Resource-limited control of stream periphyton

by

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Abstract

Stream periphyton can undergo dramatic cycles of biomass accrual, senescence, decay and export. Since deterioration of stream water quality can occur when biomass accrual reaches nuisance levels, an understanding of the abiotic and biotic factors leading to these biomass levels are important for management of stream water quality. This study sought to determine the relative importance of the abiotic factors - water velocity, nutrient flux and ratios, light and their interactions - as determinants of excessive periphyton production.

Two experiments were conducted in an outdoor, experimental stream-trough system within a southern Vancouver Island watershed. The stream-troughs provided a compromise between the need for realism with respect to natural systems and the need for a replicated experimental design. Using this system it was possible to manipulate the effects of specific parameters (e.g. velocity and nutrients) on biomass production in the presence of unmodified parameters (e.g. temperature, light), enabling the work to be more applicable to natural systems.

In the first experiment, the effects of velocity and nutrient flux (μg N and P·time^{-1}) interactions on periphyton biomass accumulation and species composition were examined at velocities below 15 cm·sec^{-1}. Periphyton biomass was positively correlated with velocity in the absence of nutrient manipulation. Treatments receiving supplemental nitrogen and phosphorus, to mimic the nutrient fluxes of the highest velocity, yielded the greatest biomass accrual. The latter periphyton community structure consisted of large, floating, chlorophyte dominated mats.

The second experiment examined the effects of N:P ratios at low and high nitrogen and phosphorus concentrations on periphyton community structure. In single nutrient manipulation treatments (either N or P added), biomass accrual was greatest at a ratio of 15 compared with that at 5, even though the latter treatment had a greater P concentration. Taxonomic composition remained relatively consistent with differing N:P ratios, except for higher numbers of a filamentous Cyanophyte at low N:P ratios

and high P concentrations. The latter results confirm the findings reported by other workers.

A third study examined the interactions of light- and nutrient-limitation on periphyton structure within a stream (Rithet Creek) whose riparian canopy had been partially harvested. The experimental design used nutrient-diffusing substrata (agar filled clay pots) at two shaded and two unshaded sites. Unlike other studies which found only light to be the limiting resource in forested streams and nutrients the limiting factor in unshaded streams, the results of this study suggested a dual resource limitation affected periphyton biomass accrual. Light was the primary factor limiting periphyton development given that biomass accrual was an order of magnitude greater at unshaded sites compared with shaded sites. Light was an important determinant of the taxonomic composition of the periphyton, with filamentous chlorophytes dominant under high light conditions. Nitrogen was found to be a secondary limiting factor as evidenced by an 8 fold increase in biomass accrual on treatments receiving added N&P compared to the controls or treatments receiving added P only. Nutrient concentration only affected the relative proportion and numerical abundance of the species present.

Examiners [redacted]

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I lift my eyes to the hills -
where does my help come from?
My help comes from the Lord,
the Maker of heaven and earth.

Psalms 121: 1-2 (*NIV*)

Chapter I. Importance of velocity, nutrient flux and N:P ratios in structuring periphyton communities within outdoor, experimental stream-troughs

Introduction

Stream periphyton is composed of bacteria, cyanobacteria, eukaryotic algae, protozoa, fungi and microinvertebrates, together with extracellular products (Hoagland *et al.*, 1993) and accumulated organic and inorganic debris (Biggs and Close, 1989). Abiotic and biotic factors may separately, or together, control stream periphyton structure (biomass, taxonomic composition, nutritional composition) and function (photosynthesis, productivity, N-fixation) (Lock *et al.*, 1984; Power *et al.*, 1988). Top-down, or biotic controls include the direct effects of herbivory and indirect effects of predation on herbivores. Bottom-up, or abiotic factors include macro- and micronutrients, quantity and spectral quality of light, temperature, substrata type and stability, water velocity and the frequency and intensity of flood events (Biggs and Close, 1989; Horner *et al.*, 1990). Although the interaction of biotic and abiotic factors in controlling periphyton communities is receiving increasing attention (Power, 1992; Rosemond, 1993) the focus of my study was limited to the importance of the abiotic parameters - water velocity, nutrient flux and nutrient ratios, and their interactions - in producing excessive periphyton development or "blooms".

Stream periphyton can undergo dramatic cycles of growth, senescence, decay, and export (Power, 1992) and can alter stream water quality when biomass production reaches "nuisance" levels (Horner *et al.*, 1983). An understanding of factors which can lead to the production of nuisance biomass levels are thus important for management of stream water quality (Horner *et al.*, 1990). Horner *et al.* (1983) suggested that a biomass of 100 to 150 mg chlorophyll $a \cdot m^{-2}$ may represent a nuisance condition. During senescence, such biomass can cause reduction in water quality, possibly leading to taste and odor problems. Increased biological oxygen demand by decomposition of excessive periphyton biomass can affect resource use (e.g. fisheries potential) through decreased oxygen concentration in salmonid rearing waters (Lucey, 1994). The importance of water velocity and nutrient concentrations as factors affecting excessive periphyton biomass accrual have received the most investigation.

Numerous studies on interactions between water velocity and periphyton have been conducted in experimental systems (Horner *et al.*, 1983; Reiter and Carlson, 1986; Steinman and McIntire, 1986; DeNicola and McIntire, 1990; Peterson and Stevenson, 1990). In both natural and laboratory streams, at velocities from 0 to 50 $cm \cdot sec^{-1}$, increased biomass accumulation (and/or productivity) has been reported (Whitford and Schumacher, 1964; McIntire, 1966; Rodgers and Harvey, 1976; Horner *et al.*, 1983; Welsh *et al.*, 1988). At velocities above 50 $cm \cdot sec^{-1}$, lower biomass accrual has been reported (Horner and Welch, 1981; Horner *et al.*, 1983). These contrasting findings have led to the understanding that flowing water has both stimulatory and inhibitory effects on periphyton (Horner and Welch, 1981).

As water flows over a surface, a gradient of decreasing velocities, known as the boundary layer, is established. Within this layer nutrients and wastes are transported by diffusion (Riber and Wetzel, 1987). Increased velocity reduces the boundary layer thickness and enhances algal growth through an increased rate of diffusion of nutrients (Whitford, 1960). Whitford and Schumacher (1964) suggested that a velocity greater than 15 $cm \cdot sec^{-1}$ is necessary to significantly reduce the diffusion gradient. Horner and Welch (1981) and Horner *et al.* (1983) however, showed that velocity increases between 5 and 25 $cm \cdot sec^{-1}$ stimulated biomass accumulation. This was presumed to be the result of an increased rate of nutrient utilization caused by a reduction in the boundary layer thickness.

In contrast to the stimulatory effect, an increase in velocity adds to the frictional force of the passing flow, impeding colonization and removing a progressively greater portion of the attached biomass (Horner *et al.*, 1990). Increases in velocity above that to which the periphyton was acclimated can lead to dramatic losses of biomass (McCormick and Stevenson, 1991; Peterson and Stevenson, 1992; Power, 1992) and in extreme cases, reduce biomass to near zero levels, initiating new cycles of biomass accrual and taxonomic succession (Fisher *et al.*, 1982; Peterson *et al.*, 1994).

Periphyton taxonomic composition can also be influenced by velocity (Raven, 1992). McIntire (1966) found that diatoms were more abundant in laboratory streams with velocities of 38 $cm \cdot sec^{-1}$, whereas filamentous chlorophytes dominated at 9 $cm \cdot sec^{-1}$. Peterson and Stevenson (1990) examined periphyton recovery after a simulated spate (flood) in fast (29 $cm \cdot sec^{-1}$) and slow (12 $cm \cdot sec^{-1}$) water velocities in outdoor experimental stream channels and found that the adnate diatom, *Achnanthes minutissima*

was the initial dominant alga, due to its resistance to scouring, but was later out competed by *Synedra* spp. and dense floating mats of Zygnematalean filamentous green algae.

In addition to velocity, nutrient resources are important parameters in determining periphyton structure. It has been widely documented that increased nutrient enrichment of flowing water typically results in increased growth and biomass of periphytic algae (Stockner and Shortreed, 1978; Grimm and Fisher, 1986; Perrin *et al.*, 1987; Lohman *et al.*, 1991; Peterson and Grimm, 1992). These observations have been used to support the concept of nutrients as a limiting resource for stream periphyton. Odum (1971) defined a limiting factor to biological activity as "a material available in quantities most closely approaching the critical minimum required to sustain activity". The importance of resource limitation in natural systems as a major regulating factor for populations is widely debated and there is little agreement about the frequency and importance of resource limitation in streams (Hart and Robinson, 1990). The potential for nutrient limitation in periphyton is a complex function of boundary-layer hydrodynamics and absolute nutrient concentrations (Lock and John, 1979; Riber and Wetzel, 1987; Hart and Robinson, 1990).

The majority of evidence supporting the theory of nutrient limitation of stream periphyton is the correlation between increasing maximum biomass and increasing nutrient concentrations. Unlike planktonic systems where reliable models exist relating phytoplankton biomass and nutrients (e.g. Dillon and Rigler, 1974), there are few clear relationships between periphyton biomass and nutrient concentrations (Lohman *et al.*, 1992). This lack of correlation has been attributed to the complexity of parameters which have been documented to affect periphyton structure and function.

One environmental factor which has received little attention despite its postulated importance in structuring periphyton communities is nutrient ratios. Redfield (1958) proposed an atomic ratio of 16:1 as a transition point from N to P limitation, based on cellular proportions of nitrogen and phosphorus in marine phytoplankton. He postulated that when the N:P ratio in the surrounding environment exceeds 16:1, phosphorus potentially limits algal growth and when less than 16:1, algae are potentially nitrogen-limited. It is now known that the optimum N:P ratio for algae, (the cellular ratio of nutrients at which neither is growth limiting (Borchardt, 1994)), is species specific (Rhee and Gotham, 1980) and may play an important role in determining phytoplankton competitive interactions and successional patters (Tilman *et al.*, 1982).

N:P ratios have been primarily used to indicate N- or P-limitation of periphyton growth. In bioassays of river periphyton communities, Schanz and Juon (1983) found N limiting at N:P values <10:1, P limiting at N:P >20:1, and at ratios between these limits, either element might be limiting. This broad range of uncertainty is not surprising (Grimm and Fisher, 1986) because periphyton consists of many species having potentially different optimal N:P requirements (Rhee and Gotham 1980; Borchardt, 1994). Experimental enrichment studies have generally confirmed these estimates, with P additions enhancing periphyton growth at N:P >20:1 (Stockner and Shortreed, 1978; Elwood *et al.*, 1981; Peterson *et al.*, 1983; Pringle, 1987) and response to N enrichment reported at N:P <10:1 (Gregory, 1980; Grimm and Fisher, 1986; Hill and Knight, 1988). There has been little work however, examining how the N:P ratios might effect taxonomic composition and biomass of periphyton.

A factor complicating examination of resource limitation on periphyton is the development of large benthic algal mats under conditions of enhanced nutrient supply and high light intensities (Biggs and Close, 1989; Feminella *et al.*, 1989; Power, 1992). These high biomass communities can dramatically alter the surrounding physical / chemical environment (Stevenson and Glover, 1993; Mulholland *et al.*, 1994). Not only is light penetration into the mat reduced, but diffusion of nutrients into the mat may be limited by the formation of large zones of stagnant or near stagnant water around the mats (Mulholland *et al.*, 1994). The importance of velocity in maintaining high periphyton biomass levels was demonstrated by Stevenson and Glover (1993). They determined that the filamentous algal canopy can limit diffusion of nutrients to the understory, but an increase in velocity from 3 to 10 $cm \cdot sec^{-1}$, enhances nutrient diffusion within the community. Several studies have documented that nutrient limitation and nutrient cycling within periphyton communities increase with higher biomass (Bothwell, 1989; Mulholland *et al.*, 1991; Peterson and Grimm, 1992). Riber and Wetzel (1987) have shown that within periphyton mats, internal cycling of nutrients can support the established community, and that biomass accrual requires only a small influx of nutrients from the water column. Periphyton-induced changes in environmental conditions may be an important factor leading to the senescence of communities, especially if nutrient cycling is insufficient for maintenance. Thus, there may be critical biomass levels where limited supplies of nutrients and/or light would result in deterioration of the periphyton from senescence and sloughing (Horner *et al.*, 1990).

Various approaches have been taken to examine resource-limitation of stream periphyton from laboratory microcosms (McIntire, 1966; Steinman *et al.*, 1987) to whole stream manipulations (Elwood *et al.*, 1981; Perrin *et al.*, 1987). The former approach frequently suffers from a lack of realism with respect to natural benthic habitats (Manual and Minshall, 1980; Weevers *et al.*, 1988) while the latter are difficult to replicate, making it difficult to determine whether variation in upstream and downstream communities are caused by natural variation or by treatment effects (Hurlbert, 1984). Outdoor, experimental mesocosms (e.g. Mundie *et al.*, 1983, Bothwell, 1985; Grimm and Fisher, 1986) are being used with increasing frequency as a compromise between the need for realism and the need for a replicated experimental design (Hart and Robinson, 1990). For my research, an established outdoor, mesocosm system (experimental stream-troughs) (Ridley-Thomas, 1989; Lucey, 1994) located within the Greater Victoria Water District was used. Concerns over the potential deleterious effects on water quality from excessive algal biomass development in streams feeding drinking water reservoirs (Figure 1) led to the examination of the importance of velocity and nutrient flux, concentrations and ratios in structuring periphyton communities. By using such a system it was possible to manipulate specific parameters (e.g. velocity and nutrients) in attempts to partition out their interactions. By leaving other parameters unmodified it was hoped that the work would be more applicable to natural systems.

The objectives of the first experiment (velocity / nutrient flux experiment) were to examine the effects of maintaining a similar nutrient flux (μg N and P·time^{-1}) at differing velocities below 15 cm·sec^{-1} on periphyton biomass accumulation and species composition. Other studies have examined biomass accrual at velocities less than 15 cm·sec^{-1} with and without added nutrients (Horner *et al.*, 1983; Horner *et al.*, 1990). These studies were done in laboratory streams and their nutrient manipulations did not result in an examination of a similar nutrient flux under different velocities.

The objective of the second experiment reported here was to determine if N:P ratios at low and high N and P concentrations resulted in alteration of the structure of periphyton communities. Many studies have examined the effects of nutrient enrichment on periphyton but there has been little examination of the effects of specific nutrient ratios.

Methods and Materials

Study Site

Greater Victoria Water District watershed

The Greater Victoria Water District (G.V.W.D.) watersheds comprise 8 catchment areas, with the major four (Sooke, Goldstream, Waugh and Humpback) owned and managed by the Water District (Figure 2). Sooke and Goldstream catchment areas comprise the majority (approximately 75%) of the Water Supply Area (Terrasol, 1992). Water bodies within the G.V.W.D. watershed include six major lakes (Sooke, Goldstream, Butchart, Lubbe, Mavis and Jack Lake) that were impounded to function as storage reservoirs. In addition, Japan Gulch and Humpback are artificial, surge reservoirs with downstream treatment and distribution facilities (Terrasol, 1992) (Figure 2).

Sooke Reservoir

Sooke is the primary reservoir of the G.V.W.D. watershed and its catchment area encompasses 7,070 hectares, including the 615 hectare reservoir. This reservoir accounts for approximately 80% of the District's water supply capacity (Terrasol, 1992). The reservoir is 7.26 km long, has a maximum width 1.47 km, and its main axis running NNE; the reservoir consists of two major basins. The much larger, northern basin has a maximum depth of 68 m and depth generally decreases from north to the shallow south basin (Lucey 1994). Kapoor Tunnel, the intake for the water delivery system to Victoria and its surrounding municipalities, withdraws water from the shallow, southern basin of the reservoir. Sooke Reservoir is consistently oligotrophic with extremely low turbidity (Secchi Disk depths 6 -10 m) (Lucey, 1994). The major water source for Sooke Reservoir is Rithet Creek, with lesser input from Whiskey and Judge Creeks and Horton, Deer, and Begbie Lakes (Figure 2).

Humpback Reservoir

Humpback Reservoir, located at the downstream end of the district's catchment basins (Figure 2), was the water source for the experimental stream system reported here. Water for Humpback Reservoir principally originates in Sooke Reservoir, but other

sources (Goldstream and Waugh Creek watersheds) are often diverted into the Reservoir. Water from Sooke Reservoir is fed into Kapoor Tunnel, which flows into Japan Gulch Reservoir, and then through a short flume to the northern corner of Humpback Reservoir (Figure 2).

Humpback Reservoir is oligotrophic (Lang and Austin, 1984), with high water clarity and Secchi Disk values (6 m mean annual depth) and low total hardness, total alkalinity, and macro-nutrients (including total phosphorus, soluble reactive phosphorus and nitrate) (Lucey, 1994). The water quality is dependant upon the sources of water discharged into Humpback Reservoir, which can vary greatly from that of Sooke Reservoir.

Experimental stream-trough system

The experimental stream-trough system was located adjacent to Humpback dam, below the water intake tower. The major components of the system were siphons, header box, water delivery system to the experimental stream-troughs, PVC experimental stream-troughs, and exhaust-water collection system (Figure 3). Details of the original stream-trough system can be found in Ridley-Thomas (1989) and Lucey (1994). Several modifications to the original system were made prior to initiation of the studies described below. The objectives of the modifications were to: (1) increase the number of stream-troughs to increase replication and permit more complex experimental designs; (2) increase the independence of variables examined in the experimental design (e.g. velocity, nutrient manipulations); and (3) decrease the variability among replicate stream-troughs by increasing the uniformity of unmodified variables (e.g. light exposure).

Water was siphoned through four, 3.8 cm diameter PVC pipes, which drew water from three depths (0.5, 1.0 and 1.5 m). The siphons were attached to a float, anchored at a set distance from the dam face, maintaining the siphoning depth independent of fluctuating water levels.

Water siphoned over the dam entered a PVC-lined, plywood header box (dimensions 1.0 x 1.0 x 1.5 m deep) (Figure 3). A constant water level in the header box was required to maintain a uniform pressure in the water delivery system. This was accomplished by siphoning more water than was required for the stream-troughs, with the

excess being discharged through two, 3.8 cm PVC stand-pipes located inside the header box. Water flow into the header box was regulated by PVC gate valves on the siphon lines, positioned just preceding the header box.

Water was delivered to paired experimental stream-troughs through a single 3.8 cm diameter PVC pipe which was split into two inlet pipes prior to discharge into the stream-troughs. Water flow to 24 stream-troughs was regulated by twelve PVC gate-valves located at the base of the header box. To obtain independent control of water velocity in each stream-trough, PVC ball valves were added to the water supply pipe in front of each trough. Thus, each gate valve was adjusted to deliver the volume of water required for paired stream-troughs, and independent control of velocity in each stream-trough was possible by adjustment of the ball valve. This modification was chosen instead of an independent water supply line to each stream-trough, because the number of PVC through-hull (bulk-head) fittings that could be installed in the header box limited the number of supply lines to twelve.

Twenty-four experimental stream-troughs were used, positioned in groups of four, on six (1.22 x 2.44 m x 0.9 m approximate height) plywood tables. The tables were arranged in two rows oriented approximately north to south providing the stream-troughs with approximately uniform exposure to sunlight. The stream-troughs were fabricated from 4.5 mm grey PVC sheets, cut into 4, 0.3 m x 2.44 m strips. The PVC strips were heat bent to produce a rhomboid trough (dimensions: base 18 cm, open top 26 cm, sides 11 cm, and length 244 cm) (Figure 3). The angle of base to sides (114^{O}) was designed to minimize shading of periphyton communities on the stream bed (Lucey, 1994). The PVC end plates (6 mm) were heat welded to the troughs. Each end plate was drilled to accept either a 3.8 cm threaded male / slip PVC adaptor (for the water supply inlet), or five, 2.5 cm PVC 90^{O} elbows in two staggered rows (for water exhaust) (Figure 3).

Water discharged from the experimental stream-troughs was collected in a gutter trough connected to a common drainage system. Water from the streams and header box overflow, flowed into the overflow exhaust discharge system for Humpback Reservoir and was discharged into an adjacent wetland forming the headwaters of Bilston Creek.

Nutrient Delivery System

The nutrient delivery system, designed by Lucey (1994), was modified to improve replication of nutrient manipulations in the experimental stream-trough system. The nutrient delivery systems consisted of 22-L nutrient reservoirs which contained stock solutions of nitrogen ($NaNO_3$), phosphorus (Na_2HPO_4), or both. Nutrients were added to stream-troughs through a modified, medical intravenous (I.V.) administration set (Venisystems; Nonvented I.V. Administration Set 200-SL). The I.V. set was mounted to the bottom of the nutrient reservoir with a 1-cm diameter neoprene rubber O-ring. The I.V. line was shortened to a length that discharged directly into the inlet of the stream-trough. The nutrient delivery systems were mounted on raised platforms 30 cm above the front of the stream-troughs and delivered stock nutrient solutions to individual stream-troughs (Figure 3).

The nutrient concentrations in experimental stream-troughs were estimated on the basis of flow rates through the stream-troughs, nutrient drip rate, concentration of stock solutions in the nutrient reservoirs and background nutrient concentration in the water supply. The drip rate was determined by selection of a hypodermic needle bore diameter yielding a drip rate that would drain the 22-L nutrient reservoir in approximately 7 days. The drip rate was dependant upon the head of solution in the nutrient reservoir volume ($1.8 \pm 10\% mL \cdot min^{-1}$). Variation in drip rate was reduced by refilling the nutrient reservoir every four days (approximately 10-L discharge).

Periphyton substrata

A variety of artificial substrata have been used for periphyton colonization and sampling in artificial streams: glass microscope slides (Clark *et al.*, 1979; Ridley-Thomas, 1989; Lucey, 1994); unglazed ceramic tiles (Lamberti *et al.*, 1989; DeNicola and McIntire, 1990; Hill *et al.*, 1992; Peterson and Stevenson, 1992) and cylinders (Steinman *et al.*, 1990; Mulholland *et al.*, 1991); natural stream rock (Hill and Harvey, 1990), stream gravel (Mundie *et al.*, 1991; Gardner, 1993); Plexiglass (Horner *et al.*, 1990); and Styrofoam sheets (Bothwell, 1985). Glass slides have been the most commonly used artificial substrata for studying periphyton colonization and biomass development on stream and lake periphyton sampling systems (Aloi, 1990).

Glass slides were the artificial substrata used in the experimental stream system because they are inert, inexpensive, readily available, accrued periphyton growth may be easily removed, and were used in previous studies conducted within this experimental system. A greater surface area for periphyton development was also possible with vertically, rather than horizontally, oriented glass slides. Vertically-positioned slides have been demonstrated to colonize more slowly than horizontal slides, but algal populations on both sides of vertically-positioned slides are quantitatively and qualitatively similar (Aloi, 1990). Vertical orientation of slides also decreases sediment accumulation and generally results in a more firmly attached periphyton (personal observation, this system).

Glass microscope slides (75 x 50 mm) were positioned vertically, on long edge, parallel to the velocity in the artificial streams. The slides were arranged in six rows with 8 to 10 slides per row held in thin, slotted strips of PVC siliconed (Dow Corning 999) to the PVC stream bed. The first series was positioned 30 cm from the front of the trough and the remaining series 30 cm apart. The first series were located sufficiently downstream from the water inlet to ensure establishment of uniform velocity conditions and adequate mixing of nutrient treatments. This was established by adding drops of methylene blue dye at the stream-trough inlet and determining the distance downstream where the dye appeared to be uniformly mixed. This distance corresponded to a zone of limited periphyton colonization, presumably from turbulent scouring of the water flowing into the experimental stream-troughs.

Stream-trough experimental design

Velocity / nutrient flux experiment

Treatments consisted of experimental stream-troughs with velocities of 6, 9, and 12 $cm \cdot sec^{-1}$ without nutrients manipulation (designated V6, V9, and V12). Two additional treatments (V6N+ and V9N+) had nitrate and phosphate added to stream-troughs with velocities of 6 and 9 $cm \cdot sec^{-1}$ to approximate the nutrient flux occurring in the 12 $cm \cdot sec^{-1}$ treatment. Concentrations of NO_3-N and PO_4-P in treatments V6N+ and V9N+were increased by 2 and 1.3 times ambient concentrations, respectively. Thus treatments V6, V9, V12 had different velocities, different nutrient fluxes, and the same ambient NO_3-N and PO_4-P concentrations. But treatments V6N+, V9N+ and V12 had different velocities, different NO_3-N and PO_4-P concentrations, and the same nutrient flux

(Figure 4). The five treatments were randomly assigned amongst 20 experimental stream-troughs.

N:P ratio experiment

Treatments consisted of manipulating the NO_3-N and PO_4-P concentrations in the stream-troughs (by addition of $NaNO_3$ or Na_2HPO_4) to obtain N:P ratios of 5, 15, 30, and 45 (designated NP5, NP15, NP30 and NP45). Unless otherwise specified, the N:P ratios refer to the ratio of NO_3 to PO_4 only. The control stream-troughs received no added nutrients. To determine the effects of N:P ratios at elevated NO_3-N and PO_4-P concentrations, three additional treatments were used (Controlx2, NP15x2 and NP30x2). The NO_3-N and PO_4-P concentrations were modified in these latter treatments to be twice the concentrations in the Control and the NP15 and NP30 treatments. These eight treatments were randomly assigned amongst 24 experimental stream-troughs.

Field procedures

Prior to the beginning of each experiment the system was shut down and cleaned. The stream-troughs and header box were drained and scrubbed to remove periphyton and bacterial biofilm accumulation. Gate valves on the siphon lines located just above the header box permit interruption of the water flow without having to restart the siphons. Empty substrata holders removed from the stream-troughs during cleaning were replaced and cleaned glass slides added.

Velocity manipulation

For the velocity / nutrient flux experiment, velocities of 6, 9 and 12 $cm \cdot sec^{-1}$ were randomly assigned to each of the stream-troughs. For the N:P experiment a velocity of 9 $cm \cdot sec^{-1}$ was used. These velocities were chosen because they were the highest possible given the siphon volumes available.

Since flow rate (volume of water per unit time) was less time consuming to measure than velocity (distance per unit time), the relationship of these two parameters was established prior to the start of the experiment. Velocity was measured by timing the movement of dye (0.5 mL methylene blue) over 0.5 m intervals in the stream-troughs.

Flow rate in each trough was estimated by measuring the time required to fill a 22-L bucket. All measurements were done in triplicate and averaged.

Velocity in each stream-trough was established by the following procedure. First, the gate valve at the base of the header box was adjusted to give the flow rates required by paired stream-troughs fed by a single line. This was repeated for all twelve gate valves to ensure a constant water depth inside the header box. Second, the ball valves for each stream-trough was adjusted to the correct flow and locked. Once set the valves did not need further adjustments throughout the experiments. During the velocity / nutrient flux experiment the flow rates were checked twice (after 3 and 6 weeks) and it was found that the velocities had not changed from the original setting.

Water chemistry analysis

Manipulation of nutrient concentration in the stream-troughs was necessary for both the velocity / nutrient flux and the N:P ratio experiment. This required determination of the ambient nutrient concentrations in the stream-troughs. Water samples for chemical analysis were collected weekly from the header box into 2-L polyethylene bottles, (supplied by either the G.V.W.D. or the laboratory conducting the analysis). Water sampling was done concurrently with periphyton sampling (see below) and the water samples were delivered to the analytical lab (MB Research, Sidney) the same day. Additional outside laboratory analysis was required given the potentially low nitrogen and phosphorus concentrations of the oligotrophic water supply.

Periphyton sampling

Periphyton sampling was initiated one week after the beginning of the experiment and was repeated weekly thereafter. A stratified sampling design was used in which one glass slide was randomly chosen from each of six series in each stream-trough. The six glass slides were placed in a labelled 250-mL glass jar with stream water. The jars were put on ice in a cooler for transport to the laboratory.

Laboratory Procedures

Periphyton was scraped off the glass slides with a razor blade into the 250-mL sample jars and the volume of water in each jar made up to 250 mL with distilled water. To increase the homogeneity of the periphyton for improving subsampling replication, the periphyton was blended for up to 30 seconds in a kitchen food blender (Osterizer). Biggs (1987) found that sample blending generally resulted in a significant increase in the mean values of the community measurements: number of taxa, Shannon-Weaver diversity, total number of cells and chlorophyll *a*, compared to samples which were simply shaken. Biggs (1987) also found blending resulted in considerable improvement in subsampling precision (subsamples of chlorophyll *a* and AFDM had errors as low as 5 - 10 % and coefficients of variation of 4 - 8%). Algal cell damage was only observed in very large or long narrow cells (eg. *Spirogyra* and *Synedra* spp. respectively) when blending exceeded 2 min (Biggs, 1987).

Periphyton biomass measurements

Newman and McIntosh (1989) have cautioned periphyton workers about the need to distinguish between aufwuchs (synonymous with periphyton) and procedurally-defined aufwuchs. Procedurally-defined aufwuchs is defined as (all) material accumulating on submerged surfaces and includes entrapped planktonic biota, dead organisms, previously suspended organic and inorganic material, and other surface-derived abiotic material in addition the organisms growing on these surfaces (Newman and McIntosh, 1989). If this distinction is not made, periphyton biomass measurements may be overestimated if considerable exogenous organic and inorganic material is entrapped within the periphyton. The method of biomass determination is important in determining the potential for overestimation. Periphyton biomass has been measured by a variety of procedures. The most common methods include: gravimetric measurement, dry mass (DM), organic matter or ash-free dry mass (AFDM) and organic carbon; phytopigment content (generally chlorophyll); ATP; and caloric values (Clark *et al.*, 1979). Each of these techniques have specific advantages, limitations and equipment requirements and by using more than one method for measuring periphyton biomass the possibility of partitioning periphyton into autotrophic/heterotrophic and living/nonliving matter has been demonstrated (Clark *et al.*, 1979).

Two methods of determining periphyton biomass were employed in my research; phytopigment (chlorophyll *a*) and gravimetric (AFDM).

Chlorophyll *a*

Unlike AFDM, measurements of chlorophyll refer only to the algal component of the periphyton. The ratio of algal chlorophyll to biomass however, varies with algal species, physiological state, and light and nutrient levels (Clark *et al.*, 1979). Contamination from detrital chlorophyllous pigments may also be a problem (Aloi, 1990). The latter problem can be minimized by correcting the chlorophyll *a* values for phaeophytin concentration.

Subsamples (50 - 150 mL) of scraped, blended periphyton were filtered onto 5.5 cm glass fiber filters (Whatman GF/F) using a multi-filter unit Millipore filtration apparatus and Millipore vacuum pump (vacuum setting of 300 mm of Hg). Stockner *et al.*, (1989) found no increase in cell rupture of phytoplankton samples filtered at vacuum pressures between 50 - 500 mm Hg. Several milliliters of a magnesium carbonate solution (10 $g \cdot L^{-1}$ $MgCO_3$) were added at the end of filtering to prevent acidification and the consequent formation of phaeophytin during storage and extraction (Lang and Austin, 1984; APHA, 1985). Samples were stored frozen at -20 ^{o}C until analysis.

The ease with which chlorophyll is extracted from cells varies with different algal taxa, and requires cell disruption to achieve complete pigment extraction (APHA, 1985). Chlorophyll was extracted by grinding for less than 1 min, in cold, 90% acetone with a variable speed Teflon / glass homogenizer. The homogenate was left to steep for 24 hr, refrigerated in the dark at 2 ^{o}C (APHA, 1985), followed by filtering through a 2.2 cm glass fiber filter (Whatman GF/F) into a side arm test tube.

