonal (Jordan 1996) or even diel (King and Wrubleski 1998) movements of invertebrates, while coarse patterns may influence dispersal across the landscape (e.g., Gilpin and Hanski 1991; Delettre and Morvan 2000; Palmer et al. 2000). Moreover, fine-scale heterogeneity may sustain unique, local assemblages and, consequently, increased species diversity (e.g., MacArthur and Wilson 1967). Therefore, alteration to this characteristic mosaic due to P inputs could be a substantial perturbation to invertebrate assemblages across all levels of the spatial hierarchy. Only a hierarchical perspective (sensu Urban et al. 1987) could reveal all of the possible implications of P enrichment to invertebrate assemblages across this large wetland landscape.

11.2 Methods

11.2.1 Study Area and Sampling Design

Sampling was conducted in Water Conservation Area 2A (WCA-2A) in the northern Everglades (Fig. 11.2). A detailed description of the study area is presented in Chaps. 5 and 9, and King et al. (2004). The spatial component of this study used the sampling design described by King et al. (2004). All vegetation plots (n = 126 plots, n = 14 plot-clusters) were included in this component of the study. Data collection for the spatial study was conducted on 20–29 October 1998. In addition to the October 1998 collection, a temporal study was conducted as well, but space does not allow presentation of those results in this chapter (but see King 2001). In the temporal study, plots within 3 of the 14 clusters (one cluster in each of the P-impacted, transition, and reference zones, respectively) were sampled during February 1999 (low water, dry season), July 1999 (immediately after reflooding following an extensive period of no surface water), and October 1999 (deep water, wet season, 1 year after first collection).

11.2.2 Abiotic Variables

Spatial, soil/sediment chemical, hydrological, and fire frequency variables were considered to be potentially important dimensions of vegetation and invertebrate assemblage organization along the P gradient (King et al. 2004). In October 1998, values of 14 spatio-environmental variables were estimated from each of 126 plots

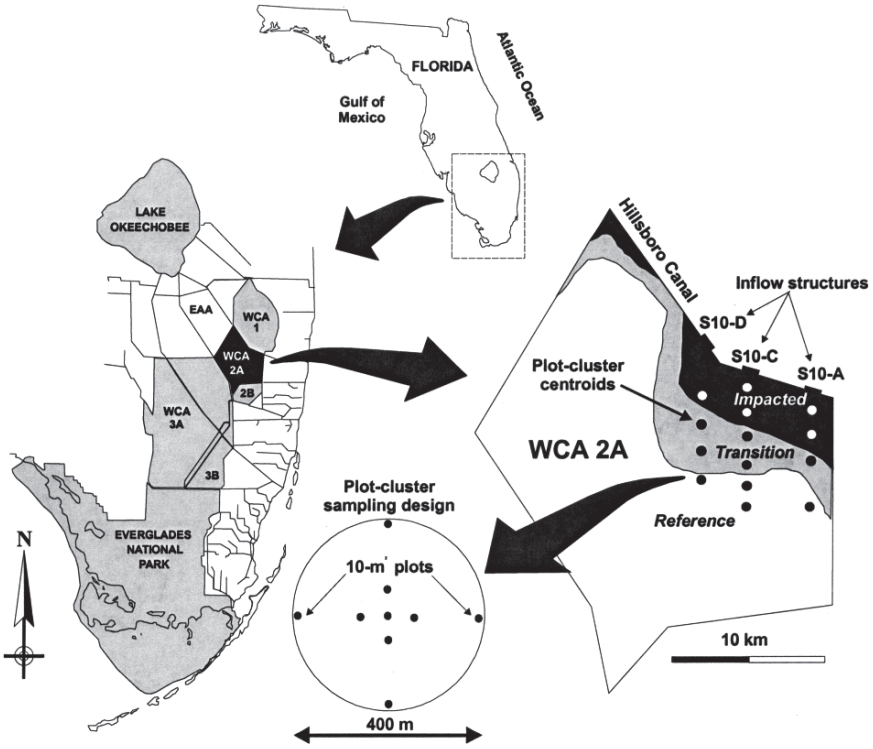

Fig. 11.2 Map of south Florida showing the location of Water Conservation Area 2A (WCA-2A); impacted, transition, and reference landscape zones; locations of S-10 water-control structures; centroids of sampling clusters; and plot-cluster sampling design. *EAA* Everglades Agricultural Area

in the spatial study (see Table 11.1). Greater details on the rationale and methods of measurement for all of these variables are provided in King et al. (2004).

Of the abiotic variables, hydrology was the only one expected to change markedly over time (soil chemistry along the P gradient has remained similar over the past decade) (see Chap. 6; no fires occurred at the three temporal clusters during the study). Water depth at each plot within each cluster was estimated using a hydrological model developed by Romanowicz and Richardson (1997) (see Chap. 7). Depths (cm) were estimated daily through the end of the study period in October 1999 (King 2001).

11.2.3 Biotic Sampling

Vegetation species composition and cover was estimated at each plot in the spatial and temporal studies. Cover for each species was recorded using Braun-Blanquet

Table 11.1 Mean (±1 SD) environmental characteristics of impacted, transition, and unimpacted landscape zones in WCA-2A of the Everglades (from King et al. 2004)

Variable	ID	Units	Impacted (n = 45)	Transition (n = 45)	Unimpacted (n = 36)
Distance from canal inflow structures	Canal	m	2,495 ± 869	5,541 ± 914	9,050 ± 924
Total carbon (soil)	C	g kg^{-1}	435.8 ± 20.3	435.9 ± 27.2	428.2 ± 47.7
Total calcium (soil)	Ca	g kg^{-1}	37.1 ± 1.7	42.8 ± 2.1	47.0 ± 3.5
Total potassium (soil)	K	g kg^{-1}	0.6 ± 0.2	0.6 ± 0.4	0.5 ± 0.2
Total magnesium (soil)	Mg	g kg^{-1}	3.7 ± 0.8	3.9 ± 0.9	3.6 ± 0.9
Total sodium (soil)	Na	mg kg^{-1}	3,058 ± 160	2,900 ± 173	2,165 ± 113
Total nitrogen (soil)	N	g kg^{-1}	29.2 ± 2.2	29.0 ± 3.7	29.2 ± 4.4
Total phosphorus (soil)	P	mg kg^{-1}	1,430 ± 172	1,203 ± 181	578 ± 151
Water depth (1981–1998)	xDepth	cm	29.0 ± 8.7	32.3 ± 9.6	31.2 ± 11.4
Water depth (1 year)	xDepth1y	cm	35.7 ± 8.3	41.8 ± 9.6	46.4 ± 10.4
Interquartile range, water depth (1981–1998)	IQR(Depth)	cm	28.2 ± 0.9	29.7 ± 1.5	33.6 ± 0.8
Frequency, water depth <−10 cm (1981–1998)	Freq. <−10 cm	%	3.1 ± 3.0	3.1 ± 2.4	6.0 ± 4.5
Fire index (frequency 1981–1998)	Fire	Sum[a]	0.2 ± 0.4	0.4 ± 0.5	0.3 ± 0.5

[a]Sum of total number of fires during 1981–1998, weighted as $1/\log_{10}(t + 1)$, where t is the time (years) since fire, for each fire

cover classes (Phillips 1959). Additional details on vegetation sampling are provided in King et al. (2004). Presence and abundance of periphyton was hypothesized to be an important determinant of invertebrate assemblage biomass and composition. Two periphyton abundance metrics were estimated: metaphyton (floating periphyton mats) cover and epiphyton (vegetatively attached periphyton) biomass accumulation. Metaphyton cover (hereafter, metaphyton) was estimated using Braun-Blanquet cover classes. Metaphyton samples were used to estimate molar C:N and C:P ratios of periphyton, important measures of food quality and potential elemental imbalance between consumers and their food (Sterner and Elser 2002).

Macroinvertebrate sampling was based on a slight modification of protocols used by the state of Florida (FDEP 1996; FDEP SOP #BA-7) and the US EPA (US EPA 1997b; Barbour et al. 1999) for bioassessment. A D-framed dip net (0.3-m wide, 500-µm mesh) was used to collect ten sweeps of 0.5-m length within each plot (total area 1.5 m^2). Because the initial sweep may have dislodged but missed organisms, the sweeping process was repeated rapidly two additional times over the same area (US EPA 1997b; Maxted et al. 2000). Contents of all ten sweeps were composited into a 500-µm mesh sieve bucket, rinsed to remove fine particulates, placed in 4-l heavy-duty storage bags, and put on ice for return to the laboratory. In the laboratory, samples were weighed for wet mass and preserved in 5% (v/v)

buffered formalin stained with rose bengal. The method was quite repeatable and employed consistently throughout the study.

11.2.4 Macroinvertebrate Sample Processing: Subsampling and Taxonomic Resolution

Macroinvertebrate sample processing followed techniques recommended by FDEP (1996; FDEP SOP #BA-8) and Barbour et al. (1999). Samples were rinsed and homogenized in a 500-µm mesh sieve and large pieces of coarse particulate organic matter (CPOM) were discarded. Sieve contents were placed in a 20-cm wide × 45-cm long subsampling pan, and gently spread evenly throughout. The subsampling pan was gridded with numbered 5 × 5 cm^2 cells (36 cells total). Cells were selected for subsampling using a random number table. Individual cell contents were transferred to a petri dish marked with grooves into 1/8 sections. One 1/8-cell subsample of material was removed at a time, placed into a second petri dish, and a small amount of water was added to suspend all contents. Subsequently, invertebrates were picked from the subsample using a stereomicroscope at 10× magnification. The process was repeated until a target area or number of individuals was obtained.

We selected three fixed-count (100, 200, and 300 individuals) and two fixed-area (10 and 25%) levels of subsampling for comparison. We chose fixed counts and fixed areas most commonly used in other bioassessment studies. We recognized that evaluations of fixed areas, by themselves, might be of limited utility to biologists because few have agreed on a standard sample size to be used (e.g., Courtemanch 1996; Larsen and Herlihy 1998). However, evaluated in the context of average numbers of individuals per subsample and average proportions of the total sample sorted, these fixed-area subsamples were similar to the fixed-count subsamples and allowed for valid comparisons among approaches.

Upon reaching a specified number of individuals or area for a respective subsample level, specimens were placed in a vial containing 70% ethanol. Total area, time required to complete, and number of individuals were noted. Larger subsamples (e.g., 300 individuals) were actually an accumulation of specimens stored in several vials, each representing a previous stopping point for other subsamples.

We implemented a supplementary large rare (LR) search as defined by Courtemanch (1996) once 300 individuals and 25% of the total sample were subsampled. However, rather than pick all LR taxa from a sample before subsampling, as recommended by Courtemanch (1996), we picked remaining LR taxa after all subsampling was completed because to remove them prior to subsampling would have altered the composition of subsamples and prevented a valid assessment of the use of the LR search as a supplementary procedure. We defined a priori all LR taxa so that individuals included as part of a larger subsample (e.g., 25%) could be added into the pool of LR individuals for smaller subsamples that included the LR search (e.g., 100 + LR). For example, a 100 + LR subsample might only represent

5% of the total sample area for the fixed-count component. Subsequently, some LR taxa could be contained in the following subsamples of 200, 10%, 300, and 25% and would need to be counted in the final tally of additional LR organisms to be added to the 100 + LR subsample. Thus, any LR taxa in the 200, 10%, 300, and 25% subsamples would have to be added to the remaining LR search for the 100 + LR subsample to be accurate and valid. We classified large mollusks, hemipterans, hirudineans, coleopterans, decapods, all anisopteran odonates, and a few miscellaneous large taxa as LR taxa. We calculated densities for LR taxa on the basis of the total number of individuals per sample, not the fractional area of individual subsamples in which LR taxa were supplemented.

We assembled macroinvertebrate data sets using the five basic levels of subsampling (100, 200, 300, 10%, and 25%), an integrated subsample requiring a minimum fixed count and fixed area in the same subsample (100 and 10%), and a fixed-count (100) and fixed-area (10%) subsample supplemented with the LR search. Data sets also were assembled using three levels of taxonomic resolution (family, genus, and species) for each subsampling level, thus totaling 24 sets. Each level of taxonomy connoted the lowest level achieved for most identifications. Data were densities (no. m^{-2}) of each taxon for each of the 126 plots sampled.

We evaluated the importance of identifying Chironomidae beyond family level by constructing three tiered data sets (1) non-Chironomidae family-level data tiered with species-level Chironomidae data, (2) non-Chironomidae genus-level data tiered with family-level Chironomidae data, and (3) non-Chironomidae species-level data tiered with family-level Chironomidae data. These tiered sets were compared with family-, genus-, and species-level data sets. A representative midsized subsample (200 count + LR) was used.

11.2.5 Macroinvertebrate Sample Processing: Biomass Estimation

Macroinvertebrate biomass was estimated for every plot in the spatial and temporal studies. Every individual of most taxa was measured to the nearest 0.5 mm. These measurements were used in taxon-specific length–mass regression equations to estimate individual dry mass (Kushlan et al. 1986; Meyer 1989; Sample et al. 1993; Benke et al. 1999). Biomass of taxa that either did not have published length–mass equations or were very small was estimated using a biovolume technique (Smit et al. 1993). For small taxa, particularly some Chironomidae and Oligochaeta, individuals were enumerated into taxon-specific size classes, based on length and width – these size classes were used to estimate dry mass using biovolume. Biomass of Gastropoda (other than *Pomacea paludosa*; Kushlan et al. 1986) was also estimated using biovolume since few length–mass equations were published to estimate flesh mass (excluding shell mass). Proximate geometric shapes and measured dimensions of tissue of individual gastropods were used to estimate biovolume and dry mass. Densities (no. m^{-2}) of each taxon were used to calculate biomass (mg dry wt m^{-2}) for each plot.

