The interaction of ultraviolet radiation, dissolved organic carbon and primary production by Laurentian Great Lake phytoplankton communities

by

Daniel T. Hamilton

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Author's Declaration

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.

I understand that my thesis may be made electronically available to the pubic.

Abstract

Man-made chemicals are continuing to erode the ozone layer, and recovery of the ozone layer is slow. Therefore aquatic ecosystems are continuing to receive elevated ultraviolet radiation (UVR). The aim of this work was to investigate and quantify the impact of ultraviolet light on photosynthesis at various near-shore Great Lakes sites (Hamilton Harbour, Lake Ontario, Bay of Quinte, Woods Bay, Georgian Bay) using both oxygen metabolism and carbon assimilation. Enhanced UVR suppressed primary production at all stations when compared to incubations with photosynthetically active radiation (PAR) only. The average decline in net O_2 production was greater than 90% and sometime exceeded 100%; that is, enhanced UVR often produced net consumption of oxygen. Carbon was always assimilated with UVR+PAR exposure, although C-assimilation declined on average between 40 and 60% relative to PAR only, so there must have been some photosynthetic activity taking place as well as enhanced oxygen consumption. The oxygen consumption appears to be biotic, as tests for abiotic oxygen consumption failed to demonstrate any O_2 loss.

Dark respiration after 4-h light treatments with PAR or PAR+UVR was sometimes different, but not in a consistent way even at the same site. For example, at Hamilton Harbour on two occasions respiration after the PAR+ UVR exposure exceeded the PAR only treatments, while on the remaining dates dark respiration after the PAR-only treatments exceeded the PAR+UV treatment. While there is some evidence of enhanced dark respiration as a result of exposure during incubation in enhanced

ultraviolet radiation, it appears that the decline in net photosynthesis is caused mainly be the decline in gross photosynthesis rather than an increase in respiration.

Carbon assimilation was suppressed under enhanced ultraviolet radiation, and the degree of suppression was negatively related to the 14-day cumulative average of ultraviolet radiation (at 295 nm) prior to the experiment. I interpret this as evidence that phytoplankton to adapt to UVR exposure. Neither chlorophyll nor DOC appeared to be important factors.

Bacterial productivity also responded to the PAR + UVR irradiation compared to the PAR alone incubations although the response appeared to vary with season. Experimental PAR + UVR incubations carried out during the mid season appeared to have higher production during the 12 h post-treatment dark incubation compared to incubations under PAR, while those from later in the season tended to respond with suppressed productivity in the PAR + UVR incubations compared to the PAR alone.

Acknowledgments

I would like to thank my supervisor, Bill Taylor, for his support and direction. I would also like to thank Elaine Garner for her support and advice as well as financial assistance. There're so many others I'd like to thank such as Sergei and Amanda for the company during late-night titration marathons. Tim for his company during sampling sessions on Lake Ontario with thunder clouds overhead. I know I've missed many of you, so please don't be offended if your name isn't here. Last but not least I'd like to thank Kim for her support, advice and friendship during my stay at Waterloo.

Dedication

I dedicate this thesis to my wife Vicki, who supported me through all the ups and downs of this endeavor. I would never have been able to complete this thesis without you being there.

Dan

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Chapter 1

Introduction:

In 1998, David Schindler published an article entitled "A Dim Future for the Boreal Waters and Landscapes" (Schindler 1998). In this publication, he explained that our aquatic systems are being heavily impacted by human activities on a global scale. He identified the "Triple Whammy" of ozone depletion (which allows elevated levels of ultra-violet radiation (UVR) to reach the earths surface), global warming and acid deposition that act synergistically to degrade aquatic systems. While this article focused on the boreal zone, an article he co-authored (Magnuson et al.1997) examined possible influences of the "Triple Whammy" on the Laurentian Great Lakes and Precambrian shield regions of North America. It indicated that many of the aquatic systems in this region are at risk as a result of these stressors, that our understanding of the processes resulting from climate change, ozone depletion, and acid stress are poorly understood, and that models of those processes contain many uncertainties (Magnuson et al.1997)

In the ten years since Schindler's original 1998 publication, a considerable amount of research has been completed in this area, but questions still remain. Models of the interaction of these variables on the Laurentian Great Lakes remain an important area of research and while acidification is not an issue in the Great Lakes they are subjected to increased fluxes of UVR (Schindler 1997, Cockell et al.2000, Pemberton et al.2007, Urban et al.2007).

It has become apparent that dissolved organic carbon (DOC) plays a significant role in rapidly attenuating UVR in aquatic systems (Schindler et al.2003, Schindler 2001, Prairie 2008, Zepp et al.2007), and shields many aquatic organisms from the full impact of UVR exposure (Schindler 2001). DOC also affects primary production by altering overall light quantity and quality, and secondary production by providing a substrate for bacterial growth (Cole et al.2007, Hader et al.2007). A greater understanding of the interaction of DOC, UVR and primary production in aquatic systems will help in our understanding of the geochemical cycling of carbon (Zepp et al.2007).

UVR effects in an aquatic system are not limited to primary producers or to the bacterial community; zooplankton are affected by elevated UVR levels (Zellmer et al.2004), as are fish and other organisms (de Mora et al.2000). Ciliates have been shown to respond negatively to UVR, although the response appears to be genus specific (Sanders et al.2005). Ferrero et al. (2006) found that UVR impact on *Daphnia pulex* was greatest when both *Daphnia* and their food sources were exposed. UVR caused damage to the gut and they reported a decrease in amylase and cellulase, two digestive enzymes.

Autotrophic and heterotrophic microbes, along with inputs of nutrients and terrestrial organic carbon, largely determine the net metabolism of the pelagic zone of a lake. Sobek et al.(2007) suggested that input of dissolved organic carbon (DOC) results in many lakes being net heterotrophic, and as a result they are net producers of CO_2 which will be released to the atmosphere.

This thesis will focus on aquatic primary production, DOC and community metabolic response to UVR exposure in the Laurentian Great Lakes as this system provides water to 10's of millions of people, supports a commercial and sport fishery, as well as recreational use. It is important to understand how increased fluxes of UVR will impact this important chain of lakes.

Primary Production and Photosynthesis:

Photosynthesis in biological systems is the production of chemically stored energy from electromagnetic energy by living organisms (Blankenship 2002). The source of this energy is the sun, although a small fraction of primary producers use chemical energy, or heat from oceanic volcanic vents, for their production (Blankenship 2002). Some percentage of the energy "fixed" by a primary producer is consumed by respiration to support cellular activity (del Giorgio and Williams 2005).

In aquatic systems, primary production has been measured in a variety of ways, from measuring algal biomass changes, to measuring dissolved oxygen production or carbon assimilation, and is usually related to time and volume or area (Wetzel 2001). The production rates of phytoplankton are regulated by physical factors that include temperature and light, including both light quality and quantity. Temperature and light conditions of an aquatic environment are influenced by depth, solar input, light attenuation, mixing depth, mixing intervals and the depth of

the euphotic zone (Gervais and Behrendt 2003, Kohler et al.2001, Falkowski and Raven 2007).

The photosynthetic apparatus of most phototrophs relies on a linked system of H_2O oxidation and CO_2 reduction (de Mora et al.2000, Falkowski and Raven 2007). Chlorophyll (chl) is the major pigment involved in photosynthesis and comes in several forms. Chl-a is present in all algae and cyanobacteria, while chl-b, chl-c and chl-d are present in different groups of algae and have different absorption/fluorescence bands. Fluorescence is an energy dissipation method in photosynthetic organisms to release excess energy in high light environments and is also one path of relaxation of an excited electron to a ground state (Falkowski and Raven 2007). By examining the fluorescence profile of a lakewater sample using the proper excitation frequencies, an estimate of the algal biomass and community composition can be made (Beutler et al.2002).

Light excitation promotes a pigment to an excited state where it loses an electron to an acceptor molecule. Photosystems II and I (PS-II and PS-I) are responsible for capturing sunlight through a series of antenna pigments, which absorb the light and transfer the energy to the reaction centres of the two photosystems. PS-II and PS-I operate in series to produce photosynthetic electron flow used to power additional reactions within the cell (Hill et al.2004).

Photosystem II, is comprised of a number of proteins, with the D1 and D2 proteins at the core, along with others including the protein complex responsible for the oxidation of water to form O_2 and H^+ (proton). Water oxidation is a five step

process requiring 4 photons to produce 1 O_2 molecule and 4 protons, followed by a regeneration step (Blankenship 2002). The protons, by way of a proton pump, are used to produce ATP (Figure 1.1).

Light absorption in PS-II results in a rapid transfer of an electron from P680 to an electron acceptor, pheophytin. The primary charge separation is stabilized by a series of electron transport reactions both reducing and oxidizing sides of the PS-II system (Blankenship 2002). The electron from the pheophytin is transferred to quinone A (Q_A) and then to quinone B (Q_B). Q_B transfers the electron to an oxidized form of Q_B or plastiquinone from the quinone pool in the membrane of the chloroplast. The electron is then transferred to the cytochrome-b protein and, through a series of reactions, plastiquinone transfers the electron to the PS-I system. When a photon is absorbed by the PS-I system, an electron through a series of reactions is transferred to ferridoxin which is then used to reduce nicotinamide adenine phosphate-oxidase (NADPH) to $NADPH_2$ in the stroma of the chloroplast. $NADPH_2$ is used in the reduction of CO_2 to form sugars, which takes place in the Calvin cycle (Hill et al. 2004, Blankenship 2002).

Light driven electron transport generates ATP and NADPH, which are in turn used by the enzyme ribulose-bisphosphate carboxylase/oxygenase (runbisco) to fix carbon via the Calvin cycle, which produces 3-phosphoglycerate (PGA). The fixed carbon is processed for long-term storage into two forms (Blankenship 2002); starch, which is made and stored in the chloroplast, and sucrose, which is produced in the cytoplasm (Falkowski and Raven 2007, Blankenship 2002).

Rubisco may also react with oxygen instead of CO_2, and produces 2-phosphoglycolate and PGA. 2-phosphoglycolate inhibits the Calvin cycle and must be metabolized to PGA for the recovery of the Calvin cycle. The oxygenase activity of Rubisco is a wasteful process, which includes the light dependent uptake of O_2 and the release of CO_2, and is referred to as photorespiration but is different from normal mitochondrial respiration.

Aquatic respiration is the main fate of carbon fixed by photosynthesis. Del Giorgio and Williams (2005) define respiration as the process by which all organisms obtain vital energy from a variety of reduced compounds, and state that it represents the largest sink of organic matter in the biosphere. Respiration can be studied at multiple levels, but those of interest here are physiological level and the resulting ecological consequences. Physiologically, respiration is supported by the electron and proton (H+) flow through membrane-associated transport systems. The charge gradient and potential energy is coupled to ATP synthesis and can be used to drive "flagellar motors" and other metabolic processes (del Giorgio and Williams 2004). Del Giorgio and Williams (2004) also state that molecular oxygen supports aerobic respiration, while anaerobic respiration is found largely in bacteria. Additional electron acceptors found in aquatic systems include nitrate, manganese (Mn^{+4}), ferric iron (Fe^{+3}), elemental sulfur, CO_2 and quinine, functional groups found in humic acids (Falkowski and Raven 2007, del Giorgio and Williams 2004).
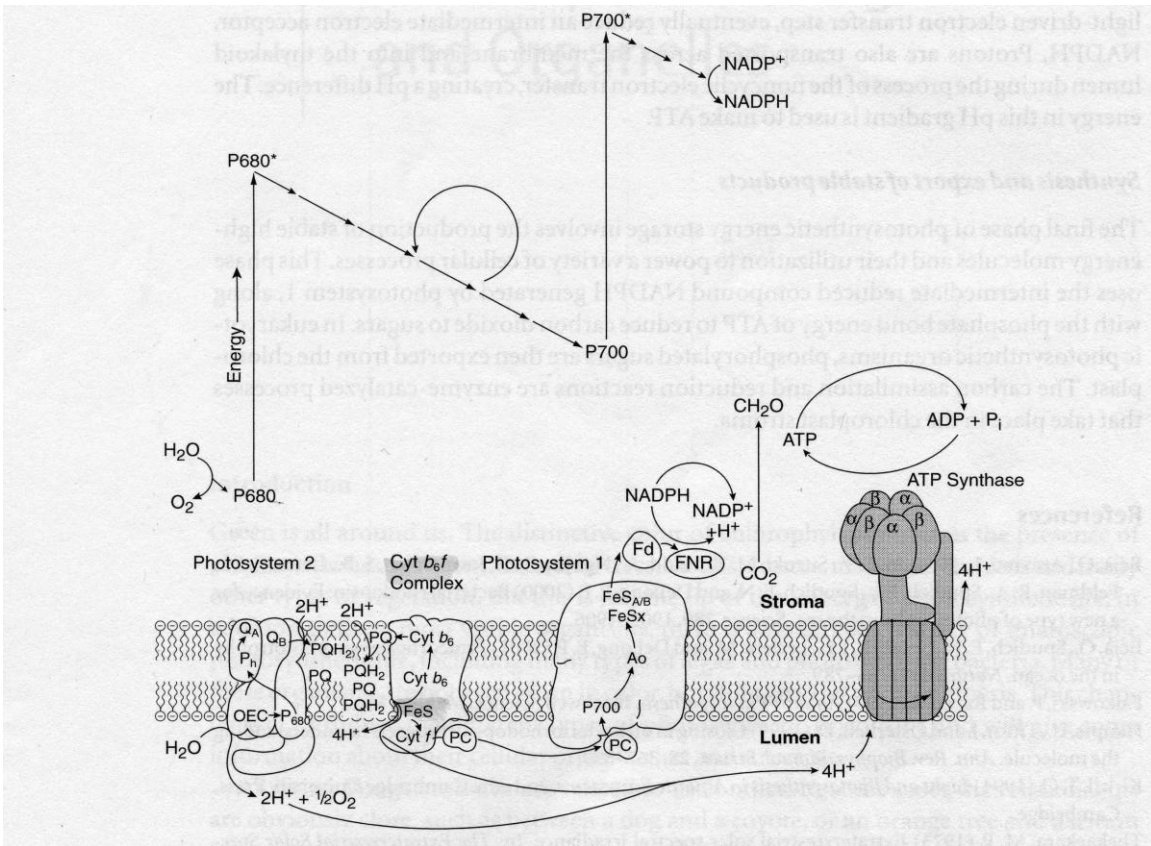
Figure 1.1 The "Z-diagram" of electron flow through the reaction centres as well as a cross sectional view of the various proteins and structures involved in light capture and energy conversion.

Pace and Prairie (2005), using data from the literature, showed that aquatic ecosystem respiration was dependent on the lake trophic condition and temperature, plus other factors such as carbon loading and community structure. When they regressed lake respiration against other factors, they found positive relationships with total phosphorus ($r^2=0.81$), Chl a ($r^2=0.71$) and DOC ($r^2=0.49$). These data suggest a stronger relationship of respiration rates to phytoplankton biomass than to DOC.

Solar Radiation and its attenuation:

Solar radiation covers a wide spectrum, from short wavelengths of less than 200 nm to very long wavelengths in the infrared range of greater than 1000 nm. The atmosphere attenuates much of this radiation and limits the shortest wavelengths (Figure 1.2). UV-B (280 to 320 nm) and UV-A (320 – 400 nm) comprise the ultra violet radiation that reaches the earth. UV-C (200 to 280 nm) is completely attenuated by the atmosphere and is largely responsible for the production of ozone which, in turn, attenuates all wavelengths of UV-R to some degree (de Mora et al.2000).

Ozone (O3) is a reactive oxygen species and is produced through a process represented by the following equations (de Mora et al.2000):

$$O_2 + h(\lambda < 240 \text{ nm}) \Rightarrow O + O$$

$$O + O_2 + M \Rightarrow O_3 + M$$
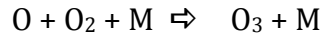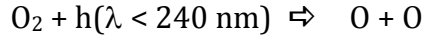
where h is light, λ is light less than 240 nm, and M is a collision chaperone that is unreactive but absorbs excess energy.

Ozone is itself destroyed by direct photolysis shown in the equation below:

$$O_3 + h(\lambda < 410 \text{ nm}) \Rightarrow O + O_2$$

Ozone may also be destroyed by:

$$O_3 + O \Rightarrow 2(O_2)$$
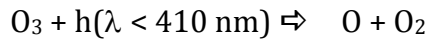
The rate of ozone destruction is slower than the rate of production, which results in the ozone layer that attenuates UVR.

Ozone may also be destroyed by reactions involving man-made chemicals:

$$O_3 + X \Rightarrow O_2 + XO$$

$$O + XO \Rightarrow O_2 + X \quad \text{or} \quad O_3 + XO \Rightarrow 2O_2 + X$$
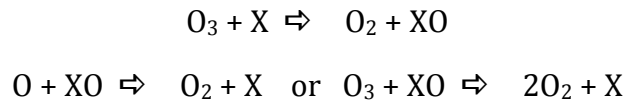
Where X is any one of NO, HO, Cl, I, Br.

Chlorofluorocarbons (CFCs) are produced anthropogenically and used as propellants and refrigerants because of their non-toxic, non-flammable and highly stable nature in the troposphere. In the stratosphere, under exposure to UVR, the chlorine-carbon bonds of the CFCs are broken and reactive chlorine species (Cl and
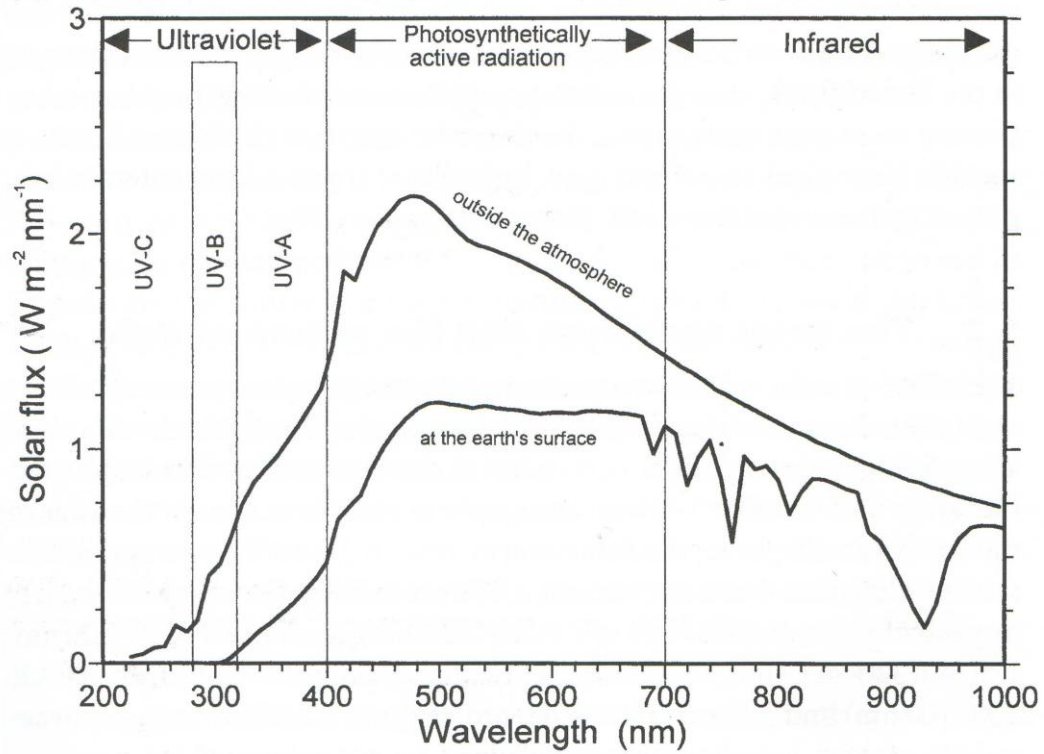
Figure 1.2 The relative energy by wavelength of the solar spectrum just outside the earth's atmosphere and at the earth's surface (from de Mora et al.2000).

ClO) are produced. These, in combination with polar stratospheric clouds composed largely of condensed nitric acid, destroy ozone. Studies by NOAA, the British Antarctic Survey and Environment Canada have shown a clear link between ozone depletion, atmospheric CFC levels and the resultant increase in UVR reaching the earth's surface (de Mora et al.2000).

McKenzie et al.(2007) state that "The Montreal Protocol is working" as concentrations of major ozone depleting substances in the atmosphere are decreasing. They caution, however, that a significant amount of the ozone recovery in the Northern Hemisphere can be attributed to changes in atmospheric circulation, and suggest that global climate change is expected to cause a decrease in temperature thus increasing the likelihood of severe ozone depletion due to the heterogeneous chemistry on the surface of polar stratospheric clouds. As the stratospheric temperature declines, for each degree Celsius a reduction of 15 Dobson units in ozone can be expected (Rex et al.2004). As approximately 50% of the ozone depletion in the mid latitudes can be attributed to the export of ozone-poor polar air, the impact of global climate change may be to reduce the rate of ozone recovery. However, there are too many variables to accurately predict long-term outcomes at the moment (McKenzie et al.2007) and the impact of higher UVR exposures on ecosystems remains an important issue.

In aquatic systems, sunlight is further attenuated by material in solution or suspended in the water column, as well as by the biota of the system. This

attenuation is biased, changing not only the quantity of light but also its spectral quality (de Mora et al.2000). As in atmospheric attenuation, the nature of the aquatic light environment is highly variable and, as reported by Zhang et al.(2007), UV-B attenuation coefficients can vary from 0.02 to 60 m^{-1}. Sommaruga (2001) studied 26 clear mountain lakes in the Pyrenees and Alps where the 1% UV-B irradiance depth varied from 8 m to 27 m. Numerous studies have demonstrated that DOC is largely responsible for UVR attenuation (Zhang et al.2007 and references cited therein).

With warmer winters possible as a result of global climate change, longer ice-free periods in many temperate and high latitude lakes will provide both greater exposure of the organisms in the water column and longer periods for UVR to photo-degrade dissolved organic matter (DOC), reducing its ability to attenuate UVR (Magnuson et al.1997, Schindler and Curtis 1997, Schindler et al.1996, Schindler et al.1990).

DOC is a major form of organic carbon in almost all aquatic ecosystems, and is defined operationally here as the fraction of organic matter that passes through a 0.45 μm filter (McDonald et al.2007 and references cited therein). Typically, the dissolved fraction is obtained by filtering the sample through a glass fiber filter such as a Whatman GF/F filter, which has an operational pore size of 0.7 um (Williams 2000), and it is highly likely that 40 – 90% of marine bacteria and perhaps 100% of viruses pass through these filters. Williams (2000) suggests that using ultrafilters with submicron pore sizes is giving a much more accurate measure of the dissolved

organic matter in a system, though many studies continue to use GF/F filters, e.g.,

McDonald et al.(2007).

DOC is a complex mixture of organic material that can be divided into humic

and non-humic forms (McDonald et al.2007). Humic substances fall into 2 different

classes of acids; humic acid which is not soluble at pH <2, and fulvic acids which are

soluble at all pH conditions (McDonald et al.2007, McDonald et al.2004). Non-humic

DOC is composed of lipids, carbohydrates, polysaccharides, amino acids and

proteins. Humic substances usually are defined as being large molecular weight

substances many of which have yet to be identified (McDonald et al.2007). There

remains considerable controversy over the bioavailability of humic DOC when not

irradiated. Boavida and Wetzel (1998) suggested that, for humic substances in

particular, the predominance of fulvic acids in an ecosystem may provide

thermodynamic stability to the metabolism of aquatic ecosystems. Water

discharges from sanitary systems appear to contain DOC primarily of the non-humic

class (Chen et al.2001).