Chlorophyll *a* extract concentrations were determined spectrophotometrically using the trichromatic method (APHA, 1985) corrected for phaeophytin. A 3 mL acetone extract subsample was removed from a measured volume of extract, and absorbance was measured at 750, 664, 640, and 630 nm using a LKB Ultraspec II UV/Vis spectrophotometer (Biochrom 4050). When necessary, pigment extracts were diluted to obtain an absorbance value within the range of 0.1 - 1 (APHA, 1985). Phaeophytin was

determining by measuring the change in absorbance (at 665 nm) after acidification of extract sub-sample with 1-2 drops of 0.1 N HCl (APHA, 1985).

Chlorophyll *a*·dm^{-2} values were calculated using the formulas of APHA (1985) and Jeffrey Humphrey (1975):

$$C_a = 11.85(OD664) - 1.54(OD647) - 0.08(OD630) \quad (1)$$

where C_a = the concentration of chlorophyll *a* in the acetone extract (mg·L^{-1}) and OD664, OD647 and OD630 = turbidity corrected optical density (OD) (with a 1-cm light path) at 664, 647, and 630 nm. The absorbances were corrected for turbidity by subtracting the OD at 730 nm.

$$\text{Chl } a = (C_a \text{ x } V)/SA \quad (2)$$

where chl *a* = chlorophyll *a*, mg·dm^{-2}; V = extract volume (L); and SA = surface area (dm^{-2})

$$\text{Ph } a = (26.7[1.7(OD665)-OD664] \text{ x } V) / SA \quad (3)$$

where Ph *a* = phaeophytin *a*, mg·dm^{-2}

The phaeophytin corrected chlorophyll *a* content was determined by subtracting the value from equation 3 from equation 2.

Dry mass and ash-free dry mass

DM and AFDM measure not only algal biomass, but also that of bacteria, fungi, invertebrates and organic detritus. Subsamples (50 - 150 mL) of scraped, blended periphyton were filtered onto pre-weighed ashless filters (5.5 cm Whatman ashless 41) for gravimetric analyses. The filters were dried (60 °C, Gallenkamp Incubator, model 1H-150) to constant weight, determined by two weighings 12 hr apart (Mettler balance AE50, weighed to nearest 0.1 mg). The biomass on filters was stored frozen at -20 °C until prior to freeze-drying.

The biomass on filters were placed in ceramic crucibles and freeze-dried (Precision Scientific Co.) to constant weight. DM values were calculated by subtracting the weight of crucible and filter and converted to mg DM·dm^{-2}. Ash content was determined by incinerating filter and periphyton organics in a muffle furnace (BlueM Electric Co.) at 450 ^{o}C for 4-6 hr (Ridley-Thomas, 1989; Ridley-Thomas *et al.*, 1989). AFDM was calculated as ash weight subtracted from the DM and converted to mg AFDM·dm^{-2}.

Taxonomic composition determination

Determinations of algal numerical abundance were made using a Carl Zeis inverted microscope using Utermöhl sedimentation chambers (Lund *et al.*, 1957). Preliminary numerical abundance analyses was accomplished on two replicate stream-trough samples (n=2) for all treatments at day 42 for both velocity / nutrient flux experiment and N:P ratio experiment.

Subsamples (50 mL) of scraped, blended periphyton were preserved with 2.5 mL formalin in opaque, plastic vials until enumeration. Each sample was gently shaken, subsampled (1-2 mL), diluted if necessary (up to 25 fold dilution) and treated with Lugol's prior to adding 3 mL of sample to the Utermöhl base. The samples were allowed to settle for a minimum of 6 hours. Organisms were enumerated (at a magnification of 200X) using randomly selected fields (Sandgren and Robinson, 1984) with usually twenty microscope fields (10 fields replicated two times using different aliquots from the same sample) required to enumerate a minimum of 500 cells (Steinman and McIntire, 1986; Rosemond, 1993). Taxa were identified to the lowest level possible with the inverted microscope (usually genus) using the following source works: Prescott (1962); Bourelly (1966, 1968, 1970); Patrick and Reimer (1966, 1975); and Foged (1981).

Due to the complex community composition, it was necessary express the taxonomic composition as numerical abundance of algal groups. The algal taxa were assigned to these groups (guilds), based on how the alga is associated with the substratum (physiognomic form) (Lowe *et al.*, 1986). Designated physiognomic groups were prostrate diatoms, prostrate to stalked diatoms (diatoms that can be attached directly to the substrata or on a stalk), erect diatoms, chain forming diatoms, and filamentous chlorophytes.

Biovolume estimates of the dominant taxa (accounting for >1% of total community biovolume) were determined. For each taxon, length, width, and depth (or diameter for filaments) were measured from a minimum of 20 cells from 4-5 different samples. Biovolume estimates were based on standard geometric formulas for shapes which best approximated the alga (Clark *et al.*, 1987). Biovolume estimates of individual taxa were presented as a percentage of the total community biovolume.

Periphyton nitrogen and phosphorus analysis

At the close of the velocity / nutrient flux experiment and N:P ratio experiment, periphyton was harvested from remaining unsampled glass slides and the PVC stream-trough bed, for determination of periphyton nitrogen (N) and phosphorus (P) content. The glass slides were removed from each trough and put into 250 mL jars (six per jar) on ice, for transport back to the laboratory. Periphyton attached to the sides and bottom of the PVC troughs was collected by draining the stream-troughs and scraping the periphyton from the PVC. The resulting periphyton slurry was poured into a 22-L bucket and transported to the laboratory.

On arrival at the laboratory, periphyton on the glass slides was removed by scraping with a razor blade into a plastic weigh dish and frozen at -20 ^{o}C. Once frozen, the periphyton was stored in labelled plastic ziplock bags at -20 ^{o}C. The periphyton slurry from the PVC trough beds was allowed to settle for approximately 6 - 8 hours at room temperature and decanted to 1 - 2 L. This was divided into 250-mL glass jars and settled a second time overnight at 2 ^{o}C, decanted, and the periphyton from each stream-trough was frozen in zip-lock bags at -20 ^{o}C.

Prior to initiating proximate composition analysis the frozen periphyton samples were freeze-dried to constant weight, ground to a fine powder in a kitchen coffee grinder, and screened through a 0.625 mm sieve. Samples were stored in sealed ziplock bags over desiccant at -20 ^{o}C.

Total (Kjeldahl) nitrogen and phosphorus content of periphyton was determined using Hach Digesdahl apparatus (model 21400) following the procedures (Hach Co., 1985) modified for periphyton by Ridley-Thomas (1989). The Digesdahl apparatus consisted of 100-mL heat-resistant digestion flask, a thermostatically controlled 25-250 W

disk element heater, and a Vigreux fractionating column fitted with a capillary tube / funnel for controlled delivery of peroxide reagent at 3 $mL \cdot min^{-1}$ (Hach *et al.*, 1987).

A 250 mg periphyton subsample was placed in a 100-mL digestion flask with 4 mL of concentrated sulfuric acid. The sample was charred at 450 ^{o}C for 5 min and 10 mL of 50% hydrogen peroxide was added slowly over 7 min through a capillary funnel. After the hydrogen peroxide has been added, the sample was heated for an additional 5 min to allow for degradation of the excess hydrogen peroxide. The flask was then removed from the heat, allowed to cool, and diluted to 100 mL with deionized water. The digested samples were then gravity filtered through 3.7 cm glass fiber filters (Gelman A/E) prior to colorimetric determination of NH_3-N and PO_4-P with Nessler's reagent (Hach Co.) and Phosphorus reagent 1 and 2 (Hach Co.) respectively (Hach Co., 1985). N and P standard curves were determined for each assay trial using Hach calibration standards (Calibration standard set, Hach Co.). Periphyton N and P content was expressed as a percentage of AFDM.

The accuracy and precision of the N and P analysis was determined in two ways. A large periphyton sample (collected from Humpback experimental stream-trough system) was designated as an intra-laboratory standard, of which a subsamplw was analyzed in every digestion trial. The sample was composed primarily of diatoms and detritus. In a total of 6 different assay trials, the percent N and P averaged 9.6% (standard error 6.6,% range 9.2 - 9.8%) and 0.15% (standard error 6.2%, range 0.15 - 0.16%), respectively. To determine the digestion efficiency in the N assay, periphyton standard samples were spiked with 50 mg of nicotinic acid (an extremely difficult to digest refractory compound; Hach *et al.*, 1987). This was done during four assay trials, with an average percent recovery of 98% (standard error 4.9%, range 96 - 101%).

Statistical analysis

Changes in periphyton biomass accrual over time was analyzed using a two-way ANOVA with repeated measures using the GLM procedure of the Statistical Analysis System (SAS, 1985). The model used in the ANOVA tested for the effects of treatment, time and their interactions. When significant interactions were found between treatment and time, no further statistical analysis of the main effects was conducted (Zar, 1984). Changes in periphyton nitrogen and phosphorus content with treatment were analyzed

using a one-way ANOVA, followed by a Student-Newman Keuls (SNK) test to determine order and significance of differences among treatments (SAS, 1985).

Results

Velocity / nutrient flux experiment

Water chemistry

Nitrate-N concentrations were consistently less than 6 $\mu g \cdot L^{-1}$ from July 17 to August 26 1991, and on two occasions were below method detection limits (MDL: 2 $\mu g \cdot L^{-1}$) (Table 1). Ortho-phosphate-P concentrations were also low, with values ranging from below MDL (<0.5 $\mu g \cdot L^{-1}$) to 3.2 $\mu g \cdot L^{-1}$. N:P ratios (nitrate-N:ortho-P) ranged from 1 to 7. The planktonic algal biomass from Humpback Reservoir, the water and biota recruitment source for the experimental streams, was consistently low with values ranging from 1-2 mg chl $a \cdot m^{-3}$ (Dillon and Rigler, 1974) (Table 1).

Periphyton biomass

Periphyton biomass was significantly ($P<0.0001$) different among treatments whether measured as AFDM or chlorophyll *a* and biomass changed significantly ($P<0.0001$) within treatments over time. There was a significant ($P<0.0001$) interaction between treatment and time, resulting from different biomass accrual patterns. This interaction limited further statistical analysis of the main effects of treatment and time and differences in biomass accrual with treatment and time are presented graphically (Figures 4 and 5).

Periphyton biomass was positively correlated with increasing velocities of 6 to 12 $cm \cdot sec^{-1}$ (Figures 5 and 6). Maximum chlorophyll *a* concentrations of 0.03, 0.05 and 0.06 mg chl $a \cdot dm^{-2}$ were reached after 28 to 35 days for velocities of 6, 9, and 12 $cm \cdot sec^{-1}$, respectively, before decreasing after that (Figure 5). Maximum ash-free dry mass (AFDM) values of 7.2 and 11 $mg \cdot dm^{-2}$ were reached by day 28 at velocities of 6 and 9 $cm \cdot sec^{-1}$ and remained constant (Figure 5). Maximum AFDM of 19 $mg \cdot dm^{-2}$ was not reached in the 12 $cm \cdot sec^{-1}$ velocity treatment until day 35.

Treatments V6N+ and V9N,+ had a greater biomass accrual during the first 21 to 28 days than did other treatments (Figures 5 and 6). Treatment V9N+ which received less additional N and P than the V6N+ treatment (Figure 4), had a maximum biomass of 0.12 mg chl *a*·dm^{-2} after 28 days, compared with 0.09 mg chl *a*·dm^{-2} for the V6N+ treatment (Figure 5). AFDM accrual in the V6N+ and V9N+ treatments differed from chlorophyll *a* accrual. Maximum AFDM of 15 mg·dm^{-2} was reached after 21 to 28 days for the V6N+ treatment, compared with 35 to 45 days (24 mg·dm^{-2}) for the V9N+ treatment.

Taxonomic composition

The algal community composition was similar in all the treatments by day 42, with prostrate/stalked diatoms numerically dominant (Figure 7). The total number of algal cells per area increased with increasing velocity. Values for treatments V6N+ and V9N+ were between those observed for treatments V9 and V12. Numerical abundance was similar for erect diatoms, chain-forming diatoms and filamentous chlorophytes (Figure 7). All treatments had the same dominant algal taxa based on biovolume estimates (Figure 8). The filamentous chlorophyte, *Mougeotia* spp. (Argardh) Wittrock contributed the majority of the biovolume in treatments V6, V9, and V12 (53-66%), whereas for the V6N+ and V9N+ treatments, *Mougeotia* contributed less (35 and 45% respectively). In these latter treatments *Tabellaria fenestrata* (Lyngb.) Kuetz. and *Tabellaria flocculosa* (Roth.) Kuetz. (chain forming diatoms) contributed the greatest biovolume percentage (45 and 38%). *Synedra ulna* (W.Sm.) Brun., *Synedra* spp Ehren. (erect diatoms) and *Achnanthes* spp. Kuetz. (prostrate/stalked) diatoms biovolume contributed in decreasing order to the biovolume (Figure 8). There were visual differences in periphyton community composition after 28 days (Figure 9). Treatments V6, V9 and V12 had a community composed primarily of diatoms while treatments V6N+ and V9N+ had an extensive, floating chlorophyte dominated community.

N:P ratio experiment

Water chemistry

During the experimental period from September 14 to October 26 1991, nutrient (NO_3-N and PO_4-P) concentrations were variable (Table 2). NO_3-N concentrations

ranged from 6 and 37 $\mu g \cdot L^{-1}$ and PO_4-P concentrations (although generally less than 1.5 $\mu g \cdot L^{-1}$), ranged from below MDL to 7.3 $\mu g \cdot L^{-1}$ (Table 2). N:P ratios ranged from 5 to 40, but were generally above 25 (Table 2). Planktonic algal biomass were consistently low and ranged from 0.5 to 2.5 mg chl $a \cdot m^{-3}$ (Table 2).

Due to the fluctuations in NO_3-N and PO_4-P concentrations in Humpback reservoir, the treatment nutrient manipulations had to be modified weekly in order to maintain a consistent N:P ratio (Table 3). Treatments NP5 received only added P while treatments NP30 and NP45 received only added N. Treatment NP15 received additional P except from September 28 to October 4, when N was added, while treatments Controlx2, NP15x2 and NP30x2 always received added N and P. Treatment NP5 received more added P than NP15 (0-4.6 $\mu g\ P \cdot L^{-1}$ and 0-0.7 $\mu g\ P \cdot L^{-1}$, respectively). Treatment NP45 received more added N than NP30 (6.5-25 $\mu g\ N \cdot L^{-1}$ and 0-7 $\mu g\ N \cdot L^{-1}$). Between September 28 and October 4 a low N:P ratio of 5, resulted in 182 and 292 $\mu g\ N \cdot L^{-1}$ being added to increase the N:P ratio to 30 and 45 respectively (Table 3). Controlx2 and NP30x2 treatments received similar N and P additions, while treatment NP15x2 generally received 2-3 times more P than either Controlx2 and NP15x2 (Table 3).

Periphyton biomass

Periphyton biomass was significantly ($P<0.0001$) different among treatments whether measured as AFDM or chlorophyll *a* and biomass changed significantly ($P<0.0001$) within treatments over time. There was a significant ($P<0.0001$ for chlorophyll *a* and $P<0.014$ for AFDM) interaction between treatment and time, resulting from different biomass accrual patterns. This interaction limited further statistical analysis of the main effects of treatment and time and differences in biomass accrual with treatment and time are presented graphically (Figures 10 and 11).

There were general patterns observed in biomass accrual which were usually consistent with either chlorophyll *a* concentration or AFDM (Figures 10 and 11). Periphyton accrual exhibited (1) a single biomass maxima followed by either (a) a reduction in biomass (NP5, NP15), (b) a stable biomass (NP30, NP30x2 chl *a* only) or (c) possibly a bimodal function (treatments NP45, Controlx2, and NP15x2); or (2) a linear increase in biomass for the duration of the experiment (Control, NP30x2 AFDM only).

Biomass accrual was lowest in the Control, NP30 and NP45 treatments with biomass maxima of 0.08 mg chl *a*·dm^{-2} and 25 mg AFDM·dm^{-2}; 0.3 mg chl *a*·dm^{-2} and 36 mg AFDM·dm^{-2}; 0.25 mg chl *a*·dm^{-2} and 30 mg AFDM·dm^{-2}, by day 42 (Figures 10 and 11). NP5 and NP15 had the greatest biomass accrual of the single nutrient addition treatments, with maximum biomass 0.36 mg chl *a*·dm^{-2}, 39 mg AFDM·dm^{-2} and 0.5 mg chl *a*·dm^{-2}, 49 mg AFDM·dm^{-2}, respectively.

Treatments Controlx2, NP15x2 and NP30x2 had the highest biomass accrual of all treatments. By day 28, NP15x2 had the greatest chlorophyll *a* concentration (0.6 mg chl *a*·dm^{-2}), followed by NP30x2 (0.47 mg chl *a*·dm^{-2}) and Controlx2 (0.41 mg chl *a*·dm^{-2}) (Figure 10). NP30x2 had the greatest AFDM (61 mg AFDM·dm^{-2}) by day 42, followed by NP15x2 (59 mg AFDM·dm^{-2}) and Controlx2 (53 mg AFDM·dm^{-2}) (Figure 11).

Periphyton N and P content

The highest periphyton nitrogen content was observed in treatments Control (8.2%), NP30 (7.6%), NP45 (7.8%) and NP15x2 (7.8%) (Figure 12). The other treatments had %N content ranging from 6-7%. The highest phosphorus content was found in Control (0.9%), NP45 (0.8%) and NP15x2 (0.8%) (Figure 12). NP15, Controlx2 and NP30x2 had the lowest %P content (0.5-0.6%).

Taxonomic composition

Chain forming diatoms were numerically dominant in the single nutrient addition treatments, with filamentous cyanophytes abundant in NP5 and NP15 treatments (Figure 13). There were more algal cells per area in treatments Controlx2, NP15x2 and NP30x2 than in the other treatments, with the filamentous cyanophyte, *Oscillatoria* sp. Vaucher, numerically dominating. Chain forming diatoms were also more abundant in Controlx2, NP15x2 and NP30x2 treatments. Erect and prostrate/stalked diatoms were present in similar numbers in all treatments (Figure 13).

Based on biovolume estimates, filamentous chlorophytes (*Mougeotia* spp., *Zygnema* sp Agardh. and *Oedogonium* sp. Link) comprised between 44 and 53% of the periphyton community in treatments Control, NP5, NP15, NP30, NP45 and Controlx2

(Figure 13). In the NP15x2 and NP30x2 treatments, however, filamentous chlorophytes comprised 12 and 76% of the community, respectively. In treatments Control, NP5, NP15, NP30, NP45 and Controlx2, *Tabellaria fenestrata* and *Tabellaria flocculosa* provided the largest biovolume contribution by diatoms (37-42%) (Figure 14). In treatment NP15x2, the diatom *Synedra ulna* contributed proportionally the largest biovolume. *Oscillatoria* sp. (filamentous cyanophyte) although numerically dominant, constituted only 1% of the biovolume in all treatments except NP15x2 (7%) (Figure 14). There were little visual differences in periphyton community composition among treatments after 28 days (Figure 15).

Discussion

Velocity / nutrient flux experiment

In the velocity range from 0 to 50 cm·sec^{-1}, increased biomass accrual (and/or productivity) has been correlated with higher velocities in both laboratory and natural streams (Whitford and Schumacher, 1960; McIntire, 1966; Horner *et al.*, 1983; Welch *et al.*, 1988). Whitford and Schumacher (1964) suggested that a velocity of >15 cm·sec^{-1} is necessary to significantly reduce the diffusion gradient. The results of the velocity / nutrient flux experiment showed increased maximal periphyton biomass accrual at velocities of 6 to 12 cm·sec^{-1} under ambient nutrient concentrations (Figures 5 and 6). This supports the results of Horner *et al.* (1983) and Horner *et al.* (1990), who found that at velocities of 5 to 25 cm·sec^{-1} and 10 to 60 cm·sec^{-1}, respectively, periphyton biomass accrual was enhanced.

In the velocity / nutrient flux experiment, treatments with velocities of 6, 9 and 12 cm·sec^{-1} with a similar nutrient flux (V6N+, V9N+, V12) produced different biomass accrual (Figures 5 and 6). Treatments V6N+ and V9N+ which received additional nitrogen and phosphorus at levels 2 and 1.3 times ambient concentrations, respectively (Table 1), exhibited greater biomass accrual when compared with the V12 treatment (Figures 5 and 6). Thus, similar nutrient fluxes at different velocities did not result in similar biomass accrual as originally hypothesized. The biomass accrual in the nutrient manipulated treatments was not correlated with absolute nutrient concentration, however. Treatment V9N+ had a maximum biomass of 0.12 mg chl *a*·dm^{-2} while V6N+ biomass maxima was 0.09 mg chl *a*·dm^{-2}. These results suggest that the nutrient flux in the bulk

water does not accurately reflect the nutrient conditions (nutrient flux and concentrations) occurring at algal cell surfaces.

The interactions among velocity, mass transport of solutes, and substrata is complex and not fully understood due to the inherent difficulties in measuring the processes occurring on such a microscale, although the development of microelectrodes is greatly enhancing our understanding (Carlton and Wetzel, 1987; Jorgensen and Marais, 1990). In the bulk water flow, mass transport takes place by eddy diffusion (Jorgensen and Marais, 1990) and the eddy diffusion coefficient (K) is roughly proportional to velocity (Borchardt *et al.*, 1994). Adjacent to all surfaces in flowing water is a thin layer (usually tens to hundreds of micrometers thick) where flow is laminar and velocity greatly reduced (Borchardt *et al.*, 1994). This results from surface friction retarding the flow until it is ultimately stagnant adjacent to the substratum surface. The stagnant layer is called the laminar sublayer (or diffusive boundary layer), while the region of decreasing velocity is called the boundary layer (Jorgensen and Marais, 1990; Carling, 1992). Mass transport through these two layers is by different mechanisms (Borchardt *et al.*, 1994). Eddy diffusion is proportional to velocity and is the dominant transport force through both the bulk water and the boundary layer. At the upper boundary of the laminar sublayer, molecular diffusion becomes of greater importance to mass transfer than eddy diffusion.

Molecular diffusion through the laminar sublayer is described by Fick's first law of diffusion (from Borchardt *et al.*, 1994):

$$J_D = h(C_b - C_0) \qquad (4)$$

where J_D = diffusion flux, with dimensions of mass·area^{-1}·time^{-1}; C_b and C_0 = nutrient concentrations in the bulk solution and at the cell surface, respectively; and h = permeability, with dimensions of distance·time^{-1}. Permeability is a function of the diffusion coefficient (a constant for the solute) and the laminar sublayer thickness (Riber and Wetzel, 1987). Permeability increases as a hyperbolic function with velocity, since velocity reduces the thickness of the laminar sublayer (and the diffusion distance) (Borchardt *et al.*, 1994).

Jorgensen and Marais (1990) demonstrated with oxygen gradients in the diffusive boundary layer (laminar sublayer) adjacent to a marine microbial mat, that at velocities

approaching 8 cm·sec^{-1} over the mat, the molecular flux of oxygen through the mat was nearly asymptotic. This indicates that at higher velocities the thickness of the laminar sublayer would decrease at a decreasing rate. These results may help to explain why treatments V6N+ and V9N+ had a greater biomass than treatment V12. If the results of Jorgensen and Marais (1990) can be extrapolated to other systems, then in the velocity / nutrient flux experiment, the increased nutrient concentrations would be expected to increase the molecular diffusion to algal cell surfaces to a greater degree than would the reduction in the laminar sublayer with increasing velocity. It is unclear however, what the relative nutrient flux at the algal cell surface would have been for treatments V6N+ and V9N+. From the biomass accrual data it may appear that treatment V9N+ received a greater nutrient flux to the algal cells in spite of a lower concentration. The possibility the V6N+ periphyton may have been limited by other factors related to velocity (e.g. inorganic carbon) cannot be ruled out. Borchardt (1994) found that at velocities from 3 to 12 cm·sec^{-1}, increases in maximum photosynthesis at nutrient saturated conditions was due to enhanced carbon uptake.

Mass transfer of solutes in flowing water is the summation of molecular diffusion and eddy diffusion (from Borchardt *et al.*, 1994):

$$J_{MT} = -(D+K)dC/dy \qquad (5)$$

where J_{MT} = mass transfer flux, D = molecular diffusion coefficient, K=eddy diffusion coefficient, and dC/dy = the solute concentration gradient in one direction. In stable laminar sublayers, eddy diffusion is minimal and molecular diffusion is the principal mode of mass transfer, whereas in the bulk fluid the opposite is true (Borchardt *et al.*, 1994).

This distinction is biologically important to benthic community structure. Low, adnate benthic communities are surrounded by both the boundary layer and laminar sublayer (Raven, 1992), while erect and filamentous forms can emerge through the boundary layer and are surrounded only by their own laminar sublayer and as such would increase the potential availability of nutrients (Raven, 1992). Erect and filamentous algae are more susceptible to loss at higher velocities however, and require a greater expenditure of energy for synthesis of structural elements (Borchardt, 1994).

In addition to differences in biomass, treatments V6N+ and V9N+ had a distinctly different community composition than the other treatments after four weeks (Figure 9). The former treatments were dominated by filamentous chlorophytes (predominantly *Mougeotia* spp., preliminary data not shown) which formed extensive floating mats (Figure 9), whereas the other treatments were diatom dominated. This provides additional evidence for increased diffusion of nutrients through the laminar sublayer at higher N and P concentrations, for filamentous chlorophytes are typically found to become dominant at elevated phosphorus concentrations and low flows (Horner *et al.*, 1990).

Attached algae in many streams undergo dramatic cycles of growth, senescence, decay and export and little is known about the factors leading to detachment and senescence (Power, 1992). By the end of the velocity / nutrient flux experiment periphyton biomass (measured as chlorophyll *a*) had decreased by approximately half for treatments V6, V9 and V12 and by approximately two thirds in treatments V6N+ and V9N+ (Figure 5). There was a decrease in periphyton AFDM in treatments V12, V6N+ and V9N+ by the end of the experiment, while AFDM biomass from treatments V6 and V9 remained unchanged after 4 weeks. The decrease in chlorophyll biomass was due in part to an increase in the proportion of phaeophytin.

Periphyton taxonomic composition had changed by the end of the experiment, and was similar among all treatments (Figure 7). Filamentous chlorophytes and chain forming diatoms comprised (usually) greater than 75% of the biovolume (*Mougeotia* spp. 35-66% and *Tabellaria* spp. 15-45%) (Figure 8). Treatments V6N+ and V9N+ tended to have a smaller proportion of *Mougeotia* spp. and a greater proportion of *Tabellaria* spp. when compared to the other treatments. This loss of chlorophyte biomass, especially pronounced in the V6N+ and V9N+ treatments, may have been the result of changed hydraulic characteristics surrounding the periphyton mats (Stevenson and Glover, 1993; Mulholland *et al.*, 1994).

Mulholland *et al.* (1994) found that periphyton biomass levels reaching 4.3 $mg \cdot cm^{-2}$ significantly decreased velocity and increased the volume of stagnant water surrounding the periphyton mats. They also found that increased streamwater P concentration increased P uptake which suggested that the periphtyon was limited by the rate of nutrient diffusion into the mat. Several other studies have shown that as periphyton biomass increases, nutrient limitation can occur at concentrations which

saturate growth rates of individual cells (Bothwell, 1989; Mulholland *et al.*, 1991). It is possible that within the extensive chlorophyte mats seen in the velocity /nutrient flux experiment, nutrient limitation led to the senescence of part of the community. As periphyton biomass and potential nutrient limitation increases, increased nutrient cycling is believed to sustain the nutrient requirements of the community (Riber and Wetzel, 1987; Paul and Duthie, 1989). It is possible that factors other than nutrient limitation led to the loss of periphyton biomass, such as carbon limitation (Borchardt, 1994) or photoinhibition of the surface mats from visible or ultraviolet light (Bothwell *et al.*, 1993).

N:P ratio experiment

Periphyton biomass responded positively to both phosphorus and nitrogen additions made to modify the N:P ratio of the bulk water (Figures 10 and 11). The ambient N:P ratios throughout the majority of the experiment were between 25 and 30 (Table 2) and indicated potential P-limitation of periphyton accrual (Schanz and Juon, 1983; Grimm and Fisher, 1986). However, there was not a linear increase in biomass accrual with increasing P concentration (Figure 16). This is not surprising since in contrast to planktonic systems (Dillon and Rigler, 1974), there have been few clear relationships between periphyton biomass and nutrient concentration (Lohman *et al.*, 1992). Under a P-limited system, increased P concentration would have been expected to have produced a greater biomass, thus it was expected that biomass accrual within treatment NP5 should have been greater than treatment NP15. However, treatment NP15 had a greater biomass accrual than NP5, by day 28 (0.50 mg chl $a \cdot dm^{-2}$ and 0.36 mg chl $a \cdot dm^{-2}$, respectively) (Figure 10). This enhanced accrual may have been the result of a more optimal N:P ratio for the periphyton community.

It is known that algae take up nutrients in specific ratios (Rhee and Gotham, 1980; Tilman *et al.*, 1982) and that the optimum nutrient ratio is the cellular ratio of two nutrients where neither is growth-limiting (Rhee and Gotham, 1980). The optimum nutrient ratio is species specific and has been shown to range from 5 to 37 (Rhee and Gotham, 1980; Wynne and Rhee, 1986; Olsen, 1989). Although N:P ratios have been used to explain competitive interactions and successional patterns in phytoplankton communities (Tilman *et al.*, 1982), little is known about the importance of N:P ratios in structuring periphyton communities. Borchardt (1994) examined photosynthesis and P-uptake under N- and P-limitation of *Spirogyra fluviatilis* Hilse. He was the first to

determine optimum N:P ratios for a charophycean alga and he found that the optimum N:P ratio ranged from 23-26, depending on velocity. Optimum N:P ratios have also been shown to be dependent on growth rate, for when growth rate changes the cellular requirement for one nutrient, requirements for other nutrients may not change proportionately (Borchardt, 1994).

Although P appeared to be the primary limiting nutrient in the N:P ratio experiment, N appeared to have an additive effect. An additive effect occurs when N and P are both at low concentrations so that when the primary limiting nutrient is supplied the secondary nutrient is rapidly depleted and becomes limiting (Grimm and Fisher, 1986). Treatments Controlx2, NP15x2 and NP30x2, which received added N and P, had a greater biomass accrual than did the Control, NP15 and NP15 treatments (Figure 16). It is worth noting that treatment NP15 had a slightly greater biomass accrual by 28 days compared to treatments Controlx2 and NP30x2 even though the N and P concentrations in the NP15 treatment were less than the others (Table 3). This was not the result of nutrient saturation, since there was a greater biomass accrual in the Controlx2, NP15x2 and NP30x2 treatments than the Control, and NP15 and NP30 treatments. This provides additional evidence for an optimum N:P ratio for periphyton communities and that at low concentrations, N:P ratios may be important in determining periphyton biomass accrual.

The nitrogen and phosphorus content of the periphyton did not appear to be related to the N:P ratio of the bulk water flow (Figure 12) or to taxonomic composition (Figure 14). Rather, N and P content appeared to be correlated with the rate of biomass accrual. When the percent change in periphyton biomass over a seven day sampling period was plotted against N and P content (Figure 17) there appeared to be a positive correlation between periphyton P content and change in biomass accrual. Biggs (1994) also found no correlation between periphyton N and P content with environmental nutrient concentrations. Growth and nutrient uptake rates of planktonic algae have been shown to be related to internal and external concentrations of nutrients (Morel, 1987). Maximum short term uptake rate has been shown to be dependant upon the previous degree of nutrient stress (Morel, 1987) and would support the correlation between accrual rate and periphyton N and P content.

