Development of harmful algal blooms in a coastal lagoon: the influence of physicochemical processes and phytoplankton ecophysiology

by

Arielle Jensen Kobryn
BSc., University of Alberta, 2008

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of

MASTER OF SCIENCE

in the Department of Biology

Supervisory Committee

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Abstract

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This study was conducted in Esquimalt lagoon, located southwest of Victoria, British Columbia, Canada. Physical characteristics of the water column, e.g. circulation and stratification, changed seasonally resulting from variations in tides, temperature, precipitation and wind. Chemical characteristics, e.g. oxygen and dissolved nutrient concentrations, also differed temporally relative to those in the lagoon's ocean source water (Juan de Fuca Strait) because of variations in local photosynthesis and nutrient use by phytoplankton. Diatom blooms occurred in the spring, and blooms of photosynthetic flagellates (*Heterosigma akashiwo* (2009) and *Akashiwo sanguinea* (2009 and 2010)) occurred in the late summer and early fall when nitrate, ammonium, and urea were depleted. Proliferation of these flagellates led to the development of harmful algal blooms (HABs) associated with oxygen depletion in the lagoon bottom waters. Increased oxygen demand from bacterial degradation of algal biomass and exudates was the likely cause for bottom water hypoxia under reduced tidal exchange.

During the spring phytoplankton bloom, maximum biomass was 27 $\mu g\ L^{-1}$ chlorophyll *a*. Diatoms dominated the phytoplankton assemblages from March to mid-June. During this period dissolved nutrients were depleted from high winter concentrations to the lowest levels observed in this study. Nitrate, ammonium, urea, and orthophosphate decreased from 25.6 $\mu mol\ L^{-1}$, 6.7 $\mu mol\ L^{-1}$, 0.31 $\mu mol\ L^{-1}$, and 2.2 $\mu mol\ L^{-1}$, respectively, to near limits of detection, and silicic acid decreased from 52.7 $\mu mol\ L^{-1}$ to 5.8 $\mu mol\ L^{-1}$.

Blooms of photosynthetic flagellates occurred from August through October in 2009 and August through September in 2010. The maximum biomass achieved during these blooms was 30 $\mu g\ L^{-1}$ chlorophyll *a*. All forms of nitrogen remained depleted throughout

this latter part of the growing season, although orthophosphate concentrations were intermediate and silicic acid was abundant.

In diatom-dominated assemblages, rates of dissolved nitrogen uptake (sum of nitrate, ammonium, and urea uptake) correlated strongly with rates of dissolved inorganic carbon uptake, but in photosynthetic flagellate-dominated assemblages, the correlation was poor. This suggests that photosynthetic flagellates were utilizing other forms of dissolved or particulate organic nitrogen to fulfill their nitrogen requirements when nitrate, ammonium and urea were low.

Oxygen depletion developed in bottom waters of the lagoon during blooms of photosynthetic flagellates, and zones of hypoxia were observed in August of 2009 and September of 2010. Senescent phytoplankton cells and phytoplankton exudates, such as microsporine-like amino acids, are labile forms of organic matter that can be produced during the decline of large blooms, stimulating bacterial growth and increasing oxygen demand. Microsporine-like amino acids were measured in 2010 and found to be present during the *A. sanguinea* bloom.

A critical factor that may have contributed to the persistence of low oxygen conditions and hypoxia was the narrowing of tidal ranges in the late summer and early fall compared to earlier in the growing season. Narrow tidal ranges reduce tidal current velocities and flushing rates.

This study demonstrates that understanding HAB development requires characterization of both phytoplankton bloom dynamics in the target system and the physicochemical processes that can affect bloom dynamics and intensify oxygen depletion.

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List of Abbreviations

C, carbon
Chl *a*, chlorophyll *a*
CO_2, carbon dioxide
D, diatom
DIC, dissolved inorganic carbon
DOC, dissolved organic carbon
DIN, dissolved inorganic nitrogen
DON, dissolved organic nitrogen
DOM, dissolved organic matter
F, photosynthetic flagellate
HCO_3^-, bicarbonate
JdFS, Juan de Fuca Strait
M, mixed phytoplankton assemblage
MAA, microsporine-like amino acid
N, nitrogen
NH_4^+, ammonium
NO_3^-, nitrate
O_2, oxygen
$[O_2]$, oxygen concentration
P, phosphorous
P*, absolute uptake rate of dissolved inorganic carbon, or photosynthetic rate
PO_4^{3-}, orthophosphate
POC, particulate organic carbon
PON, particulate organic nitrogen
ρ, absolute uptake rate
Si, silicon
$Si(OH)_4$, silicic acid
SoG, Strait of Georgia
TEP, transparent exopolymer particle
Total dissolved N, sum of nitrate ammonium and urea
V_c, specific uptake rate
W, winter

Acknowledgments

I would like to acknowledge my supervisor Diana Varela and all members of the Varela lab. Marcos Lagunas in particular from the Varela lab provided tremendous help with field work and lab work in exchange for canoe lessons! Also, to the many ocean-minded friends in SEOS and Biology, their partners, and Diana herself: thanks for helping me collect water samples and search for lost Niskin bottles, I hope you all enjoyed the tour and I'm glad everyone came out of it alive.

To the Esquimalt Lagoon Stewardship Initiative (ELSI), thank you for being an eager audience as I was gradually able to unveil the findings of this project. It was very informative to be a part of your meetings and to see all of the effort and dedication that goes into being stewards of a local ecosystem.

Thank you to the School of Earth and Ocean Sciences for providing the Quanta G and the 5 L Niskin bottles, sorry one of them spent half a year on the bottom of the lagoon! Also to Hugh McClean at the Institute of Ocean Sciences for lending the 2 L Niskin bottle, Brett Malcolm from Go Row and Paddle and Royal Roads University for the canoes, and Patrick Buntain for salvaging plentiful discarded field gear from the marina.

Thank you to the Canadian Hydrographic Service and Environment Canada for the handy tidal and weather data and to sources of funding for this project: NSERC (CGS-M awarded to AJK, Discovery Grant awarded to DEV) and the University of Victoria (President's Scholarship and King Platt Fellowship awarded to AJK).

Finally, I would like to acknowledge the members of my committee Dr. Verena Tunnicliffe and Dr. Vera Pospelova, as well as my external examiner Dr. Sophia Johannessen for their input on this thesis.

Dedication

This thesis is dedicated to water. Be it present in lagoon, wetland, river, gorge or ocean, this is what grounded me daily on my bike-ride to and from the University of Victoria. Especially on those late nights coming home and crossing the trestle bridge by Swan Lake or riding alongside the Gorge Waterway and watching the reflection of the moon on the water. Really that is what this thesis is about, one of the natural aquatic systems that keeps us connected to reality and trying to understand how it functions so we can make sure it that it remains present.

Also, to which all things should be dedicated, I dedicate this thesis to my mom and to love, which is the under-estimated companion and enemy to science in the mind of many a grad student that I know, and dare I admit… myself??

Chapter 1: General introduction

1.1 Phytoplankton and water chemistry

Within any water body, there is an interplay between chemical properties of the water and dynamics of phytoplankton growth. Phytoplankton require a variety of chemical elements in order to grow, and their demands for nitrogen, silicon (in the case of diatoms), phosphorous and carbon have been the focus of much study because these are the elements that can become limiting to phytoplankton biomass and growth (Riebesell and Wolf-Galdrow 2002). In fact, nitrogen, phosphorous and silicon can become completely depleted in the water during phytoplankton growth. Both oxygen (O_2) concentrations and parameters related to the carbonate system (i.e. pH and carbon dioxide concentrations) are also closely tied to phytoplankton growth, via the processes of photosynthesis and respiration (Borges and Frankignoulle 2002, Gürel et al. 2005, Yates et al. 2007). When photosynthesis is occurring, O_2 concentrations and pH rise while carbon dioxide (CO_2) concentrations fall, and when photosynthesis ceases, respiration processes (by phytoplankton and heterotrophic organisms) dominate and the reverse trends are observed. As a result, O_2, pH, and CO_2 vary both diurnally, as phytoplankton only photosynthesise in daylight (Gürel et al. 2005, Yates et al. 2007), and seasonally, particularly in temperate areas where phytoplankton biomass is minimal in the winter (Borges and Frankignoulle 2002, Gürel et al. 2005) due to light limitation.

1.2 Phytoplankton and physical properties of the environment

Similarly to chemical properties, physical characteristics of the water and the surrounding environment affect phytoplankton growth, but they are not readily influenced in return.

Solar irradiance, which provides the energy required for photosynthesis, has a fundamental relationship with phytoplankton growth (Harris 1986, Litchman and Klausmeier 2008). This relationship is evident in the seasonal decline of phytoplankton communities that grow in high-latitude areas, and in the limitation of phytoplankton production that occurs in waters where mixing processes can transport phytoplankton below the critical depth (Harris 1986), or where high turbidity or growth of algal mats causes shading (Harrison et al. 1983, Viaroli et al. 2008). Temperature is also relevant to phytoplankton growth because although most phytoplankton groups (particularly those present in shallow coastal areas) can tolerate a large range of temperatures (Dale et al. 2006), each species has a narrow window of optimal growth temperatures within which its physiological processes function most efficiently (Litchman and Klausmeier 2008). Physical properties or "forces" such as winds, tides, and density gradients (caused by variation in salinity and temperature in the water), control the movement of water masses, and can therefore affect phytoplankton growth. In many cases the introduction of "new" water to a system with different physicochemical properties can stimulate the growth of phytoplankton populations by delivering new nutrients; for instance during upwelling or runoff events (Seliger et al. 1979, Watanabe and Robinson 1979, Kudela and Chavez 2004, Harris et al. 2009). In addition, phytoplankton can be entrained within a water mass and concentrated at frontal or convergence zones (Seliger et al. 1979), dispersed vertically or horizontally (Figueiras et al. 2006), or advected into new areas where they can act as an inoculum for local growth under favourable conditions (Taylor and Harrison 2002). Thus, the growth of phytoplankton populations is dependent on the exogenous physical and chemical influences of the environment.

1.3 Phytoplankton bloom dynamics

Because phytoplankton growth is influenced by exogenous physicochemical factors, these factors also affect the ability of phytoplankton populations to form a "bloom". However, endogenous characteristics of phytoplankton themselves, as well as food-web interactions, are equally important in determining bloom dynamics (Steidinger and Garcés 2006). A phytoplankton "bloom" refers to an increase in phytoplankton biomass and/or cell numbers above background levels that is accompanied by an "observable or recordable effect" (Steidinger and Garcés 2006), such as discoloration of the water. For example "red tides" refer to phytoplankton blooms that cause the water to appear red. The level of biomass that constitutes a bloom depends on the type of phytoplankton present and the environment in question, however, all blooms undergo common stages of development including initiation, growth, maintenance, and dispersal/dissipation/ termination (Steidinger and Garcés 2006). Some of the critical factors controlling the progression of these stages include inoculum size, solar irradiance, temperature (especially when resting cells are involved (Genovesi-Giunti et al. 2006), nutrient availability, intrinsic population growth rates, grazing by zooplankton, life-stage transitions, stability versus turbulence in the water column and advection of populations vertically or horizontally (Figueiras et al. 2006, Steidinger and Garcés 2006, Stolte and Garcés 2006). Transport of phytoplankton cells by water movement can serve to concentrate populations to a level where they are observable and is thus also important in the manifestation of a bloom (Seliger et al. 1979).

Harmful algal blooms or "HABs" are increasing in frequency and magnitude in coastal areas worldwide, largely due to nutrient loading from human practices (Heisler et al.

2008). HABs are phytoplankton blooms that produce a negative impact on other organisms (including humans) and ecosystems. Examples of such impacts include O_2 depletion in the water leading to fish kills, chemical toxicity to higher-level organisms, and fouling of beaches (Granéli and Turner 2006).

Anthropogenic nutrient loading can cause phytoplankton blooms to be more intense and frequent, thus increasing the impact that they can have on organisms and ecosystems. However, it is not only increased phytoplankton biomass that creates HABs, but shifts in phytoplankton composition. For instance, shifts from diatom-dominated communities to dinoflagellate-dominated communities are widespread and of particular concern because many dinoflagellates are toxic (Burkholder et al. 2006). Shifts in composition are thought to be related to nutrient inputs, but in particular to the form and relative concentrations of human-supplied nutrients that can differ from those supplied naturally (Heisler et al. 2008). Different phytoplankton species are favoured under different nutrient regimes (Burkholder et al. 2006, Litchman et al. 2007, Heisler et al. 2008).

Aside from altering the quantity and composition of nutrients supplied to coastal ecosystems, humans may be indirectly contributing to the increase in dinoflagellates (and/or other photosynthetic flagellates) by increasing global temperatures. Both warming of surface waters and an increase in freshwater input cause the water column to become stratified, meaning that there is a physical force opposing nutrient renewal in the surface waters through mixing (Dale et al. 2006, Zhang et al. 2010). Photosynthetic flagellates have a competitive advantage in stratified waters because they can swim to where nutrients are high below the nutricline or to the surface where light is available for photosynthesis (Smayda 1997, Burkholder et al. 2006).

Coastal systems are susceptible to the development of HABs because of terrestrial nutrient inputs that are often enhanced by human practices, and semi-enclosed water bodies such as lagoons are especially vulnerable. This is because nutrients coming into a lagoon from the watershed are concentrated within its boundaries, and water can be more stagnant than in the open ocean. These conditions can be favourable for phytoplankton growth.

1.4 Coastal lagoons

A comprehensive definition of coastal lagoons was provided by Kjerfve (1994):

> *"Coastal lagoons are inland water bodies, found on all continents, usually oriented parallel to the coast, separated from the ocean by a barrier, connected to the ocean by one or more restricted inlets which remain open at least intermittently, and have water depths which seldom exceed a few meters. A lagoon may or may not be subject to tidal mixing, and salinity can vary from that of a coastal fresh-water lake to a hypersaline lagoon, depending on the hydrologic balance. Lagoons formed as a result of rising sea level mostly during the Holocene and the building of coastal barriers by marine processes. They are often highly productive and the ideal systems for aquaculture projects but are, at the same time, highly stressed by anthropogenic inputs and human activities." Kjerfve, 1994.*

The shallow and enclosed nature of lagoons means that physical forces play an integral role in influencing phytoplankton bloom dynamics in these systems. Therefore, the following sections describe the relevant physical processes that occur in lagoons and the interaction of these processes with water chemistry and bloom dynamics.

1.4.1 Physical processes within lagoons

Because flow of ocean water in and out of coastal lagoons is restricted, inputs from the watershed are temporarily trapped within the boundaries of the lagoon and water quality differs from that of the neighbouring ocean. Physical forces dictating water transport are key factors in the degree of difference because lagoon water quality depends largely on flushing rates and residence times (Smith 1994). Coastal lagoons are a type of estuary, and as in estuaries, currents are driven both by tides and by differences in the densities of water masses coming from the land and the ocean (Phleger 1969). The presence of tidally-driven and density-driven movement of water masses means that water quality can vary horizontally within a lagoon, because tidally-driven currents and water transport are not usually uniform from mouth to toe (Phleger 1969, Smith 1994), and vertically because of estuarine-type stratification due to density differences (Postma 1969).

Typical density-driven circulation in estuaries functions as follows: fresh water coming from land creates a light, brackish layer that exits the system at the surface and drives the influx of higher-salinity ocean waters at depth. However, estuarine circulation does not always exhibit this classic pattern in lagoon systems, and in fact Postma (1969) defined three main types of coastal lagoons based on water circulation patterns. The first, most widely observed, type of lagoon exhibits classic estuarine circulation, and the water column in such systems has lower average density than the surrounding ocean because of fresh land-derived water at the surface. The second type of lagoon has minimal vertical variation in density and the average density of such lagoons is very similar to that of the nearby ocean. Because there is no discernable freshwater layer at the surface, water movement in these lagoons is driven only by tides. The volume of water entering the

lagoon during flood tides largely equals the water exiting on ebb tides, and movement of water in and out occurs uniformly throughout the water column. The third type of lagoon exhibits anti-estuarine circulation (also known as "inverse" or "negative" circulation). This type of circulation occurs when evaporation within the lagoon increases internal salinities (and thus densities) relative to the ocean, meaning that the lighter ocean waters must enter at the surface while the lagoon waters sink to the bottom and exit at depth (Groen 1969, Postma 1969, Valle-Levinson 2010).

These classifications are useful, but deviations from such straightforward circulation patterns are common. Lagoons are typically very shallow and this means that (*a*) local wind forcing (rather than tidal forcing) is often dominant in the transport of water masses during windy periods, (*b*) wave-mixing can extend to the bottom of the water column, and (*c*) the bottom friction layer can extend up to the surface. The implication of (*a*) is that tidally-driven transport is important near the mouth of the lagoon, but is often overshadowed by wind-driven transport in the interior and at the toe. The implication of (*b*) and (*c*) is that although lagoons are most often stratified, they may not have a defined pycnocline, so currents can be quickly dampened after wind or tidal forcing ceases (Smith 1994). Overall, tidal currents dissipate towards the interior of a lagoon (Phleger 1969), and flushing can become increasingly limited further away from the mouth, unless wind is creating circulation favourable to exchange.

1.4.2 Nutrients and primary producers in lagoon ecosystems

Coastal lagoons occupy a transition zone between the continent and the ocean and they share the common features of being shallow and semi-isolated from the open ocean. This means that the physical and chemical factors that affect phytoplankton bloom dynamics

in lagoons are a product of terrestrial and marine influences, as well as influences from bottom sediments. In each individual lagoon the interplay among these influences is unique and depends on location, climate and lagoon morphometry, and thus, water quality and phytoplankton bloom dynamics can vary from system to system. However some commonalties do exist, which will be summarised below.

Nutrient enrichment and autotrophic biomass in lagoons

Terrestrial nutrients typically enter lagoon systems via streams, rivers, and groundwater, both in pristine watersheds (Herrera-Silveira et al. 2002, Drake et al. 2010) and anthropogenically-altered watersheds (Castel and Caumette 1996, Fonseca and Braga 2006, Newton et al. 2003). These nutrients support primary producers. In pristine lagoons, the dominant primary producers are sea grasses because most nutrients are retained in the sediment pool. But when nutrients become enriched in the water column, the growth of a mixture of opportunistic macroalgae and phytoplankton (including cyanobacteria) can be favoured (Castel and Caumette 1996, Viaroli et al. 2008). In lagoon systems where phytoplankton production comprises a large fraction of autotrophic production, phytoplankton biomass is largely dependent on the residence time of water in the lagoon. Even when nutrient inputs are high, chlorophyll *a* (Chl *a*) concentrations will remain low (< 5 μg L^{-1}) if flushing rates are on the order of days (Castel and Caumette 1996, Newton et al. 2003). When water residence times are longer, phytoplankton have a greater opportunity to utilize dissolved nutrients (Herrera-Silveira et al. 2002) and in some cases localised blooms of phytoplankton can achieve biomass levels higher than 1 000 μg L^{-1} Chl *a* (Fonseca and Braga 2006, Drake et al. 2010).

Temporal variability in phytoplankton dynamics in lagoons

In temperate and polar aquatic systems, periodicity of phytoplankton growth and the succession of phytoplankton groups is seasonal. In temperate coastal zones, winter growth is minimal due to light limitation even with elevated nutrient inputs from stream inflow (Glé et al. 2008, Drake et al. 2010). In tropical areas, wet seasons and dry seasons can differentially influence the type and amount of phytoplankton present because of changing contributions of freshwater versus marine water. The balance of fresh and marine waters can influence the supply of nutrients, flushing rates, and temperatures (Fonseca and Braga 2006, Varona-Cordero et al. 2010). Seasonal succession of phytoplankton groups in lagoons can also be tightly linked to fluctuations in nutrient concentrations that are mediated by phytoplankton (Glé et al. 2008, Varona-Cordero et al. 2010) and bacterial remineralisation (Glé et al. 2008, Gouze et al. 2008, Fonseca and Braga 2006, Drake et al. 2010).

Spatial variability of phytoplankton dynamics in lagoons

Phytoplankton biomass can also differ horizontally and vertically in the water column of lagoons, despite small areas and shallow depths. This is because stratification often occurs, and the tidal nature of lagoons leads to spatially variable flushing rates relative to nutrient sources like streams or sewage effluent (Fonseca and Braga 2006, Newton et al. 2003, Oliveira et al. 2006, Gouze et al. 2008, Drake et al. 2010, Varona-Cordero et al. 2010). Also, phytoplankton populations can be advected into or out of coastal embayments such as lagoons (Taylor and Harrison 2002) and concentrated in tidal convergence zones (Seliger et al. 1979, Watanabe and Robinson 1979). In lagoons,

phytoplankton blooms of very large densities are usually a sign of eutrophication, which can be associated with O_2 depletion that can occur in specific locations or seasons depending on spatial and temporal patterns of phytoplankton blooming.

1.4.3 Eutrophication and oxygen depletion in lagoon systems

A water mass is considered hypoxic when dissolved O_2 saturation is < 30 %, or about 2 mg L^{-1} (Zhang et al. 2010, Gilbert 2011), and anoxic when O_2 is absent. Although tolerances of different animals to low O_2 conditions are variable, animals tend to avoid water with saturation < ~ 40 % (Ekau et al. 2010). Periods of benthic hypoxia or anoxia occur in many lagoons, more commonly in anthropogenically-affected eutrophic systems (Bartoli 1996, Castel and Caumette 1996, Newton et al. 2003, Gouze et al. 2008, Fonseca and Braga 2006, Viaroli et al. 2008, Pereira et al. 2010) but also in naturally eutrophic lagoons (Drake et al. 2010). At first these events are manifested as short-lived periods of O_2 depletion in the sediments, but as anthropogenic nutrient inputs to the water column continue to rise, hypoxia or anoxia can become more frequent and widespread (Viaroli et al. 2008). The link between increased nutrients in the water column and intensified O_2 depletion is algal growth, as pelagic nutrients support proliferation of fast-growing macroalgae and phytoplankton. Bacterial respiration of senescent algal biomass increases benthic O_2 demand, and these pelagic primary producers also shade out slow-growing sea grasses (Viaroli et al. 2008), which also die and are respired, contributing to O_2 demand. Respiration processes affect the biogeochemical cycling of O_2 and sulphur in the sediments and the water-column. These cycles mediate the process of eutrophication, and if they are sufficiently altered, the lagoon can enter a positive feedback cycle of eutrophication that can end in dystrophy (Viaroli et al. 2008).

Dystrophy is the final stage of eutrophication that is characterized by extended periods of hypoxia or anoxia which alter the redox state in the sediments, causing the release of hydrogen sulphide into sediment pore-space. Under such conditions there is a lack of rooted sea grasses (due to shading and poisoning of the roots by hydrogen sulphide), water is turbid, acidic and rich in organics, and autotrophic biomass is dominated by phytoplankton (often picoplankton or cyanobacteria). At this stage the ecosystem has lost much of its original functionality and ecological value (Viaroli et al. 2008).

Many Mediterranean lagoons experiencing various degrees of eutrophication suffer from "dystrophic crises", also known as "anoxic crises" or "malaïgues". These events are episodic and do not indicate that the system is permanently dystrophic, but they are a sign that the capacity of sediments and sea grasses to buffer the effects of O_2 consumption is being overwhelmed, which can eventually result in permanent dystrophy. Dystrophic crises are characterised by sulphidic odours, death of aquatic organisms and transient development of milky turquoise waters that occur when release of hydrogen sulphide into the water column decreases pH, leading to the precipitation of carbonates and proliferation of phototrophic sulphur bacteria. These crises tend to occur in poorly-flushed areas of a lagoon (Bartoli 1996, Castel and Caumette 1996, Harzallah and Chapelle 2002).

1.5 Esquimalt Lagoon

1.5.1 Geological history

Esquimalt Lagoon is located on the southern end of Vancouver Island, British Columbia, Canada, adjacent to the city of Colwood, near to the capital city of B.C., Victoria (Fig. 1.1). Esquimalt Lagoon is a barrier spit lagoon or "bar-built estuary"

(Westland Resource Group, 1993), separated from the Juan de Fuca Strait by a sand-gravel spit. The lagoon is shallow, only ~ 4 m deep at high tide, and it rests in a depression that formed when a large block of ice was left behind by a retreating glacier and melted about 13 000 years ago (Capital Regional District 2012). The spit, known as Coburg peninsula, was formed by glacially-deposited gravel, sand and rock, that built up around the block of ice thousands of years ago. After the ice melted, the spit was maintained by longshore transport of sand that eroded from the coastline south of the lagoon. During the 1900s, the spit was enhanced by longshore transport of sand from a nearby gravel mine, which is no longer in operation (Westland Resource Group 1993, Capital Regional District 2012). There is evidence that in the past, the spit was occasionally breached during large storms (Dr. David Blundon, Camosun College, personal communication, November 8, 2011), but it has now been stabilized by the construction of a road running along its length.

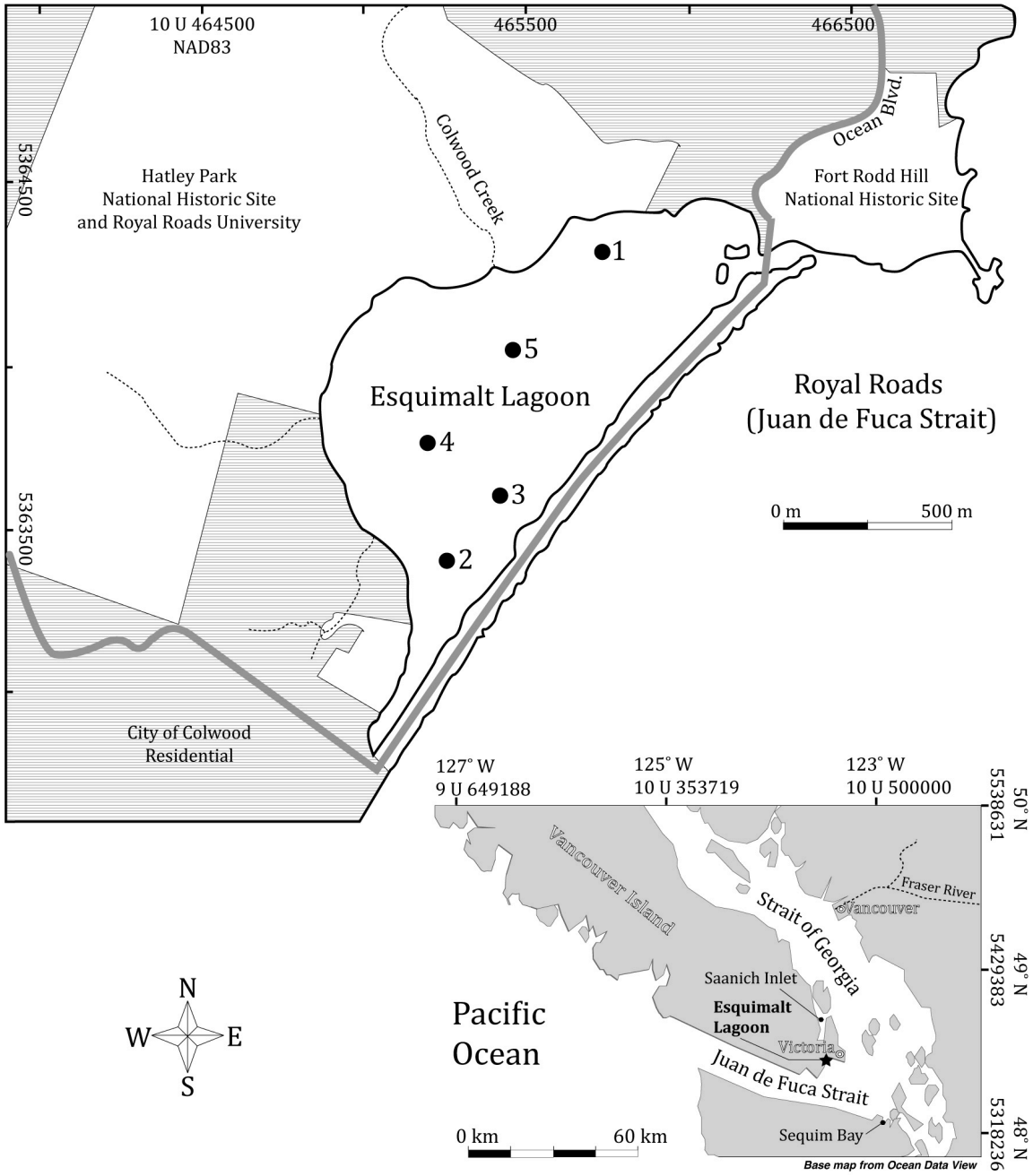


Figure 1.1 Map of Esquimalt Lagoon. Esquimalt Lagoon is located near the city of Victoria on the southern end of Vancouver Island, British Columbia, Canada. The lagoon is connected to the Juan de Fuca Strait via a narrow channel on the north eastern end entering onto the embayment known as Royal Roads. The five sampling stations visited during the current study are illustrated.

1.5.2 Oceanic water exchange

Ocean water enters Esquimalt Lagoon from the Juan de Fuca Strait via Royal Roads, a shoaling embayment of the strait (Westland Resource Group 1993). The entrance is at the north-eastern end of the lagoon and it consists of a narrow channel (20 m wide and 1.5 m deep) that is characterized by tidal water flowing in and out of the lagoon through a delta-like deposit of gravel, derived from erosion of the spit (Westland Resource Group 1993, Archipelago Marine Research Ltd. 2000). In the 1950s it was estimated that 35 % of the water volume within Esquimalt Lagoon is exchanged each day (Scrimger 1960), but since then it has been speculated by researchers at Royal Roads Military College (now Royal Roads University) that tidal waters tend to enter and exit the lagoon without much exchange (Watanabe and Robinson 1979). As a result, the flushing rate is higher near the entrance channel and decreases towards the southern end of the lagoon, further away from the entrance (Scrimger 1960, Watanabe and Robinson 1979, Westland Resource Group 1993). In addition, flushing is largely dependent on tidal ranges, tending to be higher in the winter months when large tidal ranges occur more frequently (Westland Resource Group 1993). Tidal energy is dampened as water passes through the entrance channel and it is estimated that the mean tidal amplitude in Esquimalt Lagoon is less than 50 % of that in Esquimalt Harbour, which lays adjacent to Esquimalt Lagoon (Archipelago Marine Research Ltd. 2000). Also, tides lag behind those outside the lagoon by 1-3 hours (Westland Resource Group 1993). Therefore, Esquimalt Lagoon presents water characteristics distinct from those found in the outside ocean waters.

1.5.3 Characteristics of the watershed

Esquimalt Lagoon is fairly small, with a perimeter of 6.3 km and an area of 0.82 km^2, and the area of its watershed was calculated to be 16.2 km^2 (Archipelago Marine Research Ltd. 2000). The watershed supplying Esquimalt Lagoon is a valley filled with glacial deposits of sand and gravel, bordered by a ring of higher-elevation rocky terrain (Environment Canada Forestry Service 1975, Payne Engineering Geology Ltd. 1996). About 34 % of total precipitation infiltrates and travels through these permeable sand and gravel deposits, discharging at the bottom of the lagoon basin or on the western shore via a network of about 30 seepages and springs (Payne Engineering Geology, 1995). Groundwater from the springs on the shore enters the lagoon via small creeks. About 60 % of precipitation enters the lagoon via surface flow, and Colwood Creek contributes 75-95 % of the surface flow (Payne Engineering Geology, 1995). Other contributors to surface flow include small channels and residential stormwater drains and ditches that are scattered around the perimeter of the lagoon (Westland Resource Group 1993, Capital Regional District 2008).

Esquimalt Lagoon's watershed is located in a Douglas fir bioclimatic region. Although some areas of natural or restored vegetation still exist (e.g. forest stands, salt marsh, and wetlands), the remaining vegetated areas have been altered for cultivation, institutional grounds (e.g. Royal Road University Campus and Fort Rodd Hill National Historic Site) or recreation (i.e. a golf course). The rest of the watershed has been developed into residential and commercial properties in the City of Colwood (Payne Engineering Geology Ltd. 1996, Stallard 2009).

1.5.4 Ecosystem services

Habitat

There are a variety of habitats in Esquimalt Lagoon, including relatively extensive (0.15 km^2) sea grass beds dominated by *Zostera marina* or "eel grass". Other aquatic habitats include intertidal sand and gravel flats, mud and sand benthic substrates with red, green, and brown macroalgae (Archipelago Marine Research Ltd. 2000), spit sand dunes, and shoreline marshlands (Westland Resource Group 1993). These habitats support shellfish beds, benthic invertebrates (including crabs), and most noticeably birds. Esquimalt Lagoon and the land within 100 m of the high-water mark is a federally protected migratory bird sanctuary that harbours both migratory and non-migratory birds. Bird communities in the lagoon vary seasonally, being large and diverse in the winter and smaller in the summer (Westland Resource Group 1993). Esquimalt Lagoon is also a conduit for salmon and trout that spawn in Colwood Creek and other small spring-fed creeks, and historically, the lagoon itself supported herring spawns. Mammals such as river otters, raccoons, harbour seals, ungulates (e.g. deer) and rodents are observed frequently in and around the lagoon (Westland Resource Group 1993).

Cultural and social values

Esquimalt Lagoon and its immediate surroundings are aesthetically appealing natural areas and traditional territories of the Esquimalt and Songhees Nations (Esquimalt Lagoon Stewardship Initiative, personal communication). Ancestors of these nations were able to subsist on the abundant resources provided by Esquimalt Lagoon, in particular shellfish. Shell middens have been excavated from at least five ancient village

sites surrounding the lagoon, some identified to be 2 000 to 3 000 years old (Blacklaws 1975 unpublished work by Butch Dick Songhees Nation 2010). In present day, Esquimalt Lagoon retains cultural value for First Peoples, is well suited for recreational activities, and is recognized as part of B.C.'s military heritage (Parks Canada 2012, Royal Roads University 2012). The B.C Heritage Conservation Branch has identified 12 heritage sites in the vicinity of the lagoon (Westland Resource Group 1993) and two large National Historic Sites lay adjacent to the lagoon (Hatley Park National Historic Site/Royal Roads University and Fort Rodd Hill National Historic Site).

1.5.5 Human impacts and water quality

Given the proximity of Esquimalt Lagoon to urban areas, maintaining the sustainability of lagoon habitats, wildlife and water quality is of current concern to local citizens. Impacts to the lagoon include recreational activities on the spit, off-leash dogs, boating, vehicle traffic, expansion of residential and commercial development, septic system leakage, urban and agricultural runoff, decommissioning of the gravel pit that provides sand to the spit, and other less-direct human impacts such as sea-level rise.

