The Ecology of the Nuisance Macroalga, Cladophora glomerata, and its Resurgence in Lake Ontario

by

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I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Cladophora glomerata is the dominant spring and summer epilithic macroalga in the lower Laurentian Great Lakes, and was a notorious nuisance prior to nutrient management of the early 1970s. It is an indicator of excessive nutrient supply and appears to be experiencing a resurgence in the nearshore of the lower Great Lakes. This thesis examines the ecology of *C. glomerata* in an urbanized location of Lake Ontario and addresses decadal scale environmental changes to the lake and their impact on this macroalga.

A *Cladophora* growth model (CGM) was calibrated and validated to simulate attached and sloughed *Cladophora* biomass using two years of collected input data and independent measurements of *Cladophora* biomass. The CGM was used to hindcast *Cladophora* growth using multiplicative factors of seasonal minimal tissue phosphorus concentrations (Q_P) and seasonal mean nearshore light attenuation (Kd_{PAR}) of the early 1970s and 1980s relative to contemporary data. *Cladophora* Q_P in Lake Ontario is currently lower than in the early 1980s, resulting in reduced *Cladophora* biomass at all depths in the euphotic zone. Kd_{PAR} has also declined, most strongly since the mid-1990s, following *Dreissena* mussel invasion, driving an increase in macroalgal biomass between 3.5 and 10 m depth. Combining these effects, the CGM predicted that biomass is currently lower in Lake Ontario than in the early 1980s. However, increases in Q_P in this post-dreissenid mussel period are predicted to result in greater *Cladophora* proliferation than in previous decades due to increased nearshore water clarity.

The *in situ* rates of primary production on *Cladophora*-dominated rocky substrata at 1m depth were measured through the spring and summer. Net primary production (NPP) was measured as change in dissolved inorganic carbon (using IRGA) in benthic incubation chambers flushed continuously with water. Incubations were of 15- 20 minutes duration, permitting measurements of productivity rates over diurnal and seasonal scales. Maximum biomass-specific net photosynthetic rates (P^B_{max}) were highest in the spring and late-summer/fall (2.39, 1.98 $mgC \cdot gDM^{-1} \cdot hr^{-1}$, respectively) and decreased to negative rates by early summer (-0.76 $mgC \cdot gDM^{-1} \cdot hr^{-1}$). Directly measured rates of net primary production were simulated with the CGM. Simulated depth-integrated rates of *Cladophora* primary production were compared with published depth-integrated measurements of planktonic primary production from Lake Ontario. From the shoreline to the 12 m depth contour, the benthos was

estimated to contribute 70% of the areal primary production. On a seasonal basis, attached macroalgae are an important component of the energy flux in the Lake Ontario nearshore.

This phenology of *Cladophora glomerata* growing in the western end of Lake Ontario is also described. Based on internal stoichiometric ratios (C:P and N:P), and a positive correlation between the decrease in the biomass-specific maximum photosynthetic rate (P^B_m) and phosphorus quota (Q_P), *Cladophora* productivity at shallow depths was shown to be P limited. In addition, light attenuation through the *Cladophora* canopy was estimated to be 24.1 ± 3.3 (standard deviation) m^{-1} using paired light loggers deployed *in situ*. Acclimation to lower light levels through the *Cladophora* stand was demonstrated by significantly higher *Cladophora* chlorophyll concentrations at the base of the canopy. Decreases in *Cladophora* canopy cover in the summer resulted in increased P^B_m, even when Q_P remained near the minimal cell quota, indicating potential co-limitation of *Cladophora* productivity by light during peak standing crop. *Cladophora* growing at 1m depth was also shown here to be tolerant of high irradiance, with an average decline of less than 10% in F_v/F_m at during peak midday insolation, regardless of nutrient status or ambient water temperature.

In conjunction with its role as a seasonally important nearshore primary producer, *Cladophora* appears to play a role as a seasonal nutrient regulator in the nearshore of Lake Ontario. The nutrient chemistry of nearshore lake water, *Cladophora* tissue, and a dominant tributary to western Lake Ontario were examined over the growing season of 2 years. As *Cladophora* grew and assimilated nutrients in the spring, total phosphorus (TP) and soluble reactive P (SRP) concentrations declined in the nearshore. Detachment and sloughing of *Cladophora* in the late summer was associated with increasing TP in the water column. These changes in nearshore nutrient concentrations were correlated with *Cladophora* phenology and not catchment loading. Nutrient loading from Oakville Creek was compared with the nutrient uptake of an adjacent *Cladophora* stand. The TP supply directly from the creek during the growing season was insufficient to meet the concentration of stored P in *Cladophora* tissue. It appears *Cladophora* is growing on P regulated by recycling within the lake, supporting the hypothesis that dreissenid mussels are sustaining *Cladophora* growth through recycling of TP in the lake. *Cladophora* remains P limited, however, such that increases in catchment loading would further augment its resurgence.

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Chapter 1 Introduction

Cladophora autecology

Cladophora is a uniseriate, branching filamentous green macroalga. *Cladophora* has a cosmopolitan distribution with representatives found at high and low latitudes in coastal marine environments, estuaries, rivers and freshwater lakes. Among freshwater macroalgae, it is possibly the most globally widespread genus (Dodds and Gudder 1992). Freshwater *Cladophora* is usually, although not exclusively, found growing attached to hard surfaces by a holdfast (Whitton 1970; van den Hoek 1995).

Cladophora is a member of Chlorophyta, a division distinguished by the presence of light harvesting pigments chlorophylls *a* and *b*, and the order Cladophorales, distinguished by the accessory pigments β-carotene and a host of xanthophyll pigments (van den Hoek et al. 1995). *Cladophora* contain the full xanthophyll cycle complement (violaxanthin, zeaxanthin, and antheroxanthin; Ensminger et al. 2001), which serve as photoprotection, dissipating excess light energy as heat (Demmig-Adams and Adams 1996). Generally among Chlorophytes, increasing starch is observed when the macronutrients N and P are limiting to growth. *Cladophora* possess many discoid parietal chloroplasts which contain thylakoids grouped to form lamellae (van den Hoek et al. 1995). *Cladophora* cells are multinucleate and are surrounded by cellulose cell walls (McDonald and Pickett-Heaps 1976). The cross-walls between filament cells are not perforated with plamodesmata (van den Hoek et al. 1995).

While marine species of *Cladophora* reproduce both sexually and asexually, freshwater forms have only been observed to reproduce asexually (Hoffman and Graham 1984). The formation of zoosporangia, which release biflagellate zoospores, may be induced by short days (8L: 16D; Hoffman and Graham 1984), but other drivers of zoosporogenesis are not well described. In the Baltic Sea, *Cladophora* zoospores continue to settle on suitable substrate from May to the end of August, initiating growth first in small crevices (Kiirikki and Lehvo 1997). Freshwater *Cladophora* can also reproduce by forming modified thick walled resting cells, called akinetes, which sustain *Cladophora* overwinter (Whitton 1970). Simple cell division is both apical and intercalary in *Cladophora,* although what drives one cell division strategy over another is not well known (Wong and Wainwright 1993).

The external cell walls of *Cladophora* are thick and lamellated, providing suitable substrate for epiphyte colonization (e.g. Stevenson and Stoermer 1982*a,b*). *Cladophora*

growing in lakes generally accumulate increasing abundances of diatom epiphytes through the summer (Whitton 1970; Stevenson and Stoermer 1982*a*), at times vastly dominated by *Cocconeis pediculus* (Whitton 1970; O'Connell et al. 1997).

The morphology of *Cladophora* is highly variable and the degree of branching varies widely between individuals even among the same species. In lentic water, *Cladophora* tends to have many branches, giving it a "bushy" appearance, while in fast moving lotic environments, filaments are often longer, forming "streamers" which can be in excess of 1-2 m length (John 2003). A distinct growth habit among the Cladophorales occurs in the species *C. aegagropila* (sometimes called *C. sauteri* or *Aegagropila linnaei*; Hanyuda et al. 2004). This ball-like (aegagropiloid) form grows slowly up to about 10 cm in diameter unattached on shallow lake or estuary floors (e.g. Gordon et al. 1985; Einarsson et al. 2004), and individuals are commonly referred to as "*Cladophora*-balls" or "marimo" in japanese (a popular novelty in the aquarium trade and character in various Japanese cartoons), but are not generally considered a nuisance.

Due to its thermal tolerance limits, the timing and duration of *Cladophora*'s growing season is established by water temperature. *Cladophora* overwinters as attached filaments and akinetes and germinates in early spring. Zoospores produced during the summer settle in the nearshore continuously in the Great Lakes (pers. observ.) and in the Baltic Sea (Kiirikki and Lehvo 1997). Rapid cell growth begins when water temperatures rise above approximately 10 ^{o}C (Bellis and McLarty 1967; Whitton 1970). In this way, temperature drives differences in the seasonal growth cycle between habitats. Temperature has been implicated as the most influential determinant of growth rate of *Cladophora* in lakes (Mantai 1974, Hoffman and Graham 1984) and thermal effluents have been correlated with high *Cladophora* biomass during times when biomass is minimal elsewhere in the Laurentian Great Lakes (Moore 1978). The literature reports a wide range of optimal temperatures for freshwater *C. glomerata* to achieve maximal photosynthetic rates (measured as O_2 evolution) ranging from 13 - 17 ^{o}C (Graham et al. 1982), to 24^{o}C (Wong et al. 1978) and up to 28 – 31 ^{o}C (Lester et al. 1988) in laboratory studies. These discrepancies highlight the physiological interactions of different environmental factors on photosynthetic rates. Ensminger et al. (2000*a*) found that water temperature, in conjunction with light intensity, were the most important determinants of photosynthetic efficiency of temperate riverine *C. glomerata*.

Along with substrate availability and water motion, requirements for light is a primary determinant of where *Cladophora* may grow. That is, light availability sets the limit for the deepest depths of colonization. Lorenz et al. (1991) estimated from *in situ* measurements and from laboratory experiments that a daily average light intensity of 27 μmol m^{-2} s^{-1} is required for *Cladophora* colonization. The depth to which this light intensity corresponds is closely related to turbidity. *Cladophora* is generally favoured by high light intensities (Whitton 1970), although this appears to be dependent on other habitat conditions, particularly ambient temperature. For example, laboratory experiments found that at 12°C, *C. glomerata* reached light saturation at 100 μmol m^{-2} s^{-1}, while at 30°C, light saturation was achieved at 750 μmol m^{-2} s^{-1} (Dodds and Gudder 1992).

The habitat requirements for rapid and prolific *Cladophora* growth have been the focus of much investigation. *Cladophora* germination and growth usually requires hard substrates for attachment, such as rocks, mussels, and artificial reef structures (Whitton 1970). A further physical requirement for *Cladophora* growth is water motion. The need for water motion is usually interpreted as the need for reducing the boundary layer through which nutrients and dissolved gases diffuse (Raven 1990).

Theoretical evaluation of algal physiology and some field evidence suggests that DIC speciation and availability may affect the quantum efficiency and productivity of aquatic autotrophs (Rivers and Peckol 1995; Tortell 2000), especially macroalgae (Turner et al. 1995). Investigations of phytoplankton have demonstrated that decreasing CO_2 levels are associated with decreasing quantum efficiency (Badger and Andrews 1982; Berman-Frank et al. 1998). Furthermore, among macroalgae, reduced rates of C-fixation have been observed in dense stands, potentially due to severe DIC depletion, and/or increased stability of the boundary layer (due to reduced water velocity; Beer and Eschel 1983). *Cladophora* is successful only in alkaline environments (Whitton 1970) and *C. glomerata* has an estimated optimal pH for photosynthesis of 8.2 (Mantai and Haase 1977). At this pH, more than 95% of the dissolved carbon is carbonate. *Cladophora* appears to have a very high DIC utilization efficiency, and this may be an important adaptive advantage of this alga over its competitors. *Cladophora glomerata* from the Baltic sea was shown to utilize HCO_3^- by active uptake with a cell surface proton pump or by conversion to CO_2 by carbonic anhydrase (Choo et al. 2002). Active uptake and upregulation of cell surface or periplasmic enzymes impose greater energy demand than

CO_2 diffusion (Hawes 2002), but, the quantum efficiency of HCO_3^- utilization is not known for *Cladophora*.

Cladophora has high nutrient requirements for growth (e.g. Whitton 1970). Growth limitation by inorganic nitrogen has been documented for *C. glomerata* in freshwaters (Moore 1978, Millner et al. 1982, Dodds 1991a). However, much more often, phosphorus is charged with setting the seasonal limit to biomass accumulation of *Cladophora* (Whitton 1970) and to limit photosynthetic and growth rates in freshwater lakes (Gerloff and Fitzgerald 1976, Auer and Canale 1982, Neil and Jackson 1982, Freeman 1986, Jackson 1988, Planas et al. 1996; Parker and Maberly 2000).

Due to these high nutrient requirements, growth of *Cladophora* is typically considered a sentinel of excessive nutrient conditions. In both marine and freshwaters, blooms of filamentous green algae (FGA) are symptomatic of nutrient enrichment and typically indicative of degraded ecosystem health. In particular, high biomass of the globally distributed FGA, *Cladophora*, is regarded as a sentinel of eutrophication in alkaline systems (Dodds and Gudder 1992, Lembi 2003). *Cladophora* is often a public nuisance, causing shoreline fouling and clogging water intakes in temperate and tropical systems (Bäck et al. 1994, Joska and Bolton 1996, DeJong 2000). Around the lower Laurentian Great Lakes, in proximity to littoral zones underlain by hard substrate, detached *Cladophora glomerata* (L.) Kützing washes onshore as mats of decaying algae (Wilson et al. 2006, Higgins et al. 2005*a*; Fig. 1.1), which are associated with elevated bacterial loads (Ishii et al. 2006).

A.

B.

Figure 1.1 *Cladophora* growing in Lake Ontario (A) and fouling a shoreline (B). Photo credits to the author.

The autecology and community ecology of *Cladophora* has been reviewed in several publications. Whitton (1970) focused on *Cladophora* in freshwaters, which included much of the earliest knowledge of *Cladophora* in the Great Lakes. Dodds and Gudder (1992) focused on *Cladophora* in all environments, and provided particularly detailed ecology of the genus in lotic systems. Most recently, Higgins et al. (2008) reviewed the resurgence of *Cladophora glomerata* specifically in the Great Lakes, highlighting new research and approaches to studying the ecology of the macroalga.

Cladophora glomerata ecology in the Laurentian Great Lakes

Although taxonomic identification of *Cladophora* species can be obscured by its large morphological plasticity, recent phylogenetic work using rRNA markers has resolved that the species found throughout the Laurentian Great Lakes is *C. glomerata* (L.) Kützing (Ross 2006). In these lakes, two distinct clades exist; one cluster represented by samples from Lakes Ontario and Erie (the lower Great Lakes) and the other represented by samples from Lakes Michigan and Huron (the upper Great Lakes; Ross 2006). No phenotypic differences have yet been detected between these clades, and so are herein considered a single species.

Beginning in the 1950s and increasing through the 1960s, *Cladophora* biomass reached nuisance densities in the lower Great Lakes (Bellis and McLarty 1967; Herbst 1969) and in the vicinity of major nutrient point sources in the upper Great Lakes (Bellis and McLarty 1967; Canale and Auer 1982). High *Cladophora* biomass in these decades occurred concomitantly with high nutrient loading from the catchment, high nearshore and offshore P concentrations, and a high frequency of phytoplankton blooms in the lower Great Lakes. These algal blooms provoked the promulgation of the Great Lakes Water Quality Agreement (GLWQA), first signed in 1972, which sought to reduce the lakewide concentrations of P by reducing the catchment loading. Massive investments (i.e., on the order of billions of dollars) were made to reduce P loading from wastewater effluent and to reduce phosphates supplied in many detergents (reviewed in Nicholls et al. 2001). These mitigation strategies succeeded in reducing nearshore total phosphorus (TP) concentrations in the lower Great Lakes (Nicholls et al. 2001), the offshore spring TP concentration (Mills et al. 2003), and the "growth potential" of *Cladophora,* which, by deductive inference, led to a reduction in *Cladophora* biomass (Painter and Kamaitis 1987).

In the decades since the initiation of the GLWQA, the Great Lakes, especially the lower Great Lakes, have experienced additional stressors, some of which were unforeseeable during the construction of the original agreement. Many areas of the drainage basins surrounding the lower Great Lakes have become increasingly urbanized. In addition, a continuing parade of invasive species, frequently from the Ponto-Caspian region, have become established in the Great Lakes. Among the most prolific, and arguably the species with the greatest effect on the ecology and biogeochemistry of the Great Lakes, have been the dreissenid mussels (i.e., the zebra mussel, *D. polymorpha* and the quagga mussel, *D. bugensis*). The zebra and quagga mussels were first reported from the lower Great lakes in the late 1980s (Griffiths et al. 1991, Dermott and Munawar 1993), and first detectably influenced the chlorophyll to TP ratio of western Lake Ontario in 1991 (Nicholls 2001). The mussels grow prolifically throughout the lower Great Lakes, as well as to a lesser extent in the upper Great Lakes, achieving shell-free dry mass up to 150 g m^{-2} (Fleisher et al. 2001). As filter feeders, the mussels feed on plankton and suspended detritus and excrete fully and partially digested material as feces and pseudofeces, respectively. Due to the combination of their high biomass in the lower Great Lakes and their high specific filtering capacity, mussels have been credited with reengineering nearshore nutrient distribution in these lakes (Hecky et al. 2004). By removing suspended particulate matter through filter feeding then excreting dissolved regenerated nutrients and generating organic waste in the benthos (Hecky et al. 2004), the mussels have been implicated in shifting pools of matter with attendant nutrients from the pelagia to the benthos and increasing the solar radiant flux to the littoral zone. This process has recently been termed "benthification" (Zhu et al. 2006). In the Great Lakes, long-term pelagic total P concentrations declined between the 1970s and the 1990s, due to nutrient abatement strategies (e.g., Nicholls 2001; Millard et al. 2003). However, currently, dreissenid mussels may be increasing supply of P to benthic flora (Hecky et al. 2004). Previous studies have shown that mussels can be an important source of nutrients to overlying flora (Kahlert and Pettersson 2002).

Cladophora biomass in Lake Ontario appears to be experiencing a resurgence. Increasing complaints of shoreline fouling by macroalgae in Halton Region (L. Moore, Ontario Clean Water Agency) and increasing clogging of cooling water intakes of the nuclear generating stations at Pickering and Darlington by macroalgal material (C. Gregoris, Ontario Power Generation, pers. comm.), along the north shore of Lake Ontario, point to a need to

reevaluate the productivity and biomass of macroalgal growth. In previous decades, *Cladophora* was controlled by reducing P loading from the catchment (Painter and Kamaitis 1987). Yet, the effects of the potentially major changes to nutrient cycling, foodweb dynamics and optical properties on benthic algal ecology in Lake Ontario have not been investigated since dreissenid mussels became established in Lake Ontario. The constraints of *Cladophora* productivity and biomass may be undergoing changes, and consequently, the means by which it can be controlled needs to reevaluated.

Thesis Objectives and Chapter Organization

This thesis is a study on the ecology of the dominant macroalga on hard substrata in Lake Ontario. It was undertaken to evaluate the productivity, biomass and constraints of *Cladophora glomerata* growth and how its growth may have been affected by decadal-scale changes to Lake Ontario. This thesis is comprised of four data chapters, each written as a discrete study. Following a preliminary investigation of appropriate sites for study along the Canadian shoreline from Niagara to Toronto, a single site dominated by hard substrata and maximally exposed to the open lake was chosen as the primary study location. All chapters are based on field work that was completed in this area near Oakville, Ontario, in the Halton Region on Lake Ontario in 2004 and 2005. A sampling frequency of 1 to 2 weeks was targeted for the period commencing prior to the growing season to the end of the growing season for 2 years. This was designed to complement a spatially broader, but less frequent, sampling program in the nearshore of Lakes Ontario, Erie and Huron (e.g. Houben 2007).

The first objective of Chapter 2, the first data chapter, was to quantify the density of *Cladophora* through the course of the growing season. This was primarily accomplished using quadrats and destructive sampling by snorkelers on multiple dates through the growing season. The next objective was to evaluate if the attached biomass of *Cladophora* in Lake Ontario could be predicted using a numeric growth model developed for *Cladophora glomerata* growing in other Laurentian Great Lakes. The constraints of *Cladophora* gross primary production and biomass accumulation were parameterized following empirical relationships developed in a laboratory setting. The final objective of this chapter was to quantify how anthropogenic changes in Lake Ontario over the past four decades have affected the biomass accumulation of *Cladophora glomerata*. Because directly measured biomass data since

dreissenid mussel invasion are not available, the effects of changes in phosphorus concentrations and water clarity were quantified by hindcasting the prediction of attached and sloughed *Cladophora* biomass through the growing season using historical forcing parameters. Additionally, decadal scale changes in summer water temperatures were estimated. The potential effects of increasing water temperatures were also assessed by increasing step-wise the temperature forcing data of the numeric growth model. The contents of this chapter have been accepted for publication (Malkin et al. 2008).

The main objective of Chapter 3 was to quantify the benthic primary production on hard substrata. Benthic primary production is often responsible for the majority of littoral zone carbon-fixation, and can be a major contributor to whole lake or marine coastal primary production (Wetzel 1964; Davis 1970; Charpy-Roubaud and Sournia 1990; Vadeboncoeur et al. 2002). While the littoral zone is a small proportion of Lake Ontario, this zone encompasses the space in which we interact with the lake most intensely. For example, the nearshore areas are used for water extraction for municipal and industrial users, for recreation, and provide critical fisheries habitat. *Cladophora* may play an important role in nutrient distribution and nutrient cycling processes in the nearshore area, but its role has rarely been examined quantitatively or seasonally. Measurements of benthic primary production in the Laurentian Great Lakes furthermore remain scarce or absent. To begin an assessment of the role of *Cladophora* in the nearshore, primary production on *Cladophora*-dominated hard substrata was measured. To accomplish this, a new carbon-based *in situ* method was developed and implemented. This method employed benthic chambers sealed over attached *Cladophora* filaments at 1 m depth from which water was recirculated continuously. The change in dissolved inorganic C in this recirculated water was calculated from continuous measurements of pCO_2 (using an infrared gas analyzer) and temperature and a point measurement of alkalinity. Incubations were restricted to 15-20 minutes each, to minimize calcite precipitation and to maximize the number of measurements that could be made through a diurnal period. In this way, daily and seasonal net primary production was measured. A secondary objective of this chapter was assess the magnitude of epilithic primary production relative to planktonic primary production in the nearshore. To calculate the depth-integrated epilithic primary production, the numeric growth model, from Chapter 1, was used. The direct measurements of benthic primary production at 1 m were compared with the model-predicted rates of

Cladophora primary production at this same depth. The growth model was then used to estimate the depth-integrated benthic primary production, which was compared with published rates of depth-integrated planktonic primary production from Lake Ontario.

The objective of Chapter 4 was to explore the *in situ* constraints of *Cladophora* primary productivity. Nutrient limitation was assessed by quantifying the stoichiometric ratios of macronutrients through the growing season, and comparing these values with literature values for other nutrient-constrained benthic algae. High light stress was assessed with measurements of quantum efficiency, using pulse amplitude modulated (PAM) fluorometry. Light attenuation through the *Cladophora* canopy was quantified from solar irradiance measurements made for 3-5 days at 5 minute intervals above and below a *Cladophora* bed. Along with measurements of chlorophyll concentration through the depth of the *Cladophora* canopy, these measurements were used to assess the role of light limitation and light acclimation of *Cladophora in situ*.

Based on a result of Chapter 3 that the C flux in the nearshore (down to 12 m contour) may be dominated by *Cladophora* metabolism, the objective of Chapter 5 was to assess the role of *Cladophora* in nearshore nutrient flux. The seasonal nutrient sequestration and release by *Cladophora* was examined with measurements of biweekly harvested *Cladophora* tissue nutrient concentrations and ambient water chemistry. To put the measurements of nearshore nutrient concentrations at this one site in a spatial and historical context, lake-wide nearshore and offshore Lake Ontario nitrogen and phosphorus data collected from lake-wide cruises from the 1970s to present years was graphed. Because catchment supply of phosphorus to the nearshore was the dominant source to *Cladophora* historically, a secondary objective of this chapter was to examine if catchment supply has increased in the past 15 years, coincident with the perceived *Cladophora* resurgence. An additional objective was to assess if catchment supply during the *Cladophora* growing season is sufficient to support the seasonal growth of the macroalga. Towards this objective, the supply of total phosphorus from a Halton region tributary was compared with the phosphorus demand by locally growing *Cladophora*. While phosphorus in the nearshore is ultimately supplied via the catchment, this chapter explored whether the amount supplied from the catchment during the growing season is sufficient to meet the seasonal growth of the macroalga. If the rate of supply is insufficient, then the results in this chapter would indicate that in-lake processes that regulate nutrient supply, including

dreissenid mussel metabolism, need to be further explored to understand the regulation of the *Cladophora* resurgence in Lake Ontario.

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Chapter 2 Modeling the growth response of *Cladophora* in a Laurentian Great Lake to the exotic invader *Dreissena* and to lake warming

Introduction

Eutrophication continues to be among the most critical and pervasive issues facing coastal waters – marine and freshwater alike (Rabalais and Nixon 2002; Smith et al. 2006). Blooms of annual or ephemeral attached macroalgae are symptomatic of nutrient enrichment in littoral zones underlain by hard substrata and are typically indicative of degraded ecosystem health (Raven and Taylor 2003; Smith et al. 2005; Worm and Lotze 2006). High biomass of the globally distributed filamentous green alga, *Cladophora*, in particular, is regarded as a sentinel of eutrophication in alkaline systems (Dodds and Gudder 1992; Lembi 2003). Nuisance densities of *Cladophora* have been reported from estuaries (Gordon and McComb 1989; Valiela et al. 1997), inland seas (Kiirikki 1996; Curiel et al. 2004), and hardwater rivers and lakes with moderate energy (Power 1992; Parker and Maberly 2000). In the Laurentian Great Lakes, *Cladophora* is a nuisance throughout Lakes Ontario, Erie, and Michigan, where hard substrate is available, and in isolated areas near nutrient point sources in Lake Huron (Herbst 1969).

There is a widespread perception that since the establishment of invasive dreissenid mussels (i.e., the zebra mussel *Dreissena polymorpha* and the quagga mussel *D. bugensis*) over the past 15 years, *Cladophora* biomass has resurged in the lower Great Lakes (Mills et al. 2003; Bootsma et al. 2004; Hecky et al. 2004). The degree to which this perception is accurate, however, is unclear. Due to their high biomass in these lakes (e.g., up to 150 g shell-free DM m^{-2}; Fleisher et al. 2001), dreissenid mussels have been credited with reengineering nutrient distributions by removing suspended particulate matter through filter feeding then excreting dissolved regenerated nutrients as well as generating organic waste as feces and pseudofeces in the benthos (Hecky et al. 2004). This process, recently termed "benthification" (Zhu et al. 2006), has been implicated in shifting pools of matter with attendant nutrients from the pelagia to the benthos and increasing the solar radiant flux to the littoral benthos. Mussels can be an important source of nutrients to overlying flora (Kahlert and Pettersson 2002) and furthermore may remove competitive demands for nutrients via filtration of plankton (Holland

et al. 1995). In the Great Lakes, long-term pelagic total P concentrations have been declining since the 1970s due in part to nutrient abatement strategies (e.g., Nicholls 2001; Millard et al. 2003) while currently, dreissenid mussels may be increasing supply of P to benthic flora (Hecky et al. 2004). The decadal-scale response of *Cladophora* tissue P quota (Q_P) to these counteracting pressures in the Great Lakes is not known. The lower Great Lakes have additionally experienced a significant increase in light penetration since dreissenid mussel establishment (Howell et al. 1996) in response to decreased seston concentration (Millard et al. 2003) and, in Lake Ontario, possibly a reduced frequency of whiting events (Barbiero et al. 2006). The resulting increase in euphotic depth may have increased the biomass of *Cladophora* occurring at previously light-limited depths and increased its areal extent of coverage (Zhu et al. 2006).

The effects of climate change on the limnology of the Great Lakes may also have important consequences for macroalgal blooms. Direct effects of epilimnetic temperature increases on macroalgae include increases in photosynthetic and respiration rates. An early hypothesis proposed that supraoptimal temperatures cause a metabolic imbalance in *Cladophora* beds giving rise to a commonly observed mid-summer senescence and detachment (Graham et al. 1982). However, evidence has supported the converse; *Cladophora* effectively acclimates to high temperatures (Mantai 1987), such that temperature optima for net primary production can be as high as 28-31^{o}C (Lester et al. 1988). Whether an increase in surface water temperature due to climate change may exacerbate the magnitude of *Cladophora* blooms has not yet been investigated.

Testing hypotheses about the relative effects of historical changes to nutrient supply and light penetration on benthic algal biomass accrual is limited by scant direct measures of *Cladophora* biomass over decadal scales. In the absence of direct measures, a numeric model that predicts *Cladophora* growth rate and biomass accumulation seasonally, in response to known environmental drivers including light, temperature, and nutrient concentrations, provides a framework for assessing the seasonally- and depth-resolved accumulation of *Cladophora* biomass. Through sensitivity analyses of the model, the effects of changes in water transparency, nutrient concentration, and surface water temperature can be assessed.

The goal of this study is to quantitatively assess the effects of decadal-scale changes in Lake Ontario on nuisance macroalgal biomass. Our first objective is to calibrate and validate a

growth model for an area on the north shore of western Lake Ontario, an area that has received an increasing number of public complaints of macroalgal shoreline fouling over the past decade (The Regional Municipality of Halton, pers. comm.), using direct measurements of *Cladophora* biomass collected over two growing seasons that experienced very different meteorology. The second objective is to apply the model to predict the independent and combined effects of decadal-scale changes in Kd_{PAR} and *Cladophora* Q_P on *Cladophora* biomass accumulation. Two decades are chosen for hindcasting: the early 1970s, which preceded P-abatement strategies, and the early 1980s, which followed P-abatement but preceded dreissenid mussel establishment, which was first ecologically detectable in western Lake Ontario in 1991 (Nicholls 2001). The third objective is to use the model to forecast the direct effects of climatic warming on the seasonality and magnitude of *Cladophora* growth, based on a near linear trajectory of increasing surface water temperature in Lake Ontario. In these sensitivity analyses, I employ the two modern years (2004 and 2005) of meteorology in order to predict a range of outcome scenarios. The limnological and meteorological data from the two modern years are referred to as the baseline data.

Methods

Site description

Field sampling for this study was conducted on Lake Ontario in Oakville, Ontario, Canada (43.44°N, 79.66°W). The site is underlain by boulders and cobble and supports up to 100% *Cladophora* coverage at 2.0 m depth during midsummer. The species identified throughout the Laurentian Great Lakes, using molecular markers, is *C. glomerata* (L.) Kützing (K. Muller, U. Waterloo pers. comm.).

Measurements of *Cladophora* biomass

Cladophora biomass estimates were made by snorkellers deploying 0.25 m^2 quadrats at 2.0 m depth. Five replicate quadrats were harvested. The percentage of attached algal coverage, bed height, and filament length were measured in each quadrat. All *Cladophora* biomass was harvested with scraping tools from the quadrats and collected in mesh bags. The product of bed height and algal coverage, the "stand volume", was correlated, independently

on each day, with algal biomass (mean ± SD of all days: 7.5 ± 2.6 g DM L^{-1} stand volume). In 2004, biomass was estimated either by direct harvests or using the stand volume correlation. In 2005, all biomass was estimated from direct harvests. In the lab, harvested material was rinsed in mesh sieves (pore size approximately 1 mm) and picked clean with forceps to remove larger debris and macrofauna. The rinsing process also had the effect of removing the majority of the finer filamentous alga, *Ulothrix zonata*, which was abundant early in the spring prior to *Cladophora* dominance and in late-summer between *Cladophora* seasonal cohorts, but was otherwise a marginal fraction of the macroalgal biomass. The cleaned *Cladophora* material was examined under dissecting microscope to confirm that the filamentous algae were almost exclusively *Cladophora*.

Measurements of tissue phosphorus concentrations

Tissue P concentration (Q_P) was measured of randomly sampled *Cladophora* harvested in triplicate weekly to biweekly during the growing season at 2.0 m depth. Samples were collected on 13 dates in 2004 and on 15 dates in 2005. Dried *Cladophora* tissue was combusted at 450^{o}C for 1 hour and then autoclaved for 30 minutes in distilled water with 4% potassium persulphate solution added to a final concentration of 0.16%. Following digestion, solubolized P was measured spectrophotometrically using the molybdate blue method (American Public Health Association 1998).

Cladophora growth model

To construct seasonally-resolved estimates of *Cladophora* biomass, the *Cladophora* growth model (CGM) was used. Previous versions of the model described here have successfully been validated to predict *Cladophora* biomass at discrete distances from a sewage treatment plant in Lake Huron (Canale and Auer 1982), and to predict *Cladophora* seasonal growth from 2-10 m depth in the agriculturally-dominated, oligotrophic eastern basin of Lake Erie (Higgins et al. 2005*b*).

Daily time steps of *Cladophora* were predicted as attached or as sloughed (i.e., detached) biomass. The sum of these components, the "cumulative" biomass, was also

quantified. The CGM was run using the visual interactive modeling package Stella, version 7.0.2 (2001; High Performance Systems Inc., Hanover, NH, USA).

The CGM predicts daily specific growth rate (μ; d^{-1}) as:

$$\mu = (GPP\text{-}R\text{-}S)\cdot X \qquad (1)$$

where GPP is the daily specific gross primary production (d^{-1}), R is the daily specific respiration rate (d^{-1}), S is the daily specific sloughing rate (d^{-1}), and X is the attached *Cladophora* biomass (g DM m^{-2}). GPP is the product of the gross primary production during the day (GPP_{day}) and the photoperiod. GPP_{day}, in turn, is the sum of the net primary production during the day (NPP_{day}) and the respiration during the day (R_{day}). R is the sum of R_{day} multiplied by the photoperiod, and the respiration during the night (R_{night}) multiplied by the dark period of they day (1-photoperiod). NPP_{day} and R_{day} are calculated as:

$$NPP_{day} = NPP_{max}\cdot M_{LT\text{-}NPP}\cdot M_P\cdot M_X \qquad (2)$$

$$R_{day} = R_{max}\cdot M_{LT\text{-}R}\cdot M_P\cdot M_X \qquad (3)$$

where NPP_{max} and R_{max} are the maximum NPP_{day} and R_{day} under ideal conditions; $M_{LT\text{-}NPP}$ and $M_{LT\text{-}R}$ are multipliers representing the effects of irradiance and temperature on NPP and R, respectively; M_P is the multiplier representing the effects of *Cladophora* Q_P; and M_X is the multiplier representing the effects of self-shading. All multipliers are dimensionless and expressed as proportions from zero to unity. NPP_{max} was set to 0.60 (d^{-1}; Higgins et al. 2005*b*) and R_{max} was set to 0.44 (d^{-1}; Canale and Auer 1982).