Periphyton community composition did not however, appear to be greatly affected by N:P ratios (Figures 13 and 14). By day 42, larger cell numbers accrued in treatments

NP5 and NP15 compared to other single-nutrient manipulation treatments. The greatest cell numbers were found in treatments with both added N and P (Figure 13). The main discernable difference in taxonomic composition was that the numbers of a filamentous cyanophyte (*Oscillatoria* sp.) were proportional to added P concentrations regardless of N:P ratios (Figure 14). Taxonomic composition was only determined from samples collected on day 42 and greater community composition differences may be found from examination of other dates (Figure 9).

Increased P concentrations have been observed to be important in determining the abundance of periphytic cyanophytes. Horner *et al.* (1990) found the cyanophyte, *Phormidium* sp. favoured higher P concentrations, and Elwood *et al.* (1981) found increased numbers of *Oscillatoria* and *Nostoc* under conditions of increased P. Taxonomic differences in competitive ability for nutrient uptake have been examined in planktonic systems (Tilman *et al.*, 1986; Grover, 1989) and provide insights into what may occur in lotic systems. Certain diatoms (*Synedra* spp.) have been found to be superior competitors for P (Tilman *et al.*, 1986; Grover, 1989), while some species of chlorophytes and cyanophytes are superior competitors for nitrogen (Tilman *et al.*, 1986). If this is true for attached algae, then it may help to explain the occurrence of *Oscillatoria* sp. found at both low N:P ratios, and at high P concentrations in the N:P ratio experiment, and the dominance of diatoms at low ambient N and P concentrations of velocity / nutrient flux experiment, with chlorophytes becoming dominant at higher P concentrations.

Other factors, in addition to N:P ratios, may be important in determining species composition of a periphyton community, especially under nutrient saturating conditions. The importance of nutrient uptake kinetics on growth rate and potential competitive advantage under different nutrient regimens has been well documented in phytoplankton ecology (Tilman, 1977; Sommer, 1983;Kilham and Kilham, 1984). Taxonomic differences in maximal kinetic uptake of nutrients and maximal growth rates have been demonstrated (Gotham and Rhee, 1981; Goldman and Gilbert, 1982) and may be important in determining community structure and succession under both nutrient-limited and non-limiting conditions. Fluctuating nutrient supply rates have been shown to maintain a higher species diversity compared with steady-state nutrient supplies, in laboratory phytoplankton competition experiments (Sommer, 1985). In fluctuating nutrient environments, a species with high nutrient concentration requirements may be able to out compete or coexist with species with lower nutrient concentration requirements, if the

maximum growth rate of the former species is greater than the latter (Grover, 1989). Since the nutrient concentrations in Humpback Reservoir were highly variable during the N:P ratio experiment (Table 4), this may have led to a greater taxonomic similarity among treatments than would have developed under a more constant nutrient supply.

Examination of nutrient uptake kinetics in lotic systems has lagged considerably behind research in lentic systems. Such studies would significantly assist our understanding of the linkages between periphyton biomass accrual, and taxonomic composition under different nutrient regimens. However, the effects of factors unique to lotic systems will need to be addressed, specifically, effects of velocity and the development of high biomass periphyton mats. The majority of lotic studies have examined phosphorus uptake by whole periphyton communities with respect to affects of flow (Whitford and Schumacher, 1965; Lock and John, 1979; Horner and Welch, 1990; Riber and Wetzel, 1987) and P concentration on periphyton biomass (Horner *et al.*, 1990) and cellular growth rate (Bothwell, 1989). Velocity increases as low as 0.1 $cm \cdot sec^{-1}$ can measurably increase the diffusive flux of phosphorus to a periphyton film (Riber and Wetzel, 1987). Horner *et al.* (1990) and Bothwell (1989) found P uptake increased in a hyperbolic fashion with increasing P concentration. Bothwell (1989) found that P concentrations needed to saturated high periphyton biomasses were two orders of magnitude greater than those required to saturate growth rates in thin film periphyton communities. The explanation for the disparity between these findings relates to the diffusion of nutrients through layers of a relatively thick periphyton mat compared to thin films. As algal accumulations become more dense, cells within the mat may become P limited while those closer to the surface of the periphyton matrix remain P replete. Hence, higher concentrations of P in the bulk water increase growth rates of cells deeper in the matrix by increasing the supply rate of the limiting nutrient (Perrin *et al.*, 1987; Bothwell, 1989).

The effects of velocity on nutrient uptake kinetics, N- and P-limited photosynthesis and growth rates, and optimum N:P ratios has recently been examined for the first time on a single periphytic algal species (*Spirogyra fluviatilis*) (Borchardt, 1994; Borchardt *et al.*, 1994). They found that flowing water altered short-term uptake of P when the alga was P-deficient, but not when it was P-sufficient. They also demonstrated that increased flow increased the requirement for N and P by increasing the minimum cellular N:P ratio. They concluded that the potential for flowing water to mediate nutrient partitioning among lotic algae by altering growth rates and optimum nutrient ratios provides new insights into

periphyton community biomass accrual and taxonomic composition (Borchardt, 1994; Borchardt *et al.*, 1994). Additional information of this kind from taxonomically and architecturally different algae would provide a better understanding of the relative importance of velocity, nutrient uptake kinetics and N:P ratios are in determining periphyton structure and function.

Table 1. Analysis of Humpback Reservoir chemical water quality characteristics measured during the velocity / nutrient flux experiment (July 17 to August 26 1991). Water samples were obtained from the header box of the experimental stream-trough system, unless otherwise noted. Nutrient ratios N:P and TN:TP refer to NO_3-N:PO_4-P and total nitrogen:total phosphorus ratios, respectively. Method detection limits (MDL) for NO_3-N and PO_4-P are <2 µg $N \cdot L^{-1}$ and <0.5 µg $P \cdot L^{-1}$, respectively. NA = not analyzed.

Parameter		09-Jul*	22-Jul	23-Jul*	30-Jul	12-Aug	20-Aug	26-Aug
Nutrients								
nitrate	(ug N/L)	6	<2	3	2	<2	3	3
ammonia	(ug N/L)	<1	NA	<1	NA	NA	<1	NA
total Kjeldahl nitrogen	(ug N/L)	250	200	300	NA	270	160	260
reactive phosphorus	(ug P/L)	0.9	1	1.8	<0.5	1.2	<0.5	3.2
total phosphorus	(ug P/L)	3.7	3.5	10.6	NA	8.5	5.1	6.5
Ratios								
NO3-N:PO4-P		7	<2	2	>4	<2	>6	1
TN:TP		70	60	30	NA	30	30	40
Plankton biomass								
Chl a	(mg/m3)	1.7	0.5	1.9	2.3	1.2	1.1	1.6

* water samples taken from GVWD sampling station HBR-01-01

Table 2. Analysis of Humpback Reservoir chemical water quality characteristics measured during the N:P ratio experiment (September 14 to October 26 1991). Water samples were obtained from the header box of the experimental stream-trough system. Nutrient ratios N:P and TN:TP refer to NO_3-N:PO_4-P and total nitrogen:total phosphorus ratios, respectively. Method detection limits (MDL) for NO_3-N and PO_4-P are <2 µg N.L-1 and <0.5 µg P.L-1, respectively. NA = not analyzed.

Parameter		11-Sep	18-Sep	25-Sep	2-Oct	9-Oct	16-Oct	22-Oct
Nutrients								
nitrate	(ug N/L)	15	16	37	29	16	23	6
total Kjeldahl nitrogen	(ug N/L)	220	NA	210	NA	210	NA	270
reactive phosphorus	(ug P/L)	0.6	<0.5	7.3	1.2	<0.5	1	<0.5
total phosphorus	(ug P/L)	2	NA	11.9	NA	1.7	NA	8.3
Ratios								
NO3-N:PO4-P		25	>30	5	25	>30	25	>12
TN:TP		110	NA	20	NA	125	NA	30
Plankton biomass								
Chl a	(mg/m3)	0.5	1.3	1.6	1.4	1.4	2	2.5

Table 3. Summary of nutrient manipulation of treatments in the N:P ratio experiment. N+ and P+ = nitrogen and phosphorus were added to treatment stream-troughs. Values in [ ] refer to the concentration of N and/or P ($\mu g \cdot L^{-1}$) added to manipulate the N:P ratio of the treatment stream-troughs. Ambient nitrate-N and ortho-phosphate-P concentrations used for determining N and P supplements for each manipulation interval were from water samples collected three days prior to each interval (Table 2). Ambient N:P = NO_3-N:PO_4-P ratios. NAN = no added nutrients.

Nutrient Manipulation Intervals	**Ambient N:P**	**Nutrient Manipulation of Treatments**							
		Control	NP5	NP15	NP30	NP45	Controlx2	NP15x2	NP30x2
14-Sep: 20-Sep	25	NAN	P+ [2.4]	P+ [0.4]	N+ [3]	N+ [12]	N+,[15] P+,[0.6]	N+,[15] P+,[1.4]	N+,[21] P+,[0.6]
21-Sep: 27-Sep	>30	NAN	P+ [2.7]	P+ [0.6]	NAN	N+ [6.5]	N+,[16] P+,[0.5]	N+,[16] P+,[1.7]	N+,[16] P+,[0.5]
28-Sep: 4-Oct	5	NAN	NAN	N+ [72.5]	N+ [182]	N+ [292]	N+,[37] P+,[7.3]	N+,[182] P+,[7.3]	N+,[401] P+,[7.3]
5-Oct: 11-Oct	25	NAN	P+ [4.6]	P+ [0.7]	N+ [7]	N+ [25]	N+,[29] P+,[1.2]	N+,[29] P+,[2.6]	N+,[43] P+,[1.2]
12-Oct: 18-Oct	>30	NAN	P+ [2.7]	P+ [0.6]	NAN	N+ [6.5]	N+,[16] P+,[0.5]	N+,[16] P+,[1.7]	N+,[16] P+,[0.5]
19-Oct: 26-Oct	25	NAN	P+ [3.6]	P+ [0.5]	N+ [7]	N+ [22]	N+,[23] P+,[1]	N+,[23] P+,[2]	N+,[37] P+,[1]

Figure 1. Photographs of periphyton biomass along sections of Rithet Creek showing (A) an extensive chlorophyte community and (B) a senescent chlorophyte / diatom community.

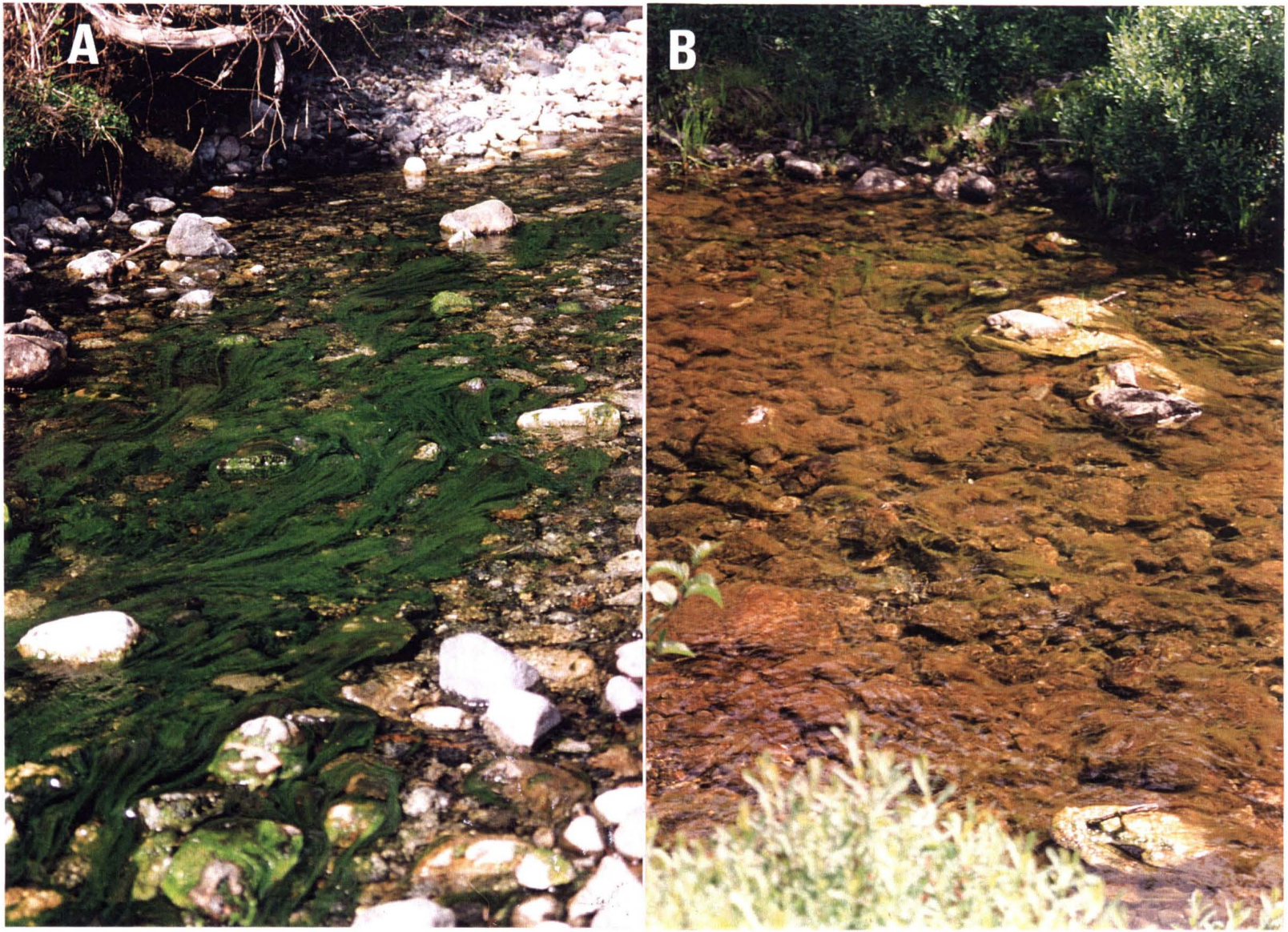

A
B

Figure 2. The Greater Victoria Water District's principal watersheds (Goldstream and Sooke), reservoirs (Deception, Goldstream, Humpback and Sooke) and location of stream-trough facility (INFLEX- IN Field Laboratory EXperiment).

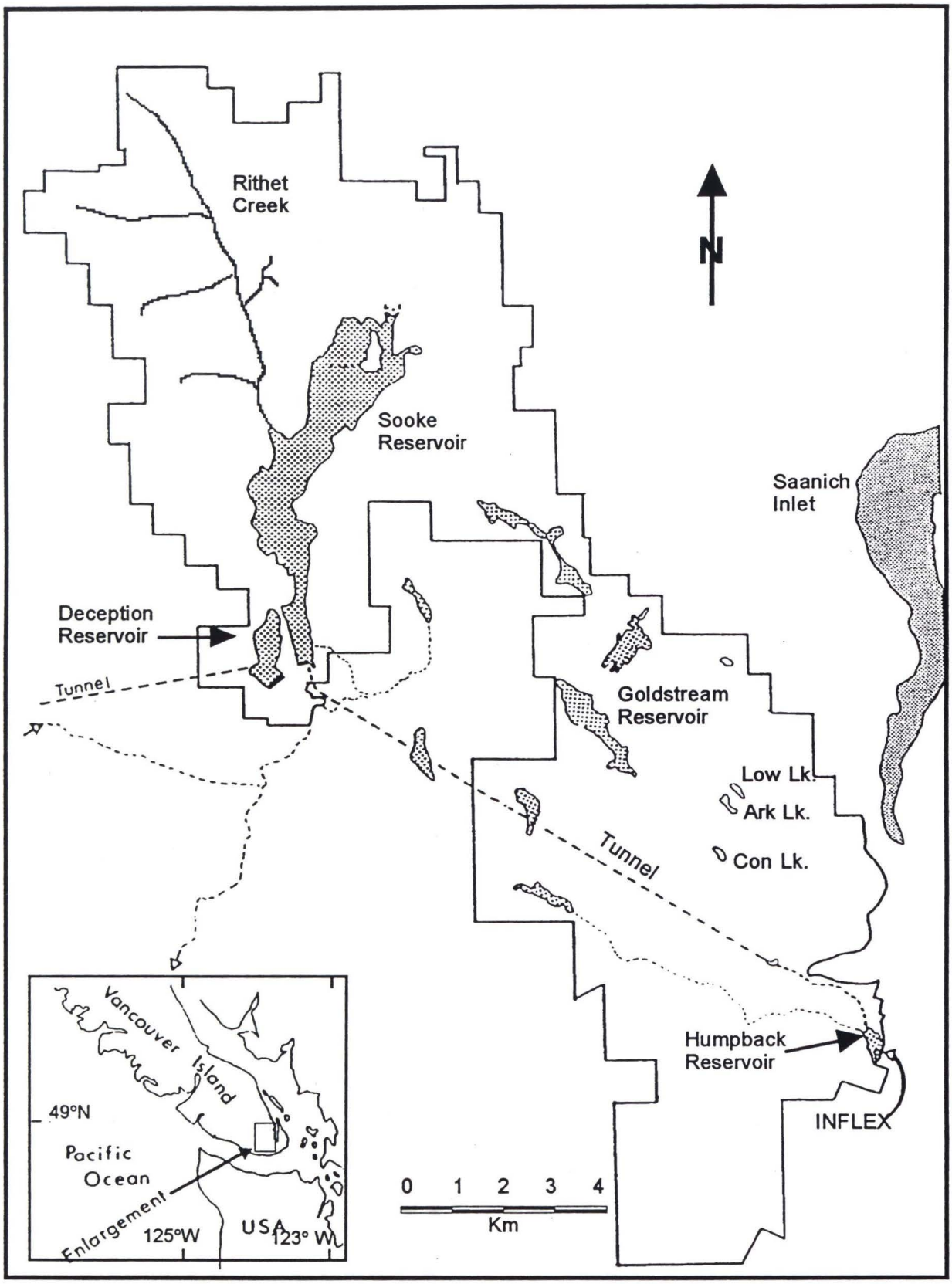
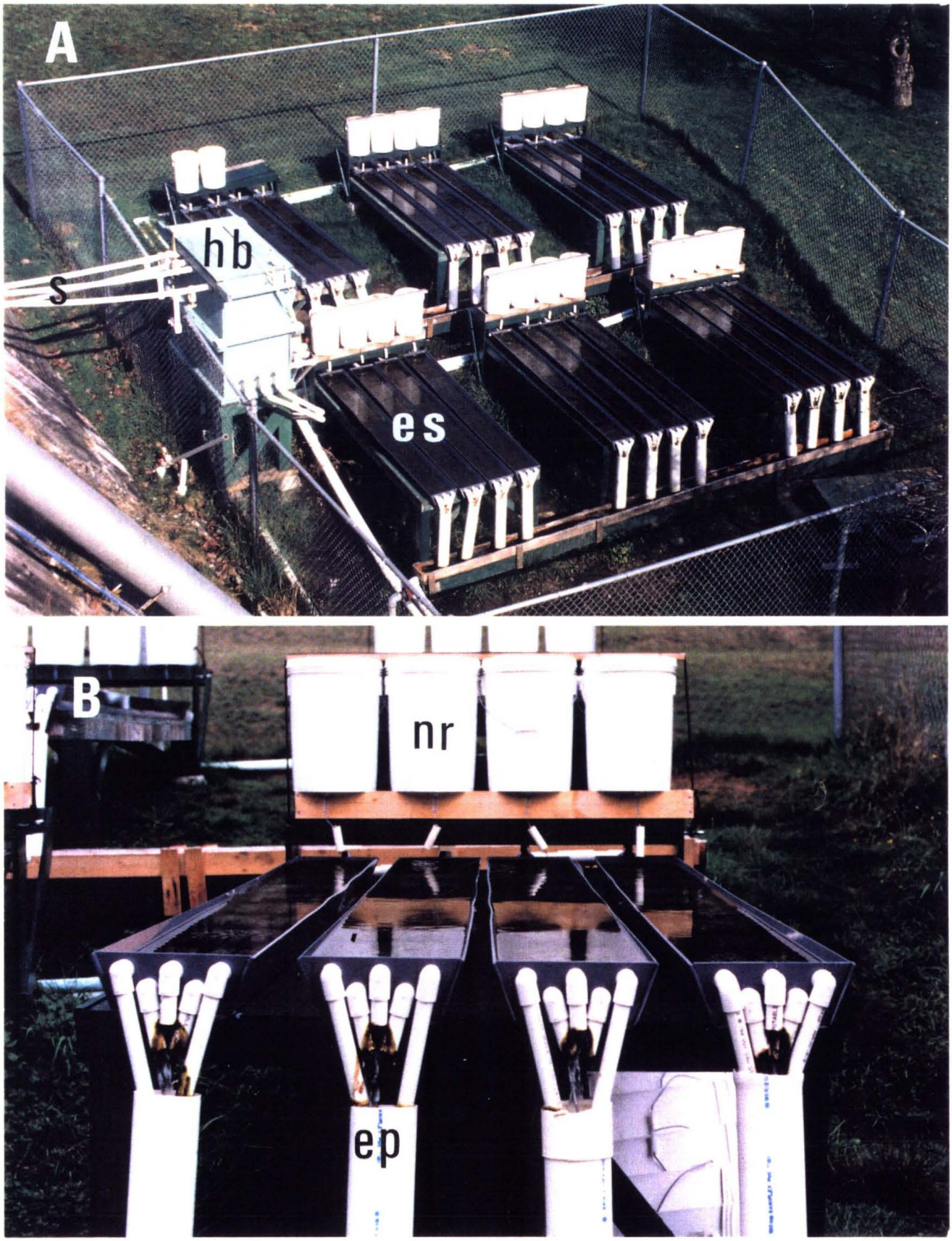

Rithet
Creek
N
Sooke
Reservoir
Saanich
Inlet
Deception
Reservoir
Tunnel
Goldstream
Reservoir
Low Lk.
Ark Lk.
Con Lk.
Tunnel
Humpback
Reservoir
INFLEX
Vancouver Island
49°N
Pacific
Ocean
Enlargement
125°W
USA
123° W
0 1 2 3 4
Km

Figure 3. Experimental stream-trough system at Humpback Reservoir. (A) Water siphoned (**s**) over the dam, flowed into the header box (**hb**) and was distributed to 24 PVC experimental stream-troughs (**es**). (B) Nutrient manipulation within each stream-trough was accomplished by adding nutrient concentrates from a 22-L nutrient reservoir (**nr**) using a modified medical I.V. administration set mounted in the bottom of the nutrient reservoir. The stream-troughs discharged into an exhaust pipe (**ep**) complex which collected water from all stream-troughs which then flowed into an adjacent wetland.


A
hb
s
es
B
nr
ep

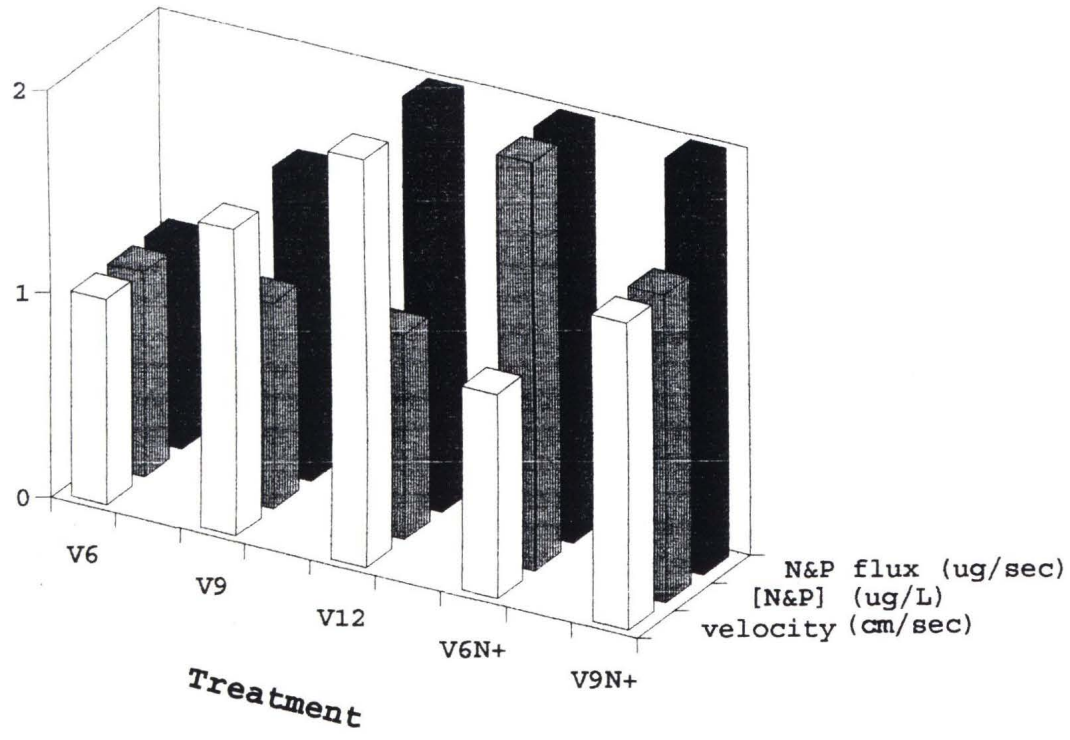
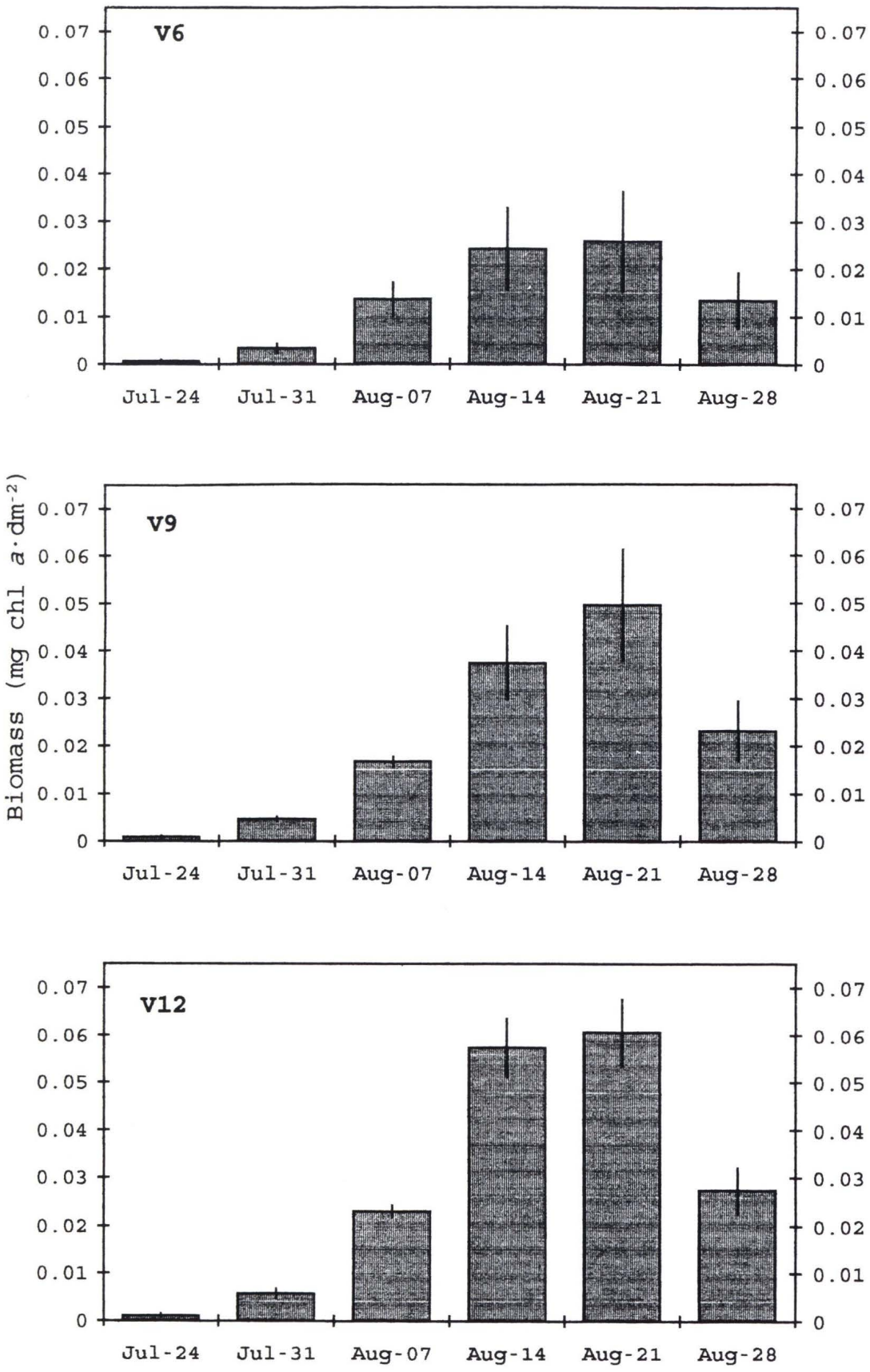


Figure 4. Summary of experimental design for velocity / nutrient flux experiment showing relative velocities, nutrient concentrations and nutrient flux (μg N and P·sec^{-1}) of experimental treatments.

Figure 5. Periphyton chlorophyll *a* biomass (mean $\pm$ SD, n=4) at velocities of 6, 9 and 12 cm·sec^{-1} (V6, V9 and V12) and at velocities of 6 and 9 cm·sec^{-1} with nutrients added (V6N+ and V9N+) to approximate the nutrient flux (μg N and P·sec^{-1}) occurring at the 12 cm·sec^{-1} velocity.


