Effects of Phosphorus and Nitrogen Enrichment on Periphyton Growth in Tenderfoot Creek, MI.

BIOS 569 - Practicum in Aquatic Biology

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1991

ABSTRACT

The effects of nutrient enrichment upon periphyton growth were studied in Tenderfoot Creek, MI. Phosphorus and nitrogen, potential limiting nutrients, were released into the stream through porous clay pot substrates. A riffle area was studied to determine the effect of nutrient addition in a benthic habitat and a pool area was studied to assess the planktonic effect. Growth was measured by determining and comparing biomass and chlorophyll-*a* concentrations of periphyton removed from the substrates. Values for each treatment were compared within and between the two study sites. Although the growth differences between treatments were not statistically significant, the overall results suggest that periphyton did respond to nutrient enrichment. P enrichment stimulated the most autotrophic growth, whereas N stimulated the most non-autotrophic accumulation in the benthic environment. All three nutrient treatments stimulated substantial autotrophic growth in the planktonic environment while P, and to some extent N, enrichment produced substantial non-autotrophic periphyton accumulation in the planktonic environment as well. The results show that periphyton growth in Tenderfoot Creek is nutrient limited and that addition of nitrogen and phosphorus positively influenced the growth and accumulation of periphyton.

INTRODUCTION

Phosphorus is the least abundant element of the major nutrients required for algal growth in the large majority of fresh waters (Wetzel 1983). As specified by the Redfield ratio, organisms require nitrogen and phosphorus for their metabolic needs in about a 15:1 molar ratio. Nitrogen, along with carbon, hydrogen and phosphorus, is one of the major constituents of the cellular protoplasm of organisms (Wetzel 1983). For these reasons, inorganic phosphorus and inorganic nitrogen are considered "limiting" nutrients that affect the productivity of freshwaters. Because phosphorus and nitrogen are limiting nutrients, they are capable of enhancing autotrophic growth when their concentrations are increased (Fairchild and Everett 1988). A previous river study has indeed shown P limitation of periphyton growth (Stanley *et al.* 1990).

This paper presents the results of a study to determine the effects of nitrogen (N) and phosphorus (P) enrichment upon periphyton growth in Tenderfoot Creek, Michigan. At the onset of the experiment, it was predicted that phosphorus enrichment would result in the most growth, because the lakes in the area, and presumably their influents and effluents as well, are phosphorus limited.

MATERIALS AND METHODS

The experimental design was based on a similar study conducted by Fairchild and Everett (1988) in a small lake. The adaptations made for this stream study were based on experiments conducted by Guiltinan (1990) and Tate (manuscript).

Tenderfoot Creek is located in Gogebic County, Michigan, on the property that constitutes the University of Notre Dame Environmental Research Center (UNDERC). The stream has a low gradient and is an effluent of Tenderfoot Lake. Because the land is privately owned, the study sites were not disturbed by the public. The effects of nutrient enrichment were studied in two areas of Tenderfoot Creek: a shallow riffle area of high current velocity and a deep pool area of lower velocity. The riffle area study demonstrated the periphyton response in a largely benthic habitat, while the pool area study demonstrated the response in a planktonic habitat.

The substrates used were sealed clay flower pots that were

porous enough to allow diffusion of nutrients. The pots were 3.5 cm in height, 8.9 cm in diameter at the base and 6.0 cm in diameter at the top. The clay used to make the pots was phosphorus-free and therefore did not increase the concentration of phosphorus released from the substrates. The pots were filled with microbiological agar containing various nutrient solutions. The four treatments per habitat were: 1) 0.5 M potassium nitrate, 2) 0.1 M potassium phosphate, 3) 0.5 M potassium nitrate plus 0.1M potassium phosphate and 4) agar without any nutrients added. A square piece of plexiglass was attached to the open end of each pot by the application of silicone sealant.

The pots used to study the effects of nitrogen and phosphorus enrichment in the riffle area of the stream were tied by monofilament to rebar strips that lay on the sediments. The pots used to study the effects of nitrogen and phosphorus enrichment in the pool area of the stream were suspended at uniform depths (38cm) in the water column by wooden supports anchored in the stream bottom. For each study site, two replicate pots for each treatment were collected on each sampling date.