Invertivorous fish were expected to be a possible determinant of invertebrate assemblage biomass and composition. Fish abundance and biomass were estimated using the same dip-net samples used to collect invertebrates. Because of dynamic hydrology, small, surface-oriented taxa dominate the fish assemblage in the Everglades (Jordan 1996; Turner et al. 1999), and the dip-net approach has been shown to be an effective technique for estimating abundance of these fishes (Rader and Richardson 1994). Fish were exhaustively picked from each sample, enumerated, measured for total length, and identified to species. Total length was used to estimate biomass (mg dry wt) (Kushlan et al. 1986). Taxa that were predaceous or omnivorous were classified as invertivorous. Densities and biomass (no. m^{-2} and mg dry wt m^{-2}, respectively) of insectivorous fish were subsequently estimated for each plot.

11.2.6 Data Analysis: Effects of Subsampling and Taxonomic Resolution on Bioassessment

We compared the magnitude of assemblage–environment relationships among subsampling approaches and levels of taxonomic resolution using the multivariate Mantel test (Mantel 1967), which measures the correlation between distance matrices. Increasing magnitude in Mantel *r*, the test statistic, reflects a stronger correlation. Mantel *r* typically ranges from 0.1 to 0.3 for assemblage–environment relationships that are ecologically significant and infrequently exceeds 0.5 because the analysis is based on the full rather than reduced dimensionality (e.g., ordination-axis scores) in the assemblage data (e.g., Leduc et al. 1992; Sanderson et al. 1995; Foster et al. 1999). We selected distance from canal inflow structures (hereafter, Canal) as predictor of macroinvertebrate assemblage composition because (1) it was a surrogate for a wide range of biogeochemical, hydrological, and habitat–structural variables that substantially change along this eutrophication gradient (Table 11.1) and (2) it was the best predictor of biological changes in this study area (King 2001). Canal (m) was converted to a distance matrix using Euclidean distance, whereas assemblage matrices used Bray–Curtis dissimilarity as the distance metric (Legendre and Legendre 1998). Bray–Curtis dissimilarity was selected because it is one of the most robust and ecologically interpretable distance metrics available (e.g., Faith et al. 1987; Legendre and Anderson 1999; Hawkins and Norris 2000). All macroinvertebrate density data were $\log_{10}(x + 1)$ transformed prior to conversion to distance matrices to give greater weight to less-abundant taxa (Legendre and Legendre 1998).

We estimated 95% confidence intervals (CIs) for each test statistic using bootstrapping, a resampling method (Manly 1997), rather than qualitatively comparing the magnitude of Mantel *r* statistics among subsamples and taxonomic levels. We resampled (with replacement) distance matrices at a level of 90%, with 1,000 resamples (Manly 1997). Mantel *r* statistics were considered significantly different if 95% CIs did not overlap (Manly 1997; Johnson 1999). We also evaluated whether Mantel *r* statistics were significantly different from 0 ($p < 0.05$) using 10,000 random

permutations (Manly 1997); however, this test was merely an antecedent to the more relevant comparison of uncertainty (95% CI) among assemblage–environment correlations (Suter 1996; Germano 1999; Johnson 1999). Mantel tests and bootstrapping were done using S-Plus 5.0 for Unix (Mathsoft, Inc., Seattle, WA, USA).

11.2.7 Data Analysis: Diversity and Biomass in Relation to P

Biomass of the complete invertebrate assemblage, as well as of the common coarse-taxonomic groups (classes or orders), was plotted and regressed against distance from canal inflow structures and sediment total phosphorus (TP) to evaluate assemblage response relationships to the P gradient. Averages of biomass among all plots ($n = 9$) within each plot-cluster ($n = 14$) were used as replicates since these represented an estimate of the biomass across a large spatial area (weighted by vegetation pattern) rather than at individual plots (Allen and Wyleto 1983; Turner et al. 1999). Distance from canal (m) was based on the centroid of each cluster, while sediment TP was an average value from all plots within each plot-cluster. Since distance from canal produced results very similar to that of sediment TP for total biomass, all regressions were subsequently based only on sediment TP. All biomass data were $\log_{10}(x)$ or $\log_{10}(x + 1)$ transformed prior to averaging and analysis.

Assemblage diversity was evaluated using species density (number of taxa/fixed-area subsample) and species richness (total number of taxa/cluster of plots) (Hurlbert 1971; Larsen and Herlihy 1998). Species density was averaged among the nine plots per cluster, while species richness was the total accumulation of unique taxa among the nine plots per cluster. Species richness was estimated using data produced from the tiered 25% fixed-area/300 fixed-count + LR search subsampling approach (Vinson and Hawkins 1996). Species density and richness were regressed against sediment TP. To characterize diversity and distribution of invertebrate species at a landscape scale, species accumulation curves were generated using species-richness data from each plot. Accumulation curves were stratified by impact zone to examine differences in diversity among landscape regions of differing vegetation and nutrient status. Curves provided a visual assessment of average fine-scale (plot) as well as broad-scale (cluster and impact zone) species richness. Asymptotic species richness (S_{jack}) was also estimated for each curve using a first-order jackknife procedure (Palmer 1990) to provide a better evaluation of the total expected number of species per zone. Accumulation curves and jackknife estimates were performed using PC-ORD 4.09.

11.2.8 Data Analysis: Abiotic and Biotic Drivers of Macroinvertebrate Community Structure

Two complementary procedures were used to determine the primary dimensions of invertebrate assemblage organization across the landscape. First, plots and species

were ordinated based on species composition using nonmetric multidimensional scaling (nMDS; Minchin 1987). Ordination provided a visual assessment of gradients in species composition among and within impact zones. Bray–Curtis dissimilarity was used as the distance metric, a coefficient shown to be one of the most robust and ecologically meaningful (Faith et al. 1987). Log-transformed density data ($\log_{10}$[no. m^{-2}]) for each taxon was used in calculation of dissimilarities rather than biomass data because preliminary results using densities indicated that it was slightly more robust (lower stress – an indicator of goodness-of-fit). Once plots were ordinated, species centroids were mapped into ordination space using weighted-averaging (Legendre and Legendre 1998). Ordinations were limited to two or three dimensions, as stress values were relatively low and exhibited small decreases at higher dimensionality.

To relate abiotic and biotic variables to gradients in composition in nMDS ordinations, rotational vector fitting was used (Faith and Norris 1989). Vector fitting was performed on all ordinations. Abiotic and biotic values from each plot were used in fine-scale vector fitting, while average values from within each plot-cluster were used in the coarse-scale analysis. For vegetation data, dominant species (e.g., cover of *Typha*, *Cladium*) and structural groups (e.g., cover of unrooted floating species) were used as predictors. Density of invertivorous fish was used instead of biomass because it showed a stronger relationship to composition. Significance (Bonferroni-corrected $p \leq 0.05$) of vectors was estimated using 10,000 random permutations. Ordination and vector fitting were performed using DECODA 2.05 (University of Melbourne, Parkville, Victoria, Australia).

To assess sensitivities or affinities of invertebrate species among impact zones and help explain patterns of diversity, Indicator Species Analysis was used (INSPAN; Dufrêne and Legendre 1997). INSPAN is a nonparametric technique used to identify species with a high fidelity for a particular group or class, as defined by the user. The three impact zones were used as classes for the analysis. Significance (Bonferroni-corrected $p \leq 0.05$) of indicator values was estimated using 10,000 random permutations (Manly 1997). INSPAN was performed using PC-ORD 4.09 (MjM Software, Gleneden Beach, OR, USA).

11.3 Results

11.3.1 Subsample Characteristics and Taxonomic Structure

Over 78,000 individuals were identified across all 126 plots in the spatial study during October 1998, and additional 66,000 individuals were identified during the temporal study. A total of 93 families, 181 genera, and 272 unique taxa (species or morphospecies; lowest level of taxonomy achievable) were identified (see Table 11.2 for a complete list of macroinvertebrates identified in the Everglades gradient and dosing studies). Coleopterans, dipterans, gastropods, odonates, and oligochaetes were the

Table 11.2 List of invertebrate taxa collected from the P gradient (spatial and temporal) and P-dosing studies in the Everglades

Group	Family	Code	Taxon	Gradient		
				Spatial	Temporal	Dosing
Amphipoda	Crangonycitidae	CRANGONY	*Crangonyx* nr. *richmondensis* Ellis	x	x	
Amphipoda	Hyalellidae	HYALAZTE	*Hyalella azteca* (Saussure)	x	x	x
Anomopoda	Chydoridae	CAMPTOCE	*Camptocercus* sp.			x
Anomopoda	Chydoridae	CHYDOSP1	Chydoridae sp. 1			x
Anomopoda	Chydoridae	CHYDOSP2	Chydoridae sp. 2			x
Anomopoda	Chydoridae	CHYDOSP3	Chydoridae sp. 3			x
Anomopoda	Chydoridae	CHYDORID	Chydoridae spp.	x	x	
Anomopoda	Daphniidae	CERIODAP	*Ceriodaphnia* sp.	x	x	x
Anomopoda	Daphniidae	DAPHNSP1	Daphnidae sp. 1	x	x	
Anomopoda	Daphniidae	SIMOCEPH	*Simocephalus* sp.			x
Anomopoda	Macrothricidae	ILYOSPIN	*Ilyocryptus spinifer* Herrick	x	x	x
Anomopoda	Macrothricidae	MACROTHR	Macrothricidae sp. 1	x	x	x
Anomopoda	Macrothricidae	OPHRYOXU	*Ophryoxus* sp.			x
Arhynchobdellida	Erpobdellidae	MOORMELA	*Mooreobdella melanastoma* Sawyer and Shelley	x		
Arhynchobdellida	Erpobdellidae	MOORMICR	*Mooreobdella microstoma* (Moore)	x	x	x
Arhynchobdellida	Erpobdellidae	MOORTETR	*Mooreobdella tetragon* Sawyer and Shelley	x	x	
Arhynchobdellida	Hirudinidae	MACRDITE	*Macrobdella ditetra* Moore	x	x	
Arhynchobdellida	Hirudinidae	PHILOBDE	*Philobdella* sp.	x		
Arynchobdellida	Haemopidae	HAEMSEPT	*Haemopsis septagon* (Sawyer and Shelley)	x		
Bivalvia	Sphaeriidae	SPHAERIU	*Sphaerium* sp.	x	x	
Coleoptera	Curculionidae	CURCULIO	Curculionidae sp.	x	x	
Coleoptera	Dytiscidae	CELIANGU	*Celina angustata* Aube	x	x	
Coleoptera	Dytiscidae	CELIIMIT	*Celina imitatrix* Young	x	x	
Coleoptera	Dytiscidae	CELISLOS	*Celina slossoni* Mutchler	x		
Coleoptera	Dytiscidae	CELINA.L	*Celina* sp. larva	x	x	x
Coleoptera	Dytiscidae	CYBIFIMB	*Cybister fimbriolatus* Wilke	x	x	
Coleoptera	Dytiscidae	DESMOPAC	*Desmopachria* sp.	x	x	

(continued)

Table 11.2 (continued)

Group	Family	Code	Taxon	Gradient		
				Spatial	Temporal	Dosing
Coleoptera	Dytiscidae	HYDROPOR	*Hydroporus* sp.	x		
Coleoptera	Dytiscidae	HYDROPOR	*Hydroporus* sp. larva	x		
Coleoptera	Dytiscidae	HYDRPUST	*Hydrovatus pustulatus compressus* Sharp	x	x	
Coleoptera	Dytiscidae	HYDROV.L	*Hydrovatus* sp. larva	x	x	
Coleoptera	Dytiscidae	LACCGENT	*Laccophilus gentilis gentilis* LeConte	x	x	
Coleoptera	Dytiscidae	LACCPROX	*Laccophilus proximus* Say	x		
Coleoptera	Dytiscidae	LACCOP.L	*Laccophilus* spp. larva	x	x	
Coleoptera	Dytiscidae	MATUOVAT	*Matus ovatus blatchleyi* Leech	x		
Coleoptera	Dytiscidae	NEOPORUS	*Neoporus* sp.		x	
Coleoptera	Gyrinidae	GYRIELEV	*Gyrinus elevatus* LeConte		x	
Coleoptera	Gyrinidae	GYRINU.L	*Gyrinus* sp. larva	x	x	
Coleoptera	Haliplidae	HALIPL.L	*Haliplus* sp. larva	x		
Coleoptera	Hydraenidae	HYDRAE.L	*Hydraena* sp. larva		x	
Coleoptera	Hydrophilidae	BEROINFU	*Berosus infuscatus* LeConte	x		
Coleoptera	Hydrophilidae	BEROSU.L	*Berosus* sp. larva	x	x	x
Coleoptera	Hydrophilidae	DERAALTU	*Derallus altus* (LeConte)	x	x	
Coleoptera	Hydrophilidae	ENOCBLAT	*Enochrus blatchleyi* (Fall)	x		x
Coleoptera	Hydrophilidae	ENOCHAMI	*Enochrus consortus* Green	x		
Coleoptera	Hydrophilidae	ENOCCONS	*Enochrus hamiltoni* (Horn)		x	
Coleoptera	Hydrophilidae	ENOCOCHR	*Enochrus ochraceus* (Melsheimer)	x	x	x
Coleoptera	Hydrophilidae	ENOCPYGM	*Enochrus pygmaeus pygmaeus* (Fabricius)	x		
Coleoptera	Hydrophilidae	ENOCSAYI	*Enochrus sayi* Gunderson	x		x
Coleoptera	Hydrophilidae	ENOCHR.L	*Enochrus* spp. larva	x	x	x
Coleoptera	Hydrophilidae	HELOLARV	*Helobata larvalis* (Horn)	x	x	
Coleoptera	Hydrophilidae	HYDRCAST	*Hydrobiomorpha casta* (Say)	x	x	
Coleoptera	Hydrophilidae	PHAEEXST	*Phaenonotum exstriatum* (Say)	x	x	
Coleoptera	Hydrophilidae	PHAEMINO	*Phaenonotum minor* Smetana	x	x	
Coleoptera	Hydrophilidae	PHAENO.L	*Phaenonotum* spp. larva	x	x	