Biological Impact of DOC, UVR and PAR interactions in Aquatic systems:

Dissolved organic carbon is also produced in aquatic environments through

excretion by organisms, cell breakage, and, in phytoplankton, extra-cellular release

of polysaccharides as a result of light stress (Panzenbock 2007). These are termed

autochthonous DOC. Allochthonous DOC is exported from catchment of the aquatic

system and can act as an external subsidy for production in the system. Sobek et

al.(2007) state that DOC is a key lake parameter that can affect microbial

metabolism, light climate and primary production. Some lakes have a distinct "tea"

colour to the water, which is fairly common in smaller Canadian Shield lakes as well

as other locations globally.

Chromophoric DOC (CDOC) can radically influence the light quality and quantity

in an aquatic system, and is dominated by terrestrially-derived organic matter

(Murphy et al.2008). It is a major absorber of PAR as well as UVR, so can reduce

primary productivity (Retamal et al. 2008). Photochemical processing of CDOC and

DOC can make it more labile, and Moran and Zepp (1997) suggest DOC may be

mineralized directly to CO_2. CO_2 may then be taken up by the autotrophs, or if there

is insufficient photosynthetic activity to use the CO_2 load, it may be released into the

atmosphere (Morn and Zepp 1997). Pahlow and Vezina (2003) found

photochemical processing made labile DOC refractory. Biddanda and Cotner (2003)

found that in water samples from the euphotic zone of Lakes Superior and Michigan,

bacterial productivity was actually reduced by 51 to 71% after the water was

exposed to sunlight for several days (compared to dark controls). However,

bacterial productivity was enhanced by 54% compared to dark controls if the water

was taken from the aphotic zone. River-water treated in the same fashion produced

enhanced bacterial growth by 229 and 266% compared to dark controls (Biddanda

and Cotner 2003).

UVR acts directly and indirectly on aquatic organisms. Its indirect action is

through changes induced in water chemistry, through the production of free radicals

such as singlet ROO*, superoxide O_3- and HO^+, as well as photo-activation of chemicals in the water column such as polycyclic aromatic hydrocarbons (PAHs) which may react with cell membranes (referred to as oxidative stress) (Kosian et al.1998). Photochemical bleaching of DOC, reducing its attenuating properties, results in UVR penetrating more deeply in the water column (Schindler 1998). Direct UVR damage may include the production of pyrimidine dimers in the DNA of exposed organisms. This damage must be repaired if the organism is to survive (de Mora et al.2000). Reduction in photosynthetic activity (Villanfane et al.1995), along with impaired motility and ability of algae to orient themselves in the water column, have been reported. UV-B, the most energetic form of UVR, is largely responsible for the damage mentioned above, however the role of UV-A in photoinhibition may be important in both freshwater and marine ecosystems since UVA intensity is much greater than UVB intensity especially below the surface (Smith et al.1998, Helbling 1992, de Mora 2000). UV-B also impacts proteins in the phytoplankton, damaging the D1 protein of the reaction centre, plastiquinone, and Rubisco. As discussed above, plastiquinone and Rubisco play important roles in electron transport chains of the photosynthetic apparatus and therefore in the productivity of the phytoplankton (de Mora et al.2000, Day and Neale 2000). Reactive oxygen species produced in the cell may react with lipids causing oxidation as well as additional cellular damage (de Mora et al.2000).

The law of reciprocity states that the number of photons absorbed results in a certain amount of effect, that is, if 15 quanta are absorbed by a molecule in 1 second it will have the same effect as if 15 of photons were absorbed over 1 minute

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(Markvart 2000). In biological systems, with active repair processes functioning within the organism, reciprocity does not usually hold (de Mora et al.2000).

Phytoplankton have evolved mechanisms to manage UVR radiation and high light environments (de Mora 2000). These adaptations include adjusting the amount of light absorbing pigment, adjusting their orientation, and releasing microsporine-like amino acids (MAAs). These are induced by exposure to UVR and offer some protection to UVR (Villafane et al.1995). Within the cell, both PAR and UVR exposure can result in the production of toxic radicals, and the chlorophylls can reach an excited triplet state. Carotenoids can function to convert the excited chlorophyll to its ground state through the xanthophyll cycle (van de Poll et al.2006 and references cites there in). Additionally, enzymes such as superoxide dismutase (SOD) scavenge reactive oxygen species (ROS) in the chloroplasts that are damaging to all biomolecules. Damage to the PS-II reaction centre is compensated for by a rapid turnover of the D1 protein. Some repair processes require light, such as the excision repair of DNA. This process results in the excision of the damaged DNA, re-synthesis of the damaged DNA sequence and integration of the new DNA to replace the photo-damaged sequence (de Mora et al. 2000).

The bacterial communities in aquatic systems are closely linked to the primary producers (Lee and Bong 2008 and references cited there in). Phytoplankton are the main producers of autochthonous DOC and bacteria are the main consumers. According to Cole et, al. (1998), bacterial biomass correlates with Chl-a and bacterial productivity correlates with primary productivity. It may be that in systems

dominated by exogenous inputs of terrestrial organic matter, bacterial and algal productivity may be less tightly coupled and bacterial respiration may dominate the community metabolism (Shiah and Ducklow 1993, Ducklow 2000). The response of bacteria to UVR is complex and appears to be linked to their nutrient status (Medina-Sanchez et al.2002). Medina-Sanchez et al.(2002) found that phosphorus-limited bacteria were negatively influenced by UVR, while phosphorus-replete bacteria were not. Xenopoulos and Schindler (2003) found that bacterial productivity in the presence of algae was suppressed by a 4 h exposure to PAR + UVR, but increased after 48 h of incubation.

Bacteria constitute a significant path for carbon and other nutrients through the microbial loop (Wetzel 2001). In addition to the uptake of nonliving organic matter, bacteria are grazed by ciliates and flagellates, which are then consumed by metazoans, passing carbon and other nutrients to high trophic levels within the ecosystem. Changes in bacterial metabolic activity in response to UVR may have a significant impact on the overall ecosystem metabolism.

Respiratory Response:

There is evidence that UVR reduces photosynthetic uptake of CO_2 and oxygen production. There is also evidence that respiratory processes tend to be higher in the light than the dark (Bender et al.1987, Grand et al.1989,Stelmakh 2000, Pringault, Tassas and Rochelle-Newall 2007). Tassas and Rochelle-Newall (2007) used highly sensitive oxygen electrodes to determine the respiratory activity in the

first few minutes after light exposure using a technique described by Falkowski et al.(1985). The authors were able to measure net community production (NCP), gross primary production (GPP) and community respiration (CR), and found that respiration in the light (R_{light}) was 640% greater than dark respiration (R_{dark}).

There are conflicting reports about the post-illumination community respiration in the literature. Beardall et al. (1997) examined both the inhibition and the recovery of photosynthesis and reported a decline in post-illumination respiration in several cultured algal species after exposure to UVR. Further, they found that UVR eliminated dark carbon loss in *Aphanizomenon flos-aquae.* Using three different species in culture, Beardall et al.(2002) reported no significant change in respiration as a result of UVR exposure.

Zooplankton respiration rates in light were found to be twice the rate of dark controls (del Giorgio and Williams 2005). Schindler (1968) and Pearcy et al.(1969) using *Daphnia magna* and *D. pacifica,* respectively, found no difference in respiration between light and dark respiration.

Hortnagl and Sommaruga (2007) studied photo-oxidative stress in 2 different strains of *Paramecium bursaria,* one containing a *Chlorella* symbiont and one without. Oxidative stress was higher in the aposymbiotic strain then the symbiotic strains. The authors suggested that symbiotic strains benefit from the light stress handling capabilites of the *Chlorella* and, while the authors did not examine respiration differences between the 2 strains, it appears likely that respiration would be greater in the symbiotic strain.

Many of the studies cited here used cultured organisms to obtain their results, while others have used natural populations incubated *in situ* in highly variable light conditions.

Objectives

The aim of this thesis is to examine on natural phytoplankton communities, the impact of elevated UVR on primary production from several locations in the lower and upper Great Lakes. The sites will provide a range of DOC concentrations, trophic status, and DOC sources.

In chapter 2, I use 2 sites, Hamilton Harbour and a near shore station in Lake Ontario, to examine the impact of elevated UVR on community oxygen and carbon metabolism, as measured using 2 standard methods; high precision Winkler technique (Carignan et al.1998) and carbon assimilation through the addition of ^{14}C-NaHCO$_3$ (Ostrom et al.2005). Hamilton Harbour is a eutrophic system and chl-a can be as high as 26 µg L^{-1} and total phosphorus 30 µg L^{-1}, while Lake Ontario is an oligo/meso-trophic system with chl-a reaching 4.1 µg L^{-1} and total phosphorus 9.22 µg·L^{-1} (Hamilton 2008 unpublished data). Aquatic community oxygen metabolism and light-driven carbon assimilation across three different light treatments, PAR only, PAR + UVR and dark, will be assessed by comparing O$_2$ concentration before and after incubation, and by measuring C-fixation with ^{14}C-HCO$_3$$^-$.

Chapter 3 will examine the impact of UVR on community oxygen and carbon metabolism as described above across a broader range of sites, including Hamilton Harbour and Lake Ontario, along with the Bay of Quinte, a eutrophic bay on Lake

Ontario and two oligotrophic sites in Georgian Bay; Woods Bay with higher levels of chromophoric DOC and an off shore location with clear water. The locations have different sources, quantities and qualities of DOC. The objective of Chapter 3 is to determine if DOC has an influence on community response to elevated levels of UVR. Hamilton Harbour is in a highly urbanized environment with significant levels of agriculture within the boundaries of the watershed, while the Bay of Quinte, though also eutrophic, is moderately urbanized and has a moderate level of agricultural usage in its watershed. Woods Bay is more pristine, with a series of interconnected oligotrophic lakes upstream providing the main water inflow of tea-coloured water. It has been suggested by Prairie (2008) that the land use in the watershed may influence the nature of the DOC entering the system.

I expect a negative response of oxygen production and carbon assimilation to the gradient of DOC concentration. However, in highly eutrophic waters such as the Bay of Quinte and Hamilton Harbour, algal biomass may be more important to attenuating the negative influence of UVR on the community.

Chapter 4 will explore the relationship of DOC sources and concentrations on post illumination bacterial productivity. The bacteria will be studied in whole lake water from each site used in Chapter 3 and will be exposed to the same light treatments. Bacterial response will measured through the assimilation of tritimized-thymidine. We will test if the DOC natural waters at the sample sites can be further processed to provide additional substrate for bacterial growth.

Chapter 2

The effects of ultraviolet light on Lake Ontario and Hamilton

Harbour phytoplankton.

Introduction:

Over the last several decades, nations of the world have stopped using chlorofluorocarbons (CFCs) as these disrupt the complex photochemistry of the ozone creation/destruction cycle. CFCs reaching the stratosphere shift the ozone cycle so that it favours ozone destruction, which caused a global reduction of ozone and a corresponding increase in the amount of ultraviolet radiation (UVR), mostly UVB, reaching the earth's surface (Schindler 1998). In the aforementioned paper, Schindler (1998) discussed the combined impacts of climate change, ozone depletion and acidification on aquatic systems, with special interest in the role of dissolved organic carbon (DOC). DOC plays a significant role in aquatic systems by attenuating electromagnetic radiation, including UVR, lessening its negative impact on organisms in the water such as phytoplankton (Schindler 2001). It also provides a substrate for bacterial growth (Lignell et al.2008).

UVR is known to disrupt photosynthetic processes in aquatic primary producers (de Mora et al.2000). The damage to these organisms usually occurs to the proteins of the reaction centres, to antenna pigments and to enzymes involved in the electron transportation mechanisms within the cell. UVR is also responsible for the production of reactive oxygen species such as singlet O_2, ROO^* and H_2O_2. Reactive oxygen species produced within the cell may react with lipids, causing their oxidation. Symptoms of cellular damage include changes in cell membrane permeability and reduction in motility (de Mora et al.2000). UVR affects most aquatic organisms, including zooplankton (Obertegger et al.2008), protozoa (Momo et al.2006, Sanders et al.2003) and fish (Olson

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and Mitchell 2006).   As phytoplankton are at the base of the food chain, it is important to fully understand changes in phytoplankton physiology, because since effects on primary productivity may be transmitted to higher trophic levels in the ecosystem. Photosynthesizing organisms also form an important carbon sink (Hader 1994) and any decrease in primary production may intensify global warming trends and result in further stratospheric ozone destruction (Schindler 1998, Whitehead et al.2000).

In autotrophs, UVR usually causes damage within PSII reaction centers at the D1 protein, which also contains the H_2O oxidation complex (Blankenship 2002). Damage to this centre must be repaired via synthesis of new D1 protein, which is then inserted into the reaction centre.   UVR (specifically UVB) damages plastiquinone, which plays an important role in the electron transport process. Rubisco, a complex enzyme that carries out the carboxylation step of carbon fixation, is also damaged by UVR (Blankenship 2002).

The DNA of UVR-exposed aquatic organisms is damaged through the production of pyrimidine dimers (Falkowski and Raven 1997, Fischer et al.2008 and references cited therein). Protein synthesis is required for the dimers to be repaired and for the cells to survive. The dimer is excised from the DNA strand, and new undamaged DNA is inserted. Some repair processes require light (Roy 2000).

Disruption of metabolism in the primary producers can cause a reduction in photosynthesis. This can be measured as a reduction in O_2 evolution, and can range from a few percent compared to phytoplankton not exposed to UVR to losses of 50% or

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higher. Rates of carbon assimilation are also suppressed, with declines of up to 50% or more (Beardall et al.1997, Schindler 1997).

Heterotrophic bacteria also play a significant role in driving the biogeochemical processes of an aquatic ecosystem. They are responsible for up to 90% of the total respiration of an aquatic system, with the highest percentage occurring in oligotrophic systems (Biddanda et al. 2001).

Bacteria and primary producers are closely linked in aquatic systems (Lee and Bong 2008 and references cited there in, Daufresne et al.2008). Phytoplankton are the main producers of autochthonous DOC and bacteria are its main consumers. Cole et al.(1998) found that bacterial biomass correlates with Chl-a and bacterial productivity correlates with primary productivity. In systems dominated by exogenous inputs of terrestrial organic matter, bacterial and algal productivity may be less tightly coupled and bacterial respiration may dominate the community metabolism (Ducklow and Shiah 1993, Ducklow 2000, Anusha and Asaeda 2008).

Heterotrophic bacteria constitute a significant path for carbon and other nutrients through the microbial loop (Wetzel 2001). In addition to the uptake of nonliving organic matter, bacteria are grazed by ciliates and flagellates, which are then consumed by metazoans, passing carbon and other nutrients to high trophic levels in the ecosystem. Metabolic changes in bacterial activity in response UVR may have a significant impact on overall ecosystem metabolism.

Some dissolved organic carbon is produced by the plankton (autochthonous DOC) and plankton may release DOC for several reasons, including as a response to light stress,

as suggested by Panzenbock (2007), through cell leakage and through cell death (Berman and Wynne 2005). Mycosporine-like amino acids (MAAs) are photo-protective molecules produced by a range of organisms (Sinha et al. 2007) including algae. MAA's are water-soluble low-molecular weight molecules that absorb light across a broad band from 310 nm to 360 nm (Karsten et al. 2007 and references cited therein, Sinha et al.2007). Karsten et al. (2007) identified MAA's from three different species of phytoplankton; *Stichoccoccus* sp., *Chlorella luteoviridis* and *Myrnecia incisa*. MAAs from the former two species absorb UV light most strongly at 324 nm, while the latter absorbs most strongly at 322 nm.

Blendow et al.(2006) found that in highly eutrophic lakes the algal biomass limits its own productivity through self-shading. The concept of phytoplankton self shading affecting their own productivity is not new. Talling et al. (1972) studied phytoplankton in Ethiopian soda lakes and found primary production was severely limited by algal biomass.

Sommaruga and Augustin (2006), in studying one alpine lake, found the attenuation of UVR had no relationship to the concentration of coloured dissolved organic matter (CDOM), but was highly correlated to the temporal changes in phytoplankton Chl-a. Further, they determined that the DOC in the ecosystem was of autochthonous origin. It follows that higher algal biomass could result in greater protection from UVR, whether because of autochthonus DOC released by the phytoplankton or because of self-shading.

In this chapter I present the results of the experiments I conducted during 2003 at a site in Hamilton Harbour and one nearby in Lake Ontario. I tested the following hypotheses:

1. The inhibitory effect of UVR on photosynthesis, at exposures known to be inhibitory from previous studies, will be of a similar magnitude regardless of the trophic status of the site (oligotrophic versus eutrophic). Alternatively, photosynthesis in water collected from Hamilton Harbour, the more eutrophic system, may not be suppressed to the same degree as photosynthesis in water collected from the more oligotrophic site in Lake Ontario (Blindow et al.2006).

2. Hamilton Harbour, with its higher allochthonous inputs, will respond with a decoupling of autotrophic and heterotrophic production. This will be observed as divergence of the effects of UVR on net oxygen evolution and on carbon uptake (^{14}C assimilation) in Hamilton Harbour. The divergence will be observable in the ratio of oxygen evolved to carbon assimilated, as bacterial respiration will be subsidized by the allochthonous inputs and will be increased by photochemical oxidative processes driven by UVR (Anusha and Asaeda 2008).

3. Incubation in PAR, PAR + UVR and in the dark will not change post- incubation respiration rates. Alternatively, dark respiration may be elevated in response to UVR damage. Many of the damage repair processes require protein synthesis, which may result in elevated respiration. This would be observed as an increase in dark respiration over the 12-h dark incubations. UVR may also increase respiration through the generation of substrate for heterotrophs.

Materials and Methods

Two sites in western Lake Ontario were selected for this study based on their different trophic status, DOC concentrations and light environments. Site 1 is located at N 43° 17' 43.4" W 79° 50' 51.5" just north of the centre of Hamilton Harbour (Figure 2.1) and is referred to in this work as "HH". Hamilton Harbour is eutrophic, with a high level of urbanization along the north shore and heavy industry along the south shore. Site 2 is located in Lake Ontario at N 43° 17' 09.6" W 79° 43' 43.8" (Figure 2.1) in a near-shore location that was easily accessible by our 18' boat, yet had characteristics of an offshore location with lower TP and Chl-a, and higher water clarity. It is referred to in this work as "LO".

Water was collected approximately every two weeks from June 3 to September 23, 2003. On each visit to the stations, a light profile was obtained with a quantum sensor (Li-COR, Inc.), measuring PAR at 1-m intervals. The light extinction coefficient (k_{PAR}) was determined for each sample date and site using the linear regression of the natural log of the irradiance versus depth (Kirk 1994). Temperature profiles were measured using either a YSI 6600 or YSI 600XLM sonde (YSI Inc., USA).

All samples were obtained from the epilimnion using an integrated sampler and were passed through a 200-μm mesh to remove large zooplankton while filling several darkened carboys. The carboys were subsequently transferred to insulated coolers for transportation to University of Waterloo where experimental incubations

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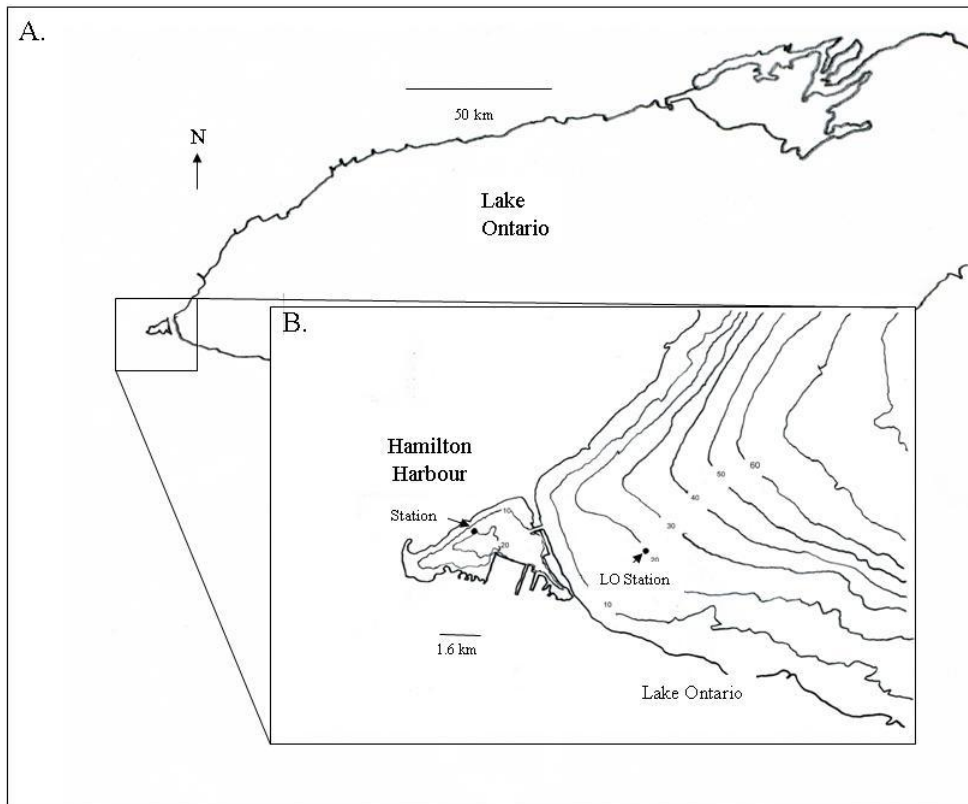
Figure 2.1 A. Map of the eastern portion of Lake Ontario. Inset B is a bathometric map showing the sample stations.

were completed. Water samples were also collected for measurements of total phosphorus (TP), dissolved inorganic carbon (DIC) and chlorophyll-a (Chl-a). Samples for phytoplankton enumeration were fixed using Lugol's Iodine.

Experimental Incubations:

All light incubations occurred under simulated solar radiation in a light chamber consisting of a metal frame lined with corrugated plastic. This was, in turn, lined with a highly reflective MYLAR ™ film. Two 1000 watt Sun Master high pressure sodium metal halide lamps (Part # 80149) provided PAR, which was supplemented with UVA and UVB (S.N.E. Ultraviolet Co.). The spectral composition of the simulated solar radiation was measured using an Oreil Instaspec diode array spectroradiometer (Oreil Corp., Stanford Conn.) equipped with an integrating sphere. The light spectrum generated by this system provided the incubating samples with light of 90% PAR, 9% UV-A and 1% UV-B when measured in μwatts cm^{-2}, with a total photon density of 1160 μM photon m^{-2} sec^{-1}. Cut-off filters were used to exclude any UV-C from the PAR + UV incubations, and UVR from the PAR only incubations. Filter cut-off efficiency was tested with the spectroradiometer.

A circulating water bath was used to maintain sample temperature during the light incubations (Figure 2.2). The samples were brought to a common temperature that was the average temperature of the 2 sites when there was a difference in temperature between the Hamilton Harbour station and the Lake Ontario station. When there was a difference, Hamilton Harbour was warmer.

The 12-h post-treatment dark incubations used a water bath in a large, upright incubator. Sample temperature was maintained within 0.5 C during the incubations. All light incubations lasted approximately 4 h, with the carbon uptake measurements starting 1 h after the start of the oxygen change measurements. Standard 300-ml BOD bottles were used for the PAR and dark incubations, while custom quartz BOD bottles were used for samples exposed to UVR. Quartz test tubes with a sample volume of 55 ml were used for C^{14} uptake. Dark incubations were wrapped with several layers of aluminum foil, but were incubated in the same chambers as all other samples. Three replicates were used for each light treatment and station (Figure 2.3).

