Does Aquaculture Impact Benthic Algal Ecology?

A study on the effects of an experimental cage aquaculture operation on epilithic biofilms

By

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"The fascination of periphyton resides in the elusive complexity of its integration"

- Robert G. Wetzel

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Abstract

Epilithic biofilms were monitored for potential impacts of an experimental rainbow trout aquaculture operation at the Experimental Lakes Area in northwestern Ontario, Canada. Metabolic and particulate samples were collected from the middle littoral zone of the experimental and reference lakes before and during the aquaculture operation. Phosphorus stocks in the experimental lake (epilimnetic and epilithic) increased as a result of both food waste and fish excretion. These cumulative P inputs were predicted to increase epilithic biomass and productivity, cause major compositional changes in the dominant groups and increase epilithic algal toxin production (microcystins). However, no major aquaculture-related changes in algal biomass, productivity, toxins or group dominance were observed in the epilithon. The only observed changes occurred at the species-level. These species-level changes were transient but did allude to an ecosystem reacting to stress.

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Chapter 1: Background

General Introduction: Freshwater aquaculture in Canada

Worldwide demand for fish products is on the rise, although wild fish stocks have drastically decreased in our oceans and lakes over the last few decades. The need for an alternative fish source has shifted interest to the global aquaculture industry. Currently, in Canada the aquaculture industry is dominated by marine production; lake-based culture accounts for only 3% of total aquaculture (Statistics Canada 2007). Considering Canada's vast freshwater resource base, there is great potential for growth in the freshwater sector. Possible rapid expansion of the industry has created public concern over actual and perceived environmental impacts associated with aquaculture practices (Yan 2005).

Starting in the mid-1990s, the Ontario aquaculture industry, Canada's largest producer of freshwater fish (2.5% of total aquaculture production (Statistics Canada 2007)), had an "unofficial" moratorium on new cage sites due to unresolved environmental questions of concern (Ontario animal research and services committee 2004). For this reason, understanding the environmental impacts of current aquaculture activities in freshwaters is emerging as both an environmental concern and as a primary management objective. Until specific scientific uncertainties are understood, not only will there be minimal growth in an economically viable industry, there is also the potential for substantial impairment of lake ecosystems where current aquaculture operations exist.

To understand and evaluate the potential impacts of in-lake cage aquaculture on the whole-lake ecosystem, an experimental aquaculture operation was initiated by Fisheries and Oceans Canada and the Ontario Aquaculture Association. The experimental operation began in 2003 at the Experimental Lakes Area (ELA) in northwestern Ontario,

Canada. Knowledge gained from this collaborative research effort is anticipated to aid in the development of an environmentally sustainable industry that would have minimal impact on freshwater ecosystems.

The team of expert scientists researching the effects of the aquaculture operation consisted of individuals, or groups of individuals, who investigated fish production, fish behaviour (Blanchfield *et al.* in prep.), zooplankton and phytoplankton production (Findlay *et al.* in press), benthic algal community changes (current study), and bacterial production (Findlay *et al.* in press). There were also further food web investigations involving stable isotopes (Kullman *et al*. in prep.), as well as, mass balance studies to determine aquaculture loads (Bristow *et al.* 2008; Azevedo *et al.* in prep.). In addition, the team consisted of researchers who routinely monitored the lakes for water chemistry parameters.

Literature Synthesis and Review: Potential impacts of in-lake aquaculture on algal communities

Aquaculture is a form of agriculture and involves the propagation, cultivation, and marketing of aquatic animals and plants in controlled environments (Swann 1992). As is the case in any agricultural enterprise, aquaculture is concerned with the production of high yields while minimizing cost and labour inputs (Lazur 2000); as a result ecosystem integrity is often compromised in the process (Beveridge *et al.* 1997a).

The practice of fish farming dates back to 2000 B.C. in China (Swann 1992), but it was not until the late 20th Century that aquaculture was firmly established in the world market (Varadi 2001). Capture methods supported the world's fish and seafood demand until the 1990s when the world market peaked (Garcia and Grainger 2005).

Over-exploitation, pollution, and habitat destruction all led to the decline of native fish populations (Swann 1992). An alternative method of fish acquisition was needed to meet global needs and aquaculture became the proposed solution. By 1996, global production from aquaculture operations grew by 150% to compensate for the global fish shortages (Yan 2005). Currently, one quarter of the world's fishery production is via aquaculture (ca. 35 mt) and this is projected to double by 2015, reaching yields equivalent to conventional capture methods by the year 2030 (ca. 80-90 mt) (Garcia and Grainger 2005).

Until recently intensive, caged fish farming occurred mainly in marine coastal environments, but focus has now shifted to the development of the freshwater cage aquaculture industry (Clerk *et al.* 2004). In cage aquaculture, fish are raised in large floating cages in outdoor waters. Pens are often used for easy access to the fish. Large amounts of fish can be raised in these cages without incurring high facility costs (Phillips *et al.* 1985b). These open cage operations pose a threat to the aquatic environment because they allow free exchange of farm-related nutrients with the natural water body (Beveridge *et al*. 1997a).

In 1998, marine operations accounted for 56% of the total aquaculture catch and freshwater production accounted for 46% (Varadi 2001). Of the freshwater production, only 10% was attributed to cage aquaculture (Clerk 2002). However, higher consumer demand, food security, global economic development, and improved technology have all made this an increasingly important industry in many parts of the world (Kwei Lin and Yi 2003).

China is the number one producer of freshwater aquaculture fish (Guo and Li 2003). China's operations account for more than one third of the total freshwater fishery production in the world (Guo and Li 2003). Europe is the second largest contributor (Aps *et al.* 2004), with Finland being the leading European country (Varadi 2001). In many other regions of the world, like Canada, freshwater aquaculture is still regarded as a small industry (Clerk *et al*. 2004). Nevertheless, as population growth continues and the demand for fish as a food protein increases, continued growth and expansion of the freshwater aquaculture industry is expected, even in these small scale industries (Garcia and Grainger 2005).

Impacts of aquaculture

The potential impacts of aquaculture operations are wide-ranging, from simply being aesthetically displeasing to potentially causing considerable environmental damage (Read and Fernandes 2003). Some of the main concerns associated with intensive aquaculture operations are habitat destruction, disease introduction, loss of species integrity and the release of large quantities of nutrients into water bodies (Beveridge *et al.* 1997b; Read and Fernandes 2003; Yan 2005).

It is inevitable that some caged fish will escape from the aquaculture operation. As a result, the risk of foreign or exotic species introduction to the lake or coastal environment is very high (Beveridge 1984). Read and Fernandes (2003) estimated the frequency of escaped farmed salmon to be between 20-30% in the Faroese Ocean. The impacts of non-native fish on indigenous fish stocks can be devastating and include the extermination of local fish populations through predation or competition, interbreeding with native fish and adulteration of the native species' gene pool, habitat destruction and

the outbreak of disease epidemics (Beveridge 1984; Crossman 1991; Read and Fernandes 2003).

Disease and pathogen transfer from an intensive fish farm facility to native stocks can also occur by ingestion of contaminated fish farm wastes (Read and Fernandes 2003). In wild fish populations, fish and disease-causing organisms are usually in a state of balance and thus mass mortalities are rare. Massive outbreaks of disease and pathogens are usually linked to external stress factors associated with fish farms (Beveridge 1984). The effects of the pharmaceutical chemicals and drugs used to control diseases, pathogens and fungi in aquaculture operations are not yet known (Yan 2005).

The high protein feed pellets used to nourish caged fish contain large quantities of oils and proteins that are derived from wild fish stocks (Folke *et al.* 1998). From 1985-1995, the world's shrimp farmers used 36 million tonnes of wild fish to produce only 7.2 million tonnes of shrimp (Frankic and Hershner 2003). This practice exacerbates the global fisheries' losses and reduces native fish stocks (Yan 2005).

With increased feed inputs, waste emissions increase, increasing nutrient loads to the system (Kwei Lin and Yi 2003). These waste materials include organic matter, nutrients, and suspended solids. Production discharges can range in form from various dissolved and particulate organic compounds, to inorganic products. Organic compounds are in the form of feces, waste food and accidental food spillage, including proteins, carbohydrates, lipids, vitamins and pigments. Inorganic products are released in the forms of ammonium and species-dependent trace quantities of bicarbonate, phosphate, and urea (Read and Fernandes 2003). There are also discharges related particularly to the fish farm

activities, such as the regulated dumping of mortalities, and fish processing wastes (detergents, antifoulants, heavy metals, and medicines) (Read and Fernandes 2003).

Marine studies have shown that nutrients and organic wastes from intensive fish farm operations can threaten water quality, algal communities, and alter local water flow and sedimentation regimes (Read and Fernandes 2003; Yan 2005), all of which can affect the balance of trophic levels within the system (Read and Fernandes 2003). For this reason, understanding of how the lower trophic levels, particularly the phytoplankton and benthic algae respond to these nutrient loads is integral to understanding how the whole-lake ecosystem will respond.

Nutrient Enrichment in Aquaculture

Nutrients are vital to all biological functions (DeAngelis ***et al.*** 1989). They are generally in small supply so that they limit production within the ecosystem (Schindler 1978; DeAngelis *et al.*1989). Nutrients such as phosphorus (P), nitrogen (N), or silicon usually limit growth in marine and freshwater ecosystems (Zhang 2000). P and N are of most concern though, as they can cause rapid eutrophication (Schindler 1974; Schindler 1978).

In freshwater, P is most often the least abundant nutrient that is needed by most photosynthetic organisms (Schindler 1974; Schindler 1978), in marine waters, N is often the limiting nutrient (Bonsdorff ***et al.*** 2002). As a result, additional nutrient inputs of either N or P can change the dynamic of an aquatic ecosystem. Potential anthropogenic sources of nutrients can include: sewage and animal wastes, atmospheric deposition, groundwater inflow, agricultural and fertilizer runoff, and of particular interest in the current study, aquaculture operations (Anderson *et al*. 2002).

Aquatic ecosystems that receive additional inputs of limiting nutrients usually experience an increase in algal biomass (Schindler 1974). The results of which can range in severity from, nuisance blooms to a phenomenon known as harmful algal blooms (Burkholder 1998; Anderson *et al.* 2002). Nuisance blooms reduce or at times destroy the pleasure associated with being near the water (Lund 1972). They can also be the cause of blocked filters and unpleasant tastes and odours in drinking water supplies (Pip 1993). Harmful algal blooms involve toxins or harmful metabolites that have been linked to wildlife deaths and human poisonings (Anderson *et al.* 2002). Some algal toxins, including potent hepatotoxins and neurotoxins, can cause poisonings at concentrations as low as a few hundred cells ($0.1 \mu g \cdot L^{-1}$) (Anderson *et al.* 2002). High algal biomass can also lead to the development of foams or scums (Anderson *et al*. 2002). Oxygen depletion is usually associated with the decay of this increased biomass. This increase can impact fish habitat, and in extreme cases even destroy it (Havens 1993).

Aquaculture has been identified as a major nutrient source in both marine and freshwater ecosystems. Nutrients inputs are via the fish feed (Beveridge *et al.*1997a), nutrients released from solid fish wastes (Cho and Bureau 2001) and to a lesser extent, the excretion of nitrogenous wastes via the gills and kidneys of the farm fish (Tovar *et al.* 2000; Glaholt and Vanni 2005). The surplus feed can be released into the water column as suspended organic solids or dissolved C, N and/or P (Tovar *et al.* 2000). Guo and Li (2003) estimated feed utilization rates of cultured fish and found N and P retention of 14.8% and 11.0%, respectively. That is, for every 1 kg fresh fish, there was a loss of 0.16 kg total nitrogen (TN) and 0.04 kg total phosphorus (TP) to the surrounding water (Guo and Li 2003).

In aquaculture operations located in large, open, coastal regions it is difficult to estimate the nutrient imports and exports because the effects from the separate nutrient sources can not be distinguished or quantified (Menge 1992). For this reason, controversy exists over the contribution of aquaculture to nutrient loads in areas subject to rapid water exchange (Johansson 2001; Read and Rernandes 2003). Sediment re-suspension, the import of nutrients from the surrounding sea, and other coastal anthropogenic sources will also influence these nutrient budgets (Johansson 2001). Black (2001) argues that aquaculture contributes only a small fraction of the total nutrients added to coastal waters and the nutrients it does add are easily assimilated by the system. Conversely, lakes and confined coastal areas have defined boundaries and identifiable connections with bordering ecosystems (Vadeboncoeur *et al.* 2002). Water exchange and the import and export of nutrients can be easily identified, for lakes especially, making changes more concentrated and apparent (Vadeboncoeur *et al.* 2002).

In confined areas, where exchange is restricted, high levels of nutrients can accumulate (Read and Fernandes 2003). Particulate waste products from these operations will quickly settle to the bottom close to their discharge points. When nutrients are released into deeper waters, or into areas with water currents, nutrients will disperse more easily (Handy and Poxton 1993). A number of factors will influence how much waste material (N and/or P) will reach the cage sediment, such as tidal flow or water current, supply of waste, depth of the site, composition, size and behaviour of the particulate matter released, temperature and salinity (of the sea water), and wind and wave action (Johansson 2001).

A substantial part of the feces and uneaten food (~ 85% Guo and Li 2003) is emitted as rapidly sinking particles that ultimately settle on the bottom sediments below the aquaculture operation. Nutrient concentrations in the sediments below the aquaculture operation can be a magnitude higher in comparison to unaffected sediments (Kelly 1993). In freshwater aquaculture operations, more than half of the loading from fish farms is particulate material in the form of feed dust, uneaten feed, fish fecal material, and fish carcasses (Beveridge 1984; Hakanson 2005). These particles sink rapidly to the bottom sediments (feed pellets typically sink at a rate of 6-14 $cm \cdot s^{-1}$ depending on size and composition (Yan 2005), while salmonid fecal material settles at a rate of 5.5-6.5 $cm \cdot s^{-1}$ (Chen *et al.* 1999)). Because of this, fish food pellets (and/or fecal wastes) can rapidly form a visible upper layer on the surface of the sediments (Hargrave *et al.* 1997). Hargrave *et al.* (1997) compared surface sediment values (organic C: 22.7 to 27.3% and N: 3.02 to 3.83%; C: N atomic ratios of 8.3 to 9.2) to dry salmon food pellets (organic C = 50.4%; N=8.5%; C: N atomic ratio of 6.92) and found that they were of similar magnitude.

When rich organic material settles it increases the sediment organic load, which leads to a number of negative effects on sediment biogeochemistry and benthic community health (Hargrave *et al.* 1997). The organic waste is broken down by bacteria and other organisms through respiration (consumption of oxygen). Surplus settling of organic matter can result in a reduction of dissolved oxygen levels and the creation of an anoxic environment (Forsberg 1996; Forsberg 1997). Holmer *et al*. (2003) explained that as long as the sediments remain oxidized (contain electron acceptors such as oxygen, manganese and/or iron) anoxia will not occur. However, once the electron acceptors are

exhausted the sediments will become highly reduced with a build-up of sulfides in the sediment pore waters. Methane-reducing bacteria (use methane as an energy and carbon source) thrive in these oxygen-depleted environments. These bacteria are also major contributors to lake anoxia, exacerbating the problem of low oxygen (Welch *et al.* 1980). These anoxic environments can be severely detrimental to the growth and survival of all benthic organisms (Holmer *et al.* 2003). In some intense aquaculture operations, organic matter mineralization can not keep up with the organic matter load (Hargrave *et al*. 1997; Holmer *et al.* 2003) and the sediments become so enriched that they act as nutrient reserves even after the fish pens have been abandoned (Hargrave *et al.* 1997; Holmer *et al.* 2003; Yan 2005).

A large portion of nutrient wastes created by in-lake aquaculture emissions rapidly settle to the bottom, although up to 60% of the wasted N and P is bioavailable to algae prior to reaching the sediments (Chen *et al.*1999; Johansson 2001). Johanson (2001) estimated that in 1999, aquaculture emissions represented 38-93% of the P loads and 13-56% of the N loads in Swedish lakes. This contributed to 11-80% of the TP and 7-40% of the TN. Loads in the high part of this range can cause rapid eutrophication and potentially large increases in algal biomass in aquatic ecosystems (Honkanen and Helminen 2000). Whether the increased nutrient load is reflected in the biomass of photoautotrophs is dependent not only on feed type (proportion of rapidly sinking particulate to dissolved material) but also on area and depth of the cage operation, annual fish production and loading rates, and the water residence time of the nutrients (Phillips *et al.*1985b; Guo and Li 2003).

Nutrient Loading: Algal Consequences

Planktonic algae

In general, an increase in nutrients results in an increase in phytoplankton biomass (Van de Bund *et al.* 2004). In lakes it is most often P that regulates the standing stocks of phytoplankton (Schindler 1978; Honkanen and Helminen 2000). Changes in nutrient conditions in shallow lakes can lead to changes in algal taxonomic composition (Tilman *et al.* 1982) and changes in the size of dominant phytoplankton species (Cottingham 1999). Changes in taxa are related to differences in nutrient uptake, storage, growth, and loss rates due to herbivory (Watson *et al.* 1997). Under low nutrient conditions small phytoplankton dominate the water column. However, when the water column becomes enriched with nutrients larger phytoplankton become more dominant (Cottingham 1999).

In oligotrophic lakes (<10 μg TP liter^{-1}), phytoplankton biomass is fairly evenly divided among cryptophytes, chrysophytes and diatoms (Watson *et al.* 1997). Eutrophic lakes (> 60 μg TP liter^{-1}) on the other hand, are dominated by fewer taxonomic groups, mainly chlorophytes and cyanobacteria (Watson *et al*. 1997). Hence, increasing nutrient loads are marked by a shift in dominance from chrysophytes and cryptophytes to chlorophytes and cyanobacteria (Jensen *et al.*1994; Van de Bund *et al.*2004).
Jensen *et al.* (1994) found that when nutrients were increased in a shallow hypereutrophic Danish lake, chlorophytes and cyanobacteria became the dominate taxanomic groups. In a later study, Watson *et al.* (1997) noted a 100-fold increase in cyanobacterial biomass from a temperate northern mesotrophic lake (10-30 μg TP liter^{-1}) to a eutrophic lake. Chlorophytes dominate the relatively few phytoplankton communities present in the hypereutrophic lakes, despite only a slight increase in biomass.

Cyanobacteria are responsible for noxious blooms in eutrophic lakes (Anderson *et al.* 2002) and are generally considered inedible to zooplankton (Haney 1987). This can be attributed to their ability to accommodate for environmental changes associated with nutrient enrichment, such as reduced N: P ratios, CO_2 availability and light (Paerl 1997). Chlorophytes, on the other hand, include a diverse group of green algae with a broad range of morphologies, with both inedible and edible forms (Watson *et al.* 1997). In eutrophic systems, it is the inedible forms that usually increase in dominance (Watson *et al.* 1997). Watson *et al.* (1997) showed that the inedible fraction of the phytoplankton community exhibits significant, nonlinear growth over the entire total phosphorus range.

In aquaculture, phytoplankton can utilize the bioavailable P prior to its sedimentation and under ideal light conditions can proliferate (Handy and Poxton 1993). It is expected that if the supply of available P increases in the lake from fish-farm emissions then the in-lake algal biomass will also increase. Similarly, the lake's phytoplankton community composition (Jensen *et al.* 1994; Watson *et al.* 1997; Van de Bund *et al*. 2004) and zooplankton-phytoplankton interactions will undergo large changes caused by an increase in inedible or unpalatable species (Jensen e*t al.* 1994).

Phytoplankton responses to aquaculture activities have been reported in Węgleńska *et al.* (1987), Diaz *et al.* (2001) and Findlay *et al.* (in press). All three studies observed changes in species composition, abundance and increases in the large-size fractions of planktonic algae. Węgleńska *et al.* (1987) observed lake-wide co-dominance by cyanobacteria and dinoflagellates in Lake Glebokie, Poland. Diaz *et al.* (2001), on the other hand, observed only localized changes in the Alicura Reservoir in Argentina. They

observed an increase in cyanobacterial abundance; however, diatoms remained the dominant group. They also saw a change in secondary phytoplankton associations from a decrease in chrysophytes and dinoflagellates to an increase in chlorophytes. Findlay *et al.* (in press) studied the phytoplankton in the current project and noted compositional changes similar to Węgleńska *et al.* (1987); lake-wide co-dominance by cyanobacteria and dinoflagellates. The greatest responses in Findlay *et al.* (in press)'s L375 phytoplankton community occurred in the early spring and late fall, during lake-mixing times. Diaz *et al.* (2001) reported only fall maximums for planktonic algae near the aquaculture operation, while Węgleńska *et al.* (1987) reported lake-wide blooms occurring in the spring and summer.

The differences in phytoplankton responses among aquaculture studies can be attributed to variation in hydrology, morphometry, and differences in physical (lake-mixing times) and chemical factors (buffering capacity) among the study lakes (Diaz *et al.* 2001). In addition differences among the aquaculture operations i.e., changes in feed composition, and fish-production rates, can cause differences in results (Findlay *et al.* in press). Both the Alicura Reservoir (6700 ha) and Lake Glebokie (47.3 ha) are much larger than L375 (23.2 ha) with faster flushing rates (0.4 yrs, 1.5 yrs and, 5.5 respectively) and greater annual fish production (100 tonnes, 18 tonnes and, 8-10 tonnes per year respectively). Findlay *et al*. (in press) explain that the differences between Lake Glebokie and L375 are likely due to: (1) differences in farming practices, (2) differences in the type of feed used, and (3) physical differences between the two lakes. Major differences in fish production, feed rates and nutrient loading between L375 and Alicura Reservoir should have also led to a much greater difference in phytoplankton

responses. However, faster flushing rates in Alicura Reservoir (0.4 yrs) enabled a higher buffering capacity for the system, leading to only minimal, localized impacts (Diaz *et al.* 2001).

At low P concentrations, freshwater lakes are usually in a clear-water stage, submerged macrophytes are in abundance, algal species are diverse, piscivorous fish are present in large numbers and predation pressure on zooplankton is relatively low (Jeppesen *et al.* 1994; Van de Bund *et al.* 2004). When P concentrations are increased in a lake, a shift to a turbid stage is experienced, the submerged macrophytes ultimately disappear, and the fish stocks shift towards dominance by planktivorous fish (Herikson *et al.* 1980; Jeppesen *et al.* 1994). An increase in zooplanktivorous fish leads to a reduction in the capacity of zooplankton to control algal growth and the lake becomes dominated by phytoplankton (Herikson *et al.* 1980; Hansson *et al.* 1987).

In addition, since increased P can increase the large-size fraction of algae, as well as inedible algae, zooplankton grazing capacity can be reduced (Jeppesen *et al.* 1997; Tilman *et al.* 1982; Menge 1992), dampening top-down control (Bohannan and Lenski 1999). This is believed to occur because the lowered edibility of the prey is acting as an anti-herbivory mechanism, which allows the prey population to respond to the nutrient enrichment (Worm *et al.* 2000). Without the presence of unpalatable species, zooplankton grazing pressure can override the effects of increased nutrients on phytoplankton populations (Worm *et al*. 2000).

Large filamentous cyanobacteria can affect zooplankton populations through a number of pathways (Haney 1987). First, they can have deleterious effects on feeding rates through poor nutritional quality and the production of toxins. Second, through

reduced zooplankton filtration capacity (Haney 1987; Jensen *et al*. 1994; Worm *et al*. 2000), large algal filaments created by chains of cyanobacterial cells interfere with the efficiency of mechanical food gathering (Haney 1987). Third, indirect effects caused by allelopathic interactions within the cyanobacteria retard other more desirable phytoplankton species, which can affect the selective grazing capacity of zooplankton species (Haney 1987) and finally, a widely recognized effect of cyanobacterial blooms is the eventual suffocation of higher trophic levels within the lake (Pick and Lean 1987; Dillon and Molot 1997). When dense blooms of cyanobacteria senesce the biological oxygen demand (BOD) of decomposing organic material increases, causing anoxic conditions in the bottom waters (Dillon and Molot 1997). The problem is usually confined there. However, in extreme conditions, the whole-water column may experience hypoxia or even anoxia (Yan 2005). Since the oxygen concentration of the water is no longer at or near saturation, a reduction of habitat for fish and other aquatic biota occurs (Jeppesen *et al*. 1994; Havens *et al*. 2001). In severe cases, massive kills of entire fish populations can be linked with the sudden collapse of large algal blooms (Pick and Lean 1987). Indirectly, decomposing organic matter can also augment the eutrophication problem by increasing the internal P load by releasing P from the sediments in anoxic water (Yan 2005).

Benthic algae

Benthic algal community responses to nutrient enrichment in general, are not as established in the literature as phytoplankton response (Winter and Duthie 2000; Vadeboncoeur *et al.* 2002). However, Vadenbocoeur *et al.* (2001) found that contrary to phytoplankton, as nutrients increased, benthic primary production often decreased in

eutrophic lakes. Initially, the relationship between periphyton and phytoplankton productivity was positive, but as the water-column became more fertilized and phytoplankton biomass increased, benthic primary production decreased and became less important to lake-energy flow.

Mannino and Sara (2007) specifically studied the effects of an in-lake aquaculture operation on periphytic assemblages. They found that the increased biodeposition from the fish-farm activities lowered species diversity and richness, but increased net productivity of the benthic algae. The farm seemed to support more opportunistic species with rapid growth rates (a high output of reproductive bodies) and a tendency to be more eurytopic. Differences in benthic algal responses between the Vadeboncoeur *et al.* (2001) and the Mannino and Sara (2007) studies are likely due to differences in the nature of the nutrient release and therefore phytoplankton availability. Aside from Mannino and Sara (2007), few studies have research benthic algal changes due to aquaculture nutrient additions; therefore, further research is needed.

Like zooplankton, zoobenthic invertebrate grazers exert a strong top-down influence on benthic algae (Vadeboncoeur *et al*. 2002). Unfortunately, there have been few quantitative estimates of the extent to which zoobenthic grazers use benthic algae as an energy resource in lakes (Vadeboncoeur *et al*. 2002). Strayer and Likens (1986) estimated that periphyton made up 50% of the diet of zoobenthos in Mirror Lake, New Hampshire. However, variation is expected under different light, substrate, pH and nutrient conditions (Lowe and Pillsbury 1995; Turner *et al*. 2005; Turner *et al*. 1991). It is expected that zoobenthic grazers will alter benthic algal assemblages, causing

community structural changes through the preferential removal or avoidance of distinct algal species and/or changes in net primary productivity and biomass (Smith *et al.* 2001).

Top-down pressure on the zoobenthos is not only exerted by benthivores but also by planktivores. Vandeboncoeur *et al.* (2001) did a survey of 15 North American fish to determine the extent of benthic consumption. They found that 65% of the diet of these fish was either directly or indirectly from the zoobenthos. Therefore, benthic algae represent an often largely neglected carbon pool in most lakes (Vadeboncoeur *et al.* 2001).

Interaction and integration: benthic and planktonic algae

In order to understand the dynamic associated with nutrient loading on trophic groups at the lake scale, the lake must be viewed as an integrated whole, starting with the primary producers. Both benthic and pelagic primary producers depend on light and nutrients. The same nutrients, N and P, can limit benthic and planktonic algae (Havens *et al.* 2001). The lake's nutrient cycle is therefore likely to include a close coupling between the two.

Benthic primary producers are often more effective than phytoplankton at sequestering sedimentary nutrients, such as P, from the interstitial water and as a result they regulate the availability of sediment nutrients to phytoplankton in oligotrophic lakes (Hansson 1988). Phytoplankton and periphyton have similar photosynthetic irradiance response curves (Havens *et al.* 2001); however phytoplankton are found circulating in the water column and can therefore intercept light before it reaches the benthic algae (Vadeboncoeur *et al*. 2001). Consequently, when high densities of phytoplankton occur as a result of P loading, the effect can be reduced light penetration to the benthic algae.

This in turn hinders the development of attached benthic algae so that they cannot achieve their net growth (Havens *et al*. 2001).

Vadeboncoeur *et al.* (2001, 2002) point out that the majority of primary production occurs in the benthic habitat in unmanipulated lakes and often only shifts to the pelagic habitat when fertilizer is added to the water column. A literature review by Lodge *et al*. (1998) found similar results in that of over half the lakes studied, benthic algal production equalled or exceeded phytoplankton production. When nutrients were added the importance of the benthic community as an energy source diminished. It was found that in shallow, eutrophic Danish lakes, periphyton contributed less than 1% to whole-lake production. However, in shallow, oligotrophic Greenland lakes, periphyton accounted for as much as 95% of the lake's productivity (Lodge *et al.* 1998).

In a healthy system fish can play a substantial role in nutrient recycling and can be important integrators for benthic and pelagic habitats (Vander Zanden and Vadeboncoeur 2002). Benthic feeding fish can stimulate nutrient transfer into the benthic food-web (Glaholt and Vanni 2005), or plantivorous-benthivorous fish can recycle it back into the pelagic food web (Jeppesen *et al.* 1997). Benthic algae are able to provide top predators with an alternative source of energy than the pelagic zone (Vander Zanden and Vadeboncoeur 2002; Glaholt and Vanni 2005). The reliance of planktivorous-benthivorous fish on the zoobenthos for much of their food supply (Vadeboncoeur *et al*. 2001) permits nutrient recycling within the lake and illustrates the linkages between benthic and pelagic food webs.

However, lake-wide trophic changes related to aquaculture, can occur without corresponding algal changes. Hankanson (2005) studied changes to lake ecosystem

structure resulting from rainbow trout fish farm emissions in Lake S. Bullaren, Sweden and found that wild fish biomass increased substantially without corresponding increases in algal biomass. The fish farm managed to influence secondary production without influencing primary production. This occurred through the direct consumption of fish farm food spillage and feces by native fish. Hankason (2005)'s work demonstrates a new food web component related to food spill and feces that can ultimately change in-lake trophic structure and dynamics.

Rate and extent of natural recovery after cage fish farming has ceased

Total phosphorus and chlorophyll concentrations have been shown to decrease in lakes after P loading is reduced (Perrow *et al.* 1994), but the severity, internal loading, and duration of historical eutrophication influences recovery rates (Yan 2005; van der Molen and Boers 2006). In aquaculture, a decrease in TP is expected following the cessation of the cage operation; however, the length of recovery time is contingent on water residence time, hydrology and morphometry of the lake (Diaz *et al.* 2001). Kelly (1995) and Axler *et al.* (1998) observed a decrease in TP from 95$\mu g \cdot L^{-1}$ to pre-farming levels of < 10 $\mu g \cdot L^{-1}$, 5 years after termination of operations in Twin City-South Pit lake. Kelly (1995) also saw a reduction in TP after the closure of a cage fish farm in study lake 5 in Scotland.

The results of a paleolimnological study of the LaCloch Channel in Lake Huron, Canada by Clerk *et al.* (2004) found that the removal of the operation did lead to improvements in surface water quality; however, there was very limited recovery of deep-water oxygen levels. It appeared that benthic communities took longer (up to several years longer) than planktonic communities to recover from past aquaculture

operations. Doughty and McPhail (1995) also showed that sediments under a closed down aquaculture site had elevated oligochaete abundances (indicator of high sediment organic loading (Verdonschot 1996)) even 3 years after fish farming activities had ceased (Doughty and McPhail 1995).

Summary

The modern view that freshwater aquaculture is the solution to the future supply and demand gap seems rather optimistic in light of the obvious environmental impacts associated with it. The greatest environmental unease is associated with the significant nutrient loadings from the farm fish wastes (uneaten fish food, feces, and urine), which cause an increase in the P and N load to the system. An increase in these limiting nutrients may allow phytoplankton populations to increase to unmanageable sizes. The inevitable increase in phytoplankton biomass can have detrimental effects on trophic structure and function. Their sudden senescence can cause an influx of organic matter to the benthos. Combined with the demand already placed on it by the organic loading from the fish farm, a collapse of the whole benthic community could occur, leading to eventual water column anoxia and massive fish kills.

The benthic algal community is an important part of the lake in that it sustains the balance between the two major lake habitats. Understanding how this community will react to changes in nutrient additions from aquaculture is important for the understanding of the whole-lake impacts. Since there is little information available on benthic community responses to anthropogenic nutrient additions, research in this area will provide long-needed insight into this vital community.

Aquaculture at the Experimental Lakes Area

The experimental aquaculture research began in 2001, with two pre-impact control years (2001 and 2002), in which all water quality parameters were tested and food web dynamics were monitored. The cage was stocked in the spring of 2003 wherein 10,000 female rainbow trout (*Oncorhynchus mykiss*) were introduced to a 100-m^2 steel framed cage in the northern portion of the study lake (Fig. 1.1). The square steel frame contained a 760-m^3 nylon mesh net with 5-cm^2 openings (Bristow 2006). The net was suspended to a depth of 10-m and installed over approximately 15-m of water (Bristow 2006).

A high nutrient density commercial trout diet of Martin Mills high energy pelletized fish feed (Profishent®) was used. This feed was partially comprised of marine fishmeal (herring in 2003-04, and 2006-07; menhaden in 2005) (Kullman *et al.* in prep). The fish were manually fed at a rate monitored by a commercial operator. The Feed Conversion Ratio (FCR: a metric used in the aquaculture industry as an indicator of farm economy), was economically viable (Table 1.1) (Bristow 2006), and minimized feed wastage. Fish harvest typically occurred after an average growing season of 5.5 months, at a point in time when the fish were approximately ten times their initial weight (mean initial weight: 0.1 kg, mean final weight: 1 kg; Table 1.1).

Following initial stocking, and for the next five years of operation, a multidisciplinary team of scientists researched the effects of the aquaculture operation on different aspects of the ecosystem to understand how the operation affected the whole lake. As part of this multidisciplinary team, my research focused on the effects of nutrient

loading from cage wastes on the littoral zone in terms of benthic algal composition (chapter 2), productivity (chapter 3) and toxins (chapter 4).

Figure 1.1: Rainbow trout aquaculture cage on L375. Photos by K. Hille, June 2006.

Table 1.1: General farm characteristics during years of operation, 2003-2007. Farm data from 2003 to 2005 from Bristow 2006; 2006 and 2007 data from A. Mcfee (pers. comm.).

	2003	2004	2005	2006	2007
Stocking Date	June 5th	May 31st	May 31st	May 29th	May[a] 7th & 28th
Harvest Date	Nov 19th	Oct 24th	Oct. 30th	Nov. 6th	Oct 29th
Initial number of fish	10640	10249	9834	9947	9681
Initial body weight (g)	94.0	101	190	61.3	69.0
Mean initial biomass (kg)	1000	1038	1868	610	668
Final number of fish	9988	9830	9161	9623	9317
Final body weight (g)	850	996	1109	809	941
Mean final biomass (kg)	8490	9792	10,159	7783	8763
Biomass gain[b] (kg)	7490	8753	8292	7173	8094
Feed[c] (kg)	8714	9672	11,125	9740	11,770
FCR[c]	1.16	1.10	1.34	1.36	1.45

[a] A Massive loss, due to fish stress, following initial stocking on May 7th required a second stocking in 2007.
[b] Biomass at harvest minus biomass at initial stocking
[c] Feed Conversion Ratio (FCR) calculated as feed divided by biomass gain. Data from P. Azevedo, pers. comm.

Benthic Algae as a Diagnostic Community

The Littoral Zone

The littoral zone is one of the most diverse and complex areas of the lake ecosystem (Turner 1993). It is often referred to as the near shore region and is the link between the catchment area of a lake and its open water (Wetzel 2001a). It includes the lake bottom, its overlying water in the euphotic zone and the interface between the water surface and the atmosphere (Loeb *et al.* 1983; Moss 2005).

Unlike the open water, which is relatively homogeneous, the littoral zone is a heterogeneous assemblage of surfaces (Fig. 1.2 a) (Turner 1993). This diverse area is capable of supporting a wide range of independent and sometimes interconnected communities, which include macrophytes, attached algae, bacteria, protozoans, sessile invertebrates, motile grazers and scrapers, seasonally important egg-laying fish, and other small, transient fish species (Moss 2005).

As the link between the catchment area and the open water, the littoral zone may be affected by, and in turn influence, what is occurring in the open water. The littoral zone acts as an interceptor or a sink for nutrients, as a source of new nutrients, and as a refuge or resource for animals to avoid predation or to feed (Moss 2005). Since it is both a sink and source (Riber *et al.* 1983), it effectively increases the residence time of nutrients in this zone. In contrast to the open-water, which typically requires a sustained input of new nutrients for algal growth (Wetzel 2001c), the littoral zone has the capacity to recycle and retain an internal nutrient load (Riber *et al.*1983; Turner *et al.* 1994).

The potential importance of this biologically abundant and diverse community can not be over-emphasized and neither can its complexity. Thus, littoral research

requires carefully crafted, easily tested predictions and theories that focus on specific characteristics and communities in the zone (Turner 1993).

The Epilithon

The particular diagnostic community chosen for this study was the epilithon (Fig. 1.2 b), which is the natural biofilm on rock surfaces. In addition to benthic algae, the biofilm consists of bacteria, fungi, metazoans, and detritus embedded in an abiotic mucopolysaccharide matrix secreted by the bacteria and algae (Wetzel 2001 a). Furthermore, this three dimensional hydrated glycocalyx is enmeshed with numerous other organic (dead algae, animals and terrestrial debris) and inorganic materials e.g., siliceous dead diatom frustules and, in high pH systems, inorganic calcium carbonate precipitated by photosynthetic processes (Wetzel 2001a).

The epilithon was chosen for this study due to: (1) the predominance of rock as an algal substratum in ELA lakes; (2) the rock substratum allowed for natural and low-impact, direct measures of community productivity to be performed; and (3) the inertness of the rock substratum ensured that nutrient inputs to the epilithon would be through the biofilm-water interface and not leached to the community through the substratum.

Epilithic communities are often thick communities (ca. 5 mm at a depth $\geq$ 1 m in ELA lakes (Turner 1993)), which are maintained by intensive internal recycling of nutrients among the organisms within the 'boundary layer' (Wetzel 2001a). The 'boundary layer' is a layer of inactive water above the algal mat (Kahlert and Petterson 2002); it can reach a thickness of up to 1 cm (Riber and Wetzel 1987). Diffusion across this boundary and the external medium slows the exchange of nutrients, gases and

metabolic products (Wetzel 2001a); hence, the thicker the layer, the slower the exchange (Riber and Wetzel 1987). In addition to boundary layer thickness, nutrient availability from the water is also dependent on: (1) the concentration of dissolved compounds in the surrounding water, (2) the hydraulic conditions at the boundary layer and (3) the metabolic activity of the attached organisms within the biofilm (Wetzel 2001a). Since the internal processes within the boundary layer change the chemical conditions within the biofilm, they can be quite different from those in the free water phase. Thick, dense and active periphytic communities in standing waters can show a significant difference between the biofilm and open water chemistry (Sand-Jensen 1983).

Most often in inland waters, P is the limiting nutrient for photosynthesis and biomass of phytoplankton (Schindler 1974; Schindler 1978). However, the role of P limitation in the epilithon is less clear (Turner *et al.*1994). Turner *et al.* (1994) showed that in the oligotrophic, low alkaline lakes of ELA, rates of periphyton productivity can be limited by low dissolved inorganic carbon (DIC) in the overlying water, rather than by P. The supply of DIC for photosynthesis is restricted by the boundary layer, which enables the biofilm to trap and recycle P. That is, even though P is ultimately the limiting nutrient for benthic algae, since P accumulates (and is recycled) within the epilithon, there may be a shift to C limitation in these low DIC lakes. When DIC is high in the overlying water, the epilithon can be P limited (Turner *et al.* 1994). In a high DIC system the resistance to diffusion is effectively diminished because of the steep concentration gradient, allowing DIC and other nutrients to easily diffuse in and out of the epilithon (Turner *et al.* 1994).

In low DIC lakes, increased P loading will favour energy flow in the pelagic zone (Turner *et al.*1994). The importance of epilithic productivity to whole-lake budgets will be reduced through competition with the phytoplankton for light and inorganic nutrients (Vadeboncoeur *et al.* 2002). In high DIC lakes where P remains limiting, an increase in P loading can initially increase benthic algal biomass and productivity (Fairchild and Lowe 1984; Cattaneo 1987), depending on the form and mechanism of P delivery to the system (Cattaneo 1987; Wetzel 2001a).

Some periphytic communities are able to obtain nutrients from their substrata. Living substrata in particular are important nutrient sources for benthic algae. For epiphytic communities, the small amounts of nutrients that leach out of macrophytes while they are actively growing are sufficient to cover the majority of the P demand of some benthic algae (Riber *et al.* 1983; Kahlert and Petterson 2002). For epilithic communities, which reside on relatively inert surfaces (rock), nutrients are obtained mostly by diffusion into the communities from the overlying water (Kahlert and Petterson 2002). Therefore, nutrient concentrations in the surrounding water influence the epilithon to a greater extent than other periphytic communities.

Regions of the littoral zone, where there is considerable water movement such as the shore-line, are more likely to exhibit P limitation. Also, epilithon developing on vertical substrata are more vulnerable to water movement and can experience P limitation (Turner *et al.*1994). Water motion alters the physico-chemical environment overlying the epilithic matrix, reducing the residence time of dissolved nutrients (Stevenson *et al.* 1982; Turner *et al.*1994), thus allowing the biofilm to be depleted of essential nutrients such as P. As a result, the response of the epilithon to nutrient additions will differ depending on

the nutrient status of both the lake and biofilm prior to the additions, water movement, geometry of the biofilm, surface roughness, and orientation of the substrata (vertical vs. horizontal orientation) (Turner *et al.* 1994).

Figure 1.2: (a) Littoral zone heterogeneity. Algal colonization can occur on rock, woody debris, macrophytes, sediment and various other surfaces. (b) Vertical epilithic algal growth in the middle littoral zone. Photo (a) by K. Hille 2007, photo (b) by M. Turner 2007.

Potential Importance of Benthic Algal Communities

Most of the world's lakes are shallow (< 20 m mean depth) and possess large littoral zones with an abundance of surfaces available for colonization (Wetzel 2001a). Since over 90% of all algal species will grow attached to a substratum (Wetzel 2001a), the magnitude of the effect that littoral productivity can have on the lake ecosystem can be substantial. Although productivity on a cellular basis is not necessarily greater in littoral algae than phytoplankton, the large surface area available for colonization allows for a very high contribution by attached algae to the total productivity pool (Wetzel 2001a). Vadeboncouer *et al*. (2002) compared studies from the International Biological Program in the 1970s and found that when annual productivity per square meter of lake area was estimated, benthic primary productivity was, on average, $58 g \cdot C \cdot m^{-2} \cdot yr^{-1}$ compared to $69 g \cdot C \cdot m^{-2} \cdot yr^{-1}$ for phytoplankton, for 29 lakes worldwide ranging in size (1.7 to 99,000 ha in surface area) and depth (0.2 to 34 m mean depth). In addition, Bjork-Ramberg and Anell (1985) observed high periphyton densities in subartic Swedish lakes that constituted 70-83% of the total lake primary production. Lodge *et al.* 1998 also found that periphyton productivity accounted for 95% of whole-lake production in oligotrophic Greenland lakes. Finally, Wetzel (2001 a; Table 19-10) provides several examples from the literature in which attached algal net productivity is similar to, or exceeds that of phytoplankton.

Benthic algae can also be a main energy source for top predators in pelagic food webs. Fish production is energetically dependent on benthic secondary production (Vadeboncoeur *et al.* 2002). A wide array of fish taxa rely on carbon fixed by both littoral attached algae and phytoplankton (Hecky and Hesslein 1995; Vadeboncoeur *et al.* 2003).

Benthic algae are therefore generally an underappreciated carbon source for higher trophic levels in most aquatic systems (Vadeboncoeur *et al.* 2001).

Benthic algal productivity is often ignored in the literature (Strayer and Likens 1986), despite its importance. Technical and logistical problems associated with sampling this community are the main reasons for its neglect (Wetzel 1965). Difficulties include: (1) the inclusion of the benthic algae in a matrix with non-algal material, bacteria, fungi and detritus; (2) benthic algal investigations usually require the use of SCUBA diving, which is both costly and time consuming; and (3) benthic algae vary significantly in time and space, both horizontally and vertically (Kahlert and Petterson 2002). Subsequently, most littoral benthic algal assumptions are based on pelagic observations, or use communities developed on artificial substrata for relatively short periods of time, both of which have undoubtedly yielded misleading conclusions regarding natural littoral productivity.

Research Objectives and Chapter Previews

Limnologists often study the effects of perturbations on whole-lake ecosystems while overlooking the littoral benthic algal community. Insight into how this community responds to a given perturbation will partly determine how the disturbance affects the whole ecosystem. Poor understanding of the effects on the benthic algal habitat and the linkages with the pelagic habitat will impede our ability to understand the effects of the anthropogenic stress being investigated. It may also result in incorrect decisions in terms of policy development (in this case for an environmentally sustainable and economically viable aquaculture industry). Hence, this study explores the potential impacts associated with nutrient loading on the benthic algal community residing in the littoral zone.

My research (2006-2007) was based on previous work done at the ELA by Dr. Michael Turner and his assistants. The majority of the methods used in this study were based on applications outlined in Turner *et al*. (1987; 1991; 1994). Data retrieved from 1992 to 2002 by littoral researchers was used as a background assessment of pre-cage conditions. In addition, littoral investigations of cage effects began prior to my involvement in the study, therefore cage operation data collected from 2003-2005 by littoral investigators was also used to supplement my thesis research.

This thesis explores the use of benthic algae as biomonitors in an experimental aquaculture operation by examining both composition and metabolic changes in the community. Specifically, In chapter 2, *Epilithic composition: does aquaculture waste change benthic algal structure and/or biomass?* I tested whether increased nutrient availability in the experimental lake, due to cage wastes (feed and excretory), affected any of the compositional characteristics of the biofilm. I predicted that, if inorganic carbon concentrations were sufficiently high, the increase in available P from the aquaculture wastes would cause a response in the epilithon. The response would be demonstrated by:

1. an increase in epilithic biomass,
2. a shift in algal species composition, from diatom dominance to cyanobacterial dominance due to a decline in the N:P
3. a decrease in the C: P conditions, which would improve the nutritional quality of the biofilm for herbivorous grazers
4. incorporating the cage wastes, as measured by an increase in the δ^{15}N-signal over-time.

In chapter 3, *Epilithic function: can fish farms increase algal productivity?*, I investigated the effects of the aquaculture operation on epilithic metabolism in the experimental lake. I tested the hypothesis that as the available P increased in the experimental lake, given sufficiently high inorganic carbon concentrations, there would be an increase in epilithic photosynthesis measured by net and gross photosynthesis and a decrease in respiration.

Chapter 4, *Epilithic toxins: what lies beneath the surface?* I focused on potential microcystin toxin production in the epilithon of the three study lakes at ELA. I also included a second, longer-running aquaculture operation in Lake Wolsey to provide a more thorough understanding of the potential for toxin production in aquaculture lakes. In the chapter, I aimed to:

1. confirm that benthic biofilms are capable of producing the toxin in Canadian lakes,
2. understand whether epilithic microcystin concentrations are linked to the P gradient, and would thus be affected by the increased P load from the aquaculture operation,
3. and investigate whether it was possible to determine which epilithic taxa were responsible for toxin production.

Finally the general discussion, chapter 5, integrates and consolidates the thesis chapters, ending with future recommendations for benthic algal research. The chapter also investigates the role of benthic algae as biomonitors for eutrophication studies by examining whether benthic algal sampling provided insight into the experimental system that other ecosystem components did not.

Chapter 2: Epilithic composition: Does aquaculture cage waste affect benthic algal structure or biomass?

Introduction

Scientific uncertainty surrounding in-lake cage aquaculture practices has delayed widespread expansion of the industry in Canada. One of the major concerns associated with an in-lake operation is the nutrient loading produced from cage wastes (feed and excretory) (Beveridge *et al.*1997b). Farm-related wastes can accumulate below the cage and have localized effects or the released nutrients can disperse directly into the water column and become available for uptake by photosynthetic algae (Read and Fernandes 2003).

In the nutrient poor lakes of the ELA, P is the primary nutrient limiting algal productivity (Schindler 1974; Schindler 1978); an increase in available P is predicted to cause an increase in algal biomass (Schindler 1974; Schindler 1978; Harris 1986). This is true for phytoplankton; however, the response for benthic algae is less clear (Turner *et al.* 1994). A number of studies have reported increased benthic algal biomass in response to an increase in total P (Sand-Jensen 1983; Shortreed *et al.* 1984; Kahlert and Petterson 2002); others, however, have shown that benthic algal responses correlate less well with P than phytoplankton (Cattaneo 1987; Turner *et al.*1994). The uncertainty surrounding the response of benthic algae to water column nutrients may be a function of the community's ability to maintain its internal chemical environment through the recycling of nutrients within the biofilm (chapter 1) (Riber and Wetzel 1987).

This unique and complex community plays an important role in the ecosystem of a lake as a primary producer, regulator and integrator (Turner *et al.* 1994; Wetzel 2001a;

Vadeboncoeur *et al.* 2002). Because it is still one of the most neglected and underappreciated communities in freshwater investigations (Hecky and Hesslein 1995; Vadeboncoeur *et al.* 2001), further study is needed regarding it's physico-chemical environment and response to nutrient inputs (Wetzel 1983). Fairchild and Lowe (1984) indicate that this community is often able to rapidly integrate novel water column nutrients. Further, Sand-Jensen (1983) explains that these nutrients can be readily reflected in short-term fluctuations in site-specific development of benthic algae. Since this community can quickly assimilate new nutrients it may be a useful biological tool for monitoring and assessing the effects of short-term inorganic pollution (Sand-Jensen 1983; Fairchild and Lowe 1984).

In the current study, I focused on the response of the epilithon to increased P and N loading due to cage wastes (feed and excretory) from an experimental aquaculture operation in northwestern Ontario, Canada. This form of nutrient loading differs from other forms of cultural eutrophication, in that not all nutrients are directly or immediately available to the algae upon loading. For example, late season delivery, hypolimnetic nutrient release, mixing and sedimentation prevent these nutrients from being readily available to epilimnetic biota (Bristow 2006). However, since the littoral zone is often a sink for P (Vadeboncoeur *et al.* 2002), epilithic algae residing in this zone may eventually accumulate the aquaculture-derived P. If the P-loading from the aquaculture operation is able to increase epilithic P to a point where it elicits a response in the benthic algae, it would confirm that the benthic algae and potentially the whole-lake food web are being impacted.

It was important to the study to identify benthic algal utilization of aquaculture nutrient inputs to understand whether any observed biomass and compositional changes were a response to the aquaculture operation. Since the nutrients were added in the form of pelletized fish feed and the fishmeal used had a high marine signal, the $\delta^{15}N$ signal of the marine fish food could be tracked throughout the lake (Kullman *et al.* in prep.). Therefore, by examining the stable isotope signature of the epilithon I could determine whether the epilithic biofilm was utilizing the fishmeal as a nutrient source.

Finally, I examined whether there was a site-specific response of benthic algal communities to the aquaculture nutrients. Benthic algal biomass, composition, and P and $\delta^{15}N$ content of the biofilm were monitored to test the potential for spatial variability as a function of distance from the cage operation.

The main objective of my study was to examine whether in-lake cage aquaculture impacted benthic algae. More specifically, I predicted that if the aquaculture P increased in the system, specifically in the epilithon, the benthic algae would respond by (1) a possible increase in algal biomass; (2) a shift in species composition, from diatom dominance to cyanobacterial dominance, if there was a concurrent decline in the N: P; and (3) a decrease in the $\delta^{15}N$- marine fishmeal signal as a function of increased distance from the cage site, if the farm-related nutrients were not well mixed in the epilimnion.

Materials and Methods

Description of Study Area

The study took place in the Precambrian Shield region of northwestern Ontario at the Experimental Lakes Area (ELA) (lat. 49º34'-49º47'N, long. 93º36'-93º52'W) (Fig. 2.1). The ELA was a nearly pristine, headwater region where public access was restricted to allow long-term manipulations and monitoring of whole-lake ecosystem (Johnson and Vallentyne 1971).

The ELA was situated on the southern extension of a massive pink granodriorite batholith (Brunskill and Schindler 1971). Because of the underlying bedrock expanse most lakes in the region were water-tight basins. Also, these lakes can be characterized as soft-water, oligotrophic lakes, as atmospheric deposition on the exposed bedrock resulted in minimal inputs of weathered chemicals (Brunskill and Schindler 1971). The hilltops and hill slopes of ELA had little or no soil, and in areas where there was soil the vegetation was dominated by pine (*Pinus banksiana* Lamb.; *Pinus resinosa* Ait.; *Pinus strobus* L.), spruce (*Picea mariana* (Mill.) BSP.; *Picea glauca* (Moench) Voss), aspen (*Populus tremuloides* Michx.) and birch (*Betula papyrifera* Marsh.). A full description of the area's geology, vegetation, soils and climate can be found in Brunskill and Schindler (1971).

Morphological and Chemical Characteristics

All the lakes in the study were small, moderately deep, mostly dimictic lakes (Table 2.1), which stratified thermally in the summer and winter. The lakes were usually ice-covered by mid-November and ice-free by late April or early May (Brunskill and Schindler 1971).

The experimental aquaculture lake 375 (Fig. 2.2), was a second-order lake that received water from the main reference system, a first-order lake, L373 (Fig. 2.3). A second headwater reference system, L239 (Fig. 2.4) was also monitored to increase understanding of regional fluctuations in epilithic characteristics that may have occurred during the study. It was located approximately 15 km south of the other two lakes, near the ELA field station (Fig. 2.1).

The three lakes were relatively similar in both size and water chemistry (Tables 2.1 and 2.2). Lake 375 was the smallest lake, with a large drainage area to lake surface area than L373 (Table 2.1). The lakes differed slightly in pH from 7.1 (L239) to 7.4 (L375) (Table 2.2). The concentrations of dissolved organic carbon (DOC) ranged from L373 on the low end (344 $\mu mol \cdot L^{-1}$) to L239 on the high end (574 $\mu mol \cdot L^{-1}$). The dissolved inorganic carbon (DIC) values were markedly higher in L375 (370 $\mu mol \cdot L^{-1}$) than in the reference lakes (180-200 $\mu mol \cdot L^{-1}$) (Table 2.2).

These unperturbed lakes had naturally low nutrient levels (Table 2.2, Appendix I Fig. 1.1-1.4), which did not support high phytoplankton populations (Appendix I Fig. 1.8). The water residence time was approximately 5.5 years in L375, 16.5 years in L373, and 6.5 years in L239 (Table 2.1). Though yearly open-water surface temperatures varied from year to year in all three lakes, temperatures varied little among lakes, and all lakes followed a similar seasonal pattern (Table 2.2; Appendix I Fig. 1.10).

All three of the lakes contained large littoral zones due to their low sloping shorelines (Table 2.1). These littoral zones consisted of various substrata available for algal colonization: rock shelves, large boulders, organic sediment, gravel, and aquatic macrophytes (Stockner and Armstrong 1971). In addition to plenty of surfaces available

for colonization, good light penetration in these lakes provided conditions that were optimal for abundant attached algal growth (Stockner and Armstrong 1971).

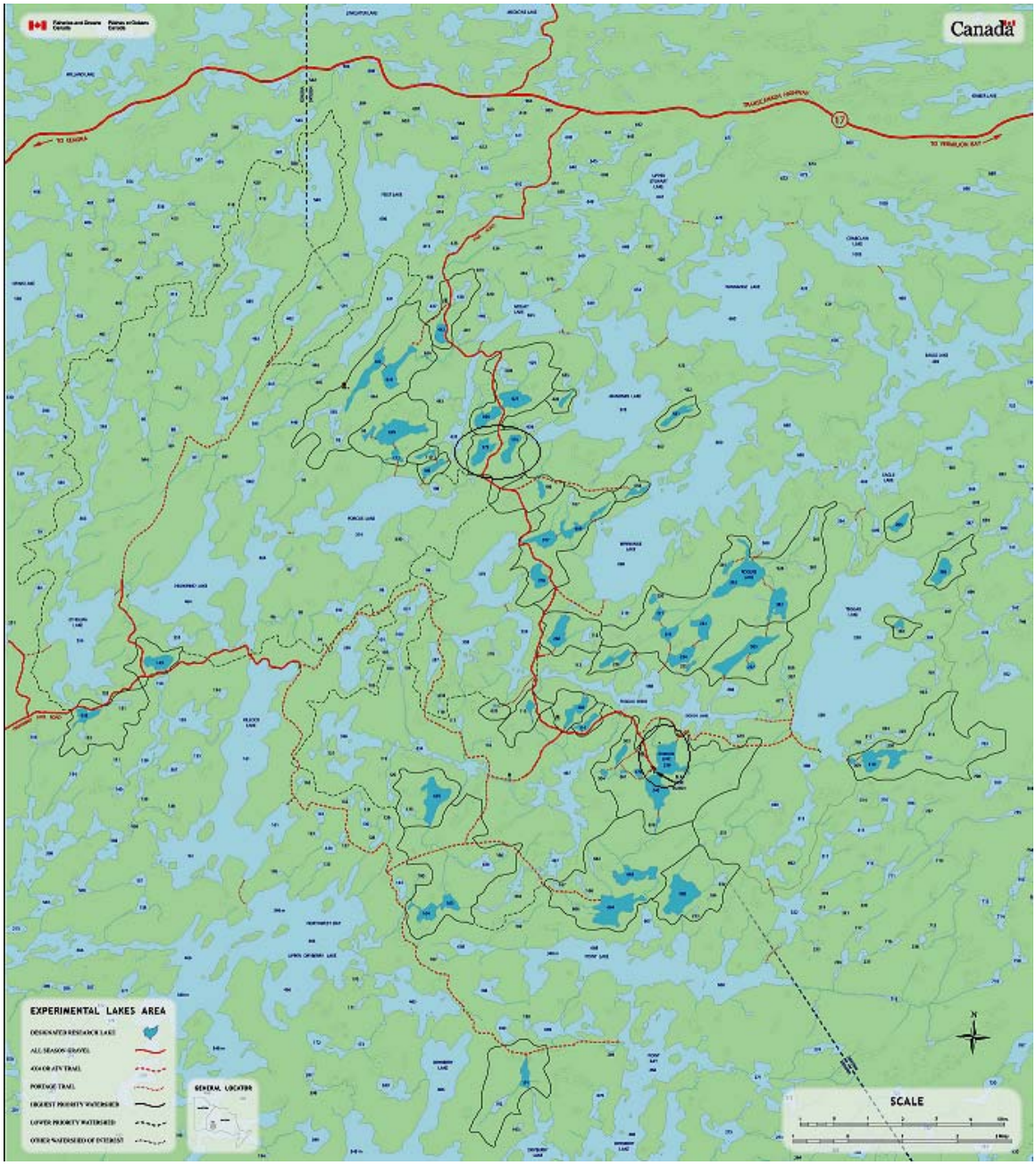

Figure 2.1: A map of the Experimental Lakes Area. Designated research lakes (past and present) are in dark blue. The upper-east encircled lake is the experimental lake 375 and the upper-west encircled lake is reference lake 373. The lower circle contains reference lake 239. Figure adapted from *ELA Data Retriever* (version 7.1).

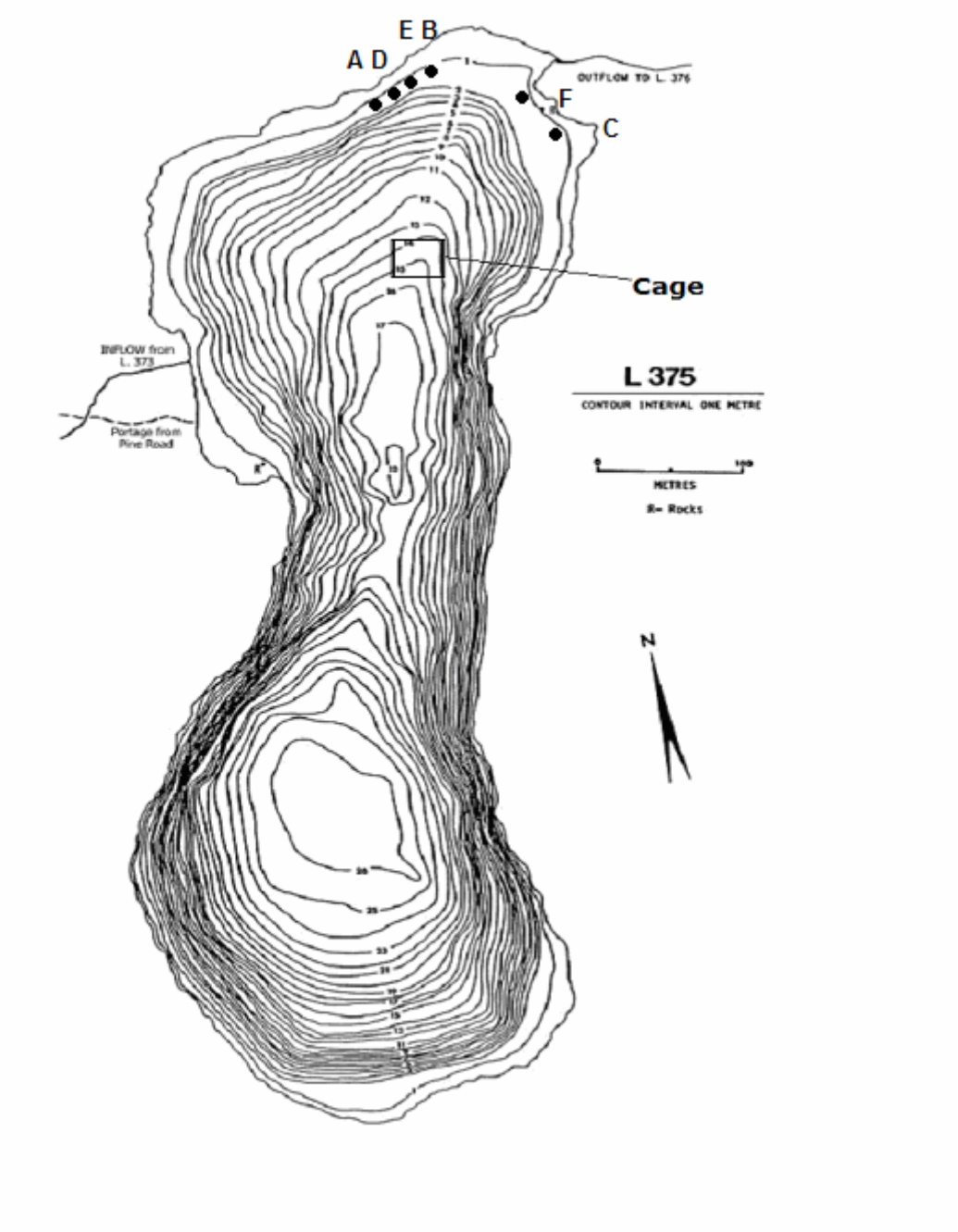

Figure 2.2: Bathymetric map of L375 showing the northwest inflow from L373 and the approximate location of the aquaculture cage and epilithic sampling sites from 2003 to 2006 (A, B, C) and 2007 (D, E, F) (modified from the *ELA Data Retriever* version 7.1).

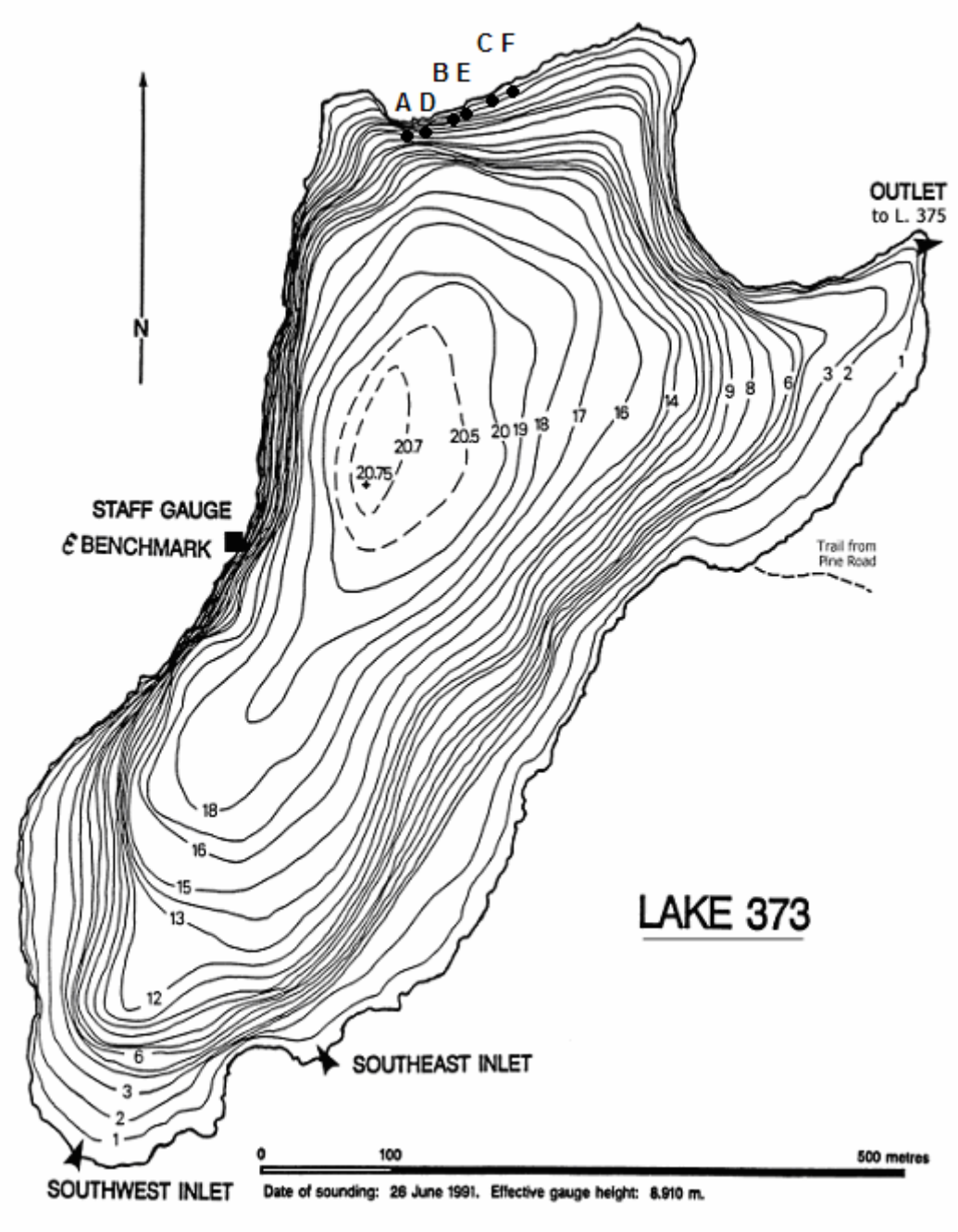

Figure 2.3: Bathymetric map of L373 showing the northeast outlet to L375 and epilithic sampling sites (A-F) adapted from *ELA Data Retriever* (version 7.1).

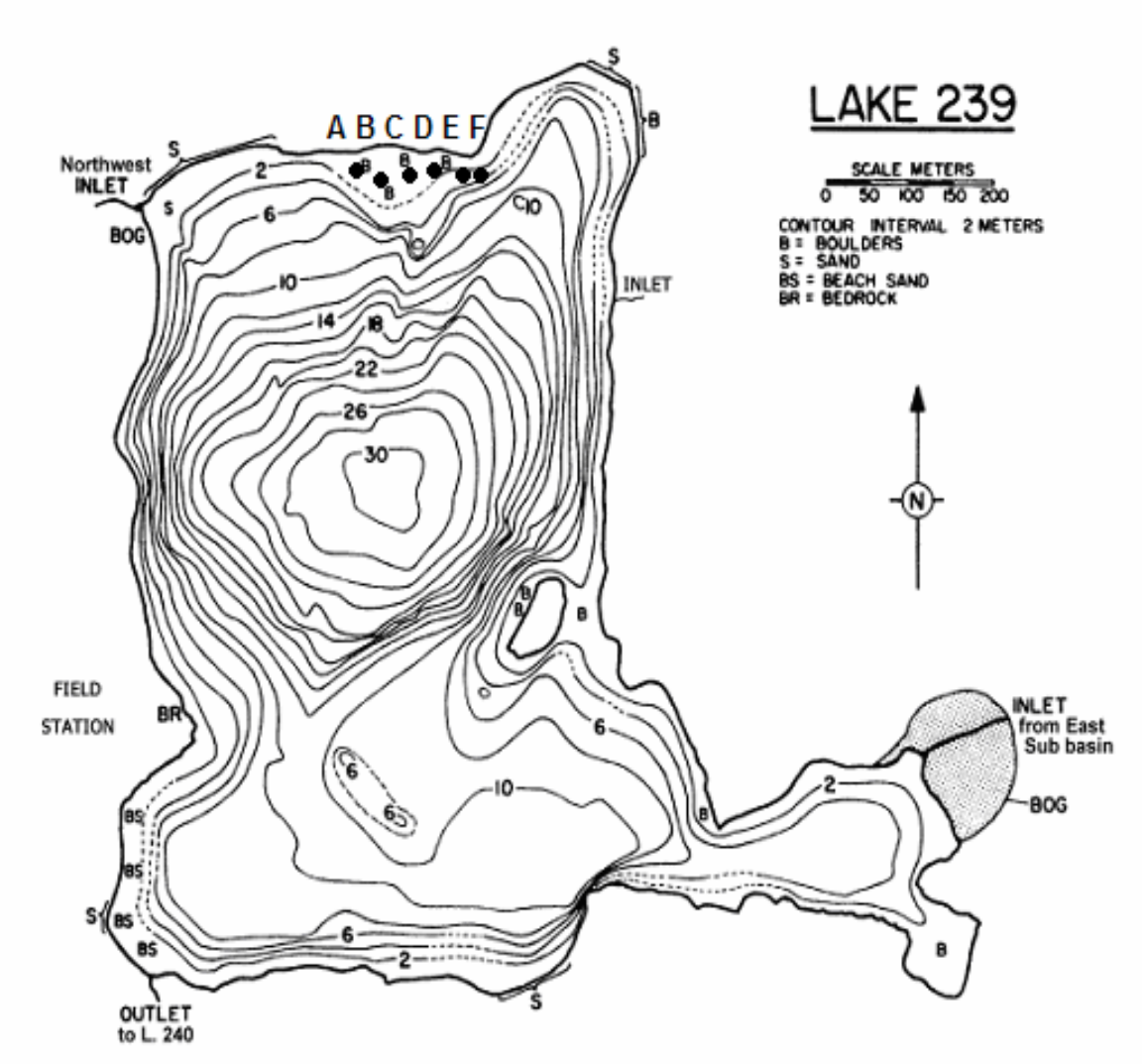

Figure 2.4: Bathymetric map of L239 showing epilithic sampling sites (A-F), adapted from *ELA Data Retriever* (version 7.1).

