PATTERNS OF HYDROGEN PEROXIDE AMONG LAKES OF THE MACKENZIE DELTA AND POTENTIAL EFFECTS ON BACTERIAL PRODUCTION

by

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Hons.B.Sc., University of Toronto, 2002

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

In the
Department
of
Geography

SIMON FRASER UNIVERSITY

Summer 2005

APPROVAL

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Degree: **Master of Science**

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ABSTRACT

Lakes in the Mackenzie Delta have complex patterns of dissolved organic carbon (DOC) ranging from low levels of coloured DOC in lakes frequently flooded with river-water to high levels of non-coloured DOC in infrequently flooded lakes. Hydrogen peroxide (H_2O_2) levels measured in 40 lakes at three times, ranging from summer solstice to late summer were highest around the solstice and in lakes of intermediate flood-frequency. Diurnal dynamics of H_2O_2, tracked for 40 hours during 24-hour sunlight in two lakes with contrasting DOC, showed cumulative build-up of H_2O_2 during multiple cloudless days. Build-up of H_2O_2, in experimental enclosures where exposure to UV-irradiance was manipulated, responded to both UVA and UVB. The effect of modestly elevated H_2O_2 levels and DOC substrate on bacterial production in enclosures from six differing lakes appeared weak during late summer and suggests a trade-off between UVB-inhibition (direct effect) and photolyzed-DOC as an additional food substrate.

DEDICATION

In memory of two phenomenal women, my *lolas*,

Maria Fabular Febria (1910 – 1993)

&

Editha Acurel Mitra (1929 – 2002),

who passed away a few short weeks before I started this project. I miss you.

Thank you for both for your wisdom and strength.

Mahal kita.

ACKNOWLEDGEMENTS

Research in the North is never accomplished entirely on one's own, and this thesis was no exception! Firstly, my sincere thanks to Dr. Lance Lesack for the financial support and intellectual encouragement, but above all, for giving me the opportunity to return to the Canadian North, certainly one of the best places on Earth! Additional thanks to the support and encouragement of my committee members, Dr. Max Bothwell and Dr. Margo Moore. Thanks to you both for contributing your individual areas of expertise and experience to this project. I sincerely appreciate your timely and extremely helpful suggestions throughout this process. Thanks to Dr. Len Hendzel (DFO Winnipeg) and Dr. Bill Cooper (UNC-W) for assistance with my hydrogen peroxide methodologies and equipment. Thanks also to funding agencies, NSTP, PCSP, NSERC and SFU Geography for funding this research.

To my colleagues in the Lesack Limnology Lab – Craig Emmerton, Jolie Gareis, Suzanne Tank and also Maggie Squires – there aren't enough 'thank you's'. Many thanks for reading my drafts, for keeping me company in the boat (and under my box!), and for always being supportive. We've certainly spread the word on "limnology"! I truly had a blast with you fabulous people and look forward to the day when we may collaborate once again. Thanks also to Bryan Spears and Andrea Riedel for advice and assistance very early on in this project.

A huge thank you to Les Kutny at the Inuvik Research Centre for unparalleled help in the field, on the river and for the fresh veggies. Thanks for assistance in the field from field assistants Nicole Vander Wal, Shannon Turvey, the Canadian helicopter pilots as well as assistance from occasional helpers (and good pals!) Raila Salokangas, Sharpie™, Duct Tape™ and Ziploc™. My appreciation also goes out to Margo Moore's lab and Linda Pinto, for being just next door and for assistance beyond the call of duty, especially with my bacterial production work. I'd also like to acknowledge the support from Marcia Crease, Dave Green, Radiation Safety (Kate, Dr. Brodovitch & Sharon), OHS (Michael Caine), and Science Stores (Jocelyn, Bob & Vince) for making graduate life and conducting field-based research as smooth as is humanly possible.

To the many friends I've met at SFU and in Vancouver – the Ocampo family, Alison, Vikki, Don, Kim and Neil – thanks for the fun times and always being supportive. My gratitude and appreciation to the Philippine Women Centre of BC, the Kalayaan Centre and Bert Monterona - thank you for the opportunity to continue working in our community and for fulfilling a passion other than science. I wish you all the best in our continued struggle. To Patrick, Eric, Rosemary, Jenny and Lydia (Chia-Wen) – thanks for the great memories, great food and great company. I'm so proud of you all and I'll really miss you guys. To Taskin Shirazi –*khudafiz* & *salamat,* chocolate cupcakes & hugs – it wouldn't be the same without you, girl!

Finally, to my partner (and rock), Peter Oabel. We've come so far and there's still so much to look forward to! You've made this ride so much more bearable, fun and unforgettable. To my loved ones back home thanks for always keeping in touch and always believing in me. To my sisters, Tessa and Nina, thank you so much! I am so proud of you both and feel so blessed because you two inspire me! Most importantly, thank you Mom and Dad! For everything. Thank you especially for choosing Canada, I am certain that it has forever changed the trajectory of my life. I know we know it was all worth it in the end...!

Merci, salamat, gracias, Muhsi choo, Thank you all!

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1 INTRODUCTION

Predictions from global climate models (GCMs) suggest that the most dramatic effects of increasing temperature will occur at the polar latitudes (Rouse et al. 1997). This is predicted to impact the length of ice-free seasons in the arctic, nutrient dynamics and aquatic food web functioning (Hobbie et al. 1999). Simultaneously, global levels of greenhouse gas emissions are predicted to increase (Shindell et al. 1998) and future volcanic eruptions at lower latitudes are predicted to combine with the positive and negative effects of climate change and ozone depletion (Rex et al. 1997; Tabazadeh et al. 2002). As a result, Arctic ozone levels are predicted to decrease up to 30 percent (Tabazadeh et al. 2000), which corresponds to an increase in ultraviolet-B radiation levels reaching the earth's surface (Kerr and Mcelroy 1993). The depletion of stratospheric ozone has been well documented since the 1990s and is of significant concern at Arctic and Antarctic latitudes.

Recent interest has been placed on the Arctic since rates of ozone depletion have matched those observed in the southern hemisphere (Austin et al. 1992). In Arctic aquatic ecosystems, the effects of enhanced ultraviolet radiation (UVR) is of particular concern during the open water season when lake productivity and biological activity is at a maximum, solar intensity is continuous and the potential for photochemical interactions are extremely high (Perin and Lean 2004).

UVR from the sun is divided into three main categories based on wavelength: ultraviolet-A (UVA, 320-400 nm), ultraviolet-B (UVB, 280-320 nm) and ultraviolet-C (UVC, 200-280 nm). UVA is the most abundant of all UVR, with the highest number of photons reaching the earth's surface (Diaz et al. 2004). UVC strongly interacts with stratospheric ozone, rarely reaching the earth's surface. Per photon, UVB is the most damaging of all UV wavelengths to biological activity (Karentz et al. 1994), negatively affecting ecosystem functioning (Bothwell et al. 1994). Indirect consequences of UVB include photoproducts such as radical oxygen species (ROS, Scully et al. 1996; Zepp et al. 1995) and hydrogen peroxide (H2O2, Cooper et al. 1994). Both the direct and indirect UVR effects impact biogeochemical cycles and food web dynamics (Mopper and Zhou 1990) and both UVA and UVB wavelengths play a role in biogeochemical processes, namely the breakdown of dissolved organic carbon (DOC).

This thesis will examine H_2O_2 formation within lakes of the Mackenzie Delta that differ in DOC content and determine the potential effects on bacterial production. Chapter 1 summarizes the general characteristics of dissolved organic carbon (DOC) as it applies to Mackenzie Delta lakes. Furthermore, this chapter will elucidate the main photochemical processes responsible for its breakdown including the simultaneous production of H_2O_2. Subsequently, a brief summary of the interaction between DOC, UVR and H_2O_2 will be provided followed by a discussion of the potential effects of H_2O_2 on bacteria. The chapter will conclude by providing the specific objectives of this research and identify the specific hypotheses that were tested. Chapter 2 summarizes the routine sampling, experimental designs and analytical methods used to conduct this study. Chapter 3 presents the results of lake surveys and outdoor incubator experiments. And finally, Chapter 4 highlights the major findings of this research and attempts to address the implications of the results to our current understanding of UVR in Arctic aquatic ecosystems.

1.1 DISSOLVED ORGANIC CARBON (DOC)

H_2O_2 formation and H_2O_2 dynamics in freshwaters are strongly linked with the composition and concentrations of DOC (Cooper et al. 1994; Scully et al. 1996). DOC is a mixture of dissolved carbon compounds of varying origin and concentration, with total concentrations ranging widely among lakes (from < 1 to over 50 mg L^{-1}, (Perdue and Gjessing 1990). DOC is highly variable among aquatic ecosystems due to differences in the physical properties of organic carbon compounds, relative ages, sources and retention times within each body of water, as well as variations in lake depth, regional climate, geology and drainage area (Curtis 1998).

DOC is a large pool of organic molecules originally from structural plant materials (lignin and cellulose) that were released by aquatic vegetation, or, transported into lakes and transformed by abiotic or microbiological processes (Mann and Wetzel 1996; Wetzel 1995). Total DOC (TDOC) in fresh waters is derived from two major sources: *autochthonous* sources (within-lake processes) and *allochthonous* sources (run-off from landscape). DOC is either formed from within-lake processes (*i.e. autochthonous* sources) or carried into lakes through run-off from the surrounding watershed (*i.e. allochthonous* sources). Differences in DOC source and age result in differing optical and physical properties, determining DOC quality and interactions with compounds and also with climate over time (Curtis 1998; Mcknight and Aiken 1998). Autochthonous

sources of DOC that include phytoplankton and macrophyte excretions are less refractory, labile sources of DOC that are readily available for microbial processing (Cole et al. 1982; Farjalla et al. 2001; Mcknight and Aiken 1998). In contrast, allochthonous sources of DOC include terrestrial vegetation and wetland runoff, which are typically more recalcitrant to microbial processing (Cole et al. 1984). DOC from these sources are more complex in chemical structure (Mcknight and Aiken 1998), also possessing molecules called *chromophores* as part of their structures. Two smaller sources of DOC also include: bacterial degradation of organic matter (Horne and Goldman 1994; Pomeroy and Wiebe 1988), and, excretions from secondary consumers such as zooplankton, nanoflagellates and fish (Wetzel 2001).

Chromophoric or coloured-DOC (herein referred to as CDOC) is effective in absorbing visible light (Wetzel 2001) and UVB (Laurion et al. 1997; Morris et al. 1995; Scully and Lean 1994) and can also be modified by sunlight (*i.e.* visible and UV light, Whipple 1914). The presence of CDOC can affect water colour from nearly transparent when present in small concentrations, to nearly black when overly abundant (Williamson et al. 1999). DOC can be photodegraded into to smaller carbon units resulting in the generation of humic and fulvic acids, nutrients and gases (Mcknight and Aiken 1998).

1.1.1 Photochemical breakdown of CDOC

As stated previously, CDOC (allochthonous DOC) is responsible for staining natural waters from nearly transparent to yellow, brown or black water colour (Williamson et al. 1999). An optical definition for CDOC is the ability to absorb UVR wavelengths greater than 290 nm (Wetzel 2001). In contrast, autochthonous DOC does not appear to affect water colour (herein referred to as non-coloured DOC, NCDOC, (Wetzel 2001). However, CDOC can also have little effect on water colour once it is photodegraded (or "photobleached"). Whipple (1914) first noted that water became "bleached" of colour after periods of light exposure. In more recent literature, water colour has become a generally accepted relative measurement that describes the absorbance of light at 320, 330 or 440 nm (Cuthbert and Del Giorgio 1994; Squires 2002; Williamson et al. 1999 , respectively). Water colour and humic DOC are therefore understood to be closely related (Birge and Juday 1934; Juday and Birge 1933) with relatively high water colour interpreted as a surrogate for humic DOC.

Additionally, the term CDOC "photobleaching" describes the progressive loss of water colour due to light exposure (Reche et al. 1999). Photochemical processes are

mainly responsible for degrading the highly complex molecular structures of DOC although groundwater and residence time can also degrade DOC (Curtis 1998). The main process involves the absorption of light energy by chromophoric molecules followed by the transfer of energy to other molecules (*e.g.* oxygen) before returning to its' original state (Diaz et al. 2004). In this process, high molecular weight compounds break down to lower molecular weight compounds (Allard et al. 1994), losing colour in the process.

CDOC is also involved in other critical photochemical processes in addition to photobleaching. CDOC photodegradation can also generate nutrients and gases (*e.g.* CO, NH_4) and converting organic carbon to inorganic carbon (CO_2, Miller and Zepp 1995). DOC photoproducts can be formed such as radical oxygen species (ROS) and superoxide radicals (O2.-, Zepp et al. 1992) and H_2O_2 (Cooper et al. 1994). These photoproducts involve a series of interconnected reactions that have implications to aquatic food webs and will be expanded upon in Section 1.2.

1.1.2 DOC and bacteria

The role of UVB in aquatic ecosystems is a widely studied issue in current literature (Williamson 1995). Photolyzed DOC as an additional food substrate for bacteria can be a considerable contribution in some lakes (Cammack et al. 2004; Hessen et al. 2004; Lindell et al. 1995; Lindell et al. 1996; Tranvik 1998). The role of UV attenuation by DOC and inhibitory effects of UV on bacteria have also been widely documented for freshwaters worldwide (Häder et al. 1998; Karentz et al. 1994; Mostajir et al. 1999; Sinha et al. 1996). This next sub-section will elucidate how DOC can both stimulate and inhibit bacterial production.

Freshwaters are classically defined by trophic structure based on the rate of organic matter supplied from either autochthonous or allochthonous sources (Rodhe 1969). In allotrophic food webs (driven by allochthonous DOC), the microbial loop in freshwaters is centered on DOC and bacteria (Horne and Goldman 1994; Pomeroy and Wiebe 1988). The microbial loop is part of a larger carbon flow in aquatic ecosystems where the conventional food chain, made up of larger consumers (phytoplankton, zooplankton and fish), through photosynthetic excretions, excrement, senescence and death, contribute to the DOC pool that is eventually utilized by bacteria (Figure 1.1).

Photoproducts may also play an integral role as a major source of energy to heterotrophic bacteria and up through the food chain (Bertilsson and Tranvik 1998;

Tranvik 1998). Some simple carbon compounds such as carbohydrate serve as the main food substrate for bacteria (Tranvik and Jørgensen 1995; Wetzel 2001) however a range of other photolyzed carbon units are also produced (e.g. acetaldehyde, formaldehyde, glyoxalate and pyruvate (Moran and Zepp 1997), easily serving as additional substrates for bacteria (Bertilsson and Tranvik 1998).

The most common measures of biological response from bacteria is growth in terms of cell abundance and increase in protein or DNA (Robarts and Zohary 1993). Recently, there has been an increasing interest in coupling bacterial respiration with the measure of bacterial production. "Bacterial growth efficiencies" take into consideration both microbial metabolism and consumption (Del Giorgio and Cole 1998; Herndl et al. 2000) and are arguably more accurate in describing bacterial communities since, in some cases, rates of bacteria growth may increase while respiration may remain constant or decrease (Del Giorgio and Cole 1998; Pullin et al. 2004). Bacterial communities have also been described in terms of community composition and the presence of specific bacterial strains specific to DOC source (Crump et al. 2003). In an Arctic lake, Crump *et al.*(2003) documented a shift in bacterial communities that were related to shifts in sources of DOC. Community composition (measured by denaturing gradient gel electrophoresis of 16S rRNA genes) reflected changes in the community as a result of seasonal influx of allochthonous DOC coupled with an introduced bacterial community as a result of advection from runoff. Rates of bacterial production were highest just after the DOC shift and molecular evidence determined that microbial communities were unique to the different DOC sources.

Regardless of the methodology used, the literature suggests that bacteria respond to both sources of DOC (Cole et al. 1982; Moran and Hodson 1990) and that the availability of labile carbon in addition to UVB protection, or "sunscreening", by CDOC is largely beneficial (Lindell et al. 1995; Lindell et al. 1996). For example, in a manipulated mesocosm experiment in a Mackenzie Delta lake, Teichreb (1999) demonstrated that bacterial production increased in response to both normal and elevated additions of CDOC. The study suggests that CDOC appeared to provide both an energy substrate as well as a UVB "sunscreen" for plankton. Moreover, in a gradient of Finnish lakes that ranged from low to high DOC concentration (and low to high water colour), Lindell *et al.* (1996) observed a gradient of responses from bacteria. The results suggest that bacteria were inhibited by UV light, especially among the clear lakes, during the day however bacteria were simultaneously stimulated by the photolyzed DOC at

night. Hence, bacteria appear to be both directly and indirectly stimulated by DOC-UVR dynamics. Hence, any analysis of DOC and bacteria must always recognize the stimulatory and inhibitory roles that UVR simultaneously exerts on aquatic food webs.

1.1.3 Direct effects of UVB on bacteria

The direct effects of UVB are largely inhibitory to bacteria leading to DNA damage and hindered metabolic processes (Herndl et al. 1993; Jeffrey et al. 1996; Müller-Niklas et al. 1995). Direct biological effects occur as certain UV wavelengths are absorbed by specific macromolecules such as: nucleic acids, proteins and membrane lipids (Jeffrey et al. 2004). One consequence of inhibition among bacteria is the production of cyclobutane pyrimidine dimers (i.e. fusion of nucleotide bases due to UVR, Jeffrey et al. 1996; Joux et al. 1999; Lyamichev et al. 1990). This inhibition is the result of dimerisation between two adjacent pyrimidine bases that combine with other photoproducts (*e.g.* pyrimidinone). This photoreaction impairs DNA synthesis and gene transcription, eventually leading to more lethal effects (Jeffrey et al. 2004).

Other responses by bacteria to direct UVB include the decreased numbers of cyanobacterial heterocysts (sites of nitrogen fixation, Sinha et al. 1996; Tyagi et al. 2003), decreased nitrogenase activity (Sinha et al. 1996), decreased $^{14}CO_2$ uptake in photosynthetic activity (Sinha et al. 1996) and reduced growth rates (Arrieta et al. 2000; Herndl et al. 1993; Lindell et al. 1996; Lund and Hongve 1994; Sommaruga et al. 1997; Xenopoulos and Schindler 2003). These responses ultimately affect the ability of bacterial cells to replicate (Bergeron and Vincent 1997; Chatila et al. 2001; Joux et al. 1999; Mostajir et al. 1999).

Responses to UV damage vary among aquatic organisms and include both sunscreening and antioxidative defense mechanisms (Shick and Dunlap 2002). For example, mycrosporine-like amino acids (MAAs) effectively screen out specific UV wavelengths and is common among cyanobacteria (Tyagi et al. 2003) and other aquatic organisms including algae, fungi and fish (Shick and Dunlap 2002). Some cyanobacteria also counteract cellular damage through the production of anti-oxidant chemicals including: L-cysteine, ascorbic acid, reduced glutathione, L-tryptophan and sodium pyruvate (Tyagi et al. 2003). In a comparative analysis of non-motile and fast-gliding cyanobacterial species, Quesada and Vincent (1997) determined that the gliding species, *O. priestleyi* relocates elsewhere in the water column in order to escape UVR. Nitrogen-fixing cyanobacteria are highly abundant in high arctic systems where UV is

enhanced (Perin and Lean 2004), suggesting that cyanobacteria are fairly UV-tolerant and important in the nitrogen budget of high arctic lakes (Quesada et al. 1999). Among planktonic bacteria, photo-enzyme repair is more common and is activated by UVA or blue radiation (360 - 430 nm, Häder et al. 1998) and photosynthetically active radiation (PAR, 400 - 450 nm, Kaiser and Herndl 1997). Some bacteria possess pigmentation to counter UV stress (Arrage et al. 1993); however, most aquatic bacteria lack UV-screening pigments (Garcia-Pichel 1994). Light-induced repair typically involves the production of photolyase enzymes combined with more passive movement of microbes to greater depths in the mixing layer of surface waters in order to avoid UV stress (Häder et al. 1998).

The most commonly observed direct response in aquatic ecosystems to UVR is decreasing rates of bacterial community production (Arrieta et al. 2000; Herndl et al. 1993; Jeffrey et al. 2004; Lund and Hongve 1994; Sommaruga et al. 1997). However, responses are highly variable among aquatic environments and bacterial communities. The responses of marine bacteria in systems characterized by low DOC concentrations are more thoroughly understood. In one case, marine isolates displayed highly variable responses to UVB and variable levels of cyclobutane pyrimidine dimers that were mostly inhibitory (Joux et al. 1999). However among the isolates, one particular isolated strain of *Sphingomonas* sp. (strain RB2256) was found to exhibit very little DNA damage and therefore possessed a higher level of UVB resistance than bacterial strains (Joux et al. 1999).

Because UVR penetrates from above the water column, CDOC can absorb UVR and attenuate it in the upper surface of freshwaters (Laurion et al. 1997; Morris et al. 1995; Scully and Lean 1994). This rapid attenuation is beneficial for bacteria as a UVB sunscreen. UVB penetration can be quite high among Arctic lakes which are typically shallow (<5m in Mackenzie Delta lakes, Marsh and Hey 1989) and lack any depth refuge (Perin and Lean 2004). However, the absorption of UVB by chromophoric molecules also leads to the formation of inhibitory photoproducts such as reactive oxygen species (ROS) and H_2O_2 which may add an additional stressor to aquatic systems, and especially bacteria.

1.2 Indirect Effects of UVR on Bacterial Production: Hydrogen peroxide

H_2O_2 is the most stable of all ROS in aquatic environments and is ubiquitous in oceans, rivers, streams and lakes. Compared to all other ROS, it is relatively long lived (*i.e.* 4 – 24 hours) and easy to measure in natural waters (Cooper et al. 1994; Cooper et al. 1989; Cooper et al. 1988). Increasing attention on H_2O_2 can be attributed to increased levels in natural waters due to smog formation, acid rain, and more recently, ozone depletion (Blough and Zepp 1995; Zepp et al. 2003). In aquatic systems, H_2O_2 formation is an indicator of photochemical processes and is intricately linked with DOC and UVR. H_2O_2 can also become involved in biogeochemical and biological interactions (Miller and Zepp 1995; Zepp et al. 2003). The primary mechanism for H_2O_2 formation is photochemical and the main mechanism for H_2O_2 decay is biological (Cooper et al. 1994). This next section will provide a review of H_2O_2 in natural waters, the main mechanisms for production and decay, H_2O_2 cycling in the environment and the potential effects of H_2O_2 on aquatic food webs, and in particular, bacterial communities.

1.2.1 Hydrogen peroxide in natural waters

H_2O_2 dynamics have been studied extensively worldwide (Cooper and Lean 1989; Häkkinnen et al. 2004; Miller and Zepp 1995; Moffett and Zafiriou 1990; Obernosterer et al. 2001; Petasne and Zika 1987; Scully and Vincent 1997; Zuo and Hoigne 1993). Estimates of H_2O_2 concentrations have been determined for groundwaters (Cooper and Zika 1983), streams (Herrmann 1996; Mostofa and Sakugawa 2003), a gradient of temperate to subarctic to high arctic lakes (Scully et al. 1996; Scully and Vincent 1997), in hot springs (Wilson et al. 2000), coastal seas (Petasne and Zika 1987), Antarctic waters (Yocis et al. 2000) and at various depths in the subtropical ocean (Obernosterer et al. 2001).

It is well-established that H_2O_2 is highly dynamic, exhibiting both diurnal and season variations due to solar intensities (Cooper et al. 1994; Herrmann 1996; Obernosterer et al. 2001; Scully and Vincent 1997). Photochemical activity (and H_2O_2 production) in lakes is limited to the top few metres as UV radiation rapidly decreases with depth (Scully et al. 1995). It is generally thought that H_2O_2 formation is greatest in clear lakes (Scully et al. 1997) with high DOC concentrations where UV wavelengths can potentially reach considerable depth compared to more humic stained waters (Scully et

al. 1996). In comparative analyses of lakes representing a DOC and latitudinal gradient, Scully *et al.*(1996) suggest that DOC concentration is proportional to H_2O_2 formation and that, as a result, H_2O_2 formation is extremely low in arctic lakes because of characteristically low DOC concentrations.

In terms of formation potential, both UVA and UVB are effective in catalyzing photochemical reactions with DOC. Because of the abundance of UVA photons, UVA has been found to be more critical in H_2O_2 formation (Jerome and Bukata 1998; Scully et al. 1997) and is thought to be the most efficient type of light for H_2O_2 production (Wong and Wong 2001). H_2O_2 formation from DOC can be influenced by the degree of acidification in lakes (Donahue 2000) and can occur in conjunction with other ROS and trace metals (Cooper and Zika 1983; Zepp et al. 1992).

1.2.2 Photochemical hydrogen peroxide formation

When CDOC absorbs UVR, a series of electron transfers occurs in the water column to eventually produce H_2O_2 (Cooper et al. 1994; Scully et al. 1996). This simplified mechanism of formation illustrates how the ground state of DOC ($_0{}^1$DOC) becomes excited by UVR to form $^1{}_1$DOC*. Through a process identified here as Intersystem crossing, another electron transfer occurs to form $^3{}_1$DOC*:

$$\begin{aligned} {}_0{}^1\text{DOC} - \text{UV radiation} &\rightarrow {}^1{}_1\text{DOC}^* - \text{Intersystem Crossing} \\ &\rightarrow {}^3{}_1\text{DOC}^*; \end{aligned} \quad (1)$$

Electron transfers from DOC to oxygen results in the superoxide radical ion ($O_2^{-\cdot}$; Equation 2a) which then combines with its' conjugate acid to form a hydroperoxyl radical ($HO_2^{-\cdot}$, Equation 2b):

$$^3{}_1\text{DOC}^* + {}^3O_2 \rightarrow \text{DOC}^{+\cdot} + O_2^{-\cdot}; \quad (2a)$$

$$O_2^{-\cdot} + H^+ \rightarrow HO_2^{-\cdot} \quad (2b)$$

Hydroperoxyl disproportionates into superoxide, oxygen or a hydroxyl ion to form H_2O_2 (Equations 3 and 4):

$$HO_2^{-\cdot} + HO_2^{-\cdot} \rightarrow H_2O_2 + O_2; \quad (3)$$

$$HO_2 + O_2^{-\cdot} + H_2O \rightarrow H_2O_2 + O_2 + OH^{\cdot}. \quad (4)$$

Both metal redox cycling and biological processes can also produce additional superoxide and therefore provide alternative pathways for H_2O_2 formation (Cooper et al. 1994; Scully et al. 1996; Wilson et al. 2000) however photochemical production of H_2O_2 is still thought to be the primary pathway.

H_2O_2 decay is primarily biological but can also decay chemically when dissolved metals are present in the water column, especially iron in the form of Fe^{2+} and Fe^{3+} (Zepp et al. 1992). A process known as Photo-Fenton reactions occurs, which depend on pH, dissolved metal and oxygen concentrations. This can result in H_2O_2 reacting with ferrous iron (Fe^{2+}) to produce ferric iron (Fe^{3+}), $HO^{\cdot}$ and $OH^{\cdot}$:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\cdot} + OH^{\cdot} \quad (5)$$

Consequently, H_2O_2 concentrations can be lower in high Fe^{2+} environments (Scott et al. 2003) but can also produce additional deleterious $OH^{\cdot}$ that can react with other ROS and organic materials in the water.

1.2.3 Biogenic hydrogen peroxide formation

Considerable amounts of biogenically-formed H_2O_2 are associated with harmful algal blooms and phytoplankton species that form extracellular ROS (Oda et al. 1997; Twiner and Trick 2000). Algae in coastal systems have been found to produce substantial amounts of H_2O_2 internally as perhaps an adaptive strategy against UV stress (Collén et al. 1995; Palenik et al. 1987; Twiner et al. 2001) however little is known about freshwater species including both phytoplankton and bacteria.

In laboratory trials, few cyanobacteria were deemed capable of both internally producing H_2O_2 and also catalyzing the decomposition of H_2O_2 when under UV stress (Palenik et al. 1987; Zepp et al. 1987). In all cases of biogenic H_2O_2 formation, it is thought to occur during photosynthesis, often as a toxic by-product of aerobic respiration (Messner and Imlay 1999) or produced by the electron transport chain within the mitochondria, producing free radicals (Cooper et al. In prep.; González-Flecha and Demple 1995). Among aerobic bacteria, NADH dehydrogenase II (a proton translocating complex) is the primary site of superoxide and H_2O_2 formation in the respiratory electron transport chain (Messner and Imlay 1999). The functioning of this complex can be influenced by the energy status of the bacterial cell and the arrangement of the

respiratory chain which can in turn be influenced by surrounding growth conditions (González-Flecha and Demple 1995). Through this process, up to nanomolar levels of H_2O_2 can be potentially produced (Palenik et al. 1987), however, in studies among freshwater bacteria, internally produced H_2O_2 does not appear to contribute significant amounts (Cooper et al. 1994; Cooper et al. 1989).

1.2.4 Hydrogen peroxide cycles

The pathways of H_2O_2 formation and decay are relatively simple compared to other biogeochemical cycles (Figure 1.2). The input of H_2O_2 in natural waters is through photochemical formation, rainwater and biological formation. Rainwater can be considerable at temperate latitudes and oceans (appox. 20 µm, Willey et al. 2004; Zuo and Hoigne 1993) whereas biologically produced H_2O_2 has been found to be negligible (Section 1.2.3). Another potential source of H_2O_2 in lakes may also be from runoff from terrestrial sources. Plant tissues of liverwort *Dumortiera hirsute* have been shown to produce significant amounts of ROS, even when unstressed (Beckett et al. 2004) and could be a source of H_2O_2, however this pathway remains unknown. Biologically-mediated processes are the main pathway for H_2O_2 decay and will be elaborated upon in the following section (Section 1.2.5).

Within lakes, H_2O_2 formation occurs near the surface of the water column in highly-chromophoric waters and at greater depths in more clear waters (Scully et al. 1996). Compared to other ROS, H_2O_2 is relatively long-lived (i.e. 4 - 24 hours, Cooper et al. 1994) and, once formed, H_2O_2 is typically distributed within a lake by mixing processes, such as wind action, leading to H_2O_2 formed near the surface to be transported to much greater depths (Häkkinnen et al. 2004; Obernosterer et al. 2001; Scully and Vincent 1997). Among Mackenzie Delta lakes, no thermal stratification is known to occur (*pers. obs.*, M. Squires, *pers. comm.*). Thus, in particularly shallow lakes, H_2O_2 produced near the surface can be easily transported to the bottom of the lakes as a result of wind action and mixing.

The effect of increasing UVB on will likely enhance H_2O_2 cycles through the acceleration of CDOC breakdown and therefore H_2O_2 production (Zepp et al. 2003). One implication of enhanced photochemical activity may be decreasing water colour as CDOC is photolyzed more rapidly. Light penetration may increase in the water column causing increased biological inhibition and enhanced photochemical activity at greater

depths. This may also affect the availability of nutrients and metals, which may have further unpredictable impacts on aquatic systems.

1.2.5 Hydrogen peroxide removal and decay

The main mechanism for H_2O_2 decay is attributed to biological processes (Moffett and Zafiriou 1990). Abiotic removal of H_2O_2 is thought to be quite small, due mostly to mixing of H_2O_2 to greater depths and interactions with manganese and iron in the water column (Häkkinnen et al. 2004; Moffett and Zafiriou 1990). Two major enzyme systems are responsible for biological H_2O_2 decomposition: peroxidase and catalase systems. These enzymes facilitate the breakdown of H_2O_2 through redox reactions. In a review by Henle and Linn (1997), catalases and peroxidases utilize other organic reductants (*e.g.* glutathione, thiols, cytochrome *c* and ascorbate) and superoxide dismutases (SOD) in H_2O_2 depletion. Both enzyme systems occur intracellularly in most plankton; however, because of surface area-to-volume ratios, H_2O_2 decay is more effectively mediated by small algae and bacteria and less so among zooplankton and large algae (Cooper et al. 1994).

1.2.6 Effects of hydrogen peroxide on bacterial production

The biological defenses of aquatic organisms to ROS and H_2O_2 include a variety of antioxidant defenses. H_2O_2 and ROS can lead to cellular damage and propagate other photochemical reactions in the water column that may indirectly inhibit bacterial growth. Similar to the processes involved with H_2O_2 decay, most microbial defenses appear to be centered on avoiding oxidative stress by producing catalases and peroxidases (Henle and Linn 1997; Ostrowski et al. 2001; Palenik et al. 1987). It has been suggested that the expression of genes that control enzyme systems (catalase and peroxidase systems) in bacteria involve cellular protection from both UVR and H_2O_2 stress (Arrage et al. 1993) confirming the close coupling of UV effects with ROS and H_2O_2 formation.

In some cases, marine bacteria can be tolerant of high H_2O_2 concentrations (~25 mM, Eguchi et al. 1996). Ostrowski et al.(2001) determined that a marine strain of ultramicrobacterium *Sphingomonas alaskensis* RB2256 has an uncharacteristically high resistance to millimolar concentrations of H_2O_2. Stress resistance (measured as differences in growth rates) did not appear to be mediated by catalase activity. This suggests that the physiology of the organism was particularly unique to this environment and perhaps better-adapted to the oligotrophic, conditions of Alaskan waters. Hence it is

plausible that, in high stress environments such as the arctic, bacteria may be physiologically adapted to UV stress and related photoproducts.

In fresh waters, extremely small additions of H_2O_2 has been shown to inhibit bacterial production (<50 nM, Xenopoulos and Bird 1997). H_2O_2 can be toxic to bacteria and algal metabolism and development (Neidhardt et al. 1984; Palenik et al. 1987), even leading to the loss of culturability for some aquatic bacteria (Arana et al. 1992). Oxidants created through Fenton reactions damage DNA bases or sugars, removing hydrogen from one of the deoxyribose carbons in the creation of H_2O_2 (Henle and Linn 1997). Furthermore, in some bacterial cells and chloroplasts, protection from H_2O_2 is primarily achieved by reducing the formation of $O_2^{-\bullet}$ through the presence of SOD, more so than from catalases or other organic reductants (Henle and Linn 1997). As seen with the mechanisms for H_2O_2 decay, catalases, sodium pyruvate and thioglycolate were critical in protecting *E. coli* cells from H_2O_2 damage (Arana et al. 1992). Additionally, the biological defenses of bacteria to H_2O_2 appear to be similar to those defenses used to counteract direct UVB inhibition including the production of antioxidants including MAAs seen in algae and cyanobacteria (Shick and Dunlap 2002).

