RESOURCE-LIMITED BACTERIOPLANKTON STIMULATED AT MID SALINITIES ACROSS THE SURFACE MIXED LAYER OF LAKE MELVILLE, CANADA.

by © Claire Moore-Gibbons

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Abstract

This study investigates dissolved organic matter (DOM) and nutrient cycling by heterotrophic bacterioplankton along a salinity gradient in the surface waters of the Lake Melville estuary, Canada. Understanding estuarine processing of DOM is essential to better constrain the potential environmental impacts of natural and anthropogenic hydrological changes to the estuarine source waters. Here, bacterial biomass and chlorophyll concentrations were compared with modelled conservative mixing, and suggests stimulation of surface bacterioplankton and primary production at mid salinities. Multi-day nutrient incubations in the dark revealed resource limitation of bacterioplankton during September when the flow rates of Churchill River are low. Changes to chemical constituents and bacterioplankton biomass suggest that a riverine-dominated site was predominantly C- and P-limited while a marine-dominated site was predominantly C- and N-limited. Results suggest that riverine inputs' control of microbial processes are relevant to future study of food webs in this boreal estuary.

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List of Abbreviations and Symbols

BA – Bacterial Abundance

BB – Bacterial Biomass

BCC – Bacterial Carbon Consumption

BGE – Bacterial Growth Efficiency

BP – Bacterial Production

BR – Bacterial Respiration

BV – Bacterial cell Volume

C – Carbon

CDOM – Coloured Dissolved Organic Matter

Chl *a* – Chlorophyll a

CTD – Conductivity Temperature Depth meter

$\delta^{13}C$ – $^{13}C/^{12}C$ isotope ratio

$\delta^{15}N$ – $^{15}N/^{14}N$ isotope ratio

DIC – Dissolved Inorganic Carbon

DIN – Dissolved Inorganic Nitrogen

DIP – Dissolved Inorganic Phosphorus

DO – Dissolved Oxygen

DOC – Dissolved Organic Carbon

DOM – Dissolved Organic Matter

DON – Dissolved Organic Nitrogen

EA-IRMS – Elemental Analysis-Isotope Ratio Mass Spectrometry

EOC – Excretion of Organic Carbon

G – 500‰-labeled ^{13}C glucose

HMW – High Molecular Weight

LCP – Lower Churchill Project

LMW – Low Molecular Weight

MAR – Marine-dominated location (of nutrient addition experiment)

N – Nitrogen

NH_4^+ – Ammonium

$NO_{2/3}^-$ – Nitrate/nitrite

OPA – Orthophthaldialdehyde

P – Phosphorus

PAR – Photosynthetically Active Radiation

PO_4^{3-} – Phosphate

POC – Particulate Organic Carbon

POM – Particulate Organic Matter

PON – Particulate Organic Nitrogen

$\delta^{13}C$-POC – stable carbon isotope signature of POC pool

$\delta^{15}N$-PON – stable nitrogen isotope signature of PON pool

psu – practical salinity units

RDL – Reported Detection Limit

RIV – Riverine-dominated location (of nutrient addition experiment)

S_R – Spectral slope Ratio

SRP – Soluble Reactive Phosphate

SUVA – Specific Ultraviolet Absorbance

TDN – Total Dissolved Nitrogen

TIC – Total inorganic carbon

δ^{13}C-TIC – stable carbon isotope signature of TIC pool

TOC – Total organic carbon

WCO-IRMS – Wet chemical oxidation isotope ratio mass spectrometry

Co-authorship Statement

Major contributions were made by Dr. Susan Ziegler and Dr. Robin Anderson to the design and identification of the research proposal, which was then articulated by me (Claire Moore-Gibbons). I prepared and conducted all fieldwork under the supervision of Dr. Ziegler and Dr. Richard Rivkin. I conducted all laboratory work (with instruction and training from lab managers) and data analysis, with the exception of chlorophyll *a* data that was provided by the Rivkin Lab at the Ocean Sciences Centre, Memorial University. I wrote the full thesis, including both manuscripts, and was assisted by comments and suggestions from my supervisor, Dr. Ziegler, and committee members Dr. Anderson and Dr. Rivkin.

CHAPTER 1

Resource-limited bacterioplankton stimulated at mid salinities across the mixed layer of Lake Melville, Canada.

Introduction and Overview

Estuaries

Estuaries are semi-enclosed bodies of water receiving riverine inputs that mix with oceanic water mass. This mixing creates physical gradients of salinity, density and temperature horizontally across the estuary, as well as vertically with increasing depth. It also has the potential to relieve resource-limiting conditions experienced by plankton in one or both environments, stimulating higher rater of production at the riverine-marine interface (Bianchi 2007). As a result, estuaries represent ideal habitat for juvenile stages of commercially important shelf species of fish and macroinvertebrates.

Estuaries receive large inputs of terrestrially-derived organic matter, however, only a small fraction of oceanic DOM is of terrestrial origin, suggesting that a great deal is processed in the coastal margins (Hedges et al. 1997; Opsahl & Benner, 1997; Hedges & Keil 1999, Middelburg & Herman 2007). Estuarine processes control fluxes of dissolved and particulate organic carbon (DOC and POC, respectively) to the coastal ocean as well as fluxes of CO_2 to the atmosphere, and therefore represent a key component in global carbon budget estimates. The processing of organic matter in estuaries is dependent on a number of factors including: the timing and quantity of allochthonous organic matter delivered to the estuary, the composition of DOM pools (which is linked to DOM source),

microbial activity, the availability of nutrients, and properties of the receiving environment including residence time and the estuary's mixing regime.

Estuaries can be characterized by their physical features, riverine inputs and type of mixing. These characteristics can provide insight into key processes occurring within the estuary. For example, stratification of an estuary will separate the pelagic and benthic bacterial communities, whereas a shallow estuary might exhibit complete mixing of the water column, and experience biochemical interactions absent from a stratified system (Almeida et al., 2001). The quantity, quality and rate of discharge of riverine inputs can also dictate the dominant processes of an estuary. Riverine inputs of carbon or nutrients to a resource-limited marine environment can stimulate production (Bratbak and Thingstad, 1985; Hecky et al., 1988; Caron,1994; Hopkinson, 1998; Cloern, 1999). The quality of the riverine inputs (i.e. its bioavailability) can influence the net metabolic state of an estuary, alternating between net autotrophy and net heterotrophy (Thottathil et al., 2008). The mixing regime of an estuary is related to the physical features and river discharge rate. The extent of water column mixing across an estuary can impact resource availability in surface waters, as well as the potential for carbon burial (Gattuso et al., 1998; Leithold et al., 2016).

Dissolved Organic Matter in Estuaries

Dissolved organic matter is an important source of carbon and nutrients to aquatic ecosystems accessed through microbial processes. In most coastal systems, DOM has both autochthonous and allochthonous sources, arising from primary production, and upstream and watershed inputs, respectively (Bianchi 2007). The source of DOM affects

its composition, which in turn determines what biogeochemical processes are most likely to act upon it. Autochthonous DOM is generally considered to be more labile or biodegradable, have lower aromaticity and lower molecular weight (LMW) (<1000 Da). Allochthonous DOM is considered more refractory, with higher aromaticity and high molecular weight (HMW) (>1000 Da) (Amon and Benner, 1996; Sun et al., 1997; Nagata, 2008). While bacteria require LMW compounds for assimilation, a LMW DOM pool is not necessarily an indicator of a labile bacterial substrate; the pool may be dominated by diagenetically altered LMW refractory DOM. Similarly, HMW DOM can be more labile than LMW but requires breakdown into its components before it can be assimilation by bacteria (Amon and Benner, 1994; Nagata, 2008).

Dissolved organic matter is a complex mixture that is difficult to characterize. Commonly measured fractions of the DOM pool include coloured dissolve organic matter (CDOM), dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) concentrations. The C:N ratio (DOC:DON) is often calculated and believed to be inversely related to the lability of the DOM pool (Balcarczyk et al., 2009). Specific ultraviolet absorbance (SUVA) is measured as an indicator of aromaticity since SUVA at 254 nm ($SUVA_{254}$) tends to increase with increasing aromaticity (Weishaar et al., 2003).

As riverine inputs enter the estuary, terrestrial DOM is exposed to longer residence times and greater light penetration that in rivers, which can enhance photochemical reactions and change the DOM pool. For example, photochemical transformations have been found to increase bioavailability of riverine DOM (Moran and Zepp, 1997; Moran et al., 2000), whereas they have been found to decrease bioavailability of autochthonous DOM

(Tranvik and Kokalj, 1998). Since assimilation by bacteria is the only way for DOM to re-enter the aquatic food web, changes to the availability of bacterial substrate can have large ramifications to the overall productivity of the system, and are described in the next section.

Bacterioplankton in the Estuarine Food Web

Heterotrophic bacteria are the most abundant organisms in aquatic systems, next to viruses. They use dissolved organic carbon as carbon (DOC) and energy source (Russel & Cook, 1995). Bacteria dominate DOM assimilation because of their high surface-to-volume ratio, which gives them an advantage in competing for dissolved compounds. The availability of organic phosphorus in DOM has been shown to regulate how bacteria use DOC (Lennon & Pfaff, 2005). Bacterial activity is also regulated by access to inorganic nutrients, and inorganic phosphorus has been frequently reported as a limiting nutrient (Chrzanowski et al. 1995; Cotner et al., 1997; Cotner et al., 2000; Jansson et al., 2006). While bacterial activity can be stimulated by inorganic nutrients, it can also be a source of inorganic nutrients through mineralization of organic matter (Kirchman, 1994). It is clear that bacteria play an important role in aquatic ecosystems for several reasons: 1) they act as mineralizers of organic substrate, providing inorganic nutrients and carbon for use in photosynthesis by primary producers (Kirchman, 1994); 2) they act as a conduit for the transfer of C from DOC to the aquatic food web through the microbial loop (Pomeroy 1974; Azam 1983); and, 3) they represent the largest contribution to community respiration (Robinson 2008).

The function of bacterial communities is especially important in terms of carbon and nutrient fluxes since the efficiency with which they convert assimilated carbon into biomass, or the bacterial growth efficiency (BGE), dictates the quantity of DOC transferred to higher trophic levels via the microbial loop. In turn BGE may in part be controlled by composition and source of DOC (Del Giorgio & Cole 1998). For example, when more refractory sources of DOM are used as bacterial substrate, a greater proportion of the carbon substrate is respired, decreasing the BGE (Kroer, 1993; Carlson et al., 1999). When bacteria use a more labile source of DOM, they tend to incorporate a greater proportion of carbon into their biomass, thereby increasing BGE and making a larger amount of carbon from the DOM pool available to the food web.

Bacterial activity clearly has the potential to alter global carbon budgets depending on its assimilation of DOC and its contribution to community respiration, but bacteria might also affect nutrient concentrations through remineralization of organic matter into inorganic nutrients (Kirchman, 1994). Bacterial remineralization of nutrients could increase nutrient-limited primary production, thereby increasing the pool of labile DOC for use as bacterial substrate. However, the uptake of inorganic nutrients by bacterioplankton has also been documented, suggesting that in some systems, bacterioplankton may be in direct competition with phytoplankton for inorganic nutrients (Bratbak and Thingstad, 1985). DOM availability and associated bacterial activity clearly play critical roles in the ecosystem processes of estuaries.

Resource limitation of bacterioplankton clearly has the potential to affect estuarine production, respiration, and the cycling of organic matter. Carbon limitation of

bacterioplankton activity has been documented in freshwater (Benner et al., 1995), marine (Kirchman 1990, Kirchman et al. 1990; Carlson & Ducklow, 1996; Cherrier et al., 1996; Caron et al., 2000; Church et al., 2000; Carlson et al., 2002) and in several estuarine studies (Findlay et al., 1992; Chin-Leo & Benner 1992). Nitrogen and phosphorus limitation of bacterioplankton activity has been suggested in rivers and river plumes (Chin-Leo and Benner, 1992; Smith and Kemp, 2003) as well as in the marine environment (Cotner et al., 1997; Rivkin and Anderson, 1997; Caron et al., 2000). Furthermore, a combination of resource limitation may exist wherein the addition of labile carbon along with inorganic nutrients could be expected to stimulate heterotrophic activity to a greater extent than carbon or nutrients alone (Pomeroy et al. 1995, Cotner et al. 1997, Rivkin & Anderson 1997, Shiah et al. 1998, Thingstad, 1998, Church et al. 2000, Donachie et al. 2001). Identifying the existence and nature of resource limitation of microbial communities can further an understanding of organic matter cycling in coastal waters, and allow for predictions to be made about the responses of bacterioplankton to changes in carbon and nutrient availability (Rivkin and Anderson, 1997; Cotner et al., 2000; Carlson et al., 2002; Hitchcock et al., 2010).

Constraining Environmental Impacts of Upstream Changes in Hydrology

Upstream changes in the hydrology of rivers and their watersheds have the potential to greatly alter downstream receiving environments. An understanding of biogeochemical cycling and the importance of allochthonous inputs in estuaries is critical to constraining the possible environmental impacts to estuaries of upstream changes. Changes in the quantity of DOM and nutrients delivered by river inputs could impact the microbial

production of the downstream system, while changes in the quality of DOM could shift the dominance from one set of processes to another. For example, delivery of more refractory terrestrial DOM could reduce microbial production while also increasing microbial respiration, thereby shifting the net metabolism of the system towards net heterotrophy (Thottathil et al., 2008). Additionally, changes to the timing of the delivery of DOM could result in major repercussions to system productivity. For example, if the bacterioplankton are nutrient limited and rely on delivery of riverine nutrients in order to assimilate autochthonous DOM, changes to the timing of the delivery of nutrients might result in the absence of autochthonous DOM when nutrients arrive. Similarly, if bacterioplankton are carbon-limited, changes to the timing of riverine DOM delivery might mean that phytoplankton have exhausted estuarine supply of inorganic nutrients by the time bacterioplankton receive their carbon substrate.

Hydrological changes to upstream environments are occurring due to climate change (Middelkoop et al., 2001; Jiang et al., 2007; Chang and Jung, 2010), but are exacerbated by activities such as hydroelectric development. These projects result in changes to the rate of freshwater discharge into the marine environment, which is often expressed as a dampening of the natural seasonal cycling of freshwater flow (e.g. reduced freshet), and therefore different water residence times. Only a few studies address downstream changes in mixing that alter the halo-, thermo- and pycnoclines of coastal water bodies (Kaardvedt and Svendsen 1990; Kaardvedt and Svendsen, 1995: as reviewed by Rosenberg et al. 1997). Changes to the hydrology also lead to erosion along rivers and lakes downstream, changing sediment loading to the freshwater, and potentially estuarine environments.

While there is great potential for hydroelectric impacts on marine ecosystems, there is relatively little known compared to hydroelectric effects on freshwater ecosystems (Rosenberg et al. 1995; Plourde et al. 1997).

Hydroelectric projects require the creation of reservoirs of varying size that can alter the concentration and composition of inorganic nutrients and DOM being delivered to estuaries (Egborge, 1979; Palmer & O'Keefe, 1990). Depending on the existing estuarine food web structure, changes in nutrient and DOM delivery can result in changes in ecological function including productivity, trophic status, and carbon flux. Reservoir creation has also been linked to the remobilization of contaminants (e.g. methyl mercury), negatively impacting lentic and lotic systems (Jackson, 1988; Anderson et al. 1995; Mucci et al. 1995; Kainz and Mazumder, 2005), as well as downstream marine environments. In freshwater, naturally occurring mercury previously trapped in sediments is thought to be methylated by sulfate-reducing bacteria under low oxygen conditions (Gagnon et al. 1996; Gilmour et al. 1992). Mercury levels in aquatic food webs, especially in fish, have been monitored over long periods of time due to their importance in human diet, and due to the fact that these levels increase with increasing trophic status, known as biomagnification (Anderson et al. 1995; Rudd 1995; Tremblay & Lucotte 1997; Kehrig et al. 1998; Waldron et al. 2000; Kainz and Mazumder, 2005). However, little effort has been invested in tracking the effect of contaminant remobilization to downstream marine ecosystems (Aleem 1972; Chen et al., 2008).

The effects of climate change will also cause changes to upstream watersheds and the delivery of allochthonous inputs to estuarine environments. Estuaries in boreal and arctic

regions receive relatively large inputs of terrestrial organic matter, but very little is known about how it is processed in the coastal margins, making it difficult to make accurate estimates of global carbon budgets and fluxes. Recent efforts have been directed towards understanding high latitude coastal systems and how they process terrestrial organic matter, in order to better constrain the potential environmental impacts of climate change (Mannino and Harvey, 2000; Berggren et al., 2010; Reader et al., 2014; Rontani et al., 2014; Tesi et al., 2014; Kulinski et al., 2016; Painter et al., 2018).

Lake Melville

Lake Melville is large (~3000 km^2) a high latitude estuarine fjord receiving inputs from the Churchill river, which drains a watershed of ~120 000 km^2 in Labrador, Canada. The Churchill river flows into Goose Bay and then Lake Melville, and is the dominant source of freshwater (50-80%) to the estuary. A second, smaller freshwater input called the Northwest River enters the system near the boundary of Goose Bay and Lake Melville. Gradients across the estuary are mainly driven by physical forcings such as salinity, temperature and wind direction and velocity with minimal tidal influence (Aquatic Environment in Goose Bay Estuary, AMEC & SNC Lavalin project); a narrow inlet from the Atlantic Ocean, and shallow sills at the mouths of Lake Melville and Goose Bay, reduce tidal range to 0.3 to 0.6 m compared to three times those values along the coast (Bobbitt and Akenhead 1982). Year-round stratification exists with freshwater from the Churchill river creating a thin surface layer with salinity generally under 10 ppt, followed by a brackish mixed layer with a sharp thermohalocline, and then a marine layer with higher salinities reaching 25 ppt (JWEL 2001: Biological Study of Goose Bay Estuary).

Temperatures in Goose Bay and Lake Melville are warmer than waters on the adjacent Labrador shelf, likely due to shallow sills (approximately 30m at its most shallow) that prevent mixing of colder ocean water with estuarine water below this depth (Bobbitt and Akenhead, 1982; JWEL 2001: Biological Study of Goose Bay Estuary). The mixing dynamics of Lake Melville estuary suggest that riverine inputs could be very influential in dictating estuarine ecosystem function.

Lake Melville is also downstream from an operational hydroelectric generating station, and a second one currently under construction, making it an ideal site to study the dominant biogeochemical processes across a high latitude estuary, with the additional potential to better constrain the impacts of hydroelectric development on a downstream marine environment. The operational hydroelectric development is located at Twin Falls in the Upper Churchill river. This development is one of the largest in the world (over 8000 MW; Baxter, 1977), and resulted in the creation of the Smallwood Reservoir (6650 km^2) in 1971 (Bruce and Spencer, 1979). In 2013, the construction of Lower Churchill Hydroelectric Generation Project, or Lower Churchill Project (LCP), began in order to develop the remaining hydroelectric potential of the Churchill River system, with generating facilities downstream from the Smallwood Reservoir at Muskrat Falls (JWEL 2001: Biological Study of Goose Bay Estuary). Further dampening of the seasonal fluctuation in Churchill river discharge is expected following the completion of the LCP, which could result in critical changes to biogeochemical cycling and the aquatic food webs of both the Churchill river and the downstream Lake Melville ecosystems.

Two approaches were taken to examine the importance of riverine inputs on microbial activity across the surface mixed layer in Lake Melville estuary. First, evidence of net production or loss of DOM and nutrients was assessed at the riverine-marine interface relative to a conservative mixing model. This was complemented by an assessment of microbial responses to changes in DOM and nutrients relative to conservative mixing. Second, a nutrient amendment experiment was conducted to determine if resource limitation of bacterioplankton existed in the surface mixed layer, and if there was a difference between a riverine- and a marine-dominated site.

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CHAPTER 2

Evidence for stimulation of bacterial production across the mixed layer of the western Lake Melville estuary, Labrador.

2.1 INTRODUCTION

Estuaries are unique bodies of water located at the interface of freshwater and marine environments where seawater is diluted by, and mixes with, a freshwater input (Bianchi 2007). Estuaries provide an essential region of mixing where gradients of salinity, density and temperature are created, which has the potential to relieve resource-limitation imposed by one or both environments (Bianchi 2007). As a result, estuaries represent areas of high biodiversity, creating suitable habitat for both freshwater and marine species, as well as providing shelter and food resources to juvenile stages of commercially important shelf species of fish and macroinvertebrates. Many estuaries have been found to be net heterotrophic, where community respiration exceeds autochthonous primary production (PP), an impossible situation to maintain unless the pool of dissolved organic matter (DOM) is subsidized by allochthonous inputs (Gattuso et al. 1998).

Coastal regions such as estuaries are particularly important with respect to global carbon fluxes because they are sites of exchange between land, sea and atmosphere. While their surface area is relatively small compared to the open ocean, these coastal water masses are enormously important in biogeochemical cycling due to the large inputs of organic matter and nutrients they receive from terrestrial sources (Gattuso et al. 1998). These

terrestrial inputs make up only a small fraction of oceanic DOM suggesting that a great deal is processed in the coastal margins (Hedges et al. 1997; Opsahl & Benner, 1997). More recently chemical evidence of terrestrially-derived DOM in the ocean has raised important questions regarding its export and processing within estuaries (Cao et al. 2018). Despite their importance in the global carbon cycle, there is still very little known about the production, consumption and transformation of organic matter in estuaries (Canuel & Hardison, 2016).

Estuaries are known to be regions of high primary production supported by terrestrial inputs of nutrients (Hopkinson, 1998; McAllister, 2006), however, many are known to be net heterotrophic (Gattuso et al., 1998). The metabolic state of estuaries is influenced by the processing of terrestrial organic matter and can vary seasonally within the same estuary, exerting great control over the amount and type of carbon, nitrogen and phosphorus that reach the coastal ocean (Raymond et al., 2000). As a result, there is significant effort being directed towards understanding how these river-influenced coastal systems regulate primary production, community respiration and carbon burial (Coffin & Sharp, 1987; Benner et al. 1991; Chin-Leo & Benner 1992; Gardner et al. 1994; Mannino & Harvey, 2000; Thottathil et al., 2000; Maranger et al. 2005; Bianchi, 2007; Fichot et al., 2014; Tesi et al., 2014; Osterholz et al, 2016; Medeiros et al., 2017).

The Microbial Loop

Dissolved organic matter is an important source of carbon and nutrients to aquatic ecosystems accessed through microbial processes. In most coastal systems, DOM has

both autochthonous and allochthonous sources, arising from primary production and upstream and watershed inputs, respectively (Bianchi 2007). As the relative contributions of DOM sources vary across estuaries, the coupling between estuarine bacterial production and algal production or riverine DOM inputs has also been found to vary (e.g. Findlay et al., 1991; Goosen et al., 1997). Investigating different fractions of the DOM pool can be useful in characterizing DOM source, composition and bioavailability (Benner, 2003; Aitkenhead-Peterson et al., 2003). Dissolved organic carbon (DOC) is the commonly measured fraction of DOM that is assimilated by bacterioplankton as a carbon and energy source.

Bacteria play an important role in aquatic ecosystems for several reasons: 1) they act as mineralizers of organic substrate, providing inorganic nutrients and carbon for use in photosynthesis by primary producers; 2) they act as a conduit for the transfer of C from DOC to the aquatic food web through the microbial loop (Pomeroy 1974; Azam 1983); 3) they represent the largest contribution to community respiration.

The function of bacterial communities is especially important in terms of carbon and nutrient fluxes since the efficiency with which they convert assimilated carbon into biomass, or the bacterial growth efficiency (BGE), dictates the quantity of DOC transferred to higher trophic levels via the microbial loop. In turn BGE may in part be controlled by composition and source of DOC (Del Giorgio & Cole 1998). The coupling of bacterial activity to DOM inputs from terrestrial origins, therefore, has the potential to alter carbon budgets through bacterial assimilation of DOC. They might also affect nutrient concentrations through mineralization of organic matter into inorganic nutrients,

which in turn can increase primary production and, therefore, autochthonous production of DOC. DOM availability and associated bacterial activity clearly play critical roles in the ecosystem processes of estuaries.

The Goose Bay and Lake Melville Estuary

The Churchill River drains a large watershed (~120 000 km^2) in Labrador, Canada, emptying into Goose Bay which is a part of the larger Lake Melville estuary. The Churchill River is the dominant source of freshwater (50-80%), to the estuary. A second, smaller freshwater input is the Northwest River, which joins the system near the boundary of Goose Bay and Lake Melville. Estuarine gradients are mainly driven by physical forcings such as salinity, tidal cycles, temperature and wind direction and velocity (Bianchi, 2007). In the case of Lake Melville, salinity, temperature and wind are the main components influencing physical mixing with minimal tidal influence (Aquatic Environment in Goose Bay Estuary, AMEC & SNC Lavalin project); a narrow inlet from the Atlantic Ocean, and shallow sills at the mouths of Lake Melville and Goose Bay, reduce tidal range to 0.3 to 0.6 m compared to three times those values along the coast (Bobbitt and Aikenhead 1982). The current at these sills is great, promoting strong localized vertical mixing at these sites. Conductivity, temperature and depth (CTD) measurements reveal three main layers with distinct physical characteristics. The freshwater input from the Churchill River creates a thin surface layer with salinity generally under 10 ppt. Below this, a brackish mixed layer exists where a sharp thermohalocline is present. Below this is a marine layer with higher salinities reaching 25 ppt (JWEL 2001: Biological Study of Goose Bay Estuary). Temperatures in Goose Bay

and Lake Melville are warmer than waters on the adjacent Labrador shelf, likely due to shallow sills (approximately 30m at its most shallow) that prevent mixing of colder ocean water with estuarine water below this depth (Bobbitt and Akenhead, 1982; JWEL 2001: Biological Study of Goose Bay Estuary). The mixing dynamics of Lake Melville estuary suggest that riverine inputs are indeed critical to its structure and function.

Goose Bay and Lake Melville represent important fishing areas, both commercially and for local Innu and Inuit communities. Additionally, fish assemblages are comprised of a variety of freshwater and marine species, indicating that this estuary is important in lifecycles of fish from both environments (JWEL 2001). While changes in DOM and nutrient delivery to the estuary may not have significant direct impacts on these fish stocks, these changes could affect plankton dynamics, altering the structure and function of the food web upon which these fish depend.

Currently, a hydroelectric generating station exists on the Upper Churchill River at Twin Falls, which resulted in the creation of the Smallwood Reservoir (6650km^2) in 1971 (Bruce and Spencer, 1979). This hydroelectric development is one of the largest in the world (over 8000 MW; Baxter, 1977) and much research has been conducted to determine the impacts of this development on the lotic, lentic and terrestrial environments immediately surrounding the Smallwood Reservoir (e.g. Brassard et al. 1971; Duthie & Ostrofsky 1974; Duthie & Ostrofsky 1975). For example, biomagnification of mercury is known to persist for over 20 years in higher trophic levels of the Smallwood Reservoir (Anderson et al. 1995, 1997; French et al. 1998). Very little is generally known regarding hydroelectric impacts on marine ecosystems, however, virtually nothing is known about

impacts on the highly productive Lake Melville estuary, apart from a focus on the downstream effects of methyl mercury remobilisation (Bajzak & Roberts, 2011; Anderson, 2011; Schartup et al., 2015; Calder et al., 2018).

In 2013, the construction of Lower Churchill Hydroelectric Generation Project, or Lower Churchill Project (LCP), began in order to develop the remaining hydroelectric potential of the Churchill River system, with generating facilities downstream from the Smallwood Reservoir at Muskrat Falls (JWEL 2001: Biological Study of Goose Bay Estuary). Given the impact of the Smallwood Reservoir on freshwater flow (i.e. dampening the seasonal variation by increasing discharge in low flow months and decreasing it in high flow months), it is reasonable to assume this second project will have similarly large effects. Reduction in the seasonal fluctuations in discharge could result in critical changes to the aquatic food web not only in the Churchill River but also in the downstream estuarine ecosystem, which depends upon the DOM and nutrient delivery (Bianchi, 2007).

Estuaries are largely influenced by their freshwater allochthonous inputs such that small changes in hydrology or chemical composition upstream, including those generated by hydroelectric development and climate change, could have disproportionately large effects on the receiving estuarine environment. Upstream changes could affect the delivery of freshwater, DOM and nutrients, both in terms of quantities and in terms of the physical and chemical processes by which they enter the estuary. An understanding of the current distribution of DOM, nutrients, and microbial biomass across Lake Melville estuary will provide a basis for interpreting the importance of allochthonous inputs and

autochthonous production in supporting the estuarine food web and directing ecosystem function.

In this paper, the focus is on examining carbon and nutrient dynamics in Goose Bay and Lake Melville, and their relationship to inputs from the discharge of the Churchill river. The physical mixing that drives this system permits the use of a conservative mixing model to investigate the distribution of organic matter and nutrients across the estuary's horizontal salinity gradient (Bianchi 2007). Deviations from a theoretical physical mixing line drawn between riverine and marine end-member values will be interpreted as the addition or removal at intermediate salinities due to biological or chemical processes, or a combination thereof. For example, DOC discharged from the Churchill River is diluted by seawater across the estuary. An increase in DOC at a mid-salinity could indicate increased algal production at that location, whereas a decrease in DOC (below the value predicted by physical mixing alone) could indicate increased bacterial metabolism or flocculation (Figure 2.1).

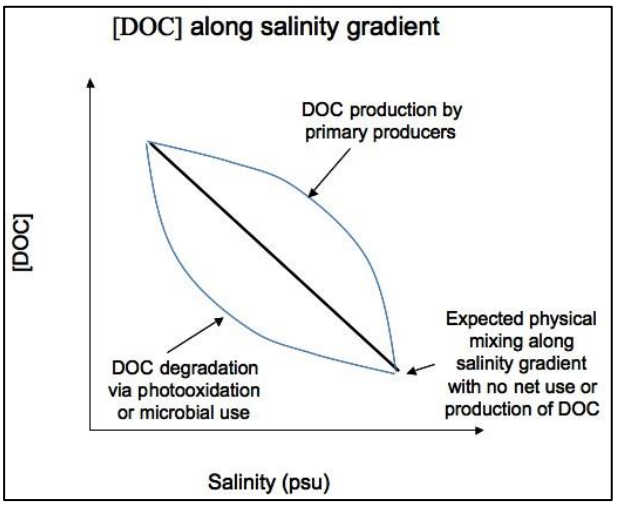


Figure 2.1. Example of conservative mixing model depicting DOC concentration across an estuarine salinity gradient (adapted from Bianchi 2007).

The objective of this research was to investigate the potential influence of hydrological changes of a large boreal river on the receiving estuarine ecosystem by investigating the delivery and transformation of DOM and nutrients, and their impacts on microbial processes. This paper seeks to achieve this objective by:

(1) Assessing evidence of net production or loss of dissolved organic matter and nutrients at the riverine-marine interface in the Lake Melville estuary; and,

(2) Assessing evidence of the stimulation of microbial processes in response to changes in DOM and nutrients across the riverine-marine interface.

2.2 METHODS

A sampling campaign was carried out in the fall of 2008 to capture the biogeochemical properties of the water column within Goose Bay and the western Lake Melville estuary. Water column properties measured with the conductivity-temperature-depth (CTD) meter include: depth, temperature, salinity, and dissolved oxygen concentration. These measurements were taken using Sea-Bird Electronics (SBE) 25 Sealogger CTD and conducted in collaboration with Fisheries and Oceans Canada. Some initial CTD casts were conducted to estimate the pynocline and to aid in selecting representative sampling stations across Goose Bay and into Lake Melville. Depth profile sampling to capture above, close to and below the pycnocline took place at 6 stations on September 23-28, 2008 along a transect beginning near the mouth of the Churchill River and extending northwest into Lake Melville (Figure 2.2). The main freshwater input was identified as the Churchill River, with a mean annual discharge estimated at $(5.72 \pm 0.72) \times 10^{10}$ m^3

(MAE, 1972-2008). The North West River also contributes freshwater to the Lake Melville estuary, with a mean annual discharge estimated at 2.83 ± 0.48) x 10^9 m^3, using Naskaupi River discharge as a proxy (MAE 1978-2011).