The nature of Esquimalt Lagoon's restricted tidal exchange leaves it vulnerable to the accumulation of nutrients and pollutants from numerous non-point sources entering via surface flow or groundwater. These sources include urban runoff into streams, fertilizers from agriculture and landscaping, septic systems, waterfowl and other animals, human garbage on the spit, and atmospheric deposition (Westland Resource Group 1993)

Water quality in Esquimalt Lagoon has been a concern since at least the 1970s, when the Coastal Marine Science Laboratory at Royal Roads Military College measured nutrient concentrations and fecal coliform (FC) bacterial counts in the lagoon and in

streams discharging into the lagoon (Watanabe and Robinson 1980) and attempted to study the dynamics of reoccurring "red tides" (Watanabe and Robinson 1979, Robinson and Brown 1983). These reports found that FC bacterial counts were sometimes very high in streams (> 5 000 FC/100 mL) and that O_2 depletion severe enough to kill benthic invertebrates developed in the autumn months of certain years in the 1970s. Elevated stream FC counts were attributed to contamination from septic systems and a small cattle operation. During and prior to the 1970s all of the residences near to Esquimalt Lagoon were on septic tanks including those built in the spring/seepage discharge area where water tables are high and the spring-fed creeks originate (Westland Resource Group 1993, Payne Engineering Geology Ltd. 1996). Oxygen depletion was attributed to decay processes within the lagoon water column during the decline of "red tides". These "red tides" can thus be considered to be HABs. The death of benthic invertebrates mentioned above often occurred in conjunction with the development of "white tides" shortly after or during the tail end of "red tides". "White tide" is a locally-used term that refers to milky turquoise waters, and the phenomenon is likely analogous to the dystrophic crises that occur in many Mediterranean lagoons (see section 1.4.3). The "red tides" themselves were believed to be sustained by nitrate (NO_3^-) entering from streams following storm events, providing nutrients at the surface of a water column that had been depleted of nitrogen during the growing season. Sewage inputs did not appear to be related to the "red tide", and although FC-contaminated streams occasionally delivered elevated levels of ammonium (NH_4^+) and phosphate (PO_4^{3-}) to the lagoon, NO_3^- concentrations in these streams were comparable to those in the other streams (Watanabe and Robinson 1980).

In the last 15 years, most of the residences near Esquimalt lagoon have been connected to the local sewer system and lower maximum FC counts have been measured, although the lagoon is still closed to shellfish harvesting (Payne Engineering Geology Ltd. 1996). A value of 14 FC/100 mL is the cutoff below which shellfish harvesting is acceptable in British Columbia and 200 FC/100 mL is the cutoff for recreational use. Occasionally levels greater than 200 FC/100 mL are measured in the lagoon or the streams connected to it (Haigh 2008, Capital Regional District 2008, Stallard 2009).

Other aspects of water quality have not improved over the years. "Red tides", "white tides", retreat of benthic crabs, and invertebrate mortalities continue to occur, appearance of floating macroalgal mats has increased, and a number of autumn fish kills have occurred since 1990 (Westland Resource Group 1993, Haigh 2008 Esquimalt Lagoon Stewardship Initiative personal communication 2009). Based on unpublished reports on water quality, NO_3^- inputs into Esquimalt Lagoon via streams have increased by an order of magnitude in the last 30 years. Watanabe and Robinson (1980) found that stream NO_3^- concentrations ranged from 42 μmol L^{-1} to 99.8 μmol L^{-1} in the late 1970s. In contrast, Haigh (2008) and Stallard (2009) reported NO_3^- values ranging from 7 μmol L^{-1} to 593 μmol L^{-1} in 2008, and from 7 μmol L^{-1} to 681 μmol L^{-1} in 2009, respectively. It should be noted that the measurements taken in the 1970s by Watanabe and Robinson (1980) were collected in spring and early summer, whereas the more recent measurements were taken in the late summer and fall, so this could affect the observed ranges. The highest NO_3^- levels in the water column of the lagoon itself 30 years ago were ~ 20 μmol L^{-1} to 30 μmol L^{-1} (Watanabe and Robinson 1979). For comparison,

high NO_3^- values in the Juan de Fuca Strait (Esquimalt Lagoon's ocean source water) tend to be around 25.6 μmol L^{-1} (Masson and Peña 2009).

1.6 Motivations for this study

In recent years there has been a lack of scientific research in Esquimalt Lagoon despite the proliferation of HABs. Furthermore, a thorough characterization of phytoplankton ecophysiology in the lagoon has not been undertaken to date. Hence, the primary motivations for this study were (a) to understand phytoplankton succession in Esquimalt Lagoon and how the ecophysiology of harmful algal groups differs from that of benign phytoplankton assemblages, and (b) to understand how HAB ecophysiology leads to development of O_2 depletion in the context of physical processes.

1.7 Outline of this thesis

My thesis project investigated the physical and chemical characteristics of the pelagic system in Esquimalt Lagoon as well as phytoplankton dynamics. Chapter 1 has presented fundamental background information on phytoplankton blooms and HABs, as well as information on physical processes and eutrophication in coastal lagoons and the characteristics of Esquimalt Lagoon itself. Chapter 2 describes the seasonal variability in tidal cycles, temperature, salinity, density, O_2 concentrations, pH, concentrations of dissolved nutrients (nitrate (NO_3^-), silicic acid ($Si(OH)_4$), orthophosphate (PO_4^{3-}), ammonium (NH_4^+), and urea), and phytoplankton biomass (total and size-fractionated) over the study period. Chapter 3 investigates phytoplankton species succession and ecophysiology, and the influence of phytoplankton blooms and physical processes on O_2

depletion events in the lagoon. Chapter 4 provides general conclusions and suggests avenues of future research.

Chapter 2: Seasonal variability in the physical, chemical, and biological characteristics of the pelagic system in Esquimalt Lagoon

2.1 Introduction

Because of the shallow, enclosed nature of lagoons, water chemistry is intimately related to the biological processes (Viaroli et al. 2008) and physical forces (Postma 1969) that act on the water column. Photosynthesis and respiration by both phytoplankton and heterotrophic bacteria can have a substantial affect on the concentrations of dissolved nutrients, O_2 and CO_2, and pH levels (Borges and Frankignoulle 2002, Riebesell and Wolf-Galdrow 2002, Gürel et al. 2005, Yates et al. 2007). The processes involved in phytoplankton growth and bacterial decomposition can in turn be influenced by physical characteristics of the environment. Wind and tides affect circulation, stratification and mixing in the water column, flushing and delivery of oceanic nutrients. Precipitation affects density-driven circulation and aids in delivery of terrestrial nutrients, while temperature affects physiological processes in aquatic organisms as well as stratification and circulation by controlling evaporation rates and influencing density gradients (Groen 1969, Postma 1969, Seliger et al. 1979, Gürel et al. 2005, Figueiras et al. 2006, Litchman and Klausmeier 2008). Esquimalt Lagoon is an ideal system for investigating temporal changes in biological, physical and chemical characteristics of a shallow, semi-isolated water body.

The Juan de Fuca Strait (JdFS) is the ocean end member that supplies the majority of Esquimalt Lagoon's water volume, and thus comparing biological, physical, and chemical characteristics of the lagoon with the JdFS and the larger Strait of Georgia

(SoG) can shed light on how ocean water is altered during its residence in the lagoon. The JdFS and the SoG separate Vancouver Island from the British Colombia mainland and Washington State, U.S.A (Fig. 1.1). These straits are separated by a sill and form a large composite estuary (~ 250 m deep in JdFS, ~ 420 m deep in SoG, and ~ 150 m deep at the sill) influenced by freshwater delivered by the Fraser River and deep ocean water from the continental shelf west of Vancouver Island that is drawn into the JdFS at depth. Waters in the JdFS are high in nutrients all year round and have low O_2 concentrations at depth, whereas the SoG has higher O_2 concentrations (temporally averaged) throughout the water column, but experiences nutrient depletion near the surface. Water properties in the straits are also influenced by upwelling and downwelling processes (Masson 2006). Saanich Inlet and Sequim Bay are two enclosed water bodies that are connected to the SoG and JdFS (respectively). Saanich Inlet is a deep (~ 215 m) eutrophic fjord that is connected on its northern end to the SoG. There is a shallow sill at the mouth of Saanich Inlet that largely restricts water flow, leading to anoxia at depth (Herlinveaux 1962). Sequim Bay is a nearby water body that is more similar in size to Esquimalt Lagoon than Saanich Inlet. This bay has weak circulation in certain areas and suffers from toxic algal blooms and localised O_2 depletion (Elwha-Dungeness Planning Unit 2005). Sequim Bay is located on the northern coastline of Washington and is connected to the JdFS via a narrow channel. Esquimalt Lagoon will be compared to these water bodies where appropriate.

2.2 Objectives of this Chapter

The objectives of this chapter are: (1) to describe the seasonal variability of biological, physical and chemical characteristics of Esquimalt Lagoon, which include tidal cycles, temperature, salinity, density, O_2 concentrations, pH, concentrations of dissolved nutrients (NO_3^-, $Si(OH)_4$, PO_4^{3-} orthophosphate, NH_4^+, and urea), and phytoplankton biomass (total and size-fractionated), (2) to compare these characteristics to nearby water bodies, (3) to discuss the interplay between phytoplankton dynamics and chemical properties of the water, and (4) to explore the nature of stratification and circulation in Esquimalt Lagoon.

2.3 Methods

2.3.1 Sampling Procedure

Samples from Esquimalt Lagoon were collected twice per month during the growing season (March through October) and once per month during the winter (November through February), from August of 2009 until January of 2011. This sampling protocol resulted in 29 field days, which are listed in Table 2.1. Initially, samples were collected at four stations (1 – 4, Fig. 1.1), but in February of 2010, a fifth station (5, Fig. 1.1) was added to cover an under-sampled area of the lagoon. Station coordinates are recorded in Appendix A. Three stations (2, 4 and 5) were chosen in order to maintain historic sampling sites used by researchers at Royal Roads Military College (now Royal Roads University), station 1 was chosen based on its proximity to the lagoon inlet, and station 3 was chosen to represent areas of the lagoon with eel grass coverage.

Table 2.1 Sampling dates and stations during the current study. Note that an additional station was sampled starting in February of 2010, until the end of the study.

Year	Field days	Stations sampled
2009	August 18 and 25	1, 2, 3, 4
	September 12 and 29	
	October 13 and 28	
	November 17	
	December 17	
2010	January 31	
2010	February 16	1, 2, 3, 4, 5
	March 9 and 23	
	April 6 and 20	
	May 7 and 27	
	June 10 and 29	
	July 16 and 26	
	August 10 and 26	
	September 14 and 27	
	October 11 and 25	
	November 29	
	December 17	
2011	January 28	

Sampling occurred via canoe and was finished before local noon during most sampling days. Water samples were taken at two depths per station: the upper or "surface" water sample was collected in a 2 L Niskin bottle from 1.0 to 1.5 m below the water surface and the lower or "bottom" water sample was taken with a 1.5 L horizontal sampler at 20 cm above the sediment-water interface. This chapter presents and discusses only the data from the surface samples. See Appendix B for figures illustrating the bottom data, and note that the raw data from both sampling depths is included in supplements to the electronic version of this thesis (see Table 2.2). After collection, water was transferred into 2 L polypropylene bottles that were previously acid cleaned and rinsed with double-deionized water. These bottles were kept on ice in the dark until they were sub-sampled

upon returning to the laboratory within and hour of leaving the study site. Sub-samples were preserved and analyzed according to procedures detailed in section 2.3.2. A complete list of measurements can be found in Table 2.2.

Table 2.2 Measurements made in Esquimalt Lagoon during the current study. Surface samples were collected at 1.0 -1.5 m below the water surface and bottom samples were collected 20 cm above the sediments. "T" means that the data figures are presented and discussed in this thesis. "A.B" means that data figures are included in Appendix B. "A.C" means that raw data are included in Appendix C. "A.D" means that raw data are included in Appendix D. "E" means that raw data are included in the supplements to the open-source electronic version of this thesis available from the online repository UvicSpace in August of 2013.

Parameter	Presentation of Data	Collection information
	A.C	Every station
Water depth		
Secchi disc depth	A.C	
Temperature	T, E	Every station, at 20 cm depth intervals
Salinity	T, E	vertically throughout the water column
Dissolved oxygen		
%	T, E	
mg L^{-1}	E	
pH	T, E	
Specific conductivity	E	
Oxidation-reduction potential	E	
Nitrate	T^{S}, $A.B^{B}$, E	Every station, at surface (1.0 -1.5 m
Orthophosphate	T^{S}, $A.B^{B}$, E	depth) and bottom (20 cm above
Silicic acid	T^{S}, $A.B^{B}$, E	sediments)
Urea	T^{S}, $A.B^{B}$, E	
Ammonium	T^{S}, $A.B^{B}$, E	
Phytoplankton species composition	T*	
Chlorophyll *a* (> 0.7 µm)	T^{S}, $A.B^{B}$, E	
Size-fractionated chlorophyll *a* (0.7 - 2 µm, 2 - 20 µm and > 20 µm)	T^{S}, $A.B^{B}$, E	
Uptake rates of nitrate, ammonium, urea, and carbon	T, E	Station 4 at surface (1.0 – 1.5 m depth)
Natural $\delta^{15}N$ and $\delta^{13}C$ of particulates	A.D	
Tidal height[§]	T	Esquimalt Lagoon harmonic station, predicted at 5 minute intervals
Precipitation[‡]	T	Total daily values
Wind speed	T	Hourly values

[S] Refers to data from surface samples
[B] Refers to data from bottom samples
*Only surface samples from station 4 were fully analysed
[§] Source: Canadian Hydrographic Service Pacific Region (Fisheries & Oceans Canada, Institute of Ocean Sciences, Sidney BC).
[‡] Source: Environment Canada, National Climate Data and Information Archive.

2.3.2 Physical, chemical and biological measurements

Temperature, salinity, and density

Temperature and salinity were measured with a Hydrolab Quanta G submersible multiprobe at 20 cm intervals throughout the water column. The probes were maintained regularly and calibrated according to directions in the manual (Hydrolab Corporation 2002). Salinity values were reported according to the practical salinity scale and were normalized to 15 °C.

Density was calculated from density routines reported in the US Department of Energy handbook on the CO_2 system in sea water (1994) with modifications described in Crawford & Harrison (1997). Density values in this thesis are reported as σ_t (sigma-t or specific gravity anomaly), which in surface waters is calculated by subtracting 1 000 from density (in kg m^{-3}).

Tidal height, precipitation and wind

Predicted tidal data were obtained from the Canadian Hydrographic Service Pacific Region (Fisheries & Oceans Canada, Institute of Ocean Sciences, Sidney BC). Esquimalt Lagoon is harmonic station # 7107 in their tidal prediction software (Canadian Hydrographic Service 2011). Tidal height predictions for harmonic stations are created by summing sinusoidal waves that represent each of the constituents contributing to the observed changes in tidal height (e.g. astronomical constituents, shallow water constituents).

Precipitation and wind data were obtained from Environment Canada's National Climate Data and Information Archive (Environment Canada 2012). Both of these parameters were measured at the Esquimalt Harbour Station (48.43° N, 123.44° W).

Oxygen and pH

O_2 and pH were also measured with the Hydrolab Quanta G submersible multiprobe. The pH probe was calibrated using commercially-available standards (pH of 7 and 10) and the O_2 probe was calibrated using the "saturated air method" described in the manual, with a programmed atmospheric pressure (at sea level) of 760 mm Hg. O_2 values are reported in terms of concentration ($[O_2]$, in mg L^{-1}) and percent saturation (%). The maximum $[O_2]$ that will dissolve in seawater based on physical processes alone varies based on temperature and salinity. Percent saturation is calculated by dividing the measured $[O_2]$ in a water mass by the maximum theoretical concentration at the *in situ* temperature and salinity. Waters above 100 % saturation have $[O_2]$s exceeding physical saturation.

Dissolved nutrients

Sub-sampling protocol

Surface water samples were collected on each field day at five stations (described in section 2.3.1) for the measurement of nitrate + nitrite (from this point forward referred to as NO_3^-), PO_4^{3-}, $Si(OH)_4$, urea, and NH_4^+. Two sub-samples from the original water sample were syringe-filtered through a combusted 0.7 μm glass fiber filter into 30 mL polypropylene bottles. These samples were stored at -20 °C until automated analysis. One extra 30 mL sub-sample was taken for manual analysis of urea. In addition, extra

sub-samples were taken from station 1 and 4 and filtered through 0.6 μm polycarbonate (PC) filters to determine if glass fibre filters were a source of Si contamination for $Si(OH)_4$ measurements. All sampling equipment was acid-washed and rinsed with double-deionized water. The majority of samples were analyzed within 6 months. Sub-samples (40 mL) for NH_4^+ analysis were taken in duplicate and transferred, unfiltered, into 50 mL borosilicate tubes to be analyzed on the same day as collection. The tubes used for NH_4^+ analysis were cleaned by filling them with active working reagent to bind any contaminant NH_4^+ and triple-rinsing them with double-deionized water before water samples were introduced.

Automated analysis of nitrate, orthophosphate, silicic acid, and urea

Before analysis, samples were thawed in a drying oven at 50°C just until the ice melted, and then they were warmed to room temperature outside the oven. All glassware used during analysis was acid-washed. All four dissolved nutrients were measured spectrophotometrically on an Astoria 2 Analyzer (Astoria-Pacific International) using reagents and procedures described in the Astoria 2 operations manual (Astoria-Pacific Inc. 2005). Protocols for NO_3^- and PO_4^{3-} are based on "Standard Methods for the Examination of Water and Wastewater" (1981), the $Si(OH)_4$ protocol is based on Truesdale & Smith (1975), and the automated urea protocol is based on Rahmatulla & Boyd (1980). One exception to the NO_3^- procedure outlined in the Astoria manual was that the "open tubular cadmium reactor" was replaced with a U-shaped cadmium tube that is similar to those commonly used in the manual NO_3^- analysis method (Grasshoff 1976). Nutrient concentrations were calculated based upon calibration curves produced by measuring nutrient standards during the sample runs. The limits of detection for NO_3^-,

PO_4^{3-}, $Si(OH)_4$, and urea were 0.1 μmol L^{-1}, 0.03 μmol L^{-1}, and 0.2 μmol L^{-1}, and 0.06 μmol L^{-1}, respectively. Duplicate sub-samples were averaged. The mean coefficient of variation (CV) and pooled standard deviation (SD) for NO_3^- sub-samples were 0.04 and 0.24 μmol L^{-1}, respectively. The mean CV and pooled SD for PO_4^{3-} sub-samples were 0.04 and 0.05 μmol L^{-1}. The mean CV and pooled SD for $Si(OH)_4$ sub-samples were 0.11 and 5.46 μmol L^{-1}.

Automated analysis of urea was only performed on samples from March 2010 onward to compare with the data obtained from manual urea analysis (see below). Glass fiber filters proved not to be a source of Si contamination and hence sub-samples from both polycarbonate and glass fiber filters were included when averaging nutrient sub-samples.

Manual analysis of urea

Urea concentrations were measured fluorometrically using the diacteylmonoxime manual method developed by Mulvenna & Savidge (1992) with slight modifications. Urea concentrations were calculated based upon calibration curves produced during every sample run. A limit of detection of 0.06 μmol L^{-1} was obtained. Only samples from March 2010 and onward were measured in duplicate (on the autoanalyzer, see above), so these values were averaged. The mean CV and pooled SD for urea sub-samples (from the two methods) were 0.16 and 0.06 μmol L^{-1}.

Manual analysis of ammonium

Concentrations of NH_4^+ were measured fluorometrically on the same day that samples were collected following the manual method of Holmes et al. (1999). Duplicate sub-

samples (40 mL) were kept in the dark with 10 mL of the o-phthalaldehyde working reagent for 4 to 6 hours. This reagent binds with NH_4^+ forming a fluorescent complex, and hence fluorescence was measured on a TD 700 Turner Designs fluorometer. Concentrations of NH_4^+ were calculated based upon a calibration curve produced for each sample run. The limit of detection for NH_4^+ analysis was 0.01 μmol L^{-1}. Duplicate sub-samples were averaged. The mean CV and pooled SD for NH_4^+ sub-samples were 0.07 and 0.13 μmol L^{-1}.

Phytoplankton biomass, as Chlorophyll *a*

Sub-samples (100 mL) for the measurement of total Chl *a* concentrations were filtered in the dark onto a 0.7 μm glass fiber filter. Sub-samples (150 mL) for the measurement of size-fractionated Chl *a* were filtered either through a cascade or a stack of filters of the following pore sizes and types: 20 μm PC, 2 μm PC, and 0.7 μm glass fiber. This allowed for the determination of microphytoplankton (> 20 μm in diameter), nanophytoplankton (2 μm - 20 μm in diameter), and picophytoplankton biomass (0.7 μm - 2 μm in diameter). Chl *a* was analyzed fluorometrically according to the protocol in Parsons et al. (1984) with slight modifications. Chl *a* fluorescence was measured on a 10-AU Turner Designs fluorometer before and after acidification with 1N HCl. Chl *a* concentrations were calculated based on a calibration curve established for the instrument and were corrected for the presence of pheopigments.

2.3.3 Data analysis

When describing seasonal data, "winter" months were considered to be November, December, January, and February, "spring" months were March, April, and May, "summer" months were June, July, and August, and "fall" months were September and October. November was included in the winter instead of the fall because temperature values were low and nutrients were high, being very similar to those in the December, January and February. Also, phytoplankton biomass dropped in November following the fall blooms. The "growing season" was considered to be March through October (spring, summer and fall).

Daily averages for nutrient concentrations and Chl *a* concentrations were calculated by averaging data from all five stations at the 1 m sampling depth. Daily averages for temperature, salinity, density, O_2 concentrations, and pH were calculated by averaging data at all five stations and all depths where measurements were taken.

2.4 Results

2.4.1 Physical characteristics of Esquimalt Lagoon

Temperature

Water temperatures in Esquimalt Lagoon ranged from 3.4 °C to 19.2 °C throughout the water column during the sampling period (Table 2.3, Fig. 2.1). In the winter, daily average temperatures ranged from 6.3 °C to 8.3 °C. On most days in the winter, temperature varied by < 2 °C vertically in the water column, and either increased or did not change with depth (Fig. 2.2A). Waters began to warm in early March, and by mid-

April conditions had changed in the water column, with warmer water at the surface and colder water at the bottom. The spring months had intermediate daily average temperatures, ranging from 7.7 °C to 11.2 °C. Temperatures at each station in the spring varied by 0 °C to 3 °C vertically in the water column (Fig. 2.1). Typical temperature profiles at this time showed either an upper layer of uniform temperature ranging from ~ 0.5 m to 1.5 m in depth, or a gradual decrease in temperature over the entire water column (Fig. 2.1). Waters were warmest in the summer months, with daily average temperatures ranging from 13.1 °C to 16.0 °C. During the summer, the warmest waters were at the surface and temperature at most stations varied vertically by 2 °C to 6 °C (Fig. 2.1). Usually, there was a layer of uniform temperature at the surface about 0.5 m to 1.5 m deep, and underneath this temperatures gradually decreased towards the bottom (e.g. July 16, 2010 in Fig. 2.2B). The fall months also had intermediate daily average temperatures, ranging from 8.7 °C to 13.6 °C. The temperature variability within profiles was between 0 °C and 4 °C vertically (Fig. 2.1). In September, the summer trend of warm surface waters and large temperature variability throughout the water column was still present, but starting in October (of both 2009 and 2010) winter conditions were re-established. The water column in the winter of 2009 - 2010 was warmer than that in the winter of 2010 – 2011, and water temperatures in September of 2009 were also warmer than those in September of 2010.

Table 2.3 Physical parameters measured in Esquimalt Lagoon during the current study (August 2009 through January 2011) and in the Juan de Fuca Strait (throughout 1967 and 1968, Crean and Ages (1971)). Juan de Fuca Strait measurements were made at two depths (0 m and 5 m) at three stations (65, 67, and 69) near Esquimalt Lagoon. These stations were sampled on periodic cruises in 1967 and 1968 (Crean and Ages, 1971). Daily averages were obtained by averaging measurements from all stations and depths for each field day. Maximum and minimum values for the entire study periods in both Esquimalt Lagoon and the Juan de Fuca Strait are also presented.

	Esquimalt Lagoon		**Juan de Fuca Strait**	
Parameter	**Max and min values**	**Max and min daily averages**	**Max and min values**	**Max and min daily averages**
Temperature (°C)	3.4 - 19.2	6.3 – 16.0	7.0 - 13.6	7.2 – 11.9
Salinity	17.0 - 32.5	28.1 - 32.1	28.5 - 31.9	29.3 - 31.4
Density as σ_t (kg m^{-3})	13.5 - 25.2	21.6 - 24.6	21.3 - 24.7	22.2 – 24.3

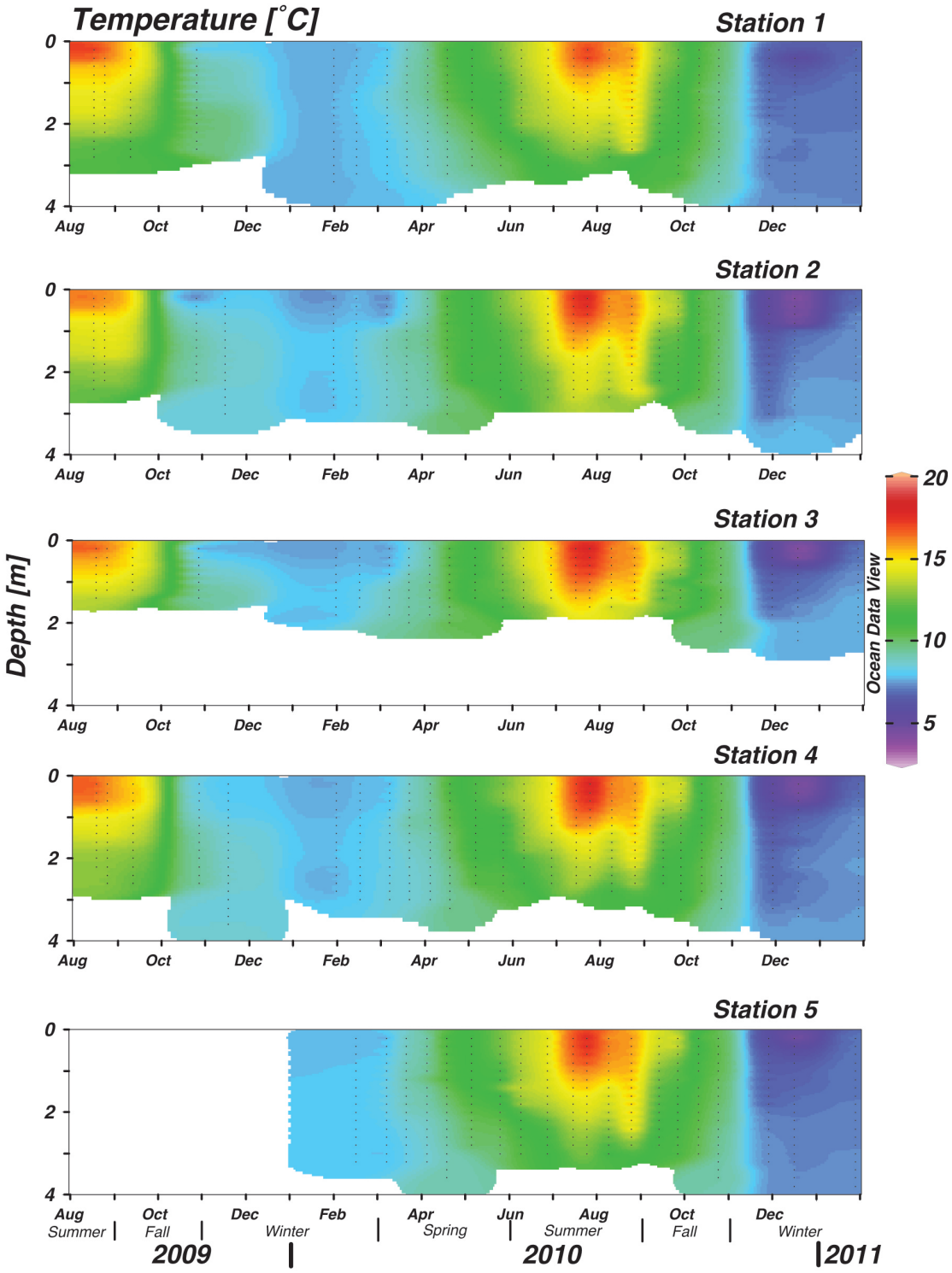


Figure 2.1 Temperature (˚C) at five stations in Esquimalt Lagoon during the current study. Each field day (listed in Table 2.1) is represented by a vertical line of dots, with the exception of certain dates at certain stations in 2009 because of instrument malfunction. These dates are September 12, September 29, October 13, November 17, and December 17. The dots represent single measurements taken at 20 cm intervals over the depth of the water column. The disparities in depths sampled on different field days are due to fluctuating tides. Ticks on the x-axis represent the first day of each month.

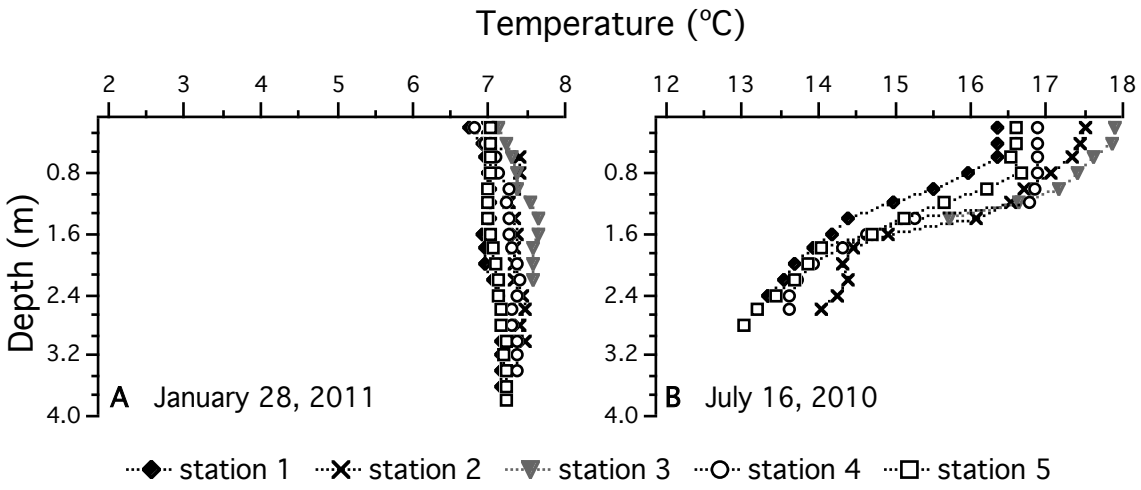


Figure 2.2 Example temperature profiles (°C) in Esquimalt Lagoon on (A) January 28, 2011 and (B) July 16, 2010. Measurements were taken at 20 cm intervals over the depth of the water column at five stations.

Salinity

The overall range in measured salinities was 17.0 to 32.5 throughout the water column and for all seasons (Table 2.3; Fig. 2.3), however, daily average salinities experienced relatively small fluctuations over the course of the year, ranging from 28.1 to 32.1 (Table 2.3; Fig. 2.3). There was one distinguishable temporal trend in salinity data: the majority of the spring-summer period (from March 23, 2010 to August 10, 2010) had higher daily average salinities (30.8 to 32.1) than the rest of study period, and daily minimum salinities were also high. In accordance with these observations, vertical salinity ranges at individual stations were also small at this time of year, with average salinity variations of 1.3 along the depth profile. The remainder of the year (from late-summer until early spring) experienced higher levels of precipitation (Fig. 2.4) and daily average salinities ranged from 28.1 to 30.8, although minimum daily salinities as low as 17.0 were

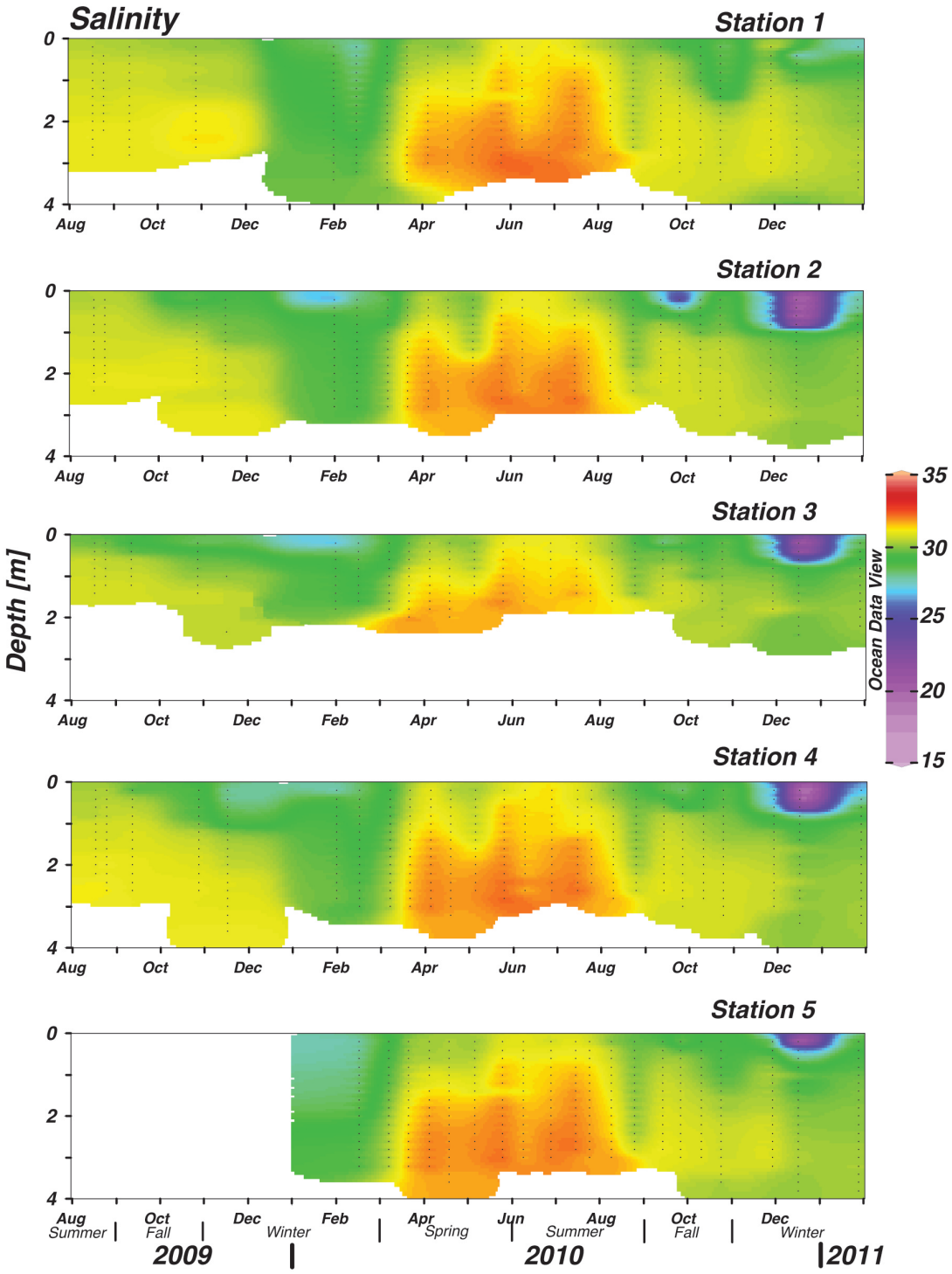


Figure 2.3 Salinity at five stations in Esquimalt Lagoon during the current study. Each field day (listed in Table 2.1) is represented by a vertical line of dots, with the exception of certain dates at certain stations in 2009 because of instrument malfunction. These dates are September 12, September 29, October 13, November 17, and December 17. The dots represent single measurements taken at 20 cm intervals over the depth of the water column. The disparities in depths sampled on different field days are due to fluctuating tides. Ticks on the x-axis represent the first day of each month.