An adaptive strategy to UV and H_2O_2 stress is for phytoplankton is through stimulated initial growth rates leading to self-shading. Barros et al.(2003) demonstrated that one mechanism for reducing H_2O_2-induced oxidative stress by phytoplankton was to undergo rapid growth rates, increasing their biomass to substantially increase self-shading. By being able to provide shade for phytoplankton, levels of SOD, catalase and peroxidase in phytoplankton significantly decreased, indicating reduced H_2O_2-induced oxidative stress. This process may also provide shading for other plankton, especially non-photosynthetic bacteria.

Another consideration is the indirect effect of H_2O_2 that is potentially stimulatory for bacteria. Photolyzed DOC provides an additional food substrate (stimulatory) yet also photobleaches simultaneously leading to both direct UVB inhibition and the production of H_2O_2 and ROS (inhibitory). In Swedish lake microcosms, Scully et al.(2003) added a known concentration of ROS to humic lake water and found that rates of photobleaching were much less rapid when irradiated with UVR. Although UVR and ROS activity was high, an increase in bacterial growth was also observed. This suggests that bacteria efficiently can utilize photolyzed carbon despite having biologically inhibitory levels of ROS present.

1.2.7 Hydrogen peroxide in Mackenzie Delta lakes

Mackenzie Delta lakes are highly productive (Spears 2002; Squires 2002) and consist of simple, yet dynamic, food webs across topographical and limnological gradients (Figures 1.3 and 1.4, Lesack et al. 1991; Lesack et al. 1998; Riedel 2002; Spears 2002; Squires 2002; Teichreb 1999). Precipitation levels in this region are near desert levels (248.4 mm yr^{-1}, Environment Canada, 2005), thus, for the >49,000 lakes within the Delta (C. Emmerton, *unpub. data*), spring flooding is critical to the replenishment of nutrients and water (Marsh and Bigras 1988). The north-flowing Mackenzie River freezes each winter, and in the spring, snow and meltwater flood over a majority of the lakes creating a gradient of limnological trends in relation to the degree of flooding (Lesack et al. 1998). Lakes range from being frequently flooded and permanently connected to a river channel to lakes that are infrequently flooded with less than annual return intervals (Marsh and Hey 1989). Climate change and other global change stressors (*e.g.* increased UVB, land-use change) are expected to have differing and complicated impacts on the gradient of lake types found in this system (Table 1.1, Lesack et al. In prep.).

It is also well-established that lakes of the Mackenzie Delta vary in DOC compositions, determined largely by frequency of flooding in relation to sill elevation (Figure 1.4). Mackenzie Delta lakes are thought to be driven by opposing gradients in DOC concentration and water colour (Lesack et al. 1991; Spears 2002; Squires and Lesack 2002; Squires et al. 2002). Lakes that are frequently flooded are more likely to have low TDOC concentrations but high CDOC. These lakes are typically connected to a river channel and are highly coloured. In contrast, Mackenzie Delta lakes that are infrequently flooded typically have high TDOC concentrations due to high macrophyte abundances. Infrequently flooded lakes are flooded less than annually, remaining disconnected from the river channel for long periods of time, and are extremely clear.

Among delta lakes, the main climate change concern is the dampening of flood intensity that will result in overall decreasing lake levels and the isolation of infrequently flooded lakes (Marsh and Lesack 1996). An additional consequence of climate change will also include the short-term overall increase in CDOC into delta lakes from river water (Rouse et al. 1997). This occurs as warming temperatures facilitate permafrost to melt and release organic substances into the watershed. The implications of these combined predictions on H_2O_2 production remains unknown.

On a global scale, increasing UVB radiation is predicted to result in increased H_2O_2 production (Zepp et al. 2003). Bergeron and Vincent (1997) suggest that ozone depletion and changes in DOC may alter microbial food webs and productivity within subarctic lakes. In a shallow sub-arctic lake, Scully and Vincent (1997) demonstrate that H_2O_2 can build up cumulatively, especially at high latitudes when summer day-lengths are long (*i.e.* up to 24 hours) and under constant cloudless conditions. In another study, Scully *et al.* (1996) found that clear arctic lakes in sparsely vegetated drainage basins have low H_2O_2 formation rates and very high UV radiation penetration. They further suggest that the formation of H_2O_2 in clear, high-latitude lakes will be highly sensitive to increases in UVR due to ozone depletion. It is plausible that the prediction by Scully et al. (1996) is unlikely the case among Mackenzie Delta lakes where DOC concentrations are markedly higher from high arctic systems and CDOC among lake can be considerable (Spears 2002; Squires 2002).

1.3 Climate Change in the Canadian Arctic

The question of how climate change and increasing UVB will impact DOC-bacteria interactions in arctic aquatic ecosystems remains poorly understood. Predicted increases in arctic temperatures correspond to permafrost thawing in areas characterized by ice and snow cover. For lakes of the Mackenzie Delta, climate change is predicted to result in a significant change in DOC concentration (*i.e.* increased CDOC), source (*i.e.* allochthonous) and related chemical changes (Perin and Lean 2004; Rouse et al. 1997; Schindler 2001).

According to the predictions of Rouse et al. (1997), a short-term consequence is that permafrost will thaw as temperatures warm and that runoff production will decrease. A consequence of this may be the import of CDOC into Delta lakes via river water (Marsh and Lesack 1996) and therefore a substantial increase in water colour. A long-term consequence is an overall decrease in precipitation and increasing temperatures (Rouse et al. 1997). Therefore, interactive responses to climate change among arctic lakes may include: reduced flooding in the Delta (Marsh and Lesack 1996); a short-term decrease in light penetration in the water column due to increased CDOC and a resulting decrease in photosynthetic activity (Pienitz and Vincent 2000); increased microbial activity due to warming temperatures (Rouse et al. 1997); long-term increase in water clarity in arctic freshwaters (Pienitz and Vincent 2000); as well as increased photochemical activity (Zepp et al. 2003).

1.4 RESEARCH OBJECTIVES

The gradient of lake types in the Mackenzie Delta provides an ideal landscape on which to test hypotheses regarding H_2O_2 and bacterial production. This research attempts to characterize patterns of H_2O_2 and addressed untested hypotheses regarding Mackenzie Delta lakes, a unique perspective from Arctic lakes currently represented in the literature. The first objective of this research was to assess the patterns of hydrogen peroxide among lakes of the delta in order to determine if H_2O_2 levels would vary temporally from the arctic summer solstice to late summer, and, spatially across a gradient of DOC compositions. To address this objective, four specific hypotheses were tested:

1. H_2O_2 levels are highest around the solstice and lower later in the summer. This was determined from three forty-lake surveys that measured for H_2O_2 at three different times during the open water season: near summer solstice, mid-summer and late summer.
2. H_2O_2 levels are highest where DOC concentration is highest, or alternatively, where CDOC is highest. This represents two opposing types of Mackenzie Delta lakes (frequently flooded or infrequently flooded) with contrasting DOC regimes. Patterns of H_2O_2 with DOC composition and water colour were determined from surveys of 40 lakes that represented a gradient of DOC concentrations.
3. H_2O_2 levels cumulatively build-up during sequences of cloudless days with 24-hour sunlight. This was determined via an *in situ* study of two lakes with contrasting DOC regimes. H_2O_2 levels and CDOC absorbencies were tracked continually for up to forty-hours shortly after the arctic summer solstice when solar intensity was at an annual maximum.
4. H_2O_2 concentrations are greatest where UVB exposure and photochemical substrate is greatest. Using two lakes with contrasting DOC regimes in an outdoor incubator experiment, simultaneous H_2O_2 build-up and CDOC photobleaching was assessed under both cloudy and sunny conditions.

Because DOC, UVR and bacterial production are closely related, the secondary objective of this research is to assess the potential inhibition of hydrogen peroxide on bacterial production. The hypothesis tested was that after controlling for grazing, DOC variability and UVB exposure, bacterial production will be lowest where H_2O_2 is highest.

In order to test this hypothesis, a series of planned experiments were necessary to assess the relative strengths of factors that also affect bacterial production:

1. Assess the strength of grazing pressures on bacterial production. This was achieved via forty lake survey where rates of bacterial production with the full plankton community present to when only the bacteria community was present.
2. Assess the impact of H_2O_2 on bacterial production. This was achieved via a forty lake survey where rates of bacterial production were analyzed with H_2O_2 and DOC concentrations.
3. Assess the strengths of direct UVB versus a general DOC effect on bacterial production. This was determined through two outdoor incubator experiments on six lakes that represented a gradient of DOC compositions. This experiment was conducted twice – once in the presence of grazing and again in their absence – to assess the response of bacterial production to direct UVB.
4. To assess the strength of photolyzed DOC, direct UVB damage and H_2O_2 on bacterial production. This was simultaneously assessed in an outdoor incubator experiment on three lakes that represent different DOC regimes. Microcosms were subject to two levels of treatment: bacteria *in situ* or removed, and, full sunlight or – UVB.

By better understanding the factors regulating H_2O_2 formation and bacterial production, we may more thoroughly understand the role that DOC exerts among Mackenzie Delta lakes. In turn, this research will help to better understand the Mackenzie Delta ecosystem and the potential response of Mackenzie Delta lakes to the combined stressors of global change.

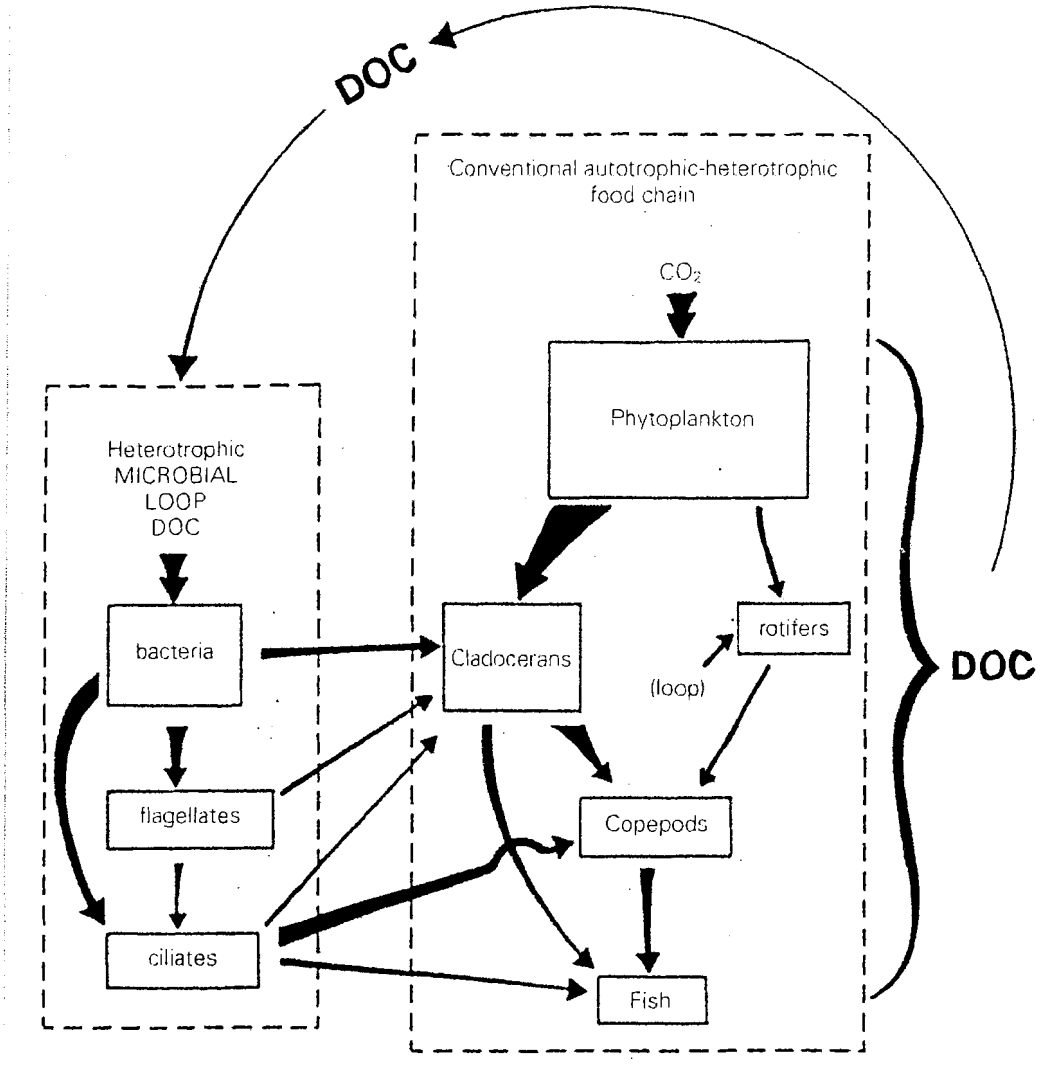

Figure 1.1 The microbial loop as part of a larger carbon flow chart depicting interactions between the conventional autotrophic-hetertrophic food chain (right) and the heterotrophic microbial loop (left). The microbial loop is the bacteria-microflagellate-ciliate part of the food web that utilizes dissolved organic carbon (DOC), which is not directly available to other plankton (adapted from Horne and Goldman 1994).

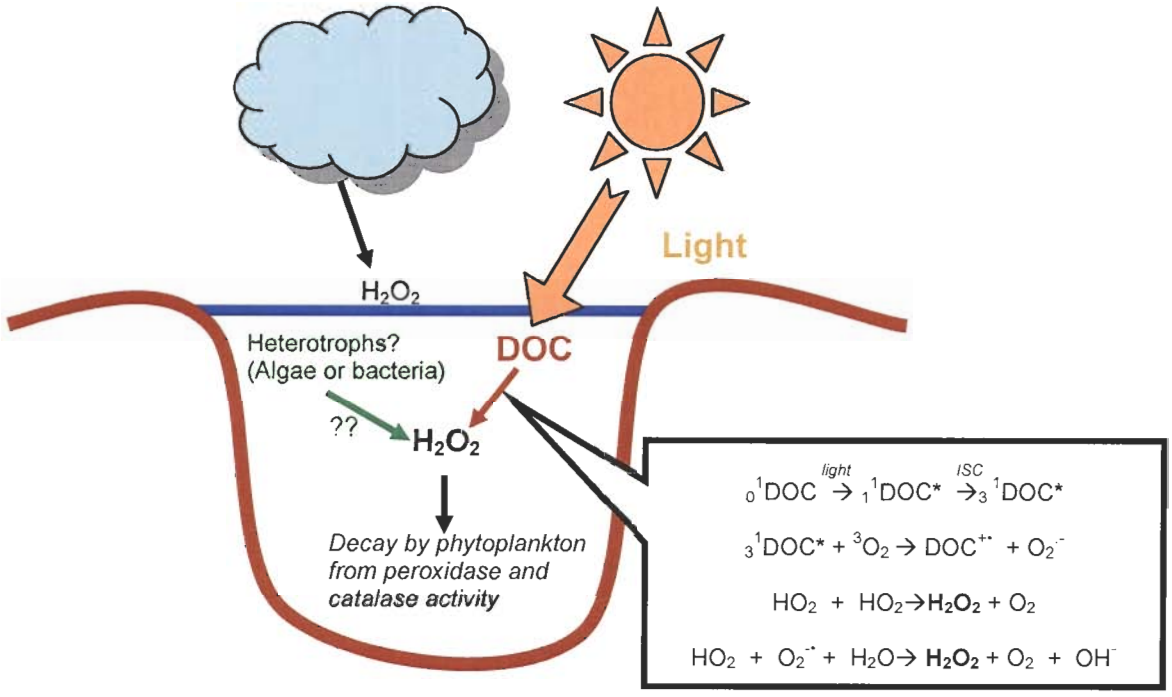

Figure 1.2 Schematic of major sources of H_2O_2 formation and decay in lakes. The main mechanism for H_2O_2 formation is photochemical and the result of the disproportionation of superoxide ($O_2^{-\bullet}$) to H_2O_2 and $O_2^{-\bullet}$. This simplified mechanism of formation illustrates how the ground state of DOC ($_0{}^1DOC$) becomes excited by UVR to an excited state ($^1_1DOC^*$). Intersystem crossing (ISC) transfers the excited DOC to a triplet state ($^3_1DOC^*$) that then interacts with superoxides or its conjugate acid (HO_2). This reaction is further propagated by the interaction with itself to produce H_2O_2 and O_2, resulting in the formation of a hydroxyl radical (OH^-), another superoxide ($O_2^{-\bullet}$) and H_2O_2. Electron transfers involving O_2 and DOC can be influenced by metals as producing $O_2^{-\bullet}$ which in turn forms H_2O_2 through further disproportionation. Another minor source of H_2O_2 is rainwater. The contribution of heterotrophs including algae, cyanobacteria and non-photosynthetic bacteria (due to respiratory chain activity) is less known. The main mechanism for H_2O_2 decay is through enzyme activity (peroxidase and catalase) by plankton and to a lesser extent, reactions with manganese and iron in the water column.

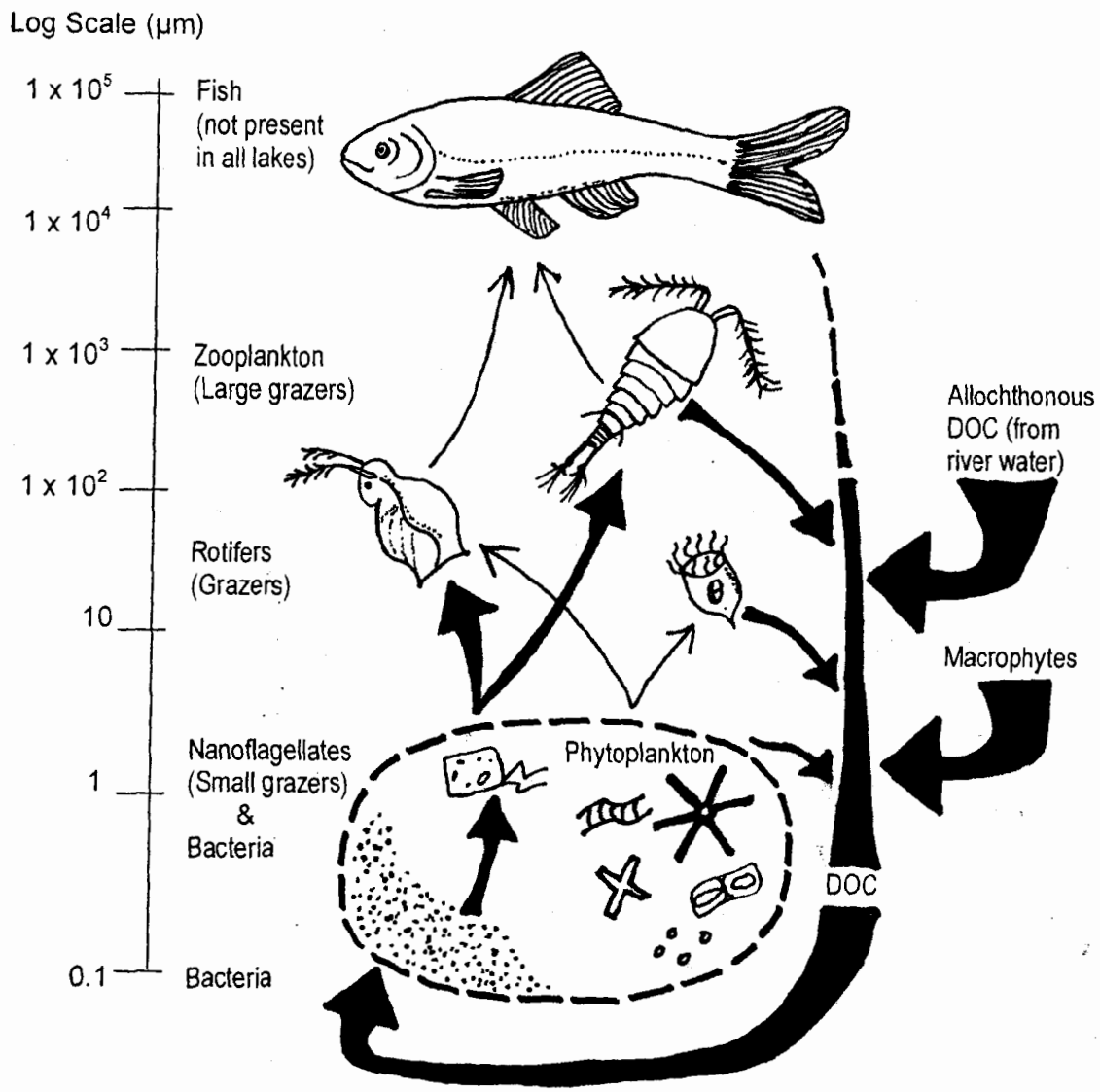

Figure 1.3 The microbial loop as it occurs in Mackenzie Delta lakes with relative sizes for each trophic level (adapted from Horne and Goldman 1994). In some lakes (infrequently flooded), fish are absent and large zooplankton are the highest trophic level (cf. Riedel 2002). Among lakes, organic matter is not lost from the planktonic habitat – extracellular products from photosynthesis continually leaks from phytoplankton cells, small fragments of organic matter and excretion are lost from fish and zooplankton, detritus and carbon (dissolved and particulate) are either consumed by grazers or utilized by small heterotrophs (nanoflagellates and bacteria). Arrows represent relative flow of soluble organic matter between trophic levels and through re-mineralization back to dissolved organic carbon (DOC) and heavy arrows show microbial loop.

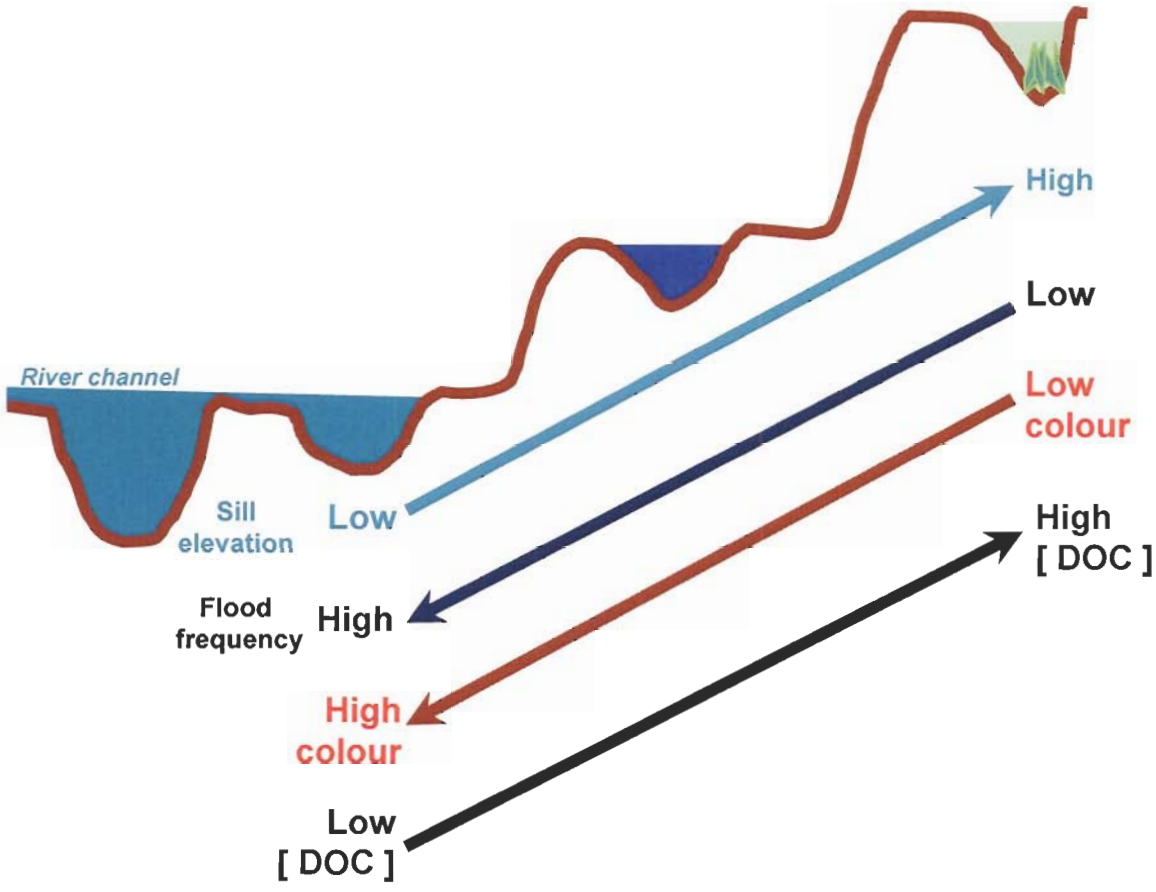

Figure 1.4 Profile of Mackenzie Delta lake types as characterized by Marsh and Hey (1989), featuring limnological gradients determined by Spears (2002) and Squires (2002). Illustrated are the three main lake types as a function of sill elevation: frequently flooded lakes (high flood frequency), intermediately flooded lakes and infrequently flooded lakes (low flood frequency). At either extremes of this gradient are frequently flooded lakes with high colour (measured as CDOC absorbency) and low total DOC concentrations (both coloured and non-coloured DOC) versus infrequently flooded lakes with low colour and high total DOC concentrations as a result of high macrophyte abundance.

STRESS		Runoff	Turbidity	CDOC	Nutrients	Phytoplankton	Bacteria
Climate Change	F	↓	↑	↑	↑	↓↑	↑
	I		↓	↑	↓	↓	↑
Land Use Change	F	↑	↑	↓	↓↑	↓↑	↓
	I		↑	↓	↓↑	↑	↓
Flow Regulation	F	↓	↓	↓↑	↓	↓↑	↓↑
	I		↓	↓↑	↓	↓	↓↑
Increased UV-B radiation	F			↓	↓↑	↑	↓
	I			↓	↓↑	↓↑	↓↑

Table 1.1 Predicted responses of abiotic and biotic variables to changes in climate, land use and flow regulation in frequently- (F) and infrequently- (I) flooded lakes of the Mackenzie Delta (Lesack et al. In prep). Highlighted is the predicted response of Mackenzie Delta lakes to increasing UVB radiation.

2 METHODS

This chapter summarizes the methods used to test the objectives presented in Chapter 1. The approach for this research was two-fold: seasonal surveys, and, field and lab experiments. In assessing spatial and temporal patterns in H_2O_2 production and its' potential effects on bacteria, larger trends were identified in large lake surveys (40 lakes) and weekly sampling of six lakes near Inuvik. This was complemented by a series of planned experiments using an outdoor incubator that specifically assessed H_2O_2 formation under differing UV regimes, diurnal H_2O_2 dynamics and finally, the potential effects of H_2O_2, UVB and grazing pressures on bacterial production.

2.1 STUDY AREA

The Mackenzie Delta is an extremely lake-rich environment (approx. 49,000 lakes, C. Emmerton, *pers. comm.*) and is the largest arctic delta in North America. The Delta lies within the zone of continuous permafrost, extending approximately 80 metres below the surface (Mackay 1963). The north-flowing Mackenzie River, the adjacent channels and lakes all become ice-covered for a large portion of the year. Extensive annual ice jamming in the delta is the main flooding event of the year, typically occurring in late-May to early-June. At this time, lakes are replenished with floodwaters and nutrients. The majority of the lakes become disconnected from the main channel during the summer months, experiencing a negative water balance due to low levels of precipitation and high evaporation rates (Marsh and Hey 1989).

Generally, Delta lakes are quite shallow (less than 5 metres), small (less than 10 hectares) and unstratified. Marsh and Hey (1989) determined that sill elevation, defined as the highest elevation along the connecting channel thalweg between the lake and distributary channel, increases as flood frequency decreases. The definitive feature of these lakes is sill elevation and the corresponding flood frequency categorization:

Frequently flooded (low sill elevation, 12% of Delta lakes): Lakes flood annually, remaining connected to the main river channel for the entire open water period with sill elevation typically less than1.5 metres above sea level.

Intermediately flooded (55% of Delta lakes): Lakes flood annually but will become disconnected from the main river channel for at least a brief period during the open water period with sill elevations typically between 1.5 to 3.5 metres above sea level.

Infrequently flooded (high sill elevation; 33% of Delta lakes): Lakes do not typically flood annually. Flooded lakes are connected very briefly during the open water period with sill elevations typically greater than 3.5 metres above sea level.

As a result, a gradient of limnological trends across the delta are apparent across the flood frequency (Figure 1.4). These biotic and abiotic trends characterize the community structure and distribution of Mackenzie Delta lakes.

2.2 SUBSYSTEM OF LAKES STUDIED

The lakes used in this study are all located in close proximity of the town of Inuvik (68°19' N, 133°29' W) on the east channel of the Mackenzie Delta (Figure 2.1). They were selected specifically because they represent a gradient of lakes with varying sill elevations and DOC compositions. These lakes have also been studied previously and were ideal for making interannual comparisons. A set of six lakes in close proximity to the Inuvik Research Centre were ideal for weekly monitoring and specific experiments whereas the forty lake set was ideal for testing large-scale hypotheses and assessing spatial and temporal patterns in H_2O_2 and DOC.

2.2.1 Weekly monitoring of six lakes

Six lakes were sampled weekly during the summer of 2003: South Lake, Lake 80, Lake 87, Lake 280, Lake 56, Dock Lake. The lakes range from low to high sill elevation, frequently to infrequently flooded, low to high DOC concentration, high to low colour (Table 2.1). They were chosen due to the availability of background data and representativeness across the DOC gradient (Riedel 2002; Spears 2002). The lakes were sampled weekly for temperature, TDOC, CDOC and bacterial abundance and are all within a one-hour boating distance from the Inuvik Research Centre where all lab analyses and microcosm experiments were conducted. Most importantly, weekly monitoring of these six lakes provided a context of system conditions for the Mackenzie Delta lakes.

Typical of floodplain systems, biotic and abiotic variables continually change as water level changes occur and it was therefore critical to document the changing parameters in each lake. This was especially important when addressing the objectives of this research since the lakes chosen provide a gradient of characteristics from which our experiments and manipulations were conducted.

In the final of three bacterial production experiments, a subset of the six lakes (South Lake, Lake 280 and Dock Lake) was used due to time and labour constraints, and still represented a gradient of DOC compositions. South Lake has the highest water colour and lowest DOC concentration out of the three lakes used in the study. It is frequently flooded and remained connected to the river channel for the entire open water period. Lake 280 was known to have a mixed DOC composition and was initially connected to the river channel after the spring melt. Water levels dropped rapidly and remained connected to the river by a small stream that eventually dried up by August, disconnecting the lake from the channel. Comparatively, Dock Lake is a deeper lake (approx. 3 metres) with high DOC concentrations and has not been connected to the river channel since at least 2001 (L.Lesack, *pers. comm*).

2.2.2 Extended surveys of 40 lakes

The forty lake set represents forty spatially discrete lakes where flood frequency is determined by sill elevation. This "sill-set" of lakes has been previously investigated and therefore provides some background data on flood frequency, DOC concentration and macrophyte abundance (Squires et al. 2002, Table 2.2). One forty lake survey was conducted in 2003 to assess patterns in bacterial production, DOC concentration and water colour. Three forty lake surveys were conducted in 2004 to assess temporal patterns in H_2O_2 concentrations, DOC concentrations and water colour.

2.2.3 Diurnal dynamics of 2 lakes

The diurnal dynamics of H_2O_2 formation was tracked in two contrasting lakes near Inuvik. The lakes chosen (Dock Lake and South Lake) represent endpoints in the DOC gradient: low colour, high DOC concentration (*i.e.* Dock Lake, approx. 1100 µM) versus high colour, low DOC concentration (*i.e.* South Lake, approx. 500 µM). Temperate latitude lakes demonstrate diurnal variability in H_2O_2 that follows solar intensity and decays away completely each night (Cooper and Lean 1989; Wilson et al. 2000). In a shallow subarctic lake during the summer, evidence of H_2O_2 build-up in over

72 hours of cloudless conditions (Scully and Vincent 1997). Therefore, I expected to find cumulative build up of H_2O_2 concentrations over multiple cloudless days around the arctic summer solstice (Figure 2.2). At this time of the year, daylengths were 24-hours long and the solar angle was at a seasonal maximum. H_2O_2 formation potential was therefore believed to be at an annual high.

2.3 WATER SAMPLING AND CHEMICAL ANALYSES

Samples for DOC, water colour, H_2O_2, bacterial abundance and bacterial production were taken from each lake. Samples were placed in darkened coolers while being transported to the Inuvik Research Centre. Samples taken for bacterial production and hydrogen peroxide were prioritized and therefore analyzed immediately upon returning to the lab whereas CDOC analyses were completed within 24 hours of sampling and TDOC samples were filtered, frozen and sent to the Freshwater Institute (Department of Fisheries and Oceans, Winnipeg, Manitoba).

2.3.1 Lake sampling protocols

Lake water samples were obtained from a central location in each lake. At the six lakes, a weighted float or fallen tree near the middle of the lake was marked with flagging tape and used as the standard sampling location. Using a 1.5 metre long Tygon™ tube sampler (2 $^5/_8$" internal diameter) with a rubber stopper, an integrated sample of a lake water column was collected and transferred to a large sampling bucket until a sufficient volume of water for the microcosms was reached (10 L). Lake water was transferred to a darkened jerry can and placed in cooler during transport to the Inuvik Research Centre for DOC analyses and any bacterial production experiments. A separate 60mL sub-surface sample was taken with an amber glass bottle and Teflon™ cap for hydrogen peroxide analyses.

2.3.2 Temperature and Conductivity

In situ temperature and conductivity measurements were taken each time a water sample was taken using a 3000 T-L-C model field conductivity probe (Y.S.I. Incorporated). A mid-lake measurement was taken by suspending the probe to about half the depth of the lake.

