Impacts of Urbanization on Phytoplankton Distribution in Southern California Coastal Regions and Other High Salinity Environments

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Impacts of Urbanization on Phytoplankton Distribution in Southern California Coastal Regions and Other High Salinity Environments

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Earth System Science

by

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Dissertation Committee:
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DEDICATION

To my family for their unconditional love and support throughout the process of my upbringing and educational pursuits. And to my wonderful wife, Jessica Walden, for inspiring me to participate in this journey. She was my greatest asset throughout the entire experience. Her endless motivation and confidence in my abilities kept me engaged even during the toughest of times. Without you, none of this would have been possible.

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VITA

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ABSTRACT OF THE DISSERTATION

Impacts of Urbanization on Phytoplankton Distribution in Southern California Coastal Regions and Other High Salinity Environments

by

Christopher McGuire

Doctor of Philosophy in Earth System Science

University of California, Irvine, 2023

Associate Professor Elizabeth Derse Crook, Chair

The Southern California Bight is representative of a highly productive coastal ecosystem that is heavily influenced by intense urbanization. Along this unique coastal ecosystem resides a dense human population. Consequently, these waters are subject to large anthropogenic pulses of nutrient species and pollutants. Such inputs may have both positive and negative consequences at nearly all trophic levels. For primary producers, nutrient enrichment means there is more biologically active resources that help facilitate growth and reproduction. This often results in shifts to community composition between the multiple classes of phytoplankton and cyanobacteria found in the region.

This body of work aims to evaluate the interaction between anthropogenic nutrient enrichment and phytoplankton community dynamics at both point and non-point source locations. Specifically, the intent was to identify and monitor seasonal variations of common SCB species and identify others capable of causing human harm and/or ecological destruction. Environmental conditions including temperature, salinity, dissolved oxygen, and nutrient concentrations were collected to elucidate possible initiators of harmful algal

bloom (HAB) events. Additionally, we performed an incubation using water samples collected from a super saline site that had been frequently seeded with SCB phytoplankton species until as recent as the 1980's. However, there was no evidence of current HAB species at the site.

In Chapter 2, we find that there was no significant difference in environmental conditions or community composition between waters collected near a wastewater outfall and waters collected at a reference site up current. In Chapter 3, we determine that the current assemblage of marine diatoms and dinoflagellates at the Salton Sea have adapted and continue to adapt to changing conditions expected for the region. Rapid additions of trace elements and salinity fluctuations resulted in a toxicity effect for many species. Smaller sized picoeukaryotes were able to adapt to changing conditions more rapidly and may suggest a shift in community composition if rapid change occurs. In Chapter 4, we show that environmental conditions are more seasonally variable in estuarine systems when compared to coastal open waters. Some HAB species have demonstrated a wider range of tolerance to these factors and might possibly utilize estuaries as refuge until conditions allow for growth in open water.

CHAPTER 1

INTRODUCTION

Coastal marine ecosystems account for some of the most biologically diverse and productive habitats throughout the oceans of the world. These near-shore shallow-water environments include continental shelves, surf zone habitat, and intertidal waterways such as estuaries. Coastal waters are generally richer in biologically active nutrients that support primary production in comparison to deeper open-water regimes (Whitney et al., 2005). Human development, ubiquitous along most coastlines and ocean-bound waterways, has been modifying the natural cycles and concentrations of chemical species in aquatic environments since the industrial revolution (Valiela et al., 1992). Many of the chemicals being discharged can influence the surrounding ecosystem substantially. For instance, highly productive marine ecosystems are typically limited by a single nutrient species. Small changes in the relative concentration of something like silicate can influence the phytoplankton community to shift towards a diatom dominated population (Conley et al., 1993; Mooij et al., 2016). This seemingly insignificant changes to community composition could impact the greater marine ecosystem in multiple ways. Following the example above, a larger partitioning of marine diatoms may result in an increased rate of carbon export due to silicate-based frustules that other phytoplankton classes lack (Treguer et al., 2018). This can have implications on the trophic transfer of carbon as many higher-level species have preferential prey (Gentleman et al., 2003). As such, it is imperative that scientist understand the magnitude of biogeochemical change associated with coastal development and the ecosystem response to such changes; given that humans will likely continue to influence these systems.

1.1 Natural Drivers of Nutrient Availability in Coastal Environments

Biologically available inorganic nitrogen, phosphorus, and other trace elements are essential to primary productivity in the marine environment. These valuable chemical constituents make their way into coastal waters via many sources. Some coastlines receive seasonal injection of nutrients when deeper, nutrient rich, water is drawn to the surface during upwelling events (Kämpf & Chapman, 2016). The deeper water becomes nutrient replete as sinking debris is remineralized while passing through the column or by decomposition that occurs in the benthic environment (Nixon, 1981). Nutrient inputs also arrive to coast environments from freshwater sources that have weathered adjacent land masses and become rich with nutrients and trace elements (Smith et al., 1999). These terrestrial inputs can be highly variable because they are often driven by the intensity of episodic rain events and seasonal snow or glacial melt (Ge et al., 2020).

1.2 Human impacts to coastal waterways

In addition to natural nutrient enrichment of coastal waters, modern industrial processes and intensified urbanization over the past few centuries have contributed considerably to the nutrient and pollutant load (Lapointe et al., 1994). The development of coastal regions continues to today as resources are typically abundant and proximity to maritime trade routes are advantageous for international commerce. Anthropogenic pollutants arrive to coastal waters via many delivery methods, some deliberate and others unintentional. With appropriate permitting, and under the guidelines determined by the Environmental Protection Agency (EPA), many industries can dispose of environmentally harmful chemicals in quantities under predetermined thresholds. This “wastewater” may require treatment or can, at times, be discharged directly into waterways and coastal ecosystems by industrial processes that result in unwanted byproducts (Sweeney et al., 1980). Even if wastewater is treated, many of these chemicals in the final effluent can impact the natural environment once being disposed. Pollutants also arrive from unassuming sources like roadways and residential landscaping. Household oils, surfactants, and detergents accumulate on streets and driveways. Additionally, fertilizers, pesticides, and herbicides, intended to accelerate growth and protect landscaping, end up in stormwater systems that eventually lead these untreated waters out to sea. Thankfully, most coastal cities in developed countries collect household sewer waste and treat it before discharging as effluent offshore. Although the effluent is conventionally processed to reduce its environmental impact, it still carries measurable amounts of nutrient chemical species along with other potentially harmful contaminants into the systems they are discharged (Chambers et al., 1997).

1.3 Nutrient Budgets and Primary Production

Budgets of bioavailable nutrients and trace minerals set up the conditions for marine primary producers to either thrive, decline, or simply maintain populations in response to relative concentrations. Like their terrestrial plant counterparts, phytoplankton require water, sunlight, and a balanced partitioning of select chemical species to grow and reproduce. Inorganic species of nitrogen, phosphorus, iron, and sometimes silicon all need to be present in certain concentrations to support primary productivity (Tozi et al., 2004). For communities to thrive and bloom, there needs to be an ideal ratio of each chemical species in the water column. In 1934, Alfred Redfield determined the ratio of carbon, nitrogen, and phosphorus within marine phytoplankton cells was, on average, 106:16:1 (Redfield, 1934). Subsequent work found that primary production was greater in regions of the ocean where the relative concentrations of nitrogen and phosphorus were found at the similar ratios within the surrounding waters (Tett et al., 1985). When these chemicals are at a similar ratio in the water column, cells can grow uninhibited and reproduce at their maximum rate. However, once concentrations in the water column deviate from the Redfield ratio, there will emerge a limiting nutrient causing growth to halt once it becomes exhausted. Natural seasonal fluctuations in nutrient availability play a large roll in controlling phytoplankton population dynamics over both short and long timescales (Widdecombe et al., 2010). Evidence suggests that human derived nutrient enrichment, originating from urban and agricultural sources, has also substantially impacted coastal ecosystems and the organism community dynamics (Pitois et al., 2001).

Within the world's oceans, there are many organisms responsible for primary production. The open ocean is mostly nutrient limited resulting in lower rates of primary production (Moore et al., 2013). However, coastal shallow water environments provide ideal habitats for many photosynthesizing organisms like vascular aquatic plants, macroalgae, and phytoplankton. Phytoplankton are the free-floating microalgae that are ubiquitous in nearly every realm of the ocean that receives sunlight. These photosynthetic organisms have evolved to natural biogeochemical cycles and physical conditions in their respective regions to establish a balanced ecosystem. Bacillariophyta and Dinophyceae, commonly referred to as diatoms and dinoflagellates commonly dominate the overall phytoplankton community composition in coastal regions (Malviya et al., 2016). Although these classes are similar in size and general ecological function, they differ in many ways. Dinoflagellates can be single or multicellular and are characterized by flagella. These hairlike appendages facilitate active mobility within the water column. Diatoms lack these appendages and instead passively drift with ocean currents. Diatoms form a silica-based frustule shell, that comes at the cost of an added nutrient requirement. The additional mass of the frustules can also influence impact carbon export fluxes by increasing the sinking rates of regional carbon should an ecosystem shift from dinoflagellate dominated to diatom. The partitioning of these two phytoplankton classes in coastal regions is predominantly driven by physical conditions and nutrient availability in the water column, and evidence suggest coastal regions have been experiencing a shift over the last several decades (Hinder et al., 2012). Portions of the northeast Atlantic and North Sea show a fundamental shift in the relative abundance of diatoms versus dinoflagellates, resulting in a marked decline in dinoflagellates.

Picoeukaryotes and cyanobacteria are additional contributors to coastal primary production and nutrient cycling. Smaller than the classes mentioned above, these classes typically range from 0.2 to 2 micrometers in diameter. Their high surface area to volume ratio makes them very efficient at nutrient uptake (Lewis, 1976). As such, they can be found thriving in oligotrophic waters and under conditions that are inhospitable for most other phytoplankton. Many have evolved to use a multitude of wavelengths to perform photosynthesis. For instance, the globally abundant cyanobacteria Synechococcus spp. has many complexes that photosynthesize utilizing a phycoerythrin light harvesting pigment in place of chlorophyl (Flombaum et al., 2013). This allows it to thrive in regimes where chlorophyll light harvesting species cannot. Certain species grow at such high rates that they can cause harm to the ecosystems they exist in.

1.4 Harmful Algal Blooms

Many urbanized lakes and coastal regions experience rapid rates of phytoplankton growth in response to anthropogenic nutrient enrichment, natural nutrient cycling, and other changes to physical drivers. In some cases, these high growth rates result in events called Harmful Algal Blooms (HABs). HABs are declared when phytoplankton species that pose significant risk to both marine ecosystems and human populations exceed predetermine allowable limits. Thresholds vary in different areas of the globe but are typically based on the overall threat of the species involved. At the ecological level, HABs can be as simple as disruptions to aquatic ecosystems when one phytoplankton species outcompetes others for nutrients or light. Large-scale blooms can lead to changes in food availability and complex trophic interactions (Burkholder et al., 2018). In severe situations, HABs create mass fish kills due to oxygen depletion resulting from the decay of phytoplankton biomass. This often disrupts the local ecosystem and can lead to economic impacts considering the loss of fish stocks. Other HAB species are monitored because they produce toxins that have harmful effects on marine life and humans. These toxins can be transferred up the trophic chain, affecting fish, shellfish, and marine mammals. Consumption of seafood containing HAB toxins may lead to various forms of shellfish poisoning in humans. Conditions include Amnesic Shellfish Poisoning (ASP), Diarrhetic

Shellfish Poisoning (DSP), Neurotoxic Shellfish Poisoning (NSP), and Paralytic Shellfish Poisoning (PSP). Exposure to these marine toxins can result in gastrointestinal distress, paralysis, and neurological effects. Severe cases have resulted in death among patients who have consumed large quantities.

In many coastal towns, beach and water access are restricted when HAB thresholds are exceeded. In some cases, high concentrations give way to water discoloration, resulting in a reddish, brownish, or greenish hue. This often creates the perception of poor water quality among the surrounding human population. When more extreme HAB events initiate fish kills, it is common practice to restrict recreational activities such as swimming, fishing, and boating. Coastal communities enforce this to protect the health of the human population. Additionally, HABs may lead to the closure of fisheries and aquaculture operations. This is often done to prevent the harvest and sale of contaminated seafood. As a result, economic losses are commonly occurred for communities that rely on coastal tourism and industries.

1.5 Southern California Bight

As one of the most populated sections of shoreline in North America, the Southern California Coast represents a unique geophysical region that has been impacted by human development since the early 1900’s. Here, the California Current brings cold water from the North Pacific along the coastline and towards the equator. At Point Conception (34.45°N) the profile of the shoreline deviates abruptly to the east and continues to the south-east to Punta Colonet, Mexico (30.97°N), marking the boundary for the Southern California Bight (SCB). Throughout the bight, geophysical drivers and the Channel Islands work to generate large and small-scale eddies that lead to increased residence times for much of the year (Warrick et al, 2004). Along this stretch of coast, the Southern California Countercurrent (SCC) brings warmer surface waters from sub-tropical ocean regions and moves them poleward along the shore. Evidence suggests that warmer waters, combined with increased residence time and nutrient enrichment likely impact phytoplankton community composition within the SCB (Howard et al., 2017).

Seasonal phytoplankton bloom dynamics in the SCB have historically shown the highest biomass during spring (MAM) months. Spring blooms occur as the result of wind driven mixing, shoaling of the stratified layer, and increased horizontal transport of nutrient rich waters brought to the surface by coastal upwelling along the California Current system (Kim et al., 2009). Summer (JJA) months are typically associated with lower primary productivity in surface waters as nutrients in the euphotic zone are depleted and surface and deep layers become stratified. Smaller blooms have been recorded during fall (SON) months as decaying biomass becomes remineralized. Winter (DJF) is characterized by lower light intensity levels and cooler surface temperatures that relax stratification and when coupled with wind driven mixing, allow for the upwelling of nutrients that initiates the subsequent spring bloom.

1.6 HABs of the SCB

The SCB houses several phytoplankton species that are monitoring as potential HAB threats. Examples of both hypoxia inducing “red tide” species and toxin producing species capable of harming human populations can be found in its’ waters. Species monitored for environmental degradation with no direct effect on humans include *Akashiwo spp.,* *Cochlodinium spp., Heterosigma spp., Linguilodinium spp., and Phaeocystis spp*. (SCCOOS, 2023). Consequences of these blooms range from surfactant production that can harm fish and birds, to chemicals capable of collapsing entire salmon stocks.

Within the SCB, there are a few HAB species that produce toxins capable of harming human populations. Several complexes in the dinoflagellate genus *Alexandrium* spp. produce a neurotoxic substance identified as a saxitoxin. This toxin acts by blocking nerve signal transmission and can result in temporary paralysis. Human ingestion of shellfish contaminated with saxitoxins can lead to serious health complications from a condition called paralytic shellfish poisoning (PSP). Another dinoflagellate identified as *Dinophysis* spp., produces a chemical called okadaic acid and other toxins that result in diarrhetic shellfish poisoning (DSP). Symptoms of DSP include vomiting, nausea, diarrhea, chills, headache, and fever.

Arguably the most hazardous SCB HAB species is the consistently abundant pennate diatom *Pseudonitzschia* spp. The complex *P. seriata* produces a chemical known as domoic acid; a potent neurotoxin that can accumulate in the tissues of various marine organisms. Consumption of these organisms by can lead to amnesic shellfish poisoning (ASP) in humans. ASP symptoms range from gastrointestinal distress and seizures to severe cases causing death in multiple cases throughout the globe. Drivers of P. seriata blooms that resulted in high concentrations of domoic acid has been linked to several environmental factors, including nutrient availability, temperature, and pH (Bates et al., 1998; Thorel et al., 2014). The investigation of monitoring and studying *Pseudonitzschia* spp. populations are important not only for understanding their ecological roles in the natural environment, but also for safeguarding human health by predicting and managing the potential risks associated with domoic acid contamination.

1.7 Investigating Anthropogenic Influence in the SCB

With global human population projected to rise over the next century, continued urbanization of coastal regions should be expected. Without changes to environmental policies and practices, persistent nutrient enrichment along these developed coastlines is likely to continue. Additional rapid warming of surface ocean temperatures and changes to pH measured over the past decades are projected to increase over the next century (Cheng et al., 2019). Consequently, it is imperative that scientist and policy makers evaluate and monitor coastal sites that have the highest anthropogenic risk potential. The overall objective of the studies contained within was to identify and investigate coastal marine ecosystems in the SCB that were perceived to be heavily influenced by external human forcings. Consideration was given to changing physical and chemical conditions of the water column and how they affect the structure of phytoplankton communities they contained.

The first study (Chapter 2) was conducted over a 24-month period near Orange County Sanitation District's outfall pipe, located 5 miles offshore near Huntington and Newport Beaches in Southern California. The intent was to capture any influence that effluent driven nutrient enrichment had on the seasonal dynamics of the contemporary phytoplankton population. Special consideration was placed on HAB species, as their presence could impact the overall health of the coastal ecosystem and regional human health.

The second study (Chapter 3) was a 60-hour incubation conducted at the Salton Sea, in Imperial and Riverside counties. Here, we analyzed a phytoplankton assemblage assumed to be seeded in previous decades by species that inhabit the SCB. The experiment evaluated phytoplankton response to manipulations of salinity, and to additions of fine dust collected from adjacent playas. The five dust additions, collected from two opposing shoreline locations, were analyzed for concentrations of trace elements that were deemed to have a potential to impact growth rates of primary production. Dust additions were administered in quantities to simulate reasonable atmospheric deposition conditions in the region.

The final study (Chapter 4) was conducted over a 12-month period that investigated biogeochemistry and phytoplankton community dynamics at Los Peñasquitos Lagoon in San Diego County. This site was chosen because it represents a protected estuary that is connected to a heavily urbanized watershed. The estuary was sampled at five stations, along the salinity gradient, at monthly intervals. The intent was to capture any spatial variability between the mouth, mid channel, and upper estuary locations. Special consideration was given to harmful algal species and the idea that estuarine systems may provide refuge for HAB populations, during seasons of open ocean decline.

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CHAPTER 2:

Characteristics of Coastal Phytoplankton Communities Near a Wastewater Outfall Site in the Southern California Bight

Adapted from:

McGuire, C. M., J. A. Walden, L. Terriquez, G. Robertson, J. Tavares, K. R. M. Mackey, J. K. Moore (*In Prep.*). Characteristics of Coastal Phytoplankton Communities Near a Wastewater Outfall Site in the Southern California Bight

2.1 Introduction

Coastal ecosystems make up some of the most biodiverse marine habitats on Earth. These regions are often nutrient-rich and provide essential shallow-water habitats capable of supporting complex ecosystems (Gray, 1997). The nearshore water receives life supporting nutrients from many sources. Natural inputs include surface run-off from adjacent land masses, and seasonal upwelling of deep-water layers (Ahn et al., 2005; Cushing, 1990). Nutrient replete coastal regions, and the resulting biological activity, also provide many important ecosystem services for coastal human populations. Services include several food sources that can be extracted from the waters, nutrient and pollutant cycling, carbon sequestration, and important cultural benefits for coastal inhabitants (Bolund and Hunhammar, 1999). In densely urbanized coastal regions, pollutant and nutrient enrichment from city and industry put these ecosystems and the services they provide at possible risk.

Changes to nutrient concentrations in the water column directly influence the rate of primary production. Phytoplankton populations and community composition have shown to shift as nutrients are manipulated within a water column (Tillman et al., 1982; Widdecombe et al., 2010). Studies indicate increased concentrations of bioavailable nitrogen, phosphorus, and silicon species have led to a shift in growing season and the rate of individual phytoplankton species (Jacquet et al., 2006). The seasonal dynamics of the phytoplankton population are important for the overall health of the ecosystem, and a shift in seasonal abundance would likely have cascading effects in higher trophic levels.

A densely populated portion of the Southern California coastline, running from Santa Barbara County to the southern tip of San Diego County (figure 1), contains an important oceanic geological feature known as the Southern California Bight (SCB). Coastal waters in this region experience seasonal nutrient enrichment from naturally occurring upwelling and land-surface runoff. Waters within the bounds of the SCB experience increased residence time in comparison to adjacent waters further offshore. Here, an abrupt change in coastline aspect creates both large- and small-scale eddies which induce a countercurrent moving from south to north for much of the year (Warrick et al, 2004). These conditions can initiate localized upwelling and have shown to retain communities of phytoplankton within the SCB for extended periods of time (Todd et al., 2009). A study in the northern portion of the California Current system showed that retentive eddy circulation systems are more likely to contain high populations of toxin producing planktonic species (MacFadyen et al., 2008). Consequently, it is critical that coastal industries and local governments evaluate human impacts to regional phytoplankton populations and carefully consider the extent to which anthropogenic nutrient enrichment may harm or benefit these organisms.

