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Macroinvertebrate and Fish Responses to Experimental P Additions in Everglades Sloughs

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19.1 Introduction

A variety of anthropogenic influences threaten the Everglades. One of the most publicized perturbations to the system has been excessive inputs of phosphorus (P). The Everglades is a P-limited ecosystem, and its biota are adapted for survival under highly oligotrophic conditions (e.g., Browder 1982; Steward and Ornes 1975a,b; Swift and Nicholas 1987; Davis 1991). Indeed, numerous experiments have demonstrated the sensitivity of Everglades biota to P enrichment. In one of the first fertilization experiments, Steward and Ornes (1983) showed that small additions of P resulted in significant increases in the productivity of sawgrass (*Cladium jamaicense* Crantz.) seedlings, the most abundant macrophyte in the Everglades (Loveless 1959). However, high levels of P actually inhibited sawgrass production (Steward and Ornes 1983). Ensuing field fertilization studies have shown that P enrichment can have profound effects on open-water slough communities, a unique and potentially critical habitat in the Everglades ecosystem (e.g., Loveless 1959). For example, Walker et al. (1989) demonstrated that continuous dosing of high concentrations of inorganic P in sloughs of Everglades National Park nearly eliminated the common submergent macrophyte *Utricularia purpurea* Walt. and attached floating mats of calcareous periphyton, but stimulated growth of several emergent macrophyte species. In the same study, Flora et al. (1988) and Hall and Rice (1990) found that periphyton accumulation on artificial substrates showed as much as a tenfold increase in biomass as a direct result of P. This biomass increase was concomitant with significant shifts in algal species composition and changes in tissue nutrient content (Hall and Rice 1990). Subsequently, other P-fertilization experiments have been conducted in slough habitats, producing similar responses for both periphyton (Vymazal et al. 1994; Craft et al. 1995; McCormick and O'Dell 1996) and macrophytes (Craft et al. 1995).

Despite this clear connection between P enrichment and changes in productivity and species composition of primary producers in the Everglades, remarkably little has been done to assess how these effects manifest themselves at higher trophic levels. To date, only papers resulting from the P-dosing experiment that is presented in this chapter (King 2001; King and Richardson 2003; Qian et al. 2003) have examined responses of invertebrates or fish to P enrichment using experimental manipulations. Moreover, relatively few observational studies have been conducted on Everglades invertebrates or fish in relation to nutrient enrichment (Terczak 1980; Urban and Koebel 1992; Rader and Richardson 1994; Jordan 1996; Turner et al. 1999; King 2001; King and Richardson 2002).

As part of the P-dosing experiment designed to refine our understanding of how specific concentrations of P affect multiple levels of the Everglades slough ecosystem, we set out to establish dose–response relationships for attributes of macroinvertebrate and fish assemblages to local-scale, long-term experimental P additions. We used flume-style mesocosms with multiple treatments to create experimental P gradients, thus providing a wide range of concentrations to meet with our primary objective.

We used the subsidy–stress model (Odum et al. 1979) as a framework to establish several hypotheses regarding macroinvertebrate and fish assemblage responses to experimental P enrichment:

1. Macroinvertebrates and fish are resource limited. Relaxation of P-limitation will result in a positive response in primary production with small doses of P. Macroinvertebrate and fish abundance will mirror responses of primary producers, such that increased in production will increase macroinvertebrate and fish standing stocks.
2. Species turnover of primary producers will result in a stress to specialized macroinvertebrate species (e.g., grazers of specific algae). Opportunistic species will proliferate with P additions, while specialists will be disadvantaged by the loss of sensitive algal taxa, resulting in a subsidy–stress dose–response curve for species richness. The opportunistic small fish assemblage, which has few species, will not exhibit a detectable change in species richness.
3. Shifts in assemblage structure (e.g., increased dominance by one or a few species) will occur for both macroinvertebrates and fish at relatively low doses of P.
4. Succession of macroinvertebrate assemblages will be amplified with P due to greater secondary production and turnover, potentially leading to lesser temporal stability in composition.
5. The direct effect of P on periphyton production and nutrient content will be the most important dimension of organization for macroinvertebrate and fish assemblages in Everglades sloughs.

Our goal in this chapter is to emphasize tests of these specific hypotheses. Here, we present a synthesis of the results of approximately 3 years of macroinvertebrate and 1 year of fish studies from the P-dosing experiment.

19.2 Methods

19.2.1 Study Area and Experimental Design

The P-dosing experiment was established in the southern interior of WCA-2A, a large, contiguous Everglades landscape. Levees surround the perimeter of WCA-2A, and water-control structures pump water in and out of the fen at irregular intervals

(SFWMD 1992). Concentrations of water-column soluble reactive phosphate (SRP) and unfiltered total phosphorus (UTP) in the vicinity of the study area are typically near 5 and 10 μg l^{-1}, respectively (Vaithiyanathan and Richardson 1998). Sediment and soil TP typically range between 250 and 400 mg kg^{-1} (Vaithiyanathan and Richardson 1998). Other detailed characteristics of WCA-2A are presented by numerous authors in this book, including geology and hydrology (Chap. 7), soil properties (Chap. 3), water chemistry (Chap. 6), and vegetation (Chap. 4).

Two dosing facilities (hereafter called "sites") were constructed in adjacent sloughs (26°15′N, 80°23′W) in fall of 1992. Each site had five-walled mesocosms, or flumes, 2-m wide × 10-m long with walls approximately 90 cm in height above the slough substrate. Flumes were oriented N–S and separated by 1 m, where permanent boardwalks were built to allow investigators access for sampling. Flumes were not sealed at the south end but were obstructed to prevent large vertebrates from entering. Each flume was randomly assigned one of five SRP treatments: ~5 μg l^{-1} (control; 0.25 g m^{-2} $year^{-1}$), ~22 μg l^{-1} (1.5 g m^{-2} $year^{-1}$), ~39 μg l^{-1} (2.75 g m^{-2} $year^{-1}$), ~57 μg l^{-1} (3.5 g m^{-2} $year^{-1}$), and ~126 μg l^{-1} (8.2 g m^{-2} $year^{-1}$). These concentrations correspond to mean doses and loads for the full 6 years of the study. An additional unwalled control area was established on the west side at both dosing sites to concurrently monitor the potential effects of placing walls around slough habitat. Originally, categorical labels (unwalled control (UC), walled control (WC), 30, 50, 75, and 150, respectively) were assigned to each treatment based on the anticipated SRP-dosing concentration, but actual doses were lower. Thus, these names are used at times in the text for convenience but are not intended to reflect the precise dose applied to flumes.

SRP was dosed from the northern end of flumes via large mixing tanks. Tanks contained natural slough water mixed with a regulated ratio of SRP concentrate. Flumes were dosed on a continuous schedule except during low- or high-water shutdowns or periodic maintenance to specific flumes. Maintenance shutdowns, particularly during 1997 and 1998, caused cumulative P loads to vary modestly among treatments, so actual loads were calculated for each flume to better estimate cumulative exposure to P. Dosing was applied from 30 November 1992 to 21 September 1998. Greater detail on the design and operation of the P-dosing experiment are presented in Chap. 14.

Water depth at the sites varied seasonally and interannually. During the course of study, water depths ranged between near 0 cm and over 100 cm, but typically were 25–75 cm. Depths were generally greatest in late summer to early fall (wet season) and reached their lowest values during spring (dry season). However, in 1998, water levels were quite high (60–90 cm) throughout the dry season.

Vegetation of the sloughs was characterized by abundant white water lily (*Nymphaea odorata* Aiton). Submergent plants were primarily bladderworts, *Utricularia fibrosa* Walt., *Utricularia foliosa* L., and *U. purpurea* Walt. *Chara* sp., a macroalga, also was locally and seasonally abundant. Spikerushes (*Eleocharis cellulosa* Torrey and *Eleocharis elongata* Chapm.) were the dominant emergent plants, with occasional stems of maidencane (*Panicum hemitomon* Schult.) also observed. A chalkish-colored mat of calcareous periphyton, composed primarily of cyanobacteria and diatoms, often covered much of the slough surface and typified this oligotrophic habitat (Swift and Nicholas 1987). Epiphytic periphyton was also locally abundant, particularly on stems of *Eleocharis* spp. Richardson et al. (Chap. 16) and Kaštovský et al. (Chap. 18) provide a synthesis of vegetation and periphyton responses to P dosing, respectively.