2.3.3 Hydrogen peroxide

H_2O_2 concentration estimates were made using a scopoletin-horseradish fluorometric technique first described by Andreae (1955) and Perschke and Broda (1961). The concentration of H_2O_2 was determined by creating a standard curve based on the method of standard additions (W.J. Cooper, pers. comm., Larsen et al. 1973). Using known amounts of hydrogen peroxide additions to the same sample and measuring further decreases in fluorescence, a standard curve was constructed that associates decreasing fluorescence to nanomolar concentrations of H_2O_2.

This method was chosen due to its' relatively simple requirements for equipment and a high sensitivity for detecting extremely low concentrations of H_2O_2 in natural waters (Cooper and Lean 1989; Cooper et al. 1989; Donahue 2000; Holm et al. 1987; Kieber and Helz 1986; Wilson et al. 2000). The basis for this method was that when H_2O_2 combines with scopoletin (7-Hydroxy-6-methylcoumarin, a highly fluorescent compound), the chemiluminescent compound fluoresces at a specific wavelength. Horseradish peroxidase (HRP) is then added to the sample, initiating oxidation of H_2O_2 and phenol. A phenoxy-radical is created that, when combined with scopoletin, decreases the fluorescence of the water sample. This decrease in fluorescence is proportional to the amount of H_2O_2 present in the sample.

A specific protocol for H_2O_2 fluorometric determination was derived for Mackenzie Delta water samples and is fully outlined in Appendix 6.1. Included in the method for H_2O_2 determination is the method for standard curve using standard additions as well as H_2O_2 standardization determination using permanganate titrations.

2.3.4 Dissolved organic carbon

Upon returning to the lab, lake water was filtered through a GF/C filter (47mm, Whatman) using a volumetric flask with sidearm. Filters were held in place with a Millipore™ filtration apparatus and metal clamp which were secured to the flask. The GF/C filtered lake water was immediately transferred to a 60 mL HDPE Nalgene™ bottle and frozen at -4° C. Samples were shipped to the Freshwater Institute (Department of Fisheries and Oceans, Winnipeg, Manitoba) for analysis. DOC was analyzed using the wet chemical oxidation technique (Stainton et al. 1979; Wetzel and Likens 2000) where a sample of the filtrate was first acidified, converting all inorganic carbon to carbon dioxide (CO_2). The sample was then sparged of inorganic CO_2 using an inert gas (N_2 or He) and any remaining DOC was treated with an oxidant (persulfate) at an elevated

temperature and pressure, resulting in the generation of CO_2. Using an infrared scanner, the generated CO_2 was measured and provides a measure of DOC in the lake water sample.

2.3.5 Coloured dissolved organic carbon

CDOC absorbences were used as a proxy for CDOC concentrations (Scully and Lean 1994). Specific absorbance is related to UV penetration in lakes whereby increased UV penetration is inversely related to CDOC concentration. Spectrophotometric analyses were performed on filtered lake water samples using a Milton Roy Spectronic 501 spectrophotometer and a 10cm long cuvette. Sixty ml of GF/C filtered lake water was re-filtered through a Millipore 0.22µm 45mm filter. CDOC absorbencies were taken at 330nm (UV wavelength) and at 740nm (for background scatter) then compared to a distilled deionized water (DDW) blank. CDOC absorbencies were accurate to 3 decimal places and used a DDW blank in between each replicate to ensure accuracy.

In temporal analysis of CDOC absorbencies, photobleaching was interpreted as the progressive loss of absorbance values observed within lake water samples with time (*i.e.* days, weeks, months).

2.4 BACTERIA SAMPLING AND MEASUREMENTS

The forty spatially discrete lakes used in previous studies (Squires 2002; Teichreb 1999) represent a gradient of DOC compositions from highly coloured to highly clear. A forty lake survey was done to identify any spatial trends in bacterial production in the presence and absence of grazers across the gradient of lake types (August 4th, 2003). Bacterial production and bacterial abundance were measured for unfiltered lake water (with grazers) and for GF/C filtered lake water (without grazers).

2.4.1 Separation of bacteria from the grazer community

Two experiments required the removal of the grazer community. When needed, lake water was filtered to remove organisms larger than 1 µm including all zooplankton and heterotrophic nanoflagellates, phytoplankton and particularly large or filamentous bacteria (GF/C, 1 µm nominal pore size, Whatman). Once filtered, filtrate with mostly bacteria was transferred to 1 litre microcosms.

2.4.2 Bacterial production per cell

Bacterial production was determined using a [methyl-^{3}H]-thymidine uptake method (Moriarty 1986; Robarts and Zohary 1993; Spears 2002; Teichreb 1999; Wicks and Robarts 1987). This method has been used in previous limnological studies (Spears 2002; Teichreb 1999) because it arguably assumes the best expression of microbial activity (Robarts and Zohary 1993). This method also assumes that cell division, together with DNA and protein synthesis are indicative of a balanced microbial growth rate.

Specifically, cell division occurs after successful DNA synthesis through one of two principal nucleotide biosysnthesis pathways: the de *novo* or salvage pathway. Theoretically, bacterial production is measured from the rate at which exogenous supplies of radiolabelled [^{3}H]-thymidine are incorporated into bacterial DNA via the salvage pathway (Moriarty 1986). It has been argued that the [^{3}H]-thymidine method is preferred for aquatic microbes since it is relatively simple, commonly used by microbial ecologists and procedurally renders equivalent measures of total microbial production when compared other methods such as [^{3}H]-leucine incorporation (Robarts and Zohary 1993). Using the method employed by Teichreb (1999) and Spears (2002), tritiated-thymidine (P576, Amersham Biosciences) was added to the water sample and allowed to incubate. Incubated [^{3}H]-thymidine incorporation was terminated by adding a chemical inhibitor (trichloroacetic acid, TCA) and stopping the reaction on ice. The radiolabelled DNA was extracted from cells as a TCA precipitate and radioactivity was measured.

Coupled with the radiolabelling of microbial DNA, bacterial abundance was achieved by slide preparation and counting using epifluorescent microscopy based on the methods of Porter and Feig (1980). The method involves the staining of cellular DNA with 4'6-diamidino-2phenylindole (DAPI; Sigma Chemicals, Cat. No. 32670). Bacterial DNA intercalates with the stain, causing cells to fluoresce a bright blue when viewed under an epifluorescent microscope when illuminated at 365 nm. This method was similar to previous studies (Riedel 2002; Spears 2002; Teichreb 1999) with a few enhancements to the method, particularly the use of phosphate buffered saline tablets (PBS, Sigma P4417) and Polyoxyethylenesorbitan monolaurate (TWEEN, Sigma P1379).

Estimates of bacterial production per cell (mol TdR cell^{-1} hr^{-1}) were determined by dividing the number of moles of [^{3}H]-TdR incorporated into cells into bacterial

abundance (Bell 1993). A detailed protocol for both bacterial production and abundance methodologies are fully outlined in Appendix 6.2.

2.5 MICROCOSM ENCLOSURES

A key component to the experimental aspect of this research was the use of an outdoor incubator and one-litre cylindrical microcosms (Figures 2.3 to 2.6). Acrylite™ OP-4 cylinders (GE Polymershape, Burlington, Ontario; Fabricated by ASI Plastics, Coquitlam, British Columbia) were stoppered with plastic rubber stoppers. Acrylite™ OP-4 allows all environmental wavelengths to pass freely through walls of the vessel. Up to 54 microcosms filled with lake water were submerged in the incubator and temperature was regulated using a pump and chiller unit.

2.5.1 Radiation screens

The use of UV radiation filters to manipulate incoming wavelengths is common for related H_2O_2 experiments (Donahue 2000; Kelly and Bothwell 2002; Kelly et al. 2003; Teichreb 1999). Different filters were used to efficiently remove specific UV wavelengths but not photosynthetically active radiation (PAR; 400-700nm). UVB wavelengths were removed using Mylar-D™ sheeting (herein referred to as Mylar, 4mil, GE Polymershape; Burlington, Ontario). UVA+UVB wavelengths were removed using Acrylite™ OP-2 plexiglass sheeting (Cadillac Plastics, Coquitlam, British Columbia). As a methodological control, polyethylene sheeting was used, which effectively transmits all UV and PAR wavelengths through. Transmission graphs for each filter were obtained using an OL-754 UV-Visible spectroradiometer (Optronics Laboratories, Inc. Orlando, Florida). Transmission scans carried out on a clear, sunny day confirm the optical properties of each filter (Figure 2.5).

Two separate experimental designs were carried out using the outdoor incubator: the hydrogen peroxide formation experiment (Section 2.6), and, a series of bacterial production experiments (Section 2.7). For consistency, the treatments will herein be referred to as full sunlight (*i.e.* no UV filter, includes UVA+UVB+PAR wavelengths), -UVB (*i.e.* Mylar filter, UVA+PAR wavelengths) and PAR (*i.e.* OP-2 filter, PAR wavelengths only), for all outdoor incubator and microcosm experimental designs.

2.5.2 Measurement of incoming solar radiation and PAR

Total incoming radiation (measured as Watts m^{-2}) was continually logged at the Inuvik Research Centre during 2003 and 2004. This provided a measure of total irradiance, including visible and ultraviolet wavelengths. When possible, integrated underwater measurements of photosynthetically active radiation (PAR, measured as $\mu mol\ s^{-1}\ m^{-2}$) were logged using a LiCor underwater radiation sensor (LI-195A, Lincoln, Nebraska) that was placed just below the surface of the water inside the incubator. Maximum, minimum and average PAR values were logged continuously using a LI-1000 Data Logger (LiCor Inc.), reporting integrated averages every 30 minutes.

2.6 EXPERIMENTAL H_2O_2 FORMATION & CDOC PHOTOBLEACHING

Photochemical formation of H_2O_2 was assessed in an outdoor microcosm experiment. Specifically, this experiment assessed the potential for H_2O_2 formation and CDOC photobleaching under different UV scenarios, testing the hypothesis that H_2O_2 production would be highest where the combination of UVB exposure and photochemical substrate is highest. Lake water samples from Dock and South Lakes were again selected for this experiment. Triplicate 1-litre microcosms were filled with unfiltered lake water and placed in the outdoor incubator, under each of three treatments: Acrylite OP-2 filter (PAR wavelengths only), a Mylar filter (UVA+PAR wavelengths) and the Control (full sunlight, UVA+UVB+PAR). A total of nine microcosms from each lake remained in the outdoor incubator for up to 12 hours on a cloudy day (July 5th 2004) and for 15 hours on the sunny day (July 9th 2004).

H_2O_2 concentrations and CDOC absorbencies were measured immediately after removing each microcosm from the incubator. Total incoming radiation (measured as Watts m^{-2}) was continually logged at the Inuvik Research Centre and average PAR values were logged continuously, reporting integrated averages every 30 minutes.

It was hypothesized that H_2O_2 concentrations would be highest under full sunlight, lower under Mylar and lowest under OP-2 filters as the wavelengths critical to H_2O_2 formation and CDOC photobleaching would be effectively removed by each of the filters. It was further hypothesized that CDOC photobleaching would be greatest under

full sunlight rather than under the Mylar or OP-2 filters where critical UV wavelengths would be effectively removed by the radiation filters.

2.7 BACTERIAL PRODUCTION WITH MANIPULATED UVB

In order to address the overall objective that bacterial production would be lowest when hydrogen peroxide was highest, a number of smaller experiments were conducted to assess the relative strengths of other factors that affect bacterial production. The bacterial production experiments were an attempt to tease out the interactive effects of H_2O_2, DOC substrate availability and grazing pressure on bacterial communities in a range of lakes with varying DOC compositions. Hence, the relative strengths of grazing, H_2O_2, DOC substrate and direct UVB inhibition were individually tested using a combination of forty lake surveys and outdoor incubator experiments. After controlling for grazing, DOC variability and UVB exposure, I expected to find that bacterial production would be inhibited when H_2O_2 levels were highest.

2.7.1 Bacterial production with the full grazing plankton community

This experiment was conducted to assess the response of bacteria to H2O2 levels and UVB radiation in a range of lakes with varying DOC compositions. The experiment was conducted with lake water that included the full grazing plankton community (e.g. nanoflagellates, phytoplankton and zooplankton). On August 3rd, 2003, lake water was collected from the six lakes and brought back to the Inuvik Research Centre. For each lake, triplicate one litre microcosms were filled and randomly placed under a UV treatment (Mylar, UVA+PAR wavelengths) or under Full sunlight (Control). A total of six microcosms for each lake were used (three in the Control; three under Mylar). Incoming solar radiation was measured for the duration of the experiment at the Inuvik Research Centre. Microcosms were sampled for H2O2, bacterial abundance and bacterial production at 0 and 8 hours.

2.7.2 Bacterial production with the bacterial community only

To assess the response of bacteria in the absence of the full plankton community, another outdoor experiment was carried out using lake water with the full plankton community removed, leaving only the bacterial community present. On August

14th, 2003, lake water was collected from the six lakes and brought back to the Inuvik Research Centre and was filtered to remove most grazers (1 µm nominal pore size) with a portion of the planktonic bacterial community remaining. For each lake, triplicate one litre microcosms were filled and randomly placed under the same treatments as in the previous experiments (full sunlight or -UVB). A total of six microcosms for each lake were used (three in the Control; three under -UVB). Incoming solar radiation was measured for the duration of the experiment at the Inuvik Research Centre. Microcosms were sampled for H_2O_2, bacterial abundance and bacterial production at 0 and 8 hours.

2.7.3 Lake water only with bacteria re-inoculated at end

The final of the outdoor experiments was conducted to assess the response of bacteria to direct UVB and H_2O_2 while controlling for grazing and DOC variability. Because of a slightly more complicated experimental set-up (Figures 2.6 and 2.7), three lakes were selected for this experiment (South Lake, Lake 280 and Dock Lake, Table 2.1) and smaller volumes were used for the microcosms (0.25 L). Microcosms were irradiated for 6 hours under two levels of treatments: bacteria *removed* from lake water, or, with bacteria left *in situ*); followed by Mylar (UVA+PAR wavelengths) or the Control (Full sunlight, UVA+UVB+PAR). The predicted effects of each treatment involved a gradient of responses by bacteria to enhanced photolyzed DOC, direct UVB inhibition and H_2O_2 inhibition (Table 2.3).

Similar to the previous experiments, lake water was collected from each lake and brought to the Inuvik Research Centre. For each lake, half of the collected lake water was set aside, placed in a darkened fridge until later in the experiment. The other half underwent a series of staged filtrations to remove specific size fractions of the planktonic community (Figure 1.1). The first treatment was the "Removal & Replacement" treatment (RR). Microcosms were subject to one of two RR treatments: remove bacteria (+RR) or to leave the bacteria *in situ* (-RR). The +RR treatment involved the removal of plankton *including* bacteria by filtering out plankton first using 1 µm filtration (GF/C, Whatman) followed by another filtration using 0.2 µm filtration (Nucleopore polycarbonate) to remove most bacteria. The –RR treatment was subject to filtration using 1 µm to remove grazers only, leaving only bacteria. Microcosms were then randomly placed in the outdoor incubator under one of two treatments: the full sunlight Control (+UVB) and Mylar (-UVB). A "dark" methodological control was also used for each lake. The dark

control consisted of an aluminum foil-covered microcosm where only the +RR treatment was carried out.

All microcosms were incubated for 6 hours in the outdoor incubator at which time the lake water initially set aside and in the dark, was retrieved. At 6 hours, a 250 mL inoculum of lake water was added to every microcosm. The inoculum consisted of a full complement of plankton (bacteria and other plankton). Once added, the microcosms were re-stoppered and placed back into the incubator. The microcosms continued to incubate for an additional two hours, for a cumulative total of 8 hours of irradiation. Samples for H_2O_2 concentration, bacterial abundance and bacterial production were taken at 0, 6, and 8 hours. Bacterial abundance and production were also measured for the added inoculums. Incoming solar irradiation was measured at the Inuvik Research Centre and integrated underwater PAR was continually monitored for the duration of the experiment.

2.8 STATISTICAL ANALYSES

All statistical analyses were completed using JMP 5.0 Software (SAS Institute). Regression analyses were carried out on some experiments to extrapolate any potential relationships between abiotic and biotic factors and sill elevation or DOC concentration. Multiple regression analyses were carried out for the forty-lake hydrogen peroxide results to identify variables that were closely related to one another (p to enter = 0.15). The main purpose was to identify if depth, sill elevation, DOC and lake area were important variables in hydrogen peroxide formation among forty lakes. The results were used to create a stepwise regression equation where only the variables significant at α=0.05 were used in the results.

Variation in H_2O_2 concentrations, CDOC absorbencies and bacterial production per cell within lakes were analyzed using one-way ANOVAs (α=0.05). In preparation for a significant single factor ANOVA result, planned comparisons for the incubator experiments included:

1. between individual treatments: Control, OP-2 (removed UVA and UVB wavelengths), Mylar (removed UVB wavelengths) and full sunlight;
2. between full sunlight and removed UVB (Mylar filter);
3. between grazers and non-grazers (filtered)

Two-way ANOVAs were planned for incubator experiments to assess any differences between the UV treatment and bacterial removal. Planned comparisons for the Removal & Replacement experiment include:

1. between full sunlight (Control), dark and Mylar (removed UVB)
2. between removal and replacement (+/- RR) treatments

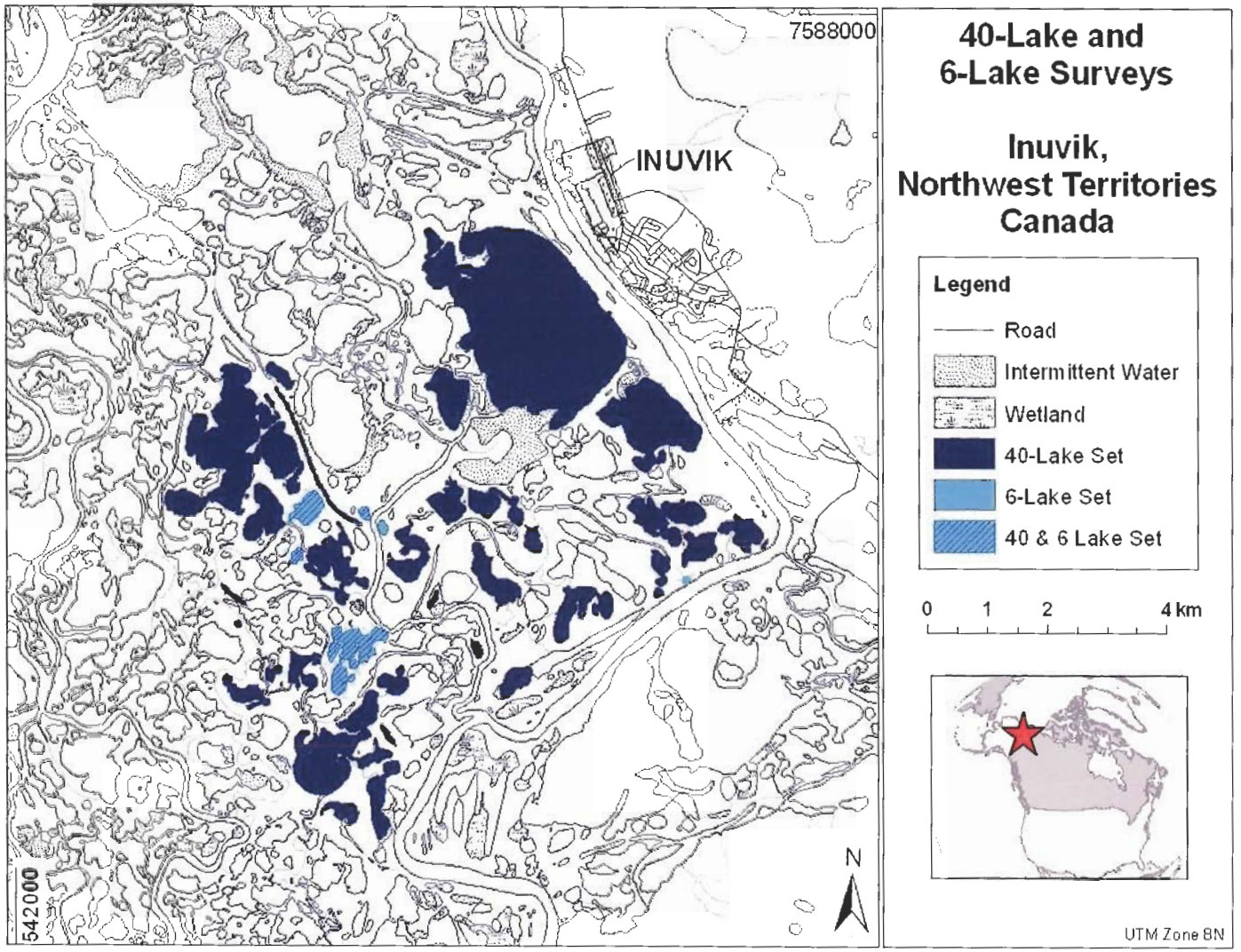

Figure 2.1 Map of Mackenzie Delta area near Inuvik (68°19' N, 133°29' W) in the western Canadian arctic. Identified are the lakes used in the seasonal six-lake surveys and in forty-lake surveys during 2003 and 2004.

Lake	Latitude (North)	Latitude (West)	Sill elevation (m)	Lake area (km^2)	Flood frequency	Flooding recurrence
South	68°18.330'	133°50.566'	2.363	0.378	Frequently	Continually connected to river channel
80	68°19.399'	133°52.324'	2.631	0.193	Frequently	Continually connected to river channel
87	68°19.047'	133°52.435'	3.389	0.039	Intermediately	At least annually
280	68°19.248'	133°50.375'	3.838	0.024	Intermediately	At least annually
56	68°19.394'	133°50.817'	4.623	0.031	Infrequently	Less than annually
Dock	68°18.816'	133°42.854'	4.913	0.002	Infrequently	Less than annually

Table 2.1 Summary of abiotic characteristics for six lakes used in seasonal surveys and used in experiments during the summers of 2003 and 2004.

Lake	Latitude North	Latitude West	Spring sill elevation (masl)	Lake area (km^2)	[TDOC] (um/L)	CDOC (abs@330nm)
4	68°20.015'	133°53.978'	2.363	3.305	571	0.411
11	68°20.612'	133°52.864'	3.838	1.054	588	0.326
15a	68°20.513'	133°48.437'	2.177	4.376	430	0.633
56	68°19.394'	133°50.817'	4.623	0.031	877	0.482
58	68°19.784'	133°52.049'	3.389	0.137	640	0.312
79a	68°19.393'	133°53.078'	2.631	0.346	567	0.473
80	68°19.428'	133°52.160'	2.631	0.193	575	0.440
85a	68°18.982'	133°51.552'	2.363	0.506	510	0.473
85b	68°19.289'	133°51.747'	2.990	0.017	584	0.467
87	68°19.015'	133°52.460'	3.389	0.039	580	0.467
107	68°18.041'	133°52.404'	2.990	0.167	612	0.481
111	68°17.964'	133°53.095'	3.671	0.053	739	0.461
115	68°18.673'	133°53.980'	4.623	0.023	646	0.502
South	68°18.238'	133°51.145'	2.363	0.378	614	0.480
131	68°18.065'	133°51.065'	4.077	0.012	1098	0.636
134	68°18.218'	133°48.047'	4.623	0.034	806	0.399
141	68°17.878'	133°50.090'	3.389	0.172	872	0.600
143	68°17.425'	133°50.205'	5.169	0.021	817	0.477
148a	68°16.928'	133°50.517'	2.631	0.284	909	0.620
148b	68°17.098'	133°52.062'	3.389	0.948	692	0.483
148f	68°16.747'	133°51.307'	4.077	0.124	817	0.444
181	68°17.298'	133°53.688'	5.169	0.008	980	0.706
184	68°17.773'	133°53.662'	3.671	0.177	640	0.442
186	68°18.418'	133°53.840'	5.169	0.010	770	0.229
261	68°17.922'	133°47.145'	4.623	0.485	780	0.497
272	68°18.772'	133°47.680'	2.990	0.273	673	0.495
272b	68°18.747'	133°46.492'	3.671	0.021	897	0.626
278	68°18.672'	133°49.112'	4.077	0.059	649	0.373
287	68°19.145'	133°46.632'	4.077	0.098	851	0.429
300	68°18.900'	133°49.630'	2.990	0.344	627	0.367
301a	68°19.487'	133°47.755'	2.990	0.366	673	0.407
302a	68°21.012'	133°47.368'	1.500	8.530	410	0.308
302b	68°19.492'	133°48.707'	2.631	0.189	630	0.355
501	68°20.254'	133°43.529'	2.631	1.269	608	0.397
511	68°19.763'	133°43.617'	3.389	0.016	1017	0.550
517	68°19.377'	133°43.662'	4.077	0.729	705	0.451
521	68°19.033'	133°41.802'	5.169	0.001	686	0.528
522	68°19.257'	133°41.518'	4.913	0.225	574	0.489
527a	68°18.957'	133°43.530'	5.169	0.085	1029	0.543
538	68°18.568'	133°45.843'	3.838	0.379	825	0.534

Table 2.2 Summary of abiotic characteristics for forty lakes near Inuvik sampled during the summers of 2003 and 2004. TDOC concentrations reported are from late summer 2003 and CDOC absorbencies are from around the arctic summer solstice in 2004.

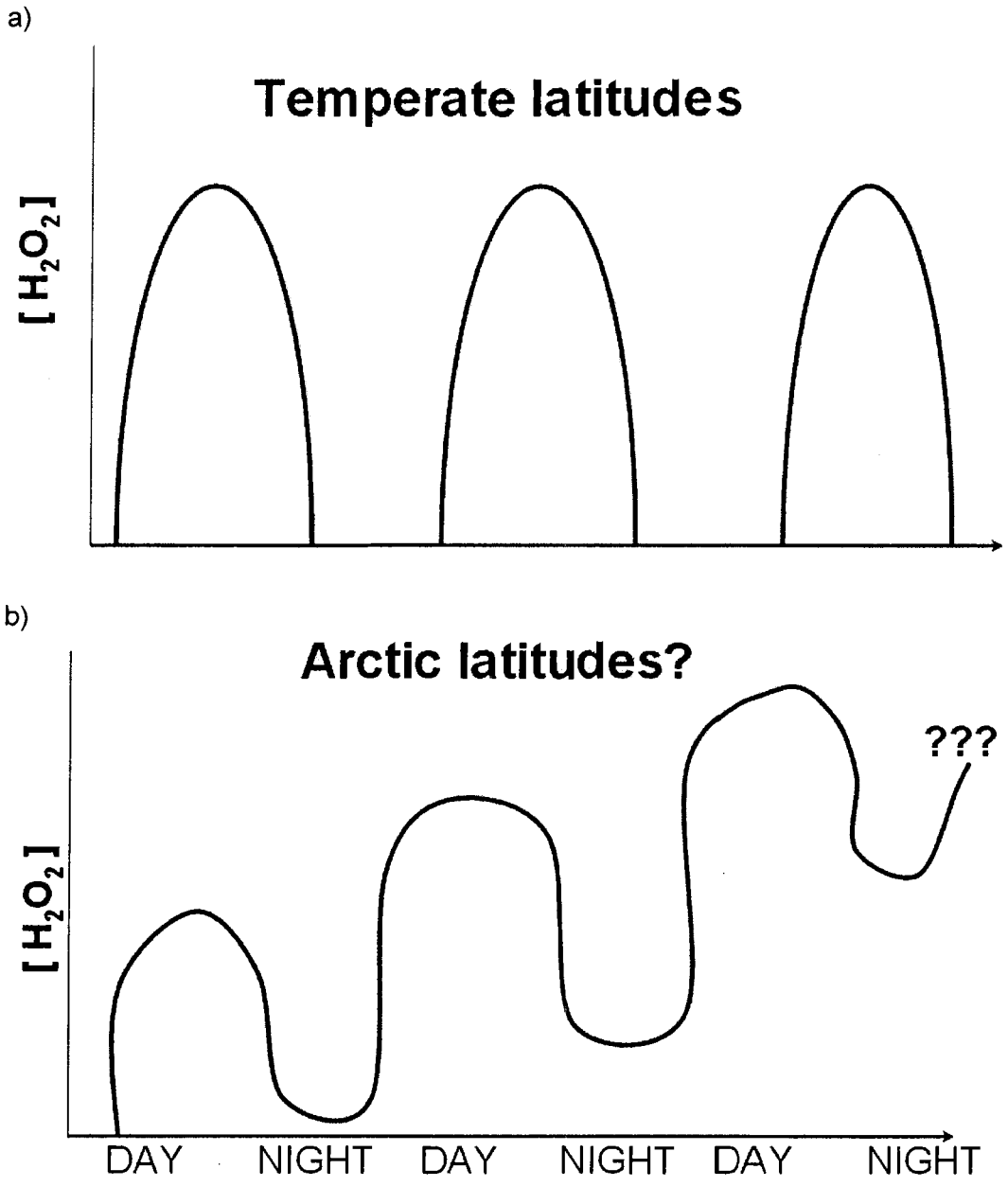

Figure 2.2 Hypotheses for diurnal H_2O_2 dynamics at a) temperate and b) arctic latitudes. At temperate latitudes, H_2O_2 builds up daily in lakes but decays completely during the night. It is hypothesized that at arctic latitudes, particularly around the summer solstice that H_2O_2 may progressively build up in Mackenzie Delta lakes during sequences of cloudless days with 24-hour sunlight.

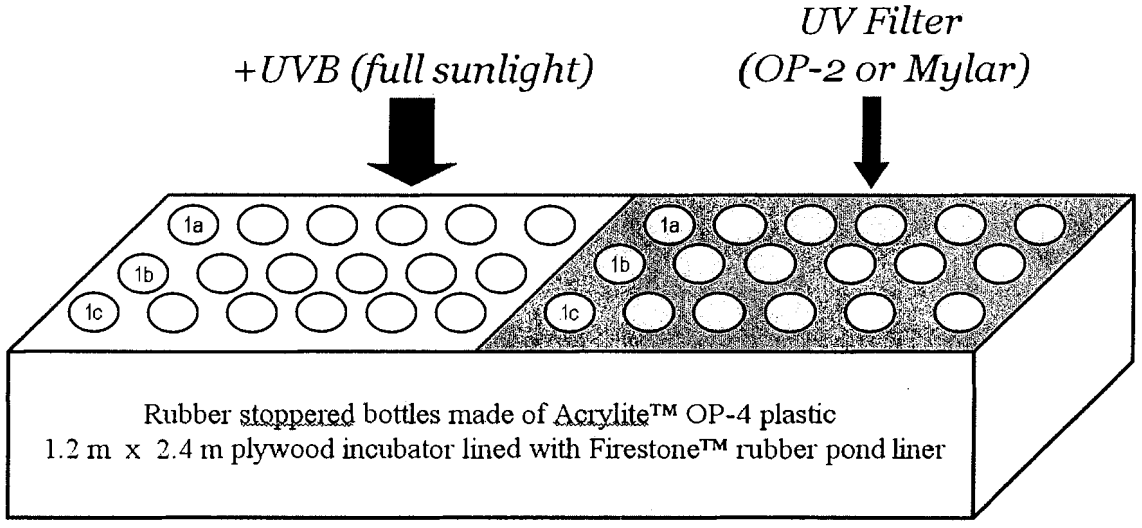

Figure 2.3 Schematic of outdoor incubator used in bacterial production and hydrogen peroxide formation experiments at Inuvik Research Centre. Incubator was fabricated from plywood and measured 1.2 m by 2.4m (4' by 8') and lined with pond liner. One-litre microcosms made of Acrylite™ OP-4 plexiglass were placed under different conditions during various experiments: full sunlight; polyethylene; Mylar™; Acrylite™ OP-2. See Figure 2.4 for photograph of experimental set-up and Figure 2.5 for transmittance properties of UV filters used in the experiment.

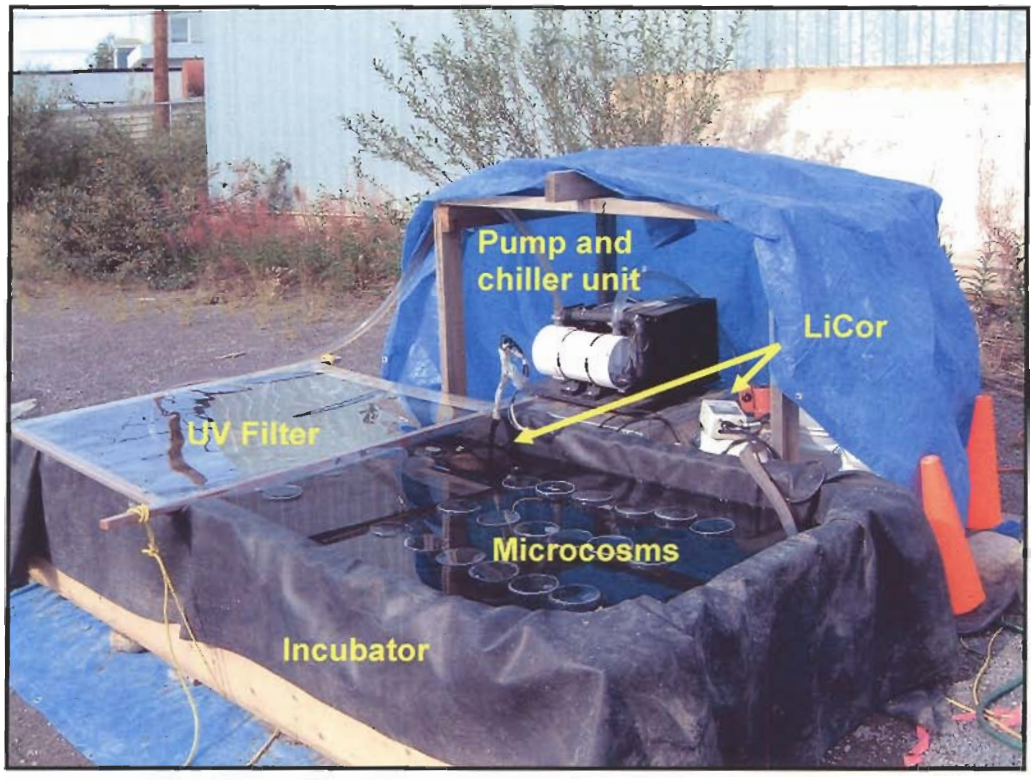

Figure 2.4 Experimental set-up of outdoor incubator with one-litre microcosms placed randomly under each treatment (full sunlight or UV radiation filter). Located at the Inuvik Research Centre, the incubator was fabricated using plywood and lined with pondliner and placed away from any possible shading from surrounding structures. Filters were held in place using a wooden support and rope. Underwater PAR in full sunlight (no filter) was measured using the LiCor radiation sensor. Water temperature was maintained using an electric-powered pump and chiller unit with the outlet feeding into the incubator on one end and the inlet removing water from the opposite end. Water temperature was determined each day to reflect average lake water temperatures.

