

Comparing King County CTD Data with Phytoplankton Biomass and Abundance in Puget
Sound's Main Basin to Determine Leading Causes of Harmful Algal Blooms

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Abstract

This study investigates the relationship between phytoplankton growth and different environmental factors, hypothesizing that an increase in the stratification of Puget Sound's Main Basin will result in an increase in phytoplankton biomass and the possibility of harmful algal blooms (HABs). CTD data was collected from 7 stations in the Main Basin by King County's Department of Natural Resources & Parks for phytoplankton biomass ($\mu\text{g C L}^{-1}$), NO_2 and NO_3 concentration (mg N L^{-1}), Chl-a ($\mu\text{g L}^{-1}$), salinity (PSS), and water temperature ($^{\circ}\text{C}$) in 2018 and 2019. There were earlier peaks in biomass in 2018 in May averaging at $700 \mu\text{g C L}^{-1}$ compared to the later 2019 peaks in June averaging at $1000 \mu\text{g C L}^{-1}$. Biomass and nutrient concentration were concluded to have a statistically significant correlation when plotted against one another and a linear regression was conducted. Salinity depth profiles indicated earlier water column stratification in 2018 and surface temperature was approx. 1.5°C greater in June of 2019 for 2 stations. A FlowCAM was used to identify phytoplankton taxa to determine the abundance of potentially toxic species. Multiple potentially toxic phytoplankton taxa were found at the 3 stations of focus including *Akashiwo sanguinea*, *Pseudo-nitzschia sp*, *Chaetoceros*, *Thalassiosira*, and *Heterosigma akashiwo*. These species accounted for the majority of the total phytoplankton abundance in 2019, indicating that later occurring blooms have greater potential to result in an HAB.

Summary

Phytoplankton play an important role in the cycle of carbon on Earth by acting as a vital carbon sink. When there is rapid growth of certain species that have the potential to release toxins harmful algal blooms can occur, which can have numerous negative impacts. When these toxins are ingested by marine organisms they can die, which affects the stability of the marine food web. Shellfish can pass them on to humans, where they pose a health risk. Increased growth also leads to eventual decomposition, a bacterial process that uses oxygen and when performed at a rapid rate can create an oxygen-poor zone. This study examines the effects of water column stratification and NO_2 & NO_3 concentrations on phytoplankton biomass and the abundance of potentially toxic species. Stratification is influenced by properties like temperature and salinity that impact the density of water. As water becomes denser i.e., the temperature decreases or the salinity increases it sinks and forms density layers that can be disrupted by wind-driven mixing and turbulence. The 7 sample sites are located in the Main Basin of Puget Sound and data was compared between 2018 and 2019 with a focus on 3 of the stations that are spread out from each other. Among the findings it was found that there is a clear relationship between these nutrients and phytoplankton biomass and trends and decreases in nutrients are followed by an increase in biomass. The 2019 biomass peak was larger and occurred a month later than the smaller 2018 peak. While there was a higher overall abundance of phytoplankton taxa in 2018, potentially toxic species made up a larger proportion of the 2019 species abundance. Both years experienced the strongest stratification in June, though the salinity profiles indicated signs of stratification beginning earlier in the year in 2018. These trends indicate that harmful algal blooms may be more likely to appear in the Puget Sound when the peak in the spring bloom occurs later in the year.

Introduction

Phytoplankton play a key role in the global carbon cycle by carrying out 40% of primary production in the oceans, resulting in an equal amount of organic carbon to all terrestrial rainforests combined (Nelson, 1995). By consuming carbon dioxide, they slow the effects of ocean acidification and provide organic material for other organisms higher in the marine food chain whose growth is dependent on phytoplankton productivity (Falkowski, 2012). Diatoms, a type of algae, serve as food for both open-ocean predators at the surface and for deep-water organisms once they sink and begin to decompose (Sarhou, 2005). Consumption and decomposition release carbon dioxide, which is either taken in by other phytoplankton or sequestered in deep water until it is upwelled via deep-ocean circulation over a period of multiple centuries. These processes create large carbon sinks and effectively reduce the amount of carbon dioxide returning to the atmosphere, which mitigates the pace of climate change (Falkowski, 2012). The pathway of atmospheric carbon into the ocean and the various forms it takes as it is passed among organisms is known as the carbon pump and is depicted in Fig.1. Phytoplankton first convert it into organic carbon and are then grazed on by zooplankton. Particulate organic carbon then sinks out of the surface layer as fecal pellets and dead organic material. Dissolved organic material is brought below the surface through the process of physical mixing and by zooplankton vertical migration (Benoiston, 2017).

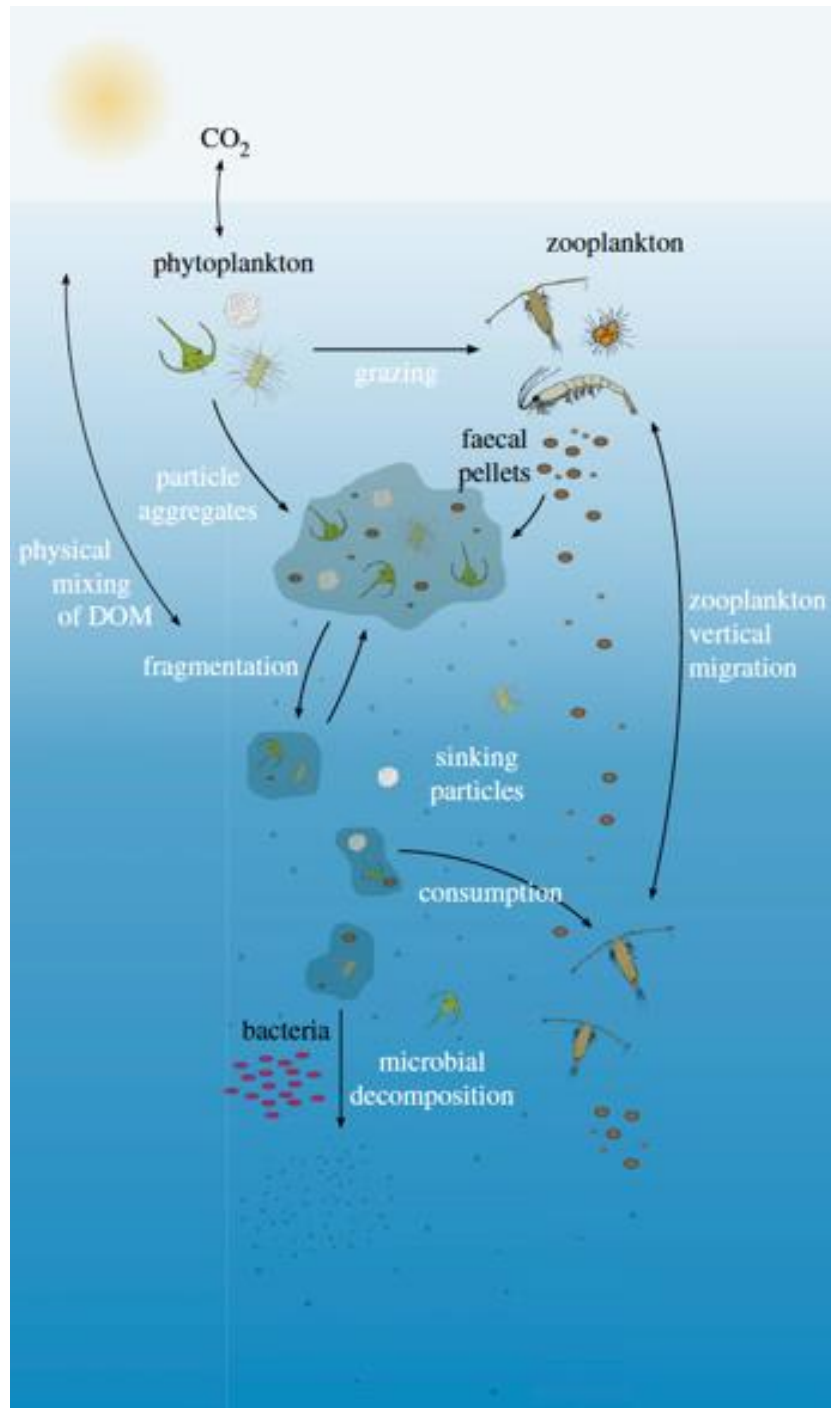


Figure 1. Diagram of oceanic carbon pump. Through photosynthesis phytoplankton convert atmospheric carbon in the form of CO₂ to organic carbon. The particulate organic carbon (POC) then may be consumed by zooplankton before sinking as fecal pellets or dead organic matter. Physical mixing of dissolved organic material (DOM) and zooplankton vertical migration also aid in moving carbon out of the surface layer. Once at the ocean floor it can be kept in the sediment for thousands of years, making the ocean a vital carbon sink (Benoiston, 2017).