Coleoptera	Hydrophilidae	TROPBLAT	*Tropisternus blatchleyi blatchleyi* d'Orchymont	x	x	
Coleoptera	Hydrophilidae	TROPLATE	*Tropisternus lateralis nimbatus* (Say)	x	x	
Coleoptera	Hydrophilidae	TROPIS.L	*Tropisternus* spp. larva	x	x	
Coleoptera	Lampyridae	LAMPYRID	Lampyridae sp. larva	x	x	
Coleoptera	Noteridae	HYDROBLO	*Hydrocanthus oblongus* Sharp	x	x	
Coleoptera	Noteridae	HYDRREGI	*Hydrocanthus regius* Young	x		
Coleoptera	Noteridae	HYDCAN.L	*Hydrocanthus* spp. larva	x	x	
Coleoptera	Noteridae	SUPHINFL	*Suphis inflatus* LeConte	x		
Coleoptera	Noteridae	SUPHGIBB	*Suphisellus gibbulus* (Aube)	x	x	
Coleoptera	Noteridae	SUPHPUNC	*Suphisellus puncticollis* Crotch	x		
Coleoptera	Noteridae	SUPHISEL	*Suphisellus* spp. larva	x	x	
Coleoptera	Scirtidae	SCIRTES	*Scirtes* sp.	x	x	
Coleoptera	Staphylinidae	STAPHYLI	Staphylinidae sp.	x	x	
Collembola	Entomobryidae	ENTOMOBR	*Entomobrya* sp.	x	x	
Collembola	Isotomuridae	ISOTOMA	*Isotoma* sp.	x	x	
Collembola	Sminthuridae	SMINTHRI	*Sminthurides* sp.	x	x	
Collembola	Sminthuridae	SMINTHRU	*Sminthurus* sp.	x	x	
Copepoda	Calanoida	CALANOID	Calanoida	x	x	x
Copepoda	Cyclopoida	CYCLPOID	Cyclopoida	x	x	x
Copepopda	Harpacticoida	HARPACTI	Harpacticoida	x	x	x
Ctenopoda	Sididae	PSEUDOSI	*Pseudosida bidentata* Herrick	x	x	x
Decapoda	Cambaridae	PROCALLE	*Procambarus* cf. *alleni* (Faxon)			x
Decapoda	Cambaridae	PROCFALL	*Procambarus fallax* (Hagen)	x	x	
Decapoda	Palaemonidae	PALAPALU	*Palaemonetes paludosus* (Gibbes)	x	x	x
Diptera	Ceratopogonidae	ATRICHOP	*Atrichopogon* sp.	x	x	
Diptera	Ceratopogonidae	BEZZSP1	*Bezzia/Palpomyia* gr. sp. 1	x	x	x
Diptera	Ceratopogonidae	BEZZSP2	*Bezzia/Palpomyia* gr. sp. 2	x	x	
Diptera	Ceratopogonidae	BEZZSP3	*Bezzia/Palpomyia* gr. sp. 3	x	x	
Diptera	Ceratopogonidae	CERATOPO	*Ceratopogon* sp.	x	x	
Diptera	Ceratopogonidae	CULICOID	*Culicoides* sp.		x	
Diptera	Ceratopogoridae	DASYNELE	Dasyhelea sp.	x	x	x

(continued)

Table 11.2 (continued)

Group	Family	Code	Taxon	Gradient		
				Spatial	Temporal	Dosing
Diptera	Ceratopogonidae	FORCMYIA	*Forcipomyia* sp.	x	x	
Diptera	Ceratopogonidae	PROBEZZI	*Probezzia* sp.	x	x	
Diptera	Chironomidae	ABLAPELE	*Ablabesmyia peleensis* (Walley)	x		
Diptera	Chironomidae	ABLARHAM	*Ablabesmyia rhampe* Sublette gr.	x	x	x
Diptera	Chironomidae	APEDELAS	*Apedilum elaschitus* Townes	x	x	x
Diptera	Chironomidae	BEARTRUN	*Beardius truncatus* gr. sp. Reiss & Sublette	x	x	
Diptera	Chironomidae	CHMUSSP	*Chironomus* sp.	x	x	x
Diptera	Chironomidae	CHIRSTIG	*Chironomus stigmaterus* Say	x	x	x
Diptera	Chironomidae	CLADOPEL	*Cladopelma* sp.	x	x	x
Diptera	Chironomidae	CLADOTAN	*Cladotanytarsus* sp.	x	x	x
Diptera	Chironomidae	CORYSPD	*Corynoneura* sp. D Epler	x	x	x
Diptera	Chironomidae	CRICSYLV	*Cricotopus sylvestris* Fabricius gr.	x	x	x
Diptera	Chironomidae	DICROMOD	*Dicrotendipes modestus* (Say)	x	x	x
Diptera	Chironomidae	DICROSIM	*Dicrotendipes simpsoni* Epler	x	x	
Diptera	Chironomidae	DICROSP	*Dicrotendipes* sp.	x		
Diptera	Chironomidae	DICROSPA	*Dicrotendipes* sp. A Epler	x		
Diptera	Chironomidae	DJALPULC	*Djalmabatista pulchra* (Johannsen)		x	
Diptera	Chironomidae	ENDONIGR	*Endochironomus nigricans* (Johannsen)	x	x	x
Diptera	Chironomidae	ENDOHESP	*Endotribelos hesperium* (Sublette)	x	x	x
Diptera	Chironomidae	FITTSERT	*Fittkauimyia serta* (Roback)	x	x	
Diptera	Chironomidae	GLYPTOSP	*Glyptotendipes* sp.		x	
Diptera	Chironomidae	GOELDCAR	*Goeldichironomus carus* (Townes)	x	x	x
Diptera	Chironomidae	GOELDNAT	*Goeldichironomus* cf. *natans* Reiss	x	x	x
Diptera	Chironomidae	GOELDHOL	*Goeldichironomus holoprasinus* (Goeldi)	x	x	x
Diptera	Chironomidae	KIEFFDUX	*Kiefferulus dux/pungens* gr. sp.	x	x	
Diptera	Chironomidae	KIEFFSPA	*Kiefferulus* sp. A Epler	x	x	
Diptera	Chironomidae	LABRBECK	*Labrundinia becki* Roback	x	x	
Diptera	Chironomidae	LABRNEOP	*Labrundinia neopilosella* Beck and Beck	x	x	x

Diptera	Chironomidae	LARSBERN	*Larsia berneri* Beck & Beck	x	x	
Diptera	Chironomidae	LARSDECO	*Larsia decolorata* (Malloch)	x	x	x
Diptera	Chironomidae	LIMNOPHY	*Limnophyes* sp.		x	
Diptera	Chironomidae	MONOBOLI	*Monopelopia boliekae* Beck and Beck	x	x	
Diptera	Chironomidae	NANOALTE	*Nanocladius alternantherae* Dendy & Sublette	x	x	x
Diptera	Chironomidae	NILOTHAU	*Nilothauma* sp.	x	x	x
Diptera	Chironomidae	PARACALA	*Parachironomus alatus* (Beck)	x	x	x
Diptera	Chironomidae	PARACDIR	*Parachironomus directus* (Dendy and Sublette)	x	x	x
Diptera	Chironomidae	PARACMON	*Parachironomus monochromus/tenuicaudatus* gr.		x	
Diptera	Chironomidae	PARAKSPC	*Parakiefferiella* sp. C Epler	x	x	x
Diptera	Chironomidae	PARAMERI	*Paramerina* sp.	x	x	
Diptera	Chironomidae	PARASPB	*Paratanytarsus* sp. B Epler	x	x	x
Diptera	Chironomidae	PARASPC	*Paratanytarsus* sp. C Epler	x		
Diptera	Chironomidae	POLYHALT	*Polypedilum halterale* (Coquillett) gr.	x	x	x
Diptera	Chironomidae	POLYILLI	*Polypedilum illinoense* (Malloch) gr.	x	x	x
Diptera	Chironomidae	POLYPSPA	*Polypedilum* sp. A Epler	x	x	x
Diptera	Chironomidae	POLYTRIG	*Polypedilum trigonus* Townes	x	x	x
Diptera	Chironomidae	POLYTRIT	*Polypedilum tritum* (Walker)	x	x	
Diptera	Chironomidae	PROCLAD	*Procladius* (*Holotanypus*) sp.	x	x	x
Diptera	Chironomidae	PSEUDOCH	*Pseudochironomus* sp.	x	x	x
Diptera	Chironomidae	TANYPCAR	*Tanypus carinatus* Sublette	x	x	x
Diptera	Chironomidae	TANYLIMN	*Tanytarsus limneticus* Sublette	x	x	x
Diptera	Chironomidae	TANYSP	*Tanytarsus* sp.	x		x
Diptera	Chironomidae	TANYSPE	*Tanytarsus* sp. E Epler		x	
Diptera	Chironomidae	TANYSPF	*Tanytarsus* sp. F Epler	x	x	
Diptera	Chironomidae	TANYSPG	*Tanytarsus* sp. G Epler	x	x	x
Diptera	Chironomidae	TANYSPJ	*Tanytarsus* sp. J Epler	x	x	
Diptera	Chironomidae	TANYSPR	*Tanytarsus* sp. R Epler	x	x	x
Diptera	Chironomidae	TANYSPT	*Tanytarsus* sp. T Epler	x	x	
Diptera	Chironomidae	XENOXENO	*Xenochironomus xenolabis* (Kieffer)	x		

(continued)

Table 11.2 (continued)

Group	Family	Code	Taxon	Gradient		
				Spatial	Temporal	Dosing
Diptera	Culicidae	AEDES	*Aedes* sp.	x	x	
Diptera	Culicidae	ANOPHSP1	*Anopheles* sp. 1	x	x	
Diptera	Culicidae	ANOPHSP2	*Anopheles* sp. 2	x	x	
Diptera	Culicidae	COQUPERT	*Coquillettidia perturbans* (Walker)	x	x	
Diptera	Culicidae	CULEX	*Culex* sp.	x	x	
Diptera	Culicidae	MANSOSP2	*Mansonia titillans* (Walker)	x	x	
Diptera	Culicidae	URANOTAE	*Uranotaenia sapphirina* (Osten Sacken)	x	x	
Diptera	Dolichopodidae	DOLICHOP	Dolichopodidae sp.	x		
Diptera	Ephydridae	EPHYDRA	cf. *Ephydra sp.*		x	
Diptera	Muscidae	MUSCIDAE	Muscidae sp.	x		
Diptera	Psychodidae	PSYCHODA	*Psychoda/Threticus* gr. sp.	x	x	
Diptera	Sciomyzidae	SCIOMYZI	Sciomyzidae sp.	x	x	x
Diptera	Stratiomyiidae	ODONTOMY	*Odontomyia* sp.	x	x	x
Diptera	Tabanidae	CHRYSOPS	*Chrysops* sp.		x	
Diptera	Tipulidae	HELIUS	*Helius* sp.	x	x	
Diptera	Tipulidae	LIMONIA	*Limonia* sp.	x	x	x
Diptera	Tipulidae	ORMOSIA	*Ormosia* sp.	x	x	
Diptera	Tipulidae	TIPULSP1	Tipulidae sp. 1	x		
Diptera	Tipulidae	TIPULSP2	Tipulidae sp. 2		x	
Ectoprocta	Plumatellidae	PLUMATEL	*Plumatella* cf. *repens* (L.)	x	x	x
Ephemeroptera	Baetidae	CALLFLOR	*Callibaetis floridanus* Banks	x	x	x
Ephemeroptera	Caenidae	CAENDIMI	*Caenis diminuta* Walker	x	x	x
Gastropoda	Ancylidae	FERRISSI	*Ferrissia* sp.	x	x	x
Gastropoda	Ancylidae	HEBEEXCE	*Hebetancylus excentricus* (Morelit)	x		
Gastropoda	Ancylidae	LAEVPENI	*Laevapex peninsulae* (Pilsbry)	x	x	
Gastropoda	Hydrobiidae	APHAOPAC	*Aphaostracon pachynotus* Thompson	x	x	x
Gastropoda	Hydrobiidae	LITTMONR	*Littoridinops monroensis* (Frauenfeld)	x	x	x
Gastropoda	Lymnaeidae	FOSSMODI	*Fossaria modicella* (Say)	x	x	

Gastropoda	Lymnaeidae	PSEUDCOL	*Pseudosuccinea columella* (Say)	x	x	
Gastropoda	Physidae	PHYSCUBE	*Physella cubensis* (Pfieffer)	x	x	x
Gastropoda	Physidae	PHYSELLA	*Physella* sp.	x	x	
Gastropoda	Pilidae	POMAPALU	*Pomacea paludosa* (Say)	x	x	x
Gastropoda	Planorbidae	GYRAPARV	*Gyraulus parvus* (Say)	x		
Gastropoda	Planorbidae	MICRDILA	*Micromenetus dilatatus avus* (Pilsbry)	x	x	x
Gastropoda	Planorbidae	PLANDURY	*Planorbella duryi* (Weatherby)	x	x	x
Gastropoda	Planorbidae	PLANSP1	*Planorbella duryi/scalaris* complex	x	x	x
Gastropoda	Planorbidae	PLANSCAL	*Planorbella scalaris* (Jay)	x	x	x
Gastropoda	Planorbidae	PLANTRIV	*Planorbella trivolvis intertexta* (Jeffreys)	x	x	x
Gastropoda	Polygyridae	POLYCERE	*Polygyra cereolus* (von Muhlfeld)	x	x	
Gastropoda	Pupillidae	VERTOVAT	*Vertigo ovatus* (Say)	x	x	
Gastropoda	Thiaridae	MELATUBE	*Melanoides tuberculata* (Muller)	x	x	x
Gastropoda	Zonitidae	ZONIARBO	*Zonitoides arboreau* (Say)	x	x	
Hemiptera	Belostomatidae	BELOLUTA	*Belostoma lutarium* (Stal)	x	x	
Hemiptera	Belostomatidae	BELO.IMM	*Belostoma* spp. immature	x	x	x
Hemiptera	Belostomatidae	BELOTEST	*Belostoma testaceum* (Leidy)	x	x	
Hemiptera	Corixidae	TRICHORX	*Trichocorixa* sp.	x	x	x
Hemiptera	Gerridae	RHEUVEGA	*Rheumatobates* cf. *vegatus* Drake and Harris	x		
Hemiptera	Hydrometridae	HYDROMET	*Hydrometra* sp.	x	x	
Hemiptera	Mesoveliidae	MESOVELI	*Mesovelia mulsaulti* White	x	x	
Hemiptera	Naucoridae	PELOFEMO	*Pelocoris femoratus* (Palisot-Beauvois)	x	x	x
Hemiptera	Nepidae	RANAAUST	*Ranatra australis* Hungerford	x	x	
Hemiptera	Notonectidae	BUENOA	*Buenoa* sp.	x	x	
Hemiptera	Pleidae	PARAPLEA	*Paraplea* sp.	x	x	
Hemiptera	Saldidae	SALDIDAE	Saldidae sp.	x	x	
Hemiptera	Veliidae	MICRVELI	*Microvelia* sp.	x	x	
Hydracarina	Arrenuridae	ARREAPOP	*Arrenurus* nr. *apopkensis* Cook	x	x	
Hydracarina	Arrenuridae	ARREZAPU	*Arrenurus* nr. *zapus* Cook			x
Hydracarina	Arrenuridae	ARRENSP1	*Arrenurus* sp. 1	x	x	x
Hydracarina	Arrenuridae	ARRENSP2	*Arrenurus* sp. 2	x	x	x