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High Pressure Sodium Lamp

Experimental Samples

UV Lamps

Water in tray

Circulating Water bath
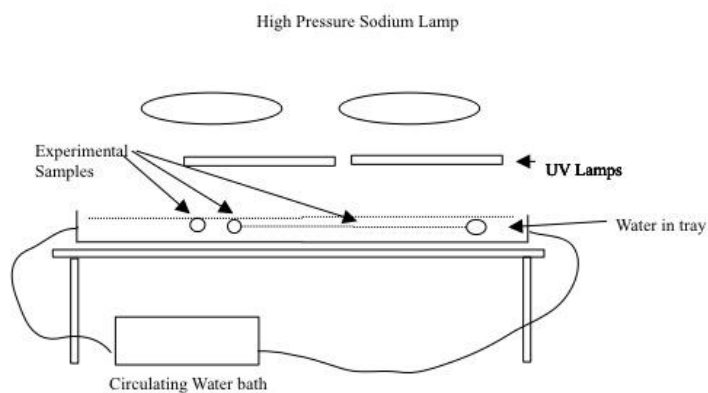
Figure 2.2: Cut away diagram showing the general configuration of the experimental chamber. The high intensity pressure sodium lamps each had a large white-lined reflector, reflecting light toward the work bench. Not shown are the walls which stood around the edge of the bench, which were covered with a highly reflective mylar. The light field was 1160 µM photons m^{-2} sec^{-1}.
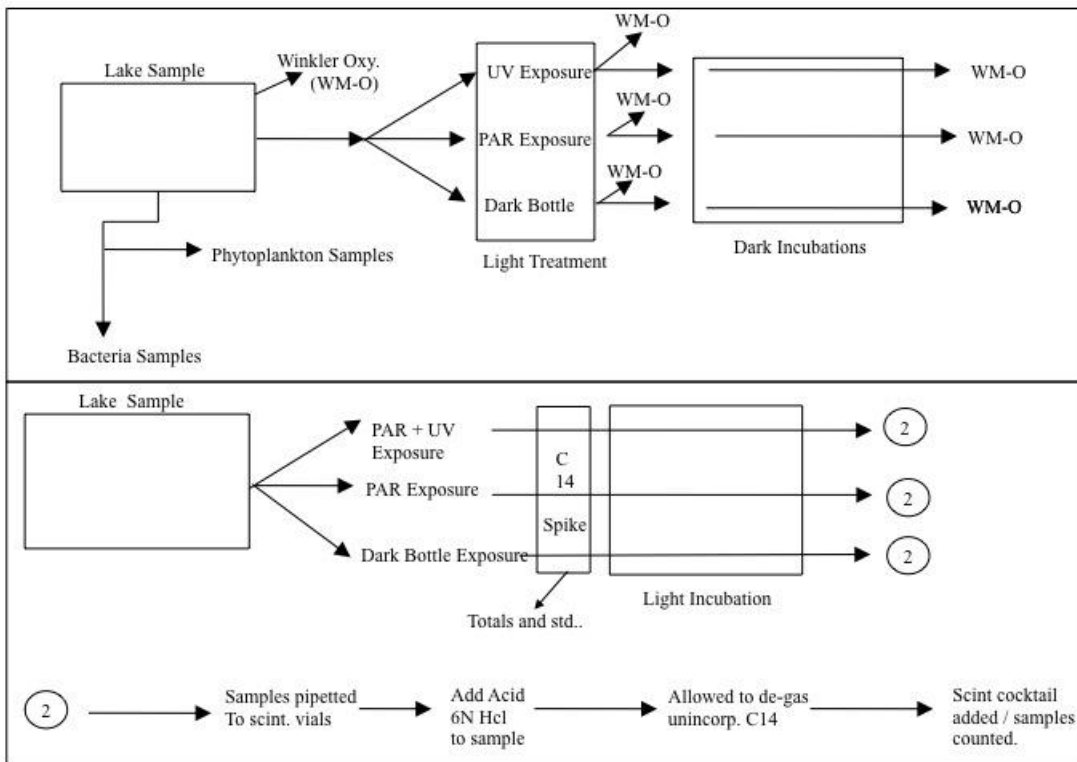
Figure 2.3: A diagram showing the activities and durations for the step followed in processing the experimental treatments. The top part of the figure shows the steps used for measuring the oxygen levels during the experiment, while the lower part of the figure shows the steps used in measuring the carbon assimilation. All light incubations were 4 h in length while the dark incubations (oxygen only) were 12 h in length.

All glassware that came in contact with the samples was washed with Extran cleaner, and well rinsed with deionized water. This was followed by an acid wash for several hours. After acid washing, the glassware was rinsed three times in de-ionized water, and three times with high purity water obtained from a Barnstead water purification system.

Metabolic Measurements

The high performance Winkler technique of Carignan et al.(1998) was used to measure dissolved oxygen concentrations. Time zero (T_0) BOD bottles were fixed immediately after all BOD bottles were filled, and titrated using a Mettler-Toledo DL 50 titrator to determine the initial O_2 concentrations. The titrations for T_0 were done as soon as the experimental incubations were started. All other titrations were completed within 2 to 3 h of the end of the experiment.

At the end of the 4-h light incubation (T_4), oxygen in the BOD bottles was fixed and titrated as above. The oxygen concentrations from each treatment were averaged and the standard deviation was determined. Oxygen evolution was determined using the following equations:

$(O_2T_{4\ light}) - (O_2T_0) =$ net O_2 evolution

$(O_2T_0) - (O_2T_{4\ dark}) =$ dark respiration

net O_2 evolution + dark respiration = gross O_2 production

$(O_2T_4) - (O_2T_{12}) =$ post illumination respiration

All measurements were expressed as hourly rates.

To test for abiotic oxygen consumption in PAR and PAR + UVR treatments, whole lake water was sterilized by filtration during the August 21st, 2003 field trip. Three T_0 samples of filtrate were fixed immediately after all the BOD bottles were filled and three bottles of filtrate were incubated under PAR, PAR + UVR and in the dark for 4 h. All 12 bottles were titrated for dissolved O_2 as described above.

Carbon assimilation experiments were completed in the same light chamber, using the same material for cut-off filters, the same circulating water bath and the same light treatments were used as in the oxygen work described above. Three replicates for each light treatment were used as well, but I took three sub-samples per replicate in order to account for pipet and scintillation counting errors. Water samples were inoculated with ^{14}C-sodium bicarbonate (ICN Biomedical; 0.02 µCι mL^{-1}) and then dispensed into the quartz test tubes, capped with Teflon stoppers and placed in the light incubator for 4 h. Total activity (200 µL) samples were collected to validate the isotope additions. At time zero, triplicate 5-mL subsamples from each treatment were acidified with 100 µL of 6 N HCl. After 24 h, 15 mL of EcoLume™ scintillation cocktail (ICN Pharmaceuticals) were added to each vial. Dark uptake was subtracted from the light uptake to produce light-driven carbon assimilation values for the PAR and PAR + UVR incubations.

At the completion of the incubations, triplicate 5 mL samples from each replicate were placed in scintillation vials and 100 µL of 6 N HCl were added to each vial. After 24 h, Ecolume™ was added to each sample as described above.

To identify if any changes that occurred in the assimilation of carbon and the production of oxygen within the community, the molar ratio of evolved dissolved oxygen and light driven carbon assimilated was used. This ratio is commonly referred to as the photosynthetic quotient.

Additional experiments to test for effects of bottle type on C-fixation were also completed using ^{14}C uptake with whole lake water. Each test was done in triplicate for each test vessel used in this work.

Statistical methods:

All data were tested for normality and heteroscedasticity. When required, data were transformed, usually with a $\log_{10}$ transformation, and then retested. SPSS™ 11.0.0 was used for ANOVAs, and SigmaStat™ 3.5 was used for simple t-tests (unless stated otherwise) and regression analyses. SigmaPlot™ 9.0 was used for some regression analyses and graph production. A significance level of 5% ($P \leq 0.05$) was used for all statistical tests. The software above were running on a Windows XP ™ virtual machine using VMWare Fusion™ on a Mac Book™ with OS X 10.5.3.

Results:

Limnological Characteristics

The limnological characteristics of each sample site varied considerably over the season, but Hamilton Harbour generally had higher total phosphorus, chlorophyll, temperature and light attenuation (Table 2.1).

The water column of both sites stayed reasonably well stratified over the sampling season, though there was a distinct thinning of the epilimnion around the time of the July 8, 2003, sampling. The epilimnion in the Harbour changed from 15 m on June 8 to less than 3 m, while in Lake Ontario the epilimnion disappeared. The metalimnion reached the surface and temperature gradually declined with depth to about 14 m and, at this point, very significant temperature change began (Bocaniov 2008).

Light attenuation was greater for Hamilton Harbour (Table 2.1) and predictable from chlorophyll a ($R^2 = 0.7$), whereas light attenuation was lower and less predictable from chlorophyll a ($R^2 = 0.2$) at the Lake Ontario site.

Gross and Net Community Oxygen Production:

Pooling the PAR-only productivity data from HH and LO, there was a highly significant relationship between ln(chl-a) and ln(Net Primary Production$_{oxygen}$) (NPP$_o$) with an adjusted R^2 value of 0.75. For ln(Gross Primary Production$_{oxygen}$) (GPP$_o$) the R^2 was 0.52. Both regressions were highly significant ($P < 0.001$, Figure 2.4). This shows a good relationship between Chl-a and the amount of oxygen evolved in the PAR incubations for Hamilton Harbour and Lake Ontario. In constrast, the relationship between ln(chl-a) and ln(NPP) with UVR was much weaker ($R^2 = 0.19$). For HH alone, the relationship between NPP-UVR and Chl-a was negative, with an adjusted R^2 of 0.664 ($P = 0.016$). NPP-UVR for LO had a non-significant positive relationship with Chl-a, with an adjusted R^2 of 0.21 ($P = 0.169$). Accordingly, analysis of covariance uncovered that station and chl-a had significant interaction as independent variables predicting ln(NPP) under UVR.

Table 2.1. Chlorophyll-a, total phosphorus, temperature and PAR attenuation (K_{par}) by station and date. The units for PAR attenuation are m^{-1}.

Station	Date	Julian Date	Chl-a µg/L	TP µg/L	Temp. C	K_{par} m^{-1}
HH	09/06/2003	159	6.362	39.3	14.4	0.6739
HH	23/06/2003	173	22.966	51.4	19	1.1311
HH	08/07/2003	188	6.840	49.0	21	0.7728
HH	22/07/2003	202	12.170	24.2	20	0.9208
HH	05/08/2003	216	14.278	22.5	22.3	0.7484
HH	21/08/2003	232	8.118	19.6	24	0.7456
HH	29/08/2003	240	9.02	-	23	-
LO	09/06/2003	159	3.67	15.5	10.0	0.2390
LO	23/06/2003	173	4.206	10.6	13	0.3491
LO	08/07/2003	188	3.192	12.9	14.9	0.4392
LO	22/07/2003	202	2.303	10.3	15.5	0.2233
LO	05/08/2003	216	1.371	12.6	17.9	0.1955
LO	21/08/2003	232	1.549	7.1	20.5	0.2378
LO	29/08/2003	240	1.21	-	19.2	-

A.
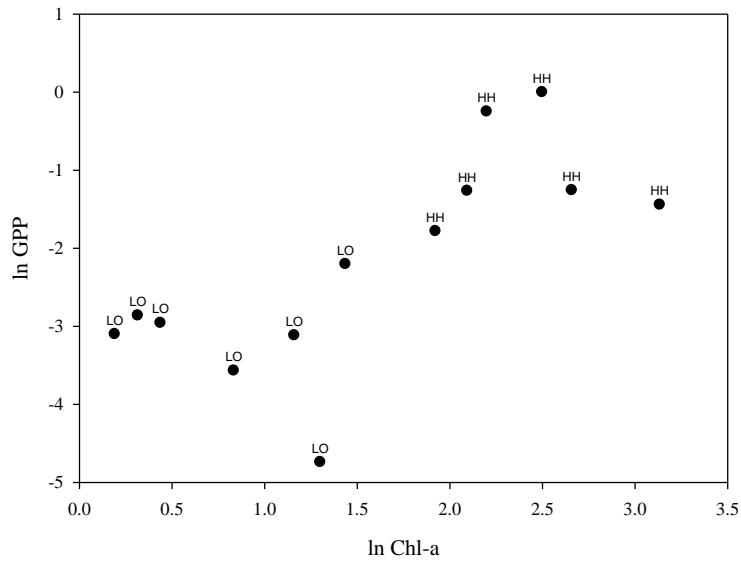
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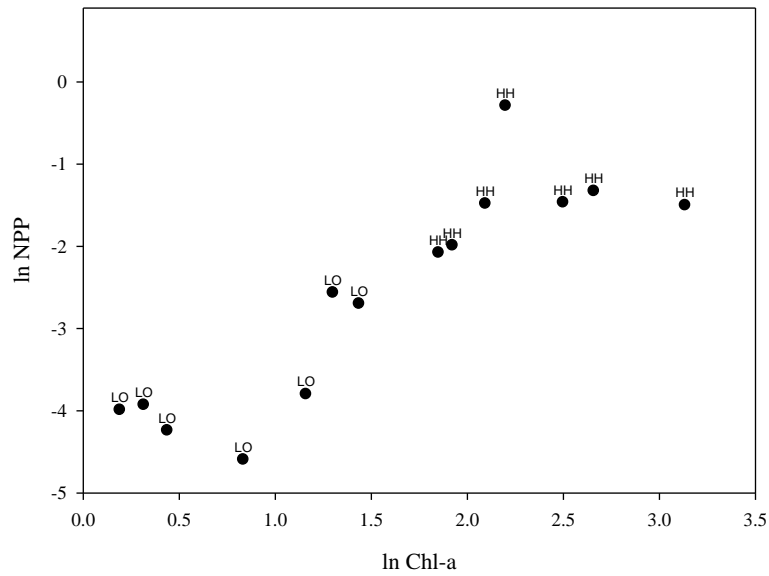
Figure 2.4 A. The relationship between (O_2) gross primary production (GPP) under PAR-only exposure and Chl-a for both the Hamilton Harbour and Lake Ontario stations. The adjusted R^2 = 0.52. Figure 2.4 B is the same relationship for net primary production (NPP). The adjusted R^2 = 0.75.

On all dates and stations, net O_2 production was lower in PAR + UVR incubations compared to PAR-only incubations (Tables 2.2, 2.3). There was a net loss of O_2 in the PAR + UVR incubations on many dates at one or both stations.

Gross community O_2 evolution was significantly reduced in the PAR + UVR treatments compared to PAR-only incubations for HH on all survey dates except June 9th (Table 2.2, Figure 2.5-A and B). On all dates, except June 9th, net oxygen production in PAR was significantly higher than in the PAR+UVR treatments and on the dates June 23rd through to August 5th, inclusive, PAR + UVR treatments consumed rather than produced oxygen (Tables 2.2-A and 2.2-B).

To test for abiotic oxygen consumption, dissolved O_2 in samples that had been filter sterilized and incubated for 4 h in PAR, PAR + UVR and dark were also incubated and showed no significant differences in dissolved O_2 among the treatments (1-way ANOVA, $P = 0.38$). A T_0 sample, which was filtered and immediately fixed without incubation, was included in this test.

Lake Ontario water also displayed a decrease in gross community O_2 production when incubated with PAR + UVR compared to PAR alone (Table 2.3 A). Statistically significant differences were found on four of the seven dates (Table 2.3 A and Figure 2.6 A). Net oxygen production was suppressed in the PAR + UVR incubations compared to PAR alone on all dates and was significantly so on four of the seven dates surveyed, (June 23rd, July 8th, August 21st and August 29th). Oxygen was consumed in PAR + UVR on the last three of those dates (Table 2.3 B and Figure 2.6 B). Again, there were no significant differences in O_2 concentration when water was filter sterilized and incubated

Table 2.2. Community oxygen production for Hamilton Harbour for each date. The mean production and standard deviation for the PAR and PAR + UVR treatments are shown together with comparisons using t-tests. Units are mg O_2 L^{-1} h^{-1}.

A. Gross O_2 Production

Gross Prod.	HH PAR O_2 Production	Std Dev.	HH PAR + UVR O_2 Prod	Std. Dev.	t	p
June 9-03	-0.00493	0.05122	-0.02600	0.04623	1.649	0.188
June 23-03	0.23606	0.01226	-0.07474	0.02264	48.562	< 0.001
July 8-03	0.16840	0.00684	0.01890	0.00616	48.688	< 0.001
July 22-03	0.99780	0.01048	0.74077	0.00246	78.630	< 0.001
Aug 5-03	0.28480	0.01856	-0.03560	0.01720	37.959	< 0.001
Aug 21-03	0.28210	0.03935	0.13820	0.10878	3.732	< 0.002
Aug 29-03	0.78020	0.00616	0.15307	0.02526	72.357	< 0.001

B. Net O_2 Production.

	HH PAR O_2 Production	Std. Dev.	HH PAR + UVR O_2 Prod.	Std. Dev.	t	p
June 9-03	0.12521	0.03129	0.10415	0.02220	1.647	0.119
June 23-03	0.22320	0.00022	-0.08761	0.01904	64.746	< 0.001
July 8-03	0.13690	0.00660	-0.01260	0.00590	50.664	< 0.001
July 22-03	0.23080	0.01040	-0.02623	0.00210	72.676	< 0.001
Aug 5-03	0.26540	0.00700	-0.05500	0.00080	136.426	< 0.001
Aug 21-03	0.22770	0.01800	0.08380	0.10300	4.129	< 0.001
Aug 29-03	0.74870	0.00590	0.12157	0.02520	72.693	< 0.001

Table 2.3. Community oxygen production for Lake Ontario on each date. The mean production plus standard deviation for the PAR and PAR + UVR treatments are shown together with the t-test results.

A.   Gross O_2 Production

Date	LO PAR O_2 Production mg/L/hr	Std. Dev.	LO PAR + UVR O_2 Prod. Mg/L/hr	Std. Dev.	T	P
June 9-03	0.08740	0.05507	0.07899	0.06034	0.309	0.761
June 23-03	0.11051	0.02968	0.06804	0.02785	2.320	0.034
July 8-03	0.04430	0.01768	-0.02540	0.01834	8.207	< 0.001
July 22-03	0.02820	0.02897	0.01500	0.03396	0.891	0.386
Aug 5-03	0.05716	0.02277	0.04737	0.01156	1.150	0.267
Aug 21-03	0.05200	0.01858	-0.02120	0.02133	7.760	0.001
Aug 29-03	0.04510	0.01524	-0.02970	0.01845	9.387	0.001

B.   Net O_2 Production

Date	LO PAR O_2 Production mg/L/hr	Std. Dev.	LO PAR + UVR O_2 Prod. Mg/L/hr	Std. Dev.	T	P
June 9-03	0.07707	0.02315	0.06866	0.03383	0.616	0.547
June 23-03	0.06737	0.02500	0.02490	0.02280	3.766	0.002
July 8-03	0.02240	0.01119	-0.04730	0.01220	12.631	< 0.001
July 22-03	0.01010	0.02270	-0.00310	0.02880	1.080	0.296
Aug 5-03	0.01969	0.02180	0.00990	0.00950	1.235	0.236
Aug 21-03	0.01440	0.00500	-0.05880	0.01160	17.385	0.001
Aug 29-03	0.01850	0.00920	-0.05630	0.01389	13.469	0.001

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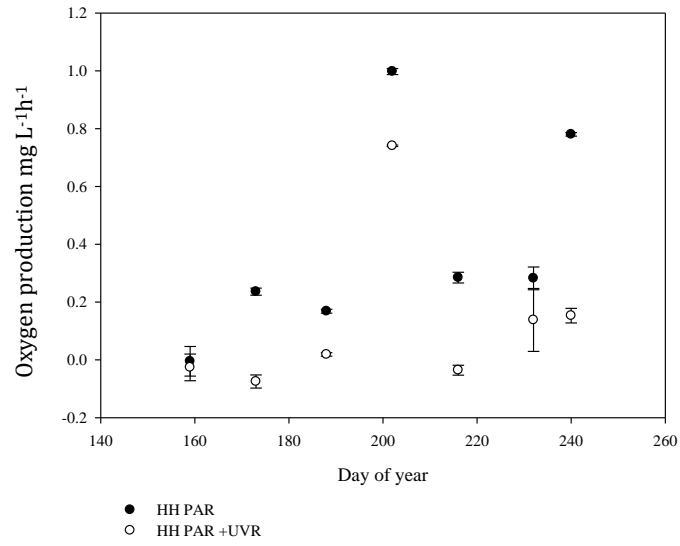
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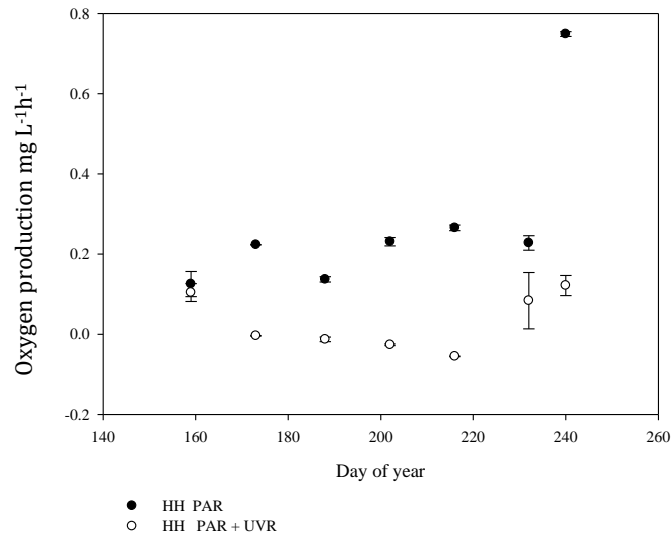
Figure 2.5 A. I GPP of Hamilton Harbour under PAR and PAR + UVR treatments. B. Is a similar graph for NPP (O$_2$) under PAR and PAR + UVR.

6 A.
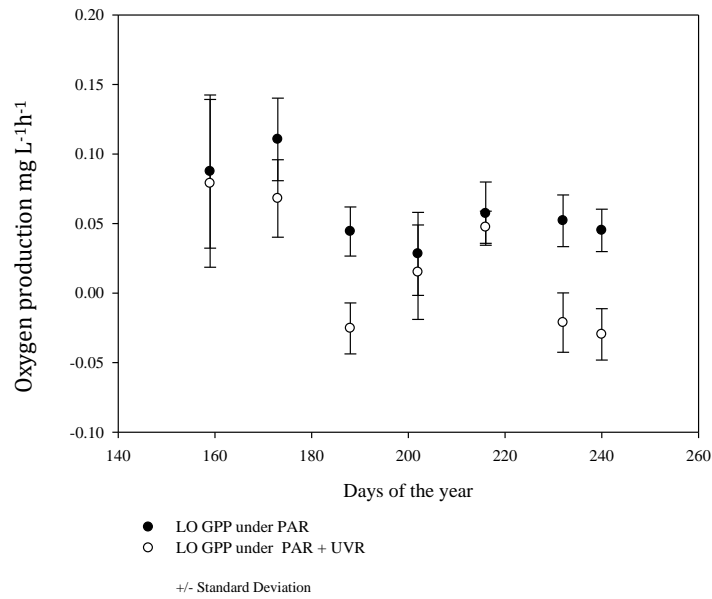
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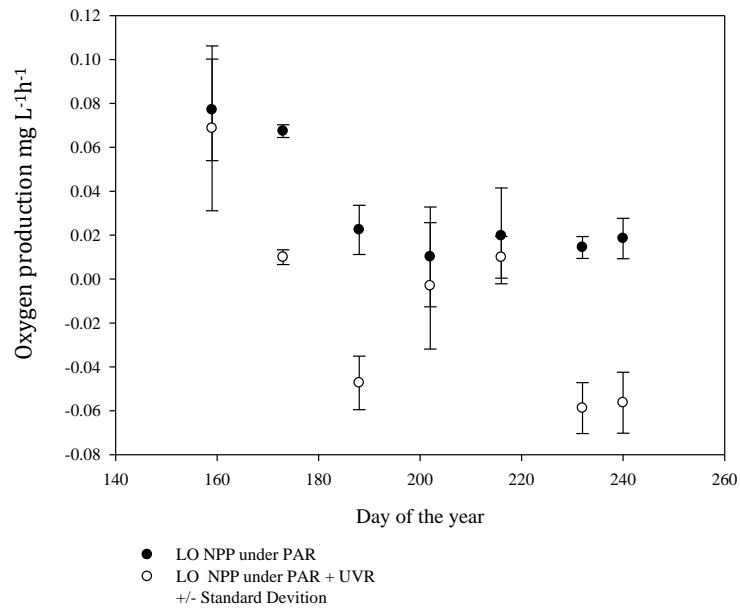
Figure 2.6. A) GPPin PAR and PAR + UVR through the sampling period; B) same for NPP (O_2). Error bars are standard deviations based on triplicates.

for 4 h in three light treatments, or compared to T_0 (F = 0.615, P = 0.572). I conclude

that there was no measurable oxygen consumption by abiotic processes.