Table 2.1: Basic physical characteristics for the experimental and reference lakes (morphometric data are from Brunskill and Schindler (1971)).

Parameter	Units	Experimental Lake	Reference Lakes	
		L375	L373	L239
Surface area	ha	23.2	27.3	54
Lake volume	m^3	$27x10^5$	$30x10^5$	$59x10^5$
Area of littoral zone[a]	%	25	25	23
Mean depth	m	11.6	11	10.9
Max depth	m	26.5	20.8	30.4
A_d:A_o[b]		6.5	3	6.3
Water residence[c]	years	5.5	16.5	6.5

[a] Percent littoral zone = 100 x ($A_{0\text{-}4m}$/ A_0); area of lake bottom in the epilimnion from approximately zero to four meters ($A_{0\text{-}4m}$) divided by the total lake surface are (A_o)
[b] A_d:A_o = ratio of drainage area (A_d) to lake surface area (A_o)
[c] Water residence time in years = lake volume divided by mean annual outflow for 1992-2006 (data retrieved from the *ELA Data Retriever* version 7.1)

Table 2.2: Physical and chemical characteristics for the epilimnia of the experimental and reference lakes prior to (1992-2002) and during manipulation (2003-2006). Data were filtered log-normalized annual averages and standard error of the mean from the ice-free season. Data was obtained from the *ELA Data Retriever* (version 7.1).

		Experimental Lake 375		**Reference Lake 373**		**Reference Lake 239**	
Parameter	**Units**	**Pre[a]**	**Post**	**Pre**	**Post**	**Pre**	**Post**
		(n= 6)	(n= 4)	(n= 10)	(n= 4)	(n= 11)	(n= 4)
Temperature	°c	16.6 ± 1	15.6 ± 1	16.6 ± 0.5	15.4 ± 1	16.5 ± 0.4	15.2 ± 0.4
Light Attenuation Coefficient	m^{-1}	0.55 ± 0.02	0.62 ± 0.03	0.37 ± 0.01	0.39 ± 0.02	0.69 ± 0.03	0.65 ± 0.04
H^+	pH	7.4 ± 0.1	7.4 ± 0.1	7.3 ± 0.03	7.2 ± 0.03	7.1 ± 0.04	7.1 ± 0.1
DIC	$\mu mol \cdot L^{-1}$	337 ± 9	353 ± 6	198 ± 2	196 ± 3	184 ± 3	178 ± 1
DOC	$\mu mol \cdot L^{-1}$	470 ± 10	486 ± 12	344 ± 5	349 ± 8	574 ± 9	579 ± 15
Chl (planktonic)	$\mu g \cdot L^{-1}$	1.5 ± 0.1	2.7 ± 0.5	1.5 ± 0.1	1.5 ± 0.1	2.5 ± 0.2	2.4 ± 0.2
Total N	$\mu g \cdot L^{-1}$	267 ± 15	326 ± 12	236 ± 2	258 ± 2	298 ± 9	323 ± 13
Total P	$\mu g \cdot L^{-1}$	4.9 ± 0.4	8.8 ± 2	5.0 ± 0.1	6.5 ± 0.4	5.3 ±0.2	5.5 ± 0.5

[a] Pre = Pre-cage years 1992-2002, years absent from L375 data set: 1995-1996, 1998-2000

Farm characteristics

A 10-m^3 aquaculture cage was installed in the north end of L375 (Fig. 2.2) in the spring of 2003. The cage was stocked every spring with approximately 10,000 rainbow trout (mean initial weight = 0.1 kg) and harvested every fall (mean final weight = 1 kg) for the five years of operation (2003-2007). The fish were fed a conventional diet (Profishient®) at a rate monitored by a commercial operator (Meeker Aquaculture, Evansville, Ontario). An approximately six to sixteen-fold increase in P loading to the epiliminion of L375 versus pre-cage conditions occurred after the aquaculture operation began (Bristow *et al*. 2008). N inputs, on the other hand, tripled over natural inputs from 2003-2006 (Avezedo *et al*. in prep.). Nutrient loading rates and epilimetic concentrations are described in more detail in Appendix I.

Epilithic particulate sampling

The epilithon was sampled during the ice-free season in each of the three study lakes at the ELA. Sampling effort varied among lakes and years for the time series. The time series data (July and/or August, 1992-2005) collected by M. Turner, excluded data from 1995-1996 and 1998-2001 in L375. In 2006 and 2007, I continued to use this sampling strategy in order to sustain the synoptic data series. In lakes 373 and 375 for the 2007 field season, the full ice-free sampling period was included, beginning in the spring after ice-off (May 8th) and ending in the fall (Oct. 17th), with four weeks between sampling occasions.

Samples were collected in unshaded sites on the north shore near the aquaculture cage in L375 (Fig. 2.2). Similar northern, unshaded sites were sampled in the reference lakes as well. Naturally occurring communities were studied in the middle littoral zone

(depths of 1 to 2 m), where there was reduced influence of wave action. The epilithic samples were obtained from natural rock or bedrock shelves in areas of low slope (<10º, assessed visually; Fig. 2.5) (Turner *et al.* 1987). Three sites were sampled regularly, allowing a four-week recovery period, even though samplers tried to minimize their impact on the littoral zone. In 2007, the number of sites was increased relative to the historical time series, i.e., from three to six sites for the July and August runs. This was done to increase our understanding of inter-site variation.

The epilithon was removed from the rock surface by a SCUBA-diver using a scraping-brush sampler (Fig. 2.6) (Turner *et al.* 1991). Four 5-cm^2 samples were collected at each of the three to six sites. These samples were returned to shore, covered with ice, stored in the dark, and returned to the lab for sample preparation.

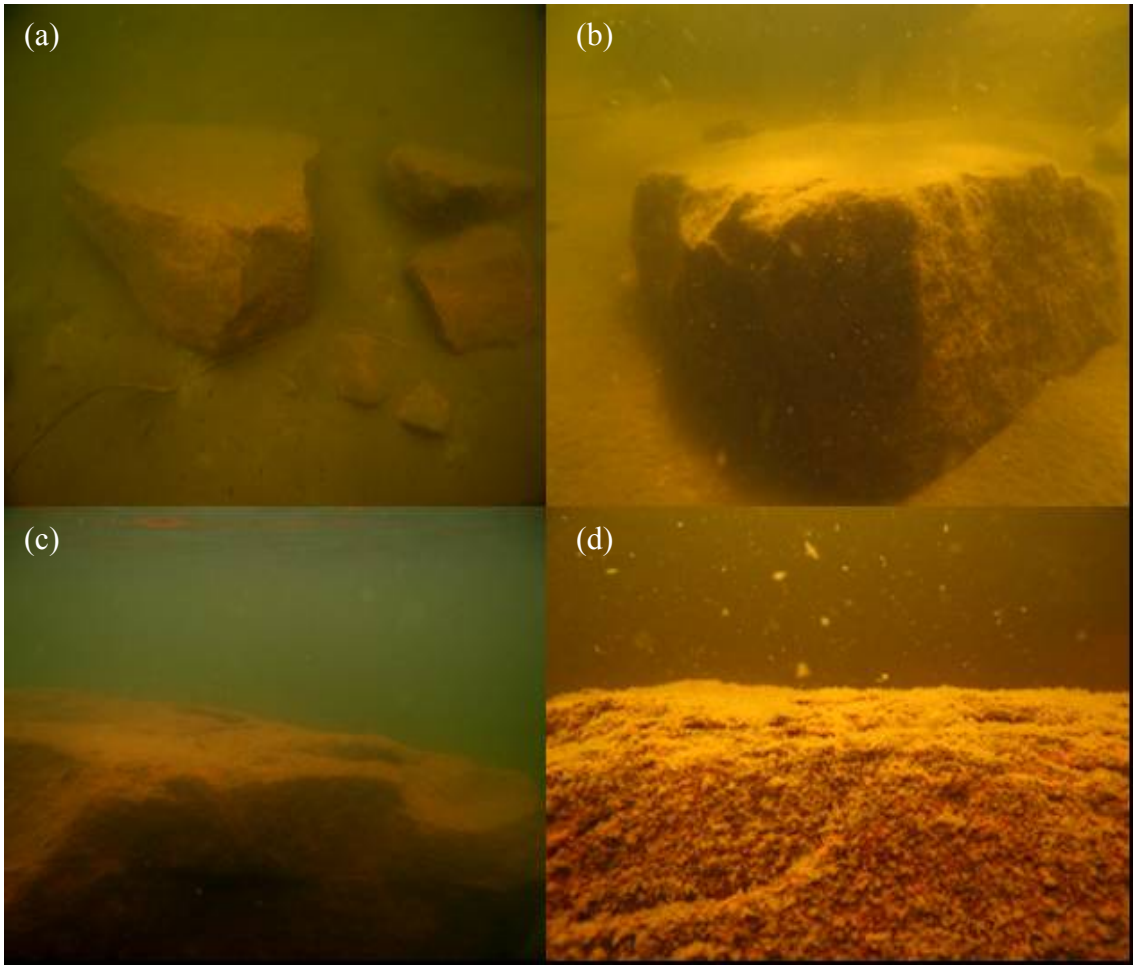

Figure 2.5: Examples of surfaces colonized by epilithon in the experimental and reference lakes at the Experimental Lakes Area. Sampling occurred on rocks or rock-shelves of low slope. Photos were taken during the summer of 2007 in lakes 375 (a, b, d) and 239 (c) by M. Turner.

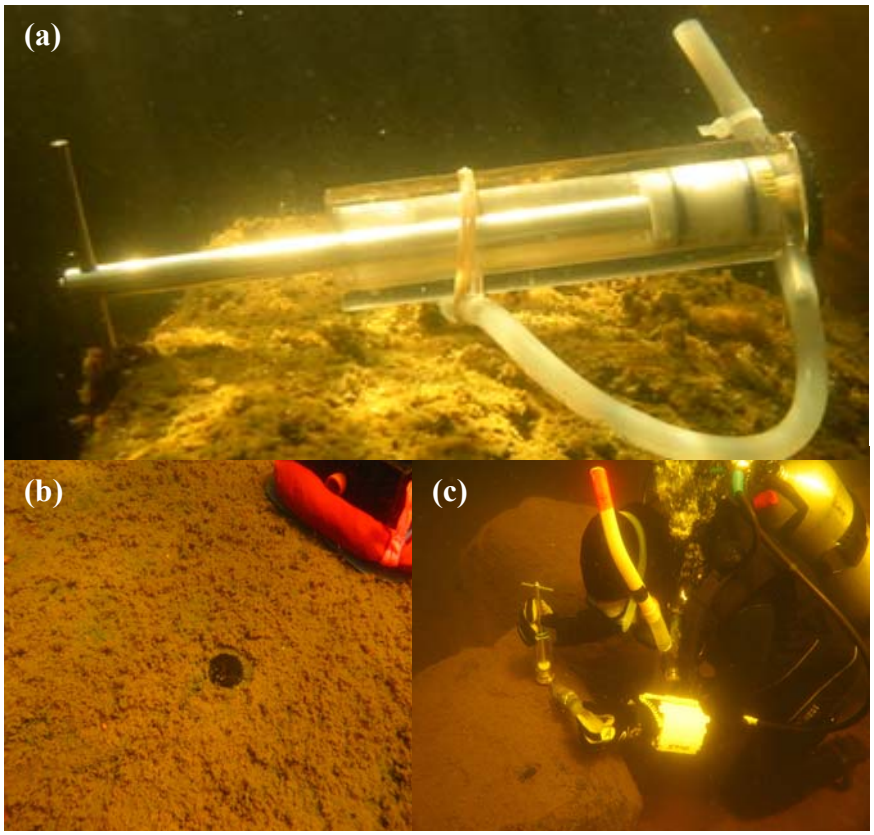

Figure 2.6: Epilithic particulate samples were collected in situ using a scraper sampler (a). The sampler was firmly kept in placed on the rock surface, while four, 5-cm^2 samples (b) were removed from each site. Samples were collected in a large bore, 60-ml syringe (c) and returned to the lab for analysis. Photos by M. Turner 2006.

Epilithic sample preparation

Sample preparation occurred within 24 hours of collection (samples were ice covered until preparation). Samples from four-sites were combined to create a site-specific sample, which was homogenized by blending for three, one-second pulses at low speed. The suspension was then transferred to a stirring beaker and subsamples were removed using a large-bore syringe. The sample suspensions were filtered onto pre-ignited Whatman GF/C filters (1.2-µm nominal pore size) and equal aliquots were removed for duplicate samples of C, N, P and HPLC analysis of Chlorophyll a (Chl a). The C/N and Chl a filters were placed in a desiccator for 12-24 hours and then frozen until analysis. The P samples were kept at room temperature in a screw cap vial until analysis.

For some analyses, the site-specific suspensions were then combined to produce a lake-composite sample. In July and August of 2007, one lake composite sample was prepared from the synoptic time series sites; a second lake composite was created from the second set of three sites. Duplicate sub-samples were removed from these composite suspensions and preserved with Lugol's solution for algal enumeration, taxonomy, biovolume and biomass estimation.

In 2006 and 2007, C/N stable isotopes were measured. In addition, archived samples from the three lakes from 1997 to 2005 were also analyzed. Two 10-ml sub-samples of the lake composites were filtered onto QM-A (2.2-µm nominal pore size) Whatman filters. Samples were desiccated for 12-24 hours, and then frozen.

Particulate chemistry

Particulate chemistry was analyzed by the Freshwater Institute Laboratory in Winnipeg. C and N samples were analyzed using an Exeter Analytical Model CE-440 Rapid Analysis Elemental Analyzer following methods outlined in Stainton *et al.* (1977). The filter paper was first combusted in an oxygen helium atmosphere at 980 ºC. The gas stream was then passed over hot copper, where oxides of N were reduced and excess oxygen removed. The remaining CO_2, H_2O and N_2 were separated chromatographically and analyzed with a single thermal conductance detector.

Suspended P samples were analyzed using a simple absorbance method (Stainton *et al.* 1977). The particulate matter collected on the filter paper was first ignited at 104ºC to destroy the organic matter. The ignited filter was then heated with diluted HCl to extract the P and convert it to ortho-phosphates. Ascorbic acid was used as a reductant. Antimony was used as a color enhancer, which when combined with ortho-phosphate produced an intensely blue coloured complex that was detected spectrophometrically at an absorbance maximum of 885 nm.

The Chl a samples were analyzed using high pressure liquid chromatography (HPLC) techniques. The particulate matter on the filters was extracted in a mixture of 68% methanol, 27% acetone and 5% water at 4 ºC in the dark for 16 hrs. After the extraction period, the mixture was inverted several times to ensure homogenization. The photosynthetic pigments were then quantitatively extracted, filtered and analyzed fluorometrically using HPLC. Calibration of fluorometer instrumentation was required using Chl a (from *Anacystis*) from Sigma Chemicals, proper calibration provided outputs

around 440 nm and detected emission around 660nm (Chl a excitation occurs between 430-450 nm and gives an emission maximum of 650-675 nm) (Stainton *et al.* 1977).

Algal Taxonomy

Subsamples of the preserved algal composites were analysed using the modified Ütermohl technique (Nauwerck 1963). All analyses were preformed by the same taxonomist, Dave Findlay. To break up detrital clumps, samples were sonicated at 20 kHz (Sonifer cell Disruptor, Model W140, Heat Systems, Ultrasonic Inc.) for two fifteen-second intervals (number of intervals varied depending on the severity of the clumps). Two, 2-mL sub-samples were allowed to settle for 24 hrs. Cells were identified to the lowest taxonomic unit using a phase-contrast inverted microscope at 125x and 400x magnification until a minimum of 100 cells of the dominant taxon was counted. Only viable cells that showed chloroplast presence were enumerated (Owen *et al.* 1978). Algal taxonomy was based on taxonomic groupings by Hustedt (1930), Patrick and Reimer (1966) and Findlay and Kling (1979). In each sample, 50 cells of the most common taxa were measured by approximating cell shapes as geometric solids (Vollenweider 1974). For less common taxa, cells were measured as they were encountered, and estimates of cell size were based on less than 50 measurements. For simplicity both algal cells and colonies were referred to in terms of algal cell size. Estimates of algal wet biomass were obtained from algal cell measurements assuming a specific gravity of 1 (Nauwerck 1963).

Percent algal carbon in the epilithon

The percent algal carbon in the epilithon was calculated by assuming that algal dry weight was approximately 10% of the algal wet biomass. Of the dry weight, I

assumed that half (50%) was algal carbon or 5% of the wet weight was algal carbon (Frost and Elser 2002). This estimate of algal carbon was then divided by the measured particulate carbon for that lake and date to determine the proportion of the epilithic C that was contained in the viable algae within the biofilm.

Species diversity

Species diversity was determined using Simpson's diversity index and the Shannon-Weaver index. For the Simpson's diversity index p_i was the fraction of organisms which belong to the *i*-th species, s was the total number of taxa in the sample:

$$D = \sum_{i=1}^{S} p_i^2$$

D was between zero and one, where values near zero correspond to highly diverse ecosystems, while values near one correspond to more homogeneous ecosystems (Krebs 1999).

The Shannon-Weaver index also uses the fraction of organisms (p_i) which belong to the *i*-th number of species (s):

$$H = -\sum_{i=1}^{S} p_i \log p_i$$

The Shannon-Weaver index examines the number of species and the evenness of the species. If the proportions of all species are the same then the evenness was equal to one but the value increases when there are more unique species or the species are very dissimilar (Krebs 1999).

Stable Isotopes

Samples were analyzed at the University of Waterloo's Environmental Isotopes Laboratory. Once received, the samples were freeze dried and analyzed using either an

Ioschrom Continuous Flow Stable Isotope Ratio Mass Spectrometer (GVInstruments/Micromass) or a Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer (Therosm Finnigan/Bremen). Both machines were coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA 1108). The results were corrected to N and C standards, generally IAEA-N1 and IAEA-N2 (both Ammonium Sulphate) for N and IAEA-CH6 (sugar), EIL-72 (cellulose) and EIL-32 (graphite) for C standards. The error for clean standard material was ± 0.2 ‰ for C and ± 0.3 ‰ for N (Drimmie and Heemskerk 2005).

C isotope ratios are generally used to identify food source, whereas the N ratio is generally used to determine relative trophic positions of organisms within a food web (Hecky and Hesslein 1995; McCutchan *et al.* 2003). C isotope ratios of consumers are usually similar to isotope ratios of their diets; the mean trophic shift for C between diet and consumer is usually about +1 ‰. For N, the trophic shift between diet and consumers is approximately + 3 ‰ (McCutchan *et al.* 2003).

Since commercial feed used in freshwater aquaculture was partially comprised of marine fishmeal and $\delta^{13}C$ and $\delta^{15}N$ are typically enriched in marine organisms compared to freshwater biota, the isotopic signature of the feed used in the experimental aquaculture operation should be enriched compared to other sources in the lake. This was indeed what Kullman *et al.* (in prep.) found in L375; both $\delta^{13}C$ and $\delta^{15}N$ were enriched in the native biota following the commencement of the cage operation.

Both $\delta^{13}C$ and $\delta^{15}N$ isotopic ratios are affected by autochthonous and allochtonous sources. The $\delta^{13}C$ fractionation in the epilithon differs depending on the source of dissolved CO_2 in the waters: carbonate rock weathering, from mineral springs,

from the atmosphere (affected by fossil fuel burning) and from respired organic matter (Petersen and Fry 1987). The $\delta^{15}N$ isotopic values in the water depend mostly on atmospheric fractionation, for example, anthropogenic emissions in highly industrialized areas can affect atmospheric deposition and precipitation change $\delta^{15}N$ isotopic values in the system (Petersen and Fry 1987)

Distance from cage study

Epilithic samples were collected in L375 as a function of distance from the aquaculture cage. Sites were selected based on increasing the distance from the cage site and the availability of rock as an algal substratum. Once sites were selected, sampling occurred in the same fashion as the long-term data series, with a SCUBA diver-borne scraping sampler. Samples were collected from the middle littoral zone in areas of low slope at four sub-locations within each site. Four 5-cm^2 samples were taken from each sub-location. Samples were analyzed for particulate P, $\delta^{15}N$, and $\delta^{13}C$. The sub-site samples were then combined to produce a site-specific sample for algal composition and biomass analyses following procedures outlined above.

Data Analysis

An analysis of covariance was used to model the data to determine whether the aquaculture operation affected epilithic nutrient concentrations and community composition in L375. All analyses were conducted using SAS PROC Mixed (SAS for Windows v.8). The dependent variables were natural log-transformed to normalize the data or to homogenize the error variance. Prior to log-transformation, a small constant (0.01) was added to variables with zero values.

Yearly data from the two reference lakes were averaged and treated as a covariate. Year was also modeled as a continuous covariate to look for temporal trends. An additional pre- vs. post-treatment effect was modeled as a categorical predictor variable. The "pre-post" variable was allowed to interact with the average values in the reference lakes and also with year to see if either the temporal trends in L375 or patterns in the reference lakes were different during the cage years versus pre-cage years. For certain variables, where anticipated effects failed to achieve statistical significance, non-significant model effects were removed and reduced models were fit to the data. The subsequent increase in error (degrees of freedom) yielded tests with increased statistical power, as was seen in the reduced model of the C: N ratio (Table 2.4).

In all cases, a p-value between $0.10 \geq p \geq 0.01$ was considered significant and $p < 0.01$ was highly significant. By increasing the p-value to 0.1 the likelihood of rejecting the possibility of an impact when there actually was one (Type I error) decreased. The variability within these natural systems can easily mask statistical significance of an impact when using a smaller p-value. The risk of failing to reject the possibility of an impact may have increased (Type II error), but I decided that it was better to err on the side of caution, than to reject an impact.

Results

Community nutrient ratios and food quality

There was no statistical association between epilithic P in L375 and the reference lakes in the pre-cage years, but there was a positive association in the cage-years (p-value = 0.10; Table 2.3). There was also a consistent increase in epilithic P over the years of the study (p-value = 0.10; Table 2.3). In three out of the five years of aquaculture cage operation, epilithic P concentrations were above those of the reference systems and above the pre-cage P mean (Fig. 2.7 a). Overall, reference lake 239 had higher epilithic P concentrations and higher variability than L375 (Fig. 2.8), however, P began to decrease in 2005 in L239 while in L375 P did not (Fig. 2.8 a).

Epilithic C and N did not show a significant difference between lakes or between pre-cage and cage years (Table 2.3). Both nutrients remained within the range of natural variability of the reference systems (Fig. 2.9 and 2.10). When L375 was compared to the average of the reference systems neither nutrient showed a sustained trend after the commencement of the aquaculture operation and both continued to show values within the variability of the pre-cage mean (Fig. 2.7 b-c).

For both N: P and C: P there were significant interactions between average values in the reference lakes and pre-cage vs. cage years (p-value = 0.08 and 0.07, respectively, Table 2.4; Fig. 2.11 a and b and Fig. 2.12 a and b). However, there was no association between the treatment and reference lakes in the pre-cage years, but positive associations during the cage years. There were no significant effects for C: N in the full model. Although there was a consistently positive association between the values in L375 and

reference lakes for the reduced model and the C: N ratio was higher during the pre-cage vs. cage years (p-value = 0.01 and 0.005, respectively, Table 2.4; Fig. 2.11 c and 2.12 c).

Table 2.3: Results of a mixed procedure analysis of covariance on cage effects on nutrient concentrations, comparing: (1) the epilithon of the experimental lake 375 to the average of the reference systems, (2) pre-cage and cage years within L375, (3) their possible interaction, (4) yearly changes within L375, and (5) whether yearly changes interacted with pre-cage and cage years in L375. A single asterisk next to a value represents significant data.

Nutrient	Effect	df	F-value	P-value
Particulate P	Reference lake particulate P	1,4	5.4	0.08*
	Pre-cage vs cage years in the experimental lake	1,4	4.1	0.11
	Interaction between reference lake particulate P and pre vs cage years	1,4	4.7	0.10*
	Year	1,4	4.7	0.10*
	Interaction between year and pre vs cage years	1,4	4.2	0.11
Particulate C	Reference lake particulate C	1,4	3.2	0.15
	Pre-cage vs cage years in the experimental lake	1,4	0.02	0.89
	Interaction between reference lake particulate C and pre vs cage years	1,4	2.5	0.19
	Year	1,4	0.03	0.86
	Interaction between year and pre vs cage years	1,4	0.04	0.85
Particulate N	Reference lake particulate N	1,4	3.6	0.13
	Pre-cage vs cage years in the experimental lake	1,4	0.4	0.59

	Interaction between reference lake particulate N and pre vs cage years	1,4	2.3	0.21
	Year	1,4	0.01	0.93
	Interaction between year and pre vs cage years	1,4	0.4	0.57

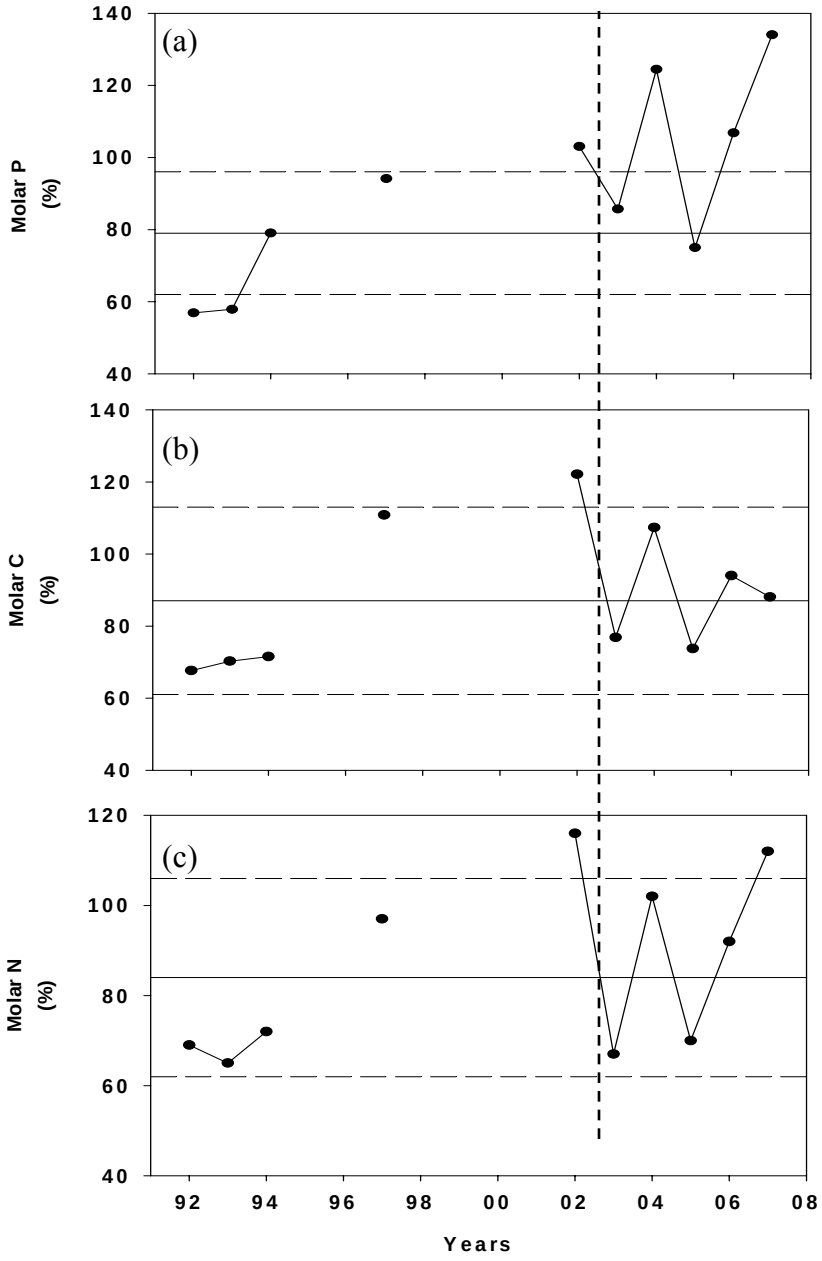

Figure 2.7: Experimental lake 375 relative to the average of the reference systems (L239 and L373) from 1992 to 2007 for epilithic molar P (a), molar C (b) and molar N (c). The solid horizontal line represents the pre-cage mean in L375; the dashed horizontal lines represent the standard deviation around the pre-cage mean. Years of cage operation are distinguished from pre-cage years by the dashed vertical line.

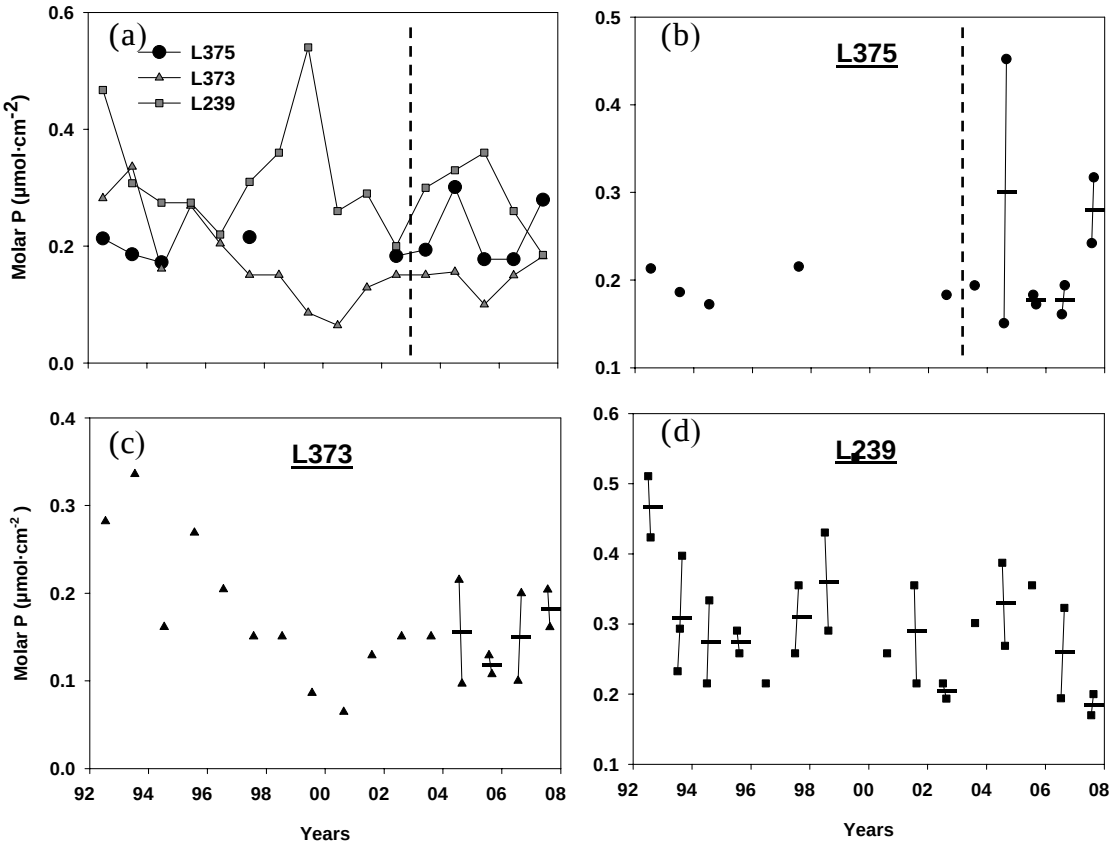

Figure 2.8: Phosphorus concentrations (µmol P·cm^{-2}) in the epilithon of the experimental lake (L375) and reference lakes (L373 and L239) from 1992 to 2007. In panel (a) the July and August means are shown for the experimental and reference lakes. The remaining panels (b-d) display July and August sampling dates connected by the solid vertical line; the horizontal line represents the means of these sampling dates for each lake. The dashed vertical line (a-b) distinguishes between pre-cage (1992-2002) and cage operation years (2003-2007).

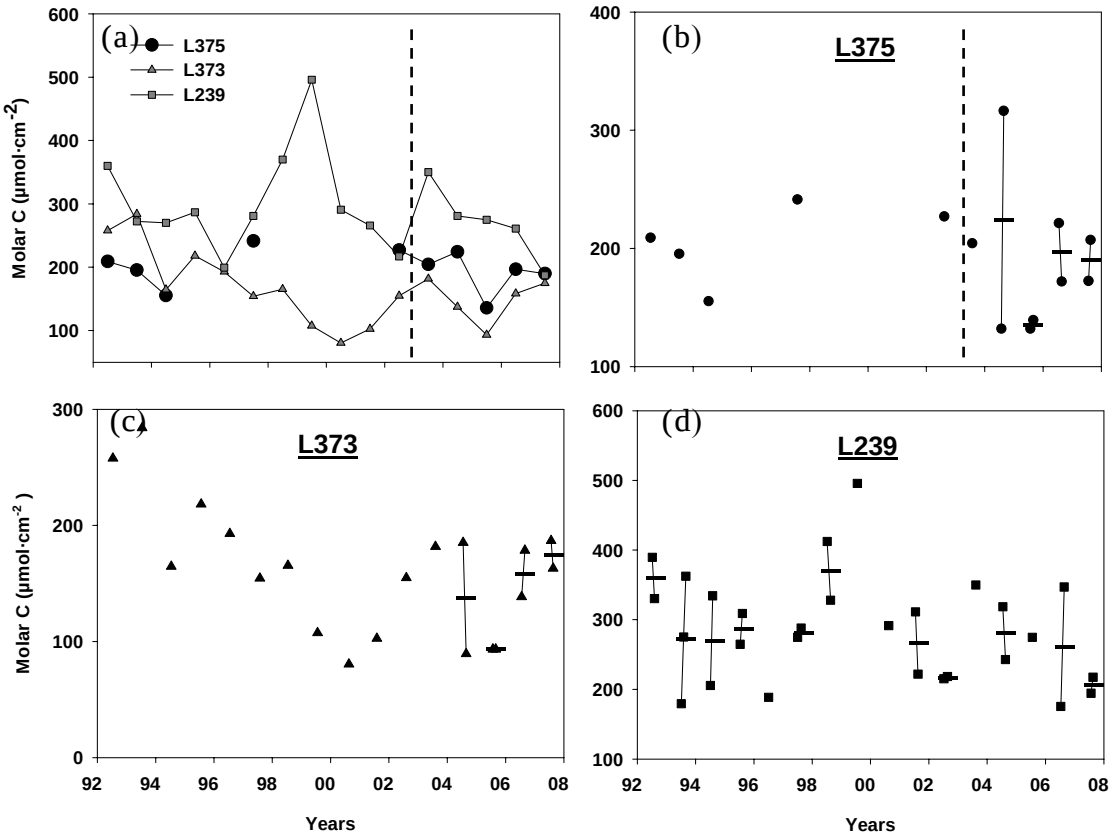

Figure 2.9: Carbon concentrations (µmol C·cm^{-2}) in the epilithon of the experimental lake (L375) and reference lakes (L373 and L239) from 1992 to 2007. In panel (a) the July and August means are shown for the experimental and reference lakes. The remaining panels (b-d) display July and August sampling dates connected by the solid vertical line; the horizontal line represents the means of these sampling dates for each lake. The dashed vertical line (a-b) distinguishes between pre-cage (1992-2002) and cage operation years (2003-2007).

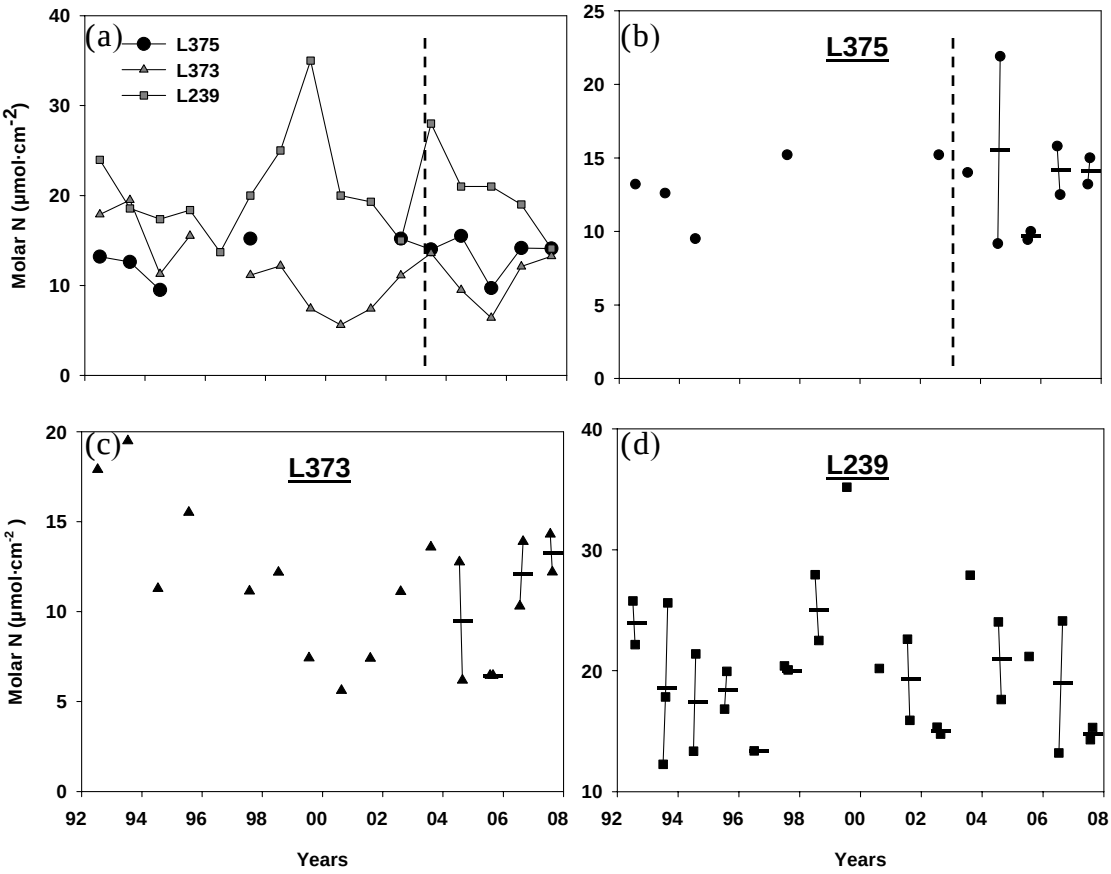

Figure 2.10: Nitrogen concentrations (µmol N·cm^{-2}) in the epilithon of the experimental lake (L375) and reference lakes (L373 and L239) from 1992 to 2007. In panel (a) the July and August means are shown for the experimental and reference lakes. The remaining panels (b-d) display July and August sampling dates connected by the solid vertical line; the horizontal line represents the means of these sampling dates for each lake. The dashed vertical line (a-b) distinguishes between pre-cage (1992-2002) and cage operation years (2003-2007).

Table 2.4: Results of a mixed procedure analysis of covariance on cage effects on nutrient ratios, comparing: (1) the epilithon of the experimental lake 375 to the average of the reference systems, (2) pre-cage and cage years within L375, (3) their possible interaction, (4) yearly changes within L375, and (5) whether yearly changes interacted with pre-cage and cage years in L375. Significant values are distinguished from non-significant values by an asterisk; highly significant values have two asterisks.

Nutrient ratio	Effect	df	F-value	P-value
N: P	Reference lake N: P	1,4	0.12	0.75
	Pre-cage vs cage years in the experimental lake	1,4	2.9	0.16
	Interaction between reference lake N: P and pre vs cage years	1,4	5.5	0.08*
	Year	1,4	0.12	0.75
	Interaction between year and pre vs cage years	1,4	3.1	0.16
C: P	Reference lake C: P	1,4	0.14	0.72
	Pre-cage vs cage years in the experimental lake	1,4	2.9	0.16
	Interaction between reference lake C: P and pre vs cage years	1,4	6.0	0.07*
	Year	1,4	0.27	0.63
	Interaction between year and pre vs cage years	1,4	3.2	0.15
C: N[1]	Reference lake C: N	1,4	0.13	0.73
	Pre-cage vs cage years in the experimental lake	1,4	0.00	0.99

	Interaction between reference lake C: N and pre vs cage years	1,4	0.03	0.86
	Year	1,4	0.05	0.83
	Interaction between year and pre vs cage years	1,4	0.00	0.98
C: N[2]	Reference lake C: N	1,4	12.81	0.01*
	Pre-cage vs cage years in the experimental lake	1,4	16.86	0.005**

[1] Full model
[2] Reduced model
Refer to the *Data analysis* section of the materials and methods in chapter 2 for a detailed explanation of the two models.

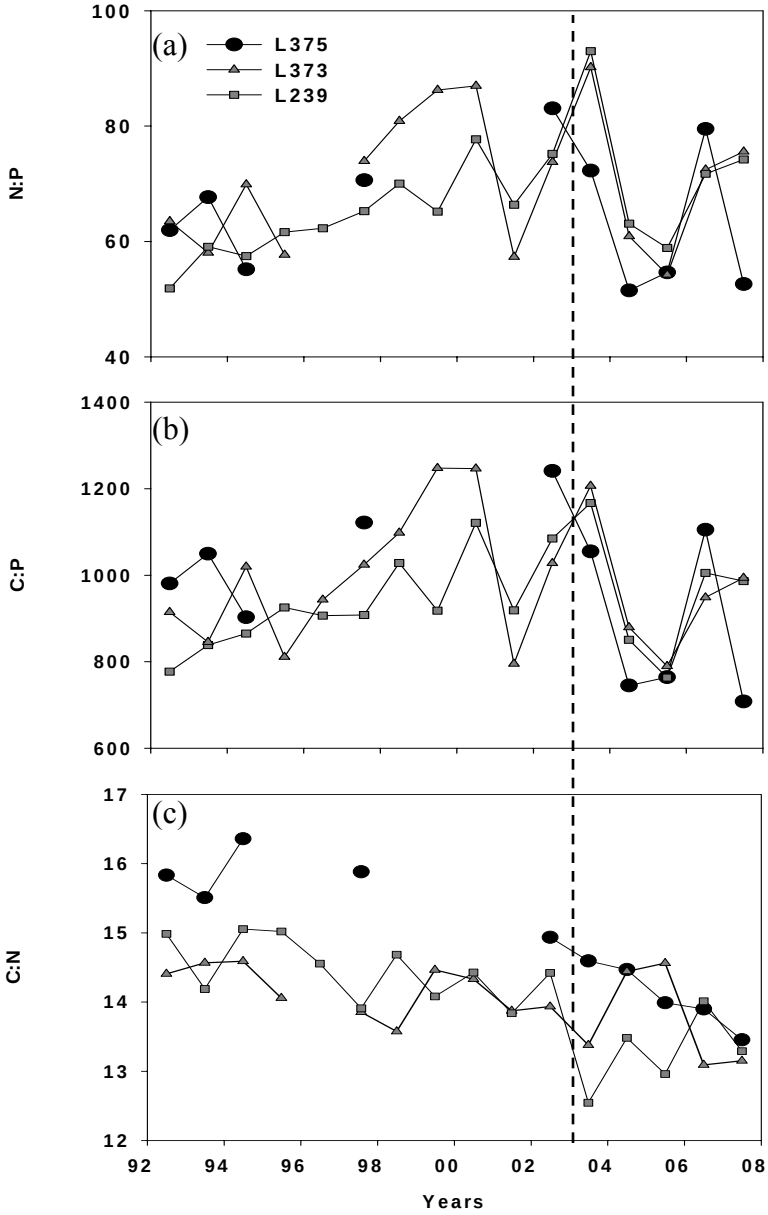

Figure 2.11: The July and August means of molar nutrient ratios (a) N: P, (b) C: P and (c) C: N in the epilithon of the experimental (L375) and reference lakes (L373 and L239) from 1992 to 2007. The dashed line distinguishes between pre-cage (1992-2002) and cage operation years (2003-2007).

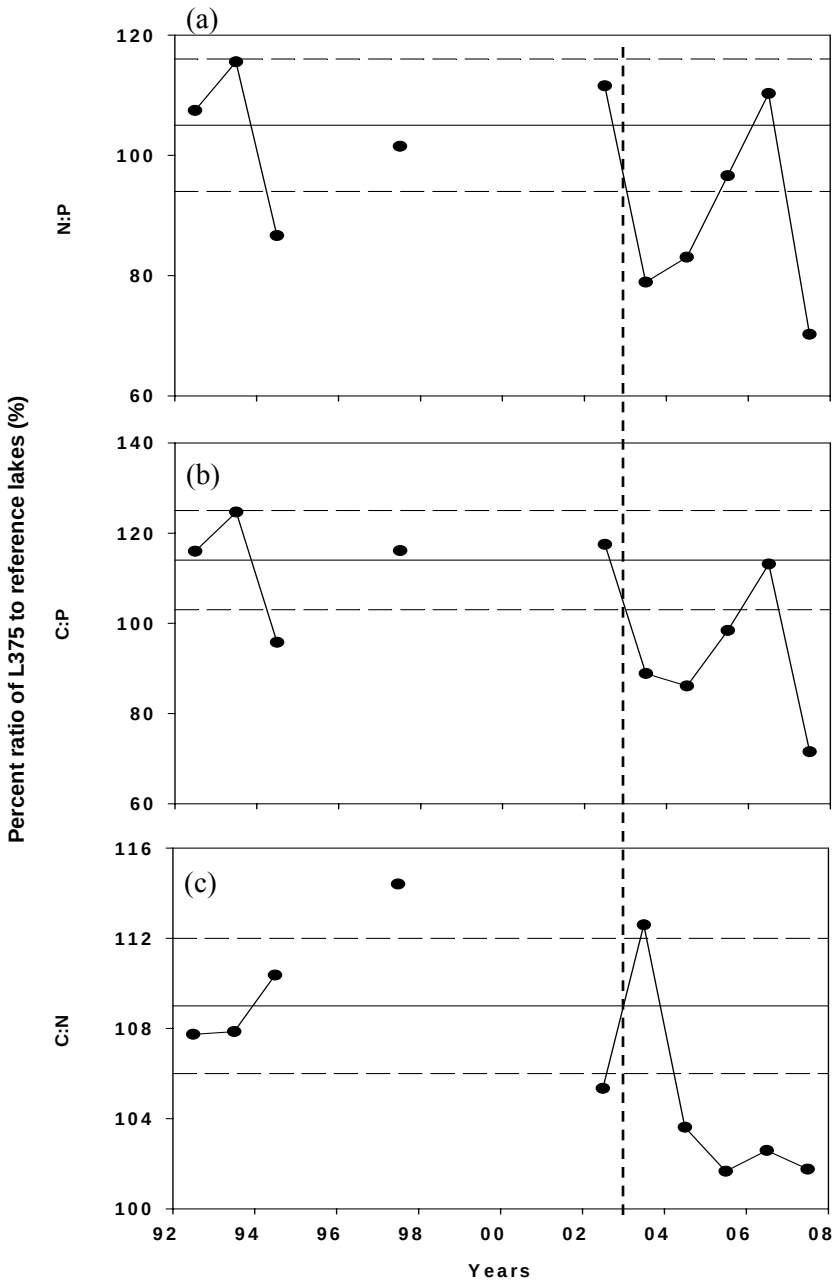

Figure 2.12: Experimental lake 375 relative to the average of the reference systems (L239 and L373) from 1992 to 2007 for epilithic molar nutrient ratios ((a) N: P, (b) C: P and (c) C: N. The solid horizontal line represents the pre-cage mean; the dashed horizontal lines represent the standard deviation around the pre-cage mean. Years of cage operation are distinguished from pre-cage years by a dashed vertical line.

Percent algae and pigments

The percent algal carbon within the epilithon can be used to understand both the portion and the nature of the algae within the biofilm. The percentage of the biofilm that can be attributed to algal carbon was low in all three systems (Fig. 2.13 a). In the reference systems approximately 1-5% of the biofilm was viable algae and in L375 approximately 5% of the biofilm was viable algae (Fig. 2.13 a). The rest of the biofilm (95-99%) is composed of non-viable algae, bacteria, detritus, metazoans, protozoans and fungi (chapter 1).

Overall, the percentage of viable algal carbon did not differ between pre-cage and cage years or between lakes (Table 2.5). There appeared to be a substantial increase in 2005 (Fig. 2.13 a); however, when compared to the average of the reference systems the increase was still within the range of variability of the relationship between L375 and the reference systems during the pre-cage period (Fig. 2.13 b).

There was no significant difference in algal Chl a ($\mu g \cdot cm^{-2}$) between cage and pre-cage years or between L375 and reference lakes (Table 2.5). Chl a concentrations in L375 remained within the range of variability of the reference systems throughout the study period (Fig. 2.14 a). However, Chl a increased above the pre-cage mean and above the reference system in 2007, when L375 was compared to the average of the reference systems (Fig. 2.14 b). Until 2007 the July and August Chl a means remained within the variability of the pre-cage mean (Fig. 2.14 b).

Table 2.5: Results of a mixed procedure analysis of covariance on percent algal carbon (%) and chlorophyll a concentrations ($\mu g \cdot cm^{-2}$), comparing: (1) the epilithon of the experimental lake 375 to the average of the reference systems, (2) pre-cage and cage years within L375, (3) their possible interaction, (4) yearly changes within L375, and (5) whether yearly changes interacted with pre-cage and cage years in L375. Neither of the measured parameters showed significance in the statistical model.

	Effect	df	F-value	P-value
Percent algal carbon	Reference lake percent algal carbon	1,4	2.2	0.21
	Pre-cage vs cage years in the experimental lake	1,4	0.01	0.92
	Interaction between reference lake percent algal carbon and pre vs cage years	1,4	3.7	0.13
	Year	1,4	0.31	0.61
	Interaction between year and pre vs cage years	1,4	0.01	0.91
Chlorophyll-a	Reference lake chlorophyll-a	1,4	0.42	0.55
	Pre-cage vs cage years in the experimental lake	1,4	0.18	0.69
	Interaction between reference lake chlorophyll-a and pre vs cage years	1,4	0.08	0.80
	Year	1,4	0.29	0.62
	Interaction between year and pre vs cage years	1,4	0.19	0.69

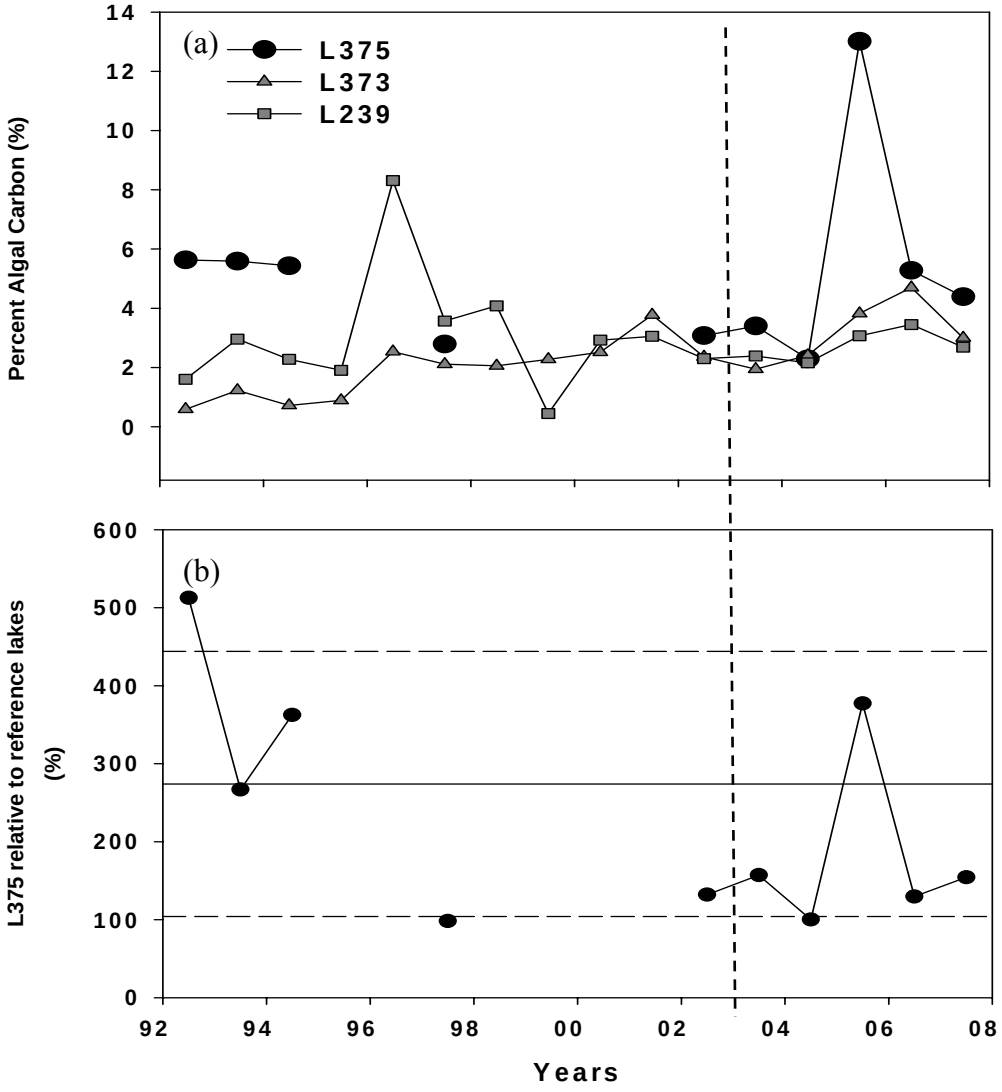

Figure 2.13: (a) The amount of epithic algal carbon as a percent of total epilithic carbon in the biofilm of the experimental lake 375 and reference lakes (L373 and L239) from 1992 to 2007 using July and August means. (b) Algal carbon expressed as the percentage of experimental lake relative to the average of the reference lakes. The solid horizontal line represents the pre-cage mean; the dashed horizontal lines are the standard deviation around the mean. The dashed vertical line distinguishes between pre-cage (1992-2002) and cage operation years (2003-2006).

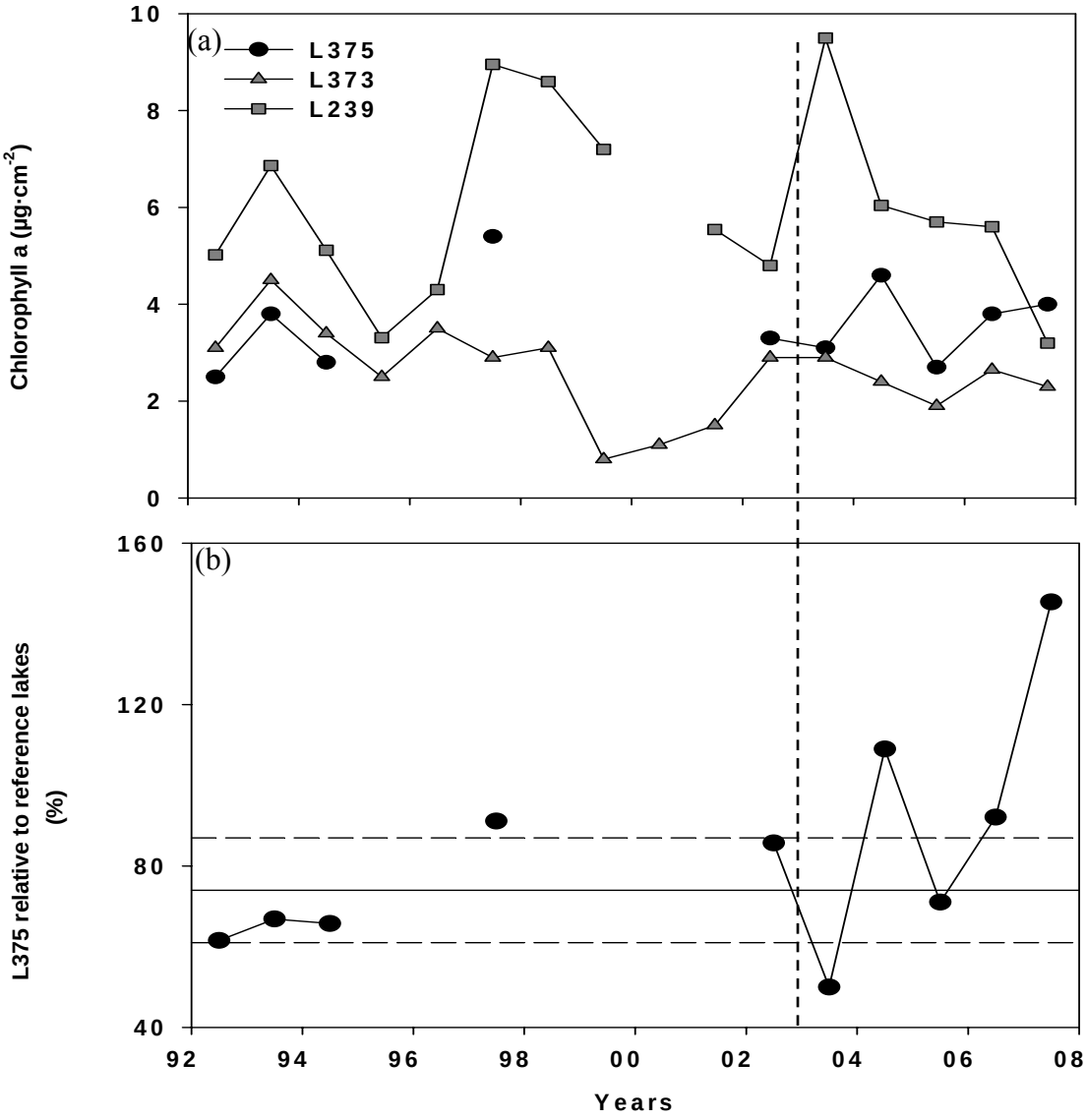

Figure 2.14: (a) Epilithic chlorophyll-a concentrations (µg·cm^{-2}) in the biofilm of the experimental lake 375 and reference lakes (L373 and L239) from 1992 to 2007 using July and August means. (b) Epilithic Chl-a concentrations in the experimental lake relative to the average of the reference lakes. The solid horizontal line represents the pre-cage mean; the dashed horizontal lines are the standard deviation around the mean. The dashed vertical line distinguishes between pre-cage (1992-2002) and cage operation years (2003-2006).

Algal biomass and composition

Total epilithic algal biomass showed no significant difference between cage years and pre-cage years or between reference lakes and L375 (Table 2.6). There was a substantial but transient increase in total epilithic biomass in L375 in 2005 (Fig. 2.15). But when compared to the average of the reference systems and the pre-cage mean (Fig. 2.21 a), the increase observed in L375 was only slightly above the variability associated with the pre-cage mean. These temporary increases in total biomass are not unusual for these study lakes; L239 revealed two occasions (1996, 1998) of high epilithic algal biomass of a similar magnitude to that documented in L375 in 2005 (Fig. 2.15). However, the peaks in L239 were due to an increase in a few large celled diatoms (Fig. 2.16) (M. Turner, pers. comm.), whereas the increase in L375 was due to a bloom in cyanobacteria (Fig. 2.17).

The total epilithic biomass in the three lakes was dominated by three main groups: diatoms, cyanobacteria and chlorophytes, which represented approximately on average between 40-80%; 20-60%; and 1-2% (respectively before commencement of the farm (Fig. 2.22)). L375 had been dominated by diatoms until sampling in 2002 revealed that cyanobacterial biomass had increased substantially in the system (Fig. 2.22 a). Algal composition in L373 was co-dominated by diatoms and cyanobacteria throughout the study period (Fig. 2.22 b). L239 showed a similar trend to L375, in that diatoms initially dominated the community but shifted towards cyanobacterial dominance throughout the study period (Fig.2.22c). Chlorophytes appear sporadically throughout the study period in all three lakes, with the highest occurrence in L239 (Fig. 2.22 c). Since 2005,

chlorophytes have continued to persist in L375 (Fig. 2.22 a), however, their appearance was not statistically related to the aquaculture cage operation (Table 2.6).

Overall, diatom biomass decreased in L375 (Fig. 2.16; Fig. 2.21 c). However this decreasing trend was occurring prior to commencement of the cage operation, therefore there was no significant difference between pre-cage and cage years (Table 2.6). Since epilithic diatom biomass in L375 was high (~2.6 $mg \cdot cm^{-2}$) early in the time series, the decrease in abundance has now placed it in the range of the reference systems (~1.0 $mg \cdot cm^{-2}$) (Fig. 2.16).

There was a consistent positive association between cyanobacteria values in L375 and the reference lakes (p-value = 0.01; Table 2.6). However, the increase was found to be statistically unrelated to the cage operation (Table 2.6), despite the apparent increase in cyanobacterial biomass since the commencement of the operation seen in Fig. 2.21 b.

The major increase in total biomass in L375 in 2005 was due to an increase in cyanobacterial biomass (Fig. 2.17). The July sampling date showed an especially high abundance of cyanobacteria (76% of total biomass) (Fig. 2.17 b). The increase in abundance was due to a bloom of a specific taxon of cyanobacteria: *Calothrix* sp. (Table 2.7; Fig. 2.23). *Calothrix* sp. biomass was over 50% of the total biomass and approximately 70% of cyanobacterial biomass in the system in 2005 (Table 2.7). In addition to the increase in biomass in 2005, community characteristics changed as well. The community became more firmly attached to the rock. Because of this, the estimates of particulate biomass were biased on the low side (M. Turner, pers. comm.). The scraper was unable to remove all attached algae in the sample area and collections under-represented actual biomass. The increase in 2005 was transient and in 2006 *Calothrix* sp.

biomass dropped from 4.5 mg·cm^{-2} to levels below those recorded for the reference systems (76 μg·cm^{-2}) (Table 2.7). With the drop in *Calothrix* sp. cyanobacterial biomass also decreased, but still remained above the pre-cage mean and the mean of the reference systems (Fig. 2.21 b).

Epilithic chlorophyte biomass was low for all three lakes and was similar among the reference and experimental lakes (Table 2.6). Although not statistically significant (Table 2.6), chlorophyte biomass appeared to increase in L375 following commencement of the cage operation (Fig. 2.18).

Neither measure of species diversity (Simpson's diversity index and the Shannon-Weaver index) changed over the study period among lakes or between pre-cage and cage years (Table 2.6; Fig. 2.19-2.20; Fig. 2.21 e-d). Over the study period, values for both indices remained within the range of variability about the pre-cage mean in L375 (Fig. 2.21 e-d), and within the range of the reference systems (Fig. 2.19-2.20). All three systems have Simpson's diversity values above 0.5; on average most values were close to 0.7, which indicates that these lakes have relatively homogenous algal communities. The Shannon-Weaver values indicate that the system was above evenness (above one), which implies that there are some rare species within these communities. In all years, on average, L375 had higher species diversity than the reference systems (Fig. 2.20, Fig. 2.21).

Changes in species composition at the fine scale included the appearance of novel and unique taxa in L375 (Table 2.8). For example, in 2007, there was a dinoflagellate bloom in the biofilm of L375 (Fig. 2.22 a); neither *Ceratium hirundinella* (Muller) Schrank nor *Peridinium pusillum* (Penard) Lemmermann had been recorded in the study

lake prior to the cage operation, although *Peridinium pusillum* had been reported earlier in the reference lakes, as well as in the phytoplankton of L375 (D. Findlay, pers. comm.). New taxa of chlorophytes, which only appeared in L375, were also noted following commencement of the cage operation: *Arthrodesmus (Staurodesmus) octocornis* (Ehrn and Ralfs) Ach.; *Paulschulzia pseudovolvox* (Schultz) Skuja.; and *Gonatozygon* spp. (Table 2.8). As well, three new taxa of cyanobacteria appeared: *Oscillatoria sancta* (Kutz.) Gomont; *Radiocystis geminata* Skuja*; Tychonema rhodenema* Skuja (Table 2.8 and Table 2.9). Five new taxa of diatoms were also noted in L375 after the cage operation; *Gomphonema trucatum* Ehrenberg; *G. angustum* Agardh; *Aulacoseira distans* (Ehrenberg) Simonsen; *Cyclotella michiganiana* Skvortsov; *Anomoeneis brachysira* Grunow and *Cymbella lapponica* Grunow ex. Cleve. The reference systems also had new taxa, unique to their system during this period; however, the numbers were much less than in L375. L239 had nine new taxa and L373 had seven new taxa in comparison to L375 which had 13 unique taxa (Table 2.8) and 39 newly reported taxa for L375 (data not shown).

Table 2.6: Results of a mixed procedure analysis of covariance on total biomass, diatom, cyanobacterial, and chlorophyte biomass and species diversity (Simpson's diversity index and Shannon-Weaver index). The analysis was used to determine whether the cage operation impacted algal structure and composition in experimental lake, comparing: (1) the epilithon of the L375 to the average of the reference systems, (2) pre-cage and cage years within L375, (3) their possible interaction, (4) yearly changes within L375, and (5) whether yearly changes interacted with pre-cage and cage years in L375. Significant values are distinguished from non-significant values by an asterisk.

	Effect	**df**	**F-value**	**P-value**
Total Biomass	Reference lake total biomass	1,4	0.71	0.45
	Pre-cage vs cage years in the experimental lake	1,4	0.06	0.82
	Interaction between reference lake total biomass and pre vs cage years	1,4	0.70	0.45
	Year	1,4	0.02	0.89
	Interaction between year and pre vs cage years	1,4	0.05	0.83
Diatom	Reference lake diatom biomass	1,4	1.1	0.35
	Pre-cage vs cage years in the experimental lake	1,4	1.6	0.28
	Interaction between reference lake diatom biomass and pre vs cage years	1,4	0.85	0.41
	Year	1,4	0.0	0.95
	Interaction between year and pre vs cage years	1,4	1.6	0.28

Cyanobacteria	Reference lake cyanobacterial biomass	1,4	18.63	0.01*
	Pre-cage vs cage years in the experimental lake	1,4	1.65	0.27
	Interaction between reference lake cyanobacterial biomass and pre vs cage years	1,4	2.49	0.19
	Year	1,4	0.35	0.59
	Interaction between year and pre vs cage years	1,4	1.55	0.28
Chlorophyte	Reference lake chlorophyte biomass	1,4	1.5	0.29
	Pre-cage vs cage years in the experimental lake	1,4	2.3	0.20
	Interaction between reference lake chlorophyte biomass and pre vs cage years	1,4	0.09	0.78
	Year	1,4	1.4	0.30
	Interaction between year and pre vs cage years	1,4	2.3	0.20
Simpson's diversity index	Reference lake diversity index	1,4	1.07	0.36
	Pre-cage vs cage years in the experimental lake	1,4	0.08	0.80
	Interaction between reference lake diversity index and pre vs cage years	1.4	0.56	0.50
	Year	1,4	0.00	0.97
	Interaction between year and pre vs cage years	1,4	0.08	0.79

Shannon-Weaver index	Reference lake Shannon-weaver index	1,4	0.58	0.49
	Pre-cage vs cage years in the experimental lake	1,4	0.27	0.63
	Interaction between reference lake Shannon-weaver index and pre vs cage years	1,4	0.56	0.50
	Year	1,4	0.00	0.96
	Interaction between year and pre vs cage years	1,4	0.26	0.64

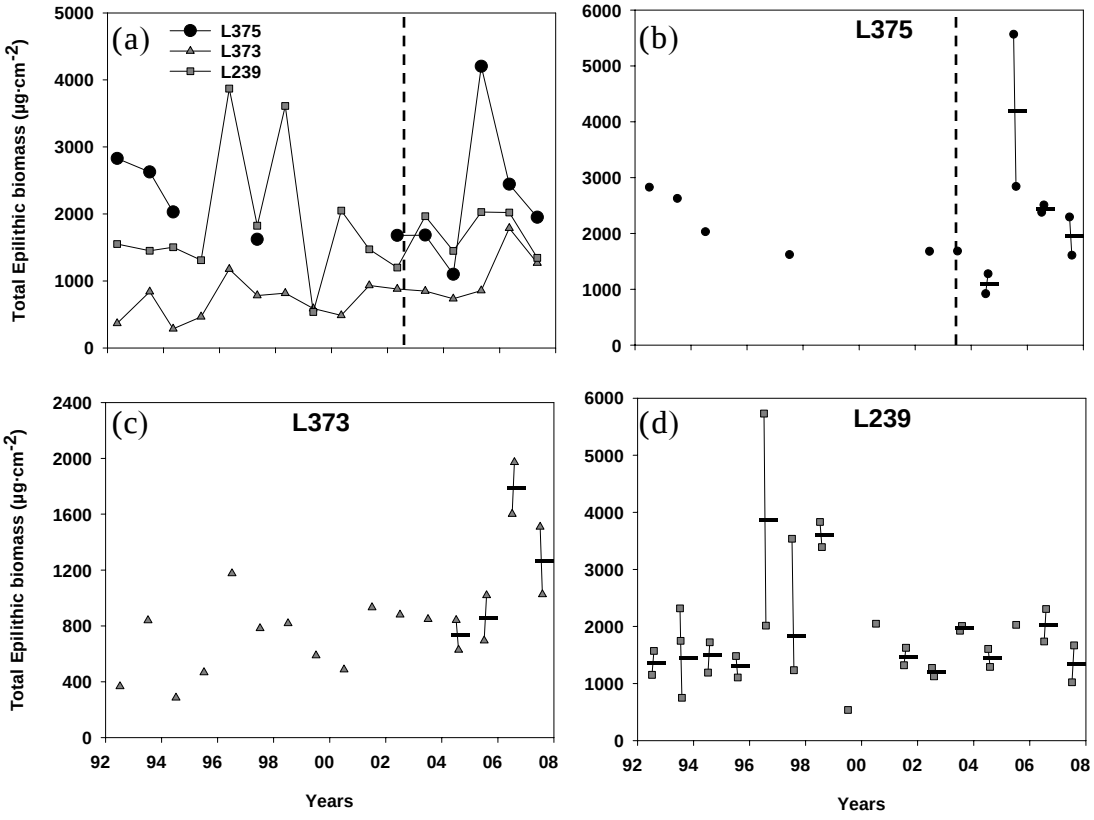

Figure 2.15: Total algal biomass (µg·cm^{-2}) in the epilithon of the experimental lake (L375) and reference lakes (L373 and L239) from 1992 to 2007. In panel (a) the July and August means are expressed for the experimental and reference lakes. The remaining panels (b-d) represent July and August sampling dates connected by the solid vertical line; the horizontal line represents the means of these sampling dates for each lake. The dashed vertical line (a and b) distinguishes between pre-cage (1992-2002) and cage-operation years (2003-2007).

