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Katherine L. (Katherine Leigh) Brown  
*Western Washington University*

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***SYNECHOCOCCUS* DISTRIBUTION AND ABUNDANCE IN THE SAN JUAN  
ARCHIPELAGO, SALISH SEA**

By

Katherine Leigh Brown

Accepted in Partial Completion  
Of the Requirements for the Degree  
Master of Science

Kathleen L. Kitto, Dean of the Graduate School

ADVISORY COMMITTEE

Co-Chair, Dr. Suzanne Strom

Co-Chair, Dr. Benjamin Miner

Dr. Jude Apple

## **MASTER'S THESIS**

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Katherine Leigh Brown

October 9, 2013

***SYNECHOCOCCUS* DISTRIBUTION AND ABUNDANCE IN THE SAN JUAN  
ARCHIPELAGO, SALISH SEA**

A Thesis  
Presented to  
The Faculty of  
Western Washington University

In Partial Fulfillment  
Of the Requirements for the Degree  
Master of Science

By  
Katherine Leigh Brown  
August 2013

## ABSTRACT

*Synechococcus*, a unicellular cyanobacterium of about one micron in size, is one of the most prolific and abundant primary producers worldwide and, hence, has an important role in the phytoplankton community. This study sought to determine 1) the distribution and abundance of *Synechococcus* in the eastern San Juan Archipelago; 2) the environmental variables related most closely to abundance; and 3) the key grazers of *Synechococcus* in this ecosystem. Two stations were chosen, East Sound near Orcas Island, WA and Rosario Strait near Lopez Pass, for their differing hydrographic conditions. Sampling was conducted from June to September 2012. Water samples were taken at three depths at both stations twice a month June through August, and then approximately every three days for three weeks in September. A CTD (Conductivity, Temperature, and Depth) was lowered at each station to obtain environmental data from the water column. Water samples were used for nutrient analysis, size-fractionated chlorophyll *a* analysis, and for the enumeration of *Synechococcus* and the protist grazer community.

*Synechococcus* abundance rose as high as  $1.5 \times 10^4$  cells  $\text{ml}^{-1}$  at both East Sound and Rosario Strait in August. *Synechococcus* abundance and depth distribution were nearly the same at both stations despite the well-mixed environment at Rosario and the more frequently stratified environment at East Sound. Both stations were abundant in nitrate+nitrite and phosphate throughout the sampling period. However, chlorophyll *a* concentrations were unusually low July through August, a season that usually exhibits variable and episodically high concentrations. Of all the environmental variables analyzed, only salinity was correlated with *Synechococcus* abundance at both stations, and that correlation was negative. The importance of salinity as a predictor of abundance may be due to a physiological effect of fresher water that allows for increased biomass production, or simply to the dominant effect of salinity on water column stratification, which may provide a preferable growth environment for *Synechococcus*. Ciliates, heterotrophic nanoflagellates, and dinoflagellates were observed with ingested *Synechococcus*. Surprisingly, nanoflagellates were rarely observed with ingested cells. Dinoflagellates seemed to be the key grazers of *Synechococcus* in the eastern San Juan Archipelago, but there was no clear temporal pattern to the level of *Synechococcus* ingestion by any of the aforementioned grazers.

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First and foremost, my warmest thanks go to my thesis advisor, Dr. Suzanne Strom, for her patience and support as well as the time she spent working with me to make this thesis the best it could be. Thanks to my co-advisor, Dr. Benjamin Miner, for his excellent ideas and open door. Another big thanks goes to my committee member, Dr. Jude Apple, for his constant encouragement and guidance through the maze of multivariate statistics. This work could not have been accomplished without the help of many people at Shannon Point Marine Center including the Strom lab: Kelley Bright and Kerri Fredrickson for teaching me all I know about epifluorescent microscopy, and Amelia Kolb for her assistance and companionship in the field, and for teaching me the lab techniques needed for this project. Thanks to Dr. Steve Sulkin and Gene McKeen for logistical and financial support for field work. Thank you to the captains of the R/V Zoea, Nate Schwarck and Jay Dimond, who gave their time and effort to providing a safe and successful field season. Thank you to Horng-Yuh Lee for her help conducting the nutrient analysis. This thesis was also supported financially by a grant from the National Science Foundation (1021189).

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## INTRODUCTION

The base of the ocean's food web is composed of photosynthetic microorganisms called phytoplankton, which are essential to ocean ecosystems because they are the major primary producers (Pomeroy 1974). Life for a population of phytoplankton in the euphotic zone involves both gains and losses. In order for a phytoplankton bloom to occur, rates of gain must exceed rates of loss. Gain processes include cell division as well as water movement, or advection from an area rich in phytoplankton (Banse 1992). Because phytoplankton are microalgae, continued cell division can occur only when both light and nutrients are available for photosynthesis and biomass production. However, even if there was an uninhibited supply of light and nutrients, population growth could also be diminished by losses. These loss processes include sinking out of the euphotic zone, dilution by moving into an area of poor phytoplankton concentration, grazing by predators, and death by age, parasites, or viruses (Banse 1992). These processes must be taken into consideration when thinking about how a bloom occurs, and which variables might be affecting its successful initiation and eventual termination. The focus of this study is on growth related to light, nutrients, and movement of water masses, along with loss from grazing by protists.

There is a food web of microbial producers and consumers called the microbial loop amid the ocean food web (Pomeroy 1992). In the microbial loop, dissolved organic matter (DOM) and inorganic nutrients released from phytoplankton by excretion, exudation, and diffusion is returned to the food chain through bacteria and grazing of bacteria by flagellates (3 to 10  $\mu\text{m}$ ) and microzooplankton (10 to 80  $\mu\text{m}$ ), including protist grazers such as ciliates (Azam et al. 1983). Production from the microbial community is either lost to trophic transfers or released as dissolved material such as ammonium, phosphate, and DOM within

the euphotic zone (Calbet and Landry 2004). Picophytoplankton, to which *Synechococcus* belongs, accounts for 40% of this ocean primary productivity (Tai and Palenik 2009). Due to this significant primary production, studies of both picophytoplankton abundance and microzooplankton grazing are of particular interest in determining the fate of carbon in the ocean's food webs, and especially in the microbial loop (Weinbauer et al. 2011).

Predator-prey relationships are a key part of the microbial loop because these interactions result in lost production and allow for organic material and nutrients to become available to higher and lower trophic levels. The fate of most phytoplankton cells, such as *Synechococcus*, is to be consumed by grazers (Strom 2002). Grazers of *Synechococcus* include protists, such as heterotrophic nanoflagellates, dinoflagellates, and ciliates (Apple et al. 2011, Dolan and Simek 1999, Frias-Lopez 2009). There is not agreement on which of these protists are the most important grazers of *Synechococcus*, although heterotrophic nanoflagellates and ciliates are typically pointed to as the primary grazers (Apple et al. 2011, Frias-Lopez 2009, Hirose et al. 2008). However, dinoflagellates are able to consume prey of diverse size including small *Synechococcus* cells (Jeong et al. 2010). Knowledge of when and if protist grazers ingest *Synechococcus* cells is important for gaining an understanding of these particular predator-prey relationships.

*Synechococcus* are unicellular, photosynthetic cyanobacteria of about one micron in size. Multiple clades of *Synechococcus* can co-exist within a small geographic region in marine habitats like coastal and oceanic waters (Strom et al. 2012, Zwirgmaier et al. 2008). These typically coccoid cells divide by binary fission and contain phycobilisomes that serve as the major light-harvesting antenna (Ting et al. 2002). *Synechococcus* also have a gram-negative cell organization with a cell envelope including a cytoplasmic membrane and a

surface protein layer (S-layer; Strom et al. 2012). *Synechococcus* are also the only bacteria known to swim without flagella (McCarren and Brahamsha 2005). The cell surface is used to create thrust and two cell surface proteins are necessary to produce this non-flagellar swimming (McCarren and Brahamsha 2009). Overall, *Synechococcus* are ecologically important contributing a significant amount of global primary production (Strom et al. 2012).

Worldwide, there have been studies on *Synechococcus* abundance and its relation to environmental factors. These studies show that *Synechococcus* abundance can range up to  $10^6$  cells  $\text{ml}^{-1}$  (Zwirgmaier et al. 2008) and that growth rates differ among ecosystems. In northern Baja California, the most productive conditions for *Synechococcus* were seen after nutrients were delivered to surface waters by upwelling events (Linacre et al. 2010). In this same area, nano and micrograzer consumption averaged 66% of the phytoplankton growth (Linacre et al. 2010). In the Mediterranean Sea, *Synechococcus* abundance varied from 0.07 to  $5.5 \times 10^4$  cells  $\text{ml}^{-1}$  (Christaki et al. 2002) while another study of a Mediterranean system showed that *Synechococcus* growth rate was maximal in the summer when temperatures and irradiance were high (Agawin et al. 1998). Thus far, little work has been done on *Synechococcus* in the coastal waters of the Salish Sea so it is uncertain how abundance varies with season in this area. *Synechococcus* is generally most abundant in summer and least abundant in winter (Li 1998). Temperature has been found to be the dominant factor affecting the growth and loss of *Synechococcus* (Li 1998). Temperature has also been found to be negatively correlated with *Synechococcus* abundance in Qingdao, China (Wang et al. 2010) and positively correlated with *Synechococcus* abundance in Virginia as well as San Francisco Bay (Moisan et al. 2010, Ning et al. 2000). Correlations between *Synechococcus* abundance and salinity have also been observed. A positive correlation between

*Synechococcus* abundance and salinity was found in Qingdao, China (Wang et al. 2010) whereas, in the Salish Sea, *Synechococcus* abundance was negatively correlated with salinity (Schanke and Strom, unpublished data). This negative correlation with salinity was of particular interest because of its uniqueness to the Salish Sea.

The Salish Sea, which includes the Strait of Georgia, Strait of Juan de Fuca, and Puget Sound, is a region with diverse oceanographic features that affect phytoplankton growth. In the Strait of Georgia, nitrate is an especially important nutrient fueling primary productivity and therefore, changes in the nitracline with tidal cycling impact the amount of production (Yin et al. 1997). Phytoplankton communities of coastal seas are usually dominated by taxa adapted to nutrient inputs driven by physical processes (Lucas et al. 2011). This is true of the Strait of Georgia where phytoplankton biomass and productivity are high in summer due to the combined forces of wind, river discharge, and tides acting to cause vertical mixing (Yin et al. 1997). The Strait of Juan de Fuca is the major entry/exit for much of the estuarine flow of the Salish Sea (Mackas and Harrison 1997). Fast tidal currents in the entry to the Pacific Ocean create strong vertical mixing resulting in weakly stratified water flowing into the region (Mackas and Harrison 1997). Mixing of these water masses with varying phytoplankton cell abundances can cause an increase or decrease in production while grazing and viral lysis lead to losses (Sosik et al. 2003). The combined interactions of nutrients and physical processes like tidal mixing have an important impact on the overall abundance of phytoplankton like *Synechococcus*.

The aim of this study was to determine the distribution and abundance of *Synechococcus* at two contrasting locations in the Salish Sea, and to examine the relationships between environmental factors and *Synechococcus* abundance and vertical

distribution. I also sought to identify the key grazers of *Synechococcus* at East Sound and Rosario. I expected that *Synechococcus* distribution and abundance would vary seasonally and that salinity would have the strongest correlation with abundance. Along with salinity, I hypothesized that tidal range would have an effect on *Synechococcus* abundance as tidal mixing is an important process in the Salish Sea. I also expected that dinoflagellates would be important grazers of *Synechococcus*. A comparison of seasonal environmental variation, as well as the differences in abundance and distribution of *Synechococcus* at these stations, provides information about how the environment affects *Synechococcus* biomass production.

## MATERIALS AND METHODS

Two stations in the eastern San Juan Archipelago were chosen for sampling—East Sound at 48°38.624" N, 122°52.851" W and Rosario Strait near Lopez Pass at 48°26.915" N, 122°45.579" W (Figure 1). These stations were chosen for their distinct oceanographic features. At Rosario, water masses from the Strait of Juan de Fuca and the Strait of Georgia join, causing mixing to occur; the station is also exposed to winds (Figure 1). The other station, East Sound, differs from Rosario South in that it is located in the center of a fjord. The sill at the entrance reduces water exchange and the surrounding hills provide partial shelter from the wind. Due to the reduced water column mixing, there are often distinct thermoclines and haloclines at East Sound (Twardowski and Donaghay 2001).

Thirteen cruises were conducted aboard the R/V Zoea from June to September 2012. At each station, a SBE 19plus V2 Conductivity, Temperature, Depth (CTD) was lowered to approximately 30-35 m to obtain depth profiles of temperature, salinity, chlorophyll fluorescence, photosynthetically active radiation (PAR), and density ( $\sigma_t$ ). Water samples were collected at three depths using a Van Dorn bottle. A depth of 5 m was always sampled to represent the near-surface community, while the other two depths were chosen based on the vertical profile on the day of sampling. Specifically, depths were chosen at, above, and below the chlorophyll maximum layer to gain a better picture of the near-surface community. However, water was not sampled below 20 m because *Synechococcus* was not expected to be found in abundance below this depth due to typical exponential decay of light. In East Sound, surface depth was from 2-5 m, mid depth was from 5-12 m, and deep depth was from 10-18 m. In Rosario, surface depth was from 1.5-5 m, mid depth was from 5-10 m,



Figure 1. Map of sampled stations, East Sound and Rosario, in the San Juan Archipelago. Shannon Point Marine Center, Anacortes, WA and Bellingham, WA are noted for reference.



and deep depth was from 10-18 m. Water samples were collected in two-liter Teflon bottles that had first been rinsed with sample water. These were then placed in a cooler with surface seawater to be brought back to the laboratory. I used samples to make slides and measure *Synechococcus* abundance and ingestion of *Synechococcus* by protists in the community, as well as for chlorophyll *a*, nitrate, and phosphate analyses. Light data (incident irradiance) was obtained via a Li-Cor 2 $\pi$  quantum sensor that continuously recorded PAR at Skyline Marina in Anacortes, WA. Tide data were obtained from Tides and Currents for Windows software program.

Upon return to the laboratory, water samples were preserved in amber glass bottles. For each depth, a sample was preserved for the enumeration of *Synechococcus* and another sample was preserved for counting protist grazers in the community. An appropriate amount of 10% glutaraldehyde and DAPI stain were added to the amber glass bottles to allow for 20 ml *Synechococcus* samples (1.5 ml 10% glutaraldehyde) and 40 ml community samples (2.5 ml 10% glutaraldehyde, 3 drops DAPI) with final concentrations of 0.5% glutaraldehyde and 1  $\mu$ M DAPI. DAPI was only added to the community samples. Both samples were pre-screened with 100  $\mu$ m mesh to dispose of chain diatoms and other large particles. Fixed samples were refrigerated overnight at 4 °C, and then filtered the next day to make slides to examine under an epifluorescent microscope. For *Synechococcus* samples, 20 ml were filtered on 0.6  $\mu$ m pore size polycarbonate membrane filters over 0.65  $\mu$ m pore size backing filters. For community samples, 40 ml were filtered on 1.0  $\mu$ m pore size polycarbonate membrane filters over 1.2  $\mu$ m pore size backing filters. Finished slides were then stored in a freezer for later analysis.

## *Enumeration of Synechococcus and the Protist Community*

*Synechococcus* slides were counted under oil immersion (1000x total magnification) and blue light excitation. In June, when *Synechococcus* abundance was low, counting consisted of four transects per slide. Counting with transects involved counting all *Synechococcus* cells along a measured diameter of the slide and using that number and the filter diameter to estimate the number of cells on the entire slide. For July-September, grid counting of approximately 250 cells per slide occurred. Grid counting involved counting all *Synechococcus* cells within multiple grids of known area. The number of grids and number of cells counted were recorded and used to estimate the total number of cells on the slide.

Community slides from July 24<sup>th</sup> to September 10<sup>th</sup> were chosen for analysis due to the high abundance of *Synechococcus* during this time. For each slide, grazers were sorted into groups of taxa and size, counted, and examined to estimate the number of ingested *Synechococcus*. Ciliates, dinoflagellates, and nanoflagellates were quantified. However, ciliates do not preserve well with glutaraldehyde so the ciliate counts may not best represent these particular grazers. Counting consisted of looking at two size fractions of grazers—those greater than 20  $\mu\text{m}$ , and those less than 20  $\mu\text{m}$ . The large size fraction was counted at a total magnification of 400x while the smaller size fraction was counted under oil at a total magnification of 1000x. For the large size fraction, transects across the slide were used to identify 100 grazers and record how many *Synechococcus* cells were ingested for each grazer. The recorded area of the transects and filter diameter were used to calculate the abundance of each grazer taxon. Taxa enumerated included ciliates, autotrophic *Gymnodinium* and *Gyrodinium*, heterotrophic *Gymnodinium* and *Gyrodinium*, thecate dinoflagellates, *Dinophysis*, *Ceratium fusus*, and other dinoflagellates. For the smaller size

fraction, grid counting was used to count 100 grazers and their ingested *Synechococcus* cells in the following taxa: nanoflagellates, cryptophytes, heterotrophic *Gymnodinium* and *Gyrodinium*, and other dinoflagellates.

Issues occurred with slides and chlorophyll samples from the August 6<sup>th</sup> cruise. When slides were analyzed, it appeared that most *Synechococcus* cells had lost their orange fluorescence. Slides were counted based on the shape and size of normal *Synechococcus* cells, but the data for this day may be inaccurate. Chlorophyll samples for Rosario depths two and three were also strange on this day with undetectable concentrations.

#### *Chlorophyll a Analysis*

Chlorophyll *a* concentration was determined using a cascade-type filtration system with three size fractions: >20  $\mu\text{m}$ , 5-20  $\mu\text{m}$ , and <5  $\mu\text{m}$ . Filters were 47 mm polycarbonate with pore sizes of 20 and 5  $\mu\text{m}$ , and 25 mm glass fiber filters (GFF) with a 0.7  $\mu\text{m}$  effective pore size. Water samples were first inverted to mix and then 250 ml were filtered through the system. Each filter was folded and added to a tube of 6 ml of 90% acetone. The tubes were stored in a -20  $^{\circ}\text{C}$  freezer for a 24-hour extraction period. The next day, the tubes were taken out, vortexed, and the filters were removed. The tubes were centrifuged for five minutes at 5000 RPM and then placed in a Turner 10-AU fluorometer to read each sample for its initial fluorescence ( $F_0$ ) value. Two drops of 1N HCl were added and the acidified fluorescence ( $F_a$ ) value was measured and used to find the chlorophyll *a* concentration.

### *Long Term PAR Data Analysis*

An analysis of Shannon Point Marine Center's long term PAR data (2002-2012) was conducted to observe PAR anomalies. Data were obtained via a Li-Cor  $2\pi$  quantum sensor that continuously recorded PAR at Skyline Marina in Anacortes, WA. Daily PAR data were averaged over two week periods for each year. Anomalies for these periods were calculated based on the difference between the period's average for all years combined and the average for that specific year. When more than five days of data were missing from the two week period that was averaged, that two week period was not used in the analysis.

### *Nutrient Analysis*

Nitrate and phosphate samples were prepared by filtering about 60 ml of sample water through a syringe with 25 mm GFF filters attached at the end into a scintillation vial. The syringe and plunger were first rinsed with sample water and then the scintillation vials were rinsed with the filtered sample water. Two vials were filled with about 20 ml for each depth, one for nitrate and one for phosphate analysis. Samples were stored in a  $-40^{\circ}\text{C}$  freezer for later processing.

Both nitrate and phosphate samples were analyzed using the Lachat procedure on a QuikChem 8500 autoanalyzer. Lachat method 31-107-04-1-G was used for nitrate+nitrite analyses for concentrations of 0.01 – 1.0 mg N/L (0.71 – 71  $\mu\text{M}$ ). Lachat method 31-115-01-1-H was used for phosphate analyses for concentrations of 1 – 1.0 mg N/L (0.16 – 12.9  $\mu\text{M}$ ). A water sample was passed through a copperized cadmium column where nitrate was reduced to nitrite (Parsons et al. 1984). Nitrite was diazotized with sulfanilamide and coupled

with N-(1-naphthyl) ethylenediamine dihydrochloride to form a light pink azo dye. This dye was then detected spectrophotometrically at 540 nm, and the nitrate+nitrite concentration was determined by comparing the absorbance signal with various standard concentrations of nitrate. For phosphate, a mixed reagent was created with ammonium molybdate, antimony potassium tartrate, sulfuric acid, and ascorbic acid. The addition of 0.5 ml of the mixed reagent to 5 ml of the standards or samples produced a blue color that was measured using a spectrophotometer at 885 nm, and the phosphate concentration was determined by comparing the absorbance signal with various standard concentrations of phosphate.

### *Statistical Analysis*

A one-way analysis of variance (ANOVA) was conducted using SPSS Software to determine whether there were any differences in the distribution of *Synechococcus* abundance with depth ( $\alpha = 0.05$ ). For the following statistical analyses, analyses were conducted separately by station. A Pearson product-moment correlation was performed using R software to examine the relationships between *Synechococcus* abundance and environmental variables. Principal components analysis (PCA) was performed using JMP software to observe relationships amongst environmental variables and *Synechococcus* abundance at East Sound and Rosario. A correlation matrix was used and the principal components were kept based on the cumulative percentage of variability reaching over 50%. Twelve parameters were used in PCA—depth, temperature, salinity, PAR, tidal range, total chlorophyll *a*, chlorophyll size fractions < 5  $\mu\text{m}$ , 5-20  $\mu\text{m}$ , and > 20  $\mu\text{m}$ , phosphate ( $\text{PO}_4$ ), nitrate+nitrite ( $\text{NO}_3+\text{NO}_2$ ), and *Synechococcus* abundance.