The benthic pots were placed in the field on May 31, 1991, and treatments were randomly sampled on days 11, 27, and 38 of the experiment. The planktonic pots were installed in the field on June 1, 1991, and were randomly sampled on days 10, 26, 37 and 44 of the experiment. In the field, a scalpel and toothbrush were used to scrape the periphyton from only the top and sides of the pots. The periphyton was stored in dark film containers, placed in a cooler and filtered immediately upon return to the lab. Half of each sample was analyzed for chlorophyll-*a* content using the Chlorophyll-*a* Analysis Procedure outlined in Methods of the Cascading Trophic Interactions Project (Soranno 1990). The other half of each sample was used to determine total biomass, measured by determining the dry weight of the periphyton. For biomass analysis, half of the sample was filtered onto a pre-weighed glass fiber GF/F Whatman filter and then frozen for the remaining time at UNDERC. Upon return to Notre Dame, the filters were placed in a drying oven at 60°C for 24 hours and then weighed.

Invertebrates that were attached to the top and sides of the pots were removed with forceps and placed in vials of 80% ethyl alcohol labeled with pot treatment and replicate number. The invertebrates were later identified and counted.

To ensure that the release of the nitrate and phosphate solutions was not detrimental to stream invertebrates, benthic samples were taken twelve days after the experiment began. A Surber

sampler was used to take two replicate samples directly upstream and two samples directly downstream from the riffle study site (Table 1). Analysis of the invertebrates in the samples did not indicate significant change in species numbers or species richness between the two areas, thereby suggesting that nutrient addition did not alter stream benthic invertebrate populations.

RESULTS

The benthic biomass values suggest that the addition of nutrients increased the rate of periphyton growth and accumulation on the pots (Fig. 1). Periphyton standing crop on the nutrient treated pots in the riffle site increased over the 38-day experiment. However, the marked decline in benthic chlorophyll-*a* concentration after day 27 indicates that sometime after this date a majority of the periphyton on the pots became non-photosynthetic (Fig. 2). The increasing benthic biomass values may be attributed to accumulation of dead algal cells and detritus, as well as live bacteria and fungi, that also require and respond to external supplies of nutrients. It has also been suggested that such a decline in specific production accompanied by an increase in standing crop can be attributed to competition between algal cells for nutrients and/or light (Fairchild and Everett 1988).

P enrichment appears to have stimulated the most autotrophic growth according to benthic biomass and chlorophyll-*a* concentration for the dates when chlorophyll was abundant. After day 27, N enrichment substantially increased benthic biomass, which suggests a possible response to N enrichment by the heterotrophic constituents of the periphyton. The N+P effect was less obvious. Although a P stimulation trend is suggested for algal growth and a N stimulation trend is suggested for heterotrophic growth, the differences in biomass among the four treatments for day 38 were not significant, as indicated by ANOVA ($F_{3,4}$=0.680, p=0.609). Differences in chlorophyll-*a* concentrations among treatments on date 37 also were not significant ($F_{3,4}$=0.611, p=0.643). It is possible that the small sample size (N=2) for each treatment was the reason the results were not statistically significant.

As with the benthic response, the periphyton community in the planktonic habitat appeared to decline sometime after day 26. Growth trends, as indicated by periphyton biomass (Fig. 3) and chlorophyll-*a* concentration (Fig. 4), suggest that all three nutrient treatments stimulated planktonic growth. However, the

difference in biomass among the treatments on day 37 was not statistically significant ($F_{3,4}$=1.031, p=0.468). The low concentration of planktonic chlorophyll-*a* on day 10 for the N+P substrate can be partially explained by the fact that in the field, a canister containing one replicate sample of periphyton scraped from an N+P pot spilled and much of the sample was lost. This considered, the N+P enrichment appears to have resulted in the most growth in the pool site, followed by P enrichment and then N enrichment. Again, this trend is not supported by statistical analysis for planktonic chlorophyll-*a* concentrations on day 37 ($F_{3,4}$=0.833, p=0.522). Overall, N, P, and N+P enrichment appears to have stimulated autotrophic growth in the planktonic habitat, while P, and to some extent N, enrichment appears to have stimulated heterotrophic growth.