I used the ratio of net-O_2 evolved in PAR + UVR to PAR alone to compare the

two stations, as there were significant differences in biomass and related productivity

between the two systems. There was no significance difference between these ratios for

the two stations, indicating that both stations responded in the same fashion when

stressed by the addition of UVR. This was true for both GPP and NPP (F=0.027,

P=0.872 and F=2.165, P=0.167, respectively).

Carbon Assimilation

Carbon uptake in the Hamilton Harbour PAR + UVR incubations was

significantly impaired compared to the PAR-only incubations on every date (Table 2.4,

Figure 2.7 A). There was variability in the community response to UVR over the season,

but the average loss of carbon assimilation was about 45%.

Carbon uptake in LO followed a similar pattern to Hamilton Harbour, but

included an elevated assimilation rate on July 8th (Julian Day 189). In the PAR + UVR

incubations, the overall loss in carbon assimilation (compared to PAR only) was greater

than in HH with an average loss of 68% in LO. All PAR + UVR

Table 2.4. Comparison of carbon assimilation for Hamilton Harbour incubations in PAR and PAR + UVR. On all dates PAR was greater than PAR+UV. DF are the degrees of freedom for the t-tests. The ratio is carbon assimilation in PAR + UVR divided by assimilated carbon in PAR only incubations.

Date	T	DF	Probability	Ratio of inhibited carbon assimilation (PAR+UVR)/PAR
June 9, 2003	3.164	4	0.034	0.19777
June 23,2003	6.979	4	0.002	0.45892
July 8, 2003	9.737	4	0.001	0.54784
July 22, 2003	25.308	4	< 0.001	0.60807
Aug. 5, 2003	8.227	4	0.001	0.52313
Aug. 22, 2003	12.298	4	< 0.001	0.39332
Aug. 29. 2003	6.89	4	0.002	0.46851

7 A.
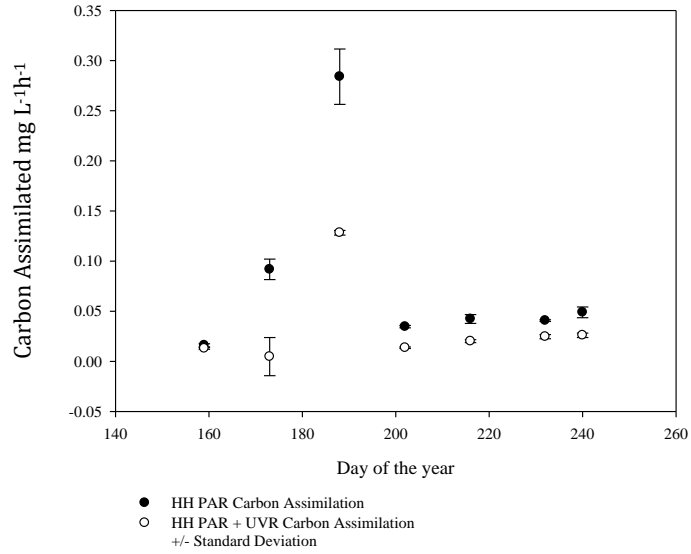
7 B.
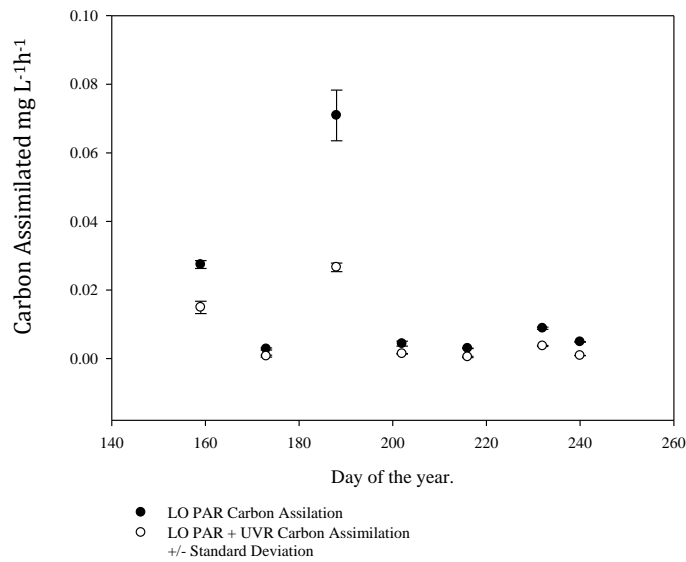
Figure 2.7. A) Light driven carbon assimilation for HH incubated in PAR and PAR + UVR; B) carbon assimilation rates (as above) for Lake Ontario.

incubations displayed significant suppression of assimilated carbon compared with the PAR only samples (Table 2.5, Figure 2.7 B).

The percent suppression of carbon assimilation in Hamilton Harbour was less than in Lake Ontario (paired t = 4.82, DF = 6, P = 0.003).

Post Treatment Dark Respiration

Dark respiration after the 4-h light treatments was significantly different (t-tests, P <0.05) on each sample date in Hamilton Harbour (Table 2.6), but not in a consistent way. On two sample dates, June 9th and 23rd, respiration in the PAR + UVR samples exceeded that in the PAR-only treatments. On the remaining dates, July 8th through August 21st, dark respiration in the PAR only treatments exceeded the PAR + UVR treatments (Figure 2.8 A).

Lake Ontario samples showed no statistically significant differences in PAR + UVR compared to PAR only post treatment respiration (Table 2.7). However, post PAR + UVR respiration for Hamilton Harbour and Lake Ontario followed a somewhat similar temporal pattern (Figure 2.8 B).

Looking within stations and treatments, Chl-a and post treatment respiration in PAR + UVR for HH had a significant positive relationship (adjusted R^2 = 0.57, P = 0.003). No such relationship was found the other treatments HH-PAR; LO-PAR or LO PAR+UVR.

Table 2.5. Carbon assimilation comparisons for Lake Ontario incubations in PAR and PAR + UVR. On all dates PAR was greater than PAR+UV. The ratio is carbon assimilation in PAR + UVR divided assimilated carbon in PAR only incubations

Date	t	DF	Probability	Ratio of inhibited carbon assimilation (PAR+UVR)/PAR
June 9, 2003	10.104	4	< 0.001	0.45555
June 23, 2003	8.973	4	< 0.001	0.74466
July 8, 2003	10.222	4	< 0.001	0.62429
July 22, 2003	7.014	4	< 0.001	0.68034
Aug. 5, 2003	19.781	4	< 0.001	0.84109
Aug. 21, 2003	25.359	4	< 0.001	0.58396
Aug. 29, 2003	66.095	4	< 0.001	0.82144

Table 2.6: Comparison of post-treatment respiration after PAR+UV relative to after PAR for Hamilton Harbour.   Units are mg L^{-1} h^{-1} of oxygen consumed.

Date	t-Test	DF	P	PAR + UVR	PAR
June 9, 2003	2.59	4	= 0.042	0.0076	0.0012
June 23, 2003	21.64	3	< 0.001	0.0874	0.0462
July 8, 2003	5.91	4	= 0.007	0.0031	0.0149
July 22, 2003	9.64	3	< 0.001	-0.0005	0.0380
Aug. 5, 2003	6.14	4	= 0.004	0.0114	0.0138
Aug, 21, 2003	5.91	3	= 0.007	0.0271	0.0525

A.
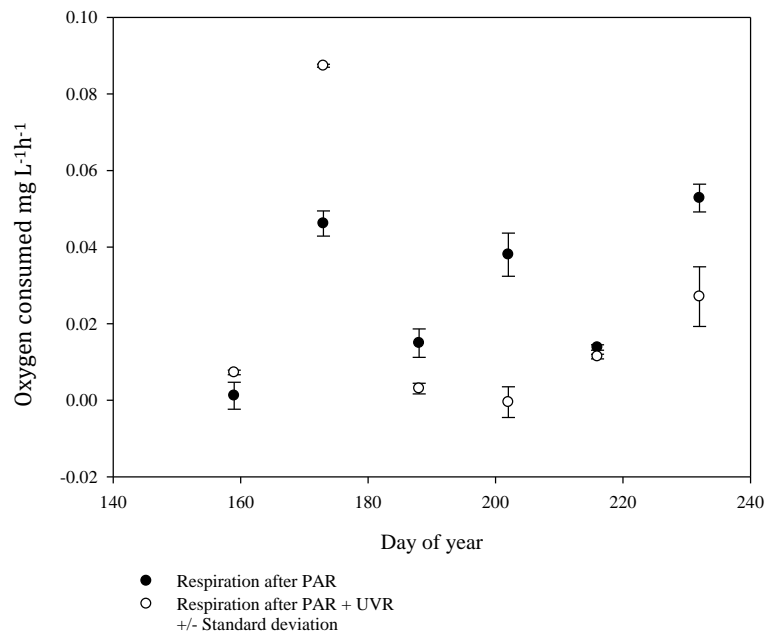
B.
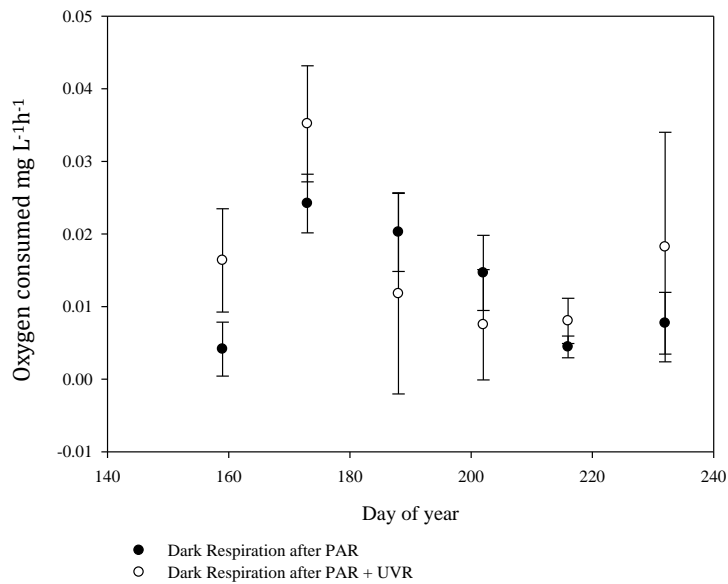
Figure 2.8. Post treatment respiration under PAR and PAR + UVR treatments for Hamilton Harbour (A) and Lake Ontario (B).

Table 2.7: Comparison of post-treatment respiration after PAR+UV relative to after PAR for Lake Ontario. Units are mg L^{-1} h^{-1} of oxygen consumed.

Julian Date	t-Test	DF	P	PAR + UVR	PAR
159	2.63	4	= 0.058	0.0164	0.0041
173	2.126	4	= 0.101	0.0352	0.0242
188	7.96	3	= 0.484	0.0118	0.0203
202	1.34	4	= 0.250	0.0075	0.0146
216	1.74	4	= 0.147	0.0080	0.0044
232	0.11	3	= 0.329	0.0182	0.0077

Discussion:

Three different hypotheses are tested in this chapter. The first hypothesis stated that the two stations used, Hamilton Harbour and Lake Ontario, would respond to enhanced UV in the same fashion regardless of the differing trophic status of the two sites. Hamilton Harbour is eutrophic and receives about 50% of its water input from waste treatment facilities (Hiriat-Baer 2007). In contrast, the Lake Ontario station represents an oligo/mesotrophic site with many characteristics of an offshore deep-water site (M.N. Charlton, NWRI, Burlington, ON, personal communication).

The PAR + UVR incubations caused a profound inhibition of oxygen production and carbon assimilation compared to PAR-only incubations. The seasonal average oxygen production was inhibited by 211 % in Lake Ontario and 98 % in Hamilton Harbour. The suppression of oxygen production in PAR + UVR treatments was expected, and has been documented by many others. However, the net oxygen consumption that was observed at both experimental stations has not been observed before. This represents a shift from net autotrophy to net heterotrophy and, in the case of Hamilton Harbour, oxygen consumption was related to algal biomass as determined by Chl-a concentration. That is, as algal biomass increased, there was a reduction in O_2 evolved, leading to a net consumption of oxygen. While Lake Ontario did display net O_2 consumption on several dates, a weakly positive but non-significant relationship was found when NPP under PAR + UVR was regressed against Chl-a.

There are numerous factors that may be contributing to the consumption of O_2 in the PAR+UVR incubations, but in this situation they must be related to the organisms in

the water column, as I was unable to measure photochemical oxygen consumption in sterilized samples from either Hamilton Harbour or Lake Ontario.

Increased oxygen consumption in the light can occur through a number of processes that include, but are not limited to, light-enhanced mitochondrial respiration. Oxygen consumption can also be a result of ATP synthesis, oxygenase of rubisco, photoreduction of oxygen by way of alternative terminal oxidases located in the thylakoid membranes and the Mehler reaction (Suggett et al.2009), all of which occur within the cell during the light cycle. Additionally, algal exudates such as MAAs and other low molecular weight DOM may be photo-oxidized during exposure to UVR. Photo-oxidation of these exudates would take place in the water column.

Pringault et al.(2009), studying the linkage between autotrophic production and heterotrophic production, found that 60% of the variation they observed in respiration in the light could be explained by variations in the primary producers. They examined light respiration in samples that had a wide range of Chl-a of 1 to 40 μg L^{-1}. They found that when Chl-a was low, <1 μg L^{-1}, bacterial respiration assumed a dominant role, representing up to 79% of community respiration, and that it was negatively correlated with Chl-a.

Carbon assimilation is suppressed under PAR + UVR (e.g., Kohler et al.2001, Sobrino et al.2005). In this work, assimilation of carbon was suppressed in samples from both stations, though at significantly different amounts between stations. Hamilton Harbour carbon assimilation under PAR + UVR was suppressed by an average of about 45% compared to the PAR-only incubations, and 65% in the Lake Ontario samples.

Kohler et al.(2001) reported suppression of between 20 and 40% in mixed water systems, when they simulated mixing depths of 2 to 10 m. Marwood et al. (2000) found a rapid loss of up to 60% of PSII efficiency in sunlight that contained UVB in Lake Erie. Although they were using pulse amplitude modulated chlorophyll fluorescence in their work, their results are in the range of suppression observed in this study for Lake Ontario. Marwood et al. (2000) had suppression that was somewhat higher than the ^{14}C assimilation suppression I observed in Hamilton Harbour. Both Marwood et al. (2000) and this work used UVB in the light-exposed incubations.

An interesting observation is that on all dates in which Hamilton Harbour samples consumed oxygen (NPP) during the PAR + UVR incubations, carbon assimilation was suppressed by 45% or more. On the last sampling date, carbon assimilation was suppressed by more than 45% but there was oxygen evolved during the incubation even though it was 84% less than occurred in the PAR-only incubations.

Assuming an intact electron transport system between the PS-II and PS-I system in the primary producers and a 1:1 ratio for O_2 production to carbon assimilation, then O_2 production should be suppressed by roughly the same amount, even on sample dates that displayed a loss of O_2 during the incubations.

Tassar and Rochelle-Newall (2007) found that light respiration can be 640% higher than respiration in the dark. Given the dark respiration measured in this work, if GPP is reduced by about 50% (comparable to the decline in C-assimilation) through exposure to UVR, only a slight increase of light respiration over the measured dark

respiration would shift the systems from O_2 production to O_2 consumption. So, observing O_2 consumption on several sample dates does not seem unreasonable.

While some UVR response characteristics of Hamilton Harbour and Lake Ontario are similar (i.e., shift to net heterotrophy, average suppression of O_2 production) the systems responded differently in that suppression of the carbon uptake in Lake Ontario was proportionally greater than in Hamilton Harbour. I conclude the results contradict the first null hypothesis of this work.

The second hypothesis tested was that higher allochthonous inputs of DOC in Hamilton Harbour compared to Lake Ontario would result in a decoupling of autotrophic and heterotrophic production. That is, bacterial production which is normally highly correlated to algal productivity (Tassas and Rochelle-Newall 2007) will be subsidized by allochthonous inputs. This would be detected by a divergence in the ratio of evolved O_2 and assimilated carbon.

A divergence in the ratio of oxygen evolved to carbon assimilated occurred under PAR + UVR compared to the PAR-only incubations in Hamilton Harbour and Lake Ontario. One assumption of this hypothesis was that the divergence would be caused by an increase in bacterial respiration as a result of additional substrate being made available through photochemical processing of recalcitrant DOC and the release of DOC by the particulate fraction in response to UVR exposure. The samples used in this work were whole lake water samples passed through a 202 μm screen and therefore contain any organisms smaller than that, which would include rotifers, small crustaceans and protozoans.

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While I cannot say with certainty that the oxygen/carbon divergence observed was caused by a positive bacterial response, it is reasonable to assume that an increase in respiratory oxygen-consuming processes did take place in the PAR + UVR incubations and is related to biological activity. I expected the response to be greatest in Hamilton Harbour, which contains considerably more biomass than Lake Ontario, but instead I found the largest change in Lake Ontario. This result may be due, in part, to the clarity of the water in Lake Ontario compared to Hamilton Harbour, though it seems unlikely given the short light path of the experimental vessels. Nonetheless, algal self-shading may be a factor in the smaller response observed in the Hamilton Harbour samples. I conclude that hypothesis 2 is true, but with the reservations outlined above. A study of bacterial responses to enhanced UVR in this system may provide some insight into the results observed here.

The 3rd hypothesis tested in this chapter is that PAR+UVR will have no impact on dark respiration. In HH, post treatment respiration (PTR) was significantly different on all sample dates. This was not the case in the Lake Ontario samples. Hamilton Harbour had elevated PTR on the first two sampling dates, June 9th and June 23rd, and suppressed PTR for the remaining dates in the PAR + UVR treated samples. In contrast, Lake Ontario had elevated PTRs for all but 2 dates, July 8th and July 22nd, but there were no dates that were statistically different from the PAR only treatments. Post treatment respiration in Hamilton Harbour was strongly related to algal biomass (Chl-a).

It is possible that a shift in species composition from spring time, largely diatoms, to a summer mix of green and blue green algae is responsible for the shift from PTR enhancement of respiration to suppression. Further, the bacterial population many have

become the dominant respiratory organisms in HH, and without many of the UVR

protective mechanisms found in algae, they were more susceptible to the UVR in the

PAR + UVR incubations. Nitrification is very light sensitive and might account for

detectable 02 uptake.

On one occasion in the PTR work, a slight O_2 increase was observed instead of

respiration. Ostrum (2005) also observed an O_2 increase in dark samples used to

determine respiration. While the circumstances are different in this case, as my samples

had been incubated in PAR + UVR, some of the causes may be similar.

It seems unlikely that algal cells, super-saturated with oxygen, released the

oxygen in the dark, as generally speaking I observed a significant suppression of oxygen

production under PAR+ UVR. It may be possible that the decay of reactive oxygen

species (ROS) and possibly H_2O_2 resulted in a measurable release of O_2. For a complete

discussion of possible causes see Ostrum (2005).

I believe the most promising approach in the future would be the application of

molecular tools such as micro-array analysis, which would show which genes are up-

regulated in the phytoplankton under UVR and those which are down regulated. Possibly

using cultures of well characterized species along with the PAM or FRRF may shed some

light on the underlying changes occurring in the UV stressed phytoplankton. By using

these tools in tandem we could determine the state of reaction centres and correlate their

status with the genetic disposition of the stressed organism.

Chapter 3

The effects of ultraviolet light and DOC on Georgian Bay and

Lake Ontario phytoplankton

Introduction:

DOC (dissolved organic carbon) is a major modulator of the structure and function of lake ecosystems (Sobek et al.2007), affecting the thermal structure and mixing depth of lakes and also affecting how quickly a lake becomes stratified (Prairie 2008). It plays a major role in determining the productivity of phytoplankton as it attenuates light (Fee et al.1996) and thereby impedes photosynthesis (Jones 1998). DOC in a lake has two origins. The DOC from the surrounding watershed is known as allochthonous, while the DOC that is produced by the organisms within the lake is known as autochthonous DOC. There has been debate about the role of allochthonous DOC (Pace at al. 2007, Cole et al. 2007, Mohamed and Taylor 2009) in lake metabolism, particularly on the question of whether it provides a subsidy to the aquatic system and, if so, to which trophic levels.

Chromophoric dissolved organic matter (CDOM or CDOC) is DOC that is coloured to varying degrees. The humic substances of CDOM are derived from terrestrial plant matter and are composed of ligna, namely cumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, as well as tannins and terpenes (Steinberg et al.2006, Wetzel 2001). The alcohols are primarily polyvalent and have functional groups that are able to react with many chemicals as well as with organisms in the water column (Steinberg et al.2006). Humic substances are primarily responsible for making the water a tea colour (Wetzel 2001).

Docherty et al.(2006) selected 3 sites, including a dystrophic lake, with varying

DOC quantity and quality. The water from the sites was filter-sterilized and 3 samples

from each site were cross-inoculated with bacteria from the other sites, to produce 9 fully

crossed treatments. Cultures with lower DOC concentration and molecular weight tended

to exhibit higher microbial production rates than cultures with high concentrations and

high molecular weight DOC. Further experimentation suggested that the source and

quality of allochthonous DOC plays an important role in determining the bacterial

community structure and productivity of a system (Docherty et, al. 2006).

Exposure to DOC with humic content has been observed to result in the

production of stress proteins in zooplankton and nematodes, and to reduce photosynthetic

release of oxygen by freshwater plants and algae (Steinberg et al.2006). Steinberg et

al.(2006) believe that quinines present in humic substances are able to enter the cells of

autotrophs and interfere directly with photosynthetic electron transport. While DOC may

constitute a subsidy to an aquatic system, DOC with high humic acid content may be

damaging to the system, at least until it is processed either photochemically or

biologically.

DOC is well known for its ability to attenuate light. UVR in particular can be

attenuated within the first few centimeters in water with high DOC and this is particularly

true for DOC with high humic content (Steinberg et al.2006). Ultraviolet light is an

important factor inhibiting aquatic primary production, and increased UV flux is a major

concern at present (Schindler 1996, Sobek et al.2007). While advances have been made

in reducing ozone-depleting chemical emissions that have led to increased UV flux,

global climate change appears be slowing the rate of ozone recovery (Rex et al. 2005).

Temperatures in the lower stratosphere over the poles have been decreasing sharply over the last few years, producing thicker lower stratospheric clouds and providing new substrates for ozone-destroying reactions, i.e., bromine and chlorine (Rox et al.2006). Rox et al.(2006) observed large ozone losses, which they attributed to chlorine/bromine reactions during the winter of 2005 in the northern hemisphere. A near-record hole in the ozone layer was observed in the southern hemisphere in spring 2006 (Qi 2007). Larger holes in the ozone layer will increase the surface level UVR that impacts aquatic systems (Schindler 2001).