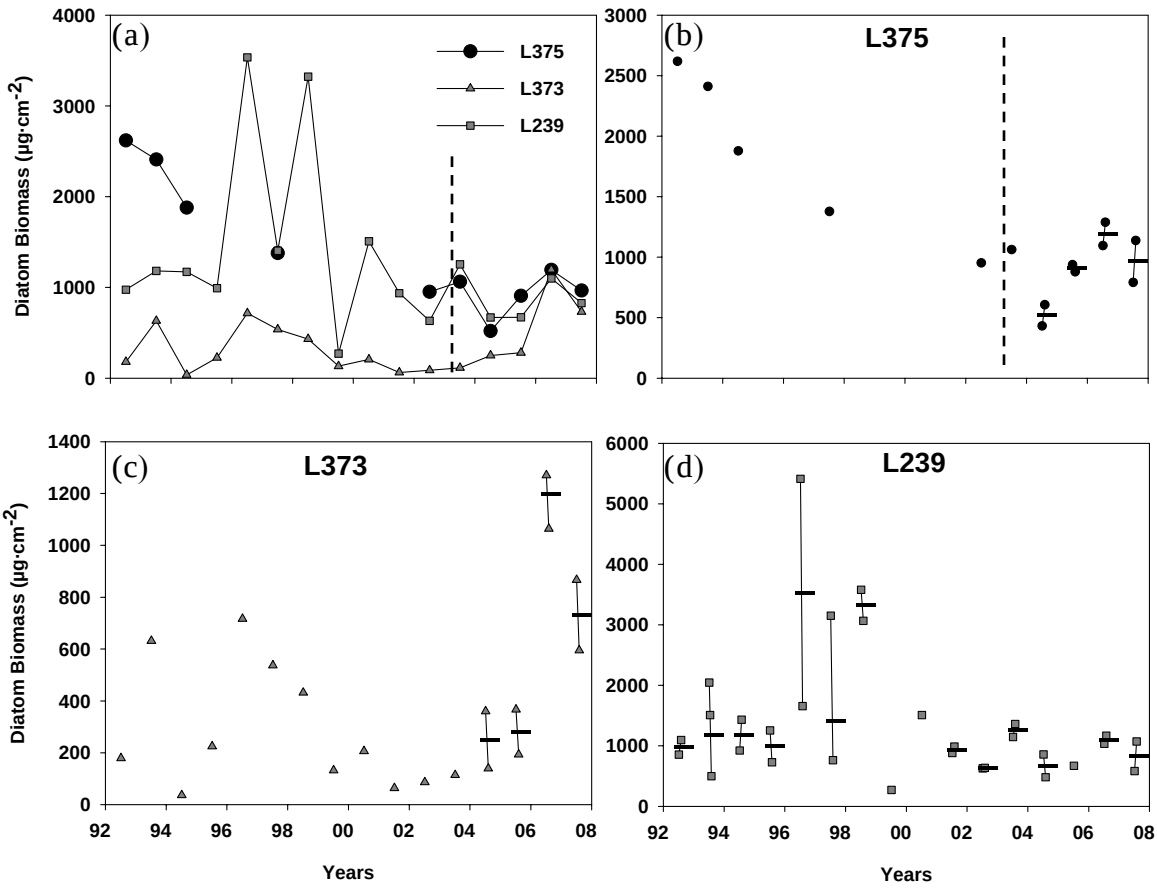

Figure 2.16: Diatom biomass (µg·cm^{-2}) in the epilithon of the experimental lake (L375) and reference lakes (L373 and L239) from 1992 to 2007. In panel (a) the July and August means are expressed for the experimental and reference lakes. The remaining panels (b-d) represent July and August sampling dates connected by the solid vertical line; the horizontal line represents the means of these sampling dates for each lake. The dashed vertical line (a and b) distinguishes between pre-cage (1992-2002) and cage-operation years (2003-2007).

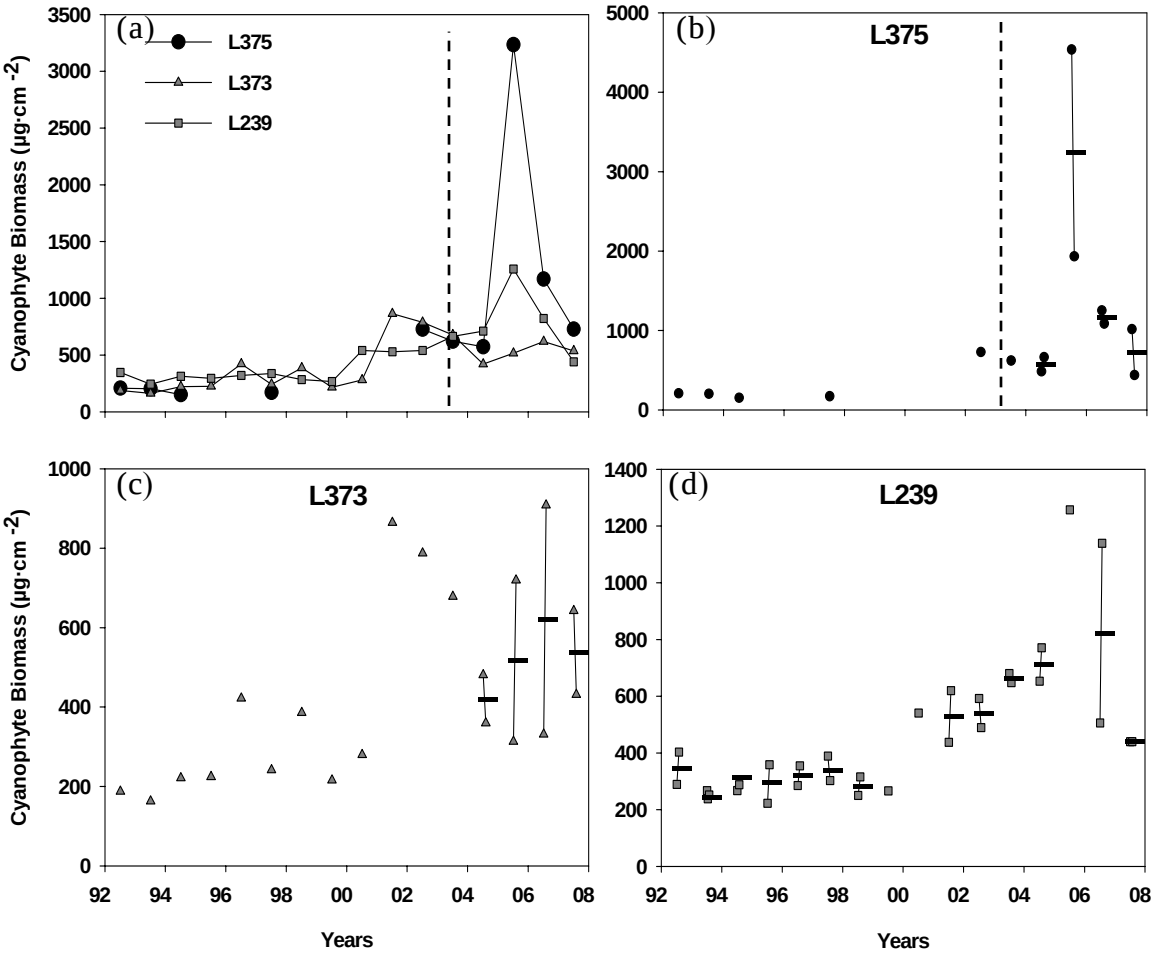

Figure 2.17: Cyanobacterial biomass (µg·cm^{-2}) in the epilithon of the experimental lake (L375) and reference lakes (L373 and L239) from 1992 to 2007. In panel (a) the July and August means are expressed for the experimental and reference lakes. The remaining panels (b-d) represent July and August sampling dates connected by the solid vertical line; the horizontal line represents the means of these sampling dates for each lake. The dashed vertical line (a and b) distinguishes between pre-cage (1992-2002) and cage-operation years (2003-2007).

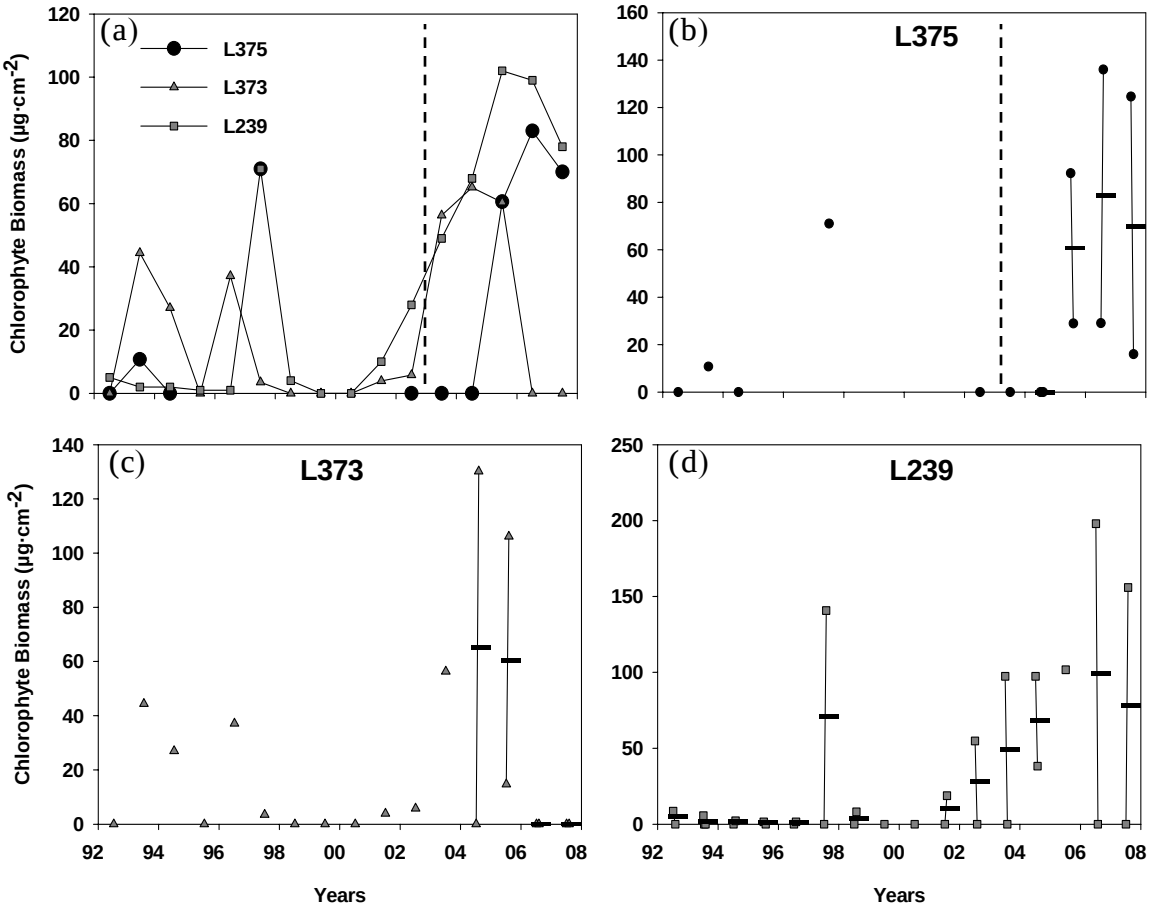

Figure 2.18: Chlorophyte biomass (µg·cm^{-2}) in the epilithon of the experimental lake (L375) and reference lakes (L373 and L239) from 1992 to 2007. In panel (a) the July and August means are expressed for the experimental and reference lakes. The remaining panels (b-d) represent July and August sampling dates connected by the solid vertical line; the horizontal line represents the means of these sampling dates for each lake. The dashed vertical line (a and b) distinguishes between pre-cage (1992-2002) and cage-operation years (2003-2007).

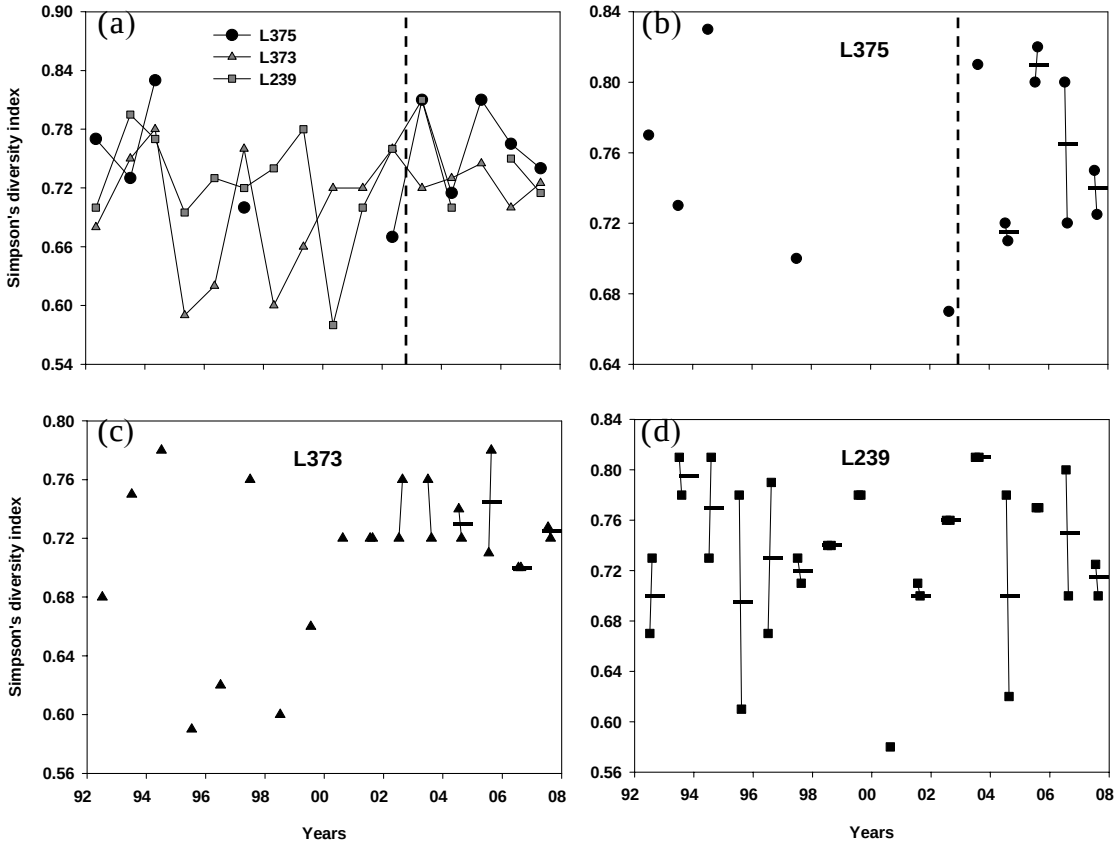

Figure 2.19: Species diversity using Simpson's diversity index in the epilithon of the experimental lake (L375) and reference lakes (L373 and L239) from 1992 to 2007. In panel (a) the July and August means are expressed for the experimental and reference lakes. The remaining panels (b-d) represent July and August sampling dates connected by the solid vertical line; the horizontal line represents the means of these sampling dates for each lake. The dashed vertical line (a and b) distinguishes between pre-cage (1992-2002) and cage-operation years (2003-2007).

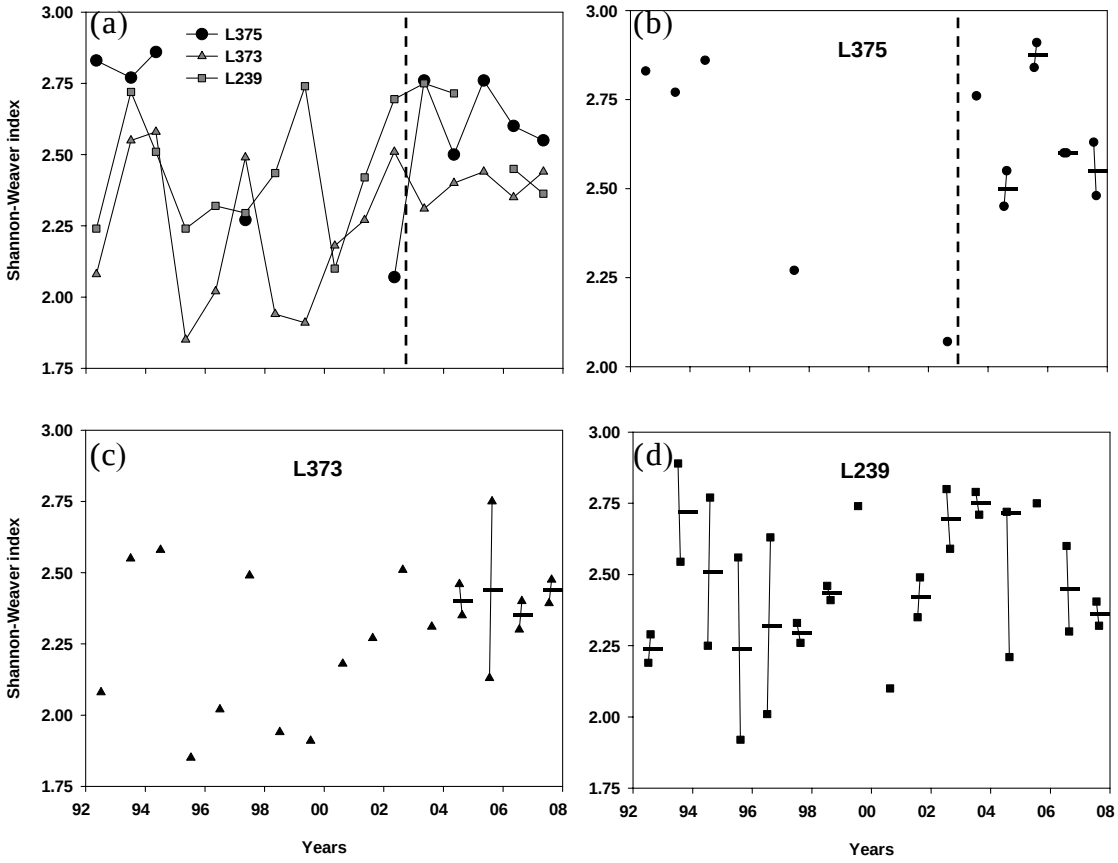

Figure 2.20: Species diversity using Shannon-Weaver index in the epilithon of the experimental lake (L375) and reference lakes (L373 and L239) from 1992 to 2007. In panel (a) the July and August means are expressed for the experimental and reference lakes. The remaining panels (b-d) represent July and August sampling dates connected by the solid vertical line; the horizontal line represents the means of these sampling dates for each lake. The dashed vertical line (a and b) distinguishes between pre-cage (1992-2002) and cage-operation years (2003-2007).

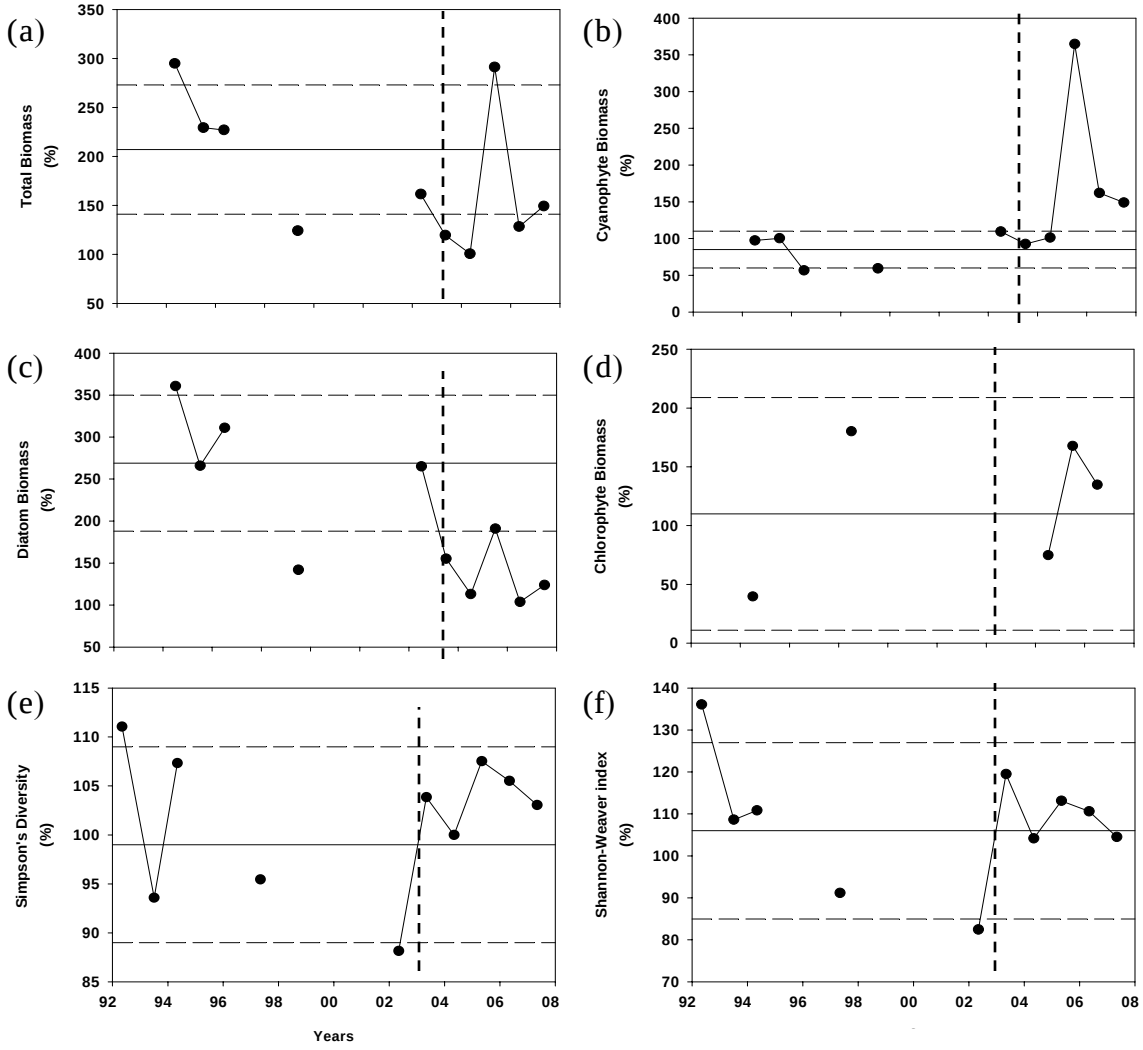

Figure 2.21: Experimental lake 375 relative to the average of the reference systems (L239 and L373) from 1997 to 2007 for (a) total biomass, (b) cyanobacterial biomass, (c) diatom biomass, (d) chlorophyte biomass, (e) Simpson's species diversity and (f) Shannon-weaver species diversity. The solid horizontal line represents the pre-cage mean in L375; the dashed horizontal lines represent the standard deviation around the mean. Years of cage operation are distinguished from pre-cage years by a dashed vertical line.

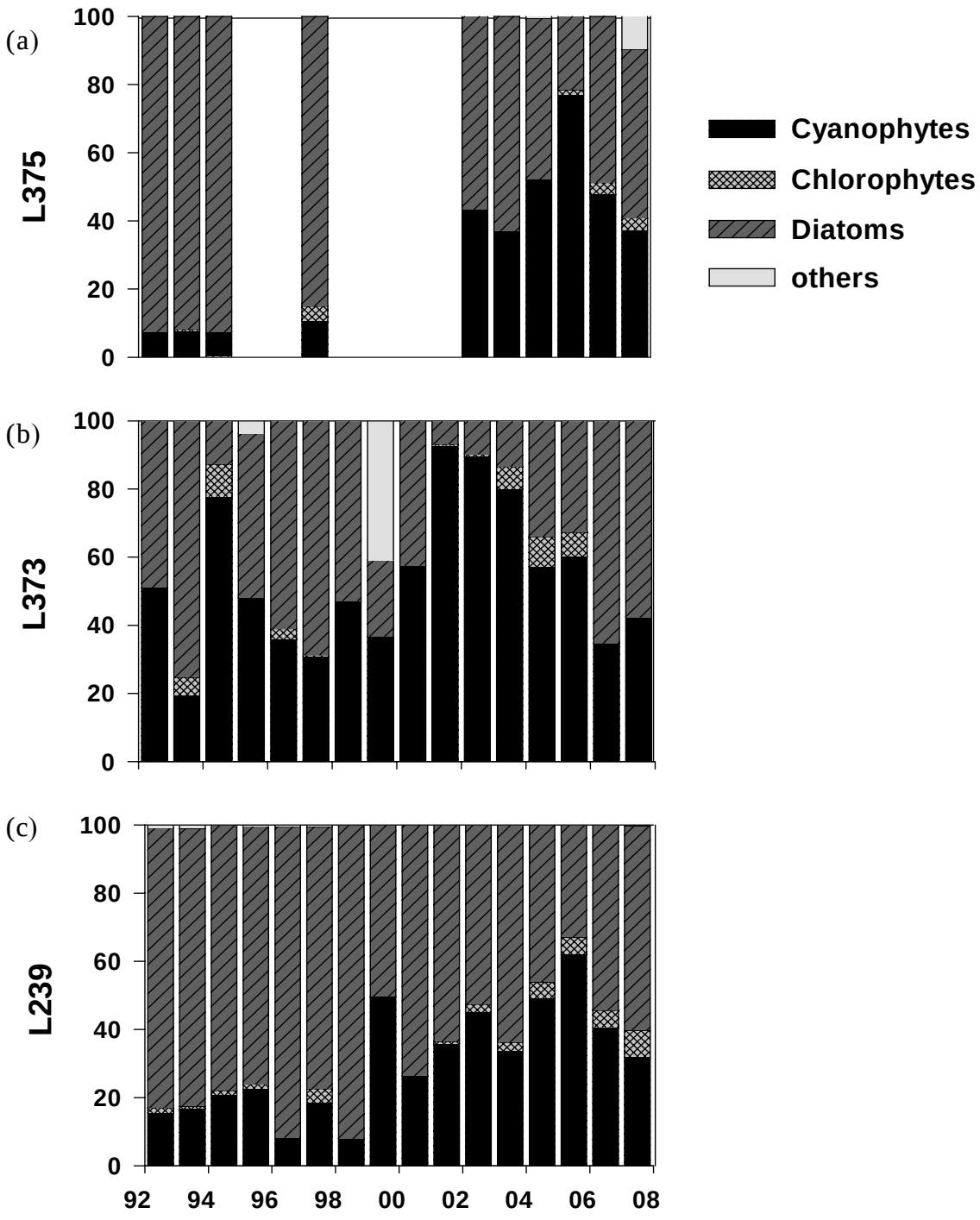

Figure 2.22: Percent algal composition in the epilithon of (a) the experimental lake (L375) and reference lakes ((b)L373 and (c) L239) from 1992 to 2007; 2003 to 2007 are years of aquaculture operation.

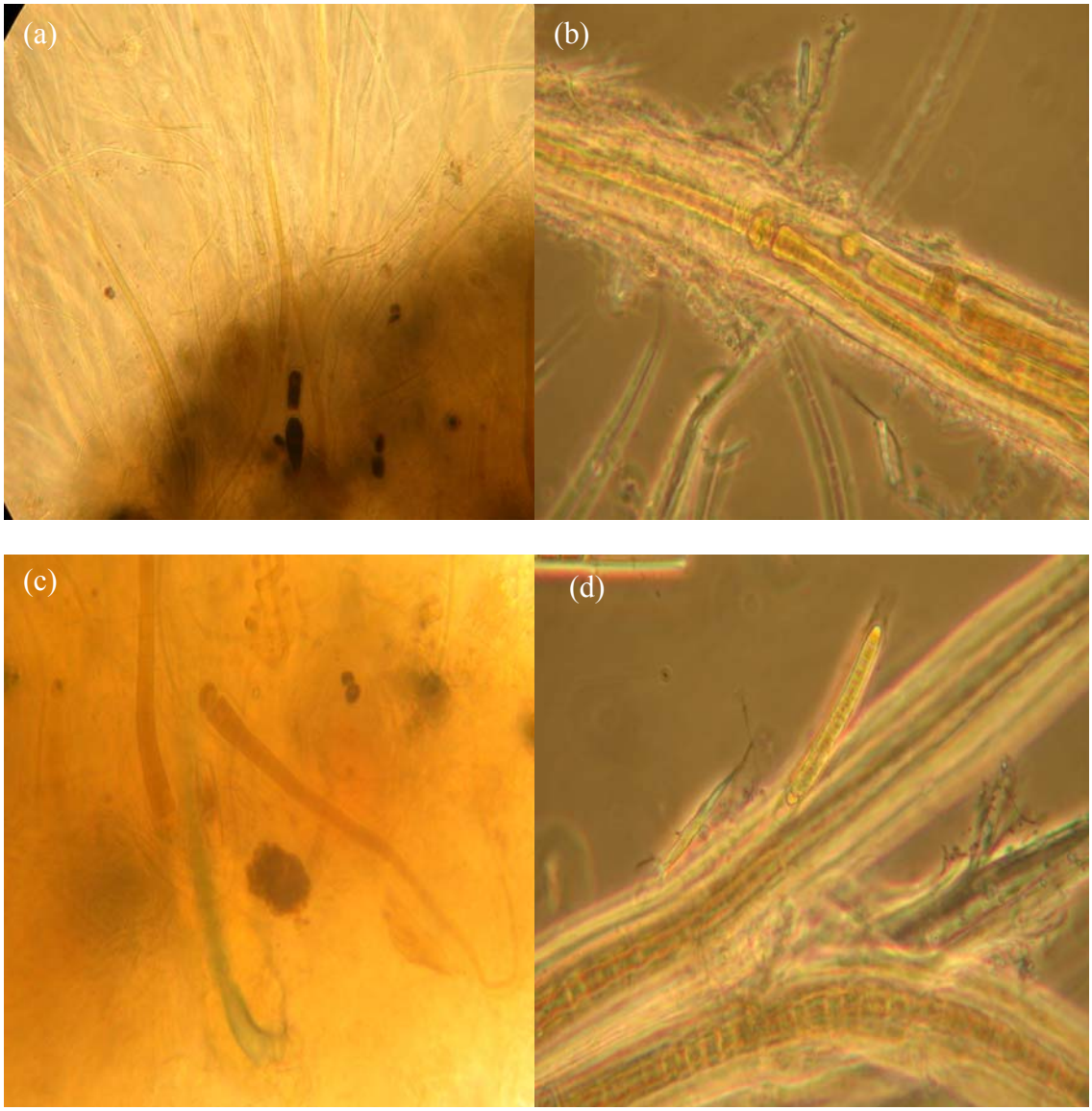

Figure 2.23: (a) Low magnification photograph, (c, b, d) high magnification photographs of *Calothrix* sp found in the epilithon of L375. Photos by K. Hille 2007.

Table 2.7: *Calothrix* sp. abundance in the epilithon of the experimental lake (L375) and reference lakes (L239 and L373) from 1999 to 2007.

Lake	Year	*Calothrix* sp. biomass ($\mu g \cdot cm^{-2}$)	Percent of total biomass (%)
375	2005	4508	54
373	2003	472	31
375	2007	322	14
373	2005	312	25
239	2004	130	8
373	2006	111	3.1
375	2006	76	1.6
239	2007	75	2.9
373	2007	42	1.5
375	2004	29	3.1
373	1999	10	1.6

Table 2.8: Unique L375 taxa. Taxa first detected in the epilithon of L375 following the commencement of the aquaculture operation in 2003. Three groups showed changes in species composition: dinoflagellates, cyanobacteria and chlorophytes.

Taxa	Sampling date	Biomass ($\mu g \cdot cm^{-2}$)	Proportion of total yearly biomass (%)	Ecology and Distribution
Chlorophytes				
Arthrodesmus (Staurodesmus) octocornis (Ehrn and Ralfs)	04-June-2007	6.08	0.7	Component of the plankton and periphyton of acidic oligotrophic systems[3]
Gonatozygon spp. De Bary	25-Aug-2007	32	2.8	Planktonic, usually in acidic, oligotrophic lakes/pond or in sphagnum bogs[3]
Paulschulzia pseudovolvox (Schultz) Skuja.	31-Aug-2005	29	1.0	Planktonic, uncommon sp. found in temperate lakes
Cyanobacteria				
Oscillatoria sancta (Kutz.) Gomont	18-July-2006	261	10	Component of benthic biofilms[3]
	22-Aug-2006	174	7.2	
Radiocystis spp. Skuja	25-Aug-2007	2.5	0.1	Mainly nanoplanktonic, apart of surface blooms in mesotrophic and eutrophic waters[3]
Tychonema rhodenema Skuja	25-Aug-2007	83	4.0	Genus has both planktonic and benthic species, possibly a cold-stenotherm of northern-temperate areas[3]

Diatoms				
Cyclotella michiganiana Skvortsov	08-May-2007	4.33	0.3	
Aulacoseira distans (Ehrenberg) Simonsen	04-June-2007	10.39	1.2	Very large, complex and widely distributed genus[3]
Anomoeneis brachysira Grunow	30-June-2007	14.3	1.6	Frequently observed in bogs[3]
Cymbella lapponica Grunow and Cleve	30-June-2007	44.74	2.6	
Gomphonema angustum Agardh	18-July-2006	22	0.8	Genus cosmopolitan, characteristic of circumneutral lakes. *G. angustum* reported to be alkaliphilous[3]
Gomphonema truncatum Ehrenberg	31-Aug-2005	20	0.7	Genus cosmopolitan, found in waters with high electrolytes[3]
Dinoflagellates				
Ceratium hirundinella (Muller) Schrank	27-July-2007	722	21	Component of the phytoplankton, capable of P-storage encysts in the sediment [1,2]

References: [1]Sigee *et al.*(1998), [2]Hansson (1996), [3]Wehr and Sheath (2003)

Table 2.9: Chlorophyte occurrence, abundance and diversification in the epilithon of L375 before and during cage operation from 1992 to 2007; 1995-1996 and 1998-2001 were absent from the record. Chlorophytes were not detected in L375 in 1992, 1994 and 2002-2004.

Year	Taxa	Biomass (µg·cm^{-2})	Percent of total biomass (%)
1993	*Chlamydomonas* sp.	10.7	0.41
1997	*Oedogonium* sp.	71.0	4.4
2005	*Paulschulzia pseudovolovox* (Schultz) Skuja.	51.9	1.1
	Oedogonium sp.	178.3	2.2
2006	*Oedogonium* sp.	136.4	5.2
	Euastrum sinuosum Lenorm.	58.6	3.0
2007	*Mougoeotia* sp.	59.2	1.7
	Bulbochaeta sp.	189.9	5.5
	Gonatozygon sp.	31.9	2.8

Incorporation of cage wastes

Epilithic $\delta^{15}N$ concentrations increased in L375 during the aquaculture operation until 2007, when $\delta^{15}N$ decreased substantially (Fig. 2.24). The difference between pre-cage and cage years did not show any statistical significance (Table 2.10), even though L375 remained above the pre-cage mean throughout the years of cage operation (Fig. 2.26 a). In addition, the $\delta^{15}N$-signal in L375 was not significantly different from that of the reference systems (Table 2.10).

Changes in $\delta^{13}C$ were significantly different between pre-cage and cage years (Table 2.10; p-value= 0.09), between lakes (p-value= 0.05) and interacting effects of pre-cage and cage years and lakes (p-value= 0.05). The $\delta^{13}C$ in L375 appeared to be within the envelope of references systems (Fig. 2.25), but examination of L375 relative to the average of the reference systems showed that the $\delta^{13}C$ was falling below the pre-cage mean (more positive) (Fig. 2.26). If the value for 2005 was ignored in Fig. 2.25 a, the increasingly positive trend was more evident.

Table 2.10: Results of a mixed procedure analysis of covariance on cage effects on stable isotopes of C and N, comparing: (1) the epilithon of the experimental lake 375 to the average of the reference systems, (2) pre-cage and cage years within L375, (3) their possible interaction, (4) yearly changes within L375, and (5) whether yearly changes interacted with pre-cage and cage years in L375. Significant values are distinguished from non-significant values by an asterisk.

	Effect	**df**	**F-value**	**P-value**
$\delta^{15}N$	Reference lake $\delta^{15}N$	1,2	0.00	0.97
	Pre-cage vs cage years in the experimental lake	1,2	0.13	0.78
	Interaction between reference lake $\delta^{15}N$ and pre vs cage years	1,2	0.05	0.85
	Year	1,2	0.02	0.91
	Interaction between year and pre vs cage years	1,2	0.14	0.77
$\delta^{13}C$	Reference lake $\delta^{13}C$	1,1	194	0.05*
	Pre-cage vs cage years in the experimental lake	1,1	51.8	0.09*
	Interaction between reference lake $\delta^{13}C$ and pre vs cage years	1,1	190	0.05*
	Year	1,1	68.2	0.08*
	Interaction between year and pre vs cage years	1,1	72.9	0.07*

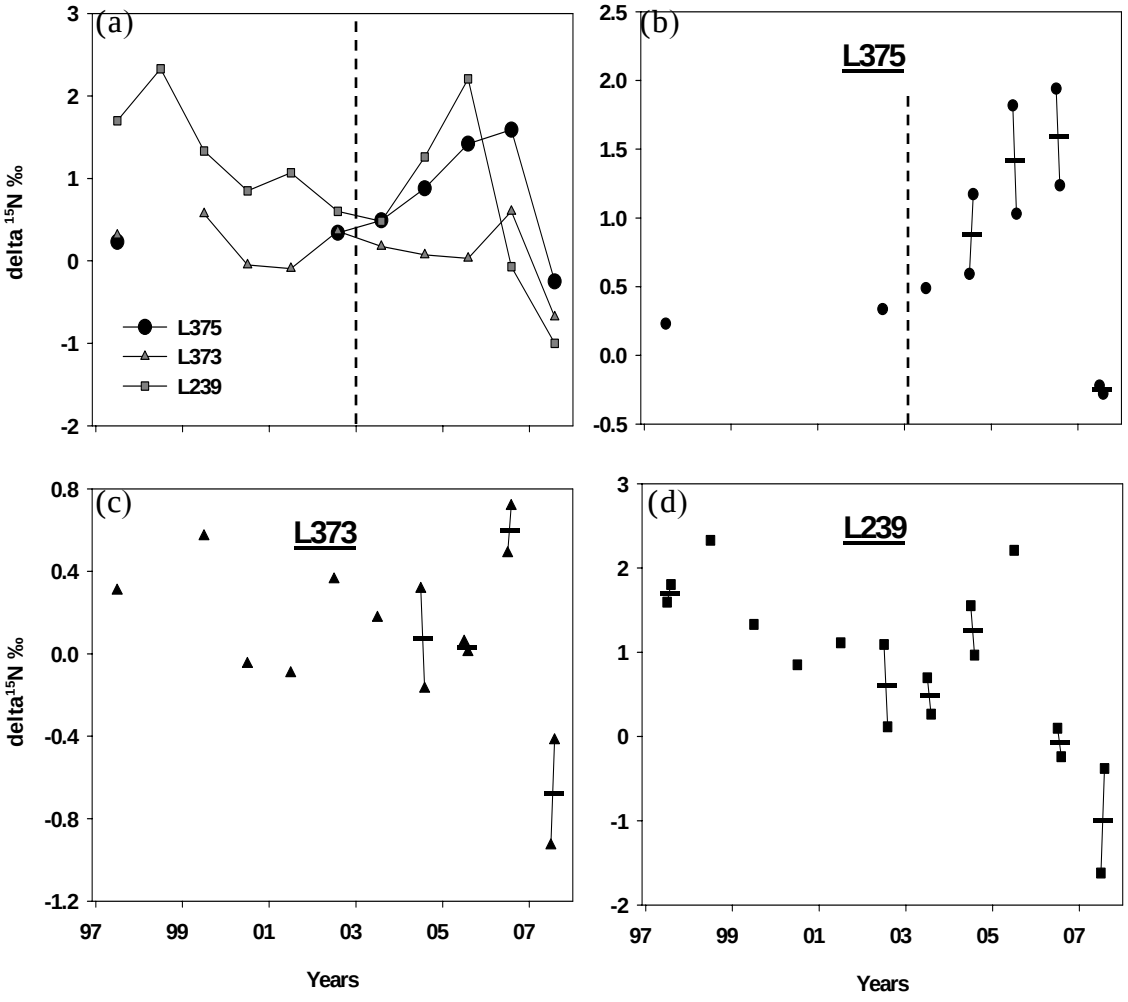

Figure 2.24: Stable isotope $\delta^{15}N$ (‰) in the epilithon of the experimental (L375) and reference (L373 and L239) lakes from 1992 to 2007. In panel (a) the July and August means are shown for the experimental and reference lakes. The remaining panels (b-d) show July and August sampling dates connected by the solid vertical line; the horizontal line is the mean of these sampling dates for each lake. The dashed vertical line (a-b) distinguishes between pre-cage (1992-2002) and cage-operation years (2003-2007).

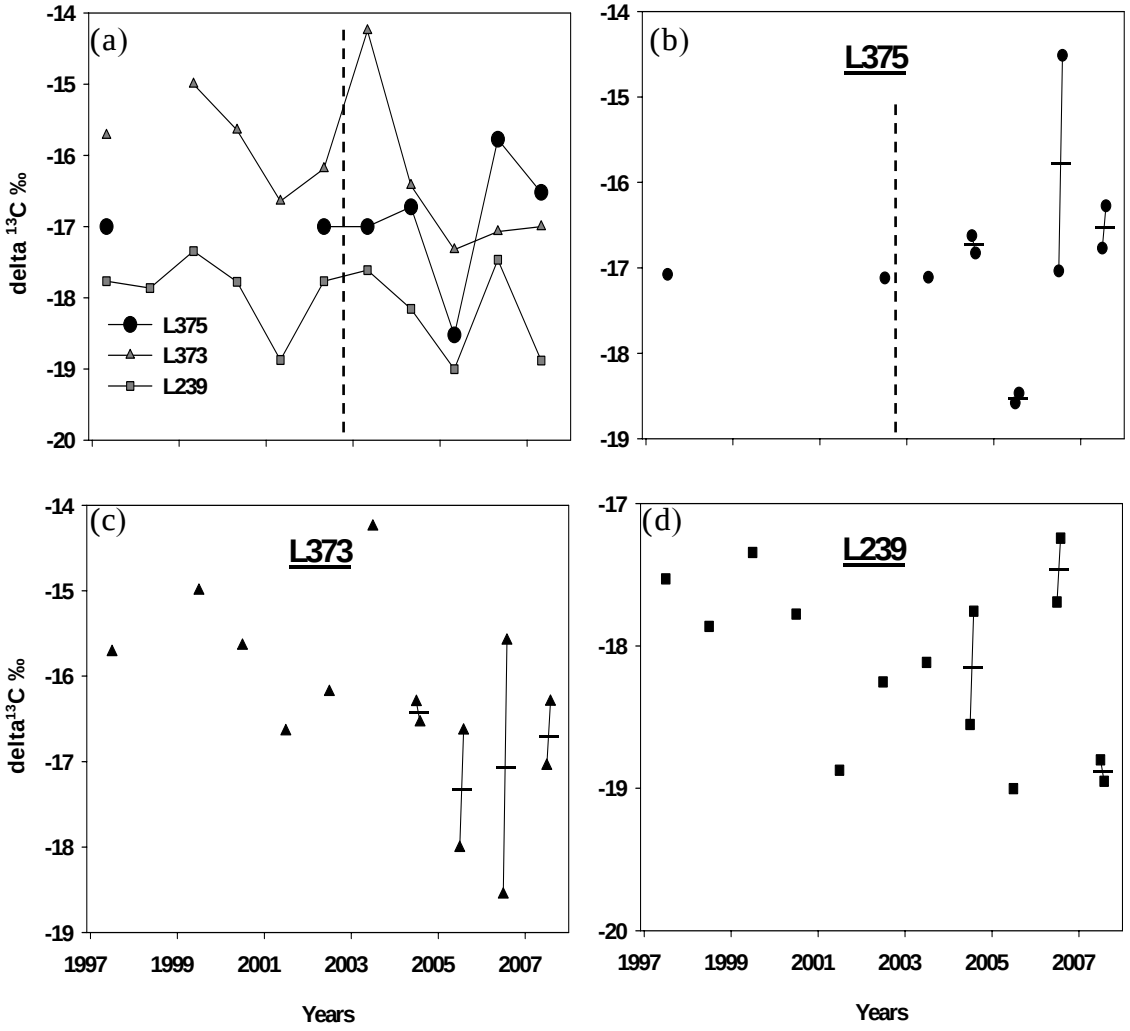

Figure 2.25: Stable isotope $\delta^{13}C$ (‰) in the epilithon of the experimental (L375) and reference (L373 and L239) lakes from 1992 to 2007. In panel (a) the July and August means are shown for the experimental and reference lakes. The remaining panels (b-d) show the July and August sampling dates connected by the solid vertical line; the horizontal line is the mean of these sampling dates for each lake. The dashed vertical line (a-b) distinguishes between pre-cage (1992-2002) and cage-operation years (2003-2007).

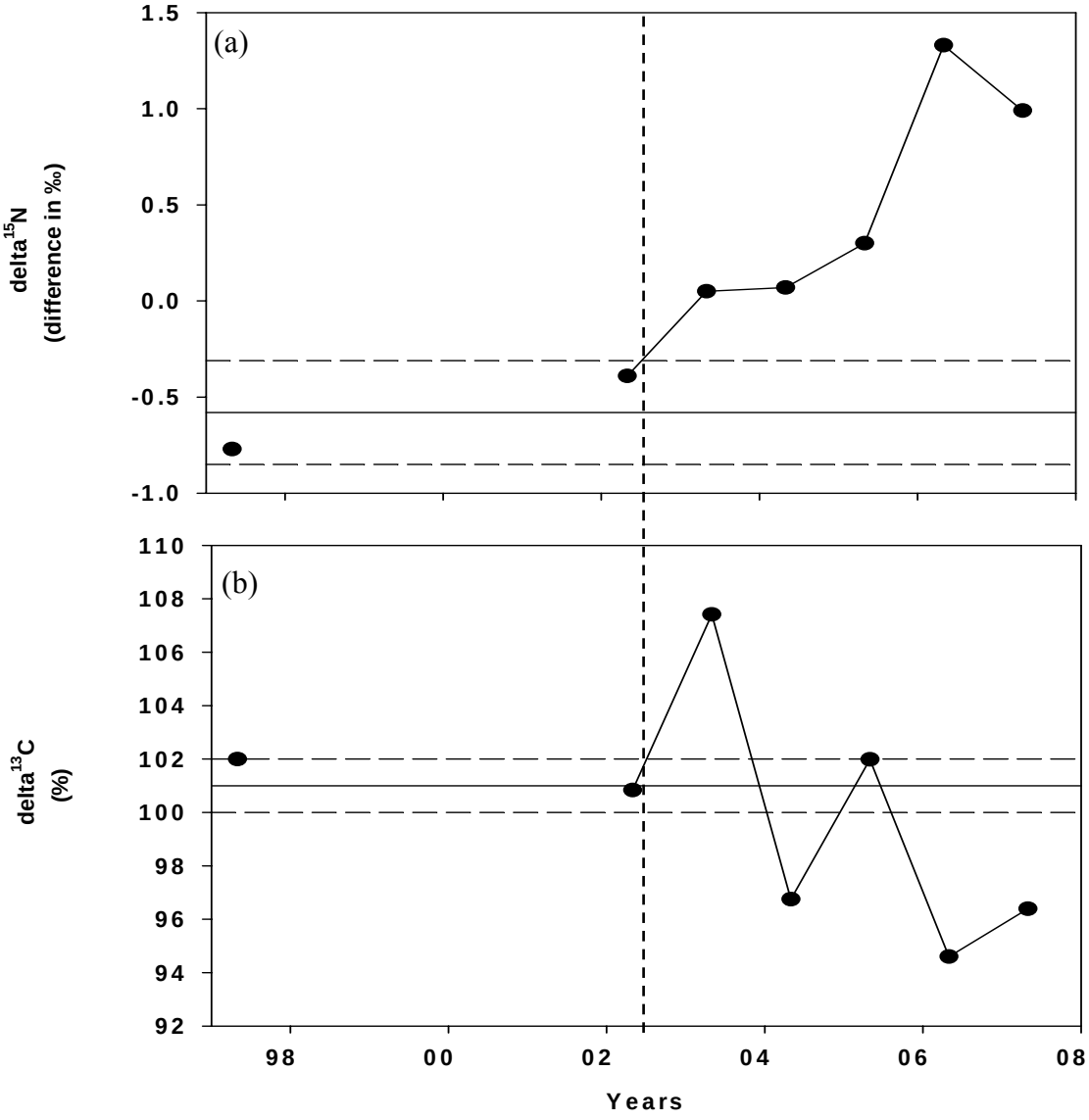

Figure 2.26: (a) The difference in epilithic $\delta^{15}N$ (‰) between experimental lake 375 and the average of the reference systems (L239 and L373), (b) epilithic $\delta^{13}C$ in the experimental lake 375 relative to the average of the reference systems (L239 and L373) from 1997 to 2007. The solid horizontal line represents the pre-cage mean in L375; the dashed horizontal lines are the standard deviations around the pre-cage mean. Years of cage-operation are distinguished from pre-cage years by a dashed vertical line.

Distance from cage

Epilithic P (μmol·cm^{-2}) and $\delta^{15}N$ (‰) were examined at six sites around the lake that differed in distance from the cage operation. While P and algal biomass appeared to show a step function increase at sites closer to the cage, $\delta^{15}N$ did not show a substantial difference between sites (Fig. 2.27). The sites also differed in algal composition (Fig. 2.28). Sites 1-4 showed a decreasing trend with increasing distance from cage (Fig. 2.27 a). The closer sites also had higher cyanobacterial biomass than sites further away (Fig. 2.28; Table 2.11). Chlorophyte biomass was higher at sites 5 and 6 than the others (Fig. 2.28; Table 2.11). Site 4, which was in the middle of the lake on the eastern shore, had the lowest biomass, while site 1, which was closest to the cage, had the highest biomass (Fig. 2.28; Table 2.11). Site 1 was dominated by two filamentous cyanobacteria: *Scytonema* sp. and *Lyngbya* sp. (Table 2.11). Site 2 was dominated by two keeled diatom taxa: *Rhopalodia* sp. and *Epithemia* sp. Site 3 had a high proportion of *Rhopalodia* sp. and *Lyngbya* sp. Site 4 was mainly *Epithemia* sp. and *Lyngbya* sp, while site 5 was mainly *Lyngbya* sp., *Rhopalodia* sp. and *Cyclotella* sp. (centric diatom). Finally, most of the biomass in site 6 was due to *Epithemia* sp.

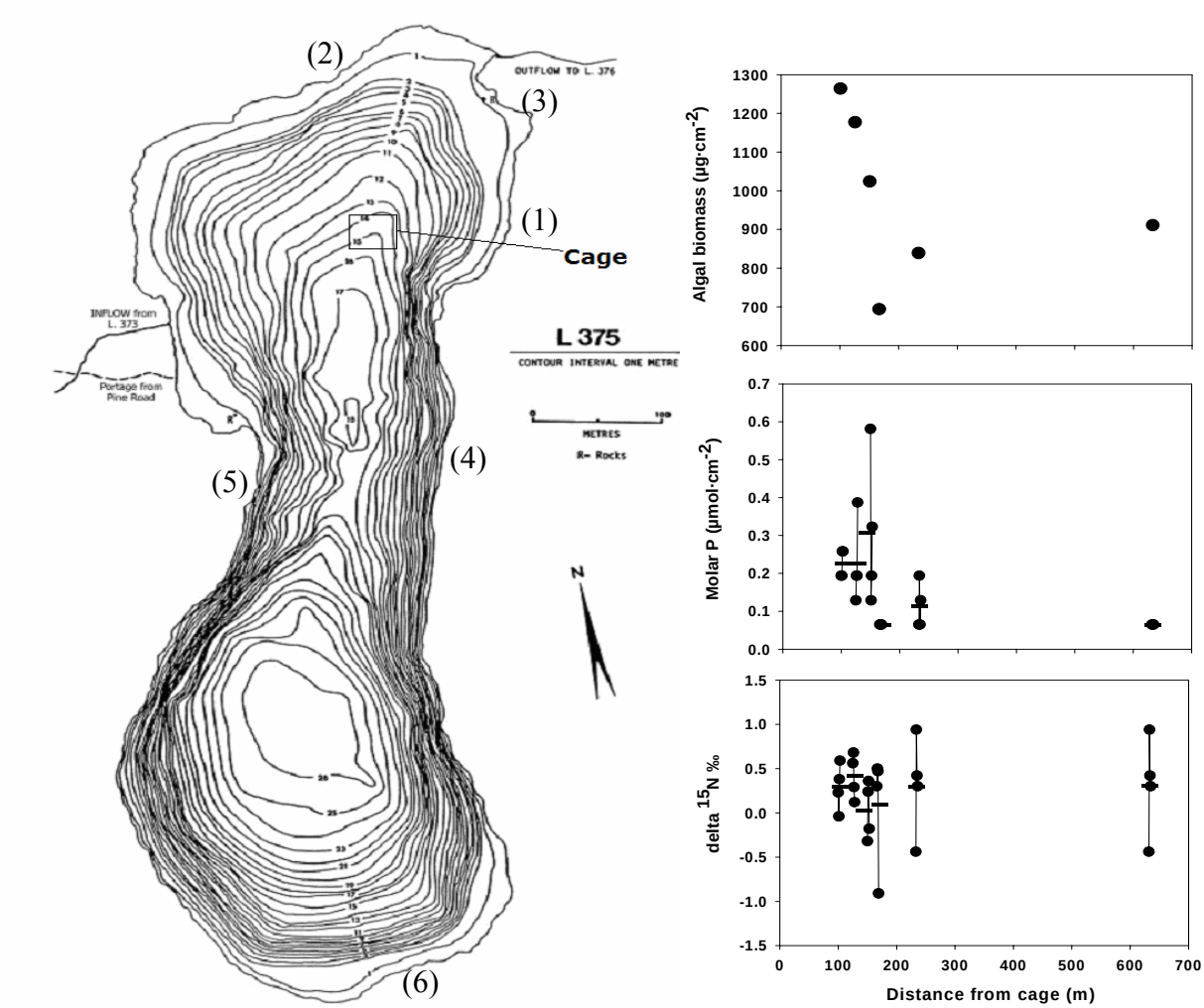

Figure 2.27: Distance-from-cage plots for (a) epilithic algal biomass (µg·cm^{-2}), (b) epilithic P (µmol·cm^{-2}) and (c) δ^{15}N (‰). The bathymetric map shows site locations; for example site 1 on the map corresponds to the first datum on the plots. (a) Algal biomass data represents site composites, (b) epilithic P and (c) δ^{15}N values represent both sub-site samples (circles) and the site means (horizontal line). The black box on the map is the approximate location of the aquaculture cage from 2003 to 2007 in the experimental lake. Map modified from the *ELA Data Retriever* version 7.1

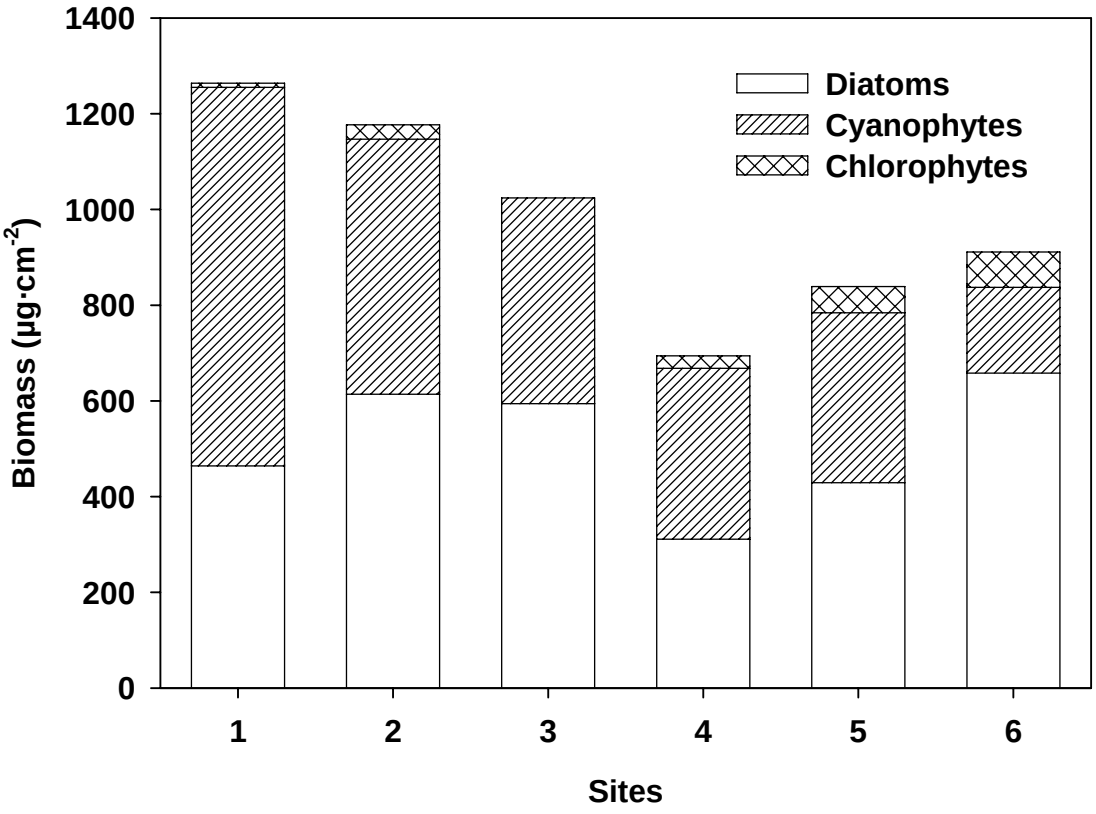

Figure 2.28: Composition and biomass (µg·cm^{-2}) of main algal groups in the epilithon of L375, sites 1-6 correspond to sites 1-6 located on the map in Figure 2.27. Only three groups of algae were present in the survey: diatoms, cyanobacteria and chlorophytes.

Table 2.11: Site composition and biomass (µg·cm^{-2}) of epilithic algal taxa in L375, sites 1-6 correspond to sites 1-6 located on the map in Figure 2.27.

Taxa	Sites					
	1	**2**	**3**	**4**	**5**	**6**
Cyanobacterial biomass (µg·cm^{-2})	**791**	**533**	**430**	**357**	**355**	**179**
Anabaenopsis sp.	2	1	1	1	2	3
Calothrix sp.	-	15	-	-	-	65
Anabaena flos-aquae (Lyngb.) Breb. and Bornet	-	-	-	19	-	-
Chroococcus limneticus Lemmermann	-	-	-	-	3	-
Chroococcus turgidus (Kutzing) Nageli	72	144	81	81	17	23
Cylindrospermum sp.	-	-	-	-	-	10
Gloeocapsa punctata Nageli	34	34	26	31	33	36
Lyngbya sp.	267	167	225	120	195	29
Merismopedia glauca (Ehrenberg) Kutzing	-	-	-	1	-	-
Nostoc spp.	-	-	97	-	-	-
Oscillatoria sancta (Kutz.) Gomont	64	-	-	-	-	-
Pseudoanabaena sp.	9	-	-	-	-	-
Scytonema sp.	343	124	-	90	92	-
Snowella sp.	-	48	-	11	13	13
Spirulina laxa G.M. Smith	-	-	-	3	-	-
Diatom biomass (µg·cm^{-2})	**464**	**614**	**594**	**311**	**429**	**658**
Achnanthes minutissima Kutzing	7	18	4	9	31	13
Asterionella formosa Hassall	-	-	1	-	-	-
Aulacoseira distans (Ehr.) Simonsen	-	-	-	-	5	-
Cyclotella bodanica Eulenst	-	-	-	-	151	-

Cyclotella stelligera Cleve and Grunow	17	68	14	-	54	59
Cymbella gracilis (Rabhorst) Cleve	-	7	17	-	28	7
Cymbella silesiaca Bleisch	8	11	42	-	-	10
Diatoma elongatum Agardh	6	-	-	-	3	2
Epithemia argus Kutzing	15	228	61	213	-	498
Fragilaria capucina Grunow	-	-	-	19	-	-
Gomphonema acuminatum Ehrenberg	9	-	-	-	-	-
Gomphonema constrictum v capitata (Ehrenberg) Cleve	20	-	-	55	-	-
Navicula radiosa Kutzing	-	14	51	10	-	-
Neidium iridis (Ehrenberg) Cleve	120	-	72	-	-	-
Nitzschia palea (Kutz.) W. Smith	-	7	-	-	-	-
Rhopalodia sp. O. Muller	204	237	325	-	144	-
Syndra acus Kutzing	-	1	-	-	-	-
Tabellaria fenestrata (Lyngbye) Kutzing	58	23	7	5	13	37
Tabellaria flocculosa (Roth) Kutzing	-	-	-	-	-	32
Chlorophyte biomass (µg·cm^{-2})	**9**	**30**	**0**	**26**	**55**	**74**
Bulbochaeta sp.	-	-	-	-	52	-
Euastrum sinuosum Lenorm.	-	-	-	-	-	16
Oocystis lacustris Chodat	1	-	-	-	3	-
Mougeotia sp.	-	-	-	26	-	58
Scenedesmus brevispina (G.M Smith) Chodat	8	-	-	-	-	-
Staurodesmus paradoxum Meyen	-	30	-	-	-	-
Total biomass (µg·cm^{-2})	**1264**	**1177**	**1024**	**694**	**839**	**911**

Seasonal changes

Seasonal changes in $\delta^{15}N$ (‰), $\delta^{13}C$ (‰), P (µmol·cm^{-2}), N: P, C: N, C: P, Chl a, algal biomass and composition were examined in the epilithon of L375 and L373 over the entire open-water period in 2007 (Fig. 2.29 and 2.30). Over the season epilithic P (µmol·cm^{-2}), $\delta^{15}N$ (‰) and Chl a increased in L375 (Fig. 2.29), closely following the aquaculture production cycle with increased nutrient supply (feed) in the fall. In the reference system (L373), these parameters remained fairly stable, $\delta^{15}N$ did show a slight increase however not to the same extent as in L375 (-1.12 to 0.06 ‰) (Fig. 2.29 a). The carbon stable isotope changed very little throughout the season in L375, until October when it decreased from -17 to -19 (became lighter). L373 showed a slight increasing trend over the season (-17 to -15.8) and similar to L 375 dropped in October (Fig. 2.29 b)

The molar ratios showed more variability in their seasonality (Fig. 2.29 d, e, and f). Both N: P and C: P followed a similar trend, both were high at the start of the season in L375 and dropped during the early summer months (June and July) and then began to increase again in August (Fig. 2.29 d and f). In L373 the ratios decreased steadily throughout the season. The ratio of C: N was similar in both lakes during the early part of the season until September when a major decrease in L375 brought it substantially lower than L373 for the remainder of the season (Fig. 2.29 e).

Total epilithic algal biomass was higher in L375 than in L373 and showed two major peaks during the open-water season in July and October (Fig. 2.29 h). L373 showed a similar peak in algal biomass in July but lacked the October peak. Algal composition was similar between lakes and months. L375 showed greatest group diversity (chlorophytes, cyanobacteria, diatoms and dinoflagellates) in June and July.

L373 showed greatest group diversity in May, July and September (chlorophytes, cyanobacteria and diatoms). Otherwise the communities were dominated by diatoms and cyanobacteria (Fig. 2.30).

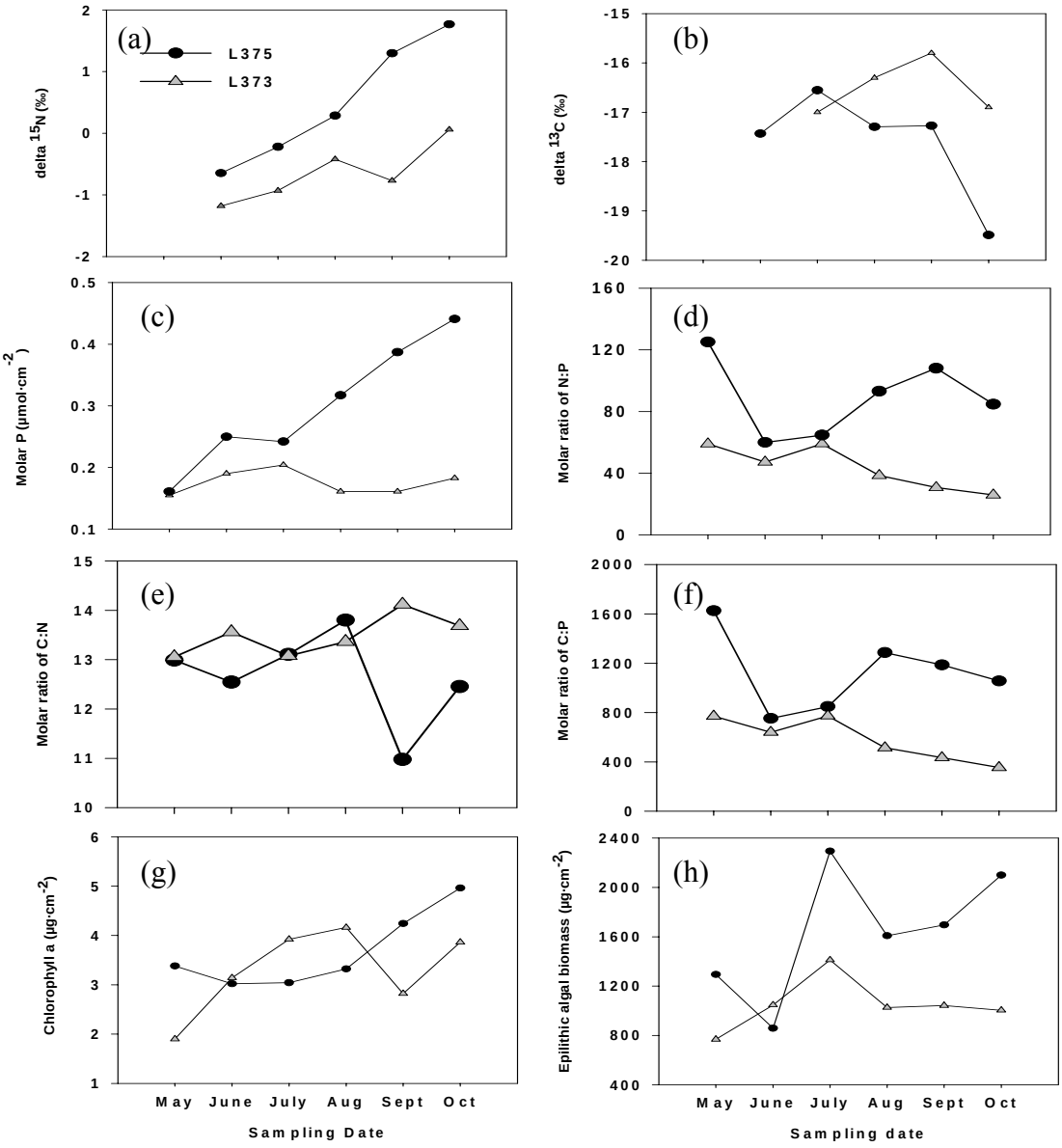

Figure 2.29: Seasonal changes in (a) $\delta^{15}N$ (‰), (b) $\delta^{13}C$ (‰), (c) molar P (µmol·cm^{-2}), (d) molar ratio N: P, (e) C: P, (f) C: N, (g) Chl a (µg·cm^{-2}), and (h) algal biomass (µg·cm^{-2}) in the epilithon of the experimental lake 375 (circles) and reference lake 373 (triangles) during the open-water season in 2007. $\delta^{15}N$ and $\delta^{13}C$ samples were insufficient for analysis in May and $\delta^{13}C$ were insufficient for analysis in June 2007.

