Benthic algal and insect responses to nutrient enrichment of an in-stream mesocosm

by

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ABSTRACT

A nutrient bioassay was conducted in the Slocan River in southeastern British Columbia to evaluate potential trophic level responses in advance of whole-stream enrichment. A mesocosm approach was used to contrast periphyton and insect responses to low-level nitrogen and phosphorus treatments over an 83 d period in late summer. The nutrient bioassay was used to describe quantitatively and qualitatively benthic algal and insect relationships to manipulations of N and P concentrations and their ratios. N:P concentrations of 1:1, 3:3 and 5:5 $\mu g \cdot L^{-1}$ and 4:1, 12:3 and 20:5 $\mu g \cdot L^{-1}$ were used for treatment group comparisons. The experimental design consisted of six, replicated (x2) treatments and control. Although significant differences in periphyton accrual (measured as chlorophyll *a* biomass) were consistently demonstrated between control and treatment groups, the 4:1 treatment group was substantially higher in algal biomass than the 1:1 treatment group. Up to 4 and 8-fold differences in periphyton accrual over background were observed for the 1:1 and 4:1 treatment groups, respectively. Differences in biomass between treatment groups suggested N limitation. With the exception of the 1:1 nutrient concentration where a cyanophyte (*Oscillatoria* sp.) became highly abundant, all other treatments were dominated by chlorophytes and diatoms.

The benthic insect response was similar to the periphyton response with up to 3-fold differences in insect abundance and a near-doubling of biomass observed in the 1:1 and 4:1 treatment groups. Differences in the amplitude and periodicity of insect diel drift cycles suggested differences in food abundance between control and treatment groups. A reduction in total insect per capita drift rate from one-half to one-third of that observed under background conditions provided further evidence in support of delayed emigration in treated channels. Taxa-specific differences in per capita drift rate between control and treatments also occurred for baetid mayflies where a drop from one-half to one-third was observed within certain nutrient ratios of each treatment group. The higher availability of food in treatment groups was further supported by larger body size of insects in both the drift and the benthos. Over the period of study, chironomid midges and baetid mayflies were the most abundant taxa. Owing to a large emigration near the end of the experiment, chironomid midges that were initially numerically dominant were replaced by mayflies, albeit at lower densities. Although insects were not considered food-limited, grazers had substantial influence on areal algal biomass. After comparing differences in taxonomic richness and trophic level response, the 4N:1P ratio was considered optimum for whole-stream application.

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1.0 Introduction

Experimental research to increase primary and secondary production in nutrient-deficient streams has evolved significantly over the last three decades. Early investigations focused on the importance of heterotrophic processes in experimental streams and artificial stream troughs by adding organic matter to augment allochthonous sources (Warren et al. 1964; Mundie et al. 1973; Williams et al. 1977; Mundie et al. 1983). By the late 1970's however, the importance of autotrophy as a critical energy pathway in sustaining lotic communities was advanced, particularly in higher order, downstream reaches of a watershed (Minshall 1978). The transition from heterotrophy to autotrophy was strongly influenced by the availability of light as a function of riparian canopy cover and the amount of transported organic matter; a concept that was later described as a continuum, from headwaters to lowlands, characteristic of temperate stream ecosystems (Vannote et al. 1980). The role of nutrients in limiting primary production was further explored by Stockner and Shortreed (1978), Elwood et al. (1981), Peterson et al. (1983), and Bothwell (1988).

Experimental manipulation of a coastal British Columbia stream comparing inorganic and organic treatments clearly demonstrated a higher rate of accrual among periphyton and a larger total biomass after inorganic nutrient introduction (Perrin et al. 1987). *In situ* periphyton response to inorganic treatment displayed a five to ten-fold increase over background during a single growing season (Johnston et al. 1990). Under mesocosm conditions in other coastal B.C. streams, a 3.5-fold increase in periphyton biomass and a 2.2-fold increase in emergent insect numbers were demonstrated over a 7 week period (Mundie et al. 1991) whereas insects responded strongly to fertilization with only a small periphyton response (Quamme 1994); the latter results suggesting that insect grazing was a factor. Moreover, inorganic nutrient enrichment contributed to a 1.4 to 2-fold increase in late summer fry weight of salmonids when compared to organic sources (Johnston et al. 1990). Improvements in food availability to at least two trophic levels have been extended to stream environments in northern latitudes as well (Peterson et al. 1985; Deegan et al. 1992). More recently, energy transfer from stream periphyton to arctic grayling was confirmed in an arctic study using C:N stable isotope ratios and provided evidence that insect grazers could control algal standing crops (Peterson et al. 1993). Collectively, this body of research has demonstrated the importance of inorganic nutrient augmentation in stimulating primary production as well as indicating energy transfer between trophic levels through the algal-insect-fish food chain in stream ecosystems.

The character of community structure and function has often been related to a combination of biotic and abiotic factors. The complexity of these linkages has been extensively reviewed

(Gregory 1983; Lamberti 1996). Several lines of experimental evidence suggest that algal stream communities are regulated by physical parameters such as nutrient availability, temperature, light, current velocity and degree of annual disturbance as well as biological interactions such as competition and predation. Moreover, there is considerable experimental evidence in support of "bottom up" control of trophic production that has been positively affected by nutrient manipulation (reviewed in Lamberti 1996). Inorganic nitrogen (N) and phosphorus (P) additions to stream ecosystems (Stockner and Shortreed 1978; Elwood et al. 1981; Peterson et al. 1983; Perrin et al. 1987; Johnston et al. 1990) and experimental troughs (Bothwell 1988; Bothwell 1989; Mundie et al. 1991; Perrin and Richardson 1998) have clearly shown exponential increases in periphyton biomass up to an order of magnitude above background, at least until grazers respond (Peterson et al. 1993; Quamme 1994). Benthic invertebrate communities have also shown a strong positive response to N and P enrichment (Hart and Robinson 1990; Mundie et al. 1991; Perrin and Richardson 1998) indicated by doubling in survival to emergence, increased benthic density or increased body size. In regard to juvenile fish, studies have either shown an increase in the availability of fish food organisms (Mundie et al. 1991; Quamme 1994; Perrin and Richardson 1998) or demonstrated an increase in fish size or abundance (Johnston et al. 1990; Deegan et al. 1992; Peterson et al. 1993). Notwithstanding, much of the experimental work has been conducted over relatively short time scales which cannot account for longer term interactions mediated by competition and predation. As a consequence, the majority of experimental results have emphasized resource-limited rather than a combination of resource-limited and consumer-controlled food web dynamics. With the exception of the unreplicated Kuparak River study in Alaska, where energy transfer extended over three trophic levels, most experiments have only described algal-insect inter-relationships. Single stream segment studies or related mesocosm experiments however, may not be broadly applicable elsewhere.

Application of research on nutrient limitation in other streams or rivers may not be transferable to a large river where salmon escapements historically supplied most of the nutrients that affect stream productivity. For example, the annual return of chinook, sockeye and coho salmon and steelhead trout in the Slocan River was eliminated in 1939 with completion of the Grand Coulee Dam in Washington State. Linkage between nutrient loss from salmon carcasses in lacustrine environments and commercial overharvesting of sockeye salmon was first suspected in oligotrophic nursery lakes in Alaska during the 1950's; an event which eventually lead to whole-lake fertilization experiments to improve the phosphorus supply (Ashley and Slaney 1997). While the contribution of salmon carcasses to stream ecology has been generally acknowledged, actual quantification of their importance was only determined recently. Up to 40% of the carbon and nitrogen content of juvenile salmonids in coastal streams has been

linked to a marine origin; its contribution to nursery streams having been conveyed by returning adult salmon spawners (Bilby et al. 1996). Similarly, the contribution of chinook salmon carcasses to nutrient concentration and eventual periphyton accrual was demonstrated in a small tributary to Lake Superior (Schuldt and Hershey 1995 reported in Ashley and Slaney 1997). These examples clearly underscore the historic value of anadromous species to the trophic ecology of river systems affected by hydro-electric development of the Columbia Basin.

Although enumerations of chinook, sockeye, and coho salmon and steelhead trout entering the Slocan River were never recorded, an estimated 0.5 to 1.0 million fish likely migrated into Canada (Netboy 1980). Historical diary accounts of David Thompson in 1810 as well as Baillie-Grohman (1900) in the 1880's lend support to this estimate. During their expeditions throughout the Kootenays, they reported 'millions of salmon at the outlet of Columbia Lake', 'chinook weights of 25 pounds', and 'salmon plentiful throughout the Slocan District'. Although run reconstruction of the Slocan River is hampered by the lack of escapement data, estimates have been calculated using comparative data from Upper Fraser River tributaries where chinook escapements vary from 150 to 300 fish per kilometre (Department of Fisheries and Oceans, Vancouver, B.C., file data). On the basis of this information, the spawning run of chinook salmon into the Slocan and Little Slocan rivers was estimated between 9,000 and 18,000 fish per year. Assuming an average weight of 8.4 kg per fish and based on 0.325% P and 3.0% N recovery per body weight (reported in Ashley and Slaney 1997), the nutrient contribution from chinook salmon carcasses to the food web likely ranged from 246 - 491 kg of P (i.e. 4.1 - 8.2 $kg \cdot km^{-1}$) and from 2268 - 4536 kg of N (i.e. 37.8 - 75.6 $kg \cdot km^{-1}$). In light of the combined escapements for all species, the loss of nitrogen and phosphorus from decaying salmon carcasses to whole-river nutrient dynamics was likely, considerable.

Given that historical escapements to upper Columbia River tributaries in B.C. no longer occur, it is conceivable that these systems have adjusted to an overall lower level of productivity in the absence of anadromous species. To this end, a nutrient bioassay study on the Slocan River was initiated to determine suitable concentrations of nitrogen and phosphorus to augment present background conditions. The benefits of fertilization are intended to increase the food supply of the resident rainbow trout population. In combination with ongoing efforts to address mainstem juvenile habitat impacts (Oliver 1997), improved food availability is expected to increase juvenile survival and recruitment to a population that has been in decline since the mid-1980's (Oliver 1996).

As a fisheries management tool, stream fertilization holds great promise for nutrient-deficient streams in British Columbia, particularly in those drainages where the nutrient status has been affected by human intervention. While stream enrichment is an attractive means to short term

recovery in trophic status, a prior knowledge of possible biological responses is desirable in advance of whole-stream manipulation. The nutrient bioassay technique, applied within an *in situ* mesocosm environment, is a proven method that allows integration of ecosystem processes (nutrient flux, species interactions, energy flow) under conditions more representative of nature (Richardson and Perrin 1990). In combination with an appropriate experimental design, mesocosm bioassays provide an opportunity to observe possible outcomes first-hand and more accurately predict responses at the whole-river scale. Mesocosm studies therefore, not only include realism but provide for an experimental approach wherein randomization, manipulation and replication are key requisites of its design (Hicks 1982). Owing to limitations in size and period of operation however, mesocosms have been criticized for their lack of spatial and temporal heterogeneity (Levin et al. 1989). Despite problems of scale, ecosystem processes observed over a range of treatment effects can often provide useful insight to avoid costly mistakes that may have otherwise occurred in the face of uncertainty.

The results of nutrient enrichment experiments outlined above have clearly demonstrated a community-wide response to nutrient augmentation. Experimental manipulations to date however, have focused on phosphorus addition due to its limited availability in most freshwater ecosystems (Wetzel 1975; Horne and Goldman 1994). Ambient concentrations to saturate specific cellular growth rates of lotic periphytic diatoms have been shown to occur at exceedingly low levels (0.3 - 0.6 $\mu g \cdot L^{-1}$ P; Bothwell 1988) whereas much higher concentrations have been required to achieve peak areal biomass ($\geq$ 5 $\mu g \cdot L^{-1}$ P; Bothwell 1989). In keeping with the strategy to augment the nutrient considered in shortest supply, experimental manipulations have included phosphorus additions over a wide range of concentrations (0 - 50 $\mu g \cdot L^{-1}$ P; Stockner and Shortreed 1978; Peterson et al. 1983, 1985; Perrin et al. 1987; Mundie et al. 1991; Quamme 1994). These same experiments have included a surplus of nitrogen (up to 400 $\mu g \cdot L^{-1}$ N) to alleviate concerns of possible N-limitation following phosphorus amendment. The majority of experimental designs have, therefore, achieved these results with relatively high concentrations of nutrient (generally $\geq$20 $\mu g \cdot L^{-1}$ N and $\geq$5 $\mu g \cdot L^{-1}$ P) but have not always considered exact N:P ratios.

The nutrient ratio approach used in the present study was predicated on the prior understanding that algae portray a variety of physiological attributes (nutrient uptake, efflux, storage and assimilation) that lead to competitive success where species are constrained by the same growth-limiting nutrient (Borchardt 1996). More importantly, no one species has been capable of meeting the suite of physiological requirements that would provide a competitive advantage under all nutrient supply regimes observed in nature. In view of these physiological differences, algae are partitioned along environmental gradients where a specific combination of nutrients

favour individual species over the range of nutrient supply ratios afforded in nature (Rhee and Gotham 1980; Tilman et al. 1982; Turpin 1988). Physical, chemical and biological processes operating at different spatial and temporal scales provide the mechanism for change in environmental gradients. In concert with the array of physiological trade-offs of individual species (e.g. cell quotas of the growth-limiting nutrient, nutrient uptake rates, etc.), nutrient ratios that change under non-equilibrium conditions often result in shifts in algal community composition (Turpin and Harrison 1979, Sommer 1985, Olsen et al. 1989 cited in Borchardt 1996). Although physical processes such as stream temperature (DeNicola 1996), light intensity (Hill 1996) and disturbance (Peterson 1996) are important, nutrient supply ratios ultimately define the successional trajectory and eventually shape benthic algal community structure (McIntire et al. 1996). The existence of spatial and temporal successional sequences of phytoplankton in lentic environments are similarly supported by resource ratio theory (Tilman 1982; Tilman et al. 1982).

Species-specific optimum ratios of nitrogen and phosphorus have been described for algae by comparing N:P atomic ratios at the cellular level; ratios that are generally high among the green algae and low among the diatoms for the species tested (Rhee and Gotham 1980, Sommer 1988). Under nutrient augmentation, benthic diatoms have been shown to respond positively to nitrogen and/or phosphorus enrichment (Pringle and Bowers 1984, McCormick and Stevenson 1989, Stevenson et al. 1991), whereas green algae have generally required high concentrations of nutrient (particularly P) to become abundant (Happey-Wood 1988; Davis et al. 1990 cited in Borchardt 1996). While phosphorus enrichment of lentic environments has usually led to dominance by nitrogen-fixing cyanobacteria (Schindler 1977), the same pattern has not been consistently observed in lotic environments (e.g. dominance, Elwood et al. 1981; low abundance, Stockner and Shortreed 1978).

Of equal importance, nutrient ratios that favour one group of algae over another will likely provide differences in nutritional quality for consumers based on the chemical composition of individual species. For example, colonial chlorophytes are considered especially nutritious for aquatic insects over filamentous forms due to their relatively higher protein and lipid content (Lamberti 1996). In contrast, the nutritional value of cyanophytes is considered poor due to their low assimilatory values as well as other cellular constituents that may affect their palatability (Paerl 1988). Although diatoms have been considered an important component of the diet of stream invertebrates (Gregory 1983), protein and lipid content are relatively low and ash content is among the highest of the algae (Lamberti 1996). Notwithstanding, the thicker cell wall and outer mucous layer of chlorophytes are less easily digested by grazers when compared to diatoms (Lamberti and Moore 1984). Moreover, high ingestion and assimilation rates of diatoms

may compensate for the lower nutritional value (Lamberti 1996). Thus, stream fertilization should be evaluated on the basis of increased algal biomass as well as nutrient ratios that promote species assemblages that are highly edible and nutritious.

Differences in food quality have also been demonstrated between benthic algae and detritus; the former having a higher food value (Lamberti 1996). The quality of food consumed has implications on growth, voltinism and size at maturity of aquatic insects that occupy distinct functional feeding groups (i.e. food acquiring mechanisms; Anderson and Cummins 1979; Merritt and Cummins 1984). Anderson and Cummins (1979) proposed a nutritional gradient, from lowest to highest value, where wood < terrestrial leaf litter < fine particulate organic matter < decomposing vascular hydrophytes and filamentous algae < living algae (primarily diatoms) < animal tissues. The relative food value suggests that food quality is highest for predators followed by grazers followed by collectors followed by shredders. Evidence for the importance periphyton over detritus in relation to larval insect growth has been demonstrated in intermediate instars of hydropsychid filter-feeding caddisflies (Fuller and Mackay 1981). Increased periphyton biomass, from nutrient augmentation studies reported in the above, has similarly been shown to increase survival of benthic invertebrates to emergence, increase emergence, increase emigration within the drift and increase the density of the total benthos.

Given the results of earlier stream fertilization experiments, there is little question that algal biomass and insect abundance will increase in oligotrophic waters in response to elevated levels of nitrogen and phosphorus above background; benefits that have accrued over three trophic levels. Yet few studies have reported both quantitative and qualitative relationships of benthic algae and insects to modest concentrations or specific ratios of nitrogen and phosphorus. Under low-level concentrations and nutrient-specific ratios, it is hypothesized that (1) quantitative differences in benthic algal biomass and insect abundance should occur in response to elevated levels above background and (2) qualititative differences in benthic algal community structure between treatment ratios may exhibit both positive and negative effects on primary consumers (grazers, collectors, shredders) despite improvements in overall algal biomass. More specifically, differences in fish growth as a result of differences in insect abundance under modest nutrient loadings should be achieved by growing:

(a) more edible, small algal cells that generate quickly at high N:P;

(b) a wide diversity of algal cells for many diverse consumers at intermediate N:P; and

(c) less edible, very large, filamentous cells that do not slough at low N:P.

Manipulation of N:P ratios should result in different algal responses where diatoms and cyanophytes are favoured under lower N:P ratios while chlorophytes (particularly small, colonial

green algae) are favoured under higher N:P ratios (Rhee and Gotham 1980). Nutrient-specific (optimum N:P) ratios for individual algal species that contribute to higher growth rates (Horner et al. 1983; Bothwell 1989) are also expected to display differences in areal-specific growth rate, higher peak biomass over a shorter duration, sloughing later in the accrual cycle due to higher nutrient diffusion gradients, and an increased rate of succession from diatoms to large green filamentous algae or cyanophytes (i.e. successional trajectories that favour species assemblages suited to the nutrient supply ratio). Differences in quantity and quality of algal communities are also expected to determine invertebrate species assemblages and growth rate. Indicator variables to track community level responses to nutrient manipulations will include algal cell type, density and biomass, insect type, density, biomass, size and drift rate.

The purpose of this study is to evaluate benthic algal and insect responses using a range of nutrient concentrations up to 20 $\mu g \cdot L^{-1}$ N and 5 $\mu g \cdot L^{-1}$ P. Specific N:P concentrations are varied at a 1:1 and 4:1 ratio and thereby compare treatment effects on abundance, biomass and taxonomic composition at both community levels, and identify time lag phases relative to algal and insect community dynamics. Specific objectives are to:

1) describe the qualitative relationship between periphyton assemblages and macro-invertebrate taxa,

2) describe the quantitative relationship between algal and macro-invertebrate abundance and biomass,

3) contrast differences in body size of aquatic invertebrate larvae in the drift and benthos within individual treatments, and

4) determine the nitrogen and phosphorus concentration and ratio that are probable in maximizing fish growth in the Slocan River.

1.1 Description of Study Area

The Slocan River is a fifth order stream (1:50,000 scale) located in the southeastern interior of British Columbia between Nelson and Castlegar (117° 31′ 30″; 49° 25′ 20″). The Slocan River drains Slocan Lake and flows south to join the Kootenay River / Brilliant Reservoir at South Slocan (Fig. 1). It is a large meandering river with a mean annual discharge of 89 $m^3 \cdot sec^{-1}$ (Anon. 1988). Mean summer flow (July 1-September 30) is 95 $m^3 \cdot sec^{-1}$ and mean summer temperature is 15.4 C (Anon. 1977; Fig. 2). Water temperatures during August have been previously reported above 20 C.

The lower reaches of the river (30 km) are typical of repeated riffle-glide sequences with

occasional large pools and a cobble/boulder-dominated bed material. Over this same distance, the channel is moderately incised and ranges in width from 40 to 50 m. The upper reaches of the river immediately downstream of Slocan Lake resemble small shallow lakes owing to an exceptionally wide channel (up to 400 m) during spring freshet. Further downstream, the channel averages 50 m in width and is relatively unconfined for approximately 15 km. The bed materials are comparatively smaller in size with sand and silts often comprising the stream bottom. The river can be described as a stable river system by virtue of its lake-headed nature, with an abundant water yield (low summer flows recorded at 64% of mean annual discharge). The river has 10 to12 minor tributaries and one major tributary (Little Slocan River) which is also lake-headed.

Water quality surveys conducted in the past indicate the river is oligotrophic (BC Environment, Environmental Protection, Nelson, B.C.; file data). Ambient dissolved orthophosphorus levels as SRP are low (< 3 $\mu g \cdot L^{-1}$) and always below the detection limit by standard analytical (i.e. wet chemical) techniques. Nitrate and nitrite-nitrogen levels can often exceed 100 $\mu g \cdot L^{-1}$ during winter but levels generally range between 20 and 40 $g \cdot L^{-1}$ during early summer. Over the period of record, NO_3-N has dropped below the detection limit (<0.020 $mg \cdot L^{-1}$) during late summer (August). Ammonia-nitrogen is always below detection (<0.005 $mg \cdot L^{-1}$). Total alkalinity is approximately 42 $mg \cdot L^{-1}$, pH ranges from 7.2 to 7.6 and total dissolved solids are approximately 50 $mg \cdot L^{-1}$.

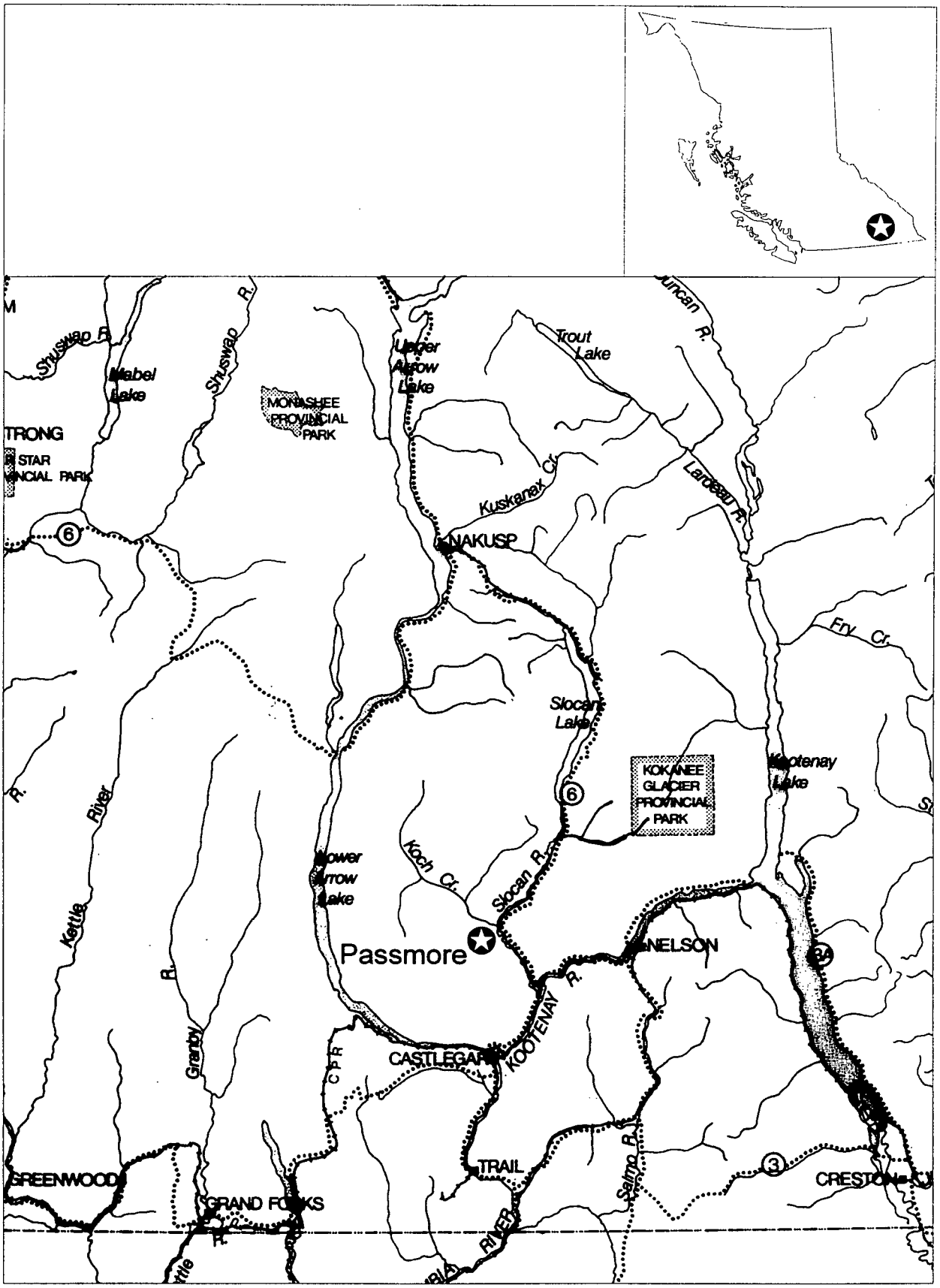

Figure 1. Location of the Slocan River in southeastern British Columbia.

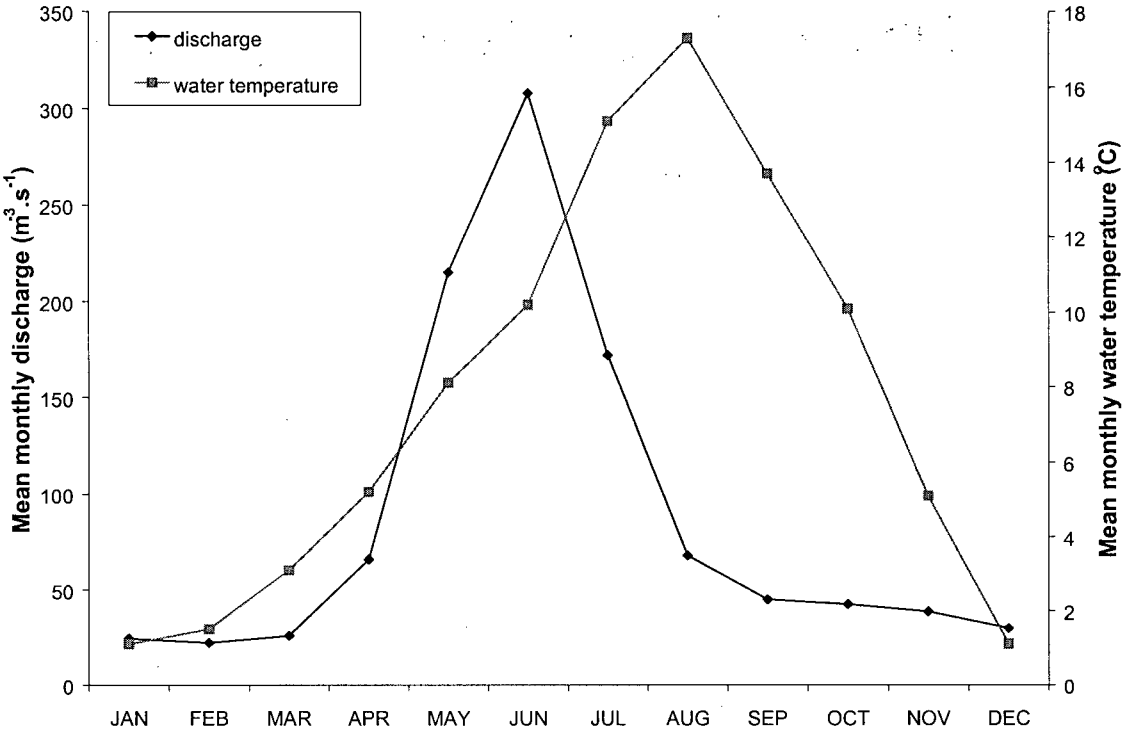

Figure 2. Mean monthly flow and water temperature (1925-1976) in the Slocan River near Crescent Valley, B.C. Water Survey of Canada Station 08NJ013.

The Slocan River supports a wide variety of fish species: rainbow trout (*Oncorhynchus mykiss*), bull trout (*Salvelinus confluentus*), mountain whitefish (*Prosopium williamsoni*), northern squawfish (*Ptychocheilus oregonensis*) , as well as catostomids, cottids and other cyprinids. Steelhead (anadromous rainbow), chinook (*O. tshawytscha*), sockeye (*O. nerka*) and coho (*O. kisutch*) salmon can no longer access this system.

A continuous-flow, instream mesocosm, used to test differences in nitrogen and phosphorus treatment effects, was located approximately 1 km downstream of the confluence of the Little Slocan and Slocan rivers (refer to Fig. 1). The mesocosm was anchored to a steel bridge crossing the Slocan River at Passmore, B.C. It was situated approximately 30 m from the east shore where river flow was laminar.

2.0 Methods

2.1 Experimental design and analysis

Review of the historical water quality data collected from the Slocan River during the 1970's (BC Environment, Environmental Protection, Nelson, B.C.; file data) revealed a high degree of

seasonal variation in nitrogen concentration. Over the period of record, nitrate-nitrogen often exceeded 120 $\mu g \cdot L^{-1}$ by mid-winter dropping, on average, to 30 $\mu g\ L^{-1}$ by late-summer; a single record <20 $\mu g \cdot L^{-1}$ occurred in 1975. In contrast, the annual level of dissolved inorganic phosphorus was consistently reported <3 $\mu g \cdot L^{-1}$. Since low-level nutrient sampling techniques were never completed during annual monitoring, dissolved nitrogen (N) and phosphorus (P) levels within periods of lowest availability were non-detectable using standard analytical procedures (Zenon Laboratories; general ion scan). Low-level nutrient sampling in the summer of 1992, however, revealed nitrate minima of 5 $\mu g \cdot L^{-1}$ while the detection limit for dissolved inorganic phosphorus was again <1 $\mu g \cdot L^{-1}$. Comparison of the cellular N:P atomic ratios of 16:1 (i.e. the Redfield ratio considered as the benchmark for community-wide optimum nutrient ratio for algal physiology (Borchardt 1996)) with ambient nutrient concentrations of the Slocan River suggest that phosphorus is growth-limiting most often. This conclusion is inferred from experimental evidence for phytoplankton in lakes where ambient N:P ratios of >20:1 are considered P-limited, <10:1 are considered N-limited and between 10 and 20 to one are regarded as indeterminate (Borchardt 1996). Notwithstanding, the results of the 1992 low level analysis imply that co-limitation of nitrogen is conceivable in some years and therefore experimental treatments to address potential nutrient deficiencies should incorporate applications of both nitrogen and phosphorus to overcome the possibility of any transitional limitation.

A nutrient bioassay study was selected to investigate algal and benthic invertebrate responses to incremental increases in nutrient concentration and ratio, above background levels. An instream, floating mesocosm was employed to test mean differences in treatment applications. Experimental N:P concentrations (in $\mu g \cdot L^{-1}$) were fixed at 1:1, 3:3 and 5:5 (group 1) and each treatment varied at a 4:1 ratio to provide alternate concentrations of 4:1, 12:3 and 20:5 (group 2). The 1:1 and 4:1 ratios are equivalent to 2.3:1 and 9.2:1 μmolar ratios, respectively. Although these ratios remain within the range considered N-limited, the intent was to evaluate modest increases in nitrogen without inducing P-limitation. Each of the six treatments and control (0:0) were replicated for a total of 14 experimental units. Two additional troughs were used to account for periphyton settlement in troughs from upstream sources. Following an initial assessment of water velocity variability among troughs, control and treatment groups were randomly assigned in pairs; each replicate trough for a single treatment having similar velocity. Although the allocation of experimental units cannot be considered as a completely randomized design, pairing of troughs was undertaken to control for measurable flow differences, minimize experimental error and maximize the effect of individual response variables. Troughs were numbered from 1 to 16 (left to right; upstream orientation) across the mesocosm. Nutrient

manipulations were monitored in troughs 2 through 14. Pairing of control and treatment troughs occurred in the following order:

N:P Concentration	0:0	1:1	3:3	5:5	4:1	12:3	20:5
Trough Number	6,11	2,4	3,15	8,9	12,14	7,10	5,13

Response variables were qualitatively and quantitatively assessed. Representative benthic algae and insects were described in terms of taxonomic richness and measured in terms of numerical abundance and biomass (Krebs 1989).

Differences in treatment effects were tested by analysis of variance using orthogonal contrasts. Contrasts were arranged *a priori* to compare differences in means between groups 1 and 2 and the control as well as within (e.g. 1:1 vs 3:3 vs 5:5 and 4:1 vs 12:3 vs 20:5) and between (e.g. 1:1 vs 4:1, 3:3 vs 12:3 and 5:5 vs 20:5) groups 1 and 2. Probability of acceptance was set at $\alpha = 0.05$ for each contrast. A null hypothesis of no significant difference between treatments was used to evaluate individual analyses for each trophic level. A logarithmic transformation of the data was applied where initial examination of residuals failed to demonstrate homogeneity of variance. Statistical analyses were completed using the statistical package provided in Systat for Windows, Version 5 (Wilkinson et al. 1992).

2.2 Experimental apparatus

Responses to nutrient manipulation employed the trough bioassay or mesocosm concept (Bothwell 1983) that has been used elsewhere in the province of British Columbia (Carnation Creek, Stockner and Shortreed 1978; Keogh River, Quamme 1994; Nechako River, Perrin and Richardson 1998). Each of these applications consisted of a shore-based microcosm supplied with water piped from an instream gravity-fed intake to a header tank. In contrast, an in-channel, suspended, flow-through microcosm was used in the present study (Fig. 3a). The apparatus consisted of 14 clear and 2 black, plexiglass troughs 18 cm in width and depth and 2.4 m in length (Fig. 3b). The fourteen clear troughs were used for nutrient manipulations whereas the two black troughs were used to measure passive settlement of algae from upstream sources. Troughs were constructed with material 65 mm in thickness. Each set of troughs was equipped with a set of baffles at the influent end to balance flow characteristics

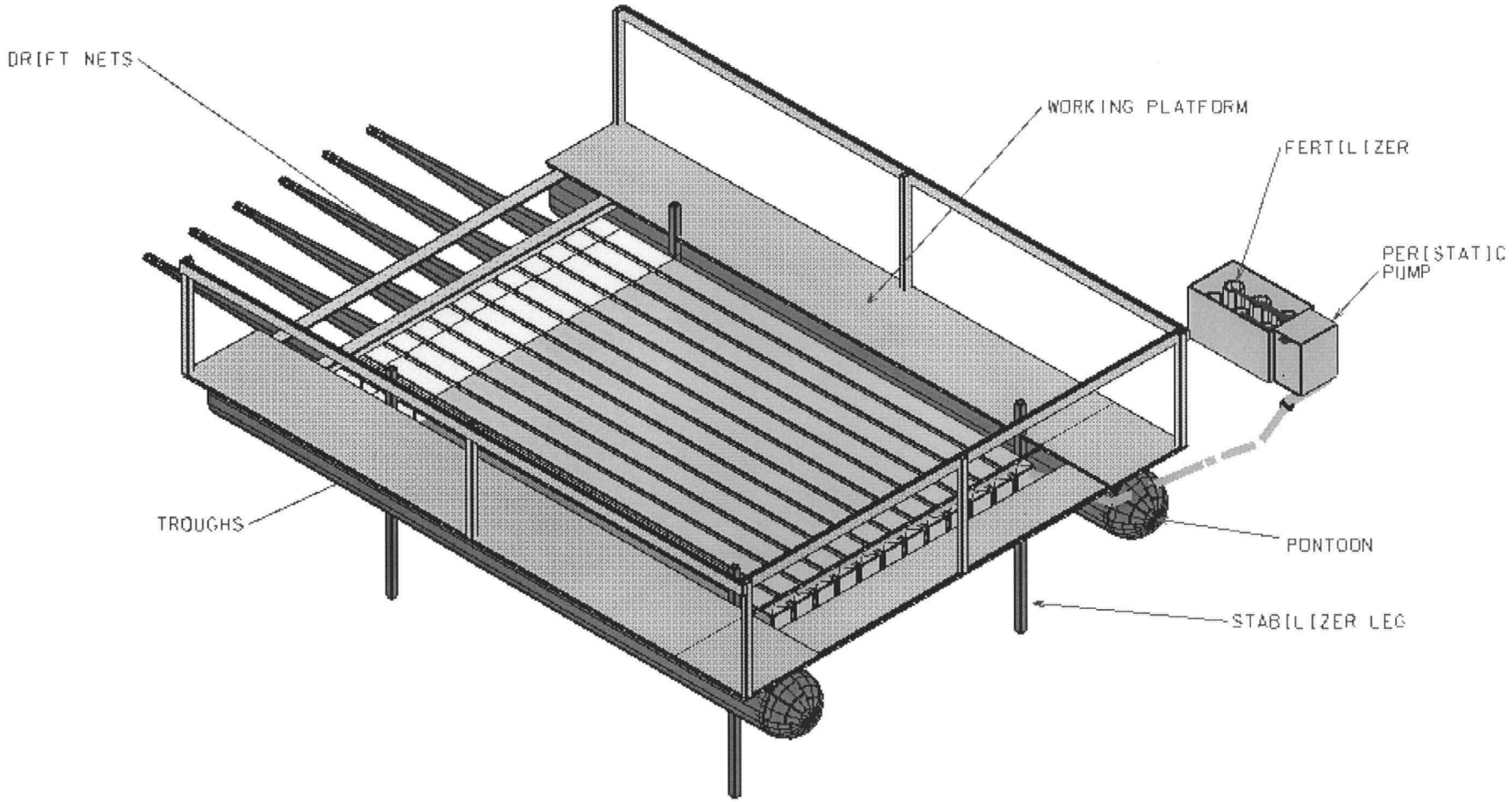

Figure 3a. Conceptual drawing of instream mesocosm .

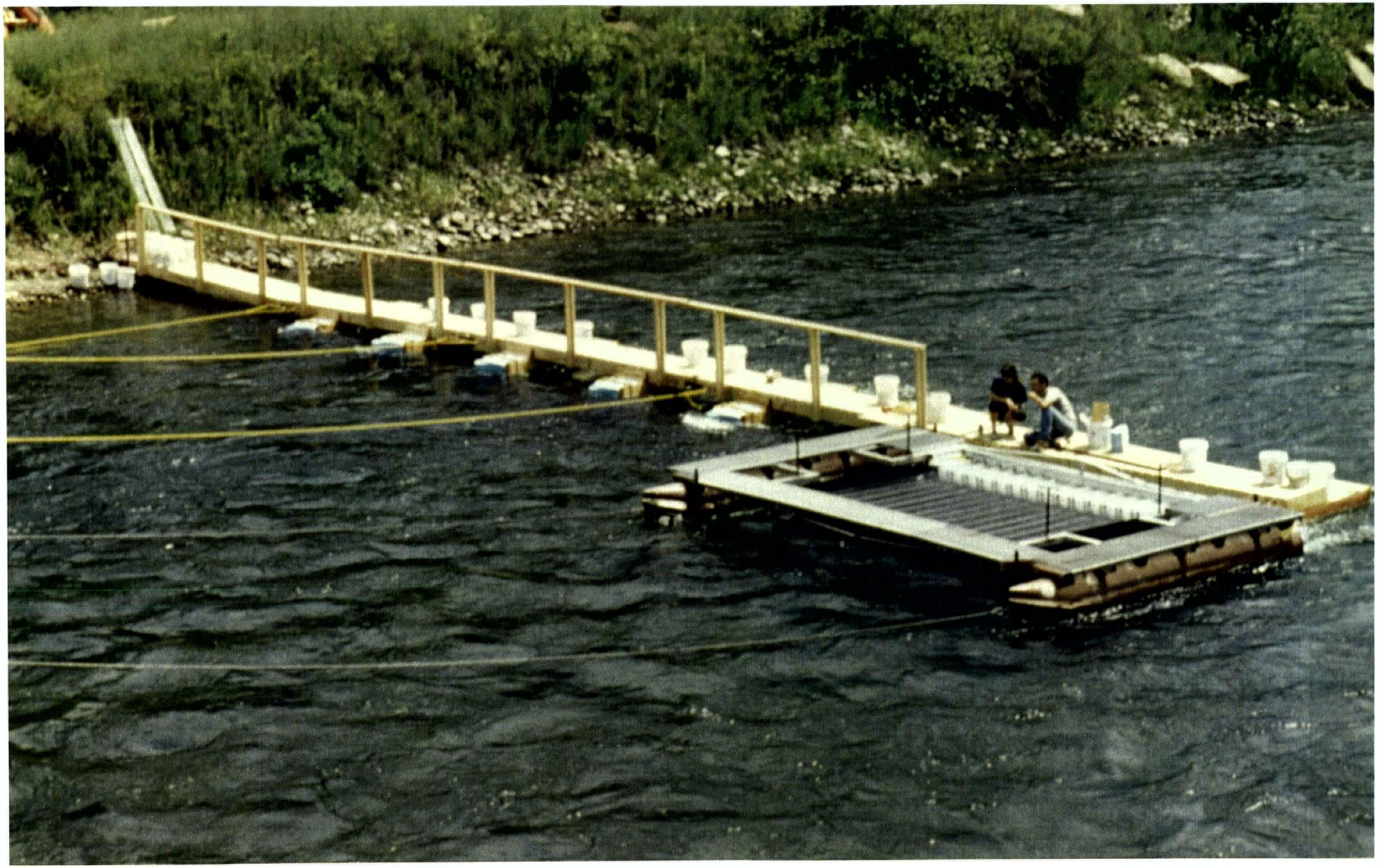

Figure 3b. Mesocosm placed in-stream at Passmore, B.C.

and ensure mixing of nutrients. Each treatment trough was similarly fitted with a clear, plexiglass lid to prevent the escape of emergent insects. Fine nylon mesh netting was placed over the influent end of the troughs between the water surface and the lid to close the entire structure. Troughs were suspended near the river surface and supported by an aluminum frame between two pontoons. Each trough was bolted to the frame to prevent its being dislodged. The apparatus was further equipped with a stabilizer leg in each corner to facilitate leveling of the raft assembly and maintenance of a constant flow within individual troughs.

Pre-screened, clean, even-sized (3-5 cm) gravel was added to each trough to simulate a natural streambed (Fig. 4a). Sand particles were avoided to maximize attachment sites for periphyton and interstitial areas for macroinvertebrates. Gravel was placed to a depth of approximately 7 cm and an average water depth of 3.5 cm was maintained. Four gravel baskets (area 100 cm^2) were installed within the lower one-half of each trough to quantify invertebrate density through time. Similarly, open cell styrofoam DB (Snowfoam Products Ltd., El Monte, California) was secured to a raised plexiglass platform (15 cm by 15 cm) immediately downstream of the gravel bed to measure chlorophyll *a* biomass. The platform elevation was continuous with the gravel bed surface and the styrofoam substratum provided a horizontal plane from which periphyton biomass was sampled through time.

A drift net measuring approximately 1.3 m in length was attached to the effluent end of each trough to intercept daily insect drift and emergence (Fig. 4b). The body of the net was conical in shape (maximum diameter approximately 30 cm) and constructed of 100 μm Nitex cloth. The drift net was reinforced with a nylon collar at each end to prevent excessive wear to the woven fabric. A side-screened collection bottle was fastened to the downstream end of the net to facilitate insect removal. The screen was similarly constructed of 100 μm Nitex cloth. The upstream end of the drift net was fitted to the effluent end of the trough and secured with stretch cord.

The raft assembly was outfitted with a work platform along two sides (i.e. over each pontoon) as well as across the upper end of the structure to facilitate monitoring and sampling activities. A floating walkway was constructed to provide access from the shore to the mesocosm. A waterproof wooden box was placed across the upper end platform to provide storage for 20 L nutrient containers and a peristaltic pump. The mesocosm was cabled to a steel bridge over the Slocan River at Passmore, B.C. and positioned in laminar flow near the east river bank.

Figure 4a. Artificial stream troughs used to monitor treatment effects. Note baffles at influent end, interim benthic baskets within the lower two thirds, and styrofoam substratum near the effluent end, of each trough. Microbore tubing for nutrient delivery is also shown adjacent to each baffle.

Figure 4b. Drift net size and shape. Note re-inforced nylon collar at each end of the Nytex fabric.

2.3 Nutrient preparation, delivery and monitoring

Reagent grade ammonium nitrate (NH_4NO_3) and potassium dihydrogen phosphate (KH_2PO_4) were used in liquid formulation for each treatment. Calculations for nutrient stock solutions were based on trough dilution rates (trough flow and drip rate) and percentages of N or P (by atomic weight) within each chemical fertilizer.

Measured quantities of each nutrient were dissolved in distilled water for a batch volume of 20 L. Mean flow rates were derived from paired trough discharge measurements since a single stock solution of target concentration supplied replicate troughs. Stock solutions were prepared every ten days over the duration of the experiment and incorporated differences in ambient trough flow (particularly velocity) relative to river discharge. Fertilizer was conveyed to each trough through 1.3 mm microbore tubing by means of a Technicon autoanalyzer pump. The fertilizer was delivered at a drip rate of 1 $ml \cdot min^{-1}$ and was dispersed immediately above the baffles at the upstream end of each trough to ensure complete mixing.

Water samples were collected from each trough bi-weekly to verify the accuracy of nutrient concentrations. A composite from three separate samples taken over a 15 s period (based on drip rate) was obtained to ensure an average concentration was measured. Trough samples were filtered through 0.7μm ashed Millipore GFF filters and sample bottles were pre-rinsed in distilled deionized water prior to filling. All samples were stored on ice and shipped to the Department of Fisheries and Oceans, West Vancouver Laboratory for processing within 24 hrs of sampling. Analytical techniques followed procedures outlined in APHA (1989) with detection limits as low as 1 $\mu g \cdot L^{-1}$ for NO_3 + NH_4 and 0.5 $\mu g \cdot L^{-1}$ for SRP (E. McIsaac pers. comm.). Drip rates were monitored four times during the experiment, twice in August and twice in September. Fertilizer from each microbore tube was collected in a graduated test tube over an average of ten minutes to determine the accuracy of the delivery rate. The microbore tubing was replaced at ten day intervals to prevent reductions in delivery rate due to fertilizer viscosity.

2.4 Physical data, experimental operations and sampling

Slocan River water temperatures at Passmore, B.C. were monitored three times daily at 08:30, 12:00 and 16:30 using a high precision mercury thermometer throughout the study. A Ryan Model J90 thermograph was installed at the mesocosm September 9, 1992 and provided continuous temperature readings until the end of the experiment. Mean water temperatures were calculated for the hand-held data series. Thermograph readings were plotted at 2 hr intervals to account for daily variation in stream temperature during late summer. Staff gauge

measurements of the Slocan River at Passmore were similarly recorded daily at 08:30 and 16:30 and averaged to monitor daytime water level differences throughout the study.

Water levels in troughs were monitored daily to maintain nutrient concentrations under constant flow and therefore, mesocosm elevation was altered daily in response to declining river flow. Depth and velocity were measured in each trough every second day. Additional days in August were also measured to closely monitor trough flow rates in relation to steadily declining river levels. Depth was measured at the upper, middle and lower section of each trough while velocity was measured at the middle and lower sections where flow was most uniform. Current velocity in each trough was measured with a Marsh-McBirney electromagnetic velocity sensor.

The raft assembly was initially installed in the Slocan River July 7, 1992. The mesocosm apparatus was operational (i.e. completed installation of troughs, fry chambers, gravel, invertebrate baskets, periphyton platforms, etc.) July 10 and natural colonization by benthic algae and insects proceeded for a period of two weeks. Fertilization of troughs was initiated July 28 and continued until September 30, 1992. Overall, the experiment continued for a total of 83 days. Owing to a marked decline in river flow along the eastern margin of the river channel, the mesocosm was shifted to west side of the river September 2, 1992 (day 55) where a higher stream velocity sustained a more suitable trough flow until the end of the experiment.

Drift nets were installed at the onset of fertilization. Drift and emergence were sampled every third day from July 29 to September 30, 1992. Individual drift nets were sprayed with a wash bottle to remove insects caught on the fabric. Basket samplers, used to estimate benthic invertebrate density, were removed from each trough August 17 and September 14, 1992. Aquatic insects were collected, stored in glass jars and immediately preserved in 70% ethanol. Chlorophyll a, used as an estimate of algal biomass was collected weekly from styrofoam plates August 4 to September 31, 1992. An initial sample was obtained July 23 to compare levels across all troughs prior to fertilization. Samples from the two black troughs were used to account for passive settlement of drifting algae from upstream sources. Individual cores were removed by compressing the open end of a plastic vial against the styrofoam plate. Styrofoam cores (5.7 cm^2) complete with periphyton sample were stored in separate plastic vials, held at -20 °C and shipped to Zenon Environmental Laboratories for processing. Duplicate cores were obtained during each sample period to provide a mean estimate of biomass owing to expected variation between cores reported in the literature (Bothwell 1983). Styrofoam plates were replaced in each trough August 12 and September 4, 1992 to track each accrual series over a given time interval, monitor weekly growth rates and estimate time to attainment of peak biomass for each treatment. The first two time-course accrual series were sampled over three weeks whereas the third series extended over a four week period. Representative algal taxa

were identified and algal cell density estimated from three rock samples collected from each trough August 13 and September 4, 1992. Periphyton samples on styrofoam substrata were collected simultaneously to assess potential differences in representative species due to differences in substrate type. Individual stones were randomly selected from stream troughs by "blind touch". Similarly, styrofoam cores were randomly sampled within the area of the styrofoam plate. All samples for taxonomic identification or cell count were stored in glass jars and preserved in Lugol's solution. Prior to identification, both gravel and styrofoam substrata were either scraped or brushed to remove attached algae.

2.5 Periphyton analysis

2.5.1 Identification and algal cell density

Identification of algal taxa within each treatment was provided by Fraser Environmental Services in Vancouver, B.C. Subsample volumes of 25 ml from gravel substrates were initially settled in Utermohl chambers for 2 hrs. Individual slides for each treatment were prepared and scanned at magnifications up to 1000x. Examination of the entire slide was completed to account for all taxa present. Individual taxa were identified to genus (in some cases, species). Three diatom slides were prepared for each sample following either pyrolysis or acid digestion to enhance imaging of frustule structure. Taxonomic richness was compared between treatments by simply enumerating all genera and/or species identified within troughs (Krebs 1989). Differences in taxonomic richness were compared over three periods spaced at 3 week intervals (August 13, September 4 and September 30). Taxa were grouped as blue-greens (Cyanophyceae), green algae (Chlorophyceae), diatoms (Bacillariophyceae) or flagellates (Chrysophyceae, Euglenophyceae, Pyrrophyceae and Cryptophyceae) for comparative purposes.

Subsamples of 25 ml volume were similarly used for algal enumeration. Cell counts incorporated 10 random fields and a minimum count of at least 100 individuals of the dominant species or genus was completed. Quantitative estimates of the entire sample were extrapolated from subsample counts using methods described in APHA. (1989). The data were reported as cells·cm^{-2} having calculated the surface area of sampled stones from each trough, colonized by periphyton. The perimeter of an impression of the stone surface, using aluminum foil, was digitized to provide an estimate of surface area.

2.5.2 Algal biomass

Chlorophyll *a* analysis was completed by Zenon Environmental Laboratories in Vancouver, B.C. Fluorometric determination of chlorophyll *a* levels, following acetone extraction, was achieved

using methods described in APHA (1989). Time-course chlorophyll *a* levels were illustrated on a semi-log plot to follow changes in accrual for the September 4 - 30 series. A test for equality of slopes was completed by analysis of covariance (time by treatment interaction) to assess statistical differences in algal accrual relative to nutrient concentration. Potential biomass differences within and between the 1:1 and 4:1 ratios were further evaluated by orthogonal contrast. Similarly, the affect of flow variation on periphyton accrual was tested by repeated measures analysis of covariance (i.e. flow by treatment interaction). Adjusted chlorophyll *a* levels, which accounted for the amount attributed to passive settlement of algae, were used in each biomass analysis following logarithmic transformation.

