

Phytoplankton Biological Community Assessment: Case Study at the Ballona Wetlands Ecological Reserve



Vanessa De Anda
Jessie Jaeger
Jacquelyn Lam
Kristine Leon
Mahsa Ostowari
Ellisa Soberon
Anthony Noren

Advisor: Dr. Rebecca Shipe
Client: Santa Monica Bay Restoration Commission
Client Advisor and Contact: Karina Johnston

June 2014

UCLA Institute of the Environment and Sustainability • Environmental Science Practicum

ABSTRACT.....2

INTRODUCTION2

FACTORS AFFECTING PHYTOPLANKTON ABUNDANCE.....3

HARMFUL ALGAL BLOOMS (HABs).....5

PHYSICAL OCEANOGRAPHY OF THE CA COAST.....6

PHYTOPLANKTON: INDICATORS OF ECOSYSTEM HEALTH.....7

PROJECT OBJECTIVES.....7

METHODS.....7

 Survey Locations.....7

 Sample Collection.....8

 Preparation and Analysis.....8

 Phytoplankton Identification.....9

 Statistical Analysis.....10

RESULTS AND DISCUSSION.....11

 Abiotic Factors.....11

 Phytoplankton Community.....13

 Harmful Phytoplankton Species.....20

APPLICATIONS TO RESTORATION.....20

CONCLUSION.....21

LITERATURE CITED.....22

APPENDIX A: PHYTOPLANKTON LIST.....25

APPENDIX B: PHYTOPLANKTON IDENTIFICATION GUIDE.....26

APPENDIX C: PCA RESULTS.....41

APPENDIX D: RAW DATASeparate Excel Spreadsheet

ABSTRACT

The Ballona Wetlands is one of the few remaining wetland areas within the coastal southern California region that is located in the heavily developed metropolitan Los Angeles area. Plans are underway to restore many of the ecosystem resources and native diversity of the system. Determining the composition of the phytoplankton community within the Ballona Wetlands Ecological Reserve (BWER) is important as they play an integral role as primary producers in the aquatic regions of the wetlands. Furthermore, certain species of phytoplankton blooms can pose serious environmental issues to local ecosystems; Harmful Algal Blooms (HABs) can release toxins, cause hypoxic conditions or clog feeding structures of organisms in higher trophic levels. This study involved the collection of water samples at three locations within the BWER (the east channel, the west channel, and the creek) and measurements of five abiotic factors including pH, temperature, dissolved oxygen (DO), salinity, and tidal height over a 24 hour period. Results indicate that the water across all sample locations was generally saline, with pennate and centric diatoms as the two dominating groups of phytoplankton. Similar communities and proportions of dominant species were found in all three sites, though phytoplankton abundances changed dramatically in all locations over the 24-hour period. A total of 53 genera of phytoplankton were identified in the BWER with *Navicula*, *Nitzschia*, *Melosira*, and *Pleurosigma* as the dominant genera. Seven of the 53 genera were identified as harmful. In the west channel and the creek, highest abundances of harmful genera were found in oceanic conditions, and the most dominant harmful genus present was *Prorocentrum*. The harmful genus, *Alexandrium*, dominated the east channel. This data may aid the SMBRC in future restoration efforts within the BWER to strengthen ecosystem services and health.

INTRODUCTION

Estuaries support the complex interactions of a food web consisting of both aquatic and terrestrial organisms, such as benthic macroinvertebrates, fish, and birds. These ecosystems are intrinsically variable as they are characterized by the constant changes in abiotic conditions including inundation, salinity, and temperature. Unfortunately, many of California's estuaries have been detrimentally affected by urbanization, causing environmental stresses on the ecosystem. This can result in changes in water flow, flow constriction, sediment deposition, and decreased wetland area. Our project area within the western tidal channels of the Ballona Wetlands Ecological Reserve (BWER) is representative of such an impacted environment.

Accounting for over half of our global primary productivity, phytoplankton form the base of many marine food webs. In estuaries, phytoplankton play an integral role and can be used as indicators of ecosystem health (US EPA 2005). Estuarine phytoplankton have a wide range of characteristics, functions and optimal environmental range allowing them to adapt to different habitats (Cloern & Dufford 2005). Further, phytoplankton blooms can also pose serious environmental issues as during harmful algal blooms (HABs). For these reasons, it is useful to identify the organisms present in a system as well as to understand the environmental factors that affect phytoplankton abundance and distribution.

The BWER was once a thriving set of coastal estuarine habitats that covered over 2,000 acres (Tsihrintzis 1996, Dark et al. 2011). Today, the size of the BWER has been reduced to roughly 600 acres, with only approximately 15 acres of tidally influenced estuarine habitat, several contiguous areas of seasonal (non-tidal) wetlands, and degraded upland habitats. This decrease in size and quality of the BWER is a direct result of years of urbanization in the Los Angeles Basin, including anthropogenic activities such as concrete channelization, fill placement, oil and gas extraction, agriculture, and housing developments (Tsihrintzis 1996, Dark et al. 2011). Ballona Creek, which used to flow through the wetland, was turned into a flood control channel due to major floods in the 1930s (Tsihrintzis 1996, Dark et al. 2011). The channelization of the Creek involved the creation of concrete levees, redirection of water inputs, and restricted tidal flows, which further contributed to the wetland's degradation.

Located within the alluvial floodplain of the LA River, the BWER touches Los Angeles' western edge, is north of LAX, south of Marina del Rey, and east of Culver City (Zedler, 2001). Ballona is comprised of three primary sections: Area A, B, and C (Johnston 2012). This partitioning of the wetlands is a result of bisecting streets including Culver Boulevard, Jefferson Boulevard, Lincoln Boulevard, and Gas Company access roads. The BWER's surrounding area is comprised of mostly urbanized land, which makes for a more challenging project in terms of restoration and infrastructure limitations. The western portion of the BWER consists of two connected intertidal estuarine channels. The curved u-shape channel pattern also has an offshoot branch channel running underneath Culver Blvd in Playa Del Rey, where freshwater runoff enters the wetlands from the surrounding residential areas. The two channels also connect to Ballona Creek, through two self-regulating tide gates on the north side of the wetlands. The tide gates are the main source of inflow and outflow of seawater in the BWER due to tidal variations, while freshwater runoff becomes a significant contributor during rainstorms. The west tide gate has a narrower, shallower channel and is a flap gate that only allows water to exit the wetlands. Ballona Creek is channelized by concrete levees, which separate it from the wetlands to the south and Marina del Rey to the north (Johnston 2012).

Taxonomic identities of the phytoplankton community of the BWER is still unknown. Therefore, we established a baseline composition of the phytoplankton community in the BWER in this project. We also sampled five specific abiotic factors including pH, dissolved oxygen (DO), salinity, temperature, and tidal height in order to assess their contributions to the phytoplankton composition and abundances.

Factors Affecting Phytoplankton Abundance

1. TIDAL INFLUENCE

Our project area focuses on the two, directly tidally influenced, channelized regions located in the northwest region of the Ballona Wetlands Ecological Reserve (BWER). The BWER, however, receives low tidal influence as a whole. The two self-regulating tide gates create a muted flow, causing low inundation across the tidal habitats (Johnston 2011). Only about 15 acres of the 600 acre BWER is directly tidally influenced, with the largest proportion occurring within the channels themselves. The wetland's main source of fresh water comes from dry and wet weather runoff from the surrounding communities such as Playa Vista, Westchester, and the Playa Del Rey Bluffs regions (Tsihrintzis 1996,

Dark et al. 2011). The majority of the wetlands, located near our project area of the BWER, do not exhibit the full mixed semi-diurnal tidal regime that other southern California estuarine ecosystems exhibit (Johnston 2011). Instead they mimic the hydrological characteristics of a non-tidal saline wetland, or seasonal wetland (Johnston 2011). The mean tidal range is 3.81 ft, and the diurnal tidal range is 5.49 ft (Johnston 2011).

This unique tidal regime may have consequences abiotically, which in turn may affect the make-up of the phytoplankton community, both directly and indirectly. For example, the tidal influence may serve as a driver for changes in abiotic factors, such as dissolved oxygen (DO). For instance, a less intense tidal regime may limit estuarine mixing, thus decreasing DO within the estuary and/or creating a more saline habitat than fully mixed estuaries. This higher saline environment, in turn, could result in a primarily marine phytoplankton community.

II. SALINITY

In channel sites, salinity within the water ranged from 0ppt to 35ppt, where average salinities were higher in areas closest to Ballona Creek and lower in areas further away from Ballona Creek (Boland & Zedler, 1991). Due to the low precipitation inputs characteristic of the Mediterranean climate, the soils may vary in salinity concentrations throughout the year (Philip Williams & Associates 2006). In turn, the distributions of plants within the wetlands are directly affected by the concentration of salinity in the soil (Philip Williams & Associates 2006). Due to the relatively low freshwater inputs and low tidal influence, estuarine water conditions are more likely to be more saline. This could result in the elimination of less tolerant species that cannot thrive in highly saline waters. Furthermore, these conditions could favor a marine phytoplankton community composition (Philip Williams & Associates 2006).

III. TEMPERATURE

Water temperature may also influence the composition of phytoplankton primary producers. California is a temperate or Mediterranean region characterized by dry summers with highs at about 80 °F with little precipitation (Cole et al. 1995). Water temperatures follow a similar pattern, with higher temperatures in the summer and lower temperatures in the spring and fall (Domingues et al. 2005). The direct effect of temperature on phytoplankton abundances is difficult to isolate. For instance, a study done by Cloern (1982) found that increasing summer surface water temperatures seem to correlate with increasing concentrations of chlorophyll a (an indicator of phytoplankton biomass). However, this increase in phytoplankton abundances is caused by the high incident radiation that accompanies the summer seasons, and is not solely due to increasing water temperatures (Cloern 1982).

With that being said, phytoplankton abundances can be directly influenced by variations in water temperature (Cebrian et al. 2001). Since phytoplankton do not regulate their internal body temperatures, variations in water temperature directly affect the phytoplankton's metabolic activity (Toseland et al. 2013). Although still debated, recent data have shown a decline of global phytoplankton abundances within the last century, due to increasing water temperatures (Toseland et al. 2013). Under high water temperatures, it is found that more resources are invested into photosynthesis (Toseland et al. 2013). This could lead to a decrease in phytoplankton abundances because climatic increases in water

temperature occur independently of light and nutrient availability. Therefore higher temperatures increase the rate of photosynthetic activity undergone by phytoplankton; however, there is not a sufficient amount of nutrients and/or light to support the increased metabolic activity, which can cause the organisms to essentially “starve” and die. Phytoplankton sensitivity to temperature varies from species to species; they express a range of temperature tolerances that is also linked to other factors, such as light and nutrient availability.

IV. DISSOLVED OXYGEN (DO)

In general, dissolved oxygen (DO) levels are dependent on the balance of respiration and photosynthetic activities occurring in aquatic systems (Smith 1988). Intermediate phytoplankton abundances result in the highest levels of dissolved oxygen within the water, (Smith 1988) whereas an overabundance of phytoplankton would result in limitation of nutrients and/or light, causing the organisms to die off. Extremely low phytoplankton abundances also result in lower DO levels simply as a consequence of low photosynthetic rates (Smith 1988). Without primary producers, simple mixing of the water column would not supply enough DO to support the many organisms that rely on oxygen for respiration.

V. pH

Changes and differences in pH can affect phytoplankton abundances and growth in a number of ways. These effects include changes in carbon availability, variation in species distributions, and changes in the availability of trace metals and nutrients (Chen & Durbin 1994). Extreme pH has the potential to cause direct physiological changes to the phytoplankton community (Chen & Durbin 1994). It is still not well known if increased CO₂ concentrations (more acidic conditions) will stimulate, inhibit, or have no effect on net community phytoplankton productivity (Berge et al. 2010). It is possible that increased CO₂ availability will increase phytoplankton primary production because dissolved CO₂ is currently the limiting supply for RUBISCO, an enzyme responsible for carbon fixation within photosynthesis (Beardall & Raven 2004, Berge et al. 2010). By the end of this century, oceanic pH is predicted to decrease and become increasingly acidic by ~ 0.4 units to ~7.8 (Berge et al. 2010). In other words, there will be an ~150% increase in hydrogen ions (H⁺), which could affect intracellular pH, membrane potential, energy distribution, and enzyme activity (Beardall & Raven 2004, Riebesell 2004, Giordano et al. 2005). In a study done by Berge et al. (2010), it was observed that marine phytoplankton exhibit no changes in cell growth and primary productivity when subjected to a pH range of ~7.8 to 8.5. In this same study, it was found that the lowest pH limit in which positive growth rates of marine phytoplankton is observed is ~6.0. The maximum pH limit, a number that is well studied, is ~9.0; however, there is a maximum pH tolerance limit that varies from species to species (Hansen 2002). Thus, pH clearly has an effect on phytoplankton community composition.

Harmful Algal Blooms (HABs)

The Southern California Coastal Ocean Observing System (SCCOOS) reports that the HAB species found along the Californian coastline include: the dinoflagellates *Akashiwo sanguinea*,

Alexandrium spp., *Cochlodinium spp.*, *Dinophysis spp.* and *Lingulodinium polyedrum* and the diatoms *Phaeocystis spp.* and *Pseudo-nitzschia spp.* These species are hazardous to ecosystems through two general mechanisms. They may produce toxic compounds such as brevetoxin, ciguatoxin, domoic acid, saxitoxin, domoic acids, and surfactants. Alternately or additionally, bloom formation can reduce dissolved oxygen concentrations and can lead to hypoxic conditions (Van Dolah, 2000). Harmful algal blooms affect organisms that feed on phytoplankton, such as birds and mammals (including humans), because the toxic compounds bioaccumulate up the food chain. For example, dinoflagellate genera *Alexandrium*, *Gymnodinium*, and *Pyrodinium*, that are usually associated with toxic outbreaks along the North American west coast, release a combination of biotoxins, collectively called paralytic shellfish toxins (PSTs) (Lewitus et al. 2012). Contaminated shellfish species along the west coast include mussel species, butter clams, geoduck clams, razor clams, and Pacific oysters, all of which are eaten by aquatic mammals and humans (Lewitus et al. 2012). Toxin production is still not fully understood, but several studies indicate that it can be influenced by the ratio of nutrient availability. Graneli et al. (2011) found that domoic acid production by *Pseudo-nitzschia spp.* varied with deficiencies of phosphate, silicic acid, and nitrate. Domoic acid production has also been linked to iron and copper stresses (Graneli et al., 2011) and lithium, selenium, and nickel variability (Cochlan et al., 2010).