19.2.2 Sampling: Water Chemistry, Periphyton, and Vegetation

Water-column SRP and UTP were measured biweekly throughout the duration of the study. Since uptake and diffusion dramatically affected concentrations down the length of the flumes, surface samples were collected at 0.5-, 1-, 2-, 3-, 4-, 6-, and 8-m stations corresponding to distances from the source of P inputs. Sediment TP (0–10 cm) was measured in 1995, 1997, and 1998 at each station. Other surface-water measurements (UTN, NH_4, NO_3, major cations) also were collected but generally were not affected by P dosing. Dissolved oxygen (DO) was measured periodically using multiprobes deployed at 2-m stations in mid-water column for 1-week durations. However, minimum DO concentrations were not sufficiently altered by P dosing to elicit negative effects on macroinvertebrates or fish and will not be considered further here. Synthesis of the dosing study water chemistry results are presented in Chap. 15.

Periphyton was collected from a variety of substrates during the study, but primarily from the floating mat and artificial substrates (Plexiglas slides). Because Plexiglas slides best estimated short-term periphyton responses to P and were similar to the substrates used to collect macroinvertebrates in this study, data from these samples were used in subsequent macroinvertebrate analyses. Plexiglas slides were deployed at 2-, 4-, and 6-m stations in flumes for a duration of 2 months. Ash-free dry mass (AFDM), % ash, and total C, N, and P were the primary periphyton analytes. Species composition was examined on some dates earlier in the study, but species data were not generated concurrently with macroinvertebrate or fish data. Periphyton results from the first 4 years of the study are presented in Chap. 18.

Macrophyte stem counts and % cover as well as floating periphyton mat % cover were surveyed quarterly using 1-m^2 quadrats. Quadrats were centered in the flumes, and macrophytes were surveyed across the entire length of the flumes at all stations. Richardson et al. (Chap. 16) describe the responses of vegetation to P additions.

19.2.3 Sampling: Macroinvertebrates

We initiated the macroinvertebrate component of the P-dosing study in 1996, 4 years after dosing had begun. We sampled macroinvertebrates in May and September 1996, January 1997, and February and September 1998. Sampling was intended to be maintained twice per year, one wet (September) and one dry (January–May) season event, but dry conditions during summer 1997 forced a shutdown of dosing and prevented a September 1997 collection. Macroinvertebrates were sampled from the dosing flumes using Hester–Dendy artificial substrates. Hester–Dendys (HDs) were used for several reasons. The state of Florida has classified the Everglades as a Class III waterbody and has mandated that water-quality assessments using macroinvertebrates in these areas will be conducted using a composite sample of a minimum of three HD artificial substrate samples (Florida Administrative Code 17-302.560[9]). Additionally, HDs are deployed for a fixed period of time to allow for colonization of the substrate, thus are passive samplers and minimize disturbance to the surrounding natural habitat (e.g., Cairns 1982). Active sampling devices (e.g., dip nets) are destructive to habitat and were inappropriate for use in this long-term experiment. Finally, HDs are standardized for surface area and help reduce the variability associated with sampling from natural substrates (Murkin et al. 1994), which were spatially heterogeneous on the relatively fine scale present in the dosing flumes.

We deployed three HDs at each of the 2-, 4-, and 6-m stations from the source of P inputs in all flumes on each date (108 per date). Since three HDs are required to form a composite sample, each station could therefore be considered as an observation. This sampling design was chosen to allow for comparisons among P-dosing treatments as well as observational analyses using water-quality measurements at each station. HDs were deployed for 28 days. HDs were suspended from fixed bars across the width of the flumes and maintained at mid-depth in the water column. Depth of HDs was adjusted on a weekly basis, if necessary, to accommodate for water-level changes. We spaced samplers 0.5 m apart on each bar, centered within the flume. After 28 days, HDs were collected by sliding a 4-l heavy-duty plastic bag under the sampler and pulling the bag to the water surface. This technique alleviated potential dislodging of mobile invertebrates resting on the sampler during retrieval. However, during May 1996, three dosed flumes were nearly completely overgrown (90–100% cover throughout the water column) with *Chara* sp., a macroalga, which prohibited this collection technique. These HDs had to be untangled from and forced through the *Chara* mats, undoubtedly agitating and dislodging attached macroinvertebrates. Therefore, HDs collected from these flumes during this event did not meet QA/QC procedures and were subsequently excluded from the final data set.

Once collected, samplers were put on ice and returned to the laboratory for processing, where material on the substrates was scraped and rinsed into a sieve, then fixed in 10% buffered formalin for storage. We defined macroinvertebrates as any invertebrate retained in the 0.5-mm sieve, which included Cladocera, Copepoda, and Ostracoda. All macroinvertebrates were sorted from associated periphyton and detritus under a stereomicroscope using 10× magnification. We identified individuals to the lowest possible taxonomic unit. Nomenclature followed Hobbs (1942), Keyser (1975), Sanderson (1982), Pluchino (1984), Thompson (1984), Berner and Pescador (1988), Daigle (1991), Dodson and Frey (1991), Williamson (1991), Daigle (1992), Epler (1995, 1996), Klemm (1995), Pescador et al. (1995), Courtney et al. (1996), and Milligan (1997). Taxa that could not be assigned a species name were identified to morphospecies, a level of taxonomy that differentiated among individuals that were likely distinct species but did not attach specific names (Oliver and Beattie 1996). Multiple specimens of each taxon were retained in a voucher collection and sent to expert taxonomists for independent verification of names.

We estimated macroinvertebrate biomass for each HD station-composite after all names had been verified. Because shell mass of snails is generally not considered to constitute biomass, all Gastropoda were separated from other invertebrates. Gastropods were soaked in Bouin's fixative, a technique used to dissolve the calcareous shells but not affect mass of the flesh (F.G. Thompson, personal communication). Macroinvertebrates were pooled together for each sample, oven-dried in preweighed aluminum pans for 48 h at 60°C, and placed in a desiccator before weighing. Pans were weighed on an analytical balance (± 0.0001 g) to estimate dry mass. Gastropoda were dried and weighed separately from other invertebrates. Many of the smaller invertebrates, particularly Chironomidae and Oligochaeta, had been previously mounted on slides for identification purposes, thus were not usable for direct weighing. These specimens were enumerated into size classes based on length and width, and dry mass subsequently was estimated using a biovolume technique (Smit et al. 1993).

19.2.4 Sampling: Fish

We initiated fish collection in October 1997, and additional collections were conducted in January and May 1998. As was the case with the macroinvertebrates, fish sampling methods had to be nondestructive to habitat in the flumes. This immediately ruled out active sampling devices, such as throw traps, that have been effectively used in Everglades marshes to sample fish (e.g., Loftus and Eklund 1994; Jordan 1996). Instead, we used small fish traps (43 × 25 × 25 cm, 5 cm funnel openings, 2 mm nylon mesh), which were passive samplers and relied on fish activity for collection rather than movement of the sampler itself (e.g., Hubert 1996). These traps functioned similarly to funnel traps and fyke nets commonly employed in wetlands to capture fish (e.g., Brazner 1997). Traps were deployed in pairs at the 2-, 4-, and 6-m distances within each flume (72 traps per date), suspended from bars across the width of the flumes. We aligned the top of each trap with the surface of the water to more effectively capture the primarily surface-oriented small fishes (e.g., mosquitofish, *Gambusia holbrooki*) found in Everglades slough habitats.

We deployed traps for 24 h on each date. Initially, we set out to identify, count, and measure all individuals in the field and release them back into the flumes, but this was deemed almost logistically impossible and statistically limiting due to the relatively poor accuracy of mass estimates made on small fish in the field. Additionally, because flumes could be recolonized by fish from the surrounding sloughs through the opening at the south ends, we assumed that any removals would be offset by recolonization. Thus, we retained fish for laboratory examination. Contents of each trap were emptied into a container where fish were anesthetized, then fixed in 10% formalin. All individuals were identified, measured for total length (mm), and weighed on an analytical balance (±0.0001 g). Taxonomic nomenclature followed American Fisheries Society (1990).

We also considered the potential that predation by large fish may have an influence on the abundance and composition of small fish in the flumes. We attempted electrofishing as a supplementary technique to the fish traps (Reynolds 1996). We used a pulsed-DC backpack electrofishing unit (Smith-Root, Inc., Vancouver, WA, USA); however, it was not effective due to interference by the heavy vegetation and abundant galvanized pipe comprising the support structure of the dosing facility.

19.2.5 Data Analyses: Macroinvertebrates

The dose–response relationship between P and macroinvertebrates was examined using a suite of assemblage-level attributes. Attributes were selected from 1 or 4 categories (1) standing stocks, (2) taxonomic richness, (3) taxonomic structure, and (4) feeding ecology. Mean or total (total number of taxa) values from each dosing flume were regressed against P load to assess a potential dose–response relationship. Load rather than dosing concentration was used because of slight variation in [PO_4] within individual mixing tanks over time and occasional maintenance shutdowns to flumes that caused the loads to vary somewhat among flumes of the same treatment. Therefore, load more accurately represented the actual P treatments and was subsequently used as a continuous predictor variable. Of note, sediment TP concentrations in the flumes during the course of the study spanned a very similar range to those observed along the P gradient in WCA-2A (250–1,800 mg kg^{-1} at dosing study; 300–1,800 mg kg^{-1} along P gradient); thus, the treatment with the highest P load was similar to the most enriched areas near canal inflow structures. Load units were standardized to total surface area of the flumes (g m^{-2} $year^{-1}$). UC flumes could not be assigned a load value because they received no P additions and thus could not be used in dose–response regressions. These flumes served as qualitative reference points to assess potential wall effects on assemblage attributes.