(continued)

Table 11.2 (continued)

Group	Family	Code	Taxon	Gradient		
				Spatial	Temporal	Dosing
Hydracarina	Arrenuridae	ARREZORU	*Arrenurus zorus* Cook	x	x	x
Hydracarina	Eylaidae	EYLAIS	*Eylais* sp.	x	x	x
Hydracarina	Hydrodromidae	HYDRODRO	*Hydrodroma* sp.	x		x
Hydracarina	Limnesiidae	LIMNESIA	*Limnesia* sp.	x	x	x
Hydracarina	Limnocharidae	LIMNOCHA	*Limnochares* sp.	x	x	x
Hydracarina	Oxidae	OXUS	*Oxus* sp.	x	x	
Hydracarina	Sperchontidae	SPERCHON	*Sperchon* sp.		x	
Hydracarina	Unionicolidae	KOENIKEA	*Koenikea* sp.	x	x	x
Hydracarina	Unionicolidae	NEUMANIA	*Neumania* sp.	x	x	x
Hydracarina	Unionicolidae	UNIONICO	*Unionicola* sp.	x	x	x
Hydroida	Hydridae	HYDRA	*Hydra* sp.	x	x	x
Isopoda	Asellidae	CAECIDOT	*Caecidotea* sp.	x	x	
Lepidoptera	Nepticulidae	NEPTICUL	Nepticulidae sp.	x		
Lepidoptera	Noctuidae	NOCTUSP1	Noctuidae sp. 1	x	x	
Lepidoptera	Noctuidae	NOCTUSP2	Noctuidae sp. 2	x	x	
Lepidoptera	Noctuidae	SIMYRA	*Simyra henrici* (Grt.)	x	x	
Lepidoptera	Pyralidae	ACENTRIA	*Acentria* sp.	x	x	
Lepidoptera	Pyralidae	PARAPONY	*Paraponyx* sp.	x	x	
Lepidoptera	Pyralidae	PYRALSP1	Pyralidae sp. 1	x	x	
Lepidoptera	Pyralidae	PYRALSP2	Pyralidae sp. 2	x		
Lepidoptera	Pyralidae	PYRALSP3	Pyralidae sp. 3			x
Nemata	Nemata	NEMATA	Nemata	x	x	
Odonata	Aeschnidae	ANAX	*Anax* sp.	x		
Odonata	Aeschnidae	CORYINGE	*Coryphaeschna ingens* (Rambur)	x	x	
Odonata	Coenagrionidae	ENALCIVI	*Enallagma civile* Hagen	x	x	x
Odonata	Coenagrionidae	ENALPOLL	*Enallagma pollutum* Hagen	x		
Odonata	Coenagrionidae	ISCHHAST	*Ischnura hastata* Say	x	x	x
Odonata	Coenagrionidae	ISCHPOSI	*Ischnura posita*	x		

Odonata	Coenagrionidae	ISCHNURA	*Ischnura* sp.	x	x	
Odonata	Coenagrionidae	TELEBYER	*Telebasis byersi* Westfall	x	x	
Odonata	Libellulidae	BRACGRAV	*Brachymesia gravida* (Calvert)	x	x	x
Odonata	Libellulidae	CELIEPON	*Celithemis eponina* (Drury)	x	x	x
Odonata	Libellulidae	ERYTHSIM	*Erythemis simplicicollis* (Say)	x	x	x
Odonata	Libellulidae	LIBENEED	*Libellula needhami* Westfall	x	x	x
Odonata	Libellulidae	PACHLONG	*Pachydiplax longipennis* (Burmeister)	x	x	x
Oligochaeta	Enchytraeidae	ENCHYTRA	Enchytraeidae	x	x	
Oligochaeta	Lumbriculidae	ECLIPALU	*Eclipidrilus palustris* (Smith)	x	x	
Oligochaeta	Naididae	ALLOPECT	*Allonais pectinata* (Stephenson)	x	x	x
Oligochaeta	Naididae	BRATUNID	*Bratislavia unidentata* (Harman)	x	x	x
Oligochaeta	Naididae	DERODIGX	*Dero digitata* (Muller) complex	x	x	x
Oligochaeta	Naididae	DEROFURC	*Dero furcata* (Muller)	x	x	x
Oligochaeta	Naididae	DEROLODE	*Dero lodeni* (Stephenson)	x	x	
Oligochaeta	Naididae	DEROPECT	*Dero pectinata* Aiyer	x	x	x
Oligochaeta	Naididae	DEROVAGA	*Dero vaga* (Leidy)	x	x	
Oligochaeta	Naididae	HAEMWALD	*Haemonais waldvogeli* Bretscher	x	x	x
Oligochaeta	Naididae	PRISAEQU	*Pristina aequiseta* Bourne	x	x	x
Oligochaeta	Naididae	PRISLEID	*Pristina leidyi* Smith	x	x	x
Oligochaeta	Naididae	PRISLONG	*Pristinella longisoma* (Harman)	x		
Oligochaeta	Naididae	PRISTINE	*Pristinella* sp.	x	x	
Oligochaeta	Naididae	STYLLACU	*Stylaria lacustris* (L.)		x	
Oligochaeta	Opistocystidae	CRUSTRIB	*Crustipellis tribranchiata* (Harman)	x		x
Oligochaeta	Tubificidae	LIMNHOFF	*Limnodrilus hoffmeisteri* Clarapede	x	x	x
Oligochaeta	Tubificidae	TUBIFSP1	Tubificidae imm. gr. 1	x	x	x
Oligochaeta	Tubificidae	TUBIFSP2	Tubificidae imm. gr. 2	x	x	x
Oligochaeta	Tubificidae	TUBIFSP3	Tubificidae imm. gr. 3	x	x	
Ostracoda	Candonidae	CANDANNA	*Candona annae* Mehes	x	x	x
Ostracoda	Cyprididae	CHLATEXA	*Chlamydotheca texasiensis* (Baird)		x	
Ostracoda	Cyprididae	CHLAUNIS	*Chlamydotheca unispinosa* (Baird)	x	x	

(continued)

Table 11.2 (continued)

Group	Family	Code	Taxon	Gradient		
				Spatial	Temporal	Dosing
Ostracoda	Cypridopsidae	CYPROKEE	*Cypridopsis okeechobei* Furtos	x	x	x
Ostracoda	Cytheridae	CYTHALOS	*Cytheridella alosa* (Tressler)	x	x	x
Ostracoda	Cytheridae	HETEPUNC	*Heterocypris punctata* (Baird)		x	
Polychaeta	Neridae	NAMALABI	*Namalycastis abiuma* (Muller)	x	x	
Porifera	Spongillidae	SPONCENO	*Spongilla cf cenota* Penney and Racek			x
Porifera	Spongillidae	SPONGILL	*Spongilla* sp.	x	x	
Rhynchobdellida	Glossiphoniidae	HELOFUSC	*Helobdella fusca* (Castle)	x	x	
Rhynchobdellida	Glossiphoniidae	HELOSTAG	*Helobdella stagnalis* (Linnaeus)	x		
Rhynchobdellida	Glossiphoniidae	HELOTRIS	*Helobdella triserialis* (Blanchard)	x	x	x
Rhynchobdellida	Glossiphoniidae	PLACPAPI	*Placobdella papillifera* (Verill)	x	x	
Trichoptera	Hydroptilidae	OXYETHIR	*Oxyethira* sp.	x	x	x
Trichoptera	Leptoceridae	OECECINE	*Oecetis cinerascens* (Hagen)	x		
Trichoptera	Leptoceridae	OECETSPE	*Oecetis inconspicua* complex sp. E Floyd	x	x	
Trichoptera	Leptoceridae	OECETSP	*Oecetis* sp.	x	x	
Trichoptera	Polycentropodidae	CERNOTIN	*Cernotina* sp.	x	x	x
Tricladida	Planariidae	PLANARII	Planariidae sp.	x	x	

Table 11.3 Comparison of selected properties of the eight subsampling approaches evaluated for wetland bioassessment (from King and Richardson 2002)

	Percentage of total sample	Number of individuals		Sorting time (min)
Subsample	Mean ± 1 SD	Mean ± 1 SD	Range	Mean ± 1 SD
100 count	7.7 ± 6.4	102.7 ± 4.7	92–118	94.2 ± 55.3
200 count	15.2 ± 12.7	203.7 ± 7.3	191–224	156.1 ± 100.0
300 count	22.6 ± 18.3	304.6 ± 10.9	283–326	206.7 ± 117.8
10% area	10.0 ± –	230.0 ± 178.3	23–1,036	94.7 ± 51.5
25% area	25.0 ± –	573.1 ± 440.3	62–2,558	250.7 ± 136.2
100 count + LR[a]	NA	121.3 ± 14.3	100–214	117.0 ± 54.0
10% area + LR	NA	247.7 ± 177.7	36–1,044	117.2 ± 51.7
100 count and 10% area	11.6 ± 4.3	238.2 ± 170.9	97–1,036	118.0 ± 64.5

NA not applicable

[a]Subsamples containing the large rare (LR) component were picked completely for all LR taxa (100% of sample area) in addition to the fixed-count or fixed-area component

most diverse of the major taxonomic groups, and contributed most to the differences among the number of families, genera, and species identified. Chironomidae was the most diverse family in the spatial study, represented by 30 and 51 genera and species, respectively.

Numbers of individuals showed tremendous variation among subsamples using the fixed-area approach (Table 11.3). Although 10% area averaged over twice the number of individuals as the 100 count, it produced as few as 23 individuals in one subsample, and had <100 individuals 27% of the time. Similarly, the 25% area averaged nearly twice the number of individuals as the 300 count despite averaging a similar percentage of the total sample subsampled. The LR search added an average of as many as 19 individuals to subsamples. Sorting times mirrored the percentage of total sample subsampled rather than number of individuals picked (Table 11.3). LR searches added an average of 23 min (100 + LR) to sorting time. The LR search added as many as 4 families, 9 genera, and 16 species, cumulatively, to any one level of subsampling (100 vs. 100 + LR). Frequencies of occurrence of many LR taxa increased as much as a factor of 10 by implementing the LR search, with the 100-count subsample performing the poorest of all in capturing LR taxa (King and Richardson 2002).

11.3.1.1 Effects of Subsampling and Taxonomic Resolution on Assemblage–Environment Relationships

Mantel r statistics were significantly different from 0 ($p \leq 0.0001$), regardless of subsample or taxonomic level. However, the magnitude of these assemblage–environment correlations varied significantly (95% CI) among subsamples and taxonomic levels (Fig. 11.3). In particular, the greatest increase in assemblage–environment relationships with increasing subsample size was observed between

100 and 200 counts – 100 performed significantly worse than 200, whereas 200 was not different from 300, regardless of taxonomic level.

Differences in the magnitude of Mantel *r* values suggested that fixed-area subsamples generally were less efficient than fixed counts. Fixed counts of 200 and 300 individuals produced significantly greater Mantel *r* values than 10% area, despite averaging similar numbers of individuals (Fig. 11.3). Similarly, 25% area assemblage–environment relationships were not significantly greater than the less labor-intensive 300 count at the genus and species levels.

Adding the LR search to 100-count and 10% area subsamples resulted in very slight increases in the strength of assemblage–environment relationships for all three levels of taxonomy (Fig. 11.3). LR taxa significantly increased the Mantel *r* value for 100-count data at the family level.

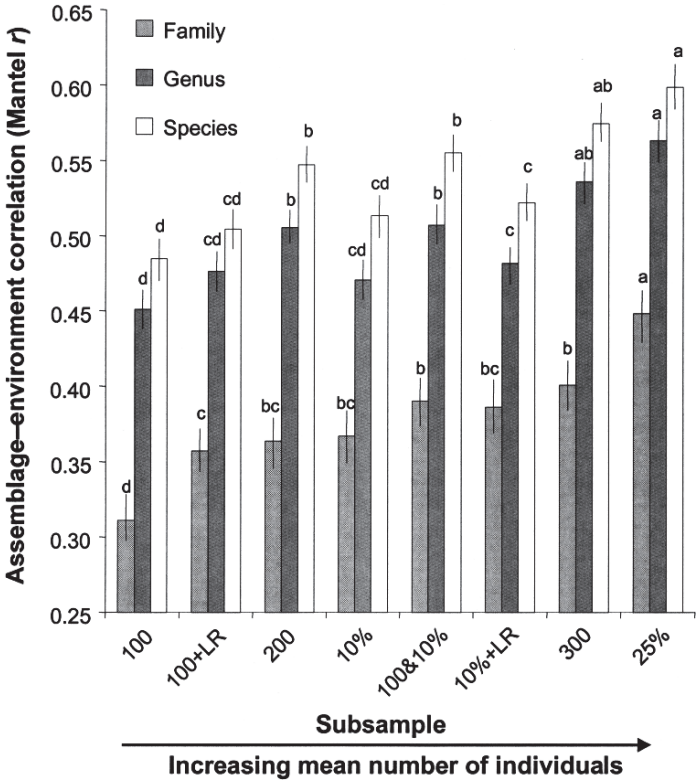

Fig. 11.3 Assemblage–environment correlations for each subsampling approach and level of taxonomic resolution, as estimated using Mantel tests. Significant differences in the magnitude of Mantel *r* values (bootstrapped 95% CI, error bars) among subsamples within taxonomic levels are indicated by the *lower-case letters*; Mantel *r* values with the same letters were not different. Among taxonomic levels, Mantel *r* values with overlapping 95% CI were not significantly different (all Mantel *r* values differed among the three levels of taxonomy within each level of subsampling)

Differences in the magnitudes of assemblage–environment correlations were more apparent among levels of taxonomic resolution than among subsampling approaches. The family level was significantly inferior to both genus- and species-level data, regardless of subsampling approach (Fig. 11.3). Species-level data showed significantly stronger relationships to the environment than the genus level, although 95% CIs were only marginally separated within each level of subsampling.