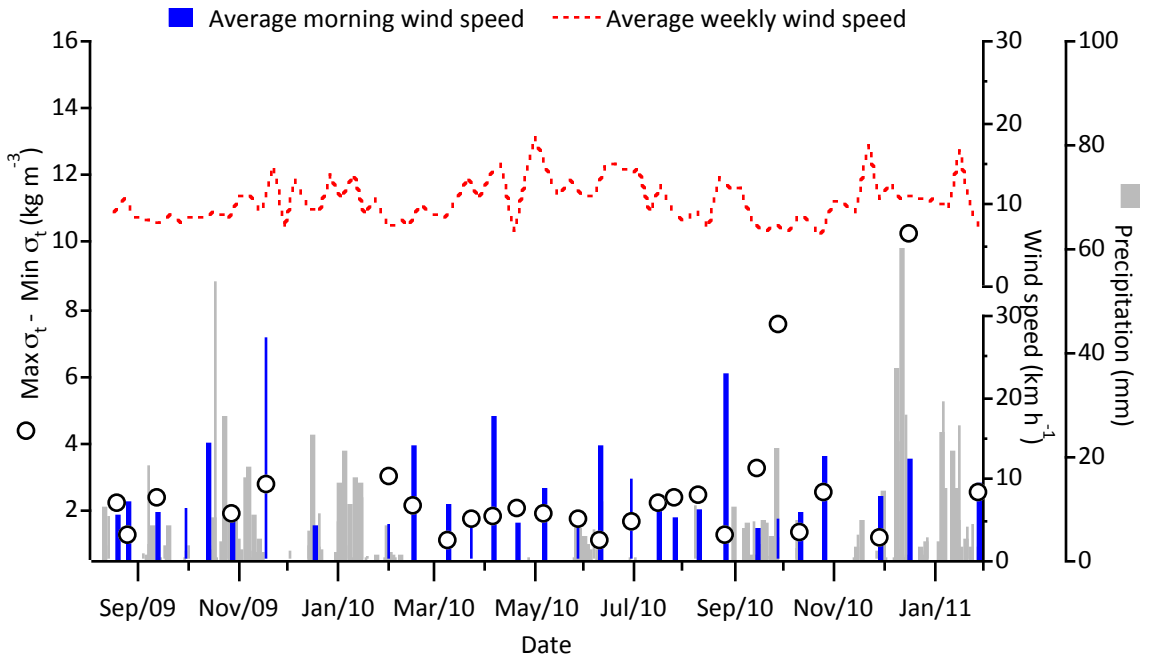


Figure 2.4 Precipitation, wind speed and daily maximum minus minimum densities (as σ_t) in Esquimalt Lagoon during the current study. "Average morning wind speed" is the average hourly wind speed from 12:00 AM to 12:00 PM on each field day (field days listed in Table 2.1) and "weekly wind speeds" are the average hourly wind speeds on a weekly basis throughout the study period. Wind and precipitation data were measured in Esquimalt Harbour by Environment Canada. Ticks on the x-axis represent the first day of each month.

observed near the surface (Fig. 2.3). Vertical salinity ranges at individual stations from late-summer until early spring varied on average by 2.3. At all times, salinities were lower near the surface and greater at depth, increasing gradually over the entire water column, or increasing gradually underneath an upper layer of uniform salinity.

Density

Maximum and minimum density measurements in Esquimalt Lagoon were 13.5 kg m^{-3} and 25.2 kg m^{-3}, respectively (Table 2.3; Fig. 2.5), and the range in daily average densities was 21.6 kg m^{-3} to 24.6 kg m^{-3} (Table 2.3). There are a couple of temporal patterns worth pointing out in the density data. Firstly, the majority of the spring-summer period (March 23, 2010 to August 10, 2010) had higher daily average densities (values in

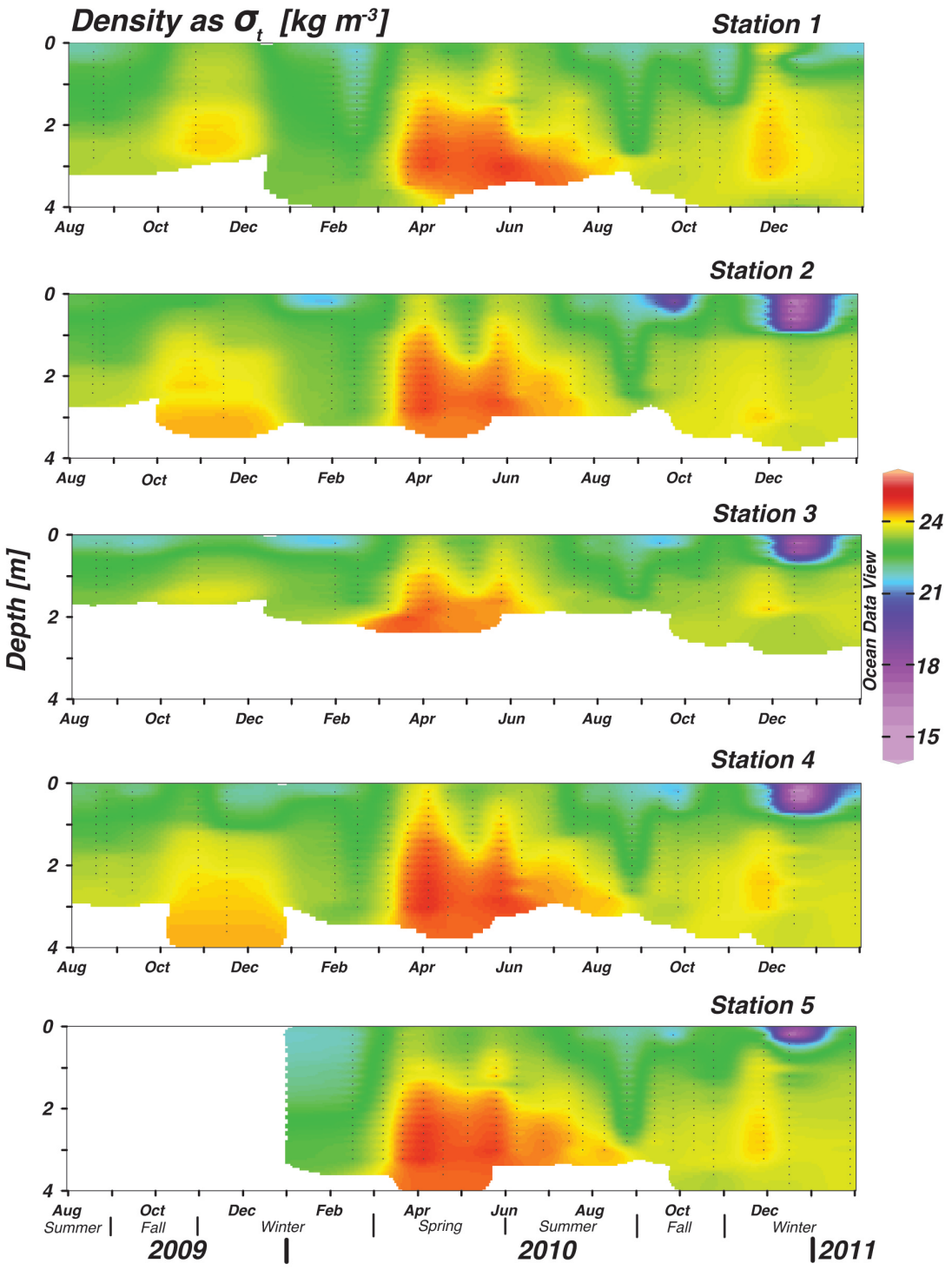


Figure 2.5 Density (kg m^{-3}) at five stations in Esquimalt Lagoon during the current study. Density was calculated from salinity and temperature measurements and is represented by σ_t (sigma-t), which at the water surface is equivalent to density minus 1,000. Each field day (listed in Table 2.1) is represented by a vertical line of dots, with the exception of certain dates at certain stations in 2009 because of instrument malfunction. These dates are September 12, September 29, October 13, November 17, and December 17. The dots represent measurements taken at 20 cm intervals over the depth of the water column. The disparities in depths sampled on different field days are due to fluctuating tides.

between 23.0 kg m^{-3} and 24.6 kg m^{-3}) than the fall and winter months (values in between 21.6 kg m^{-3} and 23.9 kg m^{-3}) and secondly, the lowest observed surface densities were achieved in the fall and winter months due to surface freshwater inputs (Figs. 2.4 and 2.5).

When conditions were calm, density profiles consisted of a concave-downward curve with no visible pycnocline, meaning that density gradually increased with depth (Fig. 2.6, A and B). However, when wind was present a mixed layer developed (Fig. 2.6, C and D) and when high wind speeds occurred in the morning prior to and during sampling (averaged over a twelve hour period, from local midnight to local noon), reduced values of maximum minus minimum densities were often observed (Fig. 2.4). Both calm-weather profiles and windy-weather profiles were observed in all seasons. Calm weather profiles that occurred in the spring and summer were distinct from those that occurred in the fall and winter. The primary differences were that (a) in the fall and winter, densities below about 1 m were quite uniform, rather than increasing gradually, and (b) despite the reduced density range in deep waters, overall vertical density ranges were still higher in the fall and winter months due to fresh waters with low densities at the surface (Fig 2.5 and Fig. 2.6B). The average vertical density range during calm weather in the fall and winter was 3.60 kg m^{-3} compared to 1.65 kg m^{-3} in the spring and summer. As mentioned previously, the concave-downward pattern appeared to break up frequently, likely due to wind. Under light winds, mixed layers ranging in depth from about 0.5 m to 1.5 m were observed (Fig. 2.6D) and density increased gradually below the pycnocline. Also under these conditions, density profiles were variable among stations, particularly in the mixed

layer. When winds were stronger, mixed layers ranged from about 1 m to 2.5 m, and the profiles showed even more variability among stations (Fig. 2.6C); densities among stations were different in the mixed layer and also in the stratified waters underneath the pycnocline.

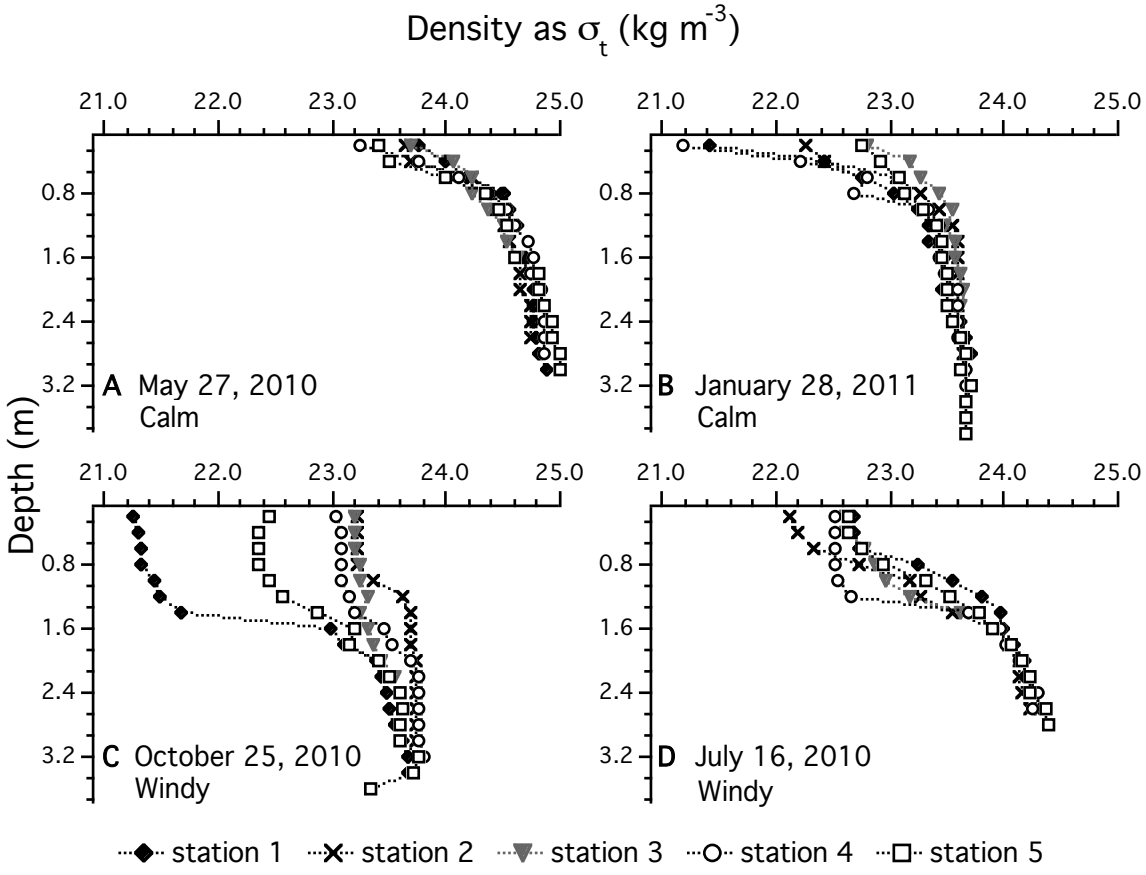


Figure 2.6 Example density profiles in Esquimalt Lagoon from (A) May 27, 2010, (B) January 28, 2011, (C) October 25, 2010, and (D) July 16, 2010. Density profiles in panels C and D were affected by wind mixing at the surface. Density was calculated from salinity and temperature measurements and is represented by σ_t (sigma-t), which at the water surface is equivalent to density (in kg m^{-3}) minus 1,000. Measurements were taken at 20 cm intervals over the depth of the water column at five stations.

A noteworthy trend that was observed in density profiles during windy conditions was that when winds had a southerly component to their direction and were greater than about 9 km h^{-1} (~5 knots), the surface waters of station 1 and 5 had deeper pycnoclines and were less dense compared to the other stations. This can be seen in the October 25th profile (Fig. 2.6C). Strong winds without a southerly component were not observed, but it appears that light winds with a northerly or easterly component may have created an opposite effect to southerly winds. In these situations, the upper layers of stations 2, 3, and 4 were moderately less dense with deeper pycnoclines (data not shown).

Tidal height, precipitation and wind

During the study period, predicted water depth ranged from 1.2 m above sea level to 0.3 m below sea level (Fig. 2.7), and predicted tidal ranges were between 0.15 m and 1.3 m. This indicates that Esquimalt Lagoon is a microtidal system (tidal range < 2 m). During the 2010 growing season, tidal ranges (particularly during neap tides) were smaller in August, September, and October than in May, June, and July.

Field days were not selected according to tidal cycle, but rather scheduled fortnightly (red dots in Fig. 2.7). Despite this, sample collection from August 2009 through March 2010 and from September 2010 through January 2011 occurred during rising tides, while from April 2010 through August 2010 sample collection occurred during falling tides.

Amount and frequency of precipitation was variable during the study period (Fig. 2.4), but precipitation was generally more frequent during the fall and winter months. The highest daily precipitation values occurred in December of both 2009 and 2010. Precipitation values were very low from February through August in 2010, with the exception of a period of moderate rainfall at the end of May and beginning of June.

Average weekly wind speeds were variable during the study period (Fig. 2.4), although four relatively calm stretches occurred from August through October in 2009, January through February in 2010, late July through early August in 2010 and September through October in 2010. Average morning wind speeds on field days did not follow any obvious seasonal patterns (Fig. 2.4), but they corresponded somewhat with average weekly wind speeds, with the highest morning wind speeds occurring during windy stretches and lower values tending to occur during calm stretches.

As mentioned in the above description of density (this section), both precipitation and wind appeared to have an influence on daily maximum minus minimum densities (Fig. 2.4). High precipitation often corresponded with increased density differences and high winds often corresponded with decreased density differences. Also, field days occurring during calm stretches often had relatively high density differences.

2.4.2 Chemical Characteristics of Esquimalt Lagoon

Oxygen

From the late winter until mid-summer (February through early August in 2010), [O_2] ranged from 4.9 mg L^{-1} (55.3 % saturation) to 11.8 mg L^{-1} (142.3 % saturation), with the majority of the water column (except for bottom waters) remaining above 100 % saturation (Fig. 2.8). For the majority of the winter period in 2009 and 2010 (November through January), the water column was less saturated with O_2 than during the remainder of the year, with O_2 saturation ranging from 47.8 % (4.88 mg L^{-1}) to a 93.2 % (10.9 mg L^{-1}). Both the highest and the lowest [O_2]s occurred during the late summer and fall of 2009 and 2010 (mid-August to October). These concentrations were 0.64 mg L^{-1} and

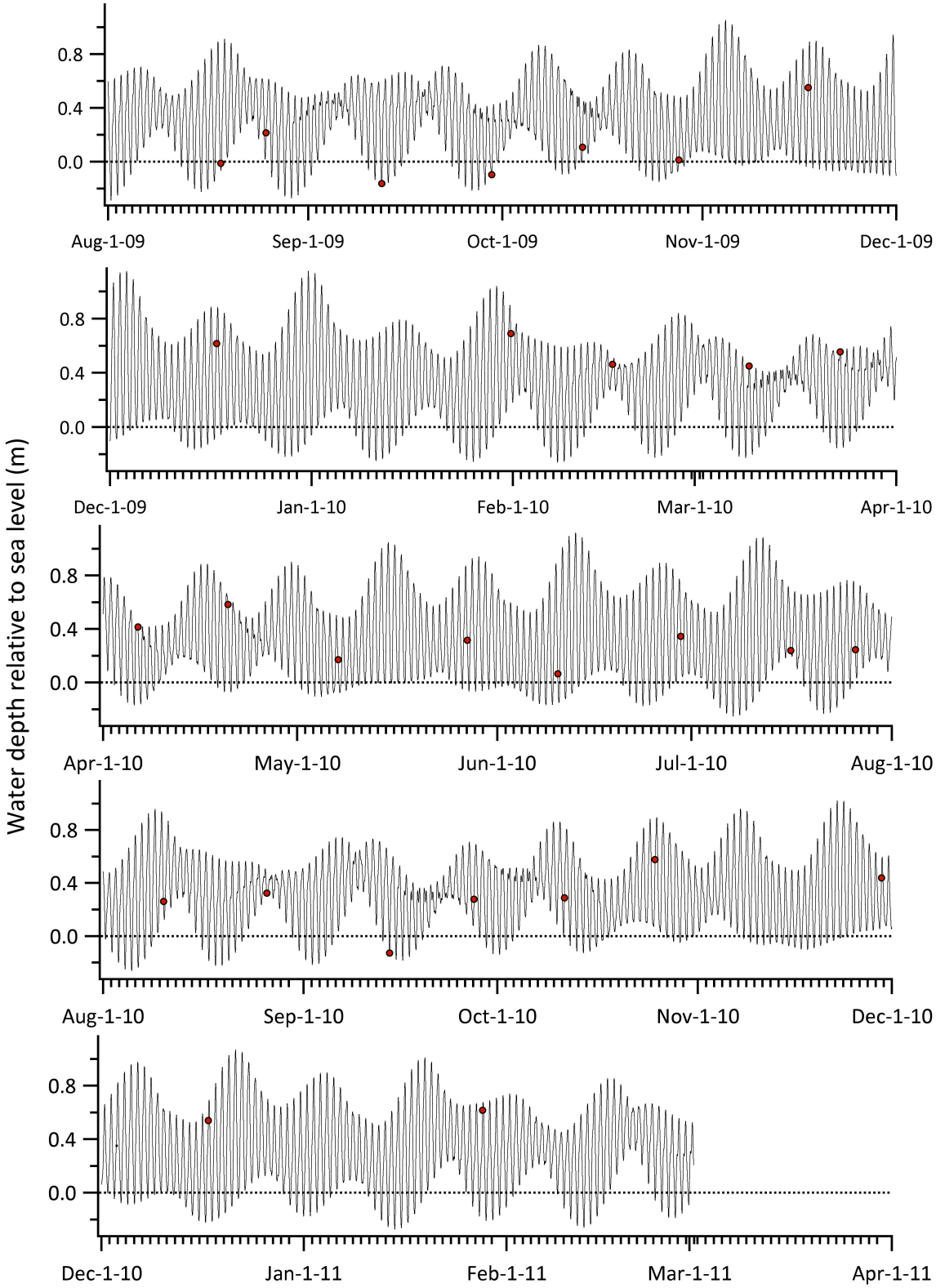


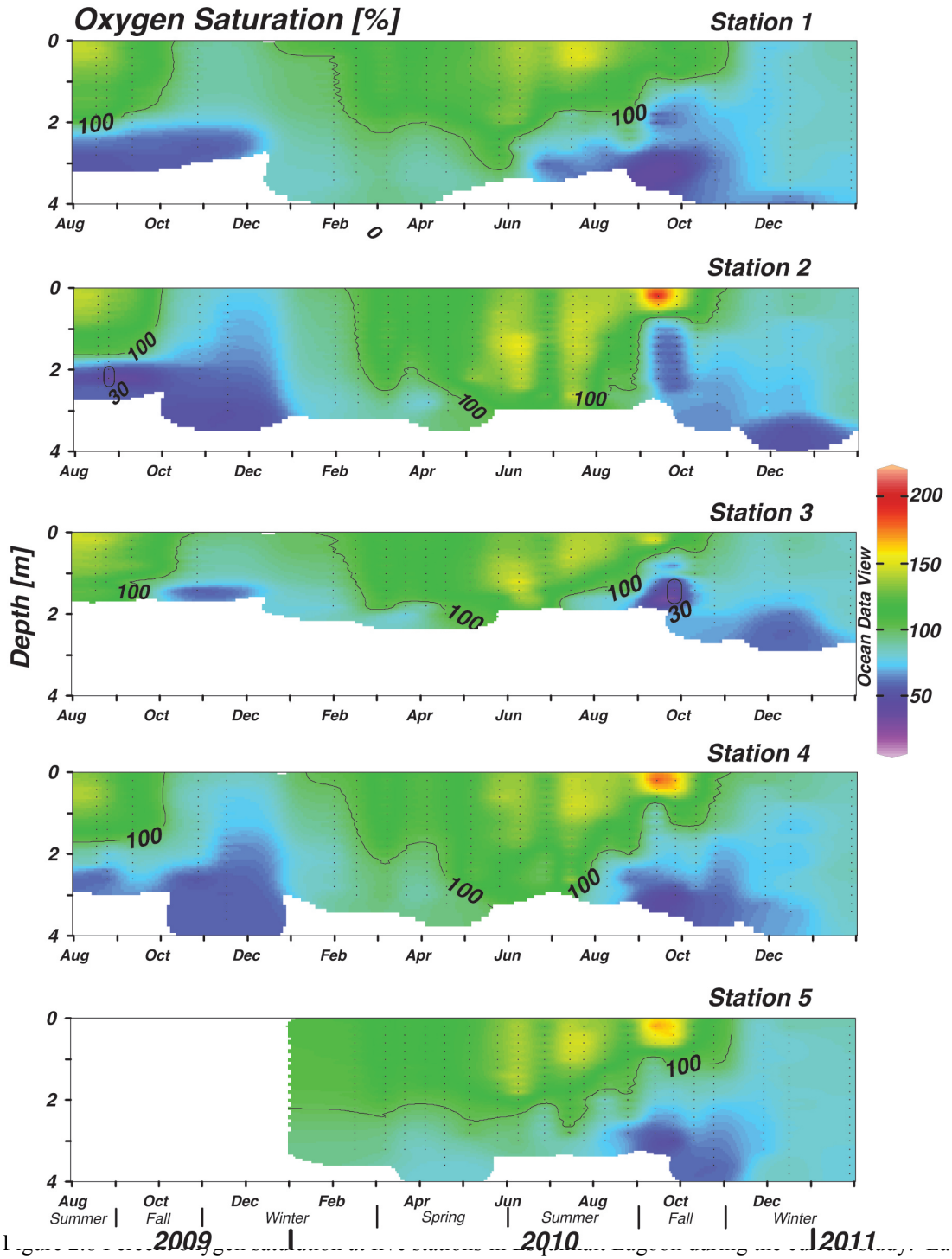
Figure 2.7 Tidal height predictions (water depth relative to sea level) for Esquimalt Lagoon during the current study. Predictions were provided by the Canadian Hydrographic Service. Red dots represent the field days listed in Table 2.1.

19.3 mg L^{-1} respectively, corresponding to 7.4 % saturation and 220.7 % saturation. The late summer and fall period was characterized by large vertical ranges in O_2 levels within the water column, with O_2 being depleted in the lower water column (40 to 60 % saturation) yet remaining above 100 % saturation near the surface. Hypoxia was measured on two occasions in the bottom waters, on August 25, 2009 at station 2, and on September 27, 2010 at station 3 (Fig. 2.8).

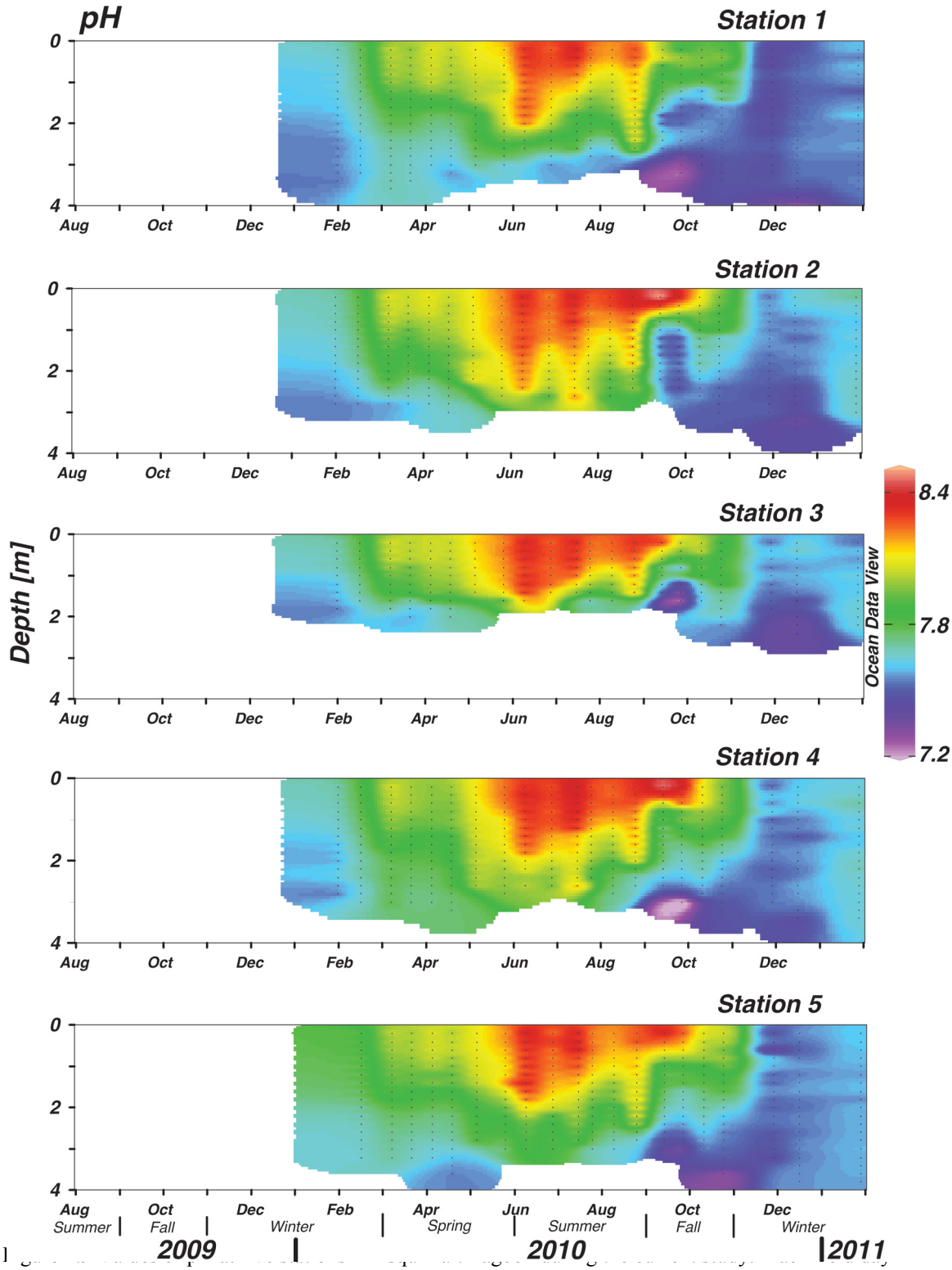
Oxygen levels at different stations were usually similar, but one notable deviation was that moderate depletion of O_2 near the bottom began as early as late June at station 1 and 5, whereas the other stations had saturations of over 100 % in bottom waters at this time (Fig. 2.8).

pH

pH measurements ranged from 7.03 to 8.60 in Esquimalt Lagoon (Fig. 2.9). pH was consistently higher near the surface and lower near the sediments. In the winter, daily average pH values were lower compared to other times of the year, particularly in November and December, with an average daily pH of 7.68. Also, changes of pH throughout the water column were small. In March, pH started to increase, particularly in surface waters, which coincided with increases in phytoplankton biomass (see section 2.4.3). This increasing trend of pH in surface waters continued throughout the spring and summer, and in May pH in the bottom water started to increase as well. The average daily pH in the spring and summer was 8.23. In the fall, bottom waters reached their minimum pH values, dropping to as low as 7.03, but the highest surface pH value (8.60) was also observed at this time. Average daily pH (8.33) was also highest in the fall.



field day (listed in Table 2.1) is represented by a vertical line of dots, with the exception of certain dates at certain stations in 2009 because of instrument malfunction. These dates are September 12, September 29, October 13, November 17, and December 17. The dots represent single measurements taken at 20 cm intervals over the depth of the water column. The disparities in depths sampled on different field days are due to fluctuating tides. Waters above the 100 % contour have saturation values > 100 % and waters within the 30 % contour have concentrations < 30 % and are thus hypoxic. Oxygen data in mg L^{-1} are included as a supplement to the electronic version of this thesis. Ticks on the x-axis represent the first day of each month.



(listed in Table 2.1) is represented by a vertical line of dots, with the exception of all dates in 2009 because of instrument malfunction. The dots represent single measurements taken at 20 cm intervals over the depth of the water column. The disparities in depths sampled on different field days are due to fluctuating tides. Ticks on the x-axis represent the first day of each month.

Nitrate

Surface water NO_3^- concentrations varied considerably during the year, but were fairly consistent among stations (Fig. 2.10A). In both 2009 and 2010, concentrations started to build up in October and remained high during the winter: the daily average NO_3^- concentration (for 2009 and 2010) was 25.57 μmol L^{-1}. After March 9, 2010, the first substantial drawdown of NO_3^- occurred, and from March 9 until May 7, intermediate values of NO_3^- were observed, with an average concentration of 10.32 μmol L^{-1}. On June 10, concentrations at all stations were depleted to the limit of detection of analysis (0.1 μmol L^{-1}) and for the remainder of the growing season during both years, concentrations remained largely exhausted, periodically reaching the limit of detection. The average NO_3^- concentration from the end of May through September in 2010 and from August through September in 2009 was 1.35 μmol L^{-1}.