The M_{LT} multipliers for NPP_{day} and R_{day} were determined in steady-state conditions as the interactive effects of irradiance and temperature on the respective metabolic rates and expressed as polynomial functions of irradiance and temperature (Graham et al. 1982). R_{day} was measured as the dark respiration rate of *Cladophora* that had been immediately previously acclimated to a given light level. R_{night} was calculated as the dark respiration rate measured in dark acclimated *Cladophora* and was a function of temperature alone (Graham et al. 1982). The M_P multiplier was based on the Droop growth model in which P is the limiting resource:

$$M_P = 1 - Q_o\cdot Q_P^{-1} \qquad (4)$$

where Q_o is the minimal cell quota (%P, as a fraction of dry mass). Q_o was set to 0.05% (Auer and Canale 1982). The M_X multiplier is a function of the attached crop of *Cladophora* which acts as a surrogate for light attenuation through the *Cladophora* canopy. M_X was calculated as:

$$M_X = 1 - X\cdot X_{max}^{-1} \qquad (5)$$

where X_{max} is the maximum attached biomass attainable and took the constant value of 500 g DM m^{-2}, based on the highest biomass recorded in the Oakville region (Kamaitis 1984). Setting the maximum biomass to a constant value was an update to earlier CGM configurations, which previously allowed it to vary with depth (Higgins et al. 2005*b*). This amendment was made because X_{max} is a function of substrate roughness which was not observed to vary with depth.

The continuous sloughing function was computed as:

$$S = S_{max} \cdot M_{shear} \cdot V_{east} \cdot V_{max}^{-1} \cdot X \cdot X_{max}^{-1} \quad (6)$$

where S_{max} is the maximum sloughing rate (d^{-1}); M_{shear} is an empirically derived dimensionless multiplier related to wave-induced shear stress as a function of depth (Higgins et al. 2005*b*); V_{east} is the wind speed weighted to the east, the direction of greatest fetch; and V_{max} is the maximum easterly-weighted wind speed, which was set to 14 m s^{-1}. Because of the depth-dependence of wave induced shear stress, sloughing estimates were greatest at the shoreline and decayed with depth.

An additional catastrophic detachment function was also added to the model. This function removed 75% of the attached biomass in one time step, triggered when the product of M_{shear}, V_{east}, V_{max}^{-1}, and X exceeded a threshold ranging from 150-200 (g DM). This threshold range was based on a catastrophic detachment that occurred in 2004 during a storm. Because only a single event was available to set the threshold, I suggest that the absolute values be employed with due caution when applied to a new situation.

Input data parameterization, calibration, and validation of the *Cladophora* growth model

All data were input as diel averages or interpolations, or in the case of surface irradiance, as diurnal averages. Input requirements to the CGM are summarized in Table 2.1. Daily Q_P values were interpolated from weekly to biweekly samples by fitting a cubic function, the curve of best fit, to the measured Q_P values.

To calculate photosynthetically active radiation (PAR) at the depth of growth (E_z; µmol m^{-2} s^{-1}), surface irradiance (E_o; µmol m^{-2} s^{-1}) and daily estimates of the PAR extinction coefficient (Kd_{PAR}; m^{-1}) were required. E_o was calculated from measurements of shortwave radiation dose (W m^{-2} 10^{-1} min) collected with a pyroheliometer on the roof of Canada Centre

for Inland Waters (Burlington, ON). The calculation of PAR fluence rate (μmol m^{-2} s^{-1}) was accomplished by multiplying the radiation dose by three constants: (1) 1.667 to account for the conversion of the integration time; (2) 5.03, the estimated conversion from shortwave radiative energy to quanta (Appendix 2; Wetzel 2001); and (3) 0.46, the estimated proportion of PAR in shortwave radiation (Kirk 1994). Each measure of E_o was corrected for surface solar reflectance as a function of solar azimuth (Walsby 1997), then averaged to compute the diurnal mean.

On 24 occasions, PAR profiles were taken at 2 m depth using a spherical quantum sensor (Li-Cor, Lincoln, Nebraska), from which Kd_{PAR} values were calculated. To estimate daily Kd_{PAR}, an empirical relationship was derived as a function of wind speed and direction. Light attenuation in the shallowest depths is attributable to wind-induced resuspension, so wind speed weighted from the direction of greatest fetch, east, was correlated with Kd_{PAR}. Weighting was accomplished as follows:

$$V_{east} = V_{absolute} \cdot (0.5 \cdot \sin(\text{wind direction}) + 1) \quad (7)$$

where north is 0^o. Winds blowing for extended periods of time were assumed to drive greater resuspension than shorter gusts, so weighted wind speeds were averaged over three-hour intervals and the maximum value was correlated with measured Kd_{PAR}. This correlation (r = 0.747) was higher than all others calculated with weighting from all other cardinal directions and unaveraged wind speeds. Wind speed and direction were recorded hourly from a surface buoy on western Lake Ontario (C45139; Environment Canada).

Temperature was measured hourly from the same surface buoy as was used for wind speed and direction (C45139; Environment Canada), from which daily mean averages were computed. For input to the sloughing algorithm, hourly windspeed weighted from the east (V_{east}) was averaged over each day.

The initial starting date of the model run was set to Julian day 125 (04 May 2004 or 05 May 2005); a day when *Cladophora* biomass was evident but had not yet come to dominate the periphyton community at 2.0 m depth. An initial date based on a threshold temperature did not result in a better model fit, measured as sum of squared errors between predicted and measured biomass during exponential phase growth. An initial seed biomass of 2.0 g DM m^{-2} yielded the best model fit.

The CGM was first calibrated at 2.0 m depth using input data from 2004 and then validated with data from 2005. The daily attached biomass output was compared with seasonal biomass collected at 2.0 m. To extend the biomass predictions of the CGM to other depths, I constructed a relationship between Q_P and depth. An exponential increase in Q_P with depth was determined, following the form:

$$Q_{P(Z)} = (Q_{P(2)} - a) + ae^{b(Z-2)} \qquad (8)$$

where $Q_{P(Z)}$ and $Q_{P(2)}$ are the Q_P values at a given depth (Z) and at 2.0 m, respectively. Using samples collected from the same study site during midsummer 2006 (A. Houben, U. Waterloo, unpubl.) a and b were calculated to be 0.00366 and 0.3388 respectively (Fig. 2.1). Additionally, Kd_{PAR} was assumed to decrease with depth (Higgins et al. 2005*b*). In order to report a depth-integrated biomass (kg DM m^{-1} shoreline), the areal biomass predicted by the CGM at 0.5 m depth increments was multiplied by the mean area of each depth contour in the Oakville region (Virden et al. 2000).

The maximum potential error in predicted peak biomass at the calibration depth, 2 m, was assessed by sensitivity gradient analysis. Four major measured input parameters (surface temperature, Kd_{PAR}, wind speed, and Q_P) were varied independently from –20% to +20% and plotted against the percentage change in peak attached biomass. Using these sensitivity gradients, and estimated coefficients of variation for each input parameter, the magnitude of the sources of variability in estimates of peak attached biomass were quantified.

Table 2.1 Input parameters to the *Cladophora* growth model.

Input parameter	Method of measurement	Source
Temperature (^{o}C)	Surface measurements reported hourly, from which daily means were calculated.	Environment Canada buoy C45139
Surface irradiance ($\mu mol\ m^{-2}\ s^{-1}$)	Diurnal mean PAR was calculated from the mean of 10 minute shortwave radiation doses measured with a rooftop pyroheliometer.	Canada Centre for Inland Water
Wind speed and direction ($m\ s^{-1}$)	Reported hourly from station buoy, from which daily means weighted from the east, were calculated.	Environment Canada buoy C45139
Kd_{PAR}, modern (m^{-1})	Daily mean values were calculated from wind velocity weighted from the east. Wind data were reported hourly.	Environment Canada buoy C45139
Kd_{PAR}, historical (m^{-1})	Seasonal mean values were calculated from historical nearshore secchi depths using a correlation between modern seasonal mean nearshore secchi depth and Kd_{PAR} at 2m.	Environment Canada nearshore Station 5, and this study
Q_P, modern (% DM)	Directly measured from triplicate samples collected weekly to biweekly. Daily values were interpolated from a curve fit to the seasonal data.	this study
Q_P, historical (% DM)	Seasonal minimum Q_P was measured directly. Daily values were calculated as a proportional fraction to modern Q_P.	Owens 1972 and Kamaitis 1984

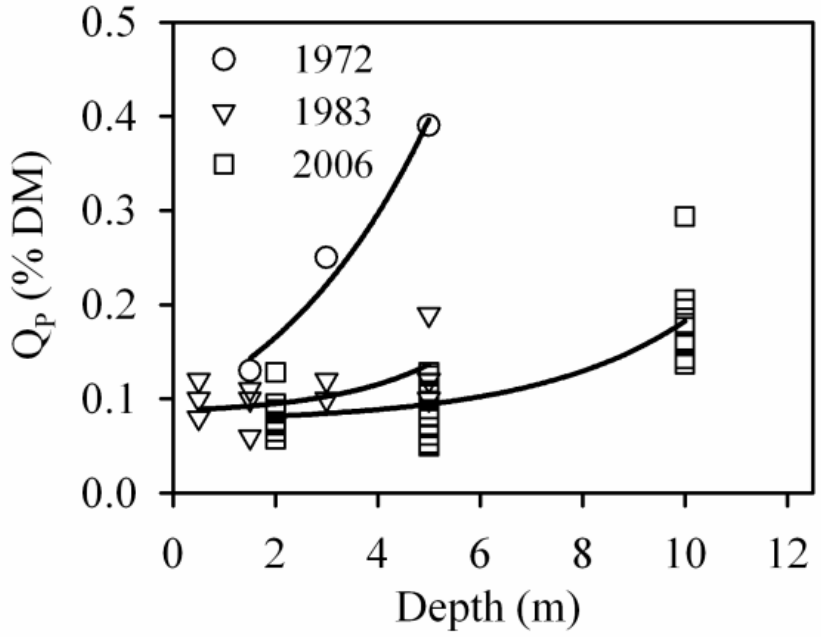

Figure 2.1 The relationship between *Cladophora* tissue phosphorus (Q_P) and depth between decades. The exponential rise in Q_P with depth decreases in each progressive decade, indicating that the switch from P-limitation to light-limitation is occurring at deeper depths than historically. Directly measured data (symbols) with exponential curves of best fit.

Hindcasting: Changes in Kd_{PAR}, Q_P, and temperature

The sensitivity of the CGM to historical Kd_{PAR} and Q_P was assessed for each factor independently and interactively. Because historical daily estimates of these variables were not available, two different approaches were taken to employ historical Kd_{PAR} values and historic Q_P in the CGM. For Kd_{PAR}, a seasonal mean historic value was used as a constant input into the model. Using a seasonal mean Kd_{PAR}, instead of a daily variable, yielded differences in peak biomass of less than 10% compared with the calibration dataset output. Historic Kd_{PAR} was estimated based on secchi depths collected from 1966-2005 from a nearshore north western buoy (Environment Canada Station 5: 43.42°N, -79.66°E). These secchi depth data were used because they comprised the largest proximal dataset available (STAR database; Environment Canada). Data collected between June and August, the main *Cladophora* growing season, were used. These data were collected from a median depth of 24 m. Data were subdivided into 3 time periods: a period prior to dreissenid mussel invasion and substantive reductions in pelagic P concentrations, 1966-1980 (n=24); a period prior to dreissenid establishment and following large pelagic P reductions, 1981-1990 (n=10); and the modern period which is characterized by dreissenid mussel establishment and low pelagic P concentrations, 1991-2001 (n=4). Secchi depth (m) was converted to Kd_{PAR} (m^{-1}) as:

$$Kd_{PAR} = c \cdot (\text{secchi depth})^{-1} \qquad (9)$$

where c is a constant. Using the relationship between mean Kd_{PAR} for 2004 and 2005 (0.454 m^{-1}) and the most recent secchi depths, c was calculated to be 2.37.

For historical Q_P, a seasonal constant was inappropriate. Instead, a ratio between the historical seasonal minimum value and the modern seasonal minimum, termed the Q_P-factor, was calculated. Historical daily Q_P values were calculated as the product of the daily baseline Q_P values and the decade-specific Q_P-factor . Historical minimum *Cladophora* Q_P from Lake Ontario were obtained from previous *Cladophora* surveys conducted in 1972 and again in 1982 and 1983 (Owens 1972 (reported in Kamaitis 1984); Kamaitis 1984; Painter and Kamaitis 1987). These surveys targeted the dates of peak *Cladophora* biomass and correspondingly to seasonal minimum Q_P. No comparable datasets were available from this study area from the 1990s.

The combined effects of historical Kd_{PAR} and Q_P were assessed using seasonal mean Kd_{PAR} and Q_P-factors for each of the two time periods (1972 and 1982-83). The relationship

between Q_P and depth was different between time periods (Fig. 2.1). Consequently, for the sensitivity analyses of the combined effects of Kd_{PAR} and Q_P, exponential relationships between Q_P and depth (equation 8), specific to each time period, were used. The relationships were calculated based on Q_P values reported in Kamaitis (1984). For 1972, a and b were 0.09209 and 0.2992 and for 1982-83 a and b were 0.00406 and 0.5146, respectively.

To assess the potential effect of changes in surface water temperature, an ecologically relevant scale of change was first defined. Climate change models, based on global circulation models, so far, have not been constructed with the spatial resolution necessary to directly predict the water temperature in the Great Lakes expected in the coming decades (Schertzer and Crowley 1999). Instead, I plotted the surface water temperature in Lake Ontario from the earliest available records to construct the surface water trend from 1966 – 2006, using data from July – September, inclusive. Data from 1966 to 1990 were taken from lake-wide surface water temperature data collected during surveillance cruises, from an airborne radiometer, or satellite data (Schertzer 2003). Years for which there were less than four data points available during the season of interest were excluded. Data from 1991 to 2006 were collected hourly from a western Lake Ontario buoy (C45139; Environment Canada). Trends in temperature over time were made by linear regression analysis in SPSS version 14.0.

The direct effects of rising temperatures on the peak biomass of attached *Cladophora* were assessed with the CGM using additive increases to baseline temperature. Seasonal surface temperature was increased by increments of 0.5^{o}C for successive model runs.

For all sensitivity analyses, the CGM was run from Julian day 125 to 208, the day of catastrophic detachment in 2004. No catastrophic detachment events were allowed in these model predictions because of the unpredictable episodic trigger for these events.

Results

Contemporary *Cladophora* growth and seasonal biomass accumulation

The two years used to calibrate and validate the *Cladophora* growth model (CGM) were very different meteorologically, as evident from the differences in the spring warming and in mean surface irradiance between the two years (Fig. 2.2). The contrast in seasonal growth patterns of *Cladophora* at 2.0 m depth is made obvious by comparing the timing of peak measured biomass, which was a full month earlier in 2005 relative to 2004 (Fig. 2.2A).

The CGM adequately predicted the timing and magnitude of attached *Cladophora* biomass at 2.0 m depth (Fig. 2.3A, B). Peak predicted biomass as a percentage of peak measured biomass was 90% in 2004 and 113% in 2005. Due to heterogeneity in physical structure at the 1 m^2 scale, there was inherently high variability in attached *Cladophora* biomass measured with quadrats. The mean coefficient of variation in measured biomass averaged across the season was 52% (n=5) and standard error for each date ranged from 1.3 – 23.4 g m^{-2} (mean standard error or all dates = 9.3 g m^{-2}).

Error around the model-predicted peak biomass using the calibration dataset was dominated by the model's sensitivity to Q_P input (Fig. 2.4). Q_P had a coefficient of variation of 20.0% (between replicates, within sampling days). Variation in Q_P of +20% and –20% resulted in an average of +75% and –79% variation in peak biomass predictions, respectively (Fig. 2.4). Changes in all other input parameters by ± 20% resulted in peak biomass variation of less than ± 20%. The large error created by variation in Q_P is due to the hyperbolic relationship between Q_P and growth rate, and the very low concentrations of Q_P currently measured in Lake Ontario. The difference in Q_P between decades, however, is approximately an order of magnitude greater than the CV between modern replicates.

A major storm event on Julian day 208 (24 July 2004) caused a synchronous detachment of *Cladophora* that resulted in extensive shoreline fouling in the region of study and caused blockage of the cooling water intake at Pickering Nuclear Generating Stations, approximately 80km to the east of the study site. By contrast, loss of attached biomass in 2005 was always gradual, and no single storm event caused massive detachment (Fig. 2.3A, B).

The seasonal peak in total attached *Cladophora* biomass integrated through 12.0 m depth were similar in 2004 and 2005 (46.0 kg DM m^{-1} shoreline in 2004 vs. 55.5 kg DM m^{-1} shoreline in 2005; Fig. 2.3C, D). The cumulative depth-integrated biomass, the sum of sloughed and attached material, on the day when attached biomass was at its peak, was also similar between years (54.3 kg DM m^{-1} shoreline in 2004 vs. 65.1 kg DM m^{-1} shoreline in 2005).

The depths of peak attached biomass were the same or deeper than the depth of peak cumulative biomass (Table 2.2), due to the depth dependency of sloughing. Taking the mean from the two calibration years, peak attached biomass was estimated at 2.5 ± 0.3 (SD) m depth.

The depth beyond which no growth occurred during the season was estimated at 10.4 ± 0.4 m depth, which experienced mean irradiance of 2.9 ± 0.2 mol m^{-2} d^{-1}.

Table 2.2 Results of sensitivity analyses testing the independent and combined effects of historical light attenuation coefficients (Kd_{PAR}) and historical *Cladophora* tissue P concentrations (Q_P) relative to the mean of 2004 and 2005. Mean averages and standard deviations are computed from two model runs using each baseline year separately. Maximal attached and cumulative (attached plus sloughed) *Cladophora* biomass integrated from 0.5 – 12.0 m depth (kg DM m^{-1} shoreline) was predicted using the *Cladophora* growth model (CGM). Maximum depth (m) is defined as the depth at which no net positive growth was predicted for any day during the model run.

		Peak attached biomass	Peak cumulative biomass	Depth of peak attached biomass	Depth of peak cumulative biomass	Maximum depth
	2004, 2005 (baseline)	55 ± 3	66 ± 5	2.5 ± 0.3	2.5 ± 0.1	10.4 ± 0.4
Kd_{PAR} effects	1982-83	24 ± 3	32 ± 4	1.6 ± 0.0	1.6 ± 0.1	6.7 ± 1.1
	1972	20 ± 3	27 ± 3	1.5 ± 0.1	1.4 ± 0.1	5.2 ± 1.1
Q_P effects	1982-83	157 ± 4	274 ± 28	3.5 ± 0.4	2.0 ± 0.2	11.3 ± 0.3
	1972	189 ± 4	356 ± 33	3.5 ± 0.4	1.8 ± 0.3	11.6 ± 0.2
Kd_{PAR} and Q_P effects	1982-83	77 ± 4	173 ± 16	2.1 ± 0.4	1.4 ± 0.1	8.1 ± 1.4
	1972	88 ± 4	236 ± 12	2.1 ± 0.2	1.2 ± 0.1	6.5 ± 0.7

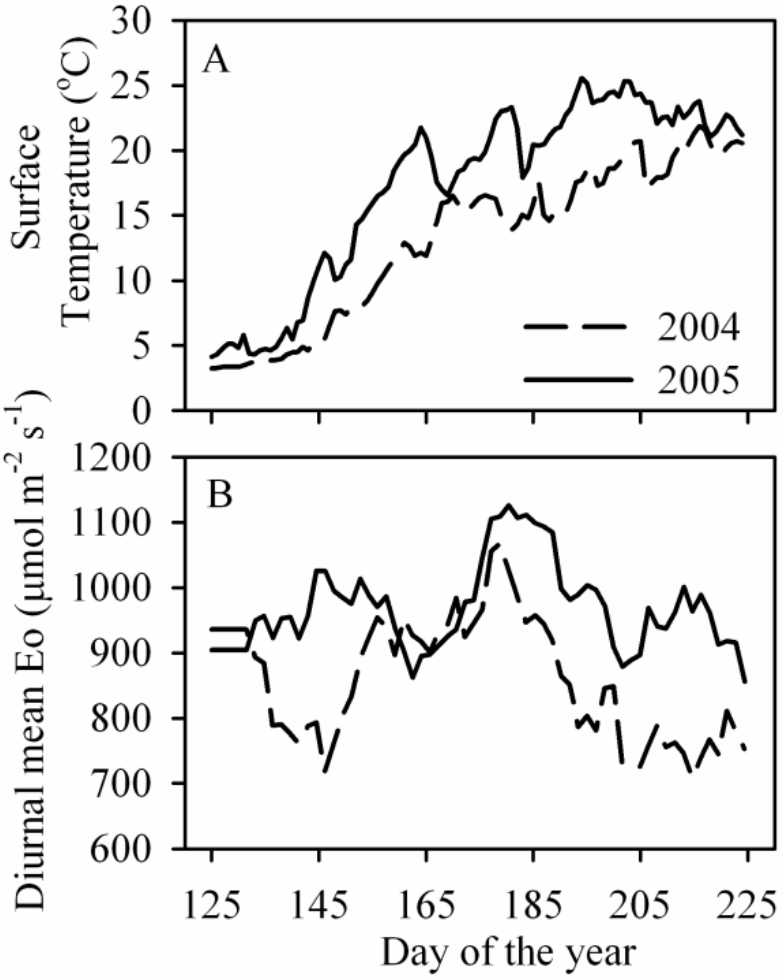

Figure 2.2 Baseline data input to *Cladophora* growth model (CGM). (A) illustrates the faster rate of spring warming and the higher peak temperature of 2005. (B) shows the higher mean surface irradiance (E_o) in 2005. For clarity, the daily mean E_o in this figure was smoothed with a nearest neighbour running average.

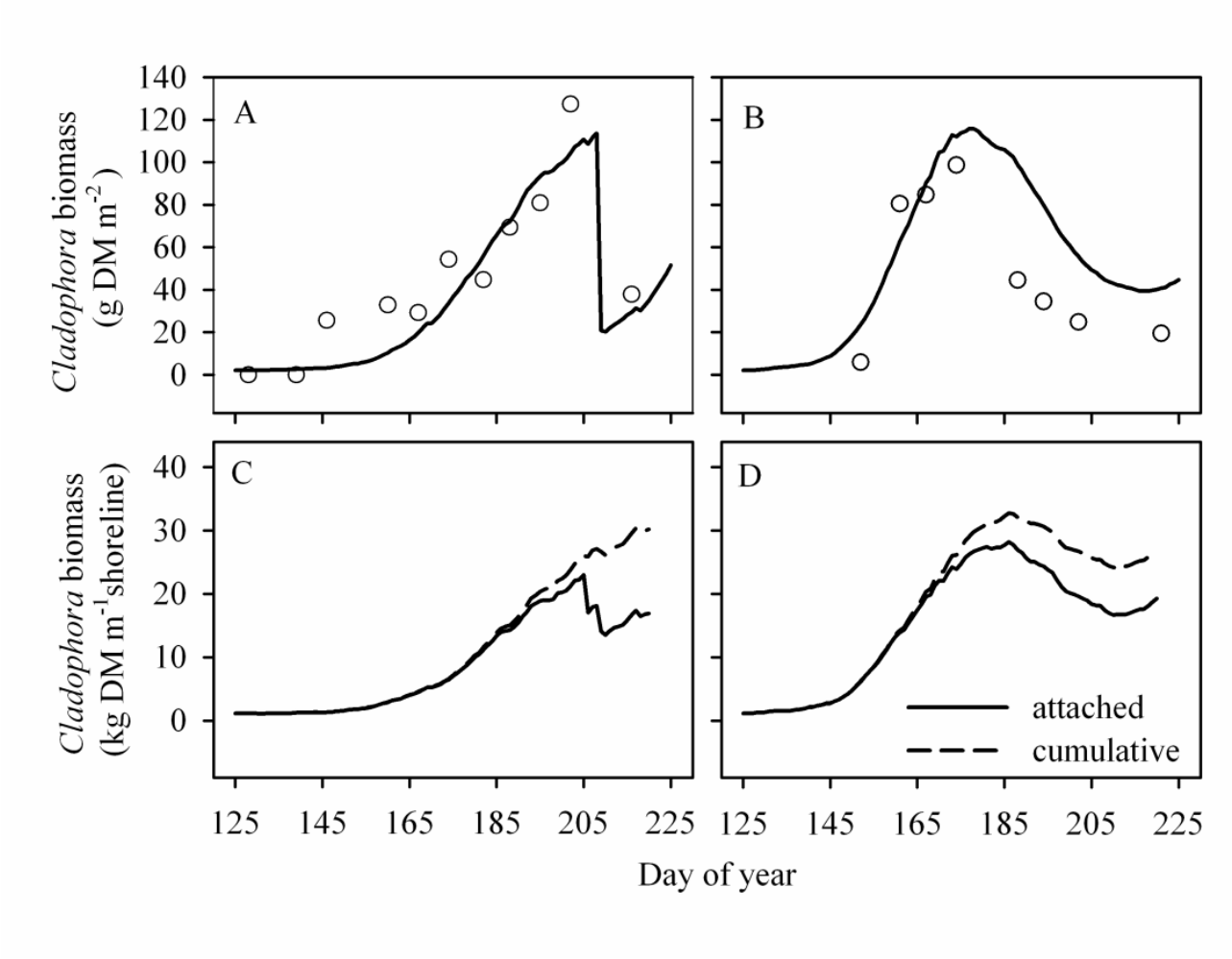

Figure 2.3 Measured (circles) and *Cladophora* growth model predicted (lines) biomass of *Cladophora glomerata* at 2.0 m depth at Oakville, Lake Ontario, in (A) 2004 and (B) 2005. Predicted depth-integrated *Cladophora* biomass in (C) 2004 and (D) 2005. Biomass was predicted at 0.5 m increments, between 0.5 m to 12.0 m depth. Cumulative biomass was calculated as the sum of attached and sloughed *Cladophora* biomass.

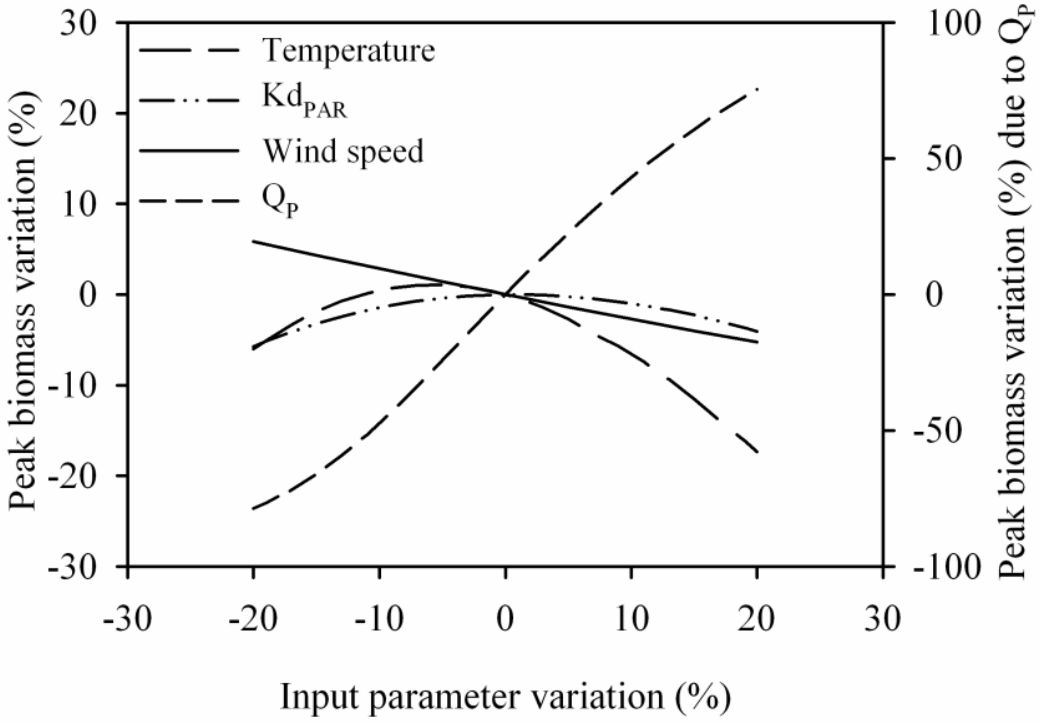

Figure 2.4 The variation in peak attached biomass in 2004 and 2005 at 2.0 m depth due to variation in the main measured input parameters. The left ordinate axis relates to all parameters except Q_P.

Hindcasting *Cladophora* biomass using historical light attenuation (Kd_{PAR}) and tissue P concentrations (Q_P)

The seasonal mean Kd_{PAR} was 0.43 m^{-1} in 2004 and 2005, 0.68 m^{-1} for the period that encompassed 1982-83, and 0.76 m^{-1} for the period that encompassed 1972. The mean seasonal minimal Q_P for the baseline years was 0.050% and the Q_P-factors (ratio of historical minimum seasonal Q_P to baseline seasonal minimum Q_P) were 1.78 for 1982-83 and 2.57 for 1972. The independent effects of changing Kd_{PAR} and Q_P led to opposing model outputs. Analyses using historical Kd_{PAR} alone yielded peak attached biomass predictions that were 42% ± 3% in 1982-83 and 36% ± 3% in 1972 of the 2004-05 peak attached biomass (Fig. 2.5A, B). Using historical Kd_{PAR}, the attached biomass at depths deeper than 2.0 m were predicted to be lower than in 2004-05 (Fig. 2.6A, B). The depths beyond which no seasonal growth was predicted were 10.4 ± 0.4 m in 2004-05, 6.7 ± 1.1 m during the 1982-83 period, and 5.2 ± 1.1 m during the 1972 period (Table 2.2). Peak attached biomass predictions using historical Q_P alone yielded 286% ± 26% in 1982-83 and 344% ± 29% in 1972, relative to 2004-05 peak attached biomass (Fig. 2.5C, D). Using historical Q_P, the depth of peak attached biomass was 1.0 m deeper than in 2004-05, due to greater sloughing at the shallowest depths (Fig. 2.6C, Table 2.2).

Using historical Kd_{PAR} and Q_P together, peak attached biomass was predicted to be higher and to occur earlier in the season relative to 2004-05 (Fig. 2.5E, F). At depths shallower than 3.5 m, peak attached and cumulative biomass was predicted to be higher in 1982-83 and 1972, relative to 2004-05 (Fig. 2.6E, F). Conversely, at depths greater than 3.5 m, peak attached and cumulative biomass was predicted to be lower in 1982-83 and 1972, relative to modern years (Fig. 2.6E, F). The overall result was that peak attached biomass for 1982-83 was 1.40 ± 0.16 times higher than 2004-05 and cumulative biomass was 2.04 ± 0.06 times higher (Table 2.2). For 1972, peak attached biomass was 1.6 ± 0.17 times higher than 2004-05 and cumulative biomass was 2.66 ± 0.20 times higher (Table 2.2).

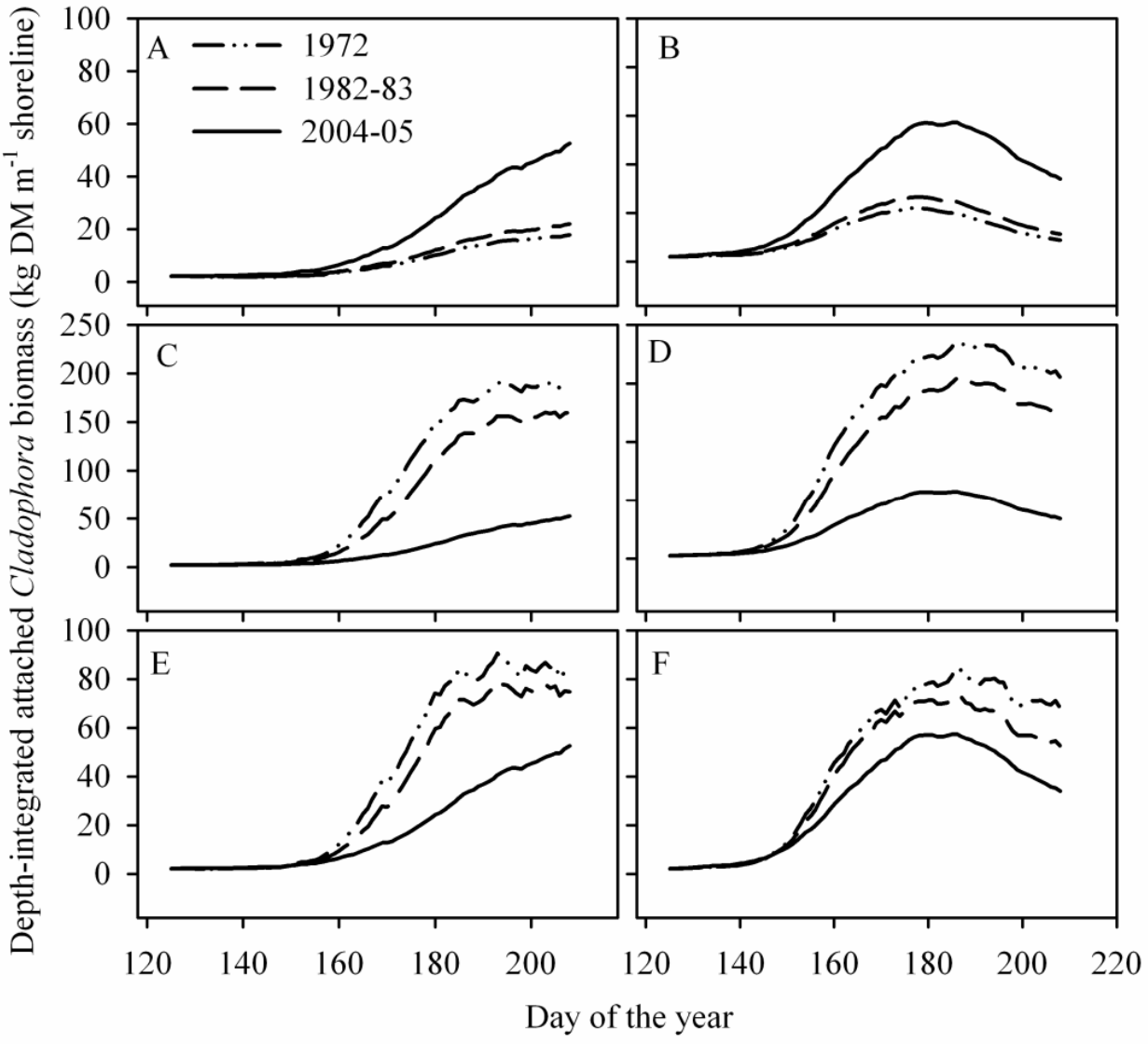

Figure 2.5 *Cladophora* growth model (CGM) predictions of depth-integrated attached *Cladophora* biomass and the independent effects of historical Kd_{PAR} using (A) 2004 and (B) 2005 baseline data; the independent effects of historical Q_P with (C) 2004 and (D) 2005 baseline data; the combined effects of historical Kd_{PAR} and Q_P using (E) 2004 and (F) 2005 baseline data. Note the differences in ordinate axis scales.