2.6 Macroinvertebrate analysis

2.6.1 Sorting, identification and sub-sampling

The procedures for sorting aquatic insects obtained in the drift differed from those acquired in the final trough benthos (i.e. total insect larvae remaining in troughs at the end of the experiment). Preserved drift samples were initially washed through a 2 mm brass sieve to remove coniferous needles, deciduous leaves and other coarse woody material. Total counts of invertebrates > 2 mm were recorded and the larger number of insects passing through the 2 mm sieve were collected in a 70 μm sieve, sorted and enumerated separately. Identification of all insects to family was completed with a binocular microscope at 10 - 25x magnification following taxonomic keys provided in Merritt and Cummins (1984). Where excessive numbers of insects were encountered, one quarter of a 500 ml volume containing individuals < 2 mm was obtained with a modified Folsom plankton splitter. The apparatus consisted of a rotating plexiglass cylinder fitted with four internal dividers over one-third of its inner circumference. The liquid sample was poured into an opening in the side-wall of the cylinder, rotated and poured out into four separate plexiglass trays. An initial trial sample was tested for randomness using the index of dispersion based on the equality of variance and mean in a Poisson series (Elliot 1977). Agreement with a Poisson distribution was achieved for 16 families of insects and hence a single sub-sample was enumerated for all other drift samples where invertebrate numbers exceeded 200. Total counts of sub-sampled individuals were expanded by a factor of 4 and added to the original count of individuals > 2 mm to provide an estimate of the total number of insects collected in the drift over a 3 d period. Invertebrate drift data was analyzed at 6 d intervals from July 30 to September 30, 1992. An additional sample was added at the beginning of the series (day 24) as well as a replacement sample date on day 74 due to incomplete sampling of a single control trough on day 77.

In contrast, final trough benthos samples were passed through a stack of brass sieves ranging from 850 to 177 μm to ensure retrieval of the various invertebrate size classes mixed with fine organic debris and sand that had accumulated in each trough over the duration of the experiment. Invertebrates were washed from the slurry with a Nalgene bottle and the procedure was repeated for each of the larger sieve sizes until recovery of the benthos was complete. Insects 3 mm and larger were sorted using a dissecting microscope and a gridded Petri dish. The remaining smaller sizes were grouped separately for enumeration and biomass processing. All samples were stored in 70% ethanol. Invertebrate samples from benthic baskets, collected on August 17 and September 14, were processed in the same manner. Taxonomic identification and total counts for the final trough benthos and reference benthic baskets were completed by Karen Needham of the Entomology Museum, Department of Zoology at UBC. The taxonomic keys provided in Merritt and Cummins (1984) were similarly followed. Reference samples from benthic baskets were used for varification of identity of larval insects collected in drift as well as benthic basket samples collected on the alternate date.

2.6.2 Body size measurement

Individuals within the Ephemeroptera, Plecoptera and Trichoptera were measured for head capsule width (mm) to contrast differences in growth between control and treatment groups. Although body length has been recommended as the measurement of choice for biomass estimation (Smock 1980), head capsule width was favoured owing to apparent shrinkage of abdominal segments in these three orders following storage in ethanol, without fixation in formalin. Alternatively, body length (mm) of chironomid larvae was measured since abdominal segments were comparatively unaffected. Measurements were completed with a binocular dissecting microsope fitted with a drawing tube, a SummaSketch III digitizing graphics tablet, a microcomputer and a software package (Zoobbiom Ver 1.3) developed by Russell Hopcroft of the Zoology Department at the University of Guelph. The stylus of the digitizer "puck" was fitted with a small red diode. The image of the diode was conveyed through the drawing tube by a series of mirrors and superimposed on an object situated on the stage of the dissecting scope. Head capsule width, measured across the widest portion of the head for each individual, was accomplished by positioning the diode image at the appropriate points on the target insect and clicking the stylus at each position. The graphics tablet simply measured the distance between points. Body length along the curvature of the abdomen of chironomids was estimated by summing short, in-line segments over the length of the abdomen. Measurements were calibrated with a metric ruler upon program initiation.

Head capsule width measurements were completed for baetid nymphs on three occasions

during the experiment (day 30, 47 and 65) due to their abundant representation in the drift. Each event corresponded to the periods of peak emigration to compare differences in growth. Similarly, measurements were completed for nine families of aquatic insect occurring in the final trough benthos at the end of the experiment (day 83). The majority of individuals within samples were measured to provide equal characterization of all size categories. Where sample numbers were exceptionally large, individuals within split samples, obtained from prior enumeration procedures, were similarly measured. Individuals from 11 families of insects within the four sub-samples obtained from the initial splitter trial were measured and tested for size difference by analysis of variance to account for possible bias in size selectivity associated with the split sampler. Of the 11 families measured, significant differences ($p<0.05$) across the four sub-samples were evident for 4 groups (Chironomidae, Hydroptilidae, Leptophlebiidae, and Heptageniidae). Due to their relative contribution to the benthos, measurements of individuals < 2 mm were provided for chironomid and hydroptilid larvae and heptageniid nymphs within sub-samples and combined with individuals > 2 mm. All measurements between replicate control and treatment troughs were then pooled for each family.

2.6.3 Biomass

Following enumeration and body measurement, single individuals of families of insects were oven dried separately at 60 °C for 24 hours. Dry mass was determined with a Cahn Microbalance sensitive to 1 μg for benthic insects in basket samples ($g \cdot 100\ cm^{-2}$) on days 47 and 65 as well as total benthos (g) in troughs on day 83. Individuals > 2 mm were dried separately from those < 2 mm and combined to provide total weights for each sampling period.

3.0 Results

3.1 Water temperature and level

Mean daytime water temperatures in the Slocan River at Passmore were highly variable over the experimental period ranging from 11 to 21.5 C (Fig. 5). The highest water temperatures were encountered from August 15 to 20 when daytime means consistently exceeded 20 C. A general decreasing trend in water temperature was observed beyond August 20. River levels displayed a steady decline and dropped approximately 0.5 m over the period of study. A storm event on September 23 raised levels about 0.2 m (refer to Fig. 5). Daily water temperatures from September 9 to 30, 1992 were again variable ranging from about 9.5 to 15 C (Fig. 6).

Diurnal variation ranged approximately 2 to 2.5 C and the largest single decline (about 5 C) coincided with increasing flows following the late September storm.

3.2 Trough flow variation and water chemistry

Under laminar flow conditions along the east margin of the river (days 15-56), mean flow across experimental units was not significantly different (repeated measures ANOVA; $p>0.05$) although somewhat variable (Fig. 7). Re-positioning of the mesocosm along the western margin of the channel (days 59-82) however, resulted in significant differences in mean flow across experimental units (repeated measures ANOVA; $p<0.01$) as well as a higher degree of variation in flow for troughs located near the centre of the apparatus. Re-positioning was necessary to improve in-trough water velocities that decreased markedly in response to declining river levels (refer to Fig. 5); the eastern one-half of the channel having considerably lower velocity than the western one-half due to location of the thalweg against the west bank of the river and increasing bed elevation toward the east bank. Similarly, significant differences in trough flow between replicates of the same nutrient treatment level were also evident in the latter half of the experiment (repeated measures ANOVA; $p<0.01$). Mean discharge levels were higher during the latter half than those measured during first half of the experiment. Initial pairing of

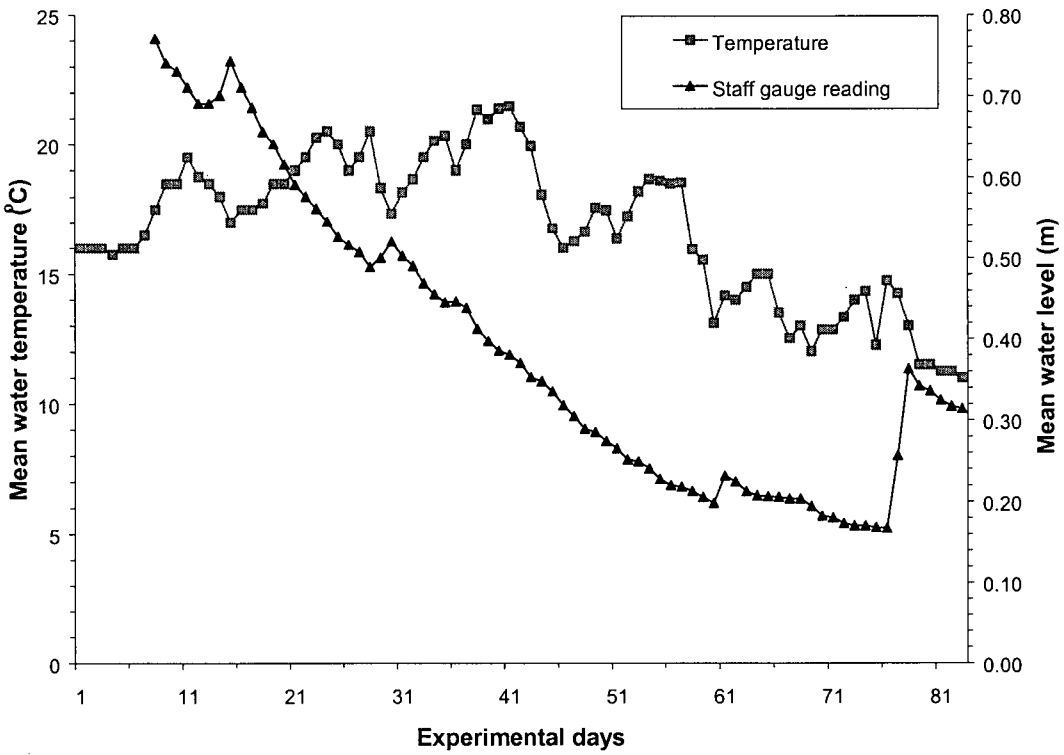

Figure. 5. Mean daytime water temperature and water level in the Slocan River at Passmore, B.C. from July 10 to September 30, 1992.

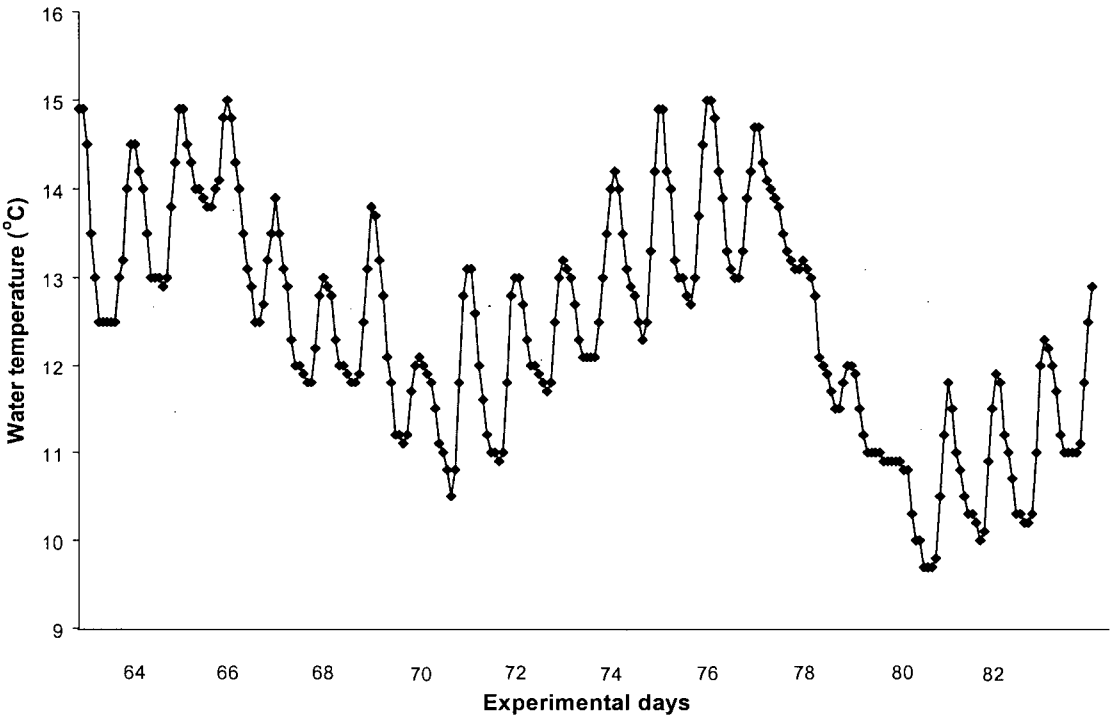

Figure 6. Diurnal variation in water temperature in the Slocan River at Passmore, B.C. from September 9 to 30, 1992. The data are based on continual measurements at 2 hr intervals from a Ryan thermograph.

replicate troughs was based on flow dynamics measured when the mesocosm was located along the eastern margin of the river channel. Comparable mean levels observed between replicates over the first half of the study (days 15-56) were only reflected in the control troughs during the second half of the experiment (days 59-82). Flow characteristics of the Slocan River therefore, had a much stronger influence on flow dynamics within individual stream troughs during the latter half of the experiment.

Mean treatment concentrations for nitrogen and phosphorus were close to the target concentrations with the exception of the 5:5 N:P ratio (Fig. 8). Considerable variation in treatment concentration however, was observed for nitrogen values associated with the 3:3, 5:5, 12:3 and 20:5 treatments. Of the five sampling periods established to monitor water chemistry, the results for nitrogen from one sampling period were excluded owing to a lack of ammonia-nitrogen information in the analysis. Background levels of ammonia and nitrate-nitrogen in the Slocan River averaged about 4 $\mu g \cdot L^{-1}$. Mean orthophosphate levels during the fertilization experiment were similarly close to target concentrations above background and variation in treatment concentration was less dramatic in all but two cases (12:3 and 20:5 ratios). Background levels of orthophosphate in the Slocan River were generally <1 $\mu g \cdot L^{-1}$. Total

dissolved phosphorus (TDP) levels, on average, were 1 to 2 $\mu g \cdot L^{-1}$ above orthophosphate levels. TDP is provided to account for organically bound forms that may be biologically available following remineralization. The largest variation in TDP was again most evident for the 12:3 and 20:5 treatment ratios. The large variation in mean nitrogen and phosphorus levels are likely attributed to differences in flow rates between replicate troughs of the same concentration because fertilizer was supplied to each replicate from the same stock solution. The use of an average flow rate between replicate troughs to calculate the amount of chemical required during preparation of the target stock solution was likely responsible, in part, for the observed variation in nutrient concentration.

Diurnal variation in river flow was also responsible for differences in trough flow within a given day (G.G. Oliver, pers. obs.). Despite efforts to maintain a constant water level in troughs by adjusting the stabilizer legs in each corner of the mesocosm to compensate for changes in river elevation, water levels in troughs did vary. In consideration of the cumulative effects of declining flow regime, diurnal variation and the use of an average flow rate at a single point in time to calculate fertilizer requirements, maintenance of a consistent nutrient concentration or ratio is somewhat doubtful. There is a strong likelihood that target concentrations were never consistently achieved but subject to daily fluctuation. Although overall N and P means were approximate, considerable variability in water chemistry values was apparent (refer to Fig. 8).

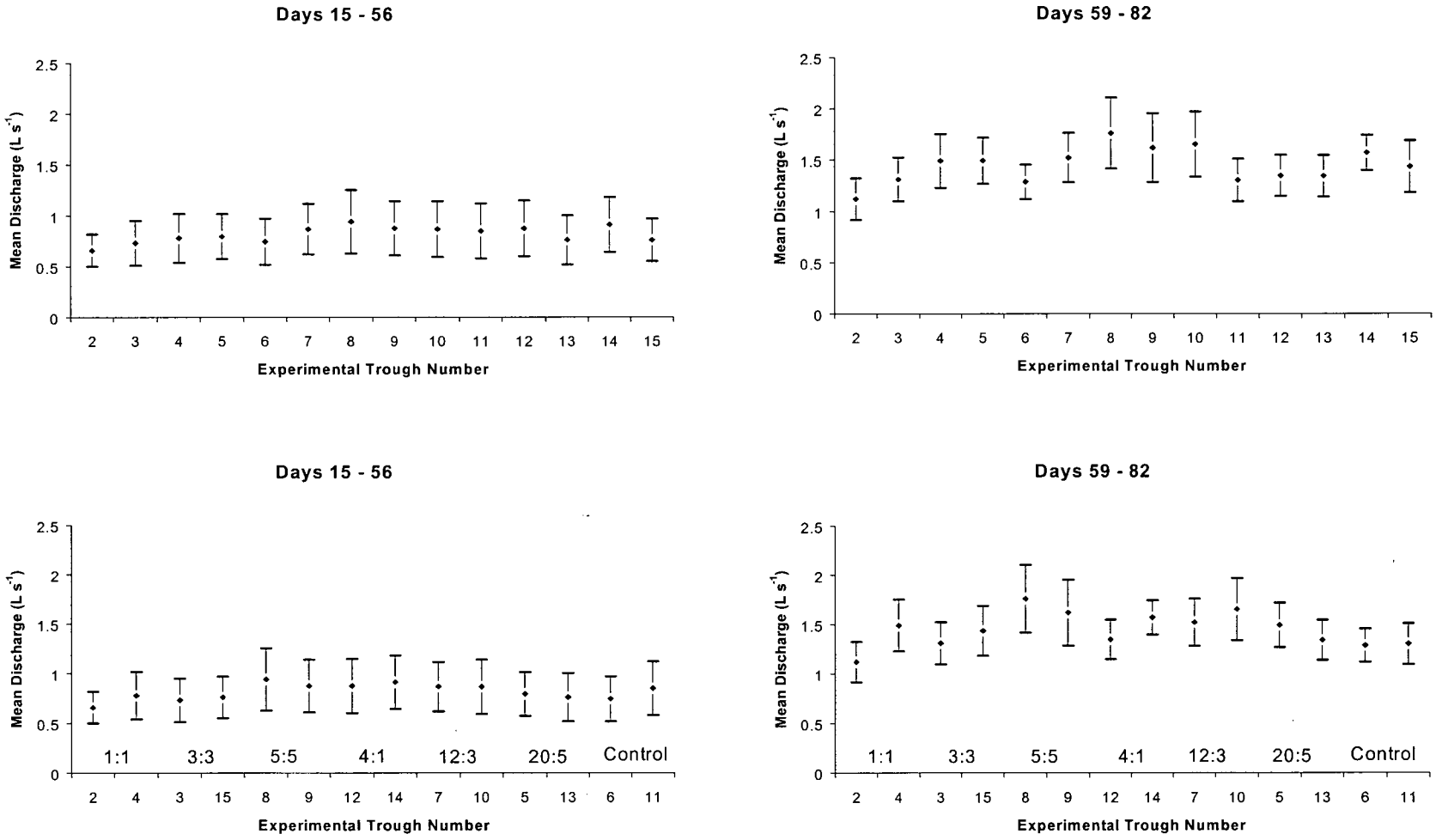

Figure 7. Variation (±1 SD) in mean flow ($L \cdot s^{-1}$) across experimental stream troughs and comparison of mean flow between troughs of similar treatment concentration. Experimental dates are partitioned to illustrate differences in trough flow relative to the instream position of the mesocosm.

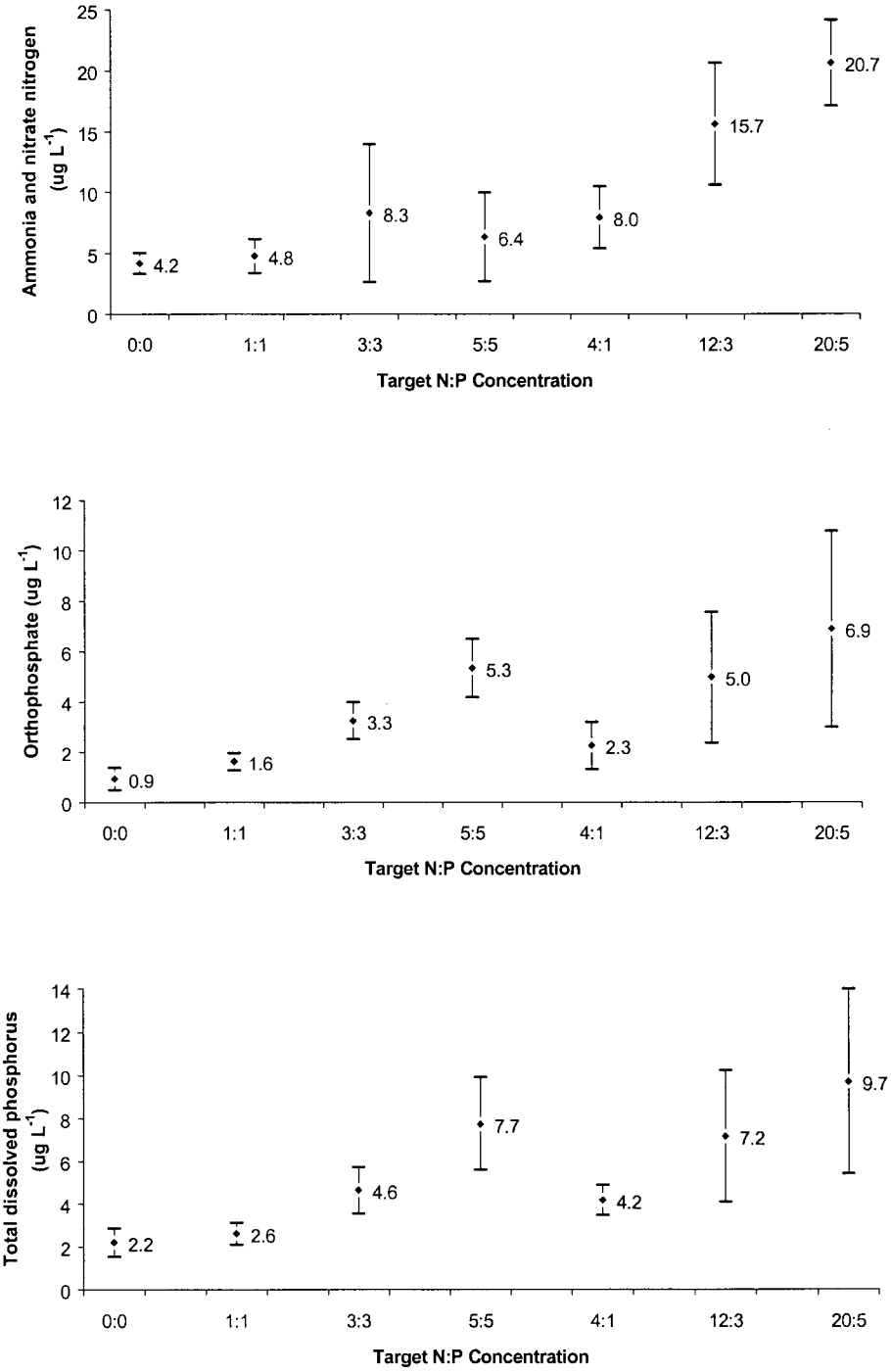

Figure 8. Variation (± 1 SD) in observed mean inorganic nitrogen (n=4) and phosphorus (n=5) from August 4 to September 29, 1992 for each treatment regime based on low level water chemistry analysis. Mean values are derived from two replicates.

3.3 Algal community response

3.3.1 Periphyton accrual and algal biomass

Three series of periphyton accrual were measured from July 11 to September 30, 1992 (Fig. 9). The first trial ran for a period of 34 days, the second for 24 days and the third for 27 days. The results of the first two series were confounded by suspended sediment (sand) in the river that was deposited in a fine layer over styrofoam plates in each trough. Although suspended sediment levels began to abate during the second series, growth inhibition of the algal mat persisted under the declining river flow regime (refer to Fig. 5). Re-positioning of the mesocosm to the western margin of the channel on day 55 of the experiment improved water velocity in each stream trough and exponential growth was observed among the six treatments of the third accrual trial. The contribution of chlorophyll *a* by passive settlement of algae on styrofoam substrata at the end of the third trial was minimal (~ 3 $mg \cdot m^{-2}$).

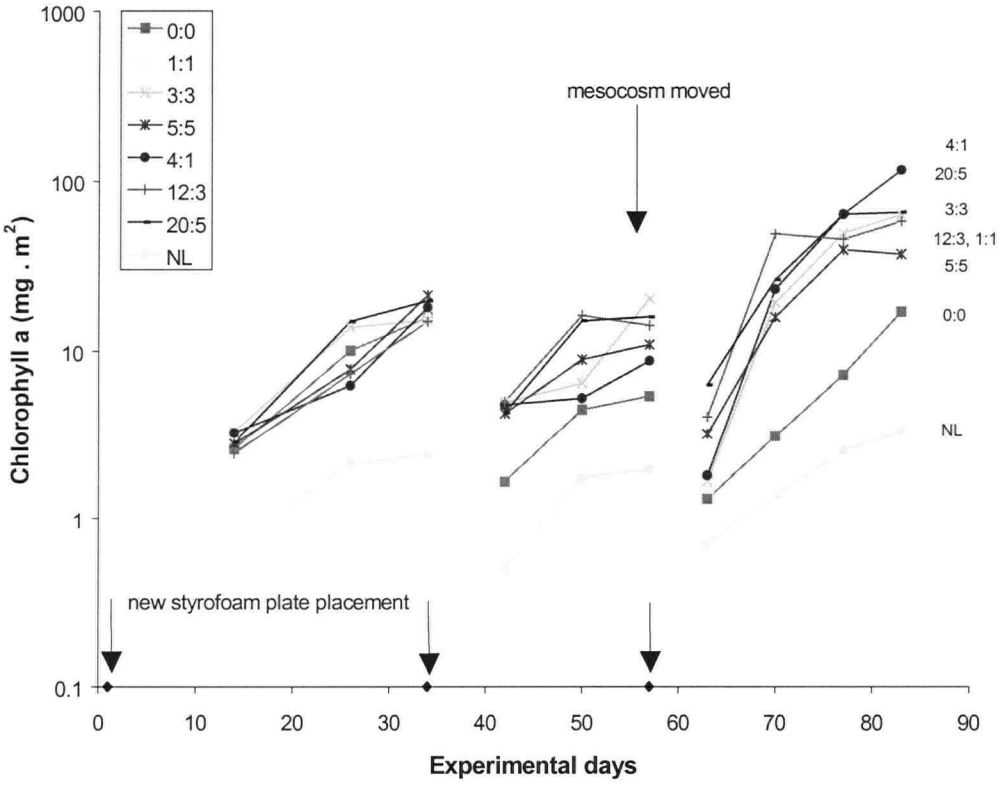

Figure 9. Time-course changes in periphyton accrual across treatment concentrations. NL (no light) indicates the level of accrual due to passive settlement of algae as measured in dark troughs. Replacement times for styrofoam plates are indicated. Time of mesocosm re-positioning is also shown.

During the first week of periphyton accrual, rapid growth rates were observed for both control and treatment groups and a progressive increase in biomass was noted with increasing concentrations and ratios of applied nutrient (Fig. 10). The 1:1 treatment group displayed the lowest rate of increase after 7d. Beyond the first week, a constant rate of accrual was detected in the control, whereas differential rates of increase were characteristic of each treatment for the remainder of the accrual cycle. Following an initially higher algal biomass in the 5:5 and 20:5 nutrient concentrations after 7d, a subsequent decline in growth rate was observed for each of these treatments, likely in response to competitive interactions (light, nutrients) within a thicker benthic mat (Bothwell 1989). In contrast, growth rates remained higher for all other nutrient concentrations during the second week of accrual before a gradual decline during the third week. By the end of the accrual trial, the 1:1 and 3:3 concentrations produced a higher algal biomass than the 5:5 concentration and the 4:1 concentration was similarly greater than the 20:5 concentration. Among the 4:1 treatment ratio group, algal biomass was lowest in the 12:3 nutrient concentration. Insect grazing or sloughing was suspected to account for the lower algal biomass response in the 12:3 concentration, however data to support this contention is lacking. The 5:5 nutrient concentration, having reached a plateau followed by a net reduction in algal biomass during the last week of the cycle, was the only treatment to reach peak biomass during the 27 d trial period.

The test for equality of slopes (treatment by time interaction) revealed a significant difference between control and treatment groups (i.e. between the first and fourth weeks of the accrual trial; ANCOVAR; $p<0.01$). Slope differences between the 1:1 and 4:1 treatment ratio groups were generally non-significant with the exception of the 3:3 and 12:3 contrast ($p<0.01$). Within treatment groups, a significant difference in slope was also evident between the 5:5 and the 1:1 and 3:3 nutrient concentrations ($p<0.01$). Similarly, a significant difference was also demonstrated between the 4:1 and 20:5 nutrient concentrations ($p<0.01$). Collectively, the 5:5 and 20:5 nutrient concentrations displayed a slower rate of periphyton accrual, following an initial higher biomass after 7 d, than all other treatments (12:3 concentration excluded).

During the third trial, N:P concentrations of both 1:1 and 4:1 treatment groups provided a higher algal biomass than background levels in the river water (Fig. 11). Mean chlorophyll *a* levels at the end of the third accrual trial ranged from 40.8 to 68.2 $mg \cdot m^{-2}$ for the 1:1 treatment group and 57.5 to 115.4 $mg \cdot m^{-2}$ for the 4:1 treatment group compared to 16.7 $mg \cdot m^{-2}$ in the control. The corresponding levels of biomass represent a 2.5 to 4 fold increase for the 1:1 treatment group and a 4.5 to 8 fold increase for the 4:1 treatment group over background. An increasing trend in biomass peaked at the 3:3 nutrient concentration in the 1:1 treatment group. A similar

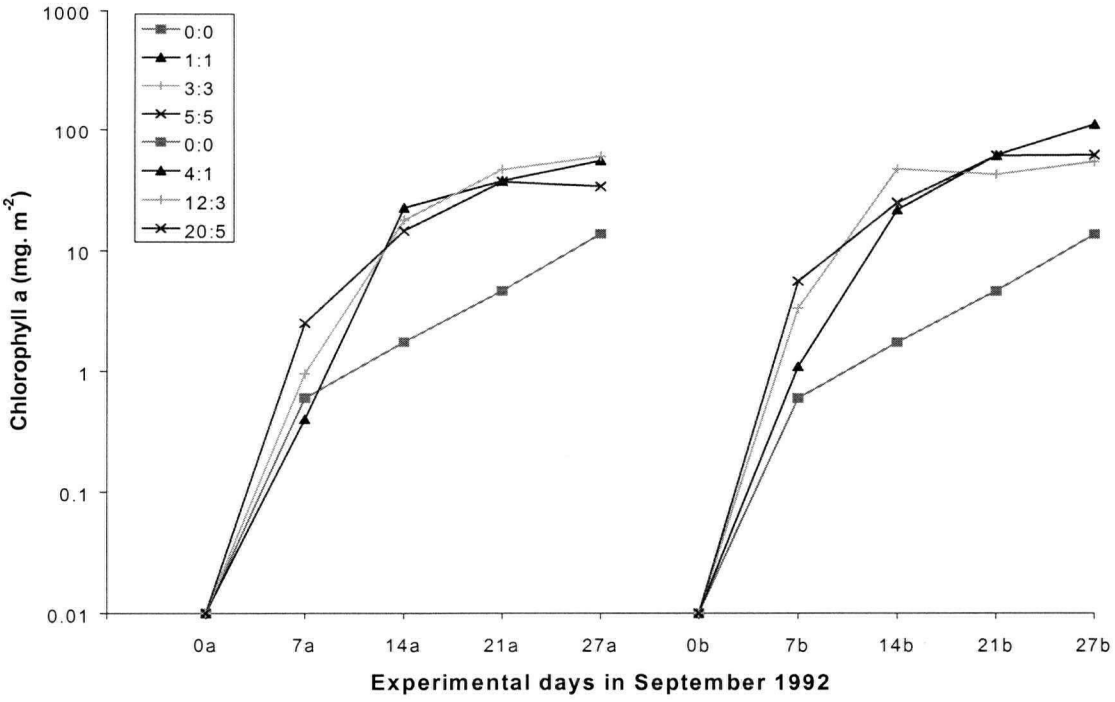

Figure 10. Time-course changes in adjusted mean chlorophyll *a* levels among treatment concentrations during the third trial in September 1992. Adjusted means account for passive settlement of algae from upstream sources. The a and b time series distinguish 1:1 and 4:1 treatment groups, respectively.

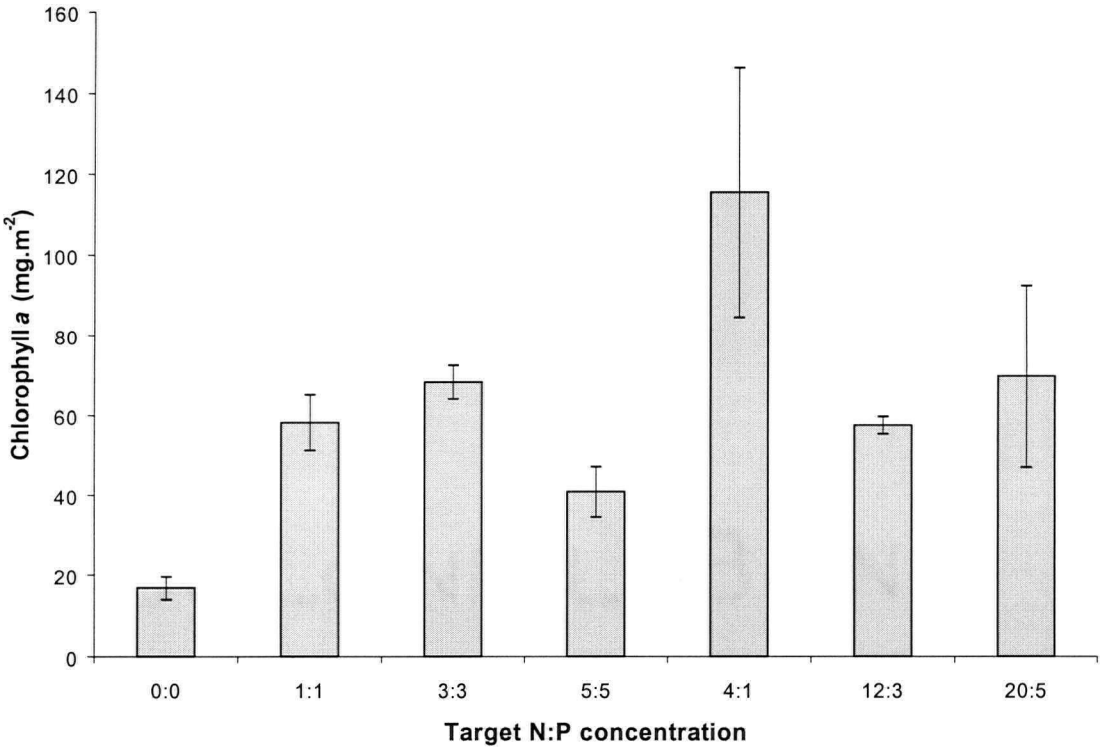

Figure 11. Mean adjusted chlorophyll *a* levels (± 95% CI) for the control and each treatment concentration at the end of the third periphyton accrual trial. The data are based on duplicate cores within replicate troughs (n=4) from styrofoam plates.

pattern was observed in the 4:1 treatment group with the exception of the 12:3 nutrient concentration where suspected grazer effects prevented attainment of peak biomass. Significant differences in biomass (as measured by chlorophyll *a*) were again evident between control and treatment groups (ANOVA; $p<0.01$) as well as between the 1:1 and 4:1 treatment groups ($p<0.05$; Table 1). Orthogonal contrasts between treatment groups of the same phosphorus level (i.e. 1:1 vs 4:1, 3:3 vs 12:3, 5:5 vs 20:5) displayed significantly higher biomass in the 4:1 than the 1:1 treatment group ($p<0.05$) with the exception of the 3:3 and 12:3 nutrient concentrations. Within treatment groups, significant differences in biomass were only demonstrated between the 3:3 and 5:5 nutrient concentrations ($p<0.05$) and between the 4:1 and the 12:3 and 20:5 nutrient concentrations ($p=0.01$); the former concentrations of each contrast having the higher biomass.

Given that a large degree of variation in flow rates was observed across stream troughs, a repeated measures ANCOVAR was used to distinguish differences in algal biomass among treatments that may have been influenced by differences in trough discharge. Although a significant difference in flow between troughs was observed over four weeks of the third periphyton accrual trial ($p<0.01$), there was no significant difference in the treatment * flow interaction ($p>0.05$). Accordingly, differences in algal biomass between treatments were unaffected by differences in trough discharge.

Table 1. Orthogonal contrasts of algal biomass (as chlorophyll *a*) within and between treatment groups at the end of the third periphyton accrual trial (September 1992). Analyses completed after logarithmic transformation (LOG_{10}) of chlorophyll *a* levels. Group 1 = 1:1 treatment ratio and Group 2 = 4:1 treatment ratio. Level of significance set at $\alpha=0.05$.

Analysis of variance	Orthogonal Contrast	Significance
Control vs treatment		p=0.001
Between treatments	Group 1 vs Group 2	p=0.021
	1:1 vs 4:1	p=0.011
	3:3 vs 12:3	p=0.410
	5:5 vs 20:5	p=0.035
Within treatments	1:1 vs 3:3 and 5:5	p=0.577
	3:3 vs 5:5	p=0.033
	4:1 vs 12:3 and 20:5	p=0.010
	12:3 vs 20:5	p=0.431

3.3.2 Algal cell density

After 35 d of nutrient enrichment, algal densities on gravel bed materials paralleled the biomass response reported on styrofoam plates in the third periphyton trial (Fig. 12). Cell densities across all treatments were significantly different than those observed in the control (ANOVA; $p<0.01$). Mean total cell density on August 13 ranged from approximately 67,000 to 155,000 cells·cm^{-2} in the 1:1 treatment group and from approximately 66,000 to 208,000 cells·cm^{-2} in the 4:1 treatment group compared to 15,000 cells·cm^{-2} in the control. The highest density within treatment groups was achieved at the 3:3 and 12:3 nutrient concentrations although little difference was observed between the 4:1 and 12:3 concentration levels. Notwithstanding, the majority of replicates showed a high degree of variation. Colonization dynamics of the dominant taxa, including cyanophytes and green algae, diatoms and flagellates, were similarly varied between control and treatment groups (Fig. 13). Whereas diatoms were significantly higher in number in the early successional stages of the control (ANOVA; $p<<0.01$), green algae were most dominant across all treatment groups with diatoms sub-dominant. Blue greens were well established in treatment groups and flagellates were least abundant across both treatment and control groups. Although highly significant differences between treatment, taxa and treatment*taxa interaction were evident between control and treatment groups

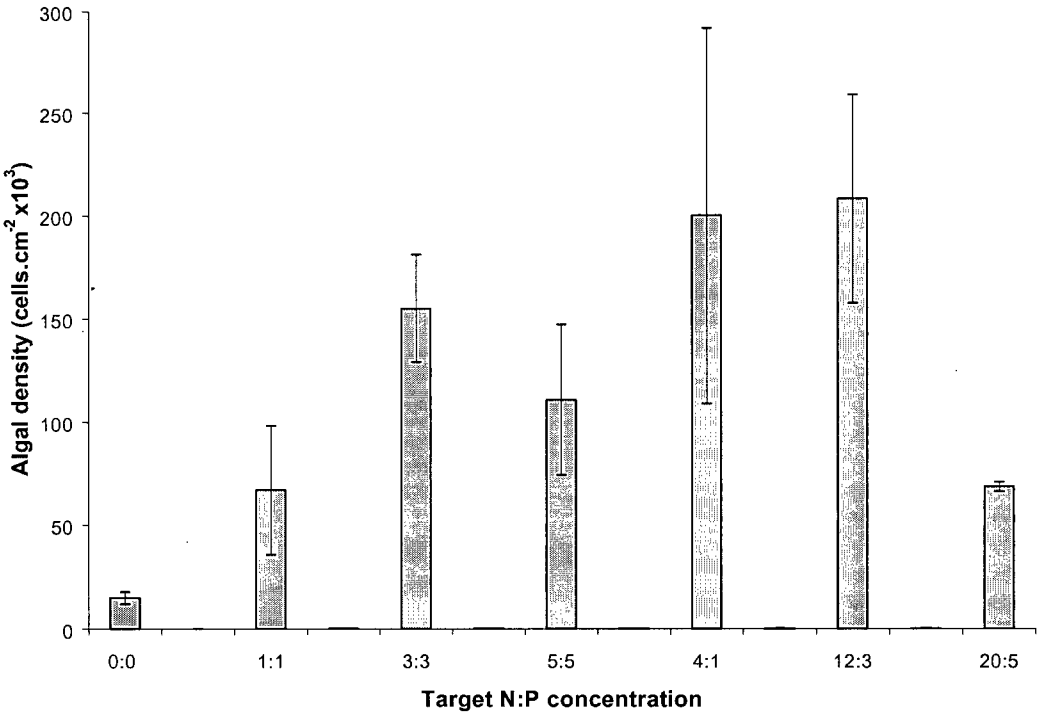

Figure 12. Mean total algal cell density measured August 13, 1992 (day 35) on gravel materials in stream troughs. The data are pooled from three samples from each trough (n=6). 95% CI is included.

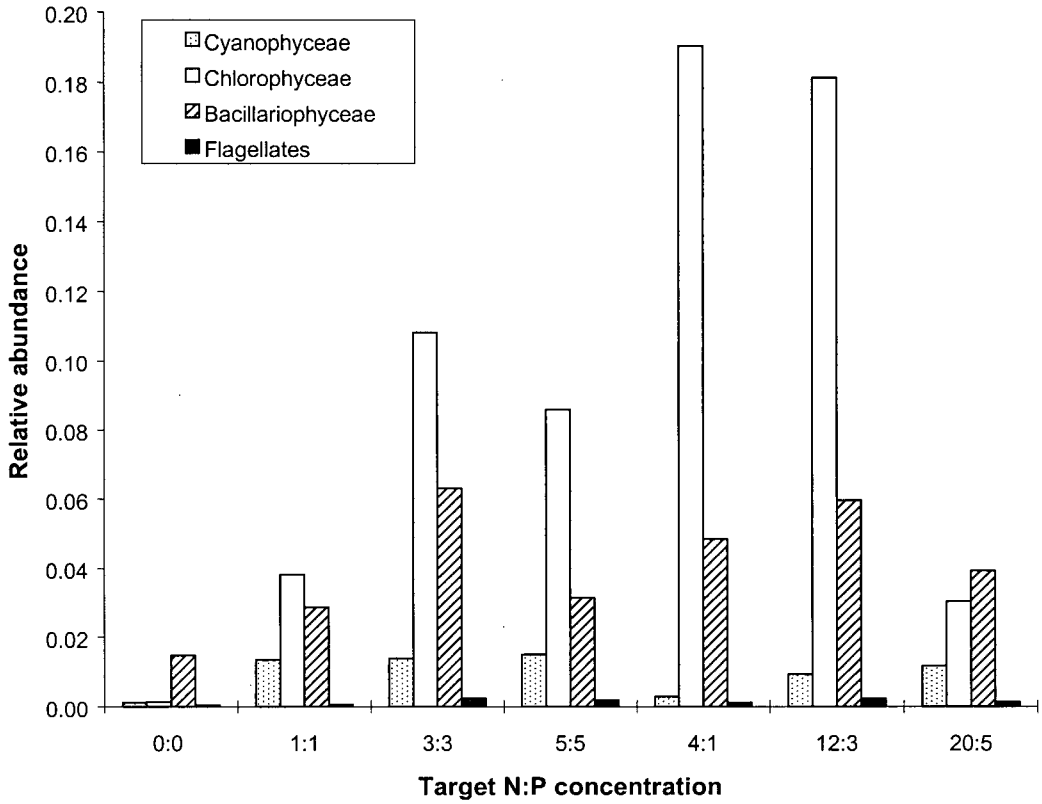

Figure 13. Relative abundance of the four major taxa sampled from gravel materials within stream troughs on August 13, 1992 (day 35). Individual treatments and control were normalized for total cell density across all experimental units.

(ANOVA; $p<0.01$), few comparisons were significant within or between treatment groups (i.e. within or between 1:1 and 4:1 ratios; Bonferroni pairwise comparisons (*a posteriori*)). The notable exception was the non-significant difference in number of diatoms between control and treatment groups ($p>0.05$). Significant differences in number within and between treatment groups were always observed between the green algae-diatom assemblage and the flagellates ($p<0.01$). The only other significant differences in numbers were observed between cyanobacteria and diatoms in the 4:1 nutrient concentration ($p<0.01$) and cyanobacteria and greens in the 12:3 nutrient concentration ($p<0.01$).

After 57 days of nutrient enrichment, algal densities remained lower in control versus treatment groups (ANOVA; $p<0.01$) however overall numbers and pattern of abundance between treatments changed dramatically (Fig. 14). Within the 1:1 treatment group, mean total cell densities on September 4 ranged from about 87,000 to 143,000 cells·cm^{-2}, peak density shifted to the 1:1 nutrient concentration and average total number of cells were similar for both 3:3 and 5:5 nutrient concentrations. Within the 4:1 treatment group, mean total cell

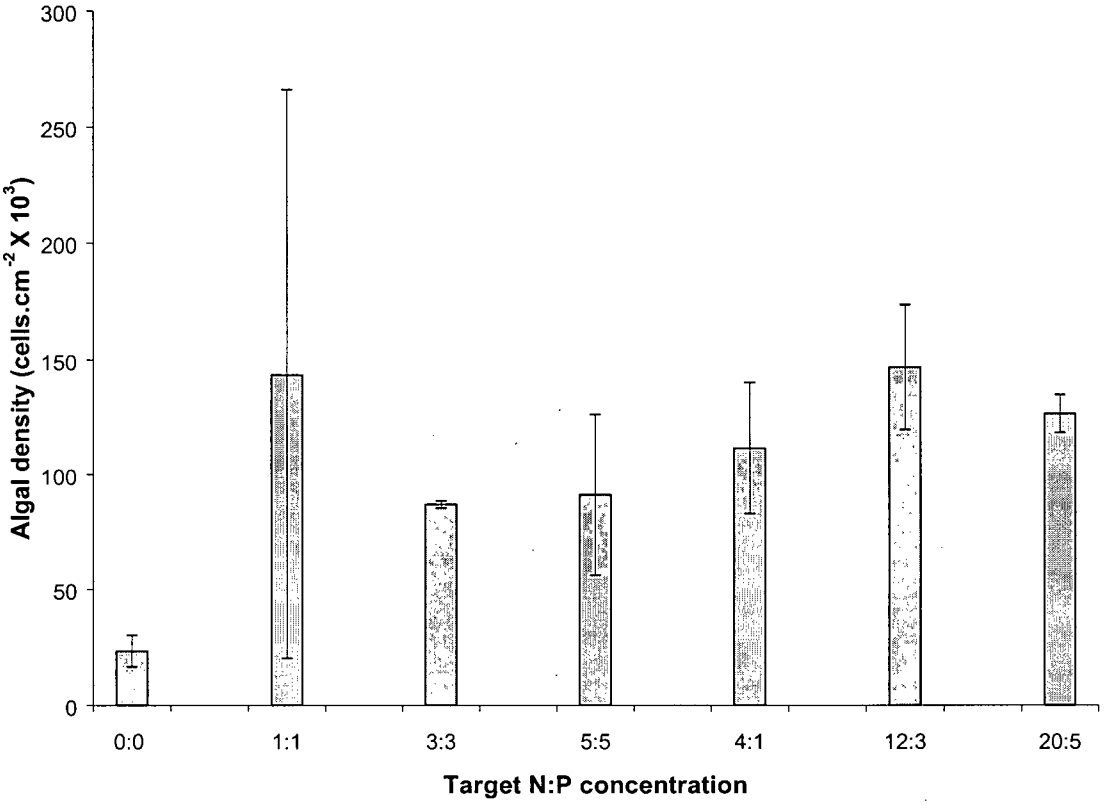

Figure 14. Mean total algal cell density measured September 4, 1992 (day 57) on gravel materials in stream troughs. The data are pooled from three samples from each trough (n=6). 95% CI is included.

densities ranged from about 111,000 to 147,000 cells·cm^{-2}. Mean total density in the control group was estimated at about 23,000 cells·cm^{-2}. The highest cell density was observed in the 12:3 concentration but at a lower maximum level than previously observed during the August 13 enumeration and average total numbers in the 20:5 nutrient concentration exceeded those in the 4:1 concentration. Overall, there was no significant difference in cell density between or within treatment groups (ANOVA; $p>0.05$; Bonferroni pairwise comparisons).

Variability in cell density was again high within and between treatment groups and exceedingly high in the 1:1 nutrient concentration due to the variation in abundance of cyanobacteria (primarily *Oscillatoria* sp.; Fig. 15). With the exception of the similar abundance of blue-greens, greens and diatoms in the 1:1 treatment, diatoms remained dominant in the control group whereas the green algae remained dominant in all other treatment groups. Diatoms were again sub-dominant across treatment groups but proportionally had a higher relative abundance than the August 13 enumeration (refer to Fig. 13). Similarly, the relative abundance of cyanobacteria was marginally higher in all nutrient concentrations of the 4:1 treatment group and slightly

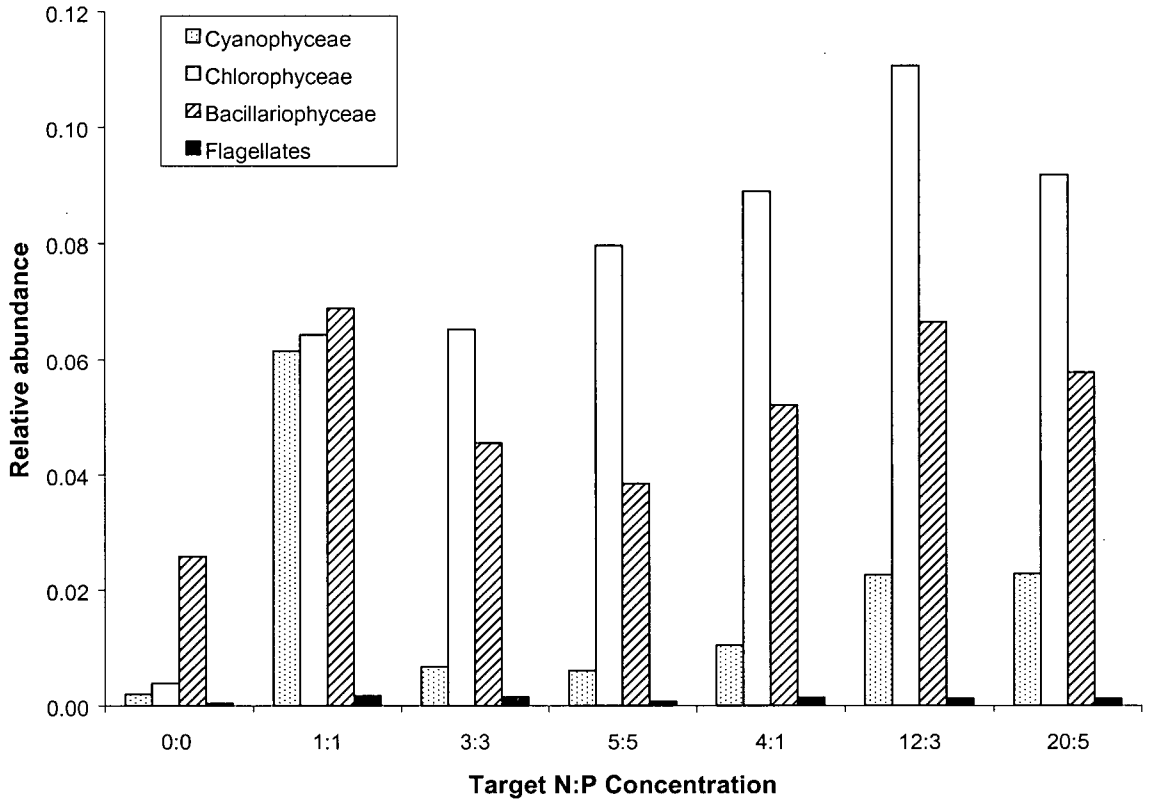

Figure 15. Relative abundance of the four major taxa sampled from gravel materials within stream troughs on September 4, 1992 (day 57). Individual treatments and control were normalized for total cell density across all experimental units.

higher in the 12:3 and 20:5 concentrations. The relative abundance of flagellates was unchanged and remained at a very low level in comparison to the other taxa. Significant differences in taxa were again evident between control and treatment groups (ANOVA; $p<0.01$) but non-significant between treatment groups ($p>0.05$).