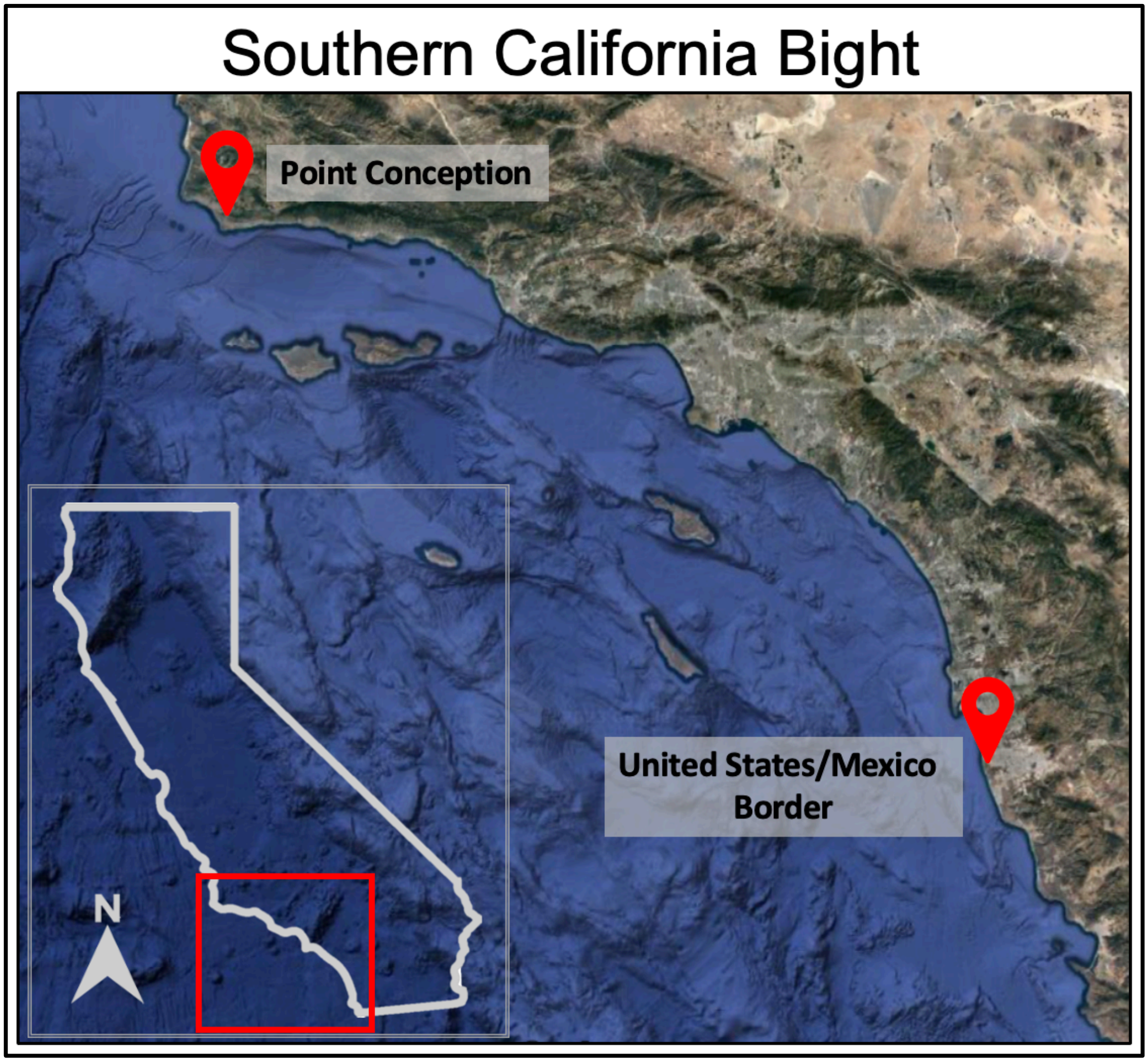
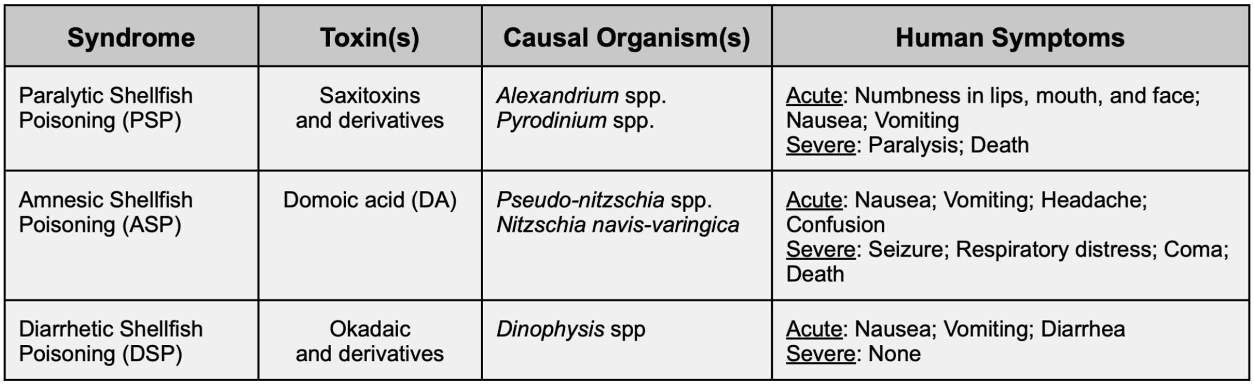


Figure 1: Map of Southern California coastline showing the bounds of the Southern California Bight within the United States.

Harmful Algal Blooms (HABs) are likely to occur when biogeochemical conditions favor the growth of potentially harmful planktonic species. Different HAB species impact the marine environment in unique ways. Certain species can rapidly reproduce and subsequently decay. The resulting hypoxic conditions can be deadly for fish and other marine organisms. Other algal species produce toxic compounds that are harmful to higher trophic levels when concentrations exceed safe thresholds. The exact ecological function of many of these chemicals remains unknown. Recent work suggests that some are likely a biological response designed to alleviate grazing pressure (Cusick and Widder, 2020). Others suggest they might play a role as pheromones during phytoplankton reproduction (Wyatt & Jenkinson, 1997). These toxins are not only harmful to other organisms in the water column. In some cases, exposure to affected water or organisms results in symptoms in human populations. The three human syndromes associated with planktonic produced toxins are Paralytic Shellfish Poisoning (PSP), Amnesic Shellfish Poisoning (ASP), and Diarrhetic Shellfish Poisoning (DSP). Each of these syndromes can be linked to species that are found frequently within SCB waters (table 1).



Syndrome	Toxin(s)	Causal Organism(s)	Human Symptoms
Paralytic Shellfish Poisoning (PSP)	Saxitoxins and derivatives	*Alexandrium* spp. *Pyrodinium* spp.	Acute: Numbness in lips, mouth, and face; Nausea; Vomiting Severe: Paralysis; Death
Amnesic Shellfish Poisoning (ASP)	Domoic acid (DA)	*Pseudo-nitzschia* spp. *Nitzschia navis-varingica*	Acute: Nausea; Vomiting; Headache; Confusion Severe: Seizure; Respiratory distress; Coma; Death
Diarrhetic Shellfish Poisoning (DSP)	Okadaic and derivatives	*Dinophysis* spp	Acute: Nausea; Vomiting; Diarrhea Severe: None

Table 1: Table syndromes and the symptoms experienced by humans due to HAB related phytoplankton species. Adapted from Anderson et al., 2021.

Many of the species responsible for HAB events are closely monitored because they impact local fisheries, public access to beaches, and marine ecosystem health. The Southern

California Coastal Ocean Observing System (SCCOOS) monitors sites within the SCB for multiple HAB species. Of particular interest in the region is the pennate diatom *Pseudonitzschia spp*. Certain complexes of this species have been shown to produce the neurotoxin domoic acid (DA). In humans, ingestion of filter feeders like mussels and clams which have accumulated the chemical in their tissue can lead to Amnesic Shellfish Poisoning (ASP). Symptoms that accompany ASP start with nausea and vomiting, but severe cases can lead to respiratory distress, coma, and even death. A study by Bates et al. (1998) considered the driving mechanisms for *Pseudonitzschia spp*. DA production and reported correlations to physical parameters, such as temperature and salinity, to increase occurrence of harmful algal blooms (HABs). Subsequent studies have shown there to be a positive correlation between elevated sea surface temperature (SST) and blooms of *P. seriata*; a complex present in the SCB that produces DA at toxic levels (Thorel et al., 2014; McKibben et al., 2016). Lundholm et al (2004) determined that the less toxic *P. delicatissima* (also a SCB species) outcompetes other species in elevated pH conditions. With these considerations in mind, it is important to develop ways to effectively predict how HAB producing species will respond as the SCB continues to experience change, and implement controls designed to mitigate any anthropogenic drivers of such blooms.

Within the confines of the SCB, Orange County operates one of the largest wastewater treatment facilities in the state. Orange County Sanitation District (OC San) processes an average of 182 million gallons of domestic sewage per day (MGD) (OC San, 2021). This is accomplished by primary and secondary water treatment methods on municipal liquid waste. Of the wastewater processed each day, roughly half of the intake volume is returned to a potable quality and injected back into the local groundwater aquifer. The other half consists of concentrated wastewater and is piped approximately five miles offshore where it is released to the ocean. The outfall is located near the edge of the continental shelf, at a nominal depth of 60 meters (figure 2). This location was strategically selected, as vertical and horizontal mixing is enhanced by wind driven upwelling and density driven internal wave action near the edge continental shelf (Lucas et al., 2011). Carefully considered geographical placement, combined with a perforated outfall releasing system, work to homogenize the effluent with regional waters. The aim is to dilute the relative concentrations of the effluent constituents and decrease any biological influence.

Orange County Sanitation District Outfall

Figure 2: Map of OCSD sampling sites, near Newport and Huntington Beaches, California, monitored during the study. Site A (red) looks to capture the effect of effluent and is a floating sample site in which the station with the highest CDOM concentration is selected for each cruise. The reference site (yellow) is spatially separated and up current from the outfall for much of the year.

This study consisted of monthly biogeochemical analysis and evaluation of phytoplankton community dynamics over a two-year period near the Orange County Sanitation District's offshore outfall site. The intent was to determine if there is a discernable impact on phytoplankton population and distribution due to inputs arriving with wastewater effluent. In addition, special consideration was given to HAB species found in the region as increased concentrations may pose a considerable threat to the near-term stability of the SCB marine ecosystem.

2.2 Methods

2.2.1 Sample Collection

To capture seasonal dynamics of Southern California Bight (SCB) phytoplankton populations and evaluate possible effects of effluent in the water column, a collaboration was established with the Orange County Sanitation District's (OC San) Ocean Monitoring Program. This partnership facilitated UC Irvine researchers' ability to collect monthly sampling cruises aboard the M/V Nerissa, and it ranged from February 2017 to January 2019. Samples were collected and compared from two open water stations (figure 2). Sample Site A was established over the outfall with the intent to capture phytoplankton dynamics near the effluent plume. Sample Site B was located approximately three miles east of the outfall site, just off the continental shelf. Site B was selected as a reference site to determine the impacts of the plume. Each cruise measured water temperature, nutrient and chlorophyll-a concentrations, and phytoplankton community composition at four stations. Discrete samples were collected using a remote-firing Niskin bottles attached to a rosette with a conductivity-temperature-depth (CTD) sonde device. Real-time onboard CTD recorders logged continuous depth profiles for temperature, salinity, oxygen, pH, colored dissolved organic matter (CDOM), and chlorophyll-a. The rosette was cast from the stern of the vessel, and vertical profile samples were collected upon retrieval. Once samples were aboard, appropriate aliquots for the analysis of chlorophyll-a, nutrient concentration, and phytoplankton population were subsampled and preserved based on laboratory analysis protocol (see below). To best capture the effects of the wastewater plume, samples from a depth of 30 meters were evaluated. This depth was determined to be where the deep chlorophyll maximum (DCM) was while also capturing meaningful values of CDOM over the course of the study. CDOM has been identified as a suitable tracer for effluent plume tracking in coastal environments (Nezlin et al., 2020). Sampling at 30 meters ensured depth would be adequate to evaluate potential plume inputs, while remaining shallow enough to contain an abundant phytoplankton community. It is important to note that lower depths did have higher concentrations of CDOM but lacked high concentrations of chlorophyll for much of the year.

2.2.2 Chlorophyll-a analysis

To measure chlorophyll-a concentration from each sample site, 200 ml of seawater was filtered through an in-line Whatman Glass Fiber Filter (GF/F) with a pore size of 0.7 um. The filters with captured suspended matter were immediately placed on dry ice for preservation. In the laboratory, GF/F were thawed then extracted in 10 mL of 90% (by volume) acetone for 24 hours at -20^{o}C. Once returned to ambient temperature, the extracted chlorophyll-a was measured for raw fluorescence on a Turner Trilogy, benchtop fluorometer (Venrick & Hayward, 1984). Raw fluorescence was converted to an absolute chlorophyll-a concentration (ug/L) using a standard curve generated using the cyanobacteria species *Anacystis nidulans*. Measurements were then validated against OCSD's 1-meter resolution CTD chlorophyll-a data.

2.2.3 Nutrient Analysis

To determine the nutrient concentrations of each sample, 50 ml of filtrate from chlorophyll-a processing was collected and placed on wet ice for lab analysis. Once thawed in the laboratory, samples were passed through a QuickChem 8500 Series Analyzer (Lachat Instruments) in accordance with manufacturers seawater specifications (Johnson and Petty, 1983; Lachat, 2003). Concentrations of nitrate/nitrite (Total N) and phosphate (Total P) were then reported in uM.

2.2.4 Microscopy

To assess the distribution and abundance of micro size class phytoplankton (> 5 um in diameter), microscopy cell counts were conducted at two stations during each cruise. The samples were retrieved from a depth of 30-meters from a Site A location (figure 2, stations 0001, 2205, or 0009) with the strongest plume signature based on CDOM levels for that day , and Site B (figure 2, station 2014). 250 ml aliquots were preserved with a 1% Logol's solution and placed on wet ice for later analysis. In the laboratory, each sample was agitated to homogenize the contents and 50ml was settled for 24 hours, following the Utermöhl method (Utermöhl, 1958). The settled slide was then visually transected for phytoplankton, where cells were identified to the genus level using the inverted microscope method of estimating algal numbers (Lund, 1958). This was accomplished using a Nikon Eclipse Ti2 inverted light microscope under 200x magnification.

2.2.5 Flow Cytometry

To facilitate the analysis of phytoplankton too small to identify via microscopy, 1 ml aliquots of 4% formalin fixed samples were retained for flow cytometry and stored on dry ice. Once in the laboratory, pico size class phytoplankton (<5 um) abundances were analyzed using a Novocyte Benchtop Flow Cytometer (Agilent). For each depth at every station, a 50μL sample was drawn and processed. Populations of picoeukaryotes and *Synechococcus spp.* were identified and enumerated based on their peridinin-chlorophyll-protein (PerCP) and phycoerythrin (PE) autofluorescence signatures, respectively (Haynes et al., 2016).

2.3 Results

2.3.1 Environmental Conditions

At the depth evaluated for this study, physical parameters of the water column followed historical seasonal trends at both sampling locations (OC San, 2016) (figure 3). Temperatures ranged from 11.5°C to 18.9°C. The highest temperatures were recorded in the fall of both years studied. Salinity remained nearly constant throughout the study, ranging from 33.27 PSU to 33.68 PSU. CDOM levels were highest in spring and decreased as the seasons progressed. No significant difference was identified between the two sampling sites for temperature, salinity, and CDOM.

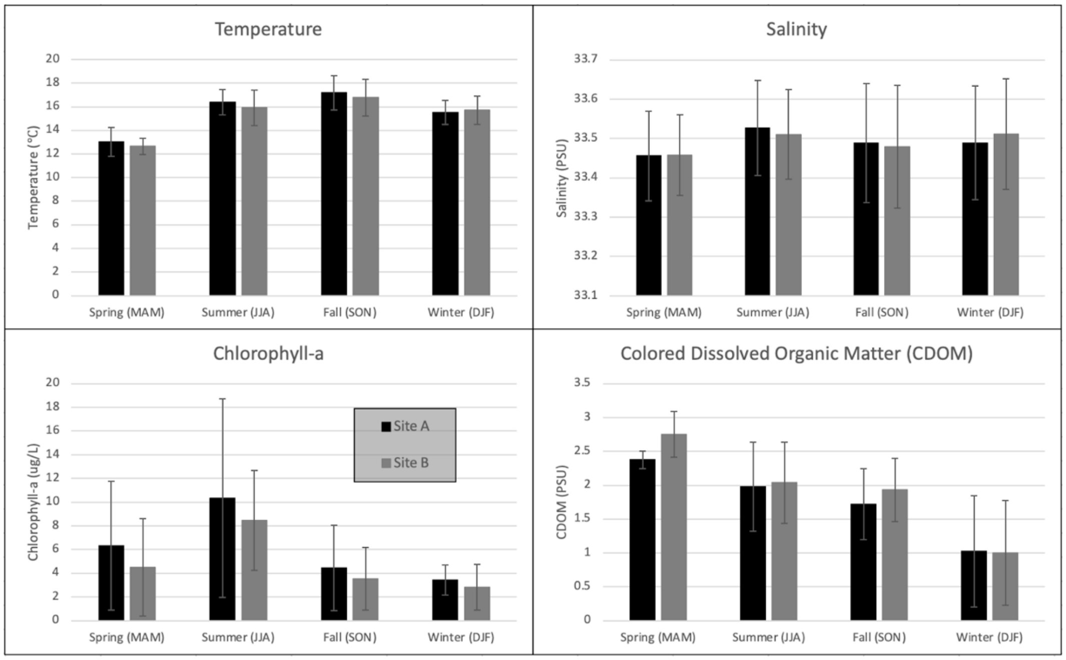


Figure 3: Seasonal averages of Site A and Site B for Temperature (°C), Salinity (PSU), Chlorophyll-a (ug/L), and CDOM (PSU).

2.3.2 Chlorophyll

Chlorophyll analysis displayed a seasonal signal that is consistent with regional trends for waters near this location (figure 4). The highest concentration at Site A was 19.58 ug/l (July 2018) and the lowest was 0.67 ug/l (November 2018). Site B measured a maximum of 15.46 ug/l (June 2018) and a minimum of 0.72 ug/l (November 2018). Summer season showed the highest concentration of chlorophyll at both sampling locations (figure 3). This would be expected as depletion of nutrients near the surface forces phytoplankton into deeper waters as spring progresses into summer. The winter season showed the lowest abundance of chlorophyll at each location. Linear regression analysis revealed a moderate strength correlation (R-squared = 0.68, p-value = 4.4 x 10-6), and a regression coefficient of 1.01, suggesting similar concentrations of chlorophyll at each sampling location throughout the study (figure 5).

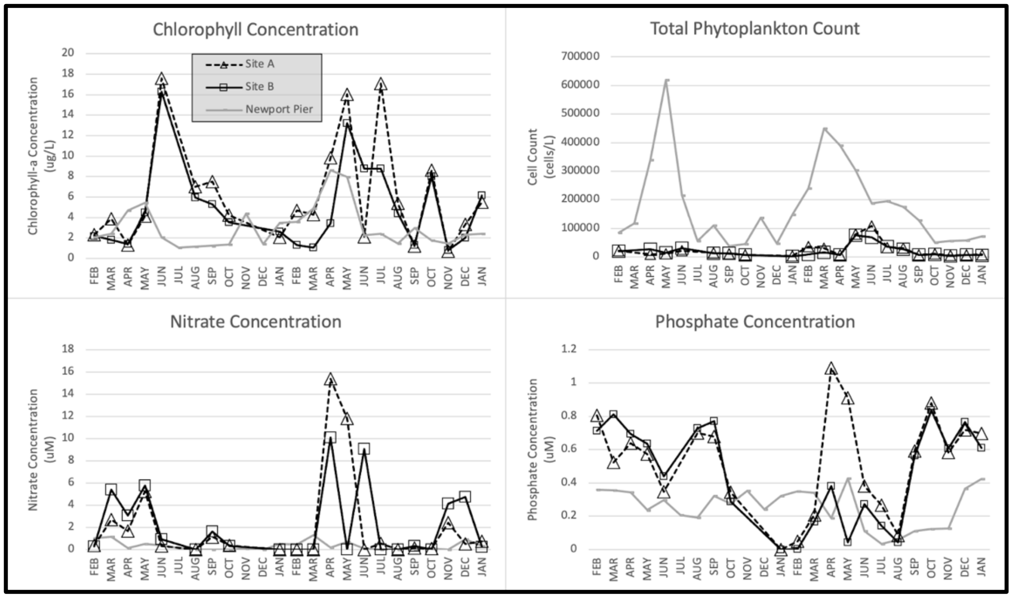


Figure 4: Monthly time series depicting Site A and Site B concentrations of chlorophyll-a (ug/L), total phytoplankton cell count (cells/L), nitrate (uM), and phosphate (uM).

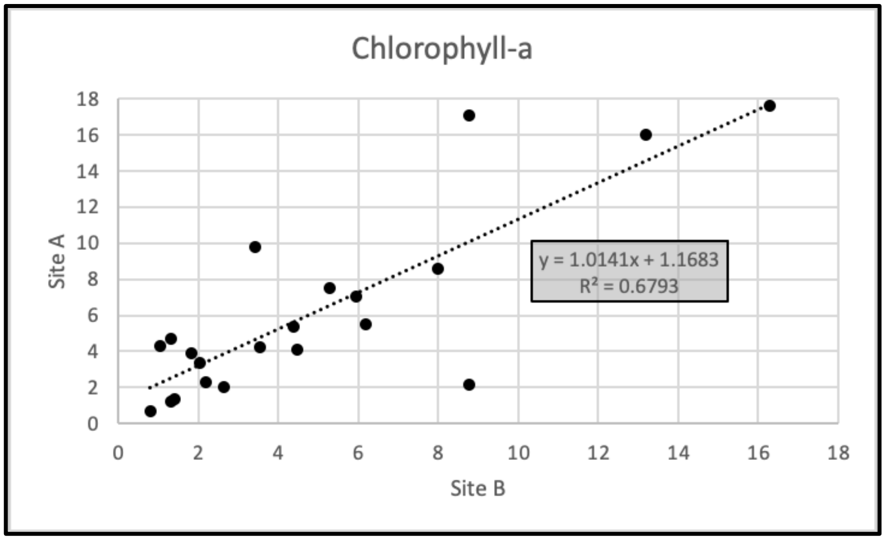


Figure 5: Linear regression plotting chlorophyll-a concentration between the two sample sites. The r-squared value would suggest a moderate relationship between the two sites. The regression coefficient of 1.01 indicates there is no difference in primary production between the sites.

2.3.3 Nutrients

Nutrient concentrations changed considerably over the course of the study. The highest concentration of nitrate/nitrite at Site A was 15.40 uM (April 2018) and the lowest was recorded below the detection threshold (August 2018). Site B had a maximum concentration of 10.10 uM (April 2018) and a minimum recorded below the detection (August 2018). Phosphate concentration at Site A was highest in April 2018 at 1.09 uM and below the detection limit in December 2017. Site B had a maximum of 0.84 uM and a minimum below detection. Nitrate/nitrite experienced large increases during spring months (figure 4). Phosphate saw smaller increases during spring before declining in summer months and rising again in the fall. N:P ratios were typically high during the spring months, however the maximum ratio of 33:1 occurred in June of 2018. Both sites measured lower ratios during late summer and early fall but are likely driven by concentrations below detection for both chemical species. Linear regressions showed no significant correlation between the two sites for nitrate+nitrite (r-squared = 0.24), and a weak correlation between the sites for phosphate (r-squared = 0.40) (figure 6).