Due to the role phytoplankton play in the carbon cycle, they are a key piece in the discussion of climate change prevention techniques. One proposed mitigation effort is to fertilize the ocean with iron in hopes that it will result in increased uptake of CO₂ (Martin, 1990). This idea is not without negative possible side effects including a reshaping of the marine food web due to increased phytoplankton growth and a rapid decrease in the ocean's pH (Denman, 2008). These results make large-scale changes like this controversial and highlight the importance of understanding the correlation between phytoplankton and excess nutrients.

This study will be focusing on two nutrients: nitrate and nitrite and their relationship to phytoplankton biomass. While it is expected for an increase in these nutrients to result in a similar increase in biomass, such a rapid increase may result in an algal bloom. There are numerous factors that influence phytoplankton production including temperature, salinity and precipitation, nutrient availability, and water column structure. Blooms have a variety of effects on the environment; sometimes the increase in planktonic biomass has just a minor effect on the food web by outcompeting other species (Wells et al., 2020). The more concerning type, known as a harmful algal bloom (HAB), has more serious consequences. Harmful algal blooms occur when a specific set of algae including diatoms, dinoflagellates, and cyanobacteria bloom and have negative impacts due to many of them producing toxins leading to contaminated food, oxygen depletion, and environmental deterioration (Erdner et al., 2008). A possible byproduct of HABs is the growth of one of the *Pseudo-nitzschia* species that produces domoic acid, which can be taken in by predators like mussels and be fatal for humans, birds, and mammals (Armbrust, 2009).

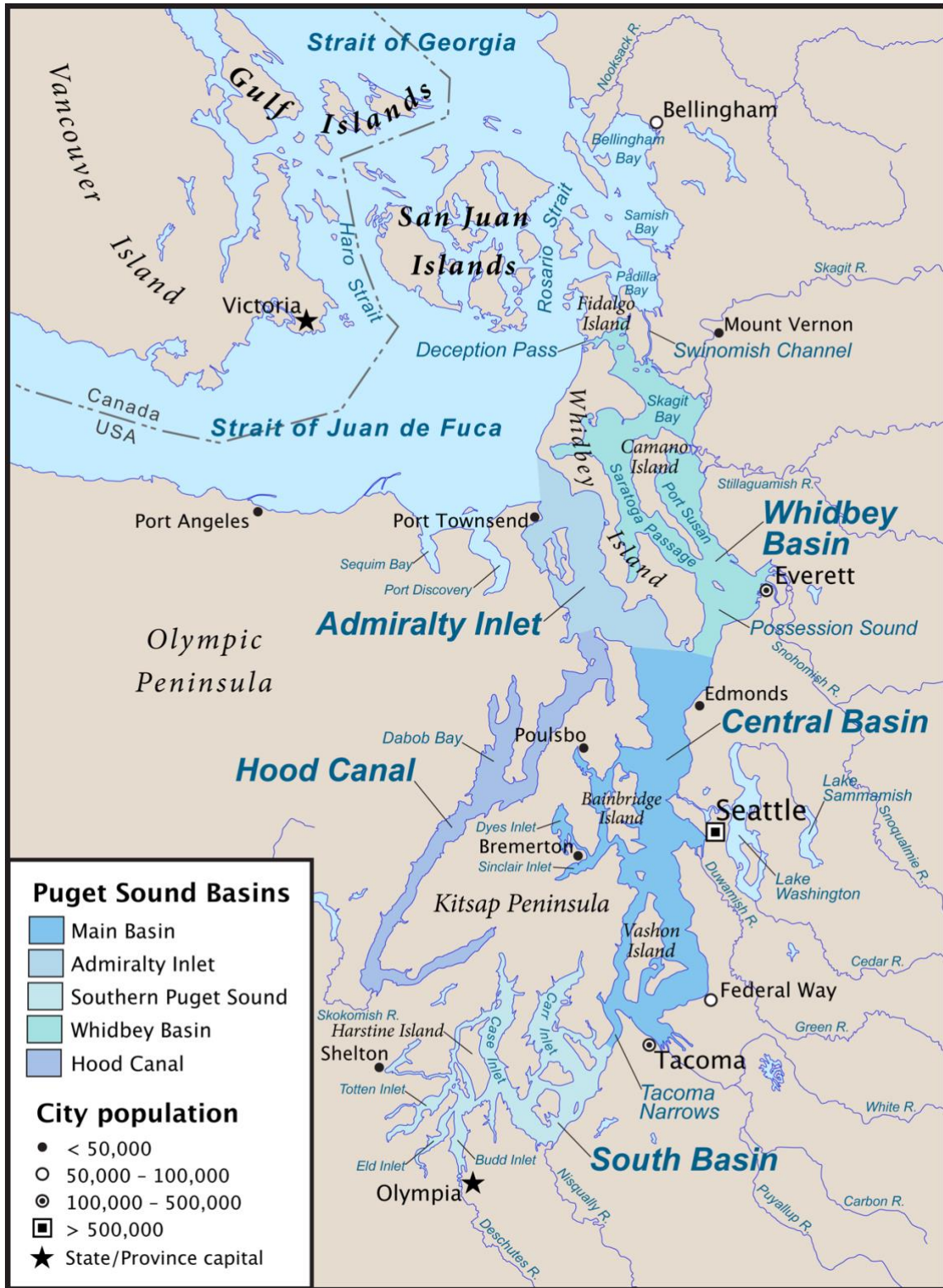


Figure 2. Map of 5 Puget Sound basins: Admiralty Inlet, Whidbey Basin, Hood Canal, Main (Central) Basin, and South Basin. (<https://www.eopugetsound.org/articles/puget-sound-uniquely-diverse-and-productive-estuary>, 2015)

By examining the phytoplankton response to nitrite and nitrate, this study will aim to create a better understanding of the mechanisms that contribute to HABs in the Main Basin of Puget Sound (Fig. 2). The exact effects of the currently warming climate on these processes in this region are unknown, so comparing nutrient and biomass trends across recent years may provide insight. There are multiple driving factors that contribute to phytoplankton growth and the timing and length of blooms. In 2018 and 2019 weak stratification was recorded which led to shortened bloom seasons for both years (Fig. 3). When stratification is strong, it allows for a well-mixed top layer and a deep layer that is poorly mixed. Biomass is expected to be greater when phytoplankton are in a shallower mixed layer that receives ample light (Mellard et al, 2011).

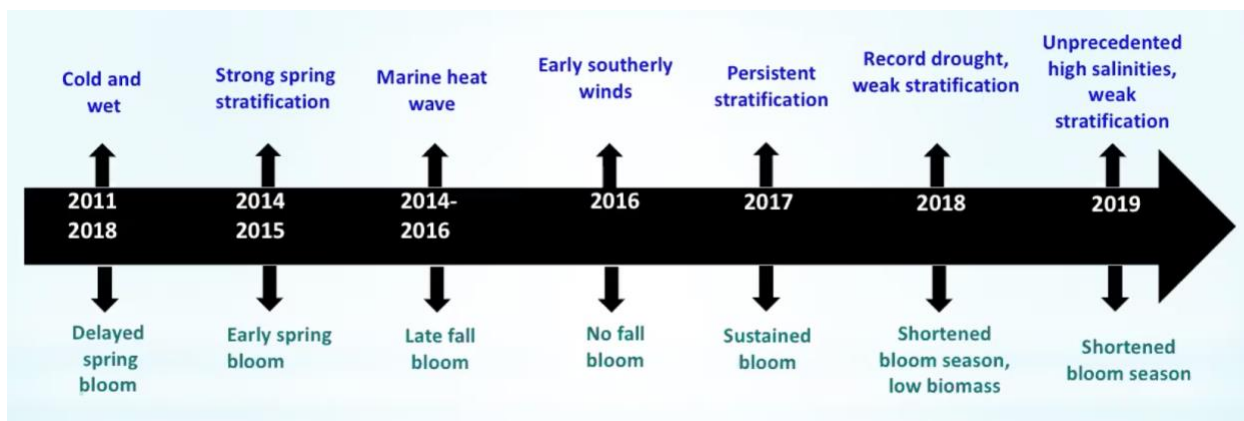


Figure 3. Environmental factors influencing primary production in the Puget Sound Main Basin by year from 2011 to 2019. Weak stratification in 2018 and 2019 led to shorter bloom seasons for both years (<https://ssec.confex.com/ssec/2020/meetingapp.cgi/Paper/4901>, 2020).

