University of Alberta

EFFECTS OF LOW-LEVEL EUTROPHICATION ON ECOLOGICAL INTEGRITY OF RIVERS IN THE ROCKY MOUNTAIN NATIONAL PARKS OF CANADA

by

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Abstract

Nutrient availability has long been considered one of the most important factors regulating production of benthic algae in oligotrophic rivers yet empirical models relating benthic algal abundance to nutrient availability have rarely yielded accurate predictions. The aim of this research was to improve the ability to predict patterns of benthic algal abundance in oligotrophic rivers by improving the understanding of processes that regulate abundance. *In situ* experiments combined with surveys of physical, chemical and biological attributes of oligotrophic mountain rivers confirmed that small amounts of anthropogenic phosphorus (P, 0.1-5.6μg/L total phosphorus) adversely impacted the ecological integrity of Rocky Mountain Rivers. Downstream of municipal wastewater treatment plants (MWWTPs), abundance of benthic algae and benthic macroinvertebrates (BMIs) increased by 4- to 30-fold. In sites upstream of MWWTPs, benthic communities were dominated by thin diatom films and mayfly scrapers but downstream abundance of diatoms and chironomids increased. Epilithic abundance was proximately controlled by P availability if light was not limiting but ultimately controlled by variation in temperature and river discharge, and was highest downstream of MWWTPs in autumn. However, at a given P concentration, there were greater increases in abundance of benthic biota downstream of MWWTPs than upstream. The variable response of benthic biota was attributed to high P bioavailability in wastewater effluent. Upstream of MWWTPs, algal accrual was limited by the availability of P, and organisms in the epilithon could cleave P with metabolically expensive enzymes. Downstream, P limitation of epilithon decreased or was eliminated, epilithic organisms did not produce phosphatase enzymes, metabolic costs decreased and therefore, more algal growth per unit P occurred. As a consequence of decreased P limitation of benthic algae

downstream of MWWTPs, P content of epilithon increased. Increased abundance of BMIs downstream of MWWTPs was attributed to improved food quality as a result of increased epilithic P content rather than increased food quantity. Measuring the degree of both epilithic nutrient limitation and producer-consumer nutrient imbalance will improve the ability to predict the magnitude of effects from eutrophication of oligotrophic rivers, and will help elucidate the causes and consequences of various patterns and processes.

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1.0 General Introduction: Effects of low-level eutrophication on the ecological integrity of rivers in Rocky Mountain National Parks of Canada

National Parks in Canada were established to protect and present outstanding representative examples of natural landscapes and natural phenomena that occur in Canada's natural regions (Parks Canada 2003). Banff National Park, established after railway workers found a cave containing hot springs in 1883, was Canada's first national park and the world's third. Banff, Jasper, Kootenay and Yoho National Parks and the British Columbia provincial parks of Hamber, Mt. Assiniboine and Mt. Robson collectively form the Rocky Mountain Parks World Heritage Site, one of the largest protected areas in the world.

The Canada National Parks Act (October 2000) states that the maintenance or restoration of ecological integrity through the protection of natural resources and natural processes, shall be the first priority when considering all aspects of park management. In the Act, an ecosystem with ecological integrity is defined as *a condition that is determined to be characteristic of its natural region and likely to persist, and includes abiotic components and the composition and abundance of native species and biological communities, rates of change and supporting processes.* Towns within national parks must provide a strategy for the management of growth within the park community that is consistent with the principles of no net negative environmental impact, and also practice responsible environmental stewardship and heritage conservation. Although measures to minimize the population growth of towns within the mountain national parks have been taken, urban development and tourism are difficult to regulate and continue to increase.

Wastewater management was one of eleven issues of concern highlighted in the framework developed to mitigate the effects of human use on environmental quality in National Parks (Parks Canada 2003). Eutrophication, the increase in algal or plant growth, and changes in species that follow addition of a limiting nutrient is of particular concern in nutrient poor ecosystems such as rivers and lakes in the Rocky Mountain National Parks. More phosphorus (P) in wastewater as a result of more people was identified as a likely threat to the ecological integrity of P-poor rivers in mountain national parks (Chambers 1996). Within the mountain national parks, four towns release wastewater to three rivers. Although the quantity of anthropogenic P released into mountain rivers is modest relative to release in larger urban areas, when P concentrations are naturally low, P often limits benthic algal growth and any available P is quickly assimilated into algal biomass (e.g., Bothwell 1985). Therefore, small additions of P may be scarcely detectable in P-poor rivers but can stimulate several-fold increases in benthic algal and consequently, invertebrate scraper abundances.

Low-level eutrophication of oligotrophic mountain rivers and its impacts on aquatic biota have been difficult to document because routine monitoring of most rivers is done primarily with measurements of chemical parameters. Earlier studies by Chambers et al. (1996) in Jasper National Park concluded small (ppb) increases in P concentrations in river water as a result of effluent discharge from the Jasper MWWTP caused nuisance levels of algal growth in the Athabasca River, and increased algal abundance would probably lead to increased densities of benthic macroinvertebrates (BMIs). If invertebrate consumers are food limited, grazing by BMIs can prevent algal accumulation and conceal the effects of eutrophication on benthic algae (e.g., Peterson et al. 1993). Low-level eutrophication of P-poor systems can also cause changes in species composition of primary producers and consumers (e.g. Bowden et al. 1994), and decrease species diversity and habitat complexity (e.g., Schindler et al. 1971). Therefore, it is necessary to consider trophic transfer of nutrients to assess the impact of low-level eutrophication on the ecological integrity of oligotrophic rivers.

To improve the understanding of stream processes that regulate abundance of stream benthos in oligotrophic rivers, we performed *in situ* experiments to determine nutrient status of algae and BMIs, and surveys of chemical, physical and biological attributes at sites upstream and downstream of each treatment plant in the mountain national parks. Based on the limited number of studies on low-level eutrophication in cold-water, mid-sized rivers, our general predictions were 1) added P would be rapidly assimilated into epilithon (autotrophs and bacteria attached to stone) at P-limited sites and consequently difficult to detect in river water, 2) bioavailable P from treatment plant discharges would reduce or eliminate epilithic P limitation, 3) the magnitude of response in algal abundance would be greater in oligotrophic rivers than in more nutrient-replete rivers, and 4) BMI abundance would be correlated with the gradient in anthropogenic P loading but grazing would diminish the response in benthic algae.

The main goals of the second thesis chapter were to define the eutrophication gradient, describe temporal and spatial patterns in algal abundance, and isolate the cause of any residual variation in algal abundance that could not be explained by the P gradient or scraper abundances among sites upstream and downstream of three MWWTPs. Identification of the primary factors that control algal abundance was used to modify sampling protocols and aid in interpretation of results of studies described in the subsequent two chapters. In chapter three, we confirmed that additional P in wastewater effluent was the cause of increased abundance of benthic organisms, characterized how the degree of epilithic P limitation changed along the eutrophication gradient, and determined the best way to measure changes in epilithic P limitation in mountain rivers. To better understand how anthropogenic nutrient additions elicit a bottom-up transfer of energy among trophic levels, we used a stoichiometric (mass balance of chemical elements) approach to study effects of changes in epilithic nutrient limitation on producer-consumer nutrient transfer in the mountain rivers, in chapter four. The concluding chapter summarizes how the degree of epilithic nutrient limitation, and changes in nutrient transfer between producers and consumers helps explain patterns in the abundance of benthic biota along natural and anthropogenic nutrient gradients in oligotrophic mountain rivers, and how this knowledge will improve the ability to mitigate the adverse effects of nutrient loading in cold-water rivers.

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2.0 Physical, chemical and biological constraints on benthic algal accrual in oligotrophic cold-water rivers impacted by anthropogenic nutrient enrichment

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Our aim was to examine the response of benthic algae in intrinsically oligotrophic rivers to natural and anthropogenic nutrient gradients. This study took advantage of a unique natural experimental design: three towns with varying amounts of waste and levels of wastewater treatment discharging varying volumes of wastewater into two mid-sized (4-5th order at 1:250000) rivers in the Rocky Mountain National Parks of Canada. Physical (turbidity, riparian shading, discharge, temperature, and velocity), chemical (soluble reactive phosphorus (SRP), total phosphorus (TP), dissolved inorganic nitrogen (DIN)) and biological (algal abundance, scraper abundance) parameters were measured upstream and downstream of nutrient point sources, in spring, summer and autumn for two years. This study design allowed us to test for variation in algal response to nutrient enrichment in oligotrophic systems, quantify the nutrient concentration associated with nuisance growths of algae in mountain rivers, and examine whether algal response to increasing nutrient concentrations in oligotrophic rivers was greater or more variable than the response in more nutrient replete systems. Quantification of the response of benthic algae along a nutrient gradient also allowed us to predict the response of algae to changes in nutrient loading that are occurring as a result of improved wastewater treatment and expanding population in the Rocky Mountain aquatic ecoregion.

Although many factors can regulate the primary productivity of streams, anthropogenic nutrient inputs are the most prevalent cause of impairment and often the most amenable to mitigation (Dodds and Welch 2000). Studies have shown that nutrient addition to streams, from point or non-point sources or experimental manipulation, can increase benthic algal abundance and affect productivity and composition of higher tropic levels (e.g., Stockner and Shortreed 1977, Mainstone and Parr 2002). Conversely, reductions in anthropogenic nutrient loading can lead to recovery of benthic stream communities (e.g., Sosiak 2002). Yet despite strong evidence of the importance of nutrient availability in controlling benthic algal abundance, simple causal relationships between nutrients and abundance have been difficult to establish for rivers (e.g. Biggs and Close 1989). Factors such as temperature (Welch et al. 1989), flow (Biggs and Close 1989), biomass accumulation period (Lohman et al. 1992), scrapers, shading (Welch et al. 1992), substrate size (Cattaneo 1997), penetration of ultraviolet light (Kelly et al. 2003), and algal community composition (Chételat et al. 1999) can modify the response of benthic algae to changes in riverine nutrient concentrations. In addition, nutrient concentrations in rivers are highly variable in space and time, are highly correlated, and can co-limit algal growth (Francoeur 2001). Empirical models relating benthic algal abundance to various forms of nutrients in streams are inadequate for general predictions (e.g., Bothwell 1989, Dodds et al. 1997, Welch et al. 1998, Scrimgeour and Chambers 2000, Dodds et al. 2002) since they are often specific to certain locations (Dodds et al. 1997, Biggs 2000). Over the range in nutrient concentrations representative of rivers throughout the world (e.g., 5μg/L<TP <1000μg/L), the relationship between total phosphorus (TP) and benthic chlorophyll is highly variable (four orders of magnitude).

The ability to predict variation in benthic algal responses to nutrient addition is more difficult in oligotrophic rivers. Dose-response studies (e.g., Bothwell 1989, Dodds 1997) have consistently shown that the relationship between in stream nutrient concentrations and algal abundance is curvilinear, with algal abundance exhibiting greatest change (three orders of magnitude) over the range in nutrient concentrations typical of oligotrophic rivers (i.e., < 30 μg/L TP or < 500 μg/L TN). Moreover, of the nutrient-chlorophyll models that have been developed for rivers, data are scarce for the most oligotrophic systems (TP<10μg/L, Dodds et al. 1997) making these models less reliable at low nutrient concentrations. Variability in nutrient-algal response to changing nutrient availability in oligotrophic systems may be affected by strong selective advantages for algal species that can fix nitrogen (N) in N-limiting conditions or cleave organically bound phosphorus (P) in P-limiting conditions (e.g., Jansson et al. 1988). In addition, tight nutrient spiraling weakens the relationship between water nutrient concentrations and algal abundance in oligotrophic systems (e.g., Paul et al. 1991). Therefore, the ability to predict the response of benthic algae to changes in nutrient availability in oligotrophic rivers is constrained by high inherent variability and lack of data.

2.1 METHODS

2.1.1. Site Description

Sampling sites (115° 35′ - 118 ° 05′ N, 51° 10′ - 53° 54′ E, 1000-1500m a.s.l.) were located in the Rocky Mountain National Parks of Alberta: the Bow River in Banff National Park, and the Athabasca River in Jasper National Park (Fig.2-1). The Bow River is one of the headwaters of the Nelson River, which discharges to Hudson Bay; and the Athabasca River is one of the headwaters of the Mackenzie River, which discharges to the Beaufort Sea. The rivers are glacially fed and flow through alpine, sub-alpine and montane terrestrial ecoregions within National Park boundaries (Holland and Coen 1983, Ricketts et al. 1999). Study sites were located in the lower sub-alpine (Lake Louise sites) and montane (Banff and Jasper sites) terrestrial ecoregions, and all sites were within the Rocky Mountain aquatic ecoregion (Abell et al. 2000). The Rocky Mountain ecoregion exhibits high aquatic endemism because parts remained unglaciated during the Wisconsin glaciation (Abell et al. 2000). Lodgepole Pine forests and eutric brunisols soils overlying calcareous rock dominate lower sub-alpine and montane regions. Mean annual air temperatures at the study sites range between 0-3°C, with monthly average temperature minimums of negative 10-15°C in January and maximums of 13-15°C in July (but instantaneous temperatures has ranged from –52-38°C, Abell et al. 2000). Average annual precipitation in the Rocky Mountain ecoregions is 465-665 mm, with approximately twice as much precipitation in winter as summer (Abell et al. 2000). Mean annual runoff is approximately 50% of mean annual precipitation.

Within the national parks, the Bow and Athabasca rivers have concave longitudinal profiles with gradients averaging 0.1-3.0 m/km, and sinuous, frequently braided river channels in wide valleys (Kellerhals et al. 1972). The mainstems of both rivers are unregulated but there are several tributaries to the Bow River that are regulated (Schindler 2000). The channel beds are composed of sand and gravel and the bank materials range from silt to boulders. On average, maximum annual discharge and turbidity (caused by abundant glacial flour) occur in July. Freeze up occurs in early to mid-November and break-up occurs in early to mid-April. Approximately 70% of mean annual river flow occurs between June and August. The study sites were located in unbraided, 4-5th order (at 1:250000 scale) river reaches within park boundaries.

Both rivers are intrinsically oligotrophic: average annual nutrient concentrations upstream of population centers are 1μg/L SRP, 4μg/L TP and 100μg/L TN in the Bow River, and 1μg/L SRP, 23μg/L TP and 130μg/L TN in the Athabasca River (Environment Canada historical data). Much of the TP in the rivers (particularly the Athabasca River) is associated with glacial flour in summer and thus is largely unavailable for use by biota (Chapter 3). During the study period, wastewater from the town of Jasper (population 4301 in 1996) was treated in aerated lagoons and discharged to the Athabasca River. The Bow River basin has two major population centres in the National Parks: Lake Louise (population 1305 in 1996) and, further downstream, Banff (population 6098 in 1996). During the study, the Lake Louise municipal wastewater treatment plant (MWWTP) was the only system that removed phosphorus from effluent (TP<0.5 mg/L via biological uptake and alum precipitation). Wastewater from Banff was treated with activated sludge and clarification processes and discharged to the Bow River, 60km downstream of the Lake Louise effluent discharge.

As a result of differences in natural nutrient loads, population, level of wastewater treatment and river discharge, there is a gradient in stream water nutrient concentrations among sampling sites (Table 2-1). Previous work (Scrimgeour and Chambers 1997) and our research (Chapter 3) indicate that algal growth in these rivers is controlled by P availability. The quantity of P added by the MWWTPs resulted in a gradient of total P concentrations in river water that varied considerably over time but during the principal growing season (August, September, October) was on average, least upstream and downstream of Lake Louise (3.9 and 7.5 μg/L, respectively) and progressively higher near Banff (8.9 and 14.4 μg/L, respectively) and Jasper (28 and 29 μg/L, respectively). Thus, wastewater discharges result in a TP gradient for testing the enrichment responses of epilithon in oligotrophic rivers.

2.1.2. Sample collection

The rivers were surveyed for physical characteristics (discharge, velocity, temperature and riparian cover), water chemistry and epiphyton and benthic macroinvertebrate (BMI) abundance at 2 sites upstream and 3-6 sites downstream of effluent discharges from the Lake Louise, Banff and Jasper MWWTPs in spring (late May-early June), late summer (August) and late autumn (late October) in 1998 and 1999. Samples were not collected in the Bow River in spring 1999. Sampling locations spanned 5km upstream and 15km downstream of MWWTPs (with the exception of sampling sites located up to 50km downstream of Lake Louise). Effluent plumes were by and large mixed in the rivers before the first downstream sampling locations, located 2-3 km downstream of each MWWTP. All sampling sites contained at least three, shallow (30cm), fast flowing (0.5m/s) riffles within a 500m reach, and substrata that were dominated by cobble (10-20cm diameter). A 1997 pilot survey confirmed that one replicate of physical and chemical parameters per site, and three replicates of biological parameters was adequate to reveal statistically significant upstream-downstream differences in algal abundance.

During each survey, physical and chemical parameters were measured once at each site. Temperature, dissolved oxygen, pH and conductivity were measured *in situ* with a Hydrolab meter (Model: Survey 3). Water samples were collected and analyzed for TP, SRP, nitrate+nitrite-N, ammonia-N and total dissolved N (TDN). Phosphorus concentrations were often near detection limits so to avoid contamination of samples, water samples (1L) were taken from every site before other sampling commenced. Samples were collected, while wearing rubber gloves, in a nalgene bottle that had been acid washed and rinsed seven times with double distilled water, and rinsed with river water three times *in situ*. Water samples contained no air, and caps were sealed tightly with electrical tape and kept cool and dark. River water samples were kept separate from MWWTP effluent samples, and all samples were processed within 24 hours of collection. Samples of MWWTP effluent were a 24-hour composite at Lake Louise and instantaneous grab samples at other MWWTPs. Samples were analyzed following modified standard methods (University of Alberta Limnology Laboratory). TP (unfiltered), TDP (filtered) (Prepas and Rigler 1982), and SRP (filtered) (Murphy and Riley 1962) were determined colorimetrically with a Varioan Cary 50 probe and a Milton Roy Model 1001 Plus Spectrophotometer. Ammonia-N (unfiltered) and nitrate+nitrite-N (filtered) were determined colorimetrically with a TechniconTM AutoanalyzerTM II using automated berthelot and cadmium reaction methods, respectively. Concentrations of ammonia-N and nitrate+nitrite-N were summed to give dissolved inorganic N (DIN).

Composite BMI and epiphyton samples were collected from three riffles at each site. To sample BMIs, a u-net was randomly placed over 10-20cm substrata at 30cm (±5cm) depth, and cobble within the sampling area were rubbed and removed. The remaining sand was disturbed for 20 seconds, and the net was held in place for an additional 20 seconds. For each riffle, the contents of three u-nets (250μm, $0.15m^2$) were combined and then preserved in 90% ethanol, for a total of three samples (1 per riffle) from each site. A random sub-sample of 300 organisms was identified to the lowest possible taxonomic level (generally species). Velocity was measured with a Pygmy current meter at the location where each u-net sample was taken, and riparian cover was measured using a Spherical Densiometer (Model A) in the middle of each riffle.

Epiphyton samples were collected from cobble taken within 1m upstream of each u-net location. To collect epiphyton for chlorophyll *a* analyses, a template was used to demarcate a $9.6cm^2$ area of maximum epiphyton abundance (generally the downstream end of the cobble), and epiphyton was removed with a scalpel; this procedure was repeated on the same cobble to obtain a sample for algal taxonomy and biovolume. Epilithon collected from each of three cobbles was combined into a composite sample (one composite for chlorophyll and another for taxonomic/biovolume analyses), for a total of three composite chlorophyll and three composite taxonomy/biovolume samples for each site. Samples for algal taxonomy and biovolume were immediately preserved in 5% formalin. Methods used for algal taxonomy are described in detail by Findlay and Kling (2003). Samples for chlorophyll *a* were immediately frozen on dry ice, and stored in the dark at -20°C until analyzed. Samples for chlorophyll *a* were blended with double distilled water for ten seconds, filtered onto GF/F filters, and extracted in boiling, 90% ethanol for seven minutes. Concentrations of chlorophyll *a* were measured with a fluorometer (Turner Designs, Model 10).

In addition to *in situ* sampling, historical data (discharge, turbidity, temperature and water chemistry) collected at Environment Canada monitoring stations from 1978 to 1999 for the Bow River upstream of Lake Louise, and the Athabasca River upstream of Jasper were compared to 1998 and 1999 data. If necessary, data were log transformed prior to statistical analyses to correct for positive correlation of variance and mean. Upstream-downstream variability in physical, chemical and biological parameters was evaluated using paired, one-tailed *t* tests. Correlation analyses were used to evaluate relationships between algal abundance, and riverine P concentrations and abundance of scrapers.

2.2 RESULTS

2.2.1. Physical Characteristics

Summer conditions during the study period were unrepresentative of long-term conditions: 1998 was warm and dry whereas 1999 was cool and wet. Discharge in the Bow and Athabasca rivers follows the seasonal pattern typical of glacially fed rivers: flows peak in June and July when annual snowpacks and glaciers melt and decline to minimum values in winter (Fig. 2-2). Mean annual (1978-1999) discharge averages 2.4 m^3/s in the Bow at Lake Louise, 40 m^3/s in the Bow at Banff, and 88 m^3/s in the Athabasca River at Jasper, peaking at 50, 127 and 265 m^3/s, respectively. During the surveys, discharge in 1998 was above average at all sites in spring and below average in summer in the Bow River at Banff and in the Athabasca River at Jasper (Fig.2-2, Table 2-2); the opposite trend occurred in 1999. Although discharge varied seasonally, current velocity did not differ ($p<0.05$) between sites from the same location (Lake Louise, Jasper and Banff) because our sampling design minimized variability in velocities to reduce variability in abundance of benthos. However, velocities were typically higher in the Bow than Athabasca River. Mean velocities at sites 1-6 km upstream or 2-6 km downstream of MWWTPs ranged from 0.44-0.78 m/s in the Bow River compared to 0.30-0.49 in the Athabasca River.

Mean monthly water temperatures in the Bow and Athabasca Rivers ranged from <1°C in December and January to 8-13°C in August (1978-1999 data, Environment Canada). Long-term temperature and turbidity data are not available for the Bow River at Banff. Water temperature in the Bow River begins to rise later in the spring, rises more quickly, and is 2-4°C higher by late summer than temperature in the Athabasca River (Fig.2-3). Water temperature in the Bow River was above average during the spring, summer and autumn 1998 surveys, and below average in late spring and summer

1999 (Fig.2-3, Table 2-2). In the Athabasca River, water temperature was higher than average from April until November in 1998, and in September in 1999.

Light levels were low in summer in the Athabasca River near Jasper because of high summer turbidity, and in spring and autumn in the Bow River downstream of Banff because of riparian shading. Mean monthly turbidity in the Bow River was 10-fold lower than in the Athabasca River (Fig.2-4). In the Bow River, turbidity was above average in summer and autumn 1998, and below average in spring and early summer 1999 (Fig.2-4, Table 2-2). In the Athabasca River, turbidity was higher than average in spring and early summer 1998 and lower than average in August. The opposite trend occurred in the Athabasca River in 1999.

In the Bow River, a near-by mountain (Mt. Rundle) blocked light from approximately 60% of the water surface at sites 3-8km downstream of the Banff MWWTP discharge (Fig.2-5, Table 2-2), significantly ($p<0.05$) more riparian cover than observed at sites further up- or downstream. As a result of riparian shading, the Bow River downstream of Banff received little direct sunlight in spring and autumn (when the sun was low on the horizon). There were no significant upstream-downstream differences in riparian shading near Lake Louise or Jasper ($p>0.05$). Riparian shading was nearly absent from the Athabasca River while the Bow River near Lake Louise was shaded by riparian vegetation across 10-40% of the water surface at upstream sites and 10-25% of the water surface at downstream sites (Fig.2-5).

2.2.2 Nutrient Chemistry

Concentrations of SRP were consistently low (< 4 μg/L) upstream of MWWTP discharges in all three rivers (with the exception of one instance in the Athabasca River in spring 1999, Fig.2-6). In contrast, SRP concentrations were higher downstream of MWWTP discharges (5-30%, 85-7200% and 0-280% increase relative to upstream for Lake Louise, Banff and Jasper, respectively). Upstream-downstream differences in SRP were most pronounced in autumn when effluent to discharge ratios increase, although differences between sites upstream and downstream of Banff were evident in all seasons. Paired t-tests comparing nutrient concentrations near Lake Louise, Banff and Jasper showed that nutrient concentrations did not vary ($p>0.05$) between 1998 and 1999, except for higher TP concentrations in 1998 than in 1999 near Lake Louise and Jasper (Fig.2-7) that tracked unusually high discharge and turbidity. Upstream-downstream differences in TP (Fig.2-7) and total dissolved P (*data not shown*) paralleled changes in SRP.

Concentrations of N were also higher downstream than upstream of MWWTPs (e.g., Fig. 2-8). In the case of DIN, concentrations increased downstream of Lake Louise and Banff in all seasons. Downstream of Banff, DIN concentrations were lowest in spring, moderate in summer and highest in the fall, consistent dilution via high river discharge. In autumn, significant ($p<0.05$) increases in total dissolved nitrogen (TDN) occurred downstream of all townsites: increases in nitrate+nitrite-N downstream of Lake Louise and Banff, and increases in ammonia-N downstream of Banff and Jasper. For a given site, there were few differences in N concentrations between years.

Our observation of elevated nutrient concentrations downstream of MWWTP discharges are consistent with predictions made with historical data (Table 2-1). However, observed increases in nutrient concentrations downstream of MWWTPs were generally less than expected downstream of Lake Louise, much higher than expected downstream of Banff and roughly as expected downstream of Jasper (Table 2-3). Higher than expected nutrient concentrations downstream of Banff were caused by frequent MWWTP malfunctions.

2.2.3. Benthic algae and scrapers

Benthic algal abundance was consistently low (e.g., mean <5.4 $\mu g/cm^2$ and maximum <11 $\mu g/cm^2$, chlorophyll *a*) at all upstream sites (Table 2-4, Fig.2-9). Conversely, mean and maximum chlorophyll *a* increased downstream of all MWWTP discharges, in spring, summer and autumn (except downstream of Banff in spring). Longitudinal downstream sampling showed at Lake Louise, high ($>10\mu g/cm^2$) algal biomass consistently occurred in summer and autumn but was limited to the first 3 km downstream of the MWWTP (Fig.2-9, Table 2-2). In contrast, algal abundance remained elevated up to 15 km downstream of Jasper in autumn and in the case of the Bow River downstream of Banff, was high at least 3 km downstream in summer and 9 km downstream in autumn. Coefficients of variation for chlorophyll *a* were, on average, significantly greater upstream than downstream of MWWTPs, and for the Bow River compared to the Athabasca River (Table 2-4). Chlorophyll *a* concentrations were higher in 1998 than 1999 at 3 km downstream of Lake Louise in summer and at 3 and 15 km downstream of Jasper in autumn (Fig.2-9, Table 2-2). Conversely, autumn chlorophyll *a* concentrations were lower in 1998 than 1999 in the Bow River 2km downstream of Lake Louise and 15 km downstream of Banff.

Trends in other measures of algal biomass followed patterns in chlorophyll *a* concentration. For example, trends in algal biovolume and number of cells (Table 2-4) corresponded to one another (with two exceptions) and to trends in chlorophyll (with one exception). The few exceptions included an increase in algal biovolume downstream of Lake Louise and Banff in the autumn when number of algal cells decreased, and an increase in chlorophyll content downstream of Lake Louise in summer when algal biovolume decreased. Trends in ash-free dry mass (1-9mg/cm^2) were very similar but more variable than trends in chlorophyll *a* (*data not shown*).