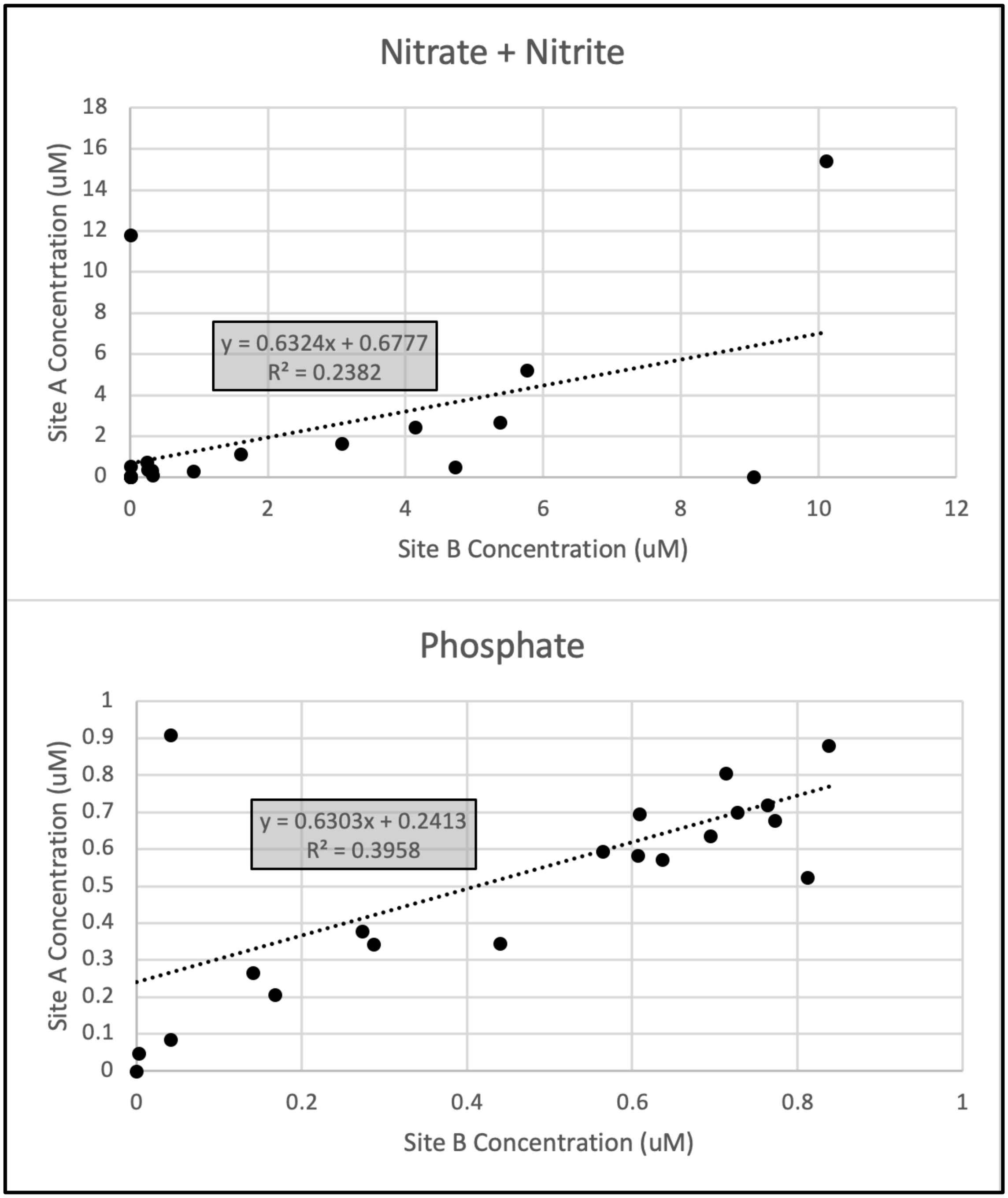


Figure 6: Linear regressions showing the relationships of nitrate + nitrite and phosphate between Site A and Site B.

2.3.4 Microscopy

Diatoms dominated community composition between the micro size-class phytoplankton for most of the two years evaluated. Site A total diatom cell counts ranged from 1300 cells/L (December 2017) to 97,000 cells/L (June 2018). Total dinoflagellate cell counts at Site A range from 640 cells/L (April 2017) to 21,760 cells/L (July 2018). Site B diatom count ranged from 1000 cells/L (December 2017) to 75,160 cells/L (May 2018). The dinoflagellates had a minimum cell count of 800 cells/L (December 2017) and a maximum of 24,440 cells/L (July 2018). Linear regression between sites A and B indicate strong relationships between the sites for both diatoms (r-squared = 0.84), and dinoflagellates (r-squared = 0.72) (figure 7). For diatoms, the linear equation slope is greater that 1 (m=1.15), which would suggesting there is a slightly higher abundance at Site A. the reverse is true for dinoflagellates with a slope that is less that 1 (m=0.82). At the studies' location and depth, the diatom bloom started in the mid-spring, while dinoflagellate blooms occurred slightly later; typically in early summer months. Of the diatom population, Pseudonitzschia spp. appeared to be the dominant genus for most of the study, having two complexes present and the highest total abundance for 13 of the 21 sampling dates. Within this genus, the non-harmful complex of *P. delicatissima* was more abundant for much of the year. The complex that is a HAB threat, *P. seriata,* dominated the distribution briefly in late spring of each year, exceeding the bloom threshold of 10,000 cells/L just once during the study (May 2018) at both sampling locations (figure 8). Present dinoflagellate HAB species included small populations of *Akashiwo sanguinea, Alexandrium cantenella, Cochlodinium polykrikoides, and Prorocentrum spp. (P. micans & P gracile)* were observed throughout the study. The only HAB threshold exceedance for dinoflagellate populations occurred between June and July of 2018, for *Akashiwo sanguinea.*

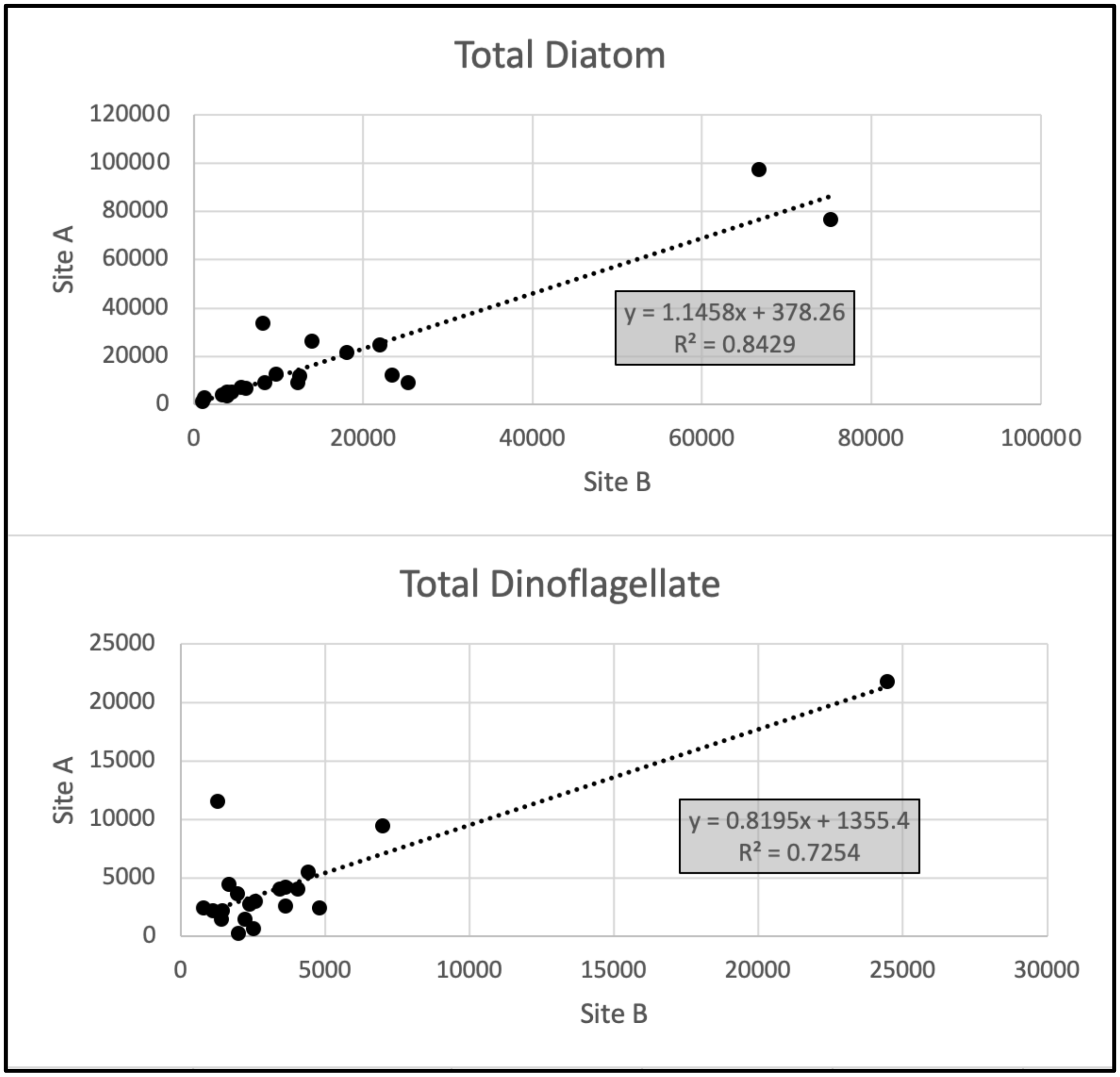


Figure 7: Linear regressions comparing Site A and Site B for total diatom and total dinoflagellate cell counts.

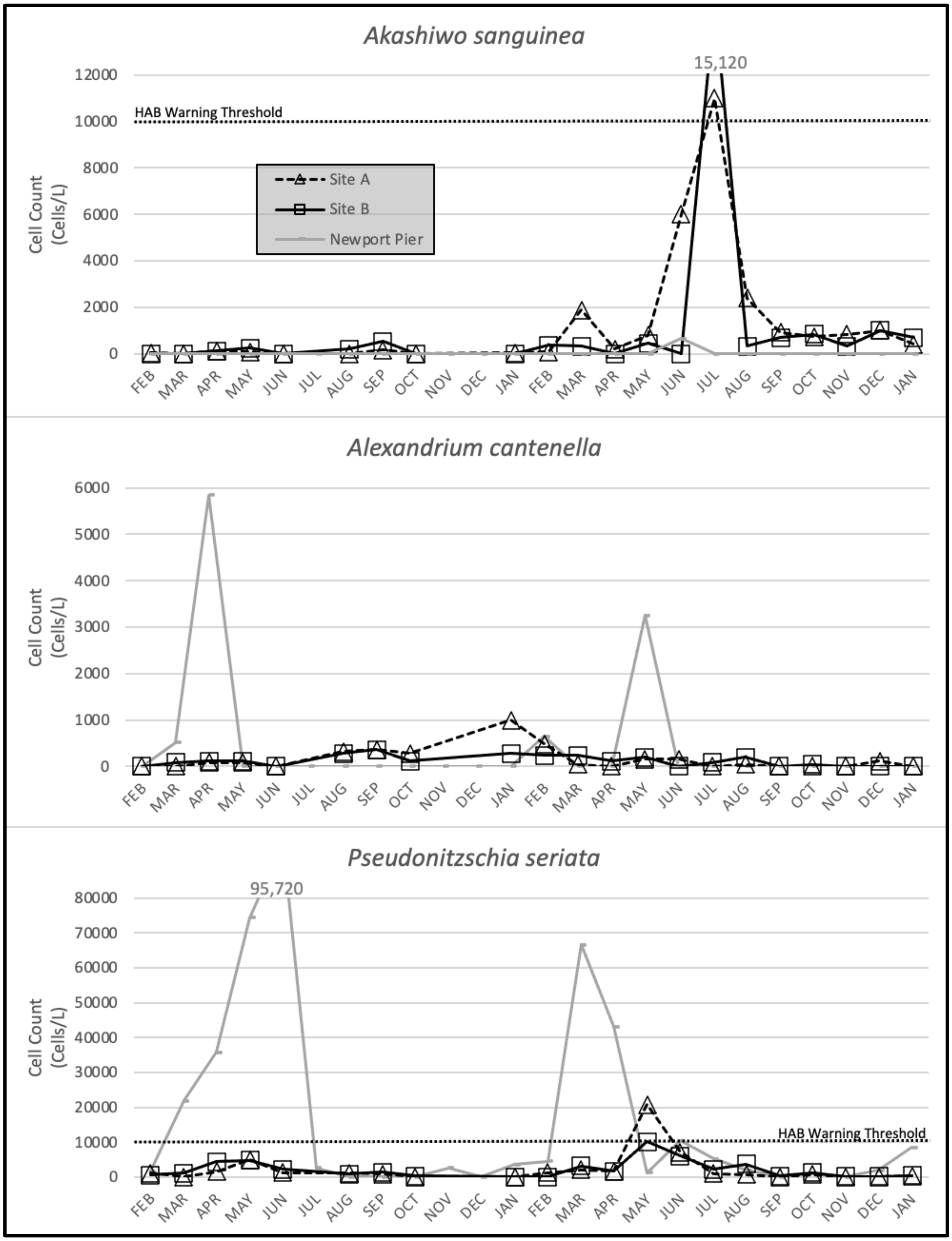


Figure 8: Time series of cell counts for significant HAB species for sites A and B, along with monthly averages of counts collected from Newport Pier (SCCOOS).

2.3.5 Flow Cytometry

Pico/nano-plankton cell counts were generally equal between sites in both communities of phycoerythrin (PE) harvesting *Synechococcus* and peridinin-chlorophyll-protein (PerCP) harvesting picoeukaryotes (figure 9). The highest *Synechococcus* count at Site A was 1.3 x 10^5 cells/ml (June 2017), and the lowest was 2.7 x 10^3 cells/ml (September 2018). Site B ranged from 3.1 x 10^3 cells/ml (April 2017) to 9.6 x 10^4 cells/ml (June 2017). Picoeukaryote populations at Site A were highest in May 2018 at 2.5 x 10^3 cells/ml and lowest in June 2017 at 900 cells/ml. Site B ranged from 660 cells/ml (June 2017) to 2.5 x 10^4 cells/ml (May 2018). Linear regression between sites A and B indicate strong relationships between the sites for both *Synechococcus spp.* (r-squared = 0.91), and picoeukaryote (r-squared = 0.92) (figure 10). For *Synechococcus*, the linear equation slope is greater that 1 (m=1.27), which would suggesting there is a slightly higher abundance at Site A. The reverse is true for picoeukaryote with a slope that is less that 1 (m=0.92). Between all size classes of phytoplankton, the picoeukaryote population was the first population to bloom each spring. However, *Synechococcus* experienced growth shortly after and during concurrent periods as the larger size class phytoplankton.

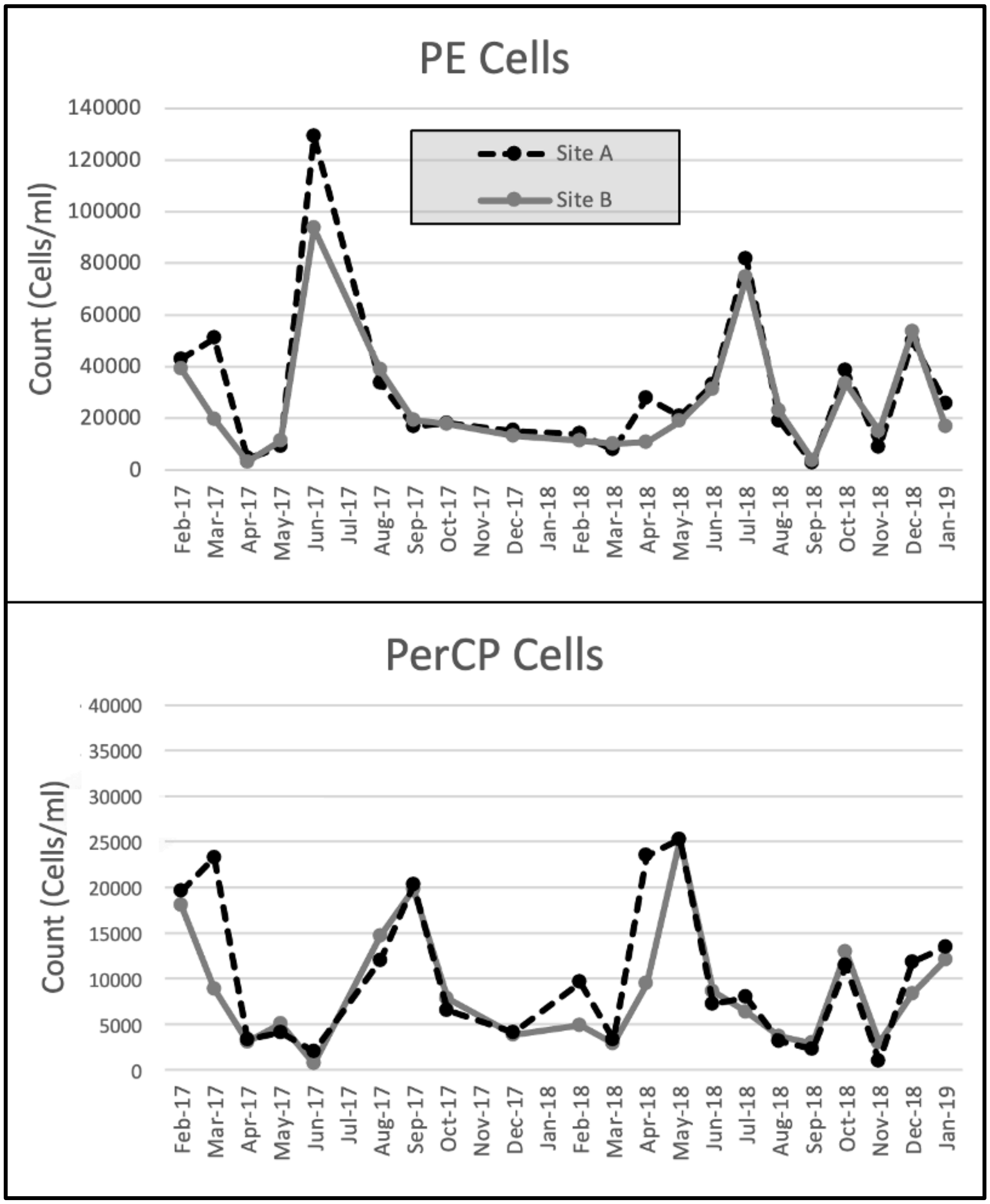


Figure 9: Monthly time series of pico-size phytoplankton populations. *Synechococcus spp.* populations are identified using phycoerythrin (PE) fluorescence and general picoeukaryotes are counted using peridinin-chlorophyll-protein (PerCP) fluorescence.

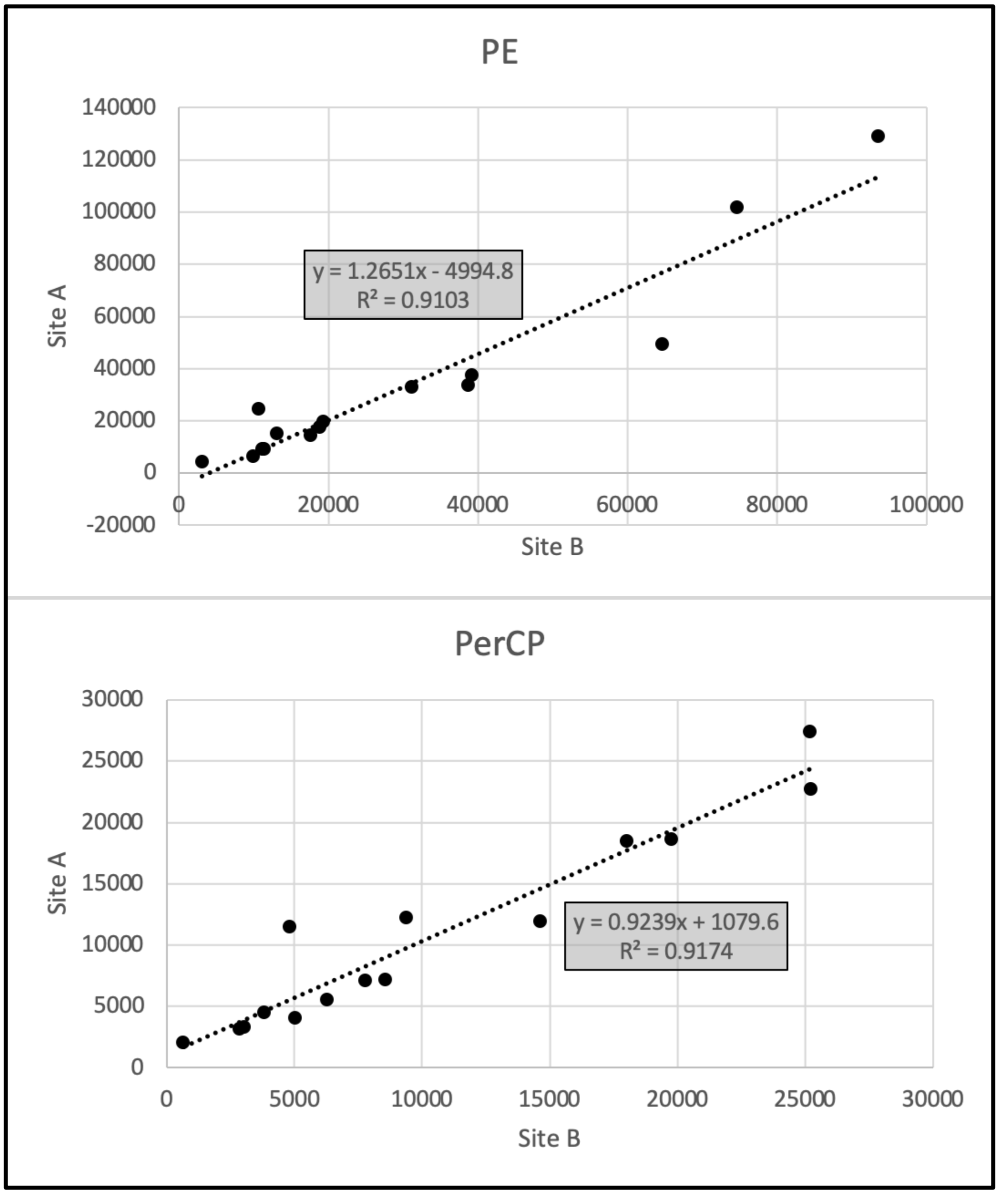


Figure 10: Linear regressions comparing Site A to Site B for populations of *Synechococcus* (top panel) and picoeukaryotes (bottom panel).

