WHO IS CHLOROPHYLL A? PHYTOPLANKTON COMMUNITY STRUCTURE IN HUMBOLDT BAY, CALIFORNIA

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ABSTRACT

WHO IS CHLOROPHYLL A? PHYTOPLANKTON COMMUNITY STRUCTURE IN HUMBOLDT BAY, CALIFORNIA

Gregory D. O’Connell

Phytoplankton community descriptions are becoming an increasingly important component in studies of oceanographic and nearshore marine ecosystems. These primary producers play a major role in the global carbon cycle and are the foundation of nutritional resources that are transferred up the food chain. The lack of a phytoplankton community description from Humboldt Bay, CA (USA) limits the understanding of primary producers within the bay and such a study would be useful to mariculture operations so that they may have a better idea of what their animals eat, including species known to form harmful algal blooms (HABs). This study seeks to describe the phytoplankton community from a long term monitoring site within Humboldt Bay along seasonal and tidal gradients and to document the occurrences of species known to produce toxins. Samples were collected from April 2007 through October 2008 nearly once per week at high and low tide. A total of 71 taxa were found in 108 samples with diatoms composing 91% of the total cell numbers (86% of total biovolume) whereas dinoflagellates made up 2% of cell numbers (17% of the biovolume). *Thalassiosira, Odontella and Noctiluca* composed 70% of the total biovolume for the entire sampling period and the top 15 contributors accounted for over 85 % of total biovolume. More
than half of the taxa accounted for less than 1% of the total biovolume. Community structure varied the most by seasonal gradients with different communities occurring in the upwelling season of 2007, the winter of 07-08 and the upwelling season of 2008. The winter had lower biovolume with a higher tychoplankton component than the upwelling season. Upwelling 2007 had lower biovolume and had a stronger dinoflagellate signal than 2008 corresponding with a transition from El Niño to La Niña conditions. The 2008 upwelling season had the highest biovolume and was primarily composed of bloom taxa such as *Thalassiosira*, *Chaetoceros* and *Odontella*. Community differences between high and low tide, although not as strong as seasonal differences, were driven by transport of nearshore oceanic taxa on the incoming tide and resuspension of benthic and epiphytic diatoms on the outgoing tide. Although major HAB events did not occur locally during the sampling period, *Alexandrium* and *Pseudo-nitzschia* abundance paralleled their respective toxin concentrations from mussel samples within the bay.
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INTRODUCTION

Phytoplankton are a major source of energy on the planet. Virtually all organic matter in the Earth’s carbon cycle originates from photosynthetic forms of life and almost half of this carbon is fixed by marine phytoplankton (Field et al. 1998). From the Greek roots *phyto* (plant) and *plankto* (to wander), these microscopic organisms are at the mercy of ocean currents and their blooms may advect to new locations, providing resources that link ecosystems at large spatial scales. Their importance has been reemphasized by the industrial age increase in atmospheric CO$_2$ and its resulting ocean acidification since phytoplankton, along with other marine photoautotrophs, may be able to partially mitigate the acidification effect by increasing the rate at which they fix aqueous CO$_2$ (Boden et al. 2009, Falkowski 2012, Koch et al. 2013). Phytoplankton communities contain taxa that are phylogenetically and nutritionally diverse, ranging from cyanobacteria to every major clade of eukaryotic algae except for most rhodophytes and phaeophytes (Falkowski and Raven 1997). They range in size from sub-micron picoplankton to cells that are hundreds of micrometers in width; the latter may combine to form even longer colonies. Blooms (i.e. rapid population growth rate) tend to occur seasonally and are favored when light and nutrients are abundant (Karentz and Smayda 1984, Cloern et al. 1985, Clarke and Leakey 1996, Casas et al. 1999). Some of these same phytoplankton species can produce poisonous secondary metabolites giving rise to harmful algal blooms (HABs).

Studies of marine phytoplankton communities have focused on identifying general patterns in the spatial and temporal distributions of species or functional group
assemblages and, secondly, in understanding the forcing variables that drive assemblage
dynamics (Cupp 1943, Verduin 1951, Odate 1996, Johnson and Costello 2002, Cloern
Horner et al. 2005). The search for general assemblage patterns of species has met with
limited success because localized oceanographic conditions, coupled with an
unpredictable taxonomic identity of dispersal propagules and resting cysts, may combine
to produce unpredictable fine scale phytoplankton community structures (Cloern and
Nichols 1985, Cloern et al. 1985, Smayda and Reynolds 2001). When comparing long
term data sets of phytoplankton species abundance, Venrick (1998) states that “the
common denominators within and among these studies are variability and complexity.”
Allen’s (1939) long term observations from the Scripps Institution of Oceanography Pier
led him to state that “our records show no two years alike in the twenty, no two months
alike, and no two weeks alike.”

There has been more success in identifying the spatial and temporal patterns of
larger taxonomic and functional groups of marine plankton. Phytoplankton communities
off the western coast of the United States can be categorized as either offshore or
nearshore communities (Venrick 2009). The California Cooperative Fisheries
Investigations (CalCOFI) has surveyed the California current system for zooplankton,
ichthyoplankton and phytoplankton for decades (Venrick 2002). CalCOFI ship transects
out to 450 km offshore reveal distinct nearshore and offshore phytoplankton
communities. Warmer waters (>13°C) located 150 km or more offshore support
phytoplankton assemblages of coccolithophorids, dinoflagellates and a few diatoms
whereas colder, upwelled coastal assemblages include large colonial diatoms such as Chaetoceros, Pseudo-nitzschia, Thalassiosira, Skeletonema and Thalassionema (Venrick 2009). The latter composition of the nearshore community also applies to Northern California and southern Oregon (Hood et al. 1990, Chavez et al. 1991, Moses and Wheeler 2004, Lassiter et al. 2006). These nearshore locations lie near the longitudinal center the California Current, which is a transitional body of water that separates the central sub-arctic near Canada and the central Pacific extending into Mexico (Venrick 1971). Temporally, nearshore assemblages are affected by seasonal upwelling and therefore shift from being diatom dominated during the spring and early summer to dinoflagellate dominance in the late summer following upwelling relaxation and water column stratification (Smayda and Reynolds 2001, Smayda and Trainer 2010, Corcoran and Shipe 2011). While these observations are useful for ecosystem generalizations about which major phytoplankton groups are likely to be present, generalizations about the spatial and temporal dynamics of genera and species have not been possible. The latter is important for assessing risks from HABs.

HABs are responsible for at least $82 million per year in negative economic affects in the U.S.A. and untold costs to noncommercial ecosystems (Hoagland and Scatassa 2006). HAB taxa are a polyphyletic group of organisms with diverse mechanisms of harm (Table 1). Many produce toxins but others cause harm by means of gill clogging, aerobic decay or surfactant production. Use of the term “red tide” is misleading as not all red tides are harmful and not all harmful blooms are red. The California Department of Public Health’s (CDPH) Marine Biotoxin Monitoring program
focuses on the dinoflagellate *Alexandrium* and the diatom *Pseudo-nitzschia*. Some species of *Alexandrium* produce saxitoxins and similar analogs responsible for paralytic shellfish poisoning (PSP) whereas species and varieties of *Pseudo-nitzschia* may produce domoic acid that causes amnesic shellfish poisoning (ASP). Recent events along the west coast warrant expanding this watch list to other taxa. For instance, a massive invertebrate die-off occurred in the shallow waters of the Sonoma County, CA coastline in August of 2011. Although a definitive cause of the mortality has not be ascertained, a coinciding bloom of the dinoflagellate *Gonyaulax spinifera* and low, but detectable, yessotoxin levels suggests that *Gonyaulax* was responsible (Rogers-Bennett *et al.* 2012). While many west coast harmful blooms originate offshore, they can be advected to nearshore and estuarine-like habitats where humans are more likely to notice their impact (Lewitus *et al.* 2012).

The degree to which the spatial and temporal patterns of nearshore phytoplankton communities, including HAB taxa, are also seen within bays and estuaries depends upon the extent of the physical connection between the two systems, the geomorphology of the inlet and its surrounding watersheds, as well as the nature of local anthropogenic influences (Cloern *et al.* 1985, Hickey and Banas 2003, Marshal *et al.* 2005). For example, Grays Harbor and Willapa Bay in Washington and Coos Bay in Oregon are shallow embayments with large tidal prisms (Hickey and Banas 2003). These characteristics result in well-mixed, tidally driven systems with high flushing rates that correlate with nearshore colder, saline oceanic water from the spring through fall (Hickey and Banas 2003). Coos Bay receives coastally derived chlorophyll on the flooding tide
coincident with the influx of water with lower water temperatures and higher salinities (Roegner and Shanks 2001). In contrast, larger and deeper bays with a more restricted tidal influence, such as San Francisco Bay, have entrances with nearshore qualities but the central and far reaches are more associated with stratified, density driven exchange, especially during seasonal increases of freshwater (Cloern and Nichols 1985).