Simple linear and curvilinear relationships were explored between attributes and P load. Several P load durations (immediately prior to and including date of macroinvertebrate collection) were considered, ranging from 28 days (during deployment), 2, 3, 6 months, 1 year, and total (complete study duration). Six-month load was selected since it approximated the aquatic lifespan of most of the long-lived taxa present at the dosing facility, and it consistently showed the strongest relationship to attribute values. Data transformations (log and arcsine) were applied when appropriate (Sokal and Rohlf 1995).

Multivariate assemblage composition was summarized and related to environmental variables using nonmetric multidimensional scaling (nMDS), an ordination technique based on ranked dissimilarities among samples (Minchin 1987; Clarke 1993; Legendre and Legendre 1998). The objective in the use of nMDS was to recover a multivariate community pattern that could potentially be attributed to an environmental gradient, such as phosphorus concentration. Bray–Curtis dissimilarity was selected as the metric for the macroinvertebrate assemblage data, as it has been shown to be one of the most robust and ecologically interpretable measures for species abundance data (Bray and Curtis 1957; Faith et al. 1987; Clarke 1993; Legendre and Legendre 1998; Legendre and Anderson 1999). Taxon abundances were standardized using a $\log_{10}(x + 1)$ transformation to provide greater weight to uncommon taxa (e.g., Faith and Norris 1989; Cao et al. 1998).

In the first nMDS analysis, data were ordinated using individual flumes (including UCs) as observational units. This analysis was done separately for each date (n = 12 per date) as well as all dates combined (n = 60), with the former analysis designed to assess the overall effect of P loads on each date, while the latter was intended to track the trajectories of each flume over time and in relation to each other (successional vectors; Legendre and Legendre 1998). Ordinations were also conducted using data at 2-, 4-, or 6-m stations within each flume, again including UCs, for the purpose of relating observational environmental data collected at each station (n = 36 per date) to assemblage composition. Measured environmental variables included macrophyte cover/stem counts (by species), periphyton AFDM, % ash, TC, TN, and TP, water-column SRP and UTP (6-month average), and sediment TP (most recent annual collection). We related these variables to species composition using vector fitting in nMDS space (Faith and Norris 1989; Growns et al. 1992).

To complement the nMDS ordinations and cleanly assess the direct and indirect effects of the environment on macroinvertebrate species composition, partial Mantel's tests were applied in conjunction with path diagrams (Leduc et al. 1992; Sanderson et al. 1995). The Mantel's test is a multivariate technique that uses distance matrices as variables and allows the investigator to partial out the effect of other variables to obtain an independent estimate of the relative contribution of each variable to macroinvertebrate species composition. Using a priori hypotheses regarding relationships among variables, a path diagram synthesizes the results of the partial Mantel's tests by depicting the significant paths of relationships (Legendre and Legendre 1998). Phosphorus (SRP, UTP, sediment TP, and periphyton TP), macrophyte (species composition weighted by untransformed cover estimates), periphyton (AFDM and C:N ratio), and macroinvertebrate distance matrices were constructed for this analysis. Individual variables used in P and periphyton distance matrices were standardized to z-scores prior to matrix calculation using Euclidean distance (Legendre and Legendre 1998). Macrophyte and macroinvertebrate matrices used Bray–Curtis dissimilarity as the distance metric.

19.2.6 Data Analyses: Fish

The Everglades small fish assemblage has relatively few common species; thus, the number of attributes for use as response variables to P additions was more limited than for the macroinvertebrates. We calculated individual species abundance and biomass, total numerical abundance and biomass, and species richness within each flume as a unit. Only six species were abundant enough to make univariate statistical inferences. Consistent with the macroinvertebrate analysis, we used 6-month P load as a predictor in linear and curvilinear regression analyses to estimate potential dose–response relationships.

Additionally, we compared the slope and the intercept of length–mass regression equations to assess whether average fish size or condition changed among P treatments. To eliminate potential flume-specific findings and increase sample size, we pooled individuals within each of the six dosing concentration "categories" (i.e., UC, WC, 30, 50, 75, and 150; see Chaps. 14 and 15). Only *G. holbrooki* had sufficient numbers to justify treatment comparisons (minimum $n > 50$ per treatment, total $n = 693$) (Sokal and Rohlf 1995; Anderson and Neumann 1996).

We ordinated multivariate species composition using nMDS separately for each date to assess potential gradients in the assemblages that could be attributed to P load or other environmental variables (e.g., vegetation, periphyton). We also examined trajectories of assemblages in each flume over time using successional vectors in nMDS space, as in the macroinvertebrate analysis.

19.3 Results

19.3.1 Dose–Response Relationships: Macroinvertebrates

Over 36,000 individuals represented by 123 taxa were collected and identified during the study period (Table 19.1). Amphipoda, Chironomidae (Diptera), Gastropoda, microcrustacea (Cladocera, Copepoda, and Ostracoda), and Oligochaeta were the most numerically dominant taxonomic groups (Table 19.1). Amphipoda, Decapoda, Gastropoda, and Odonata tended to have the largest individuals and thus made the greatest contributions to assemblage biomass. Chironomidae (at least 33 species) had the most species among all higher groups.

Many macroinvertebrate assemblage attributes changed significantly as a function of P dosing. The two primary measures of standing stocks, density and biomass, showed a positive response to increasing P load on all collection dates, with significant relationships ($p \leq 0.05$) on four of five dates for density and three of five dates for biomass (Fig. 19.1). This trend was particularly apparent during the winter (dry season) collections, January 1997 and February 1998. Water depths were markedly different between these two dates, with February 1998 having much greater depth than January 1997, yet results were remarkably similar (Fig. 19.1).

Taxon richness and taxon density were also affected by P. These diversity metrics increased consistently among dates with increased P additions, following a similar pattern to that of total abundance and biomass (Fig. 19.2). Generally, the intermediate P loads resulted in a marked increase in the number of taxa relative to the controls, with diminishing marginal increases with additional P loading.

Table 19.1 Dominant invertebrate taxa collected at the P-dosing experiment

Taxonomic group	No. collected	Rank	Dominant taxon/taxa
Amphipoda	4,304	5	*Hyalella azteca* (Saussure)
Bryozoa	41	15	*Plumatella* cf. *repens* (L.)
Cladocera	687	7	*Ceriodaphnia* cf. *reticulata* (Jurine)
Cnidaria	6	18	*Hydra* sp.
Coleoptera	16	16	*Enochrus ochraceus* (Melsheimer)
Copepoda	2,426	6	Cyclopoida
Decapoda	69	14	*Palaemonetes paludosus* (Gibbes) *Procambarus* cf. *alleni* (Faxon)
Diptera (Chironomidae)	7,239	2	*Chironomus* sp. 2 *Chironomus stigmaterus* (Say) *Cladotanytarsus* sp. *Endochironomus nigricans* (Johannsen) *Larsia decolorata* (Malloch) *Parakiefferiella* sp. C Epler *Paratanytarsus* sp. B Epler *Polypedilum halterale* group *Polypedilum* sp. A Epler *Tanytarsus* sp. G Epler *Tanytarsus* sp. R Epler
Diptera (Other)	228	9	*Dasyhelea* sp.
Ephemeroptera	454	8	*Caenis diminuta* Walker *Callibaetis floridanus* Banks
Gastropoda	5,812	3	*Aphaostracon pachynotus* Thompson *Physella cubensis* (Pfieffer) *Planorbella duryi* (Weatherby) *Planorbella scalaris* (Jay)
Hemiptera	9	17	*Belostoma* sp.
Hirudinea	205	10	*Helobdella triserialis* (Blanchard)
Hydracarina	166	11	*Arrenurus* sp. 1
Odonata	128	12	*Celithemis eponina* (Drury) *Ischnura hastata* Say
Oligochaeta	9,649	1	*Dero digitata* (complex) *Pristina leidyi* Smith
Ostracoda	5,002	4	*Cypridopsis okeechobei* Furtos *Cytheridella alosa* (Tressler)
Porifera	4	19	*Spongilla* cf. *cenota* (Penney and Racek)
Trichoptera	106	13	*Oxyethira* sp.

Dose–response relationships for taxon richness and density were significant in January 1997, and February and September 1998. Richness of other major groups (e.g., Chironomidae) generally followed this same pattern, thus no particular group of macroinvertebrates was primarily responsible for the overall increase in number of taxa.