2.4 Discussion

This two-year study near Orange County Sanitation District's offshore outfall site discovered little to no difference between sites A and B based on nutrients, chlorophyll, and phytoplankton populations at the 30-meter depth evaluated. The physical parameters and phytoplankton species followed similar cycles between sites throughout the study for most variables investigated. Temperature and salinity remained comparable at both locations throughout all seasons. CDOM concentrations only demonstrated small differences and were of no clear significance to the phytoplankton community. Evaluation of deeper waters may have displayed a more significant impact from the outfall. Nutrient concentrations followed similar trends during all seasons, except for spring when concentrations of both chemicals were higher at Site A. Possible drivers for this seasonal spike over the outfall remain unclear.

CDOM fluorescence measured slightly higher at Site B during many of the sampling campaigns. This may be due to other factors that can influence the measurement of CDOM, such as river and estuarine inputs, along with coastal upwelling (Hudson et al., 2007). This may suggest that the outfall design is adequate for mixing wastewater discharge to minimize the impact of effluent on the surrounding environment. Alternatively, it's possible that discharge in the region has such a large-scale impact that a third sampling location, further from the outfall site, is needed to study the effect of effluent. Previous studies that have investigated chlorophyll concentration and phytoplankton community composition on larger spatial scales within the bight have found there to be differences in concentration and composition as sampling moved further off the San Pedro Shelf. (Smith et al., 2018; Nezlin et al., 2018). The stations chosen for this study were of similar distance to the shoreline and separated by less than three miles. The only factor that differed significantly was the depth of the water column between the two locations. It is possible that mixing across this smaller area was more substantial than expected, and a larger zone is needed to determine if effluent impacts the phytoplankton community.

Within the sampling area, the seasonal chlorophyll signal followed the expected trend for an eastern boundary current system that experiences decreased stratification in winter months and subsequent nutrient upwelling in early spring. Nutrient-rich deep water mixed with the euphotic layer during these events provides ample resources for phytoplankton to bloom. Surface waters are the first to become colonized, due to the ample sunlight at the top of the water column. Assuming the surface and deeper water layers are stratified, when resources are depleted near the surface, phytoplankton move deeper into the column where there is a higher concentration of nutrients. Once a threshold is reached where light can no longer penetrate to an adequate depth and nutrients are depleted, the populations decline and return to background levels observed prior to the bloom event. This seasonal phytoplankton vertical migration dynamic is evident in figure 4. Although measurable amounts of chlorophyll were present throughout the study, the rapid increases associated with a bloom occurred in late-spring and early-summer at this depth. Relatively high chlorophyll concentrations were maintained throughout summer but subsided by early fall.

Beyond the overall phytoplankton biomass, individual species populations had bloom dynamics that differed considerably from each other. Overall, standard seasonal dynamics for general SBC phytoplankton populations were observed. When nutrients were abundant, both pico and micro size class cells were experiencing growth. As the phytoplankton community depleted nutrients, the smaller cells made up an increasingly larger proportion of the overall community composition. Earlier studies have shown that phytoplankton cell size can influence community composition as nutrients transition from high to low abundance (Lewis et al, 1976). As cell size decreases, the surface area to volume ratio increases along with nutrient uptake efficiency. This typically results in an increase in relative abundance of smaller phytoplankton as nutrients become scarce for larger phytoplankton species. Their ability to maintain populations under all nutrient abundance regimes ensures seed populations of pico-plankton are maintained throughout all seasons. Evidence of this exists when considering that the first spring bloom is initiated by the picoeukaryote population. It's not until a short time later that we see the rest of the larger size-class populations begin to thrive. These larger cells are important for multiple reasons. First when considering carbon export rates from surface waters to deep, larger cells contribute to a more efficient transport. Additionally, the larger cells are important food supplies for many primary consumers that cannot utilize pico-plankton as a food source. Microscopy counts showed diatoms dominated between the two micro-class phytoplankton groups throughout much of the year. However, during the late-fall and early-winter, the ratio between the two were less pronounced.

Of the diatoms observed in the study, the HAB forming species of *Pseudo-nitzschia* spp. was of significant interest. This species was one of the few diatoms that could be identified during every cruise sampled. Among all diatoms identified, it remained the dominant species throughout 13 of the 21 months evaluated. Of the competing complexes, *P. delicatissima* dominated most of the study. However, *P. seriata* became the dominant complex during April and May of each year studied. These seasonal increases are meaningful as they may result in accelerated production of domoic acid (DA) when *P. seriata* becomes the dominant complex. This suggests that most of the year, there is a low risk of Amnesic Shellfish Poisoning resulting from the higher abundance of the low-DA-producing *P. delicatissima* complex. However, the risk of DA production is constantly present, as *P. seriata* was identified during each collection. Consequently, the Pseudonitzschia spp. genus interaction will require close monitoring in the coming decades.

As waters off the California coast continue to experience localized human influence along with the impacts of broader climate change, the population dynamics between these phytoplankton assemblages will likely shift. Studies have already shown that some domoic acid producing *Pseudonitschia* complexes experience increased growth rates in warmer waters and nutrient replete conditions. Continued monitoring of these species, combined with a better understanding into the range of effluent influence, is critical to ensure a safe and clean marine environment.

2.5 Conclusion

As the overall purpose of this study aimed at determining any effect of wastewater in the coastal environment, the results would suggest no significant difference was observed between sites. However, we only consider the 30-meter depth and there could be consequences in deeper waters which are closer to the plume. It is recommended that monthly sampling continue, however a more reference sites needs to be established. It's important to acknowledge the possibility that the current study could in fact be correct and there is no significant change to biogeochemistry and its relation to phytoplankton community dynamics, but this is unlikely the case with injection of wastewater. The study would also benefit from a longer time series that captured interannual variability in the SCB.

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CHAPTER 3

Phytoplankton Community Response to Changes in Biogeochemistry at a Southern California Endorheic Basin

Adapted from:

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3.1 Introduction

Endorheic basins are considered some of the most ecologically important arid habitats on Earth. Often, the biological communities found at these geologic formations are resilient to seasonal and episodic fluctuations to physical conditions in the natural environment. Endorheic basins are characterized by closed drainage basins that lack surface outflows to external bodies of water. Lakes and inland seas that form in these basins are the terminal destinations for precipitation falling within the watershed. Rainwater is often sparse in these regions and precipitation either remains at the surface or in local underground aquifers rather than flowing out to sea. As such, they often supply essential habitat for local ecology while also providing a wide array of ecosystem services (Ragab & Prudhomme, 2002). Studies have shown endorheic basins to be highly diverse, often containing vital aquatic ecosystems which serve as stopover points during seasonal migrations of many bird species (Zadereev et al., 2020). Economically, these regions are valuable to the human population, as they often contain marketable resources such as salts, lithium, and geothermal energy potential (Grosjean et al., 2012; De Giorgi & Leucci, 2015). For example, in Central Africa, Lake Chad supplies water and food resources to one of the driest regions on Earth. Its shoreline spans between four countries, which has caused geopolitical tensions in the region as these resources have become increasingly limited. Satellite observations indicate that surface extent of Lake Chad has been declining rapidly over the past several decades and has consequently cut off access to water for many of the surrounding human populations (Coe et al., 2004).

In the United States, the Salton Trough of Southern California represents a large endorheic basin. Naturally, it should exist as an isolated dry extension of the Gulf of California formed by lateral motion and spreading between the San Andreas fault system and the Eastern Pacific Rise (Alles, 2011). Sediment deposits from the Colorado River Delta and the addition of wind-blown sand dunes have since created a barrier that today isolates the trough from the ocean. The Salton Sea is the terminal lake that currently occupies the lowest lying terrain in the trough. As of 2020, the surface level was reported to be 209 feet below sea level (figure 1). It was created by an agricultural engineering accident along the banks of the Colorado River in the early 1900's. Waters originally destined for the Gulf of California have since been partially diverted for agricultural use in the Southern California desert. Consequently, the Salton Sea has remained present as drainage of agricultural runoff within the trough is drawn to its lowest point.

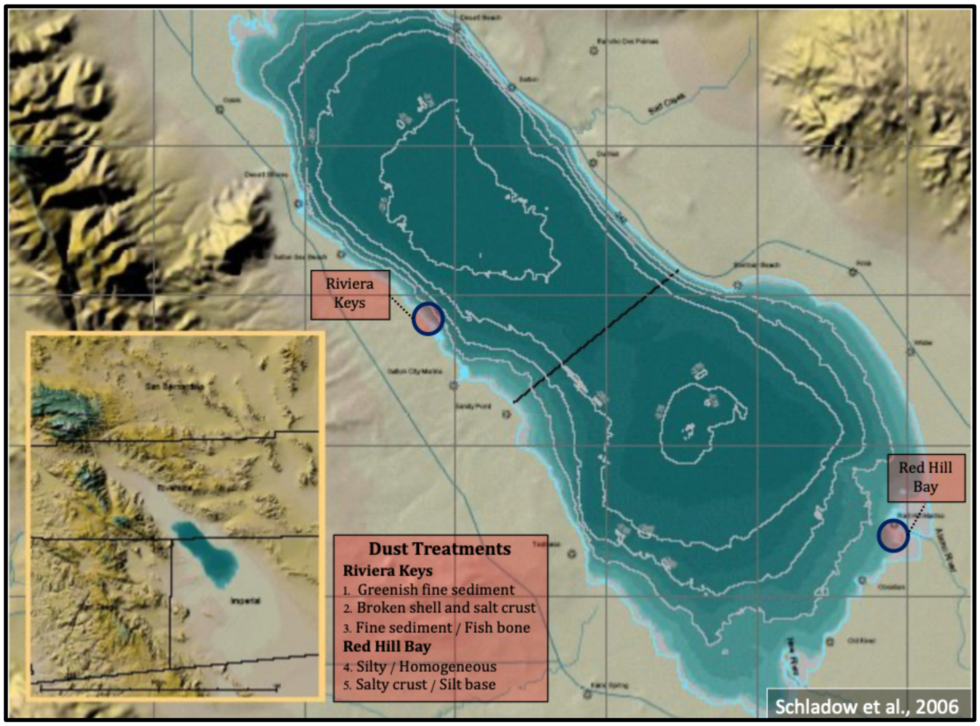

Figure 1: Map of dust sample sites at the Salton Sea, in Imperial and Riverside County.

Over the past century, the Salton Sea has experienced persistent changes in water chemistry, facilitated by both natural and anthropogenic causes. Naturally, the Colorado River contains trace amounts of heavy metals such as selenium and lead that have been weathered from the rock and soil in the Rocky Mountain range. While these metals exist at low concentrations within the river, over time they concentrate in the Salton Sea's water column due to high evaporation rates and no outflow. Within the lake, they can exceed hazardous levels and become harmful to the ecosystem (ATSDR, 2003; Cherdwongchareonsuk et al., 2003). These natural contaminants are compounded by agricultural and urban runoff from California's Imperial and Coachella Valleys. These waters often contain soil additives like macronutrients and trace minerals important for primary production, but they also contain ecologically harmful pesticides and herbicides like atrazine and glyphosate. Before being banned, these flows even contained high concentrations of dichlorodiphenyltrichloroethane (DDT) (Holdren & Montaño, 2002). These constant manipulations to water chemistry have led to many aquatic ecosystem collapses over the past several decades. When this happens, there are often cascading effects to the surrounding terrestrial ecosystem as well (Sarkar et al., 2001).

After its initial formation early in 1905, the Salton Sea was characterized by warm temperatures and low salinity (Doede & DeGuzman, 2020). Chemically, its composition was like that of the Colorado River water that fed the system. However, around the middle of the 20th century, several factors led to a steady increase in the salinity of the sea. Leaching salts from sediments and agricultural additives caused salinity concentrations to rise resulting in ocean-like conditions by the 1960's. The continuous salt inputs were exacerbated by the physical constraints governing the basin. As a terminal lake in one of North America's most arid regions, the primary outbound flux of water occurs through evaporation, which leads to increased salinity over time. Another driver causing change to salinity is a dramatic decline in the surface extent of the Sea over the past few decades due to changes in regional water policy (Jones & Fleck, 2020). To exacerbate the policy issue, restructuring of the Colorado River Compact was signed in 2023 that reduces the allocation of river water to California. The purpose of restructuring was to divide water rights up more equitably between the seven states with access to the Colorado Riverbanks. In California this is likely to have consequences on the agriculture quotas that feed the Salton Sea, leading to continued and accelerated reduction in surface extent. Consequently, current measurements of salinity at the Salton Sea are approaching 60 parts per thousand (PPT).

As the water level of the Salton Sea has decreased, there have been corresponding increases in sediment exposure along the shoreline. These exposed sediments consist of very fine silt and other small particles that are susceptible to aeolian transport. This has resulted in increased atmospheric dust deposition, causing widespread nutrient enrichment, a reduction of photosynthetically active radiation (PAR), and adverse air quality conditions for surrounding terrestrial animals and human populations. Although there have been many ecological impacts due to a rapid decline in water quality, some organisms can indeed thrive under these harsh conditions. For example, some phytoplankton populations can endure high temperatures and salinity. These resilient organisms utilize the abundant nutrients in the water column, allowing for sustained growth at the Salton Sea. Previous studies of phytoplankton assemblages conducted during the late 1980's and throughout the 1990's consistently found high concentrations of marine diatoms and dinoflagellates (Setmire et al., 1993; Tiffany et al., 2007). Many of the species identified were known to exist in Southern California coastal marine environments. It is speculated that these species were introduced via ballast water when Salton Sea was a busy recreational site between 1940-1970. During these decades, Salton Sea salinity was comparable to the open ocean at roughly 35 ppt. However, the current phytoplankton community may not be suited to adjust to the persistent increases of salinity, which today are nearly double that of the typical marine environment.

This study looks to evaluate the biogeochemical consequences of continued environmental degradation within this anthropogenically altered ecosystem and the subsequent effects to primary production. The factors that could have some of the most severe environmental consequences are evaporation driven increases in salinity and dust deposition of fine sediment particles exposed by a rapid decline in surface extent. The study mimicked possible changes to these variables during a 60-hour in-situ mesocosm incubation to determine the impacts on phytoplankton productivity and community composition.

3.2 Methods

3.2.1 Dust Treatments

To evaluate any biogeochemical influence of wind-driven dust deposition upon the aquatic ecosystem, five dust samples were collected from two sites (Riviera Keys and Redhill Bay) along the Salton Sea shores (figure 1). Sites were selected to be representative of different playa compositions found on the eastern (Redhill Bay) and western (Riviera Keys) shorelines. Dust samples from the playa regions were collected during a site survey in the spring of 2018. At both collection locations multiple samples were obtained at 50 meter intervals along a sloping gradient towards the waterline. Samples were obtained from each location by removing a thin layer of crust (~ 5 mm) and collecting 500 g of the finer grain-size substrate found below. The intent was to collect each sample from temporally different regions regarding atmospheric exposure. Dust 1(D1), Dust 2 (D2), and Dust 3 (D3) were collected near the community of Riviera Keys in Salton City along the western shoreline (figure 1), and represent a region with steeper bathymetry, resulting in a gradual sediment exposure. Dust 4 (D4) and Dust 5 (D5) were collected at Red Hill Bay, located at the south-eastern end of the lake, where the bathymetry tapers much more gently, causing large areas of sediment to become exposed with relatively small declines in depth. When equal amounts of sample were added to a set volume of MilliQ water

(0.15g/150mL MQ) average salinity of these two sample sites were 0.33 and 0.44 PSU, respectively. Each dust sample was inoculated into Salton Sea water samples in high and low concentration treatments. 2.5mg of dust was added to 250mL of Salton Sea water. Low dust treatments received 0.25mg per 250mL of water sample. Concentrations were selected to simulate to an atmospheric deposition rate of 10 g/m^2 day (high) and 1 g/m^2 day (low). Deposition rates recorded in the region range from 0.01 g/m^2 day to 0.4 g/m^2 day, and models predict this will increase as more playa is exposed (Frie et al., 2019).

3.2.2 Dust Elemental Analysis

Dust additions were subject to trace metal analysis to determine possible response of the current assemblage to increased atmospheric deposition. For each of the five dust samples collected, three replicates of 1.25 mg dust were agitated in 50 uL and subsequently filtered through a 2 um GFF for elemental analysis. An Element XR Inductively Coupled Plasma Mass Spectrometer was used to measure B, Mg, Al, P, K, Ca, Cu, V, Cr, Mn, Fe, Ni, Co, Zn, As, Cd, Pb, and U. Measurements of each sample were compared against a standard using Brooks Applied Laboratories SOP BAL-0551 and EPA 6020B methods (EPA, 2014). Apparent trends for each element will be evaluated at each site. Focus will be placed on trace metals that are considered nutrient requirements for phytoplankton growth. This includes iron (Fe), zinc (Zn), manganese (Mn), and nicke (Ni). Quotas for the marine environment are in general:

Fe ≈ Zn > Mn ≈ Ni

3.2.3 Salinity Treatment

Four Salinity manipulations were evaluated during the incubation. Two dilutions of five (Dil05) and ten (Dil10) percent were achieved by adding 12.5 mL of Milli-Q water to 237.5 mL of sample and 25 mL of Milli-Q water to 225 mL of sample, respectively (table1). Additionally, two salinity increase treatments of five (Sal05) and ten (Sal10) percent were analyzed. Salinity additions were achieved using salts isolated from Salton Sea water via evaporation. The five percent treatment added 0.75 g of the isolate salt to 250 mL of sample, while the ten percent received 1.5 g per 250 mL of sample.

Incubation Inoculation Matrix							
Atmospheric Deposition Rate	Site 1 Dust	Site 2 Dust	Site 3 Dust	Site 4 Dust	Site 5 Dust	Salinity	Dilution
Low (x 3)	1 mg/l	1 mg/l	1 mg/l	1 mg/l	1 mg/l	+5%	+5%
High (x 3)	10 mg/l	10 mg/l	10 mg/l	10 mg/l	10 mg/l	+10%	+10%

Table 1: Sample addition matrix, showing concentrations for high and low dust additions and salinity manipulations.

3.2.4 Phytoplankton Collection

To evaluate the most current assemblage of phytoplankton in the Salton Sea, 30 liters of sample water was collected the morning of the incubation at Obsidian Beach (figure 2). This location was selected as one of only a few spots along the shoreline where water can be collected without disturbing the underlying sediment. Large pieces of volcanic obsidian here allowed for phytoplankton samples to be collected in water up to a meter in depth. This site selection was intended to reduce the influence of the benthic community during the incubation. A second consideration for this particular location was its' close proximity to the incubation site (figure 2). At the incubation site, the sample water was agitated to homogenize, then divided into 180 250mL polycarbonate bottles. Treatments included the dust and salinity manipulations mentioned above and were inoculated in triplicate for each of the time points. Batches of samples were collected and analyzed at 0, 12, 36, and 60 hours.

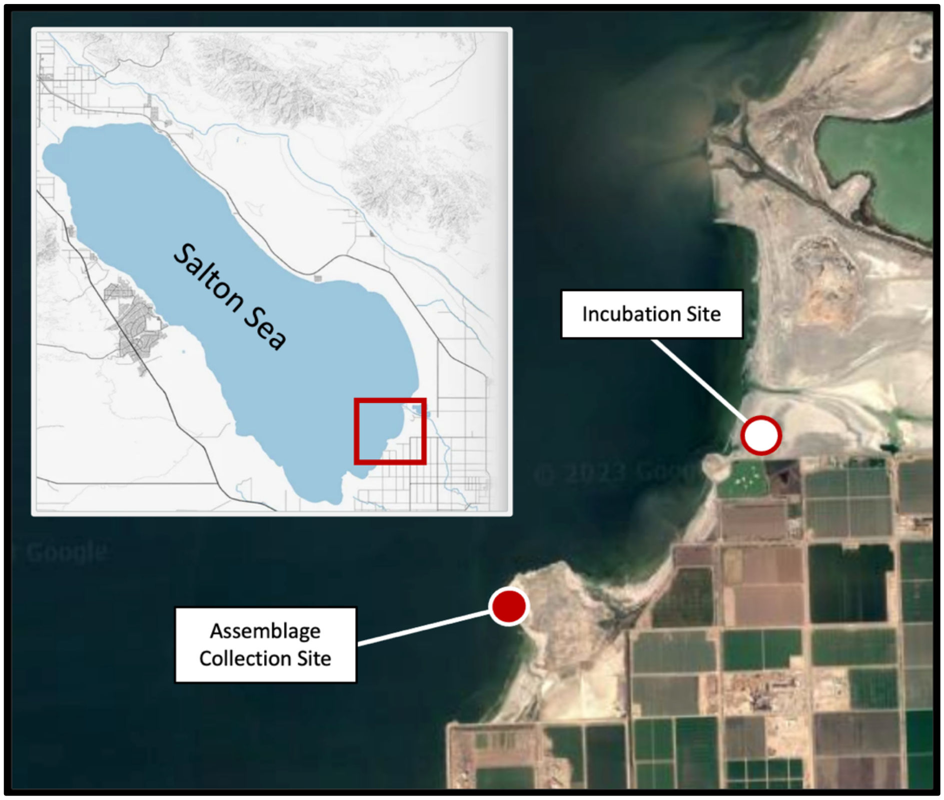

Figure 2: Map of phytoplankton collection and incubation sites along the southeastern shoreline of the Salton Sea. Site access granted by Salton Sea National Wildlife Sanctuary.

3.2.5 Chlorophyll-a

The concentration of Chlorophyll-a was determined for each sample and used as a proxy for overall phytoplankton biomass. Within 90 minutes of collection, each treatment had an aliquot of 10mL filtered through a 25mm Whatman Glass Fiber Filter (GFF) which was subsequently frozen and stored for follow up analysis. Each deposited filter was extracted in 10mL of 90% acetone for 24 hours at -20ºC. After such time, samples were returned to room temperature and the raw fluorescence (RFU) was measured. Measurements were completed using a non-acidic chlorophyll-a optical cassette placed in a Trilogy Fluorometer (Turner). The reported RFU value was subsequently converted to ug/L using a standard curve provided by the instrument manufacturer.

3.2.6 Nitrate and Phosphate

50 ml of filtrate was collected after the chlorophyll analysis and used to determine nutrient concentrations of both nitrate/nitrite (N+N) and phosphate (PO4). Samples were kept frozen between the field station and lab analysis at UC Irvine. Once thawed, samples were passed through a QuickChem 8500 Series Analyzer (Lachat Instruments) in accordance with manufacturers seawater specifications (Johnson and Petty, 1983; Lachat, 2003). Concentrations of N+N and PO4 were then reported in uM/L.