In lakes, UVR is absorbed by DOC, but that DOC is bleached in the process. This diminishes its ability to act as a UVR filter (Magnuson et al.1997, Schindler 2001, Zepp et al.2008). UVR also has the potential to free nutrients present in DOC, or to make labile nutrients unavailable (Sobek et al.2007). Obernosterer and Benner (2004) used DOC from different sources, and before and after UVR irradiation, to inoculate plankton cultures and test the response of the plankton to the changes in substrate. They were able to demonstrate the potential for competition between biological and photochemical processing of DOC, as UVR-irradiated substrate produced slower bacterial growth. DOC is the substrate for heterotrophic bacteria (Hader 2007), but Kamjunke and Tittel (2008) found evidence that a number of phytoplankton, including a chlorophyte, a diatom and a euglenophyte, can assimilate DOC directly.

The focus of this chapter will be primary production of phytoplankton at 5 locations in the Great Lakes using two standard techniques, light-driven ^{14}C assimilation and photosynthetic O_2 production. I will examine the impact of PAR and PAR+UVR on primary production and, by comparing different sites, the importance of DOC. I will

expand on the work completed in the previous chapter by adding 3 additional sites with different levels of DOC from different allochthonous sources. The systems have different watersheds with different characteristics (Fig. 3.1). Hamilton Harbour is eutrophic and is highly urbanized with 5 sewage treatment plants discharging into its waters. Heavy industry, including 2 steel plants complete with coking ovens and several blast furnaces, is present on the southern shore of the bay. The Bay of Quinte is eutrophic and has a moderate amount of urbanization, but the Trent and Moira Rivers flowing into it, and their watersheds drain moderate to low intensity agricultural land and temperate forest. The Woods Bay station has tea-coloured water, typical of many northern Ontario lakes, and its Moon River tributary drains the Muskoka and Algonquin regions. It represents a relatively pristine environment. The Georgian Bay station is a typical oligotrophic Great Lakes site, with clear water low in both DOC and Chl-a.

In this work I will test the following hypotheses:

1.   Abiotic oxygen consumption will be negligible compared to biological fluxes, but greatest at high DOC sites.

2.   Planktonic photosynthesis at these diverse sites, as measured by oxygen production and C^{14} fixation, will respond similarly across sites to UVR exposure.

Materials and Methods

Five sampling locations were selected (Table 3.1) based on watershed and land use (Figure 3.1 and Figure 3.2). Samples were collected from May 18, 2005, to

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November 11, 2005. In an effort to reduce the impact of transporting the sample water back to the laboratory in Waterloo sample water was collected as late in the day as possible, typically within 3 h of sunset. The carboys were filled with epilimnetic water using an integrated sampler, and the sampled water was passed through a 200-µm screen to remove large zooplankton and placed in insulated coolers for transportation back to the laboratory. In the lab, the carboys were placed in temperature-controlled dark incubators until the following morning.

The experimental lake water incubations followed methods previously described in chapter 2, except that Tedlar® bags were used rather than standard and quartz glass BOD bottles. The bags are UVR and PAR transparent, which was verified using an Oreil Instaspec diode array spectroradiometer (Oreil Corp., Stanford Conn.) equipped with an integrating sphere. The Tedlar bags were selected for this work as they allowed the removal of water samples and insertion of probes through an airtight septum.

Oxygen measurements were made using an Ocean Optics coated Model AL300 oxygen probe in combination with a BIF200-UV-VIS Bifurcated Optical Fiber cable and a USB2000 Spectrometer provided with an attached Ocean Optics LS-450 LED light source to drive the oxygen probe. All data were produced in real time and in mg L^{-1} using the company's OOISensor Software for Oxygen Measurement.   The probes were factory calibrated several times through the field season to ensure readings were consistent. The instrument was also calibrated several times during the experiment following the manufacturers instructions.

Table 3.1. Sampling stations used during the 2005 field season. The errors for K_{PAR} and DOC are standard deviations. The sample size for Kpar is n = 4, except for WB/GB where n=2. DOC was measured 3 times at each station except for WB/GB where n=2

Site	Identification	Longitude	Latitude	K_{PAR}* (m^{-1})	DOC ($mg\ L^{-1}$)
Woods Bay	WB	79 59' 44.93"	45 08' 21.05"	0.62 ± 0.05	4.5 ±0.4
Georgian Bay	GB	80 07' 05.67"	45 08' 04.07"	0.31 ±0.09	2.4 ±0.1
Bay of Quinte	BQ	77 15' 25.89"	44 08 57.95"	1.03 ±0.08	6.6 ±0.02
Hamilton Harbour	HH	79 50' 29.91"	43 17' 45.39"	0.71 ±0.03	4.4 ±0.05
Lake Ontario	LO	79 43' 16.25"	43 17" 58.01"	0.25 ±0.04	2.4 ±0.2

Figure 3.1. Study sites used during 2005. Inset A, Hamilton Harbour and Lake Ontario; inset B, the Bay of Quinte Station; inset C, the locations of the 2 stations on the eastern shore of Georgian Bay.
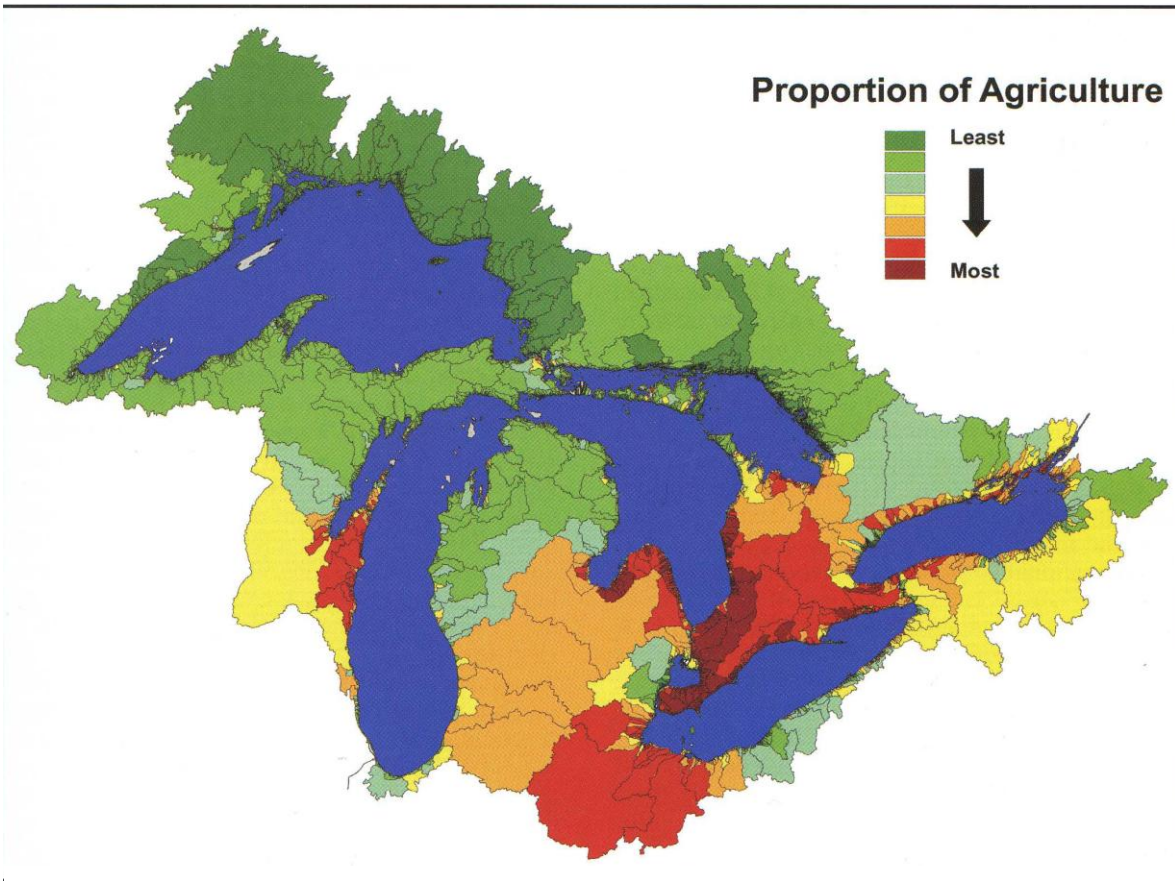
Figure 3.2. Land use map of watersheds draining into the Great Lakes from Miemi et. al (2007).

On each visit to the stations, a light profile was obtained by using a quantum sensor (Li-COR Inc.) to measure PAR at 1-m intervals. The light extinction coefficient (k_{PAR}) was determined for each sample date and site using the linear regression of the natural log of the irradiance versus depth (Kirk 1994). Temperature profiles were measured using either a YSI 6600 or YSI 600XLM sonde (YSI Inc., USA) or a Fluoroprobe (BBE Moldaenke, Germany).

Water samples were also collected for measurements of total phosphorus (TP), dissolved inorganic carbon (DIC), chlorophyll-a (Chl-a) and dissolved organic carbon (DOC). DOC samples were filtered and frozen until analysis.

Experimental Incubations:

The experimental incubations were completed in the light chamber described in chapter 2, using the same light conditions and durations for PAR and PAR + UVR incubations. When two stations were being studied simultaneously, the mean temperature of the two stations was used as the incubation temperature. Incubation temperatures were maintained using a temperature-controlled circulating water bath.

As in chapter 2, both oxygen evolution and carbon assimilation were measured under PAR and PAR + UVR at a total flux of 1160 μMol photons $M^{-2} L^{-1}$ (comprised energetically of 90% PAR; 9% UVA and 1% UVB).

Glassware coming into contact with the water samples was washed with Extran cleaner, well rinsed with de-ionized water, then placed in an acid bath for several hours. After acid washing, the glassware was rinsed three times in de-ionized water, and three times with Barnstead high purity water.

Tedlar bags were rinsed several times with deionized water, washed in Extran cleaner, rinsed well, then washed several times with 100% ethanol and again rinsed with deionized water, then rinsed 3 times with Barnstead ultra pure water.

Metabolic Measurements

Oxygen measurements used an Ocean Optics O_2 probe that was inserted through the septum. This allowed repeated direct measurements of the O_2 in a non-destructive fashion, so I was able to measure the changes in O_2 concentration of each sample at the beginning and end of the incubation for each bag. Triplicate samples were used in each light treatment and three O_2 measurements were taken at each sampling time.

The Tedlar bags were filled directly from the carboys using a peristaltic pump. The intake tube was kept well below the surface, and the water in the carboys was thoroughly mixed either with a large rod by hand or with a magnetic stirring bar. The Tedlar bags were rinsed 3 times with sample water through the filling spigot, and then filled. Any bubbles were expelled through the valve on the bag.

Light Driven Carbon Assimilation:

Although ^{14}C-bicarbonate has been used to measure primary production for decades and remains a standard metabolic measurement, there is debate surrounding what exactly is being measured, i.e., gross production versus net production, or something in between. As our incubations were 4 h in length, in a saturating light field, I will interpret my results as net, or closely approaching net productivity (del Giorgio and Williams 2005).

Carbon assimilation experiments were completed using quartz test tubes in the same light chamber using the same material for cutoff filters. Three replicates for each light treatment were used. Samples from each site were inoculated with ^{14}C-sodium bicarbonate (ICN Biomedical; 2 μCi μL^{-1}) and then dispensed into the quartz test tubes capped with Teflon stoppers and placed in the light incubator for 4 h. Total activity (200 μL) samples were collected to validate the isotope additions. At time zero, triplicate 5 mL subsamples from each treatment were acidified with 100 μL of 6 N HCl. These were subtracted along with the dark incubation values to produce the light driven ^{14}C assimilation rates which were then used to generate the light driven carbon assimilation values. After 24 h, 15 mL of EcoLume™ scintillation cocktail (ICN Pharmaceuticals) were added to each vial.

At the completion of the incubations, triplicate 5 mL aliquots from each of the 3 treatment replicates were placed in scintillation vials and 100 μL of 6 N HCl were added to each vial. After 24 h, Ecolume™ was added to each sample as described above. Dark assimilation values were subtracted from the light incubations to produce light-driven carbon assimilation values for the PAR and PAR + UVR incubations.

Abiotic Light-Driven Oxygen Consumption:

To test for abiotic oxygen consumption in PAR and PAR + UVR treatments, whole lake water from each station was sterilized by the addition of mercuric chloride and O_2 decline was measured using the high precision Winkler technique previously described (Chapter 2). Three bottles for PAR, PAR + UVR and Dark were incubated for 4 h and were then fixed and titrated to determine O_2 concentration. An ANOVA was

used to test the null hypothesis that there was no difference in the oxygen concentration among the three light treatments.

Additionally, samples from each site were incubated using ^{14}C-bicarbonate in quartz bottles of varying sizes in an effort to detect UVR attenuation, which may have been occurring in the experimental vessels during the experimental incubations.

Statistical methods:

All data were tested for normality and homoscedasticity and transformed as required, usually with a $\log_{10}$ or natural log transformation, and then retested. SPSS™ 11.0.0 was used for ANOVAs and SigmaStat™ 3.5 was used for t-tests and regression analyses. SigmaPlot™ 9.0 was used for some regression analyses and graph production. Systat 10 was used in all analysis involving general linear models (GLM) and was used to identify any relationships between the response ratio and several independent factors measured in this work. In evaluating the carbon assimilation rates, simple tests (unless stated otherwise) were used to detect differences between PAR + UVR and PAR-only incubations. To examine between-site differences, the data were plotted and error bars for the standard deviations were used. A significance level of 5% ($P \leq 0.05$) was used in all statistical tests. The software above was running on a Windows XP ™ virtual machine using VMWare Fusion™ on a Mac Book™ with OS X 10.5.3.

I attempted to predict the response ratio (the fractional decline in C-fixation under PAR+UVR relative to PAR-only) from environmental data using regression analysis (GLM in Systat 10). Data on UVR incidence was obtained from the Ministry of the

Environment station in Toronto which recorded UVR and PAR radiation daily. This data was used to determine the light history for the limnological stations used in this work.

Results:

Limnological Environment:

The sites selected provide contrasts in land use in their watersheds as well as trophic status and DOC (Tables 3.1, 3.2; Figure 3.2). DOC concentrations ranged from 2.4 mg L^{-1} in Georgian Bay to 6.6 mg.L^{-1} in the Bay of Quinte. Hamilton Harbour and Woods Bay were intermediate with 4.4 and 4.5 mg L^{-1}, respectively and Lake Ontario was similar to Georgian Bay with 2.4 mg L^2 of DOC (Table 3.1).

A strong positive relationship was found between TP and Chl-a for these stations and dates (Figure 3.3). DOC was not related to any other variables.

The 2005 field season was marked by unusually warm temperatures throughout the summer and well into the late fall. On October 23, the temperature at the 1 m depth of our Lake Ontario site was 11.7 °C and Hamilton Harbour was 20.3 °C. In contrast, the temperatures in 2004 for the same stations on October 31 were 9 °C and 13 °C, respectively.

Abiotic Oxygen Consumption:

To test for abiotic oxygen consumption, the oxygen concentration in the chemically-sterilized water samples were measured after light treatment. There were no significant differences between the PAR, PAR + UVR and dark incubations in the chemically sterilized samples for any site.

Table 3.2. Chl-a, DIC and TP for dates and stations sampled during 2005. Chl-a and TP are in μg L^{-1}, and DIC is in mg L^{-1}.

Date	Julian Date	Station	Chl-a	DIC	TP
01/06/2005	151	BQ	24.80	25.91	13.2
29/06/2005	179	BQ	2.50	26.53	-
04/08/2005	215	BQ	9.14	29.93	33.49
03/11/2005	306	BQ	13.8	24.09	27.04
15/06/2005	165	HH	9.79	29.90	23.97
14/07/2005	194	HH	8.9	29.93	27.65
18/08/2005	229	HH	19.63	24.95	30.42
17/10/2005	289	HH	9.82	26.70	-
15/06/2005	165	LO	4.04	22.53	6.15
14/07/2005	194	LO	1.46	23.03	-
18/08/2005	229	LO	0.73	22.93	7.68
17/10/2005	289	LO	3.27	23.70	9.22
18/05/2005	137	WB	14.24	2.28	-
11/07/2005	191	WB	2.87	1.87	11.40
12/08/2005	223	WB	5.33	2.24	11.35
01/10/2005	273	WB	7.33	2.50	7.37
18/05/2005	137	GB	2.04	14.27	-
11/07/2005	191	GB	0.63	15.70	5.53
12/08/2005	223	GB	1.33	15.77	6.76
01/10/2005	273	GB	0.95	17.42	5.53

Community Oxygen Metabolism:

Oxygen evolution was always less in PAR + UVR compared to PAR alone, and significantly less (t-tests, $P < 0.05$) on 6 of 18 occasions and with $P < 0.06$ on 9 occasions (Table 3.3).

Georgian Bay and Woods Bay:

These sites followed somewhat different trends through the field season. Georgian Bay had a maximum net O_2 production early in the season, while Woods Bay community net production remained relatively unchanged throughout the sampling season (Figure 3.4). For Woods Bay, net O_2 production did not differ (Table 3.3 B) significantly between the PAR + UVR incubation and the PAR-alone on any date, although net O2 production was always lower with UVR (Figure 3.4 A).

For Georgian Bay, net O_2 production was significantly less under PAR + UV relative to PAR-only on August 12th, and on this date net production was negative under PAR + UV. The other sample dates had lower net primary productivity, but the differences are not statistically significant (Figure 3.4 B, Table 3.3 A and B).
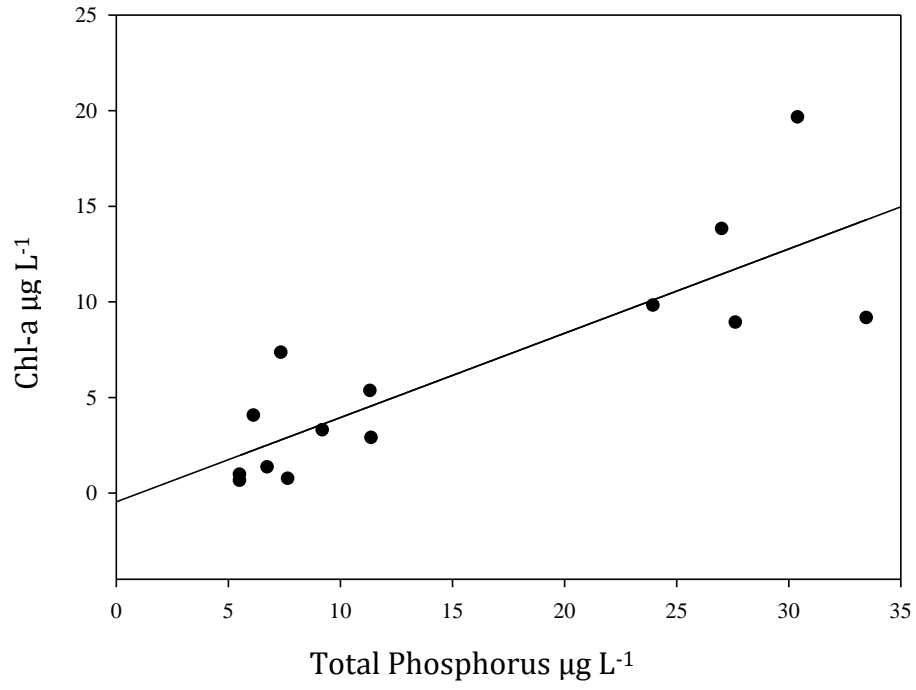
Figure 3.3. TP versus Chl-a for all stations and dates.

Table 3.3: Net community production for incubations in PAR and PAR + UVR. A through E are Georgian Bay; Woods Bay; Lake Ontario; Hamilton Harbour and Bay of Quinte respectively. Production is in mg $O_2L^{-1}h^{-1}$.

A.

Date	Julian Date	GB PAR O2 Production	Std. Dev.	GB PAR + UVR O2 Production	Std Dev.	t	P
July 11-05	191	0.058	0.064	0.038	0.088	0.32	0.76
Aug 12-05	223	0.026	0.025	-0.069	0.011	5.91	0.004
Oct 1-05	273	0.0114	0.022	-0.028	0.031	1.80	0.146

B.

Date	Julian Date	WB PAR O2 Production	Std. Dev.	WB PAR + UVR O2 Production	Std Dev.	t	P
July 11-05	191	0.024	0.084	-0.130	0.051	2.71	0.053
Aug 12-05	223	0.022	0.066	-0.013	0.089	0.17	0.872
Oct 1-05	273	0.027	0.096	-0.066	0.045	1.52	0.203

C.

Date	Julian Date	LO PAR O2 Production	Std. Dev.	LO PAR + UVR O2 Production	Std Dev.	t	P
Jun 15-05	165	0.018	0.046	-0.042	0.0566	1.4	0.225
Jul 17-05	194	0.112	0.037	-0.080	0.026	7.3	0.002
Aug 18-05	229	0.041	0.067	-0.030	0.054	1.4	0.229
Oct 17-05	289	0.018	0.040	-0.004	0.034	0.7	0.504

D.

Date	Julian Date	HH PAR O2 Production	Std. Dev.	HH PAR + UVR O2 Production	Std Dev.	t	P
Jun 15-05	165	0.133	0.096	0.027	0.020	1.9	0.136
Jul 17-05	194	0.285	0.047	0.205	0.025	2.6	0.059
Aug 18-05	229	0.110	0.055	-0.020	0.050	3.0	0.039
Oct 17-05	289	0.103	0.094	0.023	0.061	1.2	0.286

E.

Date	Julian Date	BQ PAR O2 Production	Std. Dev	BQ PAR + UVR O2 Production	Std Dev.	t	P
Jun 1-05	151	0.210	0.115	-0.075	0.130	3.06	0.38
Jun 29-05	179	0.134	0.025	-0.097	0.024	11.66	<0.001
Aug 4-05	215	0.191	0.101	-0.067	0.013	13.10	<0.001
Nov 3-05	306	0.131	0.045	0.010	0.034	3.71	0.021

A
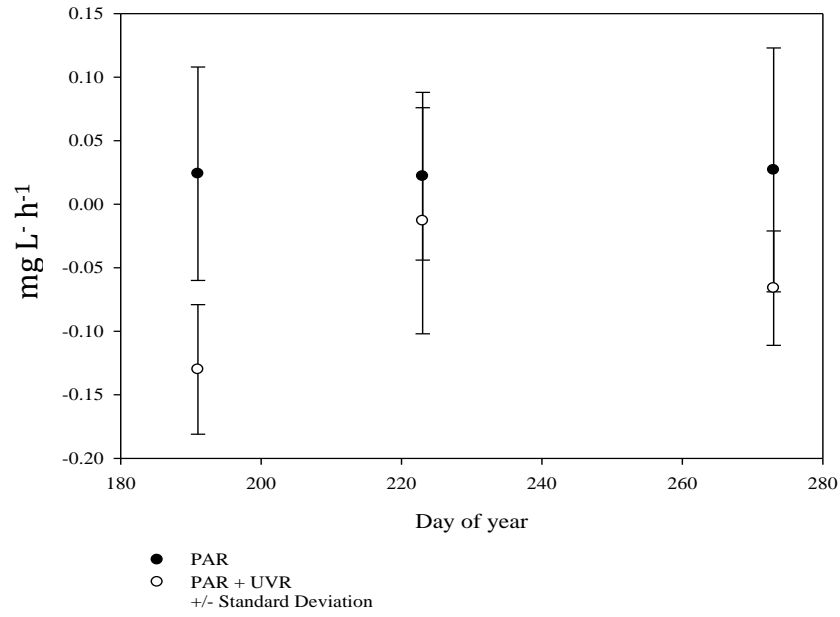
B.
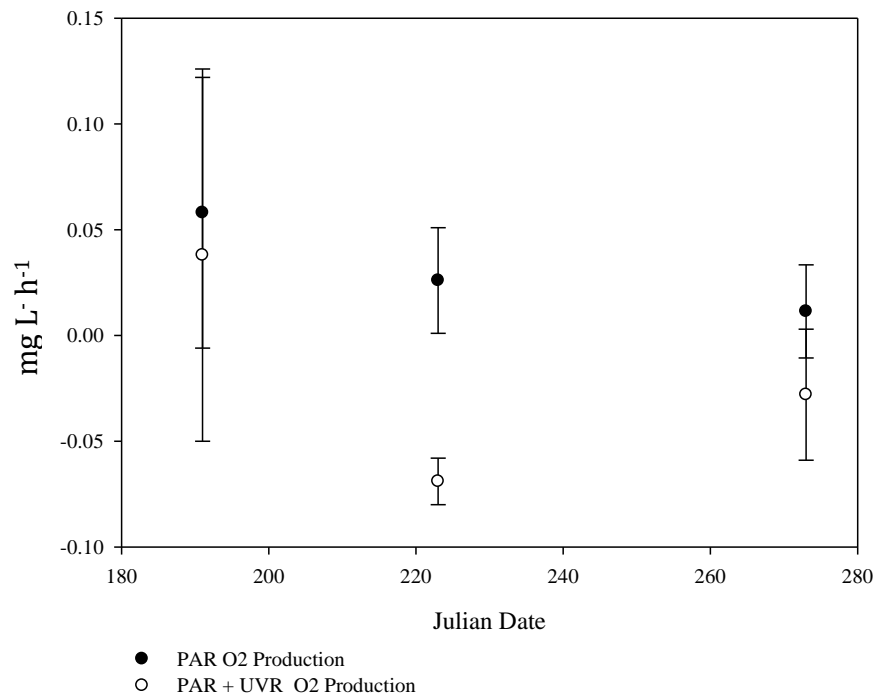
Figure 3.4: Net community oxygen production for Woods Bay (A) and Georgian Bay (B).