V6
V9
V12
Biomass (mg chl a·dm-2)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Jul-24
Jul-31
Aug-07
Aug-14
Aug-21
Aug-28

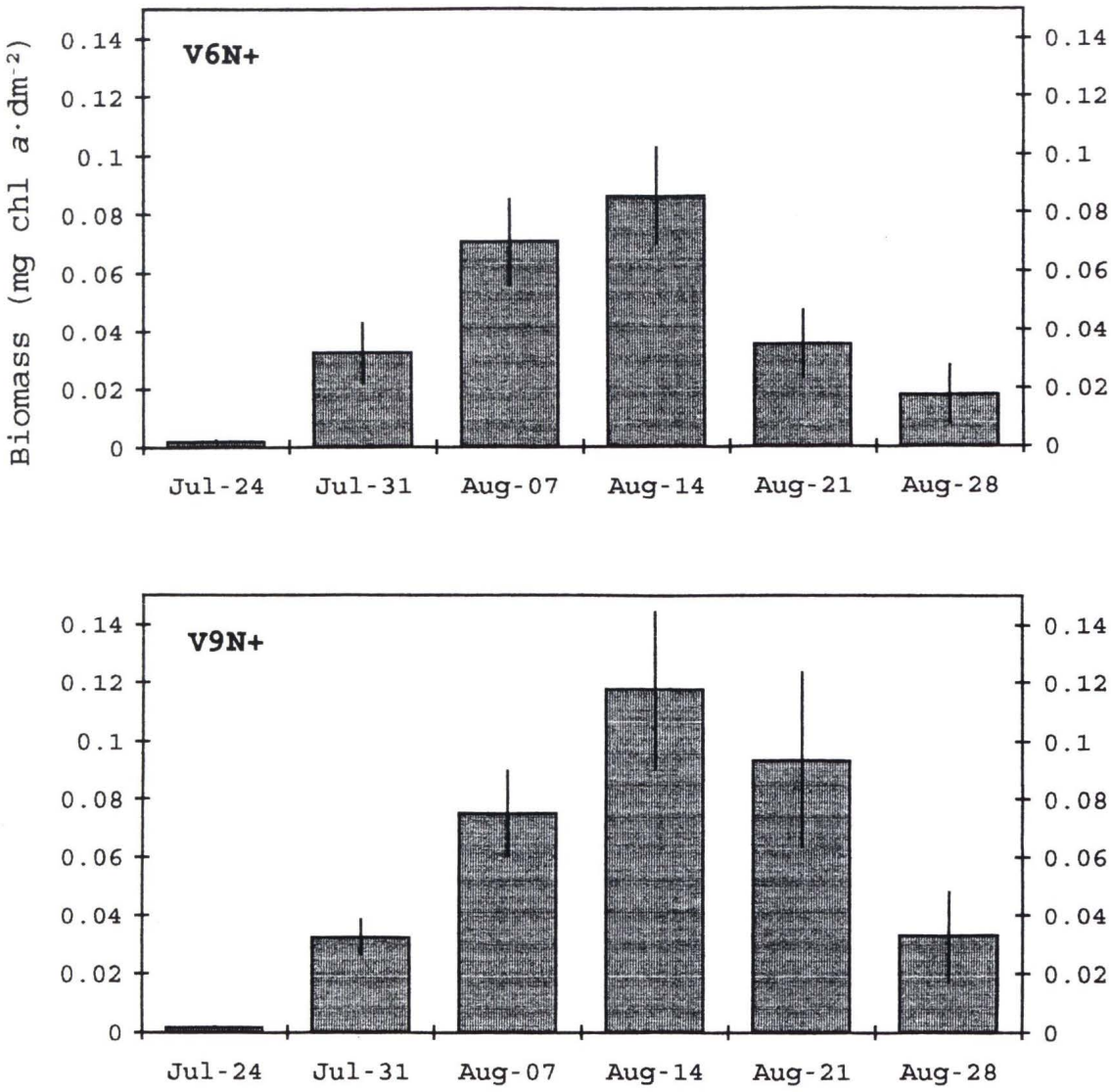
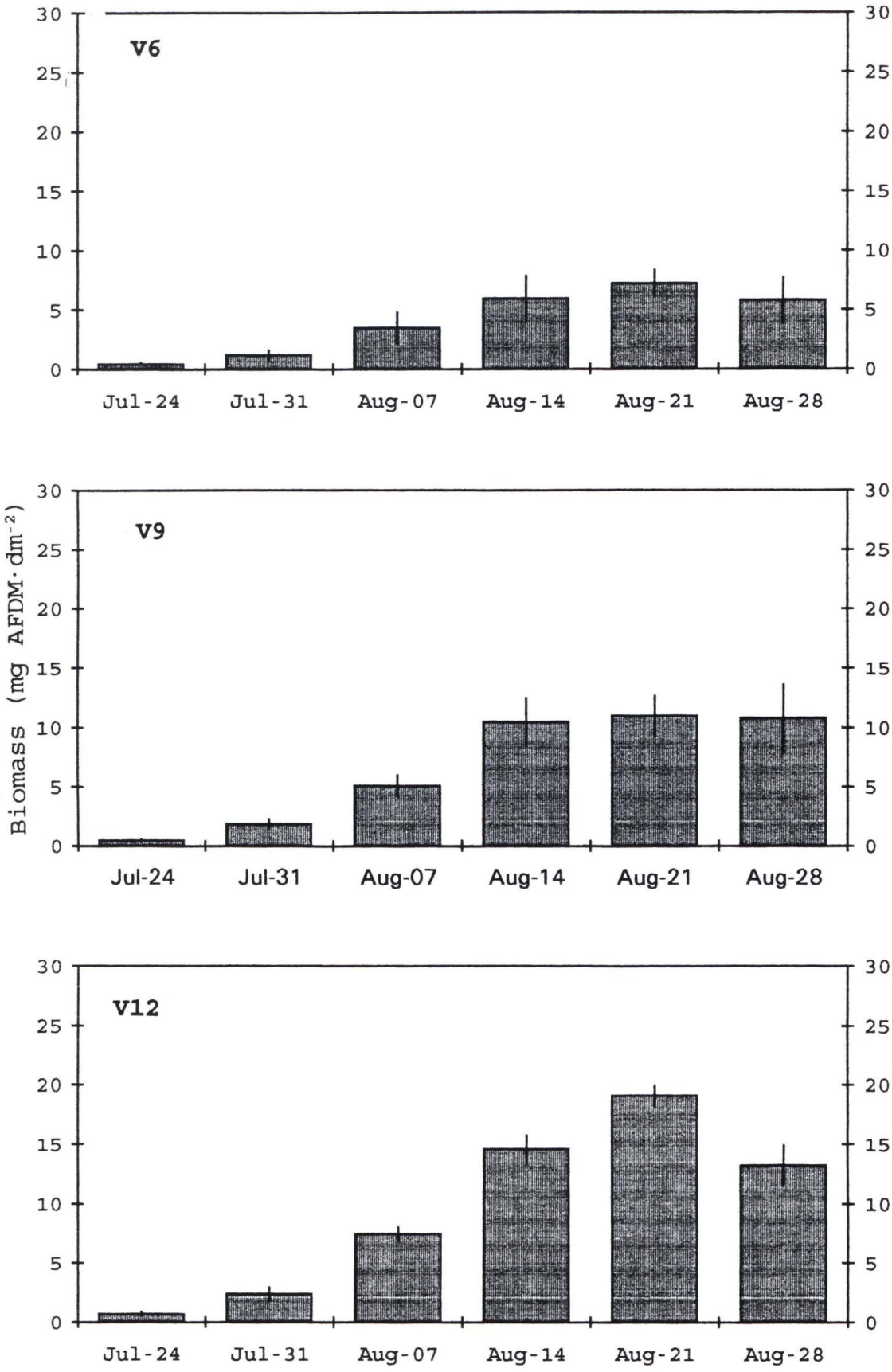
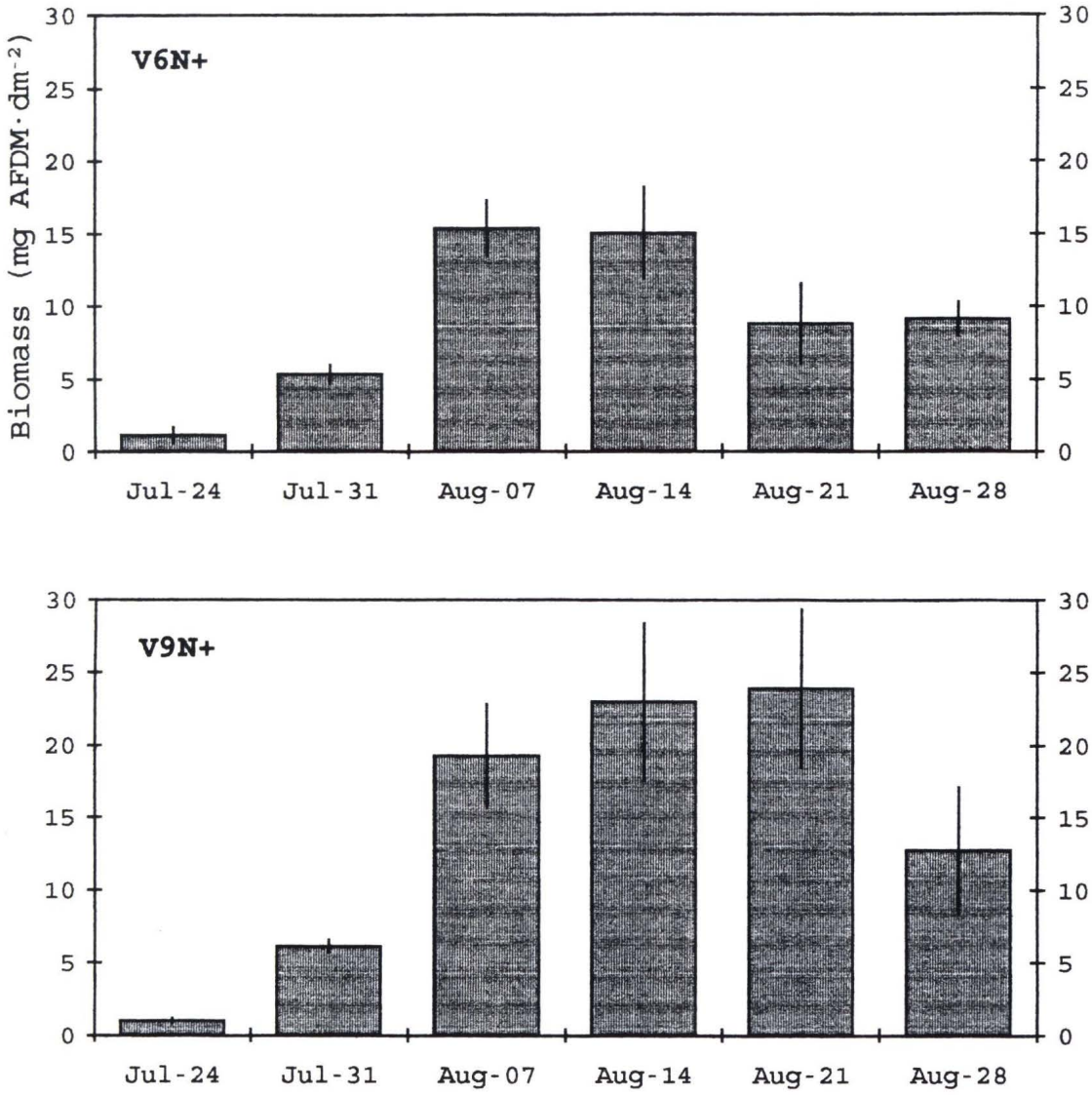

V6N+
V9N+
Biomass (mg chl a·dm⁻²)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Jul-24
Jul-31
Aug-07
Aug-14
Aug-21
Aug-28

Figure 6. Periphyton ash free dry mass (AFDM) (mean $\pm$ SD, n=4) at velocities of 6, 9 and 12 cm·sec^{-1} (V6, V9 and V12) and at velocities of 6 and 9 cm·sec^{-1} with nutrients added (V6N+ and V9N+) to approximate the nutrient flux (µg N and P·sec^{-1}) occurring at the 12 cm·sec^{-1} velocity.


V6
V9
V12
Biomass (mg AFDM·dm⁻²)
0
5
10
15
20
25
30
Jul-24
Jul-31
Aug-07
Aug-14
Aug-21
Aug-28


V6N+
V9N+
Biomass (mg AFDM·dm⁻²)
0
5
10
15
20
25
30
Jul-24
Jul-31
Aug-07
Aug-14
Aug-21
Aug-28

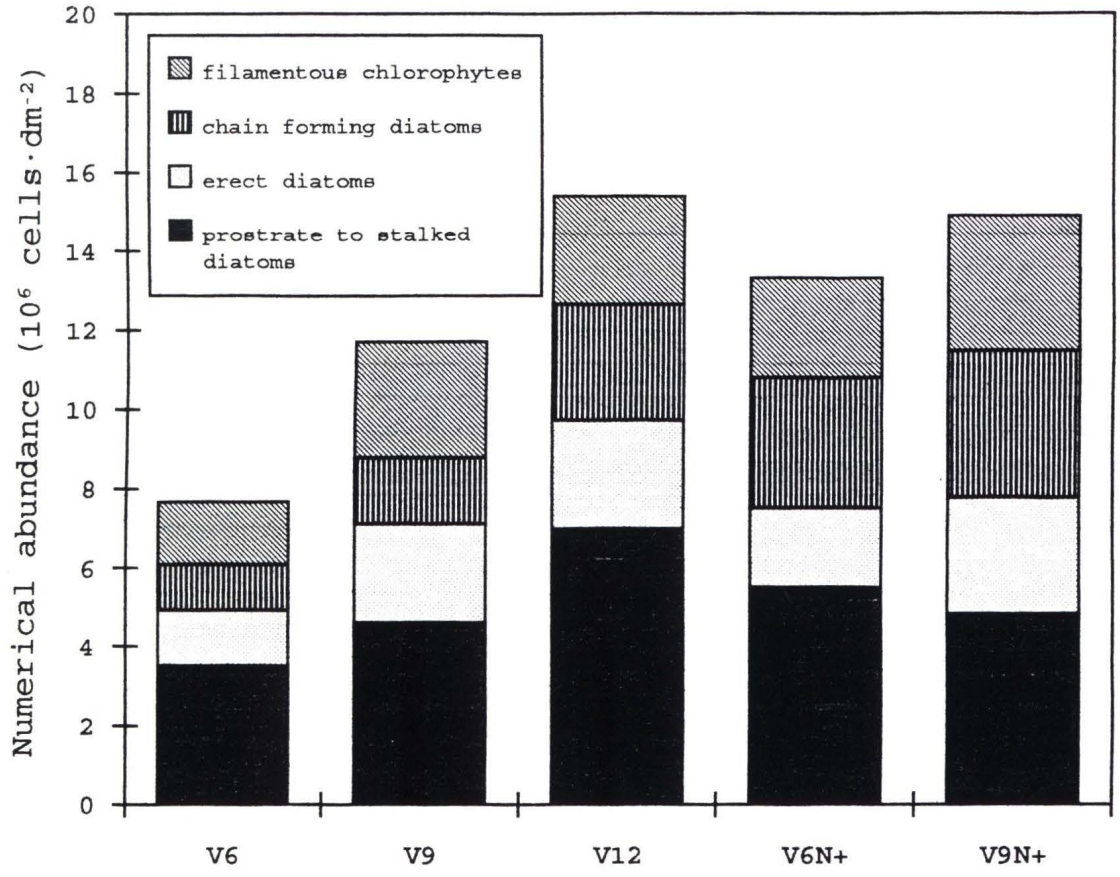


Figure 7. Numerical abundance (10^6 cells·dm^{-2}, n=2) of algal guilds. Periphyton communities were 45 days old and were developed at velocities of 6, 9 and 12 cm·sec^{-1} (V6, V9 and V12) and at velocities of 6 and 9 cm·sec^{-1} with nutrients added (V6N+ and V9N+) to approximate the nutrient flux (μg N and P·sec^{-1}) occurring at the 12 cm·sec^{-1} velocity.

Figure 8. Percent biovolume (n=2) of dominant algal taxa comprising the periphyton after 42 days. Periphyton communities were developed under velocities of 6, 9 and 12 $cm \cdot sec^{-1}$ (V6, V9 and V12) and at velocities of 6 and 9 $cm \cdot sec^{-1}$ with nutrients added (V6N+ and V9N+) to approximate the nutrient flux (μg N and $P \cdot sec^{-1}$) occurring at the 12 $cm \cdot sec^{-1}$ velocity.

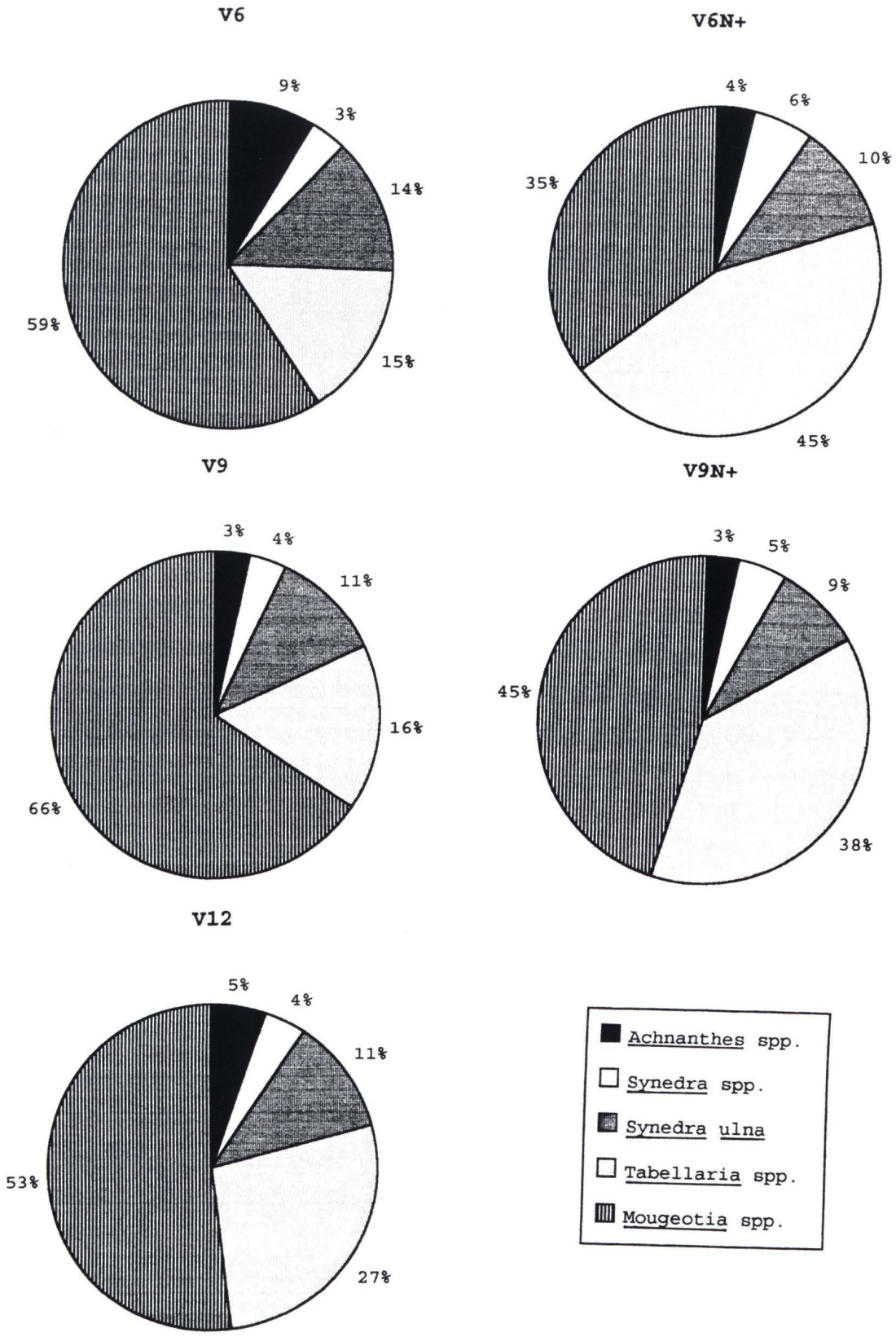

V6
9%
3%
14%
59%
15%
V6N+
4%
6%
10%
35%
45%
V9
3%
4%
11%
16%
66%
V9N+
3%
5%
9%
45%
38%
V12
5%
4%
11%
53%
27%
Achnanthes spp.
Synedra spp.
Synedra ulna
Tabellaria spp.
Mougeotia spp.

Figure 9. Photographs of periphyton communities in experimental stream-troughs showing visibly different biomass and taxonomic composition after 28 days. Lower biomass and brown diatom dominant periphyton at velocities of 6, 9 and 12 $cm \cdot sec^{-1}$ (V6, V9 and V12) and higher biomass and long, green filamentous community forming floating surface mats at velocities of 6 and 9 $cm \cdot sec^{-1}$ with nutrients added (V6N+ and V9N+) to approximate the nutrient flux (μg N and $P \cdot sec^{-1}$) occurring at the 12 $cm \cdot sec^{-1}$ velocity.

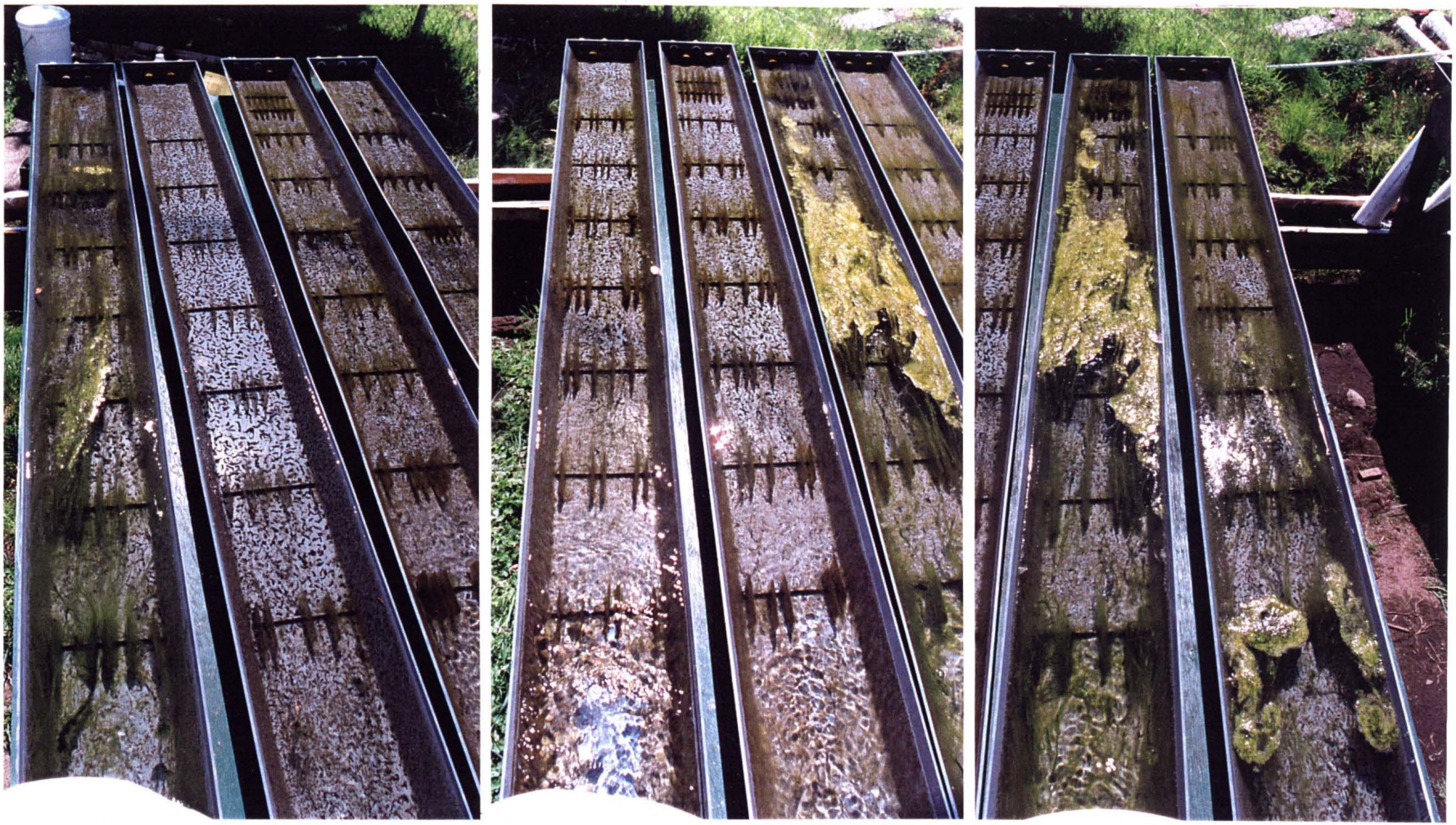
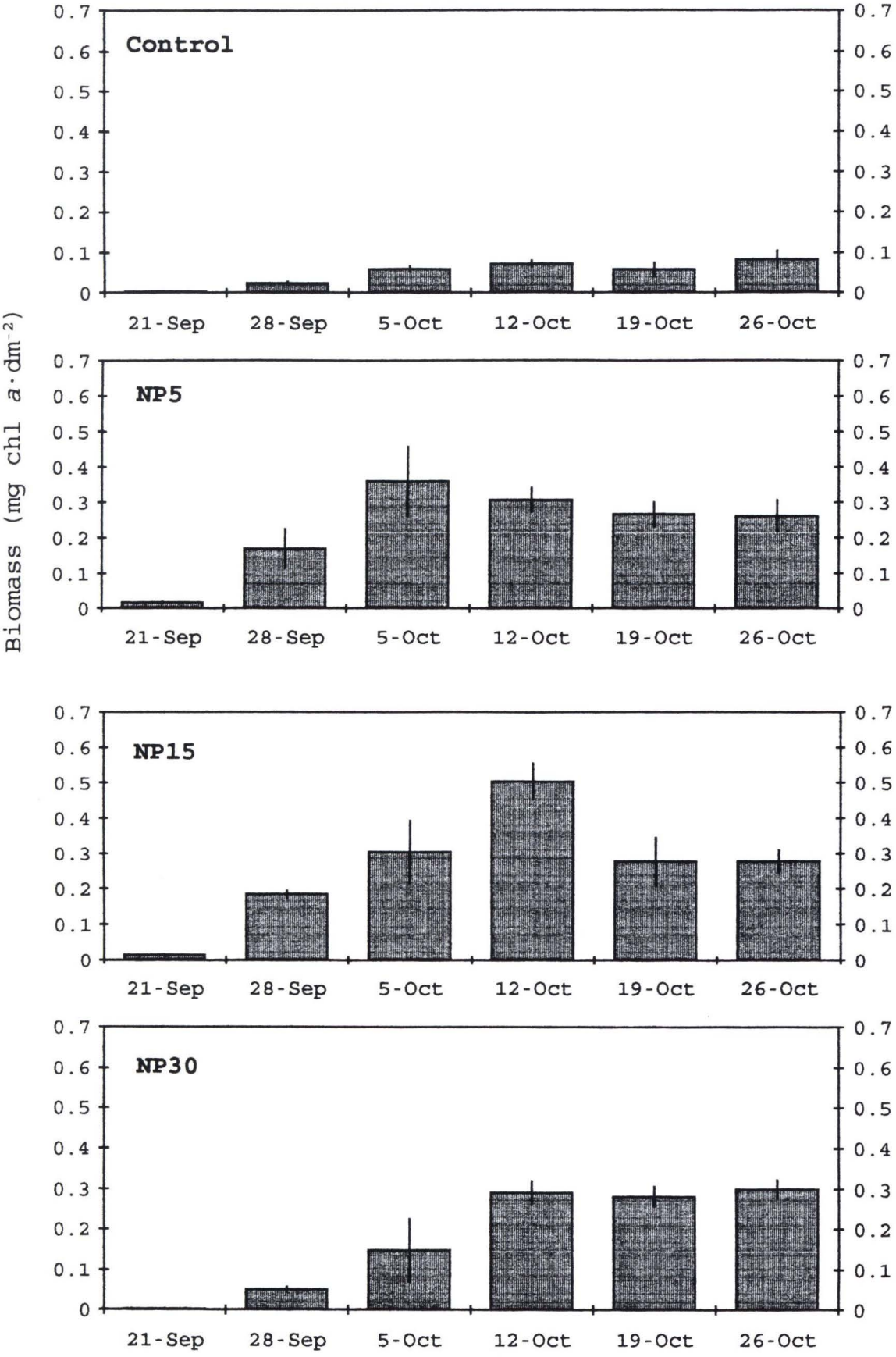

V6 N+
V6
V12
V9
V9 N+
V6 N+

Figure 10. Periphyton chlorophyll *a* biomass (mean $\pm$ SD, n=3) at NO_3-N:PO_4-P ratios manipulated to 5, 15, 30 and 45 (NP5, NP15, NP30 and NP45) or unmanipulated (Control). Treatments Controlx2, NP15x2 and NP30x2 had NO_3-N and PO_4-P concentrations twice that of the Control and NP15 and NP30 treatments, respectively.