HABs commonly occur in estuaries in the Northeast, Mid-Atlantic, and Pacific Northwest regions of the United States. However, there are fewer observations of HABs in US west coast estuaries with some exceptions, such as the presence of microcystis cyanobacteria in the San Francisco Bay estuary (Lehman et al. 2005). In any case, there is a potential for estuaries to serve as pools for “cultures” of harmful algal taxa, or even serve as a refuge for small abundances or resting spores of harmful algal taxa.

Due to the physical properties of the California coast, HAB species have the ability to propagate through upwelling events and strong winds, allowing their toxins to spread to other regions. Almeda-Juaregui et al. 2013 and the Southern California Coastal Ocean Observing System (SCCOOS) confirmed the spread and maintenance of HAB dinoflagellate, *Lingulodinium polyedrum* along the California coastline by wind drift. Climate variability due to anthropogenic sources may also stimulate HABs. Flewelling et al. (2013) experimentally observed that *Alexandrium catenella* produced more toxins with higher levels of CO₂, less phosphorus, and lower temperatures.

Physical Oceanography of the California Coast

The California Current originates from British Columbia and flows southward along the western North American coastline and ends at the Southern coast of Baja California (NOAA 2011). Along the eastern gyre boundaries in the Northern Hemisphere, northern winds veer to the right, which prompts Ekman transport of surface water offshore (NOAA 2011). As a result, upwelling occurs; cold, nutrient-rich water from below replaces the south-flowing water. Strong seasonal upwelling typically occurs from March to September. It is from this upwelling that phytoplankton (including HABs) are able to receive the nutrients needed to support growth.

There are many ecological benefits of surface blown wind drift (SBWD) on phytoplankton abundances. Phytoplankton populations will be in a less turbulent environment during cell division, which is particularly important for *L. polyedrum*, since shear flow has been found to impede cell

division. Moving shoreward is ecologically beneficial to phytoplankton species because there are more nutrients near the coast. Organisms that reach sediments will have more nutrients available, so moving towards the coast will decrease the depth required for species to access sediments and the depth to overcome to access light upon vertical ascension. Thus, shoreward wind transfer helps blooms to propagate by providing favorable environments (Almeda-Juaregui et al. 2013).

Phytoplankton: Indicators of Ecosystem Health

Phytoplankton community composition and abundances can be used as early indicators of ecosystem health. Phytoplankton play major roles in estuarine ecosystem processes, such as eutrophication, nutrient cycling, water quality, and food web dynamics (US EPA 2005). They are the major primary producers in estuarine ecosystems and are sensitive to the slightest environmental disturbances. Due to this sensitivity, changes in the phytoplankton community can serve as an early warning sign for declines in estuarine ecosystem health. For example, researchers at the University of North Carolina's Institute of Marine Sciences use chlorophyll *a* concentrations as a method to determine total maximum daily nutrient loads (TMDLs) (US EPA 2005). Additionally, in order to determine phytoplankton community responses to anthropogenic and natural environmental disturbances such as hurricanes, droughts, nutrient runoff, and pollution, diagnostic photopigments (chlorophylls and carotenoids) unique to specific phytoplankton genera, are monitored. Furthermore, initial presence of invasive and toxic phytoplankton species such as HABs, can be detected in their early colonization stages (Bortone 2005). For these reasons, it is vital for the BWER to have knowledge of the phytoplankton community composition within the Ballona Estuary and connecting creek.

PROJECT OBJECTIVES

The primary objective of our study was to determine a baseline of phytoplankton taxa present in the intertidal channels and adjacent Ballona Creek at the BWER. It is essential to establish a baseline composition of the phytoplankton community because there is no current knowledge on the makeup of this community, and potential restoration options are being proposed that may significantly alter the hydrology of the system. Our secondary focus was to determine the presence and abundance, if any, of potentially harmful algal species. Our third objective was to determine any associations between phytoplankton community composition and abundance and some relevant abiotic factors (pH, temperature, tidal influence, DO, and salinity). Finally, we evaluated differences in phytoplankton composition and abundance between the three sample sites (east channel, west channel, and creek).

METHODS

Survey Locations

Our three specific sampling locations were in the BWER tide channels adjacent to both the east, self regulating, tide gates and the west, outflow flap gate; an additional sampling location was in the estuarine portion of Ballona Creek approximately 50 meters upstream of the eastern tide gates (Figure 1). These three locations provided data on the tidal exchange between the BWER and Ballona Creek. The east channel tide gate allows inflow and outflow between Ballona Creek and BWER, while the west

channel has a flap gate that allows only outflow from the BWER into Ballona Creek. The creek location was 50 meters upstream of the east channel to evaluate the conditions in Ballona Creek with minimal influence from water exiting the BWER.



Figure 1: Aerial view of Ballona Wetlands Ecological Reserve (BWER). Sample locations are indicated by red dots.

Sample Collection

One 24-hour study of the phytoplankton species composition was conducted on the dates of March 6 to 7, 2014. Once every hour, one surface water sample was collected from each of the three sample locations. Sampling for the full tidal cycle allowed us to see variability in the composition of the phytoplankton community within the BWER during fluctuating tides and depths. Samples were collected in clean 500mL high density polyethylene plastic bottles for all samples and immediately put on ice. Water samples from the upper 2-4 centimeters of the water column were collected throughout the study as the depth of the water changes dramatically within the tidal cycle inside the BWER. Water samples were stored in a dark refrigerator and processed within three days to reduce the chances of cell degradation or growth. A handheld data sonde was used to measure temperature ($^{\circ}\text{F}$), salinity (ppt), dissolved oxygen (mg/L), and pH in the main water body (probe held in creek, or channel, not in the water bottle) for each sample taken. Additionally, a permanent YSI 6600 data sonde collected the same water quality parameters every 15 minutes at the east channel sampling location.

Preparation and Analysis

Samples were concentrated to facilitate the identification and counting by light microscopy. Each sample was passed through a 10 micron polycarbonate membrane filter. The filtration device consisted of a cup with an open bottom and a rubber O-ring to create a water-tight seal with the platform, which held the filter and was connected to a drainage tube (Figure 2). A measured amount of the sample (250 to 500 ml) was poured into the cup and allowed to drain by the force of gravity. If the filter clogged, light vacuum was carefully used. Care was taken to avoid strong suction as it would damage the phytoplankton structure and render them unidentifiable.



Figure 2: Filtration apparatus used to concentrate phytoplankton from sample water.

After filtering the initial water sample, the filter membrane and the collected material were transferred to a small preservation vial containing 1 ml of formalin and 10 ml of filtrate. Then, the samples were analyzed using a light microscope to count and identify phytoplankton.

The Sedgwick Rafter chamber is a 20 by 50mm glass slide with a rectangular inset that holds 1 ml of liquid. A pipette was used to transfer 1 ml of the solution from each bottle to a Sedgwick- Rafter

chamber and then it was placed onto the counting stage of the microscope. The sample in the chamber was divided into 8.5 horizontal transects; the number of transects in which the phytoplankton were counted was determined based on the desired level of accuracy (Zeitzschel et al. 1978). To reduce the substantial laboratory assessment time, cells within three horizontal transects of a slide were identified and counted. After the cells were counted, they were averaged to obtain the number of cells in each transect. To calculate the total number of cells, the average number of cells over the three transects was multiplied by the number of total transects (8.5), then by the total number of aliquots in each vial (11) (Equation 1). The final abundance of phytoplankton, in cells per liter, was calculated by dividing the total number of cells by a concentration factor (CF) in liters (Equation 1). The concentration factor was the total amount of the original sample that was poured through the filtration device.

$$\text{Cell abundance (cells/L)} = (\text{avg. cells/transect}) * (8.5) * (11) / (\text{CF}). \quad \text{Equation 1}$$

Phytoplankton Identification

Phytoplankton identification occurs through observation of their gross morphology. Sometimes, seasonal factors and latitudinal ranges may help narrow down the possible species that exist in the sample. For example, blue-green algae prefer brackish subtropical and tropical waters (Zeitzschel et al. 1978). While it is ideal to classify phytoplankton to the lowest taxonomic level, it is not always possible due to time-constraints, damage to cells during sample collection and analysis, and alterations in the appearance of some cells when exposed to preservatives (Zeitzschel et al. 1978). Identifying phytoplankton at the species level can be difficult due to the morphological resemblance of many species within the same genus, so phytoplankton were identified to the genus level.

The characteristics that were examined for identification include cell shape, cell length, cell chains, chromatophores and unique appendages (Verlencar and Desai 2004). Phytoplankton cells come in a variety of shapes; for instance, centric diatoms usually have a “flat discoid shape” and pennate diatoms are variations on a rod shape with tapered ends with bilateral symmetry (Verlencar and Desai 2004). Chromatophores are organelles that contain pigments and reflect light, which vary in shape and color among different species; *A. wyvillei* in the *Actinodisceae* family have disc-shaped chromatophores

and *Prorocentrum* have yellowish-brown chromatophores (Verlencar and Desai 2004). Some species possess filaments and appendages such as setae, bristles, and horns (Verlencar and Desai 2004). Although motility is usually present in flagellates it will not be observed in fixed samples. The main group of diatoms that was expected to be observed in the BWER was the pennates since they are more commonly found in brackish and freshwater environments (Shipe, personal communication). The other groups which generally reside in marine environments were centric diatoms and dinoflagellates, which have two flagella that give them swimming abilities (Verlencar and Desai 2004). Many species that have chain forming mechanisms; for example, *Asterionella japonica* is a diatom that can aggregate into spiral colonies (Verlencar and Desai 2004).

Due to the difficulty in differentiating between the exceptionally small and similarly-shaped pennate species under the light microscope, an “unknown pennates” category was created for the species that possess a general elongated oval shape without visible internal or external features. The same problem was presented in the identification of some of the centric diatoms, so in the same manner, an “unknown centrics” category was created for the species that possess a structure based on a circular valve shape without clearly identifiable internal or external features.

Statistical Analysis

Phytoplankton abundances were plotted using bar graphs with time on the X-axis and cell abundances (cells/liter) on the Y-axis for each sample location. Each bar was divided into sections representing the few most dominant phytoplankton genera, the total harmful species, and the combination of all other genera observed in the particular sample location. The graph of the east channel consists of harmful species, *Pleurosigma*, *Nitzschia*, *Navicula*, *Melosira*, *Cylindrotheca*, *Amphiprora*, other pennates, other centrics, and other species. The graph of the west channel consists of harmful species, *Stephanopyxis*, *Pleurosigma*, *Nitzschia*, *Navicula*, *Cylindrotheca*, other pennates, other centrics, and other species. The graph of the creek location consists of harmful species, *Pleurosigma*, *Cylindrotheca*, *Nitzschia*, *Navicula*, other pennates, other centrics, and other species. The “harmful species” represents the total harmful phytoplankton genera. The “other pennates” category represents the total pennates that were not dominant in the location. The “other centric” category was a total of all centrics that were not dominant in the location. The “other” category was a combination of phytoplankton groups that were present in very low abundances, including Dinoflagellates (non-harmful genera), Silicoflagellates, flagellates, Chrysophytes, Chromista, and Rhodophytes, as well as macroalgae spores present in some samples.

Phytoplankton groups were statistically analyzed to assess associations with any of the measured abiotic factors using the software Past 3.01 to conduct a multivariate Principal Component Analysis (PCA) on a correlation matrix. Since the abiotic variables were dependent on one another, performing the PCA generated new independent variables upon which the clustering of phytoplankton groups was evaluated. The abiotic factors that were analyzed in the PCA are temperature (°F), salinity (ppt), dissolved oxygen (mg/L), tide height (m), and pH. There were missing data at two times for tide height; data were interpolated to arrive at reasonable values. In order to assess if any phytoplankton characteristics were associated with the new independent variables, clustering of the groups was evaluated on a biplot of the first two principal components (PC1 on x-axis, PC2 on y-axis). Groupings

that we evaluated included (1) total phytoplankton abundances (2) abundances of harmful algal taxa and (3) relative contribution of centric diatoms to the total phytoplankton assemblage.

RESULTS AND DISCUSSION

For this project we had four goals: determine phytoplankton taxa and abundances, determine the presence of harmful phytoplankton, determine if phytoplankton communities are associated with abiotic factors over a 24-hour period, and provide SMBRC with all of this data with suggestions for further studies and how this data might contribute toward restoration efforts.

Abiotic Factors

The abiotic factors measured by the YSI data sonde probes include: tidal height, temperature ($^{\circ}\text{C}$), salinity (ppt), dissolved oxygen (%), and pH. As seen in Figure 3, tidal height varies between 0 and 3 meters, peaks around 4 AM and dips at 6 PM and 9 AM. Water at sample locations was generally very saline (17.9 to 39.8 ppt) during our study and reflects more oceanic than freshwater characteristics. Figure 4 shows that salinity concentrations in the creek were most affected by changes in tidal height, decreasing slightly before tidal height reaches its peak. At all three locations, temperature decreases to 14°C - 18°C between 11PM and 7AM as seen in Figure 5. The temperature at the three sites specifies a mesophilic environment. Disregarding the creek outlier in Figure 6, pH ranges from 6.4 to 7.6 (indicating a neutrophilic environment) with dips between 3AM-10AM for the west and east channel. Lastly, DO percentage varied between 41.8% and 115.5% over time with what seemed to be attributed to sporadic instrument sampling errors as seen in Figure 7. But in general, the DO percentage indicates that the three sites support aerobic processes like aerobic degradation of debris by phytoplankton. All these factors may contribute to the phytoplankton community composition and were used in a principal component analysis to understand relationships between the abiotic environment and phytoplankton community data.