Lake Ontario and Hamilton Harbour

Community net oxygen production in Lake Ontario and Hamilton Harbour followed somewhat similar patterns for both PAR + UVR and PAR-only light treatments (Figure 3.5). When incubated under PAR + UVR, there was less oxygen production although it was only significantly lower on July 17th in Lake Ontario and August 18th in Hamilton Harbour (Table 3.3 C and D).

Bay of Quinte

There was relatively little variation in net production over the field season compared to other sites used in this study (Figure 3.6). PAR + UVR incubations had significantly less net O_2 production on 3 of 4 sampling dates (Table 3.3 E). Net oxygen consumption was measured on 3 of the sampling dates in the PAR + UVR incubations.

Oxygen Metabolism Summary

While many of the results for oxygen production were not statistically significant, there are clear trends in the data that suggest a significant reduction in oxygen evolution that occurred in the PAR + UVR samples across dates and stations. The lack of resolution by the Ocean Optics oxygen probe is in part responsible for the lack of statistical significance.

Carbon Assimilation

Generally, there was a substantial reduction in carbon assimilation in the PAR + UVR incubations compared to the PAR-only incubations. The suppression of C assimilation was statistically significant at all stations and on all sample dates with the exception of Georgian Bay on May 18th ; Hamilton Harbour June 15 and the Bay of Quinte on June 29th (Table 3.4 A through E).

A.
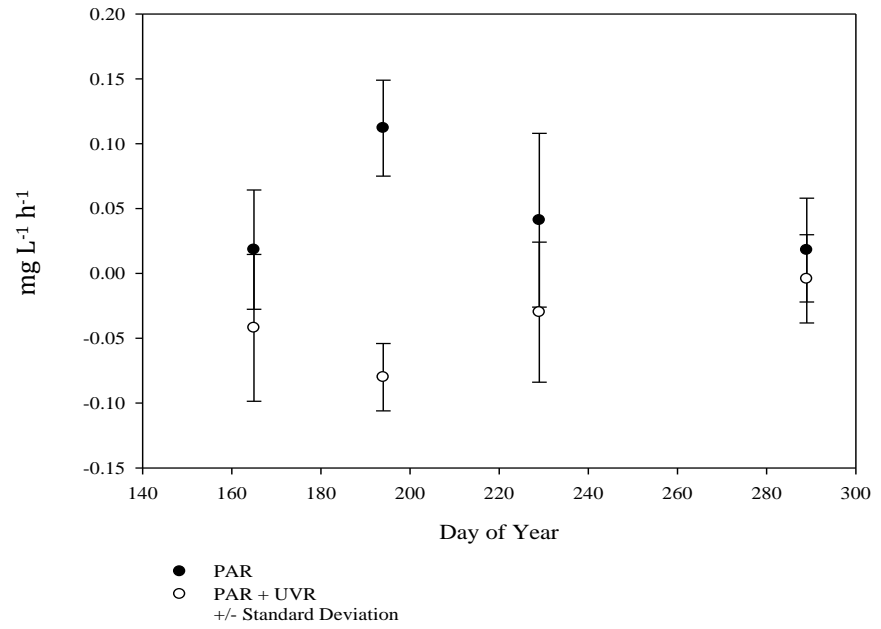
B.
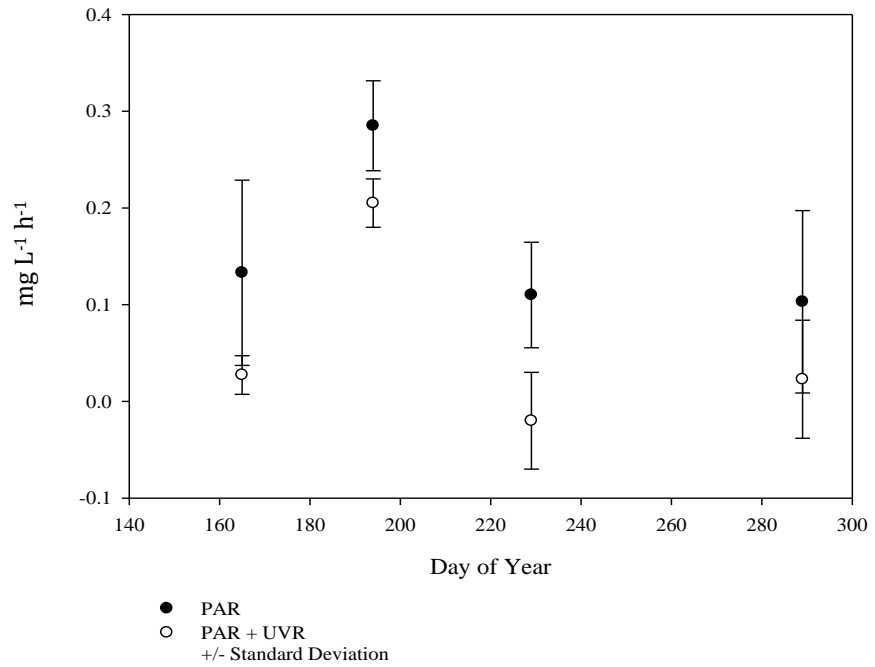
Figure 3.5: Lake Ontario (A) and Hamilton Harbour (B) community net oxygen metabolism PAR and PAR + UVR incubations.
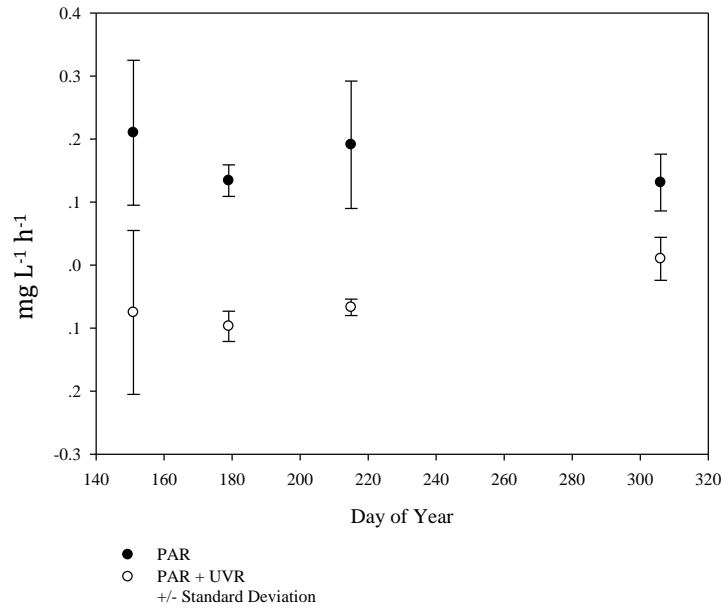
Figure 3.6: Bay of Quinte community net oxygen production PAR and PAR + UVR

incubations.

Light-driven carbon assimilation in Georgian Bay and Woods Bay differed seasonally (Figure 3.7 A and B) with assimilation being highest on July 11th (Day 191) in Woods Bay and on August 12th (Day 224) in Georgian Bay. Woods Bay under PAR only incubation was approximately 5 times as productive on a volumetric basis as oligotrophic Georgian Bay (Table 3.4 A and B). There were relationships between Chl-a and productivity in Woods Bay ($R^2 = 0.55$) and in Georgian Bay ($R^2 = 0.32$) under PAR.

When incubated in PAR + UVR, there was a statistically significant suppression of carbon assimilation at both stations and on all samples dates, except for Georgian Bay on May 18th (Day 138).

Lake Ontario and Hamilton Harbour

Suppression of carbon assimilation in the PAR-UVR incubations for Lake Ontario displayed a seasonal pattern, with the most suppression in the spring and the least in the summer. This appears to differ from the Hamilton Harbour result, where suppression appeared to reach a maximum in the summer (Figure 3.8 A and B). Light-driven carbon assimilation appears to be low in Hamilton Harbour on July 17th (Day 194). Typically, Hamilton Harbour is more productive than Lake Ontario, however, on July 17th (Day 194) the Lake Ontario PAR only incubation assimilated nearly twice as much carbon as Hamilton Harbour. Chl-a was relatively low in both Hamilton Harbour and Lake Ontario on this date (Table 3.2).

Table 3.4: Primary production via ^{14}C assimilation for incubations in PAR and PAR + UVR. A through E are: Georgian Bay; Woods Bay; Lake Ontario; Hamilton Harbour; and Bay of Quinte, respectively. Assimilation rates are in mg C L^{-1} h^{-1}. "t" =t-test value; "P"=probability.

A.

Date	Julian Date	GB PAR Carbon Assimilation	Std. Dev.	GB PAR + UVR Carbon Assimilation	Std Dev.	t	P
May 18-05	137	0.00054	0.00014	0.000135	0.00003	2.23	0.063
July 11-05	191	0.00686	0.00026	0.002885	0.00018	25.8	0.001
Aug 12-05	223	0.03456	0.00220	0.012100	0.00190	13.4	0.001
Oct 1-05	273	0.00389	0.00012	0.000060	0.00002	272	0.001

B.

Date	Julian Date	WB PAR Carbon Assimilation	Std. Dev.	WB PAR + UVR Carbon Assimilation	Std Dev.	t	P
May 18-05	137	0.00049	0.00020	0.000239	0.00008	5.4	0.006
July 11-05	191	0.11368	0.00032	0.055850	0.00027	257	<0.001
Aug 12-05	223	0.07390	0.00038	0.031444	0.00030	105	<0.001
Oct 1-05	273	0.00617	0.00018	0.000940	0.00022	50	<0.001

C.

Date	Julian Date	LO PAR Carbon Assimilation	Std. Dev.	LO PAR + UVR Carbon Assimilation	Std Dev.	t	P
Jun 15-05	165	0.04462	0.00356	0.010549	0.00360	16.9	<0.001
Jul 17-05	194	0.00435	0.00022	0.001387	0.00010	5.54	0.005
Aug 18-05	229	0.00475	0.00042	0.001424	0.00013	13.2	<0.001
Oct 17-05	289	0.00883	0.00090	0.000800	0.00050	13.5	<0.001

D.

Date	Julian Date	HH PAR Carbon Assimilation	Std. Dev.	HH PAR + UVR Carbon Assimilation	Std Dev.	t	P
Jun 15-05	165	0.08275	0.08000	0.010549	0.00360	1.53	0.203
Jul 17-05	194	0.03515	0.00011	0.013870	0.00010	11.7	<0.001
Aug 18-05	229	0.11914	0.00073	0.032137	0.00091	18.6	<0.001
Oct 17-05	289	0.04432	0.00260	0.014500	0.00147	16.7	<0.001

E.

Date	Julian Date	BQ PAR Carbon Assimilation	Std. Dev	BQ PAR + UVR Carbon Assimilation	Std Dev.	t	P
Jun 1-05	151	0.01047	0.00020	0.003190	0.00020	27.4	<0.001
Jun 29-05	179	0.02720	0.00518	0.017870	0.00344	2.60	0.060
Aug 4-05	215	0.18930	0.00650	0.142125	0.00860	3.60	0.023
Nov 3-05	306	0.01111	0.00060	0.003190	0.00010	22.7	<0.001

A.
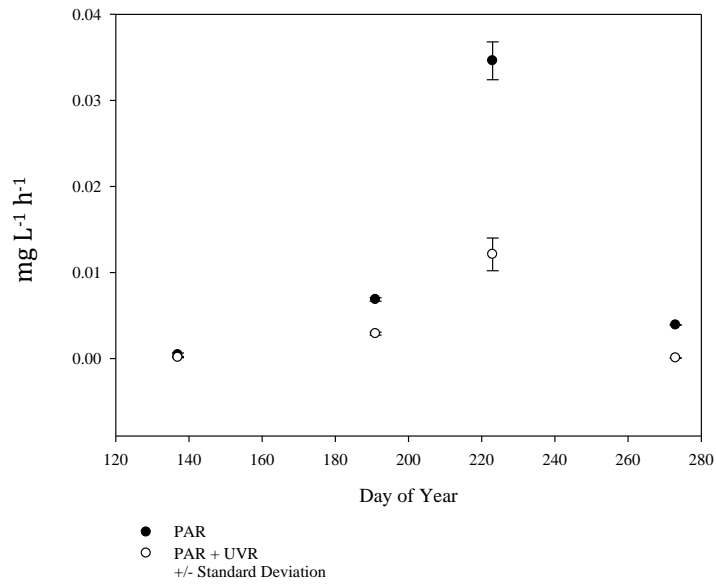
B.
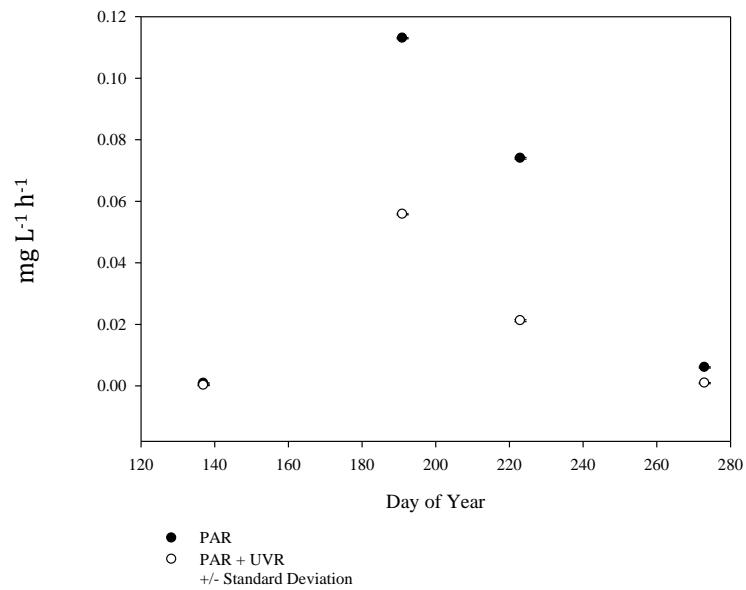
Figure 3.7: Light driven assimilation of carbon for Georgian Bay (A) and Woods Bay (B) under PAR and PAR + UVR

Bay of Quinte

The Bay of Quinte had a maximum rate of carbon assimilation of 0.713 mg L^{-1} h^{-1} on August 4th, the highest observed during this study. Productivity for Bay of Quinte samples incubated under PAR had a major seasonal component as well (Figure 3.9). The UVR incubations were not suppressed to the same extent found at the other stations.

Carbon Response Ratio:

In 2005, the assimilation of carbon provided a more sensitive probe of the physiological response of the primary producers than oxygen production. Therefore, I calculated response ratios using carbon assimilation rather than oxygen production as was done in Chapter 2.

As in Chapter 2, I used a response ratio to compare the depression in primary production between the sites: LO, HH, GB, WB, and BQ. The response ratio is a simple ratio of assimilated carbon under PAR + UVR divided by assimilated carbon in PAR-only 4 hour experimental incubations.

There were significant differences in the response ratios of HH and LO on all dates except June 15th, day 165 (Figure 3.10 A). It is interesting to note that LO had a higher response ratio (less suppression) than HH on June 15th and August 18th. This was unexpected based on previous work (Chapter 2) that suggested that HH, due to water-color and larger biomass, would be less likely to have significant suppression of carbon assimilation compared to LO.

On all dates, WB response ratio was larger than GB, indicating that PAR+UVR had less impact on primary producers in WB than GB (Figure 3.10 B).

A.
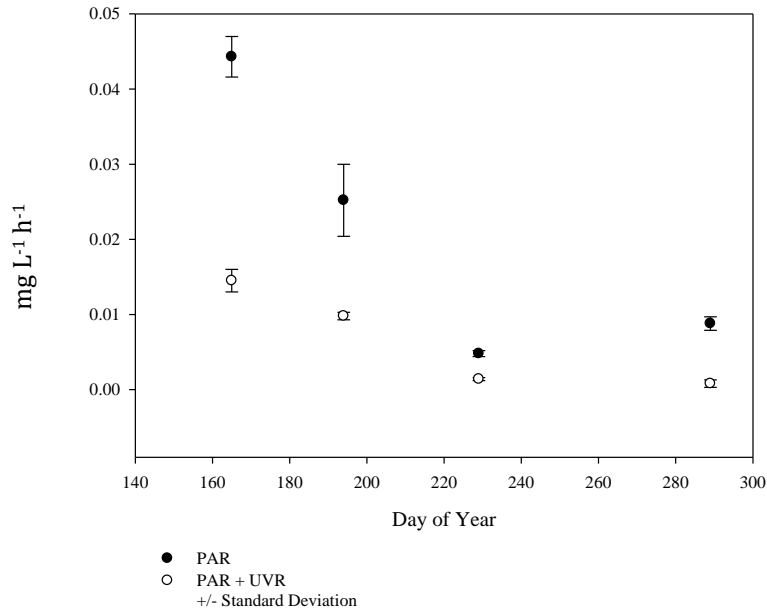
B.
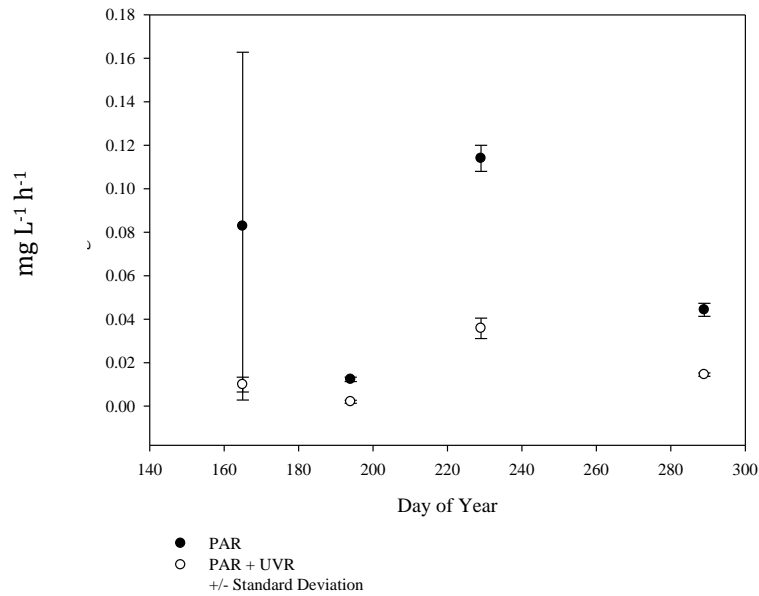
Figure 3.8. The assimilation of carbon for Lake Ontario (A) and Hamilton Harbour (B) under both PAR and PAR + UVR.
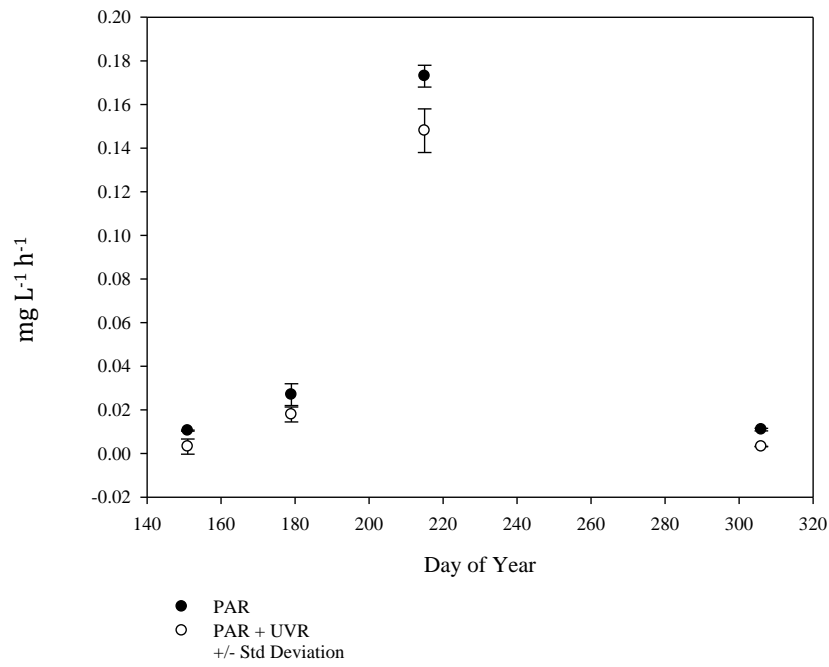
Figure 3.9: PAR and PAR+ UV driven assimilation for carbon as determined using ^{14}C, for Bay of Quinte.

The BQ response ratio indicates that during the first and last experiments UVR had the greatest negative impact on productivity (Figure 3.11). It has a very strong seasonal appearance to the data as well. It is important to note that the BQ station appears to be far less sensitive to the PAR + UVR compared to the other stations and there was also no correlation over time with the sample chl-a.

The accumulated total incidence for UVR at 295 nM for 14 days prior to the sample date, had a positive relationship with the carbon response ratio, with multiple $R^2 = 0.542$ (P = 0.024). I explored relationships between the response ratio and other wavelengths of light, temperature, DOC, attenuation coefficient, and Chl-a. None were significant. Station was added as a categorical variable, but it was not significant, nor was there a UVR-station interaction.