## RESULTS

*Synechococcus* abundance in East Sound varied over the summer from 11 cells ml<sup>-1</sup> at mid depth on June 11<sup>th</sup> to 1.56 x 10<sup>4</sup> cells ml<sup>-1</sup> at the deep depth on August 6<sup>th</sup> (Figure 2A). Abundance of cells was not significantly different with depth ( $F_{2, 75} p = 0.994$ , one-way ANOVA). On August 6<sup>th</sup> there was the largest bloom in *Synechococcus*, with concentrations ranging from 1.33 to 1.56 x 10<sup>4</sup> cells ml<sup>-1</sup> in East Sound at the three sampled depths. Within two weeks (August 20<sup>th</sup>), this bloom had quickly dissipated to 4.62 x 10<sup>3</sup> cells ml<sup>-1</sup> at the surface. On September 10<sup>th</sup>, there was a small fall bloom with abundance up to 6.87 x 10<sup>3</sup> cells ml<sup>-1</sup> at mid depth. *Synechococcus* remained at low abundances for most of September with the exception of the mid depth on September 24<sup>th</sup> with an abundance of 8.52 x 10<sup>3</sup> cells ml<sup>-1</sup>.

*Synechococcus* abundance was similar in Rosario with a bloom on August 6<sup>th</sup> from 1.36 to 1.55 x 10<sup>4</sup> cells ml<sup>-1</sup> (Figure 2B). There was also a fall bloom between 4.64 and 5.49 x 10<sup>3</sup> cells ml<sup>-1</sup> at the sampled depths. In contrast to East Sound, *Synechococcus* abundance over the month of September mostly decreased. Distribution of *Synechococcus* also appeared less variable among depths in Rosario in comparison with East Sound.

### *Environmental Data*

#### *Temperature*

Temperature varied with depth throughout the summer as the water column shifted between stratified and well-mixed in East Sound (Figure 3A). Temperatures in the upper 30 m ranged from 9.5 to 14.5 °C (Appendix A). Throughout the month of June, surface water

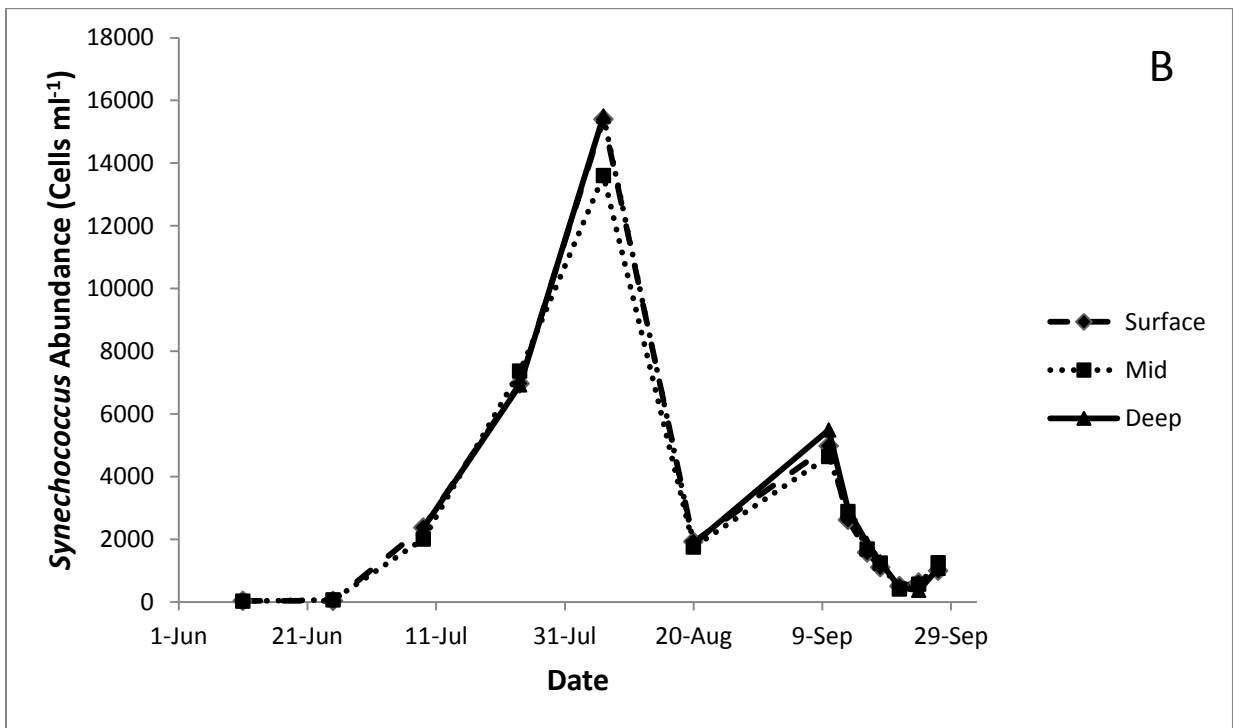
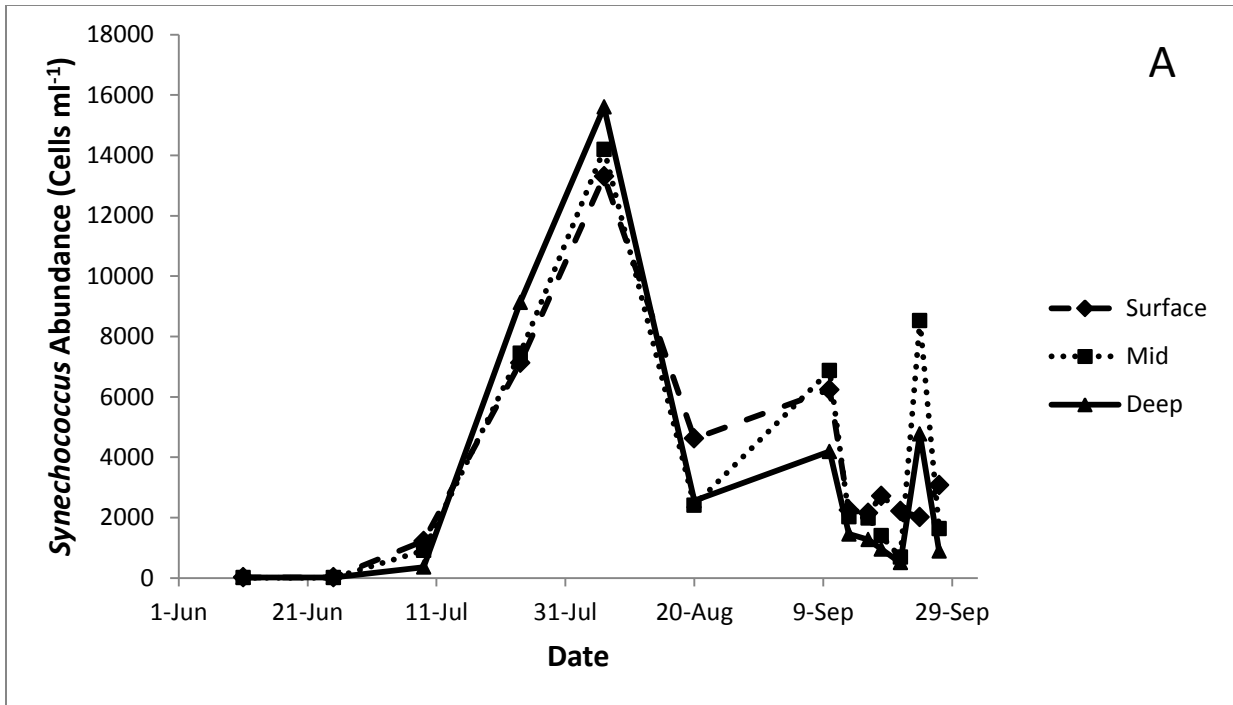


Figure 2. *Synechococcus* abundance (cells ml<sup>-1</sup>) from June 11 to September 27, 2012 in A. East Sound, and B. Rosario. There is no deep depth data for the first two sampled dates, June 11<sup>th</sup> and June 25<sup>th</sup> in Rosario.

warmed and by July 24<sup>th</sup>, a deep thermocline formed with the surface warmed to 14.8 °C. On August 6<sup>th</sup> (the day of the major bloom), there was not a clear thermocline, but there were still some small layers and by August 20<sup>th</sup>, a thermocline had formed near the surface. Surface temperatures started to decrease in the month of September. By mid-September, the water column in East Sound had become almost completely mixed.

Compared with East Sound, Rosario was well mixed more often (Figure 3B). In Rosario, temperatures ranged from 9.0 to 12.6 °C (Appendix A). The first sampling date on which stratification was readily apparent was August 6<sup>th</sup>, the day of the major bloom, with the surface water warmed to about 12.6 °C. On August 20<sup>th</sup>, the surface again cooled to 11 °C but by September 10<sup>th</sup>, the surface had warmed and there were layers in the water column. Throughout the rest of September, the water column cooled and became well-mixed once again. There was a positive correlation between temperature and *Synechococcus* abundance at Rosario (Table 1). However, temperature and *Synechococcus* abundance were not correlated at East Sound.

### *Salinity*

As the water temperature increased at the surface, surface water masses also became fresher. Consequently, the salinity was less at the surface in late July through late August in East Sound, ranging from 27.2 to 28.2 psu (Figure 4A). Water at all depths became more saline through September. Over the sampling period, salinity ranged from 27.3 to 30.4 psu (Appendix B).



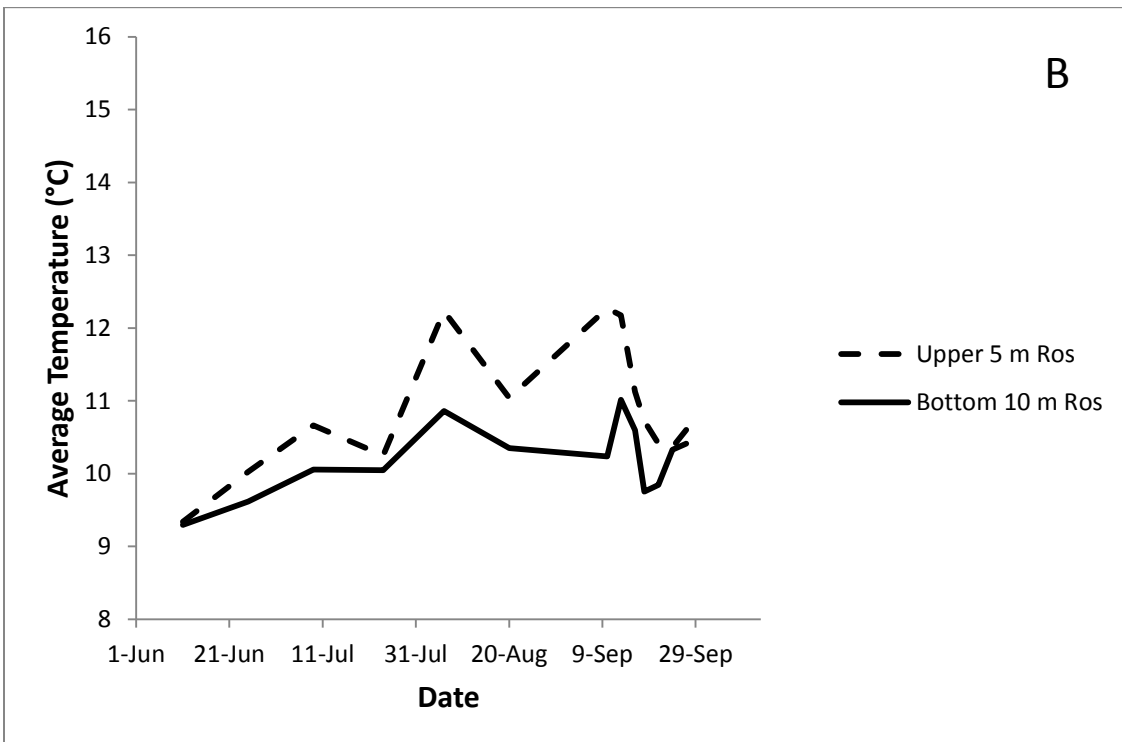
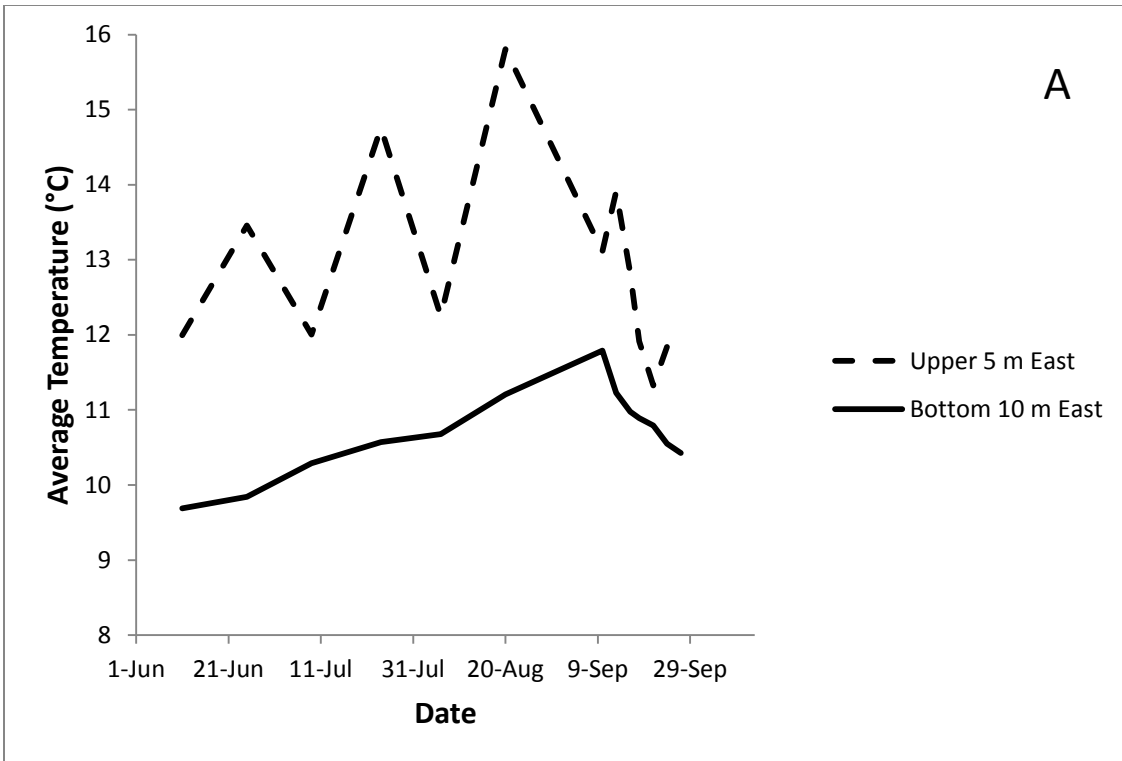


Figure 3. Average temperature (°C) from June 11 to September 27, 2012 in the upper 5 m and bottom 10 m in A. East Sound (East) and, B. Rosario (Ros). Temperature was averaged over the upper 5 m and the bottom 10 m of sampling depths.

Table 1. Correlation table showing Pearson product-moment correlation coefficients,  $r$ , for *Synechococcus* abundance and environmental variables as well as nutrients, nitrate+nitrite and phosphate, and chlorophyll  $a$  at East Sound and Rosario. P-values are also shown for the corresponding correlations. P-values less than 0.05 are in bold.

	East Sound		Rosario	
	$r$	p-value	$r$	p-value
Depth	0.135	0.412	0.285	0.087
Temperature	0.307	0.057	0.529	<b>0.001</b>
Salinity	-0.432	<b>0.006</b>	-0.715	<b>&lt; 0.001</b>
Fluorescence	-0.334	<b>0.037</b>	-0.194	0.25
PAR	-0.092	0.576	-0.233	0.165
Tidal Range	-0.305	0.059	-0.478	<b>0.003</b>
NO <sub>3</sub> +NO <sub>2</sub>	0.368	<b>0.021</b>	-0.057	0.74
PO <sub>4</sub>	0.093	0.574	-0.305	0.066
Total Chl $a$	-0.286	0.077	-0.344	<b>0.037</b>
Chl $a$ < 5 $\mu\text{m}$	0.456	<b>0.003</b>	0.304	0.075
Chl $a$ 5-20 $\mu\text{m}$	0.062	0.708	-0.064	0.715
Chl $a$ > 20 $\mu\text{m}$	-0.299	0.065	-0.208	0.23

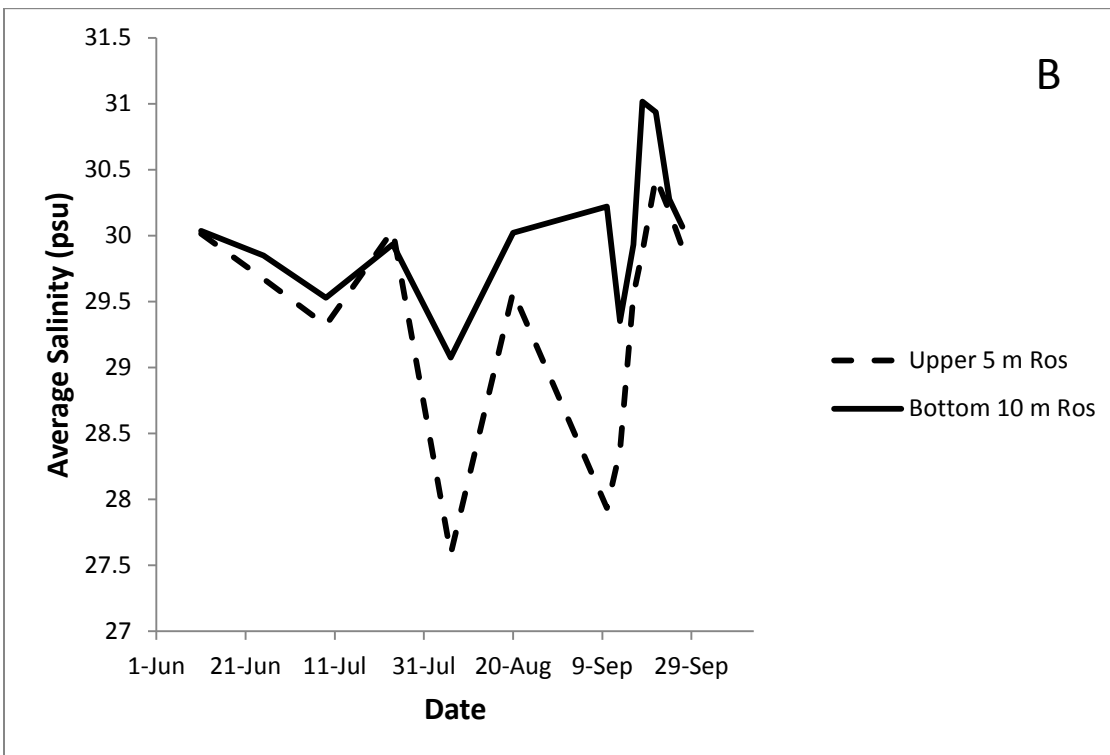
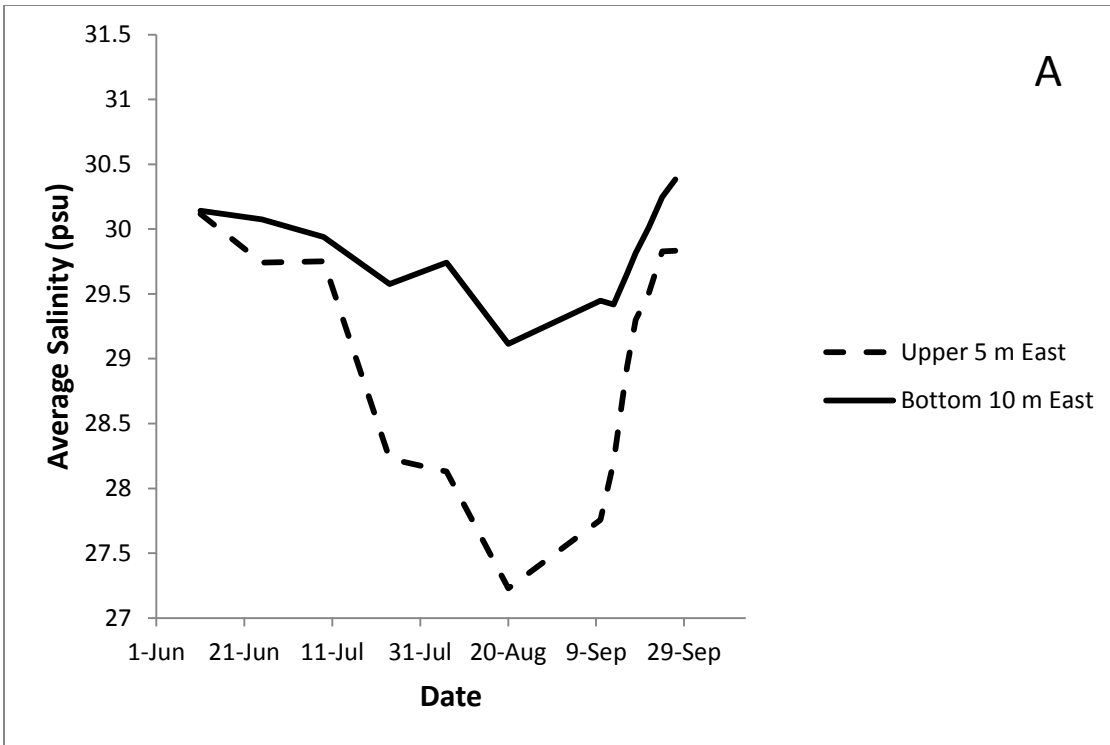


Figure 4. Average salinity (psu) from June 11 to September 27, 2012 in the upper 5 m and bottom 10 m in A. East Sound (East) and, B. Rosario (Ros). Salinity was averaged over the upper 5 m and the bottom 10 m of sampling depths.