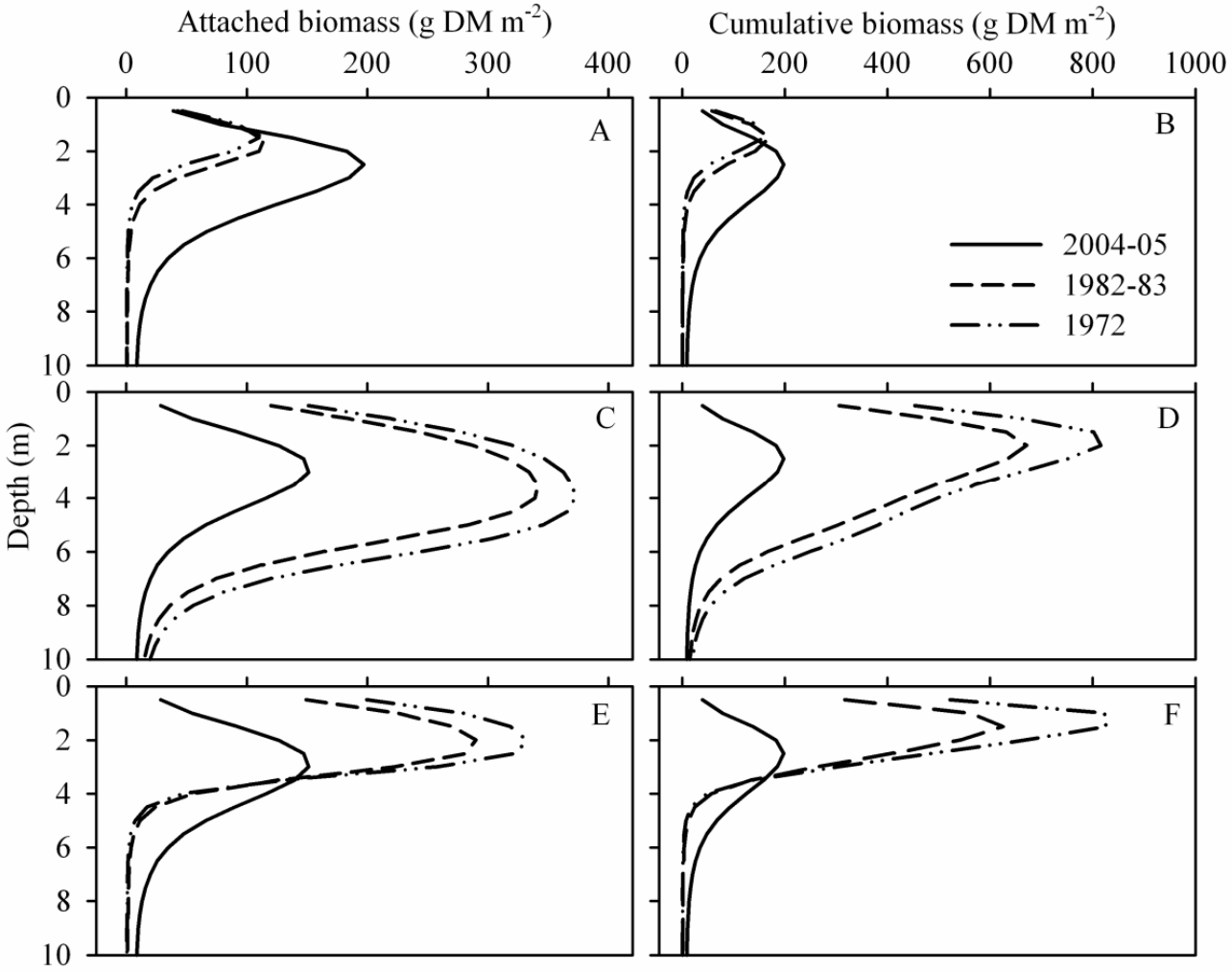

Figure 2.6 On the dates of peak biomass, *Cladophora* growth model predicted depth distribution of *Cladophora* biomass density. The independent effects of historical light attenuation (Kd_{PAR}) on (A) attached biomass density and (B) cumulative biomass density; the independent effect of historical tissue P concentrations (Q_P) relative to modern concentrations on (C) attached biomass and (D) cumulative biomass; the combined effects of historical Kd_{PAR} and Q_P on (E) attached and (F) cumulative biomass.

Forecasting *Cladophora* biomass at increasing temperatures

Data records of the mean surface water temperature from Lake Ontario averaged over July – September (days 182-273) from 1966 – 2006 indicate an overall increase at a rate of 0.049 ^{o}C yr^{-1} determined by linear regression analysis (Fig. 2.7). A warming trend appears to commence around 1980; linear regression analysis from 1980 to 2006 indicates an increase in surface temperature of 0.096 ^{o}C yr^{-1}.

The dominant predicted direct effect of increased temperatures was an earlier onset of exponential growth (Fig. 2.8A, B). There was a marginal increase in peak biomass with increasing temperature (Fig. 2.8C, D). The greatest increase in biomass was achieved with a 1.0 ^{o}C increase over baseline temperatures (at 0.5^{o}C resolution), resulting in 7.7% ± 2.5% increase in peak attached biomass.

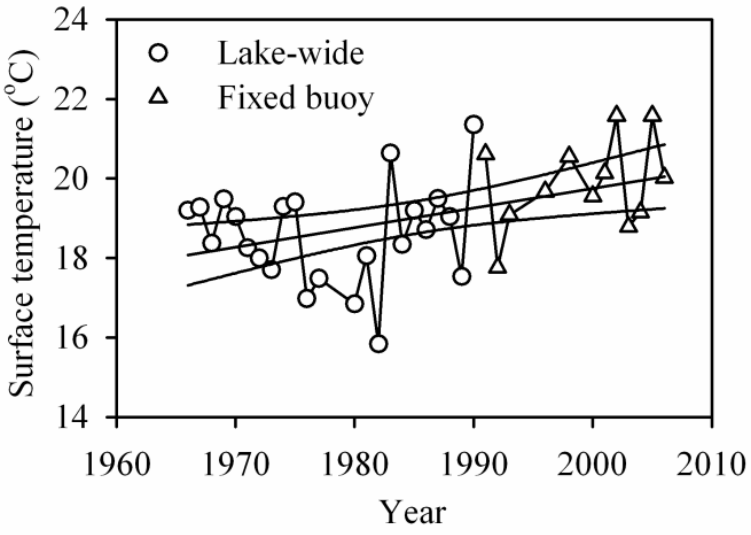

Figure 2.7 Mean surface water temperature from July – September in Lake Ontario, collected from lake wide methods (Schertzer 2003), or from a fixed buoy (C45139; Environment Canada). Linear regression for 1966 to 2000: mean surface temperature (^{o}C) = 0.0494 (year) – 79.00; r^2 = 0.21. A warming trend appears to begin in 1980. Linear regression from 1980 to 2006: mean surface temperature (^{o}C) = 0.0961 (year) – 172.24; r^2 = 0.30. The line of best fit and 95% confidence intervals about the regression are shown.

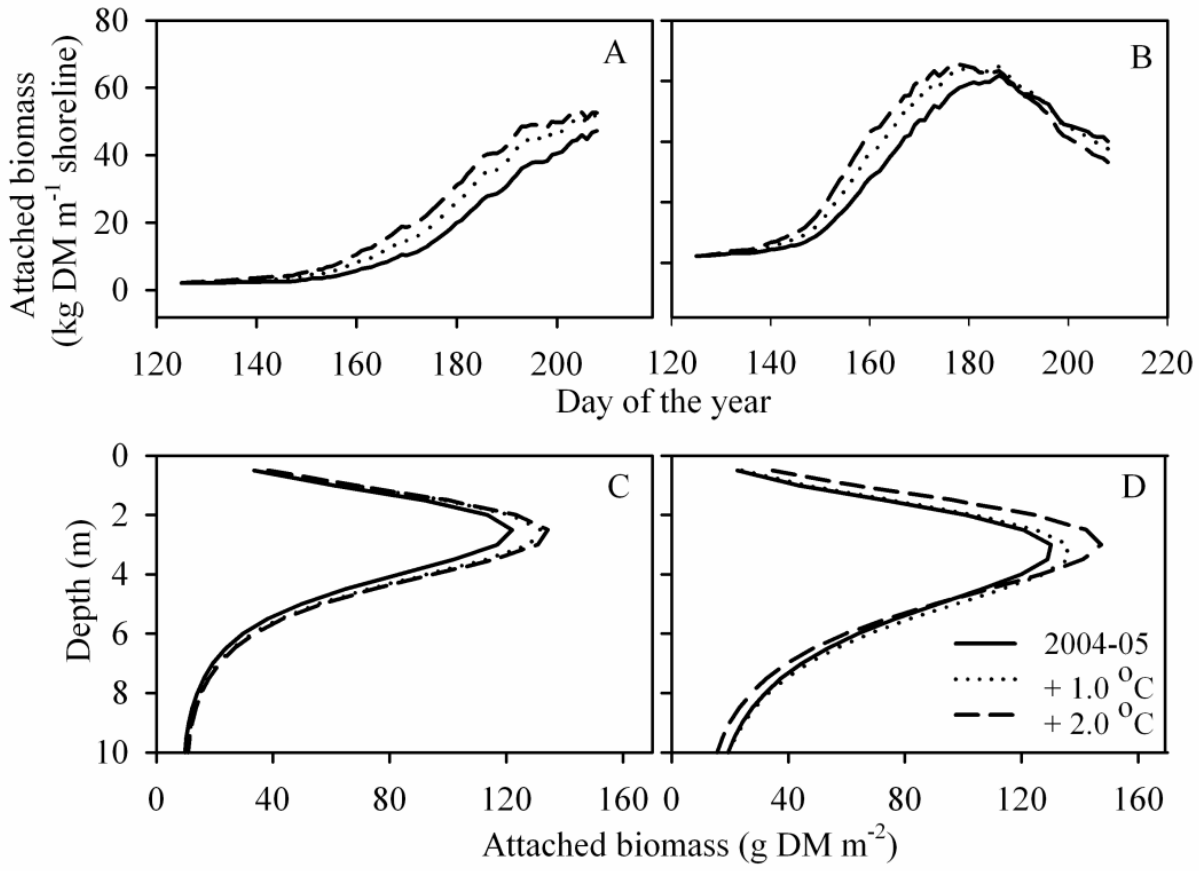

Figure 2.8 The effects of increasing temperature on the seasonality of *Cladophora* growth using (A) 2004 and (B) 2005 baseline data. The effects of temperature on the depth distribution during peak biomass using (C) 2004 and (D) 2005 baseline data. There was no further increase in depth-integrated peak attached biomass above a 2.0 ^{o}C increase in temperature (at 0.5 ^{o}C resolution).

Discussion

Proximal and global anthropogenic pressures, such as exotic species invasions and global climate change, have led to major changes to the limnology of the Laurentian Great Lakes over the last several decades. Yet, in the absence of continuous historical records, quantifying the effects of these perturbations on ecosystem processes remains a major challenge. Sensitivity analyses employing calibrated numeric models offer a practicable means to assess the response of a system to historic anthropogenic stressors, and furthermore, to predict future responses to environmental changes. In this study, I addressed the response of nuisance macroalgal biomass accumulation to decadal-scale ecosystem level changes. I analysed the effects of decreased light attenuation in the nearshore due to dreissenid mussels, changes in tissue phosphorus concentration (Q_P) due to the opposing forces of decreasing pelagic P concentrations and potentially increasing benthic P supply from mussels, and increasing summer lake water temperatures on attached and sloughed *Cladophora* biomass in Lake Ontario. Because *Cladophora* is the dominant component of the summer littoral flora on hard substrata in the lower Laurentian Great Lakes, knowledge of its production and seasonal growth dynamics is a prerequisite to constructing nearshore nutrient and carbon budgets, and, by extension, essential to assessing the relative importance of the benthos to nearshore or whole system processes.

The *Cladophora* growth model and contemporary biomass

Attached *Cladophora* biomass measured in Lake Ontario at 2.0 m depth peaked at 99 g DM m^{-2} and 130 g DM m^{-2} in 2004 and 2005, respectively. These values are near the range of direct measurements obtained in 2002 from five sites in eastern Lake Erie (range of maximum biomass measurements: 100 – 243 g DM m^{-2}; median: 152 g DM m^{-2}), with the highest *Cladophora* density found in proximity to the nutrient rich effluent of the Grand River (Higgins et al. 2005*a*). The largest deviation in attached *Cladophora* biomass between estimates predicted by the *Cladophora* growth model (CGM) and directly measured values occured in 2005 following the summer biomass peak. This deviation may reflect an underestimation of one or both of the attached biomass loss terms, sloughing and respiration, during senescence. Sloughing rates are recognized as the most difficult parameter to quantify accurately (Canale and Auer 1982). More likely, *Cladophora* respiration rates may have been

underestimated for senescing *Cladophora* because metabolic rate algorithms were based on measurements of vigorously growing *Cladophora* filaments (Graham et al. 1982). The metabolic demands of cellular repair may increase as *Cladophora* ages, leading to higher respiration following peak biomass.

In this version of the CGM, biomass loss is comprised of three components: daily respiration, daily sloughing, and a massive detachment triggered by a storm event. In 2005, respiration and sloughing alone adequately accounted for the biomass decrease of the first seasonal cohort of growth. In contrast, in 2004, these processes could not account for the rapid loss of *Cladophora* that occurred in synchrony along the north shore of Lake Ontario. Catastrophic loss events have been observed previously in the Great Lakes (e.g., Whitton 1970), but an algorithm describing the mechanism of this loss has been lacking. Therefore, I added a strictly physical mechanism to describe a catastrophic detachment of *Cladophora* related to attached biomass, a correlate of algal filament length, and storm magnitude, a correlate of shear stress imposed by waves. One consequence of a massive detachment event is an increase in irradiance and ambient nutrient concentrations relative to the remaining *Cladophora* filaments, potentially serving to enhance growth. The mechanism of detachment, therefore, could affect total seasonal biomass accrual. The implication is that with increasing frequency and magnitude of storm events predicted as a consequence of climate change in the Laurentian Great Lakes region (Kunkel et al. 1999), total seasonal *Cladophora* productivity may increase.

The CGM, originally constructed to predict macroalgal biomass density in relation to a high concentration nutrient point source in Lake Huron (Canale and Auer 1982), and then validated in a rural shoreline in the oligotrophic eastern basin of Lake Erie, was herein validated in a very different limnological setting. I applied the model in an urbanized location which experiences higher and more seasonally dynamic nutrient supply than the Lake Erie site, with numerous nutrient-rich point sources (including e.g., storm water drains and a wastewater treatment plant approximately 6 km distal to our study site). The Lake Ontario study site also allowed us to assess the capacity of the model to accurately predict *Cladophora* biomass in response to temperature differences, for use in predicting *Cladophora* biomass under future temperature scenarios. While Lake Erie is a shallow lake that warms quickly, Lake Ontario is deep and strongly stratified; the north shore experiences frequent upwelling (i.e., see frequent

cooling intervals during the warming season in Fig. 2.2). By validating the CGM in this new environment, I gained confidence that it can be applied to other systems that experience seasonal macroalgal blooms e.g., other Great Lakes with different temperature and nutrient regimes, the Baltic Sea, fluvial lakes of the St. Lawrence Seaway, etc.

Cladophora growth model under historic and forecast scenarios

In this study, I collected *Cladophora* biomass and determined Q_P over two years that experienced very different meteorology. The differences in the rate of spring warming and the maximum seasonal temperatures provided a good range of conditions to assess the effects of the target variables: Q_P, light attenuation (Kd_{PAR}), and surface water temperature. *Cladophora* Q_P declined from 1972 to 1982 (Painter and Kamaitis 1987), a period marked by declining dissolved P concentrations in Lake Ontario (Nicholls 2001). Seasonal minimal *Cladophora* Q_P in modern years are lower still than 1982 levels. Anecdotal evidence of increasing macroalgal shoreline fouling and water intake clogging since the early 1990s supports the possibility that the seasonal mean *Cladophora* Q_P was lower in the late 1980s, prior to mussel establishment, than today. If this is true, we may be currently witnessing an increase toward concentrations measured in the early 1980s. However, only continued monitoring will resolve the direction of trajectory of an interannual change in *Cladophora* biomass.

The effects of lower Q_P and higher Kd_{PAR} observed over the past three decades operate in opposing directions on seasonal *Cladophora* density and depth distribution. Biomass at depths down to 3.5 m are lower in modern years than in the early 1970s and 1980s (due to lower Q_P) while biomass at depths greater than 3.5m are now higher (due to higher Kd_{PAR}). The overall result is a lower total depth-integrated attached and cumulative *Cladophora* biomass in contemporary biomass relative to the early 1970s and 1980s. The consequence of the increased areal extent of growth, however, is that if Q_P rises to levels that were found in previous decades, due to allochthonous nutrient sources or autochthonously through nutrient regeneration by mussels, the accumulation of biomass will exceed the historical biomass previously produced at a given P concentration.

Cladophora biomass was measured in 1982 and 1983 at 3 depths in Oakville on weeks estimated to be the seasonal peak (Kamaitis 1984). The magnitude and depth-distribution of the peak *Cladophora* biomass predicted for 1982 and 1983 at Oakville were very similar to

those measured (Fig. 2.9), demonstrating that the CGM was adequately parameterized for historical Q_P and Kd_{PAR}. Historical mean seasonal Kd_{PAR} was estimated from the relationship between modern secchi depth at 25 m and light attenuation measured at 2 m. This assumption that an increase in secchi depth at 25 m is proportional to the increase in water clarity at a much shallower site leads to a conservative estimate of nearshore Kd_{PAR} (i.e., minimal interdecadal change is estimated) because the influence of dreissenid filtration is greater in shallow waters where the water column is smaller and particle resuspension is greater. But, the relationship between Kd_{PAR} and secchi depth between decades may also be affected by changes in the particle size spectrum due to size-selective filtration by the mussels and consequently changing scattering characteristics of particles in the nearshore. Despite these potential uncertainties, the Kd_{PAR} value used as model input for 1982-83 simulated the observed depth–distribution of *Cladophora* biomass with remarkable accuracy (Fig. 2.9). Direct measurements of *Cladophora* biomass lie between the predicted attached and cumulative *Cladophora* biomass, indicating that sloughing may be overestimated at depths shallower than 2 m. No comparable data are available at Oakville in 1972 because biomass was measured immediately following a major detachment event.

Changes in global climate are expected to decrease the ice cover and increase the number of nearshore ice-free days in Lake Ontario, resulting in increased temperatures and a prediction of no spring turnover (Schertzer and Croley 1999). Modeling these effects on *Cladophora* biomass will require linking the CGM to a hydrodynamic model to assess the influence of not only temperature, but also nutrient circulation, in the littoral zone. In this study, I limited our assessment to the direct effects of increasing temperatures on spring growth and peak summer biomass. As I have demonstrated, summer peak biomass is limited by available P and light. Consequently, higher temperatures are predicted to strongly influence only the timing of growth, rather than biomass accumulation. Surface temperature has increased at an average rate between 0.5 and 0.9 ^{o}C $decade^{-1}$ over the past 2-3 decades in Lake Ontario, slightly less than the increase measured in Lake Superior from 1979 – 2006 (1.1 ± 0.6 ^{o}C $decade^{-1}$; Auston and Colman 2007). The CGM predicts a modest 7.7 ± 2.5% and 7.2 ± 6.3% increase over modern years in depth-integrated peak attached *Cladophora* biomass for a 1.0 ^{o}C and 2.0 ^{o}C increase in temperature, respectively. That temperature largely affects the seasonality rather than total biomass accumulation is also illustrated by the similarity in total

biomass accumulation predicted using the two baseline datasets. The summer surface water temperature of 2004 was, on average, 4.2 ^{o}C colder than in 2005, yet peak measured and predicted biomass were very similar between years. Thus, predicting peak *Cladophora* biomass in the future will continue to depend primarily on P supply and nearshore optical properties.

Two shortcomings of the application of the CGM should be noted. First, while the change in Q_P with depth is accounted for in predicting *Cladophora* biomass at depths beyond 2 m, I predict biomass accumulation at depths that are up to 2 m deeper than the deepest direct measurements of Q_P. This extrapolation of the Q_P versus depth relationship, however, can generate only a marginal error in biomass estimates because the growth rates at these depths are constrained almost entirely by irradiance, rather than by Q_P. Secondly, I calibrated the CGM at 2.0 m depth, and then implicitly assumed that the accuracy of the model's output would be similar at all depths. The generality of the CGM is demonstrated in this study, and as a corollary, I suggest the application of the CGM to depths beyond 2.0 m at this site is well justified.

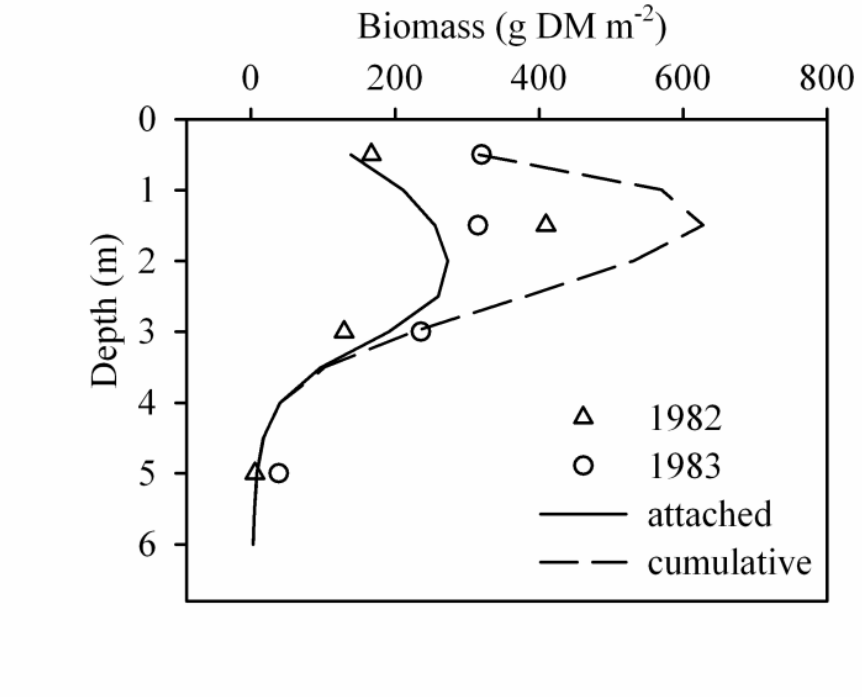

Figure 2.9 Historical direct measures of *Cladophora* biomass in 1982 and 1983 relative to model predictions for attached biomass and cumulative biomass on the date of peak predicted biomass.

Implications for dreissenid mussels and eutrophication

This study demonstrates, using historical data in a contemporary model, that critical environmental factors that are changing as a result of human activity (e.g., dreissenid mussel establishment) could result in proliferation of noxious amounts of *Cladophora* biomass equal to, or in excess of, levels reached during the decades prior to P control. It has been suggested that P loading restrictions be relaxed in the lower Laurentian Great Lakes as a means to enhance fisheries yields (e.g., Stockner et al. 2000); yet the establishment of abundant benthic filter feeders has shifted the effects of eutrophication (in terms of high algal biomass) towards the nearshore and especially to the littoral zone (Hecky et al. 2004; Zhu et al. 2006). Dreissenid mussels effectively clear the water column through filter-feeding and thus reduce the effects of eutrophication as indicated by water clarity and suspended chlorophyll concentration (e.g., Holland et al. 1995, Budd et al. 2001). However, I demonstrate how these benthic grazers, by enhancing light availability and possibly by focussing nutrients to the benthos in the littoral, enhances other undesirable effects of eutrophication, specifically, an overabundance of benthic macroalgal biomass. Other studies have similarly demonstrated that other adverse effects of nutrient enrichment, such as the frequency of (potentially toxic) cyanobacterial blooms, are not ameliorated, and perhaps even amplified, by the addition of benthic filter-feeders (Sarnelle et al. 2005; Caraco et al. 2006).

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Chapter 3 Carbon-based *in situ* measurement of benthic primary production on *Cladophora*-dominated rocky substrata in Lake Ontario

Introduction

Excessive nutrient enrichment in aquatic ecosystems leads to many undesirable consequences, including an increasing frequency in blooms of potentially noxious benthic macroalgae (Raven and Taylor 2003, Worm and Lotze 2006). In marine systems with low to moderate nutrient supply, hard substrata are generally dominated by slow growing perennial benthic macroalgae. In contrast, in freshwater systems with low nutrient supply, where phaeophytes and macroalgal rhodophytes are largely absent, hard substrata are generally dominated by adnate microbial communities. Yet, in both marine and freshwater shallow systems that are disrupted by excessive nutrient supply, there is a tendency towards proliferation of filamentous nuisance macroalgae (e.g. Whitton 1970, Dodds and Gudder 1992, Vogt and Schramm 1991, Fong et al. 1993, Valiela et al. 1997, Smith et al. 2005). Such macroalgae are commonly from the genera *Cladophora, Spirogyra, Mougeotia, Ulva*, *Pilayella*, and *Enteromorpha* and demonstrate rapid nutrient uptake kinetics and high maximal growth rates that are typical of r-strategists (Wallentinus 1984; Raven and Taylor 2003). Proliferation of fast-growing, short-lived benthic macroalgae is a particularly undesirable consequence of eutrophication because when their populations eventually senesce, they promote high bacterial production (Byappanahalli et al. 2003), detach as algal clumps that can clog water intakes, release fetid odours (Higgins et al. 2005*b*), and can create local areas of hypoxia (Turner et al. 1995).

Despite the management and ecosystem health concerns related to blooms of benthic macroalgae, studies and monitoring efforts to estimate benthic primary production are rare relative to measurements of pelagic primary production (Vadeboncoeur et al. 2002). This omission is furthermore striking because benthic primary production can be a substantial contributor to whole lake (Vadeboncoeur et al. 2002) or marine coastal (Charpy-Roubaud and Sournia 1990) C-fixation budgets. Additionally, lacustrine top predators generally assimilate carbon fixed from both pelagic and benthic habitats (Hecky and Hesslein 1995, Vander Zanden and Vadeboncoeur 2002) and benthic primary production may provide a disproportionately

large contribution to secondary consumers relative to pelagic primary production (Hecky and Hesslein 1995; Vander Zanden et al. 2006). The relative scarcity of benthic primary production measurements is certainly due in part to the difficulty associated with working in habitats with high spatial heterogeneity (e.g. variable substrata along the shoreline, variable light climate within a periphyton or macrophyte canopy, and variable light and substrata over the depth of the euphotic zone). Accounting for temporal variability of ephemeral or seasonally-blooming species also requires great effort to achieve a sufficiently high sampling frequency.

The introduction of clear benthic incubation chambers that are sealed to hard substrates, gave rise to the first *in situ* measurements of benthic primary productivity on hard substrates in lakes (e.g. Wetzel 1964, Loeb 1981). The use of such chambers circumvented the need for artificial substrates for periphyton colonization or the removal of hard benthic substrata to artificial, and potentially disturbed, experimental conditions for incubation (e.g., Turner et al. 1983). Generally, ^{14}C uptake or oxygen evolution methods have been employed with benthic chambers, for high precision estimates. Yet, several challenges with these methodologies remain. The ^{14}C uptake technique cannot quantify respiration, thereby generating a value that represents a rate somewhere between gross primary production (GPP) and NPP. This issue may be especially critical when measuring macroalgal growth because respiration may vary greatly seasonally and represent a large fraction of the C-fixed due to self-shading within the algal mat. Measuring oxygen uptake rates, in contrast, provides a means to measure dark respiration, and therefore NPP, the process of interest from a community or ecosystem-scale. However, converting oxygen rates to carbon-based NPP for comparison with biomass accumulation requires an estimate of the efficiency of C-fixation relative to oxygen evolution (i.e., the photosynthetic quotient). Photosynthetic quotients have rarely been estimated for benthic macroalgae (c.f., Davies and Hecky 2005), and can vary with light intensity and nutrient sources. Thus, a C-based estimate of NPP is advantageous. A final challenge is that benthic chambers typically restrict water movement. Extended constraints in overlying water motion may not lead to critically different water movement at the benthic (diffusion limited) boundary layer over microalgal epiphytes. However, one of the putative advantages conferred to attached macroalgae is the ability to access nutrients and C by growing up out of the benthic boundary layer (Dodds and Gudder 1992). Indeed, some macroalgae, such as *Cladophora*,

have a growth requirement for moving water (Dodds and Gudder 1992). Methodological artifacts imposed by chambers may therefore be particularly acute for macroalgae.

During the summer, in the lower Laurentian Great Lakes (Michigan, Erie and Ontario), periphyton communities on rocky substrata are dominated by the filamentous green macroalga *Cladophora glomerata* (Herbst 1969, Higgins et al. 2005*b*, Malkin et al. 2008). This species grows attached to the benthos, grows rapidly to high standing crops, and is generally considered a sentinel of nearshore or benthic eutrophication (Dodds and Gudder 1992). The seasonality of *Cladophora* growth dynamics in the Great Lakes is dictated largely by water temperature (Malkin et al. 2008). In the Great Lakes, *Cladophora* blooms occur in the spring when water temperatures approach 10^{o}C (Whitton 1970). In western Lake Ontario, accumulation to nuisance densities is achieved within approximately three weeks of detectable growth (Malkin et al. 2008). Following mid-summer senescence, *Cladophora* in the Great Lakes typically exhibits a second annual period of regrowth (Bellis and McLarty 1967, Malkin et al. 2008).

The first objective of this study is to test the utility of a novel C-based *in situ* method for measuring benthic NPP in a lake with high carbonate alkalinity. This method monitors change in dissolved inorganic carbon (DIC) concentration, based on continuously measured changes in dissolved carbon dioxide concentration, the two dissociation constants of carbonic acid as functions of continuously monitored temperature, and a single measurement of alkalinity. The method employs short-duration incubations (e.g. 15 minutes) with chambers that maintain continuously flowing water. The second objective is to report NPP of the dominant summer primary producer, *Cladophora*, on rocky substrata in Lake Ontario through the growing season. *In situ* measured rates of benthic primary production on *Cladophora*-dominated rocky substrata have previously been reported on four dates in Lake Erie, measured as changes in oxygen and DIC during 24 hour incubations (Davies and Hecky 2005). These data were the first published *in situ* benthic primary production estimates from the lower Great Lakes. The current study overcomes the uncertainties attendant with long incubations. By measuring benthic primary production biweekly from May to September, this study provides much greater temporal resolution to determine seasonal changes in NPP at a shallow site on Lake Ontario. The final objective of this study is to compare our direct measures of benthic NPP as a direct estimate of growth with the simulated growth output of a *Cladophora* growth

model (CGM), previously calibrated against seasonal biomass estimates of *Cladophora* in Lake Ontario (Malkin et al. 2008).

Methods

Study site

Measurements of *Cladophora* production, biomass, and tissue chemistry were made on the north shore of western Lake Ontario in Oakville, Ontario, Canada, to the east of 16 Mile Creek (43.44^{o}N, 79.66^{o}W). Net primary production (NPP) measurements were conducted at 1 m depth. The site is underlain by boulders and cobble and supports 100% *Cladophora* coverage at 2 m depth during midsummer and dense, although somewhat patchier coverage at 1m depth due to greater wave scouring. During the year of investigation, 2005, the study site at 1 m depth supported a patchy distribution of the invasive quagga mussel, *Dreissena bugensis*.

Background information and definitions of carbonate solution chemistry

There are four dissolved species of inorganic carbon in water ($CO_{2(aq)}$, H_2CO_3, HCO_3^-, and CO_3^{2-}) which maintain an equilibrium controlled by pH. In discussing carbonate equilibrium, the two uncharged molecules, $CO_{2(aq)}$ and H_2CO_3, are considered together as a composite species, $H_2CO_3^*$, due to the small contribution of H_2CO_3 (< 0.3% at 25^{o}C) and the difficulty in distinguishing the two species analytically (Stumm and Morgan 1996). Total dissolved inorganic C (DIC) is thus:

$$[DIC] = [H_2CO_3^*] + [HCO_3^-] + [CO_3^{2-}] \quad (1)$$

The concentrations of each species of a carbonate solution at equilibrium are described by the ion product of water and the dissociation constants of carbonic acid,

$$K_W = [OH^-]\cdot[H^+] \quad (2)$$

$$K_1 = [H^+]\cdot[HCO_3^-]\cdot[H_2CO_3^*]^{-1} \quad (3)$$

$$K_2 = [H^+]\cdot[CO_3^{2-}]\cdot[HCO_3^-]^{-1} \quad (4),$$

where the values of K_W, K_1 and K_2 are functions of temperature. Because the Laurentian Great Lakes are sufficiently dilute (< 10mM ionic strength) I equate measured activities with concentrations, making no corrections for ionic strength (Stumm and Morgan 1996). For

greatest precision, concentrations are measured as mass and reported in molality (mol kg^{-1}). Differences in water density, however, can be ignored in shallow freshwaters (Stumm and Morgan 1996). In this study, water was measured volumetrically and concentrations reported as molarity ($mol \cdot L^{-1}$).