Higher taxonomic groups were not negatively affected by P dosing in terms of absolute abundance, but some groups demonstrated greater responses than others. In particular, the primary-consuming Oligochaeta and microcrustacea were most responsive to P. These two groups were in much greater numbers in even the lowest P treatment than in the controls (Fig. 19.3). In contrast,

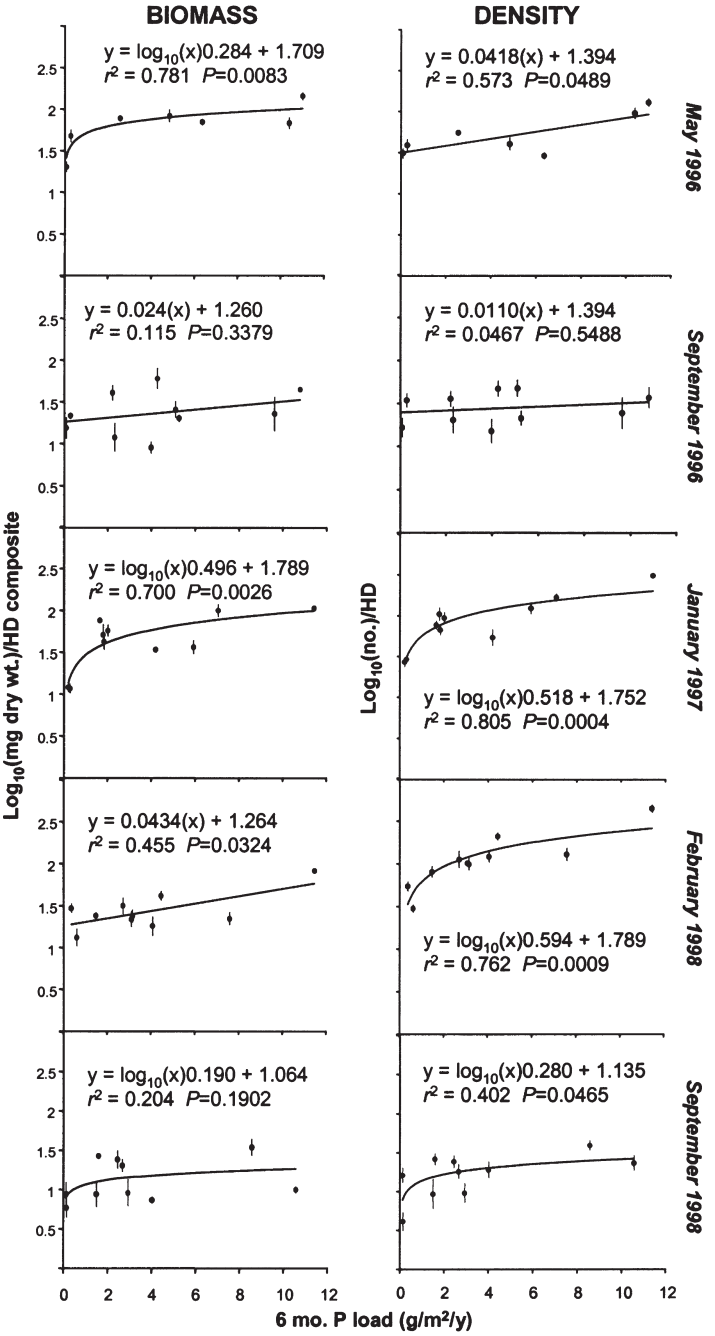

Fig. 19.1 Dose–response relationships between P load and invertebrate biomass and density for all five collection dates at the P-dosing study. Response variables are means (± 1 SE) per mesocosm

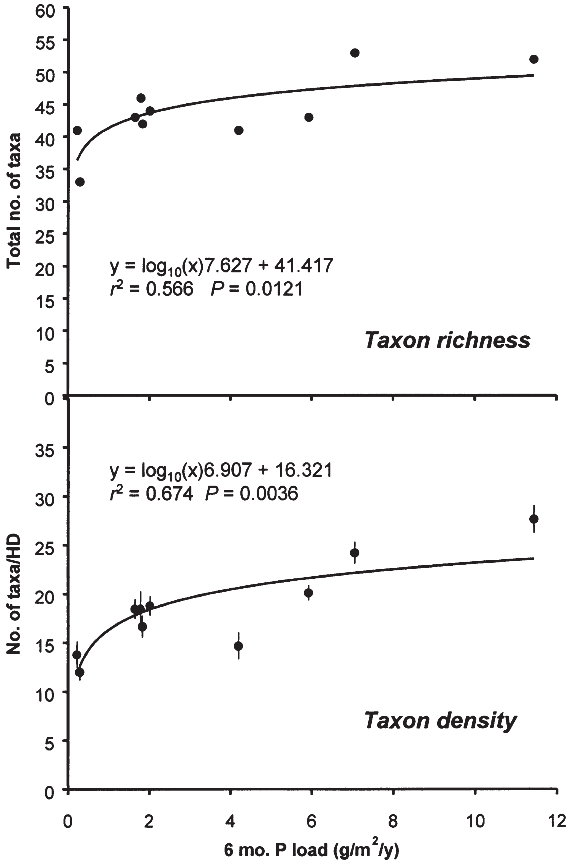

Fig. 19.2 Dose–response relationship between P load and taxon richness and taxon density during January 1997. Taxon density values are means (± 1 SE) per treatment, while taxon richness is expressed as the total number of taxa per treatment

Amphipoda (represented exclusively by the abundant *Hyalella azteca*) revealed potential seasonality in its response to P. Its numbers increased significantly ($p \leq 0.05$) and log-linearly with P during January 1997 and February 1998, but did not respond significantly to P dosing during the May and September collection dates.

Due to large increases in absolute numbers of some groups, proportional abundance measures subsequently indicated shifts in assemblage structure with P dosing. Particularly, % Gastropoda showed a negative linear or log-linear response to P additions on most dates, suggesting that enrichment had not benefited these periphyton grazers in proportion to the rest of the assemblage (Fig. 19.4). In spite of this, Gastropoda numerical abundance consistently exhibited a positive response to P additions.

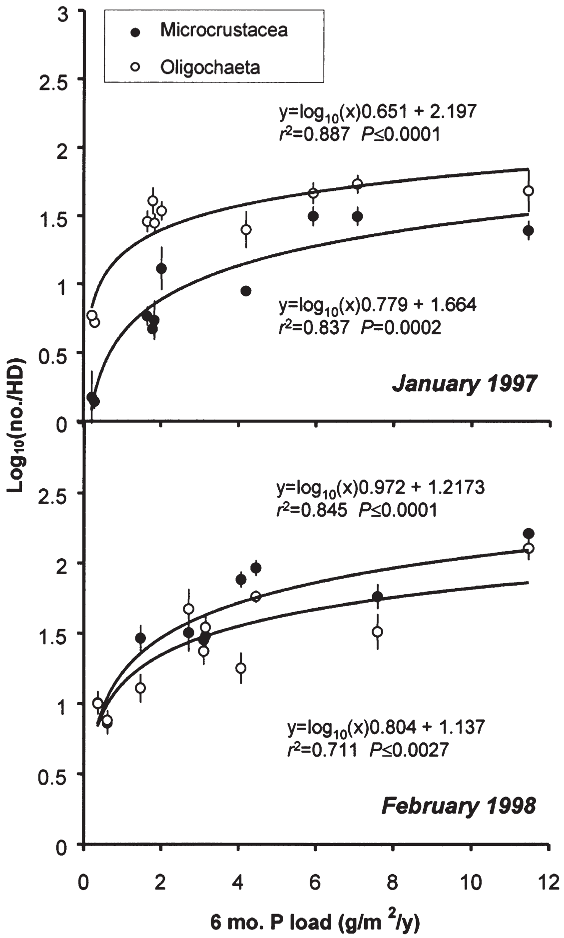

Fig. 19.3 Dose–response relationship between P load and densities of microcrustacea and Oligochaeta during January 1997 and February 1998. Response variables are means (± 1 SE) per mesocosm

Of the trophic levels, primary consumers dominated the macroinvertebrate assemblage, generally representing >90% of the total numbers collected. These periphyton, macrophyte, and detrital consumers showed positive responses to P load on all dates. Secondary and tertiary consumers, or predators, were less responsive to P additions. Their abundance either did not increase above control treatments or showed very subtle increases with increasing P load. However, their proportional abundance was negatively affected on all dates, and significantly during January 1997 and February 1998 (Fig. 19.5). On these dates, % Predators declined in a steep log-linear fashion.