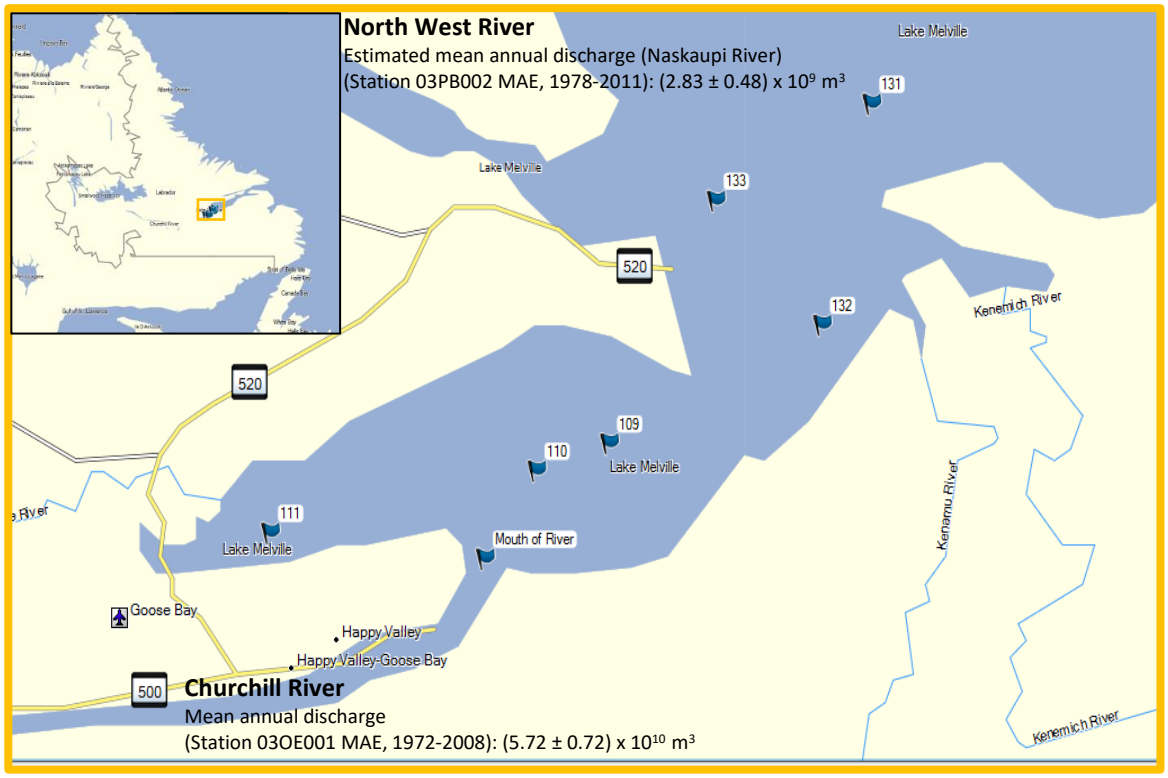


Figure 2.2. Map of sampling stations (109-111 and 131-133) across Goose bay and Lake Melville

Discrete water samples were collected at the three depths at each station. The sampling depths were selected based on the pycnocline, with the intention of capturing information from within a mixed surface layer in the euphotic zone, and from below this mixed layer. The pycnocline offers a logical boundary at which to divide these layers, allowing for the quantification of variation with depth as well as laterally across the estuary. The pycnocline depth was calculated from the greatest change in conductivity per metre. Water samples were processed for analysis of total inorganic carbon concentration

([TIC]), TIC stable carbon isotopes (δ^{13}C-TIC), dissolved inorganic phosphorus (DIP) in the form of phosphate concentration ([PO_4^{3-}]), dissolved inorganic nitrogen (DIN) in the forms of nitrate/nitrite concentration ([$NO_{2/3}^-$]) and ammonium concentration ([NH_4^+]), dissolved organic carbon concentration ([DOC]), total dissolved nitrogen concentration ([TDN]), particulate organic carbon concentration ([POC]), POC stable carbon isotopes (δ^{13}C-POC), particulate organic nitrogen concentration ([PON]), PON stable nitrogen isotopes (δ^{15}N-PON), absorbance measurements, chlorophyll *a* concentrations, and bacterial abundance and cell volume measurements (BA and BV). These measurements were used within a series of conservative mixing models to estimate possible deviations from conservative mixing and how they relate to proxies for phytoplankton and bacterioplankton abundance and biomass.

Field Sampling

Samples were collected aboard the vessel using 5-L Niskin bottles mounted individually on a cable and lowered to the predetermined depth based upon the CTD cast results. Samples were first allocated from the 5L Niskin bottle into 40mL amber glass vials with no headspace using butyl rubber septa, fixed with 400 μL of a saturated solution (7.4g/100ml) of mercuric chloride and stored at 4°C for analysis of total inorganic carbon (TIC) concentration and stable carbon isotope ratios (δ^{13}C-TIC). Following the TIC water sample collection, large volume samples were collected into 1L amber HDPE bottles and taken back to the field laboratory for immediate processing and/or filtration in the order described below:

a) Samples were filtered through pre-combusted glass fiber filters (Whatman GF/F) and collected in 60mL HDPE Nalgene containers, frozen at approximately -20°C to be analyzed for concentrations of dissolved organic carbon (DOC), total dissolved nitrogen (TDN),

b) Aliquots of the GF/F filtrate were collected into 60ml falcon tubes and frozen to be analyzed for phosphate (PO_4^{3-}), nitrite/nitrate ($NO_{2/3}^{-}$), and ammonium (NH_4^{+}).

c) A final aliquot of the GF/F filtrate was collected in dark 60mL HDPE Nalgene containers and stored at 4°C in preparation for absorbance measurements.

d) Whole water samples (15 mL) were collected into 15mL falcon tubes and fixed with 1.5 mL of GF/F filtered 37% formaldehyde (formalin) for bacterioplankton abundance and cell volume analysis.

e) Measured volumes of sample were also filtered through pre-combusted GF/F filters for the collection of particulate samples for chlorophyll *a* (0.7μm to 5μm fraction, and >5μm fraction), particulate organic carbon (POC) and particulate organic nitrogen (PON) concentrations.

f) Finally, measured samples were filtered through pre-combusted quartz filters to collect POC and PON for δ^{13}C-POC and δ^{15}N-PON analysis. All filters were stored in pre-combusted aluminum foil envelopes. Volumes filtered were all between 500ml and 1000ml and were recorded for each sample.

Conservative Mixing Model Approach

Depth profiles of salinity, temperature and density were constructed at each station established within Goose Bay and western Lake Melville to examine the mixing of riverine and marine water masses. For the purpose of visualizing changes in salinity, temperature and density across the estuary, a transect line was drawn beginning at the most riverine- dominated station, closest to the Churchill River mouth, to the most marine-dominated station; all inter-lying stations were assigned a location along the transect based on their distance from the mouth of the Churchill River. This method of assigning station locations along the transect based on distance from the mouth of the Churchill River aligned well with increasing salinity across the mixed layer of the estuary. The depth profiles collected using the CTD casts were used to identify the pycnocline at each station, that is the depth at which the greatest rate of change in density occurs. Location of the pycnocline enabled the identification of the mixed layer.

Information from the depth profiles was used to select a representative subset of stations for a comprehensive sampling regime across the range of salinities. A conservative mixing model was established for each constituent analyzed as part of the sampling regime. The model relies on measurements from two endmember stations to make predictions about the distribution of each constituent across the salinity gradient that would result from physical mixing alone. The conservative mixing model, therefore, does not account for chemical or biological processes that might also cause the constituent to vary. This model is based on that discussed by Bianchi (2007) and is similar in approach to the mixing models employed in many studies including Peterson et al. (1994), Cifuentes and Eldridge (1998), Benner and Opsahl, (2001), Raymond and Bauer (2001)

and Markager et al. (2011). Any deviations from the predicted mixing line of the model are expected to result from processes outside of physical mixing of the riverine and marine or oceanic water masses. For example, production and degradation processes leading to increases or decreases in DOC, respectively, in relationship to what is expect from estuarine mixing. The endmember stations selected for the mixing model were the most riverine- and marine-dominated sampling stations based on their salinity depth profiles, and proximity to the mouth of the Churchill River. Since a freshwater endmember of 0 practical salinity units (psu) was not a requirement of this model, the riverine endmember was the station located closest to the mouth of the Churchill River. The marine endmember was the station located furthest from the mouth of the Churchill River, out into western Lake Melville. The predictions made using the conservative mixing equation can be sensitive to changes in the endmember selection, such that a different predicted distribution of a given constituent might be produced when a different end-member is used. This will be taken into consideration when interpreting and discussing the results.

A conservative distribution of each constituent was estimated using the following equation:

$$C_{mix} = fC_R + (1-f)C_O \qquad \text{eq. 1}$$

such that the predicted concentration of a constituent in the mixed layer (e.g. DOC, TIC, inorganic nutrients) at each station was calculated as C_{mix}. The fraction of freshwater in each sample is f, calculated based on the salinity of each sample with respect to the sample of highest salinity (the marine endmember). The concentration of a constituent at

the riverine end-member (C_R) and marine end-member (C_O) were used to calculate C_{mix}. The predicted carbon stable isotope values for TIC or POC ($\delta^{13}C$) and nitrogen stable isotope values for PON ($\delta^{15}N$) were estimated based on the following equation:

$$\delta_{mix} = [f\delta_R C_R + (1\text{-}f)\,\delta_O C_O] / C_{mix} \qquad \text{eq. 2}$$

The stable isotope value of a conservatively distributed constituent is δ_{mix}, and the stable isotope values of the riverine and marine ("oceanic") end-members are δ_R and δ_O, respectively. Concentrations and stable isotope ratios determined using this simple conservative mixing model approach were directly compared with the measured values to determine the potential existence of non-conservative behavior of the measured constituent.

In order to better interpret the deviations of measured values from the conservative mixing model, 95% confidence intervals were calculated for the modeled riverine and marine endmembers using the following equation:

$$CI_{95\%} = C_{mix} \pm [t_{crit} (\frac{Smix}{\sqrt{n}})] \qquad \text{eq. 3}$$

where $CI_{95\%}$ was calculated for the lower and upper limits of the 95% confidence interval for each predicted endmember. The standard deviations used to calculate 95% confidence intervals for the modeled endmembers was S_{mix}, and was estimated using the following equation (similar equation 1 above):

$$S_{mix} = fS_R + (1\text{-}f)S_O \qquad \text{eq. 4}$$

For equation 4, as in equation 1, the fraction of freshwater in each sample is f, calculated based on the salinity of each sample with respect to the sample of highest salinity (the marine endmember). The standard deviation of the mean at the riverine endmember (S_R) and marine endmember (S_O) were used to calculated S_{mix}.

Deviations from the conservative mixing model are interpreted here as indicative of stimulation of chemical or biological processes that resulted in an increase or decrease of the given constituent. These processes could include: 1) flocculation of riverine DOM caused by dramatic change in pH and ionic strength resulting in a reduction of DOC (Sholkovitz, 1976); 2) autochthonous primary production, which might be stimulated by relief of nutrient limitation due to mixing water masses, and cause increases in DOC and decreases in TIC, DIN or DIP; 3) heterotrophic activity including respiration, which might be stimulated by the relief of nutrient limitation and/or availability of labile DOM, and cause a reduction in DOC, DON and dissolved oxygen (DO). Additionally, photochemical transformations have the potential to produce deviations from a conservative mixing line by: 1) increasing DOM bioavailability through photochemical production of small organic acids (Kieber, 2000); and 2) mineralizing DOM, thereby reducing available substrate for bacterioplankton. There is also a possibility that confounding effects from other freshwater inputs to this estuary (e.g. the Northwest River) may result in distributions that deviate from the conservative mixing line used in this study. The Northwest river is most likely to affect sampling results at station 133, where a more riverine profile of biochemical constituents might be expected compared to

other nearby stations (i.e. stations 132 and 131), and this is taken into consideration in the interpretation and discussion of the results.

Biochemical Analyses

Inorganic Carbon and Nutrients

Wet chemical oxidation isotope ratio mass spectrometry (WCO-IRMS) was used to determine concentrations and $^{13}C/^{12}C$ isotope ratios ($\delta^{13}C$) of TIC of the preserved samples collected. TIC concentrations and $\delta^{13}C$ values were determined using an AURORA 1030 TOC analyzer (O.I. Analytical, College Station, Texas, USA) coupled to a MAT252 isotope ratio mass spectrometer (Finnigan, Bremen, Germany). As reported above, values for $\delta^{13}C$ are reported in per mil units (‰) relative to the international reference standard Vienna Pee Dee Belemnite (R = 0.011237). The analytical precision for $\delta^{13}C$-TIC was <0.4‰.

Phosphate and nitrite/nitrate were analyzed using established colorimetric techniques using a Technicon AutoAnalyzer II (Mitchell et al. 2002). The analysis of dissolved inorganic phosphate (DIP) is based on the formation of a phosphomolybdenum blue complex, which measures soluble reactive phosphate (SRP). The analysis of nitrate+nitrite is based on the measurement of a diazo dye formed by the reaction between sulfanilamide and nitrite, which was produced following the reduction of nitrate to nitrite on a copperized cadmium column prior to the colorimetric analysis. Ammonium concentration was determined using a method involving the reaction of ammonia with orthophthaldialdehyde (OPA) as described by Kerouel and Aminot (1997). Standards

were prepared using Na_2HPO_4 dissolved in NaCl solutions made up to the salinity of the samples being analyzed (i.e. salinity ranging from 0 to 33 g/L).

Organic Carbon and Nitrogen Pools

Concentrations of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were determined using high temperature combustion on a total organic carbon analyzer (Shimadzu TOC-V) equipped with a chemiluminescent NOx detector (TNM-1; Shimazu, Japan). Dissolved organic nitrogen (DON) concentrations were calculated as difference between the TDN concentrations and total dissolved inorganic nitrogen (DIN) concentrations (sum of ammonium and nitrate+nitrite concentrations). Molar C:N of the DOM was calculated by dividing DOC by DON. Elemental analysis-isotope ratio mass spectrometry (EA-IRMS) was used to determine the $\delta^{13}C$ of POC ($\delta^{13}C$-POC) and $\delta^{15}N$ of PON ($\delta^{15}N$-PON), as well as POC and PON concentrations. Values for $\delta^{13}C$ are reported in per mil units (‰) relative to the international reference standard Vienna Pee Dee Belemnite (R = 0.011237). The analytical precision for $\delta^{13}C$-POC was <0.2‰. These measurements were made using a Carlo Erba NA1500 Series II elemental analyser interfaced to a MAT252 isotope ratio mass spectrometer (Finnigan, Bremen, Germany) via a Conflo II interface.

Optical Properties

Absorbance measurements of the collected filtrates were conducted using a 1cm path length cuvette at wavelengths spanning 250 to 325 nm using a UV/VIS scanning

spectrometer (Lambda 25; Perkin Elmer, Waltham, MA, USA) and a 1 cm quartz cuvette, with NanoPure-UV water as a blank. Absorption coefficients were calculated as:

$$a(\lambda)=2.303A(\lambda)/L \quad \text{eq. 5}$$

where A(λ) is the absorbance at wavelength λ and L is the path length (m) of the cuvette (Green and Blough, 1994). Specific ultraviolet absorbance (SUVA) (μM C m^{-1}) was also determined by normalizing absorption coefficients at 254 nm (a_{254}) to DOC concentration. $SUVA_{254}$ has been shown to correlate strongly with DOM aromaticity (Weishaar et al. 2003; Helms et al., 2008).

The spectral slope (S) was obtained from absorbance spectra using the equation:

$$a\lambda = a\lambda_0 \, e^{[-S(\lambda - \lambda 0)]} \quad \text{eq. 6}$$

where $a\lambda_0$ is the reference wavelength and S is the spectral slope. Spectral slope is a measure of how quickly absorption decreases with increasing wavelength. The spectral slopes for wavelength intervals 275 to 295 nm ($S_{275\text{-}295}$) were calculated using linear regression of the log transformed absorbance data (Helms et al., 2008). The use of narrow wavelength intervals, such as 275-295 nm can help to reduce variation in the spectral slope that might be exhibited by a broader interval (e.g. 280-400 nm) (Brown 1977). Furthermore, the spectral slope from 275-295 nm has been found to be a good proxy for chromophoric DOM (CDOM) molecular weight (MW) (Helms et al., 2008), varies more closely according to the type of aquatic environment and has a high chance of being accurately determined (Asmala et al., 2012).

Though not measured in this study, iron concentrations should be considered when investigating optical properties of water samples since Fe-DOM complexes have the potential to contribute to absorbance features of natural waters (e.g. Weishaar et al., 2003; Maloney et al., 2005). Iron concentrations of the Churchill river will be discussed following the presentation of the results of the optical analyses.

Chlorophyll a Analysis

Chlorophyll *a* was extracted from the GF/F and 5μm filters collected in the dark by placing them in scintillation vials with 5ml of 90% acetone and incubating overnight in the freezer (Parsons et al., 1984). Fluorometric measurements of the final, filtered extracts (>5μm fraction and >0.7μm fraction) were taken on a Turner fluorometer (model 111) at the Ocean Sciences Centre of Memorial University, calibrated with commercially prepared pure chlorophyll *a* (Sigma Chemical Co., St Louis, MO, USA).

Bacterioplankton Analysis: Acridine Orange Staining and Epifluorescence Microscopy

The water samples collected and preserved for bacterial abundance were filtered onto Irglan-black-stained 0.2 μm polycarbonate filters then stained with Acridine Orange prepared to a concentration of 0.468 g L^{-1}. Two filters were prepared from each water sample and were observed using an Olympus BH-2-RFCA epifluorescent microscope, equipped with a 120x oil immersion lens (1250x magnification total), and Acridine Orange filter. At least 1000 Acridine Orange stained cells were counted for each filter (Pernthaler et al. 1998).

To calculate bacterial biomass, a conversion factor was used to estimate mass of carbon per cell based on cell volume measurements. Cell volume estimates were made from the same slides that were prepared for abundance using ImagePro Plus v. 6.2 with measurements of length, width and aspect ratio used to calculate cell volume. The conversion factor used was $120v^{0.72}$, where v is cell volume (Norland 1993). Volume was calculated as a sphere or cylinder depending on whether the aspect ratio of the bacterial cell was less than or greater than 1.5, respectively. This mass of carbon per cell was multiplied by corresponding bacterial abundance measurements to determine biomass in terms of mass of carbon per litre.

2.3 RESULTS

Physical Description of the System

Conductivity, temperature and depth (CTD) measurements along a transect across Goose Bay and Lake Melville were conducted on September 25, 26 and 28, 2008. Surface salinity was found to increase with increasing distance from the mouth of the Churchill River ranging from 1 psu to 16 psu. Surface temperature was relatively constant across the transect, ranging from approximately 7°C to 12°C. This suggests that the surface gradient is primarily salinity-driven (Figure 2.3).

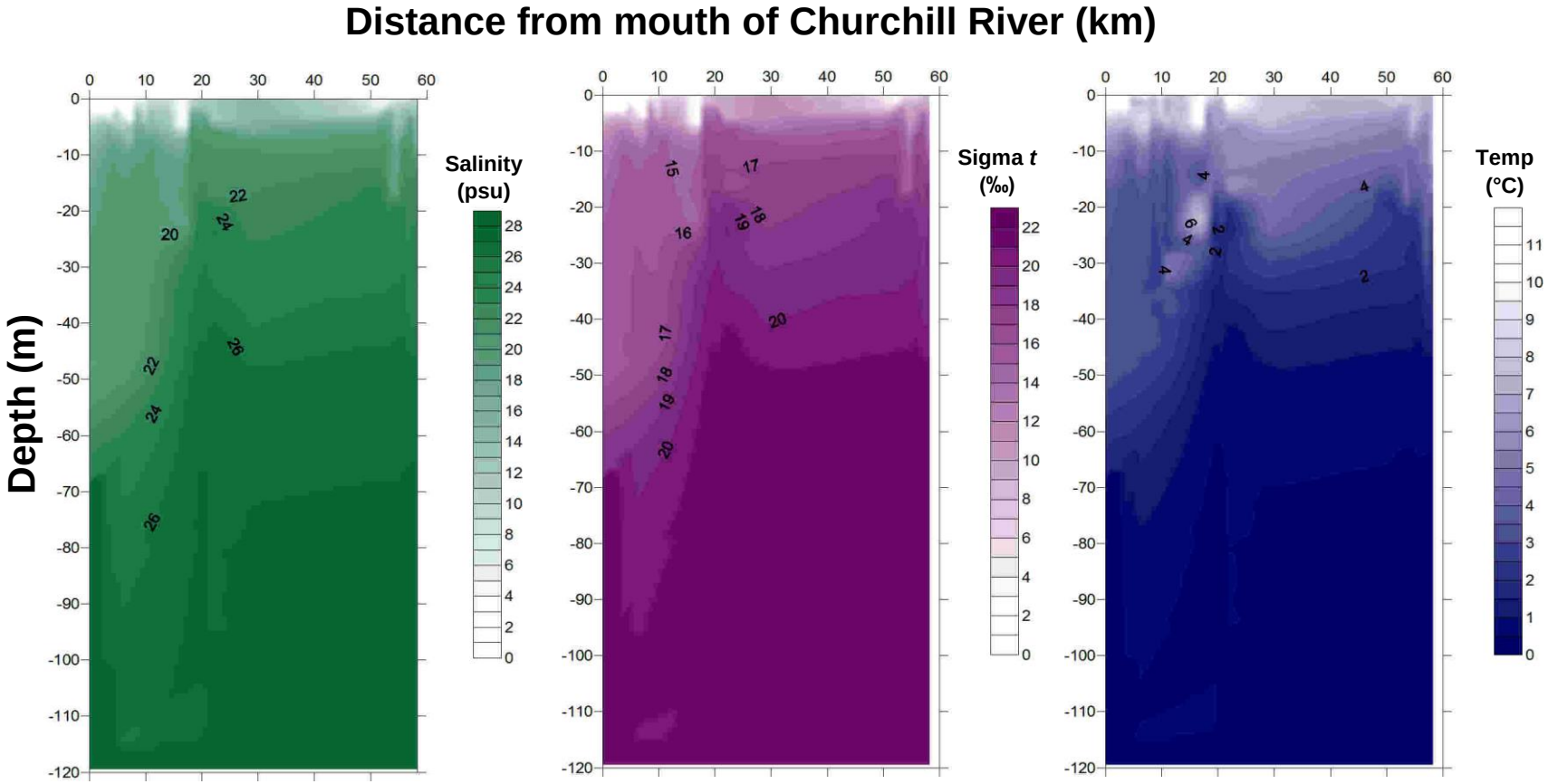


Figure 2.3. Contour depth profiles for salinity, sigma *t* and temperature (*from left to right*).

The pycnoclines were calculated at these sampling stations, and ranged from depths of 1.7 m to 6.3 m. The surface mixed layer was determined to be the portion of the water column located above the pycnocline. The waters of the surface mixed layer ranged from 7.7 to 8.7 °C, with salinities ranging from 3.0 to 14.9 psu. Dissolved oxygen concentrations in the surface mixed layer ranged from 9.3 to 10.3 mg O_2 L^{-1}, with percent saturation from 77.0% to 86.7%. Below the pycnocline, temperatures across the transect ranged from 0.3 to 8.5°C and salinity ranged from 18.0 to 27.1 psu. Dissolved oxygen concentrations below the pycnocline ranged from 6.6 to 9.8 mg O_2 L^{-1}, with percent saturation from 49.2% to 68.4% (Table 2.1).

Table 2.1. Water Column Properties: 2008 Field Season

Sample Collection					**CTD Measurements**					**Pycnocline**	
Date in 2008	**Time (local)**	**Site**	**Way-point**	**Sample Depth (m)**	**Salinity (psu)**	**Temp (°C)**	**Sigma *t* (σ_t)**	**Diss. O_2 (mg O L^{-1})**	**Diss. O_2 % Sat.**	**Depth (m)**	**Sample Above or Below**
Sep 24	09:11	GB	109	3	2.97	8.57	2.15	10.27	86.71	3.4	Above
				15	19.38	8.52	8.34	7.83	58.35		Below
				30	20.13	3.90	15.39	7.16	53.19		Below
Sep 24	11:37	GB	110	3	3.96	8.70	2.92	9.99	84.36	4.7	Above
				10	17.98	5.33	14.19	7.92	62.06		Below
				20	19.83	3.26	15.79	6.99	51.92		Below
Sep 24	14:00	GB	111	3	10.56	7.93	8.15	9.68	79.76	5.8	Above
				10	18.72	4.29	14.84	7.61	58.07		Below
				25	19.92	3.03	15.87	6.62	49.19		Below
Sep 27	09:00	LM	131	3	14.86	7.72	11.53	9.34	76.96	6.3	Above
Sep 23	*09:45*	*LM*		40	24.90	1.66	19.90	8.05	56.62		Below
CTD data from wpt 108, malfunction at 131				120	27.19	0.31	21.80	7.76	53.07		Below
Sep 27	09:55	LM	132	2	13.58	5.96	10.68	8.92	68.44	1.7	At pycno
				10	21.60	4.89	17.08	8.11	60.27		Below
				20	23.49	3.06	18.70	8.00	57.36		Below
Sep 27	11:33	LM	133	2	13.44	8.26	10.37	9.28	78.01	2.2	Above
				40	25.82	1.19	20.66	7.44	52.35		Below
				90	27.10	0.40	21.72	7.32	50.04		Below

GB=Goose Bay; LM=Lake Melville

Biochemical Description of the System

Sampling was conducted above and below the pycnocline at each station in order to compare the characteristics of the surface mixed layer across the transect, separate from those characteristics of the water mass below the mixed layer. Mixed layer sampling occurred at 2 to 3 m and ranged in salinity from 2.97 to 14.86 psu.

Total inorganic carbon (TIC) and nutrient concentrations (PO_4^{3-}, $NO_{2/3}^-$, NH_4^+) were generally lower across the mixed layer than below the pycnocline. Stable carbon isotopes of TIC (δ^{13}C-TIC) were similar above and below the pycnocline but were slightly more variable in the mixed layer than below. Both dissolved and particulate organic carbon (DOC and POC) were higher in the mixed layer. Dissolved organic nitrogen (DON) was lower in the mixed layer whereas particulate organic nitrogen (PON) was higher. Stable carbon isotopes of POC (δ^{13}C-POC) didn't appear to vary with depth nor with distance from the Churchill River, however, stable nitrogen isotope ratios of PON (δ^{15}N-PON) were more elevated closer to the river and lower further from the river out into Lake Melville. Specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) and spectral slope from 275 to 295 nm ($S_{275-295}$) were both fairly consistent across the surface mixing layer above the pycnocline, while both exhibited slightly greater variation below the pycnocline. The concentrations of both fractions (0.7μm-5μm and >5μm) of chlorophyll *a* were higher in the mixed layer compared to below the pycnocline. Bacterial abundance, cell volume and biomass were also all higher in the mixed layer.

Inorganic Carbon and Nutrients

Inorganic carbon (IC) concentrations ranged from 351.6 ±3.7 to 943.6 ±29.9 μM C in the mixing region above the pycnocline with stable carbon isotope composition ranging from -2.63 ±0.58 to 0.18 ±0.17 ‰. Below the pycnocline, 2008 IC concentrations ranged from 540.8 ±14.7 to 1887.0 ±3.5 μM C and stable carbon isotope ratios were -2.09 ±0.42 to -0.02 ±0.10 ‰ (Table 2.2). Mixed layer phosphate concentrations were below detection to 0.25 ±0.04 μM P, combined nitrite/nitrate concentrations were 0.75 ±0.01 to 5.85 ±0.35 μM N and ammonium concentrations were 0.88 ±0.01 to 2.76 ±0.23 μM N. Below the pycnocline, phosphate was 0.15 ±0.01 to 0.90 ±0.04 μM P, nitrite/nitrate was 6.54 ±0.04 to 12.16 ±0.10 μM N and ammonium was 0.50 ±0.03 to 3.28 ±0.11 μM N (Table 2.2).

Table 2.2. Inorganic Carbon and Nutrients: 2008 Field Season

Sample Collection			**Nutrients**			**Total Inorganic Carbon**	
Way-point	**Sample Depth (m)**	**Salinity (psu)**	**[PO_4^{3-}] (μM P)**	**[$NO_{2/3}^-$] (μM N)**	**[NH_4^+] (μM N)**	**[TIC] (μM C)**	**δ^{13}C-TIC (‰)**
109	3	2.97	0.15 ± 0.01	5.85 ± 0.35	1.08 ± 0.09	943.6 ± 29.9	-0.70 ± 0.66
	15	19.38	0.51 ± 0.02	11.43 ± 0.54	0.60 ± 0.03	1440.4 ± 11.0	-1.75 ± 0.08
	30	20.13	0.65± 0.03	11.19 ± 0.14	1.37 ± 0.08	1458.3 ± 1.7	-1.67 ± 0.54
110	3	3.96	0.25± 0.04	0.75 ± 0.01	2.76 ± 0.23	351.56 ± 3.7	-2.63 ± 0.58
	10	17.98	0.35± 0.01	8.05 ± 0.12	0.55 ± 0.23	1221.1 ± 10.4	-1.46 ± 0.40
	20	19.83	0.46 ± 0.02	11.57 ± 0.02	0.66 ± 0.01	1473.1 ± 2.6	-1.94 ± 0.55
111	3	10.56	BD	2.60 ± 0.10	1.77 ± 0.05	676.2 ± 18.6	-1.02 ± 0.50
	10	18.72	0.32 ± 0.01	8.80 ± 0.08	0.50 ± 0.03	1213.0 ± 0.1	-1.73 ± 0.33
	25	19.92	0.42 ± 0.02	12.16 ± 0.10	0.63 ± 0.06	1462.8 ± 28.9	-2.09 ± 0.42
131	3	14.86	BD	1.57 ± 0.05	1.85 ± 0.03	812.1 ± 13.46	-1.22 ± 0.66
	40	24.90	0.82 ± 0.02	9.24 ± 0.09	2.17 ± 0.28	1791.9 ± 3.8	-0.16 ± 0.11
	120	27.19	0.86 ± 0.06	9.98 ± 0.10	1.53 ± 0.06	1887.0 ± 3.5	-0.29 ± 0.01
132	2	13.58	BD	1.44 ± 0.04	1.66 ± 0.02	540.8 ± 14.7	-1.35 ± 0.70
	10	21.60	0.59 ± 0.01	6.54 ± 0.04	0.54 ± 0.06	1458.6 ± 17.9	-0.16 ± 0.05
	20	23.49	0.67 ± 0.03	6.82 ± 0.06	3.28 ± 0.11	1557.6 ± 1.9	-0.02 ± 0.10
133	2	13.44	0.14 ± 0.01	3.44 ± 0.05	0.88 ± 0.01	925.3 ± 23.6	0.18 ± 0.17
	40	25.82	0.88 ± 0.03	9.53 ± 0.02	0.60 ± 0.05	1734.67 ± 10.5	-0.03 ± 0.14
	90	27.10	0.90 ± 0.04	9.49 ± 0.06	1.62 ± 0.05	1847.6 ± 26.45	-0.11 ± 0.18
BD = Below Detection							

Organic Carbon and Nitrogen

Dissolved organic carbon (DOC) concentrations were also greater above the pycnocline where concentrations ranged from 250 ± 3 to 311 ± 3 μM C. Below the mixing layer, concentrations of DOC ranged from 86.2 ±0.3 to 199 ± 2 μM C. Dissolved organic nitrogen (DON) concentrations were lowest near the surface and ranged from 3.30 ±0.58 to 8.71 ±0.71 μM N while below the pycnocline, concentrations ranged from 4.44 ±0.63 to 16.76 ±0.37 μM N (Table 2.3). The C:N ratio of the dissolved organic matter (DOM) pool was clearly higher at the mixed region above the pycnocline and ranged from 30.5 ±2.5 to 87.8 ±15.4, while it ranged from 8.2 ±0.4 to 41.6 ±5.9 below the mixed surface layer (Table 2.3).

Particulate organic carbon (POC) and nitrogen (PON) concentrations were higher at the surface from 114.6 to 271.4 μM C and 11.7 to 24.3 μM N, respectively, with a C:N ratio of POM from 8.5 to 11.3. Below the pycnocline, POC concentrations ranged from 40.2 to 255.1 μM C and PON concentrations ranged from 3.1 to 22.6 μM N, with a C:N ratio of 8.5 to 17.2. Stable isotope ratios of the POC pool were similar across the estuary and ranged from -32.96‰ to -29.74‰ whereas the PON stable isotope ratios were all positive in Goose Bay from +0.75‰ to +3.58‰ and all negative in Lake Melville from -9.89‰ to -1.18‰ (Table 2.3).