At the end of the experiment (September 30, 1992), algal density was at its highest abundance for both control and treatment groups and the pattern of abundance among control and treatment groups had again been modified (Fig. 16). Mean total cell density in the control on day 83 climbed to about 155,000 cells·cm^{-2}, almost a 7 fold increase over the September 4 estimate. Within the 1:1 treatment group, mean total cell density ranged from about 173,000 to 500,000 cells·cm^{-2}. Peak density was still evident within the 1:1 nutrient concentration, owing to the large representation by cyanobacteria (i.e. *Oscillatoria* sp.) and a progressive decline in abundance proceeded in the 3:3 and 5:5 nutrient levels. A narrow range in mean total cell density was observed in the 4:1 treatment group (276,000 to 297,000 cells·cm^{-2}) however, the 20:5 nutrient concentration predominated. Cell counts were again highly variable over most

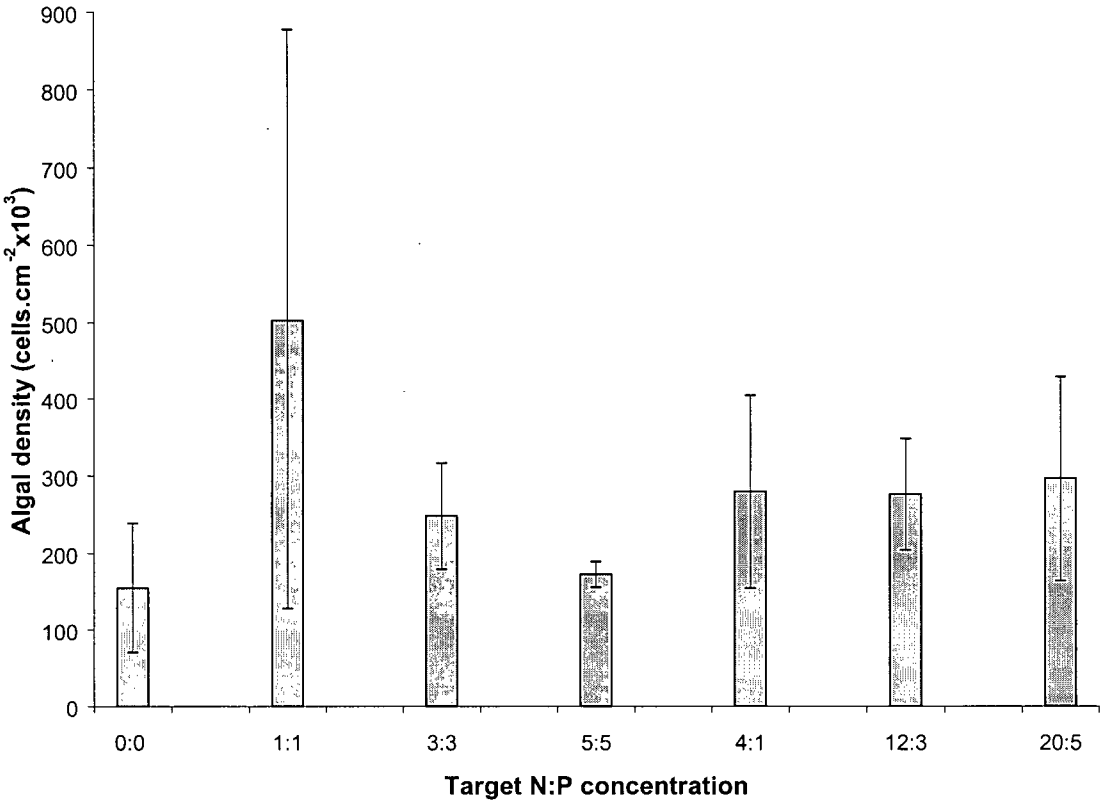

Figure 16. Mean total algal cell density measured September 30, 1992 (day 83) on gravel materials in stream troughs. The data are pooled from three samples from each trough (n=6). 95% CI is included.

groups, unusually high between control troughs and exceedingly high in the 1:1 concentration. There was no significant difference in cell numbers between control and treatment groups (ANOVA; $p>0.05$) however a significant difference between taxa was observed between control and treatment groups only ($p<0.01$).

After 83 days, green algae and diatoms were co-dominant in control troughs, and the cyanophytes and flagellates both remained low in abundance (Fig. 17). Within the 1:1 treatment group, green algae and diatoms were equally dominant but over-shadowed by super-abundant cyanobacteria (i.e. *Oscillatoria* sp.) in the 1:1 nutrient concentration. A decreasing trend in cyanophyte abundance from the 1:1 to 5:5 concentrations was also observed. Within the 4:1 treatment group, green algae remained dominant and diatoms were again sub-dominant. Cyanobacteria remained at about the same level of relative abundance. The flagellates were unchanged over both control and treatment groups maintaining the least abundance of all taxa.

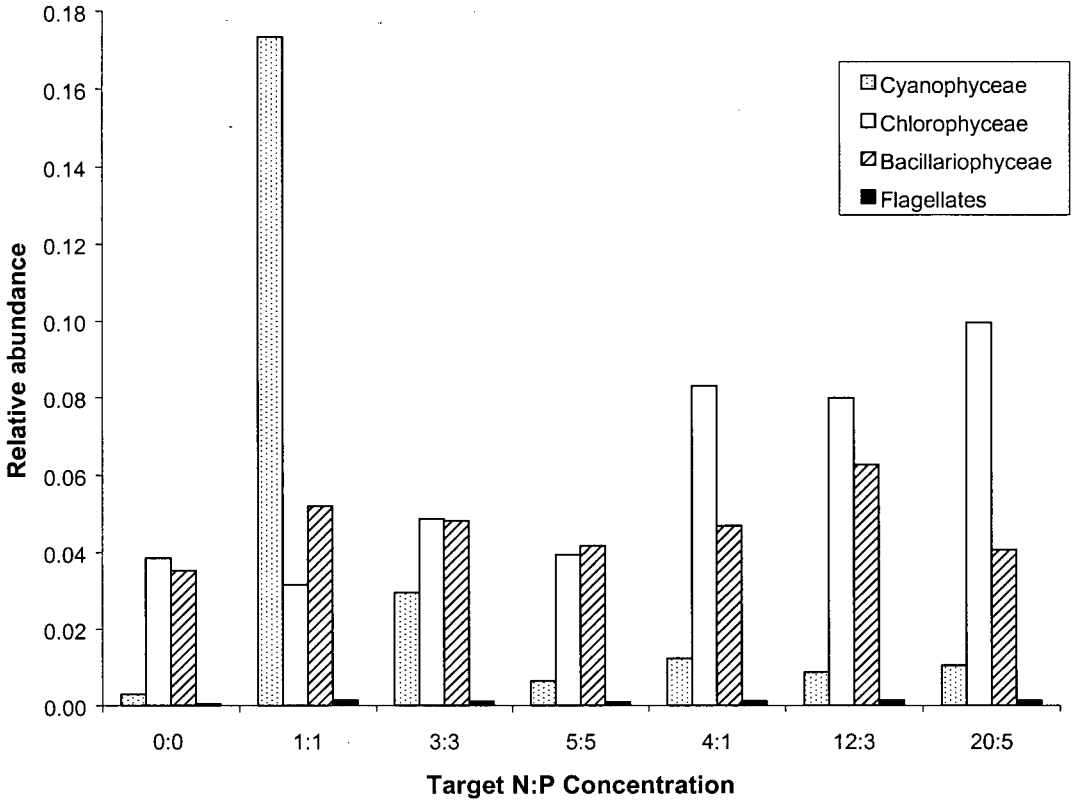

Figure 17. Relative abundance of the four major taxa sampled from gravel materials within stream troughs on September 30, 1992 (day 83). Individual treatments and control were normalized for total cell density across all experimental units.

A comparison of mean total algal density and taxa over time illustrates the changes in abundance and community structure observed over the duration of this experiment (Fig. 18). The fact that cell counts were obtained from periphyton on gravel substrates colonized by benthic insects further illustrates the response of algae subject to grazing. Although the duration of the study represents a narrow time frame in terms of algal community dynamics, five observations are apparent: 1) chlorophytes were only dominant under nutrient-replete conditions. Within the control group, the green algae were either poor competitors under ambient nutrient conditions and low N:P ratios or subject to heavier grazing pressure. Nutrient enrichment therefore appeared to promote algal succession where chlorophytes eventually became dominant; 2) under a 1:1 N:P regime, environmental conditons favoured the development of cyanophytes suggesting possible fixation of the nutrient in shortest supply (N); 3) the algal community in the 20:5 concentration responded with initial higher growth rates but displayed slower incremental growth over the remainder of the accrual cycle. A slowing in the rate of accrual was likely attributed to potential boundary layer effects (lower nutrient diffusion)

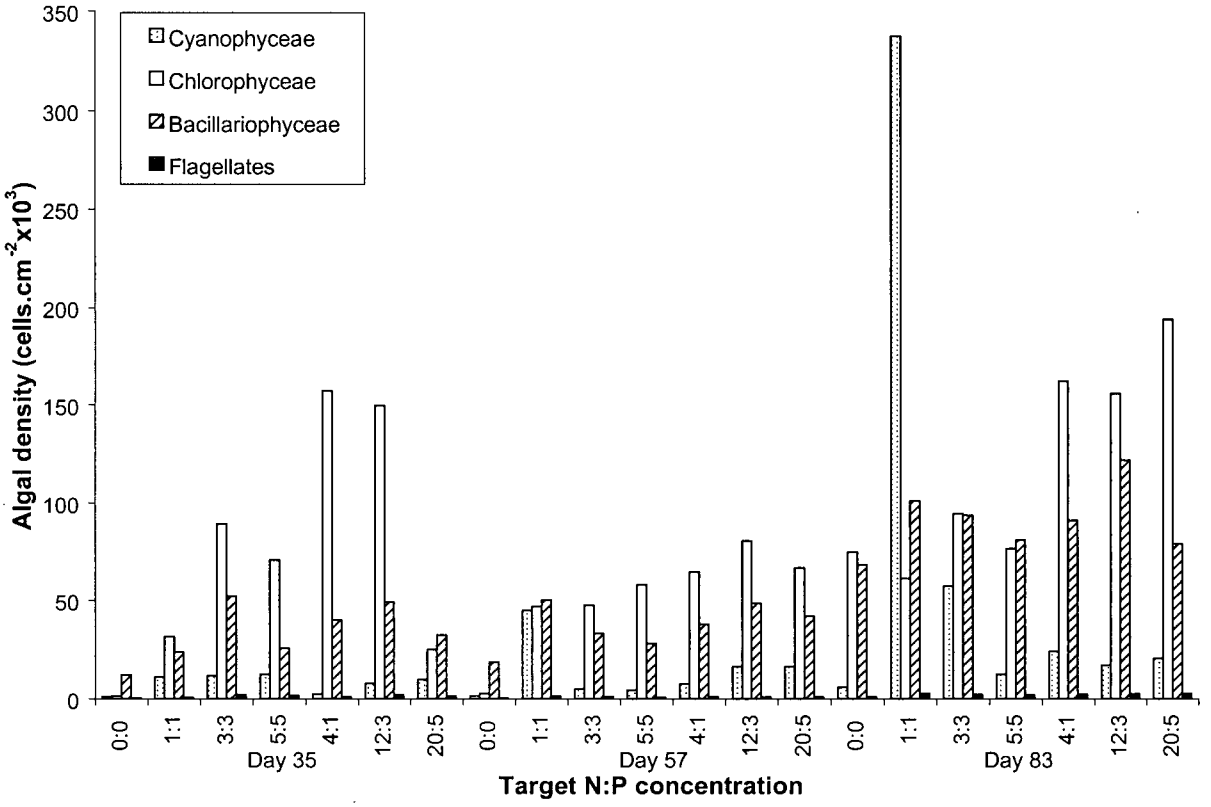

Figure 18. Changes in mean total cell density and taxa at three time intervals during the experiment.

and lower light intensity due to a more highly developed benthic algal mat (Bothwell 1989). At the end of the experiment, the 20:5 treatment level supported a marginally higher algal cell density than alternate treatments within the same treatment group (i.e. 4:1 treatment ratio); 4) the near-doubling of algal cell numbers between 1:1 and 4:1 treatment groups over the first 35 d is consistent with the near-doubling of algal biomass (measured as chlorophyll *a*) on styrofoam plates in the third periphyton trial (i.e. after 27 d; refer to Fig. 10). These results further support the case for nitrogen limitation during late summer; and 5) variation in trough flow was not responsible for the variation in algal growth between control and treatment groups.

3.3.3 Representative taxa and richness

At each of the three algal sampling periods identified in the above, algal richness was greatest in the green algae (range 30 - 33 genera) and diatoms (24 diatoms) followed by the flagellates (range 10 - 11 genera) and cyanobacteria (range 6 - 9 genera). With respect to the August 13 sampling date, taxonomic richness varied from 59 to 62 genera and/or species between control and treatment groups (Table 3.3.3.1) but overall numbers of taxa were not significantly different

Table 2. Taxonomic richness and percent composition of representative algae on gravel substrate in control and treatment troughs on August 13, 1992. Percentages based on pooled cell counts from 3 samples in each of 2 replicates. Standard error of taxonomic richness is also shown.

	Target N:P Concentration						
	0:0	**1:1**	**3:3**	**5:5**	**4:1**	**12:3**	**20:5**
Taxonomic Richness	62	60.5	62.5	60.5	59.5	60	58.5
2 SE	3.92	0.98	0.98	2.94	0.98	1.96	0.98
Cyanophyceae							
Agmenellum glauca	0.3	4.2	0.4	5.9	0.2	0.1	6.0
Anabaena	0.1	0.2	0.1	0.0	0.0	0.1	0.1
Anacystis	0.5	0.4	0.5	2.2	0.4	0.3	0.2
Gomphospheria	0.3	0.2	0.1	0.1	0.1	0.0	0.2
Lyngbya	5.9	10.5	5.4	2.6	0.4	2.3	6.8
Oscillatoria	0.4	1.3	0.8	0.4	0.2	0.8	1.0
Total	*7.4*	*16.7*	*7.5*	*11.2*	*1.2*	*3.7*	*14.3*
Chlorophyceae							
Ankistrodesmus falcatus	0.4	0.2	0.3	0.3	0.1	0.2	0.5
Botryococcus braunii	0.3	0.4	0.2	0.2	0.8	0.2	0.4
Bulbochaete	0.8	0.5	0.3	0.9	0.2	0.2	0.8
Cosmarium	0.4	0.3	0.3	0.3	0.2	0.4	0.4
Mougeotia	0.4	0.6	0.9	0.3	0.2	0.2	0.8
Oedogonium	0.3	0.0	1.1	2.2	0.5	0.2	0.3
Scenedesmus	1.5	2.2	0.6	0.3	0.2	0.6	0.8
Sphaerocystis schroeteri	0.3	0.2	1.2	2.1	0.2	3.0	0.4
Spirogyra	0.3	38.2	50.3	40.9	74.8	65.3	29.6
Spondylosium	0.5	0.4	0.2	1.2	0.2	0.3	0.5
Staurastrum	0.3	0.1	0.2	0.3	0.1	0.1	0.2
Stigeoclonium	0.2	0.1	0.2	0.1	0.1	0.2	0.4
Ulothrix	1.0	0.2	0.3	0.4	0.2	0.2	0.4
Zygnema	0.0	3.2	0.2	13.1	0.1	0.1	0.1
Other	1.3	0.4	1.4	1.2	0.7	0.8	1.0
Total	*8.1*	*47.1*	*57.6*	*64.0*	*78.4*	*71.7*	*36.6*
Bacillariophyceae							
Achnanthes flexella	0.5	0.6	0.3	0.5	0.2	0.3	0.5
Achnanthes minutissima	59.1	21.8	19.3	13.8	12.2	14.6	32.2
Amphora	0.6	0.2	0.3	0.3	0.1	0.2	0.2
Ceratoneis arcus	0.3	0.1	0.2	0.2	0.1	0.2	0.4
Cocconeis	0.4	0.1	0.3	0.3	0.2	0.2	0.4
Cymbella minuta	0.8	0.4	0.2	0.3	0.2	0.2	0.9
Eunotia	0.7	0.4	0.5	0.4	0.2	0.2	0.5
Fragilaria crotonensis	2.2	1.3	1.5	0.6	0.3	0.6	1.4
Fragilaria sp	3.1	2.7	3.3	1.1	1.6	1.5	1.6
Gomphonema	1.0	0.4	0.5	0.6	0.2	0.3	0.6
Melosira	1.1	0.6	0.3	0.2	0.2	0.4	0.6
Navicula	2.5	1.4	1.4	0.6	1.1	0.5	1.4
Synedra ulna	3.3	2.7	2.9	1.3	0.9	1.6	2.1
Tabellaria fenestra	1.4	0.7	0.3	0.4	0.7	0.7	0.5
Tabellaria flocculosa	0.9	0.2	0.3	0.3	0.2	0.2	0.4
Other	4.1	1.7	2.3	2.4	1.6	1.9	3.7
Total	*82.1*	*35.4*	*33.6*	*23.4*	*19.9*	*23.6*	*47.4*
Flagellates							
Chroomonas acuta	0.5	0.1	0.3	0.2	0.1	0.3	0.3
Cryptomonas	0.3	0.2	0.3	0.2	0.1	0.1	0.2
Eudorina	0.3	0.1	0.2	0.2	0.1	0.1	0.4
Euglena	0.3	0.1	0.2	0.1	0.0	0.1	0.2
Hydrurus foetidus	0.2	0.3	0.1	0.2	0.1	0.1	0.1
Peridinium inconspicuum	0.5	0.1	0.3	0.3	0.1	0.2	0.4
Other	0.4	0.0	0.1	0.1	0.0	0.0	0.1
Total	*2.4*	*0.9*	*1.3*	*1.4*	*0.5*	*0.9*	*1.8*

(ANOVA; $p>0.05$). *Oedogonium* sp., *Scenedesmus* sp., *Sphaerocystis schroeteri* and *Spirogyra* sp. were the most dominant genera of the green alga whereas *Achnanthes minutissima*, *Fragilaria crotonensis*, *Fragilaria* sp., *Navicula* sp. *Synedra ulna* and *Tabellaria fenestra* were dominant among the diatoms. *Agmenullum glauca*, *Anacystis* sp., *Lyngbya* sp. and *Oscillatoria* sp. were most representative of the cyanobacteria despite the few genera in comparison to other taxa. *Chroomonas acuta*, *Cryptomonas* sp., *Eudorina* sp. and *Peridinium inconspicuum* were typical of the flagellates.

After 35 d, over 80% of the algal community in control troughs was represented by diatoms whereas up to 80% of the algal community within treatment troughs was represented by green algae (Fig. 19). An inverse relationship between diatoms and green algae was evident over the range of nutrient concentrations. An increase in the occurrence of green algae corresponded to a decrease in the occurrence of diatoms in conjunction with an increase in the N:P ratio. Maximum differences between diatoms and green algae were observed in the 4:1 nutrient concentration. The 20:5 concentration had a higher occurrence of diatoms. The percentage of cyanophytes was highest in the 1:1 nutrient concentration but they comprised <20% of the total

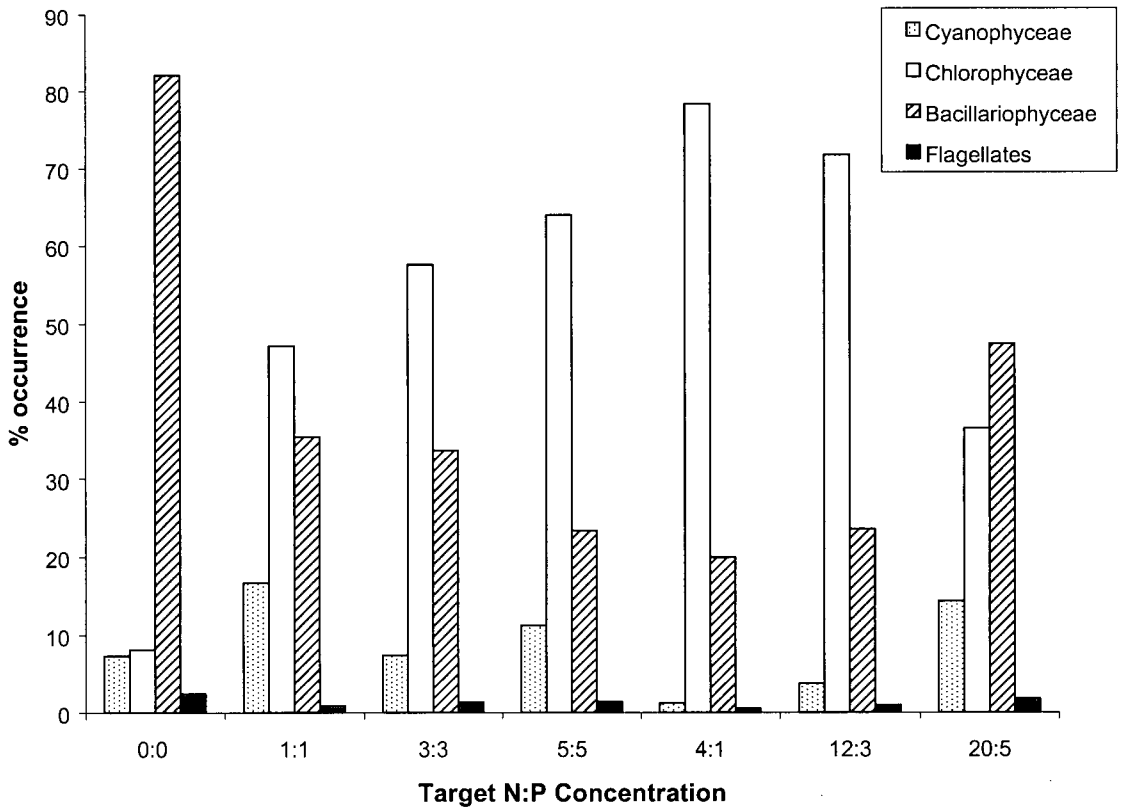

Figure 19. Percent composition of algal taxa in control and treatment troughs on August 13, 1992 (day 35). The data are based on pooled counts of 3 samples in each of 2 replicates.

cell count. The flagellates comprised <5% of the algal cells across all control and treatment groups.

After 57 d of the experiment, taxonomic richness was little changed and no significant difference in numbers of species between control and treatment groups was evident (ANOVA; $p>0.05$). A shift in species dominance was noted, however (Table 3). *A. glauca* and *Lyngbya* sp. comprised a large percentage of taxa among the cyanophytes, *Mougeotia* sp., *Oedogonium* sp., and particularly *Spirogyra* sp. were most dominant among the green algae, while *Eudorina* sp. was lower in occurrence among the flagellates. The percentage composition of the dominant diatoms remained unchanged (Fig. 20).

Diatoms continued to dominate the algal community in the control (about 80% of cells) and across the treatment groups increased to over 30% of the species present. The green algae continued to represent well over 50% of the total number of species in all treatment groups. The most notable finding was the similar occurrence of diatoms, green algae and cyanobacteria in the 1:1 nutrient concentration. The flagellates however, remained well under 5% of the total species present.

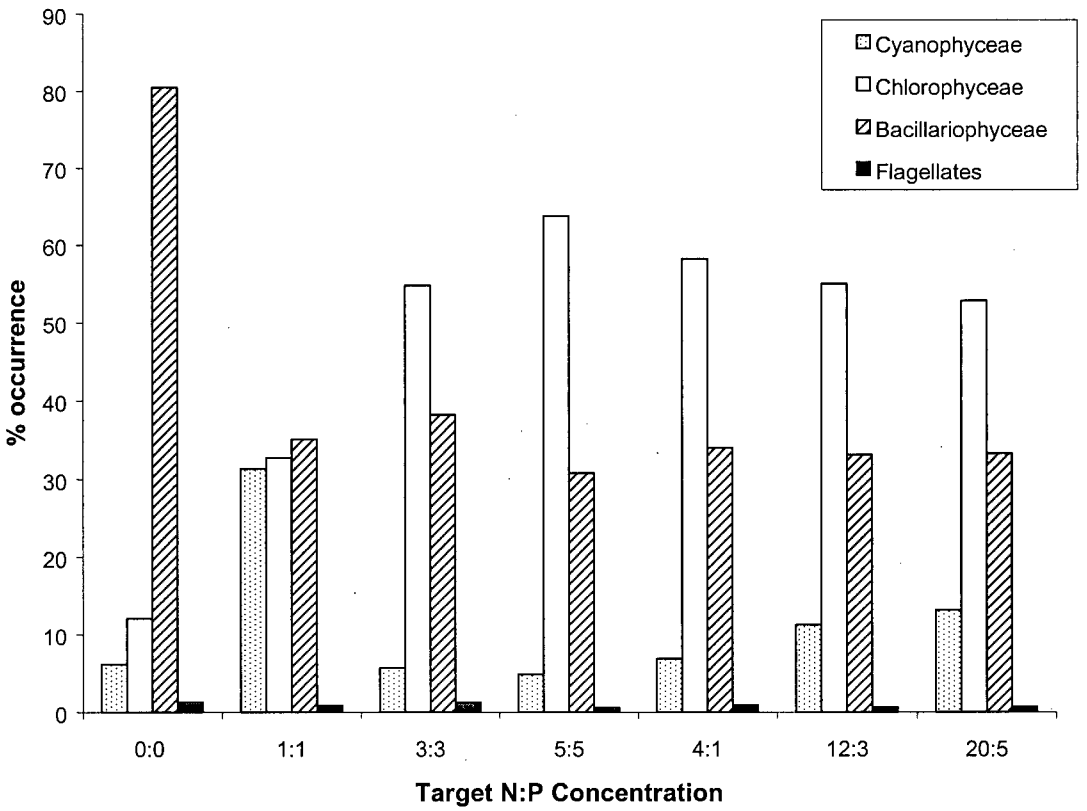

Figure 20. Percent composition of algal taxa in control and treatment troughs on September 4, 1992 (day 57). The data are based on pooled counts of 3 samples in each of 2 replicates.

Table 3. Taxonomic richness and percent composition of representative algae on gravel substrate in control and treatment troughs on September 4, 1992. Percentages based on pooled cell counts from 3 samples in each of 2 replicates. Standard error of taxonomic richness is also shown.

	Target N:P Concentration						
	0:0	**1:1**	**3:3**	**5:5**	**4:1**	**12:3**	**20:5**
Taxonomic Richness	59	61	59	59	62	62	59
2 SE	3.92	1.96	1.96	1.96	1.96	1.96	3.92
Cyanophyceae							
Agmenellum glauca	0.2	0.2	2.1	3.0	0.5	8.7	1.7
Anacystis	0.3	0.4	0.3	0.4	5.0	1.4	6.0
Lyngbya	3.4	6.6	2.8	0.7	0.7	0.5	2.4
Oscillatoria	1.9	23.9	0.3	0.6	0.3	0.3	1.8
Other	0.2	0.2	0.2	0.2	0.3	0.3	1.3
Total	*6.2*	*31.3*	*5.7*	*4.9*	*6.8*	*11.3*	*13.1*
Chlorophyceae							
Ankistrodesmus falcatus	0.4	0.2	0.5	0.3	0.3	0.5	0.9
Botryococcus braunii	1.5	0.2	0.3	0.2	0.3	0.2	0.3
Bulbochaete	0.3	0.6	0.3	1.4	0.6	0.7	0.3
Closterium	0.1	0.1	0.1	0.1	0.3	0.1	0.1
Cosmarium	0.3	0.3	0.3	0.2	0.4	0.2	0.3
Mougeotia	0.7	3.8	2.6	1.2	6.3	3.3	0.8
Oedogonium	0.5	4.3	8.3	1.2	1.8	0.4	1.0
Scenedesmus	0.4	0.4	0.4	0.3	0.4	0.8	0.4
Spirogyra	0.2	19.9	39.7	55.9	45.0	46.4	43.8
Spondylosium	0.6	0.6	0.3	0.4	0.2	0.3	0.4
Ulothrix	0.7	0.3	0.4	0.4	0.6	0.5	0.6
Zygnema	4.9	0.5	0.2	0.4	0.2	0.1	2.1
Other	1.6	1.7	1.5	1.8	2.0	1.5	2.0
Total	*12.1*	*32.8*	*54.8*	*63.8*	*58.2*	*55.0*	*52.9*
Bacillariophyceae							
Achnanthes flexella	0.4	0.3	0.4	0.4	0.4	0.6	0.5
Achnanthes minutissima	52.5	18.4	22.6	15.1	17.9	19.2	19.8
Cymbella minuta	0.9	0.3	0.4	0.3	0.4	0.3	0.3
Eunotia	1.0	0.3	0.4	0.5	0.7	0.3	0.3
Fragilaria crotonensis	2.9	2.4	1.5	2.4	1.4	1.3	1.6
Fragilaria sp	3.9	3.8	1.5	1.7	1.0	2.3	0.8
Gomphonema	0.8	0.5	0.4	0.4	0.4	0.7	0.4
Melosira	0.7	0.3	0.3	0.7	0.3	0.5	0.7
Navicula	2.4	1.5	1.2	0.9	0.8	1.1	0.9
Pinnularia	0.3	0.3	0.5	0.2	0.3	0.4	0.3
Synedra ulna	9.8	3.8	5.1	3.8	5.7	2.7	4.4
Tabellaria fenestra	1.9	0.6	0.8	1.7	0.8	1.2	0.3
Tabellaria flocculosa	0.3	0.2	0.3	0.2	0.6	0.2	0.3
Other	2.7	2.4	2.8	2.6	3.1	2.3	2.7
Total	*80.5*	*35.1*	*38.2*	*30.8*	*34.0*	*33.1*	*33.2*
Flagellates							
Chroomonas acuta	0.3	0.2	0.3	0.0	0.4	0.1	0.2
Cryptomonas	0.3	0.2	0.3	0.2	0.2	0.2	0.1
Peridinium inconspicuum	0.3	0.2	0.3	0.2	0.1	0.2	0.2
Other	0.5	0.2	0.4	0.3	0.2	0.1	0.2
Total	*1.3*	*0.9*	*1.2*	*0.6*	*0.9*	*0.6*	*0.7*

At the end of the experiment, a slight increase in taxonomic richness was observed in both control and treatment groups (Table 4) however, there was still no significant difference in number of species between groups (ANOVA; $p>0.05$). There was virtually no change in cyanobacteria composition and *A. glauca*, *Anacystis* sp., *Lyngbya* sp., and *Oscillatoria* sp. were again, most representative. Among the green algae, *Mougeotia* sp., *Oedogonium* sp., *Spirogyra* sp., and *Zygnema* sp. were dominant. *Chroomonas acuta* and *Cryptomonas* sp. were most common in the flagellates and dominance in diatom composition was again unchanged.

After 83 d, the occurrence of green algae surpassed diatoms in the control group, albeit at a marginal level (Fig. 21). A similar occurrence between diatoms and green algae was observed for each nutrient concentration within the 1:1 treatment group although an increasing trend was evident from the 1:1 to the 5:5 concentration level. Within the 4:1 treatment group, well over 50% of the species were green algae and diatoms comprised up to 45% of the total. The separation between diatoms and green algae was highest in the 20:5 ratio.

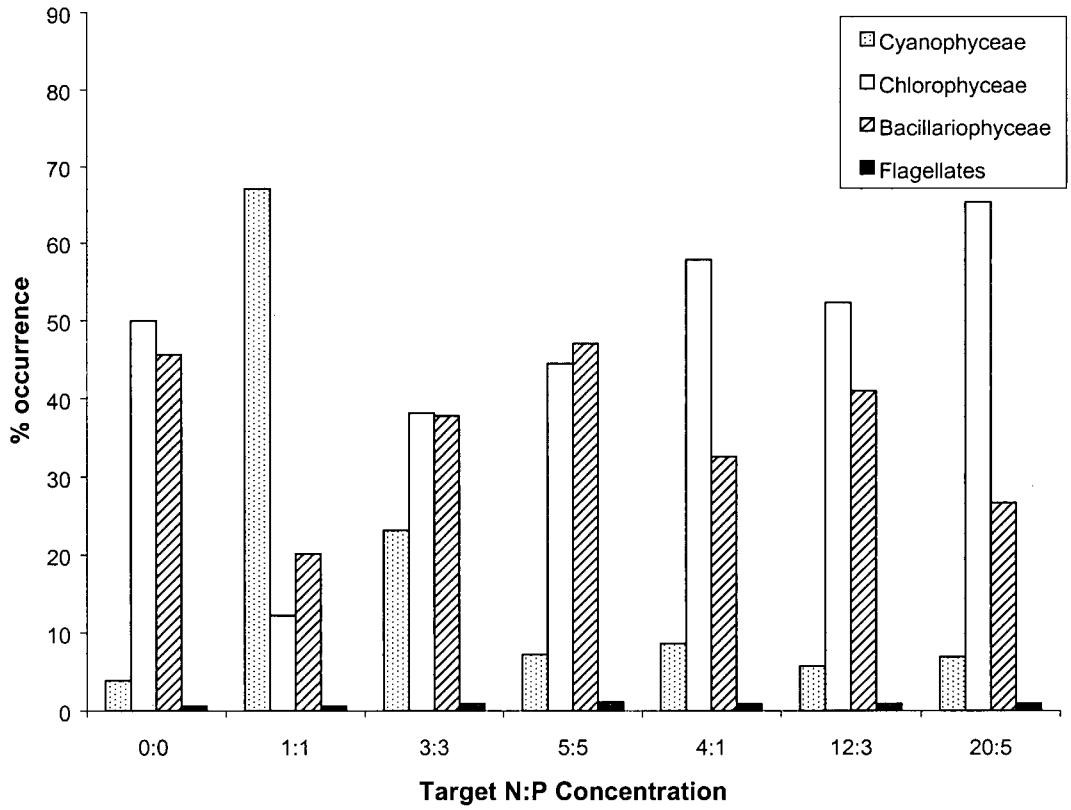

Figure 21. Percent composition of algal taxa in control and treatment troughs on September 30, 1992 (day 83). The data are based on pooled counts of 3 samples in each of 2 replicates.

Table 4. Taxonomic richness and percent composition of representative algae on gravel substrate in control and treatment troughs on September 30, 1992. Percentages based on pooled cell counts from 3 samples in each of 2 replicates. Standard error of taxonomic richness is also shown.

	Target N:P Concentration						
	0:0	1:1	3:3	5:5	4:1	12:3	20:5
Taxonomic Richness	64	64	64	63.5	63.5	63	60.5
2 SE	3.92	7.84	3.92	2.94	2.94	0.00	2.94
Cyanophyceae							
Agmenellum glauca	0.5	1.4	8.3	0.9	2.9	0.2	2.5
Anacystis	0.1	0.3	0.3	0.9	0.8	0.2	0.3
Lyngbya	1.9	0.7	8.1	2.7	1.4	0.6	2.8
Oscillatoria	1.4	63.7	6.2	2.3	2.7	4.6	1.1
Other	0.0	1.0	0.3	0.4	0.9	0.1	0.3
Total	*3.9*	*67.1*	*23.2*	*7.2*	*8.6*	*5.8*	*6.9*
Chlorophyceae							
Ankistrodesmus falcatus	0.5	0.3	0.7	0.3	0.6	0.4	0.4
Arthrodesmus	6.1	0.2	0.1	0.2	0.2	0.2	0.3
Geminella	0.6	0.3	0.9	0.3	0.3	1.6	0.8
Mougeotia	1.1	1.9	6.8	8.4	5.1	2.6	1.0
Oedogonium	2.5	1.1	3.0	3.0	3.7	3.8	5.1
Pediastrum	0.1	0.2	0.5	0.7	0.2	0.1	0.2
Scenedesmus	0.3	0.4	1.1	1.4	1.0	0.7	1.2
Spirogyra	3.6	4.9	17.6	23.9	38.8	33.9	44.6
Spondylosium	0.6	0.2	0.4	0.5	0.3	0.2	0.7
Stigeoclonium	0.1	0.3	0.2	0.5	0.2	0.2	0.3
Ulothrix	1.5	0.2	0.9	0.6	1.2	0.7	0.4
Zygnema	31.3	0.4	3.9	2.7	4.5	6.1	8.5
Other	1.5	1.9	2.0	2.1	1.7	1.8	2.0
Total	*49.9*	*12.2*	*38.1*	*44.5*	*57.9*	*52.3*	*65.4*
Bacillariophyceae							
Achnanthes flexella	0.2	0.2	0.6	0.3	0.4	0.2	0.3
Achnanthes minutissima	23.8	8.6	20.6	30.6	15.8	23.2	13.8
Cymatopleura	6.1	0.2	0.1	0.1	0.1	0.2	0.2
Cymbella minuta	0.4	0.2	0.6	0.4	0.9	0.4	0.3
Eunotia	1.1	0.3	0.6	0.4	0.5	0.4	0.4
Fragilaria crotonensis	1.6	1.7	1.8	2.1	1.7	1.7	1.3
Fragilaria sp	1.8	0.5	2.5	1.2	4.2	2.4	1.6
Gomphonema	0.2	0.3	0.4	0.5	0.6	0.4	0.5
Melosira	0.4	0.4	0.4	1.4	0.6	0.2	0.2
Navicula	0.5	1.5	1.1	1.6	0.5	0.9	0.7
Synedra ulna	3.6	2.7	3.5	4.8	3.1	6.7	3.2
Tabellaria fenestra	3.4	0.6	1.2	0.5	0.5	1.3	0.5
Tabellaria flocculosa	0.4	0.3	0.9	0.5	0.2	0.2	0.3
Other	1.9	2.6	3.5	2.8	3.5	2.9	3.3
Total	*45.6*	*20.1*	*37.8*	*47.1*	*32.6*	*41.0*	*26.7*
Flagellates							
Chroomonas acuta	0.1	0.2	0.3	0.1	0.2	0.1	0.2
Cryptomonas	0.2	0.2	0.2	0.2	0.2	0.2	0.3
Other	0.3	0.2	0.4	0.8	0.5	0.6	0.4
Total	*0.6*	*0.6*	*0.9*	*1.1*	*0.9*	*0.9*	*0.9*

The cyanobacteria approached almost 70% of the cells in the 1:1 nutrient concentration, exceeded 30% in the 5:5 nutrient concentration and represented <10% of all other treatment or control groups. The flagellates remained consistent at < 5% of the total composition across all groups.

The difference in the number of algal taxa sampled on gravel substrate between day 35 and the end of the experiment was tested to distinguish differences in diversity over time and determine if there were any differences in the rate of change between treatments. Although no significant difference in the number of taxa between treatments was observed (ANOVA; $p>0.05$), there was a significantly greater number of taxa at the end of the experiment among experimental units than after 35 d (ANCOVAR; $p<0.05$). There was no significant difference however, in the slope of the interaction lines across control and treatment groups suggesting that settlement and colonization rates were similar among all experimental units (ANCOVAR; $p>0.05$; Fig. 22).

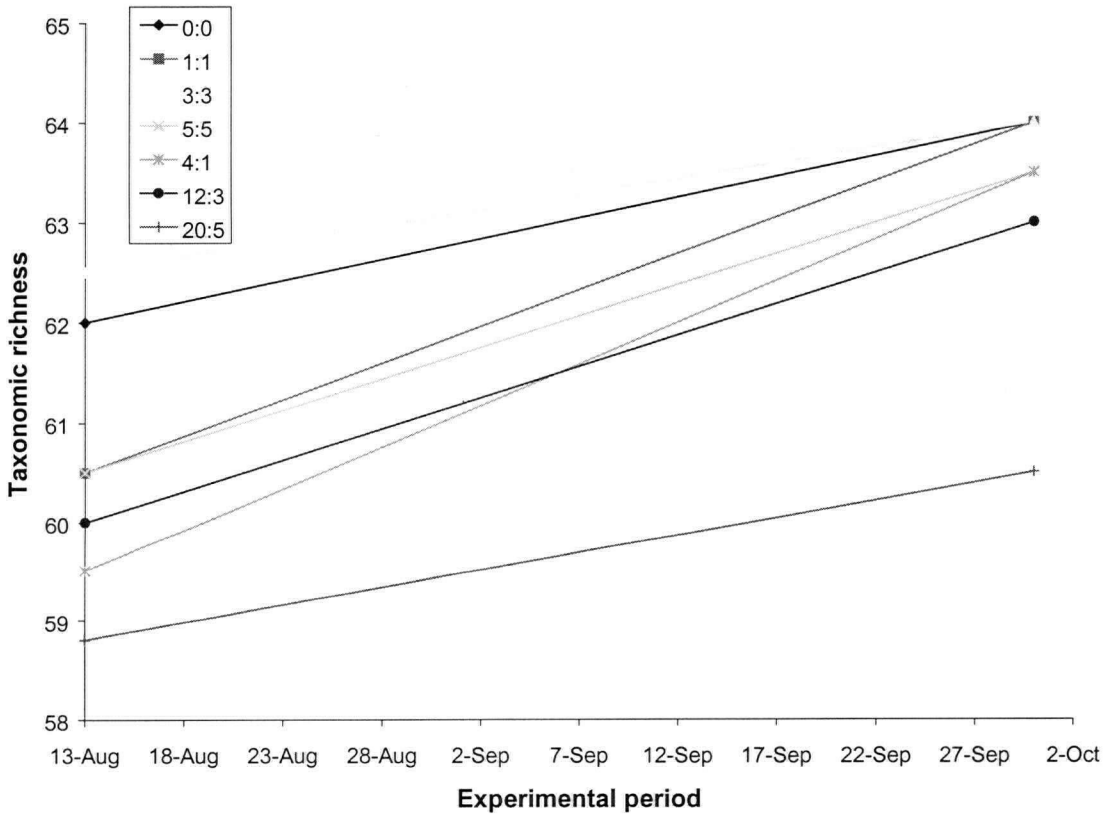

Figure 22. Taxonomic richness of algal cells measured on gravel substrate in control and treatment troughs August 13 and September 30, 1992. Interconnecting lines between data points show the treatment * time interaction. The test for equality of slopes was non-significant. Significance values are provided in text.

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Albeit that a significant difference in richness between control and treatment groups was not demonstrated, fewer taxa in the 12:3 and 20:5 nutrient concentrations were evident compared to all other treatment or control groups. This was likely associated with a lower initial richness (Table 2). Overall, fewer species of flagellates were observed at the end of the experiment.

A significant difference in the mean number of taxa on gravel in baskets after 35 d (August 13) compared with the mean number on styrofoam substrate after 27 d (September 30) was evident with fewer taxa occurring on styrofoam (Table 5; ANOVA; $p<0.01$). While the former comparison may highlight the patchy distribution of algae within troughs, the latter comparison, implying colonization preferences in substrate type, may in fact be an artifact of passive settlement due to differences in sampling dates.

Table 5. Taxonomic richness of representative algae on gravel (August 17 in baskets) and styrofoam (September 30) substrates in control and treatment troughs. Number of genera based on means from 2 replicates. Standard error of taxonomic richness is also shown.

	Target N:P Concentration						
	0:0	**1:1**	**3:3**	**5:5**	**4:1**	**12:3**	**20:5**
Basket							
Taxonomic Richness	58.0	58.5	56.5	59.0	57.0	56.0	56.5
2 SE	1.96	2.94	2.94		1.96	1.96	2.94
Styrofoam							
Taxonomic Richness	51.0	50.0	51.5	53.0	56.0	51.5	52.5
2 SE	1.96	15.68	2.94	5.88	1.96	2.94	4.90

Differences in taxonomic richness over the duration of the experiment were not only reflected in the number of species encountered but also expressed in terms of morphological diversity. The combination of unicellular, colonial and filamentous forms was characteristic of the species assemblage throughout the period of study however, among the chlorophytes a shift towards filamentous forms was favoured at the end of the experiment in treatments characteristic of higher N:P ratios. Genera such as *Spirogyra*, *Zygnema*, and *Oedogonium* were dominant under a higher nitrogen regime (e.g. 12:3 and 20:5 concentration) whereas *Mougeotia* sp. was favoured under a lower N:P ratio (e.g. 3:3 and 5:5 concentration). The development of species assemblages dominated by filamentous forms under high N:P concentrations and ratios has implications on the quality of food resources for higher trophic levels (i.e. the insect community).

3.4 Macroinvertebrate community response

3.4.1 Benthic insects, body size and taxa

3.4.1.1 Interim benthos

Benthic baskets collected from experimental units on August 17 (day 39) provided an estimate of insect density in stream troughs after 20 d of fertilization. Mean densities at this time were only marginally higher in three of six treatment concentrations compared to the control (Fig. 23). Large differences in insect numbers between replicate samples within paired troughs suggested a rather patchy distribution of the representative taxa and resulted in the high degree of variation within treatments. Not surprisingly, differences in mean insect density between control and treatment groups were non-significant (ANOVA; $p>0.05$). Benthic insects in troughs on day 39 were largely dominated by chironomids (Fig. 24). Mayflies, represented mostly by baetid nymphs, were sub-dominant albeit in low abundance. Hydropsychid caddisflies along with chloroperlid, perlodid and pteronarcyid stoneflies were present across all experimental units but

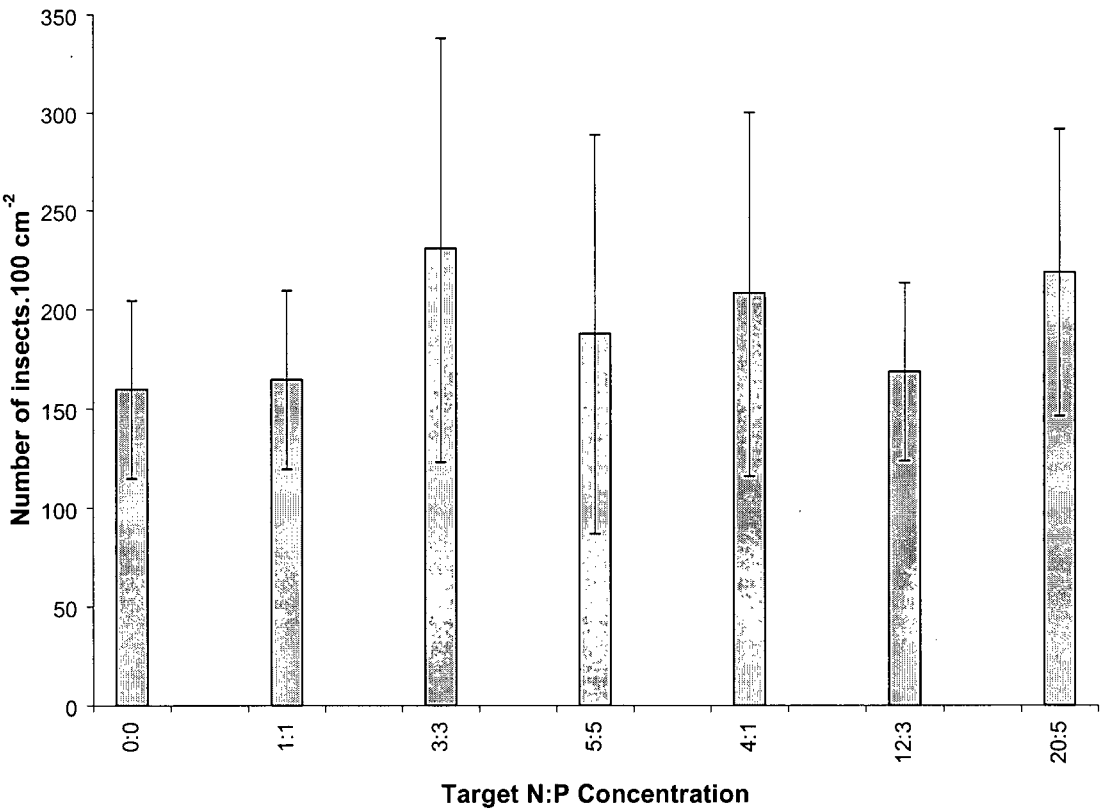

Figure 23. Mean density of benthic insects across control and treatment troughs based on basket samples removed August 17, 1992. Mean estimates were derived from pooled counts of two samples within each replicate (n=4). 95% CI is indicated.

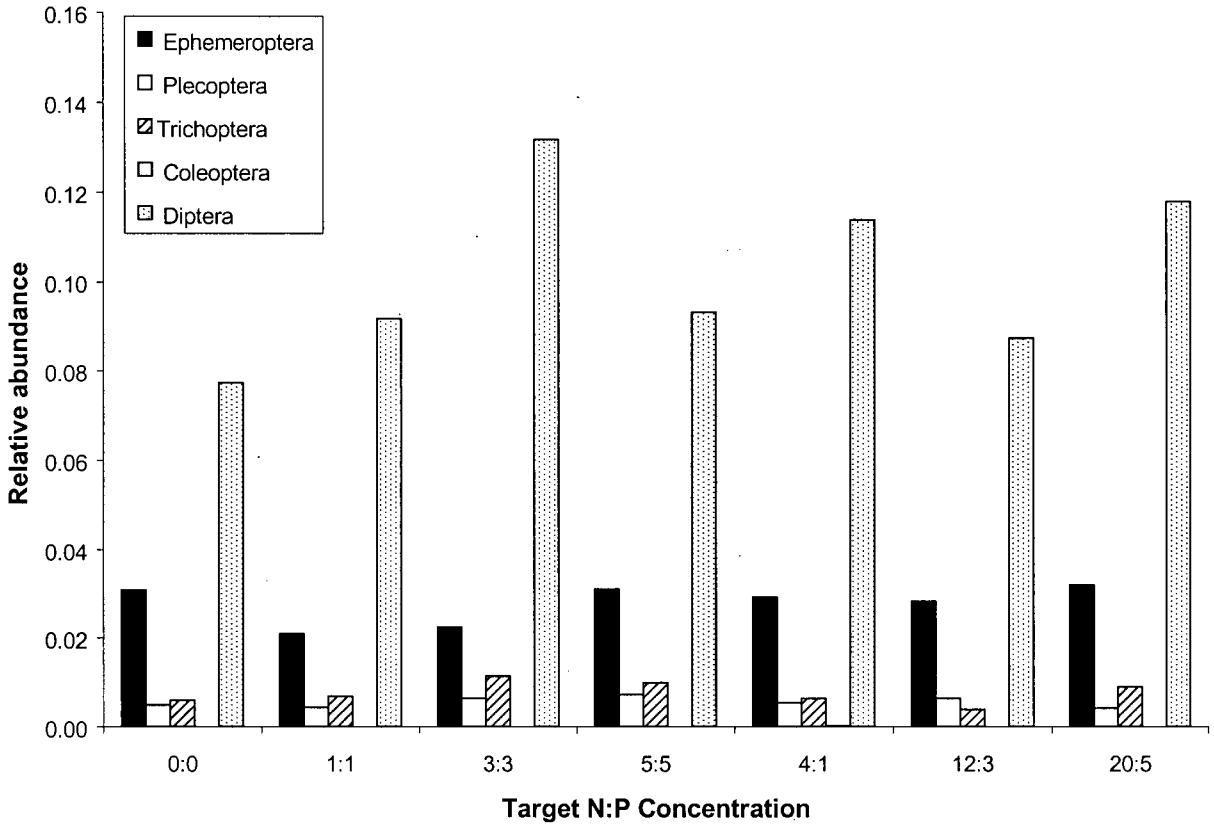

Figure 24. Relative abundance of representative taxa in stream trough benthos on August 17, 1992 (day 39). Individual treatments and control were normalized for total insect density in baskets across all experimental units.

elmid beetles were only observed in the 4:1 treatment troughs.