Common sources of nitrite and nitrate in seawater are from an anthropogenic origin. Studies have shown that in the temperate zone urban and agricultural wastes such as sewage and fertilizer have resulted in the river export of nitrogen increasing by 3- to 20-fold in

developed areas (Downing et al., 1999). The Puyallup River and the Duwamish River are the two major rivers that empty into Puget Sound's Main Basin where the data was collected (Fig. 2). These rivers flow through heavily urbanized areas and mix with the surface layer once in the Puget Sound to make a nutrient-rich brackish water (Khangaonkar, 2016). Nutrient availability along with sufficient sunlight is an important contributor to phytoplankton growth and when concentrations increase, the potential for a bloom to occur exists (Moore, 2015). Phytoplankton growth rate can also be influenced by the stability of the water column, where greater stratification leads to less upwelling, favoring fast-growing taxa (Levasseur, 1984). This study will take what is known about these relationships to phytoplankton biomass in and focus on the Main Basin of Puget Sound, a location with highly populated surrounding areas (Fig. 2). This provides ample runoff sources for nitrite and nitrate to enter the water and a population that feels the effects of drastic changes in phytoplankton biomass.

Most dinoflagellates grow best in stratified water columns with warmer surface temperatures. *Alexandrium*, a potentially toxic taxa sometimes found in the Puget Sound has been found to grow best in temperatures between 13 °C and 17 °C (Bill et al., 2016). Since Puget Sound is an estuarine system, the density gradients resulting from saline ocean water entering from Admiralty Inlet and fresh river water drive water column stratification and currents (Moore et al., 2009). This study will examine the relationship between phytoplankton biomass and water properties such as salinity and temperature. The impact of these variables on the stratification of the water column should influence the nutrient levels at the surface influencing phytoplankton biomass and the likelihood of harmful algal blooms.

Methods

I. Source of Data

Data for this project was collected from King County Department of Natural Resources & Parks' 7 offshore marine monitoring stations in the Main Basin of Puget Sound. At each station, both seawater samples for phytoplankton counts and CTD profiles including nutrients and chl-a are collected. Samples are collected at these locations twice a month from February to November and once a month in December and January for a total of 22 times per year. (<https://green2.kingcounty.gov/marine/Monitoring/Phytoplankton>, 2020) Station coordinates and descriptions are listed in Table 1 along with a map of their locations in Fig. 4.

Table 1. Sampling Station Coordinates and Descriptions. Site locators correspond with station markers in Fig. 4 and site descriptions provide a brief landmark summary of where stations are in the Main Basin. (<https://green2.kingcounty.gov/marine/Content/Reports/PDF/SiteSummaryPhyto.pdf>, 2020)

Site Locator	Latitude	Longitude	Site Description
JSUR01	47.777401	-122.4179993	Brightwater Treatment Plant Outfall
KSBP01	47.7439995	-122.4280014	Point Jefferson
KSSK02	47.6605988	-122.4469986	West Point Treatment Plant Outfall
LTED04	47.6035995	-122.3570023	Elliott Bay
LSEP01	47.6012001	-122.4260025	South Treatment Plant Outfall
LSNT01	47.5332985	-122.4329987	Point Williams/Fauntleroy
NSEX01	47.3586006	-122.387001	East Passage

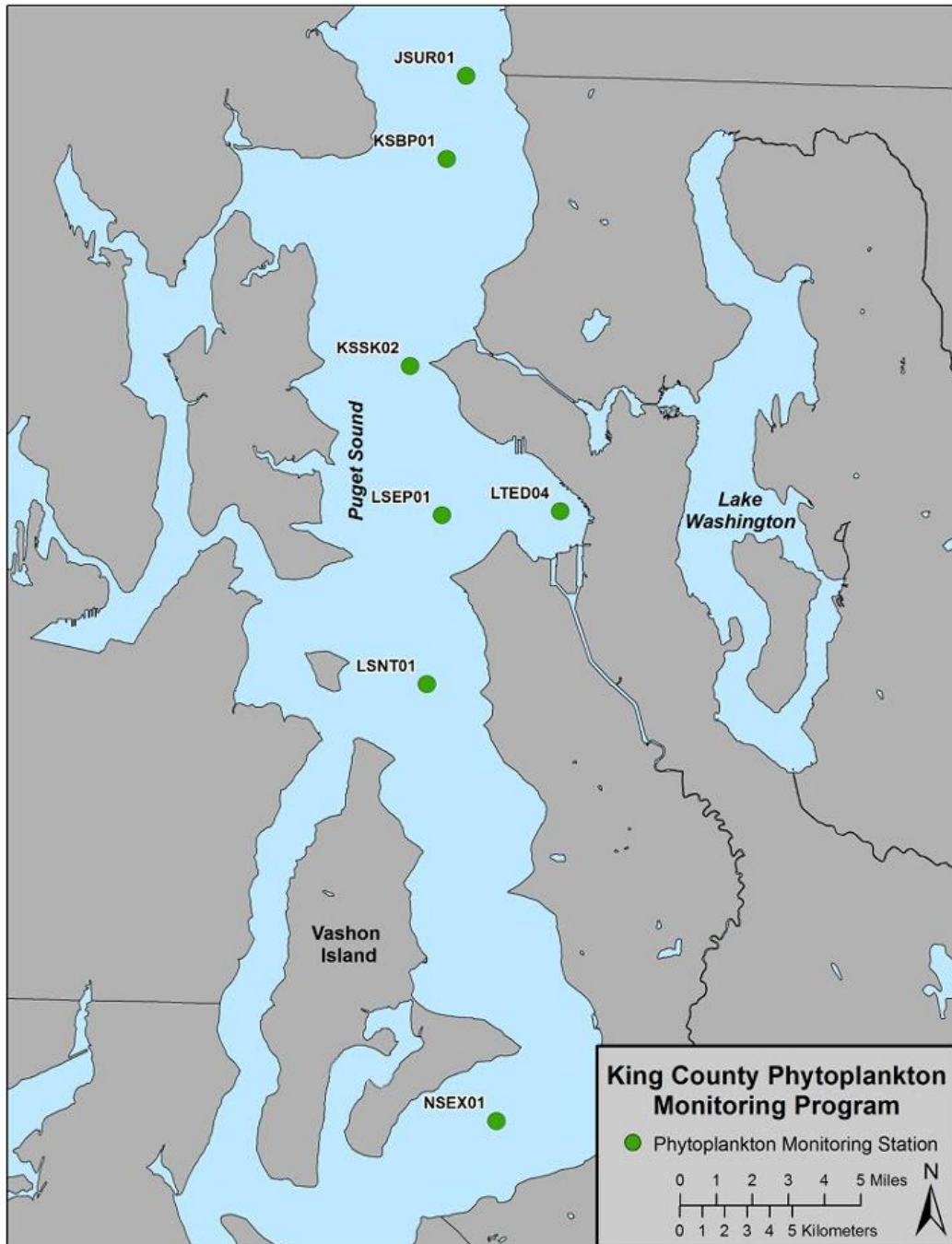


Figure 4. Locations of 7 Sampling Stations in Puget Sound. Offshore stations are distributed throughout the Main Basin and marked by green circles. The 8-character labels correspond to site locators in Table 1. (<https://green2.kingcounty.gov/marine/Content/Images/Sampling/PhytoMap.jpg>, 2020)

Seawater samples were taken at 1-meter depth and collected using a CTD rosette, with 12 5-liter Niskin bottles that were triggered to close at specific depths on the upcast when the rosette

was being brought back up to the surface. Once collected, a 1L clear plastic bottle was filled with seawater from the Niskin bottle at each station then kept on ice. The samples were analyzed using a FlowCAM, which is able to identify and quantify the abundance and size of plankton samples. Abundance (natural units/mL) and biovolume (mm^3/L) were also measured. The FlowCAM data was analyzed using Visual Spreadsheet, a software that uses pattern recognition to identify species. (<https://green2.kingcounty.gov/marine/Monitoring/Phytoplankton>, Jul. 7, 2020) The Seabird SBE CTD rosette has sensors which measure salinity, chl-a, temperature, depth, and nutrients. Water samples were brought to a laboratory for analysis and the water column data was averaged over each 0.5 meters of the total depth. A WET Labs WETStar Fluorometer was used to measure chl-a (g/L) at it was brought up the water column. Resulting CTD and phytoplankton data was obtained from the King County Marine Monitoring website. (<https://green2.kingcounty.gov/marine/Monitoring/OffshoreCTD>, 2020).