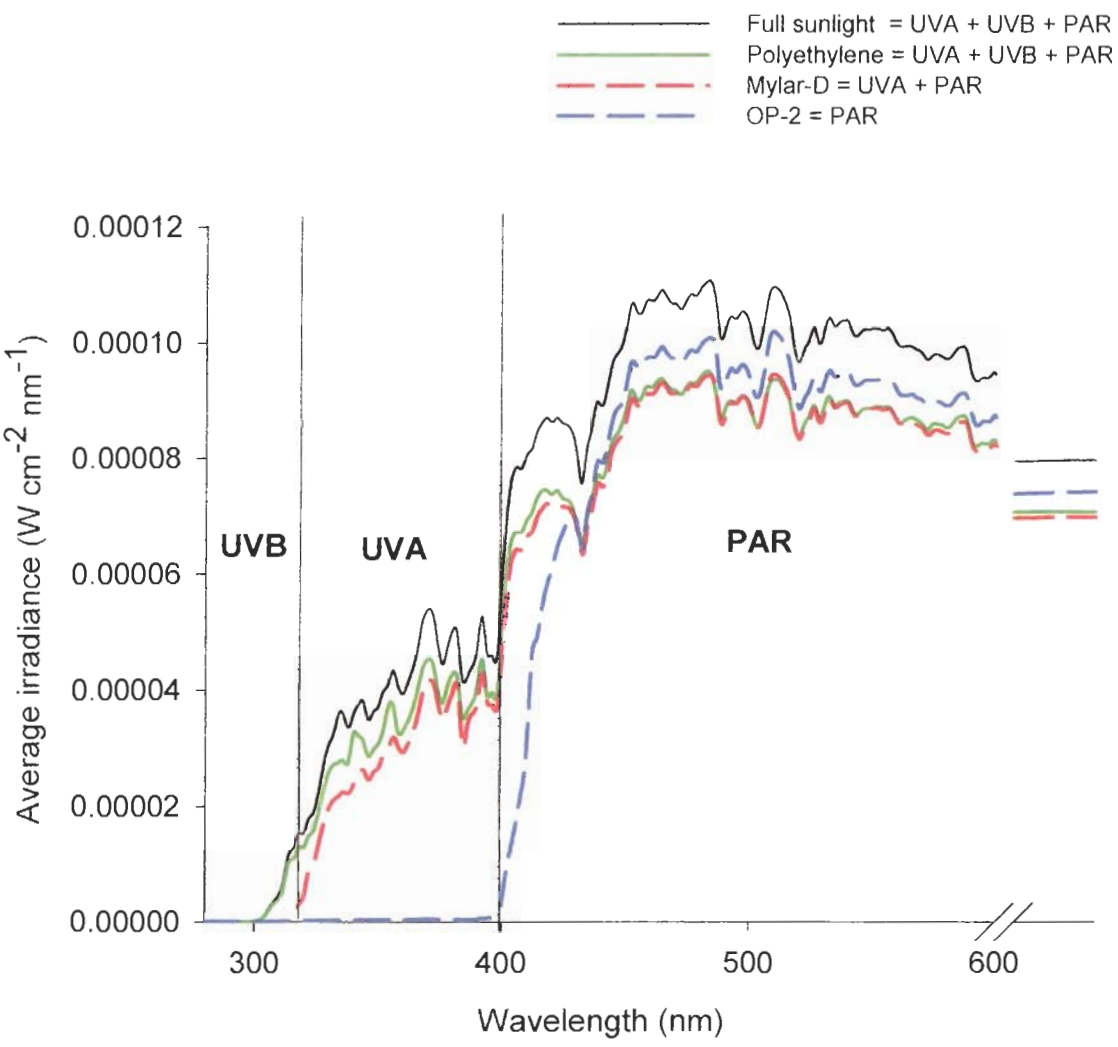

Figure 2.5 Transmittance of UV radiation filters used in outdoor microcosm experiments. Filters were chosen to effectively filter out a particular range from mid-UV (UVB, 280-320 nm), near-UV (UVA, 320-400 nm) and photosynthetically active radiation (PAR, 400-700 nm). The Polyethylene filter allowed all wavelengths through, Acylite™ OP-2 filters effectively removed UVA and UVB wavelengths, and Mylar-D™ filters effectively removed UVB wavelengths. Transmittance for each filter was determined simultaneously under clear conditions and compared to Full sunlight (PAR + UVA + UVB wavelengths. Data provided by J. Gareis (*unpub. data*). Also see Figures 2.3 and 2.4 for experimental set-up.

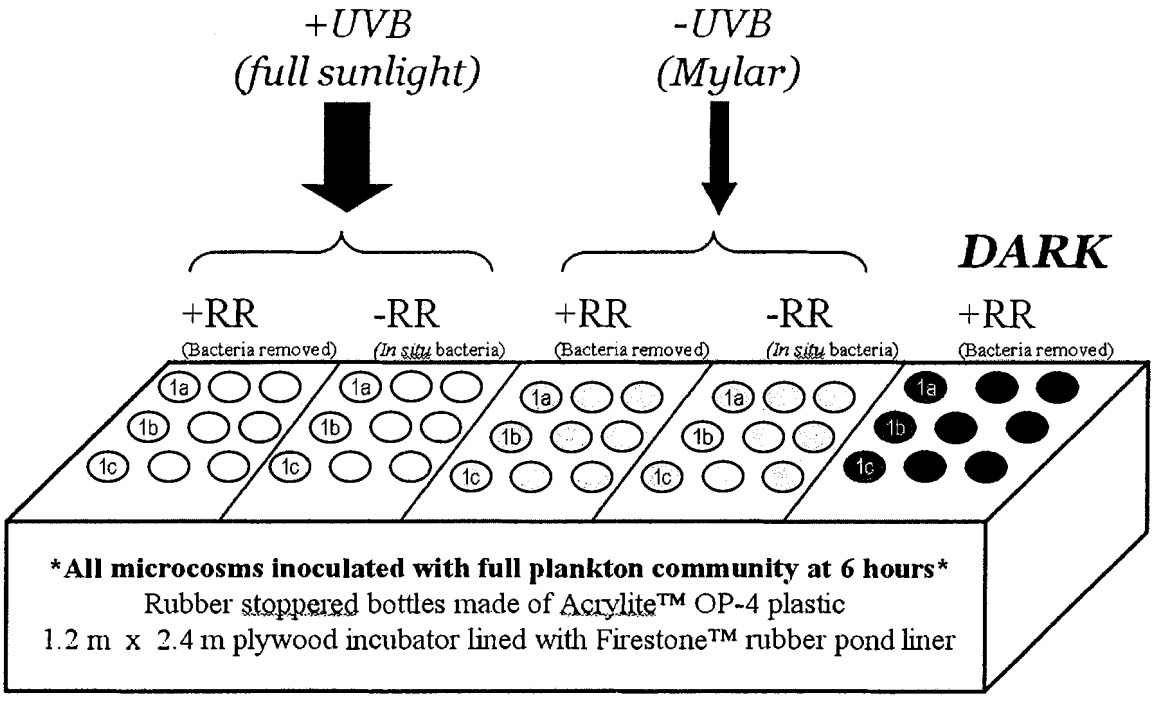

Figure 2.6 Schematic of outdoor incubator used in Removal & Replacement bacterial production experiment (August 15th, 2003). 250 mL microcosms were subject to two levels of treatments: Removal and Replacement (+ or - RR) and UVB (+ or – UVB). Three lakes were chosen for this experiment: South Lake, Lake 280 and Dock Lake. Lakewater microcosms were subject to a +RR treatment that resulted in microcosms filled with only lakewater (1 µm followed by 0.2 µm filtration to remove plankton including bacteria). Microcosms subject to the –RR treatment left only bacteria in situ with lakewater (only 1 µm filtration). Microcosms were then placed under full sunlight (UVA+UVB+PAR, Control) or under Mylar (UVA+PAR). A methodological control using aluminum-foil covered micrcocosms were used for each lake where only the +RR treatment was carried out. Microcosms were incubated for 6 hours at which time a 250 mL inoculum of fresh lakewater was added to each microcosm and incubated for another 2 hours, for a total of 8 hours of irradiation. See Figure 2.7 for schematic of experimental treatments.

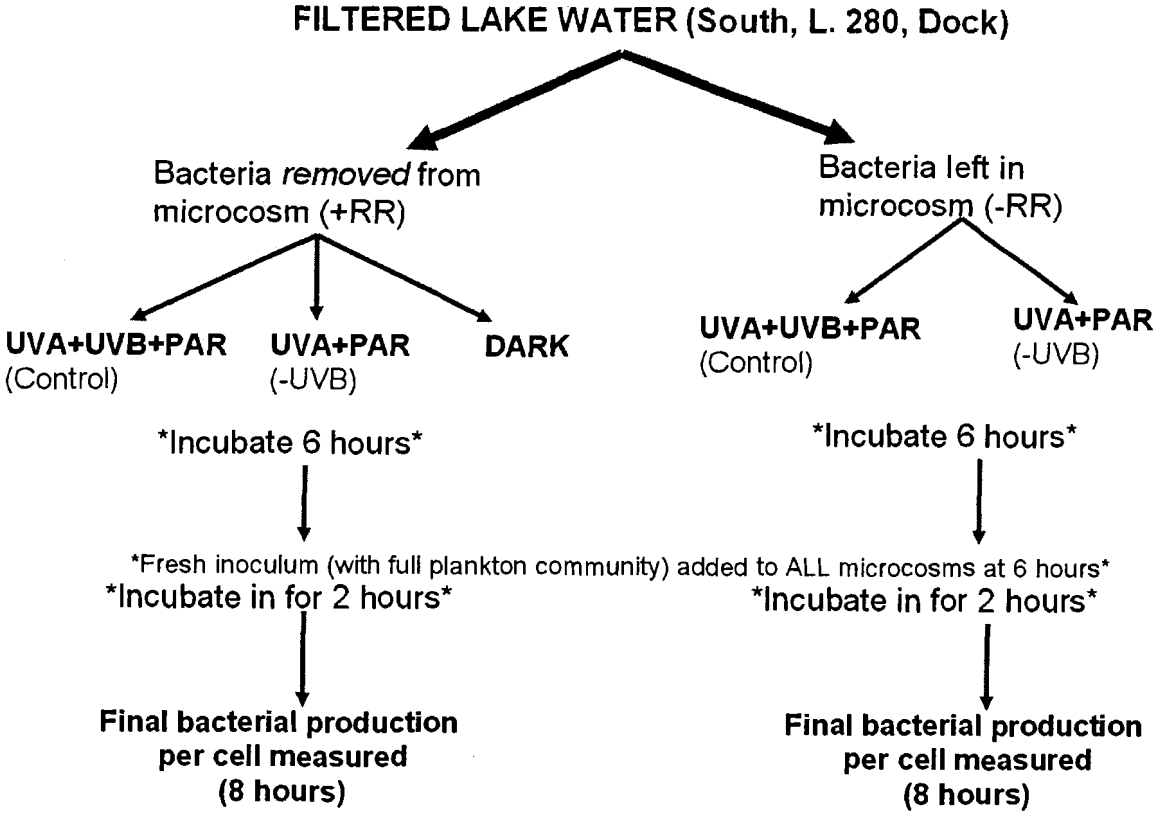

Figure 2.7 Schematic of treatments and experimental set-up for the Removal & Replacement bacterial production experiment (August 15th, 2003). See Table 2.3 for predicted effects on bacteria in each individual treatment.

TREATMENT	Predicted Effects		
	Direct physiology	Enhanced-C	H_2O_2
+RR (UVA+UVB+PAR; Full sunlight)	✓	✓ ✓	✓ ✓
+RR (UVA+PAR)		✓	✓
+RR DARK			
-RR (UVA+UVB+PAR; Full sunlight)	✓ ✓	✓ ✓	✓ ✓
-RR (UVA+PAR)		✓	✓

Table 2.3 Summary of predicted effects on bacteria in each of the treatments used in the Removal & Replacement experiment (August 14th, 2003). A single check signifies that bacteria will be moderately subjected to the effect compared to the dark control. A double check signifies that bacteria will be considerably subjected to the effect compared to the dark control.

3 RESULTS

3.1 STUDY SYSTEM CONDITIONS – WEEKLY PATTERNS AMONG SIX LAKES

3.1.1 Temperature

Mean summer surface temperature was similar for six lakes with no relationship with sill elevation (n = 7; Figure 3.1) or DOC (data not shown). Lakes were well-mixed with no evidence of thermal stratification throughout the summer. Although not statistically significant, seasonal temperatures may be somewhat related to lake depth and river connection (i.e. moderated by river channel, Lesack et al. 1998). For example, Dock Lake represents the lake with the lowest mean summer temperature. Dock Lake is a thermokarst lake (underlain by melting permafrost), located at a high sill elevation and also the deepest lake of the six-lake set (Marsh and Hey 1988). In this lake, melting of permafrost at depths may facilitate lower water temperatures. Temperatures in the other lakes may be somewhat moderated by the river channel resulting in connected lakes at lower sill elevations having lower temperatures due to river inputs in contrast to lakes at higher sill elevations.

3.1.2 Dissolved organic carbon

Weekly measurements were taken from integrated water samples between June and August 2003 (n=7). Mean summer DOC concentrations increased with sill elevation (R^2 = 0.6623; Figure 3.2a). Mean summer absorbance values (at 330 nm) for CDOC decreased with sill elevation (R^2 = 0.7946; Figure 3.2b). Seasonal DOC trends were similar to previous years in which gradients of CDOC absorbance and TDOC run counter to one another (Spears 2002, Squires 2002).

Evidence of photobleaching was apparent as CDOC absorbencies were quite high very early in the season and rapidly decreased within three weeks (Figure 3.3). The overall trend in CDOC absorbency followed sill elevation (Figure 3.2b).

3.1.3 Bacterial abundance

Mean summer abundance counts reveal no significant pattern in abundance with sill elevation (Figure 3.4). Mean abundance for all lakes is 2 x 10^6 cells mL^{-1} and appear to be higher than estimates of abundance from previous years (10^4 cells mL^{-1}, Spears 2002). It is suggested that differences in abundances are likely due to slight modifications made to the DAPI method (Appendix 6.2), rendering higher counts than in prior years. This may also be due considerable year- to year variation.

Bacterial abundances appear to fluctuate throughout the season. Similar to earlier studies (Spears 2002), bacterial production $cell^{-1}$ was higher immediately after the spring melt in all lakes but Lake 280 with no relationship with sill elevation (Figure 3.5). Generally, abundances were observed to decrease for the month of July followed by a marked increase in abundance for August. Abundances substantially decrease in the following week.

3.2 SEASONAL PATTERNS OF H_2O_2 AND RELATION WITH CDOC

For all three forty lake surveys, the highest concentrations of H_2O_2 were found at mid-DOC levels among lakes with intermediate DOC concentrations and intermediate CDOC absorbencies (Figure 3.6). This was unexpected and contrary to our proposed hypothesis that H_2O_2 levels would be highest where CDOC would be highest, or alternatively, where DOC concentrations would be highest. The results suggest that optimal conditions for photoproduction of H_2O_2 may be due to a trade-off between photochemical substrate such as chromophoric macromolecules in DOC (high in lakes at low sill elevations), versus the amount of UV penetrating through the water column (high in lakes at high sill elevations). Therefore, the results suggest that the highest H_2O_2 concentrations may be found among lakes of intermediate sill elevation.

The results also demonstrate that H_2O_2 levels were highly seasonal, highest early in the season (July 8^{th}) with a peak concentration of 4100 nM. Subsequently, H_2O_2 concentrations dropped dramatically by the end of July and remained stable into August. These results suggest a strong seasonal variation in photoproduction of H_2O_2 that is enhanced early in the open water season. This seasonality may be facilitated by abundant non-photobleached chromophoric DOC, particularly long daylengths and a favourable angle associated with the summer solstice.

Analyses of the potential relationship between H_2O_2 and lake mixing (a function of both lake area and lake depth) revealed no significant relationship with either lake area or lake depth (Table 3.1, Figures 3.7 and 3.8). Multiple regression analyses were carried out for H_2O_2 levels with lake depth, lake area, sill height and CDOC absorbency for all three surveys. No significant relationship was found for the near solstice (July 8th) or late July surveys (data not shown). A statistically significant relationship was obtained for the August 14th survey for H_2O_2 with lake depth ($p = 0.0225$), however, a visual inspection of the residuals indicated a linear model was not appropriate. Since this relationship was not found for the earlier surveys when H_2O_2 levels are enhanced, my analysis suggests that although there may some effect with depth, a linear regression may not be the best statistical test to address this question.

3.3 DIURNAL DYNAMICS OF H_2O_2 WITH 24-HOUR DAYLIGHT

The diurnal study revealed interesting patterns in H_2O_2 variability over forty hours. Dock Lake, a more transparent lake with high DOC concentrations, was tracked first from June 26 to June 28. The weather conditions during the Dock Lake study were constant, sunny and cloudless. South Lake, a highly coloured lake with typically higher CDOC absorbencies, was assessed subsequently July 2 to July 4. The weather conditions for the South Lake study started out cloudless and sunny but smoke and haze from a distant forest fire slowly began to thicken by the end of the experiment.

Total incoming solar radiation, measured at the Inuvik Research Centre, was somewhat comparable for both lakes, showing a maximum of 1180 W m^{-2} on June 27th and 1090 W m^{-2} on July 2nd (Figure 3.9) at solar noon. Underwater PAR measured at each lake also demonstrated similar irradiance patterns for both lake experiments, confirming that the highest solar intensity (solar noon) occurred at approximately 3pm (Figure 3.8). It was therefore expected that the highest concentrations would be observed at or shortly after solar noon.

The results revealed clear diurnal patterns in H_2O_2 concentrations for both lakes that track solar intensity (Figure 3.10). Peaks in H_2O_2 concentrations correspond to solar noon and never completely declined to zero. H_2O_2 concentrations fluctuated very little in Dock Lake (between 150 – 250 nM) whereas they fluctuated greatly in South Lake (between 100 – 500 nM). For Dock Lake, a smaller magnitude in H_2O_2 fluctuation is suggested to be due to less intense irradiation during that period. The results also

document CDOC photobleaching (*i.e.* progressive loss in CDOC absorbency with time) in Dock Lake that correspond to both peak H_2O_2 concentration and solar noon (Figure 3.11). CDOC appeared to progressively decrease in Dock Lake as H_2O_2 progressively increased in concentration. In Dock Lake, CDOC photobleaching appears to be greatest shortly after the observed peak in H_2O_2 concentration. In South Lake, CDOC absorbency does not appear to progressively decrease with time as seen in Dock Lake but rather CDOC absorbency fluctuated moderately. This is likely due to South Lake's connection with the river channel that provides a continual source of CDOC, potentially replacing photobleached DOC with more CDOC.

The potential for H_2O_2 build up was evident among the lakes. Water samples taken at the same time of the day were higher in Day 2 of the experiment for both lakes. Additionally, water samples taken from Dock Lake four days later reveal higher concentrations than those ever observed during the course of the experiment (500 nM, data not shown). This sample was taken on Day 1 of the South Lake experiment therefore suggesting that Dock Lake, a high DOC concentration, had greater H_2O_2 concentrations. The conditions in the days in between sampling times were clear and cloudless (Figure 3.9), demonstrating that there is strong potential for H_2O_2 build-up from one day to the next, especially when solar conditions are constant. Overall, H_2O_2 did appear to build-up among two delta lakes under constant solar conditions and only became hampered once cloud and haze arrived.

3.4 EXPERIMENTAL H_2O_2 FORMATION & CDOC PHOTOBLEACHING

Two lakes with contrasting DOC proportions were compared under differing UV scenarios, once under cloudy conditions (July 5th) and again under sunny conditions (July 9th) with lake water from South Lake (high colour) and Dock Lake (low colour). It was hypothesized that H_2O_2 production and CDOC photobleaching would be less under cloudy conditions than under sunny conditions. Underwater integrated PAR measurements confirmed that there was a three-fold difference in maximum PAR between both dates (Figure 3.12).

For both experiments, CDOC absorbencies and H_2O_2 production behaved as expected. The results revealed a pattern in CDOC photobleaching and H_2O_2 production that was lowest under cloudy conditions and enhanced under sunny conditions (Figures

3.13 and 3.14). Each lake was tested individually for statistical significance for each experiment (cloudy and sunny, Tables 3.2 and 3.3, respectively). Overall, H_2O_2 concentrations were significantly higher in Dock Lake than in South Lake ($df = 10$, $F = 6.9196$, $p = 0.0117$).

Under cloudy conditions, the highest H_2O_2 concentrations were measured in the Control (UVA+UVB+PAR) for both lakes compared to the 0-hour reference (Figure 3.13). Under sunny conditions, the same trend was apparent. However, not surprisingly, H_2O_2 levels were doubled under sunny conditions compared to cloudy conditions (Figure 3.14a). Among South Lake microcosms, H_2O_2 levels were highest in the full sunlight treatment (639 nM), followed by the next highest concentration in the UVA+PAR treatment (381 nM) and the lowest concentrations observed in the PAR and the reference treatments (83 nM and 103 nM, respectively). Significant differences were found between all treatments with the exception of the UVA+PAR and full sunlight treatments (Table 3.3a). Among Dock Lake microcosms, H_2O_2 concentrations were also considerably higher under sunny conditions. Significant differences were observed between the reference treatment and PAR, UVA+PAR and full sunlight treatments.

As designed, South Lake had higher CDOC absorbencies (hence higher water colour) than compared to Dock Lake (Figures 3.13b and 3.14b). The greatest differences in CDOC absorbences were apparent in microcosms with the highest amount of H_2O_2 production. The lowest CDOC absorbencies (and therefore the highest amounts of photobleaching) were observed in the full sunlight and UVA+PAR treatments, even under cloudy conditions. A similar pattern was observed under sunny conditions whereby the highest CDOC absorbencies were observed in the reference and PAR treatments and the lowest absorbencies (and therefore highest amounts of photobleaching) were observed in the -UVB and full sunlight treatments. For South Lake, significant differences were observed between all treatments but -UVB and full sunlight. Similar to South Lake, significant differences for Dock Lake microcosms were observed between all treatments but -UVB and full sunlight (Table 3.2b).

These results suggest that both UVA and UVB wavelengths are critical not only to CDOC photobleaching but also to H_2O_2 production. In both experiments and for all lakes, there were no significant differences between the -UVB and full sunlight treatments. UVB was only filtered out between the two treatments and UVA wavelengths were present among both treatments. This suggests that UVA wavelengths are most

critical for photochemical interactions with DOC since comparable amounts of H_2O_2 were formed.

3.5 PATTERNS IN BACTERIAL PRODUCTION VERSUS DOC AND H_2O_2

3.5.1 Bacterial production with & without grazers

Estimates of bacterial production cell^{-1} were measured in the forty lakes in early August, 2003. To assess any potential food web interactions, bacterial production cell^{-1} in the presence and absence of the full plankton community (*i.e.* grazers) was measured for each of the lakes. Results show that bacterial production cell^{-1} was significantly higher in the presence of grazers than in their absence (Figure 3.15, df = 1, F = 39.7196, $p < 0.0001$). In some cases, bacterial production cell^{-1} was over an order of magnitude higher in the presence of grazers. Bacterial abundances were checked for both full plankton community samples (non-filtered) and bacteria only samples (filtered), confirming that bacteria cells were not removed during the filtration (data not shown). These results therefore suggest an especially strong trophic interactions, possibly predator-prey interactions, between bacteria, heterotrophic nanoflagellates and possibly larger zooplankton.

3.5.2 Bacterial production versus DOC

Particularly large differences in rates of bacterial production were observed between grazing treatments in lakes with mid-TDOC levels; however; this was not statistically significant (Figure 3.16). One-way ANOVA results for bacterial production and DOC concentrations revealed no apparent trend with either the full plankton community present (df = 1, F = 2.9630, p = 0.0892) or bacterial community only (df = 1, F = 3.9921, p = 0.0492).

3.5.3 Bacterial production versus H_2O_2

Bacterial production cell^{-1} did not appear to correlate with end of summer H_2O_2 levels and were not statistically significant (Figure 3.17). One-way ANOVA results for bacterial production and H_2O_2 revealed no apparent trend with either the full plankton community present (df = 1, F = 0.612, p = 0.8053) or bacterial community only (df = 1, F

= 0.0005, p = 0.9824). It is plausible that one reason for non-significant differences is due to low H_2O_2 levels at this time of the season (mid-August). Hence, these results may suggest that, particularly at the end of the summer, DOC and H_2O_2 may not play significant roles in regulating bacterial communities. One hypothesis may be that the grazing effect is great late in the open water season when H_2O_2 is low and perhaps less inhibitory.

3.6 BACTERIAL PRODUCTION WITH MANIPULATED UVB

3.6.1 Full grazing plankton community

The response of bacteria to grazing pressures and photochemical production of H_2O_2 was variable among lakes representing a gradient of DOC compositions (Table 3.4). The experiment started when incoming solar radiation was at a daily maximum (Figure 3.18). H_2O_2 concentrations were found to be comparable for five out of the six lakes and also relatively low (less than 300 nM, Figure 3.19b). Unsurprisingly, no significant differences were observed for H_2O_2 between treatments or among lakes (Table 3.5). It was therefore concluded that H_2O_2 effects were quite weak and that any differences in bacterial production would be likely due to a response to UV rather than H_2O_2.

The only significant difference was observed in the clearest lake that week (Lake 80) where bacterial production $cell^{-1}$ was significantly lower in the -UVB treatment (F = 25.9126, p = 0.0070, Table 3.5). The results suggest that clearer lakes may be more inhibited by direct UVB and that coloured lakes are stimulated by the additional food substrate and UVB sunscreen provided by chromophoric DOC. Interestingly, the three lakes with the lowest water colour (Lakes 80, 87 and 280) revealed higher rates of bacterial production in the UVA+PAR treatment (Figure 3.19a) indicating that in clear lakes, direct UVB may be more inhibitory to bacteria. In contrast, the three lakes with higher water colour (South Lake, Dock Lake and Lake 56) revealed higher rates of bacterial production in the Control (UVA+UVB+PAR) illustrating that the additional photolyzed carbon substrate can stimulate bacterial production.

3.6.2 Bacteria community only

In the subsequent experiment with only the bacteria community present (Table 3.6), rates of bacterial production were considerably lower than for those observed in the experiment with grazers present (Section 3.6.1). Incoming solar radiation was at a daily maximum at the start of the experiment and steadily decreased (Figure 3.20). H_2O_2 levels were generally quite low in this experiment (less than 150 nM, Figure 3.21b) and therefore the effects of H_2O_2 on bacterial production were assumed to be quite weak. A significant difference was observed only in Lake 87 where levels were higher in the -UVB treatment ($F = 27.0092$, $p = 0.0065$, Table 3.7) and in Lake 280 where levels were significantly higher in the full sunlight treatment ($F = 29.3911$, $p = 0.0056$, Figure 3.21b, Table 3.6). Interestingly, the trends in low and high colour reversed with the lowest colour being measured in South Lake, a lake with typically high water colour, and the highest water colour being Dock Lake, a lake with typically low water colour. It is thought that this reversal in CDOC may be explained by the dynamic fluctuations that characterize Mackenzie Delta lakes. Weekly measures of CDOC absorbance were found to fluctuate moderately, especially after mid-June (Figure 3.3) when most photobleaching had already occurred. South Lake, a frequently flooded lake may also be influenced by CDOC brought by the river, thereby influencing water colour unpredictably throughout the season.

In contrast to the previous experiment, bacterial production $cell^{-1}$ appeared to have reversed in trend for three out of the six lakes; however; this was not statistically significant. Microcosms in which bacterial production $cell^{-1}$ was higher in the full sunlight treatment now revealed an opposite pattern where production $cell^{-1}$ was higher in the -UVB treatment (Figure 3.21a) with the most considerable differences between treatments in the -UVB treatment. Considerably large error bars for each lake suggests that non-significant results may be due to lack of statistical power rather than non-significant trends. Overall, these results suggest that the interactive effects of DOC and UV are indeed quite complex or that UV effects late in the season may be a subordinate issue to grazing or nutrient limitation. Because a reversal in pattern was observed, it is recognized that food web effects further obscure our understanding of microbial responses to H_2O_2 and UV across the DOC gradient.

3.6.3 Lake water only with bacteria re-inoculated at end

The 'Removal and Replacement' experiment revealed complex responses by bacteria to direct UVB, photolyzed food substrate and H_2O_2. Rates of bacterial production did not appear to follow any significant pattern among treatments however a few trends may be apparent. The experiment was conducted on August 15th, 2003 with solar radiation at a daily maximum at the start of the experiment and steadily decreasing (Figure 3.22). As observed in the previous experiment, lakes with typically low and high water colour (Dock and South Lakes, respectively) reversed in trend with South Lake having low water colour and Dock Lake having higher water colour (Table 3.8).

H_2O_2 concentrations were measured during the course of this experiment but showed no noticeable trend (Figure 3.23b), again suggesting that H_2O_2 effects were weak with a significant difference observed only for Lake 280 (Table 3.9). In Lake 280, a significant difference was observed between RR treatments where peroxide was higher in –RR microcosms ($F = 7.8558$, $p = 0.0231$, Table 3.9). Overall, H_2O_2 concentrations were highest in Dock lake microcosms with significant differences observed between UV treatments ($F = 8.5198$, $p = 0.0193$) and between RR treatments ($F = 18.2002$, $p = 0.0027$). A strong interaction was observed between the RR and UV treatments (two-way ANOVA, $F = 39.5277$, $p = 0.0002$). The implications of this interaction are not clear and are complicated by H_2O_2 formation under all treatments. Overall, the experimental design was not conducive to producing substantial levels of H_2O_2 across all lake microcosms to fully address the objective of this research.

Regardless, a few trends were apparent in rates of bacterial production. Based on initial values obtained in the 0 hour Reference, there may be a general increase in bacterial production with DOC concentration (Figure 3.23a). Where bacteria were left *in situ* (-RR), bacterial production cell^{-1} was higher under –UVB conditions for all three lakes, indicating that photochemical effects may be more inhibitory on bacterial production than the individual photochemical effects themselves. Bacterial production cell^{-1} in the +RR treatment (*i.e.* bacteria removed) was higher under full sunlight in the low- and mid-CDOC lakes suggesting that photochemical breakdown of DOC into more labile carbon may be more stimulatory in clear lakes.

Overall, the results were not as insightful as originally hoped. A series of assumptions based on my understanding of the system were used to carefully choose the timing of the experiment and treatments. In conducting this research, the interactions of DOC, CDOC photobleaching and H_2O_2 formation were further explored

and highlight the importance of the early open water season for photochemical activity. My bacterial production experiments further emphasize the complexity of the interactions between UV, DOC and bacterial production, demonstrating that seemingly comparable results may be the result of inhibitory effects of UV and H_2O_2 production that are counterbalanced by the stimulatory effects of coloured DOC as a UV sunscreen and additional food substrate.

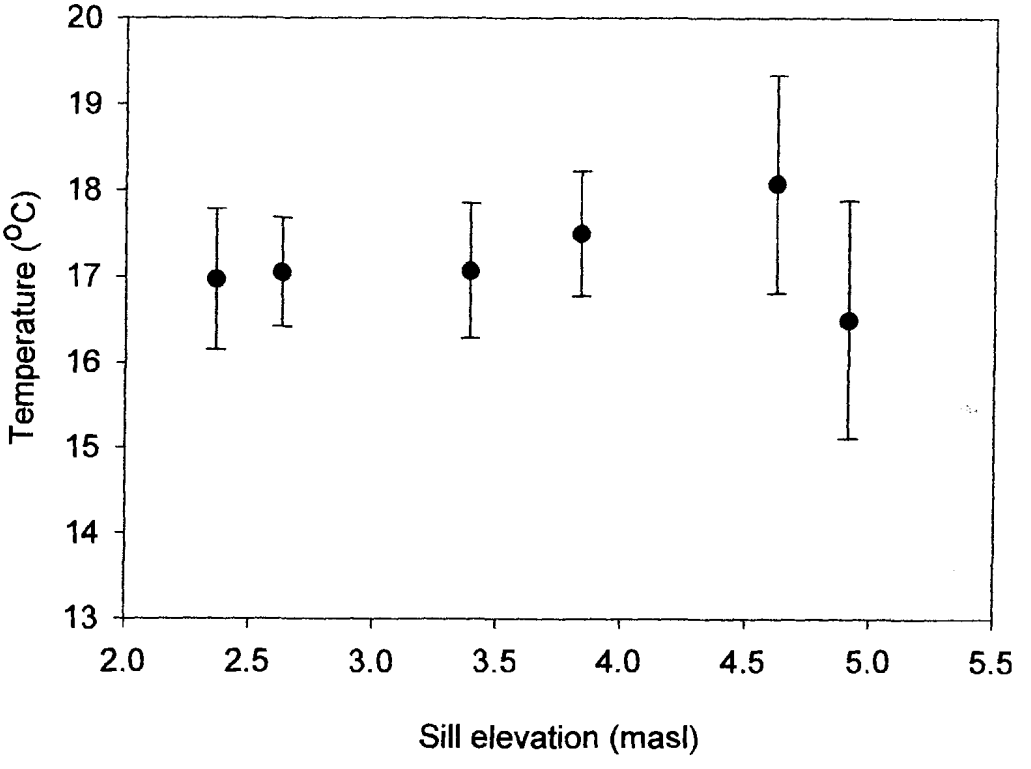

Figure 3.1 Relationship between mid-lake temperature and sill elevation for six lakes near Inuvik. Reported are mean summer values from weekly measurements made from June to August 2003 (n = 7, relationship not statistically significant). Temperature was taken at the same sampling point for each lake at approx. 0.1 m. Lakes were well-mixed and did not appear to stratify. Error bars represent one standard error of the mean.