Approximately one month later (September 14), mean densities of benthic insects in treatment troughs displayed up to a 5-fold increase over their previous levels whereas the benthos in control troughs doubled (Fig. 25). Up to a 3-fold increase in mean density within treatments was observed over background. The largest densities occurred within the 5:5 and 4:1 treatment concentrations. Although not so evident in control troughs, considerable variation among baskets within replicates was still apparent in treatment troughs. After 48 d of nutrient enrichment, a significant difference in mean insect density was demonstrated between control and treatment groups (ANOVA; $p<0.05$). A significant difference was also evident between the treatment groups ($p<0.05$). Orthogonal contrasts displayed a significant difference between the 1:1 and 4:1 and 3:3 and 12:3 concentrations (ANOVA; $p<0.05$) but failed to include the 5:5 and 20:5 comparison ($p>0.05$). The larger densities were accordingly noted in the 4:1 and 12:3 nutrient ratios. Within treatment groups, the 1:1 nutrient concentration was again significantly

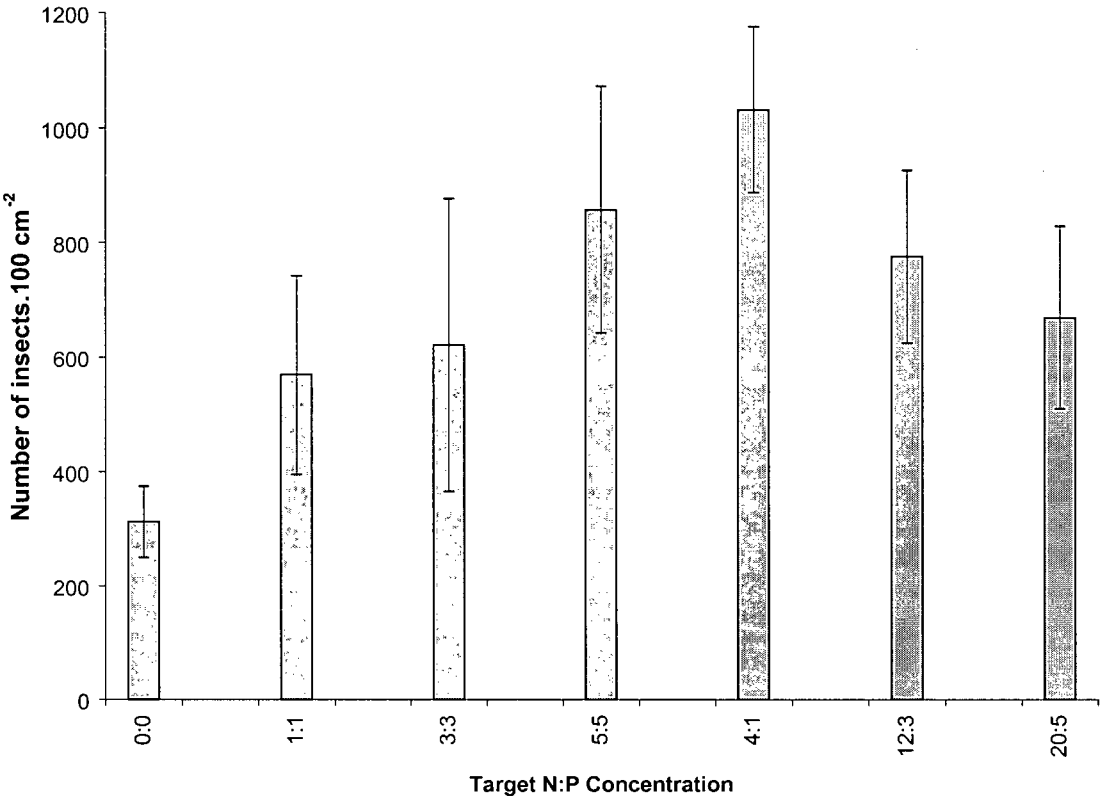

Figure 25. Mean density of benthic insects across control and treatment troughs based on basket samples removed September 14, 1992. Mean estimates were derived from pooled counts of two samples within each replicate (n=4). 95% CI is indicated.

different than the 5:5 treatment ratio (ANOVA; $p<0.05$), however, densities of insects in the 3:3 and 5:5 concentrations were not significantly different from each other (ANOVA; $p>0.05$). There was no significant difference in density among the 4:1 ratio concentrations (ANOVA; $p>0.05$).

Comparable to the August 17 density estimate, chironomids remained dominant in the benthos of the September 14 sample (Fig. 26). The relative abundance of mayflies and caddisflies increased slightly over the previous date but stoneflies remained at their previous low level. The complete absence or extremely low incidence of elmid beetles was again reflected across all experimental units.

Changes in benthic insect density between August 17 and September 14 were further tested by analysis of covariance for homogeneity of slope (Fig. 27). Density and the change in density were significantly higher in treatment groups than controls over the two dates (ANCOVAR; treatment * time interaction; $p<0.05$). Bonferroni pairwise comparisons (*a posteriori*) revealed a significantly greater slope of the interaction lines for both the 4:1 and 5:5 nutrient concentrations

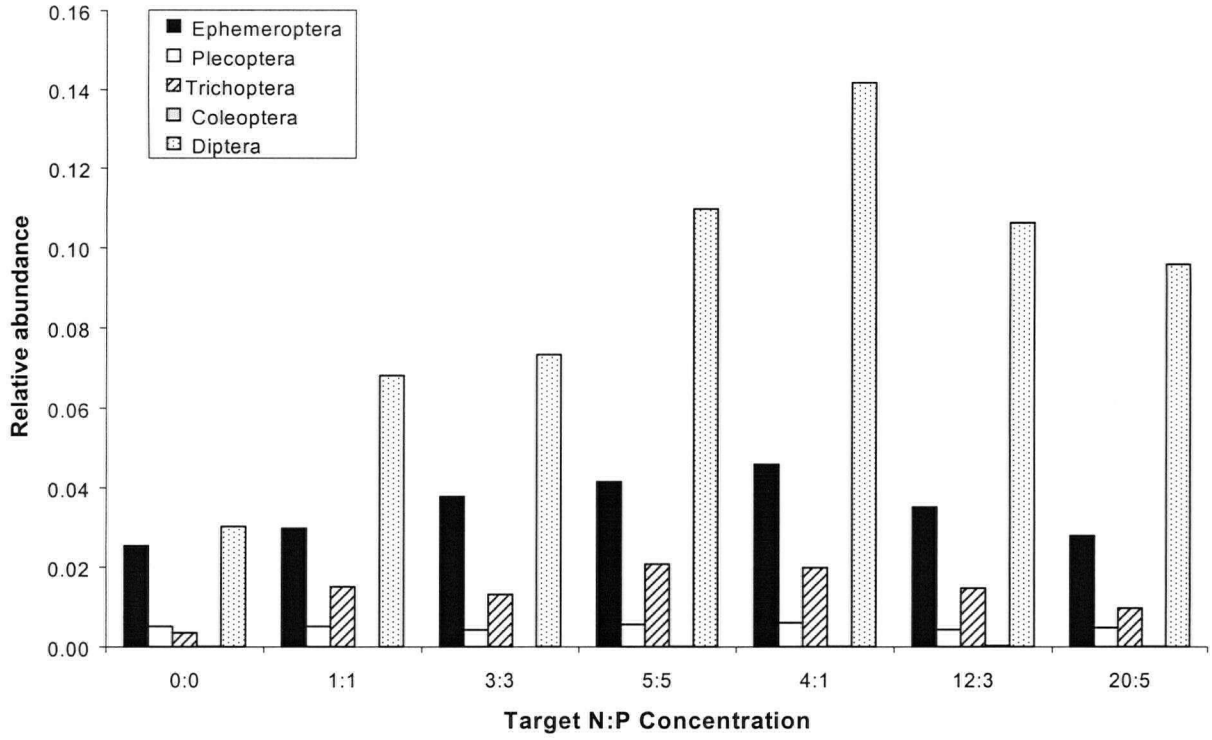

Figure 26. Relative abundance of representative taxa in stream trough benthos on September 14, 1992 (day 67). Individual treatments and control were normalized for total insect density in baskets across all experimental units.

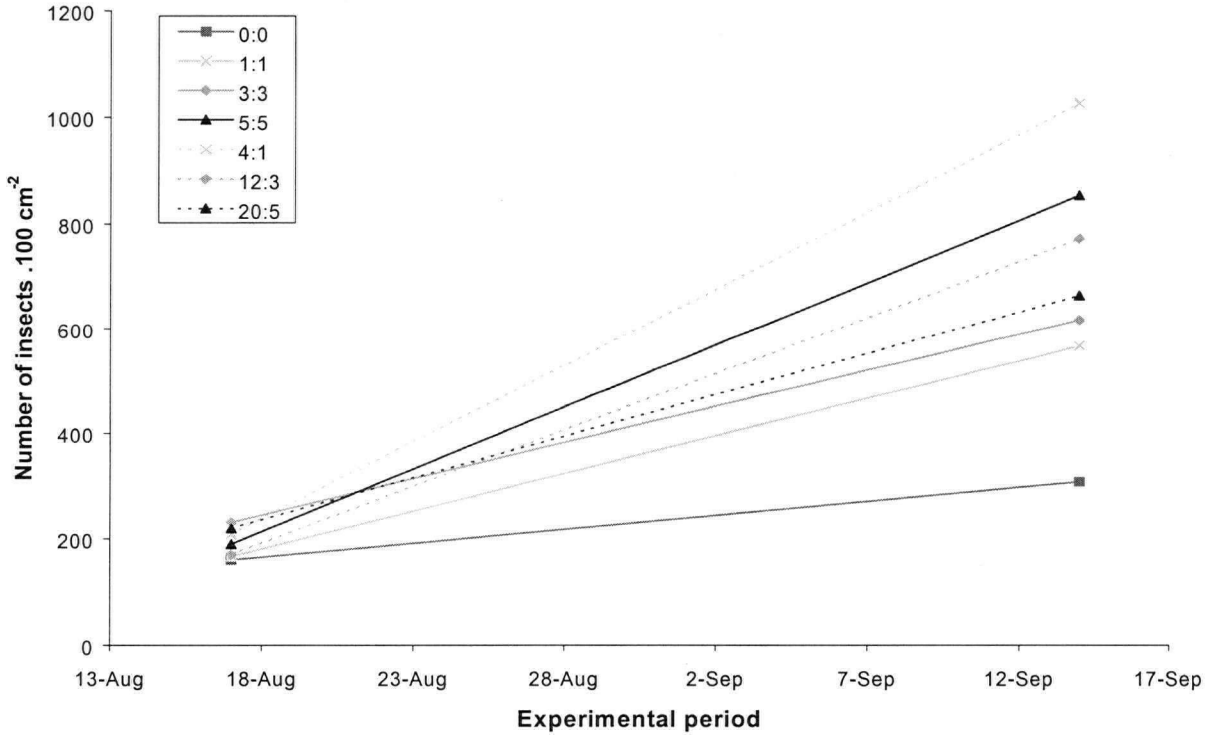

Figure 27. Differences in benthic insect density from August 17 to September 14, 1992. Treatment * time interaction lines are illustrated to show differences in the rate of change between control and treatment groups over time.

(ANCOVAR; $p<0.05$).

The total biomass of all insects in experimental troughs measured on August 17 deviated somewhat from the pattern observed for insect density (Fig. 28). Whereas the highest density of insects was measured in the 3:3 nutrient concentration, the highest biomass was observed in the 4:1 and 12:3 concentrations. Both of the latter treatments however, had the highest variation among replicated samples in the paired troughs. Differences in biomass across all experimental units for this date were also non-significant (ANOVA; $p>0.05$). Closer inspection of the relative biomass of the representative taxa on the same date indicated that a much larger proportion of mass was contributed by stoneflies in the 4:1 and especially the 12:3 treatments and accounted for the overall differences in relative biomass between treatment groups (Fig. 29). Other noteworthy differences in relative biomass between treatments include the higher proportion of chironomids in the 1:1 nutrient concentration and the higher proportion of mayflies in the 5:5 nutrient concentration. A much smaller proportion of stoneflies was also characteristic of the 20:5 concentration compared to its counterparts in the 4:1 treatment.

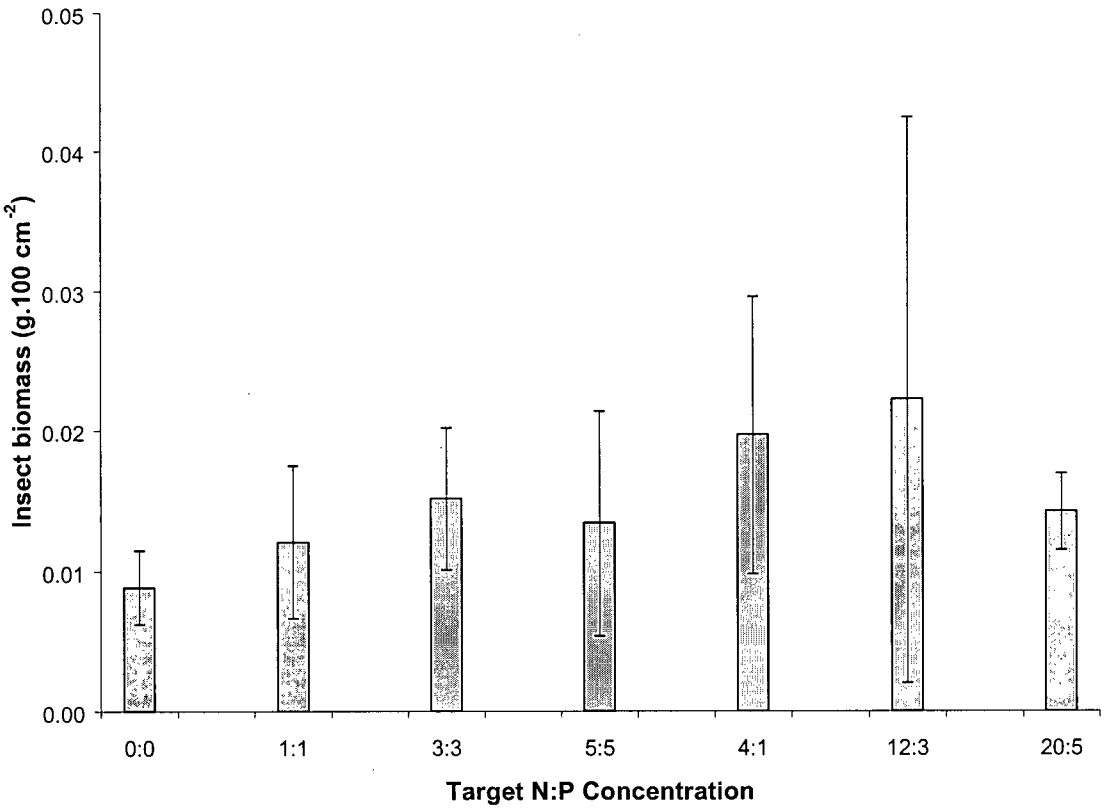

Figure 28. Mean biomass of benthic insects across control and treatment troughs based on basket samples removed August 17, 1992. Mean estimates were derived from pooled counts of two samples within each replicate (n=4). 95% CI is indicated.

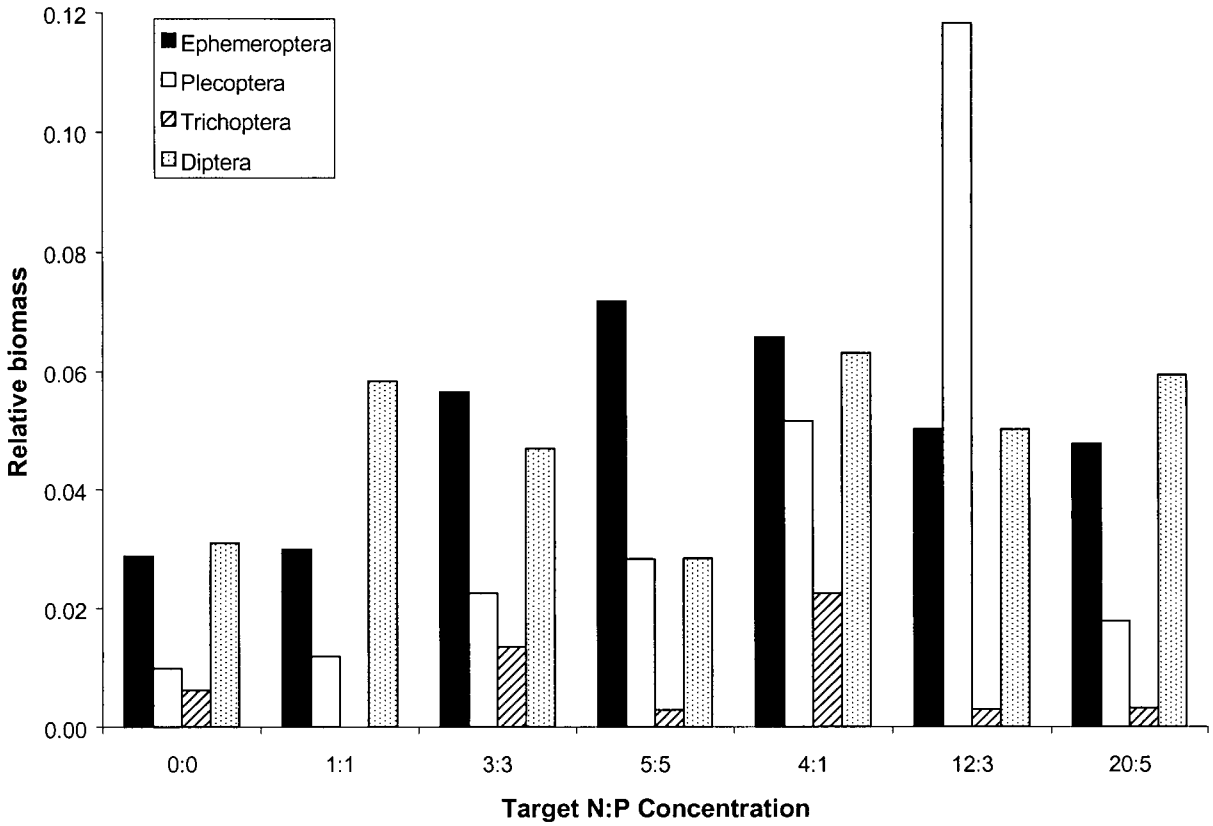

Figure 29. Relative biomass of representative taxa in stream trough benthos on August 17, 1992 (day 39). Individual treatments and control were normalized for total insect biomass in baskets across all experimental units.

A slightly higher proportion of caddisflies was also evident within the 4:1 nutrient concentration.

Differences in total biomass across experimental troughs on September 14 were much more apparent between control and treatment groups (Fig. 30). After 48 days of fertilization, a near-doubling of biomass was evident between the control and the 5:5 and 4:1 nutrient concentrations. Notwithstanding, a high degree of variation was also observed among all experimental units. As such, a significant difference in biomass between control and treatment groups was not demonstrated (ANOVA; $p>0.05$). Closer examination of the relative biomass of the representative taxa suggests that the high incidence of stoneflies (particularly pteronarcyids) in the control group may have been responsible for the notable contrast between insect density and biomass (Fig. 31). The substantial contribution of mayflies and chironomids to the total biomass measured on September 14 was further examined by partitioning both taxa. The removal of a single outlier in the analysis for mayflies demonstrated a significant difference between control and treatment groups (ANOVA; $p<0.05$) and a highly significant

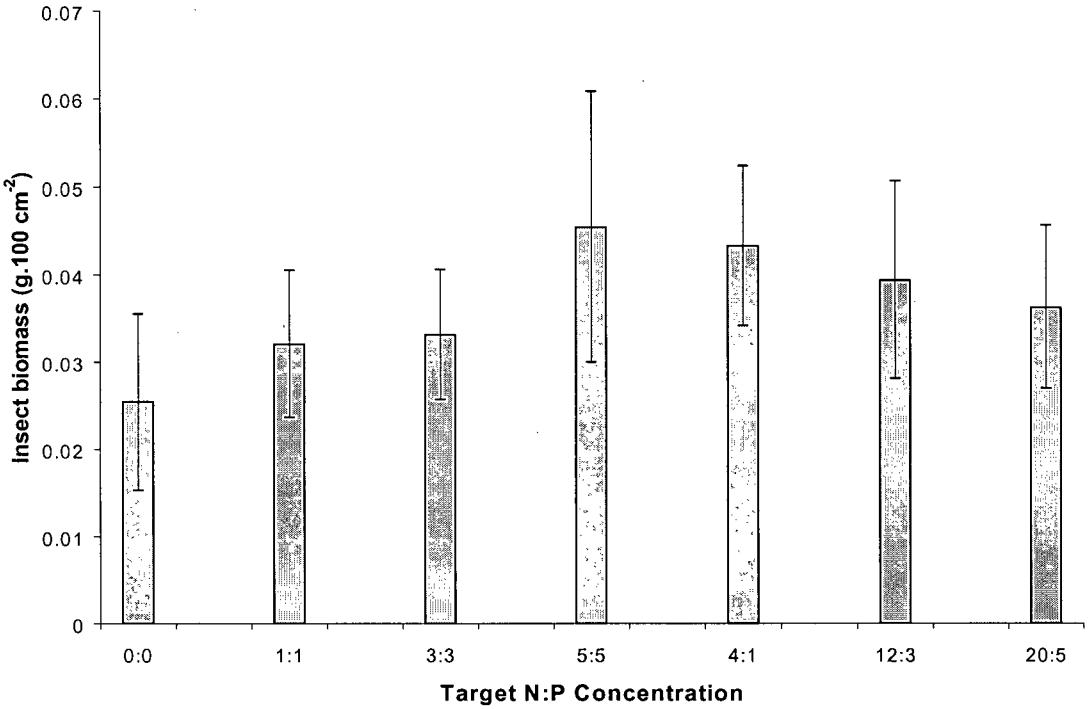

Figure 30. Mean biomass of benthic insects across control and treatment troughs based on basket samples removed September 14, 1992. Mean estimates were derived from pooled counts of two samples within each replicate (n=4). 95% CI is indicated.

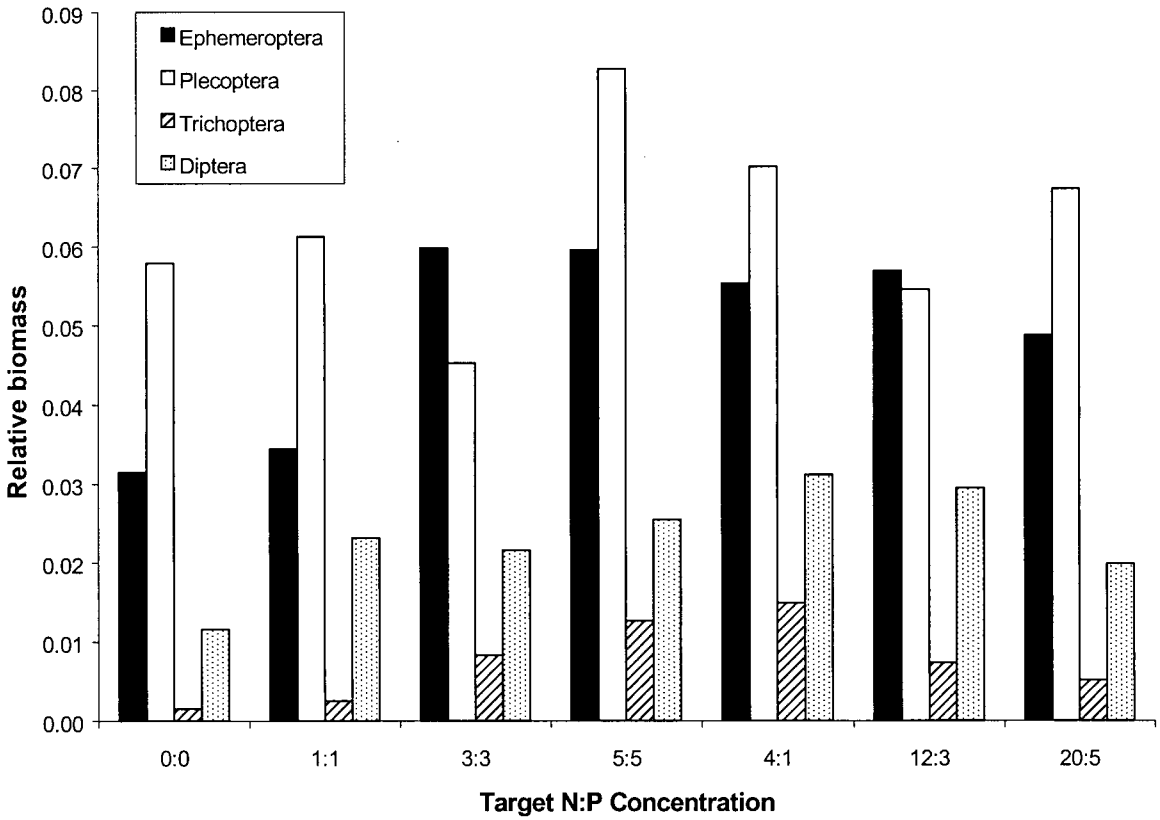

Figure 31. Relative biomass of representative taxa in stream trough benthos on September 14, 1992 (day 67). Individual treatments and control were normalized for total insect biomass in baskets across all experimental units.

difference for chironomids ($p<0.01$; Figs. 32 and 33). Orthogonal contrasts of chironomid biomass across treatments revealed significant differences between treatment groups (4:1 vs 1:1, 12:3 vs 3:3 and 20:5 vs 5:5; $p<0.05$). Within treatment groups, a significant difference was only demonstrated between the 1:1 nutrient concentration and the 3:3 and 5:5 concentrations (ANOVA; $p<0.05$). There was no significant difference however between the latter two treatments (ANOVA; $p>0.05$).

The test for homogeneity of slopes between control and treatment groups revealed a significant difference in insect biomass between the August 17 and September 14 dates ($p<0.05$) but the interaction term indicating differences in the rate of change in biomass were non-significant over the same period (ANCOVAR; $p>0.05$; Fig. 34). Bonferroni pairwise comparisons (*a posteriori*) showed a significant difference in biomass between the 4:1 and 12:3 treatment and control groups. Despite appearances, the rate of change in biomass within the 5:5 nutrient concentration was non-significant (ANCOVAR; $p>0.05$).

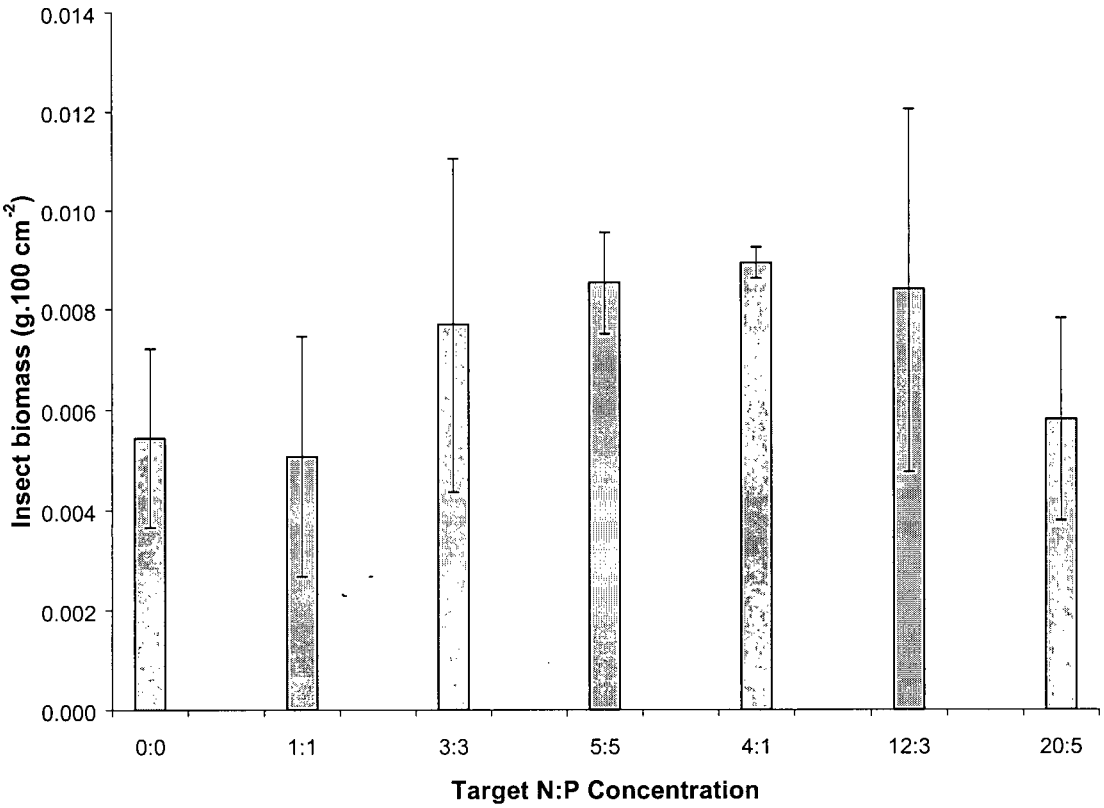

Figure 32. Mean biomass of baetid mayflies across control and treatment troughs based on basket samples removed September 14, 1992. Mean estimates were derived from pooled counts of two samples within each replicate (n=4). 95% CI is indicated.

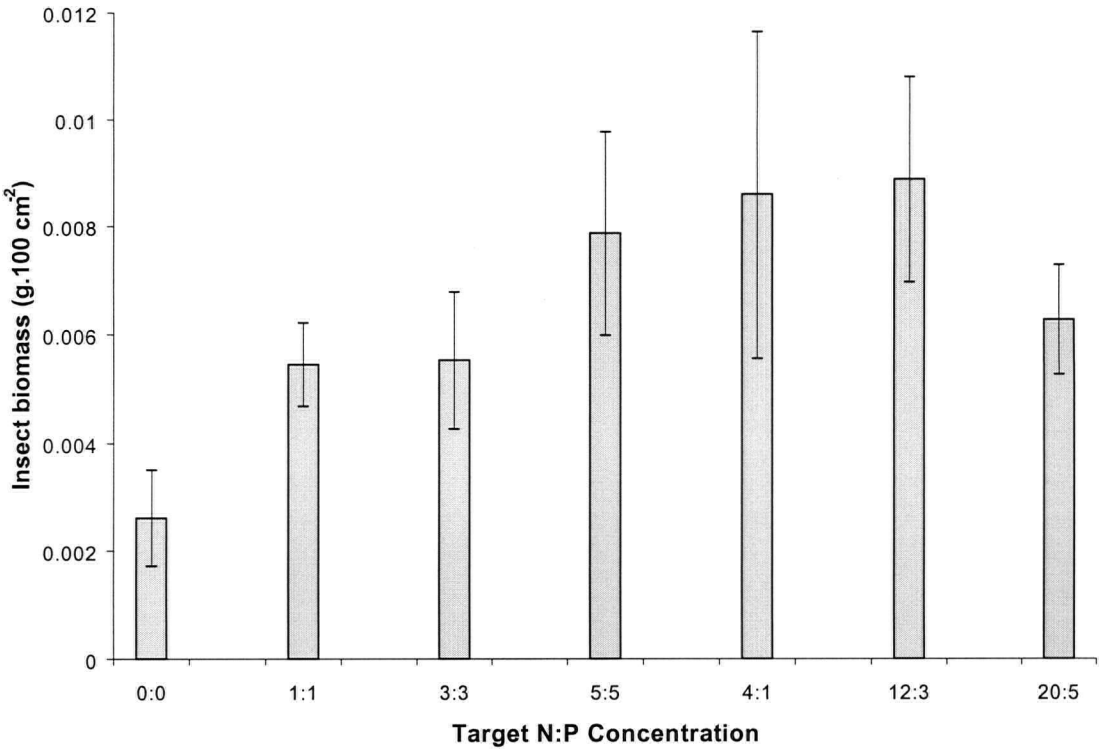

Figure 33. Mean biomass of chironomids across control and treatment troughs based on basket samples removed September 14, 1992. Mean estimates were derived from pooled counts of two samples within each replicate (n=4). 95% CI is indicated.

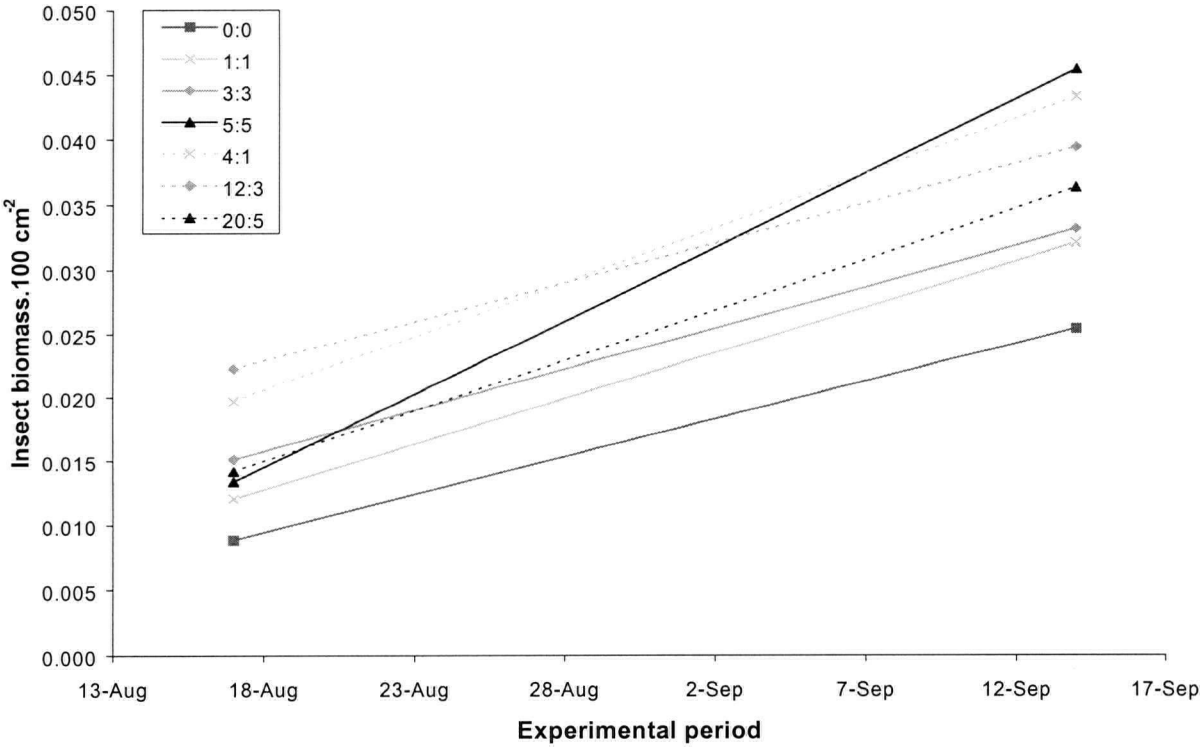

Figure 34. Differences in biomass of insects measured on August 17 and September 14, 1992. Treatment * time interaction lines are illustrated to show differences in the rate of change between control and treatment groups over time.

3.4.1.2 Final trough benthos

The total mean number of benthic insects across control and treatment troughs at the end of the experiment (September 30) varied considerably (Table. 6). The largest contrast in mean density between control and treatment groups was observed among the 5:5, 4:1, and 20:5 nutrient concentrations, although the lowest overall numbers across all experimental units were found in the 12:3 concentration (Fig. 35). Similar mean densities of the 4:1 and 20:5 nutrient concentrations displayed a 2-fold increase over the control. Due to the large variation between replicates (particularly the control), however, significant differences in mean number between control and treatment groups were not demonstrated in the benthos at the end

Table 6. Total mean counts of benthic insects from control and treatment troughs at the end of the experiment (September 30, 1992). Mean values derived from two replicates.

	Target N:P Concentrations						
	0:0	**1:1**	**3:3**	**5:5**	**4:1**	**12:3**	**20:5**
Order Ephemeroptera							
Baetidae	251	279.5	294	427	462	296	521
Ephemerellidae	20	13	32	54.5	54.5	11.5	76
Heptageniidae	71	70	82.5	135	118.5	72.5	132
Leptophlebiidae	32	25.5	29	51	56	30.5	57
Siphlonuridae	93.5	98	121	96.5	196	69	177.5
Sub-total	*467.5*	*486*	*558.5*	*764*	*887*	*479.5*	*963.5*
Order Plecoptera							
Capnidae	0	0	4.5	0	3.5	0	2
Chloroperlidae	41.5	31.5	51	48.5	57	48	41
Nemouridae	0	1	1	0	1.5	0	4.5
Perlidae	4.5	4	2.5	0.5	1.5	2.5	1
Perlodidae	32	30.5	33	50.5	50.5	27.5	46.5
Pteronarcyidae	81.5	38.5	78	59.5	55.5	41	51
Sub-total	*159.5*	*105.5*	*165.5*	*159*	*166*	*119*	*144*
Order Trichoptera							
Brachycentridae	3	4	1	12.5	6.5	2	11.5
Hydropsychidae	9	6	4.5	8	5.5	8	16
Hydroptilidae	61.5	67.5	95.5	87.5	87.5	32.5	54.5
Lepidostomatidae	62	112	143.5	514	272.5	68.5	352.5
Subtotal	*135.5*	*189.5*	*244.5*	*622*	*372*	*111*	*434.5*
Order Coleoptera							
Elmidae	4	3	4	5	3	2	2.5
Haliplidae	0	2.5	3.5	3	1	1	1.5
Sub-toal	*4*	*5.5*	*7.5*	*8*	*4*	*3*	*4*
Order Diptera							
Ceratopogonidae	4	0.5	2.5	2	1	1	2.5
Chironomidae	184	215.5	276	383.5	349.5	197.5	404.5
Chironomidae (pupae)	11.5	10.5	12	22	22.5	9.5	25
Empididae	0	0.5	0	0	1	0.5	0
Simuliidae	1	1.5	2	0	0.5	0.5	0.5
Tipulidae	0.5	0	0.5	1.5	0.5	0.5	0.5
Sub-total	*201*	*228.5*	*293*	*409*	*375*	*209.5*	*433*
Total	*967.5*	*1015*	*1269*	*1962*	*1804*	*922*	*1979*

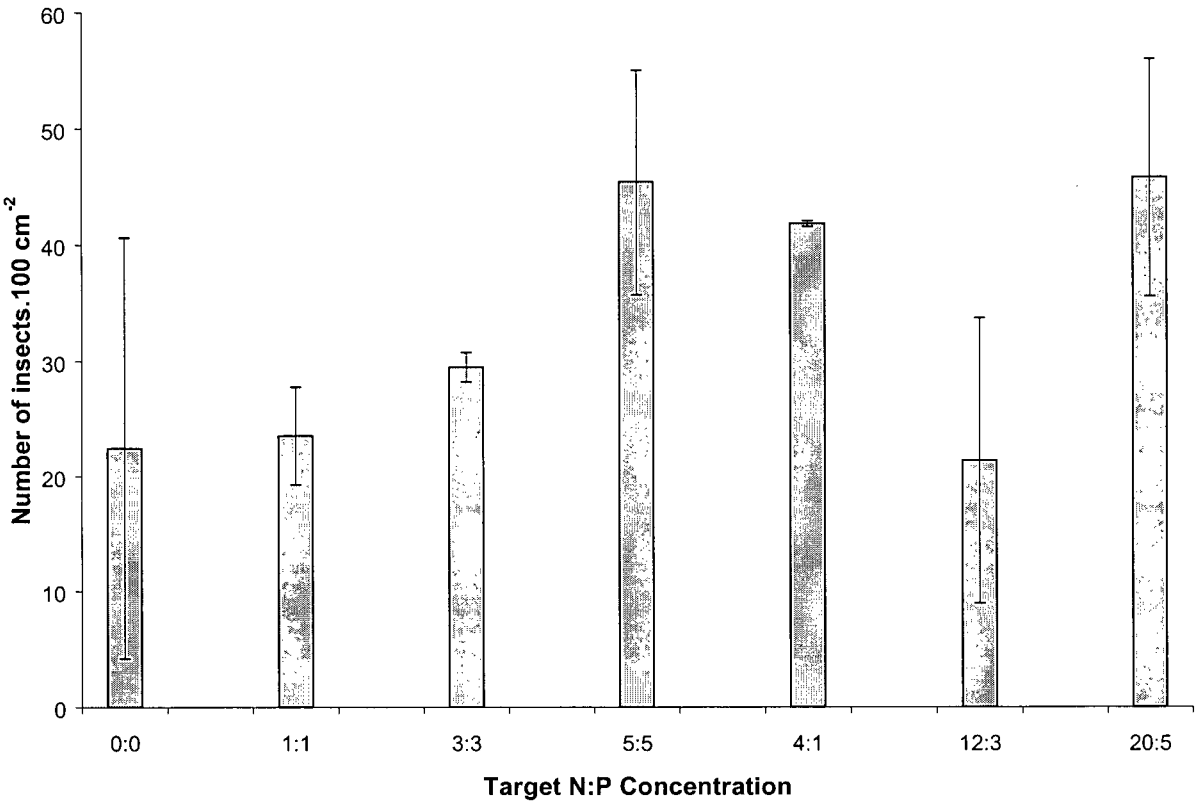

Figure 35. Mean density of insects in control and treatment troughs estimated from total trough benthos on September 30, 1992. Mean estimates were derived from two replicates. 95% CI is indicated.

of the experiment (ANOVA; $p>0.05$). In contrast to the dominance of chironomids in the mid-September benthos (refer to Fig. 33), a shift to mayfly dominance was observed across all experimental units reaching highest abundance in the 4:1 and 20:5 nutrient concentrations (Fig. 36). Chironomids were approximately one-half the relative abundance of mayflies in late September. Both caddisflies and chironomids occurred in near equal abundance across troughs followed by stoneflies. The largest abundance of caddisflies was found in the 5:5 nutrient concentration.

The low density of insects at the end of the experiment is best explained in terms of per capita drift rates observed on September 30 than earlier in the month. The highest per capita drift rate at the end of the experiment was associated with the 12:3 nutrient concentration. The lower relative abundance of chironomids at the end of the experiment also complements their higher emigration following day 55, after re-positioning of the mesocosm to the west side of the river. Per capita drift rates and the affect of mesocosm re-positioning on drift behaviour will be explored in greater detail in section 3.4.2.

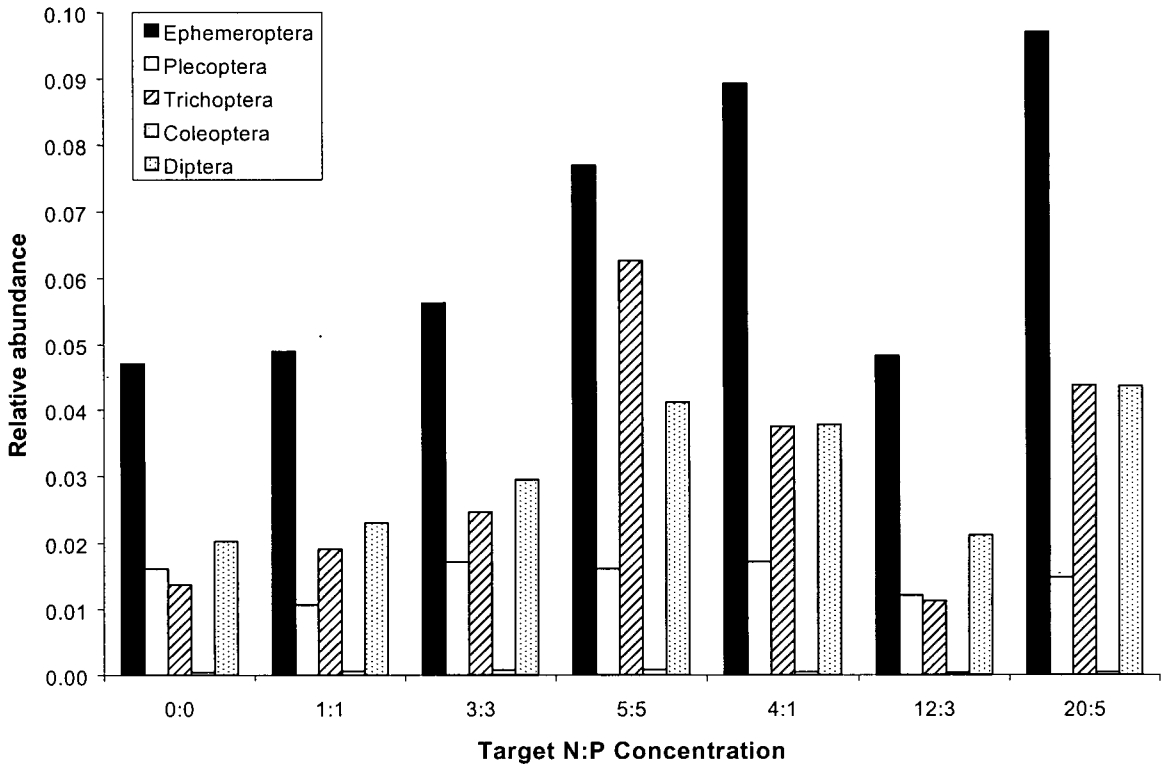

Figure 36. Relative abundance of representative taxa in stream trough benthos on September 30, 1992 (day 83). Individual treatments and control were normalized for total insect density in troughs across all experimental units.

Differences in biomass between control and treatment groups paralleled the pattern established for benthic insect density (Fig. 37). The largest contrast between control and treatment groups was only observed in the 4:1 nutrient concentration which again displayed a 2-fold increase over the control. Similarly, high variation between replicates was clearly apparent and a significant difference in biomass between control and treatment groups was unattained at the conclusion of the study (ANOVA; $p>0.05$). Whereas both 4:1 and 20:5 nutrient concentrations were similar in density, differences in biomass between these treatments were associated with a higher proportion of mayflies and stoneflies in the 4:1 concentration (Fig. 38). Hydroptilid and lepidostomatid caddisflies were noticeably higher in biomass in the 5:5 nutrient concentration at the end of the experiment and distinctly higher than biomass estimates obtained from baskets earlier in the month.

Differences in insect growth (tested by orthogonal contrast) between and within control and treatment groups, respectively, were compared for mayflies, midges, stoneflies and caddisflies sub-sampled in the final benthos (Table 7; Figs. 39 - 46). Significant differences in head capsule width size were determined for baetid nymphs between the control and treatment

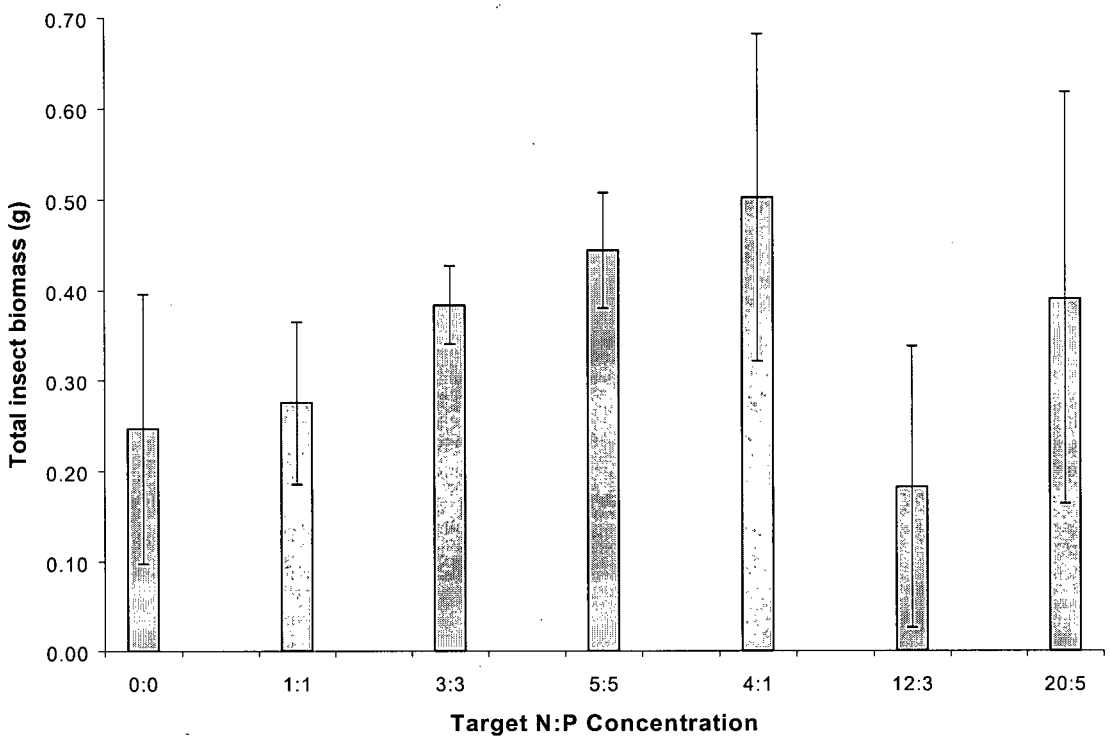

Figure 37. Mean insect biomass in control and treatment troughs estimated from total trough benthos on September 30, 1992. Mean estimates were derived from two replicates. 95% CI is indicated.