II. Methods of Analysis

The data set has phytoplankton counts by species, so an equation derived by researchers at the King County Environmental Lab specific to this FlowCAM is used to calculate biomass. With y representing measured particulate organic carbon and x representing the FlowCAM determined biovolume the equation is as follows:

$$y = 127.67x^{0.4496}$$

(<https://your.kingcounty.gov/dnrp/library/water-and-land/science/SalishSea-2018/17-2018-POSTER-Hannach-Using-FlowCAM-data.pdf>, 2020) Data Analysis and biomass calculations were carried out in Excel using the above formula. Excel was also be used to graph results for the data over time to determine yearly trends.

Results

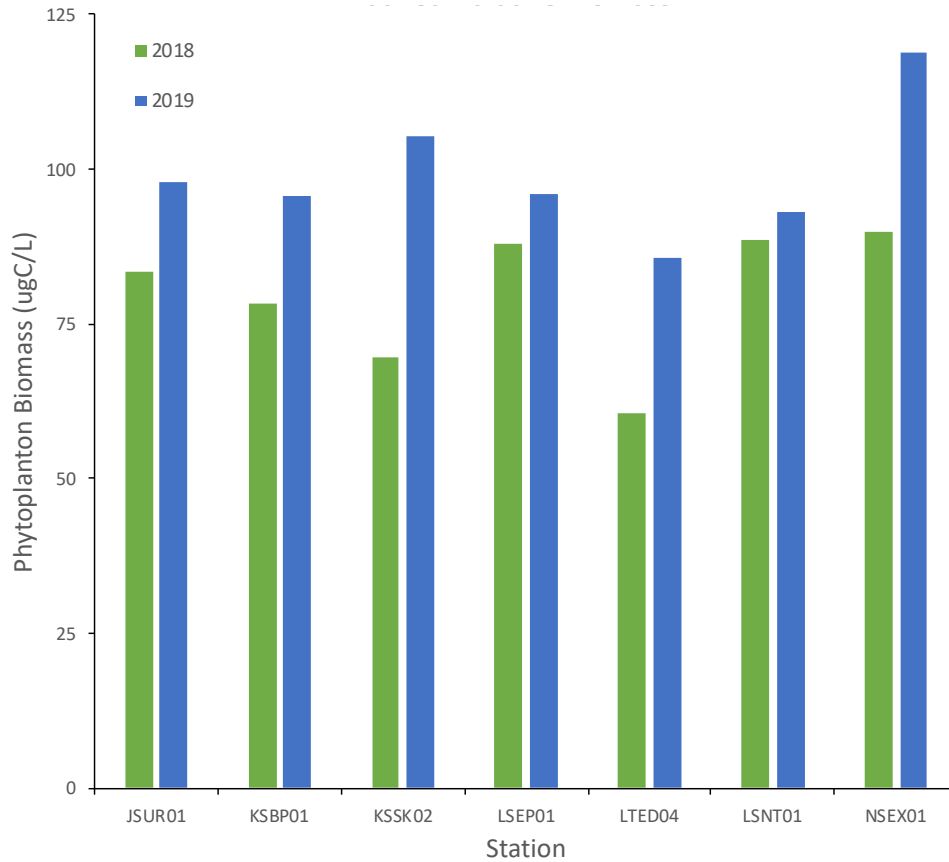


Figure 5. Cumulative annual phytoplankton biomass ($\mu\text{g C L}^{-1}$) for each station. From left to right stations read north to south.

The annual sum of biomass of all phytoplankton species for each station in 2018 and 2019 is given in Fig. 5. The 2019 biomass was greater than the 2018 biomass for all stations and there is a wide range of values between the stations. On Fig. 6 and Fig. 7 phytoplankton biomass, NO_2 and NO_3 concentration, and Chl-a for 3 sample stations are plotted. There is a decrease in nutrients during the spring and summer months and an increase in biomass during this same time period. Stations JSUR01, LTED04, and NSEX01 were chosen to depict trends in the northern, middle, and southern sections of the Main Basin, respectively.

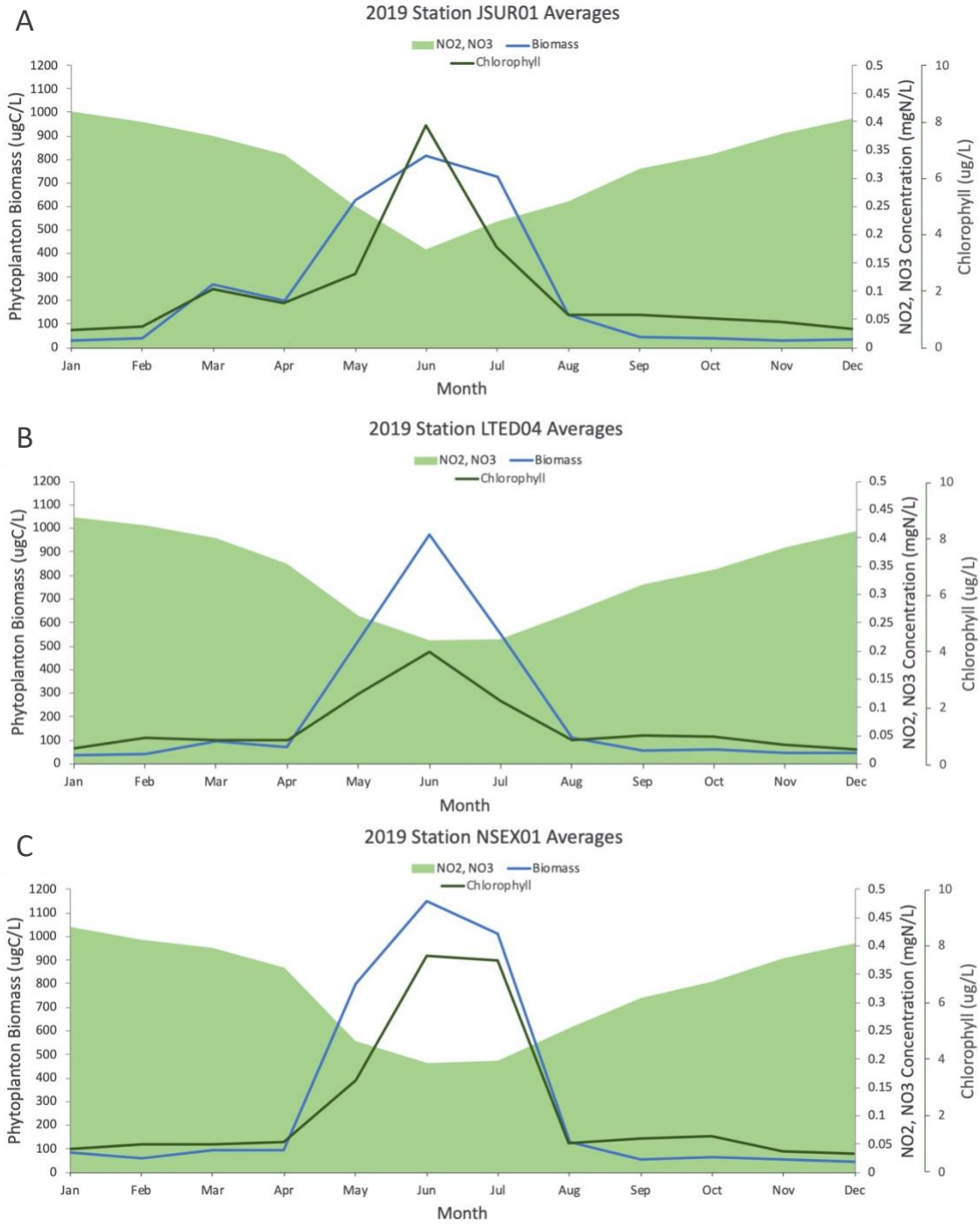


Figure 6. 2019 monthly averages of phytoplankton biomass ($\mu\text{g C L}^{-1}$), NO_2 and NO_3 concentration (mg N L^{-1}), and Chl-a ($\mu\text{g L}^{-1}$) for stations (A) JSUR01, (B) LTED04, and (C) NSEX01. Nutrient and chl-a values were averaged over the top 60 m of the CTD downcast for each month. Plots are in order of northernmost to southernmost station. Plots for remaining stations are located in Appendix 1.