A.
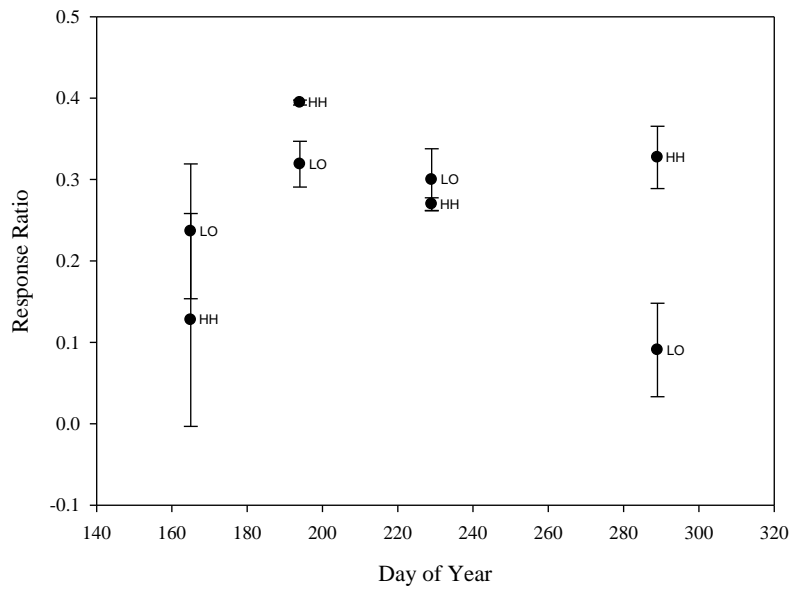
B.
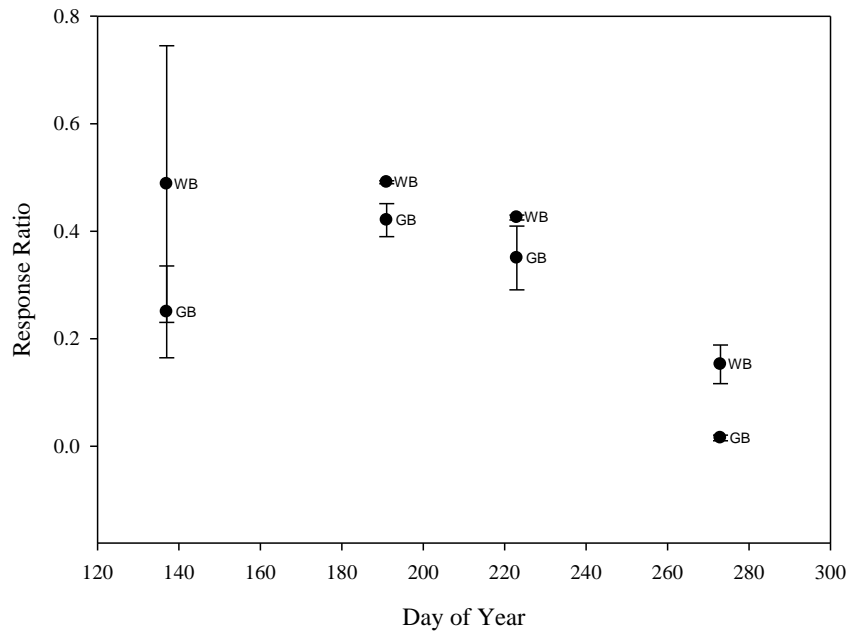
Figure 3.10. The response ratio of carbon assimilation of the paired systems (A) HH and LO and (B) WB and GB. The error bars are the standard deviations.
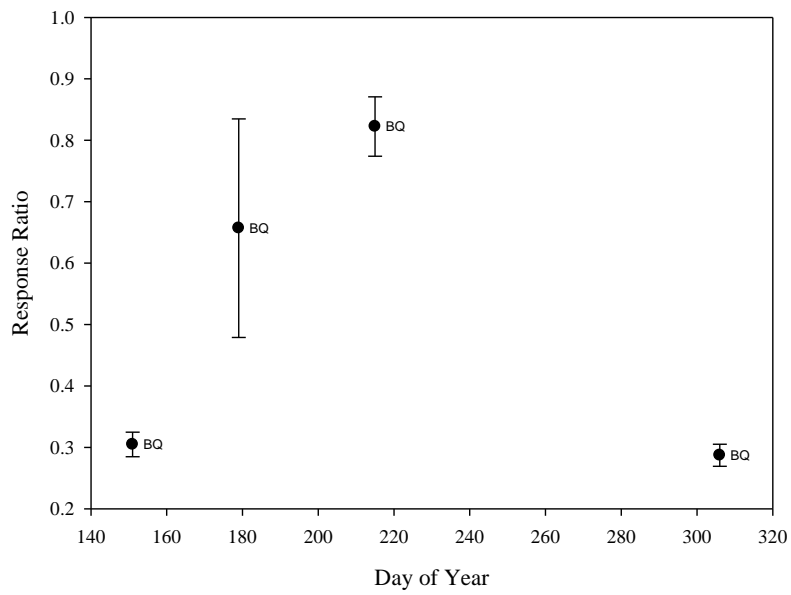
Figure 3.11. The response ratio of carbon assimilation of the Bay of Quinte. The error bars are the standard deviations.

Discussion

This chapter tested 2 different hypotheses. The first hypothesis was that any abiotic oxygen consumption during 4 hour incubations under PAR or PAR + UVR would be negligible compared to biologically-mediated changes. I was unable to detect any significant abiotic oxygen consumption in chemically sterilized samples under either PAR or PAR + UVR, and the incubated samples also did not significantly differ from their initial oxygen concentration. I conclude that the hypothesis is true. That is, that photochemical oxygen consumption was negligible compared to biological fluxes.

Abiotic light-driven oxygen consumption has been observed and measured by many authors (Estapa and Mayer 2010 and references cited there in), and it is usually associated with particulate organic carbon (POC). Estapa and Mayer (2010) used long irradiations (24 h) in their work, while my incubations were limited to 4 h. It is possible that had my incubations been longer or at higher light intensities that I may have been able to detect abotic oxygen consumption, but for my work I do not believe that it was a factor in those incubations where I measured oxygen loss.

The second part of this hypothesis "except in the highest DOC sites, where significant amounts of oxygen may be photochemically consumed" was false. There were no detectable changes in oxygen during the incubation of chemically sterilized samples, even in the BQ samples containing the highest DOC concentrations.

Dissolved organic carbon has long been recognized as an important variable in our understanding of overall lake physiology (Schindler 1998). DOC not only influences the light and temperature regime (Caplanne and Laurion 2008), but it also has direct influence on aquatic organisms by providing a substrate for biological activity (Lignell et al.2008).

DOC attenuates UVR and thereby reduces the amount of UVR aquatic organisms are exposed to (Schindler 2001). It follows that organisms in a high DOC environment, given the same PAR + UVR exposure as those in a low DOC environment, should have higher oxygen production and carbon assimilation because of the protective effect of the DOC.

My second null hypothesis states that under PAR + UVR there will be no difference in oxygen production or carbon assimilation by the primary producers relative to what occurs with PAR only incubations, despite differences in the amount or source of the DOC in the water column.

Oxygen production was always lower in the PAR + UVR incubations than in PAR-alone, though not always significantly lower. Of the 18 incubations reported in this chapter, 13 had net oxygen consumption during the 4 h incubations in PAR + UVR. On 5 of the 6 dates when the difference in oxygen production between PAR and PAR +UV was significant, net primary production was negative under PAR + UV. The consumption of O_2 was caused by the exposure of biota to UVR, as oxygen consumption did not occur in the chemically-sterilized samples exposed to PAR + UVR. PAR incubations never had net oxygen consumption and in all cases (i.e. both

PAR and PAR + UVR) there was light-driven carbon assimilation indicating that some photosynthetic activity was taking place.

I conclude that the first part of the second hypothesis, that aquatic photosynthesis at these sites, as measured by oxygen evolution, respond similarly to UVR exposure, is not true. While all sites displayed a suppression of oxygen evolution under PAR + UVR, only the Bay of Quinte had statistically significant suppression on 3 of 4 sample dates. The other sites combined had 3 significant dates of suppression out of a total 14 sample dates. All sites displayed net oxygen consumption under PAR + UVR incubations, at least on some occasions.

The lack of resolution of the oxygen probe used during this work limited my ability to compare O_2- NPP of the sites. I was unable to achieve the same degree of accuracy using the oxygen probe compared the high-precision Winkler technique used in chapter 2. The probe was extremely sensitive to touch during measurements, so if I inadvertently touched the Tedlar bag, I found it necessary to recalibrate the probe and repeat the measurement. The fiber optic cable, which connected the probe to the spectrometer, was also sensitive to movement. Even the slightest movement of the cable would cause a shift in the oxygen measurement. I went to extreme lengths to immobilize the cable with tape, but still found it necessary to recalibrate the system and repeat measurements.

The loss of oxygen during the UV incubations does not appear to be a result of photochemical oxidation of allochthonous DOC (hypothesis 1), but rather it is dependent on the organisms in the samples during incubation. The respiration of

some aquatic organisms is increased under UVR (de Mora et al.2000). If oxygen

evolution is reduced sufficiently and/or metabolic oxygen consumption is increased,

that may account for the observed net oxygen loss. Further, it is known that aquatic

organisms stressed by UVR will release a variety of compounds, e.g., MAAs and DOC

(de Mora et al.2000). These compounds may be photochemically oxidized by UVR,

consuming additional oxygen from the system. A combination of these mechanisms

may explain the net loss of oxygen I observed in the PAR+UVR incubations and may

also provide an additional source of CO_2 adding to the supersaturation observed in

the epilimnion of some lakes by Urban et al.(2009), Sobek et al. (2005) and others.

The second part of hypothesis 2 deals with carbon assimilation, which

proved to be a more precise indicator of photosynthesis. The hypothesis was that

carbon fixation, despite several differences among stations, will respond similarly to

UVR exposure.

As expected, carbon assimilation in the experimental PAR + UVR incubations

was suppressed significantly compared to the PAR-only incubations in most cases.

In order to compare the response to PAR + UVR relative to PAR only among stations

and dates, I used the response ratio. The ratio should detect a proportionally similar

or different response to UVR exposure.

DOC significantly attenuates UVR (Fee et al.1996) and it is expected that

there should be less UVR reaching the primary producers in high DOC systems

compared to low DOC systems. As a result there should be less UVR-based

suppression of carbon assimilation (de Mora et al.2000). While this may a play

lesser role in the experimental bags which provide a relatively short light path in this experiment, it would certainly be important in-situ at the stations. It seems reasonable to suggest that the effect observed in the PAR + UVR incubations compared to the PAR-only incubations is due to the degree of adaptation of the organisms rather than different exposures in the bags due to differences in the CDOC. Woods Bay and Hamilton Harbour had approximately the same amount of DOC (about 4 mg/L), but Woods Bay has a distinct tea colour, which might be expected to attenuate more UVR (Steinberg et al.2006) producing a larger response ratio than Hamilton Harbour. The larger the response ratio the smaller the suppression of carbon assimilation. The stations responded in much the same way as the field season progressed. When regression was used to relate the response ratio to UVR history, the effect of station was still not statistically significant. I conclude that the null hypothesis "that there is no difference among sites" is also false for carbon assimilation. While the response ratio did not appear to depend on season, light history, which is related to season, was found to correlate significantly with the response ratio. Additionally BQ appeared to be much more resistant to the UVR in the PAR + UVR incubations than the other stations.

It is possible that the water from the various stations had previously been exposed in situ to sufficient UVR that the DOC lost much of its ability to attenuate UVR (Zepp et al.2007, Magneson et al.1997) and the primary producers had become adapted to elevated UVR levels. This would explain to some degree why the response ratio was positively correlated with the cumulative 14 day total for 295 nM radiation incidence. It would have been ideal to have UVR measurements from

each station rather than a central location, or the mean UVR exposures for the epilimnion at each station. That would have provided more accurate data on prior UVR exposure. Overall, I conclude that differences in the inhibition of photosynthesis I observed were the result of recent weather (i.e., UVR history) as well as differences among the sites. HH had a higher response ratio than the other stations in the later part of the sampling season, which would indicate a different response to the UVR.

If this work is to be repeated, I would use the Winkler technique for oxygen measurements with the expectation of much higher resolution for the oxygen measurements. UVR measurements for a period of time prior to water sampling at each station would provide further insight into the relationship between response ratio and light history observed in this work. The addition of PAM fluorometry to provide estimates of photosynthetic capacity before and after the experimental incubation would assist in determining the changes that occur to the primary producers. This may provide a better tool allowing more precise across basin comparisons. Additionally, characterization of the DOC before and after the experiment could have provided valuable insights into photochemical processing that may be taking place during the experimental incubations. The use of $H_2^{18}O$ would assist in understanding the respiratory activity taking place during the incubations as it is thought to provide an accurate method of measuring respiration in the community.

It may also be worth considering running the experiments in-situ, which would provide a more natural light environment, though due to the distances between station locations, alternative station locations would have to be considered in order to reduce travel time. The downside to this approach is the loss of a uniform light and temperature regime that was obtained for this work.

Chapter 4

UVR and DOC impact on bacterial production.

Introduction:

Bacterioplankton play important roles in aquatic ecosystems and with changes in the environment, such as the intensification of UVR and climate change, our understanding of the impacts on these organisms is critical. They form part of the foundation of aquatic food webs by processing dissolved organic carbon (DOC) and making the carbon and nutrients available to mixotrophic algae, flagellates, ciliates and metazoan zooplankton (Wetzel 2001, Cole et al.2002, Grover and Chrzanowski 2009). Bacterioplankton also compete with autotrophs for limiting nutrients in aquatic systems (Wetzel 2001).

Climate change will bring changes in precipitation, which will affect the amount and quality of allocthonous DOC entering aquatic ecosystems (Schindler 1998). Biddanda et al.(2001) have suggested that allochthonous DOC is a major subsidy, particularly to oligotrophic systems, making bacteria and their processing of DOC of greater importance. As well, algae are an important source of DOC for the support of the heterotrophic bacterial population (Ogbebo and Ochs 2008). Linkages between pelagic bacteria and algae, along with flagellates, ciliates and the dissolved organic material, form the basis of the microbial loop (Wetzel 2001).

Solar radiation, and in particular ultraviolet light, is known to affect virtually every component of aquatic ecosystems (Falkowski and Raven 2007). Pelagic organisms are exposed to varying levels of ultraviolet light depending on their position in the water column, stratification, weather conditions, and the transparency of the water. Organisms that are higher in the water column are exposed to more intense levels of solar

radiation, while those lower in the water column receive less light and less damaging UVR.

Ultraviolet radiation has the potential to damage biological organisms and inhibit biological processes. Ultraviolet radiation can also interact with bacterial productivity by inhibiting phytoplankton production or by making recalcitrant DOC more usable to the microbial population (Cole 1999). Ultraviolet radiation is also known to cause extracellular release of compounds from a variety of organisms, including algae. The extracellular release of material by phytoplankton, and its utilization by bacteria, have been examined in an alpine lake and two high arctic lakes in Siberia, and an inverse relationship between UVR photoinhibition of the phytoplankton and DOC content was found (Panzendoeck 2007). Further, he concluded that the DOC excreted by the algal population was capable of sustaining the bacterial community.

Perez et al. (2003) studied the role of UVR in waters with highly chromophoric DOC, and concluded that UVR stimulated production of the heterotrophic pathways while inhibiting autotrophic production. Piccini et al. (2009) studied the impact of solar radiation on the growth of bacterioplankton from an ocean lagoon with a high CDOM concentration using dilution cultures and water samples exposed to 3 different light regimes (Dark, PAR + UVR, and PAR). The water samples were pre-exposed to the light treatments then dilution cultures were grown in the pre-exposed water. The cultures were incubated in the dark for a period of 5 h. They also used changes in absorption, fluorescence, and DOC concentration as proxies for CDOM photo-alteration. They found an increase in bacterial activity in the pre-exposed UVR samples, and also observed photo-bleaching of CDOM and a loss of DOC from the cultures.

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In this work, natural populations and water were exposed to 3 different light treatments, PAR, PAR + UVR and dark, for a period of 4 h. The samples were then incubated in the dark for 12 h. Bacterial productivity was measured using tritiated thymidine incorporation immediately after the light treatments and again after 12 h of incubation in the dark. The objective was to test the null hypothesis that there would be no change in bacterial production across the different light treatments and among the different stations. An alternate hypothesis is that PAR + UVR would result in enhanced production in the natural bacterial population in water higher in DOC compared to low DOC waters. Another alternate hypothesis is that there will be a suppression of bacterial productivity in the PAR + UVR treatments compared to PAR-alone or dark treatments.

Methods and Materials

Sites and sampling protocols were described in Chapter 3. Pertinent information about these sites is provided in Tables 4.1 and 4.2. The only difference here is that bacterial productivity was measured for only some sampling dates (Table 4.2) and bacterial productivity was measured at three different times: time 0 (T0) just prior to the experimental incubations; 4 h, at the completion of the experimental incubations; and 16 h, 12 h in the dark after the experimental incubations were completed. Time 0 productivity measurements were obtained directly from the carboys, and the water was then placed into the experimental test tubes for the light and following dark incubations.

Table 4.1 Sampling stations used during the 2005 field season. Attenuation is K_{PAR} (m^{-1}) and DOC is in mg L^{-1} +/- standard.

Site	Abbreviation	Longitude	Latitude	K_{PAR}	DOC
Woods Bay	WB	79 59' 44.93"	45 08' 21.05"	0.62 ± 0.05	4.5± 0.4
Georgian Bay	GB	80 07' 05.67"	45 08' 04.07"	0.31 ±0.09	2.4 ± 0.1
Bay of Quinte	BQ	77 15' 25.89"	44 08 57.95"	1.03 ±0.08	6.6± 0.02
Hamilton Harbour	HH	79 50' 29.91"	43 17' 45.39"	0.71 ±0.03	4.4± 0.05
Lake Ontario	LO	79 43' 16.25"	43 17" 58.01"	0.25 ±0.04	2.4± 0.2

Table 4.2 The dates and stations sampled during the 2005 field season. Total phosphorus and Chl-a are also shown. Chl-a is in µg L^{-1}, DIC is in mg L^{-1}, and TP is in µg L^{-1}.

Date	Julian Date	Station	Chl-a	DIC	TP
01/06/2005	151	BQ	24.80	25.91	13.2
04/08/2005	215	BQ	9.14	29.93	33.49
03/11/2005	306	BQ	13.8	24.09	27.04
14/07/2005	194	HH	8.9	29.93	27.65
18/08/2005	229	HH	19.63	24.95	30.42
17/10/2005	289	HH	9.82	26.70	-
14/07/2005	194	LO	1.46	23.03	-
18/08/2005	229	LO	0.73	22.93	7.68
17/10/2005	289	LO	3.27	23.70	9.22
11/07/2005	191	WB	2.87	1.87	11.40
12/08/2005	223	WB	5.33	2.24	11.35
01/10/2005	273	WB	7.33	2.50	7.37
11/07/2005	191	GB	0.63	15.70	5.53
12/08/2005	223	GB	1.33	15.77	6.76
01/10/2005	273	GB	0.95	17.42	5.53

Quartz test tubes were used as the experimental light incubation vessels and three different light treatments were used, as in Chapter 3. At the specified times, triplicate 5 mL aliquots were incubated in the dark with tritiated thymidine following the procedures described in Wilhelm and Smith (2000), except that only thymidine was used rather than thymidine and leucine.

Glassware Preparation:

The quartz test tubes and their stoppers were washed in a strong nitric acid solution followed by a triple rinse in de-ionized water and were subsequently placed in 50% ethanol for a period of no less than 3 h. They were then wrapped in aluminum foil, and placed in a drying oven until all alcohol had evaporated. Scintillation vials used for the sample thymidine incubation were soaked in 100% ethanol, covered in aluminium foil and dried in the drying oven.

A 20-nM working solution of thymidine was made by adding 300 µL of the thymidine stock solution (1 mC/mL) in 2 mL of distilled water. The working solution was then filter-sterilized by passing it through a 0.2-µm mixed cellulose ester filter.

At the specified times (0 h, 4 h, and 16 h), 5 mL of the treated lake water was removed from each test tube in triplicate, placed in sterilized scintillation vials and 50 µL of the working thymidine solution was added. After 2 minutes, initial samples were placed on ice and other samples were placed in the incubation chamber for 60 minutes at

the experimental temperature as explained in Chapter 2. To terminate thymidine uptake,

samples were placed on ice for a period of 5 minutes, then 5 mL of 10% tri-chloroacetic

acid (TCA) was added and the samples were allowed to sit for another five minutes

allowing the TCA to extract any unincorporated thymidine. The samples were then

filtered through 0.2-µm mixed cellulose filters. Another 5 mL of 10% TCA was used to

rinse the inside of the manifold and this was followed by additional rinsing with de-

ionized water to ensure that all labeled organisms were on the filter surface. The filter

was then carefully removed from the manifold and placed in scintillation vials along with

15 mL of scintillation cocktail. The scintillation counting of labeled material was done on

a Beckman–Coulter LS 6500 liquid scintillation counter.

Bacterial production was calculated using the methodology of Chin-Leo &

Kirchman (1988).

$V_T = [(DPM_{sample} - DPM_0)(20\ nM)/(DPM_{Total})(incubation\ time)]$

Where:　　　V_T = thymidine uptake rate in mM $L^{-1} h^{-1}$

DPM is disintegrations per minute

DPM_{sample} is DPM incorporated into DNA

DPM_0 is the time zero count

DPM_{Total} is the sample total thymidine activity

Bacterial Cell Production (cells $L^{-1} h^{-1}$):

$BP = V_T * 2 * 10^9$

Bacterial Biovolume Production ($\mu m^3 L^{-1} h^{-1}$)

$BBP = BP * 0.04\ \mu m^3\ cell^{-1}$

Bacterial Carbon Production (g C $L^{-1} h^{-1}$)

$$BCP = BPP * F$$

Where F is the assumed carbon content of $2.2 * 10^{-13}$ g C μm^{-3} for living bacterial cells.

Statistics

ANOVAs were used to assess differences among light treatment, and post-hoc Student-Newman-Keuls (SNK) tests were completed when differences were confirmed. Analysis of the data used SPSS and data were graphed using Sigma-Plot.

Results:

During the early season (June 1 to July 14) only the Woods Bay site on July 11 (day 191) bacterial productivity had a significant difference in response to the light treatments, and that occurred at 16 h, i.e., after 12 h of dark incubation. All three light treatments were different from each other (ANOVA, F = 96.0, P = 0.0004, followed by SNK) (Table 4.3). Bacterial production was highest for the PAR + UVR treatment (1.25 * 10^{-3} g C L^{-1} h^{-1}) followed by the PAR treatment (8.53 * 10^{-4} gC L^{-1} h^{-1}) and the dark treatment (6.65 * 10^{-4} g C L^{-1} h^{-1}). The divergence in treatments occurred during the post-treatment dark incubations, as the treatments were not significantly different immediately after the light treatment.

Table 4.3 Bacterial productivity for Woods Bay, July 11 (day 191) at 16 h after the start of light incubation. Production values are in g C L^{-1} h^{-1}. Means that are not significantly different by the Student Newman Keuls test are found in the same column. Production values are in g C L^{-1} h^{-1}.

Treatment	N	1	2	3
PAR	2	1.25×10^{-3}		
Dark	3		8.53×10^{-4}	
PAR + UVR	2			6.65×10^{-4}

In the mid-season experiments (August 4th through to August 18th) there were significant differences among light treatments at Bay of Quinte on August 4th (day 215), again after 12 h of dark incubation after light exposure (Table 4.4), but not immediately after the light treatments. The PAR + UVR treatment displayed the lowest carbon uptake, while the Dark treatment was more productive and the PAR-only treatment had the greatest productivity.

In Woods Bay and Georgian Bay, the light treatments were not significantly different at 4 h on August 12th. I was able to perform only 1 thymidine incubation per light treatment at 16 h for the 2 stations (Figure 4.1 A and B). So for 16 h there are no replicates. It is interesting to note that the PAR + UVR treatment had the highest productivity in Georgian Bay and the lowest in Woods Bay, while PAR-only had the lowest in Georgian Bay.

Both Lake Ontario and Hamilton Harbour on August 18th (day 229) displayed significant differences at 4 h, just after the light treatments (Figure 4.2). The difference between the samples disappeared after 12 h of dark incubation. In the Lake Ontario samples, PAR + UVR bacterial productivity was significantly higher than either the PAR alone or the dark treatment. The difference among light treatments had disappeared by 16 h, although PAR + UVR was still higher than the other treatments at the end of the experiment (Figure 4.2A).