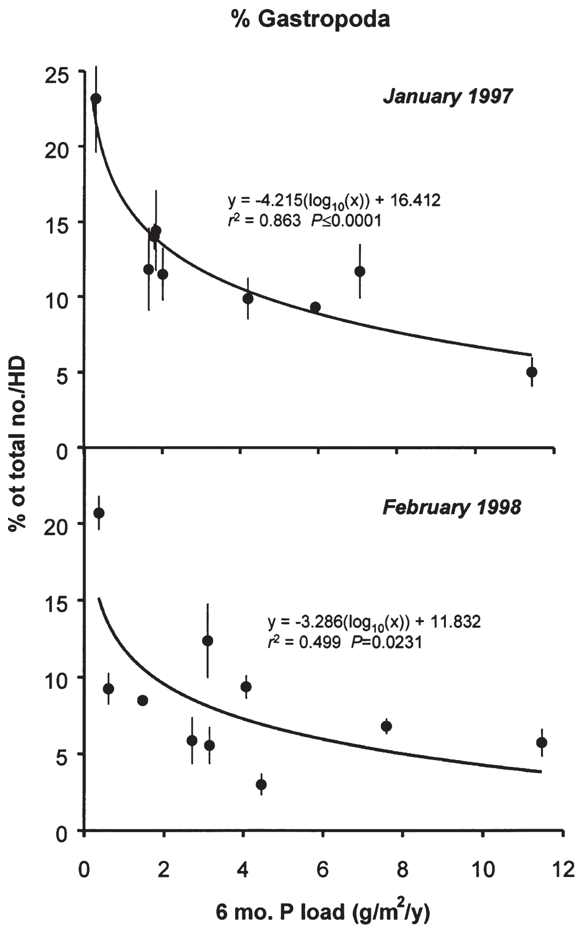

Fig. 19.4 Dose–response relationship between P load and % Gastropoda during January 1997 and February 1998. % Gastropoda values are means (± 1 SE) per mesocosm

Rate of change in composition over time was relatively similar among treatments. Examination of successional trajectories suggested that mean vector lengths, a direct measure of the magnitude of change over time, did not vary notably as a function of P load ($p > 0.05$). However, projection of these vectors in nMDS space revealed that the relative trajectory among dates was affected by P (Fig. 19.6). Assemblages in P-dosed flumes during January 1997 and February 1998 diverged from the paths of the controls, thus reflected increased dissimilarity from control assemblages. This trend was particularly apparent at the greatest loads of P. Moreover, dosed flumes were clearly separated from controls on all dates, complementing the findings from univariate analyses that dosing had not only affected abundance and biomass, but had also manifested itself as a gradient in taxonomic composition. This gradient

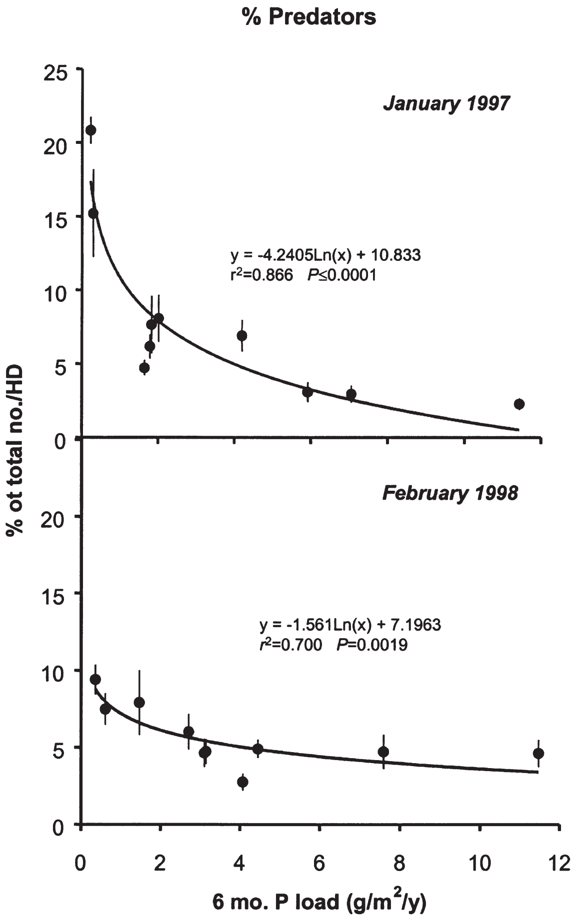

Fig. 19.5 Dose–response relationship between P load and % Predators during January 1997 and February 1998. % Predator values are means (± 1 SE) per mesocosm

in composition was directly related to the magnitude of P loading, as highest-dosed flumes were most dissimilar from controls (Fig. 19.6).

19.3.2 Composition–Environment Relationships: Macroinvertebrates

Several observational environmental variables were strongly related to macroinvertebrate assemblage composition. Vector fitting of individual variables into nMDS space showed that all four measures of P (plexislide periphyton TP, water-column

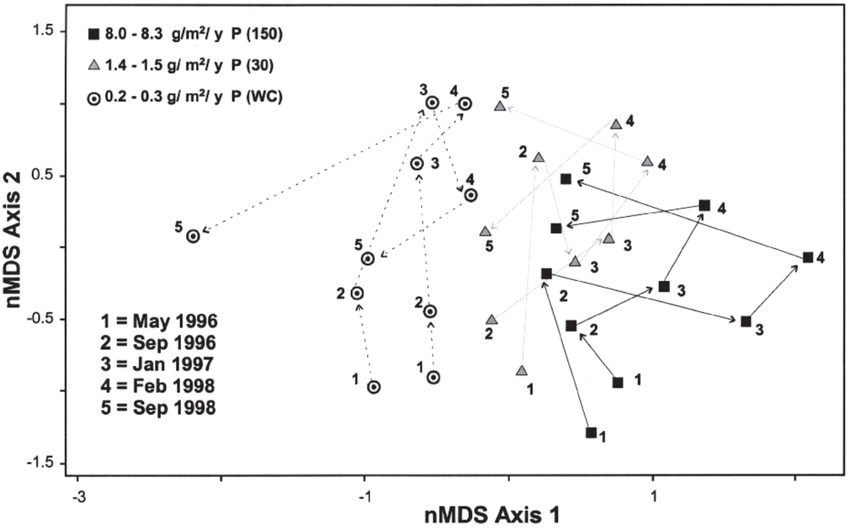

Fig. 19.6 Nonmetric multidimensional scaling (nMDS) ordination of invertebrate successional vectors during 1996–1998 at the P-dosing study. *Symbols* indicate the long-term mean P load per treatment, while *numbers* indicate the date of collection. For clarity, replicate mesocosms are shown only for each of three of the six P treatments

SRP and UTP, and sediment TP) were significantly ($p \leq 0.05$) correlated to the gradient in assemblage composition defined by nMDS Axis 1 (Fig. 19.7). Vectors for these variables were directed away from control sampling stations toward the highest-dosed flumes. Sediment TP, although measured only annually during the study period, had the strongest relationship with composition among the P metrics ($r = 0.43$–0.77, $p \leq 0.0001$ on all dates except September 1996 ($p = 0.0260$)). This trend was consistent among all five dates.

Vegetation and periphyton variables also were significantly correlated to composition. Three macrophyte species, *U. purpurea*, *U. foliosa*, and *E. elongata*, had vectors directed toward control stations (Fig. 19.7). Thus, the distribution of these macrophytes was negatively related to increasing concentrations of P, a consistent finding throughout the duration of the study. Periphyton AFDM accumulation on plexislides, however, followed a similar trajectory as the P vectors (Fig. 19.7). It was directed toward high-P stations and suggested that it played a role in the observed gradient in macroinvertebrate composition. Periphyton total carbon-to-nitrogen (C:N) ratio values decreased as AFDM values increased, typically ranging from 17–22 at control locations to as low as 9–10 at stations of high-P exposure. Consequently, the C:N vector also was related to composition and was directed toward control assemblages.