The surface at Rosario was much fresher August 6<sup>th</sup> at about 27.6 psu and shifted to a well-mixed, more saline water column August 20<sup>th</sup> (Figure 4B). On September 10<sup>th</sup>, the water column was stratified and from then on, the water column became more saline and well-mixed. Salinities in Rosario ranged from 27.5 to 31.0 psu. Both stations exhibited a negative correlation between salinity and *Synechococcus* abundance (Table 1). Also, temperature and salinity were strongly negatively correlated at both stations (East:  $r = -0.825$ ,  $p = < 0.001$ , Ros:  $r = -0.816$ ,  $p = < 0.001$ ).

### *Density*

Based on the density data, fresh water entered East Sound between July 9<sup>th</sup> and July 24<sup>th</sup> (Figure 5A). Layers of less dense (warmer, fresher) water stayed at the surface through September, but dissipated towards the end of the month. Density ranged from 19.8 to 23.3 kg m<sup>-3</sup> in East Sound (Appendix C). In Rosario, fresh water came in between July 24<sup>th</sup> and August 6<sup>th</sup> (Figure 5B). Water flushed out quickly in Rosario though, and the water column returned to its previous well-mixed state by August 20<sup>th</sup>. Water continued to become denser at the surface and throughout the water column in the month of September. Over the sampling period, density ranged from 20.7 to 23.8 in Rosario (Appendix C). Based on a comparison of the average salinity and density data, it is clear that salinity was a major driver of density, especially at Rosario where the patterns are almost identical (Figures 4B and 5B).

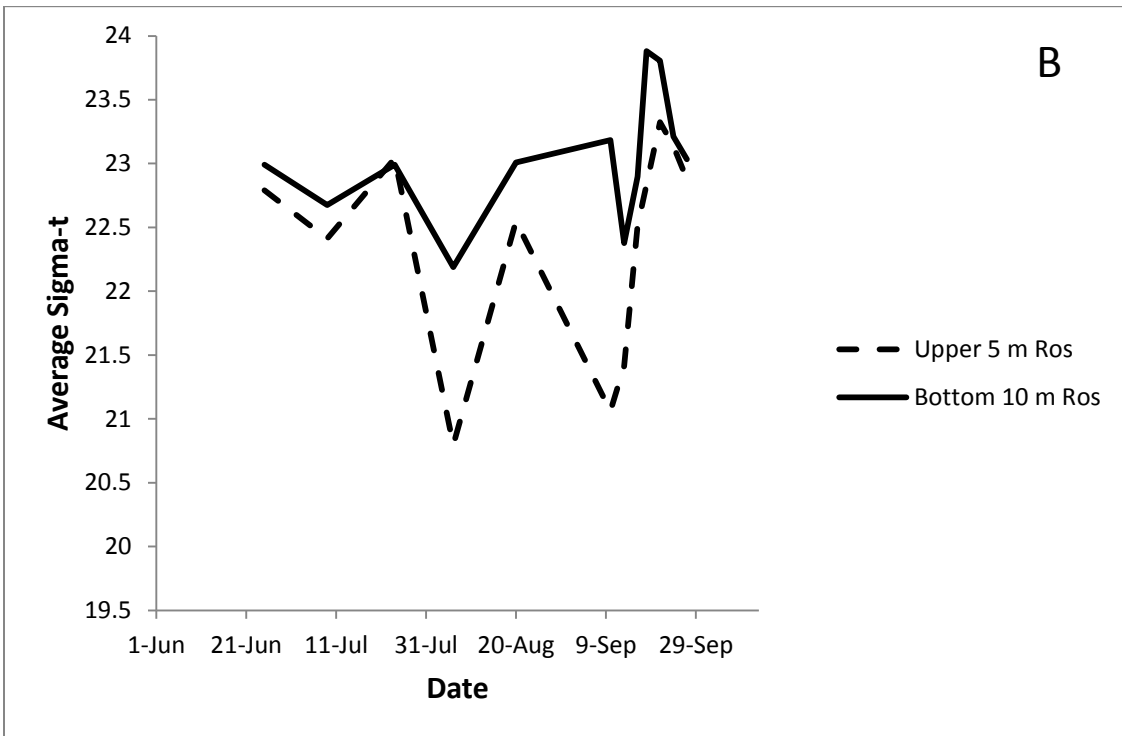
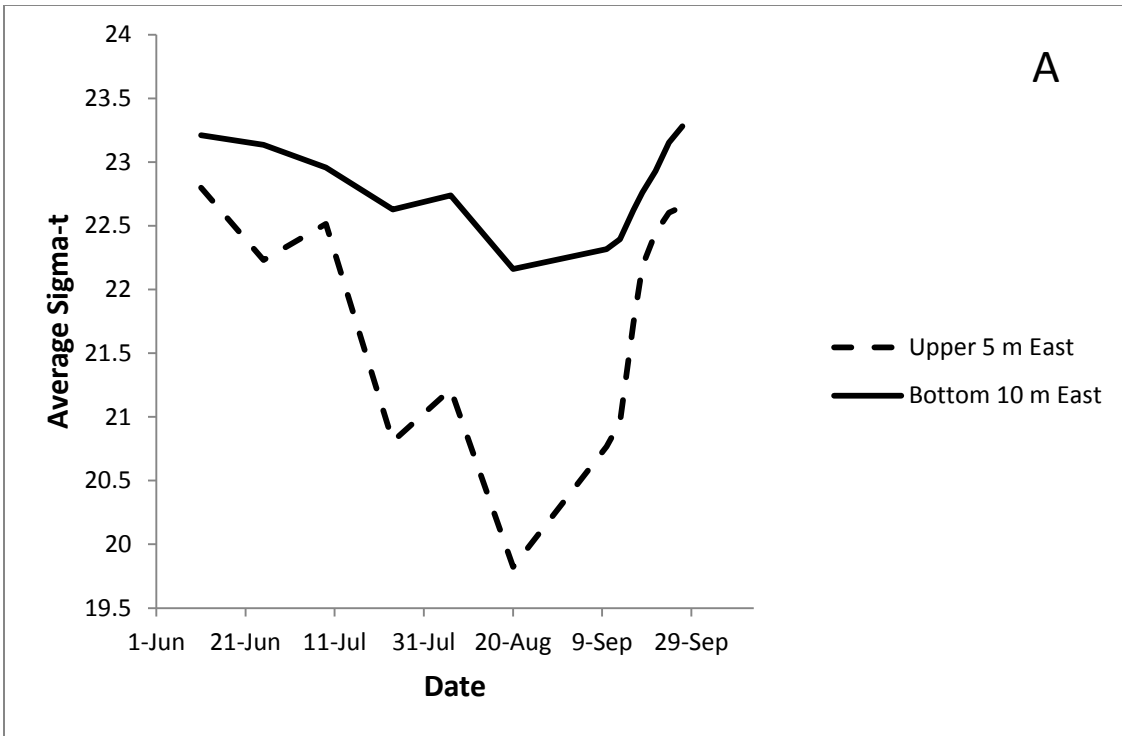


Figure 5. Average density ( $\text{kg m}^{-3}$ ) as sigma-t from June 11 to September 27, 2012 in the upper 5 m and bottom 10 m in A. East Sound (East) and, B. Rosario (Ros). June 11 data is missing from this figure. Sigma-t was averaged over the upper 5 m and the bottom 10 m of sampling depths.

### *Chlorophyll a Fluorescence*

Fluorescence profiles revealed that East Sound often had thin layers of water in the upper 10 m throughout the month of September (Figure 6, Appendix D). Rosario infrequently displayed the same level of layers in the water column, although there were occasional layers in the month of September (Figure 6). There was a clear depth with a chlorophyll maximum in June and early July, but this dissipated in late July and August. Fluorescence was negatively correlated with *Synechococcus* abundance at East Sound (Table 1). Fluorescence was not correlated with *Synechococcus* abundance at Rosario.

### *Photosynthetically Active Radiation (PAR)*

There were periods, typically about a week, of high PAR in early July and early August, and the light gradually decreased throughout September with the shortening day length (Figure 7). Although this trend would be expected toward the end of summer, there were also some decreases in PAR towards the end of July (July 20 and 22) which preceded the increase in *Synechococcus* abundance on July 24. The day of the largest bloom, August 6<sup>th</sup>, was preceded by about a week of high PAR levels (35 to 40 mol photons m<sup>-2</sup> day<sup>-1</sup>). August 6<sup>th</sup> was lower at 20.2 mol photons m<sup>-2</sup> day<sup>-1</sup>. The day of the fall bloom, September 10<sup>th</sup>, had fairly low PAR levels as well at 23.8 mol photons m<sup>-2</sup> day<sup>-1</sup>. The days preceding the fall bloom had PAR levels between 16 and 28 mol photons m<sup>-2</sup> day<sup>-1</sup>. PAR was not correlated with *Synechococcus* abundance at either East Sound or Rosario (Table 1).

June 11

July 9

August 20

September 21

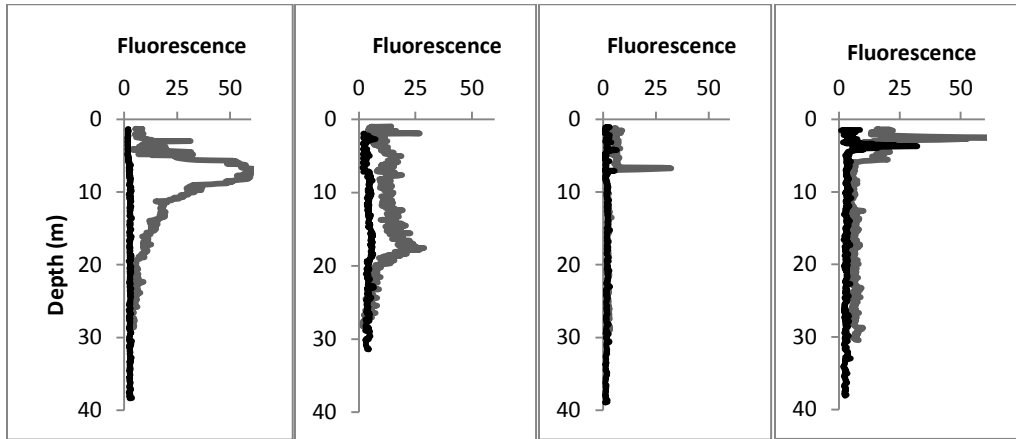


Figure 6. Chlorophyll *a* fluorescence depth profiles. East Sound is dark grey and Rosario is black.

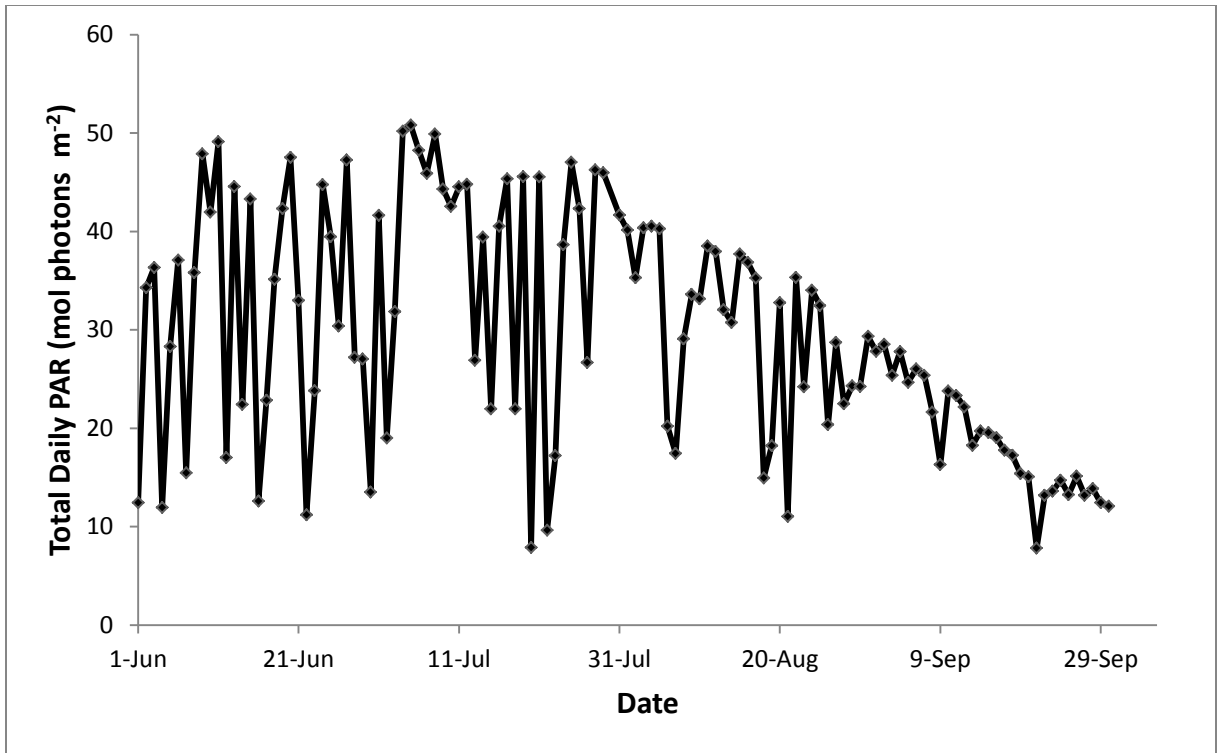


Figure 7. Total daily PAR in mol photons m<sup>-2</sup> from June 1 to September 29, 2012.



Table 2. Percentage of surface light (PAR) at East Sound and Rosario for maximum and minimum attenuation coefficients (k).

Station	Min. k (m <sup>-1</sup> )	Depth (m)	% Surface Light	Max. k (m <sup>-1</sup> )	Depth (m)	% Surface Light
East Sound	0.19	5	39	0.35	5	17
	0.19	12	10	0.35	10	3
	0.19	18	3	0.35	15	1
Rosario	0.14	5	50	0.32	3	38
	0.14	8	33	0.32	5	20
	0.14	15	12	0.32	10	4

Water column light attenuation coefficients were calculated based on vertical profiles of PAR; these revealed that light attenuated rapidly with depth at both stations (Table 2). At the surface depths, the percentage of surface light was usually between 17 and 50% of the incident irradiance, while mid depths ranged between 3 and 33%, and deep depths ranged between 1 and 12% of incident irradiance. When the attenuation coefficient ( $k$ ) was at its maximum of  $0.35 \text{ m}^{-1}$ , the surface depth only received 17% of the incident irradiance, while the mid depth received 3% and about 1% of the surface light reached the deepest sampled depth.

An analysis of long-term (2002-2012) PAR data from Shannon Point Marine Center revealed a transition from positive PAR anomalies toward negative PAR anomalies beginning about 2006. The years 2002-2005 remained positive with 2004 showing the maximum positive PAR anomaly at 14.38 on week 26, or the end of June (Figure 8A). The year 2006 was the first year with a mainly negative anomaly (Figure 8B). From then on, each year showed negative PAR anomalies with 2012 as one of the most negative PAR years overall. This same trend from positive to negative PAR anomalies was exhibited in comparing past East Sound study years (Figure 9). The year 2005 had the largest positive anomalies while 2007, 2011, and 2012 displayed mostly negative anomalies (Figure 9).

### *Nutrients*

Nitrate+nitrite and phosphate concentrations varied with depth at East Sound (Figure 10A and 11A). Other than the first sampling date (June 11), when surface concentrations were below detection limits, nitrate+nitrite concentrations were fairly high (4.6 to  $18.4 \mu\text{M}$

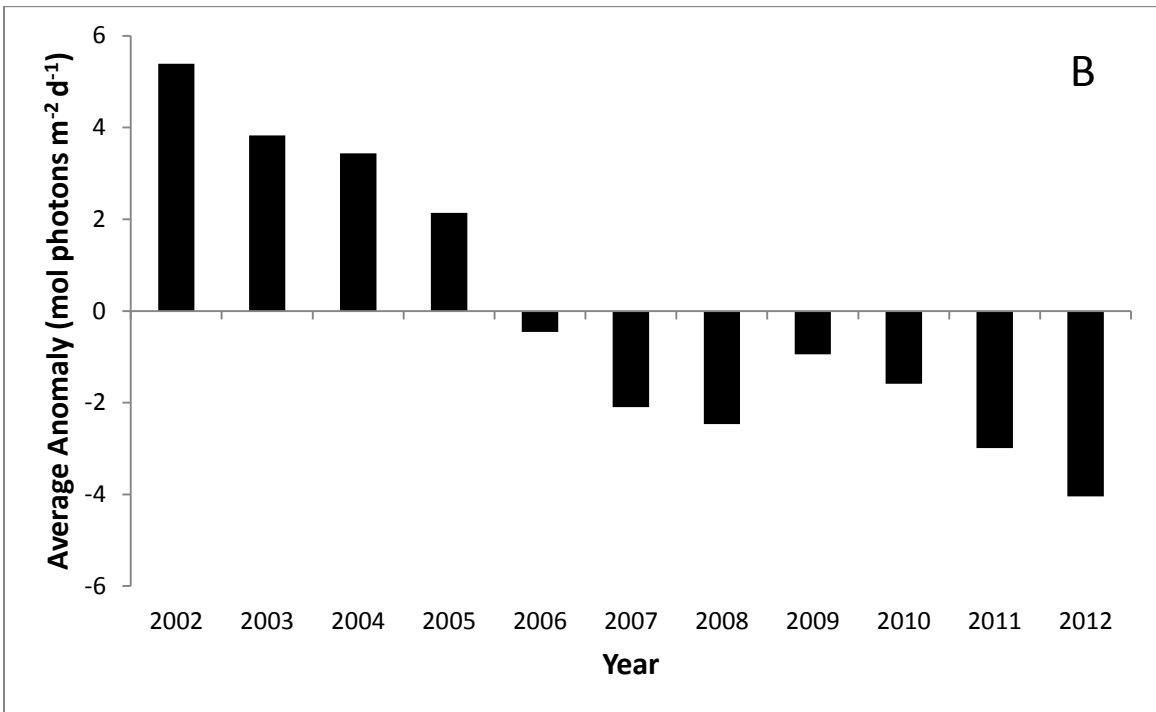
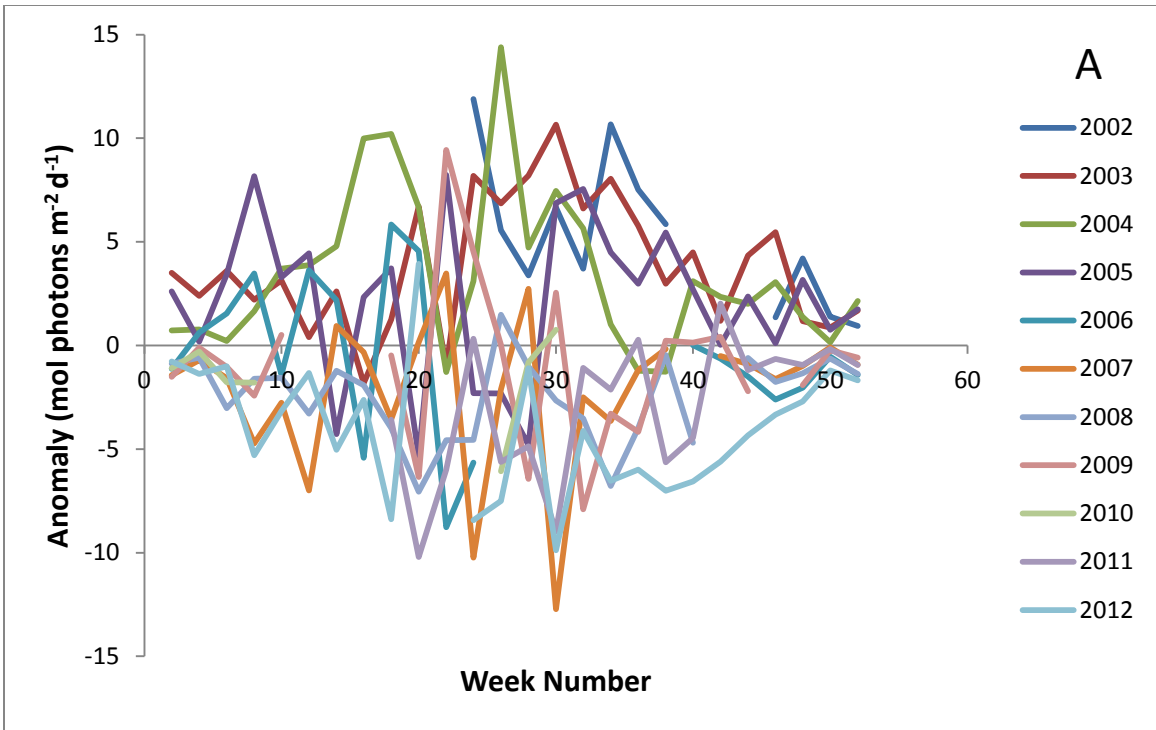


Figure 8. A) PAR anomaly data based on an average of daily PAR data for every two weeks of the years 2002-2012. There are gaps in the graph where more than five days of data were missing from the two week period that was averaged. B) The yearly average of PAR anomaly data from graph A for the years 2002-2012.