As a consequence, seasonal assemblage dynamics occurring in nearshore waters can be expected also to occur in those areas of a bay or estuary that rapidly exchange water with the open ocean. Additional assemblage patterns within bays are possible due to tidal and wind waves. The latter suspend microalgae, particularly epipelic and epiphytic diatoms, into the water column where they are known as tychoplankton (tycho-by chance) in contrast to algal cells that originate in the water column (euplankton) (Lucas et al. 2001). The potential for tychoplankton to occur in an estuary during low tide is greater than at high tide because of the higher water velocities preceding the low tide and the source of that water (Vos and Wolf 1993, Sancetta 1989). The spatial and temporal differences in the water temperature, salinity and nutrient regime form another kind of gradient that can structure phytoplankton assemblages within inlets (Cloern and Dufford 2005). For example, the chrysophyte *Aureococcus anophagefferens* blooms in Long Island Sound when supplied with anthropogenic nitrogen (Laroche et al. 1997). In comparison to other west coast inlets, phytoplankton in Humboldt Bay should be among the most affected by the seasonal dynamics in the adjacent nearshore waters, a tidal gradient, and possibly a summer lagoon effect in northern Humboldt Bay. The bay is
shallow and is not considered an estuary because it does not receive the immediate

Located 115 km south of the Pt. St. George upwelling center and 50 km north of
Cape Mendocino, Humboldt Bay is the only major port along a span of 700 km of coast.
An entrance channel to the open ocean allows rapid turnover of water in much of the bay
but longer residence time may create a lagoon effect in the northern bay. This unique
embayment is important habitat for fish, invertebrates and migratory birds (Barnhart et al.
1992) yet an understanding of its primary producers is limited. Whereas the
understanding of the eelgrass community is improving (Shaughnessy et al. 2008), there
have been few studies of phytoplankton. These have been about chlorophyll variability
in the water column, which is a proxy for phytoplankton biomass. Temporally, bay
chlorophyll is positively correlated with flood tides and often peaks at 10µg/L by the
early summer, whereas nearshore values may surpass 20µg/L during this time of the year
(Pequegnat and Butler 1981, Barnhart et al. 1992). In contrast, winter ebb tide
chlorophyll concentrations are occasionally higher in the bay than outer coast raising the
possibility that some of the phytoplankton originates in the bay rather than being
imported into the bay from the outer coast (Pequegnat and Butler 1981). The limited data
describing chlorophyll dynamics in Humboldt Bay suggest the phytoplankton
assemblage, which has never been described for the bay, could be temporally
heterogeneous. An investigation into the phytoplankton community is needed to
ascertain which organisms are producing these chlorophyll signatures and to document
the presence and patterns of species known to produce HABs. This is especially
important considering that over half of the commercially grown oysters in California are produced in Humboldt Bay, adding over $6 million each year to the local economy (Pomeroy et al. 2010).

The objective of this study is to conduct a rigorous temporal description of the phytoplankton community at a monitoring site (Dock B) operated by the Central and Northern California Ocean Observing System (CeNCOOS) in the central part of Humboldt Bay. I will be testing six hypotheses:

1. Phytoplankton community structure changes seasonally as has been described by other studies from the region (Cloern et al. 1985 and Lassiter et al. 2006).

2. Phytoplankton community structure is composed of distinct high and low tide assemblages similar to other embayments (Sancetta 1989, Miller and Harding 2007).

3. Given that the central part of the bay is close to Entrance Channel, euplankton should be a greater portion of phytoplankton biomass (as biovolume) than tychoplankton at all times during the year regardless of tidal stage (Hood 1990, Chavez et al. 1991, Lassiter et al. 2006).

4. Due to tidal resuspension of sediments, tychoplankton should be more abundant at low tide than at high tide (Sancetta 1989, Lucas et al. 2001, Miller and Harding 2007).

5. Concentrations of domoic acid and PSP-saxitoxins measured by the CDPH in Humboldt Bay parallel abundances of the HAB species known to produce those toxins.
6. The phytoplankton community is dominated by taxa larger than 10 µm (Cole et al. 1986). This hypothesis is being tested in order to determine if my collection methods are capturing the majority of the community.
METHODS

Study Site

Humboldt Bay (40°46′N, 124°12′W) is a shallow, multibasin, coastal lagoon with limited freshwater input. Its surface area spans 66 km² at high tide but at least half of that area drains during low tides revealing shallow mudflats connected by subtidal channels. Arcata Bay, Entrance Channel, and South Bay are the sub-basins that compose Humboldt Bay (Figure 1). The region has mixed semi-diurnal tides that range in excess of +2.5 m above MLLW to below -0.6 m. The continental shelf outside the bay is relatively narrow and slopes to a depth of about 150 m at 16 km offshore, then drops precipitously. There are rivers to the north (Mad River) and south (Eel River) of Humboldt Bay that deposit high sediment loads directly into the ocean during winter months (Milliman and Syvitski 1992, Syvitski and Morehead 1999). These rivers coupled with fine sediments from the Humboldt Bay mudflats create turbid nearshore and bay waters for much of the year. Dock B (also known as Commercial Dock B and the Carvelo Dock) is situated on the east side of a bottle neck that funnels the outgoing tide of Arcata Bay into the narrow passage leading to Entrance Bay (Figure 1).

Sample Collection

Water samples were usually collected once per week at Dock B at high tide and low tide during daylight hours from April 2007 through October 2008. A plankton tow (63 μm mesh) for obtaining taxonomically useful images of living organisms was obtained during most sample dates. Additionally, two 3.2 L PVC horizontal Van Dorn
water bottles were used to collect water samples for cell counts and chlorophyll a quantification. Each sample was taken at the same time that the CeNCOOS sonde (Yellow Springs Instruments, YSI mo 6600) was recording water quality parameters. Water samples were transferred to opaque 1L plastic bottles and immediately brought back to the lab in a cooler. A 1L portion of water was fixed with 20 mL of formalin and stored for phytoplankton enumeration.

*Cell Counts and Bio-volume Estimations*

Cells were counted utilizing the Utermöhl method on an Olympus CK 2 inverted microscope (Utermöhl, 1958). Between 10-50 mL, depending on the amount of sediment in the sample, was settled for 24 hours in a Hydrobios settling chamber. The entire settling plate was counted at 200x magnification and cells were viewed at 400x when necessary. Microscope images of live cells that were taken of each fresh sample which assisted with identification. Species were identified using Cupp (1943), Rines and Hargraves (1988), Tomas (1997) and Horner (2002). Current taxonomic nomenclature was achieved using algaebase.org. *Pseudo-nitzschia* spp. were categorized as either *P. delicatissima* or *P. seriata* size classes based on cell width (Seubert *et al.* 2013) because species level identification with scanning electron microscopy was unsuccessful. Due to frequent high sediment loads that obscured image resolution during cells counts, nondescript species of dinoflagellates, some pennate diatoms and members of the centric diatom *Thalassiosira* were recorded by size-class. For this same reason, cells smaller than 10 µm were not counted, therefore avoiding small flagellates that did not preserve
well. The percent of the total chlorophyll from organisms smaller than 10 µm was determined in order to know the percent of the phytoplankton community being described by this study.

The length and width of each taxon was recorded for each sample so that biovolume equations could be used to estimate total biovolume per cell, which could then be scaled up to the total biovolume (µm³/L) of that taxon in a water sample (Hillebrand et al. 1999, Cloern and Dufford 2005). Contemporary studies of this type are increasingly utilizing published formulas for taxon specific microalgal cell volumes to convert cell abundances into biovolume. Biovolume estimates are more ecologically meaningful than cell counts because they standardize the potential contribution of biomass that organisms of various sizes may contribute in a given sample (Menden-Deuerand and Lessard 2000).

Chlorophyll a Quantification

The amount of chlorophyll a in the Dock B water column was determined for two reasons. First, chlorophyll a was extracted from the water in order to validate raw fluorescence estimates made by the Dock B YSI sonde. Validation was necessary before making comparisons between the Dock B fluorescence time series versus measures of phytoplankton abundance (Appendix A). Secondly, since cells smaller than 10 µm were not counted, the amount of chlorophyll a that passed through a 10 µm net was compared to the total chlorophyll a in order to get an estimate of what percent of the total phytoplankton community would be described by only counting cells greater than 10 µm.
Therefore, three 500 mL replicate water samples were filtered through 47 mm diameter GF/F filter disks with a pore size of 0.7 μm to quantify chlorophyll a from each sample time. Three additional 500 mL water samples were passed through a 10 μm net to quantify the portion of chlorophyll that came from organisms less than 10 μm. Filter disks were placed in polypropylene centrifuge tubes and stored in a freezer for benchtop fluorometric analysis. Pheophytin corrected chlorophyll a concentrations were obtained by adding 9.0 mL of 90% acetone to the centrifuge tubes and allowing them to soak for 18-24 hours. Samples were run on a Trilogy fluorometer (Turner Designs) with a chlorophyll a acidification module that was calibrated with dilutions of spinach derived chlorophyll a from Sigma Aldrich. Fluorescence was read before and after acidifying with 2 drops of 10% HCl (Arar and Collins 1992, Lassiter et al. 2006). Chlorophyll a by size class was quantified for samples in which cells were counted but almost half the chlorophyll data were thrown away due to a questionable fluorometer calibration.

*California Department of Public Health Data*

Domoic acid and PSP toxin levels from sentinel mussel tissue samples within Humboldt Bay were obtained from the CDPH’s Marine Biotoxin monitoring program. These toxin levels were compared to cell counts of the relevant HAB taxa that occurred at Dock B.