Table 2.3. Organic Carbon and Nitrogen: 2008 Field Season

Sample Collection			Dissolved Organic Carbon and Nitrogen			Particulate Organic Carbon and Nitrogen				
Way-point	Sample Depth (m)	Salinity (psu)	[DOC] (μM C)	[DON] (μM N)	C:N ratio DOM	[POC] (μM C)	δ^{13}C-POC (‰)	[PON] (μM N)	δ^{15}N-PON (‰)	C:N ratio POM
109	3	2.97	250 ± 3	4.25 ± 0.74	58.9 ± 10.3	181.3	-31.37	16.2	1.21	11.2
	15	19.38	186 ± 3	16.0 ± 0.61	11.6 ± 0.5	72.5	-32.96	7.2	3.42	10.1
	30	20.13	152 ± 3	12.2 ± 1.00	12.5 ± 1.1	67.3	-32.26	5.8	2.52	11.6
110	3	3.96	290 ± 3	3.30 ± 0.58	87.8 ± 15.4	271.4	-30.96	24.3	1.11	11.2
	10	17.98	194± 3	10.9 ± 0.91	17.9 ± 1.5	117.7	-31.11	10.5	3.58	11.2
	20	19.83	199 ± 2	12.8 ± 0.43	15.6 ± 0.6	79.0	-32.73	4.6	1.05	17.2
111	3	10.56	311± 3	5.29 ± 0.44	58.8 ± 4.9	260.7	-30.50	23.2	0.75	11.2
	10	18.72	194 ± 4	12.3 ± 0.56	15.8 ± 0.8	102.9	-30.90	9.8	2.26	10.5
	25	19.92	183 ± 2	16.8 ± 0.37	10.9 ± 0.3	78.2	-32.06	6.2	1.23	12.7
131	3	14.86	266 ± 2	8.71 ± 0.71	30.5 ± 2.5	187.2	-29.74	22.0	-1.42	8.5
	40	24.90	154 ± 3	12.6 ± 1.07	12.2 ± 1.1	51.8	-30.94	5.2	-9.89	10.0
	120	27.19	119 ± 1	11.4 ± 0.54	10.4 ± 0.5	42.1	-30.33	4.5	-5.97	9.3
132	2	13.58	185 ± 2	4.44 ± 0.63	41.6 ± 5.9	255.1	-30.04	22.6	-1.65	11.3
	10	21.60	179 ± 0	11.7 ± 0.34	15.3 ± 0.5	50.3	-30.63	4.0	2.49	12.5
	20	23.49	86.2 ± 0	4.51 ± 0.29	19.1 ± 1.2	48.0	-29.78	5.6	-1.93	8.5
133	2	13.44	254 ± 1	4.59 ± 2.23	55.4 ± 26.9	114.6	-30.43	11.7	-2.81	9.8
	40	25.82	119 ± 0	11.9 ± 0.27	10.0 ± 0.2	40.2	-32.63	3.1	-8.36	13.1
	90	27.10	91 ± 1	11.1 ± 0.54	8.23 ± 0.41	43.3	-31.62	3.3	-1.18	13.0

Optical Properties

Absorbance was measured at every sampling depth at each sampling station. Absorbance measurements were used to calculate absorption coefficients at 254 nm (a_{254}), as well as spectral slopes for 275 to 295 nm ($S_{275\text{-}295}$). Absorbance at 254 nm was normalized to DOC concentration to calculate the specific ultraviolet absorbance (SUVA) at this wavelength.

$SUVA_{254}$ was similar across the mixing layer above the pycnocline (0.083 to 0.162 μM C^{-1} m^{-1}) with greater variation below the pycnocline (0.050 to 0.191 μM C^{-1} m^{-1}). Spectral slopes from 275 to 295 nm were also fairly similar across the mixing layer above the pycnocline (13.62 to 17.01 μm^{-1}) with slightly greater variation below the pycnocline (10.26 to 19.21 μm^{-1}) (Table 2.4).

Forms of pH-dependent aqueous iron(III) have been known to absorb light in the UV spectrum (200-400nm), and contribute to absorption by CDOM when associated with humic substances (Xiao et al., 2013; Maloney et al., 2005). Weishaar et al. (2003) studied the effects of lower concentrations of iron(III) (0-3.5 mg Fe L^{-1}) on UV absorbance, and found no interaction between iron(III) and other sample components. In 2010, the effects of Iron(III) concentrations on absorbance were studied experimentally up to 10 mg/L (0.18mM) by Doane and Howarth (2010) with concentrations above 5 mg L^{-1} affecting absorbance by up to 0.7 units decrease. In 2013, Xiao et al. found that iron(III) contributed to absorbance by CDOM when it associated with humic substances at a pH of 8. In 2014, Weyhenmeyer et al. (2014) determined that iron bound to DOC increases

absorbance in the visible spectrum (specifically, a_{420}). Similarly, the iron to organic carbon ratio (Fe:OC) in river water samples has been found to negatively correlate with the amount of iron that stays in suspension at high salinities, suggesting pH was of secondary importance (Kritzberg et al., 2014).

As discussed above, iron concentrations have been known to affect water sample absorbance values, particularly for those above 5 mg Fe L^{-1} (e.g. Doane & Howarth, 2010). Churchill river total iron concentrations ranged from 0.06 mg L^{-1} sampled at Grizzle Rapids on September 27, 2008, to 0.37 mg L^{-1} sampled above Muskrat Falls on September 28, 2008. Sampling was conducted by Newfoundland and Labrador Department of Environment and Conservation, presently called the Department of Municipal Affairs and Environment (MAE), and analyzed by Maxxam Analytics, with reported detection limit (RDL) of 0.05 mg/L (MAE, 2007; MAE, 2008). Given the low concentrations of iron in the Churchill River water samples, it is unlikely that iron had any effect on the optical data presented here.

Table 2.4. Optical Properties: 2008 Field Season

Sample Collection			Spectral Slopes		Specific Ultraviolet Absorbance	
Way-point	**Sample Depth (m)**	**Salinity (psu)**	**Mean Slope $S_{275-295}$ (μm^{-1})**	**$S_{275-295}$ st dev**	**$SUVA_{254}$ a_{254} $[DOC]^{-1}$ ($\mu M\ C^{-1}\ m^{-1}$)**	**$SUVA_{254}$ st dev**
109	3	2.97	13.62	1.81	0.101	1.15
	15	19.38	11.07[1]	-	0.143[1]	-
	30	20.13	12.58[1]	-	0.116[1]	-
110	3	3.96	14.77	3.29	0.102	0.28
	10	17.98	14.25[1]	-	0.069[1]	-
	20	19.83	19.12	5.70	0.050	0.81
111	3	10.56	17.01[2]	0.90	0.083[2]	0.55
	10	18.72	16.59[2]	1.50	0.093[2]	0.36
	25	19.92	14.52	1.29	0.079	0.12
131	3	14.86	14.92	1.41	0.105	0.89
	40	24.9	19.21[1]	-	0.052[1]	-
	120	27.19	16.70	0.31	0.051	2.09
132	2	13.58	15.21	0.40	0.162	1.02
	10	21.6	15.90	0.96	0.080	0.08
	20	23.49	11.55[1]	-	0.191[1]	-
133	2	13.44	13.78	0.90	0.108	0.62
	40	25.82	10.26[1]	-	0.139[1]	-
	90	27.1	16.43[1]	-	0.088[1]	-

1 n=1, otherwise n=3
2 n=2, otherwise n=3

Chlorophyll a

Chlorophyll *a* concentrations were higher at the surface both for samples collected on GFF filters (nominal pore size of 0.7μm) and on 5μm filters. Above the pycnocline, chlorophyll *a* collected on GFF ranged from 0.09 to 0.78 μg L^{-1} while >5μm fraction ranged from 0.07 to 0.42 μg L^{-1} and represented 31-53% of the total chlorophyll a concentration (Table 2.5).

Bacterioplankton

Bacterial abundance, cell volume and biomass were all generally higher above the pycnocline than below. In the surface mixed layer, bacterial abundance ranged from $(6.56\pm0.85)x10^8$ to $(10.29\pm0.76)x10^8$ cells L^{-1}, mean cell volume was 0.037 ±0.001 to 0.053 ±0.001 μm^3, and bacterial biomass ranged from 8.49 ±1.10 to 14.97 ±1.23 μg C L^{-1}. Below the pycnocline, bacterioplankton ranged from $(2.42\pm0.45)x10^8$ to $(9.17\pm0.74)x10^8$ cells L^{-1} with mean cell volume from 0.044 ±0.001 to 0.068 ±0.002 μm^3, and biomass ranging from 3.80 ±0.41 to 11.94 ±1.15 μg C L^{-1} (Table 2.5).

Table 2.5. Bacterioplankton and Chlorophyll *a*: 2008 Field Season

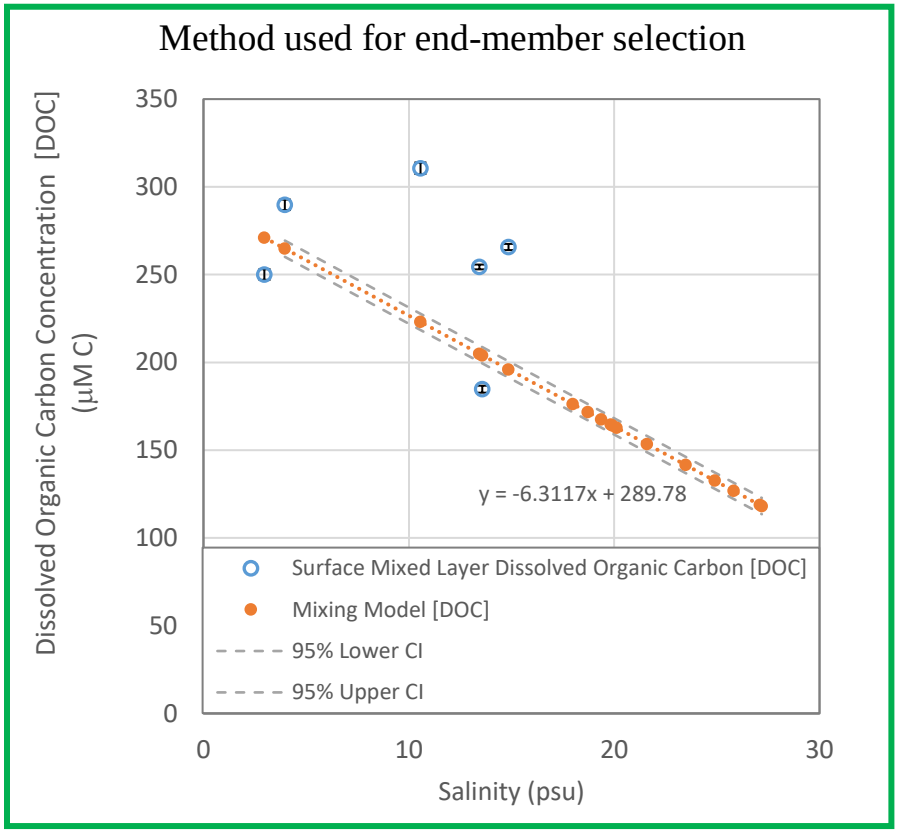
Sample Collection			**Bacterioplankton**			**Chlorophyll *a***		
Way-point	**Sample Depth (m)**	**Salinity (psu)**	**Bacterial Abundance (BA) ($x10^8$ cells L^{-1})**	**Bacterial Biomass (BB) (μg C L^{-1})**	**Bacterial Cell Volume (μm^3)**	**Chl a (GFF filter) (μg L^{-1})**	**Chl a (5μm filter) (μg L^{-1})**	**% of total Chl a >5μm**
109	3	2.97	8.17 ± 0.75	9.15 ± 0.85	0.037 ± 0.001	0.104	0.117	53
	15	19.38	3.67 ± 0.27	6.26 ± 0.48	0.067 ± 0.002	0.014	0.018	56
	30	20.13	2.63 ± 0.27	3.80 ± 0.41	0.053 ± 0.001	0.012	0.012	50
110	3	3.96	6.56 ± 0.85	8.49 ± 1.10	0.045 ± 0.000	0.089	0.073	45
	10	17.98	7.99 ± 0.75	11.94 ± 1.15	0.055 ± 0.001	0.081	0.063	44
	20	19.83	4.16 ± 0.39	6.86 ± 0.67	0.064 ± 0.002	0.017	0.015	47
111	3	10.56	9.81 ± 1.11	12.22 ± 1.41	0.043 ± 0.001	0.332	0.150	31
	10	18.72	7.18 ± 0.67	9.05 ± 0.87	0.044 ± 0.001	0.087	0.058	40
	25	19.92	N/A	N/A	N/A	0.015	0.015	50
131	3	14.86	10.29 ± 0.76	14.97 ± 1.23	0.053 ± 0.001	0.279	N/A	N/A
	40	24.90	3.46 ± 0.70	5.59 ± 0.83	0.062 ± 0.002	0.021	0.024	53
	120	27.19	2.42 ± 0.45	3.95 ± 0.42	0.062 ± 0.002	0.021	0.018	46
132	2	13.58	9.17 ± 0.74	12.65 ± 1.14	0.050 ± 0.001	0.506	0.423	46
	10	21.60	5.26 ± 0.70	7.82 ± 0.68	0.055 ± 0.001	0.080	0.031	28
	20	23.49	3.92 ± 0.43	5.91 ± 0.60	0.056 ± 0.001	0.026	0.024	48
133	2	13.44	N/A	N/A	N/A	0.778	0.401	34
	40	25.82	3.16 ± 0.81	5.14 ± 0.53	0.062 ± 0.001	0.020	0.015	43
	90	27.10	2.88 ± 0.51	4.99 ± 0.50	0.068 ± 0.002	0.011	0.016	59
N/A = no measurement available								

Comparisons of the measured versus conservative mixing model results

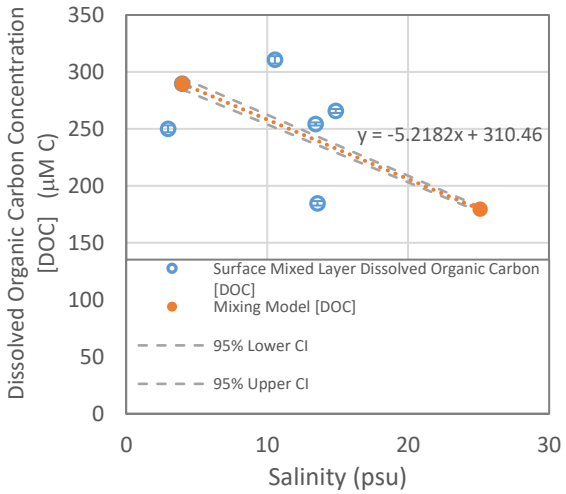
End-member Selection

Simple models such as this two end-member mixing model can be sensitive to end-member characteristics, such that changes in the end-member might alter the conservative mixing line and change the interpretation of the data. Selecting appropriate end-members is important to the integrity of the model and to the accurate interpretation of deviations of the measured constituents from the conservative mixing line it generates. End-members chosen for this study were: 1) the surface water (3 m) sample from waypoint 110, the sampling station closest to the mouth of the Churchill River (4 psu); and 2) the deep water (120 m) sample from waypoint 131, the most marine sampling station along the transect (27 psu). These sampling locations were chosen to reflect the most riverine and most oceanic water masses sampled as part of this study. The measured values plotted against the conservative mixing line generated by this model are sampled from the surface mixing layer, that is above the pycnocline that ranges from 2 m to 6 m depth along the study transect. It could be argued that, given the apparent stratification of the system and the fact that comparisons are being conducted using surface mixing layer samples, a marine end-member from a surface sample should be used to calculate a conservative mixing line. This method, along with two several others (see below), was considered and rejected because the highest surface salinity in this study is a mere 15 psu at waypoint 131. Using this surface sample as a marine end-member would potentially obscure important information about the behaviour of biological and chemical constituents at the mid salinities of the riverine-marine interface, since these measurements would no longer display any deviations from conservative mixing once they were assigned the role of marine end-member.

Other methods considered for selecting appropriate end-members included: 1) using the same riverine end-member described above (waypoint 110, 4 psu, 3 m depth) and calculating a marine end-member using a depth-integrated value for salinity at waypoint 131 and averaging the three water column measurements taken at this station (i.e. samples from 3 m, 40 m, and 120 m); and 2) calculating both end-members using a depth-integrated value for salinity and averaging the three water column measurements taken at each station (i.e. samples from 3 m, 10 m, and 20 m at waypoint 110 and samples from 3 m, 40 m, and 120 m at waypoint 131). These methods have both been illustrated in the figure below (Figure 2.4) to allow comparison to the actual method selected for use in this study. Neither alternative alters the interpretation of the data significantly, and the second alternative method requires extrapolation of the conservative mixing line down to lower salinities. Furthermore, since the Lake Melville system exhibits stratification at this time of year, integrating measurements over a water column that undergoes little mixing in order to generate an end-member is not a reasonable method to pursue. While it may not be ideal to use a deep water sample in a stratified system, it is clear from the lower salinity of this sample (relative to ~35 psu of pure seawater) that some freshwater does mix down to this depth. Additionally, this deep water sample has likely been spared some of the biological and photochemical processes that might be causing changes to the marine characteristics of seawater in the surface mixing layer.



1) Alternative Method 1 (calculating marine end-member)



2) Alternative Method 2 (calculating both end-members)

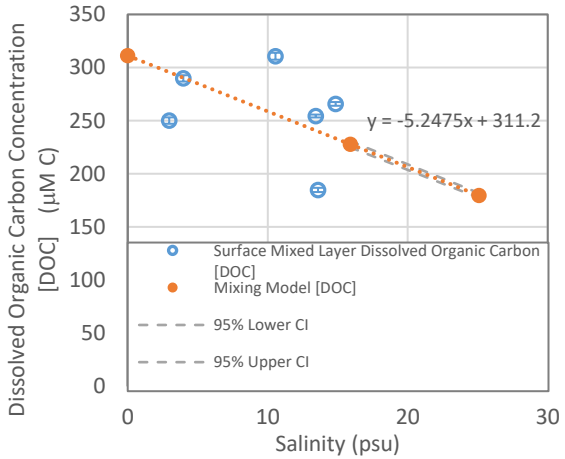


Figure 2.4: Illustrations of three methods considered for end-member selection for the conservative mixing model using dissolved organic carbon concentration plots (method selected is presented in large panel on the left and two alternatives considered presented on the right).

Inorganic Carbon and Nutrients

The conservative mixing model of total inorganic carbon (TIC) concentration illustrated a positive linear relationship between TIC concentration and salinity, as predicted. TIC concentrations deviated below a conservative distribution at mid salinities, with the exception of station 109, which had increased TIC concentration relative to the conservative distribution estimate (Figure 2.5A). The measured values fell well outside the estimate of the model's 95% confidence interval of 6.2 μM C (Table 2.6, Figure 2.5A). Measured values of TIC concentration deviated below the predicted conservative mixing values by 17% to 39%, with the value at station 109 reaching a 82% increase relative to the conservative mixing prediction (Table 2.6). Stable carbon isotope ratios of the TIC pool (δ^{13}C-TIC) appeared to be more variable at lower salinities than at mid salinities, however, stable carbon isotope signatures exhibit both positive and negative deviations from a conservative mixing model across the range of salinities sampled (Figure 2.5B). Most measured values of δ^{13}C-TIC fell within the 95% confidence interval of the mixing model, and ranged from a 0.8‰ (or 127%) decrease to a 0.6‰ (or 104%) increase relative to the predicted conservative mixing values (Table 2.6). One measured value fell outside the 95% confidence interval (wpt 110, the riverine end-member), however the standard deviation of this value overlaps with the confidence interval (Figure 2.5B).

Phosphate and nitrite/nitrate concentrations had a predicted positive linear relationship with salinity, increasing with salinity, while ammonium was predicted to have a negative relationship with salinity, decreasing with increasing salinity (Figures 2.5C, 2.5D & 2.5E,

respectively). Phosphate concentration measurements were lower than predicted conservative mixing values, especially at mid salinities. All measured values fell below the lower limit of the model's 95% confidence interval; values were 26% to 54% lower at low salinities but ranged from 76% to 100% lower than predicted values at mid salinities (Table 2.7). Nitrite/Nitrate concentrations were also lower than predicted. All values except for wpt 109 fell below the lower limit of the model's 95% confidence interval, ranging from 35% to 73% lower than predicted conservative mixing values (Table 2.6). Ammonium concentrations were mostly lower than predicted by conservative mixing, ranging from 12% to 59% lower than the predicted values. Measured values at four of the six stations fell below the model's 95% lower confidence interval (Table 2.7).

Table 2.6. Deviations from a conservative mixing model of inorganic carbon concentration and stable isotope signature.

Way Point	Depth (m)	Salinity (psu)	Total Inorganic Carbon Concentration [TIC] (μM C)			TIC Stable Carbon Isotope Signature δ^{13}C-TIC (‰)		
			95% CI Mixing Model	Deviation	% Diff	95% CI Mixing Model	Deviation	% Diff
110	3	3.96	±6.2	-224.6	-39%	±0.84	-1.12	75%
109	3	2.97		423.7	82%		1.00	-59%
111	3	10.56		-273.8	-29%		-0.20	25%
132	2	13.58		-580.4	-52%		-0.70	107%
133	2	13.44		-187.8	-17%		0.83	-127%
131	3	14.86		-381.4	-32%		-0.62	104%

Table 2.7. Deviations from a conservative mixing model of inorganic nutrient concentrations.

Way Point	Depth (m)	Salinity (psu)	Phosphate Concentration [PO_4^{3-}] (μM P)			Nitrite/Nitrate Concentration [$NO_{2/3}^-$] (μM N)			Ammonium Concentration [NH_4^+] (μM N)		
			95% CI Mixing Model	Deviation	% Diff	95% CI Mixing Model	Deviation	% Diff	95% CI Mixing Model	Deviation	% Diff
110	3	3.96	±0.08	-0.09	-26%	±0.04	-1.35	-64%	±0.10	0.18	7%
109	3	2.97		-0.17	-54%		4.09	232%		-1.55	-59%
111	3	10.56		-0.49	-100%		-1.75	-40%		-0.51	-22%
132	2	13.58		-0.56	-100%		-3.94	-73%		-0.48	-22%
133	2	13.44		-0.42	-76%		-1.88	-35%		-1.28	-59%
131	3	14.86		-0.58	-99%		-4.24	-73%		-0.24	-12%

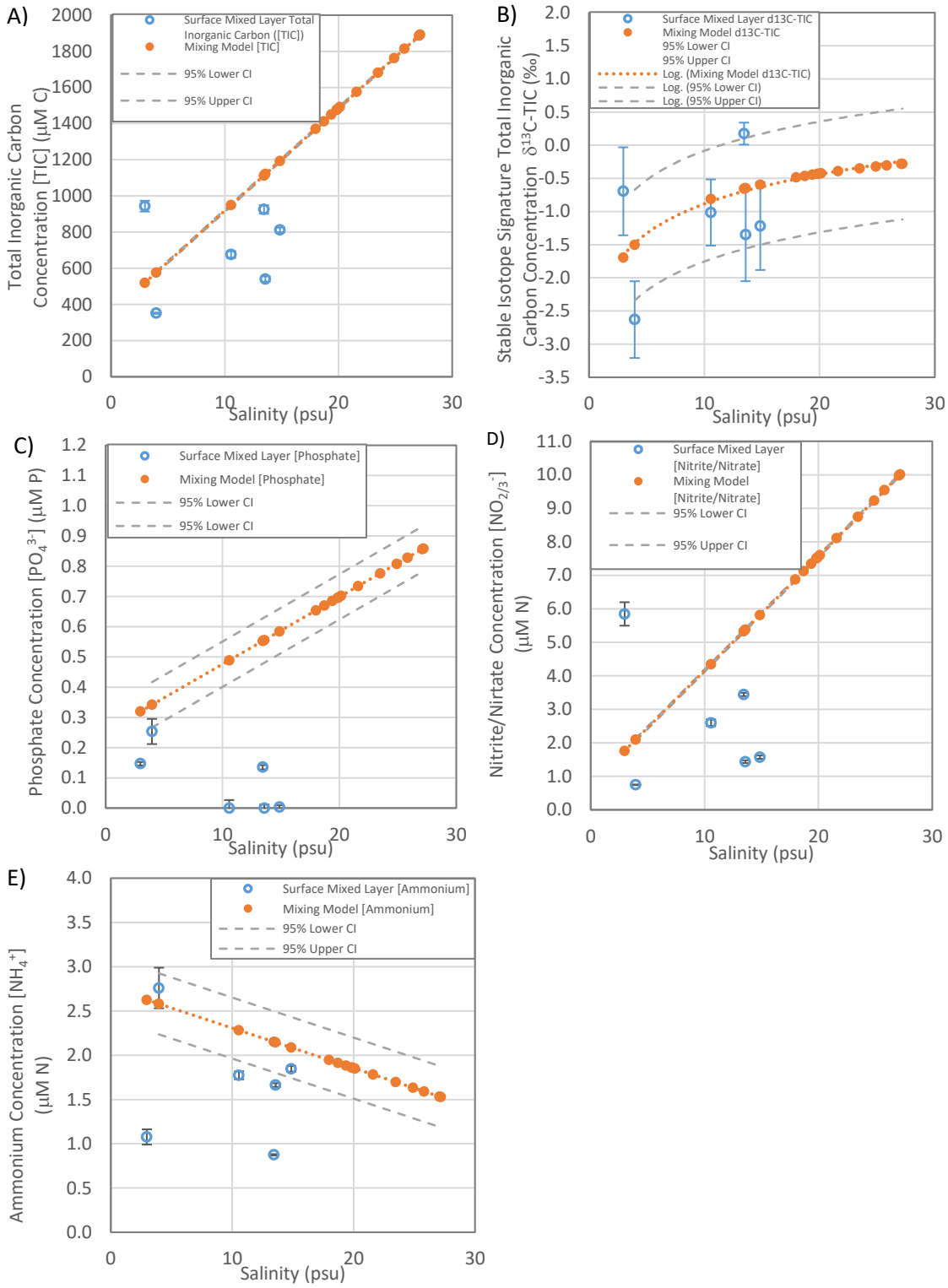


Figure 2.5 A) to E). Comparison of mixed surface layer measurements and conservative mixing models of: A) inorganic carbon concentration and B) stable isotope signature, and C) phosphate concentration, D) nitrite/nitrate concentration and E) ammonium concentration.

Organic Carbon and Nitrogen, and Organic Matter Optical Properties

Conservative mixing model of dissolved organic carbon (DOC) concentration exhibited a negative relationship with salinity, decreasing with increasing salinity, whereas the conservative mixing model of dissolved organic nitrogen (DON) concentration displayed a positive relationship with increasing salinity (Figures 2.6A and 2.6B). The relatively high concentrations of DOC compared to DON lead to a conservative mixing model that exhibited a negative relationship of C:N of the DOM with salinity, with C:N values predicted to decrease with increasing salinity (Figure 2.6C). Measured DOC concentrations all fell outside of the 95% confidence interval of the mixing model. Measured values were slightly higher than predicted values at mid salinities (at four of the six stations), ranging from a 24% to 39% increase. The remaining two stations had values below the conservative mixing line, 8% to 9% lower than the predicted values (Table 2.8, Figure 2.6A). DON concentrations were nearly all below the conservative mixing model line, ranging from 14% to 42% lower than the predicted values; all values below the mixing model line also fell outside the model's 95% confidence interval. One DON concentration was found above the conservative mixing line but was only 8% higher than the predicted value at that salinity, and fell within the upper limit of the model's 95% confidence interval (Table 2.8, Figure 2.6B). Calculated C:N generally followed the conservative mixing model and remained within the model's 95% confidence interval except at two mid salinity locations where values were 34% and 62% higher than predicted values (Table 2.8).

The conservative mixing model for $SUVA_{254}$ predicted a negative relationship with increasing salinity, and deviations at mid-salinities ranged from 41% to 112% higher than their predicted values (Figure 2.6D, Table 2.9). Spectral slope values from 275 to 295 nm ($S_{275\text{-}295}$) were close to those predicted by conservative mixing with only 2% to 12% difference from modeled values (Figure 2.6E, Table 2.9).

Conservative mixing models for particulate organic carbon (POC) and nitrogen (PON) concentrations were found to have negative relationships with increasing salinity (Figures 2.7A and 2.7B). Conservative mixing models for stable isotope signatures of POC and PON exhibited a very weak positive relationship with increasing salinity (δ^{13}C-POC) and a negative relationship with increasing salinity (δ^{15}N-PON), respectively (Figures 2.7C and 2.7D). The measured concentrations of POC and PON showed almost identical patterns with respect to percent deviations from their predicted conservative mixing model values. Both values were 14% higher than predicted closest to the mouth of the Churchill River, followed by a drop below the conservative mixing line to measurements 26% and 27% lower than predicted values, respectively. At the next two stations, measured values were 43% and 63%, and 40% and 57% greater than predicted for POC and PON concentrations respectively. The last two stations showed a drop and then an increase in measured values, first dropping to 27% and 19% lower than predicted for POC and PON concentrations at waypoint 133, then rising to values 29% and 64% greater than predicted for POC and PON at waypoint 131 (Table 2.10). Conservative mixing lines for stable isotopic signatures of the POC and PON pools (δ^{13}C-POC and δ^{15}N-PON) were curvilinear because they were plotted using weighted end-member

isotopic values versus salinity (see eq. 2 and Bianchi, 2007). Stable carbon isotope signatures of the POC pool followed a weak positive conservative relationship with increasing salinity, whereas the stable nitrogen isotope signature of the PON pool exhibited a strong negative relationship with increasing salinity. Deviations at mid-salinities were small in δ^{13}C-POC (enrichment of 0.4‰ to 1.1‰ compared to predicted values) but were twice as large in δ^{15}N-PON, and in the opposite direction (reduction of 1.2‰ to 2.8‰ compared to predicted values) (Table 2.10).

Table 2.8. Deviations from a conservative mixing model of dissolved organic carbon and nitrogen concentrations.

Way Point	Depth (m)	Salinity (psu)	Dissolved Organic Carbon Concentration [DOC] (μM C)			Dissolved Organic Nitrogen Concentration [DON] (μM N)			Dissolved Organic Carbon to Nitrogen ratio (C:N)		
			95% CI Mixing Model	Deviation	% Diff	95% CI Mixing Model	Deviation	% Diff	95% CI Mixing Model	Deviation	% Diff
110	3	3.96	±4.6	25.0	9%	±0.90	-1.00	-14%	±6.86	5.65	13%
109	3	2.97		-21.0	-8%		-1.49	-22%		3.38	8%
111	3	10.56		87.5	39%		-1.67	-19%		11.2	34%
132	2	13.58		-19.3	-9%		-3.39	-36%		1.81	6%
133	2	13.44		49.4	24%		-3.99	-42%		17.9	62%
131	3	14.86		69.8	36%		0.74	8%		-1.47	-6%

Table 2.9. Deviations from a conservative mixing model of specific ultraviolet absorbance at 254 nm ($SUVA_{254}$), and spectral slope for 275-295 nm ($S_{275\text{-}295}$).

Way Point	Depth (m)	Salinity (psu)	$SUVA_{254}$ (cm^{-1} μM C^{-1})		$S_{275\text{-}295}$ (m^{-1} μM C^{-1})	
			Deviation	% Diff	Deviation	% Diff
110	3	3.96	0.48	5%	-1.36	-9%
109	3	2.97	0.75	8%	-0.28	-2%
111	3	10.56	0.08	1%	1.49	10%
132	2	13.58	3.10	42%	-0.91	-6%
133	2	13.44	8.57	112%	-0.52	-3%
131	3	14.86	3.17	41%	-1.94	-12%

Table 2.10. Deviations from a conservative mixing model of particulate organic carbon and nitrogen concentrations and stable isotope signatures.