At upstream sites, epilithic communities were generally dominated by diatoms, and mayflies were the most abundant primary consumers. Taxonomic analysis of benthic algal samples showed that assemblages were composed mainly of diatoms at all sites (both upstream and downstream of discharges) and all sampling dates, but high abundances of other taxonomic groups occurred sporadically (Appendix 2-1). At sites upstream of MWWTPs, scrapers (feeding group of aquatic insects that scrape periphyton from mineral or organic surfaces, Merrit and Cummins 1996) in both the Bow and Athabasca Rivers were dominated by mayflies (Heptageniidae, Ephemerellidae and Baetidae) but there were also numerous caddisfly (Brachycentridae) and stonefly (Taeniopterygidae) scrapers (*data not shown).* The number of chironomids increased downstream of MWWTPs.

Algal abundance was positively correlated (r=0.78, p=0.001) with numbers of benthic scrapers: high (>10µg/cm^2) chlorophyll *a* concentrations, as occurred 2km downstream of Lake Louise, 9km downstream of Banff and 2 km downstream of Jasper, were associated with high numbers (1160, 2660, 295 individuals/m^2, respectively) of benthic scrapers (Fig.2-10). Low chlorophyll concentrations (<10µg/cm^2) at high TP concentrations (>4μg/L) were instead associated with (1) high riparian shading downstream of Banff, in the spring and autumn when the sun was low on the horizon, and (2) high turbidity (>50 NTU) downstream of Jasper in the summer (Table 2-2). Particularly high chlorophyll *a* concentrations (>30μg/cm^2) at low TP concentrations (<4μg/L) only occurred at the site 2km downstream of Lake Louise where bryophytes were abundant (Table 2-2), consistent with lower than expected P concentrations via high epilithic P uptake. Analyses on all data showed that benthic algal abundance was not correlated ($p>0.05$) with any form of P. However, when data confounded by biological and physical factors were removed, abundance was significantly correlated with P concentrations (Table 2-5).

2.3 DISCUSSION

2.3.1. Variation in algal response to nutrient enrichment

Abundance of benthic biota in the oligotrophic rivers of the Canadian Rocky Mountains is controlled by a hierarchy of physical and chemical factors. In these rivers, seasonal variation in air temperature determines river discharge (which tracks summer melt and autumn freeze of high elevation glaciers), water temperature and underwater light penetration (which is determined by discharge-controlled turbidity during open-water periods and snow and ice cover during in winter). Algal abundance in these intrinsically nutrient-poor rivers (< 3 μg/L SRP, < 10 μg/L DIN) is consistently low (< 5 μg/cm^2) and shows little seasonal variation in reaches relatively unaffected by human activity. However, despite physical constraints, addition of comparatively low volume (<1.7% wastewater to river volume), nutrient rich (0.2-3.5mg/L TP; 0.3-17.1mg/L DIN) wastewater to these rivers results in a nutrient response by benthic algae that is greatest in autumn (when discharge, temperature, light and accumulation period are conducive for production) and lowest in spring (when temperature is inhibitory and accumulation period is short). Further, correlation analyses showed no relationship between algal abundance and various forms of P when all data were considered, but when dates where physical or biological factors constrained algal productivity were eliminated (i.e., summer data from the Athabasca River when turbidity was high, spring and fall data from sites on the Bow River that were shaded, and fall data from a site near Lake Louise where primary production was dominated by bryophytes), algal abundance (chlorophyll *a,* biovolume and cell numbers) was strongly correlated to the gradient in anthropogenic nutrient concentrations. The hierarchical effects of, first, physical factors (discharge, temperature, light and accumulation period) and, then, chemical factors (nutrients) in regulating benthic algal abundance in mountain rivers is consistent with research on other temporally variable river systems. For example, models developed to identify causes of benthic algal abundance patterns in Mid-Atlantic streams also showed that physical and chemical determinants of chlorophyll *a* were hierarchical (Pan et al. 1999). In general, we found algal accrual in mountain rivers is proximately regulated by P availability, only if light is not limiting. Ultimately, seasonal and annual changes in river discharge and temperature will control algal accumulation. For example, in summer 1998, water temperatures in the Bow River near Lake Louise were at maximum historical levels, proximate constraints (P and light) were not limiting, and benthic algal abundance (expressed as chlorophyll content) downstream of the Lake Louise MWWTP was one of the highest values recorded throughout the study.

Whereas physical and chemical conditions strongly influenced benthic algal abundance in Rocky Mountain rivers, algal accrual along a gradient in nutrient availability was not masked by grazing pressure but, instead, was positively correlated with abundance of scrapers. Increased scraper abundance in response to eutrophication occurs when food is limiting and results in top-down control of periphyton biomass. For example, Bourassa and Cattaneo (1998) concluded that in streams where TP concentrations (5-60μg/L) were not correlated with periphyton biomass but positively correlated with the biomass and size of scrapers (predominately snails), periphyton abundance was controlled by top-down mechanisms. However, in the mountain rivers, both algal biomass and scraper densities were correlated with river P concentrations. Mayflies are the dominant scraper in mountain rivers both upstream and downstream of MWWTP discharges. In a review of effects of herbivores on periphyton, mayflies were found to be the only taxonomic group of benthic invertebrates that did not exert top-down control of periphyton abundance (Feminella and Hawkins 1995). It is also unlikely that mayflies and other insects in mountains rivers are limited by food abundance, particularly downstream of wastewater discharges. Deegan (et al. 1997) reported that during P fertilization of the oligotrophic Kuparuk River, algal accumulation in the absence of top-down control occurred during a year when the ratio of chlorophyll *a* (μg/m^2) to number of benthic insects (no./m^2) was ~0.075 in control and ~0.3 in fertilized reaches. In the Bow and Athabasca Rivers, average chlorophyll per insect was 1.1 μg upstream of MWWTPs and 2.3 μg downstream, indicating that insects were likely food replete and not likely to be exerting top-down control of periphyton abundance. Our observation that algal accrual in mountain rivers is proximately controlled by light or P availability but not by scrapers is consistent with current stream ecology theory which predicts that periphyton accrual is regulated by resource stress and herbivory in streams dominated by low-density diatom biofilms but is regulated by competition for light and nutrients when resources are sufficiently high for algal accumulation rates to equal grazing rates (Stevenson 1997). In the mountain rivers, this herbivory threshold occurs at very low resource conditions (< 5 μg/cm^2 chlorophyll *a*), consistent with the expectation that herbivory thresholds occur at lower resource conditions in relatively harsh habitats (e.g., cold, low light) than in more benign, stable habitats (Stevenson 1997).

2.3.2. Nutrient concentrations associated with nuisance levels of benthic algae

The range of P concentrations associated with nuisance levels of benthic algae in the mountain rivers was variable but 30-50% lower than concentrations previously reported to stimulate extensive algal mat formation. Benthic algae are generally considered to become a nuisance at 5-20μg/cm^2 chlorophyll *a* (e.g. Dodds et al. 1997). In the mountain rivers, benthic algal biomasses of 5-10 μg/cm^2 chlorophyll *a* were associated with nutrient concentrations averaging 4μg/L SRP (range 1-42) and 19μg/L TP (range 2-194). P concentrations previously reported to be in surplus of periphyton requirements in mountain-fed rivers in Alberta, Canada (including the Athabasca River) were >9μg/L SRP and >40μg/L TP (Chambers et al. 2000). Similarly, Dodds et al. (1997) concluded that nuisance levels of benthic algae (≥10μg/cm^2 chlorophyll *a*) would occur in most streams at TP concentrations >30μg/L. These values are 30-50% higher than average P concentrations we found in association with nuisance levels of benthic algae.

Although significant changes in aquatic habitat or additional point source nutrient additions prevented us from measuring the effect of MWWTP discharges at distances greater than 15km downstream, algal abundance remained elevated by approximately 5-fold (but as much as 50-fold) as far as 15 km downstream of point source nutrient inputs despite only little change in average P concentrations between upstream to downstream of MWWTP discharges (i.e., increase of 0.1-12μg/L SRP, except downstream of Banff). Similarly, a previous study of the Athabasca River near Jasper recorded an increase in SRP from 2-4μg/L upstream to 4-15μg/L downstream of the MWWTP and a 4-fold increase epilithic abundance extending up to 4-20 km downstream (Scrimgeour and Chambers 2000). In the oligotrophic Kuparuk River, fertilization increased PO_4-P concentrations by 10μg/L above background (<1-4μg/L) and also caused, on average, a 5-fold increase in algal biomass over a distance of 3-10 km (Peterson et al. 1993). Welch et al. (1989) found the abundance of riverine periphyton increased nearly 10-fold downstream of inputs from a nutrient rich aquifer even though there was no change in ambient SRP concentrations (5-15μg/L), and predicted that algal biomass would be elevated by an order of magnitude for over 10km unless SRP was below 10μg/L. Large changes (4- to 10-fold) in benthic algal abundance in response to modest changes in nutrient concentrations (0-13μg/L SRP) are characteristic of nutrient-limited systems.

2.3.3. Changes in magnitude of algal accrual along nutrient gradients

Despite growing evidence that the magnitude of benthic algal response to nutrient addition is much greater for nutrient-poor rivers, published nutrient-chlorophyll models drastically underestimated chlorophyll concentrations in oligotrophic rivers (Table 2-6). In general, the models that most closely estimated downstream chlorophyll levels overestimated upstream levels, and vice versa (e.g. Bothwell 1989, Scrimgeour and Chambers 2000 in Table 2-6). Estimated chlorophyll values were also highly variable, indicating that models are not well calibrated for oligotrophic systems (e.g. Dodds et al. 1997 & 2002) or not developed for universal predictions (e.g., Bothwell 1989), but also highlight the fact that algal accrual under low nutrient conditions is difficult to predict. The coefficient of variation in chlorophyll *a* was lower in both the most nutrient-rich sites within mountain rivers, and in the more nutrient-rich Athabasca River. Aquatic systems receiving anthropogenic nutrient loading often show reduced habitat complexity (e.g., Peterson et al. 1993).

In conclusion, our results show that despite strong influence of physical parameters on benthic algal abundance, eutrophication of strongly nutrient-limited cold-water rivers reduces variability in abundance, and induces a greater change in abundance than occurs in warm-water rivers with moderate or high nutrient concentrations. Thus, there is greater potential for even slight enrichment of the most strongly oligotrophic rivers to negatively affect aquatic biota. Because algal abundance in strongly nutrient-limited rivers is highly variable, changes in ecological integrity are difficult to predict, further warranting the need for caution when considering expansion of human activities in these catchments. Bunn and Davies (2000) have shown that faunal patterns can lack persistence and predictability, and therefore direct measures of ecosystem processes (e.g., nutrient cycling, metabolism) should be used to accurately detect changes in ecological integrity. In addition, indices of ecosystem processes are sensitive to causal factors, making strategies for remediation more obvious (Bunn and Davies 2000). Direct measures of nutrient limitation (e.g., Chapter 3) and nutrient transfer between producers and consumers (e.g., Chapter 4) will improve the ability to predict patterns in stream benthos at the lower end of the resource gradient, yield models that are indicative of ecosystem processes of concern, and will therefore improve the ability to manage and mitigate the effects of anthropogenic nutrient enrichment caused by changes in nutrient loading.

2.4 LITERATURE CITED

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Table 2-1. Mean monthly (August, September and October) river and effluent volumes, and total phosphorus (TP), soluble reactive phosphorus (SRP), and dissolved inorganic nitrogen (DIN) concentrations in wastewater effluent, study rivers upstream (u/s) of the municipal wastewater treatment plants (MWWTPs), and predicted to occur downstream (d/s) of the MWWTP discharges (estimated as the mean monthly sum of upstream nutrient concentrations, and the product of MWWTP effluent nutrient concentrations and the ratio effluent volume to river volume (EV:RV)). Upstream river concentrations are mean Environment Canada data from 1978-1999.

		← Volume →			← MWWTP →			← River u/s →					← River d/s →				
		River (m^3/s)	MWWTP (m^3/s)	%EV: RV	TP mg/L	SRP mg/L	DIN mg/L	TP µg/L	SRP µg/L	DIN µg/L	DIN: TP	DIN: SRP	TP µg/L	SRP µg/L	DIN µg/L	DIN: TP	DIN: SRP
Low P Load	L.Louise	2.6	0.043	1.67	0.2	0.02	1.3	3.9	0.9	82.8	21.1	88.1	7.5	1.2	103.7	13.9	11.4
↓	Banff	43.4	0.118	0.27	2.0	1.62	11.9	8.9	1.5	87.4	9.8	60.3	14.4	5.9	119.8	8.3	1.4
High P Load	Jasper	124.0	0.054	0.04	3.4	2.85	17.1	27.5	1.3	70.1	2.5	52.7	29.0	2.6	77.5	2.7	1.0

Table 2-2. Summary of temporal (annual), and spatial (distance upstream and downstream in autumn) trends in physical, chemical and biological parameters (98=1998, 99=1999, chlorophyll values reported in µg/cm^2).

		Annual Differences				Spatial Differences in Autumn		
		May	August	October		Upstream 1-6 km	Downstream 3-6 km	Downstream 7-15 km
L.Louise	*Chlorophyll 3km d/s*	15	10-43	21-57	*Max. Chlorophyll*	5	56	5
	Chlorophyll	-	98>99	99>98	Dominant epilithon	diatoms	Bryophytes	diatoms
	Discharge	98>99	-	-	Riparian cover (%)	10-40	10-25	10
	Temperature	99>98	98>99	98>99	Autumn TP (µg/L)	2.0-3.7	2.1-3.9	1.7-2.1
	Turbidity	98>99	98>99	98>99				
Banff	*Chlorophyll 3km d/s*	6.8	16-17	1.1-2.7	*Max. Chlorophyll*	10	8	24
	Chlorophyll	-	-	99>98	Dominant epilithon	diatoms	diatoms	diatoms
	Discharge	98>99	99>98	-	Riparian cover (%)	2-10	60	0
					Autumn TP (µg/L)	2.8-4.5	9.6-116.0	8.6-16.2
Jasper	*Chlorophyll 3km d/s*	6.6	0.9-3.5	13-45	*Max. Chlorophyll*	4	45	42
	Chlorophyll	-	-	98>99	Dominant epilithon	diatoms	diatoms	diatoms
	Discharge	98>99	99>98	-	Riparian cover (%)	0-7	0	0
	Temperature	98>99	98>99	98>99	Autumn TP (µg/L)	3.5-5.6	4.2-9.8	7.2-7.5
	*Turbidity	98>99	**99>98	-				
	Summer TP	-	98>99	-				

*Highest in summer
**Despite higher spring and summer (May-July) turbidity in 1998

Table 2-3. **Predicted and observed total phosphorus (TP), soluble reactive phosphorus (SRP), and dissolved inorganic nitrogen (DIN) concentrations 3 km downstream of the Lake Louise, Banff and Jasper wastewater effluent discharges in October 1998 and 1999. Downstream estimates were calculated following the procedure given in Table 2-1.**

			Observed Concentration (μg/L)		Estimated Concentration (μg/L)	Difference
			Upstream	Downstream	Downstream	(%)
Bow River						
L.Louise	TP	1998	2.1	3.8	2.6	-48
		1999	2.4	5.9	2.2	-169
	SRP	1998	0.8	0.8	1.4	40
		1999	0.7	0.7	0.7	-2
	DIN	1998	67	148	93	-59
		1999	59	174	109	-60
Banff	TP	1998	4.4	19.4	116.1	83
		1999	4.5	11.0	70.9	85
	SRP	1998	1.0	14.4	102.3	86
		1999	1.2	6.0	58.9	90
	DIN	1998	72	126	486	74
		1999	104	136	367	63
Athabasca River						
Jasper	TP	1998	5.1	7.3	9.7	25
		1999	5.6	8.9	9.8	10
	SRP	1998	1.1	2.8	3.6	22
		1999	1.0	3.7	3.5	-6
	DIN	1998	70	78	86	10
		1999	82	100	85	-18

Table 2-4. Mean and standard error (s.e.) of benthic algal abundance (mean and maximum chlorophyll content, coefficient of variation in chlorophyll, algal biovolume and number of algal cells) at sites 1-6km upstream (u/s) versus 2-6km downstream (d/s) of the Lake Louise, Banff and Jasper wastewater treatment plants in spring, summer, and autumn. Underlined means indicate significant differences ($p<0.05$ in bold, $0.05<p<0.1$ in italics) between upstream and downstream means for a given site and season.

		Chlorophyll *a* Mean (μg/cm^2)		Chlorophyll *a* Maximum (μg/cm^2)		CV		Biovolume (μg/cm^2)		Cells (#/cm^2)	
		mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
Spring											
L.Louise	u/s	3.3	0.8	5.8	1.3	79	13	**46**	8.3	10	0.1
	d/s	7.7	6.9	15	13	74	12	**182**	0.0	27	0.0
Banff	u/s	5.4	2.4	11	3.4	134	5.4	89	22	21	5.2
	d/s	4.0	2.8	7.2	4.5	85	24	50	42	10	8.1
Jasper	u/s	1.8	0.5	**2.7**	0.4	**72**	12	**22**	3.5	7.5	0.8
	d/s	6.6	0.0	**7.2**	0.0	**10**	0.0	**111**	21	11	2.4
Summer											
L.Louise	u/s	3.7	0.9	*6.1*	*1.8*	58	15	317	292	22	17
	d/s	26.6	16.3	*52*	*37*	69	25	81	4.1	16	7.2
Banff	u/s	**2.8**	2.3	**3.6**	2.6	72	40	55	1.2	7.0	2.4
	d/s	**11.9**	2.8	**18**	3.6	57	18	218	123	16	9.6
Jasper	u/s	1.3	0.4	2.0	0.9	51	9.3	*18*	4.6	*3.3*	0.9
	d/s	2.2	1.3	3.1	2.1	24	18	*47*	6.9	*14*	5.9
Autumn											
L.Louise	u/s	**0.7**	0.3	**1.1**	0.3	69	21	505	478	28	24
	d/s	**38.9**	17.7	**70**	39	53	3.1	654	467	20	9.0
Banff	u/s	0.6	0.1	1.6	0.4	106	8.3	8.7	0.3	2.4	0.4
	d/s	2.5	0.2	5.9	1.6	98	5.9	24	24	1.8	0.7
Jasper	u/s	**1.3**	0.6	**2.5**	1.3	**75**	14	**22**	3.4	**9.3**	1.6
	d/s	**33.7**	11.4	**43**	12	**24**	1.0	**1135**	753	**824**	767

Table 2-5. Correlation (r) between benthic algal abundance and variability (coefficient of variation (CV) in chlorophyll *a*) and phosphorus concentrations (total, total dissolved and soluble reactive) using all data (spring, summer and autumn of 1998 and 1999) and with confounded data removed (data from sites 3 km downstream of Lake Louise removed due to presence of bryophytes, data from sites 3 and 8 km downstream of Banff in the spring and autumn removed due to riparian shading, and data from sites in Jasper in summer removed due to high turbidity). Significant correlations are in bold (p<0.05 underlined and 0.01<p<0.05 in italics).

		Chlorophyll *a*			**Biovolume**	**No. Cells**
		Maximum	**Mean**	**CV**		
All Data						
TP	r	0.07	0.08	-0.10	-0.13	-0.10
	p	0.57	0.49	0.41	0.29	0.41
TDP	r	0.11	0.10	-0.03	-0.14	-0.15
	p	0.35	0.39	0.79	0.24	0.21
SRP	r	0.14	0.13	-0.01	-0.16	-0.16
	p	0.24	0.29	0.92	0.18	0.19
Data Removed						
TP	r	0.18	***0.25***	**-0.32**	0.13	0.19
	p	0.20	0.08	0.02	0.37	0.19
TDP	r	***0.23***	**0.31**	**-0.41**	**0.32**	**0.37**
	p	0.10	0.03	0.00	0.02	0.01
SRP	r	**0.36**	**0.42**	**-0.38**	**0.26**	**0.33**
	p	0.01	0.00	0.01	0.06	0.02

Table 2-6. Benthic algal abundance (expressed as chlorophyll *a* (chl*a*) content) in the Bow River upstream (u/s) and downstream (d/s) of Lake Louise and Banff, and in the Athabasca River upstream and downstream of Jasper as measured in this study and as predicted from published nutrient – chlorophyll models (using estimated nutrient concentrations downstream of the Lake Louise, Banff and Jasper MWWTPs). The difference between downstream and upstream chl*a* values is expressed as percent increase.

Reference	Model	Data Range		Lake Louise			Banff			Jasper		
		nutrient	Chl*a*	Chl*a* (μg/cm^2)		Increase	Chl*a* (μg/cm^2)		Increase	Chl*a* (μg/cm^2)		Increase
		μg/L	μg/cm2	u/s	d/s	(%)	u/s	d/s	(%)	u/s	d/s	(%)
This Study		4-60 TP	1-51	1.4	11	693	0.90	26	2778	3.4	51	1385
Bothwell (1989)	$y = 44.1 \log x + 247.4$	<30 SRP	20-35	25	25	2	26	28	10	25	27	5
Lohman et al. (1992)	$y = 41.1 \log x - 4.1$	<3000 TP	<20	2.0	3.2	60	2.9	4.0	38	5.5	5.6	2
Krewer and Holm (1994)	$y = 2.44 \log (\log x) + 3.58$	<100 TDP	<100	0.54	0.79	46	0.34	2.6	665	0.53	2.5	372
Cattaneo et al. (1997)	$y = 0.814 \log x + 0.224$	<45 TP	<15	0.07	0.09	29	0.08	0.11	38	0.14	0.14	0
Dodds et al. (1997)	$\log y = 0.279 \log x + 1.091$	$<10^2$ TP	$<10^4$	1.8	2.2	22	2.1	2.5	19	3.1	3.2	3
Chelelat et al. (1999)	$\log y = 0.91 \log x + 0.49$	6-130 TP	<47	1.1	1.9	73	1.6	2.9	81	6.2	6.5	5
Scrimgeour and Chambers (2000)	$y = 10.1 \log SRP + 17.7 \log DIN - 25.6$	<120 SRP	<100	3.8	7.8	105	12	20	69	8.3	12	45
Dodds et al. (2002)	$\text{Log } y = 0.39 \log DIN + \log SRP + 2.98$	$<10^2$ TP	$<10^4$	0.36	0.38	6	0.40	0.46	15	0.38	0.41	8

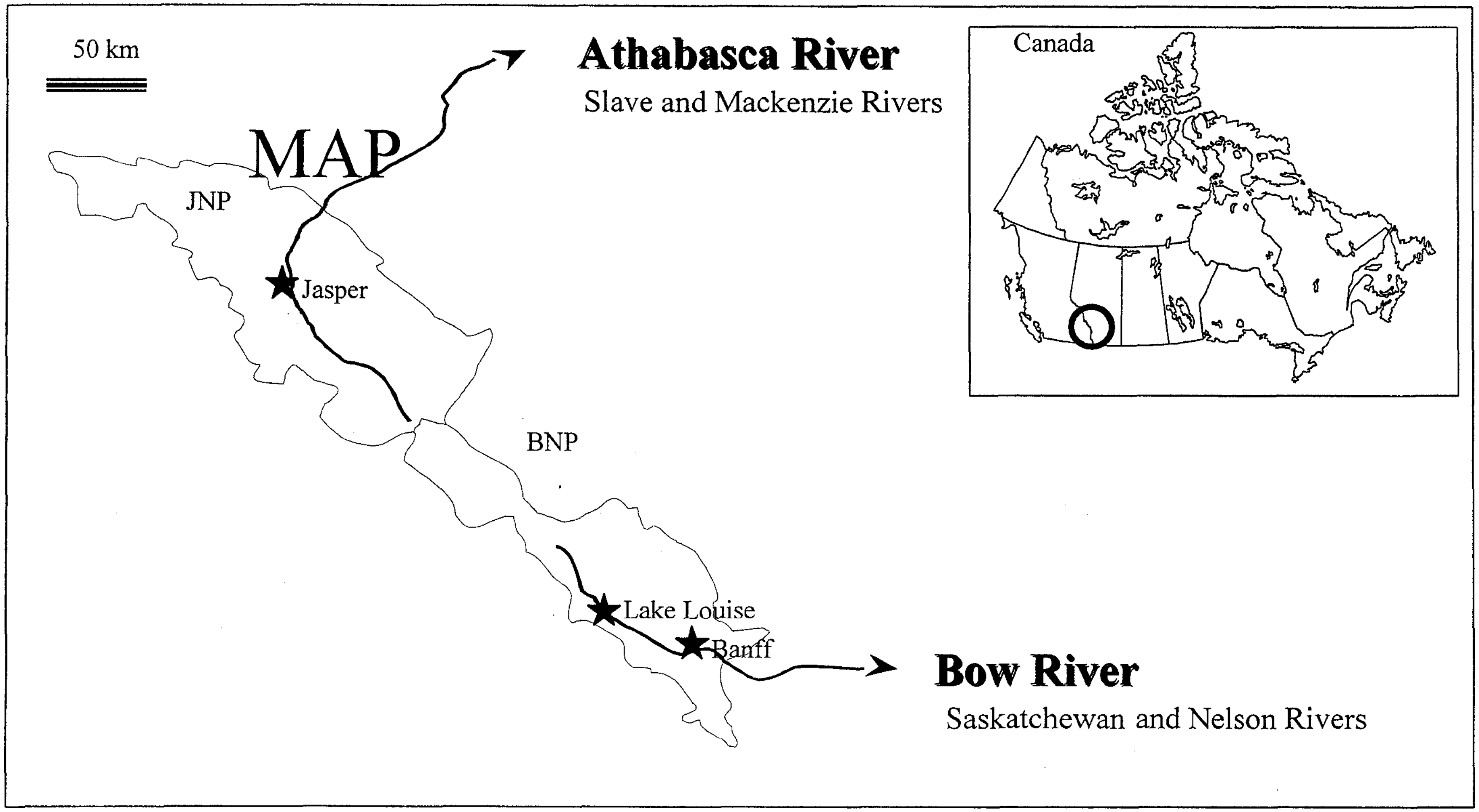

Fig.2-1. Map of national parks in the Rocky Mountains of Canada showing the Athabasca River in Jasper National Park (JNP) and the Bow River in Banff National Park (BNP), and the locations of Jasper, Lake Louise, Banff and Field townsites.