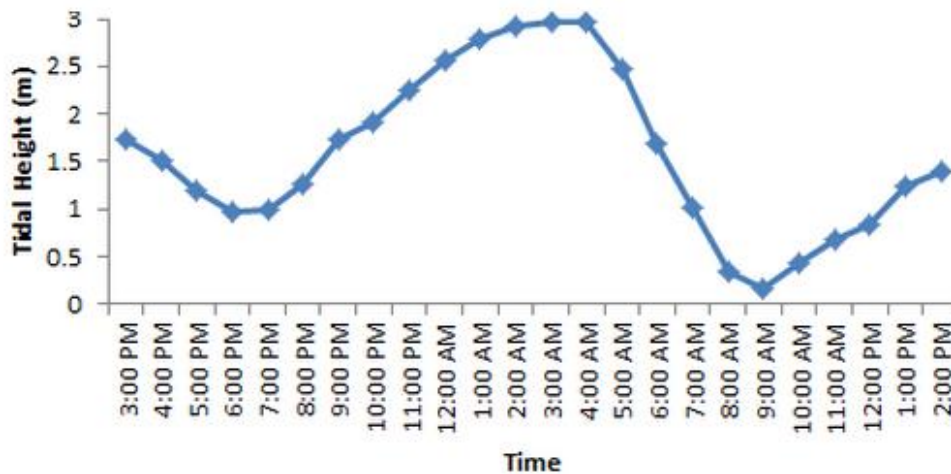


Figure 3: Tidal height with respect to time in the creek, east, and west channel locations of the BWER over time from March 6-7, 2014.

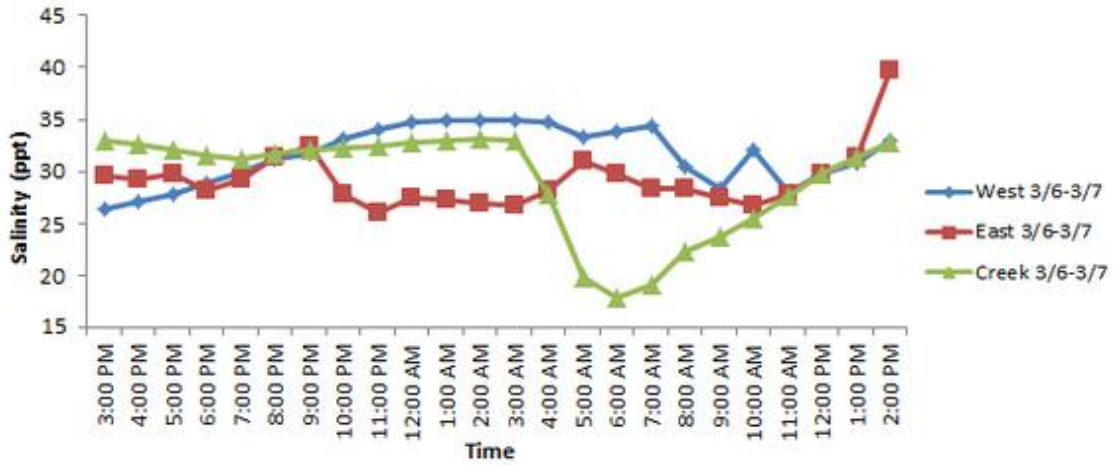


Figure 4: Salinity in the creek, east, and west channel locations of the BWER over time from March 6-7, 2014.

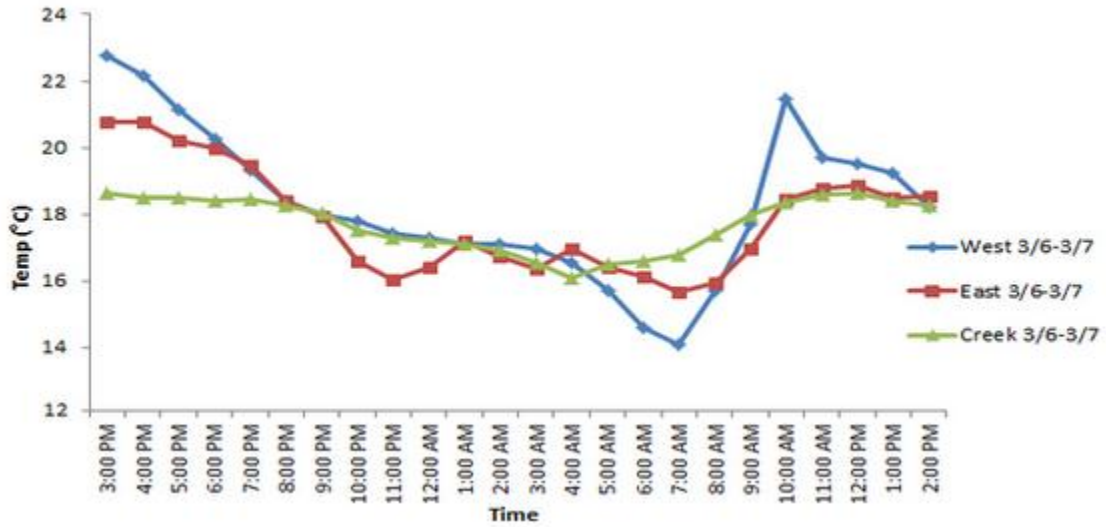


Figure 5: Temperature in the creek, east, and west channels with respect to time at the BWER from March 6-7, 2014.

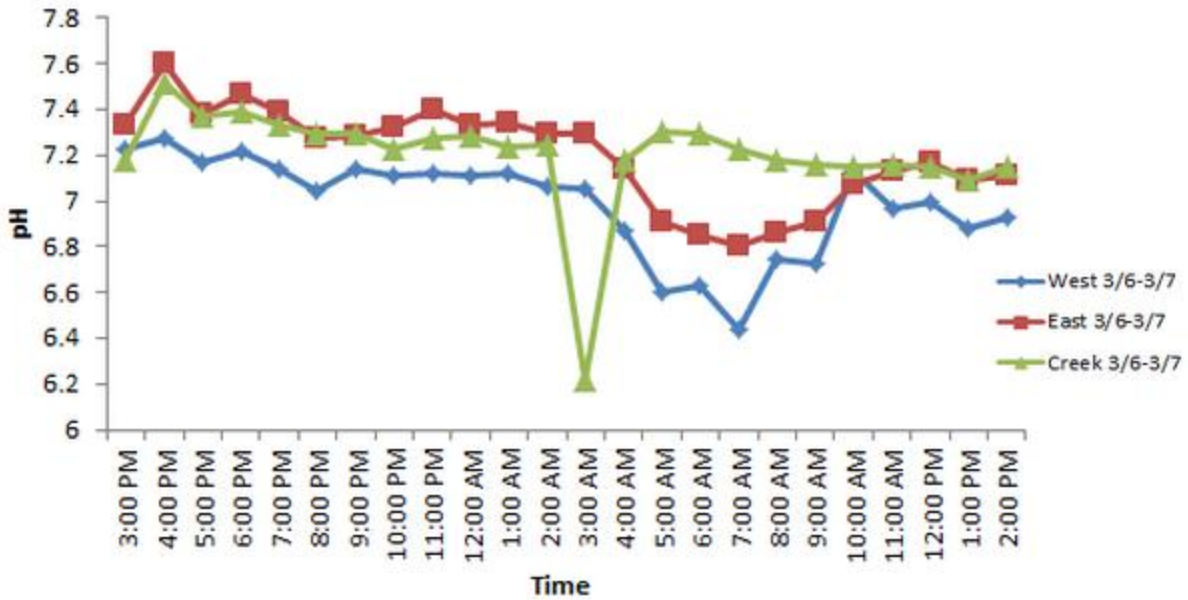


Figure 6: pH levels in the creek, east, and west channels with respect to time at the BWER from March 6-7, 2014.

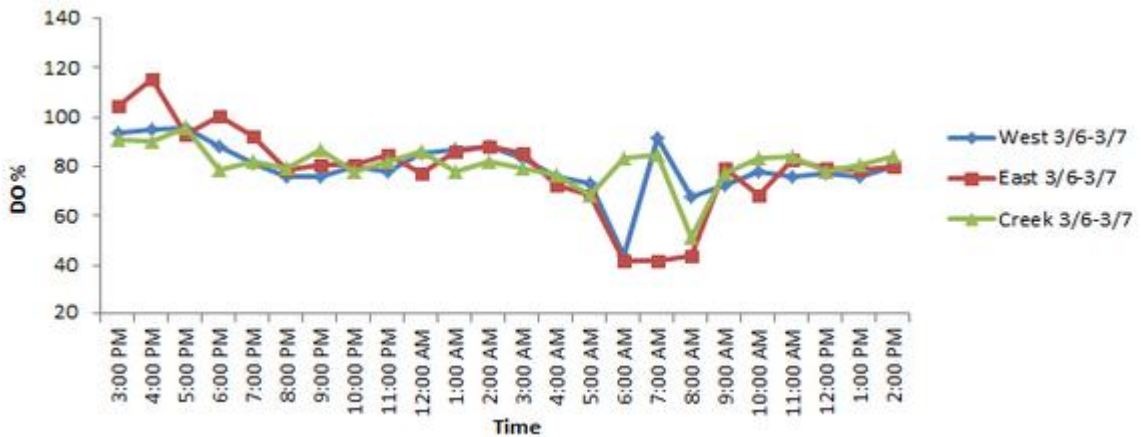


Figure 7: Dissolved oxygen levels in the creek, east, and west channel sample locations of the BWER over time from March 6-7, 2014.

Phytoplankton Community

Appendix A provides a list of the complete phytoplankton taxa identified in the Ballona Wetlands on March 6th-7th, 2014. There are 53 different genera from five classes of Protists. The majority of the genera are in the category of diatoms, which belong to the class of Bacillariophyceae. Most of the harmful genera belong to the class of Dinophyceae, which are within the phylum of dinoflagellates. Some of the less common genera and species were found to be unique to certain channels. *Alexandrium* was only found in the west and east channels. *Cerataulina* and *Hemidiscus* were only found in the east channel and the creek. *Gymnodinium* and *Manguinea* were only found in the west channel and the creek. *Amphora*, *Bacillaria*, *Chroomonas*, *Ditylum*, *Eucampia* and *Grammatophora*

were only present in the east channel. *Akashiwo*, *Asteromphalus*, *Guinardia Striata*, *Leptocylindrus*, *Lingulodinium*, *Phaeoplaca*, *Meuniera* and *Rhizosolenia* were only found in the creek.

EAST CHANNEL: The east channel exhibited phytoplankton abundances ranging from 3,000-60,000 cells per liter, with peak abundances at 10PM, 11PM and 9AM. Despite the wide range of abundances, there was fairly consistent relative proportions of dominant phytoplankton throughout the 24 hour study period, which was dominated by the two categories that include unidentified pennate and centric diatoms. Throughout the 24 hours, centric composition averaged around 19% of the total phytoplankton abundance with a peak abundance of 54% at 9AM, which corresponds to the highest tide depth. *Navicula*, *Nitzschia*, *Melosira*, and *Pleurosigma* were the secondary dominant species, as seen in Figure 8.

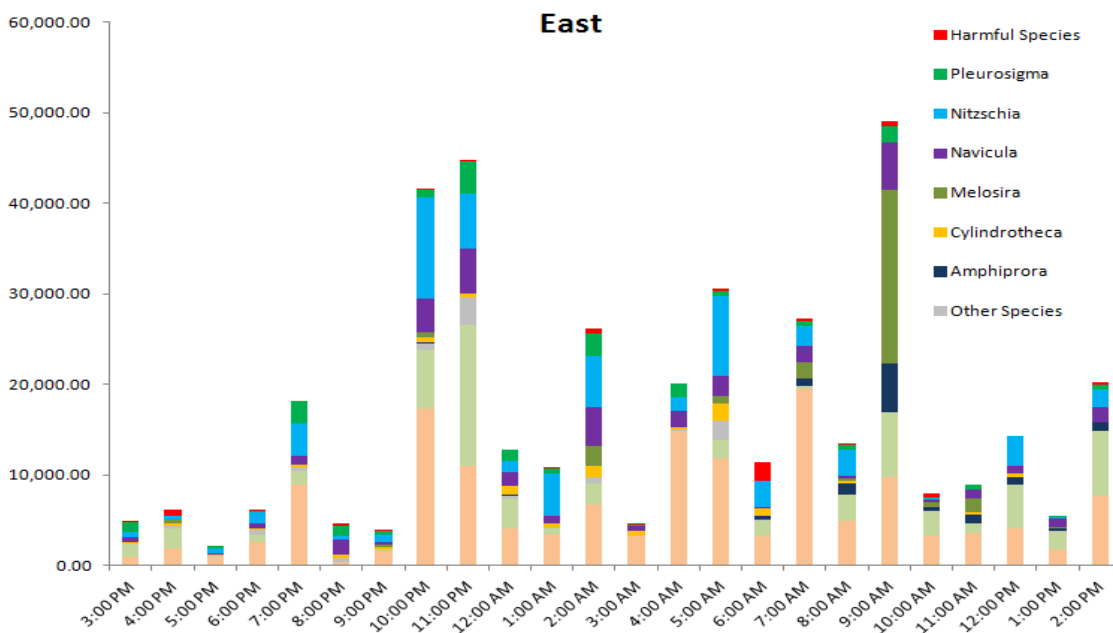


Figure 8: Phytoplankton abundances of dominant species in cells per liter over time in the east channel of the BWER from March 6-7, 2014.

A principal component analysis was run for the east channel with abiotic factors of temperature, salinity, dissolved oxygen concentration, tidal height and pH. The first principal component described 49% of the variability in these factors and the second principal component showed 29% of the variability in all of these factors, for a total of 78% of variability described by these first two principal components. PC1 is highly positively correlated with DO, pH and temperature. PC2 is positively correlated with tide depth and negatively correlated with salinity. Thus, a high value for PC2, associated with high tide but low salinity, is not clearly describing either oceanic or wetland conditions. In the biplot of PC1 versus PC2, Figure 9 shows a negative correlation between phytoplankton abundance and PC1; this means that high phytoplankton abundances are in the cooler, possibly more oceanic waters whereas low phytoplankton abundances were observed in the warm waters, which are possibly more

associated with the wetland. There were no clear correlation between total phytoplankton abundances and PC2.