Chironomidae may have been largely responsible for the observed disparity in correlations to the environment among taxonomic levels (Fig. 11.4). Results from the tiered-taxonomic analysis revealed that tiering family-level data with species-level Chironomidae data yielded assemblage–environment correlations that were not different from those obtained by identifying all taxa to genus or species. Conversely, leaving Chironomidae identifications at just the family level but identifying other taxa to genus or species produced significantly worse assemblage–environment correlations than that of genus, species, and tiered family/Chironomidae-to-species data (King and Richardson 2002).

Species accumulation curves indicated that species richness patterns among impact zones were scale dependent (Fig. 11.5). On a single-plot scale, the transition zone averaged more species than the impacted, while both of these zones averaged more than the reference zone. However, steepness of the accumulation curve was initially greater in the impacted zone than the transition, resulting in higher richness in this eutrophic region. The impacted-zone curve sharply flattened above 20 plots, while transition-zone plots continued to accumulate new species. Jackknife estimates of asymptotic richness indicated the intermediate-P, transition zone had the most species at a landscape scale, while impacted and reference zones were similar in total richness.

11.3.2 Biomass Responses to P

Macroinvertebrate assemblage biomass exhibited a significant unimodal response to both distance from canal (Fig. 11.6a) and sediment TP (Fig. 11.6b). These subsidy–stress relationships were nearly identical for both predictor variables; therefore, subsequent responses were evaluated only with sediment TP as a predictor. Twelve major taxonomic groups were evaluated for their specific responses to P. Eight groups showed subsidy–stress responses (Fig. 11.7a, b, e–g, i–k), three demonstrated significant subsidy responses (Fig. 11.7c, d, h), while one showed a significant stress response (Fig. 11.7l). Of the eight taxa responding with a subsidy–stress relationship, five were statistically significant.

Decapoda made the greatest contribution to assemblage biomass, and revealed the most obvious subsidy–stress response to P enrichment (Fig. 11.7g). Represented by only two species, *Palaemonetes paludosus* and *Procambarus fallax*, their cumulative standing stocks increased markedly with intermediate (transition zone) P enrichment, but plummeted in localities within the eutrophic, impacted zone. *P. paludosus*, in particular, was rarely collected in high-P areas.

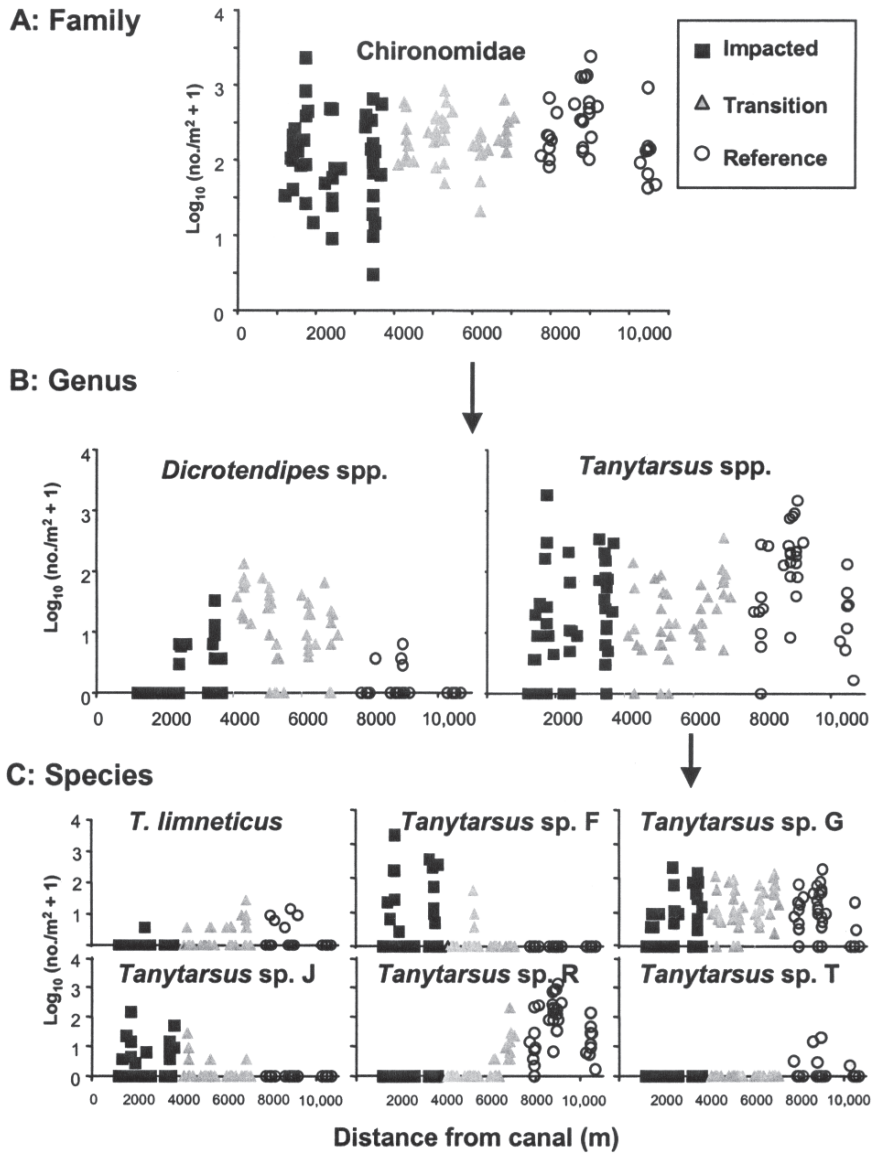

Fig. 11.4 The effect of differing levels of taxonomic resolution on the environmental signal provided by the abundance of the family Chironomidae. Scatterplots of density (no. m^{-2}) for all taxa in (**a**) Chironomidae, (**b**) two representative genera (*Dicrotendipes* and *Tanytarsus*), and (**c**) all six species within the genus *Tanytarsus* are shown as a function of distance from canal inflow structures in Water Conservation Area (WCA) 2A. *Symbols* indicate impacted, transition, and reference landscape zones

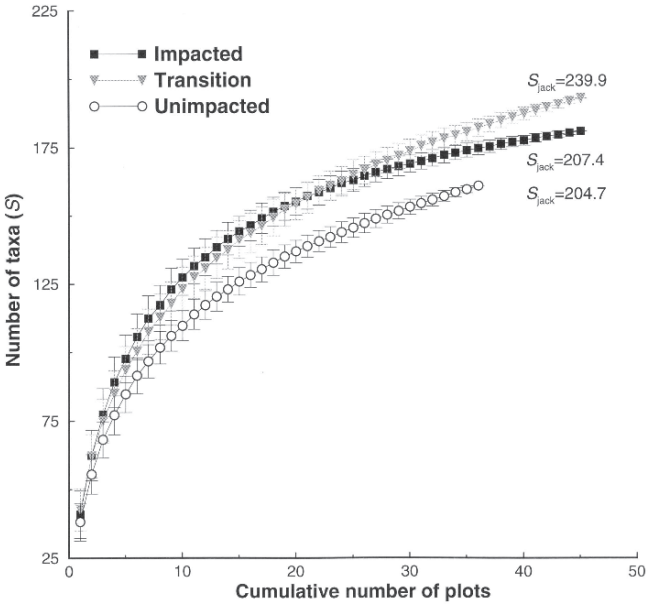

Fig. 11.5 Invertebrate species accumulation curves (mean ± 1 SD) for impacted, transition, and unimpacted zones. Jackknife estimates of asymptotic richness (S_{jack}) are shown next to each curve

Taxa that were either primarily algivores or suggested to at least be partially dependent upon periphyton all showed subsidy–stress patterns along the P gradient. Additionally, two predaceous groups, Odonata and Hirudinea, exhibited this same response (Fig. 11.7f, k). However, two other predominantly predaceous groups, Hemiptera and Coleoptera, responded favorably to high levels of P (Fig. 11.7d, h). The only other major taxon to respond positively to high P was Isopoda, represented exclusively by *Caecidotea* sp. (Fig. 11.7c). This detritivorous taxon became most abundant in dense stands of *Typha* with large quantities of decaying CPOM. Finally, Trichoptera – represented by three families and at least five different species (Table 11.2) – was the only coarse taxon to show a stress response to P enrichment (Fig. 11.7l).

Diversity responses mimicked the general pattern of biomass. Species density and richness showed subsidy–stress responses to P; however, these relationships were not statistically significant (Fig. 11.8a, b). However, species density clearly increased at intermediate levels of P when compared with low-P clusters (Fig. 11.8a). Species density and richness were variable in the high-P zone but tended to show a stress response above intermediate-P levels.

Aside from vegetation, several biotic variables hypothesized to be determinants of invertebrate biomass also were significantly related to P. Food quality of periphyton, expressed as C:N ratio, decreased linearly with increasing sediment TP

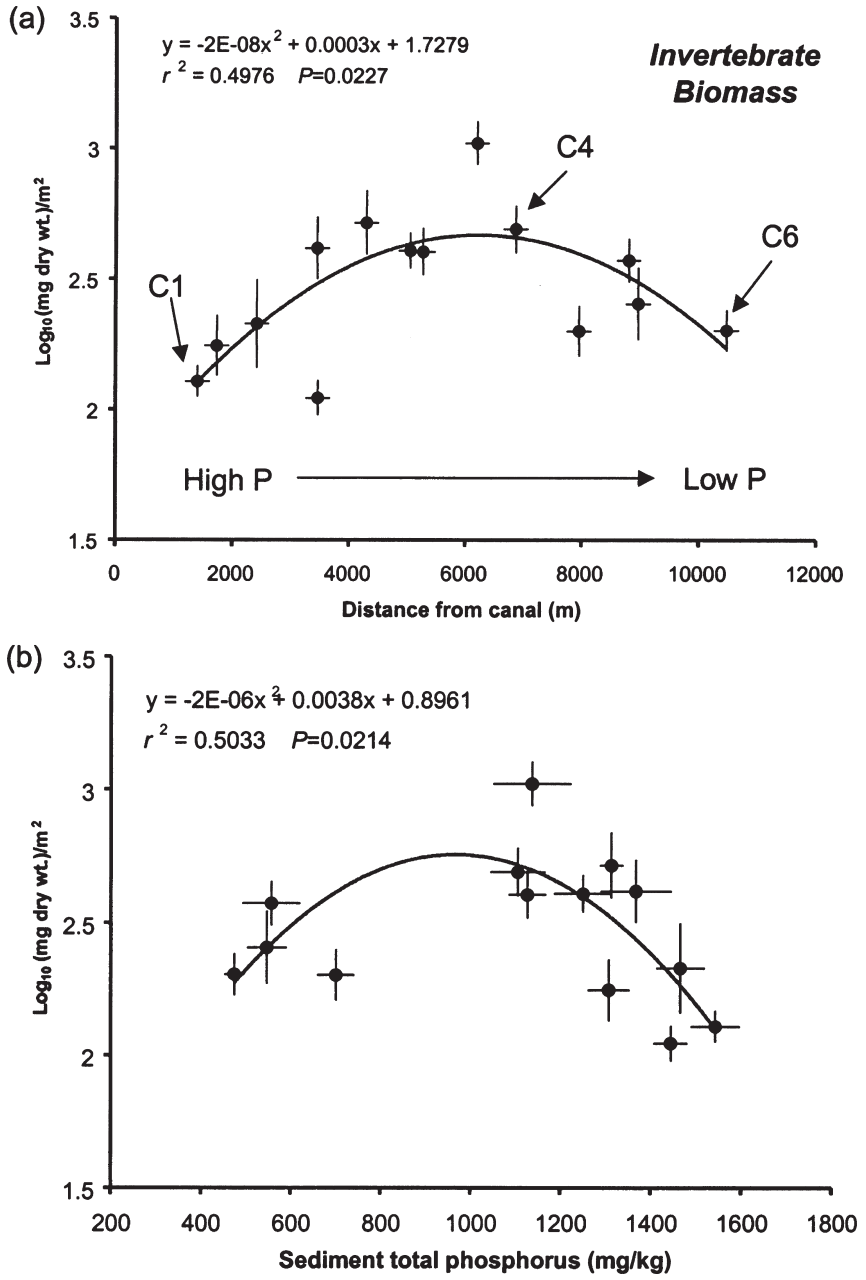

Fig. 11.6 Response of invertebrate assemblage biomass to (**a**) distance from canal inflow structures and (**b**) sediment total phosphorus (TP). *Error bars* indicate ±1 SE. Locations of plot-clusters used in the temporal study (C1, C4, C6) are indicated in (**a**)