Silicic acid

Surface water $Si(OH)_4$ concentrations varied considerably during the year, but as with NO_3^-, they were quite consistent among stations (Fig. 2.10B). The period of highest $Si(OH)_4$ concentrations lasted from October-November through February. Average $Si(OH)_4$ during the period of high concentrations was 52.71 μmol L^{-1}. On March 9, 2010, the first substantial drawdown of the growing season was measured. Following this, $Si(OH)_4$ continued to be periodically drawn down and partially replenished until it reached its minimum concentration on June 10, 2010. Average $Si(OH)_4$ on June 10 was 5.75 μmol L^{-1}. By the end of June, $Si(OH)_4$ had been largely replenished, and during the

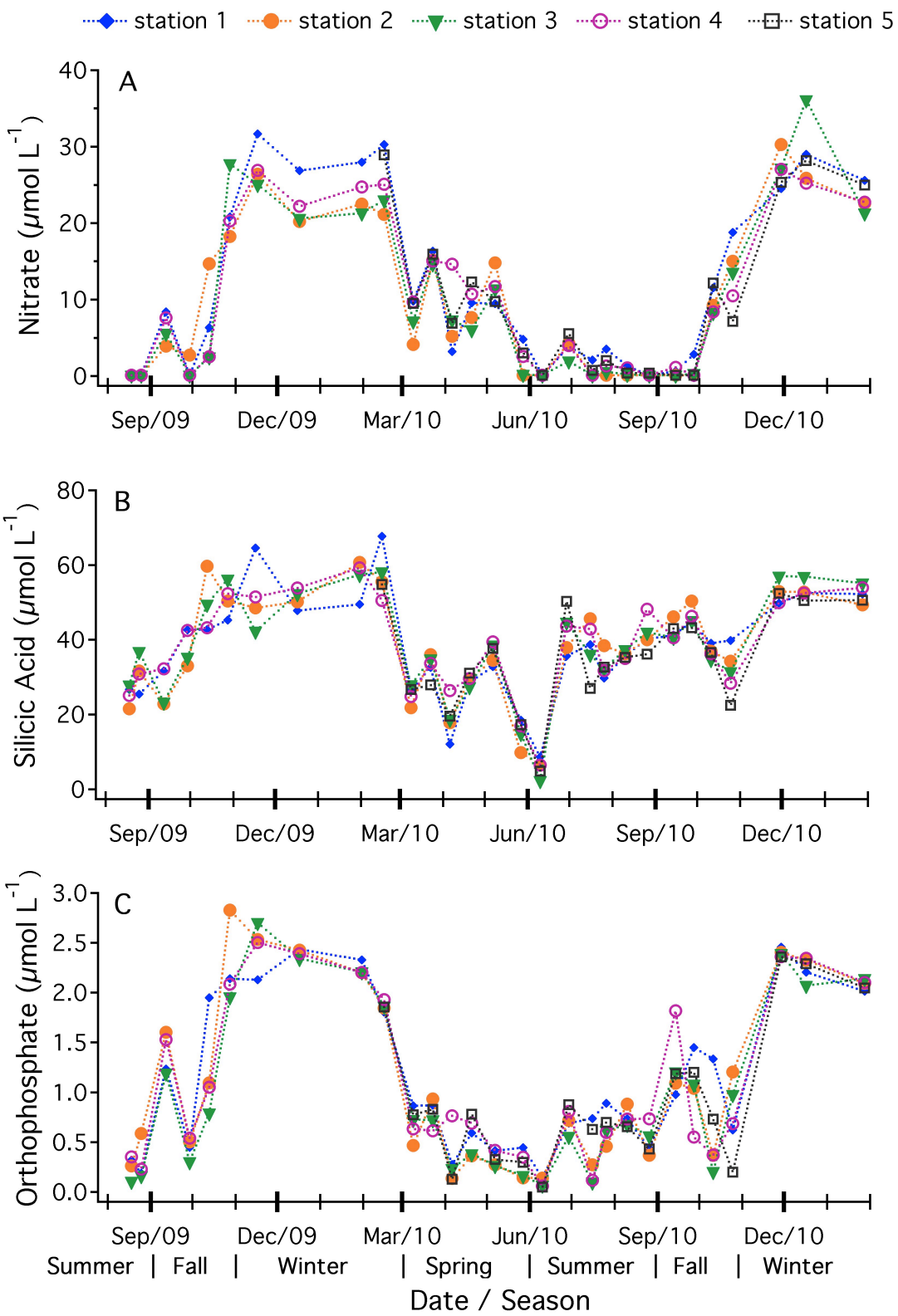


Figure 2.10 Surface water concentrations of (A) nitrate, (B) silicic acid and (C) orthophosphate in Esquimalt Lagoon during the current study. Samples were taken at five stations, 1 m below the surface on the dates listed in Table 2.1. The growing season included the spring, summer, and fall months. Ticks on the x-axis represent the first day of each month.

remaining half of the growing season, concentrations were almost as high as during the winter, with an average concentration of 36.11 μmol L^{-1}.

Orthophosphate

Surface water PO_4^{3-} concentrations were an order of magnitude lower than NO_3^- and $Si(OH)_4$. Concentrations were quite variable during the year but also consistent among stations (Fig. 2.10C). Concentrations of PO_4^{3-} were highest from the end of October-November and throughout the winter. Average PO_4^{3-} during the period of high concentrations was 2.24 μmol L^{-1}. The first substantial drawdown of PO_4^{3-} was measured on March 9, 2010, and concentrations continued in a general decreasing trend until reaching a minimum on June 10, 2010. The average concentration on this date was 0.09 μmol L^{-1}. Levels of PO_4^{3-} were partially replenished after June 10, but continued to undergo periods of drawdown until late October in 2009 and November in 2010. The average PO_4^{3-} concentration for the growing season was 0.64 μmol L^{-1}.

Ammonium

In both 2009 and 2010, surface water NH_4^+ concentrations began to build up in late October and remained high for the winter period (Fig. 2.11A). Concentrations in the winter of 2009-2010 however, were considerably higher than in the winter of 2010-2011; the average concentrations being 6.73 μmol L^{-1} and 3.88 μmol L^{-1}, respectively. Levels of NH_4^+ began to decrease in February and were substantially drawn-down by March 9, 2010. For the rest of the growing season NH_4^+ remained low, with the average concentration from March through October being 0.64 μmol L^{-1}. The lowest NH_4^+

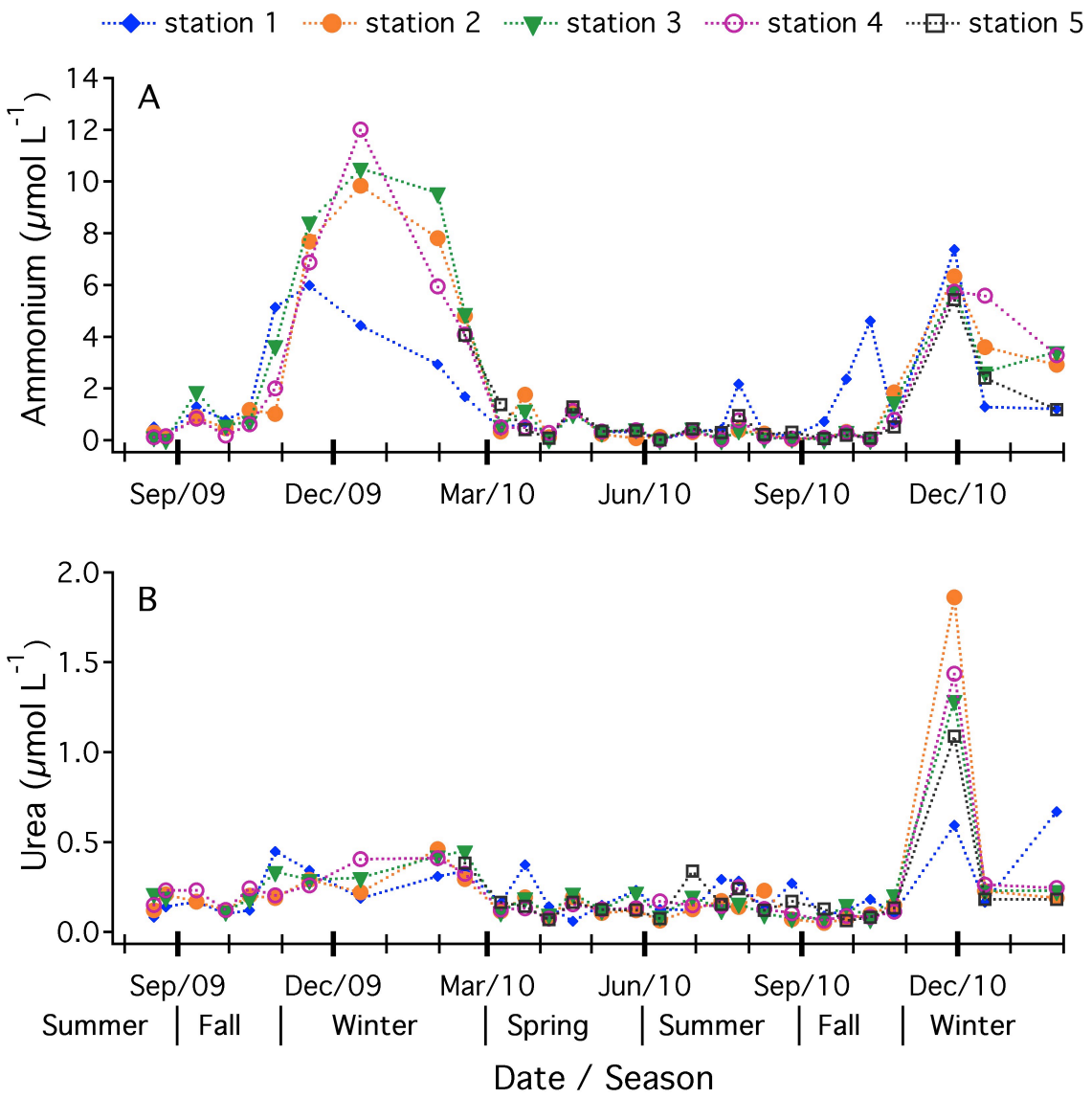


Figure 2.11 Surface water concentrations of (A) ammonium and (B) urea in Esquimalt Lagoon during the current study. Samples were taken at five stations 1 m below the surface on the dates listed in Table 2.1. The growing season included the spring, summer, and fall months. Ticks on the x-axis represent the first day of each month.

concentrations occurred on June 10, 2010. Concentrations of NH_4^+ were uniform among stations during the growing season, but became more variable when concentrations rose in the late fall and winter. Also, at times, concentrations at station 1 were distinct from those at the others stations. From December 2009 to February 2010, concentrations at

station 1 were less than half of those at the other stations, and during the growing season, station 1 occasionally exhibited spikes in NH_4^+ relative to the average of 0.64 μmol L^{-1}.

Urea

Concentrations of urea in surface waters in the winter were two orders of magnitude lower than NO_3^- (with the exception of an atypical spike in concentrations in November of 2010), and they were also quite similar among stations (Fig. 2.11B). Values were higher in the winter compared to the growing season, with average concentrations being 0.31 μmol L^{-1} and 0.15 μmol L^{-1}, respectively. The urea values from November 29, 2010 were excluded from the winter average due to the large concentrations measured at most stations. The average concentration on this date was 1.25 μmol L^{-1}.

2.4.3 Phytoplankton abundance

Total phytoplankton biomass

Phytoplankton biomass (as Chl *a* concentration) fluctuated considerably during the study period (Fig. 2.12). Daily average Chl *a* concentrations in the winter were < 3 μg L^{-1} on five of the seven winter field days, but in 2009 localized peaks in biomass occurred following the growing season on November 17 and December 17. The winter average including both 2009 and 2010 was 4.6 μg L^{-1} Chl *a*.

The first spring increase in biomass was observed on March 9, 2010. From March until late July, Chl *a* concentrations ranged from 2.2 μg L^{-1} to 14.9 μg L^{-1} (Fig. 2.12) with an average of 8.5 μg L^{-1}, and remained similar among stations. One exception to this pattern occurred on April 6, which showed the most pronounced peak in spring Chl *a*. The average Chl *a* concentration for all stations on this day was 27 μg L^{-1}.

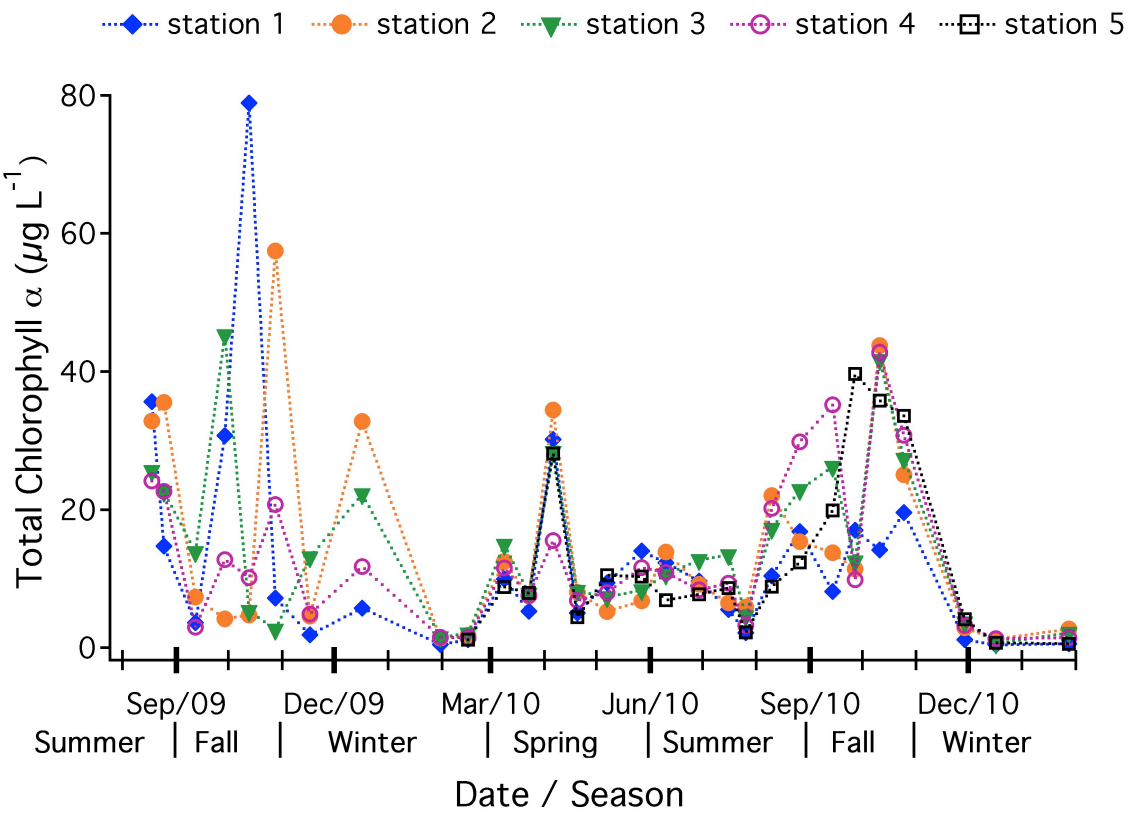


Figure 2.12 Phytoplankton biomass as chlorophyll *a* concentration (μg L^{-1}) in Esquimalt Lagoon during the current study. Samples were taken at five stations 1 m below the surface on the dates listed in Table 2.1. The growing season included the spring, summer, and fall months. Ticks on the x-axis represent the first day of each month.

In the late summer and fall (August, September, and October) in both 2009 and 2010, substantial variability emerged in the Chl *a* concentrations among stations. In 2009, Chl *a* from August through October ranged from 2.6 μg L^{-1} to 78.9 μg L^{-1} and in 2010 it ranged from 8.1 μg L^{-1} to 43.7 μg L^{-1} (Fig. 2.12). Phytoplankton biomass on average was higher at this time than during the rest of the year: the average Chl *a* concentration from August through October in 2009 and 2010 was 22.3 μg L^{-1}. The average Chl *a* concentration for all stations during the entire growing season (March through October) was 14.8 μg L^{-1}.

Size-fractionated phytoplankton biomass

The size structure of the phytoplankton assemblages varied considerably during the current study (Fig. 2.13). In general, the size structure was consistent among stations, although there were appreciable deviations on certain days. In the growing season (March through October), microphytoplankton biomass tended to be highest (> 40 % of Chl *a*). In August and September of 2009, nanophytoplankton was usually the largest fraction, and from the end of July 2010 through September 2010, Chl *a* was mostly evenly distributed among size fractions, except for on September 14, 2010 when microphytoplankton biomass was highest (Fig, 2.13). Size distribution was variable during the winter, but notably, the only days when picophytoplankton biomass was consistently high among stations occurred in the winter, in January and February of 2010 (Fig 2.13).

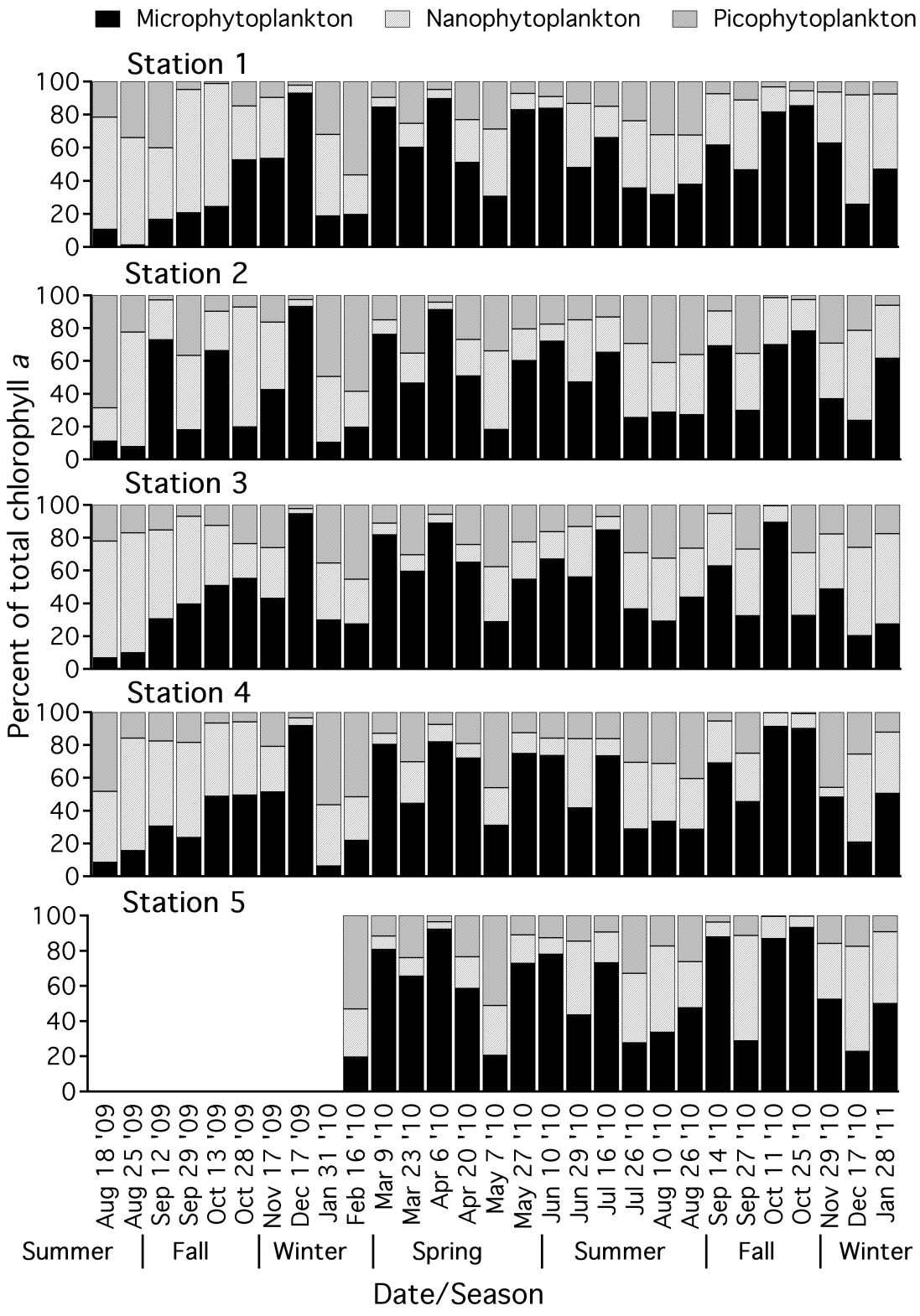


Figure 2.13 Size distribution of phytoplankton biomass in Esquimalt Lagoon during the current study. Samples were taken at five stations, 1 m below the surface. Microphytoplankton is the fraction > 20 µm in cell size, nanophytoplankton is 2 µm - 20 µm in cell size, and picophytoplankton is 0.7 µm - 2 µm in cell size. The growing season included the spring, summer, and fall months.

2.5 Discussion

2.5.1 Stratification and mixing

Stratification and mixing are opposing processes that affect the stability of the water column, and have an important influence on phytoplankton dynamics and O_2 depletion. Past descriptions of stratification in Esquimalt Lagoon by researchers at Royal Roads Military College and consulting companies have been somewhat subjective and contradictory. The lagoon has been described as "generally stratified" by Robinson and Brown (1983), "highly stratified" and "very stable" by Watanabe and Robinson (1979), and also "weakly stratified" by Westland Resource Group (1993), who reported a vertical eddy diffusion coefficient of 0.1 cm s^{-1} for the lagoon, indicative of weak mixing. Hence, in the following paragraphs I will attempt to better-characterise stratification and mixing in Esquimalt Lagoon.

Because lagoons (including Esquimalt Lagoon), often possess density gradients and circulation patterns analogous to those in estuaries, the discussion of stratification is partially rooted in estuarine theory (Postma 1969, Valle-Levinson 2010). In particular the salinity (or density) profiles in "weakly stratified or partially mixed estuaries" (Valle-Levinson 2010) are similar to the profiles observed in Esquimalt Lagoon. Valle-Levinson (2010) described these profiles as exhibiting either a weak pycnocline or "continuous stratification from surface to bottom, except near the bottom mixed layer". The latter condition applies to Esquimalt Lagoon, with density increasing gradually with depth in a concave-downward curve and with no visible pycnocline unless wind is present. I therefore propose that Esquimalt Lagoon possesses "weak gradual stratification".

Influence of salinity and temperature on stratification

In Esquimalt Lagoon, density stratification was largely dictated by salinity gradients because the density and salinity profiles were very similar, but the role of temperature in stratification should not be underestimated. Although temperature did not have a dominant affect on the overall shape of these density profiles, it either enhanced or reduced the stratification resulting from salinity gradients. When the lagoon waters began to warm in the spring, the density gradient caused by salinity was reinforced because warming further reduced surface densities. Also, warming established a temperature gradient in the deeper waters where salinity gradients were minimal; this ensured that the entire water column was stratified (see Fig. 2.5A). From the end of March to early August, daily average densities and salinities were higher than in the fall and winter months, which suggests that evaporation exceeded precipitation at that time, contributing to salinity gradients. During the winter, temperature gradients were small and thus temperature was not a strong driver of stratification, and in addition, warmer waters were located at the bottom, decreasing the density of these high-salinity waters and thus weakening the density gradient over the water column and weakening stratification. In the winter, waters below about 1 m were well-mixed, and stratification was localized at the surface where the water underwent a transition from fresher to more-saline (Fig. 2.5D). In the fall months, stratification was also localized at the surface. In summary, stratification in the spring and summer was maintained by heating and evaporation, and in the fall and winter it was maintained by freshwater inputs at the surface.

Potential influence of the 2009-2010 El Niño

In late 2009 and early 2010, the "strongest [El Niño] event of this century" occurred in the Equatorial Pacific Ocean (Department of Fisheries and Oceans Canada 2010). Given the warming influence of El Niño events on ocean temperatures, it is important to address whether or not warming from this event could have influenced the patterns of stratification in Esquimalt Lagoon.

In the JdFS, positive temperature anomalies of about 1 ºC were observed from January 2010 through April 2011 that were associated with the El Niño conditions (Department of Fisheries and Oceans Canada 2011). It is possible that the warmer lagoon temperatures in the winter of 2009 - 2010 compared to the winter of 2010 – 2011, were due to these warm waters in the JdFS. Also, warmer lagoon temperatures in September of 2009 compared to September of 2010 could have been related to higher than average warming of B.C. coastal waters in September 2009 following a shift away from the La Niña conditions that existed earlier in the year (Department of Fisheries and Oceans Canada 2010). However, even if water coming into Esquimalt Lagoon from the JdFS was 1 ºC warmer than normal, any effect that this temperature anomaly had on stratification would have depended on how JdFS water was altered during its residence in the lagoon. This is because what appears to influence stratification in Esquimalt Lagoon most strongly are local factors that control temperature and salinity gradients like precipitation and surface warming (see previous section), and wind (see following section). The variability in maximum minus minimum daily densities presented in Fig. 2.4 illustrates that the degree of density stratification is not highly predictable, given the interplay of so many contributing factors.

The stratification patterns discussed thus far are underlying conditions and can be disrupted by wind-derived mixing as presented in section 2.4.1. Almost 50 % of the field days (14 out of 29) showed water columns affected by surface wind-mixing. However, even on the windiest days of sampling, the bottom 40 cm of the water column did not mix with the upper water column, suggesting that density gradients were resilient enough to maintain isolation of the bottom waters. Although, it is possible that full mixing occurs when winds are stronger than those experienced during sampling (maximum speeds observed were ~ 30 km h^{-1}). During strong wind, density gradients increased in downwind stations and decreased in upwind stations (Fig 2.6C), meaning that stratification could be variable at different locations in the lagoon.

2.5.2 Circulation

In semi-enclosed basins such as lagoons, water quality and O_2 concentrations in bottom waters are related to patterns of circulation and water exchange, which affect residence times and flushing rates of water within the basin (Smith 1994, Zhang et al. 2010). The biological basis of O_2 depletion is discussed in Chapter 3 of this thesis, but here I will describe the circulation patterns in Esquimalt Lagoon that result from differences in density between the lagoon and the JdFS, and the strength of water circulation in Esquimalt Lagoon.

Estuarine and anti-estuarine circulation

Physical characteristics in Esquimalt Lagoon are comparable to those in the upper layers of the JdFS, but Esquimalt Lagoon is more sensitive to changes in ambient temperature and precipitation, due to its smaller volume and larger surface area to volume ratio. This assertion can be demonstrated by comparing temperature, salinity and density in the two systems (Table 2.3). Data for the JdFS comes from three mid-strait stations (Crean and Ages 1971) located near Esquimalt Lagoon.

Due to the sensitivity of physical characteristics in Esquimalt lagoon to variations in temperature and precipitation, these characteristics can differ from the JdFS in either surface or bottom waters, depending on the season. In the current study, these vertical differences led to an apparent switch in the circulation pattern from estuarine circulation in the wet fall and winter months, to anti-estuarine circulation in the dry spring and summer months.

The occurrence of anti-estuarine circulation from late March until mid August is supported by the fact that surface densities in Esquimalt Lagoon closely matched surface densities from the JdFS (top 5 m), whereas the average density of the lagoon was higher than that of the JdFS (note maximum densities in Table 2.3). These patterns could be explained if JdFS water was entering at the surface of Esquimalt Lagoon, becoming saltier and denser during its residence period due to evaporation, then sinking and exiting at depth. The suggestion of higher evaporation in the spring-summer period in Esquimalt Lagoon is supported by the high temperatures in the water column (Fig. 2.1), which were up to 5.6°C warmer than those in the JdFS (note maximum temperatures in Table 2.3).

Also, precipitation events were infrequent from March through July in the region (Fig. 2.4).

The establishment of anti-estuarine circulation in the summer is also supported by Scrimger (1960), who measured temperature variations and water circulation in the northern end of Esquimalt Lagoon. In July and August, Scrimger (1960) observed that, during flood tides, surface waters within Esquimalt Lagoon moved from the entry channel towards the southern reaches of the lagoon, and simultaneously, waters at mid depths and bottom depths moved "outwards" in the direction of the channel, but more slowly. During ebb tides, the surface waters switched directions and moved towards the channel, bottom waters moved very slowly towards the channel, and mid-depth waters moved slowly but with no consistent direction (Scrimger 1960).

Hence, it appears that in the summer, waters from JdFS are less dense and move in and out of the lagoon on flood and ebb tides (respectively). This action results in mid-depth waters moving outwards on flood tides, and bottom waters moving slowly and continually towards the mouth of the lagoon. The portion of JdFS water that remains in the lagoon longer than one flood-ebb cycle likely becomes more saline during its residence in the warm lagoon, thus increasing in density as it ages.

Unfortunately, Scrimger (1960) did not describe any winter circulation patterns, and therefore the occurrence of classical estuarine circulation in the wet months cannot be confirmed, however, it is suggested by the fall and winter density patterns in the current study. From September through December, the bottom water in Esquimalt lagoon had densities that closely matched those in JdFS surface waters (note maximum densities in Table 2.3) suggesting that these deep dense waters (below about 1 m) were "newer"

ocean waters coming in from JdFS, while fresh waters at the lagoon surface (Fig. 2.5) were exiting.

Effect of wind on circulation

Both estuarine and anti-estuarine circulation in Esquimalt Lagoon could be altered by wind-driven circulation regardless of established density gradients. The influence of wind on shallow lagoons can be dramatic, and can include vertical-mixing of the water column and transport of water masses downwind or upwind, depending on bathymetry (Groen 1969, Zeigler 1969). In the current study, southerly winds greater than ~ 10 km h^{-1} appeared to push low density surface waters to the northern end of the lagoon and increase the depth of vertical mixing at this end (see the effect of wind on density profiles described in section 2.4.1). The effect of wind could thus enhance or counteract circulation patterns and thus flushing rates, depending on whether estuarine or anti-estuarine circulation was occurring.

Tidal currents

Circulation patterns and current velocity have an impact on the water quality of lagoon systems. The limited available information on tidal current velocities in Esquimalt Lagoon indicates that they are low (Scrimger 1960).

The highest water velocity observed in Scrimger (1960)'s work was 21 cm s^{-1} (0.4 knots) at the surface on a flood tide, but values around 5 cm s^{-1} (0.1 knots) or less were more typical in the water column. More recently, the Westland Resource Group (1993) report suggested that current velocities (at an unspecified depth) were normally ~ 1 to 2 cm s^{-1}, at times reaching 10 cm s^{-1}, and up to 25 cm s^{-1} in the entrance channel, but data

were not presented in their report. For comparison, the highest surface velocities in the main channel of JdFS are ~ 100 cm s^{-1} (~ 2 knots), but they decrease to 0 cm s^{-1} during slack tides (Herlinveaux and Tully 1961).

Tidal current velocities depend on tidal ranges (Postma 1969), and the highest tidal range prediction for Esquimalt Lagoon during the period of this study was only 1.3 m (the smallest was 0.15 m). Microtidal systems like Esquimalt Lagoon have weak tidal currents, a slow rate of water exchange and a high likelihood of becoming stratified (Postma 1969). In terms of lagoons worldwide, the tidal ranges and current velocities in Esquimalt Lagoon fall on the lower end of observations (Bartoli 1996, Castel and Caumette 1996, Newton and Icely 2006, Mitchell et al. 2007, Malhadas et al. 2009, Varona-Cordero et al. 2010), but circulation is not as sluggish as in intermittently closed and open lagoons (Gale et al. 2006) or in non-tidal lagoons where currents are driven only by wind (Castel and Caumette 1996). For reference, the average tidal range near the entrance to the JdFS is ~ 3.4 m (Tully and Dodimead 1957). In the latter part of the growing season in Esquimalt Lagoon (August through October), tidal ranges (particularly during neap tides) were smaller than earlier in the summer (Fig. 2.7). Given that small tidal ranges reflect slow tidal currents and reduced flushing, tidal ranges in Esquimalt Lagoon could help to explain why O_2 concentrations dropped in the bottom waters in the late summer and fall.

2.5.3 The Influence of phytoplankton on chemical characteristics in Esquimalt Lagoon

In order to understand how water chemistry in Esquimalt Lagoon is affected by bloom dynamics occurring within its boundaries, it is necessary to contrast chemical

characteristics and phytoplankton biomass in Esquimalt Lagoon with those in the water entering from the JdFS. It is also informative to compare nutrient concentrations to those in nearby eutrophic water bodies such as the well-studied Saanich Inlet, and Sequim Bay, which is more similar in size and location to Esquimalt Lagoon than Saanich Inlet is. In the following sections I will first discuss relevant data from these nearby systems (section 2.5.3.1), and then I will compare and contrast the characteristics of phytoplankton populations and water chemistry in order to explain how they are linked (section 2.5.3.2).

2.5.3.1 Comparing Esquimalt Lagoon with the Juan de Fuca Strait and other nearby water bodies

Chemical Characteristics

Oxygen Concentrations

O_2 concentrations in the JdFS were lower and less variable than in Esquimalt Lagoon. In Crean & Ages (1971), $[O_2]$ in the JdFS ranged from 3.44 mg L^{-1} to 8.87 mg L^{-1} and in the current study, $[O_2]$ in Esquimalt Lagoon ranged from 0.64 mg L^{-1} to 19.3 mg L^{-1}. Average daily $[O_2]$s in Esquimalt Lagoon were 1.2 mg L^{-1} higher than average surface values in the JdFS (9.1 mg L^{-1} versus 7.85 mg L^{-1}, respectively), and this pattern held during all seasons. Low $[O_2]$s in Esquimalt Lagoon were only recorded near the benthos, and aside from O_2 depletion occurring in the late summer and fall, minimum daily $[O_2]$s were also higher (1.0 mg L^{-1} on average) in Esquimalt Lagoon than in the bottom waters of the JdFS. However, at the time when hypoxia was measured in the bottom waters of Esquimalt Lagoon, $[O_2]$s were ~ 3 mg L^{-1} lower than those in the bottom waters of the JdFS.

Dissolved nutrient concentrations

The peak concentrations of NO_3^-, $Si(OH)_4$, and PO_4^{3-} in Esquimalt Lagoon occurred in the winter and were comparable to, or slightly higher than, those measured in the adjacent JdFS (Lewis 1978, Masson 2006, Masson and Peña 2009, unpublished data from Peña 2011) and in Saanich Inlet (Grundle et al. 2009). During the growing season however, interesting differences emerged between these systems. In Saanich Inlet, the spring patterns of nutrient drawdown observed by Grundle *et al.* (2009) were similar to those in Esquimalt Lagoon, and nutrients were largely exhausted in both systems by May or June. However, when each nutrient is considered separately, differences in timing of replenishment are evident. In Saanich Inlet, all nutrient concentrations had increased by September, but in Esquimalt Lagoon NO_3^- was still depleted until October and was not fully replenished until November. Also, although PO_4^{3-} underwent partial replenishment after June in both systems, there continued to be occurrences of PO_4^{3-} drawdown until November in Esquimalt Lagoon. Concentrations of $Si(OH)_4$ in Esquimalt Lagoon began to build up in late June, reaching concentrations higher than 30 μmol L^{-1}, which were very similar to the summer average in JdFS (36.4 μmol L^{-1} (unpublished data from Peña 2011)). In Saanich Inlet, concentrations of $Si(OH)_4 > 30$ μmol L^{-1} were not achieved until September. In contrast to both Esquimalt Lagoon and Saanich Inlet, JdFS nutrient concentrations were only moderately lower in the growing season compared to the winter (Lewis 1978, Masson 2006, Masson and Peña 2009, unpublished data from Peña 2011), although NO_3^- can become depleted to a higher degree in Royal Roads, the shallow embayment external to Esquimalt Lagoon (Watanabe and Robinson 1979).