Way Point	Depth (m)	Salinity (psu)	Particulate Organic Carbon Concentration [POC] (μg C L^{-1})		Stable Carbon Isotope Signature of [POC] δ^{13}C-POC (‰)	Particulate Organic Nitrogen Concentration [PON] (μg N L^{-1})		Stable Nitrogen Isotope Signature of [PON] δ^{15}N-PON (‰)
			Deviation	% Diff	Deviation	Deviation	% Diff	Deviation
110	3	3.96	33.54	14%	-0.016	2.90	14%	0.22
109	3	2.97	-64.92	-26%	-0.422	-5.99	-27%	0.26
111	3	10.56	78.69	43%	0.403	6.63	40%	0.39
132	2	13.58	98.63	63%	0.835	8.20	57%	-1.65
133	2	13.44	-43.05	-27%	0.447	-2.81	-19%	-2.83
131	3	14.86	41.51	29%	1.120	8.55	64%	-1.23

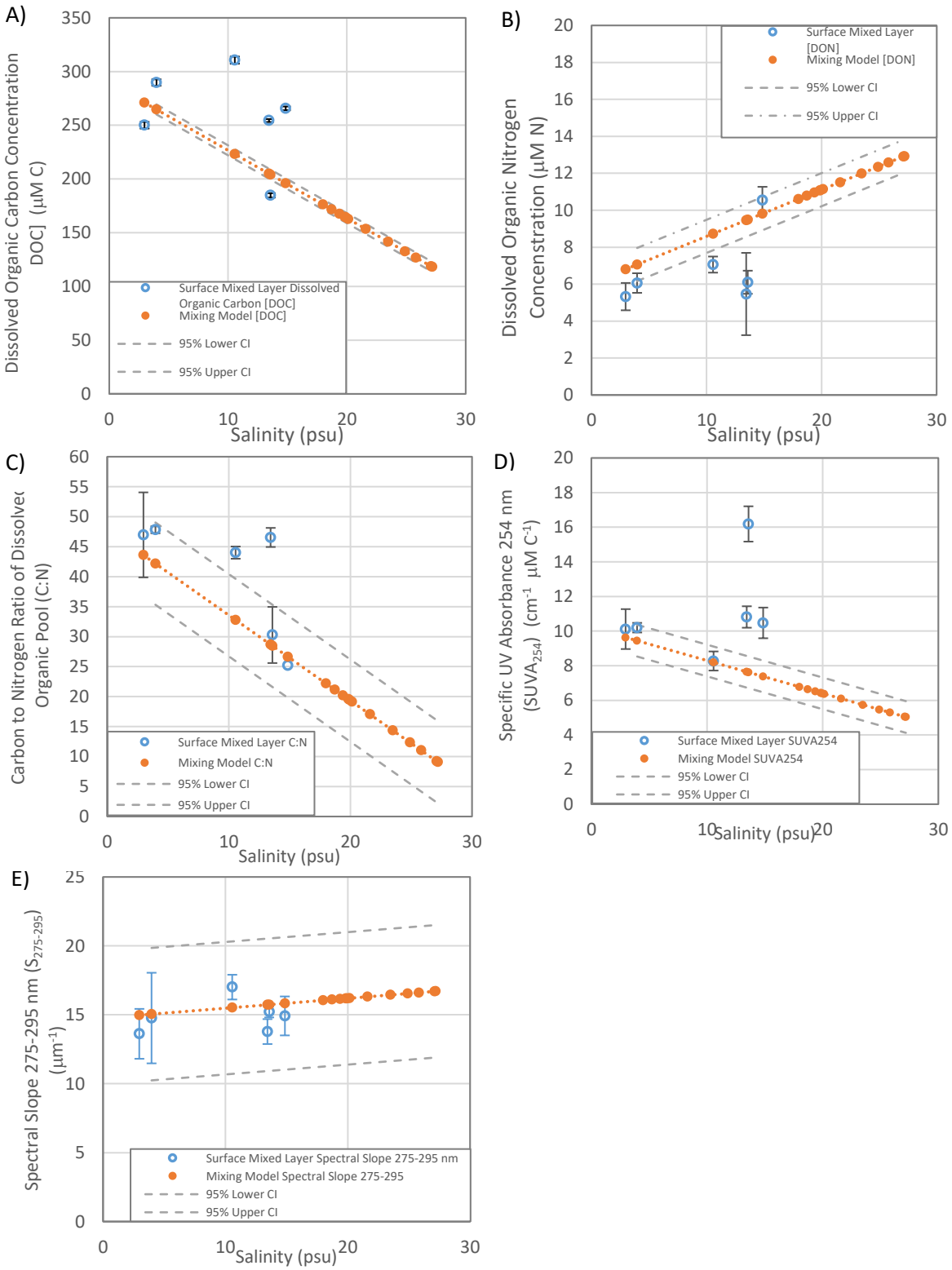


Figure 2.6 A) to E). Comparison of mixed surface layer measurements and conservative mixing models of: A) DOC, B) DON, C) C:N ratios, D) $SUVA_{254}$, E) $S_{275\text{-}295}$.

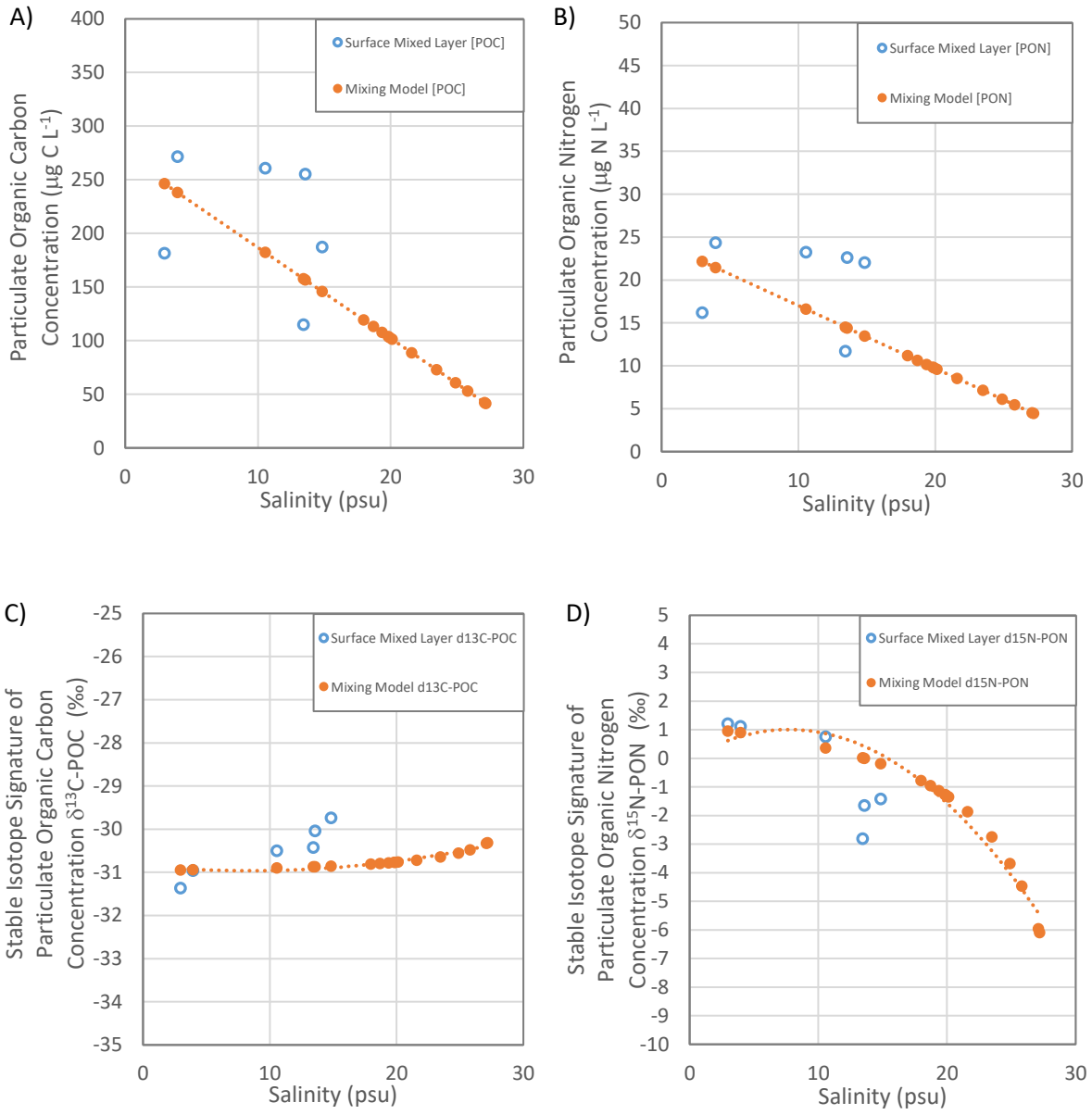


Figure 2.7 A) to D). Comparison of mixed surface layer measurements and conservative mixing models of: A) particulate organic carbon and B) nitrogen concentrations, and C) stable isotope signatures δ^{13}C-POC and D) δ^{15}N-PON.

Chlorophyll a and Bacteria

Chlorophyll *a* concentrations, which were measured in two fractions (GFF 0.7μm to 5μm, and >5μm), both exhibited weak negative conservative mixing relationships with salinity; predicted values decreased slightly with increasing salinity (Figures 2.8A and 2.8B). Measured values of both fractions of chlorophyll *a* concentrations showed large positive deviations from conservative mixing predictions at mid salinities; the GFF fraction ranged from 431% to 1308% increase of the predicted values, and the >5μm fraction ranged from 191% to 831% increase of the predicted values (Table 2.11).

Bacterial abundance and biomass were predicted to increase with increasing salinity, according to the conservative mixing model illustrated in Figure 2.8C and 2.8E, while bacterial cell volume was predicted to decrease slightly with increasing salinity (Figure 2.8D). Measurements of bacterial abundance and biomass were much higher at mid salinities relative to their predicted conservative mixing values and fell far above the 95% confidence interval, ranging from 98% to 140% higher than predicted values (Table 2.12). Bacterial biomass, which is calculated using abundance and cell volume, shows a similar trend at mid salinities, falling well above the 95% confidence interval and ranging from 82% to 150% higher than the predicted biomass values at mid salinities (Table 2.12). Bacterial cell volume measurements were close to the conservative mixing line but were slightly below their predicted values, ranging from 2% to 21% lower than predicted values (Table 2.12). All cell volume measurements fell outside the 95% confidence intervals of the mixing model.

Table 2.11. Deviations from a conservative mixing model of chlorophyll *a* concentrations.

Way Point	Depth (m)	Salinity (psu)	Chlorophyll *a* Concentration (GFF) (μg L^{-1})		Chlorophyll *a* Concentration (>5μm) (μg L^{-1})	
			Deviation	% Diff	Deviation	% Diff
110	3	3.96	0.010	13%	0.008	12%
109	3	2.97	0.022	28%	0.050	75%
111	3	10.56	0.270	431%	0.098	191%
132	2	13.58	0.451	821%	0.378	831%
133	2	13.44	0.723	1308%	0.355	777%
131	3	14.86	0.227	440%	N/A	N/A
N/A = sample analysis not available						

Table 2.12. Deviations from a conservative mixing model of bacterial abundance, cell volume and biomass.

Way Point	Depth (m)	Salinity (psu)	Bacterial Abundance (cells x 10^6 L^{-1})			Cell Volume (μm^3)			Bacterial Biomass (μg C L^{-1})		
			95% CI Mixing Model	Deviation	% Diff	95% CI Mixing Model	Deviation	% Diff	95% CI Mixing Model	Deviation	% Diff
110	3	3.96	±133.9	60.5	10%	±0.000	-0.002	-5%	±1.69	0.66	8%
109	3	2.97		206.4	34%		-0.010	-21%		1.16	15%
111	3	10.56		486.5	98%		-0.009	-17%		5.51	82%
132	2	13.58		468.9	105%		-0.004	-8%		6.44	104%
133	2	13.44		N/A	N/A		N/A	N/A		N/A	N/A
131	3	14.86		600.0	140%		-0.001	-2%		8.98	150%
N/A = sample analysis not available											

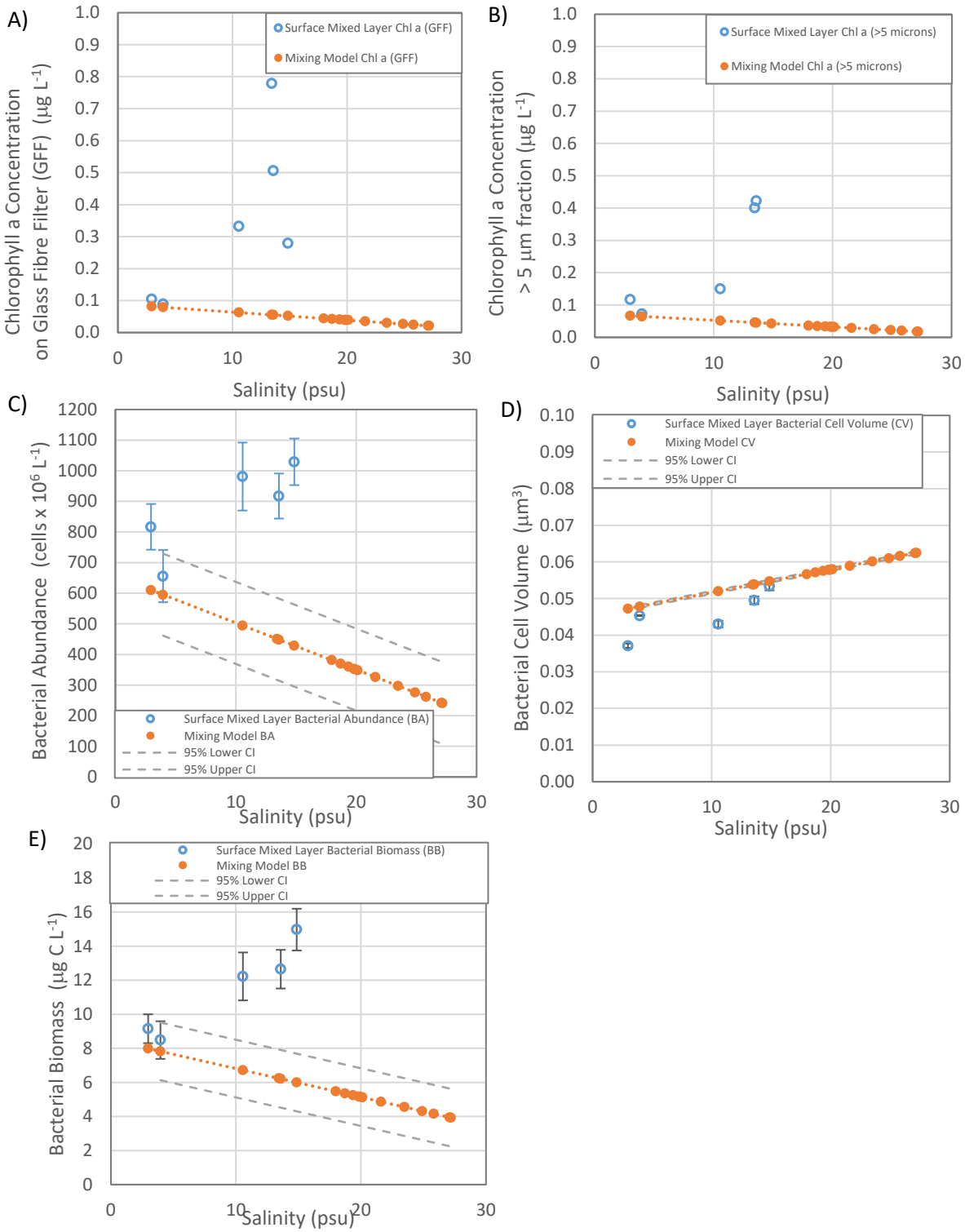


Figure 2.8 A) to E). Comparison of mixed surface layer measurements and conservative mixing models of: A) chlorophyll *a* concentrations <5µm and B) chlorophyll *a* >5µm, and C) bacterioplankton abundance, D) bacterioplankton cell volume and E) bacterioplankton biomass.

2.4 DISCUSSION

The processes that govern the production, removal and transformation of DOM in estuaries remain largely unknown. An understanding of the factors that govern the quantity and quality of DOM and nutrients delivered by rivers to coastal margins is key to developing accurate models of the global carbon budget. Studying high latitude river inputs to the estuarine environment, such as the Churchill river discharging into Lake Melville estuary, is therefore extremely useful. It also has the potential to improve predictions of the impacts of climate change since these high latitude regions are likely to be among the first to manifest its effects. The net production and removal of chemical and biological constituents were examined in the Lake Melville estuary using a comparison of field data to conservative mixing lines to investigate deviations across estuarine salinity gradients. Several studies employ this type of comparison (Peterson et al., 1994; Cifuentes and Eldridge, 1998; Benner and Opsahl, 2001; Raymond and Bauer, 2001; Markager et al., 2011) and mixing patterns are generally dominated either by conservative mixing or by variability produced by internal estuarine processes, as was observed in Lake Melville (e.g., stimulation of phytoplankton and bacterioplankton production). For example, in Lake Melville there was evidence of a net loss of TIC and inorganic nutrients, and of a net production of DOC and POM, which, coupled with increases of chlorophyll a, suggest stimulation of primary production. However, there was also evidence that the net production of DOC was accompanied by changes to its composition, as was seen in measurements of $SUVA_{254}$ and the C:N ratio of the DOM pool. This, coupled with the net increases in bacterial biomass, suggest that bacterial production was

also being stimulated at the riverine-marine interface. The fact that these net changes were observed at mid salinities in the surface mixed layer of Lake Melville indicates the possibility that resource limitation in one or both water masses might have been alleviated through mixing.

Stimulation of Primary Production

There was strong evidence of stimulated primary production at mid salinities based on the net changes in chlorophyll *a*, POM, inorganic nutrients and TIC, relative to conservative mixing. Changes in these variables provide evidence of primary production since chlorophyll *a* is a key pigment for the absorption of light energy used in photosynthesis, POM is the pool of material to which phytoplankton biomass belongs, inorganic nutrients are required in the cellular processes of primary producers, and TIC (i.e., dissolved CO_2) is the form of carbon fixed by photosynthesis into carbohydrate molecules. As predicted by conservative mixing, chlorophyll *a* decreased with increasing depth and salinity in Lake Melville, but showed distinct net increases at mid salinities in the surface mixed layer indicating an increase in phytoplankton biomass. At these mid salinities, there were concurrent increases in POM, which confirmed the interpretation of the chlorophyll a data, as well as net decreases in nutrients and TIC, indicating net growth of photosynthesizers that were fixing inorganic carbon into organic biomass while taking up inorganic nutrients in the process. Furthermore, δ^{13}C-TIC shows evidence of possible enrichment, consistent with uptake and fractionation of TIC through photosynthetic activity, since the lighter ^{12}C isotope would have been preferentially fixed to POC during

photosynthesis, increasing the relative proportion of the heavier ^{13}C isotope left behind, and resulting in higher $\delta^{13}C$ values of the TIC pool at mid salinities.

Phosphate and nitrate/nitrite concentrations increased with increasing salinity, whereas ammonium concentrations show a slight decrease as salinity increases, however, all nutrients exhibit net losses at mid salinities in Lake Melville surface waters. The net removal of phosphate at mid salinities, and the generally low concentrations of phosphate in the surface mixed layer, suggested this nutrient was important in stimulating primary production at the riverine-marine interface of Lake Melville estuary. Since inorganic nutrients may also be taken up by bacterioplankton, it is possible that net decreases in phosphate, ammonium and nitrite/nitrate could also have been due to the stimulation of secondary production at mid salinities, which is discussed in further detail below.

A net increase in DOC concentration was also observed at mid salinities relative to conservative mixing, which provided further evidence of the stimulation of primary production since phytoplankton exude autochthonous DOM. However, given the higher aromaticity of the DOM pool at mid salinities, as indicated by the high C:N and $SUVA_{254}$, there were likely other processes contributing to net changes in the DOM pool besides primary production, such as bacterial assimilation of labile DOM. If this was the case, it would be useful to investigate the relative contribution of DOM from autochthonous sources compared to allochthonous sources, in order to better constrain the potential impacts that changes to DOM source and composition might have on processes that govern carbon cycling and production in the Lake Melville estuary.

Estimates of allochthonous DOM inputs from the Churchill River were found to be within the range of autochthonous DOM generated by PP in Lake Melville, therefore it was unclear which source of DOM might be of greater relative importance to microbial processes. Markager et al. (2011) suggested that the contribution of PP to the DOC pool could be estimated by assuming that 50% of PP is grazed and 25% of that grazed biomass is converted to DOM. Since the present study provided *net* measurements, an estimate of new production of organic carbon (i.e. primary production) in the Goose Bay and Lake Melville estuary was taken from Kamula (1993), which ranged from 0.57 mol POC m^{-2} $year^{-1}$ in Goose Bay to 4.90 mol POC m^{-2} $year^{-1}$ in western Lake Melville. The production of DOC from PP in Lake Melville was therefore estimated to range from 0.07 to 0.61 mol C m^{-2} $year^{-1}$. An estimate of DOM loadings from Churchill River discharge was made using DOC concentrations measured near the mouth of the river during the present study, and mean annual flow rates near Muskrat Falls over a 10-year period (from 1998-2008, Environment Canada), which was the closest station to the mouth of the river that had available flow data. By this method, annual loading of allochthonous DOC has been estimated at 0.18 ± 0.01 mol C m^{-2} $year^{-1}$. While this appeared to be low based on values from other boreal estuaries (e.g. 0.16 to 0.45 mol C m^{-2} $year^{-1}$ in three Finnish boreal estuaries in Asmala et al., 2012), the estimate is likely fairly accurate since this study was conducted during low flow conditions (September 2008), as opposed to spring melt conditions in May or June when DOC loadings from the Churchill River were likely much higher. Since the estimated allochthonous DOM inputs (0.18 ± 0.01 mol C m^{-2} $year^{-1}$) fall within the range of autochthonous DOM concentrations (0.07 to 0.61 mol C m^{-2} $year^{-1}$), it is not possible to predict with any certainty what the dominant source of DOM

might be and how changes to the source of DOM might impact biogeochemical cycling in Lake Melville. However, it should be noted that, while DOM source was not traceable in the present Lake Melville study, Barber et al. (2017) analyzed δ^{13}C-DOC samples from Lake Melville in September 2015, and found a terrestrial signature (-25.8 ±0.1 ‰) in surface water >100km seaward from the most marine station used in this study. This suggests that surface DOC maintained a distinctly terrestrial profile across Lake Melville estuary, and that changes to allochthonous DOC from the Churchill River might have far-reaching effects throughout the surface waters of the estuary.

Stimulation of Bacterioplankton Production

Bacterioplankton biomass exhibited net increases at mid salinities, indicating that bacterial production was stimulated by mixing of riverine and marine water masses at mid salinities. This interpretation is supported by the non-conservative behaviour of inorganic nutrients, of the concentrations and optical properties of the DOM pool, and of the concentrations and stable isotope ratios of the POM pool.

As discussed above, net decreases in phosphate, ammonium and nitrite/nitrate concentrations were observed at mid salinities, which could have been in support of PP or BP. The form of inorganic nitrogen appeared to differ at lower salinities compared to mid salinities, suggesting that microbially-mediated nitrification-denitrification processes might have been at play in surface waters of Lake Melville (York et al., 2007) which favoured net removal of ammonium at low salinities relative to removal of nitrite/nitrate.

The disparate behaviour of DOC and DON components of the DOM pool suggested that, in addition to stimulation of primary production, bacterial processing of DOM also occurred in the surface waters of Lake Melville. While DOC exhibited net increases at mid salinities (attributed to stimulated PP and discussed above), DON exhibited slight net decreases, suggesting that N-limited bacterioplankton might have used DON to fulfill their N requirements instead of inorganic forms of nitrogen. The stimulation of PP at mid salinities discussed above could have caused N-limiting conditions for bacterioplankton since phytoplankton have overlapping nutrient requirements and might have outcompeted bacterioplankton for inorganic forms of nitrogen (Caron et al., 1994; Lovdal et al., 2007). It should also be acknowledged that net increases in DOC at mid salinities could have been from viral lysis causing the release of bacterial DOC (Gobler et al., 1997; Kawasaki and Benner, 2006), which might also have released other resources important in stimulating planktonic processes. For example, viral lysis of bacterial cells might also have released inorganic phosphorus which can be an important contribution in a P-limited system (Middelboe et al., 1996; Riemann et al., 2009). If this was the case in Lake Melville, bacterial lysates might have played a role in stimulating primary and secondary production observed at the riverine-marine interface.

The optical properties of the DOM pool were in line with the interpretation of the DOM concentrations discussed above, providing further evidence of stimulation of bacterial activity at mid salinities in Lake Melville. At mid salinities, $SUVA_{254}$ was higher than predicted by conservative mixing, falling well above the model's 95% confidence interval, while $S_{275\text{-}295}$ did not deviate from conservative mixing predictions. This

supported the interpretation that bacterial production was being stimulated at mid salinities since heterotrophic degradation of DOM has been found to cause an increase in $SUVA_{254}$ (Asmala et al., 2013). Microbial degradation has also been found to decrease $S_{275\text{-}295}$, however, this was not observed at mid salinities in Lake Melville. Instead, $S_{275\text{-}295}$ did not exhibit any net change compared to conservative mixing, which could be explained by photochemical effects (i.e. photobleaching) since these have been found to increase $S_{275\text{-}295}$ (Fichot and Benner, 2012).

Net increases in POM at mid salinities relative to conservative mixing, measured as POC and PON concentrations and interpreted as increases in plankton biomass, supported the conclusion that primary and secondary production were stimulated at the riverine-marine interface in Lake Melville. Given the lack of estimated error for the POM conservative mixing models, it was difficult to interpret the deviations from predicted values of POC and PON, however, measurements of δ^{13}C-POC and δ^{15}N-PON offered further information about the sources contributing to the POM pool. While δ^{13}C-POC across Lake Melville did not appear to vary with salinity, there was a slight net increase of the δ^{13}C-POC at mid salinities relative to conservative mixing. This could have been the result of bacterioplankton uptake of DOC from autochthonous primary production, which typically has a higher δ^{13}C (-18 to -24‰) relative to terrestrially-derived, allochthonous DOC (-30 to -24‰) (Coffin and Cifuentes, 1999; Bianchi, 2007). Alternatively, increases in δ^{13}C-POC could have been the result of primary production, where dissolved inorganic carbon ($\delta^{13}C = -7‰$) is fixed into photosynthetic organic carbon by phytoplankton, thereby increasing the δ^{13}C of POC. Both interpretations of the δ^{13}C-POC

data support the conclusion that PP and BP are stimulated at mid salinities in Lake Melville.

The δ^{15}N-PON also deviated from conservative mixing at mid salinities but in the opposite direction (i.e., net decreases in δ^{15}N-PON), suggesting that biological processes (e.g., PP and BP) were driving fractionation of δ^{15}N at these locations as well. The δ^{15}N-PON is generally lower in marine phytoplankton compared to riverine phytoplankton (Bristow et al., 2013), however, the highest values of δ^{15}N-PON measured in Lake Melville are among the lowest reported in other systems (e.g. as low as 0.2‰ reported for sedimentary POM by Thornton and McManus (1994) in the Tay estuary, Scotland), suggesting that the δ^{15}N-PON in Lake Melville could not be wholly attributed to phytoplankton, and was likely influenced by other POM sources such as bacterioplankton biomass. For example, assimilation of DON, or other terrestrial sources of N, by marine bacterioplankton has been found to lead to a decrease in δ^{15}N-PON of up to -12 ‰ depending on the substrate on which they were grown in a laboratory setting (Macko and Estep, 1984). If bacterioplankton in Lake Melville were sourcing N from DON or terrestrial inputs, it could help explain the depleted signatures of δ^{15}N-PON observed at mid-salinities, and further support the conclusion that bacterioplankton production contributed to increases in POM at the marine-riverine interface.

Coupling of Primary and Bacterioplankton Production

There was strong evidence for the stimulation of both PP and BP at mid salinities in the surface waters of Lake Melville, as discussed above, and therefore a possibility of

interdependence or coupling of these biological processes. Bacterial production can be closely coupled with primary production, even in systems with large allochthonous inputs (Kirchman and Hoch, 1988), such as Lake Melville estuary which receives large inputs from the Churchill River. In Lake Melville, mixing of Churchill River inputs with the estuarine water mass was likely stimulating bacterioplankton production across the surface mixed layer, both directly through the alleviation of nutrient and carbon limitation, and indirectly through the stimulation of primary production which provides labile substrate to bacterioplankton through algal exudates. It has been shown that while excretion of organic carbon (EOC) from phytoplankton is important for bacterial production and often selectively used by bacterioplankton (Jensen et al., 1983; Lignell, 1990), the DOC produced is not always rapidly nor completely used (Norrman et al., 1995). This could account for the net increase in DOC that was observed at mid-salinities in Lake Melville despite clear evidence of bacterial production which relies on assimilation of labile DOM. Alternatively, weak relationships between bacterial and primary production can be created in estuaries where high allochthonous inputs of DOM have been shown to support net heterotrophy (i.e., where high rates of community respiration surpass net primary production) (Findlay et al., 1991; Thottathil et al., 2000), and it was not clear to what degree these biological processes might have been coupled during the study period of September 2008.

Conclusions and Future Directions

There was strong evidence that estuarine mixing stimulated both primary production and bacterial production in the surface waters of Lake Melville, supported by the distribution

of chemical and biological constituents across the surface salinity gradient which revealed net increases and losses greater that any that were predicted by physical mixing alone. The evidence strongly suggested that the combination of riverine and marine water masses was an important driver in relieving resource limitation that might have existed in one or both environments. While there was evidence of stimulated production at mid salinities, there was still a lack of clarity around the relative importance of allochthonous riverine inputs in supporting the base of the estuarine food web in Lake Melville. Direct measurements of phytoplankton and bacterioplankton production in Lake Melville could be useful in future studies to elucidate their relative contributions to the net increases in biomass that were observed in this study, and to provide insight into how planktonic production is stimulated, and possibly interconnected, at the riverine-marine interface in a high latitude boreal estuary. Since upstream hydrological changes are anticipated as a result of both climate change and hydroelectric development on the Churchill River, there is clearly a need to better understand the links between estuarine PP, microbial processing of DOM, and the quality and timing of riverine inputs of DOM and nutrients, in order to better constrain the potential environmental impacts of upstream hydrological changes on downstream coastal environments and global carbon budgets.

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CHAPTER 3

Response of bacterioplankton to organic carbon and inorganic nutrient additions in high latitude estuary, Lake Melville, Canada.

3.1 INTRODUCTION

Estuaries are at the interface of freshwater and marine environments, where mixing of freshwater and seawater creates horizontal and vertical gradients of salinity, density and temperature. Estuarine mixing has the potential to relieve resource-limiting conditions experienced by plankton through the delivery of organic matter and inorganic nutrients by river discharge, which can stimulate high rates of primary production at the marine-riverine interface (Bianchi 2007). As a result, estuaries are considered ideal habitat for juvenile species of fish and macroinvertebrates, creating areas of high biodiversity.

Coastal regions such as estuaries are particularly important with respect to global carbon (C) fluxes because they are sites of exchange between land, sea and atmosphere. While estuaries receive large inputs of terrestrially-derived organic matter, only a small fraction of oceanic dissolved organic matter (DOM) is of terrestrial origin, suggesting that a great deal is processed in the coastal margins (Hedges et al. 1997; Opsahl & Benner, 1997; Hedges & Keil 1999, Middelburg & Herman 2007). Despite their importance in the global carbon cycle, there is still very little known about the production, consumption and transformation of organic matter in estuaries (Canuel & Hardison, 2016). It is clear that more information is needed, especially in light of climate change, since upstream hydrological changes and increased precipitation are likely to increase the delivery of DOM and nutrients to the

downstream marine environment, and impact how that DOM is processed (Figueroa et al., 2016; Soares et al., 2018).

Heterotrophic bacterioplankton are responsible for biological activity that links the pool of DOM to the microbial food web, and the higher trophic levels it supports. Bacterioplankton have key roles in aquatic ecosystems: 1) they mineralize organic substrate, providing inorganic nutrients and carbon for use in photosynthesis by primary producers; 2) they transfer C from dissolved organic carbon (DOC) to the aquatic food web through the microbial loop (Pomeroy, 1974; Azam, 1983; Azam, 1998; Cotner and Biddanda, 2002; Fenchel, 2008); and, 3) they generally represent the largest contribution to community respiration (Schwaerter et al., 1988; Findlay et al., 1992; Kirchman, 2010; Smith and Kemp, 2001). The degradation, assimilation and transformation of the DOM pool by bacterioplankton has been found to be influenced by a number of factors such as DOM quality, nutrient availability, temperature and salinity (Amon and Benner, 1996; del Giorgio and Cole, 1998; Apple et al., 2006; Apple et al., 2008; Blanchet et al., 2017), as well as top-down controls such as grazing and viral lysis (Sherr et al., 1992; Middelboe et al., 1996; Middelboe and Lyck 2002; Agis et al., 2007; Weitz et al., 2015). These factors can influence the amount of carbon processed by bacterioplankton, or the bacterial carbon demand (BCD), as well as the bacterial growth efficiency (BGE). The BGE is the proportion of the of the carbon utilized by bacteria (BCD) that is incorporated into biomass, and therefore enters the aquatic food web. It is calculated as BGE = BP/(BP+BR), where BP is bacterial production and BR is bacterial respiration (Kirchman, 2010). Therefore, BGE is critical to controlling the fate of carbon cycling

through the microbial loop. Since bacterial growth efficiency is difficult to calculate accurately in aquatic systems (it requires a measurement of BR which cannot easily be measured *in situ*) (del Giorgio and Cole, 1998), much effort has been directed towards understanding how BGE is controlled. An understanding of the factors that control BGE will ultimately better constrain the role of bacterioplankton with respect to global carbon budgets and to the microbial food web.