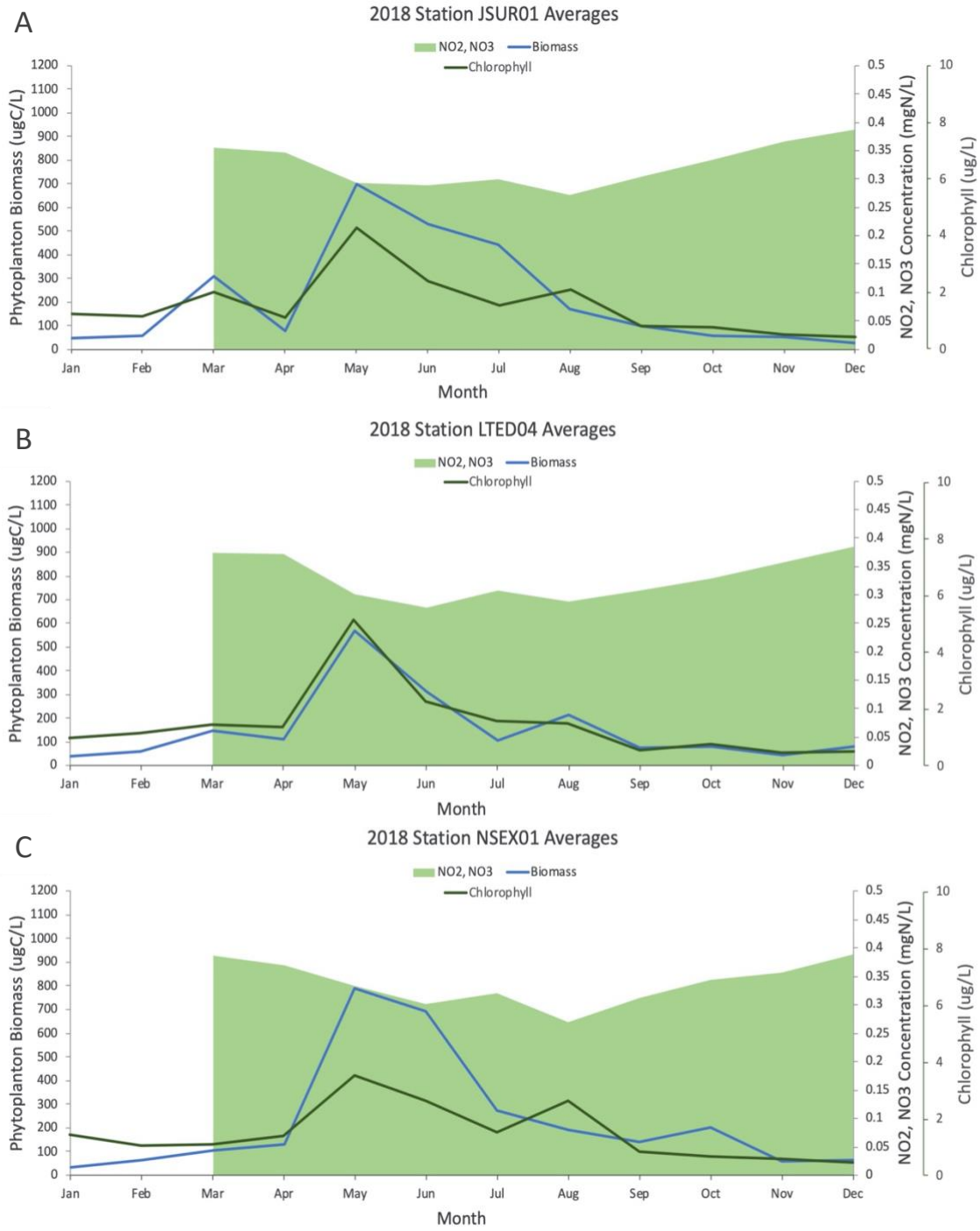


Figure 7. 2018 monthly averages of phytoplankton biomass ($\mu\text{g C L}^{-1}$), NO_2 and NO_3 concentration (mg N L^{-1}), and Chl-a ($\mu\text{g L}^{-1}$) for stations (A) JSUR01, (B) LTED04, and (C) NSEX01. Nutrient and chl-a values were averaged over the top 60 m of the CTD downcast for each month. Plots are in order of northernmost to southernmost station. Data was unavailable for February. Plots for remaining stations are located in Appendix 1.

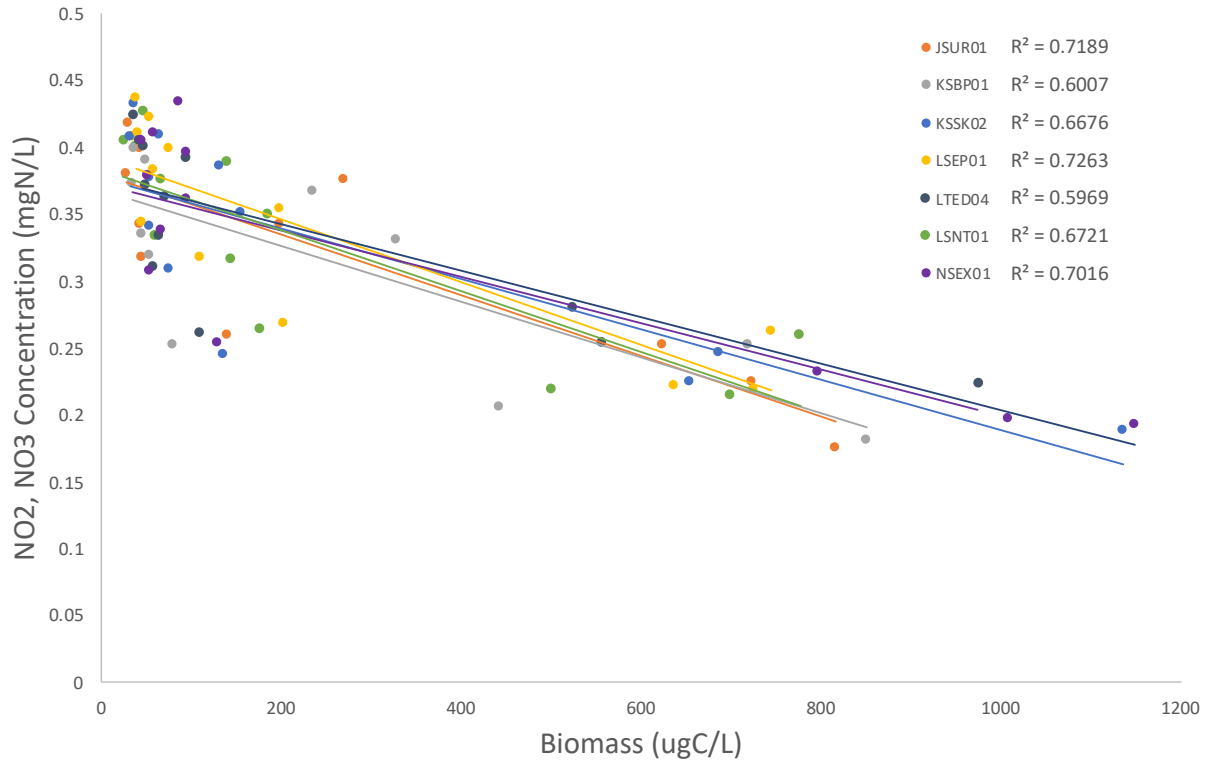


Figure 8. Scatterplot with linear regression and R² values of the biomass and nutrient concentration for all 7 stations in 2019 to determine the variables' correlation. Stations are listed in legend from north to south.

The R² values in Fig. 8 represent how much of the variation in NO₂ & NO₃ can be explained by the variation in biomass. An R² value of 1 would indicate 100% correlation. Station LTED04 had the lowest R² value of 0.5969 and station LSEP01 had the largest R² value of 0.7263. Despite this range all values are high enough to be considered statistically significant. Total phytoplankton abundance at stations was greater in 2018 for all three stations and a higher proportion of potentially toxic species was present in 2019 (Fig. 9). *Chaetoceros* was the most abundant taxa while *Akashiwo sanguinea* was the least. *Chaetoceros* and *Thalassiosira* are phytoplankton genera, while the other taxa are specific species so these two groups contain both toxic and non-toxic species.