At pH levels of Lake Ontario (7.8-8.9), the dominant DIC species is HCO_3^-. [$H_2CO_3^*$] ranges from 4 to 25 μM between May and August contributing to less than 1.5% of the DIC pool (Leggett et al. 1999, and this study). Measuring changes in [$H_2CO_3^*$] can therefore be up to 100-fold more precise than measuring changes in [DIC], assuming a similar coefficient of variation. [$H_2CO_3^*$] can be measured by equilibrating lake water $pCO_{2(aq)}$ with an airstream, and measuring the $pCO_{2(g)}$ in the airstream. The measured concentration of $pCO_{2(g)}$ to the original $H_2CO_3^*$, is calculated by the gas-water partitioning equilibrium given by Henry's law:

$$[H_2CO_3^*] = K_H \cdot pCO_{2(g)} \quad (5)$$

where K_H is the solubility constant of CO_2 ($mol \cdot L^{-1} \cdot atm^{-1}$), and $pCO_{2(g)}$ is the partial pressure of CO_2 in the gas phase (atm). The equation is rigorously applicable to fugacity and activity, but the difference between pCO_2 and *f*CO2 in shallow freshwater is negligible (i.e. the ratio of *p*CO2 to *f*CO2 was found to differ from unity by less 10^{-6} using equations of Weiss 1974). pCO_2, by definition, is the product of the molar fraction of CO_2 (xCO_2) and the pressure of that gas (atm):

$$pCO2 = xCO_2 \cdot P \quad (6)$$

Total alkalinity (A_T) is defined as the number of moles of protons equivalent to the excess of proton acceptors over proton donors (Dickson 1981) and is operationally defined as the concentration of titratable base by a strong acid, such as HCl, to a pre-selected endpoint.

$$A_T = [HCO_3^-] + 2[CO_3^{2-}] + [OH^-] - [H^+] + [\sum \text{species that add alkalinity}] - [\sum \text{species that remove alkalinity}] \quad (7)$$

Carbonate alkalinity (A_C) is the subset of alkalinity due to carbonates alone:

$$A_C = [HCO_3^-] + 2[CO_3^{2-}] \quad (8)$$

A_C can thus be determined from A_T and pH and the equilibrium coefficient K_W (equation 2), assuming that all other molecules that increase alkalinity (e.g. $B(OH)_4^-$, HPO_4^-, $2HPO_4^{3-}$, $SiO(OH)_3^-$) or decrease alkalinity (e.g. NH_3, HS^-, HSO_4^-, HF, and H_3PO_4) are present in negligible concentrations. Rearranging equations 7 and 8 yields:

$$A_C = A_T - [OH^-] + [H^+] \quad (8b).$$

Rationale and overview of DIC uptake methodology

Algae meet their C demands for photosynthesis by the passive diffusion of $CO_{2(aq)}$ into cells, by active uptake of HCO_3^-, and by catalytic conversion by plasmalemma-bound carbonic anhydrase of HCO_3^- to $CO_{2(aq)}$ at the cell surface, followed by passive diffusion of $CO_{2(aq)}$ into cells (Axelsson et al. 1995, Falkowski and Raven 2007). These mechanisms for DIC acquisition are widespread among eukaryotic algae and have been demonstrated specifically for *Cladophora glomerata* (Choo et al. 2002). Regardless of the DIC species acquired, pH increases with photosynthetic uptake of DIC. With regard to $CO_{2(aq)}$ uptake, an increase in pH is directly attributable to a shift in the carbonate equilibrium which lowers $[H^+]$ with the removal of $CO_{2(aq)}$ from the bulk medium. HCO_3^- uptake is met with molar equivalent release of OH^- (through membrane-bound ATPases or anion exchangers), causing an increase in pH. Carbonic anhydrase-mediated interconversion of $CO_{2(aq)}$ and HCO_3^- reflects an accelerated drive towards equilibrium, not a change in the direction of balance (Coleman 2000). Uptake of $CO_{2(aq)}$, subsequent to its catalytic conversion, however, causes a shift in the carbonate equilibrium, increasing the pH of the bulk medium.

In contrast to the ambient pH which changes with metabolic activity, A_C is not affected directly by photosynthetic DIC uptake or respiratory $CO_{2(aq)}$ production (Stumm and Morgan 1996). Because a shift in pH caused by photosynthetic uptake of either $CO_{2(aq)}$ or HCO_3^- leads to a shift in the carbonate equilibrium, by knowing the value of A_C, the values of the dissociation constants K_1 and K_2, and by monitoring $[H_2CO_3^*]$, the concentration of all other DIC species can be calculated.

In the method introduced here, I estimated net primary production (NPP) as a change in [DIC] by monitoring the change in $[H_2CO_3^*]$ in benthic chambers during short-term incubations, coupled with a single measurement of A_C for each incubation, and the values of K_1 and K_2 calculated by continuously monitoring temperature. An illustration of how DIC flux varies with $H_2CO_3^*$ flux is given in Fig 3.1. To calculate DIC from A_C and $[H_2CO_3^*]$, pH was first determined through an iterative calculation, given the following rearrangements and substitutions of equations 3 and 4 and the definition of A_C (Eqn 7):

$$[HCO_3^-] = [H_2CO_3^*] \cdot K_1 \cdot [H^+]^{-1} \quad (9)$$

$$[CO_3^{2-}] = [H_2CO_3^*] \cdot K_1 \cdot K_2 \cdot [H^+]^{-2} \quad (10)$$

Values of $[H^+]$ were iteratively estimated until measured and calculated A_C (equation 8) converged to within 1µequiv. DIC concentration was then calculated from the definition in equation (1) and DIC flux (mgC g DM *Cladophora*$^{-1}$ hr^{-1}) was calculated using the linear $H_2CO_3^*$ flux and assuming constant A_C during the short incubation.

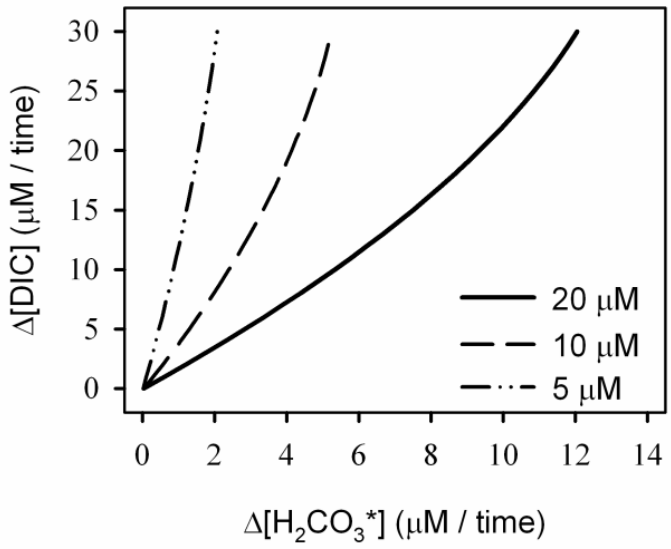

Figure 3.1 The relationship between $H_2CO_3^*$ flux and dissolved inorganic carbon (DIC) flux at a constant carbonate alkalinity (1500 µequiv) and a constant temperature (20°C). Data are shown for three initial $H_2CO_3^*$ concentrations (5 µM, 10 µM and 20 µM), which, at the given temperature and alkalinity, correspond to initial pH values of 8.25, 8.55, and 8.84, respectively.

In situ primary production measurements

Net primary production estimates were carried out by deploying a clear benthic incubation chamber, similar to that of Davies and Hecky (2005), over flat surfaces of rocky substrate covered with *Cladophora*. Rocks colonized by dreissenid mussels were avoided. Chambers were made of acrylic cylinders (internal diameter: 10.1 cm; height: 25.4 cm), with a clear acrylic lid securely fitted with a single rubber gasket, and a neoprene skirt extending outward from the base (Fig. 3.2). To secure the chamber to the substrate, the chamber bottom was lined with weather stripping and the neoprene skirt was weighted down with a lead shot-filled sock. Care was taken to prevent filaments from being held down by the sides of the chamber which would inhibit their movement. The lid had two holes, through which tygon tubing hoses were passed. These hoses served as inflow and outflow to and from the chamber. The hoses were secured and sealed in place through the duration of each incubation with rubber stoppers. The chamber was deployed at 1m depth by a snorkeller.

The inflow and outflow hoses of the chamber were connected to a measuring apparatus, maintained on a tethered surface vessel. The measuring apparatus is modified from a design by Morris Holoka and Ray Hesslein (Department of Fisheries and Oceans, Winnipeg, Canada). The apparatus at the surface, semi-autonomously controlled by a person aboard the vessel, circulated water through the system, measured atmospheric and dissolved pCO_2 and water temperature at 5 second intervals, and recorded data to a data logger (CR10X, Campbell Scientific, Edmonton, AB, Canada). Water was pumped from the chamber with a peristaltic pump. Water from the chamber flowed first past a thermistor (P107, Campbell Scientific) then through a membrane contactor (Liqui-Cel, Membrana, Charlotte, NC, USA) which extracted dissolved gas by increasing the pressure of the air stream. From the membrane contactor, the water continued back to the chamber so that the water was circulated in a closed-loop system. Water was pumped at a rate of approximately 1.2 $L \cdot min^{-1}$, varying slightly with battery voltage. The volume of water in the chambers was 2.04 L and water in the tygon tube hosing was 0.02 L, thus flushing was approximately 0.75 min^{-1}.

Gas extracted by the membrane contactor was circulated using an air pump via a separate system, first through Nafion tubing (Perma Pure, Toms River, NJ, USA) to remove moisture, then via tygon tubing, through a non-dispersive infrared gas analyser (IRGA; Li-820, Li-Cor Inc., Lincoln, NB, USA) which measured the molar fraction of CO_2 (xCO2) and gas

pressure (atm). The IRGA was calibrated in a range from zero to 1000 ppm CO_2. Gas removed from solution, once passed through the IRGA, was reintroduced to the moving water via the membrane contactor. The gas flow system was therefore also a closed-loop. In this way, consumption and production of CO_2 could be attributed to processes occurring in the benthic chamber. Each measurement (water temperature, gas pressure, xCO_2) was recorded to the data logger. The data logger was also connected electrically to a solenoid which directed either ambient air or the airstream from the membrane contactor to the IRGA, a reverser which designated the direction of the water flow, and a pair of mechanical switches to control these components.

Incubations were 15-20 minutes in duration. Incubation measurements were repeated from sunrise as long as weather and water quality conditions permitted, which was typically 18:00 in the spring and early summer, and 13:00 in late summer/autumn. Each incubation was made on a different rock in order to gain the best estimate of NPP for the study site on each day. Following each incubation, all macroalgal biomass under the chamber was harvested by hand with a scraping tool into a mesh bag (porosity < 1.0mm). These samples were brought back to the lab where they were rinsed clean of debris and macroinvertebrates, dried for 24 hrs at 65^{o}C, and weighed. Sub-samples were further analysed for tissue P (Q_P) concentration and chlorophyll *a* (Chl*a*) concentration.

A water sample (120mL) for alkalinity measurements was collected at the beginning of each incubation. These samples were collected by pumping water from the chamber to a dark polyethelyne bottle (to overflowing) and sealed with gas exclusion caps. These samples were kept on ice until brought back to the lab, where they were allowed to warm to room temperature just prior to measurements of pH and alkalinity. These measurements were made within 24 hours of sampling. pH was measured with a combination Ag/AgCl electrode (precision ± 0.01) with an external automatic temperature-correction. Alkalinity ($A_{T;}$ $\mu equiv \cdot l^{-1}$) was derived from Gran titrations (Stumm and Morgan 1996) in which a 50.0 mL aliquot of sample was titrated with 0.1N HCl using 10 µL aliquots in an open cell. The HCl was titrated independently against a HCO_3^- standard to calibrate its concentration to within 0.01 N. The volumes of titrant required to drop the pH to five step-points between pH 4.0 and 3.5 were recorded, from which Gran functions were plotted and the equivalence point calculated. The value of A_C was calculated from initial pH and A_T following equation 8b.

Rates of $H_2CO_3^*$ flux were measured as the slope of $[H_2CO_3^*]$ against time starting from the period of steady state $H_2CO_3^*$ removal and re-addition (within 5 minutes) until the end of the incubation. Accidental incursions of ambient water due to leakage around chamber seal (occurring approximately once every 40 incubations) were seen as high fluctuations in $[H_2CO_3^*]$. Data from these incubations were discarded.

Empirical measurements of pK_W, pK_1 and pK_2 as functions of temperature at zero ionic strength were conducted by Harned and Owen (1958), Harned and Davis (1943), and Harned and Scholes (1941), respectively. Based on these data, polynomial equations describing the temperature dependencies of these constants as given in Stumm and Morgan (1996; for pK_W), and Maberly (1996; for pK_1 and pK_2) were used:

$$pK_W = -4470.99/T - 0.01706T + 6.0875 \quad (14)$$

$$pK_1 = 3404.71/T + 0.03279T - 14.84 \quad (15)$$

$$pK_2 = 2902.39/T + 0.02379T - 6.50 \quad (16)$$

where T is absolute temperature (K). At 20°C, $pK_W = 14.17$, $pK_1 = 6.38$ and $pK_2 = 10.38$.

Between 10:00 and noon, a light profile was measured at 2.5 m depth at 0.5 m intervals from which the PAR light attenuation coefficient (Kd_{PAR}) was calculated. Surface irradiance was measured with a pyroheliometer at 10 minute intervals at a site less than 20 km away (Canada Centre for Inland Waters; Burlington, ON). Conversion of the short-wave radiation dose measured with the pyroheliometer to instantaneous PAR irradiance is described elsewhere (Malkin et al. 2008).

Nighttime respiration of *Cladophora* was calculated using equations of Graham et al. (1982) which predict respiration based on temperature and photoperiod. Measurements of dark respiration as CO_2 evolution using these methods were attempted with black opaque shielding over the chambers, but these rates were strongly correlated with ambient $[H_2CO_3^*]$, rather than temperature or other metrics of metabolism. Thus these data are not reported.

A.

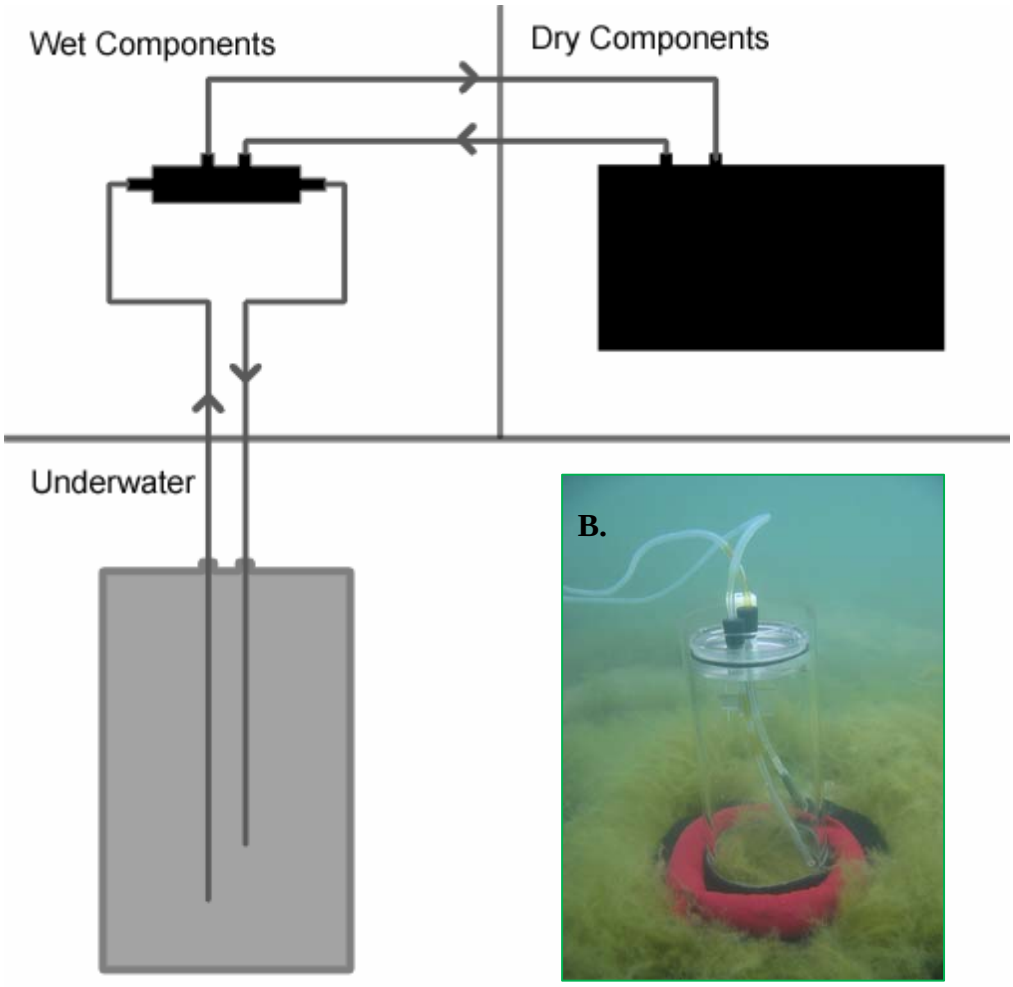

Figure 3.2 Gas and water flow during benthic incubations (A). "Wet" and "dry" components are kept separate in watertight boxes held on a surface vessel. Wet components include a peristaltic water pump, a thermistor, solenoids, a membrane contactor (shown), and a naphion drier. Dry components include an air pump, an infrared gas analyser (shown) and a data logger. Water is pumped between the benthic incubation chamber (underwater) and the box of wet components. Air is pumped between the boxes of wet and dry components. The boxes are also connected electrically (not shown). A photograph (B) illustrates a mid-summer bed of attached *Cladophora* on hard substrate and a benthic incubation chamber. The cylindrical acrylic chamber has a single gasket lid penetrated by inflow and outflow hoses. The chamber is sealed to the bottom with a lead-filled sock sitting on top of a neoprene skirt that extends from the base of the chamber.

Cladophora biomass and tissue chemistry

For areally expressed rates of *Cladophora* primary production, biomass was measured by harvesting 0.25 m^2 quadrats deployed randomly approximately every 2 weeks from May to October. Details of harvesting are given in Malkin et al. (2008). The harvested macroalgal material was rinsed in a sieve (mesh size < 1.0 mm) with de-ionized water to remove debris and was picked clean of macroinvertebrates with forceps. Every sample of macroalgae was observed under a dissecting microscope (4-25X magnification) to ensure its identity was *Cladophora* (van den Hoek 1995). Using molecular markers, all *Cladophora* in the Laurentian Great Lakes have recently been identified as *Cladophora glomerata* (L.) Kützing (Ross 2006). Macroalgal material collected from benthic incubations were dried in a lyophilizer for 48 hours prior to weighing.

Cladophora Growth Model

The calibration of the *Cladophora* growth model (CGM), which simulated biomass accumulation for this site on Lake Ontario, is described elsewhere (Malkin et al. 2008) and is conceptually similar to Higgins et al. (2005*a*). The CGM predicts daytime NPP as the product of a maximum NPP and three growth multipliers related to (1) irradiance and temperature, (2) tissue P concentration and (3) self-shading (Graham et al. 1982, Canale and Auer 1982). Nighttime respiration is calculated as a function of temperature alone (Graham et al. 1982). Diel NPP is calculated as the daytime NPP less the nighttime respiration, accounting for the duration of photoperiod. The model predicts biomass accumulation (i.e. growth) as the difference between diel NPP and physical loss processes (e.g. sloughing). Here, I compare the model-predicted diel NPP to direct measurements. For the purposes of this study, C was assumed to comprise a constant 30% of the *Cladophora* dry mass, a mid-range measured value (see Chapter 4).

Results

Cladophora biomass-specific net primary production: Diurnal and seasonal patterns

$H_2CO_3^*$ drawdown rates in the incubation chambers were nearly linear following a 5 minute equilibration of the measuring system (Fig. 3.3). Rates of *Cladophora* biomass-specific net primary production (NPP) at 1m depth, calculated as dissolved inorganic carbon (DIC) uptake from benthic chambers, demonstrated high seasonal variability (Fig. 3.4). Data in Fig. 3.4, divided into panels for clarity, were used to infer three seasonal phases of growth of the *Cladophora* population: (A) rapid spring growth rate, (B) summer declining growth rate and senescence, and (C) fall regrowth. Irradiance at 1m depth is shown also for each date of measurement. Cloud-free mornings were common during our spring and summer dates of measurement, but there was greater cloud cover during late summer and autumn measurement dates. During spring and summer, all incubations appear to have achieved light saturation by the first observation (3.4*A*,*B*). Additionally, there was no evidence for photoinhibition at the highest irradiances measured.

Based on data in Fig. 3.4, in the spring and summer, the light saturated biomass-specific maximum photosynthetic rate (P^B_m) for each day was taken as the mean of all light saturated incubations. During the fall regrowth, some incubations were performed at light limiting insolation (Fig. 3.4*C*). On fall dates (Julian days 228 and 235), the first morning incubations were excluded from the calculation of P^B_m.

P^B_m was greatest on the earliest date of sampling, 26 May (Julian day 146; 2.3 ± 0.8 SD mgC g DM^{-1} hr^{-1}) and remained high during the second date of measurement (Julian day 153; 1.1 ± 0.7 mgC g DM^{-1} hr^{-1}; Fig. 3.5). This period was marked by a rapid increase in *Cladophora* biomass (Fig. 3.5). P^B_m declined in the summer to nearly zero by 24 June (Julian day 175; 0.05 ± 0.06 mgC g DM^{-1} hr^{-1}). The *Cladophora* summer senescence coincided with peak and then with declining attached biomass (Fig. 3.5). The *Cladophora* fall regrowth exhibited mean P^B_m rates ranging between 1.3 and 1.9 mgC g DM^{-1} hr^{-1}. Unlike during the spring, this high photosynthetic rate was not matched with high biomass accumulation: In the autumn, *Cladophora* biomass was maintained at less than 30 g DM m^{-2}.

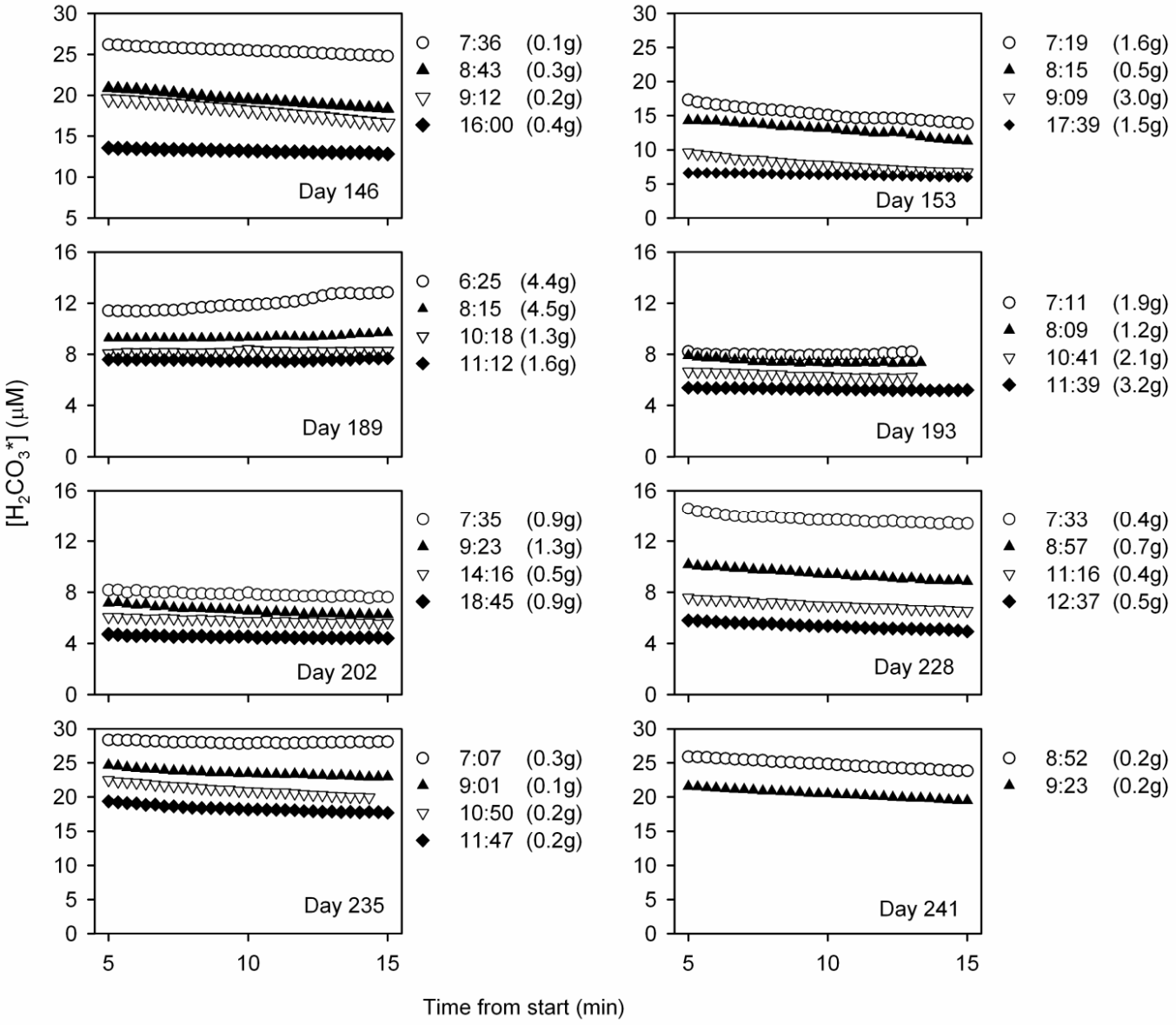

Figure 3.3 Examples of $H_2CO_3^*$ flux during incubations. Legends indicates the initial time of incubation and dry mass of *Cladophora* harvested from the chamber following incubation (in parentheses). Note the scale on the ordinate axis is different between panels.

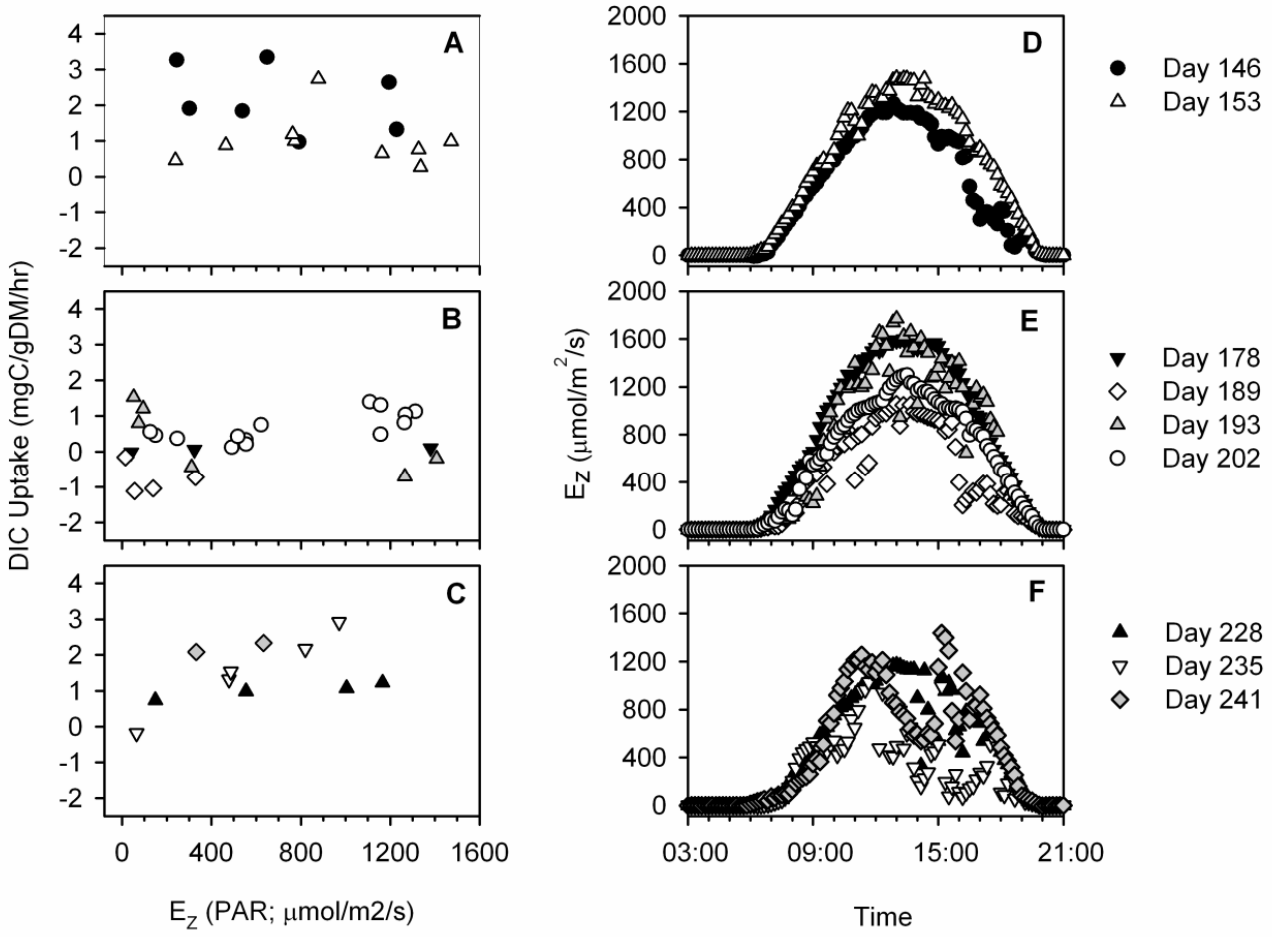

Figure 3.4 Net primary production (NPP) measured as dissolved inorganic carbon (DIC) removal from incubation chambers during 15 minute incubations versus PAR. For clarity, data are divided into panels based on season. The panels correspond to the periods of (A) spring growth, (B) summer senescence, and (C) fall regrowth. All measurements were taken at 1m depth. Panels D, E, and F correspond to irradiance at 1m depth.

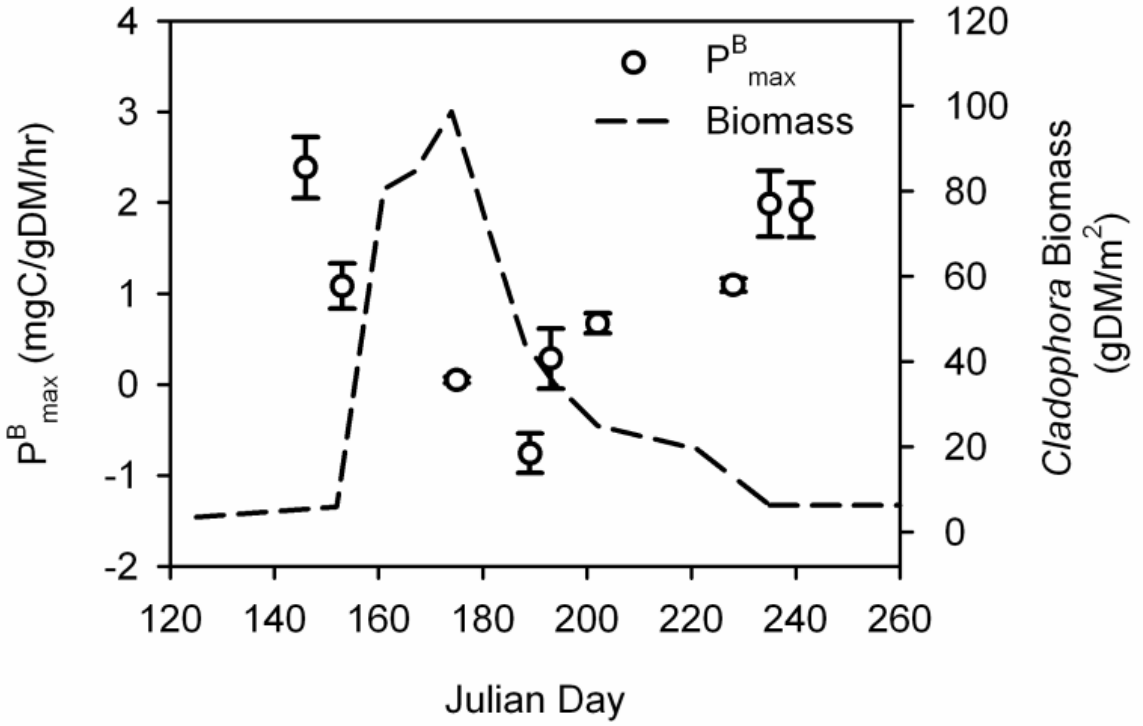

Figure 3.5 Seasonal *Cladophora* biomass-specific maximum realized photosynthetic rate (P^B_m) with standard error and harvested *Cladophora* biomass. All data from 1m depth, Oakville, Lake Ontario, 2005.

Measured versus modeled daily NPP estimates

NPP rates, calculated from P^B_m, estimates of nighttime respiration, and the photoperiod, are shown in Fig. 3.6. In the spring, measured NPP was as high as 28.1 mgC g DM^{-1} d^{-1} on 26 May (Julian day 146) and declined to negative rates by 24 June (Julian day 175; -8.8 mgC g DM^{-1} d^{-1}), reaching a minimum rate of –21.9 mgC g DM^{-1} d^{-1} on 8 July (Julian day 189). During fall regrowth, NPP was again high and positive reaching a peak of 14.2 mgC g DM^{-1} d^{-1} on 23 August (Julian day 235).

The *Cladophora* growth model (CGM) simulated well the seasonality of *Cladophora* NPP at 1 m depth (Fig. 3.6). The model predicted high rates of NPP in the spring, peaking on Julian Day 146 (26 May; 45 mgC g DM^{-1} d^{-1}; Fig. 3.6). Following this spring peak, NPP declined to negative rates reaching a minimum –15 mgC g DM^{-1} d^{-1} on Julian Day 202 (21 July). A period of prolonged negative diel NPP was predicted by the CGM from 25 June to 9 August (Julian days 176- 221). A fall regrowth was also simulated, achieving a period of prolonged high NPP (maximum 33 mgC g DM^{-1} d^{-1}) between Julian days 232 to 260 (20 August and 17 September).

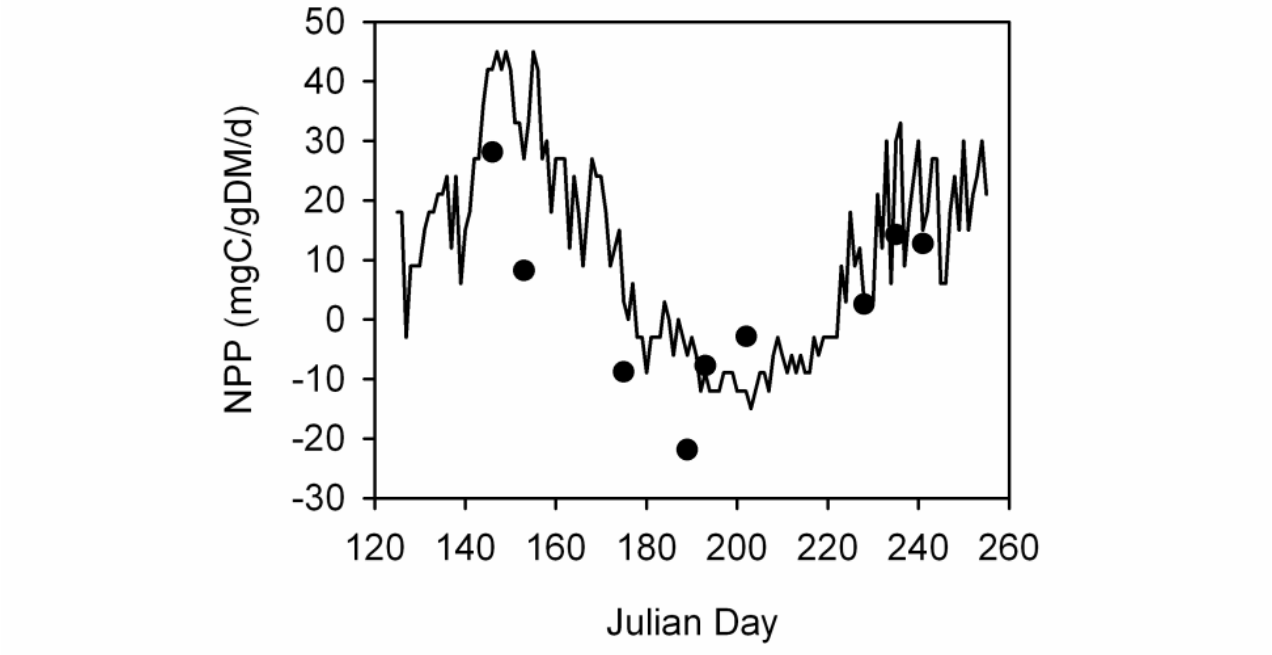

Figure 3.6 Measured (points) and *Cladophora* growth model (CGM) – predicted (line) rates of diel net primary production (NPP) at 1m depth.