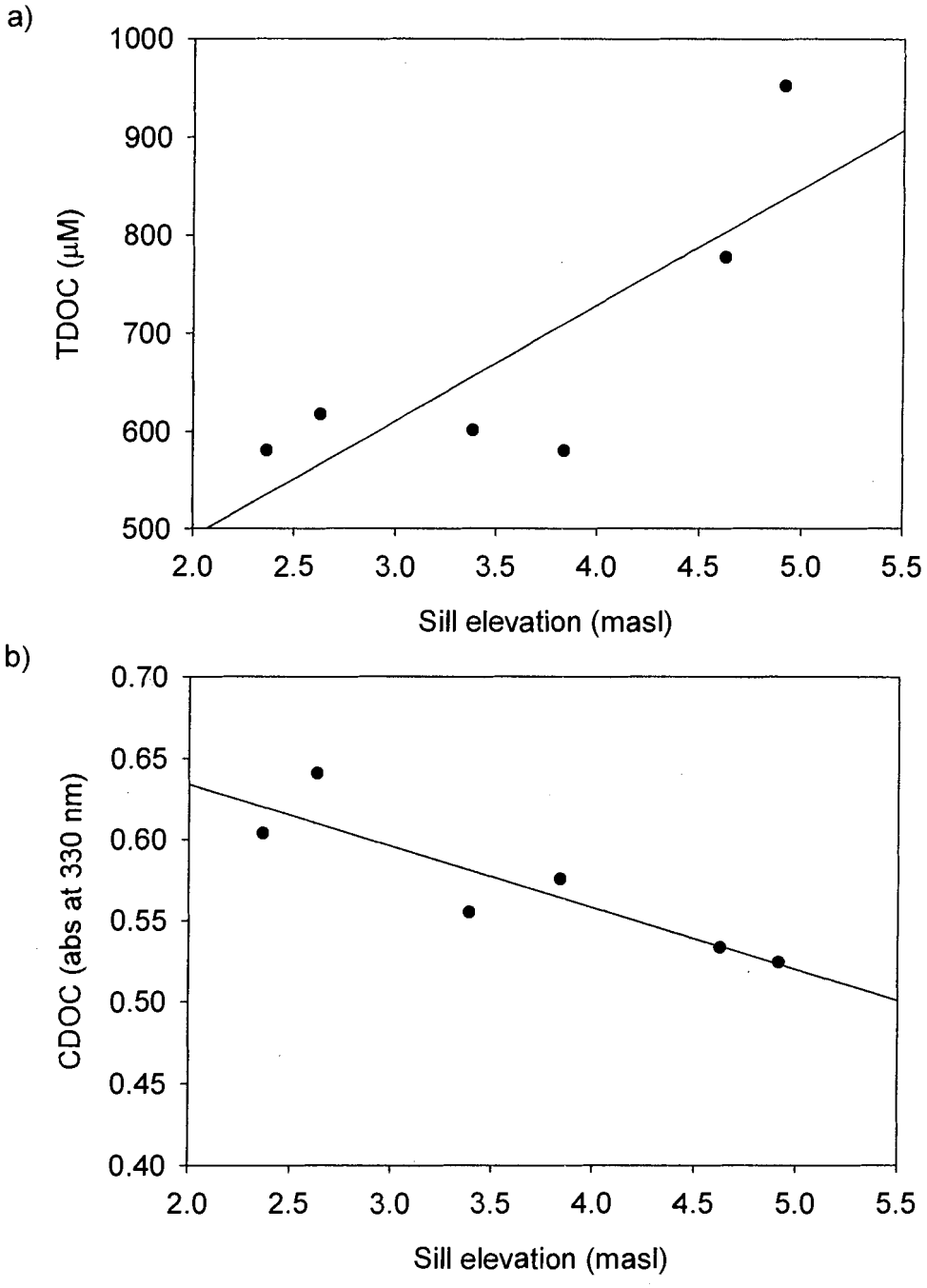

Figure 3.2 Relationship between: a) TDOC concentration (μM), and b) CDOC absorbency (at 330 nm), with sill elevation for six lakes near Inuvik. Reported are mean summer values from integrated mid-lake samples. Weekly measurements were taken between June and August 2003 (n = 7, TDOC R^2 = 0.6623, CDOC R^2 = 0.7946).

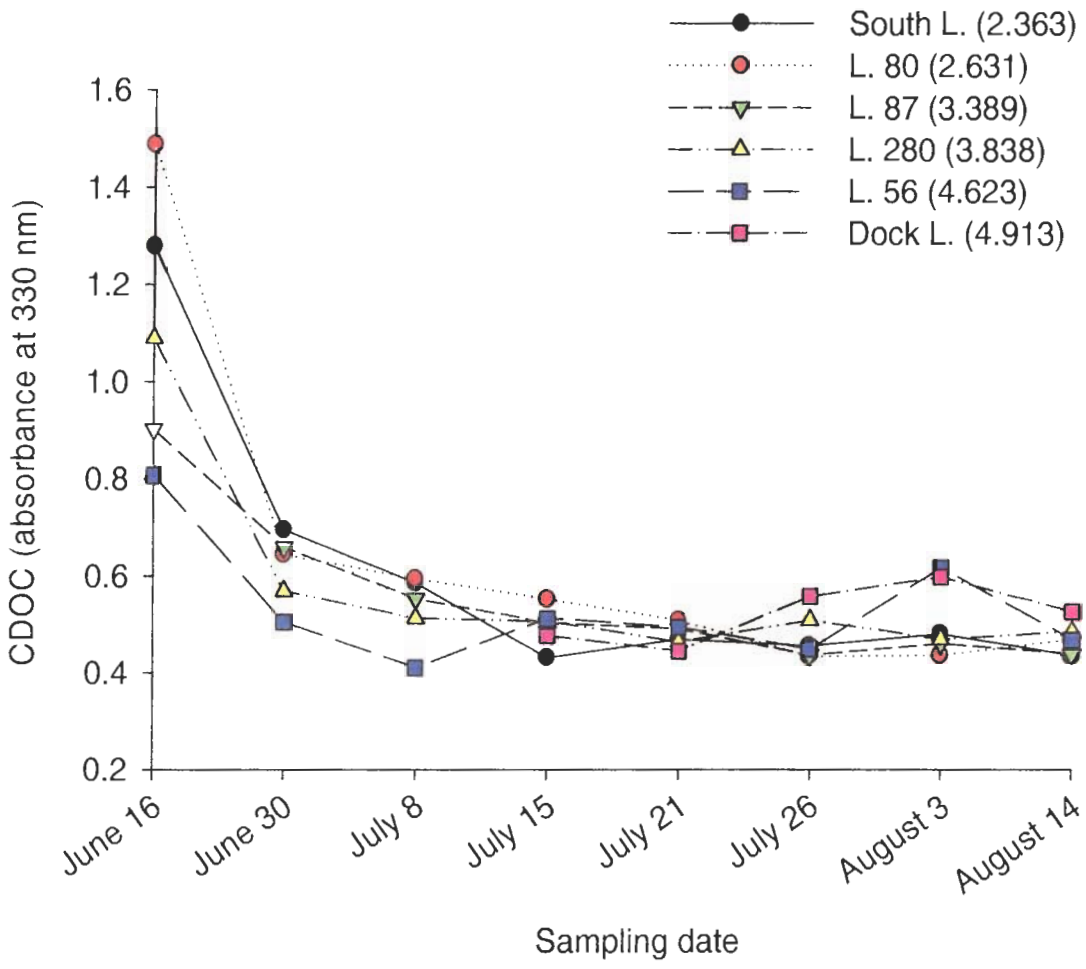

Figure 3.3 Temporal variation in CDOC absorbencies for six lakes near Inuvik (2003). Lakes represent a gradient of lake types from low to high sill elevation (in metres above sea level). Measurements for CDOC absorbencies were taken one week after 2003 flooding for all lakes except Dock Lake, when sampling began on July 15.

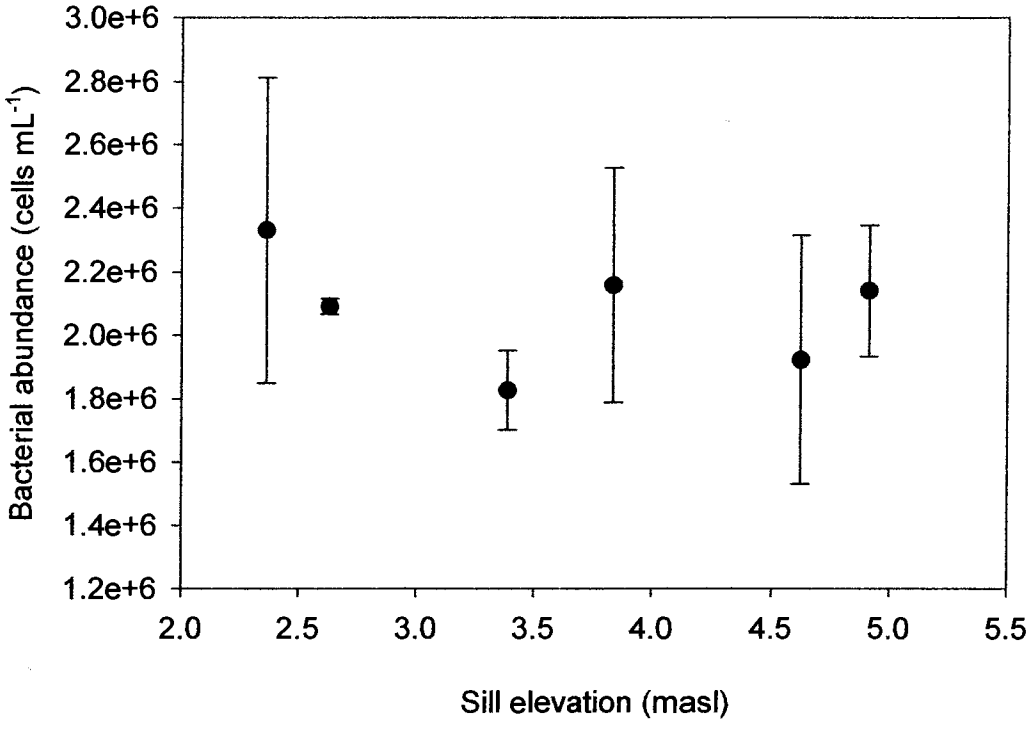

Figure 3.4 Relationship between bacterial abundance and sill elevation for six lakes near Inuvik. Reported are mean summer values from weekly measurements made from June to August 2003 (n = 7, relationship not statistically significant). Water samples for bacterial abundance were taken at the same mid-lake sampling point. Error bars represent one standard error of the mean.

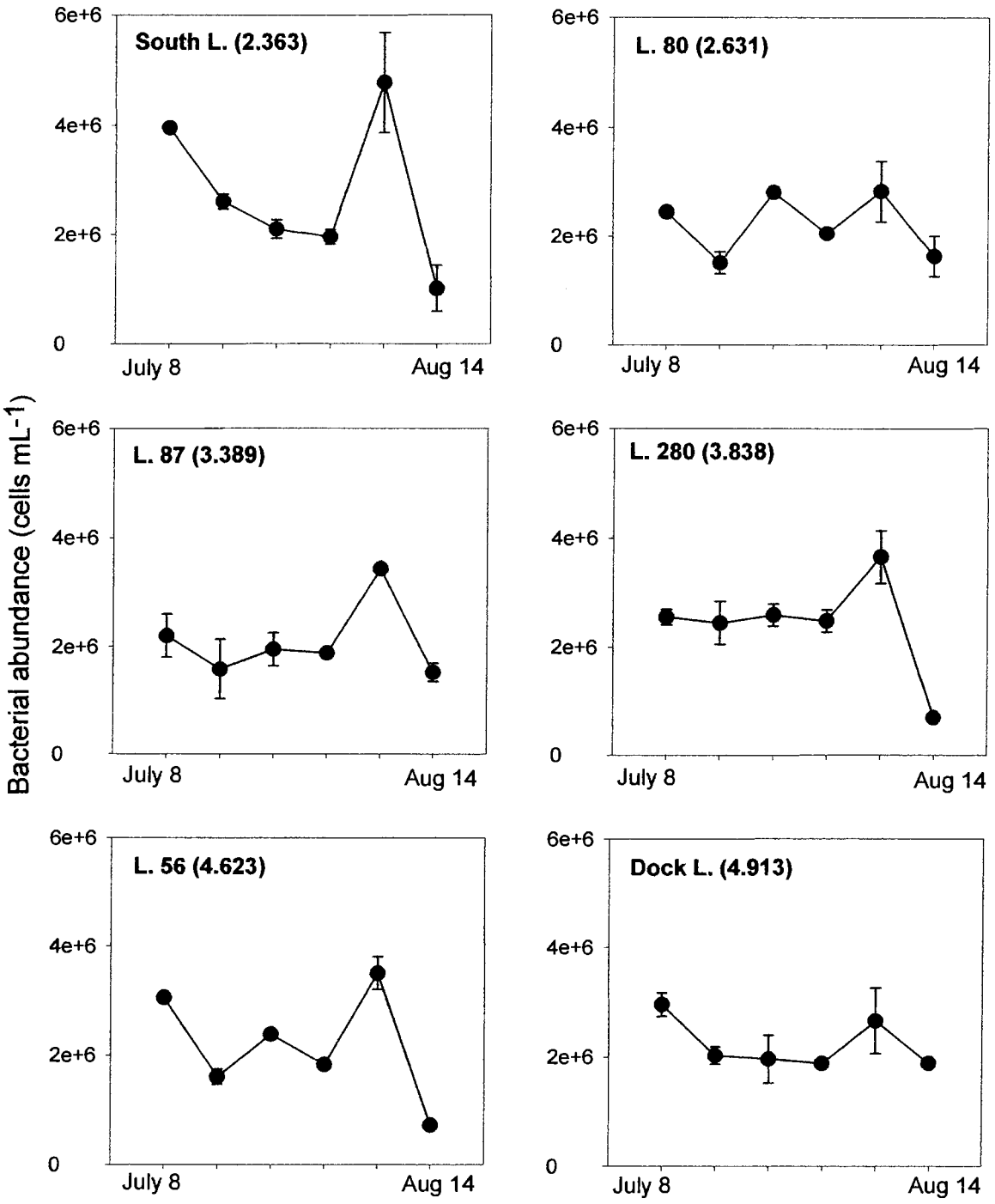

Figure 3.5 Seasonal pattern of bacterial abundance (cells mL^{-1}) among six lakes near Inuvik with sill elevation provided in parentheses (in metres above sea level). Abundances were determined on a weekly basis from July 8 to August 14, 2003 from mid-lake integrated water samples (n=6). Error bars represent one standard error of the mean.

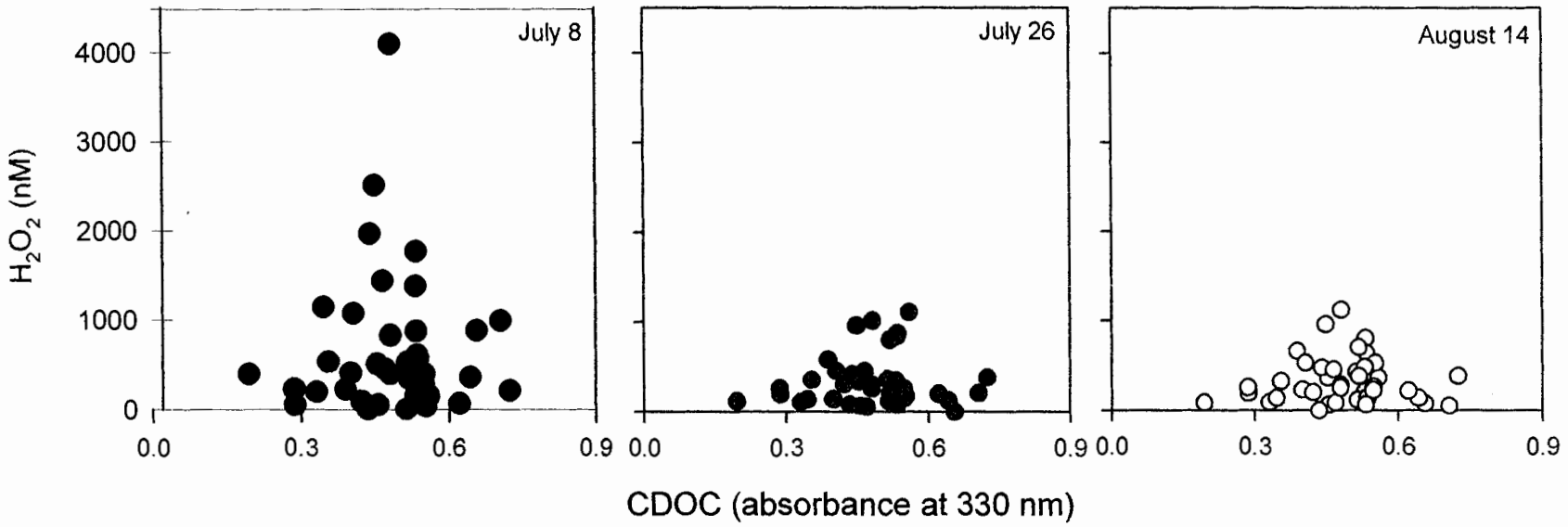

Figure 3.6 Seasonal variation in H_2O_2 concentrations with CDOC absorbency in 40 spatially discrete lakes near Inuvik. Estimates of H_2O_2 concentrations were taken at three different times in 2004. Concentrations were highest shortly after the arctic summer solstice (July 8th), decreased markedly by July 26th and remained stable thereafter.

Lake	Latitude North	Latitude West	Spring sill elevation (masl)	Lake area (km^2)	Depth (m)	[TDOC] (um/L)	Hydrogen peroxide (nM) Near solstice July 8th	July 26th	Aug 14th
15a	68°20.513'	133°48.437'	2.177	4.38	0.68	430	876.93	0.00	75.76
56	68°19.394'	133°50.817'	4.623	0.03	1.08	877	514.10	337.89	359.91
80	68°19.428'	133°52.160'	2.631	0.19	1.52	575	830.25	296.44	278.65
85b	68°19.289'	133°51.747'	2.990	0.02	1.51	584	534.15	116.03	116.03
87	68°19.015'	133°52.460'	3.389	0.04	1.31	580	5.63	357.75	428.17
107	68°18.041'	133°52.404'	2.990	0.17	1.03	612	1772.39	839.55	802.24
111	68°17.964'	133°53.095'	3.671	0.05	1.10	739	2513.69	956.31	956.31
South	68°18.238'	133°51.145'	2.363	0.38	1.29	614	150.74	1108.19	363.51
134	68°18.218'	133°48.047'	4.623	0.03	1.40	806	224.60	581.75	661.11
143	68°17.425'	133°50.205'	5.169	0.02	2.00	817	459.04	53.64	84.82
148a	68°16.928'	133°50.517'	2.631	0.28	0.33	909	32.97	178.38	528.02
181	68°17.298'	133°53.688'	5.169	0.01	1.70	980	989.63	206.90	52.13
184	68°17.773'	133°53.662'	3.671	0.18	0.75	640	4095.74	1010.64	1117.02
278	68°18.672'	133°49.112'	4.077	0.06	0.72	649	409.09	136.36	227.27
287	68°19.145'	133°46.632'	4.077	0.10	1.36	851	89.57	304.62	197.10
301a	68°19.487'	133°47.755'	2.990	0.37	0.95	673	1072.09	451.94	529.46
302b	68°19.492'	133°48.707'	2.631	0.19	0.91	630	535.15	353.33	323.03
521	68°19.033'	133°41.802'	5.169	0.00	0.98	686	396.67	261.54	251.14
522	68°19.257'	133°41.518'	4.913	0.23	1.75	574	404.26	156.73	255.74
527a	68°18.957'	133°43.530'	5.169	0.09	0.97	1029	343.61	800.30	704.43

Table 3.1 Summary of variables for nineteen lakes near Inuvik. Data presented here were used in multiple regression analyses carried out on hydrogen peroxide levels.

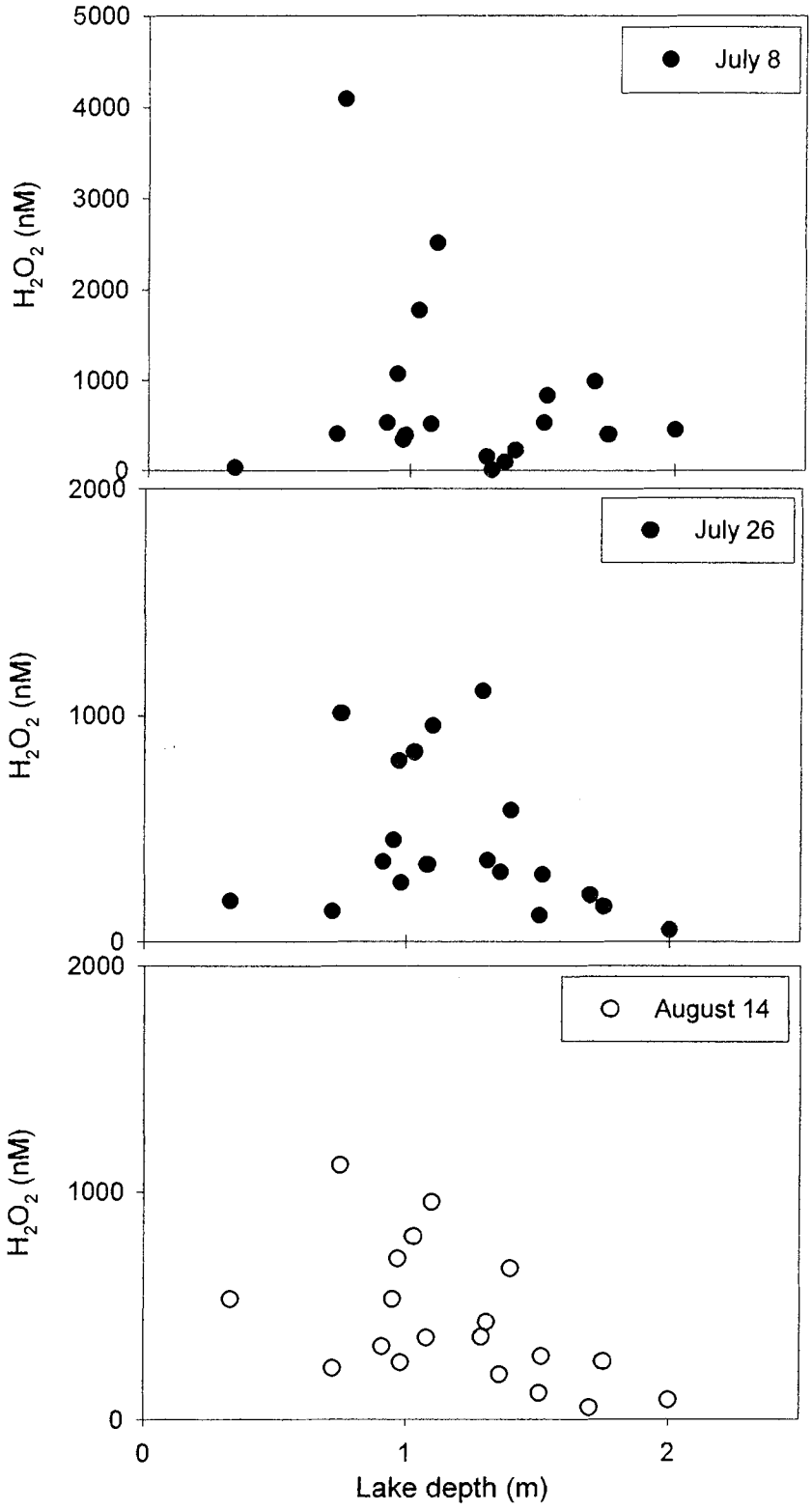

Figure 3.7 Scatterplot of H_2O_2 concentration with lake depth for 19 lakes near Inuvik (summer 2004).

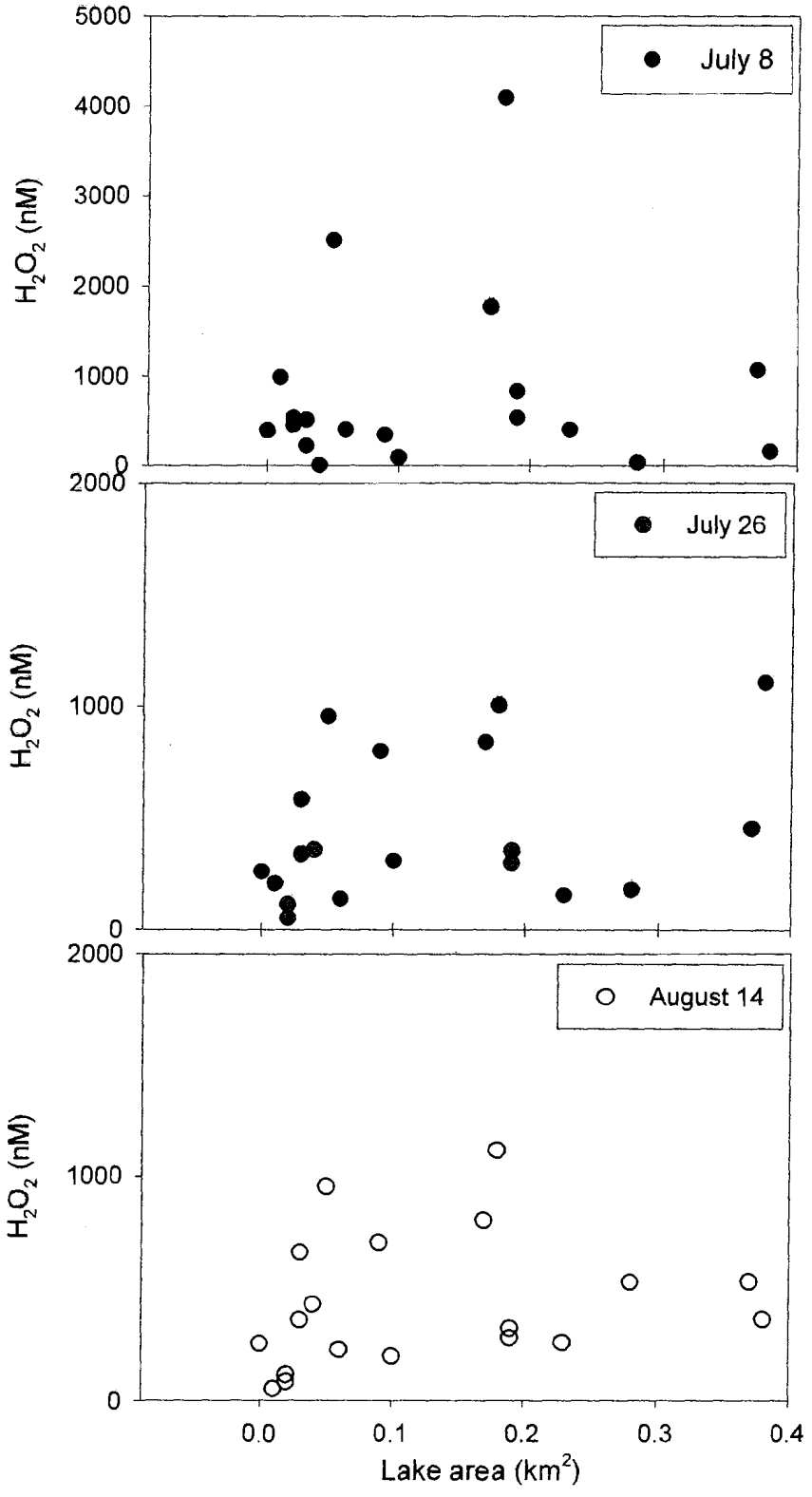

Figure 3.8 Scatterplot of H_2O_2 concentration with lake area for 19 lakes near Inuvik (summer 2004).

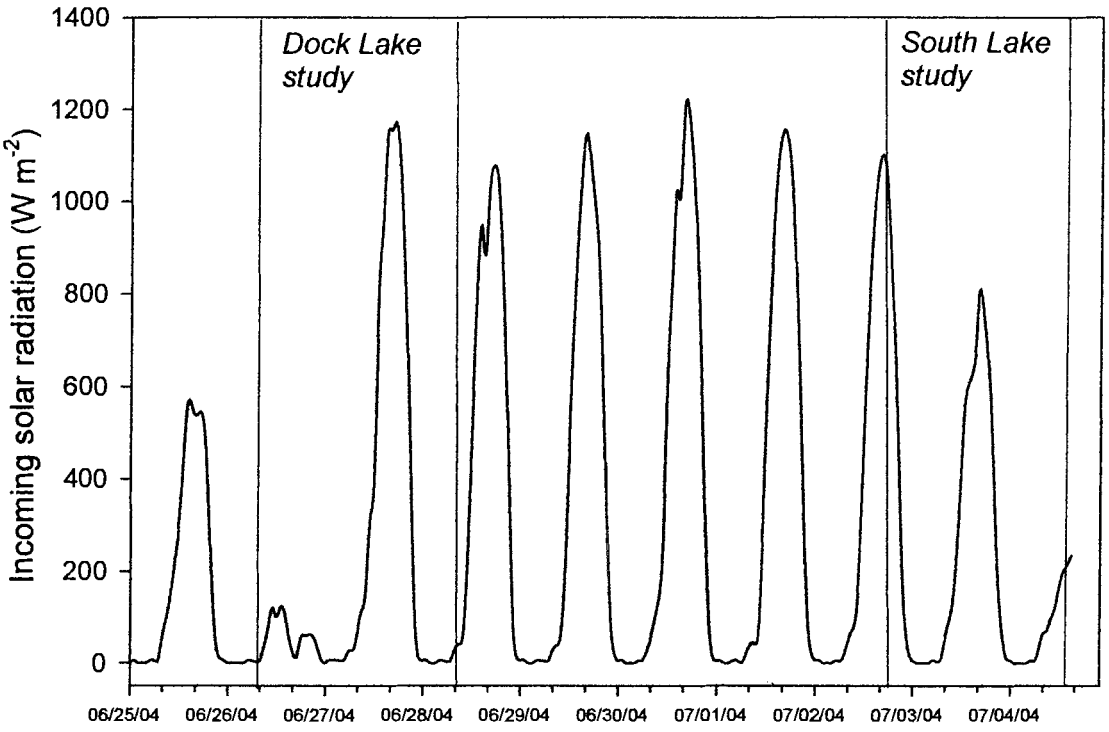

Figure 3.9 Incoming solar radiation (measured as Watts m^{-2}) taken at the Inuvik Research Centre from July 25th to July 4th, 2004. Diurnal variability studies were conducted on Dock Lake from June 26th to June 28th and on South Lake from July 2nd to 4th. Extremely cloudy weather preceded the Dock Lake experiment (July 27th). The following days were reasonably cloudless and constant leading into the South Lake experiment (July 2nd). Irradiation was reduced on July 4th by the onset of haze due to a regional forest fire.

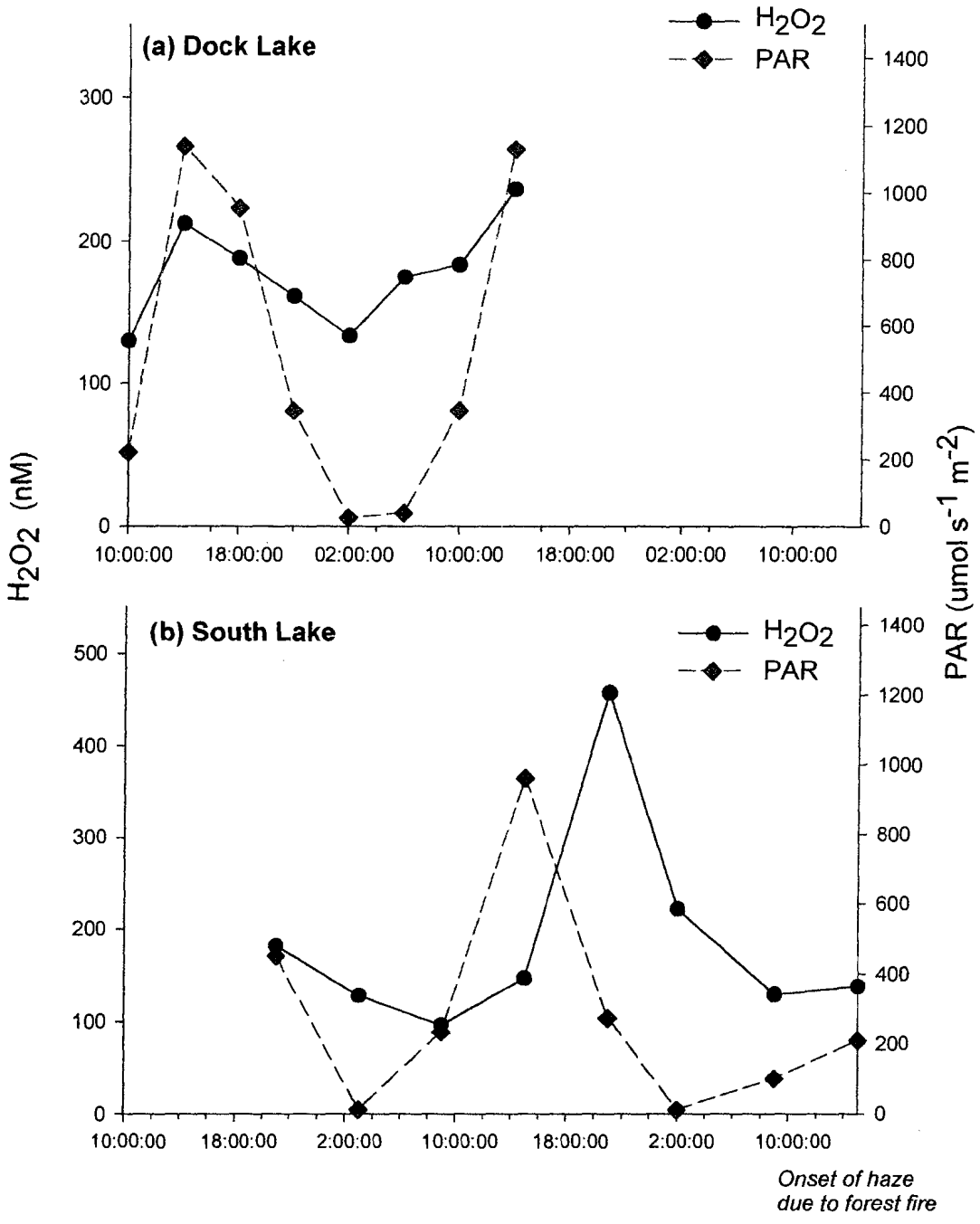

Figure 3.10 Diurnal variations in H_2O_2 concentrations and photosynthetically active radiation (PAR) at: (a) Dock Lake (June 26th to 28th) and (b) South Lake (July 2nd to 4th). H_2O_2 concentrations tracked solar intensity and PAR with peak concentrations at mid-afternoon, just after Solar Noon (15:00:00) and reached minimum concentrations during the night.