*Environmental Data*

Water quality parameters recorded by the YSI sonde operated by CeNCOOS at Dock B were correlated to phytoplankton community dynamics. These parameters
included water temperature, salinity, depth, turbidity and chlorophyll fluorescence; each variable has been recorded every 15 minutes since 2003. Quality control of the 2007 and 2008 sonde data, which spans the period when water samples were taken for the phytoplankton community description, included the check that all time stamps were in GMT; removal of values when it became apparent that probes for specific variables were not functioning correctly (e.g., broken wipers on the optical probes of turbidity and chlorophyll fluorescence); corrections to water depth so that depth was always expressed in feet relative to Mean Lower Low Water (MLLW); removal of values recorded when the sonde was taken out of the water; and conservative corrections of turbidity when it drifted below 0.0 NTU. Missing data occurred due to data quality control, equipment failure and when the sonde was removed every three weeks for calibration. Procedures for filling in these missing data depended upon the water quality parameter. Since observed water depths at the NOAA station on the North Spit of Humboldt Bay (ID: 9418767) parallel those at Dock B, North Spit depths were used in place of missing Dock B depths. Turbidity values recorded by the YSI sonde operated by the Wiyot Tribe on the NE corner of Indian Island also qualitatively parallel those at Dock B and so were inserted as necessary. Regression relationships were used to estimate missing data for water temperature ($\ln(\text{Dock B Water Temperature}) = 0.453 + (0.800 \times \ln(\text{Indian Island Water Temperature}))$, $R^2 = 0.94$) and salinity (Dock B Salinity = -20.390 + (2.647 * Indian Island Salinity) - (0.0315 * Indian Island Salinity $^2$), $R^2 = 0.84$). There was no reliable method for deriving missing values of chlorophyll fluorescence.
It was necessary to define the transitions among the oceanic seasons as they occurred during 2007 and 2008 so that these seasons could be compared in subsequent statistical analyses. A local upwelling index, based primarily on nearshore winds (NOAA buoy 46022), was developed for this purpose. These transitions were also compared to the chlorophyll fluorescence time series from Dock B in order to see if the seasons as identified by the upwelling index corresponded to the timing of actual phytoplankton production. Water temperature and salinity data from Dock B were also presented in order to understand their seasonal and tidal variability.

Statistical Analyses

_Hypothesis 1:_ The community metrics of richness, evenness and Shannon-Wiener diversity were calculated for each season. Non-metric multidimensional scaling (NMS) ordination in PC-Ord 5 (McCune and Mefford 2011) was used to describe assemblage structure. Prior to running the ordination, taxa that occurred in less than 10% of the samples were removed and outliers of two or more standard deviations were also removed. The remaining matrix was transformed using Beals Smoothing Function and the Bray-Curtis dissimilarity measure was used to assess sample similarity (McCune _et al._ 2002, De Cáceres and Legendre 2008). In order to interpret the identities of ordination axes, the primary species matrix was joint plotted using a secondary matrix that included the same species plus derived water quality variables. An NMS axis potentially representing seasonal structure would, in a joint plot, be expected to correlate with turbidity, water temperature and salinity all of which have strong seasonal signatures.
in the bay. All three of these variables were calculated as means from two time scales; 24 and 120 hrs prior to each water sample time. Finally, multi response permutation procedure (MRPP) from PC-Ord 5 was used to test if significant assemblage differences existed among seasons and the indicator species analysis (ISA) was applied to determine if each season was typified by distinct species.

Hypothesis 2: The community metrics of richness, diversity and evenness were calculated for each of the two possible tidal stages. The ordination and MRPP approach used for Hypothesis 1 was also used to determine if tidal stage was an important structuring gradient for the assemblage. Water depth was used to describe the different ways that the tidal exchange in the bay could structure the phytoplankton community. The bay exchanges a greater volume of water with the ocean during spring tides than during neap tides. Two tidal range variables each at a different time scale (i.e., 120 hrs and 24 hrs prior to a sample time) were therefore calculated in order to describe the strength of the oceanic connection prior to sampling. The third variable was the depth at the time of sampling but this variable distinguished between the depth of flooding tide water, potentially carry more oceanic taxa, versus the depth of ebbing waters that could contain more taxa suspended from the mudflats in the northern bay. The last depth variable was the depth at the time of sampling because it is possible that greater water depths contain more oceanic taxa and lower depths contain more suspended taxa regardless of whether the tide is flooding or ebbing. These four depth variables were among the environmental variables included in the secondary ordination matrix. ISA and MRPP analyses were used to test for the effects of tidal stage on species assemblage.
**Hypothesis 3**: Species were assigned to either the euplankton or tychoplankton functional groups based on their described habitats (Cupp 1943, Tomas 1997, Horner 2002) in order to test the hypothesis that euplankton are most abundant regardless of season or tidal stage. Two sample $t$-tests were used to compare the abundance, as biovolume, of plankton to tychoplankton by each season and tidal stage.

**Hypothesis 4**: A two sample $t$-test, using only sample dates with paired high and low tides, was run to determine if tychoplankton make a greater contribution to total biovolume at low tide than they do at high tide.

**Hypothesis 5**: HAB taxa abundances from this study were compared with toxin concentrations from the CDPH using only descriptive graphics because CDPH samples did not necessarily occur at the same time as the water sampling in the present study.

**Hypothesis 6**: A $t$-test was used to determine if a greater portion of the photosynthetic community, as described by chlorophyll $a$, is composed of species larger than 10 um. All $t$-tests and assumptions of normality and equal variances in this study were performed using Sigma Plot (Systat Software 2008. San Jose, CA).
RESULTS

Oceanic Seasons

The cumulative upwelling index shows a positive slope beginning in March 2007 about six weeks before water sampling began in the present study (Figure 2A). Upwelling conditions persisted until early October 2007. From this time until mid March 2008, the cumulative curve plateaus with a no net gain in offshore flow. Upwelling resumed in late March of 2008 and continued to late October 2008. These three oceanic seasons will be referred to hereafter, respectively, as Upwelling 2007, Winter 07-08 and Upwelling 2008. The higher peaks of chlorophyll fluorescence usually occurred during each upwelling season and, within a tidal cycle, fluorescence levels were usually higher on the flood than on the ebb tide.

The salinity from Dock B varied during the sampling period from a high around 33 ppt to a low of about 14 ppt (Figure 2B). Salinity is positively correlated with tidal height for the entire sampling period, with high tide containing the highest salinity and low tide containing the lowest salinity. The salinity at Dock B is most variable during the winter months with the lowest salinity values associated with precipitation events and discharge from tributaries into the bay. The salinity at Dock B during the upwelling seasons had the least variation with salinity values staying above 30 ppt during all phases of the tide.

Water temperatures at Dock B varied during the sampling period with a high over 20°C and a low around 7°C (Figure 2B). Temperature during both upwelling seasons
was more variable than the winter season. Spring and especially summer water temperatures are more variable across a tidal cycle because flooding waters are cold whereas ebbing waters are much warmer. The reverse occurs during the winter when temperatures of flooding water are often warmer than ebbing waters, but the temperature difference between the two stages of the tide are not as great as during the spring and summer.

El Niño conditions started building during early 2006, they peaked during the fall of 2006, and then transitioned to La Niña conditions during the early summer of 2007 (Figure 2C, McClatchie et al. 2008, McClatchie et al. 2009). La Niña conditions still existed by the end of 2008.

**Community composition and abundance**

Seventy-nine species and taxa were observed in 108 water samples during the 18 month sampling period. Diatoms composed 91% of total cell numbers and 86% of total biovolume whereas dinoflagellates made up 2% of cell numbers and 17% of the biovolume. *Thalassiosira, Odontella and Noctiluca* composed 70% of the total biovolume for the entire sampling period and the top 15 contributors account for over 85% of total biovolume (Table 2, Figure 4). More than half of the taxa accounted for less than 1% of the total biovolume (Table 2).

**Season and species assemblages**

Upwelling 2007 had the highest species richness by season; Winter 07-08 and Upwelling 2008 were less and nearly identical to each other (Figure 3). While species
evenness was similar throughout the seasons, Winter 07-08 had the highest evenness and Upwelling 2008 had the lowest. Diversity (H’) was highest in Upwelling of 2007 and lowest in Upwelling of 2008 (Figure 3). Upwelling 2007 and Winter 07-08 had lower total biovolume compared to Upwelling 2008 (Figure 4). *Chaetoceros*, *Thalassiosira* and *Noctiluca*, were more abundant by biovolume during the upwelling seasons whereas pennate diatoms, such as *Dictyocha*, *Rhizosolenia* and *Proboscia*, were more abundant during winter than they were during either upwelling season (Figure 4).

The NMS ordination resulted in three axes describing 89.8% of the total assemblage variation with the Beals smoothed data set and a final stress of 12.4 after 155 iterations. For axis one (37.7% of total variation), high scores were positively correlated to dinoflagellates, high water temperatures and salinities, richness and most of the Upwelling 2007 assemblage (Figures 5A, 5B). In contrast, low axis one scores were correlated with pennate diatom taxa in the tychoplankton, low salinities and temperatures, and almost all of the Winter 07-08 assemblage. Axis three (32.5% of total variation) was not correlated with water quality variables that vary seasonally. Instead, low axis scores were associated with Upwelling 2007 and taxa from the tychoplankton whereas high scores were associated with most of the Upwelling 2008 assemblage and the planktonic, chain-forming colonies of *Chaetoceros*. All three seasons were significantly different from each other (Table 3) and there were unique indicator species for each season (Table 4). Upwelling 2007 had the largest number of indicator species and included all three size classes of nondescript dinoflagellates as well as *Pseudo-nitzschia delicatissima* and a mixture of planktonic and tychoplanktic forms. *Grammatophora marina* and the smallest
size class of pennate diatoms were the only indicators of Winter 07-08. Upwelling 2008 indicators included several species of *Chaetoceros*, *Thalassiosira* and *Odontella* (Table 4).