Discussion

Periphytic biomass on rocky substrata in Lakes Erie, Michigan, and Ontario, is dominated by *Cladophora* from late spring into autumn (Higgins et al. 2005; Bootsma et al. 2005; Malkin et al. 2008). Here, I report the first *in situ* measurements of *Cladophora* primary production in Lake Ontario through the growing season. The *Cladophora* growth model (CGM; Malkin et al. 2008) adequately simulated the daily rates of net primary production (NPP) measured *in situ* in Lake Ontario (Fig 3.6). This independent validation of simulated rates of net primary production allowed me to use the CGM to simulate depth-integrated rates of primary production to compare with independent measurements of planktonic primary production. Using this approach, I estimated that *Cladophora* dominates the total carbon-fixation in the water column to beyond the 12 m depth contour. The comparison between benthic and planktonic primary production was facilitated by the C-based approach employed in this study, by avoiding the potentially large uncertainty associated with using a photosynthetic quotient for benthic primary production. Simulated and measured biomass-specific rates of NPP were also in reasonably good agreement during the second seasonal growth cohort. However, the model overpredicted accumulated *Cladophora* biomass during this time, identifying that additional loss processes are likely underestimated in the late summer and autumn.

Carbon-based method to measure benthic net primary production

Some assumptions inherent to this new methodology are worth noting explicitly. First, I assumed that carbonate alkalinity was equivalent to total alkalinity; and second, I assumed that alkalinity was constant during the incubations. The contribution of phosphates to alkalinity is negligible in the nearshore Lake Ontario during the growing season where summer SRP is currently around 0.05 μM (S. Malkin, unpubl. data). During the summer, nitrates plus nitrites (which add alkalinity) are below 10 μM (Chapter 5) and dissolved silicon (adds alkalinity) is less than 10 μM (D. Depew, University of Waterloo, unpubl. data). Ammonium concentration (removes alkalinity) was always less than 1.5 μM (Chapter 5). Of greater potential importance is the assumption of constant alkalinity which implies there is no $CaCO_3$ precipitation and that uptake and removal by *Cladophora* of non-carbonate molecules that contribute to alkalinity are negligible. During the summer, the epilimnion of Lake Ontario is typically supersaturated with

respect to dissolved Ca^{2+} (Hodell et al. 1998), although the concentration is decreasing, potentially due to Ca demand by dreissenid mussels in upstream Lake Erie (Barbiero et al. 2006). As CO_2 is removed biologically, the potential for $CaCO_3$ to precipitate increases. However, spontaneous precipitation of calcite does not occur until the degree of saturation exceeds the threshold for supersaturation (e.g. Stabel 1986). Davies et al. (2003) measured changes of alkalinity during incubations of the cultured phytoplankter *Chlamydymonas reinhardtii* in chemostat experiments and found that C removed as alkalinity accounted for less than 10% of the gross primary production. The severity of the consequences of assuming constant alkalinity is constrained by employing short incubation times. Future work should assess the change in alkalinity due to nutrient uptake (and possibly $CaCO_3$ precipitation).

A separate issue with this system is that only one incubation can be measured at a time (per IRGA). In this study, my goal was to measure NPP at depths from which I previously calibrated and applied a *Cladophora* growth model (Malkin et al. 2008). I had anticipated that by commencing measurements within 30 minutes following sunrise I would be able to describe rates of NPP as a function of light and calculate light response curve parameters. However, only on 2 dates, with high cloud cover was NPP measured at light limiting irradiance. This simplified our calculations of daily NPP and provided the further benefit of allowing us to compare variability in P^B_m rates between samples.

Rates of *Cladophora* primary production

Biomass-specific rates of *Cladophora* primary production reported here are generally lower than other published estimates for *Cladophora* spp. (Table 3.1). The minimum rates reported in this study are within the previously reported range of minimum NPP rates for attached *Cladophora,* while the maximum rates reported here are below all of the maximum NPP rates previously reported (Table 3.1). The higher rates reported in the literature may reflect that most previous measurements of *Cladophora* production were made on small fragments, rather than whole attached stands. Unless light attenuation through the macroalgal canopy is taken into account, this leads to overestimates of *in situ* rates of net primary production to a *Cladophora* stand. A systemic bias in population or community level estimates of benthic primary is widespread in studies of angiosperm macrophytes (Sand-Jensen et al. 2007). Even among thin, uniseriate macroalgae, self-shading imposes an important constraint on the net photosynthetic rates of whole macroalgal stands at high stand density (Chapter 4).

Table 3.1 Light saturated rates of biomass-specific *Cladophora* net primary production (P^B_m; $mgC \cdot gDM^{-1} \cdot hr^{-1}$). For measurements made as oxygen exchange, a photosynthetic quotient of 1.1 was used (Davies and Hecky 2005). Unless otherwise stated, all studies listed were conducted under artificial lights at saturating photon flux density, using nutrient replete artificial media that was not moving continuously. The precision reported here is taken from its original publication.

Methods	Range	Species	P^B_m	Source
DIC; *in situ* at 1m; closed-system circulating chambers; [$H_2CO_3^*$] monitored continuously	Diel: sunrise to 14:00, often 18:00 Seasonal: 9 dates from 25 May to 29 Aug	*C. glomerata*	−0.8 - 2.3	This study
O_2; ex situ, closed-system circulating chambers; Winkler measures; incubation duration not given; mass of *Cladophora* not given	Seasonal: 5 dates from 28 Jun to 9 Sept	*C. glomerata*	9.4 - 14.2	Lester et al. 1988
^{14}C; ex situ; 4 mgDM *Cladophora* in borosilicate vials with 6mL river water; 6hr incubation under midday sunlight using a range of light filters	Spatial: 2 rivers, on 22 Aug	*Cladophora* sp.	4.7 - 10.1	Dodds 1991
O_2; ex situ, 2 mgDM *Cladophora* in sample chamber with 4mL lake water; continuous monitoring with Clark-type electrode	Seasonal: 7 dates from 24 May to 7 Jul	*Cladophora* sp.	4.1 - 11.7	Mantai 1974
DIC; 100-200 mgDM in 125mL glass bottles suspended in lake at range of depths for 30-60 minutes commencing at 13:00; measured change in pH	Seasonal: 1 date in “autumn”, 1 date in “spring”	*C. glomerata*	3.5 - 4.5	McMillan and Verduin 1953

O_2; ex situ; 1-5mgWM in reaction chamber with 8mL media; monitored continuously with Clark-type electrode	Spatial: 2 sites	*C. albida*	2.7 - 12.3	Gordon et al. 1980
^{14}C; incubated in lake at approximately 10cm depth in glass bottles; 2hr incubation	Diurnal: 8:00 until at least 14:00, often 18:00 Seasonal: 4 dates from 8 Jun to 19 Aug Spatial: 3 sites at varying distances from nutrient-rich river mouth	*C. glomerata*	2 - 12	Adams and Stone 1973
^{14}C; "loose clumps" of *Cladophora* in 125 mL glass bottles incubated for 1 hr suspended in the lake; "occasional swirling"	Diurnal: 1 to 3 measures per day Seasonal: 9 dates from 17 May to 17 Oct	*C. fracta*	0.5 - 5.4	Cheney and Hough 1983
^{14}C; ex situ; 20-30mgDM incubated in 300mL BOD bottle for 5 hrs under midday sunlight	Spatial: 2 bays around Bermuda	*C. prolifera*	0.5 - 0.6	Bach and Josselyn 1979

Table 3.2 Areal rates of *Cladophora* productivity: a literature review.

Method	Species	Site	Areal NPP ($gC\ m^{-2}\ d^{-1}$)	Source
DIC removal	*C. glomerata*	Lake Ontario, 1m depth, spring and summer 2005	−0.9 - 0.1	This study
CGM; validated against seasonal change in biomass	*C. glomerata*	Lake Ontario, 1m depth, spring and summer 2005	−0.4 - 0.5	This study
Change in biomass; measured every 6-9 days in Plexiglas tubes; and quantitative biomass samples at one site; assumes top 1cm of mat is productive	*C. prolifera*	Tucker's Bay, Bermuda, 2.7-4.9m depth, summer 1977	2.7	Bach and Josselyn 1979
Change in biomass and numeric modeling	*C. montagneana*	Peel-Harvey Estuary, Western Australia, full year, 1966-1979	−0.2 - 1.5	Gordon and McComb 1989
O_2; *in situ* incubations over diel and diurnal periods; measured change in DIC and/or O_2	*Cladophora*-dominated rocky substrata	Lake Erie, 2.2m depth, summer 1997 and 1998	−0.1 - 0.4	Davies and Hecky 2005

Implications of *Cladophora* productivity

By avoiding many of the problems associated with previous methodologies, and by validating the CGM against primary productivity of *Cladophora*, I now have the means to compare the seasonal primary productivity of *Cladophora* with other published datasets on primary productivity of Lake Ontario. Millard et al. (1996) presents depth-resolved estimates of ^{14}C-derived planktonic primary production at two offshore stations on Lake Ontario over the period 1987-1992. *In situ* incubations were 3-5 hrs, so I assumed these values were close to

GPP. Using data from the station with higher primary production (Station 81), to err on the side of greater contribution of planktonic production, I compared the planktonic GPP estimates (Fig. 10 in Millard et al. 1996) with our estimates of *Cladophora* daytime gross primary production (Fig. 3.7). Depth-resolved data in Millard et al. (1996) is presented as a cumulative from May to October. I estimated the cumulative C-fixation of phytoplankton from May-July – the main season of *Cladophora* accumulation – by assuming constant monthly planktonic production rates. Because phytoplankton production rates tend to increase through the spring and plateau in the autumn (Millard et al. 1996), this assumption also errs on the side of overestimating planktonic production. I used the CGM and estimated daytime respiration using a temperature relationship for *Cladophora glomerata* (Graham et al. 1982) to estimate daily C-specific GPP. I used the light attenuation estimates and the relationship between tissue P and depth from Malkin et al. (2008). Our results indicate that C flux is dominated by *Cladophora* at depths less than the 6 m depth contour (Fig. 3.7). The maximal peak of benthic gross primary production is close to 3 m depth because there is an estimated greater loss of biomass from the shallowest depths by wave-induced shear stress. By integrating the area under each curve and multiplying the GPP calculated at each depth contour by the relative proportional area of each depth contour in the Oakville area (Virden et al. 2000), I estimated that cumulative C-fixed from May to July from the shoreline down to the 12m depth contour was 241 kgC m^{-1} shoreline. I calculated that 70% of this total was contributed by benthic (i.e., *Cladophora* production) and the remaining 30% was contributed by planktonic primary production. Using the same data, *Cladophora* contributes to approximately half the GPP in the nearshore (defined as depth < 20m) of Oakville area, Lake Ontario. Similarly, in Lake Erie, Davies and Hecky (2005) estimated that benthic production of *Cladophora*-dominated rocky substrata dominated areal water column C flux at depths less than 5m. My estimates relate to nearshore areas with rocky substrates, such as the Oakville area (Rukavina 1976). The comparison presented here should be viewed as preliminary because the planktonic dataset was not ideal in terms of distance from shore and year of study. Nonetheless, this comparison identifies for the first time that benthic macroalgae on hard substrata is a seasonally substantive component of the nearshore carbon budget in a lower Laurentian Great Lake.

A potential consequence of the establishment of invasive dreissenid mussels in the lower Great Lakes is an increase in the energy flux through the benthos (Hecky et al. 2004, Zhu et al.

2006). This is hypothesized based on an increase in water clarity and potentially an increase in nutrient recycling in the benthos. A corollary of increased benthic primary production due to dreissenid infestation, is the potential effect on secondary production, including pelagic fish production. Benthic foodwebs may be more efficient in transferring energy to higher trophic levels (Azim et al. 2005; Vander Zanden et al. 2006). But, alternatively, macroalgal blooms are generally considered noxious and grazer-resistant. The only animal consumer, so far, demonstrated to feed directly on attached *Cladophora* in the Great Lakes is the native amphipod *Gammarus fasciatus* (Johnson 2004). *Cladophora* in the lower Great Lakes generally demonstrates two periods of growth, commencing in late spring and again in late summer, as quantified in this study. While the first cohort of *Cladophora* growth in early summer achieved high biomass in Lake Ontario (e.g. 120 g m^{-2} in 2005), the second cohort again demonstrated high productivity (both measured and simulated), but did not accrue beyond densities of 30 g m^{-2}. I hypothesize that the second cohort, unlike the first, is controlled by benthic invertebrate grazing. The abundance of *Gammarus fasciatus* typically increases in the late summer (Bousfield 1958; Johnson 2004), which is consistent with grazer control of the second cohort of *Cladophora*. Preliminary estimates of the amphipods' grazing on *Cladophora* from field collected *Gammarus* and cultured *Cladophora* (i.e. free of epiphytic diatoms) in nutrient rich media in 6 replicates over 4 days were 0.15 ± 0.06 mg *Cladophora* DM amphipod^{-1} d^{-1} (S. Malkin, unpubl data). At this rate, approximately 2000 amphipods m^{-2} would be needed to control the production of the second cohort of *Cladophora*. In the eastern basin of Lake Erie, *Gammarus fasciatus* abundance at 2 m depth in 2002 averaged 3040 m^{-2} from June to October. Further work on the pathways for energy and nutrient transfer in the benthos need to be examined to assess the role benthic macroalgae play in the nearshore foodweb.

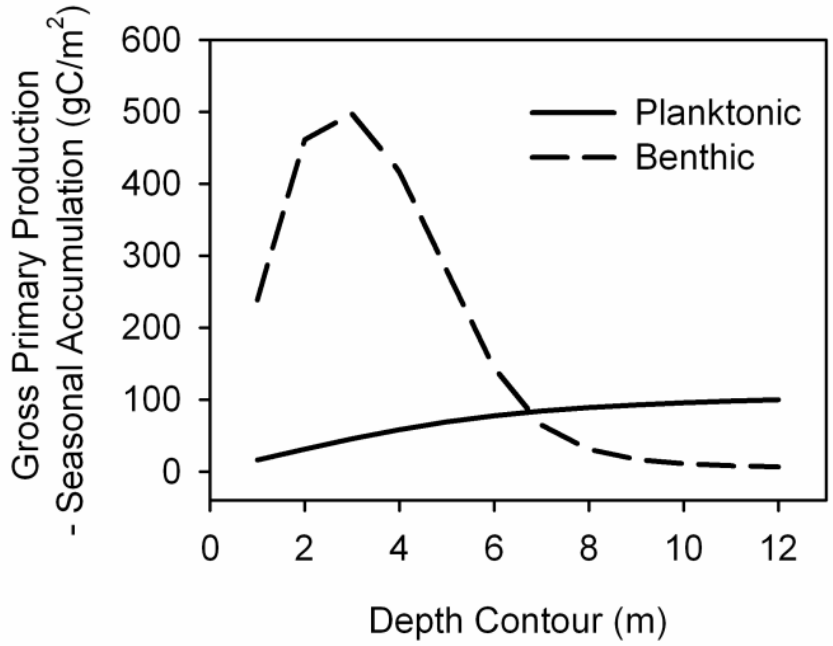

Figure 3.7 Areal rates of cumulative seasonal gross primary production (GPP); a comparison of benthic (i.e. *Cladophora*) and planktonic rates. Benthic estimates, using the *Cladophora* growth Model (CGM) simulations of daily GPP in 2005, are cumulative for the first cohort of growth, May-July. Planktonic estimates are from Millard et al. (1996; Stn 81), cumulative from May-July, assuming constant monthly production from May to October.

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Chapter 4 Seasonal adjustments in the photosynthetic rates of *Cladophora glomerata* in response to nutrient and light availability

Introduction

The rates and controls of benthic algal metabolism have responded to a unique set of selective pressures through their evolutionary history, distinct from those pressures on phytoplankton. For example, phytoplankton fitness is, in part, a function of their ability to maintain suspension in the euphotic zone and their ability to acclimate to large and rapid changes in irradiance due to mixing. Attached benthic algae, in contrast, are adapted to the restricted fluxes of nutrients and dissolved gases imposed by a benthic boundary layer and to sustain productivity despite increasing self-shading with its growth. Constraints on photosynthetic rates are generally assumed to be the same for benthic and for planktonic primary producers, but this assumption bears little quantitative evaluation (Loeb et al. 1983, Turner et al. 1994).

Cladophora grows ephemerally in some habitats (e.g. Smith et al. 2005), but in the lower Laurentian Great Lakes its growth occurs every spring (Bellis and McLarty 1967). However, its biomass accumulation has varied greatly in response to decadal-scale environmental changes (Chapter 2). A contemporary description of its phenology is important as a baseline for future comparisons. Based on data collected since 1980, mean summer surface water is becoming warmer, and spring warming is occurring earlier (Chapter 2). Nutrient loading and nearshore nutrient cycling are also changing in response to alterations in watershed management and nearshore ecological processes. Since the widespread establishment of dreissenid mussels in the late 1980s-early 1990s, nutrient flux within and between limnetic zones has been altered (Hecky et al. 2004), potentially affecting the coupling between pelagic and benthic nutrient cycles. An assessment of how the seasonality of *Cladophora* growth compares with other environmental events, such as the timing of spring runoff or the life history of macroalgal grazers, and how the timing of *Cladophora* growth and senescence will be affected by ongoing changes to the ecosystem, is warranted. Hecky et al. (2004) hypothesized that the dreissenid mussel invasion would augment benthic algal and particularly *Cladophora* production in the lower Great Lakes by supplying phosphorus and

carbon dioxide to the benthos. Testing these hypotheses demands an assessment of the *in situ* constraints on *Cladophora* photosynthesis *in situ*. An assessment of the seasonal constraints of *Cladophora* production is necessary to predict how environmental changes could be affecting interactions between phytoplankton and benthic primary producers. The first objective of this study is to describe the seasonality of *Cladophora* internal stoichiometry, in conjunction with biomass and measured rates of primary productivity. The second objective is to examine the *in situ* constraints on photosynthetic *Cladophora* rates in Lake Ontario.

Methods

Cladophora collection and preservation

This study was conducted on the north shore of western Lake Ontario in Oakville, Ontario, Canada (43.44^{o}N, 79.66^{o}W). The site is described in greater detail elsewhere (Chapters 2, 3). Qualitative and semi-quantitative observations of *Cladophora* characteristics, such as colour, texture, relative epiphyte load were recorded from the beginning of its growing season, at the end of May, until October. This study was conducted in 2005, a year of typical meteorology for this region.

Cladophora filaments were harvested from their basal holdfasts from rocks at 1-2 m depth by a snorkeler and kept in a cooler until being transferred to a refrigerator within 6 hours of collection. *Cladophora* samples were cleaned and prepared for nutrient analyses within 24 hours. Filaments were cleaned in a sieve (pore size approximately 1 mm) using deionized water and were picked free of invertebrates and large detritus with forceps. All samples were observed under a dissecting microscope at 4-25X to confirm it was *Cladophora*. Aliquots to be analysed for tissue phosphorus (Q_P) were dried in an oven at 60^{o}C for at least 24 hours. Aliquots destined for tissue carbon (Q_C) and nitrogen (Q_N) or chlorophyll analyses were temporarily frozen until being dried in a lyophilizer.

Additional triplicate *Cladophora* samples, harvested from their holdfasts, were sampled and cleaned as previously described but then cut into segments from the basal cells (but with holdfasts removed) at 2 cm, 5cm, 10 cm, and every 10 cm after that. These segments were homogenized individually and prepared and analysed for Q_P and chlorophyll concentrations.

Nutrient analyses

For Q_P analysis, dried *Cladophora* was combusted at 450°C for 1 hour. Tissue P was oxidized to orthophosphate (PO_4^-) by autoclaving the samples for 30 minutes in distilled water with 4% potassium persulphate solution added to a final concentration of 0.16%. Solubilised PO_4^- was then measured spectrophotometrically using the molybdate blue method (APHA 1998).

For Q_C and Q_N analyses, *Cladophora* samples were ground to a powder using a Retsch MM 2000 ball mill grinder (F. Kurt Retsch GmbH & Co., Haan, Germany). Approximately 1 mg samples of dried, pulverized *Cladophora* tissue were packed into nickel sleeves and weighed to an accuracy of 0.01 mg. The concentrations of C and N were measured in an elemental analyzer (Exeter Analytical Inc. CEC-440; combustion 980°C, reduction 700°C).

Cladophora chlorophyll *a* and *b* concentrations were measured following the trichromatic methods of the American Public Health Association (1998), employing a Cary 100 Bio spectrophotometer and a 10 cm pathlength cuvette. Preweighed samples of 3-5 mg DM were ground for a maximum of 90 seconds in cold 90% acetone using a motorized tissue grinder with a ground glass pestle and mortar. Ground samples were immediately transferred to a freezer (-18°C) for 18-20 hours. Following extraction, samples were gently filtered (8-µm porosity paper filter; < 10 mm Hg) to remove turbidity. Absorbance was read at 664, 647, and 630 nm from which Chl*a* and Chl*b* were calculated. A reading at 750nm was also made and subtracted from readings at all other wavelengths, as a correction for remaining turbidity.

Nutrient limitation was assessed by comparing mean stoichiometric ratios of C:N:P of *Cladophora* on each date with benthic algal nutrient limitation thresholds defined by Kahlert (1998). Phosphorus limitation was defined by molar C:P > 369 and N:P > 32. Nitrogen limitation was defined by molar C:N > 11.5.

Light measurements

Irradiance was calculated from measurements of surface irradiance, depth of sampling, and measured daily light attenuation. Surface irradiance was calculated from measurements of shortwave radiation dose ($W \cdot m^{-2} \cdot 10^{-1}$minutes) collected with a pyroheliometer on the roof of Canada Centre for Inland Waters (Burlington, ON). The calculation of PAR fluence rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$) was described previously (Chapter 2). Light attenuation (Kd_{PAR}) was calculated

from PAR profiles at 2.5 m depth using a spherical quantum sensor (Li-Cor, Lincoln, Nebraska). PAR measurements that were collected with a 2.5 mm flat plate collector, an accessory to the PAM fluorometer (described below), were not used due to problems with its calibration and persistent low bias.

Irradiance through the *Cladophora* canopy was estimated by affixing a pair of cosine-corrected flat plate PAR logging sensors (Odyssey Light Loggers, Christchurch, New Zealand) above and below a 10 cm canopy of attached *Cladophora*. Irradiance was logged every 5 minutes for 3 or 5 days on separate deployments. Light attenuation was calculated through the canopy based on the natural logarithm-transformed difference between the sensor readings.

Benthic primary productivity

Cladophora net primary production was measured *in situ* as carbon flux in benthic incubation chambers, as described in Chapter 3. Incubations were 15-20 minutes in duration, and repeated through the day from within 30 minutes of sunrise. *Cladophora* from each incubation was harvested, and production was normalized to biomass. From these data a daily biomass-specific rate of maximum photosynthesis (P^B_m) was calculated.

Quantum yield of fluorescence – rationale and methodology

A small, but variable, proportion of light energy absorbed by the light harvesting complex of photosystem II is re-emitted as fluorescence (approximate range: 0.6 - 3 %; Krause and Weis 1991). The proportion depends on how much energy is quenched by the primary electron acceptor, Q_A, of the photosynthetic electron transport chain (i.e., photochemical quenching), and how much is quenched by various processes that result in nonradiative energy dissipation (collectively termed nonphotochemical quenching). Minimum fluorescence (F_o) is observed when all PSII reaction centers are open (oxidized), that is, following dark acclimation and in the presence of only a weak (i.e., not actinic) measuring light. Maximum fluorescence (F_m) is observed when all PSII reaction centers are closed (reduced), which can be measured when providing a pulse of light of sufficient intensity and duration to reduce all primary electron acceptors and the plastoquinone pool, typically following a measurement of F_o. The maximum variable fluorescence, F_v, is the difference between F_m and F_o and, when normalized

to maximum fluorescence, indicates the potential quantum yield of fluorescence originating from PSII. When F_v/F_m is measured around dawn, assuming no sustained photodamage, it represents the maximum potential quantum efficiency of PSII.

Decreases in the maximum F_v/F_m indicate increases in nonphotochemical quenching. There are several processes which contribute to nonphotochemical quenching, and these are partially distinguishable based on relaxation kinetics (Krause and Weis 1991). Quenching that relaxes rapidly, on the order of minutes, is due to thylakoid energization (qE), the process whereby a proton gradient is built up across the thylakoid membrane, and by state transitions (qT), a phosphorylation-initiated process whereby antenna pigments associated with PSII migrate to PSI. These processes, qE and qT, are considered photoprotective. Nonphotochemical quenching that relaxes slowly, typically on the order of hours, is considered part of photoinhibition (qI) and includes quenching by photoprotective processes, such as xanthophyll pigment cycling (Demmig-Adams and Adams 1996) and quenching due to photodamage arising from photooxidative stress or direct damage to proteins or nucleic acids.

In the presence of continuous actinic irradiance (i.e. at steady-state), the effective quantum yield of fluorescence is a measure of the actual, or effective, photosynthetic electron transport efficiency. This value is calculated as $(F_m'-F)/F_m'$, where F is the steady state fluorescence and F_m' is the maximum fluorescence at a given irradiance. The effective quantum yield of fluorescence is commonly written as $\Delta F/F_m'$ (or sometimes, Φ_{PSII}). This parameter is related to photosynthetic electron transport rate (ETR), which may be linearly related to the quantum yield of photosynthesis under nonphotoinhibiting conditions (Genty et al. 1989), although this is rarely the case among aquatic autotrophs (Franklin and Badger 2001). The ETR is a product of $\Delta F/Fm'$ and the flux of light energy absorbed by the light harvesting complex associated with photosystem II (LHCII). Because of difficulties in quantifying the amount of energy absorbed by *Cladophora* (and the assumptions about what fraction of this light is absorbed specifically by LHCII) a relative ETR (rETR) can be calculated instead, as the product of incident irradiance and $\Delta F/F_m'$.

The quantum yield of fluorescence was measured by the pulse-saturation technique using a portable pulse amplitude modulated fluorometer (Diving PAM; Walz; Effeltrich, Germany) equipped with a peripheral accessory (Universal Sample Holder, Walz) to secure *Cladophora* filaments in place, either in the dark or in incident solar actinic light.

Fluorescence was measured with a weak red modulated light (~0.15 μmol m^{-2} s^{-1}) and PSII reaction centers were transiently closed with a 800 ms saturating pulse (~9000 μmol m^{-2} s^{-1}) of white light provided with an internal halogen light source. Measurements of quantum yield of fluorescence of *Cladophora* at 1m were made in ambient actinic light during the day ($\Delta F/F_m'$) and following 30 minutes dark acclimation (F_v/F_m). Dark acclimation was achieved by placing a *Cladophora* specimen with about 70 mL of lake water in a sealed 100 mL HDPE cup in an insulated cooler. Samples that were dark acclimated for several hours under these conditions had lower F_v/F_m values than those dark acclimated for 30 minutes, and so were not used in analysis.

Measurements of quantum yield commenced at sunrise. The effect of background fluorescence in lake water was removed using the "autozero" function at the start of each day. At approximately each hour following sunrise, *in situ* measurements of *Cladophora* ΔF/Fm' were made by a snorkeler, with *Cladophora* filaments oriented at a 45^o angle relative to the measuring light and carefully exposed to the incident solar irradiance. Only measurements in which F was greater than 100 (FU) were kept due to low accuracy at low fluorescence values. Measurements were taken until there were four replicates with F above this threshold. Measurements were taken at the top third of the apical end. Four replicate samples were subsequently harvested and dark acclimated for measurements of F_v/F_m. The PAR irradiance at the time of harvesting was noted for the F_v/F_m measurements.

Statistical analyses

All the seasonal values of F_v/F_m and ΔF/Fm' were plotted together against irradiance. For F_v/F_m the irradiance was that at the time of harvesting. An ordinary least squares linear regression was computed for the measures of quantum efficiency as a function of irradiance at the time of harvesting. Residuals of this linear model was assessed for correlations with ambient water temperature, *Cladophora* Q_P, and height and biomass of the *Cladophora* canopy (as surrogates for self-shading).

The maximum F_v/F_m was defined as the intercept of the linear regressions of F_v/F_m against irradiance. The degree of photoinhibition (qI) was calculated as the proportion of F_v/F_m out of maximum F_v/F_m and was plotted against irradiance at the time of sample harvesting.

Relative ETR was plotted against irradiance and fit with an exponential curve: rETR = rETRm · $(1 - e^{-\alpha \cdot E/ETRm})$, where E is the irradiance incident to the macroalgal thallus, rETRm is the maximum rETR (relative units) and α is the initial slope of the curve (Jassby and Platt 1976). The light saturation parameter for fluorescence, Ek, was calculated as rETRm/α.

Results

Seasonal observations

In western Lake Ontario, at depths scoured by ice and thus where *Cladophora* holdfasts do not overwinter, *Ulothrix zonata* was observed to be growing luxuriantly during the first spring sampling in early May, most densely on the sides and in fissures of boulders. *Cladophora* was first visible growing within beds of *Ulothrix zonata* in late May. *Cladophora* filaments continued to elongate and new filaments filled in on the tops of, and between, boulders and displaced *Ulothrix* entirely by late May, at which time, *Cladophora* coverage at 1-2 m depth was up to 100% on hard substrates. Following peak biomass in June, *Cladophora* filaments, which were originally bright green, became a dull yellow, and their texture became more fragile. A second cohort of growth began as the first cohort was sloughing. New *Cladophora* filaments began growth in patches of senescing filaments. New filaments were visible by their bright green colour, and at 25X magnification under a dissecting microscope, new apical cells were readily observed.

Macronutrient stoichiometry

The earliest collected samples of *Cladophora* had the highest cellular P and N quota (Q_P and Q_N, respectively) and the highest rates of biomass-specific photosynthesis (P^B_m; Fig 4.1). As *Cladophora* biomass accrued, mean Q_P, Q_N, P^B_m, and chlorophyll *a* content of *Cladophora* (Chl*a*) declined (Fig 4.1). Importantly, while Q_P was maintained near minimal concentrations (~0.05% DM), P^B_m increased in concert with a decrease in *Cladophora* biomass (Fig 4.1). The second *Cladophora* cohort began with high Q_P and P^B_m, which were maintained during the remainder of the sampling period. The second cohort of biomass never accumulated to densities as high as the first.

Based on the nutrient ratios C:P and N:P, attached *Cladophora* was characterised as P limited through the entire growing season. C:P ratios ranged from 630-1557 and N:P ratios ranged 46-130, always well above the thresholds defining P limitation (C:P ≥ 369 and N:P ≥ 32; Fig 4.2). *Cladophora* was secondarily N limited during the period following peak biomass, based on C:N ratios which ranged 5.5 – 18 (threshold for N limitation; C:N ≥ 11.5; Fig 4.2). The C:Chla ratio remained at low, but variable, through the spring (range: 250-600), and became much higher (> 1000) at the end of the first seasonal cohort, following decline of the *Cladophora* biomass, and visible as yellowing tissue (described above).

Light attenuation and distribution of chlorophyll and Q_P in the *Cladophora* canopy

Mean light attenuation measured through the *Cladophora* canopy ($Kd_{PAR\text{-}canopy}$) was 24.1 ± 3.3 (SD; m^{-1}; Fig 4.3). High frequency variation in light attenuation is illustrated in Fig 4.3A. Because light attenuation is exponential, cells deeper in the *Cladophora* canopy are predicted to receive a smaller range in diurnal irradiance (Fig 4.3B). At an irradiance of 1000 µmol m^{-2} s^{-1} on the surface of the *Cladophora* canopy, the irradiance at 1 cm, 10 cm, and 20 cm depths in the canopy would be 789, 93, and 9 µmol m^{-2} s^{-1}, respectively.

Cladophora segments through the canopy analysed for Q_P revealed no significant differences in concentration between segments (one-way ANOVA; $F_{3, 93} = 2.330$; $P = 0.079$). *Cladophora* Chl*a* and Chl*b* concentrations, in contrast, were significantly different through the canopy (one-way ANOVA; Chl*a*: $F_{3, 112} = 10.657$; $P < 0.0005$; Chl*b*: $F_{3,112} = 11.864$; $P < 0.0005$). The basal segments (0-2 cm from holdfast) had the highest Chl*a* and Chl*b* concentration, and segments more distal had increasingly lower concentration (Fig. 4.4). For Chl*a*, a post-hoc Tukey test indicated that the 0-2 cm segments had uniquely high concentrations, and the 2-5 cm segments were also different from the 10-20 cm segments. For Chl*b*, the 0-2 cm segments had uniquely high concentrations, while the other segments were not significantly different from each other.

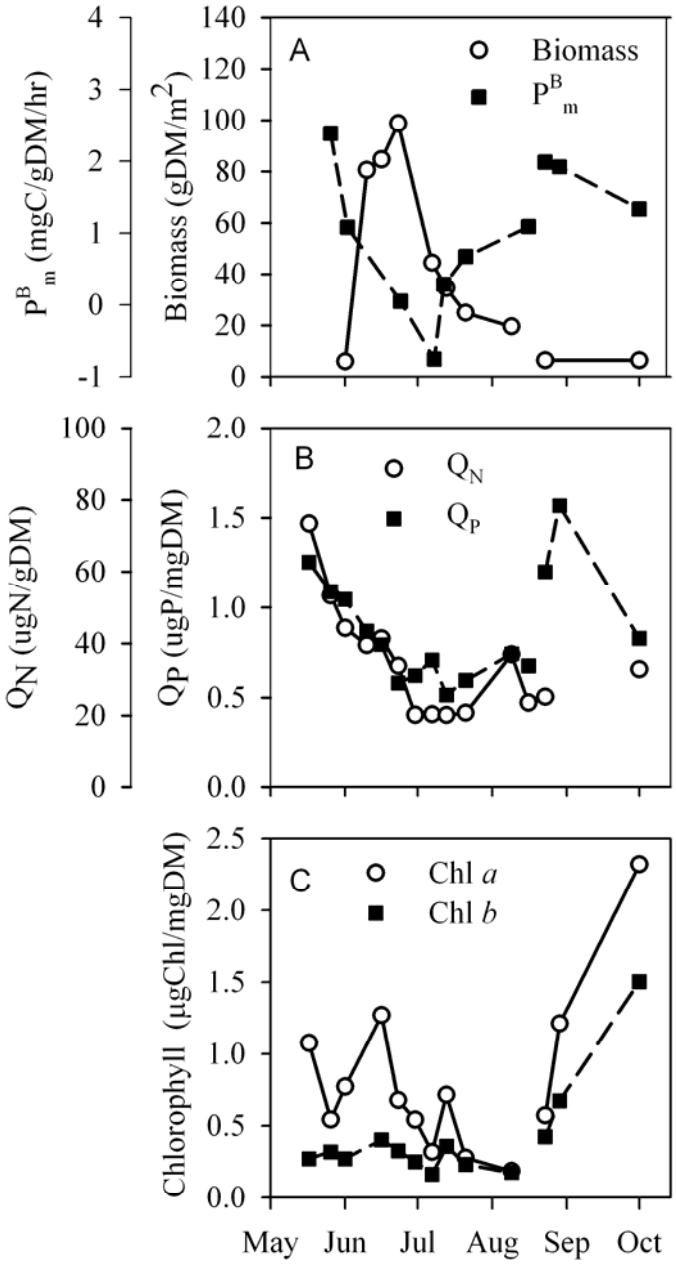

Figure 4.1 Seasonal variation in *Cladophora* light saturated photosynthetic rate normalized to biomass (P^B_m; mg C gDM^{-1} hr^{-1}) measured *in situ* at 1m, *Cladophora* biomass at 2m, and *Cladophora* nutrient quotas (Q_P and Q_N; µg $mgDM^{-1}$) and chlorophyll concentrations at 1-2 m depth. Connected points within a series represent samples taken from the same cohort.