Partial Mantel's tests, in conjunction with path analysis, were useful in assessing the relative indirect and direct contribution of the environmental variables used in the nMDS analyses on multivariate composition. Although the magnitude of relationships varied slightly among dates, the paths of relationships did not. Periphyton and vegetation both were strongly influenced by P, but the relationship was stronger for

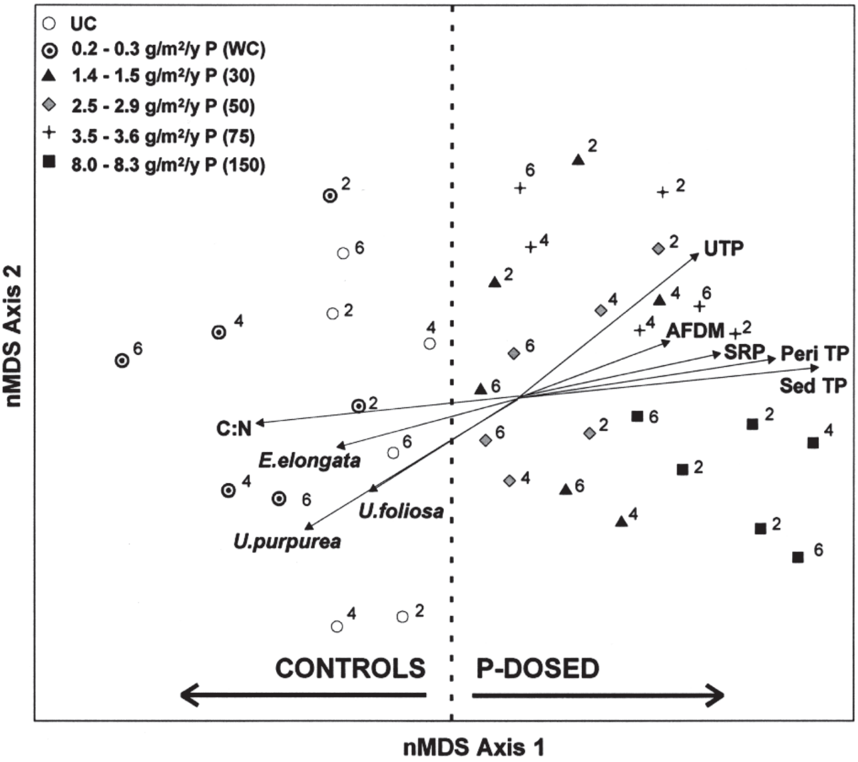

Fig. 19.7 Nonmetric multidimensional scaling (nMDS) ordination of macroinvertebrate assemblage composition at each sampling station from all mesocosms (including unwalled controls) during January 1997. *Numbers* adjacent to symbols indicate station distance (2, 4, or 6 m) down the flumes. Environmental vectors indicate the direction and magnitude of significant ($p \leq 0.05$) correlations to assemblage composition

periphyton (partial Mantel $r = 0.181$–0.512, all $p \leq 0.05$ for periphyton; simple Mantel $r = 0.020$–0.2725, $p \leq 0.05$ only during May 1996 and September 1998 for macrophytes) (Fig. 19.8). Periphyton also was influenced weakly by vegetation structure, even after accounting for the effects of P (partial Mantel $r = 0.088$–0.195, $p \leq 0.05$ only in February 1998). Macroinvertebrate composition appeared to be most influenced by the direct effect of periphyton, in terms of both AFDM and C: N ratio (partial Mantel $r = 0.147$–0.314, all $p \leq 0.05$). Vegetation also accounted for variation in assemblage composition that could not be accounted for by either P or periphyton; this direct effect was not as strong as that for periphyton (partial Mantel $r = 0.107$–0.228, $p \leq 0.05$ during January 1997, and February and September 1998). Surprisingly, a weak, but significant indirect effect of P on composition also was detected (partial Mantel $r = 0.0628$–0.4910, $p \leq 0.05$ in May and September 1996, and January 1997). This residual suggested that the selected measures of periphyton and vegetation could not account for all P-related variation in composition, thus indicating the potential importance of other P-mediated variables in structuring the macroinvertebrate assemblages at the dosing facility.

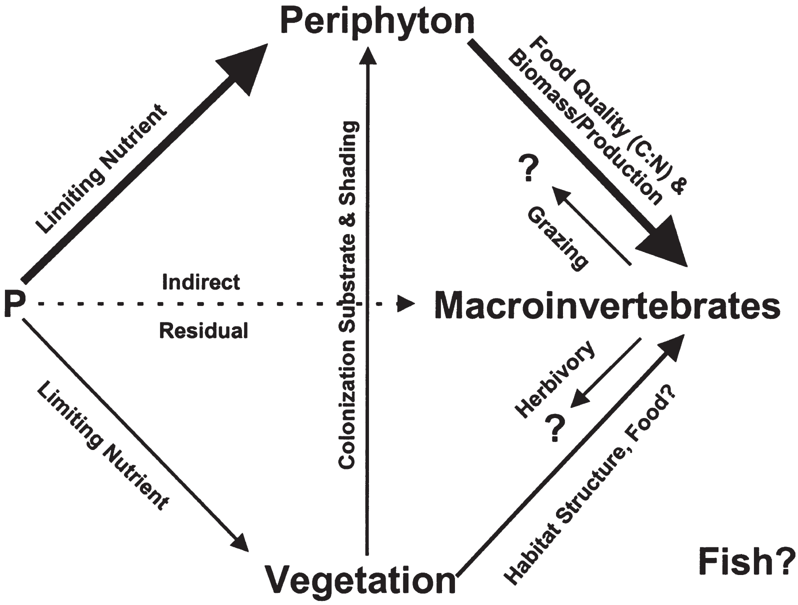

Fig. 19.8 Path diagram synthesizing relationships among P, periphyton, vegetation, and invertebrates based on results of partial Mantel's tests. Relative strength of relationships is indicated by the magnitude of *arrows*

19.3.3 Fish Responses to P Additions

We collected a total of 2,785 individuals and identified 15 species, but only six were common (Table 19.2). Rank order of species abundance was consistent with data in the literature on fish assemblages in Everglades sloughs (Table 19.2), suggesting our fish traps were not excessively selective toward particular species. However, in contrast to the macroinvertebrates, our data did not reveal a pattern in abundance, biomass, or number of species that could be attributed to P dosing. Abundance and biomass were highly flume specific and very noisy among treatments. Regressions for individual species abundances and biomass also yielded nonsignificant relationships.

Assessment of mass response of the most abundant fish in our study, *G. holbrooki*, among P-treatment categories also showed no detectable effect. Despite having high statistical power and precision in length and mass estimates, comparison of both slopes and intercepts of length–mass equations among treatments showed that P had not significantly affected condition of this species.

Species composition was not affected by P. Rather, temporal variation sorted assemblages among the three dates. October was dominated by *Gambusia*, *Poecilia latipinna*, *Heterandria formosa*, and *Fundulus chrysotus*, all small fishes. By January, these three species were less common. January brought about a noted increase

Table 19.2 Common and scientific names of fish collected at the P-dosing experiment, shown by rank order of abundance

Common name	No. collected	Scientific name
Eastern mosquitofish	1,164	*Gambusia holbrooki* Girard
Sailfin molly	549	*Poecilia latipinna* (Lesueur)
Bluefin killifish	226	*Lucania goodei* Jordan
Least killifish	106	*Heterandria formosa* Agassiz
Golden topminnow	98	*Fundulus chrysotus* (Günther)
Flagfish	98	*Jordanella floridae* Good and Bean
Spotted sunfish	22	*Lepomis punctatus* (Valenciennes)
Largemouth bass	7	*Micropterus salmoides* (Lacépède)
Redear	5	*Lepomis microlophus* (Günther)
Yellow bullhead	3	*Ameiurus natalis* (Lesueur)
Bluegill	2	*Lepomis macrochirus* Rafinesque
Lake chubsucker	2	*Erimyzon sucetta* (Lacépède)
Bluespotted sunfish	1	*Enneacanthus gloriosus* (Holbrook)
Seminole killifish	1	*Fundulus seminolis* Girard
Redfin pickerel	1	*Esox americanus* Gmelin

in the abundance of 1+ and adult age-class sunfish, particularly *Lepomis punctatus*. While primarily invertivores, adult *Lepomis* were observed actively ambushing individuals of the small fish assemblage at and near the water surface. This species also was captured in most flumes, and so it is unlikely that predation was treatment or flume specific. In May, young-of-the-year (YOY) *Micropterus salmoides* and *Lepomis* spp. were common. Additionally in May, greater numbers of *Lucania goodei* and *Jordanella floridae* contributed to differences in assemblage structure over time. None of the successional vectors from nMDS indicated that P had a temporal influence on the fish assemblage.

Analysis of other environmental variables in relation to fish assemblages indicated that microhabitat structure might be more important than P-status, at least on a local scale. A weak relationship was found between total abundance and biomass of small fishes and the combined abundance of two macrophyte species, *E. elongata* and *E. cellulosa* (spikerush). Flumes that had the greatest abundance of fish generally had high stem densities of these plants.