*Tidal Stage and Species Assemblages*

Richness, evenness and diversity between high and low tide assemblages were similar (Figure 3). None of the axes in the NMS ordination, including axis two (19.6% of total variation), were correlated to any of the four depth variables used to describe different aspects of a possible tidal effect on species assemblage structure. Although the MRPP analysis comparing all high to all low tide samples was significantly different (Table 3), high tide samples did not consistently associate with low or high axis scores of any axis (Figures 5A, 5B). This lack of clear structure in ordination space also applied to low tide samples. The indicator species analysis identified the dinoflagellates *Noctiluca scintillans* and *Protoperidinium* sp. as the only indicators of high tide (Table 4). All of the low tide indicators were members of the tychoplankton functional group (Table 4).

*Functional Group Responses*

Except for Winter 07-08 when euplankton and tychoplankton comprised the same proportion of total biovolume, euplankton were significantly more abundant regardless of whether the comparison was made within a season or within a tidal stage (Figure 6). The mean planktonic biovolume calculated from all samples was 70.5% of the total biovolume. The upwelling seasons had the highest euplanktonic contributions with 76.9% and 86.3% during Upwelling 2007 and Upwelling 2008, respectively. High tide
and low tide samples were 82% and 57% planktonic, respectively (Figure 6). Focusing only on tychoplankton, they made up a significantly higher percent of low tide (43.2%) than high tide (17.9%) samples (Figure 6).

**HAB and Toxin Patterns**

*Alexandrium* and *Pseudo-nitzschia* were loosely correlated to their respective toxins concentrations from nearby mussels (Figures 7, 8). Two water samples from the summer of 2007 contained *Alexandrium* with their highest abundance being close to 200 cells/L. This level corresponds to the lowest detection limit of PSP toxins used by CDPH, which is about 35μg of saxitoxins/100 g mussel tissue. A few samples in spring of 2008 also contained *Alexandrium* at about the same concentration as 2007 with similar lower detection limits of PSP toxins. Samples from both high tide and low tide in the summer and fall of 2008 had the highest numbers of *Alexandrium* cells reaching near 500 cells/L with corresponding PSP concentrations between 60 and 70 μg saxitoxins/100 g tissue (Figure 7). *Alexandrium* was never a common or abundant component of the community.

*Pseudo-nitzschia* was present at low numbers at high tide and low tide throughout much of the sampling period but absent from December 2007 through March 2008. The highest abundances occurred from high tide samples around August of both years with 2007 reaching over 5,000 cells/L and 2008 reaching over 4,000 cells/L. Domoic acid concentrations for most of the study were either below or near the detection limit when samples were taken. The only elevated level of domoic acid (~2.5 μg/g) corresponds
with the August 2008 cell abundance of 4,300 cells/L (Figure 8), but this toxin concentration was nearly 10 times below the public health alert level (Table 1).

**The Phytoplankton Community > 10 µm**

Overall, the proportion of chlorophyll $a$ that came from organisms less than 10 µm was less than the proportion that came from larger organisms (Figure 9). The periods of high chlorophyll concentration (i.e. high tide and upwelling seasons) contained the greatest percent chlorophyll from larger organisms. Although there wasn’t a significant proportional difference between the two size classes during the winter or at low tide, these categories contained low chlorophyll concentrations. Seasonally, the upwelling periods had the greatest percent chlorophyll larger than 10 µm with 65.2% and 68.1% in Upwelling 2007 and Upwelling 2008, respectively. This percent was less during Winter 07-08 with 48% of the chlorophyll coming from organisms larger than 10µm and 52 % coming from organisms smaller than 10µm. By tidal state, 67.4 % and 53.2% of the chlorophyll was from organisms larger than 10 µm at, respectively, high and low tides (Figure 9).
DISCUSSION

Several hypotheses were tested in this first community description of phytoplankton in Humboldt Bay. Community structure was expected to differ by seasons as well as tidal stage. The euplankton habitat group was predicted to be more abundant than tychoplankton throughout the seasons and tides, but tychoplankton would be more abundant at low tide than they would at high tide. HAB abundances were expected to parallel their associated toxin concentrations. Lastly, the sampling procedure used was expected to capture the majority of algal cells in the water column.

Species assemblages and ecological gradients

The high ordination $r^2$ values indicate that much of the variance in community composition was described by axes one and three. Samples from Upwelling 2007, Winter 07-08 and Upwelling 2008 form separate clusters on the plot of axes one and three, the MRPP analysis indicates that all three seasons were different from each other, and there were unique indicator species for all three seasons.

The relationships between axis one and temperature as well as salinity demonstrate that this axis is related to seasonal changes that occur within the winter and upwelling seasons of the study. The low range of axis one contains almost all of the ordination scores of the winter phytoplankton community from the primary matrix. This overall low biovolume season is characterized by Grammatophora and the smallest pennate size class (<150 μm), both of which scored low on this axis and were also the only significant indicator species of Winter 07-08. Additionally, low salinity and low
temperatures from the secondary matrix also score low on axis one due to winter precipitation and occasional snow melt from the local watershed, which enters the bay 2°C colder than local upwelled ocean water. Strong southerly winds that often accompany winter precipitation events cause brief periods of downwelling that advect offshore waters closer to the coast (Checkley and Barth 2009). The silicoflagellate Dictyocha is associated with these offshore waters (Fischer et al. 1983) and its highest abundance was recorded in the winter. Offshore, nearshore, and brackish waters, each with different physical characteristics and communities, converge in Humboldt Bay during the winter.

The high range of axis one contains most of the primary matrix sample scores from Upwelling 2007 and many from Upwelling 2008. Higher salinity and warmer temperatures also score high on axis one and are representative of the upwelling seasons. It may seem odd that the upwelling season is correlated with warmer temperatures but solar warming of the bay mud flats at low tide during the spring and summer warms the incoming flood tide water by as much as 7°C. With little or no precipitation during the upwelling seasons, the salinity remains high and parallels the nearshore ocean water outside the bay. Dinoflagellates, which are known to favor these warmer saline conditions, score high on axis one (Figures 5A, 5B; Ryan et al. 2009) as do samples that contained high proportions of upwelling taxa, which matches studies during the upwelling season off Bodega Bay (Lassiter et al. 2006) and waters north the Oregon border (Hood et al. 1990, Chavez et al. 1991). While community ordinations of both upwelling seasons score high on axis one, there is some segregation between these two
seasons with 2007 having higher axis scores and being more positively correlated to species richness (figure 5A).

While axis one is a seasonal gradient, axis three forms an interannual gradient that separates the two upwelling seasons. The mechanism producing the axis three gradient may be the El Niño Southern Oscillation (ENSO). This global process, as indexed by the Multivariate ENSO Index (MEI; Wolter 1987, Wolter and Timlin 1993), appears to be having a time lagged effect on phytoplankton community composition and biovolume at Dock B (Figure 2C). The El Niño conditions of warmer water and lower nutrient concentrations, which dominated during the year prior to this study and partially into the Upwelling 2007 season, were typified by Dock B taxa such as Licmorpha and several groups of dinoflagellates. Warmer, lower nutrient waters are known to be associated with dinoflagellates and smaller cells (Pahlow et al. 1997, Smayda and Reynolds 2001, Smayda and Trainer 2010, Corcoran and Shipe 2011) and is consistent with the finding in this study. However, a connection between higher dinoflagellate abundance during El Niño events has not been established from studies in California or Mexico (Chavez et al. 2002, Franco-Gordo et al. 2004) but another study from California documents an increase in dinoflagellates one to two years following El Niño conditions, suggesting a lag effect (Anderson et al. 2008). More local ship transects across the California Current during this period are consistent with the global MEI since the former show the spring 2007 chlorophyll levels as being one of the lowest on record (McClatchie et al. 2008). In contrast, these same transects describe a transition from El Niño to La Niña conditions during the summer of 2007 and by summer of 2008 cold water with high primary
production dominated (McClatchie et al. 2008, 2009). The MEI describes the same transition to La Niña conditions. The Upwelling 2008 taxa that were recorded at Dock B, such as *Thalassiosira*, *Chaetoceros* and *Odontella* are consistent with historical observations during La Niña conditions (Esparza-Alvarez et al. 2007). Distinct El Niño and La Niña phytoplankton assemblages, which are produced offshore, may not be completely synchronized with the MEI curve because: (1) the switch from El Niño to La Niña water conditions is gradual, (2) a particular assemblage that exists offshore may take weeks or months to appear at Dock B depending upon the frequency of downwelling conditions and relaxation events.

The ordination of species supports the hypothesis that there is a seasonal shift of the phytoplankton community in Humboldt Bay. There was also an unanticipated shift in ENSO conditions that appears to have structured the phytoplankton assemblages in the bay. Tidal conditions were expected to be another important ecological gradient that could structure species assemblages at several time scales. Despite the distinction of high and low tide communities by the MRPP analysis and the presence of indicator species for each tidal stage, none of the axes were correlated with the four variables that were designed to describe the ways that tidal flows could potentially structure the phytoplankton community. The magnitude of difference among samples categorized by tide is small ($A = 0.004$) as compared to differences by season ($A = 0.024-0.043$) revealing that differences by tide, while significant, may be tenuous. A sample size affect (more samples per tide group than by season) could allow for more significant $p$-values for groups based on a larger sample size.
The high tide assemblage was expected to contain primarily taxa that originate in the water column of the ocean, like *Chaetoceros*, because Dock B is in the middle of Humboldt Bay which places it relatively close to Entrance Channel and because chlorophyll fluorescence values are generally higher during times of flooding rather than ebbing water (Shaughnessy et al. 2008). Even so, the tidal variable indicating if a sample was taken when maximum water volumes enter the bay (spring tides) versus when minimal volumes enter the bay (neap tides) was not explanatory. This was surprising because, while there were reasons for assuming that high tide collections would reflect an oceanic assemblage, there were also reasons for thinking that the low tide assemblage would be unique and not just a reduced version of the high tide community that had been grazed down while in the bay. In particular, the qualitative observation that, at least on the northern mudflats, ebbing tidal velocities looked higher than flooding velocities, led to the idea that epiphytic and mudflat diatom species would get resuspended and therefore be better represented in the low tide assemblage at Dock B. But neither the tidal variable that described whether the water at the time of sampling was flooding or ebbing, or the variable just indicating the depth of the water, were correlated with any axis.