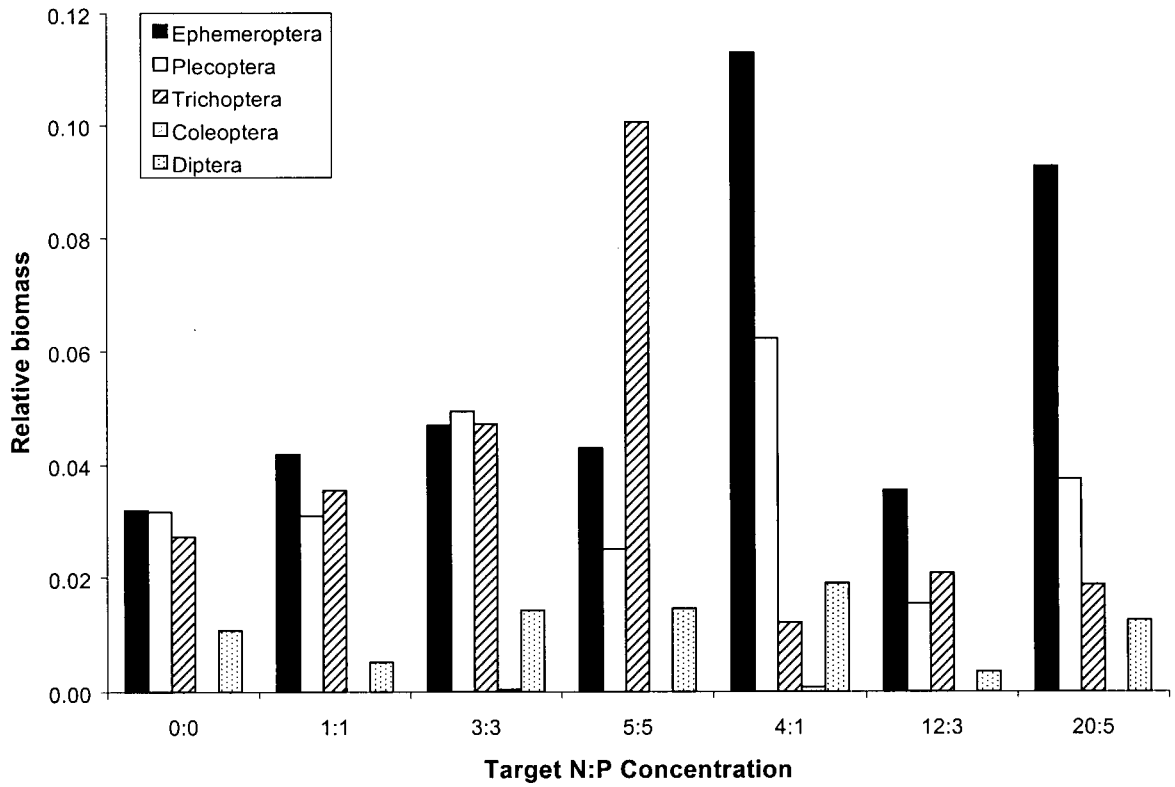

Figure 38. Relative biomass of representative taxa in stream trough benthos on September 30, 1992 (day 83). Individual treatments and control were normalized for total insect biomass in troughs across all experimental units.

concentrations (ANOVA; $p<0.05$; Figs. 39 and 40) with a higher frequency of the larger size categories occurring among treatments. Size differences were also significant between treatment groups with the 4:1 treatment ratio group (ANOVA; $p<0.05$) and generally displayed a wider distribution of sizes both smaller and larger. Within treatment groups, the only significant differences demonstrated occurred between the 12:3 and 20:5 nutrient concentrations with a higher proportion of larger size categories in the 12:3 concentration. Significant differences in chironomid head capsule width size categories were also demonstrated but may have been related to sampling bias associated with the sub-sampling apparatus (ANOVA; $p<0.05$; Figs. 41 & 42). In this instance, size distributions of the 1:1 and 4:1 treatment ratios were slightly skewed to the smaller size categories compared to the control. Significant differences between treatment groups were also determined between the 1:1 and 4:1 and 3:3 and 12:3 nutrient concentrations (ANOVA; $p<0.05$) but not between the 5:5 and 20:5 concentrations ($p>0.05$). Differences within both treatment groups (i.e. 1:1 and 4:1 treatment ratios) were non-significant (ANOVA; $p>0.05$). With respect to stoneflies, significant differences in size classes between control and treatment groups were demonstrated in perlodid nymphs (ANOVA; $p<0.05$; Fig. 43 & 44). This outcome is likely based on wider, more unimodal distribution of size classes in treatment groups compared to the more truncated, bimodal distribution of the control. There was no significant difference between treatment groups (ANOVA; $p>0.05$) and the only within group difference was distinguished between the 1:1 and the 3:3 and 5:5 nutrient concentrations (ANOVA; $p<0.05$) where a higher proportion of larger size classes was evident in the latter two concentrations. The results of the analysis for chloroperlid stoneflies also displayed a significant difference between control and treatment groups (ANOVA; $p<0.05$; Fig. 45 & 46). The 1:1 treatment ratio group showed a wider overall distribution and a slightly higher proportion of larger size classes whereas the 4:1 treatment ratio group was split; the 4:1 nutrient concentration was skewed to the lower size classes and the 12:3 and 20:5 concentrations were skewed to the higher size classes when compared with the control. Contrasts between treatment groups were only significant for the 3:3 and 12:3 and the 5:5 and 20:5 nutrient concentrations (ANOVA; $p<0.05$). The higher nitrogen ratios of the latter comparisons were slightly skewed towards the larger size classes and displayed a narrower distribution than the lower nitrogen ratios. Within treatment group comparisons showed significant differences between the 3:3 and 5:5 nutrient concentrations as well as the 12:3 and 20:5 concentrations (ANOVA; $p<0.05$). Whereas the 5:5 concentration had a higher proportion of larger size classes than the 1:1 concentration, the similar pattern between the 12:3 and 20:5 concentrations may be confounded by a lower sample size in the 12:3

Table 7. Orthogonal contrasts of three dominant orders of insects demonstrating differences in head capsule width size within and between treatment groups based on measurements from samples collected in the final trough benthos. Analyses completed after logarithmic transformation (LOG_{10}) of the data. Group 1 = 1:1 treatment ratio and Group 2 = 4:1 treatment ratio. Level of significance set at α=0.05.

Order / Family	Analysis of Variance	Orthogonal Contrast	Significance
Ephemeroptera			
Baetidae	Control vs treatment		p<0.001
	Between treatments	Group 1 vs Group 2	p<0.001
		1:1 vs 4:1	p>0.050
		3:3 vs 12:3	p>0.050
		5:5 vs 20:5	p=0.008
	Within treatments	1:1 vs 3:3 and 5:5	p>0.050
		3:3 vs 5:5	p>0.050
		4:1 vs 12:3 and 20:5	p>0.050
		12:3 vs 20:5	p=0.007
Diptera			
Chironomidae	Control vs treatment		p<0.001
	Between treatments	Group 1 vs Group 2	p<0.001
		1:1 vs 4:1	p=0.010
		3:3 vs 12:3	p=0.018
		5:5 vs 20:5	p>0.050
	Within treatments	1:1 vs 3:3 and 5:5	p>0.050
		3:3 vs 5:5	p>0.050
		4:1 vs 12:3 and 20:5	p>0.050
		12:3 vs 20:5	p>0.050
Plecoptera			
Perlodidae	Control vs treatment		p<0.001
	Between treatments	Group 1 vs Group 2	p>0.050
		1:1 vs 4:1	p>0.050
		3:3 vs 12:3	p>0.050
		5:5 vs 20:5	p>0.050
	Within treatments	1:1 vs 3:3 and 5:5	p=0.008
		3:3 vs 5:5	p>0.050
		4:1 vs 12:3 and 20:5	p>0.050
		12:3 vs 20:5	p>0.050
Plecoptera			
Chloroperlidae	Control vs treatment		p<0.001
	Between treatments	Group 1 vs Group 2	p>0.050
		1:1 vs 4:1	p>0.050
		3:3 vs 12:3	p=0.016
		5:5 vs 20:5	p=0.046
	Within treatments	1:1 vs 3:3 and 5:5	p>0.050
		3:3 vs 5:5	p=0.025
		4:1 vs 12:3 and 20:5	p>0.050
		12:3 vs 20:5	p=0.044

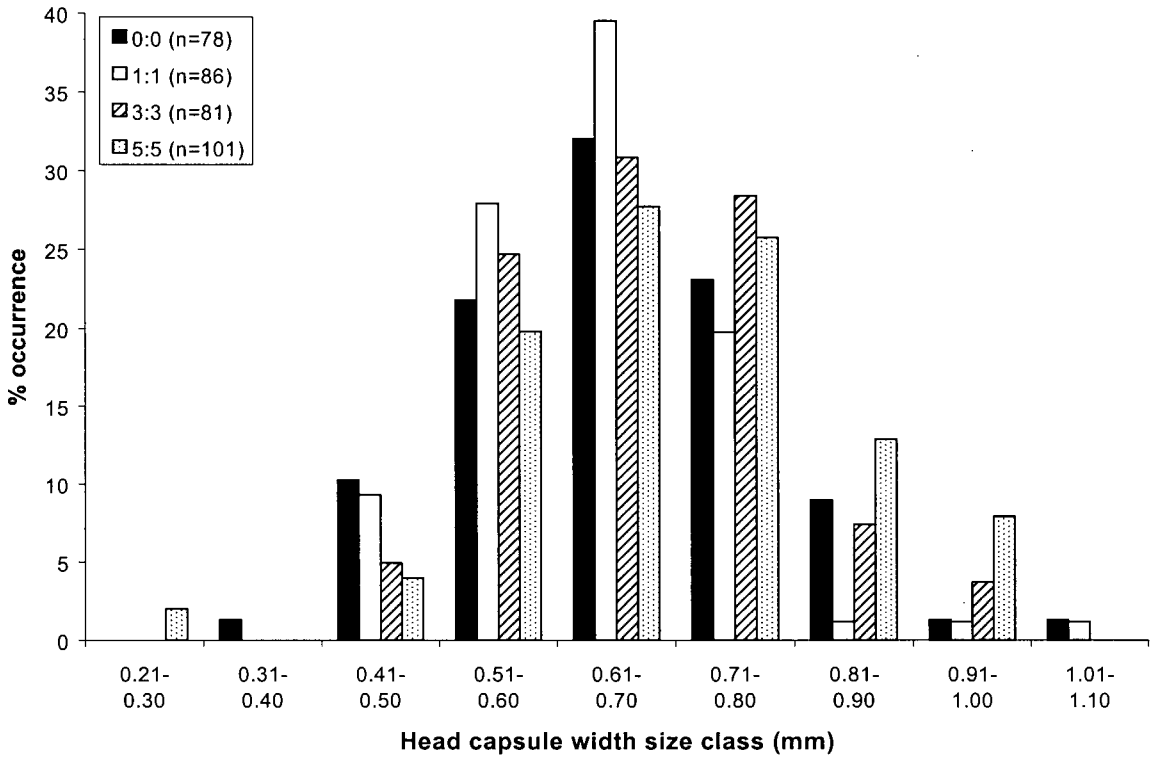

Figure 39. Size comparisons of baetid mayfly nymphs sampled in the final benthos of control and 1:1 treatment ratio troughs on September 30, 1992. Measurements are pooled between two replicates.

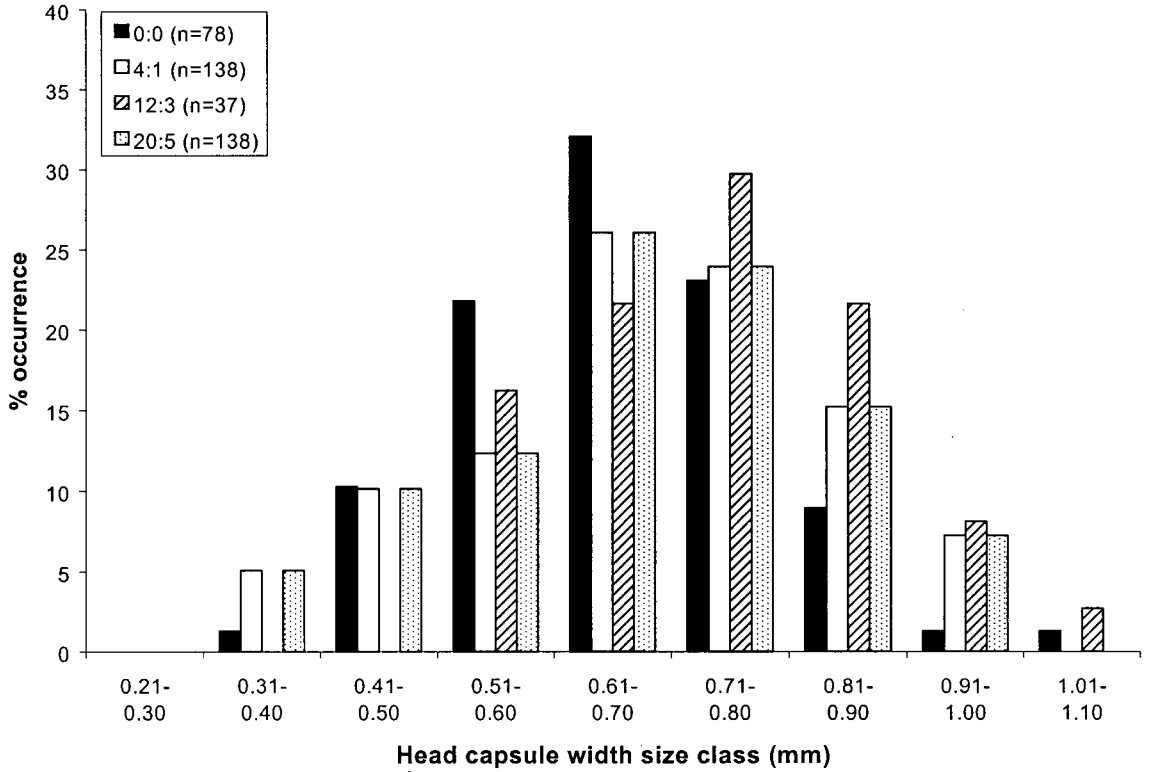

Figure 40. Size comparisons of baetid mayfly nymphs sampled in the final benthos of control and 4:1 treatment ratio troughs on September 30, 1992. Measurements are pooled between two replicates.

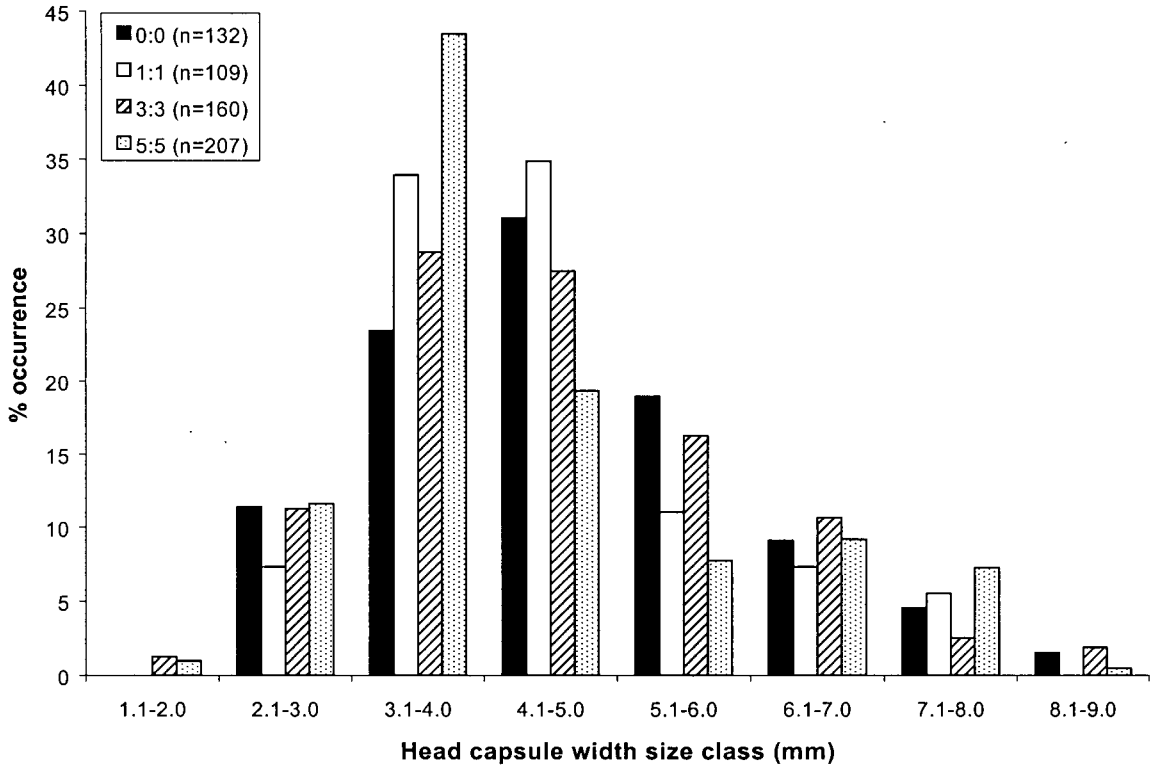

Figure 41. Size comparisons of chironomid larvae sampled in the final benthos of control and 1:1 treatment ratio troughs on September 30, 1992. Measurements are pooled between two replicates.

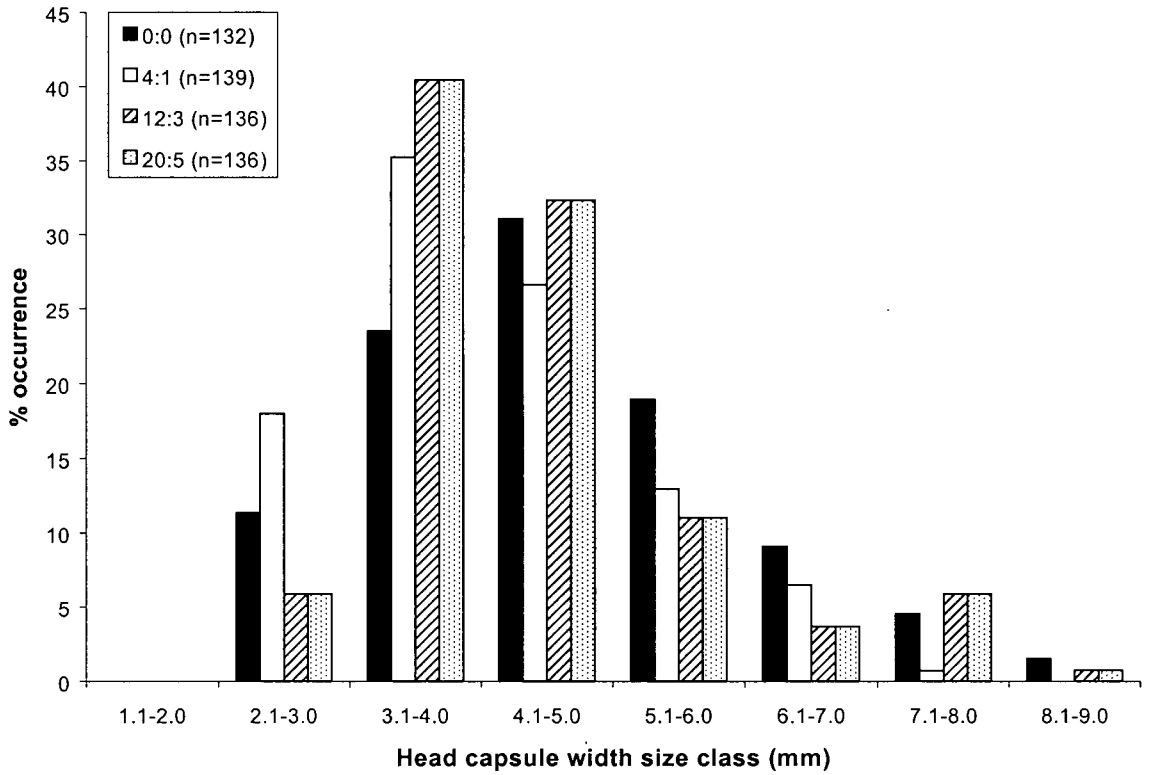

Figure 42. Size comparisons of chironomid larvae sampled in the final benthos of control and 4:1 treatment ratio troughs on September 30, 1992. Measurements are pooled between two replicates.

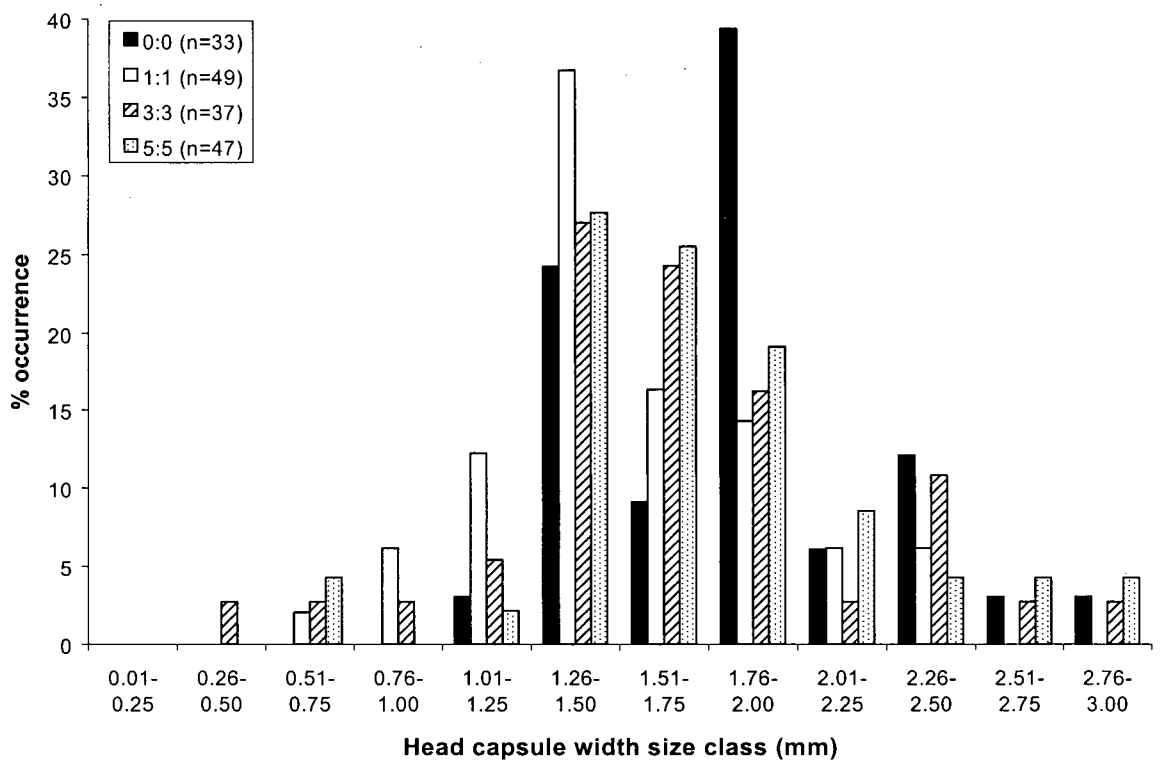

Figure 43. Size comparisons of perlodid nymphs sampled in the final benthos of control and 1:1 treatment ratio troughs on September 30, 1992. Measurements are pooled between two replicates.

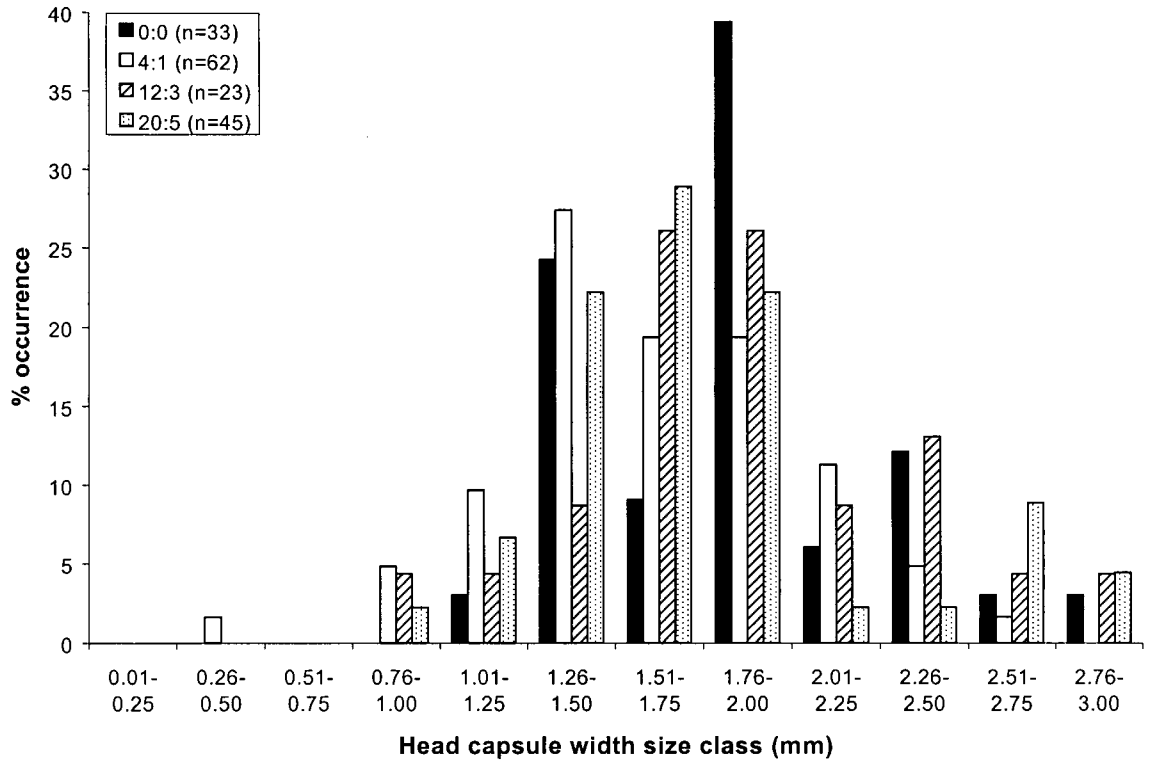

Figure 44. Size comparisons of perlodid nymphs sampled in the final benthos of control and 4:1 treatment ratio troughs on September 30, 1992. Measurements are pooled between two replicates.

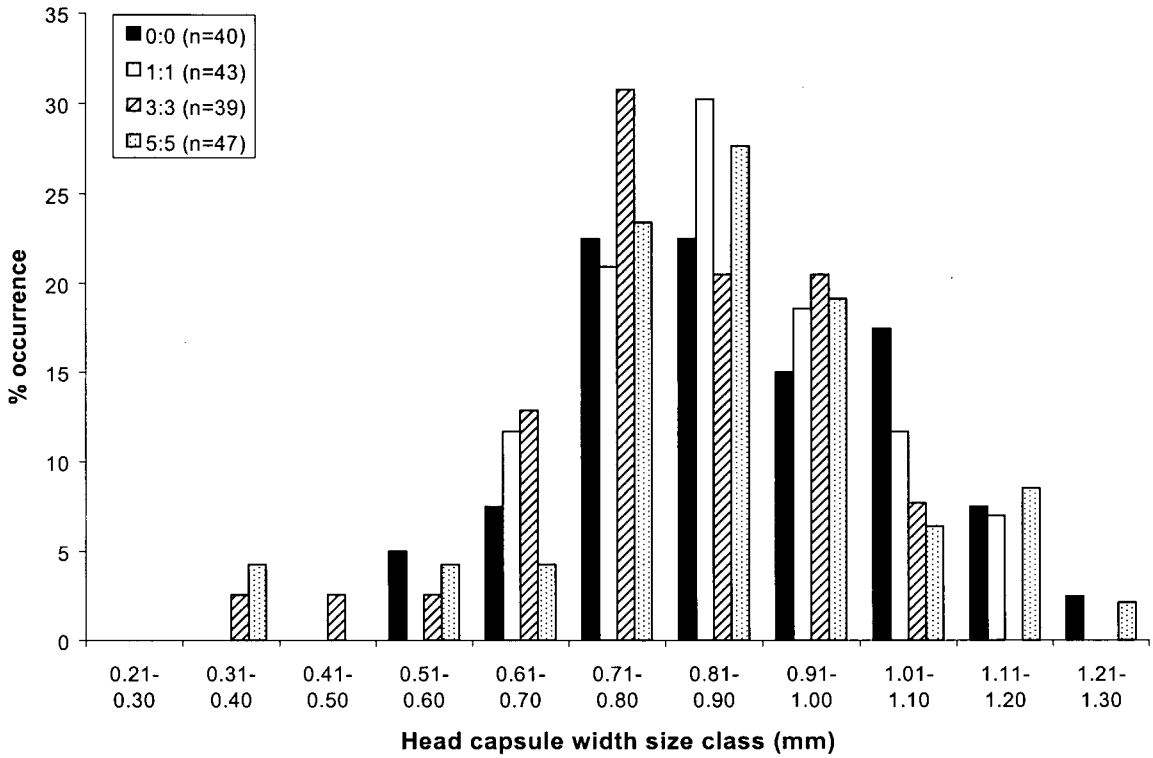

Figure 45. Size comparisons of chloroperlid nymphs sampled in the final benthos of control and 1:1 treatment ratio troughs on September 30, 1992. Measurements are pooled between two replicates.

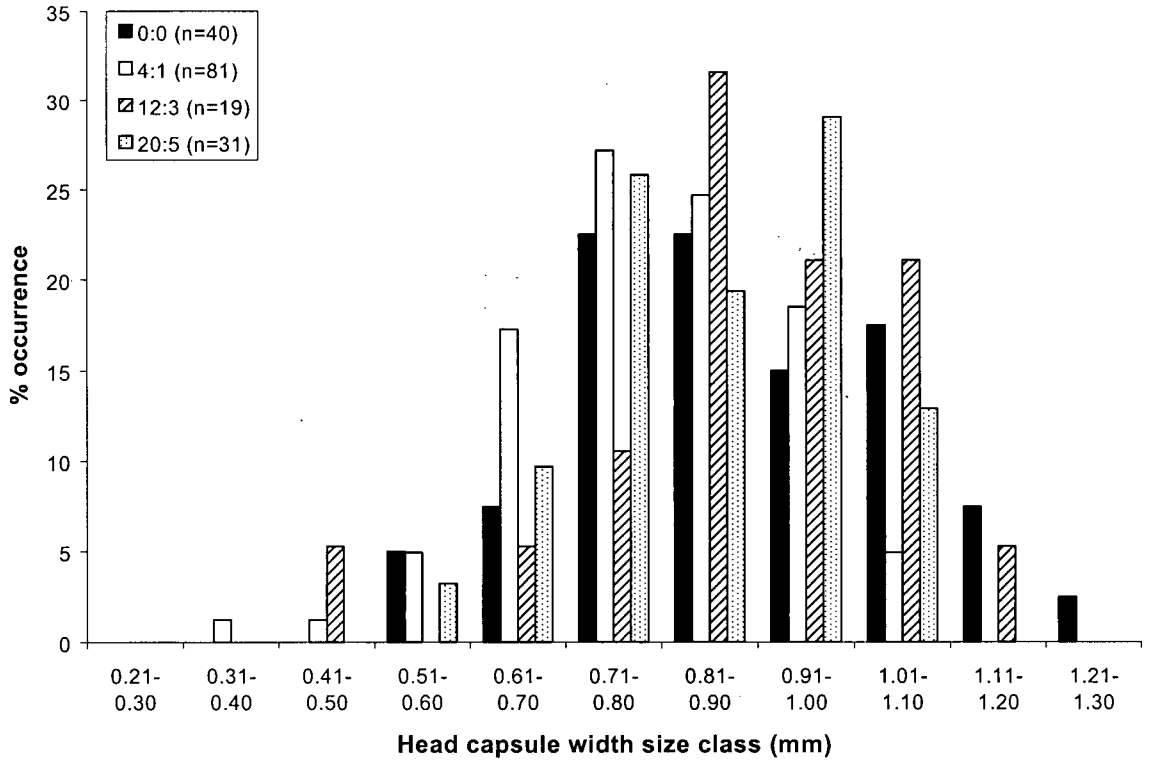

Figure 46. Size comparisons of chloroperlid nymphs sampled in the final benthos of control and 4:1 treatment ratio troughs on September 30, 1992. Measurements are pooled between two replicates.

concentration.

The analysis was also completed for hydroptilid caddisflies which showed relatively high abundance at the end of the experiment however differences between control and treatment groups were non-significant (ANOVA; $p>0.05$). This family of insects was also recognized as having sampling bias during the initial trial for size comparisons between sub-samples and hence these results may be an artifact of the sub-sampling methodology.

3.4.1.3 Representative taxa and richness

A comparison of representative taxa provided in the benthos on days 39 and 67 with the final benthos on day 83 indicated that 5 orders of aquatic insects remained unchanged over the duration of the experiment but the number of families increased progressively over time (Tables 8 - 10). The lowest diversity was observed on day 39 and taxonomic richness varied from 10 to 14 families across experimental units but the overall composition was not significantly different (ANOVA; $p>0.05$; Table 8). After 39 d, over 65% of the insect community was comprised of chironomids in control and treatment troughs alike (Fig. 47). Mayflies, made up mostly of baetid nymphs, ranged from about 15 to 25% and stoneflies and caddisflies ranged from 5 to 7%. Pteronarcyid and perlodid stoneflies were the most recurrent plecopterans. Hydroptilid caddisflies were most frequent among the trichopterans. Taxonomic richness increased marginally over the next 28 d (i.e. day 67) ranging from 12 to 15 families however differences between control and treatment groups were again non-significant (ANOVA; $p>0.05$; Table 9). The 1:1 nutrient concentration had the lowest diversity of all experimental units at this time. Chironomids continued to dominate the composition of insects across control and treatment groups however the difference between chironomid and mayfly occurrence was much more evident in the treatment group than the control (Fig. 48). Whereas about 40% and 47% of the control composition was comprised of mayflies and chironomids, respectively, well over 50% and up to 70% of the composition was represented by chironomids in treatments. Over the range of treatment ratios, an increasing trend in chironomid composition was followed by a decreasing trend in mayfly composition. Caddisflies were also higher in frequency across treatments and stoneflies were relatively unchanged, with the largest increase noted in the control group. Baetid mayflies were still the most frequent of the ephemeropterans but a larger frequency in lepidodstomatid caddisflies was observed with the highest composition occurring in the 1:1 nutrient concentration. Pteronarcyid stoneflies were again most recurrent of the plecopteran composition.

Table 8. Taxonomic richness and percent composition of representative insects in the benthos collected from control and treatment troughs on August 17, 1992. Percentages based on mean counts of 2 replicates. Standard error of the diversity index also shown.

	Target N:P Concentrations						
	0:0	**1:1**	**3:3**	**5:5**	**4:1**	**12:3**	**20:5**
Taxonomic Richness	11.5	11.5	12.5	11	12.5	10.5	12.5
2 SE	2.08	0.69	2.08	1.39	2.08	3.46	0.69
Order Ephemeroptera							
Baetidae	16.0	9.3	6.1	14.5	10.7	16.5	11.9
Ephemerellidae	4.7	3.0	3.0	2.8	1.4	1.2	3.0
Heptageniidae	4.2	3.3	2.8	3.9	5.4	3.4	2.6
Leptophlebiidae	0.6	0.6	0.9	0.1	0.1	0.9	1.0
Siphlonuridae	0.5	0.6	0.2	0.7	1.2	0.4	1.1
Sub-total	*26.0*	*16.9*	*13.0*	*22.0*	*18.8*	*22.5*	*19.6*
Order Plecoptera							
Capnidae	0.0	0.0	0.1	0.0	0.0	0.0	0.1
Chloroperlidae	0.2	0.6	0.2	0.3	0.4	0.1	0.2
Nemouridae	0.5	0.2	0.2	0.1	0.2	0.3	0.2
Perlidae	0.0	0.0	0.0	0.1	0.1	0.0	0.1
Perlodidae	1.1	0.8	0.7	1.7	0.7	1.0	0.6
Pteronarcyidae	2.3	2.0	2.5	2.9	2.0	3.5	1.4
Sub-total	*4.1*	*3.5*	*3.7*	*5.1*	*3.5*	*5.0*	*2.5*
Order Trichoptera							
Brachycentridae	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hydropsychidae	1.3	0.5	0.2	0.3	0.7	0.9	0.2
Hydroptilidae	2.3	1.7	4.8	1.8	2.4	1.0	1.6
Lepidostomatidae	1.4	3.4	1.6	4.9	1.0	1.2	3.7
Subtotal	*5.0*	*5.5*	*6.6*	*7.0*	*4.1*	*3.1*	*5.5*
Order Coleoptera							
Elmidae	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Haliplidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sub-total	*0.0*	*0.0*	*0.0*	*0.0*	*0.1*	*0.0*	*0.0*
Order Diptera							
Ceratopogonidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chironomidae	64.9	74.2	76.7	65.9	73.5	69.4	72.3
Chironomidae (pupae)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Empididae	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Simuliidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tipulidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sub-total	*64.9*	*74.2*	*76.7*	*65.9*	*73.5*	*69.4*	*72.3*

Table 9. Taxonomic richness and percent composition of representative insects in the benthos collected from control and treatment troughs on September 14, 1992. Percentages based on mean counts of 2 replicates. Standard error of the diversity index also shown.

	Target N:P Concentrations						
	0:0	**1:1**	**3:3**	**5:5**	**4:1**	**12:3**	**20:5**
Taxonomic Richness	14.5	12.5	13.5	14	15	14	14
2 SE	0.69	0.69	0.69	1.39	0.00	1.39	0.00
Order Ephemeroptera							
Baetidae	25.7	13.6	18.2	13.1	12.0	12.4	10.8
Ephemerellidae	3.9	4.7	5.1	4.2	4.7	4.0	3.0
Heptageniidae	4.1	3.4	2.2	2.8	2.5	2.5	2.8
Leptophlebiidae	2.8	1.4	1.9	1.5	1.4	1.4	1.8
Siphlonuridae	3.2	2.2	2.0	1.8	0.9	1.5	1.8
Sub-total	*39.6*	*25.2*	*29.4*	*23.3*	*21.5*	*21.8*	*20.2*
Order Plecoptera							
Capnidae	0.1	0.0	0.0	0.0	0.1	0.0	0.0
Chloroperlidae	0.7	0.7	0.4	0.3	0.4	0.3	0.3
Nemouridae	0.3	0.1	0.3	0.2	0.1	0.1	0.1
Perlidae	0.2	0.0	0.1	0.1	0.1	0.0	0.1
Perlodidae	0.2	0.5	0.4	0.2	0.1	0.1	0.1
Pteronarcyidae	6.3	2.9	2.2	2.4	2.0	2.2	2.9
Sub-total	*7.9*	*4.3*	*3.3*	*3.1*	*2.8*	*2.7*	*3.4*
Order Trichoptera							
Brachycentridae	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hydropsychidae	1.0	1.0	1.7	0.9	1.5	0.7	0.9
Hydroptilidae	1.0	1.7	1.1	1.1	1.3	1.0	1.7
Lepidostomatidae	3.4	10.0	7.4	9.6	6.5	7.4	4.4
Subtotal	*5.4*	*12.7*	*10.2*	*11.7*	*9.3*	*9.1*	*7.0*
Order Coleoptera							
Elmidae	0.1	0.0	0.0	0.1	0.0	0.1	0.0
Haliplidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sub-total	*0.1*	*0.0*	*0.0*	*0.1*	*0.0*	*0.1*	*0.0*
Order Diptera							
Ceratopogonidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chironomidae	47.1	57.8	57.2	61.8	66.4	66.2	69.4
Empididae	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Simuliidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tipulidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sub-total	*47.1*	*57.8*	*57.2*	*61.8*	*66.4*	*66.2*	*69.4*

Table 10. Taxonomic richness and percent composition of representative insects in the benthos collected from control and treatment troughs on September 30, 1992. Percentages based on mean counts of 2 replicates. Standard error of the diversity index also shown.

	Target N:P Concentrations						
	0:0	1:1	3:3	5:5	4:1	12:3	20:5
Taxonomic Richness	17	18.5	19.5	17	19.5	17	18
2 SE	0.00	0.69	0.69	1.39	2.08	1.39	0.00
Order Ephemeroptera							
Baetidae	25.9	27.5	23.2	21.8	25.6	32.1	26.3
Ephemerellidae	2.1	1.3	2.5	2.8	3.0	1.2	3.8
Heptageniidae	7.3	6.9	6.5	6.9	6.6	7.9	6.7
Leptophlebiidae	3.3	2.5	2.3	2.6	3.1	3.3	2.9
Siphlonuridae	9.7	9.7	9.5	4.9	10.9	7.5	9.0
Sub-total	*48.3*	*47.9*	*44.0*	*38.9*	*49.2*	*52.0*	*48.7*
Order Plecoptera							
Capnidae	0.0	0.0	0.4	0.0	0.2	0.0	0.1
Chloroperlidae	4.3	3.1	4.0	2.5	3.2	5.2	2.1
Nemouridae	0.0	0.1	0.1	0.0	0.1	0.0	0.2
Perlidae	0.5	0.4	0.2	0.0	0.1	0.3	0.1
Perlodidae	3.3	3.0	2.6	2.6	2.8	3.0	2.3
Pteronarcyidae	8.4	3.8	6.1	3.0	3.1	4.4	2.6
Sub-total	*16.5*	*10.4*	*13.4*	*8.1*	*9.4*	*12.9*	*7.4*
Order Trichoptera							
Brachycentridae	0.3	0.4	0.1	0.6	0.4	0.2	0.6
Hydropsychidae	0.9	0.6	0.4	0.4	0.3	0.9	0.8
Hydroptilidae	6.4	6.7	7.5	4.5	4.9	3.5	2.8
Lepidostomatidae	6.4	11.0	11.3	26.2	15.1	7.4	17.8
Subtotal	*14.0*	*18.7*	*19.3*	*31.7*	*20.6*	*12.0*	*22.0*
Order Coleoptera							
Elmidae	0.4	0.3	0.3	0.3	0.2	0.2	0.1
Haliplidae	0.0	0.2	0.3	0.2	0.1	0.1	0.1
Sub-total	*0.4*	*0.5*	*0.6*	*0.4*	*0.2*	*0.3*	*0.2*
Order Diptera							
Ceratopogonidae	0.4	0.0	0.2	0.1	0.1	0.1	0.1
Chironomidae	19.0	21.2	21.7	19.5	19.4	21.4	20.4
Chironomidae (pupae)	1.2	1.0	0.9	1.1	1.2	1.0	1.3
Empididae	0.0	0.0	0.0	0.0	0.1	0.1	0.0
Simuliidae	0.1	0.1	0.2	0.0	0.0	0.1	0.0
Tipulidae	0.1	0.0	0.0	0.1	0.0	0.1	0.0
Sub-total	*20.8*	*22.5*	*23.1*	*20.8*	*20.8*	*22.7*	*21.9*

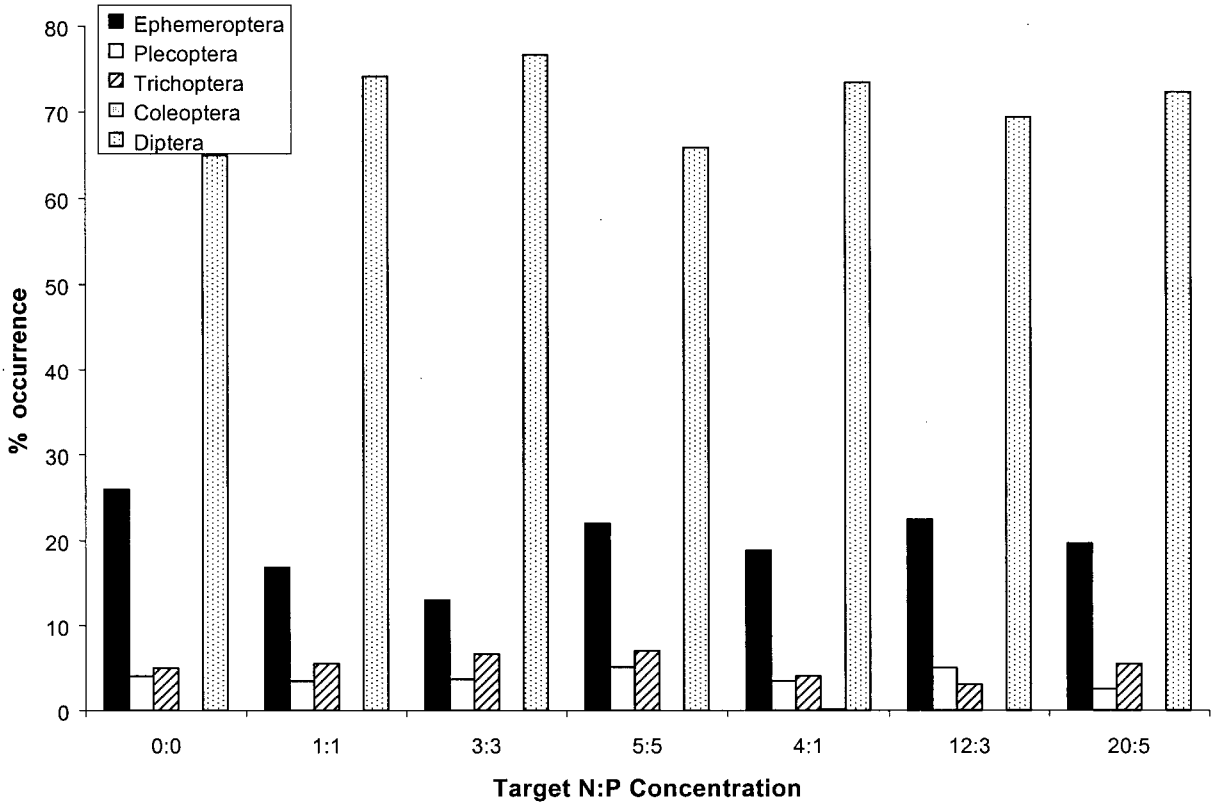

Figure 47. Percent composition of benthic insect taxa in control and treatment troughs on August 17, 1992. The data are based on mean counts of 2 replicates.

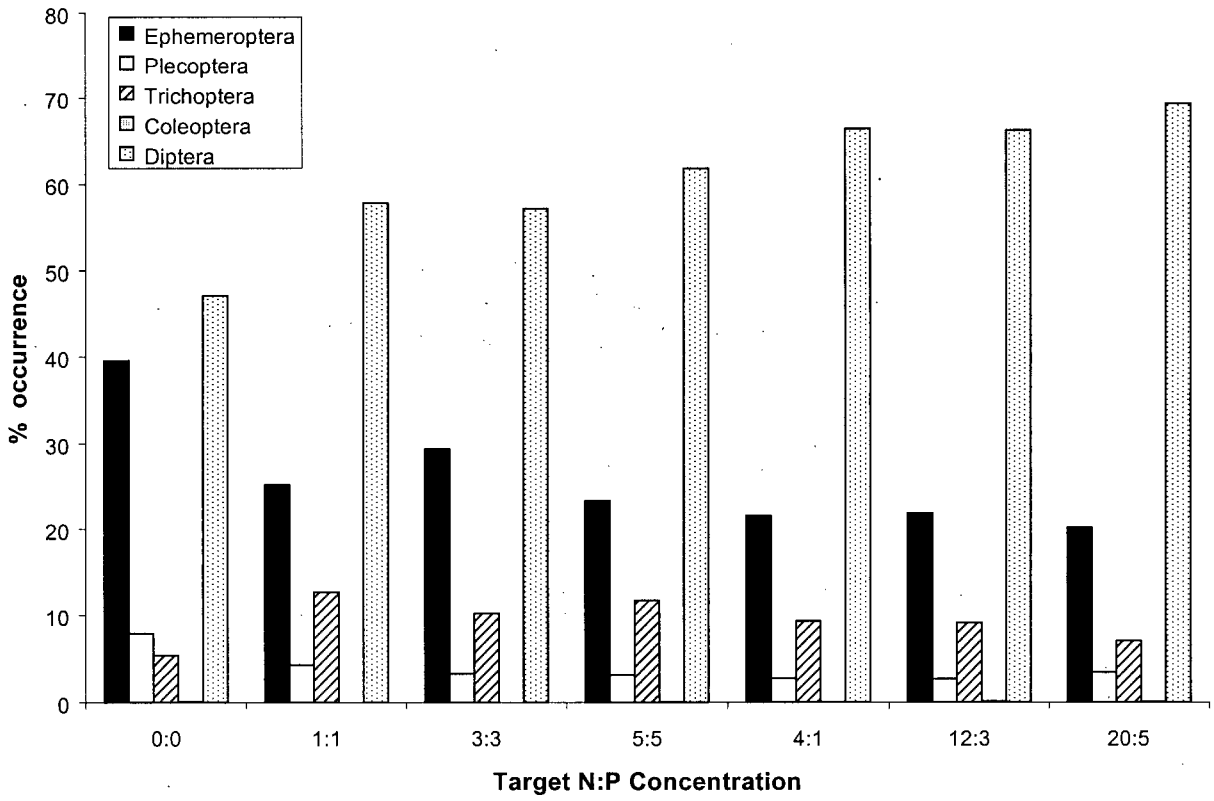

Figure 48. Percent composition of benthic insect taxa in control and treatment troughs on September 14, 1992. The data are based on mean counts of 2 replicates.

At the end of the experiment (day 83), the taxonomic richness of the benthos increased to its highest level ranging from 16 to 21 families across experimental units (Table 10). Differences between control and treatment groups however, were non-significant (ANOVA; $p>0.05$), but the highest diversity was observed in the 4:1 nutrient concentration. In contrast to the previous two sample periods, a shift in species dominance to mayflies was evident during the final census. Mayflies ranged from a low of 39% in the 5:5 nutrient concentration to a high of 52% in the 12:3 concentration (Fig. 49). Chironomids averaged approximately 20% in composition across all experimental units. The lower frequency of mayflies in the 5:5 nutrient concentration was balanced by a higher occurrence of caddisflies. Stoneflies were highest in percent composition in the control and overall, elmid beetles were consistently least frequent in both control and treatment groups. At the end of the experiment, baetid mayflies were again most frequent although siphlonurid and heptageniid families varied from about 7 to 11% of the mayfly composition. Lepidostomatid caddisflies displayed the highest frequencies in treatment groups and exceeded 25% of the composition in the 5:5 nutrient concentration. Pteonarcyid stoneflies remained the most frequent of plecopteran families with perlodid and chloroperlid stoneflies

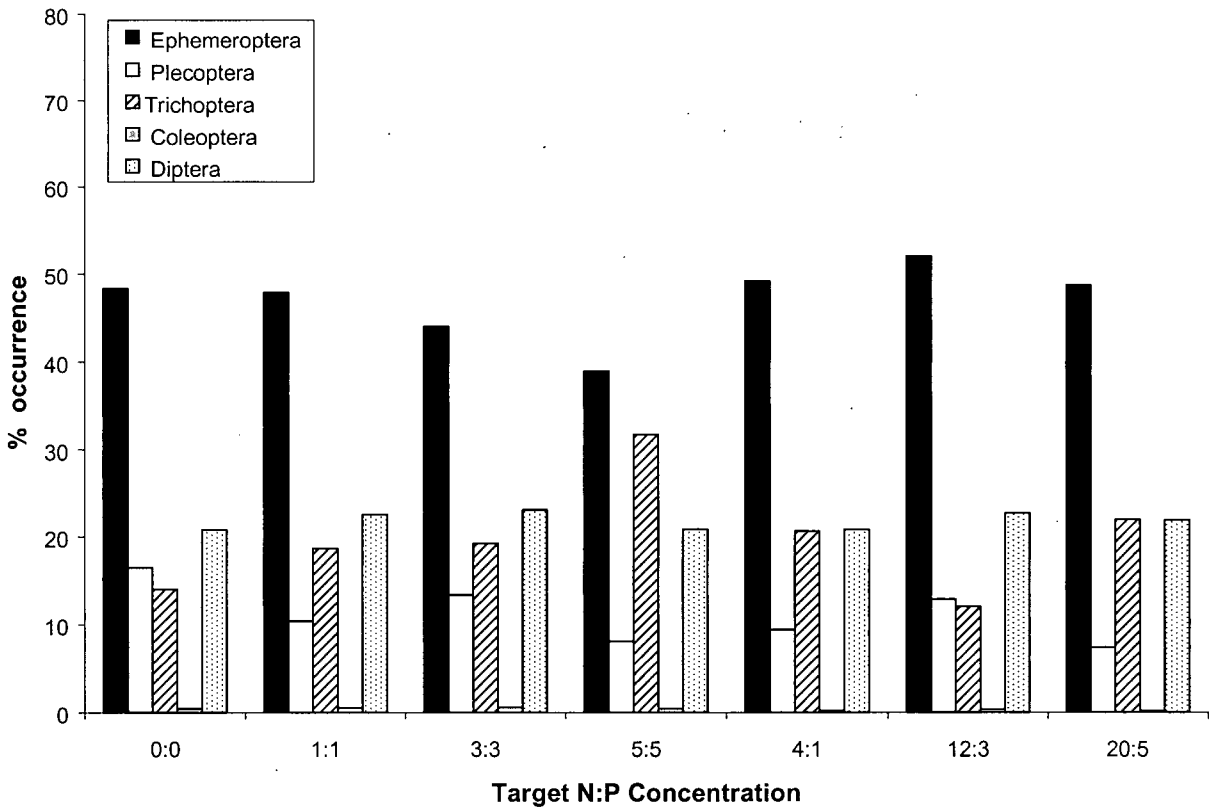

Figure 49. Percent composition of benthic insect taxa in control and treatment troughs on September 30, 1992. The data are based on mean counts of 2 replicates.

making up about 3-4% of the final composition.

Differences in taxonomic diversity were analyzed from day 39 to day 83 to account for significant changes in the benthic community. Although the interaction term for a difference in the rate of change in diversity between control and treatments was non-significant (ANCOVAR; $p>0.05$), a significant difference in diversity was demonstrated between the two dates ($p<0.05$; Fig. 50). A higher diversity of insects was evident after 45 d of operation but there was no significant difference in diversity between control and treatment groups (ANCOVAR; $p>0.05$).