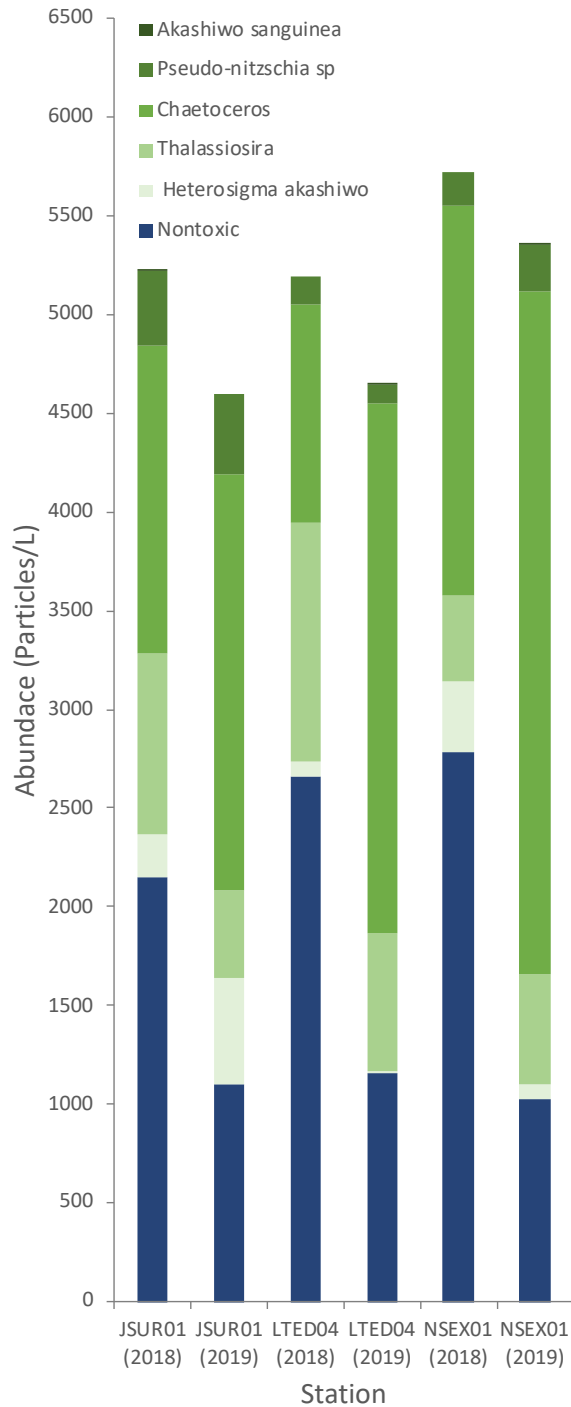


Figure 9. 2018 and 2019 phytoplankton species abundance (particles/L) at stations JSUR01, LTED04, and NSEX01. Potentially toxic taxa *Akashiwo sanguinea*, *Pseudo-nitzschia sp*, *Chaetoceros*, *Thalassiosira*, and *Heterosigma akashiwo* are in shades of green. *Alexandrium catenella* were not present at these stations. Taxa were identified by the King County Department of Natural Resources & Parks using FlowCAM technology.

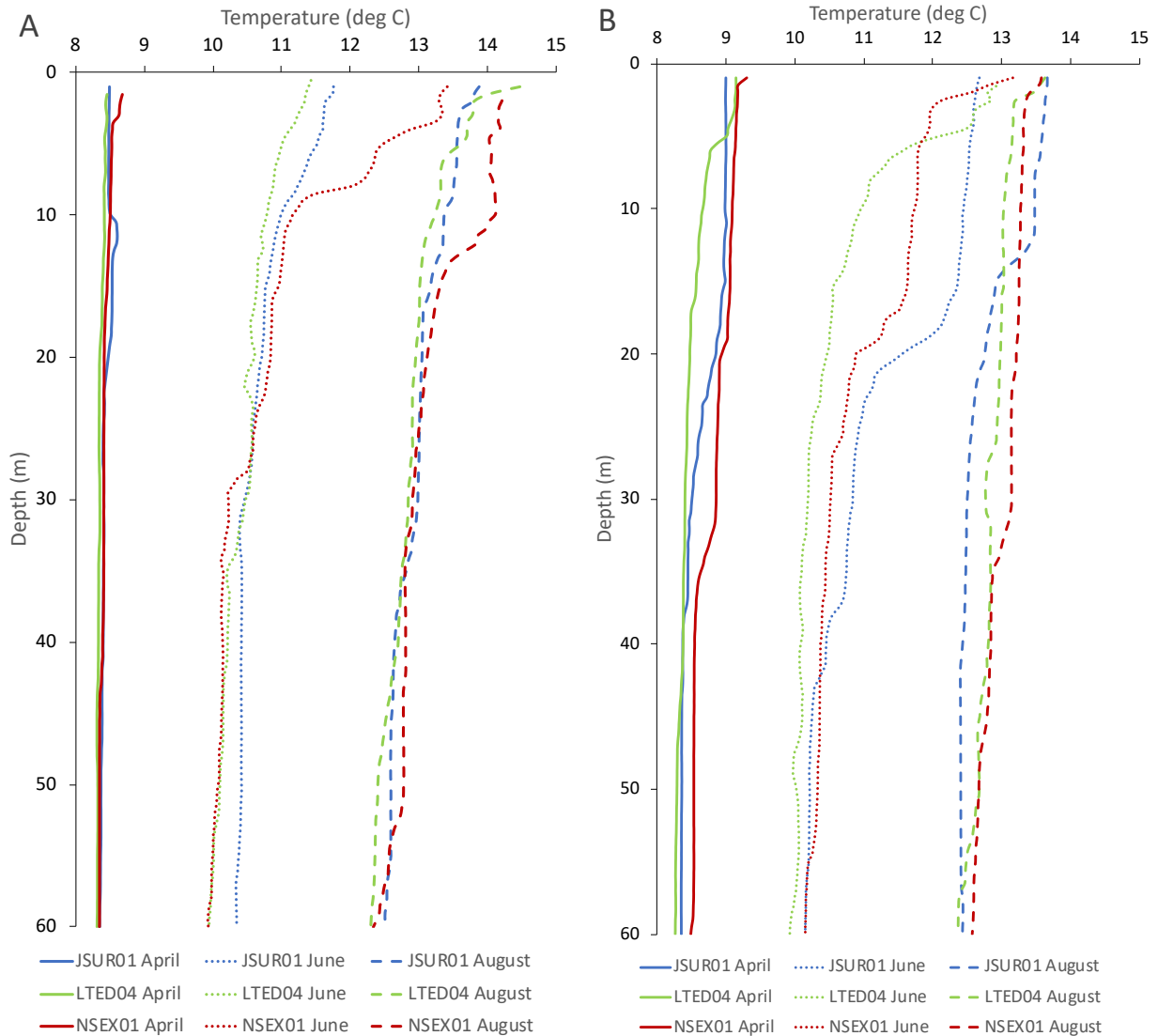


Figure 10. Temperature profiles across top 60 meters at the beginning (April), middle (June), and end (August) of the spring bloom at stations JSUR01, LTED04, and NSEX01 in (A) 2018 and (B) 2019. Temperature data taken from the first sample data for each month.

Spring bloom temperature profiles in Fig. 10 became warmer from April at approx. 9 °C to August between 13 °C and 14 °C with June temperatures covering the largest range from 10 °C to 13 °C. In June and August of 2018 station NSEX01 had higher overall surface temperatures whereas the following year station JSUR01 had higher temperatures. 2019 profiles show a greater range than the previous year with deeper thermoclines in June and August of that

year. Salinity profiles during the spring bloom in Fig. 11 show stratification most prominent at station LTED04 for both 2018 and 2019. April and August of have relatively well-mixed water columns compared to the June profiles with deeper haloclines.