Dissolved organic matter quality has been has been identified as a key factor in regulating heterotrophic bacterioplankton activity and BGE in coastal systems (del Giorgio and Cole, 1998; Apple et al., 2006; Apple and del Giorgio, 2007; Asmala et al., 2013; Kaartokallio et al., 2016). The quality or bioavailability of the DOM pool has been described in many terms, including: diagenetic state or age; molecular weight fractions, namely low molecular weight (LMW, <1000 Da) and high molecular weight (HMW, >1000 Da) (e.g. Amon and Benner, 1996); elemental ratio (C:N); and aromaticity (e.g. Weishaar et al., 2003; Kellerman et al., 2018). The quality of DOM as bacterial substrate has generally been found to decrease with increasing age or diagenetic state, and to increase with increasing molecular weight (Amon and Benner, 1996). Since diagenetic processes tend to reduce the molecular weight of the DOM compounds, it follows that fresher HMW DOM would be a more labile bacterial substrate than older LMW DOM. For example, allochthonous terrestrially-derived DOM delivered by rivers, which comprises primarily HMW DOM, has been found to be the primary source of organic carbon substrate for bacteria in some estuaries (Coffin and Cifuentes, 1999, Tranvik, 1992; Kritzberg et al., 2004; Berggren et al., 2010). Conversely, while overall rates of

carbon consumption may be greater for HMW DOM substrate, Amon and Benner (1996) showed that BGE may in fact be higher for the LMW fraction of the DOM pool in both riverine and marine samples. This is likely because of a greater increase bacterial respiration relative to the increase in bacterial production when HMW DOM substrate is used, resulting in a lower BGE compared to LMW DOM. The C:N and aromaticity of the DOM pool have also been used as indicators of bioavailability since bacterioplankton have been shown to favour DOM with low C:N and low aromaticity (Kroer, 1993; Weishaar et al., 2003). In addition to DOM quality, but to a lesser extent, heterotrophic bacterioplankton activity and BGE have been shown to respond to changes in nutrient availability, temperature, grazing pressure and viral lysis (del Giorgio and Cole, 1998).

In addition to DOM quality, several bottom-up and top-down controls of bacterial activity and growth efficiency have been identified, the most important of which is the availability of inorganic nutrients. Nutrient supply influences bacterial activity and BGE in many coastal systems by reducing the energetic cost of acquiring nutrients (del Giorgio and Cole, 1998; Pinhassi et al., 2006; Hoikkala et al., 2009; Asmala et al., 2013; Asmala et al., 2014b), though other studies have shown no clear role of inorganic nutrients in regulating bacterial activity (e.g. Apple and del Giorgio, 2007). Similarly, evidence for temperature control of bacterial activity and BGE is mixed, with bacterial production (BP) and respiration (BR) both increasing with temperature (del Giorgio and Cole, 1998; Kritzberg et al., 2010a; Kritzberg et al., 2010b), and BGE decreasing with increasing temperature, usually due to a larger relative increase in BR (Apple et al., 2006; Kritzberg et al., 2010a; Kritzberg et al., 2010b). Top-down controls on bacterioplankton such as

grazing pressure or viral lysis have both been shown to influence bacterial activity and BGE. Grazing pressure has been shown to alter the way bacteria utilize the DOM pool by selectively grazing on certain types of bacterial cells, for example by favouring the removal of dividing cells (Sherr et al., 1992). Viral lysis has been found to increase activity and production of noninfected bacterioplankton, which utilize the organic matter and inorganic nutrients released as viral lysates (Middleboe et al., 1996). Furthermore, Middelboe and Lyck (2002) showed that bacterial net growth and BGE were greater in cultures where viral load was reduced, suggesting that the abundance of viruses could be an important control of bacterial processing of DOM.

An understanding of the controls on bacterial processing of DOM, and how those controls may vary across coastal systems, is critical to constraining the potential environmental impacts of upstream hydrological changes on the downstream estuarine food webs and carbon fluxes. While estuaries in boreal and Arctic regions can receive relatively large inputs of terrestrial DOM, very little is known about how it is processed by bacterioplankton and about the controls that govern that processing. These high latitude regions become especially important in the context of predicting ecosystem response to climate change (Mannino and Harvey, 2000; Berggren et al., 2010; Reader et al., 2014; Rontani et al., 2014; Tesi et al., 2014; Kulinski et al., 2016; Painter et al., 2018).

Lake Melville is a 3000 km^2 high latitude boreal estuarine fjord located in Labrador, Canada. Lake Melville, and the adjacent Goose Bay, receive inputs from the Churchill (Grand) River and other smaller tributaries, mixing those riverine inputs with ocean water that enters from the Labrador Sea. While the Churchill River's drainage area is estimated

close to 100 000 km^2, (~25% of the Labrador land mass) (Anderson, 1985), there is little information on the delivery and processing of riverine DOM and inorganic nutrients by bacterioplankton in Lake Melville.

Lake Melville, like most high latitude systems, experiences seasonal river flow; winter snow and ice cover represents an important control on river inputs to the estuary. As a result, there is a high flow spring freshet that enters Lake Melville, which reduces the time that riverine DOM might be exposed to biological and photochemical transformations before entering the estuary. This lack of exposure of DOM to photodegradation highlights the importance of understanding how heterotrophic bacterioplankton process DOM in Lake Melville, since they may play a large role in determining carbon fluxes within the system. Additionally, an understanding of how bacterioplankton in Lake Melville respond to changes DOM quality and the availability of nutrients would help constrain potential environmental impacts of hydrological changes to the Churchill River and its watershed.

Carbon, nitrogen and phosphorus often limit bacterial activity in aquatic systems (del Giorgio and Cole, 1998). Carbon limitation of bacterioplankton activity has been documented in freshwater (Benner et al., 1995), marine (Kirchman 1990, Kirchman et al. 1990; Carlson & Ducklow, 1996; Cherrier et al., 1996; Caron et al., 2000; Church et al., 2000; Carlson et al., 2002), arctic (Thingstad et al., 2008) and in several estuarine studies (Findlay et al., 1992; Chin-Leo & Benner 1992), including high latitude estuaries such as those in the Baltic Sea (Hoikkala et al., 2009; Figueroa et al., 2016; Soares et al., 2018). Nitrogen and phosphorus limitation of bacterioplankton activity has been suggested in

rivers and river plumes (Chin-Leo and Benner, 1992; Smith and Kemp, 2003), in the marine environment (Cotner et al., 1997; Rivkin and Anderson, 1997; Caron et al., 2000), and in coastal regions such as the Arctic (Cota et al., 1996; Thingstad et al., 2008), the Mediterranean Sea (Pinhassi et al., 2006; Kritzberg et al., 2010b) and the Baltic Sea (Hoikkala et al., 2009; Asmala et al., 2013; Soares et al., 2018). Furthermore, evidence suggests that the addition of labile carbon in combination with inorganic nutrients could be expected to stimulate heterotrophic activity to a greater extent than carbon or nutrients alone (Pomeroy et al. 1995, Cotner et al. 1997, Rivkin & Anderson 1997, Shiah et al. 1998, Thingstad et al. 1998, Church et al. 2000, Donachie et al. 2001).

Carbon limitation is expected to occur across the Lake Melville estuary in both freshwater- and marine-dominated areas. Studies in other boreal estuaries have indicated C-limited BP and BR (e.g. Hoikkala et al., 2009; Figueroa et al., 2016) and *in situ* concentrations of DOC in Lake Melville (surface DOC: 185 to 311 μM C) are comparable (316 to 408 μM C in Hoikkala et al., 2009; 416 to 916 μM C in Figueroa et al., 2016; 260 to 480 μM C in Kaartakallio et al., 2016; 343 to 458 μM C in Soares et al., 2018), suggesting that C limitation of bacterial activity might also be detected in this study. Inorganic nutrient limitation of bacterioplankton activity is expected in Lake Melville, but would be more likely to be detected during a spring phytoplankton bloom when C limitation is most likely to be alleviated from algal DOM and increased river inputs from spring melt (Norrman et al., 1995), and when competition with phytoplankton for inorganic nutrients might also be present (Caron, 1994). In this study of Lake Melville, nutrient limitation is expected to be detected in combination with C limitation

(i.e. once C limitation is relieved, nutrient limitation becomes apparent). However, nutrient availability has also been shown to enhance DOC consumption by bacteria, suggesting that some response by bacterioplankton to increased nutrient availability alone might be expected in Lake Melville (Zweifel et al., 1995; Asmala et al., 2013)

The objective of this study was to characterize the response of bacterioplankton to increases in organic and inorganic nutrients in a high latitude estuary in Labrador, Canada. This objective was addressed by determining: 1) if the remineralization of *in situ* DOM substrates was stimulated by the addition of inorganic nutrients; 2) if bacterioplankton responded differently to the addition of a labile DOC source (i.e. glucose) either alone or in combination with inorganic nutrients; and, 3) if there was a difference in bacterioplankton response between a riverine-dominated site and a more marine-dominated site.

3.2 METHODS

Nutrient Addition Experimental Design and Execution

Potential nutrient limitation of bacterioplankton growth (i.e. changes in abundance, cell volume and biomass) was investigated through nutrient addition and subsequent dark incubation of water sampled from Goose Bay and Lake Melville estuary. In order to compare bacterioplankton response from different salinities in the estuary, two locations were selected for sampling: a riverine-dominated location (RIV) and a more marine-dominated location (MAR) (Figure 3.1). Both locations were sampled in the surface mixed layer, as determined by CTD casts conducted across the system, in order to capture

the response of bacterioplankton in the region of the water column where mixing of autochthonous and allochthonous DOM and nutrients occurred.

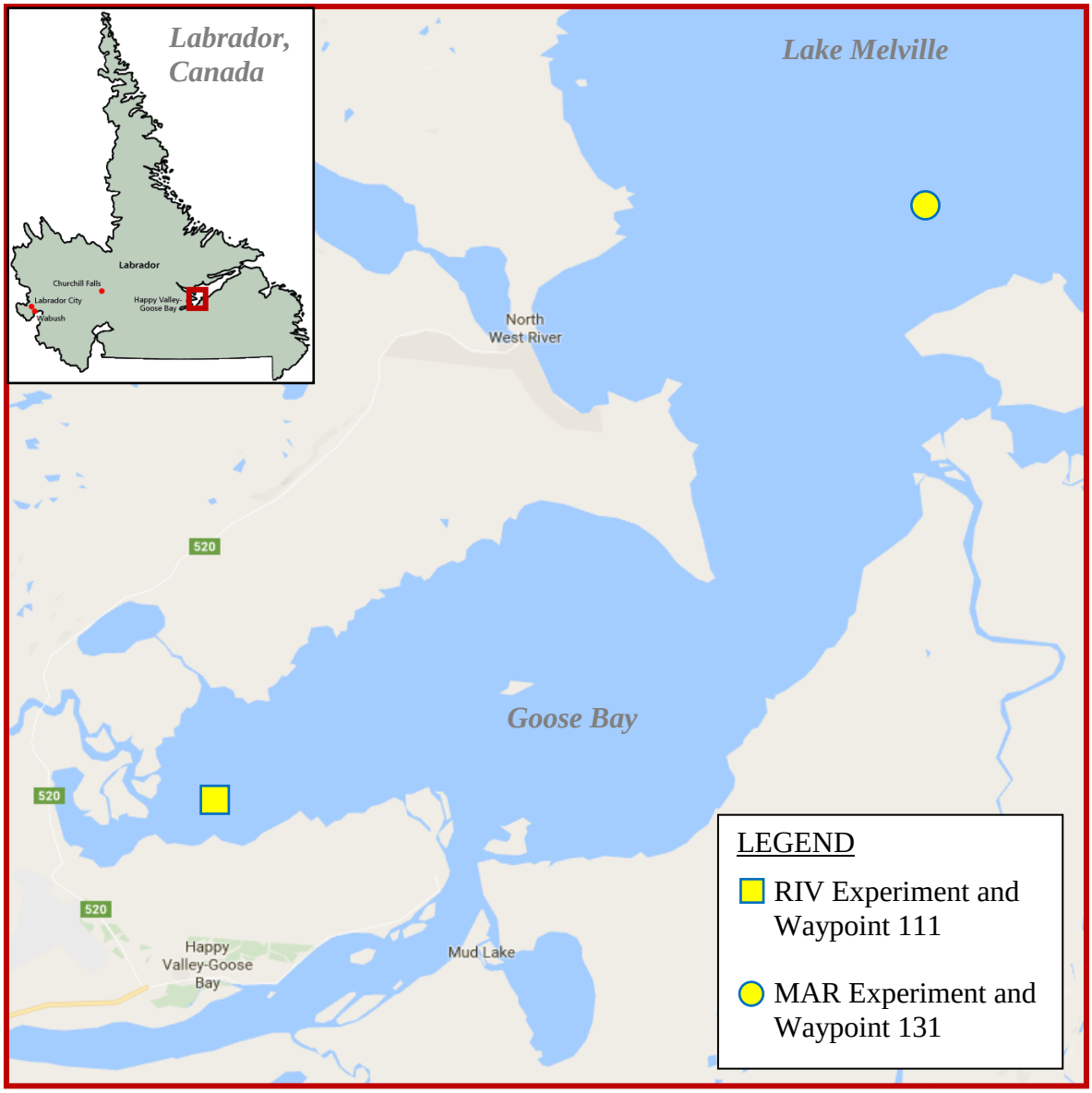

Figure 3.1. Experiment locations in Goose Bay and Lake Melville estuary, Labrador.

The nutrient amendment consisted of the addition of sources of labile organic carbon, and the inorganic nutrients ammonium and phosphate, followed by a 72-hour dark incubation. In order to reduce the potential for confounding effects from grazing on the bacterioplankton during the incubation, the concentration of grazers was reduced through filtration prior to nutrient addition. This 4:1 dilution of the grazer abundance is common (e.g. Dolan et al., 2000, Agis et al., 2007) and reduces the grazing mortality on prey populations (Landry and Hassett, 1982).

Fifty litres of water were collected at the surface from each experimental location using a 5-L Niskin bottle mounted on a cable and lowered to the predetermined depth based on the thickness of the surface mixed layer. Forty litres were filtered through 0.2μm cartridge filter using a peristaltic pump, producing particle-free seawater. The remaining 10 litres of water was filtered through a 1.0μm cartridge filter to remove particles. The <1 μm filtrate was used as the bacterioplankton inoculum that was added to the 40L of < 0.2μm filtrate. The seawater culture (10 L inoculum plus the 40 L of <0.2μm filtrate) was distributed into three 20L high density polyethylene (HDPE) carboys (A, B and C) for each of the two sampling locations in preparation for nutrient additions.

Organic carbon, inorganic nitrogen and inorganic phosphorus were used in this nutrient amendment experiment. The labile organic carbon source used was glucose enriched with stable carbon isotopes such that it's $\delta^{13}C$ was 500‰. This 500‰-labeled glucose (G) was added to achieve a final concentration of 20μM C, and allowed for the tracing of this labile carbon source as it was respired into the TIC pool causing an increase in the $\delta^{13}C$-TIC instead of the usual decrease that accompanies respiration. The source of inorganic

nitrogen (N) used was ammonium (NH_4Cl), with an intended final concentration of approximately 4μM N. The source of inorganic phosphorus (P) used was potassium phosphate (KH_2PO_4) with an intended final concentration of 0.25μM P. Nutrient additions followed a full factorial matrix design such that every combination was represented: G, N, P, GN, GP, NP, and GNP.

Nutrients were added to carboys A, B and C in sequence followed by subsampling into smaller 1L HDPE round, brown Nalgene bottles to create all treatments listed above as well as untreated controls (pre- and post-incubation). Figure 3.2 depicts the addition and sampling designed to achieve these nutrient combinations. Pre-incubation sampling (initials and GNP initials) was conducted from 1L bottles from Carboy A filled prior to any nutrient addition and again from 1L bottles from Carboy B filled after all three nutrients (glucose (G), ammonium (N) and phosphate (P)) were added in a molar ratio of approximately 80:16:1. For example, prior to any additions to Carboy A, three 1L bottles were filled for initials and three more for control finals. Then, following the addition of glucose to Carboy A, three 1L bottles were filled for G finals. Inorganic nitrogen was then added to Carboy A and three 1L bottles were filled for GN finals.

The selection of nutrients (glucose, ammonium and phosphate), their concentrations (20μM, 4μM and 0.25μM, respectively), incubation duration (72 hours) and methodology for the filtration and dilution of grazers were all informed by relevant publications on the subject (e.g. Chin-Leo and Benner, 1992; Rivkin and Anderson, 1997; Jorgensen et al., 1999; Sala et al., 2002; Smith and Kemp, 2003; Neddermann and Nausch, 2004).

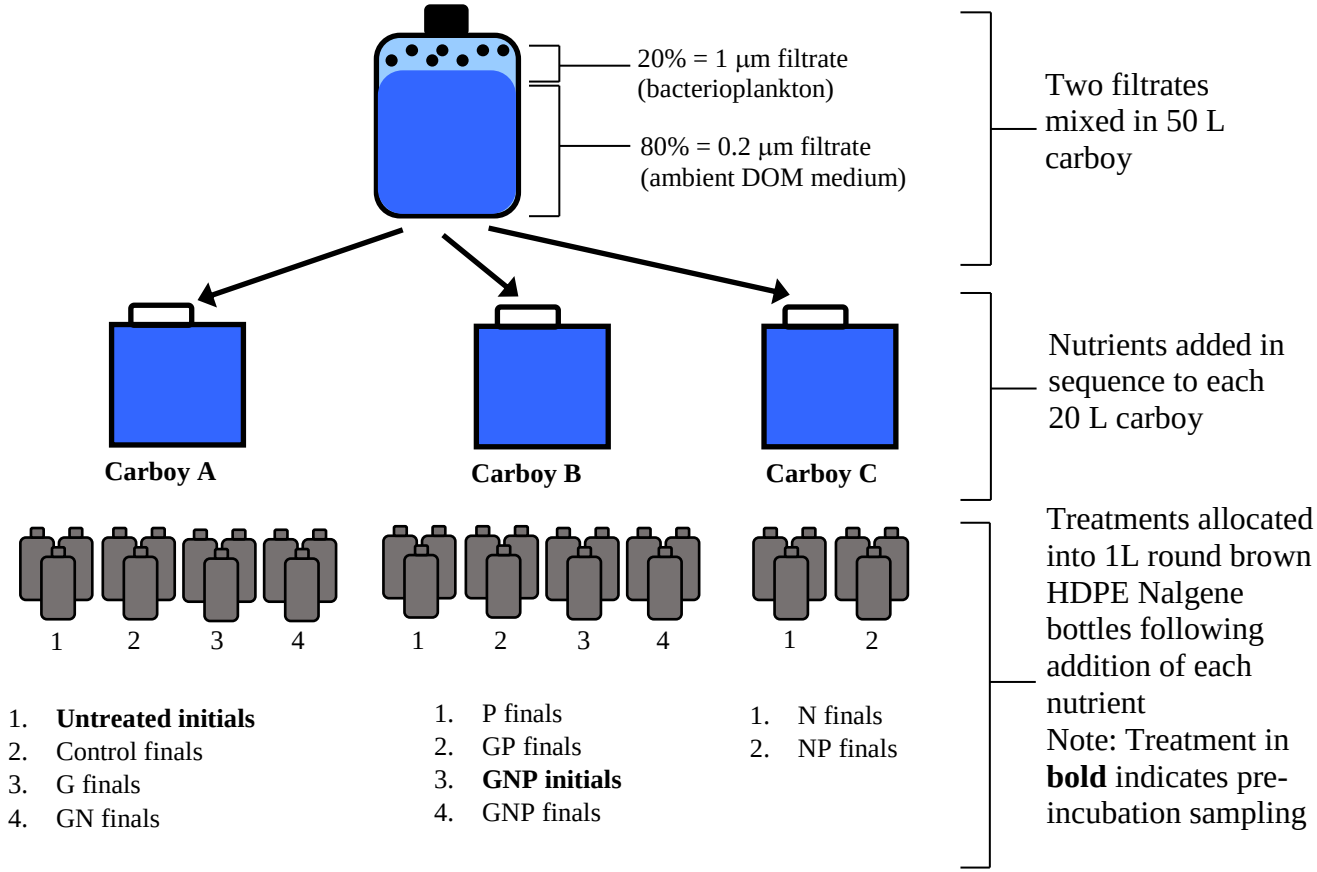

Figure 3.2. Illustration of the experimental setup for the nutrient amendment experiments; nutrients were added in sequence indicated by numbers and included glucose (G), ammonium (N), and phosphate (P).

Sample Collection

Depth profiles were generated using Sea-Bird Electronics (SBE) 25 Sealogger CTD to measure depth, salinity, temperature, sigma t, dissolved oxygen (DO), and photosynthetically active radiation (PAR). The data resolution down the water column was approximately 3-4 measurements per metre. At each location, the surface mixed layer was identified by calculating the depth of the pycnocline (depth at which the greatest change in sigma *t* was observed) using the results of the CTD casts. Water samples were collected from the surface mixed layer (i.e. above the pycnocline) at both the riverine and marine experimental sites.

All pre-incubation initial, and post-incubation final samples were collected from 1 L round brown HPDE Nalgene bottles. Samples were first collected into 40mL amber glass vials with no headspace using butyl rubber septa, fixed with 400 μL of a saturated solution (7.4g/100ml) of mercuric chloride and stored at 4°C for analysis of total inorganic carbon (TIC) concentration and stable carbon isotope ratios (δ^{13}C-TIC). Following the TIC water sample collection, remaining samples were processed and/or filtered in the order described below:

a) Samples were filtered through pre-combusted glass fiber filters (Whatman GF/F) and collected in 60mL HDPE Nalgene containers, frozen at approximately -20°C to be analyzed for concentrations of dissolved organic carbon (DOC), total dissolved nitrogen (TDN).

b) Aliquots of the GF/F filtrate were collected into 60ml falcon tubes and frozen to be analyzed for soluble reactive phosphorus (SRP), hereafter referred to as phosphate (PO_4^{3-}), nitrite/nitrate ($NO_{2/3}^-$), and ammonium (NH_4^+).

c) A final aliquot of the GF/F filtrate was collected in dark 60mL HDPE Nalgene containers and stored at 4°C in preparation for absorbance measurements.

d) Whole water samples (15 mL) were collected into 15mL falcon tubes and fixed with 1.5 mL of GF/F filtered 37% formaldehyde (formalin) for bacterioplankton abundance and cell volume analysis.

Laboratory Analysis

Organic Carbon and Nitrogen Pools

Concentrations of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were determined using a high temperature combustion elemental analyzer (Shimadzu TOC-V) with a chemiluminescent NOx detector (TNM-1; Shimadzu, Japan). Dissolved organic nitrogen (DON) concentrations were calculated as difference between the TDN concentrations and total dissolved inorganic nitrogen (DIN) concentrations (the combined ammonium and nitrate/nitrite concentrations). Molar C:N of the DOM was calculated by dividing DOC by DON.

Inorganic Carbon and Nutrients

Wet Chemical Oxidation – Isotope Ratio Mass Spectrometry (WCO-IRMS) was used to determine concentrations and $^{13}C/^{12}C$ isotope ratios ($\delta^{13}C$) of total inorganic carbon (TIC)

of the preserved seawater samples. Samples were preserved in 40mL amber glass vials with butyl rubber septa and no headspace and fixed with 400μL of mercuric chloride. The TIC concentrations and δ^{13}C-TIC values were analyzed using an AURORA 1030 TOC analyzer (O.I. Analytical, College Station, Texas, USA) coupled to a MAT252 isotope ratio mass spectrometer (Finnigan, Bremen, Germany). Values for δ^{13}C are reported in per mil units (‰) relative to the international reference standard Vienna Pee Dee Belemnite (R = 0.011237). The analytical precision for δ^{13}C-TIC was <0.4‰.

Nutrients (phosphate, nitrate/nitrite and ammonium) were analyzed using established colorimetric techniques using a segmented-flow autoanalyzer, a Technicon AutoAnalyzer II (Mitchell et al. 2002). The analysis of dissolved inorganic soluble reactive phosphorus is based on the formation of a phosphomolybdenum blue complex, and is largely orthophosphate but can include any polyphosphates. The analysis of nitrate is based on the measurement of a diazo dye formed by the reaction between sulfanilamide and nitrite, which in turn has been produced by the reduction of nitrate to nitrite on a copperized cadmium column. Because the analysis is based on the reduction of nitrate to nitrite, the method determines the sum of the nitrate and nitrite concentrations. Ammonium concentration was determined using a method involving the reaction of ammonia with orthophthaldialdehyde (OPA) as described by Kerouel and Aminot (1997). Nutrient analysis was conducted on filtered samples and standards were prepared in NaCl solutions with concentrations appropriate to the salinity of the samples being analyzed (i.e. matching salinity of the riverine-dominated experiment and salinity of the marine-dominated experiment).

Acridine Orange Staining and Epifluorescence Microscopy of Bacterioplankton

The water samples collected and preserved for bacterial abundance were filtered onto Irglan-black-stained 0.2 μm polycarbonate filters with a diameter of 25 mm, then stained with Acridine Orange prepared to a concentration of 0.468g L^{-1}. Volumes of 10 or 11 mL were used for each filter prepared. Duplicate filters were prepared from each water sample and were observed using an Olympus BH-2-RFCA epifluorescent microscope, equipped with a 120x oil immersion lens (1250x magnification total). At least 1000 bacteria were counted for each filter (Pernthaler et al. 1998).

Cell volume estimates were made from the same slides that were prepared for abundance using ImagePro Plus v. 6.2 with measurements of length, width and aspect ratio used to calculate cell volume. To estimate bacterial biomass, the equation $120v^{0.72}$ was used to estimate mass of carbon per cell based on cell volume measurements, where v is cell volume (Norland 1993). Volume was calculated as a sphere or cylinder depending on whether the aspect ratio of the bacterial cell was less than or greater than 1.5, respectively. Carbon per cell was multiplied by corresponding bacteria abundance measurements to determine biomass in terms of mass of carbon per litre.

Optical Characterization of DOM

Absorbance measurements of the collected filtrates were conducted using a 1cm path length cuvette at wavelengths spanning 250 to 325 nm using a UV/VIS scanning spectrometer (Lambda 25; Perkin Elmer, Waltham, MA, USA) and a 1 cm quartz cuvette, with NanoPure-UV water as a blank. Absorption coefficients were calculated as:

$$a(\lambda)=2.303A(\lambda)/L \quad \text{(eq. 1)}$$

where $A(\lambda)$ is the absorbance at wavelength λ and L is the path length (m) of the cuvette (Green and Blough, 1994). Specific ultraviolet absorbance (SUVA) ($\mu M\ C\ m^{-1}$) was also determined by normalizing absorption coefficients at 254 nm (a_{254}) to DOC concentration. $SUVA_{254}$ has been shown to correlate strongly with DOM aromaticity (Weishaar et al. 2003; Helms et al., 2008).

The spectral slope (S) was obtained from absorbance spectra using the equation:

$$a\lambda = a\lambda_0\, e^{[-S(\lambda - \lambda 0)]} \quad \text{(eq. 2)}$$

where $a\lambda_0$ is the reference wavelength and S is the spectral slope. Spectral slope is a measure of how quickly absorption decreases with increasing wavelength. The spectral slopes for wavelength intervals 275 to 295 nm ($S_{275\text{-}295}$) were calculated using linear regression of the log transformed absorbance data (Helms et al., 2008). The use of narrow wavelength intervals, such as 275-295 nm can help to reduce variation in the spectral slope that might be exhibited by a broader interval (e.g. 280-400 nm) (Brown 1977). Furthermore, the spectral slope from 275-295 nm has been found to be a good proxy for DOM molecular weight (MW) (Helms et al., 2008), varies more closely according to the type of aquatic environment and has a high chance of being accurately determined (Asmala et al., 2012).

Statistical Analyses

Three replicates were sampled from each treatment for each constituent that was measured, with one replicate sampled from each 1L incubation bottle. Means and

standard deviations were calculated and used to identify changes occurring during the 72-hour incubation. Student *t*-test was used to compare initials to post-incubation finals. An alpha value of 5% ($p < 0.05$) was used to identify significant differences among treatments. For some comparisons, a hypothesis about the direction of expected change can be formulated. For example, in a dark incubation, the absence of photosynthesis is likely to result in net increases in TIC concentration generated by respiration. As a result, a one-tailed test was used when analyzing changes in TIC to increase the power to detect an effect of the nutrient addition treatments. However, on most occasions where the direction of net change was not clear, a two-tailed test was used (e.g. bacterioplankton response). The *t* statistic was calculated as follows:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{{s_1}^2}{n_1} + \frac{{s_2}^2}{n_2}}} \qquad \text{(eq. 3)}$$

where x_1 and x_2 are the two means being compared, s_1 and s_2 are the standard deviations of those means, and n_1 and n_2 are the respective samples sizes.

In cases where a significant difference between means existed, the t-test effect size was examined. The effect size was measured using Cohen's d value, calculated as the difference in means over the pooled standard deviations (Cohen, 1988). The treatment finals were compared to one of two initial samples: the untreated pre-incubation initial, hereafter referred to as "initial", or the GNP initial, depending on the variable being analyzed. For example, comparison of P final to the initial is not appropriate for analysis of phosphate concentration, since any change detected could simply be from the

experimental addition of phosphate. In this case, P final must be compared to GNP initial to account for any changes from the addition. A comparison matrix is presented in the table below to identify comparisons of finals to their appropriate initial or GNP initial samples for each analyte (Table 3.1.). In order to distinguish specific treatment effects from universal incubation effects, an untreated final was included in the study, hereafter referred to as the control final. In cases where a variable in the control final was significantly different from the initial, changes in the treatment finals were compared to changes in control final instead of to the initial.

Table 3.1. Matrix of comparisons of incubation finals to appropriate initial based on the variable being analyzed.

	TREATMENT (Carboys A, B & C)							
ANALYTE	**Control Final** A	**G Final** A	**GN Final** A	**P Final** B	**GP Final** B	**GNP Final** B	**N Final** C	**NP Final** C
[DOC]	No Trt	GNP	GNP	No Trt	GNP	GNP	No Trt	No Trt
[DON]	No Trt	No Trt	No Trt	No Trt	No Trt	No Trt	No Trt	No Trt
[TIC]	A	A	A	B	B	B	C	C
δ^{13}C-TIC	A	A	A	B	B	B	C	C
[PO_4^{3-}]	No Trt	No Trt	No Trt	GNP	GNP	GNP	No Trt	GNP
[$NO_{2/3}^{-}$]	No Trt	No Trt	No Trt	No Trt	No Trt	No Trt	No Trt	No Trt
[NH_4^{+}]	No Trt	No Trt	GNP	No Trt	No Trt	GNP	GNP	GNP
Absorbance	No Trt	No Trt	No Trt	No Trt	No Trt	No Trt	No Trt	No Trt
Bacterio-plankton	No Trt	No Trt	No Trt	No Trt	No Trt	No Trt	No Trt	No Trt

No Trt = Untreated pre-incubation initial
GNP = GNP initial (after addition of glucose, ammonium and phosphate)
A, B or C = TIC initial from each carboy (A, B or C)

Respiration will increase the dissolved TIC concentration during the 72-hour dark incubation. However, given the large size of the TIC pool, small increases in TIC concentration might not have been detectable. However, respiration directly supported by the added glucose would have been detected as an increase in the stable carbon isotope signature ($\delta^{13}C$) of the TIC due the mineralization of the isotopically-enriched glucose (500‰ glucose) that was added as the labile carbon source in the nutrient addition experiments. The fraction of the TIC pool derived from this labile labeled carbon source was examined using the follow formula:

$$\Delta[TIC]_{500‰} = \left(\frac{\delta^{13}C\text{-}TIC_{FINAL} - \delta^{13}C\text{-}TIC_{INITIAL}}{500\ ‰ - \delta^{13}C\text{-}TIC_{INITIAL}} \right) \times [TIC]_{FINAL}$$

where $\Delta[TIC]_{500‰}$ is the change in TIC concentration in a treatment final due to mineralization of the 500‰ enriched glucose addition. The stable isotope signatures of the treatment final and untreated initial are represented by $\delta^{13}C\text{-}TIC_{FINAL}$ and $\delta^{13}C\text{-}TIC_{INITIAL}$, respectively. The treatment final TIC concentration is represented as $[TIC]_{FINAL}$.