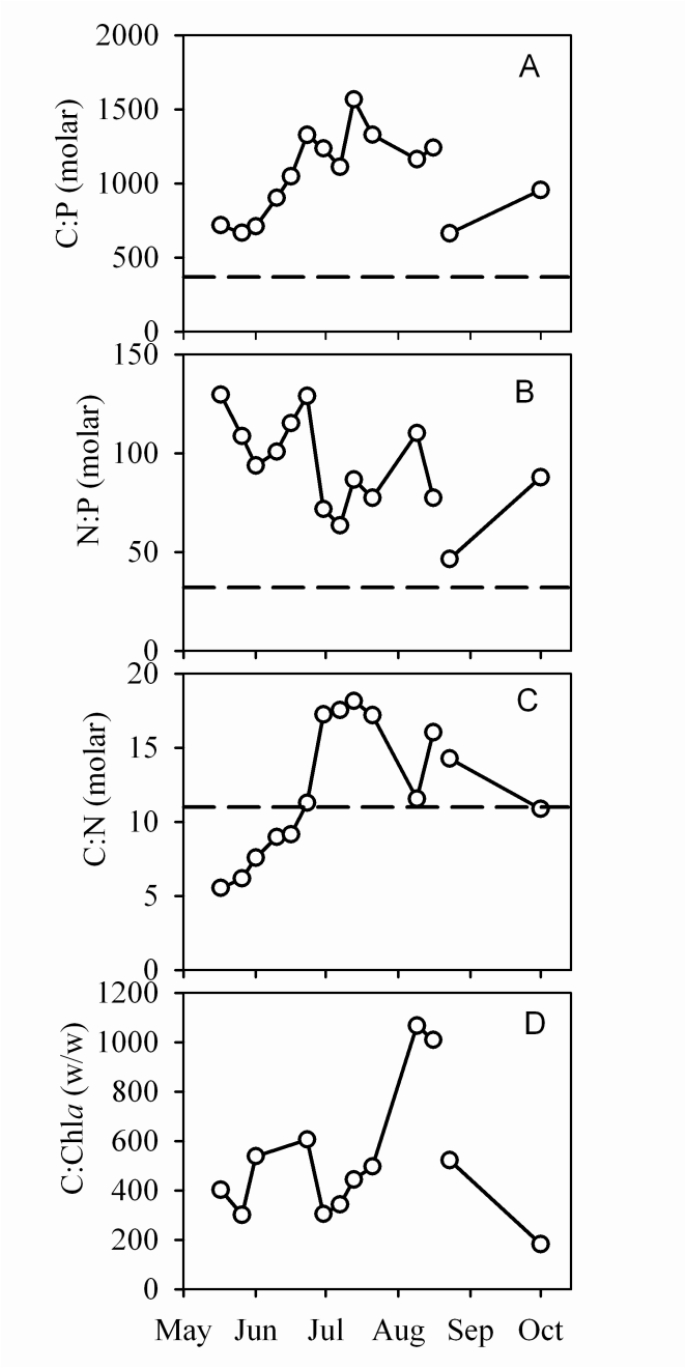

Figure 4.2 *Cladophora* tissue nutrient ratios at 1-2 m depth. For every series, the data are divided into first and second cohort of growth. Threshold ratios indicating P or N limitation are indicated as a dashed line in each panel. In panels A and B, data above the line indicates P limitation, while in panel C, data above the line indicates N limitation.

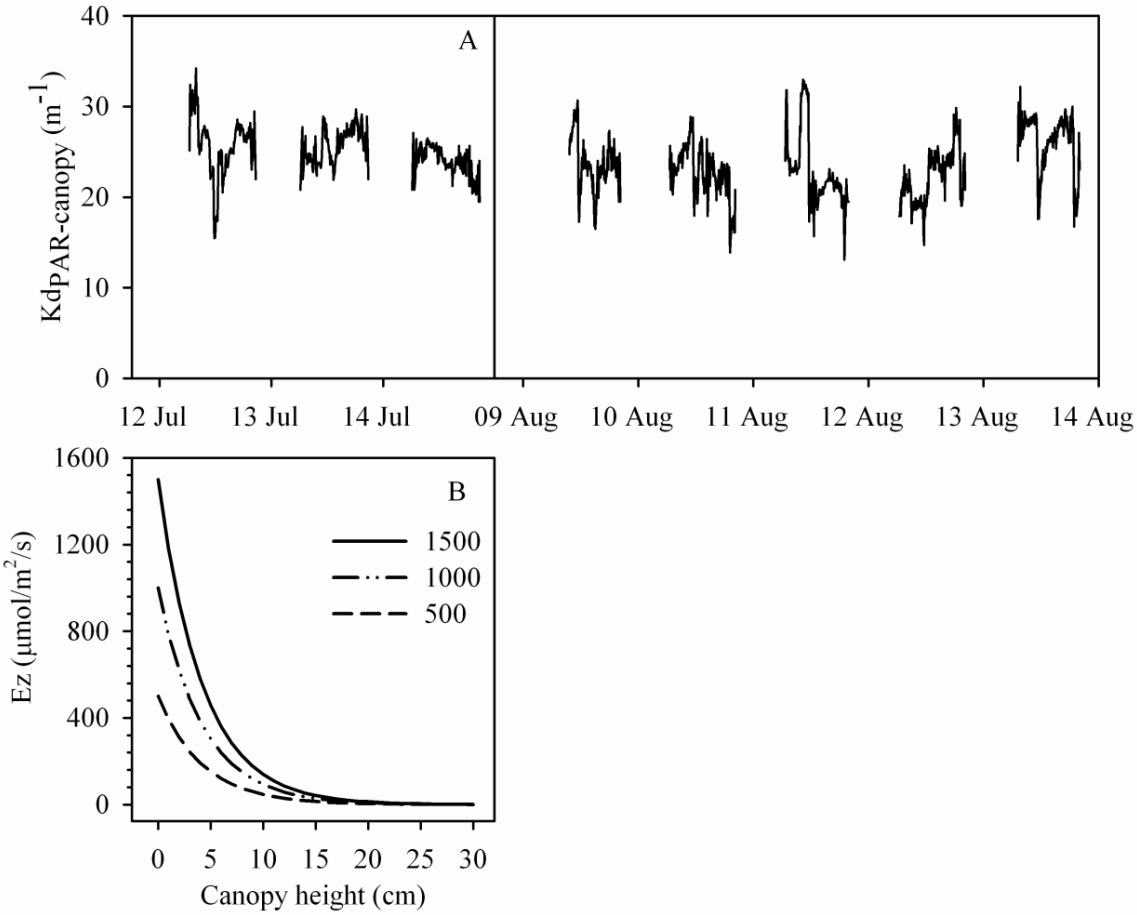

Figure 4.3 Light extinction coefficients measured *in situ* through a *Cladophora* canopy. A: The two boxes are data from 2 separate deployments of a pair of PAR sensors. Mean $Kd_{PAR\text{-}canopy}$ was 24.1 ± 3.3 (SD; m^{-1}). B: shows light in the canopy given the mean Kd_{PAR} and an irradiance of 1500, 1000, and 500 µmol m^{-2} s^{-1} striking the surface of the canopy.

Table 4.1 One-way ANOVA tests to assess differences in mean Chl*a*, Chl*b* and Q_P between sections harvested from increasing depths from basal holdfast.

Variable	Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Chl *a* (µg $mgDM^{-1}$)	Between Groups	4.961	3	1.654	10.657	.000
	Within Groups	17.378	112	.155		
	Total	22.339	115			
Chl *b* (µg $mgDM^{-1}$)	Between Groups	2.435	3	.812	11.864	.000
	Within Groups	7.661	112	.068		
	Total	10.096	115			
Q_P (µg $mgDM^{-1}$)	Between Groups	.399	3	.133	2.330	.079
	Within Groups	5.309	93	.057		
	Total	5.708	96			

Table 4.2. Mean concentration of chlorophyll *a* and chlorophyll *b* of sectioned *Cladophora*, which were found to be different in a one-way ANOVA. Results from post-hoc Tukey tests of differences between sections of *Cladophora* indicate which subsets of sections were different from one another.

Variable	Section (from base in cm)	Mean (µg $mgDM^{-1}$)	Standard Error	Homogeneous subsets
Chl *a*	0-2	.92	.10	a
	2-5	.65	.07	b
	5-10	.50	.06	bc
	10-20	.37	.04	c
Chl *b*	0-2	.60	.07	a
	2-5	.38	.04	b
	5-10	.30	.04	b
	10-20	.21	.03	b

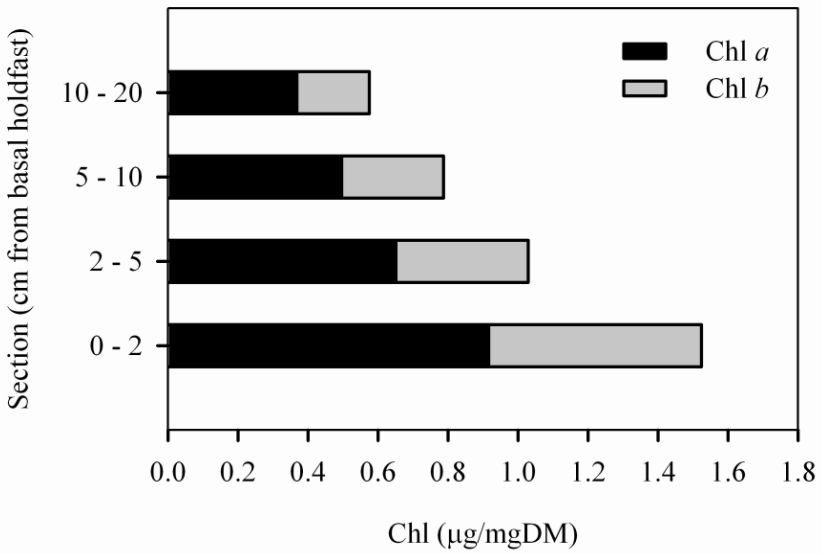

Figure 4.4 Mean chlorophyll concentration through the canopy. Data pooled from 5 dates.

In situ fluorescence response to irradiance by *Cladophora*

There was no detectable trend in maximum F_v/F_m through the season. The effective quantum yield of fluorescence in actinic light ($\Delta F/F_m'$) and the maximum quantum yield of fluorescence following 30 minutes dark acclimation (F_v/F_m) were linearly related to irradiance at the time of measurement, and the irradiance at the time of harvesting, respectively (Fig 4.5A). From ordinary least square regression, maximum F_v/F_m was 0.687 and slope was -5.37×10^{-5} and $r^2 = 0.435$. The residuals about the regression were not related to temperature, *Cladophora* Q_P, canopy height, or biomass.

The relative electron transport rate (rETR) calculated from all *in situ* measurements of quantum yield through each day and through the season when plotted against irradiance, was well described by the exponential curve of Jassby and Platt (1976), $rETR_m = 859 \cdot (1-e^{-0.641 \cdot E/859)})$; $r^2 = 0.811$. From this curve, Ek, the light saturation parameter, was calculated to be 1340 μmol m^{-2} s^{-1}.

Photoinhibition quenching (qI) of fluorescence yield (daytime F_v/F_m / maximum F_v/F_m) was plotted as a function of irradiance at harvesting (Fig 4.5C). For context, a range of hypothetical diurnal irradiances calculated as the cloud-free irradiance on the first day of summer, Jun 21, at the seasonal average $Kd_{PAR} \pm 1$ SD (0.454 ± 0.182 m^{-1}) at 1 m depth are also illustrated (Fig 4.5C). As an example, on a cloud-free day on the summer solstice at the mean water column light attenuation (Kd = 0.454 m^{-1}), the irradiance at 1m depth is expected to be 1160 μmol m^{-2} s^{-1}. At this irradiance, qI is equal to 9.1% of the light energy absorbed.

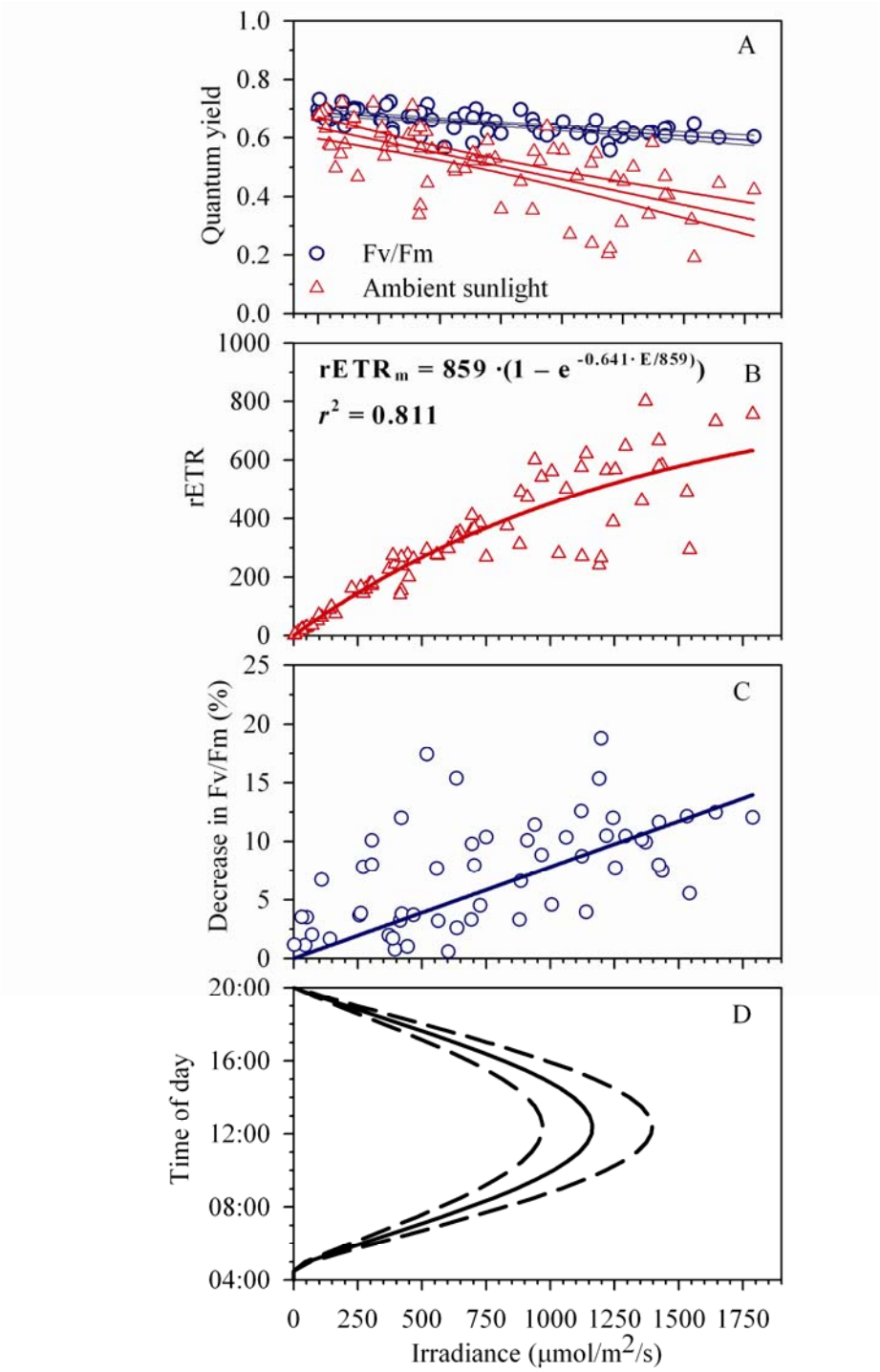

Figure 4.5 Quantum yield of fluorescence of all samples collected through the growing season in 2005. A: Irradiance at top of *Cladophora* canopy (at 1 m depth) vs. quantum yield of fluorescence in full irradiance ($\Delta F/F_m'$; red triangles), and following 30 minutes of dark acclimation (F_v/F_m; blue circles). B: rETR vs. irradiance, fit with an exponential curve. C: Decrease of F_v/F_m measured during the day as a percentage of the maximum F_v/F_m plotted as a function of irradiance at time of harvesting. D: Irradiance at 1 m depth on a cloud free day on the summer solstice (21 June, 2005), using the seasonal mean light attenuation coefficient (0.454 m^{-1}; solid line) ± 1 SD (0.182 m^{-1}).

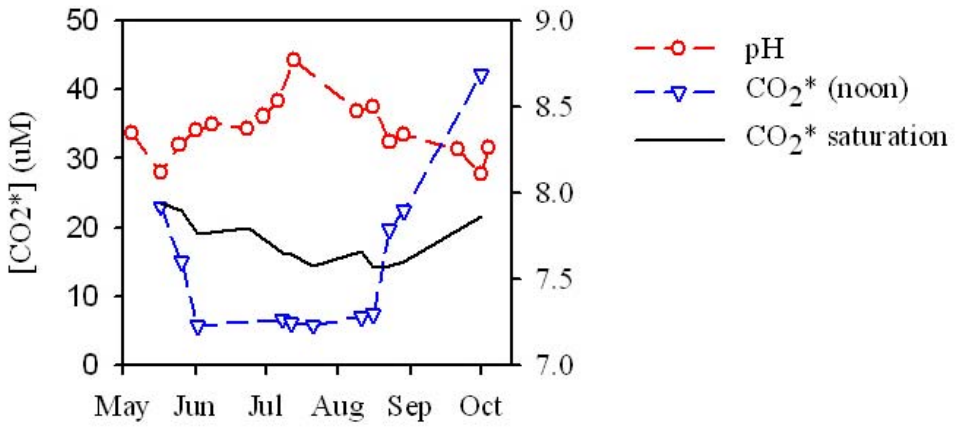

Figure 4.6 Seasonal changes in pH and dissolved CO2 concentration in 2005 measured at 1-2.5 m depth.

Discussion

This study describes the seasonal growth of *Cladophora glomerata* in the western end of Lake Ontario. Previous studies of *Cladophora* life history in the Great Lakes identified that initial growth occurs when the lake warms to about 10 ^{o}C and documented two seasonal growth cohorts (Neil and Owen 1964, Bellis and McLarty 1967, Herbst 1969, Whitton 1970). Here, I've expanded on this work by describing the changes in the nutrient stoichiometry and chlorophyll concentrations of *Cladophora*, in conjunction with its seasonal biomass and *in situ* productivity. The seasonal description of *Cladophora* stoichiometry serves to highlight the need to design sampling strategies and interpret collected field data within a seasonal context.

The photosynthetic rates of *Cladophora* at shallow depths in Lake Ontario are currently primarily limited by phosphorus and potentially co-limited by light during the period of dense growth. Stoichiometric macronutrient ratios of *Cladophora* tissue (C:P and N:P), and a coherent decrease in P^{B}_{m} and Q_{P}, are consistent with P limitation of *Cladophora* productivity. Light limitation was inferred by an increase in P^{B}_{m} as self-shading decreased due to a loss of

biomass, even while Q_P remained near the estimated minimal quota. In previous work (Chapter 3), I demonstrated that diurnal increases in irradiance at the canopy surface from within an hour of sunrise did not increase photosynthetic rates of *Cladophora* on most dates. These two seemingly contradictory results (light limited primary production rates for the stand versus light saturated for the canopy surface) are reconciled by considering light attenuation through the *Cladophora* canopy. Because light attenuation through the canopy was high ($Kd_{PAR\text{-}canopy}$ = 24.1 ± 3.3 m^{-1}), increases in irradiance at the surface of the canopy had no detectable effect on the production by the entire integrated *Cladophora* bed. A range of 0-1000 μmol m^{-2} s^{-1} of surface irradiance is attenuated to a range of only approximately 0-90 μmol m^{-2} s^{-1} at 10 cm depth within the canopy. While the effect of the diurnal range in irradiance on rates of *Cladophora* productivity was not detectable (Chapter 3), removal of canopy increased the irradiance received by the entire *Cladophora* thallus by up to 2 orders of magnitude, leading to an increase in the P^B_m rates.

Nutrient limitation

In the upper Laurentian Great Lakes, both historically and recently, *Cladophora* productivity has been demonstrably P limited, and consequently confined to areas with direct nutrient inputs (Neil and Owen 1964, Herbst 1969, Auer et al. 1982). But, this has not always been the case in the lower Laurentian Great Lakes. Prior to P loading reduction strategies (1970s) and dreissenid mussel establishment (early 1990s), *Cladophora* P quota at shallow depths in Lake Ontario, for example were not strongly P limited (Painter and Kamaitis 1987). Even today, despite reduced P loading and oligotrophic conditions in the pelagia, *Cladophora* can generally be found in Lake Ontario, Michigan and Erie wherever there is suitable substrate, sufficiently energetic water, and sufficient solar irradiance (pers. observation). Based on the threshold nutrient ratios of Kahlert (1998) to describe nutrient deficiency among benthic primary producers, *Cladophora* was shown here to be always P limited at shallow depths. During the latter part of the summer, *Cladophora* was defined as N limited based on the C:N molar ratio (Kahlert 1998). The C:N ratio did not exceed the threshold to define N limitation in the previous year of study (2004) which had more storm events and consequently, higher inorganic N (both NO_3^- and NH_4^+ concentrations; data not shown). However, the physiological basis of a higher C:N ratio, and whether it does indicate N limitation of

productivity, remains uncertain. Low inorganic nitrogen supply, or low iron supply, which is necessary for NO_3^- reduction and assimilation, may have led to temporary N limitation during the summer. Alternatively, the low N concentration may have reflected low resource allocation to chlorophyll production (because of P limitation, for example) and thus a decreased N demand and cell quota for N (Healey and Hendzel 1979; Guildford et al. 1994). Evidence from Lake Erie indicates that secondary N limitation, mediated by Fe limitation during times of strong thermal stratification, is possible, at least for phytoplankton in the Great Lakes (North et al. 2007). But, the demand for P is still far more out of balance than the demand for N in *Cladophora* in Lake Ontario, as indicated by the *Cladophora* N:P ratios.

Using other stoichiometric thresholds of nutrient limitation, such as that suggested for freshwater benthic algae (Allen 1995) or freshwater *Cladophora* in rivers in Ontario (*C. fracta*; Wong and Clark 1976) would also lead to the conclusion of chronic and acute P limitation of shallow growing *Cladophora* in western Lake Ontario. Allan (1995) suggested P limitation at N:P above 30 and N limitation at N:P below 10. Wong and Clark (1976) expressed their estimated critical P and N concentrations in terms of dry weight. Assuming that dry mass is 30 - 35% C, Wong and Clark's (1976) C:P threshold ratios would be in the range of 484 – 565. These are below the ratios I measured on any given date. The lowest C:P ratios of *Cladophora* reported here, at the beginning of the seasonal growth cohorts (around 660) are similar to those reported for marine benthic primary producers (macroalgae plus seagrasses, mean = 700, median = 550; Atkinson and Smith 1983). However, the N:P ratios reported here are generally higher (range 46-130), and the C:N ratios are generally lower (5.5 – 18.1), than those reported for the marine benthic flora (median N:P = 30, median C:N = 18.3), indicating greater P deficiency among the freshwater samples and greater N limitation among the benthic marine flora, consistent with expectations based on nutrient limitation of nearshore (or coastal) planktonic communities.

At my study site, there was additionally no correlation between CO_2 concentration and *Cladophora* productivity on a seasonal or diurnal scale (Fig 4.6), from which I infer there was no C limitation of benthic primary production. This contrasts with other systems, such as those found on Precambrian Shield topography at the experimental lakes area (ELA), Ontario, Canada, where benthic primary production was limited by DIC supply, even when phytoplankton were demonstrably P limited (Turner et al. 1994). This discrepancy is most

likely attributable to differences in total DIC supply to the benthos. There is a tremendous range in CO_2 and DIC concentration in freshwater environments, and DIC concentrations and its speciation can fluctuate significantly on a seasonal and diurnal basis due to metabolic processes. While CO_2 concentrations were likely similar between the systems studied by Turner et al. (1994) and that reported here, the total DIC was an order of magnitude different. The lower Laurentian Great Lakes have high buffering capacity and a slightly alkaline pH. In Lake Ontario between May and September in the very nearshore (<3m depth), measured midday CO_2 concentrations ranged 5.7 to 25 μM, morning pH was in the range of 8.1 to 8.8, and DIC concentration ranged from 1400 to 1740 μM (Fig 4.6). In the lakes not experimentally manipulated that are described in Turner et al. (1994; n=10 lakes), pH was in the range of 7.0 – 7.5 and median DIC was 127 μM. Based on carbonate equilibrium at standard temperature and pressure, these pH values and DIC concentrations equate to CO_2 concentrations in the range of 9.4 – 26 μM. Thus, while CO_2 was lower than the saturation concentration for the primary carboxylation enzyme Rubisco (Tortell 2000) in both systems, DIC was low only in the ELA lakes, accounting for the C limitation of the benthos. A high demand for C likely restricts the spatial distribution of *Cladophora*, allowing it to thrive only in alkaline, high DIC environments.

Many algae are adapted to acquire C in high DIC, low CO_2 aquatic systems with high efficiency. Even so, there is a greater energy requirement for acquiring and/or utilizing HCO_3^- instead of CO_2, which can passively diffuse into cells. HCO_3^- can be taken up actively with proton pumps, or be converted to CO_2 for uptake at the cell surface with periplasmic carbonic anhydrases. The production of carbonic anhydrases are generally a response to low CO_2, indicating that there is an energetic requirement involved. Indeed, investigations of cyanobacteria (Badger and Andrews 1982) and a freshwater mixed assemblage dominated by a dinoflagellate bloom (Berman-Frank et al. 1998) showed that decreasing CO_2 concentrations were associated with decreasing quantum efficiency of oxygen evolution and C-fixation, respectively. And, Jones (2005) determined that the cost of using HCO_3^- over CO_2 amounted to 69 quanta mol^{-1} C for the macrophyte *Elodea nuttallii*.

This metabolic cost of bicarbonate utilization may explain why two other studies found DIC limitation of *Cladophora* spp. in high nutrient and high DIC waters. *In situ* experiments demonstrated that the primary productivity and growth rates of marine *Cladophora vagabunda*

(Rivers and Peckol 1995) and freshwater species *C. fracta* (Cheney and Hough 1983) were limited by DIC concentration and not by availability of inorganic macronutrients. However, *Cladophora* has been shown to be efficient at utilizing of HCO_3^-, compared with other macroalgae (Choo et al. 2002, 2005). And, *Cladophora glomerata* and *C. fracta* have a low CO_2 compensation, in the range of 2.7-4.1 μM in alkaline media, suggesting that C limitation is unlikely (Birmingham and Colman 1979; Cheney and Hough 1983).

Given that P supply limits *Cladophora* productivity at well lit depths in Lake Ontario, the determinants of the rate of P supply to benthic macroalgae could give rise to important secondary constraints of *Cladophora* productivity. One of these determinants is the size of benthic boundary layer and the position of the flora within it. Flux of nutrients and dissolved gases between bulk water and the benthos is a function of the size of the benthic boundary layer and the concentration gradient across it. The size of the benthic boundary layer is a function of hydrodynamics and morphology of the benthos (Vogel 1994). In lotic systems at least, the rate of nutrient uptake by attached periphyton is not limited by the diffusive rates of transfer to the cells (Dodds 1990). Dense *Cladophora* biomass restricts water movement through the canopy (e.g. Dodds 1991), potentially restricting replenishment of depleted resources such as CO_2 (Raven et al. 1982). But, *Cladophora*'s morphology, growing above the laminar boundary layer (Dodds and Gudder 1992), allows it greater access to nutrients, facilitating more rapid nutrient and dissolved gas flux.

Light acclimation, light limitation, and photoinhibition

Cladophora grows at greatest density in shallow well-lit waters (Chapter 2). Yet, even at these depths, *Cladophora* can suffer light limitation due to self-shading. Shading by optically thick macroalgae, such as kelp species, has been shown to be an important factor structuring macroalgal community composition (Toohey et al. 2005). Here, I measured light attenuation through the canopy of a filamentous macroalga and found that it was also high, but due to the density of the canopy, rather than the thickness of the thallus. Recent studies have highlighted that light attenuation by benthic attached macroalgae and macrophyte angiosperms has not been sufficiently accounted for in estimates of benthic primary production, leading to overemphasis of nutrient limitation (Middleboe and Binzer 2004; Binzer et al. 2006). Even

among optically thin macroalgal species, such as densely growing *Cladophora*, light limitation appears to be underappreciated.

Light limitation can be equated with energy limitation. Because the energy demand of an autotroph is dependent upon the substrates it utilizes for metabolism, light limitation can be modified by the chemistry of its environment. As previously discussed, a decrease in CO_2 availability with a concomitant increasing dependency on HCO_3^- is estimated to incur a energetic cost to photosynthesizing cells. Likewise, reliance on oxidized N substrates incurs a cost of reductant necessary for protein synthesis. Thus, as light attenuation through the *Cladophora* canopy increases while its biomass accumulates, and CO_2 and NH_4^+ becomes increasingly scarce in the aquatic medium, the demand for light energy may increase at the same time that light availability decreases. During the summer, decreases in the seasonal rates of P^B_m indicate that the total light harvested by dense *Cladophora* stands fails to meet its energy demand, constraining the rate of biomass-specific *in situ* photosynthesis. In light gradient incubation experiments, only P limitation would likely be detected. While one of the hypotheses of Hecky et al. (2004), that CO_2 supply by dreissenid mussel respiration is relieving energy limitation of *Cladophora*, does not appear to be substantiated on a macroscale (i.e. CO_2 is brought down to less than 5 μM), investigations at the microscale would be required to adequately test this hypothesis.

Previously, I did not detect any mid-day depression in photosynthetic rates in *Cladophora* beds (Chapter 3). In this chapter, I examined photoinhibition using *in situ* fluorometric techniques. These techniques were employed because decreases in phytosynthetic efficiency generally precede decreases in photosynthetic capacity with increasing photon flux density (Hanelt 1996). Changes in F_v/F_m are a good diagnostic indicator of photoinhibition (Valladares and Pearcy 1997). *Cladophora* was shown in this study to experience a low degree of photoinhibition at high irradiance. For example *Cladophora* exhibited less than a 10% decrease in 30 minute dark-acclimated F_v/F_m relative to maximal F_v/F_m when previously exposed to 1000 μmol m^{-2} s^{-1}. Another filamentous green alga, *Chaetomorpha linum*, was shown to have a decrease of 36% in 15 minute-dark acclimated F_v/F_m when exposed to full irradiance (Bischof et al. 2006). By comparison, available evidence indicates that natural phytoplankton assemblages can suffer a greater degree of photoinhibition. When exposed to surface irradiance, Lake Erie phytoplankton experienced

a 75% decrease in 30 minute dark-acclimated F_v/F_m (Marwood et al. 2000). While the phytoplankton would not have been previously acclimated to surface irradiance in these measurements, *Cladophora* in this study was exposed to increasing light prior to measurements, so these differences reflect realistic *in situ* exposure scenarios to high light. Furthermore, *Cladophora*, along with *Ulothrix zonata* and *Bangia atropurporea*, thrive on hard substrates up to the splash zone in the Great Lakes, demonstrating success at high irradiance. *Cladophora* growing in the splash zone differs from deeper growing samples in being devoid of epiphyton (pers. observ.), possibly related to the spectral quality or intensity of light at the water line. Other studies have reported low rates of photoinhibition among macroalgae as a general feature of these organisms (Loeb et al. 1983; Turner et al. 1995).

The maximum F_v/F_m did not change seasonally, indicating that daytime photoinhibition is completely recoverable each night. I made no distinction in this study between photooxidative stress due to high light intensity or direct UV damage. At shallow depths, both are potential stressors for which *Cladophora* is evidently well adapted. Adaptations to high light exposure involve complementary mechanisms. *Cladophora* was shown to have a functional xanthophyll cycle in which the pool size of the xanthophyll cycle pigments increase in *Cladophora* in response to growth irradiance (Ensminger et al. 2001), which serves to dissipate excess absorbed energy in response to changing diurnal light conditions (Demmig-Adams and Adams 1996). *Cladophora glomerata* also has higher activities of oxygen radical scavenging enzymes, such as catalase and ascorbate peroxidase, relative to other filamentous green algae, and was shown to suffer less photooxidative stress than both *Enteromorpha ahlernerania.* and *Ulva procera*, putatively as a result of these enzyme activities (Choo et al. 2004, 2005). Other photoprotective agents known from benthic microalgae, such as scytonemin-like compounds (Donahue et al. 2003), have not been specifically investigated in *Cladophora*.

Surprisingly, neither nutrient status nor temperature (within the range in this study, 10-25 ^{o}C) were related to the effective or maximum quantum yield of fluorescence, $\Delta F/Fm'$ or F_v/F_m. Studies of phytoplankton have demonstrated F_v/F_m as an indicator of nutrient deficiency (Kolber et al. 1994; Behrenfeld et al. 1996; Boyd et al. 1998; Lippemeier et al. 1999), including P deficiency, specifically (La Roche et al. 1993). Yet, some studies have found no relationship between F_v/F_m and nutrient status among phytoplankton growing in

steady-state (Cullen et al. 1992, Parkhill et al. 2001), and others still have found an unexpected positive relationship between F_v/F_m and increasing nutrient deficiency (Cabello-Pasini and Figueroa 2005). As *Cladophora* depletes its internal stores of P as it grows, and its rate of primary productivity decreases, it maintains the same maximum F_v/F_m indicating an effective balance between light absorption capacity and downstream dark reaction rates. Lower temperatures are associated with greater excitation energy pressure (Huner et al. 1998) because lower temperatures slow enzyme-mediated dark reactions and the physical transport of reduced compounds in the photosynthetic electron transport chain associated with membranes, while photochemical reactions are not affected by temperature. Contrary to the results presented here, Ensminger et al. (2001) found that *Cladophora* F_v/F_m was lower in the early spring (about 10^oC) than in the summer (about 18^oC), and suggested this was attributable to a measurable increase in the xanthophyll cycle pigment pool. The reasons for this discrepancy between our results are not obvious.