Control
NP5
NP15
NP30
Biomass (mg chl a·dm⁻²)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
21-Sep
28-Sep
5-Oct
12-Oct
19-Oct
26-Oct

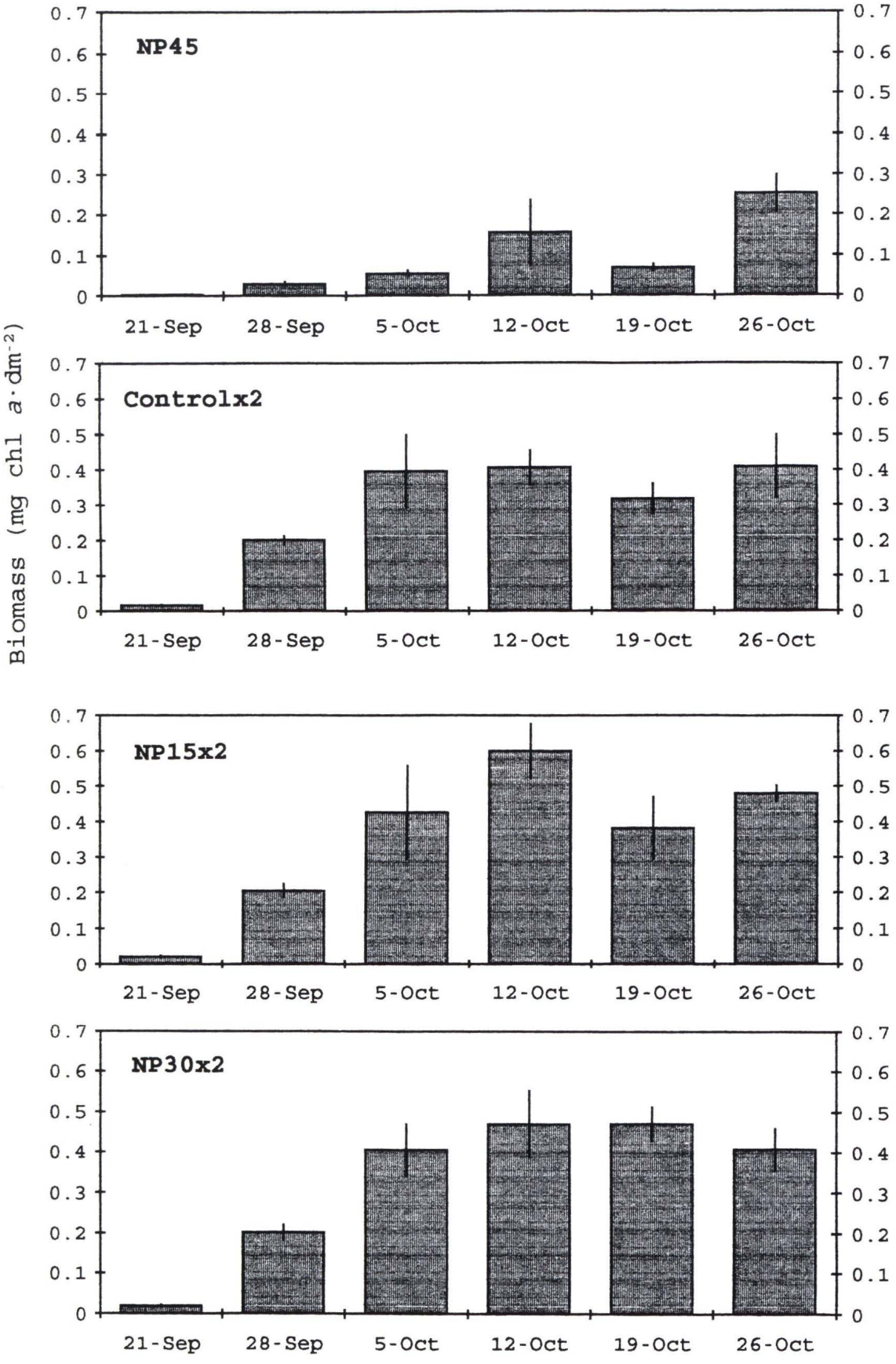
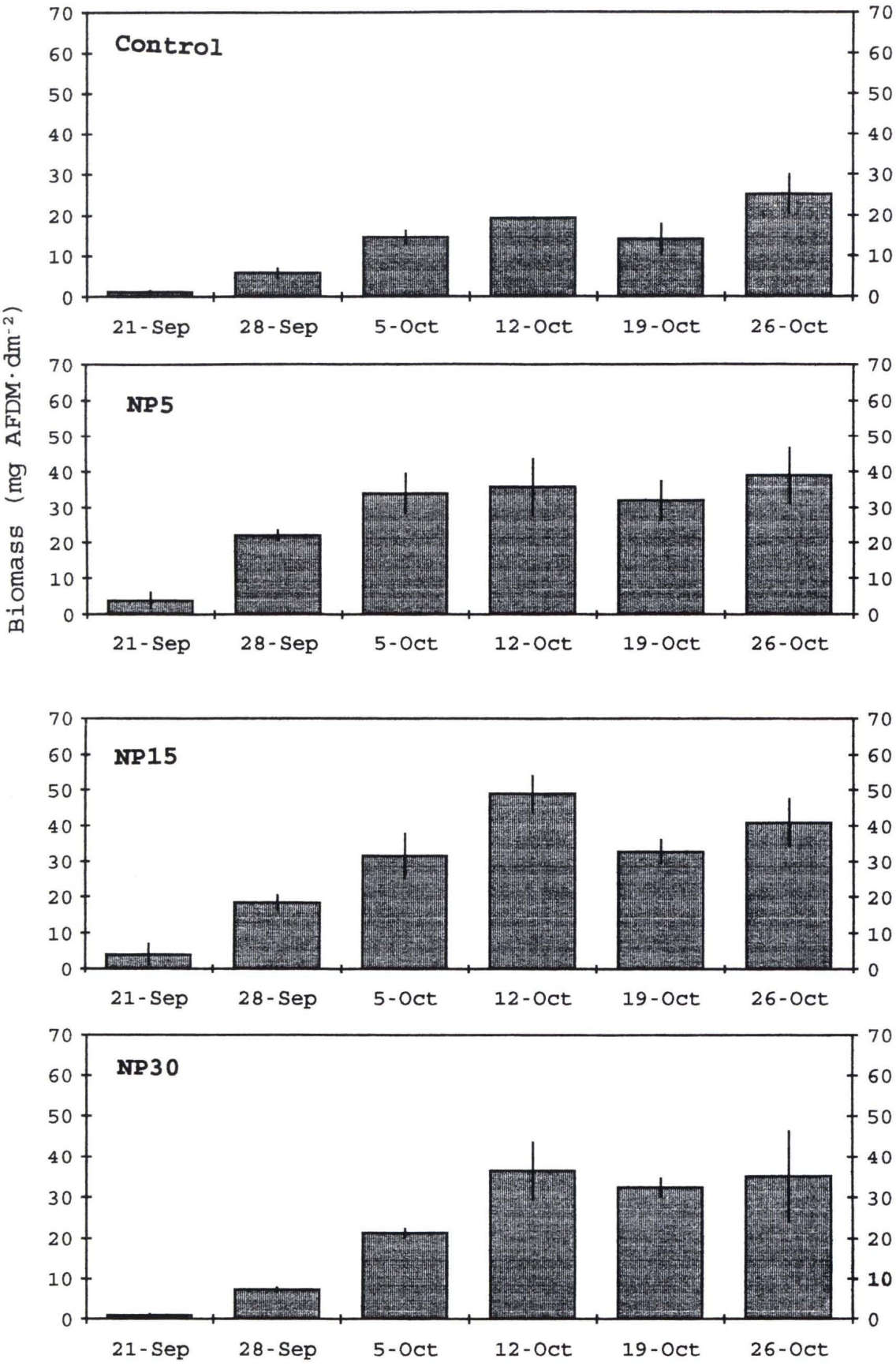
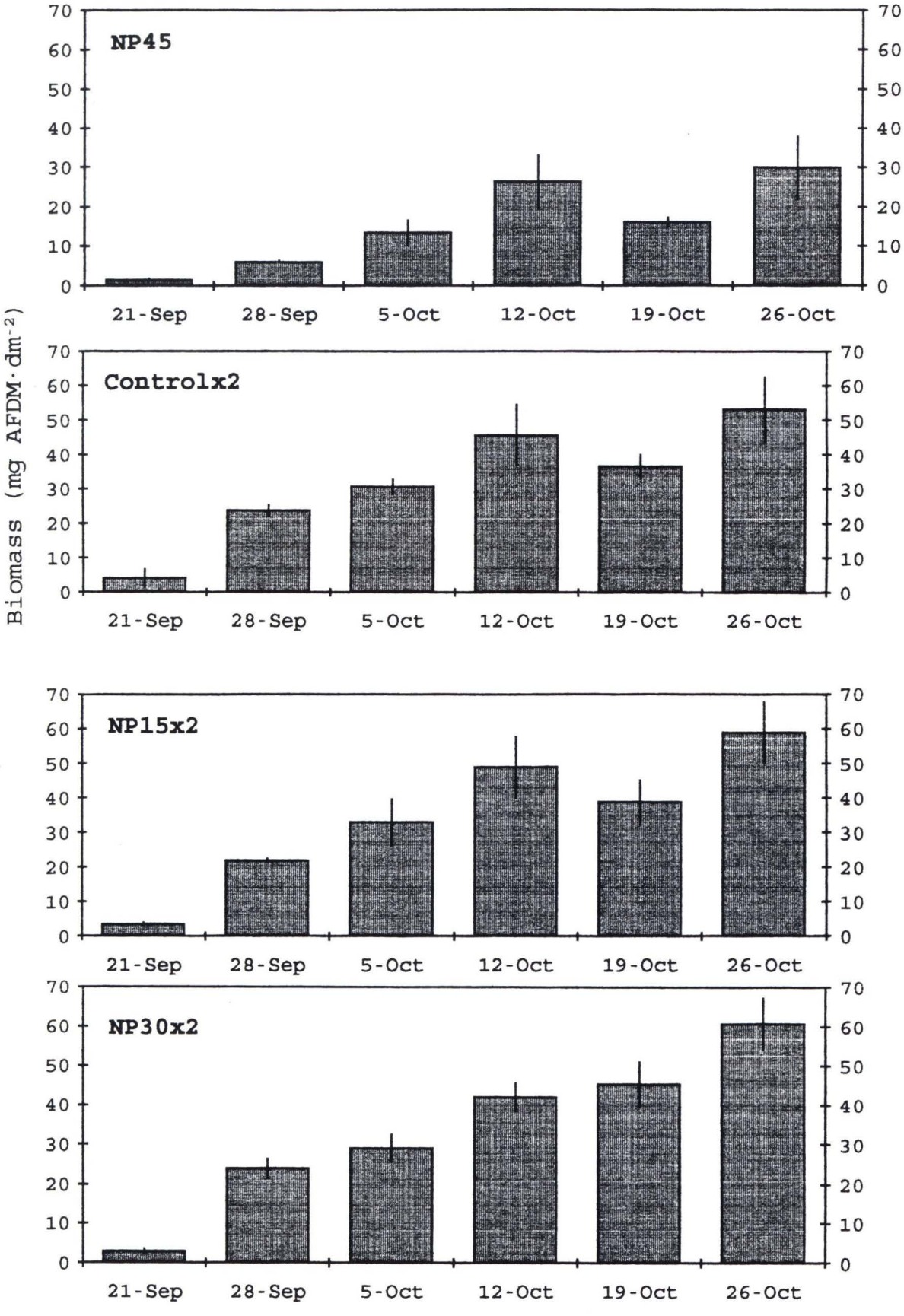

NP45
Controlx2
NP15x2
NP30x2
Biomass (mg chl a·dm⁻²)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
21-Sep
28-Sep
5-Oct
12-Oct
19-Oct
26-Oct

Figure 11. Periphyton ash free dry mass (AFDM) (mean $\pm$ SD, n=3) at NO_3-N:PO_4-P ratios manipulated to 5, 15, 30 and 45 (NP5, NP15, NP30 and NP45) or unmanipulated (Control). Treatments Controlx2, NP15x2 and NP30x2 had NO_3-N and PO_4-P concentrations twice that of the Control and NP15 and NP30 treatments, respectively.


Control
NP5
NP15
NP30
Biomass (mg AFDM·dm⁻²)
0
10
20
30
40
50
60
70
21-Sep
28-Sep
5-Oct
12-Oct
19-Oct
26-Oct


NP45
Controlx2
NP15x2
NP30x2
Biomass (mg AFDM·dm⁻²)
0
10
20
30
40
50
60
70
21-Sep
28-Sep
5-Oct
12-Oct
19-Oct
26-Oct

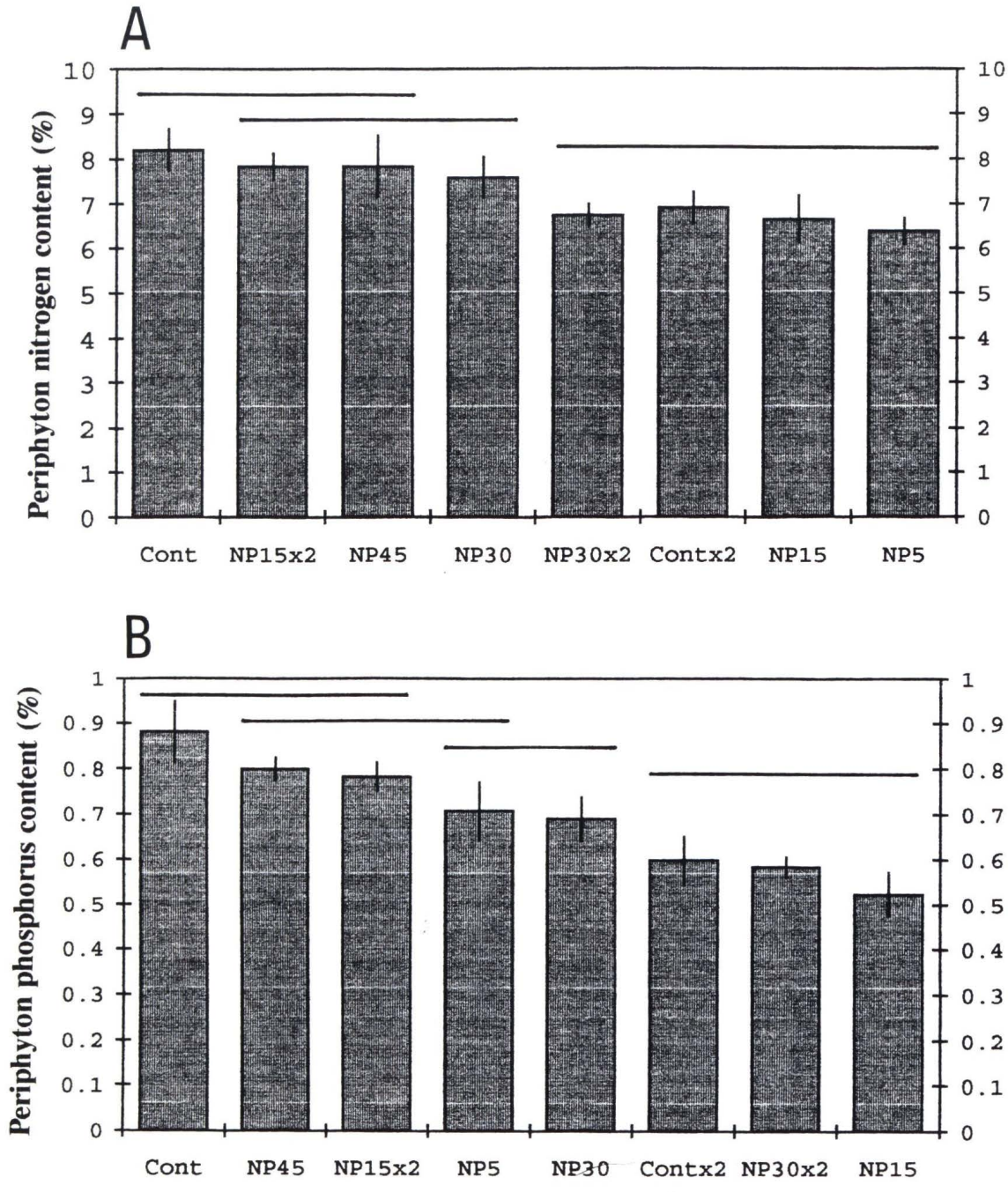


Figure 12. Periphyton nitrogen (A) and phosphorus (B) content (mean $\pm$ SD, n=3) after 42 days at NO_3-N:PO_4-P ratios manipulated to 5, 15, 30 and 45 (NP5, NP15, NP30 and NP45) or unmanipulated (control). Treatments controlx2, NP15x2 and NP30x2 had NO_3-N and PO_4-P concentrations twice that of the control and NP15 and NP30 treatments, respectively. Horizontal bars link treatments that were not significantly different ($P > 0.05$).

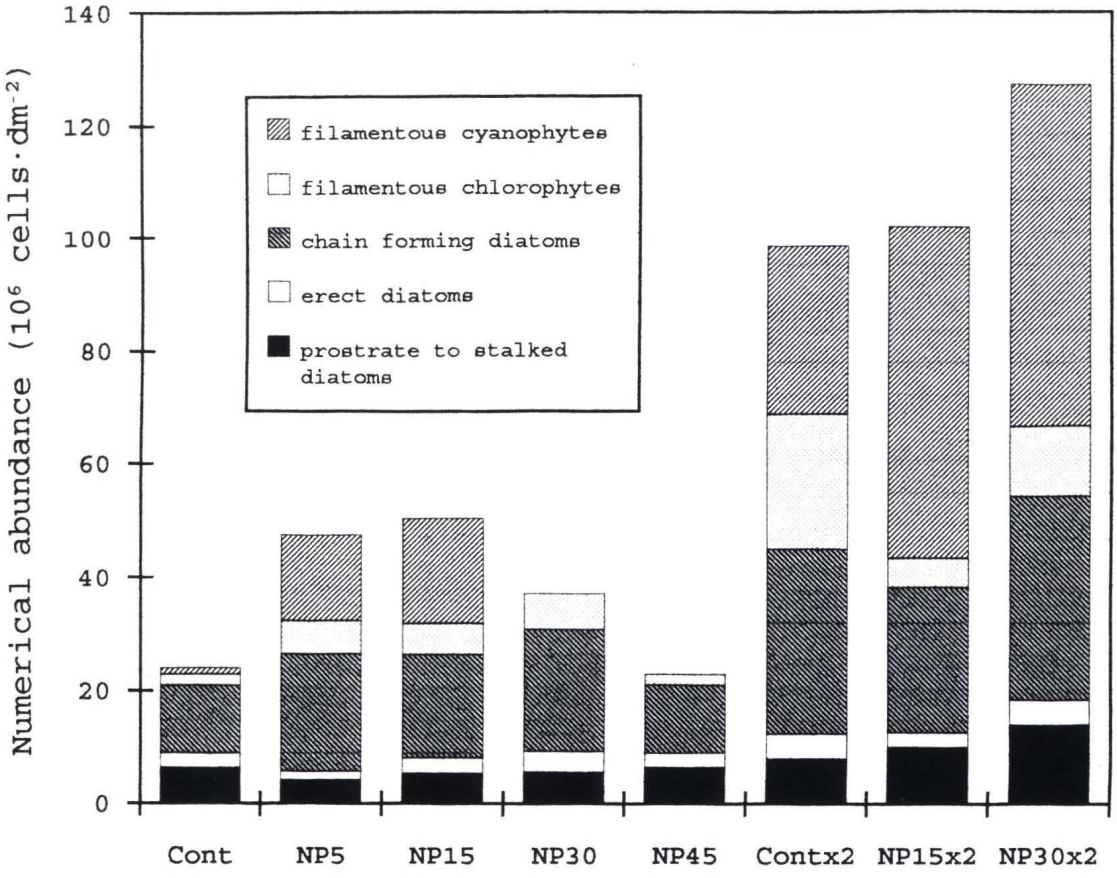
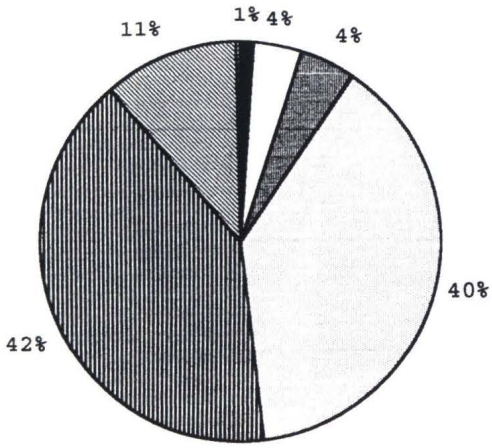


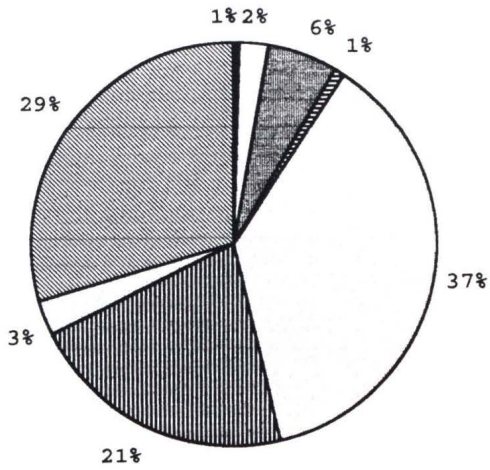
Figure 13. Numerical abundance (10^6 cells·dm^{-2}, n=2) of algal guilds. Periphyton communities were 45 days old and were developed at NO_3-N:PO_4-P ratios manipulated to 5, 15, 30 and 45 (NP5, NP15, NP30 and NP45) or unmanipulated (control). Treatments controlx2, NP15x2 and NP30x2 had NO_3-N and PO_4-P concentrations twice that of the control and NP15 and NP30 treatments, respectively.

Figure 14. Percent biovolume (n=2) of dominant algal taxa at day 42. Periphyton communities were developed at NO_3-N:PO_4-P ratios manipulated to 5, 15, 30 and 45 (NP5, NP15, NP30 and NP45) or unmanipulated (Control). Treatments Controlx2, NP15x2 and NP30x2 had NO_3-N and PO_4-P concentrations twice that of the Control and NP15 and NP30 treatments, respectively.

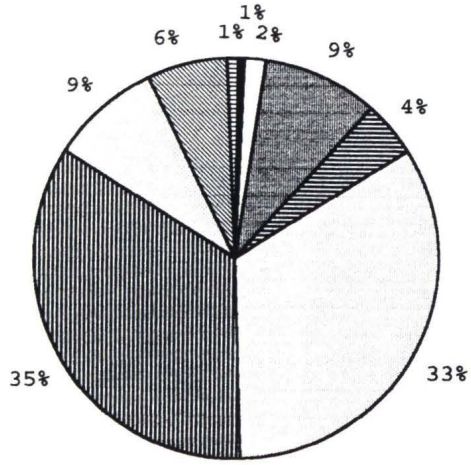
Control



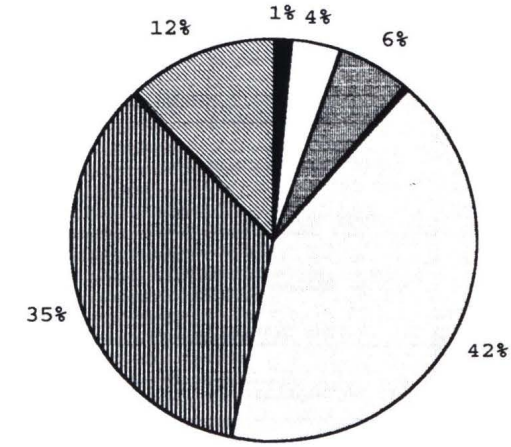
NP30



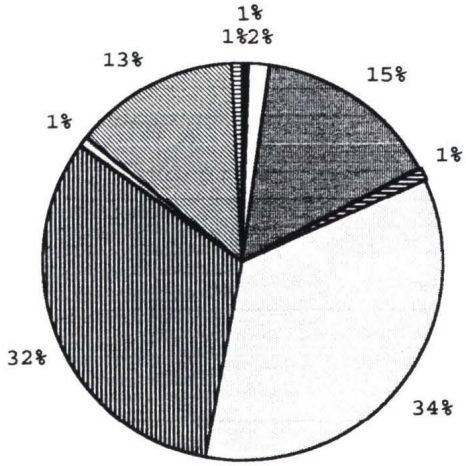
NP5

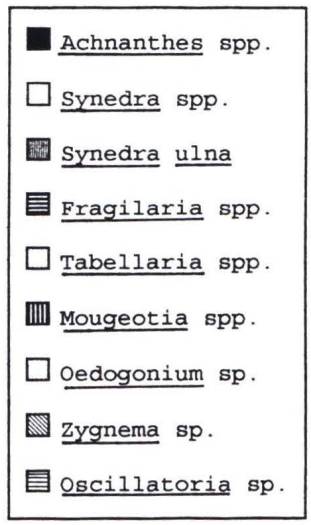


NP45

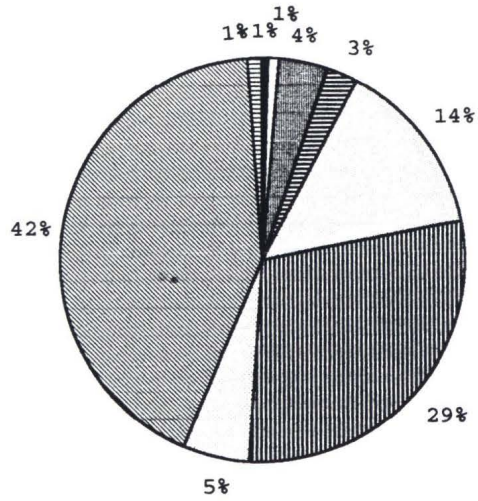


NP15

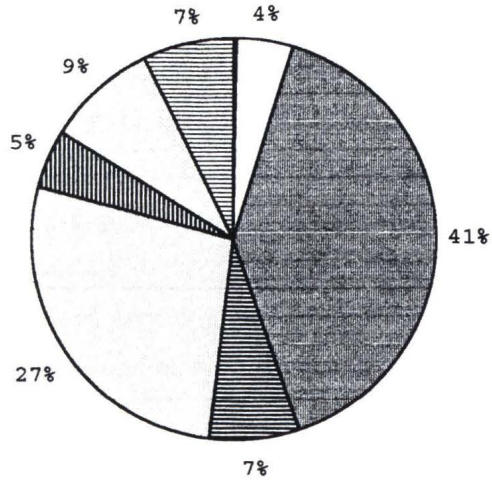




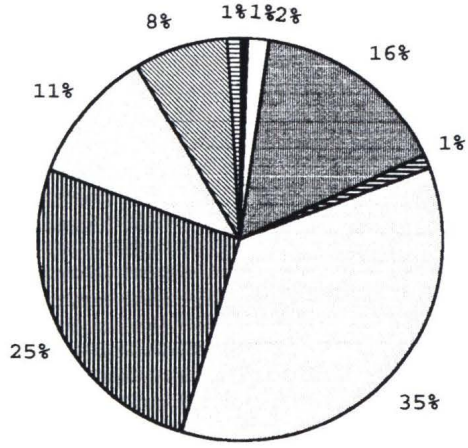
NP30x2



NP15x2



Controlx2



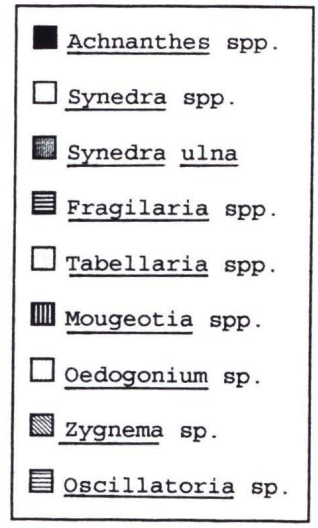


Figure 15. Photographs of experimental streams showing periphyton communities after 28 days at NO_3-N:PO_4-P ratios manipulated to 5, 15, 30 and 45 (NP5, NP15, NP30 and NP45) or unmanipulated (Control). Treatments Controlx2, NP15x2 and NP30x2 had NO_3-N and PO_4-P concentrations twice that of the Control and NP15 and NP30 treatments, respectively. Note relatively low biomass and brown diatomaceous community at NP45 and Control stream-troughs and heavy dark green filamentous chlorophyte community at NP15 and NP15x2.

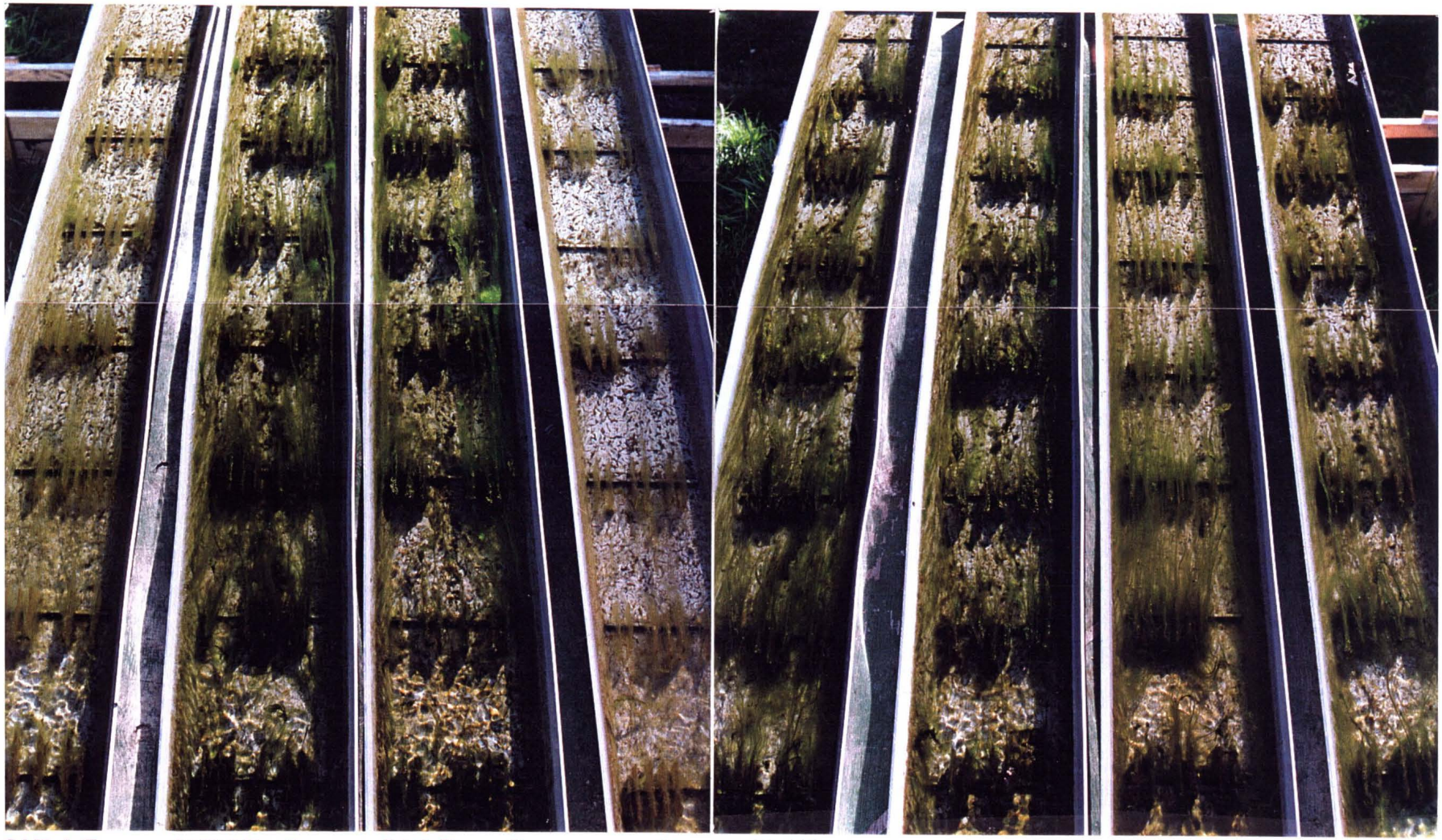
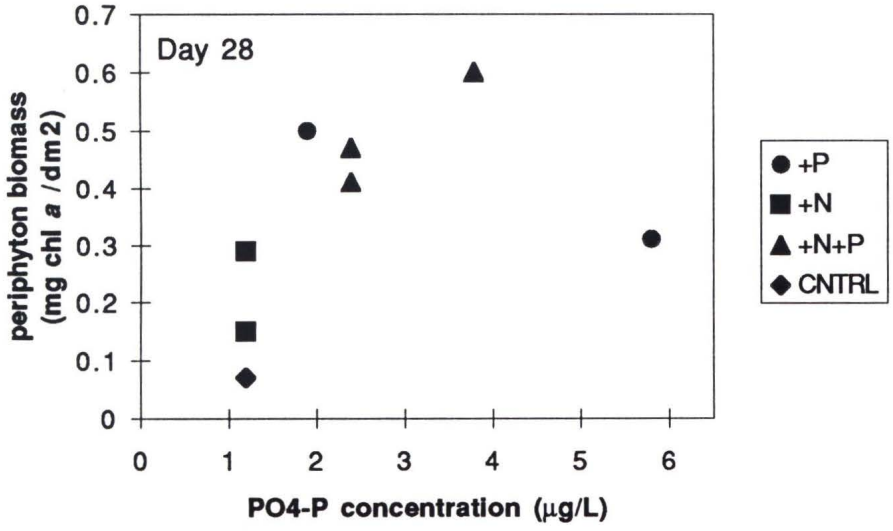
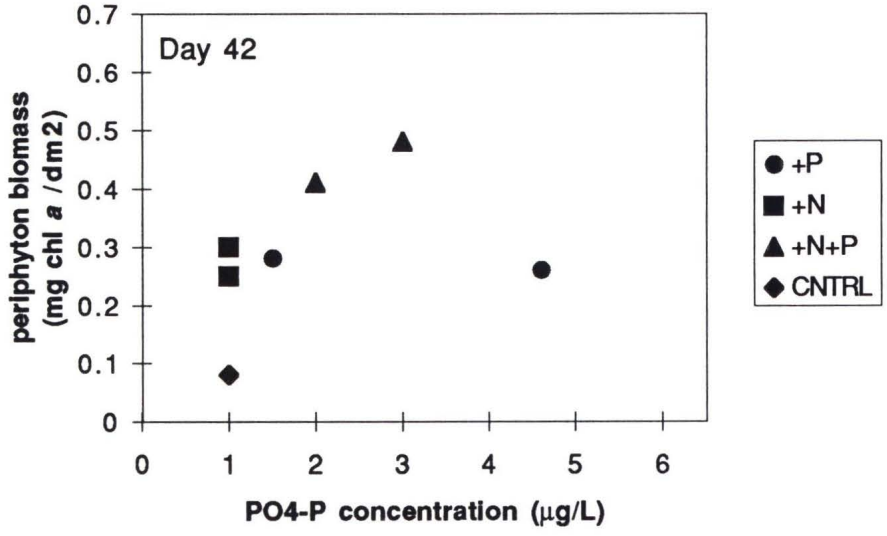

NP45
NP15x2
NP30x2
C
NP15
Cx2
NP30
NP5

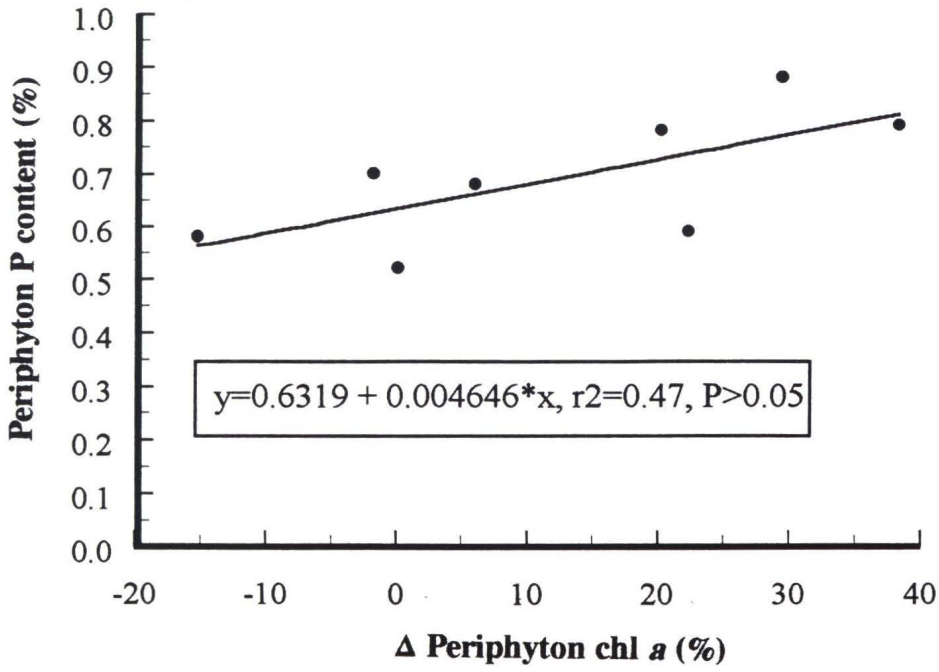
Figure 16. Relationship between orthophosphate concentration (μg $P \cdot L^{-1}$) and periphyton biomass (mg chl $a \cdot mg^{-2}$) for periphyton at (A) day 28 and (B) day 42. Treatments received added phosphorus (+P), added nitrogen (+N) and added nitrogen and phosphorus (+N+P); Cntrl =Control.