Ammonium concentrations

Concentrations of NH_4^+ for the JdFS have not been published, so it is not possible to unequivocally determine whether elevated concentrations of NH_4^+ in the fall and winter in Esquimalt Lagoon are associated with replenishment from the JdFS or due to autochthonous remineralisation processes and/or terrestrial inputs. Sediments are likely a source of NH_4^+ to the water column, a phenomenon that is common in shallow lagoons (Castel and Caumette 1996, Ma and Whereat 2006, Glé et al. 2008), and which was evident in Esquimalt Lagoon due to higher concentrations of NH_4^+ near the sediments than at the surface (see Appendix B). One nearby water body that is also fed by the JdFS is Sequim Bay, which is located on the northern coastline of Washington (Fig. 1.1). In Sequim Bay, Trainer *et al.* (2007) measured NH_4^+ concentrations 2 m below the surface that were 4.5 times higher than in Esquimalt Lagoon, and about 2 times greater than in the adjacent JdFS. The average NH_4^+ concnetration from June through October in Sequim Bay was 2.9 μmol L^{-1} versus 0.64 μmol L^{-1} in Esquimalt Lagoon. Sequim Bay has a number of potential nutrient sources, including a waste water treatment plant in the bay, that could be responsible for its higher NH_4^+ concentrations, but it is also possible that lower concentrations in Esquimalt Lagoon could be due to higher demands by phytoplankton. Growing season NH_4^+ concnetrations in Esquimalt Lagoon were more similar to those measured at the surface of Saanich Inlet; from April to October, NH_4^+ remained below 1.5 μmol L^{-1} in the upper 5 m of the inlet (Grundle and Juniper 2011). However, Saanich Inlet is much deeper than Esquimalt Lagoon, and it has a largely persistent zone of high NH_4^+ concnetrations (1 - 5 μmol L^{-1}) at a depth of ~ 10 m to 60 m,

which was attributed to remineralisation of phytoplankton and ammonification at depth (Grundle and Juniper 2011).

Urea concentrations

Price *et al.* (1985) reported urea concentrations ranging from 0.18 μmol L^{-1} to 0.6 μmol L^{-1} in July in the SoG, which are similar to those observed during the growing season in Esquimalt Lagoon. Published records of urea concentrations in the JdFS appear to be lacking, so it is not possible to compare urea concentrations in the lagoon relative to its ocean source-water.

Phytoplankton Biomass

Chlorophyll *a* concentrations in Esquimalt Lagoon are much higher than those in the adjacent JdFS, and they are higher than or similar to those in the SoG. In the JdFS, Chl *a* are highest in the summer, with an average concentration of 1.3 μg L^{-1} in the top 30 m over a seven year period (Masson and Peña 2009). In the SoG, Chl *a* is elevated in the spring, averaging about 4.6 μg L^{-1} (Masson and Peña 2009), but sometimes reaching concentrations above 20 μg L^{-1} (Fox and Gower 2009). In Esquimalt Lagoon, Chl *a* is elevated for the whole growing season, with the average concentration in 2010 being 14.8 μg L^{-1}, but reaching concentrations as high as 30 μg L^{-1}. These comparisons imply that phytoplankton biomass in the lagoon is largely autochthonous, and not simply brought in by tidal waters, because concentrations of Chl *a* in the JdFS are lower than in Esquimalt Lagoon.

As in Esquimalt Lagoon, Chl *a* concentrations in Saanich Inlet were elevated throughout an extended growing season, but they tended to be lower than in the lagoon, with average

concentrations being no greater 8 μg L^{-1} in the spring, summer or fall (Grundle et al. 2009).

The above comparisons of nutrients, O_2, and Chl *a* concentrations among Esquimalt Lagoon, the JdFS and other nearby water bodies enable the examination of how chemical characteristics of the water that enters Esquimalt Lagoon from the JdFS can be altered by phytoplankton bloom dynamics within the lagoon. This alteration is discussed below.

2.5.3.2 Linking phytoplankton dynamics with chemical properties of the water column in Esquimalt Lagoon

It can be argued that the major fluctuations in chemical characteristics of the water column in Esquimalt Lagoon are linked to phytoplankton dynamics.

Beginning in January of 2010, phytoplankton biomass was minimal, nutrients were high, O_2 saturation was below 100 % (values around 8 mg L^{-1}), and pH was low. Characteristics within Esquimalt Lagoon were very similar to those in its oceanic source water, the JdFS. The first spring peak in phytoplankton biomass was detected on March 9, 2010, and at this point marked changes in all chemical parameters occurred; nutrients decreased to intermediate levels, the majority of the water column was now over 100 % saturated in O_2, and pH was increasing. Such changes are to be expected because phytoplankton use nutrients for growth (Riebesell and Wolf-Galdrow 2002) and influence the carbonate system (and pH) through uptake of dissolved inorganic carbon and production of O_2 during photosynthesis (Gürel et al. 2005, Yates et al. 2007). Phytoplankton biomass rose and fell throughout the spring, and by the beginning of the summer (June 10, 2010), all nutrients with the exception of urea reached their minimum concentrations. On June 10, the water column was fully saturated in O_2 and pH was at

the highest value measured during this study. For the remainder of the growing season, from June through October, phytoplankton biomass continued to fluctuate, dissolved nitrogen forms remained low, PO_4^{3-} underwent partial replenishment (with some instances of drawdown), and $Si(OH)_4$ was replenished to levels very close to those in the JdFS. The latter suggests that the JdFS was delivering $Si(OH)_4$ to Esquimalt Lagoon, which was not being utilised to any great degree. Given the high summer concentrations of NO_3^- in the JdFS, NO_3^- supply to the lagoon should also have been high, but there was no evidence of NO_3^- replenishment in the lagoon until October. This means that phytoplankton in the late summer and fall in Esquimalt Lagoon must have been efficiently using any inputs of new NO_3^-. Recycled forms of nitrogen, NH_4^+ and urea, were persistently low throughout the growing season, suggesting that they were also being taken up readily.

The divergent patterns in NO_3^- and $Si(OH)_4$ concentrations during the latter half of the growing season (low N and high Si) in Esquimalt Lagoon suggest that autotrophic organisms other than diatoms were dominant from the end of June through September in 2010 and from August through October in 2009. Size fractionated Chl *a* data from these same periods strengthen this hypothesis because phytoplankton biomass was a mixture of picophytoplankton, nanophytoplankton, and microphytoplankton, rather than being mostly composed of microphytoplankton. Also, because O_2 saturation in the upper half of the water column became even higher (> 150 %) starting at the end of June, it is expected that vertically migrating photosynthetic flagellates (that were producing oxygen through photosynthesis) may have been present. Further evidence to support these hypotheses will be provided in Chapter 3.

Decreasing O_2 levels near the sediments in Esquimalt Lagoon in August and September (of 2009 and 2010) were probably related to bacterial decomposition of the high-biomass blooms that occurred at this time. The circumstances leading to O_2 depletion will be addressed in Chapter 3.

A different situation occurred in Saanich Inlet, where $Si(OH)_4$ was not fully replenished until September (as opposed to late June in Esquimalt Lagoon) and fluctuations in NO_3^- and $Si(OH)_4$ were quite similar throughout the growing season (Grundle et al. 2009), which can be indicative of diatom growth (Brzezinski 1985, Dugdale et al. 1995). Furthermore, data from Grundle's M.Sc. thesis (referenced in Grundle et al. 2009), documented that phytoplankton biomass was dominated by diatoms during the study period in Saanich Inlet, even in the latter part of the season. Therefore, summer phytoplankton assemblages in Saanich Inlet in 2005 and 2006 were likely different than those during the current study in Esquimalt Lagoon.

By November, phytoplankton biomass in Esquimalt Lagoon had decreased, and nutrients, O_2 and pH values had returned to winter levels, which were similar to those in the JdFS (Crean and Ages 1971, Lewis 1978, Masson 2006, Masson and Peña 2009, unpublished data from Peña 2011).

2.6 Conclusions

Chemical and physical properties in Esquimalt Lagoon are largely influenced by its oceanic source water, the JdFS. However, because the narrow entrance channel to the lagoon restricts tidal movement of water in and out of its shallow basin, properties of the ocean water are altered during their residence in the lagoon. Water temperatures,

salinities, densities, and thus patterns of circulation, stratification and mixing are influenced by freshwater inputs from land, ambient temperatures and wind. In the spring/summer period, weak gradual stratification is maintained by salinity and temperature gradients, and in the fall/winter period, stratification is dependent on surface freshwater inputs that create salinity gradients. However, at any time of the year, this gradual stratification can be disrupted during wind events that mix the upper water column and create a pycnocline. Patterns of lagoon circulation also appear to change in fall/winter versus spring/summer, with classic estuarine circulation likely occurring in the former and anti-estuarine circulation occurring during the latter.

Chemical characteristics of the water, most notably dissolved nutrient and O_2 concentrations, and pH, are altered by biological processes occurring within the boundaries of the lagoon. Activity by phytoplankton during the growing season depletes nutrients relative to JdFS concentrations. Dissolved N forms, $Si(OH)_4$ and PO_4^{3-} are largely exhausted by diatom growth in the spring, and although N forms are held at very low levels until the end of the growing season, $Si(OH)_4$ and PO_4^{3-} are partially replenished during the summer and early fall. The reasons for this discrepancy in nutrient concentrations will be explored in the next chapter, but may be due to a decline in diatom dominance in the phytoplankton assemblages. Also, patterns of nutrient replenishment are different in Esquimalt Lagoon and Saanich Inlet, which suggests that phytoplankton succession may differ in the two water bodies. O_2 and pH in Esquimalt Lagoon respond to the opposing processes of photosynthesis and respiration. Towards the end of the growing season, O_2 levels are super-saturated near the surface where photosynthesis is occurring, but near the sediments O_2 approaches hypoxic levels most

likely due to a combination of increased O_2 demand by bacterial respiration and low flushing rate.

Chapter 3: Phytoplankton ecophysiology and harmful algal blooms in Esquimalt Lagoon

3.1 Introduction

3.1.1 The ecophysiology of diatoms and photosynthetic flagellates

Diatoms and photosynthetic flagellates are two widespread and distinct groups of phytoplankton. Diatoms have a high requirement for silicic acid ($Si(OH)_4$), which they use for the formation of their silica cell wall (Sarthou et al. 2005). In contrast, photosynthetic flagellates, which include mostly members of the dinoflagellates and also groups such as raphidophytes, do not have a high silicon requirement and can swim with the help of their flagella. In addition, many photosynthetic flagellates have mixotrophic nutrition (Burkholder et al. 2006). Mixotrophy is a combination of autotrophy and heterotrophy i.e. the use of inorganic or organic nutrients, respectively, to provide chemical energy for growth. Organic nutrients can be either particulate or dissolved.

The seasonal succession of phytoplankton in coastal upwelling systems, including those on the west coast of Canada, often involves diatom blooms in the spring followed by blooms of photosynthetic flagellates in summer or fall (Taylor and Harrison 2002, Smayda and Trainer 2010). These two groups have distinct physiological and anatomical characteristics that give them competitive advantages under different environmental conditions, which contribute to such patterns of succession. For instance, although diatoms and photosynthetic flagellates can use the same forms of inorganic nutrients for growth, diatoms have a much higher affinity for inorganic nutrients and have higher maximum growth rates than dinoflagellates (Sarthou et al. 2005, Litchman et al. 2007). Thus, diatoms are often dominant in systems where inorganic nutrients are abundant

(Sarthou et al. 2005, Litchman et al. 2007). In contrast, blooms of photosynthetic flagellates can often be detected when waters are nitrogen-limited and have low ratios of dissolved inorganic nitrogen to phosphorous, and when organic nutrients are available (Heisler et al. 2008, Burkholder et al. 2008).

In systems where concentrations of inorganic nitrogen are low and those of organic nitrogen are high, the growth of mixotrophic phytoplankton, which include many photosynthetic flagellates, can be favoured (Bronk et al. 2006). Mixotrophy is considered an important feeding strategy responsible for supporting coastal phytoplankton blooms of high biomass and duration (Bronk et al. 2006, Heisler et al. 2008).

The fact that photosynthetic flagellates can swim gives them a competitive advantage in stratified waters. This is because they can actively seek dissolved nutrients below the nutricline or high light levels near the surface to drive photosynthesis (Smayda 1997, Burkholder et al. 2006). As a result, photosynthetic flagellates may thrive when inorganic nutrients are depleted and the water column is stable and stratified, whereas diatoms may thrive when the water column is well mixed and inorganic nutrients are abundant.

The ecophysiology of photosynthetic flagellates is of interest on the B.C. coast because these phytoplankton are responsible for a number of reoccuring HABs (Taylor and Harrison 2002). Dinoflagellates of the genus *Alexandrium* dominate many of these HABs, but members of the dinoflagellate genera *Dinophysis* and *Prorocentrum* also create HABs (Taylor and Harrison 2002), as well as the species *Akashiwo sanguinea.* The raphidophyte *Heterosigma akashiwo* also forms extensive reoccurring blooms in

B.C. coastal waters that can be harmful to fish in aquaculture operations and possibly to wild salmon (Taylor and Harrison 2002, Rensel et al. 2010).

3.1.2 Feeding strategies in the photosynthetic flagellates *Akashiwo sanguinea* and *Heterosigma akashiwo*

Both *Akashiwo sanguinea* (K. Hirasaka) G. Hansen & Ø. Moestrup (syn. *Gymnodinium sanguineum, Gymnodinium splendens, Gymnodinium nelsonii*) and *Heterosigma akashiwo* (Y. Hada) Y. Hada ex Y. Hara & M. Chihara (*syn. Entomosigma akashiwo*) have been documented in Esquimalt Lagoon previous to the current study. *A. sanguinea* has formed reoccurring "red tides" in lagoon since at least the 1970s (Robinson and Brown 1983, Haigh 2008) and *H. akashiwo* has been present in low numbers (Waters et al. 1992). Both of these species are adapted to neritic coastal environments (Litchman et al. 2007, Kudela et al. 2008) and utilize dissolved inorganic nitrogen in the form of NO_3^- and NH_4^+, as well as dissolved organic nitrogen in the form of urea (Levasseur et al. 1993, Herndon and Cochlan 2007, Kudela et al. 2008). These species can also use other forms of dissolved organic matter (DOM). *A. sanguinea* has the capability to use proteins after breaking them down enzymatically at the cell surface (Stoecker and Gustafson 2003) and *H. akashiwo* is able to use nitrogen derived from refractory humic substances, although the latter may be facilitated by some degree of bacterial decomposition before uptake (Bronk et al. 2006, See et al. 2006). Both species can also consume particulates. *A. sanguinea* populations from Chesapeake Bay can obtain 11.6 % of their body carbon and 18.5 % of their body nitrogen daily by feeding on nanociliates (Bockstahler and Coats 1993) and can also consume cryptomonads (Li et al. 1996) and small to mid-sized phytoplankton (up to ~ 20 μm diameter), including *H. akashiwo* cells

(Jeong et al. 2005). The flagellate *H. akashiwo* has the ability to feed on single-celled cyanobacteria and heterotrophic bacteria, with ingestion rates of up to ~ 4 cells h^{-1} and ~12 cells h^{-1}, respectively (Seong et al. 2006, Jeong et al. 2010a).

Given their mixotrophic capabilities, it is important to take into account alternative nutrient sources when investigating blooms of photosynthetic flagellates like *A. sanguinea and H. akashiwo*, particularly when they occur under low ambient concentrations of inorganic nutrients.

3.1.3 Photosynthetic flagellates and harmful algal blooms

"Red tides" like those formed by *A. sanguinea* and *H. akashiwo* often have harmful effects on other organisms or coastal ecosystems, and are thus considered to be HABs. HABs (regardless of coloration) are often dominated by photosynthetic flagellates, which can release potent toxins (in the case of numerous dinoflagellate taxa) (Burkholder et al. 2006), or form intense blooms that lead to O_2 depletion when they are decomposed by bacteria. A recent review of the HAB literature by Burkholder *et al.* (2008) revealed that in eutrophic coastal systems, mixotrophy is a ubiquitous trait in harmful algal species, many of which are photosynthetic flagellates. Thus, high concentrations of DOM characteristic of eutrophic systems are increasingly implicated in the sustenance of HABs dominated by photosynthetic flagellates (Bronk et al. 2006, Burkholder et al. 2008).

Another factor that can favour HABs dominated by photosynthetic flagellates is stratification in the water column (Dale et al. 2006, Zhang et al. 2010). Stratified systems are particularly vulnerable to HABs related to O_2 depletion, because stratification prevents renewal of O_2 in the bottom waters (Zhang et al. 2010). So, respiration of phytoplankton detritus in the benthic environment can reduce O_2 to hypoxic levels.

Therefore, the type of water body that would favour the development of flagellate-dominated HABs would be eutrophic and have a stratified water column in which nutrient pools may be best accessed by swimming. Many lagoon ecosystems fit this description well. Firstly, coastal lagoons tend to have elevated nutrient inputs that are concentrated within the lagoon boundaries for a period of time, allowing phytoplankton blooms to develop (Kjerfve 1994, Gamito et al. 2005), and secondly, water quality can differ horizontally (Phleger 1969, Smith 1994) and vertically in lagoons and the water columns are often stratified (Groen 1969, Valle-Levinson 2010). In fact, episodes of benthic hypoxia and even anoxia are common in lagoon ecosystems (Bartoli 1996, Newton et al. 2003, Gouze et al. 2008, Fonseca and Braga 2006, Viaroli et al. 2008, Drake et al. 2010, Pereira et al. 2010), and this study will link such events to HABs dominated by photosynthetic flagellates in a local lagoon.

3.1.4 Study site: Esquimalt Lagoon

Esquimalt lagoon (which is described in detail in Chapter 1) is a unique local case study in which physical, chemical and biological factors that contribute to the development of HABs can be examined.

3.1.5 Objectives

The objectives of this chapter are to (1) determine what environmental factors lead to the succession of phytoplankton assemblages from those dominated by diatoms to those dominated by photosynthetic flagellates, and (2) explore how this shift in phytoplankton groups in combination with seasonal changes in the physicochemical properties of the water column may induce events of O_2 depletion in the late summer and early fall.

3.2 Methods

3.2.1 Water sampling procedure

Samples from Esquimalt Lagoon were collected twice per month during the growing season (March through October) and once per month during the winter (November through February), from August 2009 through January 2011. This sampling plan yielded 29 field days, which are listed in Table 2.1. Although samples for Chapter 2 were collected at five stations, nutrient uptake experiments were only performed at station 4 (Figure 1.1), and thus this chapter will only refer to data collected at station 4. This location was chosen because it is situated in a deep area of the lagoon that is distal to the channel that connects the lagoon to the JdFS. This station also has no submerged vegetation and was historically sampled by researchers at Royal Roads Military College. The coordinates of station 4 are recorded in Appendix A.

Sampling occurred via canoe and was finished before local noon. Samples were collected in a 2 L Niskin bottle from 1 to 1.5 m below the surface. After collection, water was transferred into 2 L polypropylene bottles that had been acid-washed and triple-rinsed with double-deionized water and *in situ* lagoon water. These bottles were kept on ice in the dark until they were sub-sampled in the laboratory on the same day as collection. Sub-samples were preserved and analyzed according to procedures outlined in the sections below.

3.2.2 Physical, chemical and biological measurements

Sub-sampling from the 2 L water sample collected at station 4 is explained in sections 2.3.1 and 2.3.2. Measurement of temperature, salinity and O_2, calculation of density, and analysis of NO_3^-, NH_4^+, urea, $Si(OH)_4$, PO_4^{3-}, and Chl *a*, are explained in section 2.3.2.

Nutrient ratios

Ratios of N:P, Si:P and Si:N were calculated for dissolved nutrients. The nitrogen concentrations used in these calculations consisted of the sum of NH_4^+, NO_3^- and 2 x urea concentrations (urea has two molecules of nitrogen). This sum is referred to as "total dissolved N" in this document.

Phytoplankton biomass, as chlorophyll *a* concentrations

Sub-samples for measurement of total Chl *a* and size-fractionated Chl *a* were drawn from the 2 L water sample collected at station 4 and analyzed according to procedures explained in section 2.3.2.

Tidal height predictions

Predicted tidal data was obtained from the Canadian Hydrographic Service Pacific Region (2011), as described in section 2.3.2.

Phytoplankton identification

Phytoplankton samples were preserved with unbuffered Lugol's iodine and imaged with a FlowCAM® in autoimage mode. Water samples were divided into winter samples

(collected November through February) and samples from the growing season (March through October). Samples from the growing season were further categorized based on whether the phytoplankton assemblage was dominated by diatoms, photosynthetic flagellates, or mixed phytoplankton groups. Two samples from growing season (September 12, 2009 and July 26, 2010) were dominated by non-photosynthetic ciliates and had low Chl *a* concentrations, so these samples were not assigned to a category. Identification was performed using FlowCam images and was verified at higher magnifications (usually 400 x) with an Olympus IX71 inverted microscope. In some cases, size fractionated Chl *a* concentrations aided in the determination of the dominant group. Cell counts and volumes were calculated using VisualSpreadsheet© particle analysis software and were also used in the categorisation of water samples.

A sample was considered to be dominated by either diatoms or photosynthetic flagellates when the following conditions were met:

1) the cell type represented ≥ 30 % of the total phytoplankton cell volume

2) the opposing cell type (either diatoms or photosynthetic flagellates) was less than 15 % of total cell volume

A sample was considered to be composed of mixed assemblages when diatoms, dinoflagellates and filamentous cyanobacteria were present, but neither diatoms nor photosynthetic flagellate cell volume was > 30 %.

Nutrient uptake rate experiments

After returning to the laboratory, water for uptake rate experiments was transferred into nine 250 mL polycarbonate bottles. Bottles were spiked with $^{15}NH_4Cl$, $^{15}NH_2CO^{15}NH_2$ (urea), or both $Na^{15}NO_3$ and $NaH^{13}CO_3$ (sodium bicarbonate) in triplicate. Enriched

isotope salts were obtained from Cambridge Isotope Laboratories, Inc. and contained at least 99 % of the heavy isotope. Sample enrichment was performed at ≤ 10 % of ambient nutrient concentrations by adding nutrients to the incubation bottles at the following concentrations: 0.1 μmol L^{-1} for $^{15}NO_3^-$ and $^{15}NH_4^+$, 0.2 μmol L^{-1} for ^{15}N-urea, and 160 μmol L^{-1} for $H^{13}CO_3^-$. However, when an ambient nutrient concentration was below the limit of detection, enrichment was > 10 %. Samples were incubated for ~ 4.5 hours at the temperature measured in the lagoon at 1 m and at an optimal irradiance of 180 μmol photon m^{-2} s^{-1}. At the end of the incubation period, samples were filtered under vacuum at 5 mmHg onto pre-combusted 0.7 μm glass fiber filters and oven-dried at 60 °C for three days. Dried samples were stored in a dessicator until analysis at the University of California Davis Stable Isotope Facility. Isotopic composition and particulate carbon and nitrogen (POC and PON) content were measured using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (IRMS).

Both absolute and specific uptake rates were calculated. The absolute uptake rate of a nutrient describes the amount of nutrient taken up by all of the biomass present, in a given time, but specific uptake rate of a nutrient is normalized to the mass of C or N (for C uptake and N uptake respectively).

Absolute and specific uptake rates of ^{15}N (ρ and V_c respectively) for each nitrogen form, as well as V_c for ^{13}C, were calculated based on equation 3 and equation 6 in Dugdale & Wilkerson (1986), as presented below. Note that absolute uptake is referred to as "transport" in the referenced paper. Absolute uptake rates of ^{13}C, or "photosynthetic rate" (P*) were calculated according to equation 4 in Hama *et al.* (1983), as presented below.

Ambient dissolved inorganic carbon (DIC) concentrations are required for the calculation of carbon uptake rates. DIC was estimated according to methods in Crawford & Harrison (1997) that use pH and alkalinity values. Alkalinity values were estimated from salinity based on a mathematical relationship established for California coastal waters (Gray et al. 2011).

Absolute uptake of nitrogen, ρ, equation 3 in Dugdale & Wilkerson (1986)

$$\rho = \frac{^{15}N_{XS}}{(^{15}N_{enr} - F) \times T} \times \text{PON}$$

Where "$^{15}N_{xs}$" is the "excess" ^{15}N incorporated into particulates during the incubation as calculated by $^{15}N_s - F$, "$^{15}N_s$" is the atom percent of ^{15}N in the particulates after incubation with the enriched target N form, "F" is the natural atom percent of dissolved ^{15}N, "$^{15}N_{enr}$" is the atom percent of dissolved ^{15}N (of the target N form) in the water sample after it was spiked with enriched N, "PON" is the concentration of PON back-calculated from the PON measured after the incubation, and "T" is the incubation time.

Specific uptake, V_c, equation 6 in Dugdale & Wilkerson (1986)

$$V_c = \frac{1}{T} \times \ln \frac{(^{15}N_{enr} - F)}{(^{15}N_{enr} - {}^{15}N_s)}$$

Symbols are defined above for the equation that calculates absolute uptake of N.

Absolute uptake of DIC, P, equation 4 in Hama et al. (1983)*

$$P^* = \frac{C\,(a_{is} - a_{ns})}{t\,(a_{ic} - a_{ns})}\,f$$

Where "C" is the concentration of POC in the particulates of the incubated sample, "a_{is}" is the atom percent of ^{13}C in particulates of the incubated sample, "a_{ns}" is the atom percent of ^{13}C in the particulates of a natural sample, "a_{ic}" is the atom percent of ^{13}C in DIC after the water sample is spiked with enriched C, "t" is the incubation time, and "f" is a discrimination factor of 1.025.

Concentrations of particulate organic carbon and nitrogen

POC and PON concentrations were measured on the same 9 filters where isotopic composition was measured. As these filters were analysed after incubations were performed (see section "Nutrient uptake rate experiments" above), they were corrected for N and C accumulated during the incubation. In addition, two samples for ambient PON and POC were collected, analysed, and combined with the corrected values. Thus, POC and PON concentrations presented in this thesis represent averages of 11 sub-samples for each field day.

3.2.3 Data analysis

When describing seasonal trends, "winter" months were considered to be November, December, January, and February, "spring" months were considered to be March, April, and May, "summer" months were June, July, and August and "fall" months were

September and October. The growing season was March through October (spring, summer, and fall).

For the purpose of determining if phytoplankton physiology differed among phytoplankton assemblages, nutrient uptake, Chl *a*, PON, and POC data were grouped according to days dominated by diatoms (n = 10) or those dominated by photosynthetic flagellates (n = 9). Data from field days in the winter, field days with mixed assemblages of phytoplankton, and field days when heterotrophic ciliates dominated the plankton, were not included in these groups (10 field days in total). Within groups of photosynthetic flagellates and diatoms, means were computed and compared and regressions linking relevant variables were performed. Linear regressions and t-tests were performed in Microsoft® Excel® 2008 for Mac.

3.3 Results

3.3.1 Physicochemical characteristics of the water column

Gradients in temperature, salinity, and density

Vertical gradients in temperature, salinity, and density were present throughout most of the year in Esquimalt Lagoon (Fig. 3.1A, B, and C), indicating that the water column was stratified to some degree during all seasons. Temperature gradients were stronger in the summer than the winter due to surface warming (Fig. 3.1A), but salinity gradients were stronger in the winter than in the summer due to freshwater inputs at the surface (Fig. 3.1B). Stratification was generally weak to moderate as indicated by typical vertical

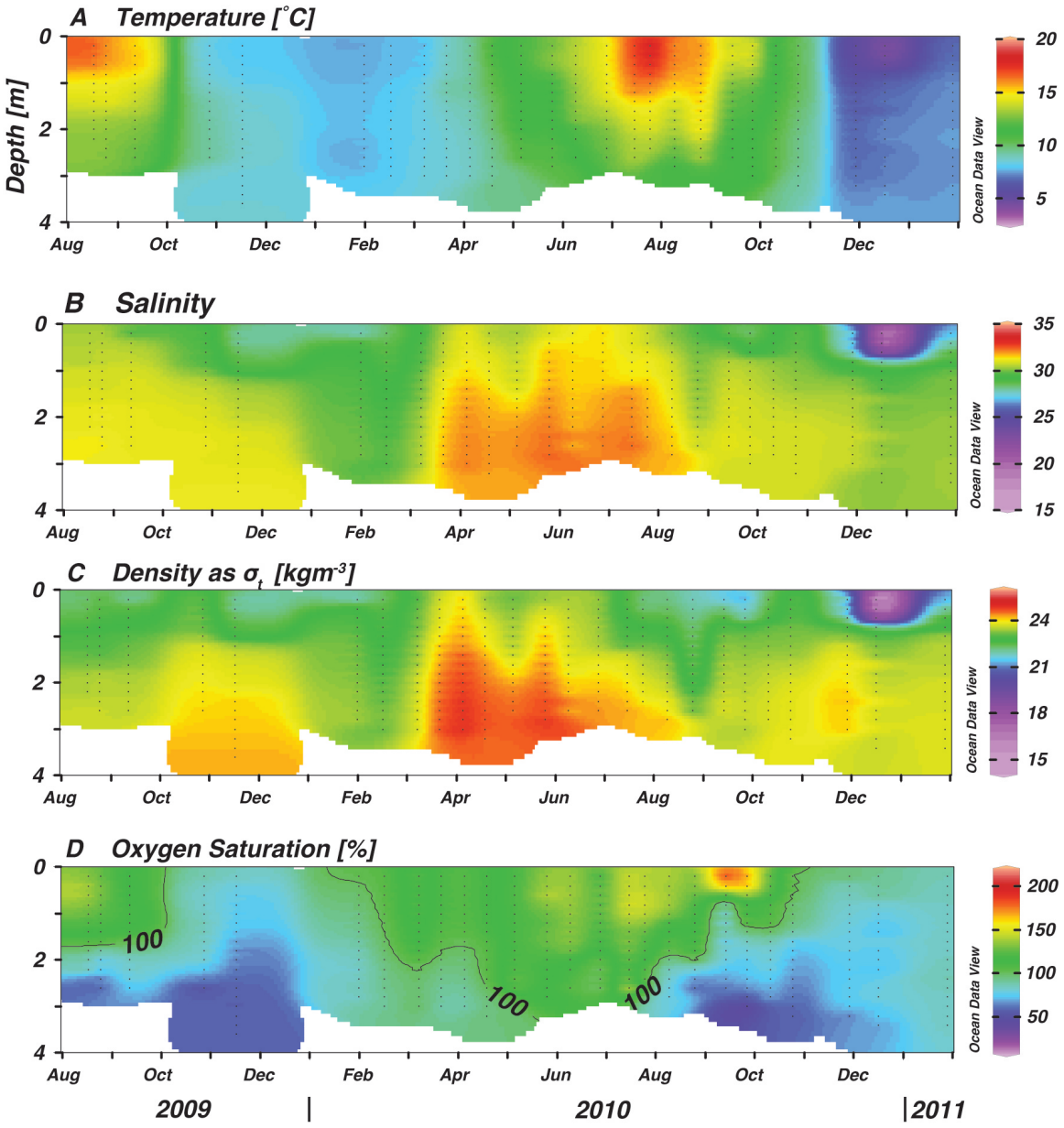


Figure 3.1 Vertical variations of physicochemical properties at station 4 during the current study: (A) temperature, (B) salinity, (C) density (σ_t), and (D) percent oxygen saturation; the 100 % contour line delineates waters with oxygen concentrations exceeding physical saturation at *in situ* temperatures. These panels were presented previously in Figs. 2.1, 2.3, 2.5, and 2.8, respectively. Each field day is represented by a vertical line of dots, with the exception of certain dates in 2009 when the instrument was not operational. The dots represent measurements taken at 20 cm intervals over the depth of the water column. The disparities in depths sampled on different field days are due to fluctuating tides. Winter months are Jan - Feb, spring months are Mar – May, summer months are Jun – Aug, and fall months are Oct – Sep. Ticks on the x-axis represent the first day of each month.

density differences ~ 2 kg m^{-3} (Fig. 2.4) and a gradual increase of density with depth (Fig. 3C). The upper water column became mixed on windy days. Chapter 2 presents a more detailed description of patterns of mixing and stratification at 5 locations (including station 4) in Esquimalt Lagoon, and the data from station 4 represented in Fig. 3.1 were drawn from Chapter 2, but repeated here for more direct access and comparison between parameters.

Oxygen levels

The minimum O_2 concentration measured at station 4 in Esquimalt Lagoon was 3.41 mg L^{-1}, and the maximum concentration was 17.11 mg L^{-1}, corresponding to 38 % saturation and 196 % saturation respectively. O_2 levels in the water column were high for the majority of the growing season in 2010 (Fig. 3.1D). From March through August, much of the water column had an O_2 saturation of greater than 100 %, and saturation remained above 50 % at all depths. During August however, O_2 began to decrease in the bottom waters and reached its minimum in late September. In September, hypoxia (O_2 saturation < 30 %) in the bottom waters was observed in localized areas of the lagoon (see station 2 and 3 in Fig. 2.8). Hypoxia was also observed in August of 2009. Maximum O_2 concentrations in surface waters occurred at the same time as minimum concentrations were measured near the benthos. In the winter, O_2 levels remained moderate to high throughout the water column, but were lower than during the remainder of the year (Fig. 3.1D).

Tidal height predictions, precipitation and wind

During the current study, predicted tidal ranges were between 0.15 m and 1.3 m. This means that Esquimalt Lagoon can be classified as a "microtidal system" (tidal range < 2 m). During the 2010 growing season, tidal ranges (particularly during neap tides) were smaller in August, September, and October, than in June, July, and August (Fig. 2.7).

Precipitation and wind data are presented in Figure 2.4 of Chapter 2.