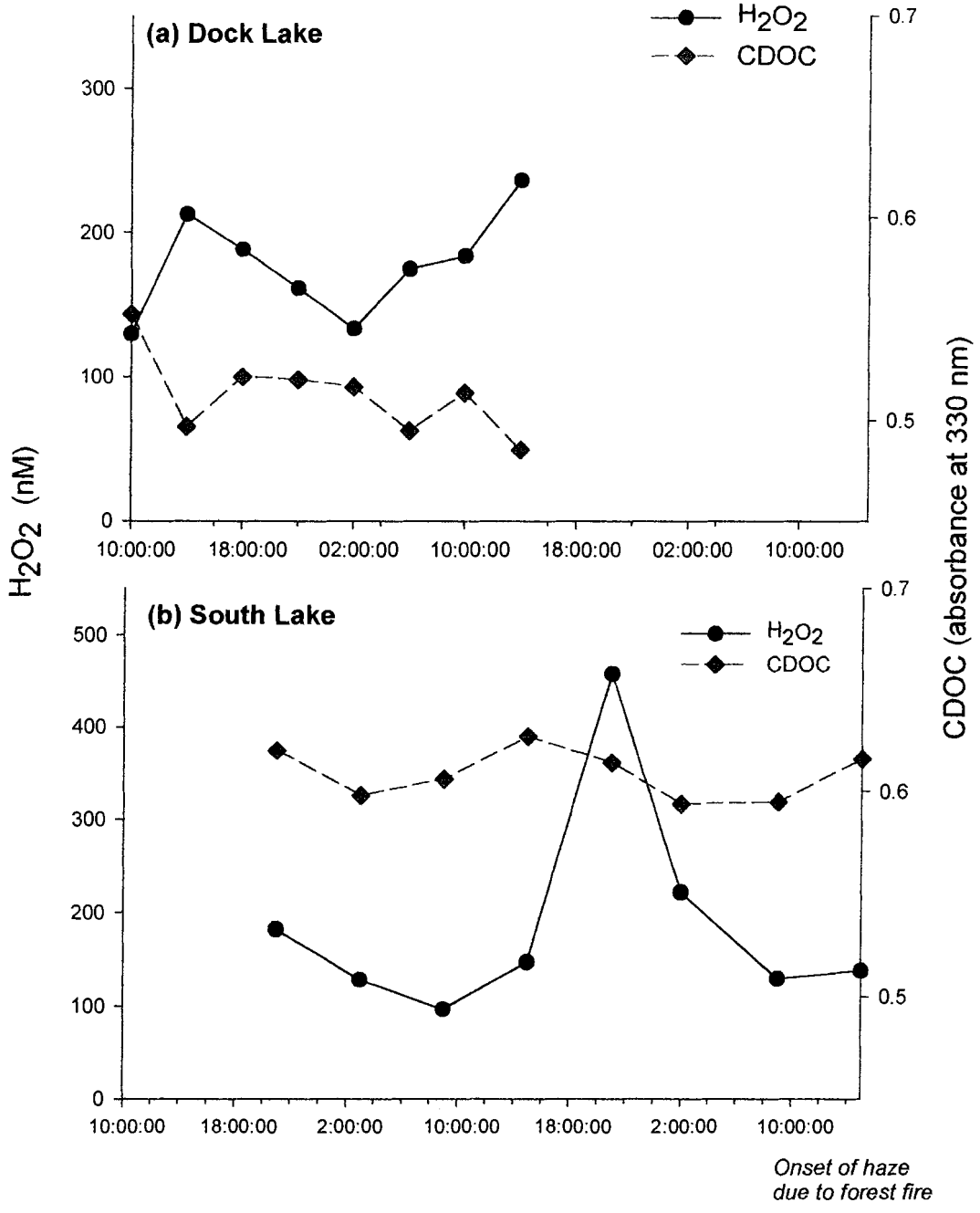

Figure 3.11 Diurnal variations in H_2O_2 concentrations and CDOC absorbency (at 330 nm) at: (a) Dock Lake (June 26th to 28th) and (b) South Lake (July 2nd to 4th). H_2O_2 concentrations exhibit diurnal variation with solar intensity in both lakes however patterns of CDOC depletion is most pronouced at Dock Lake, whereas CDOC depletion is likely replaced by CDOC brought in from river water at South Lake.

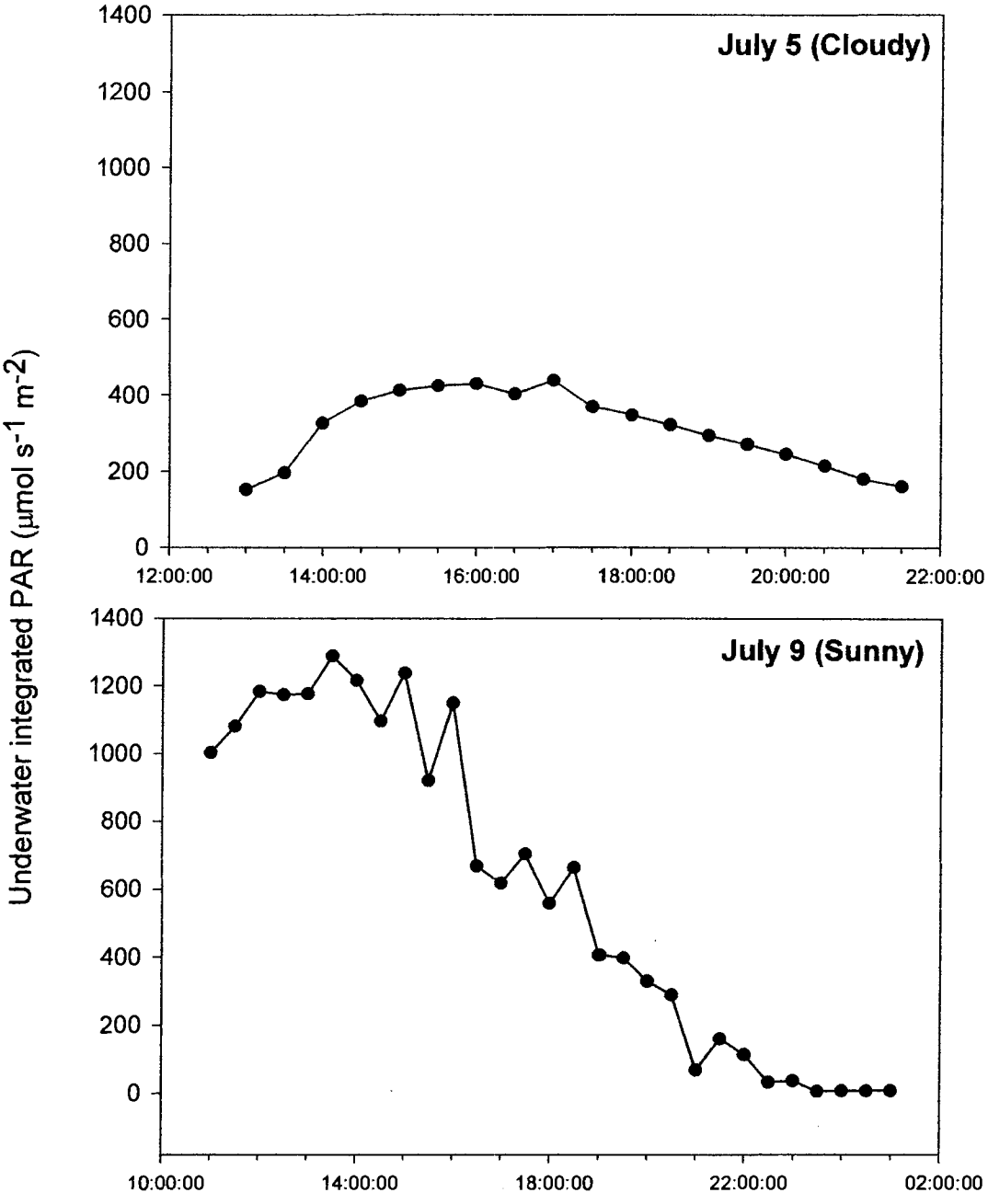

Figure 3.12 Underwater integrated photosynthetically active radiation (PAR) conditions for outdoor incubator experiments conducted on July 5th (cloudy conditions) and July 9th, 2004 (sunny conditions). PAR measurements were taken just below the surface of the water inside the outdoor microcosm. Integrated measurements were averaged for every half-hour interval.

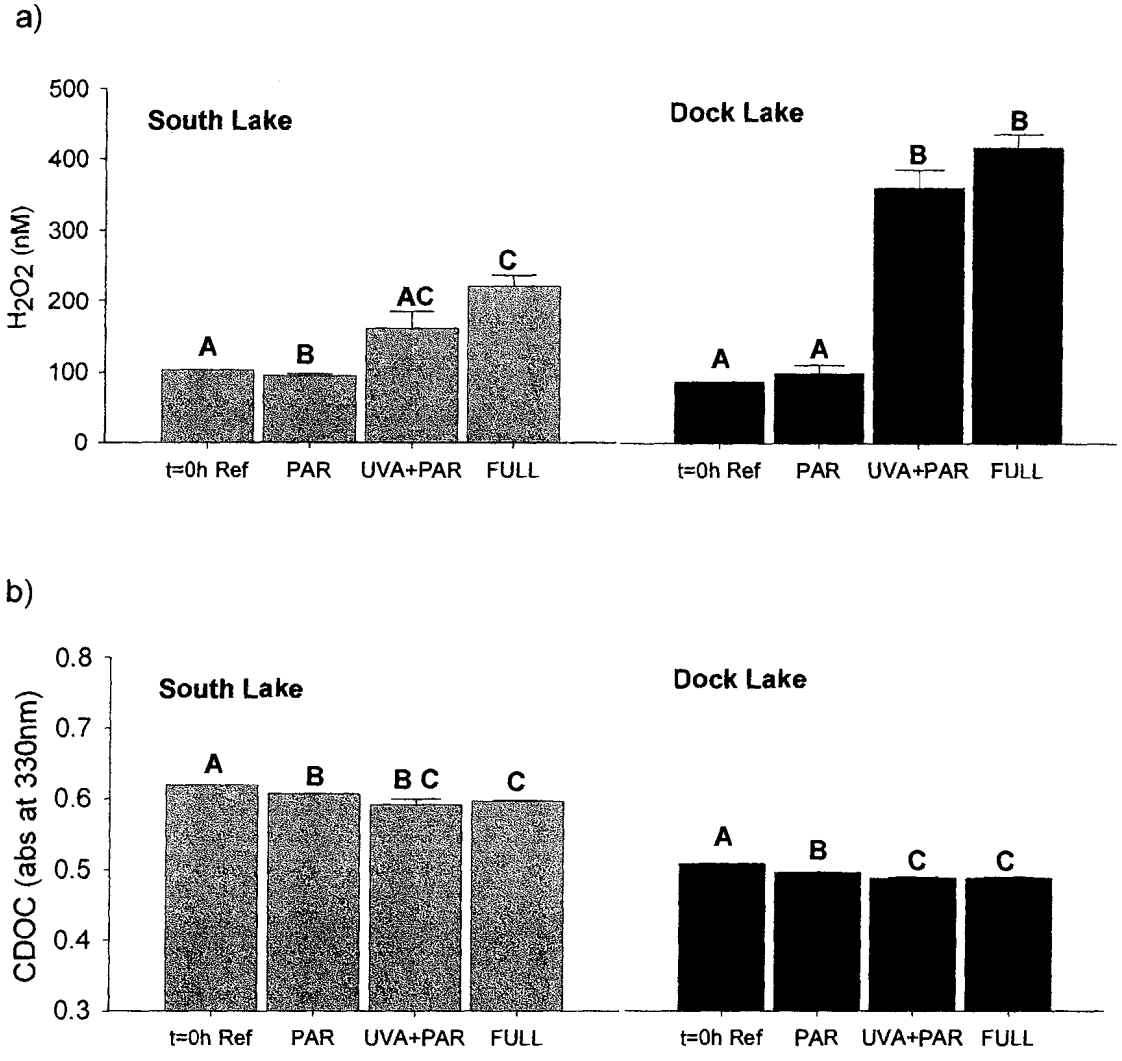

Figure 3.13 Results from the outdoor incubator experiment under cloudy conditions, carried out on July 5th showing: (a) final H_2O_2concentrations and (b) average CDOC absorbencies for microcosms in each treatment after 12 hours of irradiation. Treatments were: PAR only (OP-2), UVA+PAR (Mylar) and Full sunlight (UVA+UVB+PAR). Treatments were compared to the 0 hour Reference. Error bars are one standard error of the mean. Significant differences are indicated by different letters ($n = 3$, $\alpha = 0.05$).

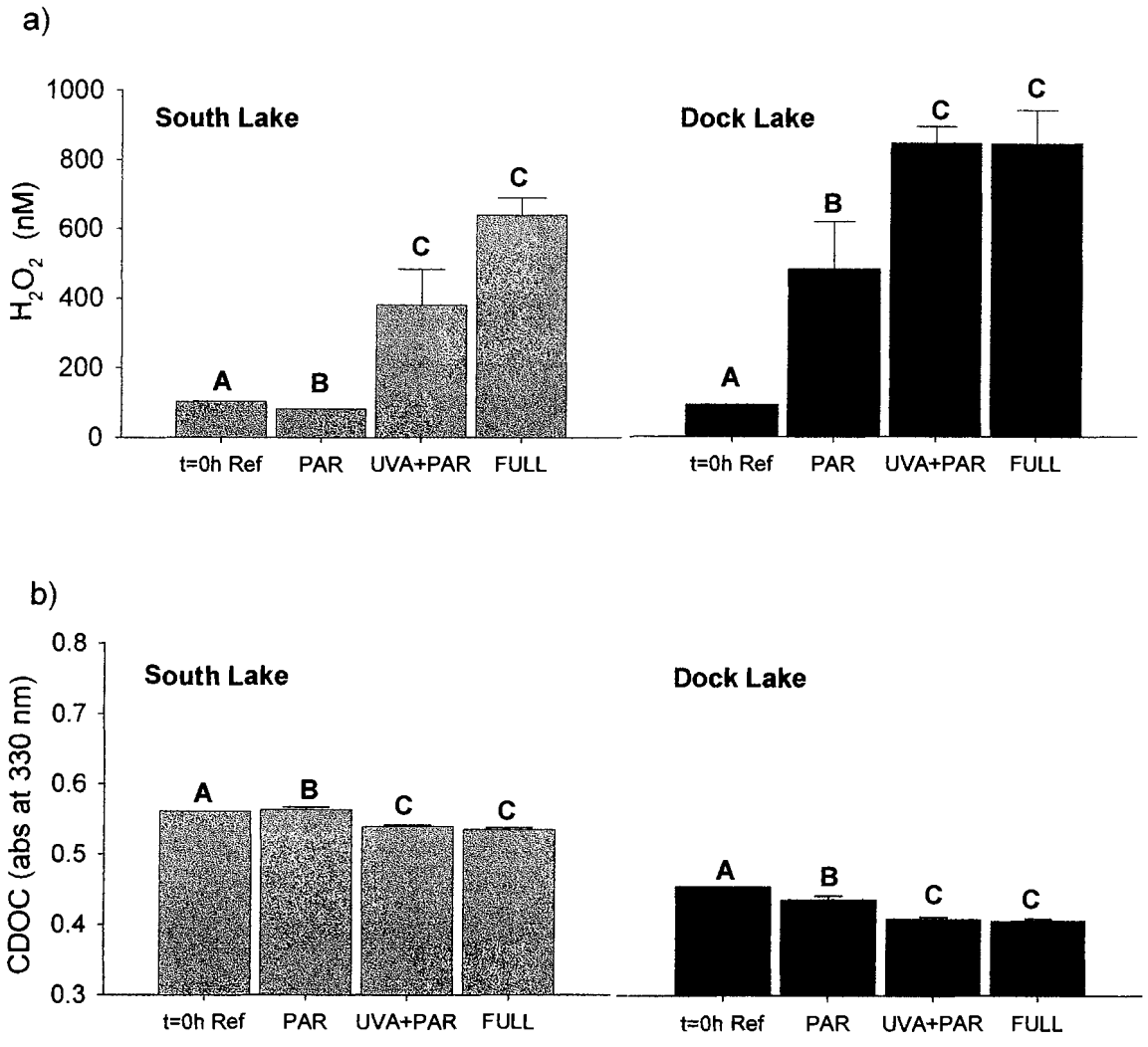

Figure 3.14 Results from the outdoor incubator experiment under sunny conditions, carried out on July 9th showing: (a) final H_2O_2 concentrations and (b) average CDOC absorbencies for microcosms in each treatment after 15 hours of irradiation.Treatments were: PAR only (OP-2), UVA+PAR (Mylar) and full sunlight (UVA+UVB+PAR). Treatments were compared to the 0 hour Reference. Error bars are one standard error of the mean. Significant differences are indicated by different letters (n = 3, α = 0.05).

a)

H_2O_2	South Lake			Dock Lake		
Treatments	**df**	**F**	***p***	**df**	**F**	***p***
Reference*PAR	1	9.1363	***0.0391***	1	0.9709	0.3802
Reference*UVA+PAR	1	6.0955	0.0690	1	114.0129	***0.0004***
Reference*Full sunlight	1	58.0352	***0.0016***	1	298.4466	***<0.0001***
PAR*UVA+PAR	1	7.7649	***0.0495***	1	85.5356	***0.0008***
PAR*Full sunlight	1	64.2547	***0.0013***	1	198.4453	***0.0001***
UVA+PAR*Full sunlight	1	4.4170	0.1035	1	3.0384	0.1563

b)

CDOC	South Lake			Dock Lake		
Treatments	**df**	**F**	***p***	**df**	**F**	***p***
Reference*PAR	1	51.5714	***0.0020***	1	588.0000	***<0.0001***
Reference*UVA+PAR	1	10.5471	***0.0314***	1	196.0000	***0.0002***
Reference*Full sunlight	1	226.7143	***0.0001***	1	226.7143	***0.0001***
PAR*UVA+PAR	1	3.0359	0.1564	1	28.0000	***0.0061***
PAR*Full sunlight	1	19.6122	***0.0114***	1	30.3750	***0.0053***
UVA+PAR*Full sunlight	1	0.3261	0.5985	1	0.0217	0.8899

Table 3.2 One-way ANOVA results for H_2O_2 outdoor incubator experiment under cloudy conditions (July 5th, 2004). Results show differences for a) H_2O_2 concentrations, and, b) CDOC absorbencies, between UV treatments (OP2, Mylar and Full sunlight) compared to the Reference at 0 hours. Significant differences are identified in bold ($\alpha = 0.05$).

a)

H_2O_2	South Lake			Dock Lake		
Treatments	**df**	**F**	***p***	**df**	**F**	***p***
Reference*PAR	1	1.73E+13	***<0.0001***	1	9.3249	***0.0379***
Reference*UVA+PAR	1	8.7990	***0.0413***	1	262.9256	***<0.0001***
Reference*Full sunlight	1	124.6951	***0.0004***	1	63.0392	***0.0014***
PAR*UVA+PAR	1	8.4580	***0.0438***	1	6.2418	0.0669
PAR*Full sunlight	1	122.0639	***0.0004***	1	4.5742	0.0992
UVA+PAR*Full sunlight	1	5.1172	0.0865	1	0.0005	0.9831

b)

CDOC	South Lake			Dock Lake		
Treatments	**df**	**F**	***p***	**df**	**F**	***p***
Reference*PAR	1	18.9055	0.0122	1	13.0000	***0.0226***
Reference*UVA+PAR	1	560.3333	***<0.0001***	1	320.3333	***<0.0001***
Reference*Full sunlight	1	366.4490	***<0.0001***	1	322.5806	***<0.0001***
PAR*UVA+PAR	1	35.5584	***0.0040***	1	20.2500	***0.0108***
PAR*Full sunlight	1	41.0511	***0.0030***	1	25.1419	***0.0074***
UVA+PAR*Full sunlight	1	1.5921	0.2756	1	0.8448	0.4100

Table 3.3 One-way ANOVA results for H_2O_2 outdoor incubator experiment under sunny conditions (July 9th, 2003). Results show differences for a) H_2O_2 concentrations, and, b) CDOC absorbencies, between UV treatments (OP2, Mylar and Full sunlight) compared to the Reference at 0 hours. Significant differences are identified in bold ($\alpha = 0.05$).

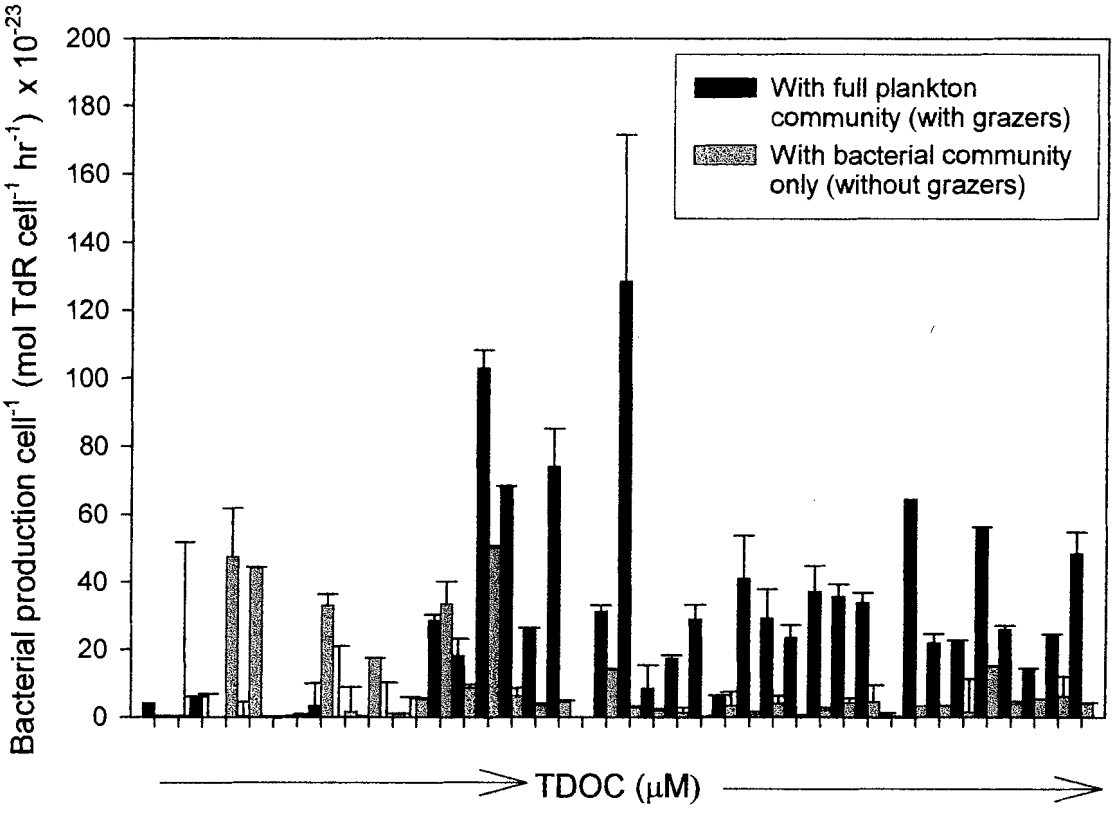

Figure 3.15 Bacterial production cell^{-1} for forty lakes near Inuvik on August 4th, 2003. Results show bacterial production cell^{-1} in the presence and absence of grazers across a gradient of TDOC concentrations. Error bars show one standard error of the mean.

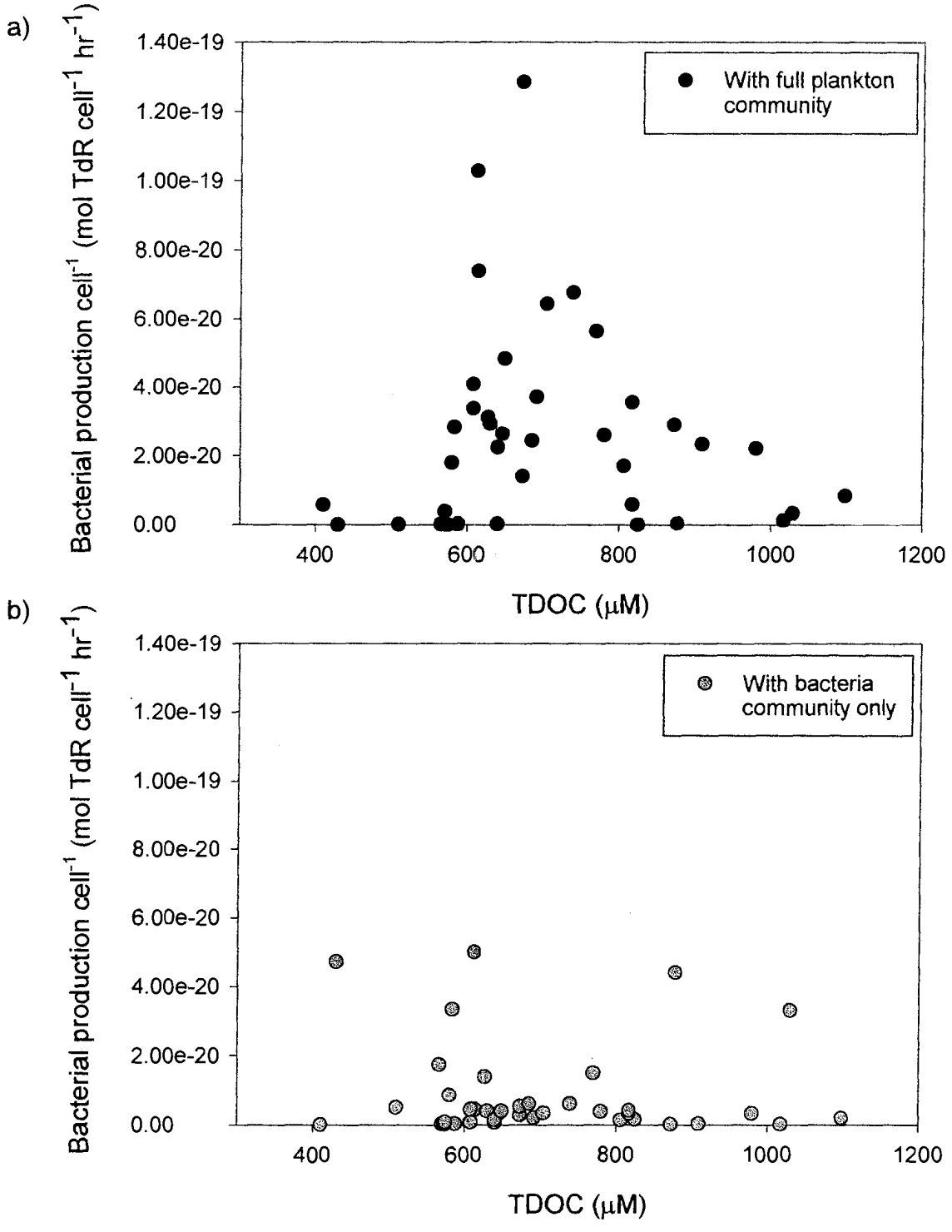

Figure 3.16 Patterns in bacterial production cell^{-1} for forty lakes near Inuvik with DOC concentration. Reported are rates of bacterial production: a) in the presence of the full plankton community, and, b) with the bacteria community only, from one survey conducted in August 2003 (no significant statistical relationship determined).

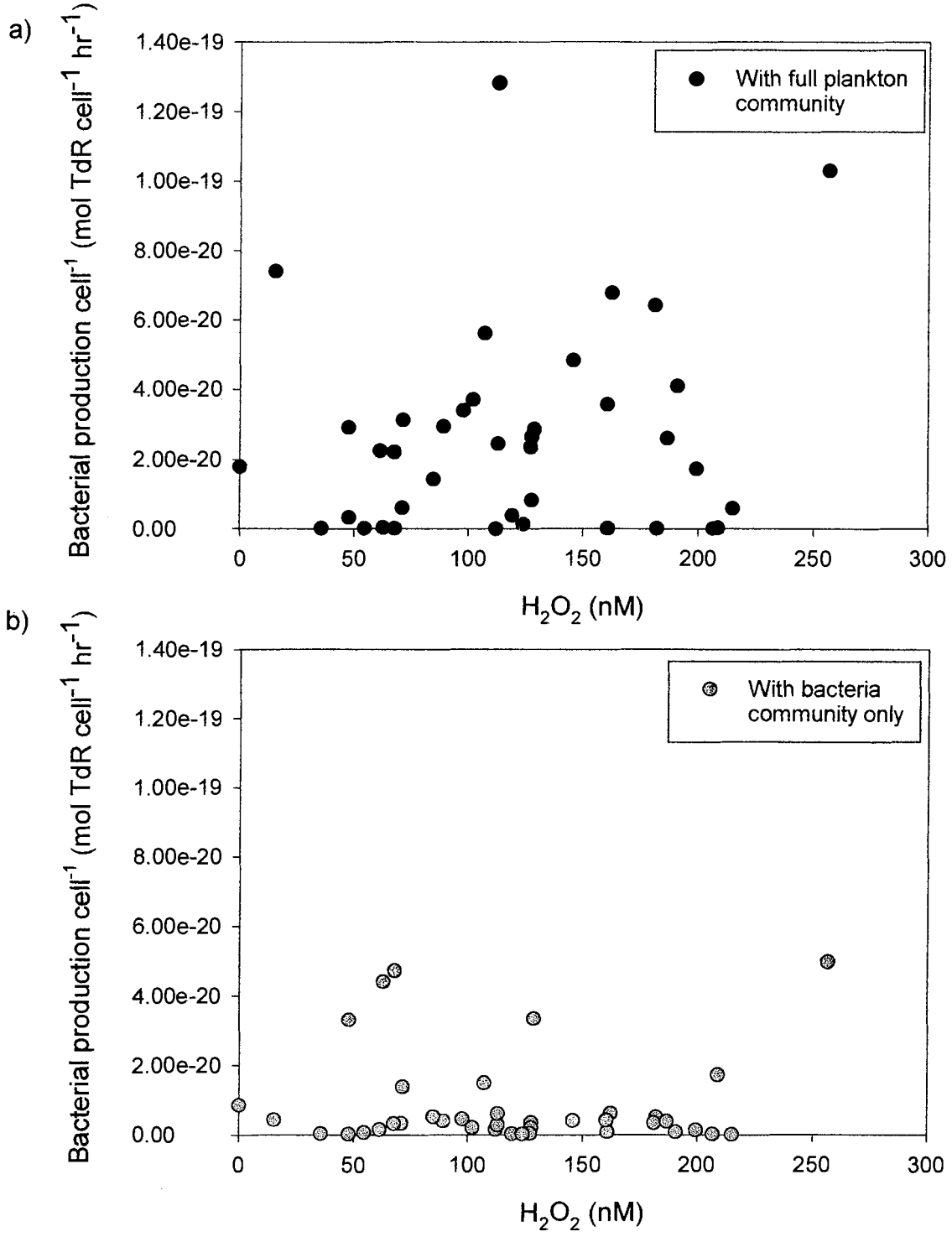

Figure 3.17 Patterns in bacterial production cell^{-1} for forty lakes near Inuvik with H_2O_2 concentration. Reported are rates of bacterial production: a) in the presence of the full plankton community, and, b) with the bacteria community only, from one survey conducted in August 2003 (no significant statistical relationship determined).