Harmful phytoplankton abundances ranged from 0-14% of the total composition with an average of 2% throughout the day. The highest abundance of harmful genus, *Alexandrium*, occurred at 6 AM. The PCA, however, did not show a strong correlation between harmful phytoplankton presence and the abiotic factors measured and thus the biplot with harmful taxa is not shown. Centric diatom relative abundance ranged from 0-54% of the total phytoplankton abundance with an average of 19%. The highest abundance of centrics was observed at 9 AM, which corresponds to the lowest tide height. The PCA, however, did not show any clear correlation between centric abundance and the abiotic factors measured.

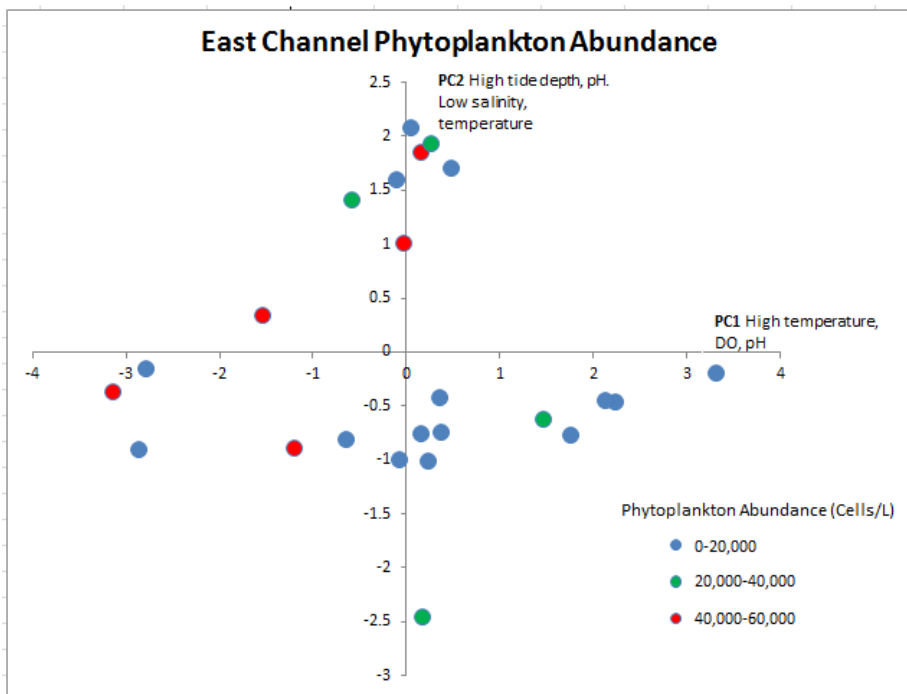


Figure 9: Phytoplankton abundances mapped onto the biplot of PC1 and PC2 for a principal component analysis of the 5 abiotic factors in the east channel.

WEST CHANNEL: The west channel sample location contained higher phytoplankton abundances than the east channel. Their abundance ranged from 4,500-95,500 cells/L with unknown pennates and centric diatoms as the dominant species. *Amphiprora*, *Navicula*, and *Nitzchia* were the secondary dominant species as seen in Figure 10. The West channel had similar relative proportions of dominant species as the east channel but with less variability in abundances throughout the day. This is likely due to the structure of the west channel, which only allows for an outflow of water, resulting in minimal disturbance of the water.

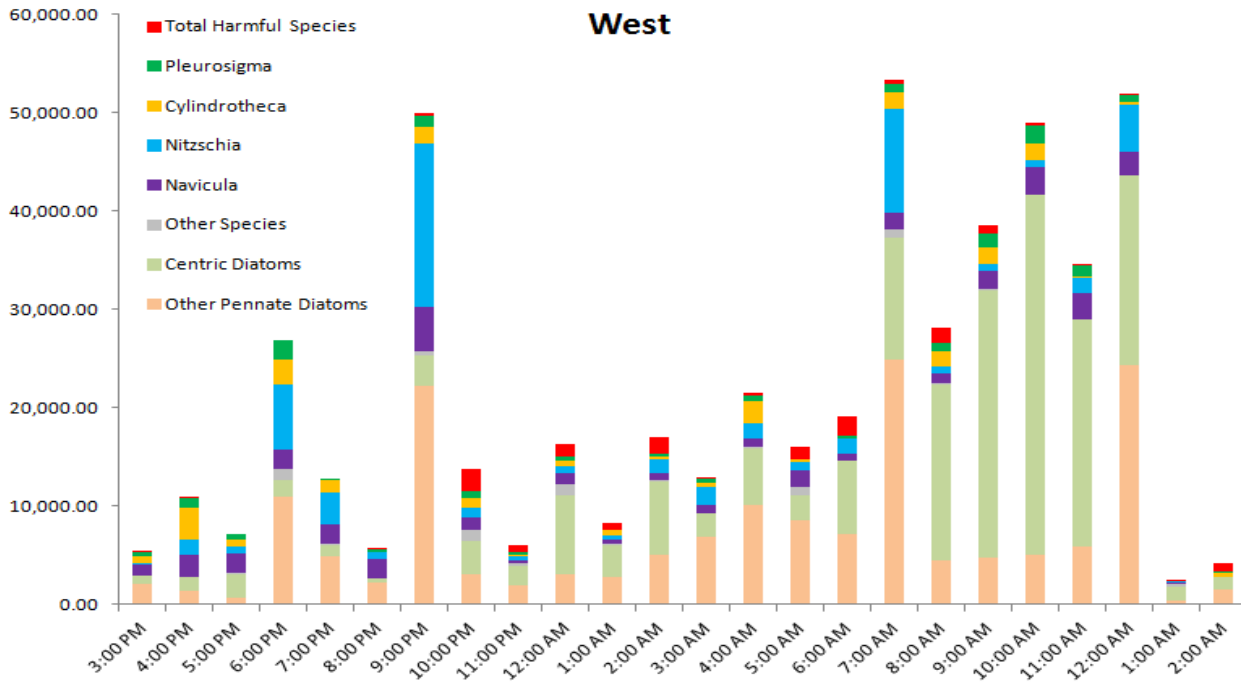


Figure 10: Phytoplankton abundances of dominant species in cells per liter over time in the west channel.

The multivariate analysis for the west channel shows that PC1 accounts for 53% of the variability in the abiotic data and PC2 accounts for 31% of the variability, amounting to a total of 84% of variability. PC1 is positively correlated with temperature, pH and DO and negatively correlated to salinity and tide height. PC2 is strongly correlated to tide height and moderately correlated to DO, pH and salinity. Furthermore, Figure 11 shows that higher abundances of phytoplankton were negatively correlated with PC2, while PC1 doesn't show clear correlation with the level of phytoplankton abundance. These high abundances were observed at 7 AM and 12 PM when the West channel abiotic conditions resembled those of wetlands (high PC2 values).

Centric phytoplankton composition ranged from 4-40%, with peak abundances at 10 AM. There was no correlation, however, between the contribution of centric diatoms to the total phytoplankton abundance and abiotic factors. Meanwhile, harmful phytoplankton abundances ranged from 0-12% of the total phytoplankton abundance with an average of 3%. The highest percent contribution was observed at 2 PM with *Prorocentrum* as the most abundant species. Figure 12 shows a negative correlation between harmful phytoplankton abundance and PC1, which reflects low salinity and tide height and a positive correlation with PC2, which reflects an increase in tide depth, salinity, DO, and pH, closely resembling in oceanic conditions. Thus, harmful phytoplankton seem to be associated with oceanic conditions in this channel.

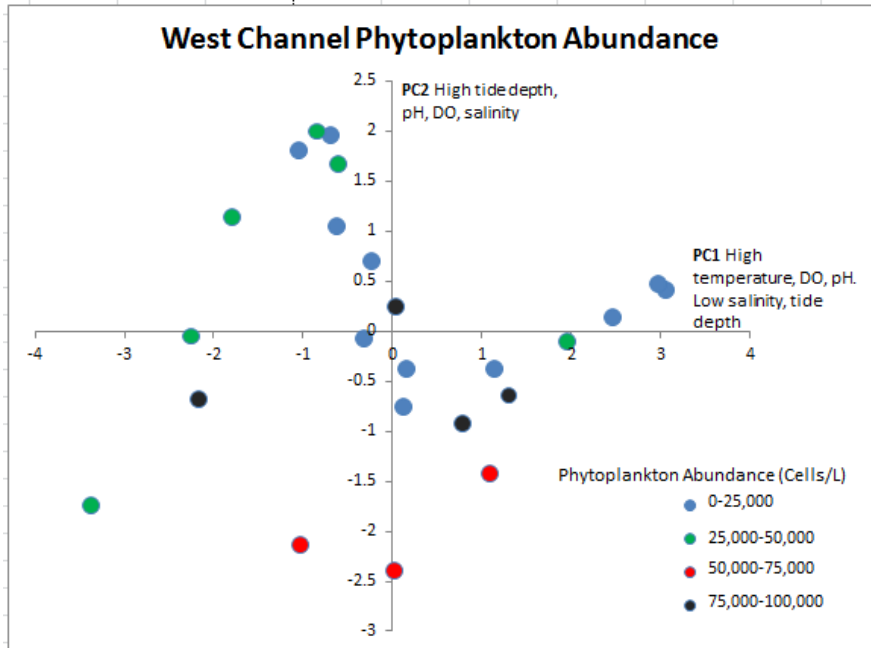


Figure 11: Phytoplankton abundances mapped onto the biplot of PC1 and PC2 for a principal component analysis of the 5 abiotic factors in the west channel.

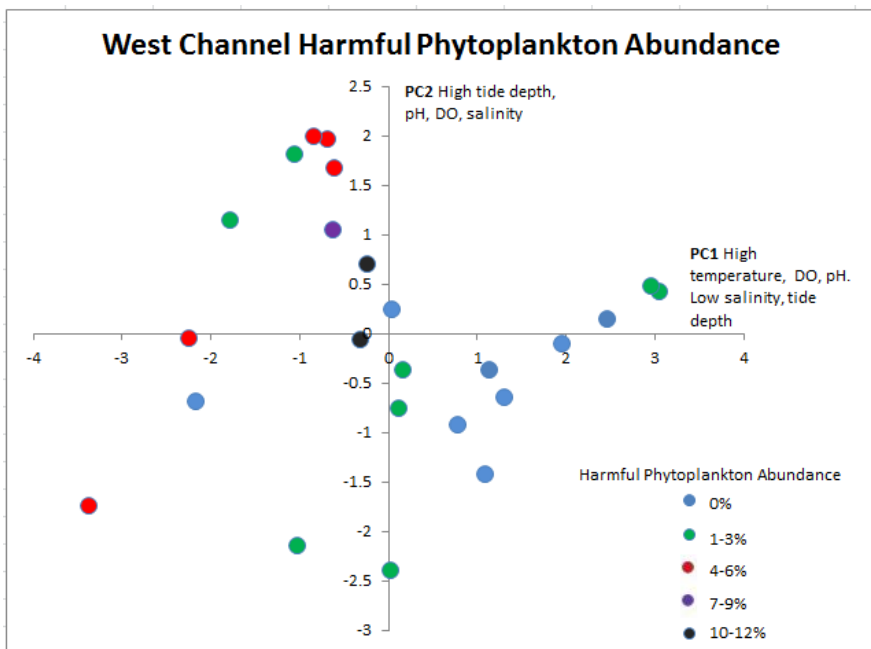


Figure 12: Potentially harmful phytoplankton abundances mapped onto the biplot of PC1 and PC2 for a principal component analysis of the 5 abiotic factors in the west channel.

CREEK: The creek had the lowest phytoplankton abundances, ranging from 250-48,000 cells/L. Like the East and west channels, the categories of unidentified pennate and unidentified centric diatoms were the dominant species. *Navicula*, *Nitzschia*, and *Stephanopyxis* were the secondary dominant species as seen in Figure 13.

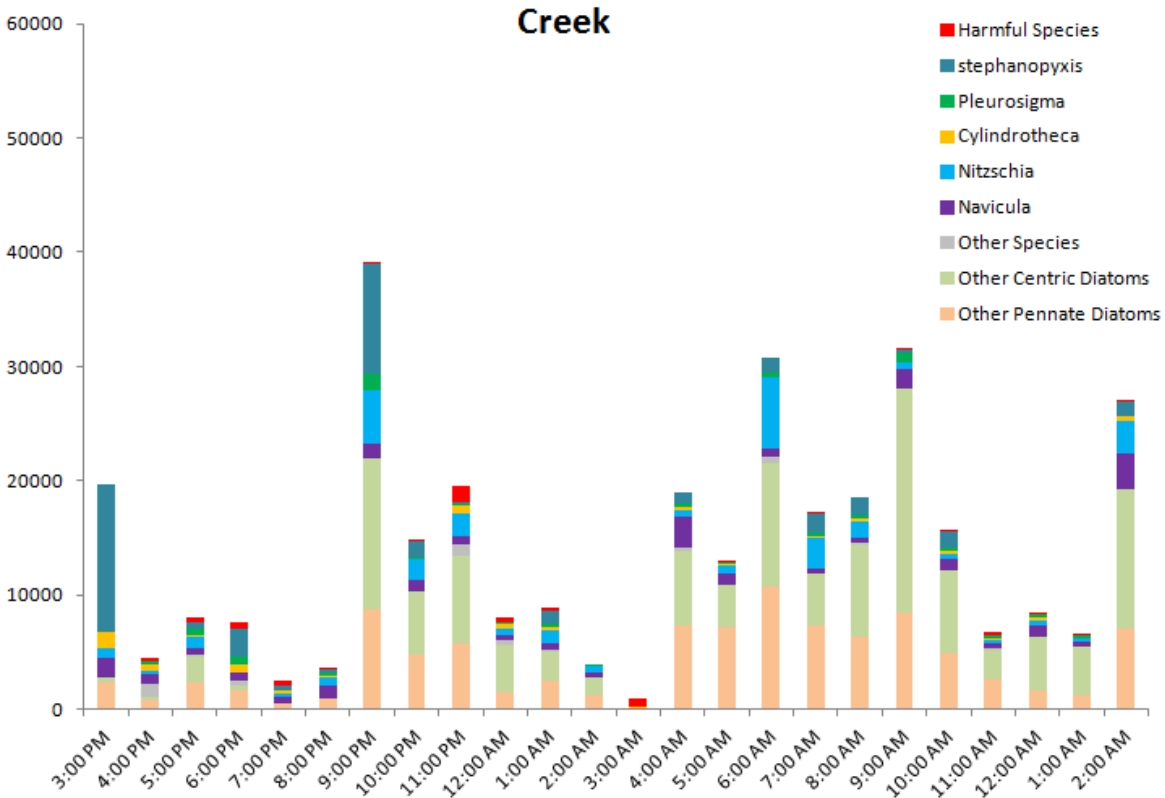


Figure 13: Phytoplankton abundances of dominant species in cells per liter over time in the creek.