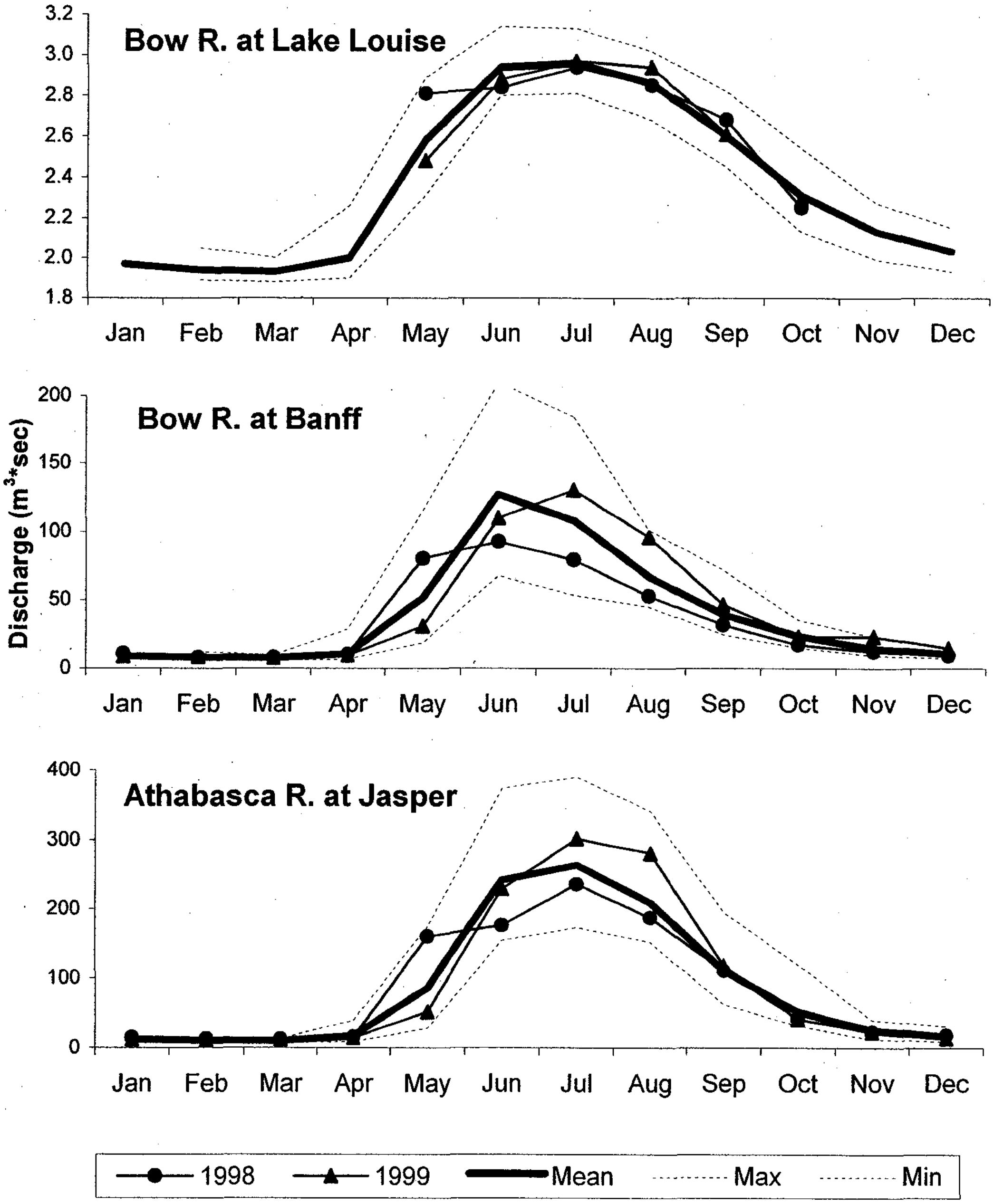

Figure 2-2. Mean monthly discharge of the Bow River and Athabasca River in 1998 and 1999 relative to mean, maximum and minimum values from 1978-1999 (Environment Canada). Note change in scale of y-axis.

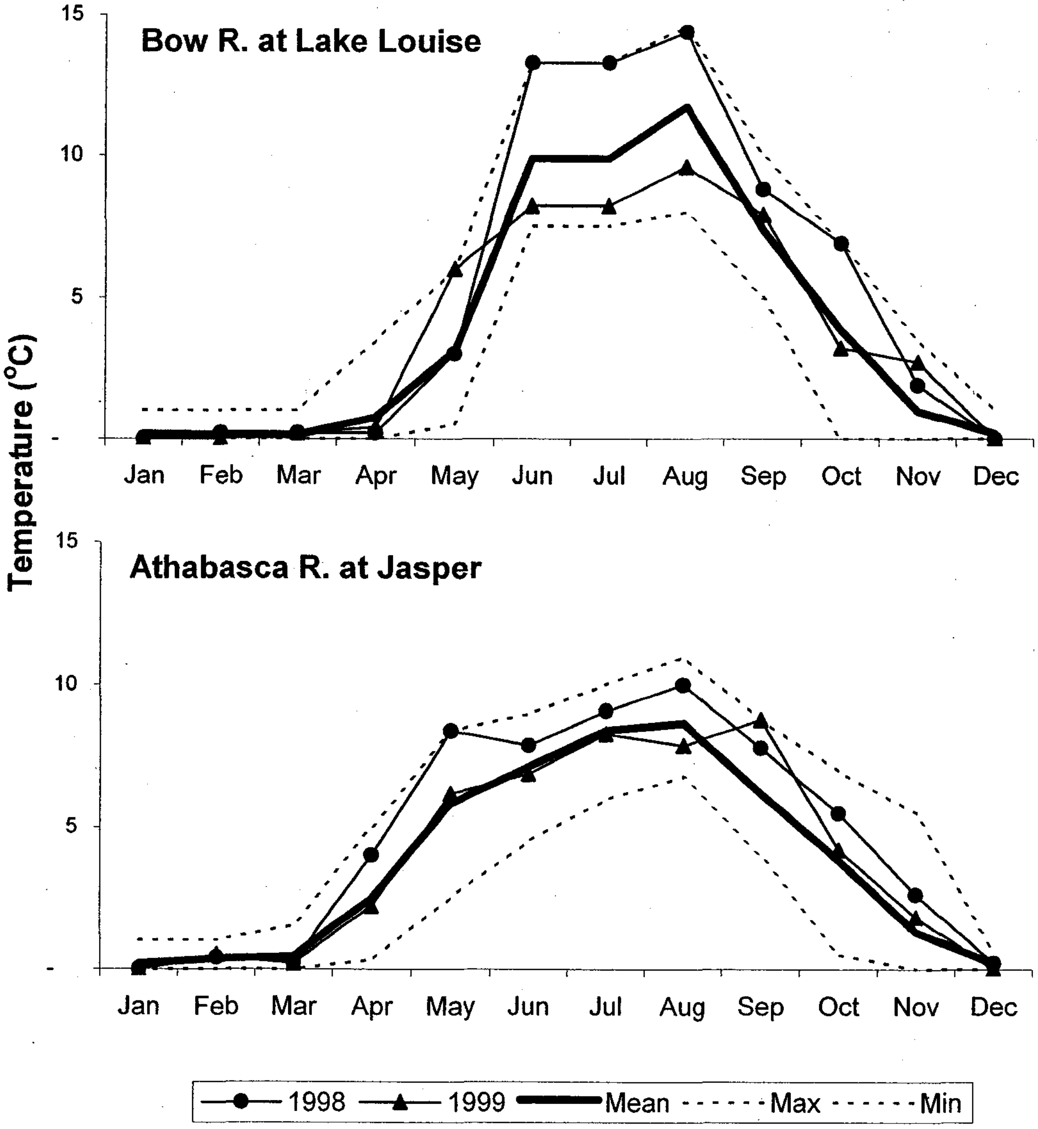

Figure 2-3. Mean monthly water temperature of the Bow River and Athabasca River in 1998 and 1999 relative to mean, maximum and minimum values from 1978-1999 (Environment Canada).

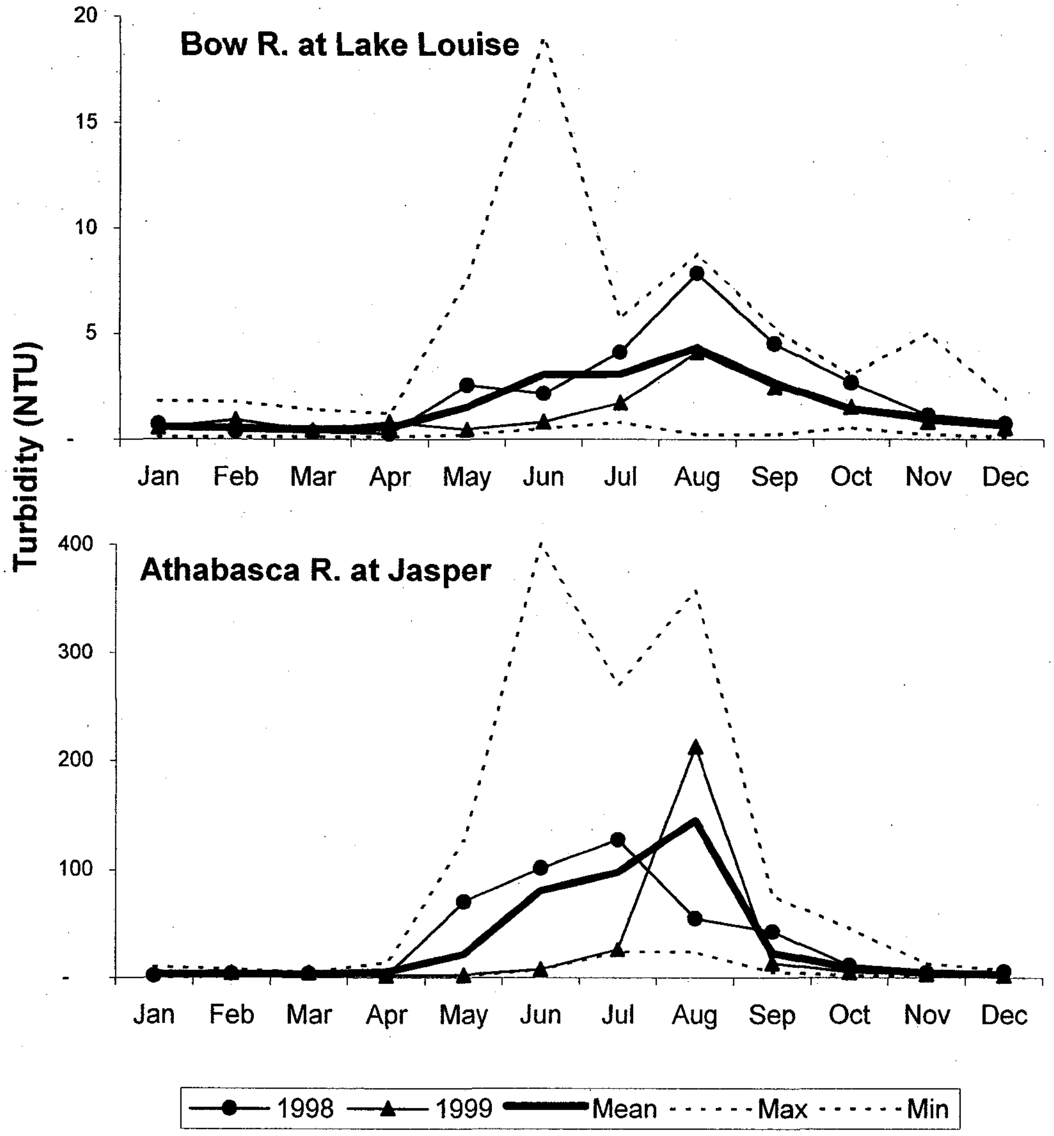

Figure 2-4. Mean monthly turbidity (in nephelometric turbidity units) of the Bow River and Athabasca River in 1998 and 1999 relative to mean, maximum and minimum values from 1978-1999 (Environment Canada). Note change in scale of y-axis.

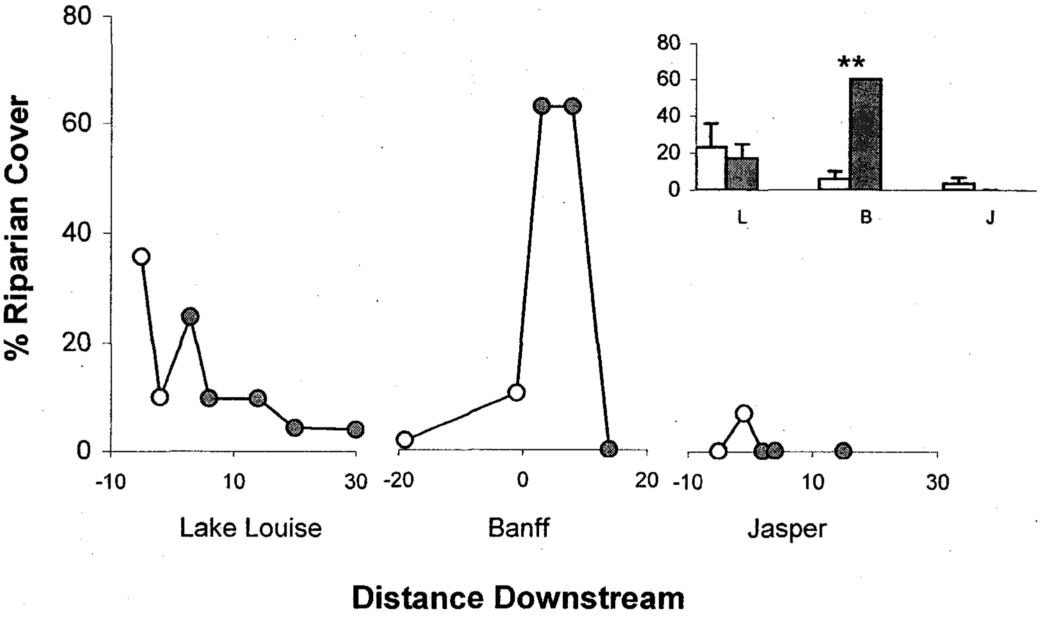

Figure 2-5. Percentage of water surface shaded by riparian vegetation or mountains at sites upstream (open symbols) and downstream (closed symbols) of MWTPs. Inset graphs show amount of riparian shading (mean + one standard error) at sites 1-6 km upstream (open bars) and 3-6 km downstream (closed bars) of the Lake Louise (L), Banff (B) and Jasper (J) MWTPs. Asterisks identify difference between sites 1-6 km upstream and sites 3-6 km downstream (**$p<0.05$, *$0.05<p<0.10$).

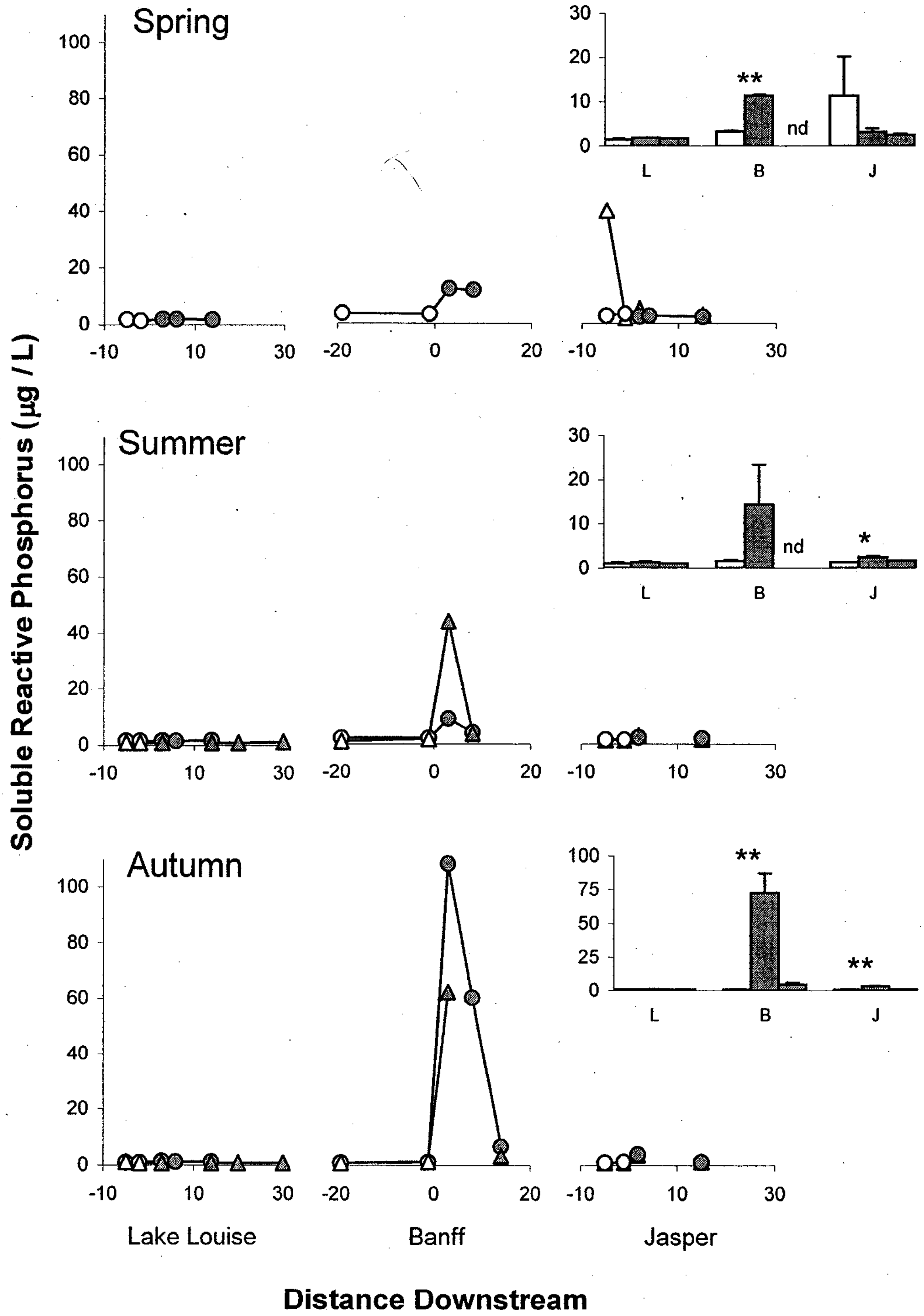

Figure 2-6. Soluble reactive phosphorus (SRP) concentrations at sites upstream (open symbols) and downstream (closed symbols) of MWTPs in 1998 (•) and 1999 (▲). Inset graphs show SRP (mean + one standard error) at sites 1-6 km upstream (open bars) and 3-6 km or 7-15 km (nd = no data) downstream (closed bars) of the Lake Louise (L), Banff (B) and Jasper (J) MWTPs. Asterisks identify difference between sites 1-6 km upstream and sites 3-6 km downstream ($p<0.05$, *$0.05<p<0.10$).**

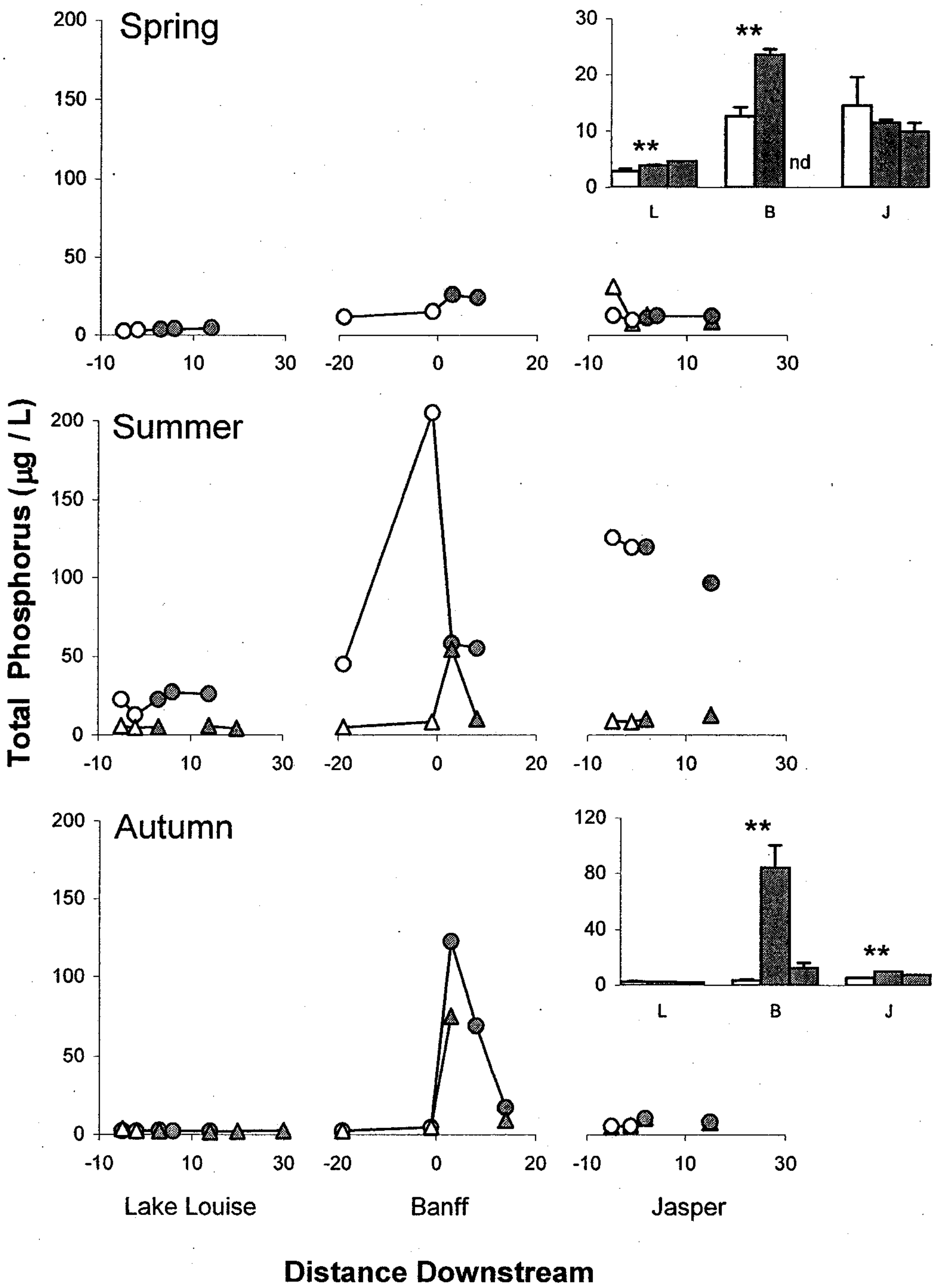

Figure 2-7. Total phosphorus (TP) concentrations at sites upstream (open symbols) and downstream (closed symbols) of MWTPs in 1998 (•) and 1999 (▲). Inset graphs show TP (mean + one standard error) at sites 1-6 km upstream (open bars) and 3-6 km or 7-15 km (nd = no data) downstream (closed bars) of the Lake Louise (L), Banff (B) and Jasper (J) MWTPs (summer TP concentrations upstream and downstream of MWTP were not averaged due to significant ($p>0.5$) differences between concentrations in 1998 and 1999). Asterisks identify difference between sites 1-6 km upstream and sites 3-6 km downstream (**$p<0.05$, *$0.05<p<0.10$).

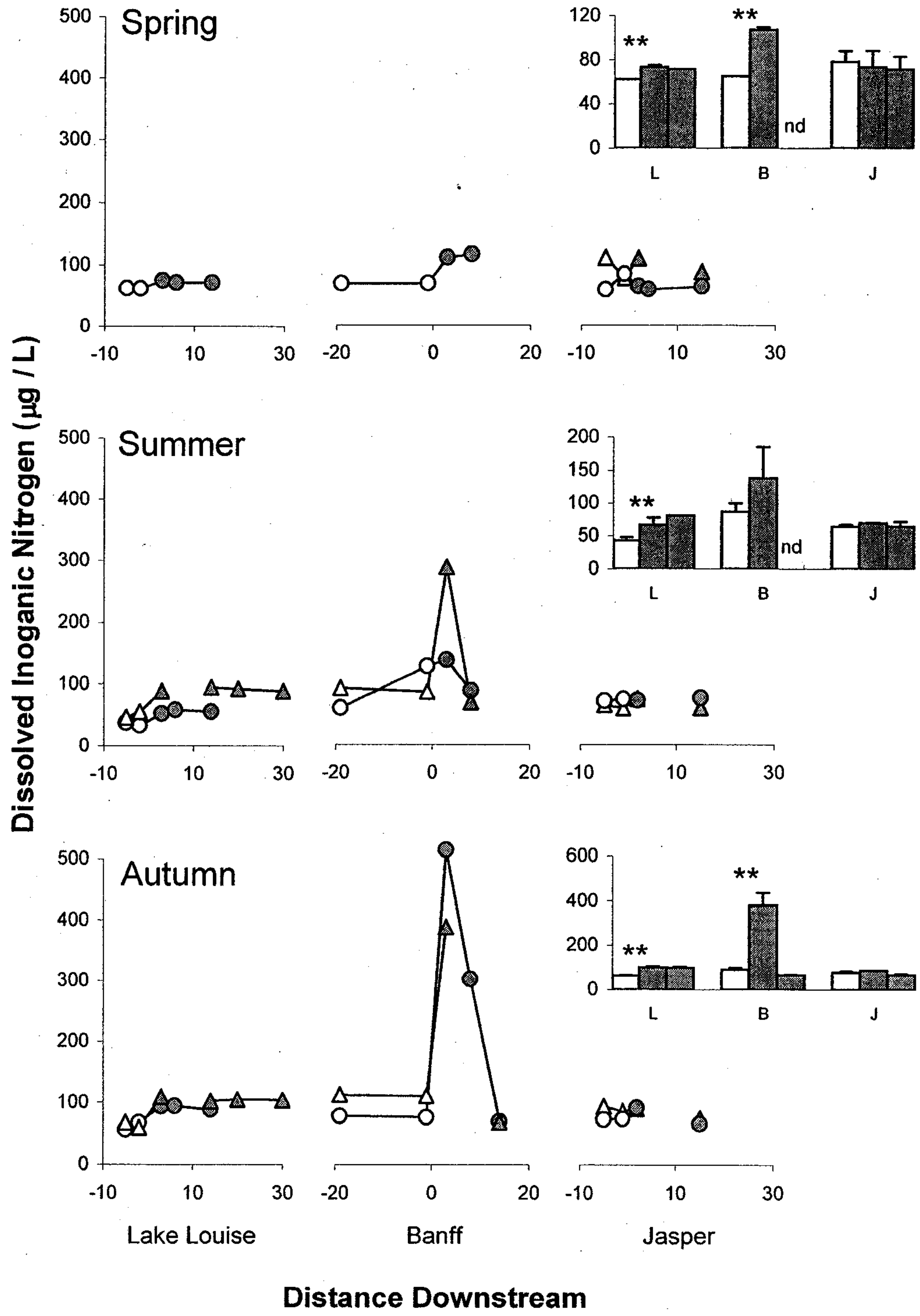

Figure 2-8. Dissolved inorganic nitrogen (DIN) concentrations at sites upstream (open symbols) and downstream (closed symbols) of MWTPs in 1998 (•) and 1999 (▲). Inset graphs show DIN (mean + one standard error) at sites 1-6 km upstream (open bars) and 3-6 km or 7-15 km (nd = no data) downstream (closed bars) of the Lake Louise (L), Banff (B) and Jasper (J) MWTPs. Asterisks identify difference between sites 1-6 km upstream and sites 3-6 km downstream (p<0.05, *0.05<p<0.10).**

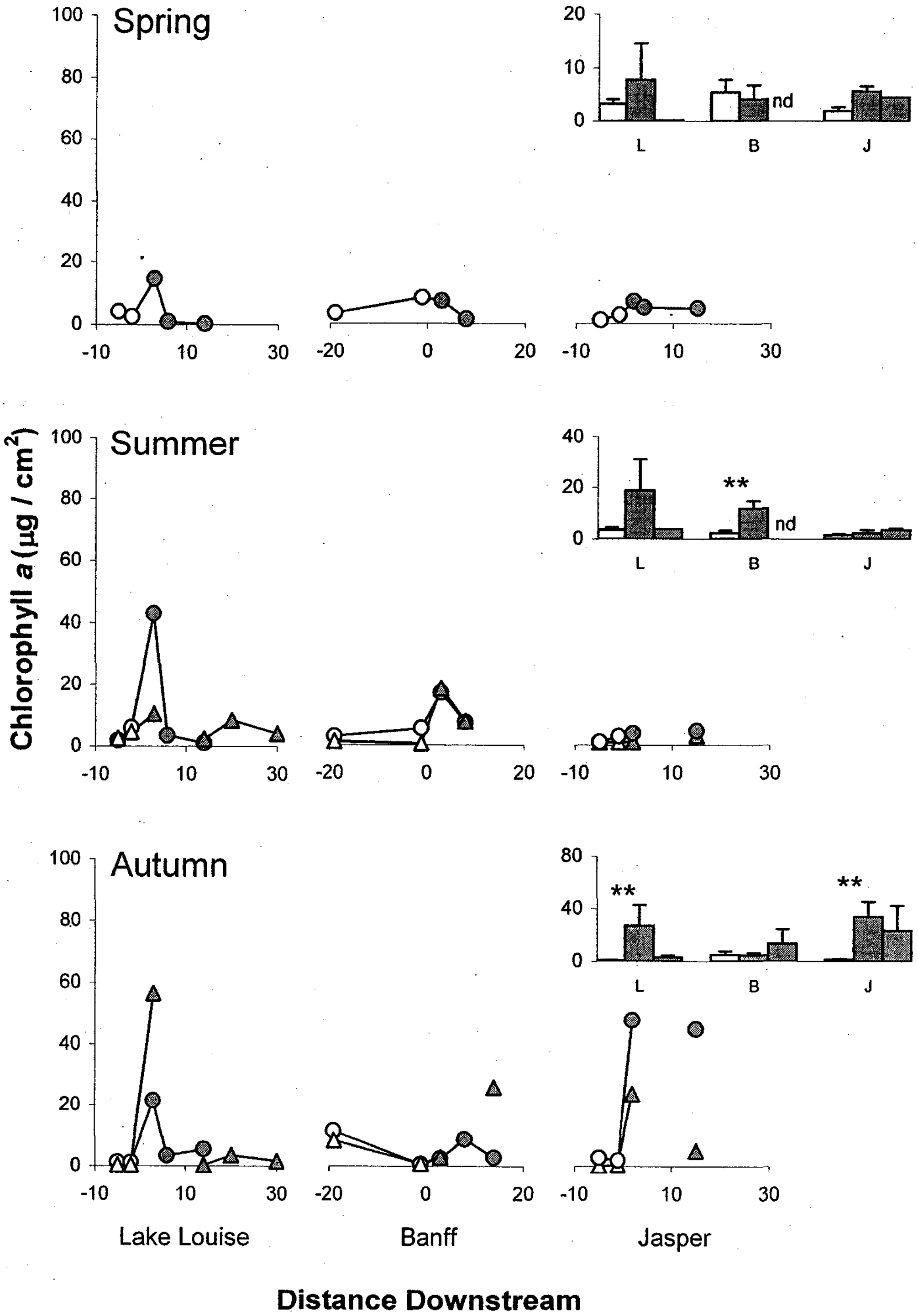

Figure 2-9. Chlorophyll *a* concentrations at sites upstream (open symbols) and downstream (closed symbols) of MWTPs in 1998 (•) and 1999 (▲). Inset graphs show chlorophyll (mean + one standard error) at sites 1-6 km upstream (open bars) and 3-6 km or 7-15 km (nd = no data) downstream (closed bars) of the Lake Louise (L), Banff (B) and Jasper (J) MWTPs. Asterisks identify difference between sites 1-6 km upstream and sites 3-6 km downstream (**p<0.05, *0.05<p<0.10).