Lake No.	Sill elevation (masl)	CDOC (abs at 330nm)	TDOC (µM)
South	2.363	0.48	599
80	2.631	0.44	553
87	3.389	0.46	577
280	3.838	0.47	776
56	4.623	0.62	877
Dock	4.913	0.60	1020
Weather conditions:		Clear, cloudless, sunny	
Average water temperature:		14 ° C	
Average conductivity:		0.211 µohms/s	

Table 3.4 Summary of conditions at six lakes used in bacterial production *with* grazers experiment (August 3rd, 2003)

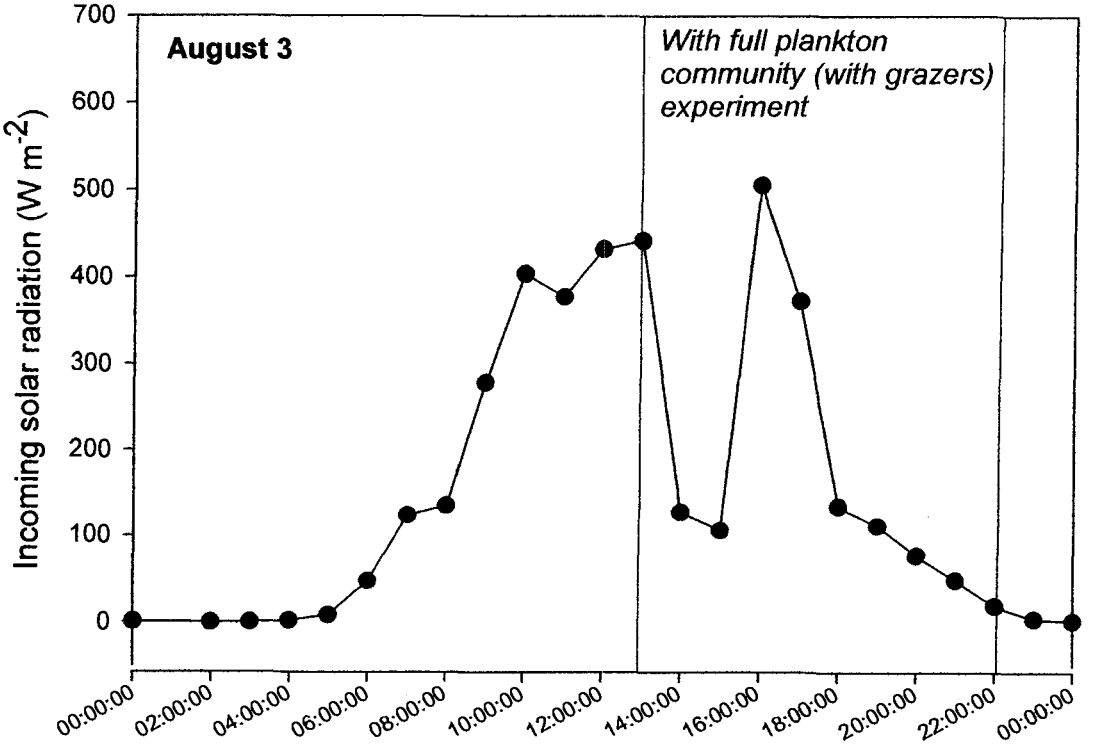

Figure 3.18 Incoming solar radiation for August 3rd, 2003 at the Inuvik Research Centre. Bacterial production experiment with the full plankton community (with grazers) was conducted between 13:00:00 and 22:00:00. Heavy cloud cover during the early part of the experiment was observed. No PAR data were available for this experiment (due to mechanical error).

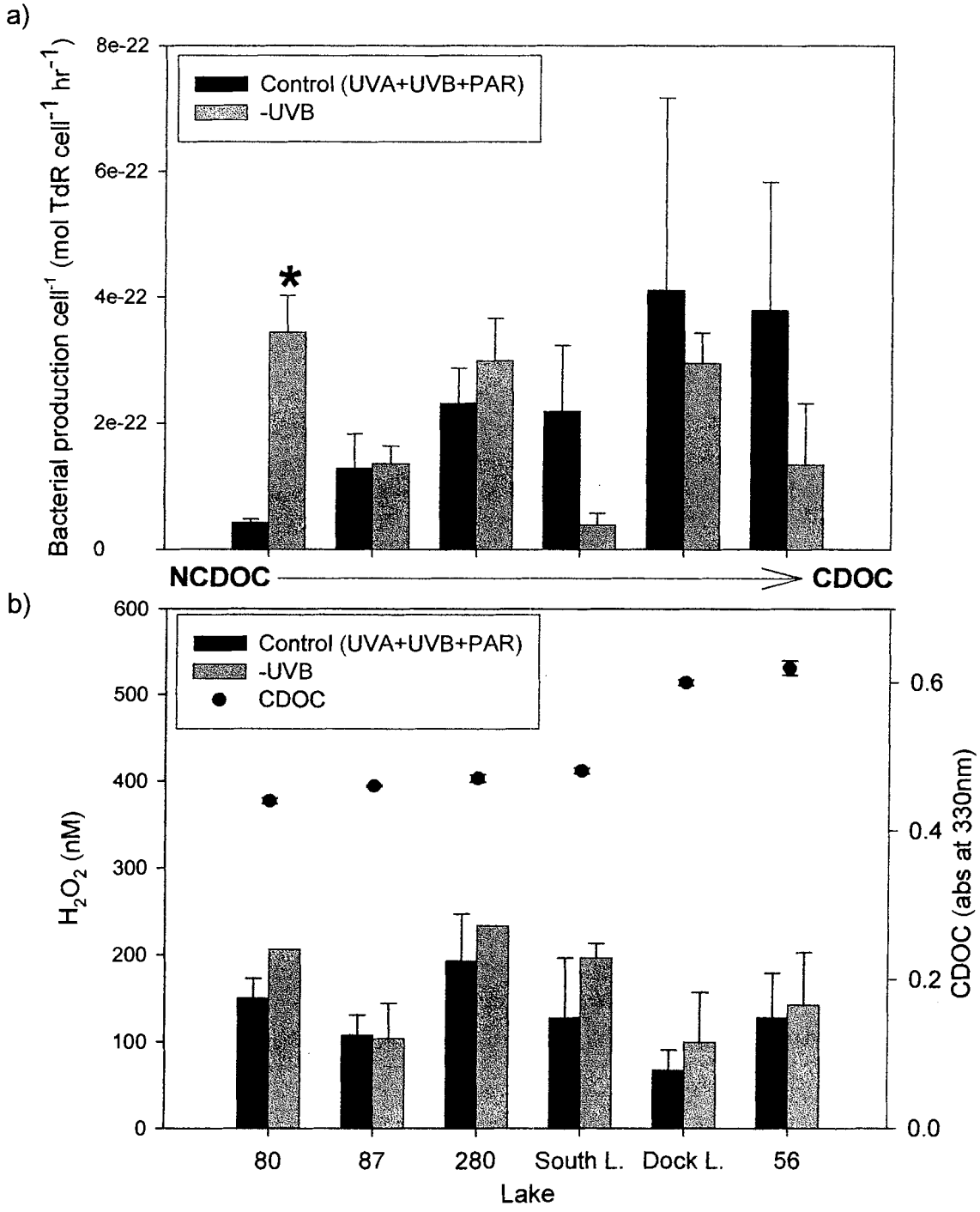

Figure 3.19 Results from bacterial production experiment *with* grazers, carried out on August 3rd, 2003. Results show: a) bacterial production cell^{-1} and b) final H_2O_2 concentrations and CDOC absorbencies under full sunlight (Control) and with UVB removed (-UVB, using Mylar). Significant differences between treatment (-UVB) and Control are marked by an asterisk (*, n=3, α = 0.05). Error bars represent one standard error of the mean.

a)

	Bacterial Production		
Lake	**df**	**F**	***p***
South	1	2.835	0.168
80	1	25.913	***0.007***
87	1	0.015	0.908
280	1	0.609	0.479
56	1	1.180	0.339
Dock	1	0.142	0.726

b)

	H_2O_2		
Lake	**df**	**F**	***p***
South	1	0.959	0.383
80	1	6.177	0.068
87	1	0.008	0.935
280	1	0.550	0.500
56	1	0.035	0.860
Dock	1	0.272	0.629

Table 3.5 Results from t-tests for a) bacterial production $cell^{-1}$ and b) H_2O_2 concentration between control and -UVB treatments in bacterial production *with* grazers experiment (August 3rd, 2003). Significant differences are in bold ($\alpha = 0.05$).

Lake No.	Sill elevation (masl)	CDOC (abs at 330nm)	TDOC (µM)
South	2.363	0.44	570
80	2.631	0.47	558
87	3.389	0.44	549
280	3.838	0.49	796
56	4.623	0.47	852
Dock	4.913	0.53	1060
Weather conditions:		Foggy	
Average water temperature:		11 °C	
Average conductivity:		0.141 µohms/s	

Table 3.6 Summary of conditions at six lakes used in bacterial production *without* grazers experiment (August 14th, 2003)

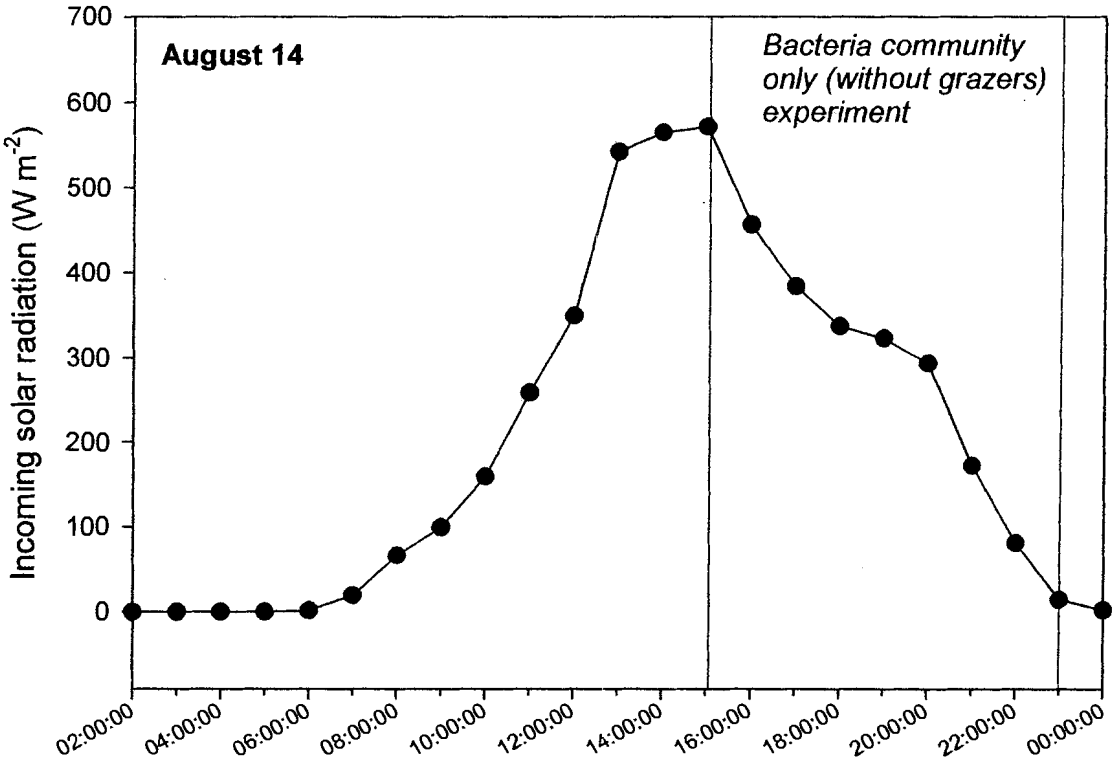

Figure 3.20 Incoming solar radiation for August 14th, 2003 at the Inuvik Research Centre. Bacterial production experiment *without* grazers was conducted between 15:00:00 and 23:00:00. No PAR data were available for this experiment (due to mechanical error).

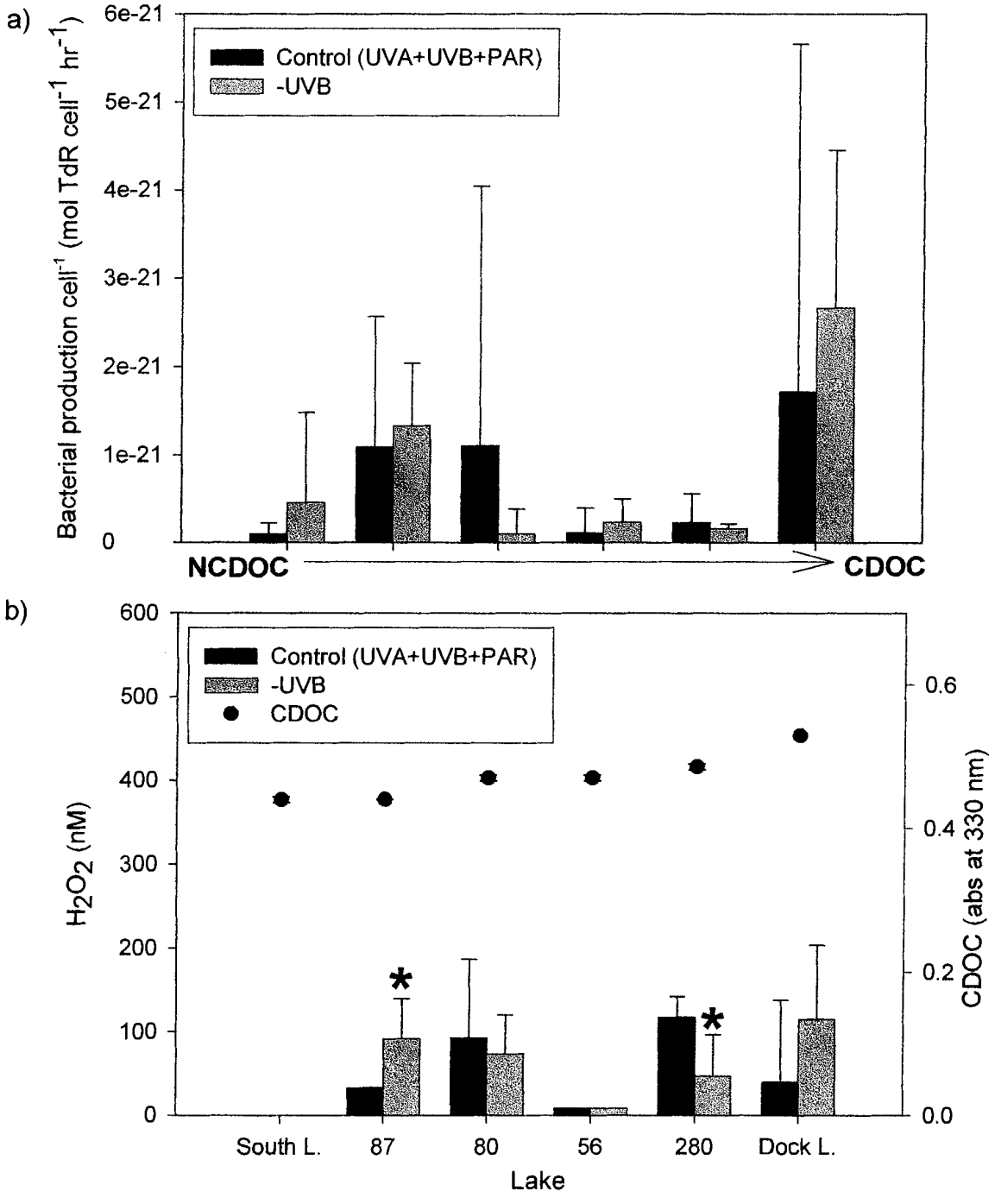

Figure 3.21 Results from bacterial production experiment with the bacteria community only (*without* grazers), carried out on August 14th, 2003. Results show: a) bacterial production cell^{-1} and b) final H_2O_2 concentrations and CDOC absorbencies under full sunlight (Control) and with UVB removed (-UVB). Significant differences between treatment (-UVB) and Control are marked by an asterisk (*, n=3, α = 0.05). Error bars represent one standard error of the mean.

a)

	Bacterial Production		
Lake	**df**	**F**	***p***
South	1	2.238	0.209
80	1	2.154	0.216
87	1	0.413	0.555
280	1	1.385	0.305
56	1	0.764	0.432
Dock	1	0.891	0.399

b)

		H_2O_2	
Lake	**df**	**F**	***p***
South	1	n/a	n/a
80	1	0.600	0.482
87	1	27.009	***0.007***
280	1	n/a	n/a
56	1	23.391	***0.006***
Dock	1	5.984	0.071

Table 3.7 Results from t-tests for a) bacterial production $cell^{-1}$ and b) H_2O_2 concentration between control and -UVB treatments in bacterial production *without* grazers experiment (August 14th, 2003). Significant differences are in bold ($\alpha = 0.05$).

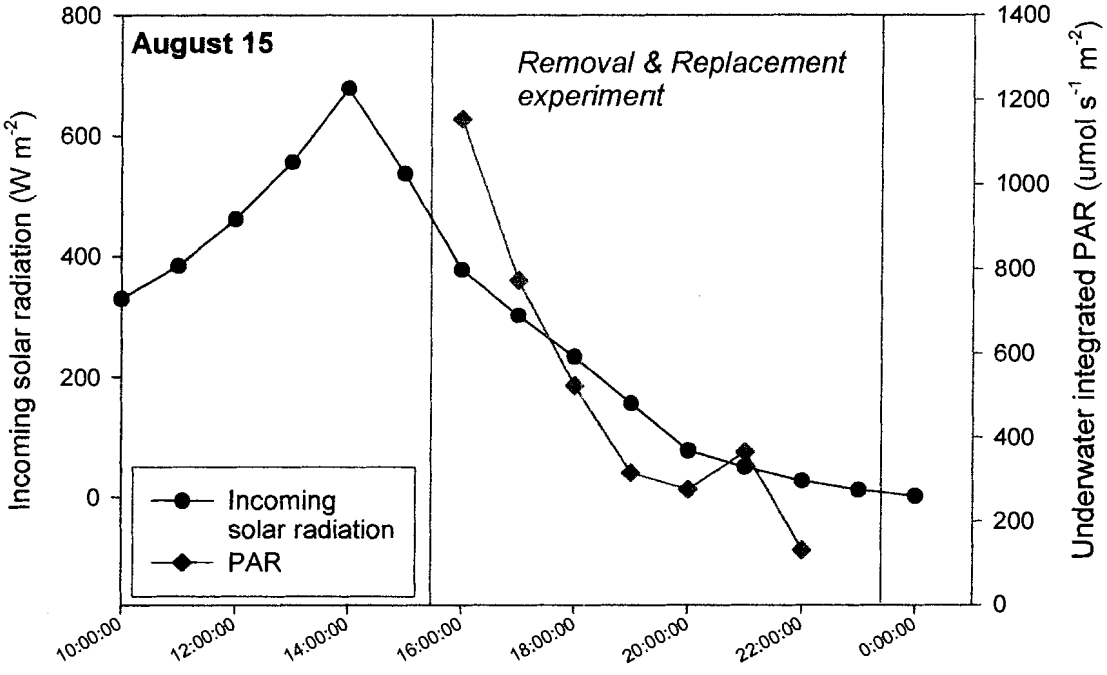

Figure 3.22 Incoming solar radiation and underwater integrated PAR in outdoor incubator on August 15th, 2003 at the Inuvik Research Centre. The 'Removal & Replacement' experiment was conducted between 13:30:00 and 23:30:00. Integrated PAR measurements were taken just below the surface of the water inside the outdoor microcosm.

Lake No.	Sill elevation (masl)	CDOC (abs @ 330nm)	TDOC (µM)
South	2.363	0.402	570
280	3.838	0.428	796
Dock	4.913	0.5265	1060
	Weather conditions:	Foggy, few sunny breaks	
	Average water temperature:	11 °C	
	Average conductivity:	0.250 µohms/s	

Table 3.8 Summary of conditions at lakes used in removal & replacement experiment (August 15th, 2003)

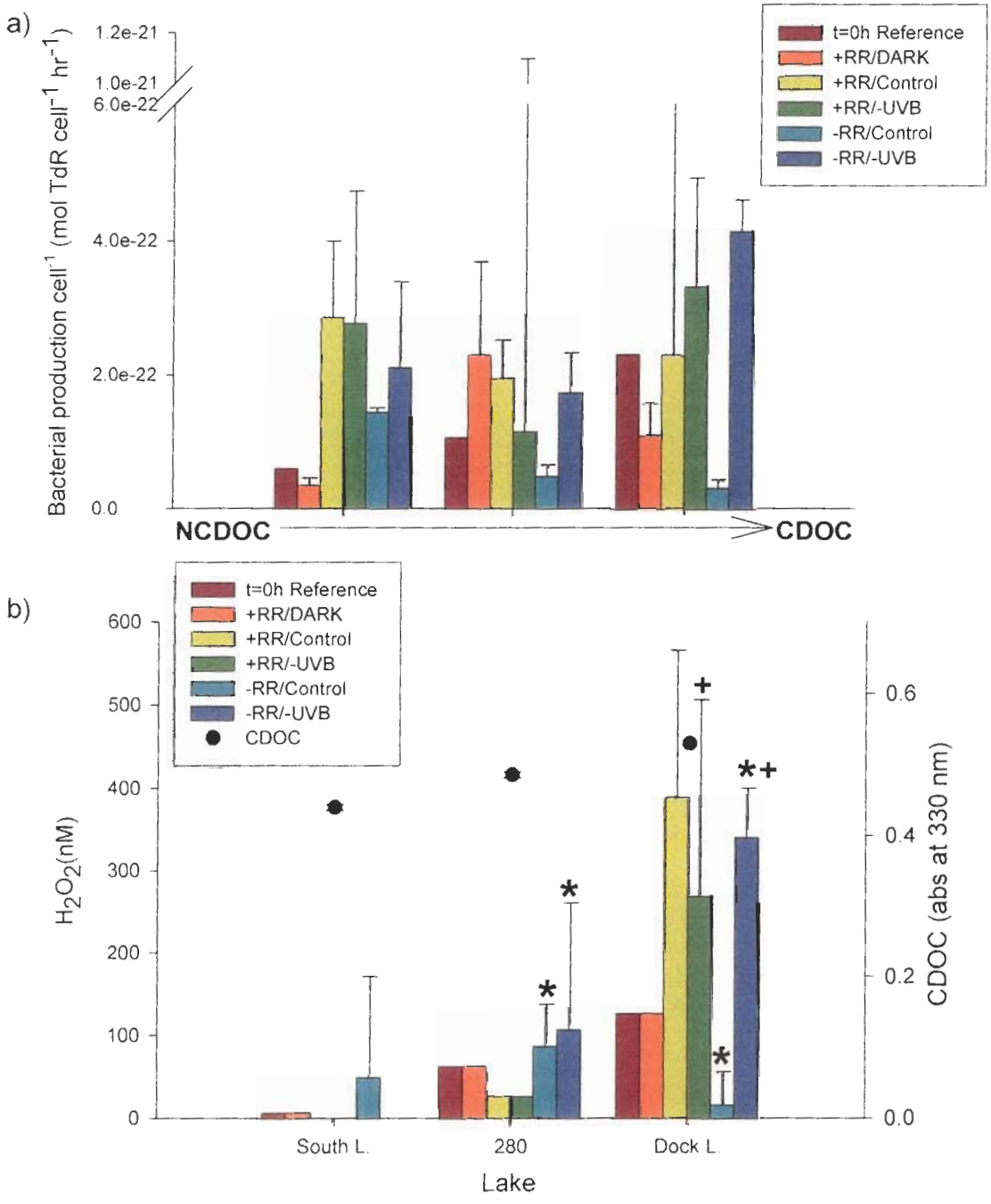

Figure 3.23 Results from Removal & Replacement experiment (August 15th 2003). Results show: (a) bacterial production cell^{-1} and (b) final H_2O_2 concentrations and CDOC absorbency under full sunlight (Control) and removed UVB (-UVB, using Mylar) as well as a 'Removal & Replacement' treatment (+RR or -RR). Significant differences were determined using a two-way ANOVA (n = 3, α = 0.05). Bacterial production was overall non-significant among treatments. Significant differences between +RR and -RR treatments are marked by an asterisk (*), between UVB treatment (-UVB) and the Control are marked by a cross (+). For Dock Lake only, a strong interaction was determined between the RR and UV treatments (p=0.0193). Error bars represent one standard error of the mean.

a)

South Lake Effect Tests	Bacterial Production df	F	*p*	South Lake Treatments	df	H_2O_2 F	*p*
UV	1	0.612	0.827	UV	1	3.000	0.122
RR	1	0.638	0.448	RR	1	3.000	0.122
UV*RR	1	0.086	0.777	UV*RR	1	3.000	0.122

b)

L. 280 Effect Tests	Bacterial Production df	F	*p*	L. 280 Treatments	df	H_2O_2 F	*p*
UV	1	0.949	0.359	UV	1	0.172	0.689
RR	1	0.936	0.362	RR	1	7.856	***0.023***
UV*RR	1	0.491	0.491	UV*RR	1	0.172	0.689

c)

Dock Lake Effect Tests	Bacterial Production df	F	*p*	Dock Lake Treatments	df	H_2O_2 F	*p*
UV	1	0.021	0.889	UV	1	8.520	***0.019***
RR	1	1.403	0.270	RR	1	18.200	***0.003***
UV*RR	1	2.155	0.190	UV*RR	1	39.528	***0.000***

Table 3.9 Results from two-way ANOVA for Removal and Replacement experiment for a) South Lake, b) Lake 280 and c) Dock Lake. Results compare differences between UV treatment and control, removal & replacement treatment (+/- RR) and interactions UV and RR treatments. Significant differences are identified in bold ($\alpha = 0.05$).

4 DISCUSSION AND CONCLUSIONS

This chapter presents an analysis of the trends and significant results presented in Chapter 3. This chapter will demonstrate how the results of this research addressed the specific hypotheses presented in Chapter 1 regarding H_2O_2 formation among delta lakes and arctic floodplains with an attempt to put the results in the context of the scientific literature. Finally, a discussion will be presented on how DOC, H_2O_2 and bacterial production in arctic delta lakes may be respond to global change scenarios with suggestions for future research.

4.1 MACKENZIE DELTA SYSTEM CONDITIONS

4.1.1 Dissolved organic carbon

General patterns of DOC were consistent with results from prior years however some aspects slightly differed. The range in DOC concentrations in this study compare reasonably with summer values for various Alaskan lakes (~58 to 3800 µM, Satoh et al. 1992), North American freshwaters (~400 to 1600 µM, Perdue and Gjessing 1990) and global freshwaters between (<100 and 4000 µM, Pace and Cole 2002). Mean TDOC concentrations were observed to increase with increasing sill elevation (R^2 = 0.6623). This general pattern was evident in two forty-lake surveys conducted in 2003 (data not shown) and also previously identified by Lesack et al. (1991). Increasing TDOC concentrations with sill elevation were attributed to the increasing autotroph biomass in infrequently flooded lakes (Squires et al. 2002). Squires et al. (2002) demonstrated that NCDOC (and TDOC) increased with macrophyte abundance as well as with sill elevation. Spears (2002) also demonstrated that CDOC:TDOC ratios were quite low for lakes with high sill elevation. The results presented here, in combination with prior work done in the delta are generally consistent with other freshwater systems in that a large portion of TDOC consists of non-coloured sources from macrophytes (Farjalla et al. 2001; Mann and Wetzel 1996; Squires 2002).

Highly coloured lakes are atypical for high arctic systems where little vegetation is present (Scully et al. 1996) but are a common feature in arctic tundra and floodplain

systems where substantial amounts of peat are present in the surrounding landscape and contribute considerable amounts of CDOC to lakes through drainage runoff (Perin and Lean 2004). An interesting exception is the Mackenzie Delta floodplain where river flooding is unequivocally the main contributor of CDOC (and water colour) to delta lakes. In contrast to TDOC, mean CDOC absorbencies appeared to increase with decreasing sill elevation (R^2 = 0.7946) as a function of lakes and a connection to the river channel, which is common among lakes at low sill elevations. River water continually brings in CDOC into lakes permanently connected to the river channel (*e.g.* South Lake) whereas disconnected lakes at higher sill elevations receive CDOC from river flooding which is typically infrequent.

Seasonal studies by Riedel (2002) and Spears (2002) found a similar pattern where CDOC absorbencies were highest in frequently flooded lakes, although a late-summer snap-shot study by Teichreb (1999) did not. Teichreb's study likely did not capture any patterns of CDOC with sill elevation because of the late summer timing of sampling since, in this particular case, most photobleaching occurred in the weeks prior to the time of Teichreb's experiments (mid-August). Nonetheless, all of the aforementioned studies based in the Mackenzie Delta acknowledged that CDOC was temporally variable among lakes and throughout the season.

4.1.2 CDOC Photobleaching

CDOC rapidly declined following ice-out due to one of two main mechanisms: flushing via river water or photobleaching. Other possible mechanisms may be due to variations in photon flux, steady state conditions within an individual lake, and perhaps, lake mixing. CDOC photobleaching was observed to be a likely mechanism for CDOC decline among lakes and especially in microcosms. Evidence of photobleaching was apparent as weekly measurements of six lakes showed high absorbance values (at 330 nm) just after ice-out followed by a considerable decrease in the weeks following. Similarly, Riedel (2002) noted that CDOC absorbencies were highest early in the open water season among the same six lakes. Absorbencies measured during the summer of 2001 were found to decrease quickly and were largely comparable by early August. In this study (2003), CDOC absorbencies decreased much more rapidly, reaching comparable levels by early July. However, these weekly measurements were not able to isolate out the extent of river water flushing from CDOC photobleaching in CDOC decline.

The other likely mechanism of CDOC decline, photobleaching, was experimentally tested and found to be highest under full sunlight and UVA+PAR (Mylar) treatments. CDOC photobleaching under full sunlight (UVA+UVB wavelengths) and under UVA+PAR confirm the important role of UVA photons among Mackenzie Delta lakes. The role of UVA in H_2O_2 formation has been previously identified and modeled for temperate latitude freshwaters (Scully *et al.*1997, Jerome and Bukata 1998), however it had previously remained unconfirmed for this system. The importance of UVA in this system was especially interesting since prior work among delta lakes focused on the role UVB in biological processes (Teichreb 1999). Therefore, in terms of CDOC photobleaching and concomitant changes to DOC among delta lakes, UVA photons are the most critical in the formation of H_2O_2, other ROS, leading to more rapid rates of photobleaching. Future research on DOC dynamics among delta lakes are therefore encouraged to incorporate both UVA and UVB in future experimental designs through the use of both Mylar and OP-2 UV filters.

The results of this research provide an interesting story of CDOC photobleaching however it still remains incomplete. CDOC decline is likely due to photobleaching rather than flushing by the river. My results did not find H_2O_2 to be a statistically significant function of lake mixing (lake depth of lake area), and it is also not thought to a major factor in CDOC photobleaching as well. Photobleaching rates may differ due to short-term variations (e.g. solar intensities, Gibson et al. 2001; Zagarese et al. 2001) or long-term variations (*e.g.* DOC compositions, trophic conditions). This study demonstrates the DOC compositions may be one factor influencing photobleaching rates, specifically the amount of CDOC present. My results also suggest that differences in the degrees of photobleaching between Dock and South Lakes were likely due to the varying DOC compositions. The amount of chromophoric DOC present in South Lake in addition to the continual input of CDOC to the lake was quite different from the isolated, low colour Dock Lake where CDOC is already quite low and there are no other inputs of CDOC after ice-out.

Photobleaching may also be influenced by within-lake interactions with photochemical by-products such as H_2O_2 and other free radicals (Zepp et al. 1992) or internal mixing processes within lakes that stratify (Gibson et al. 2001). It is plausible that photobleaching rates may also stabilize after reaching a level of steady state conditions when CDOC is completely photolyzed. However, among Mackenzie Delta lakes, it seems that the role of flushing in providing CDOC in lakes connected to the river

channel is more important but was not addressed in this scope of the study. Although evidence of CDOC in lakes appears to be a balance between both photodegradation processes and river inputs, I did not specifically test this among the lakes. It can be hypothesized that changes in CDOC among delta lakes may also be a function of a lakes connection to the river channel.

4.1.3 Bacterial abundance and production

Contrary to all prior work in Mackenzie Delta lakes, seasonal bacterial abundances revealed no significant patterns with sill elevation. Abundances varied between $1.8x10^6 - 2.3x10^6$ cells mL^{-1} among the six lakes and between $4x10^5 - 7x10^6$ cells mL^{-1} in the forty lake surveys. Average rates of bacterial production ranged between $1x10^{-19}$ - $1x10^{-21}$ mol TdR $cell^{-1}$ hr^{-1} in the microcosm experiments and forty lake surveys. Non-normalized rates of production were comparable to earlier studies (Spears 2002; Teichreb 1999) however normalized rates of production were two to three orders of magnitude lower than both Teichreb (1999) and Spears (2002) possibly because cell counts slightly different values than in previous years. A slight refinement was made to the bacterial enumeration technique used by Teichreb (1999), Riedel (2002) and Spears (2002). Specifically, the use of TWEEN and phosphate buffered saline tablets (PBS) diluted in sterile water were used to rinse away humic materials and to prevent clumping of bacterial cells (M. Bahr, pers. comm., Appendix 2).

Comparatively, abundances were slightly less that those observed in Toolik lake in northern Alaska (10^6 - 10^8 cells mL^{-1}, O'brien et al. 1992), equivalent to a high arctic lake on Ziegler Island, (10^5 cells mL^{-1}, Panzenbock et al. 2000) and slightly greater than abundances in the Mackenzie Delta - Beaufort sea estuary (10^4 cells mL^{-1}, Parsons et al. 1988). Moreover, bacterial production $cell^{-1}$ was considerably lower compared to values reported for Toolik Lake (~10-14 mol TdR cell-1 mL-1, O'brien et al. 1992) and were comparable to values reported by Parsons *et al.* (1988) however, all studies reported bacterial production to fluctuate considerably within the season.

Bacterial abundances fluctuated weekly with evidence of a peak in August. A substantial increase may be indicative of either a storm event that may have resulted in increased run-off from the watershed, or alternatively, evidence of predator-prey interactions between bacteria and grazers (i.e. heterotrophic nanoflagellates). This is largely speculative since neither climate data nor samples for grazer abundance were collected in 2003. Seasonal and temporal fluctuations in microbial abundance and

production have been linked to DOC (Crump et al. 2003; Hessen et al. 2004; Parsons et al. 1988; Teichreb 1999), salinity (Parsons et al. 1988), depth (Crump et al. 2003; O'brien et al. 1992), nutrients (Bahr et al. 1996; O'brien et al. 1992; Spears 2002) and grazing pressure (O'brien et al. 1992; Panzenbock et al. 2000; Riedel 2002). Coupled with the findings of Riedel (2002) of zooplankton and heterotrophic nanoflagellates and seasonal bacterial production by Spears (2002), this study suggests that grazing effects are quite strong among delta lakes, particularly at the end of the season when H_2O_2 effects were observed to be quite weak. This will be explored in greater detail in a following section.