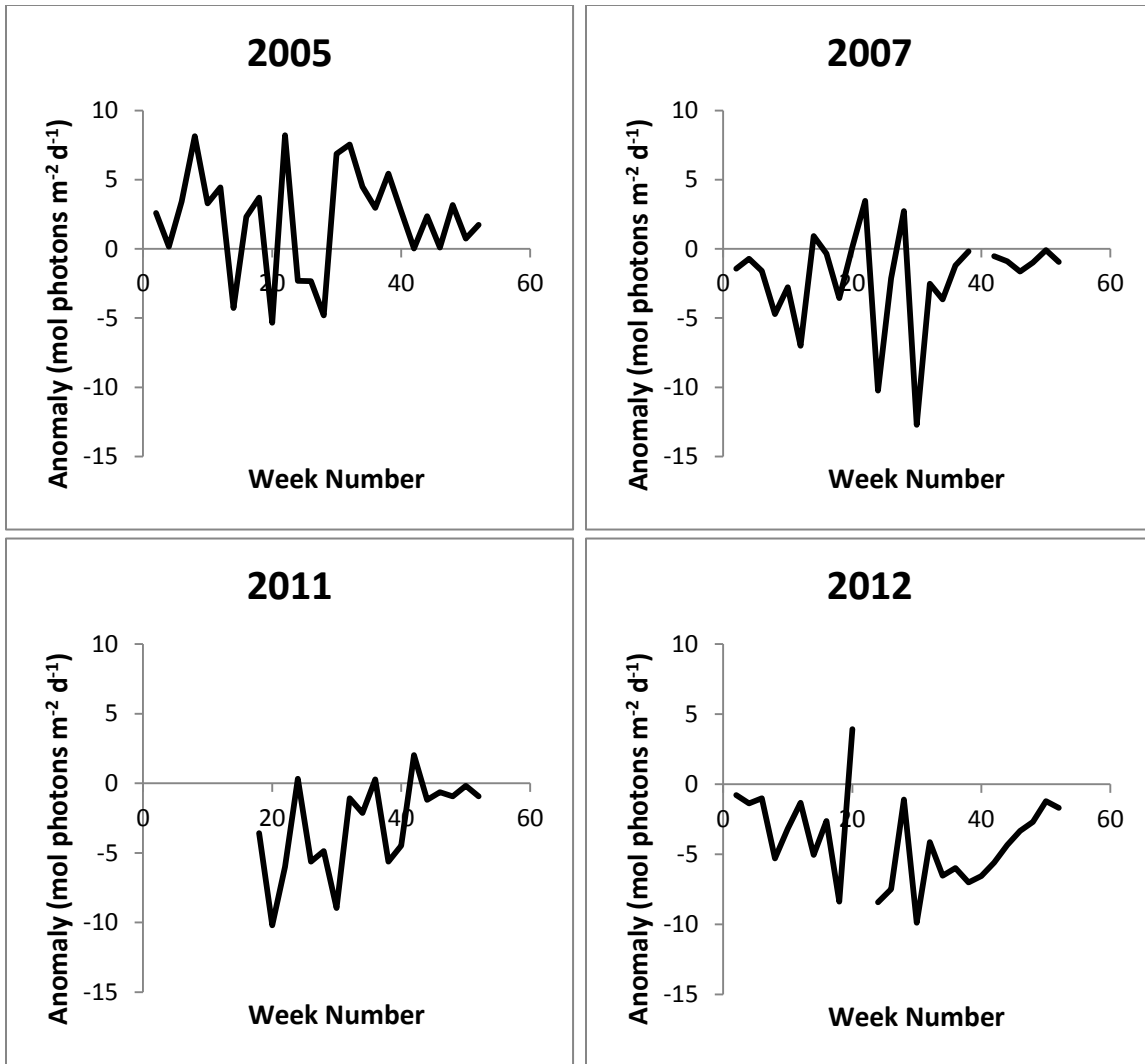


Figure 9. PAR anomaly data based on an average of daily PAR data for every two weeks of the years 2002-2012. This graph is a subset of the data set showing only East Sound study years (2005, 2007, 2011, and 2012). There are gaps in the graph where more than five days of data were missing from the two week period that was averaged.

N). As with nitrate+nitrite, the lowest phosphate concentration at the surface was observed on June 11 (0.4  $\mu\text{M P}$ ) while the concentration stayed between 0.8 and 2.5  $\mu\text{M P}$  for the rest of the summer. Usually the deep depth had the highest concentrations of nitrate+nitrite and phosphate (10.8 to 18.4  $\mu\text{M N}$ , 1.2 to 2.5  $\mu\text{M P}$ ). Nitrate+nitrite concentrations were especially high July 24-August 6 at all depths (10.4 to 18.4  $\mu\text{M N}$ ) at the time of the largest *Synechococcus* bloom and then again September 16-21 (7.5 to 17.1  $\mu\text{M N}$ ). On September 24<sup>th</sup>, a day when *Synechococcus* abundance increased, the phosphate concentration increased at the deep depth (to 2.0  $\mu\text{M}$ ), but not at the other depths.

Nitrate+nitrite and phosphate concentrations were more similar with depth at Rosario in contrast to East Sound (Figure 10B and 11B). Unlike East Sound, there was never a low concentration of nitrate+nitrite or phosphate in Rosario over the sampling period.

Concentrations did range from 12.9 to 22.3  $\mu\text{M N}$  and from 1.4 to 2.1  $\mu\text{M P}$ . September 10<sup>th</sup> was of particular interest because the concentration reached its low at the surface (12.9  $\mu\text{M N}$ , 1.4  $\mu\text{M P}$ ), while *Synechococcus* abundance rebounded at all depths with the fall bloom. From September 10<sup>th</sup> to the 27<sup>th</sup>, the phosphate concentration increased strikingly. During June through August, phosphate was never at low concentrations, and the low that was reached in early September (1.4  $\mu\text{M P}$ ) was still higher than the lowest value observed in East Sound (0.4  $\mu\text{M P}$ ). Later in September (the 24<sup>th</sup>), the nitrate+nitrite concentration peaked at 20.5  $\mu\text{M N}$  while *Synechococcus* abundance increased once again at mid and deep depths.

Nitrate+nitrite concentration was positively correlated with *Synechococcus* abundance in East Sound, but not at Rosario (Table 1). Nitrate+nitrite concentration was

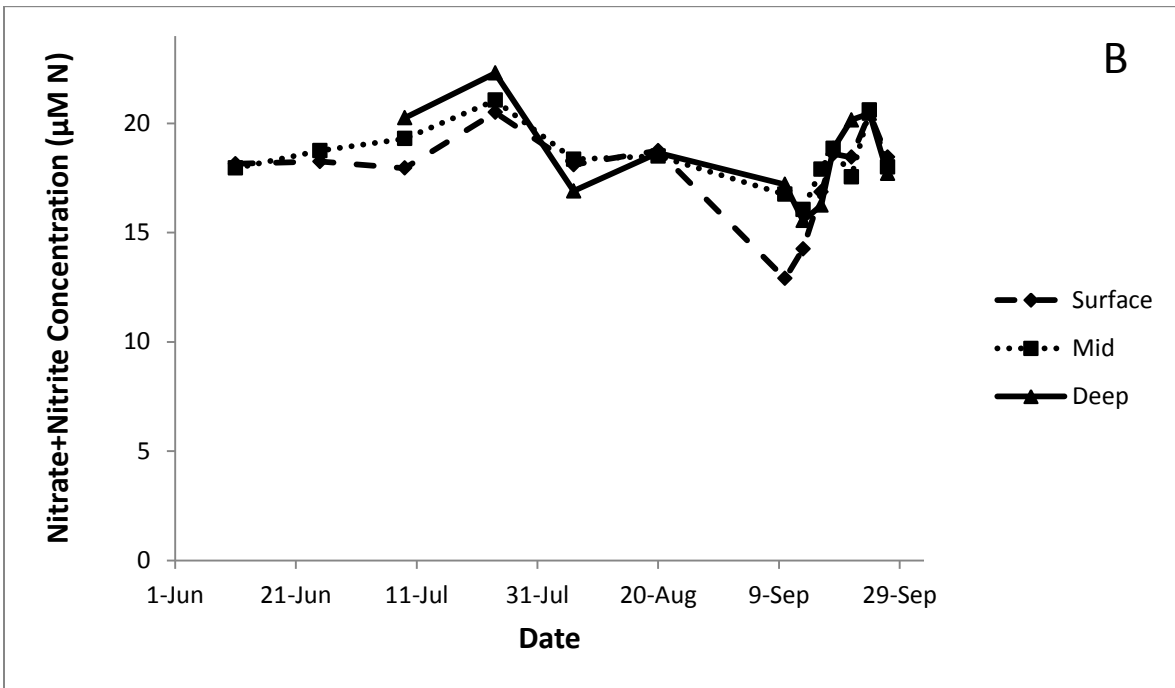
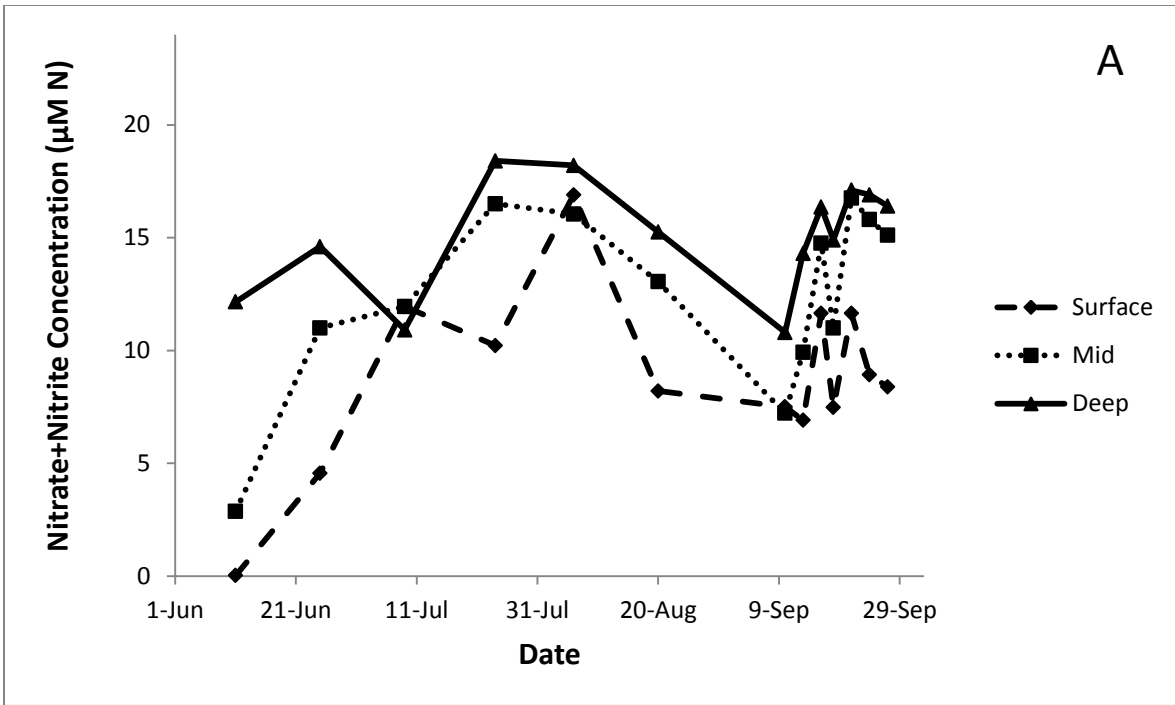


Figure 10. Nitrate+nitrite concentrations in  $\mu\text{M N}$  from June 11 to September 27, 2012 at A. East Sound, and B. Rosario. There are no deep N+N data for the first two sampled dates in Rosario, June 11<sup>th</sup> and June 25<sup>th</sup>.

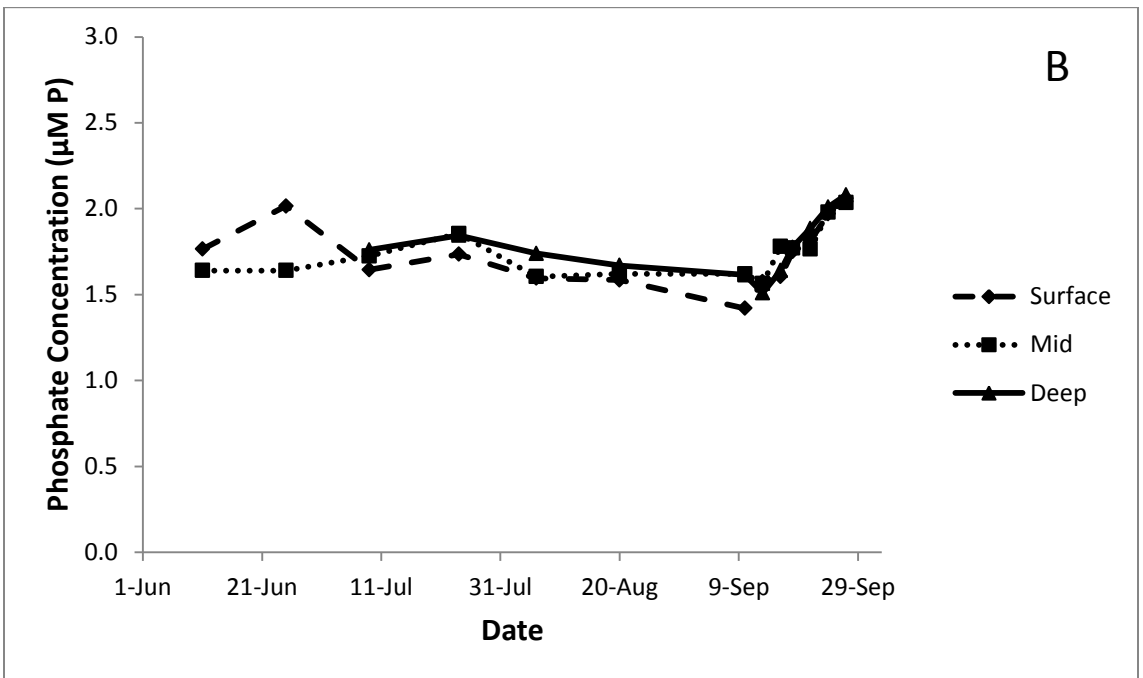
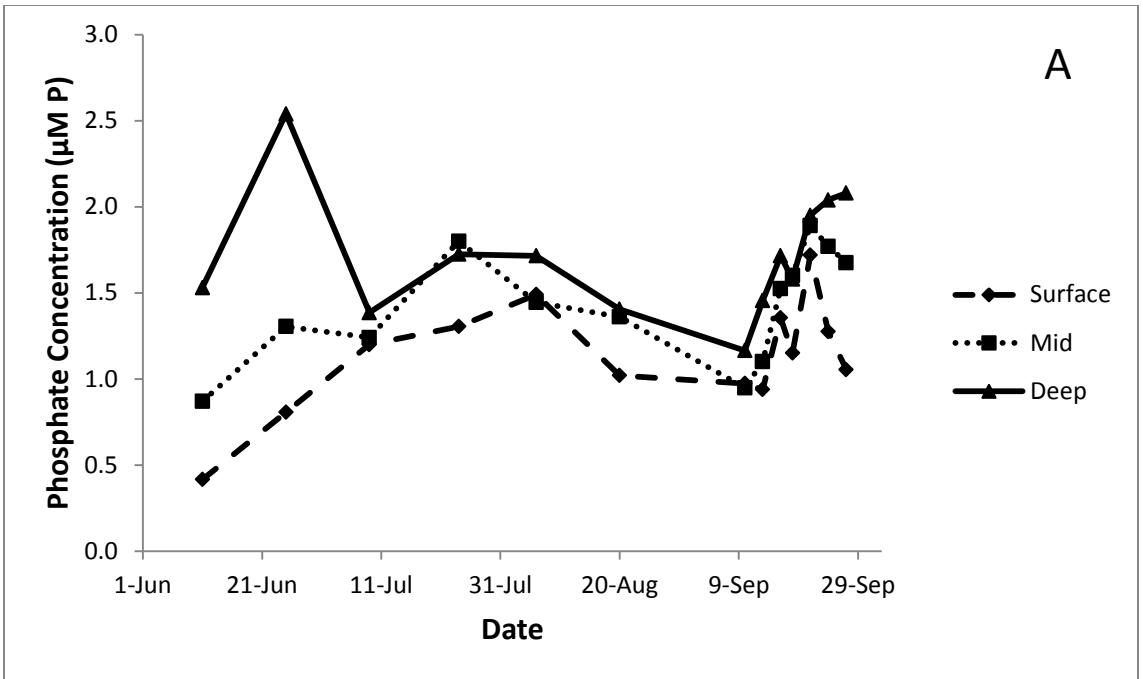


Figure 11. Phosphate concentrations in  $\mu\text{M P}$  from June 11 to September 27, 2012 at A. East Sound, and B. Rosario. There are no deep P data for the first two sampled dates in Rosario, June 11<sup>th</sup> and June 25<sup>th</sup>.

negatively correlated with temperature ( $r = -0.662$ ,  $p = <0.001$ ) and positively correlated with salinity ( $r = 0.596$ ,  $p = < 0.001$ ) at Rosario, but not at East Sound. Phosphate concentration was not correlated with *Synechococcus* abundance at either station (Table 1). However, phosphate concentration at East Sound was negatively correlated with temperature ( $r = -0.432$ ,  $p = 0.006$ ) and positively correlated with salinity ( $r = 0.35$ ,  $p = 0.029$ ). Also, phosphate concentration at Rosario was strongly negatively correlated with temperature ( $r = -0.545$ ,  $p = < 0.001$ ) and strongly positively correlated with salinity ( $r = 0.635$ ,  $p = < 0.001$ ).

### *Chlorophyll a*

Chlorophyll *a* concentrations in East Sound were high on the first sampling date, June 11<sup>th</sup>, ranging from 8.2 to 27.8  $\mu\text{g L}^{-1}$  (Figure 12A). However, chlorophyll *a* levels were very low July through August, ranging from 0.2 to 1.9  $\mu\text{g L}^{-1}$ . In September, chlorophyll *a* increased to a peak of 34.1  $\mu\text{g L}^{-1}$  at the surface on the 24<sup>th</sup> of September. While total chlorophyll *a* levels were low during mid-summer, *Synechococcus* rose to its peak in August and decreased at the end of August. *Synechococcus* abundance followed a pattern similar to chlorophyll *a* in September.

Overall, Rosario had much lower levels of chlorophyll *a* than East Sound, never reaching a concentration higher than 6.4  $\mu\text{g L}^{-1}$  (Figure 12B). Chlorophyll *a* concentrations were between 0.1 and 1.4  $\mu\text{g L}^{-1}$  until September 10<sup>th</sup> when chlorophyll *a* rapidly increased (2.2 to 3.6  $\mu\text{g L}^{-1}$ ). In September, chlorophyll *a* varied more intensely than seen in the previous months. Chlorophyll *a* at mid depth decreased from 5.7  $\mu\text{g L}^{-1}$  to 0.4  $\mu\text{g L}^{-1}$  over the course of 11 days. Although total chlorophyll *a* levels at Rosario were high in September, *Synechococcus* abundance gradually decreased at that time.



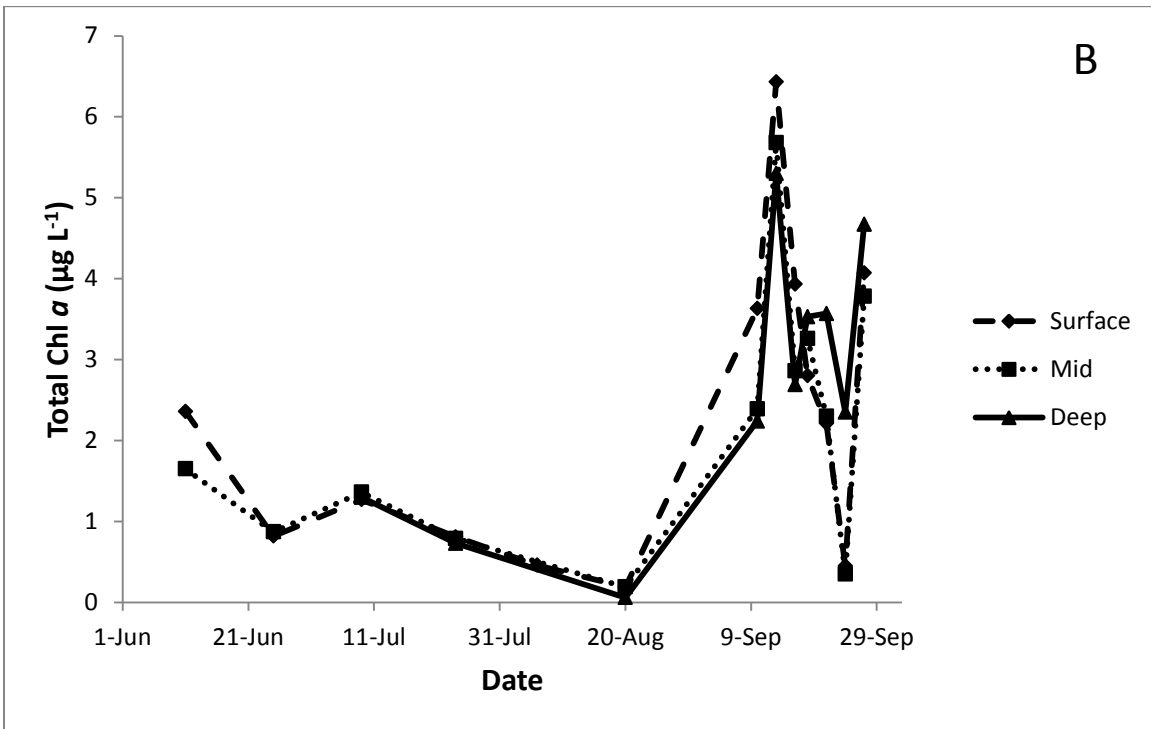
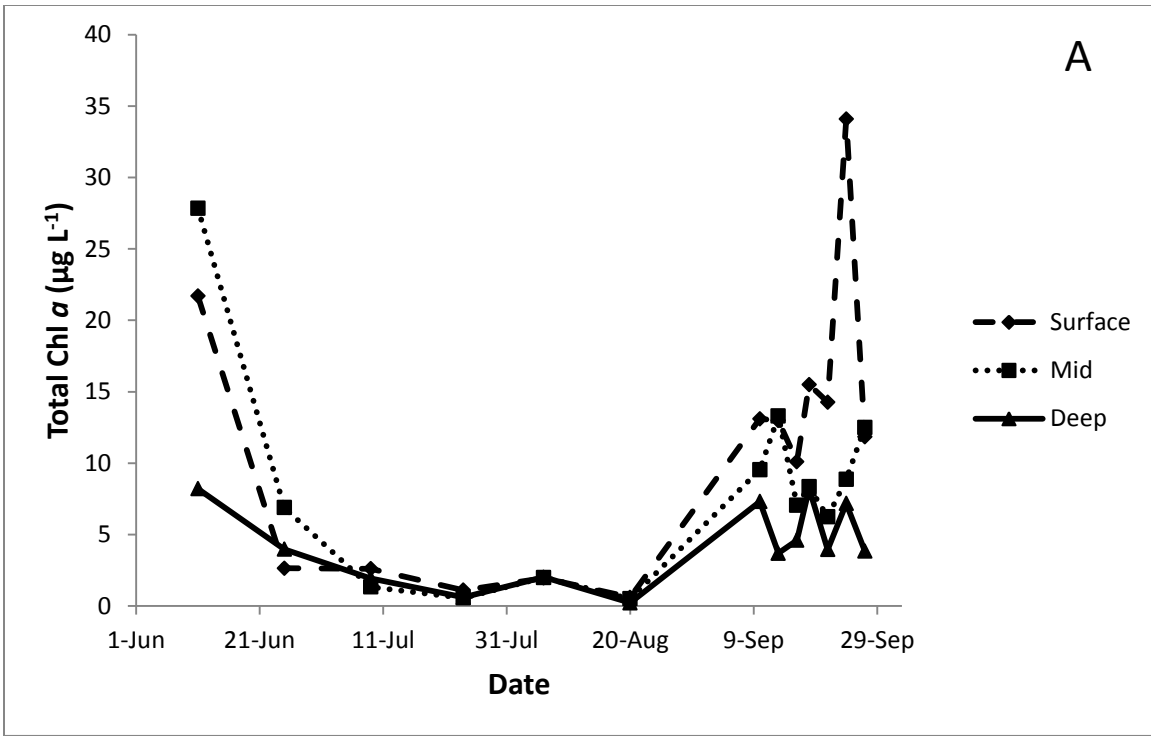


Figure 12. Total chlorophyll *a* ( $\mu\text{g L}^{-1}$ ) from June 11 to September 27, 2012 in A. East Sound, and B. Rosario. There were no deep depth data for the first two sampled dates, June 11<sup>th</sup> and June 25<sup>th</sup>. August 6<sup>th</sup> data are missing from this graph. Notice that the scale on the y-axis of Figure 8B differs from the scale on Figure 8A.