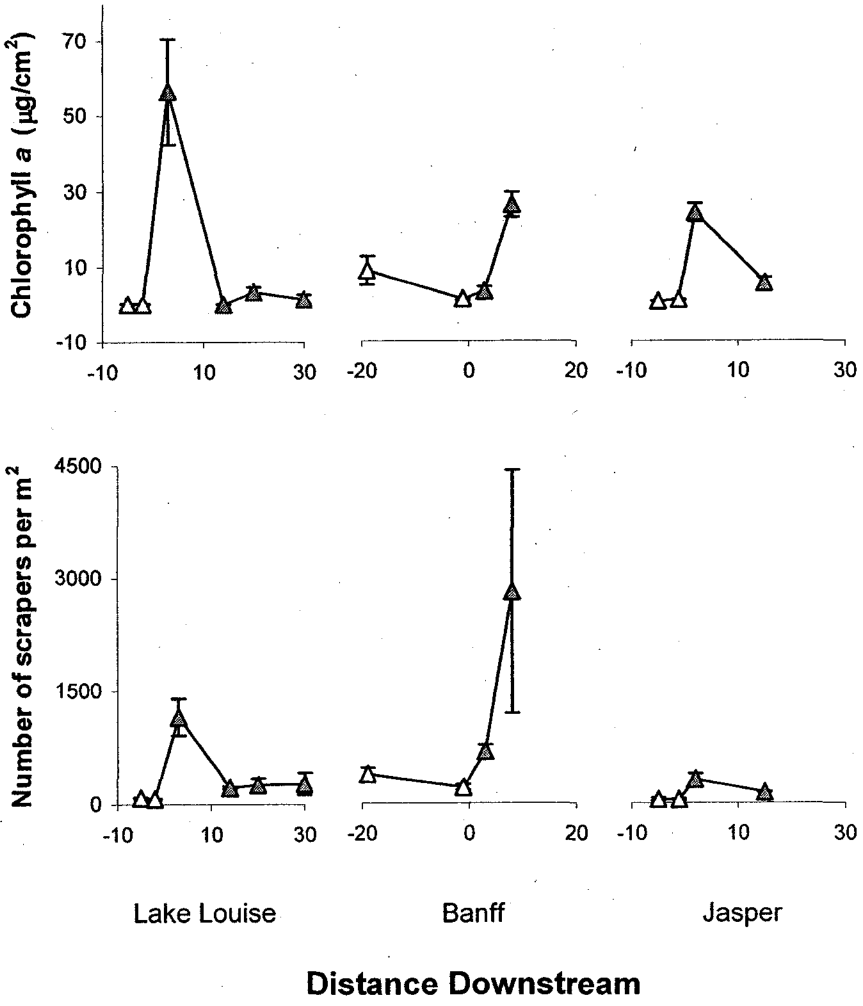

Figure 2-10. Changes in epilithic chlorophyll *a*, and the number of invertebrates that are scrapers in riffles, with distance upstream (open symbols) and downstream (closed symbols) of the Lake Louise, Banff and Jasper municipal wastewater treatment plants in autumn 1999.

Appendix 2-1. Species composition, density and biomass of benthic algae in sites 2-3 km upstream and downstream of the Lake Louise, Banff and Jasper Municipal Wastewater Treatment Plants in autumn 1998 and 1999.

Autumn 1998				Autumn 1999			
Species name	**Group**	**Density** (cells/cm^2)	**Biomass** (µg/cm^2)	**Species name**	**Group**	**Density** (cells/cm^2)	**Biomass** (µg/cm^2)
Lake Louise Upstream							
Cymbella microcephala Grunow	diatom	3953	2.98	*Rivularia* sp.	cyanophyte	3701	5.23
Diatoma elongatum Agardh	diatom	2439	0.39	*Cymbella microcephala* Grunow	diatom	336	0.22
Anomoenies vitrea Ross	diatom	2271	0.90	*Gomphonema minutum*	diatom	202	0.10
Achnanthes minutissima Kutzing	diatom	1009	0.08				
Pseudoanabaena sp.	cyanophyte	673	0.00				
Gomphonema minutum	diatom	589	0.26				
Fragilaria crotonensis Kitton	diatom	505	0.14				
Cymbella prostata (Berkeley) Cleve	diatom	252	0.69				
Eucocconeis sp.	diatom	84	0.49				
Synedra ulna (Nitzsch) Ehrenberg	diatom	84	0.17				
Stauronies sp.	diatom	84	0.15				
Gomphonema angustum Agardh	diatom	84	0.07				
Eunotia sp.	diatom	84	0.07				
Neidium sp.	diatom	84	0.03				
Spondylosium planum (Wolle) W. and G.S. West	chlorophyte	84	0.02				
Lake Louise Downstream							
Achnanthes minutissima Kutzing	diatom	69983	6.16	*Achnanthes minutissima* Kutzing	diatom	41410	2.77
Stigeoclonium sp.	chlorophyte	57197	34.50	*Diatoma elongatum* Agardh	diatom	16564	2.08
Diatoma elongatum Agardh	diatom	31627	4.24	*Pseudoanabaena* sp.	cyanophyte	9938	0.21
Cymbella microcephala Grunow	diatom	30954	23.34	*Cymbella microcephala* Grunow	diatom	7454	5.62
Didymosphenia geminata Schmidt	diatom	26244	979.46	*Cymbella cymbiformis* Agardh	diatom	5797	9.11
Gomphonema minutum	diatom	16150	8.12	*Gomphonema angustum* Agardh	diatom	5797	4.47
Ulothrix sp.	chlorophyte	14131	29.60	*Gomphonema minutum*	diatom	4969	2.50
Cymbella cymbiformis Agardh	diatom	14131	17.02	*Anomoenies vitrea* Ross	diatom	4969	1.92
Gomphonema angustum Agardh	diatom	12112	8.52	*Didymosphenia geminata* Schmidt	diatom	4141	137.86
Anomoenies vitrea Ross	diatom	9421	3.55	*Stigeoclonium* sp.	chlorophyte	4141	2.91

Synedra ulna (Nitzsch) Ehrenberg	diatom	4037	3.55	*Cymbella prostata* (Berkeley) Cleve	diatom	1656	13.13
Eunotia pectinalis (Kutzing) Rabenhorst	diatom	1346	0.89	*Cymbella gracilis* (Rabhorst) Cleve	diatom	828	3.79
Neidium sp.	diatom	673	0.16	*Cocconies* sp.	diatom	828	1.46
Nitzschia filiformis (W. Smith) Hustedt	diatom	673	0.10	*Neidium* sp. a	diatom	828	0.56
Banff Upstream							
Gloeocapsa sp.	cyanophyte	8793	0.12	*Phormidium* sp	cyanophyte	7716	0.22
Stigeoclonium sp.	chlorophyte	4307	5.41	*Stigeoclonium* sp.	chlorophyte	5383	4.33
Achnanthes minutissima Kutzing	diatom	2602	0.20	*Achnanthes minutissima* Kutzing	diatom	3858	0.29
Diatoma elongatum Agardh	diatom	1705	0.26	*Cymbella microcephala* Grunow	diatom	2512	0.82
Anomoenies vitrea Ross	diatom	987	0.45	*Anomoenies vitrea* Ross	diatom	1974	0.63
Synedra ulna (Nitzsch) Ehrenberg	diatom	538	0.47	*Cymbella cymbiformis* Agardh	diatom	1615	0.75
Gomphonema angustum Agardh	diatom	449	0.47	*Rivularia* sp.	cyanophyte	1436	4.06
Cymbella microcephala Grunow	diatom	359	0.26	*Chroococcus limneticus* Lemmermann	cyanophyte	1256	0.18
Fragilaria crotonensis Kitton	diatom	269	0.13	*Phormidium autumnale* Agardh	cyanophyte	1166	3.63
Phormidium autumnale Agardh	cyanophyte	179	0.61	*Diatoma elongatum* Agardh	diatom	807	0.12
Phormidium sp	cyanophyte	179	0.02	*Synedra ulna* (Nitzsch) Ehrenberg	diatom	538	0.27
Eucocconeis sp.	diatom	90	0.35	*Cymbella* sp.	diatom	359	0.38
Neidium sp.	diatom	90	0.28	*Gomphonema angustum* Agardh	diatom	359	0.31
				Gomphonema minutum	diatom	359	0.14
				Euastrum denticulatum Gay	chlorophyte	90	0.50
				Cocconies sp.	diatom	90	0.14
				Eunotia pectinalis (Kutzing) Rabenhorst	diatom	90	0.06
				Neidium sp.	diatom	90	0.04
Banff Downstream							
Phormidium autumnale Agardh	cyanophyte	12235	34.59	*Gloeocapsa* sp.	cyanophyte	8748	0.07
Cymbella microcephala Grunow	diatom	2080	0.79	*Phormidium* sp	cyanophyte	1750	0.05
Fragilaria crotonensis Kitton	diatom	1958	0.72	*Achnanthes minutissima* Kutzing	diatom	471	0.03
Merismopedia punctata Meyen	cyanophyte	1958	0.03	*Phormidium autumnale* Agardh	cyanophyte	135	0.34
Diatoma elongatum Agardh	diatom	1223	0.16	*Anomoenies vitrea* Ross	diatom	67	0.02
Gomphonema minutum	diatom	1101	0.48				
Pseudoanabaena sp.	cyanophyte	1101	0.01				
Cocconies sp.	diatom	979	1.84				
Gomphonema angustum Agardh	diatom	612	0.41				
Achnanthes minutissima Kutzing	diatom	612	0.04				
Eucocconeis sp.	diatom	245	1.27				
Synedra ulna (Nitzsch) Ehrenberg	diatom	245	0.21				

Synedra ulna (Nitzsch) Ehrenberg	diatom	4037	3.55	*Cymbella prostata* (Berkeley) Cleve	diatom	1656	13.13
Eunotia pectinalis (Kutzing) Rabenhorst	diatom	1346	0.89	*Cymbella gracilis* (Rabhorst) Cleve	diatom	828	3.79
Neidium sp.	diatom	673	0.16	*Cocconies* sp.	diatom	828	1.46
Nitzschia filiformis (W. Smith) Hustedt	diatom	673	0.10	*Neidium* sp. a	diatom	828	0.56
Banff Upstream							
Gloeocapsa sp.	cyanophyte	8793	0.12	*Phormidium* sp	cyanophyte	7716	0.22
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Diatoma elongatum Agardh	diatom	1705	0.26	*Cymbella microcephala* Grunow	diatom	2512	0.82
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Synedra ulna (Nitzsch) Ehrenberg	diatom	538	0.47	*Cymbella cymbiformis* Agardh	diatom	1615	0.75
Gomphonema angustum Agardh	diatom	449	0.47	*Rivularia* sp.	cyanophyte	1436	4.06
Cymbella microcephala Grunow	diatom	359	0.26	*Chroococcus limneticus* Lemmermann	cyanophyte	1256	0.18
Fragilaria crotonensis Kitton	diatom	269	0.13	*Phormidium autumnale* Agardh	cyanophyte	1166	3.63
Phormidium autumnale Agardh	cyanophyte	179	0.61	*Diatoma elongatum* Agardh	diatom	807	0.12
Phormidium sp	cyanophyte	179	0.02	*Synedra ulna* (Nitzsch) Ehrenberg	diatom	538	0.27
Eucocconeis sp.	diatom	90	0.35	*Cymbella* sp.	diatom	359	0.38
Neidium sp.	diatom	90	0.28	*Gomphonema angustum* Agardh	diatom	359	0.31
				Gomphonema minutum	diatom	359	0.14
				Euastrum denticulatum Gay	chlorophyte	90	0.50
				Cocconies sp.	diatom	90	0.14
				Eunotia pectinalis (Kutzing) Rabenhorst	diatom	90	0.06
				Neidium sp.	diatom	90	0.04
Banff Downstream							
Phormidium autumnale Agardh	cyanophyte	12235	34.59	*Gloeocapsa* sp.	cyanophyte	8748	0.07
Cymbella microcephala Grunow	diatom	2080	0.79	*Phormidium* sp	cyanophyte	1750	0.05
Fragilaria crotonensis Kitton	diatom	1958	0.72	*Achnanthes minutissima* Kutzing	diatom	471	0.03
Merismopedia punctata Meyen	cyanophyte	1958	0.03	*Phormidium autumnale* Agardh	cyanophyte	135	0.34
Diatoma elongatum Agardh	diatom	1223	0.16	*Anomoenies vitrea* Ross	diatom	67	0.02
Gomphonema minutum	diatom	1101	0.48				
Pseudoanabaena sp.	cyanophyte	1101	0.01				
Cocconies sp.	diatom	979	1.84				
Gomphonema angustum Agardh	diatom	612	0.41				
Achnanthes minutissima Kutzing	diatom	612	0.04				
Eucocconeis sp.	diatom	245	1.27				
Synedra ulna (Nitzsch) Ehrenberg	diatom	245	0.21				

3.0 Epilithic abundance in relation to anthropogenic changes in phosphorus bioavailability and limitation in cold-water rivers

In this study, we assessed the efficacy of various indicators of nutrient limitation in explaining patterns in epilithic abundance in oligotrophic, mid-sized (4-5th at 1:250000 scale) mountain rivers, upstream and downstream of four anthropogenic nutrient point sources. The amount of nutrient loading relative to river discharge varied among rivers and point sources and therefore provided both natural and anthropogenic gradients of nutrient loading to test nutrient limitation measures. Mid-sized, high latitude rivers offer advantages for studying the effects of nutrient limitation on the productivity of lotic ecosystems because they are often intrinsically oligotrophic and relatively free of anthropogenic pollution, are not generally light limited, and have simple food webs (Peterson et al. 1985). The goals of this study were to 1) compare the type and magnitude of epilithon nutrient limitation inferred from nitrogen to phosphorus ratios (N:P) in water and in epilithon tissue, alkaline phosphatase activity (APA) and nutrient diffusing substrata (NDS), 2) determine whether the type or degree of limitation changes as a result of low-level (ppb) eutrophication, and 3) determine whether nutrient limitation measures are related to the gradient in anthropogenic nutrient additions and epilithic accrual.

Eutrophication, as a result of excess inputs of N and P to aquatic systems, commonly results in algal abundance that exceeds that desired aesthetically or for maintenance of ecological integrity. The ability to predict, explain and mitigate changes in benthic algal abundance in rivers is inexact relative to capabilities for phytoplankton in lake ecosystems. Studies on the limitation of riverine benthic algae have concluded that, in the absence of over-riding physical or biological controls, N or P or both generally limit abundance (Chapter 2, Borchardt 1996). However, despite strong evidence for nutrient control of benthic algal growth in many rivers, it is not clear how much variation can be explained by nutrient availability, and which nutrient(s) will limit growth in a given location (Dodds et al. 2002).

The use of nutrient limitation indicators to predict the response of periphyton to changes in nutrient availability in rivers can have poor explanatory capability because N and P concentrations in rivers are highly variable in space and time, are highly correlated, and can co-limit algal growth (Francoeur 2001). Methods used to identify and quantify the nutrient status of algae include measurements of nutrient concentrations in stream water or algal tissue, physiological indicators or growth rates, and artificial enrichment of water or substrate. Each method has advantages and potential drawbacks (Table 3-1). For example, N:P ratios in water or algae are often compared to the theoretical, ideal molar ratio of 16:1 (e.g. Redfield 1958) but precise ratios have been shown to be species specific (e.g., Kahlert 1998). APA measures the ability of organisms (including bacteria and algae) to cleave organically bound phosphorus and is usually indicative of the degree of phosphorus limitation, but starvation for certain proteins can elicit an APA response (e.g., Jansson et al. 1988). Relative algal growth on NDS filled with agar that has been impregnated with nutrients and incubated *in situ* are often used to determine the type (but not the degree) of nutrient limitation in a given location (e.g., Pringle 1987).

In addition to potential problems in interpreting results of a single indicator, measures of nutrient limitation may identify different types or degrees of limitation because of differences in the scale of limitation or nutrient sources. For example, APA reflects cellular nutrient limitation whereas NDS experiments infer community limitation. The concentration of P found to saturate thin biofilms was two orders of magnitude less than the concentration required to saturate benthic diatom mats (Bothwell 1988, 1989). Also, nutrient limitation indicators can reflect nutrient availability in overlying water (water N:P), substrate (NDS) or both (APA, tissue N:P). Periphyton can respond to variations in nutrient supply in the water column or in the substrate alone, or synergistically (Pringle 1987). A comparison of methods is needed to highlight practical limitations and facilitate the use of appropriate methods for various situations and study objectives.

In oligotrophic systems, the P-algal abundance relationship is particularly weak because there is a strong selective advantage for organisms that can use organically bound phosphorus, and tight nutrient spiraling weakens the relationship between water nutrient concentrations and algal abundance. For example, assessment of algal nutrient status from concentrations of total phosphorus (TP) and total nitrogen (TN) in water can overestimate nutrient availability because measures include organically bound fractions that cannot be used by biota. Conversely, the use of soluble nutrient forms (soluble reactive phosphorus (SRP) and dissolved inorganic nitrogen (DIN)) can underestimate bioavailability because certain organic nutrients can be utilized. Furthermore, available limiting nutrient(s) are quickly assimilated by organisms in oligotrophic systems, and therefore may not be detected in measures of river nutrient concentrations. Paul et al. (1991) concluded that because epilithon in rivers with the least bioavailable P re-assimilated the P metabolites most quickly, empirical P-abundance relationships would underestimate algal development in the most oligotrophic rivers.

3.1 METHODS

3.1.1 Study sites

Methods to determine the type (N or P) of nutrient potentially limiting epilithon growth were performed at sites (115° 35′ - 118 ° 05′ N, 51° 10′ - 53° 54′ E, 1000-1500m a.s.l.) upstream and downstream of municipal wastewater treatment plant (MWWTP) discharges to the Athabasca, Bow and Kicking Horse rivers in the Rocky Mountain National Parks of Alberta and British Columbia. The Athabasca River flows through Jasper National Park and receives wastewater from the Town of Jasper. The Bow River is located in Banff National Park and receives wastewater from the towns of Lake Louise and Banff. The Kicking Horse River is located in Yoho National Park and receives wastewater directly from the Town of Field and indirectly from the Emerald Lake resort, which discharges effluent to the Emerald River upstream of its confluence with the Kicking Horse River. Detailed descriptions of the Athabasca and Bow Rivers, MWWTPs, subalpine and montane ecoregions, and sampling protocols are given in Chapter 2. All sampling sites contained at least three, shallow (30cm), fast flowing (0.5m/s) riffles within a 500m reach, and substrate that was dominated by cobble (10-20cm diameter).

In late September (in the Bow River) and early October (in the Athabasca and Kicking Horse Rivers) 2000, when reduced light from riparian shading or high turbidity did not limit algal growth (Chapter 2), one epilithon sample was collected for abundance, one for nutrient content and one for enzyme activity, in each riffle. Three riffles were sampled at each site. Sampling sites were located approximately 2 km upstream and 3 km downstream of each MWWTP effluent discharge. In the case of the Kicking Horse River, downstream samples were collected below the confluence of the Emerald River and reflect impacts from both the Field and Emerald Lake MWWTPs, except for APA that was sampled above the confluence. Water chemistry samples were collected once in August, and once in September or October 1998-2001. Nutrient diffusing substrata experiments in the Athabasca and Bow Rivers were performed in October 1998 and 1999, at the same riffle sites.

3.1.2. Water Chemistry

Water samples were collected and analyzed for TP, SRP, nitrate+nitrite-N, and ammonia-N following modified standard methods (University of Alberta Limnology Lab 2003, Chapter 2). Concentrations of nitrate+nitrite-N and ammonia-N were summed to given dissolved inorganic N (DIN). Expected water chemistry concentrations in rivers were derived by multiplying mean, monthly (August-October) concentrations in MWWTP effluent by the MWWTP effluent to river discharge ratio, and adding upstream river concentrations. We calculated expected water chemistry concentrations because a high proportion of N and P in MWWTP effluent is bioavailable, rapidly assimilated by biota in oligotrophic systems, and therefore may be difficult to detect at downstream sites. Measured and expected molar N:P ratios in river water were compared with previously published nutrient limitation thresholds (all ratios are reported on a molar basis). The results of numerous nutrient enrichment studies have confirmed that benthic algae are P-limited at DIN:SRP >20, and N-limited at DIN:SRP < 10 (See Borchardt 1996). There are no DIN:TP nutrient limitation thresholds published for benthic algae but the growth of phytoplankton in a mountain lake in Colorado was P-limited at lake water concentrations of DIN:TP > 12, N-limited below 0.5, and co-limited by N and P between (Morris and Lewis Jr. 1988).

3.1.3. Elemental composition

Composite 20mL samples of epilithon were removed with a scalpel from three randomly chosen rocks in a riffle, at three riffles per site (for a total of three samples per site). Bryophytes were only abundant at the site downstream of Lake Louise, interfered with APA analysis, and were therefore excluded from samples. Epilithon samples for tissue nutrient content were freeze dried at -60°C (Edwards Super Modulyo Freeze Drier), ground to a uniform powder with a mortar and pestle, dried at 90°C to a constant weight, and stored in a desiccator. Between 0.1-0.2g (±0.0001g) of epilithon sample was weighed for analysis of percent phosphorus and 1-4μg (±0.0001μg) of epilithon sample was used for analyses of percent nitrogen and carbon. Phosphorus was analyzed using persulfate oxidation followed by analysis using the acid molybdate technique (APHA 1992). The amount of carbon and nitrogen in samples was measured using a Model 440 Elemental Analyzer (Control Equipment Corporation). Benzoic acid and SRM NIST 1575 apple leaves (U.S. National Institute of Standards and Technology) were used as quality control standards for elemental analyses. All epilithon elemental ratios are expressed on a molar basis. Because there is a lack of data relating benthic algal nutrient content to nutrient status (Kahlert 1998), we compared molar N:P of epilithon to values for derived for lake phytoplankton found to have severe P deficiency with tissue $N:P > 44$, moderate P deficiency $44 > N:P > 22$, and no deficiency $N:P < 22$ (Healy and Hendzel 1980).

3.1.4. Alkaline phosphatase activity

A composite sample of epilithon taken from a 9.6cm^2 area on each of three cobbles was used to measure APA and chlorophyll *a*; as with samples for epilithic nutrient content, three samples per site were collected, and bryophytes were excluded. APA was measured following the phytoplankton method of Waiser and Bothwell (1988, *unpublished*) modified for use with epilithon samples, using information in the literature (Perry 1972, Healy and Hendzel 1979, Jansson et al. 1988, Pettersson 1980, Steinman and Mulholland 1996, Johnson et al. 1998). The epilithon sample was suspended in sterile filtered river water, refrigerated, and analyzed within six hours. Samples were slowly agitated with a syringe to break up clumps, and filtered on to GF/C filters (to collect bacteria, *unpublished data*). Next, one half of the sample was re-suspended in sterile filtered river water, and heated to 35°C in a water bath. Epilithon samples and blanks of filtered river water were saturated with 10uM 4-methylumbelliferyl phosphate (MUP). Fluorescence occurs when phosphate is enzymatically cleaved from MUP. Fluorescence was measured at increasing time intervals, with a fluorometer (Turner Designs 10-AU-005), until APA production over time stabilized. The fluorometer was set up with stacked emission filters (top - dominant λ 479.8, middle - dominant λ 569.5, bottom - 10% neutral density) and the ultra-violet lamp was set up with an excitation filter and a reference filter.