The data indicate that the tidal gradient does have an effect on phytoplankton community structure, but at the Dock B location the assemblages get mixed together in the relatively shallow Humboldt Bay. One water sample can therefore contain species that only originate in the water column plus other species that only originate on mudflats. While this mixing appears to make tidal stage assemblages of species less distinct, tidal
stage does have a clear affect on biovolume. The high tide community had a higher phytoplankton biovolume containing nearshore species and this was most apparent during nearshore bloom events outside Humboldt Bay. This tidal import trend has also been reported from Coos Bay and Willapa Bay (Roegner and Shanks 2001, Newton and Horner 2003). The dinoflagellate *Noctiluca* was the only taxon found to be a significant indicator of high tide; the dinoflagellate *Protoperidinium* was close to meeting the significance threshold. The affinity of these dinoflagellates for high tide samples is indicative of their association with saline nearshore oceanic waters (Cloern and Dufford 2005) that are transported into the bay with the waters leading to high tide. The low tide community contained lower phytoplankton biovolume and a greater proportion of species that were from benthic or epiphytic habitats.

*Functional Group Responses*

The dominance of, likely, ocean produced euplankton during the two upwelling seasons is consistent with other shallow, west coast inlets (Roegner and Shanks 2001, Newton and Horner 2003) that import nearshore oceanic phytoplankton. During the winter, the nearshore contribution of phytoplankton to the estuary declines due to lack of light, more turbidity and downwelling conditions with fewer nutrients (Margalef 1978, Cloern and Nichols 1985, Figure 2A). However, while euplankton and tychoplankton each made up about 50% of the winter biovolume, tychoplankton absolute biovolume was 7 to 27 times lower than the euplankton absolute biovolume during the 2007 and 2008 upwelling seasons, respectively. The large difference in 2008 was due to the large
biovolume of euplankton which was likely driven by stronger upwelling and La Niña
conditions.

Both high and low tide communities had a greater proportion of euplankton than
tychoplankton, again indicating the dominance of the nearshore contribution to Humboldt
Bay, but tychoplankton were proportionately more abundant during the low rather than
the high tide. Similar patterns due to benthic resuspension are reported from New
England (Baillie and Welsh 1980) and the Ems estuary along the northern border of
Germany and the Netherlands (De Jonge and Van Beuselom 1992). Additionally, studies
in San Francisco Bay report tidal and wind induced suspension, especially over shoals on
the periphery of deeper channels (Powell et al. 1989, Cloern et al. 1989). While
euplankton dominate the bulk of the biovolume, tychoplankton are an important
component of the community during periods of low biovolume, whether it be the winter
season or low tide.

HAB and Toxin Patterns

The CDPH’s monitoring program tested mussel tissues for the toxins that
Alexandrium and Pseudo-nitzschia are known to produce and these toxin concentrations
were expected to parallel the abundance of these organisms at Dock B. Alexandrium
never reached a high abundance and its toxin levels never surpassed the public health
alert level during the study period. A more rigorous statistical comparison between my
cell counts and saxitoxin concentrations could therefore not be made. Other limitations
to the comparison were that the two variables were not sampled at exactly the same time
and, in Humboldt Bay, not all the sampling by CDHP was done in the same place. However, the presence of *Alexandrium* that I documented was consistent with the months it is expected to occur during the annual sport harvest mussel quarantine imposed by the CDPH (April – October). The presence of *Alexandrium* during this time period indicates that this organism is associated with warmer ocean waters. Over decades of monitoring throughout California the CDPH has learned that, when this dinoflagellate is present, the saxitoxins (and associated analogs) responsible for PSP are usually detected by bioassays. Sometimes saxitoxins are detected when *Alexandrium* cell counts are very low, indicating that a full bloom is not required in order to produce harmful levels of saxitoxins or their analogs (G. Langlois, pers. comm.). For this reason their monitoring program tests for PSP saxitoxins on a weekly basis year round.

The same issue of low cell abundances for *Pseudo-nitzschia* and low domoic acid concentrations during this study also did not allow a rigorous comparison between the two. *Pseudo-nitzschia* was present in low numbers through the fall of 2007, beyond the standard months of the annual short harvest shellfish quarantine. Not all species within this genus are known to produce domoic acid and even those species with the genes for toxin production do not always produce it (Terseleer *et al.* 2013). This variability coupled with difficult species identification, which requires scanning electron microscopy, adds further to the difficulty of correlating cell abundance to domoic acid concentration. The CDPH has noticed that when shellfish assays for domoic acid are positive, *Pseudo-nitzschia* has been dominant phytoplankton community for weeks or longer (G. Langlois, pers. comm.). For this reason the CDPH tests for domoic acid only
if *Pseudo-nitzschia* is common (i.e., 10% or more of total cells). The presence of *Pseudo-nitzschia* throughout most of my study suggests that a wide range of conditions can support this organism but that it will be most abundant during the summer months.

Two other HAB dinoflagellates known to occur on the west coast were observed in low numbers during the sampling period, *Dinophysis* and *Gonyaulax*. *Dinophysis* was found only in six samples and occurred from July to August of 2007. Two thirds of these occurrences were from high tide samples. *Gonyaulax* was only found in one high tide sample taken in mid-July of 2007. Given the *Gonyaulax* implicated Sonoma invertebrate die off in 2010 and recent documentation of diarrhetic shellfish poisoning toxins in Washington, public health officials may expand their monitoring and regulation efforts to include these genera (Lewitus *et al.* 2012).

*The Phytoplankton Community > 10 µm*

The sampling methods used in the present study did capture the majority of the planktonic algae at Dock B. Organisms greater than 10µm composed a greater percent contribution to total chlorophyll a than smaller organisms during the two upwelling seasons and at high tide. While winter and low tide chlorophyll was not dominated by organisms greater than 10 µm, these categories had the lowest absolute amounts of chlorophyll. Therefore larger organisms compose the bulk of chlorophyll when values are high and small organisms contribute as much to total chlorophyll a as larger organisms when total chlorophyll is low.
What part of the community was not described by analyzing only that part of the community greater than 10\(\mu\)m in diameter? The percent of the community less than 10\(\mu\)m was greatest during Winter 07-08 and Low tide, suggesting species of ultra- or picoplankton. Similarly, San Francisco Bay has a greater percent of nanoplankton (5-22\(\mu\)m) and ultraplankton (<5 \(\mu\)m) from samples over shoals than deeper channels (Cole et al. 1986) and the percent differences between these small size classes and bigger ones becomes less during winter months. Given that much of Humboldt Bay’s surface area is composed of tidal mud flats, tidal stirring and transport from the flats to Dock B may explain the higher percent of chlorophyll smaller than 10 \(\mu\)m from samples taken at low tide.
CONCLUSIONS

Annual seasonal differences in the phytoplankton community at Dock B are driven by nearshore bloom events that advect into Humboldt Bay and the large colonial diatoms that dominate during such conditions are consistent with regional studies during the upwelling season (Lassiter et al. 2006, Hood et al. 1990, Chavez et al. 1991). During the winter, when upwelling halts for several months and there is a greater mixing of offshore and estuarine-like waters, a more diverse group of organisms is present. The two upwelling seasons were also different from each other and those differences appear to be linked to a lagged ENSO effect.

While the community differed between high and low tide, that difference is not as strong as the seasonal and ENSO signals. Communities supported by different sources of water were expected to be correlated with tidal state but this signal may have been weakened by mixing of these source waters with the continual tidal ebb and flow. Perhaps the development of a variable to describe mixing potential within Humboldt Bay will allow future analyses to capture stronger tidal effects. Through a functional group approach I have been able to establish that euplankton are more abundant than tychoplankton at both high and low tide and, secondly, that tychoplankton are more abundant at low tide than they are at high tide. Tychoplankton were associated with samples containing low overall biovolume, suggesting that they are an important component of the community when heterotrophic organisms do not have an ample supply of oceanic food.
HAB taxa abundance paralleled their associated toxins concentrations but strong correlations were not possible due to the lack of a major HAB event during the sampling period. The total biovolume of the phytoplankton community at Dock B is dominated by organisms larger than 10 μm. Organisms less than 10 μm contribute as much to total chlorophyll as larger organisms only when total chlorophyll is low.
LITERATURE CITED


National Laboratory, US Department of Energy, Oak Ridge, TN., USA. doi


University of California Press. Berkeley, CA.


Table 1. Syndromes and known toxins from U.S. West Coast harmful algal bloom taxa (Hallegraeff et al. 2003, Lewitus et al. 2012).