In Hamilton Harbour, samples incubated under PAR + UVR were suppressed compared to the PAR alone and dark treatment (Figure 4.2 B). The PAR and dark treatments were not different. At 16 h, the light treatments were not different.

Table 4.4 Student Newman Keuls results for the bacterial productivity of Bay of Quinte August 4 (day 215) at 16 hr. Means that are not significantly different are found in the same column. Production values are in g C L^{-1} h^{-1}.

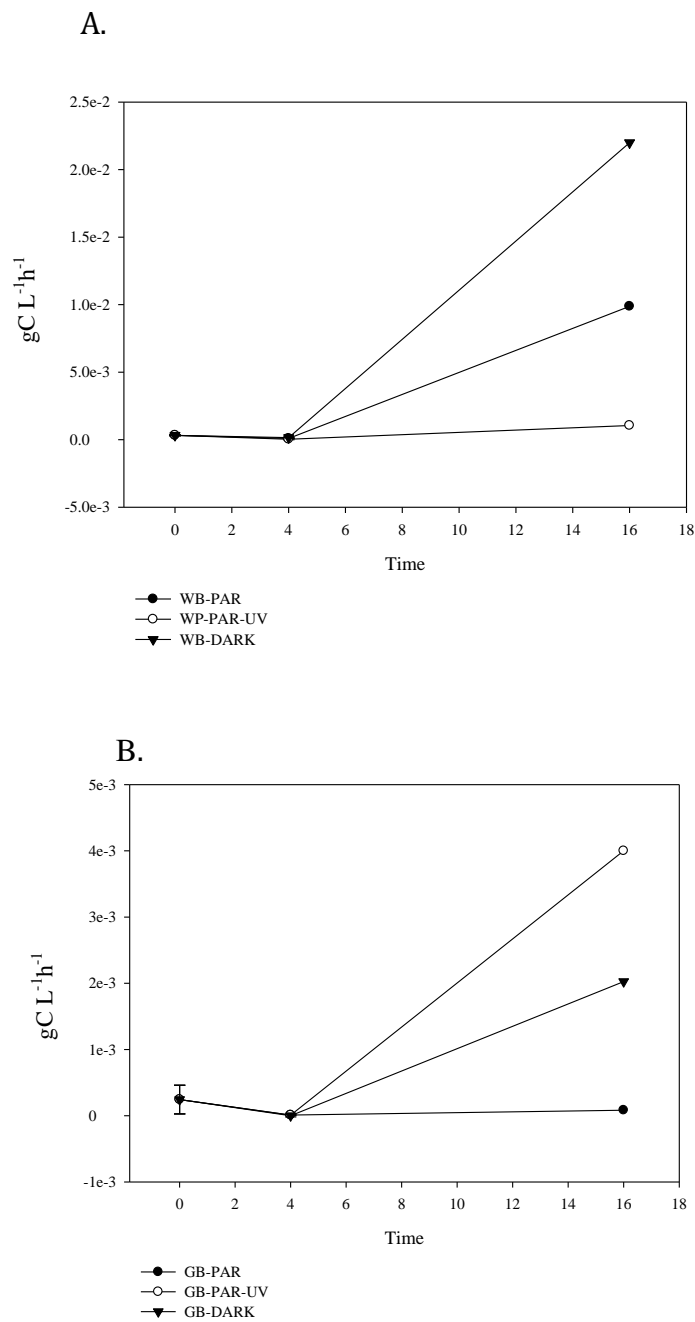| Treatment	N	1	2
PAR + UVR	3	6.321x10^{-3}	
Dark	3	7.691x10^{-3}	7.691x10^{-3}
PAR	3		9.129x10^{-3}

A.

B.

Figure 4.1 Bacterial productivity in Woods Bay on August 12th (A) and in Georgian Bay on August 12th (B) Times is in hours from the start of the light treatments.
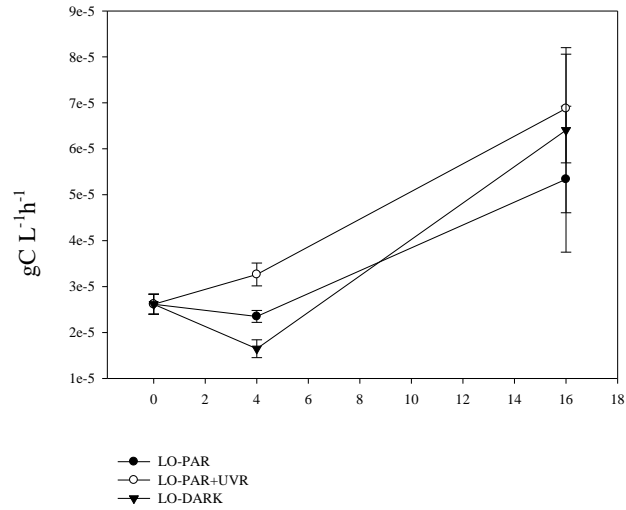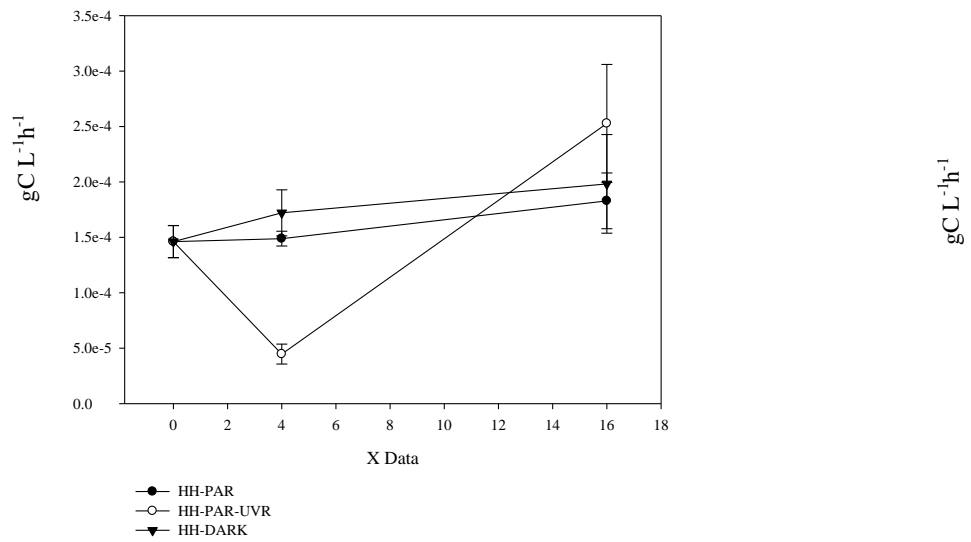
Figure 4.2 Bacterial productivity on August 18th, after light treatment and a 12 hour dark incubation period for Lake Ontario (A) and Hamilton Harbour (B). Time is in hours from the start of the light treatments.

During the late-season sampling (October through to November) Woods Bay was not sampled due to equipment failure. All other stations were sampled in the normal fashion.

On October 1st (day 273) the Georgian Bay dark and PAR treatments had lower bacterial production than PAR-alone at 4 h (Figure 4.3). At 16 h the differences among treatments were no longer significant.

On October 17th, for Lake Ontario and Hamilton Harbour, PAR + UVR suppressed productivity compared to the other two treatments at 4 h (Figure 4.4 A and B). All treatments for Hamilton Harbour at 4 h were different from each other, while by 16 h PAR + UVR was significantly different than the PAR and the dark treatments.

On November 3rd for the Bay of Quinte station, no differences were found among the light treatments at 4 h or 16 h. However, the PAR + UVR treatment had the lowest productivity (Figure 4.5).
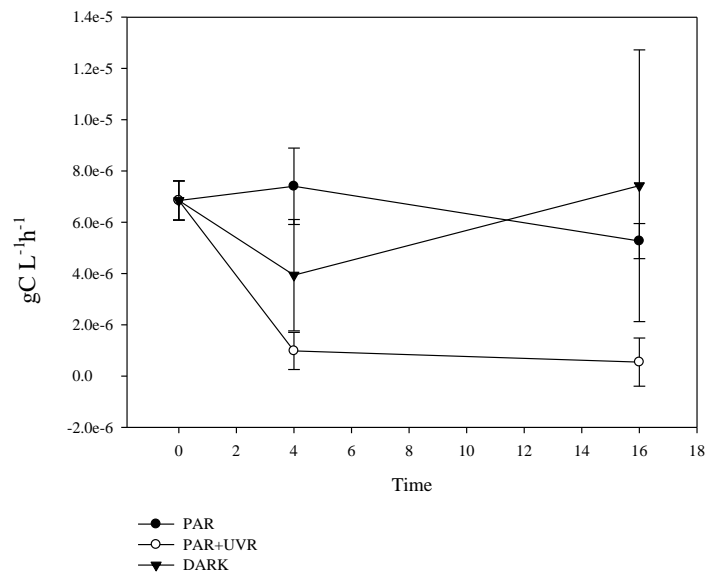
Figure 4.3 Bacterial productivity in Georgian Bay for October 1. Time is in hours from the start of the light treatments.

A
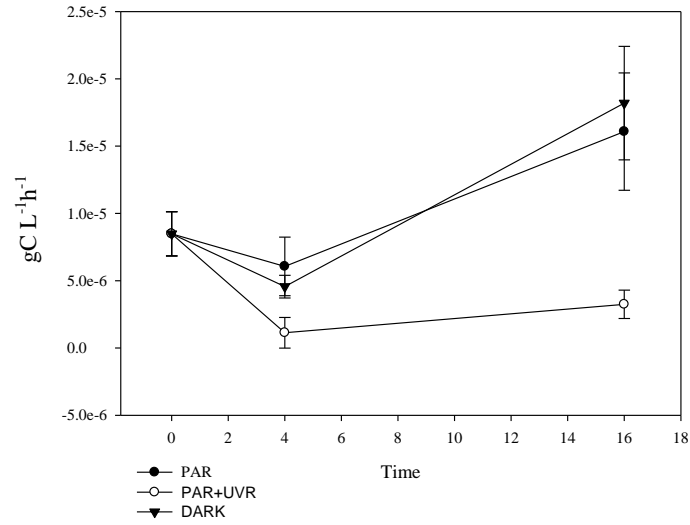
B

Figure 4.4 Bacterial productivity on October 17th, for Lake Ontario (A) and Hamilton Harbour (B). All times are in hours from the start of the light treatments.

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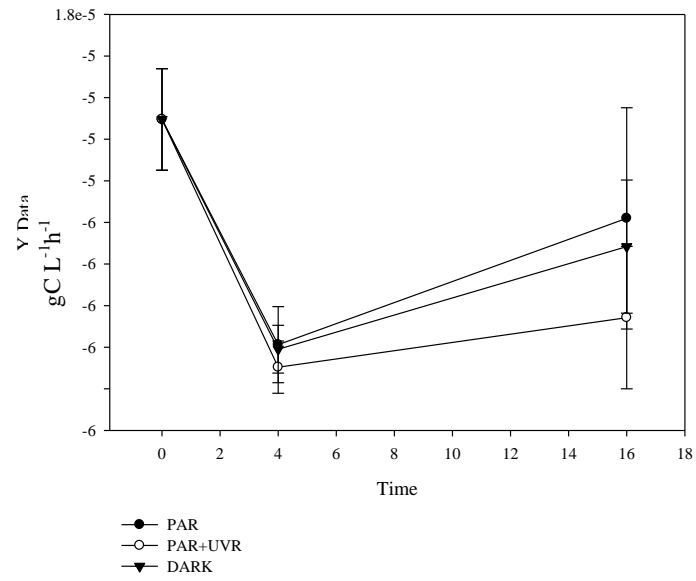
Figure 4.5 Bacterial productivity for the Bay of Quinte station on November 3. Time is in hours from the start of the light treatments.

Discussion:

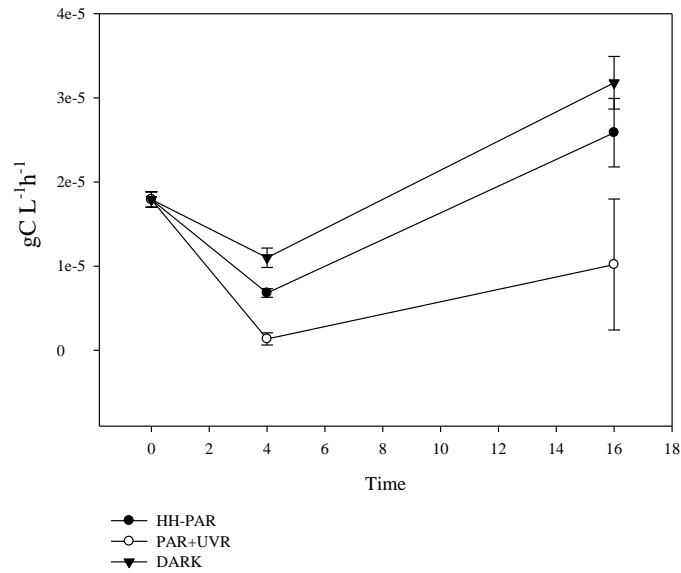Generally, bacterial productivity measured at 4 h after the light incubations did not differ among treatments. The exception was four experiments in Lake Ontario and Hamilton Harbour in August and in October. Of this group of 4 experiments, Lake Ontario on August 18th demonstrated an increase in bacterial production following the PAR+UV incubation. In the other three cases, there was a suppression of bacterial production by UVR. Many of the treatments, including the dark control, demonstrated a decline in bacterial productivity from time zero to the 4 h point at the end of the light treatment incubations. The decline in productivity occurred in the dark treatment as well as the light treatments, but was uneven over the field season occurring on some dates and not on others. This could possibly be a pause in bacterial productivity as they adapted to the experimental vessels. The comparison of the light treatments at 4 and 16 h after incubation will be the main focus below.

I would have expected Lake Ontario bacterioplankton to display a negative response to the PAR+UVR irradiance, as the low DOC and high clarity of the water would predict that the bacteria were exposed to higher levels of UVR than those in the Hamilton Harbour water. The higher exposure could be expected to cause more damage to the bacteria causing slower growth during PAR + UVR and post light treatment incubation. The only occasion on which this was observed was on October 17. One possible reason for apparent resistance to UVR exposure in Lake Ontario may be that bacterioplankton in clear waters are actually pre-adapted to tolerate UV exposure.

An interesting observation occurred for Hamilton Harbour on August 18th; the 4-h PAR + UVR sample was severely suppressed compared to the PAR and dark samples but

fully recovered at 16 h. This response might suggest that the bacteria in the PAR + UVR treatments derived significant benefit from newly available nutrients from the light treatment, but could take advantage of them only after the light treatment ended. However, this result was not consistently observed.

In the early to mid-season experiments, only one station on one date (Woods Bay on July 11) showed a significant increase in bacterial production when exposed to PAR + UVR compared to the other light treatments, and then only at 16-h. This station has highly coloured water with a moderate amount of DOC (seasonal average of 4.5 mg L^{-1}) compared with the low DOC stations, which had roughly half this DOC concentration. Piccini et al. (2009) found a rapid change in the bacterial population in high CDOC waters following UVR exposure, which the authors attributed to photo-alteration of the CDOC. Ogebo and Ochs (2008) found that only with the addition of phosphorus and nitrogen was there a response to UVR irradiation and the response was negative. They found a 20% reduction in bacterial productivity.

During August, of my mid-season experimental series, only the Bay of Quinte (August 5th) had significant differences among treatments at 16 h, and it displayed a significant suppression of bacterial productivity in the PAR+UVR treatment. At 3 of the 5 sites, bacterial production in the PAR + UVR incubations was higher than either the PAR only or dark incubations. While the results were not statistically significant, they may lend some support to the hypothesis that PAR + UVR causes the production of material, either through photo-alteration of existing DOM or by stimulating the release of new DOM, that is available to bacteria enhancing their production.

It is interesting to note that Woods Bay and Bay of Quinte, both with higher DOC and coloured water, were the 2 stations which had a reduction of bacterial production compared to the PAR-only treatments. This is an unexpected result, as several previous studies (Perez et. al 2003 and references cited therein) suggest that bacterial production at higher DOC sites should respond positively or in a neutral fashion to UVR. The coloured waters of the 2 sites would be expected to attenuate UVR to a much higher degree than the colourless waters of Lake Ontario and Georgian Bay, thereby causing less damage to the bacteria present. If this occurred as expected, then the PAR + UVR treatments would have had bacterial productivity similar to or exceeding the PAR-only treatments. That assumes that the there are no damaging chemicals being produced in the PAR + UVR treatments.

In the late field season, there was one consistent feature in the bacterial response to the light treatments. Regardless of the station or date, bacterial production in the PAR + UVR treatments was lower at the 16 h measurement. Further, 2 of the production measurements at 4 h were significantly lower (Lake Ontario and Hamilton Harbour) than either the dark treatment or the PAR-only treatment.

Phytoplankton photosynthesis was suppressed in the presence of PAR + UVR (previous chapters), but it is known that UVR can cause the extracellular release of carbon (Panzenbock 2007). This release may be feeding the higher bacterial production in the clear water samples where elevated bacterial productivity was observed. Lower bacterial production observed in the humic samples under PAR+UV (Woods Bay August 12 and Bay of Quinte August 5th) may be a response to UV-generated photoproducts or, more likely, reflect that the bacteria were not acclimated to the higher UV levels they

were exposed to in the experimental vessel. The samples were taken with an integrated sampler in the epilimnion; the UV levels lower in the water column would be significantly lower than those in the experimental chamber. There is also some evidence for a role for light history in that all sites in the late fall sample displayed a negative response to PAR + UVR. Earlier in the season there were both positive and negative responses to the increased UVR treatment.

In summary, while the results of light exposure on bacterial production were inconsistent among dates and sites, there is evidence that UVR can cause a positive bacterial response at diverse Great Lakes sites. This raises the possibility that bacterial respiration was also increased, providing an additional mechanism for the reduced community NPP documented in chapters 2 and 3, other than an increase in respiration or a decrease in photosynthesis on the part of the phytoplankton. While the evidence for enhanced bacterial production is not conclusive, additional work on community production that includes estimates of bacterial respiration may prove useful in determining which group of organism is responsible for the apparent increase in respiratory activity. The use of dual labeling, using both leucine as well as thymidine, may have provided higher resolution than the single labeling used in this work.

Chapter 5

Summary and conclusions

Summary

The primary goal of this research was to characterize the effects of incident levels of ultraviolet light and dissolved organic carbon on phytoplankton communities of the Great Lakes as measured by oxygen production and carbon assimilation. Man-made chemicals are continuing to erode the protective ozone layer in the atmosphere causing elevated levels of ultraviolet light to reach the surface of the earth and stress aquatic organisms, particularly during the spring season.

A first field season examining two stations, one in Hamilton Harbour and the other a nearshore station in Lake Ontario, provided insight into the response of the phytoplankton to elevated UVR compared to PAR-only exposures. Samples incubated in a PAR plus elevated UVR environment showed significant suppression of photosynthetic activity compared to samples incubated under PAR-only. Comparing oxygen evolution under PAR+UVR and PAR-only, I found that oxygen production was significantly reduced and that net oxygen consumption was observed on several experimental dates. While there was carbon assimilation taking place even in those samples that demonstrated a net consumption of oxygen, these results suggest that photosynthesis was suppressed to a rate below that of community respiration.

 In post-treatment dark incubations of 12 hours, the UVR incubations demonstrated an increase in respiration compared to the PAR-only treatments from Hamilton Harbour. A similar increase was not observed in samples from Lake Ontario. Though there was some variation in these results, they provide evidence for an increase in respiration after irradiation, possibly because recalcitrant DOC was converted to more

labile forms that could be used by the bacterial population present in the sample. Further, bacterial productivity may have been enhanced by the release of material by the stressed phytoplankton community.

During the second field season, the number of stations was increased by three to a total of five stations. The added stations along with the original two stations of Hamilton Harbour and Lake Ontario added a spectrum of DOC quality and quantity as the Bay of Quinte is surrounded by primarily agricultural land and Woods Bay and Georgian Bay have relatively pristine watersheds. These contrast sharply with the highly urbanized watershed of Hamilton Harbour.

Oxygen production was again significantly suppressed during the PAR+UVR incubations compared to the PAR-only incubations and, on some dates, net oxygen consumption was observed in the PAR+UVR incubations. Photosynthetic activity was taking place in these samples, demonstrated through the assimilation of carbon, even when there was net consumption of oxygen. Again, this would seem to suggest that respiratory processes were consuming more oxygen than was being produced through photosynthesis.

Carbon assimilation for the second field season was also suppressed in the PAR + UVR incubations when compared to the PAR-only incubations. The amount of suppression varied across stations, and appeared to be positively correlated with the cumulative amount of UVR at 295 μm over the previous 14 days. That is, as exposure to this wavelength increased, the suppression of photosynthesis increased. There was no direct correlation with any other measured variable.

Unfortunately, the resolution of the oxygen measurements was not as great in the 2005 field season as it was in the previous field season due to the use of the Ocean Optic Foxy Oxygen Probe. As a result, during the second field season there were no post-treatment incubations measuring dark respiration. However, measurements of bacterial productivity under the various light conditions were performed during the second field season. Bacterial productivity during the early and mid-season experiments either had no significant differences between light treatments, or the PAR + UVR treatments tended to have higher productivity, usually after the 12 h dark incubation. In the late season experiments, bacterial productivity was suppressed after PAR + UVR compared to the other treatments. Therefore, there is some evidence in this work that bacterial productivity benefitted from community UVR exposure, though it appears to be limited to early to mid-season.

In this study, the light environment was controlled and constant between and during the experiments. Previous research using ^{14}C to measure productivity *in situ* have used a variety of incubation methods. In some cases, incubations were in-situ at different depths, including the surface, but the was no indication of the type of glass vessels used during the incubations (Berman et al. 1995). This means we have no idea if UVR inhibition played a role in the reported productivity values. Others used Pyrex bottles for their in-situ incubations and included surface and near surface incubations(Dodson et al. 2000). Pyrex attenuates roughly 25% of UVB and about 10% of UVA between 320 and 360 μM. Xenopoulos and Schindler (2003) used UV transparent materials for the incubation vessels along with cut off filters and reported the productivity values for PAR alone, PAR + UVR and PAR+A and PAR+UVB.

Conclusions

UVR causes a suppression of photosynthesis as measured by O_2 production or carbon assimilation. Suppression of photosynthesis was observed for both GPP and NPP as estimated by O_2 changes. NPP may be negative under modestly increased UV irradiance. Even when NPP is negative, there is still active carbon assimilation taking place. The reduction in GPP and NPP at nearshore Great Lakes sites is not due to abiotic oxygen consumption.

Carbon assimilation was suppressed in the PAR + UVR incubations when compared to the PAR-only incubations and tended to vary somewhat by basin, time of year and the light history of the sample being incubated.

In further work on UVR effects on phytoplankton, I would recommend the use of standard BOD bottles rather than Tedlar bags and the use of the high precision Winkler technique rather than probes. As well, with the new molecular technology that is currently available, it may prove beneficial to do parallel experiments with well-defined phytoplankton species in culture measuring not only the oxygen production/consumption and carbon assimilation, but also using microarrays to determine which genes are being up-regulated and which are being down-regulated. Additionally, the use of fast repetition rate fluorometry to study the state of the photosystem could prove valuable in studying the impact of elevated UVR exposure. This would provide both a functional view of the organism (i.e., oxygen production and carbon assimilation) as well as a molecular view of the mechanisms taking place within the cell to manage the added ultraviolet light the cell is exposed to. As this

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study provided evidence that pre-exposure to ultraviolet light played a significant role in phytoplankton's ability to adapt to enhanced ultraviolet light, it may be worthwhile growing cultures under UV combined with the tests outlined above, in an effort to understand how they adapt to the elevated ultraviolet light.

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