Enzyme activity was normalized to chlorophyll *a*. To measure chlorophyll *a*, the remaining half of the composite epiphyton sample was frozen and subsequently thawed, blended with double distilled water for ten seconds, filtered onto GF/F filters, and extracted in boiling, 90% ethanol for seven minutes. Concentrations of chlorophyll *a* were measured using a fluorometer (Turner Designs, Model 10). Based on comparisons between P-sufficient and P-deficient phytoplankton assemblages, Healy and Henzel (1979) concluded that APA (nmole per μg chlorophyll *a* per hour) >5 was indicative of severe P deficiency, 3-5 was indicative of slight deficiency, and <3 was indicative of P replete conditions.

3.1.5. Nutrient diffusing substrata

Clay pots were filled with agar made with phosphorus (0.5M KH_2PO_4), nitrogen (0.5M $NaNO_3$, in 1998 only), or phosphorus plus nitrogen solutions, or unamended agar, and sealed (For detailed methods see Scrimgeour and Chambers 1997). Ten replicates of each NDS treatment were incubated *in situ* for the month of October (except for only 15 days near Jasper, in 1998, due to a rapidly falling hydrograph), at sites both upstream and downstream of the MWWTPs. Epilithon samples were removed for chlorophyll *a* analyses and a subset of samples were analyzed for C:N:P content and APA. Hourly water temperature was recorded during each experiment using a thermister (Onset), light attenuation was measured at the start and end of each experiment with a light meter (Licor), and velocity at each NDS was recorded with a current meter (Pygmy).

3.1.6. Statistical analyses

Data were log transformed prior to analyses to correct for positive correlation of variance and mean, if necessary. Significant declines in C:P, C:N, and N:P composition and APA of epilithon between upstream and downstream of MWWTPs, were identified using paired, one-tailed *t* tests. Results of the NDS experiments were evaluated using one-way ANOVAs. Correlations were calculated for the various measures of nutrient limitation with one another, to chlorophyll *a* and algal biovolume, and to nutrient concentrations in river water (measured and estimated TP, SRP and DIN).

3.2 RESULTS

At upstream sites, average TP concentrations over the period of algal accrual (August-October) ranged from 3.1-30μg/L and were lowest near Field, and progressively higher near Lake Louise, Banff and Jasper (Fig.3-1). Much of the TP at sites near Jasper is associated with periods of high turbidity in summer and would not be biologically available (e.g. bound with glacial flour), but concentrations of SRP at all sites were highly correlated with concentrations of TP ($r=0.8$, $p<0.05$). Concentrations of TP and DIN, both measured and estimated, were generally slightly elevated with respect to upstream sites (by 0.0-5.6μg/L), with the exception of the site downstream of Banff which was greatly elevated (Fig.3-1). With the exception of the site downstream of Banff, TP concentrations measured downstream of MWWTPs were within 17-23% of estimated values. Estimated DIN concentrations were significantly different from those measured, except near Jasper. On average, ammonia-N was 33.0, 24.4, and 51.8 percent of DIN upstream, and 30.6, 43.0 and 58.3 percent downstream of Lake Louise, Banff and Jasper, respectively.

As expected, there were significant ($p<0.05$) increases in chlorophyll *a* downstream of all MWWTP effluent discharges (Fig.3-2a). With the exception of the site downstream of Field, there were also increases in algal biovolume at all downstream sites (Fig.3-2a). However, at a given TP (or SRP) concentration, downstream sites always had higher chlorophyll concentrations (or algal biovolume) than upstream sites

(Fig.3-2b). Comparisons of upstream molar DIN:TP and DIN:SRP, and reported P limitation threshold values (DIN:TP>12:1, Morris and Lewis Jr. 1988; DIN:SRP>20:1, Borchardt 1996) indicated that epilithon at all upstream sites should be limited by availability of P (Fig.3-1, Table 3-2). Both N:P ratios also indicated that epilithon at sites downstream of Field and Lake Louise would remain P-limited (Fig.3-1). However, downstream of the Banff and Jasper MWWTPs, N:P ratios were consistently low (with one exception) and indicative of P saturation (Fig.3-1). The one exception was downstream of the Jasper MWWTP where DIN:SRP indicated slight P limitation: DIN:SRP was closer to the threshold value of 20 (95% confidence interval 23-27) than at other P-limited sites (60-137 DIN:SRP).

Internal measures of nutrient limitation (tissue C:N:P, APA) also showed that epilithon accrual was more limited by P availability at sites upstream of MWWTPs than at downstream sites. Carbon to phosphorus (C:P), carbon to nitrogen (C:N), and nitrogen to phosphorus (N:P, except near Jasper) ratios of epilithon were higher at sites upstream than downstream of MWWTPs (Fig.3-3, Table 3-3). Nutrient ratios in epilithon collected from cobble and NDS were consistent. C:P and N:P were 4-6 fold lower for epilithon collected from substrata emitting P and N+P than for epilithon collected from control NDS (Fig.3-3). However, comparison of tissue N:P with reported threshold values for phytoplankton (22:1, Healy and Hendzel 1980) suggested that all sites were P-limited (N:P = 40-435), except the site upstream of Jasper (N:P = 8, Table 3-2).

Average APA of epilithon at upstream sites was high relative to sites downstream of the Lake Louise, Banff and Jasper MWWTPs (Fig.3-4a, Table 3-3). APA of epilithon from all upstream sites and the site downstream of Field was >5μg nmole per μg chlorophyll *a* per hour, indicative of severe P limitation (Fig.3-4, Table 3-2). APA downstream of Lake Louise was in the 3-5μg nmole per μg chlorophyll *a* per hour range indicative of slight P limitation while APA at sites downstream of Banff and Jasper were <3, indicative of P replete conditions. As expected, epilithon grown on NDS emitting P or N+P had APA in the range considered P-replete (Fig.3-4). There was not enough algae on control NDS to measure APA. Consistent with trends in algal abundance, APA at a given TP concentration was higher upstream than downstream of MWWTPs (Fig.3-4b).

NDS experiments showed algal growth was consistently N and P saturated at sites downstream of Jasper and Banff (Fig.3-5). Upstream of Jasper and Banff, inavailability of P or N+P limited algal abundance in 1999; the same trend, although not statistically significant, was also observed, in 1998. Algal accrual upstream and downstream of Lake Louise was also constrained by insufficient P or N+P, except upstream in 1999 when there was very little algal growth on NDS. The results of the NDS experiment near Field were inconclusive due to high variability within treatments and very low algal growth at upstream sites, both of which limited statistical resolution. Phosphorus limited algal abundance downstream of Field and the Emerald River confluence; the same trend, although not statistically significant, was also observed downstream of Field but above the Emerald River confluence. During incubations of NDS, water temperatures were 3-8°C in 1998, 3-5°C in 1999, and 3-8°C in 2000. There was no variation in light attenuation or temperature between sites upstream and downstream of nutrient point sources in 1998-1999, and average velocity did not vary among NDS treatments within a site ($p>0.05$, *unpublished data*).

Measures of algal abundance (chlorophyll *a* and biovolume) were highly correlated ($r > 0.7$) with DIN:SRP, estimated DIN:TP, C:P and C:N of epilithon, and APA (Table 3-4). Within categories (measures of algal abundance, nutrient concentrations, nutrient limitation measures), there were significant correlations between 1) the two measures of algal abundance, 2) TP and all other water chemistry measures except DIN, and 3) tissue C:P and N:P; NDS C/P and C/NP; and APA and tissue C:P and NDS C/P.

3.3 DISCUSSION

Nutrient concentrations in river water and epilithic nutrient content, enzyme activity, and abundance on artificially enriched substrata showed that benthic algal accrual in three oligotrophic mountain rivers was constrained by insufficient P at sites upstream of significant pollution sources. In contrast, sites downstream of MWWTPs showed a variable response, from severe P limitation to P saturation. Our findings that algal growth in relatively pristine Rocky Mountain Rivers is P-limited and that addition of P alleviates nutrient constraints are consistent with earlier findings (e.g., Bothwell 1989, Scrimgeour and Chambers 2000). Our results are also consistent with reviews of freshwater nutrient limitation (Borchardt 1996, Franceour 2001) that conclude the likelihood of P or N limitation or saturation of periphyton is dependant on watershed geology and land use. We found algal abundance in oligotrophic mountain rivers increased and the degree of nutrient limitation decreased along a natural gradient in P (upstream of MWWTPs) as well as in response to anthropogenic P loading.

Nutrient concentrations in river water and their ratios (with one exception) accurately predicted whether algal abundance at a site would be P-limited or P-saturated (Table 3-2). The one exception was DIN:SRP, where values indicative of P limitation occurred downstream of Jasper when three other measures of nutrient limitation suggested nutrient saturation. The site downstream of Jasper was the most nutrient rich site. These results are consistent with the findings of Borchardt's (1996) review of nutrient limitation studies. Borchardt concluded that water nutrient ratios are indicative of nutrient limitation provided absolute nutrient concentrations are less than growth-limiting levels. However, absolute concentrations of SRP (mean = 7.0μg/L, s.e. = 5.6) at all sites were considerably less than concentrations found by others to saturate benthic periphyton mats (>30-50μg/L, Bothwell 1989; >25-35μg/L, Chambers et al. 2000). In contrast, concentrations of DIN at all sites (mean=55-276μg/L) were in excess of those shown to saturate periphyton in nearby river reaches (DIN>50μg/L, Chambers et al. 2000). Because high N concentrations would cause elevated N:P at all sites, the ability to detect a shift between P limitation and saturation using N:P would not be precise in N-replete systems such as the mountain rivers. Consistent with previous findings in lake ecosystems (Axler et al. 1994, Morris and Lewis 1988), nutrient limitation predictions based on DIN:TP agreed with other measures of nutrient limitation, and may be more indicative of bioavailable fractions of N and P than DIN:SRP.

APA was the only measure of nutrient limitation studied that 1) directly measured the propensity of benthic organisms to cleave organically bound P, 2) accurately discriminated various degrees of P limitation, and 3) strongly and consistently correlated to both P concentrations in river water and algal abundance (Table 3-4). Although most knowledge of APA as a P-deficiency indicator comes from work on phytoplankton in lakes and oceans (e.g., Healey and Hendzel 1979), it has been shown experimentally that APA increases P supply to algae in streams (Klotz 1985, 1991), and APA of epilithon is inversely related to stream water N:P (Klotz 1992). Consistent with a general review of APA as a P-deficiency indicator (Jansson et al. 1988), our results showed that APA was inversely related with SRP and TP concentrations, and cellular P. Healy and Henzel (1980) experimentally evaluated nine indicators of nutrient deficiency in lake phytoplankton and concluded that APA and algal N:P would be useful for evaluating the degree of P limitation and therefore, the effectiveness of potential strategies to mitigate nutrient pollution.

In contrast with algal nutrient status identified by water chemistry and APA, predictions based on epilithic N:P at the three most P-rich sites (average water DIN:SRP 8-60) were not consistent with other measures (Table 3-2). Francoeur et al. (1999) also found that cellular nutrient content of periphyton was of limited use for predicting the nutrient that limited NDS bioassays in relatively P-rich rivers (water DIN:SRP, 7-57). Conversely, in experiments using extremely P-poor river water (DIN:SRP, 100-231), the degree of P limitation based on periphytic N:P was consistent with other measures of nutrient limitation, including APA and cellular C:P (Bothwell 1985).

In contrast with N:P, epilithic C:P was highly correlated with algal abundance, river water P concentrations, and APA of epilithon. C:P ratios of benthic algae identified as P-replete were substantially higher for mountain rivers (C:P<440) than for benthic algae from other systems (C:P<158, Kahlert 1998; C:P<119 Hillebrand and Sommer 1999) but C:P ratios of epilithon identified as P-limited (C:P>720) were not inconsistent with the C:P of phytoplankton collected in a highly oligotrophic lake (250-1750, Elser and George 1993). Thus, algal communities adapted to lower nutrient concentrations will have sufficient nutrient supplies at higher C:P than algal communities in more P-replete systems (e.g., Hecky et al 1993).

Patterns in epilithic C:N ratios in mountain rivers are also consistent with strong P-limitation of both epilithon and scrapers. Scrapers can increase the N content and thus decrease the C:N of periphyton (e.g., Hunter 1980) by preferentially excreting higher proportions of non-limiting nutrients (Sterner 1990). Our observation that C:N content of epilithon was negatively correlated with abundance of algae (Table 3-5) and dominant scrapers (r = -0.63, p = 0.03, Chapter 2) but not correlated with water N concentrations nor measures of nutrient limitation suggests periphyton-scraper interactions likely affect nitrogen cycling in strongly nutrient limited rivers.

The nutrient content of epilithon grown on NDS was consistent with our view that 1) changes in C:P, N:P and APA but not C:N were caused by nutrient enrichment, and 2) the response of epilithon to nutrients supplied in the substrate and water column were additive. The observation that epilithon on P and N+P NDS treatments had low C:P, N:P and APA relative to control NDS confirms that epilithon respond to substrate nutrients. Conversely, epiphytic C:N ratios were the same on control and treatment NDS, and were the same or higher on NDS incubated downstream of MWWTPs relative to upstream. The nutrient content of epilithon accrual on NDS was consistent with the response of epilithon receiving nutrients from the water column in that epilithic C:P and N:P on both natural substrata and NDS (especially control NDS) were lower downstream of MWWTPs. Similarly, Pringle (1990) found that the response of diatoms receiving nutrients from both the substrate and water column was additive.

Although the nutrient content of epilithon on NDS was consistent with other observations, the amount of epilithon on control relative to treatment NDS was not always consistent with other measures of nutrient limitation, and was not highly correlated to P concentrations in river water or algal abundance. Discrepancies occurred at the sites downstream of Lake Louise and upstream of Banff, when NDS experiments suggested co-limitation by P and N, but all other nutrient limitation measures indicated various degrees of P limitation (Table 3-2). These sites were mid-way in the gradient of river P concentrations, representing the transition between P limitation and P saturation. The fact that the site downstream of Lake Louise was slightly P-limited (based on APA), and the site upstream of Banff was moderately P-limited (based on tissue N:P) supports the idea that these sites are closer to the P limitation threshold than other sites. Therefore, like N:P ratios (Borchardt 1996), the use of NDS near nutrient limitation thresholds may not be as precise as other measures of nutrient limitation.

However, growth of epilithon on NDS highlights the fact that the response of multi-species communities to enrichment with a single nutrient is less frequent and detectable than the response to enrichment by both N and P (Elser et al. 1990, Francoeur

2001). In our study, average accrual was greatest on N+P NDS treatments in 11 of 16 experiments. Similarly, instances where periphyton communities respond to combined N+P enrichment more frequently and more substantially than to addition of N or P alone have been documented in sub-alpine rivers in the U.S. (Wellnitz et al. 1996, Toetz 1999) and Australia (Chessman et al. 1992), and in glacially fed rivers in Canada (Scrimgeour and Chambers 2000). This is expected in multi-species communities because nutrient ratio optimums are species specific (Rhee and Gothman 1980). Simultaneous limitation of different species in the same benthic algal community by different nutrients is thought to be common (Francoeur 2001).

In conclusion, estimated DIN:TP and APA thresholds most accurately identified P limitation of epilithon, and were consistently correlated with the nutrient gradient and algal abundance. Both DIN:TP and APA reflect the ability of epilithic organisms to use organically-bound P, when P is limiting. Given the low concentrations of bioavailable P in mountain rivers (< 2 µg/L SRP), use of indicators that incorporate the full range of P available to primary producers is critical when assessing nutrient status of these systems. This study also illustrated that the thresholds of DIN:TP and APA developed to predict nutrient status of phytoplankton are appropriate for assessing nutrient limitation thresholds of benthic algae. APA is particularly useful because it identified the degree of P limitation along the eutrophication gradient. More accurate calibration of other measures of nutrient limitation, such as internal nutrient ratios, would also be valuable (e.g. epilithic C:P for detection of P limitation). Because algal abundance was correlated with the degree of P limitation, mitigation of P loading is expected to decrease algal abundance in the mountain rivers.

However, the observation that for a given nutrient concentration, benthic algae were more abundant, had lower APA and exhibited less nutrient limitation at sites downstream than upstream of effluent discharges shows that concentrations of P alone are not predictive of the abundance of benthic biota. Because the use of phosphatase enzymes is metabolically costly, organisms produce APA only when bioavailable P is scarce. Therefore, lower APA at downstream sites suggests P is more bioavailable downstream than upstream of effluent discharges. When P is more available to organisms, the need to produce APA decreases, less P per unit growth is needed because organisms have higher growth efficiencies (lower metabolic costs), and the degree of P limitation declines. More bioavailable P downstream of MWWTPs explains why higher algal abundances were observed downstream of effluent discharges even though the concentrations of nutrients were comparable to sites above MWWTPs. Previous work on phytoplankton in lakes and oceans has also clearly shown that 'new' nutrients in a system can stimulate 'new' productivity (e.g. Caraco et al 1992). In addition, when nutrient limitation declined, epilithic C:P decreased, and the growth efficiencies of consumers also increased (Chapter 4). These results suggest that invertebrate consumers could enhance P limitation of epilithon at the most P-limited sites by preferentially retaining P relative to N but would not enhance P limitation at the most nutrient rich sites. Therefore, an improved understanding of nutrient bioavailability, limitation and trophic transfer will improve the ability to explain patterns in periphyton accrual along nutrient gradients in oligotrophic rivers.

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Table 3-1. Main advantages and potential considerations when using ratios of nitrogen to phosphorus (N:P) concentrations in water and algal tissue, alkaline phosphatase activity (APA), or nutrient diffusing substrata (NDS) to determine the nutrient limiting benthic algal abundance.

Measure	Main Advantages	Considerations
Water N:P	Predictable hyperbolic response of algal growth potential to changes in N:P ratio (e.g. Schanz and Juon 1983)	Not relevant when nutrient replete, may not reflect cellular nutrient ratios, small changes undetectable (Borchardt 1996) Does not reflect tight periphyton-substratum nutrient cycling (Pringle 1987) Can be only weakly predictive (Francoeur et al. 1999)
Tissue N:P	Robust community optimum (Redfield 1958) Reflects food quality for higher trophic levels (e.g. Sterner and Hessen 1994, Stelzer and Lamberti 2002)	Optimum is species specific (Rhee and Gothman 1980) Grazing can change ratios (Hunter 1980) Growth rate dependent (McMohon et al. 1974) Can be only weakly predictive (Francoeur et al. 1999)
APA	Inverse function exists between APA and PO_4, TP and cellular TP (See Jansson et al. 1988)	Generally measures maximum potential APA production, difficult to completely separate from APA produced by bacteria and lysed cells, variable among species, also occurs in response to starvation for pyrimidines or for guanine, diurnal cycles can occur (Jansson et al. 1998)
NDS	Used successfully to address diverse theoretical and applied questions in benthology (Scrimgeour and Chambers 1997) Early pulse mimics allochthonous decay (Pringle and Bowers 1984, Pringle 1987)	Diffusion low and inconsistent, pores may clog, sorb P, contain P binding agents (Pringle 1997) Difficult to partition species along nutrient supply ratios (Borchardt 1996)

Table 3-2. The type of epilithon nutrient limitation (P-phosphorus, N-nitrogen, co-limitation, or N and / or P saturation) concluded from molar nitrogen to phosphorus ratios (N:P) in water and epilithon tissue, alkaline phosphatase activity (APA) and nutrient diffusing substrata (NDS) experiments. Discrepancies are italized.

Site	**DIN:TP**	**DIN:SRP**	**tissue N:P**	**APA**	**NDS**[e]
P-limitation Threshold	>12[a]	>20[b]	>22[c]	>3[d]	
Field u/s	P	P	severe P	severe P	***sat.***
Field d/s	P	P	severe P	severe P	P
L. Louise u/s	P	P	severe P	severe P	P
L. Louise d/s	P	P	severe P	slight P	***co-lim.***
Banff u/s	P	P	moderate P	severe P	***co-lim.***
Banff d/s	sat.	sat.	***moderate P***	P sat.	sat.
Jasper u/s	P	P	***P sat.***	severe P	P
Jasper d/s	sat.	***P***	***moderate P***	P sat.	sat.

[a] Morris and Lewis Jr. (1988)
[b] Borchardt (1996)
[c] Healy and Hendzel (1980), Hillebrand and Sommer (1999)
[d] Healy and Hendzel (1979)
[e] Significant in Tukey's test

Table 3-3. Paired, one-tailed t-tests comparing molar ratios of carbon:phosphorus (C:P), carbon:nitrogen (C:N), nitrogen:phosphorus (N:P), and alkaline phosphatase activity (APA) in epilithon collected in October 2000, upstream and downstream of the Field, Lake Louise, Banff and Jasper wastewater treatment plants.

		Mean	Variance	t	df	p
C:P	u/s	4991	2336	3.83	4	0.016
	d/s	840	304			
C:N	u/s	69.1	25.6	2.68	4	0.037
	d/s	18.6	5.4			
N:P	u/s	148	97.5	0.56	4	0.308
	d/s	43.1	5.8			
APA	u/s	13.6	2.6	2.09	4	0.064
	d/s	6.9	5.3			

Table 3-4. Correlations between measures of algal abundance (chlorophyll *a*, algal biovolume), measured and estimated water nutrient concentrations, elemental composition of epilithon (C:P, C:N, N:P), amount of chlorophyll on control divided by treatment nutrient diffusing substrata (C/P, C/NP), and alkaline phosphatase activity (APA). Correlations with $p<0.05$ are in bold. Sign indicates expected relationship with eutrophication gradient. Multiplying row and column sign gives expected sign of relationship between parameters.

				Measured					Estimated					Tissue			NDS	
		Chla.	Biovol.	TP	SRP	DIN	DIN :SRP	DIN :TP	TP	SRP	DIN	DIN :SRP	DIN :TP	C:P	C:N	N:P	C/P	C/NP
		+	+	+	+	+	-	-	+	+	+	-	-	-	-	-	+	+
Biovolume	+	**0.92**																
Measured in River Water																		
TP	+	0.66	**0.72**															
SRP	+	0.62	0.54	**0.77**														
DIN	+	0.49	0.35	0.51	**0.88**													
DIN:SRP	-	**-0.71**	-0.69	**-0.88**	**-0.92**	-0.64												
DIN:TP	-	-0.55	-0.58	**-0.93**	**-0.75**	-0.38	**0.91**											
Estimated for River Water																		
TP	+	0.61	**0.76**	**0.87**	0.37	0.10	-0.60	**-0.76**										
SRP	+	0.67	0.67	**0.88**	**0.96**	**0.79**	**-0.96**	**-0.84**	0.57									
DIN	+	0.39	0.49	0.22	0.29	0.33	-0.23	0.01	0.18	0.31								
DIN:SRP	-	-0.44	-0.36	**-0.70**	-0.70	-0.50	**0.76**	**0.81**	-0.47	**-0.74**	0.41							
DIN:TP	-	**-0.72**	**-0.73**	**-0.86**	-0.56	-0.25	**0.78**	**0.89**	**-0.85**	-0.70	0.13	**0.80**						
Tissue Nutrient Content																		
C:P	-	**-0.76**	**-0.86**	**-0.80**	-0.56	-0.52	0.60	0.55	**-0.80**	-0.70	-0.47	0.39	0.66					
C:N	-	**-0.71**	**-0.76**	-0.36	-0.44	-0.31	0.54	0.31	-0.33	-0.52	-0.39	0.28	0.42	0.50				
N:P	-	-0.33	-0.40	-0.64	-0.30	-0.37	0.27	0.38	-0.67	-0.41	-0.24	0.24	0.43	**0.78**	-0.15			
Nutrient Diffusing Substrata																		
C/P	+	0.60	0.50	0.57	**0.74**	0.48	**-0.82**	**-0.73**	0.31	**0.71**	0.05	-0.65	-0.62	-0.26	-0.31	-0.01		
C/NP	+	0.49	0.32	0.38	0.61	0.41	**-0.65**	-0.57	0.12	0.53	-0.15	-0.62	-0.51	-0.08	-0.16	0.09	**0.96**	
Alkaline Phosphatase Activity																		
APA	-	**-0.90**	**-0.91**	**-0.85**	**-0.76**	-0.51	**0.88**	**0.79**	**-0.74**	**-0.84**	-0.44	0.53	**0.79**	**0.77**	0.59	0.42	**-0.76**	-0.59

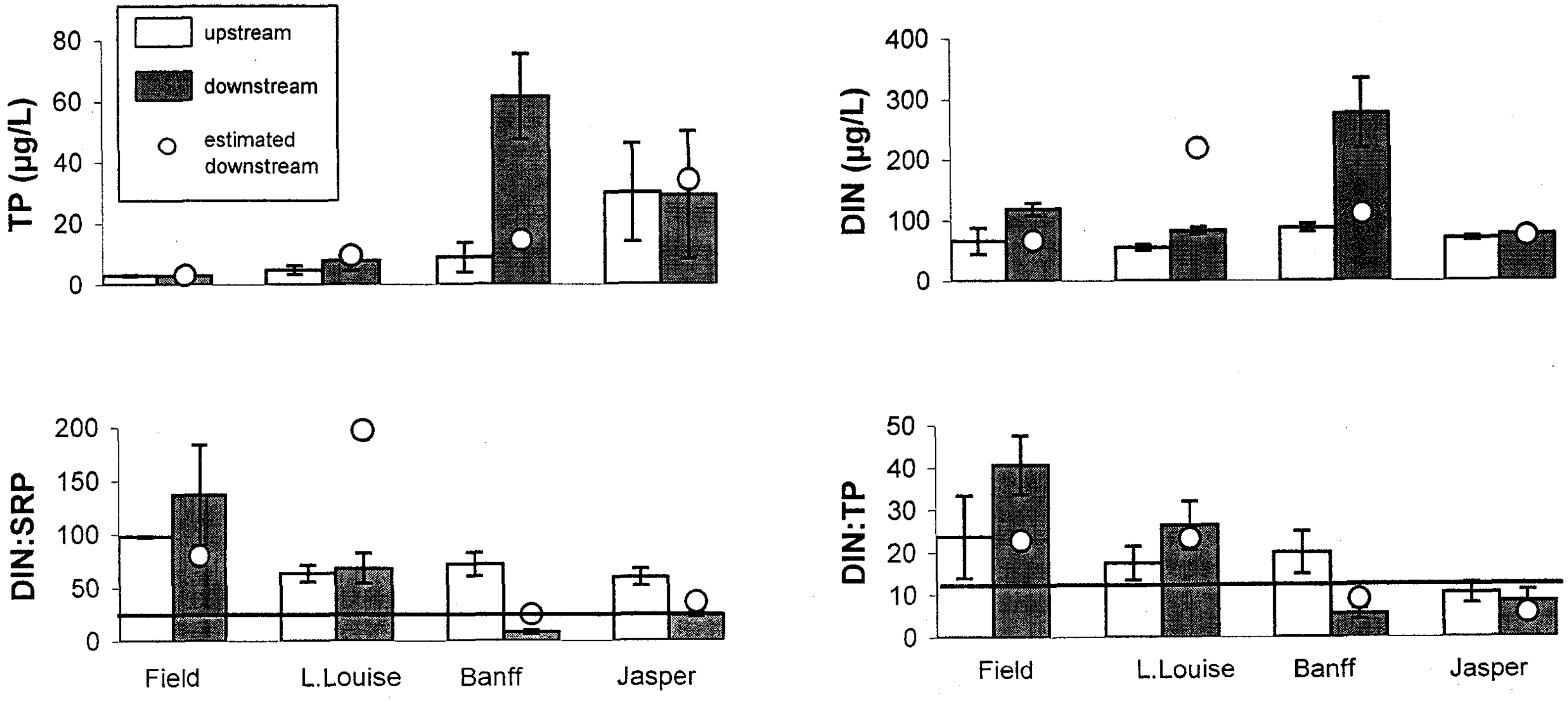

Figure 3-1. Measured (± one standard error) and estimated total phosphorus (TP), dissolved inorganic nitrogen (DIN), DIN:TP, and DIN:SRP upstream and downstream of wastewater discharges from Field, Lake Louise, Banff and Jasper. Means calculated from monthly samples from August-October 1998-2001. Horizontal lines are nutrient limitation thresholds for DIN:SRP and DIN:TP.