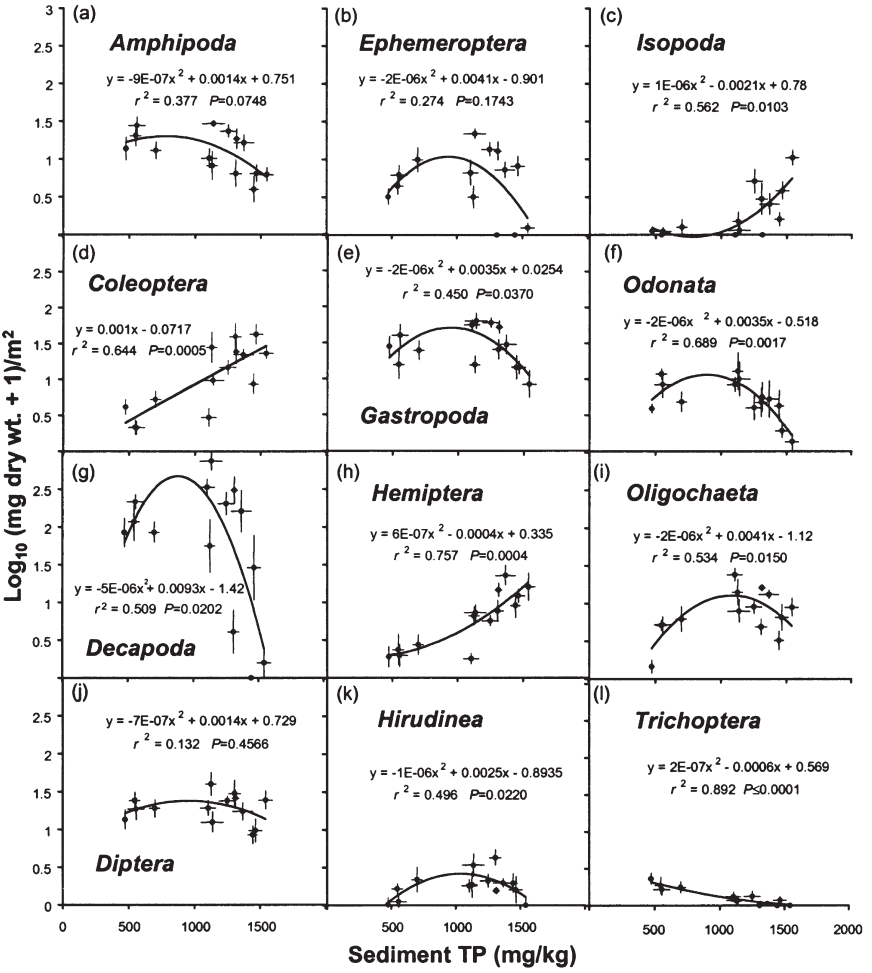

Fig. 11.7 (**a–l**) Response of biomass of 12 coarse-taxonomic invertebrate groups to sediment TP. *Error bars* indicate ±1 SE

(Fig. 11.9a). Despite the apparent increase in potential food quality with P, metaphyton cover decreased markedly above 1,200 mg kg^{-1} sediment TP (Fig. 11.9b). Invertivorous fish density and biomass were not significantly related to P, but both exhibited subsidy–stress relationships that were nearly significant (second-order polynomial, $r^2 = 0.38$, $p = 0.055$ and $r^2 = 0.31$, $p = 0.120$, respectively). Although it appeared that invertivorous fish density and biomass were greatest at intermediate levels of P, subsequent decreases at high levels of P were much less apparent than those exhibited by invertebrate biomass. Since small fish density showed the best relationship, it was retained as a potential predictor of invertebrate composition in the multivariate analysis.

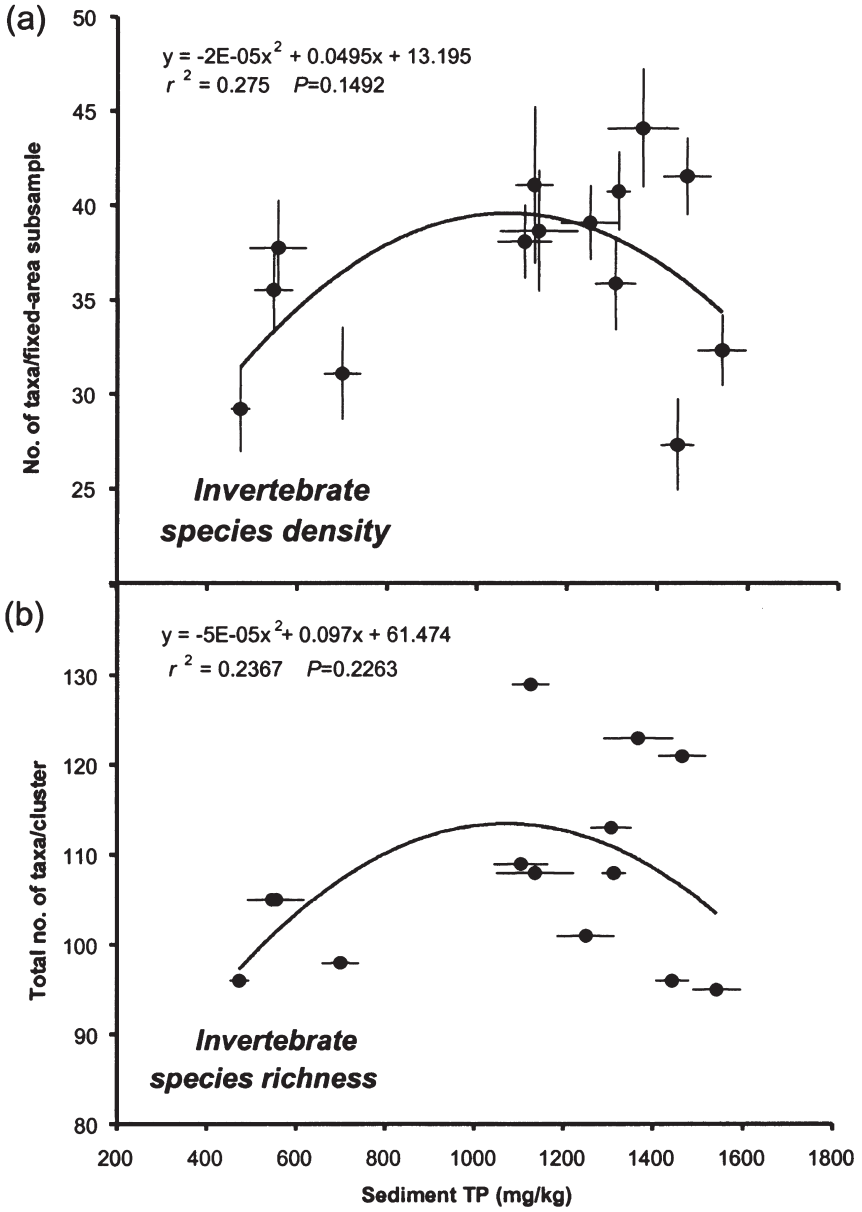

Fig. 11.8 Response of invertebrate (**a**) species density and (**b**) species richness to sediment TP. *Error bars* indicate ±1 SE

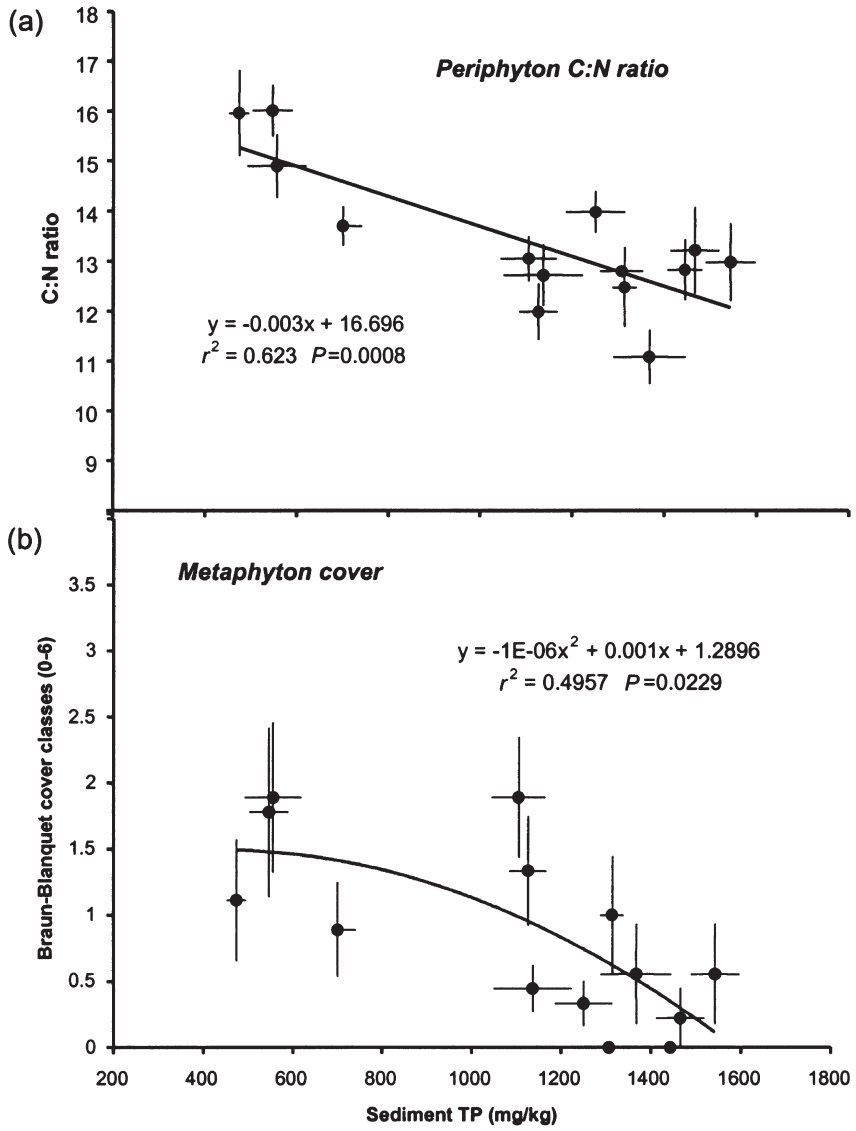

Fig. 11.9 Response of (**a**) periphyton C:N ratio and (**b**) metaphyton cover to sediment TP. *Error bars* indicate ± 1 SE

11.3.3 Determinants of Assemblage Composition

Ordination of invertebrate species composition revealed distinct separation of plots among impact zones (Fig. 11.10a). The primary axis was a landscape-scale gradient significantly associated with spatial/abiotic variables such as Canal, P, and interquartile

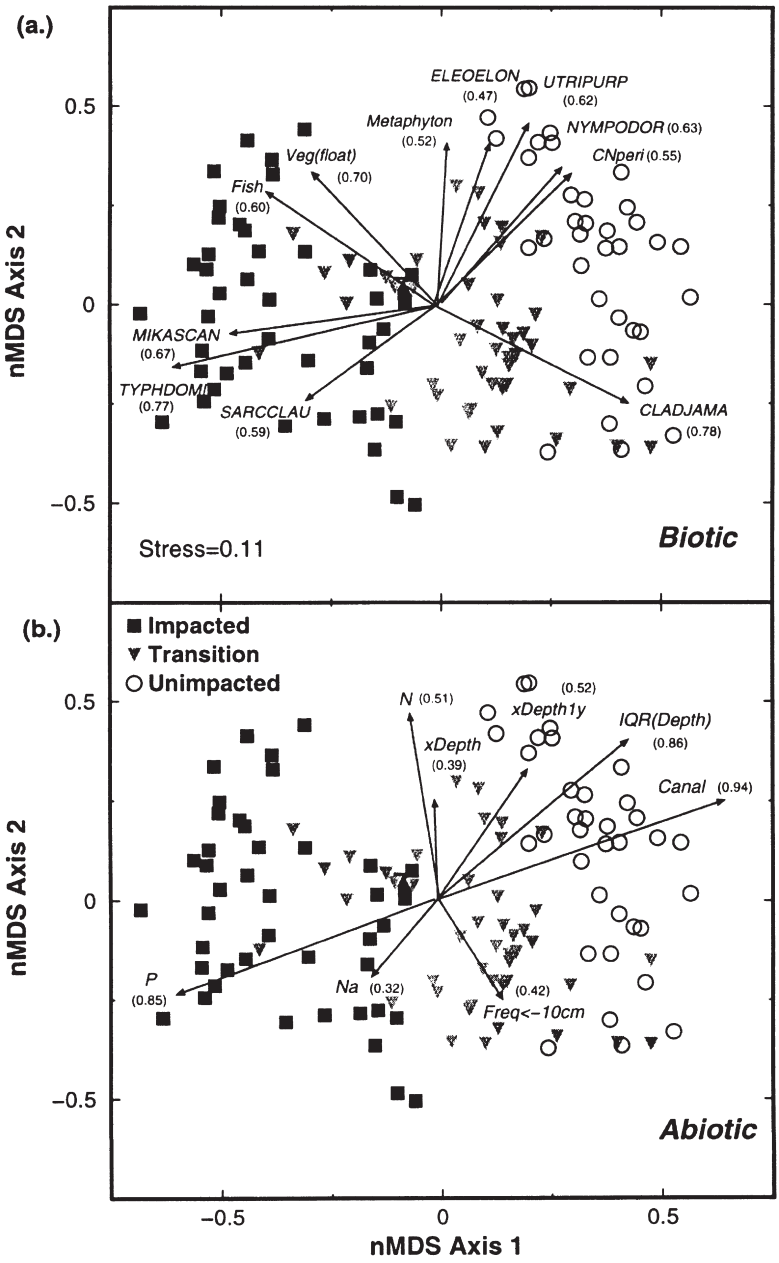

Fig. 11.10 Nonmetric multidimensional scaling ordination of fine-scale invertebrate assemblage composition (three-dimensional configuration; only two dimensions shown – third dimension added to reduce stress but it was not significantly related to abiotic or biotic variables). Significant (**a**, **c**) biotic and (**b**, **d**) abiotic (including canal) vectors are shown in relation to (**a**, **b**) plots and (**c**, **d**) indicator/important taxa in ordination space (see Table 11.2 for species codes; Tables 11.1