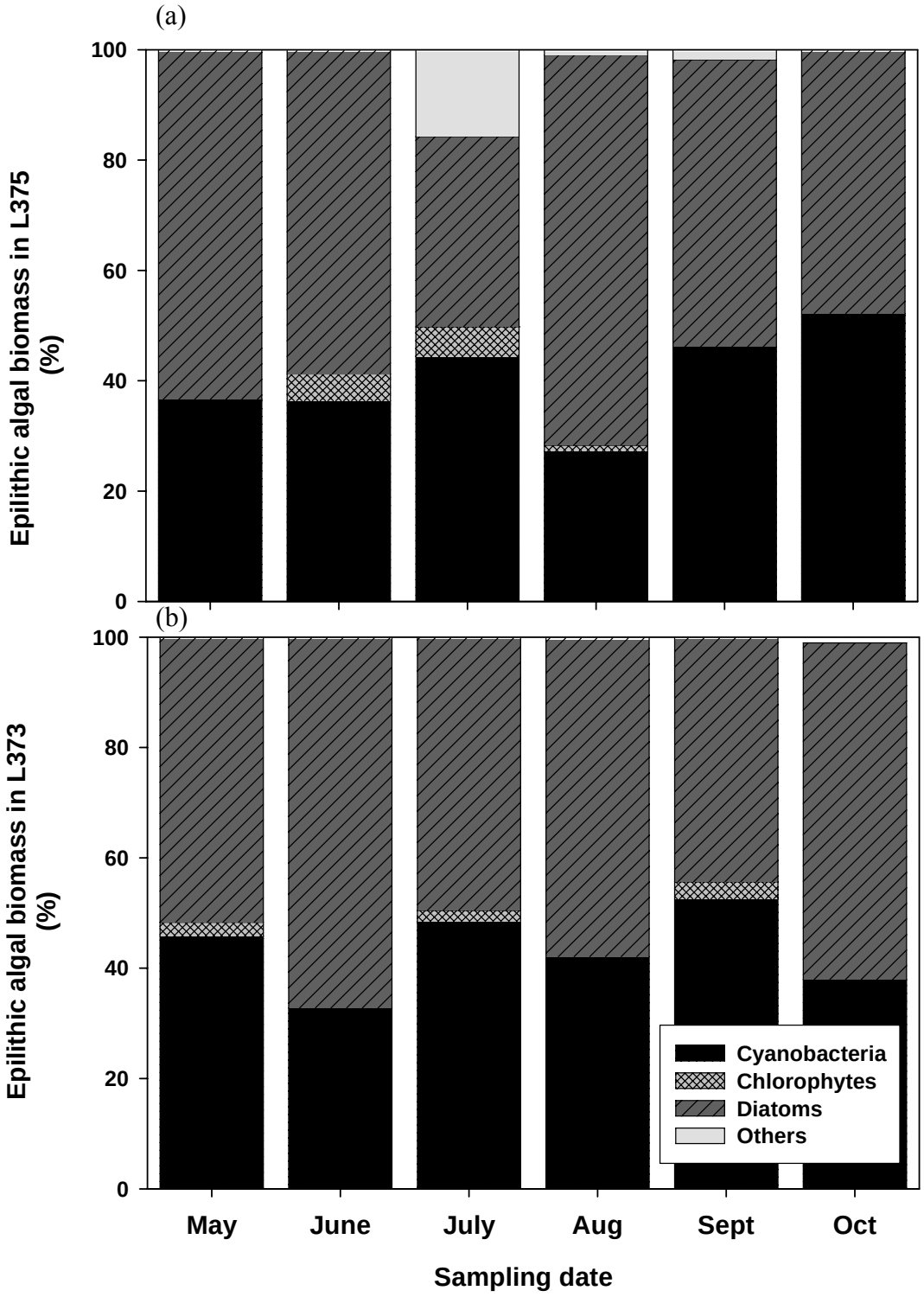

Figure 2.30: Seasonal changes during 2007 in epilithic algal composition in (a) the experimental lake and (b) reference lake 373.

Discussion

Epilithic nutrients

Like epilimnetic P (Appendix I Fig. I.1), epilithic P concentrations were significantly higher in L375 during the cage period (Table 2.3, Fig. 2.7, 2.8 a), and were significantly higher than the average of the reference systems (Table 2.3, Fig. 2.7, 2.8 a). Concentrations prior to the cage operation were relatively stable and low (0.2 ± 0.02 $\mu g \cdot P \cdot cm^{-2}$), with an N: P ratio showing severe P-limitation (70-80) (Fig. 2.11). Hillebrand and Sommer (1999) suggest that N: P >22 indicates P-limitation. Following commencement of the cage operation, epilithic P increased above the pre-cage mean by > 30%, in three out of the five years of operation (2004, 2006 and 2007) (Fig. 2.7). These increases disrupted the stability previously seen in the system and caused a decrease in the N: P ratio (~52; Fig. 2.11).

Epilithic P concentrations in the L239 epilithon were higher than in L375 throughout the historical record (Fig. 2.8 a). The L239 community was an example of an epilithic community with excellent P-trapping abilities since the lake itself was P-limited with relatively low DIC concentrations (Table 2.2). The only year in the study where epilithic P concentrations in L375 exceeded those of L239 was in 2007 (Fig. 2.8 a), after five years of aquaculture practices. Prior to this increase, epilithic P in L375 usually remained within the reference envelope (Fig. 2.8 a).

Following commencement of the aquaculture operation in L375, increases of epilimnetic total P occurred during spring and fall mixing. Larger cumulative increases occurred each consecutive year (Appendix I Fig. I.1). Alternatively, epilithic P concentrations showed an incremental increase over the open-water season (Fig. 2.29 c),

closely following aquaculture loading patterns of increased feed in the fall. This fall increase indicates that the epilithon may have been acting as a P sink, accumulating P throughout the season.

Aquaculture operations have the potential to increase nitrogen concentrations primarily through the excretion of nitrogenous wastes via the gills and kidneys of the farm fish (Tovar *et al.* 2000; Glaholt and Vanni 2005). Given that the epilithon was severely P-limited prior to the aquaculture operation (Fig. 2.11), if nitrogen had increased; the effects of the slight increase in epilithic P may have had little impact on the benthic algae. This was because increased nitrogen concentrations would continue to pull the community towards P-limitation. However, nitrogen did not increase substantially in L375 and remained well within the range of variability of the reference lakes (Fig. 2.7 c, 2.10).

In attached algal communities, the cellular C: P ratio is a good index of P limitation, the cellular N: P ratio distinguishes between N or P limitation, and the C: N ratio indicates limitation in general (Hillebrand and Sommer 1999). The N: P and C: P ratios in the current study, which lessened in L375 with continued aquaculture loading, indicated P limitation (Fig. 2.11 and 2.12). The N: P and C: P ratios have not decreased below the optimum values for P-sufficiency reported by Hillebrand and Sommer (1999), Kahlert (1998), or the Redfield ratio (Wetzel 2001b), but there was a cage-related decrease in N: P in 2007 in L375 (Table 2.4, Fig. 2.11 a, 2.12 a). A similar decrease was not observed in the reference lakes. Perhaps with continued operation of the aquaculture farm, the ratio of N: P would have declined further, which was what Temporetti *et al.*

(2001) observed in the periphyton of an Argentinean reservoir that received seven years of nutrient inputs from an intensive salmonid fish farm.

Concentrations of C in the epilithon of L375 did not significantly change following commencement of the cage operation (Fig. 2.7 b; 2.9). However, the molar ratio of C: N revealed a downward trend in all the systems (Fig. 2.11), especially in L375 (Table 2.4; Fig. 2.12). The decrease may have been unrelated to the aquaculture operation (Full model Table 2.4), as it appears to have been occurring since the beginning of the time series record (Fig. 2.11). The reduced model does indicate, though, that there has been a significant drop in the C: N ratio following treatment (Table 2.4; p-value = 0.005). The decrease in C: N (Fig. 2.11 c) may be an indication of an overall decrease in nutrient limitation in the system (Hillebrand and Sommer 1999)

Benthic algal biomass

Overall there was no statistical change in abundance of total benthic algal biomass (based on direct algal counts) or in Chl a concentrations in L375. Chl a remained within the envelope of the reference lakes throughout the time series (Fig. 2.14 a), showing no response. However, when the average of the reference systems was compared to L375 in 2007, Chl a concentrations in L375 substantially increased above the average of the reference lakes and the pre-cage mean (Fig. 2.14 b). Seasonal trends in Chl a concentrations corresponded well with seasonal changes in epilithic P and the aquaculture nutrient loading regime (increased P at the end of the season) (Fig. 2.9). Seasonal sampling of Chl a also suggested that due to the nature of the synoptic mid-summer sampling protocol, the annual Chl a peak may have been missed in earlier sampling (Fig. 2.29 g).

MacIsaac and Stockner (1995) (in Findlay *et al.* in press) documented increases in littoral epilithic chlorophyll with continued cage operation. Likewise, Temporetti *et al.* (2001) reported an increase in periphytic biomass (as Chl a) resulting from increased nutrient concentrations from an in-lake fish farm. Although there was an increase in Chl a in the current study, there was no sustained increase in total epilithic algal biomass above the pre-cage mean following commencement of the cage operation (Fig. 2.21). The major increase that was observed in 2005 (Fig. 2.15 a and b) was not statistically significant because reference lake 239 had experienced two major blooms of similar magnitude in the past (Fig. 2.15 a and d), albeit of a different algal group (diatoms rather than cyanobacteria; Fig. 2.16). Unlike Chl a and epilithic P, epilithic algal biomass did not increase incrementally over the open-water season. For algal biomass, peaks were observed in July and October (Fig. 2.29 h).

Interpretation of these Chl a increases as biomass increases should be done with caution, as pigments can be affected by changes in environmental conditions and algal community composition (Baulch *et al* in press.). Because of this, Chl a is not always a useful indicator of algal biomass. Some degree of error was associated with direct algal counts (used in the measure of total algal biomass), however, it was still considered a more reliable metric of community change (Baulch 2002). That is, the current algal biomass estimates are a better metric for interpreting change-related impacts than is Chl a.

In addition to epilithic sampling, Findlay *et al.* (in press) used tethered artificial periphyton trays to measure changes in attached algal biomass (based on direct algal counts) in the pelagic zone of L375 during the cage operation. The periphyton trays were

placed 1 m below the surface at varying distances from the cage (0 m, 50 m, 100 m and 150 m). The trays were sampled from June to Sept of each year (missing spring and fall mixing, similar to the epilithic synoptic sampling). A single tray placed inside the cage in 2004 showed an immediate increase in biomass and change in species composition. However, it was not until the fourth year of cage operation (2006) that an increase in attached algal biomass on the trays outside the cage (50 m, 100 m and 150 m) was observed. Of these, the most notable increase in algal biomass was observed on northern trays in July.

These periphyton trays represent another attached algal community in an area near the cage operation, upon which comparisons can be made. The fact that a response was not observed on the artificial trays outside the cage until the fourth year of operation suggests that an increase in epilithic algae in the middle littoral zone is possible after a longer lag. That said, it should be noted that these trays are different from the natural epilithon in several respects. First, the clean smooth tiles differ from the natural epilithon in terms of colonization rates, biofilm thickness, heterogeneity and species composition. Second, the clean smooth tiles are newly added each spring, which means that new conditions would influence growth more easily than was the case in the pre-existing biofilm. Moreover, the time required for colonization of the artificial substratum will create an attached algal community that does not mimic the natural substratum (Robinson 1983).

Algal composition: large-scale

With the observed decrease in the N: P ratio it was expected that there would be a shift in community composition from a diatom to a N-fixing cyanobacteria-dominated

community (Wetzel 2001b; Noges *et al.* 2007). The increase in cyanobacteria was predicted because N-fixing cyanobacteria are capable of easily fixing atmospheric N_2 and are therefore not constrained by a relative decrease in N-availability (Wehr and Sheath 2003), which was predicted with the increase in available P. The hypothesized shift in group dominance was observed in L375, but the change likely started prior to the commencement of the operation (Fig.2.22 a). However, a gap in the record between 1997 and 2002 in L375 leads to some uncertainty as to when the trend began. Prior to- and including 1997, cyanobacterial counts were less than 10% of the total biomass. An increase in cyanobacterial biomass in 2002 to 43% suggests that the shift in group dominance occurred prior to the commencement of the cage operation in 2003. A similar shift also occurred in reference system L239 (Fig. 2.22), although not to the extent of L375 (Table 2.6, Fig. 2.17, 2.21 b).

Less obvious compositional changes (i.e. increased presence of chlorophytes), also appeared to have occurred in the epilithon of L375, however, these changes were not statistically significant and fluctuations in chlorophyte biomass were still well within the range of variability around the pre-cage mean (Table 2.6, Fig. 2.21 d). Prior to the cage operation, chlorophyte presence in L375 had been uncommon (only two out of five recorded occurrences of green algae prior to the cage). However, following the cage operation, chlorophytes were present for three consecutive years (2005-07), with abundance values similar to those seen in the higher P lake, L239 (Fig. 2.18).

Algal compositions: fine-scale

Though biomass did not substantially increase in L375, and the major community changes that were occurring did not appear to be directly related to the aquaculture

operation, there were meaningful ephemeral changes occurring at the species-level related to the aquaculture cage operation. Following the commencement of the cage operation, a number of new taxa (39; data not shown) appeared for the first time in L375, as well as a number of taxa unique to L375 (13) appeared (Table 2.8). The number of chlorophyte taxa also minimally increased and diversified with each additional year of cage operation (Table 2.9). Caution should be taken when interpreting these results because these novel taxa may have been overlooked in earlier counts as efforts were not made to seek out new individuals. In addition, biomass estimates were based solely on a sample count effort that was achieved when 100 cells of the most dominant taxa were seen therefore; some species may not have been observed in the sample.

Species diversity and evenness indices (Simpson's diversity and Shannon-Weaver index) demonstrated that no major changes from pre-cage conditions (Fig. 2.19, 2.20, 2.21 e and f) occurred in the L375 community, however, the community did appear to be becoming more diverse, resembling that of L239. This was in contrast to Mannino and Sara (2007) who studied the effects of fish-farm biodeposition on artificial periphyton assemblages and found lower species diversity and richness. In their study, species richness was inversely correlated to fish farm effluent; the greater the organic enrichment the lower the number of taxa. Mannino and Sara's Sicilian fish farm seemed to select for more opportunistic species with rapid growth characteristics (high output of reproductive bodies) and a tendency to be more eurytopic. Their operation, however, was situated in the Gulf of Castellammare in the Southern Tyrrhenian Sea and consisted of a greater number of cages (six floating and three submerged) than L375, in an area characterised by a dominnat mono-directional current (average speed of 12-20 $cm \cdot s^{-1}$).

The L375 periphyton trays showed dominance by two filamentous taxa, a chlorophyte: *Mougeotia* sp. (Agaard) Wittrock, and a cynanophyte: *Cylindrospermum* sp. Kutzing (Findlay *et al.* in press). Both are common taxa, found epiphytically (Wehr and Sheath 2003) and both species were present in the epilithic biofilm of L375, but generally did not dominate. In June 2007, *Mougeotia* sp. was observed as a thick band (from the surface to approximately 0.5 m below the surface) attached to a rock near the lake's outflow (near epilithic sampling site C). These filaments had not been observed in previous years and were not seen during a survey of the lake's near-shore region after the rock occurrence. Turner *et al.* (1995) reported massive blooms of *Mougeotia* sp. in the littoral zone of a heavily perturbed lake (undergoing acidification), identifying the taxa as a species of interest in disturbed systems. Presence of *Mougeotia* sp. at site C and dominance of the taxa on the periphyton trays may have been an early indication of a disturbance-related phenomenon, which may have increased in severity, had the aquaculture operation continued.

Two substantial transient changes occurred during the course of the aquaculture operation in the benthic algae. The first and most considerable of the two was an increase in an attached heterocyst-forming cyanobacterium: *Calothrix* sp. (Fig. 2.23). *Calothrix* sp. abundance increased substantially in one year (2005) in L375 (4.5 $mg \cdot cm^{-2}$ or 54% of total biomass; Table 2.7) but dropped the following year to 76 $\mu g \cdot cm^{-2}$ (1.6% of total biomass). *Calothrix* sp. was not seen in L375 before 2004 and was first recorded in the study lakes in 1999 in L373 (1.6% of total biomass). After a few years of being absent from the record it reappeared in 2003 and remained a fixture of the L373 algal assemblage throughout the sampling period (average annual biomass of 1.9 $mg \cdot cm^{-2}$ from

1999 to 2007). Studies on *Calothrix elenkinii* Kosinskaja suggest that the genus is able to rapidly accumulate P and store it for long periods (Mateo *et al.* 2006). Mateo *et al.* (2006) suggest that the species is suited to nutrient poor environments that experience episodic pulses of abundant P (inorganic and/or organic). These pulses favour its ability for luxury uptake and storage strategies. The species is probably displaced by more competitive taxa in waters with high levels of P. The increase in P in L375 during the first few years of cage operation may have been sufficient to cause a response in *Calothrix* sp. to accumulate P and thus increase in abundance. However, continued P loading from the aquaculture operation may have enabled displacement of *Calothrix* sp. by more competitive taxa.

Schlegel *et al.* (1998) have also shown that *Calothrix* sp. has algicidal properties (bioactivity) against green algae. This was not directly evident in the algal assemblage of L375 as chlorophyte abundance increased in 2005 during the *Calothrix* sp. bloom. However, it may be that the chlorophyte response to the aquaculture operation would have been greater had *Calothrix* sp. been absent from the algal assemblage.

The second transient change that occurred in the epilithon of L375 was due to the occurrence of a 2007 July bloom of *Ceratium hirundinella* (O. F. Muller) Schrank (7.2 $mg \cdot cm^{-2}$ or 21% of total biomass; Table 2.8). *Ceratium hirundinella* is a prominent member of the phytoplankton in most temperate freshwater lakes. It is present over a wide range of trophic conditions and is often identified as being responsible for taste and odour problems in drinking water (Wehr and Sheath 2003). *Ceratium hirundinella* presence was never previously recorded in the epilithon of any study lake at ELA but has appeared in the phytoplankton.

Inorganic nutrients, particularly high levels of nitrates and phosphates can trigger blooms of *C. hirundinella*. In addition, light will affect the vertical distribution of *C. hirundinella* (Wehr and Sheath 2003). For example, in culture, *C. hirundinella* preferred high light intensity (Wehr and Sheath 2003), but in nature the compromise between nutrient supply and light drove them deeper in the water column (downward migration up to four meters, to the upper hypolimnion has been noted (James *et al.* 1992)). In the current study, it may be that since there was minimal shading by phytoplankton in July (Findlay *et al.* in press), *C. hirundinella* migrated downward in the water column and proliferated where nutrient concentrations were more concentrated. A second, more likely possibility, was that *C. hirundinella* presence in the epilithon in July was the result of a cell cystation period. The species is known to have a sediment-resting stage, when conditions are favourable the species blooms (Wehr and Sheath 2003). A final, and most likely, possibility is that the presence of *C. hirundinella* in the epilithon in July was the result of increased deposition from a senescing phytoplankton bloom.

Incorporation of cage wastes

Natural epilithic $\delta^{15}N$ and $\delta^{13}C$ levels at ELA (L240) were measured in a previous study by Hecky and Hesslein (1995). They reported $\delta^{15}N$ and $\delta^{13}C$ values of 1.03 ± 0.18 ‰ and -16.93 ± 0.44 ‰, respectively. Given the high variability of $\delta^{15}N$ in the reference lakes (the lake average from 1997-2002 for L373 was 0.12 ± 0.30 ‰ and for L239 was 0.93 ± 1.08 ‰; Fig. 2.24) values could not be distinguished from the L240 samples reported in Hecky and Hesslein (1995). Prior to the operation, L375 on the other hand, had lower $\delta^{15}N$ values (0.28 ± 0.08 ‰; Fig. 2.24) than any of the reference lakes,

including L240. The $\delta^{13}C$ values for all the study lakes (pre-cage; 1997-2002; Fig. 2.25) were similar to those reported by Hecky and Hesslein (1995).

Following commencement of the aquaculture operation the $\delta^{13}C$ and $\delta^{15}N$ of the fish feed and feces were measured in L375 (Kullman *et al.* in prep.). The $\delta^{15}N$ of the feed was 6.69 ± 0.12 ‰ and the feces was 7.18 ‰ (n=1) (Kullman *et al.* in prep.). Incorporation of feed and fecal waste by the epilithon was expected to increase the stable isotope of $\delta^{15}N$. This expected increase was observed in the epilithon in L375 following commencement of the cage operation (Fig. 2.24). However in 2007, after four years of increasing (0.28 to 2.1 ‰) the $\delta^{15}N$ signal decreased (0.38 ‰). This considerable drop was seen in all three of the study lakes (Fig. 2.24). The cause of the drop was unknown. Even after the decrease in 2007, $\delta^{15}N$ values in L375 were still substantially higher then the average of the reference lakes and the pre-cage mean (Fig. 2.26).

The measured $\delta^{13}C$ in L375 fell within the envelope of the reference lakes prior to the aquaculture operation. After a year of operation, the isotopic signal began to increase. In three out of the five years of aquaculture operation (2004, 2006 and 2007), the $\delta^{13}C$ signal was significantly more positive than the pre-cage mean and the average of the reference systems. The $\delta^{13}C$ of the feed and feces in the aquaculture lake was -20.04 ± 0.22 ‰ and -21.47 ‰, respectively (Kullman *et al.* in prep.). Kullman *et al.* (in prep.) found that the C isotope signatures of littoral invertebrates (epilithic algal grazers) did not show a directional shift away or towards (negative or positive) the aquaculture wastes. They concluded that the aquaculture-derived carbon was not significantly contributing to the pool of DIC in the lake. The current study observed a positive shift (heavier; 2004, 2006 and 2007) and a shift towards cage wastes (2005) in the epilithic biofilm in L375

(Fig. 2.25 and 2.26). It is not known whether this shift was aquaculture related. This is because $\delta^{13}C$ fractionation is dependent on both autochthonous and allochthonous sources of dissolved CO_2. Since allochthonous material (terrestrial detritus) is also heavier than epilithon (Hecky and Hesslein 1995), higher concentrations of terrestrial detritus may have caused the observed positive shift in the epilithic biofilms in L375. However, the 2005 negative shift, toward aquaculture wastes may have been aquaculture-related.

In the current study, dissolved nitrogenous waste appeared to be distributed evenly throughout the lake's epilithon (Fig. 2.27). Similarly, Kullman *et al.* (in prep.) found that the nitrogenous aquaculture wastes were incorporated by native organisms throughout the lake. Grey *et al.* (2004) also observed no difference in $\delta^{15}N$ values between cage and reference sites. They suggested that this lack of difference was because fish excretory products would be isotopically lighter than the trout pellets and indistinguishable from natural sources of the nutrient. However, both their reference sites and cage sites were located in the same study lake (Esthwaite water, Cumbria UK). Their explanation is unlikely. Rather, complete mixing and even distribution of dissolved nitrogenous wastes throughout the lake is a more likely reason for the inability to differentiate between reference and cage sites.

Contrary to the N isotope signal, which showed that waste was dispersed evenly throughout the lake, epilithic P and algal biomass showed that both increased with proximity to the aquaculture cage (Fig. 2.27). In comparison, Findlay *et al.* (in press) found no significant relationship between periphyton biomass and distance from cage on the artificial trays outside the cage. However, a biomass estimate for a periphyton tray

inside the aquaculture cage demonstrated values ten times higher than at any of the transect stations.

Algal group composition also differed, albeit only slightly, among distance from cage sites (Fig. 2.28). Cyanobacteria were most abundant closer to the cage, while greens were more abundant at sites furthest from the cage. *Lyngbya* sp. and S*cytonema* sp. dominated the cyanobacterial biomass at the sites close to the cage, while *Bulbochaeta*e sp. and *Mougeotia* sp. dominated green assemblages at sites furthest from the cage (Table 2.11).

Conclusions

The aquaculture operation did not cause any major changes in the large-scale parameters investigated. However, fine-scale, transient changes did appear in the system. Other studies have also shown ephemerality in periphytic communities at fish farm sites (Diaz *et al.* 2002; Mannion and Sara 2007). Such small scale changes should not be ignored and are perhaps a prelude of greater impacts to come had the operation continued.

Compared to other types of cultural eutrophication the immediate impacts observed in the current study were small due to the delayed nature of nutrient release. However, the current study has shown that epilithic biofilms are capable of incorporating cage wastes, which was suggested, but not demonstrated by Diaz *et al*. 2002 and Mannion and Sara 2007. The current study also confirms that the cage operation was capable of causing compositional changes in epilithic algae, which is likely to increase with sustained incorporation of cage wastes. Even these small changes in epilithic

composition provide useful information for evaluation of impacts and suggest that benthic algae can be informative, albeit complicated, biomonitors (chapter 5).

The present study provided a brief depiction of what changes to expect in epilithic characteristics in a system receiving nutrient inputs from an in-lake cage aquaculture operation. Given the limited study period of the cumulative aquaculture impacts, it was difficult to confidently say whether the benthic algal community would have been permanently affected, causing changes to higher trophic levels. The species-level changes suggest that the community was being disturbed. However, since no persistent large changes have occurred in the community, it must be concluded that the aquaculture operation did not permanently affect the benthic algae. Longer running studies are needed to decisively determine whether benthic algae are impacted by the cumulative effects of in-lake aquaculture operations.

Chapter 3: Epilithic Function: Can fish farms alter algal productivity?

Introduction

Presently, insight is limited into how a lake ecosystem will respond to nutrient additions from an intensive aquaculture operation (Yan 2005). In addition, studies of the effects of cultural eutrophication on benthic algal metabolism are uncommon compared to those that measure phytoplankton photosynthesis (Turner *et al.* 1994; Hecky and Hesslein 1995; Vadeboncoeur *et al.* 2001). As a result, when an experimental aquaculture operation was initiated at the Experimental Lakes Area to study the effects on the whole-lake ecosystem, efforts were also made to examine benthic algal community responses. Specifically for this chapter, the aim was to assess whether benthic algal metabolism would be affected by an increase in nutrient loading as a consequence of the experimental aquaculture practices.

Since photosynthesis sustains the majority of life on earth and supports our planet's biodiversity (Larkum 2003), the need to understand where, to what extent and how fluctuations in photosynthetic rates occur is essential to ecosystem studies. In the lake environment, both open-water and attached algal assemblages are photosynthetically active. Most research has focused on rates of phytoplankton productivity even though rates of benthic algal production can be similar or at times even greater than those of phytoplankton (chapter 1; Wetzel 2001b).

Benthic algae also represent a largely neglected carbon pool (Wetzel 2001b). This source of carbon production can be a substantial contributor to whole-lake C-fixation budgets (Vadeboncoeur *et al.* 2001). At times, benthic algae provide a large contribution to the lake when compared with pelagic C-fixation (Vander Zanden and Vadeboncoeur

2002). Top predators in pelagic food webs can also be highly dependent on this form of carbon as their main energy source (Hecky and Hesslein 1995). Neglecting to measure metabolic rates in benthic algae underestimates whole-lake productivity and may also cause misinterpretations in analyses of how a lake responds to a given manipulation, in this case, aquaculture.

A major concern of an in-lake aquaculture operation is the P loading produced from the cage wastes, both feed and excretory, (Beveridge *et al.* 1997a; Beveridge *et al.* 1997b). Given that P has been recognized as the nutrient limiting algal productivity in ELA lakes (Schindler 1974; Schindler 1978), an increase in this limiting nutrient is predicted to increase algal productivity and growth (Schindler 1974; Schindler 1978; Harris 1986). While this may be true for phytoplankton (Schindler 1974; Schindler 1978), the response of benthic algae is more complex (Chapter 1). The littoral zone is often a sink for P (Vadeboncoeur *et al.* 2002). Because of this P can accumulate and be recycled within the epilithon, which can potentially cause a shift to C-limitation in low DIC lakes. Therefore, given inorganic carbon concentrations are sufficient, if P loading from the aquaculture operation increases epilithic P to a point where it elicits a response in the benthic algae, it would demonstrate that the aquaculture operation was having an impact.

In this study, *in situ* epilithic algal metabolism was measured in a lake that contained an experimental aquaculture operation at the Experimental Lakes Area in northwestern, Ontario, Canada. Because the experimental lake represented a nutrient poor system (low in P) prior to the aquaculture operation, it was predicted that an increase in available P from the operation would increase littoral productivity by enhancing the production of the benthic algae. In addition, since L375 had relatively high DIC

concentrations compared to other ELA lakes (Fig. 3.1; Table 2.2), it was predicted that if a benthic algal response was to be detected it would be detected in L375 over other lower DIC ELA lakes.

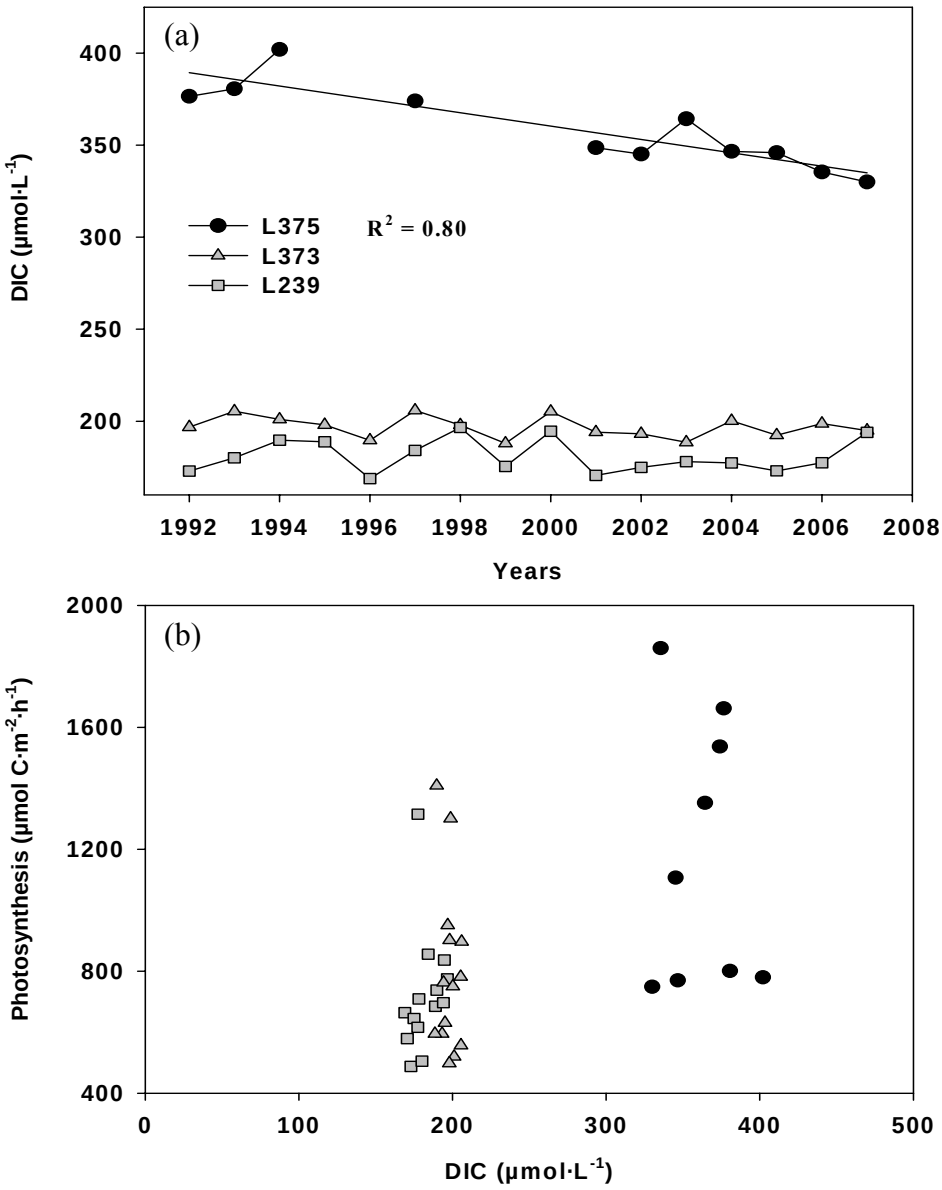

Figure 3.1: (a) Epilimnetic DIC concentrations in L375, L373 and L239 during the open-water season from 1992 to 2007. DIC concentrations appear to be decreasing ($r^2 = 0.80$) in the experimental lake. (b) Rates of gross photosynthesis expressed as a function of DIC concentrations in each lake from 1992 to 2006. Data from the *ELA Data retriever* version 7.1.

Materials and Methods

Description of Study Area

The study took place at the Experimental Lakes Area in northwestern, Ontario, Canada (lat. 49º30'-49º45'N, long. 93º30'-94º00'W) (Fig. 2.1). A full description of the area's geology, vegetation, soils and climate can be found in Brunskill and Schindler (1971). All the lakes in this study are small, moderately deep, oligotrophic, mostly dimictic lakes, which stratify thermally in the summer and winter. The experimental lake, L375 (Fig. 2.2), was a second-order lake and the two reference systems (long-term ELA monitoring lakes: L239 (Fig. 2.4) and L373 (Fig. 2.3)) were both first-order lakes. The two reference systems were studied to understand regional fluctuations in environmental conditions at the ELA during the experiment. For a more detailed description of lake characteristics and chemistry refer to chapter 2, especially Table 2.1.

Farm characteristics

A 10-m^3 aquaculture cage was installed in the north end of the experimental lake in the spring of 2003. The cage was stocked every spring with approximately 10,000 rainbow trout (mean initial weight = 0.1 kg) and harvested every fall (mean final weight = 1 kg) for the five years of operation (2003-2007). The fish were fed a conventional diet (Profishient®) at a rate monitored by a commercial operator (Meeker Aquaculture, Evansville, Ontario). An approximately six to sixteen-fold increase in P loading over pre-cage conditions was experienced in the epiliminion of L375 after the aquaculture operation began (Bristow *et al.* 2008). N inputs, on the other hand, tripled over natural inputs from 2003-2006 (Avezedo *et al.* in prep.). A more thorough description of the nutrient loading rates can be found in Appendix I.

Algal metabolism

In studying algal metabolism three ecologically significant parameters are often of interest: gross (Pgross), net photosynthesis (Pnet) and respiration (R) (Davies *et al.* 2003). Photosynthesis proceeds according to the minimal equation:

$$CO_2 + 2\ H_2O + \text{Light Energy (8 photons)} \rightarrow^{\text{catalysts}} \rightarrow [CH_2O] + O_2 + H_2O$$

Carbon assimilation occurs through a series of steps in which CO_2 is reduced to organic carbon, while utilizing the energy from solar radiation. The major by-product of the process is the evolution of free oxygen (Frank and Loomis 1949; Rothemund 1953; Rabinowitch and Govindjee 1969). Current measurements of photosynthesis are concerned with the rate of the evolved oxygen and/or the rate of consumed CO_2 (Wetzel 1965).

Photosynthesis in algae is governed not only by the response of the organisms to variability in the availability of the substrates (CO_2, light) and build-up of products (O_2, carbohydrate) but also by the variability in the availability of resources that are required to produce the catalysts used in the photosynthetic reactions (Raven and Geider 2003). Of direct importance to this process is photophosphorylation. It is the primary step in converting energy from light-excited electrons into energy (ADP→ATP) for photosynthesis. For this reason, an increase in available P links directly to the photosynthetic process.

Respiration consumes the organic carbon gained in photosynthesis (Raven and Beardall 2003). This light-independent process is essential in algal growth. The conversion of photosynthate (carbohydrate) into cellular components (nucleic acids, proteins, lipids, and structural polysaccharides) occurs mainly in this light-independent

phase. These reactions are also vital to the manufacturing of certain C skeletons, which are essential for growth (Raven and Beardall 2003).

Respiration is known to also occur in the light through photorespiration (Beardall *et al.* 2003). Difficulties, identified by others, in measuring photorespiration *in situ* prevented estimates of these respiratory processes in the current study. Because measures of respiration were based solely on dark respiration (Rd), the current method would likely have underestimated gross photosynthesis by not accounting for any photorespiration (Graham and Turner 1987).

Gross (Pgross) and net photosynthesis (Pnet), respiration (Rd) and the ratio of respiration to gross photosynthesis (Rd: Pgross) are of interest in aquatic studies as they represent the important measures in understanding trophic relations (Wetzel 2001b). Gross photosynthesis is a measure of the amount of carbon fixed during the photosynthetic process, while net photosynthesis represents the actual quantity of organic matter added to the environment (Ryther 1956). Pnet is defined as the difference between Pgross and Rd, and is often used as a surrogate measurement for instantaneous rate of growth (Turner *et al.* 1994). It can also elicit vital information about the internal cellular stress levels that total biomass estimates can not. This is because changes in the nutrient limitation can alter algal growth rates without affecting final biomass (Turner *et al.* 1994). Finally, the measures of dark respiration, in this study, represent the amount of fixed carbon that is respired and assumes that dark respiration approximates respiration in the light (Graham and Turner 1987).

Metabolic chambers

Rates of net photosynthesis and community dark respiration were measured *in situ* by incubating the natural epilithic community in chambers of differing transparency. For a detailed description of the metabolic chambers and method refer to Turner *et al.* 1983. Chambers were constructed as boxes from acrylic of differing transmisivities, and were encircled with neoprene skirts (Fig. 5.1a in Turner *et al.* 1991) (Fig. 3.2). Each side of the acrylic box contained septa that were used for sample collection. The chambers (0.41 L) enclosed approximately 100-cm^2 of rock surface and a lead-weighted 'sock' was placed on the neoprene skirt to keep the chamber firmly in place (Turner *et al.*1983).

Figure 3.2: Metabolic chambers used in epilithic sampling from 1992 to 2007. From front to back: two dark chambers, 11% transmittance, 40% and 100% transmittance chambers. In 2007, only duplicate dark and 100% chambers were used at each site. Photo by M. Turner 2006.

Sample approach

In the time series data from M. Turner, four to five chambers were monitored for each of three sites per run within each lake. This approach was maintained in 2006, however, in 2007 the number of sites was increased to six to increase understanding of inter-site variability. In addition, maximum photosynthetic rates were of more interest than rates at the intermediate light intensities; therefore, the number of 100% chambers was increased to two per site. In order to continue single-day runs, the intermediate irradiated chambers (10 and 40% chambers) were eliminated. Generally, three sites were measured in the morning and three sites in the afternoon during full sunlight hours (occasionally only three sites could be monitored per day due to weather conditions). Since the irradiance curve was not used as a check for reaching Pmax (i.e., mean net photosynthesis at 100% irradiance), sunny sampling dates were chosen to ensure the communities were receiving full light. Occasionally this objective was not achieved because of persistent cloud cover. Turner *et al.* 1983 showed that light saturation was reached at approximately 200 $\mu E \cdot m^{-2} \cdot S^{-1}$ using carbon data. In both 2006 (~650 $\mu E \cdot m^{-2} \cdot S^{-1}$ in all lakes) and 2007 (~600 $\mu E \cdot m^{-2} \cdot S^{-1}$, ~250 $\mu E \cdot m^{-2} \cdot S^{-1}$ in the reference lakes and L375, respectively) irradiance values were higher than 200 $\mu E \cdot m^{-2} \cdot S^{-1}$, confirming that Pmax should have been reached.

Beginning in 2006, the method of measuring photosynthesis and respiration changed from *infrared gas analysis* (IRGA) to a *gas chromatographic* (GC) approach. In the IRGA approach 1.0 to 2.5-mL samples were injected into the column and carried to the analyzer using CO_2-free nitrogen gas. The peak heights were calibrated against external gas standards and then DIC concentrations were calculated. For a detailed

explanation of the IRGA technique and sampling strategy, refer to Turner *et al.* (1987; 1991). The advantages of the new GC technique over the previously used IRGA were: (1) the ability to measure both O_2 and DIC simultaneously, rather than only DIC and (2) an increase in the shelf life of samples (up to eight weeks compared to same day sample processing). With the decrease in time required to process samples in the GC approach, time and energy could be reallocated to an increase in the number of sampling events and to better understanding epilithic inter-site variability. The latter also proved to be a draw-back of the technique. With IRGA immediate sample processing was required, which provided immediate feedback on sample integrity. If re-sampling was required it could occur rapidly. The delayed sample processing in the GC-technique meant that re-sampling could not occur in a timely fashion, which meant a greater number of omitted samples (25% of samples; refer to *methodological recommendations* section in the discussion).

Vial preparation for gas chromatography sampling

Each 12-mL vial used in sampling was washed, oven dried and acidified with 10-µL concentrated phosphoric acid (85% H_3PO_4) before being sealed with a rubber septum. Each vial was then evacuated and filled three times (each cycle taking approximately 2 min) with helium (He) in 2006 and a He mixture containing 1% neon, and 1% acetylene in 2007. To avoid contamination vials were over-pressurized (6 psi) with the above inert gases and sent to ELA in this state. The vials had been acidified to immediately kill the sample and to ensure that all the DIC in the water phase of the sample would be converted to gaseous carbon dioxide (CO_2) for analysis by the GC. The over-pressurized vials were adjusted to atmospheric pressure by piercing the septum

briefly with a 26-gauge needle on the morning of sampling. They were then consecutively ordered in a shotgun belt for ease of handling and organization while underwater (Fig. 3.3 a).

In situ measurements of photosynthesis and respiration

Three sample sites were selected in the middle littoral zone of each study lake that corresponded to the particulate sampling sites (Fig. 2.2-2.4). Areas of low slope (<10º, assessed visually) were chosen to prevent chamber movement, on bedrock shelves or large boulders. These three sites, located on the north shoreline to avoid shading, were the same from 1992 to 2007. In 2007 three additional sites were included on the northern shore, usually in the same general region as the previous sites.

Prior to the placement of the chambers, four 4-mL samples of water just overlying the biofilm (Fig. 3.3b) were taken for measurement of initial concentrations of DIC and O_2. The collection syringe was first filled and then flushed with lake water before sampling to ensure that all air bubbles were eliminated from the syringe. The syringe was then carefully swept over the biofilm to collect the overlying water (Fig. 3.3 b). All samples were collected with the same modified syringe that had a stop in place to limited filling to 4-mL. After each sample collection, the needle of the syringe was inserted into the 12-mL acidified vial. The sample in the syringe was ejected into the vial; the syringe was rinsed with vial gas at least three times; finally the vial and syringe were then inverted and the needle removed from the vial. The vial and syringe were inverted prior to removing the syringe needle to prevent gas from escaping.

A SCUBA diver then carefully placed the chambers upon the rock surface, minimizing disturbance of the epilithon (Fig. 3.3 c). The lead socks were placed over the

neoprene skirts to seal the chambers to the rock surface (Fig. 3.2). Start time and chamber depth were recorded for each chamber.

Following an approximately 90-minute (maximum range of 60-150 min) incubation time, final water samples were collected from the chambers (Fig. 3.4 b and 3.4c). To mix the water within the chambers prior to sampling, two 10-mL syringes were inserted through the septa on opposite sides of the chamber; approximately 5-mL of water from within the chamber was drawn into each syringe (Fig. 3.4 a). These two 5-mL aliquots of water were ejected while rotating the syringe tip to mix the waterphase within the chambers. After approximately 5 min when all chambers were mixed and any suspended material had settled, triplicate 4-mL samples were removed from each chamber (Fig. 3.4 b and c). The syringe's needle was inserted into each chamber through a septum (Fig. 3.4 c). The needle was removed from the chamber septum and the collected sample was injected into a 12-mL pre-evacuated acidified vial, as for the initials (Fig. 3.4 d). The filled vials were stored under pressure and shipped to the Freshwater Institute laboratory. Samples were left pressurized to prevent sample contamination; any small leakage that may have occurred was expected to exit the vial.

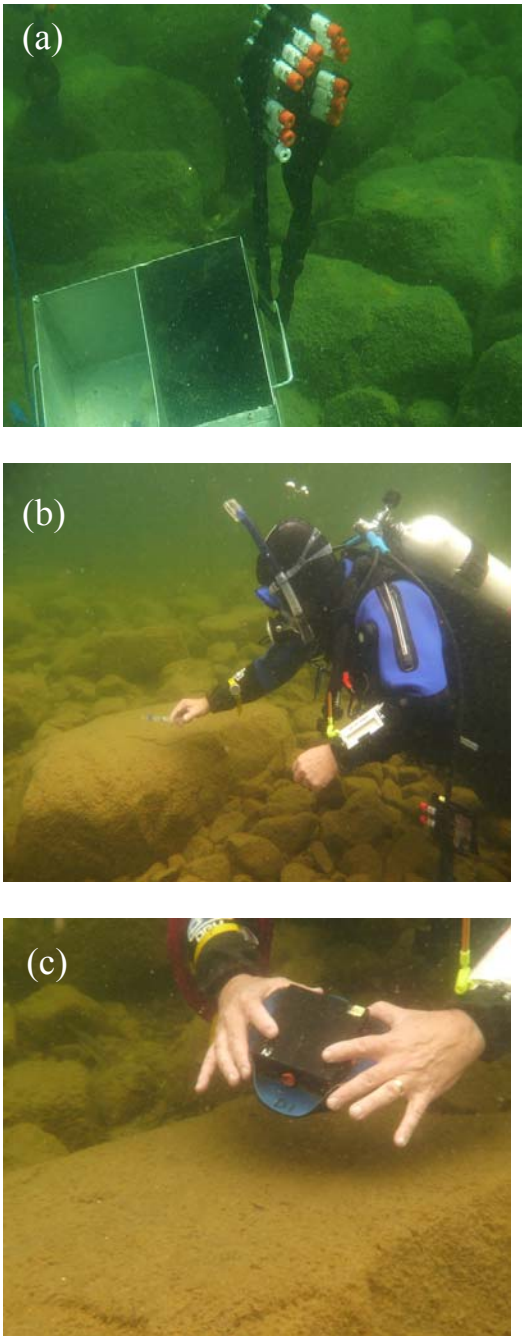

Figure 3.3: (a) vials transported to epilithic sampling sites in shotgun belts, (b) collection of initial DIC and O_2 water samples overlying undisturbed epilithon, (c) placement of dark incubation chamber onto rock surface, Photos by J. Webb 2007.

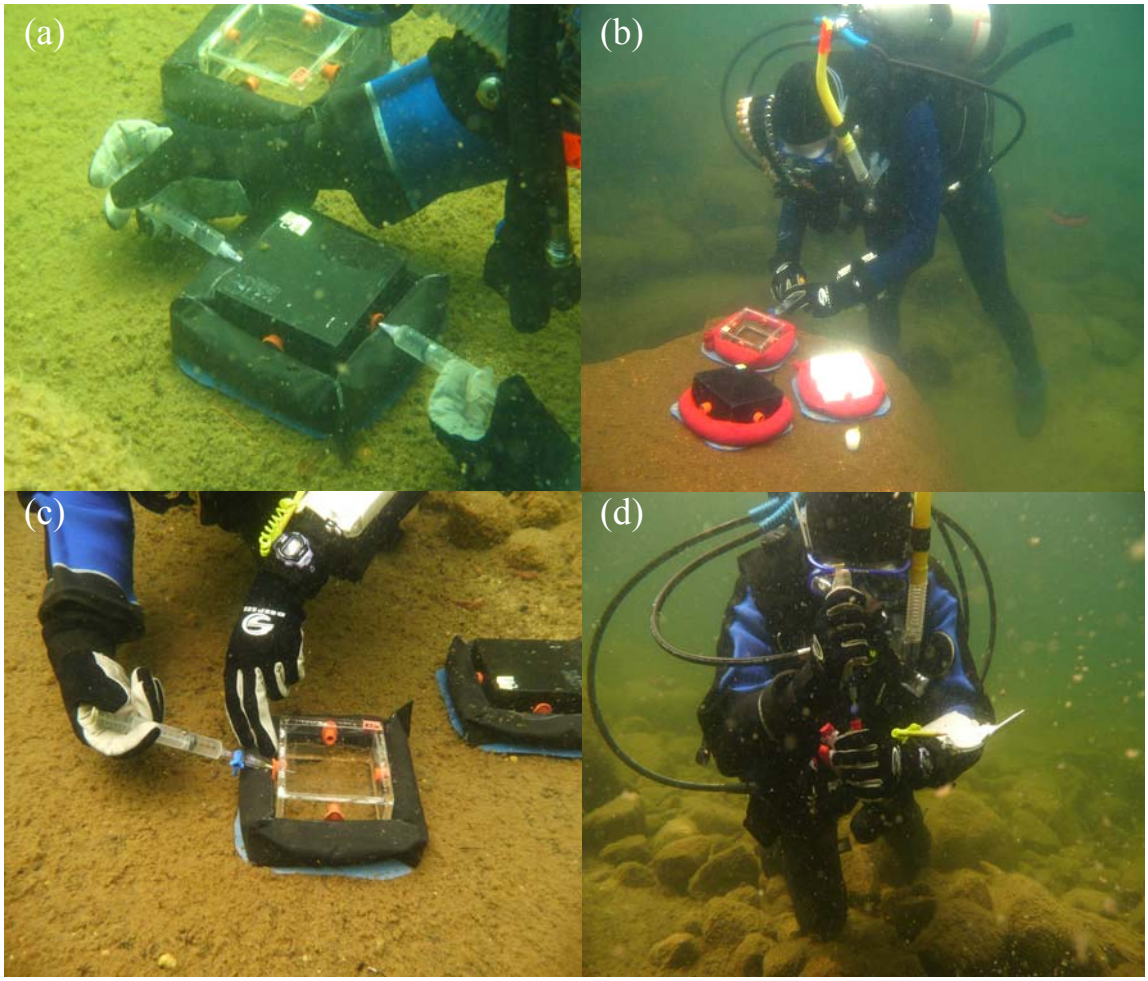

Figure 3.4: (a) Chamber mixing following 90-min incubation, (b) sample collection, (c) sample collection; syringe inserted into septum of 100% light transmittance chamber, (d) transferring the sample from collection syringe to 12-ml sample vials. Photos by M. Turner 2007.

Calculation of rates

Photosynthetic rates were determined using the rate of evolved oxygen and consumed DIC in the water overlying the benthic biofilm ($\mu mol \cdot L^{-1}$). Rates of consumed DIC were measured using the mean of the initial (i) samples (DIC_i) and subtracting the final (f) samples (DIC_f) (EQ 1). For the rate of evolved oxygen, the initial values (O_{2i}) were subtracted from the finals (O_{2f}) (EQ 2). These equations provided an estimate of community net photosynthesis for a known area (A, 0.0096-m^2), volume (V, 0.41 L) and measured incubation time (t, ~1.5 h):

$$Pnet\ (\mu mol \cdot C \cdot m^{-2} \cdot h^{-1}) = ([DIC]_i - [DIC]_f) \times V / (A \times t) \quad (1)$$

$$Pnet\ (\mu mol \cdot O_2 \cdot m^{-2} \cdot h^{-1}) = ([O_2]_f - [O_2]_i) \times V / (A \times t) \quad (2)$$

The two dark incubation chambers estimated community respiration in the dark (Rd) for a known area, volume and measured incubation time. For DIC and O_2, equations (1) and (2) respectively, the expectation was that the equation would yield a negative value for dark respiration. In the event of a positive value, it was set to zero on the assumption that this occurred due to analytical error.

Measures of gross photosynthesis (Pgross) were estimated using the following equation:

$$Pgross = Pnet - Rd \quad (3)$$

where Rd represents the corrected value for dark respiration.

Gas Analysis

Samples were sequentially ordered on a Gilson autosampler and injected into two chromatographic columns: one column for separating CO_2 and methane (CH_4), and a second for separating N, O_2 and argon (Ar). Samples were transported through the

columns using a He carrier gas. The GC was calibrated using gas standards. Samples were analyzed and peaks calculated using the *Varian Star Workstation Advanced Application GC Software*, Version 6. The concentrations in the 4-mL samples were then calculated by applying water solubility calculations for each of the gases (Sarmiento and Gruber 2006).

In 2006, the O_2: Ar ratio was used to calculate O_2 concentrations, based on a constant amount of Ar in the vials. In 2007, absolute concentrations for O_2 and CO_2 (2006 and 2007) were calculated. Since it was assumed that all gas in the vials was originally in the liquid phase, absolute concentrations were based on calculation of the liquid concentration. Each 12-mL sample vial contained an 8-mL headspace ($V_{headspace}$) and 4-mL water sample (V_{liquid}). The headspace and the water in the vial were assumed to be in equilibrium. The GC measured gas concentrations in the headspace ([headspace]) of the vials, the analysis temperature was known (GC temperature), and the liquid concentration ([liquid]) at that temperature was calculated based on the gas solubility for each of the gases. The final gas concentration ($[Gas_{final}]$) in the liquid phase was calculated using the following equation:

$$[Gas_{final}] = \frac{([headspace]/V_{headspace}) + ([liquid]/V_{liquid})}{V_{liquid}}$$

Establishing outliers (2006)

Sample contamination by air was of greatest concern in epilithic sampling. Air contamination has little effect on the measurement of CO_2 because air contains only about 0.04% carbon dioxide. However, O_2 samples can be severely compromised by air contamination. Typical water samples (roughly in equilibrium with the atmosphere)

contain approximately 300 μmol·L^{-1} oxygen; air contains 20.5% oxygen (Stanitski *et al.* 2003). Based on this solubility calculation, contamination with 100μL air could result in contamination of about 75% of the expected oxygen signal.

Since air is mostly comprised of nitrogen (78%; Stanitski *et al.* 2003), the Ar: N_2 ratio was used to evaluate for the presence of air contamination. Based on the amount of Ar added, the expected Ar: N_2 ratio in water is 0.220. For air, the Ar: N_2 value is approximately 0.0128 (Stanitski *et al.* 2003). Hence, sample values less than 0.220 indicate potential air contamination. The oxygen data obtained from this strategy showed a precision of < 1% (percent coefficients of variation (% CV) ranging from 0.6-1.16% (number of vials (n) in analysis = 18) Table 3.1).

The carbon data could not be inspected in a similar way[1]. If a vial showed potential air contamination it was completely removed from analyses of fluxes for both gases. This strategy did not remove all outlier vials from the DIC data. Precision of the DIC data was > 2% (% CV ranging from 1.9-3.5% (n=18)). Therefore, following removal of vials suspected of air contamination, vials were visually examined for outliers based on a statistical evaluation (± 2.5 standard deviations of the mean) for that chamber (a strategy used in previous years for outlier trapping, which yielded coefficients of variation of ~ 2% (n = 20) (Turner *et al.* 1983)). Using this strategy, in combination with the Ar: N_2 ratio, the percent coefficient of variation dropped below 2% (% CV = 1.4-2.1% (n = 18) Table 3.1).

[1]The GC was optimized for oxygen rather than carbon analysis in 2006. In 2007, the GC was optimized for both analyses.

Establishing outliers (2007)

In 2007, Ne was added as a tracer gas during the He filling of the sample vials, which allowed verification of both the DIC and O_2 data as well as verification of GC efficiency and sample contamination. Neon is a very insoluble gas therefore, the Ne to He ratio in the vial is nearly unaffected by sample volume. That is, the GC always sees the same amount of Ne unless the sample has been contaminated by another insoluble gas i.e. air or the pressure in the vial has dropped due to leakage, preventing the GC column from being entirely filled. These Ne concentrations can therefore be used as a check for contaminated sample vials

Prior to checking the Ne values of a sample the samples were first evaluated based on a statistical approach (± 3.0 standard deviations of the mean (n= 6)). In 2007, vials from both chambers at each site were combined to yield a single site value (i.e. for the lights, all six vials were averaged rather three vials per lightchamber). For this reason the allowable variance within the chamber was increased from ± 2.5 to ± 3.0 standard deviations (an increase in allowable observations around the mean from 99.0 to 99.7%). If there was a vial that appeared outside of the ± 3.0 standard deviations of the mean, the Ne concentrations were examined.

Most vials (~90%) had Ne values above $2.8x10^6$ μvolt·s; those below this value were removed as contaminated vials (~10% of samples). This limit was determined based on the frequency distribution of Ne, which showed that the greatest number of vials occurred above $2.8x10^6$ μvolt·s (Fig. 3.5 a). If a vial appeared to have a Ne value above the accepted value, but still remained outside the ± 3.0 standard deviations of the mean, the air and nitrogen saturation (Nsat) values (both essentially used to check for air

contamination) were examined. Raw GC peaks were used to examine air values (since >90% of air components are in the headspace of the vials). Air values greater than $3.9x10^6$ μvolt·s were flagged as potentially contaminated vials, as well as values less than $3.3x10^6$ μvolt·s caused by inexplicable vial issues (together ~26% of samples) (Fig. 3.5 b). The Nsat value was based on the expected nitrogen concentrations relative to observed concentrations based on field temperatures. Observed values well above the expected value indicated air contamination, while values below the expected level indicated inexplicable vial issues. Therefore Nsat less than 0.90 and greater than 1.06 were flagged as vial outliers (~40% of samples; Fig. 3.5 c), if Nsat outliers corresponded with air outliers the vial was removed (~13% of samples).

The same procedure was used for both oxygen and carbon data. Even though the effect of air contamination on carbon was minimal, air contamination was a sign of a compromised sample. No risk was taken: if a vial proved to be an outlier based on either the statistical evaluation and/or the technical system, it was omitted. This resulted in less variable data for both CO_2 and O_2. The coefficients of variation prior to removal for CO_2 and O_2 were 6.4 ± 1.3% (n = 9) and 3.7 ± 0.7% (n = 9) respectively. Following removal, coefficients of variation for CO_2 and O_2 were 2.2 ± 0.8% (n = 9) and 1.4 ± 0.7% (n = 9), respectively.

Increased confidence in the error trapping techniques for the DIC data in 2007 provided data for which a photosynthetic quotient could be determined, for conversion of O_2 to CO_2 data. Vial precision in the oxygen data, for 2006 and 2007 respectively 0.8% and 1.4% was superior to the carbon data (1.8% and 2.2%; Table 3.2). This placed higher confidence in the use of oxygen fluxes in determining photosynthesis and respiration. For

this reason, both the 2006 and 2007 O_2 data were converted to provide an estimate of DIC flux for both years in the time series.

Table 3.1: Precision (% C.V.) of the technical approach to removing vial outliers in 2006 and 2007, as described in the methods. Vial outliers determined by the statistical evaluation were removed prior to calculations of precision using the technical approach. Precision in the table therefore represents the measure of change using the technical approach and overestimates variability at each site.

Lake	Date	Site	Outlier Trapping Technique	Frequency of vial outliers (%)	Precision (% CV)			
					Prior to outlier trapping		Following outlier trapping	
					CO_2	O_2	CO_2	O_2
L375	Jul-06	ABC	Ar: N_2 ratio	6.4	1.70	0.79	1.43	0.69
	Aug-06	ABC			1.86	1.09	1.52	0.85
	Jul-07	ABC	Neon	0.69	20.2	18.7	4.49	3.04
		DEF			-	-	-	-
	Aug-07	ABC			-	-	-	-
	Jul-07	ABC	Nsat/ Air[a]	14	4.49	3.04	2.17	2.24
		DEF			5.62	2.57	2.99	1.77
	Aug-07	ABC			5.58	3.10	1.86	1.72
L373	Jul-06	ABC	Ar: N_2 ratio	12	1.76	0.83	1.42	0.83
	Aug-06	ABC			13.5	1.71	2.05	0.61
	Jul-07	ABC	Neon	17	5.63	4.09	5.80	4.09
		DEF			6.88	4.92	6.65	4.86
	Aug-07	ABC			7.32	2.94	4.22	2.07
	Jul-07	ABC	Nsat/ Air[a]	11	5.80	4.09	2.83	2.16
		DEF			6.65	4.86	4.13	3.60
	Aug-07	ABC			4.22	2.07	2.43	1.51
L239	Jul-06	ABC	Ar: N_2 ratio	9	2.15	1.73	1.74	0.77
	Aug-06	ABC			2.68	1.21	1.59	1.16

Jul-07	ABC	Neon	13	-	-	-	-
	DEF			6.53	4.67	5.68	4.19
Aug-07	ABC			6.64	3.73	4.96	2.94
Jul-07	ABC	Nsat/ Air[a]	13	4.79	2.94	3.10	2.13
	DEF			5.68	4.19	3.06	2.59
Aug-07	ABC			4.96	2.94	2.60	2.12

[a] The Nsat/ Air precision was based on values following removal outliers determined by the Neon criteria.

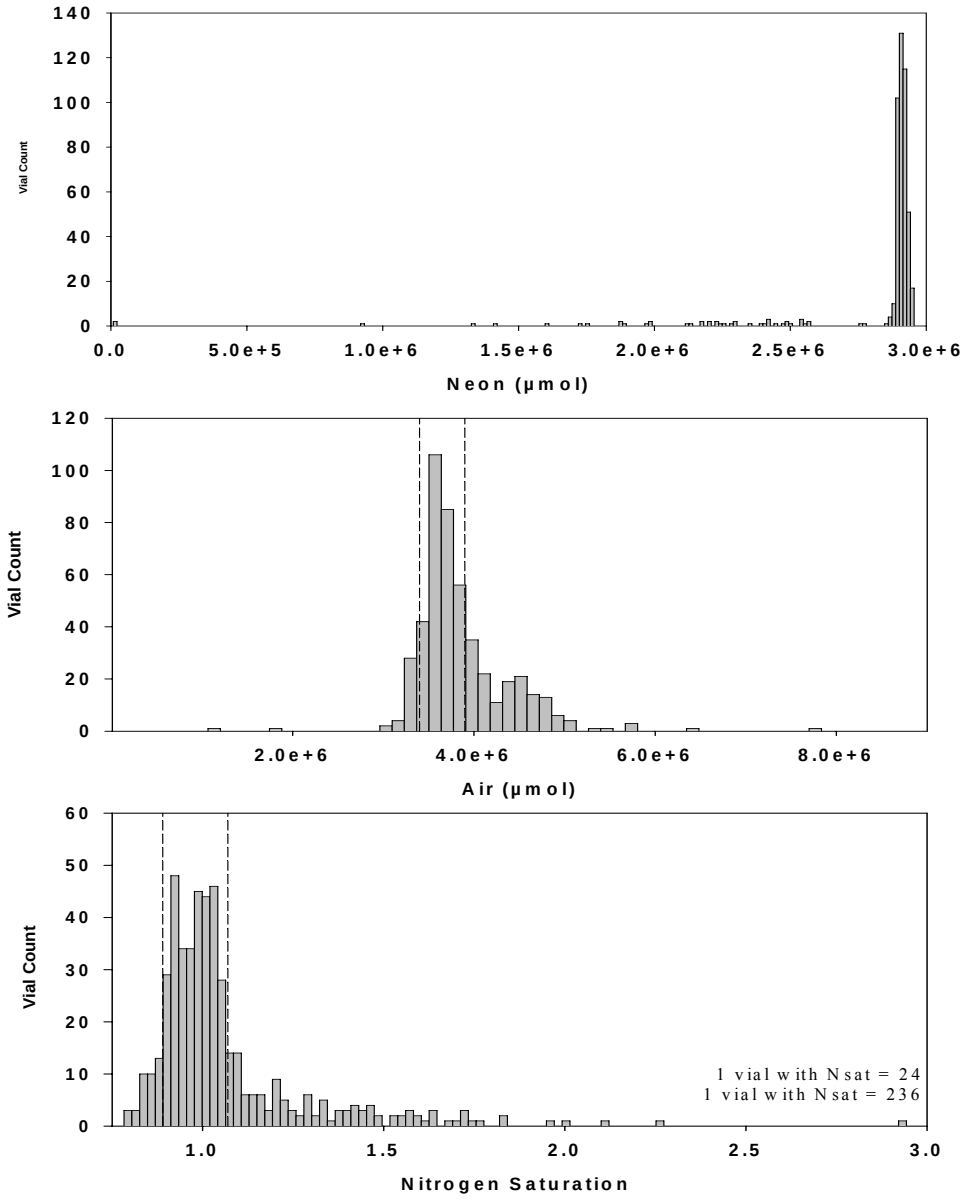

Figure 3.5: (a) Frequency distribution of neon (µvolt·s), (b) air (µvolt·s) and (c) Nitrogen saturation (Nsat) in the 2007 gas chromatography vials. The more frequent vial counts were used in analyses. Values of Ne less than $2.8e^{+6}$ µvolt·s, Air < $3.3e^{+6}$ and >$3.9e^{+6}$ µvolt·s and Nsat <0.90 and >1.06 (limits indicated by dashed vertical lines) were not used in analyses because of sample contamination and were removed as outliers.

Importance of vial precision

It was important for the analysis of metabolic rates for vial precision to be high and percent coefficients of variation (% CV) to be low. High coefficients of variation in the concentrations (vials) (Appendix III, Table III.1-III.2) translate into uncertainty with the calculated rates (the value of interest in the current study) (Appendix III, Table III.3-III.4). If analytical variability propagates in this way through the calculations of metabolic rates it can cause misinterpretations of the algal ecology in the system and preclude detection of experimental effects (Table 3.2 and 3.3). For example; a sample with a mean concentration of 100 $\mu mol \cdot L^{-1}$ (n =5), standard deviation = 1.0, % CV = 1.0% and a rate of 10, has a standard deviation of the rate equal to 1.4 and a % CV = 14%.

Mean sample concentration	100 $\mu mol \cdot L^{-1}$			
Sample size	n = 5			
Standard deviation (SD)	1.0	2.0	3.0	5.0
% CV_{sample} = (SD/ mean) x 100%	1.0%	2.0%	3.0%	5.0%
Difference between initial and final	10 $\mu mol \cdot L^{-1}$			
$SD_{rate} = \sqrt{(SD_{initial}^2 + SD_{final}^2)}$	1.4	2.8	4.2	7.1
% CV_{rate} = (SD_{rate} / rate) x 100%	**14%**	**28%**	**42%**	**71%**

Variation in the concentration causes a much greater percent coefficient of variation in the rate. Also, if the difference between the initial and final concentrations increases, the percent coefficient of variation of the rate will decrease. For example; using the same scenario but decreasing the difference between initial and final concentrations to 5 $\mu mol \cdot L^{-1}$, and increasing the difference to 20 $\mu mol \cdot L^{-1}$ gives the following % CVs:

Difference between initial and final = 5.0 $\mu mol \cdot L^{-1}$				
Standard deviation (SD)	1.0	2.0	3.0	5.0
$SD_{rate} = \sqrt{(SD_{initial}^2 + SD_{final}^2)}$	1.4	2.8	4.2	7.1
% CV_{rate} = (SD_{rate} / rate) x 100%	**28%**	**57%**	**85%**	**141%**

Difference between initial and final = 20.0 $\mu mol \cdot L^{-1}$				
Standard deviation (SD)	1.0	2.0	3.0	5.0
$SD_{rate} = \sqrt{(SD_{initial}^2 + SD_{final}^2)}$	1.4	2.8	4.2	7.1
% CV_{rate} = (SD_{rate} / rate) x 100%	**7%**	**14%**	**21%**	**35%**

Table 3.2: Variation in inter-site estimates of Pnet (100% light transmittence), Rd (0% light transmittence) and Pgross for the oxygen data ($\mu mol \cdot O_2 \cdot cm^{-2} \cdot h^{-1}$). The inter-site variation was based on calculations of the rate-level variation and vial/chamber-level variation as described in the methods and presented in Table III.1 and III.3 in Appendix III.

Lake	Date		100%			0%			Pgross		
		n	Mean	SD	% CV	Mean	SD	% CV	Mean	SD	% CV
L375	Jul-06	3	1759	257	14.6	-188	84	44.7	1799	513	28.5
	Aug-06	3	1302	244	18.7	-39	233	59.7	1527	346	22.7
	Jul-07	6	487	203	41.7	-341	222	65.1	828	165	19.9
	Aug-07	3	846	71	8.39	85	56	65.9	846	71	8.39
L373	Jul-06	3	1676	432	25.8	-226	61	27.0	1902	494	25.9
	Aug-06	3	1260	236	18.7	-128	88	68.8	1388	230	16.6
	Jul-07	6	588	456	77.6	-290	369	127	888	412	46.4
	Aug-07	3	533	236	44.3	-84	4	4.76	617	239	38.7
L239	Jul-06	3	1189	122	10.3	-335	57	17.0	1524	97.0	6.36
	Aug-06	3	1086	99	9.12	-126	162	129	1234	267	21.6

Jul-07	6	693	250	36.1	-6	256	4270	771	221	28.7
Aug-07	2	216	118	54.6	-101	126	125	317	8.50	2.68

Table 3.3: Variation in inter-site estimates of Pnet (100% light transmittence), Rd (0% light transmittence) and Pgross for the carbon data ($\mu mol \cdot CO_2 \cdot cm^{-2} \cdot h^{-1}$). The inter-site variation was based on calculations of the rate-level variation and vial/chamber-level variation as described in the methods and presented in Table III.2 and III.4 in Appendix III.

Lake	Date		100%			0%			Pgross		
		n	Mean	SD	% CV	Mean	SD	% CV	Mean	SD	% CV
L375	Jul-06	3	1743	606	34.8	918	1217	133	1855	725	39.1
	Aug-06	3	826	97	11.7	-538	134	24.9	1363	219	16.0
	Jul-07	6	697	267	38.3	-226	310	13.7	948	300	31.6
	Aug-07	3	480	99	20.6	-252	78	30.9	733	71.0	9.72
L373	Jul-06	3	1133	812	72.9	613	557	90.9	1190	921	77.4
	Aug-06	3	583	168	28.8	-241	379	157	840	177	21.1
	Jul-07	6	429	674	151	-360	402	112	1015	514	50.6
	Aug-07	3	376	86	22.9	-115	103	89.6	491	45.0	9.08
L239	Jul-06	3	670	167	24.9	-367	98	26.7	1037	103	9.93
	Aug-06	3	548	157	28.6	-146	221	151	725	368	50.1

Jul-07	6	521	279	55.6	-105	111	106	752	108	14.4
Aug-07	2	327	48	14.7	-89	33	37.1	416	16.0	4.00

Determination of the photosynthetic quotient

The values for the photosynthetic quotient ($PQ = O_2 / CO_2$) were based on estimates of gross photosynthesis for 2007 (Fig. 3.17, 3.19 and 3.21). PQs were determined for each lake and date combination (Table 3.5). A PQ was determined first for each site within a lake/date combination. A mean and standard deviation were determined for each date. The lake mean was then determined by averaging all sites for both months. The lake mean was used in the conversion of the O_2 to CO_2 data for each corresponding lake.

Light measurements

To determine the mean irradiance (I_c) in $\mu Em^{-2}s^{-1}$ received by the chamber during sampling, surface irradiance (I_o) was monitored continuously throughout each run using a LiCor-1000 data logger connected to a Li-192S Underwater Quantum Sensor near the lake surface. The output was integrated over 10-minute intervals. The extinction coefficient (ε) for each chamber was determined using light-attenuation profiles measured in the euphotic zone of each lake, measured usually within one week of the experimental run (*ELA Data Retriever* version 7.1). Grouping these data with chamber depth (z) and diffuser transmittance (D), the mean irradiance within each chamber was calculated for the experimental period using the following equation:

$I_c = D \times I_z = D \times I_o \times e^{-\varepsilon z}$.

Data analysis

An analysis of covariance was used to model the data to determine whether the aquaculture operation affected productivity in the experimental lake. All parameters were examined, Pgross, Pnet, Rd and Rd: Pgross. Most often it was necessary that the

dependent variables be natural log-transformed to normalize the data and homogenize the error variance. Yearly data from the two reference lakes were averaged and treated as a covariate. Year was modeled as a continuous covariate to look for temporal trends. Also, an additional pre vs. post-treatment effect was modeled as a categorical predictor variable. The "pre-post" variable was also allowed to interact with the average values in the reference lakes and with year to see if either the temporal trends in the experimental lake or patterns in the reference lakes were different during the cage years versus pre-cage years. Analyses were conducted using SAS 2008 PROC Mixed. In all cases, a p-value between $0.10 \geq p \geq 0.01$ was considered significant and $p < 0.01$was deemed highly significant (chapter 2).