Interestingly, data from the size-fractionated chlorophyll reveal that the ultraplankton, such as *Synechococcus*, in the < 5  $\mu\text{m}$  size fraction did not exhibit the same temporal variation as total chlorophyll *a*. While total chlorophyll *a* was low in both East Sound and Rosario July through August, the percentage of total chlorophyll *a* in the < 5  $\mu\text{m}$  fraction was relatively high on July 24<sup>th</sup>, comprising up to 50% of total chlorophyll *a* at the surface (Figure 13). On July 24<sup>th</sup>, the nanoplankton in the 5-20  $\mu\text{m}$  size fraction also exhibited high percentages of total chlorophyll *a* (25-40%) at East Sound (Figure 13A) and from 30-35% at Rosario (Figure 13B). The majority of the time, the microplankton in the > 20  $\mu\text{m}$  size fraction dominated the total chlorophyll *a* at both stations. However, it is interesting to note that the nanoplankton in Rosario (5-20  $\mu\text{m}$ ) more often experienced variation throughout September when there were spikes in the total chlorophyll *a*. The ultraplankton in the < 5  $\mu\text{m}$  size fraction also displayed variation throughout September in Rosario, although recall that it was in East Sound that the September spikes in *Synechococcus* abundance occurred. Overall, the variation in *Synechococcus* abundance did not match the variation in the < 5  $\mu\text{m}$  size fraction.

Total chlorophyll *a* was negatively correlated with *Synechococcus* abundance at Rosario, but not at East Sound (Table 1). Chlorophyll *a* < 5  $\mu\text{m}$  was positively correlated with *Synechococcus* abundance at East Sound, but not at Rosario. None of the other size fractions were correlated with *Synechococcus* abundance.

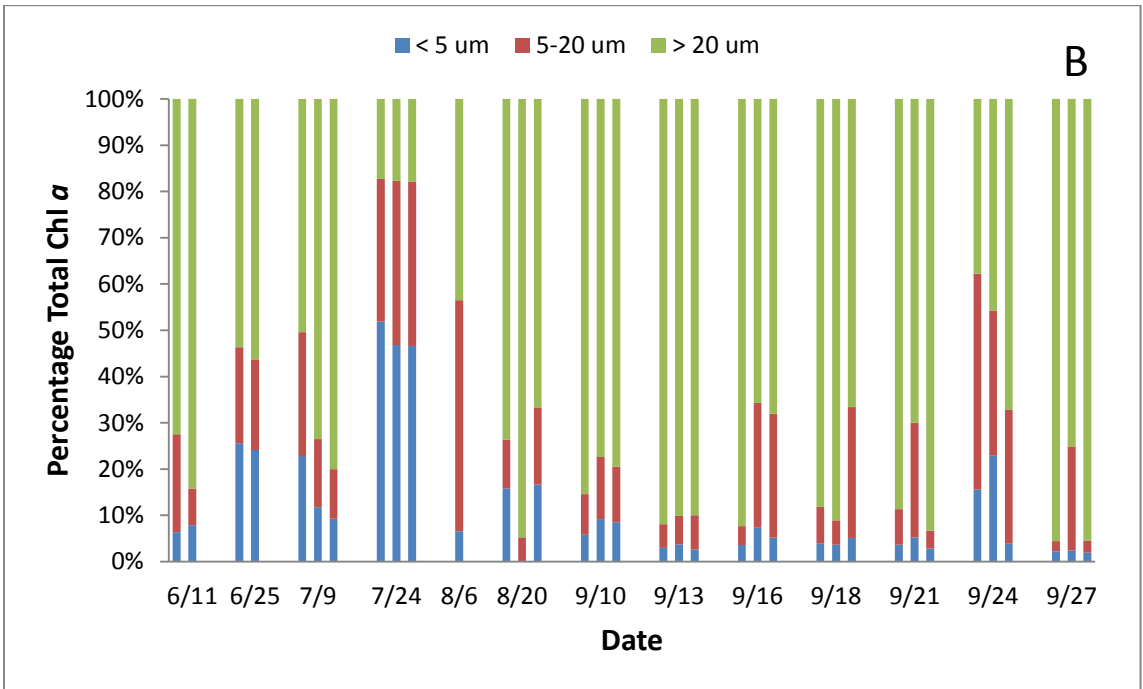
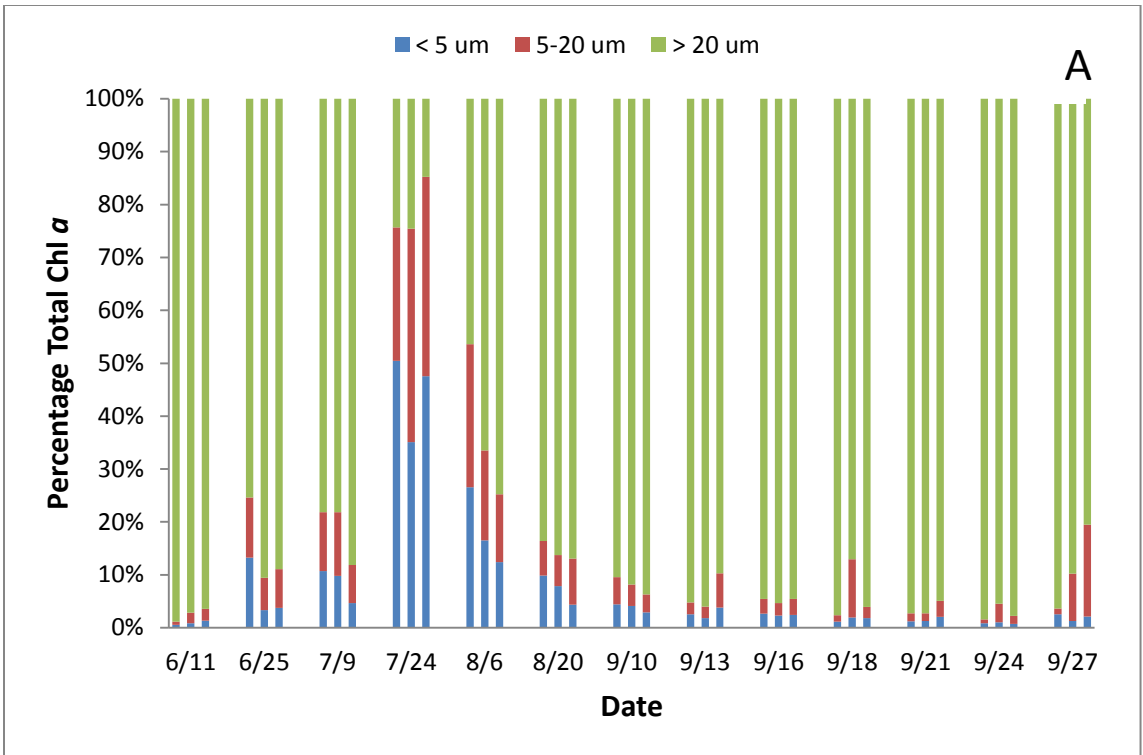


Figure 13. Percentage of total chlorophyll *a* based on size-fractionated chlorophyll from June 11 to September 27, 2012 in A. East Sound, and B. Rosario. For each date, there are up to three bars for each sequential depth (surface, mid, and deep). Note that in Rosario, there are no deep data for June 11<sup>th</sup> and June 25<sup>th</sup>, and that August 6<sup>th</sup> is missing mid and deep data.

### *Tidal Range*

Tidal range was high in June and July and began to decrease in mid-August at both East Sound and Rosario (Figure 14). East Sound tidal ranges tended to be higher than Rosario tidal ranges by one or two feet. Tidal range was weakly correlated with *Synechococcus* abundance at Rosario (Table 1). At East Sound, tidal range was not correlated with *Synechococcus* abundance.

### *Upwelling Index*

The upwelling index from the Strait of Juan de Fuca entrance region did not show a positive regime of upwelling until the month of July (Figure 15). Even during this time, there were multiple instances of near-zero upwelling intensity. The minimum index was -57 in early June and the maximum index was 57 at the end of September. The month of September had a mostly positive upwelling index.

### *Fraser River Discharge*

Fraser River discharge increased from June 1<sup>st</sup> to June 22<sup>nd</sup> when it reached its peak of the sampling period at  $11,725 \text{ m}^3 \text{ s}^{-1}$  (Figure 16). From that point on, discharge slowly decreased. By the end of summer, discharge had reached its low at  $1,529 \text{ m}^3 \text{ s}^{-1}$ . Compared with previous East Sound study years (2005 and 2007), 2011 and 2012 had higher average Fraser River discharge rates May through August (Figure 17). However, the month of September had higher discharge rates in 2011 than in 2012. In June of 2012, there was an increased average discharge rate at  $9,378 \text{ m}^3 \text{ s}^{-1}$  compared with  $6,105 \text{ m}^3 \text{ s}^{-1}$  in 2005 and

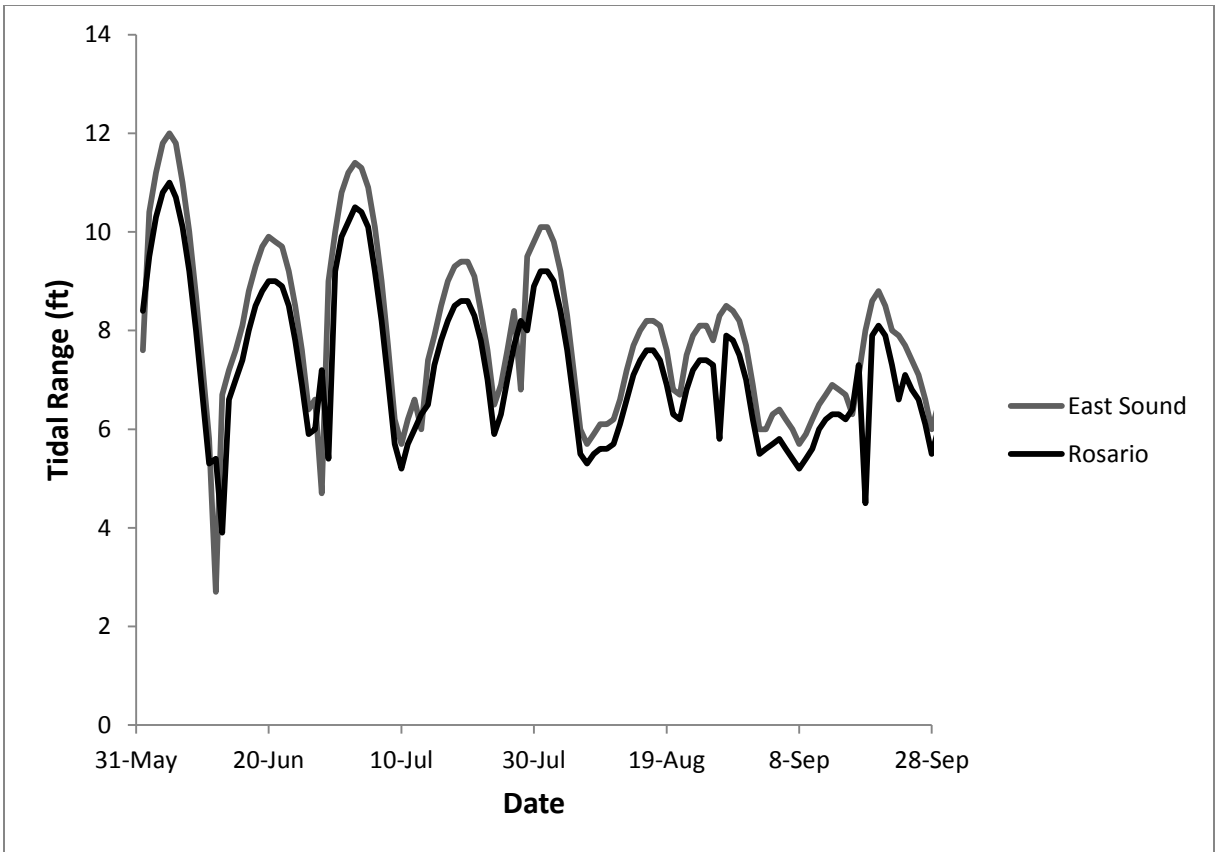


Figure 14. Tidal range in feet from May 31 to September 28, 2012. Tide charts for East Sound are from Rosario, Eastsound, Orcas Island and from Aleck Bay, Lopez Island for Rosario.

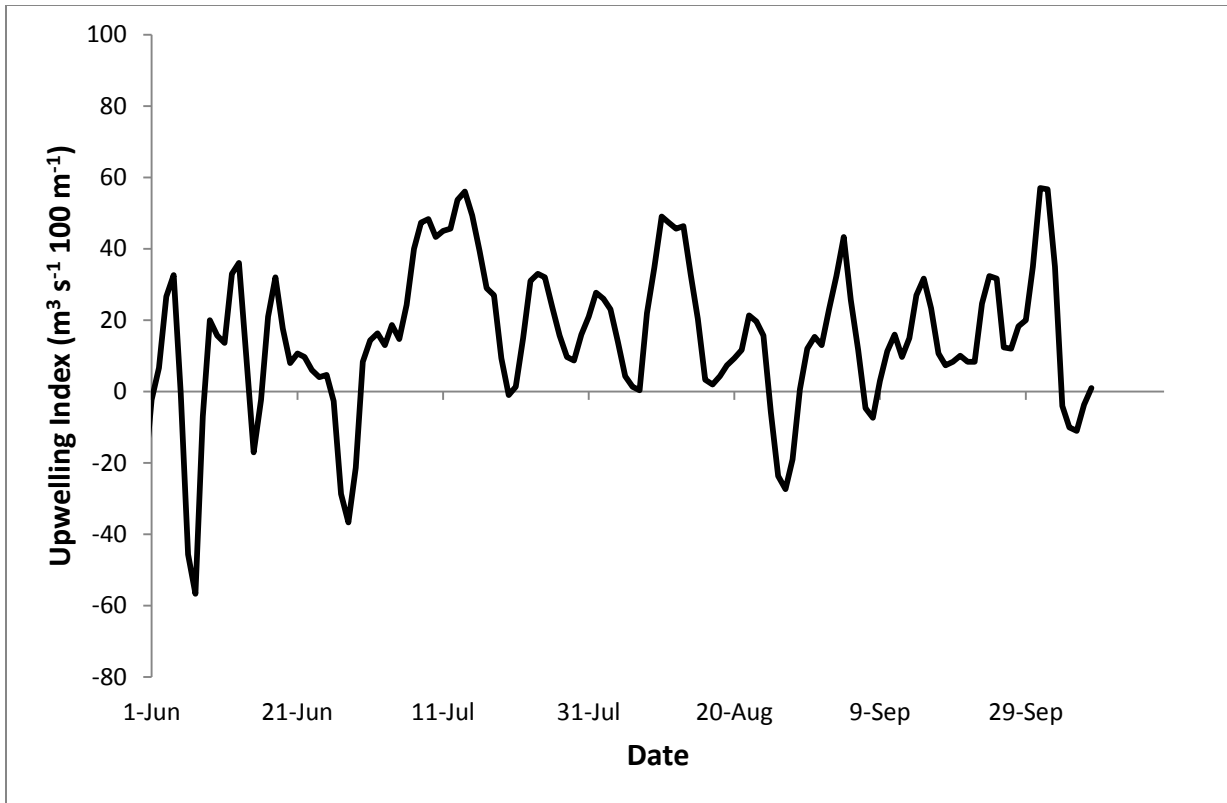


Figure 15. Upwelling index in  $\text{m}^3$  water per second per 100 meters of coastline averaged over three day periods from May 1 to October 8, 2012. Data come from  $48^\circ\text{N}$ , south of the mouth of the Strait of Juan de Fuca.

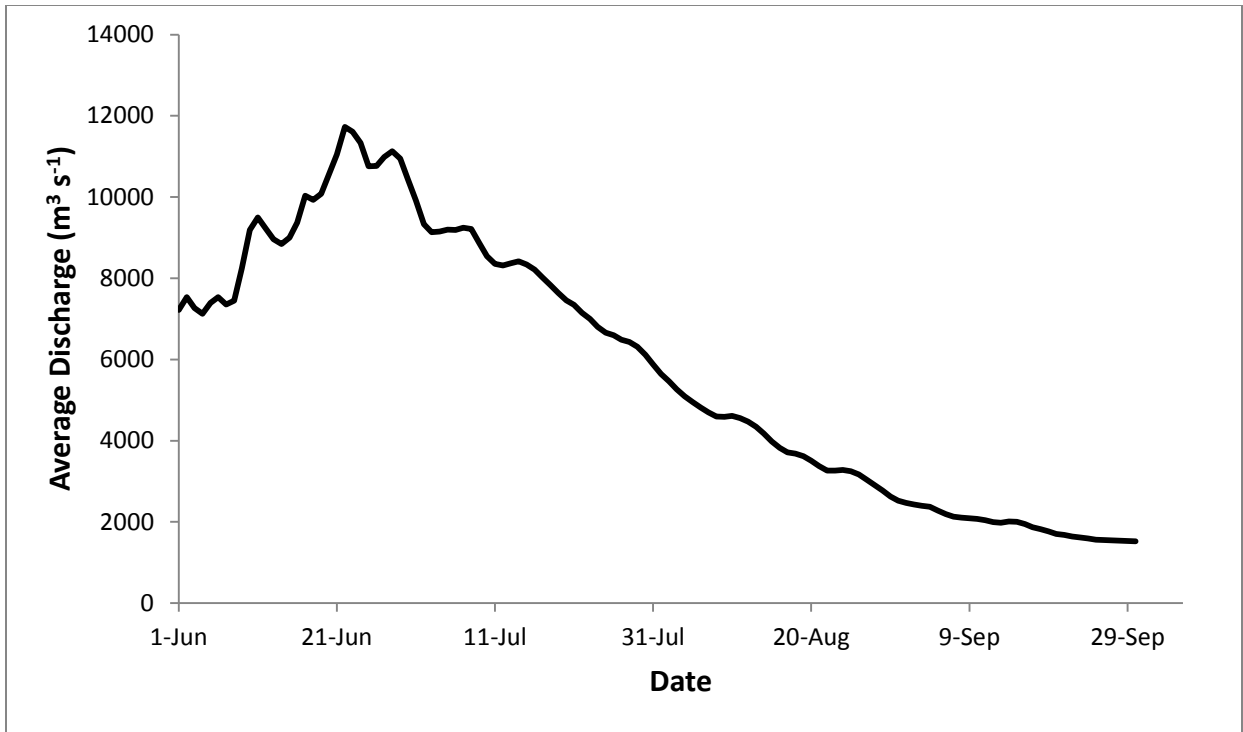


Figure 16. Average daily discharge from the Fraser River ( $\text{m}^3 \text{s}^{-1}$ ) from June 1 to September 29, 2012. Data from the Hope, BC station and the Wateroffice web page of Environment Canada, ([www.wateroffice.ec.gc.ca](http://www.wateroffice.ec.gc.ca).)

between 8,000 and 9,200 m<sup>3</sup> s<sup>-1</sup> in 2007 and 2011, respectively.

#### *Protist Grazer Community Composition*

East Sound and Rosario had different compositions of protist grazers (Table 3). Generally, there were greater abundances of grazers at East Sound as compared with Rosario. This was seen in the smaller size fraction with the heterotrophic nanoflagellate abundances at East Sound approximately one order of magnitude greater than those at Rosario. There were always ciliates present at both stations as well as heterotrophic dinoflagellates, *Gymnodinium* and *Gyrodinium*. *Ceratium fusus* bloomed in East Sound on August 20<sup>th</sup>.

Not all grazers present ingested *Synechococcus* cells even during periods of high *Synechococcus* abundance (Table 4). Ciliates and larger (> 20 µm) heterotrophic *Gymnodinium* and *Gyrodinium* were most often observed with ingested *Synechococcus* cells (seen in Figure 18). On August 20 in East Sound, all of the observed heterotrophic *Gymnodinium* and *Gyrodinium* cells > 20 µm had ingested *Synechococcus* cells. However, thecate dinoflagellates were never seen to ingest *Synechococcus* cells although they were present in every sample that was counted (seen in Figure 19). When *C. fusus* bloomed on August 20<sup>th</sup> in East Sound, 3.3% of the population were observed with ingested cells. In the smaller size fraction, nanoflagellates were observed in the greatest abundance, but were infrequently observed with ingested *Synechococcus* cells. Other dinoflagellates in the < 20 µm size range did occasionally feed on *Synechococcus*.