3.3 RESULTS

Nutrient addition experiments were conducted in the month of September (2008), when Churchill River flow is historically the lowest (Figure 3.3), in order to identify potential nutrient limitation of bacterial activity that might be seasonally alleviated during high flow conditions.

Surface water was sampled for the MAR (53° 34.20’ N, 59° 54.74’ W) and RIV (53° 21.67’ N, 60° 19.32’ W) incubation experiments on September 23 and 28, 2008, respectively. Experimental sampling locations were approximately 36 km apart with the RIV location 10 km from the mouth of the Churchill River and the MAR location 29 km from the river (Figure 3.1). The surface water for the RIV experiment had a salinity ~6 psu, temperature of 10 °C, and pycnocline was calculated at approximately 3 m depth where the greatest change per metre depth occurs for density (sigma *t*) (Figure 3.4).

Surface water for the MAR incubation had a salinity ~14 psu, temperature of 8 °C, and pycnocline was calculated at approximately 6 m (Figure 3.5).

The experimental design and interpretation of the results were put into context using *in situ*, or ambient, concentrations from Lake Melville determined at stations that closely approximated experimental sampling locations. Waypoint 111 in Goose Bay (10.5 psu, 7.9 °C) was sampled on September 24, 2008 in close proximity (53° 21.64' N, 60° 19.34' W) to the RIV experimental sampling location. Waypoint 131 in Lake Melville (15 psu, 7.7 °C) was sampled on September 27, 2008 in close proximity (53° 34.61' N, 59° 54.54' W) to the MAR experimental sampling location. The samples at Waypoints 111 and 131 were taken at 3 m depth. Results are presented in the Tables 3.2 and 3.3 below along with the initial (pre-incubation) values for the RIV and MAR incubation experiments, respectively.

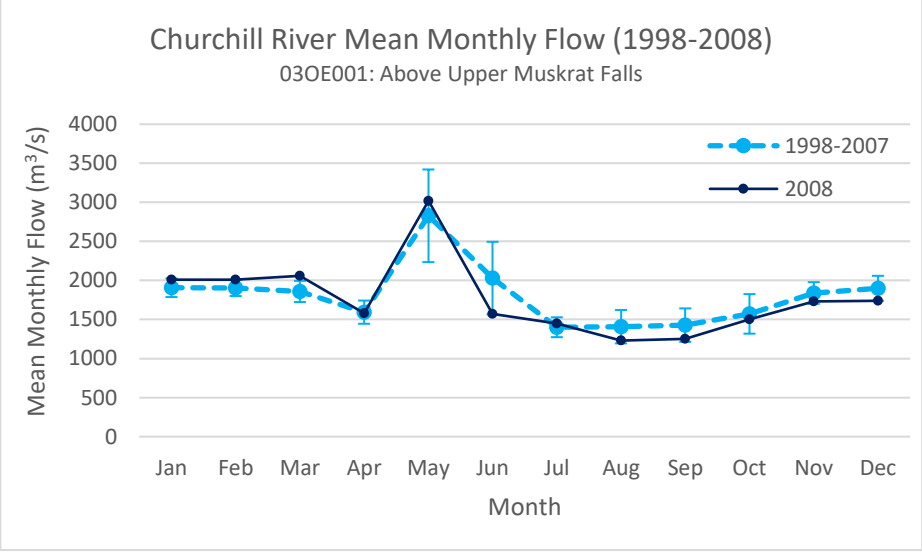

Figure 3.3: Historic data of the mean monthly flow of the Churchill river (1998-2008).

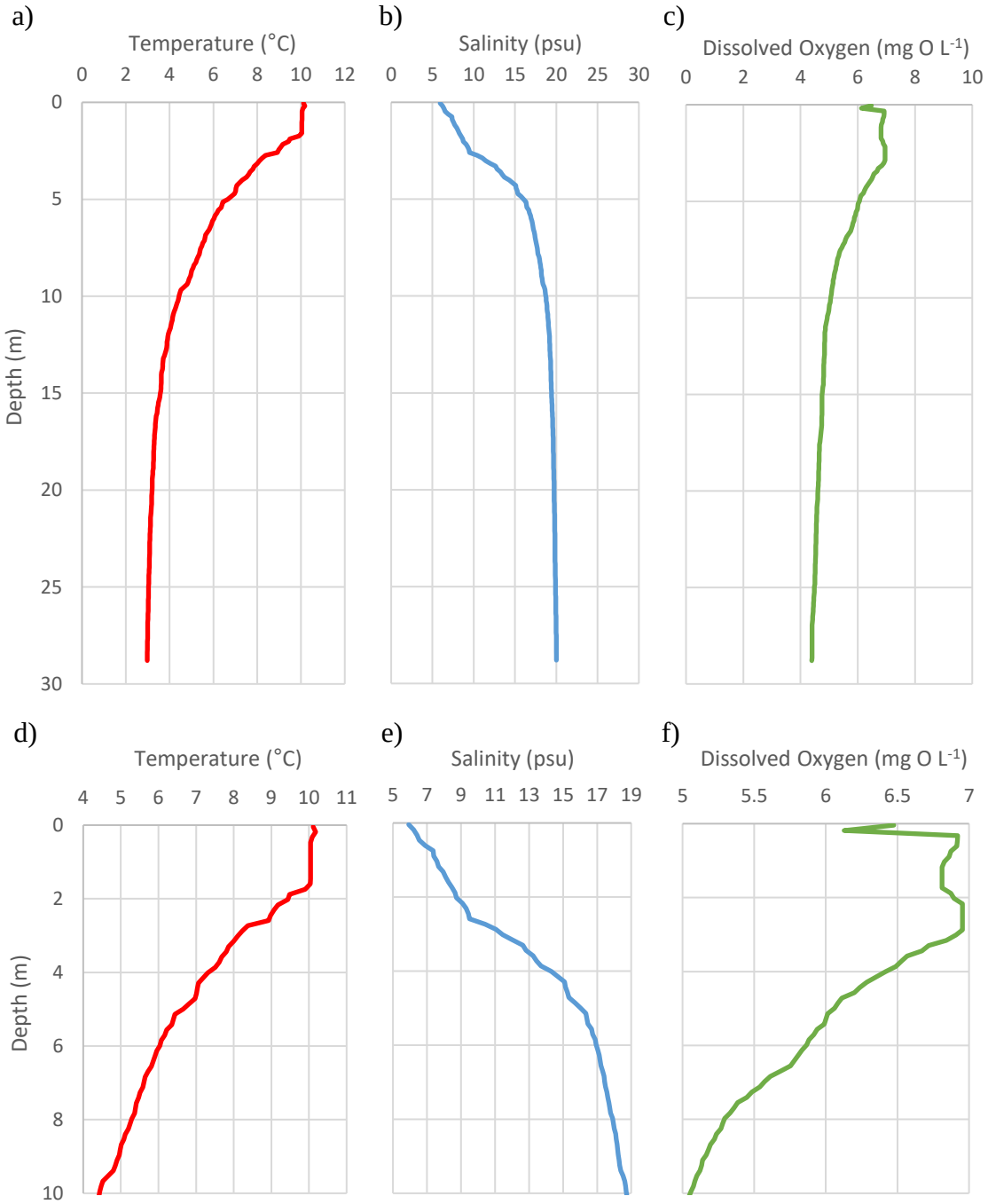

Figure 3.4. CTD depth profiles at RIV experiment sampling location including a) temperature, b) salinity, c) dissolved oxygen, d) surface temperature, e) surface salinity, and f) surface dissolved oxygen.

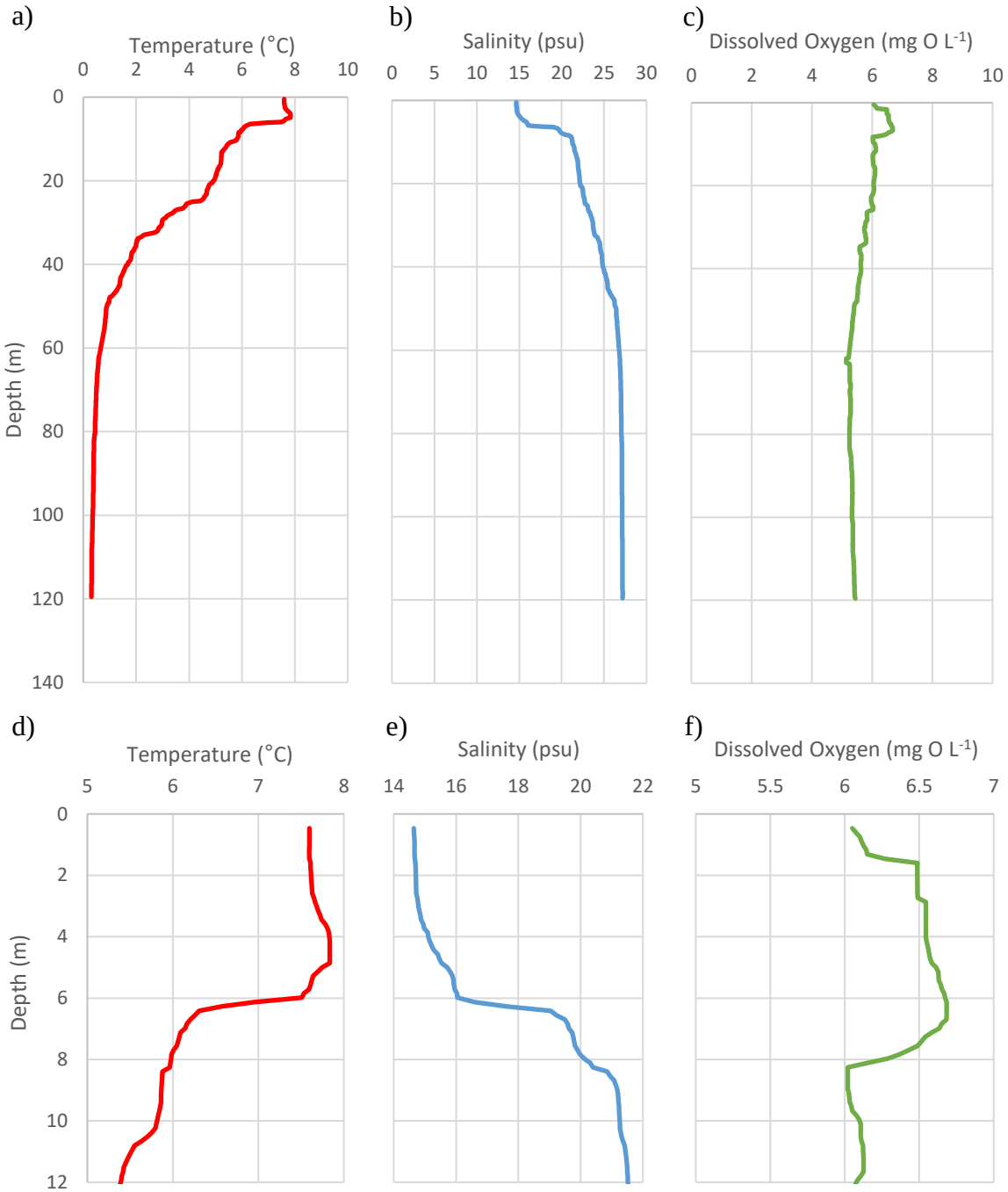

Figure 3.5. CTD depth profiles at MAR experiment sampling location including a) temperature, b) salinity, c) dissolved oxygen, d) surface temperature, e) surface salinity, and f) surface dissolved oxygen.

Pre-incubation Initials and Ambient Conditions

DOM quality

The DOM pool of the pre-incubation untreated initials of the RIV experiment exhibited attributes consistent with lower bioreactivity compared to the MAR experiment. In the RIV experiment, dissolved organic carbon (DOC) mean concentration in the initials was higher, while dissolved organic nitrogen (DON) was lower, compared to the MAR experiment. As a result, C:N of the DOM pool was over twice as high in the RIV initials compared to the MAR initials. DOC analysis of the GNP treated pre-incubation initials was not conducted, therefore values were estimated based on an intended experimental addition of 20 μM C of glucose (Tables 3.2 and 3.3).

Surprisingly, absorbance coefficient at 254 nm normalized to DOC concentration ($SUVA_{254}$) was lower in the RIV experiment compared to the MAR experiment, suggestive of lower aromaticity of the RIV DOM. Congruent with this, the spectral slope from 275 to 295 nm ($S_{275\text{-}295}$) was greater in the pre-incubation initials of the RIV experiment than in the MAR experiment.

Ambient concentrations were measured at waypoints close to locations for the RIV and MAR experiments. At waypoint 111, ambient concentrations of DOC and DON were similar to the experimental initials. The ambient $SUVA_{254}$ at waypoint 131 and in the MAR experiment initials were the same, and were higher than ambient values at waypoint 111 and in the RIV experiment. Ambient values of $S_{275\text{-}295}$ were higher at waypoint 111

than at 131, very similar at waypoint 131 and the MAR experiment initials, but much lower at waypoint 111 than in the RIV experiment initials (Tables 3.2 and 3.3).

Table 3.2. RIV experiment pre-incubation initials and Waypoint 111 ambient measurements.

		Initial Pre-incubation Sample Means									
ANALYTE	Wpt 111 (*in situ*)	No Trt (Carboy A)		GNP (Carboy B)		Carboy A		Carboy B		Carboy C	
		Mean	St Dev	Mean	St Dev	Mean	St Dev	Mean	St Dev	Mean	St Dev
[DOC] (μM C)	311 ±3	319.32	2.67	339.32[2]	2.67	-	-	-	-	-	-
[DON] (μM N)	5.29 ±0.44	6.09	0.45	-	-	-	-	-	-	-	-
C:N of DOM	44.0 ±2.8	52.4	3.9	55.7	8.1						
[TIC] (μM C)	676.2 ±18.6	-	-	-	-	233.22	3.67	236.39	7.91	243.33	4.78
δ^{13}C-TIC (‰)	-1.02 ±0.50	-	-	-	-	-5.38	0.35	-5.55	0.43	-5.53	0.20
[PO_4^{3-}] (μM P)	BD	BD	BD	0.20	0.13	-	-	-	-	-	-
[$NO_{2/3}^-$] (μM N)	2.60 ±0.10	0.26	0.21	0.11	0.07	-	-	-	-	-	-
[NH_4^+] (μM N)	1.77 ±0.05	1.43	0.01	9.02	0.50	-	-	-	-	-	-
$SUVA_{254}$ (μM $C^{-1}m^{-1}$)	0.083 ±0.006	0.066	0.004	-	-	-	-	-	-	-	-
Spectral Slope $S_{275-295}$ (μm^{-1})	17.0 ±0.9	27.2	1.7	-	-	-	-	-	-	-	-
Bacterioplankton Biomass (μg C L^{-1})	12.22 ±1.41	23.86	3.80	-	-	-	-	-	-	-	-
Bacterioplankton Abundance (x10^6 cells L^{-1})	981.1 ±111.0	1179.3	90.1	-	-	-	-	-	-	-	-
Bacterioplankton Cell Volume (μm^3)	0.043 ±0.001	0.084	0.010	-	-	-	-	-	-	-	-

No Trt = untreated initial (pre-incubation)
GNP = GNP initial (after addition of glucose, ammonium and phosphate)
A, B or C = TIC initial from each carboy (A, B or C)
BD = Below Detection
[1] n=2, otherwise n=3 for all means
[2] [DOC] for GNP pre-incubation initial is estimated.

Table 3.3. MAR experiment pre-incubation initials and Waypoint 131 ambient measurements.

		Initial Pre-incubation Sample Means									
	Wpt 131 (*in situ*)	No Trt (Carboy A)		GNP (Carboy B)		Carboy A		Carboy B		Carboy C	
ANALYTE		**Mean**	**St Dev**	**Mean**	**St Dev**	**Mean**	**St Dev**	**Mean**	**St Dev**	**Mean**	**St Dev**
[DOC] (μM C)	266 ±2	223.12	1.98	243.12[2]	1.98	-	-	-	-	-	-
[DON] (μM N)	8.71 ±0.71	10.98	0.37	-	-	-	-	-	-	-	-
C:N of DOM	25.2 ±1.7	20.3	0.7	22.1	0.8						
[TIC] (μM C)	812.1 ±13.46	-	-	-	-	1058.96	29.28	1046.72	5.19	1052.38	10.78
δ^{13}C-TIC (‰)	-1.22 ±0.66	-	-	-	-	-0.55	0.04	-0.45	0.15	-0.59	0.07
[PO_4^{3-}] (μM P)	BD	0.39	0.01	0.17	0.01	-	-	-	-	-	-
[$NO_{2/3}^-$] (μM N)	1.57 ±0.05	3.50	0.18	3.56	0.08	-	-	-	-	-	-
[NH_4^+] (μM N)	1.85 ±0.03	1.07	0.15	5.22	0.04	-	-	-	-	-	-
$SUVA_{254}$ (cm^{-1}μM C^{-1})	10.5 ±0.9	10.7[1]	0.7	-	-	-	-	-	-	-	-
Spectral Slope $S_{275-295}$	14.9 ±1.4	14.6	NA	-	-	-	-	-	-	-	-
Bacterioplankton Biomass (μg C L^{-1})	14.97 ±1.23	29.50	2.20	-	-	-	-	-	-	-	-
Bacterioplankton Abundance (x10^6 cells L^{-1})Spectral Slope Ratio (S_R)	1029.1 ±76.11. 0	1977.6 2.31	155.3-	-	-	-	-	-	-	-	-
Bacterioplankton Cell Volume (μm^3)	0.053 ±0.001	0.0552 9.50	0.0032. 20	-	-	-	-	-	-	-	-

BD = below detection
No Trt = untreated initial (pre-incubation)
GNP = GNP initial (after addition of glucose, ammonium and phosphate), i.e. GNP_{low}
A, B or C = TIC initial from each carboy (A, B or C)
NA = data not available (n=1)
[1] n=2, otherwise n=3 for all means
[2] [DOC] for GNP pre-incubation initial is estimated.

Heterotrophic Respiration

Total inorganic carbon (TIC) concentration and stable carbon isotope signature of the total inorganic carbon pool (δ^{13}C-TIC) were used as a proxy for heterotrophic respiration during the nutrient amendment experiments. Pre-incubation initials were analyzed for each of the three Carboys (A, B and C) (Tables 3.2 and 3.3) and an analysis of variance (ANOVA) revealed that initials were not significantly different within each experiment

(RIV experiment TIC: F=2.432, df=8 p=0.17; δ^{13}C-TIC: F=0.172, df=8. p=0.85; and MAR experiment TIC: F=0.338, df=8, p=0.73; δ^{13}C-TIC: F=1.585, df=8, p=0.28).

The TIC concentrations in the MAR experiment initials were 4.5 times as high as those in the RIV experiment. At waypoint 111, ambient TIC concentrations were over 2.5 times as high as the RIV experimental initials, suggesting that the higher salinity of waypoint 111 (10.5 psu) might be resulting in slightly more marine characteristics of the ambient TIC pool than at the RIV experimental location where salinity was around 6 psu (Table 3.2). At waypoint 131, ambient TIC concentration was slightly lower than that of the MAR initials (~20% lower) (Table 3.3).

The MAR experiment pre-incubations initial δ^{13}C-TIC values were an order of magnitude higher than those of the RIV experiment, however the ambient δ^{13}C-TIC values at waypoints 111 and 131 were similar to each other, and only slightly lower than the MAR experiment initials (Tables 3.2 and 3.3). Again, this lower ambient δ^{13}C-TIC could be a reflection of a more marine signature of the ambient TIC pool at waypoint 111 compared to the RIV experimental sampling location.

Inorganic Nutrients

Phosphate concentrations in the RIV experiment were found to be below detection in the untreated initials and 0.20 ± 0.13 μM P in the GNP treated initials, which was close to the intended addition of 0.25 μM P (Table 3.2). Phosphate concentration in the MAR experiment pre-incubation, untreated initials was found to be much higher than expected (0.39 ± 0.01 μM P; n=3). The MAR initials were expected to reflect *in situ* concentrations

of phosphate, which were all below detection in Lake Melville surface waters, in contrast to the GNP pre-incubation initials where nutrients were intentionally added (Table 3.3.). The intentional addition of phosphate in the MAR experiment GNP initials was 0.17 ± 0.01 μM P (n=3) whereas the untreated initial has 0.39 ± 0.01 μM P (n=3), which was thought to be due to contamination during sample transfer to the carboy; that is, phosphate contamination occurred pre-incubation in Carboy A as illustrated in Figure 3.6 below. As a result, treatments have been renamed in the MAR experiment to reflect this contamination such that Control = P_{high}, G = GP_{high}, and GN = GNP_{high}. To distinguish them from the intentional phosphate addition, the other treatments have also been renamed P_{low}, GP_{low}, GNP_{low}, and NP_{low}. This contamination of Carboy A effectively negated an untreated control final for the MAR experiment (now called P_{high} treatment), but has inadvertently created an opportunity for comparison between treatments with low and high phosphate concentrations. As a consequence of a lack of control final, results from MAR treatments were compared to the initials (untreated and GNP) and to each other in order to appropriately interpret the effects of each treatment in the absence of a control final.

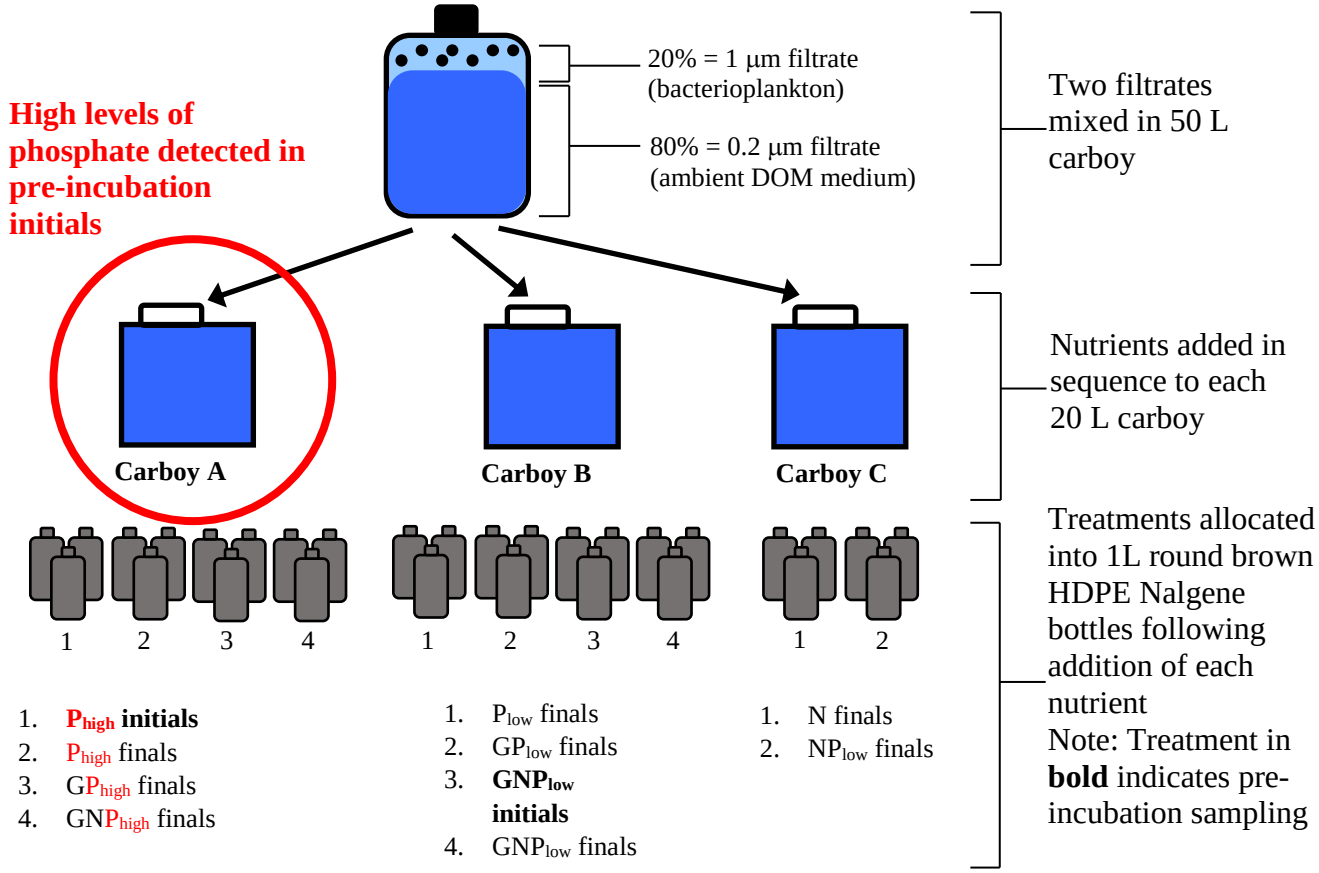

Figure 3.6. Contamination occurring in the MAR experiment's Carboy A.

Ammonium concentrations in initial samples were found to be slightly higher in the RIV experiment (1.43 ± 0.01 μM N; n=2) than in the MAR experiment (1.07 ± 0.15 μM N; n=3). Ambient concentrations of ammonium were similar for both sampling locations and were slightly higher than the concentration of the RIV initials(Tables 3.2 and 3.3). A comparison of the untreated pre-incubation initials to the GNP treated pre-incubation initials showed that ammonium additions were approximately 7.59 μM N in the RIV experiment and 4.15 μM N in the MAR experiment, additions of more than 2 to 4 times ambient levels of ammonium, respectively.

Initial pre-incubation nitrate/nitrite concentrations were an order of magnitude lower in RIV experiment than in the MAR experiment. Since no nitrate was added to the experiments, the GNP pre-incubation initials were expected to, and did, show the same trend (Table 3.3.). At waypoint 111, ambient nitrate concentrations were higher than expected based on the RIV initials concentrations, but this could be due to a more marine signature supported by the higher salinity at waypoint 111 than at the RIV experimental location. Ambient nitrate concentrations at waypoint 131 were slightly lower than pre-incubation initials from the MAR experiment (Table 3.3.).

Bacterioplankton

Bacterial abundance (BA) initial pre-incubation measurements from the RIV experiment were found to be much lower than for the MAR experiment. Bacterial cell volumes (BV) followed the opposite trend with RIV experiment initials exhibiting larger BV than MAR experiment initials. Bacterial biomass (mg C L^{-1}) was calculated using measurements of

abundance and cell volume, and was found to be fairly similar between experiments. Measurements of ambient BA and BV were similar at both stations, with a slightly higher abundance and cell volume, and therefore biomass, at waypoint 131 than at waypoint 111. Ambient BA and BV were also lower than the pre-incubation initials in both experiments. Since the experimental initials underwent a 1:4 dilution intended to reduce grazing pressure on bacterioplankton, it was expected that bacterial abundance measurements of the initials would reflect this dilution and yield values closer to 20% of ambient BA. The dilution was important for two reasons: 1) to ensure that bacterial growth could continue exponentially during the 72-hour incubation and would not approach carrying capacity; and, 2) that grazing pressure would be negligible as a result of a reduction in the concentration of grazers. Instead of a reduced bacterial abundance, experimental initials had higher BA than waypoints 111 and 131, with MAR initial BA almost twice that of waypoint 131 (Tables 3.2 and 3.3). It is clear from this comparison of ambient and experimental initial bacterial measurements that the dilution was unsuccessful, and that, while no grazer data was collected, grazing pressure must now be considered in the interpretation of the results of these experiments.

Grazing pressure

The effects of grazing pressure must be considered in the interpretation of bacterioplankton data since the proposed 1:4 dilution described in the methods did not appear successful based upon bacterial abundance (BA) measures (post-dilution, pre-incubation BA was greater than ambient BA measurements). With grazers present, changes in bacterial abundance, biomass, dissolved carbon and the uptake of nutrients

must all be considered as *net* community level changes in rates and responses. For example, grazing most likely resulted in the dampening or obscuring of measurable uptake of DOC and inorganic nutrients by bacterioplankton through messy feeding. Similarly, direct predation by grazers likely reduced measurable increases in bacterial abundance and biomass.

RIV Experiment – Post-Incubation Results

Significant net uptake of DOC compared to control was observed in GP and GNP while net production of DOC occurred in GN. There was no net change in DON in the control but GNP and N exhibited net uptake of DON compared to pre-incubation initials. There was no significant net change in TIC nor in the δ^{13}C-TIC control, but significant net decrease in TIC concentration was observed in NP and net change in δ^{13}C-TIC was observed in GN, GP and GNP (net increases) and in N and NP (net decreases). Net uptake of phosphate was observed in GP and N compared to initials, and net uptake of ammonium was observed in GNP compared to initials. There was no net change in $SUVA_{254}$ nor in the spectral slope from 275-295 nm ($S_{275\text{-}295}$).There was a net decrease observed in bacterial abundance compared to controls and a net increase in bacterial biomass in GP compared to initials (Table 3.4).

Table 3.4. Comparison of treatment finals to initials and controls: RIV experiment. Treatments were compared to pre-incubation initial when control final was not significantly different from initial.

ANALYTE	Changes Observed in Treatment Finals							
	Control	**G**	**GN**	**P**	**GP**	**GNP**	**N**	**NP**
[DOC] (μM C)	↓*	↓	↑**	↓	↓**	↓**	↓	↓
[DON] (μM N)						↓*	↓*	↓
[TIC] (μM C)								↓*
δ^{13}C-TIC (‰)			↑*		↑*	↑*	↓*	↓*
[PO_4^{3-}] (μM P)					↓*		↓*	
[$NO_{2/3}^{-}$] (μM N)								
[NH_4^+] (μM N)						↓*		
$SUVA_{254}$ (cm^{-1}μM C^{-1})								
Spectral Slope $S_{275-295}$								
Bacterioplankton Biomass (mg C L^{-1})					↑*			
Bacterioplankton Abundance (x10^6 cells L^{-1})	↑*					↓**		↓**
Bacterioplankton Cell Volume (μm^3)					↑	↑		

* = Significantly different from Untreated Pre-incubation Initial
** = Significantly different from Control Final
↑ = Increase compared to Untreated Pre-incubation Initial or Control
↓ = Decrease compared to Untreated Pre-incubation Initial or Control
Note: Blank boxes in table indicate no detectable change.

DOM quality

There was a significant decrease in DOC concentration in the control finals for both MAR and RIV experiments, therefore the changes in treatment finals were compared to the change in control DOC concentration to determine the existence of a significant treatment effect. There was a significant decrease in DOC concentration in the GP and GNP finals (DOC uptake of 24.9 ± 3.1 μM C; df=2, p = 0.024 and 22.4 ± 3.1 μM C; df=, p = 0.037, respectively), compared to the control final (DOC uptake of 13.8 ± 3.0 μM C) (Table 3.4;

Figure 3.7a). Since the net uptake of DOC in GP and GNP was close to the added glucose concentration (20μM C), it was not possible to determine if the uptake of ambient DOC occurred in these treatments, nor was it possible to determine if the DOC was allocated to biomass or respired by bacterioplankton.

An increase in DOC concentration was detected in the GN final based upon initial DOC measurements of GNP initial (DOC increase of 44.1 ± 4.2 μM C; df=4, $p < 0.00001$) (Table 3.4; Figure 3.7a). While the mechanism for DOC production is unclear, one possibility is release of DOC through viral lysis of bacterioplankton in the GN treatment. Viral lysis has been found to release bacterial DOC to the water column, providing carbon substrate for bacterioplankton (Gobler et al., 1997; Kawasaki and Benner, 2006; Middelboe et al., 1996; Riemann et al., 2009). It is possible that the DOC released via cell lysis accumulated in the GN treatment final due to P-limiting conditions, which limited microbial degradation of the DOC supply.

There was no significant net change in control final DON concentrations or C:N, therefore post-incubation treatment finals were compared to pre-incubation initials. Dissolved organic nitrogen uptake was stimulated in GNP and N treatments, resulting in significant decrease in DON compared to the initial concentration (DON uptake of 2.77 ± 0.95 μM N; df=4, $p = 0.007$ and 3.56 ± 0.62 μM N; df=4, $p = 0.0006$, respectively). However, the NP treatment did not exhibit significant DON (Table 3.4; Figure 3.7b). It would appear that the addition of ammonium can stimulate bacterial activity that leads to processing of the ambient DON pool. This is supported by C:N values of the utilized DOM (3.8 to 8.1), which were found to be much lower than the C:N of pre-incubation

initials of the whole DOM pool (52.4 to 55.7) (Table 3.2, Figure 3.7c). Though the GN treatment doesn't support this hypothesis, it is possible that, if DOC was released via viral lysis in the GN treatment, DON might also have been released in this way, obscuring DON uptake that might otherwise have been observed; however, N cycling pathways during viral lysis are unclear (Gobler et al., 1997).