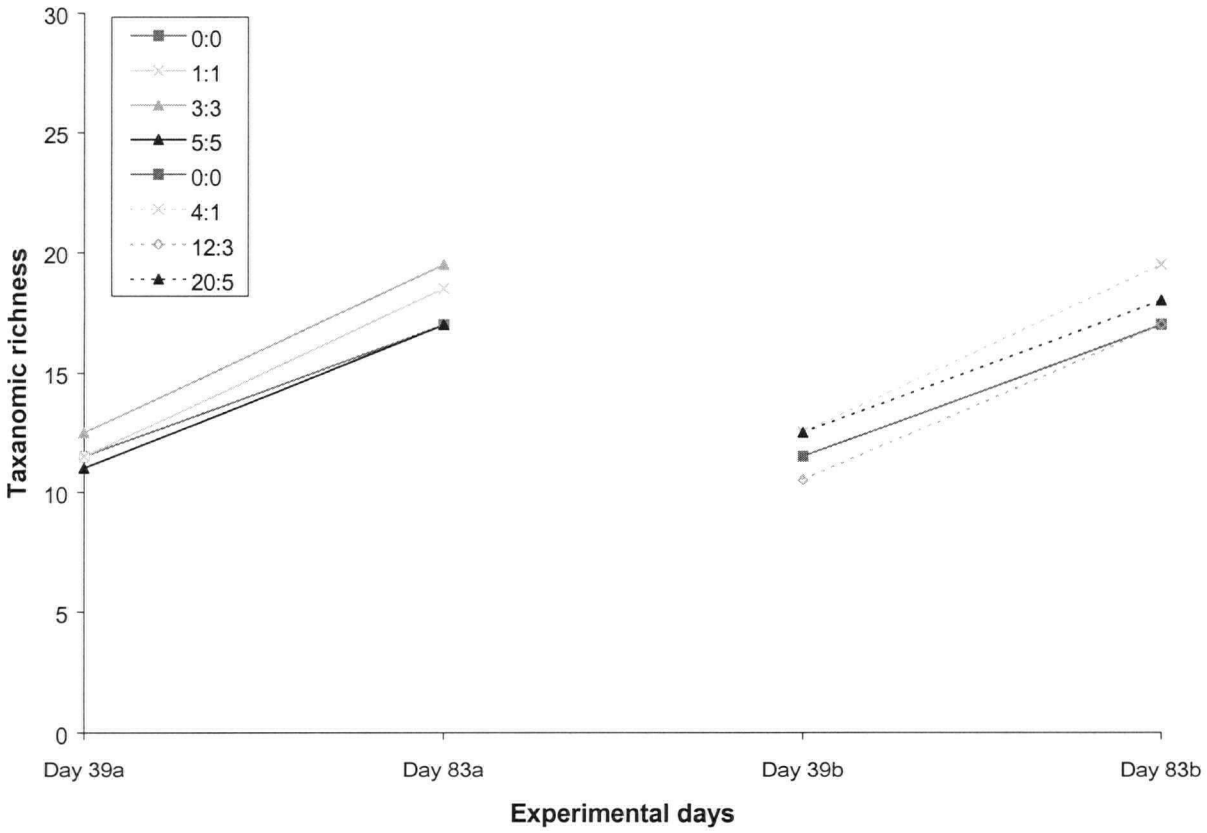

Figure 50. Differences in taxonomic diversity in benthic insects from August 17 to September 30, 1992. Treatment * time interaction lines are illustrated to show differences in the rate of change between control and treatment groups over time. The a and b series depict differences between the 1:1 and 4:1 treatment groups, respectively.

3.4.2 Insect drift and body size

3.4.2.1 Gross and per capita drift of all taxa

Three major cycles of aquatic insect behavioural drift were monitored through control and treatment troughs at approximately 18 - 20 d intervals (Fig. 51). Within the 1:1 treatment group, the first peak was measured on day 30 of the experiment and the mean total number of emigrants representing a three day collection period ranged from about 170 to 280; the 1:1 treatment ratio displaying the lowest emigration and the control and 5:5 treatment ratio showing the highest emigration. The second peak in control troughs was observed on day 47 and the mean number of 3 d totals nearly doubled. The same pattern was observed in control troughs during the third cycle on day 65 attaining a peak level consistent with the previous cycle. Peak insect emigration in the 1:1 treatment group was delayed a further 6 d beyond the peaking period observed for control troughs and the same pattern was repeated in the third drift cycle. Whereas a constant peak in amplitude was established in control troughs after the second cycle, a progressive increase in the number of emigrants was apparent across treatment troughs. Differences in both amplitude and periodicity of drift cycles between the control and

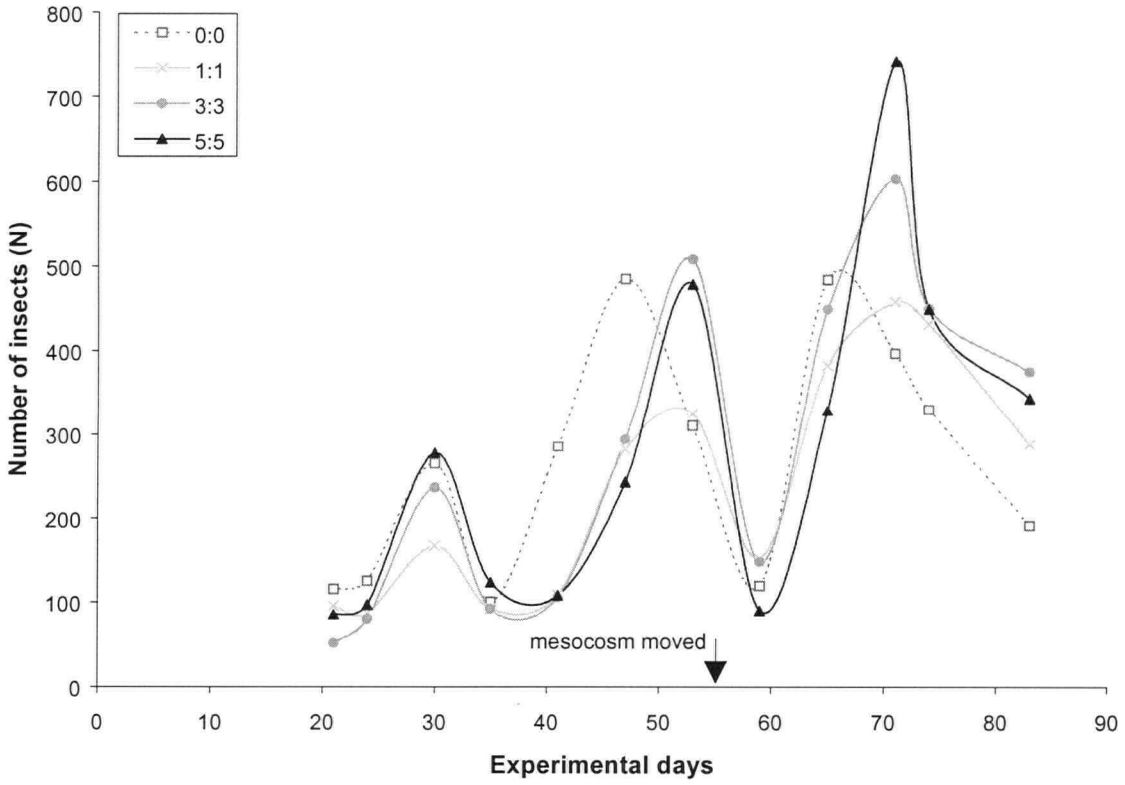

Figure 51. Mean number of aquatic insects captured as emigrants in control and the 1:1 treatment ratio group from July 30 to September 30, 1992. Numbers represent 3 d totals for all taxa. Counts are the mean of two replicates.

the 1:1 treatment group suggest differences in resource abundance (i.e. algal cell density; refer to Figs. 12 and 14) that may have altered insect behaviour. Within the 1:1 treatment group, differences were also evident between the three nutrient ratios. A higher turnover of aquatic insects was observed in the 3:3 and 5:5 nutrient concentrations without any indication of stabilizing after three cycles. Approximately one week before the end of the experiment, the number of insects leaving both control and treatment troughs remained high relative to the previous two cycles and was consistently higher in the treatment troughs compared to the control.

A similar pattern in the amplitude and periodicity of behavioural drift cycles was observed in the 4:1 treatment group (Fig. 52). In contrast to the 1:1 treatment group however, the number of emigrants within the first cycle remained much lower than the control but displayed the same progressive increase over the three cycles. Both 4:1 and 20:5 nutrient treatments contributed more insects at the crest of the third drift cycle than the 12:3 concentration, reaching a high of about 720 over a 3 d collection period. A slightly higher level of background drift between the

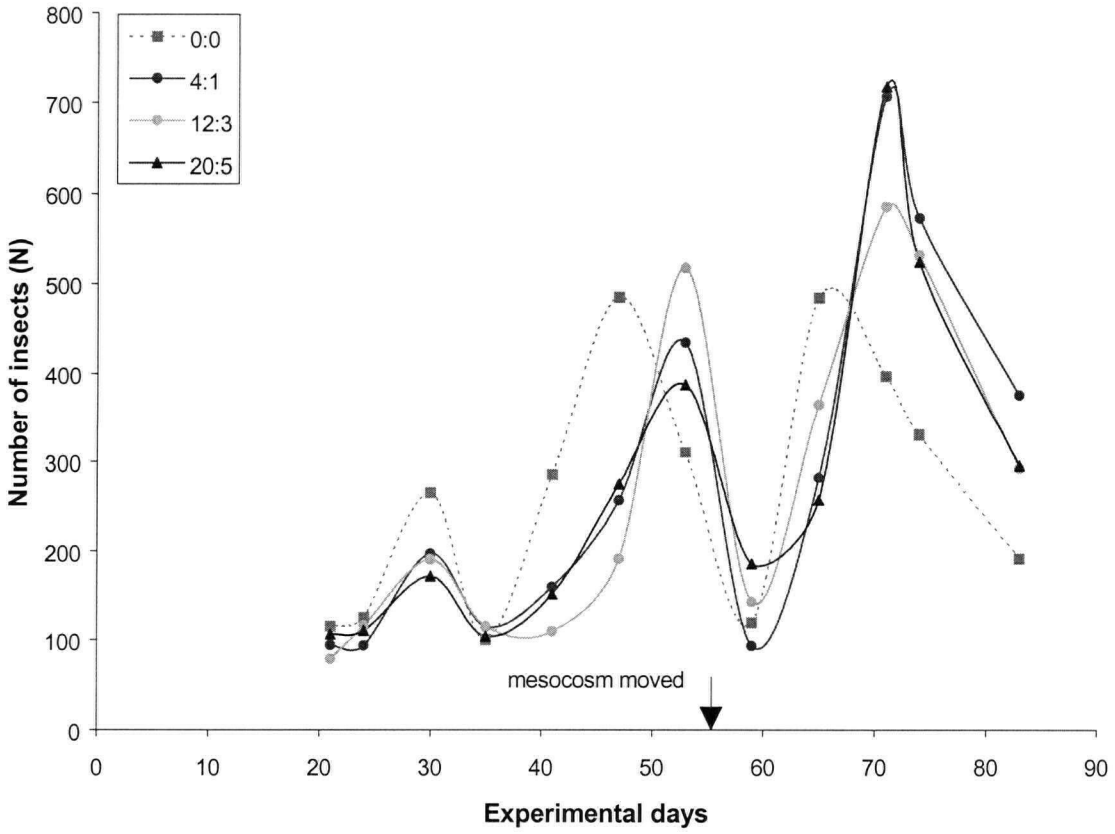

Figure 52. Mean number of aquatic insects captured as emigrants in control and the 4:1 treatment ratio group from July 30 to September 30, 1992. Numbers represent 3 d totals for all taxa. Counts are the mean of two replicates.

second and third peaks (i.e. the minimum level between peaks) was evident for the 20:5 treatment concentration suggesting a higher continual turnover within the benthos following the second peak. Near the end of the experiment all three 4:1 treatment ratios had a higher number of emigrants than the control and similarly maintained a higher level of drift during the last week.

To test the effects of flow on insect behaviour, a repeated measures analysis of covariance between insect drift and trough discharge was undertaken to account for re-positioning of the mesocosm on day 55. Differences in flow between treatments, over the period of drift monitoring, were non-significant ($p>0.05$). As well, there was no significant difference in the rate of drift within treatments (i.e. drift*flow interaction; $p>0.05$). The results of this analysis in addition to graphical inspection of the drift cycles, illustrating week long delays in periodicity (refer to Figs. 51 and 52), suggest that insect behaviour was not influenced by mesocosm relocation.

Closer inspection of the peaks in drift between control and treatment groups indicates that both the control and the 3:3 and 12:3 treatment concentrations reached a plateau whereas a steady increase was displayed in all other treatment concentrations (Fig. 53). The 3:3 and 12:3 concentration were the only treatments to surpass the level of drift observed in the control during the second cycle. With the exception of the 1:1 concentration, all other treatments had a higher level of drift than the control at the peak of the third cycle. Despite appearances, there was no significant difference between control and treatment groups in the contribution of drift or the change in drift rate (i.e. treatment and treatment*time interaction; $p> 0.05$) although a significant difference in number was evident over the period of monitoring (ANCOVAR; $p < 0.01$). Within treatment groups, a significant difference in drift rate (treatment*time interaction) was evident between the 1:1 and 5:5 nutrient concentrations ($p<0.05$).

A comparison of peak to peak differences in amplitude between control and treatment groups further illustrates differences in trends across experimental units (Table 11). The progressive increase in drift in the 5:5, 4:1 and 20:5 treatment concentrations ranged 1.6 - 1.9x higher than control or all other treatments by the third cycle. Similarly, these same treatments showed the largest increase (1.5x as large) over background conditions at the end of the third cycle.

Notwithstanding differences in emigration pattern between control and treatment groups, the lack of insect immigration data in the above analysis makes it extremely difficult to distinguish the response of insects to nutrient treatment within the mesocosm and the variation in background drift in the Slocan River. The untimely loss of drift nets associated with the black

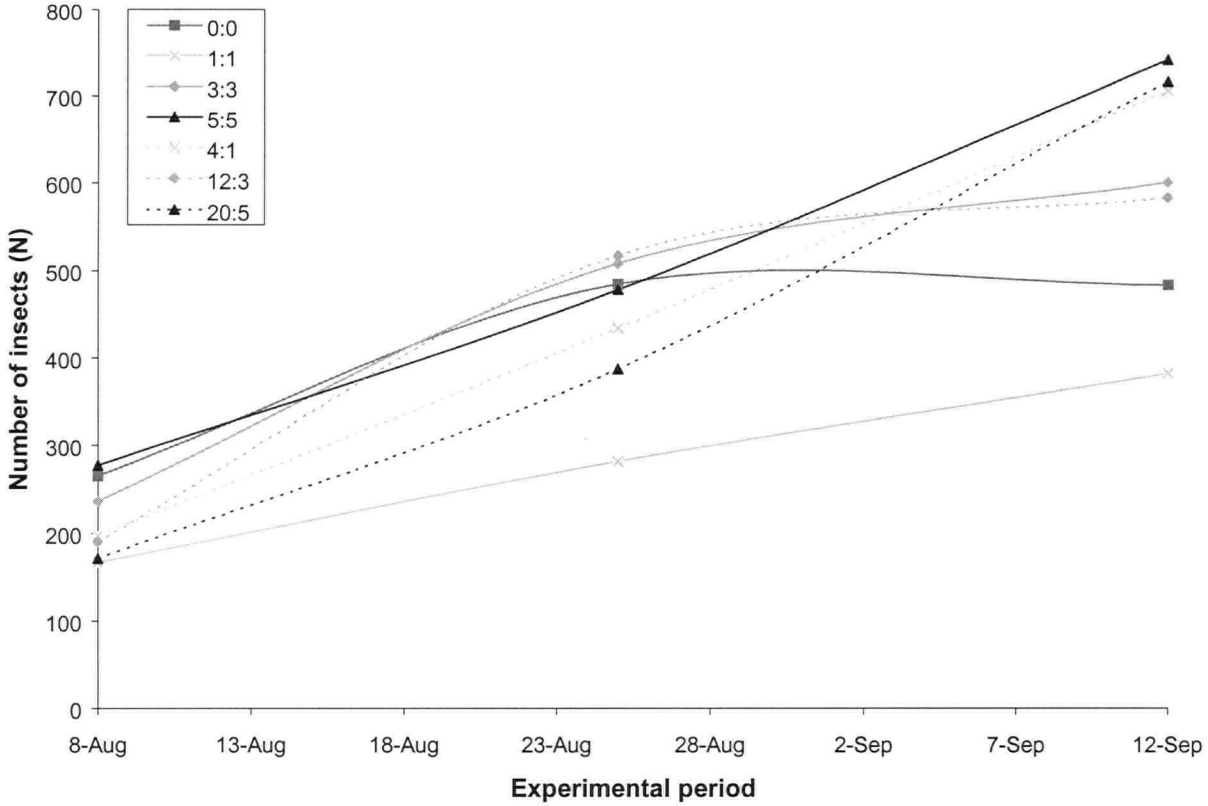

Figure 53. A comparison of mean peak counts between control and treatment groups during three drift cycles from August 8 to September 12, 1992. Peak counts are the mean of two replicates.

Table 11. The response of peak aquatic insect drift within control and nutrient treatment troughs. Values are expressed as the ratio between cycles for individual treatments and the ratio between control and treatments over the latter two cycles.

N:P Treatment concentration ($ug.L^{-1}$)	Difference in peak drift between cycles		Difference in peak drift between control and treatments	
	Cycle 2/1	Cycle 3/2	Cycle 2	Cycle 3
0:0	1.8x	1x		
1:1	1.9x	1.4x	0.7x	0.9x
3:3	2.2x	1.2x	1.1x	1.2x
5:5	1.7x	1.6x	1x	1.5x
4:1	2.2x	1.6x	0.9x	1.5x
12:3	2.7x	1.1x	1.1x	1.2x
20:5	2.3x	1.9x	0.8x	1.5x

troughs at the start of the experiment, whose intended use was to monitor immigration rates, precluded tracking of background drift conditions. Therefore, inclusion of a per capita drift rate is provided to contrast differences in control and treatment groups relative to benthic densities within each trough. Unlike gross drift rate, per capita drift rate gives an estimate of what the individual animal perceives in terms of a suitable environment (i.e. food resources) regardless of total numbers available to drift (W.E. Neill pers. comm.). Per capita drift rates, thus, provide further insight about the redistribution of benthic insects in response to competition for food and space (Williams and Feltmate 1992) and an insect's perception of habitat quality (Kohler 1985).

Per capita drift rates are based on benthic densities measured on days 39 and 67 of the experiment. The time series of benthic data was provided in section 3.4.1. The former measurement corresponds to an early point on the ascending limb of the second drift cycle within the control and treatment groups while the latter measurement corresponds to a 2 d post-drift peak in control troughs and a 4 d pre-drift peak in treatment troughs (refer to Figs. 51 and 52). Since a drift sample corresponding to the same date as the benthic sample was unavailable, a non-linear regression model, to account for exponential increases in drift over time, (Quasi-Newton or Simplex; Systat, Ver. 5; Wilkinson 1992) was fitted to the available drift data to predict missing values on days 39 and 67. Estimated values were graphically inspected for fit by plotting predicted numbers with actual numbers obtained on specified sampling dates.

The relationship of number of emigrants in the drift relative to total benthic insect density (i.e. all species combined) demonstrated a significant difference in mean daily drift rate between control and treatment groups (ANOVA; $p < 0.01$; Fig. 54). Per capita drift rates estimated on August 17 were, on average, twice as high in the control than that observed in treatment groups. The difference in per capita drift rates between control and treatment groups on September 14 were even more dramatic. Up to a 3-fold difference in daily per capita drift rate was observed between the 4:1 concentration and the control; the latter experimental unit having the higher drift rate.

The effect of trough discharge was further tested as a covariate of insect drift using flow data collected on days 40 and 68. Differences in flow across experimental units were non-significant on both dates (ANCOVAR; $p > 0.05$). These data further suggest that the lower mean daily per capita drift rate across treatments are likely related to more suitable environmental conditions based on food availability (i.e. algal biomass) rather than a behavioural response to fluctuations in trough discharge.

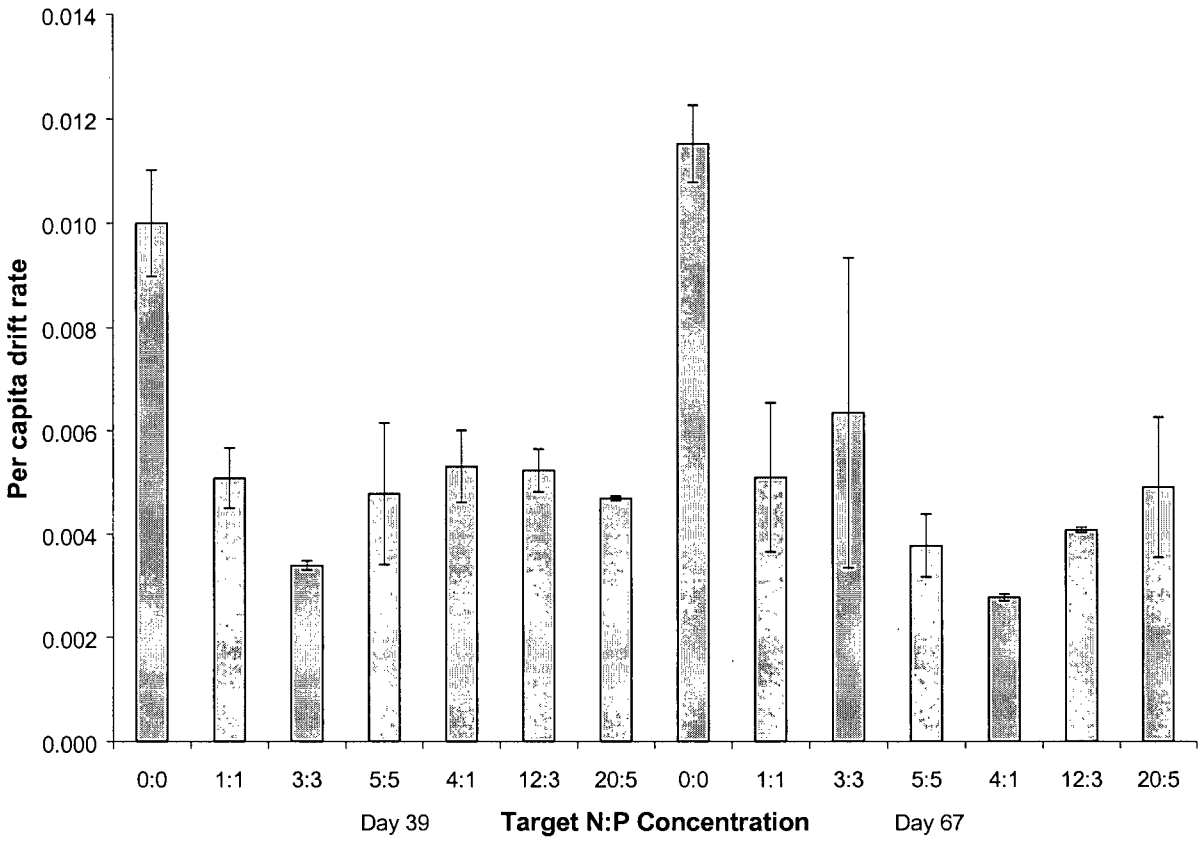

Figure 54. A comparison of per capita drift rate between control and treatment groups on days 39 and 67 (August 17 and September 14, 1992, respectively). Mean rates are based two replicates.

Larger differences in per capita drift rate within treatment groups during the September 14 measurement were also evident. The 5:5 concentration had the lowest rate among the 1:1 treatment group while the 4:1 concentration was lowest among all treatment groups. Differences in the rate of change in per capita drift rate between control and treatment groups from August 17 and September 14 however, were non-significant (ANCOVAR; $p>0.05$; Fig. 55).

3.4.2.2 Gross and per capita drift of major taxa

The relative abundance of 5 orders of aquatic insect (Ephemeroptera, Plecoptera, Trichoptera, Diptera and Coleoptera) varied considerably during the peaks of the three drift cycles (Fig. 56). Mayflies, comprised almost exclusively of baetid nymphs, dominated the drift during the first drift cycle on August 8. Chironomids, consisting of three sub-families (Orthocladiinae, Tanytarsinae and Tanypodinae) were the second most common drifters over the same period. Stoneflies represented largely by perlodid, pteronarcyid and chloroperlid nymphs were the next highest in abundance, followed by hydropsychid caddisflies. Elmid beetles were least

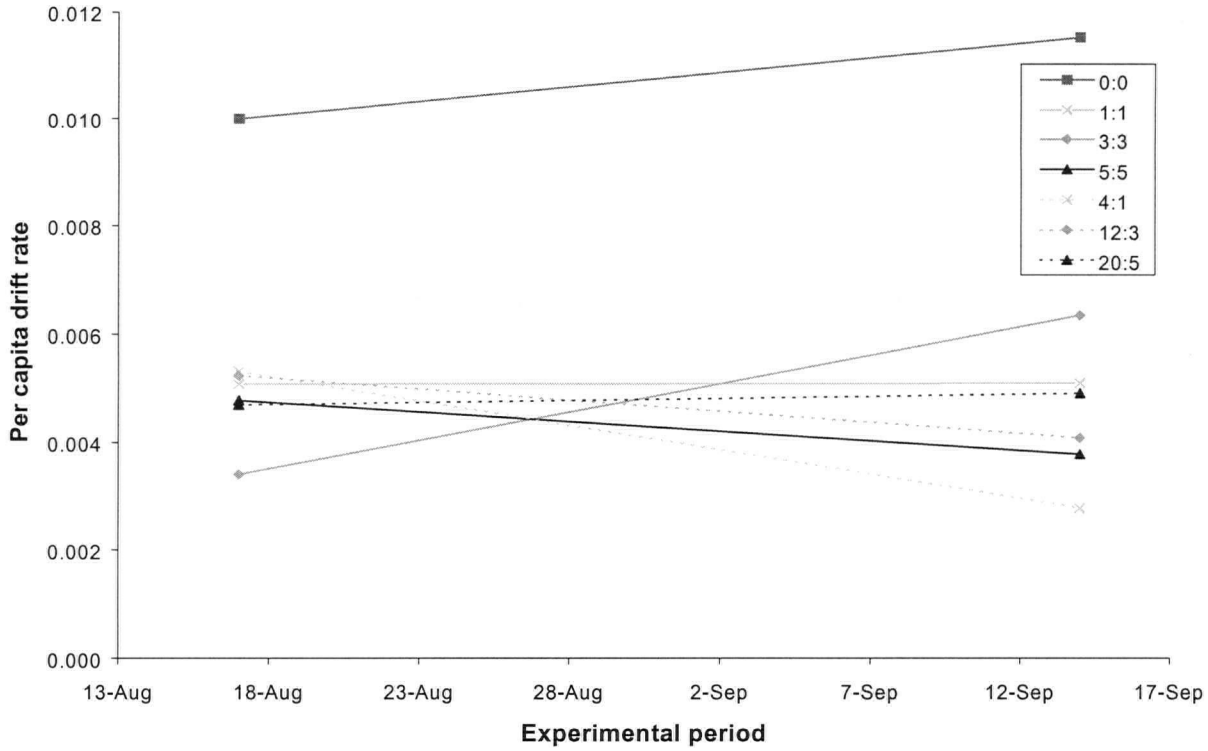

Figure. 55. Per capita drift rates estimated on day 39 and 67 (August 17 and September 14, 1992, respectively. Treatment * time interaction lines are illustrated to show differences in the rate of change between control and treatment groups over time.

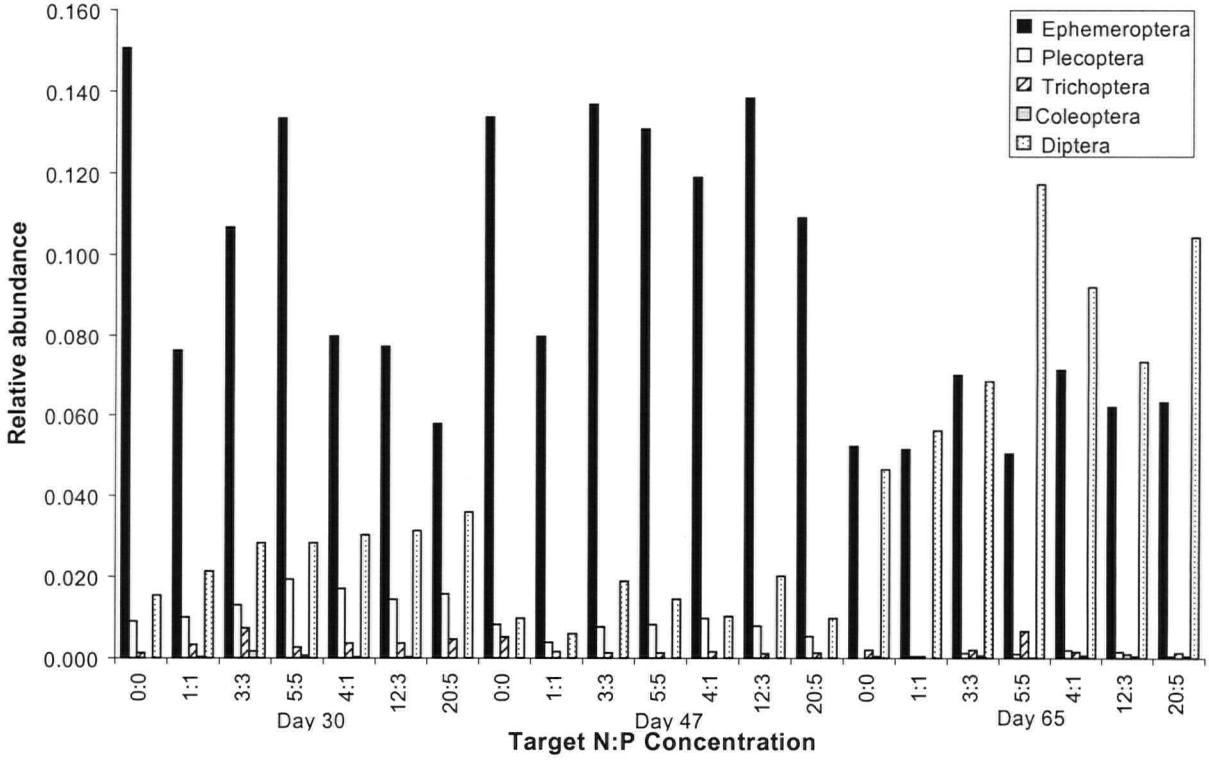

Figure 56. Relative abundance of the five major taxa of aquatic insects observed during peaks of the three drift cycles on August 8 (d 30), August 25 (d 47)and September 12 (d 65), 1992. Individual treatments and control were normalized for total insect drift density in troughs across all experimental units.

frequently seen in drift samples of all insects observed and never consistently represented across all experimental units. An increasing trend in mayfly numbers in the drift was evident with increasing nitrogen and phosphorus concentrations in the 1:1 treatment group whereas a decreasing trend was noted across the 4:1 treatment groups. Mayflies remained highly dominant during the second drift cycle and all other invertebrate taxa were represented in limited abundance, although chironomids were proportionately higher than stoneflies, caddisflies or beetles. With the exception of the 3:3 and 12:3 treatment groups, the relative abundance of mayflies in treatment troughs remained lower than control troughs. A large shift in relative abundance of the drift occurred during the third peak. Mayflies dropped to approximately one-half their previous abundance across all experimental units and were exceeded by chironomids in almost all treatments. With the exception of hydropsychid caddisflies in the 3:3 nutrient concentration, all other taxa were exceedingly low during the third cycle.

Given the relative importance of mayflies and chironomids in the drift during all three cycles, time-course changes in drift pattern for all taxa were largely echoed by baetid nymphs (Fig. 57) but strongly contrasted with chironomids (Fig. 58). Mayflies within the 1:1 treatment group followed a similar pattern of delayed emigration during the second drift cycle but amplitude and periodicity varied considerably across treatments during the third cycle. Whereas the 5:5 nutrient concentration was the only treatment that mimicked the general pattern for all taxa, the peaks of the 1:1 and 3:3 concentrations were more synchronous with the control. Variations over the duration of the third cycle were clearly evident however, in that a bimodal pattern was observed in the 1:1 concentration while the peak of the 3:3 concentration extended well beyond that of the control. In contrast to the general pattern observed in Fig. 48, gross numbers of emigrating mayflies across both control and 1:1 treatment group declined sharply after the peak of the third cycle. This pattern may have occurred in response to the dramatic increase in chironomid emigration noted during the third drift cycle (refer to Fig. 58). Whereas a minor oscillation in gross chironomid numbers was observed over the first two cycles, their largest contribution to the drift was most evident during the third cycle and most noticeable within the 5:5 nutrient concentration. The latter event is considered more catastrophic than behavioural (Williams and Feltmate 1992) however, in that the drift response of chironomids was likely induced by re-positioning of the mesocosm to the west side of the river where higher trough velocities may have encouraged outmigration.

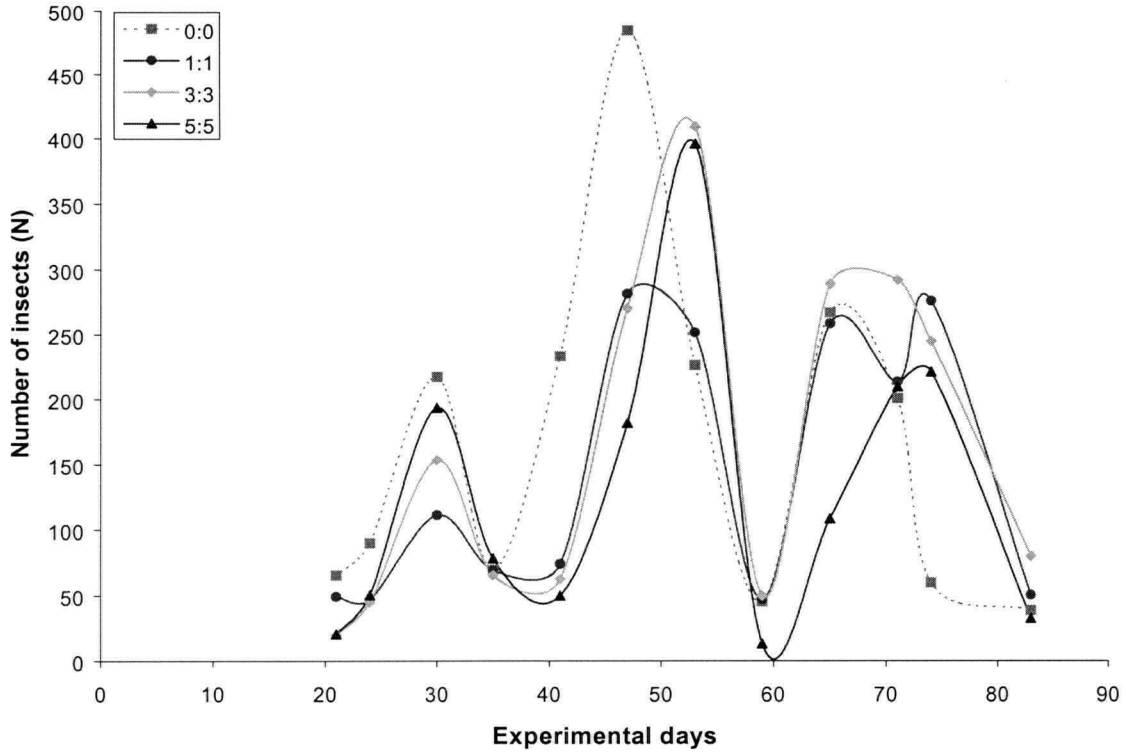

Figure 57. Mean number of baetid nymphs captured as emigrants in control and 1:1 treatment ratio troughs from July 30 to September 30, 1992. Numbers represent 3 d totals. Counts are the mean of two replicates.

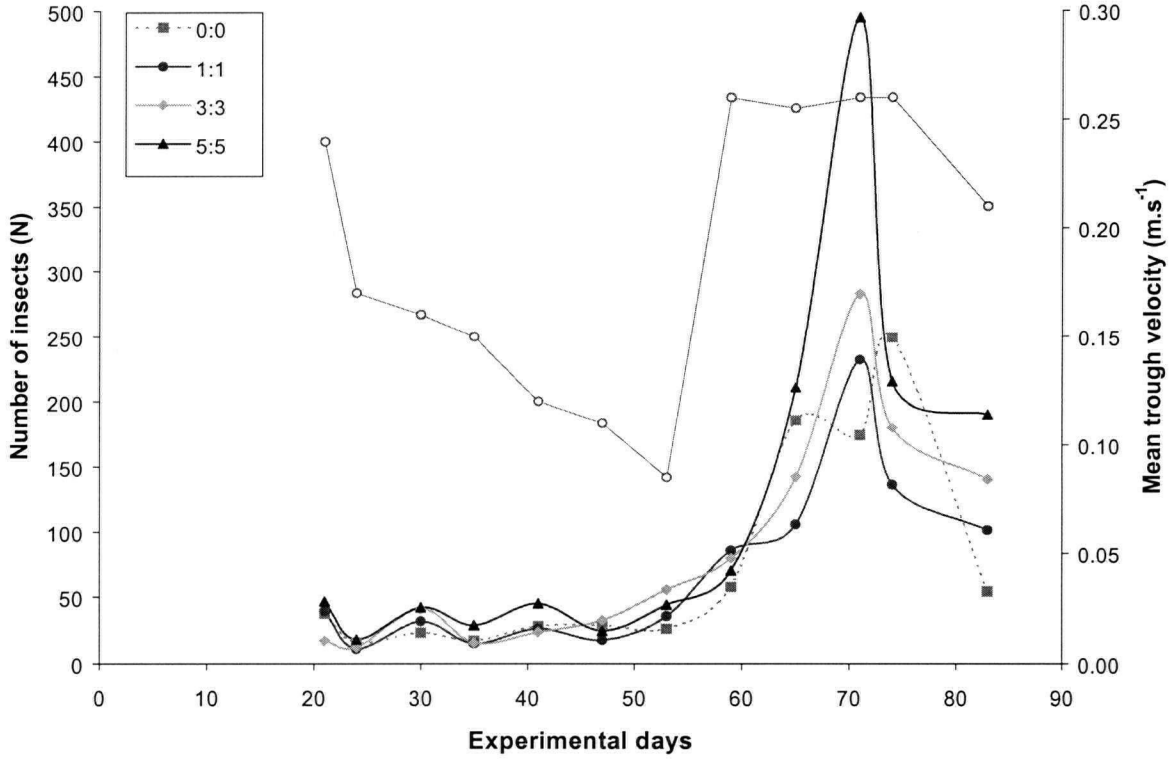

Figure 58. Mean number of chironomid larvae captured as emigrants in control and 1:1 treatment ratio troughs from July 30 to September 30, 1992. Numbers represent 3 d totals. Counts are the mean of two replicates. Note shift in trough velocity at day 55.

Within the 4:1 treatment group, an asynchronous pattern of drift in mayflies paralleled earlier observations of the general pattern for all taxa (Fig. 59). Peak numbers within treatment troughs remained below peak numbers in control troughs over the first two cycles but were more similar during the last cycle. Consistent within observations of the 1:1 treatment group, a decline in gross numbers of mayflies in the 4:1 treatment group was again evident during the peak of the third drift cycle. This similar response also corresponded with the higher number of chironomid emigrations over the same period (Fig. 60). Under higher nutrient ratio applications, gross numbers of chironomids among treatments exceeded those of the control.

Closer inspection of taxa-specific per capita drift rates for baetid nymphs and chironomid larvae on days 39 and 67 however, revealed few differences in emigration rate between control and treatment groups (Fig. 61 and 62). Albeit that a lower rate of drift was observed in treatment groups, significant differences in per capita drift rate were only demonstrated for mayflies on

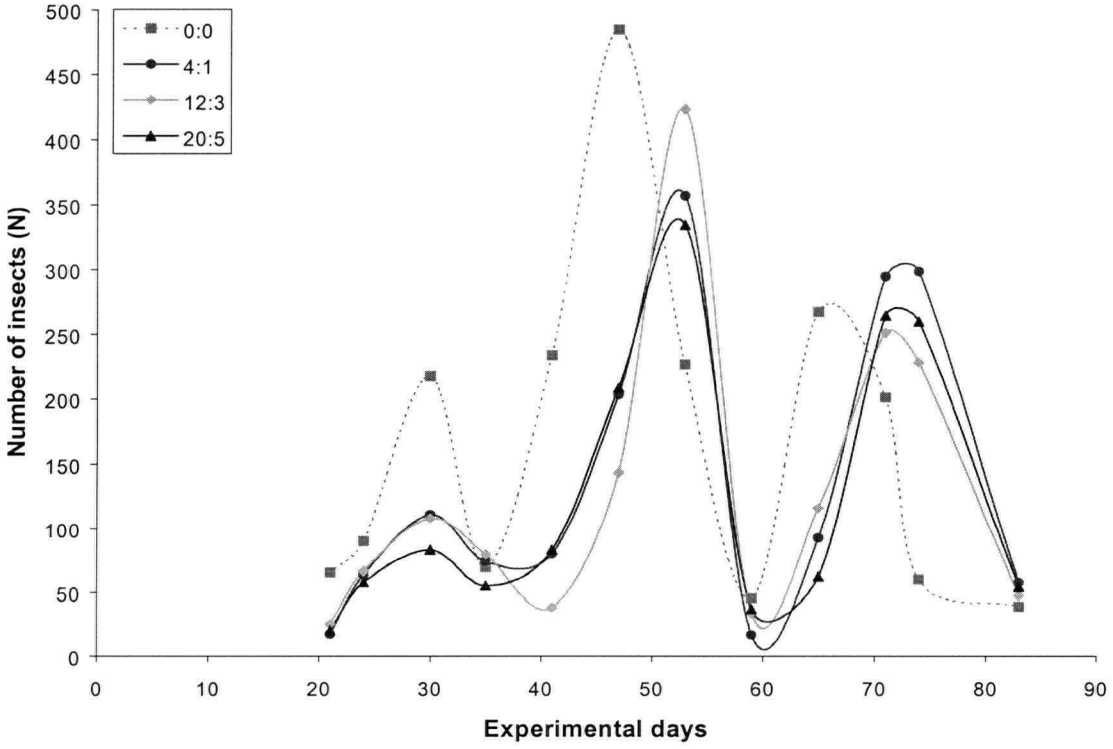

Figure 59. Mean number of baetid nymphs captured as emigrants in control and 4:1 treatment ratio troughs from July 30 to September 30, 1992. Numbers represent 3 d totals. Counts are the mean of two replicates.

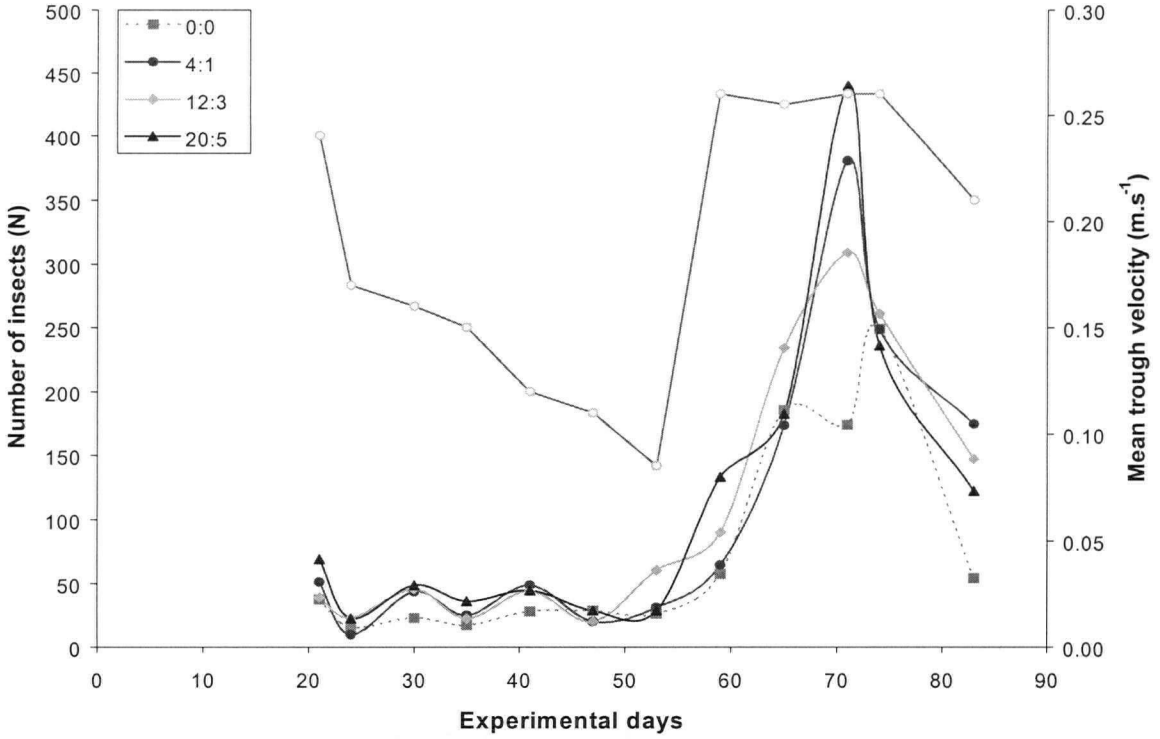

Figure 60. Mean number of chironomid larvae captured as emigrants in control and 4:1 treatment ratio troughs from July 30 to September 30, 1992. Numbers represent 3 d totals. Counts are the mean of two replicates. Note shift in trough velocity on day 55.

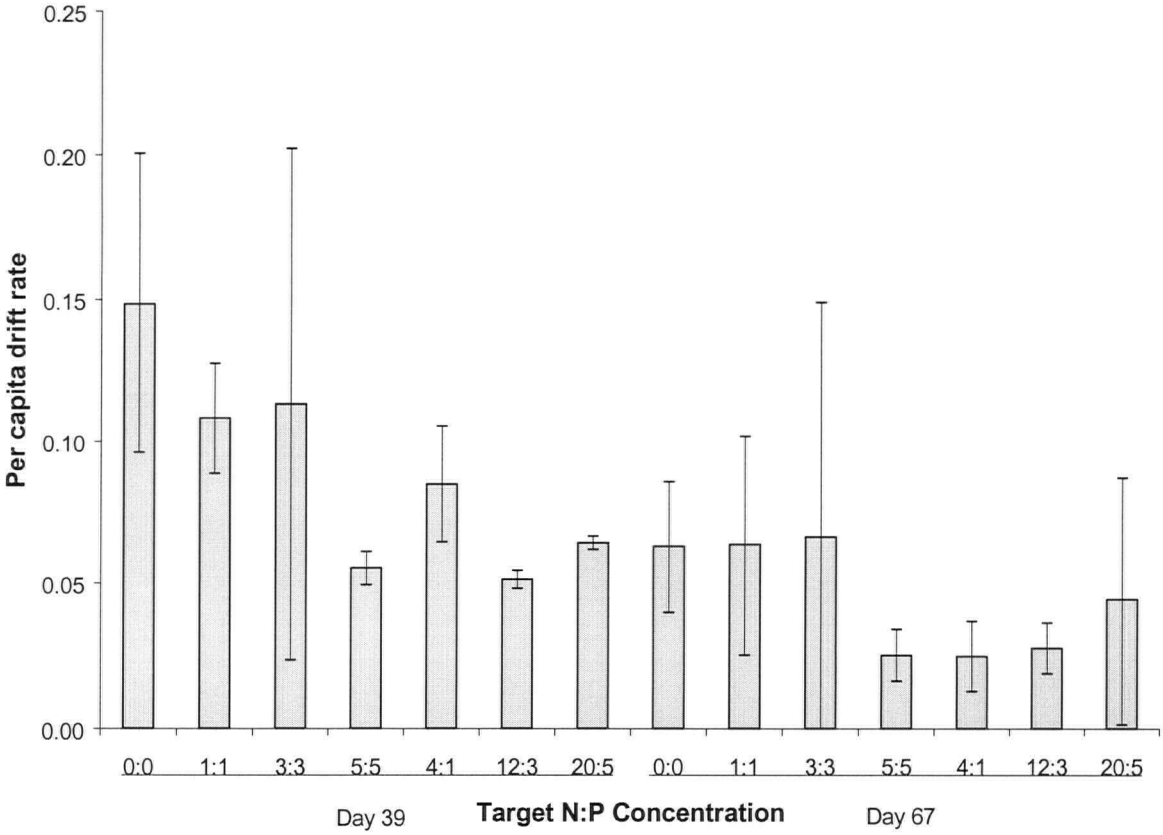

Figure 61. Baetid mayfly per capita drift rates for control and treatment groups on days 39 and 67. Mean rates are based two replicates. 95% CI is indicated.

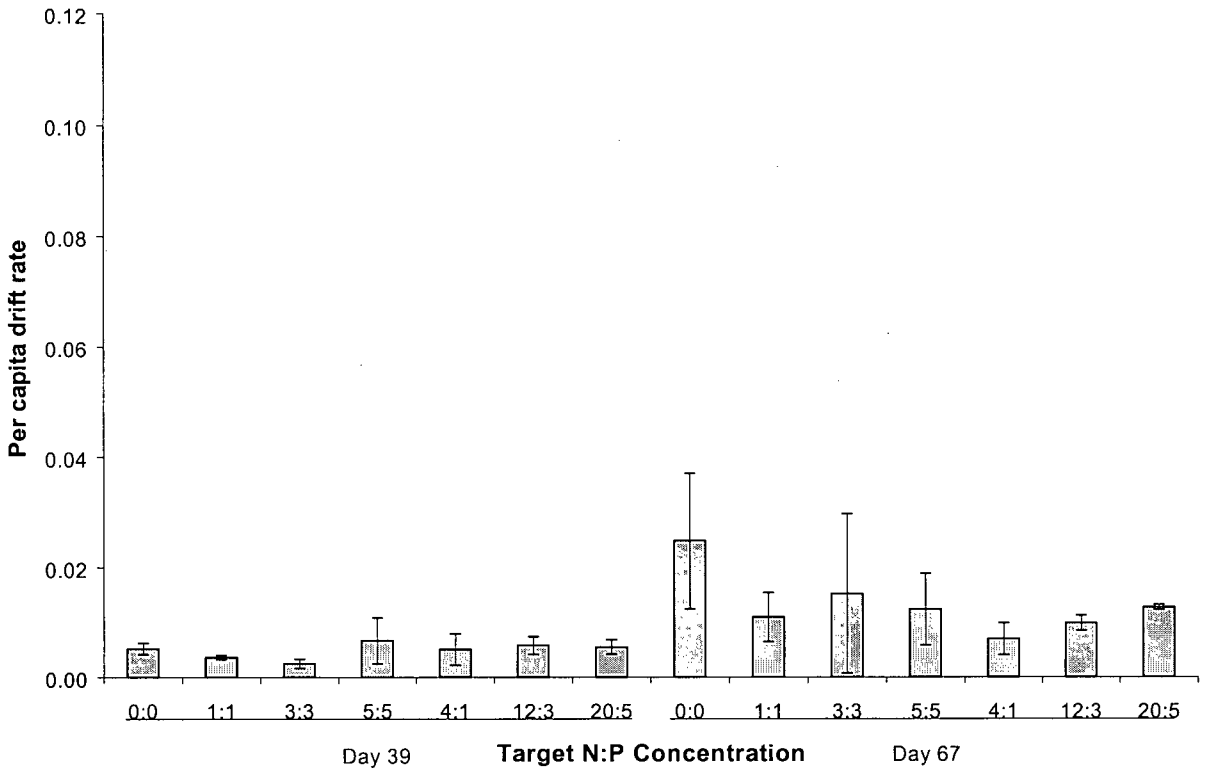

Figure 62. Chironomid larvae per capita drift rates for control and treatment groups on days 39 and 67. Mean rates are based two replicates. 95% CI is indicated.

day 39 within the 5:5, 12:3 and 20:5 nutrient concentrations (ANOVA; $p<0.05$). A significant difference in per capita drift rate between the 1:1 and 4:1 treatment groups was only observed between the 3:3 and 12:3 concentrations (ANOVA; orthogonal contrast; $p<0.05$) despite the rather large variation between replicates in the 3:3 concentration. Within treatment groups, a significant difference in per capita drift rate was only evident between the 4:1 vs 12:3 and 20:5 concentrations as well as a significant difference between the 12:3 and 20:5 concentration (ANOVA; orthogonal contrast; $p<0.05$). There was no significant difference in per capita drift rates however, between control and treatment groups for baetid nymphs on day 67 (ANOVA; $p>0.05$). Similarly, significant differences in per capita drift rate for chironomid larvae, between control and treatment groups, were not demonstrated on either of these dates (ANOVA; $p>0.05$). Significant differences in per capita drift rates between dates (i.e. between days 39 and 67) were evident however, for both mayflies and chironomids (ANCOVAR; $P<0.05$; Figs. 63 and 64, respectively). Although drift rates remained higher for mayflies compared to chironomids, an overall decrease in per capita drift rate was evident for mayflies whereas an overall increase was detected for chironomids over the period of monitoring.