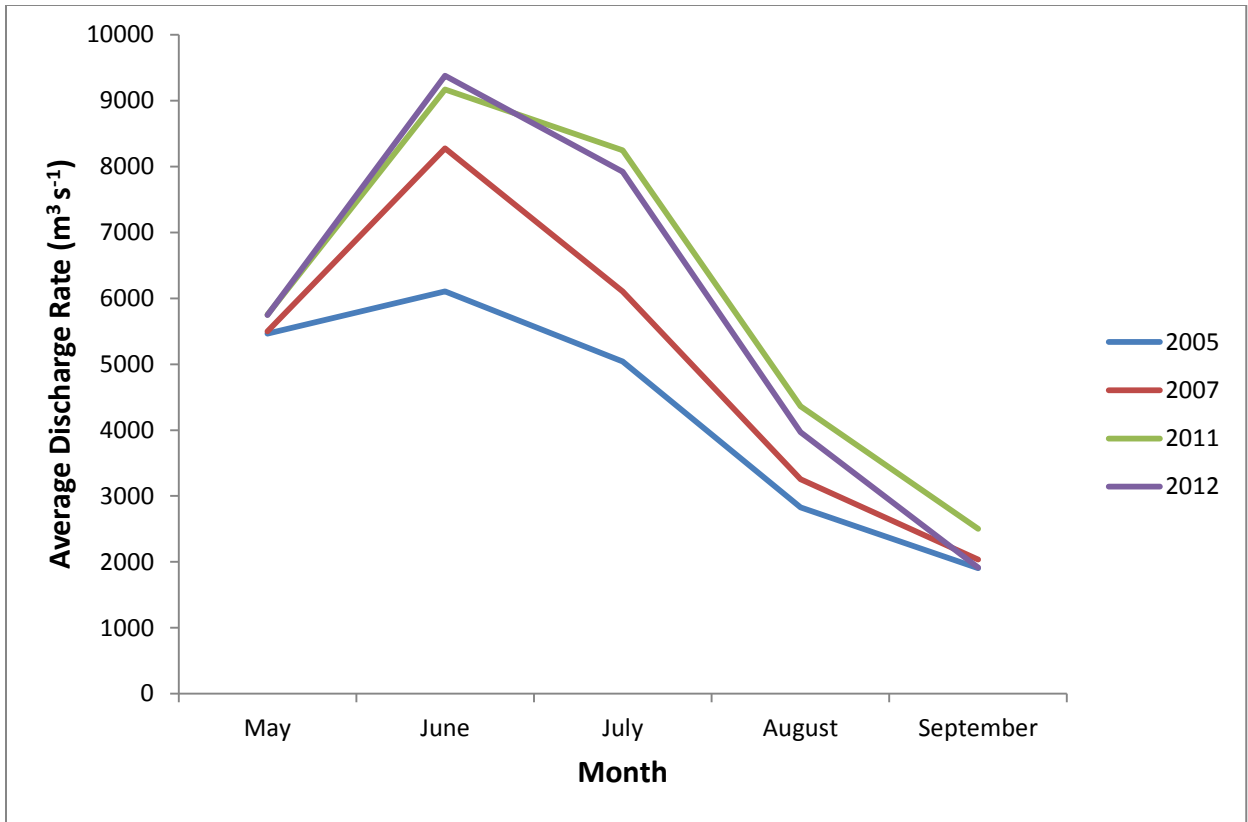


Figure 17. Average Fraser River discharge rate ( $\text{m}^3 \text{s}^{-1}$ ) from May to September in East Sound study years (2005, 2007, 2011, and 2012). Data from the Hope, BC station and the Wateroffice web page of Environment Canada, ([www.wateroffice.ec.gc.ca](http://www.wateroffice.ec.gc.ca).)

Table 3. Abundance (cells ml<sup>-1</sup>) of various protist grazer taxa at East Sound (East) and Rosario (Ros) from July 24<sup>th</sup> to September 10<sup>th</sup>, the sampling dates with the greatest *Synechococcus* abundances. Abbreviations include: Auto Gym-Gyr for autotrophic *Gymnodinium* and *Gyrodinium* (two dinoflagellate genera), Hetero Gym-Gyr for heterotrophic *Gymnodinium* and *Gyrodinium*, thecate dinos for thecate dinoflagellates, other dinos for dinoflagellates not included in the previous categories, nanos for nanoflagellates, and cryptos for cryptophytes. *Dinophysis* and *Ceratium fusus* (*C. fusus*) are both dinoflagellates as well. “nd” is used in this table as an abbreviation for “not detected.”

Date and Stn.		> 20 µm taxa							< 20 µm taxa				
		Ciliates	Auto Gym-Gyr	Hetero Gym-Gyr	Thecate Dinosaurs	<i>Dinophysis</i>	<i>C. fusus</i>	Other Dinosaurs	Total Dinosaurs	Nanos	Cryptos	Hetero Gym-Gyr	Other Dinosaurs
July 24	East	9	nd	3	2	11	4	8	28	1292	nd	nd	666
	Ros	7	2	8	1	6	nd	10	27	574	nd	nd	461
Aug. 6	East	43	nd	14	8	9	23	8	62	3176	nd	nd	478
	Ros	66	7	27	4	3	7	22	70	612	nd	47	911
Aug. 20	East	42	nd	17	8	8	763	8	804	1920	112	nd	223
	Ros	17	1	15	5	2	83	6	112	2066	nd	nd	230
Sept. 10	East	68	15	41	4	6	62	17	145	1552	340	nd	255
	Ros	32	1	9	2	1	16	3	32	920	85	nd	411

Table 4. Percentage of observed grazers feeding on *Synechococcus* at East Sound (East) and Rosario (Ros) from July 24<sup>th</sup> to September 10<sup>th</sup>, the sampling dates with the greatest *Synechococcus* abundances. Abbreviations include: Auto Gym-Gyr for autotrophic *Gymnodinium* and *Gyrodinium*, two dinoflagellates, Hetero Gym-Gyr for heterotrophic *Gymnodinium* and *Gyrodinium*, thecate dinos for thecate dinoflagellates, other dinos for dinoflagellates not included in the previous categories, nanos for nanoflagellates, and cryptos for cryptophytes. *Dinophysis* and *Ceratium fusus* (*C. fusus*) are both dinoflagellates as well. “nd” is used as an abbreviation for “not detected.”

Date and Stn.		> 20 µm taxa						< 20 µm taxa				
		Ciliates	Auto Gym-Gyr	Hetero Gym-Gyr	Thecate Dinos	<i>Dinophysis</i>	<i>C. fusus</i>	Other Dinos	Nanos	Cryptos	Hetero Gym-Gyr	Other Dinos
July 24	East	4	nd	12.5	0	17.2	8.3	4.5	0	nd	nd	0
	Ros	9.1	40	20.8	0	5.9	nd	3.4	0	nd	nd	6.7
Aug. 6	East	0	nd	0	0	0	0	0	0	nd	nd	0
	Ros	24.5	20	35	0	0	20	0	0	nd	0	3.5
Aug. 20	East	40	nd	100	0	0	3.3	0	0	0	nd	0
	Ros	7.1	0	0	0	0	4.4	0	0	nd	nd	0
Sept. 10	East	3.1	42.9	31.6	0	0	0	50	0	0.1	nd	0.6
	Ros	30	0	14.3	0	0	8	0	0.1	0	nd	0

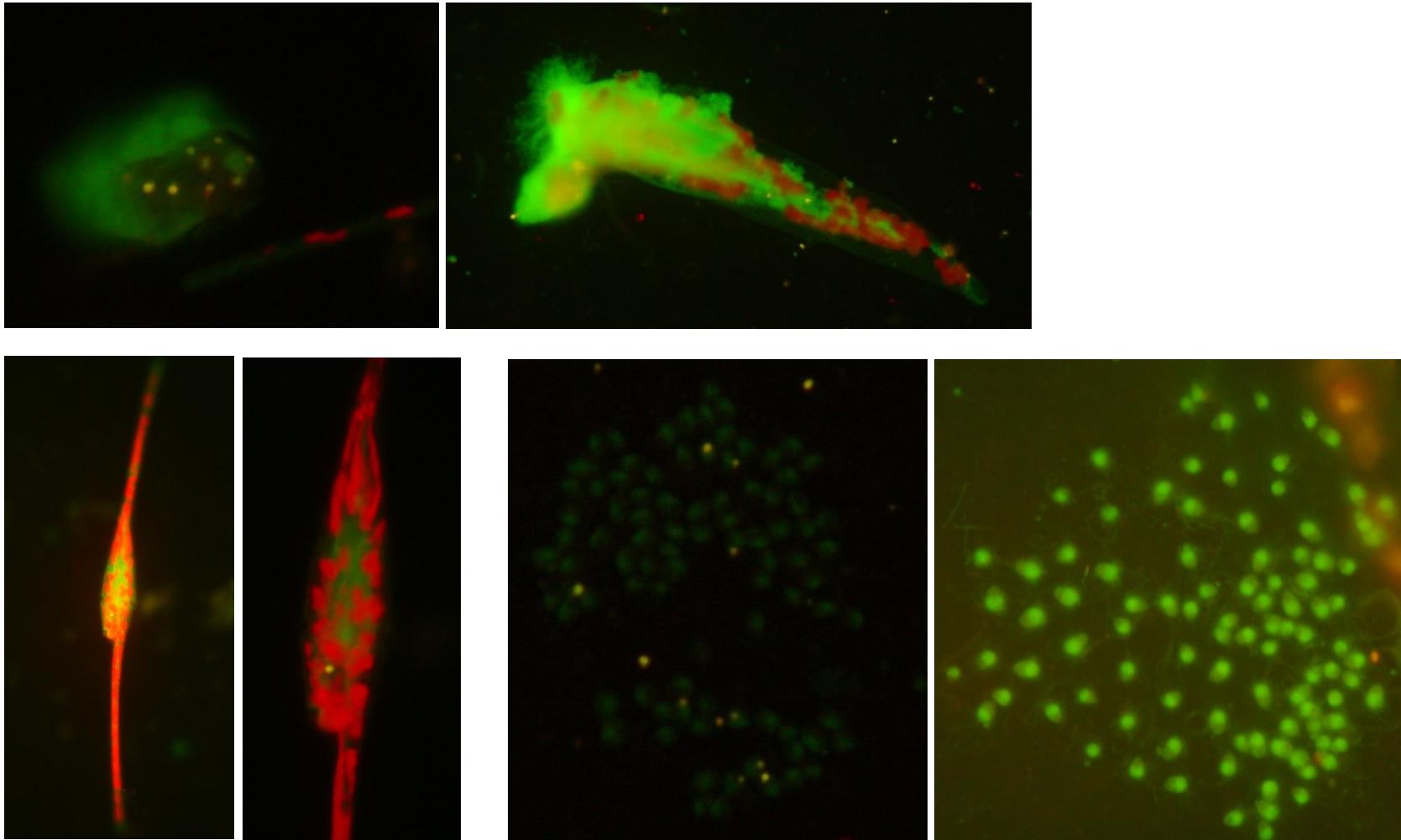


Figure 18. Examples of grazers with ingested *Synechococcus* cells. Clockwise from upper left: Heterotrophic *Gymnodinium* with eight cells (400x), ciliate with two cells (400x), heterotrophic nanoflagellates (no cells) (1000x), heterotrophic nanoflagellates (with cells) (1000x), and *C. fusus* (400x) with a close up of the ingested cell (400x). Photos taken under blue light excitation with an epifluorescent microscope.

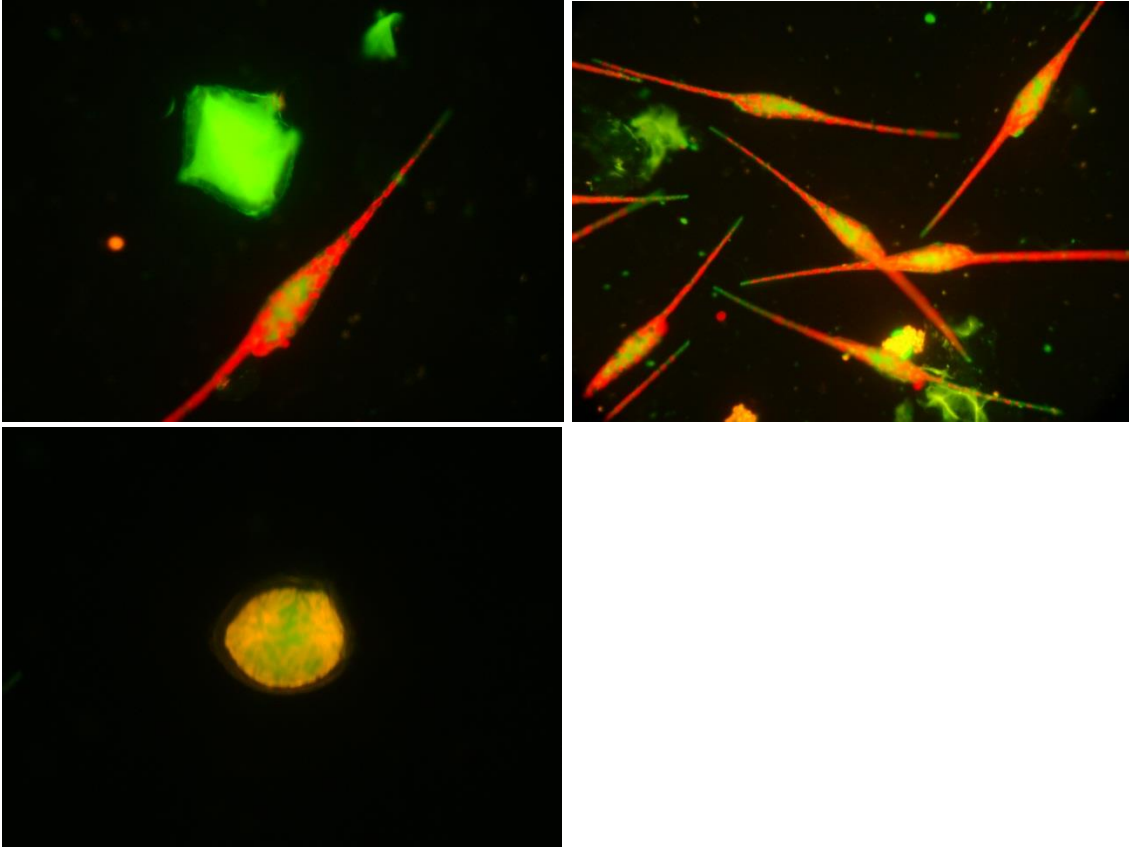


Figure 19. Examples of grazer taxa, clockwise from upper left: a thecate dinoflagellate with one *C. fusus* cell (400x), *C. fusus* bloom (400x), and *Dinophysis* (400x). Photos taken under blue light excitation with an epifluorescent microscope.

### *Principal Components Analysis*

Principal components analysis (PCA) identified two composite variables (PC1 and PC2) that together explained 57% of the variability in the East Sound dataset with 31% and 26% attributed to PC1 and PC2, respectively (Figure 20A). In East Sound, *Synechococcus* abundance appeared alone in the lower right quadrant of the graph. Phosphate, nitrate+nitrite, and depth were all clustered in the lower left quadrant while temperature, PAR, and chlorophyll  $a < 5 \mu\text{m}$  grouped together with a high loading on PC1 in the upper right quadrant (see Table 5 for specific Eigenvectors). Salinity fell out alone with a low loading on PC1 in the upper left quadrant. Total chlorophyll  $a$  and chlorophyll  $a > 20 \mu\text{m}$  shared very similar variability.

PCA identified two composite variables that explained 53% of the variability in the Rosario dataset with 30% and 23% attributed to PC1 and PC2 (Figure 20B). *Synechococcus* abundance and salinity shared similar loadings on PC1, but were separated out by PC2 in the lower right quadrant. Temperature and PAR appeared to covary in Rosario. Total chlorophyll  $a$  and chlorophyll  $a > 20 \mu\text{m}$  shared very similar variability in Rosario as well.

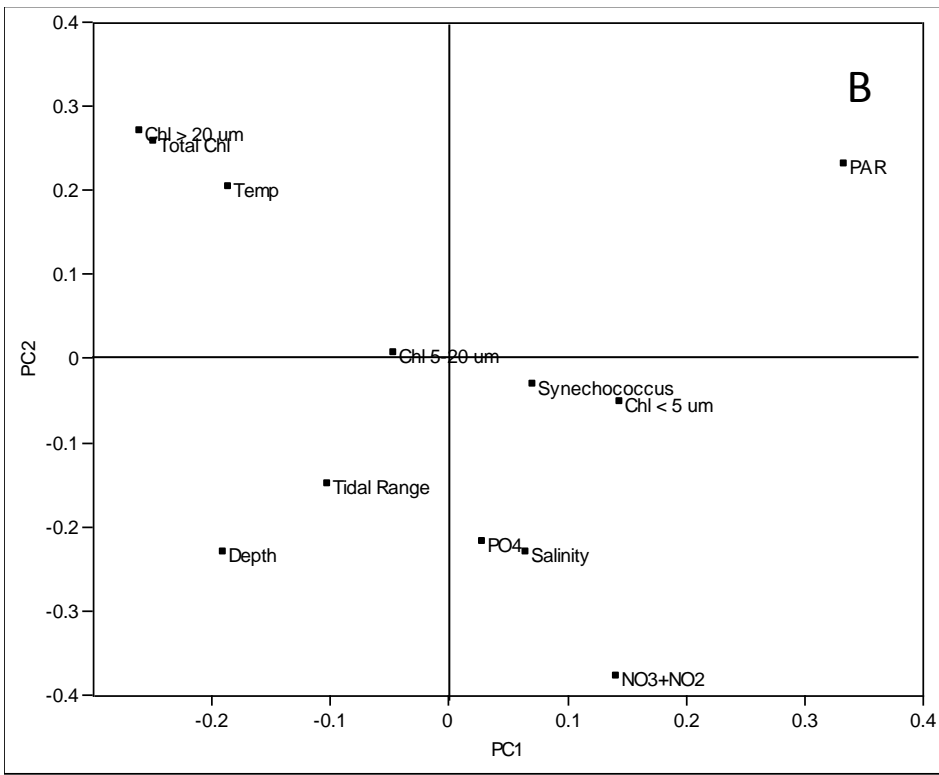
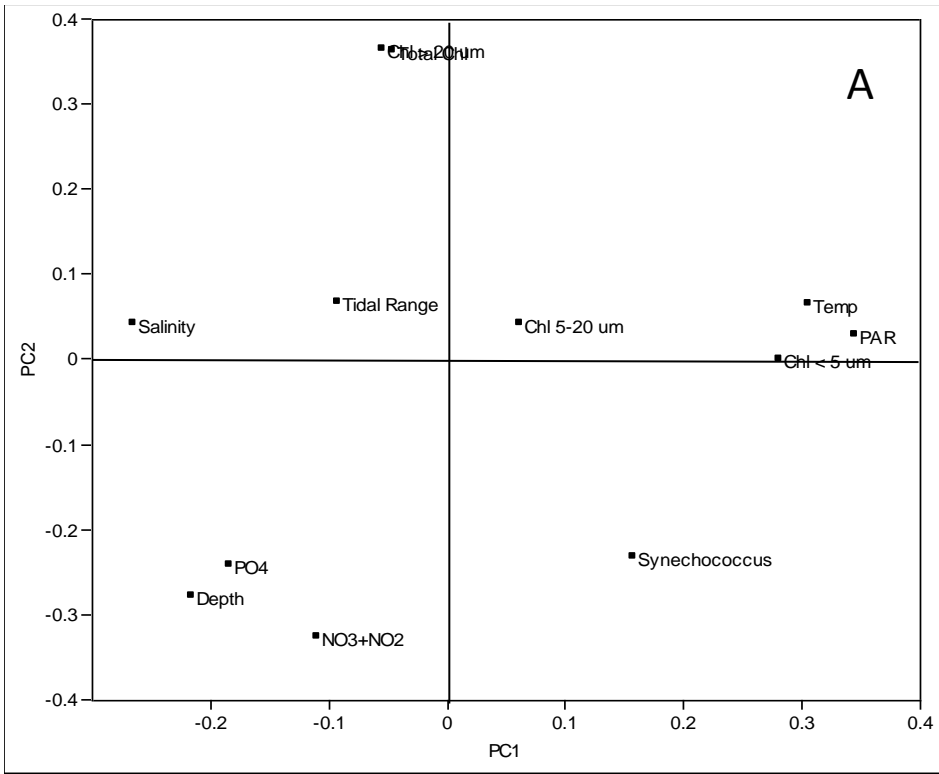


Figure 20. PCA graphs of Eigenvectors on PC1 and PC2 for East Sound (A) and Rosario (B).

Table 5. Eigenvectors from Principal Components Analysis.