3.3.2 Phytoplankton biomass and composition

During the winters (November through February) of 2009 and 2010 phytoplankton biomass was low (< 5 μg L^{-1}) except for a high value of 12 μg L^{-1} on December 17, 2009 (Figs. 3.2A and 3.3A). Winter phytoplankton biomass was composed of a mixture of picophytoplankton, nanophytoplankton and microphytoplankton, but microphytoplankton was less than 50 % in all cases except for December 17, 2009. Phytoplankton biomass in the growing season was usually dominated by microphytoplankton (Fig. 3.2B). During the spring (March through May) and early summer (early June) assemblages were composed of mixtures of diatoms (microphytoplankton), predominantly chain-forming *Thalassiosira* spp., which was replaced by *Skeletonema* spp. and then *Chaetoceros* spp. (Fig. 3.2C; Table 3.1). For most of the diatom-dominated period (March 9 to June 10) in 2010, phytoplankton biomass was at intermediate levels (< 13 μg L^{-1}, which is the average value in the lagoon for the entire period of this study). From June 10 until the end of July, mixed phytoplankton assemblages and filamentous cyanobacteria were present (Fig. 3.2C; Table 3.1). In August and September photosynthetic flagellates dominated the phytoplankton assemblages, but different species were present in 2009 and

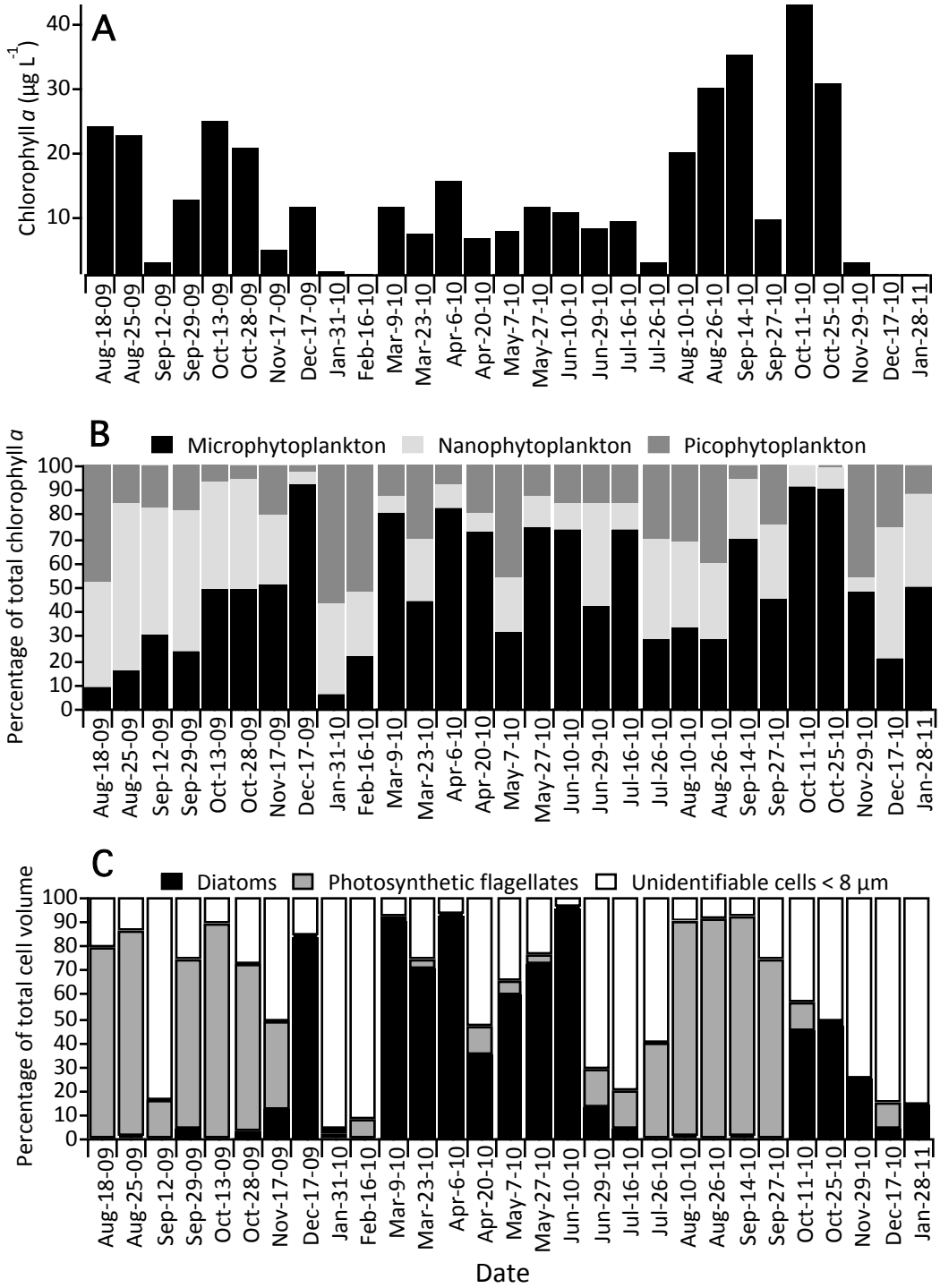


Figure 3.2 Temporal variations in phytoplankton biomass and composition at station 4 in Esquimalt Lagoon; (A) Total chlorophyll *a* concentrations (> 0.7 µm), (B) percentage of total chlorophyll *a* from each of the following size fractions: microphytoplankton (cells with diameter > 20 µm), nanophytoplankton (2-20 µm diameter), and picophytoplankton (0.7-2 µm diameter), and (C) percentage of total cell volume attributable to diatoms, photosynthetic flagellates, or unidentifiable cells < 8 µm in diameter, as determined by microscopy and from FlowCam images. Data from panels A and B were presented previously in Figs. 2.12 and 2.13, respectively. Note that dates are spaced "categorically" along the x axis, not along a continuous timescale. Winter months are Jan - Feb, spring months are Mar – May, summer months are Jun – Aug, and fall months are Oct – Sep.

Table 3.1 Dominant phytoplankton species or groups during the current study. The column "Category" displays the classification that was assigned for each field day. Water samples were first assigned a category of either "winter" (W) or "growing season" and then depending on the dominant type of phytoplankton, samples from the growing season were categorized as either photosynthetic flagellates (F), diatoms (D), or mixed phytoplankton (M). N/A indicates the days during the growing season with low phytoplankton biomass (≤ 3 μmol L^{-1}) and dominance of small zooplankton (ciliates and rotifers) based on FlowCAM images and microscopy. When the dominant species or groups are described as microphytoplankton, nanophytoplankton or picophytoplankton, size-fractionated chlorophyll *a* data was used for the determination of dominance. Microphytoplankton are > 20 μm in cell size, nanophytoplankton are 2 μm - 20 μm in cell size, and picophytoplankton are 0.7 μm - 2 μm in cell size.

Date	**Dominant species or group**	**Chl a (μg L^{-1})**	**Category**
August 18, 2009	*Heterosigma akashiwo*	24	F
August 25, 2009	*Heterosigma akashiwo* and mixed dinoflagellates	23	F
September 12, 2009	Ciliates, rotifers, and nanophytoplankton	3	N/A
September 29, 2009	*Akashiwo sanguinea*	13	F
October 13, 2009	*Akashiwo sanguinea*	10	F
October 28, 2009	*Akashiwo sanguinea*	21	F
November 17, 2009	Nanophytoplankton	5	W
December 17, 2009	*Thalassiosira spp.*	12	D
January 31, 2010	Picophytoplankton	2	W
February 16, 2010	Picophytoplankton,	1	W
March 9, 2010	Mix of chain diatoms, *Thalassiosira spp.* dominant	12	D
March 23, 2010	Mix of chain diatoms, *Skeletonema spp.* dominant	8	D
April 6, 2010	*Skeletonema spp.*	16	D
April 20, 2010	*Skeletonema spp.*	7	D
May 7, 2010	*Chaetoceros spp.*	8	D
May 27, 2010	Mix of chain diatoms	12	D
June 10, 2010	Mix of chain diatoms,, *Chaetoceros spp.* dominant	11	D
June 29, 2010	Mix of ciliates, *Chaetoceros spp*., dinoflagellates, and filamentous cyanobacteria	8	M
July 16, 2010	Filamentous cyanobacteria, small chain diatoms	9	M
July 26, 2010	Ciliates, nanophytoplankton, picophytoplankton, and mixed dinoflagellates including *Akashiwo sanguinea,*	3	N/A
August 10, 2010	*Akashiwo sanguinea*	20	F
August 26, 2010	*Akashiwo sanguinea*	30	F
September 14, 2010	*Akashiwo sanguinea*	35	F
September 27, 2010	*Akashiwo sanguinea*	10	F
October 11, 2010	*Thalassiosira spp.*	43	F
October 25, 2010	*Thalassiosira spp.* and *Skeletonema spp.*	31	F
November 29, 2010	Microphytoplankton and picophytoplankton	3	W
December 17, 2010	Nanophytoplankton	1	W
January 28, 2011	Microphytoplankton	1	W

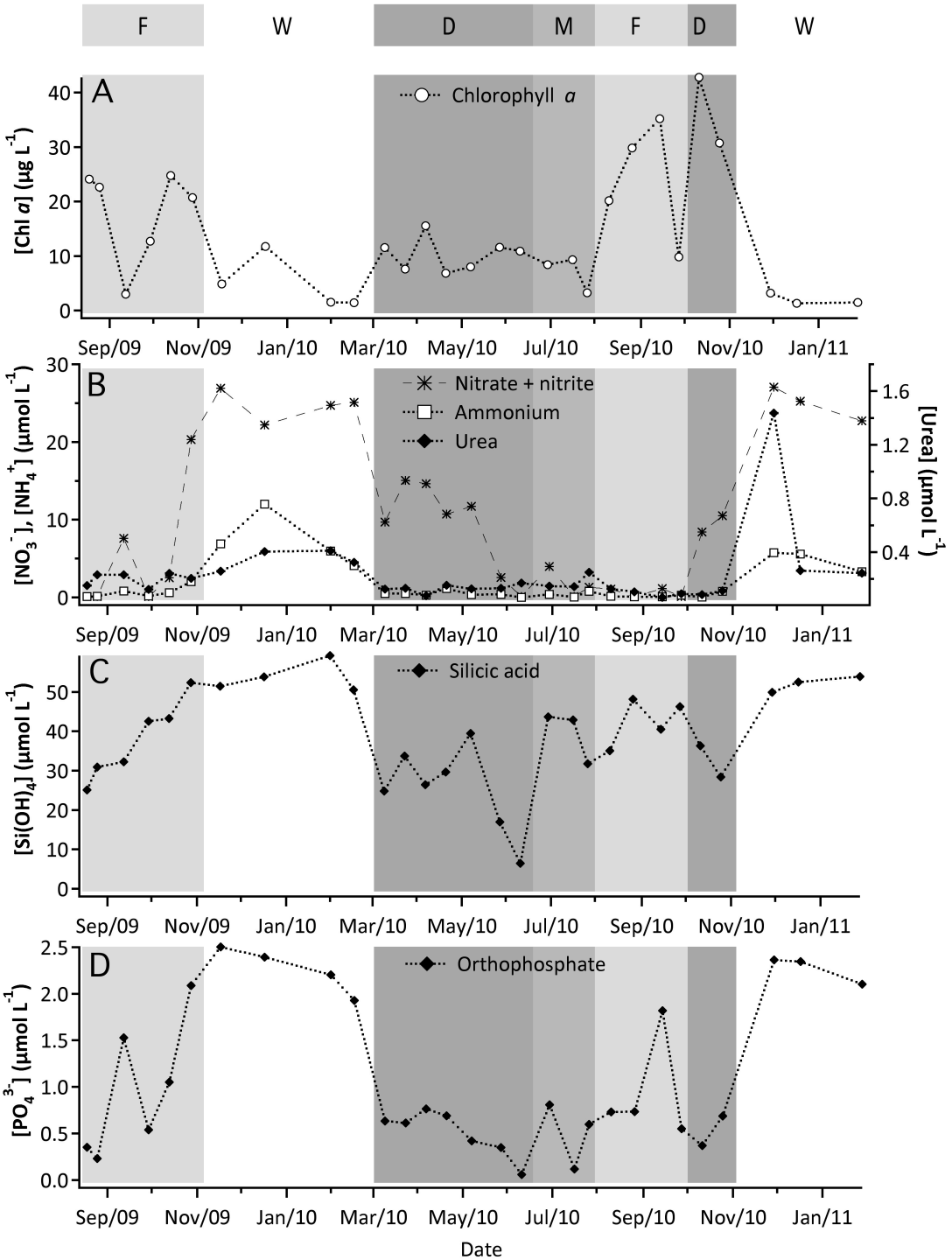


Figure 3.3 Temporal variations in phytoplankton biomass and dissolved nutrient concentrations at station 4 in Esquimalt Lagoon. (A) Chlorophyll *a*, (B) nitrate, ammonium and urea, (C) silicic acid, and (D) orthophosphate. This data has been presented previously in Figs. 2.10, 2.11 and 2.12. Shaded areas indicate the periods in which photosynthetic flagellates (F), diatoms (D) or mixed phytoplankton assemblages (M) were the dominant groups in the phytoplankton assemblages. During winter (W), phytoplankton biomass was < 5 µg L^{-1} with the exception of December 17, 2009. Winter months are Jan - Feb, spring months are Mar – May, summer months are Jun – Aug, and fall months are Oct – Sep. Nutrient concentrations are averages of two sub-samples; differences were small enough that only the averages were plotted (CV ≤ 0.11 except for urea CV = 0.16). Ticks on the x-axis represent the first day of each month.

in 2010. In August of 2009 the red-tide raphidophyte *Heterosigma akashiwo* formed a substantial bloom (maximum Chl *a* = 24 μg L^{-1}) for at least two weeks followed by a brief decline in biomass at the end of September. A second red-tide followed the *H. akashiwo* bloom, but this time it was dominated by the dinoflagellate *Akashiwo sanguinea* and lasted for at least five weeks until November 29 when biomass started to decrease to winter levels. The following year in 2010, an *A. sanguinea* bloom began in August and once again lasted for at least 5 weeks, but in contrast to 2009, *H. akashiwo* was not observed in 2010. In October of 2010 diatoms formed a fall bloom of *Thalassiosira spp.* and *Skeletonema spp.* that lasted for 3 weeks.

3.3.3 Dissolved nutrient concentrations

Concentrations of all dissolved nutrients were highest during the winter period from October through February (Fig. 3.3B, C and D). Winter average values were 25.6 μmol L^{-1}, 6.7 μmol L^{-1}, 0.31 μmol L^{-1}, 52.7 μmol L^{-1} and 2.2 μmol L^{-1} for NO_3^-, NH_4^+, urea, $Si(OH)_4$ and PO_4^{3-}, respectively. NH_4^+ was the first nutrient to be drawn down from winter levels and by March 9, 2010, NH_4^+ was already very low (0.50 μmol L^{-1}). Substantial decreases of NO_3^- and $Si(OH)_4$ began in early March, and from then until June patterns of drawdown for these two nutrients were similar (Fig. 3.3B and C). Urea and PO_4^{3-} also decreased from March until June (Fig. 3.3B and D). By early June, all nutrients had been depleted near to their limits of detection, except for $Si(OH)_4$, which was not fully depleted, but was still lower (6.5 μmol L^{-1}) than at any other time during the study. From the end of June through September, $Si(OH)_4$ and PO_4^{3-} were partially replenished and by November winter values were fully restored (Fig. 3.3C and D).

Levels of NO_3^- remained low (< 1.5 μmol L^{-1}) for the majority of the growing season, until undergoing partial replenishment in October and a more substantial replenishment in November, while NH_4^+ and urea remained fully drawn down until November (Fig. 3.3 B). In the late summer and fall of 2009 trends were quite similar to those in 2010. The average of total dissolved N (sum of NO_3^-, NH_4^+ and urea) from June through September in 2009 and 2010 was 1.9 μmol L^{-1}.

3.3.4 Dissolved nutrient ratios

N:P

In the winter period N:P ratios were lower than the Redfield ratio of 16 (Redfield 1934) ranging from 12.6 - 15.5 (Fig. 3.4A). In the spring, N:P increased from late March until early May, with ratios that ranged from 17.6 - 29.4. Following this period of increased ratios, N:P decreased steadily until the end of September, ranging from 9.2 – 0.5, with ratios being lowest in August and September. In October of 2010, two brief increases in N:P (16.8 and 23.2) preceded the re-establishment of winter ratios.

Si:P

Si:P ratios in Esquimalt Lagoon were higher than the value of 16 predicted by Brzezinski (1985) at all times during this study (Fig. 3.4B). Winter values were the lowest, ranging from 20.6 – 26.9. Si:P began to rise in March 2010, remaining elevated and fluctuating throughout the growing season. The range of ratios observed during the growing season was 21.1 – 134.2, with an average of 73.9, excluding one

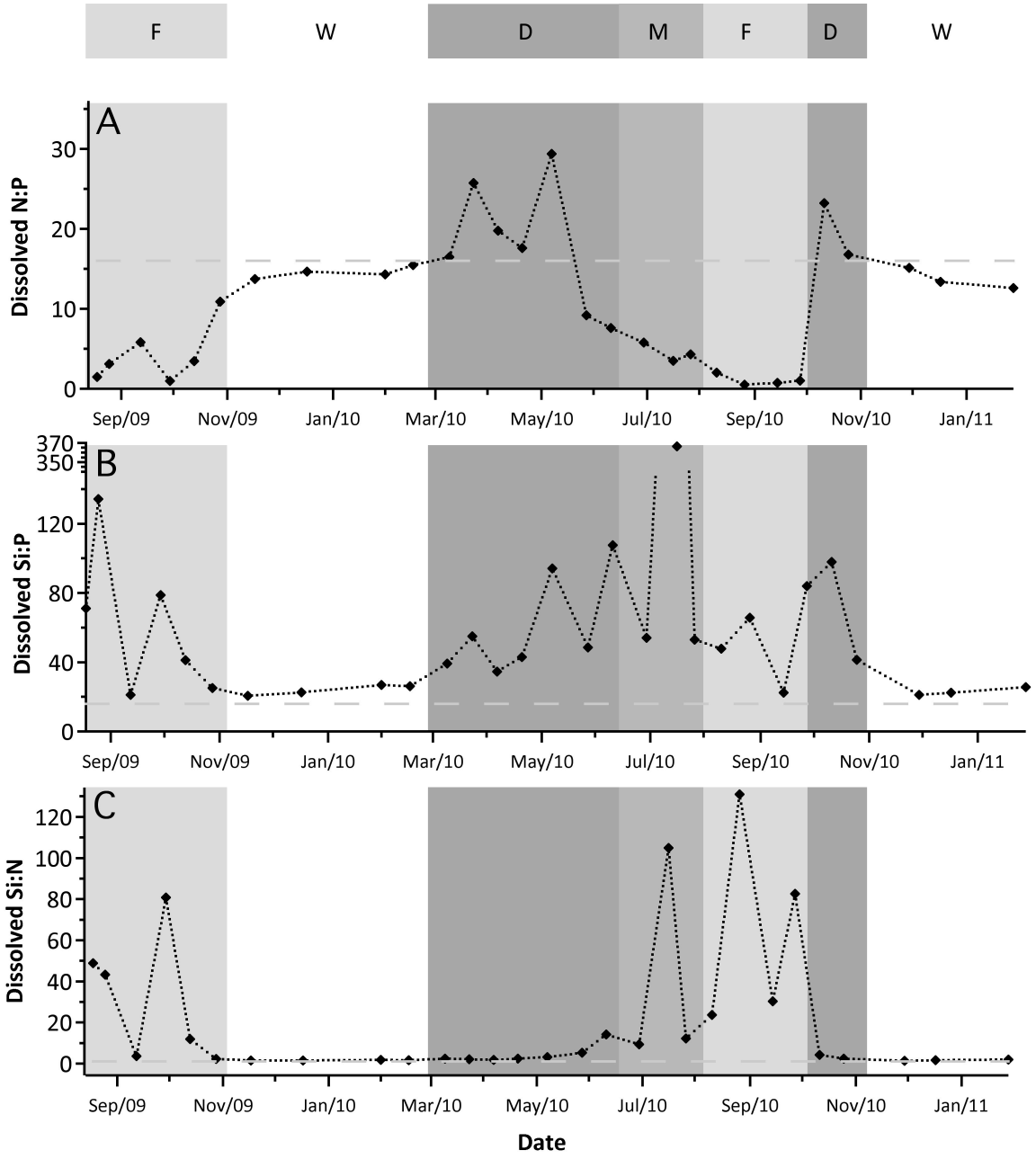


Figure 3.4 Temporal variations in dissolved nutrient ratios at station 4 in Esquimalt Lagoon. (A) Ratio of total dissolved N (sum of nitrate, ammonium, and urea) to phosphorus (orthophosphate), (B) ratio of silicon (silicic acid) to total dissolved N, and (C) ratio of silicon to phosphorus. The horizontal dashed lines represent ratios of 16 for N:P (Redfield 1934), 1 for Si:N, and 16 for Si:P (Brzezinski 1985). Shaded areas indicate the periods in which photosynthetic flagellates (F), diatoms (D) or mixed phytoplankton (M) were dominant in the phytoplankton assemblages. During winter (W), phytoplankton biomass was < 5 μg L^{-1} with the exception of December 17, 2009. Note that the y-axis scale for panel B has been extended to display the high Si:P value on July 16, 2010. Winter months are Jan - Feb, spring months are Mar – May, summer months are Jun – Aug, and fall months are Oct – Sep. Ticks on the x-axis represent the first day of each month.

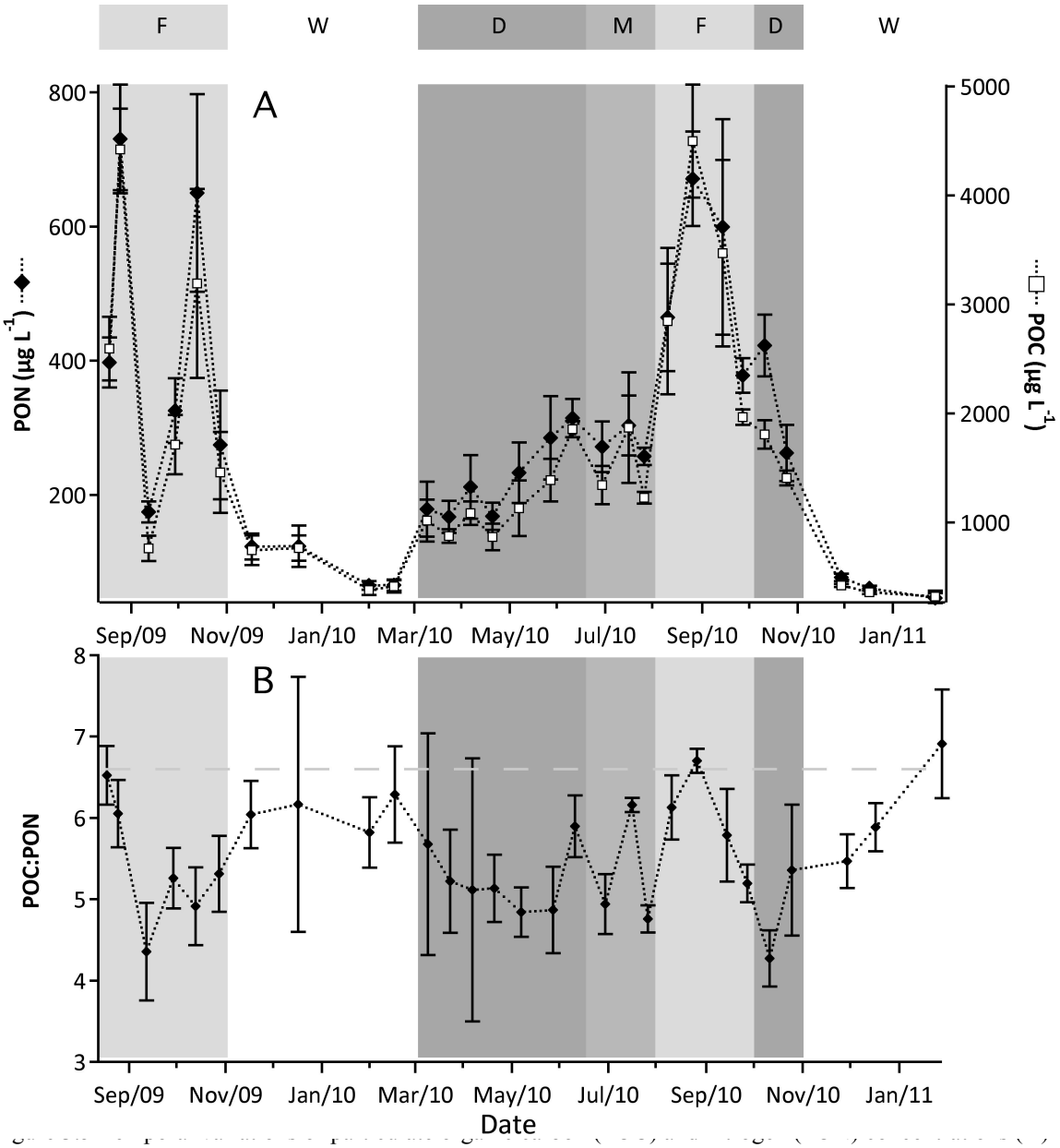
exceptionally high value of 366.4 on July 16, 2010. Si:P gradually diminished to winter values during October and November.

Si:N

Ratios of Si:N in Esquimalt Lagoon were higher than the value of 1 predicted Brzezinski (1985) at all times during this study (Fig. 3.4C). Ratios were lowest during the winter and spring, ranging between 1.4 and 5.3, with an average of 2.2. During the summer and fall, Si:N ratios were high (42.8 on average) and variable, ranging from 2.3 – 131.0 from June until the end of September in 2010 and October in 2009.

3.3.5 Concentrations and ratios of particulate organic carbon and nitrogen

The abundance of POC and PON rose during the growing season, peaking in the late summer/early fall of both 2009 and 2010, and returning to low winter values by November (Fig. 3.5A). PON followed closely with POC over the duration of the study ($R^2 = 0.96$, slope = 0.16, linear regression, data not shown). Despite the tight association of POC and PON, the ratio between the two was not constant (Fig. 3.5B). Ratios of POC:PON were typically high (~ 6) during the winter, but still generally lower than the Redfield ratio of 6.6 observed in marine particulates (Redfield 1934). In the spring POC:PON dropped to ~ 5 and during the summer and fall it was variable, ranging between high and low values. POC and PON will be described and quantified in the context of phytoplankton assemblages in section 3.3.7.



and POC:PON ratios (B) at station 4 in Esquimalt Lagoon. Values are averages of 11 sub-samples and error bars represent 1 S.D. In panel B the horizontal dashed line represent the Redfield C:N ratio of 6.6. Shaded areas indicate the periods in which photosynthetic flagellates (F), diatoms (D) or mixed phytoplankton (M) were dominant in the phytoplankton assemblages. During the winter (W), phytoplankton biomass was < 5 µg L^{-1} with the exception of December 17, 2009. Winter months are Jan - Feb, spring months are Mar – May, summer months are Jun – Aug, and fall months are Oct – Sep. Ticks on the x-axis represent the first day of each month.

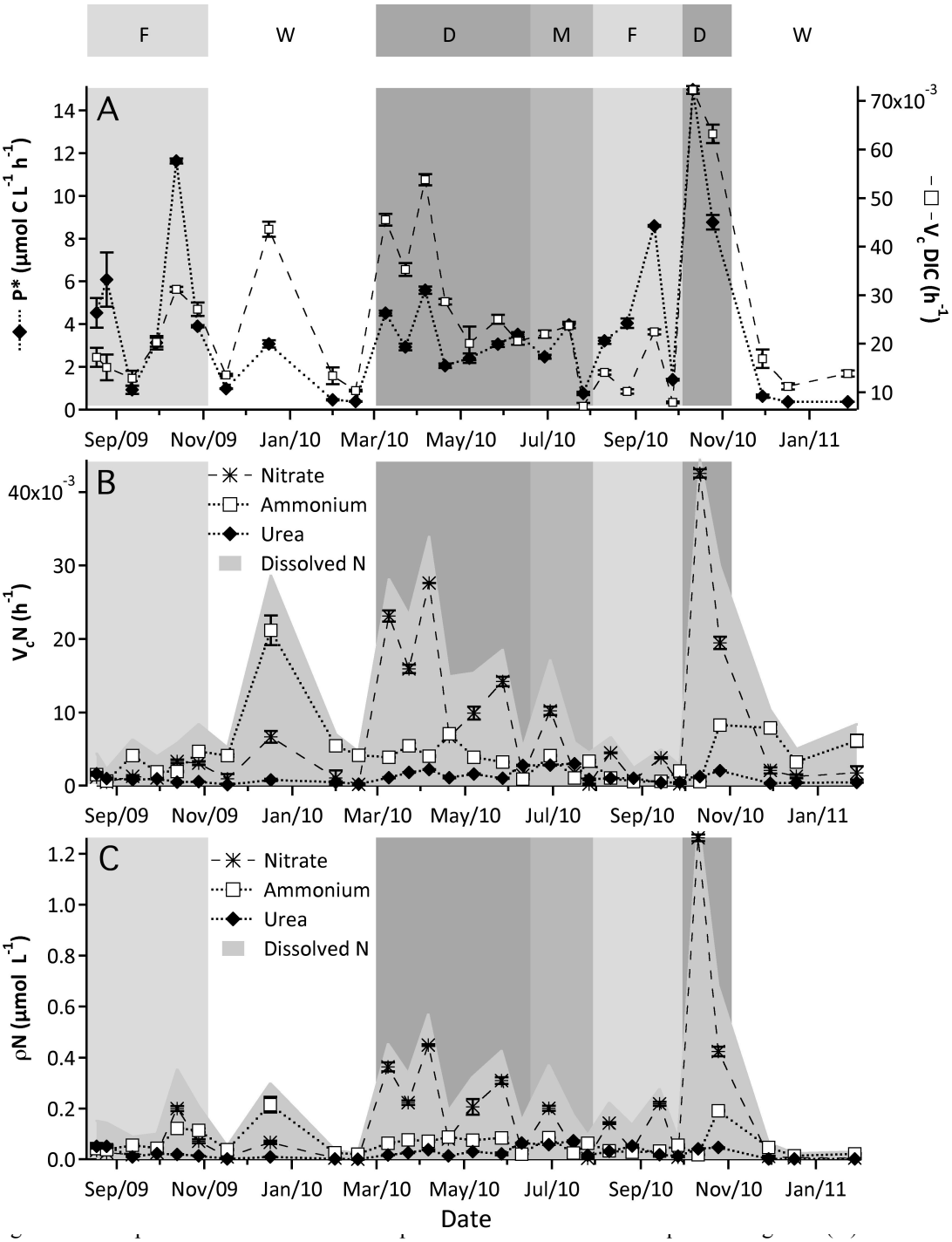
3.3.6 Nutrient uptake rates

Dissolved inorganic carbon uptake rates

Absolute and specific uptake rates of DIC (Fig. 3.6A) were generally low in the winter relative to rest of the year (aside from a high value in both of these rates on December 17, 2009). Rates were higher but variable during the growing season. DIC uptake will be described and quantified in the context of phytoplankton assemblages in section 3.3.7.

Dissolved nitrogen uptake rates

Uptake rates of NO_3^-, NH_4^+, urea, and total dissolved N showed fluctuating temporal patterns that were similar for specific and absolute uptake rates (Fig. 3.6B and C). The form of dissolved N that was most abundant in the water was taken up most readily unless NH_4^+ was higher than ~ 0.75 μmol L^{-1}. In the latter circumstance, NH_4^+ was taken up most readily. High NH_4^+ levels occurred mainly during the winter, but they did exceed 0.75 μmol L^{-1} on a couple of occasions during the growing season. In the winter, absolute uptake rates of total dissolved N were low relative to other times of the year (aside from a high value on December 17, 2009). In the spring, absolute and specific uptake rates of NO_3^- and total dissolved N were higher than most other times of the year. In the summer and early fall dissolved N uptake rates were lower and the form of N taken up most readily varied from day to day. The highest specific and absolute uptake rates of NO_3^- and total dissolved N occurred in the fall of 2010. N uptake will be described and quantified in the context of phytoplankton assemblages in section 3.3.7.



and specific uptake rates of dissolved inorganic carbon (P* and V_cDIC, respectively), (B) specific uptake rates (V_c) for nitrate, ammonium, urea, and total dissolved N (sum of nitrate, ammonium, and urea uptake rates), (C) absolute uptake rates (ρ) for nitrate, ammonium and urea and total dissolved N. Error bars represent 1 S.D. of three replicates; if they are not visible, it is because deviations were smaller than the symbol size. Vertically shaded areas indicate the periods in which photosynthetic flagellates (F), diatoms (D) or mixed phytoplankton (M) were dominant in the phytoplankton assemblages. During winter (W), phytoplankton biomass was < 5 µg L^{-1} with the exception of December 17, 2009. Winter months are Jan - Feb, spring months are Mar – May, summer months are Jun – Aug, and fall months are Oct – Sep. Ticks on the x-axis represent the first day of each month.

3.3.7 Comparison of nutrient uptake rates and composition of particulates during dominance by diatoms and photosynthetic flagellates

Particulate organic carbon and nitrogen and chlorophyll *a*

Mean POC was significantly higher during periods of dominance by photosynthetic flagellates than those by diatoms (Table 3.2; Fig. 3.7A). Mean PON was also significantly higher for flagellate-dominated assemblages, but mean Chl *a* was not (Table 3.2).

Throughout the period of photosynthetic flagellate dominance POC:PON was frequently elevated (Fig. 3.5B), and was significantly higher during this period compared to the period of diatom dominance (Table 3.2; Fig. 3.7B).

Ratios of POC:Chl *a* were significantly higher for photosynthetic flagellate assemblages than diatom dominated-assemblages (Table 3.2; Fig. 3.7C), but PON:Chl *a* was not (Table 3.2).

Dissolved inorganic carbon and nitrogen uptake rates

Although absolute DIC uptake was lower for photosynthetic flagellate-dominated assemblages than for diatom-dominated assemblages, the difference was not statistically significant (Table 3.2; Fig. 3.7D). In contrast, specific DIC uptake was significantly lower in assemblages dominated by photosynthetic flagellates (Table 3.2; Fig. 3.7E). Absolute DIC uptake normalised to Chl *a* was also significantly lower in assemblages dominated by photosynthetic flagellates (Table 3.2; Fig. 3.7F).

Absolute and specific uptake rates of total dissolved N were significantly higher for diatoms than for photosynthetic flagellates (Table 3.2; Fig. 3.7G and H). Specific uptake

rates of NO_3^-, NH_4^+, and urea were all significantly higher for diatom assemblages than for photosynthetic flagellates assemblages (Table 3.2).

Table 3.2 Parameters related to t-tests comparing means for phytoplankton assemblages dominated by diatoms (n = 10) and photosynthetic flagellates (n = 9) at station 4 in Esquimalt Lagoon from August 2009 through January 2011. Values in round brackets are standard deviations. Abbreviations for the variables are defined on page xiv of this thesis. Square brackets on variable abbreviations indicate that they are concentrations. One-tailed two sample t-test = "1 t, 2 s", two-tailed two sample t-test = "2 t, 2 s", "unequal" = unequal variances, "equal" = equal variances. The symbol * indicates that the difference between the means for diatom and photosynthetic flagellate-dominated assemblages was statistically significant.