Optical properties of the DOM pool did not change during the 72-hour incubation. There was no net change in $SUVA_{254}$ ($p > 0.09$) nor in $S_{275\text{-}295}$ ($p > 0.17$) across all treatments (Table 3.5).

Table 3.5. RIV Experiment: Optical properties of the DOM pool including $SUVA_{254}$ and $S_{275\text{-}295}$.

RIV treatments	**$SUVA_{254}$ ($\mu M\ C^{-1}m^{-1}$)**	**$SUVA_{254}$ st dev**	**$S_{275\text{-}295}$ (μm^{-1})**	**$S_{275\text{-}295}$ st dev**
Initial	0.066	0.004	27.1	1.7
Control	0.075	0.012	24.9	3.9
G[1]	0.067	0.003	26.5	1.1
GN	0.058	0.005	25.5	2.0
P	0.066	0.002	28.0	1.0
GP	0.086	0.018	21.1	5.1
GNP	0.070	0.005	26.2	2.1
N	0.067	0.002	27.5	0.8
NP	0.068	0.000	27.0	0.2

[1] n=2; otherwise n=3 for all means

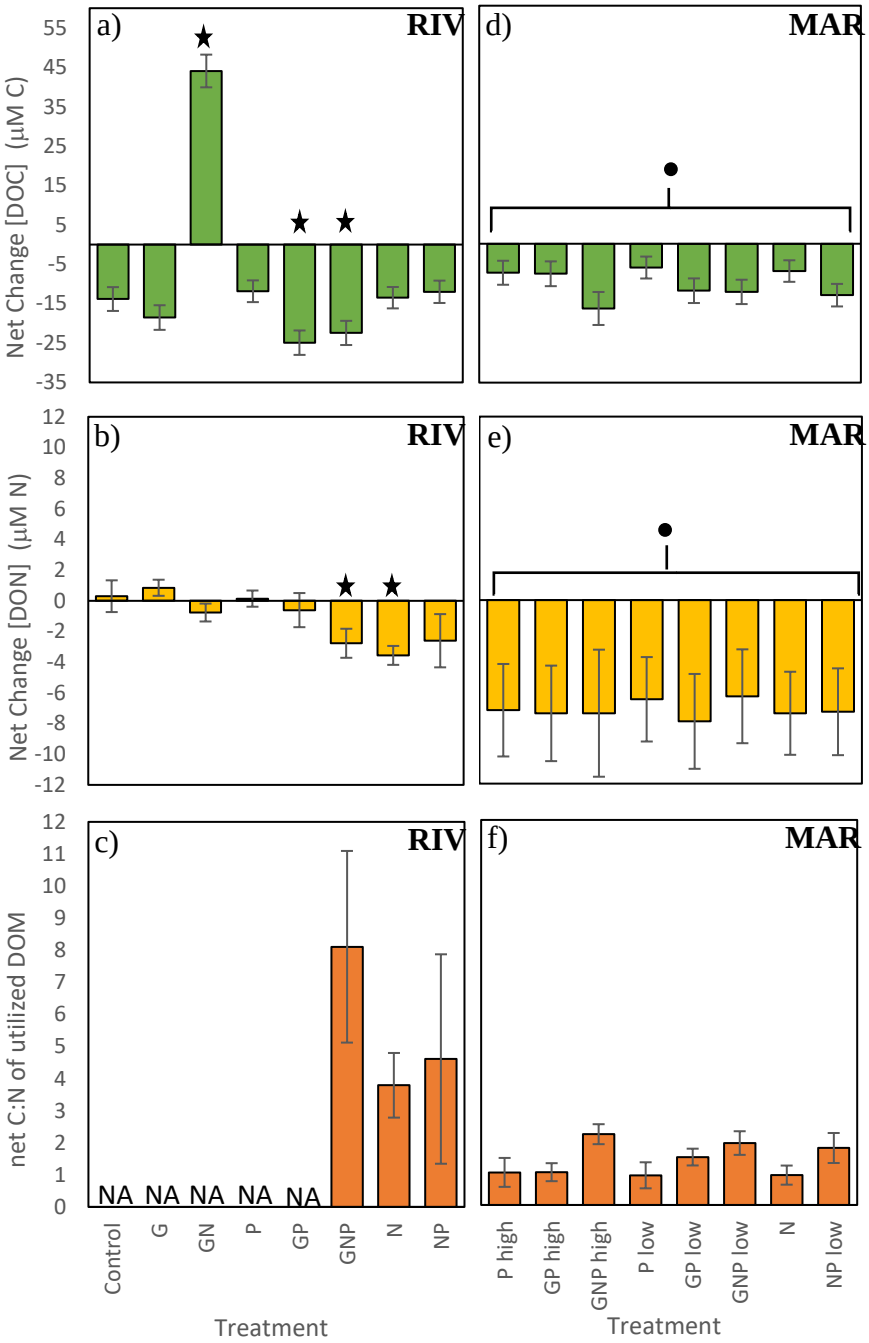

Figure 3.7a to f. Net changes in DOM over 72-hour incubations. RIV experiment, significant net change (★): a) net change in DOC, GP (-24.9 ± 3.1 µM C; df=2, p=0.024), and GNP (-22.4 ± 3.1 µM C; df=2, p=0.037), GN (production of 44.1 ± 4.2 µM C; df=2, p=0.001); b) net change in DON, GNP (2.77 ± 0.95 µM N; df=4, p = 0.007), and N (3.56 ± 0.62 µM N; df=4, p = 0.0006); c) C:N of utilized DOM (Δ DOC: Δ DON). MAR experiment, significant net change (●): d) net change in DOC (decreases of 6.0 to 16.3 µM C; df=4, 0.0002 < p < 0.02); e) net change in DON (decreases of 6.29 to 7.93 µM N; df=4, p < 0.0001) ; f) C:N of utilized DOM. P = Phosphate, G = Glucose, N = Ammonium, NA = C:N not available, (i.e., not available due to ΔDOC ≤ 0 and/or ΔDON ≤ 0).

Heterotrophic respiration

There was no measurable net increase in TIC concentration to indicate heterotrophic respiration given the high standard deviation of the difference between the pre-incubation initial and post-incubation final means, therefore the minimum detectable change (MDC) was calculated to assist with interpretation of the results (Spooner et al., 2011). The MDC was calculated for each carboy (A, B and C) using a power of 0.80 and an alpha of 0.05, and was determined to be 5.69, 6.04 and 6.02 μM C for carboys A, B and C, respectively. There was a small but statistically significant net decrease in the NP treatment (decrease of 10.70 ± 5.73 μM C ; df=4, p = 0.03) (Table 3.4; Figure 3.8a).

While heterotrophic respiration was not evident in the TIC concentrations, decreases in the $\delta^{13}C$ of TIC can be more easily detected to provide evidence of mineralization of DOC. Since 500‰ $\delta^{13}C$–labeled glucose was added in some treatments (i.e. G, GN, GP and GNP), mineralization of that glucose would increase the $\delta^{13}C$ of TIC. Significant decreases (mineralization of ambient DOC) and increases (mineralization of glucose) in the $\delta^{13}C$ of TIC of treatment finals were found compared to pre-incubation initials. Comparison to initials was conducted since controls were not significantly different from initials (p = 0.17). Increases in the $\delta^{13}C$ of TIC were found in GN treatment (0.59 ± 0.36 ‰; df = 4, p = 0.023), GP treatment (2.15 ± 0.49 ‰; df = 4, p = 0.0007) and GNP treatment (2.92 ± 0.73 ‰; df = 4, p = 0.001), while decreases in the $\delta^{13}C$ of TIC were found in N treatment (-0.42 ± 0.23 ‰; df = 4, p = 0.017) and NP treatment (-0.79 ± 0.34 ‰; df = 4, p = 0.008) (Table 3.4; Figure 3.9).

The fraction of the TIC pool derived from the 500‰–labeled glucose addition was examined, and while glucose (G) addition alone did not significantly enrich the $\delta^{13}C$ of the TIC pool, the combination of glucose and ammonium (GN) stimulated the mineralization of 0.38 ± 0.026 μM C of the labeled glucose (or 0.005 μM C hr^{-1}), and the addition of glucose and phosphate (GP) stimulated the mineralization of 1.16 ± 0.13 μM C (or 0.016 μM C hr^{-1}). The combination of all three additions (GNP) yielded greatest amount of glucose-derived TIC, 1.52 ± 0.29 μM C (or 0.021 μM C hr^{-1}) (Figure 3.10). Treatment finals that included glucose and phosphate (GP and GNP) exhibited greater increases compared to the GN treatment. The TIC stable isotope depletion in NP treatment was greater than in N treatment. This suggests that mineralization of ambient DOC was stimulated by a combined N and P addition, regardless of the addition of a labile carbon source (glucose).

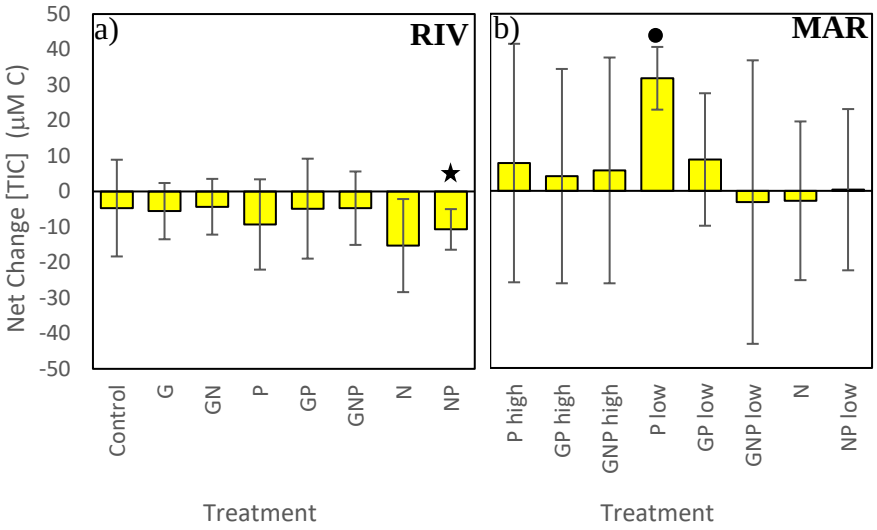

Figure 3.8a & b. Net changes in TIC over 72-hour incubations. RIV experiment, significant net change (★): a) net change in TIC, NP (-10.7 ± 5.7 µM C; df=4, p=0.03). MAR experiment, significant net change (●): b) net change in TIC, P_{low} (31.8 ± 8.9 µM C; df=4, p=0.002). P = Phosphate, G = Glucose, N = Ammonium.

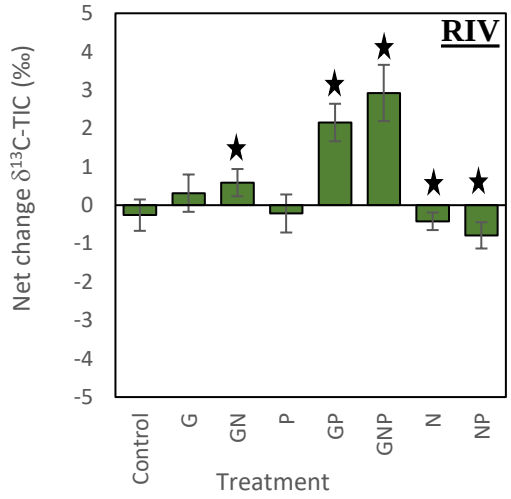

Figure 3.9. RIV experiment: net change in the δ^{13}C-TIC of the TIC pool over 72 hours. Significant net enrichment or depletion is indicated by a star (★). Significant enrichment GN (0.59 ± 0.36 ‰; df=4, p=0.046), GP (2.15 ± 0.49 ‰; df=4, p=0.002), and GNP (2.92 ± 0.73 ‰; df=4, p=0.002). Significant depletion in N (-0.42 ± 0.23 ‰; df=4, p=0.03) and NP (-0.79 ± 0.34 ‰; df=4, p=0.02), P = Phosphate, G = Glucose, N = Ammonium.

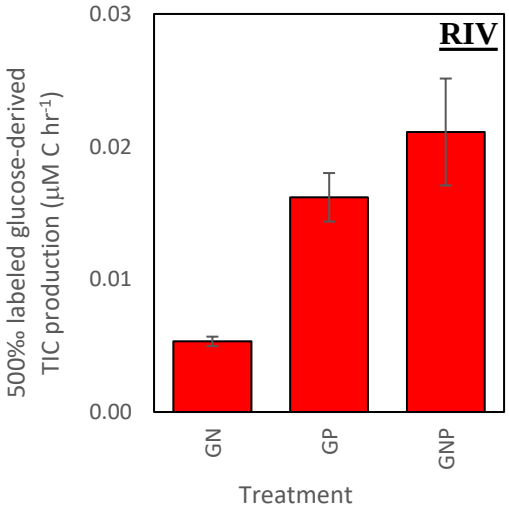

Figure 3.10. RIV experiment: Calculated concentration of TIC derived from mineralization of added glucose over 72 hour incubation. GN treatment = 0.005 ± 0.000 µM C hr^{-1}, GP treatment = 0.016 ± 0.0012 µM C hr^{-1} and GNP treatment = 0.021 ± 0.004 µM C hr^{-1}. P = Phosphate, G = Glucose, N = Ammonium.

Inorganic Nutrients

Post-incubation controls in the RIV experiment were not significantly different from pre-incubation initials, therefore all treatment finals were compared to initials. Phosphate uptake was the highest in the GP and GNP treatments , however, net change in PO_4^{3-} was not found to be significant in any treatment ($p=0.083$ to 0.879) (Table 3.5; Figure 3.11a).

The only significant net uptake of NH_4^+ was seen in the GNP treatment where 2.2 μM N was consumed over 72 hours for a rate of 0.031 ± 0.0004 μM N hr^{-1} ($p=0.012$) (Table 3.4; Figure 3.11b).

In the RIV experiment, nitrate/nitrite concentrations did not significantly differ from initial measurements of 0.18 ± 0.13 μM N ($0.153 \leq p \geq 0.452$), however, this could be due to greater variability in nitrate concentrations of the riverine-dominated experiment (final concentrations ranged from 0.01 ± 0.44 to 0.25 ± 0.04 μM N).

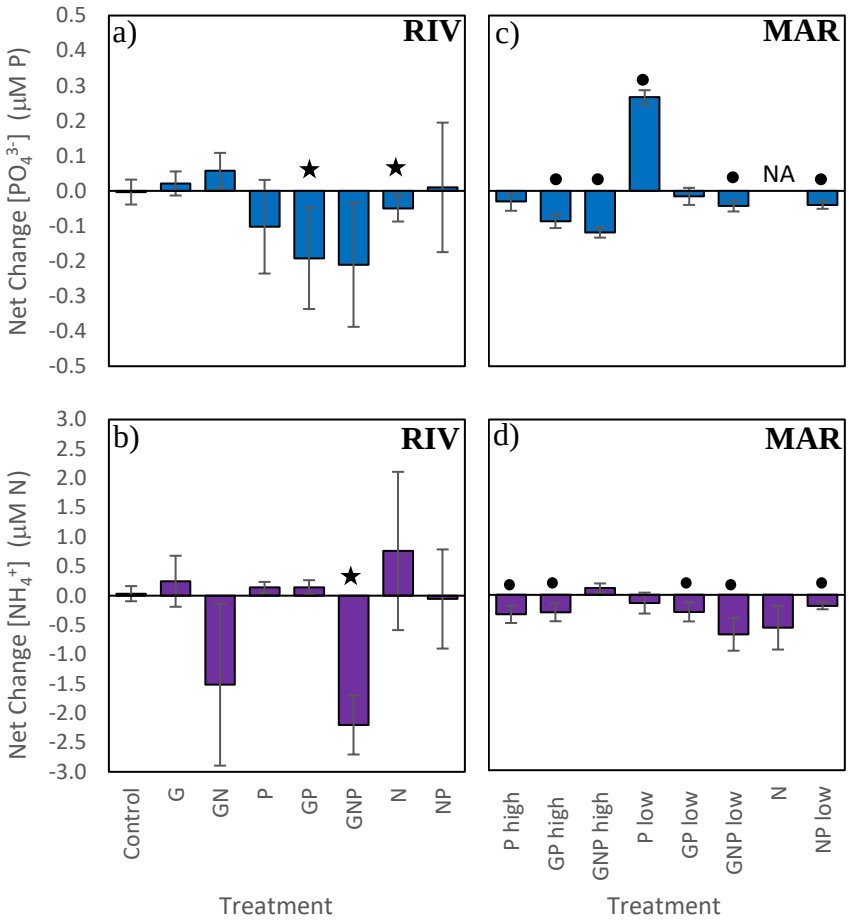

Figure 3.11a to d. Net changes in nutrients over 72-hour incubations. RIV experiment, significant net change (★): a) net change in PO_4^{3-}, GP (0.19 ± 0.06 µM P; df=4, p=0.041) and N (0.05 ± 0.04 µM P; df=4, p=0.048); b) net change in NH_4^+, GNP (2.2 ± 0.05 µM N; df=4, p=0.0125). MAR experiment, significant net change (●): c) net change in PO_4^{3-}, GP_{high} (0.09 ± 0.02 µM P; df=4, p<0.001), , GNP_{high} (0.12 ± 0.01 µM P; df=4, p<0.0001), P_{low} (0.27 ± 0.02 µM P; df=4, p<0.0001), GNP_{low} (0.04 ± 0.01 µM P; df=4, p<0.005)and NP_{low} (0.04 ± 0.01 µM P; df=4, p<0.002); d) net change in NH_4^+, P_{high} (0.33 ± 0.02 µM N; df=4, p=0.009), GP_{high} (0.30 ± 0.04 µM N; df=4, p=0.014), GP_{low} (0.29 ± 0.07 µM N; df=4, p=0.018), GNP_{low} (0.67 ± 0.28 µM N; df=4, p=0.007), and NP_{low} (0.19 ± 0.04 µM N; df=4, p=0.003). P = Phosphate, G = Glucose, N = Ammonium.

Bacterioplankton

Since the 1:4 dilution designed to reduce grazing pressure did not appear successful, as described above with the pre-incubation initial results, the response of bacterioplankton is reported as a *net* change in abundance and biomass rather than as a gross change.

There was a significant net increase in bacterial abundance in the control (from initial 1.179 ± 0.090 x10^9 cells L^{-1} to final 1.453 ± 0.111 x10^9 cell L^{-1}; df=4, $p = 0.023$) therefore final BA across treatments were compared to BA in the control final. Decreases in BA were found in the GNP and NP finals (Table 3.4; Figure 3.16) suggesting that the combination of nutrients (N and P) in these treatments might have stimulated rapid bacterial growth that was then grazed upon, reducing numbers below initial values (i.e. bacteria grew faster in these treatments which prompted an earlier response of grazers than in other treatments). Mean cell volume (BV) on the other hand was not significantly different in the control finals compared to the pre-incubation initials, therefore treatment finals were compared to initials instead of to the control finals. While BV appeared to increase in the GP and GNP treatments, the changes were not significantly different from the initial BV (Table 3.4; Figure 3.17).

The bacterial biomass in the control final was not different from the pre-incubation initials, likely due to an increase in BA coupled with a slight decrease in BV, therefore treatment finals were compared to pre-incubation initials. Net increase in bacterial biomass was observed in GP treatment based upon changes related to initials (GP final biomass of 0.033 ± 0.003 mg C L^{-1}, $p \leq 0.018$; initial biomass 0.024 ± 0.004 mg C L^{-1}) (Table 3.4; Figure 3.15). The net rate of bacterial production (BP) in the GP treatment can be estimated based on the increase in biomass over 72 hours of incubation and was found to be 0.128 μg C L^{-1} hr^{-1}.

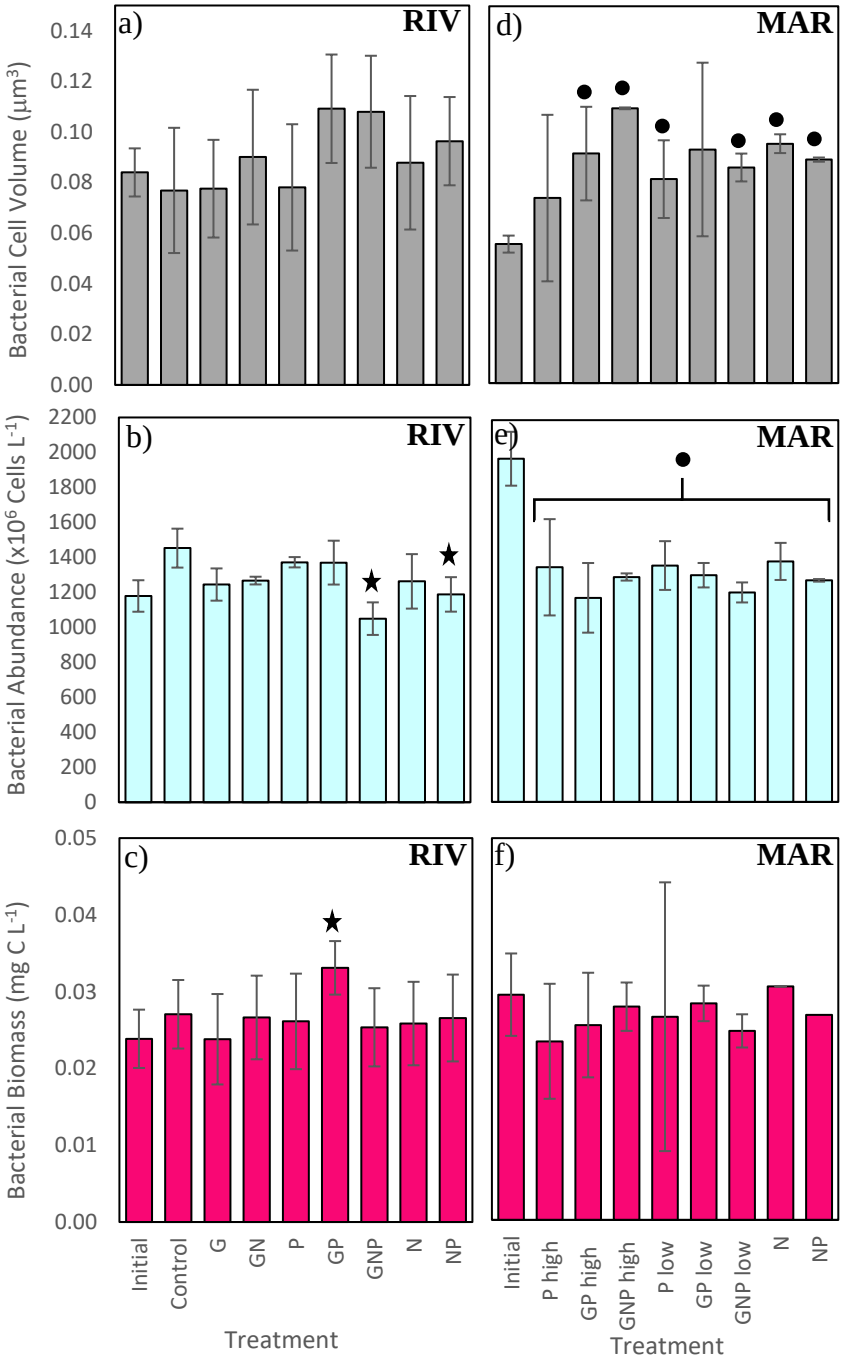

Figure 3.12a to f. Net changes in bacterial response over 72-hour incubations. RIV experiment, significant net change (★): a) no net change in cell volume; b) net decrease in abundance, GNP ($p < 0.009$) and NP ($p < 0.037$; c) net change in biomass, GP (0.033 ± 0.003 mg C L^{-1}, $p \leq 0.018$). MAR experiment, significant net change (●): d) net change in cell volume, GP_{high} (df=4, p=0.030), GNP_{high} (df=4, p=0.0001), P_{low} (df=4, p=0.047), GNP_{low} (df=4, p=0.001), N (df=4, p=0.0002), NP_{low} (df=4, p=0.0001); e) net change in abundance across all treatments (decreases of (592 to 802) $x10^6$ cells L^{-1}, $p < 0.03$); f) no net change in biomass. P = Phosphate, G = Glucose, N = Ammonium.

MAR Experiment – Post-Incubation Results

All net changes are compared to pre-incubation initials in the MAR experiment due to a lack of control final. Significant net uptake of DOC and DON was observed across all treatments. Net production of TIC was observed in P_{low}. Net uptake of phosphate was observed in GP_{high}, GNP_{high}, GNP_{low} and NP_{low} and net uptake of ammonium was observed in P_{high}, GP_{high}, GP_{low}, GNP_{low} and NP_{low}. There was no net change in $SUVA_{254}$ nor in the spectral slope from 275-295 nm ($S_{275\text{-}295}$). There was a net decrease observed in bacterial abundance across all treatments and a net increase in bacterial cell volume in GP_{high}, GNP_{high}, P_{low}, GNP_{low}, N and NP_{low}. (Table 3.6).

Table 3.6. Comparison of treatment finals to pre-incubation initials: MAR experiment (no control available).

ANALYTE	Changes Observed in Treatment Finals							
	P_{high}	**GP_{high}**	**GNP_{high}**	**P_{low}**	**GP_{low}**	**GNP_{low}**	**N**	**NP_{low}**
[DOC] (μM C)	↓*	↓*	↓*	↓*	↓*	↓*	↓*	↓*
[DON] (μM N)	↓*	↓*	↓*	↓*	↓*	↓*	↓*	↓*
[TIC] (μM C)				↑*				
δ^{13}C-TIC (‰)								
[PO_4^{3-}] (μM P)	↓	↓*	↓*	↑*		↓*		↓*
[$NO_{2/3}^-$] (μM N)								
[NH_4^+] (μM N)	↓*	↓*	↑		↓*	↓*	↓	↓*
$SUVA_{254}$ (cm^{-1}μM C^{-1})								
Spectral Slope $S_{275-295}$								
Bacterioplankton Biomass (mg C L^{-1})								
Bacterioplankton Abundance (x10^6 cells L^{-1})	↓*	↓*	↓*	↓*	↓*	↓*	↓*	↓*
Bacterioplankton Cell Volume (μm^3)	↑	↑*	↑*	↑*	↑	↑*	↑*	↑*

* = Significantly different from Untreated Pre-incubation Initial
↑ = Increase compared to Untreated Pre-incubation Initial
↓ = Decrease compared to Untreated Pre-incubation Initial
Note: Blank boxes in table indicate no detectable change.

DOM quality

There was a significant net uptake of DOC and DON across all treatments compared to pre-incubation initials (Table 3.6; Figure 3.7d and 3.7e, respectively). The DOC uptake in the GNP_{high} final was significantly higher compared to single nutrient additions of P_{high}, P_{low}, and N ($0.006 < p < 0.015$; df=4). The DOC uptake in GP_{low} and NP_{low} were both significantly higher than in P_{low} ($p = 0.036$ and 0.046, respectively; df=4; Figure 3.7d). This suggested that the addition of G or N, or both, had an effect on DOC utilized by bacterioplankton as signified by the net uptake of DOC observed here. There was a

significant decrease in DON across all treatment finals compared to pre-incubation initials, however, none of the treatments were significantly different from the others.

In the absence of an experimental control, the following comparisons were made for DOC and DON in the MAR experiment to compare the effect sizes of treatments on net use of DOM: GP_{high} vs P_{high}, GNP_{high} vs P_{high}, GP_{low} vs P_{low}, GNP_{low} vs P_{low}, NP_{low} vs N and NP_{low} vs P_{low}. Largest effect sizes (calculated as Cohen's *d* as described in the methods section) for net uptake of DOC appeared when G was added to both high and low P treatments, supporting the conclusion that C-limitation of bacterial utilization of DOC existed in the surface waters in Lake Melville; the effect sizes of N and P additions without glucose are not as large. The addition of G to P_{low} treatment yielded the largest effect size for DON, indicating that the net uptake of DON by bacterioplankton might be carbon-limited as well (Table 3.7 below).

The net uptake of DON across the MAR experiment finals resulted in a significant increase in C:N across all treatments (final C:N ranged from 42.19 ± 3.33 to 62.50 ± 3.36; df=4, $p \leq 0.0004$). An increase in C:N of the DOM pool suggested a decrease in the bioavailability of the pool, and that some of the more labile fraction has been removed by bacterioplankton. The C:N of the removed fraction of the DOM pool is illustrated in Figure 3.7f and effect size (Cohen's *d*) revealed a higher C:N whenever the treatment included the combined addition of N and P, suggesting use of higher C:N DOM is limited by N and P (see comparisons involving GNP_{high}, GNP_{low} and NP_{low} in Table 3.7 below).

Table 3.7. Cohen's *d* effect sizes calculated for uptake of DOC and DON, and for C:N of the DOM utilized across treatments. Values are typically interpreted as d = 0.2, 0.5 and 0.8 corresponding to small, medium and large effects, however, smaller sample sizes may display a larger range of values.

Treatment comparisons	**Cohen's *d* for DOC**	**Cohen's *d* for DON**	**Cohen's *d* for C:N of utilized DOM**
GP_{high} vs P_{high}	10.8	0.9	0.0
GNP_{high} vs P_{high}	5.9	0.8	3.1
GP_{low} vs P_{low}	11.9	4.5	1.7
GNP_{low} vs P_{low}	10.3	0.6	2.6
NP_{low} vs N	3.0	0.5	2.2
NP_{low} vs P_{low}	3.1	2.4	1.9

As with the RIV experiment, optical properties of the DOM pool in the MAR experiment did not exhibit net change in $SUVA_{254}$ ($p > 0.26$) nor in $S_{275\text{-}295}$ across all treatments (Table 3.8).

Table 3.8. MAR Experiment: Optical properties of the DOM pool including $SUVA_{254}$ and $S_{275\text{-}295}$.

MAR treatments	**$SUVA_{254}$ ($\mu M\ C^{-1}\ m^{-1}$)**	**$SUVA_{254}$ st dev**	**$S_{275\text{-}295}$ (μm^{-1})**	**$S_{275\text{-}295}$ st dev**
Initial	0.107[2]	0.007[2]	14.5[1]	NA
P_{high}[1]	0.097	NA	15.9	NA
GP_{high}	NA	NA	NA	NA
GNP_{high}	0.096	0.013	12.0[2]	0.9[2]
P_{low}[1]	0.093	NA	15.4	NA
GP_{low}	NA	NA	NA	NA
GNP_{low}	0.101	0.003	13.7[2]	1.2[2]
N[1]	0.087	NA	16.9	NA
NP_{low}[1]	0.088	NA	16.2	NA

NA = data not available
[1] n=1; otherwise n=3 for all means
[2] n=2; otherwise n=3 for all means

Heterotrophic respiration

As with the RIV experiment, a minimum detectable change (MDC) was calculated to assist with interpretation of the TIC results (Spooner et al., 2011). The MDC was calculated for each carboy (A, B and C) using a power of 0.80 and an alpha of 0.05, and was determined to be 8.41, 10.18 and 8.83 μM C for carboys A, B and C, respectively. The only significant increase in TIC compared to pre-incubation initials was found in the P_{low} treatment (increase from 1052.38 ± 10.78 μM C to 1078.49 ± 7.17 μM C; df=4, p = 0.0017) with a rate of increase of 0.441 μM C hr^{-1} (Figure 3.10b). Despite the measurable change in [TIC] in the P_{low} treatment, there were no significant changes found in TIC

stable isotope signature of the TIC pool (δ^{13}C-TIC) across the MAR treatments (one-tailed p values ≥ 0.07).

Inorganic nutrients

Significant net uptake of phosphate was found in almost all treatments where P was combined with other additions, suggesting that a co-limitation might exist with respect to heterotrophic use of phosphate: GP_{high} (0.09 ± 0.02 μM P; df=4, p=0.0007), GNP_{low} (0.04 ± 0.01 μM P; df=4, p=0.0045), GNP_{high} (0.12 ± 0.01 μM P; df=4, p=0.00007), and NP_{low} (0.04 ± 0.01 μM P; df=4, p=0.0019) (Table 3.5; Figure 3.11c.). P limitation might not have been adequately relieved by lower additions of P (those including P_{low}) since treatments that included P_{high} displayed the highest net uptake of P (i.e. net uptake of P was higher in both GNP_{high} and GP_{high} than in GNP_{low} and NP_{low}).