Results

Effects of aquaculture on algal metabolism

The aquaculture operation did not statistically affect algal metabolism in any of the measured parameters (Table 3.4): Pgross (Fig. 3.6), Pnet (Fig. 3.7), Rd (Fig. 3.8) or the ratio of Rd to Pgross (Fig. 3.9). All parameters remained within the range of natural variability of the reference lakes and, for the most part, of the long-term mean. Throughout the time series Pgross and Pnet were higher in L375 than the reference systems (Fig. 3.6 a; 3.7 a), but neither has shown any major change from the pre-cage mean and remain within the range of natural variability (Fig. 3.6 b; 3.7 b). Likewise, respiration in L375 remained within the range of variation of the reference lakes and the pre-cage mean (Fig. 3.8; Table 3.4). Finally, there has not been a major shift in the ratio of Rd: Pg since the commencement of the aquaculture operation (Fig. 3.9; Table 3.4).

Table 3.4: Results of a mixed procedure analysis of covariance of potential cage effects in the experimental lake on gross (Pgross) and net (Pnet) photosynthesis, respiration (Rd) and the ratio of Rd: Pgross, comparing: (1) the epilithon of L375 to the average of the reference systems, (2) pre-cage and cage years within L375, (3) their possible interaction, (4) yearly changes within L375, and (5) whether yearly changes interacted with pre-cage and cage years in L375. Significant values are distinguished from non-significant values by an asterisk.

	Effect	df	F-value	P-value
Gross Photosynthesis (Pgross)	Reference lake Pgross	1,3	4.36	0.09*
	Pre-cage vs cage years in the experimental lake	1,3	0.14	0.85
	Interaction between reference lake Pgross and pre vs cage years	1,3	0.00	0.59
	Year	1,3	0.16	0.84
	Interaction between year and pre vs cage years	1,3	0.14	0.86
Net Photosynthesis (Pnet)	Reference lake Pnet	1,3	4.94	0.11
	Pre-cage vs cage years in the experimental lake	1,3	0.11	0.76
	Interaction between reference lake Pnet and pre vs cage years	1,3	0.25	0.65
	Year	1,3	0.19	0.70
	Interaction between year and pre vs cage years	1,3	0.12	0.75

Respiration (Rd)	Reference lake Rd	1,3	0.40	0.57
	Pre-cage vs cage years in the experimental lake	1,3	0.96	0.40
	Interaction between reference lake Rd and pre vs cage years	1,3	0.31	0.62
	Year	1,3	0.53	0.52
	Interaction between year and pre vs cage years	1,3	0.96	0.40
Ratio of Rd: Pgross	Reference lake ratio of Rd: Pgross	1,3	0.01	0.92
	Pre-cage vs cage years in the experimental lake	1,3	0.26	0.65
	Interaction between reference lake Rd: Pgross and pre vs cage years	1,3	0.04	0.86
	Year	1,3	0.54	0.52
	Interaction between year and pre vs cage years	1,3	0.26	0.65

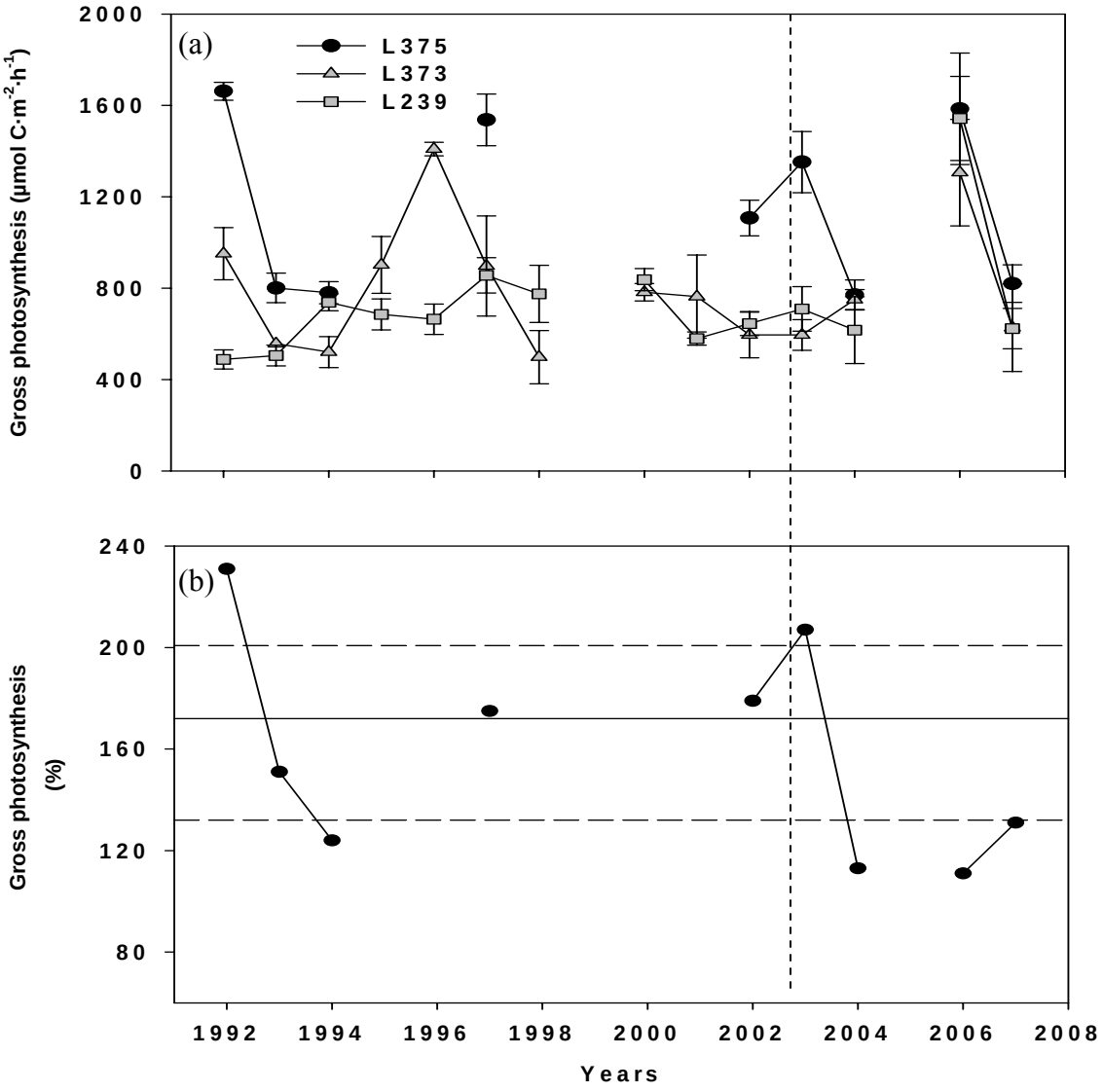

Figure 3.6: (a) Gross photosynthesis in the experimental lake (L375) and reference lakes (L239 and L373) from 1992-2007. The 2006 and 2007 data are estimates based on O_2 fluxes converted to CO_2 fluxes. The vertical dashed line distinguishes between cage and pre-cage years. (b) Gross photosynthesis in L375 is represented as a percent of the mean of the experimental lakes, the solid horizontal line represents the pre-cage mean and the dashed horizontal lines represent the standard deviation around the mean.

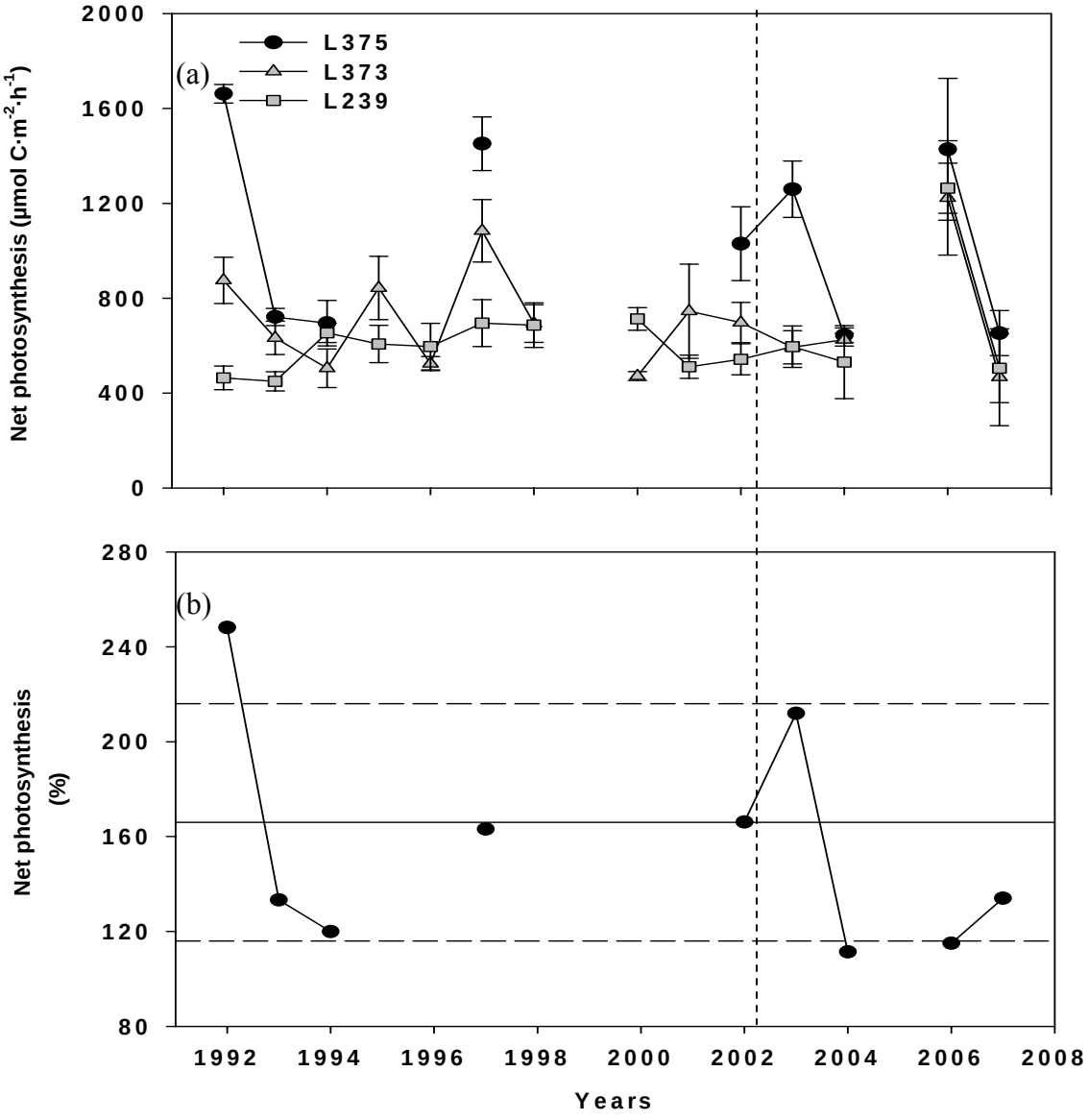

Figure 3.7: (a) Net photosynthesis in the experimental lake (L375) and reference lakes (L239 and L373) 1992-2007. The 2006 and 2007 data are estimates based on O_2 fluxes converted to CO_2 fluxes. The vertical dashed line distinguishes between cage and pre-cage years. (b) Net photosynthesis in L375 is represented as a percent of the mean of the experimental lakes, the solid horizontal line represents the pre-cage mean and the dashed horizontal lines represent the standard deviation around the mean.

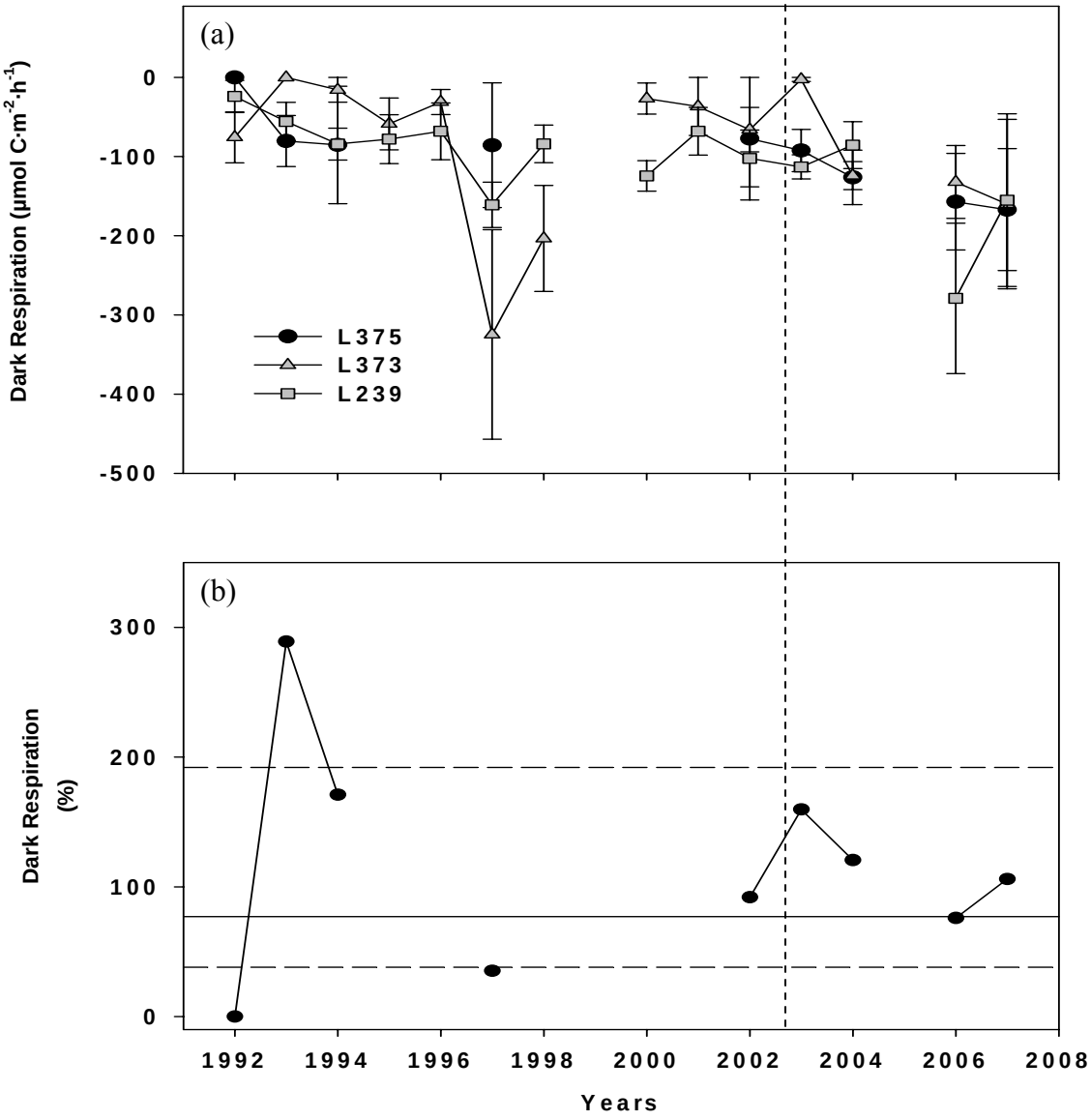

Figure 3.8: (a) Dark respiration in the experimental lake (L375) and reference lakes (L239 and L373) 1992-2007. The 2006 and 2007 data are estimates based on O_2 fluxes converted to CO_2 fluxes. The vertical dashed line distinguishes between cage and pre-cage years. (b) Dark Respiration in L375 is represented as a percent of the mean of the experimental lakes, the solid horizontal line represents the pre-cage mean and the dashed horizontal lines represent the standard deviation around the mean.

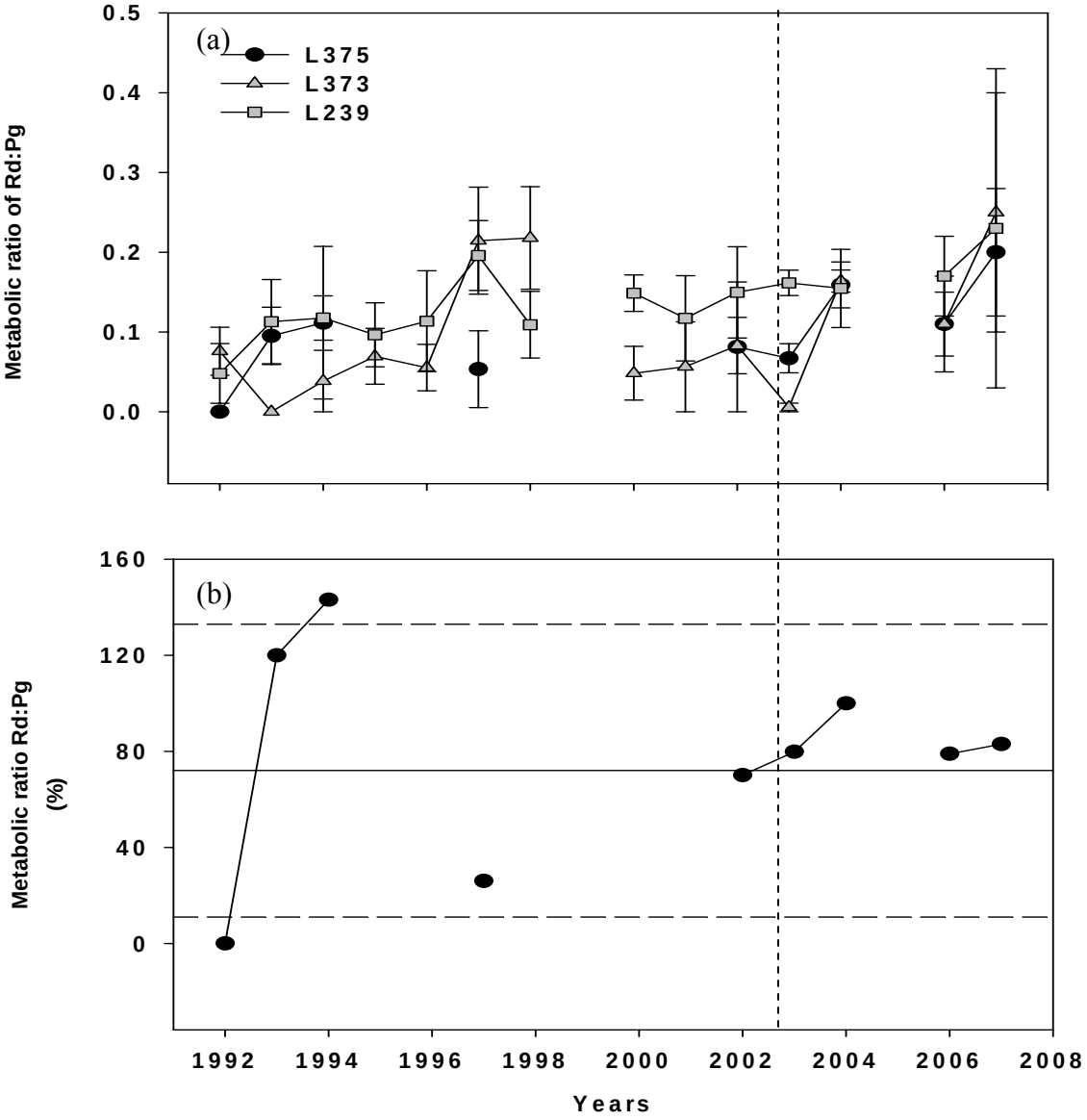

Figure 3.9: (a) The ratio of dark respiration to gross photosynthesis in the experimental lake (L375) and reference lakes (L239 and L373) 1992-2007. The 2006 and 2007 data are estimates based on O_2 fluxes converted to CO_2 fluxes. The vertical dashed line distinguishes between cage and pre-cage years. (b) The ratio of Rd: Pgross in L375 is represented as a percent of the mean of the experimental lakes, the solid horizontal line represents the pre-cage mean and the dashed horizontal lines represent the standard deviation around the mean.

Oxygen and carbon fluxes

In 2006, photosynthesis-irradiance curves were used to represent both O_2 and CO_2 fluxes for net photosynthesis and dark respiration (Fig. 3.10-3.12). The July L239 photosynthesis-irradiance curves demonstrated small intersite (Fig. 2.4) variability for both Pnet and Rd in the O_2 data and CO_2 data (Fig. 3.10 a, b and c). However, in August, CO_2 showed high inter-site variability, especially in Rd (Fig. 3.10 d). In both July and August, in L239, maximum photosynthesis (Pmax, i.e. mean Pnet at 100% irradiance) was 1189 ± 149 and 1086 ± 121 μmol· O_2 · m^{-2}· h^{-1}, respectively for the O_2-derived data, and 670 ± 205 and 548 ± 192 μmol· C· m^{-2}· h^{-1}, respectively for the CO_2-derived data. Oxygen data in L373 also showed small variation between sites (Fig. 2.3) in the photosynthesis-irradiance curves for Pnet and Rd during July and August (Fig. 3.11 a and b).

The CO_2 data, on the other hand, inexplicably demonstrated high inter-site variability (Fig. 3.11 c and d). Variability in the July CO_2-derived data was particularly high; both site C and site B showed substantial CO_2 consumption in the dark. Both sites also exhibited high CO_2 uptake values in the light, although in the 100% transmittance chamber CO_2 uptake at site B dropped below that measured in the dark chambers. The photosynthesis-irradiance curve at site A revealed a low productivity site (Fig. 3.11c). Finally, Pmax in L373 was achieved for July and August respectively at 1676 ± 529 and 1260 ± 289 μmol· O_2 · m^{-2}· h^{-1} and 1733 ± 995 and 583 ± 206 μmol·C·m^{-2}· h^{-1}.

L375 also showed similar variability among sites (Fig. 2.2) for all sampling occasions, for both O_2 and CO_2 data, except for CO_2 in the July sampling run (Fig. 3.12). Maximum photosynthesis was achieved in L375 for July and August respectively at

1611 ± 564 and 1302 ± 299 µmol· O_2 · m^{-2}· h^{-1} and 1743 ± 742 and 826 ± 118 µmol·C·m^{-2}· h^{-1}. The July sampling run demonstrated a highly productive system, especially site B in the CO_2 data (Fig. 3.12 c); however both sites B and C showed positive CO_2 flux in the dark. The photosynthesis-irradiance curves for sites A and C indicated sites that had not yet achieved Pmax. Site B however, showed a Pmax with a CO_2 uptake value below that measured in the dark chamber.

Gross photosynthesis values were also computed for both O_2 and CO_2 in July and August of 2006 (Fig. 3.13-3.15). In L239, O_2 and CO_2 values compared well with a mean PQ of 1.6 ± 0.33 (n = 6) (Fig. 3.13). Similarly, for L373, for the most part O_2 and CO_2 values compared well, except for July site A (PQ = 3.2). The mean PQ for L373 was also 1.6 ± 0.85 (n = 6) (Fig. 3.14). The mean PQ for L375 was 1.2 ± 0.12 (n = 6) (Fig. 3.15).

In 2007, metabolic activities were shown for each sampling site (Fig. 3.16 to 3.21) to facilitate easier inter-site comparisons. In L239 Pmax varied among sites and between months (Fig. 3.16); O_2-derived estimates of Pmax were 693 ± 251 and 216 ± 118 µmol·O_2 · m^{-2}· h^{-1} in July and August, respectively. On the other hand, CO_2-derived data for July (excluding site F) and August were 622 ± 144 and 327 ± 48 µmol· C· m^{-2}· h^{-1}, respectively (Fig. 3.16). The CO_2 values for site F were extremely low yielding a PQ of 27.4 (Table 3.5; Fig. 3.17). Since the discrepancy between the two measures was inexplicably large, this value was excluded from the calculation of a mean PQ for L239, resulting in a PQ of 0.90 ± 0.20 (n = 6). Respiration was generally low in L239 (Fig. 3.16). Three sites showed O_2 release in the dark and one site showed CO_2 uptake in the dark. A flux of < 200 µmol·m^{-2}· h^{-1} may be a function of analytical variability and therefore can not be distinguished from zero. Therefore all the

positive Rd values, except site C, in the O_2 data which exceeded 200 μmol·m^{-2}· h^{-1} were assumed to be zero when estimating respiration. Finally, because of vial failures in August, there was no measure of photosynthesis for site C.

Net photosynthesis in L373 was similar between months in 2007 but differed amongst sites (Fig. 3.18). Excluding site C in July, Pmax was 588 ± 456 μmol· O_2 · m^{-2}· h^{-1}. Similarly for CO_2, Pmax was 636 ± 496 μmol· C· m^{-2}· h^{-1}. Site C showed O_2-derived Pnet values that could not be distinguished from zero and negative CO_2-derived Pnet values (Fig. 3.18). Because of this negative Pnet value, Pgross was negative. This value was corrected to zero in Fig. 3.19. A PQ could not be calculated for this site (zero in the denominator) and it was excluded from the calculation of the mean PQ. The mean PQ for the remaining observations in L373 was 1.22 (Table 3.5). In July in both the O_2- and CO_2- derived data, site D showed high rates of respiration and low Pnet. In August, all sites showed higher rates of photosynthesis than respiration, with Pnet values achieved for O_2 and CO_2 at approximately 533 ± 236 μmol·O_2 · m^{-2}· h^{-1}; 376 ± 86 μmol· CO_2 · m^{-2}· h^{-1}, respectively.

In L375, Pmax for the O_2-derived data was 487 ± 203 μmol· O_2 · m^{-2}· h^{-1} at the six sites in July and 846 ± 71 μmol· O_2 · m^{-2}· h^{-1} at the three sites in August (Fig. 3.20 a and b). The CO_2 data demonstrated even greater variability (Fig. 3.20 c and d). In July Pmax varied 697 ± 267 μmol· C· m^{-2}· h^{-1}, while in August variance was less (480 ± 99 μmol· C· m^{-2}· h^{-1}) (Fig. 3.20 c and d). All instances of CO_2 uptake less than 200 μmol· C· m^{-2}·h^{-1} in the dark in L375 were analytically indistinguishable from zero.

Pgross in L375 ranged from 948 ± 300 to 733 ± 71 μmol·C·m^{-2}·h^{-1} for the CO_2-derived data for July and August, respectively and 828 ± 165 to 846 ± 71 μmol·O_2·m^{-2}· h^{-}

[1] O_2-derived data in July and August, respectively (Fig. 3.21). All values were used in the calculation of PQ in L375. The mean PQ was very close to unity for O_2 and CO_2 (PQ = 1.02 ± 0.12; n = 6; Table 3.5).

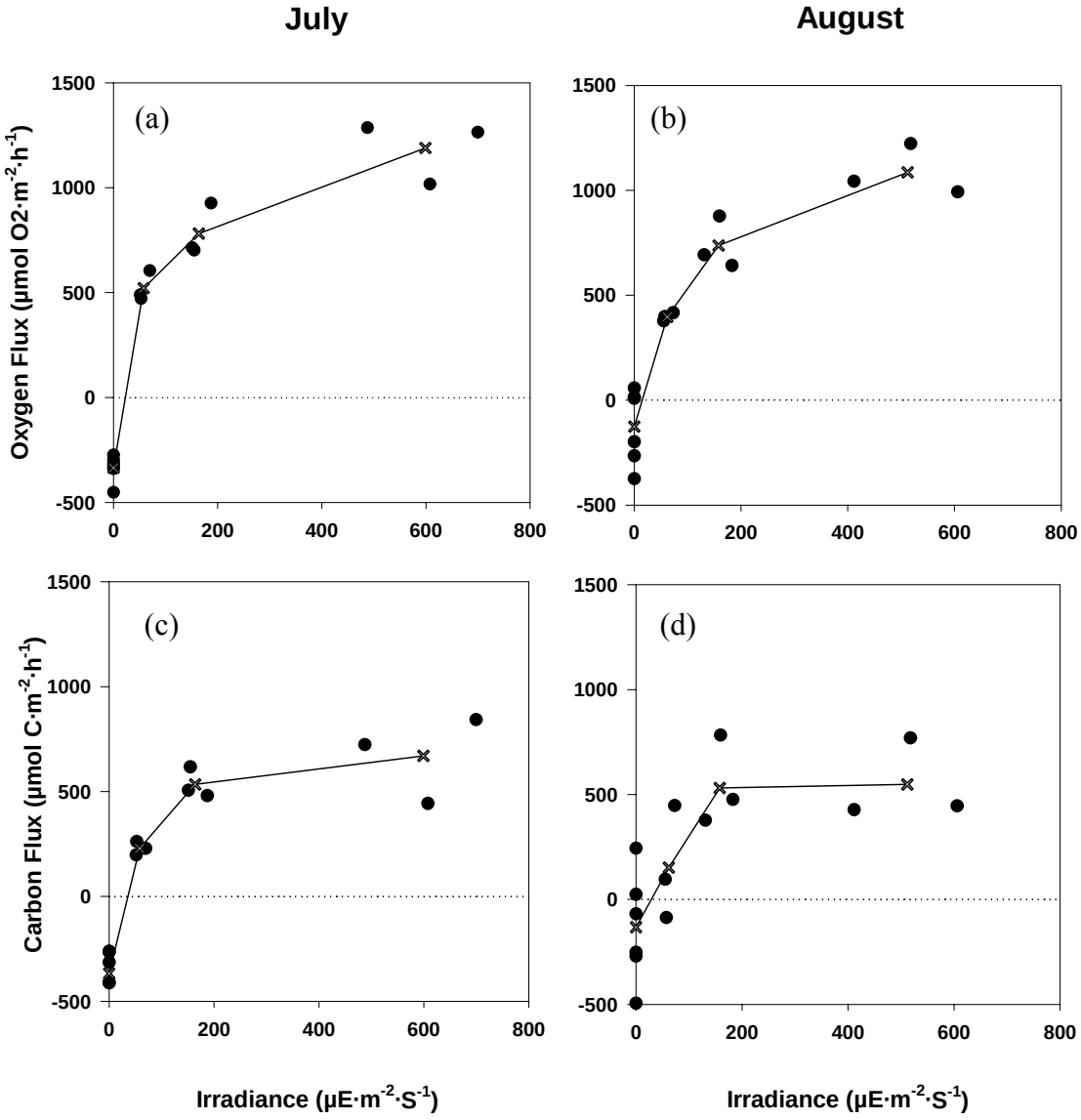

Figure 3.10: Photosynthesis-irradiance curves of oxygen (a, b) and carbon fluxes (c, d) for epilithon in L239 during July and August 2006. The curves connect site means of the dark and irradiated chambers (11%, 40% and 100%). Sites A-C on the plots correspond to sites A-C in Fig. 2.4.

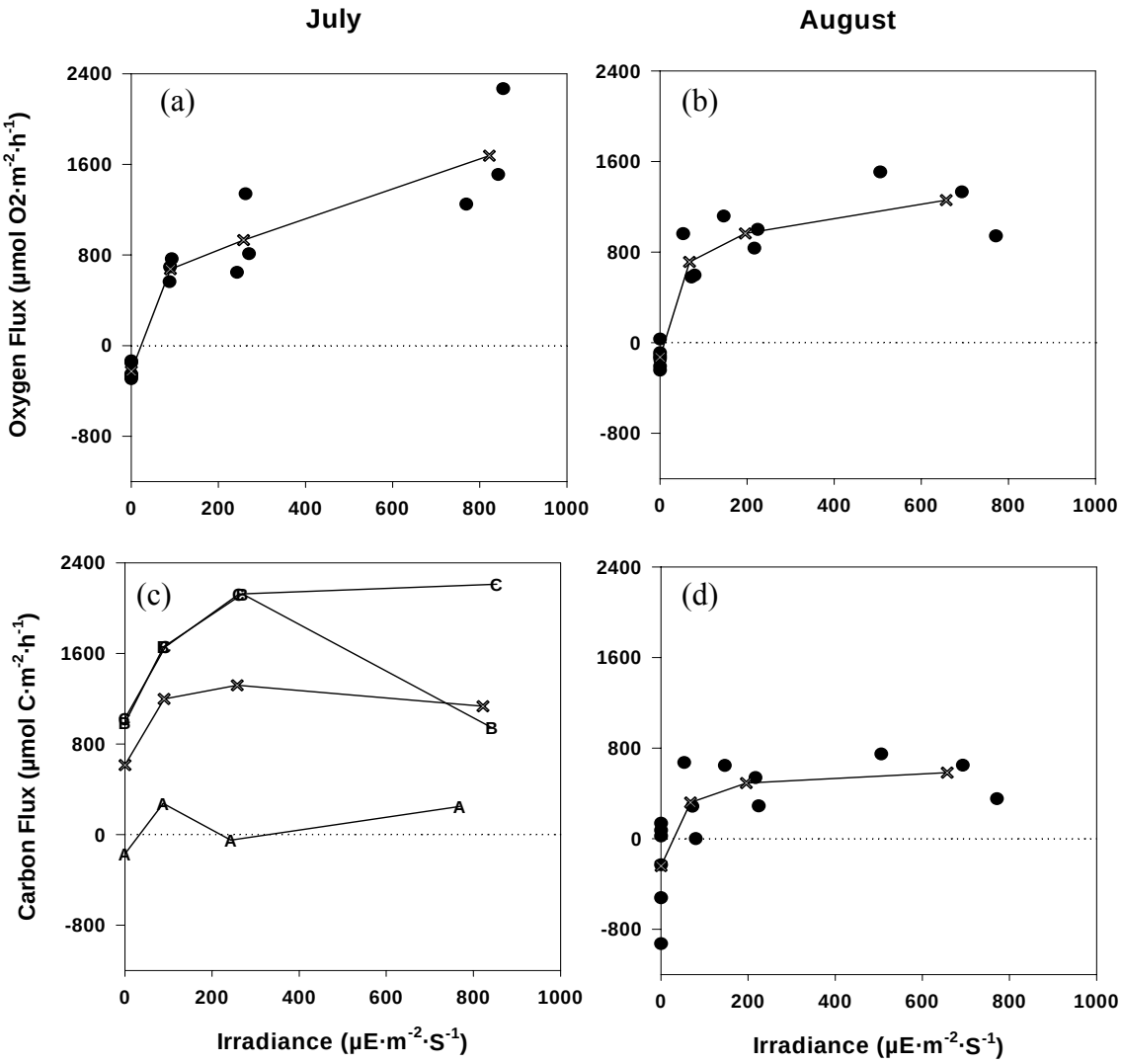

Figure 3.11: Photosynthesis-irradiance curves of oxygen (a, b) and carbon fluxes (c, d) for the epilithon in L373 during July and August 2006. The curves connect site means of the dark and irradiated chambers (11%, 40% and 100%). (c) A representation of a sampling run in which each site responded differently, distinction of sites by site name (A, B, C) on the figure provides a visual understanding of the intersite variability for this run. The site means are represented by an 'x'. Sites A-C on the plots correspond to sites A-C in Fig. 2.3.

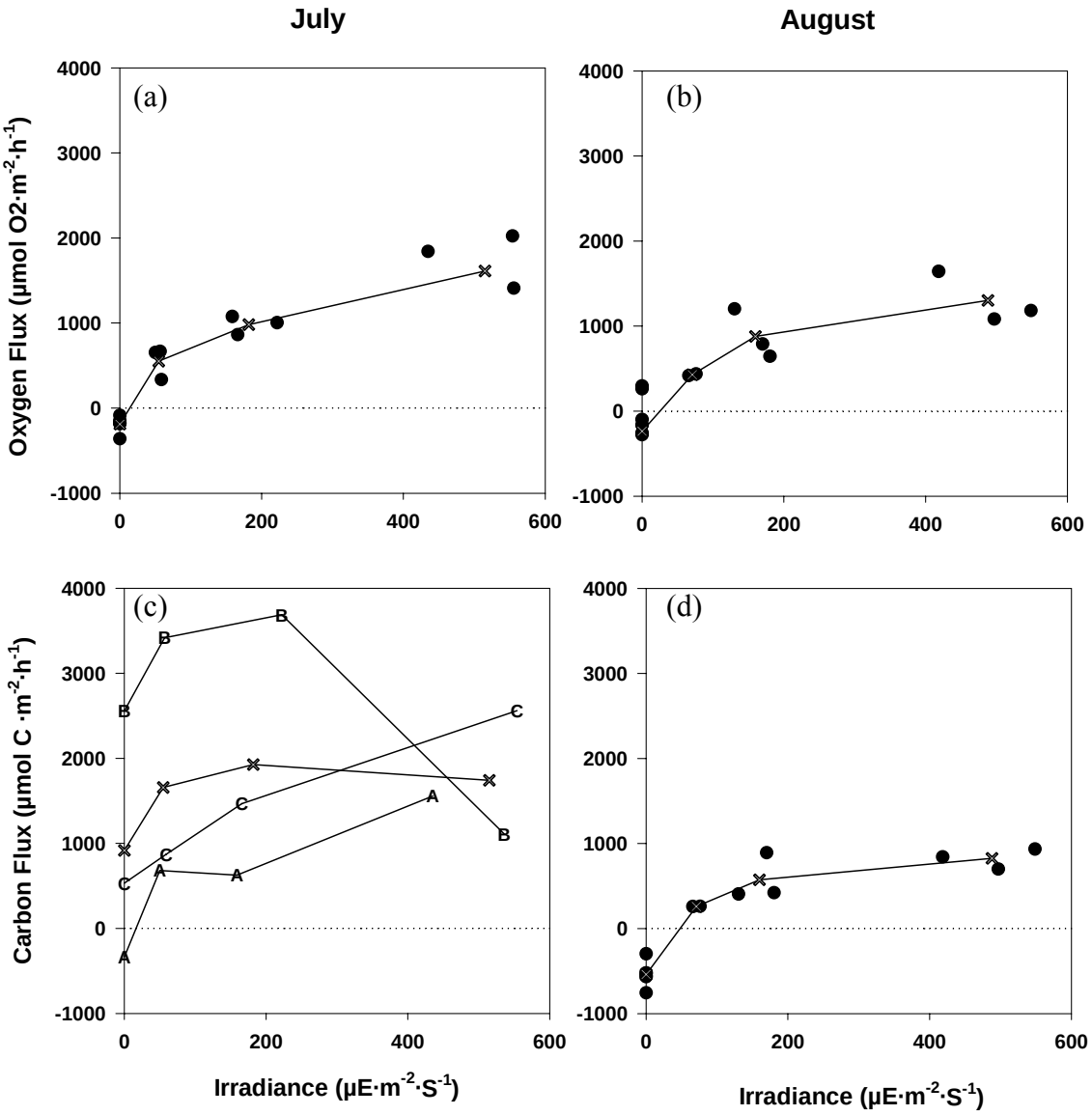

Figure 3.12: Photosynthesis-irradiance curves of oxygen (a, b) and carbon fluxes (c, d) for the epilithon in L375 during July and August 2006. The curves connect site means of the dark and irradiated chambers (11%, 40% and 100%). (c) A representation of a sampling run in which each site responded differently, distinction of sites by site name (A, B, C) on the figure provides a visual understanding of the intersite variability during this run. The site means are represented by an 'x'. Sites A-C on the plots correspond to sites A-C in Fig. 2.2.

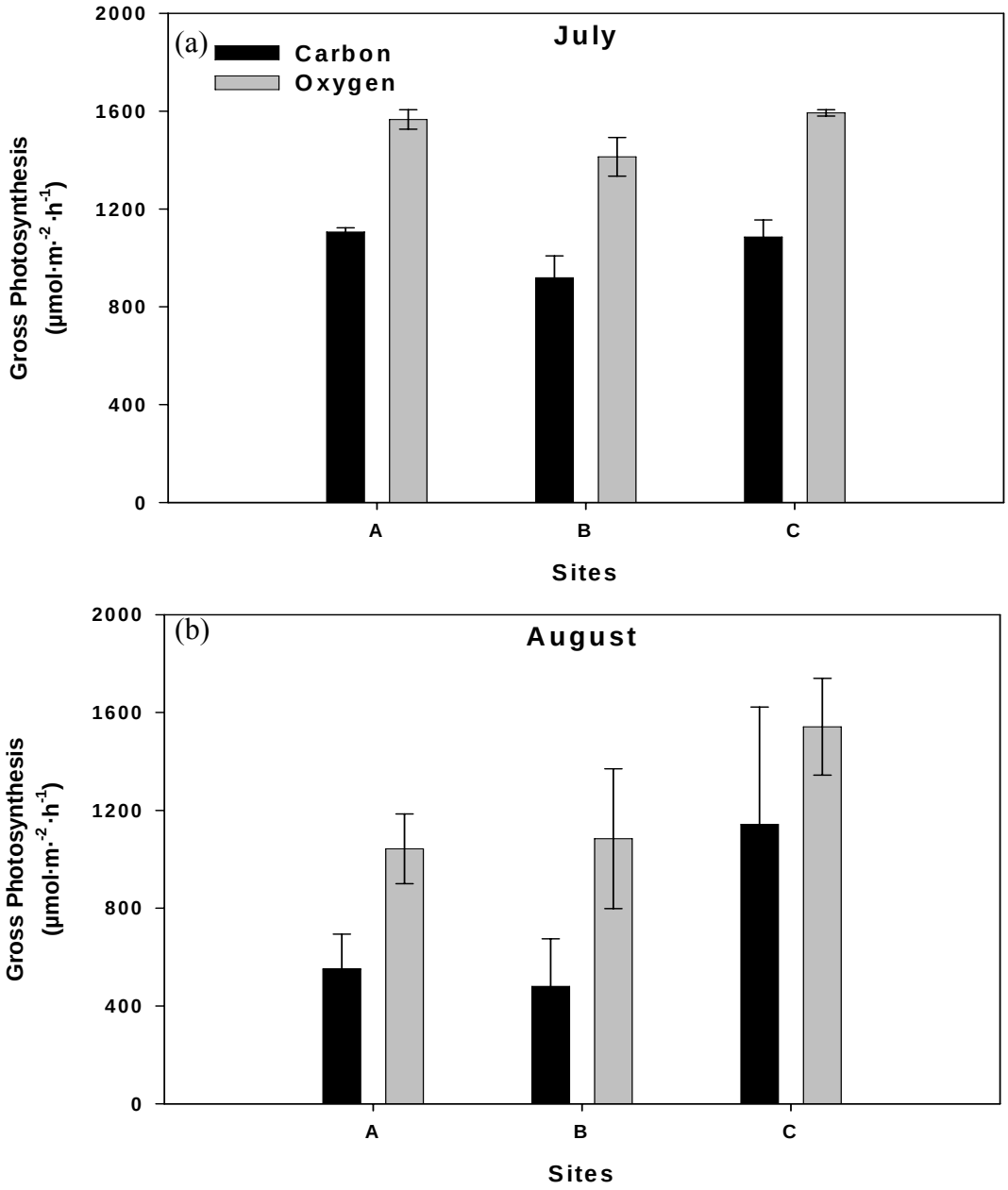

Figure 3.13: Gross photosynthesis (Pgross = Pnet-Rd) measured by oxygen and carbon fluxes in the epilithon of L239 during (a) July and (b) August 2006. Error bars represent standard deviation of the Pgross estimate based on the standard deviation of the vials (n = 3) for Pnet and standard deviation of the two dark chambers for Rd. Sites A-C on the plots correspond to sites A-C in Fig. 2.4.

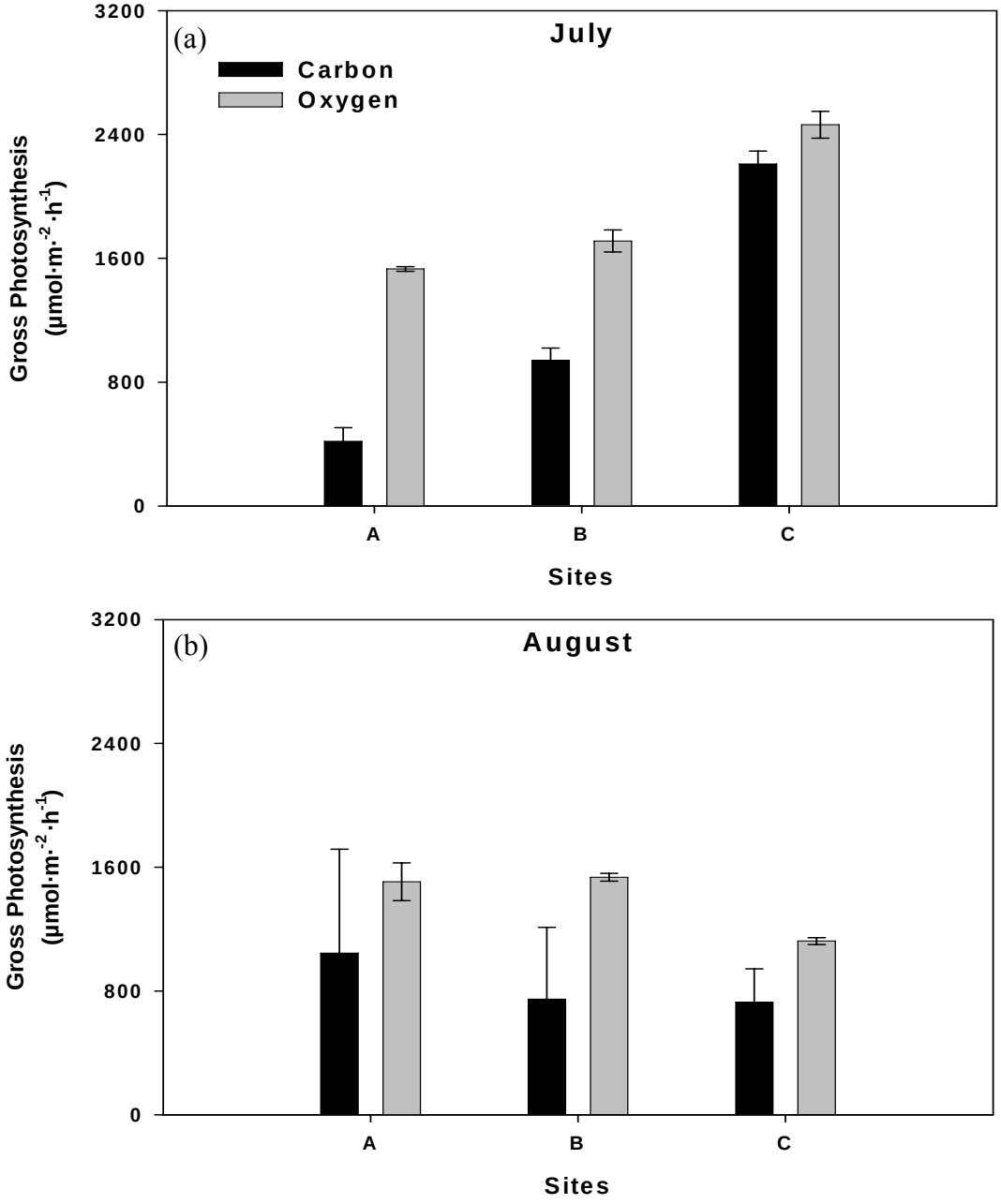

Figure 3.14: Gross photosynthesis (Pgross = Pnet-Rd) measured by oxygen and carbon fluxes in the epilithon of L373 during (a) July and (b) August 2006. Error bars represent standard deviation of the Pgross estimate based on the standard deviation of the vials (n = 3) for Pnet and standard deviation of the two dark chambers for Rd. Sites A-C on the plots correspond to sites A-C in Fig. 2.3.

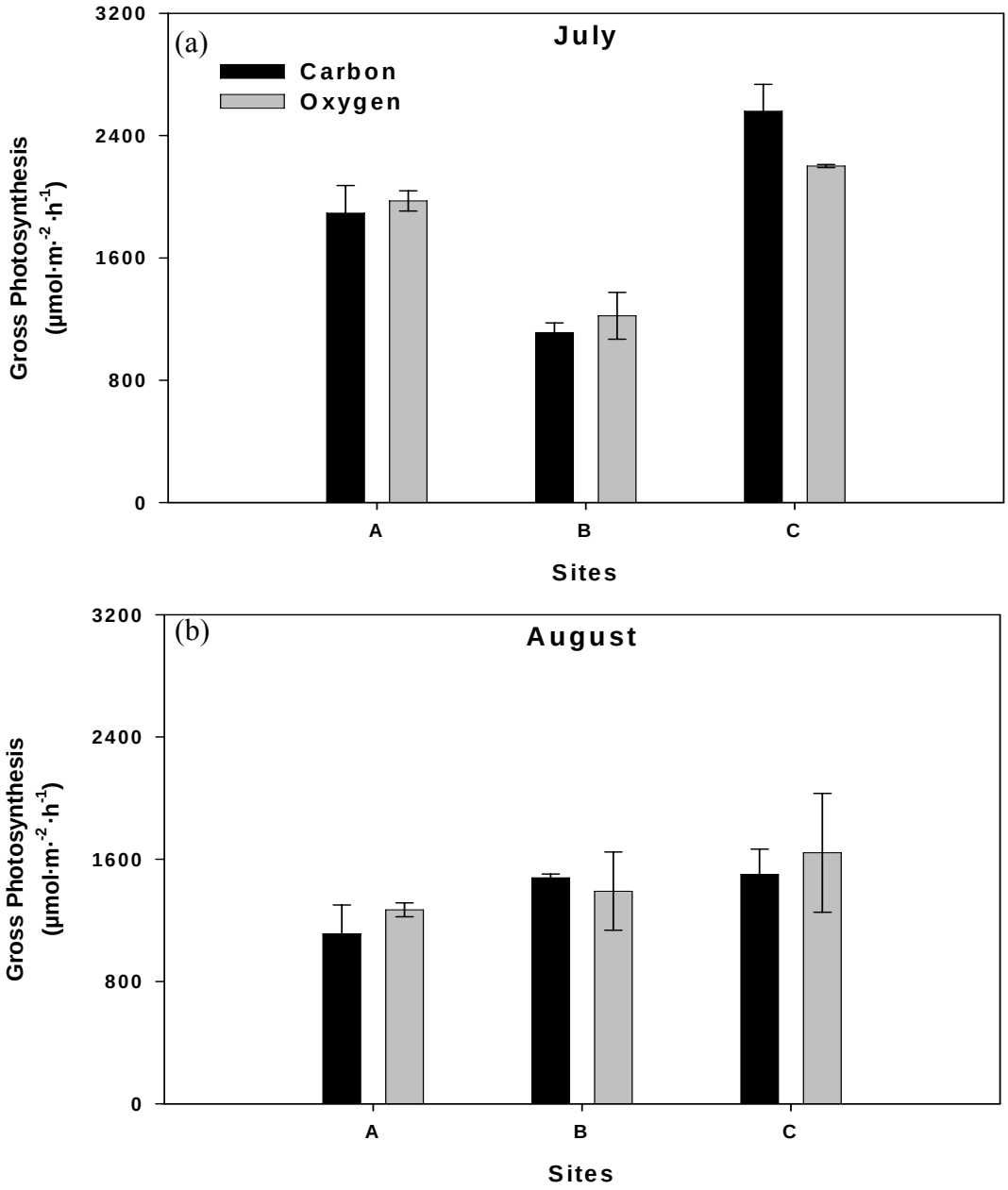

Figure 3.15: Gross photosynthesis (Pgross = Pnet-Rd) measured by oxygen and carbon fluxes in the epilithon of L375 during (a) July and (b) August 2006. Error bars represent standard deviation of the Pgross estimate based on the standard deviation of the vials (n = 3) for Pnet and standard deviation of the two dark chambers for Rd. Sites A-C on the plots correspond to sites A-C in Fig. 2.2.

Table 3.5: Photosynthetic quotients (PQ) based on rates of gross photosynthesis for the experimental lake (L375) and reference lakes (L239 and L373) for July and August 2007. The mean for each lake was used to convert oxygen fluxes to carbon fluxes. Sites with unreliable PQs (i.e. L373 site C, L239 site F) were not used in the calculations of month or lake means. Standard deviations (SD) around of the monthly mean are shown for each lake.

Month	Site	L375	L373	L239
July	**A**	1.25	1.13	1.60
	B	0.68	2.21	0.83
	C	0.76	N/A[a]	1.14
	D	1.34	0.94	0.93
	E	0.92	1.09	0.70
	F	0.65	0.63	27.4[b]
	Mean ± SD	0.87 ± 0.30	1.20 ± 0.60	1.04 ± 0.35
August	**A**	1.10	1.58	0.80
	B	1.30	0.82	0.73
	C	1.08	1.30	N/A[c]
	Mean ± SD	1.16 ± 0.13	1.23 ± 0.38	0.76± 0.05
Lake[d]	**Mean ± SD**	**1.02 ± 0.20**	**1.22 ± 0.02**	**0.90 ± 0.20**

[a] Value omitted from calculations of monthly or lake means due to negative value for photosynthesis in carbon data, when corrected to zero, PQ could not be determined (zero in the denominator).
[b] Value omitted from calculation of monthly or lake means due to major discrepancy between carbon and oxygen data.
[c] Value could not be calculated, missing photosynthesis data for site C
[d] Lake Mean PQ used to convert O_2 to CO_2 fluxes for 2006 and 2007.

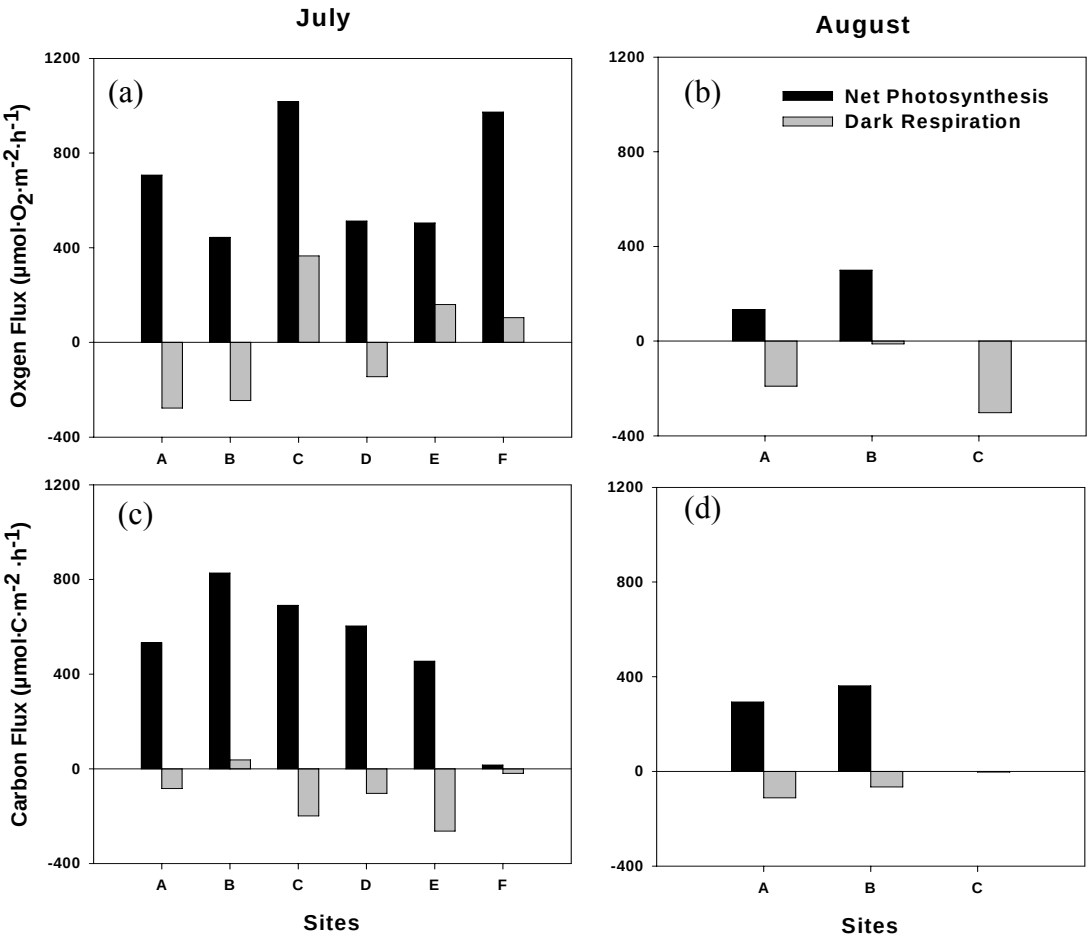

Figure 3.16: Photosynthesis and respiration in the epilithon of L239 during July and August 2007 represented by oxygen (a, b) and carbon fluxes (c, d). Only three of the six sites were sampled during the August run. (d) August, site C, photosynthesis could not be computed due to chamber vials laying outside of the accepted limits for Ne, Air and Nsat. Sites A-F on the plots correspond to sites A-F in Fig. 2.4.

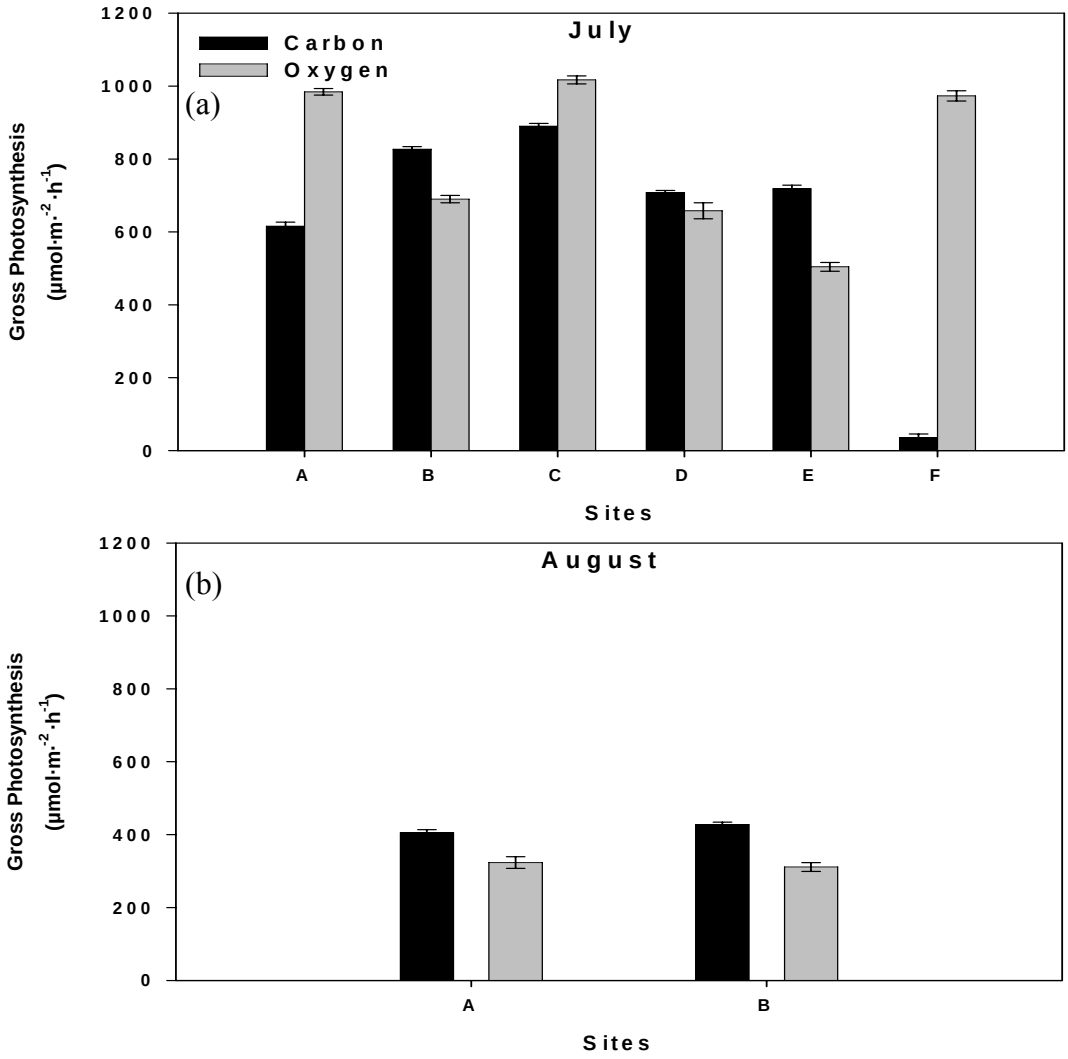

Figure 3.17: Gross photosynthesis (Pgross = Pnet-Rd) measured by oxygen and carbon fluxes in the epilithon of L239 during (a) July and (b) August 2007. Error bars represent standard deviation of the Pgross estimate based on the standard deviation associated with the vials (n = 6) for both Pnet and Rd at each site. (a) July, site F carbon and oxygen values were greatly different, therefore samples were not used in determination of PQ. (b) August, site C, photosynthesis could not be computed due to chamber vials outside of the accepted limits for Ne, Air and Nsat. Sites A-F on the plots correspond to sites A-F in Fig. 2.4.

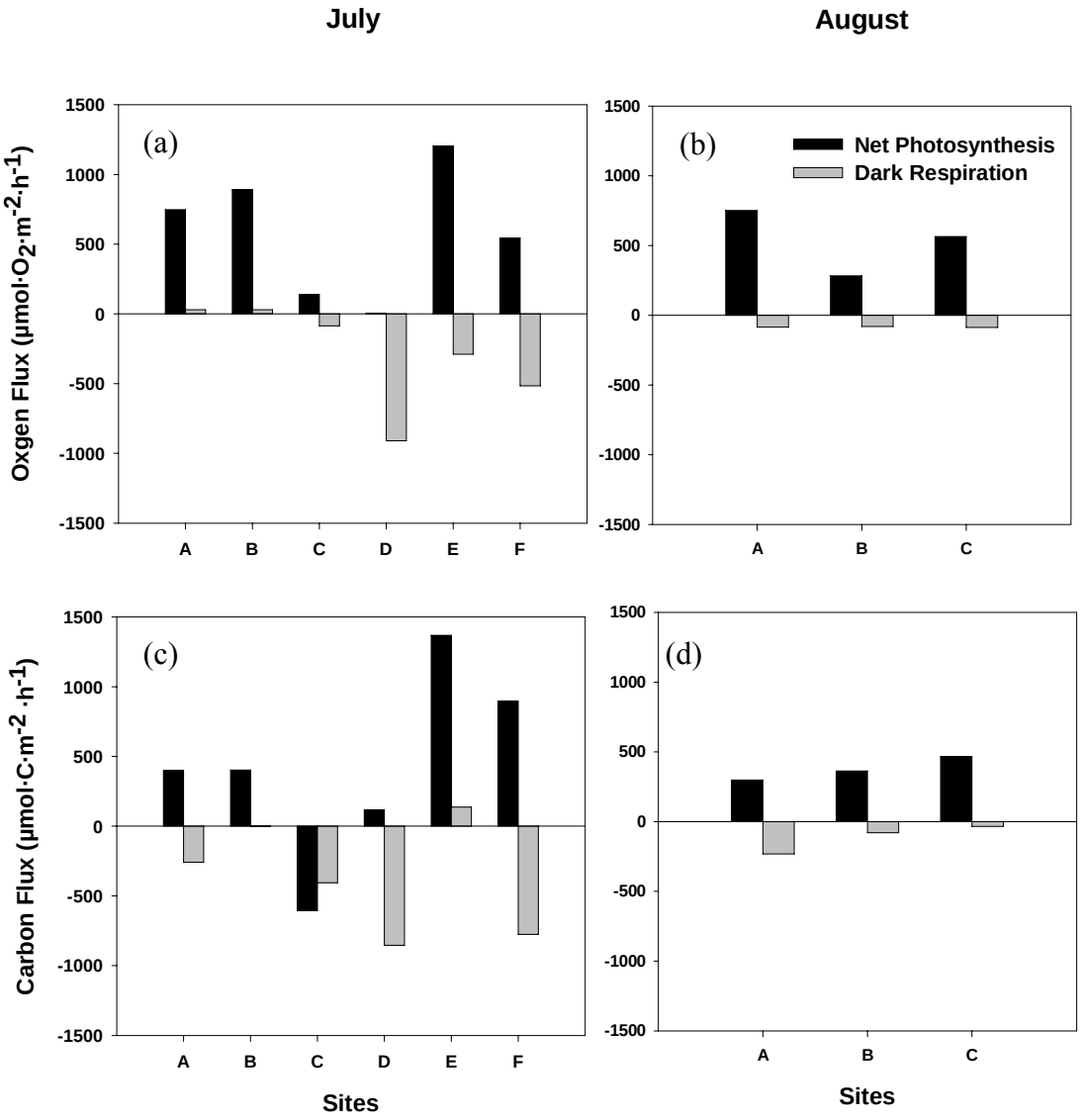

Figure 3.18: Photosynthesis and respiration in the epilithon of L373 during July and August 2007, represented by oxygen (a, b) and carbon fluxes (c, d). Only three of the six sites were sampled during the August run. Sites A-F on the plots correspond to sites A-F in Fig. 2.3.

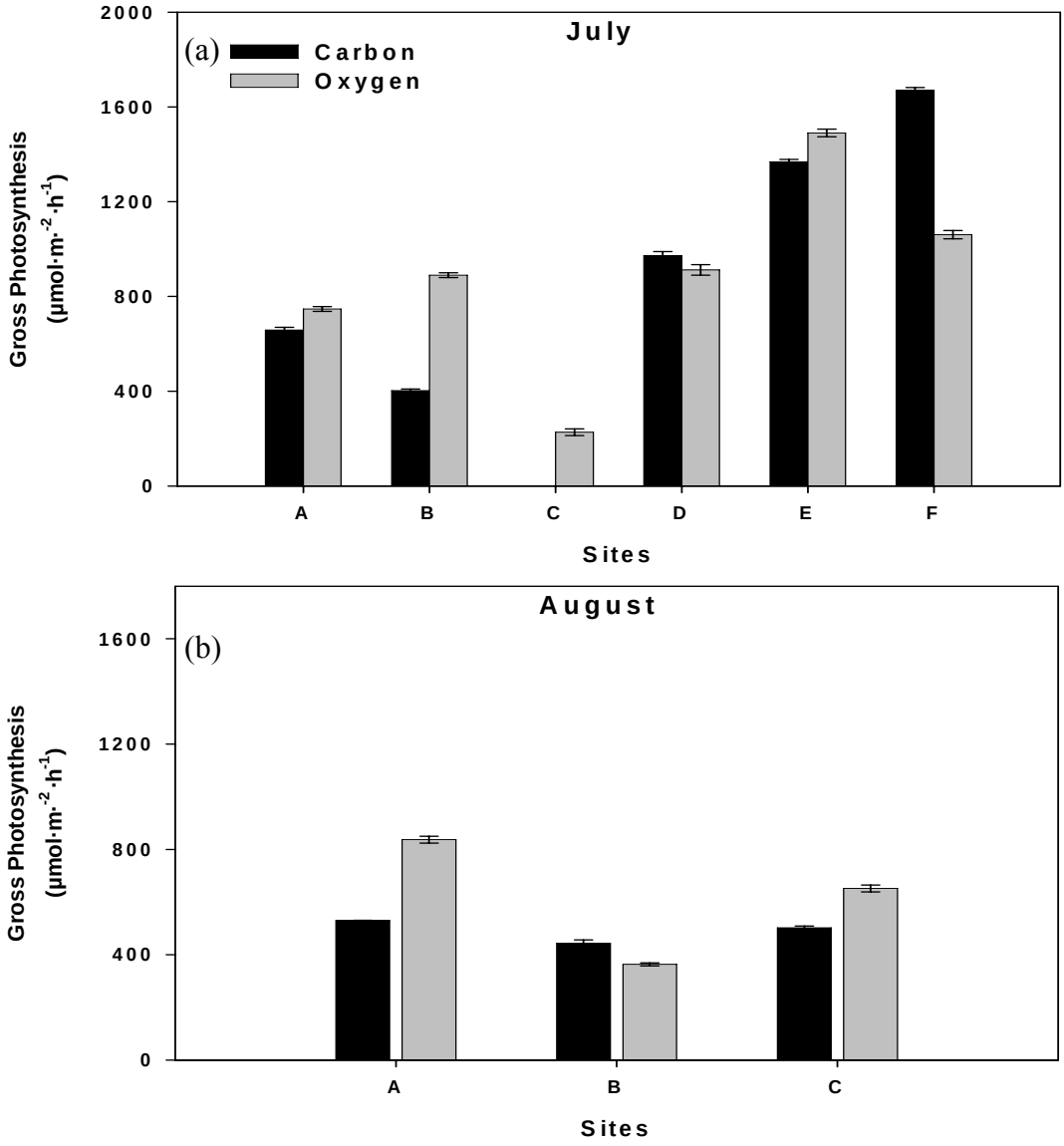

Figure 3.19: Gross photosynthesis (Pgross = Pnet-Rd) measured by oxygen and carbon fluxes in the epilithon of L373 during (a) July and (b) August 2007. Error bars represent standard deviation of the Pgross estimate based on the standard deviation associated with the vials (n = 6) for both Pnet and Rd at each site. (a) July site C, carbon fluxes showed a negative photosynthesis value, represented as a zero value in the current plot. Carbon and oxygen data for July site C were not used in determination of the PQ. Sites A-F on the plots correspond to sites A-F in Fig. 2.3.