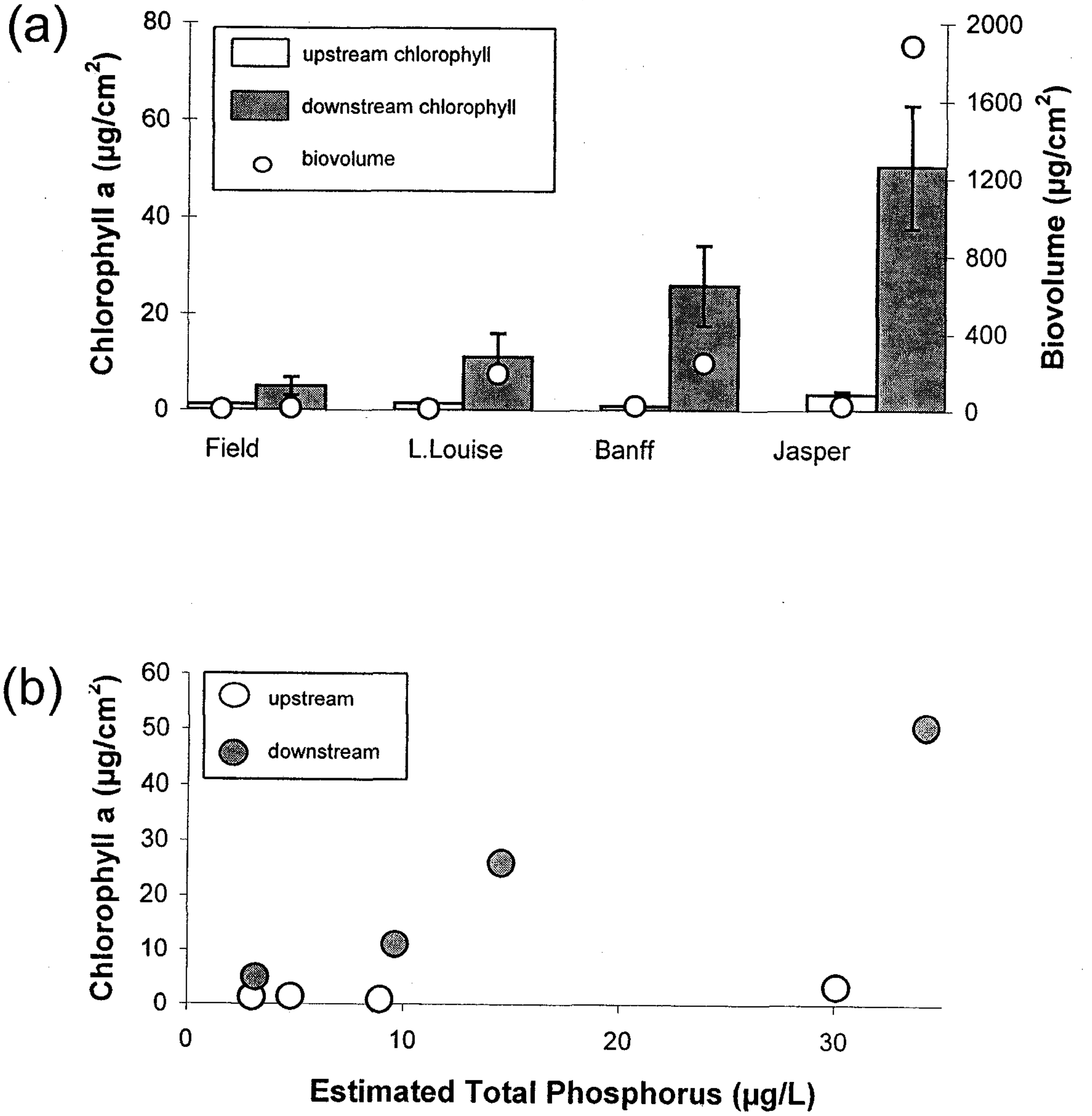

Figure 3-2. (a) Epiphytic chlorophyll *a* (± one standard error) and biovolume at sites upstream and downstream of MWWTPs in Field, Lake Louise, Banff and Jasper in autumn 2000. (b) Chlorophyll *a* versus estimated total phosphorus concentrations at upstream and downstream sites in autumn 2000.

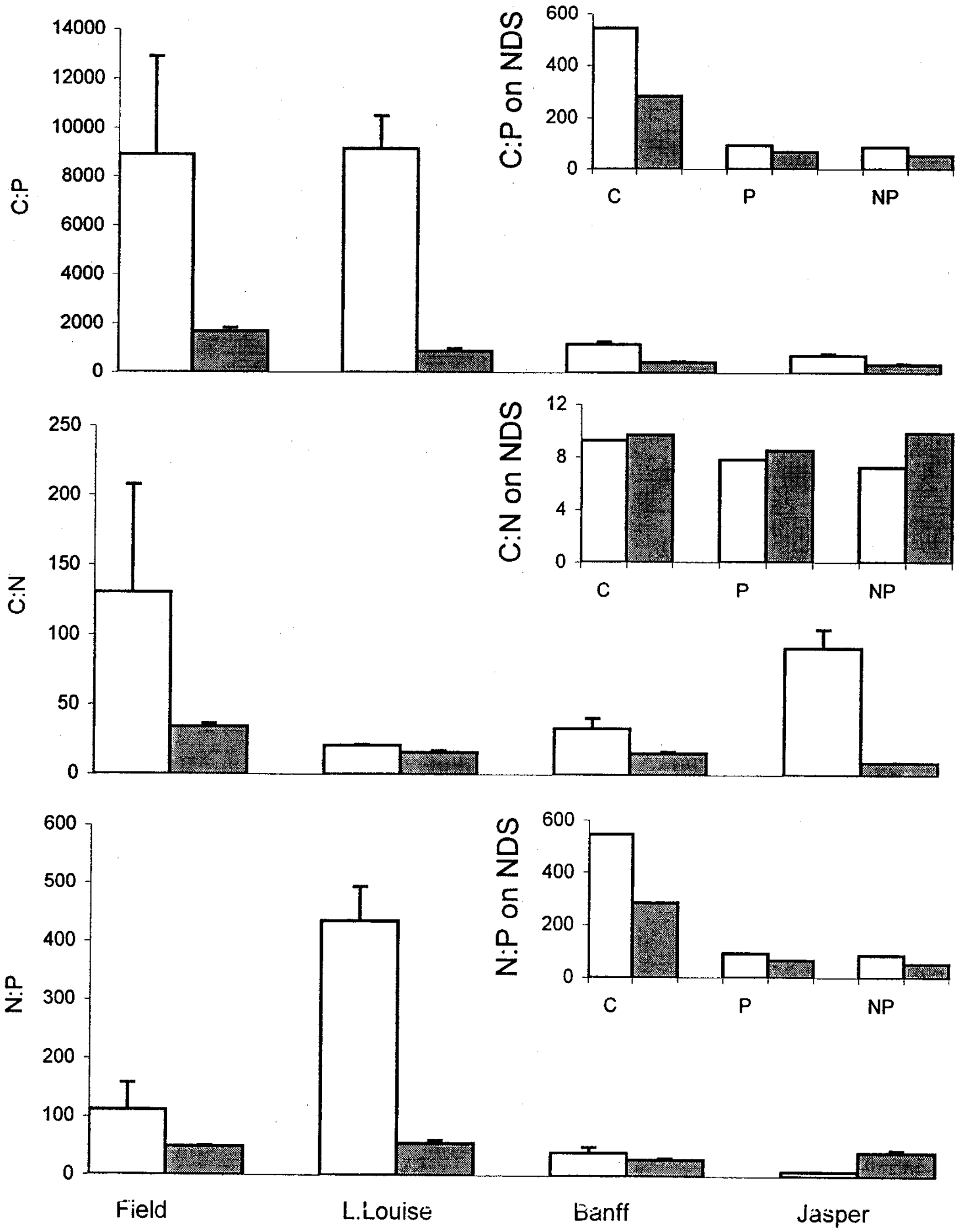

Figure 3-3. Molar ratios (+ one standard error) of carbon to phosphorus (C:P), carbon to nitrogen (C:N), and nitrogen to phosphorus (N:P) of epilithon sampled upstream (open bars) and downstream (shaded bars) of wastewater discharges, on natural substrates in autumn 2000. Inset graphs are epilithon on control (C), phosphorus (P) and N plus P (NP), nutrient diffusing substrata (NDS) treatments.

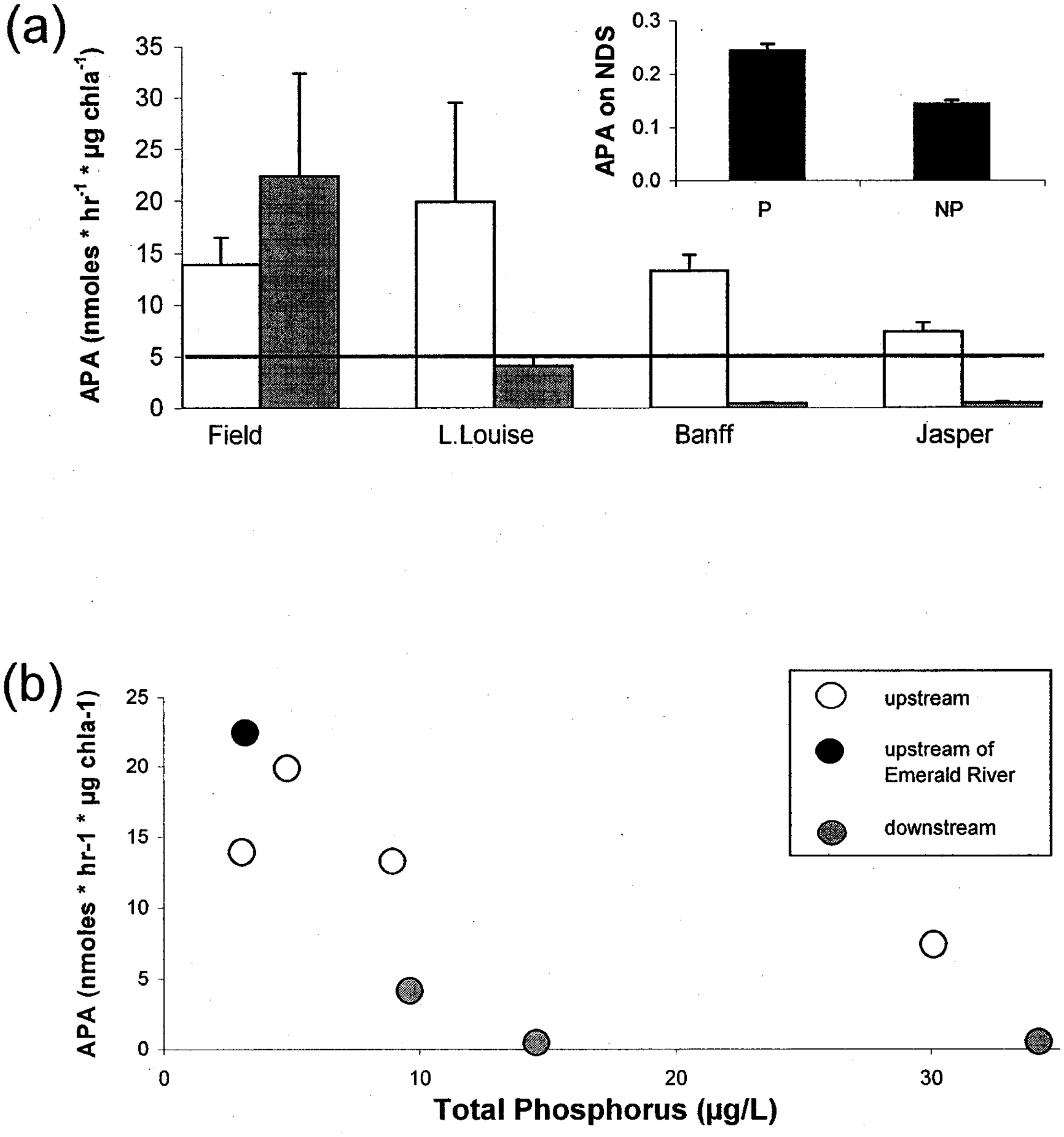

Figure 3-4. (a) Alkaline phosphatase activity (APA) of epilithon (+ one standard error) sampled upstream (open bars) and downstream (shaded bars) of wastewater discharges, on natural substrates in autumn 2000. Horizontal line is the P limitation threshold. Inset graphs are epilithon on phosphorus (P) and N plus P (NP), nutrient diffusing substrata (NDS) treatments. (b) APA versus estimated total phosphorus concentrations at upstream and downstream sites.

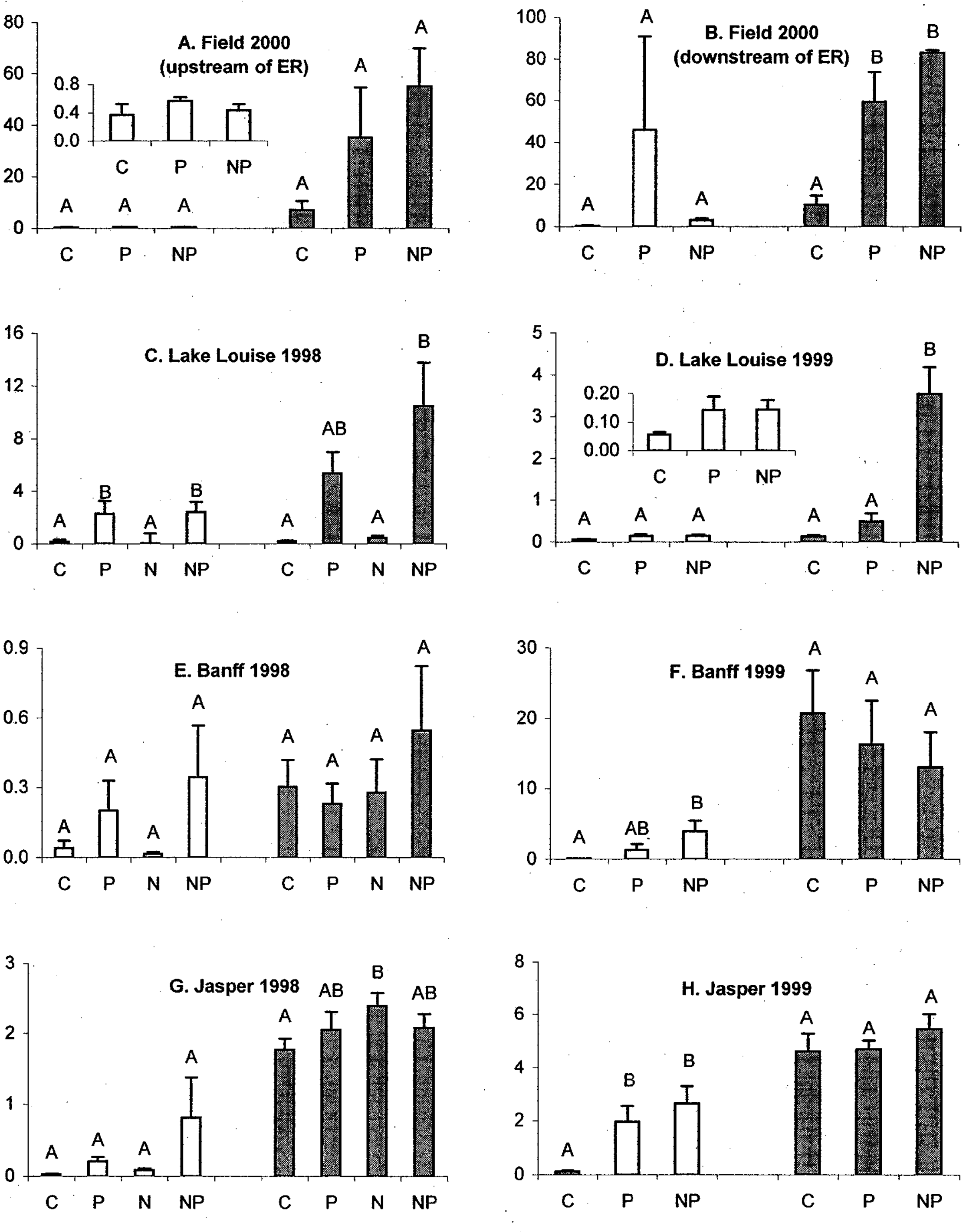

Figure 3-5. Mean chlorophyll *a* concentrations (+ one standard error) in µg/cm^2 on control (C), phosphorus (P), nitrogen (N), and P plus N (NP) nutrient diffusing substrata incubated in rivers upstream (open bars) and downstream (shaded bars) of wastewater discharges (ER = Emerald River). Inset graphs are an enlargement of upstream data when chlorophyll *a* values are low.

4.0 Changes in stoichiometric constraints on epilithon and benthic macroinvertebrates caused by low-level eutrophication of cold-water rivers

This study compares patterns in abundance of benthic organisms along a nutrient gradient in cold-water rivers to patterns in nutrient content of epilithon and benthic macroinvertebrates (BMIs). Epilithon in these rivers are inherently P limited except during periods of high summer discharge when light limits algal accrual (Chapter 2). Discharges from municipal wastewater treatment plants (MWWTPs) alleviate P limitation of epilithon in some locations, thereby providing an *in situ* gradient in nutrient availability (Chapter 3). By comparing elemental composition of epilithon and BMIs in nutrient-limited and nutrient-replete sites, we were able to test whether 1) changes in nutrient content of epilithon are correlated with changes in nutrient availability, 2) the assumption of homeostasis in the elemental composition of invertebrates is valid for stream BMIs, 3) elemental composition of epilithon and BMIs are within the ranges previously reported for other aquatic autotrophs and invertebrates at like trophic levels, 4) producer-consumer elemental imbalances in oligotrophic sites are higher than in nutrient-enriched sites, 5) increased food quality is correlated with increased abundance and size of scrapers, and 6) high producer-consumer nutrient imbalances could result in a decrease in stream productivity through preferential recycling of N relative to P by scrapers.

Ecological stoichiometry is an approach in which the constraints and consequences of the mass balance of multiple chemical elements in ecological interactions are analyzed (Elser and Urabe 1999). Stoichiometric relationships are important because the fecundity, growth and development of organisms rely on the supply of chemical elements and the first law of thermodynamics (Elser et al. 1996). The *Law of the Minimum* by Leibig, *Elements of Physical Biology* by Lotka, *Biological Control of Chemical Factors* by Redfield, *Energy Flow in Biology* by Morowitz, and *Complimentary Models for Ecosystems* by Reiners each outline the importance of stoichiometry in ecological interactions (See Sterner et al. 1992). Yet despite a long period of awareness, the stoichiometric properties of benthic organisms in aquatic ecosystems have rarely been studied and the food web implications are not understood (e.g., Sterner et al. 1992, Sterner and Elser 2002).

A stoichiometric framework has long been used to study interactions between marine or freshwater plankton and ambient nutrients (Elser and George 1993, Elser and Hassett 1994). Since Redfield's classic paper (1958) on the relatively constant elemental composition of marine plankton and therefore, seawater, the Redfield Ratio (molar ratio of 106:16:1, Carbon: Nitrogen: Phosphorus, C:N:P) has been used extensively as a benchmark for evaluating nutrient limitation of primary producers. A N:P > 16 implies P limitation of producer growth, and a ratio below this threshold indicates N limitation. Although on average this is generally true, the precise threshold between N and P limitation depends on the species of algae (Rhee 1978, Healy and Henzel 1980, Hecky et al. 1993, Lynn et al. 2000).

The elemental composition of different species of crustacean can also deviate significantly from one another, but unlike seston, individual species of crustacean each possess a relatively rigid stoichiometry (See reviews by Sterner and Hessen 1994, Sterner and Elser 2002). For example, Anderson and Hessen (1991) found little variation in C:N:P among seasons or due to starving or feeding within a single zooplankton species, but large differences among species. These interspecific differences appear to be the result of high relative amounts of P in some crustaceans that are related to greater amounts of P-rich, ribosomal RNA and higher growth rates (Main et al. 1997). For example, *Daphnia* reach sexual maturity within days of birth and contain up to 10% RNA by weight whereas slower maturing copepods are generally about 2% RNA by weight (Elser et al. 1996).

Sterner (1990) developed an empirically based Strict Homeostasis Model in which zooplankton grazers maintain their tissue N:P by accumulating the element in shortest supply in their food with higher relative efficiency. Food sources of P-rich (N:P≈12:1) organisms such as *Daphnia* tend to have comparatively higher N:P so *Daphnia* preferentially retain P and release N (Elser et al. 1996). Conversely, organisms with lower P requirements (N:P>30:1) such as calanoid copepods recycle nutrients with lower N:P. The nutrients (predominately soluble reactive P and ammonia) recycled by zooplankton are readily available to phytoplankton. Consequently, zooplankton can modify the nutrient environment of their food (Urabe 1993). Therefore, a predominance of calanoid copepods may favour N limitation of phytoplankton growth, whereas in an environment that favours cladocerans (especially *Daphnia*), phytoplankton become increasingly P limited (Elser et al. 1996). Strong effects of food quality on zooplankton, especially if grazers have high nutrient demands, have also been well documented (Sterner and Hessen 1994). A producer-consumer imbalance occurs when the nutrient content of the producer is different from the requirements of the consumer. For example, Elser et al. (2001) showed that the rate of zooplankton growth was reduced by almost 60% in the presence of P-limited algae.

The use of stoichiometric perspectives in plankton ecology has improved the ability to predict and explain ecosystem processes such as nutrient limitation, life history traits of primary consumers, succession and the likelihood of a trophic cascade (Elser et al. 1996). Recently, researchers have concluded that analysis of variation in the elemental composition of algae and benthic macroinvertebrates (BMIs), and of the environmental factors that influence this variation, could improve understanding of lotic ecosystem processes (Sterner and Hessen 1994, Elser and Urabe 1999, Hillebrand and Sommer 1999, Stelzer and Lamberti 2002, Sterner and Elser 2002). However, explicit use of a stoichiometric framework in stream ecology is rare. For example, Stelzer and Lamberti (2001) showed N:P content of stream periphyton tracked stream water N:P, consistent with current understanding of ecological stoichiometry. Also, the elemental composition of *Elimia* snails in experimental streams was not affected by food quantity but constraints on growth occurred because of a producer-consumer nutrient imbalance (Stelzer and Lamberti 2002). Stelzer and Lamberti (2002) suggested that the limitation of primary consumer growth, caused by an imbalance with the elemental composition of periphyton may be widespread in oligotrophic streams.

4.1 METHODS

4.1.1. Study sites

Epilithon and BMIs were collected at sites (115° 35′ - 118 ° 05′ N, 51° 10′ - 53° 54′ E, 1000-1500m a.s.l.) approximately 2km upstream and 3km downstream of MWWTP discharges to rivers in the Rocky Mountain National Parks of Alberta and

British Columbia. The Kicking Horse River receives most P loading indirectly from the Emerald Lake Resort (including, but not only from, a small wastewater treatment plant) via the Emerald River but also directly from MWWTP effluent from the town of Field, British Columbia. Hereafter 'downstream of Emerald River' refers to the site in the Kicking Horse River, downstream of the confluence of the Emerald and Kicking Horse Rivers. The Bow River receives MWWTP effluent from the towns of Lake Louise and Banff, and the Athabasca River from the town of Jasper, Alberta. The quantity of P added by the MWWTPs varies with changes in the effluent to river discharge ratio but results in a gradient of total P concentrations in river water that is on average least upstream and downstream of the Emerald River (3.0 and 3.3 μg/L, respectively) and progressively higher near Lake Louise (3.9 and 7.5 μg/L, respectively), Banff (8.9 and 14.4 μg/L, respectively) and Jasper (28 and 29 μg/L, respectively) during periods of autumn algal accrual (August-October). In the autumn, epilithon accrual is limited by availability of P at all sites upstream of MWWTPs and at sites downstream of the Emerald River and Lake Louise MWWTPs, but not downstream of the Banff and Jasper MWWTPs (Chapter 3). This eutrophication gradient enabled us to evaluate the effects of addition of a limiting nutrient on stoichiometric constraints on primary and secondary benthic production.

Detailed descriptions of the subalpine and montane ecoregions, rivers, MWWTPs and sampling protocols are presented in Chapter 2. Briefly, the mountain rivers are mid-sized (4-5th at 1:250000 scale) at the sampling sites. Sampling sites contained at least three, shallow (30cm), fast flowing (0.5m/s) riffles within a 500m reach, and substrate dominated by cobble (10-20cm diameter). Riffles were approximately 100m in length. Previous nutrient manipulation experiments (Chapter 3) and biological surveys (Chapter 2) showed that increased P loading to these rivers caused increased abundance of benthic algae and macroinvertebrates at sites downstream of MWWTP effluent discharges. Diatoms dominate the epilithon at all sites except 3km downstream of the Lake Louise MWWTP where bryophytes are abundant. The dominant pattern in the invertebrate community is a decrease in the relative (but not total, except downstream of Jasper) abundance of mayflies downstream of MWWTPs due to an increase in the number of chironomids. Except for the site downstream of Jasper, mayflies in Heptageniidae and

Ephemerellidae families (hereafter referred to as mayflies) were the most abundant scrapers in the rivers (*unpublished data*).

4.1.2. Sample collection and measures of abundance

Epilithon samples were collected in summer (late August) 2000, and autumn (late September and early October) 2000 and 2002. In summer 2000, epilithon samples were collected from two sites about 2 km upstream and two sites about 3 km downstream of both Lake Louise and Jasper. Downstream of Lake Louise, epilithon composed primarily of diatoms was sampled separately from epilithon dominated by bryophytes. Because epilithon dominated by bryophytes contained twice as much P (1.7%) as epilithon dominated by diatoms (0.8%), bryophytes were excluded from this analysis so changes in nutrient content of epilithon could be compared among sites.

In autumn 2000 and 2002, epilithon samples were collected at one site upstream and one site downstream of each of the four MWWTP discharges. Six epilithon samples (three for elemental and three for chlorophyll analysis) were collected from three riffles at every site in autumn 2000; two replicate epilithon samples (one for elemental and one for chlorophyll analysis) were collected in autumn 2002 from every site. Also in autumn 2000, epilithon was sampled from flumes treated with concentrations of Banff MWWTP effluent equivalent to concentrations downstream of Banff. Each 20mL epilithon sample was a composite of material removed with a scalpel from cobbles taken randomly from the start, mid-point and end of a riffle. Epilithon for analysis of chlorophyll *a* content was sampled from a known area (30cm^2), and all samples were frozen until analysis.