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Toxin</th>
<th>Genus</th>
<th>Alert level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paralytic shellfish poisoning (PSP)</td>
<td>Saxitoxins</td>
<td><em>Alexandrium, Gymnodinium,</em> and <em>Pyrodinium</em></td>
<td>80 µg 100 g⁻¹ shellfish meat</td>
</tr>
<tr>
<td>Amnesic shellfish poisoning (ASP)</td>
<td>Domoic acid</td>
<td><em>Pseudo-nitzschia</em></td>
<td>20 ppm</td>
</tr>
<tr>
<td>Diarrhetic shellfish poisoning (DSP)</td>
<td>Okadaic acid</td>
<td><em>Dinophysis</em></td>
<td>16 µg 100 g⁻¹ shellfish meat</td>
</tr>
<tr>
<td>Tumor promoter</td>
<td>Yessotoxins</td>
<td><em>Gonyaulax, Protoceratium,</em> and <em>Lingulodinium</em></td>
<td>1 µg g⁻¹ of shellfish meat</td>
</tr>
<tr>
<td>Fish kills</td>
<td>Various</td>
<td><em>Cochlodinium, Heterosigma,</em> and <em>Chattonella</em></td>
<td>N/A</td>
</tr>
<tr>
<td>Bird deaths</td>
<td>Surfactant-like proteins</td>
<td><em>Akashiwo</em></td>
<td>N/A</td>
</tr>
<tr>
<td>Sea otters deaths</td>
<td>Hepatotoxins</td>
<td><em>Microcystis</em></td>
<td>1500 µg kg⁻¹ of mussel</td>
</tr>
<tr>
<td>Invasives, hypoxia, secondary metabolites</td>
<td>N/A</td>
<td>Macroalgal blooms</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Table 2. Taxa sorted by percent contribution to total biovolume for the entire sampling period (4/26/2007 – 10/28/2008). Cell numbers are also presented for making comparisons to older studies and to HAB monitoring.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Functional group</th>
<th>% of Total</th>
<th>Biovolume</th>
<th>% of Total</th>
<th>Cell Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Mean</td>
<td>Maximum</td>
<td>Total</td>
</tr>
<tr>
<td>Thalassiosira 30-49 µm diam.</td>
<td>euplankton</td>
<td>23.151</td>
<td>100,700,000</td>
<td>1,826,000,000</td>
<td>23.466</td>
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<td>Odontella longiciraris</td>
<td>euplankton</td>
<td>20.645</td>
<td>89,830,000</td>
<td>3,800,000,000</td>
<td>0.460</td>
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<td>Noctiluca scintillans</td>
<td>euplankton</td>
<td>14.376</td>
<td>62,550,000</td>
<td>2,262,000,000</td>
<td>0.082</td>
</tr>
<tr>
<td>Thalassiosira ≥ 50 µm diam.</td>
<td>euplankton</td>
<td>5.024</td>
<td>21,860,000</td>
<td>364,900,000</td>
<td>3.703</td>
</tr>
<tr>
<td>Thalassiosira ≤ 29 µm diam.</td>
<td>euplankton</td>
<td>3.505</td>
<td>15,250,000</td>
<td>367,900,000</td>
<td>7.103</td>
</tr>
<tr>
<td>Other Pennates 151-300 µm</td>
<td>tychoplankton</td>
<td>3.273</td>
<td>14,240,000</td>
<td>516,400,000</td>
<td>0.220</td>
</tr>
<tr>
<td>Other Pennates &gt; 300 µm</td>
<td>tychoplankton</td>
<td>2.988</td>
<td>13,000,000</td>
<td>540,000,000</td>
<td>0.085</td>
</tr>
<tr>
<td>Rhizosolenia sp.</td>
<td>euplankton</td>
<td>2.645</td>
<td>11,500,000</td>
<td>1,243,000,000</td>
<td>0.013</td>
</tr>
<tr>
<td>Coscinodiscus spp.</td>
<td>euplankton</td>
<td>2.468</td>
<td>10,740,000</td>
<td>244,200,000</td>
<td>0.302</td>
</tr>
<tr>
<td>Other Pennates ≤ 150 µm</td>
<td>tychoplankton</td>
<td>2.364</td>
<td>10,290,000</td>
<td>78,750,000</td>
<td>4.282</td>
</tr>
<tr>
<td>Pleurosigma sp.</td>
<td>tychoplankton</td>
<td>2.275</td>
<td>9,901,000</td>
<td>212,800,000</td>
<td>0.791</td>
</tr>
<tr>
<td>Chaetoceros decipiens</td>
<td>euplankton</td>
<td>1.688</td>
<td>7,344,000</td>
<td>303,900,000</td>
<td>0.661</td>
</tr>
<tr>
<td>Chaetoceros debilis</td>
<td>euplankton</td>
<td>1.571</td>
<td>6,837,000</td>
<td>267,200,000</td>
<td>4.023</td>
</tr>
<tr>
<td>Odontella aurita</td>
<td>euplankton</td>
<td>1.535</td>
<td>6,680,000</td>
<td>183,200,000</td>
<td>0.490</td>
</tr>
<tr>
<td>Tropidoneis sp.</td>
<td>euplankton</td>
<td>1.403</td>
<td>6,107,000</td>
<td>405,000,000</td>
<td>0.075</td>
</tr>
<tr>
<td>Protoperidinium spp.</td>
<td>euplankton</td>
<td>1.261</td>
<td>5,488,000</td>
<td>353,400,000</td>
<td>0.100</td>
</tr>
<tr>
<td>Lichmophora flabellata</td>
<td>tychoplankton</td>
<td>1.222</td>
<td>5,317,000</td>
<td>74,090,000</td>
<td>0.240</td>
</tr>
<tr>
<td>Taxon</td>
<td>Functional group</td>
<td>% of Biovolume</td>
<td>Biovolume</td>
<td>Cell Number</td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------------</td>
<td>----------------</td>
<td>-----------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>% of Total</td>
<td>Mean</td>
<td>Maximum</td>
<td>% of Total</td>
</tr>
<tr>
<td><strong>Grammatophora marina</strong></td>
<td>tychoplankton</td>
<td>1.027</td>
<td>4,468,000</td>
<td>56,270,000</td>
<td>0.791</td>
</tr>
<tr>
<td><strong>Ditylum brightwellii</strong></td>
<td>euplankton</td>
<td>0.899</td>
<td>3,911,000</td>
<td>275,000,000</td>
<td>0.165</td>
</tr>
<tr>
<td><strong>Proboscia sp.</strong></td>
<td>euplankton</td>
<td>0.735</td>
<td>3,200,000</td>
<td>219,900,000</td>
<td>0.024</td>
</tr>
<tr>
<td><strong>Dictyocha sp.</strong></td>
<td>euplankton</td>
<td>0.728</td>
<td>3,168,000</td>
<td>81,810,000</td>
<td>0.020</td>
</tr>
<tr>
<td><strong>Melosira moniliformis</strong></td>
<td>tychoplankton</td>
<td>0.667</td>
<td>2,903,000</td>
<td>28,630,000</td>
<td>0.756</td>
</tr>
<tr>
<td><strong>Chaetoceros affinis</strong></td>
<td>euplankton</td>
<td>0.625</td>
<td>2,718,000</td>
<td>111,300,000</td>
<td>2.192</td>
</tr>
<tr>
<td><strong>Isthmia nervosa</strong></td>
<td>tychoplankton</td>
<td>0.434</td>
<td>1,887,000</td>
<td>106,100,000</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>Chaetoceros diadema</strong></td>
<td>euplankton</td>
<td>0.412</td>
<td>1,790,000</td>
<td>78,340,000</td>
<td>1.265</td>
</tr>
<tr>
<td><strong>Corethron hystrix</strong></td>
<td>euplankton</td>
<td>0.393</td>
<td>1,710,000</td>
<td>81,770,000</td>
<td>0.024</td>
</tr>
<tr>
<td>Other Dinoflagellates 31-49 μm</td>
<td>euplankton</td>
<td>0.372</td>
<td>1,618,000</td>
<td>33,240,000</td>
<td>0.353</td>
</tr>
<tr>
<td>Other Dinoflagellates ≤30 μm</td>
<td>euplankton</td>
<td>0.279</td>
<td>1,216,000</td>
<td>24,090,000</td>
<td>1.087</td>
</tr>
<tr>
<td><strong>Chaetoceros vanheurckii</strong></td>
<td>euplankton</td>
<td>0.254</td>
<td>1,107,000</td>