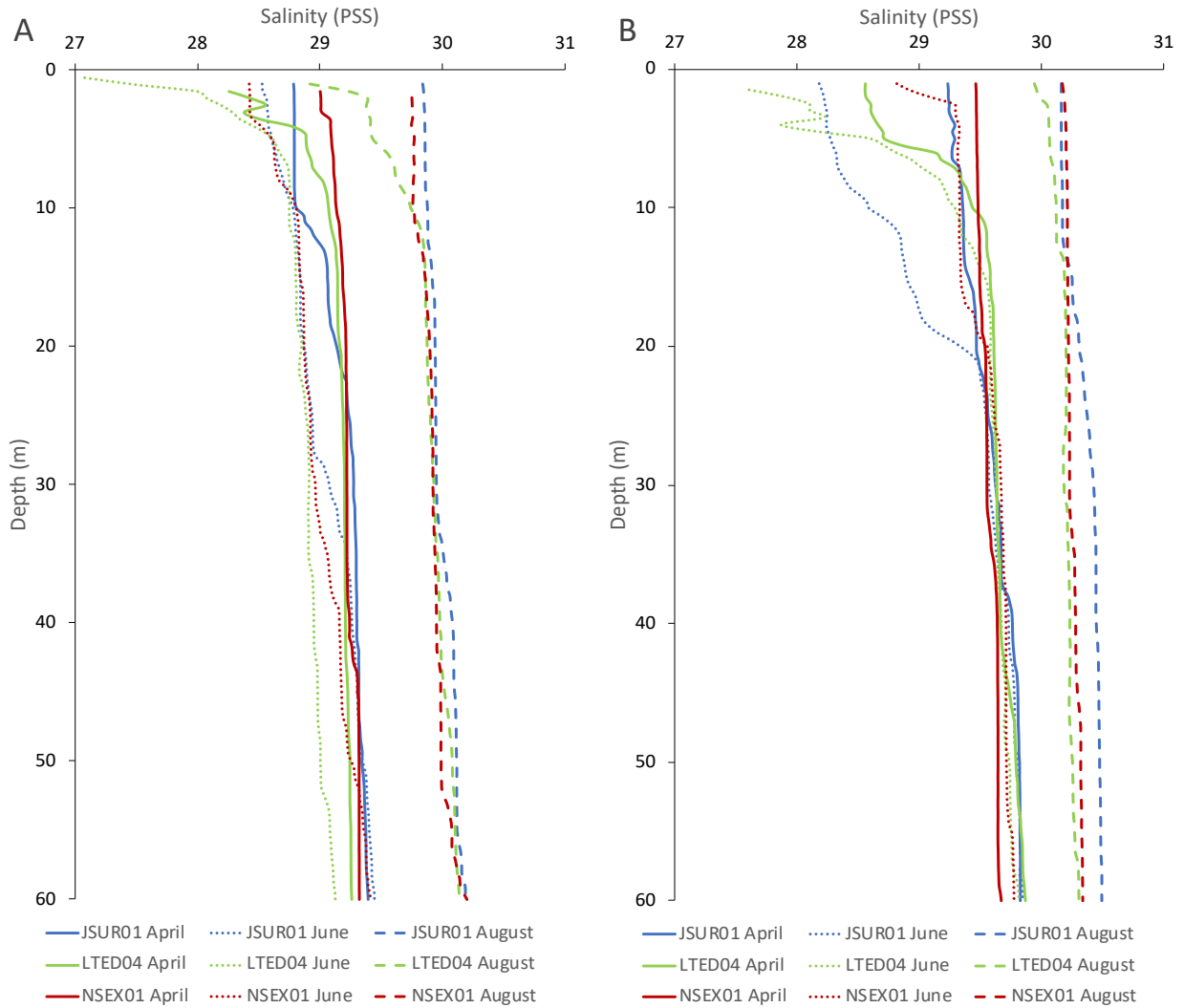


Figure 11. Salinity profiles across top 60 meters at the beginning (April), middle (June), and end (August) of the spring bloom at stations JSUR01, LTED04, and NSEX01 in (A) 2018 and (B) 2019. Salinity data taken from the first sample data for each month.

Discussion

The motivation for determining the main factors contributing to phytoplankton biomass is the potential for harmful algal blooms that arises when rapid increases in growth occur. Six main potentially toxic taxa were identified by the King County Department of Natural Resources and Parks Environmental Lab. These taxa included *Heterosigma akashiwo*, *Thalassiosira aestivalis*., *Chaetoceros convolutus*, *Alexandrium catenella*, *Pseudo-nitzschia* sp., and *Akashiwo sanguinea* (<https://ssec.confex.com/ssec/2020/meetingapp.cgi/Paper/4746>, 2020). All but *Alexandrium catenella* were found at the sample stations, and in 2019 they made up the majority of the overall phytoplankton abundance. Temperature is another contributing factor to phytoplankton growth and along with salinity it shows the strength of the stratification of a water column.

There is an inverse relationship between phytoplankton biomass and nutrients in both 2019 and 2018 with the correlation slightly more pronounced in 2019. Each station shows a statistically significant correlation between the two variables with the R^2 values lying between 0.5917 and 0.7258 (Fig. 8). After the phytoplankton began to use up the nutrients in April both years the biomass peaked in different months. While the phytoplankton biomass peaks were much higher in 2019 a greater overall phytoplankton abundance was recorded in 2018 (Fig. 9). This year there was also earlier signs of stratification of the water column resulting in an earlier peak. While there was lower species abundance in 2019, potentially toxic species made up a larger proportion of the total abundance. This would suggest that when phytoplankton blooms occur later in the year, there is a greater possibility of the formation of a harmful algal bloom.

Comparing the total annual biomass for 2018 and 2019 in Fig. 5, there was consistently a greater biomass in 2019 for all 7 stations. This corresponds with a higher surface temperature in

June for the same year and higher biomass maximums. The temperature increase from the previous year is evidence that this factor has an influence on phytoplankton biomass and the timing of the peak. There is also a decrease of approx. 1 °C in August surface temperatures from 2018 to 2019 at stations LTED04 and NSEX01. In Fig. 7, there is a rise in August biomass for both stations in 2018. This further highlights the importance of water temperature to phytoplankton growth in the Puget Sound.

The salinity profiles in Fig. 11 indicate water column stratification, a structure determined by water density that has less salty water flow on top of more salty water. Both years June was the most heavily stratified month with more pronounced haloclines and a wider temperature range than the other months. April of 2018 had more indications of the water column beginning to stratify than 2019 with the exception of station LTED04, where there was strong stratification at the surface both years. This station is located in Elliot Bay, where the mouth of the Duwamish River empties into the Main Basin of Puget Sound. This influx of freshwater is likely the reason for the stronger stratification and less saline surface water at this station.

The original hypothesis predicted that water column stratification would impact biomass due to surface nutrients being a known contributor to phytoplankton growth. The plots in Fig. 6 and Fig. 7 show evidence supporting a slightly different relationship, where the increasing biomass is followed by a decrease in nutrients. The stratification trends in Fig. 11 also support increases in biomass in June and the earlier peak that occurred in 2018.

There are some limitations to acknowledge in this study; a majority of the potentially toxic phytoplankton taxa found at the stations were either *Chaetoceros* and *Thalassiosira*, genera which contain both toxic and non-toxic species. This may mean the threat of a non-harmful algal

bloom is exaggerated by this data. Only two years' worth of data were compared so a future study might benefit from analyzing the trends of additional years.

Conclusion

Harmful algal blooms have detrimental impacts on other species, the marine food web, and humans. There are 6 potentially toxic taxa that can be found in the Puget Sound and this paper sought to determine correlation between the abundances of these species and water properties like temperature and salinity that impact stratification. In 2019 the biomass maximum occurred later in the year, corresponding with a later start in water column stratification and an increased percentage of potentially toxic taxa. This implies that there may be a link between later blooms and the potential for HAB formation in the Main Basin of the Puget Sound and possibly other similar estuarine systems. While there were differences in temperature and salinity profiles between the two years, I expected them to be more extreme due to the large variation in biomass. This could indicate there are other driving factors for HABs in this location that could serve as topics for future study.