A principal component analysis was run for the creek sample with abiotic factors of temperature, salinity, dissolved oxygen concentration, tidal height and pH. The first principal component described 39% of the variability in these factors and the second principal component showed 32% of the variability in all of these factors, for a total of 71% of variability described by these first two principal components. PC1 is highly positively correlated with temperature and negatively correlated with tide depth. Thus, a high value for this PC may indicate waters of high temperature and low tide depth, perhaps associated with wetland conditions. PC2 is positively correlated with tide depth, salinity, and dissolved oxygen. Thus, a high value for PC2, associated with high tide and high salinity, could perhaps describe oceanic conditions. In the biplot of PC1 versus PC2, Figure 14 shows a weak negative correlation between total phytoplankton abundances and PC1; this means that high phytoplankton abundances are in the cooler, possibly more oceanic waters whereas low phytoplankton abundances were observed in the warm waters, which are possibly more associated with the wetland. However, there was no clear correlation between PC2 and phytoplankton abundances in the creek.

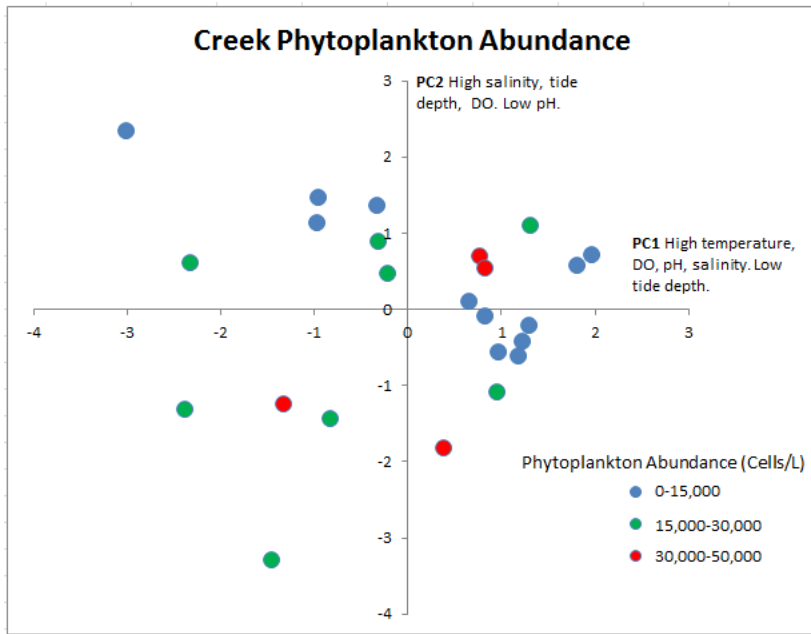


Figure 14: Phytoplankton abundances mapped onto the biplot of PC1 and PC2 for a principal component analysis of the 5 abiotic factors in the creek.

Centric abundances ranged from 6-60% of the total phytoplankton abundance, with an average of 33%. Figure 15 shows a positive correlation between positive values of PC1 and

high abundances of centric diatoms. This implies that centric diatom abundances are linked with low tide depths and higher temperatures, which are associated with wetland conditions. PC2 shows no correlation with centric diatom relative abundance. Finally, harmful phytoplankton composition ranged from 0-11%, with an average of 2%. The highest harmful species abundance was observed at 2 AM with *Prorocentrum* as the dominant species. Figure 16 shows a positive correlation between harmful phytoplankton species and PC1, which suggests oceanic conditions with high tide depth, salinity, and DO. There is no clear correlation between harmful taxa and PC2.

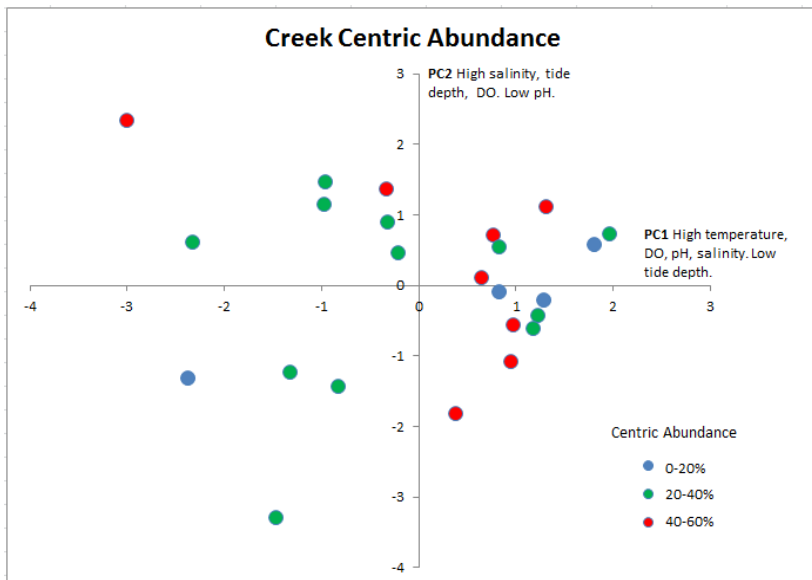


Figure 15: Percent contribution of centric diatoms to the total phytoplankton mapped onto the biplot of PC1 and PC2 for a principal component analysis of the 5 abiotic factors in the creek.

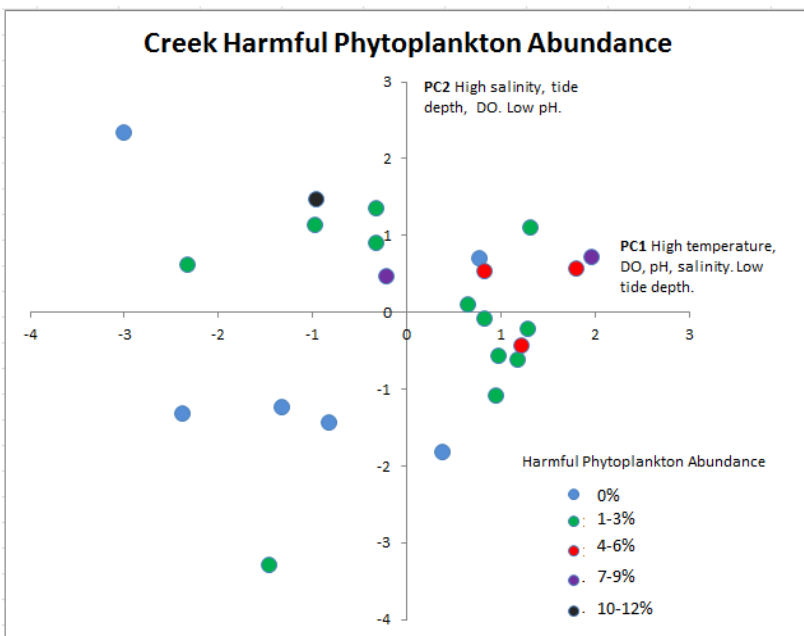


Figure 16: Potentially harmful phytoplankton abundances mapped onto the biplot of PC1 and PC2 for a principal component analysis of the 5 abiotic factors in the creek.

Harmful Phytoplankton Species

Alexandrium and *Prorocentrum* were the dominant harmful species found in all three sampling locations. Other harmful species observed included *Akashiwo*, *Dinophysis*, *Gymnodinium*, *Lingulodinium*, and *Pseudo-nitzschia*. Although *Prorocentrum* was the most common harmful algal genera with abundances ranging from 90 to 2,100 cells/L in the west channel and the creek, all harmful species abundances were relatively low. However, even at these low abundances, their presence indicates that under the right conditions, they may multiply, which can lead to devastating effects to the wetlands. Thus these low populations in the wetlands could possibly indicate the potential for higher abundances. Since most of the harmful species were in the west channel, where the tide gates allow for outflow only, this indicates that during freshwater events such as storms, these harmful species can be flushed out. However, there may still be risk for the adjacent coastal ocean ecosystem.

Since the scope of this project only considered the phytoplankton community changes across a twenty-four hour period, future studies tracking the changes over a larger temporal and/or spatial scale could be very useful. Evaluation of phytoplankton community changes over a period of months or even seasons, could provide a better understanding of the interactions between the abiotic environment and the phytoplankton community composition. This study also only took samples at the East and west channel close to the tide gates where the water is greatly influenced by the tide gates. Future studies may want to sample further into the wetlands to see if species compositions and abundances differ more inside the wetlands than those closer to the creek. Unfortunately this study could not consider the effects of storms and incoming stormwater on the phytoplankton compositions, but future studies may want to evaluate this possible forcing. Finally, determining the phytoplankton's response to changes in the nutrient concentrations would be of interested as this is a major control of phytoplankton biomass and productivity, but not a feasible measurement during our study.

APPLICATIONS TO RESTORATION

The purpose of this project was to provide baseline phytoplankton data to the SMBRC to aid restoration efforts in the BWER. As stated by the U.S. Geological Survey, there are two primary goals for wetland restoration that are also applicable to BWER's restoration plans: (1) increased production

and export of phytoplankton and (2) mitigation of wastewater-derived nutrients. Since centric diatoms tend to be chain formers and are at the base of the food chain, they can lead to a more efficient energy transfer within the food chain, allowing for higher production at higher trophic levels (Shipe, personal communication). Centrics varied in abundances over the tidal cycle, but their constant presence suggests their ability to support the marine food web in the BWER. Harmful phytoplankton species were present in all three locations, but the west channel had noticeably higher abundances than the east channel or creek locations. The presence of harmful species indicates the potential for HABs, but implementing mitigation solutions such as the prevention of wastewater-derived nutrients from entering the wetlands may reduce the chances of large blooms.

CONCLUSION

This report provides the Santa Monica Bay Restoration Commission with baseline data on the composition of phytoplankton genera in the BWER. In addition, the study determined how the phytoplankton community at the BWER is associated with abiotic factors over a full tidal cycle and information on the harmful phytoplankton species that are present at low relative abundances in the wetlands. The three sampling locations were characterized by diurnal temperature patterns, neutral pH, DO concentrations that supported aerobic processes, a mixed semidiurnal tidal cycle, and a nearly oceanic salinity. High abundances of phytoplankton were present in the west channel under conditions that could be considered more characteristic of wetland waters: high temperature and low tide. High abundances of centric diatoms were seen in the creek under wetland-like conditions, whereas the east channel showed no clear links between abiotic factors and phytoplankton abundances. A total of 53 phytoplankton genera were identified in the BWER, seven of which are harmful. Harmful phytoplankton in the west channel and creek show high abundances in conditions that are characteristic of oceanic waters. In the west channel and the creek, the most dominant harmful species present is *Prorocentrum*, whereas *Alexandrium* dominated the east channel.

LITERATURE CITED

- Almeda-Jauregui C.O., Maske, H., Ochoa, J., & Ruiz-de la Torre M.C. (2013). Maintenance of coastal surface blooms by surface temperature stratification and wind drift. *PLoS One*, **8**:4.
- "Ballona Wetland Existing Conditions FINAL Report." (2006). *Ballona Wetlands Restoration Project*. Philip Williams & Associates, Ltd.
- Beardall, J., Raven, J.A. (2004). The potential effects of global climate change on microalgal photosynthesis, growth and ecology. *Phycologia*, 26–40.
- Berge, T., Daugbjerg, N., Balling, A.B., Hansen, P. (2010). Effect of lowered pH on marine phytoplankton growth rates. *Marine Ecology Progress Series*, **416**, 79–91. doi: 10.3354/meps08780
- Bortone, S.A., (2005). *Estuarine Indicators*. Boca Raton, FL: CRC, Print.
- "California Current." (2011). Southwest Fisheries Science Center. NOAA Fisheries Service, Web. 26 Nov. 2013.
- Chen, C.Y., Durbin, E.G. (1994) Effects of pH on the growth and carbon uptake of marine phytoplankton. *Marine Ecology Progress Science*, **109**, 83–94.
- Chislom, S.W., (1992) Phytoplankton size in: Falkowski PG, Woodhead AD (eds) primary productivity and biogeochemical cycles in the sea. Plenum Press, New York, 213-237.
- Cloern, J.E., Cole, B.E., Wong, R.J.L., and Alpine, A.E (1985) Temporal dynamics of estuarine phytoplankton: A case study of San Francisco Bay. *Hydrobiologia* **129**, 153-176.
- Cloern, J.E., and Dufford, R. (2005) Phytoplankton community ecology: principles applied in San Francisco Bay. *Marine Ecology Progress Series*, **285**, 11–28.
- Cochlan, W.P., Kudela, R.M., and Seeyave, S. (2010) The role of nutrients in regulation and promotion of harmful algal blooms in upwelling systems. *Progress in Oceanography*, **85** (1-2), 122-135.
- Cohen, T., Que Hee, S.S., and Ambrose, R.F. (2001) Trace metals in fish and invertebrates of three California coastal wetlands. *Marine Pollution Bulletin*, **42**, 224-232.
- Cole, J., Doering, P., Frithsen, J., Nowicki, B., Oviatt, C., and Reed, L. (1995) An ecosystem level experiment on nutrient limitation in temperate coastal marine environments. *Marine Ecology Progress Series*, **116**, 171-179.
- Dark, S., et al. (2011). "Historical Ecology of the Ballona Creek Watershed." *Southern California Coastal Water Research Project: Technical Report*.
- Domingues, R.B., Barbosa, A., Galvão, H. (2005) Nutrients, light and phytoplankton succession in a temperate estuary (the Guadiana, south-western Iberia). *Estuarine, Coastal and Shelf Science*, **64**, 249–260. doi: 10.1016/j.ecss.2005.02.017