Variable	**Test**	**Mean for diatom-dominated assemblages**	**Mean for photosynthetic flagellate-dominated assemblages**	**p value**
POC ($\mu g\ L^{-1}$)	1 t, 2 s, unequal	1219 (385)	2906 (1103)	< 0.001*
PON ($\mu g\ L^{-1}$)	1 t, 2 s, unequal	237 (88)	499 (167)	< 0.001*
Chl *a* ($\mu g\ L^{-1}$)	1 t, 2 s, unequal	16 (12)	22 (8)	0.171
POC : PON	1 t, 2 s, equal	5.25 (0.55)	5.76 (0.63)	0.039*
POC : Chl *a*	1 t, 2 s, equal	99 (43)	136 (42)	0.035*
PON : Chl *a*	1 t, 2 s, equal	19 (8)	24 (8)	0.092
$V_cNO_3^-$ (h^{-1})	1 t, 2 s, unequal	0.017 (0.012)	0.002 (0.002)	0.002*
$V_cNH_4^+$ (h^{-1})	1 t, 2 s, unequal	0.006 (0.006)	0.002 (0.001)	0.027*
V_cUrea (h^{-1})	1 t, 2 s, unequal	0.002 (0.0006)	0.001 (0.0004)	0.004*
V_cN (h^{-1})	1 t, 2 s, unequal	0.024 (0.011)	0.005 (0.002)	< 0.001*
VcDIC (h^{-1})	1 t, 2 s, unequal	0.041 (0.018)	0.018 (0.008)	0.002*
VcDIC : V_cN	2 t, 2 s, unequal	1.86 (0.79)	4.29 (1.47)	< 0.001*
ρ_N ($\mu molN\ L^{-1}\ h^{-1}$)	2 t, 2 s, unequal	0.46 (0.34)	0.17 (0.09)	0.026*
P* ($\mu molC\ L^{-1}\ h^{-1}$)	2 t, 2 s, unequal	5.09 (3.99)	5.17 (3.16)	0.962
P* : Chl *a*	1 t, 2 s, unequal	0.32 (0.05)	0.23 (0.10)	0.013*
P* : ρ_N	1 t, 2 s, unequal	11.97 (6.28)	30.51 (11.37)	< 0.001*
[Total dissolved N] ($\mu mol\ L^{-1}$)	1 t, 2 s, unequal	12.45 (9.29)	3.54 (7.26)	0.016*
$[NO_3^-]$ ($\mu mol\ L^{-1}$)	1 t, 2 s, equal	10.55 (6.26)	2.84 (6.60)	0.009*
$[NH_4^+]$ ($\mu mol\ L^{-1}$)	1 t, 2 s, unequal	1.60 (3.68)	0.41 (0.62)	0.169
[Urea] ($\mu mol\ L^{-1}$)	1 t, 2 s, unequal	0.15 (0.09)	0.15 (0.06)	0.468

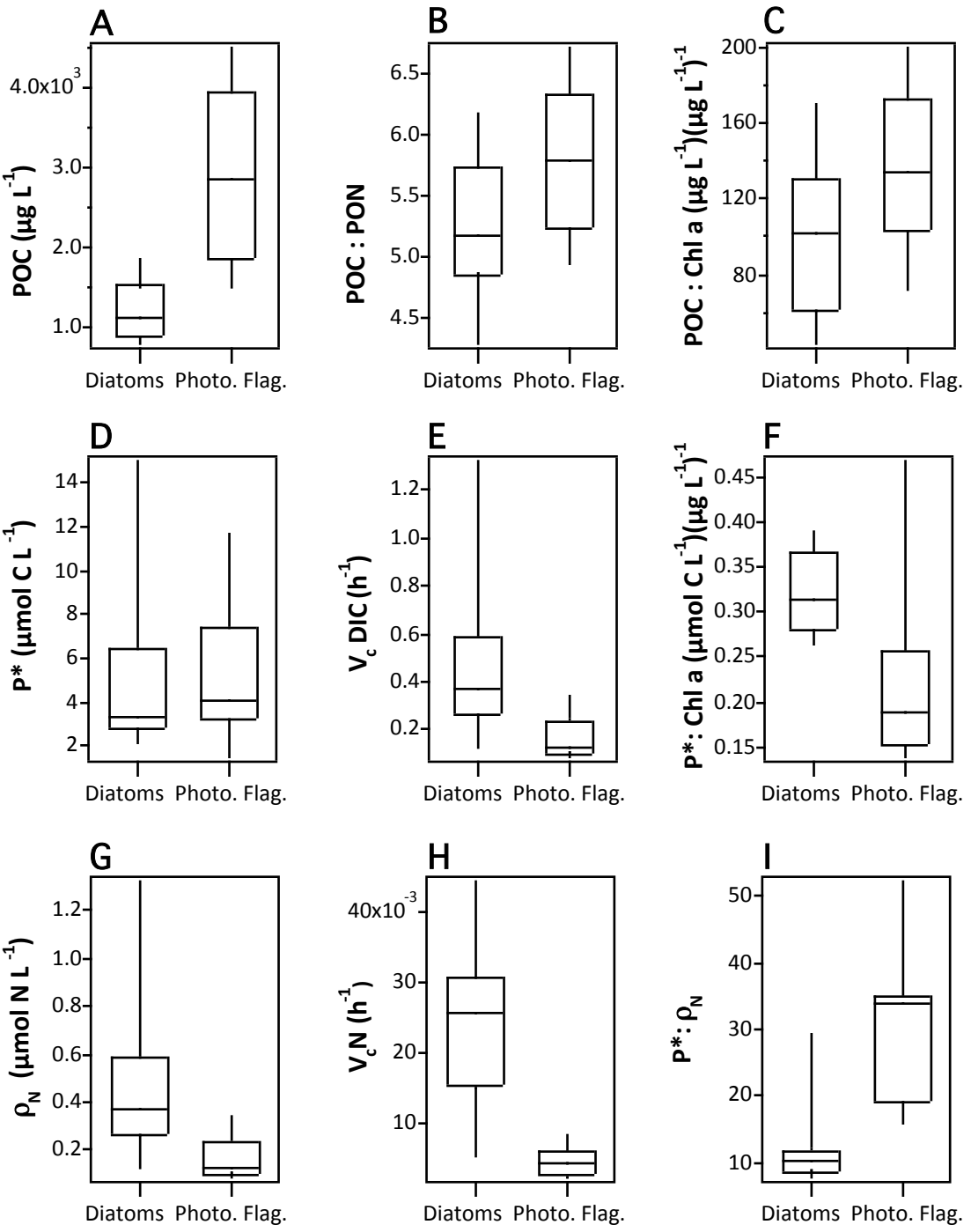


Figure 3.7 Box plots showing differences in particulate C and N concentrations, and nutrient uptake rates at station 4 in Esquimalt Lagoon in diatom-dominated assemblages and photosynthetic flagellate-dominated (Photo. Flag.) assemblages. The upper and lower whiskers represent maximum and minimum values and the boxes represent the upper quartile, the median value, and the lower quartile. Means and standard deviations can be seen in Table 3.2. Abbreviations are defined on page xiv of this thesis. (A) POC concentrations, (B) ratios of POC to PON, (C) ratios of POC to Chl *a* concentration, (D) absolute uptake rates of DIC, (E) specific uptake rates of DIC, (F) DIC uptake rates normalised to Chl *a* concentration, (G) absolute uptake rates of total dissolved N, (H) specific uptake rates of total dissolved N, (I) ratios of absolute DIC uptake to absolute uptake of total dissolved N.

The ratio of absolute carbon uptake to absolute nitrogen uptake was significantly higher in assemblages dominated by photosynthetic flagellates than those by diatoms (Table 3.2; Fig. 3.7I). The ratio of V_cDIC to V_cN was also significantly higher in photosynthetic flagellate dominated assemblages (Table 3.2).

In addition, when diatoms were dominant, V_cN was strongly associated with V_cDIC (slope = 0.57, R^2 = 0.86), whereas for photosynthetic flagellates, the relationship was weak (slope = 0.18, R^2 = 0.45) (Fig. 3.8A and B).

Relationships of nitrogen and carbon uptake with chlorophyll *a*

Absolute uptake rate of DIC was correlated with Chl a concentration for both diatom and photosynthetic flagellate-dominated assemblages (Fig. 3.8C and D) although the relationship was much stronger for diatoms (slope = 0.33, R^2 = 0.97) than for photosynthetic flagellates (slope = 0.25, R^2 = 0.39). However if one outlier is removed when computing this relationship for flagellates, it becomes stronger (R^2 increases to 0.70, Fig. 3.8D). There were no strong correspondences between V_cDIC and Chl *a*, but a weak positive relationship existed for diatom assemblages (slope = 0.0013, R^2 = 0.37, data not shown), probably because spikes in diatom biomass were accompanied by high V_c. Absolute uptake rates of total dissolved N were strongly related to Chl *a* in diatom dominated assemblages (slope = 0.03, R^2 = 0.86) but poorly correlated in assemblages dominated by photosynthetic flagellates (slope = 0.006, R^2 = 0.29) (Fig. 3.8E and F). There were no strong correspondences between V_cN and Chl *a*, but a weak positive relationship existed for diatoms (slope = 0.0007, R^2 = 0.54, data not shown).

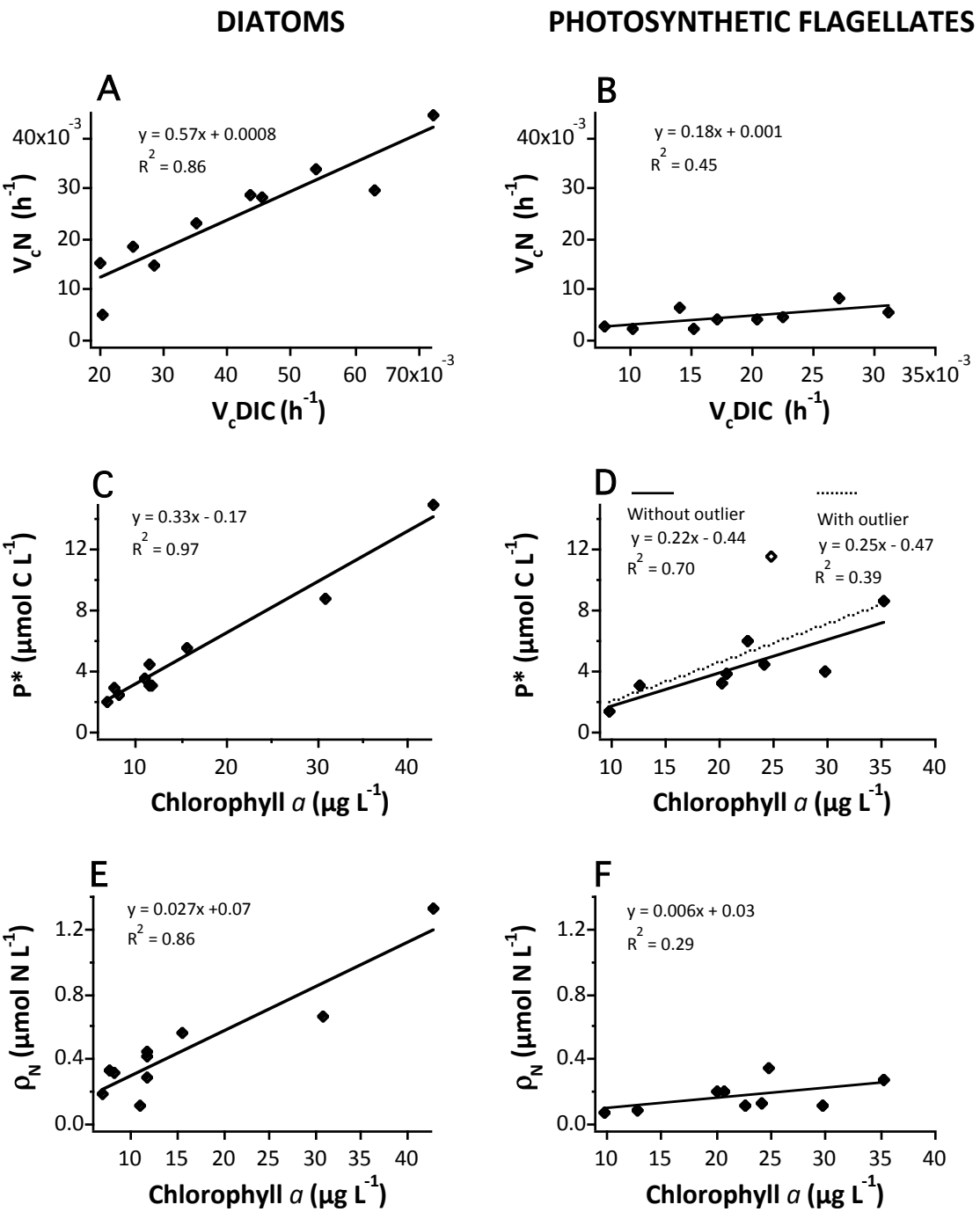


Figure 3.8 Correlation analyses relating to physiological characteristics of phytoplankton assemblages dominated by diatoms and photosynthetic flagellates during the growing season at station 4 in Esquimalt Lagoon. (A) and (B) linear regressions of specific uptake rates of total dissolved N (V_cN) with specific uptake rates of DIC (V_cDIC). (C) and (D) linear regressions of absolute DIC uptake (P*) with chlorophyll *a* concentrations. (E) and (F) linear regressions of absolute uptake of total dissolved N (ρ_N, where N is the sum of nitrate, ammonium, and urea) with chlorophyll *a* concentrations.

3.4 Discussion

3.4.1 Relationships between phytoplankton succession and nutrient dynamics

In the winter, phytoplankton biomass was low and consequently nutrients levels were high in Esquimalt Lagoon, with concentrations similar to, or slightly higher than those observed in the lagoon's ocean source water, the JdFS (see Chapter 2). In the growing season, nutrients decreased due to utilization by phytoplankton, but the patterns of draw down differed temporally depending on the phytoplankton group that was dominant in the pelagic assemblages. Therefore, changes in absolute and relative abundance of dissolved nutrients that set the stage for a phytoplankton succession throughout the growing season were mediated by phytoplankton themselves.

Spring diatom blooms

In Esquimalt Lagoon, diatom populations thrived when all dissolved nutrients were abundant. Because diatoms have high maximum uptake rates for nutrients (Sarthou et al. 2005, Litchman et al. 2007), they have a competitive advantage when nutrients are in non-limiting concentrations and can establish dominance in the water column (Sarthou et al. 2005, Litchman et al. 2007).

In the spring, diatoms dominated the assemblages and nutrient concentrations were reduced from high to intermediate levels until finally PO_4^{3-} and all forms of dissolved N decreased below or near to limits of detection and $Si(OH)_4$ was also substantially depleted. During most of the summer and early fall, diatoms were not an important component of phytoplankton assemblages, but in the fall of 2010 and the winter of 2009

diatoms formed short-lived blooms once nitrogen levels had recovered from the demands of the preceding photosynthetic flagellate blooms.

Diatoms and nitrogen

The most abundant form of dissolved N during periods of diatom dominance was NO_3^- and it was taken up most readily, however the fact that NH_4^+ and urea were almost entirely drawn down during diatom dominance indicates that diatom growth was also supported by these recycled nutrients. The fact that NH_4^+ was taken up most readily during the winter bloom of *Thalassiosira* spp. on December 17, 2009 also indicates that when NH_4^+ concentrations were high, this nutrient was important in supporting diatom growth and could potentially inhibit NO_3^- uptake. (McCarthy et al. 1977, L'Helguen et al. 2008).

Diatoms and phosphorous

Diatom assemblages in Esquimalt Lagoon also demonstrated a higher requirement for phosphorus than photosynthetic flagellates. At the first appearance of spring diatom blooms, total dissolved N and PO_4^{3-} were drawn down to intermediate levels, but the draw down of PO_4^{3-} was disproportionally larger than that of N, indicated by dissolved N:P ratios that rose temporarily above 16. Elevated N:P ratios for dissolved nutrients were also measured during the fall diatom blooms in 2010. The periods of elevated N:P ratios in surface waters suggest that diatoms were limited by phosphorous except during their last month of dominance (end of May and early June), when they were N-limited. The phytoplankton communities that experienced the highest degrees of P limitation were those that were recently established, i.e. when spring diatom assemblage switched from

dominance by *Thalassiosira* spp. to dominance by *Skeletonema* spp. there was a spike in N:P, again when S*keletonema* spp. was replaced by *Chaetoceros* spp., and then in the fall when photosynthetic flagellates were replaced by *Thalassiosira*-dominated assemblages. Phosphorous limitation could be explained if diatom communities were undergoing exponential growth at saturating levels of nutrients, as phytoplankton in this growth stage have a high requirement for P because they are investing in cell machinery that maximises optimal growth (Klausmeier et al. 2004). The occurrence of nutrient-saturated exponential growth may be supported by the fact that the highest specific carbon uptake rates occurred during rises in diatom biomass above mean concentrations.

Diatoms and silicon

Diatoms have an absolute requirement for $Si(OH)_4$ (Sarthou et al. 2005), and during the spring period of diatom growth, $Si(OH)_4$ concentrations in Esquimalt lagoon were reduced dramatically. Despite replenishment of $Si(OH)_4$ during the summer, diatoms did not regain dominance until nitrogen levels increased in the fall.

Early summer assemblages of mixed phytoplankton

As previously indicated, at the end of the diatom-dominated period in early June, surface waters became N-limited because diatom assemblages almost entirely exhausted dissolved N. It was at this point that there was a switch in the dominant phytoplankton group.

Following the crash in nutrient concentrations in early June, 2010 Chl *a* concentrations were maintained at intermediate levels until the end of July and assemblages were composed of a mixture of dinoflagellates, diatoms, and filamentous cyanobacteria. All

forms of dissolved N during June and July were very low and the phytoplankton communities were most likely N-limited (i.e. low N:P ratios).

Results from the nitrogen uptake experiments indicate that recycled forms of dissolved N (NH_4^+ and urea), as well as NO_3^-, played an important role in supporting the mixed assemblages of phytoplankton. This is because following the depletion of NO_3^-, NH_4^+ and urea were often as high or higher in abundance than NO_3^-, and the form of nitrogen that had the highest ambient concentration was taken up most readily. A similar pattern was observed by McCarthy *et al*. (1977) when phytoplankton were N-limited.

It is not surprising that cyanobacteria were present during this period when dissolved N forms were depleted because this type of phytoplankton can fix atmospheric nitrogen, giving them a competitive advantage at low nitrogen levels (Agawin et al. 2007). Also, certain species of cyanobacteria have the ability to use amino acids, which were not measured in this study, but are a form of recycled dissolved organic nitrogen (DON) that could be important in supporting phytoplankton growth when dissolved inorganic nitrogen (DIN) becomes depleted (Paerl 1991, Herrero and Muro-Pastor 2001).

Late summer and fall blooms of photosynthetic flagellates

In 2009 sampling began when biomass of *H. akashiwo* was already high, and after a crash in phytoplankton biomass to ~ 3 μg L^{-1}, an *A. sanguinea* population was established. In 2010 the *A. sanguinea* bloom followed a similar crash in photosynthetic biomass at the tail end of the period dominated by mixed phytoplankton.

As in the case with mixed phytoplankton assemblages, recycled forms of dissolved N were likely important in supporting populations of photosynthetic flagellates in 2009 and 2010, because concentrations and uptake rates of NH_4^+ and urea were often higher than

those of NO_3^-. That being said, levels of total dissolved N were still largely exhausted at this time. The most striking aspect of the *A. sanguinea* and *H. akashiwo* blooms was their ability to reach high levels of biomass (35 μg L^{-1} at peak concentrations) for extended periods of time when total dissolved N remained below 1.5 μmol L^{-1} and N:P was below 3. It could thus be inferred then that these two phytoplankton species must be able to utilize very low levels of dissolved N effectively, however this inference is not supported by studies on these organisms. Both of these species are adapted to neritic coastal environments (Kudela et al. 2008), and have average affinities for NO_3^-, NH_4^+ and urea among phytoplankton groups adapted to these eutrophic environments. Half saturation constants for NO_3^-, NH_4^+ and urea in these groups are all ~ 1 - 2 μmol L^{-1}, which are intermediate values (Dugdale and Wilkerson 1986, Herndon and Cochlan 2007, Litchman et al. 2007, Kudela et al. 2008). Also, *A. sanguinea* blooms may require alleviation of nutrient stress in order to gain dominance. Blooms of *A. sanguinea* in both 2009 and 2010 were only established after periods when phytoplankton biomass had dropped to ~ 3 μg L^{-1} in the growing season and short-lived spikes in NO_3^- (8 μmol L^{-1} in 2009 and 4 μmol L^{-1} in 2010) were observed.

So, if *A. sanguinea* and *H. akashiwo* were not able to readily take up very low concentrations of dissolved N, how did these groups sustain extended blooms of high biomass? This question cannot be entirely resolved without considering the potential that *A. sanguinea* and *H. akashiwo* were using a mixotrophic mode of nutrition, and were taking advantage of their ability to swim to reach localised pools of nutrients inaccessible to phytoplankton without flagella.

3.4.2 Alternative feeding strategies of photosynthetic flagellates in Esquimalt Lagoon

Mixotrophy

Both *A. sanguinea* and *H. akashiwo* are mixotrophs known to use DOM and consume other microbes (Bockstahler and Coats 1993, Stoecker and Gustafson 2003, Jeong et al. 2005, Seong et al. 2006, Kudela et al. 2008, Jeong et al. 2010a, 2010b). Therefore, it could be hypothesised that these phytoplankton were using alternative forms of organic nitrogen in Esquimalt Lagoon to meet their N requirements, given the low concentrations of dissolved N and low uptake rates of dissolved N forms during their period of dominance.

The strongest evidence to support the hypothesis that mixotrophy was occurring is that specific uptake rates of total dissolved N in assemblages dominated by photosynthetic flagellates did not correlate strongly with specific uptake of DIC. In diatom assemblages this correlation was strong. If photosynthetic flagellates require C and N in relatively constant proportion, then these organisms must have been utilizing additional sources of N not measured during this study, such as amino acids or particulate N. Also, the ratios of POC:PON and POC:Chl *a* were significantly higher in photosynthetic flagellate-dominated assemblages than those dominated by diatoms, phenomena that were observed when Levasseur *et al.* (1993) cultured *A. sanguinea* and other phytoplankton on the organic nutrient urea. Levasseur *et al.* suggested that high POC:PON was due to delayed assimilation of N relative to C because organic urea had to be converted to inorganic NH_4^+ prior to assimilation. This limited the phytoplankton cells' ability to accumulate a normal quota of nitrogen internally (Levasseur et al. 1993).

Another complementary piece of evidence suggesting that unmeasured nitrogen uptake was occurring is that absolute uptake of total dissolved N by photosynthetic flagellates was not strongly correlated with phytoplankton biomass; normally it would be expected that a larger biomass of phytoplankton would be utilizing a larger amount of nitrogen, but this was not the case. On the other hand, absolute uptake of total dissolved N was strongly related to biomass in diatom-dominated assemblages.

As opposed to uptake of total dissolved N, uptake of DIC did appear to be positively related to Chl *a* in photosynthetic flagellate assemblages as well as diatoms assemblages. So, DIC was still likely the dominant form of carbon supporting photosynthetic flagellate and diatom biomass. However, the relationship was certainly less strong for photosynthetic flagellates, so the incorporation of organic carbon obtained mixotrophically could reduce the dependence of phytoplankton biomass accumulation on DIC uptake.

Advantages of swimming for nutrient acquisition

The second characteristic that may have allowed *A. sanguinea* and *H. akashiwo* to thrive at low dissolved N is their ability to swim with the use of their flagella. Specifically, this ability could have allowed them to access dissolved N in areas of the water column with heightened concentrations: near the sediments and at depths < 1 m.

In the 1970s, Robinson & Brown (1983) observed pulses of NO_3^- ~ 15 μmol L^{-1} to 60 μmol L^{-1} in stream inputs to Esquimalt lagoon in the late summer and fall when the rest of the water column was below 5 μmol L^{-1}. These pulses yielded significant increases in *A. sanguinea* biomass given a lag time of 2.5 weeks. In both 2009 and 2010 in Esquimalt Lagoon, occurrence of precipitation events was higher in the late summer and fall

compared to the rest of the growing season (see Fig. 2.4). Any fresh water coming in from streams would theoretically have stayed at the surface of the water column at this time because the water was dense (salty) and stratified. This combination of environmental conditions would create a situation where photosynthetic flagellates would have an advantage over non-flagellated phytoplankton because they could concentrate near the surface and utilize this nutrient pool. In fact, at the peak of the *A. sanguinea* bloom in 2010, cell counts in the top 20 cm of Esquimalt Lagoon were up to 50 times higher than those collected at 1 m (data not shown). This strongly suggests that *A. sanguinea* was migrating vertically to waters that were less saline (Fig. 3.1B).

Robinson & Brown (1983) and Katanao *et al.* documented vertical migration of *A. sanguinea* to mid or bottom waters at night. *H. akashiwo* is also known as a vertical-migrator (Bearon and Grünbaum 2004). Downward vertical migration suggests that these species may swim to take advantage of nutrient efflux from the sediments, which can be important in sustaining phytoplankton biomass in lagoons (Castel and Caumette 1996, Ma and Whereat 2006, Glé et al. 2008). In Esquimalt Lagoon, nutrients levels are generally higher near the sediments (see Appendix B), which strengthens the hypothesis that nutrients released from the sediments could be an important source of dissolved N for swimming photosynthetic flagellates in Esquimalt Lagoon.

In summary, dominance by diatoms in Esquimalt Lagoon occurred when dissolved nutrients were abundant, but the nutrition of photosynthetic flagellates was likely more complex, as the water column appeared to be impoverished in dissolved N. Photosynthetic flagellates may have been migrating vertically to profit from localized pools of dissolved N and utilising DOM or POM to supplement their nitrogen quotas.

3.4.3 Oxygen depletion in Esquimalt Lagoon

Although phytoplankton can consume DOM, they are also major contributors to the DOM pool in marine environments, excreting ~ 10 - 20 % of their fixed carbon as dissolved organic carbon (DOC) (Gobler and SA 2003, Maranon et al. 2004, 2005, Romera-Castillo et al. 2010). DOM is an essential substrate supporting heterotrophic bacterial production (Chen and Wangersky 1996, Romera-Castillo et al. 2011) during and following phytoplankton blooms (Kamiyama et al. 2000, Ramaiah and Furuya 2002, Gobler and SA 2003, Suksomjit et al. 2009). Also, senescent phytoplankton are a source of labile particulate detritus that can support bacterial respiration in the sediments (Gürel et al. 2005). Thus phytoplankton blooms can stimulate bacterial activity both in the pelagic zone, by releasing DOM, and in the benthos when they sink. During the night, pelagic phytoplankton respire O_2 alongside heterotrophic bacteria instead of producing it (Gürel et al. 2005), and thus O_2 concentrations can drop in the surface waters as well as the bottom waters (Pereira et al. 2010), sometimes leading to hypoxia or anoxia if tidal exchange is highly restricted (Gamito et al. 2005).

Release of DOM by photosynthetic flagellates and diatoms

It has been shown in both field and laboratory studies that photosynthetic flagellates may release a larger percentage of their fixed carbon as DOC than diatoms do (Lancelot 1983, Ramaiah and Furuya 2002, Romera-Castillo et al. 2010), especially when dissolved inorganic N is depleted (Lancelot 1983).

However, elevated periods of DOC exudation by diatoms can also occur, primarily when the population is transitioning from exponential growth to stationary phase and

senescence. Chen & Wangersky (1996) attributed this increase to cell lysis, however Engel *et al*. (2002) argued that it is triggered by nitrogen limitation. Under these conditions, diatoms over-consume carbon and excrete it as high molecular weight DOC (specifically carbohydrates) which can form aggregates. These particles are known as TEPs (transparent exopolymer particles). Diatoms are thought to be the major producers of TEPs in marine environments (Engel et al. 2002), but contributions by other phytoplankton groups such as *H. akashiwo* may be even more substantial in some locations (Ramaiah and Furuya 2002).

In the current study, we did not measure DOM concentrations, but it is quite well established that *H. akashiwo* populations exude DOM (Ramaiah and Furuya 2002, Suksomjit et al. 2009). In addition, *A. sanguinea* is known to produce large quantities of microsporine-like amino acids (MAAs) that help protect the cells from UV light (Litchman et al. 2002). These N-rich compounds are released into the water most readily during cell senescence (Jessup et al. 2009). In the current study MAAs were present during the peak of the *A. sanguinea* red tide in 2010 (see Appendix E). Also DOC levels tripled in Esquimalt Lagoon during a bloom of *A. sanguinea* in 1978 (Robinson and Brown 1983).

Higher POC:PON during periods of photosynthetic flagellate dominance than during diatom dominance in Esquimalt Lagoon may indicate that photosynthetic flagellates were excreting higher levels of DOC because increases in POC:PON have been associated with accumulating DOM in mesocosm and field studies (Engel et al. 2002, Gobler and SA 2003). However, these high ratios could be also be due to reduced internal nitrogen quotas caused by retarded assimilation of organic nitrogen forms that must be deaminated

prior to use (Levasseur et al. 1993) (see section 3.4.2). Also, the higher ratios of DIC uptake to uptake of total dissolved N during dominance by photosynthetic flagellates suggest that these assemblages could be fixing excess carbon, much of which could be exuded as DOC, possibly triggered by nitrogen limitation (Lancelot 1983).

It is likely that diatoms in Esquimalt Lagoon were releasing moderate quantities of DOM (~ 10 – 20 % of fixed carbon, as mentioned above), a phenomenon that has been well-documented in a number of phytoplankton communities in recent studies (Maranon et al. 2004, 2005, Romera-Castillo et al. 2010). However, the fact that diatom growth mainly occurred in nutrient-replete conditions would suggest that elevated excretion of DOM is unlikely (Engel et al. 2002). Interestingly, a spike in POC:PON occurred on the last day of diatom dominance when nutrients were exhausted, and this could reflect nitrogen-limited excretion of fixed carbon as TEPs by diatoms.

In summary, it can be hypothesized that DOM is higher at the time of dominance by photosynthetic flagellates than during growth of diatoms, and under such circumstances, bacterial activity could be stimulated, creating the potential for O_2 depletion to occur.

Stimulation of bacterial respiration by dissolved organic matter and effects on O_2 depletion

High levels of DOM associated with blooms of *H. akashiwo* (Ramaiah and Furuya 2002, Suksomjit et al. 2009), and mixed blooms of *A. sanguinea*, *H. akashiwo*, and *Prorocentrum spp*. (Kamiyama et al. 2000) have been linked to increasing bacterial populations in field studies. Also, Gobler & Sañudo-Wilhelmy (2003) observed a significant positive correlation between low molecular weight DON (likely produced by the mixtotrophic species *Aureococcus anophagefferens*) and bacterial densities. This

relationship was attributed to the presence of labile nitrogen-rich compounds like amino acids that can support robust bacterial growth. The latter point may be relevant in Esquimalt Lagoon due to the production of MAAs during blooms of *A. sanguinea*.

Even though diatoms in Esquimalt Lagoon were likely releasing normal amounts of DOM, it may have been less available to bacteria than that released by photosynthetic flagellates. DOM released by diatoms does not always stimulate bacterial growth (Suksomjit et al. 2009) as it can be refractory in nature (Pete et al. 2010). Even in cases where diatom exudates have been shown to increase bacterial growth rates, they did not stimulate growth to the same degree that dinoflagellate exudates did (Romera-Castillo et al. 2010).

According to FlowCAM imaging, bacterial abundance could have been high during the summer and fall phytoplankton assemblages (data not shown). The number of unidentifiable cells < 8 μm in diameter rose following the decline of spring diatom populations in 2010 and remained elevated through the remainder of the growing season. However it cannot be confirmed that these particles were heterotrophic bacteria.

In summary, the growth and decline of photosynthetic flagellate blooms can be expected to be favourable for bacterial activity and for inducing O_2 depletion. Indeed, it is during the period of dominance by photosynthetic flagellates that O_2 levels started to fall in the bottom waters in 2009 and 2010.

Oxygen depletion and physical characteristics

Large and persistent photosynthetic flagellate blooms likely played a particularly important role in O_2 depletion in the late summer and fall in Esquimalt Lagoon because of their timing in relation to certain physical processes. Although the water column was

stratified to some degree year-round, stronger stratification was evident in spring and summer, due to warming. Reduced vertical mixing from strong summer stratification and the probable occurrence of anti-estuarine circulation (see Chapter 2) could have been responsible for reduced flushing of bottom waters and, thus, reduced O_2 renewal. In addition, in late summer and fall (August, September and October), tidal ranges were narrow compared to spring and early summer (May, June and July), particularly during neap tides. Small tidal ranges are normally associated with weak tidal currents that dissipate with distance from the mouth of a lagoon, leading to reduced water exchange, longer residence times of lagoon water, and thus longer periods of exchange between the water column and the sediments (Postma 1969, Smith 1994, Glé et al. 2008).

Therefore, physical properties in the water column of Esquimalt Lagoon primed the system for bottom O_2 depletion. These physical effects in combination with rising phytoplankton biomass in late summer (August) may have been the cause of O_2 depletion occurring in the bottom waters throughout the lagoon during in both years (considering not only station 4 but all five stations described in Chapter 2). As phytoplankton biomass peaked, O_2 depletion in the bottom waters became more severe as senescent cells were likely sinking to the benthos and stimulating bacterial growth. Also, as discussed above, the late summer and fall phytoplankton blooms were likely releasing abundant DOM, stimulating pelagic respiration. Although pelagic respiration was not a problem when phytoplankton were photosynthesising during the day (as indicated by supersaturated O_2 levels in the upper water column), during the night respiratory demands by phytoplankton (Gürel et al. 2005) would have been added to those by heterotrophic bacteria using DOM,

and O_2 depletion could have occurred in the upper water column as well as in the bottom waters (Pereira et al. 2010).

Oxygen depletion events in Esquimalt Lagoon

In the 1970s, O_2 depletion events occurred in Esquimalt Lagoon that lead to hypoxia/anoxia and harmed aquatic organisms. The ultimate motivation for this research project was to investigate the periodic reoccurrence of these events being suggested by anecdotal evidence.

In the 1970's, periodic occurrences of what appeared to be dystrophic crises like those experienced in a number of Mediterranean lagoons (Bartoli 1996, Castel and Caumette 1996, Harzallah and Chapelle 2002) were detected in Esquimalt Lagoon (Robinson and Brown 1983). These locally-termed "white tides" have also been observed in recent years by members of the Esquimalt Lagoon Stewardship Initiative and the Capital Regional District Harbours and Watersheds Division (personal communications). These events coincided with "red tides" of *A. sanguinea* and developed in the toe of the lagoon, which occupies the furthest point from the mouth when ocean water enters and thus would experience the lowest flushing (Scrimger 1960, Postma 1969, Robinson and Brown 1983).

A probable O_2 depletion event also occurred at the beginning of this study, indicated by a fish kill on the night of September 5, 2009 (Esquimalt Lagoon Stewardship Initiative, personal communication). At this time, O_2 levels near the benthos were approaching hypoxia, but they were still high in the upper water column during the day. However, the fish kill occurred at night when respiration processes by autotrophic and heterotrophic organisms are dominant (Gürel et al. 2005). The fish kill also coincided with the

narrowest tidal range of the season (20 cm) indicating that reduced flushing was involved.