Competitive advantages of *Cladophora* in the nearshore

In the nearshore of Lake Ontario, integrated down to at least 12 m depth, areal benthic production dominates over planktonic production throughout the productive period of the growing season (Chapter 3). High light and high nutrient conditions could favour benthic over planktonic production for several possible reasons. Benthic primary producers are generally better adapted to high light, as previously discussed, while phytoplankton tend to suffer greater photoinhibition.

In this era of abundant dreisseinid mussels, benthic primary production likely succeeds over phytoplankton production in the nearshore due to both increases in downwelling irradiance, causing increased photoinhibition of phytoplankton and more benthic production at light limiting depths, increases in pulses of nutrients in close proximity to the benthos, and possibly grazing effects on phytoplankton by the mussels (e.g. Depew et al. 2006). In the Baltic Sea, competition for limiting P appears to determine which group – bloom forming planktonic diatoms or macroalgae – is successful each spring (Kiirikki et al. 1999). Currently, in the lower Great Lakes, *Cladophora* thrives every year. Mussels may have tipped the balance in favour of benthic primary production by providing a large source of nutrients, favouring *Cladophora* production in moderate to high physical energy environment, possibly

due to faster nutrient uptake kinetics than other macroalgae (Wallentinus 1984) or tolerance to high photon fluence rates (Choo et al. 2000).

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Chapter 5 The importance of the filamentous green alga, *Cladophora glomerata*, to the nearshore nutrient dynamics of Lake Ontario

Introduction

Nearshore macroalgal growth is one of the most publicly visible manifestations of eutrophication in shallow waters. *Cladophora glomerata* is a fast-growing macroalga that grows attached to hard substrate and is considered a nuisance in the Laurentian Great Lakes, as elsewhere, due to its habit of rapidly growing in the spring and sloughing during the summers. Detached and decaying *Cladophora* filaments foul the nearshore environment from early summer, following its peak biomass, through the autumn. The initiation of *Cladophora* accumulation is strongly determined by water temperature (Chapter 2), with growth commencing in the western part of Lake Ontario in late May, and earlier in eastern Lake Ontario and in Lake Erie where spring warming occurs earlier. There are typically two growth cohorts in the Laurentian Great Lakes (Neil and Owen 1964; Chapter 3); the second cohort begins growth following a clearing of the first cohort, but is not generally observed to reach biomass concentrations as high as the first growth (Chapter 3). Based on the output of a numerical growth model, validated against *in situ* carbon-based measurements, net primary production (NPP) rates of *Cladophora* in Lake Ontario were estimated to dominate nearshore carbon sequestration down to 12 m depth in areas with suitable substrate for *Cladophora* cohort (Chapter 3). High *Cladophora* NPP rates lead to high biomass accrual, measured in excess of 100 g DM m^{-2}, for example, at depths less than 2 m (i.e., well-lit) during the seasonal peak. From a growth model simulation, peak biomass down to 12 m depth was estimated at 54.3 and 65.1 kg DM m^{-1} shoreline for 2004 and 2005, respectively (Chapter 2). This macroalgal biomass accumulation represents a large pool of sequestered nutrients, an amount that has yet to be quantified, but is imperative for constructing nearshore nutrient flux budgets (Crowder and Painter 1991).

Anecdotal evidence indicates that *Cladophora* biomass has been increasing throughout the lower Laurentian Great Lakes since the widespread establishment of dreissenid mussels in the late 1980s/early 1990s. For example, municipal complaints of shoreline fouling have increased in Halton Region on Lake Ontario (L. Moore, Ontario Clean Water Agency, pers. comm.) and the frequency of water intake facilities closing due to macroalgal fouling has

increased on the north shore of Lake Ontario (C. Gregoris, Ontario Power Generation, pers. comm.). Yet, the timing of the perceived increase in *Cladophora* biomass with dreissenid mussel expansion is correlative. During the same time period, greater proportions of the landscape have become urbanized and there has been substantive population growth around the Great Lakes Basin which may potentially be fuelling greater allochthonous nutrient loading to the lakes. Halton Region (the location of this study), for example, experienced an increase in population of 40.3% from 1991 to 2006, and over the same time, the municipality of Milton, Ontario, which lies in the Oakville Creek (also known as 16 Mile Creek) drainage basin in Halton Region, boasted a population growth of 68.0% (Government of Canada 2002; Regional Municipality of Halton 2007). Whether the nutrient demand by *Cladophora* can be met by catchment loading and whether the apparent increase in *Cladophora* biomass could be attributable to changes in landuse in this region has not been previously quantified.

The magnitude and seasonality of nutrient sequestration by *Cladophora*, and how this macroalga's growth and senescence may be modifying the nearshore nutrient environment of Lake Ontario, are the major foci of this study. Towards a better understanding of the nearshore nutrient dynamics, this study will describe the seasonal nutrient concentrations in suspension and quantify the amount of nutrients sequestered by *Cladophora* using direct measurements of tissue nutrient stoichiometry, empirical relationships of nutrient concentration with depth, and predicted growth rates. These data will be presented in a historical context. A second objective of this study is to assess if direct catchment supply of nutrients is sufficient to meet the seasonal demands of *Cladophora* growth. If direct catchment P supply is currently greater than or equivalent to the concentration sequestered by proximal *Cladophora*, and the loading from the catchment has increased since the time of dreissenid mussel establishment, then the perceived increase in *Cladophora* biomass coincident with dreissenid mussel invasion may be spurious. If conversely, catchment loading is insufficient to meet the uptake estimated for *Cladophora* growth, and has not evidently increased over the same time period, then in-lake processing of phosphorus may be indirectly implicated as increasing the supply of P to the nearshore.

Methods

Study site

The study site for this investigation was located in Oakville, Ontario, Canada, on the north shore of western Lake Ontario (Fig 5.1). This site (43.44°N, 79.66°W) lies 500 - 600 m east from the mouth of an inflow locally known as 16 Mile Creek. To avoid ambiguity with other creeks of a similar name, however, this inflow is herein referred to as Oakville Creek, following the nomenclature of the Ontario Provincial Water Quality Monitoring Network (PWQMN). The site is underlain by rocky substrata and experiences up to 100% *Cladophora glomerata* (L.) Kütz. coverage at 1-2 m depth in the early summer. The identity of this macroalga was confirmed using molecular markers and is the same species that occurs throughout Lakes Ontario and Erie (K. Muller, U. Waterloo, pers. comm.).

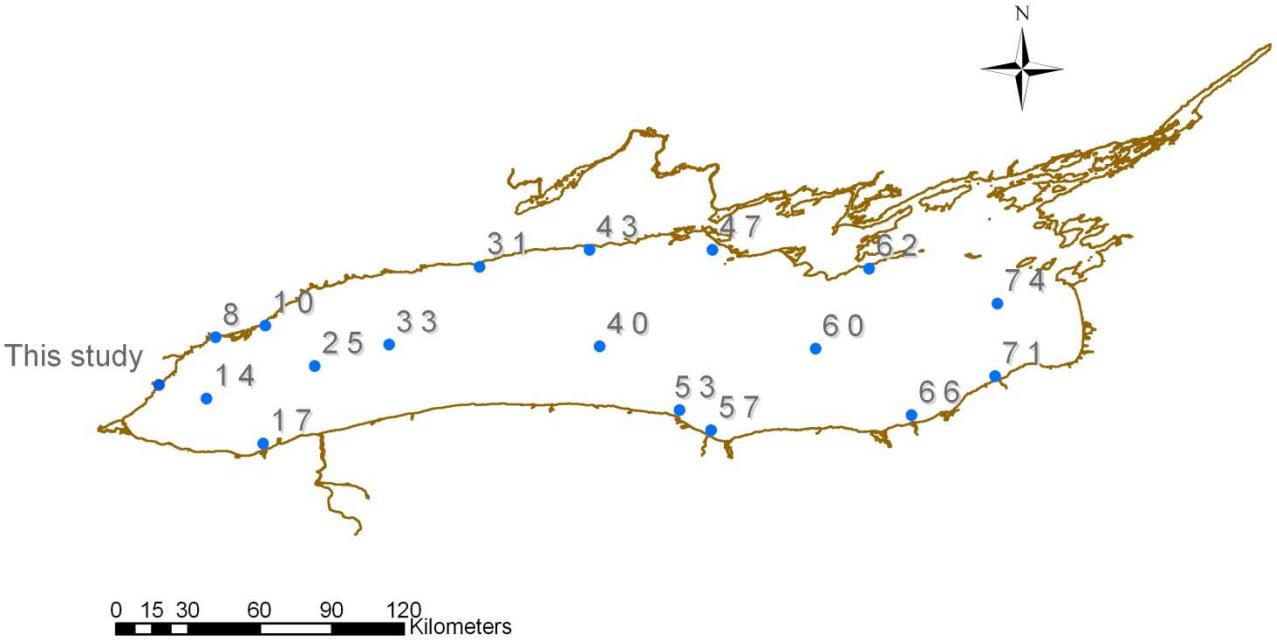

Figure 5.1 Lake Ontario lakewide survey stations chosen to represent the nutrient conditions of the north shore (nearshore < 20m), south nearshore, and offshore (> 50m). The numbers are given from Environment Canada synoptic surveys.

Figure 5.2 Map of Halton region nearshore. Credit for the satellite photo in the bottom panel goes to Google Earth, accessed 7 Dec, 2007. Locations of storm sewers and outfalls were obtained from the Ontario Ministry of Environment.

Historical offshore and nearshore nutrient concentrations in Lake Ontario

To place data collected for this study within a historical and spatial context, historical nutrient concentrations from nearshore (< 20 m) stations and offshore (>50 m) were collated from Environment Canada synoptic surveys conducted from 1967 to 2005 (the most recent available data; STAR database; Environment Canada). Six stations from the north shore (Stations 8, 10, 41, 43, 47, 62) and 5 stations from the south shore (17, 53, 57, 66, 71) were chosen to represent the nearshore environment (Fig. 5.1). Another 6 stations were chosen from offshore to span the length of the lake (Stations 14, 25, 33, 41, 60, 74; Fig. 5.1). The data representing the first 5-7 years of surveys were inconsistently labelled with respect to station location. Only data from 1972 onwards were therefore considered. The georeferencing from 1972-1975 was carefully assessed to ensure that the stations were closely located to the most recent station locations. Only data within 4 km of the current station location and within the correct depth category (< 20m or > 50 m) were used. Data from all profile depths and all seasons were included; no consistent differences between epilimnetic and hypolimnetic samples were evident.

Nutrient Loading from Oakville Creek

Nutrient loading to Lake Ontario from Oakville Creek was calculated based on daily monitored water discharge and monthly sampled water nutrients. Water discharge was monitored by Water Services of Canada (WSC) at Milton (43.5139^{o}N, 79.8797^{o}W; Station 02HB0050) using a gauge-type recorder and recorded hourly. Water quality metrics, including total phosphorus (TP), soluble reactive P (SRP), nitrate (NO_3^-–N), and ammonium (NH_4^+–N) were monitored at 13 stations on Oakville Creek by the Ontario Ministry of the Environment (MoE). Samples were analysed colorimetrically using a Technicon AutoAnalyzer (Ministry of Environment 2007*a*, *b*). The monitoring station closest to the lake (Station 06006300102; 43.4429 ^{o}N, -79.6712 ^{o}W), was sampled approximately once a month throughout the year for the period 1964 to 2007. These data were maintained by the Provincial Water Quality Monitoring Network (PWQMN), a branch of the MoE.

Nutrient loading for each date of measured nutrient concentration from 1991 to 2004 was calculated as a product of concentration and discharge. Loading was plotted as a function

of discharge. Calculated loading-discharge functions (Sigmaplot version 8) were used to compute daily loading based on daily discharge for the years of intense study, 2004 and 2005.

In order to assess if nutrient concentrations from the catchment to the study site have been increasing over the decades, calculated loading versus measured discharge relationships were plotted. A change in P concentration appeared to commence by 1976. To test whether changes occurring in the lake could be due to changes in catchment loading or due to authochthonous processes (see below), loading was compared before and after mussel establishment. Loading data were therefore divided into three time periods: 1964 – 1975; 1976 – 1990; and 1991–2007.

Upwelling events

Temperature was measured from 14 May to 16 Aug and 21 Sep to 10 Oct in 2004 and from 10 Jun to 9 Aug in 2005 using thermistors (Onset, StowAway TidbiT; Bourne, MA, USA) tethered at 2.75 m depth in 3 m of water in order to record the timing and frequency of hypolimnetic upwelling. Upwelling events were recorded as days, after spring warming to greater than 10 ^{o}C, when temperature fell to below 10 ^{o}C in less than 24 hours.

Water chemistry in Lake Ontario

Water samples for nutrient analyses were collected at approximately biweekly intervals from early May, before *Cladophora* growth was evident, and continued until October. Water was collected using a 3-L Nisken sampler from 0.5 m above the bottom at 2.5 m depth and transferred to an acid-washed 4-L carboy. Triplicate carboys were collected on each date. All water was subsequently screened through a 200 μm Nitex (nylon) screen to remove large particles.

For TP analysis, whole water (pre-screened) was collected in acid washed 60 mL high density polyethylene (HDPE) bottles. Soluble reactive P, NO_3^-–N, and NH_4^+–N samples were filtered through acid-washed inline 0.2 μm polycarbonate filters into clean 60 mL HDPE bottles. Water for particulate P analysis was filtered onto pre-combusted, acid-washed, glass fiber filters (GF/F; nominal porosity 0.7 μm). Water for particulate N analysis was also filtered onto pre-combusted glass fiber filters (GF/F). Soluble reactive P samples were kept at

4°C for not more than one week before analysis (Griesbach and Peters 1991). Inorganic N and TP samples were kept frozen (-18°C) until analysis.

Phosphorus was measured as orthophosphate using the spectrophotometric molybdate blue method (American Public Health Association 1998). For SRP, reagents were added to 20 mL aliquots and read at 885 nM in a Cary 100 Bio spectrophotometer using a 10-cm pathlength cuvette. Total P samples were first treated with potassium persulphate solution (0.6 mL of 4% solution to 20 mL aliquots) and autoclaved for 30 minutes to oxidize all organic P to phosphate.

Nitrate was analysed colorimetrically using the cadmium reduction method (APHA 1998). Nitrite concentration was first quantified in each sample by acidifying it to nitrous acid to which aromatic amine reagent compounds (sulphanilamide then N-1-naphtylethylenediamine-dihydrochloride; NNED) were added sequentially to form a pink azo dye which was quantified spectrophotometrically at 543 nm using a 1-cm pathlength. Aliquots of 20 mL were also passed through an alkaline-buffered cadmium-copper column, reducing nitrate to nitrite, almost quantitatively (APHA 1998). These samples were then analysed for nitrite, yielding the nitrate + nitrite concentration. Nitrate was calculated by difference.

Ammonium was analysed fluorometrically using the orthophthaldialdehyde (OPA) method (Holmes et al. 1999; Kumar et al. 2007). Standard curves were made separately for low (range: 0-20 $\mu g\ L^{-1}$) and for high range: 0-100 $\mu g\ L^{-1}$) concentrations. Following the addition of reagents (OPA, sodium sulfite, and sodium borate), samples were kept in the dark for a minimum of 3 hours and then analyzed fluorometrically (Turner Designs 700 fluorometer). Background fluorescence, originating from autoflourescent material in the sample, was subtracted as a sample blank. Contamination by atmospheric NH_4^+ was minimized by adding reagents directly to the original sampling bottles, rather than transferring them, and by preparing all standards and reagents in a laminar flow hood.

Cladophora tissue chemistry

Cladophora tissue was collected in Whirlpak bags by a snorkeller at a sampling frequency of approximately every 2 weeks throughout the growing season. Samples were collected in triplicate from 2 m depth. *Cladophora* samples were subsequently rinsed thoroughly with de-ionized water in a sieve (porosity approximately 1 mm) to remove debris

and other fine macroalgae which were sometimes present (e.g., *Ulothrix zonata*). Using forceps, the samples were also picked free of larger contaminants such as macroinvertebrates and dreissenid mussel shell fragments. Subsequent to washing the *Cladophora*, each sample was observed under a dissecting microscope at 25 X magnification to ensure its identity. Prior to *Cladophora*'s establishment in the early spring, and during the first sampling following a massive detachment event in 2004, the majority of the macroalgae present was *U. zonata.* Samples that were not almost entirely *Cladophora* (plus attached microalgal epiphyton) upon initial examination were discarded.

For measurements of P quota (Q_P), *Cladophora* tissue was first dried at 60^{o}C for a minimum of 24 hours. Dried *Cladophora* was combusted at 450^{o}C for 1 hour and subsequently autoclaved for 30 minutes in distilled water with 4% potassium persulphate solution added to a final concentration of 0.16%. Following this digestion procedure, orthophosphate was measured spectrophotometrically using the molybdate blue method (APHA 1998). In trial runs with increasing concentrations of potassium persulphate, no additional P was oxidized.

For measurements of nitrogen quota (Q_N), *Cladophora* was dried in a lyophilizer in 20 mL glass scintillation vials. Freeze dried *Cladophora* was ground to a powder using a Retsch MM 2000 ball mill grinder (F. Kurt Retsch GmbH & Co., Haan, Germany). Approximately 1 mg of dried, pulverized *Cladophora* tissue was packed Ni sleeves, weighed to an accuracy of 0.01mg. The concentrations of C and N were measured in an elemental analyzer (Exeter Analytical Inc. CEC-440; combustion 980 ^{o}C, reduction 700 ^{o}C).

The areal concentration of nutrients was calculated as the product of *Cladophora* nutrient quota (Q_P or Q_N) and biomass.

Cladophora P sequestration versus catchment loading

The rate of *Cladophora* P uptake was compared with daily catchment P loading supplied by Oakville Creek during the *Cladophora* growing season in 2004 and 2005. The rate of P uptake by *Cladophora* (gP m^{-2} d^{-1}) was calculated as the product of daily simulated *Cladophora* biomass per area (gDM m^{-2}), depth-specific simulated *Cladophora* growth (NPP: d^{-1}), and daily interpolations of depth-specific *Cladophora* Q_P (gP gDM^{-1}). *Cladophora* biomass and growth were simulated at daily time steps using the *Cladophora* growth model

(CGM; Canale and Auer 1982; Higgins et al. 2005; Chapter 2). This model has previously been calibrated and validated to simulate attached *Cladophora* biomass (Chapter 2) and independently validated against direct *in situ* carbon-based measurements of net primary production (Chapter 3), at this study site. The CGM simulates attached biomass accrual based on predictions of daytime biomass-specific net primary production, nighttime respiration, and mechanical or other loss processes. The biomass-specific metabolic rates are predicted based on growth constraints by Q_P, i.e., daily light dose, temperature, and density-dependent self-shading. Daily *Cladophora* Q_P at 2 m was interpolated from direct measurements. Q_P is best described as increasing exponentially with depth: $Q_{P(Z)} = (Q_{P(2)} - 0.00366) + 0.00366\ e^{0.3388\ (Z-2)}$, where $Q_{P(Z)}$ and $Q_{P(2)}$ are the Q_P values at a given depth (Z) and at 2.0 m, respectively (A. Houben, University of Waterloo, pers. comm.; Chapter 2).

The concentration of P sequestered by *Cladophora* was calculated per depth contour down to 12 m, based on the nearshore slope of the Oakville area, estimated from bathymetric maps (Virden et al. 2000). The area expected to be directly influenced by P loading from Oakville Creek varies with the magnitude of discharge. As a conservative estimate, minimizing the areal P demand by *Cladophora*, the *Cladophora* P uptake rate was summed over the minimum distance from the mouth of Oakville Creek to the study site, 500 m. As a furthermore conservative assumption, *Cladophora* coverage across that chosen area was estimated at 20%. Although up to 100% *Cladophora* coverage was visible at the study site, the coverage closer to the creek mouth was lower than this (D. Depew, U. Waterloo, pers. comm). There is heterogeneity in hard substrate distribution and stochasticity in *Cladophora* propagule settlement, and consequently bare patches were visible between 2 and 5 m depth along the Halton shoreline, based on hydroacoustic surveys (D. Depew, U. Waterloo, pers. comm.).

Results

Historical nearshore and offshore nutrient concentrations in Lake Ontario

Total phosphorus (TP), soluble reactive P (SRP) and NH_4^+ concentrations have declined since the 1970s, while NO_3^- concentrations have increased on average, to what appears to be a new asymptote (Fig. 5.3). Offshore TP exhibited a smaller variability than nearshore TP in every year. In the late 1970s and early 1980s, measurements of TP above 40 µg L^{-1} occurred every year. By 2000, very few nearshore TP concentrations exceed 20 µg L^{-1}.

The lowest limit of both nearshore and offshore TP decreased from the first year of sampling to about 1990, and since then the minimum measured concentrations have remained the same. Nearshore and offshore TP concentrations have been in the same range since about 2000. The pattern for SRP concentrations between the nearshore and offshore were different than for TP. Offshore SRP concentrations had a greater range in the years preceding 1988. The minimum concentration measured for all stations has remained the same, while the maximum concentrations measured each year declined sharply from 1972 to about 1989, but with high offshore SRP measured in 1991. Nearshore and offshore NO_3^- concentrations have generally increased in parallel. Offshore concentrations were generally more constrained than north nearshore concentrations until the 1990s. The lowest NO_3^- concentrations were usually measured in the north nearshore zone. The south nearshore exhibited much greater variability, and typically the highest NO_3^- concentrations. These high measurements were primarily due to two stations located near (< 1.8 km) to the mouths of the Irondequoit (Stn 57) and Oswego (Stn 71) inflows (Fig. 5.1). Ammonia concentration was always, and continues to be, lower offshore than nearshore. In contrast to NO_3^- concentrations, the highest concentrations of NH_4^+ were collected from the northern nearshore zone.

Data measured at the Oakville study site (at less than 3.0 m) exhibited a greater variation than the lakewide measured samples in general, but had similar mean averages. Higher variability is likely a result of the shallow depth, close proximity to Oakville Creek, and higher sampling frequency. Mean nutrient concentrations collected in this study were not different than mean nearshore nutrient concentrations from all the other stations. Nitrate concentrations at Oakville demonstrated greater variability about the mean relative to other stations, although the extreme concentrations were within the range seen for other stations near inflows in previous years (Fig 5.3).

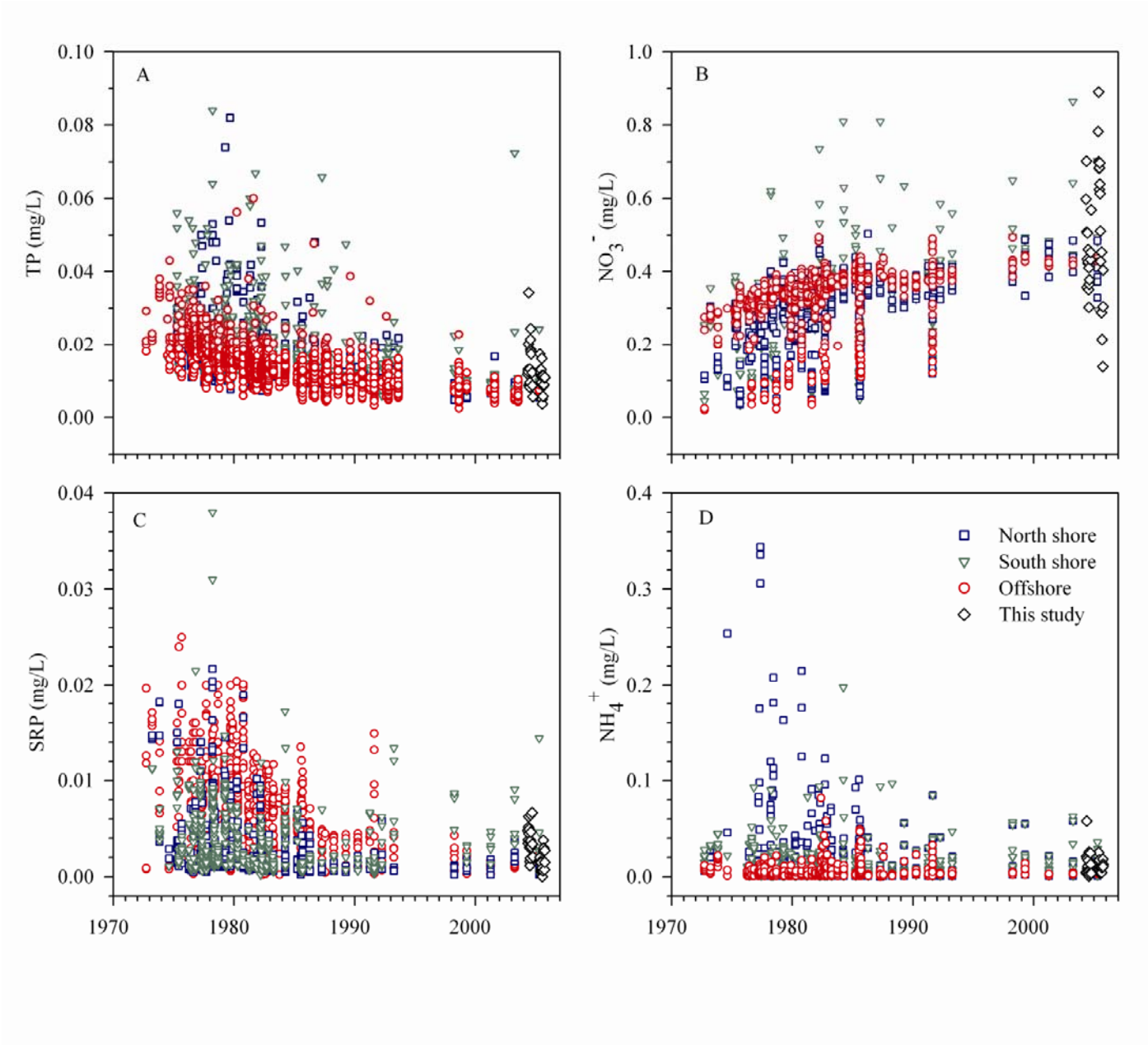

Figure 5.3 Historical and contemporary nearshore nutrient concentrations in Lake Ontario. South shore, north shore, and offshore data were collected by Environment Canada during synoptic surveys. Data from "This study" was collected in Oakville east of Oakville Creek at 2.5 m depth.

Catchment loading – historical and contemporary

Nutrient concentrations, and therefore nutrient loading of TP, SRP and NH_4^+, as a function of water discharge, has decreased over the past 4 decades (Fig. 5.4). Prior to the 1990s, there was no detectable relationship between TP or SRP concentration and flow rate. Consequently, a linear equation best describes the relationship between TP or SRP loading and water discharge. Using data from 1991–2007, the relationship between TP and SRP loading and water discharge was exponential. Thus, P loading was lower in the 1990s than in the 1970s and 1980s, at low discharge rates. For example, TP loading was, on average, lower in the 1990s than in the 1976-1990 period at discharge rates below 2.4 $m^3 s^{-1}$ and lower than in the 1964-1975 period at discharge rates below 4.4 $m^3 s^{-1}$. Importantly, the relationship between discharge and P loading was less variable in recent years (for TP: 1964-1975, r^2= 0.479; 1991-2004, r^2= 0.814; for SRP: 1964-1975, r^2= 0.313; post 1991, r^2= 0.729). At discharge rates higher than 5 $m^3 s^{-1}$, there were insufficient data to allow comparison between decades (e.g., 1 datum in the post 1991 period). The relationship between NH_4^+ loading and discharge rate was best described with a linear equation for each time period. The slopes of these regressions were higher in the 1964–1976 period than in the antecedent periods. The relationship between NO_3^- loading and discharge rate was also best described with a linear equation, but there were no differences between slopes for the different time periods. Likewise, total Kjeldahl N (TKN), and nitrite loading versus discharge rate were best described with linear relationships and demonstrated no differences between decades (data not shown). There was no evidence of a decrease in mean annual discharge over the years and, on a monthly basis, only April demonstrated a decrease in discharge at a rate of 0.033 $m^3 s^{-1} yr^{-1}$ between 1964 and 2007. This slight decline over time may be indicative of a declining snowpack under a warming climate. Thus, the changes in nutrient loading between decades is almost entirely due to changes in nutrient concentrations.

Daily loading in 2004 and 2005 was calculated from daily discharge in these two years and loading relationships based on contemporary (i.e., post-dreissenid) data. The relationship between discharge and phosphorus loading using data from 1991 - 2004 are best described by exponential curves (Fig. 5.4C, F). For TP, Loading = 39.0 e $^{(0.68\ \text{Discharge})}$ (r^2= 0.780). For SRP, Loading = 10.3 e $^{(0.64\ \text{Discharge})}$ (r^2= 0.678). The relationship between inorganic nitrogen loading and discharge rates were best described with linear equations. For NO_3^-, Loading =

51.1 + 1101 Discharge (r^2= 0.672) and for NH_4^+, Loading = 11.3 + 45.8 Discharge (r^2= 0.339) where loadings are in units of mg nutrient s^{-1} and discharge in m^3 s^{-1}.

Nearshore nutrient concentrations and *Cladophora* nutrient quotas

Peak discharges from Oakville Creek were recorded in March and April, reaching 10.1 and 10.3 m^3 s^{-1} in 2004 and 2005, respectively (Fig. 5.5). Base flow conditions, as low as 0.1 and 0.2 m^3 s^{-1} in each year, respectively, were measured as early as late May in 2005, but storm events and high discharges were measured in May and June in 2004 and base flow was not reached until July in 2004.

Coherent patterns between P loading from Oakville Creek, ambient nutrient concentrations, and *Cladophora* nutrient quotas were evident during the spring growing period in both years, 2004 and 2005 (Fig. 5.6). Storm events, and thus discharge, were large and frequent in spring 2004 during the initiation of *Cladophora* growth, while in 2005, base flow conditions were established before the start of *Cladophora* biomass accumulation and persisted through the growth of the first cohort. High discharge rates led to P loading rates (TP and SRP) that were up to an order of magnitude higher in spring 2004 than in spring 2005 (Fig. 5.6). Immediately following a large P loading event on 24 May 2004, P concentrations increased, with TP concentrations reaching a peak of 34 μg L^{-1}. *Cladophora* P quota (Q_P) was higher on the next 2 subsequent sampling dates, reaching 0.90 %P (by DM) on 9 June and 0.14 %P on 15 June 2004. During the spring *Cladophora* growing period (i.e., May to mid-June), no spikes in ambient P concentrations, or *Cladophora* Q_P, occurred in the absence of a large catchment loading event. In both years, upwelling events were never associated with peaks in TP or SRP (Fig. 5.6).

Other patterns in ambient P concentration were not temporally associated with catchment loading, but were consistent with the timing of *Cladophora* growth and senescence. In both years, as *Cladophora* grew, Q_P concentrations and ambient P concentrations generally declined (Fig. 5.6). Following the brief spike in Q_P in spring 2004, tissue concentrations declined rapidly towards the minimal cell quota, and in spring 2005, *Cladophora* Q_P declined steadily from a high of 1.3 % on 17 May 2005 to a low of 0.6% by 23 June 2005. Ambient total P and SRP concentrations declined throughout the growing season of the first *Cladophora* cohort, apart from the spring spike in 2004, until *Cladophora* detached or began to slough. On

an areal basis, the peak P concentration retained in *Cladophora* was much higher in 2005, despite higher loadings of P from the catchment in 2004, indicating that currently catchment loading is not strongly correlated with P retention, between years.

Following a major storm event in summer 2004, *Cladophora* biomass became detached on 24 June 2004 (Chapter 2). Following this detachment, TP concentrations climbed to 24.4 $\mu g\ L^{-1}$ on 28 July 2004 and SRP climbed to 6.4 $\mu g\ L^{-1}$ by 10 Aug 2004. In 2005, *Cladophora* did not detach suddenly, but instead sloughed gradually as the tissue senesced (Chapter 2). Similar to 2004, however, TP and SRP concentrations increased following peak *Cladophora* biomass; from 3.7 $\mu g\ L^{-1}$ to 11.5 $\mu g\ L^{-1}$ for TP and from below detection limits (<0.02 $\mu g\ L^{-1}$) to 3.8 $\mu g\ L^{-1}$ for SRP.

In contrast to seasonal P dynamics, relationships between inorganic N loading, dissolved inorganic N lake concentration, and *Cladophora* N quota (Q_N) were not strong (Fig. 5.7). Inorganic N loading from Oakville Creek was dominated by NO_3^-. As with P loading, inorganic N loading in May and June was much greater in 2004 than 2005. Dissolved NO_3^- concentration in 2004 initially declined from a high of 702 $\mu g\ L^{-1}$ on 18 May 2004 to a low of 256 $\mu g\ L^{-1}$ on 1 Jun 2004, but then remained above 300 $\mu g\ L^{-1}$ for the rest of the sampling period. In contrast, in 2005 NO_3^- concentrations declined throughout the entire sampling season from 782 $\mu g\ L^{-1}$ on 5 May 2005 to 139 $\mu g\ L^{-1}$ on 21 Sept 2005. Ammonium concentrations did not have a consistent seasonal trend, but did generally decrease between 25 May and 13 July in 2004 and between 25 May and 21 July in 2005. In 2005, NH_4^+ concentrations rose again through the rest of the sampling period. Upwelling events were not associated with spikes in nitrate or ammonium concentrations.

Cladophora Q_N, in general, declined in parallel with Q_P through the growth of the first cohort in both years (Fig. 5.7). The second growth cohort in 2004 exhibited high Q_N, concurrent with high Q_P. While seasonal changes in Q_N were consistent with seasonal changes in Q_P, there were differences in Q_N between years that did not appear to be associated with differences in Q_P. The lowest Q_N concentrations in 2004 were nearly 2-fold higher in 2005.