- Dorsey, J.H., Carter, P.M., Bergquist, S., Sagarin, R. (2010) Reduction of fecal indicator bacteria (FIB) in the Ballona Wetlands saltwater marsh (Los Angeles County, California, USA) with implications for restoration actions. *Water Research*, **44**, 4630–42. doi: 10.1016/j.watres.2010.06.012
- Engel, A., Piontek, J., Grossart, H.P., et al. (2014) Impact of CO₂ enrichment on organic matter dynamics during nutrient induced coastal phytoplankton blooms. *Journal of Plankton Research*, **36**, 641–657. doi: 10.1093/plankt/fbt125
- Flewelling, L.J., Fu, F., Granholm, A.A., Hutchins, D.A., and Tatters, A.O. (2013) High CO₂ promotes the production of paralytic shellfish poisoning toxins by *Alexandrium catenella* from Southern California waters. *Harmful Algae*, **30**, 37-43.
- Giordano, M., Beardall, J., Raven, J.A. (2005) CO₂ concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. *Annual Review of Plant Biology*, 99–131.
- Graneli, E., Hagstrom, J.A., Moreira, M.O.P., and Odebrecht, C. (2011) Domoic acid production and elemental composition of two *Pseudo-nitzschia* multiseriales strains, from the NW and SW Atlantic Ocean, growing in phosphorus- or nitrogen-limited chemostat cultures. *Journal of Plankton Research*, **33**(2), 297-308.
- Gruber, N., Leinweber, A., Shipe, R.F. (2008) Abiotic controls of potentially harmful algal blooms in Santa Monica Bay, California. *Continental Shelf Research*, **28**, 2544-2593.
- Hansen, P.J. (2002) Effect of high pH on the growth and survival of marine phytoplankton: implications for species succession. *Aquatic Microbial Ecology*, 279–288.
- Havens, K.E., Hauxwell, J., Tyler, A.C., et al. (2001) Complex interactions between autotrophs in shallow marine and freshwater ecosystems: implications for community responses to nutrient stress. *Environmental Pollution*, **113**, 95–107.
- Hickey, B.M., Banas, N.S. (2003) Oceanography of the U.S. Pacific Northwest Coastal Ocean and estuaries with application to coastal ecology. *Estuaries*, **26**, 1010–1031. doi: 10.1007/BF02803360
- Johnston, K. "Ballona Wetlands Restoration Project: Baseline Reports." *Ballona Wetlands Restoration Project*. N.p., June 2012. Web. 26 May 2014.
<<http://ballonarestoration.org/baseline-reports/>>.
- Johnston, K. (2012). "Chapter 11: Physical Conditions." *Ballona Wetlands Ecological Reserve, Los Angeles, California Santa Monica Bay Restoration Commission*.
- Kimmerer, W.J. (2002) Physical, biological, and management responses to variable freshwater flow into the San Francisco Estuary. *Estuaries*, **25**, 1275–1290. doi: 10.1007/BF02692224
- Kennison, R.L. and Fong, P. (2013) Extreme eutrophication in shallow estuaries and lagoons of California is driven by a unique combination of local watershed modifications that trump variability associated with wet and dry seasons. *Estuaries and Coasts*, **37**(1), 164-179.
- Lehman, P.W., Boyer, G., Hall, C., Waller, S., and Gehrts, K. (2005) "Distribution and Toxicity of a New Colonial *Microcystis Aeruginosa* Bloom in the San Francisco Bay Estuary, California." *Hydrobiologia* **541**(1), 87–99. doi:10.1007/s10750-004-4670-0.

- Lewitus, Alan J., Horner, R.A., Caron, D.A., Garcia-Mendoza, E., Hickey, B.M., Hunter, M., Huppert, D.D., et al. (2012) "Harmful Algal Blooms along the North American West Coast Region: History, Trends, Causes, and Impacts." *Harmful Algae* **19**, 133–159. doi:10.1016/j.hal.2012.06.009.
- Riebesell, U. (2004) Effects of CO₂ enrichment on marine phytoplankton. *Oceanography*, **607**, 19–729.
- Smith, D.W., Piedrahita, R.H. (1988) The relation between phytoplankton and dissolved oxygen in fish ponds. *Aquaculture* **68**, 249–265. doi: 10.1016/0044-8486(88)90357-2
- Toseland, A., Daines, S.J., Clark, J.R., et al. (2013) The impact of temperature on marine phytoplankton resource allocation and metabolism. *National Climate Change*, **3**, 979–984. doi: 10.1038/nclimate1989
- Tsihrintzis, V.A., Vasarhelyi, G.M., & Lipa, J. (1996) Ballona Wetland: a multi-objective salt marsh restoration plan. *Proceedings of the Institution of Civil Engineers Water Maritime and Energy*, **118**, 131-144.
- "New Indicators of Coastal Ecosystem Condition." *The United States Environmental Protection Agency*, no. EPA/600/S-05/004 (2005).
- Van Dolah, F.M. (2000) Marine algal toxins: origins, health effects, and their increased occurrence. *Environmental Health Perspectives*, **108** (1), 133-141.
- Verlencar, X.N. and Desai, S. (2004) Phytoplankton identification manual. *National Institute of Oceanography* [Internet]. [cited 25 Nov 2013], 1-22. Available from: <http://drs.nio.org/drs/bitstream/2264/97/1/Phytoplankton-manual.PDF>
- Zedler, J.B. (2001) Handbook for restoring tidal wetlands. CRC Press. Boca Raton, Florida, USA.
- Zeitzschel, B., Margalef, R., Venrick, E.L. et al. (1978) Phytoplankton manual. Unesco [Internet]. [cited 5 Nov 2013], 1-7, 33-50, 125-128, 160-164. Available from: <http://unesdoc.unesco.org/images/0003/000307/030788eo.pdf>

Appendix A : List of Phytoplankton Taxa Identified in Samples taken March 6-7, 2014 in East Channel, West channel and Ballona Creek sample locations





**Ballona Wetlands Phytoplankton Community
Composition Baseline Report**

Surveys of the wetland showed an observed 53 different genera of phytoplankton from many different Class level groups. March 6-7, 2014







<p><u>Diatom, Centric</u> Amphiprora Amphora Asteromphalus Bacillaria Bacteriastrum Biddulphia Cerataulina Chaetoceros Coscinodiscus Cymbella Dactyliosolen Ditylum Eucampia Fragilaria Fragilariopsis Grammatophora Guinardia cylindrus Guinardia flaccida Guinardia striata Hemidiscus Lauderia Leptocylindrus Melosira Meuniera Rhizosolenia Skeletonema Stephanopyxis Thalassionema Thalassiosira</p>	<p><u>Diatom, Pennate</u> Cylindrotheca Diploneis Gyrosigma Haslea Manguinea Navicula Nitzschia Pleurosigma Rhaphoneis Synedra Tropiconeis</p> <p><u>Harmful Algae Taxa</u> Akashiwo Alexandrium Dinophysis Gymnodinium Lingulodinium Prorocentrum Pseudonitzschia</p> <p><u>Dinoflagellate</u> Chroomonas Pyrocystis Ceratium Karlodinium</p>	<p><u>Silicoflagellate</u> Dictyocha</p> <p><u>Rhodophyte (macroalgae)</u> Rhodosorus</p> <p><u>Chyrsophyte</u> Dinobryon Phaeoplaca</p>
---	---	---


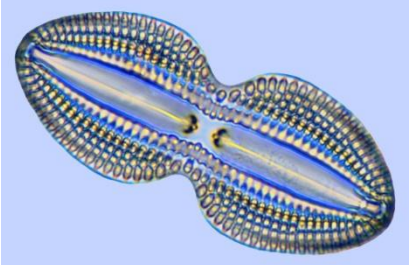

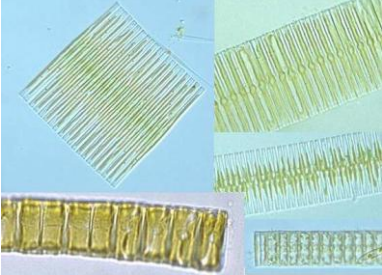
**Appendix B: Phytoplankton Identification Guide for Phytoplankton
Taxa from the BWER**

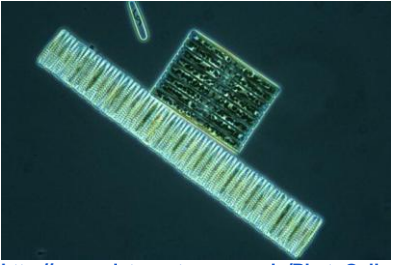

Kingdom: Chromista > Phylum: Ochrophyta > Class: Bacillariophyceae




Name (Order)	Picture
<p>Actinoptychus (Coscinodiscales)</p>	 <p>King County http://green.kingcounty.gov/marine/IndividualPhoto.aspx?SpeciesID=355&TypeId=1</p>
<p>Amphipora (Naviculales)</p>	 <p>http://www.serc.si.edu/labs/phytoplankton/guide/diatoms/amphipaldpx.aspx</p>
<p>Amphora (Thalassiophysales)</p>	 <p>http://www.planktonforum.eu/index.php?id=33&no_cache=1&tx_pydb_pi1%5Bdetails%5D=2621&tx_pydb_pi1%5Bimage%5D=28334&L=1&cHash=84e3de74b09083cf93ac857c1b90ac08</p>
<p>Asteromphalus (Asterolamprales)</p>	 <p>King County http://green.kingcounty.gov/marine/IndividualPhoto.aspx?SpeciesID=357&TypeId=1</p>

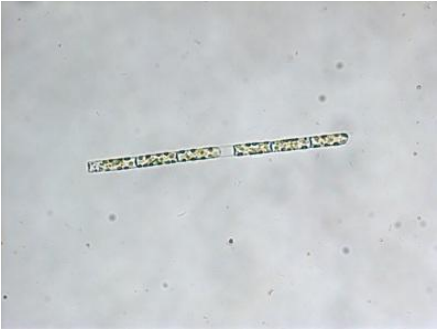
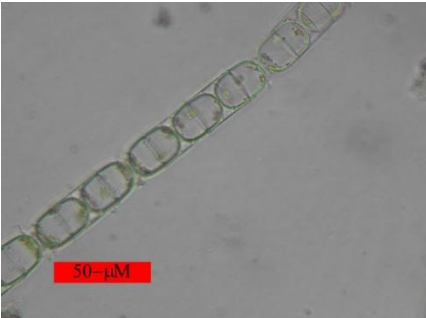
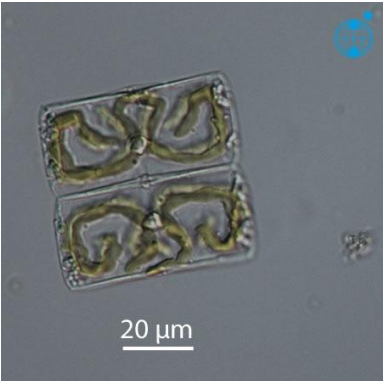

<p>Bacillaria (Bacillariales)</p>	 <p>http://westerndiatoms.colorado.edu/taxa/genus/Bacillaria</p>
<p>Bacteriastrum (Chaetocerotanae)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Diatoms/Bacteriastrum.html</p>
<p>Biddulphia (Biddulphiales)</p>	 <p>http://www.mbari.org/staff/conn/botany/phytoplankton/dia_Biddulphia_sp.htm</p>
<p>Cerataulina (Hemiaulales)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Diatoms/cerataulina.html</p>

<p>Chaetoceros (Chaetocerotanae incertae sedis)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Diatoms/Chaetoceros.html</p>
<p>Coscinodiscus (Coscinodiscales)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Diatoms/Coscinodiscus.html</p>
<p>Cylindrotheca (Bacillariales)</p>	 <p>http://phytoplanktonguide.lumcon.edu/display.asp?dtype=image&ID=2407</p>
<p>Cymbella (Cymbellales)</p>	 <p>http://protist.i.hosei.ac.jp/pdb/images/heterokontophyta/Raphidineae/Cymbella/Cymbella.jpg</p>
<p>Dactyliosolen (Rhizosoleniales)</p>	 <p> http://green.kingcounty.gov/marine/IndividualPhoto.aspx?SpeciesID=377&T</p>

	ypelD=1
Ditylum (Lithodesmiales)	 http://cimt.ucsc.edu/hab%20id/phytolist_diatoms/15ditylum.html
Diploneis (Naviculales)	 http://www.diatomloir.eu/Site%20Diatom/Cretdiatros.html
Eucampia (Hemiaulales)	 http://oceandatacenter.ucsc.edu/PhytoGallery/Diatoms/Eucampia.html
Fragilaria (Fragilariales)	 http://protist.i.hosei.ac.jp/pdb/images/Heterokontophyta/Araphidineae/Fragilaria/Fragilaria.jpg

<p>Fragilariopsis (Bacillariales)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Diatoms/thalassionema.html</p>
<p>Grammatophora (Striatellales)</p>	 <p>http://university.uog.edu/botany/474/diatoms/grammatophora.htm</p>
<p>Guinardia (Rhizosoleniales)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Diatoms/guinardia.html</p>
<p>Gyrosigma (Naviculales)</p>	 <p>http://upload.wikimedia.org/wikipedia/commons/c/cb/Gyrosigma_sp.jpeg</p>