19.4 Discussion

19.4.1 Standing Stocks and Trophic Levels

The importance of resource limitation to biotic communities is a source of much contention among ecologists, with many asserting that competition or other constraints are the chief determinants regulating populations (reviewed by Cohen et al. 1990; Pimm et al. 1991), while others suggest that its significance is dependent upon the trophic level an organism belongs to (e.g., Abrams 1993) or the disturbance frequency of the environment (Connell 1975; Schoener 1982). In the dynamic, nonequilibrium (Reice 1994) environment of the Everglades, we hypothesized that interspecific competition and predation would not play a strong role in regulating abundances of macroinvertebrates and that this would allow standing stocks to benefit from nutrient additions. An alternative hypothesis was that productivity would accumulate at the top of the food web due to top-down control of macroinvertebrate standing stocks by invertivorous fish (e.g., Hairston et al. 1960; Oksanen et al. 1981). The log-linear response of macroinvertebrate abundance and biomass to increasing levels of P enrichment, particularly primary consumers, supported the former hypothesis. This finding is also consistent with several nutrient addition studies conducted in other aquatic systems. For example, Hart and Robinson (1990) demonstrated that resources limited stream insects, as P additions to in situ flumes generated significantly greater densities, biomass, and developmental rates of grazing caddisflies in an oligotrophic stream. Similarly, Hershey et al. (1988) and Peterson et al. (1993) showed that whole-stream N and P enrichment of the Kuparuk River in arctic Alaska dramatically altered food webs, consistently increasing secondary production of grazing insects and generally increasing standing stocks. Other mesocosm studies have reached the same conclusion – nutrient and resource limitation can be significant determinants of secondary production and standing stocks in many aquatic systems (e.g., Mundie et al. 1991; Perrin and Richardson 1997).

Experimental evidence of resource limitation is less clear in wetland habitats, particularly outside the Everglades. Hann and Goldsborough (1997) used both press and pulse treatments of N and P to small enclosures in the Delta Marsh to conclude that microcrustaceans responded variably over time to enrichment, but responses were tightly coupled to both primary production and taxonomic succession of algae. Murkin et al. (1994) found little to no effect of added nutrients to invertebrates in an enclosure study, but they conceded that nutrient concentrations used were too low to elicit a response in primary productivity. Two other studies conducted in Canadian wetlands, however, demonstrated highly positive responses of invertebrates to fertilization, yielding greater secondary production and standing stocks (Campeau et al. 1994; Gabor et al. 1994). These somewhat equivocal wetland results may be partly due to the inconsistent scales and durations among their studies, most of which were conducted for no more than a few months, as well as the degree of nutrient limitation in their study areas. This study benefited from being long term and continuous, with dosing conducted for over 6 years and macroinvertebrate sampling beginning after nearly 4 years of enrichment already had occurred. We also had replicate sites, each with five P treatments, thus allowed estimates of dose–response relationships rather than just comparing "enriched" vs. "control" treatments. Subsequently, these results provide compelling evidence that the macroinvertebrate community of Everglades sloughs is resource limited, and this limitation is relaxed through the addition of P. Moreover, all levels of P enrichment resulted in a subsidy effect rather than a stress to overall assemblage standing stocks (Odum et al. 1979).

Our results conflict with the observational surveys of Terczak (1980), Urban and Koebel (1992), and Turner et al. (1999), all of whom conducted studies in P-enriched and -unenriched locations in the Water Conservation Areas (WCAs) of the northern Everglades. Terczak (1980) used several samplers to compare macroinvertebrate communities at two enriched and two unenriched sites in the WCAs of the northern Everglades, finding markedly lower abundance at one of the enriched sites while little change at the other. Urban and Koebel (1992) used litter bags in dense stands of vegetation to conclude that samples at a eutrophic site had greater abundance of Oligochaeta and Chironomidae but depressed abundance of other taxa relative to an oligotrophic site in WCA-2A. Alternatively, Turner et al. (1999) found no effect of enrichment on large-invertebrate biomass, although they used throw traps that primarily collected decapods. Aside from differences in collection techniques among these studies, we suspect that differences in vegetation were primarily responsible for their results. The highly enriched areas near canal inflows in WCA-2A are dominated by cattail (*Typha domingensis* Pers.), while interior WCA-2A is a mosaic of sawgrass stands laced with open-water sloughs (King et al. 2004). Periphyton production and biomass have been shown to be much greater in unenriched sloughs than in stands of cattail in enriched areas (McCormick et al. 1998), mostly from shading caused by high stem densities (Grimshaw et al. 1997). Consequently, dissolved oxygen levels are also much lower in the eutrophic cattail stands sampled by these researchers due to a shift from autotrophic to heterotrophic aquatic production (McCormick et al. 1997). Thus, it is not surprising that authors of these previous studies concluded that P enrichment either had no effect or was detrimental to invertebrate standing stocks in the Everglades. Here, primary production has shifted heavily to emergent macrophytes, thus P and other abiotic variables contributing to the spread of cattail likely acts as a stress rather than a subsidy to the invertebrate community in this eutrophic habitat. Accordingly, King (2001) demonstrated a clear subsidy–stress (sensu Odum et al. 1979) relationship between P and invertebrate biomass along the 10-km P gradient in WCA-2A when area weighted by the coarse-scale vegetation pattern of the landscape. Here, intermediate levels of enrichment with at least small sloughs or patches of open water yielded the greatest biomass.

In addition to results reported by King (2001), results from Rader and Richardson (1994) support the notion that vegetation was an important factor governing the findings of the previous studies. Sampling with a sweep net and core sampler in patches of open water rather than dense stands of cattail along a 10-km P gradient in WCA-2A, they found the highest densities of macroinvertebrates at sites with the greatest enrichment of P. This trend mirrored the dose–response curve of macroinvertebrate standing stocks to our experimental P gradient. However, contrary to this study, it is important to note that they did not estimate biomass.

Small fish did not respond to P additions as expected. No detectable increase of either standing stocks or mean condition was documented in the fish assemblage. This is somewhat surprising given that macroinvertebrate standing stocks increased with P, but several plausible explanations exist for this apparent disparity. Emigration and immigration to and from surrounding slough habitat may have masked P effects since the flumes had small openings at their southern ends. Predation also may have played a role. While highly speculative since we were unable to accurately estimate presence and abundance of piscivorous fish in the flumes, the small fish assemblage may have been affected by a few large predators, especially on this highly local scale. Consequently, biomass of piscivores, such as largemouth bass (*M. salmoides*), may have benefited from nutrient additions to the flumes.

An interaction between predation and habitat also may have influenced fish abundance. The only statistically detectable effect was a weak correlation between stem densities of *Eleocharis* spp. and total abundance of small fish. High densities of this emergent macrophyte may have provided refuge from predators in the flumes, a factor that may have been more important than nutrient status. This is partially supported by the work of Jordan (1996) in WCA-1, where he illustrated the importance of high habitat complexity to most of the small fish assemblage of the Everglades. However, Jordan (1996) as well as Turner et al. (1999) found that small fish densities were as much as five times greater in nutrient-rich cattail stands than in any habitat in the oligotrophic interior fen. Turner et al. (1999) hypothesized that increases in productivity cascaded up through the food chain and accumulated as high standing crops of small fishes and consequently, as predicted by food web theory (e.g., Oksanen et al. 1981), resulting in no appreciable increase in invertebrate standing stocks. Interestingly, cattail stands in WCA-2A are unlikely to have few, if any, large predator fish due to the very high stem densities and generally lesser water depth than peripheral canals and airboat trails. Thus, contrary to our slough sites, the small fish assemblage in most large, eutrophic stands of cattail in the Everglades may receive little pressure from predators. This could partially explain the discrepancy between our experimental results and these previous observational studies. Clearly, more experimental data are needed to make inferences regarding mechanisms regulating production, standing stocks, and species composition of the small fish community in the Everglades.

19.4.2 Assemblage Richness and Composition

A subsidy effect also was clearly evident for macroinvertebrate taxonomic richness and density on some dates; however, this dose–response relationship did not completely support our hypothesis. We expected to see a unimodal productivity–diversity relationship (e.g., Tilman 1982; Abrams 1995), largely based on reported sensitivity of many algal taxa, particularly diatoms, to high concentrations of phosphorus (e.g., Swift and Nicholas 1987; Stevenson and Richardson 1995; McCormick and O'Dell 1996; McCormick et al. 1996; Kaštovský et al., see Chap. 18). We hypothesized that grazing specialists would be disadvantaged by the reduction or complete exclusion of potentially preferred diatom or other P-sensitive algal taxa (Lamberti and Moore 1984). On the contrary, few of common macroinvertebrate taxa appeared disadvantaged by P additions (although there was tremendous species turnover along the 10-km P gradient in WCA-2A where vegetation changed markedly in response to high levels of P enrichment; King 2001; King and Richardson

2002). This may suggest that few Everglades invertebrate species are truly "specialists." Indeed, virtually all species documented from the Everglades are well adapted for harsh, often temporary conditions (Gunderson and Loftus 1993), hence have limits of tolerance (sensu Shelford 1913) that far exceed challenges presented by changes in food resources. Additionally, the south Florida landscape was formed very recently (~5,000 YBP) and has very few endemic species. Thus, Everglades macrofauna may best be characterized as an assemblage of rapidly dispersing, opportunistic species rather than a coevolved community uniquely adapted to the Everglades (Jordan 1996). This is not to say that Everglades macroinvertebrates are not sensitive to their environment; the sharp increase in the number of taxa at the lowest P loads above background clearly indicates to the contrary. Nevertheless, this study highly suggests that local-scale P additions to oligotrophic Everglades sloughs will likely increase species richness of macroinvertebrates.