Acknowledgements

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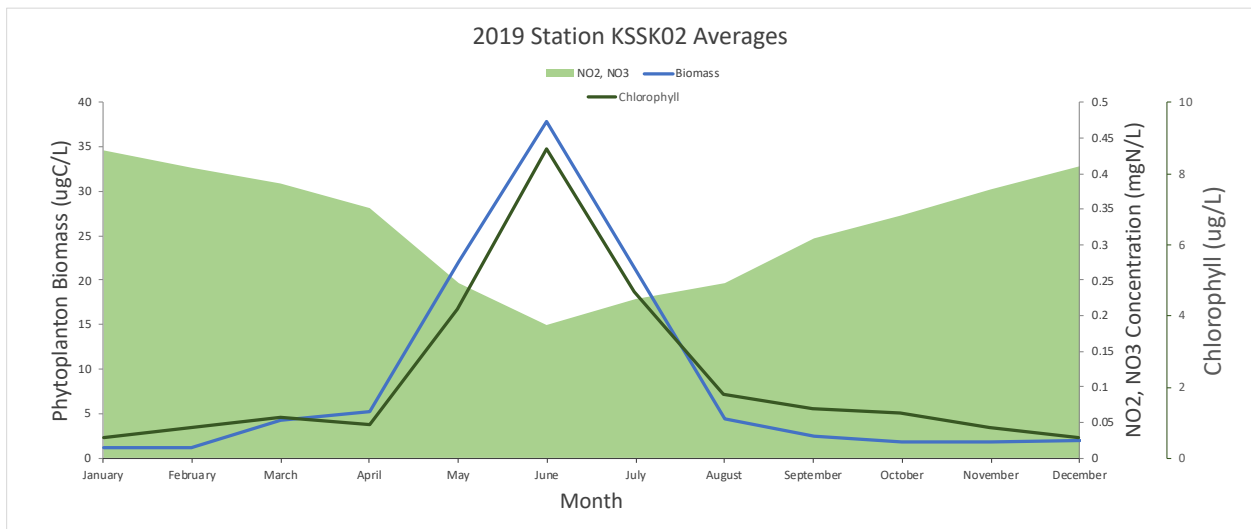
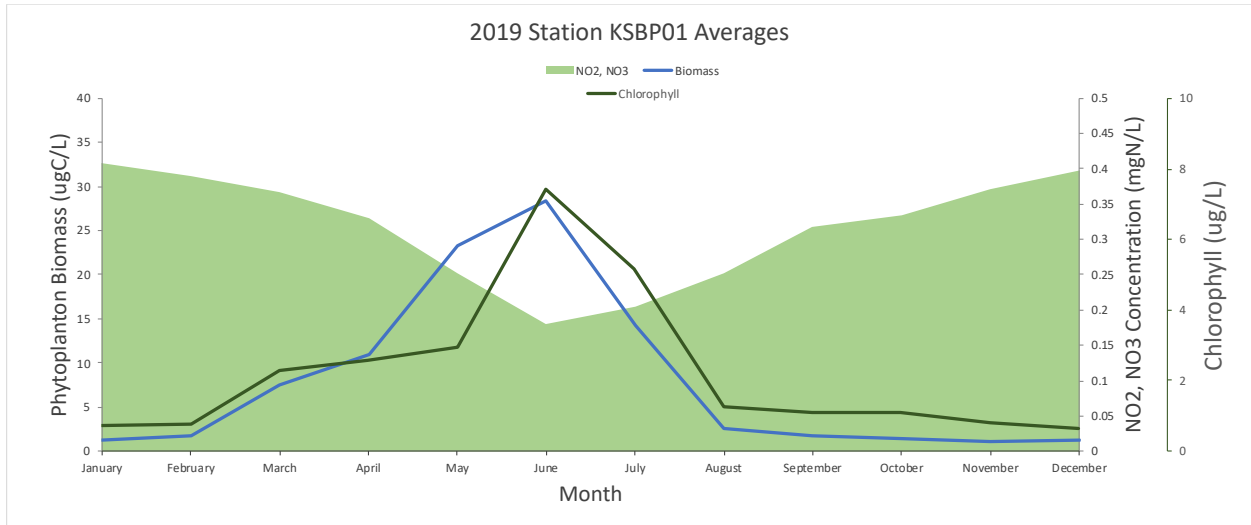
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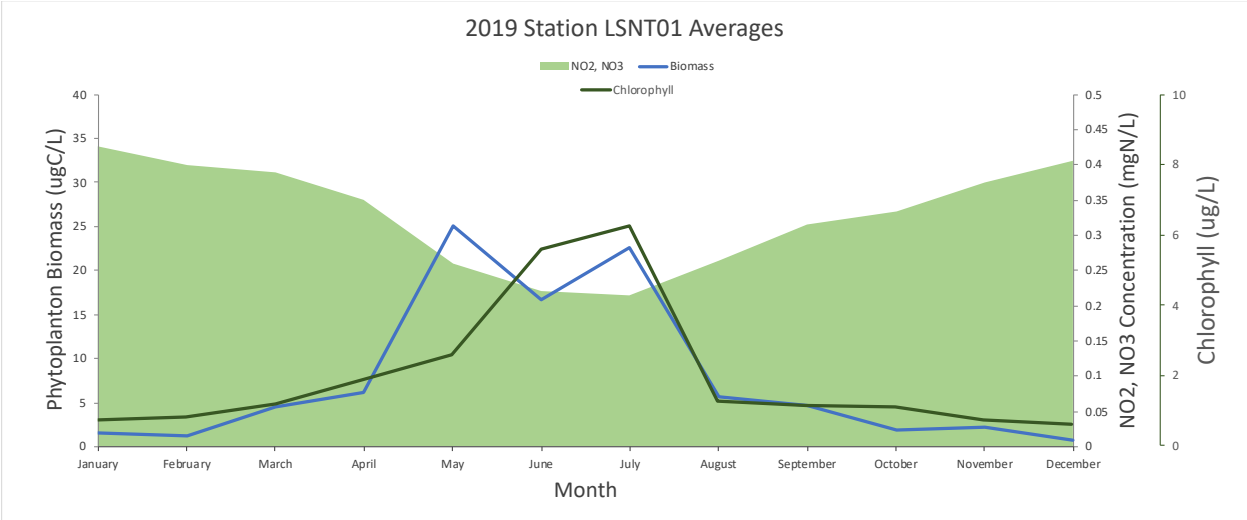
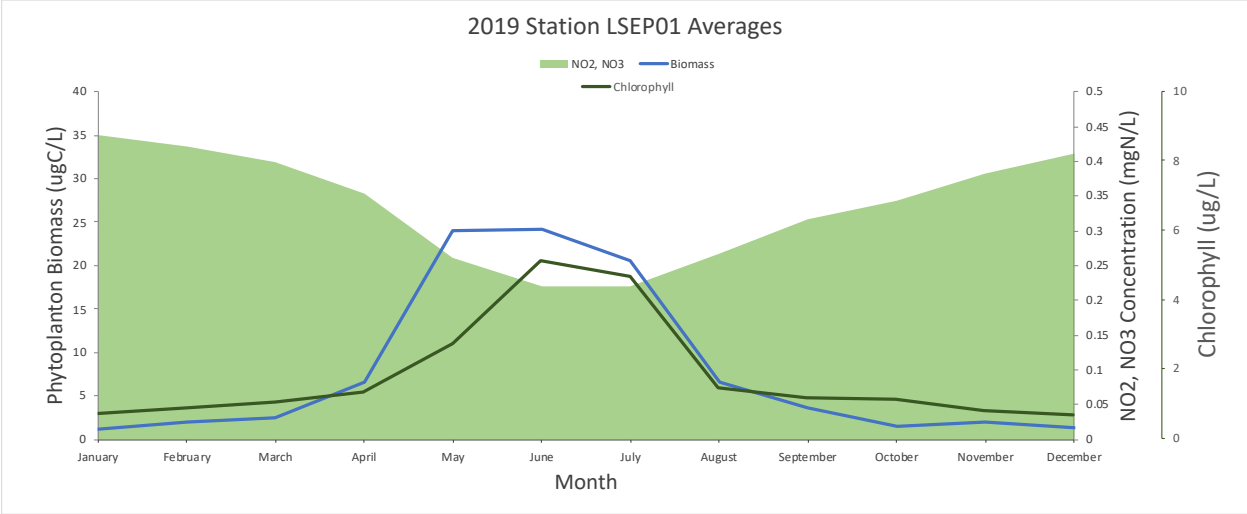
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Appendix 1

2019 monthly averages of phytoplankton biomass ($\mu\text{g C L}^{-1}$), NO_2 and NO_3 concentration (mg N L^{-1}), and Chl-a ($\mu\text{g L}^{-1}$) for stations KSBP01, KSSK02, LSEP01, and LSNT01.





Appendix 2

2018 monthly averages of phytoplankton biomass ($\mu\text{g C L}^{-1}$), NO_2 and NO_3 concentration (mg N L^{-1}), and Chl-a ($\mu\text{g L}^{-1}$) for stations KSBP01, KSSK02, LSEP01, and LSNT01. Data was not collected in February.