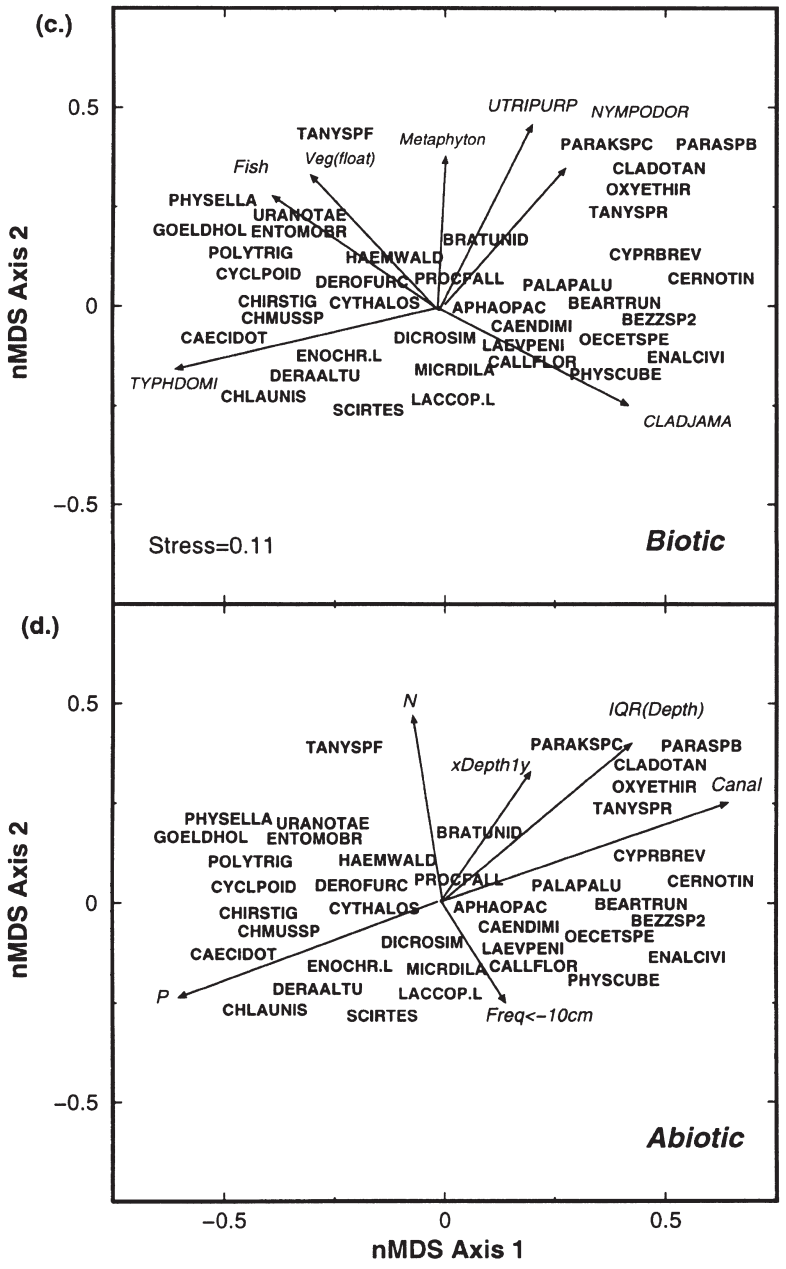

Fig. 11.10 (continued) and 11.3 for abiotic and biotic variable codes). Magnitude of vector correlations (*r*) is shown in *parentheses* next to variable codes. *Symbols* indicate membership of plots to landscape zones. Codes for variables not defined previously: *Veg(float)* cover of unrooted floating vegetation; *CNperi* C:N ratio of periphyton

range of water depth (IQR[Depth]) (Fig. 11.10b), and, in response, biotic variables such as cover of invasive vegetation (*Typha*, *Mikania*, *Sarcostemma*), cover of *Cladium*, C:N ratio of periphyton(CNperi) and abundance of invertivorous fish (Fish) (Fig. 11.10a). The second axis was a local, or fine-scale gradient driven primarily by water depth (freq. <−10 cm, xDepth1y, xDepth), but also related to soil chemistry (N, Na). Metaphyton, cover of floating vegetation, and cover of slough macrophyte species (*Eleocharis*, *Utricularia*, *Nymphaea*) all were related to this secondary axis.

Table 11.4 List of invertebrate taxa significantly associated with impacted, transition, or unimpacted landscape zones, as estimated using Indicator Species Analysis (INSPAN)

Impacted		Transition		Unimpacted	
Anopheles sp. 1[a]	29.2	*Aphaostracon pachynotus*[b]	49.3	*Ablabesmyia rhampe* gr. sp.[c]	23.1
Caecidotea sp.[d]	52.9	*Bratislavia unidentata*[e]	44.5	*Beardius truncatus*[c]	46.3
Chironomus stigmaterus[c]	33.8	*Caenis diminuta*[f]	43.6	*Bezzia/Palpomyia* gr. sp. 2[g]	42.9
Chlamydotheca unispinosa[h]	36.8	Calanoida[i]	32.2	*Cernotina* sp.[j]	41.7
Entomobrya sp.[k]	48.8	*Dero furcata*[e]	40.5	*Cladotanytarsus* sp.[c]	34.8
Cyclopoida[i]	47.0	*Dicrotendipes modestus*[c]	27.9	*Cypretta brevisaepta*[h]	36.1
Desmopachria sp.[l]	30.3	*Dicrotendipes simpsoni*[c]	51.6	*Enallagma civile*[m]	60.7
Enochrus spp. (larvae)[l]	54.6	*Haemonais waldvogeli*[e]	54.4	*Nanocladius alternantherae*[c]	16.7
Zonitoides arboretum[b]	25.2	*Laccophilus* spp. (larvae)[l]	38.7	*Nilothauma* sp.[c]	27.8
Goeldichironomus holoprasinus[c]	66.8	*Kiefferulus dux*[c]	33.9	*Oecetis* sp. E[j]	30.8
Physella sp.[b]	37.8	*Laevapex peninsulae*[b]	45.8	*Oxyethira* sp.[j]	52.2
Planorbella duryi[b]	26.0	*Micromenetus dilatatus*[b]	53.4	*Parachironomus alatus*[c]	24.6
Polypedilum trigonus[c]	43.2	*Pseudochironomus* sp.[c]	28.9	*Parakiefferiella* sp. C[c]	77.5
Scirtes sp.[l]	35.2			*Paraponyx* sp.[n]	30.6
Tanytarsus sp. F[c]	27.4			*Paratanytarsus* sp. B[c]	54.4
Uranotaenia sapphirina[a]	41.6			*Physella cubensis*[b]	44.0
				Polypedilum halterale[c]	25.3
				Polypedilum sp. A[c]	28.0
				Procladius sp.[c]	22.2
				Spongilla sp.[o]	22.7
				Tanytarsus sp. R[c]	84.5

Indicator values (IVs; % of perfect indication) are shown next to each taxon. All taxa shown had IVs with $p \leq 0.0002$ (Bonferroni-corrected $p \leq 0.05$)

Class/order – [a]Diptera:Culicidae; [b]Gastropoda; [c]Diptera:Chironomidae; [d]Isopoda; [e]Oligochaeta; [f]Ephemeroptera; [g]Diptera:Ceratopogonidae; [h]Ostracoda; [i]Copepoda; [j]Trichoptera; [k]Collembola; [l]Coleoptera; [m]Odonata; [n]Lepidoptera; [o]Porifera

Centroids of species identified as indicators of the impacted zone were, accordingly, ordinated on the eutrophic end of nMDS Axis 1, near the ends of vectors for P and *Typha* (Fig. 11.10c, d; see codes in Table 11.2). At the oligotrophic end of the gradient, taxa were separated along Axis 2 according to vegetation and hydrological affinities – a continuum of species restricted primarily to deep-water slough habitats (e.g., *Parakiefferiella* sp. C, *Oxyethira* sp., *Paratanytarsus* sp. B, *Cladotanytarsus* sp.) to those almost exclusively found in shallower, dense stands of *Cladium* (e.g., *Enallagma civile*, *Beardius truncatus*, *Oecetis* sp. E). The two large-bodied decapods, *P. paludosus* and *P. fallax*, occupied slightly different locations in species space, with *P. paludosus* bordering the transition and unimpacted zones, while *P. fallax* was proximate to the center of the ordination.

INSPAN analysis indicated that a number of these species had significant associations with specific impact zones (Table 11.4). The reference zone had the greatest number of indicator species (21) – over half of these were members of the family Chironomidae (Diptera). Three of the five trichopteran taxa collected also were indicators of this zone. Several species belonging to Gastropoda, Ostracoda, Lepidoptera, and Porifera were also sensitive and primarily found here.

Several of the best indicators of the transition zone were either gastropods or naidid oligochaetes (Table 11.4), primarily grazers or collectors of periphyton. Impacted-zone indicators included many detritivorous taxa, such as *Caecidotea* sp., *Scirtes* sp., and filter-feeding Culicidae (Diptera), *Anopheles* sp. 1, and *Uranotaenia sapphirina*. Two chironomids often associated with organic pollution, *Chironomus stigmaterus* and *Goeldichironomus holoprasinus*, also were found mostly in this eutrophic region of the landscape.

11.4 Discussion

11.4.1 Biomass Response to P

Results from the spatial study support the hypothesis that invertebrate assemblage biomass is resource limited, and this limitation is relaxed with P enrichment. The subsidy–stress model (sensu Odum et al. 1979; Fig. 11.1) served admirably as a theoretical framework for predicting assemblage-level responses along the P gradient (Fig. 11.6). We anticipated that a myriad of factors, mostly linked to changes in landscape pattern (i.e., vegetation), would cumulatively act as a stressor to standing stocks in high-P, eutrophic areas relative to areas of intermediate-P enrichment. Indeed, biomass showed a significant subsidy–stress relationship with P, and was lower in a high-P region of the wetland than an intermediate-P area on three of four collection dates. Moreover, this subsidy–stress pattern was evident for most of the major taxa collected. However, what factors were directly contributing to observed patterns along the P gradient?

King (2001) hypothesized that changes in periphyton abundance and nutrient content would be an important direct determinant of invertebrate biomass because it has been suggested to be a significant pathway in food webs in wetlands (e.g., Murkin 1989; Keough et al. 1996; D.A. Wrubleski and N.E. Detenbeck, unpublished data; Wissinger 1999). Patterns of metaphyton cover and C:N ratios, across the landscape and through time, lend credence to this hypothesis. Metaphyton was reduced to very low cover in high-P areas during the spatial study, yet remained relatively high at intermediate levels of enrichment (Fig. 11.9b). Meanwhile, C:N ratios steadily declined with increased P, implying greater nutritional value of periphyton and its detritus (e.g., Sterner and Elser 2002). Consequently, localities of intermediate enrichment had relatively high quantities of periphyton but also higher protein (inferred) content than low-P areas, a fact that may have contributed to a subsidy effect for invertebrate standing stocks.

Top-down regulation of invertebrates by invertivorous fish is a mechanism that can limit invertebrate biomass accumulation (e.g., Hairston et al. 1960; Oksanen et al. 1981). Turner et al. (1999) suggested that greater biomass and densities of small fish in eutrophic than oligotrophic areas of the Everglades may explain their finding of no increase in invertebrate biomass between these same two nutrient regimes. Although invertivorous fish abundance clearly is an important consideration, findings from this study do not provide sufficient evidence to imply that predation was the primary factor limiting invertebrate biomass in high-P areas (although it may have played a role in structuring composition). In the spatial study, biomass of invertivorous fish followed a similar subsidy–stress P–response curve to that of invertebrate biomass, while densities of small fish were generally similar or slightly lower at high-P locations than in the intermediate-P region of the gradient. In a temporal study (not reported here), fish abundance followed this similar pattern, with C1 and C4 exhibiting similar densities and biomass of fish, but greater than C6 (King 2001). Thus, it did not appear that invertebrate production was accumulating as invertivorous fish biomass to a degree that would explain patterns in invertebrate assemblage biomass. The fact that Turner et al. (1999) only sampled low- and high-P habitats, while not sampling the intermediate-P zone, may have contributed to their conclusion.

11.4.2 Diversity Relationships with P Enrichment

Estimation of species diversity (number of species) is dependent upon two important factors: sampling area (Arrhenius 1921) and a "sampling effect" related to the number of individuals sampled (Preston 1948; May 1975) – these factors are particularly influential at small spatial scales (Larsen and Herlihy 1998). Increasingly, scale of measurement has become recognized as one of the most important factors in relating diversity to nutrient or productivity gradients because of the confounding dependency of community density upon productivity (e.g., Oksanen 1996; Waide et al. 1999; Weiher 1999). In this study, the similarity between estimates of species

density (mean plot-level α-diversity within clusters) and species richness (cluster-level α-diversity) along the P gradient suggested that coarse-scale richness was at least partially due to fine-scale species density – clusters with higher mean densities of species tended to have higher total numbers of species (Fig. 11.8). Both of these diversity measures followed a subsidy–stress relationship with P enrichment in the spatial study, and predominantly subsidy–stress patterns among enrichment categories in the temporal study. Although not statistically significant in the spatial study, these patterns imply that the unimodal productivity–diversity relationship, often used as a model for plant communities, may also be a useful model for wetland invertebrate assemblages (Rosenzweig and Abramsky 1993).

At larger spatial scales, however, species accumulation curves and jackknife estimates of richness within impact zones (surrogates for nutrient categories) indicated that the cluster-scale relationship between richness and P was partially confounded by community density. This was further illustrated through the examination of species accumulation within low-, intermediate-, and high-P clusters through time. Here, differences in richness between low- and intermediate-P areas appeared to be exaggerated at smaller scales (plots and clusters). Importantly, these curves illustrated that lower invertebrate biomass in the oligotrophic, unimpacted landscape resulted in a greater incidence of missing "rare" species at smaller scales relative to enriched locations (Fig. 11.5). A conservative interpretation of these results may be that a weak subsidy–stress relationship exists between diversity and nutrients in the Everglades, but the magnitude of this relationship is scale dependent.

Results of this study conflict with those of Rader and Richardson (1994) regarding the response of species diversity to P enrichment. Sampling at many of the same locations used in this study, they concluded that P enrichment resulted in a dramatic subsidy effect for invertebrate species richness. A number of factors may have contributed to the discrepancy between studies.

First, their sampling was limited to open-water (short-emergent, floating, and submergent vegetation only) patches along the P gradient – a habitat that was and is rare in high-P areas of the gradient. These patches, while likely harboring much periphyton, also may have represented an uncharacteristic refugium among the dense stands of invasive vegetation in such locations. Moreover, this stratification removed potentially relevant heterogeneity in the landscape and subsequently missed taxa that were found associated with other types of vegetation in this study.