To extract chlorophyll *a,* epiphyton samples were frozen and subsequently thawed, blended with double distilled water for ten seconds, filtered onto GF/F filters, and boiled in 90% ethanol for seven minutes. Concentrations of chlorophyll *a* were measured using a fluorometer (Turner Designs, Model 10). Benthic metabolism was estimated by measuring oxygen evolution by epiphyton over time in light and dark re-circulating chambers, and correcting for amount of algae at each site (For detailed methods, Dodds and Brock 1998). We assumed the amount of chlorophyll in epilithon at a site was indicative of primary productivity because benthic metabolism (2.7-24.5μg oxygen per μg chlorophyll *a*) was not significantly different (df.=3, *t*=0.95, p=0.41) upstream (mean (s.e.) = 9.5 (5.0)) and downstream (mean (s.e.) = 4.6 (1.4)) of MWWTPs.

Mayflies (the dominant scraper at most sites) were collected in autumn 2000 and samples of the dominant family of BMI collector (dominant food is decomposing fine particulate organic matter), scraper (dominant food is periphyton and associated material) and predator feeding groups were collected in autumn 2002. BMIs were collected with a u-net (250 micron mesh) from 3-6 locations spanning the length of each riffle. Samples were sorted on site until a composite 0.5mL sample for each feeding group was obtained. In 2000, three composite mayfly samples (each from a separate riffle) were collected from one site upstream and one site downstream of Field, Banff and Jasper. We were unable to obtain 0.5mL mayfly samples downstream of Jasper because of low abundance. In 2002, one sample of each dominant feeding group was collected from one site upstream and one site downstream of Lake Louise, Banff and Jasper. At each riffle sampled, the BMIs collected in three u-nets were preserved in 90% ethanol and at least 300 organisms were randomly sub-sampled and identified to the lowest taxonomic level possible (generally species).

4.1.3. Elemental composition

Epilithon and mayfly samples for tissue nutrient content were immediately frozen on dry ice, stored at -20°C, freeze-dried at -60°C (Edwards Super Modulyo Freeze Drier), ground to a uniform powder with a mortar and pestle, dried at 90°C to a constant weight, and stored in a desiccator. Between 0.10-0.20g (±0.0001g) of epilithon sample and 0.01-0.03g (±0.0001g) of mayfly sample (less for the site downstream of Jasper) were weighed for analyses of percent P, and 1-4μg (±0.0001μg) of epilithon and mayfly sample were used for analyses of percent N and C. The amount of C, N, or P in tissues was expressed as a percentage of dry mass. P was extracted with persulfate oxidation and analyzed using the acid molybdate technique (APHA 1992). C and N were determined using a Model 440 Elemental Analyzer (Control Equipment Corporation). Benzoic acid and SRM NIST 1575 apple leaves (U.S. National Institute of Standards and Technology) were used as quality control standards for elemental analyses. All elemental ratios are expressed on a molar basis.

4.1.4. Data analyses

Data were log transformed prior to statistical analyses to correct for positive correlation of variance and mean, if necessary. Significant differences between C, N and P content and C:P, C:N and N:P in epilithon, number and size of mayflies, and nutrient imbalances, upstream and downstream of MWWTPs, were identified using paired *t* tests. One-tailed tests were used to determine if P content increased or C:P and N:P decreased downstream of MWWTP discharges. Two-tailed tests were used to determine whether C, N or C:N changed between upstream and downstream of MWWTP discharges.

Elser and Hassett (1994) calculated the elemental imbalance ($X{:}Y_I$) between producers and consumers as $X{:}Y_{\text{seston}} - X{:}Y_{\text{consumers}}$. This measure of elemental imbalance assumes no variation in the nutrient content of consumers with a change in the nutrient environment. There is strong evidence of homeostasis of nutrient content in certain zooplankton species (Sterner and Elser 2002). However, in a grazing community comprised of multiple species (e.g. mayflies in the Heptagenidae family), changes in the relative abundance of species could alter elemental composition of the community. Therefore, we propose an alternate definition of imbalance to allow for non-homeostasis in consumer nutrient content: we define 'community' imbalance as the perpendicular distance between the producer versus consumer C:P, C:N or N:P, and the 1:1 line (See Results and Discussion). We used empirical data describing the degree of homeostasis in consumer nutrient content to evaluate which definition most accurately described epilithon-mayfly nutrient imbalances in cold-water rivers. Geometric mean regressions (GMR, Ricker 1984) were used to determine the relationship between food quantity (chlorophyll *a*) and quality (producer-consumer nutrient imbalance) and the abundance of mayflies.

To evaluate the potential stoichiometric constraints on primary and secondary benthic productivity, we estimated the N:P recycling ratios of mayflies and stoneflies. We calculated recycling ratios following the definition of Sterner (1990):

When $f > b$ $\quad s = f / (1 - bL / f) / (1 - L)$

When $f < b$ $\quad s = f (1 - L) / (1 - Lf / b)$

where f is the epilithon N:P ratio, b is the consumer N:P ratio, s is the N:P recycling ratio, and L is the accumulation efficiency of the limiting nutrient (and is assumed to be 0.8 for all calculations, See Sterner 1990).

4.2 RESULTS

The abundance of benthic algae increased downstream of MWWTP discharges and, at a given P concentration, was higher at sites downstream than upstream of MWWTPs (Fig.4-1a). The amount of P in epilithic tissue was also greater downstream than upstream of all MWWTP discharges (Fig.4-2). Consequently, average C:P at all downstream sites and N:P at three of four downstream sites was lower than at upstream sites. Similarly, epilithic N content increased and C:N decreased between upstream and downstream of three of four MWWTP discharges. The P (but not C or N) content of epilithon was significantly higher in summer (mean %P = 0.07) than in the autumn (mean %P = 0.55, p=0.01, *data not shown*).

Average abundance of mayflies increased at sites downstream of three of four MWWTPs (Fig.4-1b). The most abundant and ubiquitous mayfly that could be identified to species was *Drunella doddsi.* The average biovolume of *Drunella doddsi* was greater downstream than upstream of all except the Emerald River (Fig.4-1c). The P content of mayfly tissue increased significantly between upstream and downstream of the Emerald River and Banff MWWTPs, and C:P and N:P decreased significantly downstream of Banff (Fig.4-3). However, relative to changes in epilithon, average C:P, C:N and N:P of dominant collector (caddisfly), scraper (mayfly) and predator (stonefly) families changed little between sites upstream and downstream of MWWTPs (Fig.4-4).

When nutrient ratios of epilithon were compared to nutrient ratios of mayflies, upstream sites were generally further away from the 1:1 line than downstream sites; indicating that there were greater nutrient imbalances at upstream sites (Fig.4-5). In all cases, the average nutrient imbalance at upstream sites was larger than the nutrient imbalance at downstream sites (Table 4-1). There was a stronger relationship between abundance of mayflies and C:P, C:N, and N:P nutrient imbalance than between mayfly abundance and concentrations of chlorophyll *a* (Fig.4-6). Estimates of mayfly N:P recycling ratios were higher than average N:P of epilithon at sites upstream of the Emerald River, Lake Louise and Banff MWWTPs, and downstream of the Emerald River and Jasper MWWTPs (Fig.4-7a). However nutrient concentrations in river water downstream of Jasper were higher that at other sites (average fall concentrations of 30 µg/L total P) and epilithon are nutrient saturated. Estimated stonefly recycling ratios were lower than the N:P of mayflies (Fig.4-7b).

4.3 DISCUSSION

4.3.1. Elemental composition of epilithon

Point source additions of N and P to P-limited mountain rivers resulted in an increase in the P content and a decrease in C:P in benthic algae. At the most oligotrophic sites (i.e. near Emerald River and Lake Louise), epilithic N:P was also 2-10 times less downstream of point source nutrient additions. Our results are consistent with those of Peterson et al (1993) who found epilithic C:P and N:P decreased by approximately half when comparable concentrations of P (10µg/L) were added to an arctic river. Analogous to the response of algal abundance, the magnitude of change in epilithic nutrient content was associated with the amount of 'new' (more bioavailable), anthropogenic nutrients rather than with total instream nutrient concentrations.

Consistent with the elemental composition of seston and periphyton in oceans, lakes and streams, the C:P and N:P of epilithon in mountain rivers yielded a broad range of nutrient ratios that included the Redfield Ratio (Table 4-2A-E). The C:P and N:P ratios in epilithon from upstream, ambient conditions in this study were considerably higher than ratios previously reported for periphyton. However, seston molar C:P ratios (645-4500) in a small, meso-oligotrophic, sub-alpine lake also greatly exceeded both elemental compositions previously reported, and reported threshold values for elemental constraints on growth and reproduction of many primary consumer zooplankton species (Elser and George 1993). The range of nutrient ratios of epilithon from sites affected by anthropogenic nutrients was similar to ranges previously reported for periphyton (Table 4-2D-E).

Unlike the changes observed in epilithic P, C:P and N:P that were a direct result of anthropogenic nutrient loading, increased epilithic N content and decreased C:N downstream of nutrient point sources were likely a result of indirect effects of increased grazing pressure. Results from experiments conducted at our sites with nutrient diffusing substrata (NDS) showed the C:N content of epilithon was the same on NDS releasing N and control NDS, and on control NDS incubated upstream and downstream of nutrient point sources, indicating that increased supply of N had no effect on epilithon N content (Chapter 3). Scrapers can increase the N content and thus decrease the C:N of periphyton (Hunter 1980) by preferentially excreting higher proportions of non-limiting nutrients (Sterner 1990). Our finding that C:N was not correlated with river water N concentrations but, instead, was correlated with scraper abundances (Chapter 3) is also indicative of scraper control of epilithic N content. Preliminary results ($n=3$, $p<0.1$) from grazing experiments are consistent with the hypothesis that grazed epilithon is more N-rich (%N: 0.5-0.7, C:N: 17-20, N:P: 20-24) than un-grazed epilithon (%N: 0.4-0.5, C:N: 20-25, N:P: 17-19) in P limiting conditions *(unpublished data).*

4.3.2. Elemental composition of BMIs

Consistent with stoichiometric theory, the elemental composition of BMIs showed little variability between upstream and downstream of nutrient point sources, relative to epilithon, and was similar to zooplankton and benthic macroinvertebrates (BMIs) with comparable growth rates. Many of the BMIs in our samples were too small to identify beyond family level. Therefore, it is possible that the small variations in nutrient content we observed upstream and downstream of nutrient discharges was caused by a shift in the relative abundance of various BMI species or life stages. However, it is likely that the stoichiometry of a given BMI species is not strictly homeostatic. The elemental composition of mayflies was similar to that reported for calanoid copepods, carnivorous zooplankton and other benthic macroinvertebrates (Table 4-2F-G). However, the C:P ratios of our mayflies were approximately twice as high as mayflies (predominately

Caenis) from nearby lakes (C:P=133, Frost et al. 2003) and P-rich cladocerans (C:P=85, Anderson and Hessen 1991). The finding that mayfly species in mountain rivers have lower P requirements than those in nearby lakes may be caused by slower growth rates in riverine systems. Even at sites receiving the highest nutrient loads, average mayfly C:P ratios were lower than those of epilithon (Table 4-2E&G). The range of mayfly C:N ratios did not overlap with the range in epilithon C:N ratios (Table 4-2E&G). In this situation, there is a high probability of trophic feedbacks due to a strong elemental imbalance between producers and consumers.

4.3.3 Producer-consumer elemental imbalance

Comparison of food source (epilithon) and consumer (mayfly) nutrient ratios showed a greater producer-consumer nutrient imbalance at sites upstream than downstream of anthropogenic nutrient sources. On average, epilithon-mayfly nutrient imbalances were 12 times greater for C:P and 5 times greater for C:N and N:P for upstream versus downstream sites. Producer-consumer imbalances have not been previously investigated for rivers but Dobberfuhl and Elser (2000) reported C:P and C:N inbalances between phytoplankton and zooplankton in Wisconsin and Alaskan lakes that were considerably lower than epilithon-mayfly C:P and C:N imbalances in mountain rivers for upstream sites (Table 4-1). C:P imbalances at sites downstream of MWWTPs were, however, within the range found in Wisconsin Lakes. Average N:P imbalances at sites upstream of MWWTPs were similar to N:P imbalances in lakes in Alaska and Wisconsin as well as for lakes of varying size, trophic status and food web structure in northern Ontario, Canada (N:P=18) and Wisconsin and Michigan, U.S.A (N:P=20, Hassett et al. 1997).

The assumption of strict consumer-nutrient homeostasis underlying the existing definition of producer-consumer imbalance (Elser and Hassett 1994) was generally valid for C:P and N:P, but not C:N, epilithon-mayfly imbalances in our P-limited rivers. For example, average epilithic C:P decreased downstream of Lake Louise whereas mayfly C:P remained constant (Fig.4-5a), an example of relatively strict homeostasis of consumer nutrient C:P. Strict homeostasis has been documented in organisms such as bacteria and *Daphnia* but homeostasis (not strict) is more common (Sterner and Elser 2002). Sterner and Elser (2002) define homeostasis as a narrowing of variation in the chemical content in an organism relative to the resources it consumes. For example, C:N of both epilithon and mayflies changed downstream of Banff but mayflies had lower C:N ratios than epilithon both upstream and downstream (Fig.4-5b). Our modification of the producer-consumer imbalance will be useful in situations where the nutrient ratios of consumer communities (single or multiple species) changes appreciably.

4.3.4. Potential effects of nutrient imbalance on life history traits of scrapers

Increased abundance and size of mayflies in mountain rivers downstream of MWWTPs was likely due to increased food quality and not increased food availability. Sterner and Hessen (1994) suggested that even if all essential substances are within food, an animal with a homeostatic elemental composition suffers growth and reproductive penalties because it is energetically costly to utilize a resource base different from its needs. Frost and Elser (2002) concluded that lentic mayfly growth should be P limited at C:P > 310, a threshold very similar to where constraints on growth and reproduction of *Daphnia* occur (C:P > 300, Elser and George 1993),. Söderström (1988) concluded that two species of lotic *Parameletus* mayflies attained highest growth rate, largest adult size and highest fecundity when the C:N of their food was 6, and development time was shortest at a C:N of 6-13. Our observation that average epilithic C:P and C:N were well above the C:P (300) and C:N (6-13) growth and development limitation thresholds at sites upstream of MWWTPs but close to these thresholds at the most nutrient rich downstream sites suggests that mayfly abundance in inherently nutrient-poor mountain rivers is constrained by food quality (Table 4-5E).

4.3.5. Implications of changing consumer recycling ratios in stream processes

Producer-consumer nutrient imbalances not only constrain consumer life history traits but also result in consumers that preferentially retain the scarcest element in their food and thereby release waste products that accentuate nutrient limitation in producers (e.g., Urabe 1993, Sterner and Hessen 1994). We found predicted mayfly N:P recycling ratios were higher than epilithic N:P at oligotrophic sites (i.e. upstream and downstream of Emerald River, upstream of Lake Louise). Therefore, producer-consumer imbalances likely resulted in nutrient recycling by scrapers that would increase P limitation in epilithon. At more nutrient-rich, but still P-limited, sites (i.e. downstream of Lake Louise and upstream of Banff), the N:P of nutrients recycled by mayflies was comparable to epilithic N:P, and therefore, P recycling by mayflies would not increase P limitation of epiphyton. Therefore, nutrient poor sites with high producer-consumer nutrient imbalances would have lower epilithic productivity than sites with the same nutrient concentrations but lower producer-consumer imbalances. Because benthic invertebrates can supply a substantial proportion of nutrient demands of primary producers (e.g., up to 70%, Vanni 2002), epilithon-scraper imbalances in mountain rivers likely influenced nutrient recycling ratios of consumers and in turn, population and ecosystem level processes such as nutrient cycling and productivity. In systems where nutrient recycling by scrapers supplies a significant proportion of available nutrients, identification of the nutrient imbalance-balance threshold will be important in accurately predicting the effects of eutrophication on ecosystem productivity.

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Table 4-1. Results of paired *t* tests comparing epilithon-mayfly nutrient imbalance at upstream sites with nutrient enriched downstream sites (n=4). Producer-consumer imbalances (assuming homeostasis in consumer nutrient content) between epilithon and mayflies (mean (standard error (s.e.)) are compared to mean imbalances between phytoplankton and zooplankton in oligotrophic lakes (from Dobberfuhl and Elser 2000).

Type of Nutrient Imbalance	Upstream or Downstream	p	Mean Epilithon-mayfly Imbalance (s.e.)	Alaska Lakes	Wisconsin Lakes
Carbon: Phosphorus	upstream	0.001	3817 (2587)	125 (25)	275 (75)
	downstream		607 (334)		
Carbon: Nitrogen	upstream	0.104	77 (39)	2 (0.5)	5 (0.5)
	downstream		16 (6)		
Nitrogen: Phosphorus	upstream	0.104	25 (30)	15 (5)	18 (3)
	downstream		5 (3)		

Table 4-2. Representative percent of dry weight that is carbon (C), nitrogen (N), and phosphorus (P), and molar C:P, C:N and N:P in the tissue of 1) ocean and lake seston, 2) ocean, lake and stream periphyton, and 3) primary consumers in lakes and streams. Data from this study presented as mean (and range in brackets) for sites upstream and downstream nutrient discharges (E-Emerald, L-L.Louise, B-Banff, J-Jasper) to mountain rivers.

Reference	Nutrient Environment	%C	%N	%P	C:P	C:N	N:P
A. OCEAN SESTON							
Redfield Ratio	optimal				106	6.6	16
B. LAKE SESTON							
Range (all data)					**49-4500**	**4-73**	**4-86**
Healy and Hendzel (1980)	severe deficiency				>258	>14.6	>22
	moderate deficiency				129-258	8.3-15.0	
	no deficiency				<129	<8.3	<22
Hecky et al. (1993)	worldwide survey				110-540	6-20	10-50
Elser and George (1993)	meso-oligotrophic				645-4500		
Dobberfuhl and Elser(2000)	Alaska and Wisconsin				55-963	4-73	6-86
Lynn et al. (2000)	P limitation				104	9	12
(*Stephanodiscus minutulus*)	No limitation				49	7	7
C. OCEAN PERIPHYTON							
Range (all data)					**100-180**	**4-10**	**13-22**
Hillebrand & Sommer (1999)	P limitation				>180	<4	>22
	optimum				119	7	17

Table 4-2. continued.

Reference	Nutrient Environment	%C	%N	%P	C:P	C:N	N:P
D. LAKE PERIPHYTON (diatom dominated)							
Range (all data)		**7-20**			**31-470**	**4-16**	**3-51**
Kahlert (1998)	review of various				31-439	4-16	3-51
Frost et al. (2002)	low P	7			470		
	high P	20			60		
E. STREAM PERIPHYTON (diatom dominated)							
Range (all data, except this study)		**7-15**	**0.6-1.5**	**0.05-0.14**	**89-500**	**8-23**	**8-40**
Kalhert (1998)	review of various				89-500	8-23	8-40
Stelzer and Lamberti (2001, 2002)	N:P = 50:1	15	1.5	0.1	500	12	40
	N:P = 4:1	15	1.5	0.4	100	12	10
	ambient	8	0.6	0.05	400	16	25
	high P	7	0.6	0.14	200	16	13
	high N&P	8	0.7	0.10	200	14	13
This study	**Range**	**13-22**	**0.6-2.6**	**0.1-0.3**	**300-3900**	**13-66**	**30-120**
	upstream	13	0.6	0.1	3900	66	120
		(7-24)	(0-1.4)	(0-0.6)	(34-16500)	(16-280)	(0-535)
	downstream (E&L)	18.5	1.3	0.3	760	20	30
		(13-26)	(0.4-2.6)	(0-1.7)	(30-1950)	(12-38)	(2-64)
	downstream (B&J)	22	2.6	0.3	300	13	30
		(8-35)	(0.3-52)	(0.1-1)	(25-500)	(8-31)	(0-46)

Table 4-2. continued.

Reference	Nutrient Environment or type of invertebrate	%C	%N	%P	C:P	C:N	N:P
F. LAKE ZOOPLANKTON							
Range (all data)		**48**			**85-250**	**5-6**	**14-43**
Anderson and Hessen (1991)	calanoid copepod	48			212	5	39
	cladoceran	48			85	6	14
Sterner and Hessen (1994)	herbivores				100-150		15-25
	carnivores				250		40-43
G. *BENTHIC MACROINVERTEBRATES							
Range (all data except this study)		**33-54**	**6-14**	**0.4-2**	**63-324**	**4-8**	**10-60**
Frost et al. (2003)	all (lentic)	33-54	6-14	0.4-2	63-324	4-8	10-60
	mayflies	47	9.8	0.9	133	5.6	24
Stelzer and Lamberti (2002)	*Elimia* (lotic)	8-9	0.5			38	
Mayflies in this study	**Range**	**42-47**	**8.6-9.7**	**0.5-0.7**	**190-250**	**5-6.4**	**31-44**
	upstream sites	42	9.2	0.5	210	5	40
		(39-47)	(8.6-10)	(0.4-0.6)	(165-300)	(5.1-5.6)	(31-60)
	downstream (E&L)	47	9.7	0.5	250	5.6	44
		(45-48)	(9.4-9.9)	(0.4-0.6)	(200-300)	(5.4-5.8)	(35-55)
	downstream (B&J)	47	8.6	0.7	190	6.4	31
		(42-51)	(8-10)	(0.5-1)	(125-240)	(5.8-7.2)	(20-40)

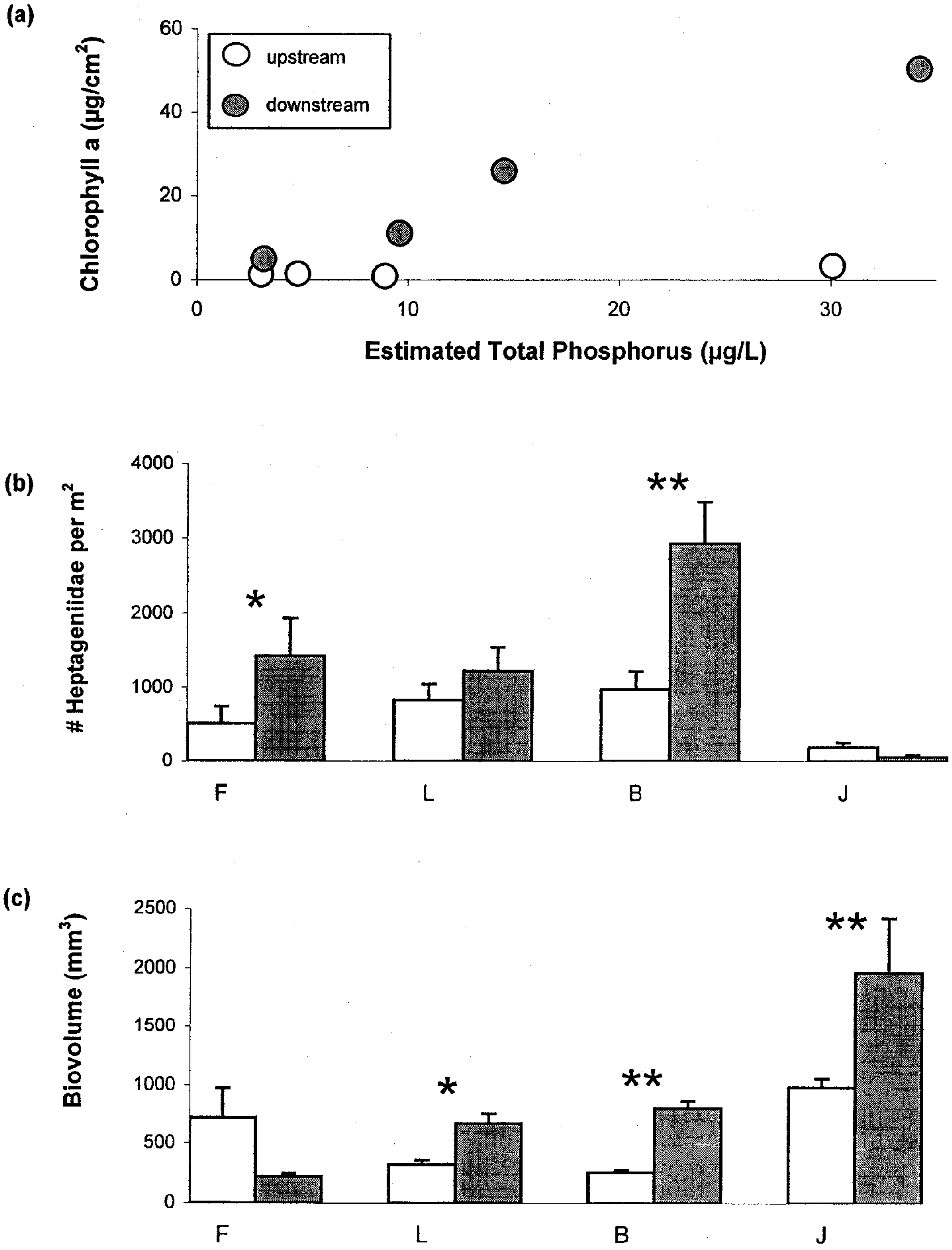

Figure 4-1. Autumn 2000 patterns in (a) epilithic abundance for a given phosphorus concentration, (b) abundance (mean + one standard error) of heptagenid mayflies and (c) size (mean + one standard error) of the mayfly *Drunella doddsi* upstream and downstream of Emerald Lake (E), Lake Louise (L), Banff (B) and Jasper (J) MWWTPs. Asterisks identify significant differences between upstream and downstream sites for a given location (p<0.05, *p<0.1).**