3.5 Conclusion

Phytoplankton succession in Esquimalt Lagoon appeared to be largely dictated by the availability of dissolved N. In the spring, NO_3^- was the most abundant form of dissolved N. Diatoms appeared to be limited by phosphorous until their last month of dominance, and they utilized the abundant NO_3^- readily. Spring diatom populations almost entirely depleted NO_3^-, NH_4^+ and urea, which led to dominance of mixed phytoplankton assemblages composed of dinoflagellates, diatoms, and filamentous cyanobacteria, that were able to exist in N-limited conditions. Later in the growing season blooms of photosynthetic flagellates developed, with *Heterosigma akashiwo* and *Akashiwo sanguinea* being the dominant species in 2009, and *Akashiwo sanguinea* in 2010. High biomass and persistence of photosynthetic flagellates at a time when concentrations of NO_3^-, NH_4^+, and urea were low indicates that these flagellates could have exploited a diverse array of nutrient sources in Esquimalt Lagoon, through the processes of mixotrophy and vertical migration. The lack of strong correlation between total dissolved N uptake and DIC uptake rates in photosynthetic flagellate assemblages suggests that these phytoplankton were using dissolved or particulate organic nitrogen to fulfill their N requirements. The presence of a high biomass of phytoplankton in the late summer and fall when waters were warm and well-stratified and tidal ranges were small, created favourable conditions for O_2 depletion. O_2 depletion in bottom waters occurred during blooms of photosynthetic flagellates, likely when senescent phytoplankton cells were accumulating on the sediments, supporting bacterial respiration, and increasing O_2

demand in the bottom waters. Elevated ratios of POC:PON and the detection of MAAs suggest that photosynthetic flagellates could have been over-consuming carbon and exuding DOM, which could have stimulated bacterial activity in the pelagic zone as well near the benthos. At night, pelagic respiration would have been occurring in both bacterial and phytoplankton populations and O_2 depletion throughout the water column could have been occurring in the late summer and early fall. This hypothesis was supported by the occurrence of a fish kill on the night of September 5, 2009. Historically, "white tides" accompanied by mortality of aquatic organisms have periodically occurred in conjunction with "red tides", and these phenomena were likely indicative of severe O_2 depletion during dystrophic crises. Therefore, late summer and fall phytoplankton blooms in Esquimalt Lagoon are considered harmful because of the negative effects that their senescence imposes on the local ecosystem. In addition, restricted tidal exchange in the late summer and fall could lead to stagnation of water in the lagoon and isolation of bottom waters, impeding O_2 replenishment and allowing the development of hypoxia.

Chapter 4: General conclusions and future research

4.1 The current study

This study documents how physicochemical properties of the water column in Esquimalt Lagoon change seasonally and how nutrient dynamics relate to the succession of phytoplankton groups. However, understanding the occurrence of HABs in Esquimalt Lagoon proved to be complex. Given the low concentrations of NO_3^-, NH_4^+, and urea during the late summer and fall, simple relationships between levels of dissolved N and biomass of HAB species did not exist. The fact that these populations were highly N-limited explains the observation that dissolved N forms remained largely exhausted. Dissolved N, whether it was NH_4^+ or urea being recycled in the water column and sediments or NO_3^- being delivered by streams and the ocean, was likely being taken up so readily by the HAB species that concentrations could not be replenished, even though uptake rates of all dissolved N forms were low.

Despite low concentrations of NO_3^-, NH_4^+, and urea at this time, nitrogen could have been abundant in dissolved and particulate organic pools (Bronk et al. 2006) and there was indirect evidence to suggest that mixotrophy was occurring when *A. sanguinea* and *H. akashiwo* were dominant. However, the relative importance of mixotrophy was not quantified in this study.

The coincidence of narrow tidal ranges with oxygen depletion events supports the idea that reduced flushing was important in allowing oxygen levels to drop, but rates of circulation were not quantified. Regarding biological processes involved in oxygen depletion, direct and indirect evidence supported the idea that HABs could be exuding

labile DOM that could stimulate pelagic bacterial activity, but neither DOM nor bacterial activity were measured.

Thus, there is much opportunity for novel research that could confirm how HABs are sustained in Esquimalt Lagoon and provide useful information for other coastal systems that experience HABs dominated by similar phytoplankton species.

4.2 Future research

4.2.1 Understanding the role of dissolved organic matter in sustaining harmful algal species

What made the harmful "red tides" in Esquimalt Lagoon different from diatom blooms is that they occurred under strong nutrient limitation and they may have been using organic matter to satisfy their nitrogen deficits, meanwhile "over-consuming" carbon and exuding DOM that could stimulate bacterial activity.

DOM concentrations can be elevated during blooms of mixotrophic raphidophytes (Ramaiah and Furuya 2002), mixotrophic pelagophytes (Gobler and SA 2003), and mixotrophic dinoflagellates (Lancelot 1983), but to my knowledge, no studies have attempted to demonstrate that there is a causal link between DOM exudation and mixotrophic feeding in nutrient-limited environments. I believe this knowledge is a critical step in understanding HABs linked with oxygen depletion and can envision a set of experiments that could test for such a relationship. These experiments would monitor concentrations of DOM in cultures where mixotrophic phytoplankton were provided with either inorganic nutrients or a combination of inorganic and organic nutrients under nutrient replete and nutrient-limited conditions. Uptake experiments (using uptake of

fluorescently-labelled bacteria and isotopically-labelled amino acids and urea for instance, as well as isotopically-labelled inorganic nutrients) could be performed in conjunction with the measurement of DOM in attempt to quantify heterotrophy versus autotrophy.

4.2.2 Understanding eutrophication and oxygen depletion in Esquimalt Lagoon

There are also at least two gaps in knowledge in Esquimalt Lagoon that could be addressed in order to understand what is causing eutrophication and HABs in this system. Although it is evident that Esquimalt Lagoon is in some intermediate state of eutrophication, the contribution that anthropogenic nutrient inputs have made to the process of eutrophication is not clear, especially because nutrients are high in the lagoon's ocean source, the JdFS. Monitoring levels of nutrients in stream sources by the Capital Regional District (CRD) is a step in the right direction, but unfortunately, monitoring has been performed at intervals that may be too infrequent to resolve patterns in nutrient delivery. The observation that nutrients in stream inputs may have increased dramatically in the last 30 years suggests that use of fertilisers may be increasing nutrient inputs to the lagoon (Haigh 2008, Stallard 2009), but nutrient concentrations in the CRD samples were highly variable, even those collected within a short time span. Also, monitoring did not occur during all seasons when patterns of nutrient delivery may have been different. If a convincing argument is to be made that the delivery of anthropogenic nutrients supports HABs and eutrophication, monitoring would need to occur more frequently, consider patterns of rainfall, and also quantify nutrient concentrations in stream-derived water masses at the surface of the lagoon.

In regards to HABs and oxygen depletion, *in situ* measurement of DOM throughout the year in Esquimalt Lagoon and measurement of bacterial numbers or bacterial respiration during the day and the night would be informative, especially in conjunction with experiments such as those described above, and could be useful in advancing the understanding of oxygen depletion events in Esquimalt Lagoon.

Given the fact that the trophic state of a lagoon ecosystem is intimately linked with flushing rates (Gamito et al. 2005), I would also suggest that circulation processes in Esquimalt Lagoon be re-examined. In the 1950s Scrimger (1960) suggested that the water residence time in Esquimalt Lagoon was only 3 days, but if this was the case the lagoon would not likely experience intense algal blooms associated with oxygen depletion. A three-day water residence time would imply that Esquimalt Lagoon has a water exchange rate that is similar to lagoons like the Ria Formosa in Portugal or Arcachon Bay in France, and these lagoons consistently have phytoplankton biomass less than 5 μmol L^{-1} Chl *a* and 15 μmol L^{-1} Chl *a*, respectively (Newton et al. 2003, Newton and Icely 2006, Glé et al. 2008). Srimger's measurements were likely representative of the amount of exchange that occurs in proximity to the mouth of the lagoon, but were not able to resolve the probable stagnation of water occurring within the interior of the lagoon and at the toe where anoxic or dystrophic crises occur. Especially given recent changes in the geology of the lagoon's entrance channel (Esquimalt Lagoon Stewardship Initiative, personal communication) a thorough quantification of tidal currents throughout the lagoon, separately during neap tides and spring tides, in the summer versus the winter, and in the upper and lower water column would be extremely valuable.

In terms of lagoon biology, I think the next priority would be to examine the "health" of the sea grass communities. Sea grasses are able to buffer perturbations in the oxidation state of the sediments and prevent dystrophic crisis, but if their capacity to do this is overwhelmed by development of anoxia, their roots can be poisoned by hydrogen sulphide. If such events occur frequently enough and persistent phytoplankton populations shade this submerged vegetation, sea grass communities can decline (Viaroli et al. 2008). Sea grasses are critical habitats that support extremely diverse biological communities, and their conservation should be a focus in Esquimalt Lagoon.

Esquimalt Lagoon is a unique local ecosystem providing a diversity of habitats for plants and animals. It is also an intriguing and picturesque location that is readily accessible to humans in an increasingly urban landscape. So, it is worthwhile to appreciate and understand this system and what we can do to allow it to function in its natural state.

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Appendices

Appendix A: Station coordinates

Table A.1 presents the geographic coordinates of sampling stations in Esquimalt Lagoon. Coordinates in are presented in both Universal Transverse Mercator and Latitude/Longitude.

Table A.1 Geographic coordinates of sampling stations for the current study in Esquimalt Lagoon. UTM stands for Universal Transverse Mercator.

Station	**UTM** Zone, easting, northing	**Latitude and Longitude** Degrees, minutes, seconds	Decimal degrees
1	10 U 465730 5364295	48 25 51.13 N 123 27 47.82 W	48.43087 -123.46328
2	10 U 465250 5363400	48 25 22.05 N 123 28 10.91 W	48.42279 -123.46970
3	10 U 465394 5363609	48 25 28.85 N 123 28 3.96 W	48.42468 -123.46777
4	10 U 465220 5363775	48 25 34.19 N 123 28 12.48 W	48.42616 -123.47013
5	10 U 465480 5364010	48 25 41.85 N 123 27 59.90 W	48.42829 -123.46664

Appendix B: Figures representing nutrient and chlorophyll *a* concentrations from the bottom sampling depth

The following figures present nutrient concentrations (Figs. B.1 and B.2) and chlorophyll *a* concentrations (Fig. B.3 and B.4) 20 cm above the sediments in Esquimalt Lagoon measured from August 2009 through January 2011 (current study) at five stations. The methods of acquisition of these data are described in section 2.3 of this thesis. Raw data can be accessed in supplements to the open-source electronic version of this thesis available from the online repository UvicSpace after August of 2013.

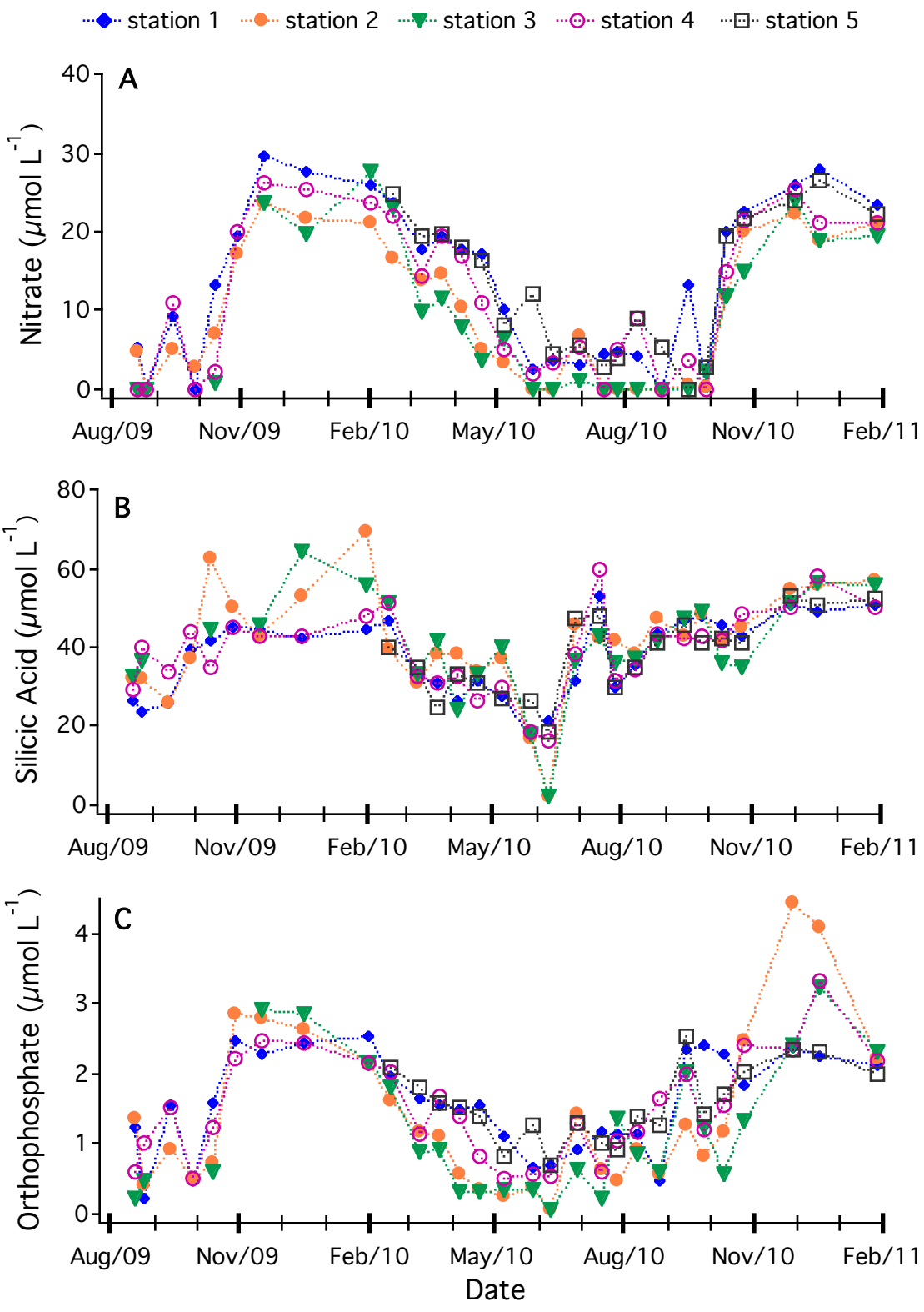

Figure B.1 Nitrate (A), silicic acid (B) and orthophosphate (C) concentrations 20 cm above the sediments at five stations in Esquimalt Lagoon during the current study.

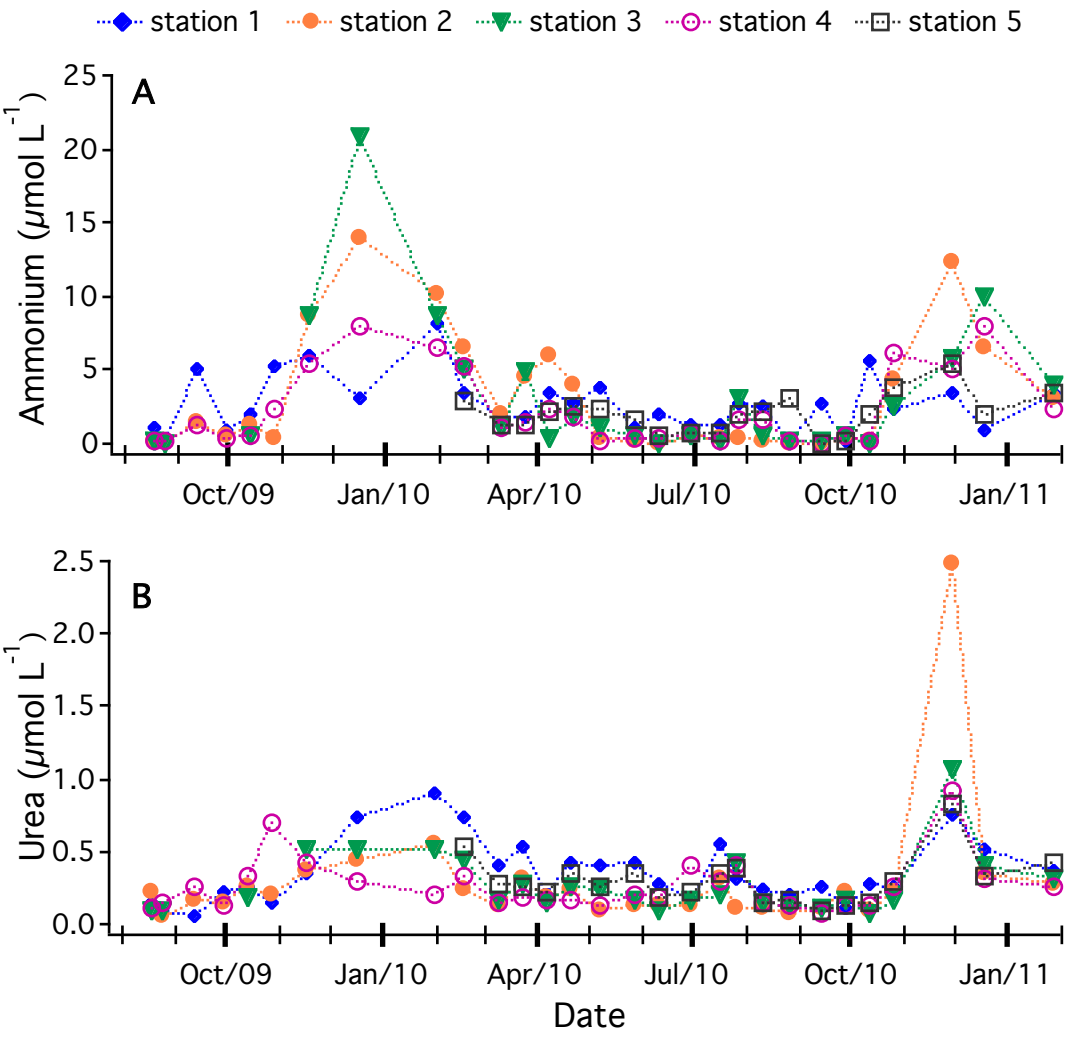

Figure B.2 Ammonium (A) and urea (B) concentrations 20 cm above the sediments at five stations in Esquimalt Lagoon during the current study.

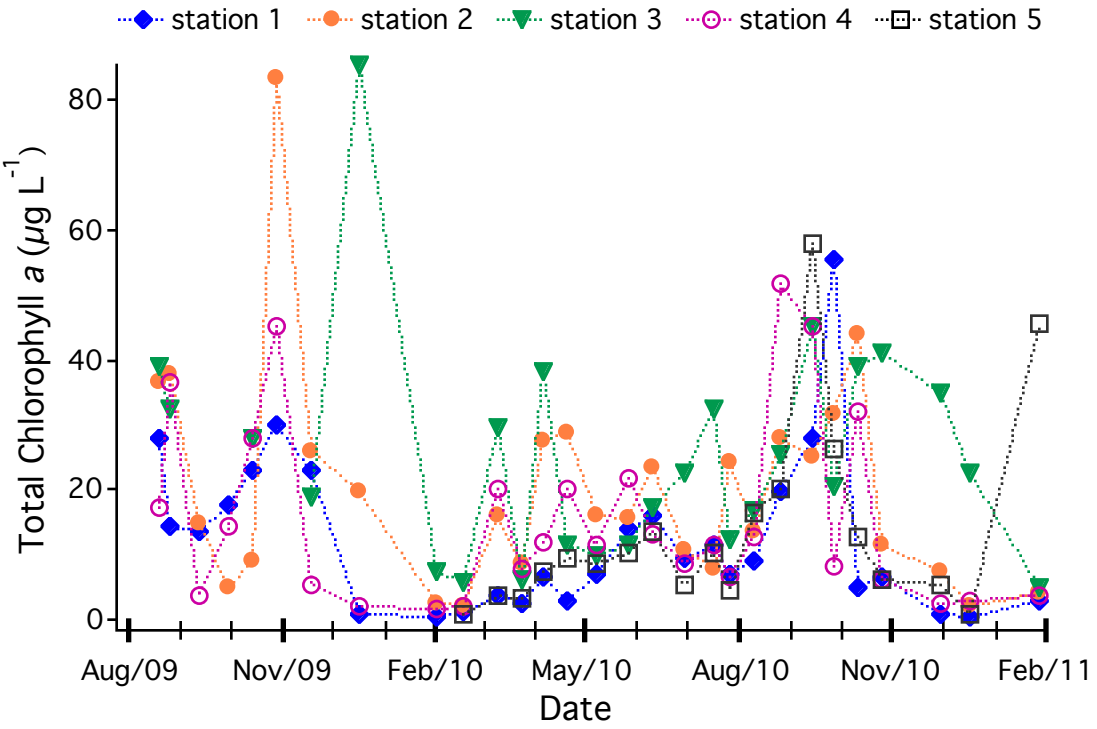

Figure B.3 Chlorophyll *a* concentrations 20 cm above the sediments at five stations in Esquimalt Lagoon during the current study.

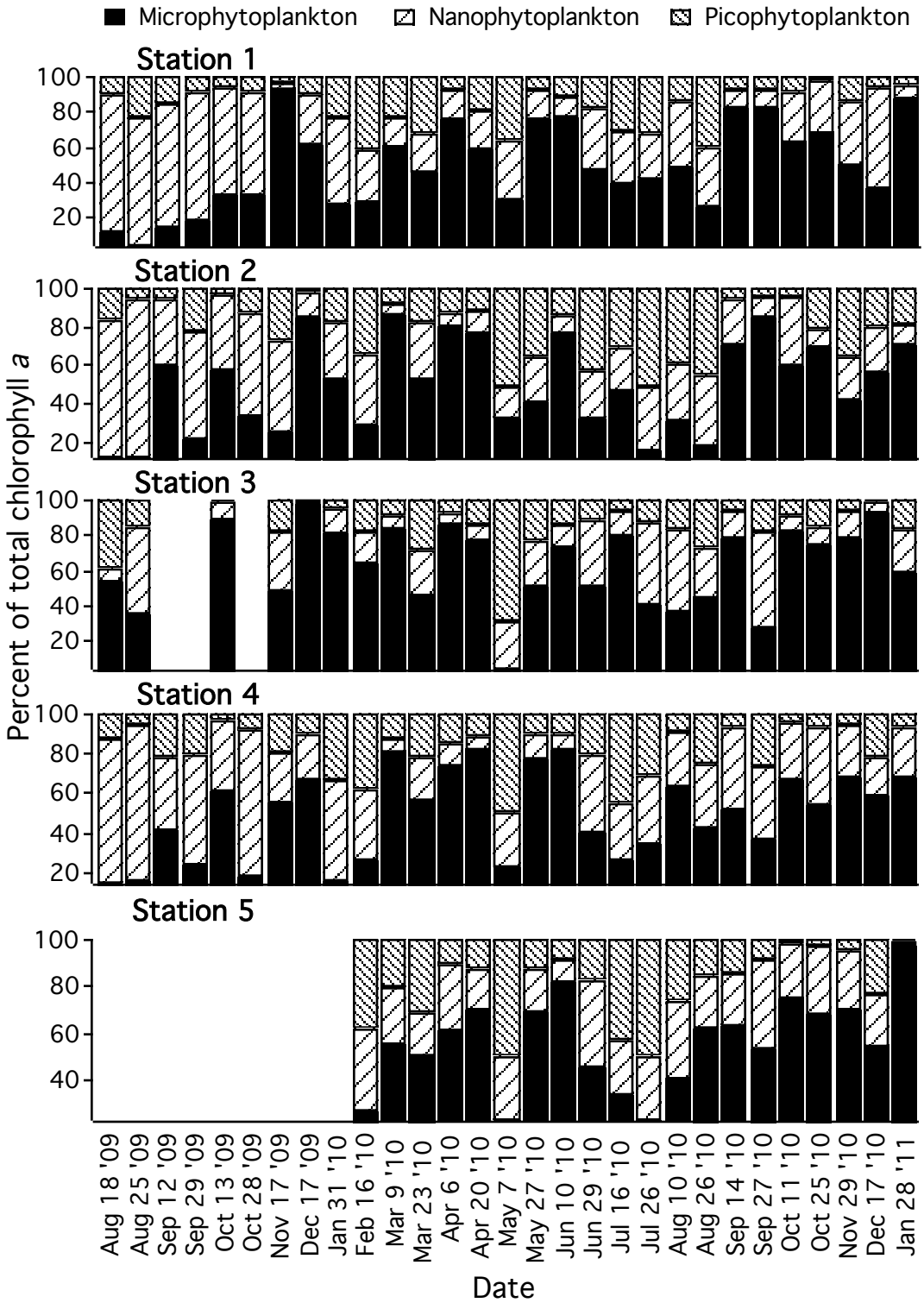

Figure B.4 Size fractionated chlorophyll *a* concentrations 20 cm above the sediments at five stations in Esquimalt Lagoon during the current study.

Appendix C: Water depths and Secchi disc depths

The methods of acquisition of the data presented in Table C.1 are described in section 2.3 of this thesis. Secchi disc depth was considered to be the depth of visual disappearance/reappearance of the Secchi disc, which was lowered on the side of the boat where glare coming off the surface of the water was minimal. The Secchi disc had a 20 cm diameter and alternating quadrants of black and white.

Table C.1 Water depths ("Depth") and Secchi disc depths ("Secchi") at five stations in Esquimalt Lagoon during the current study. When Secchi disc depth is the same value as water depth in the table, this means that the Secchi disc was visible at the bottom and that theoretically the Secchi disc depth would be greater than the water depth.

Date	Station 1		Station 2		Station 3		Station 4		Station 5	
	Depth	Secchi	Depth	Secchi	Depth	Secchi	Depth	Secchi	Depth	Secchi
18-Aug-09	300	170	240	90	160	90	240	160		
25-Aug-09	240	180	230	110	140	90	270	130		
12-Sep-09	310	300	240	200	140	140	270	270		
29-Sep-09	173	75	234	234	145	115	264	248		
13-Oct-09	300	50	240	240	165	165	285	285		
28-Oct-09	260	259	229	178	138	138	260	220		
17-Nov-09	285	180	305	180	245	180	370	210		
17-Dec-09	390	380	320	310	240	220	360	320		
31-Jan-10	320	320	290	290	195	195	305	305		
16-Feb-10	355	340	280	280	180	180	300	300	330	330
9-Mar-10	365	340	280	260	190	190	310	280	340	340
23-Mar-10	340	340	290	270	200	200	310	310	335	320
6-Apr-10	320	160	280	160	190	160	310	240	340	160
20-Apr-10	340	320	310	240	210	200	340	280	370	320
7-May-10	310	248	244	240	160	160	280	260	300	260
27-May-10	320	240	265	200	180	180	270	220	320	260
10-Jun-10	280	200	260	180	180	180	283	220	320	200
29-Jun-10	320	200	265	220	160	160	290	260	310	280
16-Jul-10	290	225	260	210	155	125	280	210	300	240
26-Jul-10	310	290	270	220	180	150	280	280	310	300
10-Aug-10	310	230	270	180	180	180	290	200	310	230
26-Aug-10	280	190	250	190	160	100	280	170	300	200
14-Sep-10	290	220	240	70	160	80	270	80	290	180
27-Sep-10	320	160	275	160	180	140	300	135	317	170
11-Oct-10	290	200	270	210	170	170	300	200	320	220
25-Oct-10	370	300	318	200	220	180	340	190	370	160
29-Nov-10	320	300	290	290	200	200	320	280	330	330
17-Dec-10	399	399	340	340	256	256	360	360	384	384
28-Jan-11	380	380	320	320	230	230	360	360	390	390

Appendix D: Natural $\delta^{15}N$ and $\delta^{13}C$ of particulates

The natural isotopic signature of planktonic particulates was measured from water samples collected on the main field day, or on the day following or previous to the main field day, at station 4 in Esquimalt Lagoon at 1.0 – 1.5 m below the water surface during the current study (Table D.1). Therefore, the date of collection is not always the same as the date of the main field day presented in Table 2.1. A large volume of water (20 L to 80 L) was filtered onto 0.7 μm glass fiber filters and two sub-samples (representing volumes anywhere from 100 mL to 1 400 mL) of lagoon particulates were taken from these filters and analysed at the University of California Davis Stable Isotope Facility.

Table D.1 Natural isotopic signatures ($\delta^{15}N$ and $\delta^{13}C$) of planktonic particulates from Esquimalt Lagoon measured at station 4, 1 m – 1.5 m below the surface, during the current study. Date of collection is not always the same as the date of the main field day presented in Table 2.1. Two sub-samples "Rep" were collected.

Date Collected	Rep	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
18-Aug-09	1	8.22	-21.75
	2	8.13	-21.50
25-Aug-09	1	8.35	-21.22
	2	8.46	-20.46
12-Sep-09	1	6.48	-21.74
	2	4.04	-22.63
29-Sep-09	1	9.67	-20.13
	2	9.88	-20.46
13-Oct-09	1	9.43	-22.63
	2	9.45	-22.72
29-Oct-09	1	4.89	-24.14
	2	4.94	-24.43
16-Nov-09	1	2.55	-23.53
	2	6.78	-23.97
18-Dec-09	1	5.96	-21.84
	2	4.07	-22.28
30-Jan-10	1	2.82	-22.86
	2	1.58	-23.28
17-Feb-10	1	2.34	-23.85
	2	4.57	-23.68
08-Mar-10	1	7.99	-19.35
	2	7.94	-19.40
24-Mar-10	1	7.61	-21.85
	2	8.58	-21.86
07-Apr-10	1	8.71	-19.43
	2	9.46	-19.09
19-Apr-10	1	8.80	-19.24
	2	9.20	-19.00
06-May-10	1	7.78	-21.47
	2	8.52	-21.20
28-May-10	1	8.84	-19.03
	2	8.60	-18.77
11-Jun-10	1	8.48	-17.56
	2		
30-Jun-10	1	8.79	-19.15
	2	8.70	-19.22
15-Jul-10	1	8.54	-19.59
	2	8.40	-19.15
27-Jul-10	1	8.99	-18.95
	2	9.71	-18.97
11-Aug-10	1	10.18	-20.52
	2	10.45	-20.54
27-Aug-10	1	7.56	-19.65
	2	7.99	-19.56
13-Sep-10	1	8.77	-19.85
	2	8.19	-19.84
28-Sep-10	1	8.53	-21.40
	2	7.84	-21.21
12-Oct-10	1	9.22	-19.46
	2	8.94	-19.44
26-Oct-10	1	8.58	-19.57
	2	8.05	-19.81
30-Nov-10	1	4.45	-22.02
	2		-22.09
29-Jan-11	1	5.64	-23.38
	2	5.29	-23.91

Appendix E: Presence of microsporine-like amino acids

Samples to test for microsporine-like amino acids (MAAs) were collected in the late summer and fall of 2010, in the top 20 cm of the water column at station 4 and near the western shoreline of the lagoon. Presence of foam on the water surface is an indication that MAAs could be present (Jessup et al. 2009), and the reason that samples were collected near the western shoreline was that foam was visible here on certain dates. Station 4 is a relatively central and deep station in the lagoon (see Fig. 1.1). Water samples were filtered with a 0.2 μm polycarbonate filter into a 1 cm quartz cuvette and absorbance was scanned spectrophotometrically at wavelengths between 240 nm – 500 nm. Presence of MAAs is suggested by a "shoulder" in the ultra violet range (R.M. Kudela, University of California Santa Cruz, personal communication 2010), and in the current study, this can be seen in the September 13 spectrum and to a lesser extent in the September 28 spectrum (Fig. E.1). This shoulder was not pronounced on the other dates, although the similarity of the spectra from August 27 to the that of September 28 could suggest that MAAs were present in low abundance.

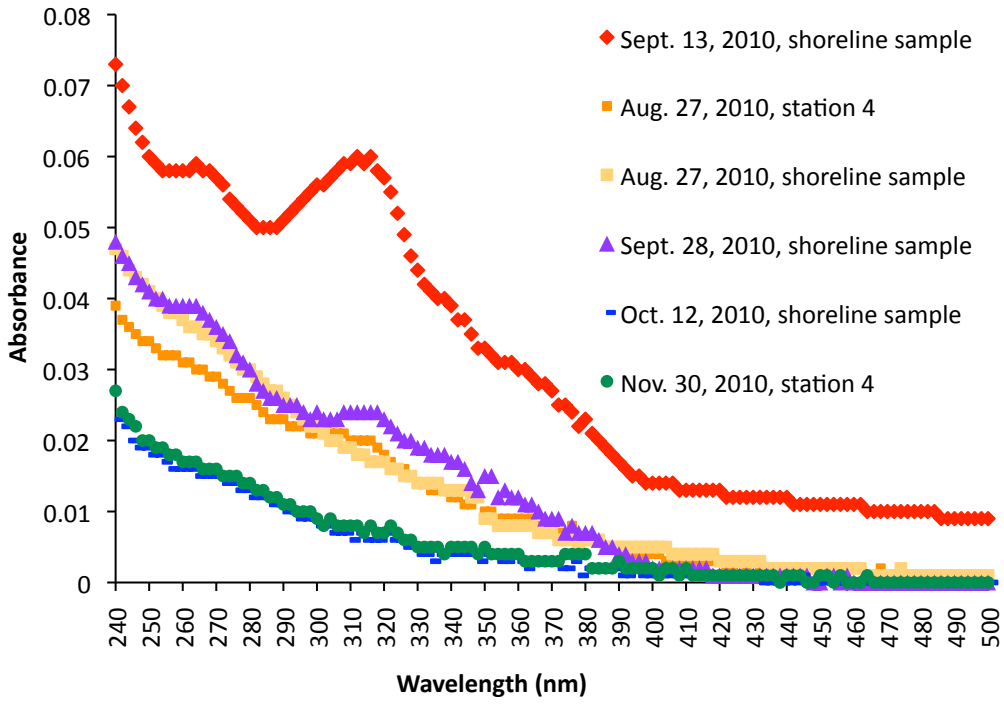

Figure E.1 Absorbance spectra testing for the presence of microsporine-like amino acids in Esquimalt Lagoon. Samples were collected in 2010 on August 27, September 13, September 28, October 12, and November 30 at station 4 and on the western shoreline of the lagoon, in the top 20 cm of the water column. Only samples that were successfully measured are displayed.