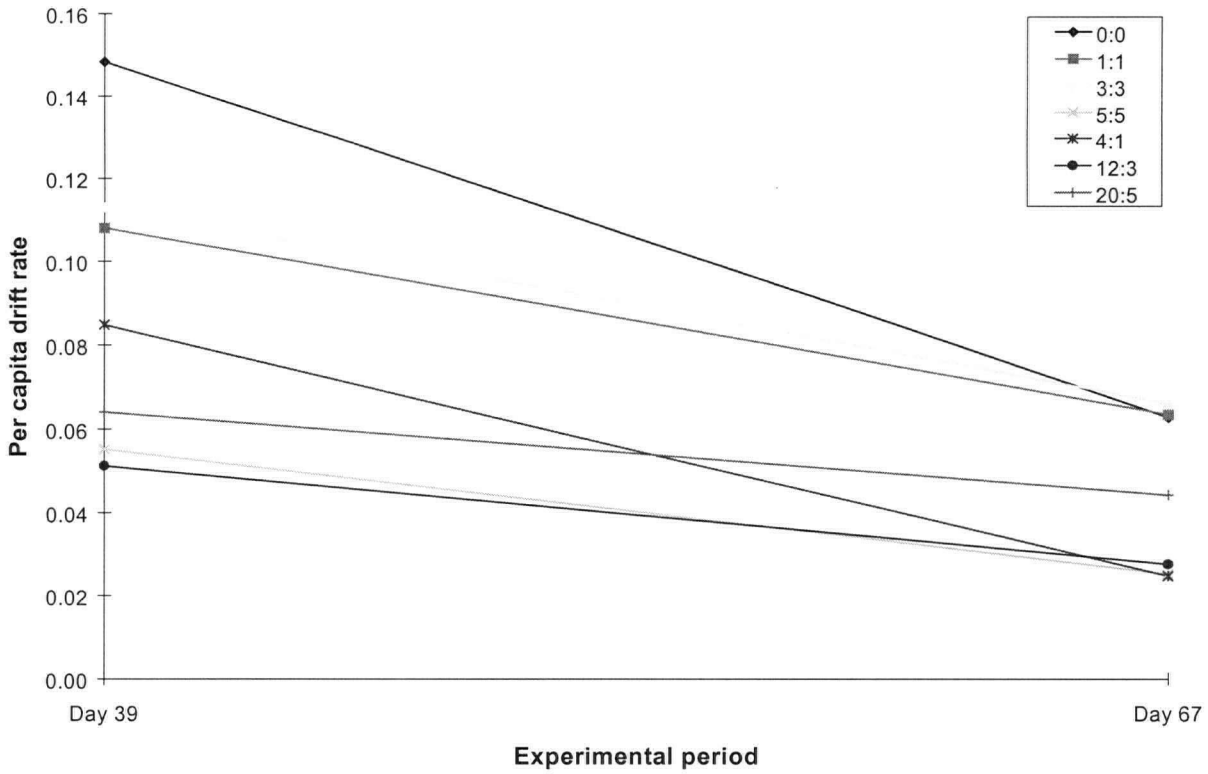

Figure. 63. Baetid mayfly per capita drift rates estimated on day 39 and 67. Treatment * time interaction lines are illustrated to show differences in the rate of change between control and treatment groups over time.

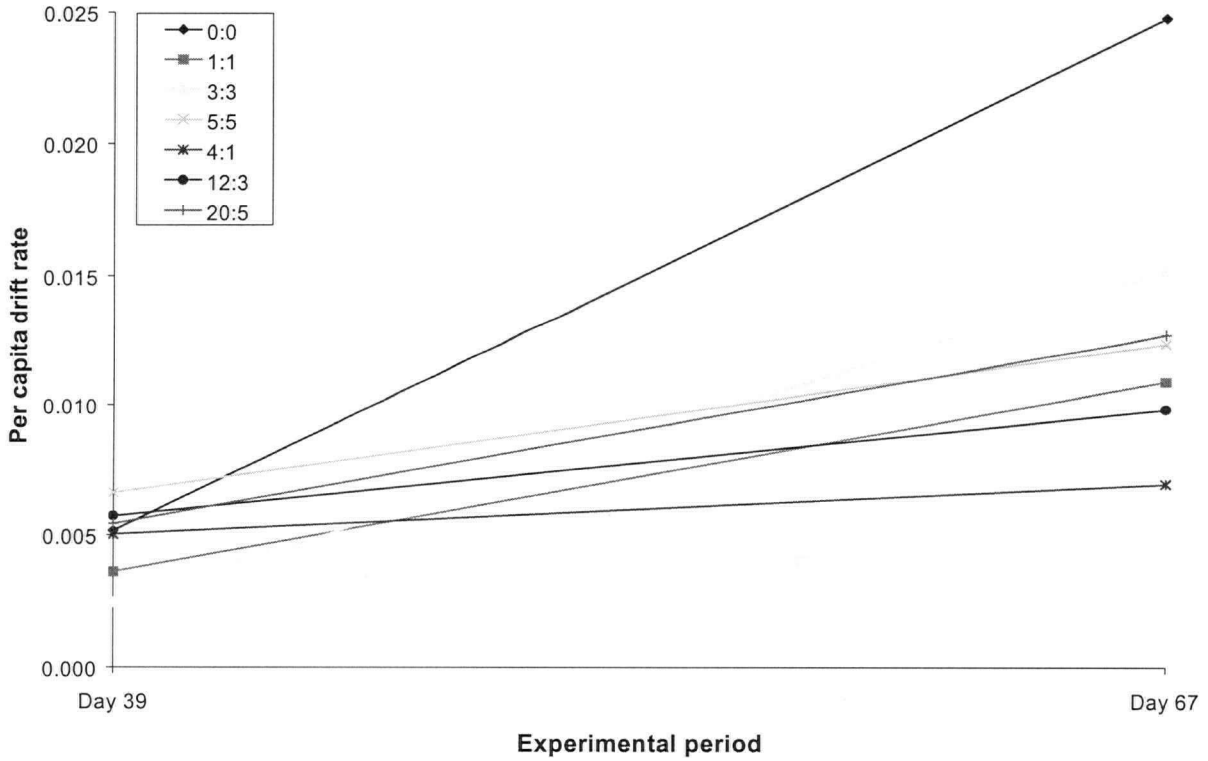

Figure. 64. Chironomid larvae per capita drift rates estimated on day 39 and 67. Treatment * time interaction lines are illustrated to show differences in the rate of change between control and treatment groups over time.

Whether or not the trend towards a lower emigration rate with higher nutrient ratios for mayflies equates to higher resource abundance or the higher emigration rate among chironomids equates to higher trough velocities remains equivocal due to estimation at single points in time.

Drift patterns observed in perlodid stoneflies over the same period were much less definitive due to the low number of individuals encountered (Fig. 65). Within the 1:1 treatment group, higher levels of drift were characteristic of the first cycle than that observed within the control. Patterns were sharply contrasted during the second cycle however, with much larger numbers of drift observed in the control. With the exception of the 5:5 nutrient concentration which experienced an earlier peak, both 1:1 and 3:3 concentration peaks were synchronous with the control. Similar patterns between the 1:1 and the 5:5 treatments were evident during the third cycle although the latter concentration was again delayed. A steady increase in emigration was further characteristic of both the control and treatments over the same period and no final peak

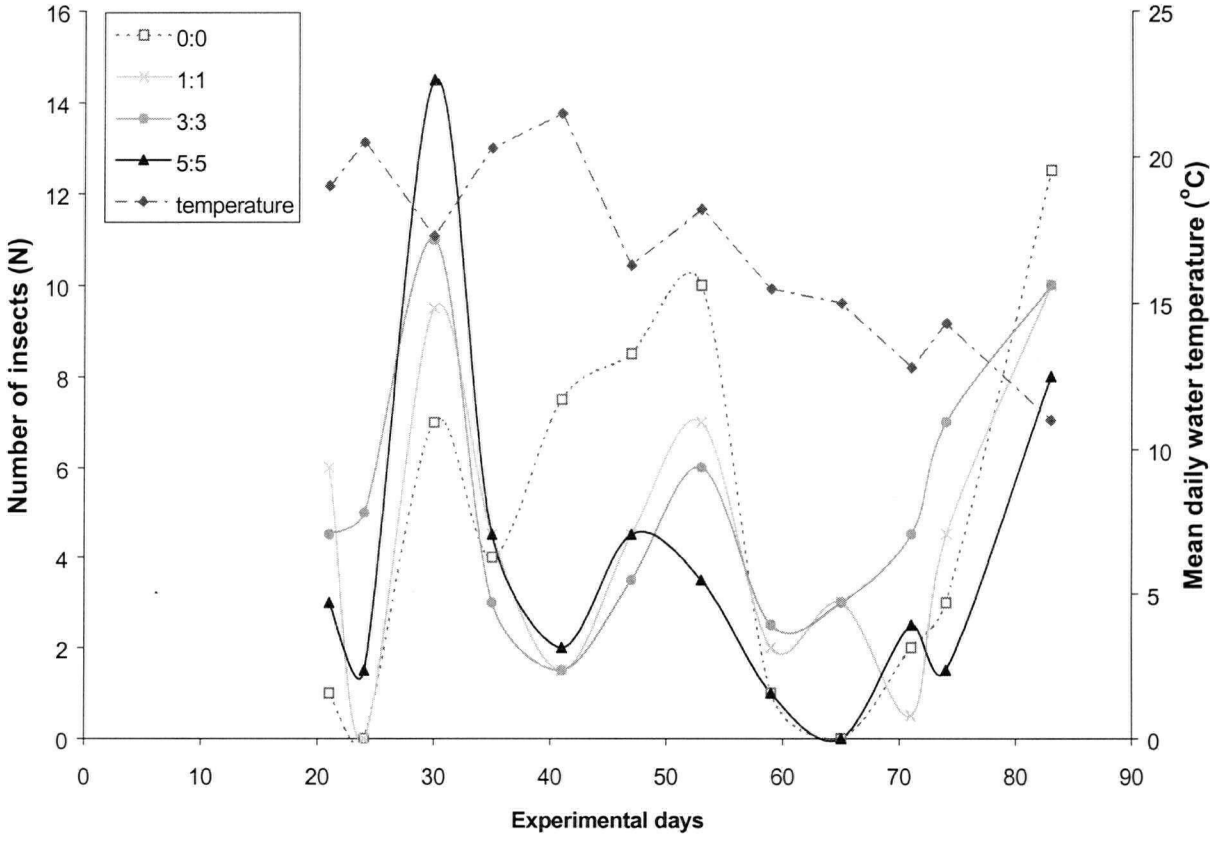

Figure 65. Mean number of perlodid stonefly nymphs captured as emigrants in control and 1:1 treatment ratio troughs from July 30 to September 30, 1992. Numbers represent 3 d totals. Counts are the mean of two replicates.

was observed. The major outmigration of stonefly nymphs showed little relationship with water temperature. Within the 4:1 treatment group, a similar pattern of drift observed in the 1:1 treatment group was contrasted by similar levels of drift in treatment troughs of the first cycle and an earlier peak than the control during the second cycle (Fig. 66). Variation in timing among treatment concentrations in relation to initiation of the third cycle was also noticeable. Differences in periodicity started with the 4:1 concentration followed by the 12:3 concentration and later yet followed by the 20:5 concentration. Emigration pattern was more tightly linked to water temperature in the 4:1 treatment group with peak counts coinciding with temperature minima over the first two drift cycles but showed no relationship during the last cycle. Stonefly outmigration appeared more closely related to chironomid larval dispersal during the third drift cycle.

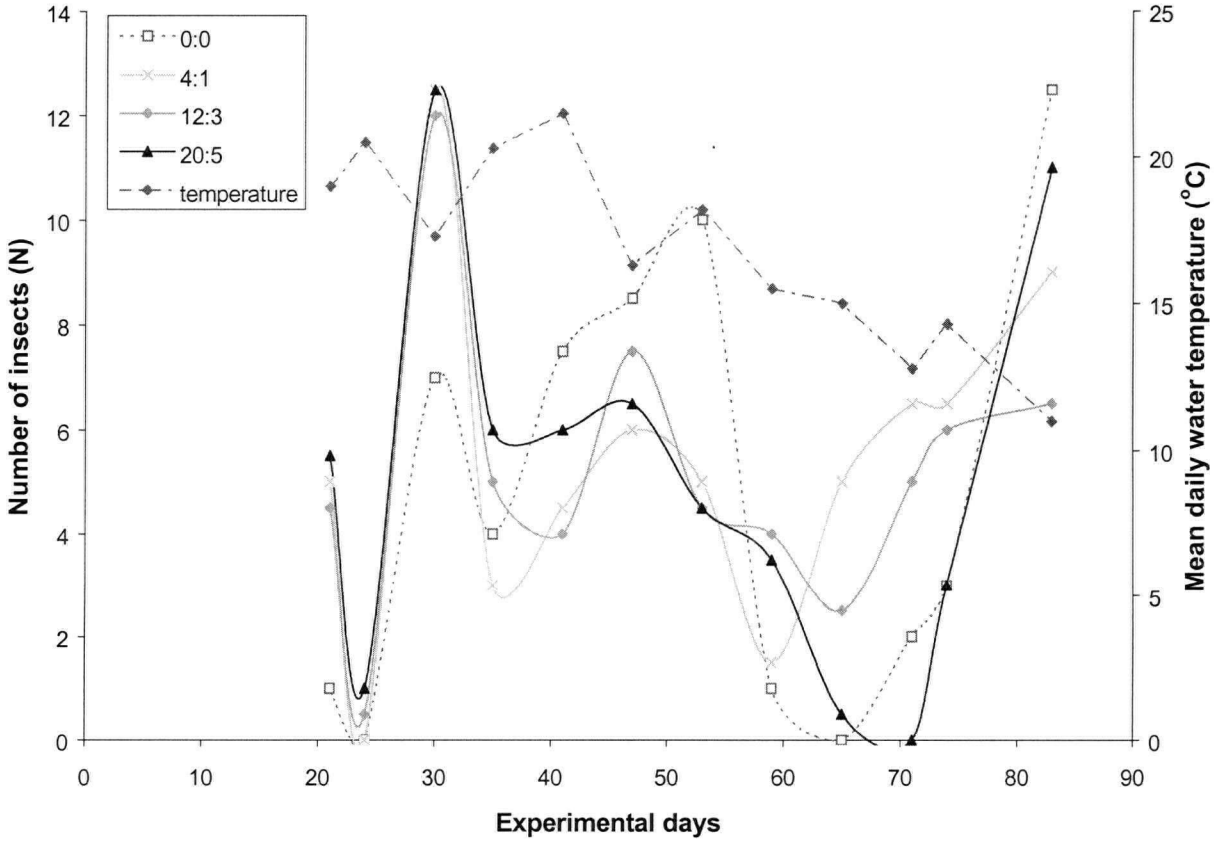

Figure 66. Mean number of perlodid stonefly nymphs captured as emigrants in control and 4:1 treatment ratio troughs from July 30 to September 30, 1992. Numbers represent 3 d totals. Counts are the mean of two replicates.

3.4.2.3 Body size relationships of drifting mayflies

Differences in head capsule width measurements of baetid nymphs sampled during the peak of the three successive drift cycles across all experimental units were used to contrast differences in growth attributed to level of fertilizer concentration. Owing to differences in timing of peak drift between control and treatment groups described in the above, measurements were completed for the corresponding dates within control and treatment groups when peak counts occurred. The three dates provided (August 8, August 25 and September 12) reference the timing of peak counts observed in control troughs but measurements from adjacent collection dates were used to account for the observed peak timing delay in treatment troughs.

Significant differences in baetid nymph head capsule widths between control and treatment groups were demonstrated for each of the three drift cycles (ANOVA; $p<0.05$; Figs. 67 - 72). Throughout each of the three cycles, head capsule widths were generally larger in treatment groups than the control. Orthogonal contrasts have been provided to show exceptions or compare differences within and between treatment groups (Table 12). Within the first drift peak (day 30), significant differences in size between treatment ratio groups were observed; the larger size categories were equated with the 4:1 treatment ratio group (ANOVA; $p<0.05$). Within the 1:1 treatment ratio group, all three concentrations were significantly different from one another, with the largest size categories recorded in the 3:3 and 5:5 concentrations. There was no significant difference in size among the three concentrations of the 4:1 treatment ratio group. Significant differences in size were again demonstrated between control and treatment groups (ANOVA; $p<0.05$) with the exception of the 1:1 concentration during the second drift peak ($p>0.05$). Between treatment group comparisons were only non-significant for the 1:1 and 4:1 concentrations during this cycle (ANOVA; $p>0.05$). The 12:3 and 20:5 concentrations had more baetid nymphs in the larger size categories. Within treatments, the 1:1 concentration was again significantly smaller from the 3:3 and 5:5 concentrations (ANOVA; $p<0.05$) however the latter treatments were not significantly different from each other ($p>0.05$). The same relationship was identified for nutrient concentrations within the 4:1 treatment ratio group where the 4:1 concentration was significantly different than the 12:3 and 20:5 concentrations (ANOVA; re observed for the 1:1 and 4:1 concentrations as well as the 5:5 and 20:5 concentrations; $p<0.05$) with no change in the latter two concentrations. The latter two concentrations were represented by larger size categories than those found in the 4:1 concentration. Size differences were significant between treatment groups (ANOVA; $p<0.05$) during the third drift cycle with the exception of the 5:5 and 20:5 comparison ($p>0.05$). Treatments within the 1:1 ratio group were significantly different from each other with the highest distribution of the larger

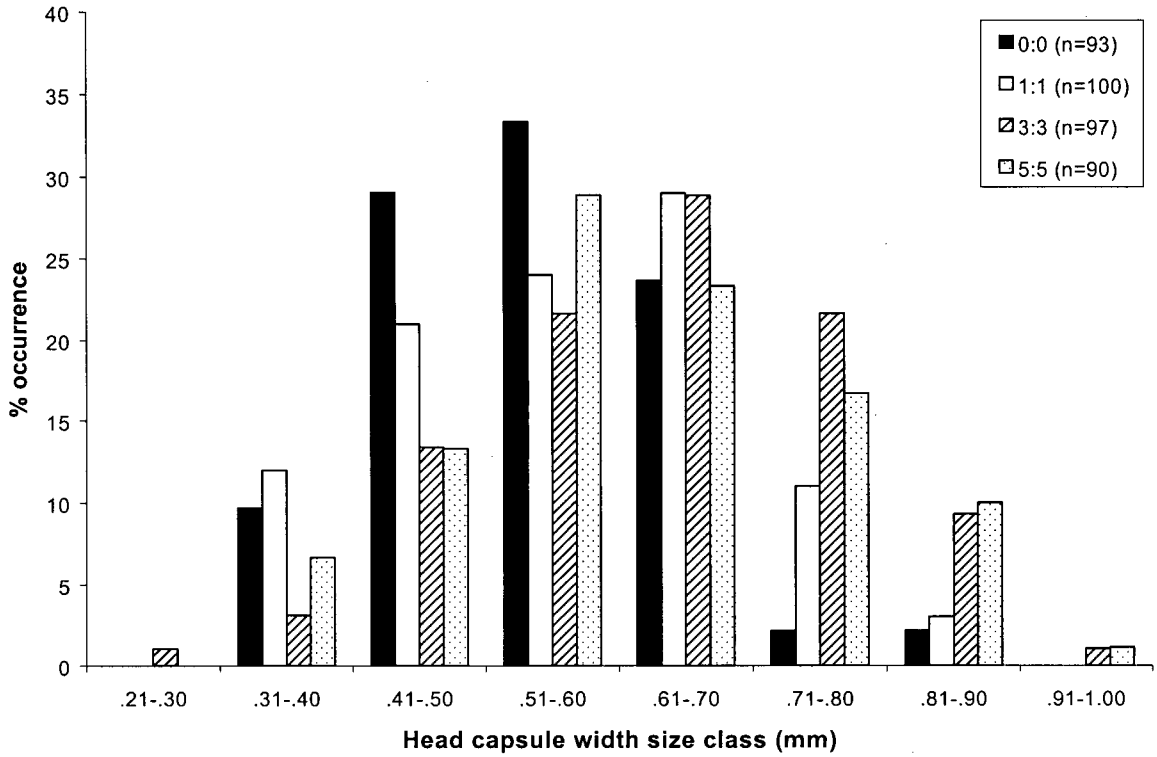

Figure 67. Size comparisons of baetid mayfly nymphs captured as emigrants in control and 1:1 treatment ratio troughs on August 8, 1992 during the peak of the first drift cycle. Measurements are pooled between two replicates.

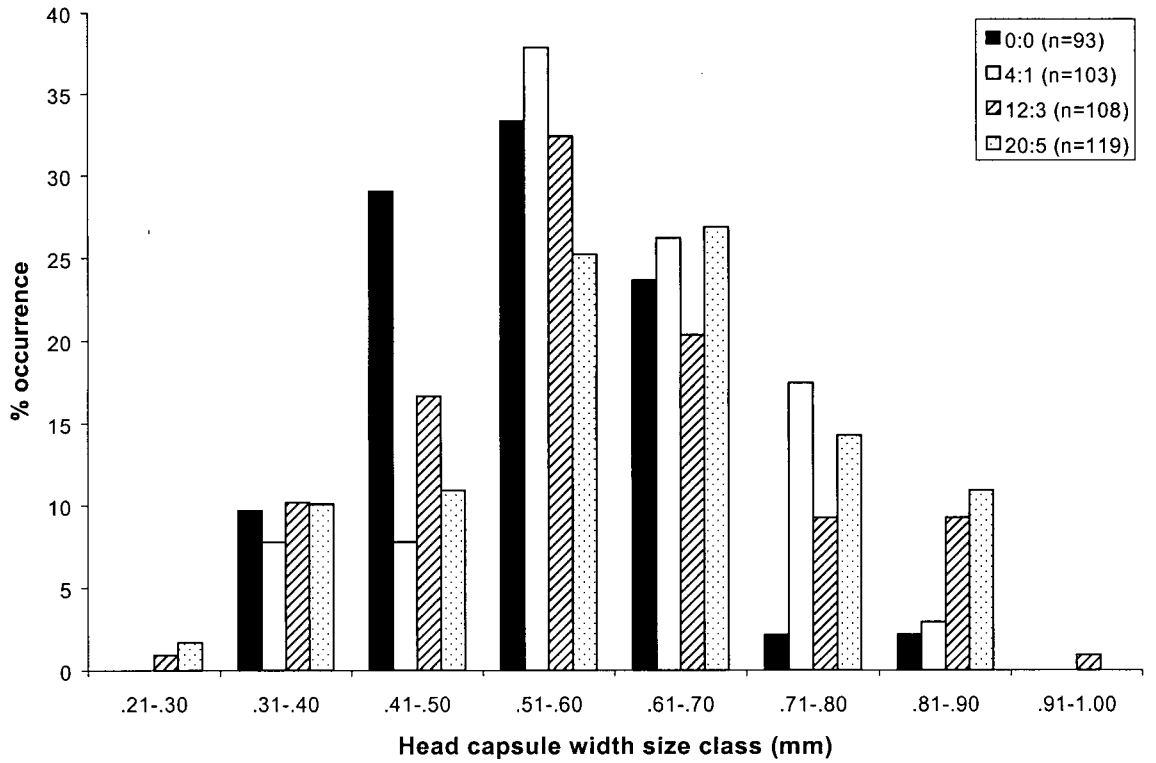

Figure 68. Size comparisons of baetid mayfly nymphs captured as emigrants in control and 4:1 treatment ratio troughs on August 8, 1992 during the peak of the first drift cycle. Measurements are pooled between two replicates.

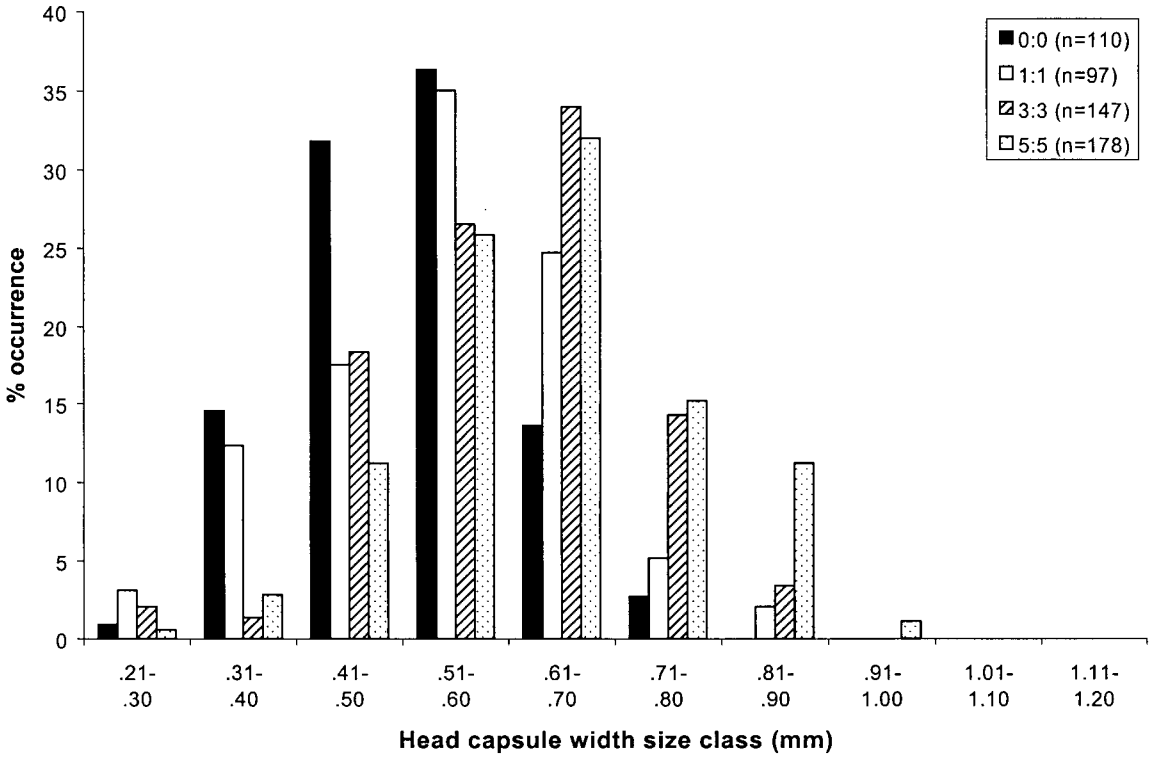

Figure 69. Size comparisons of baetid mayfly nymphs captured as emigrants in control and 1:1 treatment ratio troughs on August 25, 1992 during the peak of the second drift cycle. Measurements are pooled between two replicates.

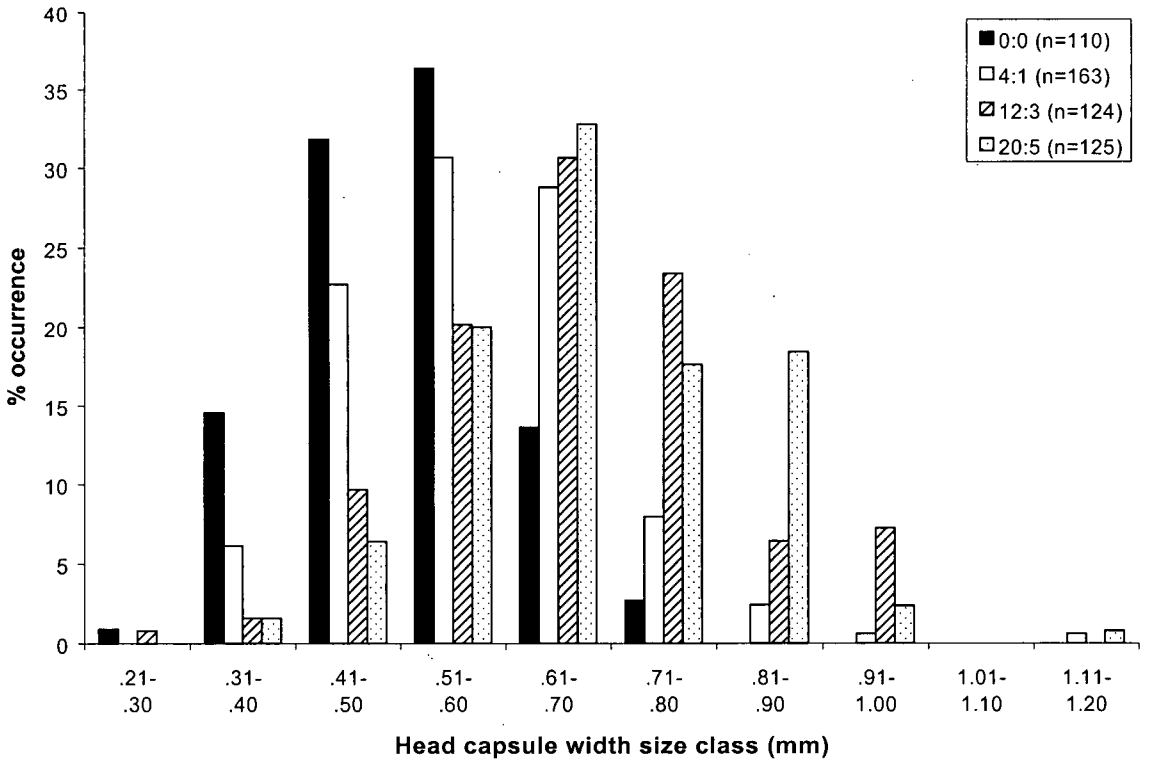

Figure 70. Size comparisons of baetid mayfly nymphs captured as emigrants in control and 4:1 treatment ratio troughs on August 25, 1992 during the peak of the second drift cycle. Measurements are pooled between two replicates.

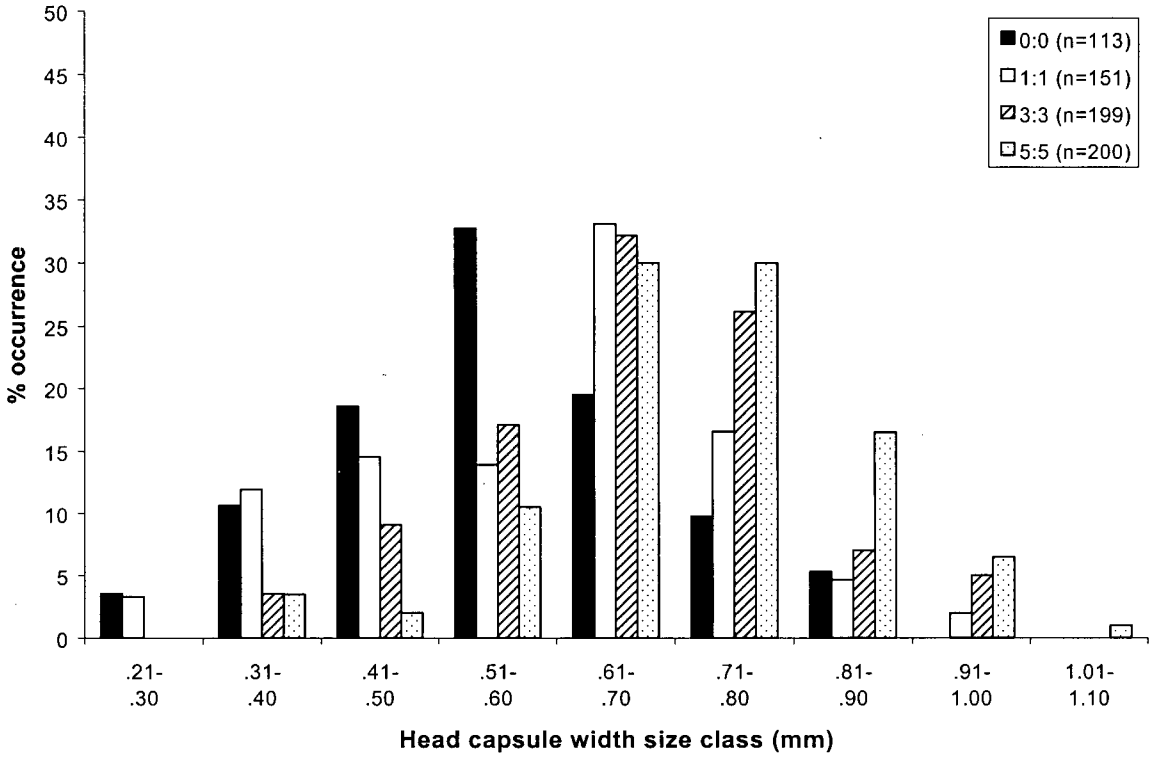

Figure 71. Size comparisons of baetid mayfly nymphs captured as emigrants in control and 1:1 treatment ratio troughs on September 12, 1992 during the peak of the third drift cycle. Measurements are pooled between two replicates.

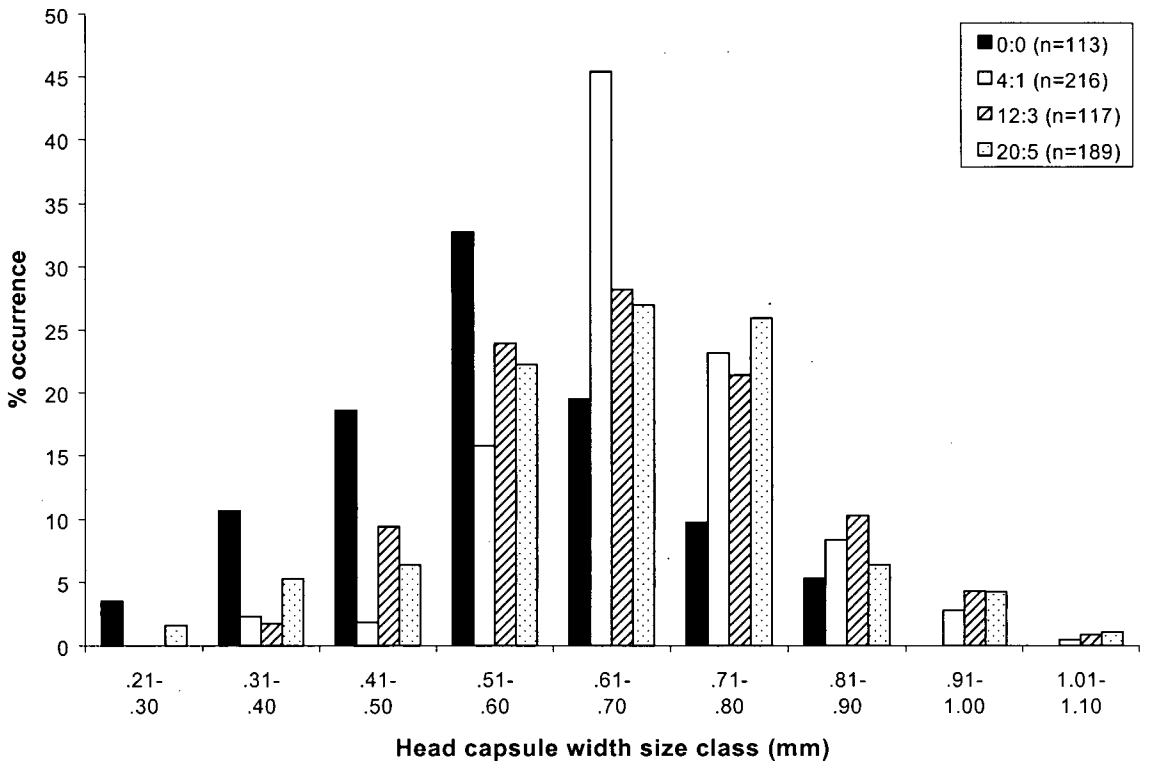

Figure 72. Size comparisons of baetid mayfly nymphs captured as emigrants in control and 4:1 treatment ratio troughs on September 12, 1992 during the peak of the third drift cycle. Measurements are pooled between two replicates.

Table 12. Orthogonal contrasts of baetid mayfly nymph head capsule width size categories within and between treatment groups based on measurements from samples collected during the peaks of three drift cycles. Analyses completed after logarithmic transformation (LOG_{10}) of the data. Group 1 = 1:1 treatment ratio and Group 2 = 4:1 treatment ratio. Level of significance set at α=0.05.

Drift Cycle	Analysis of Variance	Orthogonal Contrast	Significance
1	Control vs treatment		p<0.001
(Day 30)	Between treatments	Group 1 vs Group 2	p>0.050
		1:1 vs 4:1	p=0.000
		3:3 vs 12:3	p>0.050
		5:5 vs 20:5	p=0.007
	Within treatments	1:1 vs 3:3 and 5:5	p=0.001
		3:3 vs 5:5	p=0.001
		4:1 vs 12:3 and 20:5	p>0.050
		12:3 vs 20:5	p>0.050
2	Control vs treatment		p<0.001
(Day 47)		CTRL vs 1:1	p>0.050
	Between treatments	Group 1 vs Group 2	p>0.050
		1:1 vs 4:1	p>0.050
		3:3 vs 12:3	p=0.003
		5:5 vs 20:5	p=0.049
	Within treatments	1:1 vs 3:3 and 5:5	p=0.014
		3:3 vs 5:5	p>0.050
		4:1 vs 12:3 and 20:5	p<0.001
		12:3 vs 20:5	p>0.050
3			
(Day 65)	Control vs treatment		p<0.001
	Between treatments	Group 1 vs Group 2	p<0.001
		1:1 vs 4:1	p<0.001
		3:3 vs 12:3	p<0.001
		5:5 vs 20:5	p>0.050
	Within treatments	1:1 vs 3:3 and 5:5	p<0.001
		3:3 vs 5:5	p<0.001
		4:1 vs 12:3 and 20:5	p=0.014
		12:3 vs 20:5	p>0.050

size categories occurring in the 5:5 nutrient concentration. The 4:1 ratio group also demonstrated a significant difference between the 4:1 concentration and the 12:3 and 20:5 concentrations (ANOVA; $p<0.05$) however differences between the latter two concentrations were non-significant ($p>0.05$). Differences were due to a lower representation of the smaller size categories in the overall distribution.

3.5 Algal / insect dynamics

The dynamics of both primary and secondary producers have been considered, thus far, in isolation of one another when in fact the fate of each group, is strongly influenced by concurrent biotic interactions (Lamberti 1996; Steinman 1996). In support of these inter-relationships, a simplified description of algal and insect fluxes is presented.

Colonization by algae and insects proceeded in troughs simultaneously over a period of 19 d prior to fertilization (Figs. 73 and 74) Significant differences in algal cell density were observed on day 35 after two weeks of nutrient treatment. At that time, diatoms made up about 80% of the cell composition in the control whereas chlorophytes comprised up to 80% of the algal composition across treatments. Insect densities continued to build over the same period as evidenced by the low amplitude of the first drift cycle on day 30. In contrast to the algal densities however, measurements of benthic insect density across control and treatment groups on day 39 were similar and relatively low. Per capita drift rates estimated on the same day also suggested that a higher number of insects were continuing to aggregate in all treatment troughs. Chironomid midges were the most abundant of all insects sampled in the benthos at this time.

On day 57 a decline in algal cell density was observed albeit that differences between control and treatment groups were still significant. Diatoms continued to dominate (> 50%) the control group whereas green algae comprised over 50% of the algae in the treatment group and diatoms over 30%, on average. Within the chlorophytes, *Spirogyra* sp. was most dominant whereas *Achnanthes minutissima* was most dominant among the diatoms. Similar abundances of greens, cyanobacteria and diatoms had developed in the 1:1 nutrient concentration after 57 days. *Oscillatoria* sp. was most dominant of the cyanophytes. The lower abundance of algae was followed ten days later by a high abundance of insects that displayed significant differences between control and treatment groups. In fact, the rate of increase in benthos from day 39 to day 67 was also significantly greater in the 4:1 and 20:5 nutrient concentrations than the control. Accordingly, per capita drift rates for all insects estimated on day 67 remained low in treatment groups compared to the control and chironomid midges continued to dominate benthic insects. Differences in per capita drift rate however, were only weakly established for mayflies on day 39 whereas per capita drift rates did not differ among treatments for both mayflies and chironomids on day 67. Movement of the mesocosm to the west side of the channel on day 55 was followed by a peak in chironomid outmigration on day 71.

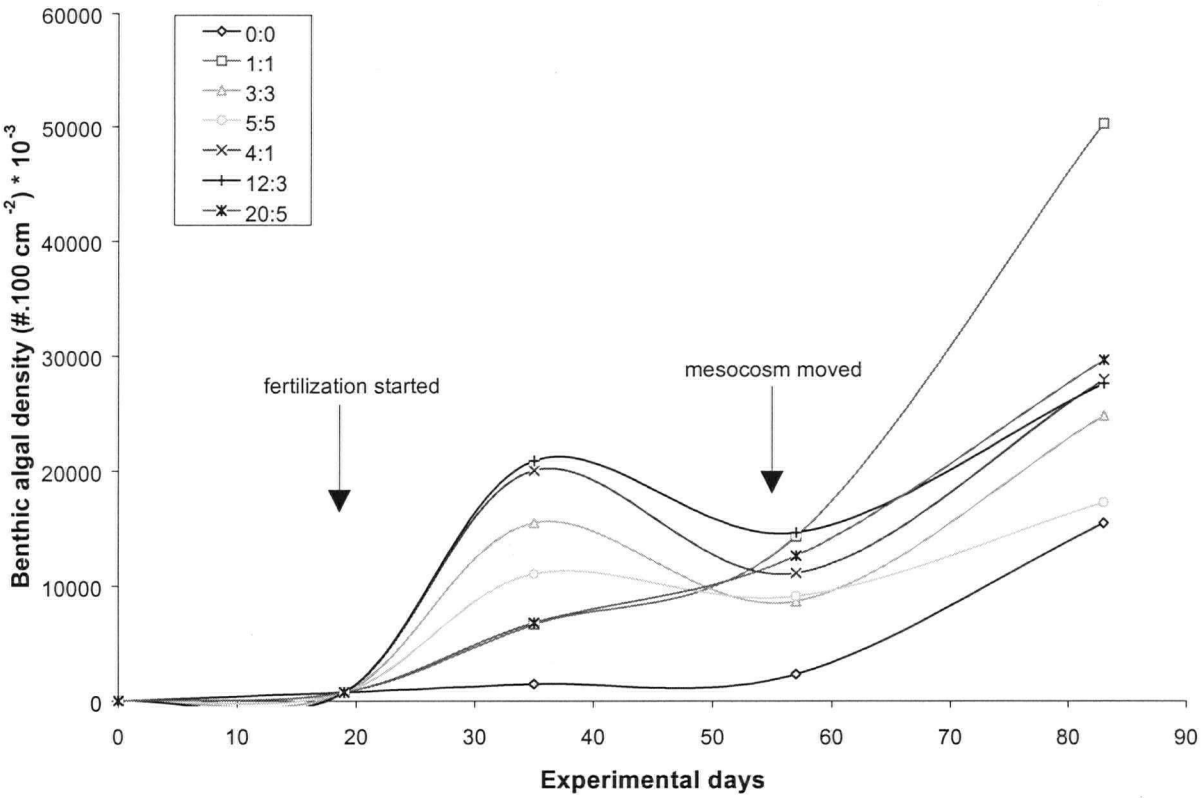

Figure 73. Changes in benthic algal density from July 28 to September 30, 1992. Connecting lines are included to illustrate trends over time and do not imply actual maxima or minima.

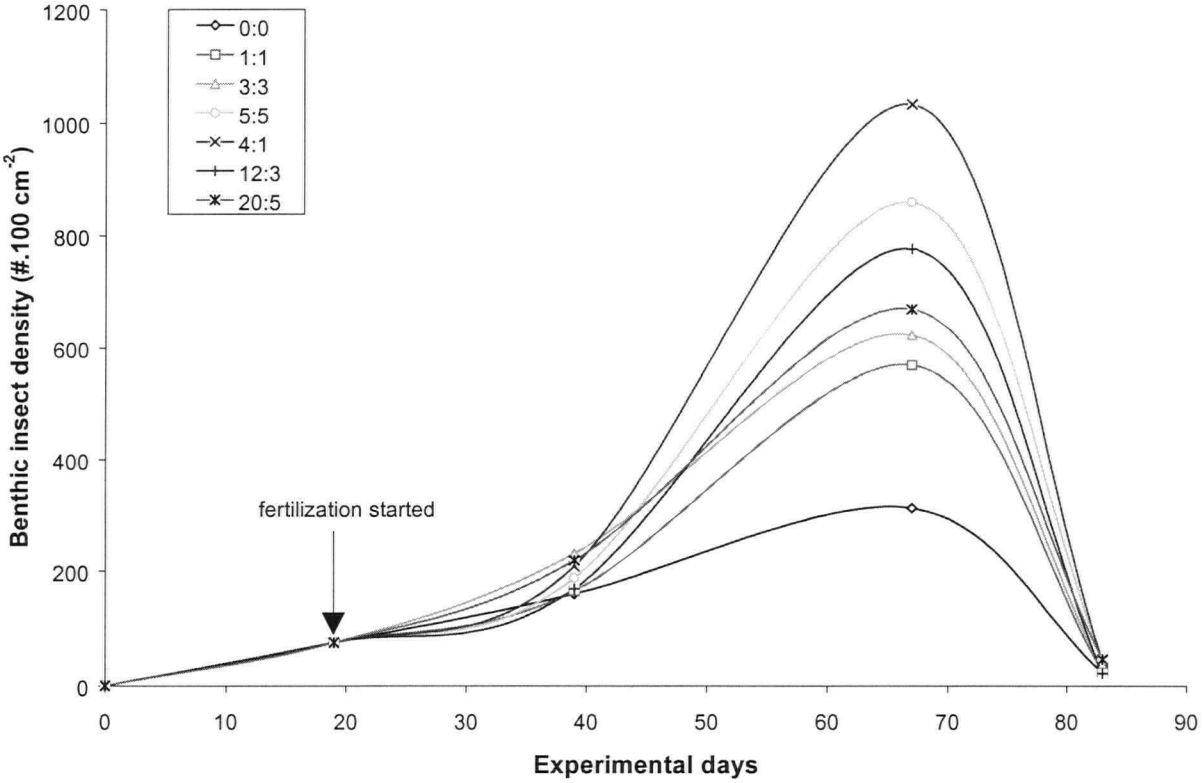

Figure 74. Changes in benthic insect density from July 28 to September 30, 1992. Connecting lines are included to illustrate trends over time and do not imply actual maxima or minima.

By the end of the experiment (day 83), algal densities had greatly exceeded their previous numbers on day 57. A 7-fold increase in cell density was observed in the control and about doubling of the cell density occurred, on average, in treatments. After 83 d, a similar occurrence of green algae and diatoms was observed in the control. Whereas *Achnanthes minutissima* was the most dominant diatom, *Zygnema* sp. was the most dominant chlorophyte. Within treatments, the green algae and diatoms comprised about 50 and 45 % of the algal composition, respectively; *Spirogyra* sp. and *Achnanthes minutissima* represented the dominant species within each phylum. A super-abundance of cyanobacteria (*Oscillatoria* sp.) was evident in the 1:1 nutrient concentration and fewer taxa among treatments were observed in the 12:3 and 20:5 treatment concentrations. Corresponding numbers of benthic insects on this date were the lowest recorded during the experiment. Unlike the previous measurement on day 67, differences between control and treatment groups were non-significant. Peaking levels of drift on day 71 together with the highest estimated total insect per capita drift rate on day 83 were responsible for the low numbers observed in the benthos at the end of the experiment. Previously high numbers of chironomid midges on day 67 fell to about half their abundance on day 83 at which time baetid mayflies became dominant. The species shift also included lepidostomatid caddisflies which comprised up to 26% of the benthos at the end of the study.

Although measurements of algal and insect density were never concurrent, increasing and decreasing trends in abundance at each trophic level appear strongly inter-related (Fig. 75). The cumulative effect of nutrient addition and grazing pressure are possible mechanisms for the higher algal density observed on day 35 followed by the lower density observed on day 57, respectively. Again the lack of direct correspondence in measurement between trophic levels is not conclusive, yet time lag changes in abundance of the benthic insect community from day 39 to day 67 (best illustrated in Fig. 74) suggest that steadily increasing numbers leading to a higher abundance on the latter date were supported by algal resource levels that declined from days 35 to 57. Despite the measured decline, algal densities ranged 3.7 - 6.3x higher across treatment concentrations compared to the control. Similarly, insect densities measured on day 67 ranged 1.8 - 3.3x higher across treatment groups relative to densities achieved in the control. The progressive decline in insects (displayed by progressively increasing levels of gross drift) to their lowest recorded level at the end of the experiment was likely responsible for the higher algal densities observed on day 83.

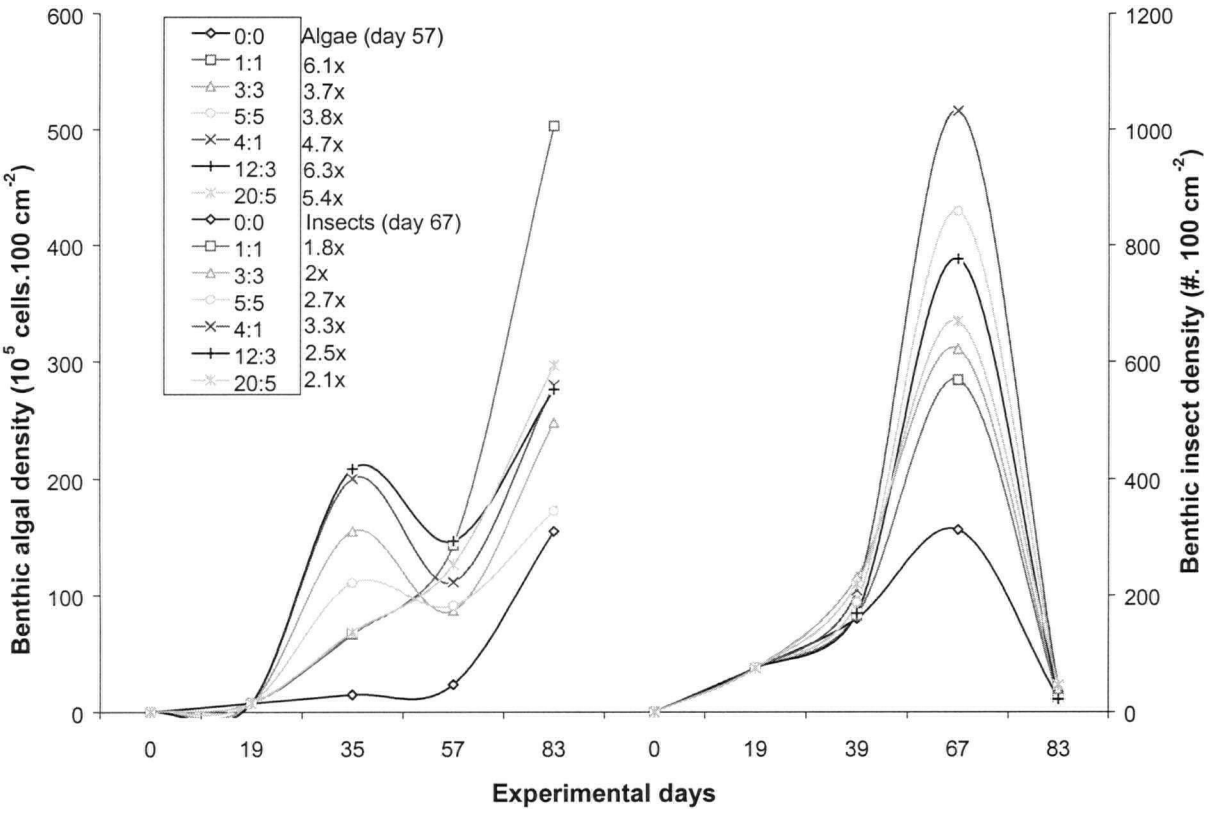

Figure 75. Combined benthic algal and insect response from July 28 to September 30, 1992. Time series is exaggerated but the contrast is provided to illustrate trophic interaction. Actual differences in algal (day 57) and insect (day 67) density over background levels are included in the legend.