A


Day 28
periphyton biomass (mg chl a /dm2)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
PO4-P concentration (µg/L)
+P
+N
+N+P
CNTRL

B


Day 42
periphyton biomass (mg chl a /dm2)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
PO4-P concentration (µg/L)
+P
+N
+N+P
CNTRL



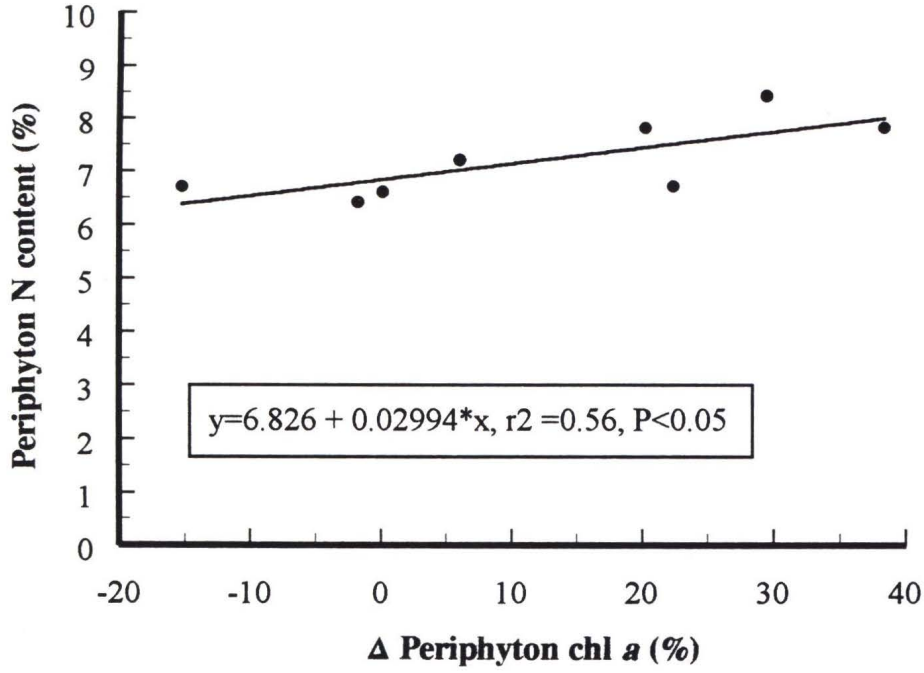


Figure 17. Correlation between the relative change in periphyton chlorophyll *a* biomass (%) from day 35-42 with periphyton nitrogen and phosphorus content.

Chapter II. Nutrient- and light-limited periphyton development in shaded and unshaded sections of a coastal watershed stream

Introduction

Many factors can limit the productivity and biomass of primary producers in headwater streams. Among these constraints are herbivory and reduced nutrients and light intensity (Rosemond, 1993). Fewer studies have examined the importance of light limitation of periphyton than nutrient limitation. Typically limiting factors have been examined separately although the possibility of multiple levels of control of periphyton is receiving increasing attention (Rosemond, 1993).

In heavily shaded streams, most of the chemical energy entering the system is of allochthonous origin (material produced and transported from outside the stream) (Lowe *et al.*, 1986). Fisher and Likens (1973) found that less than 1% of the biologically fixed energy was of autochthonous origin (material produced within the stream) in the stream of the Hubbard Brook Experimental Forest. However, when forest canopy development over streams is negligible, as a result of logging or stream geomorphology, primary production may provide a substantial contribution to community metabolism (Minshall, 1978). Shade from forest (riparian) canopy in headwater streams can reduce light intensity in the streambed to less than 5% of full sunlight (Hill and Harvey, 1990). Shading by riparian vegetation also depletes solar radiation in the blue and red wavelengths, relative to green and far-red (Raven, 1992), which may favor algal taxa with the accessory pigmentation capable of using these wavelengths.

Despite the integral role of riparian shade in the river continuum concept (Vannote *et al.*, 1980), few studies have investigated light as a factor limiting lotic primary production (Hill and Harvey, 1990). The majority of the studies which have examined light limitation were based on temporal or spatial comparisons, rather than direct manipulation of light levels within streams. Most of the former studies examined periphyton biomass development and species composition (1) before and after forest harvesting or canopy defoliation (Shortreed and Stockner, 1983; Noel *et al.*, 1986; Sheath *et al.*, 1986; Ulrich *et al.*, 1993) or (2) in forested and logged streams (open and closed canopy) (Hansman and Phinney, 1973; Lowe *et al.*, 1986; Hill and Knight, 1988; Hill and Harvey, 1990; Winterbourn, 1990). Gregory (1980) and Steinman (1992), however,

measured periphyton biomass and productivity in shaded streams following artificially increased light levels. Triska *et al.* (1983) and Hepinstall and Fuller (1994) artificially shaded either experimental channels and/or stream samplers in open canopy (both studies) and closed canopy (latter study only) stream sections.

Nutrient limitation of stream periphyton has received considerable attention, with most studies having focused on streams with little riparian forest canopy development. Investigations into nutrient limitation in streams has been advanced through the use of specific nutrient manipulation of natural (Elwood *et al.*, 1991; Perrin *et al.*, 1987) and artificial streams (Stockner and Shortreed, 1978; Bothwell, 1985) and through the development of nutrient-diffusing artificial substrata (Fairchild and Lowe, 1984; Pringle and Bowers, 1984). Chessman *et al.* (1992) noted the general biogeographical patterns in nutrient limitation that have begun to emerge, particularly for North America. Phosphorus limitation in streams appears to be common in the northern and eastern United States (Elwood *et al.*, 1981; Pringle, 1987; McCormick and Stevenson, 1989; Hart and Robinson, 1990), whereas nitrogen limitation has been documented in the central and western U.S. (Manuel and Minshall, 1980; Grimm and Fisher, 1986; Hill and Knight, 1988; Lohman *et al.*, 1991). In Canada, phosphorus limitation has been documented in both coastal (Stockner and Shortreed, 1978) and interior British Columbia (Bothwell, 1985). Only rarely have other resources been documented as limiting (Raven, 1992) including; inorganic carbon (Peterson *et al.*, 1983) and trace elements (Wurhmann and Eichenberger, 1975; Pringle *et al.*, 1986).

The importance of multiple limiting factors has received increasing attention, and their interaction in controlling periphyton structure may be more important than their singular effects (Rosemond, 1993). Multiple limiting factors controlling periphyton have been documented in streams where several resources are low in availability. For example Lowe *et al.* (1986) and Hill and Knight (1988) found that nutrient addition resulted in a greater increase in algal biomass in sunlit than in shaded streams, suggesting multiple limitation by nutrients and light. Similarly, abiotic parameters (e.g. light) and biotic interactions (herbivory) can limit periphyton biomass (Rosemond, 1993). This was shown by Feminella *et al.* (1989) and Steinman (1992) who found that algal biomass was stimulated under high light conditions only when grazing was suppressed. Rosemond (1993) determined that algal biomass from a small forested stream was limited by a combination of nutrients, irradiance and herbivores.

An understanding of factors which limit periphyton growth is important for successful rehabilitation of stream ecosystems that have been dramatically altered through human activities (e.g. logging, road building). Such perturbation may make previously limiting resources available in greater quantities. Current logging practices in B.C., which involve harvesting without adequate buffer strips being left along streams, can lead to significant increases in incident light reaching the streams. This frequently results in several fold increases in algal biomass, together with changes in the algal community structure from an adnate and erect diatom community to a filamentous chlorophyte dominated community (Hansman and Phinney, 1973; Shortreed and Stockner, 1983; Lowe *et al.*, 1986). The causes of this shift in algal community structure are difficult to identify, however, because of the many parameters which co-vary with increased light following clearcutting, such as changes in water chemistry (Scrivener, 1982; Murphy and Hall, 1981; Likens, 1984), temperature (Hansman and Phinney, 1973; Sheath *et al.*, 1986), flow patterns (Borman *et al.*, 1974; Troendle and King, 1985) and introduction of sediment and organic material (Borman *et al.*, 1974; Hartman and Scrivener, 1990). It is important to know how these parameters may effect periphyton because the dramatic increase in periphyton biomass, frequently observed following logging, can lead to degradation of fish habitat and water quality and a loss of aesthetic quality.

The general objective of the experiment reported here was to determine whether stream periphyton structure was limited by single or multiple abiotic resources under high and low light conditions. Specifically, two hypotheses were tested to determine whether light was the single limiting factor in shaded forested streams. The first hypothesis tested whether periphyton was limited only by light, then manipulation of secondary factors (e.g. nutrients) would not be expected to affect biomass and taxonomic composition. The second hypothesis tested whether under high light conditions, periphyton biomass would be nutrient limited and increased nutrient supply would increase periphyton biomass by altering the taxonomic composition of the community.

Although the methods and experimental design of this research was similar to others (Lowe *et al.*, 1986; Hill and Knight, 1988; Chessman, 1992; Hepinstall and Fuller, 1994), it was unique in several aspects. (1) There has been little research examining nutrient limitation of periphyton in coastal streams. Hill and Knight (1988), using nutrient-diffusing clay pots, examined nutrient limitation in two northern California

streams and Stockner and Shortreed (1978) examined nutrient enhancement of periphyton in wooden troughs in Carnation Creek, Vancouver Island. It is important to replicate similar studies under conditions of differing climatic and biogeochemical conditions for not only an understanding of ecological processes but also for devising management solutions to problems of nuisance biomass blooms. (2) Most of the studies examining light limitation of stream periphyton have compared biomass and taxonomic composition of periphyton from logged and unlogged streams (Lowe *et al.*, 1986; Hill and Knight, 1988; Chessman 1992) with potentially different stream water quality. The study reported here examined different sites along one stream (Rithet Creek) with a mosaic of forest types. This also permitted assessment of light limitation at two different high light and low light sites. (3) The nitrogen and phosphorus content of periphyton is a useful measure of both the nutrient status (Biggs, 1994) and potential nutritional value to higher trophic levels (Steinman *et al.*, 1988; Hambrook and Sheath, 1987). Analysis of nitrogen and phosphorus content of periphyton developed on nutrient-diffusing substrata has only been poorly studied. The present study therefore analyzed biomass, taxonomic composition and nitrogen and phosphorus content of periphyton grown on nutrient-diffusing substrata.

Methods and Materials

Site description

Study area

The study was conducted in Rithet Creek, a second order stream located in the Greater Victoria Water District (GVWD) watershed (Figure 2). The stream is the principal water source discharging into Sooke Reservoir. Rithet Creek drains a mountainous area (maximum elevation is approximately 550 m) of a mixed-species evergreen forest dominated by Douglas fir (*Pseudotsuga menziesii* Mirb), Western hemlock (*Tsuga heterophylla* Raf Sarg), red cedar (*Thuja plicata* Donn) with sub-dominant deciduous red alder (*Alnus rubrus* Bong) and bigleaf maple (*Acer macrophyllum* Purch). Large sections adjacent to Rithet Creek were harvested in the mid-1960's following severe windstorm-induced blowdown (Irwin, 1993 personal communication). More recent harvesting along Rithet Creek occurred in 1987, with replanting in 1988. This has lead to a forest mosaic along Rithet Creek, with age class stands ranging from cutover (<5 years) to old growth (>161 years) (Figure 18).

Sampling locations

Four sites along Rithet Creek were selected such that two sites were heavily shaded and two sites received full sunlight. Site 1 (Figure 18) was heavily shaded with 24-48 year old second growth conifers (crown closure 56-65%) (Table 4), and stream-side deciduous *A. rubrus*. Site 2, was heavily shaded, by a mature 141-250 year old Douglas fir (*P. menziesii*) community with a crown closure of 56-65 % (Table 4). Sites 4a and 4b (Figure 18) were located above and below, respectively, a previously established water sampling location (Site 4) (Grant *et al.*, 1993). At these sites, the harvested forest had been recently replanted with Douglas fir and Hemlock, yielding a canopy closure of 0-5% (Table 4). Site 4b received full sunlight for most of the day whereas Site 4a received full sunlight from noon on.

Experimental design

Nutrient-diffusing substrata, similar to those of Fairchild *et al.* (1985), were used to examine nutrient (nitrogen or phosphorus) and light limitation of periphyton biomass accrual in shaded and unshaded stream sections. Clay pots were filled with 2% agar solution made with (a) distilled water (Control), (b) 0.1 M phosphate-P and (c) 0.5 M nitrate-N and 0.1 M phosphate-P. The pots were deployed at four sites along Rithet Creek and were subsequently collected in triplicate, three times during the study, at two week intervals from all sites after an initial 14 days of colonization and growth.

Construction of nutrient-diffusing substrata

Nutrient-diffusing substrata were constructed from unglazed, clay flower pots (approximate shape a truncated cone: 10.0 cm height, 5.5 cm outside diameter at the base, 10.5 cm at the top, and an external surface area of 460 cm^2) (Figure 19). Agar solutions were prepared by dissolving agar (20 $g \cdot L^{-1}$) in hot distilled water (control) or nutrient treatment stock solutions (0.1 M phosphate-P (14.2 $g \cdot L^{-1}$ Na_2HPO_4), and 0.1 M phosphate-P and 0.5 M nitrate-N (42.4 $g \cdot L^{-1}$ $NaNO_3$)). The treatments were designated C (Control), P and N+P. The solutions were poured, while hot, into the clay pots which had the bottom aperture sealed with a small cork. A 9.0 cm diameter plastic petri plate was placed on top of the agar solution inside the clay pot, yielding an internal agar volume

of approximately 250 mL. The petri plates fitted tightly and did not require sealant. Once the agar had cooled the pots were individually bagged to minimize evaporation of the gelled agar. The petri plates were pre-drilled in the center to accommodate securing of the clay pot by wooden dowels (see below) to the anchoring frame for placement in the stream (Figure 19).

Field procedures

Nutrient-diffusing clay pots (108) were deployed equally at the four sites on Rithet Creek on 30 April 1992. The pots were anchored to untreated Douglas fir boards (1.83 m x 3.5 cm x 9 cm) (Figure 19). The boards held vertical, wooden dowelling (10 mm dia., 62 mm long) drilled and secured at 62 mm intervals along the board's central axis, upon which the pots were placed. The wooden frame was then anchored to the stream bed by placing rocks (approximately 30-60 cm dia) on two wooden planks (2 cm x 25 cm x 61 cm long) secured at right angles to the frame ends. Nine clay pots constituting one treatment were affixed to each board. Deployment of the treatments consisted of the control pots being furthest upstream, followed by the phosphorus enriched pots, and the nitrogen and phosphorus enriched pots being furthest downstream. This arrangement ensured that an upstream clay pot would not leach nutrients to potentially affect downstream treatments that were not enriched with that nutrient.

Periphyton sampling

Clay pot substrata were recovered randomly, in triplicate, from each site at 2, 4, and 6-week intervals. The clay pots were removed from the stream by enclosing the submerged clay pot in a plastic bag. By approaching the pot from downstream, it was possible to enclose the trailing (up to 0.5 m) filamentous chlorophytes. Progressing upstream while sampling also minimized possible sampling biases of collecting periphyton sloughed from the stream rocks through disturbance of the streambed and entrapped on the clay pots. The bags were then sealed, placed in individual small plastic buckets, and held in coolers for transport back to the lab.

Natural periphyton was sampled from stream rock at duplicate sites, 2 m above and below each sampling site. Two to four stream rocks, from each sample site, were

placed into 250-mL sample jars, filled with stream water, and transported to the lab, on ice, in the dark.

Water depth and velocity were measured adjacent to the substrata during placement and retrieval of the pots. Velocity was estimated by timing (in triplicate) the travel of a 1-cm diameter cork over a known distance (Peterson and Stevenson, 1990) above the middle of the substrata frame for each treatment. Water temperature and dissolved oxygen concentration (YSI model 57 Oxygen / Temperature meter, Yellow Springs Instrument Company, Yellow Springs, Ohio) and irradiance measurements (Biospherical Instruments Inc., Model QSL-100, San Diego) were taken at each site during both substrata placement and retrieval. Irradiance levels were measured in Quanta·sec^{-1}·cm^{-2} and were subsequently converted to mE·sec^{-1}·m^{-2} with the following equation (Lee, 1992)

$$1 \text{ mE·sec}^{-1}\text{·m}^{-2} = 6.02 \text{ X } 10^{17} \text{ Quanta·sec}^{-1}\text{·m}^{-2} \qquad (6)$$

Similarly, water samples were collected for analysis of ortho-phosphorus (PO_4-P) and nitrate (NO_3-N) in 2 L polyethylene bottles and analyzed by MB Research (Sidney, B.C.). Due to the expense of nutrient analyses water samples were collected at Site 4 for the two adjacent sites (4a and 4b) (Table 4).

Laboratory procedures

Sample preparation

Periphyton was removed with a small brush from the outside of the clay pots into a plastic tray. The resulting periphyton slurry was made up to a fixed volume and blended in a kitchen blender (Osterizer) for approximately 30 seconds. Aliquots from the blended periphyton slurry were taken for species composition, biomass determination (chlorophyll *a* and AFDM) and periphyton N and P content. There was insufficient biomass for subsampling for all assays from all sites. Aliquots for species composition and chlorophyll *a* were obtained for all treatments and sites for all dates. AFDM subsamples were obtained from Sites 4b and 4a for all sampling dates and from Sites 1 and 2 only after 42 days of growth (except for the +N&P treatment which had sufficient biomass after 4

weeks). Subsamples for periphyton N and P analysis were obtained only from Sites 4b and 4a after 28 and 42 days of periphyton accrual.

Taxonomic composition determination and biomass measurements

Subsamples for species composition (50 mL) were preserved with 2.5 mL formalin in opaque, 55-mL plastic vials. Preliminary determination of the algal numerical abundance from one shaded (Site 1) and one unshaded (Site 4b) site were made on a Carl Zeis inverted microscope using Utermöhl sedimentation chambers (procedures described in detail in Chapter I). For the unshaded site, algal taxa were grouped according to physiognomic form (see Chapter I) (Lowe *et al.*, 1986), while the simpler community architecture at Site 1 permitted presentation of numerical abundance of separate algal taxa. Biovolume estimates for algal taxa from the unshaded site were determined to examine the contribution of individual taxa to community biomass (see Chapter I).

Subsamples for chlorophyll analysis (100-500 mL) were filtered onto glass fiber filters (Whatman GF/F) with approximately 5 mL of $MgCO_3$ (10 $g \cdot L^{-1}$) and stored frozen at -20 ^{o}C. Subsample volume varied to provide sufficient biomass for extraction of chlorophyll *a* to yield concentrations within a range of 0.1-1 absorbance units (with an extract volume of approximately 10 mL). This reduced the need for further dilution of the extract prior to spectrophotometric analysis. Subsamples for AFDM (100-250 mL) were filtered onto pre-weighed, ashless filter papers (Whatman ashless 41) and stored at -20 ^{o}C. Details of chlorophyll *a* and AFDM analysis have been previously described in Chapter I.

Periphyton N and P analysis

Periphyton not required for biomass determination was used for N and P analysis. Aliquots of 300 mL were allowed to settle, refrigerated and in the dark for 12 hours. The supernatant was decanted, and the sedimented biomass was placed into ziplock bags and frozen at -20 ^{o}C. Periphyton samples were freeze-dried and processed according to the procedures outlined in Chapter I. The triplicate samples for each treatment had to be pooled prior to analysis to provide sufficient mass for the analyses. There was sufficient biomass from only Sites 4b and 4a for these analyses.

Statistical analysis

Biomass data was not analyzed statistically due to the absence of true replication (*sensu* Hurlbert, 1984) required for most statistical analyses. Hurlbert (1984) argues that stream studies consist of single replicates and that sample sites within the stream are pseudoreplicates. Additionally, since treatments were not randomly assigned to sites within the stream, but rather were located in unshaded and shaded sections, the study was not controlled in an objective and exact manner. Given these deviations from the mathematical assumptions underlying the use of an inferential statistical assessment, Hurlbert (1984) recommends that the data be plotted with the mean values and the variability of the data be presented.

Results

The forest canopy, crown closure (56 - 65%) at Site 1 and 2 (Table 4), resulted in variable light intensities. The maximum and minimum values were measured in sunlight (through unshaded sections of canopy) and shade, respectively (Table 4). Maximum light levels at Site 1 were 65-75% of the irradiance reaching the unshaded sites (Sites 4b and 4a), while minimum light levels were 2.5-4.5% that of the unshaded sites. Site 2 received approximately half as much light as Site 1 (Table 4).

In 1987, the riparian forest at Sites 4b and 4a was harvested and replanted in 1988 with mixed conifers, however, the canopy closure was less than 5% (Table 4). At Site 4a, conifers on the eastern bank shaded the stream for approximately half the day, reducing light levels by 15-25% compared with full sun values (Table 4), whereas Site 4b was unshaded throughout the day.

During the fall and winter, Rithet Creek is fed primarily by runoff from many ephemeral streams which dry up during the summer (Figure 18). This results in low base flows from ground water throughout the summer and in exceptionally dry summers, certain sections of Rithet Creek have no above-ground water flow (S. Irwin, personal communication). Stream width decreased with distance upstream. Sites 1 and 2 were widest (8.5 and 5 m respectively) while Sites 4b and 4a were 4 and 4.5 m across (Figure 20). Maximum stream depth varied, with Site 4a the deepest (0.7 m) and Site 2 the shallowest (0.3 m). Water depth to the base of the pots varied from as deep as 0.35-0.45

m (Site 4a) to as shallow as 0.2-0.25 m (Site 2) at the beginning of the experiment (Figure 20). Both water depth and velocity decreased during the experiment, with water depth dropping 5-7 cm by the end of the experiment.

Velocity also varied between sites, with Sites 4b and 4a having the highest and lowest velocity values (17-21 $cm \cdot sec^{-1}$ and 9-14 $cm \cdot sec^{-1}$, respectively) (Figure 21). Sites 1 and 2 had similar velocities with initial velocities of 12-14 $cm \cdot sec^{-1}$ and 13-16 $cm \cdot sec^{-1}$, respectively. Velocity decreased during the experiment. Velocity at Site 1 evidenced the largest decrease and by the end of the experiment was 4-4.5 $cm \cdot sec^{-1}$, or approximately 65% of the initial velocity. Decreases of approximately 50% were also observed at the other sites during the experiment to final velocities of 6-8 $cm \cdot sec^{-1}$ at Site 2; 9-14 $cm \cdot sec^{-1}$ at Site 4b; and 3.5-7 $cm \cdot sec^{-1}$ at Site 4a (Figure 21).

Nutrient (NO_3-N and PO_4-P) concentrations were low in Rithet creek during the experiment. NO_3-N concentrations were generally less than 10 $\mu g \cdot L^{-1}$ and were below method detection limits (MDL) (<2 μg NO_3-$N \cdot L^{-1}$) on three dates at Site 2 and 4 (Table 4). PO_4-P concentrations were usually MDL (<0.5 PO_4-P $\mu g \cdot L^{-1}$) throughout most of experiment except for 14 May 1992 when Sites 1, 2 and 4 had PO_4-P concentrations of 0.7 $\mu g \cdot L^{-1}$, 2.3 $\mu g \cdot L^{-1}$, and 0.7 $\mu g \cdot L^{-1}$, respectively. N:P ratios in the shaded sites, ranged from 6->20 (at Site 1) and <1->22 at Site 2. At Site 4, the N:P ratios ranged from <3->10 (Table 4).

Periphyton biomass accrual increased at all sites with added nitrogen and phosphorus (+N&P treatment) but not to phosphorus enrichment alone (Figures 22 and 23). There was little difference in biomass accrual, either measured as chlorophyll *a* or ash free dry mass (AFDM), between Control and +P treatments. The differences between biomass on +N&P containing pots and the Control and +P containing pots was similar in shaded and unshaded sites, with chlorophyll *a* concentrations 5-9 times greater on +N&P enriched substrata than Control or +P substrata (Figure 22). At the unshaded sites, AFDM on +N&P containing pots was 1/3 (Site 4a) to 3 times (Site 4b) greater than AFDM on Control and +P enriched pots (Figure 23). Biomass for all treatments in unshaded sites was approximately 10 times greater than at shaded sites (Figures 22 and 23).

At the unshaded sites, the maximum biomass accrual was reached on all treatments by day 28 (Figures 22 and 23). Chlorophyll *a* concentration declined thereafter on all treatments. There was a large decrease in AFDM for the +N&P treatment at Site 4b while AFDM on the other treatments either did not change (Site 4a Control and +N&P; Site 4b Control) or decreased only slightly (Site 4a +P; Site 4b +P). At the shaded sites, maximum periphyton biomass also occurred on the +N&P enriched substrata by day 28. At Site 2, chlorophyll *a* concentration had decreased by day 42, but remained unchanged at Site 1. Chlorophyll *a* concentration on the Control and +P pots increased throughout the 42 day duration of the experiment.

Chlorophyll *a* concentration on stream rocks in the unshaded sites increased from April 30 through May 14 1992 but remained unchanged after that (Figure 22). At the shaded sites, periphyton chlorophyll *a* biomass on stream rocks increased throughout the experimental duration at Site 1, while at Site 2 biomass accrual did not change after May 28 1992. Biomass on stream rocks at the shaded sites was similar to biomass on the +N&P pots.

The nitrogen (N) and phosphorus (P) content of the periphyton from the unshaded sites closely paralleled the nutrient manipulations (Figure 24). Controls evidenced the lowest N content (2.5-3.1%) and cellular P (0.4-0.5%) content. Periphyton receiving +N&P had the highest N content (Site 4b, 4.3%; Site 4a, 4.3-5.0%). The periphyton N content from +P pots was similar to the Control pots (Site 4b, 3.0-3.6%; Site 4a, 2.4-2.6%). Periphyton P content increased 2-3.5 times above Control levels on substrata with +P and +N&P. At Site 4b periphyton P content varied between 1.0-1.2% for +P and +N&P treatments, whereas at Site 4a, periphyton P varied from 1.0-1.5% for treatments with +P and 1.5-1.7% for treatments with +N&P.

Although, diatoms numerically dominated most of the treatments in both shaded and unshaded sites examined. Algal community composition in shaded sections of Rithet Creek differed markedly from that observed in unshaded sites (Figures 25 and 26). At Site 1, periphyton from Control and +P treatments was numerically dominated by *Achnanthes* spp. Kuetz. (Figure 25), while at Site 4b, prostrate/stalked and erect diatoms were the most abundant diatoms. *Synedra ulna* (W.Sm.) Brun., had the largest biovolume contribution of any diatom and comprised 67-69% of the periphyton receiving +N&P at

Site 4b, while on the Control and +P treatments, *S. ulna* contributed lesser proportions of 51-64% and 34-63%, respectively (Figure 27).

At both shaded and unshaded sites, substrata with +N&P had the greatest number of algal cells per unit area, and the greatest number of non-diatom algae. *Chroococcus minutus* (Kuetz.) Naegeli occurred in the greatest numbers at Site 1 on the +N&P treatments, with maximum numerical abundance reached at day 28. By day 42 the numerical abundance of *C. minutus* declined on +N&P treatments, while on the Control and +P substrata numerical abundance had increased (Figure 25).

At Site 4b, filamentous chlorophytes were numerically, most abundant on the +N&P pots but comprised approximately the same proportion of the community as the chlorophytes on the Control and +P treatment (45-50% of the total number of algal cells) due to the relative increase in the abundance of diatoms (Figure 26 and 27). *Binuclearia tatrana* Wittrock, *Mougeotia* spp. (Agardh.) Wittrock, *Oedogonium* sp. Link, *Spirogyra* sp Link. and *Zygnema* sp. Agardh. contributed between 31 and 49% of the algal biovolume from all treatments at Site 4b, except the +P pots on June 11 1992. Here filamentous chlorophytes comprised 66% of the biovolume (Figure 26). *Mougeotia* spp. contributed the largest proportion of the filamentous chlorophyte biovolume in most of the treatments. Periphyton community composition was visually different from shaded and unshaded sites (Figure 28).

Discussion

Both irradiance and nutrient supplementation affected periphyton biomass accrual in Rithet Creek. Periphyton biomass accrual increased with the addition of N and P regardless of light levels, whereas biomass on +P substrata was not different from the Control substrata (Figures 22 and 23). Throughout the experimental period of April 30 to June 11 1992, NO_3-N and PO_4-P concentrations were low (<2-11 $\mu g \cdot N \cdot L^{-1}$ and <0.5-2.3 $\mu g \cdot P \cdot L^{-1}$) within Rithet Creek. N:P ratios were generally less than 15 indicating that in Rithet Creek periphyton was potentially nitrogen limited during the nutrient-diffusing experiment (Shanz and Juon, 1983). This may be primarily a spring, early summer phenomenon, because water chemistry analyses conducted during an earlier, year long study, showed that from July through November NO_3-N concentrations were generally above 10 $\mu g \cdot L^{-1}$ and PO_4-P concentrations were >0.5 $\mu g \cdot L$ (Grant *et al.*, 1994,

unpublished data). The findings of Grant *et al.* (1994) were consistent with other studies showing that streams on Vancouver Island are typically believed to be P-limited (Stockner and Shortreed, 1978; Perrin *et al.*, 1987; Mundie *et al.*, 1991). Suttle and Harrison (1988), however, indicated that for two central Vancouver Island lakes, P was limiting but co-occurred with low N concentrations, thus only a small increase in PO_4-P was required to shift the lakes into N-limitation. Rithet Creek may be similar to the latter systems, being low in both N and P and may fluctuate seasonally between N and P limitation. There has been little documentation of streams systems which vary temporally between N and P limitation. Hill and Knight (1988) also found N-limitation in coastal, shaded and unshaded, streams in northern California evidenced by low N:P ratios and enhanced biomass accrual in unshaded streams on nitrate enhanced substrata.