The numbers of invertebrates collected from the pots differed between the two habitats (Fig. 5). The density of invertebrates on the benthic pots increased during the course of the experiment. In contrast, the number of invertebrates present on the planktonic pots decreased, with the exception of the last sampling date. Difficulty was encountered in removing the invertebrates because when the pots were lifted from the water, many invertebrates detached themselves from the pots and were swept away by the current. Some also migrated down to the lips of the pots to hide in crevices in the pot/silicone seal interface. The planktonic pots had many attached snails (Hydrobiidae) and planarians (Table 2), while the benthic pots had few of those organisms (Table 3). The benthic pots overall were populated by filter-feeding blackflies (Simuliidae) and also by species of caddisflies (Trichoptera).

DISCUSSION

Although the treatment effects were not statistically significant, the addition of nutrients to Tenderfoot Creek suggested that N, P, and N+P stimulated periphyton growth, as indicated by analysis of periphyton biomass and chlorophyll-*a* concentration. On day 27, when the maximum chlorophyll-*a* concentrations were measured in the benthic habitat, P stimulation was indicated by both the benthic biomass and chlorophyll-*a* concentration values. In a previous river study, pots enriched with P also had significantly higher chlorophyll-*a* concentrations (Stanley *et al*. 1990). On day 38, N stimulation resulted in increased biomass, presumably due to the accumulation of non-heterotrophic components of the periphyton.

When the maximum chlorophyll-a concentrations were examined in the planktonic habitat, growth trends are not as conclusive. On day 26, when the biomass values suggest that periphyton responded to N+P enrichment, the chlorophyll-a concentrations suggest a response to all three treatments. On days 37 and 44, P enrichment increased the accumulation of non-autotrophic biomass. These results suggest that periphyton growth in Tenderfoot Creek is P limited, and possibly N limited as well. Similar nutrient release studies in lakes suggest that periphyton growth is not limited by one nutrient alone (Fairchild and Everett 1988). It is possible that the Hach water chemistry analysis, which indicated that N was the major limiting nutrient in Tenderfoot Creek, was incorrect. It is also possible that the high levels of phosphorus detected, ranging from 0.06 mg/l PO_4^{3-} to 0.13 mg/l PO_4^{3-}, were overestimates (Lamberti, pers. comm.). Therefore, it is indeed a possibility that the system was phosphorus limited, as originally hypothesized.

There are many possible reasons why the effects of nutrient enrichment differed between the two study sites. Overall, the periphyton in the benthic habitat did not respond to N enrichment or N+P enrichment until late in the experiment, compared to the periphyton in the planktonic habitat which responded to both forms of N enrichment early in the experiment. It is possible that the benthic periphyton received enough N that diffused from the sediments in the form of NH_4^+, so that the local environment was not N limited. The planktonic periphyton grew on substrates that were raised from the sediment and therefore was less influenced by the N diffusing from the sediment.

Another factor that could account for the different responses in each habitat is that different taxa of periphyton could have colonized the benthic and planktonic substrates. Such different taxa could have different nutrient demands and would therefore produce different growth trends.

Numbers and types of invertebrates grazing in the two habitats could also have differed, resulting in the appearance of different periphyton growth trends. Many of the invertebrates removed from the pots were grazers. In addition, unidentified algal-grazing minnows were observed consuming the periphyton on the benthic pots. A study conducted in the riffle area by a fellow UNDERC student indicates that the most common fish present in the site was the blacknose shiner, *Notropis heterolepis* (M. Huie, pers. comm.). Many fish were also present in the pool site, but algal grazing was never observed there. Intensity of grazing also could have differed within a habitat, resulting in differences in growth and

accumulation among treatments.

The decline in stream discharge and depth over the course of the experiment also could have affected periphyton growth. There were days when the benthic pots were partially exposed to air because the water level of the stream dropped substantially as the season progressed. The pots, located near the bank, had to be relocated to deeper areas of the stream. Such exposure could have resulted in desiccation and death of periphyton, which would alter potential growth trends that would otherwise have occurred.