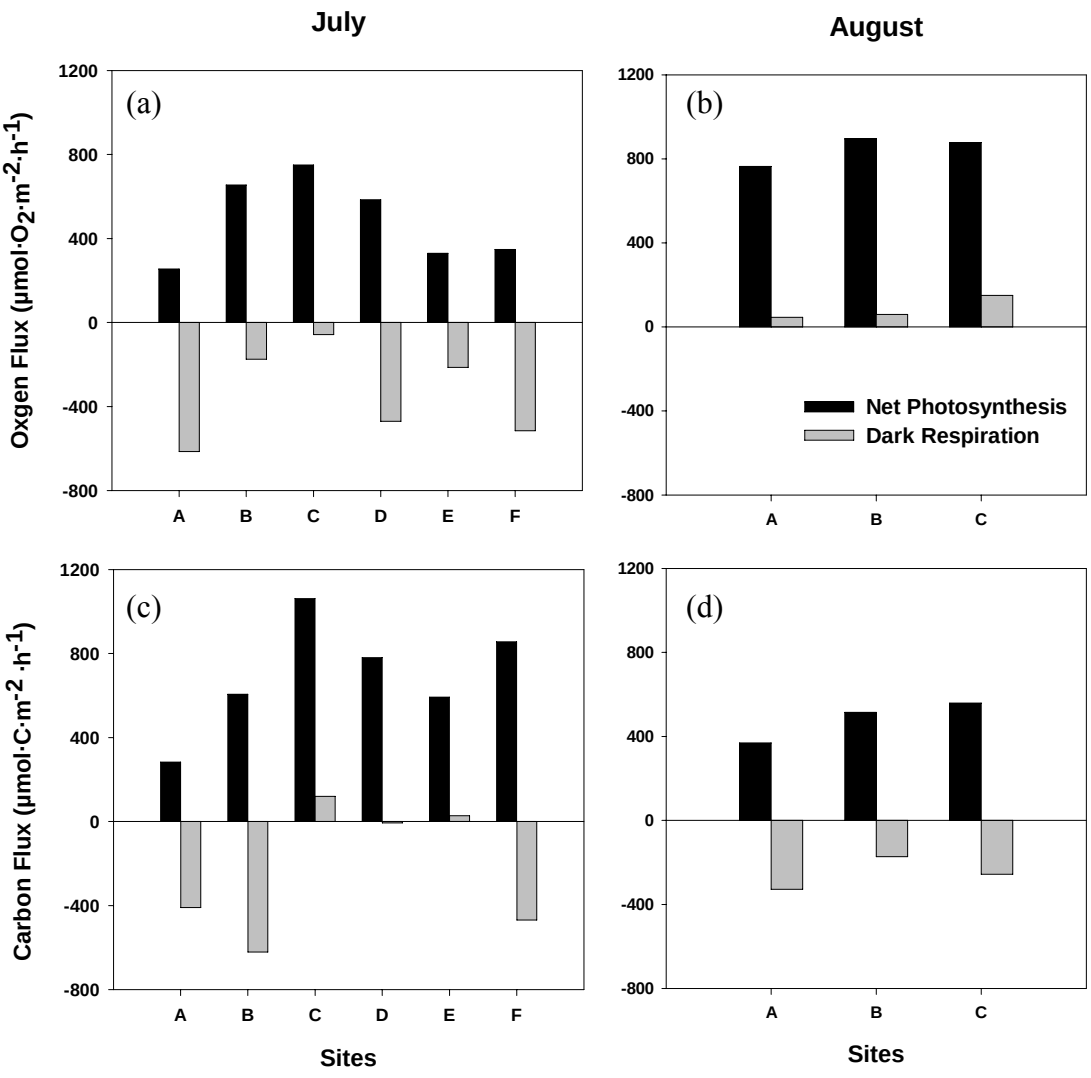

Figure 3.20: Photosynthesis and respiration in the epilithon of L375 during July and August 2007 represented by Oxygen (a, b) and carbon fluxes (c, d). Only three of the six sites were sampled during the August run. Sites A-F on the plots correspond to sites A-F in Fig. 2.2.

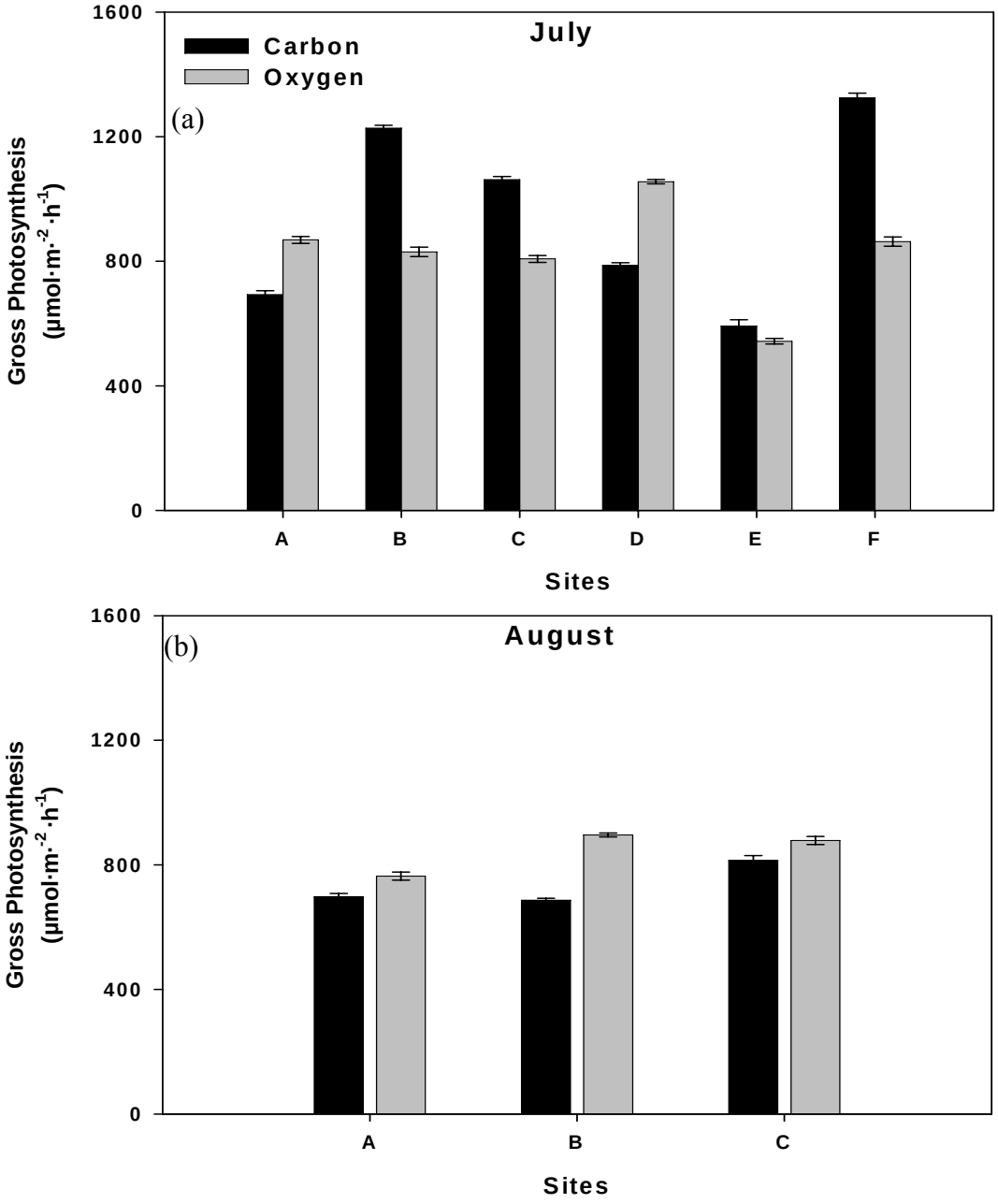

Figure 3.21: Gross photosynthesis (Pgross = Pnet-Rd) measured by oxygen and carbon fluxes in the epilithon of L375 during July and August 2007. Error bars represent the standard deviation of the Pgross estimate based on the standard deviation associated with the vials (n = 6) for both Pnet and Rd at each site. Sites A-F on the plots correspond to sites A-F in Fig. 2.2.

Inter-site variability

Inter-site comparisons in 2007 used gross photosynthesis, which removes the uncertainty associated with the initials (see *methodological recommendations* section in the discussion). Variability among sites, shown as percent coefficient of variation (% CV) in L239 was low, with the exception of site F, for which there was poor agreement among methods (Fig. 3.17). The variability around the mean in July, excluding site F, for CO_2 was 14% and for O_2 was 29%. In August variability was even lower for the CO_2 and O_2 data respectively, 3.8% and 2.5%. The major discrepancy between the CO_2 and O_2 data at site F was inexplicable. If the data does represent a true measure of photosynthesis for both CO_2 and O_2, the O_2 values would be demonstrating a site with the highest productivity, while the CO_2 values demonstrate a site with the lowest productivity (Fig. 3.17 a).

Inter-site variability in L373 was much greater than L239, especially in July (Fig. 3.19). Productivity at site C was very low for both CO_2 (negative value, corrected to zero) and O_2 (227 $\mu mol \cdot m^{-2} \cdot h^{-1}$). Excluding site C, O_2 data appeared to be more similar among sites (% CV = 29%) than the CO_2 data (% CV = 51%). Inter-site variation in August was less for CO_2-derived estimates (% CV = 8.9%) than for O_2 (% CV = 39%).

In L375 in July, sites B, C and F showed the highest productivity, while site E showed the lowest in the CO_2 data (Fig. 3.21). Variability in the July CO_2-derived data (% CV = 32%) was higher than the O_2 data (% CV = 20%). In the O_2 data, site D showed the highest productivity and site E the lowest. The other four sites were very similar for the O_2-derived data (% CV = 3%). The O_2 (% CV = 9%) and CO_2 data (% CV = 10%) were similar in August.

Discussion

The present method underwent several developmental changes during the course of thesis preparation. Estimates of productivity were difficult due to the highly complex nature of the community in addition to the complicated nature of the epilithic sampling method. For this reason, determination of a benthic algal response to aquaculture was challenging. The following section outlines the problems that were encountered during methodological development, each of which should to be considered when interpreting benthic algal metabolic responses (see *effects of aquaculture operations on benthic algal productivity* section).

Methodological recommendations

At the outset, a great deal of emphasis was placed on organization and management of the samples in this method. Efforts were made to improve underwater vial management through the use of vial carriers; however, even then vial mistakes and mix-ups were suspected and non-recoverable. A simpler approach underwater may have been to have separate and clearly marked carriers for each chamber, at each site. Each case could contain three vials, clearly and largely labelled, possibly in different colors, indicating sample sequence. The case could be placed next to the chamber before and after sampling to ensure agreement between chambers and vials. Major changes to the data management process should also be considered. First, fewer intermediary steps in the rate calculations are needed. Second, correspondence between original vial tables and those used to calculate metabolic rates should be considered. Third, fewer linkages within the rate-calculating workbook are essential to simplify data management.

Uncertainty in vial integrity in both 2006 and 2007 led to the greatest concerns with the new GC technique. Error trapping techniques in 2006 relied on the use of the Ar: N_2 ratio. However, the ratio only checked for air contamination and did not detect CO_2-compromised samples. As a result, outlier removal for CO_2 was largely based on a statistical evaluation of the data. Continued discrepancies in the site-to-site variation in the photosynthesis-irradiance curves for the oxygen and carbon data (Fig. 3.10 -3.12), especially July runs for L373 (Fig. 3.11 c) and L375 (Fig. 3.12 c), placed doubt on the integrity of the results using the current methodology.

In 2007, outlier removal was based on a statistical evaluation of the data (± 3.0 standard deviations of the mean) in combination with a technical approach using the tracer gases: Ne, Air and Nsat. The Ne, Air and Nsat limits were somewhat arbitrarily chosen based on frequency distributions of the vials for each of the gases (Fig. 3.5). The assumption was that the greater the number of vials with a certain tracer gas concentration, the more likely that was the expected concentration for all the vials. Vial omission using this criterion for removal of potentially contaminated samples was objective and stringent. Vials were removed for legitimate reasons using Ne (sample integrity), Air and Nsat (air contamination). However, had all the vials with values outside the limits set for Ne (10%), air (26%) and Nsat (38%) been removed, the number of samples left would have been very low (56%). For this reason, vials were first examined for deviation from the ± 3.0 standard deviations of the mean prior to using the Ne, Air and Nsat criteria. Even with this added step, the percentage of vials removed from analysis was high (25%), and in four cases only one vial remained for the estimation of gas fluxes within a given chamber. The end result was vial precision with coefficients

of variation for CO_2 and O_2 of 2.2 % and 1.4 %, respectively (Table 3.1). Although many vials were discarded using this technique, it was believed that this was needed to achieve the vial precision necessary for proper calculation of metabolic rates. As described in the methods, vial precision was essential because high coefficients of variation in the vials (Appendix III Table III.1-III.2) propagate into even greater variability in the calculated rates (Appendix III Table III.3-III.4).

Earlier metabolic measurements, using the IR-technique (1992-2004), provided an estimate of expected production rates and gas concentrations, especially for the CO_2 data in the reference systems. In addition, it provided an approximate level of precision to achieve (% CV < 2.0% for the CO_2 data). When values substantially exceeded this expectation, it was clear that there were vial issues. For this reason, the statistical system of outlier trapping was also employed. Even if a sample did not show errors associated with the technical parameters, but proved to be an outlier based on statistical evaluation, it was removed. Only 2.5% of samples (12 vials) were removed for statistical reasons. Coupling the statistical approach with the technical system for outlier trapping, vial precision (2.2% and 1.4% for CO_2 and O_2, respectively) approximated the precision achieved in the IR-technique, though spurious values still seemed to be embedded in the data set.

There was increased confidence in the error trapping techniques used in 2007 compared to 2006; however, the number of vials omitted due to sample contamination (25%) was troubling. The reasons for vial failures and sample omissions are not known but possibilities can be put forward. First, issues with loosened caps may have caused problems in analyses. During June metabolic runs (all data discarded), integrity of the

vial seals comprised over half of the vials. As a result, the compromised vials were contaminated by air and other gases. Subsequently, careful inspection of the vials occurred but, a few loosened caps may have found their way into circulation. Second, air bubbles trapped in the sampling syringe, despite purging, may have contributed to air contamination of the vials. Due to the nature of the metabolic sampling strategy (see *in situ measurements of photosynthesis and respiration* section), bubbles should only have been a problem for the initial sample vials and for sample vials taken at the start of a chamber sequence at each site. Third, although less likely, pressurization issues may have allowed for samples to become compromised. A number of vials (6.7%) appeared to be at atmospheric pressure prior to the gas releasing step, which may have permitted contamination. However, there was little correspondence between these vials and compromised samples, although in some cases they did match (38% of the compromised vials matched the atmospheric pressure vials).

A further issue with the method, not directly related to the new GC method but to epilithic sampling in general, was that the measurements are of community metabolism, not benthic algal productivity perse (Robinson 1983). Both Pnet and Rd are a measure of community net processes and are being affected by gas exchange caused by bacteria and other micro-organisms in addition to algae (Robinson 1983). If the light and dark chambers were populated by different micro-organism it would prevent interpretation of net fluxes as being due only to algal metabolism. For example, if a large grazer was trapped inside a light chamber during the incubation, the chamber values would show high rates of respiration, even though photosynthesis was occurring. Separation of the algal from the non-algal material was impossible in these *in situ* experiments, therefore

these community measurements should be interpreted as such. For example, in 2007, the L373 July site C estimate of productivity was based solely on one chamber (the second chamber was omitted due to vial outliers), this estimate was low for both O_2 and CO_2 (showing a negative value). It may be that the light chamber contained highly respiring organisms that concealed the algal signal and thus provided a miscalculation of productivity.

The method of determining Pnet and Rd was based on the determination of O_2 and CO_2 fluxes, which required an estimate of initial concentrations. These samples were taken from water overlying the area where the chambers would soon be placed. If by chance a light chamber was set on an area of stagnant water, the water inside the chamber might have been lower in CO_2 than the overlying water in which the initials were taken, due to CO_2 uptake. This would cause a shift downward on the y-axis for the plots, causing an underestimate of photosynthesis. Also, the physical act of placing the chamber onto the rock surface may have been enough to cause a release of trapped gases from within the biofilm, resulting in an overestimate of photosynthesis. For the O_2 measurements particularly, some of the evolved O_2 might have remained trapped in the algal layer during the initials sampling due to the relatively low solubility of the gas in water (Wetzel 1965). The physical act of mixing within the chamber would have allowed the release of this sequestered O_2. Tus, the combination of the "true" chamber productivity with the release of sequestered O_2 could have overestimated the measured productivity.

Since it was not known whether photosynthesis was being under or over represented in Pnet, the use of Pgross values were important for removing the uncertainty

associated with the initials. That is, only irradiated and dark-chamber final samples were used to compute Pgross (Eq 3). Hence, Pgross was used to assess whether there were aquaculture impacts. Subsequent studies should consider taking initial concentrations from within the chamber upon chamber placement, rather than before.

The next issue with the method was that of scale. In the current method, incubations occurred in a number of small (2 x 100cm^2) chambers at each site (6). Due to the heterogeneous nature of the epilithon, each of these chambers can be thought of as its own sub-site. Pooling chambers within a site to increase the signal-to-noise ratio was risky, as this eliminated understanding of intra-site variability. However, increasing the signal-to-noise ratio was required to provide a more accurate estimate of productivity, especially for respiration. In addition, there was uncertainty in the use of only a small number of sites (three to six) to determine lake-wide changes. The amount of measured area compared to total surface area of the lake was very small ($< 0.5x10^{-4}$% for L375), and may not have been as representative as one might have hoped. However, epilithic sampling is a high cost and high energy method, due to the SCUBA required to obtain the sample. Since very little research has been done on this area of the lake ecosystem (Turner *et al.* 1994; Hecky and Hesslein 1995; Vadeboncoeur *et al.* 2001), even these small snapshots of productivity are useful in providing insight into the habitat.

A final methodological recommendation would be to increase the number of sites, size of chambers and number of sampling events. Also, the use of continuous gas monitoring boxes with flow-through systems, would ensure that the nutrients do not become depleted within the chambers, and allow longer running cycles. The chambers could be left in place for a number of days or weeks (limited by algal wall growth) to

measure diurnal and seasonal changes in benthic algal productivity. Finally, by increasing the size of the chamber (e.g., up to 100 m^2) it would allow coverage of a greater area of the lake bottom and decrease the problem of intra-site variability. However, one would first have to overcome the challenge of finding a way to seal the chambers to the rock surface. Furthermore, new measures of productivity should be explored, rather than using O_2 and/or CO_2 evolution and/or consumption, the use of other substrata in the photosynthetic process should be explored. For example, investigate the use of $\delta^{32}P$, by measuring $\delta^{33}P$ incorporation into cellular energy (ADP→ATP).

Effects of aquaculture operations on benthic algal productivity

It was expected that the increased epilithic P from the aquaculture operation (Fig. 2.7-2.8) would play a role in an early increase in benthic algal productivity (Wetzel 2001a). However, even though available P increased (chapter 2), no metabolic parameter (Pgross, Pnet, or Rd) detected a response. All parameters remained within both the range of natural variability of the reference lakes and the pre-cage mean (Fig. 3.6-3.9; Table 3.4).

R. Hesslein (pers. comm.), monitored whole-lake primary productivity during the aquaculture operation in L375 using continuous CO_2 monitoring boxes position on floating rafts in the middle of the lake. He found that there was a general increase in whole-lake productivity over the course of the aquaculture operation, seen as larger diurnal changes in CO_2 than in other ELA lakes. A similar increase in productivity was not observed in the epilithon.

There are a few possibilities that could account for a lack of epilithic response. Epilithic metabolism may not have been significantly contributing to the lake-wide

increase (no response) or perhaps the current methods were unable to detect a response. The latter is a likely explanation, as the increases in available P in L375 may have been too small to stimulate detectable increases in algal productivity, given the substantial background P in the biofilm (chapter 1). The latter is also possible given the number of potentially interacting factors acknowledged in chapters 1 and 2. Primarily, competition with the phytoplankton for nutrients and light may have played a role in the lack of a detectable response (Vadeboncoeur *et al.* 2001). Also, increased phytoplankton deposition from the early spring blooms (Findlay *et al.* in press) may have temporarily buried the epilithon, reducing available light. Furthermore, changes in grazing pressure due to the potential relocation of littoral grazers away from the littoral zone to the aquaculture cage may have hindered detection of an effect (Vadeboncoeur *et al.* 2003). A reduction in predation of invertebrate grazers would increase consumption of the benthic algae effecting algal cell densities composition and potentially achange benthic physiognomy (Frost *et al.* 2002; chapter 5). Finally, provided the biofilm P-levels were sufficient to overcome P-limitation, the biofilm may have moved towards DIC-limitation (chapter 1) (Turner *et al.* 1994).

Other studies that have looked at algal productivity in response to increased P from aquaculture operations have used measures of biomass and Chl a (Diaz *et al.* 2001; Temporetti *et al.* 2001; Mannino and Sara 2007), rather than primary productivity estimates. Similarly, studies on the effects of increased P in general on benthic algal metabolism are few (Turner *et al.* 1994; Hecky and Hesslein 1995; Vadeboncoeur *et al.* 2001). Future research should focus on providing a more comprehensive understanding of metabolic responses to anthropogenic perturbations in

benthic algae. As well, further aquaculture research should attempt to refine our understanding of whole-lake nutrient and energy pathways for both benthic and pelagic algae.

Comparisons of oxygen and DIC measurements

Past productivity studies have noted problems in measuring photosynthesis by changes in O_2 concentrations ((Mountford 1969; Bott *et al.* 1997; Moigis and Gocke 2003). The two main drawbacks include: (1) O_2 units have to be converted to CO_2 units for comparisons to other studies and the carbon budget (Moigis and Gocke 2003; Davies *et al.* 2003; Graneli 1979) and (2) in supersaturated conditions there is the possibility of bubble formation in bottles/chambers causing an underestimate of productivity (Bott *et al.* 1997). For these reasons, the main method of measuring productivity prior to 2006 was via changes in DIC in the water overlying the epilithon. However, the introduction of the new GC technique, which measured O_2 evolution and CO_2 fixation simultaneously from the same sample, allowed for a better understanding of both processes and therefore enabled comparisons between the two types of measurements. The two measurements of primary productivity generally agreed, though in some cases there was a very poor relationship. The occasionally very large inconsistencies between the O_2- and CO_2-derived data are inexplicable and are potentially reflections of methodological issues.

Discrepancies between measured fluxes can occur in highly productive sites where photosynthetic uptake exceeds replacement of CO_2 in incubation chambers. In these cases, it is possible for CO_2 limitation to result in incorrect measurements of algal productivity due to the limitation of a key photosynthetic substrate. Further inconsistencies in O_2 and CO_2 fluxes can occur because of issues in both the O_2 and CO_2

measurements. Carbon measurements are troubled by the possible re-fixation of intracellular respiratory CO_2 by the algae themselves (Robinson 1983) and potential drawdown of free CO_2 within the incubation chambers (M. Turner, pers. comm.). As stated earlier, O_2 measurements are troubled by possible bubble formation, though bubble formation was generally not observed in the current study, it should be noted, that bubbles have been detected in the past, adhering to the surface of the community in L239 and occasionally in incubation chambers (Turner *et al.* 1983).

Early investigations of the photosynthetic process show that the volume of O_2 liberated approximates the volume of CO_2 assimilated (Ryther 1956). It was therefore expected that in the current study, the PQ values would approximate one (Ryther 1956). For the most part, measurements provided by the current method yielded PQs close to unity (1.06 ± 0.40; Table 3.5), although variability existed between lakes, months and sites. Colijn *et al.* (1983) noted PQs ranging from 1.4 to 2.1 depending on the method of O_2 measurement (Winkler method vs oxygen electrode method) and incubation time. Also, the PQ can be affected by the form of nitrogen (NO_3 or NH_3) available (Colijn *et al.* 1983), the atomic ratio of C: N in the algae (Williams and Robertson 1991), as well as the general physiological conditions of the organisms in the community. Finally, Schindler *et al.* (1973) also suggested an overall asynchrony in diel CO_2 uptake and O_2 release, which may affect PQ calculations.

In previous studies done at ELA, Turner *et al.* (1983) found photosynthetic quotients that were near the mean reported in the current study (1.08 ± 0.14). They noted that these values were consistent with values reported for phytoplankton in Strickland 1960 and Fogg 1974. Schindler *et al.* (1973) also reported PQs for the epilithon in ELA

lakes; however, their benthic values were much higher and more variable, 2.7± 1.7 (using direct measures of DIC and O_2). Similarly, Bott (1978) compared methods of measuring periphyton photosynthesis in streams and estimated PQ to be 1.89 (measured using galvanic oxygen sensors and DIC Gran titrations with a pH meter). Finally, Williams and Robertson (1991) abstracted a number of PQs from the literature and found that for natural phytoplankton communities, quotients ranged from 0.5 to 3.5. These sources covered a range of environmental situations (freshwater and marine; oligotrophic to eutrophic). By accounting for a number of systematic errors in analyses (calibration errors, physiological errors and analytical imprecision) they were able to derive PQs that were contained within a range of 1.0 to 1.36, which was consistent with most of the observations made in the current study.

Often, PQs are determined from separate measurements of O_2 and CO_2 (Williams and Robertson 1991). The current GC method used the same sample and the same techniques to determine O_2 and CO_2, which should provide a more accurate determination of PQ. However, greater refinement of the technique is needed to decrease analytical imprecision. In addition to increased sample sizes, greater spatial and temporal resolution on samples would provide a more accurate representation of the PQ. Moreover, in order to gain further insight into natural variations in PQ values, more research is required on the influence of O_2 pressure, availability of nutrients, light history of the cells (Colijn *et al.* 1983), and natural decoupling of O_2 and CO_2 fluxes within benthic biofilms.

Inter-site variability

It was not surprising to see variation in metabolic rates between sites for the epilithon in the three lakes, as benthic communities are known for their heterogeneous nature (Wetzel 1965; Robinson 1983). The greatest variability in epilithic metabolism was observed in reference lake L373, % CV = 30% for CO_2-derived data and 34% for O_2 data (excluding site C; Fig. 3.19), the least in L239, % CV = 9 % for CO_2-derived data and 16 % for O_2 (excluding site F; Fig. 3.17). From the perspective of oxygen fluxes, sampling sites in L375 appeared to be quite similar (% CV = 15%. The CO_2 data, however, showed two clusters (sites A, D, E versus sites C, B, F) (Fig. 3.21) with a % CV = 21%. The overall degree of inter-site variability, excluding site C in L373 and site F in L239, was actually low with a % CV = 21% ± 11%.

These natural communities are a combination of extremely diverse microhabitats (Wetzel 2001a), which likely range in developmental stages (Robinson 1983), as well as compositionally among sites (chapter 2). In the early stages of community development, colonizing organisms spread randomly from the clump, creating variability among sites (Robinson 1983). In the late stages of development, variance increases with community density and thickness (Robinson 1983; Wetzel 2001a). Compositional differences among sites also translate into variation in metabolic rates, as different algal groups and species metabolize at different rates (Wetzel 2001a). Differences in light availability within a periphyton canopy and patchy distribution of light through the euphotic zone can also cause variability in metabolic rates between sites (Wetzel 2001a). Finally, variation can be caused by changes in the influences of the non-algal components (bacteria and microscopic grazers) in the epilithic community at each site (Robinson 1983).

The current study has provided a provisional measure of natural variability in the epilithon (% CV = 21%), which can be used in the interim to explain the extent of natural variability in the data. Further research with greater spatial and temporal (seasonal and diurnal fluctuations) resolution would provide a more accurate representation of natural variability.

Conclusions

All the measured parameters indicated that the aquaculture operation did not affect benthic algal metabolism. Agreement between primary productivity measurements and biomass estimates (chapter 2) point to the likelihood that there was no major permanent effect on the benthic algae caused by the aquaculture operation, in the timeframe of this experiment. The most likely explanation for this conclusion is that the increases in available P in L375 were too small to stimulate a major increase in algal productivity. However, several issues with the recently adopted methodology placed doubt on the ability to detect an aquaculture impact. Acknowledgment of these issues allowed for a number of recommendations to be made for improvement in future studies. Mainly, future research into benthic algal metabolic responses to anthropogenic perturbations should consider using a simpler approach to measuring benthic algal productivity.

Chapter 4: Epilithic Toxins: What lies below the surface? Understanding microcystin-LR in epilithic biofilms of Canadian Shield lakes

Introduction

Microcystins are potent liver toxins produced by several species of cyanobacteria (Lindholm *et al.* 2003). These toxins have been implicated worldwide in the poisonings and deaths of wildlife, livestock and humans, following consumption of water containing the toxin (Kotak *et al.* 1995; Kotak *et al.* 1996). In addition to these potent hepatotoxic effects, acute exposure to the toxin can also result in kidney damage and gastrointestinal distress (Kotak and Zurawell 2007). More than 70 microcystin analogues or variants have been structurally identified worldwide (Kotak and Zurawell 2007). Of these, microcystin-LR (MC-LR) is the most common (Kotak and Zurawell 2007) and toxic analogue, with an LD_{50} of 50 μg/kg in mice (Kotak *et al.* 1996).

Blooms of certain cyanobacterial genera, (e.g., *Anabaena, Anabaenopsis, Aphanizomenon, Oscillatoria (Planktothrix) Microcystis, and Nostoc*) have been linked to increased microcystin concentrations (Zurawell *et al.* 2005) (Table 4.1). Cyanobacteria are commonly found in productive water bodies. The ubiquitous distribution of cyanobacteria and their ability to dominate phytoplankton communities is related, in part, to the concentration and ratio of nutrients such as phosphorus and nitrogen in a water body (Kotak *et al.* 2000). When the N:P ratios are low in the water body, conditions are conducive to cyanobacterial growth (Wetzel 2001b). Since microcystin concentrations are closely linked to species abundance, and planktonic cyanobacterial biomass is

strongly linked to water column nutrient concentrations, there is a positive correlation between TP and microcystin concentrations in the water column (Kotak *et al.* 1995).

Current microcystin research has focused on the mobilization of the toxin to the food web through these pelagic cyanobacterial blooms (Prepas *et al.* 1997; Zurawell *et al.* 2005; Kotak and Zurawell 2007). Generally, microcystin production is perceived to occur primarily from planktonic cyanobacterial species, even though there are a number of the same toxin-generating species present in benthic biofilms (Table 4.1) (Stevenson *et al.* 1982).

Anthropogenic nutrient additions in two of the lakes in the current study were due, in large part, to aquaculture operations. Understanding toxin production in these lakes has both environmental and management implications. Of greatest concern to fish farmers is whether these toxins will affect their commercially important fish. The main route of exposure for caged fish is through feeding on organisms fouling the net-pens (Kotak *et al.* 1996). Kent *et al.* (1988; 1990) and Anderson *et al.* (1993) (in Kotak *et al.* (1996)) reported severe liver lesions and mortality in net-pen reared Atlantic salmon. When tested the liver lesions were chromatographically indistinguishable from those induced by MC-LR in laboratory experiments. Phillips *et al.* 1985 observed that chronic exposure of the toxin manifests itself as focal, multifocal, or massive zones of coagulative or liquifactive hepatocyte necrosis in rainbow trout exposed to MC-LR by intraperitoneal injection (Phillips *et al*. 1985). This can lead to severe necrosis of hepatocytes, loss of liver function and perhaps liver tumour promotion in caged fish (Kotak *et al.*1996).

Though current husbandry practices include ridding the cages of algal growth by mechanical means, these practices may be escalating the problem by releasing

microcystins into a concentrated area where the fish can not escape the toxins (Phillips *et al.* 1985a). The alternative to using mechanical anti-fouling is to use nets and structures treated with anti-fouling agents, however there are several environmental concerns associated with these nets (Burridge 2003).

A less critical pathway for microcystins to enter the food chain of caged fish is through the consumption of native fish species which have been feeding in the littoral zone. Aboal and Puig (2005) provided evidence for this phenomenon when they detected microcystin producing algae in cyprinids in Spain. There is still little understanding of the capability of microcystins to bioaccumulate in aquatic animals, although a study by Chen *et al*. (2005) suggests that microcystins can bioaccumulate in the hepatopancreas of snails.

Since aquaculture wastes (feed and excretory) have the potential to increase P in the system, there may be a decrease in the ratio of N: P conducive to cyanobacterial growth and microcystin production. However, unlike in phytoplankton, benthic algae correlate less strongly with water column nutrients (chapter 1) and may therefore be influencing water bodies unsuspectingly by producing microcystins in lakes deemed to be harmless based on pelagic criteria. Blaha and Marsalek (2003) describe a situation in the Czech Republic where raw water samples from water intake lines were measured and significant concentrations of microcystins were detected. No apparent pelagic cyanobacterial blooms were present and concentrations were determined to be caused by periphyton. Mez *et al*. (1997) also reported significant microcystin concentrations in benthic biofilms of oligotrophic Switzerland lakes, attributing a number of cattle deaths in the last 20 yrs to high benthic levels of the toxin. If the benthic algal community is responsible for toxin

production in the systems it may be occurring without any visible signs (Blaha and Marsalek 2003), unsuspectingly affecting farm health, management and yield.

Neglecting to measure microcystin concentrations in this community overlooks a potentially large pool of the toxin. The current study aims to: (1) confirm that benthic biofilms can produce the toxin in Canadian lakes; (2) understand whether epilithic microcystin concentrations are linked to the P gradient and (3) investigate which epilithic taxa are responsible for producing the toxin.

Table 4.1: Potential microcystin producing genera and species, based on the published literature.

Genera/Species [1]	Reference (s)
Microcystis spp.	Kenefick *et al.* 1993; Chorus and Bartram 1999; Zurawell *et al.* 2005; Kotak *et al.* 2001
M. aeruginosa (Kutz.) Kutz.	Chorus and Bartram 1999; Zurawell *et al.* 2005; Kotak *et al.* 2001
M. viridis (A. Braun) Lemm.	Chorus and Bartram 1999; Znachor *et al.* 2006
M. flos-aquae (Wittr)	Zurawell *et al.* 2005; Znachor *et al.* 2006
M. wesenbergii (Lemm.) Lemm.	Zurawell *et al.* 2005; Znachor *et al.* 2006
M. ichthyoblabe (Kutz.) Kutz.	Znachor *et al.* 2006
Anabaena spp.	Kenefick *et al.* 1993; Chorus and Bartram 1999; Blaha and Marsalek 2003
A. flos-aquae (Lyngb.) Born. et Flah.	Chorus and Bartram 1999; Znachor *et al.* 2006
A. torulosa [2] (Carm.) Lagerh	Blaha and Marsalek 2003
A. circinalis Ravenh. Ex Born. et Flah.	Willen and Mattsson 1997; Zurawell *et al.* 2005; Znachor *et al.* 2006
A. lemmermannii Richt.	Zurawell *et al.* 2005; Znachor *et al.* 2006
A. viguieri Denis et Fremy	Zurawell *et al.* 2005
A. crassa (Lemm.) Kom.-Legn. et Cronb.	Znachor *et al.* 2006
A. spiroides (Kleb.)	Znachor *et al.* 2006
A. mendotae Trel.	Znachor *et al.* 2006
A. planctonica Brunn.	Znachor *et al.* 2006
Oscillatoria [2] spp.	Kenefick *et al.* 1993; Chorus and Bartram 1999; Blaha and Marsalek 2003
O. limosa Agardh	Willen and Mattsson 1997
O. sancta (Kutz.) Gom.	Willen and Mattsson 1997
O. aghardii (Planktothrix aghardii) [2]	Chorus and Bartram 1999; Hamill 2001;

Lemm.	Blaha and Marsalek 2003
O. formosa [2] Bory ex. Gomont	Hamill 2001
Phormidium spp.[2]	Izaguirre *et al.* 2007; Blaha and Marsalek 2003
Phormidium vulgare Kutz.[2]	Blaha and Marsalek 2003
Nostoc spp.	Kenefick *et al.* 1993; Blaha and Marsalek 2003; Zurawell *et al.* 2005
Nostoc muscorum [2](Agardh)	Blaha and Marsalek 2003
Aphanizomenon spp.	Zurawell *et al.* 2005; Znachor *et al.* 2006
A. ovalisporum Forti.	Znachor *et al.* 2006
A. klebahnii (Elenk.) Pech et Kal.	Znachor *et al.* 2006
A. yezoense M. Wat.	Znachor *et al.* 2006
A. flos-aquae (Linn.) Ralf. ex Born. et Flah.	Znachor *et al.* 2006
A. issatschenkoi Pros.-Lavr	Znachor *et al.* 2006
Woronichia naegeliana (Ung.)	Znachor *et al.* 2006
Lyngbya[2] spp.	Izaguirre *et al.* 2007
Pseudoanabaena[2] spp.	Herry *et al.* 2007
Gleotrichia echinulata (Smith) Richter	Willen and Mattsson 1997

[1]Potential genera/species, more genera/species may exist
[2]Reported in the literature as benthic microcystin producing taxa

Materials and methods

Description of study area

Epilithic microcystin concentrations were examined in four lakes of differing nutrient status, in two separate locations. The first location included three naturally nutrient poor, soft-water lakes at the Experimental Lakes Area in northwestern, Ontario, Canada (lat. 49º30'-49º45'N, long. 93º30'-94º00'W) (Fig. 2.1). A full description of the area's geology, vegetation, soils and climate can be found in Brunskill and Schindler (1971).

Of the three ELA lakes, two (L239 (Fig. 2.4) and L373 (Fig. 2.3)) were long-term ELA reference lakes and have not been manipulated for experimental purposes. The third lake (L375; Fig. 2.2) was experimentally enriched with nutrients from an aquaculture operation. In this experimental operation, a 10-m^3 aquaculture cage was installed in the north end of the lake in the spring of 2003. For five years of operation (2003-2007) the cage was stocked every subsequent spring with approximately 10,000 rainbow trout (mean initial weight = 0.1 kg), fed a high energy diet (Profishent ®), and harvested in the fall (mean final weight = 1 kg). A more detailed explanation of nutrient loading rates can be found in chapter 2 and Appendix I.

All three of the study lakes were small, moderately deep, naturally oligotrophic, dimictic lakes, which stratified thermally in the summer and winter (Table 2.2). The experimental lake, L375, was a second-order lake that received stream input from upstream L373. The two reference systems were both first-order lakes with similar water chemistry (Table 2.2). During the years of the aquaculture operation (2003-2007), the

reference systems had TP values ranging from 5.5-6.5 $\mu g \cdot L^{-1}$ and the experimental system had a mean annual average of ~8.0 $\mu g \cdot L^{-1}$ (Tables 2.2; Appendix I Fig. I.1).

The second location, Lake Wolsey (lat. 45º, 50'N, long. 82º, 32'W) (Appendix II Table II.1; Fig. II.1; Fig. II.2), included a nutrient-enriched lake (Gale 2000 cited in Clerk 2002) located along the north-western shoreline of Manitoulin Island (Appendix II Fig. II.1). Lake Wolsey was not a true inland lake; rather, it was a large bay connected to the North Channel of Lake Huron by a narrow opening at Campbell Bay (Clerk 2002). A full account of the area's geology, vegetation, history and land use can be found in Clerk (2002).

Lake Wolsey was also the site of a long-term aquaculture operation, which began in 1986 (Clerk 2002). The inclusion of this lake in the study stemmed from the fact that like L375, it received nutrient inputs from aquaculture practices. Currently, the farm consists of 18 functioning cages, with production estimates approximating 295 tonnes of fish per year (C. Podemski, pers. comm.).

Lake Wolsey was a large lake, 100-times larger than the lakes at ELA; although, it was similar in depth (mean depth =11m) (Appendix II Table II.1). It had no significant stream inputs and its only outflow was to Campbell Bay. However, bidirectional flow through the causeway at Campbell Bay allowed inflow from Lake Huron into Lake Wolsey. Water quality measurements taken from 1998-99 indicate TP values of 9 and 20 $\mu g \cdot L^{-1}$ for spring and fall turnover, respectively (Clerk 2002).

Epilithic particulate sampling

Epilithic samples were collected during the ice-free season in each study lake, though sampling details varied between lakes and years. For the ELA lakes, microcystin

sampling occurred in July and August in 2006 and May to October in 2007. Archived frozen epilithic slurries from 1997-2005 from M. Turner were also analyzed. All these samples were based on a long-term epilithic monitoring system and were restricted to July and August sampling, although, some years were absent from the data series (1998-2001 for L375). Historical data for all other particulate parameters (epilithic chemistry; C, N, P and algal taxonomy) were also available using the *Epilithic Data Retriever* in the *ELA Data Retriever* version 7.1. Both the historical data and the analyzed archived slurries were based on the same samples.

The archived and current samples were collected in the same fashion from the same sites in the ELA lakes. Unshaded sites on the north shore were used in each lake, which in the experimental lake were near the aquaculture cage. Naturally occurring communities were studied in the middle littoral zone (depths of 1 to 3 m). The epilithic samples were obtained from natural rock or bedrock shelves in areas of low slope (<10º, assessed visually) (Turner *et al.* 1987). Three sites were sampled regularly, allowing a four-week recovery period between sampling occasions. In 2007, the number of sites was increased from three to six, in order to increase understanding of inter-site variation.

A SCUBA diver removed the epilithon from the rock surface using a scraping brush sampler (chapter 2; Turner *et al.* 1991). Four 5-cm^2 samples were collected at each of the three to six sites. These samples were then placed on ice, kept in darkness and brought back to the lab for sample preparation.

The procedure at Lake Wolsey was slightly different. Sampling occurred over several days during a single visit to Lake Wolsey during September 2006. Samples were removed from two depths, at each of six sites varying in distance from the aquaculture

cage along the western shoreline. Since most available surfaces in the middle littoral zone were colonized by zebra mussels (*Dreissena polymorpha* Pallas; Appendix II), I adapted the epilithic sampling technique by sampling in shallower locations with fewer zebra mussels. Two depths were sampled to assess possible depth-related bias. The deeper depth, termed the *transition zone* for its change in zebra mussel density from high to low (based on a visual assessment) closely approximated our ELA epilithic middle littoral sampling depth of 1-3 m below the surface. However, the transition zone was not free from zebra mussel influence; therefore, a shallower depth was added in the wave zone, which was approximately 0.5-0.8 m below the lake surface and essentially free of zebra mussels.

In order to decrease the variability in sampling, effort was increased at each site to include four sub-sites. At each of the four sub-sites, four 5-cm^2 samples were collected in the same manner as at ELA with the SCUBA-borne scraper sampler. Soon after collection samples were immersed in ice and brought back to the lab for sample preparation. Samples were prepared for epilithic particulate chemistry, algal taxonomy and microcystin analyses, as was done for the ELA lakes.

Epilithic sample preparation

Sample processing occurred within 24 hours of sampling (samples were kept immersed in an ice bath until preparation). For the ELA samples, the four 5-cm^2 samples were pooled to create a site-specific sample. These were blended for three, 1-s pulses at low speed to homogenize the samples. Similarly in Lake Wolsey, four 5-cm^2 samples were combined at both depths. The suspension was then transferred to a stirring beaker and subsamples were removed using a large-bore syringe. The ELA suspensions were

then combined to produce a lake-composite sample. In 2007, one lake composite based on the synoptic time-series sites was prepared; in addition, a second lake composite was created based on inclusion of the second set of three sites. Duplicate subsamples were removed from these composite suspensions in order to examine analytical replication. The Lake Wolsey sub-site samples were combined to produce a composite for each site, keeping the shallow and transition zone samples distinct. From each of the suspensions, 20-mL subsamples were removed using a large-bore syringe and frozen in darkness until microcystin analysis. Also, duplicate 20-ml subsamples were removed and preserved with 4% Lugol's solution for algal enumeration, taxonomy and biovolume estimation. Algal composite samples were analyzed following the modified Ütermohl technique (Nauwerck 1963), as described in chapter 2.

Microcystin Analysis

All microcystin analyses were performed by AlgalTox International (Pine Falls, Manitoba). The epilithic samples were thawed and ultrasonicated for 10-20 seconds at ½ of maximum probe output to disrupt the algal cells. The homogenized samples were then filtered through 0.45 μm nitrocellulose filters and the filtrate analyzed directly for microcystin-LR (B. Kotak, AlgalTox International, Pine Falls, Manitoba, pers. comm.).

The samples were analyzed using protein phosphatase analysis outlined in An and Carmichael (1994). This colorometric assay was based on the principle that protein phosphatase-1c catalyzes the removal of phosphate (colorless) to p-nitrophenyl phosphate to produce a yellow endpoint. Microcystins inhibit this reaction and an increase in concentration of microcystins corresponds to a decrease in color. All samples were analyzed in triplicate, with duplicate controls (B. Kotak, Algal Tox International, Pine

Falls, Manitoba, pers. comm.). The protein phosphatase assay measured total microcystins; the reported values however were based on standardization of the value to microcystin-LR equivalents. The detection limit for microcystins was equal to 2 $ng \cdot cm^2$. Values below the detection limit were set to ½ the detection limit and used in calculations.

Microcystin concentrations were expressed on a per area basis (i.e.; microcystin concentration in the epilithon per cm of rock surface), rather than on a volumetric (Kotak *et al.* 1996) or per gram basis (Aboal and Puig 2005). Where comparisons were required with other studies conversions were made to per grams dry weight. Since approximately 54% of dry weight can be expected to be carbon (O'Sullivan and Reynolds 2004), the particulate carbon values determined for each corresponding sample were multiplied by 2.2. This value approximated the dry weight of the samples, which was then converted from $\mu g \cdot C \cdot cm^{-2}$ to $g \cdot C \cdot cm^{-2}$ and used to calculate $\mu g \cdot g^{-1}$ microcystin.

Data Analysis

Analysis of the least square means was used to analyze whether microcystin concentrations were higher in the treatment lakes compared to unperturbed reference systems. Data from the three ELA lakes (L239, L373, and L375) were treated as repeated measures data. That is, values within a year were averaged and then the available data from the years 1997 – 2007 were utilized in the analyses; standard errors were also computed. All samples from Lake Wolsey in 2006 were averaged and a standard error computed. A difference was determined when there was no overlap in the ± 1 standard error bars corresponding to the different lakes.

Also, 2006 and 2007 samples were examined independently for microcystin, cyanobacterial biomass and for potential microcystin producer biomass. Since the multiple values taken in the Lake Wolsey area were only subject to spatial variance while the values from ELA incorporate both spatial and temporal variance, the Lake Wolsey values were not compared using the aforementioned technical analysis.

In order to determine whether the aquaculture operation affected microcystin concentrations in the experimental lake at the ELA an analysis of covariance was used to model the data. The dependent variables were natural log-transformed to normalize the data and homogenize the error variance. Yearly data from the two reference lakes were averaged and treated as a covariate. Year was also modeled as a continuous covariate to detect temporal trends. An additional pre vs. post-treatment effect was modeled as a categorical predictor variable. The "pre-post" variable was allowed to interact with the average values in the reference lakes and also with year to see, if either the temporal trends in the treatment lake or patterns in the reference lakes were different during the cage years versus pre-cage years. Analyses were conducted using SAS PROC Mixed (SAS for Windows V.8). In all cases, a p-value between $0.10 \geq p \geq 0.01$ was considered significant and $p < 0.01$was highly significant (chapter 2). All correlation analyses were performed using the Spearman Rank Order Correlation analysis in Sigma Plot (version 9.0) software.

Results

Microcystin concentrations

Microcystins were detected in 80% of the samples in L375 (n=25), 25% of samples in Lake Wolsey (n=13), 94% of samples in L239 (n=18) and 100% of samples in L373 (n=20). The analysis of the least square means revealed a significant lake effect ($F(2, 8.75) = 5.11$; $p = 0.034$, for values from 1997-2007; Fig. 4.1). Microcystin levels were higher in the reference lakes than in Lake 375. In 2006, there was a difference between the reference systems; however, in 2007 no difference could be detected (Fig. 4.1). In both 2007 and 2006 there was a significant difference between the reference lakes and L375 (Fig. 4.1). There was also a significant difference between Lake Wolsey and the reference systems; although L375 and Lake Wolsey were not statistically different (Fig. 4.1).

At the ELA, concentrations of the toxin were higher in the oligotrophic reference lakes than in the experimental aquaculture lake. Concentrations in L239 for 2006 and 2007 were 7.7 ± 2.4, 7.0 ± 1.0 $ng \cdot cm^{-2}$, respectively, and in L373 they were 9.4 ± 2.4, 12.5 ± 2.5 $ng \cdot cm^{-2}$, respectively (Fig. 4.2). The experimental aquaculture lake had mean microcystin concentrations respectively in 2006 and 2007 of 3.2 ± 1.1 to 3.5 ± 0.5 $ng \cdot cm^{-2}$, respectively. The overall range for benthic microcystin levels at the ELA from 1997 to 2007 was 2.0 to 24.0 $ng \cdot cm^{-2}$ or 4.6 $\mu g \cdot g^{-1}$ to 60.0 $\mu g \cdot g^{-1}$ dry wt (Fig. 4.2).

Concentrations of the toxin in the long running aquaculture operation at Lake Wolsey were even lower with many samples below the detection limit (75% of samples n=13). The mean concentration of samples was below the detection limit at 2 $ng \cdot cm^{-2}$ (Fig. 4.1)

For all the years of aquaculture operation microcystin concentrations were above the pre-cage mean of 2 $ng \cdot cm^{-2}$ (Fig. 4.2). However, concentrations remained below the average of the reference systems throughout the study period at ELA, with the exception of 2005, in which microcystin concentrations were especially low in the reference systems (Fig. 4.2). Concentrations in the reference systems were generally 40-60% higher than the experimental lake (Fig. 4.2).

The aquaculture operation did not statistically affect microcystin concentrations and there was no significant difference between pre-cage years and years of cage operation (Table 4.2; $p>0.1$). There may have been a year effect occurring in the experimental lake(Table 4.2; $p = 0.1$), however, it was unrelated to the cage operation ($p = 0.24$).

Table 4.2: Results of a mixed procedure analysis of covariance on microcystin concentrations in the experimental lake 375 compared to the average of the reference lakes (L239 and L373) before (1997-2002) and during cage operation (2003-2007). Significant values are distinguished from non-significant values by an asterisk.

	Effect	df	F-value	P-value
Microcystin concentrations	Experimental lake vs reference lakes	1,4	9.5	0.20
	Pre-cage vs cage years in the treatment lake	1,4	6.2	0.24
	Interaction between lakes and pre vs cage years	1,4	0.5	0.61
	Year	1,4	39	0.10*
	Interaction between year and pre vs cage years	1,4	6.2	0.24

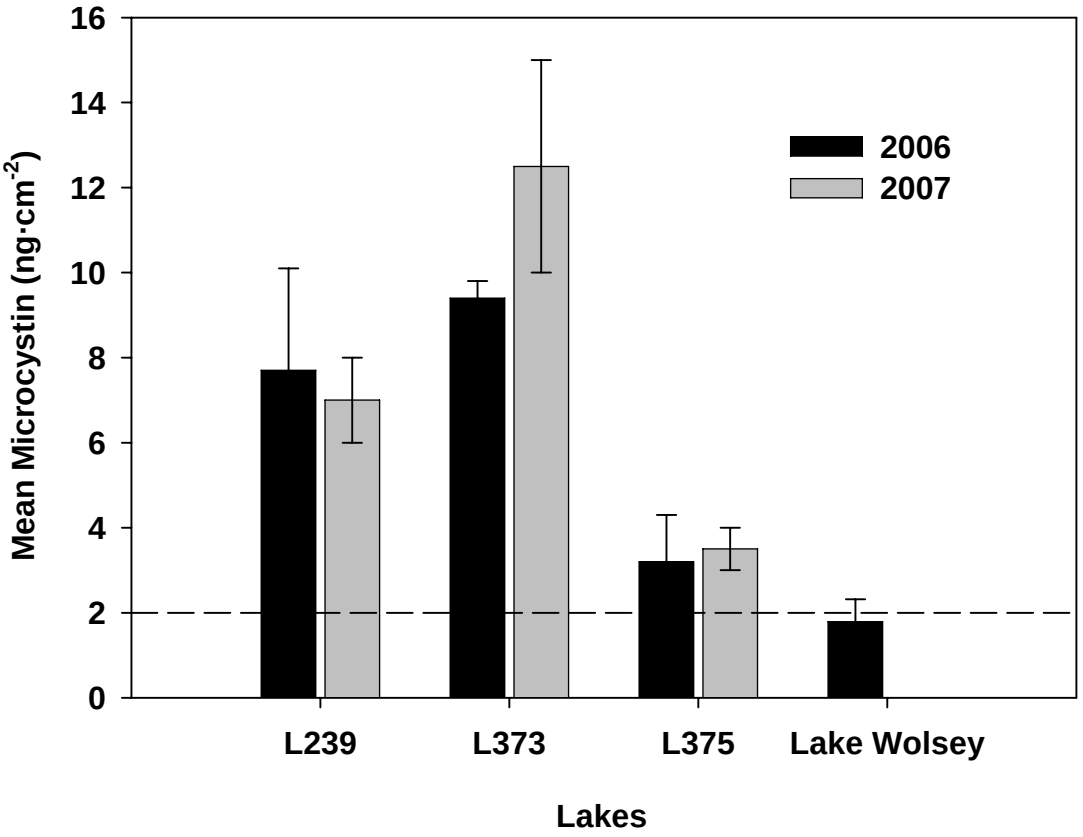

Figure 4.1: Mean microcystin concentrations in the experimental Lake 375 (n = 25), reference lakes (L239 (n = 18) and L373 (n = 20)) and Lake Wolsey (n = 13) in 2006 (black) and 2007 (grey). The standard error bars are based on the standard error of the mean. The horizontal dashed line represents the microcystin detection limit; values below the detection limit were set to half the detection limit in calculations.

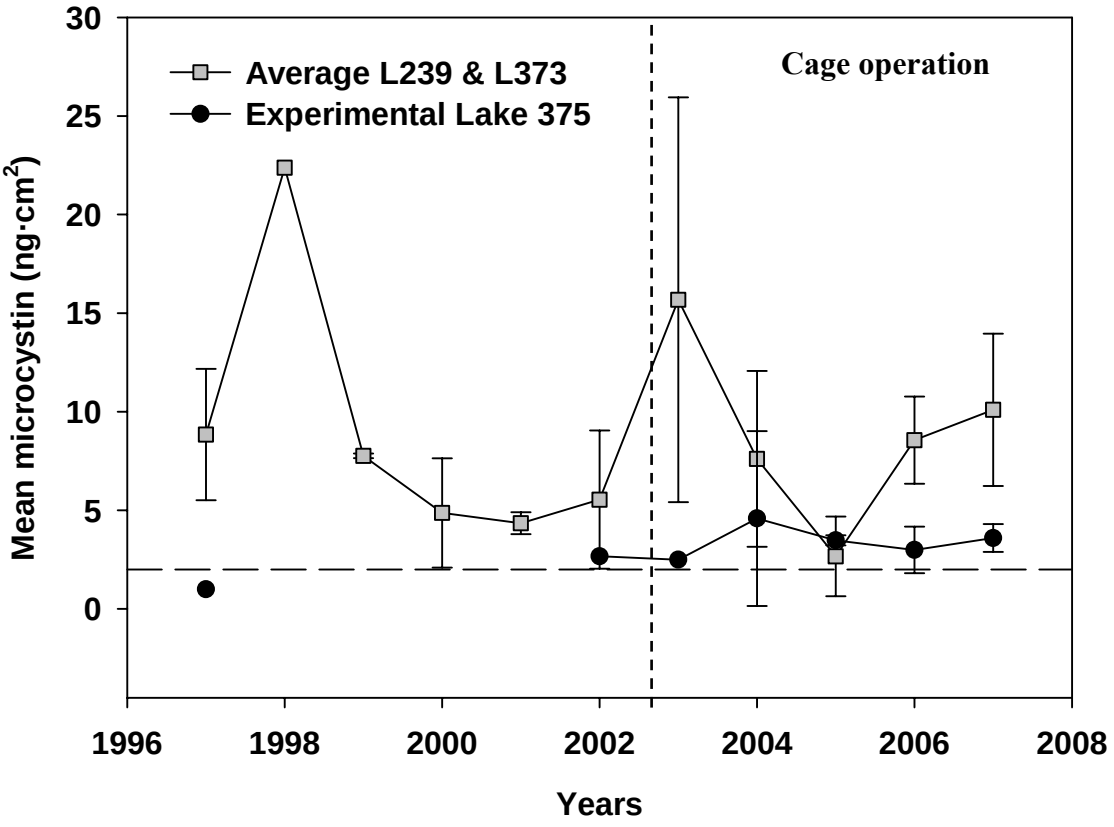

Figure 4.2: Microcystin concentrations ($ng \cdot cm^{-2}$) in the experimental lake (L375) and reference lakes (L239 and L373) from 1997 to 2007. The detection limit for microcystin ($2\ ng \cdot cm^{-2}$) is shown as the horizontal dashed line in the figure. The vertical dashed line distinguishes pre-cage years from cage years in the time series.

Biomass and composition

Cyanobacterial biomass was higher in the experimental lake relative to the reference systems during the farm operation (Fig. 2.17; Table 2.6 p = 0.01). However, pre-cage and during cage operation biomass estimates were not significantly different, despite the substantial and sustained increase above the reference systems in 2005 (Fig. 2.17). In 2006 and 2007, in all three ELA lakes, cyanobacterial biomass was less than 50% of the total algal biomass and in 2006 in Lake Wolsey it was less than 10% of the total biomass (Fig. 4.3).

In the experimental lake, cyanobacterial biomass was primarily composed of three main taxa in 2006, *Scytonema* sp., *Oscillatoria sancta* (Kutz.) Gomont, and *Chroococcus turgidus* (Kutz.) Nageli (Table 4.3). In 2007, the main taxa were *Nostoc* spp., *Calothrix* sp. and *Scytonema* sp. (Table 4.3). The reference systems were dominated by *Scytonema* sp. and *Lyngbya* sp. in both years. The main cyanobacterial species in Lake Wolsey was *Phormidium autumnale* Agarh (Table 4.3).

Only a small portion the total cyanobacterial biomass was composed of microcystin producing taxa (Fig. 4.4). In 2006, only 19% of total L239 cyanobacterial biomass was potential microcystin producers, in L373: 6% and L375: 10% (data not shown). In 2007 in L239, 14% of biomass was potential microcystin producers, in L373, 17% and in L375, 18%. The percentage of potential microcystin producers was higher in Lake Wolsey (44%), even though microcystin production was low (Fig. 4.1). There was no significant relationship between microcystin concentrations and biomass of potential microcystin producers (Table 4.4: correlation coefficient = - 0.61, p-value = 0.12

(n = 20)) in 2006 or 2007. Similarly, when each individual producer was examined separately there was no correlation with toxin production (Table 4.4).

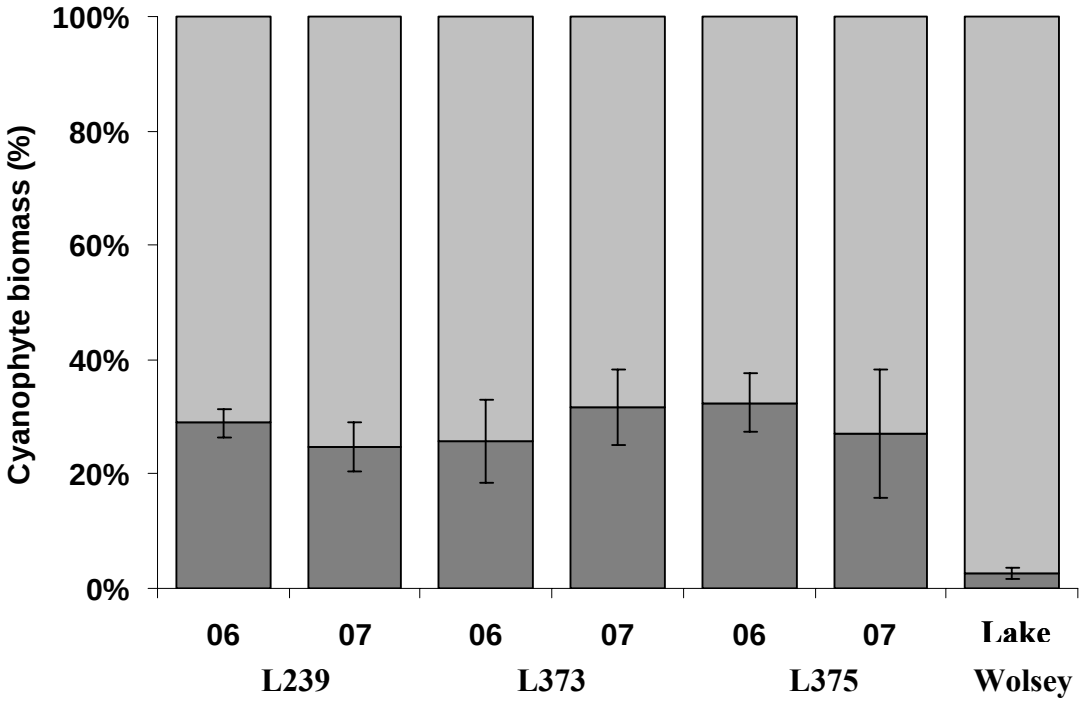

Figure 4.3: Cyanobacterial biomass (dark grey) relative to total algal biovolume (light grey) in the epilithon of L239, L373, L375 (n = 4) and Lake Wolsey (n = 6) in 2006 and 2007. The error bars represent standard error of the cyanobacterial mean.

Table 4.3: Cyanobacterial composition in lakes 375, 373 and 239 at the Experimental Lakes Area (July and August 2006 and 2007) and in Lake Wolsey (September 2006).

		L375 Biomass (µg·cm^2)			**L373 Biomass (µg·cm^2)**			**L239 Biomass (µg·cm^2)**			***Lake Wolsey* (*µg·cm^2*)**		
Species	**Year**	**No. of samples**	**Mean**	**Range**	**No. of samples**	**Mean**	**Range**	**No. of samples**	**Mean**	**Range**	**No. of samples**	**Mean**	**Range**
Anabaena sp.	2006	1	2	-	-	-	-	-	-	-	-	-	-
	2007	-	-	-	-	-	-	-	-	-	-	-	-
Anabaena cylindrical Lemm.	2006	-	-	-	-	-	-	-	-	-	-	-	-
	2007	-	-	-	1	1	-	-	-	-	-	-	-
Anabaena flos-aquae (Lyngb.) Breb. et Bornet	2006	-	-	-	-	-	-	-	-	-	1	161	-
	2007	-	-	-	1	1	-	-	-	-	-	-	-
Anabaenopsis sp.	2006	3	12	4-28	4	12	7-21	2	2	1-3	2	33	23-44
	2007	3	4	1-6	6	2	1-3	2	1	0-3	-	-	-
Calothrix sp.	2006	1	76	-	4	55	27-86	-	-	-	5	95	17-277
	2007	1	322	-	6	2	1-3	2	1	0-3	-	-	-

Chroococcus limneticus Lemm.	2006	-	-	-	2	2	-	2	2	-	-	-	-
	2007	4	6	1-8	5	3	2-4	4	1	1-3	-	-	-
Chroococcus turgidus (Kutz.) Nageli	2006	4	171	103-233	-	-	-	-	-	-	2	15	4-26
	2007	4	39	6-79	-	-	-	-	-	-	-	-	-
Cyanodictyon sp.	2006	1	1	-	-	-	-	-	-	-	-	-	-
	2007	-	-	-	-	-	-	-	-	-	-	-	-
Cylindrospermum sp.	2006	2	46	26-66	-	-	-	-	-	-	-	-	-
	2007	1	5	-	-	-	-	-	-	-	-	-	-
Gleocapsa punctata Nageli	2006	4	83	50-118	2	14	12-15	-	-	-	5	14	4-30
	2007	-	-	-	3	5	3-7	2	6	3-8	-	-	-
Gleothece sp.	2006	-	-	-	2	1	-	2	12	10-13	-	-	-
	2007	5	69	5-279	6	11	4-22	4	10	6-13	-	-	-
Heteroleibeinia profunda Komarek	2006	-	-	-	1	13	-	-	-	-	-	-	-
	2007	-	-	-	-	-	-	-	-	-	-	-	-

Lyngbya sp.	2006	4	180	62-349	4	146	91-257	4	550	364-721	6	12	2-21
	2007	2	194	110-571	6	273	221-351	4	250	175-329	-	-	-
Nostoc sp.	2006	2	31	18-43	-	-	-	2	55	50-61	-	-	-
	2007	2	340	-	2	99	95-103	-	-	-	-	-	-
Oscillatoria sancta (Kutz.) Gomont	2006	4	218	138-383	-	-	-	-	-	-	-	-	-
	2007	-	-	-	-	-	-	-	-	-	-	-	-
Phormidium autumnale Agardh	2006	-	-	-	-	-	-	-	-	-	5	552	44-1948
	2007	-	-	-	-	-	-	-	-	-	-	-	-
Planktothrix agardhii (Gom.) Anagnostidis et Komarek	2006	-	-	-	-		-	-	-	-	-	-	-
	2007	-	-	-	1	3	-	-	-	-	-	-	-
Pseudoanabaena sp.	2006	1	55	-	-	-	-	1	3	-	4	5	2-9
	2007	-	-	-	-	-	-	1	2	-	-	-	-
Radiocystis geminate Skuja	2006	-	-	-	-	-	-	-	-	-	-	-	-

	2007	1	3	-	-	-	-	-	-	-	-	-	-
Rhabdogloea lineare Scmidle and Lauterborn	2006	-	-	-	-	-	-	-	-	-	-	-	-
	2007	1	1	-	-	-	-	-	-	-	-	-	-
Rivularia sp.	2006	-	-	-	-	-	-	-	-	-	-	-	-
	2007	3	114	63-162	-	-	-	-	-	-	-	-	-
Scytonema sp.	2006	4	425	202-586	4	394	70-793	4	236	58-477	-	-	-
	2007	3	208	112-398	6	207	93-524	3	233	170-284	-	-	-
Snowella sp.	2006	2	24	22-26	-	-	-	-	-	-	1	9	-
	2007	3	27	22-32	-	-	-	-	-	-	-	-	-
Tychonema rhodonema Skuja	2006	-	-	-	-	-	-	-	-	-	-	-	-
	2007	1	83	-	-	-	-	-	-	-	-	-	-

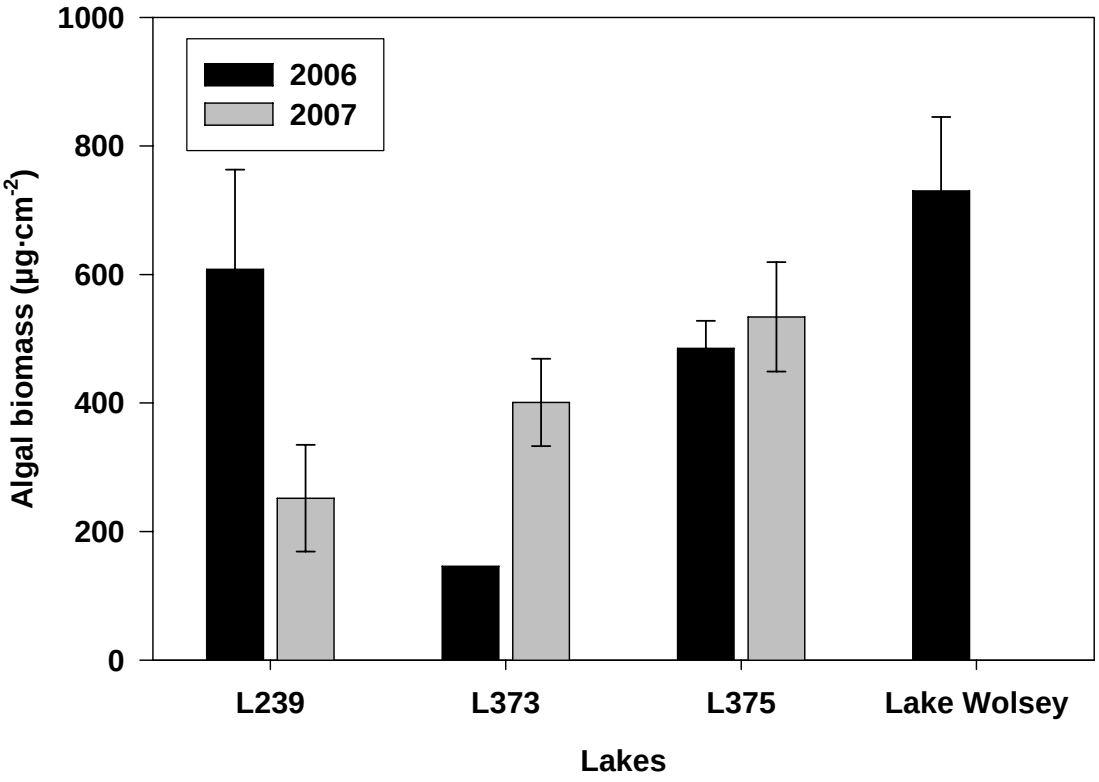

Figure 4.4: Mean biomass of potential microcystin producing taxa in the experimental lake 375 (n = 4), reference lakes (L239 and L373 (n = 4)) and Lake Wolsey (n = 6) in 2006 (black) and 2007 (grey). The standard error bars are based on the standard error of the mean. No error could be computed for L373 (2006) since only one estimate of biomass was available for potential microcystin producing taxa.

Table 4.4: Spearman Rank Order Correlation comparing microcystin concentrations to known microcystin producing taxa in the reference systems (L239 and L373), experimental lake (L375) (2006 and 2007) and Lake Wolsey (2006). There was no correlation between any of the potential microcystin producing taxa and microcystin production

Taxa	Correlation to Microcystins		
	No. of samples	Correlation coefficient	P-value
Anabaena sp.	3	-0.50	1.00
Lynbya sp.	7	0.57	0.15
Nostoc sp.	4	0.40	0.75
Oscillatoria sp.	1	1.00	1.00
Phromidium sp.	1	1.00	1.00
Pseudoanabaena sp.	4	-0.60	0.42
Total	20	-0.61	0.12

Microcystin concentrations relative to limnological variables

The Spearman Rank Order Correlation showed that there was no significant relationship between microcystin concentrations and either epilimnetic of epilithic P in the reference lake 239 or in the experimental lake during July and August sampling from 1997 to 2007 (Table 4.5; p values > 0.10). Reference Lake 373 did, however, show a positive correlation between microcystins and epilithic P (Table 4.5; p-value = 0.002). There was no significant relationship between microcystins and epilithic cyanobacterial biomass in any of the study lakes (Table 4.5). Also, neither of the reference systems showed a correlation between N: P and microcystin concentrations. The experimental lake did, however, show a negative correlation to N: P (Table 4.5: correlation coefficient = -0.68, p-value = 0.07).