Although in this study, periphyton biomass accrual was stimulated by an increase in N (the limiting nutrient), light was the dominant limiting factor at the shaded sites. Light limitation of periphyton biomass accrual has been demonstrated by many researchers (Shortreed and Stockner, 1978; Triska *et al.*, 1983; Lowe *et al.*, 1986; Sheath *et al.*, 1986; Hill and Knight, 1988; Feminella *et al.*, 1989; Hill and Harvey, 1990). In the nutrient-diffusing experiment, maximum biomass accrual differed by an order of magnitude between all treatments in shaded and unshaded sites (Figures 22 and 23). The differences between biomass accrual in shaded and unshaded sites were similar to those reported by Lowe *et al.* (1986), but are considerably greater than those of other studies (Hill and Knight, 1988; Winterbourne, 1990; Hepinstall and Fuller, 1994).

Unlike most studies where periphyton does not respond to added nutrients under shaded conditions (Lowe *et al.*, 1986; Hill and Knight, 1988; Winterbourne, 1990; Hepinstall and Fuller, 1994) periphyton biomass accrual in the present study was increased with added N&P in shaded sites (Figures 22) with periphyton chlorophyll *a* biomass on +N&P pots was approximately 8 times greater than the biomass on Control and +P pots.

Maximum biomass was reached in the unshaded sites by 28 days, and declined thereafter for all treatments, while in the shaded sites biomass continued to increase on the Control and +P substrata throughout the experiment, but declined after 28 days on the N and P enriched substrata. Fairchild *et al.* (1985) examined nutrient diffusion rates from clay pots (of similar size and nutrient concentration used in this experiment) and found a linear decrease in nutrient diffusion from the substrata for 23 days. Fairchild *et al.* (1985)

also analyzed NO_3-N and PO4-P remaining in the agar after 23 days and found that approximately 1/3 and 2/3 of the NO_3-N and PO_4-P remained. The decrease in biomass observed after 28 days in this study (Figures 22 and 23), may have been due to decreasing nutrient diffusion from the artificial substrata. However, observations from velocity / nutrient flux and N:P ratio experiments of a loss of biomass under continued nutrient supply implies other factors such as carbon limitation (Borchardt, 1994) or photoinhibition of floating mats from visible or ultraviolet light (Bothwell *et al.,* 1993) may contribute to the decreased biomass observed.

Biomass on stream rocks was analyzed to determine the biomass accrual of periphyton on natural substrata (Figure 22). In the unshaded sites chlorophyll *a* on the stream rocks increased until May 14 1992 and remained unchanged thereafter, while at the shaded sites, chlorophyll *a* on stream rocks increased throughout the experiment at Site 1, but remaining constant at Site 2 after May 14 1992. Stream rock biomass values in the unshaded sites were similar to the biomass on the Control and +P pots, while in the shaded sites, stream rock biomass was similar to the biomass on +N&P pots. Periphyton measured from natural stream rock was of an unknown colonization period. The relatively high biomass in the stream rocks from the shaded sites compared to the unshaded sites may have been the result of a slow biomass accruing periphyton community developing over a longer period of time under primarily light-limitation, whereas, periphyton accrual under higher light is faster but rapidly constrained by nutrient limitation.

At Site 1, adnate diatoms were dominant on the Control and P-enriched substrata. On the N&P enriched substrata, the numerical abundance of prostrate diatoms increased and the community was dominated by a cyanophyte (*Chroococcus minutus*) (Figure 25). By 42 days the numerical abundances of algal species were similar on Control and +P treatments. Higher numbers of *C. minutus* and a prostrate chlorophyte were found on the N&P enriched substrata. Diatoms have been found to be dominant in many heavily shaded streams examined (Lowe *et al.*, 1986; Hill and Knight, 1988; Winterbourn, 1990; Steinman, 1992; Rosemond, 1993).

At Site 4b filamentous chlorophytes and the large erect diatom, *Synedra ulna*, were dominant for all treatments but exhibited higher numbers on the N&P enriched substrata (Figure 26). Analysis of the biovolume contribution of the dominant algal

species to community structure revealed little difference in community composition (Figure 27). Lowe *et al.* (1986) also found that algal community structure was significantly different between shaded and unshaded streams but not between nutrient treatments. They also observed that algal physiognomies were significantly different between clear-cut streams dominated by filamentous chlorophytes forested streams dominated by erect diatoms.

Periphyton nitrogen and phosphorus content from unshaded sites closely paralleled nutrient manipulation (Figure 24). Ambient concentrations of N and P in Rithet Creek were insufficient to maintain the periphyton N and P content above the nutrient-limitation threshold of 5% and 0.5% (Biggs, 1994) on the Control pots. Additional N&P, increased periphyton N to between 4-5%, thus indicating the periphyton may have still been N-limited. Periphyton P content +N&P and +P substrata increased to between 1.0 and 1.7%. Thus, although periphyton biomass did not increase on +P enriched substrata, this treatment resulted in an increase in the P content of the periphyton. Luxury uptake has been demonstrated in planktonic algae with a large storage capacity (Rhee and Gotham, 1980; Thompson *et al.*, 1994), and this might be an important mechanism used by periphytic algae found in a fluctuating nutrient environment to optimize their cellular nutrient content and growth (Morel, 1987).

Unlike other studies which found only light to be the limiting resource in forested streams (Lowe *et al.*, 1986, Hill and Knight, 1988) and nutrients the limiting factor in unshaded streams (Shortreed and Stockner, 1983; Hill and Knight, 1988), results in this study indicates that there can be dual resource limitation affecting periphyton biomass accrual. Light appeared to be responsible for primarily determining the architecture of the periphyton, whereas nutrient concentration affected the relative proportion and numerical abundance of the species present, thus affecting biomass.

Table 4. Dominant riparian vegetation, irradiance, water temperature and nutrient concentrations at Rithet Creek sample sites on between April 30 to June 11 1992. NA = not analyzed.

Site	**Riparian vegetation** (dominant spp. & age of stand*)	**Crown closure****	**Light** (max./min.) (uE/m2/sec)	**Temp.** (C)	**Nutrient concentration** (ug/L)	30-Apr	14-May	28-May	11-Jun
1	Douglas fir, Hemlock, W. Red Cedar 24-48 years	56-65%	215-250 80-150	9.0-12.2	NO3-N PO4-P N:P	10 <0.5 >20	4 0.65 6	6.6 <0.5 >13	7.2 <0.5 >14
2	Douglas fir 141-250 years	56-65%	80-170 30-80	10.0-12.0	NO3-N PO4-P N:P	3.6 <0.5 >7	<2.0 2.3 <1	5.8 <0.5 >12	11.1 <0.5 >22
4b	Douglas fir, Hemlock <5 years	0-5%	300	12.5-13.5	NA	NA	NA	NA	NA
4	Douglas fir, Hemlock <5 years	0-5%	NA	NA	NO3-N PO4-P N:P	5.3 <0.5 >10	<2.0 0.65 <3	3.6 <0.5 >7	<2.0 <0.5 ?
4a	Douglas fir, Hemlock <5 years	0-5%	300 50-80	13.0-14.0	NA	NA	NA	NA	NA

*Source: Figure 18
**Source: GVWD (1993)

Figure 18. Map of Sooke watershed showing forest type and sampling stations along Rithet Creek.

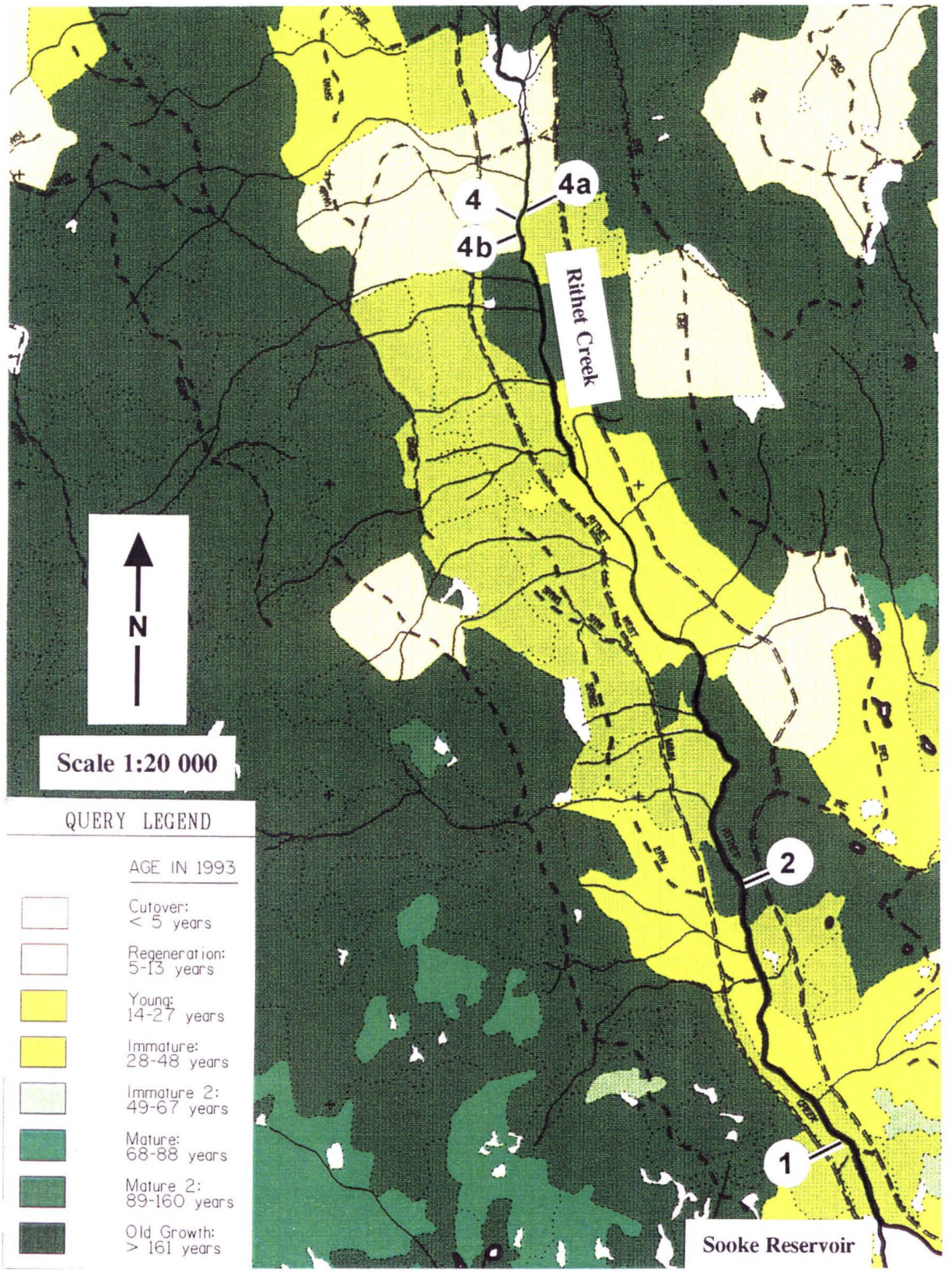
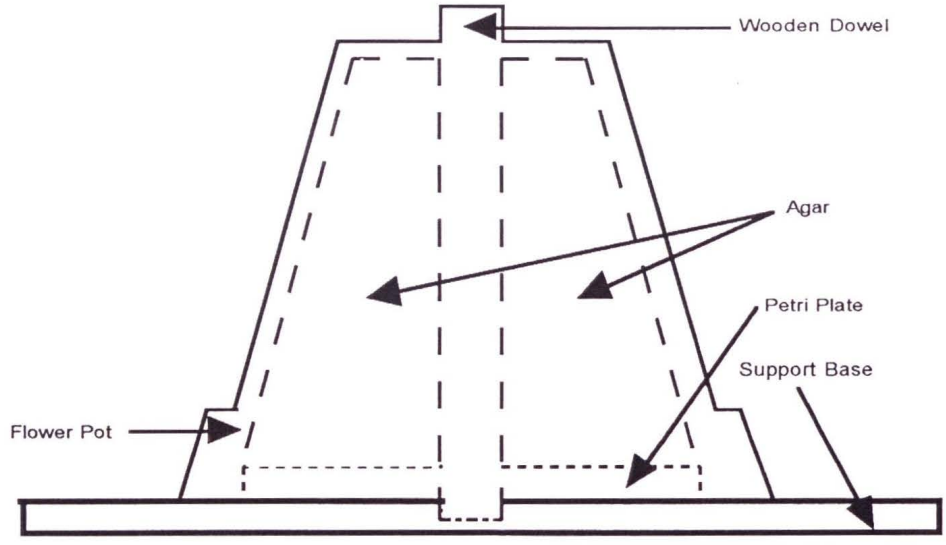

4
4a
4b
Rithet Creek
N
Scale 1:20 000
QUERY LEGEND
AGE IN 1993
Cutover:
< 5 years
Regeneration:
5-13 years
Young:
14-27 years
Immature:
28-48 years
Immature 2:
49-67 years
Mature:
68-88 years
Mature 2:
89-160 years
Old Growth:
> 161 years
2
1
Sooke Reservoir

Figure 19. Nutrient-diffusing substrata system. (A) Schematic drawing showing cross-section of nutrient-diffusing clay pot. (B) Photograph showing placement of nutrient-diffusing substrata system in Rithet Creek. The substrata system used in the experiment had nine clay pots and two 60 cm wooden planks secured perpendicular to the ends of the substrata base for placement of anchoring rocks.

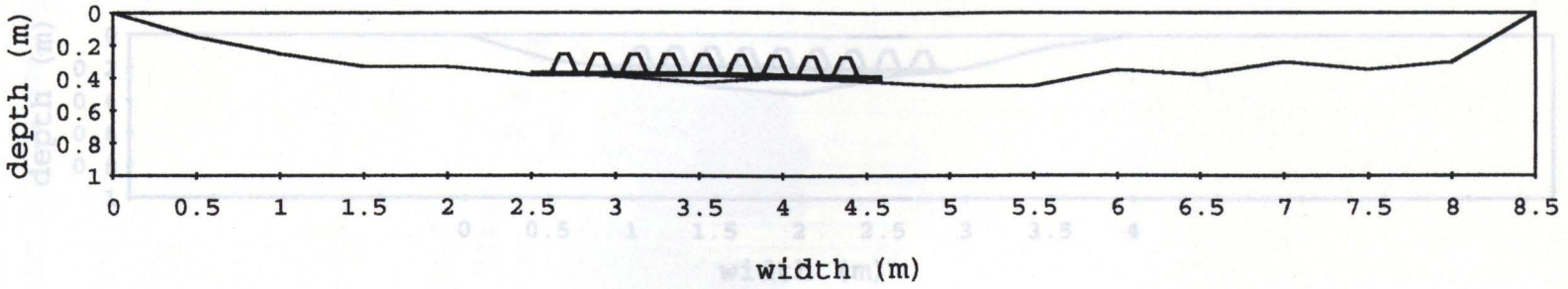
A



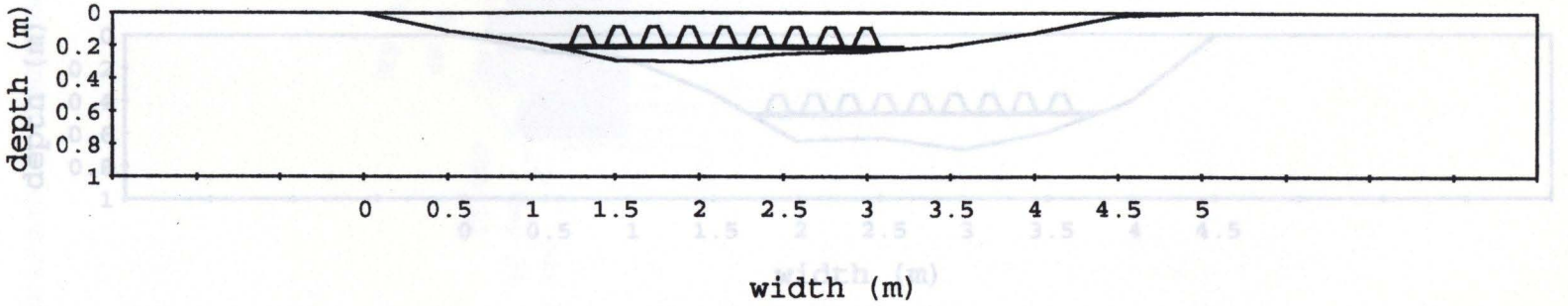
B

Figure 20. Stream cross-section showing depth profile measured at 0.5 m intervals and placement of nutrient-diffusing clay pots.

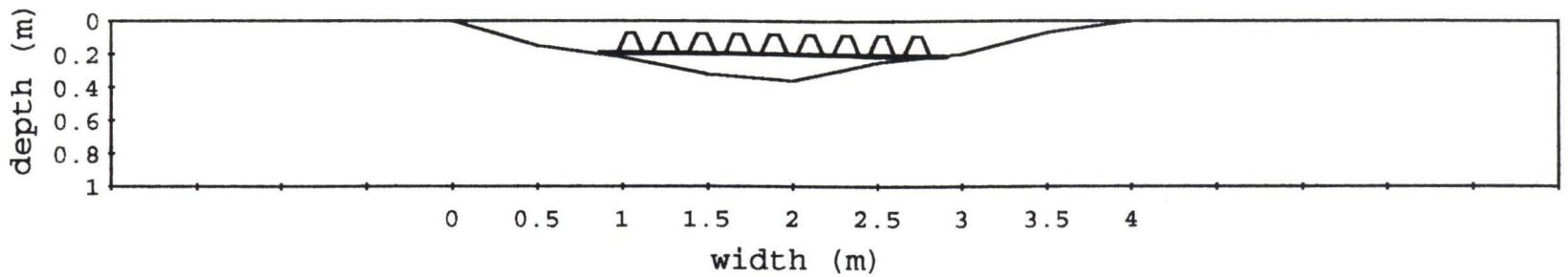
Site 1


depth (m)
0
0.2
0.4
0.6
0.8
1
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5
width (m)

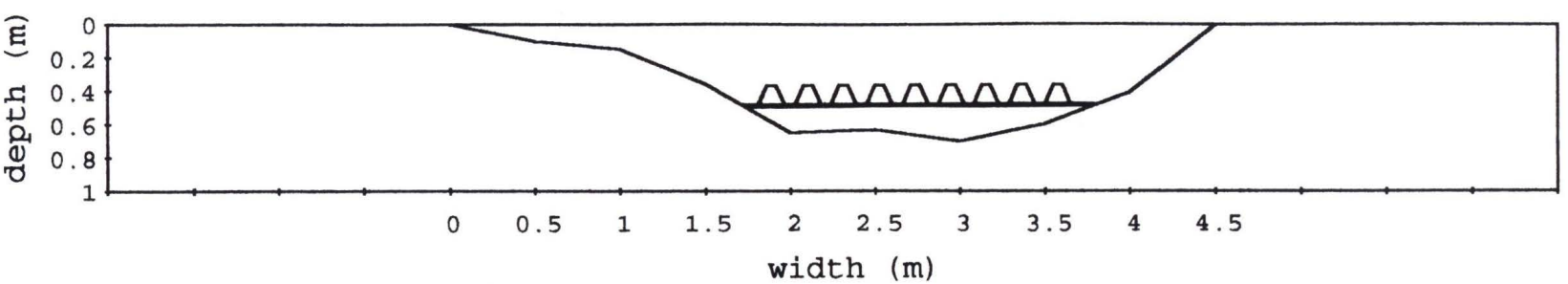
Site 2


depth (m)
0
0.2
0.4
0.6
0.8
1
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5
width (m)

Site 4b



Site 4a



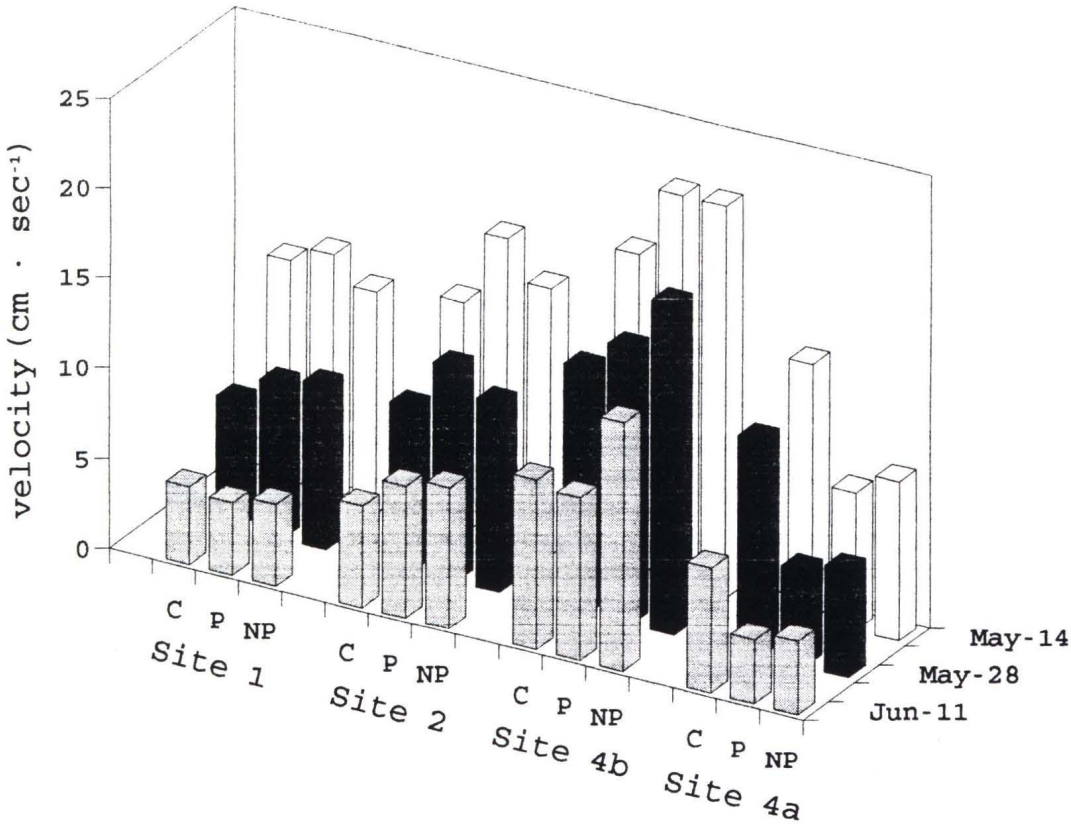
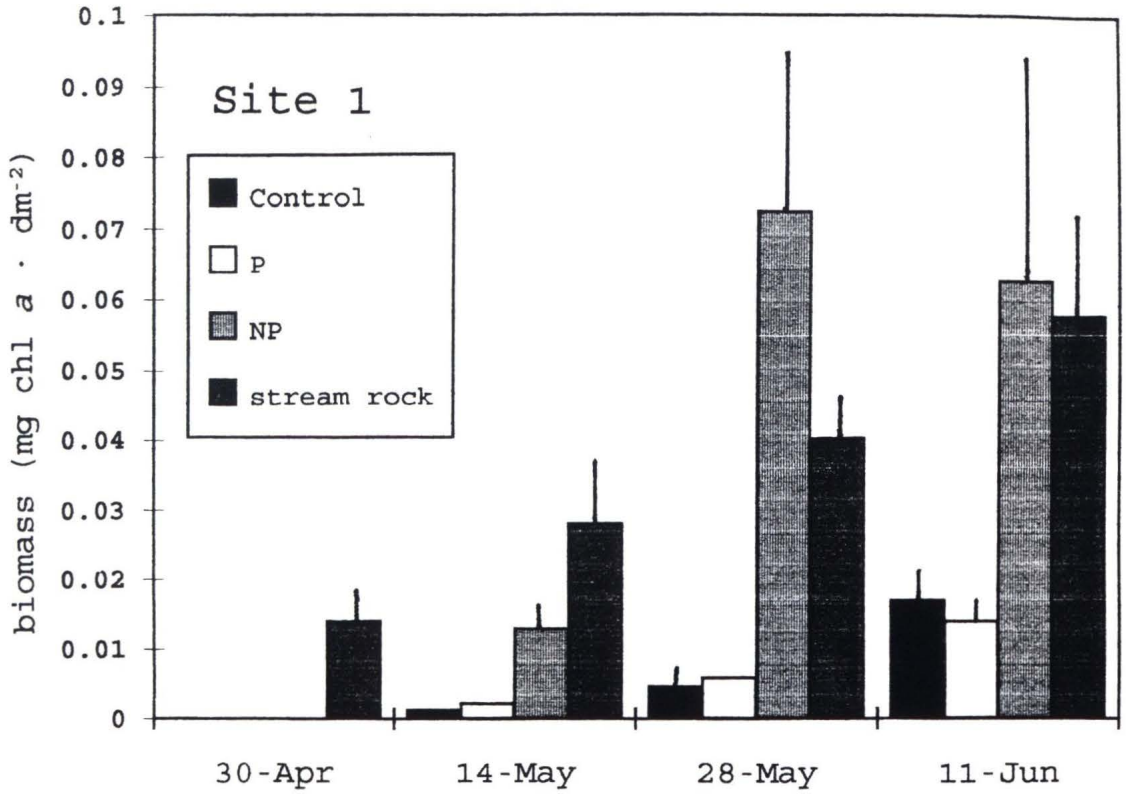
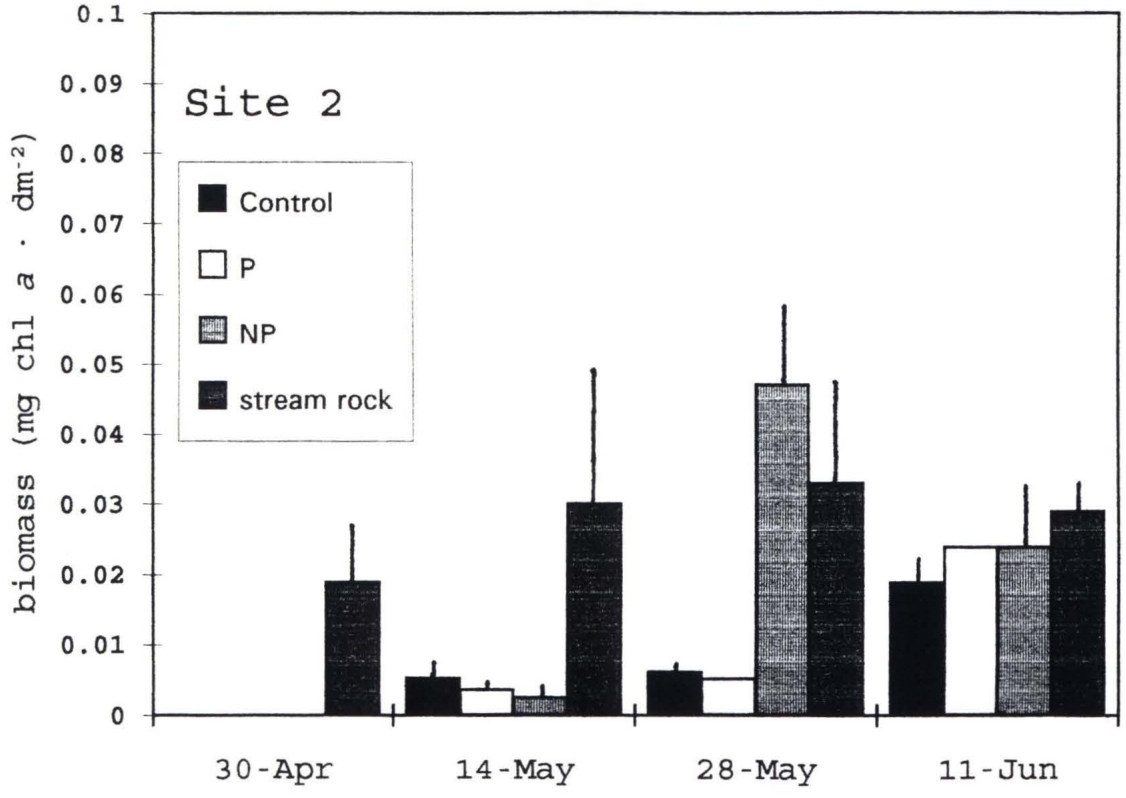
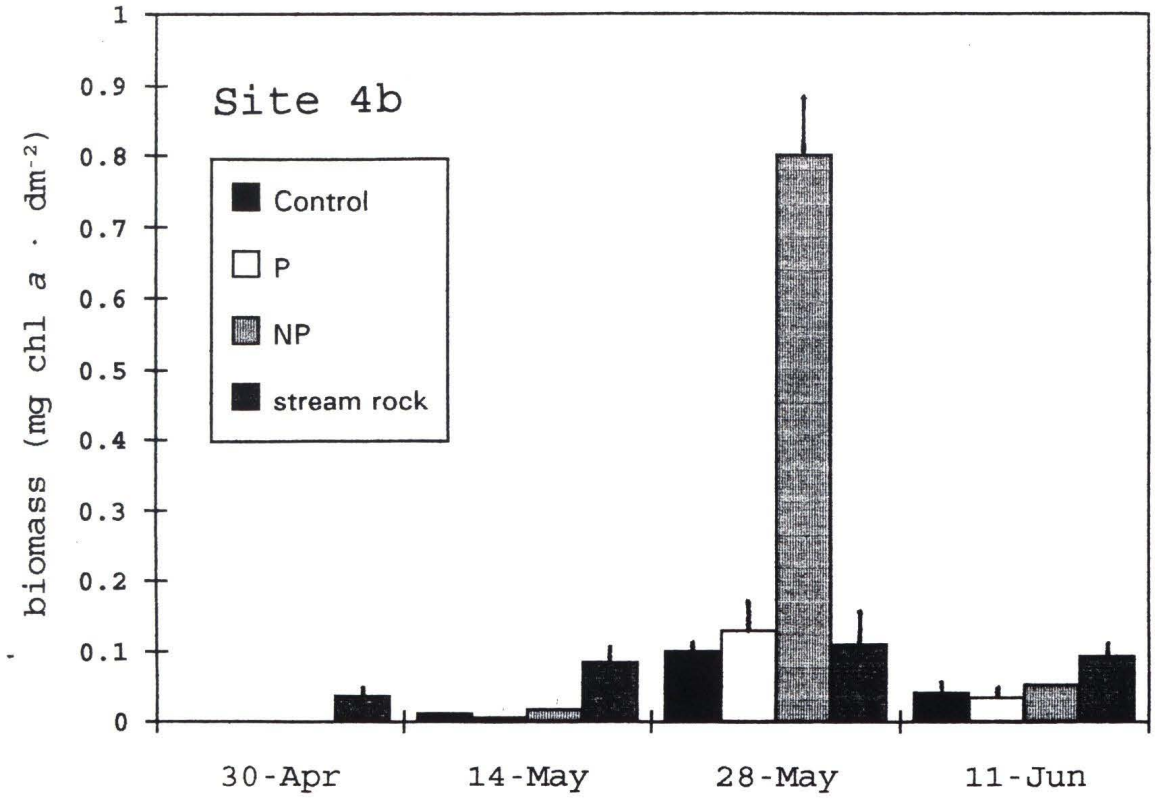


Figure 21. Surface velocity measured above each treatment at four sampling sites from May 14 to June 11 1992. Note that dates along Z-axis are reversed to show current values more clearly.

Figure 22. Periphyton chlorophyll *a* biomass (mean ± SD, n=3 for clay pots, n=4 for stream rock) after 14, 28 and 42 from nutrient-diffusing clay pots containing added phosphorus (+P), added nitrogen and phosphorus (+N&P), and no additions (Control), and from natural stream rock at two shaded (Sites 1 and 2) and unshaded (Sites 4b and 4a) sections of Rithet Creek.