Parameter	East Sound		Rosario	
	PC1	PC2	PC1	PC2
Depth	-0.22	-0.28	-0.19	-0.23
Temperature	0.31	0.06	-0.19	0.20
Salinity	-0.27	0.04	0.07	-0.23
PAR	0.34	0.03	0.33	0.23
Tidal Range	-0.09	0.07	-0.10	-0.15
NO <sub>3</sub> +NO <sub>2</sub>	-0.11	-0.33	0.14	-0.38
PO <sub>4</sub>	-0.18	-0.24	0.03	-0.22
Chl $\alpha$ < 5 $\mu\text{m}$	0.28	0.00	0.15	-0.05
Chl $\alpha$ 5-20 $\mu\text{m}$	0.06	0.04	-0.05	0.00
Chl $\alpha$ > 20 $\mu\text{m}$	-0.05	0.36	-0.26	0.27
Total Chl $\alpha$	-0.05	0.36	-0.25	0.26
Syn Abundance	0.16	-0.23	0.07	-0.03



## DISCUSSION

*Synechococcus* vertical distribution and abundance were quite similar between East Sound and Rosario throughout much of the summer of 2012, despite the hydrographic differences between the stations. *Synechococcus* abundance rose as high as  $1.5 \times 10^4$  cells ml<sup>-1</sup> at both East Sound and Rosario in August (Figure 2). There was also a small fall bloom in early September with abundances up to  $6.9 \times 10^3$  cells ml<sup>-1</sup>. Both stations experienced an alternation between stratified and well-mixed water columns, but the distribution of *Synechococcus* remained nearly the same with depth throughout the entire sampling period regardless of stratification intensity. However, temporal variation of *Synechococcus* abundance in the Salish Sea was negatively correlated with salinity at both stations and positively correlated with temperature at Rosario. Both stations also maintained high concentrations of nitrate+nitrite and phosphate and experienced similarly low levels of chlorophyll *a* July through August followed by an increase in September. Protist grazers included ciliates, dinoflagellates, and nanoflagellates with greater abundances of grazers at East Sound than Rosario.

The salinity correlation is an effective explanation for the changes in *Synechococcus* distribution and abundance over the sampling period. The correlation may be due to a physiological effect of salinity on *Synechococcus* cells, or it may be due to the effects of salinity on stratification of the water column that in turn affects *Synechococcus* with differing levels of nutrients and light. Since both stations experienced a similar level of *Synechococcus* abundance with differing temperatures and degrees of stratification, salinity might be an important, yet often overlooked, influence on abundance in the Salish Sea.

### *Salinity Correlation: A Physiological Effect of Salinity?*

The negative correlation between salinity and *Synechococcus* abundance found in this study may be related to a physiological preference for fresher water. However, this correlation may simply be related to the transport and success of *Synechococcus* in fresher water rather than a direct physiological effect. This negative correlation was also detected in a previous year at East Sound and Rosario ( $r^2 = 0.53$ ) (Schanke and Strom, unpublished data), which leads me to believe that there is something different about either the eastern San Juan Archipelago or *Synechococcus* in this ecosystem relative to other studied coastal regions.

Other studies have found correlations between *Synechococcus* abundance and environmental factors that differ from those found in the San Juan Archipelago. In Qingdao, China, *Synechococcus* abundance was positively correlated with salinity and negatively correlated with temperature (Wang et al. 2010). In the Chesapeake Bay, *Synechococcus* abundance was positively correlated with water temperature ( $r^2 = 0.78$ ) (Wang et al. 2011). Studies from Virginia and San Francisco Bay also found a positive correlation between *Synechococcus* abundance and temperature ( $r^2 = 0.44$ ;  $r^2$  between 0.55 and 0.91 between April and August) (Moisan et al. 2010, Ning et al. 2000). Li (1998) proposed that *Synechococcus* abundance was positively correlated with temperatures below 14 °C over an annual scale after comparing studies from all over the world alongside the study conducted in Bedford Basin, Canada. Although a positive correlation with temperature was only found in Rosario in my study, it is clear that temperature has an important role to play in the growth and loss processes of *Synechococcus* in general. However, in the above studies, no mechanism was suggested for why temperature may impact *Synechococcus* abundance.

Salinity also was not correlated with *Synechococcus* abundance except in Qingdao, China where, in contrast to results for the San Juan Archipelago, a positive correlation was found (Wang et al. 2010). No mechanism for the salinity correlation was given here either.

A clade analysis of *Synechococcus* in the Salish Sea was conducted in summer 2011 to see whether the observed *Synechococcus* cells were freshwater or marine. Clade analysis revealed that the cells were from coastal clades I and IV (Suzanne Strom, personal communication, 2013). These clades are most common in temperate and higher latitudes in coastal regions (Zwirgmaier et al. 2008). When considering the effect of salinity on *Synechococcus*, we can be certain that the *Synechococcus* in this area are from marine clades.

Changes in salinity can affect an organism in several ways that can cause physiological stress. A change could cause osmotic stress that impacts the cellular water potential, or it could cause ionic stress caused by the uptake or loss of ions (Kirst 1989). Lastly, a change in salinity can cause a shift in cellular ionic ratios due to the selective ion permeability of the membrane (Kirst 1989). Ions such as  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $K^+$ ,  $Cl^-$ , and  $Na^+$  are needed to maintain the metabolism of marine cyanobacteria like *Synechococcus* (Mackay et al. 1983). However, ionic imbalances can occur in cells when  $Na^+$  and  $Cl^-$  are excessively accumulated, consequently reducing the uptake of these other essential mineral nutrients (Sudhir and Murthy 2004). This imbalance from ionic stress disrupts the basic functions of the cell, until it can equilibrate once more.

Physiologically, cyanobacteria, such as *Synechococcus*, are fundamentally different from eukaryotic phytoplankton. Although they have a gram-negative structure like other bacteria, cyanobacteria, particularly *Synechococcus*, have a thick peptidoglycan layer of

about 10 nm (Hoiczky and Hansel 2000). These peptidoglycan layers are important components of the cell wall. Cyanobacteria also have a cytoplasmic membrane inside of the cell wall and some strains even have an additional surface layer, S-layer, outside of the outer membrane (Strom et al. 2012). A major function of the cell envelope is to allow the transport of nutrients and metabolites in and out of the cell (Hoiczky and Hansel 2000). Cyanobacteria have transport proteins located on the cytoplasmic membrane that have been found to mostly recognize inorganic ions (47% relative to 23% for eubacteria) (Paulsen 1998). Only a small amount of transporters in cyanobacteria are involved in carbon uptake (Paulsen 1998). This suggests that cyanobacteria like *Synechococcus* require inorganic ions such as those that compose salt,  $\text{Na}^+$  and  $\text{Cl}^-$ , more than organic molecules. If that is the case, then osmoregulation must be an essential process for *Synechococcus*, and recovering rapidly from osmotic or ionic stress is necessary for its survival in a marine environment.

Most studies looking at the effect of salinity on *Synechococcus* are focused on freshwater strains. A Florida study found that *Synechococcus elongatus*, a freshwater cyanobacterium, preferred fresher water (Badylak and Philips 2004). The study took place in Indian River Lagoon, Florida where water retention can be from one day to one year depending on the location (Badylak and Philips 2004). In the north-central region of the lagoon, salinity varied from 7 to 25, and this area was dominated by cyanobacteria with *Synechococcus elongatus* forming blooms (Badylak and Philips 2004). This species of *Synechococcus* has a wide range of salinity tolerance in other estuarine environments as well (Badylak and Philips 2004). The Salish Sea does not experience these same levels of salinity change (range of 27 to 31 psu), but it is interesting to note that some *Synechococcus* species are abundant in less saline water.

Salt stress has also been shown to cause significant interruption of the basic functioning of *Synechococcus* cells. One study observing freshwater *Synechococcus* strain 6311 found that hyperosmotic shock (up to 57 psu) actually damaged the membrane of cells (Blumwald et al. 1983). The cells were seen to shrink in the hyperosmotic treatment and could not fully reverse the effect when placed in a hypoosmotic treatment (Blumwald et al. 1983). Although this study also focused on a freshwater strain, the membrane damage of cells is a physiological effect worth noting for *Synechococcus* in general.

Beyond osmotic shock, salt stress can also reduce the efficiency of photosystems involved in photosynthesis. *Synechococcus* have phycobiliproteins attached to the surface of their thylakoid membranes that serve as light-harvesting antennae for photosystem II (Sudhir and Murthy 2004). Salt stress is known to disrupt the energy transfer from the phycobiliproteins to the photosystem II reaction center (Sudhir and Murthy 2004). This stress may be a reason why *Synechococcus* in the Salish Sea are abundant in less saline environments.

The physiological effects of salinity on phytoplankton, and more specifically, *Synechococcus*, are important to note and understand in considering the negative correlation found in this study. However, the salinity range observed in the eastern San Juan Archipelago was only from 27 to 31 psu, which is not likely to induce salt stress in marine *Synechococcus*. Salinity ranges were much more dramatic in the aforementioned studies. These studies also focused on freshwater *Synechococcus* which are likely to tolerate less saline water much better than marine *Synechococcus*. In this study, salinity seemed to be the main driver of density at the two stations but especially at the well-mixed environment of Rosario (Figures 4B and 5B). It is possible that the mechanism behind the negative

correlation is the effect of stratification on *Synechococcus* rather than a direct physiological effect of salinity. The availability of increased light and nutrients at the surface when the water column stratifies provides a favorable environment for phytoplankton (Gargett 1997). Light and nutrients alongside warmer water temperatures allow for phytoplankton to bloom as seen with *Synechococcus* in August and September. The strong alternation between well-mixed and stratified environments at East Sound and Rosario also suggests that water masses were transported in and out of these stations. *Synechococcus* may have been transported into areas with fresher water and been able to succeed in these environments. Overall, the effects of stratification and the covariates of salinity (light, nutrients, and temperature) seem to be better explanations for the negative correlation between *Synechococcus* abundance and salinity than a physiological effect alone. Salinity can still be considered an important predictor for *Synechococcus* abundance in the eastern San Juan Archipelago due to its role in driving density in the water column.

#### *Mixing at East Sound and Rosario*

The hydrography of East Sound and Rosario, and the ability for the water column to stratify, was a major factor in the success of the *Synechococcus* blooms in August and September. The shift from turbulent weather to sunnier days with slackened wind in summer caused stability in the water column: freshwater outflow, especially from the Fraser River into the Salish Sea, also stratified the water column (Yin et al. 1996). Fraser River discharge is known to increase in March, reach its maximum in June, and gradually decrease from July through September (Yin et al. 1997). In the summer of 2012, the maximum discharge was

11,725 m<sup>3</sup> s<sup>-1</sup> in late June (Figure 18). As this freshwater moved into the eastern San Juan Archipelago, the fresher water formed a layer that warmed at the surface and became more difficult to mix with the cooler, more saline water underneath it. There were times throughout the summer when the water column switched between stratified and well-mixed. These alternations could have been caused by wind events which increase mixing and therefore, reduce stratification (Yin et al. 1996). Because East Sound is surrounded by mountains on each side of the embayment, wind periodically came through causing choppy conditions as was observed in the field in June, July, and even mid-September. Rosario also experienced swells due to wind throughout the sampling period. Rosario was well-mixed more often than East Sound due to this wind mixing in combination with more overall tidal mixing as water flushed through the strait.

The alternation between a stratified and well-mixed water column was mirrored in the temperature, salinity, and density profiles (Appendix A, B, C). Because temperature, salinity, and density are closely related in terms of how water masses interact in the ocean, the halo/pycnocline forms along with the thermocline as freshwater enters the system, heats up and causes the water column to stratify. East Sound presented more layers in the water column than Rosario, and stayed stratified with a clear thermocline for longer periods of time (July-August). Rosario did not stratify until early August, and alternated more strongly between well-mixed and stratified conditions. In early September, there were visible layers at Rosario but these quickly diminished within a few days. These statements also hold true for salinity and density.

### *The Nutrient Environment*

Essential inorganic nutrients for phytoplankton growth, such as nitrate and phosphate, were plentiful throughout the majority of the sampling period (Figures 10 and 11). The shift from a well-mixed environment to a stratified one generally leads to changes in the concentration of nutrients. Any events that mix the water column, like wind or tides, also bring nutrients up from deeper waters and consequently, turbulent winter mixing makes nutrients available in the spring throughout the water column (Yin et al. 1997). Once the water column stratifies, there is less vertical movement, allowing phytoplankton at the surface to receive the light they need for growth (Gargett 1997). However, this comes with a price since growing phytoplankton also require nitrate and phosphate to successfully divide, and less vertical water movement means that these nutrients cannot be resupplied to the euphotic zone (Gargett 1997). When the water column once again becomes well-mixed, phytoplankton are mixed deeper and acquire less light, but also more nutrients (Gargett 1997). Since this situation of stratification and nutrient depletion in the upper layer is common in a spring to summer season (Mackas and Harrison 1997), it was surprising that neither nitrate+nitrite nor phosphate experienced any significant depletion during most of my study. In 2005 and 2007 in East Sound, both nitrate+nitrite and phosphate concentrations diminished at some point in the sampling period (Table 6). In 2005, nitrate concentrations were below detection in late July and phosphate concentrations dipped between 0.1 and 0.5  $\mu\text{M}$  from late July through September (Jensen 2007). East Sound in 2007 saw low concentrations of nitrate+nitrite and phosphate (0.0-0.1  $\mu\text{M}$ ) in the month of June and in mid-July (Paul 2010). In 2011, nitrate+nitrite concentration was never below 2.0  $\mu\text{M}$  and the lowest phosphate concentration was 0.9  $\mu\text{M}$  (Table 6). In comparison, nitrate+nitrite



concentrations in 2012 stayed above 5  $\mu\text{M}$  and phosphate concentrations remained above 0.5  $\mu\text{M}$  after the first sampling date on June 11 in East Sound (Figures 10A, 11A). This lack of nutrient depletion is quite strange, but may be related to the small amounts of total chlorophyll *a* present in late June to early August of 2012.

Upwelling is an important source of inorganic nutrients to coastal regions, but did not appear to have an effect on phytoplankton growth in the eastern San Juan Archipelago during my sampling period. In many oceanic systems, upwelling is often a cause for shifts in distribution and abundance of phytoplankton due to the influx of nutrients to the surface that can be used for phytoplankton growth (Neuer and Cowles 1994). Based on the upwelling index for 2012 (Figure 15), there was a generally positive upwelling regime over the summer but there were no periods of intense upwelling events as might be expected in a typical summer season (Neuer and Cowles 1994). If upwelling were to have an effect on growth in an ecosystem, we would expect to see an increase in nutrient concentrations and a spike in phytoplankton growth. However, in this system upwelling did not appear to have a major effect because both nitrate+nitrite and phosphate concentrations were always high with the exception of the beginning of June (Figures 10 and 11). Based on East Sound data from 2005 and 2007 (Jensen 2007, Paul 2010), limiting concentrations would be below 5  $\mu\text{M}$  for nitrate and below 1.0  $\mu\text{M}$  for phosphate. Paul (2010) conducted nutrient addition experiments and compared intrinsic growth rates of phytoplankton with the growth rates of phytoplankton where nutrients had been added. Those results showed that on sampling days when nitrate concentrations were below 5  $\mu\text{M}$  and phosphate concentrations were below 1.0  $\mu\text{M}$ , growth rates were frequently below 0.2  $\text{day}^{-1}$  and were stimulated by added nutrients. Mackas and Harrison (1997) also described limiting nitrate concentrations for phytoplankton growth at <

5  $\mu\text{M}$  in the Strait of Georgia. Overall, nutrients were not likely to have limited growth rates in this ecosystem, and upwelling did not appear to have an important effect on growth and production.

Tidal range also did not appear to have an effect on *Synechococcus* abundance. Tidal range was only weakly, negatively correlated with abundance and only at Rosario (Table 1). Tidal mixing is an important mixing process at both stations, but especially at Rosario where tidal flushing occurs more drastically. Hence, the fact that there was a correlation, albeit a weak one, between *Synechococcus* abundance with tidal range at Rosario makes sense due to the greater degree of tidal mixing that occurs at Rosario.

#### *Chlorophyll a and the Anomalous Decline of Light in 2012*

Total chlorophyll *a* concentrations in July and August of 2012 were surprisingly low with levels from 0.2 to 1.9  $\mu\text{g L}^{-1}$  at East Sound and from 0.1 to 1.4  $\mu\text{g L}^{-1}$  at Rosario (Figure 12). In previous years in East Sound, chlorophyll *a* showed a wider range of concentrations. The 2005 data ranged between 0.2 and 2.0  $\mu\text{g L}^{-1}$  for nanoplankton ( $< 20 \mu\text{m}$ ) alone from June through September (Jensen 2007, Table 6). However, the microplankton ( $> 20 \mu\text{m}$ ) reached 30  $\mu\text{g L}^{-1}$  in early June and plummeted to nearly 0  $\mu\text{g L}^{-1}$  in early July when the nanoplankton were at their highest chlorophyll *a* concentration (Jensen 2007). This same event of the microplankton crashing and the nanoplankton reaching their maximum in early July occurred in 2007 (Paul 2010). In 2007, total chlorophyll *a* ranged from 2.5 to 17.0  $\mu\text{g L}^{-1}$  from June through September (Paul 2010, Table 6). Similarly, the 2011 data from East Sound showed a range of 0.5 to 7.5  $\mu\text{g L}^{-1}$  from June through August (Schanke and Strom,

unpublished data, Table 6). It is important to note that in these past studies, there were periods of time when low nutrient concentrations coincided with high chlorophyll *a* concentrations. However, this was not seen in the summer of 2012 when nutrients were generally high and chlorophyll *a* remained quite low.

Size-fractionated chlorophyll data from 2012 revealed a similar pattern as that of 2005 and 2007 but with the microplankton crashing in late July to a level of  $0.1 \mu\text{g L}^{-1}$ , or approximately 20% of the total chlorophyll *a* (Figure 13). In 2005, microplankton were at lower levels ( $3\text{-}10 \mu\text{g L}^{-1}$ ) in late July alongside the nanoplankton at levels of  $0.4 \mu\text{g L}^{-1}$  (Jensen 2007). Microplankton in 2007 were at levels of  $0.1 \mu\text{g L}^{-1}$  in mid-July while the nanoplankton were high at  $10 \mu\text{g L}^{-1}$  (Paul 2010). In 2011, the nanoplankton levels rose above that of microplankton in late July up to  $1.3 \mu\text{g L}^{-1}$  (Schanke and Strom, unpublished data). The nanoplankton in late July 2012 were at levels between  $0.5$  and  $0.8 \mu\text{g L}^{-1}$ , or approximately 80% of the total chlorophyll *a*. This was a period of time when *Synechococcus* was increasing in abundance. *Synechococcus* did not reach its peak abundance until two weeks later, however, when microplankton appeared to make a comeback composing upwards of 46% of the total chlorophyll *a* concentration. In September, the microplankton dominated the total chlorophyll *a* despite the small fall bloom of *Synechococcus* in early September.

Since *Synechococcus* rely on light for growth, changes in PAR would be a candidate explanation for the variation in abundance. As one would expect, daily PAR levels were relatively high in July and started to decrease through the months of August and September (Figure 7). More consistent days of light alongside increased stratification may be a reasonable explanation for the spike in *Synechococcus* abundance on August 6<sup>th</sup>. However,

Table 6. Compilation of data from past studies in East Sound, Orcas Island, WA. “nd” means “not determined.”

Study Years	Author	Sampling Period	Max <i>Synechococcus</i> Abundance (Cells/ml)	NO <sub>3</sub> +NO <sub>2</sub> Range (µM)	PO <sub>4</sub> Range (µM)	Total Chl <i>a</i> Range (µg/L)	> 20 µm Chl <i>a</i> Range (µg/L)	< 20 µm Chl <i>a</i> Range (µg/L)
2005	Jensen 2007	Feb. 16- Oct. 11	nd	0-38.0	0-2.8	0.2-33.4	0-31.0	0.2-2.4
2007	Paul 2010	May 7- Oct. 5	nd	0-44.9	0-1.6	2.5-17.0	0.1-13.0	1.5-11.0
2011	Schanke (unpublished REU report)	June 29- Aug. 3	3.4 x 10 <sup>3</sup>	2.0-14.5	0.9-4.1	0.5-7.5	0.1-8.1*	0.2-1.3*
2012	Brown (this study)	June 11- Sept. 27	1.5 x 10 <sup>4</sup>	0.03-18.4	0.4-2.5	0.2-34.1	0.04-33.6	0.01-1.3

\*Total chlorophyll *a* fractionated into < 10 and > 10 µm size classes in this study.

PAR alone does not readily explain the increase in abundance on September 10<sup>th</sup> when light levels were decreasing. PAR was not correlated with *Synechococcus* abundance at either East Sound or Rosario (Table 1).

The variation in chlorophyll *a* among years may be due to the amount of light available in a particular year. In the years with East Sound data, 2005 was a year of positive PAR anomalies whereas the PAR anomaly became increasingly negative in later years (Figure 9). This may help explain why there are differences between years with regard to chlorophyll *a* levels, but 2007 still saw high levels of chlorophyll *a* even though PAR levels were relatively low. Thus PAR alone does not seem to be an adequate explanation for why total chlorophyll *a* levels were so low in the summer of 2012.

### *Synechococcus and a Diverse Microzooplankton Community*

#### *Synechococcus Distribution*

The distribution of *Synechococcus* with depth was surprisingly uniform, and remained so throughout the sampling period. Moreover, the distribution displayed similar patterns at both stations with the exception of September when East Sound experienced some changes with depth (Figure 2). Rosario, however, maintained its uniform depth distribution throughout the month of September. Considering the hydrographic differences between East Sound and Rosario, I had expected to see more marked differences in vertical distribution because stratification was stronger at East Sound and layers form more readily there in comparison with Rosario (Twardowski and Donaghay 2001). Since light attenuates with depth, I also had expected that *Synechococcus* would grow best near the surface where light

levels were highest. If growth rates did decrease with depth, this was not reflected in abundance patterns as the deep depth had the highest abundance on August 6<sup>th</sup> and mid depth abundances were occasionally highest in September in East Sound (Figure 2A). Deep depth abundances were often highest in Rosario (Figure 2B). This occurred despite the deep depths often only receiving between 1 and 12% of the total surface light (Table 2).