In Lake Wolsey microcystin concentrations correlated poorly with all examined limnological variable in the shallow zone (Table 4.6). However in the transition zone microcystin concentrations showed a negative correlation to N: P (Table 4.6: correlation coefficient = -0.76, p-value = 0.10), similar to L375.

Table 4.5: Spearman Rank Order Correlation comparing microcystin concentrations to total epilimnetic P, epilithic P, epilithic N: P and total epilithic cyanobacterial biomass for the average of the reference systems (L239 and L373 (n=10)) and the experimental lake (L375 (n=7)) from 1997 to 2007. The pair of variables with positive correlation coefficients and P values below 0.10 increased together. Pairs with negative correlation coefficients and P values below 0.10 have one variable increasing while the other decreases. Significant values are distinguished from non-significant values by an asterisk; highly significant values have two asterisks.

	Reference systems				**Experimental Aquaculture Lake**	
	L239		**L373**		**L375**	
	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value
Total Epilimnetic P	0.12	0.73	0.42	0.24	0.77	0.10
Total Epilithic P	0.10	0.76	0.82	0.002**	0.32	0.49
Ratio of N: P	0.24	0.47	0.29	0.4	-0.68	0.07*
Total Cyanobacterial Biomass	-0.47	0.13	0.22	0.5	0.43	0.30

Table 4.6: Spearman Rank Order Correlation comparing microcystin concentrations to epilithic P, N: P and cyanobacterial biomass in the shallow and transition zones of Lake Wolsey (n=6) in 2006. The pair of variables with positive correlation coefficients and P values below 0.10 increases together. Pairs with negative correlation coefficients and P values below 0.10 have one variable increasing while the other decreases. Significant values are distinguished from non-significant values by an asterisk.

	Shallow zone		Transition zone	
	Correlation Coefficient	P-value	Correlation Coefficient	P-value
Total Epilithic P	-0.13	0.80	0.03	0.92
Ratio of N: P	-0.13	0.80	-0.76	0.10*
Total Cyanobacterial biomass	0.66	0.18	0.70	0.14

Seasonal changes in microcystin

Throughout the open-water season (May-October), L373 had higher microcystin concentrations than L375 (Fig. 4.5). Microcystin concentrations in L373 remained stable throughout the season (11.9 ± 2.2 ng·cm^{-2}); in contrast, in L375, microcystin concentrations increased steadily. Because only single samples were analyzed, no standard deviation could be computed for these samples. The standard deviation of the mean of similar samples analyzed in L373 (standard deviations = 0.1-7.0 µg·cm^{-2}) and L375 (standard deviations = 0.3-5.0 µg·cm^{-2}) showed that variability around the mean ranged from sample to sample. Linear regression analysis resulted in an $r^2 = 0.05$ for L373 (Fig. 4.5); agreeing with the inference of no seasonal trend. However, regression analysis in L375 was less clear with an $r^2 = 0.5$. The potential seasonal trend seen in Fig. 4.5 may be due to analytical variability.

The seasonal microcystin trend was compared to seasonal trends in epilithic P and N: P in the experimental lake and L373 (Table 4.7), it was discovered that the increases in microcystins in the experimental lake correlated well with the increases seen in P over the season (Table 4.7; correlation coefficient = 0.79, p-value = 0.03); however correlated poorly with N: P ($p > 0.1$). There was also no correlation between microcystin concentrations and epilithic P or N: P in the reference lake 373 ($p > 0.1$).

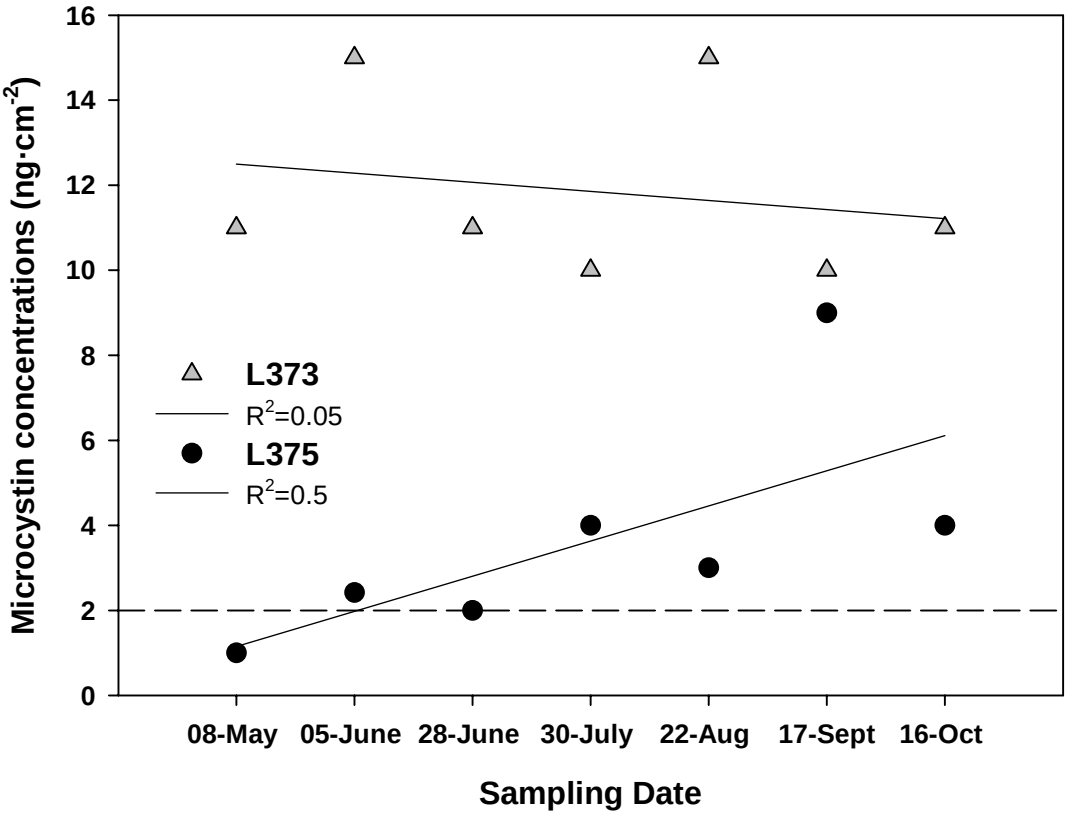

Figure 4.5: Seasonal changes in epilithic microcystin concentrations (ng·cm^{-2}) in the experimental aquaculture lake (L375; circles) and reference system (L373; triangles) during the open-water period in 2007. The microcystin detection limit (2 ng·cm^{-2}) is represented by the horizontal line.

Table 4.7: Spearman Rank Order Correlation comparing seasonal microcystin concentrations to seasonal changes in epilithic phosphorus from May to October 2007 in L375 and L373 (n=7). The pair of significant variables with positive correlation coefficients which increase together are distinguished from non-significant values by an asterisk.

	Reference System Lake L373		**Experimental Aquaculture Lake 375**	
	Correlation Coefficient	P-value	Correlation Coefficient	P-value
Epilithic P	-0.29	0.49	0.79	0.03*
Ratio of N: P	-0.15	0.71	-0.46	0.36

Discussion

Evidence of epilithic microcystins in Canadian lakes

Each study lake contained detectable concentrations of microcystin. Surprisingly, concentrations of the toxin were higher in the oligotrophic reference lakes than in the experimental aquaculture lake at the ELA (Fig. 4.1 and 4.3). Furthermore, concentrations of the toxin in Lake Wolsey were even lower with several undetectable samples.

Total benthic microcystin levels were relatively high in the lakes at ELA (4.6 to 60.0 $\mu g \cdot g^{-1}$ dry wt) compared to values reported for littoral benthic algae in south-eastern Spanish reservoirs by Aboal and Puig (2005) (0.098 to 8.6 $\mu g \cdot g^{-1}$ dry wt MC-LR). Blaha and Marsalek (2003) also provide a range of values (8.4 to 1288 $\mu g \cdot g^{-1}$ dry wt MC-LR) for Czech Republic drinking water reservoirs; the lower values are within the range of those found in the ELA study lakes. However, the higher values far exceed anything observed in the current study. The ELA concentrations were also similar to those reported by Izaguirre *et al.* (2007) for southern California drinking water reservoirs (1.23 $\mu g \cdot g^{-1}$ to 32.4 $\mu g \cdot g^{-1}$ dry wt total microcystins). However, caution should be taken when interpreting these comparisons, as it was assumed that the communities were similar in algal composition, growth and production of the toxin. Given the heterogeneity of benthic algal communities both within systems and between systems (chapter 2 and 3), it is unlikely that these communities were similar. However, comparisons to other studies provide an understanding of the overall distribution of these benthic toxins.

Disconnect from the P gradient

As predicted, epilimnetic P correlated poorly with epilithic microcystin concentrations. Kotak *et al.* (1995, 2000) described a positive correlation between TP and

microcystin concentrations in phytoplankton. Unlike in the phytoplankton, modest increases in epilimnetic P may not have detectable effects on nutrient concentrations in benthic biofilms (Turner 1993). The Spanish reservoirs studied by Aboal and Puig (2005) also showed no link between benthic microcystin concentrations and nutrient concentrations. They found that microcystin presence was more common in oligotrophic waters and was often undetected in eutrophic reservoirs, akin to the current study.

The observed increase in epilithic P was predicted to influence epilithic cyanobacterial biomass and microcystin production; however, the correlation was less clear than expected. In general, epilithic P did not appear to correlate with microcystin concentrations (L239, L375, and Lake Wolsey), although reference L373 did appear to show a strong correlation to epilithic P (Table 4.5, p-value = 0.002). The results from L373 allude to the possibility of a link to epilithic P-gradient; however, the small sample sizes in the current study were likely too small and indicate that increased sampling effort is needed.

Microcystin concentrations showed a negative correlation to the ratio of N: P in L375 and Lake Wolsey (Table 4.5 and 4.6). Both reference lakes, on the other hand, did not show a link between the ratios of N: P and microcystin concentrations. Kotak ***et al.*** (1995, 2000) described a similar negative correlation between microcystins and N: P for phytoplankton in Alberta lakes. Though, the range of N: P ratios was much greater (<1:1 to > 100:1) than those in the current study lakes (~50:1 to 90:1). The inconsistency between epilithic microcystin concentrations and N: P may have been due to the small range in N: P in the current study.

Overall there did not appear to be a link between microcystin concentrations and P or N: P. Giani *et al.* (2005) also reported a poor correlation between microcystin production and N: P ratios in the phytoplankton of Quebec lakes. Similarly, Willen and Mattson (1995) reported no correlation between toxin concentrations in the phytoplankton and various concentrations of P and N in Swedish lakes. They reported a stronger relationship with light intensity and pH than to other environmental variables. Aboal and Puig (2005) also found no link between nutrient levels and toxin production, but did find a correlation among silica, diatom decline and an increase in toxin concentrations. This was likely related to a change in species dominance from diatoms to cyanobacteria caused by the decline in silica, rather than by a direct link between silica and microcystins. Environmental factors such as temperature, light intensity, nutrient concentrations (N and P), pH and trace metals, have all proven to influence microcystin production in laboratory (Znachor *et al.* 2006). Therefore, attempting to correlate microcystin concentrations to only one variable will likely be inconclusive.

Microcystin concentrations may be influenced more by the occurrence and abundance of the toxin-producing species, rather than by environmental factors, such as nutrients (Giani *et al.* 2005). Further to this, it may be that toxin production was more strongly linked to dominance of specific toxin-producing species rather than to total cyanobacterial biomass, which would explain the poor correlation between microcystins and total cyanobacterial abundance seen in the current study (Table 4.5).

Variability in microcystin concentrations and the relationship to known microcystin producers

All of the potential microcystin producing genera found in the epilithon of the study lakes have known benthic and/or periphytic microcystin-producing species: *Anabaena flos-aquae* (Lyng.) Breb and Bornet; *Lyngbya* sp.; *Nostoc* sp.; *Pseudoanabaena*; *Planktothrix agardhii* (Gom.) Anagnostidis and Komarek and *Oscillatoria* sp. (Table 4.3). Many studies reported a strong correlation between the presence of *Microcystis aeruginosa* (Kutz.) Kutz. and high microcystin concentrations (Kenefick *et al.* 1993; Willen and Mattson 1995; Kotak *et al.* 1996), however, it was not observed in the epilithon of any of the study lakes. Willen and Mattson (1997) found that the genera *Microcystis* sp. has high nutrient requirements and were generally only detected as a toxin-producing taxa in eutrophic lakes. Nutrient levels in the oligotrophic reference lakes of the current study may have been insufficient to support abundant *Microcystis* sp. growth. However, nutrient concentrations in the experimental lake must have been conducive to *Microcystis* sp. growth, as it was present in the phytoplankton in small quantities in 2004 (2.3 $\mu g \cdot mL^{-1}$) and 2006 (0.2 $\mu g \cdot mL^{-1}$) (*ELA Data Retriever* version 7.1).

Blaha and Marsalek (2003) reported benthic microcystin production by primarily four genera: *Phormidium* sp., *Oscillatoria* sp., *Anabaena* sp. and *Nostoc* sp. All four genera were present in the epilithon of the study lakes: *Oscillatoria* sp., *Anabaena* sp. and *Nostoc* sp. were found in small amounts in the epilithon of L375 and *Nostoc* sp. in the epilithon of L373 in 2006 and 2007 (Table 4.3). Historically all genera were found in L375 and L373: *Phormidium* sp., *Anabaena* sp. and *Nostoc* sp. were found in L239, but

Oscillatoria sp. was not present in the record. *Phormidium* sp. dominated the epilithic cyanobacteria of Lake Wolsey; however the other three genera did not appear to be present in 2006 (Table 4.3). Izaguirre *et al.* (2007) and Aboal and Puig (2005) reported benthic microcystin production by species of *Oscillatoria* sp. and *Phormidium* sp. but both studies also included *Lyngbya* sp. as a potential producer. *Lyngbya* sp. co-dominated with *Scytonema* sp. in the epilithon of the ELA reference lakes. When *Lyngbya* sp. biomass was compared to MC-LR levels there was little correlation between the two (Table 4.4 p-value = 0.15). Baker and Humpage (1994) suggest that, contrary to earlier reports, not all benthic genera have toxin producing species, especially benthic forms of *Nostoc* sp., *Oscillatoria* sp. and *Lyngbya* sp.

None of the potential microcystin-producing taxa correlated well with microcystin concentrations in the current study (Table. 4.4). Even when the total biomass of potential microcystin producers was compared to microcystin concentrations the correlation was low (Table 4.4, p-value = 0.12). L375 had high potential microcystin-producer biomass and low microcystin concentrations, while the reference lakes had high microcystin concentrations and low potential microcystin-producer biomass. Lake Wolsey had the highest biomass of potential microcystin producers, the majority of which was *Phormidium* sp., even though microcystin concentrations were near or below the detection limit.

Aboal and Puig (2005) explain that the ability to produce microcystins is not a taxonomic characteristic; different strains of the same species behave differently under different circumstances. Microcystin production is regulated at the genetic and cellular level and by many environmental factors (Zurawell *et al.* 2005). Often, conditions

optimal for growth differ from those optimal for toxin production (Zurawell *et al.* 2005). Therefore, estimating the abundance of potential toxin producing species was insufficient to understand which species are producing the toxin. This was especially the case for the benthic taxa in this study, some of which (*Nostoc* sp., *Oscillatoria* sp. and *Lyngbya* sp.) may not have been toxin-producing species, let alone toxin-producing strains. Also, since the list of species and strains that can produce the toxin continues to expand, the toxin producing species in this study may have been excluded from the correlation analysis. Different species and strains may have also been producing the toxin in the different systems and thus would not show a strong link to any particular species. Knowledge of the particular species and strain would have helped to identify the specific toxin producers in each system as opposed to identification to only genus, as was often the case in this study. Future research should allocate time to collecting and culturing benthic species from ELA lakes to see specifically which species can produce the toxin under culture conditions.

Microcystins in aquaculture

Microcystin concentrations were above the pre-cage mean (2 $ng \cdot cm^{-2}$) for years of aquaculture (Fig. 4.2), although, understanding whether this trend was aquaculture related was difficult, since only two years of pre-cage data for L375 were available for analysis. Throughout the study period microcystin concentrations in the epilithon of reference lakes exceeded those in the experimental lake. Only in 2005 did microcystin concentrations in the experimental lake surpass the average of the reference lakes, although values in L373 were still above those in L375 (Fig. 4.2). Based on this, the aquaculture operation did not affect microcystin concentrations (Table 4.4; $p>0.1$), which

was not surprising, given that the July and August microcystin concentrations in the experimental lake correlated poorly with epilithic P. However, when benthic microcystin concentrations were examined throughout the open-water season (May-Oct.) they seemed to correlate more closely with epilithic P in the experimental lake (Table 4.7 p-value = 0.03).

Aquaculture waste releases of P normally increase during the production cycle, as the size of the fish population increases and amount of feed applied increases (Bristow *et al*. 2008). The production of epilithic microcystins over the season in L375 may have been related to this increased P load from the aquaculture operation. Since similar increases were not observed in the reference lake (L373), as in the experimental lake, it was less clear that there was not an aquaculture affect. However, Kotak and Zurawell (2007) and Herry *et al*. (2007) explain that, in phytoplankton, toxin production can be highly seasonally and temporally variable, at times showing changes within a 24hr period. In which case, the seasonality seen in Fig. 4.5 may be a representation of variability in microcystin concentrations normally observed in a system. A weak linear regression of r^2= 0.5 and variability of similar samples ranging from 0.3-5.0 $\mu g \cdot cm^{-2}$ agree with this possibility. Whether the microcystin increases were due aquaculture activities or not, it can be concluded from this study that sampling during "peak" bloom times (July and August) appear to be insufficient to understand the community's production of the toxin. Therefore, greater sampling effort spanning the entire open-water season is needed, until variability in benthic production is more fully understood.

Thus far it has only been considered that aquaculture operations increase production of microcystins, it should also be noted that the opposing process, degradation

of the toxin, is also a possibility. Again, since only two years of pre-cage data in L375 were available for analysis (Fig. 4.2), the pre-cage trend could not be determined with certainty. Therefore, it may be that the microcystin levels in the aquaculture lake have decreased in the system since the introduction of the aquaculture operation.

It is also possible, that as a result of nutrient addition, a shift from benthic to pelagic microcystin production has occurred. It may be that levels of the toxin are lower in the open-water than in the benthic algae in nutrient poor lakes, when nutrients are added, a shift occurs that causes higher concentrations of the toxin to accumulate in the pelagic zone and thus lower levels in the benthic algae. Potential evidence for such a shift can be seen in the occurrence of *Microcystis* sp. in the phytoplankton of L375 following commencement of the aquaculture operation. Prior to the operation *Microcystis* sp. was absent from the record; however, following aquaculture activities and nutrient loading *Microcystis* sp. appeared. As stated earlier, *Microcystis* sp. presence has a strong correlation to high MC-LR concentrations (Kenefick *et al.* 1993; Willen and Mattson 1995; Kotak *et al.* 1996), indicating that toxin production may be occurring to a greater extent in the open-water in the aquaculture lakes than in the epilithon.

Conclusions

The current study provides the first systematic examination of epilithic microcystin production in Canadian freshwater systems. Given the outcome, a survey of diverse systems including differing benthic algal communities is essential to better understand microcystin-producing dynamics. Especially noteworthy in this study was the presence of the toxin in the epilithon of Canadian Shield lakes with low nutrient levels.

This supports Mez *et al.* (1997)'s view that benthic species may be producing toxins more commonly and unexpectedly than previously recognized.

Finding these toxins in the near-shore region has potential ramifications for those mammals that use lakes and reservoirs as sources of drinking water and for recreation. Microcystin intoxication has already been reported in domestic livestock (cattle (Mez *et al.* 1997), pigs and sheep (Zurawell *et al.* 2005)), wildlife (Kotak *et al*. 1996) including protected species, pets (dogs) and birds (Aboal and Puig 2005). Easy access to these attached algal blooms in the littoral zone allows ingestion of the benthic microcystin producers as a whole (Mez *et al.* 1997). Also, disturbance of these biofilms when entering the water may release the otherwise sequestered extracellular toxins into the water column where they can more easily be ingested.

The majority of samples measured in the current study did not exceed guidelines set by the World Health Organization (1 $\mu g \cdot L^{-1}$ or 0.02 $\mu g \cdot cm^{-2}$) (Blaha and Marsalek 2003) although, three epilithic samples from the more nutrient poor reference systems did. This is unsettling as most Canadian monitoring and sampling strategies have typically involved only pelagic sampling in eutrophic water bodies (Kotak and Zurawell 2007). The current study provides evidence for the need for integrated benthic-pelagic sampling in a wider range of ecosystems until benthic production of microcystin is better understood.

For humans, greatest exposure to the toxin occurs through direct consumption of untreated water. Though, recreational contact, bathing, swimming, diving and microcystins present in water destined for irrigation may also pose a problem (Herry *et al*. 2007). Because of this, extracellular toxins found circulating in the water

column cause the highest risk for human intoxication. But the contribution of the benthic toxin pool in lake water concentrations is not yet known, and may be substantial.

Further research is needed to understand whether this community supplies an important proportion of the toxin to the open water through integrated benthic-pelagic sampling. In addition, understanding rates of benthic algal (toxin) consumption and bioaccumulation are essential. Kotak *et al.* (1996) detected microcystins in three species of gastropods collected from Alberta lakes. These studies focused on phytoplankton as the source of microcystins in the gastropods. However, since gastropods generally prefer to graze on periphyton (Kotak *et al.* 1996), attention should be paid to microcystins found in attached algal communities, such as the epilithon. Finally, the current study examined static concentrations of the toxin; additional work should focus on understanding rates of production and/or destruction of the toxin within benthic algal communities.

Chapter 5: General Discussion

The major concern associated with the experimental aquaculture operation was whether nutrient loading produced from the cage wastes would affect trophic interactions and whole-lake productivity. The observed increase in epilimnetic P concentrations (Appendix I Fig. I.1), led to the hypothesis that changes in productivity, composition and biomass would occur throughout the algal community, potentially affecting all trophic levels. However, the benthic algal response was not that straightforward.

Summary of Chapters

Chapter 2

Epilithic incorporation of cage wastes ($\delta^{13}C$ and $\delta^{15}N$) appeared to be occurring throughout the lake. The aquaculture wastes increased epilithic P concentrations, but had little effect on bulk C and N. As a result, both N: P and C: P ratios indicated that P limitation lessened as aquaculture loading continued, but remained above the optimum value for P-sufficiency.

The increase in epilithic P in the system did not cause any gross changes in benthic algal biomass or composition. The observed shift in group dominance from diatoms to cyanobacteria was likely unrelated to aquaculture wastes, as it appeared to be occurring prior to the commencement of the operation, as well as in L239.

Despite the fact that persistent aquaculture-related changes in biomass and group dominance were not detected in L375, there were meaningful transient changes occurring at the species-level. Following the commencement of the cage operation, a number of new taxa appeared for the first time in L375, as well as a number of taxa unique to the ELA region. The presence of *Mougeotia* sp. in 2007 may have been an early indication of

a disturbance-related phenomenon. In addition, the year-long bloom in P-storing *Calothrix* sp., suggests that the nutrient-poor system had experienced episodic pulses of abundant P. Displacement, following continued P loading, suggests that it was displaced by more competitive taxa. Finally, the transient increase in the motile, planktonic, *Ceratium hirundinella,* points towards potential migration of the species to a zone in the water column where nutrients were more concentrated.

Chapter 3

The increase in epilithic P was expected to increase benthic algal productivity. However, the observed P increases were likely too small to stimulate a detectable increase in algal productivity. All measured metabolic parameters (Pgross, Pnet, or Rd) remained within the range of natural variability of both the reference lakes and the pre-cage mean.

The present method underwent several developmental changes during the course of thesis preparation. Lake-wide estimates of epilithic productivity were inherently difficult given the heterogeneity of the community between sites and variation in measurements (O_2 evolution or CO_2 fixation). However, acknowledgment of both the methodological issues in the study and the difficulty associated with benthic algal sampling, allowed for several recommendations to be made for improvement in future studies.

The introduction of a new GC technique, which measured O_2 evolution and CO_2 fixation simultaneously from the same sample, assured a more thorough examination of cage-related changes, as well as a better understanding of both processes. Comparisons between the two measurements of primary productivity agreed for the most part, but in

some cases a poor relationship was observed. Determination of the photosynthetic quotient in 2006 and 2007 showed photosynthetic quotients close to unity, although variation did exist between lakes, months and sites.

Chapter 4

The detection of microcystin in the epilithon of oligotrophic Canadian Shield lakes is of considerable importance, as it demonstrates that benthic species are capable of producing toxins more commonly than previously recognized. Each of the study lakes contained detectable concentrations of the toxin, with higher measured concentrations in the reference lakes than in either L375 or Lake Wolsey.

Benthic microcystin concentrations correlated poorly with all measured parameters: epilimnetic P, N: P and epilithic P, N: P, cyanobacterial biomass, and potential microcystin producer biomass. Low sample sizes throughout the study may have prevented recognition of true correlations between microcystins and any of the measured parameters. Also the lack of consistent correlation between epilithic microcystins and N: P was likely the result of the small range in N: P in the current study. Finally, the lack of correlation between microcystins and potential microcystin producers was likely due to currently incomplete knowledge of the particular species and toxin-producing strain.

Microcystin concentrations in the experimental aquaculture lake did not change from pre-cage conditions, although the toxin was still present in the system. Of greatest concern is how this unexpected source of microcystins is affecting the aquactic system.

Benthic Algal Responses to Aquaculture

The ELA experiment was a model system in which ecosystem processes were studied in detail to determine whether the aquaculture operation impacted any of the important ecosystem processes or interactions. Since it was a model system, the results in L375 should not be taken as a direct measure of aquaculture effects in other systems. In some respects, the L375 study potentially represents a worst-case scenario because of its longer water-residence time (5.5 yrs) than other larger operations, its small lake size (23.2 ha), and high stocking rates (Findlay *et al.* in press). However, generally impacts were less than would be expected from nutrient loading rates based simply on standard feed and waste loading estimates. This was likely due to late season delivery, hypolimnetic release and sequestration of nutrients, mixing and sedimentation (Bristow *et al.* 2008). Because most of the aquaculture P from one season was unavailable to the algae until the following spring mixing (see *reactivity, seasonality and bioavailability of P* section), the study did not attempt to delineate between years, but rather approached understanding the effects of aquaculture as a cumulative investigation over the last five years. Since the results to date suggest that the impacts were cumulative, continued loading in the system could likely have resulted in larger, more severe impacts than presently measured.

Since the establishment of the cage farm, the phytoplankton (Findlay *et al.* in press.), benthic invertebrates (Rooney and Podemski in prep. a), sediments (Rooney and Podemski in prep. b), and the native fish (Blanchfield *et al.* in prep.) in L375 have all responded to the added nutrients. The responses of the benthic algae have been less clear, showing only transient species-level changes.

The current study has shown that epilithic biofilms are capable of incorporating aquaculture cage wastes ($\delta^{15}N$) throughout the lake. In addition, it has been shown that increased P loading from the aquaculture wastes can be reflected in the epilithon. It was therefore expected that the observed increase in epilithic P would play a role in early increases in benthic algal productivity, biomass, algal toxins and changes in group dominance (Wetzel 2001a). However, none of these parameters were affected.

Conversely, the aquaculture operation did cause species-level compositional changes. These fine-scale, transient changes should not be ignored and may be an indication of an ecosystem responding to stress. Greater impacts, with increased severity would likely have occurred had the experimental aquaculture operation continued. The fact that a response was not observed on artificial periphytic trays located near the cage until the fourth year of the cage operation (chapter 2) suggests that an increase in epilithic algal biomass and productivity in the middle littoral zone may occur after a longer lag.

Additional Influences and Forces

Several physico-chemical forces affect epilithic nutrient uptake and thus benthic algal composition and growth. These forces include water movement, biofilm geometry, surface roughness, orientation of the substrata (vertical vs. horizontal orientation), as well as existing chemical conditions within the biofilm (Turner 1993). In addition to these boundary-layer forces, general aquaculture bioavailability of P, and phytoplankton and grazer responses could also have affected how the benthic algae responded.

Reactivity, seasonality and bioavailability of P

In the current aquaculture operation, very little of the added P (in the form of soluble orthophosphates) was readily available for uptake by algae. Of the total

aquaculture wastes less than 1% was in the form of wasted feed pellets, the majority > 95% was in the form of fish feces (Azevedo *et al.* in prep.). When dispersed solid fish feces quickly settle to the bottom sediments (Azevedo *et al.* in prep.). In the current study, over 55% of the TP in the experimental aquaculture feed was in the form of apatite-P (phosphate rock), which is heavy and unavailable to algae (Azevedo *et al.* in prep.).

Five years after commencement of the aquaculture operation, signs of P accumulation were evident in the deep water of L375 (C. Podemski, pers. comm.). Because the hypolimnion was thermally and chemically separated from the epilimnion, P resuspension was generally restricted to wind-mixing events, during the spring and fall. At these times, a surge of P was observed in the water column and in the epilimnion in particular (Appendix I Fig. I.1). Along with this surge in P, phytoplankton biomass increased (Findlay *et al.* in press). These deep-water P levels have the potential to impact future flows of P to the surface waters and increase the availability of P to the algae (Bristow *et al.* 2008). Currently though, it appears that sediment P availability has been restricted to these mixing events.

Aquaculture releases of P usually increased as the production cycle continued because the size of the fish population increased the amount of feed applied and the waste increased (Bristow *et al.* 2008). The largest inputs of P were near the end of each production cycle in the fall, sometimes after mixing (Appendix I Fig. I.1). Prior to the aquaculture operation, total P loading followed a similar pattern throughout the season, with a decrease in the fall (Appendix I Fig. I.1). Following commencement of aquaculture operation, a noticeable change in the seasonal distribution of total P was

apparent, with increases in the spring and fall and a drop during the summer months. These increases were likely a result of lake mixing and P resuspension. Contrary to epilimnetic total P, epilithic P concentrations showed a cumulative increase over the open-water season (chapter 2), closely following aquaculture loading patterns of increased feed (and thus increased P loading) in the fall. These fall increases indicate that the epilithon may be acting as a P sink, accumulating P throughout the season.

It is also important to discuss P reactivity in terms of water renewal and P retention following cessation of the farm. In theory, a lake exposed to a new nutrient input regime will be in equilibrium in approximately one water renewal period. The reactivity of P following this period should remain relatively stable, provided there was a reduction in the P input (Wilander and Persson 2001). However, equilibrium with sediment P stores may take longer than in the water column (Wilander and Persson 2001). In shallow systems, after a decrease in the P load, a net release from the P-saturated sediments is seen (Wilander and Persson 2001). The water-renewal period for L375 is approximately 6 years; this suggests that L375 should stabilize within six years of the end of the aquaculture operation. However, if the lake experiences sediment release, the final equilibrium state will take longer than this. That is, the equilibrium state in L375 will not be reached until all surplus P has left the water column and sediments (Wilander and Persson 2001). An estimate of this potential time frame is rather unpredictable and the effects on the benthic algae are likely to continue with the sustained incorporation of these cage wastes.

Phytoplankton interactions

Since phytoplankton are able to intercept light and cage-related nutrients before they reach the benthic algae, aquaculture nutrient loadings that result in phytoplankton blooms may reduce total benthic algal productivity (chapter 3) and biomass (chapter 2). Also, increased deposition of senescing phytoplankton blooms can cause benthic algal shading and increased decomposition rates in the biofilm (Vadeboncoeur *et al.* 2002)

The phytoplankton community in L375 prior to commencement of the aquaculture operation was typical of oligotrophic ELA lakes, with seasonal biomass estimates ranging from 0.2-0.5 $g \cdot m^{-3}$ and assemblages co-dominated by chrysophytes and cyanobacteria (Findlay *et al.* in press). After one year of aquaculture, the average ice-free biomass increased significantly by 50%, with a major spring bloom increasing in biomass from 0.5 to 4.5 $g \cdot m^{-3}$ in 2004. The increase in algal biomass observed in 2006 exceeded the 2004 values by another 50%, making it the highest recorded increase seen over the study period. Summer biomass estimates were elevated compared to pre-cage estimates (2-fold), however the major increases occurred in the spring (Findlay *et al.* in press). Epilithic sampling generally occurred during July and August, at times when the L375 phytoplankton community seemed to be relatively inactive (Findlay *et al.* in press). Competition for light and nutrients during this time should not have been an issue for the benthic algae in L375. However, senescence of the early spring phytoplankton blooms may have increased the deposition onto the benthic biofilms, temporarily burying them. Phytoplankton burial of the epilithon could have caused shading of the community or, with greater decomposition rates (O_2 consumption), potentially led to misinterpretations of benthic responses.

Grazer interactions

Changes in grazing pressure were likely to have occurred in L375 due to (1) the new food web component related to food spill and feces consumption (Hankanson 2005) and (2) the influence of escapees on native fish populations (Read and Fernandes 2003). Kullman *et al.* (in prep.) provided evidence for the former, showing that the behaviour of some of the grazers (minnows) was influenced by the presence of the cage. A stable isotope investigation of L375 by Kullman *et al.* (in prep.) demonstrated that predation pressure on littoral invertebrate grazers may have been reduced as minnows began to use waste fish feed (and/or feces) as an important component of their diet rather than littoral invertebrates. That is, minnows migrated to the cage to consume wasted feed rather than meeting their dietary needs in the littoral zone. With lowered predation pressure, littoral invertebrate biomass and thus grazing would increase. Hence, an increase in grazing could have dampened a benthic algal response to the aquaculture operation, generating erroneous interpretations of aquaculture impacts on benthic algae.

In addition, Blanchfield *et al.* (in prep.), deliberately released a number of acoustically tagged rainbow trout each year to mimic escapee-related changes and to monitor escapee behaviour in the system. This means that grazing pressure could have been affected either by a response to the cage operation (i.e. increased nutrients) or by a response to the top-down influence from the "escapees".

Grazing can influence algal composition, abundance, productivity, and nutrient stoichiometry (Hunter 1980; Hillebrand and Kahlert 2001). A major limitation of this study was that grazer effects were not studied. During the developmental stage, the idea of using exclosure boxes to explore the role of grazers was discussed. However, time and

resources were limited and it was decided that I would rely on zoobenthic investigations performed by others in the project. However, near the end of the project it was revealed that only very limited attention was paid to the littoral grazers and was restricted to stable isotope investigations (Kullman *et al.* in prep.). Whether the changes in grazing pressure were natural or aquaculture related, would have affected the interpretation of how the cage impacted the benthic algae. Since it was largely unknown whether grazers influenced the epilithon of the study lakes, it is recommended that further research should attempt to (1) separate the effects of nutrients and grazing on epilithon and (2) differentiate natural changes in grazing pressure from disturbance-related changes.

Challenges in the Interpretions

The epilithon is composed not only of benthic algae but also of bacteria, fungi, metazoans and detritus embedded in an abiotic matrix. In the current study, the viable algal C component ranged from only 1-5% (chapter 2). The remaining portion of the biofilm was non-algal material. This raised concern for the interpretation of the epilithic responses; in particular, as to the contribution of epiphytic algae to the bulk parameters measured in this investigation. Since there was no way to state unequivocally that the changes in the epilithon were due to benthic algae and the supplemental information needed to make the judgement call was lacking, it must simply be recognized that alternate interpretations of the measured effects are possible. That is, it must be understood that the changes in the $\delta^{15}N$, for example, may not have been entirely due to benthic algal incorporation but rather to an increase in settled detritus.

To simplify matters in the current study, all interpretations of results were based on the assumption that the measured changes in the biofilm were due to the algae,

because they were the major viable component in the epilithon. This speculation was complicated by the interaction of other viable components; the bacteria and settled viable phytoplankton. However, neither of these components was measured and for the most part, algae found in the biofilm were considered benthic in origin even if they were settled from the phytoplankton.

The only way to explicitly determine benthic algal response would be to separate the viable algae from the remainder of the epilithon and grow it in a laboratory setting. In my opinion, this is not a realistic measure of benthic algal response since this is not how benthic algae are naturally found. They are embedded in a glycocalyx with various other organic and inorganic materials (chapter 1), all of which are connected to form the epilithon and all of which influence how the benthic algae respond. I recognize that the epilithic community cannot be adequatedly understood without understanding the metabolism and interactions of the integrating components, such as the bacteria and other microflora in close association to the algae (Wetzel 1983). However, understanding these interactions was not within the scope of the current study. This is an area worthy of additional research and as detailed knowledge of the functioning of each of these components increases, we will approach something closer to the true nature of this integrated community (Wetzel 1983).

Benthic Algae as Biomonitors

Sand-Jensen (1983) and Fairchild and Lowe (1984), recommend that benthic algae are useful biological tools for monitoring and assessing the effects of short-term inorganic pollution because of their ability to assimilate new nutrients. In addition, Turner (1993) describes how periphyton can be used as early warning detectors of

ecosystem stress, particularly for acidification. The current study also examined the value of benthic algae as biomonitors for aquaculture impacts. As a whole-lake monitor of eutrophication, benthic algae did not appear to provide any major insights into the experimental system that were overlooked by other ecosystem components. For example greater changes in biomass and composition were seen in the phytoplankton (Findlay *et al.* in press), and fish communities (Blanchfield *et al.* in prep.), than were observed in the benthic algae. In addition, changes in profundal sediment biogeochemistry (Rooney and Podemski in prep. a; Rooney and Podemski in prep. b) provided a more straightforward indication of lake-ecosystem stress than did the benthic algae. However, it was assumed that the epilithon would be the slowest community to respond to an impact, because of higher background concentrations of P than the phytoplankton (chapter 1). Since the community did show a response in transient, species-level changes it did provide useful insight into lake-impacts overlooked by other ecosystem components.

The fact that the community did show species-level responses after only five years of aquaculture operation is of value and indicates that the littoral zone is being affected. In addition, these species-level compositional changes should not be ignored as they may be an early indication of potentially larger-scale impacts if the operation were to continue, such as changes in grazer selectivity and further changes in benthic food web-dynamics.

However, benthic algal use as an impact-monitor is complicated by the nature of the epilithon as a heterogeneous matrix and by an assortment of interactions with the phytoplankton and grazer communities. Without knowledge of these interactions there are limits to the use of benthic algae as biomonitors for aquaculture impacts. One solution

to minimize the variability associated with the natural community would be to supplement the study of the natural epilithon with the inclusion of suitable artificial substrata for the assessment of the pre-, during-, and post- aquaculture impacts. This would provide an understanding of community changes in "new" epilithon in response to the aquaculture operation. It would also remove some of the uncertainty caused by variability in existing natural biofilms, allowing the researcher to concentrate on responses. A second solution to remove the uncertainty caused by the littoral grazers would be to include invertebrate-exlosure chambers.

Recommendations and Future Research

The current findings suggest that since no major persistent changes have occurred, up till now in the community; the aquaculture operation has not permanently affected the benthic algae. However, despite not showing any changes in the major parameters (algal biomass, productivity, toxin production, and group dominance); the results of transient species-level changes suggest that the community was being disturbed by the aquaculture activities. Continued incorporation of cage wastes by epilithic biofilms, as well as an increase in the concentration of a major limiting nutrient (P), is likely to cause eventual changes in community characteristics that may only be seen after continued cage operatoin. The small scale, transient changes that were observed may be providing evidence of greater impacts to come, if the operation were to continue.

Perhaps the current lack of larger-scale responses was an indication of the adaptability of the community. It may be that the epilithon is resilient to change, capable of enduring considerable stress. Another possibility is that the increases in available epilithic P in L375 were too small, compared to background levels, to stimulate major

changes. Moreover, it is also possible that the resolution of the method was such that it could not detect the major aquaculture-related responses. Finally, it is important when considering the response of key benthic algal processes to remember that they may have been concealed by a number of potentially interacting and interfering forces: phytoplankton and/or grazer influences.

Given the relatively short time frame of this cumulative impact study, it was difficult to conclusively determine whether the benthic algal community would have been permanently affected by the aquaculture waste loading. In spite of this, the current study did show a number of valuable findings. In response to aquaculture operations epilithic biofilms are: (1) capable of incorporating cage wastes, (2) experiencing compositional changes at the species-level, and (3) more adaptable and resilient than I previously thought.

This study also provided especially noteworthy information for toxin research, especially the discovery of toxins (microcystins) in the epilithic biofilms of nutrient poor Canadian Shield lakes. This finding was unsettling given that benthic species are capable of producing toxins more commonly than previously recognized, especially in water bodies deemed to be harmless using only pelagic criteria.

Further research is needed in both aquaculture impact-related studies as well; further investigations are needed to understand simple benthic community processes.

Additional Aquaculture-related Research

(1) This study shows that the impacts are proving to be cumulative and had the operation continued, greater impacts may have been detected. Longer-running studies are needed to fully understand the magnitude of these impacts.

(2) More integrated studies are needed that incorporate benthic-pelagic trophic coupling and responses.

(3) Trophic energy transfers elucidate essential information about ecosystem health. Filling in the current gaps that exist in in-lake trophic cascades, e.g., littoral invertebrates and their interaction with benthic algae, would help to fully describe the potential extent of aquaculture-related impacts.

(4) Though nutrient concentrations decrease after the removal of aquaculture operations, recovery is not observed throughout the whole-lake (Doughty and McPhail 1995; Clerk *et al.* 2004). Understanding how the benthic algal community will react following cessation of the aquaculture operations is important to understanding the duration of whole-lake impacts. Future studies should examine recovery in both short- and long- running operations.

(5) Understanding primary productivity is important to understanding whole-lake energy transfers, however, it must be kept in mind that aquaculture operations create a new lake dynamic different from changes caused by other perturbations. With aquaculture secondary production can be affected without affecting primary production (Hankanson 2005; Kullman *et al.* in prep.). This new food web component, which is related to farm spillage and wild fish consumption, needs further and more stringent investigation.

Benthic Algal Sampling-related Recommendations

(1) Since benthic algae can be major contributors to whole-lake productivity, future research should focus on providing a more comprehensive understanding of metabolic responses to anthropogenic perturbations. Future research should

concentrated on developing new and simpler approaches to benthic algal productivity measurements by providing techniques that decrease analytical imprecision, systematic errors, and uncertainty associated with benthic algal heterogeneity.

(2) An adequated understanding of the epilithic community cannot occur without understanding the metabolism and interactions of the integrating components, such as the bacteria and other microflora in close association to the algae, additional research which investigates the functioning of each of these components is necessary.

(3) Impact studies should supplement natural investigations of the epilithon with the inclusion of suitable artificial substrata for the assessment of pre-, during-, and post- impacts.

(4) A simple suggestion to decrease variability associated with the heterogeneity of the epilithon is to increase sample sizes, the number of sampling events, and the area of lake sampled. However, this is difficult using the current method due to the high cost and energy required to obtain the samples through SCUBA. A complete re-design of the sampling techniques, increased number of researchers using the current technique, or a change in focus is required.

(5) The discovery of microcystins in the nutrient poor lakes of the Canadian Shield was important, in part because the discovery raises more questions and concerns. A survey of diverse ecosystems, including differing algal communities is essential to better understand microcystin producing dynamics. Further research is also needed to understand whether this community supplies an important

proportion of the toxin found in the open-water. In addition, understanding rates of consumption of the toxin-producing strains and bioaccumulation is essential.

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Appendix I: Experimental aquaculture: Epilimnetic Nutrients

I.1: Nutrient concentrations and utilization rates:

Waste feed and excretory wastes from aquaculture operations can play a significant role in the nutrient dynamics and loading of a lake. Feed wastage in the L375 experimental operation was minimal; the greatest nutrient load came from fish excretory wastes (Azevedo *et al.* in prep). Total feed applied to the aquaculture fish varied amongst years, with the greatest quantity occurring in 2007 (Table 1.1). The greatest fish biomass gain occurred in 2004, a year in which minimal feed was applied and minimal feed wastage occurred. Throughout the study period the fish were fed an economically viable feed that minimized wastage. The Feed Conversion Ratio (FCR), a measure which is often used in the aquaculture industry as an indication of farm economy (Bristow 2006), was low over the four years of operation (1.21) (Table 11). The FCR is calculated by dividing the quantity of feed applied to the farm (kg) by the quantity of wet weight gained by the livestock (kg). A lower FCR indicates better nutrient utilization (Azevedo *et al.* in prep). Utilization efficiency was highest in 2004, and lowest in 2006. Azevedo *et al.* (in prep) explains that rearing conditions, feed ingredients, feed quality and management practices may have differed amongst years leading to varying FCR ratios.

For every 1,000 kg of fish produced, 8.7 kg of TP was released to the lake in 2003, 8.1 kg in 2004, 11.5 kg in 2005, and 12.6 kg in 2006 (Table I.1; Fig. I.1) (Azevedo *et al.* in prep), showing an increase in loading with each consecutive year (with the exception of 2004). An average of 10.3kg TP waste was produced over the four production years. The TP waste comprised both dissolved and solid forms (Fig. I.2). The

particulate P concentrations were closer to TP loading from the fish farm wastes than dissolved P concentrations, in all years (Fig. I.2). Since the majority of the P loaded to the system was in a solid form, it quickly settled to the bottom of the lake (approx. 62.5% TP was distributed to the hypolimnion (Bristow *et al.* 2008)). Approximately, 25% was soluble in the epilimnion and 7.5% fine solids (which eventually slowly settles out) (Bristow *et al*. 2008).

Natural P loads in L375 averaged 7 kg TP annually, while farm loads from feed averaged 101 kg per year from 2003-2007. According to Bristow *et al.* (2008), 70% of the feed P applied to the farm was lost to the lake and 30% was retained in the fish. Taking this into account 71 kg P was loaded to the lake per year from the farm. Thus, there was an approximately 10-fold increase in P loading over pre-cage conditions in the epiliminion of L375 after the aquaculture operation began.

The TN in L375 (Fig. I.3) was mostly comprised of dissolved nitrogen in the form of NH^{4+} (Table I.1, Fig. I.4). Annually, 767 kg N was released as feed pellets, approximately 90% of this was lost to the lake. Thus 690 kg N was loaded on average to the lake per year of operation. The natural N loads averaged around 262 kg N per year. Nitrogen loads nearly tripled over natural loads in L375 due to farm activities.
Feed pellet ratios, based on Bristow (2006), of N: P were 8:1 (by weight), indicating P limitation in the feed (Redfield ratio values of N: P >7:1 suggests P limitation for phytoplankton (Wetzel 2001)). Water column N: P concentrations showed a decrease after the commencement of the cage operation indicating an improvement in P conditions in the water column (pre-cage; 55:1, during cage; 37:1 (by weight) Fig. I.7).
Azevedo *et al.* (in prep.) investigated N:P ratios in 2003 and 2004 and found that water

column ratios were on average similar to aquaculture nutrient loads, suggesting that cage nutrients were affecting lake concentrations and therefore supporting phytoplankton biomass.

Data retrieval:

All data herein was obtained either by personal communication or retrieval from the *ELA Data Retriever* versions 7.1 and 7.6. Raw data for each month in the open-water season was obtained; monthly and yearly means were calculated. The nutrient partitioning graphs (Fig. I.2, I.4, I.6) and the nutrient ratio graph (Fig. I.7) only used annual means. Assumed outliers were removed from the plot. The assumed outlier value was then written in above the sampling date as a placeholder. Caution should be taken when interpreting the following figures, as 2006-2008 chemistry data was loaded into the *ELA Data Retriever* prior to the final verification test.

Table I.1: Estimated waste outputs (kg·1000 kg^{-1} of fish produced) of rainbow trout growing from 100g to 1000g for years of production (2003-2006 from Azevedo *et al.* in prep.); 2007 data unavailable at time of distribution.

		2003	2004	2005	2006
Total solid waste		267	236	248	264
P loading:	solid form	6.1	5.7	6.8	8.2
	dissolved form	2.6	2.4	4.7	4.4
N loading:	solid form	13.6	11.3	9.2	11.4
	dissolved form	39.3	37.3	56.7	60.1
Total P waste		8.7	8.1	11.5	12.6
Total N waste		52.9	48.6	65.9	71.5
Total Pa in water column (µg·L-1)		5.4	8.2	8.6	13.0
Total Na in water column (µg·L-1)		302	314	316	356

[a] Epilimnetic values

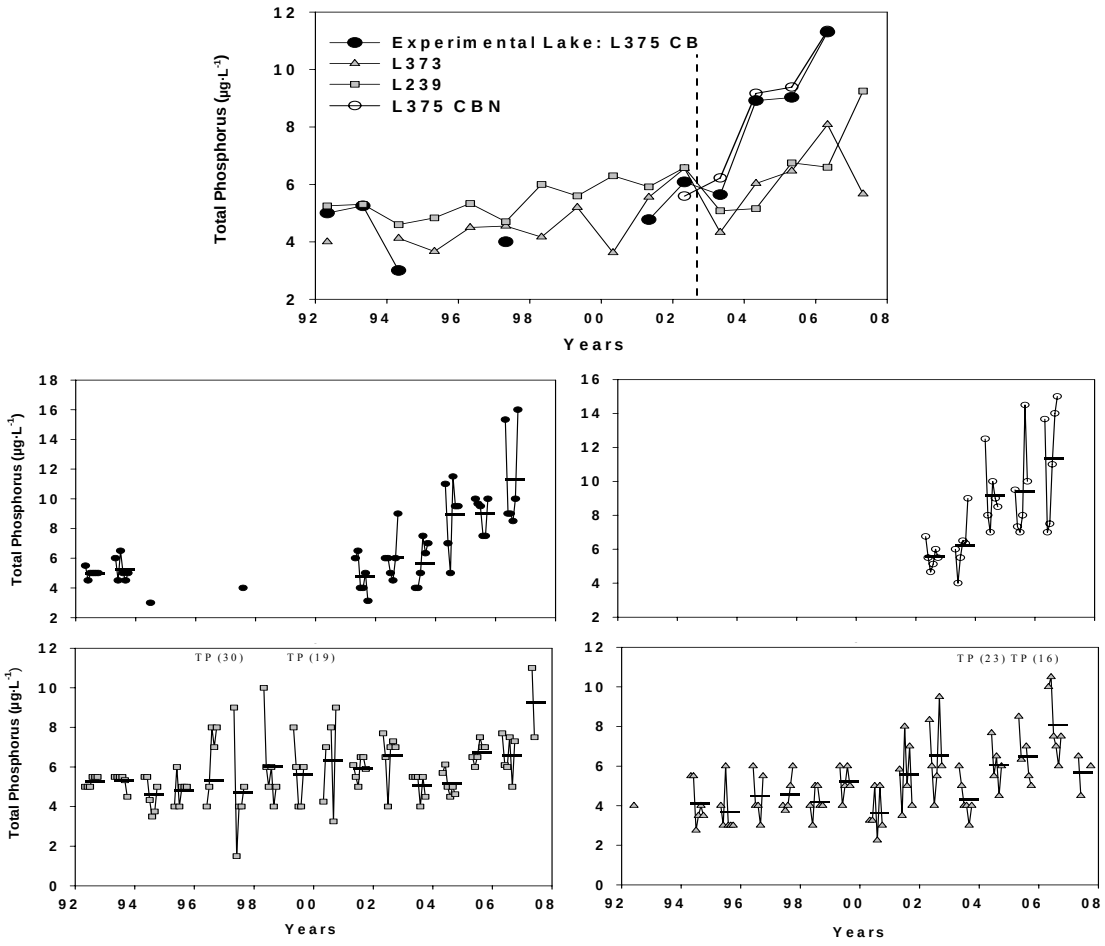

Figure I.1: Total phosphorus (TP) in the water columns of the experimental and reference lakes from 1992 to 2006 (2007 data for L375 where available). Data were retrieved from the ELA Data Retriever version 7.6. The top panel represents yearly means of TP in the experimental (center buoy (CB; deep-water location) and north buoy (CBN; near aquaculture cage operation)) and reference lakes (L239 and L373); the dashed line distinguishes between pre-cage (1992-2002) and cage operation years (2003-2007). The remaining panels represent monthly (connected by solid vertical lines) and yearly means (solid horizontal line) for each lake. Outliers were removed from L239 Oct 1997 (30 µg/L); July 2000 (19µg/L), and L373 May 2004 (37µg/L); July 2005 (22µg/L)).

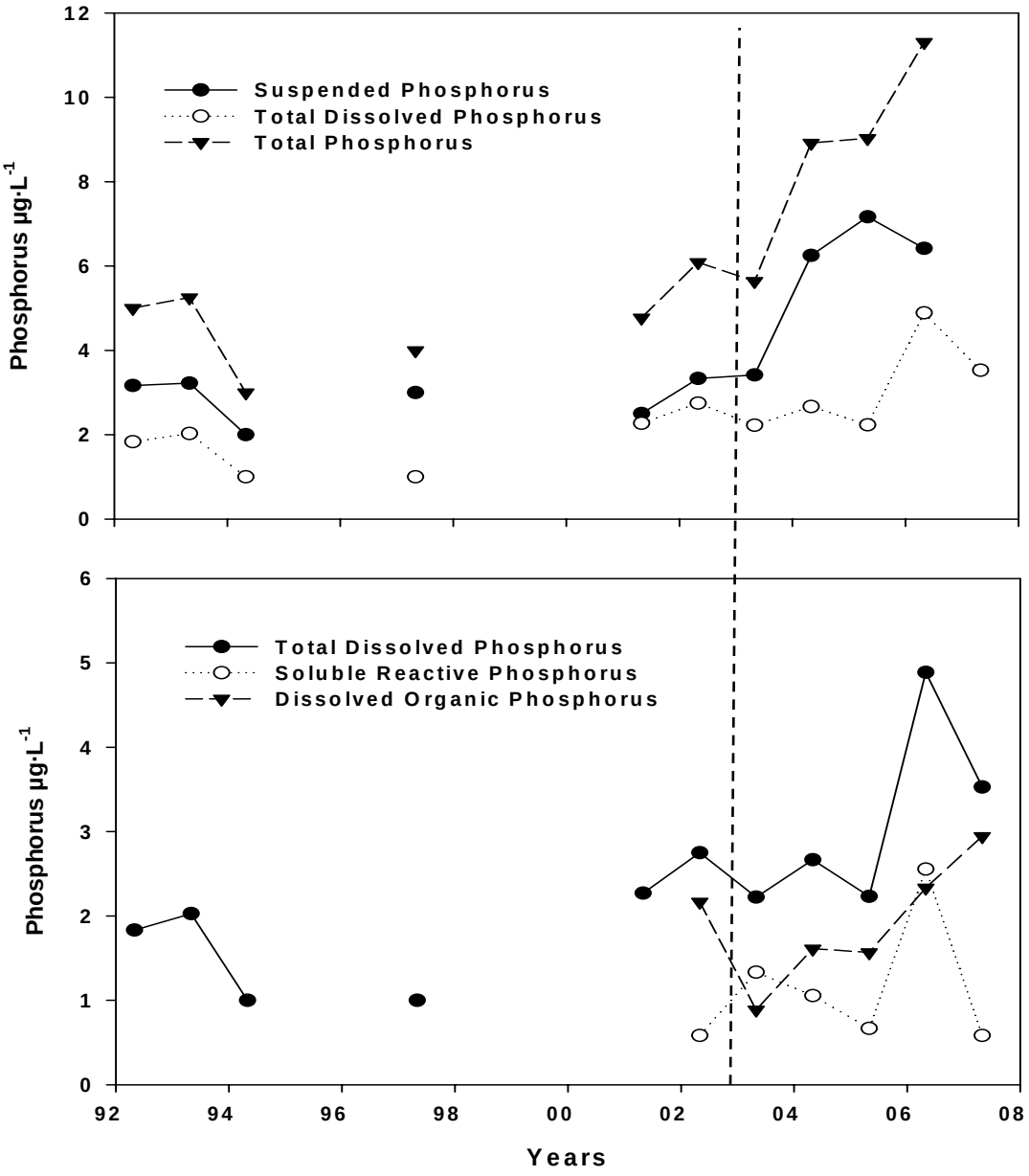

Figure I.2: Phosphorus partitioning in L375 from 1992 to 2006. Data retrieved from the ELA Data Retriever version 7.6. Top panel shows the yearly means for TP, particulate P and dissolved P, the dashed line distinguishes between pre-cage (1992-2002) and cage operation years (2003-2006). The bottom panel shows partitioning of the total dissolved fraction as yearly means.

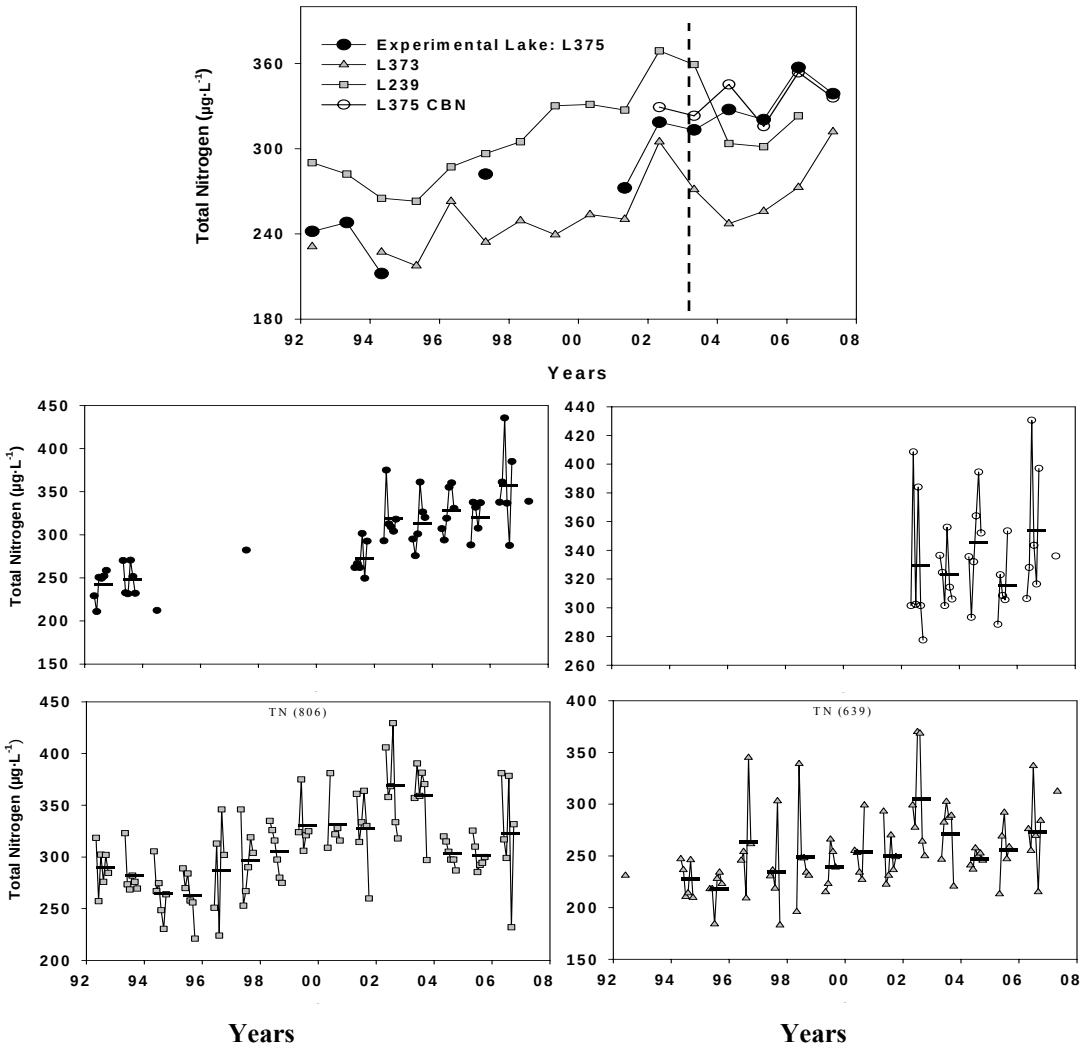

Figure I.3: Total nitrogen (TN) in the water columns of the experimental and reference lakes from 1992 to 2007. Data were retrieved from the ELA Data Retriever version 7.6. The top panel represents yearly means of TN in the experimental (center buoy (CB; deep-water location) and north buoy (CBN; near aquaculture cage operation)) and reference lakes (L239 and L373); the dashed line distinguishes between pre-cage (1992-2002) and cage operation years (2003-2007). The remaining panels represent monthly (connected by solid vertical lines) and yearly means (solid horizontal line) for each lake. Outliers removed for L239 July 2000 (806µg/L) and L373 October 2000 (639 µg/L)

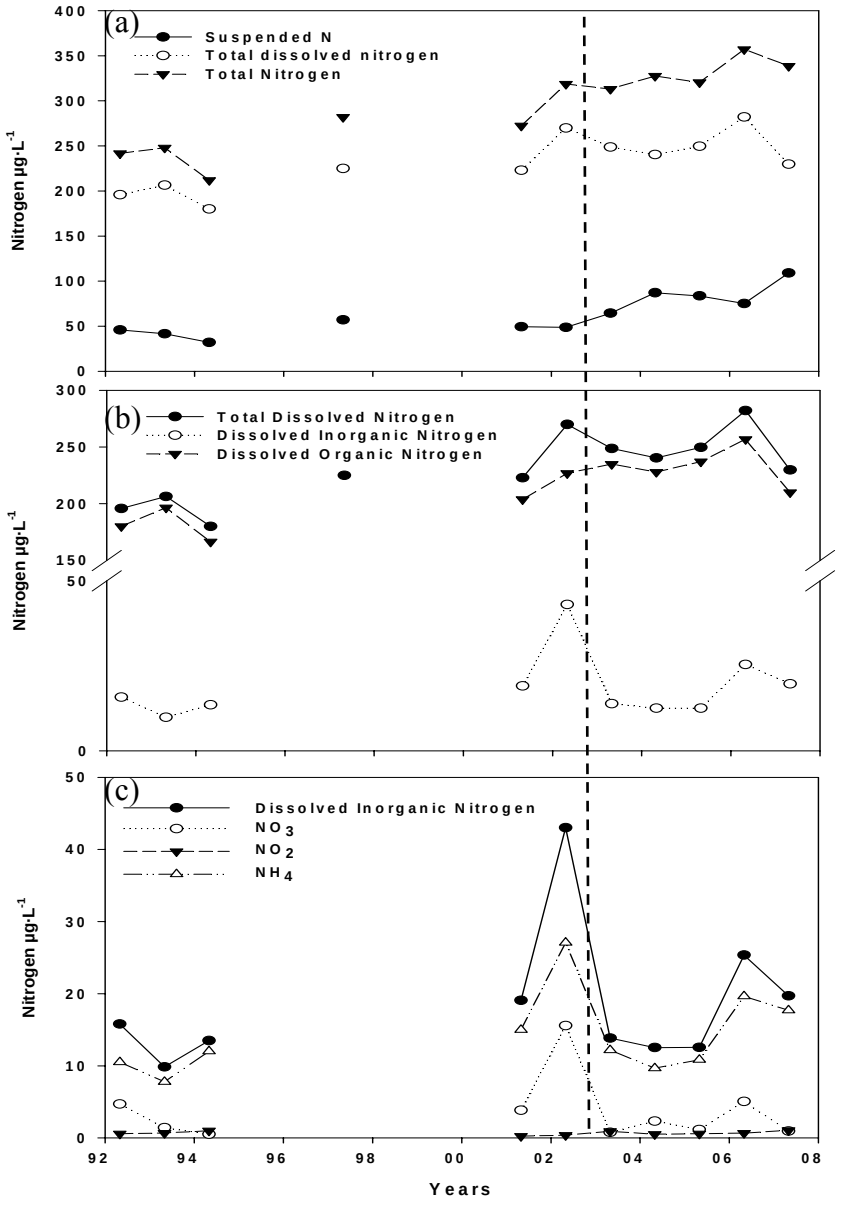

Figure I.4: Nitrogen partitioning in L375 from 1992 to 2007, (a) yearly means for TN, Suspended N and dissolved N; (b) partitioning of the total dissolved fraction as yearly means (y-axis break begins at 50 and ends at 150); (c) partitioning of dissolved inorganic nitrogen. The dashed line distinguishes between pre-cage (1992-2002) and cage operation years (2003-2007). Data retrieved from the ELA Data Retriever version 7.6.

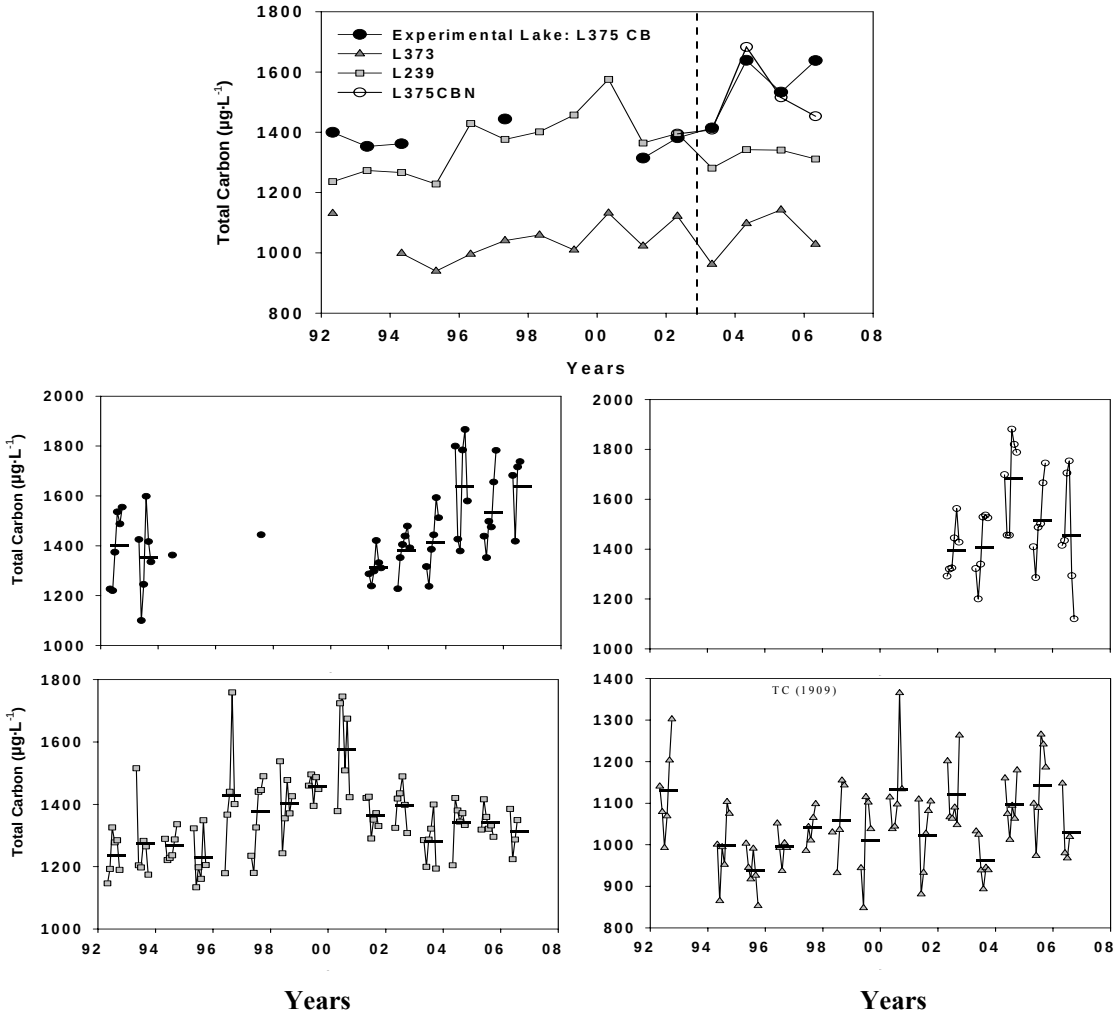

Figure I.5: Total carbon (TC) in the water columns of the experimental and reference lakes from 1992 to 2006 (2007 data unavailable). Data were retrieved from the ELA Data Retriever version 7.6. The top panel represents yearly means of TC in the experimental (L375 center buoy (CB; deep-water location) and north buoy (CBN; near aquaculture cage operation)) and reference lakes (L239 and L373); the dashed line distinguishes between pre-cage (1992-2002) and cage operation years (2003-2007). The remaining panels represent monthly (connected by solid vertical lines) and yearly means (solid horizontal line) for each lake. Outliers removed from L373 June 1998 (1909 µg/L).

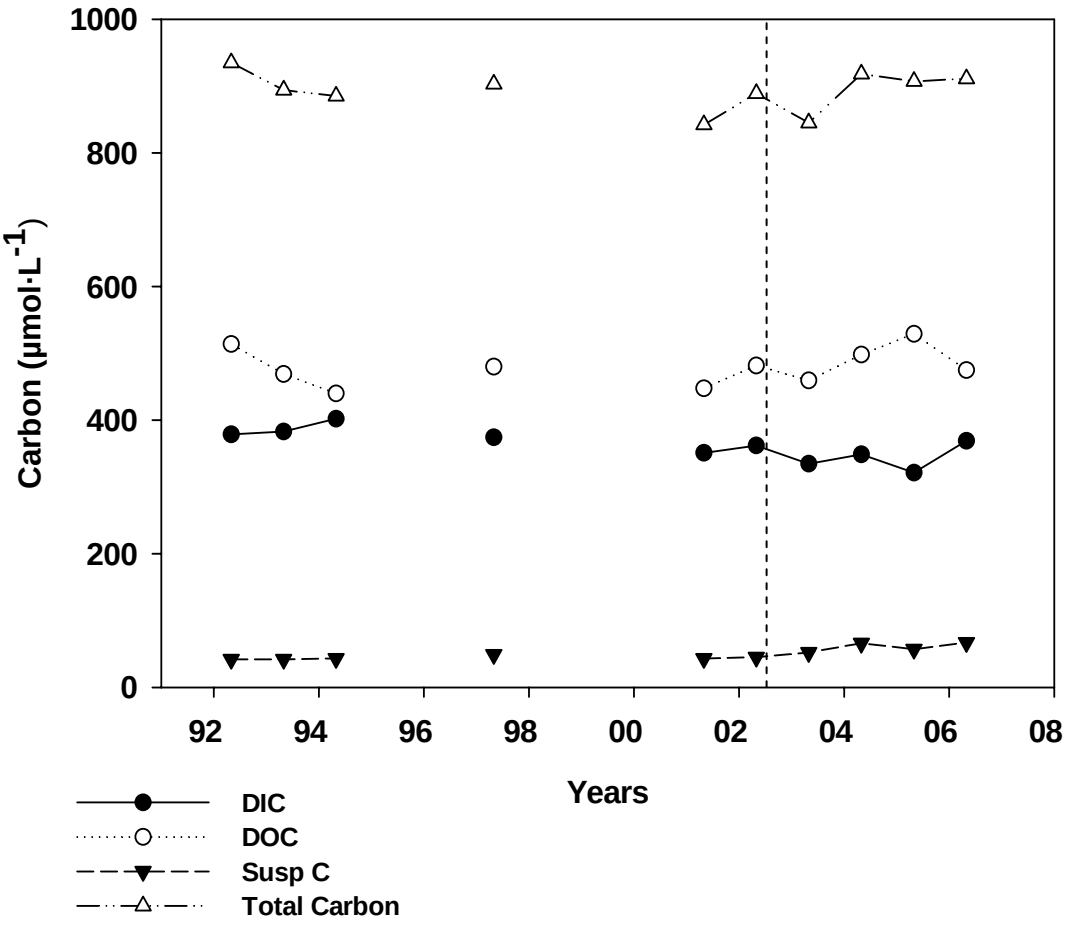

Figure I.6: Carbon partitioning in L375 from 1992 to 2006 showing yearly means for TC, suspended C and dissolved C (DIC and DOC), the dashed line distinguishes between pre-cage (1992-2002) and cage operation years (2003-2006). The y-axis break begins at 820 and ends at 1300. Data retrieved from the ELA Data Retriever version 7.6.