3.2.7 Microscopy

Phytoplankton species identification and enumeration was accomplished by inverted light microscopy. Upon sample collection, 50mL aliquots were fixed in 1% Lugol's solution and stored under dark conditions to prevent photodegradation. In the lab, samples were homogenized by gentle agitation and 1mL was immediately transferred into a settling slide, in accordance with the Utermöhl method (Utermöhl, 1958), utilizing a volume that allowed for consistent counting. Settling took place over a 24-hour period before each sample was examined under the microscope. Twenty random frames, from throughout the chamber were digitally recorded using a Nikon Eclipse Ti2 inverted light microscope under 200x magnification. Photographs of slides were subsequently counted to provide species enumeration counts in cells per milliliter.

3.2.8 Flow Cytometry

To quantify picophytoplankton and cyanobacteria populations, flow cytometry was conducted using a NovoCyte Benchtop Flow Cytometer (Agilent). Each sample had a 1.5 mL aliquot preserved in solution with 4% formalin and was stored at -80 ºC until ready for lab analysis. Analysis was accomplished by homogenizing the fixed samples and passing 50 uL through the instrument. Populations of picoeukaryotes and *Synechococcus spp.* were identified and enumerated based on their peridinin-chlorophyll-protein (PerCP) and phycoerythrin (PE) autofluorescence signatures, respectively (Haynes et al., 2016).

3.3 Results

3.3.1 Dust Elemental Analysis

Elemental analysis of playa dust samples revealed there to be no distinct chemical characteristics between the two collection locations. However, there were differences between the individual sample sites. For the trace metals required during phytoplankton growth (Fe, Zn, Mn, and Ni), concentrations in some samples were of particular interest (figure 3). Dust 1 (D1) collected at the Riviera Keys location had statistically significant (p-value = 1.79×10^{-5}) differences in manganese concentration. However, Iron, zinc, and nickel were not significant. No other samples had concentrations of these metals at concentrations statistically valuable for investigation.

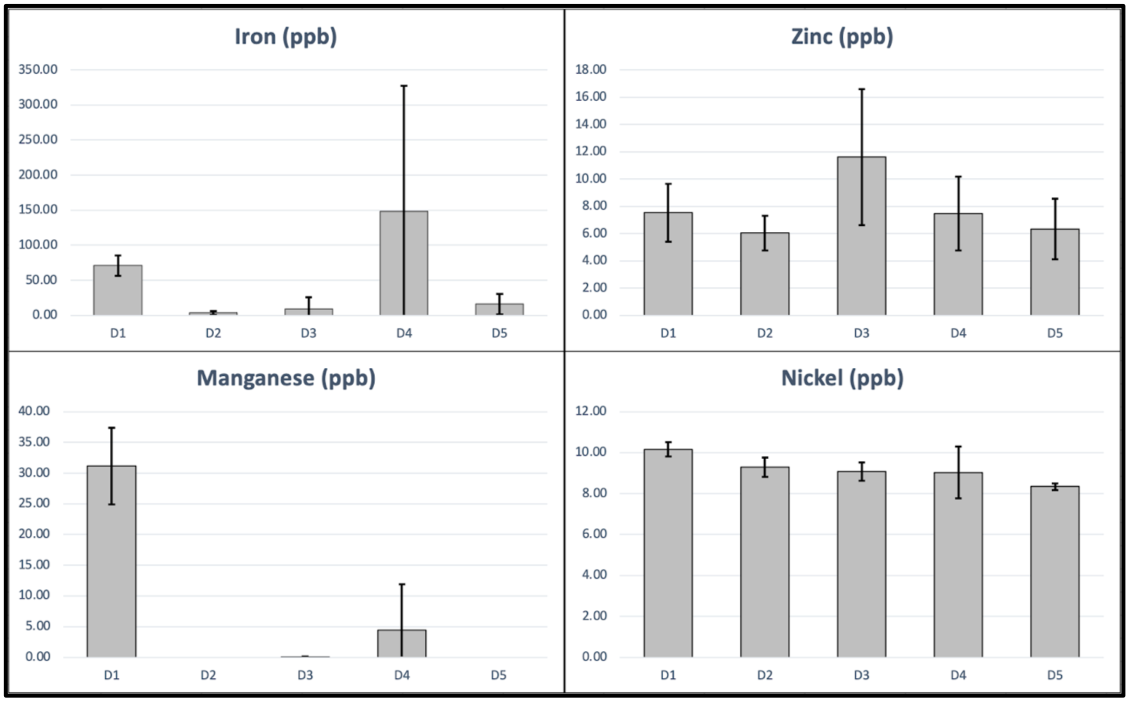

Figure 3: Dust sample trace metal analysis. Fe, Zn, Mn, Ni represent significant metalloids required by phytoplankton for photosynthesis and growth.

In addition to the trace metals required for phytoplankton growth, other elements that interact with biology were found at increased concentrations. Again, Dust 1 (D1) had significantly higher concentrations of phosphorus (p-value = 6.31×10^{-7}) and arsenic (p-value = 5.09×10^{-6}) (figure 4). Phosphorus was also measured at higher concentration in Dust 2, but no difference in arsenic was observed.

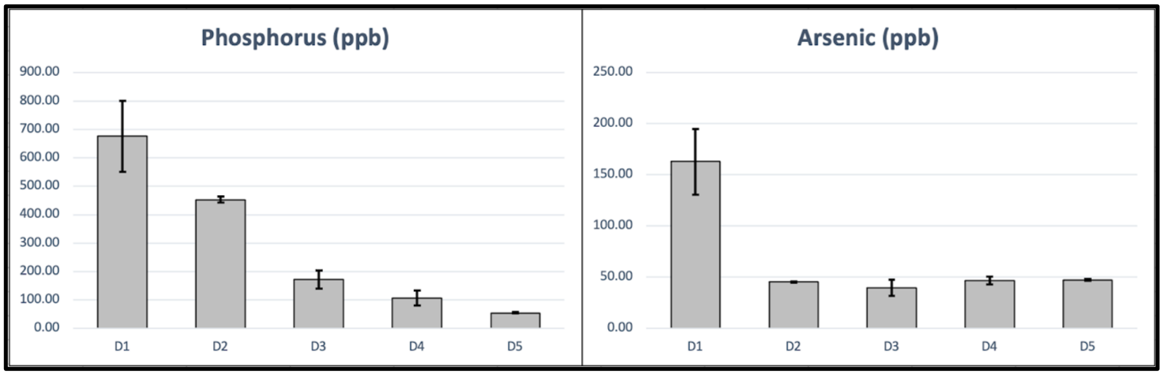

Figure 4: Elemental analysis of phosphorus (P) and arsenic (As). Phosphorus was determined to be an element of interest as biologically available species are required by phytoplankton. Arsenic was studied do to potentially toxic effects.

Based on the differences observed in elemental analysis, it was determined that the Dust 1 (D1) sample was of the most interest to the study, due to its significant levels of manganese, phosphorus, and arsenic. The higher concentration of phosphorus in Dust 2 was considered as well.

3.3.2 Chlorophyll-a

Throughout the incubation, chlorophyll-a concentration ranged from 90.25 ug/L (D1L:12hr) to 491.45 ug/L (Control:60hr) (figure 5). The experimental control began the incubation with a concentration of 205.90 ug/L and increased to 491.45 ug/L, signifying the most dramatic increase of chlorophyll-a among all treatments. The largest change within the dust treatments was D1L, increasing from 208.71 ug/L to 479.84 ug/L over 60 hours. The dust treatment with the smallest 60-hour increase was D5L, with measurements starting at 207.86 ug/L and ending with 215.81 ug/L. However, it is important to note that the 36-hour average of the D5L treatment reached higher concentrations than the 60- hour, with 290.83 ug/L. The greatest chlorophyll-a increase within the salinity manipulations was the 10% dilution, increasing from 223.66 ug/L to 432.08 ug/L. The 5% salinity increase was the only treatment that experienced overall reduction in chlorophyll-a concentration, declining from 227.33 ug/L to 197.35 ug/L.

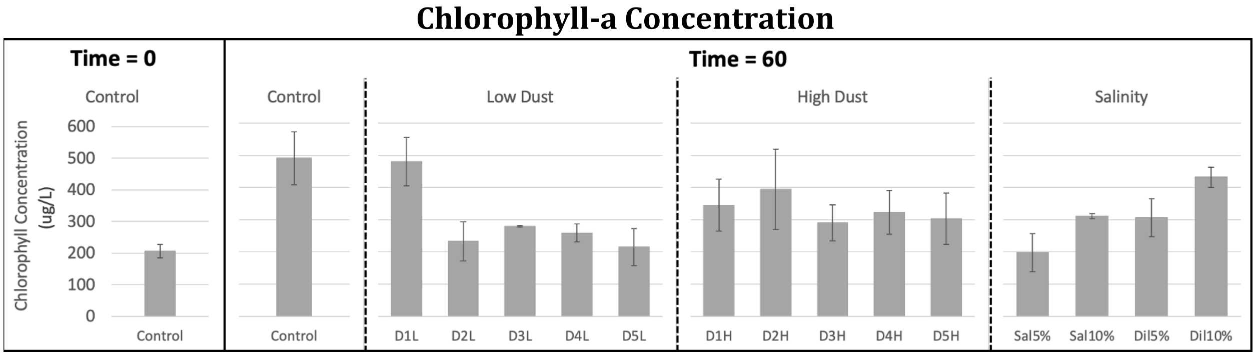

Figure 5: Change in chlorophyll-a concentration (ug/L) between time 0 and 60 hours for control, dust, and salinity treatments.

3.3.3 Nutrients

Nitrate/nitrite concentration range was between 73.73 uM (Dil10:12hr) and 170.27 uM (D5L:0hr) (figure 6). Overall, samples experienced a significant (p-value = 6.66 x10-6) decline in nitrate/nitrite over the course of the study. The mean value at time zero was 125.88 uM, and 101.97 uM at time sixty. The experimental control decreased from 122.20 uM to 96.00 uM. The largest decline in nitrate concentration was the 5% salinity increase (Sal5) treatment, which decreased from 158.53 uM to 95.27 uM. The smallest decline occurred in the DH3 treatment, starting at 109.60 uM and declining to 107.40 uM. The only nitrate/nitrite treatment to experience an increase in concentration was the low dust addition to site 3 (D3L).

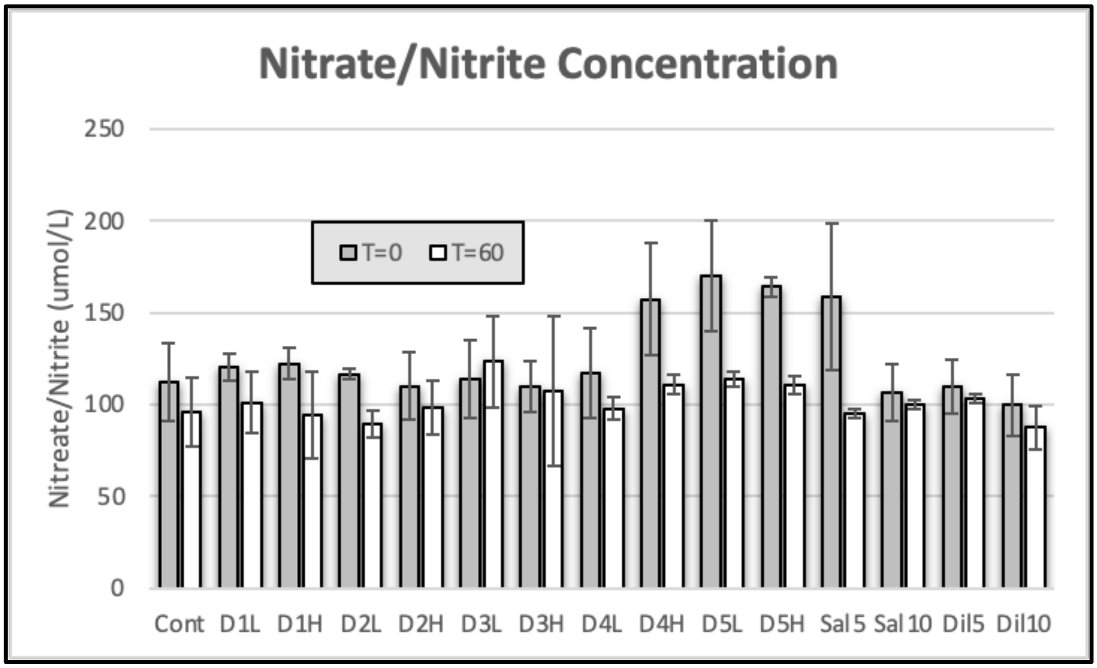

Figure 6: Change in nitrate/nitrite concentration (uM) between time 0 and 60 hours for all dust and salinity treatments.

Phosphate concentration during the incubation ranged between 0.16 uM (Sal5:12hr) and 5.92 uM (Sal5:36hr)(figure 7). Analysis revealed there to be inconsistent interaction with phosphate between samples. Many of the treatments resulted in a decline of phosphate, while others showed substantial increases. Overall, the change in concentration between time points indicates a significant (p-value = 0.04) difference in phosphate. As the study progressed, the control declined from 2.32 uM at the start of the incubation, to 0.19 uM of phosphate at 60-hours. The largest decline was found in the high dust site 2 (D2H) treatment, which experienced a decrease from 2.72 uM to 0.18 uM. Some treatments experienced an increase in phosphate. Most notably, the 5% salinity increase (Sal5) rose from 0.23 uM to 2.07 uM.

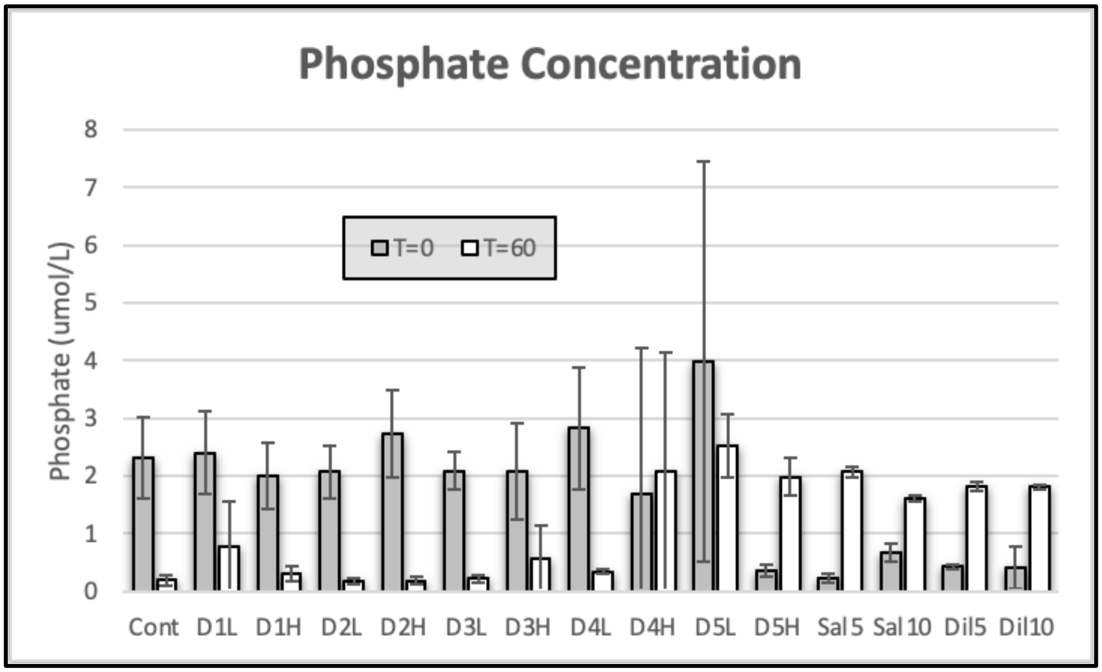

Figure 7: Change in nitrate/nitrite concentration (uM) between time 0 and 60 hours for all dust and salinity treatments.

Nitrate to phosphate ratios were substantially higher than the Redfield ratio of 16:1. Average ratio recorded throughout the study was 257:1 (figure 8). This suggests that the system was phosphate limited at the time of the study. The highest ratio measured was 782:1 in the 5% salinity increase (Sal5: 0hr) sample. The lowest ratio was recorded at 60 hours in for the same treatment (Sal5) sample, at 46:1. No discernible trends were identified using N:P ratios, which challenges the validity of nutrient analysis as a whole.

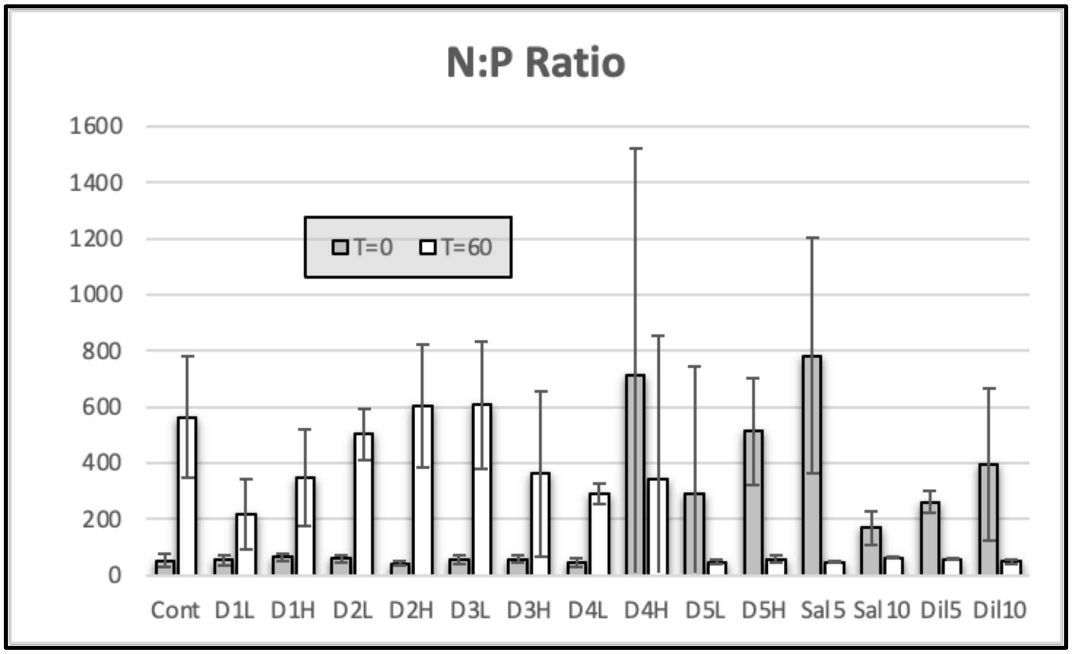

Figure 8: Results of nitrogen to phosphorous ratios (unitless).

3.3.4 Microscopy

Microscopy revealed 6 dominant genera of dinoflagellates and 7 dominant diatoms. The species identified from this study are similar to many marine phytoplankton found in coastal waters along the Southern California coast. The dinoflagellate population was minimal, but species identified consisted of *Gymnodinium spp., Gyrodinium spp., Scrippsiella spp., Gonyaulax spp., Chattonella spp.*, and *Eutreptia spp*. Diatoms were identified in much higher quantities and species included *Pleurosigma spp., Nitzschia spp., Navicula spp., Chaetoceros spp., Thalassionema spp., Diploneis spp.,* and *Cyclotella spp*. The most abundant species throughout all treatments was the diatom *Nitzschia spp*, which was, on average 3.40 times more abundant than the next most populous species, *Navicula spp*. All other species were found in substantially fewer numbers. As such, the species were separated into four groups to complete analysis. These groups were *Nitzschia spp., Navicula spp.,* Dinoflagellates, and Other Diatoms (which exculded *Nitzschia* and *Navicula*) (figure 9). The Nitzschia control count decreased from 34700 cells/mL to 2360 cells/mL over the span of the incubations. The highest Nitzschia count was the 60hr D1L treatment with 43900 cells/mL. The lowest Nitzschia count was the 60hr D4H treatment with 23100 cells/mL. The control for Navicula increased during the incubation from 12300 cells/mL to 14600 cells/mL. The highest Navicula count was the 60hr Dil10 treatment with 17400 cells/mL. The lowest concentration was the D1H treatment with 9750 cells/mL. Total Dinoflagellate population increased from 990 cells/mL to 11000 cells/mL under control conditions. The minimum and maximum counts were 470 cells/mL (Sal05:60hr) and 1650 cells/mL (D4L:60hr), respectively. Other diatoms increased from 2810 cells/mL to 3850 cells/mL within the control. Minimum and maximum counts were 1890 cells/mL (Dil05:60hr) and 4800 cells/mL (Dil10:60hr).