4.0 Discussion

A review of the historical water quality data available since the 1970's (BC Environment, Environmental Protection, Nelson, B.C., file data) in conjunction with water chemistry data provided in the present study indicate co-limitation of nitrogen and phosphorus in the Slocan River on an annual basis. Phosphorus occurs in shortest supply throughout most of the year, shifting to nitrogen limitation by late summer (August). Timing of nutrient availability is likely mediated by phytoplankton dynamics in Slocan Lake (i.e. the headwaters of the Slocan River) due to nutrient retention. Similar nutrient dynamics have been reported in the Nechako and Blackwater river systems in B.C., both of which are lake-headed (Ashley and Slaney 1997). Corroborative evidence was provided by a series of water chemistry analyses completed at sequential upstream stations in the Slocan River above Passmore, B.C. in August 1985. Nitrate-nitrogen concentrations at that time were consistently reported <0.02 $mg \cdot L^{-1}$ using standard wet chemistry techniques (Griffith 1986).

Implicit in the experimental design, then, was a need to consider both nitrogen and phosphorus augmentation to off-set limitations provided in nature. The nutrient bioassay study conducted on the Slocan River not only provides an example of benthic algal and insect response in a southeast interior B.C. watershed, but provides a further example of nitrogen and phosphorus concentrations at specific N:P ratios and below those reported in other coastal and central interior B.C. systems (e.g. Adam River, Slaney et al. 1993; Keogh River, Johnston et al. 1990; Mesilinka River, Paul et al. 1996; Nechako River, Perrin and Richardson 1998; Salmon River, Slaney et al. 1994).

4.1 Trough flow variation, nutrient supply and treatment effects

The application of an in-river mesocosm to a nutrient bioassay was not without its complications where maintenance of uniform nutrient concentrations was dependent upon a constant flow rate. Although declining river flow regimes have been shown to affect the water supply to shore-based mesocosms (Perrin pers. comm.), in-trough flow variability, due to declining river discharges and diel variation, was problematic in achieving a uniform nutrient supply rate in the present study. Overall mean N and P concentrations were close in comparison to experimental target concentrations however, weekly variation associated with river dynamics likely pre-empted the maintenance of constant nutrient levels. Nutrient concentrations and ratios were less likely to achieve steady-state and more likely to occur in a state of flux. Despite the design limitations of the present apparatus, differences in treatment effects were demonstrated and trough discharge, although significantly different before and after re-positioning of the mesocosm, was not implicated as a covariate (background variable) responsible for affecting the outcome of the experiment. The question remains as to whether or not systematic differences in discharge across individual troughs introduced a bias that affected some treatments more than others. The direct effects of higher water velocity in the latter half of the experiment were limited to physical disturbance that led to increased sloughing. Flow variability leading to fluctuations in nutrient supply rates or enhanced sloughing however, has no doubt contributed to experimental error in the present study. Improvements in the trough intake design are recommended to maintain even flow and will be discussed in greater detail at the end of the discussion.

4.2 Nutrient treatment effects on periphyton

4.2.1 Periphyton abundance and biomass

Exponential periphyton accrual across the 1:1 and 4:1 treatment groups, leading to biomass differences of up to 4 and 8 times that of the control, respectively, are consistent with responses

of periphyton to nitrogen and phosphorus addition reported in the literature (Stockner and Shortreed 1978; Peterson et al. 1983; Perrin et al. 1987; Bothwell 1988; Johnston et al. 1990; Mundie et al. 1991; Perrin and Richardson 1998). With the exception of the Thompson River research in which requirements for orthophosphate saturation of cellular growth rate were distinguished from community growth rate (Bothwell 1988 and 1989), all other studies attained comparable results with nutrient additions equal to or exceeding 10 or 20 $\mu g \cdot L^{-1}$ N and 5 $\mu g \cdot L^{-1}$ P. Similar results were achieved with levels up to 20 $\mu g \cdot L^{-1}$ N and 5 $\mu g \cdot L^{-1}$ P in the present study. In fact, the highest rate of late summer chlorophyll *a* accrual on styrofoam substrate was observed with as little as 4 $\mu g \cdot L^{-1}$ N and 1 $\mu g \cdot L^{-1}$ P in trough experiments in the Slocan River. A higher level was expected for the 12:3 nutrient concentration, but periphyton accumulation was compromised by suspected insect grazing or sloughing or by systematic error during the last accrual trial. The doubling in peak biomass between the 1:1 and 4:1 treatment groups (i.e. from 60 to 120 $mg \cdot m^{-2}$ Chl *a*) is similar to differences in late summer biomass (42 to 98 $mg \cdot m^{-2}$ Chl *a*) between two different levels of N and P applied to the Keogh River on Vancouver Island (Perrin et al. 1987). It is important to note however, that for all treatments encountered in the Slocan study, an actual peak in biomass was only achieved in the 5:5 nutrient concentration. Closer comparison of the 27 d periphyton accrual trial in the present study with the late fall biomass accumulation trial on the Thompson River (Bothwell 1989) suggests that algal biomass was very close to peak levels at the end of the Slocan experiment. Periphyton accrual trials in the Thompson study reached a plateau between 30 and 40 d for concentrations from 0.1 to 5 $\mu gP \cdot L^{-1}$ (Bothwell 1989). Peak biomass would have probably occurred within an additional 7 d because of the warmer ambient water temperatures (10 C) in the Slocan River at the end of the study.

Differences in maximum biomass between treatment groups in the third accrual trial suggested that the lower accumulation associated with the 1:1 treatment group was due to nitrogen limitation. N limitation was similarly observed in the P alone additions to background river water in experimental troughs in the Nechako River study (Perrin and Richardson 1998). Higher levels of chlorophyll *a* due to the combined effects of N + P were also noted in other *in situ* flow-through trough experiments (Stockner and Shortreed 1978; Lohman et al. 1992; Rosemond et al. 1993) as well as *in situ* stream experiments (Pringle and Bowers 1984; Pringle 1987; Winterbourn 1990; Burton et al. 1991 cited in Borchardt 1996) when compared to the effects of single-nutrient addition. A comparison of background with the mean treatment N:P supply ratios across the 1:1 and 4:1 treatment groups, ranging from 2.3N:1P to 9.2N:1P (molar) in the present study, suggest N limitation for the majority of treatments based on optimum supply ratios for selected green algae and diatoms that average 17:1 (Rhee and Gotham 1980). Similarly, the

range of ambient N:P ratios (DIN:SRP) reviewed by Borchardt (1996) suggest that ratios <10:1 (μg:μg) are considered N-limiting. Differences in algal biomass between the 1:1 and 4:1 treatment groups strongly suggest that the latter treatment ratios, having a larger contribution of nitrogen, produced a higher level of biomass than the former treatment ratio group. Differences in maximum biomass within nutrient concentrations of the 4:1 treatment group are likely related to differences in accrual rates among individual treatment ratios. A higher initial periphyton growth rate in the 20:5 nutrient concentration during the first week of the third accrual cycle was surpassed by the 4:1 nutrient concentration and eventually led to a higher maximum biomass in the 4:1 treatment. The initially higher rate of growth in the 20:5 concentration may have contributed to boundary layer effects (i.e. nutrient diffusion gradients) in the developing mat at an earlier stage, which, in turn, may have changed nutrient uptake kinetics during the latter stages of the accrual cycle (Borchardt 1996).

Parallel experimental evidence was also observed with algal cell density on gravel substrates, particularly during the first 35 d of the study. After an initial 19 d of colonization followed by 16 d of fertilization, differences in algal cell density between control and treatments were not only statistically significant, but densities in specific nutrient concentrations (3:3, 4:1 and 12:3) exceeded those in the control by an order of magnitude. The high degree of variability in cell counts among treatments relative to the control over the remaining sampling periods however, may be related to small scale sampling of contagious distributions (Elliot 1977; Shiozawa 1983) rather than variability in nutrient concentrations between replicate experimental units. Wide variation in algal numbers on individual stones within the same trough were particularly evident in the 1:1 nutrient concentration on days 57 and 83 (refer to Figs. 14 and 16). Large variations in trough discharge, that could alter nutrient concentrations produced from a common stock solution, were not encountered until after day 55 of the experiment.

In contrast to the increasing trend in algal density over the first 35 d, the dramatic decline in algal cell density on day 57 was likely attributable to a combination of sloughing and increased grazing pressure by insects. The massive decline that shortly follows attainment of peak biomass has similarly been reported in other studies (Perrin et al. 1987; Bothwell 1989; Perrin and Richardson 1998). Due to boundary layer interference during development of the algal mat, a diffusion gradient develops between cells in the basal layer and the overlying water that ultimately reduces the supply of nutrients (Borchardt 1996). In the face of nutrient depletion and lower levels of light, required metabolic and growth factors are restricted (i.e. the nutrient diffusion rate is slower than the cellular uptake rate) and eventual senescence of the basal layer leads to sloughing of the mat. Although an increase in water velocity has been shown to improve nutrient delivery to underlying cells (Horner et al. 1983; Stevenson and Glover 1993), a

balance exists between velocities necessary to affect nutrient transfer and those that exceed the hydrodynamic shear stress or tensile strength causing mat displacement (Biggs 1996). Re-positioning of the mesocosm on day 55 was responsible for partial sloughing of filamentous species in treatment groups due to increased water velocity (G.G. Oliver pers. obs.) and no doubt explains some of the observed decline in algal cell density. Despite the effects of increased water velocity on day 55, chlorophytes remained dominant among treatment groups on day 57 (refer to Fig. 15) and either dominant (4:1 treatment group) or co-dominant (1:1 treatment group) on day 83 (refer to Fig. 17).

Notwithstanding induced losses, a large body of experimental evidence has linked reductions in algal biomass in streams with invertebrate grazing (Gregory 1983; Lamberti and Resh 1983; McAuliffe 1984; Hill and Knight 1987; Feminella et al. 1989; Feminella and Hawkins 1995 cited in Steinman 1996). In a recent review of 93 studies investigating algal-grazer interactions, algal biomass was reduced 71 times by grazer activity (Steinman 1996). In addition, grazer control of nutrient-amended streams has been demonstrated in previous whole-stream applications (Elwood 1981; Peterson et al. 1993). While definitive evidence for grazer control was absent in the present study, there is indirect evidence for it. Time-course fluctuations in algal density from day 19 to 83 (refer to Fig. 73) were mirrored by time lag differences in the periodicity and amplitude of gross insect drift (refer to Figs. 51 and 52), decreased per capita drift rates on the ascending limbs of the second and third drift cycles (refer to Figs. 51 and 52 as well as 54 and 61), significantly higher density (refer to Fig. 25) and biomass (refer to Figs. 32 and 33) of benthic insects and significant differences in insect growth in both drift (refer to Figs. 67-72) and benthos (refer to Figs. 39-46) between control and treatment groups. These results strongly imply differences in resource abundance that would account for the higher aggregation of insects across treatment groups. It is suggested that delays in emigration that lead to higher insect numbers in the benthos should eventually lead to grazer control of algal biomass below the upper limit of periphyton accrual ultimately established by nutrient availability. In the process of algal biomass reduction, a concomitant increase in growth and development of insects would be expected; responses that have been identified in related studies under conditions of nutrient enrichment (Hart and Robinson 1990; Mundie et al. 1991; Hill et al. 1992; Perrin and Richardson 1998). Steinman (1996) provides three alternative conditions why algal biomass reduction may be unaffected by herbivory:

1) biomass reduction is a density-dependent response and grazer density and ingestion rate are insufficient to impart grazer control;

2) feeding morphology of the grazer is ill-suited to handle the dominant algal type; and

3) resource-limited periphyton growth is the fundamental constraint, irrespective of grazer presence or absence.

These conditions are unlikely to have occurred in the present study since benthic insect density displayed an increasing trend until at least the middle of September; collector, gatherer and shredder functional feeding groups (sensu Merritt and Cummins 1984) were well-represented and matched to dominant algal types (e.g. chironomids, baetids, siphlonurids, heptageniids and pteronarcyids with massive mouthparts capable of handling macroalgae); and ultra-oligotrophic nutrient conditions were not encountered within treatment groups.

An additional line of supporting evidence was provided by the taxonomic response. Algal communities subject to grazing pressure are generally represented by adnate forms that find refuge close to the substrate (DeNicola et al. 1990; Hill et al. 1992). The shift from a higher to a lower relative abundance of vertically upright forms (chlorophytes) and a lower to higher relative abundance of prostrate forms (diatoms) between days 35 and 57 (refer to Figs. 13 and 15) suggests enhanced cropping of overstory species. To this end, colonial or filamentous green algae such as *Stigeoclonium* sp., *Oedogonium* sp and *Spirogyra* sp. that occupy middle to upper structural (vertical) layers in the benthic mat were likely more vulnerable to grazing by chironomids, mayflies and caddisflies (DeNicola et al. 1990; Steinman 1996; Stevenson 1996). These collective explanations remain speculative, however. The inclusion of experimental exclosures that eliminate grazing (Lamberti and Resh 1983; Hill et al. 1992; Quamme 1994) are required to establish the affect of herbivory on periphyton accrual and is an important consideration for future experiments of this nature.

4.2.2 Algal community dynamics

An important feature of the difference in cell numbers between control and treatment groups during the first 35 d of the experiment is that algal colonization and subsequent community development responded to nutrient supply ratios in a predictable manner. Differences in nutrient availability between control and treatment groups likely provided environmental conditions that favoured distinct successional trajectories among the representative algae. Under low ambient nutrient conditions in the control, early colonization followed the expected developmental pattern whereby adnate forms of diatoms such as *Achnanthes minutissima* became quickly established (Stevenson 1996). This species has been shown to have a competitive advantage in low nutrient environments where a relatively high surface area to volume ratio contributes to a greater biomass-specific P uptake rate (Steinman et al. 1991). Alternatively, as explained by McCormick (1996) a high intrinsic rate of growth may be the only prerequisite for competitive success in benthic mats when the resource environment is favourable for growth. In a Kentucky

stream, McCormick found that *Acnanthes minutissima* remained the dominant form for several weeks simply by its ability to divide more quickly than either stalked or filamentous forms. In contrast, the higher level of nutrients in treatment groups created an environment that permitted both early (diatom) and late (chlorophyte) colonizers to develop simultaneously in the absence of nutrient limitation that would otherwise affect the rate of succession (Stevenson et al. 1991). Filamentous green algae have been shown to respond favourably to relatively high levels of N and P and sunlight (Borchardt 1996). By virtue of these distinct successional pathways, differences in biomass between control and treatment groups are likely related to differences in algal community structure as much as differences in community growth rate due to enrichment. Diatom-dominated assemblages elsewhere have not been equated with high biomass whereas filamentous assemblages were normally associated with chlorophyll *a* levels exceeding 100 $mg \cdot m^{-2}$ (Welch et al. 1988 cited in Lohman et al. 1992).

At the end of the experiment, both *A. minutissima* and *Spirogyra* sp. were the two dominant taxa across all treatments; similar relative abundances occurred in the 1:1 treatment group and slightly larger relative abundances of *Spirogyra* sp. developed in the 4:1 treatment group. Over the 83 d period, diatoms (especially *A. minutissima)* proved to be a formidable competitor in both amended and unamended river water. Within treatments, an apparent inverse relationship in cell density existed between these two taxa whereby an increase in the abundance of one was mirrored by a decrease in abundance of the other. This inter-relationship is likely related to both morphological and grazer-resistant characteristics of each group (Steinman 1996). The filamentous green algae have the ability to escape potential nutrient limitation within the algal mat by extending into the water column. Yet, these same species also have a higher susceptibility to grazing. Removal of the overstory layer by grazing, restores both nutrients and light to the understory providing more suitable environmental conditions (nutrients, light) for growth. The dynamics of each group is therefore highly influenced by herbivory, nutrient availability and seral structure (DeNicola et al. 1990). These characteristics were likely responsible for the competitive interactions observed between these groups.

The more dramatic taxonomic response was observed in the 1:1 nutrient concentration where *Oscillatoria* sp. out-competed all other species by an order of magnitude. This occurrence was of particular interest due to low nitrogen availability within the treatment and the non-heterocyst forming character (non-N_2 fixing ability) of the Oscillatoriaceae (Wetzel 1975). The development of epibiont relationships between host non-N_2 fixing cyanobacteria and microheterotrophs (Paerl 1992) is one mechanism by which nutrient limitation may have been avoided and provided the cyanophytes with a competitive advantage. Non-N_2 fixing cyanobacterial bloom genera such as *Anacystis* and *Oscillatoria* sp. are known to excrete organic compounds instrumental in

attracting eubacterial epibionts (Paerl 1992). The provision of organic matter (photosynthate or excretion products) by cyanobacteria supports the growth of microheterotrophs which, in turn, accelerate rates of mineralization and CO_2 regeneration. Within the immediate microenvironment of the host, rapid exchange of inorganic nitrogen, phosphorus and trace metals would provide a steady supply of nutrients that would otherwise be unavailable due boundary layer effects. Accordingly, *Oscillatoria* sp. may have been tightly coupled with bacterial heterotrophic metabolism. The higher rate of respiration by microheterotrophs may also create sufficient reducing conditions within the microenvironment of the host to allow nitrogen fixation directly. The latter process would require phosphorus however (P.J. Harrison, UBC, pers. comm.) which may have been in sufficient quantity via the 1:1 treatment application. Alternatively, host cyanobacteria may reassimilate their own excretion products either heterotrophically or photoheterotrophically (Tucker 1988 cited in Paerl 1992) which may have provided an additional means of sequestering nutrients in short supply. The similar relative abundance of cyanobacteria and green algae and diatoms on day 57 in comparison to the gross differences in relative abundance on day 83 suggests that under low nutrient conditions, cyanophytes may have gained a competitive advantage by virtue of these adaptive mechanisms. Due to their lower palatability among invertebrates (Lamberti 1996) resistance to grazing would also be facilitated.

Algal community response in the control group after 83 d adds a degree of uncertainty to the interpretation of the results. The seven-fold increase in algal density and shift in species composition reflecting co-dominance between diatoms and chlorophytes may have been related to nutrient cycling mechanisms. Since the experiment continued over a period of approximately 3 months, eventual sloughing of the algal mat would have increased bacterial activity. Under low ambient nutrient concentrations in supply waters (particularly N under late summer conditions), remineralized nutrients provided by bacterial decomposition of algal cells, grazer wastes or detrital materials, may have been recycled over an extremely short distance. The concept of nutrient spiralling has been previously demonstrated in stream environments (Newbold et al. 1983; Mulholland 1996). In this instance, tight spatial coupling between bacterial remineralization and algal nutrient uptake would explain the dramatic response in algal density due to improved nutrient availability. Similar examples of tight spatial coupling have been provided in nutrient depletion experiments in troughs (Mulholland et al. 1991; Steinman et al. 1991) and in a woodland stream (Mulholland et al. 1985). Nutrient cycling may have improved the nutrient regime allowing chlorophytes (*Zygnema* sp.) to become co-dominant in control troughs for the first time since experiment initiation. Alternatively, the opposite mechanism may have prevailed wherein a reduction in heterotrophic activity may have decreased competition for the available phosphorus supply between algae and bacteria. This mechanism borrows from

examples in the pollution literature where decreased bacterial activity in acidified systems resulted in a shift in community structure (Planas 1996). A decrease in microheterotrophic activity under lower pH was linked to a buildup of Zygnemataceae due to the greater availability of P for algal growth (Stokes 1986 cited in Planas 1996). A shift in the balance between autotrophic and heterotrophic processes (i.e. higher growth, lower decompostion rates) in the latter stages of the present study may have reduced the competitive interaction between microbial and algal groups. It remains unclear however, why *Zygnema* sp. was so highly favoured among the chlorophyte community.

4.2.3 Nutrient concentration and ratio effects

The results of low-level phosphorus addition in the present study are consistent with the findings of P-limited studies in coastal and interior British Columbia streams: exponential growth of periphyton communities is possible with as little as 1 $\mu g \cdot L^{-1}$ above background (Bothwell 1988, 1989; Quamme 1994). The important distinction in this study however, is that differences in algal response were related to specific N:P ratios. The contribution of nitrogen to algal dynamics was particularly important in light of nitrogen limitation in the Slocan River under late summer conditions. Differences in algal dynamics between the 1:1 and 4:1 treatment ratios were most striking in relation to biomass and density and less revealing in terms of species composition and taxonomic richness. Up to a doubling of algal biomass and cell density was observed between the 1:1 and 4:1 ratios with larger values noted within the 4:1 treatment group which further emphasize the relative importance of nitrogen. The dominance of chlorophytes across treatment groups however, was inconsistent with the results of several related mesocosm studies where diatoms always predominated (Stockner and Shortreed 1978; Mundie et al. 1991; Perrin and Richardson 1998). Diatoms were always of secondary importance in treatment groups in the present study. While this outcome is not surprising in the higher N:P treatment concentrations, chlorophyte dominance within the 4:1 nutrient concentration is of particular interest in that the relatively low ratio of N:P should have favoured diatoms (Rhee and Gotham 1980; Sommer 1988). A further distinction occurred within the 1:1 nutrient concentration where cyanophytes, under a higher P concentration and suspected N-fixing capability, became abundant. The development of a cyanobacterial bloom under a low N:P ratio is consistent with the prediction of Stockner and Shortreed (1978) that suggested blue-green dominance at N:P ratios <5:1. In terms of species richness, there was little difference between control and treatments although a slight increase in the number of species was evident over the duration of the experiment. The maintenance of high species diversity between control and treatment groups was again consistent with related studies (Stockner and Shortreed 1978; Lohman et al. 1991; Peterson et al. 1993).

Given that higher nitrogen values are requisite to the selection of the most suitable nutrient supply ratio for the Slocan River, the 4:1 treatment group is favoured over its counterpart. The choice of the more desirable nutrient ratio within the 4:1 treatment group however, is less clear because benthic algal biomass and growth rate are not as well correlated with nutrient levels in lotic environments as they are with phytoplankton in lentic environments (Borchardt 1996). The relationship with nutrients and attached algae is often confounded by the effects of light, disturbance and grazing (Cattaneo 1987 cited in Borchardt 1996). Light intensity was not expected to have a growth-limiting effect on periphyton owing to the nature of the open canopy of the Slocan River at Passmore, B.C. Disturbance and grazing however, were likely two important mechanisms that strongly influenced either chlorophyll *a* biomass on styrofoam plates or algal cell density on gravel substrates. The degree of disturbance caused by re-positioning of the mesocosm and the effects of induced sloughing on day 55 likely interrupted algal community dynamics and influenced algal cell density estimates on day 57. Moreover, the degree to which grazing reduced algal cell density and influenced estimates at each of the three sampling periods remains unknown. Nevertheless, the effect of grazing on algal biomass reduction is highly suspected. To this end, the simple comparison of algal cell density across treatments at specific intervals is not considered fair evaluation of treatment effects on areal-specific productivity in light of the above-mentioned abiotic and biotic factors. Similarly, the small differences in species composition and taxonomic richness between nutrient ratios of the 4:1 treatment group make the choice equally difficult. It seems apparent that larger differences in algal biomass among the 4:1 treatment group may have been precluded due to insufficient levels of nitrogen (i.e. algal growth dynamics remained N-limited). The selection of the more appropriate nutrient ratio should therefore consider treatment effects associated with higher trophic levels.

Finally, the majority of nutrient enrichment studies previously reviewed have alluded to the importance of an improved diatom assemblage based on their importance in the insect diet. Yet, nutrient concentration and ratios within the present study favoured the development of chlorophytes. Since the intent of nutrient manipulation is to improve both algal food quantity and quality, these objectives were likely achieved with the transition to chlorophyte species. For example, in a limited gut content analysis of insect larvae, Mundie et al. (1991) found a higher proportion of filamentous algae than diatoms despite the fact that diatoms predominated in both control and experimental troughs. Although a gut analysis was not performed in the present study, the contribution of colonial and filamentous green algae to insect growth was likely substantial in view of their dominance in treatment groups throughout the experiment and in consideration of the significant differences in insect size between control (diatom-dominated) and treatment (chlorophyte-dominated) groups. This conjecture in no way precludes the

importance of diatoms in the diet of insects or suggests that diatoms did not contribute to insect growth but merely emphasizes the potential value of green algae in providing a nutritional and edible food source. At the same time, it is also recognized that differences in insect growth between control and treatment groups may have been more closely aligned with density-dependent effects wherein herbivore growth under background conditions was likely resource-limited (Hart and Robinson 1990). Colonial chlorophytes have been shown to be highly nutritious based on protein and lipid content whereas filamentous forms are considered nutritionally poor due to low protein and lipid content and high ash (Lamberti 1996). These latter characteristics are also applicable to diatoms yet a lower nutritive value may be unimportant where higher rates of consumption and assimilation prevail (Lamberti 1996). These same caveats may hold for filamentous forms as well.

4.3 Nutrient treatment effects on benthic invertebrates

Benthic insects followed similar community dynamics displayed by periphyton albeit at different time intervals and levels of response. Up to 3-fold differences in insect density (refer to Fig. 25) and up to 3-fold differences in taxon-specific biomass (e.g. chironomid larvae; Fig. 33) between control and treatment groups were within the range of observations of similar nutrient manipulation studies (Hart and Robinson 1990; Johnston et al. 1990; Mundie et al; 1991; reviewed in Slaney and Ward 1993; Perrin and Richardson 1998). Consistent with short term mesocosm studies, dominant benthic insects were comprised of both baetid mayflies and chironomid midges (Mundie et al. 1991; Perrin and Richardson 1998). Sharing similar functional feeding groups, wherein baetid mayflies have been described as collector-gatherers and scrapers and chironomids as collector-gatherers (Merritt and Cummins 1984), both groups are highly dependent upon algae or algal-derived detritus as their principal food source (Lamberti and Moore 1984). The positive responses of mayflies, chironomid midges, and other benthic insects were likely related to the higher abundance of algal resources due to nutrient augmentation. Positive responses included lower emigration, lower per capita drift rate, higher benthic insect density and biomass, and greater insect growth of treatment groups compared to controls.

Colonization patterns of stream troughs were consistent with patterns associated with new or recently disturbed stream channels. The early arrival of baetid mayflies and chironomid midges was likely associated with their ability to browse fine epilithic detritus (reviewed in Mackay 1992). The influence of trough substrate particle size on taxonomic composition (and ultimately density) however, cannot be discounted. The wider size distribution of natural stream sediments has been shown to provide shelter for a greater variety of insect functional feeding groups as well as

effect a higher accumulation of fine particulate organic matter ((FPOM); Shaw and Minshall 1980; Culp et al. 1983) The larger interstitial area provided by a more uniform size of gravel in this study may have biased the colonizing fauna. The provision of high interstitial space was intentional however, because Hart (1979) and Reice (1980) suggested that high surface area within the substrate is a requisite of stream invertebrate community diversity (cited in Peckarsky 1983). The higher surface area was also intended to provide suitable habitat for stream periphyton. In the end, the deposition of river-supplied suspended sediment (fine sand) likely created an environment more similar to the streambed of the Slocan River. Alternatively, the nature of the narrow stream troughs within the mesocosm were more typical of riffle habitat which may have selected for riffle-specific species (Mundie et al. 1991) and influenced taxonomic composition.

4.3.1 Algal-insect interaction

Support for bottom up control of benthic insects was conveyed through two lines of experimental evidence. First, differences in amplitude of the initial drift cycle followed by a week long delay in periodicity in later drift cycles suggest differences in resource abundance in the 1:1 and 4:1 treatment groups that favoured increased colonization; a process mediated through reduced emigration (Richardson 1991). A highly significant reduction in drift in response to higher food abundance has been demonstrated experimentally for *Baetis* nymphs (Kohler 1985). Similarly, improved larval survival has been documented in autotrophically-driven systems (Hart and Robinson 1990; Mundie et al 1991) and heterotrophically-driven systems subject to an enhanced food supply (Richardson and Neill 1991). In the present study, variations in food supply among control and treatment groups were reflected in differences in total insect per capita drift rates. The lower per capita drift rate in treatment groups on day 39 (refer to Fig. 55) loosely coincided with a higher algal density on day 35 (refer to Fig. 73). Only baetid mayflies followed this pattern however (refer to Fig. 61). Statistically significant differences in per capita drift rate between control and treatment groups for chironomids were not evident on days 39 or 67, although extremely low per capita drift rates suggested high colonization over the same period (refer to Fig. 62). Differences were also non-significant for baetid mayflies on day 67 but a higher level of colonization was evident in treatment groups. The lack of simultaneous sampling of both algal and insect density prevents direct correlation however, oscillations in per capita drift should lag behind oscillations in food supply. This relationship was at least apparent mid-way through the present study. Like algal dynamics discussed in the previous section, differences between treatments were independent of trough flow variation over the period of study.

The magnitude of the drift during the third cycle may have been overshadowed by a behavioural response to declining seasonal temperatures that accompanied higher run-off due to local storms in late September (refer to Fig. 5). Elsewhere experimental increases in streamflow during autumn caused active entry of chironomid larvae into the drift in a regulated river (Poff and Ward 1991). It is possible that chironomid larvae leaving the mesocosm were responding to extrinsic factors resulting in movement to over-wintering habitats that can often include the hyporheic zone (Williams and Feltmate 1992). Beyond density-independent events, drift has been described as a mechanism to relocate organisms from unsuitable to suitable regions of the stream environment as an integral part of their benthic search behaviour for food and shelter (Ciborowski 1987). Active abandonment of the feeding site has been linked to perception of habitat quality that falls below some threshold level (Kohler 1985). The progressive increase in amplitude of the drift cycles remains equivocal whether a reduction in patch quality or environmental factors triggered the larger emigration during the third cycle or the increase in total insect per capita drift rate at the end of the experiment.

Second, while an increase in intra- and interspecific competition for food and space leading to density-dependent drift over time is likely (reviewed in Mackay 1992), the higher algal biomass associated with treatment groups did support a significantly higher interim (September 14; refer to Figs 32 and 33) density and biomass of mayflies and chironomids. Lower total insect and taxa-specific per capita drift rates in treatment groups were further testimony to increasing numbers of insects supported in the benthos. Owing to the lack of information beyond the two intervals of benthic density estimation (day 39 and 67) however, it is difficult to assess whether per capita drift rates responded in a similar manner as total drift (i.e. detection of a grazing cycle that resulted in a redistribution of animals following an algal decline).

Higher insect densities were only detected after a period of time following the initial peak in algal biomass. This same time lag effect however, has been observed in the Keogh and Nechako rivers and was due to differences in the rate of change in density and biomass between insects and periphyton (Perrin and Richardson 1998). The slower rate of change among insects explains the response time differential between periphyton and insects (estimated between 3 and 4 weeks in the present study; refer to Fig. 75) that accounted for the delay in grazer control of algal biomass. In more northern latitudes (e.g. Kuparak River), a period of up to two years has been observed prior to intermediate regulation of the periphyton community (Peterson et al. 1993). Hence, the faster turn-over rate of algae compared to the slower turn-over rate in insects further explains why an apparent small biomass of periphyton can support a relatively large biomass of consumer organisms (McIntyre 1973; Lamberti and Resh 1983). Nevertheless, linkages between whole-river and mesocosm studies are not directly comparable where

numerical responses are driven by two different mechanisms. At the whole-river scale, numerical responses are a function of reproduction and occur over a longer duration whereas at the mesocosm scale, numerical responses are directly related to rates of immigration and emigration and generally occur more rapidly (W.E. Neill pers. comm.).

A more plausible explanation of why insects do not colonize at rates equal to algae is related to the sequence of events surrounding colonization of bare substrates. The period of time required to establish a stable insect population has been related to the timing of substrate conditioning whereby changes in epilithic texture (bacterial and algal) or interstial accumulation (FPOM) create attractive sites that are recognizable by colonizers (Rosenberg and Resh 1982). The period of colonization has been described as early as 4-6 d in some experiments but more often it occurs over 10-25 d (reported in Mackay 1992). Timing differences between early (scrapers) and late (collector-gatherers) colonizers are therefore related to their individual abilities to perceive suitably conditioned surfaces. For some species, substrate conditioning that changes the texture or irregularity of the substrate surface may be equally important as a site for organism attachment (reported in Mackay 1992).

4.3.2 Insect body size

Differences in insect body size between control and treatment groups were demonstrated in baetid mayflies in the drift as well as baetid mayflies, chironomids, and perlodid and chloroperlid stoneflies in the final benthos. Similar results have been demonstrated in related nutrient enrichment (Peterson et al. 1985; Hershey et al. 1988; Hart and Robinson 1990) and food-supplemented studies (Richardson and Neill 1991). Non-significant differences in size however, have also been shown (Mundie et al. 1991). Since aquatic insects display resource-limited, density-dependent growth (Lamberti 1996), particularly r-selected species characteristic of small size, short generation times and simple life cycles (Williams and Feltmate 1992), the larger sizes of insects produced under nutrient amended conditions likely occurred at densities less than carrying capacity. As a consequence, increased growth would be expected when coupled with an increase in the quantity of high quality food (Hart and Robinson 1990) leading to higher per capita food availability. Due to the dynamics (turnover) of individual populations, this condition likely persisted throughout the duration of the experiment wherein r-selected benthic species never became strongly resource-limited.

Contrary to these results, hydroptilid and lepidostomatid (G.G. Oliver, pers. obs.) caddisflies did not respond to higher food availability. Non-significant differences in size between control and treatment groups may have been related to functional group type and density or differences in food quantity or quality. These latter conditions may be more applicable to lepidostomatid

caddisflies feeding on detrital materials because differences in nutritive and caloric content are generally lower for dead matter (Lamberti and Moore 1984). The lower quality of bacterially-conditioned detritus under declining stream temperatures would support the case for lower growth rate compared to species feeding directly on living algae (Sweeney 1984) although it has been shown that many shredders experience their major growth period in late autumn and winter in concert with leaf litter supply (Anderson and Cummins 1979). A numerical response leading to density-dependent suppression of growth or a short period of exposure to treatment effects may be the more important factors controlling growth of late colonizers under mesocosm conditions.

The most important aspect of growth differences in the final benthos however, is the difference in the overall size composition of the various taxa. The higher frequency of smaller individuals indicates a higher colonization of smaller instars that ultimately contributed to differences in the numerical response between control and treatment groups in the final benthos (September 30; refer to Figs. 39-46). The contribution of smaller sized organisms to the total biomass may have also been partly responsible for the non-significant difference in biomass between control and treatment groups.

4.3.3 Predator effects

Albeit that improved growth was observed among stonefly families, there is no evidence to suggest "top down" control of benthic insects by stonefly predators over the duration of the experiment. The fact that dominant predators tend to immigrate later than initial colonizers (Shaw and Minshall 1980; Ciborowski and Clifford 1984; Peckarsky 1986; Mackay 1992) in combination with the low relative abundance of predatory stoneflies across control and treatment troughs, may have reduced interactions between predators and lower trophic levels (i.e. prey species). The duration (83 d) and seasonality (late summer/early fall) of the present study are likely factors affecting colonization dynamics of predators as well. For example, recovery of a newly constructed stream channel in Wyoming, with macroinvertebrate densities similar to those encountered in upstream areas, was achieved over 70 d however, a similar species richness was not observed until approximately 300 d (Gore 1982). In an Idaho stream, numerical recovery and species richness of a previously dewatered channel was achieved over 375 and 700 d, respectively (Minshall et al. 1983). Moreover, colonization appeared to be more rapid in an Alberta stream during spring than in late summer although species at different life history stages precluded direct comparison (Ciborowski and Clifford 1984). In regard to the present study, densities of stoneflies in the benthos at the end of the experiment were similar for both control and treatment groups. Despite differences in stonefly numbers in the benthos

across experimental units on day 39, the larger density in the 12:3 concentration was due to the presence of pteronarcyids (shredders-detritivores).

Whether direct interaction between stoneflies and chironomids and mayflies was responsible for prey dispersal remains undetermined. Kohler (1985) provided two central reasons for active entry of baetid mayflies into the drift: 1) relationship between food availability and demand for food and 2) interaction with predators. The former mechanism seems more appropriate in the present study. Predator interactions however, could have contributed to baetid entry into the drift as a result of a behavioural avoidance (Williams and Feltmate 1992). Peckarsky (1983) provided a model for stream invertebrate community structure that suggested under low predation pressure, competition for available food and shelter was a stronger determinant of prey numbers. Competitive interaction rather than predation again seems more appropriate in this experiment where an increase in per capita drift rate of chironomids was countered by a decrease in per capita drift rate of mayflies. Notwithstanding, it remains uncertain whether the numerical response by mayflies at the end of the study can be considered cause and effect or merely opportunistic.

The short duration of the experiment was likely insufficient to enable predators to build to levels capable of reducing prey numbers. These results are consistent with another food-supplemented, short-term experimental manipulation study (Richardson 1991). Alternatively, high prey exchange rates (i.e. high immigration and emigration) have been linked to a low magnitude of predator effects (Cooper et al. 1990) and the high gross turnover of prey species during initial colonization (up to day 55) and following re-positioning of the mesocosm (differences in flow after day 55) may have affected the rate of predator colonization as well. The higher outmigration towards the end of the experiment may have been coupled with chironomid dispersal (i.e. prey base) or strictly related to extrinsic factors causing dispersal to over-wintering sites.

4.3.4 Nutrient concentration and ratio effects

Dominance of the species composition by chironomid midges and baetid mayflies across control and treatment groups, was likely influenced by the characteristics of a superior colonizer (small body size, semelparity, and short life span; reviewed in Mackay 1992) and the dynamics of short term mesocosm experiments that lack temporal and spatial heterogeneity (Levin et al 1989). Moreover, the observed changes in species composition were likely affected by induced abiotic factors (i.e. increased velocity due to re-positioning of the mesocosm) as much as direct biotic interaction. Differences in current velocity over the latter one-half of the experiment likely favoured development of the mayflies. Notwithstanding these inherent difficulties, a positive

response of benthic insects to treatments was observed and those taxa present in troughs were similar in diversity to taxa collected from *in situ* basket samplers at Passmore, B.C. (BC Environment, Fisheries Branch, Nelson, B.C., file data). While chironomid midges and baetid mayflies were a dominant feature of the taxonomic structure, caddisflies and to a lesser extent, stoneflies demonstrated a numerical response within the 1:1 and 4:1 treatment groups. Although differences were not so dramatic as those typified by periphyton, the 4:1 treatment group displayed a greater response than the 1:1 treatment group. Within the 1:1 treatment group however, numerical response was greatest in the 5:5 nutrient concentration. Determination of the best response among treatments within the 4:1 treatment group, is equally difficult as it was for periphyton, based on differences in response variables at different time intervals. In consideration of the higher taxonomic richness, higher interim benthic insect biomass, and higher insect biomass contributed through the drift however, the 4:1 nutrient concentration appears to have provided an optimum overall response.

Finally, the notable absence of insect emergence during this experiment is not surprising in light of the time span tested (i.e. 9 weeks), the univoltine or bivoltine nature of the colonizing insects and small size of initial colonizers. Since the life cycles of most animals are geared to seasonal differences in photoperiod and temperature (Sweeney 1984; Williams and Feltmate 1992), timing of the experiment and stage of development of juvenile insects likely precluded an emergence response. Although adult mayflies and caddisflies were observed in the drift, their low numbers were likely indicative of only background drift in the river. The low number of adults bore no relation to the high number of insects observed in the benthos. These results were particularly evident despite the higher abundance of high quality food that clearly improved growth rate. The short duration and seasonal timing of the experiment likely affected complete development (i.e. voltinism). Differences in larval weight gain have been highly correlated with differences in food quality (Fuller and Mackay 1981). Similarly, food quality has been shown to override the metabolic effects of temperature (Scriber and Slansky 1981) and variation in food supply has also been shown to alter generation time (Anderson and Cummins 1979). Nevertheless, Mundie et al. (1991) have shown that the proportion of benthic insects nearing emergence constitutes a very small fraction of the total benthos and that an initial emergence response to fertilization should not be detectable until after one month of treatment. It is therefore possible that due to the temporal limitations of this study, early colonizers represented by early instars had insufficient time to reach pupation or a high turn-over rate (low residence time) of some species precluded an emergent response. This aspect was particularly evident among chironomids based on the low incidence of individuals showing the pre-pupal "hump" (Merritt and Cummins 1984) within benthos samples. The overriding effects of increased flow and declining water temperature in September may have further pre-empted ontogenic

processes or triggered an outmigration to secure more suitable habitat prior to the onset of winter. The combination of increased emigration in the third drift cycle and closer overlap in mean size of individuals between control and treatment groups in the final benthos provide supportive lines of evidence.

4.4 Trophic interactions

While trophic interactions between microbial and algal communities remain speculative, interactions between algal and insect communities in the present study seem clearer. Differences in algal density, insect drift rate and benthic insect density and growth between control and treatment groups suggest "bottom up" control of benthic insects due to differences in resource abundance. Yet, over the course of the experiment insect abundance also appears to have reduced areal algal biomass. Direct experimental manipulation of both nutrients and grazers by Steinman et al. (1991) and Rosemond et al. (1993) implies that areal-specific algal productivity is primarily regulated by abiotic factors (nutrients, light, temperature and disturbance) but grazing imparts secondary control over algal biomass (Lamberti 1996). This growing body of evidence has demonstrated dual control of algae by biotic and abiotic factors, and "intermediate regulation" as proposed by Lamberti (1996) seems more appropriate. Lamberti suggests that dominant consumers intermediate in the food web have far greater control than top predators in regulating food web structure. While this linkage was most evident in the present study, both spatial and temporal limitations of the mesocosm must be considered in terms of the longer-term production benefits and biotic interactions observed at the whole-river scale. On the basis of the mesocosm results, there is little doubt that within-year responses of benthic algae to fertilization are directly beneficial to proximate trophic levels.

4.5 Recommended whole stream nutrient application for the Slocan River

The importance of autochthonous energy pathways to food webs in stream environments (Minshall 1978) has been widely acknowledged over the past two decades based on experimental evidence (Stockner and Shortreed 1978; Lamberti and Resh 1983; Perrin et al. 1987; Bothwell 1988, 1989; Hart and Robinson 1990; Johnston et al. 1990; Mundie et al. 1991; Peterson et al. 1993; Perrin and Richardson 1998). Within temperate streams in North America, the importance of autotrophic production has been shown to increase with stream order as a function of decreasing canopy closure and increasing light availability (Vannote et al. 1980). The importance of autotrophy to middle order streams such as the Slocan River is crucial, since up to 80% of the energy budget in 5th order stream ecosystems has been attributed to primary

production (Lamberti 1996). Experimental manipulation of streams by nutrient enrichment has provided further evidence in support of autochthonous energy transfer to higher trophic levels (Slaney et al. 1986; Johnston et al. 1990; Deegan and Peterson 1992).

The importance of salmon carcasses as a source of marine-derived carbon and nutrients in terrestrial and freshwater ecosystems has been recently demonstrated in the literature (Willson and Halupka 1995; Bilby et al. 1996). Given historical salmon spawning escapements in the Slocan River, a higher level of productivity at four trophic levels (bacterial, algal, insect and fish) would have been expected prior to 1936 (Grand Coulee Dam) than is presently available. Bacterial decomposition of salmon carcasses and remineralization of nitrogen and phosphorus would have provided an exogenous supply of nutrient for direct algal uptake or by later spiralling through the meiofaunal community. The importance of the "microbial loop" as an integral component of nutrient cycling has been demonstrated in lakes (Weisse and Stockner 1992 reviewed in Ashley and Slaney 1997) and may provide a similar role in streams. Energy transfer through the algal-insect-fish food chain would have been expected as a consequence of nutrient supplementation. The omnivorous behaviour of aquatic insects (Merritt and Cummins 1984) suggests that certain feeding groups probably benefited from direct consumption of decaying carcasses as well. Similarly, the downstream release of eggs from redd sites during salmon spawning would have provided resident fish populations with an annual source of high energy food. The Slocan River therefore represents a unique opportunity in southeastern British Columbia to demonstrate restoration of historic levels of productivity through nutrient augmentation. Unlike coastal systems, the Slocan River, due to its low annual streamflow variation, is a highly stable system (Oliver 1997). As a result, numerical responses of long-lived univoltine insects in the first year of fertilization are anticipated to carry-over and contribute to a higher level of productivity in consecutive years. This same benefit is often precluded in coastal environments due to annual winter floods that often scour streambeds and displace macroinvertebrates if suitable refugia are not available. Annual disturbances such as spring freshet that "reset" food webs in streams (Lamberti 1996) are not expected to have the same negative influence in this lake-headed system. This same level of stability is expected to minimize the effect of flow extremes on periphyton assemblages as well.

On the basis of the experimental evidence in temperate streams (Mundie et al. 1983; Johnston et al. 1990), the use of inorganic nitrogen and phosphorus seems an obvious choice to stimulate primary productivity and speed ecosystem recovery. Selection of the best nutrient ratio however, is predicated on a number of criteria based on bio-assay results that can be applied to the whole-river environment. The more desirable nutrient ratio should provide an algal community with the following inherent characteristics: a high species diversity, high quality,

edible forms, and a high biomass-specific growth rate. Subject to grazing, the periphyton assemblage should allow a low, stable biomass to accrue capable of sustaining a larger, diverse consumer community. The 1:1 and 3:3 nutrient concentrations failed to provide adequate nitrogen. A comparison of the 5:5 nutrient concentration and treatment ratios within the 4:1 treatment group all met with variable levels of success. In consideration of the above four criteria however, satisfactory primary and secondary responses showed the highest consistency in the 4:1 nutrient concentration. Application of the 4N:1P ratio at the whole-river scale is therefore recommended to meet late summer nutrient limitations, stimulate primary productivity and increase invertebrate biomass. Selection of this treatment ratio meets a primary concern of any stream fertilization program which is considered critical to achieving fisheries management objectives: maintenance of low algal biomass that contributes to higher insect biomass without creating a nuisance algal condition. The development of nuisance algae has been associated with nutrient enrichment leading to a maximum periphyton biomass of 100 -150 $mg \cdot m^{-2}$ chl *a* (Horner et al. 1983). Application of the 4:1 ratio is expected to sustain low algal biomass (in the presence of insect grazing) at a level less than the 50 $mg \cdot m^{-2}$ chl *a* guideline for recreational use on behalf of BC Environment (Nordin 1985). The more conservative approach is recommended in light of present water-based recreational use of the Slocan River and prevailing public attitudes.

Calculations to estimate the concentration of nutrients for whole-stream application (Ashley and Slaney 1997) were based on the recommended 4:1 ratio over a 60 d application period (mid-July to mid-September) and commercial grade fertilizer formulations of 10-34-0 and 28-0-0. Applications to support the total annual liquid fertilizer requirement for the Slocan River are estimated at approximately 1150 kg of phosphorus and 5200 kg of nitrogen. The current price of $385 per metric ton of phosphorus and $228 per metric ton of nitrogen places annual liquid fertilizer costs at approximately $1650 per year, not including transportation, capital costs of dispensing equipment or dispensing fees. The more desirable approach would make use of slow release briquettes described in Ashley and Slaney (1997). Since manufacturing costs of special orders are expensive at this time, pelletized slow release fertilizer to meet Slocan River requirements would likely range from $10,000 to $13,000 per year.

While fertilization efforts are geared to elevate overall ecosystem productivity, anticipated benefits are expected to target the resident rainbow trout population. The provision of higher benthic invertebrate densities and invertebrate drift densities are expected to improve growth and survival of adult and juvenile salmonids (Wilzbach 1986; Johnston et al. 1990; Paul et al. 1996 reviewed in Ashley and Slaney 1997). Therefore, in order to realize maximum benefits, fertilizer release sites should coincide with the more productive trout water. In particular, release

sites should correspond with mainstem channel locations noted for high juvenile rearing. These areas will likely include mainstem areas immediately downstream of suspected spawning and rearing areas in mainstem or tributary habitats. Recommended candidate sites include mainstem areas downstream of Winlaw Creek and the Little Slocan River based on the results of rainbow trout population surveys conducted during the early 1990's (Oliver 1996). Since fertilizer costs completed in the above were estimated using discharge measurements associated with the lower basin (Crescent Valley, B.C.), additional cost savings will be expected for release sites having lower drainage basin area and hence, lower flow.

Finally, the Slocan River basin has witnessed a variety of environmental impacts that have no doubt affected ecosystem productivity. The loss of salmon spawning escapements have likely reduced the nutrient status of the river while historic log driving activities and more recent private and industrial activities have reduced habitat complexity. In view of these cumulative impacts, nutrient augmentation, in isolation of habitat improvement, cannot be expected to restore resident fish populations to historic levels. Examples of well-intended manipulations exist, where higher summer fish production was lost due to a paucity of suitable over-wintering habitat (Murphy and Meehan 1991). While fertilization holds great promise, concurrent improvements in habitat to meet all life history needs are considered critical.

4.6 Mesocosm limitations

As previously outlined, stream mesocosms have been criticized for their lack of realism due to differences in spatial and temporal heterogeneity between stream environments and their natural processes and artificial stream troughs and their experimental intent. Specifically, mesocosms lack habitat diversity, operate over short time scales and depending upon water intake designs, may or may not experience diel flow variation. The flow-through design employed in the present study functions as a close parallel to the open stream system whereby an exchange of nutrients, organic materials and immigration and emigration processes of organisms is permitted (Richardson and Perrin 1990). Mesocosms therefore provide an inexpensive means of evaluating system perturbations. The prohibitive costs of replicated channels, logistical difficulties and long-term committment of whole-stream manipulations often preclude their consideration as a reasonable alternative. Despite these limitations, experimental manipulations employing the mesocosm approach provide insight into ecological processes, permit an understanding of biological outcomes relative to the type of perturbation invoked and avoid costly mistakes due to the uncertainty of outcomes at the whole-stream scale. Mesocosm studies therefore offer scientists or resource managers an opportunity to test actual effects of a specific perturbation on biological communities and predict their outcome with reasonable

accuracy.

Operation of an in-stream mesocosm however, is not without its complications, particularly where maintenance of a uniform flow to facilitate a constant nutrient concentration is interrupted by diel variation in water level. There is little question that increased flow variability during the latter one-third of the experiment contributed to higher nutrient variability and may explain some of the differences in response variables between replicates. Future use of this type of installation would benefit from a sub-surface, piped intake. Attachment of a 75 mm dia pipe to the influent end of each trough, at a 45° angle and to a depth of 30 cm below surface, would likely provide adequate and more even flow to the mesocosm. It would also reduce flow restrictions attributed to the accumulation of deciduous leaf and conifer needle litter during autumn.

4.7 Possible sources of error

Storage of drift and benthic insect samples in 70% ethanol resulted in partial dissolving of internal organs of some insects (particularly mayflies). Individual weights were less than actual fresh dried weights and therefore, reporting of total biomass is not entirely accurate. Since the problem was consistent across all samples, the adjusted weights were not assumed to affect relative differences in biomass between control and treatment groups. While non-significant differences in biomass between control and treatments were observed in this study, differences were considered to have been more strongly influenced by changes in taxa than differences in true weight. Notwithstanding these assumptions, the lack of absolute weight contrasts cannot be discounted relative to the outcome of statistical testing. Exoskeletons appeared unaffected however, and measurements of head capsule width are not considered biased. In future, initial fixation in a 5% solution of formaldehyde is recommended prior to final storage in ethanol.

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