<td>52,480,000</td>
<td>1.172</td>
</tr>
<tr>
<td><strong>Neoceratium tripos</strong></td>
<td>euplankton</td>
<td>0.251</td>
<td>1,093,000</td>
<td>64,390,000</td>
<td>0.012</td>
</tr>
<tr>
<td><strong>Stephanopyxis sp.</strong></td>
<td>euplankton</td>
<td>0.208</td>
<td>903,100</td>
<td>19,510,000</td>
<td>0.156</td>
</tr>
<tr>
<td><strong>Chaetoceros convolutus</strong></td>
<td>euplankton</td>
<td>0.188</td>
<td>817,500</td>
<td>35,560,000</td>
<td>0.237</td>
</tr>
<tr>
<td><strong>Skeletonema costatum</strong></td>
<td>euplankton</td>
<td>0.164</td>
<td>714,500</td>
<td>14,500,000</td>
<td>22.237</td>
</tr>
<tr>
<td><strong>Chaetoceros didymus</strong></td>
<td>euplankton</td>
<td>0.141</td>
<td>612,200</td>
<td>13,940,000</td>
<td>1.318</td>
</tr>
<tr>
<td>Other Dinoflagellates ≥50 μm</td>
<td>euplankton</td>
<td>0.080</td>
<td>348,500</td>
<td>5,575,000</td>
<td>0.029</td>
</tr>
<tr>
<td><strong>Ceratium divaricatum</strong></td>
<td>euplankton</td>
<td>0.061</td>
<td>266,300</td>
<td>28,760,000</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Alexandrium catenella</strong></td>
<td>euplankton</td>
<td>0.059</td>
<td>257,400</td>
<td>9,619,000</td>
<td>0.095</td>
</tr>
<tr>
<td><strong>Cocconeis scutellum</strong></td>
<td>tychoplankton</td>
<td>0.058</td>
<td>250,800</td>
<td>2,205,000</td>
<td>0.452</td>
</tr>
<tr>
<td><strong>Scrippsiella sp.</strong></td>
<td>euplankton</td>
<td>0.050</td>
<td>216,700</td>
<td>7,585,000</td>
<td>0.186</td>
</tr>
<tr>
<td>Taxon</td>
<td>Functional group</td>
<td>% of Total</td>
<td>Biovolume</td>
<td>% of Total</td>
<td>Mean</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------------------</td>
<td>------------</td>
<td>-----------</td>
<td>------------</td>
<td>------</td>
</tr>
<tr>
<td>Chaetoceros radicans</td>
<td>euplankton</td>
<td>0.048</td>
<td>210,300</td>
<td>1.281</td>
<td>219</td>
</tr>
<tr>
<td>Ceratium furca</td>
<td>euplankton</td>
<td>0.045</td>
<td>194,100</td>
<td>0.009</td>
<td>1</td>
</tr>
<tr>
<td>Chaetoceros socialis</td>
<td>euplankton</td>
<td>0.044</td>
<td>190,700</td>
<td>4.156</td>
<td>710</td>
</tr>
<tr>
<td>Asterionellopsis glacialis</td>
<td>euplankton</td>
<td>0.042</td>
<td>183,400</td>
<td>0.191</td>
<td>33</td>
</tr>
<tr>
<td>Raphoneis amphiceros</td>
<td>tychoplankton</td>
<td>0.041</td>
<td>180,500</td>
<td>0.893</td>
<td>153</td>
</tr>
<tr>
<td>Dinophysis sp.</td>
<td>euplankton</td>
<td>0.032</td>
<td>141,400</td>
<td>0.052</td>
<td>9</td>
</tr>
<tr>
<td>Pseudo-nitzschia seriata</td>
<td>euplankton</td>
<td>0.031</td>
<td>133,400</td>
<td>1.562</td>
<td>267</td>
</tr>
<tr>
<td>Gymnodinium sp.</td>
<td>euplankton</td>
<td>0.029</td>
<td>124,900</td>
<td>0.011</td>
<td>2</td>
</tr>
<tr>
<td>Alexandrium catenella</td>
<td>euplankton</td>
<td>0.029</td>
<td>124,700</td>
<td>0.056</td>
<td>10</td>
</tr>
<tr>
<td>Eucampia zodiacus</td>
<td>euplankton</td>
<td>0.028</td>
<td>122,500</td>
<td>0.435</td>
<td>74</td>
</tr>
<tr>
<td>Ceratium sp.</td>
<td>euplankton</td>
<td>0.028</td>
<td>120,300</td>
<td>0.022</td>
<td>4</td>
</tr>
<tr>
<td>Chaetoceros sp.</td>
<td>euplankton</td>
<td>0.023</td>
<td>98,960</td>
<td>0.364</td>
<td>62</td>
</tr>
<tr>
<td>Thalassionema nitzschioides</td>
<td>euplankton</td>
<td>0.013</td>
<td>55,220</td>
<td>1.052</td>
<td>180</td>
</tr>
<tr>
<td>Achnanthes longipes</td>
<td>tychoplankton</td>
<td>0.013</td>
<td>54,810</td>
<td>0.109</td>
<td>19</td>
</tr>
<tr>
<td>Licmophora argenscens</td>
<td>tychoplankton</td>
<td>0.012</td>
<td>50,390</td>
<td>0.219</td>
<td>37</td>
</tr>
<tr>
<td>Pseudo-nitzschia delicatissima</td>
<td>euplankton</td>
<td>0.011</td>
<td>49,690</td>
<td>5.818</td>
<td>994</td>
</tr>
<tr>
<td>Cylindrotheca closterium</td>
<td>euplankton</td>
<td>0.011</td>
<td>47,160</td>
<td>0.133</td>
<td>23</td>
</tr>
<tr>
<td>Detonula sp.</td>
<td>euplankton</td>
<td>0.007</td>
<td>31,770</td>
<td>0.145</td>
<td>25</td>
</tr>
<tr>
<td>Chaetoceros concavicorne</td>
<td>euplankton</td>
<td>0.006</td>
<td>28,270</td>
<td>0.013</td>
<td>2</td>
</tr>
<tr>
<td>Neoceratium lineatum</td>
<td>euplankton</td>
<td>0.006</td>
<td>24,840</td>
<td>0.002</td>
<td>0</td>
</tr>
<tr>
<td>Leptocylindrus sp.</td>
<td>euplankton</td>
<td>0.006</td>
<td>24,300</td>
<td>4.414</td>
<td>754</td>
</tr>
<tr>
<td>Lauderia sp.</td>
<td>euplankton</td>
<td>0.005</td>
<td>23,630</td>
<td>0.054</td>
<td>9</td>
</tr>
<tr>
<td>Taxon</td>
<td>Functional group</td>
<td>% of Total</td>
<td>Mean</td>
<td>Maximum</td>
<td>% of Total</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------</td>
<td>------------</td>
<td>----------</td>
<td>----------</td>
<td>------------</td>
</tr>
<tr>
<td><em>Gonyaulax</em> sp.</td>
<td>euplankton</td>
<td>0.004</td>
<td>19,440</td>
<td>2,099,000</td>
<td>0.002</td>
</tr>
<tr>
<td><em>Bacillaria paxillifera</em></td>
<td>euplankton</td>
<td>0.003</td>
<td>12,590</td>
<td>880,000</td>
<td>0.037</td>
</tr>
<tr>
<td><em>Pyrophacus</em> sp.</td>
<td>euplankton</td>
<td>0.003</td>
<td>10,910</td>
<td>589,000</td>
<td>0.009</td>
</tr>
<tr>
<td><em>Chaetoceros laciniosus</em></td>
<td>euplankton</td>
<td>0.002</td>
<td>7,125</td>
<td>508,939</td>
<td>0.035</td>
</tr>
<tr>
<td><em>Amylax</em> sp.</td>
<td>euplankton</td>
<td>0.001</td>
<td>4,712</td>
<td>508,939</td>
<td>0.002</td>
</tr>
<tr>
<td><em>Chaetoceros danicus</em></td>
<td>euplankton</td>
<td>0.001</td>
<td>2,825</td>
<td>113,098</td>
<td>0.030</td>
</tr>
<tr>
<td><em>Chaetoceros costatum</em></td>
<td>euplankton</td>
<td>0.001</td>
<td>2,793</td>
<td>301,594</td>
<td>0.004</td>
</tr>
<tr>
<td><em>Euglena</em> sp.</td>
<td>euplankton</td>
<td>0.000</td>
<td>776</td>
<td>83,776</td>
<td>0.007</td>
</tr>
<tr>
<td><em>Zygnematophyte</em></td>
<td>tychoplankton</td>
<td>0.000</td>
<td>589</td>
<td>63,617</td>
<td>0.173</td>
</tr>
<tr>
<td><em>Lyngbya</em> sp.</td>
<td>tychoplankton</td>
<td>0.000</td>
<td>0</td>
<td>45</td>
<td>0.027</td>
</tr>
</tbody>
</table>
Table 3. Group comparisons of species assemblages using the multi response permutation procedure (MRPP). Values are given for test statistics ($t$), homogeneity within groups ($A$) and level of significance ($p$).