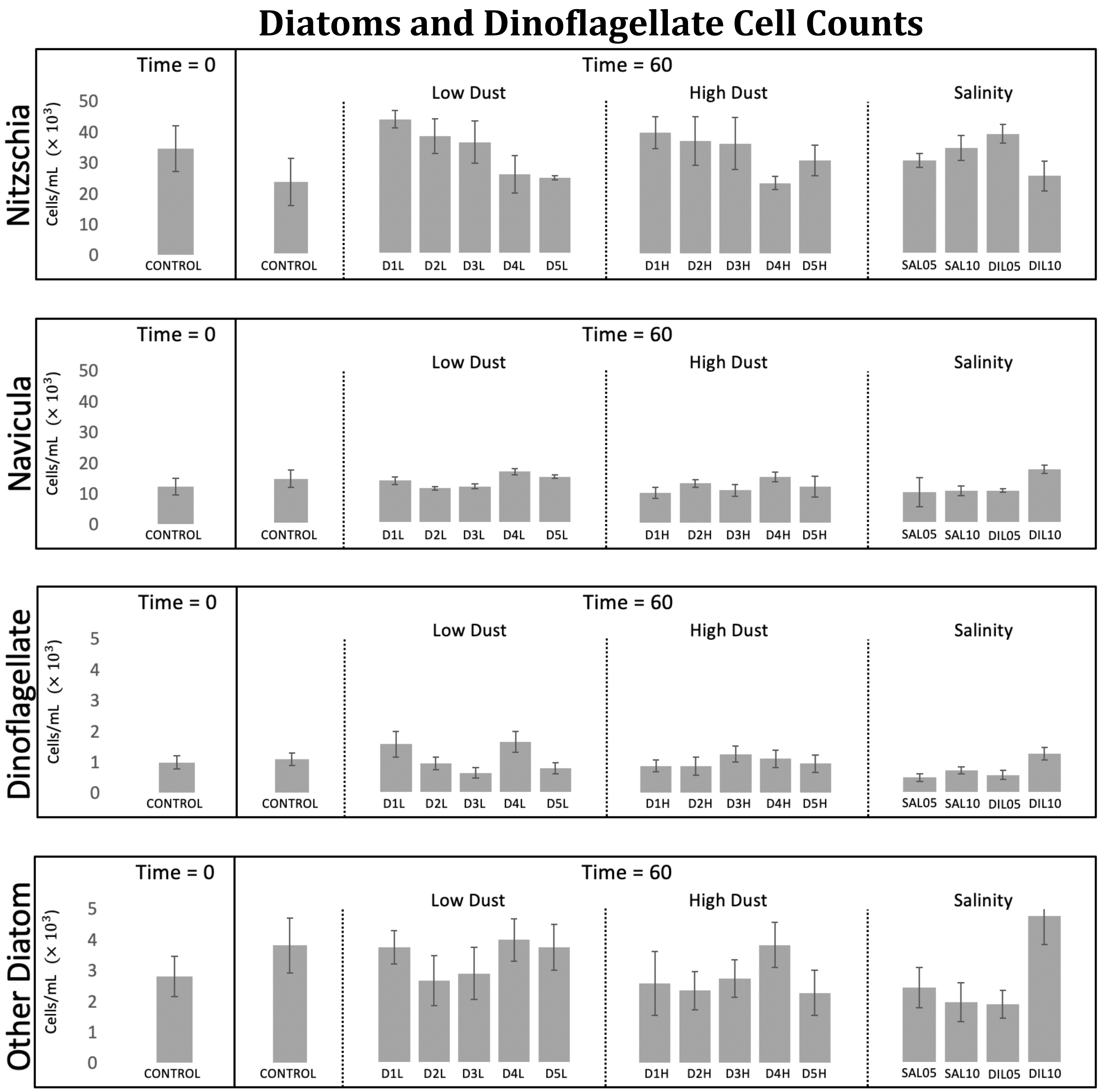

Figure 9: Microscopy cell count results for abundant species identified in the micro size class. Top panel reports the most abundant species, *Nitzschia spp.*, for the control, low and high dust treatments, and salinity manipulations. Second, third, and last panels are Navicula spp., total dinoflagellates, and other diatoms, respectively.

3.3.5 Flow Cytometry

Flow cytometry analysis revealed there to be a substantial picoeukaryote population that was identified and gated using peridinin-chlorophyll-protein (PerCP) excitation and emission. The instrument was also set up to perform counts on Synechococcus spp., by gating for phycoerythrin (PE) harvesting cells. However, none were identified during analysis. The experimental control picoeukaryote count increased from 2.35×10^5 cells/mL to 5.48×10^5 cells/mL, accounting for the smallest increase of picoeukaryotes (figure 10). The largest growth occurred in treatment D3L with an increase of 6.61×10^5 cells/mL. The growth rate throughout all treatments were substantial as the overall average increase grew by a factor of 3.39.

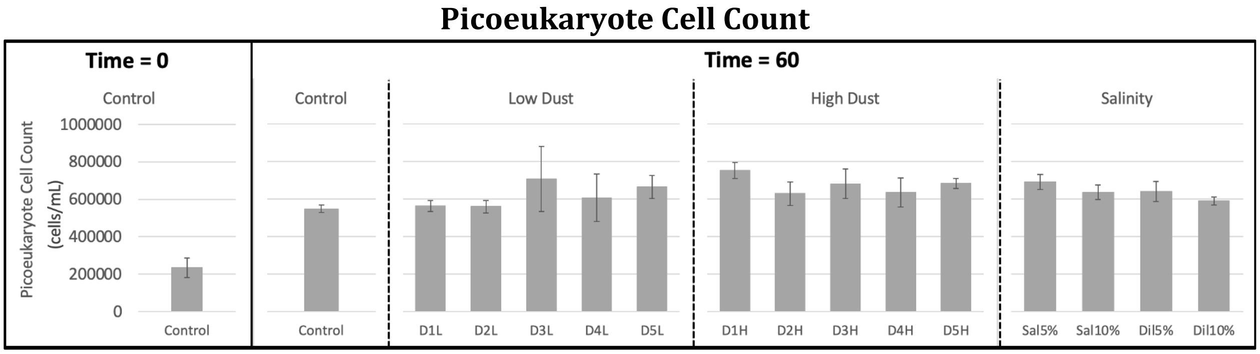

Figure 10: Flow Cytometry cell counts of picoeukaryote phytoplankton. Graph shown for species containing chlorophyll and have been identified using peridinin-chlorophyll-protein (PerCP).

3.3.6 Data Analysis

Data analysis was not performed on the nutrient data obtained during this study. While the nitrate/nitrite values appear to be valid, phosphorus measurements varied considerably. This study revealed no direct correlation between individual groups of phytoplankton and the measured chlorophyll concentration. Multiple linear regression (MLR) analysis of all phytoplankton groups combined returned a coefficient of determination (R-squared value) of 0.36, suggesting a weak linear relationship controlling chlorophyll. The most significant group identified was the picoeukaryote population, with a p-value of 6.34 × 10-4. The most significant micro size-classed drivers were *Nitzschia spp.* and *Navicula spp*., with p-values of 0.005 and 0.030, respectively. Total dinoflagellates and other diatoms were not of significant contribution to chlorophyll, with p-values of 0.29 and 0.08.

3.4 Discussion

The goal of this study was to evaluate several pathways of future ecosystem decline that might be anticipated at the Salton Sea. Focus was given to the contemporary phytoplankton community as they have been able to endure the severe and consistent changes at this site. Based on the composition of trace elements in the dust additions, we expected to see a growth response in samples inoculated with dusts containing high amounts of bioavailable nutrient species, and a toxicity response from additions containing heavy metals. Additionally, it was anticipated that phytoplankton growth rates would increase under dilute conditions and decline under conditions of increased salinity.

Chlorophyll-a concentrations suggest there was a toxicity effect resulting from most of the treatments (except for D1L, D2H, and Dil10) when evaluated against the experimental control. There was no discernable trend when considering the difference between high and low dust treatments, suggesting that there was little effect on growth rates driven by constituents of the dust. Most samples experienced a slight decline in chlorophyll concentration between T-0 and T-12. Some dust treatments also showed a decline between the 36 and 60-hour samples. Overall, the trend was a marginal increase in chlorophyll over the duration of the study. Some of the initial declines in chlorophyll may be attributed to factors associated with the methods of incubation. Changes in temperature and light regimes between the collection and incubation sites may have influenced growth. The incubation site was shallower than the collection site and sample bottles were kept at the surface. Salinity manipulations indicate consistent increases in chlorophyll concentration throughout the duration of the experiment for all but the 5% salinity increase (Sal5). Overall, the highest increase in chlorophyll, and phytoplankton abundance, was experienced when present conditions were maintained within the experimental control. This would suggest that the current assemblage of phytoplankton can adapt to continued changes in biogeochemistry at the site. However, during this study, most species were best suited when conditions were left at ambient levels.

Enumeration of the micro-size class phytoplankton revealed a toxicity effect on most species resulting from dust and salinity manipulations. One exception was the diatom *Nitzschia.* This species experienced a higher growth rate in 6 of the 10 dust treatments over the control. This may suggest that of the larger sized class planktonic species, *Nitzschia* may become more competitive as the local environment continues to change. Previous work at the Salton Sea identified many of the same phytoplankton as this study and found that dinoflagellates were consistently recorded in higher numbers than diatoms throughout every season (Tiffany et al., 2007). During this collection, microscopy revealed much higher numbers of diatoms. This could indicate a seasonal or episodic bloom was occurring at the time of collection, or that there has been an ecological shift towards diatoms at the Salton Sea.

Picoeukaryote growth rates favored all changing conditions over the control at the final time point. The 5% increase in salinity (Sal5) resulted in the highest increase in picoeukaryote abundance. These results suggest that the current assemblage of picoplankton within the Salton Sea can adapt to rapid and continued changes in water chemistry. However, it is important to note that other forcings may have contributed to this increase in abundance. With the possibility that temperatures inside the incubation bottles differed from that of the collection site, the picoeukaryotes may have benefited. Studies have shown that picoeukaryote growth rates increase with temperature (Chen et al., 2014). In either case, it suggests that rapid changes may favor the growth of the smaller picoeukaryote species at the Salton Sea. This result may have implications that affect nutrient cycling, carbon uptake, and trophic transfer efficiency at this location.

3.5 Conclusion

The scope of this incubation study may have been too large to identify any significant results based on the playa dust additions. It is recommended that future studies that pursue the affects of localized atmospheric deposition to aquatic environments modify the methods followed for this study. If this research group were to attempt another cycle, we might perform additions of singular trace elements identified in high abundance from local samples. In this instance, it would be easier to infer what elements in fact drive a response in phytoplankton. Additionally, we would recommend the incubation be extended past 60 hours. While this amount of time may be sufficient to evaluate smaller phytoplankton cell sizes, slower reproductive rates of diatoms and dinoflagellate species may not have been fully captured.

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CHAPTER 4

Seasonal Harmful Algal Bloom (HAB) Species Dynamics in a Southern California Estuary

Adapted from:

McGuire, C. M., E. Derse Crook, J. K. Moore, S. M. Becker (*In Prep.*). Seasonal Harmful Algal Bloom (HAB) Species Dynamics in a Southern California Estuary

4.1 Introduction

Lagoons, mangroves, and other tidal wetlands provide vital habitat for many species living in coastal environments. Marine fish species commonly dwelling in deeper open water utilize estuaries as breeding and nursery grounds when rearing young (Franco et al., 2008). Estuarine ecosystems help juvenile fish and other organisms reach maturity as they provide protection from many of the dangers present in open water systems. Growing fry also benefit from diverse and ample food sources (Brinda et al., 2010). In addition to the many benefits to the natural environment, estuaries accomplish several ecosystem services that provide for human populations living near the land-sea interface. Examples of such services include provisioning, erosion management, and pollution control (Barbier et al., 2011). Most estuaries are well suited to buffer external inputs when they arrive via natural sources from within the watershed. However, over the past century, human development has been pressuring these systems with increased inputs derived from anthropogenic sources.

Studies suggest that estuarine ecosystems are highly sensitive to accelerated inputs from outside the system (Worm et al., 2006, Halpern et al., 2008). Events like heavy episodic rain and seasonal snowmelt can create conditions of increased streamflow in the waterways that eventually lead to estuaries. The freshwater inputs from these events often deliver additional nutrient species and sediment to the system (turbidity). If the nutrient species coming into the system was previously limited, a subsequent increase in primary production may be expected. Alternatively, increased turbidity can cause a reduction in the transmission of photosynthetically active radiation (PAR). This would impact the amount of energy available from the sun, causing a decrease in primary production (Lin et al., 2009). From the marine environment, seasonal increases in nutrient concentrations can arrive from coastal upwelling, wind driven mixing, and shoreline erosion (Bricker et al., 2008). These coastal marine nutrients can be brought into estuarine system via tidal exchange. Anthropogenic inputs, which have intensified over the past century, have impacted the health of these intertidal waterways by elevating concentrations of many chemical species (Lotze et al., 2006).

Watershed modifications, driven by industrialization and urbanization, have now created many avenues of nutrient enrichment. Anthropogenic nutrients arrive from both point sources (sewage outfalls) and others are non-point sourced (road surfaces). In addition to nutrient chemical species, they often supply other pollutants not previously present in the systems they impact. Major contributing sources from the urban landscape include surface runoff, treated wastewater disposal, and raw sewage discharge (Kennison and Fong, 2014). Heavy and extended accumulation occurring near intensive agriculture often leads to severe nutrient enrichment and in many cases results in highly eutrophic ecosystems (Cloern and Jassby, 2012). Elevated nutrient concentrations in these systems can lead to accelerated rates of algae and cyanobacteria growth. Phytoplankton species capable of rapid reproduction in high nutrient conditions can lead to detrimental effects on other organisms and the overall ecosystem (Codd, 2000).

The Los Peñasquitos Lagoon (LPL), part of Torrey Pines State Reserve in San Diego County, California, may provide valuable insight on the dynamics between anthropogenically derived chemical inputs and phytoplankton species dynamics common in coastal California waterways (figure 1). A state-protected coastal wetland in Southern California, LPL represents a preserved wetland within a highly urbanized watershed. The watershed area covers 95 square miles. Of that, 54% has been developed as residential (27%), transportation (12%), and industrial/commercial/agricultural (15%). The remaining 46% is dedicated to open space or recreational land. Human impacts associated with the watershed and higher trophic level organisms like fish, benthic communities, and vascular aquatic plants have been well studied during previous research (Zedler J.B., 1996). However, very little work has been completed that characterizes planktonic primary producers and their seasonal succession within these waters. Of particular concern are planktonic species capable of producing HABs, many of which are toxin-producing and can impact water quality, posing a threat to the surrounding coastal ecosystem. High concentrations of species like *Alexandrium spp.* and *Pseudo-nitzschia* spp. can result in severe illness and sometimes death for marine mammals that have consumed filter feeders or fish that have accumulated the toxins in their tissue (Benjarano et al., 2008). Ingestion of affected organisms can even harm the human population. Consequently, aquaculture and wild harvest stocks are often unfit for human consumption.

Changes to environmental conditions can impact the partitioning of these harmful species. Previous studies have shown that *Pseudonitzschia* spp., tends to become increasingly abundant when seasonal warming increases water temperature within the euphotic layer of open-water systems (Thorel et al., 2014). Other species that have been identified as harmful have also shown tolerance to the wide ranges of temperature and salinity that commonly occur in intertidal waters (Lundholm et al., 2010; Ou et al., 2015.) Coastal ocean dynamics are likely to be impacted by estuary populations via tidal exchange and growth may be accelerated with elevated temperatures and increased nutrient concentrations that are typically present in urban-impacted estuarine environments.

Figure 1: Map of Los Peñasquitos Lagoon in San Diego County, CA. Station 1 (S1) was chosen to represent the mouth of the estuary. Stations 2 (S2), 3 (S3), and 4 (S4) are representative of mid channel dynamics. Station 5 (S5) is the upper estuary zone.

There are many risks associated with allowing estuarine systems to remain heavily impacted by anthropogenic forcings. Economically, these coastal cities could suffer losses in tourism revenue is poor water quality associated with HAB events causes closures. Beach closures are often issued when decaying biomass causes unpleasant conditions or when toxin production in the water column is deemed unsafe for recreation. Many of the toxins produced by HAB species have yet to have exposure thresholds established for the human population. With climate change influencing coastal water temperatures and the continued urbanization of coastal regions affecting nutrient budgets, it is imperative to evaluate and monitor these communities within estuarine systems.

Comprehensive biogeochemical analysis and phytoplankton community characterization of Los Peñasquitos Lagoon should be consistently conducted and evaluated against previous findings and taxonomic identification from adjacent estuaries (Zedler & Nordby, 1986). Additionally, open-water time series along the Southern California coastline that monitor for these species should also be considered (SCCOOS, 2021). The evaluation of community composition will focus on high concentrations species that produce harmful chemicals, such as *Akashiwo* spp., *Alexandrium* spp., and *Pseudonitzschia* spp. Additionally, baseline characterization of cyanobacteria, such as blue-green algae, will be conducted to determine the overall contribution toward primary production and for the presence of any harmful species in this class. Picoplankton and cyanobacteria have recently been identified as species associated with HAB events. These communities pose a risk to the ecosystem due to accelerated reproduction rates and the capability of some to produce toxins. These smaller classes are important to consider as they have been shown to contribute substantially to primary production in estuarine waters. Research in multiple estuaries throughout the United States have also shown high variability of these communities regarding overall contribution to primary production. A study in Pensacola Florida found that the average contribution to overall chlorophyll concentration of cyanobacteria within a regional estuary was as high as 43% (Murrell & Caffrey, 2005). A higher latitude study determined the average contribution was much less at 7% (Ray et al., 1989). Both studies found that concentrations roughly doubled in the warm summer months, peaking at 90% and 14%, respectively.

Ultimately, the purpose of this study is to determine if coastal estuaries provide refuge systems for HAB species along the Southern California coastline. By analyzing factors associated with biogeochemistry, it will attempt to establish causative relations between environmental variables and bloom dynamics. Sampling this estuary will provide insight on the seasonal succession of primary producers and the distribution between the present hazardous and non-hazardous phytoplankton species. Additionally, it will quantify the contribution that picoeukaryotes and cyanobacteria have towards primary production on a seasonal timescale. The information provided by this study may aid local governments and stakeholders in identifying potential periods of high risk to water quality driven by HABs in and around the region.

4.2 Methods

4.2.1 Sampling Locations

To evaluate the seasonal biogeochemistry and phytoplankton community dynamics of Los Penãsquitos lagoon, monthly surface water samples were collected from five stations (S1, S2, S3, S4, S5) along the salinity gradient moving from the mouth of the estuary (S1) to the surface and sub-surface freshwater inputs further up the system (S5). To maintain consistency, sampling was conducted on dates near the end of each month when early morning coincided with low tide. A mid-channel site (S2) and the uppermost accessible region of the estuary (S5) were selected to leverage real-time data loggers that were previously placed in the system to monitor bottom-water physical parameters (temperature, salinity, pH, dissolved oxygen). At each site, surface conditions were measured using a YSI multiparameter water quality sonde to record temperature, salinity, pH, and dissolved oxygen of surface waters. Following these measurements, 500ml of surface water was collected in polycarbonate bottles and subsequently divided for processing and analysis of chlorophyll and nutrient concentrations, along with phytoplankton and cyanobacteria enumeration.

4.2.2 Chlorophyll-a analysis

To measure chlorophyll-a concentration, 200 ml of water was filtered through an in-line Whatman Glass Fiber Filter (GF/F) with a pore size of 0.7 um. The filters and captured suspended matter were immediately placed on dry ice to prevent sample degradation until analysis. In the laboratory, GF/Fs were thawed, and chlorophyll-a was extracted using 10 mL of 90% (by volume) acetone for 24 hours at -20^{o}C. Once complete and returned to ambient temperature, the extracted chlorophyll-a was measured for raw fluorescence on a Turner Trilogy benchtop fluorometer using a Chl-a non-acidic (NA) cartridge (Venrick & Hayward, 1984). Raw fluorescence was subsequently converted to absolute concentration in mg/L using a linear regression standard based on the Jeffery Method (1997) utilizing cyanobacteria species *Anacystis nidulans* to create chlorophyll standards (Turner Designs).

4.2.3 Nutrient Analysis

To determine nutrient concentrations within each sample, 50 ml of filtrate from the above chlorophyll-a processing was collected and placed on wet ice for lab analysis. Nutrient analysis was conducted by Scripps Institute, University of California, San Diego. Once returned to ambient temperature in the laboratory, samples were passed through a five-channel QuickChem 8500 Series Analyzer (Lachat Instruments) in accordance with manufacturers seawater specifications (Johnson and Petty, 1983; Lachat, 2003). Concentrations of nitrate/nitrite (N+N), orthophosphate (PO4), silicate (SIL), ammonium (NH4) and nitrogen dioxide (NO2) were measured at each location. Values for each of the chemical species were reported in mM.

4.2.4 Microplankton

Larger size-class phytoplankton identification and enumeration was accomplished using a Nikon Eclipse Ti2 inverted light microscope under 200x magnification. Upon collection, samples were stained and fixed using a 1% Lugol's solution and stored in amber bottles to minimize photodegradation. In the laboratory, each sample was gently agitated to homogenize the contents and 50ml was transferred to a settling chamber for a span of

24 hours (Utermöhl, 1958). The settled slides were visually transected and phytoplankton cells were identified to the genus level in accordance with SCB taxonomy publications, utilizing Lund's method of estimating algal numbers (Lund et al., 1958). Species were then binned together as groups of diatoms, dinoflagellates, HAB species (tHAB; as identified by the Southern California Coastal Ocean Observing System (SCCOOS)), and significant HAB species (sigHAB; those which exude chemicals harmful to other biology into the water column).

4.2.5 Picoplankton

To facilitate the enumeration of picoplankton and cyanobacteria, 1 ml aliquots of 4% formalin fixed samples were retained for flow cytometry and stored on dry ice. Once in the laboratory, pico-size classed (<5 mm) organism abundances were analyzed using a Novocyte Benchtop Flow Cytometer (Agilent). 50μL of water was drawn and processed for each of the samples. Populations of picoeukaryotes and *Synechococcus* spp. were binned and enumerated based on cellular concentrations of the light harvesting cells peridinin-chlorophyll-protein (PerCP) and phycoerythrin (PE), using their autofluorescent signatures (Haynes et al., 2016).

4.2.6 Data Analysis

To determine the seasonal dynamics of lagoon biogeochemistry and the phytoplankton community composition, variables were averaged over three-month periods, corresponding to spring (MAM), summer (JJA), fall (SON), and winter (DJF) months. A correlation matrix was generated to determine the strength of single variable drivers on phytoplankton species partitioning. Multiple linear regression (MLR) was performed between each group of phytoplankton and the environmental variables to determine the strength of overall biogeochemical drivers. The resulting p-values from the MLR will be used to determine the effects of individual drivers given all others remain constant.

4.3 Results

4.3.1 Environmental Conditions

Environmental conditions within Los Peñasquitos Lagoon resemble documented seasonal cycles for other estuaries within the region. Water temperature ranged from 51.08°F in February (S5), to 82.58°F in May (S3) (figure 2). Seasonal average temperature was highest in the summer (70.21°F), and lowest in the winter (55.51°F). Dissolved oxygen (DO%) measurements indicate the lagoon surface waters were supersaturated for much of the study period. DO% ranged from 57.23% (October at S5) to 265.12% (May at S3). Seasonal average was highest in winter, at 210.33%, and lowest during summer, at 104.38%. Surface salinity ranged from 0.90 PSU, (December at S1) near freshwater inputs of the upper estuary, to 33.4 PSU (October at S5) at the mouth. Seasonal salinity averages were highest in the fall (27.49 PSU) and lowest in winter (14.09 PSU).