<p>Haslea (Naviculales)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Diatoms/stephanopyxis.html</p>
<p>Hemidiscus (Coscinodiscales)</p>	 <p>http://www.niobioinformatics.in/images/pytoplankton/Hemidiscus%20hardmanian.jpg</p>
<p>Lauderia (Thalassiosirales)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Diatoms/lauderia.html</p>

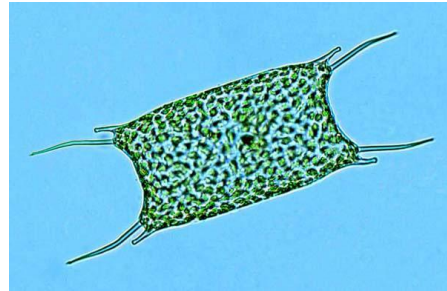
<p>Leptocylindrus (Leptocylindrales)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Diatoms/leptocylindrus.html</p>
<p>Manguinea (Naviculales)</p>	<p>No image found</p>
<p>Melosira (Melosirales)</p>	 <p>http://arch.ced.berkeley.edu/hiddenecologies/?page_id=170</p>
<p>Meuniera (Naviculales)</p>	 <p>http://planktonnet.awi.de/index.php?contenttype=image_details&itemid=60211#content</p>
<p>Navicula (Naviculales)</p>	 <p>http://protist.i.hosei.ac.jp/pdb/images/heterokontophyta/Raphidineae/Navicula/sp_3.jpg</p>

Nitzschia (Bacillariales)



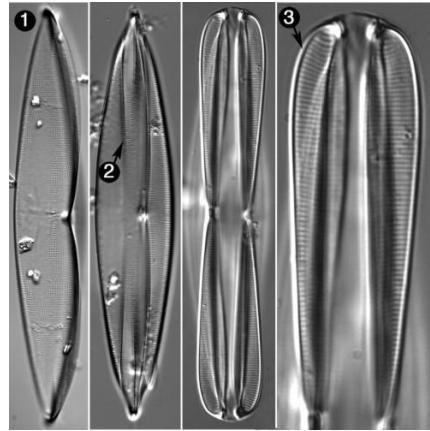
http://protist.i.hosei.ac.jp/PDB/Images/heterokontophyta/raphidineae/nitzschia/lorenziana/sp_03.jpg

Odontella (Triceratiales)




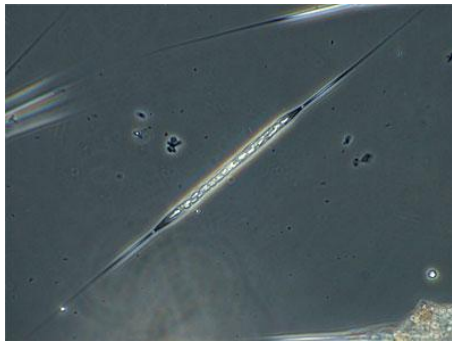


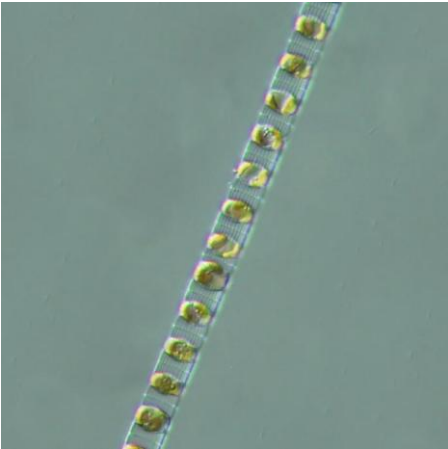
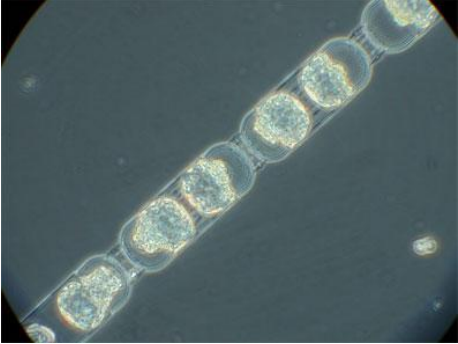

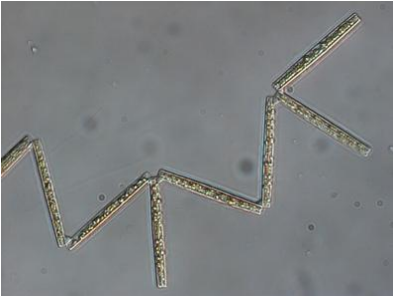
http://cfb.unh.edu/phycokey/Choices/Bacillariophyceae/Centric/Centric_ Uni cells/ODONTELLA/Odontella_Image_page.html

Plagiotropis (Naviculales)




<http://westerndiatoms.colorado.edu/taxa/genus/Plagiotropis>

<p>Pleurosigma (Naviculales)</p>	 <p>http://biology.missouristate.edu/phycology/asian%20carp/pleurosigma%20(20x)-w.jpg</p>
<p>Pseudonitzsca (Bacillariales)</p>	 <p>http://biology.missouristate.edu/phycology/asian%20carp/pleurosigma%20(20x)-w.jpg</p>
<p>Rhaphoneis (Rhaponeidales)</p>	 <p>http://botany.natur.cuni.cz/skaloud/Bac_araphid/images/PNrhaamp1.jpg</p>
<p>Rhizosolenia (Rhizosoleniales)</p>	 <p>http://cimt.ucsc.edu/hab%20id/phytolist_diatoms/24rhizosolenia.html</p>


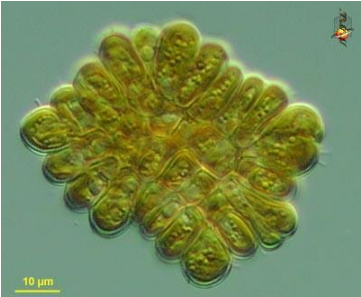
<p>Skeletonema (Thalassiosirales)</p>	 <p>http://nordicmicroalgae.org/taxon/Skeletonema%20marinoi?media_id=Skeletonema%20marinoi_1.JPG</p>
<p>Stephanopyxis (Melosirales)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Diatoms/stephanopyxis.html</p>
<p>Synedra (Fragilariales)</p>	 <p>http://www.ohio.edu/plantbio/vislab/algaeimage/pages/Synedra.html</p>
<p>Thalassionema (Thalassionematales)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Diatoms/thalassionema.html</p>

<p>Thalassiosira (Thalassiosirales)</p>	 <p>http://www.marinespecies.org/photogallery.php?album=1033&pic=39660</p>
<p>Tropidoneis (Lithodesmiales)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Diatoms/tropidoneis.html</p>

Kingdom: Chromista > Phylum: Ochrophyta > Class: Dictyochophyceae

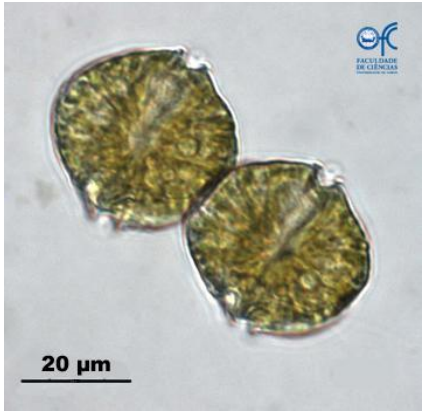


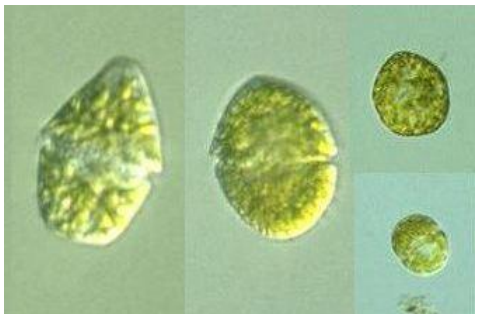
Name (Order)	Picture
<p>Dictyocha (Dictyochaales)</p>	 <p>King County http://green.kingcounty.gov/marine/IndividualPhoto.aspx?SpeciesID=253&TypeID=3</p>




Kingdom: Chromista > Phylum: Ochrophyta > Class: Chrysophyceae


Name (Order)	Picture
Dinobryon sociale (Chromulinales)	 <p>http://cfb.unh.edu/phycokey/Choices/Chrysophyceae/colonial_chrysophyceae/flagellated/DINOBRYPON/Dinobryon_Image_page.htm</p>
Phaeoplaca (Chromulinales)	 <p>http://pinkava.asu.edu/starcentral/microscope/portal.php?pagetitle=assetfactsheet&imageid=2631</p>

Kingdom: Chromista > Phylum: Myzozoa > Class: Dinophyceae


Name (Order)	Picture
Akashiwo (Gymnodiniales)	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Dinoflagellates/akashiwo.html</p>

<p>Alexandrium (Gonyaulacales)</p>	 <p>http://planktonnet.awi.de/index.php?contenttype=image_details&itemid=35204#content</p>
<p>Ceratium (Gonyaulacales)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Freshwater/Ceratium.html</p>
<p>Dinophysis (Dinophysiales)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Dinoflagellates/dinophysis.html</p>
<p>Gymnodinium (Gymnodiniales)</p>	 <p>http://protist.i.hosei.ac.jp/pdb/images/Mastigophora/Gymnodinium/Gymnodinium.jpg</p>

<p>Karlodinium (Gymnodiniales)</p>	 <p>King County http://green.kingcounty.gov/marine/IndividualPhoto.aspx?SpeciesID=480&TypeID=2</p>
<p>Lingulodinium (Gonyaulacales)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Dinoflagellates/lingulodinium.html</p>
<p>Noctiluca (Noctilucales)</p>	 <p>http://www.imas.utas.edu.au/_data/assets/image/0007/271636/noctiluca_full.jpg</p>
<p>Prorocentrum (Prorocentrales)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Diatoms/thalassionema.html</p>

<p>Pyrocystis (Pyrocystales)</p>	 <p>http://oceandatacenter.ucsc.edu/PhytoGallery/Dinoflagellates/pyrocystis.html</p>
---	---

Kingdom: Chromista > Phylum: Cryptophyta > Class: Cryptophyceae

Name (Order)	Picture
<p>Chroomonas (Pyrenomonadales)</p>	 <p>http://protist.i.hosei.ac.jp/pdb/images/mastigophora/Chroomonas/sp_03.jpg</p>

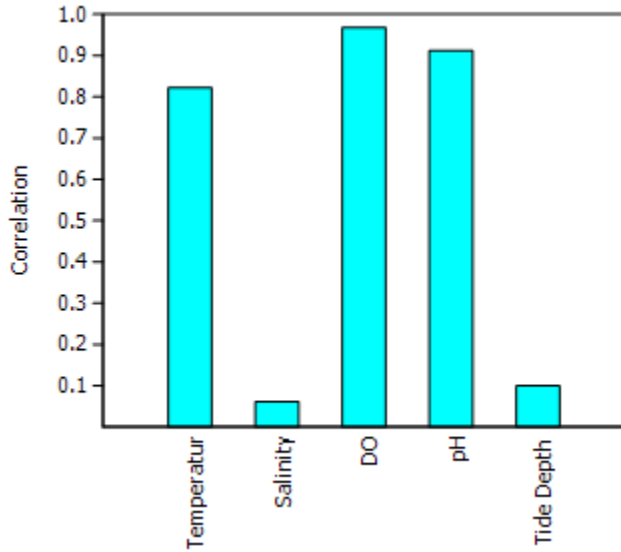
Kingdom: Plantae > Phylum: Rhodophyta > Class: Stylonematophyceae

Name (Order)	Picture
<p>Rhodorus (Stylonematales)</p>	 <p>https://ncma.bigelow.org/ccmp1338</p>

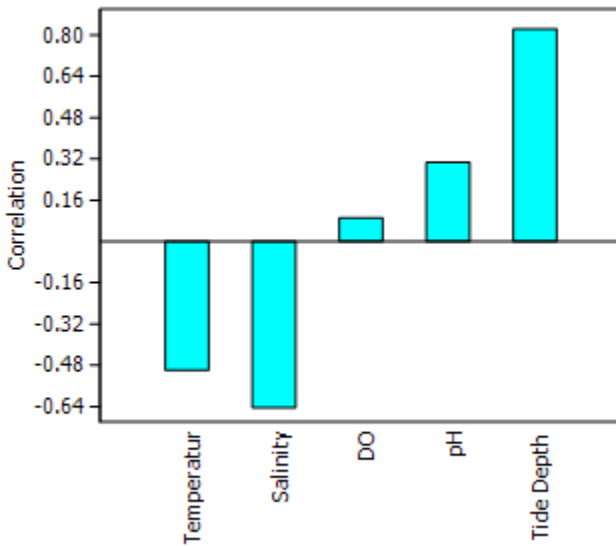
Appendix C: PCA Results

East Channel

East Channel PCA Loadings:

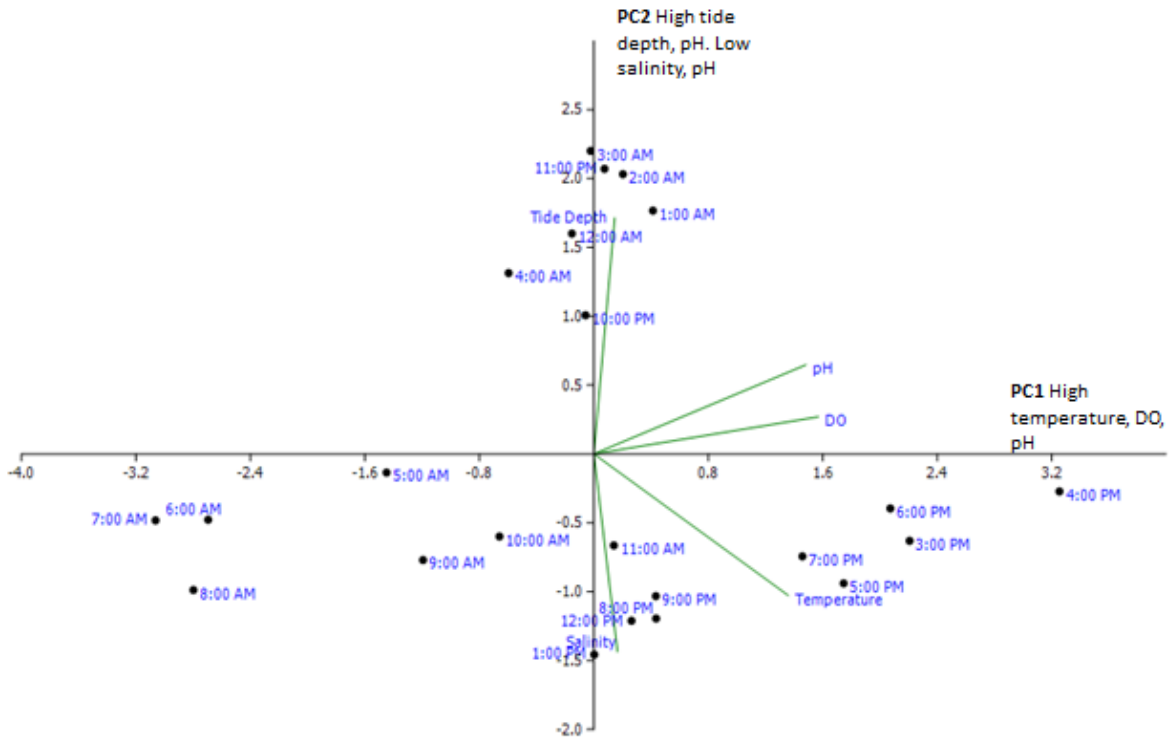


Principal Component 1: Accounts for 49% of the variability in the abiotic data.