Rader and Richardson (1994) also indicated that species richness increased with increasing levels of P enrichment based on observational data they collected in WCA-2A. They found that most taxa that were common to oligotrophic sloughs became more abundant in intermediately and highly enriched locations, while many new taxa were added to the community. These findings parallel these experimental results. However, other authors have suggested that richness has declined due to P enrichment in the Everglades (Terczak 1980; Urban and Koebel 1992). Their work primarily was conducted in heavily vegetated habitats rather than the open-water habitats sampled by Rader and Richardson (1994). Although results were not entirely conclusive, King (2001) indicated that taxon richness changed modestly along the P gradient on a landscape scale, reaching greatest richness in the intermediately enriched transition zone. Because slough-like patches of open water are not common in enriched areas of the Everglades, this rare habitat may have represented an uncharacteristic refugium amongst vast stands of cattail and other invasive macrophyte vegetation in Rader and Richardson's study.

Increases in species richness with P dosing also contributed to increases in taxonomic dissimilarity (β-diversity) relative to control treatments. Shifts in composition closely corresponded to the magnitude of P loads. Dramatic increases in the abundance of ostracods, copepods, cladocerans, oligochaetes, and many chironomids overwhelmed more modest increases of other taxa, such as gastropods, thus changed the proportional structure of taxonomic composition. The apparent decrease in % Gastropoda was of particular interest since these taxa are primarily grazers of algae. However, when calculated as a proportion of total biomass, this negative dose–response relationship was not evident. Because of the relatively small individual mass of most taxa collected on the samplers, a few large specimens (particularly *Planorbella duryi* and *Planorbella scalaris*) contributed to noise and subsequently occluded a pattern. While relative biomass is probably a more meaningful measure of structural change in the assemblage, gastropods are a numerically dominant group in Everglades invertebrate assemblages and their relative abundance may be a good indicator of ecological integrity of oligotrophic sloughs.

While macroinvertebrate species composition diverged from controls with P additions, successional trajectories generally did not. Small fish trajectories also were not affected by P additions. We hypothesized that P-induced increases in secondary production would result in more rapid emergence or turnover of some species, thus contributing to greater temporal variation in composition. Successional vectors of macroinvertebrates in P-dosed flumes did diverge somewhat from the paths of the controls during January 1997 and February 1998, but the mean rate of change was similar in all flumes. Hann and Goldsborough (1997) reported comparable findings, as the magnitude of temporal variation in cladoceran assemblages in the Delta Marsh differed little among nutrient treated and untreated mesocosms. The highly dynamic abiotic environment of the Everglades, and most wetlands for that matter, is probably the most important dimension governing the direction and magnitude of community succession (e.g., Batzer and Wissinger 1996). Seasonal variation in hydrology, water temperature, dissolved oxygen, and structure of primary producers likely were important forcing factors organizing the Everglades assemblages over time.

19.4.3 Composition–Environment Relationships

A multitude of variables measured within the flumes were correlated to macroinvertebrate assemblage composition. All observed measures of P (water-column SRP and UTP, periphyton TP, and sediment TP) were highly related to a gradient in assemblage composition. The strongest vector among all P measurements was for sediment TP, and this was consistent throughout the duration of the study. This seemed puzzling since HD samplers were suspended within the water column, not the sediment, thus P in the water should have had the greatest influence on biota attached to the samplers. However, SRP was heavily influenced by biological uptake and was often indistinguishable from the controls at low-P loads, especially during midday collections when photosynthetic activity was at its peak. Conversely, water-column UTP had relatively linear relationship with dose up to the 75 treatment but actually declined slightly in the 150 treatment. Sediment TP reached much higher values in the 150 treatments than any other, thus maintained a linear relationship with dosing concentrations from the controls to the highest treatment. Pan et al. (2000) also found a stronger relationship between algal species composition and sediment TP than with UTP in these same experimental flumes and along the P gradient in WCA-2A. Because it is unfiltered and includes particulates, UTP may often contain large amounts of living or dead plant tissue, thus may have been influenced by the coverage of periphyton mat on the water surface. Stevenson and Richardson (1995) and Richardson et al. (2000) both reported significant reductions in floating periphyton cover at the highest dosage of P; perhaps “cleaner” samples were collected in the absence of high mat coverage. Regardless of the mechanism, sediment TP appeared to be a better indicator of actual P loads than did UTP, at least at the highest load.

Macrophyte species composition covaried with P doses and also may have played a role in structuring the macroinvertebrate assemblage. Macrophytes had a significant "direct" effect on macroinvertebrates, as indicated by partial Mantel's tests. Two macrophytes, *U. purpurea* and *U. foliosa*, exhibited high sensitivity to P and thus were highly correlated to a gradient in assemblage composition. These bladderworts are largely carnivorous, relying on microorganisms as their primary source of nutrients. Coincident with the decline of *Utricularia* was a sharp increase in the abundance of microcrustacea, particularly the ostracods *Cytheridella alosa* and *Cypridopsis okeechobei*, and cyclopoid copepods. While the increase in microcrustacea was probably largely due to greater quality and productivity of grazing resources, loss of *Utricularia* also may have contributed. Microcrustacea often were observed within *U. purpurea* bladders when they were incidentally retrieved with HD samplers.

There is an increasing body of evidence that periphyton forms the base of the food web in wetland habitats (e.g., Neill and Cornwell 1992; Keough et al. 1996; D.A. Wrubleski and N.E. Detenbeck, unpublished manuscript), a contradiction to the prevailing view that macrophyte detritus is most important to secondary production (e.g., Murkin 1989; Batzer and Wissinger 1996). This study indirectly supports this theory. Consistent with our hypothesis, periphyton exhibited a strong direct effect on macroinvertebrate assemblage composition. The highly significant partial Mantel's coefficients for periphyton, based on biomass and nutrient content on plexislides, suggest that P-induced changes to periphyton had an influence on macroinvertebrate assemblages in the flumes. One of the most notable changes to the periphyton was the substantial decrease in molar total C:N ratios with increasing P concentrations. Ratios often exceeded 20:1 on plexislides collected from control flumes but were as low as 9:1 at high-P loads. High C:N ratios in controls may reflect poor nutritional value, as ratios > 17:1 (organic C:N) are considered unpalatable and inefficient to algivores (e.g., Russell-Hunter 1970; Jones et al. 1998). Although we did not correct for inorganic carbon, % ash on plexislides did not consistently vary with P, suggesting that relative differences in C:N among P treatments would be similar if expressed in terms of organic or total C.

Browder (1982), however, suggested that periphyton represented a good source of protein for primary consumers in the Everglades. After correcting for inorganic carbon, she found that periphyton from oligotrophic areas in Everglades National Park had C:N ratios of 4.84–8.0 (but C:N ratios based on total C ranged from 19 to 30). It seems unlikely that periphyton from unenriched locations is an efficient source of energy because the Everglades is characterized as having unusually high standing stocks of periphyton, yet unusually low invertebrate and fish biomass (Goldsborough and Robinson 1996; Turner et al. 1999). What makes this even more mysterious is that periphyton grows very slowly in oligotrophic sloughs (e.g., Swift and Nicholas 1987; Vymazal and Richardson 1995; Browder et al. 1994; see Chap. 18) thus must accumulate over very long time periods. Such high biomass accumulation despite low productivity suggests that it receives relatively little grazing pressure from primary consumers due to one or a combination of factors (Murdoch 1966). These may include low nutritional value, unpalatable species composition (e.g., Jones et al. 1998), chemical deterrents (Lodge et al. 1998), or unavailability due to calcification and structure of the periphyton matrix (e.g., Turner et al. 1999). Certainly, more research is needed on the interactions among nutrient additions, periphyton, and grazers in the Everglades. However, these data suggest that C:N ratios may be a good indicator of periphyton quality as food to primary consumers, and that P additions may increase the nutritional value of periphyton to macroinvertebrates.

19.5 Conclusions and Lessons for Restoration

Macroinvertebrates are resource limited in Everglades sloughs. Long-term P additions significantly increased macroinvertebrate standing stocks and species richness, but also altered the relative taxonomic and trophic structure of the assemblage. Dramatic responses often occurred at relatively low doses of P but diminished in magnitude with increasingly greater P loads, thus resulting in log-linear dose–response relationships for most attributes of the macroinvertebrate assemblage. Fish, however, did not respond to P additions, possibly due to predation or to local-scale heterogeneity in habitat among the P-dosing experiment flumes. P-induced changes to both macrophytes and periphyton influenced macroinvertebrate species composition; however, periphyton productivity and nutrient content had the greatest direct effect.