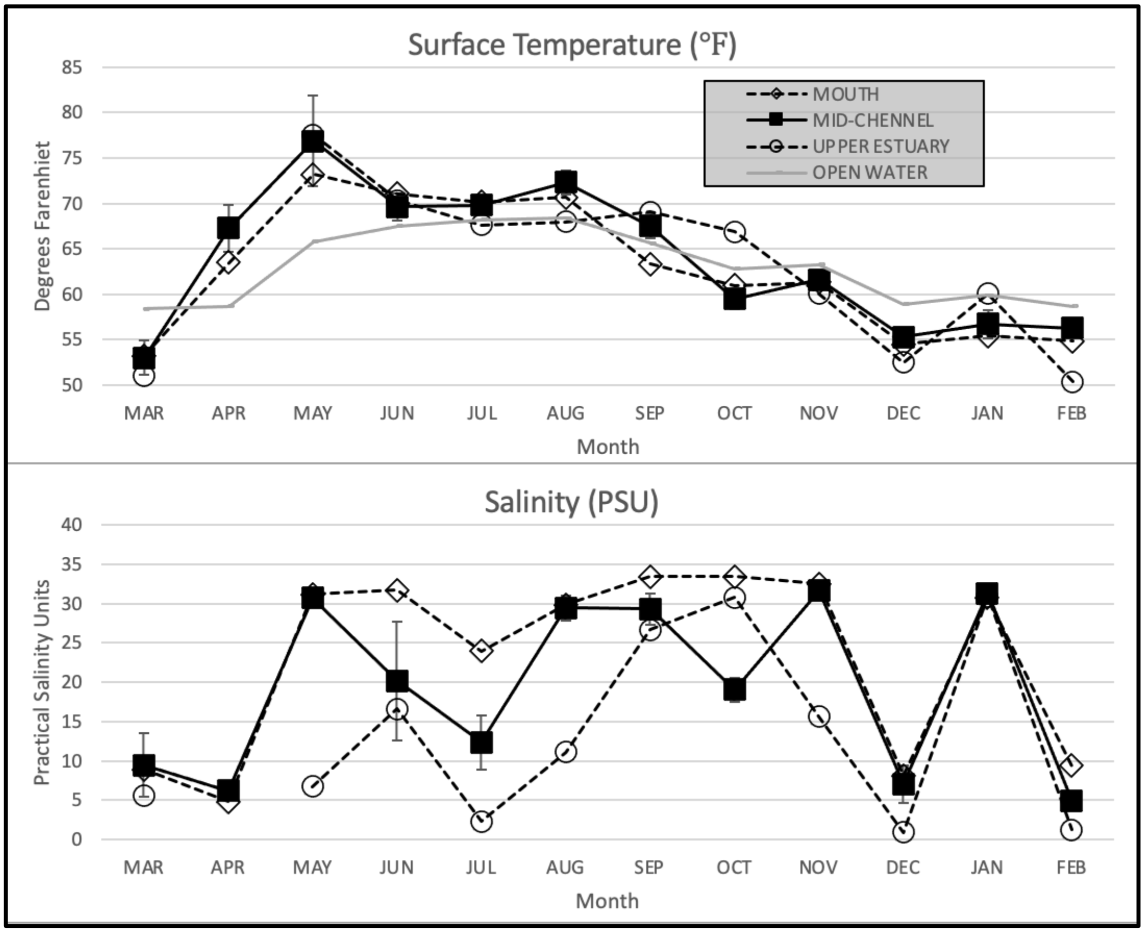


Figure 2: Time series of temperature (°F) and salinity (PSU). The diamond dashed line represents the mouth of Los Peñasquitos Lagoon, the boxed solid line represents the average of the three mid channel sites, and the circled dashed line is the upper estuary sample site. Grey line is monthly average temperature (°F) measured at Scripps Pier.

4.3.2 Chlorophyll-a Analysis

Chlorophyll analysis revealed a seasonal trend that is to be expected for waters within the Southern California Bight. Higher average concentrations were recorded in spring (4.17μg/L) and are consistent with seasonal bloom periods driven by upwelling in nearby coastal waters (figure 3). Minimum average concentration was recorded during the winter at 1.22μg/L. The highest concentrations of chlorophyll were consistently reported in the mid-estuary (S2, S3, S4) sample sites. The maximum chlorophyll concentration for the study was recorded during May at 8.36 μg/L (S3). The lowest concentration was recorded in December at S5 with 0.34 μg/L.

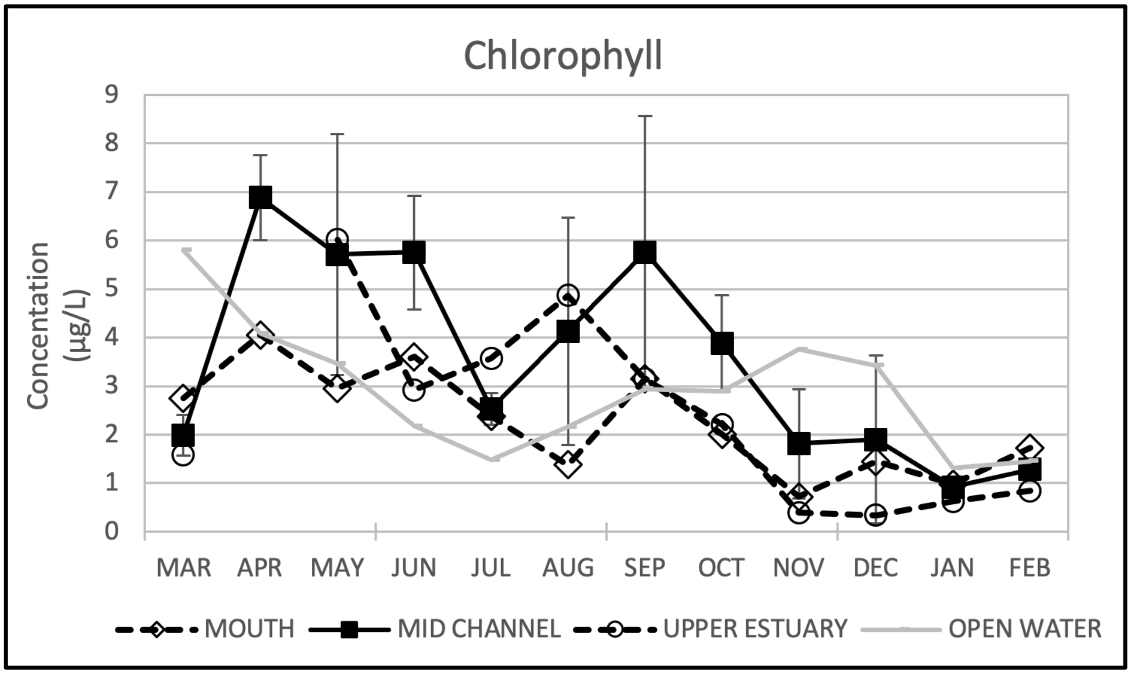
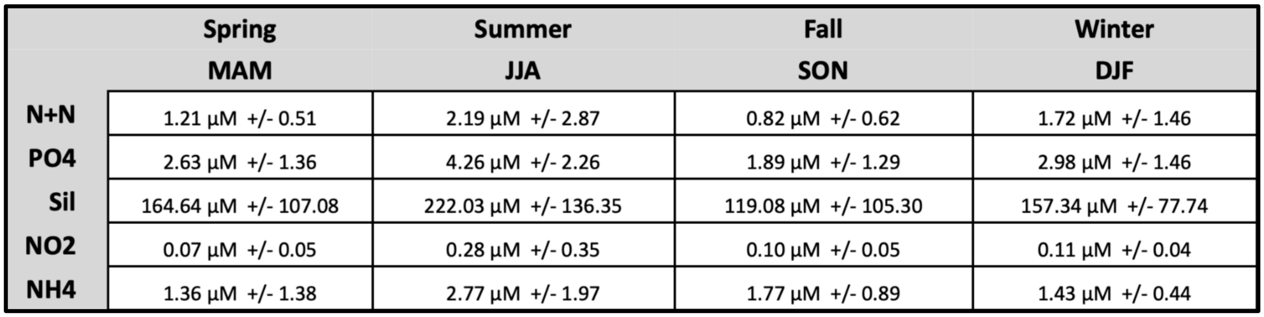


Figure 3: Chlorophyll-a concentration time series comparing single sample plots from the mouth and upper estuary. Mid-channel represents the average of the three mid estuary stations (S2, S3, & S4). Open water line (grey) represents averaged monthly data from Scripps Pier processed by SCCOOS for the period of the study.

4.3.3 Nutrient Analysis

Nutrient analysis averaged over the entire estuary showed consistent seasonal trends among all chemical species. Nitrate/nitrite (N+N) concentration was highest in summer (JJA) months with a total estuary average of 2.19 μM (table 1). Lowest measurements were taken during the fall (SON) with a mean concentration of 0.82 μM. The highest single-site N+N concentration was recorded in July at a mid-channel station (S2) with a value of 9.80 μM, while the lowest concentration of the study was 0.12 μM recorded at the same station during April 2021 collection. In general, nitrate/nitrite concentrations were lowest at the mouth (S1) of LPL and increased as sampling moved toward the upper estuary (S5). This profile reversed when coastal waters experienced reduced stratification and subsequent upwelling during late winter and early spring months, leading to higher concentrations of N+N near the mouth of the estuary that decreased as sampling progressed up the estuary. Phosphate (PO4) concentrations were notably higher within the estuary when compared to seasonal averages of concentrations in nearby open water coastal environments. Similar to N+N, PO4 was highest in summer months with an average of 4.26 μM and lowest in the fall at 1.89 μM. Concentrations were consistently highest in the upper region of the estuary and declined as sampling moved towards the mouth. This trend in the gradient did not change with coastal upwelling as displayed with N+N. The highest single value of PO4 was 8.64 μM; collected in August at the upper-most site (S5). The lowest single concentration was recorded at the mouth (S1) during October sampling at 0.28 μM. Silicate (SIL) was the nutrient species found in the highest abundance throughout the study. Concentrations were remarkably higher in the estuary than compared to measurements taken in nearby open water regions. Measured concentrations ranged from 2.4 μM at the mouth (S1) of the estuary in September, to 448.8 μM in the upper estuary (S5) during July. Silicate concentrations were consistently high in the upper estuary and declined towards the mouth. This would indicate there may be a source of silicate near the upper estuary. Seasonal averages for the estuary were highest in summer (222.03 μM) and lowest in fall (119.08 μM). Nitrogen dioxide (NO2) had the highest average concentration during the summer season with 0.28 μM and the lowest seasonal average in the spring at 0.07 μM. The highest single site concentration was recorded in July with a value of 1.29 μM in a mid-channel station (S2). The lowest recorded value was also recorded mid-channel (S4) in May at 0.02 μM. Ammonium (NH4) ranged from 0.21 μM near the back of the estuary to 8.02 μM at the mouth, with the average of the study being 1.84 μM.



	Spring **MAM**	**Summer** **JJA**	**Fall** **SON**	**Winter** **DJF**
N+N	1.21 μM +/- 0.51	2.19 μM +/- 2.87	0.82 μM +/- 0.62	1.72 μM +/- 1.46
PO4	2.63 μM +/- 1.36	4.26 μM +/- 2.26	1.89 μM +/- 1.29	2.98 μM +/- 1.46
Sil	164.64 μM +/- 107.08	222.03 μM +/- 136.35	119.08 μM +/- 105.30	157.34 μM +/- 77.74
NO2	0.07 μM +/- 0.05	0.28 μM +/- 0.35	0.10 μM +/- 0.05	0.11 μM +/- 0.04
NH4	1.36 μM +/- 1.38	2.77 μM +/- 1.97	1.77 μM +/- 0.89	1.43 μM +/- 0.44

Table 1: Full estuary (S1, S2, S3, S4, S5) seasonal averages of nutrients concentrations.

Nitrogen to phosphorus ratios (N:P) suggests the full estuary system was nitrogen limited for the duration of the study (figure 4). The average N:P ratio across all sites and dates was 1.53:1. Average ratio at the mouth of the estuary was 2.90, while the average in the upper estuary was 0.73. This would suggest a source of phosphate in the upper estuary that is undergoing phytoplankton uptake.

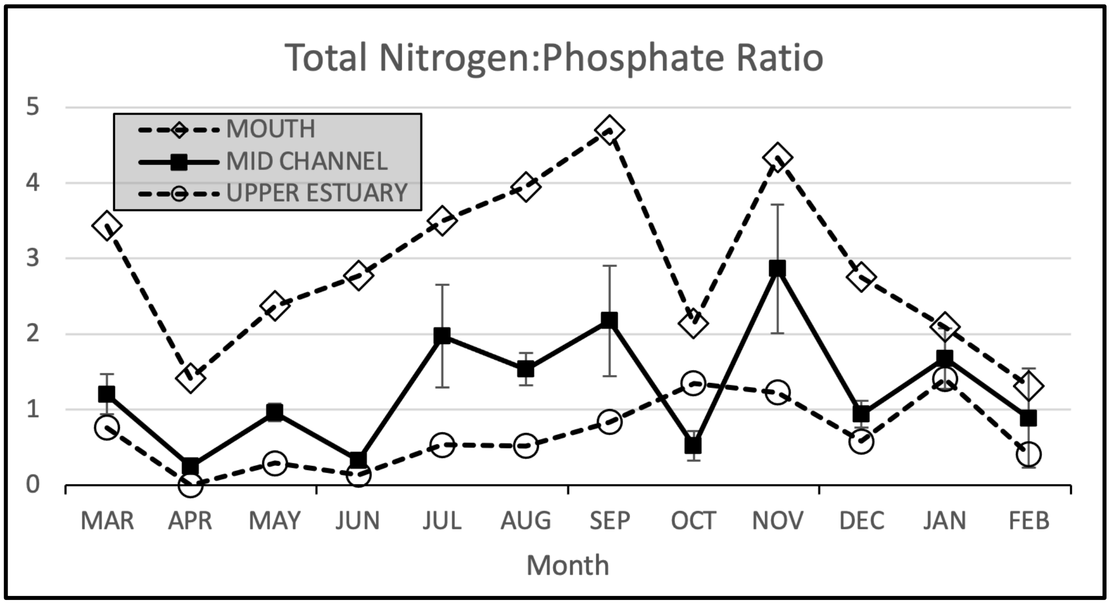


Figure 4: Time series showing total nitrogen to total phosphorus (tN:tP). Hollow diamond with dashed line represents the mouth of the estuary. Black squares with solid line show the average of the three mid channel sites. Empty circles with dashed line are the upper estuary.

4.3.4 Microplankton

Inverted light microscopy revealed the phytoplankton population to be dominated by six diatom species and six dinoflagellate species (figure 5). Abundant diatom genera consisted of *Cylidrotheca* spp., *Melosira* spp., *Navicula* spp., *Nitzschia* spp., *Pleurosigma* spp., and *Pseudo-nitzschia* spp. Of the identified diatom community, only *Pseudonitzschia* spp. is considered a species of concern for harmful algal blooms in the SCB (figure 6). Abundant dinoflagellates included *Akashiwo sanguinea*, *Alexandrium cantenella*, *Ceratium* spp., *Cochlodinium polykrikoides*, *Linguilodinium* spp., and *Prorocentrum* spp. Five of these dinoflagellates have been identified as harmful algal species. *Cochlodinium polykrikoides*, *Linguilodinium* spp., and *Prorocentrum* spp., are often associated with red tide and fish kill events. *Akashiwo sanguinea* and *Alexandrium cantenella* can produce chemical substances that can be harmful to the ecosystem and cause considerable impacts to higher trophic levels when present in high concentrations.

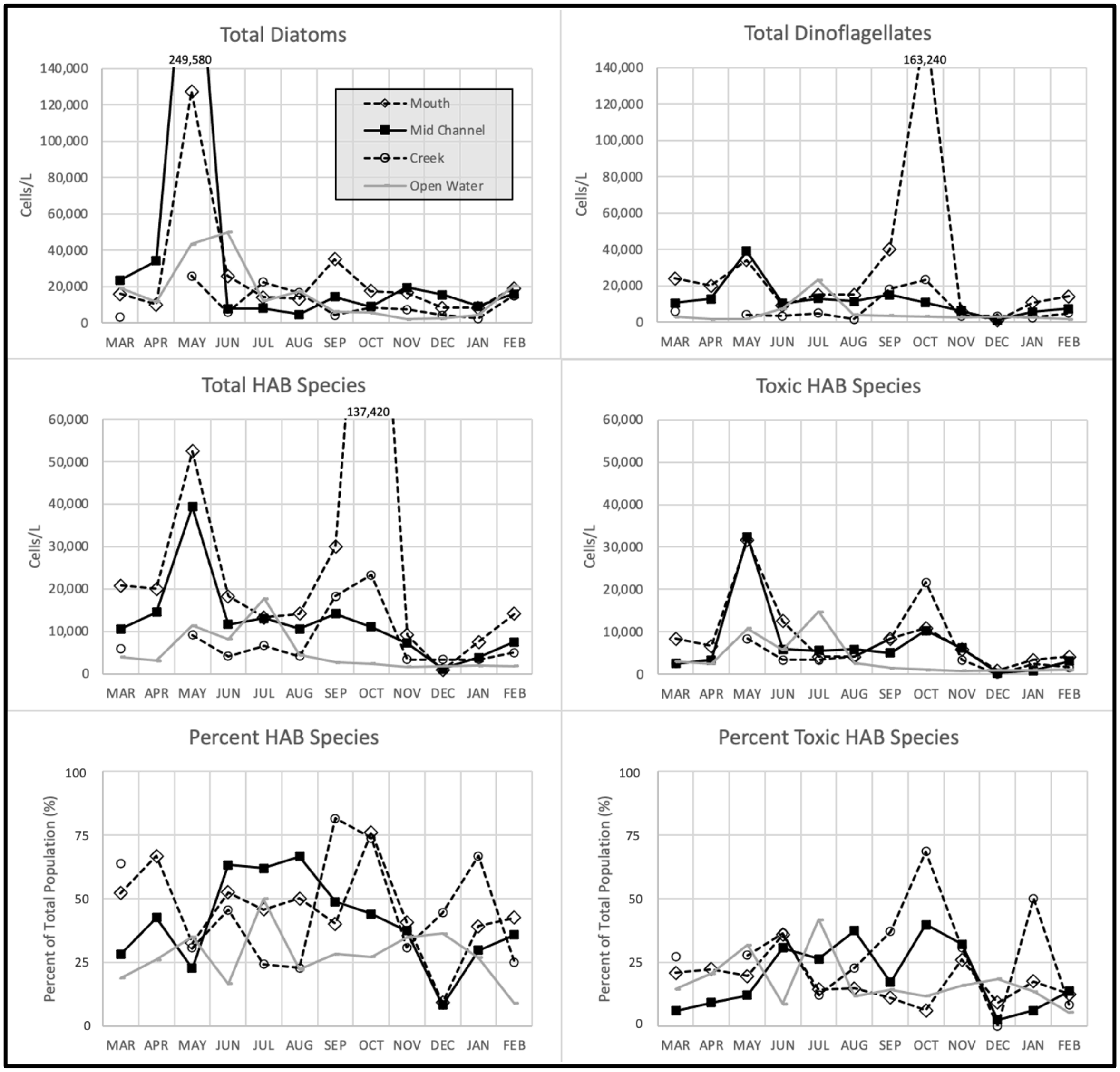


Figure 5: Time series of microscopy cell counts, including total diatoms, total dinoflagellates, total HAB species, and total toxic HAB species for LPL mouth, mid-channel average, and upper estuary creek sites. Open water annual average is included from OC Sanitation station. Percentage of HAB species of total phytoplankton population and percentage of toxic HAB species are also displayed.

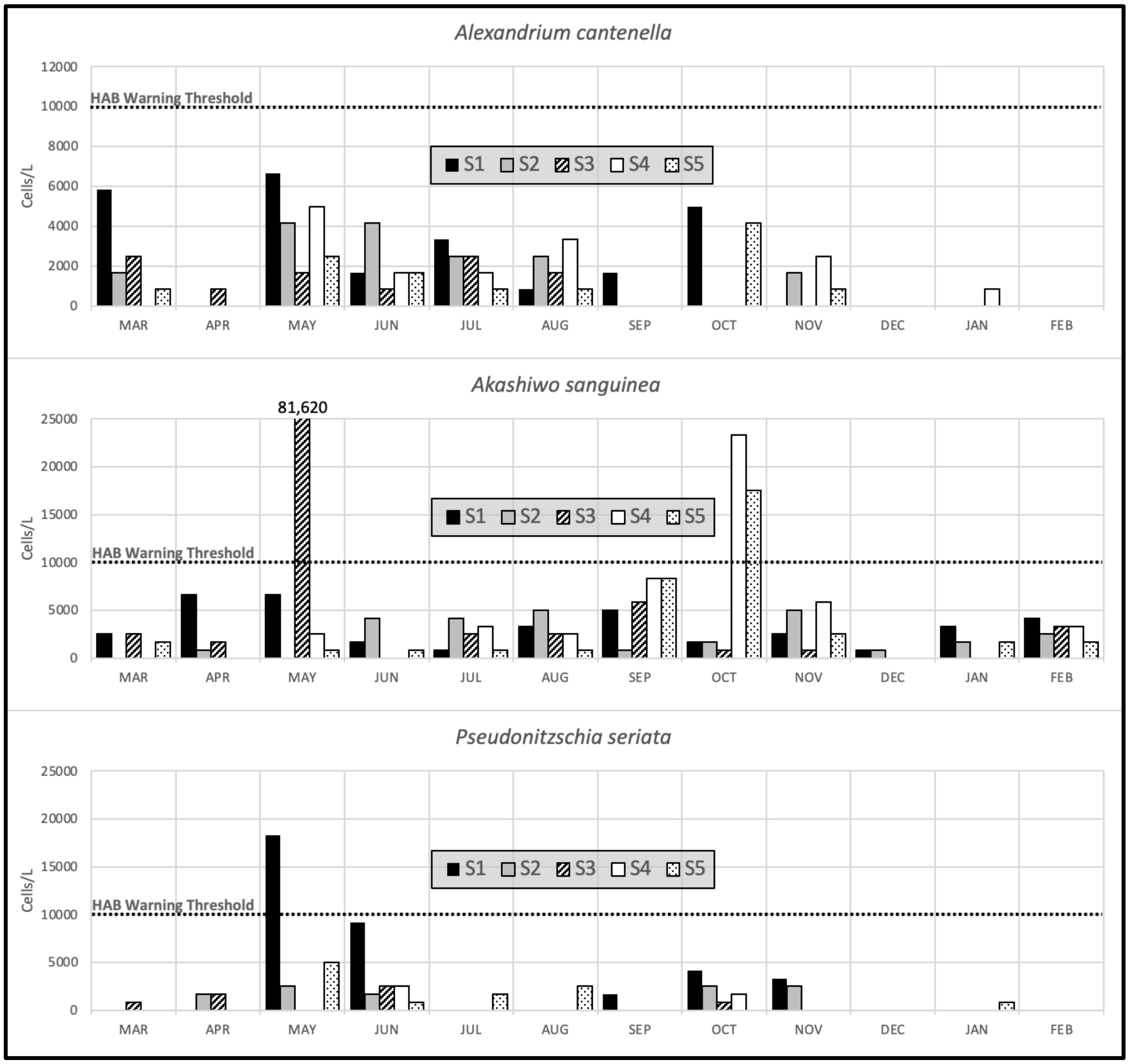


Figure 6: Time series of harmful algal bloom (HAB) species known to produce toxins identified during the study period. Black bars represent the mouth (S1) of the estuary. The gray (S2), diagonal lined (S3), and white (S4) bars represent the mid channel sites. The spotted bar is the upper estuary (S5) site.