Second, they identified 137 invertebrate taxa, in aggregate, which was approximately 1/2 of the number identified in this study (272 taxa identified in spatial and temporal studies combined; King 2001). This may have been partly due to a sampling effect, as they identified approximately 11,000 individuals compared with over 144,000 in King (2001).

Third, and directly related to the disparity of total taxa between studies, they used a dip net with 2-mm mesh compared with the standard 0.5-mm mesh used in this study and typically used for macroinvertebrate studies (e.g., Barbour et al. 1999). Numerous taxa, particularly many of the sensitive, indicator taxa from the unimpacted landscape (e.g., Chironomidae) were too small to be reliably

collected with such a coarse device. Collectively, these three factors probably contributed to the differences between the Rader and Richardson (1994) findings and those of this study. Nevertheless, if limited to local-scale, open-water habitats, their conclusion that P additions increase species richness (i.e., species density) is probably robust. However, our results imply that this pattern should not be expected when considering the broad vegetation pattern of wetland landscapes impacted by nutrients.

11.4.3 Determinants of Assemblage Composition

Results from ordination implied that invertebrate assemblages along the P gradient were organized by two spatio-environmental dimensions (1) a coarse/landscape-scale dimension best explained by distance from canal, sediment TP, variability of water depth, periphyton C:N ratio, and broad-scale vegetation pattern and (2) local-scale dimension related to mean water depth, frequency of severe dry down, density of small invertivorous fish, cover of metaphyton, sediment Na, and fine-scale vegetation pattern. Since effectively summarized as two dimensions, this suggests that wetland invertebrate communities may be assembled in a predictable way in response to nutrient enrichment. However, the great diversity of significant pure–partial relationships between candidate spatial, abiotic, and biotic predictor variables and invertebrate composition suggests that this assembly is dependent upon numerous factors that may act independently or synergistically, vary among levels of nutrient enrichment, and vary across the spatial hierarchy (King 2001).

Vegetation (expressed as cover-weighted species composition) was consistently the most important determinant of invertebrate species composition, regardless of scale or nutrient status. Reasons for this may be numerous, but may be best summarized simply: vegetation forms the physical template for other biota in wetlands. In this study, vegetation integrated numerous spatial and abiotic sources of variation directly attributable to the coarse/landscape-scale P gradient, yet manifested these sources of variability as both local- and coarse/landscape-scale variation in its compositional pattern (King et al. 2004; see Chap. 9). Consequently, associations between invertebrate species and specific plant assemblages were reflected on these same scales across the landscape. For example, the chironomid *Beardius* cf. *truncatus* was almost always collected in plots hosted by the macrophyte *Cladium* – distribution of *Cladium* varied both at local, fine scales (topographical variation) and at a coarse/landscape scale (associated with P enrichment).

Vegetation also played a direct role in controlling cover of metaphyton and other periphyton that may have been important to many invertebrates as food (e.g., McCormick et al. 1998). Similarly, small fish densities were highly correlated to vegetation pattern, a trend that may have had predator–prey implications for invertebrates (Batzer and Resh 1991; Jordan 1996). Subsequently, vegetation explained variation directly related to its function as habitat to certain invertebrate taxa, and indirectly related to other biotic determinants such as food resources and predation.

Therefore, vegetation may be expected to be the primary, direct factor driving invertebrate assemblage responses to nutrient enrichment in wetlands.

Despite the strong influence of vegetation, several other variables explained residual variation in composition that could not be explained by vegetation alone (see King 2001). Considering sediment chemistry, both Na and P were significantly related to invertebrates on a fine scale (King 2001). The influence of Na was most apparent within the impacted landscape zone. Although predominantly a P gradient, cations such as Na have been shown to be elevated in canal water that enters the wetland (Craft and Richardson 1997). Insects, in particular, are sensitive to salinity and have an extremely limited distribution in estuarine and marine habitats (Williams and Feltmate 1992). Although no taxon typically associated with estuarine environments appeared related to Na, its small but significant correlation to the invertebrate assemblage suggests that it may have played a minor role in localized differences in composition.

Similar to P, distance from canal was also an important correlate of composition. Because it was the source of the P gradient, canal had a direct effect on sediment TP, and thus an indirect effect on most biotic variables (King 2001; King et al. 2004). However, even after variation from these and all other variables was removed, canal remained a significant correlate of composition; in fact, the magnitude of its partial correlation was second only to vegetation. This residual dependency of invertebrate composition on canal mimics the same mysterious dependency exhibited by vegetation. In King et al. (2004), several plausible explanations are provided for this phenomenon; these may also apply to invertebrates here. Regardless of the mechanism, a safe conclusion is that the canal–levee system of south Florida plays a significant role in changes observed in structure of the Everglades ecosystem, and its influence is reflected in both vegetation and macroinvertebrate levels of organization.

Spatial differences in hydrology were implied to be important to invertebrates. The most obvious pattern was related to local differences in water depth, particularly in the reference and transition zones. Here, open-water slough habitats were typically situated lower on the landscape than adjacent stands of *Cladium*. However, local pattern in vegetation was not perfectly related to water depth since both were significant pure–partial correlates of composition (King et al. 2004). Thus, hydrology probably also played a direct role in organizing species. For example, many taxa were consistently associated with plots with a high frequency of severe dry down (depth <-10 cm). This may have indicated a greater tolerance to drought resistance or that these taxa were more effective at recolonizing hydrologically unstable environments (Wiggins et al. 1980).

Consistent with patterns observed in assemblage biomass, periphyton variables (metaphyton cover and C:N ratio of periphyton) were significantly related to composition. Metaphyton, particularly the calcareous form characteristic of low-P areas of the Everglades, has been indicated to be important not only as a food resource but also as a unique habitat to invertebrates (e.g., Browder 1982). Indeed, several indicator taxa such as *Paraponyx* sp., *Tanytarsus* sp. R, *Cypretta brevisaepta*, *Parakiefferiella* sp. C, and *Cladotanytarsus* sp. were almost always found associated

with mats of calcareous periphyton in the unimpacted zone. Loss of these calcareous mats has been experimentally documented in response to P additions (e.g., Walker et al. 1989; Richardson et al. 2000; see Chap. 25), thus it can be inferred that loss of these mats may have been responsible for the reduction or elimination of some taxa deemed indicators of the low-P, unimpacted zone. The importance of calcareous metaphyton in structuring invertebrate assemblages is an area of research in need of direct investigation in the Everglades.

Density of invertivorous fish was indicated to be a determinant of invertebrate assemblage composition. Although fish density or biomass did not appear to explain the subsidy–stress pattern of biomass along the P gradient, fish may have been structuring composition through selective predation of particular invertebrate taxa (top-down control). Alternatively, fish density may have been associated with greater numbers of particular taxa that were more readily available as food (bottom-up control). For example, high densities of surface-feeding *Gambusia holbrooki* (mosquitofish) and *Heterandria formosa* (least killifish) were found among floating macrophytes in high-P areas; here, mosquito larvae such as *Uranotaenia sapphirina*, *Mansonia titillans*, and *Coquillettidia perturbans* were often in great abundance. Thus, it is difficult to know whether fish were influencing assemblage composition through predation, or responding to specific invertebrate assemblages. Both mechanisms are likely to be tightly coupled, and the extent to which one is predominant is quite likely to be dependent on an interaction among multiple factors through time and space (Batzer and Resh 1991; Jordan 1996).

11.4.4 Implications for Bioassessment

Two schools of thought persist in the scientific community regarding the best approach for bioassessment in streams (Reynoldson et al. 1997). The first school is the multimetric approach, an assessment framework that relies on an aggregated index of biological integrity (IBI; sensu Karr 1981) composed of multiple "metrics" to score sites. By definition, metrics are attributes that represent key elements of structure or function of biotic assemblages, and show a monotonic response to increasing levels of human influence or specific environmental stressors (Barbour et al. 1995). Typically, metrics are developed from one of four categories (1) taxonomic richness, (2) taxonomic structure, (3) feeding ecology, and (4) tolerance/intolerance. Results from this study suggest that three of four categories (richness, structure, and feeding) may not be consistently effective for assessing detrimental nutrient enrichment in wetlands. The primary reason is that the vast majority of invertebrate assemblage attributes from these categories exhibited unimodal responses to P enrichment. Such responses are problematic for multimetric indexes, as sites at opposite ends of an environmental continuum would be considered equivalent for a metric responding in a unimodal fashion.

The second school of bioassessment, the multivariate approach, may circumvent this unimodal-response problem. This approach relies on the identification of reference

conditions (just as in the multimetric approach) to characterize the natural range of variability expected in minimally impacted aquatic habitats. However, rather than extracting coarse attributes from the assemblage to use as metrics, the multivariate approach uses taxonomic compositional data and multivariate data analysis techniques to assess a test site relative to a collection of reference sites (e.g., Reynoldson et al. 1997; Hawkins et al. 2000). On the basis of the findings in this study, this approach may be a more effective than multimetrics for assessing nutrient impacts. Changes in species-level composition in response to P were evident, as plots were clearly sorted according to impact zones in nMDS ordination space. Distance-based multivariate approaches like nMDS and Mantel tests are insensitive to the shape of species responses to environmental gradients, capturing a multitude of monotonic or unimodal responses along such gradients and expressing them as increased dissimilarity (β-diversity) relative to the reference condition.

The central theme of these findings is that simple monotonic patterns in relation to nutrients are not likely to emerge for many of the invertebrate assemblage attributes commonly used in stream bioassessment today (e.g., species richness). This is not to say that the multimetric approach will not work for wetland systems. However, it implies that compositional metrics based on family-, genus-, or species-level taxonomy may be more effective than those based on coarse taxonomy since few wetland invertebrate groups are inherently sensitive to nutrient-related stressors (e.g., dissolved oxygen).

11.5 Conclusions and Lessons for Restoration

Our results suggest that effectiveness of subsampling depended more upon the minimum number of individuals retained than minimum area or proportion of the sample picked. Fixed-area subsamples were generally less efficient than fixed counts, with 200- and 300-individual fixed counts resulting in significantly greater assemblage–environment relationships and much higher accuracy in detecting impairment than 10% fixed area, despite averaging similar numbers of individuals. The greatest improvement with increasing subsample size was observed between fixed counts of 100 and 200 individuals; detecting impairment, in particular, was not markedly improved with subsample sizes >200 individuals. Supplementing subsamples with a LR search resulted in only very slight improvements in assemblage–environment relationships, but was effective in improving prediction accuracy, particularly for family-level data. However, family-level assemblage–environment relationships and abilities to detect impairment were inferior to genus- and species-level data, regardless of subsample size. Species-level data performed best, primarily because of the large proportion (>20%) of total species belonging to Chironomidae. The potential importance of Chironomidae to wetland bioassessment was further revealed through an evaluation of a tiered-taxonomic approach, which showed that non-Chironomidae family-level data tiered with species-level Chironomidae data produced results very similar to those obtained using genus- or

species-level data exclusively. Our results suggest that fixed counts ≥200 or integrated fixed-area/fixed-count approaches that consistently obtain a minimum of 200 individuals should be considered as minimum subsample sizes for wetlands. We additionally advocate LR searches and recommend genus- or species-level taxonomy, particularly for the Chironomidae.

From a bioassessment perspective, the most significant conclusion may be that wetland invertebrate assemblages are sensitive to nutrient enrichment, and that they respond in predictable ways. However, many of the usual approaches and assemblage attributes used as metrics are not conducive to developing indices of nutrient impairment – subsidy–stress relationships using coarse levels of taxonomy, feeding ecology, or diversity were not satisfactory for this purpose. Rather, our results suggest that fine levels of taxonomic resolution (i.e., genus- or species-level data) may be necessary for bioassessment to be accurate. Compositional metrics that used species-level data were the most sensitive to P enrichment, and their responses were mediated largely through vegetation and periphyton. This begs the question: if invertebrate assemblage organization is tightly coupled to primary-producer response to nutrients, why not assess indicator groups such as periphyton or macrophytes instead?

Clearly, periphyton and macrophytes are excellent indicators of nutrient status, and should be considered for wetland bioassessment. However, the appropriateness of an indicator group may be most dependent upon temporal scales of interest. In wetlands, microbes are the first to respond to enrichment, followed by periphyton, invertebrates, and finally macrophytes. Invertebrates have an advantage in bioassessment because of their dependence both on levels that respond faster (microbial, periphyton) and those that respond more slowly (vegetation) to pollution. Their intermediate position along this continuum integrates the effects of both episodic and cumulative stressors in aquatic systems. Indicators that respond quickly may also recover too quickly for detection if pollution is episodic, while slower indicators may not respond or recover quickly enough if water quality is cumulatively degraded or subsequently restored. Ultimately, the decision on the most appropriate indicator group or groups to use will depend on both the spatial and temporal scales of interest; our data suggest that invertebrate assemblages may be robust indicators in many situations.

Anthropogenic inputs of nutrients into the Everglades are significant and ongoing, and our results suggest that the implications of such inputs go beyond just changes in primary productivity or proliferation of weedy species typically associated with enrichment. Rather, changes in pattern (e.g., arrangement of patches of vegetation) have profound effects on process (e.g., population dynamics through time and space), which subsequently affect pattern. It can be inferred that the spatial ecology of higher organisms, such as invertebrates, fish, birds, etc., can be significantly affected by these alterations in a variety of ways that may depend largely upon landscape connectivity and critical scales in their individual life histories. The field of landscape ecology has already begun to address many of these scaling issues for terrestrial wildlife (e.g., spotted owl); such management approaches could be extended to aquatic systems as well. Importantly, this research illustrates

an important deficiency in aquatic research specifically that greater attention needs to be given toward evaluating the implications of spatial pattern and scale. Such research will be critical for the successful management and restoration of wetland and other aquatic ecosystems.

Finally, one of the central conclusions of this research is that invertebrate assemblages are organized through a myriad of spatial, temporal, abiotic, and biotic pathways that are a product of differing nutrient levels and processes that operate at a variety of scales across the spatial hierarchy. From an ecological perspective, perhaps the most significant finding was the hierarchical spatial relationship between landscape pattern and invertebrate assemblage composition, and how this relationship varied among differing nutrient regimes. The finding that canals were a major influence on the macroinvertebrate community at the larger scale suggests that natural populations are highly influenced by these man made structures.