Increase in phosphate was observed in P_{low} (0.27 ± 0.02 μM P; df=4, p=0.00007), which could have been due to bacterial mineralization of organic P from the DOM pool. The ratio of TIC to phosphate produced in the P_{low} treatment was estimated in order to determine if the mineralization of DOM might account for the high phosphate values in this treatment. The C:P of the mineralized DOC and DOP was ~97 (i.e. TIC:PO_4^{3-} = 26.1μM C / 0.27 μM P = 97), which was lower than estimates from the Churchill River in September 2008 and May 2010 (DOC:TP = 384 and 227, respectively), but was likely close to C:P of ambient DOM in Lake Melville, based on lower DOC concentrations observed in the estuary compared to Churchill River DOC (MAE, 2008; MAE, 2010; Tables 3.2 and 3.3).

While ammonium uptake was highest in the GNP_{low} treatment, it was only significantly greater than the uptake in the NP_{low} treatment ($p=0.04$; $df=4$), indicating that G addition might have played a role in stimulating bacterial use of inorganic N. Significant net uptake of NH_4^+ was detected in P_{high} (0.33 ± 0.02 μM N; $df=4$, $p=0.02$), GP_{high} (0.30 ± 0.04 μM N; $df=4$, $p=0.03$), GP_{low} (0.29 ± 0.07 μM N; $df=4$, $p=0.04$), GNP_{low} (0.67 ± 0.28 μM N; $df=4$, $p=0.01$), and NP (0.19 ± 0.04 μM N; $df=4$, $p=0.005$) treatments (Figure 3.11d). Given the trend of net uptake of ammonium (N) across most treatments, the net production of ammonium observed in the GNP_{high} treatment suggests that N was remineralized in this treatment concurrently with, and to a greater extent than, N uptake yielding a net production of ammonium.

In the MAR experiment, nitrite/nitrate concentrations did not change significantly during incubation and remained close to pre-incubation values of 3.5 ± 0.13 μM N ($0.115 \leq p \geq 0.445$).

Bacterioplankton

As with the RIV experiment, the 1:4 dilution designed to reduce grazing pressure did not appear successful in the MAR experiment, therefore the response of bacterioplankton is reported as a *net* change in abundance and biomass rather than as a gross change.

There was a net decrease in bacterial abundances across all treatments ($p<0.03$, Figure 3.12d). A decrease in bacterial abundance is likely due to top down controls such as grazing pressure or viral lysis. Since only net measurements of bacterial abundance were taken, the effect of nutrient additions cannot be determined in this experiment, however,

there appeared to be measurable effects on cell volume that were not obscured by grazing. The bacterial cell volume, on the other hand, exhibited a significant net increase across almost all treatments (final volumes ranged from 0.081 ± 0.015 μm^3 to 0.109 ± 0.0004 μm^3; $p \leq 0.024$; Figure 3.12e). The highest cell volumes were found in the GNP_{high} treatment (nearly 2x size of the initials), suggesting that combined nutrient additions might have increased the allocation of carbon to cell growth.

Since abundance and cell volume experienced changes in opposite directions and are both used to calculate biomass, there was no net increase in bacterioplankton biomass detected in the MAR experiment. None of the incubation finals, which ranged from 0.023 ± 0.005 to 0.032 ± 0.0004 mg C L^{-1}, were found to be significantly different from the initials (0.029 ± 0.002 mg C L^{-1}) (Figure 3.12f).

3.4 DISCUSSION

An understanding of the factors constraining bacterial processing of DOM in estuaries is essential to estimating global carbon budgets. Bottom-up controls on bacterial activity such as DOM quality and nutrient availability can impact C flux through estuaries by influencing BGE and the fate of carbon processed by the microbial loop (del Giorgio and Cole, 1998; Fenchel, 2008). This is especially important in light of climate change which produces upstream hydrological changes that are likely to affect the quantity, timing and rate of riverine discharge, as well as the quantity and quality of dissolved the constituents therein. In Lake Melville in September 2008, full factorial matrix nutrient addition experiments were conducted to characterize the net response of bacterioplankton to

increases in organic and inorganic nutrients in a high latitude estuary. These additions were intended to simulate increases in nutrient inputs delivered by the Churchill River in order to better constrain the possible impacts of these increases with respect to bacterial response and the net processing of DOM in the estuary. Additionally, the experiments were carried out at a RIV (riverine-influenced) site and a MAR (marine-influenced) site to identify differences in bacterial response based on location along the estuary's horizontal salinity gradient.

In the RIV experiment, the *combination* of nutrient additions (i.e. GNP) stimulated net uptake of DOM and inorganic nutrients by the heterotrophic community, whereas in the MAR experiment, *all* nutrient additions (single and in combination) stimulated this net uptake by the heterotrophic community. The net effects of grazing (i.e. a net decrease in BA) were apparent in the RIV experiment in treatments that combined inorganic nutrients (i.e. NP, GNP), whereas net grazing effects in the MAR experiment were observed across *all* treatments. Though not directly observed, these experimental results suggest possible stimulation of heterotrophic bacterial production that likely increased grazing activity. This stimulation of bacterial production and subsequent grazing was more ubiquitous in the MAR experiment, with a lower C:N of the utilized DOM, suggesting greater availability of DOM to microbial communities at this more marine location in Lake Melville.

Nutrient limitation during low flow of Churchill River

It is common in estuarine waters for differences in nutrient limitation to exist along a salinity gradient, based largely on the proximity to riverine DOM inputs that dissipate

with increasing salinity until autochthonous DOM is the dominant source of bacterial substrate (Soares et al., 2018). Lower salinity sites tend to be primarily C-limited, whereas mid- salinity sites tend to be limited by inorganic nutrients, especially P (Chin-Leo and Benner, 1992; Cotner et al., 2000; Smith and Kemp, 2003; Soares et al., 2018). And in more marine environments, bacterial activity has been found to be co-limited by organic C and inorganic nutrients (Donachie et al., 2001; Rivkin and Anderson, 1997). It was expected that the surface waters in Lake Melville in September 2008 would exhibit some C limitation, which would be observed as uptake of DOC in treatments where a labile organic carbon sources was added. Nutrient limitation was also anticipated, and would be indicated by uptake of phosphate and ammonium in treatments where these nutrients were added. Co-limitation of C and P was expected at the RIV experimental location while co-limitation of C and N were expected at the MAR location. Since grazers were present in both experiments, some recycling of DOM and nutrients likely occurred meaning that measurements were of *net* uptake rather than gross uptake, and therefore underestimated the true uptake of DOM and nutrients in both experiments.

In the RIV experiment, co-limitation of labile organic C and inorganic N and P was evident from net uptake of DOC and DON. However, as expected, it was the combined additions of C and P that often produced the greatest net uptake of DOC, as observed in the GP and GNP treatments, which also exhibited the highest rates of community respiration (as interpreted from the increases to thein $\delta^{13}C$ -TIC due to the mineralization of the added glucose), and high net uptake of phosphate and ammonium. In the MAR experiment, the expected co-limitation of labile organic C and inorganic N was evident

from changes in the DOM pool; there was significant net uptake of DOC and DON compared to initials across all treatments, suggesting a greater availability of DOM in the MAR location than in the RIV location. In addition, the higher DON uptake and lower DOC uptake resulted in a lower C:N of the DOM used in the MAR experiment further suggesting that the MAR DOM used might have been more labile than the RIV. Any P limitation in the MAR experiment was difficult to identify clearly since phosphate was added to most treatments and there was no untreated control against which to compare net changes.

Nutrient limitation has been found to influence bacterial growth efficiency (BGE) such that P-limiting conditions tend to promote higher respiration compared to production and C-limiting conditions tend to favour bacterial production over respiration. This has been found in freshwater systems (e.g., Jansson et al., 2006), as well as in the coastal marine environment (e.g., in Chesapeake Bay by Smith and Kemp, 2003). Community respiration was expected to be higher in the RIV experiment where P-limiting conditions were expected to occur. However, C-limiting conditions were expected in both experiments, therefore a difference in net respiration between experiments was not necessarily expected. As anticipated, the RIV experiment showed net decreases (from mineralized ambient DOC) and net increases (from mineralized glucose) to the δ^{13}C-TIC pool, indicating net community respiration. Net changes to δ^{13}C-TIC occurred in all treatments where N was added (N, NP, GN, GNP) suggesting that an increase ammonium might be important in stimulating net community respiration. Additionally, when N was added without a labile carbon source (i.e. N and NP treatments), net respiration was

stimulated relative to the control while net DOC uptake remained the same as the control, which could suggest that BGE decreased with the addition of inorganic nutrients. However, it is more likely that, since grazing was likely occurring in this experiment, DOC uptake was offset by simultaneous grazing (recycling of DOC) resulting in no net change in DOC.

Aromaticity of the DOM pool was expected to be higher for pre-incubation measurements at the RIV experimental location compared to the MAR location. Similarly, the $S_{275\text{-}295}$ was expected to be higher in the MAR experiment, which is further from the mouth of the Churchill River, since higher $S_{275\text{-}295}$ has been linked to decreasing concentrations of the terrestrially-derived lignin molecule (Fichot and Benner, 2012). However, the opposite trend was seen for both $SUVA_{254}$ and $S_{275\text{-}295}$, suggesting that other processes (e.g. photochemical) might have influenced optical properties of DOM in the surface mixed layer of the estuary.

In the nutrient addition experiments, it was expected that the $SUVA_{254}$ would increase and $S_{275\text{-}295}$ would decrease in the treatments where nutrient limitation was alleviated, since $SUVA_{254}$ has been correlated with high aromaticity of the DOM pool (Weishaar et al., 2003) and biodegradation of DOM has been shown to increase $SUVA_{254}$ (Hansen et al., 2016) and decrease $S_{275\text{-}295}$ (Fichot and Benner, 2012). However, grazing was expected to recycle DOM which might have released bioavailable DOC and reduced the value of $SUVA_{254}$. Therefore, it is not surprising that no net changes were detected in $SUVA_{254}$ in either experiment. The lack of net change in $S_{275\text{-}295}$ suggests that either bacterioplankton use of CDOM was fairly uniform relative to bulk DOM across the

absorption spectra, or that the microbial removal of compounds absorbing in a given range of wavelengths was balanced by the release of compounds absorbing in another range of wavelengths, for a net effect that was negligible.

Bacterial abundance and biomass were expected to increase when glucose, phosphate and ammonium were added to the resource-limited environment of Lake Melville's surface waters. Conversely, it was anticipated that grazing pressure would counteract this increase through direct predation, resulting in a lower net increase of BA and BB, or possibly no net change or a net decrease, in treatments where limiting nutrients were added. Bacterial cell volume was expected to remain constant, or to increase in cases where size-selective grazing took place (e.g. Sherr et al., 1992) or where cells responded to glucose by synthesizing cell storage products (Carlson & Ducklow, 1996). In the RIV experiment, changes in BA showed evidence of combined N and P limitation based on significant net decreases in BA in the GNP and NP treatments. This net decrease could indicate that BA increased more quickly in these treatments and was therefore reduced to a great extent by grazers by the end of the 72-hour incubation. Net increases in bacterial biomass were evident in the GP treatment, suggesting that bacterial production might also be stimulated by the combined addition of phosphate and glucose. In the MAR experiment, there was a net increase in bacterial cell volume across most treatments and a net decrease in BA across all treatments, which resulted in no net change in BB. The greatest increase in BV occurred when nutrients were all combined in the GNP_{high} treatment and coincided with the largest net uptake of DOC, suggesting that bacterial production was stimulated by the combined additions of G, N and P and was

subsequently reduced by preferential grazing on small or dividing cells. The interpretation of the bacterioplankton data would have benefitted from information on the fate of the DOC used in each treatment since they would provide more information on the relative importance of the source of DOC to the stimulation of bacterial activity. However, it was not possible to distinguish net uptake of ambient DOC from net uptake of the added 500‰-labeled glucose since TIC concentrations did not increase significantly over the course of the incubation, and the amount of ambient DOC respired cannot be traced to δ^{13}C-TIC (δ^{13}C-DOC was not measured in this study).

Bacterioplankton response to Churchill River inputs

It is possible that C-, P-, and/or N-limitation of bacterial activity is seasonally alleviated during high flow conditions of the Churchill River. Seasonal increases in the concentrations of DOC and nutrients have been found to stimulate bacterial activity (Bratbak and Thingstad, 1985; Caron, 1994), and possibly primary production (Hecky et al., 1988; Bratbak and Thingstad, 1985; Caron, 1994; Cloern, 1999) which could indirectly stimulate bacterioplankton production by supplying labile DOM substrate in the form of algal exudates (Lignell, 1990). Seasonal ambient DOM and nutrient concentrations reported in other studies of Lake Melville support the hypothesis of seasonal variation of inputs to this estuary by the Churchill River, such as Schartup et al. (2015) who reported values of DOC and nutrients for the month of September (2012) that were comparable to those found in the present study, with higher values reported for the month of June (2013) (96 to 441 μM C, with a mean of 238 ± 120 μM C; n=16) when Churchill River discharge was high. These high DOC concentrations in June could reflect

increases due to riverine inputs, which is further supported by high Churchill River DOC concentrations of 400 to 641 μM C in May and June (2007-2010) when river discharges were at their annual high (Figure 3.3) (MAE, 2007; MAE, 2009; MAE, 2010). While increased DOC was likely delivered by Churchill River inputs in May and June as described above, it's also possible that high DOC in Lake Melville surface waters was due to autochthonous DOM generated from a phytoplankton bloom, which could itself have been stimulated by increased delivery of nutrients from Churchill River discharge. Regardless of the origin of DOM, DOC concentrations in June 2013 were ~25% and ~75% higher than the ambient DOC concentrations reported near the RIV and MAR experimental locations in September 2008, respectively, suggesting that C limitation might be alleviated seasonally in Lake Melville.

Nutrient concentrations in Lake Melville exhibited similar seasonal variation to DOC concentrations, suggesting that Churchill River inputs were key in providing these limiting resources on a seasonal basis. In the present study, ambient phosphate concentrations were below detection (Tables 3.2 and 3.3), as were nutrient concentrations measured in the Churchill River (PO_4^{3-}, NO_2^-, NO_3^-, NH_3 were all below detection at both Grizzle Rapids and above Muskrat Falls; RDL 0.05 to 0.1 mg L^{-1}) (MAE, 2008). Nutrient concentrations measured in the Churchill River (at stations above Muskrat Falls 03OE001 and below Muskrat Falls NF03OE0050) in May and June (2009 & 2010) included nitrate and nitrite, which were both below detection (RDL 0.06 mg L^{-1} in 2009 and 0.1 mg L^{-1} in 2010 for both nitrate and nitrite) (MAE, 2009; MAE, 2010). Total phosphorus (TP) concentrations were also measured and found to be below detection in June (2009, RDL

0.01 mg L^{-1}; and 2010, RDL 0.1 mg L^{-1}) but on May 20, 2010, TP was 0.02 mg L^{-1} (0.6 μM P) above Muskrat Falls and 0.06 mg L^{-1} (1.9 μM P) below Muskrat Falls (RDL 0.01 mg L^{-1}), suggesting that P limitation in Lake Melville might be alleviated in May when the Churchill River discharge is at its highest (Figure 3.3). This is further supported by ambient phosphate concentrations measured in June (2013) by Schartup et al. (2015), who reported ranges of 0.06 to 0.25 μM P with a mean of 0.19 ± 0.08 μM P (n=5). Given the similarity to phosphate additions made in this present study (~0.2 μM P), it is possible that during the month of June, bacterioplankton may exhibit a similar net response *in situ* to that observed in these nutrient addition experiments. The seasonal variation in the availability of DOM and nutrients, and its similarity to patterns of seasonal discharge from the Churchill River, make it clear that changes to the timing, quality and quantity of DOM and inorganic nutrients from Churchill River discharge could have significant effects on the secondary production in the Lake Melville estuary, both directly in terms of availability and quality of substrate, and indirectly in terms of effects on primary production and the role of the microbial loop (Jensen, 1983; Norrman et al., 1995).

The availability of both an organic carbon substrate and inorganic nutrients have been shown to be important limiting factors to bacterial activity in Lake Melville in this study, however, the effects of grazing pressure had to be considered in the interpretation of the net changes in bacterioplankton and dissolved constituents. Grazing pressure was most apparent in the MAR experiment where there was a net decrease in bacterial abundance across all treatments, whereas in the RIV experiment, there was only a net decrease in BA in the GNP and NP treatments. It is possible that a net decrease in BA was indicative of

treatments where BA was greatly stimulated and then substantially grazed upon, masking an intermediate increase in biomass due to final measurements of *net* changes (Jorgensen et al., 1999). If that was the case, then bacterioplankton at the MAR experimental location might have exhibited a more dramatic response to nutrient additions than those at the RIV location, which is in keeping with the findings that estuaries exhibit differences in resource limitation along a horizontal salinity gradient (Soares et al., 2018).

Upstream hydrological changes caused by development of the watershed (e.g. damming and reservoir creation) or due to climate change could have cascading effects through the base of the aquatic food web in Lake Melville, possibly causing a reduction in BGE of the microbial loop (Goldman et al., 1987; Kroer, 1993; Eiler et al., 2003; Pomeroy et al., 2007; Alonso-Saez et al., 2008; Fenchel, 2008) and affecting the extent of the coupling of bacterioplankton production to primary production **(**Boschker et al., 2005; Alonso-Saez et al., 2008). For example, while bacterioplankton may be in competition with phytoplankton for inorganic nutrients (Caron, 1994), they may also utilize autochthonous DOC from algal exudates as a carbon substrate (Morán et al., 2002). Grazers may also be impacted by upstream hydrological changes if they depend on bacterioplankton as a food resource since temporal shifts in peak bacterial production or reductions in overall production will be reflected in predator populations (Agis et al., 2007**)**. It is clear that an understanding of the response of bacterioplankton to changes in DOM and nutrient availability is essential to constraining the environmental impacts of hydrological changes, and that conducting experiments such as those presented in this study is a useful first step in addressing this challenge.

Future Direction

Organic matter loading of the Churchill river is likely to increase beginning in 2019 as the Lower Churchill Project (LCP) prepares to flood the terrestrial environment adjacent to Muskrat Falls, creating a reservoir for the hydroelectric project. Given the carbon limitation, and possible nutrient co-limitation, of bacterioplankton that has been shown in the present study to exist in the surface waters of the Lake Melville estuary, it is clear that changes to the DOM pool will impact secondary production in the estuary with potential for cascading effects through the aquatic food web.

The present study examined bacterial response to increased organic carbon and nutrient input in September 2008, however, the key findings of this work would benefit from a temporal expansion to include a comparison with high flow conditions in May and June. Natural variation in Churchill River inputs of DOC and inorganic nutrients has been demonstrated above, and a broader examination of bacterial response to such inputs would improve predictions made about the environmental impacts of the hydrological changes associated with LCP reservoir creation and dam operation. Such future work could reveal more information about the duration and cycles of nutrient limitation in the Lake Melville estuary, and provide a baseline for measuring ecosystem response to upstream changes in the Churchill River.

This study provided clear evidence of bacterial response to organic carbon and inorganic nutrient additions at both the RIV and MAR locations through measurement of net changes in biological and chemical constituents. However, future work could incorporate the measurement of additional variables that would provide information on *how* DOM

was being used by the heterotrophic community (i.e. the fate of DOM processed by bacterioplankton) in addition to the fact that is was being used. For example, analysis of the stable carbon isotope signature of the particulate organic carbon pool (δ^{13}C-POC) could help elucidate which carbon source bacterioplankton favour for assimilation into biomass (i.e. ambient DOC or the added glucose). While it was evident from this experiment that the 500‰-labeled glucose was respired by bacterioplankton at the RIV location, measuring δ^{13}C-POC would indicate how much of the labeled glucose was taken up as bacterial biomass. This could allow for rough estimates of BGE, providing critical information about the function of the microbial loop at the base of the aquatic food web, and how that function might change with changing river inputs.

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CHAPTER 4

Resource-limited bacterioplankton stimulated at mid salinities across the mixed layer of Lake Melville, Canada.

Summary and Conclusions

The flow of terrestrial organic matter to the coastal ocean is a significant component of the global carbon cycle that has been shown to be increasing with a changing climate (Schlunz & Schneider, 2000). The development of accurate models of the global carbon budget, and how those models might change with a changing climate, is dependent upon a clear understanding of how carbon is processed in coastal margins around the world. High latitude systems are especially important to study for several reasons: 1) they represent relatively large volumes of freshwater discharge compared the volume of the receiving oceans (Shiklomanov et al., 2000); 2) the relative importance and fate of terrestrial dissolved organic matter (DOM) inputs to these estuaries are not well constrained (Mannino & Harvey, 2000; Tesi et al., 2014; Medeiros et al., 2017; Painter et al., 2018), and 3) high latitude systems are expected to manifest the earliest signs of climate change (Stroeve et al., 2007; Rontani et al., 2014).

Characterizing dissolved organic matter and its role in aquatic systems has been a key focus in the attempt to constrain coastal ecosystem response to climate change. DOM, whether from allochthonous river inputs or autochthonous algal inputs, is uniquely accessed by bacteria through assimilation, providing a pathway for DOM to re-enter the aquatic food web known as the microbial loop (Pomeroy 1974; Azam 1983). In addition

to acting as a conduit for the transfer of carbon from DOM to the aquatic food web, bacteria serve as mineralizers of organic substrate, providing inorganic nutrients and carbon for use in photosynthesis by primary producers. Bacteria also represent the largest contribution to community respiration, releasing inorganic carbon into the surrounding environment. Differences in DOM quality and nutrient availability have been shown to exist across estuarine gradients (Soares et al., 2018), and to exert bottom-up control on bacterial production, respiration and growth efficiency (del Giorgio and Cole, 1998; Fenchel, 2008; Pinhassi et al., 2006; Hoikkala et al., 2009; Asmala et al., 2013; Asmala et al., 2014b). Therefore, variation in DOM and nutrients, such as that generated by variable river flow in high latitude regions, can create periods of resource limitation that affect bacterial activity in the coastal environment, which consequently can affect the processing and transfer of carbon through the estuary.

This research focused on examining biogeochemical cycling at the riverine-marine interface in the high latitude boreal estuary of Lake Melville, Canada, and identifying the existence of resource limitation of natural bacterial assemblages. This was accomplished by: 1) an examination of the net production and removal of chemical and biological constituents using a two-endmember mixing model to identify non-conservative behaviour across the salinity gradient; and, 2) conducting a nutrient addition experiment to examine bacterioplankton response to different combinations of labile dissolved organic carbon (DOC), inorganic nitrogen (N) and inorganic phosphorus (P). The first paper in this body of work presented the results from the examination of the Lake Melville estuary through the lens of a conservative mixing model, while the second paper

presented the results from nutrient addition experiment, conducted at both a riverine-dominated site and a marine-dominated site in the estuary.

Stimulation at Mid Salinities

The conservative mixing model used in this study assumed the dominance of physical mixing along a salinity gradient of two end-members with constant concentrations. Deviations from a conservative mixing line implied the existence of net production or degradation of a constituent at some intermediate salinity along the gradient. A relationship with salinity may also have been absent, suggesting that other factors were influencing the distribution of the constituent, such as high variability in end-member concentrations or a large degree of uncertainty around the measurement.

Non-conservative behaviour of the organic matter pools, and inorganic carbon and nutrients, indicated net production and loss of constituents relative to a conservative mixing model. There was evidence of a net loss of total inorganic carbon (TIC), inorganic nutrients and dissolved organic nitrogen (DON), and of a net production of dissolved organic carbon (DOC), and possibly particulate organic carbon and nitrogen (POC and PON). Additionally, it appeared that production of DOC was accompanied by changes to its composition, as evidenced by increases in specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) and the carbon to nitrogen ratio (C:N) of the DOM pool. The changes to these constituents in the mixed layer at mid salinities were suggestive of some form of resource limitation in one or both water masses, which might be alleviated during mixing at the riverine-marine interface.

A non-conservative biological response was also observed at mid salinities where microbial production was evident from the spike in bacterial abundance and biomass above a conservative mixing line. This was supported by changes to the DOM pool (e.g. increased aromaticity and C:N), the loss of nutrients and DON, and the production of POM described above. A concurrent spike in chlorophyll *a* concentrations at mid salinities suggested that primary and secondary production might have been linked at the riverine-marine interface in Lake Melville, wherein primary production might have been stimulating microbial production. Estuarine mixing in Lake Melville appeared to be stimulating bacterioplankton production across the mixed layer, both directly through the alleviation of nutrient and carbon limitation, and indirectly through the stimulation of primary production which could have provided labile substrate to bacterioplankton through algal exudates. It was clear that, even during the Churchill river's low flow conditions in the month of September 2008, microbial production in Lake Melville relied directly on allochthonous inputs of DOM and nutrients, and possibly autochthonous DOC from stimulated primary production. It followed that an investigation of possible nutrient limitation in the estuarine surface mixed layer would complement the findings of this study.

Carbon and Nutrient Limitation of Bacterioplankton

Carbon and nutrient limitation affect the role of bacterioplankton in aquatic ecosystems, and have been shown to be alleviated by the mixing of riverine inputs with marine waters (Carlson and Ducklow, 1996; Donachie et al., 2001; Sala et al., 2002; Rejas et al., 2005). Identifying resource limitation, and the response of bacterioplankton when resources

become available, is therefore very useful to understand how estuarine food webs and biogeochemical cycling are influenced by the delivery of DOM and nutrients from allochthonous riverine inputs. This understanding can also help constrain possible environmental impacts of upstream hydrological changes, such as those from climate change or upstream development. Concurrent nutrient addition experiments were conducted in Lake Melville estuary to investigate the influence of increased carbon and nutrient availability on bacterioplankton assemblages using water and bacterioplankton from the surface mixed layer at: 1) a riverine-dominated (RIV) location in Goose Bay, and 2) a marine-dominated (MAR) location in western Lake Melville. The timing of the nutrient addition experiments in Lake Melville was chosen to correspond with low flow conditions of the Churchill river (September) (MAE, 2018) to increase the likelihood of detecting any nutrient limitation in surface waters that might subsequently be alleviated by riverine inputs during high flow regimes.

Nutrient limitation of Lake Melville's bacterioplankton activity was suggested based on the results of the field data presented in the context of a conservative mixing model above. Evidence of this nutrient limitation was found in the nutrient amendment experiments conducted in September 2008 at both RIV (C- and P-limited) and MAR (C- and N-limited) experimental locations.

At the RIV location, carbon and inorganic nutrient limitation was apparent, however, the combination of G and P often produced the greatest changes in measured constituents. Heterotrophic respiration was stimulated by nutrient additions and appeared to be greatest when all three resources were added (glucose carbon – G, ammonium – N, and phosphate

– P), followed by when GP were added. The changes to the TIC pool (including δ^{13}C-TIC, which indicated respiration) in these two treatments coincided with the highest P uptake detected, suggesting a link between P demand and catabolic reactions of the bacterioplankton at the RIV location. Furthermore, bacterial biomass increased significantly in the GP treatment alone, highlighting the importance of these two nutrients at the RIV location.

In the MAR experiment, co-limitation of labile organic C and inorganic N was evident in net uptake of DOM across all treatments, suggesting a greater availability of DOM in the MAR location than in the RIV location. In addition, the higher DON uptake and lower DOC uptake resulted in a lower C:N of the DOM used in the MAR experiment further suggesting that the MAR DOM used might have been more labile than the RIV. Any P limitation in the MAR experiment was difficult to identify clearly since phosphate was added to most treatments and there was no untreated control against which to compare net changes.

Relief from nutrient limitation was observed as net uptake of DOM and inorganic nutrients by the heterotrophic community in the RIV experiment when nutrients were combined (i.e. glucose (G), ammonium (N) and phosphate (P)) and in the MAR when any single nutrient or combination of nutrients was added, suggesting greater availability of DOM at the MAR location. Since filtration to dilute grazer density was unsuccessful, experiments were assumed to include grazer predation, resulting in measurements of *net* changes throughout each experiment rather than gross changes. Grazing and viral lysis were likely responsible for the release of DOM from affected cells, thereby providing

additional labile bacterial substrate and dampening the magnitude of uptake of DOM and nutrients that might otherwise have been observed. Despite the fact that changes were likely underestimated in both experiments, measurable net changes were still observed with the addition of nutrients, supporting the conclusion that surface waters across Lake Melville experienced resource-limiting conditions in September 2008.

The Role of Allochthonous Inputs in Lake Melville Estuary

Evidence of bacterial response to allochthonous inputs was found in both the field study and in the nutrient addition experimental results. In the field study, stimulation of bacterial production was evident at mid salinities (~13 to 14 psu) relative to conservative mixing, suggesting that resource-limiting conditions were alleviated through the delivery of allochthonous DOM and nutrients from Churchill River discharge. Similarly, changes to bacterial cell volume were observed across treatments in the MAR experiment (salinity of 14 psu), suggesting that growth-limiting conditions were alleviated with the addition of nutrients (Del Giorgio and Cole, 1998). A net decrease in bacterial abundance was also observed in the MAR experiment, suggesting that stimulation of heterotrophic bacterial production might have occurred which in turn increased grazing activity. The same response might have occurred in the RIV experiment but might have been obscured in the experimental results due to differences in grazing pressure or type of resource-limitation. For example, grazing pressure in the RIV experiment might have obscured increases in cell volume if grazers were preferentially preying upon larger cells.

Allochthonous inputs of DON appeared to be important to heterotrophic activity in Lake Melville surface waters based on evidence of DON loss in both the field study, relative to

conservative mixing, and in the RIV nutrient addition experiment, specifically when N was added (N, GN, NP, GNP treatments). The concept of a priming effect has been explored in other studies (i.e. where the addition of a labile carbon stimulates further uptake of existing material than would otherwise have been consumed) (Fontaine et al., 2003; Guenet et al., 2010), however, it was not clear if that was occurring with the addition of inorganic nutrients in the Lake Melville system. Regardless, it was clear in both studies that the net loss of DON was occurring and that it was likely due to heterotrophic activity.

It is clear that allochthonous inputs delivered by the Churchill river can affect bacterial activity in the surface waters of Lake Melville estuary. Furthermore, organic matter loading of the Churchill river is likely to increase beginning in 2019 as the Lower Churchill Project (LCP) prepares to flood the terrestrial environment adjacent to Muskrat Falls, creating a reservoir for the hydroelectric project. Given the carbon limitation, and possible nutrient co-limitation, of bacterioplankton was observed in the surface waters of the Lake Melville estuary, these changes to the DOM pool are likely to influence secondary production in the estuary. At present, DOM and nutrient inputs from high flow regime of the Churchill river might stimulate bacterioplankton cell growth, providing important food resources to bactivorous grazers in the surface waters. Reservoir creation and dam operation may cause changes in the quantity, quality or timing of these inputs, thereby changing the dynamics at the base of the aquatic food web (Agis et al., 2007). In general, changes to the DOM pool delivered by the Churchill river could have cascading effects through the base of the aquatic food web in Lake Melville, causing major changes

in the cycling of carbon through the microbial loop (Goldman et al., 1987; Kroer, 1993; Eiler et al., 2003; Pomeroy et al., 2007; Alonso-Saez et al., 2008; Fenchel, 2008) and affecting the extent of the coupling of bacterioplankton production to primary production **(**Boschker et al., 2005; Alonso-Saez et al., 2008).

Future Directions

In order to better constrain the importance of allochthonous DOM and nutrient inputs to Lake Melville, direct measurements of phytoplankton and bacterioplankton production in would be useful. This would also serve to elucidate the extent of any coupling of PP and BP in the surface mixed layers across the estuary. Additionally, an expansion to include seasonal data and different hydrological regimes would be useful for identifying the ranges of concentrations and quality of DOM and nutrients delivered to Lake Melville by the Churchill river, as well as the responses of phytoplankton and bacterioplankton assemblages, over a full annual cycle. This temporal expansion could prove useful for the nutrient addition experiments and permit an assessment of the extent of seasonal variability in resource limitation in Lake Melville. Additionally, an increase in the geographic range of these studies (spread further across Lake Melville) could improve the conservative mixing model such that the mixed layer data could include a larger range of salinities and add more data points to allow for improved certainty of net production and loss at mid salinities. The nutrient addition experiment results would also benefit from additional sampling sites to allow for samples from across a salinity gradient rather than only a riverine- and a marine-dominated site.

While there are several directions to pursue based on the results of these studies, the information presented here begins to address the factors driving the assimilation, production and transformation of DOM and nutrients across Lake Melville. This research provides a basis for making and testing predictions about the potential impacts of upstream hydrological changes on the biogeochemical cycling of the downstream receiving environment.

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