4.3.5 Picoplankton

Enumeration of pico-sized organisms reveal there to be 3 distinct populations. Peridinin-chlorophyll-protein (PerCP) excitation and emission revealed there to be two populations (PerCP-1 & PerCP-2) of picoeukaryotes. A population of *Synechococcus* was identified using phycoerythrin (PE) fluorescence signatures. PerCP-1 and PerCP-2 both experienced maximum populations in April, with counts of 698,740 cells/mL (S3) and 173,940 cells/mL (S2), respectively (figure 7). These groups had minimum populations in the upper estuary (S5) during November with counts of 860 cells/mL and 3300 cells/mL, respectively. Synechococcus count was highest in May at the mouth (98,780 cells/mL) and lowest in February in the upper estuary (60 cell/mL). Highest concentrations for all groups were highest in the spring months, but smaller blooms were also recorded in late summer and fall months.

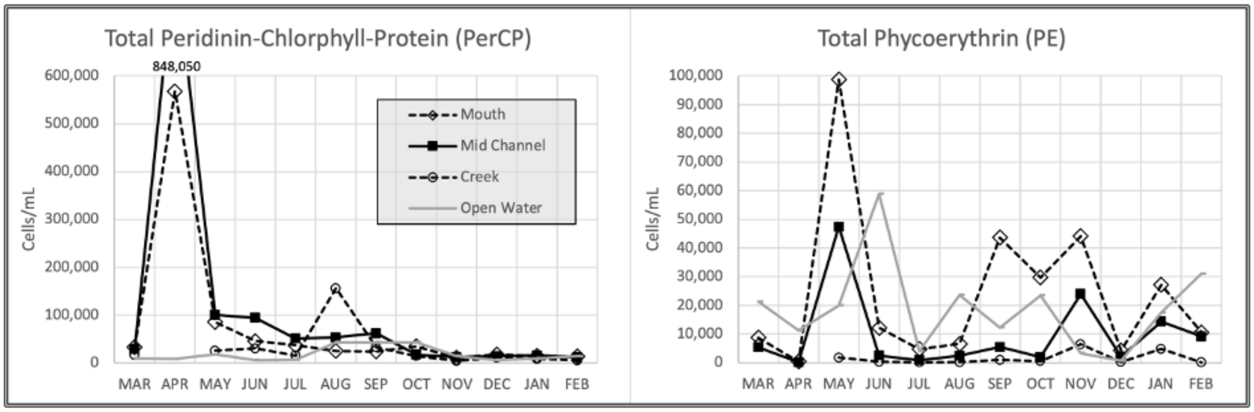


Figure 7: Picoeukaryote and cyanobacteria cell count time series comparing single sample plots from the mouth and upper estuary creek. Mid-channel represents the average of the three mid estuary stations (S2, S3, & S4). Open water represents monthly average measured at Scripps Pier. Picoeukaryotes are binned and identified utilizing PerCP excitation and emission, while PE signifies the presence of *Synechococcus*.

4.3.6 Data Analysis

This study revealed no significant correlation between single variable biogeochemical drivers and community composition of phytoplankton. Multiple linear regression (MLR) analysis of all measured physical parameters combined returned a coefficient of determination (R-squared value) of 0.39, suggesting a weak linear relationship. Of the individual independent variables evaluated for this MLR, temperature and dissolved oxygen (DO%) had the highest significance with p-values of 6.52 10-5 and 0.023, respectively. MLR also indicates a weak relationship between significant HAB (sigHAB) species when analyzed against biogeochemical drivers (R-squared = 0.37). In this regression, only temperature displayed high significance (p-value = 0.0001). Total HAB (tHAB) species has a very weak relationship to drivers with an R-squared of 0.25. Again, temperature was the only significant contributor (p-value = 0.027). When evaluated as a percentage of the total phytoplankton population, both tHAB and sigHAB had weak relationships to combined physical parameters. The tHAB, as a percentage, R-squared value was 0.31 and the sigHAB was 0.33. In these instances, DO% was the only significant driver for tHAB (p-value = 0.033), while salinity was the dominant driver for sigHAB (p-value = 0.003).

4.4 Discussion

Los Peñasquitos Lagoon displayed seasonal cycles of environmental conditions that followed closely with similar bodies of water in the region. Temperatures in the estuary were hottest during the summer season and lowest in the winter. However, when compared to open water seasonal variations, the high and low temperature range is greater in the estuary. This may prove advantageous for species that can adapt to warmer temperatures such as *Pseudonitzschia* spp. and *Akashiwo s*pp., which have demonstrated increased growth rates in warmer waters when compared to other phytoplankton species in the SCB region (Lundholm et al., 2010; Thorel et al., 2014; Ou et al., 2015). However, at the time of this collection, the highly monitored *Pseudonitzschia* spp. only presented a HAB threat on one occasion near the mouth of the estuary. Domoic acid analysis was not conducted during this study, so any impacts of DA production is unclear. Of greater concern during the course study, was the dinoflagellate *Akashiwo spp*., which produces surfactants in the water that can harm fish and bird species. However, no evidence of foaming was apparent on collection days where concentrations exceeded warning thresholds.

Chlorophyll concentrations within the estuary display similar trends to other regional waters as well. The highest concentrations were recorded in spring months, while the lowest concentrations were in winter. Concentrations declined in summer, but moderate increases occurred during the fall. When compared to open water measurements, both of the spikes in chlorophyll occurred roughly one month before similar increases offshore. This may suggest the estuary provides refuge for phytoplankton during seasons of low chlorophyll in open water. The communities that adapt best to the estuary conditions may be best suited as seed stock for open water blooms.

Nutrient concentrations followed a gradient that would be anticipated for a heavily urbanized watershed that is limited by nitrogen. Both phosphate and silicate were consistently highest in the upper estuary, and concentrations tapered moving towards the mouth. Consequently, salinity demonstrated a weak relationship with both PO4 (r-squared = 0.37) and SO4 (r-squared = 0.49). However, no consistent trend was observed in relation to nitrate across the spatial and salinity gradients of the estuary. During times of seasonal upwelling of coastal water, concentrations were the highest near the mouth. When offshore waters became nutrient-depleted, nitrate appeared to arrive from the freshwater sources in the upper estuary.

No strong correlations were identified between the total microplankton population and the independent environmental variables. MLR analysis showed a weak relationship (r-squared = 0.39) between the total phytoplankton population and the combined effect of all environmental variables. Although the correlation to environmental variables was statistically weak, temperature and dissolved oxygen had the strongest influence of them with p-values less than 0.05. Results indicate that as temperature increases in the estuary, phytoplankton populations are likely to experience growth, which in turn causes higher DO% as the more abundant numbers of phytoplankton photosynthesis. As long as nutrients are being provided to the estuary, it can be assumed that these regions will provide sanctuary for phytoplankton communities until seasonal changes favor conditions in open waters.

Picoeukaryote populations identified by PerCP demonstrated substantially higher numbers in the estuary when compared to open water cell count averages. The HAB advisory threshold of 20,000 cells/mL was exceeded at all sites for much of the study. Additionally, the caution threshold of 100,000 cells/mL was exceeded during the study. Counts at the estuary mouth and mid-channel stations were greater than five times the caution limit in April, and the upper-estuary site exceeded the threshold in August. These events were consistently succeeded by blooms of larger size class phytoplankton species in the following months. Based on this relationship, rapid picoeukaryote blooms may be a precursor to larger size classed blooms that often include HAB species, and therefore warrant continued monitoring.

4.5 Conclusion

This study was aimed at evaluating biogeochemical characteristics and phytoplankton community composition in an estuarine ecosystem. Chlorophyll concentrations showed to be higher than open water measures during the warm summer months (JJA) but were lower in colder months (DJF). The most compelling evidence was based on high concentrations of *Akashiwo* spp. in upper estuary waters which may suggest estuaries provide refuge and may play a part in seeding coastal waters with HAB species. Higher variability in temperature and nutrient availability have potential to support select HAB species that have a wider range of tolerances to conditions in the physical environment. Concentrations of HAB species enumerated for this study differed considerably from counts reported at Scripps pier. For this reason, continued monitoring of these species should be conducted in estuaries near urban development. And the added measurement of toxin production would be beneficial in future studies.

The stations selected appeared sufficient to evaluate the spatial gradient in surface waters at the LPL. It is recommended that future studies move beyond surface samples to evaluate the full water column. Dynamics between the surface and benthic environment are likely to differ considerably. Furthermore, it is recommended that collections continue through multiple years. In this way, researchers may be able to identify seasonal and interannual variability in these communities. Lastly, monitoring of HAB toxin production would be valuable in future studies to validate the thresholds determined for the individual species.

4.6 References

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CHAPTER 5

Conclusion

The goal of this dissertation was to fill in knowledge gaps and further understand the dynamics of regional environmental factors within the Southern California Bight (SCB) and how they affect phytoplankton community composition in the area. The study sites were chosen to investigate areas that have been impacted heavily by anthropogenic forcings over the past 100 years. Special consideration was given to harmful algal bloom (HAB) species as they pose a considerable threat to current and future health of marine ecosystems around the globe. The results of this body of work should help build a better understanding of the interaction between coastal urban communities and their adjacent marine ecosystems. Additionally, it might increase temporal resolution of phytoplankton community dynamics in the southern California region. At present, methods for monitoring phytoplankton species level sample surface waters from relatively shallow water pier casts. While this provides valuable insight on dynamics along the coast in the surf zone, it doesn't tell the story of deeper offshore waters or intertidal waterways. Those are the concepts these studies intended to shed light on.

5.1 Summary of Results

5.1.1 Effects of Point-Source Wastewater Effluent on Coastal Phytoplankton Communities in the Southern California Bight

(Chapter 2)

Wastewater effluent is known to contain high concentrations of chemical species that interact with coastal biology. As such, in Chapter 2 we investigated the effluent outfall site operated by the Orange County Sanitation District (OC San). The intent was to determine if nutrient enrichment supplied by wastewater effluent has an impact on the abundance and partitioning of primary producers. Furthermore, species identified to be HAB threats were carefully evaluated as they represent pathways of ecological decline in the SCB.

We determined that physical parameters (temperature, salinity, dissolved oxygen, and nutrient concentrations) and the identified primary producers (diatoms, dinoflagellates, picoeukaryotes, and cyanobacteria) were not significantly different between sites A and B during the collection period. One exception was the variable of colored dissolved organic matter (CDOM). CDOM concentrations were recorded at higher values at site B, suggesting there may be a larger phytoplankton population further away from the effluent. However, this signature was not apparent when evaluating for chlorophyll and the enumeration of individual species. Nutrient concentrations followed similar trends during all seasons, except for spring when concentrations of nitrate/nitrite and phosphate were slightly higher at Site A. Possible drivers for this seasonal spike over the outfall remain unclear, however we speculate that it could be tied the physical dynamics present near the edge of the continental shelf. Similarities between the two sites would suggest that the outfall site has no impact on regional biogeochemistry or phytoplankton community dynamics. Alternatively, it's possible that wastewater effluent has such a large spatial scale impact, that an additional sampling location, further from the effluent, is needed to study the effect of the outfall effectively. The stations chosen for this study were of similar distance to the shoreline and separated by less than three miles. The only factor that differed significantly was the depth of the water column between the two locations. It is possible that mixing across this smaller area was more substantial than expected, and a larger zone may be needed to determine the effects of effluent discharge.

Individual species populations had bloom dynamics that differed considerably from each other. Overall, seasonal phytoplankton community dynamics followed previously observed trends for the SBC. When nutrients were abundant, both pico- and micro-size class cells experienced greater abundance. As nutrients became limited, the smaller celled picoeukaryotes and cyanobacteria made up an increasingly larger proportion of the overall community. This partitioning of small versus large cell size is important for multiple reasons. For example, larger cells are important food stock for consumers in lower trophic levels that are unable to utilize pico-plankton as a food source. Additionally, the larger cells contribute to a more efficient transport of carbon containing cells to deep water.

Microscopy counts showed diatoms dominated micro-class phytoplankton groups throughout much of the study period. However, during the late-fall and early-winter, the diatom dominance was less pronounced. Of the diatoms observed in this study, the HAB forming species of *Pseudonitzschia* spp. was of particular interest. This species was persistent throughout the study, being identified at relatively high concentrations during every cruise sampled. Of the identified *Pseudonitzschia* spp. complexes, *P. delicatissima* dominated the partitioning for most of the study. The HAB threat *P. seriata* became the dominant complex during April and May of both years studied. These seasonal increases are meaningful as they may result in an increased production of domoic acid (DA) when *P. seriata* becomes the dominant complex. During this collection period, there was a low risk of Amnesic Shellfish Poisoning resulting from the relatively low abundance of DA-producing *P. seriata* complex. However, the risk of DA production is constantly present, as this species was identified during each collection.

5.1.2 Phytoplankton Community Response to Changes in Biogeochemistry at a Southern California Endorheic Basin

(Chapter 3)

The Salton Sea is one of the most anthropogenically influenced ecosystems in California. The unintentional formation of the Salton Sea led the site to become the largest lake in the state. Over the past 100 years, the system has shifted from fresh, to brackish, to its current state of hyper salinity; measuring over 60 PSU. The phytoplankton species that have managed to persist in this system are truly a testament to the resilience of primary producers. In Chapter 3 we tested the plasticity of the contemporary phytoplankton community regarding changes in salinity and localized atmospheric deposition.

Based on the composition of trace elements in the dust additions, we expected to see a growth response in samples inoculated with dusts containing high amounts of bioavailable nutrient species, and a toxicity response from additions containing heavy metals. Additionally, it was anticipated that phytoplankton growth rates would increase under dilute conditions and decline under conditions of increased salinity. However, this hypothesis was not supported by the data.

Overall, the highest increase in chlorophyll, and phytoplankton abundance, was experienced when present conditions were maintained within the experimental control. Chlorophyll-a concentrations suggest there was a toxicity effect resulting from most of the treatments (excluding D1L, D2H, and Dil10) when compared to the experimental control. Additionally, there was no discernable trend when evaluating the difference between high and low deposition rates of the dust treatments. This suggests that there was little effect on growth rates driven by the constituents of the dust samples. Salinity manipulations indicate consistent increases in chlorophyll concentration throughout the duration of the incubation for all but the 5% salinity increase (Sal5). This would suggest that the current assemblage of phytoplankton, while capable of adapting to continued changes in biogeochemistry at the site, is best suited when conditions are left at contemporary background levels.

Enumeration of the micro-size class phytoplankton revealed a toxicity effect on most species resulting from dust and salinity manipulations. One exception was the diatom *Nitzschia spp.* This species experienced a higher growth rate in most dust treatments when compared to the experimental control. This may suggest that of the larger sized class planktonic species, *Nitzschia spp.* may become more competitive, and increasingly abundant, as the local environment continues to change. This collection revealed much higher numbers of diatoms than had been found during previous investigations of this location. This might suggest that there has been an ecological shift towards diatoms at the

Salton Sea. Although it is also plausible that there was a seasonal or episodic bloom occurring at the time of collection.

Picoeukaryote growth rates favored all changing conditions over the experimental control at the final time point. The 5% increase in salinity (Sal5) resulted in the highest increase in picoeukaryote abundance. These results suggest that the current assemblage of picoplankton found at the Salton Sea can efficiently adapt to rapid and continued changes in water chemistry. However, it is important to note that other forcings may have contributed to this increase in abundance. With the possibility that temperatures inside the incubation bottles differed from that of the collection site, the picoeukaryotes may have benefited. Studies have shown that picoeukaryote growth rates increase with temperature (Chen et al., 2014). In either case, it suggests that rapid changes may favor the growth of the smaller picoeukaryote species at the Salton Sea. The shift to smaller planktonic classes could have implications that affect nutrient cycling, carbon uptake, and trophic transfer efficiency at this location.

5.1.3 Seasonal Harmful Algal Bloom (HAB) Species Dynamics in a Southern California Estuary

(Chapter 4)

Estuaries perform many important ecosystem services throughout the coastlines of the world. Human development along the Southern California coast over the past century have impacted many of the systems located within the boundaries of the SCB. Climate change is projected to further influence many changes in the global oceans. Some anticipated trends are increased temperatures, decreased pH, and continued anthropogenic nutrient enrichment. As such, it is imperative that the scientific community monitor phytoplankton community dynamics along both pristine and urbanized coastal waterways.

In this study, we showed that seasonal cycles of environmental conditions in Los Peñasquitos Lagoon followed closely with similar ecosystems in the SCB region. Temperatures in the estuary were hottest during the summer season and lowest in the winter. However, when compared to open water seasonal variations, the high and low temperature range is greater in the estuary, meaning the estuary was warmer than open water in summer, and colder than open water in winter. This may prove advantageous for species capable of rapidly adapting to warmer temperatures. Examples of regional species that have demonstrated this ability include *Pseudonitzschia* spp. and *Akashiwo s*pp. (Lundholm et al., 2010; Thorel et al., 2014; Ou et al., 2017).

Chlorophyll concentrations within the estuary display similar trends to other regional waters as well. The highest concentrations were recorded in spring months, while the lowest concentrations were in winter. Concentrations declined in summer, but moderate increases occurred during the fall. When compared to open water measurements, both spikes in chlorophyll occurred roughly one month before similar increases offshore. This may suggest the estuary provides refuge for phytoplankton during seasons of low chlorophyll in open water. The communities that adapt best to the estuary conditions may be best suited as seed stock for open water blooms.

Nutrient concentrations followed a gradient that would be anticipated for a heavily urbanized watershed that is limited by nitrogen. Both phosphate and silicate were consistently highest in the upper estuary, and concentrations tapered moving towards the mouth. Consequently, salinity demonstrated a weak relationship with both PO4 and SO4. However, nitrate remained low throughout the study and no consistent trends were observed across the spatial and salinity gradients of the estuary. During times of seasonal upwelling of coastal water, nitrate concentrations were the highest near the mouth. When offshore waters became nutrient-depleted, nitrate appeared to arrive from freshwater sources in the upper estuary.

No strong correlations were identified between the total microplankton population and the independent environmental variables. MLR analysis showed a weak relationship between the total phytoplankton population and the combined effect of all environmental variables. Results indicate that as temperature increases in the estuary, phytoplankton populations are likely to experience growth, which in turn causes higher DO% as the more abundant numbers of phytoplankton perform photosynthesis. If nutrients are being provided to the estuary, it can be assumed that these regions will provide sanctuary for phytoplankton communities until seasonal changes favor growth in open waters.

Throughout the duration of this study HAB species were consistently present, but rarely in concentrations high enough for local governments to prompt action. The regionally monitored diatom population of *P. seriata*. only presented a HAB threat on one occasion near the mouth of the estuary. The dinoflagellate *A. sanguinea*, which produces surfactants in the water column that can harm fish and birds, was found at much higher abundance during each of the monthly collections. This species exceeded the HAB warning threshold on two separate occasions. The first exceedance was recorded in May and was 8x over the threshold limit. Another exceedance occurred at two stations during the October collection. In both instances, the exceedances were recorded at sites further up the estuary gradient. This could suggest that *A. sanguinea* utilizes estuaries and intertidal waters as refuge sites and subsequently provide seed stocks to open waters when conditions allow.

Picoeukaryote populations demonstrated substantially higher numbers in the estuary when compared to open water cell count averages. The HAB advisory threshold was exceeded at all sites for much of the study. Additionally, the more concerning caution threshold was exceeded several times. Counts at the estuary mouth and mid-channel stations were greater than five times the caution limit in April, and the upper-estuary site exceeded the threshold in August. These events were consistently succeeded by blooms of dinoflagellates and diatoms class in the months that followed. Consequently, rapid picoeukaryote blooms in estuarine systems might offer a biomarker indicator for HAB warnings associated to blooms of the larger class in coastal waters. Continued monitoring of this potential relationship may be warranted in estuarine ecosystems.

5.2 Future Research Directions

As waters off the California coast continue to experience the impacts of global climate change in tandem with localized human influence, the population dynamics between SCB phytoplankton assemblages will likely shift as new equilibrium states are achieved. With the threat of harmful algal blooms becoming ever more present, it is imperative that we study these organisms in every possible environment they may exist in. In coastal California waters, it is forecasted that some HAB species will have a competitive advantage within the overall phytoplankton community. Recent work has shown that certain domoic acid producing *Pseudonitschia* complexes experience increased growth rates with warmer waters and/or nutrient replete conditions. The HAB species of *Alexandrium* spp. and *Akashiwo s*pp. have also shown tolerance to a wide range of temperature and salinity in lab incubations. Trends of increased intensity of certain HAB species have been observed in the SCB over the past several decades (Anderson et al., 2021). In part, this could be due to better and more consistent reporting. However, other contributing factors include invasive HAB species, newly identified species and toxins, or other stimulatory anthropogenic effects such as global warming or nutrient enrichment. Continued monitoring of these species and the environmental factors that influence should be conducted in all coastal regimes. Currently, monitoring is mainly conducted from surf zones and from pier sampling along the coast. More research should be conducted on how these species interact in and look for possible seeding sources such as deeper water and estuary systems, combined with a better understanding of the range of effluent influence. This combined effort may help to ensure a safe and clean coastal environment for decades to come.

5.3 Concluding Remarks

Overall, these studies achieved the goal of characterizing the phytoplankton communities in their respective regions. Both monthly monitoring experiments could have been strengthened by utilizing longer sampling intervals. While we were able to witness seasonal dynamics for the years evaluated, the data would be much more relevant if we could capture interannual variability. Furthermore, these studies would have benefited from the analysis of toxins produced by Southern California HAB species. This would have provided insight on when these chemicals are produced. In many cases, these species can be identified in high abundance in the water column with no indications of toxin production. At other times, there have been relatively low abundance of cells, with high concentrations of toxins simultaneously. It is important that researchers continue to investigate the ecological functions of these substances along with their mechanisms of production.

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