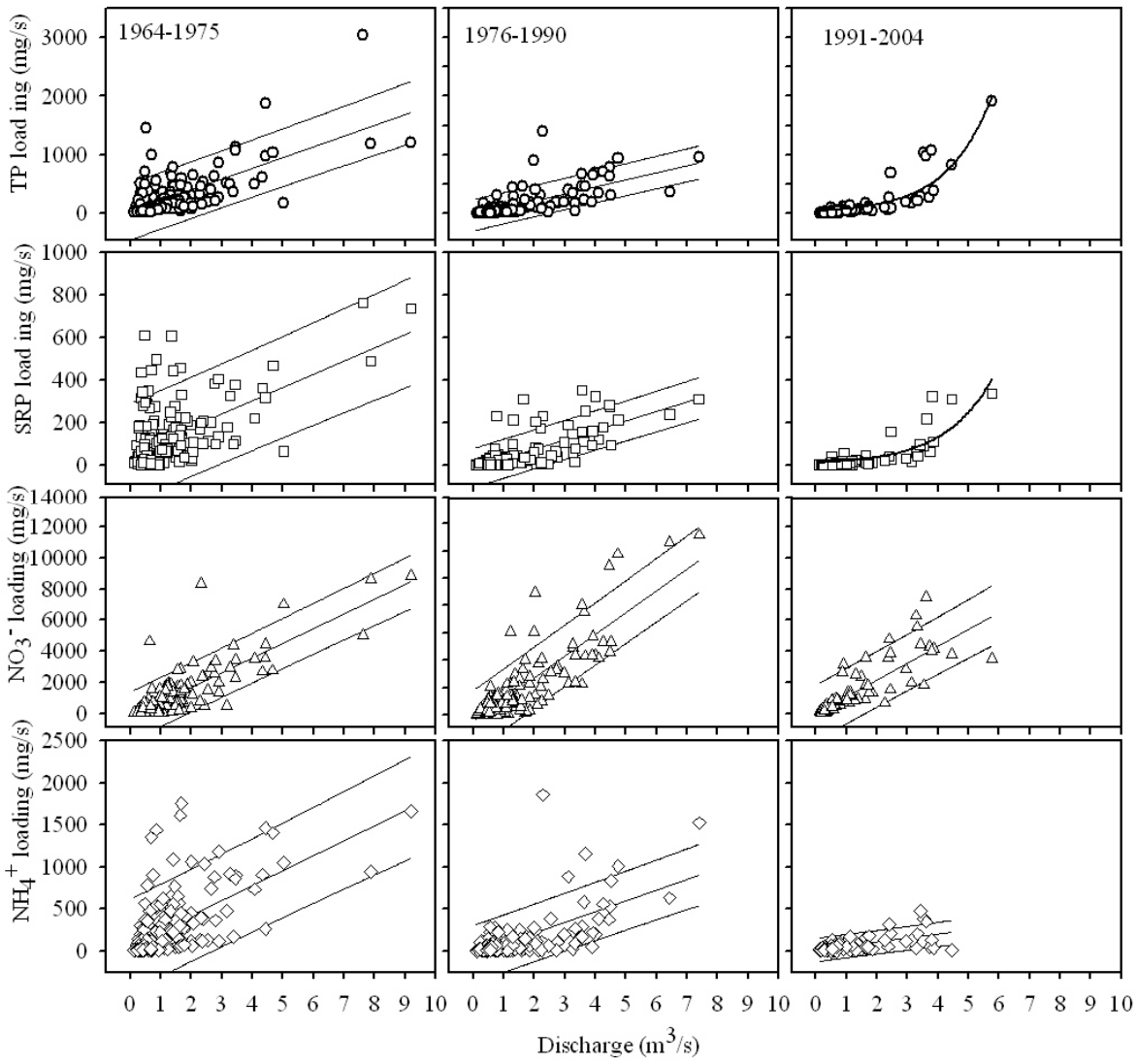

Figure 5.4 Discharge of Oakville Creek versus nutrient loading to Lake Ontario, with curves of best fit and 95% prediction intervals. The curves of best fit were linear regressions for all data except phosphorus relationships in the post 1991 datasets which were defined by exponential growth equations. Note that the ordinate scales are different between panels.

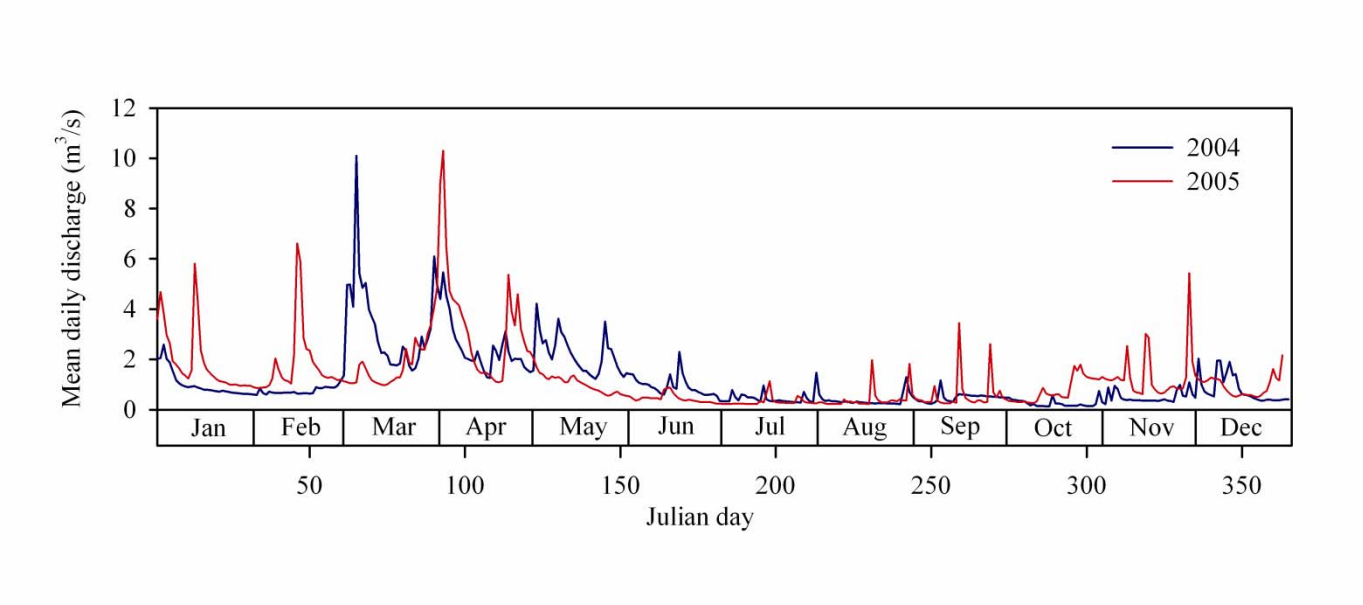

Figure 5.5 Discharge of Oakville Creek (16 Mile Creek) at Milton during the 2 years of study. Data are from Water Services of Canada.

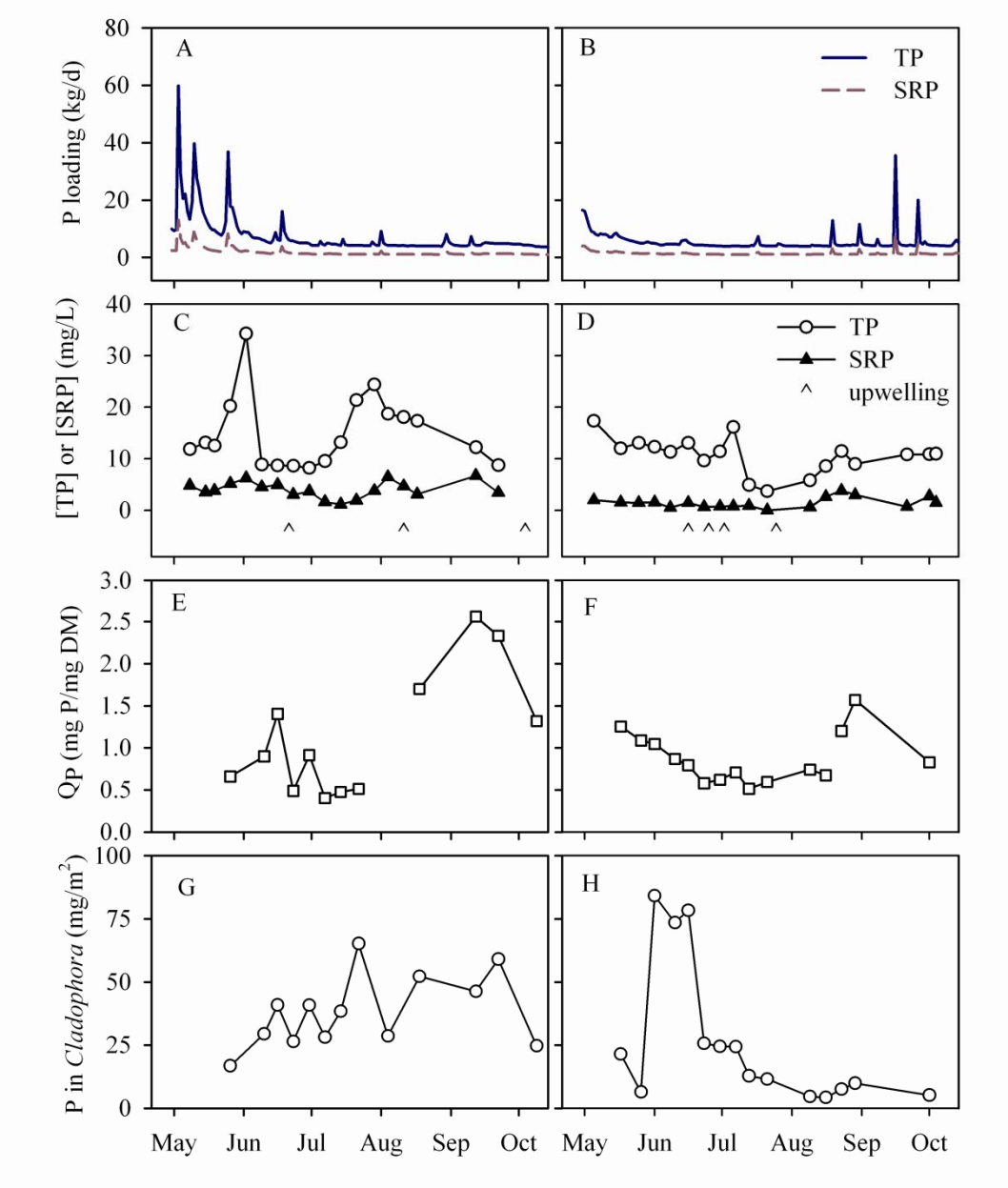

Figure 5.6 Phosphorus loading from Oakville Creek (A, B), P concentrations suspended in the water column measured at 2.5 m depth (C, D), biomass-specific P concentration in *Cladophora* tissue at 2 m (E, F) and the areal P concentrations (G, H). In panels E and F, data are divided into 1st and 2nd cohorts. Panels on the left side are 2004 data and panels on the right are 2005 data.

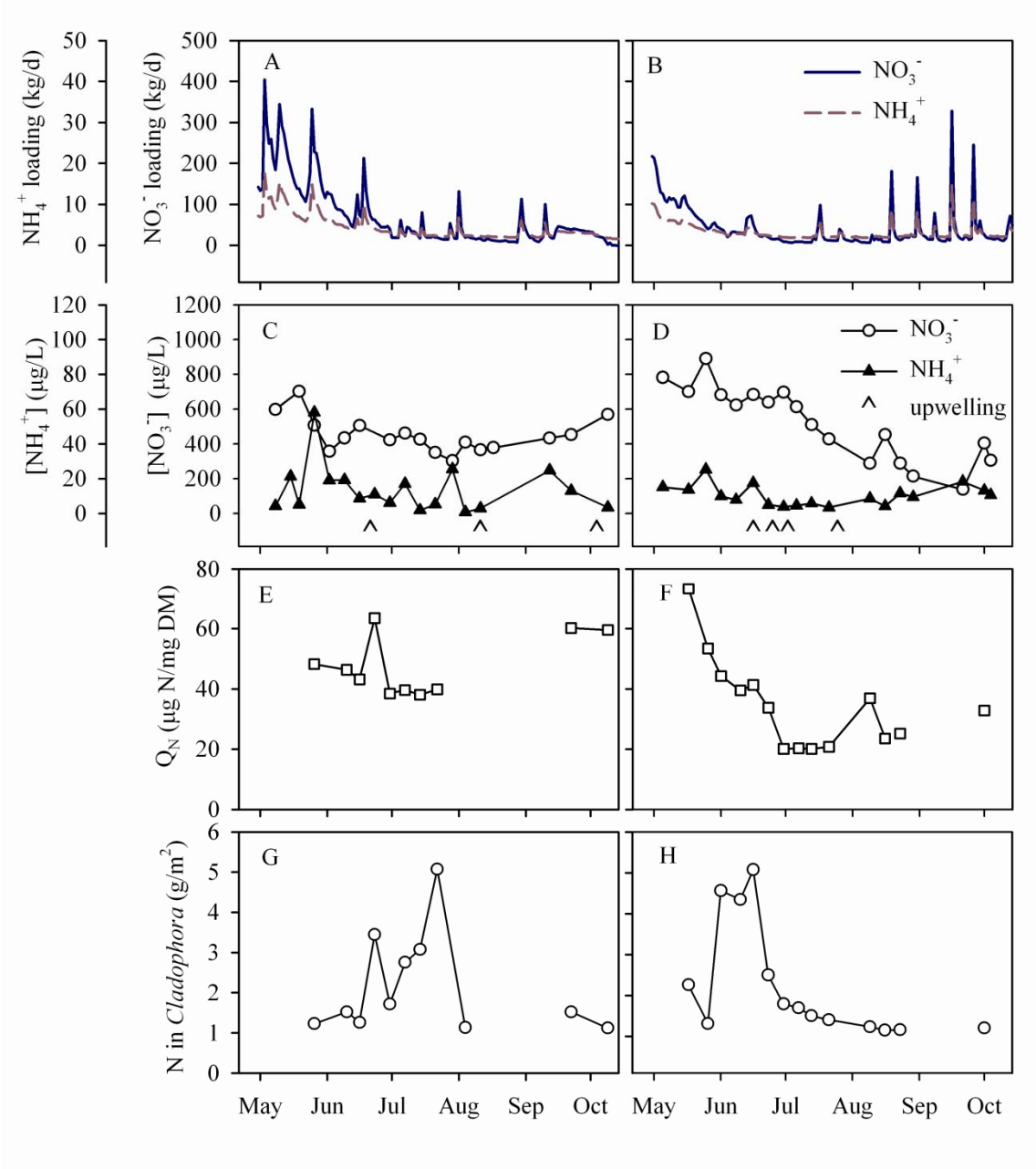

Figure 5.7 Nitrogen loading from Oakville Creek (A, B), N concentrations suspended in the water column measured at 2.5 m depth (C, D), biomass-specific N concentration in *Cladophora* tissue at 2 m (E, F) and the areal N concentrations (G, H). In panels E and F, data are divided into 1st and 2nd cohorts. Panels on the left side are 2004 data and panels on the right are 2005 data.

Cladophora P uptake versus catchment P influx

Estimated daily P supply from Oakville Creek did not meet the simulated daily P demand of the nearby defined area of *Cladophora* cover, except during spring storm events occurring in 2004 (Fig. 5.8). Due to the slower *Cladophora* growth in 2004 relative to 2005, P uptake rates by *Cladophora* during the first cohort of growth were predicted to occur later in 2004, and to peak at a lower magnitude than in 2005 (Fig. 5.8). The depth of maximum P uptake in both years was predicted at the depth of predicted maximum biomass, between 2 and 4 m. The influx of TP to Lake Ontario from Oakville Creek was estimated to average 4.5 kg P d^{-1} during baseflow conditions in 2004 and 2005. Periods of high loading caused by large precipitation events resulted in estimated peak P inflow of 39.7 kg P d^{-1} during the spring 2004 *Cladophora* growing season. The predicted P demand of proximal *Cladophora*, peaked at 63.7 kg P d^{-1} in 2004 and 128 kg P d^{-1} in 2005.

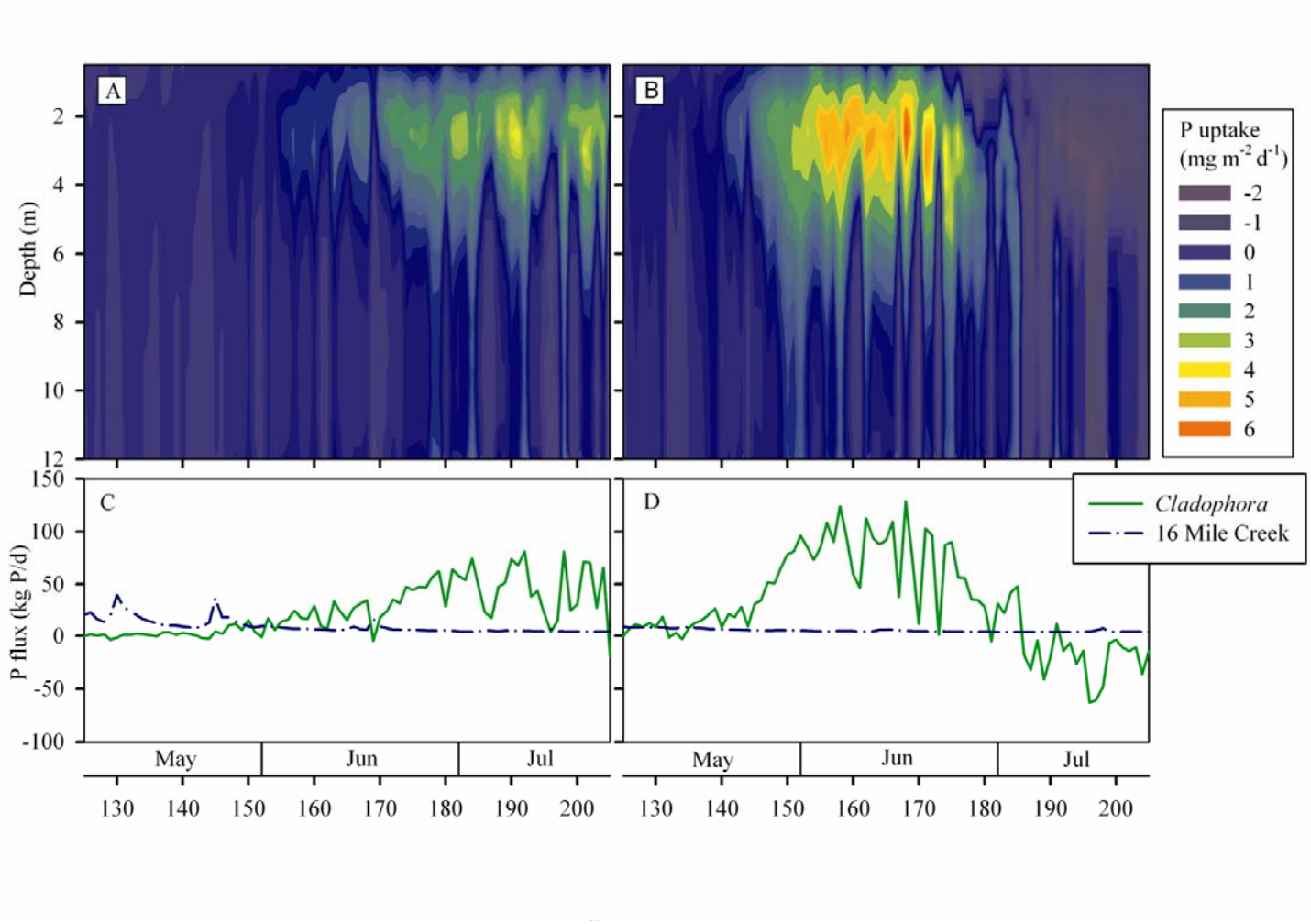

Figure 5.8 Depth-resolved predicted daily P uptake rates by *Cladophora* across the growing season of the first cohort (A, B) and predicted P uptake by a 500 m wide stretch of *Cladophora* (at 20% coverage) down to 12 m depth plotted against measured total P loading from Oakville Creek. Left panels are 2004 data and right panels are 2005 data.

Discussion

Historical changes to nearshore and offshore Lake Ontario phosphorus and nitrogen concentrations

Nutrient abatement, mandated by the binational Great Lakes Water Quality Agreement of 1972, led to decreases in catchment phosphorus loading through the 1970s and 1980s primarily due to removal of phosphate from laundry detergents and P removal at waste treatment plants. The effects of these changes are readily seen in the decreases in nearshore and offshore total phosphorus (TP) and soluble reactive P (SRP) concentrations prior to 1990, presented in Fig. 5.3. These data complement the decreases in nearshore P concentration, attributable to nutrient loading reductions, shown by Nicholls et al. (2001) for Lakes Ontario, Erie, Michigan and Huron during the 1970s and 1980s using concentrations measured from municipal drinking water intake pipes around the lakes. The highest TP concentrations were consistently found nearshore, but since dreissenid mussel establishment in the early 1990s, nearshore and offshore concentrations have become very similar (Fig. 5.3). This observation is consistent with the hypothesis that mussels are effectively retaining particulate matter, including catchment sources, in the benthos (e.g., Hecky et al. 2004). Interestingly, while post-dreissenid SRP concentrations are also very similar between nearshore and offshore stations, in the 1970s and early 1980s, this fraction of the P pool was higher offshore than the nearshore, consistent with biological assimilation of SRP in the nearshore during these decades (Depew et al. 2006). In contrast, the highest concentrations of inorganic nitrogen were found nearshore. The highest NO_3^- concentrations were found near the mouths of tributaries on the south shore of Lake Ontario, near agriculturally dominated catchments, and the highest NH_4^+ concentrations were found prior to the mid 1980s along the north shore of Lake Ontario, primarily near urbanized catchments. At 3 m depth at Oakville, as expected, there was greater variability in nutrient concentrations than at 20 m Environment Canada nearshore stations. The high variability in NO_3^-, particularly, highlights that this site is likely strongly influenced by tributary inputs.

Cladophora: a seasonal regulator in nearshore nutrient budgets?

Despite TP concentrations remaining below the targets of the 1972 Great Lakes Water Quality Agreement (GLWQA) of 10 μg L^{-1} for Lake Ontario during much of the summer, the accumulation of the fast-growing nuisance macroalga, *Cladophora glomerata*, is evidence of excessive nutrient supply to the littoral zone. The effect of *Cladophora* growth on the nearshore nutrient environment appears to be important on a seasonal scale, acting alternately as a sink and then a source of nutrients each year. *Cladophora* growth begins in the late spring in Lake Ontario, at which time ambient nutrients and *Cladophora* P quota (Q_P) are at, or near, their highest observed concentrations. As *Cladophora* grows, C-fixation exceeds P uptake, causing Q_P to decline. At the same time, the P uptake of the increasing *Cladophora* biomass leads to a decline in TP and SRP concentrations. Following the spring/early summer rapid growth period, *Cladophora* becomes a nutrient source when it senesces and detaches. This was most evident in 2004 when there was a rapid loss of *Cladophora* (and inflow from the adjacent Oakville Creek remained at baseflow conditions), followed by an increase in ambient TP to above 30 μg L^{-1}, and subsequently an increase in SRP to above 6 μg L^{-1}, concentrations that qualify as characteristic of a eutrophic system (Chapra 1997).

Both catchment loading and attached macroalgal growth exert an influence on the seasonal dynamics of nearshore nutrient concentrations in Lake Ontario. During years with typical May/June meteorology for this region (e.g., 2005), storm events are rare and thus runoff and nutrient loading from the catchment during the growing season is minimal. However, in particularly wet years (e.g., 2004), storms can provide a surge in discharge volume with attendant increased P loading to the nearshore, which was manifest in 2004 as an increase in suspended TP and then in *Cladophora* P quota (Q_P). This increase in Q_P, putatively due to a catchment influx, however, was only transient during the growing season. Despite higher catchment loading, biomass accumulation in 2004 was not different than in 2005 (Chapter 2). This evidence indicates that local nutrient sources can be locally but temporarily important in increasing the growth potential of *Cladophora,* but only if higher nutrient concentrations are provided continuously or at regular intervals by other sources.

The upper limit of *Cladophora* biomass accumulation in Lake Ontario is set by light availability and P supply (Chapter 2). Nitrogen is currently present at concentrations in excess of demand by *Cladophora* in Lake Ontario. Ambient NH_4^+ and NO_3^- did not correlate strongly

with *Cladophora* growth, indicating that the ambient concentrations of inorganic N are predominantly governed by other processes. In contrast, ambient SRP and TP decreased through the spring and early summer when *Cladophora* was rapidly growing, indicating that *Cladophora* growth has a significant influence on P concentration in the nearshore of Lake Ontario.

Is catchment P supply sufficient to meet *Cladophora* seasonal P demand?

In order to assess if P supply from the catchment is sufficient to meet the P demand of benthic algal growth, the loading from Oakville Creek was compared with the P sequestration by an adjacent area of *Cladophora*. The comparison was designed to assess if catchment P was sufficient to meet the *Cladophora* demand, and so the maximum potential influence of the tributary was considered. The area of *Cladophora* growth considered potentially under the influence of Oakville Creek was a minimal estimate, to err on the side of underestimating the benthic demand for P. The zone of influence indicated by *Cladophora* Q_P concentrations that were measured at incremental distances away from a wastewater treatment plant at Harbor Beach, Michigan, Lake Huron, in 1979 appears to have been between 250 m and 500 m (their Figure 10; Canale and Auer 1982). During Oakville Creek low flow conditions (e.g., median flow May-Aug 2005 = 0.4 $m^3 s^{-1}$; minimum flow = 0.2 $m^3 s^{-1}$), typical of the *Cladophora* growing season, I estimated that the maximum range of prolonged influence was 500 m along shore. And, finally, to make an minimal estimate of P demand, I assumed only a 20% coverage of *Cladophora* although rocky substrate predominates in the area and higher cover is expected.

While the drainage basin for Oakville Creek (37400 ha.) is an estimated 78% rural (Aquafor Beech Ltd 2005), the creek passes through two substantial and growing urban areas, the towns of Milton and Oakville, and the shoreline of Lake Ontario is also highly urbanized. As such, there are many potential catchment sources of P to any given study site along the shoreline. There were 3 storm sewers in proximity to the study site: one 110m to the west, and another two at 260m and 460 m distance to the west (Fig. 5.2). These were not considered major sources in the summer because they only run during major storm events, which are generally infrequent during the *Cladophora* growing season. Furthermore, in an inventory of P supply to Halton Region (from Joshua's Creek in the east to Burlington Beach in the west),

storm sewers were found to supply less than 5% of the total catchment P during summer months (Aquafor Beech Ltd. 2005). The three closest potential nutrient point sources are the Ford Motor Company outfall (3 km to the east), the Southeast municipal wastewater treatment outfall (5 km to the east), and the Southwest municipal wastewater treatment outfall (5 km to the west of the study site). These were all expected to have minor direct influence on this study site, relative to Oakville Creek due to their relative distance and lack of detectable nutrient gradients beyond the immediate vicinity of those outfalls to the Oakville study site (Houben 2007).

Catchment loading during the growing season was shown here to be generally insufficient to meet the P demands of *Cladophora*. In addition, P loading from the catchment has tended to decrease over the time since the mussels became established in Lake Ontario (Fig. 5.4). Phosphorus loading was higher from Oakville Creek in 2004 than the following year, but the peak amount of P retained in *Cladophora* was much higher in 2005. This further substantiates that there is currently a disconnect between direct catchment supply and *Cladophora* retention, despite the temporary effects exerted by large storm events (e.g., spring 2004). These data show that warm and sunny years are more conducive to *Cladophora* growth, rather than years with large catchment loading (within the concentration ranges currently observed). Autochthonous recycling of P must be able to sustain the higher growth when meteorological condition are favourable for growth, despite the low measured concentrations of phosphorus in the water column.

This is further evidence to implicate dressinid mussels as sources of P to *Cladophora*. Other studies directly testing the mussels' potential as a nutrient source have shown consistently high P supply (although with high variability between studies). Conroy et al. (2005), for example estimated that *Dreissena bugensis* (i.e., quagga mussel) can supply 6.8 µg SPR gDM^{-1} hr^{-1} and its cousin *D. polymorpha* (i.e., zebra mussel) can supply 12 µg SPR gDM^{-1} hr^{-1}. Arnott and Vanni (1996) contend that *D. polymorpha* excretes 97 µg SPR gDM^{-1} hr^{-1}. The results presented here are consistent with the hypothesis that dreissenid mussels in the Great Lakes are serving to recycle suspended nutrients (as phytoplankton and detritus) into dissolved excretions (for uptake by *Cladophora*) as defecated feces and rejected pseudofeces, causing the pelagic pool of TP to decrease and the benthic pool of cycling P to increase (Hecky et al. 2004) and enable the resurgence of *Cladophora*.

This conclusion does not imply that point sources cannot have a substantial local impact. On the contrary, during high discharge events in 2004, P supply by the Creek was in excess of predicted demand, which was demonstrated by a transient increase in *Cladophora* tissue P (Fig. 5.3). Furthermore, it is obvious from Figure 5.1 that long term annual catchment P loading is the ultimate source of total P to the lake. High external loading has historically been a driver of nuisance macroalgal biomass. However, the recent resurgence of *Cladophora* along Oakville shorelines does not appear to be directly driven by catchment loading. This study indicates that in the current nearshore lake environment, nuisance densities of *Cladophora* may not be sustained directly by catchment sources of P. Rather, recycled P from within the nearshore appears to be necessary to account for the P retained in *Cladophora* biomass during its growing season. Currently, *Cladophora* remains P limited throughout its growing season at shallow depths (Chapter 4). All additional nutrient sources in proximity to well lit, shallow waters with suitable substrate, can cause even greater biomass accumulation of this nuisance macroalga. Because of the increased clarity of the dreissenid dominated nearshore, any future increases in P loading would aggravate the *Cladophora* problem. If Oakville Creek is illustrative of what is occurring in other urban catchments around Lake Ontario, then it appears that the nutrient sources driving a *Cladophora* resurgence are now different than historically.

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Chapter 6 Summary of Conclusions

Throughout the lower Laurentian Great Lakes, during the season in which lake water temperatures are above 10 ^{o}C, hard substrata in well-lit and well-flushed areas are generally dominated by the nuisance filamentous green alga, *Cladophora glomerata*. The depth to which this macroalga can thrive has increased over the past decade, in concert with dreissenid mussel invasion. Yet, in the absence of continuous historical records, quantifying the effects of perturbations on ecosystem processes remains a major challenge. To examine quantitatively how decadal scale anthropogenic changes to the Lake Ontario have affected the biomass accumulation of *Cladophora*, a numeric growth model was applied (Chapter 2). The model was calibrated and validated with direct measurements of seasonal biomass made over two years. The growth rate and biomass accumulation of *Cladophora glomerata* in Lake Ontario were well described by a *Cladophora* growth model (Chapter 2). The model was then used to analyse the effects of decreased light attenuation in the nearshore due to dreissenid mussels, changes in tissue phosphorus concentration (Q_P) due to the opposing forces of decreasing pelagic P concentrations and potentially increasing benthic P supply from mussels, and increasing summer lake water temperatures on the biomass of attached and sloughed *Cladophora* biomass in Lake Ontario. From an analysis of monitoring records of Secchi depth at a nearshore station in Lake Ontario (by Environment Canada), a slight increase in light transparency was shown between the 1970s and 1980s, concomitant with decreases in phosphorus loading and planktonic primary production, and a large increase in nearshore light transparency was observed from the 1980s and the first decade of 2000, concomitant with dreissenid mussel establishment. The effects of increasing light transparency were opposed by the effects of currently lower tissue P concentrations than in 1982-'83 and 1972. Currently, *Cladophora* biomass in western Lake Ontario is higher at depths greater than 3.5 m, but lower overall, per length of shoreline. Two major gaps in data were evident in conducting this research. Historical and contemporary nearshore optical properties remain grossly under examined. And, critically, quantitative estimates of *Cladophora* biomass and seasonal tissue phosphorus concentrations over the 20 year span between 1983 and 2003 in Lake Ontario make it difficult to assess directly if dreissenid mussels have been causing an increase in biomass since the early 1990s. Only continued monitoring will be able to confirm the magnitude of the current perceived resurgence.

Rates of benthic primary production are poorly quantified in limnology, in general, and in the Laurentian Great Lakes, in particular. Because *Cladophora* is the dominant component of the summer littoral flora on hard substrata in the lower Laurentian Great Lakes, knowledge of its production and seasonal growth dynamics is a prerequisite to constructing nearshore nutrient and carbon budgets, and, by extension, essential to assessing the relative importance of the benthos to nearshore or whole system processes. The objectives of Chapter 3 were to estimate diurnal and seasonal epilithic net primary production in western Lake Ontario and to compare these rates on an areal basis with planktonic productivity. Rates of net primary production were measured *in situ* employing a carbon-based methodology with constantly flushing water, which alleviated issues associated with artificially stagnant water conditions and the error associated with using a photosynthetic quotient when calculating net C-fixation based on oxygen exchange measurements. This method constrained issues associated with calcite precipitation by measuring primary production during short incubation times (e.g., 15 minutes). Contrary to my initial expectations, rates of photosynthesis, which were measured at 1m depth, were sustained at biomass-specific maximal rates (P^B_m) from within 30 minutes of sunrise on most dates. Seasonal *Cladophora* P^B_m rates were high in the spring, and rapidly declined to net negative rates within 3 weeks, as peak biomass was achieved. The directly measured rates of NPP were well simulated by the *Cladophora* growth model (Chapter 2) during the first cohort of growth. Using the model to simulate depth-integrated *Cladophora* primary production, and using the best available depth-dependent estimates of planktonic primary production in Lake Ontario, I estimated that benthic primary production represented 70% of the total primary production from the shoreline to the 12 m depth contour.

The rates of maximum *in situ* photosynthesis were highly dynamic, ranging from –21.9 to 28.1 mgC g DM^{-1} d^{-1} through the growing season (Chapter 3). Based on stoichiometric ratios (C:P and N:P), *Cladophora* was shown to be P limited during its entire growing season (Chapter 4). The degree of limitation increased through the season as C was fixed at a faster rate than P was taken up. Light limitation was inferred from the observation that following biomass decline, biomass-specific photosynthetic rates increased, even while still exhibiting the same degree of phosphorus limitation. While changes in surface irradiance through the day did not detectably affect the achieved photosynthetic rates measured *in situ* (Chapter 3), decreases in canopy cover did result in increased biomass-specific rates of photosynthesis

(Chapter 4). Light limitation was caused by light attenuation through the canopy ($K_{d\text{-canopy}}$), which was measured to be 24.1 ± 3.3 SD m^{-1}. Thus, increases in surface irradiance have an lower affect on biomass-specific *in situ* productivity than does removal of canopy cover. Pulse amplitude modulated (PAM) fluorometry supported previous evidence that this macroalga is well adapted to high irradiance, exhibiting much lower photoinhibition of photosynthetic efficiency than phytoplankton in general (Chapter 4). Fluorometric measurements however, were not responsive to nutrient limitation, indicating that this alga achieves a successful balance between light absorption and utilization under nutrient deficiency *in situ*, consistent with other results for cultured phytoplankton in balanced growth, and in contrast with phytoplankton experiencing pulses of limiting nutrients in fertilization experiments.

Nearshore and offshore P concentrations have declined sharply since the 1970s. In the early 1970s, offshore concentrations of total phosphorus (TP) were lower than nearshore concentrations, while at the same time, concentrations of soluble reactive phosphorus exhibited the opposite pattern (Chapter 5). Since the early 1990s, both TP and SRP concentrations have decreased and are no longer detectably different between nearshore and offshore, based on synoptic lakewide surveys of Lake Ontario. An examination of a tributary in Halton region to indicated that the drivers of nuisance benthic macroalgal production may have changed since the 1950s-1970s when *Cladophora*'s biomass was closely linked with nutrient point sources and excessive nutrient loading from non-point sources. A comparison of estimated P supply from the Oakville Creek and estimated P demand from adjacent growing *Cladophora*, demonstrated that direct catchment supply could not account for the P retained by the standing stock of proximal *Cladophora* (Chapter 5). If Oakville Creek is illustrative of what is occurring in other urban catchments around Lake Ontario, then the nutrient sources driving a *Cladophora* resurgence are now different than historically. Further investigation of phosphorus cycling in the nearshore, with attention to dreissinid mussel metabolism, is clearly needed.