4.2 HYDROGEN PEROXIDE DYNAMICS

Overall, the H_2O_2 values reported in this research compare reasonably with values reported by Scully et al. (1996) along a gradient in DOC and latitude from 43 – 75° N. However, one marked difference from that study is the Mackenzie Delta lakes themselves and their physical characteristics. The Arctic lakes assessed in the study by Scully et al. (1996), were predominantly high Arctic lakes, characterized by typically low DOC and low H_2O_2 concentrations as a result of little surrounding vegetation. In stark contrast, lakes of the Mackenzie Delta are uniquely high in DOC concentration and influenced by substantial river inputs, macrophyte abundances and melting permafrost. H_2O_2 patterns among Mackenzie Delta lakes are currently underrepresented in the scientific literature.

4.2.1 H_2O_2 levels across the DOC gradient

H_2O_2 levels across the DOC gradient were initially the most surprising. My findings were contrary to what was proposed in the original hypothesis. Initially, the outdoor incubator experiments revealed a pattern where H_2O_2 concentrations were highest in clear lakes (e.g. Dock Lake) and lower in coloured lakes (e.g. South Lake). This made intuitive sense as TDOC concentrations were highest in clear lakes and also confirming findings from other clear lakes (Scully et al. 1997). For the clear and shallow lakes of the Mackenzie Delta, UVR has been noted to penetrate completely to the bottom to the lake (*e.g.* Dock Lake, J. Gareis, unpub. data). Among highly coloured lakes, sufficient photochemical substrate is available due to the presence of

chromophoric DOC but UVA and UVB wavelengths necessary for H_2O_2 production are quickly attenuated in the upper surface layers (Scully et al. 1996).

In contrast to expectations, the seasonal results from this research demonstrate that H_2O_2 concentrations appear to be highest at mid-TDOC (equivalently, mid-CDOC) levels, or equivalently, in lakes at intermediate sill elevations. Although there was considerable variability in H_2O_2 concentrations (between 0 and 4000 nM), the pattern of peak concentrations in intermediately flooded lakes was evident among all three surveys. This trend was not explained by lake depth nor any other simple parameters. Therefore, it appears that H_2O_2 concentrations do not follow DOC concentration per se. In lakes such as the Mackenzie Delta which do not stratify and have fairly similar depths, I had expected to find some relationship with lake area or depth to explain this pattern. Instead, a significant relationship was evident only for the late summer survey when H_2O_2 levels were low. It is therefore proposed that, in the Mackenzie Delta system, peak H_2O_2 levels can be explained by a trade-off between DOC as a photochemical substrate and UVB penetration in the water column. Extremely clear lakes of the Mackenzie Delta have high TDOC concentrations but largely comprised of non-chromophoric DOC from autochthonous sources (Spears 2002; Squires 2002). It is therefore further suggested that there is little photochemical substrate available to react with and produce H_2O_2. By contrast, highly coloured delta lakes may have sufficient chromophoric DOC but UV wavelengths get attenuated quickly in the upper surface. Hence, the potential for H_2O_2 production may be optimal in intermediately flooded lakes.

Patterns of H_2O_2 across the DOC gradient deserve additional consideration. An additional factor is that the pattern of H_2O_2 formation across Mackenzie delta lakes may also be explained by the connection type to the river channel in addition to sill height and flood frequency. As Squires et al.(2002) demonstrate, the connection type of a lake to the river channel can influence macrophyte distribution. As in the case of lakes with a "direct" connection to the river channel, this connection translates to a regime of high turbidity, low water clarity and consequently, low macrophyte abundance. The opposite trend with "indirect" connection to the river channel was also observed with lakes of "indirect" connection characterized by low turbidity, high water clarity and high macrophyte abundance. Thus H_2O_2 formation among Delta lakes may also be attributed to the connection type of the lakes to the river channel. However this was not incorporated into the scope of my study.

A limitation with the large survey results is that measurements of H_2O_2 are reported as instantaneous concentrations around solar noon. Cognizant that H_2O_2 is extremely dynamic within delta lakes, single point measurements from lakes with varying DOC compositions and water colour are believed to be reasonable estimates of H_2O_2 concentration. The estimates are indicative of large-scale dynamics since all sampling was done at solar noon, when concentrations were observed to be the highest, and from sub-surface depths and mid-lake locations.

However, there is still much to learn regarding H_2O_2 dynamics in the Mackenzie Delta system. For example, the use of apparent quantum yields of H_2O_2 (the number of moles of H_2O_2 formed per mole of photons absorbed at a specific UV wavelength, Cooper and Lean 1989; Scully et al. 1996) or areal H_2O_2 production (milligrams of H_2O_2 formed on an surface areal basis, Scully et al. 1997) may be more accurate descriptors of H_2O_2 patterns and distribution. Daily H_2O_2 production has also been shown to be a function of depth and wavelength (Scully et al. 1997) and DOC (Scully et al. 1996). Because Mackenzie Delta lakes represent a wide array of DOC concentrations, varying sizes and depths, quantum yields of H_2O_2 throughout the range 295 – 700 nm or areal concentrations may provide a more accurate prediction of H_2O_2 formation potential. Therefore, future work along this vein should more closely consider H_2O_2 formation as a function of UV wavelength, depth and area in addition to DOC.

4.2.2 Seasonal H_2O_2 pattern

Consistent with aquatic ecosystems worldwide, seasonal variations in H_2O_2 concentrations were observed to be highest in the summer however my results demonstrate an especially considerable peak occurring near the arctic summer solstice. This suggests that seasonal variability may be more important in this system rather than diurnal variability. Peak concentrations of over 4000 nM were observed in early July with concentrations soon falling to levels below 1000 nM. Reported values of 4000nM are rare for freshwater ecosystems, especially arctic waters. Concentrations can be as high as 25,000 nM (Blough and Zepp 1995) but most studies report values below 1000 nM in fresh and marine waters (Cooper and Lean 1989; Cooper et al. 1994; Obernosterer et al. 2001; Scully et al. 1995; Scully et al. 1996; Scully and Vincent 1997; Scully et al. 1997; Yocis et al. 2000), with up to 3000 nM reported for some temperate latitude lakes (Scully et al. 1996). In the Mackenzie Delta system, the presence of H_2O_2 may be primarily due to photochemical activity and not due to other factors such as rain or biogenic formation.

Although rainwater or biological H_2O_2 generation were not directly measured, this study has adequately demonstrated substantial photochemical formation of H_2O_2 in lakes with contrasting DOC regimes.

The seasonal pattern of H_2O_2 is especially interesting in the Mackenzie Delta compared to other aquatic systems. Few studies have tracked the seasonal dynamics of H_2O_2 in aquatic ecosystems. For most of the year, Mackenzie Delta lakes are ice-covered and in the dark. However at ice-out in the spring, runoff and snowmelt provide the largest inputs of DOC to lakes. At the same time, the solar flux is at an annual maximum and irradiance is continuous for many weeks. Consequently, H_2O_2 concentrations and CDOC absorbencies were observed to be their highest for the year.

It is plausible that H_2O_2 may have been produced biologically as a result of increased productivity by both phytoplankton and bacteria that may be associated with the spring flood and an influx of nutrients. H_2O_2 concentrations were quite low in comparison to the first survey. Comparable H_2O_2 concentrations in the July and August surveys suggest that substantial amounts of CDOC are still present in most lakes. CDOC in the water column may be due to small rain events that may advect DOC from the landscape as well as melting permafrost that may leach DOC into the water column. The factors contributing to H_2O_2 production appear to dampen as DOC photobleaches and Arctic daylengths shorten, thereby reducing photochemical activity.

My results support the hypothesis that seasonality plays a more important role than diurnal variability in arctic systems. My results are also consistent with the hypothesis that H_2O_2 ought to build up during continuous periods of irradiation during the summer months. Consistent periods of irradiation at arctic latitudes facilitate uninterrupted opportunities for H_2O_2 photoproduction and continuous CDOC photobleaching. This research demonstrates that the early weeks after ice-out are the most critical not only for biological activity (Riedel 2002; Squires 2002) and distribution of inorganic nutrients (Spears 2002) but for photochemical activity as well. Seasonal patterns in H_2O_2 may have implications on other biological and chemical interactions in the water column. It is hypothesized that intense photoproduction of H_2O_2 may be biologically inhibitory in early weeks after ice out. Subsequently, plankton may become less inhibited by H_2O_2 but rather more inhibited by direct UVR as lakes become increasingly clear due to photobleaching.

4.2.3 H_2O_2 diurnal dynamics and H_2O_2 build-up

The diurnal experiments demonstrate clear pattern of H_2O_2 formation that follow solar intensity. Similar to other diurnal studies (Cooper and Lean 1989, Scully *et al.*1995, Wilson *et al.*2000), H_2O_2 production responded to irradiance (including PAR and UV fluxes) with peak concentrations occurring up to a few hours after the peak irradiance flux. This pattern had not been previously investigated for Mackenzie Delta lakes. The evidence suggests that continuous irradiance enhances photochemical production of H_2O_2, potentially building up on a daily basis since there little opportunity for considerable biological decay. To further investigate diurnal H_2O_2 dynamics, long-term monitoring is recommended over scales of perhaps one week with multiple lakes being monitored at or around the same time to provide more accurate comparisons among lakes with varying DOC compositions and water colour.

4.3 Effects of grazing, UVR and H_2O_2 on bacterial production

Bacteria responded dramatically to grazing pressures and less so to the effects of UV and H_2O_2. The response of bacteria to grazers was consistent with prior work and additional evidence suggests that UV and H_2O_2 may exert a stronger influence on bacteria earlier in the open water season.

4.3.1 Effect of grazing

Bacterial production $cell^{-1}$ was significantly higher in the presence of grazers than in their absence in a late-summer forty lake survey. The results presented in this study capture a pattern where bacterial production was significantly higher in the presence of grazers. Bacterial production $cell^{-1}$ dropped two orders of magnitude when in the absence of grazing pressures. This suggests that trophic interactions between grazers (heterotrophic nanoflagellates) and possibly zooplankton on bacteria facilitate significantly high production rates by bacteria. Because the metabolism by grazers was not measured in this study, it is uncertain as to whether or not this likely occurred. However, it is hypothesized that phytoplankton may also play a role in enhanced bacterial growth. Although grazers were present, phytoplankton were present as well. Bacteria may have been stimulated by phytoplankton growth and their release of organic

substrates as a food substrate (Cole et al. 1982). Reduced rates by bacteria in the absence of grazing can be up to 50% per day (Wetzel 2001) associated with starvation conditions, especially under UV stress (Pace 1988; Wetzel 1995).

The effects of grazing on bacteria in Mackenzie Delta lakes were first addressed by Teichreb (1999) then indirectly by Spears (2002) and Riedel (2002). Arctic food webs are typically simple and quite short due to low fish abundance and low nutrient inputs (Kling et al. 1992; Panzenbock et al. 2000) with large zooplankton and invertebrates serving as the top predator in lakes at high sill elevation where fish are absent (Riedel 2002). Spears (2002) demonstrated that increasing bacterial abundances were found in lakes with higher sill elevations (infrequently flooded lakes). Riedel (2002) identified evidence of grazing interactions where high heterotrophic nanoflagellate (HNF) abundances were found to be coupled with extremely low bacterial abundances. It is plausible that what was captured in this survey was heavy grazing on bacteria when bacterial abundances were very low. It was further demonstrated that grazing pressures by particularly large zooplankton in infrequently flooded lakes and HNF in frequently flooded lakes were the likely cause for low bacterial abundances (Riedel 2002).

In a neighbouring arctic system, Toolik Lake, a long-term study (2 years) revealed a clear response by bacteria to grazing pressures and nutrients (O'brien et al. 1992). Bacteria were not nutrient limited in the study and therefore subject to grazing pressures only. O'brien et al. (1992) confirmed that the highest rates of bacterial production occurred when severe predation by microheterotrophs was also observed. Direct measurement of grazers and phytoplankton were not included in the scope of my study, hence it is plausible that grazing in Mackenzie Delta lakes may exert a stronger influence on bacterial production than previously thought. Furthermore, additional investigation on the role of DOC and bacteria is encouraged to pursue the role of DOC on bacteria assimilation and respiration. One limitation of measuring only bacterial production is that the conversion of organic carbon to inorganic carbon bacteria was not taken into account. Interestingly, BGE rates may differ across lakes of the Mackenzie Delta as the source and quality of organic matter, nutrient availability and energetic demands of each lake vary between highly autotrophic to highly heterotrophic, or equivalently, highly productive to highly respiratory. To better understand the food webs of Mackenzie Delta lakes, more comprehensive, multi-layer surveys that are conducted various times during the season are recommended.

4.3.2 Effect of UVR

The experimental response of bacteria to UV was complex but still consistent with findings from other freshwater habitats. Rates of bacterial production were expected to both increase (due to DOC breakdown) and decrease (due to direct UV inhibition). The results from my experiments illustrate both types of responses that may also be coupled with DOC compositions among lakes. In the bacterial production experiment with grazers, bacterial production $cell^{-1}$ increased under full sunlight (Control) compared to the -UVB treatment for half of the microcosms. This suggests that DOC as a food substrate may be more important in some of these lakes, making direct UV inhibition secondary to food substrate. However, when the bacterial production experiment was repeated without grazers (bacteria community only), rates of bacterial production decreased substantially and no trend was apparent. Hence, stimulatory effects of UV on bacterial production still remains poorly understood among Delta lakes.

In contrast, evidence of bacterial inhibition by UV was apparent in our experiments. For example, in both of the bacterial production experiments, bacterial production $cell^{-1}$ was higher in a half of the -UVB treatments. A pattern in direct UV effects was also evident in the Removal & Replacement experiment where bacteria were left *in situ* in the microcosms. In this treatment, bacteria were left in the microcosms during incubation. As a result, bacterial production $cell^{-1}$ was higher in the –UVB treatment for all lakes. These indicate an inhibitory response to direct UVB since rates of production were higher under the –UVB treatment (Mylar filter), especially since bacteria are apparently more susceptible to UV stress due to their smaller size and shorter generation times (Karentz *et al.*1994).

One expectation from the Removal & Replacement experiment was that rates of bacterial production would differ under full sunlight and –UVB despite the removal or incubation of bacteria. Lund and Hongve (1994) found bacterial production $cell^{-1}$ to decrease significantly when bacteria were introduced to UV-irradiated water. This study revealed that rates of bacterial production were comparable and largely non-significant among treatments. Non-significant differences may have been due to the concomitant production of peroxides that may have occurred during under treatments at high enough levels to inhibit production. UVA wavelengths were present among both treatments and therefore photochemical processes (and the production of H_2O_2) were occurring throughout the course of the experiment. However, this relationship still remains ambiguous since H_2O_2 levels were quite low in South Lake and Lake 280 but significant

in Lake 520. The complexities of interacting photochemical factors across the DOC gradient continue to be a difficult story to unravel. Future work should continue to address the question of the effects of UV irradiated water on in situ versus inoculated bacteria, however it is recommended that the use of an OP2 filter which removes all UVA and UVB wavelengths instead of Mylar (UVA+PAR wavelengths). It is also recommended that future experiments be carried out earlier in the season when photochemical activity is high and CDOC is still highly photoactive.

4.3.3 Potential effects of H_2O_2, ROS and other photoproducts

The response of bacteria to H_2O_2 was relatively weak compared to grazing effects. This was likely due to the experiments being conducted late in the season (August) when photochemical activity was dampened. As shown from the seasonal H_2O_2 results, concentrations are lower late in the season as CDOC becomes increasingly photobleached with time. It has been demonstrated that seasonal H_2O_2 dynamics are considerable in this system. Therefore it is proposed that the effects of H_2O_2 on bacterial production may be more critical early in the open water season when both CDOC and photochemical activity is at an annual maximum.

Overall, the effects of ROS on bacteria are variable among microbial species and among aquatic habitats. Although difficult to determine from these results, it is plausible that H_2O_2 may be inhibitory for bacteria in Mackenzie Delta lakes since small concentrations can inhibit bacterial production (<50 nM; Xenopoulous and Bird 1997). It is also possible that some bacteria may produce H_2O_2; however; our experiments were not designed to test for this. A simple experiment may be able to use sterile peroxide-free water injected with an inoculum of bacteria under light and dark conditions, measuring changes in H_2O_2 with time. In lakes of the Mackenzie Delta, less is known on the role of other photochemically produced products on bacteria including carboxylic acids (Bertilsson and Tranvik 1998), the hydroxyl radical (Pullin *et al.*2004), carbohydrates and amino acids (Tranvik and Jørgensen 1995). At our current level of understanding, the role of other photoproducts may also play a role in both stimulating and inhibiting bacterial production and should be further explored in future research.

4.4 CONCLUSIONS

This study assessed the patterns of H_2O_2 formation across lakes of the Mackenzie Delta and the potential effects on bacterial production. H_2O_2 levels were higher around the solstice with the highest concentrations in intermediately flooded lakes. This evidence is consistent with an optimal trade-off between UV penetration and chromophoric substrate that is found in intermediately flooded lakes. Unlike temperate latitude lakes where H_2O_2 decays at night, diurnal dynamics in two contrasting lakes provide evidence of H_2O_2 build-up from one day to the next. This research highlights the importance of the Arctic summer solstice when day-lengths are 24 hours long and photochemical by-products of DOC are critical. The potential for H_2O_2 formation was also found to be closely coupled with CDOC photobleaching, highlighting the importance of UVA wavelengths in these processes. Both H_2O_2 production and CDOC photobleaching were maximized under sunny conditions, under both full sunlight and UVA+PAR conditions. The potential effects of H_2O_2 and UVR on bacterial production were shown to be both stimulatory (increased rates in higher CDOC lakes) and inhibitory (decreased rates in low CDOC lakes). The experimental results suggest that plankton in clear lakes may be most susceptible to direct UV damage and that direct UV inhibition is likely to occur late in the summer season when photochemical activity appears to be dampened compared to earlier in the open water season.

Aquatic food webs in Mackenzie Delta lakes are largely influenced by DOC and therefore H_2O_2 and other photochemical transformations. Northern freshwaters are extremely sensitive to environmental change (Vincent and Pienitz 1996), therefore the impact of changes in DOC and UVR regimes cannot be underestimated. UVR penetration is increasing in lakes, UVR flux is increasing and H_2O_2 can also be expected to increase, causing additional concern. Predictions for arctic lakes identify clear lakes with low DOC as being most sensitive to the direct effects of increasing UVB (Laurion et al. 1997; Schindler 2001; Yan et al. 1996) however, in contrast, the Mackenzie Delta ecosystem is a system strongly influenced by DOC and water colour.

Prior work in the Mackenzie Delta confirm that climate change will be primarily responsible for increasing temperatures and more importantly, a reduction in ice-jamming in the delta which will dampen the intensity of flooding in delta lakes (Marsh

and Lesack 1996, Rouse *et al.*1997). The response of the Mackenzie Delta ecosystem will be largely dominated by changes to the DOC regime. Temperatures are predicted to increase in the delta which may stimulate bacterial production, however changes in the flooding regime and DOC input may have more serious consequences to aquatic food webs. These consequences may involve increased photochemical activity among Delta lakes.

This thesis has demonstrated the critical role that DOC plays in H_2O_2 production with additional insight into the complexities of DOC interactions with bacterial communities. A reduction in flooding may have serious implications to DOC dynamics, photochemical activity and also bacterial production. The consequences are complex with both costs and benefits to bacteria and in the cycling of DOC within aquatic food webs. Future research is therefore highly encourage to consider the dynamic role of DOC among Mackenzie Delta lakes in an attempt to predict how both the biotic and abiotic components of the ecosystem may respond to the multiple stressors of global change.

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6 APPENDICES

6.1 Hydrogen peroxide methodology

Scopoletin-horseradish peroxidase fluorometric technique

A standard Turner Model 111 filter fluorometer was used for this analysis. The excitation filter was a Corning 7-60 filter (range, 320-390nm; primary wavelength, 365 nm) and the emission filter was a Wratten 65A coupled with a 2A filter (range, 460-540nm; primary wavelength, 495 nm). An opaque rod bar was used to zero the machine and round borosilicate cuvettes (Barnstead Thermolyne Model No.11F06-07) were used for measuring the fluorescence of the samples.

Distilled deionized water (DDW) used for reagents, blanks and rinsing was sparged of any trace amounts of peroxide by the addition of approximately 3mg/L of bovine catalase (Sigma-Aldrich, C9322) and buffer solution and keeping the DDW cool and in the dark for at least 24 hours. The following solutions were created prior to lake sampling: a standard stock solution of 10^{-5} M H_2O_2 from a 30% solution (Fisher Scientific, reagent grade), 0.5 M phenol solution and a 0.5 M phosphate buffer solution (ph 7; using 13.34 g KH_2PO_4 and 21.58 g of Na_2HPO_4 in 500mL of DDW). The H_2O_2 stock solution was verified by permanganate titration and was stable for up to one week.

Upon returning from sampling, scopoletin (Sigma-Aldrich, S2500) and HRP (Sigma-Aldrich, P-8250) solutions were prepared. A scopoletin solution was prepared by adding 4.8 mg to 500 mL of DDW in an aluminum foil-covered flask. A small amount was transferred to a darkened 20 mL glass scintillation vial for easier pipetting. An HRP solution was prepared by dissolving 20 mg in 5 mL of buffer and 500 µl of the phenol solution.

With the fluorometer zeroed using the opaque rod and the sensitivity setting at its highest, the samples were ready for analysis. Five mL of lake water was transferred to a clean, darkened scintillation vial. Twenty µl of 0.5 M buffer was added and the sample was vortexed for 10 seconds before being poured into a cuvette and placed into the

fluorometer. Using the dial, the fluorometer was zeroed and the sample was returned to the vial. Twenty µl of scopoletin solution was added to the water sample and vortexed for 10 seconds. The fluorophore was transferred to the cuvette and placed into the fluorometer. The dial was set to maximum ("100") and the fluorophore was returned to the vial. Ten µl of HRP solution was added to the sample which was then vortexed for 10 seconds. The fluorophore was transferred to the cuvette and the instantaneous reading was recorded. A decrease in fluorescence was observed if any H_2O_2 was present in the sample. A replicate measurement was taken for each lake water sample.

Standard curve method of standard additions

A standard curve was derived in order to convert loss of fluorescence into nanomolar H_2O_2 concentrations using the same reagents as for the scopoletin-HRP fluorometric technique. A new standard curve was generated for each individual lake and for each sensitivity setting if more than one was used for each lake.

The fluorometer was zeroed with the opaque rod and set to the sensitivity setting at which the samples were analyzed (typically maximum, 30X setting). Five mL of peroxide-free lake water (DDW with buffer added) was pipetted into a clean, darkened scintillation vial. Twenty µl of 0.5 M buffer was added and the sample was vortexed for 10 seconds. The contents were poured into the fluorometer cuvette, placed into the cuvette holder and the fluorometer was zeroed. The sample was returned to the vial from the cuvette, 10 µl of HRP solution was added and vortexed for 10 seconds. The contents were poured into the fluorometer cuvette and used to zero the fluorometer. This quenches any remaining H_2O_2 in the sample. The sample was returned to the vial, 20 µl of the scopoletin solution was added and then vortexed for 10 seconds. The fluorophore was poured into the cuvette and placed into the cuvette holder. At this stage, the sample is fully fluorescing and the dial was turned to "100". Three 50 µl spikes of a known and verified H_2O_2 stock solution were added incrementally. The concentration of the H_2O_2 stock solution was determined using a permanganate titration.

The first of three 50 µl H_2O_2 spikes was added to the sample in a scintillation vial and vortexed for 10 seconds. The contents were poured into a cuvette and fluorescence was measured. There should have been a decrease in fluorescence from "100" and the reading was recorded. The fluorophore was returned to the vial, a second 50 µl H_2O_2 spike was added and vortexed for 10 seconds. Contents were poured into the cuvette and fluorescence was measured. Again, a decrease should have been observed and the

reading was recorded. The fluorophore was returned to the vial and a third 50 µl H_2O_2 spike was added and the sample was vortexed for 10 seconds. The contents were poured into the cuvette and fluorescence was measured and recorded. The process was repeated for replicate peroxide-free lake water sample.

Fluorescence data were plotted against concentration, where increasing concentrations correspond to increasing loss of fluorescence. A standard curve complete with an approximated equation of the slope was obtained and H_2O_2 concentrations in the lake sample were approximated.

Permanganate titration for hydrogen peroxide determinations

This method was used to determine the exact concentration of H_2O_2 stock solution used in the standard curve for the fluorometric determination of hydrogen peroxide concentrations. Potassium permanganate solution (0.02 M; $KMnO_4$) was diluted four times, from 25 mL up to 100 mL, in separate graduated cylinders using peroxide-free DDW, resulting in a final concentration of 0.005 M solution. Ten mL aliquots of the H_2O_2 solution to be standardized was transferred to a 100 mL beaker. Five mL of sulfuric acid (0.75 M) was added to the solution. The beaker was then titrated with the 0.005 M KMnO4 solution until the solution turns pink. This was repeated with the other 0.005 M solutions. The average titrant amount was used in the calculations.

The balanced equation for this reaction is:

$$2MnO_4^- + 5\,H_2O_2 \rightarrow 2Mn^{2+} + 10\,H^= + 5O_2 \quad (1)$$

The exact concentration of the H_2O_2 solution was then calculated using the following equation:

$$(M\ KMnO_4) \times (Volume) = (M\ Peroxide) \times (Volume) \times {}^5/_2 \quad (2)$$

This method was accurate to approximately 0.25 M and the stock solution was stable for up to one week. The titration was performed whenever a fresh stock solution was prepared.

6.2 Bacterial production cell^{-1} methodology

Bacterial production - Reagent preparation

Sterile water was prepared daily and used in all aspects of reagent preparation and rinsing of glassware. Distilled deionized water (DDW) was filtered through a 0.22 µm filter (Whatman) and autoclaved at 130°C for 1 hour. All glassware was acid washed and autoclaved at 130°C for 1 hour. A stock solution of [^{3}H] TdR was prepared by diluting a known amount of thymidine (185 MBq; Amersham Biosciences Cat. No. TK758) with 5% ethanol solution in a sterile glass ampoule and sealed. A number of ampoules were filled with the [^{3}H] TdR stock solution in advance and stored in the fridge until needed. A new ampoule was used for each experiment.

New reagents were also made for each new experiment. 100% TCA solution was made by dissolved 100 g of TCA in 100 mL of sterile water. 5% TCA solution was prepared by dissolving 5 g of TCA in 100 mL of sterile water. 80% ethanol was prepared by adding 80 mL of 100% reagent grade ethanol to 20 mL sterile water. Other reagents, 37% A.C.S. grade formaldehyde (Anachemia Canada), sodium hydroxide (reagent grade NaOH; Fisher Scientific), phenol-chloroform solution (Sigma-Aldrich) and scintillation cocktail (Filter-Count, Packard BioScience) were purchased directly and stored accordingly until use. Prior to each experiment, cellulose nitrate filters (0.22 µm; Whatman) were placed in a sterile petri dish, soaked in sterile water and stored in the fridge for 2 hours prior to use.

Standard stock solution

A standard was prepared for each ampoule of [^{3}H] TdR used. 100 µl of the [^{3}H] TdR stock solution was added to a 5 mL sterile water sample in a glass scintillation vials. In duplicate, one 100 µl aliquot was transferred from that solution into new, clean glass scintillation vials. Next, 900 µl of sterile water was added and finally, 9 mL of Filter-Count scintillation cocktail was added. The vials were then stored, transported and counted for radioactivity using a liquid scintillation counter at Simon Fraser University.

Sample collection and analysis

From each microcosm, three 10 mL water samples were collected in new glass scintillation vials and brought back to the lab. Ten mL of sterile water was collected in a new scintillation vial as a blank control. A new glass ampoule of [^{3}H]-TdR working solution was opened and 100 µl of [^{3}H]-TdR was pipetted to each sample and to the control, making the final concentration of [^{3}H]-TdR in solution 20 mL. In the control sample, 0.5 mL of 37% A.C.S. grade formaldehyde was then added. The remaining lake water samples were left to incubate for thirty minutes at room temperature. After the incubation period, 0.5 mL of 37% A.C.S. grade formaldehyde was again added to the samples in order to cease all uptake of radiolabelled thymidine. To raise the pH of the samples hence killing bacteria and preventing further [^{3}H]-TdR uptake, 0.25 mL of 10N NaOH was added to each sample and allowed to incubate for 20 minutes. Three mL of 100% TCA solution was then added to the samples and placed in an ice bath for 15 minutes. This step allows the bacterial cells to lyse and for macromolecules including DNA to precipitate.

While samples sat in the ice bath, a 6-place filtration manifold, vacuum pump and sterile glassware were set up. Pre-soaked 0.22 µm cellulose nitrate filters were placed at the base of each filtration tower. Controls and samples were filtered through the filtration tower and rinsed with 3 mL of 5% TCA in order to precipitate any left over DNA and macromolecules. Then, to remove any labeled proteins, 5 mL of phenol-chloroform solution was rinsed through. Finally, to remove any labeled lipids, 5 mL of 80% ethanol solution was rinsed through.

Each filter was trimmed using a clean blade and standard plastic core (diameter = 17mm), removed with clean forceps and placed in a clean, unused 20 mL glass scintillation vial and 10 mL of Filter-Count scintillation cocktail was added. Filters were stored for up to four weeks and then measured for radioactivity using a liquid scintillation counter at Simon Fraser University.

Gross bacterial production was then estimated using the following formula (Wetzel and Likens 2000):

$$TdR_{\text{moles L}^{-1}\text{ hr}^{-1}} = \frac{(dpm_{\text{sample}} - dpm_{\text{blank}})\,(1000_{\text{mL/L}})\,(60_{\text{min/hour}})\,(D)}{(2.22 \times 10^{6}_{\text{dpm/Ci}})\,(A)\,(V)\,(I)\,(1000_{\text{mmol/mol}})} \quad (3)$$

where:

dpm_{sample} = disintegrations min^{-1} for sample
D = Dilution factor for formalin to sample volume
A = specific activity of isotope (Ci $mmol^{-1}$)
V = volume of sample filtered (mL)
I = incubation time (min)

To obtain estimates of bacterial production $cell^{-1}$, gross bacterial production was divided by bacterial abundance estimates (cells mL^{-1}).

Bacterial enumeration

Bacteria were enumerated using a standard method where aquatic bacteria were collected on a membrane filter, stained with a fluorescing dye (4'6-diamidino-2-phenylindole; DAPI) and counted under an epifluorescent microscope (Porter and Feig 1980). Living bacteria are distinguished from non-living cells and detritus by fluorochrome stains where bacteria fluoresce a bright blue and detritus fluoresces a weak yellow when viewed at 365 nm. This method is similar to bacterial enumeration methods used by Teichreb (1999), Riedel (2002) and Spears (2002), however, the use of TWEEN, a surfactant, and phosphate buffered saline, are new contributions to the method.

Reagent preparation

All glassware and reagents were prepared in advance and sterile water was prepared daily. New glass vials were used to collect water samples. A 1 mg mL^{-1} stock solution of 4'6-diamidino-2-phenylindole (DAPI; Sigma Aldrich Cat. No. 32670) was prepared by diluting a known amount with sterile water in an aluminum foil-covered flask. Because DAPI is extremely light sensitive, the flask remained covered and stored in the fridge at -4°C until needed. The stock solution remained stable for several weeks and was used to prepare a daily working solution. A working solution was prepared by diluting a known volume of the 1 mg mL^{-1} solution down to a necessary concentration of 0.1 µg/L with sterile water in an aluminum foil covered glass flask. A 5% TWEEN stock solution was prepared by adding 5 mL of polyoxyethylenesorbitan monolaurate

(TWEEN; Sigma Aldrich, P1379) in 95 mL of sterile water and storing in a clean glass beaker. A phosphate buffered saline (PBS; Sigma Aldrich, P44177) solution was prepared by diluting 1 tablet in 200 mL of sterile water in a clean glass beaker and filtering the solution through a 0.2 µm filter. Other reagents were bought and stored accordingly until use. Autoclaved glassware and sterile water were used in all aspects of slide preparation.

Sample collection and slide preparation

From each microcosm, three 10 mL water samples were collected in new glass scintillation vials and brought back to the lab where 1 mL of glutaraldehyde (25% in H_2O, Fisher) was added to each vial to a final concentration of 2%. Once added, samples can be stored at room temperature for up to six months prior to further analysis. Samples were stored and transported back to Simon Fraser University for further analysis.

All slide preparation was conducted in a darkened lab. First, 50 µl of the 5% TWEEN solution was added to each vial, enabling the separation of humic particles and cells. Next, 1 mL of the DAPI working solution (0.1 µg L^{-1}) was added to each sample, vigorously shaken and set aside in the dark for at least 5 minutes to incubate.

One pre-stained 0.22 µm polycarbonate filter (25mm, black; Osmonics Poretic) was placed atop a pre-wetted GF/C filter (25mm; Whatman) and secured under a glass filtration tower. Two mL of PBS solution was gently filtered through to ensure the even distribution of cells on the filter. Next, 2 mL of DAPI-stained sample was filtered through followed by as much PBS solution as needed in order to wash away other humics normally found in fresh waters (at least 2 mL).

The polycarbonate filter needed to be completely dry prior to transferring to a glass slide containing a drop of immersion oil (Cargille Type A). The filter was placed on the slide followed by another drop of oil. Then a square 25 mm cover slip (No.2 glass, Fisher) was placed and secured with clear nail polish.

As a methodological control, a blank was created using sterile water and was prepared in the same way as the lake water samples.

Epifluorescent microscopy

Bacterial abundance was counted using a Carl Zeiss Axoiplan epifluorescent microscope in a darkened room to prevent fading of the DAPI stained slides. The microscope was equipped with a BP365/10 excited filter, FT 390 chromatic beam splitter, LP 395 barrier filter and a Neofluar 100/1.30 oil objective. Under a 1000x magnification, a minimum of 100 individuals or 10 field were counted.

Total bacterial abundance was calculated according to Sherr *et al.*(1993):

No. bacteria mL^{-1} = ((Y)(A)) / ((A*f*)(D)(V)) (4)

where:
- Y = mean number of cells per field of view
- A = effective filtration area of membrane filter (mm2)
- Af = area of field of view (mm^2)
- D = Dilution factor of glutaraldehyde to sample
- V = Volume of sample filtered (mL)