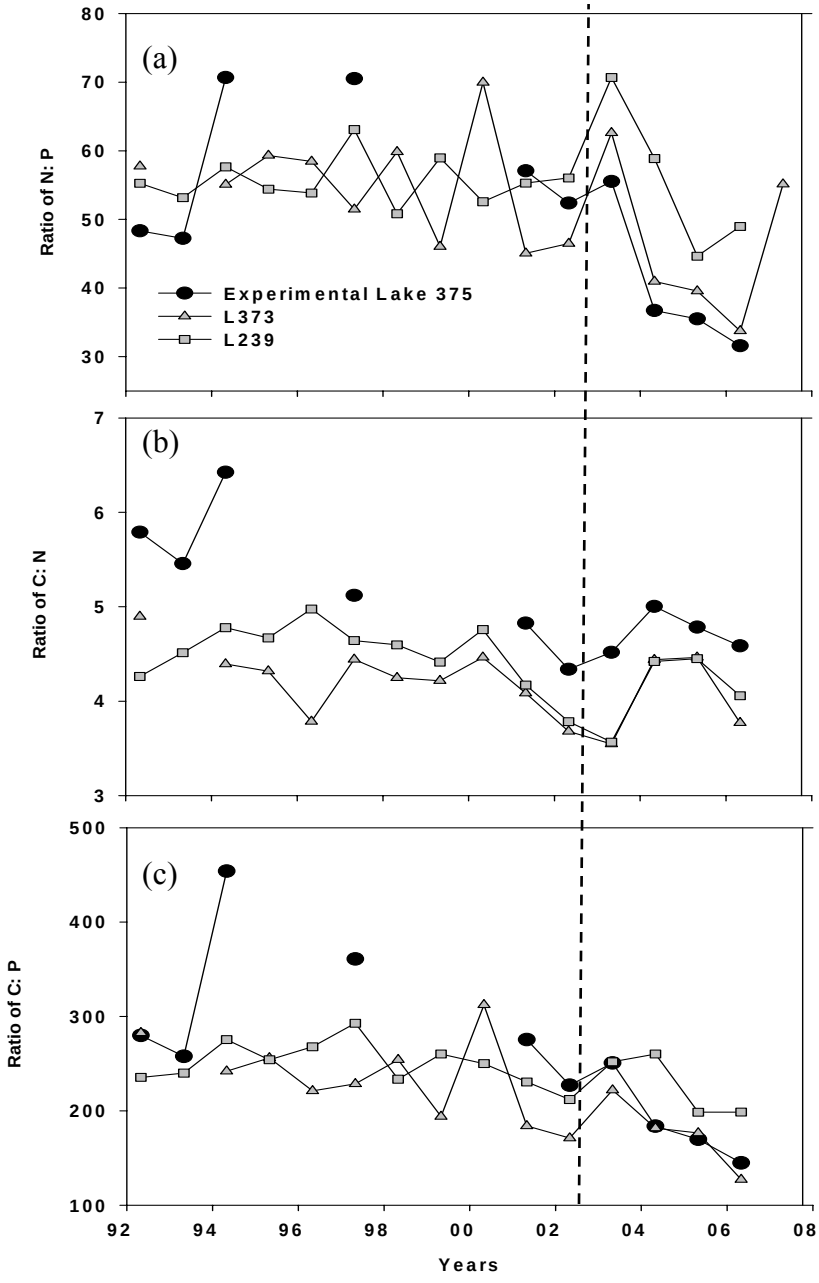

Figure I.7: Nutrient ratios (a) N: P, (b) C: N, (c) C: P in the experimental lake from 1992 to 2006. The dashed line distinguishes between pre-cage years (1992-2002) and years of cage operation (2003-2006).

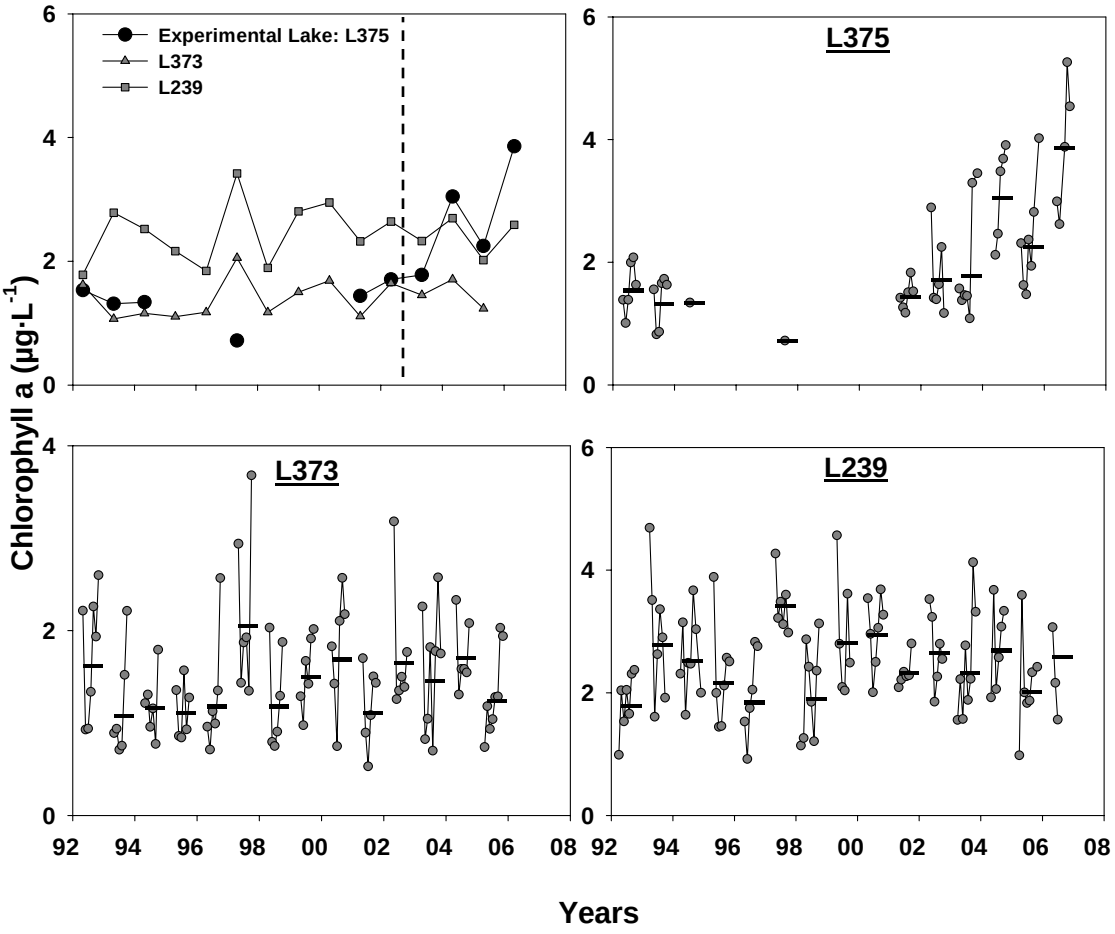

Figure I.8: Chlorophyll a concentrations in the water columns of the experimental and reference lakes from 1992 to 2006. Data retrieved from the ELA Data Retriever version 7.1. Top left panel represents yearly means of Chl a in the experimental and reference lakes, the dashed line distinguishes between pre-cage (1992-2002) and cage operation years (2003-2006). The remaining panels show monthly (connected by solid vertical lines) and yearly means (solid horizontal line) for each lake.

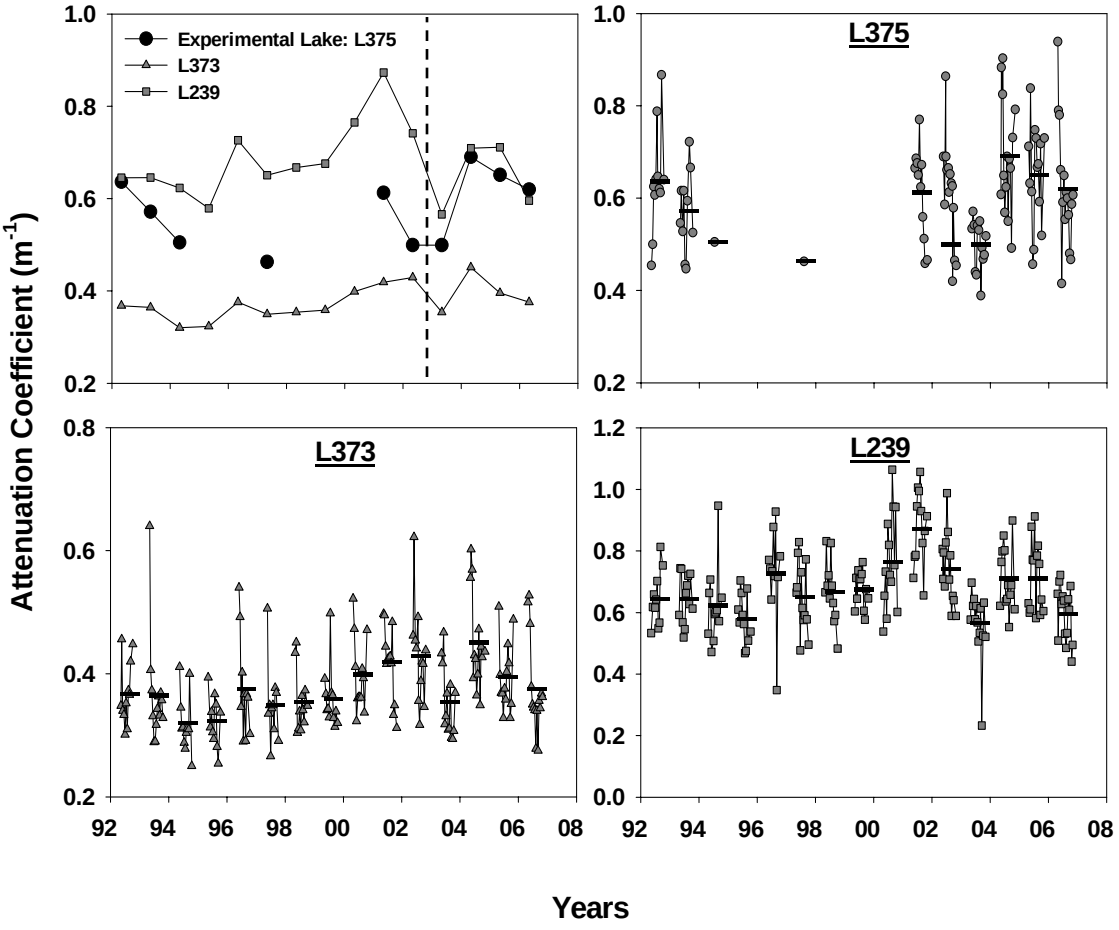

Figure I.9: Light attenuation coefficients in the water columns of the experimental and reference lakes from 1992 to 2006. Data retrieved from the ELA Data Retriever version 7.1. Top left panel represents the yearly means for light attenuation in the experimental and reference lakes, the dashed line distinguishes between pre-cage (1992-2002) and cage operation years (2003-2006). The remaining panels show monthly (connected by solid vertical lines) and yearly means (solid horizontal line) for each lake.

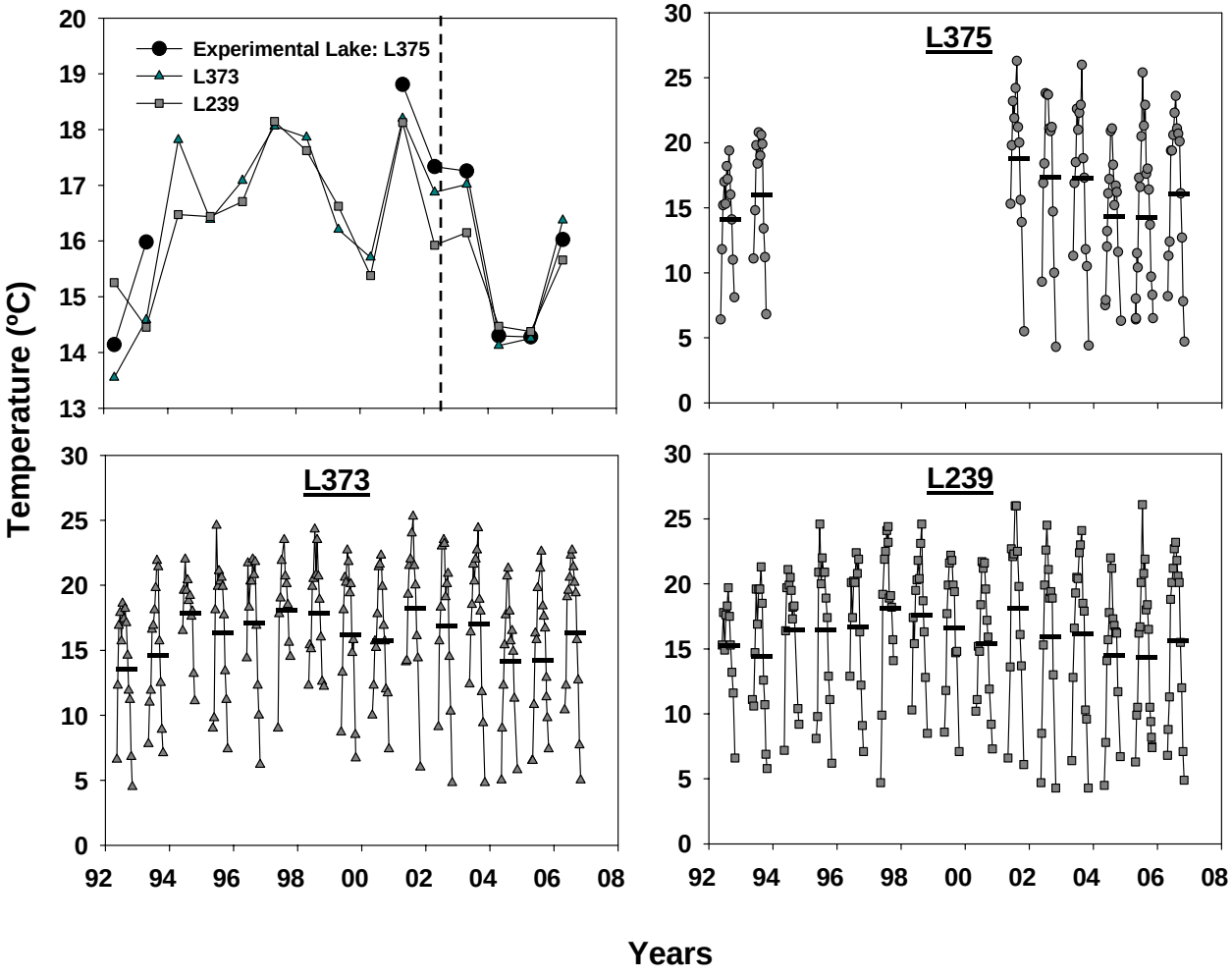

Figure I.10: Surface water temperature changes in the experimental and reference lakes from 1992 to 2006. Data retrieved from the ELA Data Retriever version 7.1. Top left panel represents the yearly means for temperature in the experimental and reference lakes, the dashed line distinguishes between pre-cage (1992-2002) and cage operation years (2003-2006). The remaining panels show monthly (connected by solid vertical lines) and yearly means (solid horizontal line) for each lake.

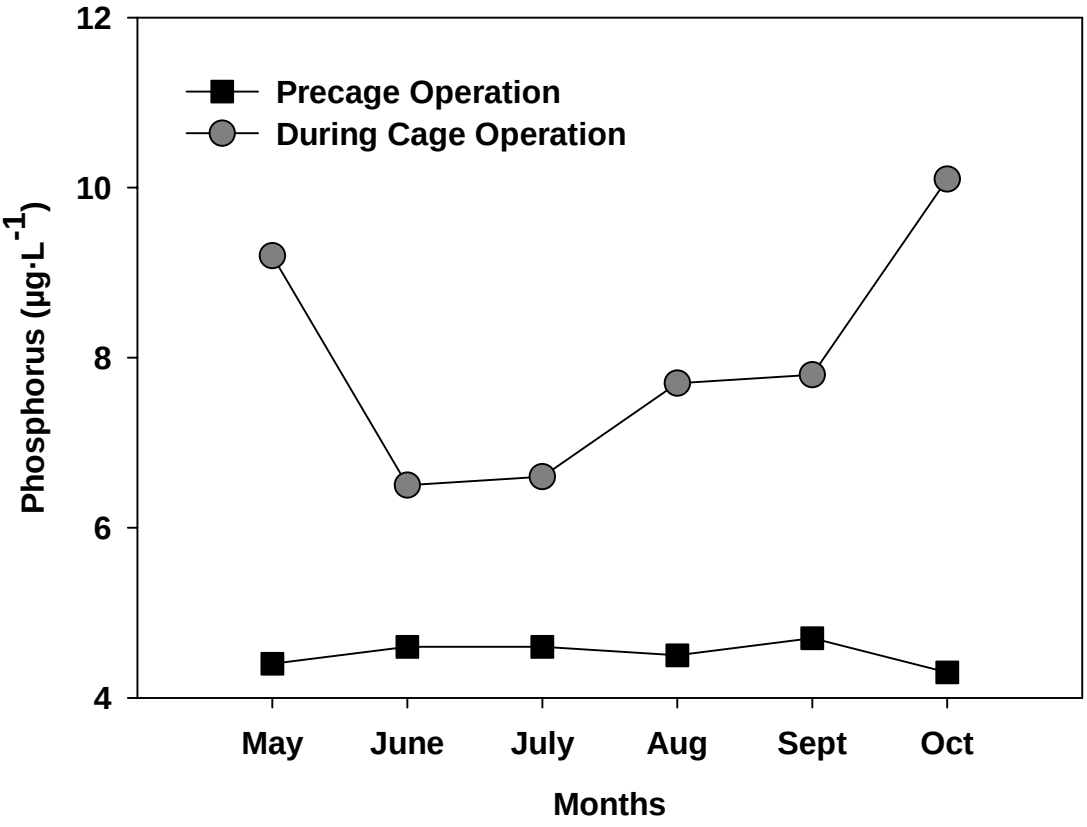

Figure I.11: Seasonal P changes in the experimental lake L375. Monthly means were taken for years prior to cage operation (1992-2002) and years of cage operation (2003-2006). Data retrieved from the ELA Data Retriever version 7.1.

Appendix II: Aquaculture in Lake Wolsey

II.1: Introduction:

Since aquaculture operations produce cumulative impacts on their receiving environments (Beveridge *et al.* 1997), it was expected that sampling at a long-running aquaculture operation would provide insight into which parameters used in the L375 experimental aquaculture operation were best at detecting potential aquaculture impacts. Also, I intended to test the transferability of the knowledge gained and techniques employed from the L375 situation to larger systems.

The stable isotope of $\delta^{15}N$ was used as an indicator of aquaculture influence on the system. An elevated $\delta^{15}N$ signal was predicted in the system given the inputs of marine fishmeal from the aquaculture feed (described in chapter 2) (Kullman *et al*. in prep.). It was expected that, all other factors being equal, the signal would decrease with increasing distance from the cage operation. This presupposes that there was not complete mixing in the lake. Similarly, it was predicted that if the aquaculture operation was supplying appreciable P to the system, it would cause changes in species composition and biomass of benthic algae close to the cage.

Site Description:

Lake Wolsey (lat. 45º, 50'N, long. 82º, 32'W) (Table II.1; Fig. II.1, Fig. II.2) is a nutrient-enriched lake (Gale 2000 cited in Clerk 2002) located along the north-western shoreline of Manitoulin Island (Fig. II.1). Lake Wolsey is not a true inland lake; rather, it is a large bay connected to the North Channel of Lake Huron by a narrow opening at Campbell Bay (Clerk 2002). The lake is underlain by Silurian limestone bedrock and the surrounding vegetation consists of swamp, second-growth forest and marsh (Clerk 2002).

A full account of the area's geology, vegetation, history and land use can be found in Clerk (2002). Unlike the pristine lakes at ELA, the shoreline of Lake Wolsey is also influenced by seasonal cottagers, campgrounds, permanent homes and pasture land for cattle. It is adjacent to a small village and has a history of timber transport and mill activities. It is also the site of a long-term aquaculture operation, which began in 1986 (Clerk 2002). Currently, the farm consists of 18 functioning cages, with production estimates approximating 295 tonnes of fish per year (C. Podemski, pers. comm.; Fig. II.3).

Lake Wolsey is a large lake, 100-times larger than the lakes at ELA; although, it is similar in depth (mean depth =11m) (Table II.1). It has no significant stream inputs and its only outflow is to Campbell Bay. Bidirectional flow through the causeway at Campbell Bay allows inflow from Lake Huron into Lake Wolsey (personal observation). Water quality measurements taken from 1998-99 indicate TP values of 9 and 20$\mu g \cdot L^{-1}$ for spring and fall turnover, respectively (Clerk 2002). According to Gale (1999), historical phosphorus levels in Lake Wolsey were not typically below 10 $\mu g \cdot L^{-1}$. Sampling prior to the commencement of cage operations in May 1986 showed TP levels of 11 $\mu g \cdot L^{-1}$.

Epilithic sampling

The sampling procedure at Lake Wolsey was slightly different then at the ELA lakes, although care was taken to make the sampling as similar as possible. Sampling occurred on a single visit to Lake Wolsey during September 2006. Samples were removed from two depths, at each of six sites varying in distance from the aquaculture cage along the western shoreline. Three sites were north of the aquaculture cages; varying in distance (45, 90 and 239 m) and three sites were south (75, 224 and 523 m). Since

most available surfaces in the middle littoral zone were colonized by zebra mussels (*Dreissena polymorpha* Pallas; Fig. II.4 and Fig. II.5), I adapted the epilithic sampling technique by sampling in shallower locations with fewer zebra mussels. Two depths were sampled to assess possible depth-related bias. The deeper depth, termed the *transition zone* for its change in zebra mussel density from high to low (based on a visual assessment) closely approximated our ELA epilithic middle littoral sampling depth of 1-3 m below the surface. However, the transition zone was not free from zebra mussel influence; therefore, a shallower depth was added in the wave zone, which was approximately 0.5-0.8m below the lake surface.

In order to decrease the variability in sampling, effort was increased at each site to include four sub-sites. At each of the four sub-sites, four 5-cm^2 samples were collected, in the same manner as at ELA with the SCUBA-borne scraper sampler. Soon after collection, samples were immersed in ice and brought back to the lab for sample preparation. Samples were prepared for epilithic particulate chemistry, algal taxonomy and microcystin analyses.

Since zebra mussels often formed the substrata for algal attachment, a number of zebra mussels were removed from a known area (Fig. II.5) and scrapped for algal composition, particulate chemistry, stable isotopes and microcystin analysis. An approximately 10-cm diameter ring (circumference = 64 cm^2) was randomly placed in the littoral zone at depths ranging from 2 to 6 m. Collections were made by removing all zebra mussels within or partially within the ring by scooping the mussels with a spoon. The remaining material was removed from the area with a large-bore 60-ml syringe. Both

zebra mussels and syringe material were placed into whirlpak, on ice until sample preparation. Five such collections were made.

Epilithic sample processing

Sample processing occurred within 24 hours of sampling (samples were kept immersed in an ice bath until preparation). The four 5-cm^2 samples were combined for both depths for a sub-site sample suspension. These were blended for three 1-s pulses at low speed to homogenize the samples. The suspension was then transferred to a stirring beaker and subsamples of these suspensions were removed, using a large-bore syringe, for C, N, P, Chl a and stable isotope analyses. The sub-site samples were then combined to produce a composite for each site, keeping the shallow and transition zone samples distinct. From each of the suspensions, 20-mL subsamples were removed using a large-bore syringe and frozen in darkness for microcystin analysis. Also, duplicate 20-ml subsamples were removed and preserved with 4% Lugol's solution for algal enumeration, taxonomy and biovolume estimation.

Zebra mussel processing

Lake water was added to the collection bag (whirlpak) containing the mussels, the bag was shaken and mussels were rinsed and decanted until the supernatant was reasonably clear. The zebra mussels were then brushed and scraped using a small brush and tweezers to remove all the attached algae. These particulates were then added to the mussel suspension. The samples were processed at a volume of 1.6 L, to adjust the suspension to the concentration used for epilithic samples (0.05 $cm^2 \cdot ml^{-1}$). The samples were analyzed for C, N, P, Chl a, stable isotopes and microcystins. Of the five sample

collections made, three were used in determination of the technique and were not used in sample analysis. Therefore all results were based solely on two samples.

II.2 Results:

The following section summarizes the quantitative (Table II.2; Fig. II.8 -II.11) and qualitative results from the Lake Wolsey 2006 epilithic sampling trip. The major challenge of the sampling trip was that every hard surface (rock, branches, and other mussels) in the littoral zone of Lake Wolsey was colonized by zebra mussels (Fig. II.4 and II.5) from ~ 1 to 10 m. Because of this the littoral habitat was completely altered. In addition, to changing the character of the littoral habitat, zebra mussel colonization increased the surface area available for algal attachment. Zebra mussel colonization appeared to be greater at site A in the south than sites B or C or in northern sites. It may have been that the aquaculture operation provided decreased wave energy that allowed shallower colonization in this area.

Generally, zebra mussels in Lake Wolsey were covered by thick tufts of dark algae (diatoms and cyanobacteria Fig. II.11). However, Lake Wolsey also had abundant metaphytic filamentous green algal (FGA) growth in some of the areas sampled (Fig. II.6). FGA growth generally occurred around < 0.5 m depth, although growth deepened in the south with increased proximity to the cage; site A had greater abundance and deeper growth of FGA than B or C. North of the cage FGA growth was only noted at site A at approximately 2.6 m.

On Sept 14th 2006, the first day of sampling, a phytoplankton bloom occurred in the near-shore region of Lake Wolsey (Fig. II.7). The bloom created suboptimal conditions for littoral sampling as it caused decreased visibility in the region. Initially the

bloom began at the surface but as the bloom intensified it became more prominent throughout the water column. It was suspected that the bloom was likely due to a blue-green alga, samples were taken but they were not analyzed. Secchi depth readings prior to the bloom ranged from 6.5 to 7 m (M. Meeker, Meeker Aquaculture, Evansville Ontario, pers. comm.). However on the day of the bloom, I could not see my hand below the surface. By the following day the bloom had dissipated and the water appeared clear, a likely result of zebra mussel filtering, as zebra mussels have the capacity to filter the equivalent of an entire lake water column in a matter of days. They can, therefore, drastically reduce phytoplankton concentrations and increase water clarity (Fanslow *et al.* 2007).

II.3 Conclusions:

The effects of the aquaculture operation in Lake Wolsey could not be separated from other influences in the lake. There were too many forces affecting nutrient concentrations and algal growth in Lake Wolsey for the techniques employed to detect any potential impacts from the aquaculture operation. In addition to a change in littoral habitat caused by the invasion of the zebra mussels, Lake Wolsey was also invaded by Round Gobbies (*Neogobius melanostonus* Pallas) (Fig. II.5) and the Spiny-water Flea (*Bythotrephes cederstroemi* Schoedler). All three of which can have major implications on littoral energy transfers and food web dynamics. Also, early cottage and farm developments have added nutrients to the system for decades longer than the aquaculture operation and have probably caused changes in the natural community long before the start of the aquaculture operation.

It was likely that the contribution from aquaculture was only a small fraction of the total nutrient load to the system. Also, since Lake Wolsey is a large system, with a connection to Lake Huron, it was probable that the nutrients it was receiving from the aquaculture operation were easily assimilated (Black 2001). However, even though the aquaculture operation may not be the main impacting agent on the system, it still plays a part. Every new invasion, every added nutrient and every physical change to the system adds stress to this already highly disturbed system.

Figure II.1: Google Earth© Image of Lake Huron showing Manitoulin Island and the location of Lake Wolsey, Tele Atlas ©2008, Image © 2008 TerraMetrics http://earth.google.com.

Figure II.2: Google Earth© Image of Lake Wolsey showing causeway at Indian point linking Lake Wolsey to Campbell Bay and the location of the aquaculture cage on the southwest bank of Lake Wolsey, Tele Atlas ©2008, Image © 2008 TerraMetrics http://earth.google.com.

Figure II.3: Aquaculture cage at Lake Wolsey. Photo by K. Hille September 2006.

Table II.1: Physical and chemical characteristics in Lake Wolsey (Clerk 2002).

Parameter	Units	Value
Surface area	Ha	2315
Drainage area	Ha	7043
Mean depth	m	11
Maximum depth	m	25
Total volume	m^3	$79.7x10^6$
Water residence[a]	days	215
Avg. spring Total P	$\mu g \cdot L^{-1}$	9.3[a]; 9.0[b]
Avg. fall turnover Total P	$\mu g \cdot L^{-1}$	7.3[a]; 20 [b]

a Data from C. Podemski, pers. comm.
b Data from 1998-99 (Gale 2000 unpublished data cited in Clerk 2002)
c Data from 1986-88 (Gale 2000 unpublished data cited in Clerk 2002)

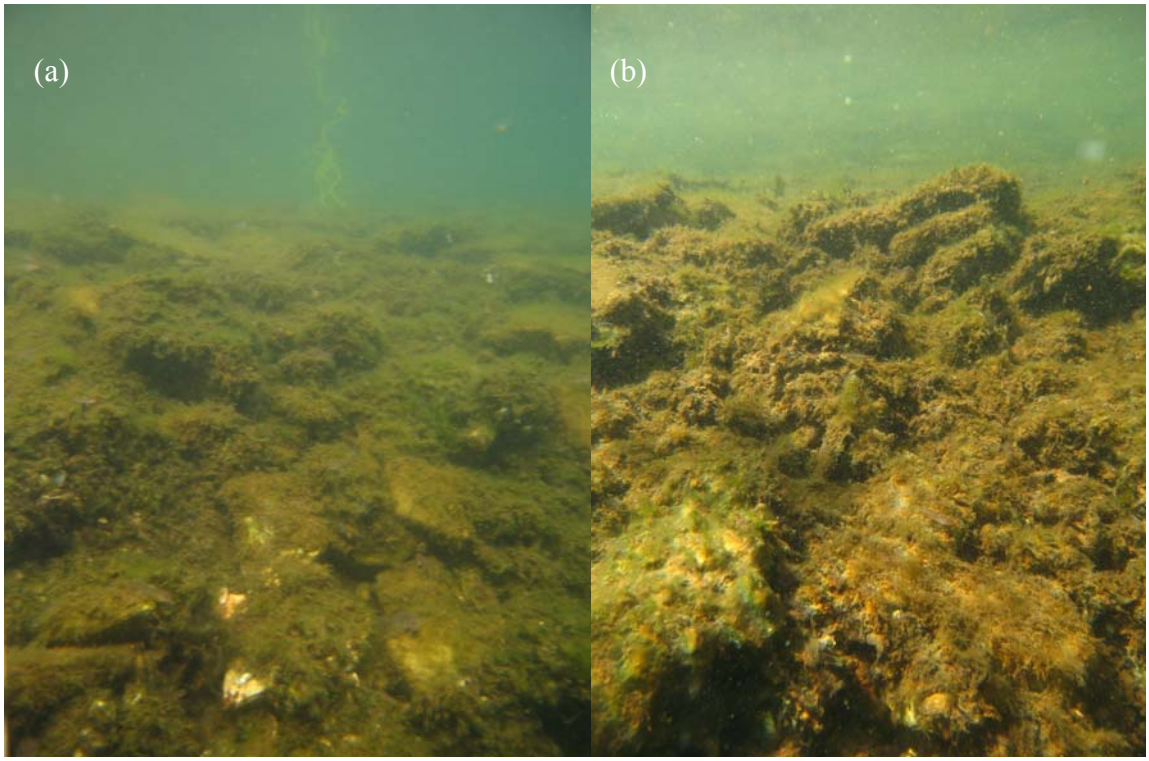

Figure II.4: Waterscape of the littoral zone in Lake Wolsey (a) distant view, (b) close-up view from below looking up towards the surface. Photos by M. Turner September 2006.

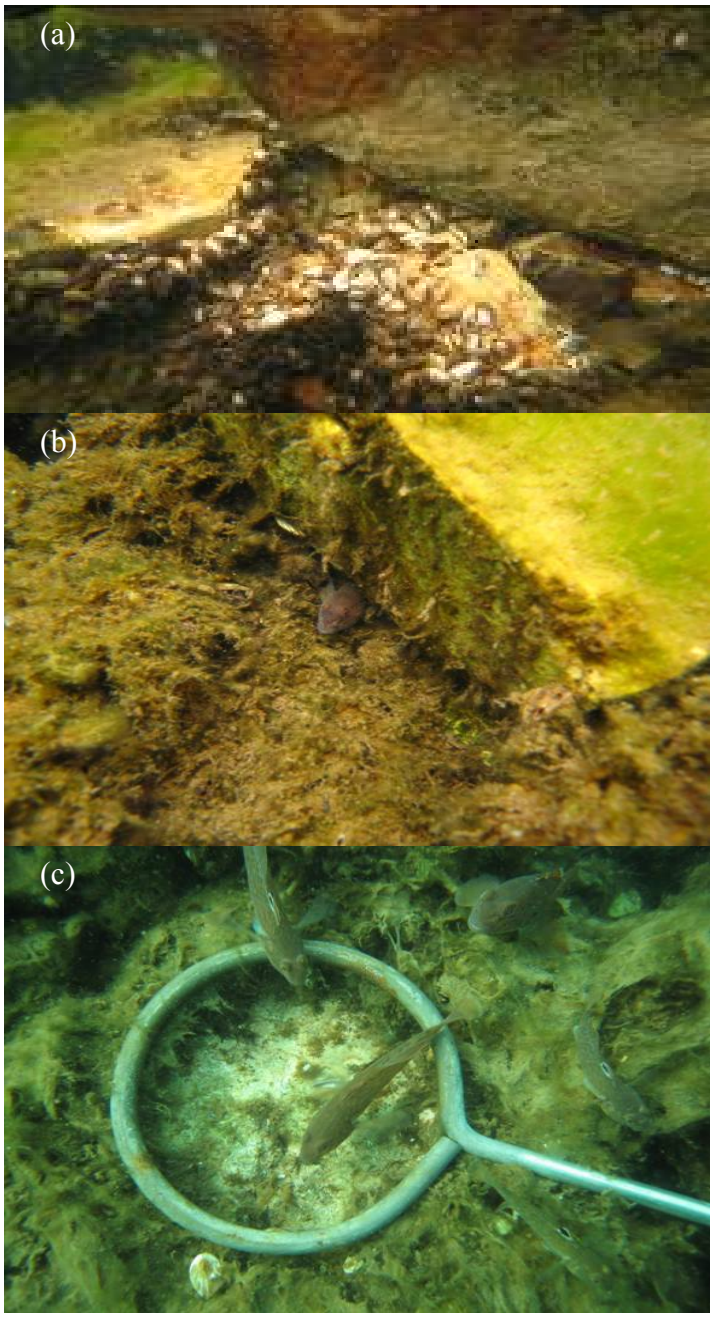

Figure II.5: Zebra mussel colonization in Lake Wolsey, September 2006. (a) Zebra mussels found in the littoral zone un-colonized by algae. (b) Zebra mussels colonized by thick tufts of benthic algae. (c) Zebra mussel sampling ring. All zebra mussels were removed from within the area of the ring exposing the rock surface below. Note the Round Gobies in (b) and (c). Photos by M. Turner September 2006.

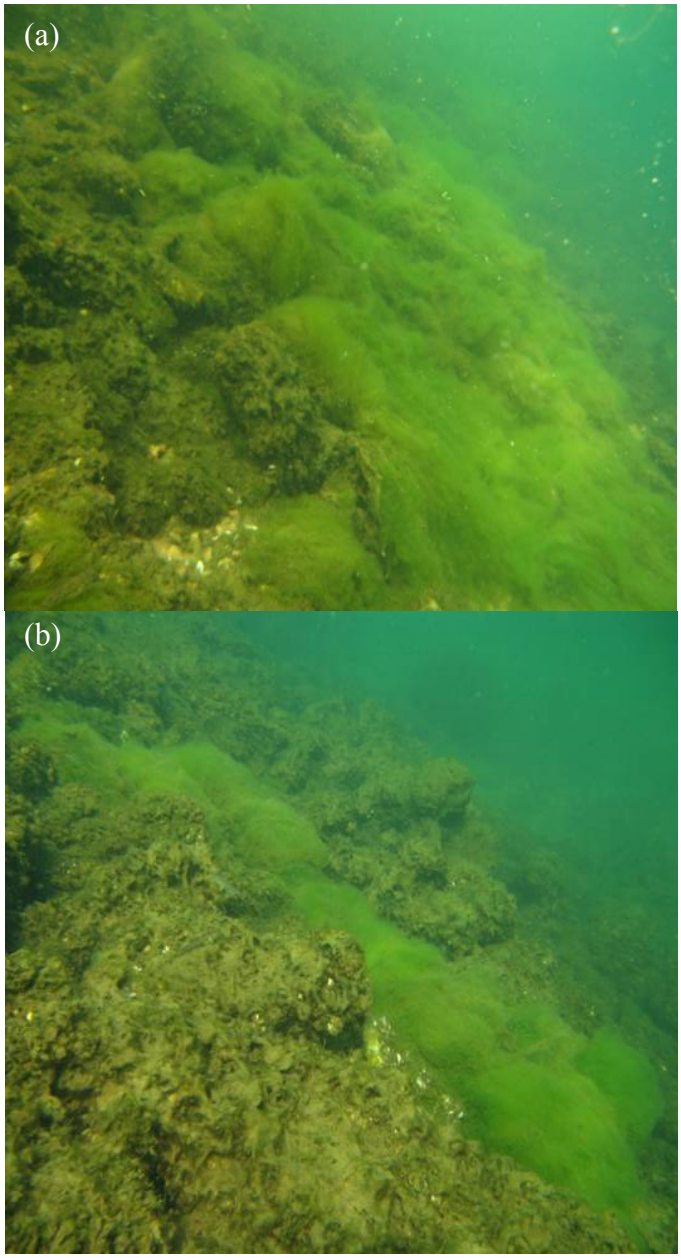

Figure II.6: Metaphytic filamentous green algal growth photo taken from (a) near and (b) far in the littoral zone of Lake Wolsey. Photos by M. Turner September 2006.

Figure II.7: Phytoplankton bloom in the near-shore region of Lake Wolsey. Photo by K. Hille September 2006.

Table II.2: Summary table of all chemical and biological parameters monitored in Lake Wolsey in September 2006. All values represent the site mean with standard error (n = 6). Only two epizoan samples were analyzed therefore no standard deviation was computed.

Parameter	Epilithon (µg·cm-2)		Epizoan (µg·cm-2)
	Shallow zone	Transition zone	Zebra mussels
Particulate P	0.55 ± 0.05	0.60 ± 0.10	1.95
Particulate C	210 ± 17.7	232 ± 28.4	273
Particulate N	15.3 ± 1.02	17.1 ± 1.85	28.8
C: N	13.6 ± 0.74	13.8 ± 0.81	9.5
C: P	420 ± 58.1	391 ± 23.0	167
N: P	30.5 ± 2.93	28.4 ± 1.01	8.1
Chl a	11.6 ± 1.41	10.5 ± 1.86	12.7
Particulate algal carbon	11.0 ± 1.80	10.3 ± 2.70	8.9
Microcystins	0.001 ± 0.0004	0.003 ± 0.002	0.005
Total algal biomass	5420 ± 747	4890 ± 717	5798
Simpson's diversity	0.733 ± 0.03	2.54 ± 0.20	2.44
Shannon-weaver	0.748 ± 0.03	2.63 ± 0.18	0.76

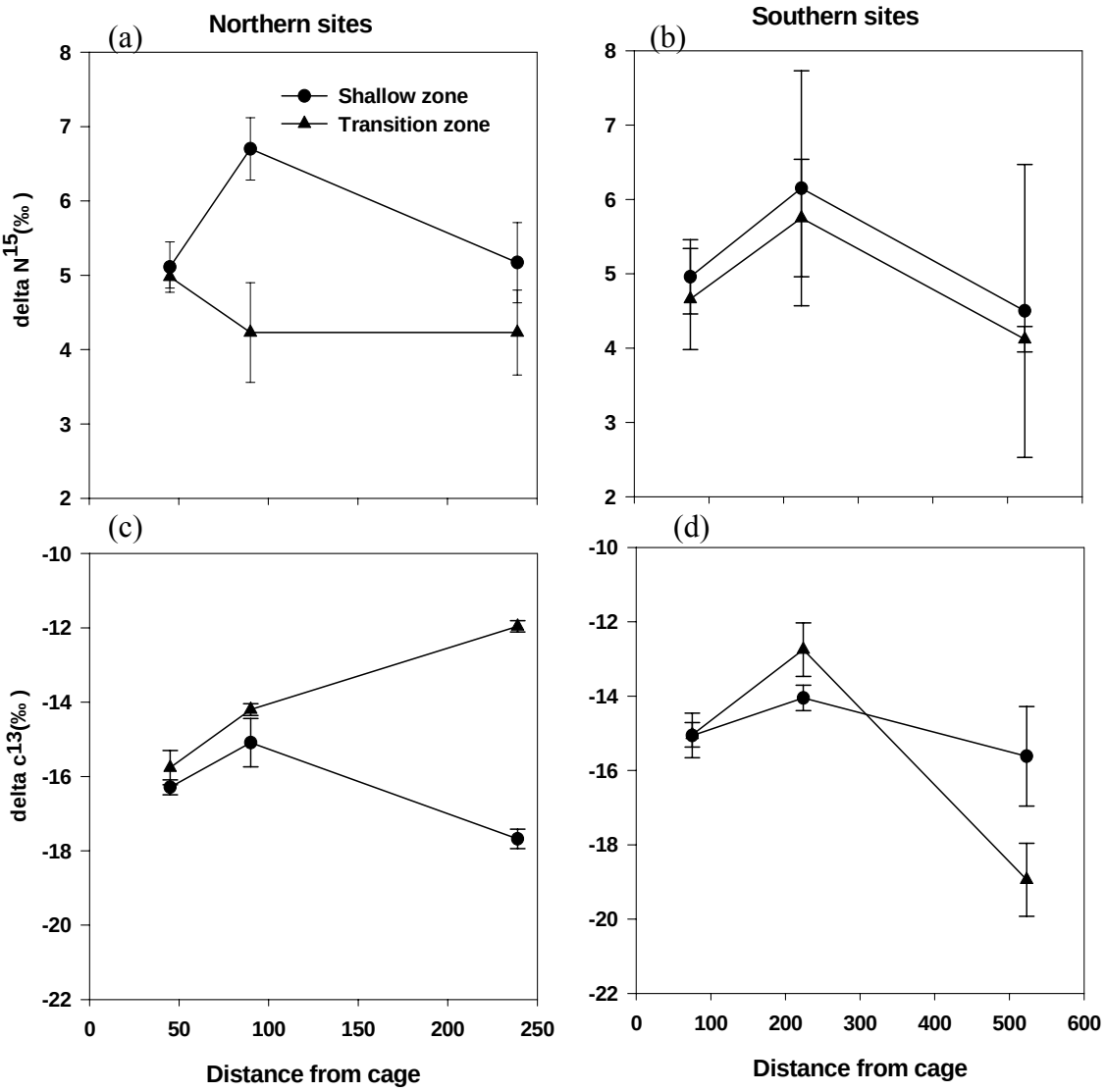

Figure II.8: Stable isotopes of (a), (b) δ15N (‰) and (c), (d) δ13C (‰) in the epilithon of Lake Wolsey north and south of the aquaculture operation in September 2006. The means and standard errors of both the transition zones and shallow zones are shown (n = 4).

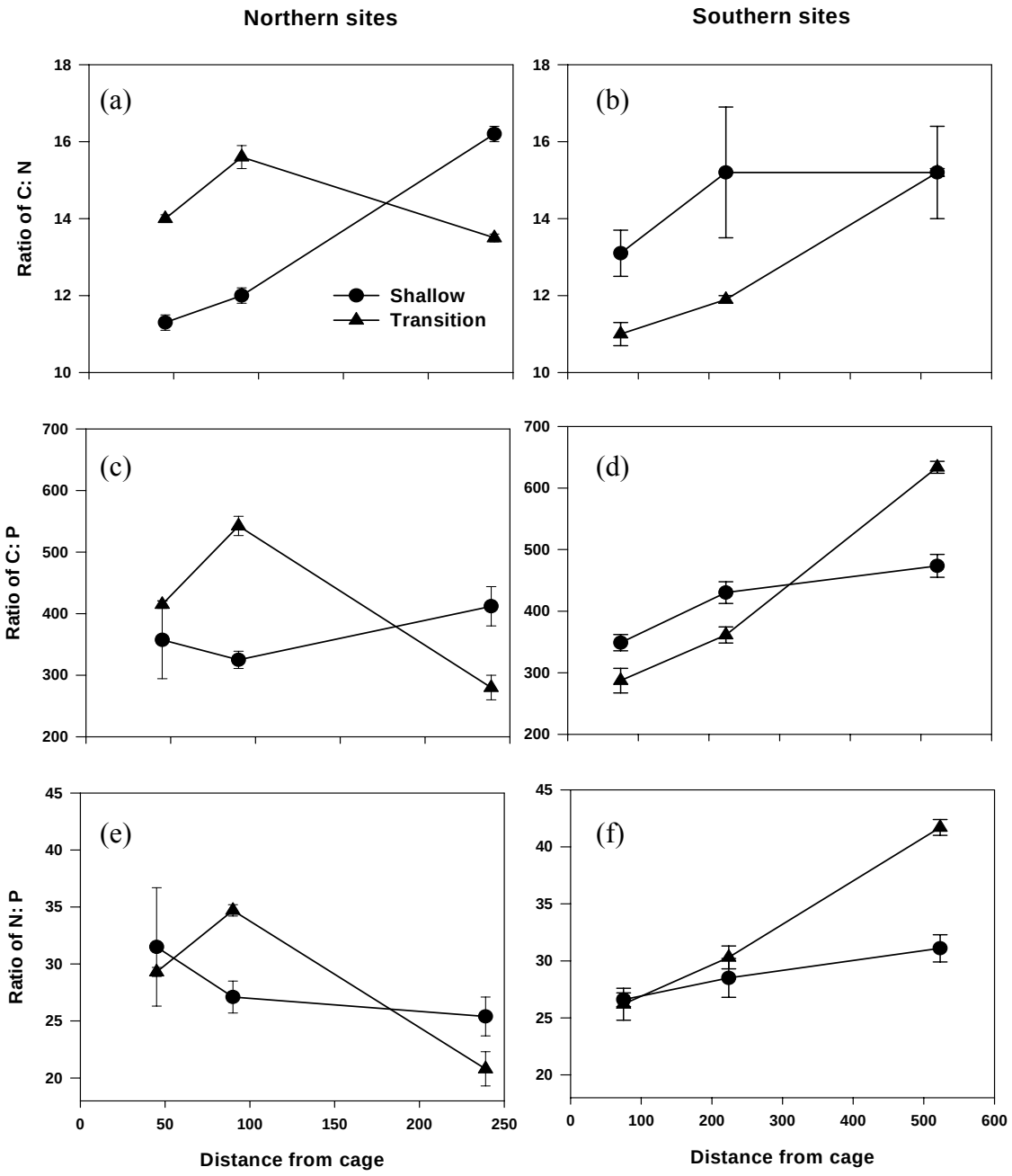

Figure II.9: Nutrient ratios of (a), (b) C: N; (c), (d) C: P; and (e), (f) N: P in the epilithon of Lake Wolsey north and south of the aquaculture operation in September 2006. The means and standard errors of both the transition zones and shallow zones are shown (n = 4).

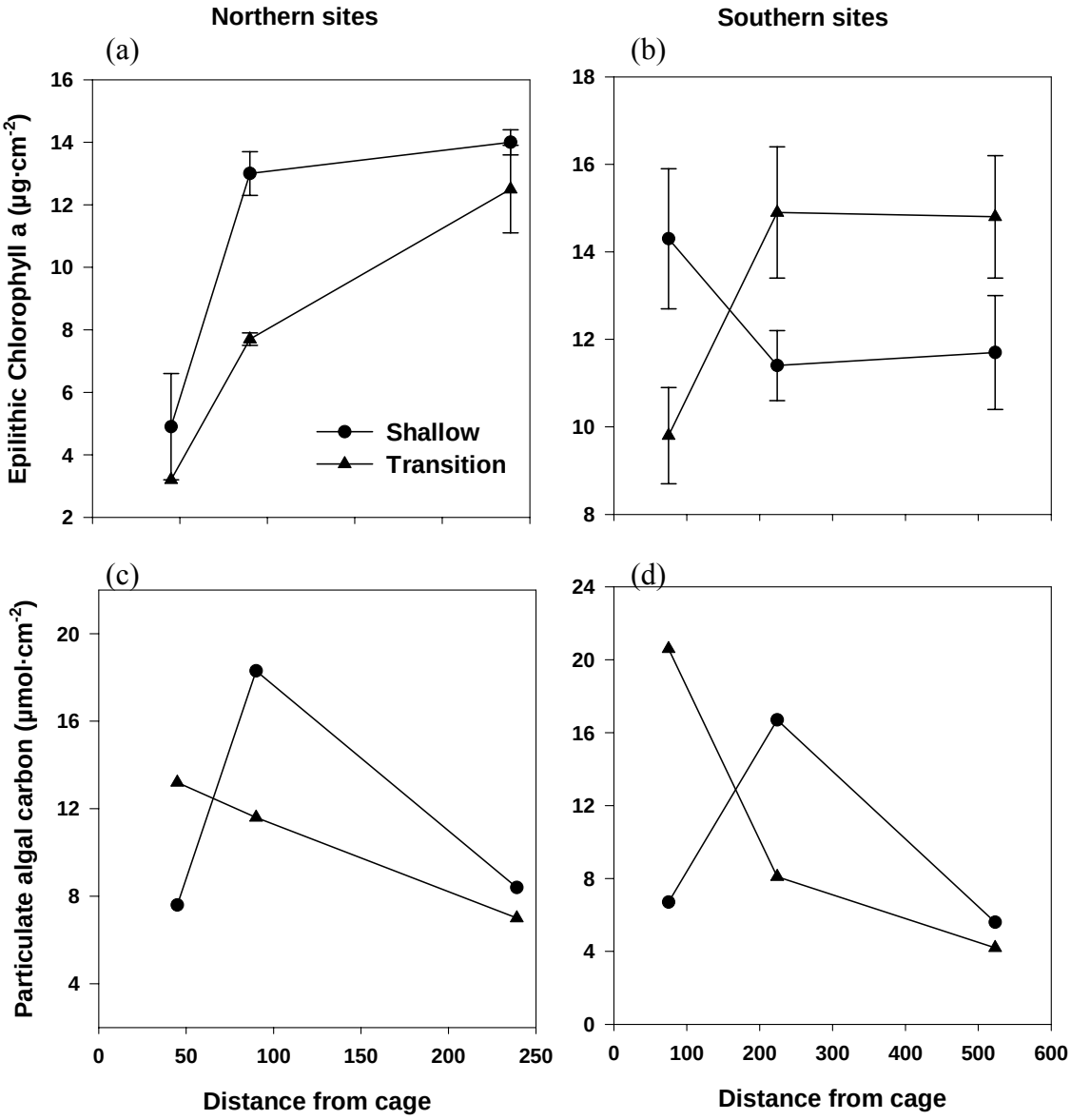

Figure II.10: (a) Chlorophyll a and (b) particulate algal carbon in the epilithon of Lake Wolsey north and south of the aquaculture operation in September 2006. The means and standard errors of both the transition zones and shallow zones are shown (n = 4).

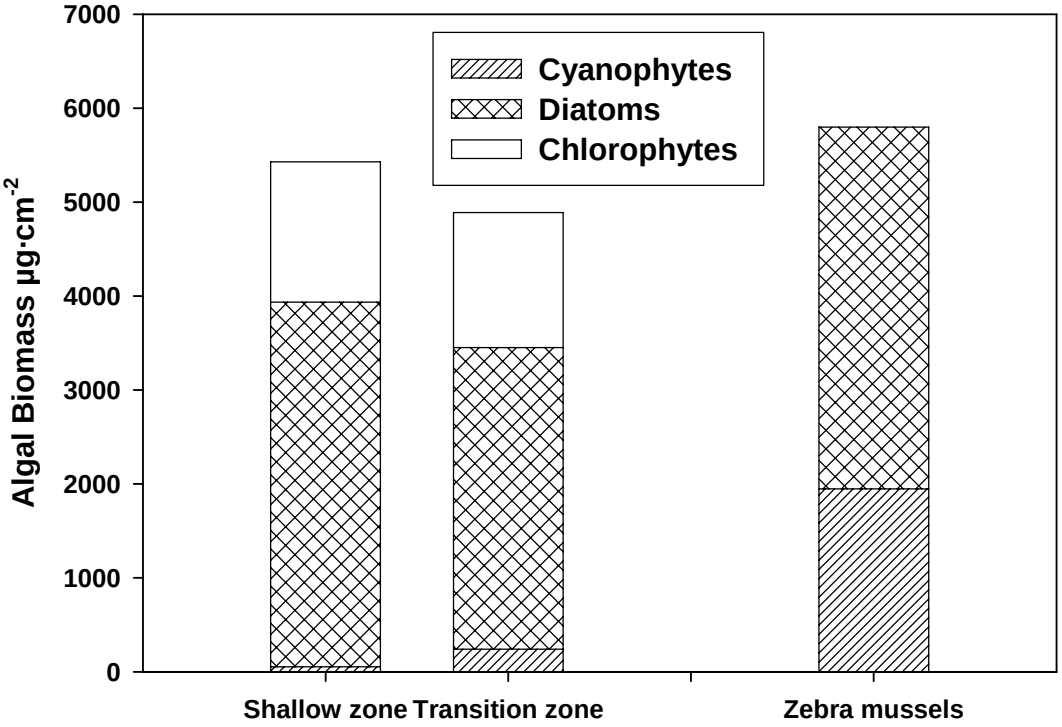

Figure II.11: Algal biomass and composition in the shallow zone, transition zone and in algae attached to zebra mussels in the littoral zone of Lake Wolsey, September 2006.

Appendix III: Vial, Chamber, and Rate Variation

As described in the methods of chapter 3, it was important in the analysis of metabolic rates for vial precision to be high and percent coefficients of variation (% CV) to be low. High coefficients of variation in the concentrations (Appendix III, Table III.1-III.2) translate into inaccuracies in the calculated rates (Appendix III, Table III.3-III.4). If analytical variability propagates in this way through the calculations of metabolic rates it can cause misinterpretations of the algal ecology in the system. The lake-wide variation estimates from Tables 3.2 and 3.3 are based on calculations of the rate-level variation and vial/chamber-level variation as outlined here.

Table III.1: Site and chamber level variation in the oxygen data ($\mu mol \cdot O_2 \cdot cm^{-2} \cdot h^{-1}$) for L375, L373 and L239 in July of 2006 and 2007

2006 Sites A, B and C

Lake	Site																					
	A										B											
	Initial			Irradiated			Dark 1			Dark 2			Initial			Irradiated			Dark 1			Dark 2		
	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV
L375	279	4	1.53	363	3	0.42	269	2	1.06	275	3	0.43	279	3	1.00	351	2	0.33	271	3	0.46	258	3	1.04
L373	268	4	1.13	239	3	0.47	258	2	0.95	257	3	0.47	274	4	0.93	338	3	0.93	259	3	0.51	264	3	0.79
L239	280	4	1.91	337	3	1.64	265	2	0.17	268	3	0.44	280	4	1.30	325	3	0.45	265	3	2.34	261	3	0.94

Lake	Site										
	C										
	Initial			Irradiated			Dark 1			Dark 2		
	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV
L375	279	4	1.67	387	3	0.31	268	3	0.38	304	3	0.86
L373	274	3	1.51	379	3	0.74	258	3	0.94	264	3	1.28
L239	280	3	0.39	336	2	0.37	266	3	0.96	267	2	0.14

2007 Sites A, B and C

Lake	Site																					
	A									B									C			
	Initial			Irradiated			Dark			Initial			Irradiated			Dark			Initial			Irradiated			Dark		
	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV
L375	261	4	1.59	291	4	2.43	258	4	2.82	272	4	2.23	308	3	1.38	274	3	4.10	284	3	0.77	301	6	3.2	279	6	1.62
L373	268	4	1.30	298	3	2.45	271	6	1.56	273	3	3.66	293	6	1.33	271	5	1.14	285	4	2.16	275	3	2.84	268	6	3.00
L239	263	2	0.45	291	6	209	253	6	2.53	264	4	3.53	278	6	1.73	256	6	2.85	273	2	1.51	290	6	2.56	274	5	1.96

2007 Sites D, E and F

Lake	Site																					
	D									E									F			
	Initial			Irradiated			Dark			Initial			Irradiated			Dark			Initial			Irradiated			Dark		
	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV
L375	272	2	1.14	299	6	1.36	259	6	1.33	268	2	0.46	290	6	1.20	268	6	2.93	286	3	1.70	286	4	1.02	262	6	4.81
L373	273	3	3.06	274	5	6.12	239	5	3.02	290	4	2.02	304	4	4.55	266	1	na	275	3	1.26	290	6	2.88	257	6	5.88
L239	279	4	4.90	283	5	2.80	257	6	2.69	260	2	0.17	278	6	1.56	268	5	3.96	262	4	2.32	292	4	1.01	266	5	3.88

Table III.2: Site and chamber level variation in the carbon data ($\mu mol \cdot CO_2 \cdot cm^{-2} \cdot h^{-1}$) for L375, L373 and L239 in July of 2006 and 2007

2006 Sites A, B, and C

Lake	Site																					
	A												B									
	Initial			Irradiated			Dark 1			Dark 2			Initial			Irradiated			Dark 1			Dark 2		
	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV
L375	481	4	0.89	390	3	2.72	487	2	1.21	472	3	2.85	486	3	0.13	405	2	0.37	310	3	0.73	312	2	1.52
L373	269	4	0.66	231	3	2.18	246	2	2.61	254	2	0.29	266	4	1.32	200	3	2.64	194	3	1.37	187	3	2.07
L239	152	4	3.49	112	3	0.81	163	2	0.04	162	2	1.81	146	3	2.26	131	3	1.05	169	3	3.29	173	3	1.07

Lake	Site											
	C											
	Initial			Irradiated			Dark 1			Dark 2		
	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV
L375	417	2	0.88	325	3	1.36	436	3	1.58	420	2	0.96
L373	190	3	1.98	137	3	1.14	184	3	1.02	189	3	0.36
L239	154	3	1.76	119	2	2.44	168	2	0.07	164	2	5.12

2007 Sites A, B, and C

Lake	Site																					
	A									B									C			
	Initial			Irradiated			Dark			Initial			Irradiated			Dark			Initial			Irradiated			Dark		
	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV
L375	239	4	1.15	225	4	4.41	251	4	1.56	233	4	0.58	211	3	1.83	261	3	2.89	234	4	1.77	208	6	2.01	232	6	2.98
L373	153	4	3.74	139	5	3.24	164	6	3.22	154	6	2.53	144	6	1.23	154	5	0.85	155	4	1.48	172	3	4.64	167	6	4.59
L239	154	3	3.20	126	6	4.32	150	6	4.96	144	4	2.31	121	6	3.43	145	5	2.19	141	2	0.56	129	6	1.39	152	5	5.51

2007 Sites D, E, and F

Lake	Site																					
	D									E									F			
	Initial			Irradiated			Dark			Initial			Irradiated			Dark			Initial			Irradiated			Dark		
	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV	mean	n	%CV
L375	241	2	1.27	209	6	1.27	238	6	2.31	244	2	4.23	214	6	1.59	237	6	6.03	237	4	1.88	216	4	3.86	252	5	4.46
L373	158	3	3.53	163	5	6.97	199	5	4.90	174	3	1.24	132	5	7.38	163	3	1.16	154	3	2.32	140	5	1.85	192	3	4.87
L239	147	3	1.00	119	6	4.64	147	6	1.07	137	2	2.29	129	6	2.72	150	5	5.72	141	4	2.39	142	4	5.84	143	3	1.82

Table III.3: Variation in the calculated rates for the oxygen data ($\mu mol \cdot O_2 \cdot cm^{-2} \cdot h^{-1}$) in L375, L373 and L239 in July of 2006 and 2007.
The 2006 dark data used two chambers that were averaged following the calculation of respiration rates (Ave. of Dark chambers).
The 2007 data were derived from the combination of two chambers at each site (for further details refer to ch. 3 methods section).

2006 Sites A, B and C

Lake	Site										
	A										
	Irradiated			Dark 1			Dark 2			Ave. of Dark chambers	
	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.
L375	1840	5.4	0.29	-178	6.3	3.54	-85.0	5.1	6.00	-131.5	65.8
L373	1250	3.9	0.31	-272	5.1	1.88	-293	3.8	1.30	-282.5	14.8
L239	1260	9.2	0.73	-329	6.2	1.88	-274	6.4	2.34	-301.5	38.9

Lake	Site										
	B										
	Irradiated			Dark 1			Dark 2			Ave. of Dark chambers	
	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.
L375	1410	3.8	0.27	-145	3.7	2.55	-361	4.7	1.30	-253	153
L373	1510	4.9	0.32	-252	3.3	1.31	-151	3.8	2.52	-202	71
L239	1020	4.6	0.45	-340	8.7	2.56	-452	5.1	1.13	-396	79

Lake	Site										
	C										
	Irradiated			Dark 1			Dark 2			Ave. of Dark chambers	
	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.
L375	2020	5.6	0.277	-184	5.5	2.99	-172	6.2	3.60	-178	8.5
L373	2270	6.1	0.269	-256	5.8	2.27	-135	6.5	4.81	-196	86
L239	1290	2.3	0.178	-317	3.4	1.07	-298	1.5	0.50	-308	13

2007 Sites A, B and C

Lake	Site																	
	A						B						C					
	Irradiated			Dark			Irradiated			Dark			Irradiated			Dark		
	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV
L375	255	8.3	3.25	-614	8.4	1.37	655	7.5	1.15	-175	12.8	7.31	750	9.9	1.32	- 57.0	5.1	8.95
L373	747	8.1	1.08	29.0	5.5	19.0	890	7.3	0.82	30.0	6.9	23.0	140	10.1	7.21	- 87.0	10.1	11.6
L239	707	6.2	0.88	-277	6.5	2.35	444	6.3	1.42	-246	8.4	3.41	1020	8.5	0.83	356	6.8	-1.91

2007 Sites D, E and F

Lake	Site																	
	D						E						F					
	Irradiated			Dark			Irradiated			Dark			Irradiated			Dark		
	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV
L375	585	5.1	0.87	-471	4.6	0.98	330	3.7	1.12	-214	7.9	3.69	348	5.6	1.61	-515	14	2.62
L373	3.00	18.7	623	-909	11.1	1.22	1200	15.1	1.26	-288	5.8	2.01	544	9.1	1.67	-516	16	3.00
L239	513	15.8	3.08	-145	15.3	10.6	504	4.4	0.87	160	11	6.63	973	6.8	0.70	104	12	11.4

Table III.4: Variation in the calculated rates for the carbon data (µmol·CO_2.cm^{-2}·h^{-1}) in L375, L373 and L239 in July of 2006 and 2007.
The 2006 dark data used two chambers that were averaged following the calculation of respiration rates (Ave. of Dark chambers).
The 2007 data were derived from the combination of two chambers at each site (for further details refer to ch. 3 methods section).

2006 Sites A, B and C

Lake	Site										
	A										
	Irridiated (Pnet)			Dark (Rd) 1			Dark 2			Ave. of dark chambers	
	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.
L375	1550	14	0.90	-463	17	3.61	-209	17	7.99	-336	180
L373	248	6.5	2.62	-108	7.8	7.22	-232	2.4	1.03	-170	88
L239	843	17	1.99	-256	8.6	3.36	-261	7.3	2.80	-259	3.5

Lake	Site										
	B										
	Irriadiated			Dark 1			Dark 2			Ave. of Dark chambers	
	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.
L375	1110	2.3	0.21	2600	3.1	0.12	2510	6.1	0.24	2555	64
L373	942	7.6	0.81	930	5.2	0.56	1041	6.2	0.60	986	78
L239	443	4.4	0.99	-412	7.9	1.92	-539	4.6	0.85	-476	90

Lake	Site										
	C										
	Irridated			Dark 1			Dark 2			Ave. of Dark chambers	
	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.
L375	2560	7.5	0.29	408	10.4	2.55	656	7.9	1.20	532	175
L373	2210	5.1	0.23	1080	5.1	0.47	962	4.7	0.49	1021	83
L239	724	5.3	0.73	-411	3.3	0.80	-314	12	3.95	-363	69

2007 Sites A, B and C

Lake	Site																	
	A						B						C					
	Irradiated			Dark			Irradiated			Dark			Irradiated			Dark		
	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV
L375	284	10.6	3.73	-410	5.3	1.29	606	4.2	0.69	-621	8.1	1.3	1060	5.9	0.56	120	8.2	6.83
L373	400	7.3	1.83	-258	7.8	3.02	402	4.3	1.07	-1.00	4.1	410	-606	8.3	1.37	-406	8.1	2.00
L239	532	7.4	1.39	-83	8.9	10.7	827	5.3	0.64	38.0	4.6	12.1	691	1.9	0.27	-199	8.4	4.22

2007 Sites D, E and F

Lake	Site																	
	D						E						F					
	Irradiated			Dark			Irradiated			Dark			Irradiated			Dark		
	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV	mean	Std dev.	%CV
L375	780	4.1	0.53	-7	6.8	97.1	592	10.4	1.76	28.0	17	61.4	856	9.2	1.07	-469	11.9	2.54
L373	117	12.6	10.8	-855	11.3	1.32	1370	10.1	0.74	137	2.9	2.12	895	4.4	0.49	-776	10.1	1.30
L239	604	5.7	0.94	-104	2.2	2.12	455	4.7	1.03	-263	9.1	3.46	16.0	9.1	56.9	-20.0	4.3	21.5

Appendix IV: List of Commonly Used Abbreviations

Abbreviation	Meaning	Units
P	Phosphorus	$\mu g \cdot L^{-1}$ or $\mu g \cdot cm^{-2}$
N	Nitrogen	$\mu g \cdot L^{-1}$ or $\mu g \cdot cm^{-2}$
C	Carbon	$\mu g \cdot L^{-1}$ or $\mu g \cdot cm^{-2}$
TP	Total P	$\mu g \cdot L^{-1}$
TN	Total N	$\mu g \cdot L^{-1}$
TC	Total C	$\mu g \cdot L^{-1}$
Chl a	Chlorophyll a	$\mu g \cdot L^{-1}$ or $\mu g \cdot cm^{-2}$
DOC	Dissolved Organic Carbon	$\mu mols \cdot L^{-1}$
DIC	Dissolved Inorganic Carbon	$\mu mols \cdot L^{-1}$
$\delta^{15}N$	Nitrogen Stable Isotope	‰
$\delta^{13}C$	Carbon Stable Isotope	‰
GC	Gas Chromatography	
IRGA	Infrared Gas Analysis	
O_2	Oxygen	$\mu mol \cdot O_2$
CO_2	Carbon	$\mu mol \cdot CO_2$
He	Helium	$\mu volt \cdot S$
CH_4	Methane	$\mu volt \cdot S$
Ar	Argon	$\mu volt \cdot S$
Ne	Neon	$\mu volt \cdot S$
NSat	Nitrogen Saturation	$\mu volt \cdot S$
Pgross	Gross photosynthesis	$\mu mol \cdot CO_2 \cdot cm^{-2} \cdot h^{-1}$ or $\mu mol \cdot O_2 \cdot cm^{-2} \cdot h^{-1}$
Pnet	Net photosynthesis	$\mu mol \cdot CO_2 \cdot cm^{-2} \cdot h^{-1}$ or $\mu mol \cdot O_2 \cdot cm^{-2} \cdot h^{-1}$
Rd	Dark respiration	$\mu mol \cdot CO_2 \cdot cm^{-2} \cdot h^{-1}$ or $\mu mol \cdot O_2 \cdot cm^{-2} \cdot h^{-1}$

PQ	Photosynthetic quotient	
ADP	Adenosine diphosphate	
ATP	Adenosine triphosphate	
SD	Standard Deviation	
% CV	Percent Coefficient of Variation	%
MC-LR	Microcystin-LR	$\mu g \cdot cm^{-2}$ or $\mu mols \cdot L^{-1}$

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