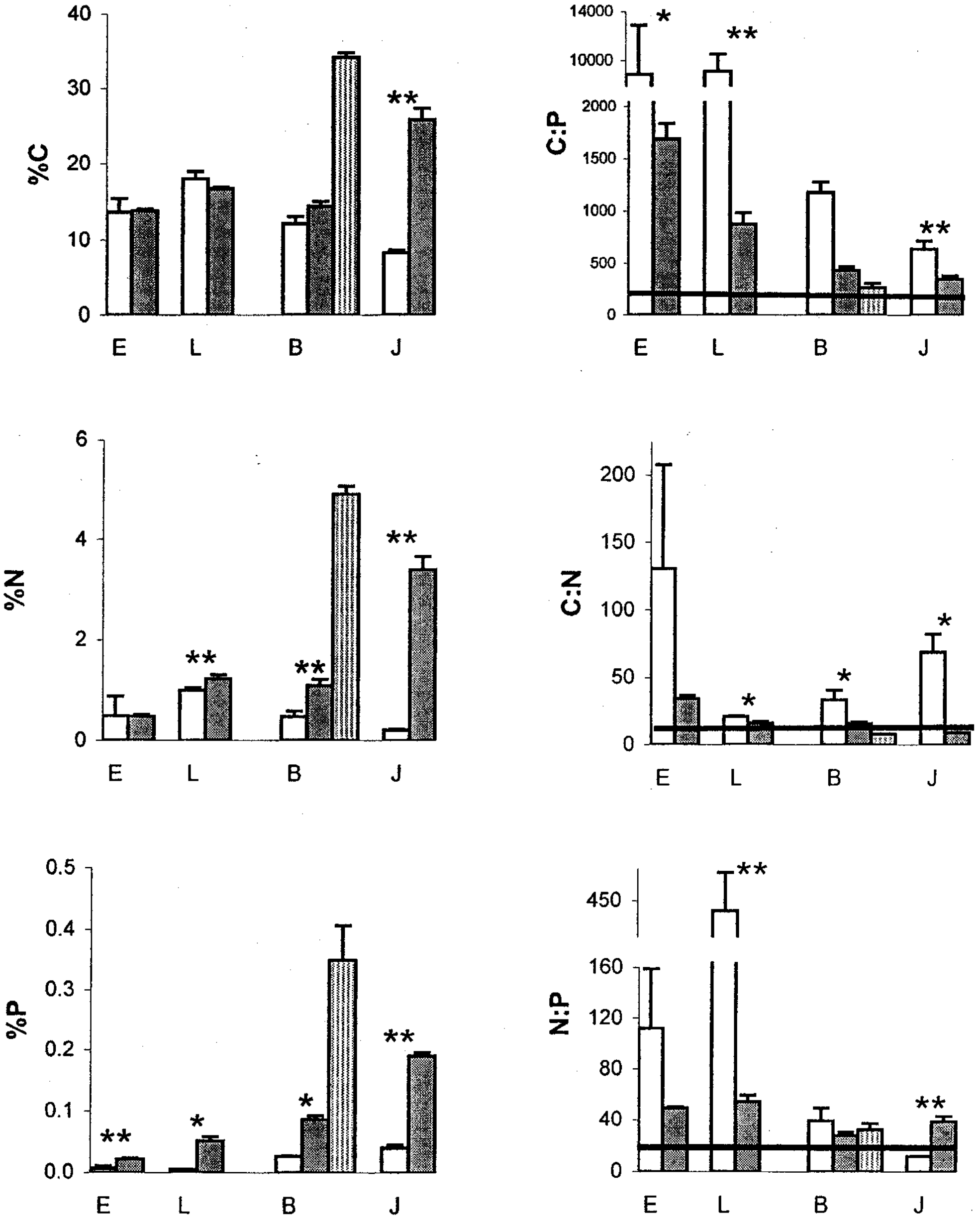

Figure 4-2. Carbon (C), nitrogen (N), phosphorus (P), molar C:P, C:N, and N:P content (mean + one standard error) of epilithon collected upstream (open bars) and downstream (closed bars) of wastewater treatment plants in Emerald Lake (E), Lake Louise (L), Jasper (J), and Banff (B), and in flumes (stippled bars) in autumn 2000. Horizontal lines on the C:P, C:N and N:P plots represent Redfield proportions. Asterisks identify significant differences between upstream and downstream sites for a given location (**p<0.05, *p<0.1)

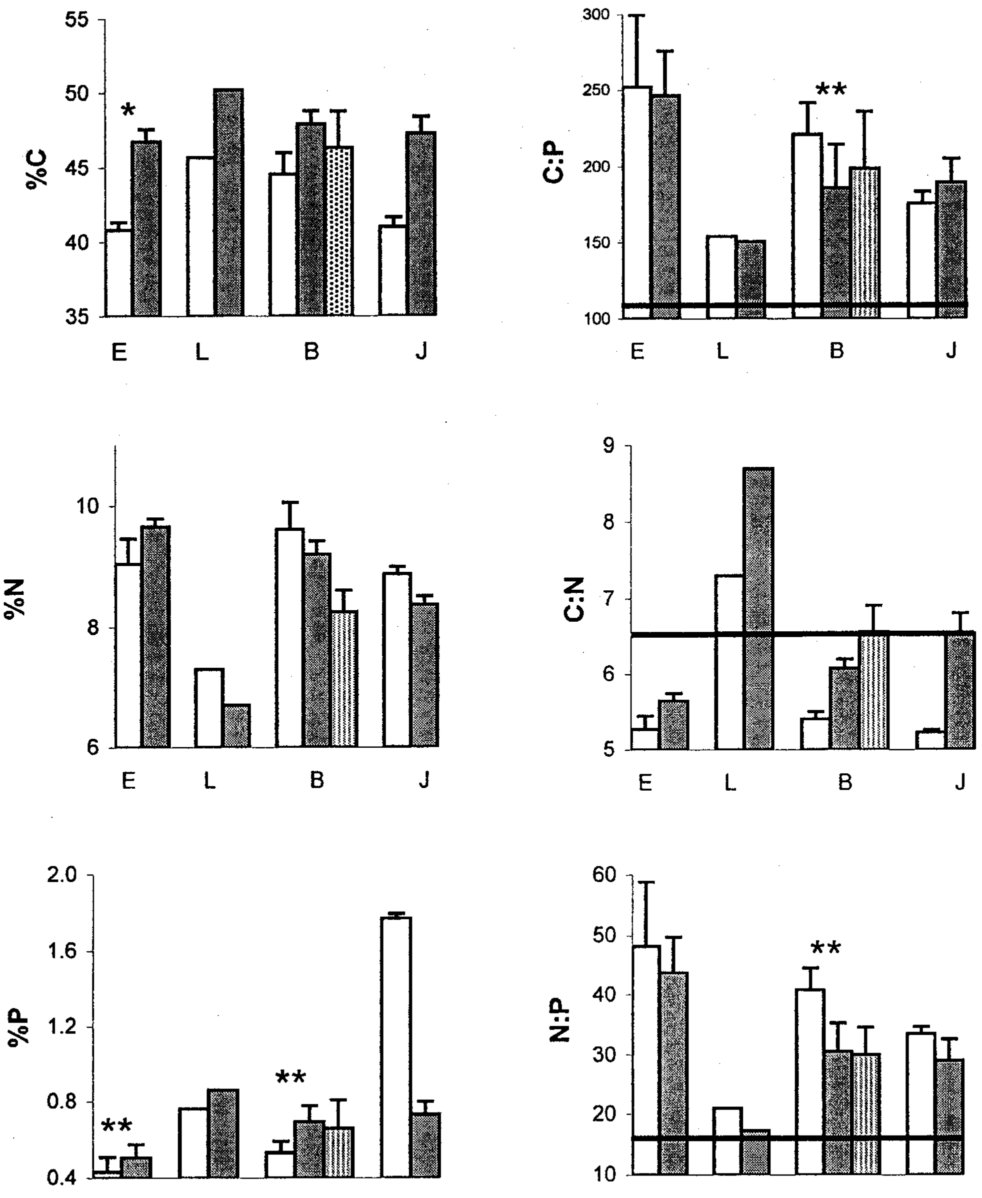

Figure 4-3. Carbon (C), nitrogen (N), phosphorus (P), molar C:P, C:N, and N:P content (mean + one standard error) of mayflies collected upstream (open bars) and downstream (closed bars) of wastewater treatment plants in Emerald Lake (E), Lake Louise (L), Banff (B) and Jasper (J) in autumn 2000 (E, B and J) and 2002 (L). Horizontal lines on the C:P, C:N and N:P plots represent Redfield proportions. Asterisks identify significant differences between upstream and downstream sites for a given location (**p<0.05, *p<0.1).

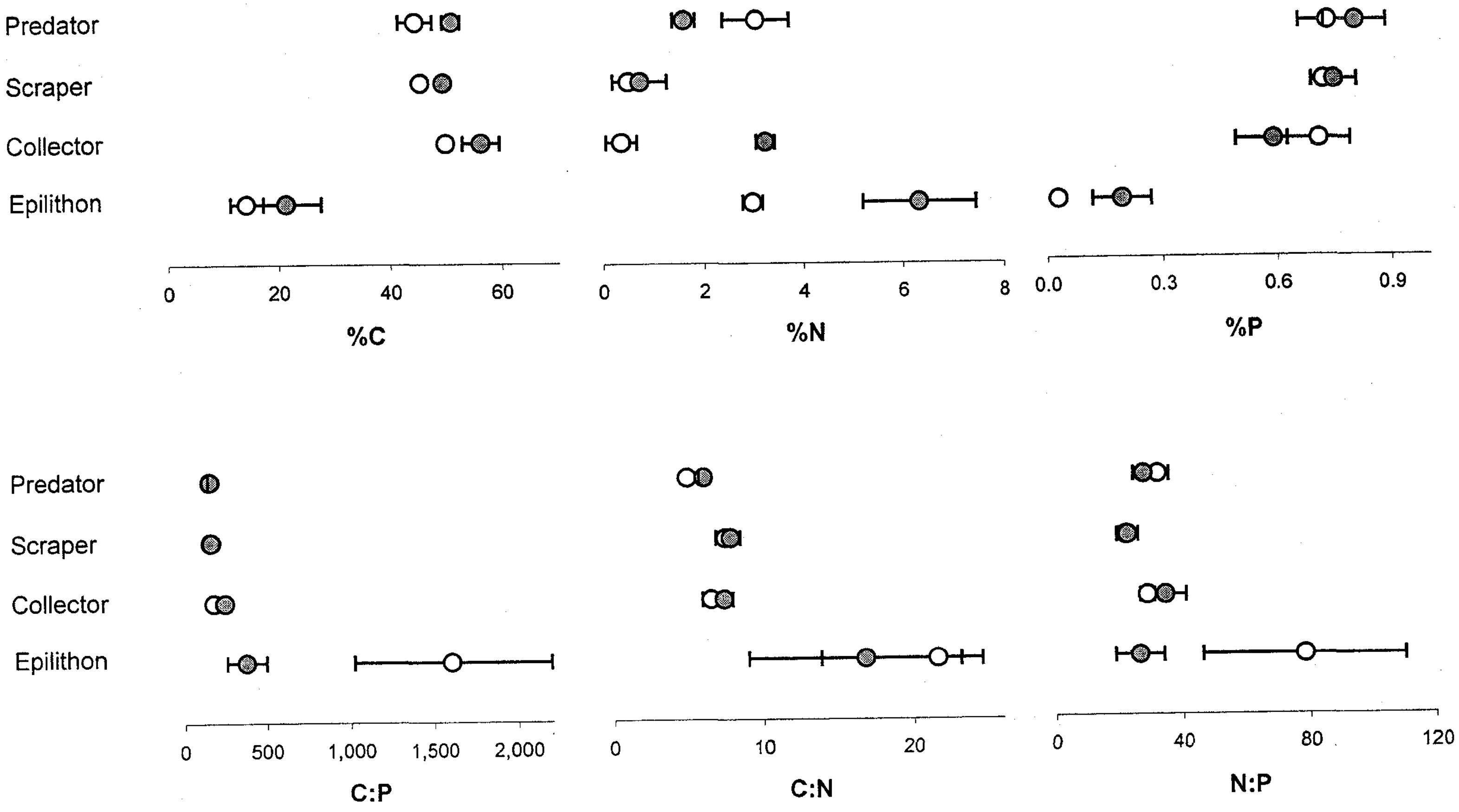

Figure 4-4. Average (± one standard error) carbon (C), nitrogen (N), phosphorus (P), C:P, C:N and N:P content of epilithon and benthic macroinvertebrate collectors (Hydropsyicidae), scrapers (Heptageniidae) and predators (Perlidae) collected upstream (open circles) and downstream (closed circles) of wastewater treatment plant discharges at Lake Louise, Banff and Jasper in autumn 2002.

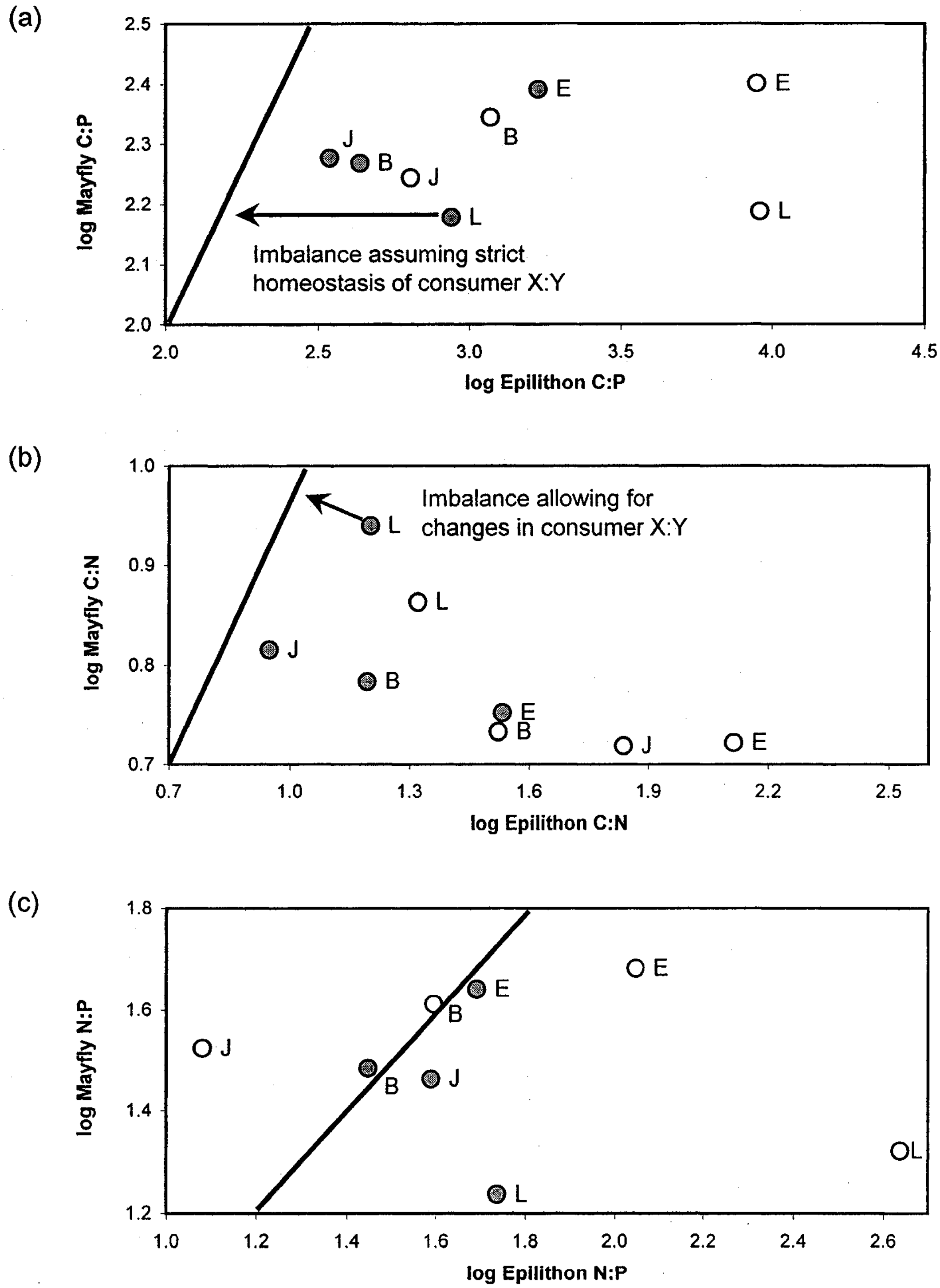

Figure 4-5. Relationship between average C:P, C:N and N:P molar nutrient ratios of epilithon and mayflies collected upstream (open circles) and downstream (closed circles) of the Emerald Lake (E), Lake Louise (L), Banff (B) and Jasper (J) MWWTPs in autumn 2000 (E, B and J) and 2002 (L). The producer-consumer nutrient imbalance allowing for changes in consumer X:Y is the perpendicular distance from a given point to the 1:1 line, and imbalance assuming strict consumer homeostasis is the horizontal distance.

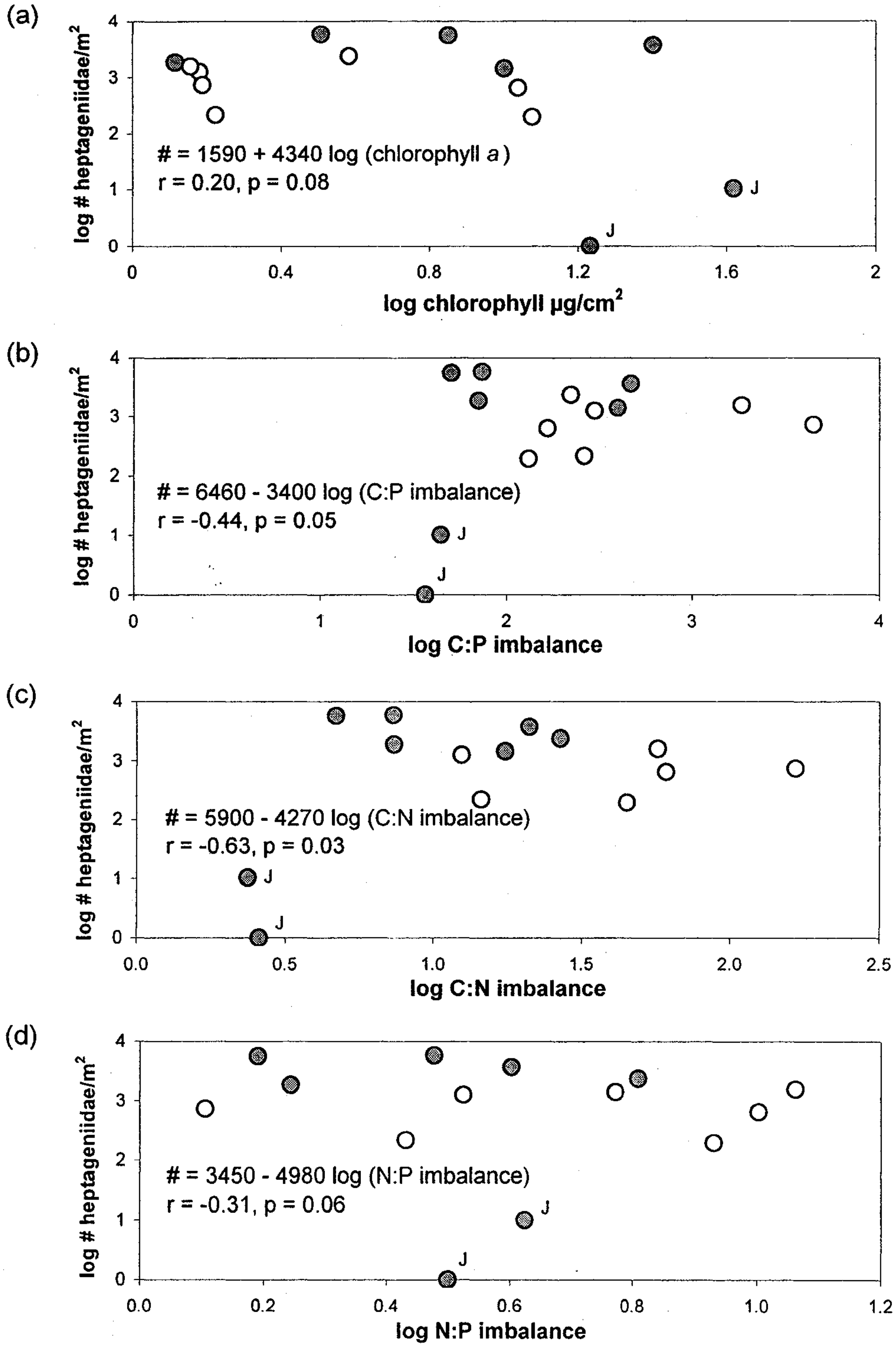

Figure 4-6. Relationships between the abundance of heptagenid mayflies and chlorophyll *a* concentration, and producer-consumer C:P, C:N and N:P imbalances upstream (open circles) and downstream (closed circles) of MWWTPs in autumn 2000. (Data collected downstream of Jasper were excluded from regression calculations because chironomids rather than mayflies were the dominant scrapers).

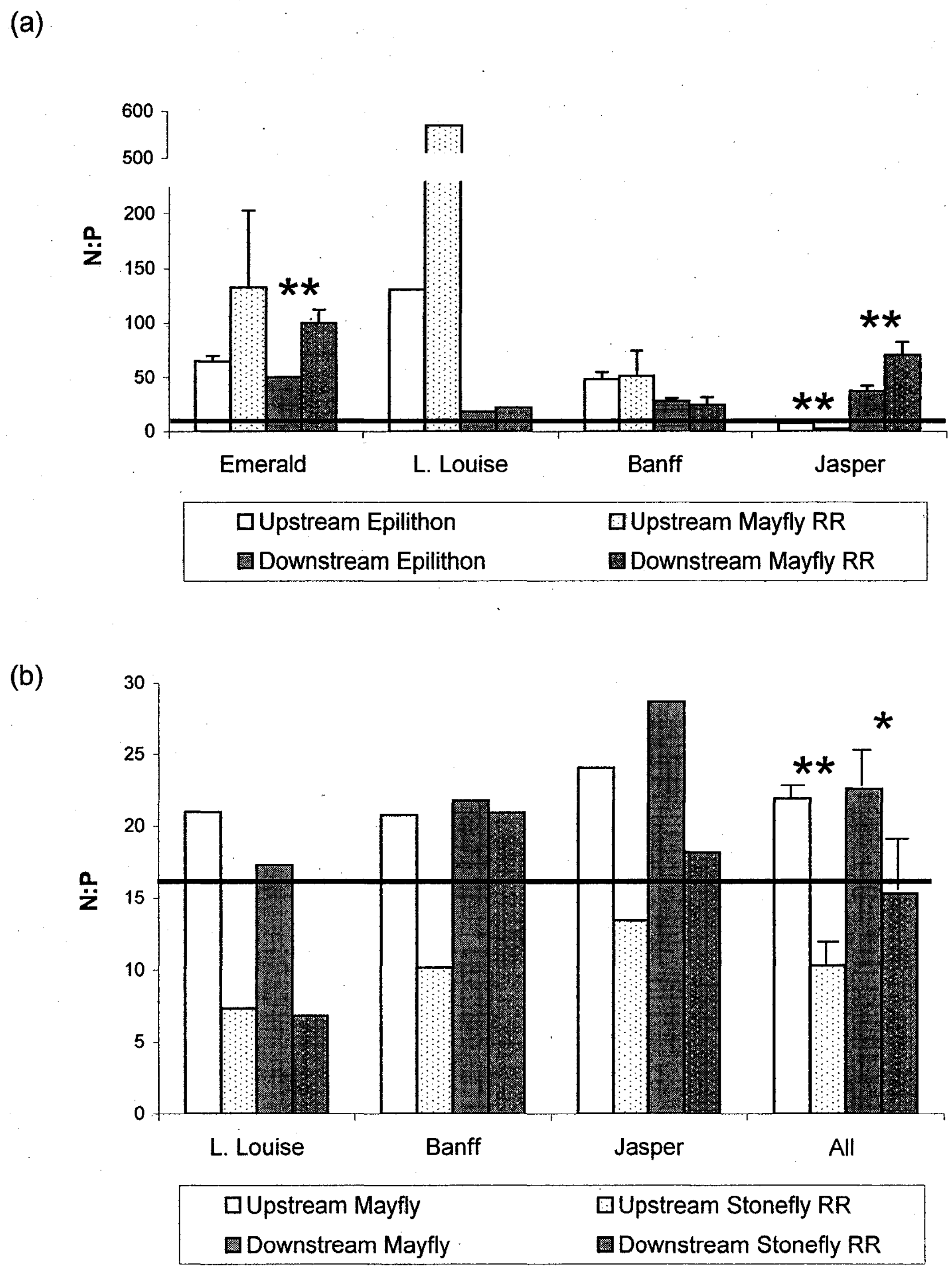

Figure 4-7. Comparisons between the (a) N:P content of epilithon and the estimated N:P recycling ratio (RR) of mayflies (+ one standard error) in autumn 2000 (E, B and J) and 2002 (L), and (b) N:P content of mayflies and the estimated N:P recycling ratios of stoneflies (+ one standard error) collected upstream and downstream of wastewater treatment plants in Emerald Lake, Lake Louise, Banff and Jasper in autumn 2002 (Horizontal lines represent Redfield N:P). Asterisks identify significant differences between epilithon N:P content and mayfly RR for a given site ($p<0.05$, *$p<0.1$).**

5.0 General Conclusions: Effect of low-level eutrophication on the ecological integrity of cold-water rivers in the Rocky Mountain National Parks of Canada

Consistent with our expectations, 1) abundance of benthic algae and macroinvertebrates increased, and species composition changed along the anthropogenic nutrient gradient as a result of diminishing P limitation of benthic algae, 2) concentrations of P in P-limited downstream sites were less than predicted from anthropogenic nutrient loadings due to uptake by epilithon, and 3) the magnitude of response in benthic algae to like changes in nutrient concentrations was greater in mountain rivers than responses previously reported in more nutrient-replete rivers. However, contrary to our predictions, increased abundance of BMIs at downstream sites was due to increased food quality rather than food quantity; therefore, grazing pressure did not mask the response of benthic algae to the gradient in nutrient loading.

Dissemination of direct evidence to Parks Canada representatives that bioavailable P in wastewater discharge caused nuisance levels of algae and changed the species composition of benthic organisms resulted in upgrades to all MWWTPs within the mountain National Parks in winter 2002-3. MWWTPs within the parks are now equipped with the best economically achievable phosphorus removal processes currently available. In addition, Parks Canada 1) has developed leadership targets to ensure nutrient concentrations downstream of MWWTPs will be returned to background levels, as the technology becomes available, and 2) requires MWWTP operating budgets to include the costs of ongoing monitoring of effects of wastewater effluent discharge on stream water chemistry, and abundance and composition of benthic algae and macroinvertebrates. Preliminary results of monitoring conducted in autumn 2003 suggest that nuisance algal blooms no longer occur downstream of Jasper and Banff. Parks Canada is trying to isolate the source of anthropogenic nutrient additions to the Emerald River and is investigating alternative methods of wastewater disposal in Lake Louise (e.g., using treated effluent to make snow at the local ski hill).

Although improvements in water quality are encouraging, declining river flows as a result of climate warming as well as increased human use of the parks will continue to jeopardize the ability to achieve the Parks Canada mandate of no-net environmental impact on the ecological integrity of mountain rivers as a result of P loading. In addition, the ability to predict responses of benthic organisms to mitigation measures from knowledge of changes in nutrient loadings alone, is limited in mountain rivers because benthic organisms are near the transition from nutrient-limiting to nutrient-replete conditions. Patterns in composition and abundance of benthic biota in mountain rivers are related not only to bioavailability of P in river water but also to degree of epilithic nutrient limitation, and changes in degree of producer-consumer nutrient imbalance. Increased alkaline phosphatase activity in epilithon at sites upstream relative to downstream of treatment plants confirmed that P was more bioavailable downstream. Therefore, nutrient limitation of epilithon was reduced or eliminated downstream of treatment plants, and the P content of epilithon increased. At upstream sites, invertebrate scrapers would excrete nutrients with high N:P, exacerbating epilithic nutrient limitation. At downstream sites, the growth efficiencies of algae and scrapers increased due to reduced nutrient limitation. These results show that the abundance and community composition of benthic organisms can change rapidly with small changes in nutrient concentrations if there is a shift between nutrient-limiting and nutrient-replete conditions.

As a result of our findings on the rapid changes in the abundance of benthic biota near the nutrient limitation threshold, Parks Canada has funded an ongoing study of benthos nutrient content in mountain rivers to confirm and calibrate the relationship between 1) epilithic C:P content and sudden shifts in benthic algal abundance, and 2) producer-consumer imbalance and scraper abundance. The addition of epilithic C:P and producer-consumer imbalance metrics to the suite of more traditional biomonitoring indices has improved our ability to predict and explain the effects of changing nutrient bioavailability on the composition and abundance of benthic organisms in cold-water rivers.