The sediment type of the two habitats was quite different. The sediment in the riffle site was rocky whereas the pool site sediment was more flocculent and less rocky. Disturbance of the pool stream bottom resulted in distribution of clouds of sediment and also possible nutrient circulation as well. Many fine sediment particles were found on the pots, which could have contributed to a mismeasurement of planktonic periphyton biomass. Also, in the pool site, floating mats of algae became attached to the apparatus that suspended the pots in the water column. Such algal mats are common seasonally in pool areas of streams (Power 1990). As the summer progressed, mats of algae partially, and sometimes completely, engulfed and shaded the pots. The planktonic pots may have been shaded for part of the experiment, whereas the benthic pots were always exposed to full sunlight. Therefore, there could potentially have been less growth of planktonic periphyton due to light limitation. It is also possible that some filaments from the of floating algal mats grew attached to the pots and were mistakenly considered to be periphyton accumulation. Such an error would have increased planktonic biomass and chlorophyll-*a* values.

The method by which the nutrients were administered to the periphyton could have resulted in trends that would not normally be observed in natural settings. In stream environments, most of the nutrients supplied to and utilized by periphyton are present in the water column, with the nutrients supplied by the sediments comprising a secondary source. In this experiment, the major nutrient source presented to the periphyton diffused from a substrate, as if arising from the sediment. As periphyton grow and accumulate, the oldest algal cells, located closest to the substrate surface, die. Living periphyton continues to grow and accumulate on top of the dead cells, and are consequently located closer to the light source, but increasingly further away from the experimental nutrient source. This situation is different from what would be observed in natural settings, in which the living cells generally are in contact with the principal aqueous nutrient source. Such an

"unnatural" experimental design could have therefore resulted in unpredictable trends (as noted by Fairchild *et al.*, 1989), such as lack of consistency between treatments from the lag period of growth to the growth peak.

It is important to note that treatment placement was not completely random. Although the position of N and P treatments were alternated, the control pots were always located upstream from other treatments and the N+P treatments were always located downstream. Growth patterns on each pot could have been influenced by adjacent nutrient diffusion and positional effects of stream current that cannot be accounted for by random variation.

There was considerable variation in biomass and chlorophyll-*a* values for replicate samples, which is especially apparent when comparing the differences in planktonic biomass and chlorophyll-*a* values. The method by which the algal sample for each replicate was divided in two for analysis could account for the difference. The algal sample was homogenized by shaking before it was divided in half, with each half being used for either biomass or chlorophyll-*a* analysis. Even with shaking and use of glass stirring rod, some clumps of algae could not be broken up. Therefore, the halved samples often did not contain equal amounts of algal material. This lab error would alter the results because the extrapolated biomass and chlorophyll-*a* values for the replicate would not represent the actual amount of periphyton present in that complete sample.

My inability to detect a statistically significant difference in periphyton growth was probably related to the small sample size. For future studies, more than two replicate samples for each treatment should be collected on each sampling date. The substrates should also be randomly positioned where possible. The toothbrush and scalpel did not always remove all of the periphyton. Perhaps a better tool, or some type of solvent, could be employed, as could a better method of homogenizing the sample in lab. It is also necessary that the benthic pots be placed in an area of the riffle that is deep enough to accommodate drops in water level. It would have been interesting to see the growth trend for a date between the first two sampling dates, while the periphyton was undergoing exponential growth. Performing a more accurate water chemistry analysis on each sampling date is also needed to generate data on nutrient levels throughout each phase of growth, which would be helpful in analysis of the periphyton growth trends.

Although there were many potential sources of error during the experiment, the study does indicate that the periphyton in Tenderfoot Creek responded to nutrient addition provided by

nutrient-enriched substrates. Chlorophyll-a concentration and biomass analyses indicated that periphyton not only responded to P enrichment, as initially hypothesized, but to N enrichment as well. Growth trends in the benthic and planktonic habitats demonstrated different responses to nutrient addition and other environmental factors. Overall, periphyton in Tenderfoot Creek appears to be co-limited by phosphorus and nitrogen.

ACKNOWLEDGEMENTS

I thank Dr. Gary A. Lamberti for his guidance and assistance in designing this experiment and interpreting the results, and Eric Willman for his field and laboratory assistance. To Dr. Marty B. Berg, I am grateful for his immense help and guidance at UNDERC. Jeff Runde contributed much to the graphing of the results. I wish to particularly thank the Bernard J. Hank Family Endowment, which made my research opportunity possible.