<table>
<thead>
<tr>
<th>Group</th>
<th>$t$</th>
<th>$A$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upwelling 2007 vs Winter 07/08</td>
<td>-13.30</td>
<td>0.043</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Upwelling 2007 vs Upwelling 2008</td>
<td>-7.13</td>
<td>0.024</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Winter 07/08 vs Upwelling 2008</td>
<td>-9.15</td>
<td>0.027</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High Tide vs Low Tide</td>
<td>-5.18</td>
<td>0.004</td>
<td>0.029</td>
</tr>
</tbody>
</table>
Table 4. Indicator species analyses for each season and tidal stage. Only those species that were significantly different from other seasons or tides are presented with the exception of *Protoperidinium* in high tide. Dinoflagellate and *Thalassiosira* size classes are measured by width and pennate diatoms by length.

<table>
<thead>
<tr>
<th>Group</th>
<th>Taxon</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upwelling 2007</strong></td>
<td><em>Chaetoceros vanheurckii</em></td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Dinoflagellates $\leq$ 30 μm</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td><em>Achnanthes longipes</em></td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td><em>Chaetoceros didymus</em></td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Dinoflagellates 31 – 49 μm</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td><em>Licmormpha argenscens</em></td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td><em>Melosira moniliformis</em></td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td><em>Skeletonema costatum</em></td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td><em>Licmormpha flabellata</em></td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td>Dinoflagellates $\geq$ 50 μm</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td><em>Pseudo-nitzschia delicatissima</em> class</td>
<td>0.032</td>
</tr>
<tr>
<td><strong>Winter 07 - 08</strong></td>
<td>Pennates $\leq$ 150 μm</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td><em>Grammatophora</em> sp.</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Upwelling 2008</strong></td>
<td><em>Thalassionema</em> sp.</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td><em>Chaetoceros affinis</em></td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td><em>Chaetoceros decipiens</em></td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td><em>Thalassiosira</em> 30-49 μm</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td><em>Chaetoceros socialis</em></td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td><em>Odontella aurita</em></td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td><em>Thalassiosira</em> $\geq$ 50 μm</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td><em>Odontella longicurris</em></td>
<td>0.029</td>
</tr>
<tr>
<td><strong>High tide</strong></td>
<td><em>Noctiuluca scintillans</em></td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td><em>Protoperidinium</em> sp.</td>
<td>0.057</td>
</tr>
<tr>
<td><strong>Low tide</strong></td>
<td>Pennates $\leq$ 150 μm</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td><em>Licmormpha flabellata</em></td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td><em>Pleurosigma</em> sp.</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td><em>Achnanthes longipes</em></td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td><em>Cocconeis scutellum</em></td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td><em>Melosira moniliformis</em></td>
<td>0.037</td>
</tr>
</tbody>
</table>
Figure 1. The location of the sampling site (Dock B) in Humboldt Bay, California.
Figure 2. Offshore upwelling from NOAA buoy 46022 and chlorophyll fluorescence at Dock B in Humboldt Bay (A), water temperature (blue) and salinity (red) from Dock B (B) and the Multivariate ENSO Index (C). The Dock B variables were recorded by NOAA CeNCOOS and the Multivariate ENSO Index calculated by NOAA’s Earth Science Research Laboratory. Sampling period abbreviations are Upwelling 2007 (UW2007), Winter 07-08 (W07-08) and Upwelling 2008 (UW2008).
Figure 3. Average species richness, evenness and diversity for the seasons and tidal stages.
Figure 4. Biovolume by season and tide for genera that composed > 1% of total biovolume for the entire study period.
Figure 5. NMS ordination comparing axes 1 and 3 (A) and axes 1 and 2 (B). Triangle symbols represent the consolidated community for each sample scored in ordination space. Taxa and environmental variables are overlaid as a joint plot with an $R^2$ cutoff of 0.1 and scaled to 400 percent for easier interpretation.
Figure 6. Functional form (i.e. euplankton, tychoplankton) comparisons within season and tidal stage groups of the percent of each form relative to the total biovolume of each group (e.g. all Upwelling 2007 samples). The percent of tychoplankton forms relative to the total biovolume of all high tide samples is also compared to the percent of these forms relative to the total biovolume from all low tide samples. The results of each paired T-test are below each comparison.
Figure 7. *Alexandrium* spp. (A) and *Pseudo-nitzschia* spp. (B) abundances from the water samples taken in the present study compared to toxin concentrations in shellfish meat measured by the California Department of Public Health (CDPH) in Humboldt Bay, CA.
Figure 8. Comparisons of the percent of total chlorophyll $a$ between algae larger and smaller than 10µm for each season and tidal stage. The overall comparison is for the entire study. The results of each paired T-test are below each comparison.
Appendix A: Procedures for collecting, measuring and validating chlorophyll $a$ in water samples

Humboldt State University CeNCOOS Chlorophyll Sample Collection and Filtration Protocol

Last updated: January 29, 2013 by Greg O’Connell

Summary:
Water samples are collected next to sonde (data logger) while its recording data. These water samples are brought back to the lab and vacuum filtered onto glass fiber filters under low vacuum for subsequent chlorophyll $a$ quantification. These data will then be used for comparison/validation of data logger’s in situ chlorophyll values.

Materials:
- Van Dorn Water Sampler (Beta Horizontal PVC) with long line
- Messenger (stainless steel)
- Cooler for sample transportation.
- 5 brown plastic bottles (250-540mL) with unique labels
- GF/F filters (47mm diam)
  - [http://www.fishersci.com/ecommservlet/itemdetail?storeId=10652&langId=-1&LCID=19030984&catalogId=-1&productId=3513966&distype=0&highlightProductsItemsFlag=Y&fromSearch=Y&crossRefData=null](http://www.fishersci.com/ecommservlet/itemdetail?storeId=10652&langId=-1&LCID=19030984&catalogId=-1&productId=3513966&distype=0&highlightProductsItemsFlag=Y&fromSearch=Y&crossRefData=null)
- Vacuum funnel fitted for 47mm filter
- Vacuum source capable of 10 psi or ~500mm Hg
- Squirt bottle with filtered sea water.
- 15mL polypropylene centrifuge tubes.
- Forceps
- Sharpie
- Ziplock bags

Procedure:
Collecting water at data logger:
- Instruments take readings every 15 minutes (:01, :16, :31, :46 of each hour). Take samples at exactly these times.
• Lower the water sampler so that it is as close to the data logger as possible.
  o Humboldt Bay, Dock B
    ▪ Data logger is 2 feet above the bottom.
• Secure and release messenger when ready.
• Pull sampler up and transfer to brown bottles after 3x rinse, then place in cooler.
• Record the following information for each sample taken:
  o Brown sample bottle numbers, date, time (indicate PST, PDT, GMT-preferred), location, tidal stage, weather conditions, and any other ancillary info you can think of.
• Bring samples back to lab for filtration immediately.

Filtration of samples back at the lab:
• Secure vacuum funnel to vacuum source.
• Place GF/F filter in filtration funnel and screw funnel back on so it is snug and secure to the manifold it sits in. Record the volume of water in each sample bottle (500mL ideal) and take note if any portion of the sample is spilled or lost.
• Pour ~½ of each sample into funnel, turn on the vacuum to begin filtration. Swirl the remaining ½ then pour entire contents into funnel. Vacuum pressure should not exceed 10 psi or ~500mm Hg.
• Wait for the entire volume to be filtered before rinsing the funnel wall with a quick squirt of filtered sea water (~2mL).
• Gently tap the funnel to allow water droplets to fall, and then close the funnel valve and turn off the vacuum motor a few seconds after the filter is dry. The filters should not be excessively wet because this sea water will dilute the solvent used for lab chlorophyll extraction and could produce poor results.
• Remove the funnel and use clean forceps to fold the filter in half with the filtrate on the inside.
• Place the sample into a label 15mL centrifuge tube and then in a Ziploc bag for storage in a freezer. Each sampling date and location should have its own labeled Ziploc bag.
• Filtered chlorophyll samples should be run on a fluorometer within 30 days of freezer storage.
• Take note if supplies are running low or if there are issues that need to be cleared up before the next sampling period.

CeNCOOS Chlorophyll Fluorometric Analysis Protocol
Last updated: June 11, 2012
Summary:
GF/F filters containing chlorophyll samples are soaked in acetone overnight to bring chlorophyll pigments into solution. The acetone solution is then read on a
fluorometer, once before acidification and once after. Fluorescence values before and after acidification are used to calculate concentrations of chlorophyll $a$ and phaeophytin-a in the original water sample.

Materials:
- Nitrile gloves
- Safety glasses
- Kim wipes
- Fluorometer with acidified chlorophyll module and solid standard
- 90% acetone
- 10% HCl
- Bottle top dispenser capable of delivering 10mL of acetone
- 10mL Graduated cylinder
- Forceps
- 15mL polypropylene centrifuge tube for each sample
- Centrifuge tube holding tray
- Light barrier (cardboard box) to contain centrifuge tube holding tray
- Spark-free freezer
- Squirt bottle with filtered sea water.
- Sharpie
- Container for acetone waste

Procedure:
Setting up samples to soak overnight:
- Remove samples from freezer and record info into spreadsheet while arranging samples in centrifuge tube holding tray.
- Check precision and accuracy of bottle top dispenser with 10mL graduated cylinder.
- Dispense 10mL of 90% acetone into each centrifuge tube. Make sure that the GF/F filter is completely submerged. Cap and return to tray.
- Place tray in light barrier box and place box in spark-free freezer for 24 hours.

Running samples on the Fluorometer:
- DO NOT use stored calibrations saved on the fluorometer. Instead, enter raw fluorescence values into your spreadsheet and use your own standard curve to calculate chlorophyll concentrations.
- Remove box from freezer and carefully invert tray several times slowly, then allow samples to come to room temperature (~30min).
- While samples are warming, turn on the fluorometer and check the fluorescence reading of the solid standard. Record this number in your spreadsheet.
- Once samples are at room temperature, pour…
• Pour ~ ½ of each sample into funnel, turn on the vacuum to begin filtration. Swirl
the remaining ½ then pour into funnel. Vacuum pressure should not exceed 10
psi or ~500mm Hg.
• Wait for the entire volume to be filtered before rinsing the funnel wall with a
quick squirt of filtered sea water (~ 2mL).
• Gently tap the funnel to allow water droplets to fall, and then close the funnel
valve and turn off the vacuum motor a few seconds after the filter is dry. The
filters should not be excessively wet because this sea water will dilute the solvent
used for lab chlorophyll extraction and could produce poor results.
• Remove the funnel and use clean forceps to fold the filter in half with the filtrate
on the inside.
• Place the sample into a label 15mL centrifuge tube and then in a Ziploc bag for
storage in a freezer. Each sampling date and location should have its own Ziploc
bag.
• Filtered chlorophyll samples should be run on a fluorometer within 30 days of
freezer storage.
• Take note if supplies are running low or if there are issues that need to be cleared
up before the next sampling period.
Appendix B: Chlorophyll $a$ validation of \textit{in situ} fluorescence by pheophytin corrected \textit{in vitro} chlorophyll $a$ extraction for samples from high (A) and low tide (B) samples.

\begin{align*}
\text{A} & : \quad y = 0.8358x + 1.0516 \\
& \quad R^2 = 0.7052
\end{align*}

\begin{align*}
\text{B} & : \quad y = 0.5418x + 1.7781 \\
& \quad R^2 = 0.3169
\end{align*}