Principal Component 2: Accounts for 29% of the variability in the abiotic data.

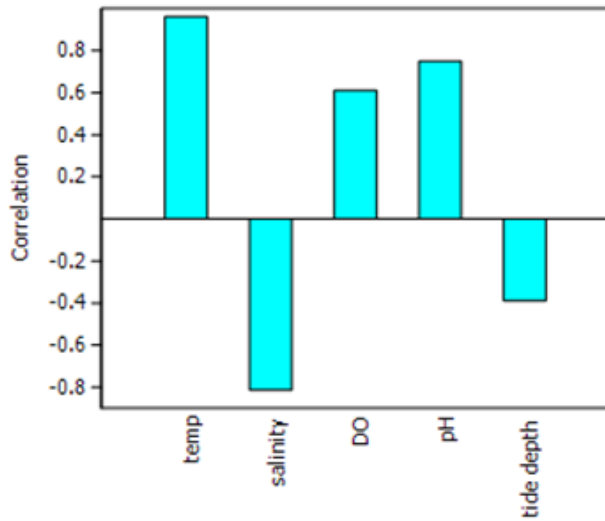
East Channel



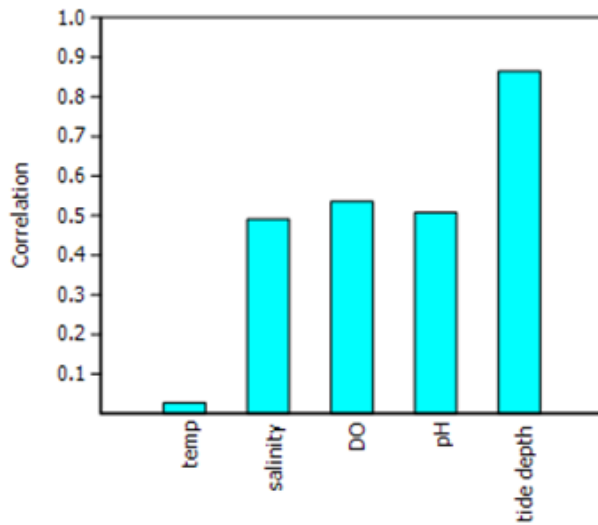
Biplot of the correlation between the times at which samples were obtained in the East Channel and the abiotic factors present at that time. Abiotic factors shift with time in a counter-clockwise direction with respect to PC1 and PC2. Beginning at 3PM until about 7PM, high DO, pH, temperature, and salinity appeared to dominate abiotic conditions, followed by decreasing temperatures and high tidal depth with low salinity around midnight to early morning. During the morning hours, the east channel experienced low DO, pH, and temperature with moderate salinity levels and low tidal depth. Finally, DO, pH, and temperature appeared to increase in the afternoon again.

West Channel

West Channel PCA Loadings:

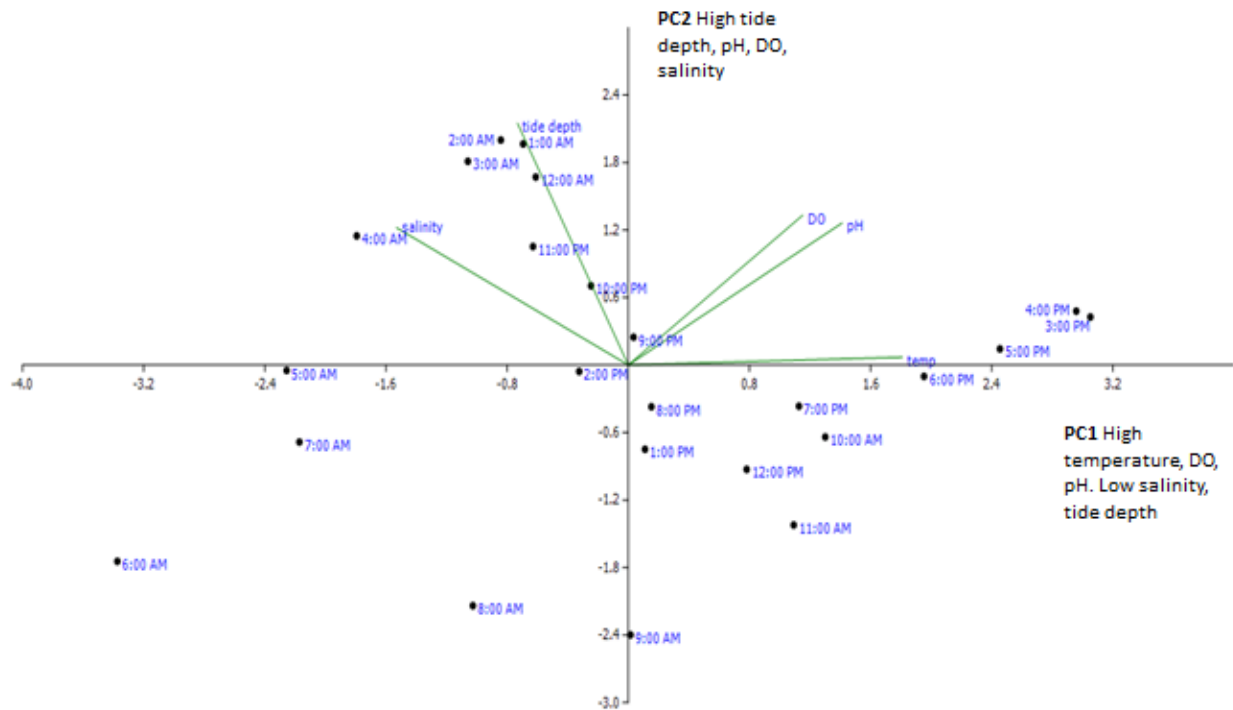


Principal Component 1: Accounts for 53% of the variability in the abiotic data.



Principal Component 2: Accounts for 31% of the variability in the abiotic data.

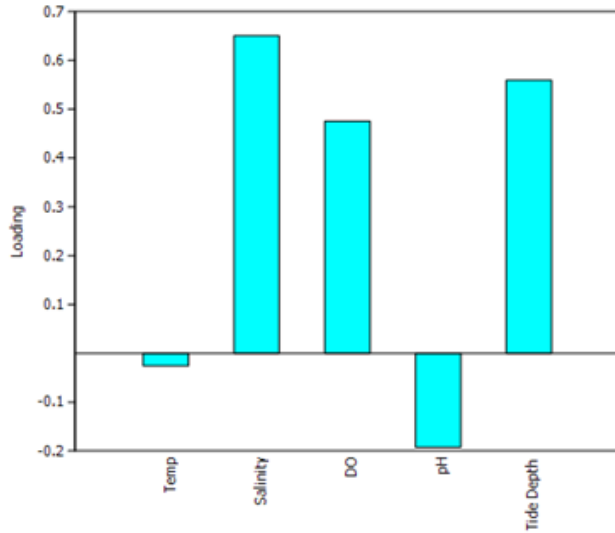
West Channel



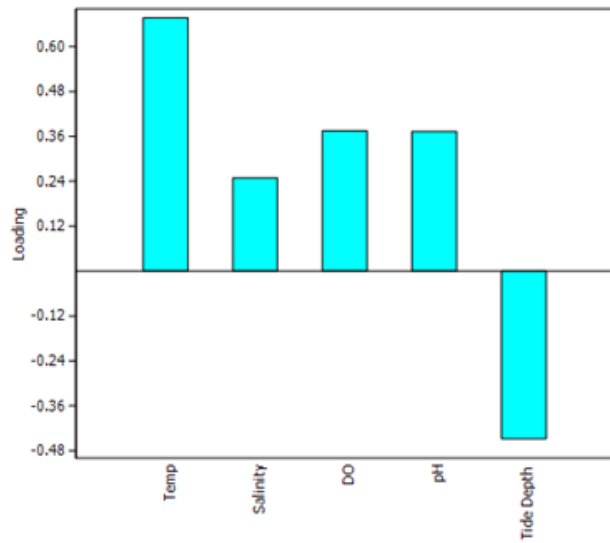
Biplot of the correlation between the times at which samples were obtained in the West Channel and the abiotic factors present at that time. At the initial sampling times during the late afternoon to early evening, high temperature, DO, pH and low salinity and low tidal depth dominated abiotic conditions. During midnight to early morning, the west channel had low temperature, low pH, and low DO concentrations and high tidal depth and high salinity. The abiotic conditions gradually then returned to the initial sampling conditions.

Creek

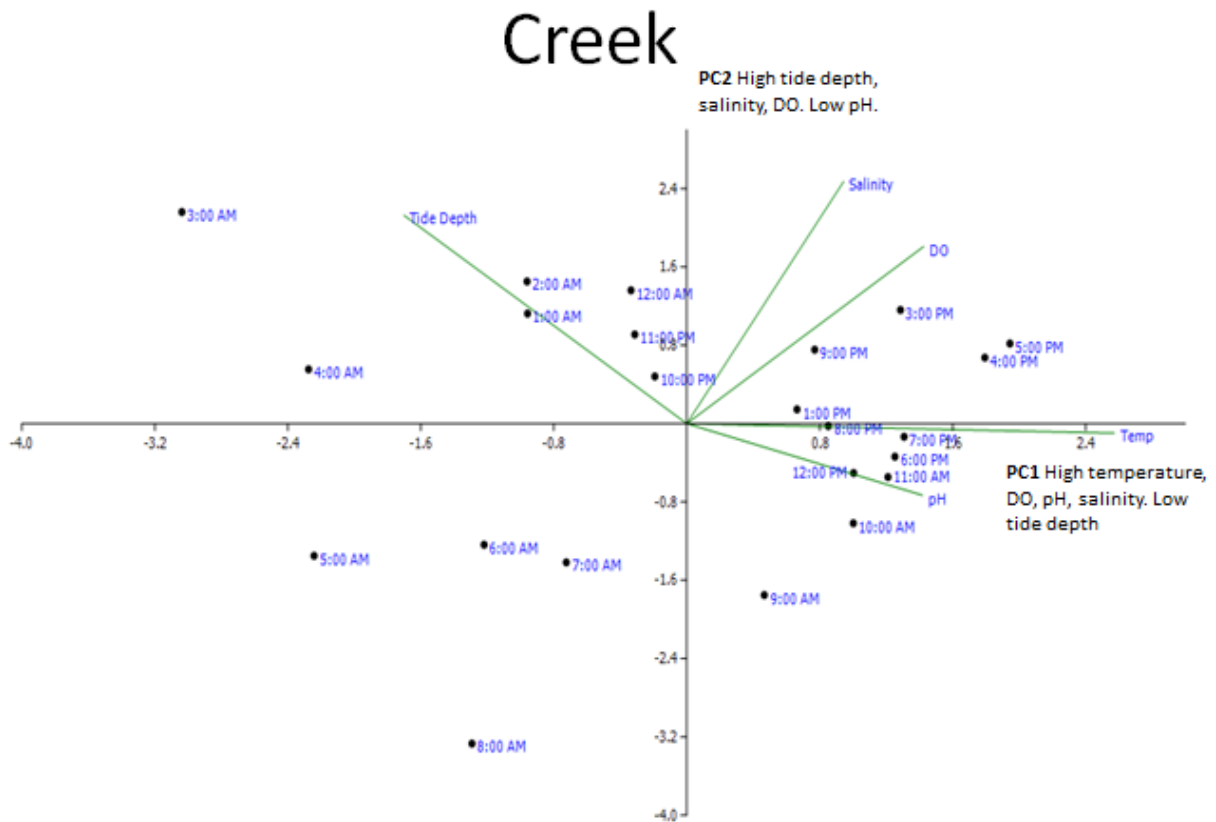
Creek PCA Loadings:



Principal Component 1: Accounts for 39% of the variability in the abiotic data.



Principal Component 2: Accounts for 32% of the variability in the abiotic data.



Biplot of the correlation between the times at which samples were obtained in the Creek and the abiotic factors present at that time. Abiotic factors shift with time in a counter-clockwise direction with respect to PC1 and PC2. At the initial sampling times during the late afternoon and evening, conditions were characterized by high temperature, DO, pH, and moderately low salinity and low tidal depth. During the early morning, tidal depth increased and DO concentrations, pH, temperature and salinity were low. Later in the morning, tidal depth decreased followed by a gradual return of the other abiotic conditions to initial conditions.

Appendix D
Raw data- Separate Excel file