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Invertebrate Taxon	Upstream (#/0.1 m²)	Downstream (#/0.1 m²)
Hirudinea	7.0	22.0
Insecta		
Ephemeroptera		
Siphlonuridae		
Ameletus sp.	0.5	0.0
Baetidae		
Baetis spp.	14.0	5.5
Oligoneuriidae		
Isonychia sp.	1.5	0.0
Heptageniidae		
Stenacron interpunctatum	0.5	0.0
Stenonema spp.	4.5	1.0
Ephemerellidae		
Danella sp.	0.5	0.0
Serratella sp.	1.0	0.0
Odonata		
Gomphidae		
Hylogomphus spp.	5.0	3.0
Ophiogomphus sp.	3.0	6.0
Plecoptera		
Perlidae		
Acroneuria sp.	3.0	1.5
Paragnetina media	0.5	0.0
Trichoptera		
Philopotamidae		
Chimarra spp.	41.5	3.0
Hydropsychidae		
Cheumatopsyche sp.	0.5	0.0
Diplectrona modesta	0.0	0.5
Hydropsyche spp.	118.5	43.0
Hydroptilidae		
(early instar)	0.5	0.0
Lepidostomatidae		
Lepidostoma sp.	2.5	8.0
Limnephilidae		
Pseudostenophylax uniformis	0.0	0.5

Leptoceridae *Oecetis* sp.	0.5	0.0
Helicopsychidae *Heliocopsyche borealis*	0.0	0.0
Coleoptera		
Elmidae *Dubiraphia* sp. *Opitoservus* sp. *Stenelmis* sp.	0.5 0.5 0.5	0.0 0.5 1.0
Diptera		
Tipulidae *Antocha* sp.	0.5	0.0
Chironomidae	26	74.5
Empididae *Heterodromia* sp.	0.5	0.0
Bivalvia	13.5	82.5
Total	247.0	252.5

TABLE 1. Density of invertebrate taxa in upstream and downstream Surber samples.

Taxon	C	N	P	N+P
Turbellaria	1.0	12.0	2.0	6.0
Insecta				
Ephemeroptera				
Baetidae				
Baetis spp.	2.0	1.0	2.0	3.0
Tricorythidae				
Tricorythodes sp.	0.0	0.0	0.0	2.0
Plecoptera				
Perlidae				
Acroneuria sp.	0.0	0.0	0.0	1.0
Trichoptera				
Hydropsychidae				
Hydropsyche spp.	2.0	0.0	2.0	1.0
Diptera				
Chironomidae	0.0	0.0	1.0	2.0
Gastropoda				
Hydrobiidae	24.0	18.0	18.0	5.0
Total	29.0	31.0	25.0	20.0

TABLE 2. Invertebrate densities (number of invertebrates/6.87m^2) on planktonic pots.

Taxon	C	N	P	N+P
Hirudinea	0.0	0.0	0.0	1.0
Insecta				
Ephemeroptera				
Baetidae				
Baetis spp.	2.0	2.0	2.0	3.0
Plecoptera				
Perlidae				
Acroneuria sp.	0.0	0.0	0.0	1.0
Trichoptera				
Hydropsychidae				
Hydropsyche spp.	13.0	8.0	5.0	4.0
Diptera				
Simuliidae	58.0	42.0	31.0	38.0
Chironomidae	2.0	1.0	1.0	10.0
Total	75.0	53.0	39.0	57.0

TABLE 3. Total invertebrate densities (number of invertebrates/5.15 m^2) removed from benthic pots (n=6) throughout the 38-day experiment.

Fig. 1. Benthic biomass. Data are means (n=2).