Site 1
Control
P
NP
stream rock
biomass (mg chl a · dm-2)
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
30-Apr
14-May
28-May
11-Jun


Site 2
Control
P
NP
stream rock
biomass (mg chl a · dm-2)
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
30-Apr
14-May
28-May
11-Jun


Site 4b
Control
P
NP
stream rock
biomass (mg chl a · dm-2)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
30-Apr
14-May
28-May
11-Jun

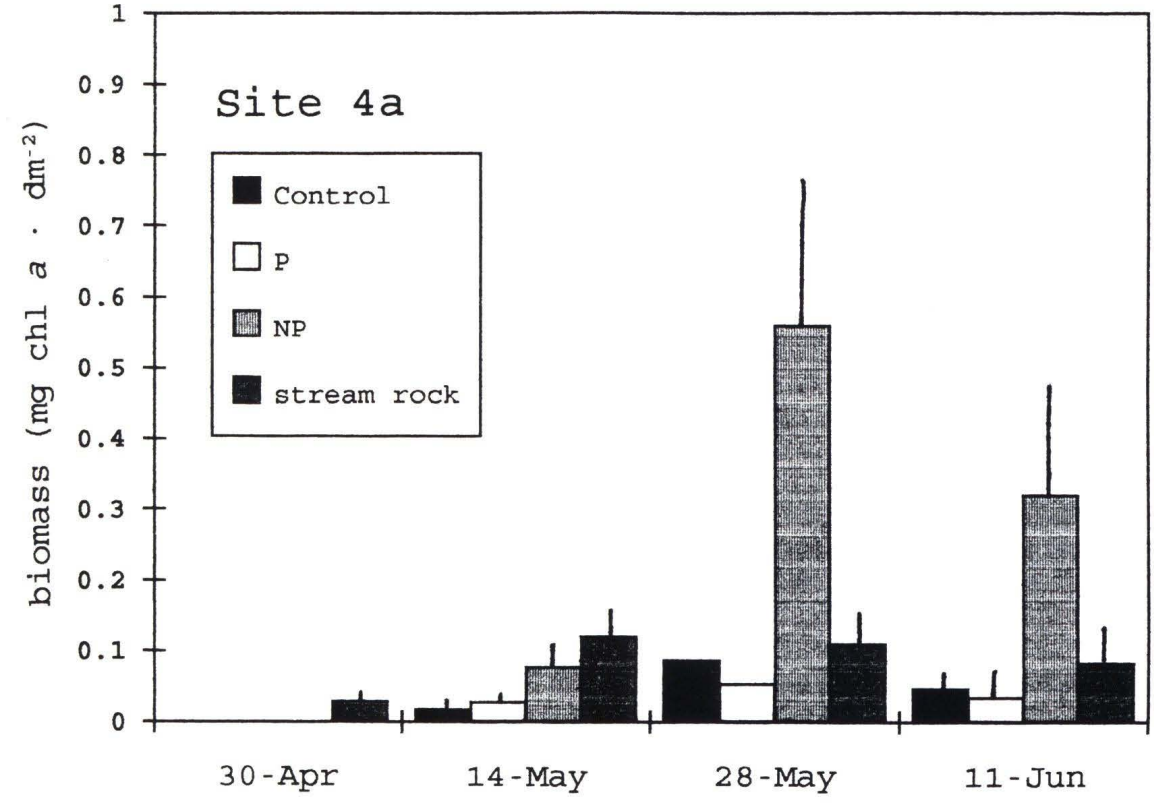

Site 4a
Control
P
NP
stream rock
biomass (mg chl a · dm-2)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
30-Apr
14-May
28-May
11-Jun

Figure 23. Periphyton ash free dry mass (AFDM) (mean $\pm$ SD, n=3) after 14, 28 and 42 days from nutrient-diffusing clay pots containing added phosphorus (+P), added nitrogen and phosphorus (+N&P), and no additions (Control) at two shaded (Sites 1 and 2) and unshaded (Sites 4b and 4a) sections of Rithet Creek. Insufficient biomass was collected to determine AFDM for all but the +N&P treatments from Sites 1 and 2 on May 14 and May 28 1992.

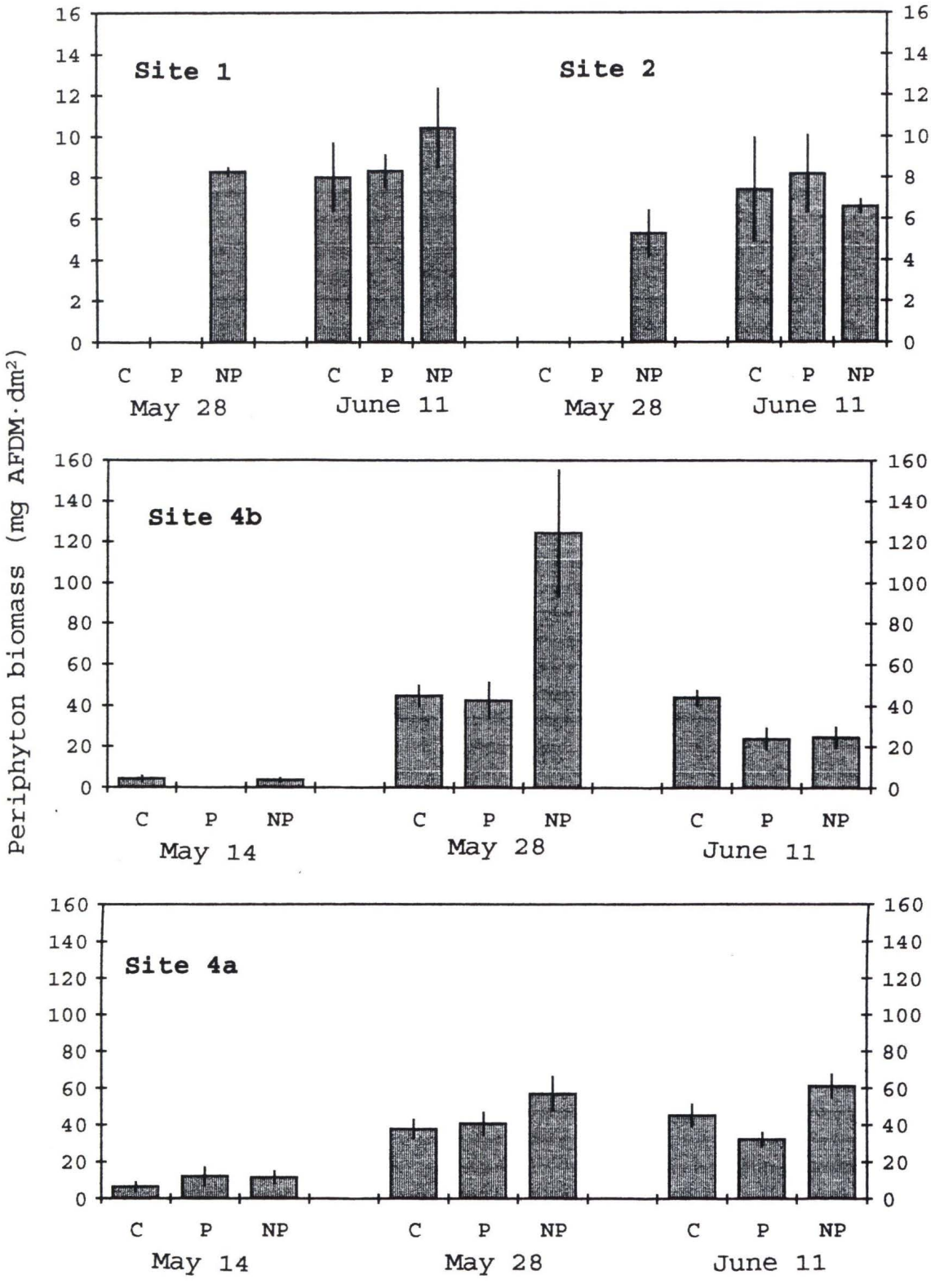
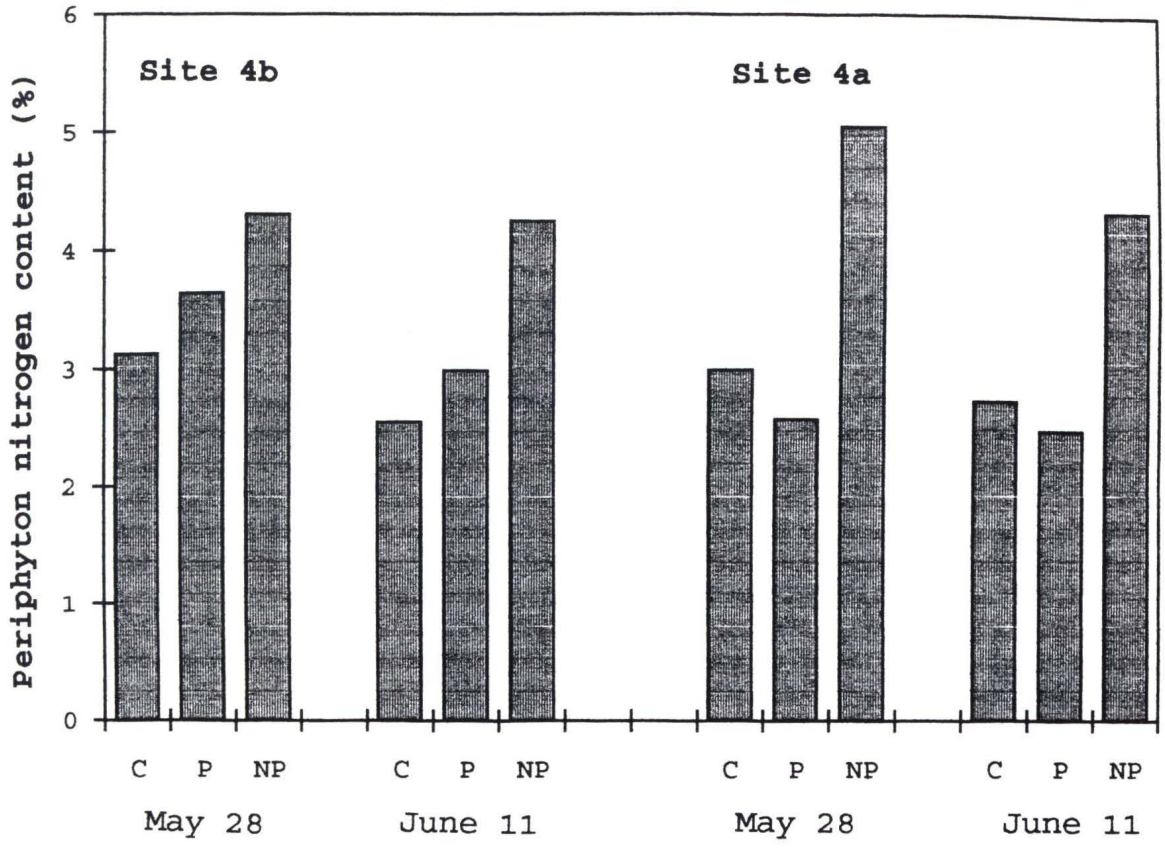
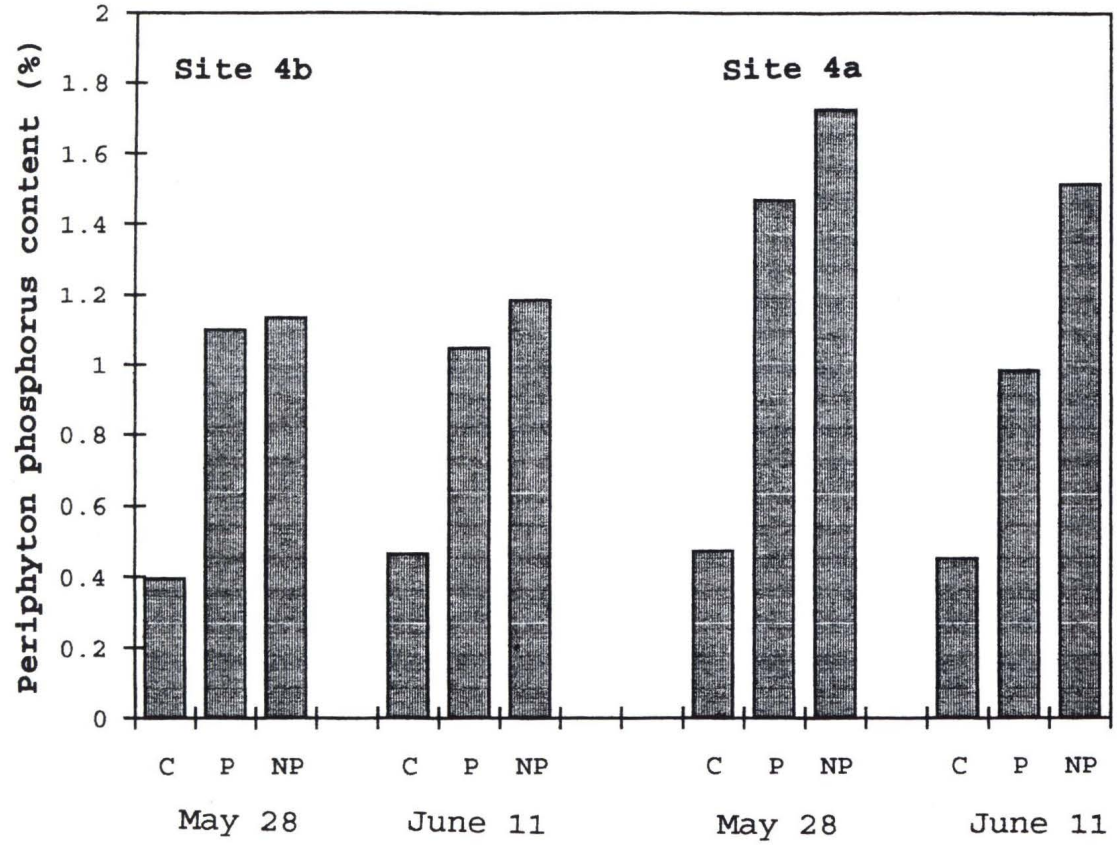

Site 1
Site 2
Site 4b
Site 4a
Periphyton biomass (mg AFDM·dm²)
C P NP
May 28
June 11
May 14

Figure 24. Periphyton nitrogen and phosphorus content (n=1, biomass from replicate pots was pooled to provide sufficient biomass for analysis) after 28 and 42 days from nutrient-diffusing clay pots containing added phosphorus (+P), nitrogen and phosphorus (+N&P), and no additions (Control), at shaded (Sites 4b and 4a) sections of Rithet Creek.


Periphyton nitrogen content (%)
Site 4b
Site 4a
0
1
2
3
4
5
6
C P NP C P NP C P NP C P NP
May 28 June 11 May 28 June 11


Periphyton phosphorus content (%)
Site 4b
Site 4a
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
C P NP C P NP C P NP C P NP
May 28 June 11 May 28 June 11

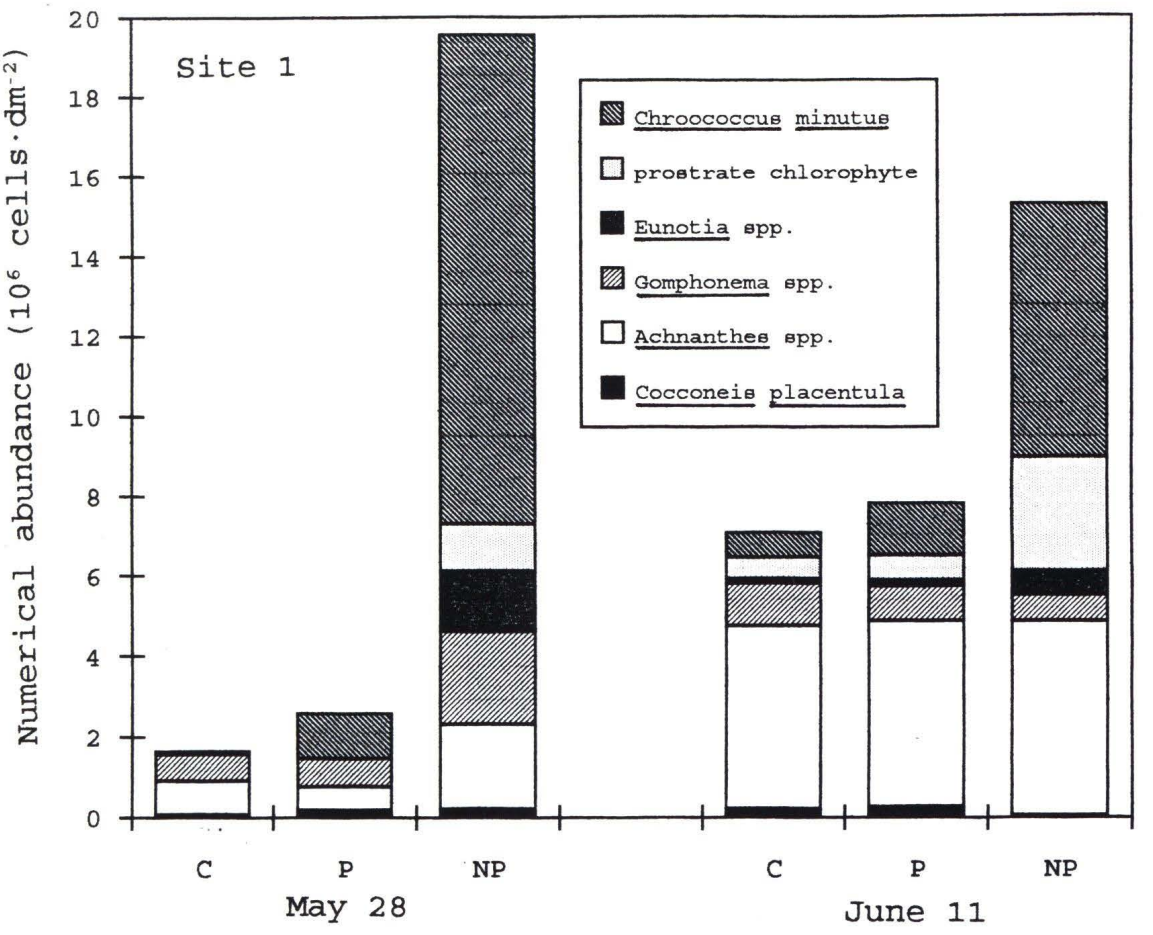


Figure 25. Numerical abundance (10^6 cells·dm^{-2}, n=2) of dominant algal taxa observed at day 28 and day 42 on nutrient diffusing clay pots containing added phosphorus (+P), nitrogen and phosphorus (+N&P), and no additions (control) from Site 1.

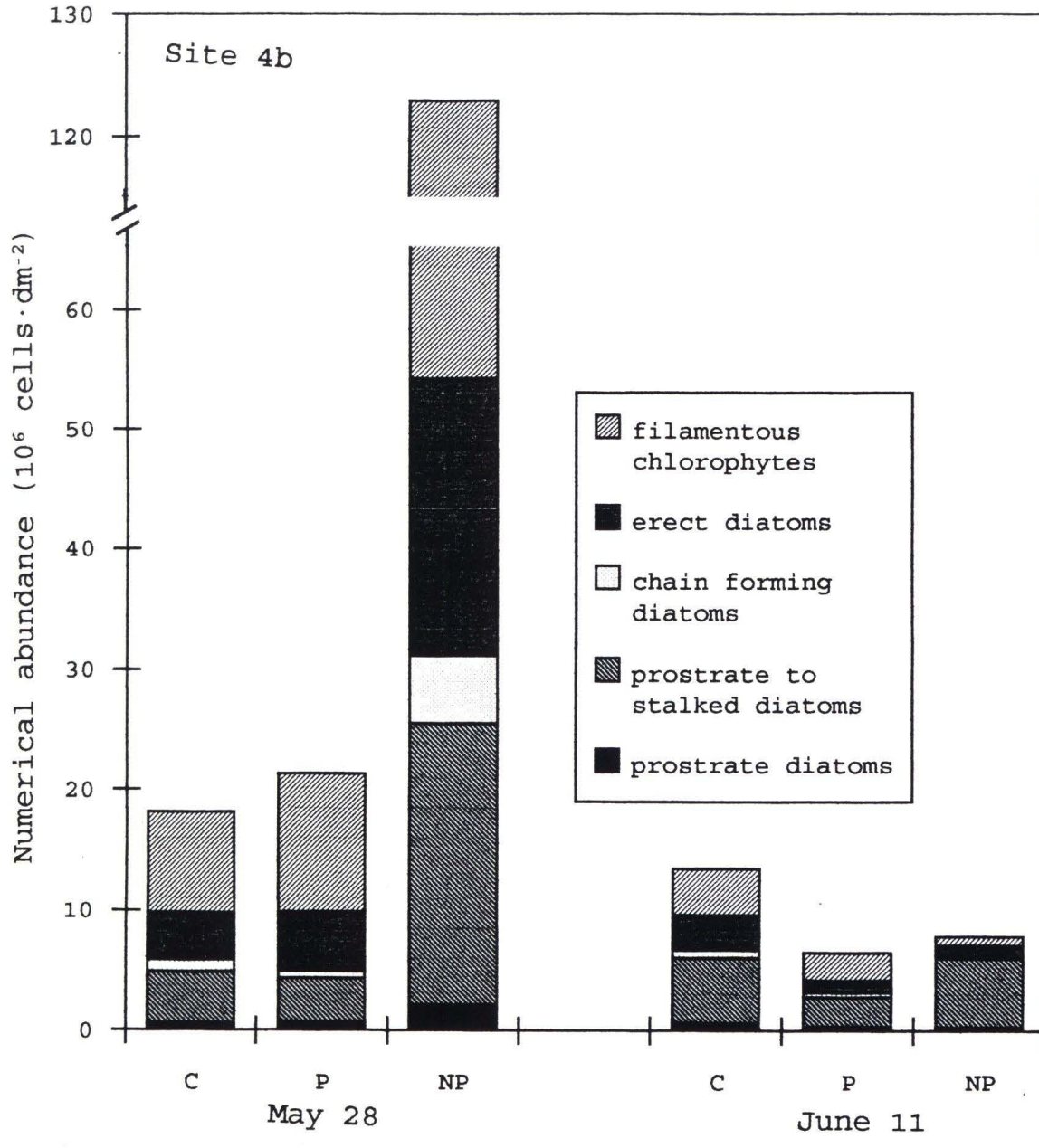
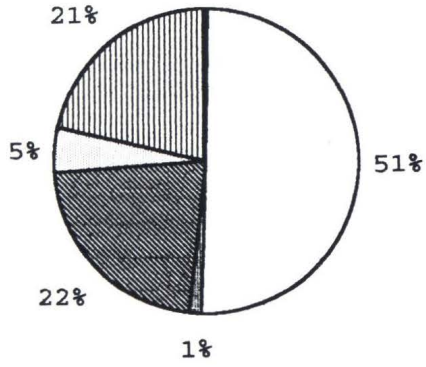


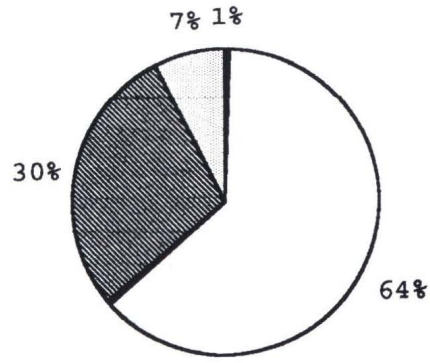
Figure 26. Numerical abundance (10^6 cells·dm^{-2}, n=2) of algal guilds observed at day 28 and day 42 on nutrient diffusing clay pots containing added phosphorus (P), nitrogen and phosphorus (NP), and no additions (control) from the harvested Site 4b.

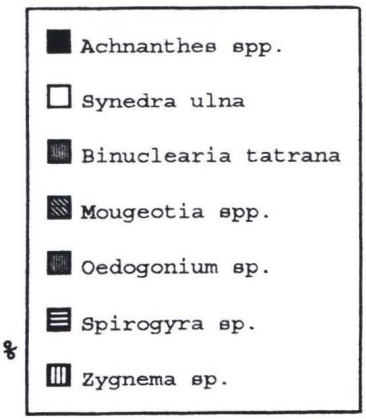
Figure 27. Percent biovolume (n=2) of dominant algal taxa at day 28 and day 42 on nutrient-diffusing clay pots containing added phosphorus (+P), nitrogen and phosphorus (+N&P), and no additions (Control) from Site 4b.

Site 4b C 28-May

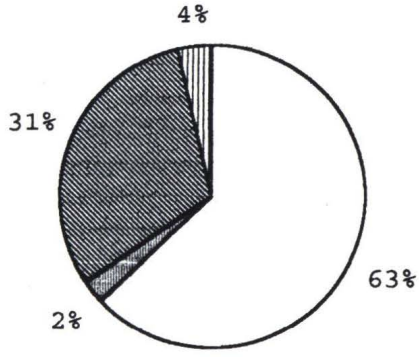


Site 4b C 11-Jun





Site 4b P 28-May



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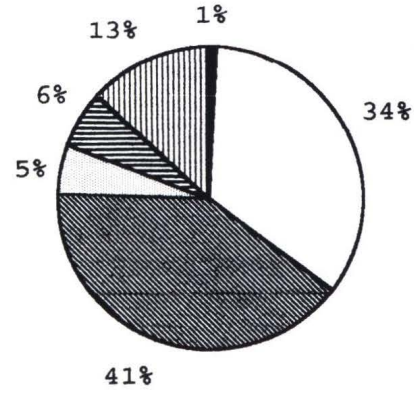

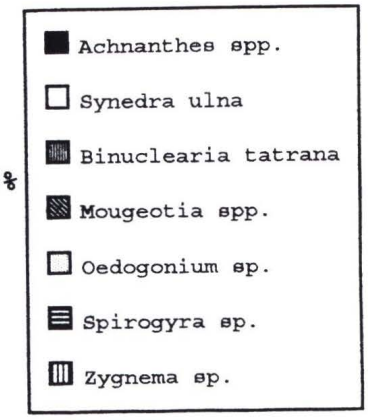

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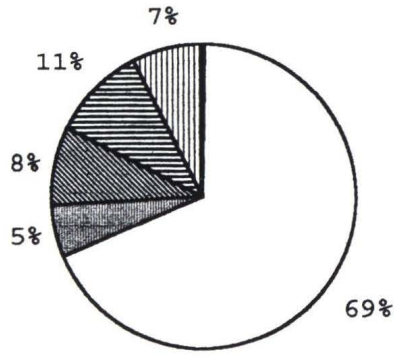

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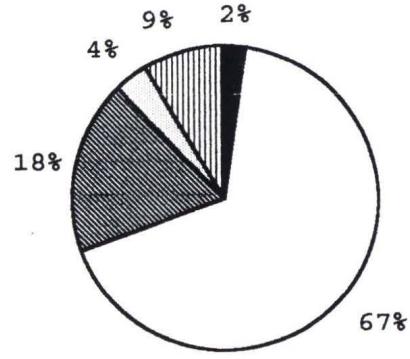


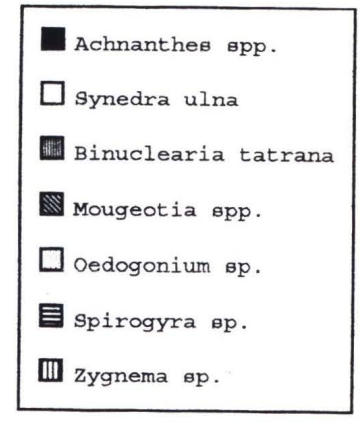
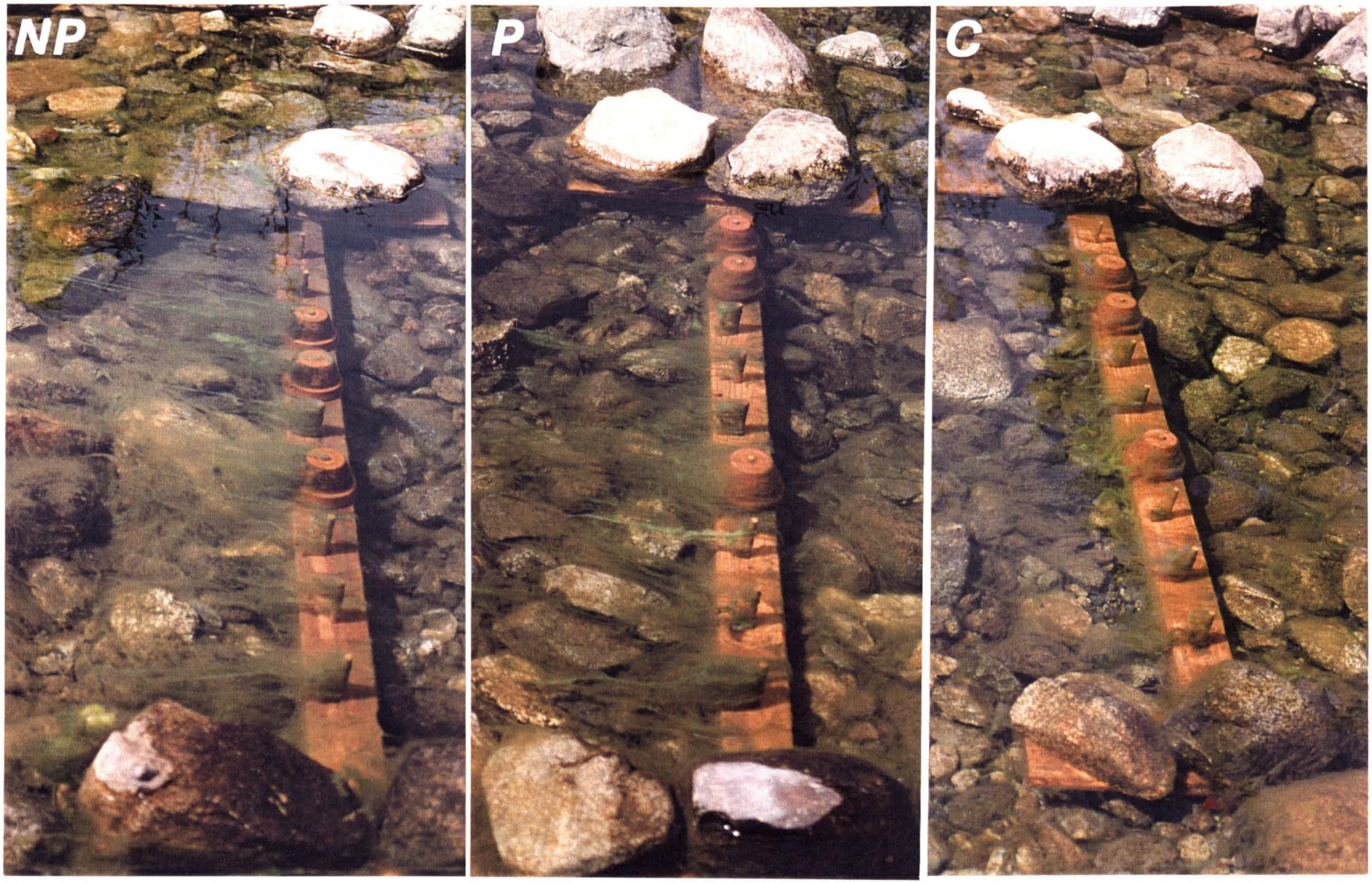


Figure 28. Photographs of periphyton communities on nutrient-diffusing clay pots containing added phosphorus (+P), nitrogen and phosphorus (+N&P), and no additions (Control) after 29 days from Site 1 and Site 4b. The photographs were taken the day after the May 28 1992 sampling.

Site 1

Site 4b



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