These distributions could be explained by vigorous horizontal transport of water masses. The Salish Sea has a positive estuarine circulation that is driven by annual freshwater input from the Fraser and Skagit Rivers as well as a small direct input from precipitation (Mackas and Harrison 1997). The San Juan Islands are in a unique position to have water masses flowing in from both the Strait of Juan de Fuca and the Strait of Georgia. The Strait of Juan de Fuca is the major entry and exit of estuarine flow to the Pacific Ocean while the Strait of Georgia is largely affected by the Fraser River plume, tides, and wind mixing (Mackas and Harrison 1997). The combination of oceanic deep water entering in through the Strait of Juan de Fuca along with the fresher surface water from the Strait of Georgia could make for a high level of estuarine circulation in the eastern San Juan Archipelago (Mackas and Harrison 1997). Vertical mixing by wind is also a possible culprit for distribution shifts but based on the increase in Fraser River flow over the past few years (Figure 17), horizontal mixing is a more likely explanation for the similarities in *Synechococcus* distribution between East Sound and Rosario.

### *Comparison of Synechococcus Abundance*

*Synechococcus* abundances vary widely in other coastal regions. The maximum abundance seen in East Sound and Rosario during my study was  $1.5 \times 10^4$  cells ml<sup>-1</sup> whereas, in 2011, the maximum abundance in East Sound was  $3.4 \times 10^3$  cells ml<sup>-1</sup> and up to  $4.85 \times 10^4$  cells ml<sup>-1</sup> at the nearby station of NE Guemes Island (Schanke and Strom, unpublished data). In the Chesapeake Bay, *Synechococcus* abundance reached  $3.3 \times 10^6$  cells ml<sup>-1</sup> in June (Wang et al. 2011). In Qingdao, China, *Synechococcus* abundance was comparable with the Salish Sea at a maximum of  $1.0 \times 10^4$  cells ml<sup>-1</sup> (Wang et al. 2010). On the Virginia coast, *Synechococcus* abundance peaked at  $1.1 \times 10^5$  cells ml<sup>-1</sup> and in San Francisco Bay, *Synechococcus* abundance reached  $5.2 \times 10^5$  cells ml<sup>-1</sup> (Moisan et al. 2010, Ning et al. 2000). This variation in abundance may be due to the fact that all of the aforementioned areas are very different ecosystems. For example, East Sound is a fjord-like embayment at high latitude that consistently had high nutrient concentrations in 2012. Contrast this with an estuarine ecosystem like the Chesapeake Bay where temperature varies from 0 to 30 °C annually (Wang et al. 2011) or the New Jersey coast where wind-driven upwelling is a major source of physical complexity (Sosik et al. 2003). These differences point to the fact that the ecosystems themselves are likely to affect *Synechococcus* abundance.

### *Microzooplankton Community Assemblage*

The euphotic zones of East Sound and Rosario had very different microzooplankton community compositions, likely due to the differing oceanographic features of the two stations. East Sound is a fjord-like embayment chiefly affected by wind and tidal mixing,

while Rosario experiences high flushing rates as water masses from the Strait of Georgia and Strait of Juan de Fuca meet (Rines et al. 2002). Each station had an abundance of ciliates, dinoflagellates and nanoflagellates, as well as ultraplankton such as *Synechococcus*. However, East Sound generally had many more ciliates than Rosario. East Sound also had a greater abundance of dinoflagellates overall than Rosario and experienced a *Ceratium fusus* bloom in late August. This may be because stratification tends to favor the growth of dinoflagellates like *C. fusus* that are not very tolerant of turbulent conditions (Margalef 1997). At the nano scale, East Sound tended to have more nanoflagellates, but Rosario also had similar abundances of small dinoflagellates. Overall, because of East Sound's hydrography, it may have acted as a better incubator for delicate phytoplankton such as ciliates in comparison with Rosario where circulation was more turbulent.

#### *Protist Grazing Impacts*

Although many environmental variables have an impact on the growth of *Synechococcus*, protist grazers represent a potentially important loss term for *Synechococcus* in the Salish Sea. As I hypothesized, dinoflagellates were important consumers of *Synechococcus* in this study (Table 3). Ciliates also seemed to be major consumers of *Synechococcus* but, as previously stated, ciliates were only counted using epifluorescent microscopy which is not a reliable source for ciliate enumeration because the glutaraldehyde used as a fixative does not preserve them well. The cells tend to lyse during fixation and therefore, a more accurate enumeration of ciliates would involve using Lugol's solution.



Unfortunately, this method is not effective for determining the ingestion of *Synechococcus* because the cells lose their autofluorescence.

The most unexpected result was that, although heterotrophic nanoflagellates were abundant in both East Sound and Rosario, they were rarely observed to contain *Synechococcus*. This was unusual considering that nanoflagellates are cited as one of the primary consumers of marine bacteria such as *Synechococcus* (e.g., Frias-Lopez et al. 2009). Ciliates and nanoflagellates together are known as the main grazers controlling *Synechococcus* biomass and production (Chan et al. 2009). Since nanoflagellate abundance was so much higher than ciliate abundance (100x more abundant), it is reasonable that grazing pressure from nanoflagellates should be higher than that of ciliates (Chan et al. 2009). However, my results revealed just the opposite. *Synechococcus* is known as a poor food source for nanoflagellates in terms of carbon transfer efficiency (Guillou et al. 2001). However, cyanobacteria have been thought to be a major carbon source for pelagic ciliates (Guillou et al. 2001, Christaki et al. 1999). Perhaps ciliates selectively ingested *Synechococcus* for this reason. Another important aspect to consider is that ciliates and nanoflagellates have different feeding strategies. Nanoflagellates may use raptorial or interception feeding to handle each prey item separately, while ciliates are effective at removing prey by filter feeding using their cilia to transport water into their oral structures (Boenigk and Arndt 2002). This feeding behavior allows ciliates to ingest many more cells overall than nanoflagellates, despite high nanoflagellate abundance.

Dinoflagellates, especially in the  $> 20 \mu\text{m}$  category, had an apparent grazing impact on *Synechococcus*. The abundance of dinoflagellates in the protist grazer community was higher than that of ciliates, and therefore, many dinoflagellates were counted and observed

with ingested *Synechococcus*. Heterotrophic *Gymnodinium* and *Gyrodinium* were the main culprits of *Synechococcus* ingestion. *Gyrodinium* spp. feeds on a single bacterium cell by creating feeding currents with its flagella and engulfing the cell (Jeong et al. 2010). This feeding strategy appears to work well for ingesting small *Synechococcus* cells. Other than the *Ceratium fusus* bloom on August 20<sup>th</sup> (Table 3), it did not appear that dinoflagellate abundance followed any particular pattern of growth and loss. There were certainly more dinoflagellates in these samples than there were ciliates, but that also had much to do with the method of enumeration i.e. not all ciliates survived fixation. It is difficult to say why dinoflagellates may have ingested more *Synechococcus* than both ciliates and nanoflagellates other than the fact that the larger dinoflagellates were more effective at capturing these small cells.

Overall, ingestion of *Synechococcus* by protists did not exhibit a clear temporal pattern from July 24<sup>th</sup> to September 10<sup>th</sup> (Tables 3 and 4). Each sampling date was very different from the last for each station. On August 20<sup>th</sup> in East Sound, it did appear that the ciliates, autotrophic and heterotrophic *Gymnodinium*/*Gyrodinium* were the most active grazers on *Synechococcus*. Heterotrophic *Gymnodinium*/*Gyrodinium* were the main dinoflagellate grazers on *Synechococcus* in both East Sound and Rosario throughout this period of time when *Synechococcus* abundance was highest. These particular dinoflagellates are capable of consuming small cells like *Synechococcus*. We cannot determine whether these dinoflagellates were preferentially consuming *Synechococcus* but, based on this study they did appear to effectively ingest *Synechococcus* cells.

### *Principal Components Analysis*

The appearance of *Synechococcus* abundance alone in the lower right quadrant of the East Sound graph (Figure 20A) most likely means that none of the explanatory variables were closely related to the variation in abundance and that another unmeasured factor, such as grazing mortality, may be an important factor to consider. The cluster of phosphate, nitrate+nitrite, and depth in the lower left quadrant pointed to an effect of stratification on the concentration of nutrients with depth. Temperature and salinity varied inversely, as expected, while the ultraplankton (< 5  $\mu\text{m}$ ) as a whole may be affected by temperature and PAR levels. This points to the fact that the ultraplankton as a whole tended to flourish in a stratified environment. However, it is interesting to note that *Synechococcus* did not group with the ultraplankton. Total chlorophyll *a* and chlorophyll *a* > 20  $\mu\text{m}$  shared very similar variability in both East Sound and Rosario, furthering the understanding that the microplankton dominated the total chlorophyll *a* levels at both stations.

In Rosario, the relationships among variables were not quite as clear, perhaps because this was a more well-mixed environment. *Synechococcus* abundance and salinity shared the same loading on PC1, but were separated out by PC2 (Figure 20B). *Synechococcus* abundance and salinity were also strongly negatively correlated at Rosario. In this environment, salinity may be the main driver of stratification since any fresh water entering the system causes stratification. Therefore, the relationship in variability seen here may be due to stratification. Temperature and PAR appeared to covary in Rosario which might be because warming surface layers did not last long before being flushed out of the area.

## *Conclusion*

Variability in light, salinity, and tidal mixing create the complex environments in East Sound and Rosario in which *Synechococcus* bloomed in August and September. Although these two stations had different oceanographic features, *Synechococcus* distribution and abundance reacted in the same manner throughout the sampling period. Based on the results of the PCA, it is clear that these stations are indeed differing in environmental variability and that *Synechococcus* may be more influenced by salinity in the well-mixed environment of Rosario where salinity acts as a proxy for stratification. Salinity is an important predictor of *Synechococcus* abundance in the eastern San Juan Archipelago due to its role in driving density in the water column consequently affecting stratification.

Consistently high nutrient and low chlorophyll *a* concentrations were an unusual trait of the summer of 2012. Although 2012 was a negatively anomalous year for PAR, previous years with a negative anomaly still exhibited a range of both nutrient and chlorophyll *a* concentrations. This suggests that another wide scale process was at work that was not measured in this study such as the advection of rich phytoplankton stocks out of the area by unusually high summer runoff rates.

Protist grazers of *Synechococcus* were active in a diverse community of ciliates, dinoflagellates, and nanoflagellates. Dinoflagellates were important grazers of *Synechococcus* in the eastern San Juan Archipelago, more so than even the ciliates or nanoflagellates that are typically thought of as the main grazers of *Synechococcus*. Dinoflagellates are significant members of the phytoplankton assemblage in the Salish Sea

and should be considered in any field or lab study involving the ingestion of ultraplankton like *Synechococcus*.

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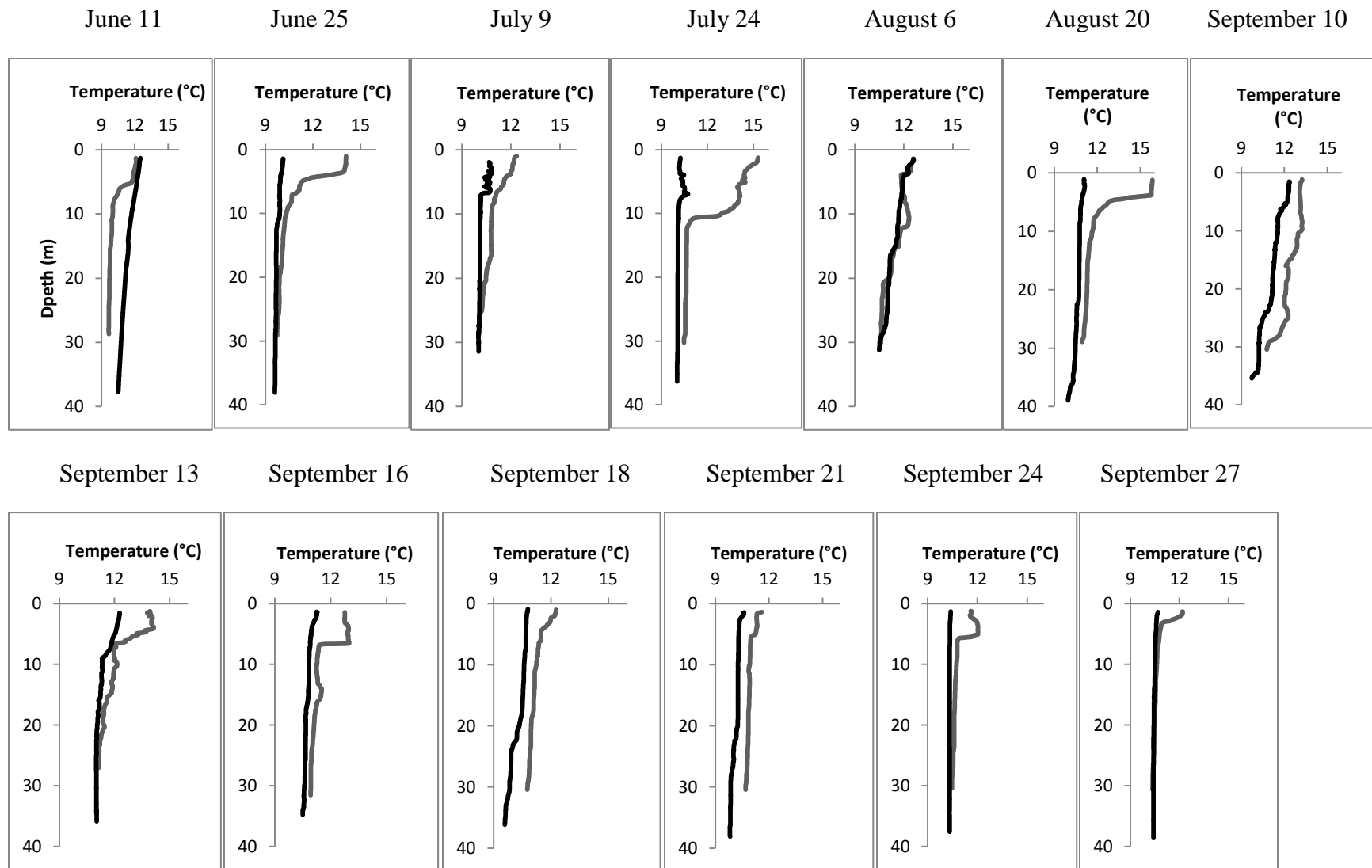
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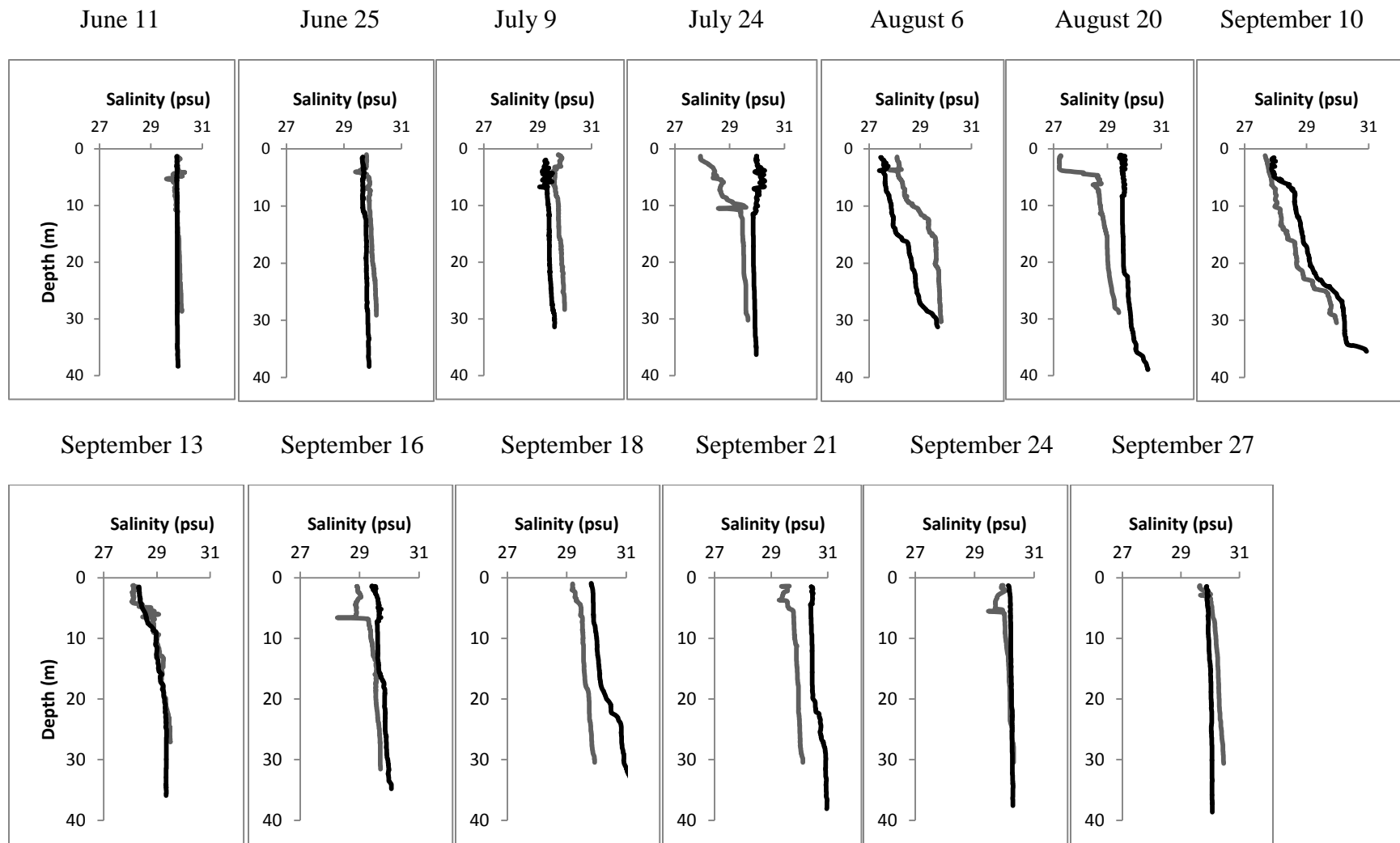
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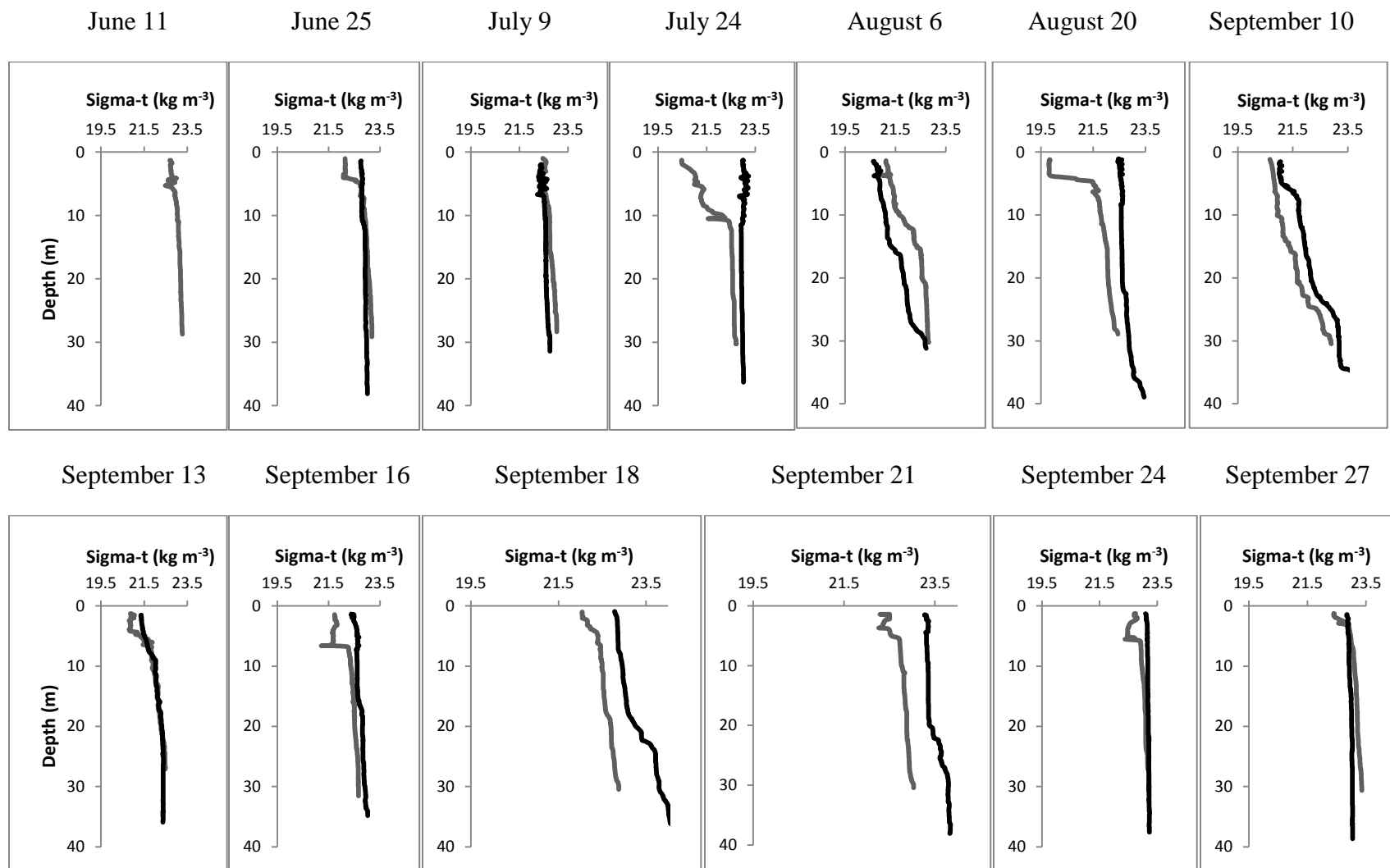
Appendix A. Depth profiles of temperature from June 11 to September 27, 2012. East Sound is dark grey, and Rosario is black.



Appendix B. Depth profiles of salinity from June 11 to September 27, 2012. East Sound is dark grey, and Rosario is black.



Appendix C. Depth profiles of density from June 11 to September 27, 2012. East Sound is dark grey, and Rosario is black. Rosario data is missing for June 11 due to irregularities in the density data for that day.



Appendix D. Depth profiles of chlorophyll *a* fluorescence from June 11 to September 27, 2012. East Sound is dark grey, and Rosario is black.