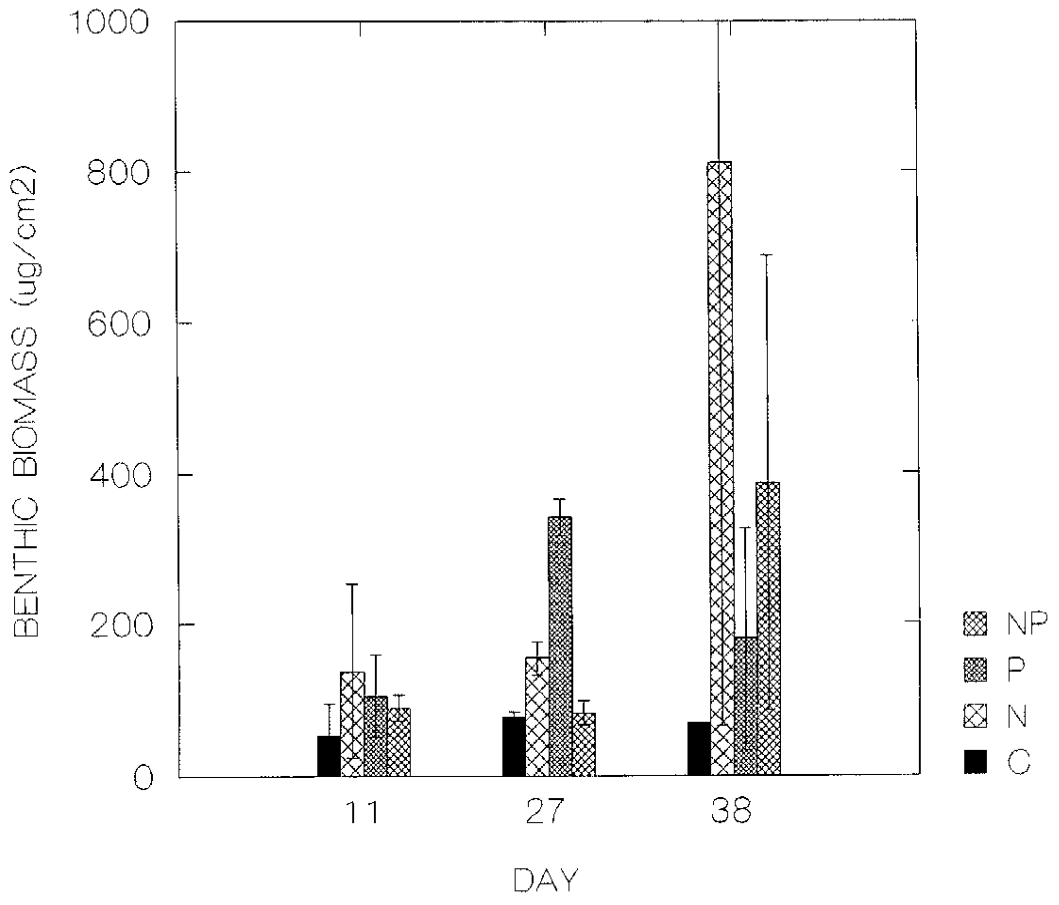

Fig. 2. Benthic chlorophyll-a. Data are means (n=2).

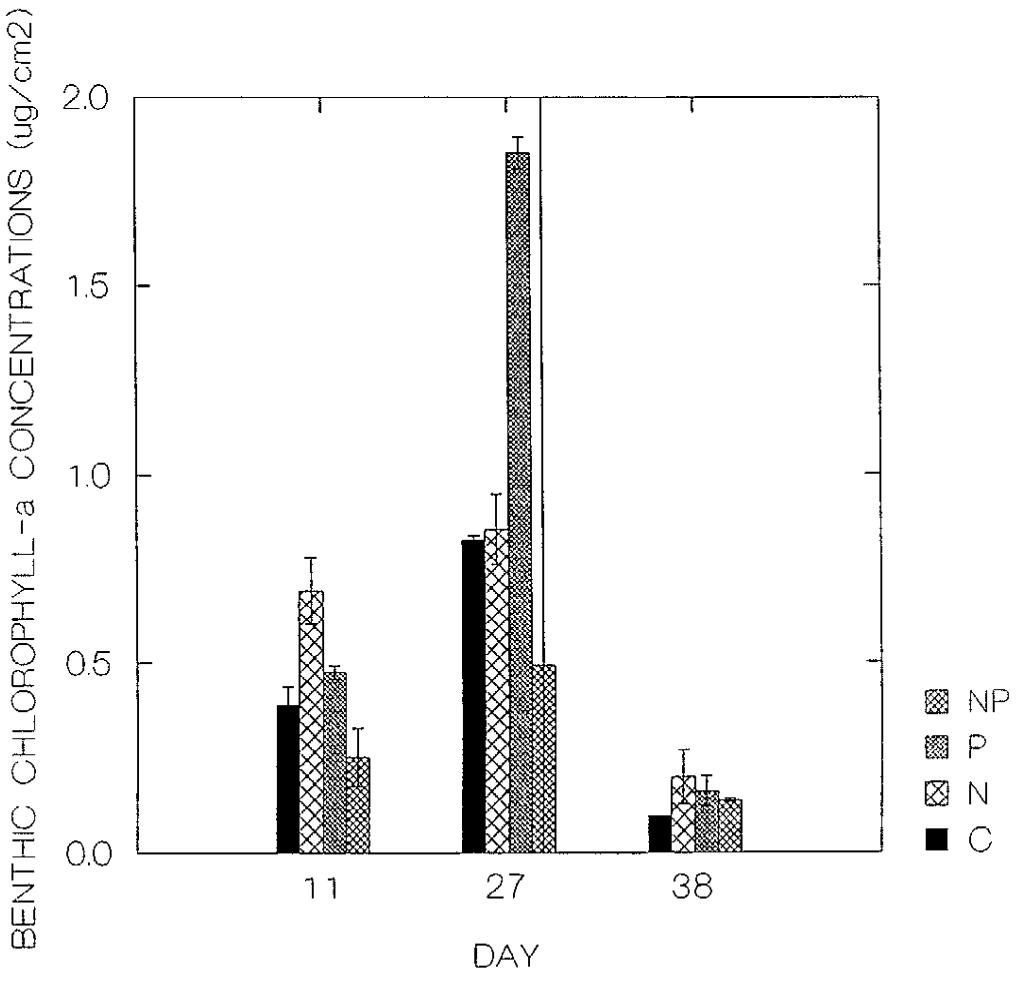

Fig. 3. Planktonic biomass. Data are means (n=2).

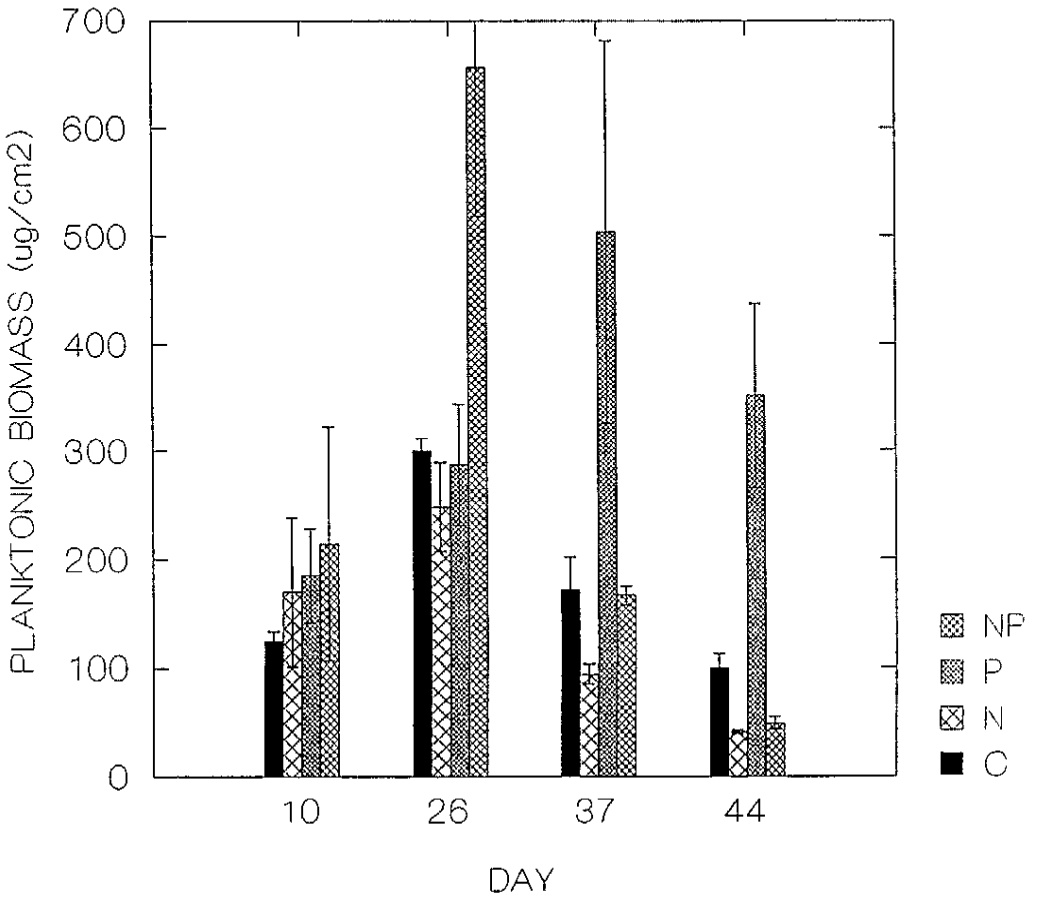

Fig. 4. Planktonic chlorophyll-a. Data are means (n=2).

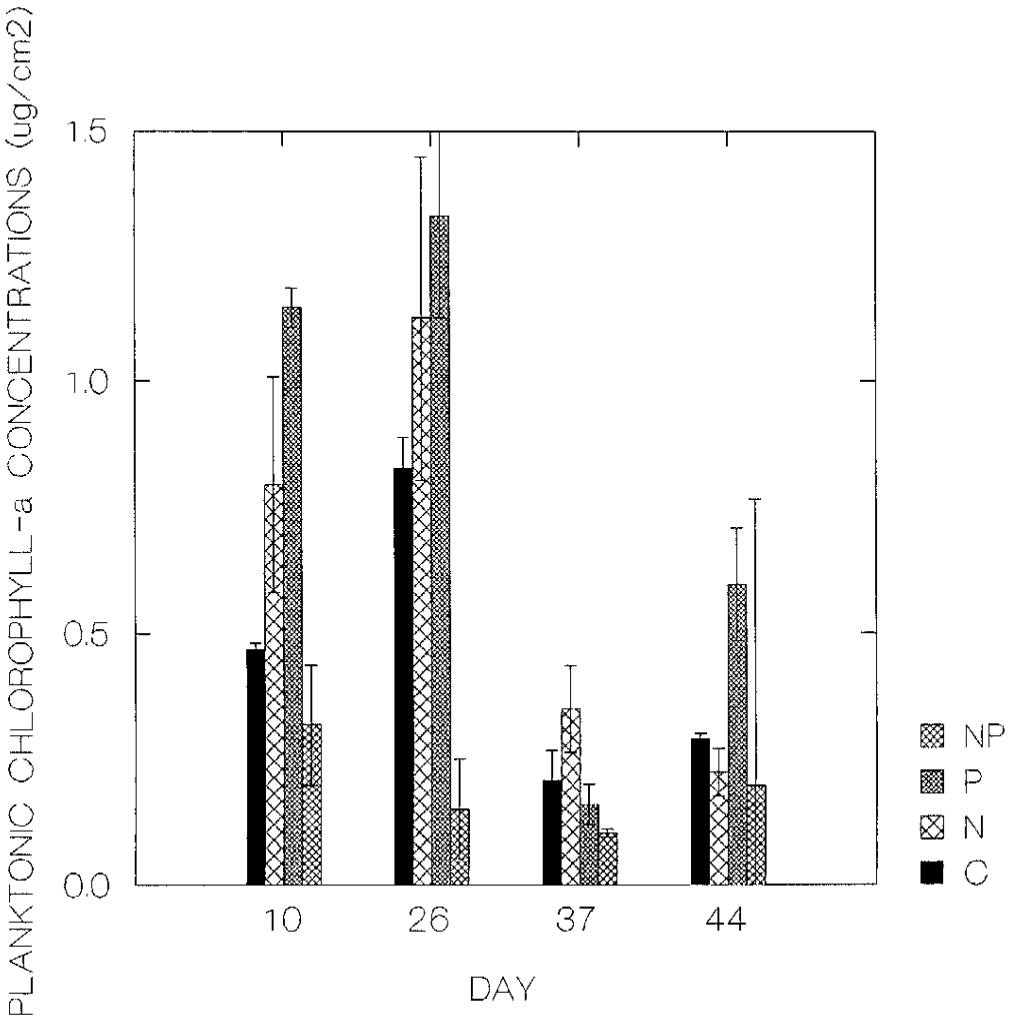

Fig. 5. Total number of invertebrates per habitat.